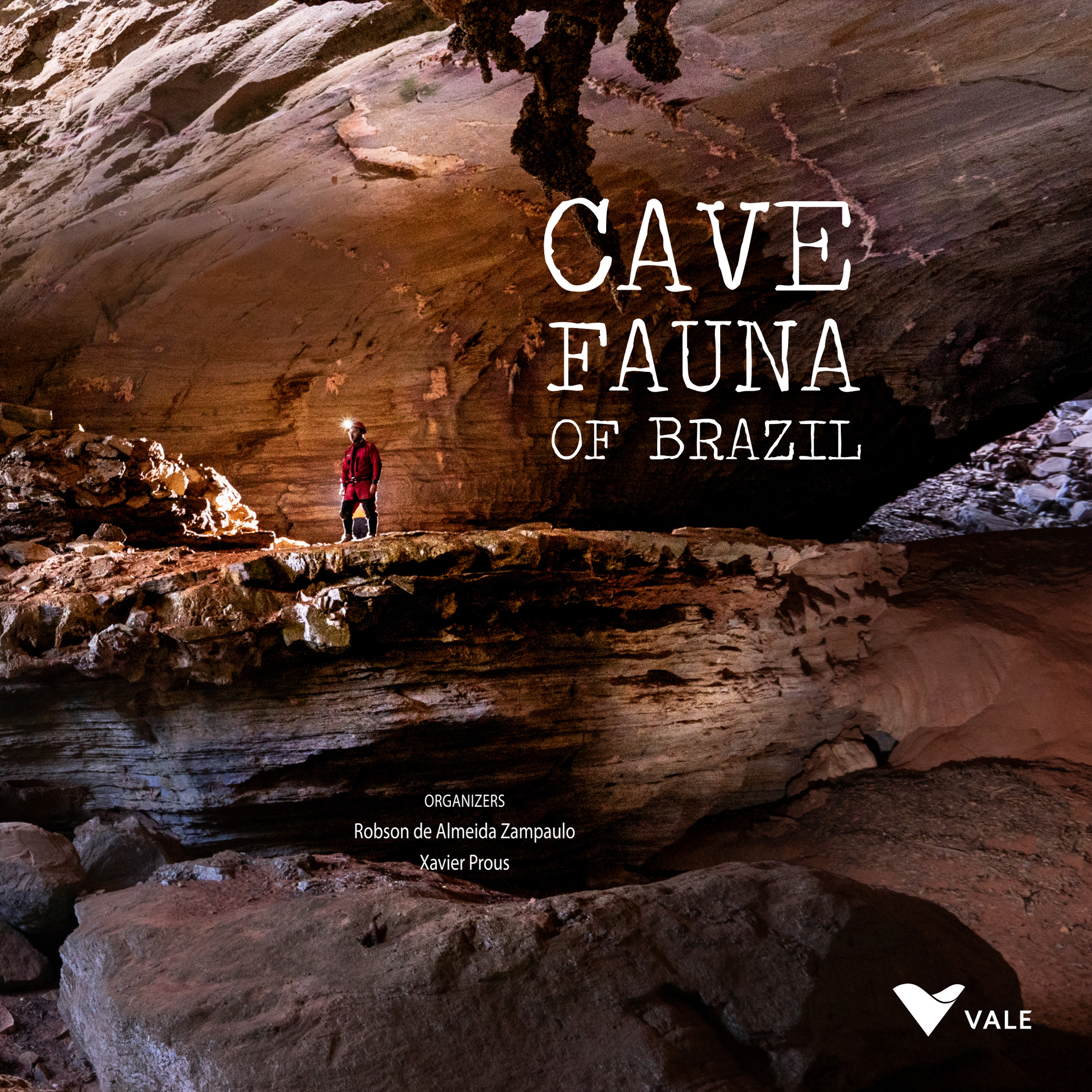


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Thecadactylus rapicauda
Robson de Almeida Zampaulo

presentation

Biological research in caves, of different lithologies, has provided significant advances in the knowledge of the biodiversity and functioning of subterranean ecosystems. Such advances are crucial for the evolution of environmental analysis relevant to speleology, whether in the context of environmental licensing or that of scientific research in general.

In this sense, Vale, in partnership with dozens of researchers from different institutions, promoted the organization of this book, which includes the main taxonomic groups and a significant part of the diversity of known species of subterranean fauna in Brazil. Throughout this precious work, it is possible to perceive the evolution of the theme in recent years, expressed by the dedication of everyone in the construction of each chapter and in the search for excellence portrayed in high quality photographs in scenarios of extreme ecological value and, many times, even untouched, which reflect the valuable biodiversity existing in the subterranean universe.

The results of years of research resulting from speleological monitoring and taxonomic studies, as well as cave relevance analysis work, are reported here. The result of a highly specialized effort that shares knowledge about the subterranean fauna, combined with beautiful images of several species in their natural environment (many of which are endemic, rare and unique), this wonderful book allows us to "dive" into the subterranean world and get to know a little more of the life present in these environments devoid of light and with few resources for the survival of these extremely adapted living beings.

In this way, and to perpetuate and disseminate the knowledge achieved through the arduous effort of scientists, consultants and private initiatives, Vale, through its technical staff, promotes with this work another step towards advancing the training of new professionals and specialists in Subterranean Biology. Undoubtedly, we have important information here that will certainly contribute to environmental licensing processes and to the evolution of this science in the country, corroborating the commitment to promote effective actions for environmental preservation and sustainable mining.

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preface

The publication of the work "*Essai sur les problèmes biospéologiques*", in 1907, by the Romanian zoologist Emil G. Racovitza, is considered an important milestone in the establishment of world Biospeleology (from the Greek *bios* = life; *spelaion* = cave; *logos* = study). The term Subterranean Biology is currently most common and can be defined as the science that is dedicated to the study of organisms that live in the subterranean environment, as well as the interactions between species and the relationships between organisms and the environment. This is because the subterranean environment is complex and vast, with caves being our main "window", but only one of the environments that make up this mosaic of ecosystems. Thus, the subterranean environment comprises the set of interconnected subsoil natural cavities, of variable sizes, formed by large networks of heterogeneous spaces that can be filled by air or water.

Approximately 22 thousand caves have been cataloged in the Brazilian territory to date, with the potential for more than one million, according to the most recent estimates. At least 591 species of fauna have been described from holotypes collected in these environments, of which 283 are considered troglobites, that is, endemic species that have evolved and have specific adaptations to survive in these unique ecosystems. However, there are still hundreds of species already known and waiting for scientific description, in addition to an incalculable number of species to be discovered.

In this context, we have gathered in 22 chapters the most up-to-date information on the main groups of cave fauna in Brazil, in addition to five initial chapters that bring historical aspects, legislation, ecosystem dynamics and areas of science still little explored, such as molecular biology and microbiology. Consolidating all the knowledge available in this work was an intricate task, in view of the significant increase in the number of professionals who are specializing and dedicating themselves to the study of our subterranean biodiversity, which, consequently, has resulted in a significant increase in publications on new species. The production capacity of our researchers is indisputable, and they certainly have a notable prominence internationally. Therefore, it is essential to invest financial resources in the training of specialists and in the implementation and maintenance of the infrastructure of current and new research centers, as well as in the maintenance of our valuable scientific collections. Only then will we be able to continue producing high quality knowledge about our caves and other subterranean environments.

Thus, we hope that this work will be an important reference for all those who are dedicated to the study of cave fauna in Brazil, whether in the context of academic research, environmental licensing or activities with speleology groups. We also hope that it will provide all readers a beautiful journey into a small part of our wonderful caves and the life that coexists there.

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Caverna Terra Ronca – São Domingos (GO)
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1

History and evolution of knowledge about the cave fauna of Brazil

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The beginning of Subterranean Biology in a global context

The history of Subterranean Biology in Brazil is recent when compared to that of other countries, especially those of Europe and North America (BARR, 1968; CAMACHO, 1992; PACKARD, 1888; ROMERO, 2009; SHAW, 1992; VANDEL, 1964). The first written records of cave species in the world correspond to some species of amphipods and fish that, despite dating from the 16th century, were described only recently, such as the Chinese fish *Sinocyclocheilus hyalinus*, discovered in 1540 and described only in 1994 (CHEN *et al.*, 1994; MA, ZHAO, YANG, 2019). Another important note is that of a prehistoric record of a cave cricket with morphology very similar to species of the genus *Troglophilus* Krauss, 1879, precisely carved in a bison bone found in the La Grotte des Trois-Frères cave, France (CHOPARD, 1928).

However, the most emblematic cave animal ever recorded corresponds to a species of blind and depigmented salamander, discovered in the 17th century by Baron Janez Vajkard Valvasor (VALVASOR, 1689) and described almost 80 years later in 1768 as *Proteus anguinus*, by the Austrian physician and naturalist Josephus Nicolaus Laurenti. This salamander corresponds to the first cave-dwelling species (troglobite or troglobionte) described for the world. More than three centuries since its description, numerous studies have been carried out involving this species, a symbol of global Subterranean Biology and an important part of the scientific research of renowned naturalists such as Carl Linnaeus, George Cuvier, Jean-Baptiste de Lamarck and Charles Darwin (ALJANČIČ, 2019).

While studies on cave fauna were driven by the exploration of large systems in some regions of the world (e.g., Mammoth Cave, United States; Postojna-Planina System, Slovenia) and by the publication of new species, such as *Dolichopoda palpa* (Sulzer, 1776); *Eurycea lucifuga* Rafinesque, 1822; *Leptodirus hochenwartii* Schmidt, 1832; *Amblyopsis spelaea* DeKay, 1842; *Stalita taenaria* (Schiödte, 1847) and *Niphargus stygius* (Schiødte, 1847), unusually the first scientific reference of a cave species in South America is also contemporary to this period, and corresponds to the description of the guácharo (oilbird) *Steatornis caripensis* Humboldt, 1817, a bird described from caves in Venezuela. It is the only representative of the family Steatornithidae Bonaparte, 1843, which, like bats, can orient itself in the dark by echolocation, allowing it to have nocturnal habits and shelter and reproduce in aphotic areas of caves (ROJAS *et al.*, 2004). Its distribution is now known to include other countries, such as Bolivia, Colombia, Ecuador, Guyana, Panama, Peru, Trinidad and Tobago and Brazil (BRINKLØV *et al.*, 2013; CISNEROS-HEREDIA *et al.*, 2012).

In the 18th and 19th centuries, dozens of cave species were

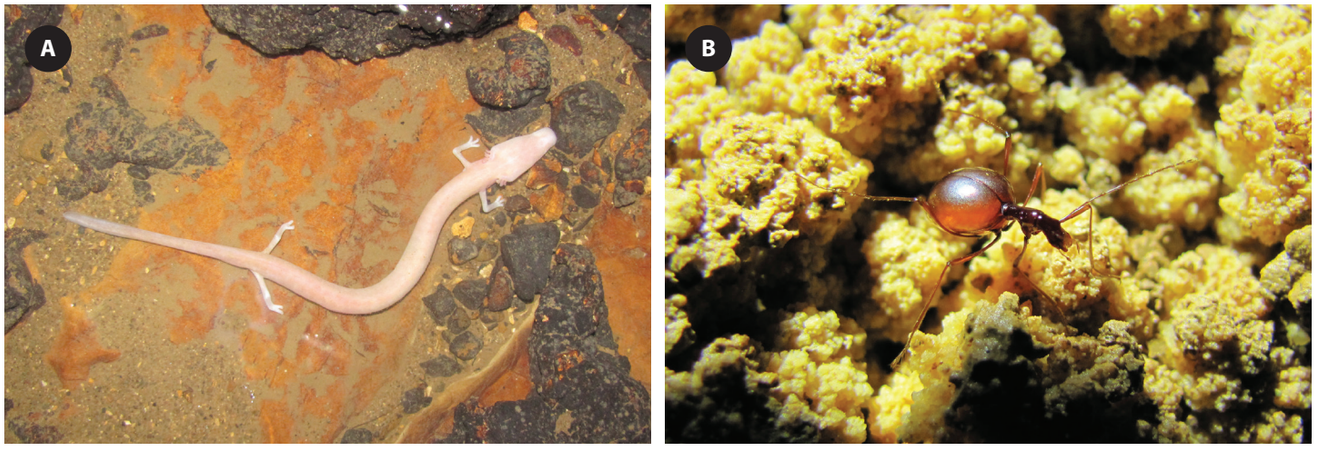


Figure 1. A) *Proteus anguinus* Laurenti, 1768, first troglobitic species described in the world; **B)** *Leptodirus hochenwartii* Schmidt, 1832, first troglobitic invertebrate species described in the world. Photos: (Rodrigo L. Ferreira).

described for Europe and North America and, although the first proposals for classifying cave fauna were created by Schiödte (1849), Schiner (1854) and Joseph (1882), the term biospeleology would only be introduced in 1904 by the Frenchman Armand Viré (VIRÉ, 1904). Armande Viré (1869-1951) was a great speleologist and collaborator of Édouard-Alfred Martel (father of speleology), having participated in explorations in different karst regions of Europe. Three years later, in 1907, the Romanian lawyer and zoologist Emil Gustave Racovitza (1868-1947, "father of modern biospeleology") published one of the most important works in global biospeleology – "*Essai sur les problèmes biospéologiques*" (Essay on biospeleological problems) – bringing, at that moment, a modern approach to this science and demystifying a series of incorrect generalizations in force at the time (MARTÍNEZ-GIL *et al.*, 2007).

Another important milestone in the history of this science corresponds to the creation of the *Laboratoire Souterrain de Moulis* (Centre National de la Recherche Scientifique - CNRS), in 1948 in France, which is probably the most famous example of a support structure for *in loco* research in the world (MAMMOLA *et al.*, 2021). Conceived by René Jeannel (1879-1965) and implemented by Albert Vandel (1894-1980), it is a large research infrastructure associated with the cave of the same name that allowed the study of different cave organisms in their natural environment, including research on the development and reproductive cycle of *P. anguinus*. Other models of subterranean laboratories were implemented in different regions of the world, including Brazil, whose experiment was carried out in the Areias System (Sistema Areias cave), in the state of São Paulo. Finally, in 1965, Vandel published the work "*Biospéologie, la biologie des animaux cavernicoles*" (Biospeleology: The biology of cavernicolous animals), which remains a reference work to this day.

The first studies on cave fauna in Brazil

In Brazil, the first publication of a cave species corresponds to a Passeriformes – *Scytalopus speluncae* (Ménétries, 1835) (Rhinocryptidae) – collected in a limestone cave (Gruta da Pedra or Casa de Pedra) in São João del-Rei, state of Minas Gerais (RAPOSO *et al.*, 2012). In turn, among invertebrates, the oldest record is the troglophilic harvestman *Eusarcus hastatus*, described in 1884 by the Danish arachnologist William Emil Sørensen (1848-1916) and collected in Lapa Vermelha cave in the municipality of Pedro Leopoldo, also in Minas Gerais. However, it was only in the first half of the 20th century that a greater number of species collected in caves were described, including the fish *Pimelodella kronei* (Miranda Ribeiro, 1907); the dipterans *Lutzomyia cavernicola* Costa-Lima, 1932, *Bruchomyia brasiliensis* Alexander, 1940 and *Micropygomyia peresi* (Mangabeira-Filho, 1942); the crickets *Eidmanacris speluncae* (Mello-Leitão, 1937) and *Endecous cavernicolus* Costa-Lima, 1940; the amblypygian *Trichodamon froesi* Mello-Leitão, 1940; the millipedes *Leodesmus yporangae* Schubart, 1946 and *Yporangiella stygius* Schubart, 1946; the spider *Ctenus fasciatus* Mello-Leitão, 1943; the harvestman *Daguerreia inermis* Soares & Soares, 1947 and the heteropteran *Amilcaria lapinhaensis* (Wygodzinsky, 1950). In addition to these, it is worth mentioning the description of the catfish *Phreatobius cisternarum* Goeldi, 1905, which, despite not being cave-dwelling, is an underground species found in shallow cisterns on the outskirts of the city of Belém and on Marajó Island, in the Amazon Basin (MURIEL-CUNHA; PINNA, 2005).

Although *P. cisternarum* was in fact the first subterranean animal described for Brazil, the "blind fish of Iporanga" – *Pimelodella kronei* – certainly corresponds to the most expressive discovery and is considered a milestone in the country's Subterranean Biology. This species is still treated as the first troglobitic (troglobite) cave animal described for South America, the study having been published in the same year that Emil Racovtiza would publish the work "*Essai sur les problèmes biospéologiques*". Blind and depigmented, this fish is endemic to one of the most important subterranean systems (set of caves) in Brazil, the Areias System. With more than 7 km of extension and more than one hundred years of research (TRAJANO, 2006), the Areias System, located in the municipality of Iporanga in the south of the state of São Paulo, currently has 28 troglobitic species and is considered a subterranean biodiversity hotspot in South America (SOUZA-SILVA; FERREIRA, 2016; TRAJANO, 2006).

Still in relation to *P. kronei*, an important part of the discovery of this species is attributed to one of the precursors of Brazilian

speleology, the German Sigismund Ernst Richard Krone (1861-1917), who was responsible for collecting the specimens used in its description. Richard Krone was a German naturalist who lived in the region of Vale do Ribeira (state of São Paulo), in the late 19th and early 20th centuries, and was responsible for the systematization of the first registry of Brazilian caves, organizing a list of 41 caves of this region between the years 1897 and 1909 (KRONE, 1898, 1909). Later, in the first half of the 20th century, this species was the target of an important study conducted by the geneticist Crodowaldo Pavan who, in his doctoral thesis, studied morphological, behavioral and evolutionary aspects of the species (PAVAN, 1946).

Subsequently, a very interesting study carried out in the 1950s was that of the researcher Augusto Ruschi who studied carbonate caves in the state of Espírito Santo – Grutas do Limoeiro (municipality of Castelo) and Monte Líbano (municipality of Cachoeiro do Itapemirim). In addition to carrying out an inventory and presenting illustrations of the bat species found in these caves, Ruschi also evaluated aspects related to shelter fidelity and the occurrence of other vertebrate species (including birds) and dealt with geological aspects of these environments, as well as providing comments on tourist potential and the state of conservation of the caves (RUSCHI, 1952). Augusto Ruschi (1915-1986) is considered "Patrono da Ecologia do Brasil" (Patron of Ecology of Brazil) and was an important naturalist. Researcher at the National Museum of Rio de Janeiro (MNRJ) and creator of the Museum of Biology Prof. Mello Leitão (MBML), in Santa Teresa (state of Espírito Santo), he collected bats in dozens of caves in that state, even contributing to studies on epidemiological aspects related to the transmission of rabies (MAIA; FRANCO, 2021).

From the second half of the 20th century onwards, numerous taxonomic works were carried out, resulting mainly from the study of biological material collected during expeditions undertaken by foreign biospeleologists in caves in the Southeast Region of Brazil, such as Tapagem cave (Caverna do Diabo cave) and the Areias System, both located in Vale do Ribeira. Among them, the Swiss biospeleologist Pierre Strinati stands out, who visited caves in approximately 70 countries and discovered hundreds of new species, publishing several scientific articles and books, being responsible for the first publications on the composition of the fauna of some Brazilian caves (SOUZA *et al.*, 2019; STRINATI, 1971; 1975).

Between 1950 and 1975, approximately two dozen cave species were described in Brazil, of which at least half were collected by Pierre Strinati, including some named in his honor. Among the species described for this period are the millipedes *Obiricodesmus rupestris* Schubart, 1956, *Sandalodesmus stramineus* Schubart, 1956, *Leptodesmus ravus* Schubart, 1956, *Peridontodesmella alba* Schubart, 1957 and *Pseudonannolene strinatii* Mauries, 1974; the harvestmen *Eugyndes patellaris* Soares, 1966, *Eusarcus maquinensis* Soares, 1966, *Spaeleoleptes spaeleus* Soares, 1966 and *Pachylospeleus strinatii* Silhavy, 1974; the cricket *Strinatia brevipennis* Chopard, 1970; the pseudoscorpion *Pseudochthonius strinatii* Beier, 1969; the beetle *Schizogenius ocellatus* Whitehead, 1972; the aeglids *Aegla cavernicola* Turkay, 1972 and *Aegla strinatii* Turkay, 1972, and the amphipod *Spelaeogammarus bahiensis* Brum, 1975. It is also important to note that at this stage there was the description of a third troglobitic fish species for the country, *Stygichthys typhlops* Brittan & Bohlke, 1965 (Characiformes), which, despite not being a cave-dwelling species, like *P. cisternarum*, is an extremely specialized fish for the subterranean environment and is found in cisterns in the northern region of Minas Gerais. This species is considered "Data Deficient" by the International Union for Conservation of Nature (IUCN) and "Endangered" by the official list of endangered species in Brazil (BRASIL, 2022; IUCN, 2022; MOREIRA *et al.*, 2010; ROMERO; MCLERAN, 2000).

Another important milestone in the 1970s was the attempt to implement the first and, so far, only subterranean laboratory installed in Brazil. This experiment was built in the resurgence of the Areias System (Gruta Laboratório cave), in Parque Estadual Turístico do Alto Ribeira (PETAR), in 1974, and was coordinated by the French speleologist Guy-Christian Collet (1929-2004) and inspired by international experiences such as the Laboratório de Moulis in France (COLLET, 1985). In addition to biological studies, this laboratory aimed to carry out research on, for example, microclimate and speleothem genesis.

Finally, for this period, it is still worth mentioning the publication of two scientific works related to the spider fauna of Brazilian caves. The first, published by Brignoli (1972), brings a compilation of spiders collected by Pierre Strinati, in caves in South America (Argentina, Brazil, Uruguay and Venezuela). The second, published in the journal "Memórias do Instituto Butantan", in 1975, brings a compilation of records of three species of spiders collected by members of the Centro Excursionista Universitário (CEU), in limestone caves in the region of Iporanga (state of São Paulo) and includes the redescription of *Ctenus fasciatus* Mello-Leitão, 1943 (EICKSTEDT, 1975).

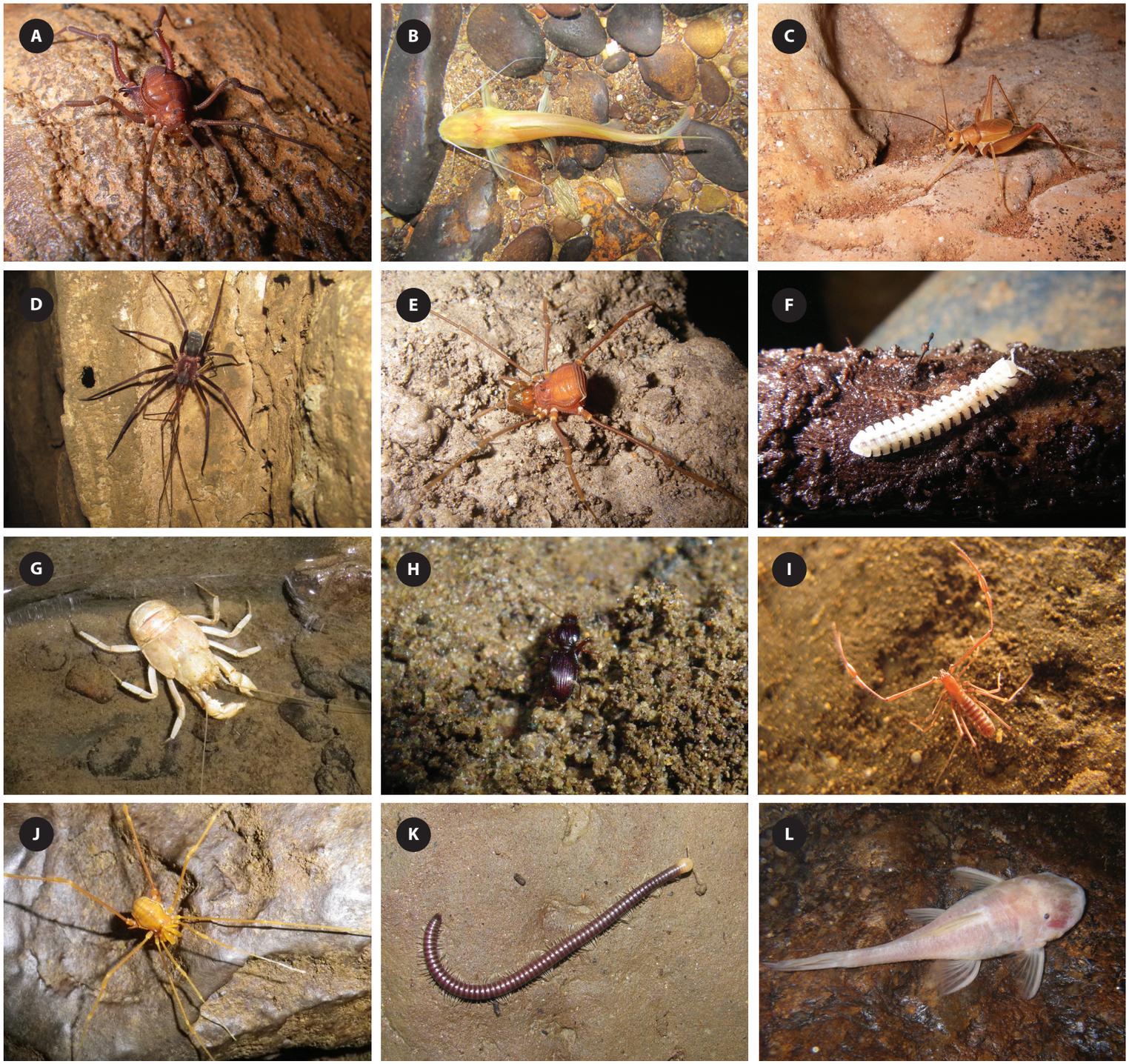


Figure 2. Historical species described for Brazilian caves. **A)** *Eusarcus hastatus* Sorensen, 1884; **B)** *Pimelodella kronei* (Miranda Ribeiro, 1907); **C)** *Endecous cavernicolus* Costa-Lima, 1940; **D)** *Ctenus fasciatus* Mello-Leitão, 1943; **E)** *Daguerreia inermis* Soares & Soares, 1947; **F)** *Yporangiella stygius* Schubart, 1957; **G)** *Aegla cavernicola*, Turkay, 1972; **H)** *Schizogenius ocellatus* Whitehead, 1972; **I)** *Spelaeobochica muchmorei* Andrade & Mahnert, 2003; **J)** *Pachylospeleus strinatii* Šilhavý, 1974; **K)** *Pseudonannolene strinatii* Mauries, 1974; **L)** *Ancistrus cryptophthalmus* Reis, 1986. Photos: A, C, E-H, J, L (Robson de A. Zampaulo); B, I, K (Rodrigo L. Ferreira).



Figure 3. Historical images of the implantation and general appearance of the underground laboratory built in the resurgence of the Areias System (Gruta Laboratório cave) in Parque Estadual Turístico do Alto Ribeira (PETAR) during the 1970s. Photos: Acervo Guy-Christian Collet/ Sociedade Brasileira de Espeleologia (SBE).

The 1980s and 1990s and the implementation of the main research centers in the country

In the 1980s, the publication by Eliana Maria Beluzzo Dessen and collaborators from the Centro Excursionista Universitário (CEU) is considered an important milestone in Brazilian Subterranean Biology as it gathers information on the fauna of 28 caves in five karst regions of the country (DESSEN *et al.*, 1980). It is also interesting to note that, in 1979, this same author published a note in the magazine "Espeleo-Tema", of the Brazilian Sociedade Brasileira de Espeleologia (SBE), highlighting the importance of organizing biospeleology in the country. Among its main recommendations are: i) Dissemination and scientific publication for training new professionals interested in this science; ii) Organization of a unified database that would allow adequate planning of future research; iii) Exchange and collaboration between different research groups; and iv) The participation of taxonomists in research on subterranean fauna (DESSEN, 1979).

Subsequently, relevant studies on the composition and structure of cave communities were carried out in different karst areas of the country (CHAIMOWICZ, 1984, 1986; GNASPINI-NETO, 1989; GNASPINI; TRAJANO, 1994; GODOY, 1986; PINTO-DA-ROCHA, 1994, 1995; TRAJANO, 1984, 1987, 1989; TRAJANO; GNASPINI, 1991; TRAJANO; GNASPINI-NETO, 1986; TRAJANO; MOREIRA, 1991). During this period, approximately 40 species of different taxa were described, including species of amphipods, beetles, springtails, crustaceans, millipedes, dipterans, scorpions,

isopods, fish, crickets and moths.

In the 1990s, important research groups were consolidated at some of the main institutions in the country, among which it is worth mentioning those linked to the Instituto de Biociências (IB) of the Universidade de São Paulo (USP), coordinated by Eleonora Trajano, Pedro Gnaspini and Ricardo Pinto-da-Rocha. This triad was responsible for guiding dozens of academic works on cave fauna, especially in the 1990s and 2000s, and for the training of renowned biospeleologists and taxonomists. Among them, we can highlight those who continued this line of research, such as Maria Elina Bichuette, currently coordinator of Laboratório de Estudos Subterrâneos (LES) (https://www.lesbio.ufscar.br/) at the Universidade de São Carlos (UFSCar) and Leila Aparecida Souza, now a researcher at the Universidade Estadual do Ceará (UECE).

Later, other groups were also consolidated, such as the Laboratório de Sistemática de Collembola e Conservação, coordinated by Douglas Zeppelini, at the Universidade Estadual da Paraíba (UEPB) and the Laboratório de Coleções Zoológicas of the Instituto Butantan, coordinated by Antonio Domingos Brescovit. Although these are not specific subterranean fauna research groups, they stand out for the expressive number of cave species published in the last 20 years. Finally, it is worth mentioning the Centro de Pesquisas em Biologia Subterrânea (CEBS) of the Universidade Federal de Lavras (UFLA), coordinated by researchers Rodrigo Lopes Ferreira and Marconi Souza-Silva, today one of the main research centers in Subterranean Biology with publications in the areas of ecology, microbiology, taxonomy, paleontology and environmental education (https://www.biologiasubterranea.com.br/pt/). Currently, CEBS and LES are the two main research centers in Subterranean Biology in the country, responsible for the discovery and description of an expressive portion of Brazilian cave fauna and training of numerous specialists in this line of research. In addition, among the main zoological collections, the Coleção de Invertebrados Subterrâneos de Lavras (ISLA) and the Museu de Zoologia da USP (MZUSP) currently have approximately 50% of the holotypes of the species described for caves in Brazil. Considering the collections of Instituto Butantan and Universidade Estadual da Paraíba (UEPB), these institutions together hold 70% of the country's cave holotypes. In this way, investment in the consolidation and maintenance of these and other collections that safeguard a significant part of Brazil's subterranean biodiversity is considered essential.

Also for this period, it is important to highlight the valuable contribution of Eleonora Trajano and Maria Elina Bichuette to knowledge of the cave ichthyofauna of Brazil. Thirty-seven species of troglobitic fish are currently known for the country, of which 22 have been described, representing the second largest diversity of subterranean fish in the world, behind only China (BICHUETTE, 2021; NIEMILLER *et al.*, 2019).

In addition to the establishment of these and other research groups, changes in speleological legislation in the last 15 years were fundamental for a significant increase in speleological studies in the context of environmental licensing, as well as investment in research, which has provided a significant evolution of knowledge about the country's cave fauna, as will be discussed below.

Changes in Brazilian speleological legislation and its influence on the knowledge of cave fauna

Despite caves being an integral part of the environment and that their use should have been regulated since the first regulatory acts in Brazil, it wasn't until the 1960s and 1970s that environmental preservation began to gain greater importance. During this period, the country became a signatory to international agreements on the subject and published legislation to protect these assets, such as Decree Nº 58.054, of 23 March 1966, which "Promulga a Convenção para a proteção da flora, fauna e das belezas cênicas dos países da América" (Enacts the Convention for the protection of the flora, fauna and scenic beauty of the countries of America; BRASIL, 1966), the Lei de Proteção da Fauna (Fauna Protection Law) Nº 5.197, of 3 January 1967 (BRASIL, 1967) and participation in the Stockholm Convention of 1972, as examples.

In 1987, Resolution Nº. 005 of the Conselho Nacional do Meio Ambiente (CONAMA) was published. Considering the need to implement the Programa Nacional de Proteção ao Patrimônio Espeleológico (National Speleological Heritage Protection Program), prepared by a Comissão Especial (Special Commission) instituted through CONAMA Resolution Nº. 009, of 24 January 1986, Resolution Nº. 005 established ten fundamental actions for the protection of Brazilian speleological heritage. Included among these were actions to protect the blind catfish of Iporanga (*P. kronei*), the obligation to prepare environmental impact studies in cases of projects potentially harmful to national speleological heritage and the execution of speleological surveys in the region of Carajás and in Altamira (state of Pará) to protect the, until then, "rare iron ore and sandstone caves existing in these two regions". Later, the caves came to be considered as assets of the Union

and Patrimônio Cultural Brasileiro (Brazilian Cultural Heritage), according to the Federal Constitution of 1988 and Federal Decree 99.556 of 1990 (BRASIL, 1988; 1990).

It is worth mentioning that some important goods of economic interest (water, iron ore and limestone, for example) are located in regions with high potential for the occurrence of caves and/or in lithologies that were still little studied at that time, such as caves in ferruginous rocks, for example. Although the first report on the biology of caves in the Serra dos Carajás region, state of Pará, was presented in 1988 (MOREIRA; PAIVA, 1988), there have been no additional robust studies in Brazil on the fauna of caves in ferruginous lithology. The first more consistent publication was carried out by Ferreira (2005) for the Quadrilátero Ferrífero (Iron Quadrangle) region, in Minas Gerais, as a result of studies for environmental licensing in areas of the former Minerações Brasileiras Reunidas (MBR). This work described a highly diverse fauna, with dozens of troglobitic species, and was, in a way, unexpected, since caves in this lithology have small dimensions and few actually have aphotic zones (relative to the ca. 3,000 currently-known caves; CECAV, 2020). Therefore, the implementation of alternatives that allowed reconciling the preservation of speleological heritage and socioeconomic development became necessary.

Thus, in the following years, almost a dozen regulatory acts were published. The main change was introduced by Federal Decree 6.640 of 2008, which reworded Federal Decree 99.556 of 1990. In this decree, the possibility of irreversible negative impacts on natural subterranean cavities (caves) was established. The document required prior studies to classify caves according to their degree of relevance, following guidelines presented in normative instructions published later. In this context, only caves classified as of Maximum Relevance remained protected. This change was harshly criticized and considered a setback for cave conservation in the country (FIGUEIREDO *et al.*, 2010). In fact, it is worth considering that the new legislation became "permissive" and, at that time, it was not possible to know what the true consequences of this decision would be for Brazilian speleological heritage. From the point of view of subterranean biodiversity, the loss of habitats with the suppression of caves and rock massifs became one of the points of greatest concern because, until that time, it was not possible to quantify the impacts resulting from this change.

However, on the other hand, the obligation to study caves made knowledge about Brazilian cave fauna evolve exponentially, mainly due to the greater availability of resources (financial and human) for licensing studies, research and the description of new species. Normative Instruction MMA Nº. 2, of 30 August 2009 (BRASIL, 2009), which regulated the application of Federal Decree 6.640, of 2008, required seasonal sampling during at least two periods (dry and rainy) and established the parameters that should be evaluated, such as the presence of troglobitic species and the presence, richness and abundance of new taxa and rare and endangered species.

Thus, these changes in legislation and the need for incentives from the private sector may have, directly or indirectly, accelerated the discovery and description of new species, since Normative Instruction Nº. 2 of 2009 even prohibited irreversible negative impacts in caves with the presence of new taxa until their formal description, through the publication of a scientific article. Therefore, the occurrence of new taxa required the description of these new species, which increased the incentive of the private sector both for the publication of species, and for the formation of specialists on the different taxonomic groups, the organization of scientific collections and greater speed in publication.

By consulting the articles describing the species listed in this chapter (Table 2), it can be seen that until 1990 the description of a new species took, on average, 10 years to be published. Between the 1990s and 2010s, the time shrunk to about six years. In the last two decades, new species began to be described, on average, three years after their discovery. In addition, some articles have described several species in the same publication, as a result of organized collections and a large volume of material deposited in these institutions. As an example, Cipola *et al.* (2020) described 23 species of the order Collembola (genus *Pseudosinella* Schaeffer, 1897) in a single publication. Another taxonomic group that had a high number of species described by works of this nature is the spiders. Brescovit *et al.* (2018), Brescovit and Cizauskas (2019) and Passanha *et al.* (2019) described, respectively, seven species of the genus *Ochyrocera* Simon, 1892, seven of the genus *Matta* Crosby, 1934 and six of the genus *Tonton* Passanha, Cizauskas & Brescovit, 2019.

Another way of generating knowledge through the public-private initiative is the contribution of financial resources from speleological compensation aimed at studies on Brazilian subterranean fauna, either through public notices for research projects or postgraduate scholarships. Many species have been described from actions of this nature, in addition to more general research with ecological approaches. One of the recent examples is the study of Rabelo *et al.* (2018), who, based on vulnerability mapping of some areas, proposed 48 caves in north-central Minas Gerais as priorities for conservation.

Furthermore, in this work, the authors identified 86 troglobitic species, most of which were unknown at that time.

However, in January 2022, the last relevant change took place in the legislation dealing with speleological heritage. The publication of Federal Decree Nº. 10.935 made possible the irreversible negative impact (including suppression) of Maximum Relevance caves, that is, caves with the most unique attributes provided for in the legislation. This decree, however, has been partially suspended by the Supremo Tribunal Federal (STF; Federal Supreme Court). In this new context, only caves with rare or endangered species would be protected, since this new decree does not consider the net loss of species, that is, their extinction, permissible (BRASIL, 2022).

Evolution in the number of species described for Brazil

Given this context of organization of research groups in Subterranean Biology and changes in legislation over time, here we present a systematic survey of the number of species described from holotypes collected in Brazilian caves, including troglobitic and non-troglomorphic species that can be classified in other ecological-evolutionary categories. Thus, species that have been proven to be subterranean, but that were not necessarily collected in caves, were also included in this survey, such as fish of the genera *Phreatobius* and *Stygichthys*, for example. Although this survey represents the most refined scenario on Brazilian cave fauna since the publication of Gallão and Bichuette (2018), we obviously do not intend to exhaust the subject. Here we only intend to provide a general scenario in the country from a literature review that considers publications until July 2022.

Regarding the level of dependence of these species on the subterranean environment, it is worth remembering that cave organisms can be classified according to their ecological-evolutionary status. In general, all proposed classifications are derived from the Schiner-Racovitza System. In this sense, Sket (2008) presented a review of the history, concepts and applicability of the different categories.

Although there are new approaches on the subject (e.g., TRAJANO 2017; TRAJANO & CARVALHO, 2017), the concepts used by American researchers (e.g., BARR, 1968) have been applied in Brazil, including in speleological legislation. Thus, cave species are generally divided into four categories: troglobites, troglophiles, trogloxenes, and accidental. These categories have equivalences with the categories listed by Sket (2008), as shown in Table 1.

Troglobitic species are known to have morphological, physiological and behavioral adaptations related to life in subterranean environments. Such morphological adaptations were called troglomorphisms by Christiansen (1962), with the main changes found in the most different taxa being the reduction or absence of pigmentation, eyes and wings and

Table 1. Classifications and definitions most used for subterranean fauna in Brazil.

Sket (2008)		Brazil	
Troglobiont	Strong connection with hypogean environments	Troglobite (Troglobionte)	Occurrence restricted to the subterranean environment
Eutroglophile	Essentially epigean species able to maintain a permanent ubterranean population	Troglophile	Capable of completing their entire life cycle in the hypogean and/or epigean environment
Subtroglophile	Species inclined to perpetually or temporarily inhabit subterranean habitat, but needs epigean habitat for some biological functions (*e.g.*, feeding)	Trogloxene	They must necessarily leave caves to complete life cycle
Trogloxene	Species only occurring sporadically in a hypogean habitat and unable to establish a subterranean population	Accidental	Penetrate the cave environment, but do not show any pre-adaptation that provides for survival inside the caves

the elongation of locomotor and sensory appendages (legs, antennae and other sensory organs). It is important to note that several troglobitic species are troglomorphic, but not all, as mentioned by Christiansen (2012). Thus, it is important to know the actual distribution and habitat occupied by the species before defining its ecological-evolutionary status, as well as compare possible troglomorphisms of cave-dwelling species with their epigean phylogenetic relatives – for review, see Deharveng and Bedos (2018).

Due to the morphological, physiological and behavioral specializations derived from the selective pressures of the subterranean environment, and to their restricted distribution and low population abundance, any troglobitic species should be considered, at least, as Vulnerable to extinction, according to the International Union for Conservation of Nature (IUCN). However, as there are very few studies on the natural history of cave species and not all troglobitic species present evident troglomorphisms, we must recognize that the classification of a species can be a complex activity and that it may require more investment in research before its categorization becomes possible (SKET, 2008). Therefore, in this chapter, the evolutionary-ecological classification of species considered only the information available in the original description or in the chapters of this book.

In total, we identified 591 species described for caves in Brazil, of which 283 (47.9%) are categorized as troglobites and 308 (52.1%) as in the other categories. Considering the evolution of descriptions, it is possible to identify two important milestones that considerably increased the list of new species. The first is the increase in the number of species described during the 1990s and early 2000s, a reflection of the formation of the first research groups in Subterranean Biology in the country and, consequently, an increase in the number of sampled caves. Previous to this time, there were only 82 described cave species. The second milestone was the change in Brazilian legislation dealing with the protection of caves (Federal Decree 6.640, of 2008). Prior to 2008, there were 153 described species; a number that has tripled in the subsequent 14 years. The evolution of

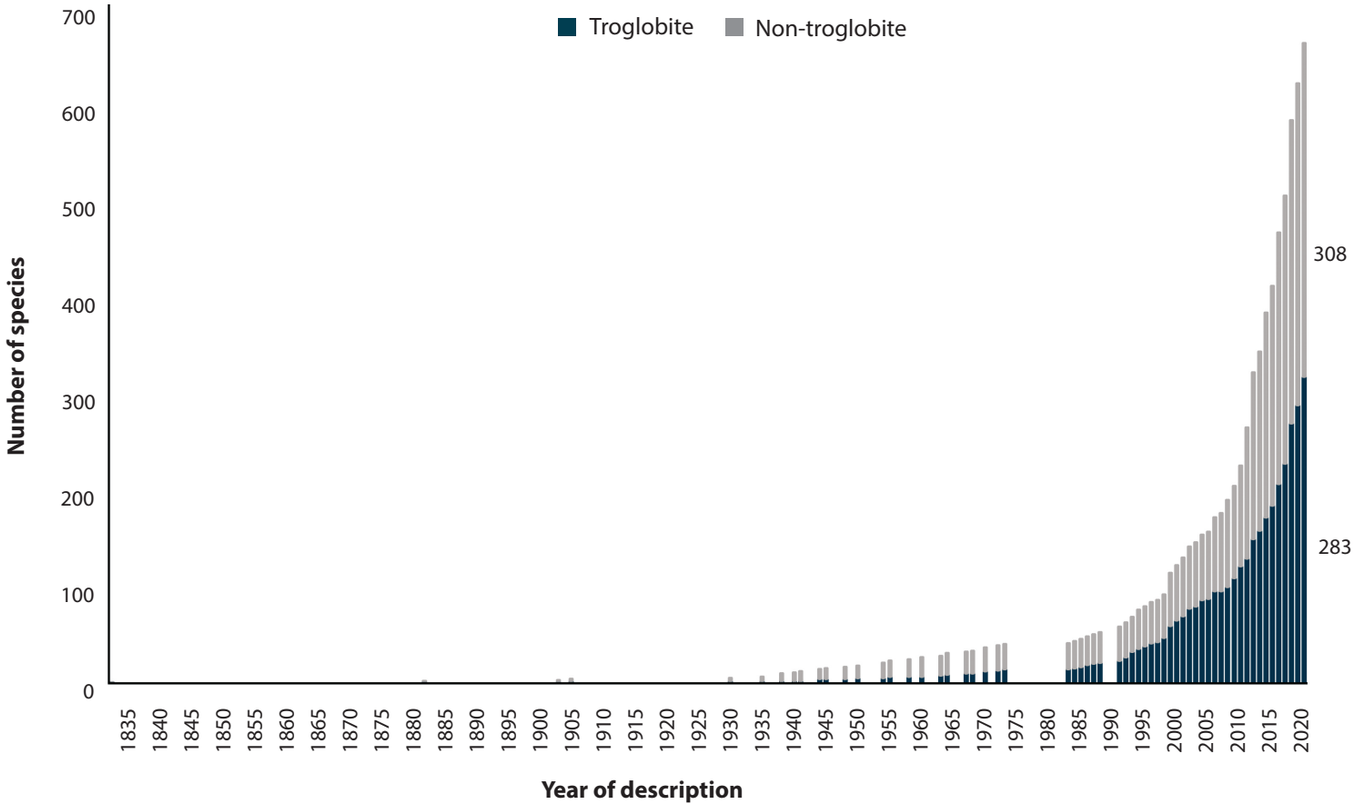


Figure 4. Evolution of the number of described subterranean species in Brazil over more than a century of research. The graph shows the increase in descriptions since the 1990s and after 2008, after the publication of Federal Decree 6.640.

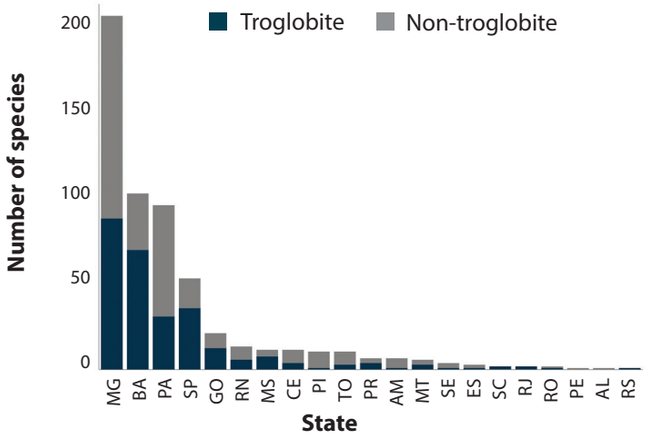


Figure 5. Number of cave species described by Brazilian state and proportion of troglobite and non-troglobite species.

cave taxa descriptions each year since the description of the first species can be seen in Figure 4.

The species described so far were collected in caves in 21 of the 27 Brazilian states. The states with the highest number of described species are Minas Gerais and Bahia, with 213 (36%) and 106 (17.9%), respectively (Figure 5). These are also the two states with the highest number of troglobitic species, with 91 and 72, respectively.

Figure 6 shows the result of the Kernel density estimator (KernelDensity) for the distribution of type localities of cave species in Brazil. It is possible to observe that the highest concentration of the described species overlaps with the main karst regions of Brazil, especially the Açungui group, in the southern region of the state of São Paulo, and the Bambuí and Una groups (including the Serra do Ramalho), in the states of Minas Gerais and Bahia. In addition to these, it is worth mentioning the iron regions of Carajás, in the southeast of the state of Pará, and the Quadrilátero Ferrífero, in the south center of Minas Gerais. These iron regions together represent about 25% of the species described for Brazil, despite having a small extension when compared to carbonate regions.

Regarding lithology, 368 species (62.3%) were described for caves inserted in carbonate rocks, 141 (23.9%) in ferruginous rocks, 69 (11.7%) in siliciclastic formations, only 11 species (1.9%) in granitic caves (Figure 7) and two (0.3%) in caves of an unidentified lithotype. The Areias System, one of the three subterranean biodiversity hotspots in Brazil, has the highest number of described species with 19, a number much higher than the other caves. The caves with the next highest number of described species are Gruta Olhos D'Agua cave, in Itacarambi, Minas Gerais, and Toca do Gonçalo cave, in Campo Formoso, Bahia, with seven species each (Figure 8).

Among the taxonomic groups with the greatest diversity of described species, whether troglobitic or non-troglobitic species, are Araneae, Collembola and Isopoda, with 74 (31 troglobites), 55 (28 troglobites) and 54 (40 troglobites) species, respectively (Figure 9). Figure 9 shows the richness of species described for different taxonomic groups, as well as the proportion of endangered species, according to MMA Ordinance Nº. 148, of 7 June 2022 (BRASIL, 2022). Among the 591 species, 166 (28.1%) are on the list of threatened species of Brazilian fauna, with 12.7% being Critically Endangered (CR), 8.3% Endangered (EN) and 6.9% Vulnerable to extinction. The species *Hyalella imbya*, endemic to the municipality of Roque Gonzales, state of Rio Grande do Sul, was classified as Probably Extinct (CR-PEX). In addition, the species *Yporangiella stygius, Trogolaphysa hauseri, Troglorhopalurus lacrau* and *Coarazuphium bezerra*, despite not being included in the list, were considered as Data Deficient (DD) during the species evaluation process, and thus also have some degree of threat, according to CONABIO Resolution Nº. 8, of 8 December 2021 (BRASIL, 2021).

Knowledge gaps to be filled

Although Brazil has an expressive number of species described with holotypes from its caves, there are still several knowledge gaps to be filled. Hundreds of species already collected and deposited in scientific collections are still waiting for description, even those from taxonomic groups for which there are specialists in full swing. A good example of this deficit is the harvestmen, one of the groups with the highest diversity of cave species and with excellent researchers working in the country and dedicating themselves to studying Brazilian species. In a checklist on the diversity and distribution of species of the superfamily Gonyleptoidea, Ázara and Ferreira (2018) concluded that at least 30% (46 species) of the species collected and deposited in scientific collections have yet to be described.

Inventories of cave fauna in Brazil have revealed a high diversity of groups that are still poorly studied. This large number of still unknown species has been recurrent for groups such as insects of the orders Archaeognatha, Blattodea, Diptera, Formicidae, Isoptera, Lepidoptera and Zygentoma; the entognates Diplura; some groups of centipedes and millipedes; and species of Acari, Clitellata, Gastropoda, Nematomorpha and even land planarians are other examples (BICHUETTE *et al.*, 2019;

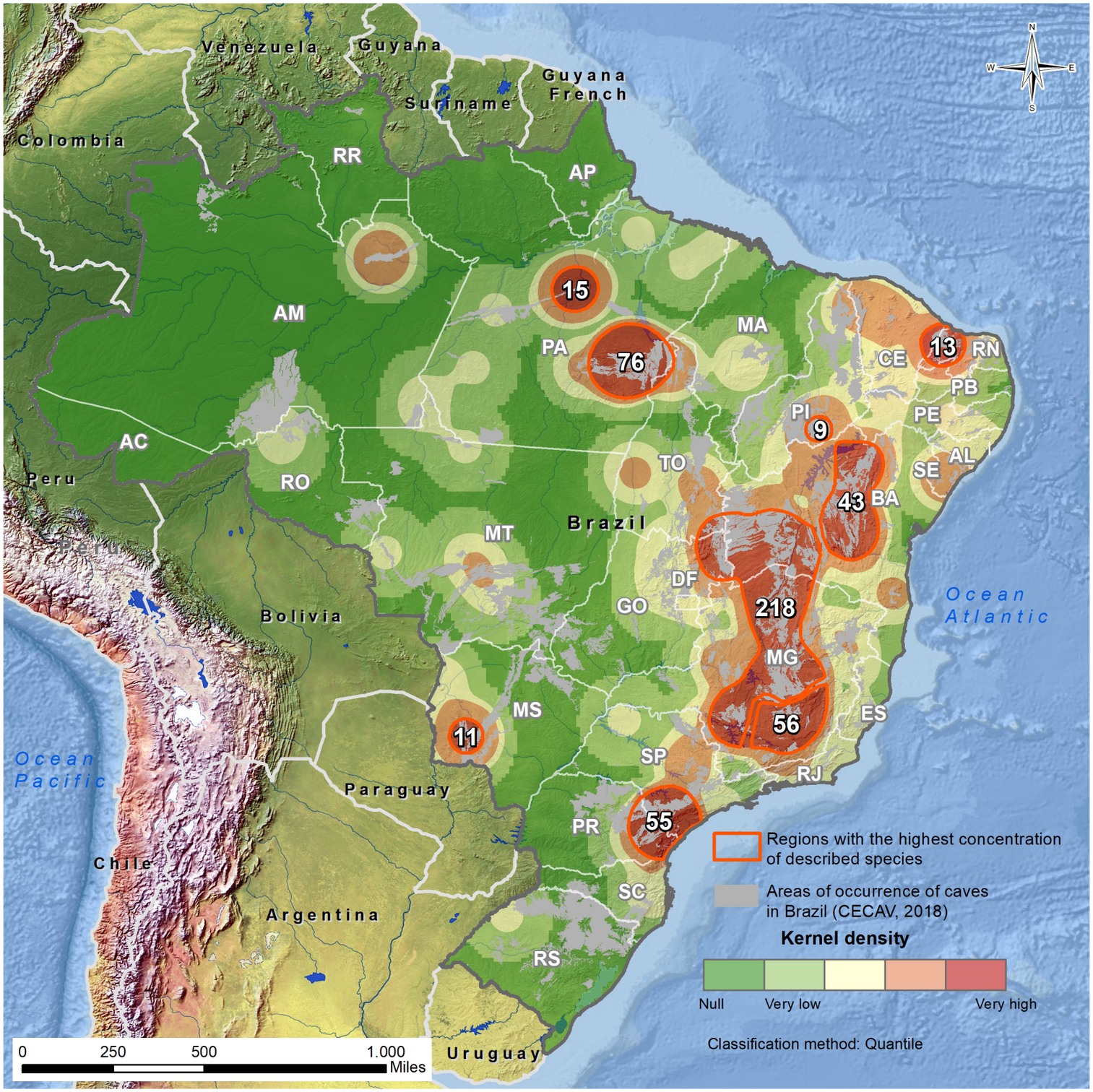


Figure 6. Heat map (Kernel Density) representing the number of species described by region for Brazilian caves.

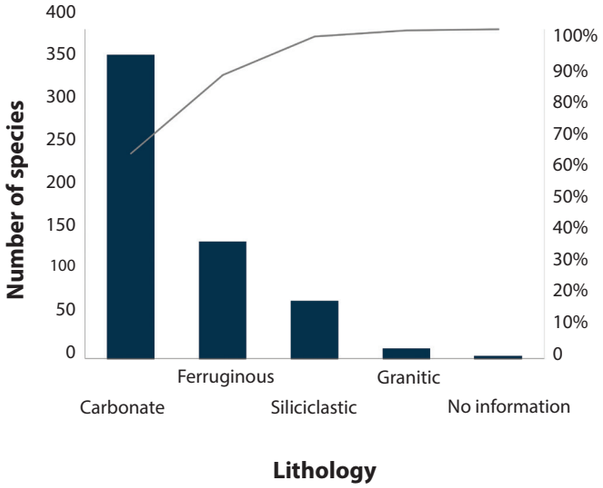


Figure 7. Number of described species by lithology where the cave (type locality) is located.

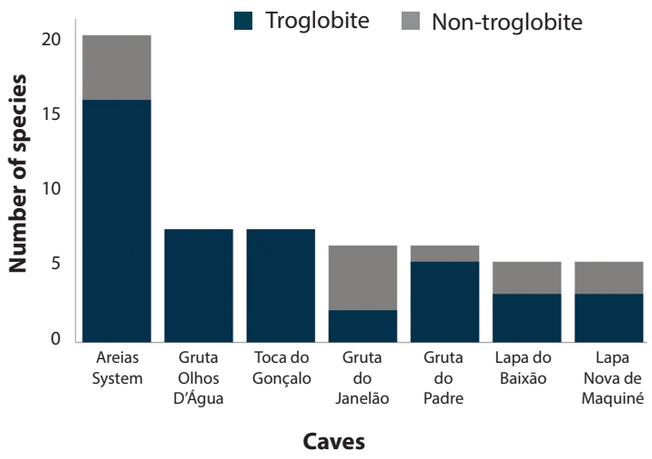


Figure 8. Caves with the highest number of described species and proportions of troglobitic and non-troglobitics species.

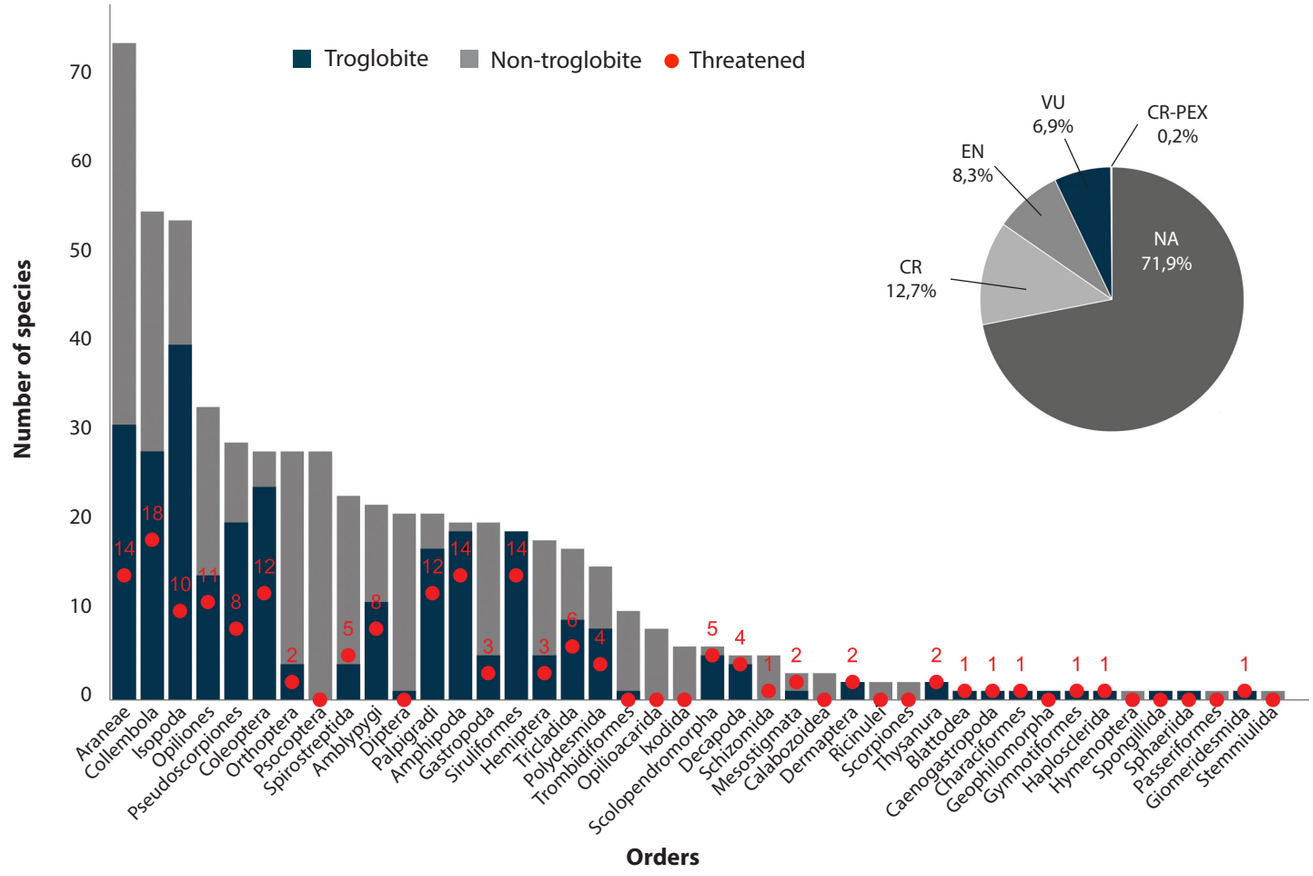


Figure 9. Number of species described for each taxon, indicating the proportion of troglobites and non-troglobites. The value in red indicates the total number of species classified in one of the IUCN threat categories. The pie chart indicates the percentage of species Not Evaluated or Not Threatened (NA), Critically Endangered (CR and CR/PEX), Endangered (EN), Vulnerable (VU) and Data Deficient (DD) according to the Ordinance MMA No. 148 of June 8, 2022 (BRAZIL, 2022).

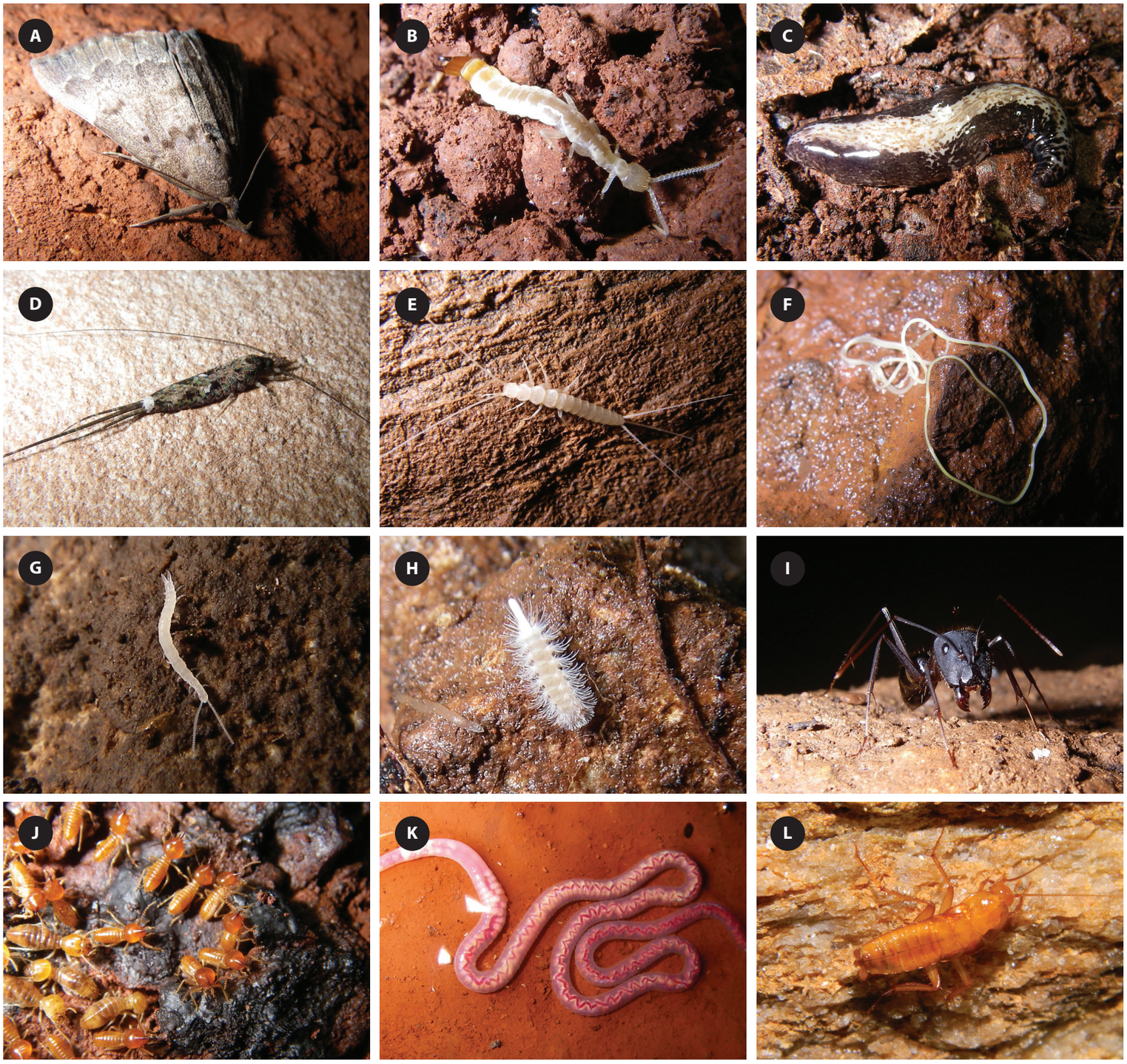


Figure 10. Some specimens of taxonomic groups little studied in Brazilian caves. **A)** Lepidoptera (Noctuidae); **B)** Diplura (Japygidae); **C)** Tricladida (Geoplanidae); **D)** Archaeognatha (Meinertellidae); **E)** Zygentoma (Nicoletiidae); **F)** Gordioidea; **G)** Symphyla; **H)** Polyxenida; **I)** Hymenoptera (Formicidae); **J)** Blattodea (Termitidae); **K)** Clitellata; **L)** Blattodea. Photos: A-K (Robson de A. Zampaulo); L (Matheus H. Simões).

CHAGAS-JR; BICHUETTE, 2018; FERREIRA *et al.*, 2016; SIMÕES *et al.*, 2015; SOUZA-SILVA; FERREIRA, 2015; TRAJANO *et al.*, 2016) (Figure 10). When we consider only troglobitic species, the scenario is also challenging. A good example is the three current Brazilian subterranean biodiversity hotspots. Of the 28 troglobitic species in the Areias System, only 15 (53%) are described; of the 22 species in Toca do Gonçalo, only seven (31.8%) are described; and among the 30 species already collected in the Águas Claras System, only eight are described (26.6%) (SOUZA-SILVA; FERREIRA, 2016; SOUZA- SILVA *et al.*, 2021).

Another relevant fact to be highlighted is that most troglobitic species described in Brazil were collected in caves, and only five (0.9%) were described from samplings in other subterranean habitats. The existence of a great diversity of subterranean habitats and micro-habitats is notorious, often inaccessible to humans, yet they harbor a high diversity of species (CULVER; PIPAN, 2013; HOWARTH; MOLDOVAN, 2018). Aquatic habitats (e.g., the epikarst, water table, lakes or even rivers and streams) and micro and mesocaves, in most cases, are not part of the sampled area of studies on Brazilian subterranean fauna. In iron formations in Australia, for example, the number of species described through collections carried out in geological drilling holes is high (GUZIK *et al.*, 2010; HALSE *et al.*, 2014; HALSE; PEARSON, 2013). Although the first subterranean species in Brazil was found in artesian wells (cisterns) connected to the water table (*Phreatobius cisternarum* Goeldi, 1905), these environments remain practically unknown and only recently we published a troglobitic species collected in Shallow Subterranean Habitats (SSH), through samplings in drilling holes in the Quadrilátero Ferrífero region in Minas Gerais (ZEPPELINI *et al.*, 2018).

In addition to the existing knowledge gaps on Brazilian cave fauna, it is important to mention that for other taxonomic groups (fungi and bacteria, for example) studies are even scarcer (see chapter on Microbiome Diversity). A recent study showed the existence of a high diversity of fungi of the genus *Cladosporium* associated with a bat cave in Northeast Brazil, including the discovery and description of two new species (PEREIRA *et al.*, 2022).

In fact, there are still a few thousand undescribed species of taxonomic groups that are little explored and without specialists. Therefore, the existence of sensitive gaps for the preservation of Brazilian subterranean fauna is evident, since the discovery and description of a species is the first step towards its conservation (CARDOSO *et al.* 2011). In addition, the absence of studies in other subterranean environments, and the fact that Brazilian legislation exclusively protects caves, makes it impossible to measure the biodiversity that is threatened in these environments. Thus, the conservation of Brazilian subterranean fauna remains challenging, even with the great advance of knowledge in recent decades. Overcoming knowledge gaps, weaknesses in legislation and the need for economic development are major challenges, as effective fauna conservation strategies require robust studies with adequate, long-term methodological projects that consider dialogue between all parties involved (researchers, government agencies, society and decision makers), and the best technical and scientific information available (MAMMOLA *et al.*, 2022). Therefore, we consider it important that initiatives and public-private partnerships be stimulated and directed to fill these gaps and that, with government incentive in basic and applied research, it is possible to increase knowledge about subterranean biodiversity and the sustainable development of the country.

Table 2. List of species described from material collected in Brazilian caves. * indicates species described as troglobites. The acronym in parentheses after the author of the description indicates the degree of threat: Critically Endangered (CR), Probably Extinct (CR/PEX), Endangered (EN), Vulnerable (VU) and Data Deficient (DD).

INVERTEBRATES				
	Taxon	Family	Species	Locality
Acari	Mesostigmata	Ameroseiidae	*Ameroseius mineiro* Narita, Bernardi & Moraes, 2013	Curvelo (MG)
		Macronyssidae	*Chiasmanyssus cavernícola* Gomes-Almeida & Pepato, 2021	Conceição do Mato Dentro (MG)
		Reginacharlottiidae	*Reginacharlottia braziliensis* Walter, 2013	Carinhanha (BA)
	Ixodida	Argasidae	*Antricola delacruzi* Estrada-Pena, Barros-Battesti & Venzal, 2004	Itabaiana (SE)
			Antricola guglielmonei Estrada-Pena, Barros-Battesti & Venzal, 2004	Itabaiana (SE)
			Antricola inexpectata Estrada-Pena, Barros-Battesti & Venzal, 2004	Araripe (CE)
			Nothoaspis amazoniensis Nava, Venzal & Labruna, 2010	Porto Velho (RO)
			Ornithodoros cavernicola Dantas-Torres, Venzal & Labruna, 2012	Ubajara (CE)

	Taxon	Family	Species	Locality
Acari	Ixodida	Ixodidae	*Ixodes paranaenses* Barros-Battesti, Arzua, Pichorim & Keirans, 2003	Quatro Barras (PR)
	Trombidiformes	Bdellidae	*Cyta troglodyta* Hernandes, Bernardi & Ferreira, 2011	Itacarambi (MG)
		Erythraeidae	*Callidosoma cassiculophylla* Bernardi, Wohltmann, Lorenzon & Ferreira, 2017	Buenópolis (MG)
			Lasioerythraeus jessicae Costa *et al.*, 2019	Conceição do Mato Dentro (MG)
			Leptus flechtmanni Bassini-Silva, Jacinavicius & Barros-Battesti, 2020	Parauapebas (PA)
			**Leptus sidorchukae* Costa *et al.*, 2019	Pedro Leopoldo (MG)
		Microtrombidiidae	*Eutrombidium carajas* Noei, Šundic, 2020	Parauapebas (PA)
		Neotrombidiidae	*Birjandtrombella minae* Noei, Šundic, Bernardi, 2022	Pains (MG)
			Birjandtrombella pataxo Noei, Šundic, Bernardi, 2022	Matozinhos (MG)
		Teneriffiidae	*Neoteneriffiola xerophila* Bernardi, Pellegrini & Ferreira, 2012	Aurora do Tocantins (TO)
		Trachytidae	*Uroseius subterraneus* Conceição & Pepato, 2021	Conceição do Mato Dentro (MG)
	Opilioacarida	Opilioacarida	*Caribeacarus brasiliensis* Bernardi, Silva, Zacarias, Klompen & Ferreira, 2013	Canaã dos Carajás (PA)
			Neocarus caipora Bernardi, Klompen & Ferreira, 2014	Canaã dos Carajás (PA)
			Neocarus coronatus Araújo & Feres, 2018	São Desidério (BA)
			Neocarus jonasi Araújo & Duarte, 2021	Monjolos (MG)
			Neocarus potiguar Bernardi, Zacarias & Ferreira, 2012	Baraúna (RN)
			Neocarus proteus Bernardi, Klompen, Zacarias & Ferreira, 2014	Mariana (MG)
			Neocarus simmonsi Bernardi, Zampaulo & Oliveira, 2020	São Gonçalo Rio Abaixo (MG)
			Neocarus spelaion Bernardi, 2018	Matozinhos (MG)
	Amblypygi	Charinidae	*Charinus acarajé* Pinto-da-Rocha, Machado & Weygoldt, 2002 (VU)	Santa Luzia (BA)
			Charinus bichuetteae Giuponi & Miranda, 2016	Vitória do Xingu (PA)
			**Charinus caatingae* Vasconcelos & Ferreira 2016 (CR)	Várzea Nova (BA)
			Charinus carajas Giuponi & Miranda, 2016	Parauapebas (PA)
			**Charinus cearensis* Miranda *et al.*, 2021	Ubajara (CE)
			**Charinus diamantinus* Miranda *et al.*, 2021	Andaraí (BA)
			**Charinus eleonorae* Baptista & Giupponi, 2003 (EN)	Itacarambi (MG)
			**Charinus euclidesi* Miranda *et al.*, 2021	Cantagalo (RJ)
			**Charinus ferreus* Giupponi & Miranda, 2016 (CR)	Canaã dos Carajás (PA)
			Charinus iuiu Vasconcelos & Ferreira, 2016	Iuiú (BA)
			Charinus jibaossu Vasconcelos, Giupponi & Ferreira, 2014	Pains (MG)
			Charinus mysticus Giupponi & Kury, 2002	Gentio do Ouro (BA)
			Charinus orientalis Giupponi & Miranda, 2016	Curionópolis (PA)
			Charinus potiguar Vasconcelos, Giupponi & Ferreira, 2013 (VU)	Felipe Guerra (RN)
			**Charinus puri* Miranda *et al.*, 2021	Cambuci (RJ)
			**Charinus renneri* Miranda *et al.*, 2021	Campo Formoso (BA)
			Charinus ricardoi Giuponi & Miranda, 2016	Presidente Figueiredo (AM)
			Charinus santanensis Vasconcelos & Ferreira, 2017	Santana (BA)
			**Charinus spelaeus* Vasconcelos & Ferreira, 2017 (VU)	Presidente Juscelino (MG)
			**Charinus taboa* Vasconcelos, Giupponi & Ferreira, 2016 (CR)	Sete Lagoas (MG)
			**Charinus troglobius* Baptista & Giupponi, 2002 (EN)	Carinhanha (BA)

Taxon	Family	Species	Locality
Amblypygi	Phrynidae	*Trichodamon froesi* Mello-Leitão, 1940	Ituaçu (BA)
Amphipoda	Artesiidae	**Spelaeogammarus bahiensis* Brum, 1975 (VU)	Curaçá (BA)
		***Spelaeogammarus ginae* Bueno & Penoni, 2022	Coribe (BA)
		**Spelaeogammarus sanctus* Bastos-Pereira & Ferreira, 2015 (CR)	Bom Jesus da Lapa (BA)
		**Spelaeogammarus santanensis* Koenemann & Holsinger, 2000 (CR)	Santa Maria da Vitória (BA)
		**Spelaeogammarus spinilacertus* Koenemann & Holsinger, 2000 (EN)	Iraquara (BA)
		**Spelaeogammarus titan* Senna, Andrade, Castelo-Branco & Ferreira, 2014 (CR)	Santa Maria da Vitória (BA)
		**Spelaeogammarus trajanoae* Koenemann & Holsinger, 2000 (EN)	Campo Formoso (BA)
		**Spelaeogammarus uai* Bastos-Pereira & Ferreira, 2017	Itacarambi (MG)
	Bogidiellidae	*Bogidiella neotropica* Ruffo, 1952	Santarém (PA)
		**Megagidiella azul* Koenemann & Holsinger, 1999 (VU)	Bodoquena (MS)
	Hyalellidae	**Hyalella caeca* Pereira, 1989 (VU)	Iporanga (SP)
		**Hyalella epikarstica* Rodrigues, Bueno & Ferreira, 2014 (CR)	Iporanga (SP)
		**Hyalella formosa* Cardoso, Bueno, Araújo & Ferreira, 2014 (CR)	Ponta Grossa (PR)
		Hyalella imbya Rodrigues, Bueno & Ferreira, 2012 (CR-PEX)	Roque Gonzales (RS)
		**Hyalella spelaea* Cardoso, Bueno & Ferreira, 2011	Itirapina (SP)
		Hyalella troglofugia Bastos-Pereira & Ferreira, 2018	Nova Lima (MG)
		**Hyalella veredae* Cardoso, Bueno, Araújo & Ferreira, 2014 (CR)	Presidente Olegário (MG)
	Mesogammaridae	**Potiberaba porakuara* Fišer, Zagmajster & Ferreira, 2013 (VU)	Felipe Guerra (RN)
	Seborgiidae	**Seborgia potiguar* Fišer, Zagmajster & Ferreira, 2013 (CR)	Dix-Sept Rosado (RN)
	Spelaeogriphacea	**Potiicoara brasiliensis* Pires, 1987	Bodoquena (MS)
Araneae	Caponiidae	**Carajas Paraua* Brescovit & Sánchez-Ruiz, 2016 (EN)	Parauapebas (PA)
		**Tisentnops mineiro* Brescovit & Sánchez-Ruiz, 2016	Moeda (MG)
		**Tisentnops onix* Brescovit & Sánchez-Ruiz, 2016 (CR)	Sete Lagoas (MG)
	Ctenidae	*Ctenus fasciatus* Mello-Leitão, 1943	Iporanga (SP)
		**Ctenus igatu* Polotow, Cizauskas & Brescovit, 2022	Andaraí (BA)
		Isoctenus corymbus Polotow, Brescovit & Pellegatti-Franco, 2005 (CR)	São Domingos (GO)
		Parabatinga danielae Brescovit, Cizauskas & Polotow, 2022	Canaã dos Carajás(PA)
	Dipluridae	**Harmonicon cerberus* Pedroso & Baptista, 2014 (CR)	Parauapebas (PA)
		Tonton emboaba (Pedroso, Baptista & Bertani, 2015)	Caeté (MG)
		Trechona diamantina Guadanucci, Fonseca-Ferreira, Baptista & Pedroso, 2016	Diamantina (MG)
	Drymusidae	*Drymusa spelunca* Bonaldo, Rheims & Brescovit, 2006 (CR)	Parauapebas (PA)
	Filistatidae	*Misionella aikewara* Brescovit, Magalhaes & Cizauskas, 2016	São Geraldo do Araguaia (PA)
		Misionella carajas Brescovit, Magalhaes & Cizauskas, 2016	Parauapebas (PA)
	Gnaphosidae	**Brasilomma enigmatica* Brescovit, Ferreira, Silva & Rheims, 2012 (EN)	Nova Lima (MG)
		**Indiani gaspar* Rodrigues, Cizauskas & Lemos, 2020	Prados (MG)
		**Paracymbiomma bocaina* Rodrigues, Cizauskas & Rheims, 2018	Canaã dos Carajás (PA)
		**Paracymbiomma caecus* Rodrigues, Cizauskas & Rheims, 2018	Canaã dos Carajás (PA)
		Paracymbiomma carajas Rodrigues, Cizauskas & Rheims, 2018	Parauapebas (PA)
		**Paracymbiomma pseudocaesus* Rodrigues, Cizauskas & Rheims, 2018	Canaã dos Carajás (PA)

Taxon	Family	Species	Locality
Araneae		*Tricongius ybyguara* (Rheims & Brescovit, 2004) (VU)	Cordisburgo (MG)
	Idiopidae	*Idiops carajas* Fonseca-Ferreira, Zampaulo & Guadanucci, 2017	Parauapebas (PA)
	Microstigmatidae	**Tonton itabirito* Passanha, Cizauskas & Brescovit, 2019	Itabirito (MG)
		**Tonton matodentro* Passanha, Cizauskas & Brescovit, 2019	Conceição do Mato Dentro (MG)
		Tonton queca Passanha, Cizauskas & Brescovit, 2019	Itabirito (MG)
	Ochyroceratidae	*Ochyrocera aragogue* Brescovit, Cizauskas & Mota, 2018	Parauapebas (PA)
		Ochyrocera atlachnacha Brescovit, Cizauskas & Mota, 2018	Parauapebas (PA)
		Ochyrocera brumadinho Brescovit & Cizauskas, 2018	Brumadinho (MG)
		Ochyrocera charlotte Brescovit, Cizauskas & Mota, 2018	Parauapebas (PA)
		**Ochyrocera dorinha* Brescovit, Zampaulo, Pedroso & Cizauskas, 2021	Itabirito (MG)
		Ochyrocera ibitipoca Baptista, Gonzalez & Tourinho, 2008 (EN)	Lima Duarte (MG)
		Ochyrocera laracna Brescovit, Cizauskas & Mota, 2018	Parauapebas (PA)
		Ochyrocera magali Brescovit, Zampaulo, Pedroso & Cizauskas, 2021	Caeté (MG)
		Ochyrocera misspider Brescovit, Cizauskas & Mota, 2018	Parauapebas (PA)
		Ochyrocera monica Brescovit, Zampaulo, Pedroso & Cizauskas, 2021	Barão de Cocais (MG)
		**Ochyrocera ritxoco* Brescovit, Zampaulo & Cizauskas, 2021	Parauapebas (PA)
		**Ochyrocera ritxoo* Brescovit, Zampaulo & Cizauskas, 2021	Canaã dos Carajás (PA)
		**Ochyrocera rosinha* Brescovit, Zampaulo, Pedroso & Cizauskas, 2021	Morro do Pilar (MG)
		Ochyrocera ungoliant Brescovit, Cizauskas & Mota, 2018	Parauapebas (PA)
		Ochyrocera varys Brescovit, Cizauskas & Mota, 2018	Parauapebas (PA)
		**Speocera eleonorae* Baptista, 2003 (EN)	Bodoquena (MS)
		**Speocera pinima* Brescovit, Zampaulo, Cizauskas & Pedroso, 2022	Parauapebas (PA)
		**Speocera babau* Brescovit, Zampaulo, Cizauskas & Pedroso, 2022	Parauapebas (PA)
		**Speocera piquira* Brescovit, Zampaulo, Cizauskas & Pedroso, 2022	São Félix do Xingu (PA)
	Pholcidae	**Metagonia diamantina* Machado, Ferreira & Brescovit, 2011 (CR)	Itaetê (BA)
		Metagonia potiguar Ferreira, Souza, Machado & Brescovit, 2011 (CR)	Felipe Guerra (RN)
	Scytodes	*Scytodes eleonorae* Rheims & Brescovit, 2001	São Domingos (GO)
	Segestriidae	*Ariadna aurea* Giroti & Brescovit, 2018 (VU)	Morro do Chapéu (BA)
	Sicariidae	*Loxosceles cardosoi* Bertani, von Schimonsky & Gallão, 2018	Carinhanha (BA)
		Loxosceles carinhanha Bertani, von Schimonsky & Gallão, 2018	Carinhanha (BA)
		Loxosceles ericsoni Bertani, von Schimonsky & Gallão, 2018	Itacarambi (MG)
		Loxosceles karstica Bertani, von Schimonsky & Gallão, 2018	Itacarambi (MG)
		Loxosceles niedeguidonae Gonçalves-de-Andrade, Bertani, Nagahama & Barbosa, 2012	Coronel José Dias (PI)
		**Loxosceles troglobia* Souza & Ferreira, 2018 (VU)	Malhada (MG)
	Symphytognathidae	**Anapistula guyri* Rheims & Brescovit, 2003	São Domingos (GO)
		Symphytognatha carstica Brescovit, Alvares & Ferreira 2004	Iguatama (MG)
	Tetrablemmidae	**Matta cambito* Brescovit & Cizauskas, 2019	Itabirito (MG)
		**Matta humhum* Brescovit & Cizauskas, 2019	Caeté (MG)
		**Matta humrrum* Brescovit & Cizauskas, 2019	Matozinhos (MG)
		**Matta nuusga* Brescovit & Cizauskas, 2019	Pains (MG)

Taxon		Family	Species	Locality
	Araneae	Tetrablemmidae	**Matta pititinha* Brescovit & Cizauskas, 2019	Prados (MG)
			**Matta teteia* Brescovit & Cizauskas, 2019	Itacarambi (MG)
			Matta zuiuda Brescovit & Cizauskas, 2019	Pains (MG)
		Theraphosidae	*Guyruita metallophila* Fonseca-Ferreira, Zampaulo & Guadanucci, 2017	Curionópolis (PA)
			Hapalopus serrapelada Fonseca-Ferreira, Zampaulo & Guadanucci, 2017	Curionópolis (PA)
			**Tmesiphantes hypogeus* Bertani, Bichuette & Pedroso, 2013 (CR)	Andaraí (BA)
		Theridiidae	*Cryptachaea pilar* Santanna & Rodrigues, 2019	Morro do Pilar (MG)
		Theridiosomatidae	*Baalzebub acutum* Prete, Cizauskas & Brescovit, 2016	Matozinhos (MG)
			Cuacuba mariana Prete, Cizauskas & Brescovit, 2018	Santa Bárbara (MG)
			Cuacuba morrodopilar Prete, Cizauskas & Brescovit, 2018	Morro do Pilar (MG)
			Cuacuba ribeira Prete & Brescovit, 2019	Iporanga (SP)
			Plato ferriferus Prete, Cizauskas & Brescovit, 2018	Parauapebas (PA)
			Plato novalima Prete, Cizauskas & Brescovit, 2018	Nova Lima (MG)
			Plato striatus Prete, Cizauskas & Brescovit, 2018	Parauapebas (PA)
		Titanoecidae	*Goeldia zyngierae* Almeida-Silva, Brescovit & Dias, 2009	Itabaiana (SE)
	Blattodea	Blattellidae	**Litoblatta camargoi* Gutiérrez, 2005 (EN)	Iraquara (BA)
Chilopoda	Scolopendromorpha	Scolopocryptopidae	*Cryptops hephaestus* Ázara & Ferreira, 2013	Mariana (MG)
			**Cryptops iporangensis* Ázara & Ferreira, 2013 (EN)	Iporanga (SP)
			**Cryptops spelaeoraptor* Ázara & Ferreira, 2014 (CR)	Campo Formoso (BA)
		Cryptopidae	**Newportia potiguar* Ázara & Ferreira, 2014 (VU)	Apodi (RN)
			**Newportia spelaea* Ázara & Ferreira, 2014 (CR)	Campo Formoso (BA)
			**Scolopocryptops troglocaudatus* Chagas-Jr & Bichuette, 2015 (EN)	Andaraí (BA)
	Geophilomorpha	Schendylidae	**Schendylops janelao* Nunes, Chagas-Jr & Bichuette, 2019	Itacarambi (MG)
	Coleoptera	Carabidae	**Ardistomis ferreirai* Balkenohl, Pellegrini & Zampaulo, 2018	Parauapebas (PA)
			**Coarazuphium amazonicus* Pellegrini & Ferreira, 2017 (CR)	Parauapebas (PA)
			**Coarazuphium auleri* Pellegrini, Bichuette & Vieira, 2021	Nobres (MT)
			**Coarazuphium bambui* Pellegrini & Vieira, 2022	Feira da Lapa (BA)
			**Coarazuphium bezerra* Gnaspini, Vanin & Godoy, 1998 (DD)	São Domingos (GO)
			**Coarazuphium caatinga* Pellegrini & Ferreira, 2014 (EN)	Campo Formoso (BA)
			**Coarazuphium cessaima* Gnaspini, Vanin & Godoy, 1998 (EN)	Itaetê (BA)
			**Coarazuphium formoso* Pellegrini & Ferreira, 2011 (CR)	Campo Formoso (BA)
			**Coarazuphium kayapo* Pellegrini, Ferreira & Vieira, 2022	Canaã dos Carajás (PA)
			**Coarazuphium lundi* Pellegrini *et al.*, 2020	Prudente de Morais (MG)
			**Coarazuphium pains* Alvares & Ferreira, 2002 (EN)	Pains (MG)
			**Coarazuphium ricardoi* Benà & Vanin, 2014 (CR)	Doutor Ulysses (PR)
			**Coarazuphium spinifemur* Pellegrini & Ferreira, 2017 (CR)	Curionópolis (PA)
			**Coarazuphium tapiaguassu* Pellegrini & Ferreira, 2011 (EN)	Curionópolis (PA)
			**Coarazuphium tessai* (Godoy & Vanin, 1990) (CR)	Santana (BA)
			**Coarazuphium xikrin* Pellegrini, Ferreira & Vieira, 2022	Parauapebas (PA)
			**Coarazuphium xingu* Pellegrini, Ferreira & Vieira, 2022	São Félix do Xingu (PA)
			**Perigona spelunca* Pellegrini, Ferreira & Vieira, 2022	Pains (MG)
			**Schizogenius ocellatus* Whitehead, 1972 (EN)	Iporanga (SP)

Taxon	Family	Species	Locality
Coleoptera	Cholevidae	*Adelopsis leo* Gnaspini, 1993	Iporanga (SP)
		Adelopsis piruapuera Gnaspini, 1993	Campo Formoso (BA)
	Dyticidae	**Copelatus cessaima* Caetano, Benà & Vanin, 2013 (EN)	Parauapebas (PA)
	Scarabaeidae	*Isacanthon mariaelinae* Pacheco & Vaz-de-Mello, 2019	Diamantina (MG)
	Staphylinidae	**Metopiellus painensis* Asenjo, Ferreira & Zampaulo, 2017 (EN)	Pains (MG)
		**Metopioxys carajas* Asenjo, Petrobon & Ferreira, 2019	Parauapebas (PA)
		**Oxarthrius aurora* Asenjo, Zampaulo & Ferreira, 2018	Aurora do Tocantins (TO)
		**Oxarthrius inexpectatus* Asenjo, Zampaulo & Ferreira, 2018	Nova Lima (MG)
	Tenebrionidae	*Discopleurus bondezani* Aloquio, Flores & Lopes-Andrade, 2019	Montes Claros (MG)
Collembola	Arrhopalitidae	**Arrhopalites alambariensis* Zeppelini, 2006 (VU)	Iporanga (SP)
		**Arrhopalites amorimi* Palacios-Vargas & Zeppelini, 1995 (VU)	Iporanga (SP)
		**Arrhopalites botuveraensis* Zeppelini, 2006 (VU)	Botuverá (SC)
		Arrhopalites glabrofasciatus Zeppelini, Brito & Lima, 2018 (EN)	Brumadinho (MG)
		**Arrhopalites gnaspinii* (Palacios-Vargas & Zeppelini, 1995) (CR)	Iporanga (SP)
		**Arrhopalites heteroculatus* Zeppelini, 2006 (VU)	Ribeirão Grande (SP)
		**Arrhopalites lawrencei* Palacios-Vargas & Zeppelini, 1995 (VU)	Eldorado (SP)
		**Arrhopalites mendoncae* Brito, Lima & Zeppelini, 2019	Rio Acima (MG)
		**Arrhopalites paranaensis* Zeppelini, 2006 (CR)	Rio Branco do Sul (PR)
	Cyphoderidae	*Cyphoderus mucrominimus* Oliveira, Alves & Zeppelini, 2017	Curionópolis (PA)
		Cyphoderus mucrostrimenus Oliveira, Alves & Zeppelini, 2017	Parauapebas (PA)
	Dicyrtomidae	*Ptenothrix dalii* Zeppelini, Ferreira & Oliveira, 2020	Caeté (MG)
	Entomobryidae	*Coecobrya phoenix* Brito, Lima & Zeppelini, 2019	Igarapé (MG)
		Pseudosinella acantholabrata Cipola, 2020	Conceição do Mato Dentro (MG)
		**Pseudosinella alfanjeunguiculata* Cipola, 2020	Morro do Pilar (MG)
		Pseudosinella ambigua Zeppelini, Brito & Lima, 2018 (VU)	Pedro Leopoldo (MG)
		Pseudosinella aphelabiata Cipola, 2020	São Sebastião do Rio Preto (MG)
		**Pseudosinella brumadinhoensis* Cipola, 2020	Brumadinho (MG)
		**Pseudosinella cearensis* Cipola, 2020	Santa Quitéria (CE)
		Pseudosinella chimerambigua Cipola, 2020	Vespasiano (MG)
		Pseudosinella diamantinensis Cipola, 2020	Diamantina (MG)
		**Pseudosinella guanhaensis* Zeppelini, Brito & Lima, 2018 (CR)	Dores de Guanhães (MG)
		**Pseudosinella keni* Cipola, 2020	Rio Acima (MG)
		Pseudosinella labiociliata Cipola, 2020	Caeté (MG)
		Pseudosinella labruspinata Cipola, 2020	Itabirito (MG)
		Pseudosinella macrolignicephala Cipola, 2020	Itabirito (MG)
		Pseudosinella marianensis Cipola, 2020	Mariana (MG)
		**Pseudosinella mitodentunguilata* Cipola, 2020	Luislândia (MG)
		**Pseudosinella neriae* Cipola, 2020	Pedro Leopoldo (MG)
		Pseudosinella paraensis Cipola, 2020	Canaã dos Carajás (PA)
		Pseudosinella parambigua Cipola, 2020	Nova Lima (MG)
		**Pseudosinella phyllunguiculata* Cipola, 2020	Barão de Cocais (MG)

Taxon	Family	Species	Locality
Collembola	Entomobryidae	**Pseudosinella prelabruscervata* Cipola, 2020	Rio Acima (MG)
		Pseudosinella pusilla Cipola, 2020	Curionópolis (PA)
		**Pseudosinella serpentinensis* Cipola, 2020	Morro do Pilar (MG)
		Pseudosinella spurimarianensis Cipola, 2020	Mariana (MG)
		Pseudosinella taurina Cipola, 2020	Curionópolis (PA)
		Pseudosinella unimacrochaetosa Cipola, 2020	Rio Acima (MG)
		Seira pietata Oliveira, Ferreira & Zeppelini, 2020	Caeté (MG)
	Hypogastruridae	**Acherontides eleonorae* Palacios-Vargas & Gnaspini-Netto, 1993 (VU)	Iporanga (SP)
		Acherontides serrasapoensis Lima, Estievano & Zeppelini, 2019	Conceição do Mato Dentro (MG)
	Paronellidae	*Cyphoderus palaciosi* Oliveira, Brito & Zeppelini, 2021	Prudente de Morais (MG)
		Cyphoderus pataxo Oliveira, Brito & Zeppelini, 2021	Nova Lima (MG)
		**Troglobius brasiliensis* Palacios-Vargas & Zeppelini, 1995 (CR)	Medicilância (PA)
		**Troglobius ferroicus* Zeppelini, Da Silva & Palacios-Vargas, 2014 (CR)	Itabirito (MG)
		**Trogolaphysa aelleni* Yoshii, 1988 (CR)	Iporanga (SP)
		**Trogolaphysa hauseri* Yoshii, 1989 (DD)	Eldorado (SP)
	Sminthuridae	*Keratosminthurus calamitosus* Zeppelini, Brito & Lima, 2020	Ubaí (MG)
		Keratosminthurus tapigu Zeppelini, Brito & Lima, 2020	Canaã dos Carajás (PA)
		**Pararrhopalites papaveroi* (Zeppelini & Palacios-Vargas, 1999) (EN)	Bonito (MS)
		Pararrhopalites queirozi Brito, Lima & Zeppelini, 2019	Matozinhos (MG)
		**Pararrhopalites sideroicus* Zeppelini & Brito, 2014 (CR)	Itabirito (MG)
		**Pararrhopalites ubiquum* Zeppelini *et al.*, 2017 (VU)	Itabirito (MG)
		**Pararrhopalites wallacei* (Palacios-Vargas & Zeppelini, 1995) (CR)	Iporanga (SP)
		**Troglobentosminthurus luridus* Souza, Medeiros & Bellini, 2022	Carinhanha (BA)
Decapoda	Aeglidae	**Aegla cavernícola* Turkay, 1972 (CR)	Iporanga (SP)
		**Aegla charon* Bueno *et al.*, 2017 (CR)	Guapiara (SP)
		**Aegla leptochela* Bond-Buckup & Buckup, 1994 (CR)	Iporanga (SP)
		**Aegla microphthalma* Bond-Buckup & Buckup, 1994 (CR)	Iporanga (SP)
		Aegla strinati Turkay, 1972	Iporanga (SP)
Dermaptera	Diplatyidae	*Cylindrogaster cavernicola* Kamimura & Ferreira, 2017	Jandaíra (RN)
		Mesodiplatys falcifer Kamimura & Ferreira, 2018	Carinhanha (BA)
Diplopoda	Polydesmida	Chelodesmidae	**Leodesmus yporangae* (Schubart, 1946) (EN)	Iporanga (SP)
			Leptodesmus ravus Schubart, 1956	Lagoa Santa (MG)
			Obiricodesmus rupestris Schubart, 1956	Lagoa Santa (MG)
			Parastenonia carajas Bouzan & Iniesta, 2020	Canaã dos Carajás (PA)
			Rotundotergum elevatum Golovatch, Bouzan & Gallo, 2022	São Desidério (BA)
			Sandalodesmus stramineus Schubart, 1956 (VU)	Lagoa Santa (MG)
			**Strongylosomides troglobius* Golovatch, Bouzan & Gallo, 2022	São Desidério (BA)
		Cryptodesmidae	**Peridontodesmella alba* Schubart, 1957 (VU)	Iporanga (SP)
		Dobrodesmidae	**Dobrodesmus mirabilis* Shear, Ferreira & Iniesta, 2016 (CR)	Ituaçu (BA)
		Furhmannodesmidae	**Phaneromerium cavernicolum* Golovatch & Wytwer, 2004	Santana (BA)
		Paradoxosomatidae	*Iulidesmus silvestrii* Golovatch & Gallo, 2022	Central (BA)
			** Onciurosoma troglobium* Golovatch & Gallo, 2022	São Desidério (BA)

	Taxon	Family	Species	Locality
Diplopoda	Polydesmida	Pyrgodesmidae	**Yporangiella stygius* Schubart, 1946 (DD)	Apiaí (SP)
		Trichopolydesmidae	*Moojenodesmus schubarti* Golovatch & Gallo, 2022	Iuiú (BA)
		Trichopolydesmidae	**Phaneromerium troglopterygotum* Golovatch & Gallo, 2022	Coribe (BA)
	Glomeridesmida	Glomeridesmidae	**Glomeridesmus spelaeus* Iniesta, Ferreira & Wesener, 2012 (EN)	Curionópolis (PA)
	Spirostreptida	Pseudonannolenidae	**Pseudonannolene ambuatinga* Iniesta; Ferreira, 2013 (EN)	Pains (MG)
			Pseudonannolene anapophysis Fontanetti, 1996	Lençóis (BA)
			Pseudonannolene caatinga Iniesta & Ferreira, 2014	Ourolândia (BA)
			**Pseudonannolene canastra* Gallo & Bichuette, 2020	São Roque de Minas (MG)
			Pseudonannolene chaimowiczi Fontanetti, 1996	Lagoa Santa (MG)
			Pseudonannolene erikae Iniesta & Ferreira, 2014	Sete Lagoas (MG)
			Pseudonannolene fontanettiae Iniesta & Ferreira, 2014	Tiradentes (MG)
			Pseudonannolene gogo Iniesta & Ferreira, 2013	Mariana (MG)
			Pseudonannolene imbirensis Fontanetti, 1996 (EN)	São Domingos (GO)
			Pseudonannolene leopoldoi Iniesta & Ferreira, 2014	São João da Lagoa (MG)
			Pseudonannolene longissima Iniesta & Ferreira, 2014	Sete Lagoas (MG)
			**Pseudonannolene lundi* Iniesta; Ferreira, 2015 (CR)	Luislândia (MG)
			Pseudonannolene marconii Iniesta & Ferreira, 2014	Pau Brasil (BA)
			Pseudonannolene microzoporus Mauries, 1987	Pedro Leopoldo (MG)
			Pseudonannolene robsoni Iniesta & Ferreira, 2014	Pains (MG)
			Pseudonannolene rolamossa Iniesta & Ferreira, 2013	Nova Lima (MG)
			Pseudonannolene rosineii Iniesta & Ferreira, 2014	Pains (MG)
			Pseudonannolene saguassu Iniesta & Ferreira, 2013	Pains (MG)
			**Pseudonannolene spelaea* Iniesta; Ferreira, 2013 (CR)	Parauapebas (PA)
			Pseudonannolene strinatii Mauries, 1974	Iporanga (SP)
			Pseudonannolene taboa Iniesta & Ferreira, 2014	Sete Lagoas (MG)
			Pseudonannolene tocaiensis Fontanetti, 1996 (CR)	Itirapina (SP)
			Pseudonannolene xavieri Iniesta & Ferreira, 2014	Iraquara (BA)
	Stemmiulida	Stemmiulidae	*Stemmiulus brasiliensis* Iniesta & Ferreira, 2015	Curionópolis (PA)
	Diptera	Psychodidae	*Bruchomyia brasiliensis* Alexander, 1940	Crato (CE)
			Bruchomyia mineira Bravo & Barata, 2012	Diamantina (MG)
			**Deanemyia maruaga* Alves, Freitas & Barrett, 2008	Presidente Figueiredo (AM)
			Edentomyia piauiensis Galati, Andrade Filho, Silva & Falcão, 2003	Picos (PI)
			Evandromyia corumbaensis (Galati, Nunes, Oshiro & Rego, 1989)	Corumbá (MS)
			Evandromyia spelunca Carvalho, Brazil, Sanguinette & Andrade Filho, 2011	Lassance (MG)
			Lutzomyia almerioi Galati & Nunes, 1999	Bonito (MS)
			Lutzomyia cavernicola (Costa Lima, 1932)	Cordisburgo (MG)
			Lutzomyia diamantinensis Barata, Serra-e-Meira & Carvalho, 2012	Diamantina (MG)
			Lutzomyia elizabethrangelae Vilela, Azevedo & Godoy, 2015	Palmeirópolis (TO)
			Lutzomyia forattinii Galati, Rego, Nunes & Oshiro, 1985	Corumbá (MS)
			Lutzomyia ischnacantha Martins, Souza & Falcão, 1962	Januária (MG)
			Lutzomyia renei (Martins, Falcão & Silva, 1957)	Lagoa Santa (MG)

Taxon	Family	Species	Locality
Diptera	Psychodidae	*Martinsmyia reginae* Carvalho, Brazil, Sanguinette &Andrade Filho, 2010	Arraias (TO)
		Micropygomyia acanthopharynx (Martins, Falcão & Silva, 1962)	Amaro Leite (GO)
		Micropygomyia peresi (Mangabeira-Filho, 1942)	Januária (MG)
		Micropygomyia petari Galati, Marassá & Andrade, 2003	Iporanga (SP)
		Nemapalpus spinosus Bravo & Barata, 2012	Diamantina (MG)
		Pintomyia gruta (Ryan, 1986)	Parauapebas (PA)
		Tonnoira distincta Bravo, Alves & Chagas, 2008	Presidente Figueiredo (AM)
		Tonnoira robusta Bravo, Alves & Chagas, 2008	Presidente Figueiredo (AM)
Gastropoda	Bulimulidae	*Anctus prolatus* Simone & Casati, 2013	Coronel José Dias (PI)
		Kora nigra Simone, 2015	Carinhanha (BA)
		Kora iracema Simone, 2015	São Desidério (BA)
	Diplommatinidae	*Habeas corpus* Simone 2013	Carinhanha (BA)
		Habeas data Simone 2013	Carinhanha (BA)
		**Habeastrum omphalium* Simone, 2019	Bonito (MS)
		**Habeastrum parafusum Simone, 2019*	Bonito (MS)
		**Habeastrum strangei Simone, Cavallari & Salvador, 2019*	Presidente Olegário (MG)
	Gastrocoptidae	*Gastrocopta sharae* Cavallari & Simone 2017	Mambaí (GO)
	Tateidae	*Potamolithus karsticus* Simone & Moracchioli, 1994 (CR)	Apiaí (SP)
		**Potamolithus troglobius* Simone & Moracchioli, 1994 (CR)	Iporanga (SP)
	Megaspiridae	*Thaumastus lundi* Pena, Salgado & Coelho, 2005 (CR)	Lagoa Santa (MG)
	Odontostomidae	*Bahiensis ribeirensis* Salvador, Cavallari & Simone, 2016	Iporanga (SP)
		Clinispira insolita Salvador, Simone & Casati, 2013	Coronel José Dias (PI)
		Cyclodontina capivara Simone & Casati, 2013	Coronel José Dias (PI)
	Pomatiopsidae	**Spiripockia punctata* Simone, 2012 (CR)	Serra do Ramalho (BA)
		Spiripockia umbraticola Simone & Salvador, 2021	Carinhanha (BA)
	Simpulopsidae	*Rhinus gilbertus* Simone & Casati, 2013	Coronel José Dias (PI)
	Streptaxidae	*Streptartemon molaris* Simone & Casati, 2013	Coronel José Dias (PI)
	Strophocheilidae	*Gonyostomus elinae* Simone, 2016	Iporanga (SP)
	Subulinidae	**Lavajatus moro*i Simone, 2018	Santa Quitéria (CE)
Haplosclerida	Spongillidae	**Racekiela cavernícola* Volkmer-Ribeiro, Bichuette & Machado, 2010 (VU)	Morro do Chapéu (BA)
Hemiptera	Cixiidae	**Ferricixius davidi* Hock & Ferreira, 2012 (CR)	Itabirito (MG)
	Hydrometridae	**Cephalometra pallida* Polhemus & Ferreira, 2018	Natividade (TO)
		**Spelaeometra gruta* Polhemus & Ferreira, 2018	Itacarambi (MG)
	Kinnaridae	**Iuiuia caeca* Hock & Ferreira, 2016 (VU)	Iuiú (BA)
		**Kinnapotiguara troglóbia* (Hoch & Ferreira, 2013) (VU)	Felipe Guerra (RN)
	Reduviidae	*Amilcaria lapinhaensis* (Wygodzinsky, 1950)	Lagoa Santa (MG)
		Dohrnemesa kuarajucassaba Gil-Santana & Ferreira, 2017	Pains (MG)
		Dohrnemesa oliveirai Gil-Santana & Ferreira, 2016	Parauapebas (PA)
		Phasmatocoris catarinae Gil-Santana, 2015	Mariana (MG)
		Phasmatocoris galvaoi Gil-Santana, 2015	Canaã dos Carajás (PA)

Taxon	Family	Species	Locality
Hemiptera	Reduviidae	*Phasmatocoris xavieri* Gil-Santana, Alves, Barrett & Costa, 2007	Presidente Figueiredo (AM)
		Polauchenia paraprotentor Gil-Santana & Ferreira, 2017	Parauapebas (PA)
		Quasitagalis afonsoi Gil-Santana, Oliveira & Zampaulo, 2020	Lavandeira (TO)
		Zelurus gerevatinga Ferreira, Ferreira & Gil-Santana, 2016	Pains (MG)
		Zelurus tambejua Ferreira, Ferreira & Gil-Santana, 2016	Dianópolis (TO)
	Veliidae	*Paravelia cunhai* Rodrigues & Moreira, 2016	Vitória do Xingu (PA)
		Paravelia digitata Rodrigues & Moreira, 2016	Santa Maria da Vitória (BA)
		Rhagovelia kararao Floriano & Moreira, 2015	Altamira (PA)
Hymenoptera	Formicidae	*Stegomyrmex bensoni* Feitosa, Brandão & Diniz, 2008	Canaã dos Carajás (PA)
Isopoda	Armadillidae	*Ctenorillo ferrarai* Campos-Filho, Araujo & Taiti, 2014	Canaã dos Carajás (PA)
		**Gabunillo aridicola* Souza, Senna & Kury, 2010	Aiuaba (CE)
	Brasileirinidae	**Brasileirinho cavaticus* Prevorcnik, Ferreira & Sket, 2012 (CR)	Paripiranga (BA)
	Calabozoidae	**Pongycarcinia xiphidiourus* Messana, Baratti & Benvenuti, 2002 (EN)	Campo Formoso (BA)
	Dubioniscidae	*Novamundoniscus altamiraensis* Campos-Filho, Araujo & Taiti, 2014	Altamira (PA)
	Philosciidae	**Alboscia jotajota* Campos-Filho, Aguiar & Taiti, 2020	Iporanga (SP)
		Androdeloscia akuanduba Campos-Filho, Cardoso & Taiti, 2020	Parauapebas (PA)
		**Benthana iporangensis* Lima & Serejo, 1993	Iporanga (SP)
		**Benthana xiquinhoi* Campos-Filho, Bichuette & Taiti, 2019	Andaraí (BA)
		Leonardoscia hassalli Campos-Filho, Araujo & Taiti, 2014	Altamira (PA)
		Metaprosekia caupe Campos-Filho, Araujo & Taiti, 2014	Altamira (PA)
		**Metaprosekia igatuensis* Campos-Filho, Fernandes & Bichuette, 2020	Andaraí (BA)
		Metaprosekia quadriocellata Campos-Filho, Araujo & Taiti, 2014	Altamira (PA)
	Platyarthridae	*Trichorhina anhanguera* Campos-Filho, Araujo & Taiti, 2014	Morro do Pilar (MG)
		Trichorhina cipoensis Campos-Filho, Bichuette & Taiti, 2016	Itacarambi (MG)
		Trichorhina curupira Campos-Filho, Araujo & Taiti, 2014	Altamira (PA)
		Trichorhina guanophila Souza-Kury, 1993	Campo Formoso (BA)
		Trichorhina kaingangi Campos-Filho, 2015	Adrianópolis (PR)
		Trichorhina pataxosi Campos-Filho, Bichuette & Taiti, 2016	Pedro Leopoldo (MG)
		Trichorhina yara Campos-Filho, Araujo & Taiti, 2014	Altamira (PA)
	Pudeoniscidae	**Iansaoniscus georginae* Campos-Filho, Araujo & Taiti, 2017 (CR)	Paripiranga (BA)
		**Iansaoniscus Iraquara* Campos-Filho, Araujo & Taiti, 2017 (CR)	Iraquara (BA)
		**Iansaoniscus leilae* Cardoso, Bastos-Pereira & Ferreira, 2022	Campo Formoso (BA)
		**Iansaoniscus paulae* Cardoso, Bastos-Pereira & Ferreira, 2022	Itaeté (BA)
	Scleropactidae	**Amazoniscus eleonorae* Souza, Bezerra & Araujo, 2006	Altamira (PA)
		**Amazoniscus leistikowi* Campos-Filho, Araujo & Taiti, 2014 (CR)	Altamira (PA)
		**Amazoniscus spica* Campos-Filho, Aguiar & Taiti, 2020	Canaã dos Carajás (PA)
		**Circoniscus buckupi* Campos-Filho & Araujo, 2011 (CR)	Parauapebas (PA)
		**Circoniscus carajasensis* Campos-Filho & Araújo, 2011 (EN)	Canaã dos Carajás (PA)
	Styloniscidae	**Chaimowiczia obybytyra* Cardoso, Bastos-Pereira & Ferreira, 2022	Coribe (BA)
		**Chaimowiczia tatus* Cardoso, Bastos-Pereira, Souza & Ferreira, 2022	Santana (BA)
		**Chaimowiczia uai* Cardoso, Bastos-Pereira, Souza & Ferreira, 2022	Itacarambi (MG)

Taxon	Family	Species	Locality
Isopoda	Styloniscidae	*Cylindroniscus flaviae* Campos-Filho, Araujo & Taiti, 2017	Iporanga (SP)
		**Cylindroniscus platoi* Fernandes, Campos-Filho & Bichuette, 2018	Pedro Leopoldo (MG)
		**Iuiuniscus iuiuensis* Souza, Ferreira & Senna, 2015 (VU)	Iuiú (BA)
		**Pectenoniscus carinhanhensis* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	Serra do Ramalho (BA)
		**Pectenoniscus iuiuensis* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	Iuiú (BA)
		**Pectenoniscus juveniliensis* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	Juvenilia (MG)
		**Pectenoniscus liliae* Campos-Filho, Bichuette & Taiti, 2019	Coribe (BA)
		**Pectenoniscus montalvaniensis* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	Montalvânia (MG)
		**Pectenoniscus morrensis* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	Morro do Chapéu (BA)
		**Pectenoniscus santanensis* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	Santana (BA)
		**Spelunconiscus castroi* Campos-Filho, Araujo & Taiti, 2014, (CR)	Matozinhos (MG)
		**Spelunconiscus septemlacuum* Bastos-Pereira, Souza, Sandi & Ferreira, 2022	Sete Lagoas (MG)
		**Pectenoniscus pankaru* Campos-Filho, Torres & Bichuette, 2022	Feira da Mata (BA)
		**Pectenoniscus fervens* Campos-Filho, Torres & Bichuette, 2022	Coronel José Dias (PI)
		**Xangoniscus aganju* Campos-Filho, Araujo & Taiti, 2014 (EN)	Carinhanha (BA)
		**Xangoniscus ceci* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	São João da Ponte (MG)
		**Xangoniscus dagua* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	Montes Claros (MG)
		**Xangoniscus ibiracatuensis* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	Ibiracatu (MG)
		**Xangoniscus itacarambiensis* Bastos-Pereira, Souza & Ferreira, 2017 (CR)	Itacarambi (MG)
		**Xangoniscus lapaensis* Campos-Filho, Gallo & Bichuette, 2022	Feira da Mata (BA)
		**Xangoniscus loboi* Campos-Filho, Gallo & Bichuette, 2022	Feira da Mata (BA)
		**Xangoniscus lundi* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	Montes Claros (MG)
		**Xangoniscus odara* Campos-Filho, Bichuette & and Taiti, 2016	Itacarambi (MG)
		**Xangoniscus santinhoi* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020	Itacarambi (MG)
Opiliones	Cosmetidae	*Paecilaema batman* Pinto-da-Rocha & Yamaguti, 2013	São Domingos (GO)
		Roquettea troguloides Medrano & kury, 2018	Canaã dos Carajás (PA)
	Escadabiidae	**Spaeleoleptes spaeleus* Soares, 1966 (EN)	Cordisburgo (MG)
	Gerdesiidae	*Gonycranaus androgynus* Bragagnolo, Hara & Pinto-da-Rocha, 2015	Conceição do Mato Dentro (MG)
		**Gonycranaus pluto* Bragagnolo, Hara & Pinto-da-Rocha, 2015 (VU)	Morro do Pilar (MG)
	Gonyleptidae	*Daguerreia inermis* Soares & Soares, 1947	Tunas do Paraná (PR)
		**Discocyrtus pedrosoi* Kury, 2008 (VU)	Andaraí (BA)
		Eugyndes patellaris Soares, 1966	Pedro Leopoldo (MG)
		Eusarcus capixaba Santos Júnior, Azara & Ferreira, 2021	Ecoporanga (ES)
		Eusarcus cavernicola Hara & Pinto-da-Rocha, 2010	São Domingos (GO)
		**Eusarcus elinae* Kury, 2008 (EN)	Iraquara (BA)
		Eusarcus hastatus Sorensen, 1884	Pedro Leopoldo (MG)
		Eusarcus maquinensis Soares, 1966	Cordisburgo (MG)
		Eusarcus marmoreus Santos Júnior, Azara & Ferreira, 2021	Vargem Alta (ES)
		Eusarcus xambioa Santos Júnior, Azara & Ferreira, 2021	Xambioá (TO)

Taxon	Family	Species	Locality
Opiliones	Gonyleptidae	**Giupponia chagasi* Perez & Kury, 2002 (EN)	Carinhanha (BA)
		Heteromitobates harlequin Da Silva & Gnaspini, 2009	Santo André (SP)
		**Iandumoema cuca* Ázara, Hara & Ferreira, 2020	Itacarambi (MG)
		**Iandumoema gollum* Ázara, Hara & Ferreira, 2020	Presidente Juscelino (MG)
		**Iandumoema setimapocu* Hara & Pinto-da-Rocha, 2008 (VU)	Montes Claros (MG)
		**Iandumoema smeagol* Pinto-da-Rocha, Fonseca-Ferreira & Bichuette, 2015 (EN)	Monjolos (MG)
		**Iandumoema stygia* Ázara, Hara & Ferreira, 2020	Montes Claros (MG)
		**Iandumoema uai* Pinto-da-Rocha, 1996 (CR)	Itacarambi (MG)
		Mitogoniella mucuri Ázara, DaSilva & Ferreira, 2013	Novo Oriente de Minas (MG)
		Mitogoniella taquara Da Silva & Gnaspini, 2009	Pains (MG)
		**Pachylospeleus strinatii* Šilhavý, 1974 (CR)	Iporanga (SP)
		Serracutisoma pseudovarium Da Silva & Gnaspini, 2009	Iporanga (SP)
		**Spinopilar moria* Kury & Perez-Gonzalez, 2008 (CR)	Cordisburgo (MG)
	Kimulidae	**Relictopiolus galadriel* Perez-Gonzalez *et al.*, 2017 (CR)	Itacarambi (MG)
	Neogoveidae	*Canga renatae* DaSilva, Pinto-da-Rocha & Giribet, 2010	Parauapebas (PA)
	Stygnidade	*Stenostygnoides caliginosus* Pinto-da-Rocha, 1990	Medicilância (PA)
	Stygnidae	*Protimesius lucifer* Villarreal, Azara & Kury, 2019	Parauapebas (PA)
		Protimesius orcus Villarreal, Azara & Kury, 2019	Santa Quitéria (CE)
Orthoptera	Phalangopsidae	*Adelos gryllus endogeus* Merlo, Castro-Souza, Junta & Ferreira, 2022	Pains (MG)
		Adelosgryllus ferratilis Merlo, Castro-Souza, Junta & Ferreira, 2022	Curionópolis (PA)
		Adelosgryllus lucifugus Merlo *et al.*, 2021	Piripiri (PI)
		Adelosgryllus megapterus Merlo, Castro-Souza, Junta & Ferreira, 2022	Conceição do Mato Dentro (MG)
		Adelosgryllus xambioa Merlo, Castro-Souza, Junta & Ferreira, 2022	Xambioá (TO)
		Bambuina bambui Mello, Horta & Bolfarini, 2013	Catas Altas (MG)
		Eidmanacris alboannulata (Piza, 1960)	Itirapina (SP)
		Eidmanacris lencionii Bonfarini, 2016	Brumadinho (MG)
		Eidmanacris speluncae (Mello-Leitão, 1937)	Santa Bárbara (MG)
		Endecous alejomesai Zefa, 2010	Vila Propício (GO)
		**Endecous apterus* Bolfarini & Souza-Dias, 2013 (EN)	Ituaçu (BA)
		Endecous bahiensis Castro-Souza, Zefa & Ferreira, 2017	Campo Formoso (BA)
		Endecous betariensis Mello & Pellegatti-Franco, 1998	Iporanga (SP)
		Endecous cavernicolus Costa-Lima, 1940	Lagoa Santa (MG)
		Endecous didymus Castro-Souza, Zefa & Ferreira, 2020	Luislândia (MG)
		Endecous painensis Castro-Souza, Junta & Ferreira, 2020	Pains (MG)
		**Endecous peruassuensis* Bolfarini & Bichuette, 2015 (EN)	Itacarambi (MG)
		Endecous potiguar Castro-Souza, Zefa & Ferreira, 2017	Felipe Guerra (RN)
		**Endecous troglobius* Castro-Souza, Zefa & Lopes Ferreira, 2020	Luislândia (MG)
		**Erebonyx catacumbae* Mello & Ferreira, 2021	Campo Formoso (BA)
		Phalangopsis araguaia Junta, Castro-Souza & Ferreira, 2020	São Geraldo do Araguaia (PA)
		Phalangopsis arenita Mews & Sperber, 2008	Presidente Figueiredo (AM)

Taxon	Family	Species	Locality
Orthoptera	Phalangopsidae	*Phalangopsis bauxitica* Mews & Sperber, 2008	Viseu (PA)
		Phalangopsis ferratilis Junta, Castro-Souza & Ferreira, 2020	Canaã dos Carajás (PA)
		Phalangopsis kyju Junta, Castro-Souza & Ferreira, 2020	São Félix do Xingu (PA)
		Phalangopsis kysuia Junta, Castro-Souza & Ferreira, 2020	Apiacás (MT)
		Phalangopsis quartzitica Junta, Castro-Souza & Ferreira, 2020	São Geraldo do Araguaia (PA)
		Strinatia brevipennis Chopard, 1970	Iporanga (SP)
Palpigradi	Eukoeneniidae	**Allokoenenia canhembora* Souza & Ferreira, 2022	Campo Formoso (BA)
		**Allokoenenia stygia* Souza & Ferreira, 2022	Canaã dos Carajás (PA)
		**Eukoenenia audax* Souza, Mayoral & Ferreira, 2020	Nova Roma (GO)
		**Eukoenenia cavatica* Souza & Ferreira, 2016 (CR)	Arcos (MG)
		**Eukoenenia eywa* Souza & Ferreira, 2018 (EN)	Caeté (MG)
		Eukoenenia ferratilis Souza & Ferreira, 2011	Brumadinho (MG)
		**Eukoenenia ibitipoca* Souza & Ferreira, 2019	Lima Duarte (MG)
		**Eukoenenia igrejinha* Souza & Ferreira, 2019	Ouro Preto (MG)
		**Eukoenenia jequitai* Souza & Ferreira, 2020	Jequitaí (MG)
		**Eukoenenia jequitinhonha* Souza & Ferreira, 2016 (CR)	Caraí (MG)
		**Eukoenenia lundi* Souza & Ferreira, 2020	Luislândia (MG)
		**Eukoenenia magna* Souza & Ferreira, 2020	Montes Claros (MG)
		**Eukoenenia maquinensis* Souza & Ferreira, 2010 (VU)	Cordisburgo (MG)
		**Eukoenenia navi* Souza & Ferreira, 2018 (CR)	Barão de Cocais (MG)
		**Eukoenenia neytiri* Souza & Ferreira, 2018 (EN)	Mariana (MG)
		Eukoenenia potiguar Ferreira, Souza, Machado & Brescovit, 2011 (VU)	Felipe Guerra (RN)
		**Eukoenenia sagarana* Souza & Ferreira, 2012 (CR)	Cordisburgo (MG)
		**Eukoenenia spelunca* Souza & Ferreira, 2011 (CR)	Vargem Alta (ES)
		**Eukoenenia virgemdalapa* Souza & Ferreira, 2012 (CR)	Vazante (MG)
		Leptokoenenia pelada Souza & Ferreira, 2013 (CR)	Curionópolis (PA)
		Leptokoenenia thalassophobica Souza & Ferreira, 2013 (EN)	Parauapebas (PA)
Pseudoscorpiones	Bochicidae	**Spelaeobochica allodentatus* Mahnert, 2001 (EN)	Palmeiras (BA)
		**Spelaeobochica goliath* Viana, Souza & Ferreira, 2018	Ibiracatu (MG)
		**Spelaeobochica iuiu* Ratton, Ferreira & Mahnert, 2012 (EN)	Iuiú (BA)
		**Spelaeobochica mahnerti* Viana & Ferreira, 2020	Monjolos (MG)
		**Spelaeobochica muchmorei* Andrade & Mahnert, 2003 (CR)	Iporanga (SP)
	Cheiridiidae	*Cheiridium brasiliense* Mahnert, 2001	Morro do Chapéu (BA)
	Chernetidae	**Maxchernes iporangae* Mahnert & Andrade, 1998 (CR)	Iporanga (SP)
		Maxchernes kapinawai Bedoya-Roqueme, 2021	Tupanatinga (PE)
		**Spelaeochernes altamirae* Mahnert, 2001	Medicilância (PA)
		**Spelaeochernes armatus* Mahnert, 2001	Botuverá (SC)
		**Spelaeochernes bahiensis* Mahnert, 2001	Itaetê (BA)
		**Spelaeochernes dentatus* Mahnert, 2001	Almirante Tamandaré (PR)
		**Spelaeochernes dubius* Mahnert, 2001	Analândia (SP)
		**Spelaeochernes eleonorae* Mahnert, 2001	Altinópolis (SP)

Taxon	Family	Species	Locality
Pseudoscorpiones	Chernetidae	**Spelaeochernes gracilipalpus* Mahnert, 2001 (EN)	Iporanga (SP)
		**Spelaeochernes pedroi* Mahnert, 2001	Pau Brasil (BA)
		**Spelaeochernes popeye* von Schimonsky & Bichuette, 2019	Laranjeiras (SE)
		Zaona cavicola Mahnert, 2001	Jardim (MS)
	Chthoniidae	**Pseudochthonius biseriatus* Mahnert, 2001 (EN)	Itacarambi (MG)
		Pseudochthonius gracilimanus Mahnert, 2001	Iraquara (BA)
		**Pseudochthonius olegario* Schimonsky, 2022	Presidente Olegário (MG)
		**Pseudochthonius ramalho* Assis, Schimonsky & Bichuette, 2021	Serra do Ramalho (BA)
		P*seudochthonius ricardoi* Mahnert, 2001	Iporanga (SP)
		**Pseudochthonius strinatii* Beier, 1969	Iporanga (SP)
	Ideoroncidae	**Ideoroncus cavicola* Mahnert, 2001 (EN)	Iporanga (SP)
	Olpiidae	*Progarypus gracilis* Mahnert, 2001	Matozinhos (MG)
		Progarypus liliae Mahnert, 2001	Morro do Chapéu (BA)
		Progarypus nigrimanus Mahnert, 2001 (CR)	Iporanga (SP)
		Progarypus setifer Mahnert, 2001	Itacarambi (MG)
Psocoptera	Amphientomidae	*Lithoseopsis brasiliensis* García Aldrete, Neto & Ferreira, 2018	Parauapebas (PA)
	Prionoglarididae	*Neotrogla aurora* Lienhard, 2010	Aurora do Tocantins (TO)
		Neotrogla brasiliensis Lienhard, 2010	Itacarambi (MG)
		Neotrogla curvata Lienhard & Ferreira, 2013	São Félix do Coribe (BA)
		Neotrogla truncata Lienhard, 2010	Campo Formoso (BA)
	Psyllipsocidae	*Psyllipsocus angustipennis* Lienhard, 2014	Itacarambi (MG)
		Psyllipsocus clunioventralis Lienhard, 2014	Chapada dos Guimarães (MT)
		Psyllipsocus clunjunctus Lienhard, 2014	Damianópolis (GO)
		Psyllipsocus didymus Lienhard, 2014	Paranaíta (MT)
		Psyllipsocus falcifer Lienhard, 2014	Pains (MG)
		Psyllipsocus fuscistigma Lienhard, 2014	Tejuçuoca (CE)
		Psyllipsocus llipsocus marconii Lienhard, 2014	Montalvânia (MG)
		Psyllipsocus proximus Lienhard, 2014	Apuí (AM)
		Psyllipsocus punctulatus Lienhard, 2014	Coronel José Dias (PI)
		Psyllipsocus radiopictus Lienhard, 2014	Murici (AL)
		Psyllipsocus serrifer Lienhard, 2014	Pains (MG)
		Psyllipsocus similis Lienhard, 2014	Itambé do Mato dentro (MG)
		Psyllipsocus spec. cf. *punctulatus* Lienhard, 2014	Itacarambi (MG)
		Psyllipsocus spinifer Lienhard, 2014	Ubajara (CE)
		Psyllipsocus subtilis Lienhard, 2014	Felipe Guerra (RN)
		Psyllipsocus thaidis Lienhard, 2014	Coronel José Dias (PI)
	Ptiloneuridae	*Brasineura troglophilica* Silva-Neto & Aldrete, 2015	Altamira (PA)
		Loneura cavernicola Cutrim, 2022	Pains (MG)
		Triplocania brancoi Silva-Neto, Aldrete, Rafael & Ferreira, 2021	Conceição do Mato Dentro (MG)
		Triplocania ferratilis Silva-Neto, Aldrete, Rafael & Ferreira, 2021	Nova Lima (MG)
		Triplocania mariaelinae Silva-Neto, Aldrete & Rafael, 2016	Altamira (PA)

Taxon	Family	Species	Locality
Psocoptera	Ptiloneuridae	*Triplocania pains* Silva-Neto, Aldrete, Rafael & Ferreira, 2021	Lagoa da Prata (MG)
		Triplocania zairae Silva-Neto, Aldrete, Rafael & Ferreira, 2021	Lagoa Santa (MG)
Ricinulei	Ricinoididae	*Cryptocellus canga* Pinto-da-Rocha & Andrade, 2012	Canaã dos Carajás (PA)
		Cryptocellus tarsilae Pinto-da-Rocha & Bonaldo, 2007	Parauapebas (PA)
		Cangazomus xikrin Pinto-da-Rocha, Andrade & Moreno-González, 2016	Parauapebas (PA)
Schizomida	Hubbardiidae	*Naderiore carajas* Pinto-da-Rocha, Andrade & Moreno-Gonzalez, 2016	Canaã dos Carajás (PA)
		Rowlandius pedrosoi Giupponi, Miranda & Villarreal, 2016	Santa Quitéria (CE)
		Rowlandius potiguar Santos, Ferreira & Buzzato, 2013	Felipe Guerra (RN)
		Rowlandius ubajara Santos, Ferreira & Buzzato, 2013 (CR)	Ubajara (CE)
Scorpiones	Buthidae	*Ananteris infuscata* Lourenço, Giupponi & Leguin, 2013 (EN)	Grão Mogol (MG)
		Troglorhopalurus lacrau (Lourenço & Pinto-da-Rocha, 1997) (DD)	Itaetê (BA)
		**Troglorhopalurus translucidus* Lourenço, Baptista & Giuponni, 2004 (EN)	Lençois (BA)
Sphaeriida	Sphaeriidae	**Eupera troglobia* Simone & Ferreira, 2022	Lagoa da Confusão (TO)
Spongillida	-	**Arinosaster patriciae* Volkmer-Ribeiro *et al.*, 2021	Diamantino (MT)
Thysanura	Campodeidae	**Oncinocampa trajanoae* Conde, 1997 (CR)	Iporanga (SP)
	Nicoletiidae	**Cubacubana spelaea* (Galán, 2000) (VU)	Campo Formoso (BA)
Tricladida	Dimarcusidae	**Hausera hauseri* Leal-Zanchet, Souza & Ferreira, 2014 (VU)	Felipe Guerra (RN)
	Dugesiidae	**Girardia arenicola* Hellmann & Leal-Zanchet, 2018 (CR)	Iporanga (SP)
		Girardia asymmetrica Hellmann & Leal-Zanchet, 2020	Lontra (MG)
		**Girardia corumbataiensis* Morais & Leal-Zanchet, 2021	Itirapina (SP)
		**Girardia desiderensis* Souza *et al.*, 2016, (CR)	São Desidério (BA)
		Girardia ibitipoca Hellmann & Leal-Zanchet, 2020	Lima Duarte (MG)
		**Girardia multidiverticulata* Souza *et al.*, 2015 (CR)	Bodoquena (MS)
		**Girardia nobresis* Morais & Leal-Zanchet, 2021	Nobres (MT)
		**Girardia paucipunctata* Hellmann & Leal-Zanchet, 2018 (CR)	Iporanga (SP)
		Girardia pierremartini Souza & Leal-Zanchet, 2016	Presidente Olegário (MG)
		**Girardia spelaea* Hellmann & Leal-Zanchet, 2020	Carinhanha (BA)
	Geoplanidae	*Difroehlichia elenae* Leal-Zanchet & Marques, 2018	Conceição do Mato Dentro (MG)
		Paraba pankaru Amaral & Leal-Zanchet, 2019	Serra do Ramalho (BA)
		Pasipha carajaensis Amaral & Leal-Zanchet, 2019	Curionópolis (PA)
		Pasipha ferrariaphila Leal-Zanchet & Marques, 2018	Conceição do Mato Dentro (MG)
		Pasipha tutameia Amaral & Leal-Zanchet, 2019	Cordisburgo (MG)
	Uteriporidae	**Sluysia triapertura* Souza *et al.*, 2018 (CR)	Felipe Guerra (RN)

VERTEBRATES			
Taxon	Family	Species	Locality
Characiformes	Characidae	**Stygichthys typhlops* Brittan & Böhlke, 1965 (EN)	Jaiba (MG)
Siluriformes	Callichthyidae	**Aspidoras mephisto* Tencatt & Bichuette, 2017	Posse (GO)
	Heptapteridae	**Phreatobius cisternarum* Goeldi, 1905	Ilha de Marajó (PA)
		**Phreatobius dracunculus* Shibatta, Muriel-Cunha & Pinna, 2007	Rio Pardo (RO)
		**Pimelodella kronei* (Ribeiro, 1907) (EN)	Iporanga (SP)
		**Pimelodella spelaea* Trajano, Reis & Bichuette, 2004 (EN)	São Domingos (GO)

Siluriformes	Loricariidae	**Rhamdia enfurnada* Bichuette & Trajano, 2005	Coribe (BA)
		**Rhamdiopsis krugi* Bockmann & Castro, 2010 (VU)	Itaetê (BA)
	Trichomycteridae	**Ancistrus cryptophthalmus* Reis, 1987 (EN)	São Domingos (GO)
		**Ancistrus formoso* Sabino & Trajano, 1997 (VU)	Formoso (BA)
		**Glaphyropoma spinosum* Bichuette, Pinna & Trajano, 2008 (VU)	Andaraí (BA)
		**Ituglanis bambui* Bichuette & Trajano, 2004 (CR)	São Domingos (GO)
		**Ituglanis boticario* Rizzato & Bichuette, 2014	Mambai (GO)
		**Ituglanis epikarsticus* Bichuette & Trajano, 2004 (VU)	São Domingos (GO)
		**Ituglanis mambai* Bichuette & Trajano, 2008 (EN)	Posse (GO)
		**Ituglanis passensis* Fernandez & Bichuette, 2002 (VU)	São Domingos (GO)
		**Ituglanis ramiroi* Bichuette & Trajano, 2004 (VU)	São Domingos (GO)
		**Trichomycterus dali* Rizzato, Costa, Trajano & Bichuette, 2011 (VU)	Bonito (MS)
		**Trichomycterus itacarambiensis* Trajano & de Pinna, 1996 (CR)	Itacarambi (MG)
		**Trichomycterus rubbioli* Bichuette & Rizzato, 2012 (VU)	Carinhanha (BA)
Passeriformes	Rhinocryptidae	*Scytalopus speluncae* (Ménétriés, 1835)	São João Del Rey (MG)
Gymnotiformes	Sternopygidae	**Eigenmannia vicentespelaea* Triques, 1996 (VU)	São Domingos (GO)

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Gruta Brejal
Ataliba Coelho

2

Trophic Dynamics In Cave Environments

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General introduction

Living organisms process and assimilate different types of organic or inorganic resources in search of energy to build molecules, cells and other structures and to maintain organs and systems. Among the most required elements are carbon, nitrogen and phosphorus. For surface ecosystems, the energy of solar radiation represents the main driver of the production of organic compounds by photosynthetic, autotrophic or primary producer organisms. The accumulation of organic matter by these producers represents, for the most part, the first step in capturing, storing and transferring energy in surface ecosystems (PHILLIPSON, 1969).

Not unlike surface ecosystems, one of the great paradigms of ecology in subterranean environments is understanding the energetic functioning of these ecosystems, which tend to be devoid of sources of photosynthetic productivity and, consequently, oligotrophic. Issues related to supply routes, retention and storage capacity and processing of organic resources are frequently encountered. Identifying the origins and destinations of the various types of food available to fauna in subterranean environments is a crucial task, since energetic dynamics represents one of the great modulators of ecological and evolutionary patterns. Dynamics of the availability of organic resources can change interactions between organisms, processing rates and the dynamics of energy transfer (LINDEMAN, 1942; MOORE *et al.*, 2004).

Discussions about this topic for oligotrophic environments are not recent, with Hutchinson (1965) being one of the pioneers by looking into the subject and creating the concept of "allobiosphere" as a set of peripheral environments on earth, which are devoid of primary production and dependent on external donors of organic matter. Examples of allobiospheres are alpine and polar environments or the deep sea. Danielopol *et al.* (1995) included subterranean ecosystems in this category. Por (2008) coined the term "deuterobiosphere" to designate peripheral environments that are based on chemoautotrophic energy production performed by microorganisms (anchialine caves and chemotrophic aquatic environments). Dudich (1933) was the first to distinguish two possibilities for the origin of organic resources of cave environments: "allochthonous", that is, brought from outside, and "autochthonous", produced inside the cave itself. Polis and Strong (1996) argued that resources of allochthonous origin generally result in communities comprising predominantly omnivorous species and dependent on organic resources donated from environments of higher productivity. In addition, trophic interactions (food webs) vary in the extent to which detritus (decomposing non-living organic resources) is derived from allochthonous or autochthonous sources and in the particular way by which detritus is distributed in the environment. Some food webs, such as those seen in caves and small streams in forested watersheds, are based almost entirely on detritus.

Considering subterranean ecosystems, organic resources are predominantly allochthonous and enter the system mainly in the form of detritus and/or dissolved organic matter (DOM) (Figure 1). Organic detritus or organic residues are non-living parts of animals or plants, which have different compositions and origins, such as leaves, fruits, roots, trunks, branches, feces and carcasses, among others. Organic detritus represents the food base for communities of microorganisms, invertebrates and some groups of terrestrial and aquatic vertebrates.

Detritus is often categorized based on its origin, its physical structure (e.g., particle size) and its biochemical composition. The relative importance of the different forms of detritus influences the interactions and dynamics of the living species that depend on them as a source of energy. Organic resources can be separated according to size into the categories of coarse particulate organic matter (particle size >1mm – CPOM), fine particulate organic matter (particle size ≤1mm to ≥5µm – FPOM) and dissolved organic matter (particle size <5µm – DOM).

Detritus includes complex elements that relate to specific polymers, associated with plant cell walls (cellulose, hemicellulose, lignin), fungi (chitin, melanin) and animal body components (keratin, dentin, carbonates, phosphates), as well as biomolecules, such as tannins, fats/oils, nucleic acids,

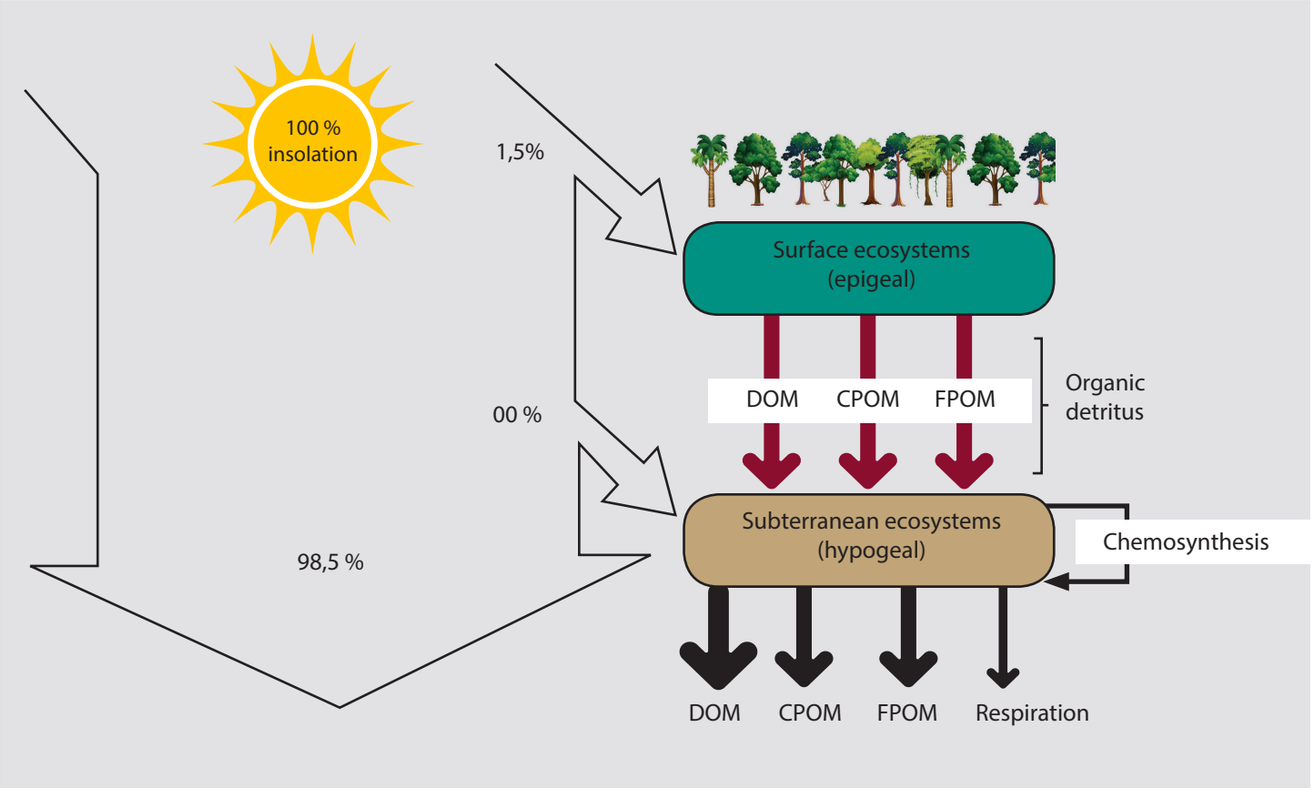


Figure 1. Schematic model of input (red arrows) and output (black arrows) of detritus in subterranean environments. Coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM) and dissolved organic matter (DOM). Photosynthetic efficiency in surface ecosystems is approximately 1.5%. Source: Modified from Graening (2000) and Phillipson (1969).

proteins, polysaccharides and the monomeric constituents of these polymers: fatty acids, sugars, amino acids, nucleotides and other aliphatic and aromatic compounds. Certain properties of detritus necromass that reflect chemical composition — C:N ratio, lignin content, and nutrient content — are useful predictors of the rate of consumption (decomposition) of newly deposited detritus (MOORE *et al.*, 2004; RAVN *et al.*, 2020).

The speed of processing different components of detritus can vary considerably over time (Figure 2). Elements that are more soluble and palatable are quickly lost (leaching) or assimilated by microorganisms and decomposer animals (consumption). On the other hand, elements that are more resistant to biological action and the action of physical weathering, persist for long periods of time in the environment (recalcitrant).

Another issue to be considered is that detritus serves as habitat and a place of reproduction for numerous groups of organisms, being essential components for "ecosystem engineering" activities, since the action of burrowing and fragmenting animals modifies the physical structure and conditions of habitats, such as humidity, temperature and air and/or water flow. Organisms manipulate detritus in both terrestrial and aquatic systems, transforming them into complex and stable environments from an ecosystem point of view (MOORE *et al.*, 2004).

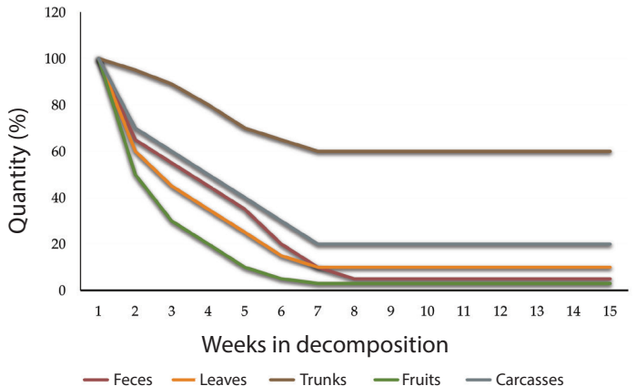


Figure 2. Hypothetical model of variation in the rate of decomposition of different types of detritus over time. Decomposition or consumption rates can be directly related to the presence of recalcitrant elements.

Thus, detritivorous organisms play a central role in the processing of organic resources. The most effective are microorganisms (fungi, bacteria, protozoa), although nematodes, mollusks, earthworms, mites, isopods, springtails and other invertebrates, are also key players in the processes of detritus decomposition. Microorganisms convert organic waste into a source of energy and nutrients for other organisms, as well as serve as food for other microorganisms, invertebrates and vertebrates.

Input of organic matter (detritus)

The components and amount of detritus in subterranean environments vary depending on the biotic or abiotic routes of production and/or transport and the distance from the donor source (Table 1, Figure 3). Sunlight, wind and gravity allow the production or input of organic resources through different vertical or horizontal openings. Watercourses, such as rivers, streams and floods, in addition to water that percolates through rock through pores or fractures, represent transport routes of particulate or dissolved organic matter to caves (SIMON *et al.*, 2007; SOUZA-SILVA *et al.*, 2007; SOUZA-SILVA *et al*., 2007). In addition, carcasses and, especially, feces of animals that use caves as shelters can also accumulate in the environment, becoming important resources for fauna, especially in permanently dry caves (CULVER, 1982; FERREIRA; MARTINS, 1999). In this case, bats are particularly important, because guano deposition can be continuous, especially in tropical and subtropical caves (FERREIRA; MARTINS, 1999; ZAGMAJSTER, 2019) (PIMENTEL *et al.* 2022). Superficial subterranean environments can receive additional increments of organic resources from roots of the surrounding external vegetation that access fractures, interstices, canaliculi and cave conduits. Such imported organic materials often form deposits that provide resources and shelter for subterranean fauna. However, the composition and constancy of the supply of organic matter can determine differences in the structure and dynamics of terrestrial and aquatic detritivorous invertebrate communities (SCHNEIDER *et al.*, 2011; SOUZA-SILVA *et al.*, 2011).

Only in a few exceptional cases are communities that inhabit subterranean environments independent of allochthonous input of food resources (ENGE, 2007). The most famous example is the Movile Cave in Romania (SARBU *et al.*, 1996). The energy base for the maintenance of invertebrate communities in Movile Cave is provided by chemoautotrophic bacteria, through chemosynthesis.

Therefore, the dynamics of the availability of food resources in subterranean environments can be assumed to depend on factors related to geographic location, morphology of entrances and conduits, extension, slope, drainage areas and the characteristics of biotic and abiotic vectors.

Caves with large areas of contact with the surface and without physical obstacles that prevent the input of materials from the epigean environment can receive greater amounts of organic resources than those with a lower degree of connectivity. Water from streams that enter caves can provide a constant input of increased organic matter during flood pulses. On the other hand, large colonies of bats provide significant amounts of organic resource to cave fauna in the form of guano, as will be detailed later (PIMENTEL *et al.* 2022).

Illuminated entrances and primary productivity via photosynthesis

Although permanent darkness represents a barrier to the development of photosynthetic organisms, specialized plant groups can populate entrance and penumbra zones in caves. Illuminated locations located primarily at the interface between surface and subterranean environments harbor cyanobacteria, algae, mosses, ferns and even flowering plants (Figure 4).

The extent of these illuminated zones (photic and dysphotic) is mainly determined by the morphology of entrances and

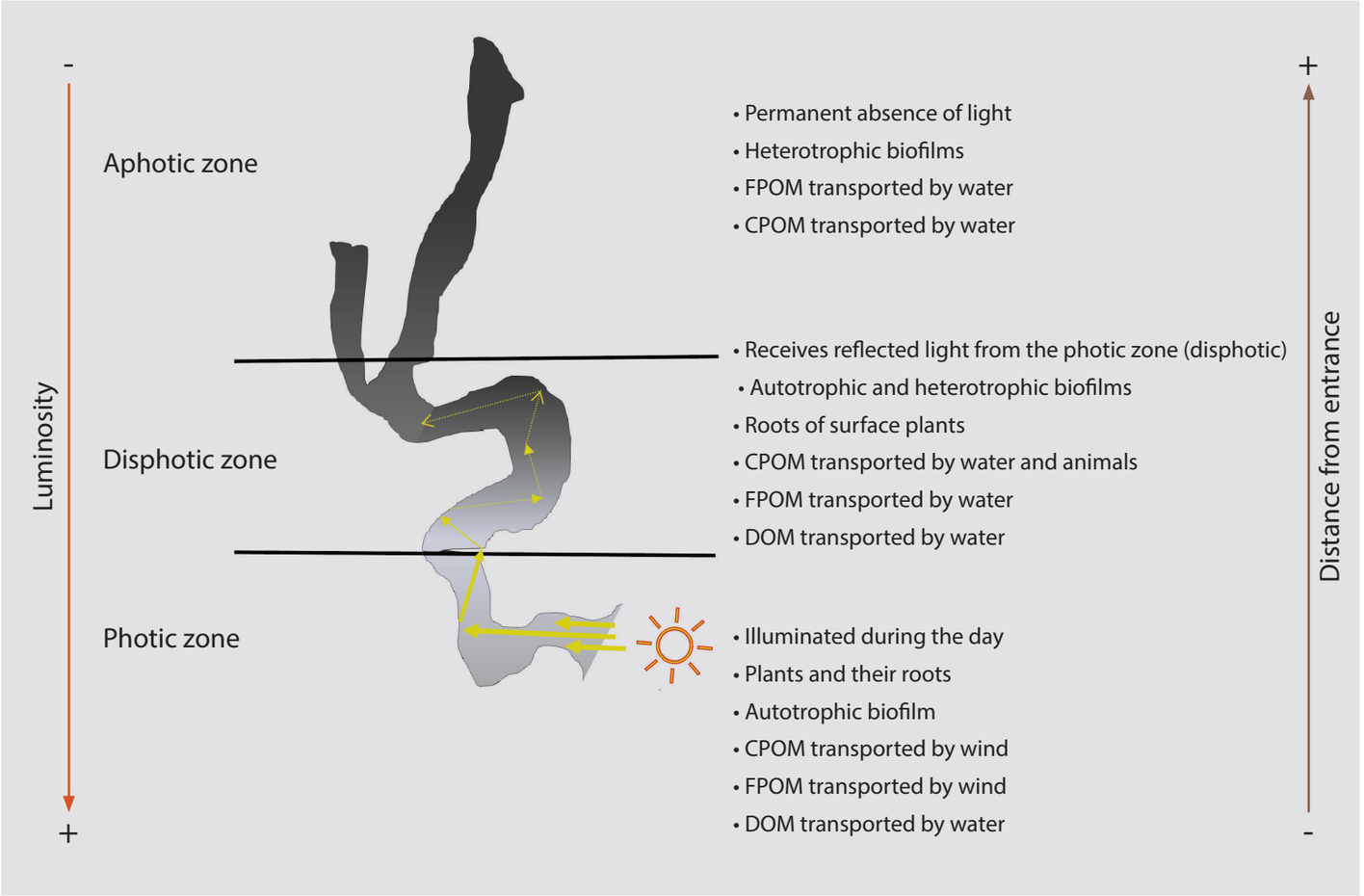


Figure 3. Hypothetical model of luminosity distribution and organic resources inside caves, based on the main dispersion vectors. The yellow arrows indicate an attenuation in luminosity between the photic and disphotic zones as a function of the distance from the entrance (brown arrow on the right). Coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM) and dissolved organic matter (DOM).

conduits, the ability of the rock to reflect light, the time of day and the seasons of the year (PENTECOST, 2004; SKET, 2004). Such characteristics, associated with soil roughness, depth and moisture, allow the formation of a compositional gradient of different plant groups, with different requirements and tolerances in relation to the amount of light present (MONRO *et al.*, 2018; MULEC; KOSI, 2009).

Groups of cryptogams (mosses and ferns) and algae (diatoms, cyanobacteria and chlorophytes) may be less demanding and more tolerant of low light availability (Figure 5). These organisms can serve not only as food resources for fauna, but also as shelter for invertebrates (isopods, springtails, psocopterans, dipterans and tardigrade, among others). The dispersion of these "plants" occurs mainly by wind (anemochory) and, eventually, by water (hydrochory).

It is important to mention that electric lighting in tourist caves allows for the occurrence of lampenflora in deep places that would naturally be aphotic. The term "lampenflora" refers to the process of proliferation of photosynthetic organisms near artificial light sources where they would not occur under natural circumstances (Figure 6). The "lampenflora" is distinguished by from plants that lives near cave entrances the fact that they colonize natural and artificial substrates in aphotic zones and are totally dependent on artificial lighting (MULEC; KOSI, 2009). Eventually, these microalgae mats of "lampenflora" also serve as food resources for invertebrates (MEYER *et al.*, 2017; MULEC; KOSI, 2009).

Table 1. Input routes of organic resources to subterranean environments. Coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM) and dissolved organic matter (DOM). Source: Modified from Culver and Pipan (2009).

Vector/Source	Type	Origen	Components	Destination habitat
Chemosynthesis	Autotrophic biofilms	autochthonous	Bacteria and fungi	Caves, interstices, fractures, canaliculi. Wet aquatic or terrestrial environments
Sunlight or artificial electric lighting	Photosynthetic organisms	autochthonous	Algae, mosses, ferns, angiosperms	Vertical or horizontal entrances Generally limited depending on the daily amount of light
Roots of epigean vegetation	Root growth	autochthonous	Live and dead roots	Caves, interstices, fractures, canaliculi. Usually limited by the superficiality of subterranean environments
Permanent flowing waters	Percolation and drip water, autogenic streams	autochthonous	Dissolved organic matter and small animals, fungi, bacteria	Caves, interstitial habitats, surface subterranean habitats Generally limited by rock porosity and water storage capacity
Wind	Gravitational	autochthonous	Plant and animal detritus of varying sizes, spores	Caves, interstitial habitats, surface subterranean habitats Generally limited by rock porosity and water storage capacity
Temporary and permanent flowing water	Allogeneic streams and floods	autochthonous	Plant and animal detritus of varying sizes, fungi, bacteria	Caves, fractures Generally limited by epigean productivity, seasonality, catchment area of watershed, presence of obstacles
Animal movement	Birds, bats, amphibians and rodents	autochthonous	Feces, guano, carcasses, uneaten dietary remains	Caves Generally limited by animal abundance and epigean seasonality



Figure 4. Growth of vegetation at the entrance to caves. **A)** Algal biofilm; **B, E and F)** ferns; **C)** mosses; **D and G)** flowering plants. Photos: (Rodrigo Lopes Ferreira).

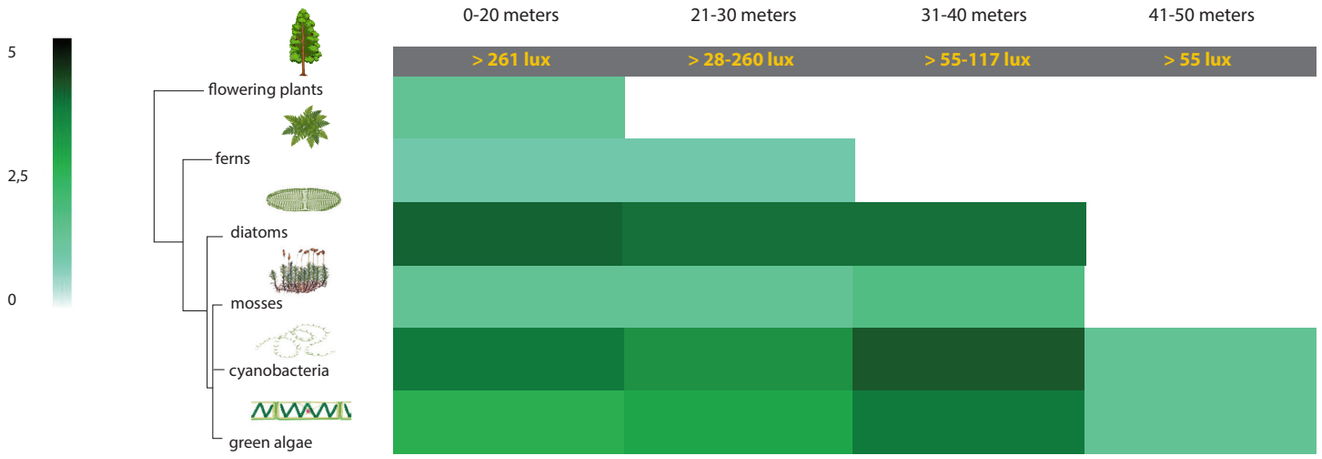


Figure 5. Distribution of groups of photosynthetic organisms limited by distance (meters) and amount of luminosity (lux) in the region of the cave entrance. The intensity of the greenish coloration in the figure reflects the actual number of species. The dendrogram shows the association in the distribution of groups. Source: Abdullin (2011).

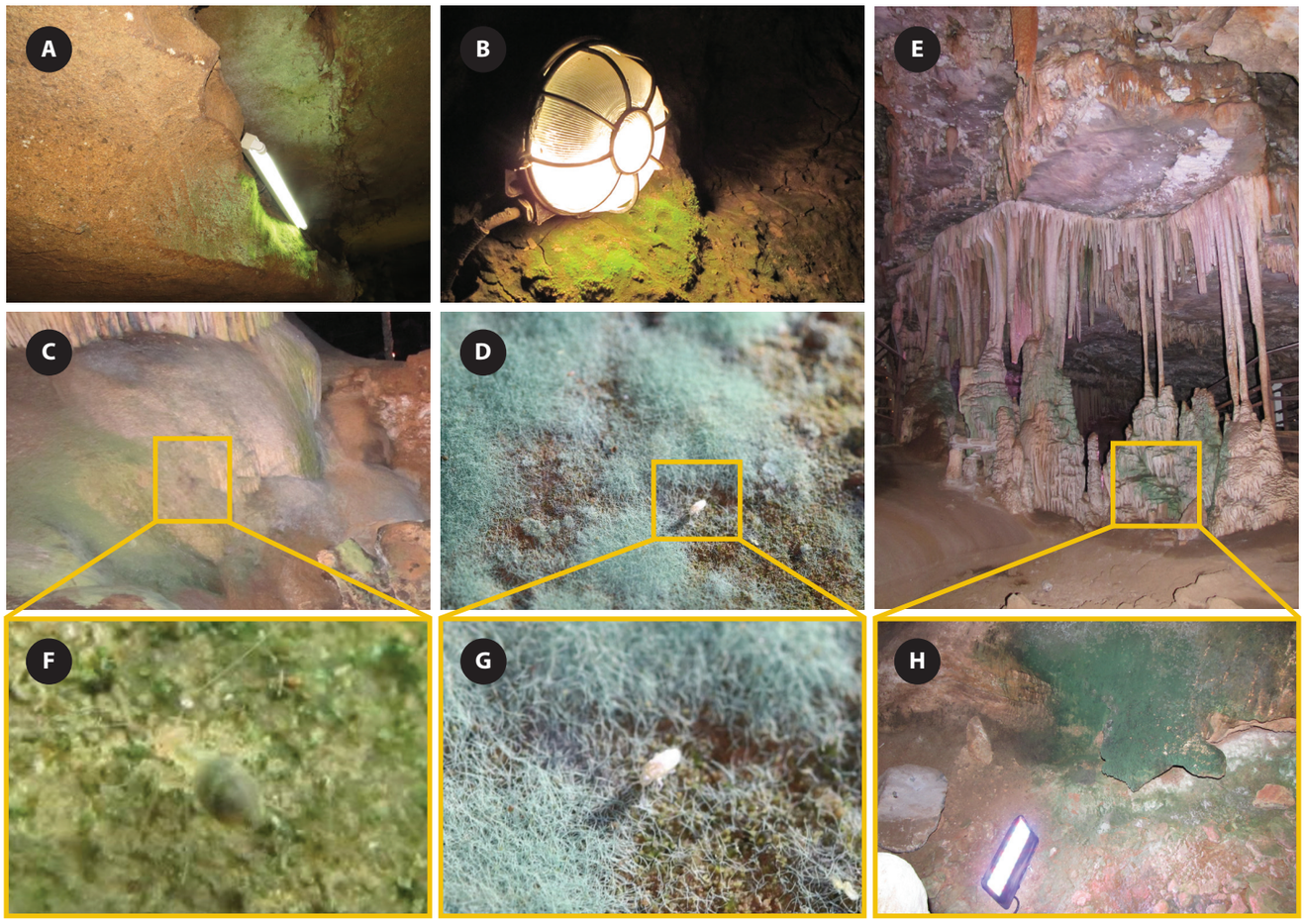


Figura 6. Growth of **(A, B)** mosses and **(C, D, E, H)** green algae under artificial lighting (lampenflora) on cave walls and speleothems; **F and G)** yellow squares indicate individuals of Psocoptera and Collembola. Photos: (Rodrigo Lopes Ferreira).

Input of detritus by wind and gravity

The high primary productivity of tropical surface ecosystems, associated with periods of high deciduousness in plant phenology, provide a large amount of organic matter that can be moved by wind or gravity. In karst regions, the vertical and horizontal openings of caves work as traps for collecting this plant organic matter. Eventually, small invertebrates that disperse in the wind can also be captured. Much of this precipitated material accumulates near cave openings or entrances. However, depending on the floor morphology of conduits, part of this material can be transported to deeper places in caves (Figure 3). Since the organic matter transported by these routes is predominantly plant fragments, it is of low nutritional quality and its processing depends on colonization by fungi and bacteria. In addition, the presence of greater moisture is a crucial factor for this material to be used as a food resource by other organisms.

Studies that seek to understand the dynamics and importance of resources transported by wind and gravity into caves are still scarce. However, it can be hypothetically assumed that the amount of transported vegetal organic matter is dependent on epigean productivity, the degree of climatic seasonality and the location and size of cave entrances.

Souza-Silva *et al.* (2013) used litter collection funnels to evaluate the transport of detritus by wind and gravity in vertical and horizontal entrances of quartzite caves in Parque Estadual do-Ibitipoca (Ibitipoca State Parl), state of Minas Gerais, Brazil, and found leaves and fragments of branches to be the main materials transported to the entrance of the caves. The structure of epigean vegetation surrounding cave entrances (forest or field) and the distance from the entrance were determining factors of the amount of organic matter deposited and the places reached inside the caves (Figure 7). In addition, the water mantainance of the valleys where the caves were located did not allow high plant deciduousness in the dry periods of the year, causing the greatest production and transport to occur in the rainy period (Figure 8).

Over the course of 23 months, Schneider *et al.* (2011) performed an experiment involving the removal of plant material from the floor of caves below skylights and separately adding standardized amounts of leaves and rodent carcasses to assess colonization and changes in invertebrate community structure. The authors showed that the species composition associated with leaves differed from that observed for carcasses, although the latter supported greater abundance compared to leaf-dominated organic deposits.

Input by growing roots (tropism)

The factors that drive the growth of root systems are related to genetic, hormonal and environmental control. Growing roots tend to follow fractures and channels and, preferably, explore porous soils (MAEGHT *et al.*, 2013). In tropical environments, roots of shrubs and trees can develop for great depths, including accessing cracks and crevices in rocks and cave conduits (DU PREEZ *et al.*, 2015). The development of cone-shaped root "stalagmites" has been described in caves in the Czech Republic, the United States and Brazil (FERREIRA, 2005; LAMONT; LANGE, 1976; WINKELHOFER, 1996) and subsequently observed in many other tropical areas (DU PREEZ *et al.*, 2015; FERREIRA, 2005).

Roots can develop in the penumbra zone or in total darkness and serve as an additional food resource for cave fauna. Although it is well known that fauna in the upper soil horizons and litter layers use roots as shelter and food resource, the depth to which roots can influence the vertical distribution of fauna is still little known. In cave environments, deep roots are important sources of energy and shelter for both troglobites and non-troglobites (CULVER; PIPAN, 2009; MAEGHT *et al.*, 2013). In Brazil, as well as in other parts of the world, there are species of terrestrial phytophagous troglobitic invertebrates that are obligatorily dependent on roots, such as the leafhoppers *Ferrixicius davidi* (HOCH; FERREIRA, 2012), *Kinnapotiguara troglobia* (HOCH; FERREIRA, 2013) and *Iuiuia caeca* (HOCH; FERREIRA, 2016).

Roots are eaten alive or dead, providing resources for a wide diversity of organisms in the terrestrial and aquatic environments of caves, including phytophages, detritivores and their predators. In addition to protozoa, fungi, mycorrhiza, nematodes, flatworms, annelids and mollusks, a wide diversity of arthropods (mites, insects, amphipods, isopods) and even fish use roots as shelter or food resource in aquatic cave environments (Figure 9). Thus, the energy provided by these entanglements of roots sustains a wide diversity of organisms (JASINSKA *et al.*, 1996, SOUZA-SILVA *et al.*, 2011; SENNA *et al.*, 2014). However, terrestrial cave communities that use roots may show less diversity and abundance than those supported by roots in aquatic habitats (FERREIRA, 2005; SOUZA-SILVA *et al.*, 2011; FONG, 2019).



Figure 7. Litter collection funnels used to assess the transport of plant organic matter by wind and gravity: **A)** horizontal openings; **B and D)** vertical openings; **C)** Graph demonstrating the total amount and diversity of organic resources imported in this experiment. Source: Modified from Souza-Silva *et al.*, 2013. Photos: A–B (Rodrigo Lopes Ferreira); D (Marconi Souza Silva).

Ferreira (2005), a pioneer in Brazil and worldwide in the description of trophic relationships between plant roots and terrestrial cave fauna, studied ferruginous caves in Nova Lima, state of Minas Gerais, Brazil, and reported that roots and exudates from their decomposition are consumed directly by a considerable number of phytophagous and detritivorous species, and considered them to be one of the main sources of nutrients in the studied cave systems. The roots grew in different configurations, being able to hang from the ceiling or the sides of cave galleries, and also grew on the floor, forming root reticles. In addition, columnar structures formed by the anastomosing growth of roots at drip sites, called rhizothems, were described (Figure 9A and D). Such root tufts develop by seeking out moisture from drips coming from the ceiling, similar to the growth of speleothems.

Trophic relationships in aquatic communities associated with roots were described by Souza-Silva *et al.* (2011) in an allogenic stream in a carbonate cave in the municipality of Mambaí, state of Goiás, Brazil (Figure 10). Although roots did not represent the most abundant form of organic resource, they proved to be important as a resource and shelter for fauna, since more than 111 species of invertebrates were associated with this material. The study also found root biomass and number of species to be positively related. Thus, it can be assumed that the presence

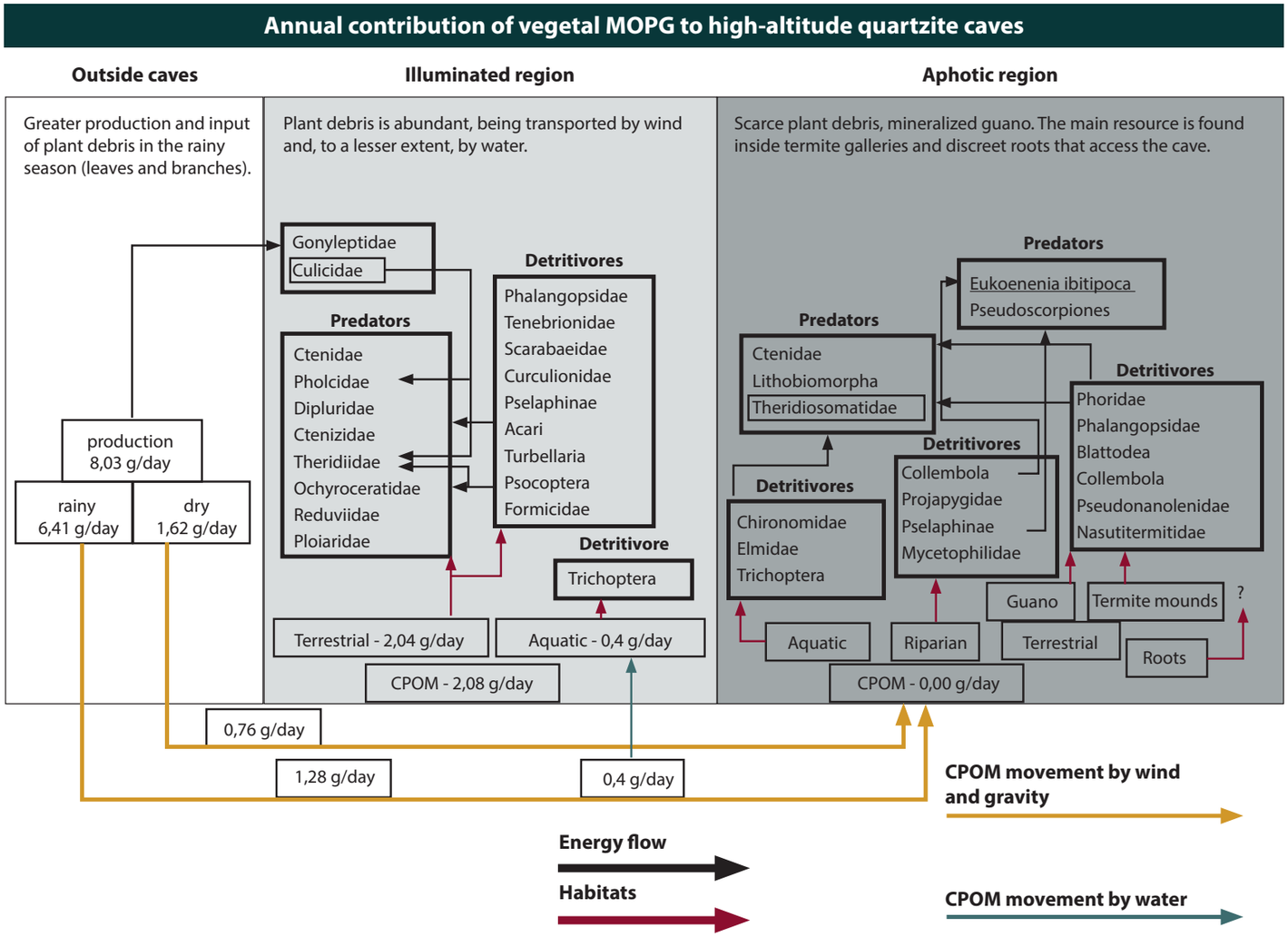


Figure 8. Input of plant detritus by wind and gravity in the Casas cave, Parque Estadual do Ibitipoca, state of Minas Gerais, Brazil. The fauna associated with different habitats and the energy flow in the invertebrate community are also represented, with weight/day being the amount of coarse particulate organic matter (CPOM >1mm) transported daily. Source: Souza-Silva *et al.* (2013).

of roots in cave environments, despite being unpredictable, supports a rich and abundant fauna, including troglobitic species. Especially in subterranean streams, roots offer a great diversity of food resources and micro-habitats with structures different from those offered by sediment (JASINSKA *et al.*, 1996). The simple presence of roots alters water flow and allows greater sediment stability, thereby increasing nutrient capture from the water column and sediment porosity, and, thus, better aeration, allowing unrestricted access to fauna. These differentiated conditions imposed by the presence of roots allow a high abundance and richness of distinct functional groups (Figure 10).

Detritus input via aquatic routes

Epigean waters that reach subterranean environments generally act as links between the two ecosystems, allowing for the exchange of organic detritus to the transit of living organisms. Subterranean ecosystems receive organic matter from the region upstream of these drainages, which is retained, transformed and exported downstream. Thus, water serves as a means of transport and acts in the movement of large amounts of organic matter in karst aquifers (Figure 11).

Such aquifers are formed by a network of subterranean spaces in a drainage basin with input and output of river or rainwater. The permeability of bedrock, fractures and conduits (>1cm) represent the functional units (porosities) that allow water circulation in karst. Differences in aquifer porosities are be determined by the action of water flow and rock types. Thus, temporal and spatial variations determine the hydrological functioning of subterranean environments. In addition, there are at least three water circulation zones in karst: (1) the unsaturated vadose zone of vertical water circulation; (2) the intermittently saturated or epiphreatic zone; and (3) the phreatic zone. In addition to these, there is the epikarst where water is stored in subsurface compartments. This diversity of compartments makes the characteristics of aquatic environments in the subterranean environment highly heterogeneous in terms of location, volumes and velocities of flow (SACCÒ, *et al.* 2022).

The water that percolates vertically in the vadose zone and epikarst compartments mainly carries dissolved organic matter (DOM) and fine particulate organic matter (FPOM). These access cave conduits through dripping or runoff by speleothems on the ceiling and walls (Figure 12A, C, D). Although the DOM concentration of drip water is generally low, it represents an important source of organic carbon available to support microbial biofilm (Figure 12C and D). Furthermore, the presence of a large volume of water dispersed in these compartments can supply water and organic matter to small autogenic streams or feed travertine reservoirs (Figure 12A, B, C). The vadose movement and dripping water of the epikarst also carry small organisms, including annelids and microarthropods, among others (CULVER; PIPAN, 2009).

On the other hand, allogeneic streams that access subterranean environments through large vertical and/or horizontal openings carry mainly fine particulate organic matter (FPOM) and a large



Figure 9. Tufts of roots growing inside caves and accessing terrestrial habitats (**A, D, E, F)** and aquatic habitats **(B, C)**. A and D are rhizothems (negative geotropism towards dripping percolation water). Photos: (Rodrigo Lopes Ferreira).

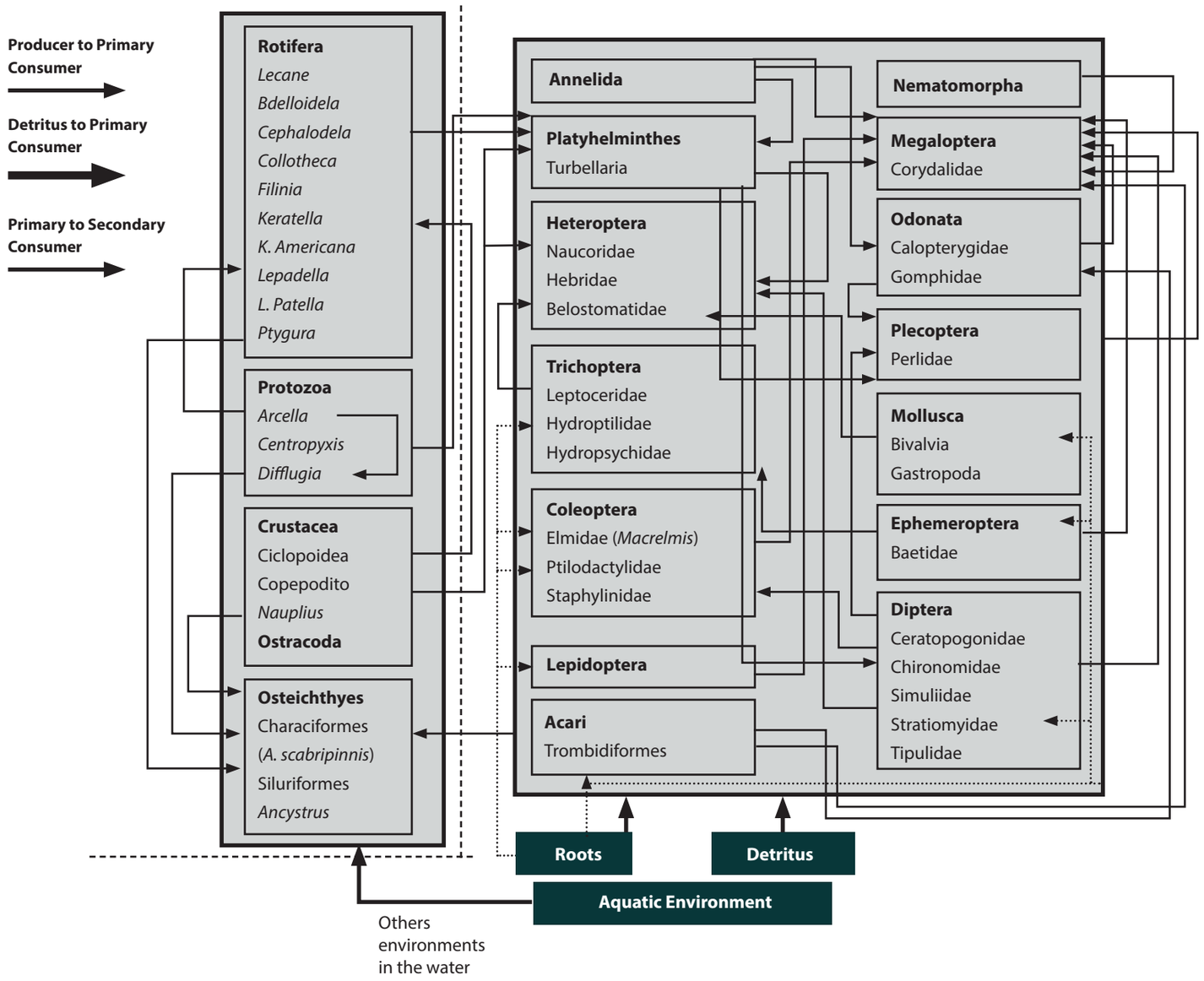


Figure 10. Importance of roots and detritus as shelter and resource for aquatic invertebrate fauna in Lapa do Ribeirão dos Porcos, Mambaí, state of Goiás, Brazil. Broad arrows indicate habitats and thin arrows indicate likely energy transfer interactions. Source: Souza-Silva (2003).

amount of coarse particulate organic matter (CPOM), including leaves, trunks and branches (SIMON; BENFIELD, 2001; SOUZA-SILVA *et al.*, 2011). The nutritional quality contained in CPOM is low and can be quickly leached by the abrasive action of water or transformed (by aquatic insect larvae and some crustaceans) into FPOM. The banks of streams inside caves represent another important compartment where organic matter is deposited. Such sites can be colonized by fungi and bacteria, flatworms, nematodes, annelids, mollusks and arthropods. Although the occurrence of these deposits is both spatially and temporally unpredictable, many terrestrial cave organisms utilize these ephemeral resources (Figure 12E).

In addition, aquatic fauna in groundwater can be highly dependent on energy from the biofilm that coats rocks and sediments. The microorganisms that compose biofilm are limited by the availability of DOM, and not by the availability of

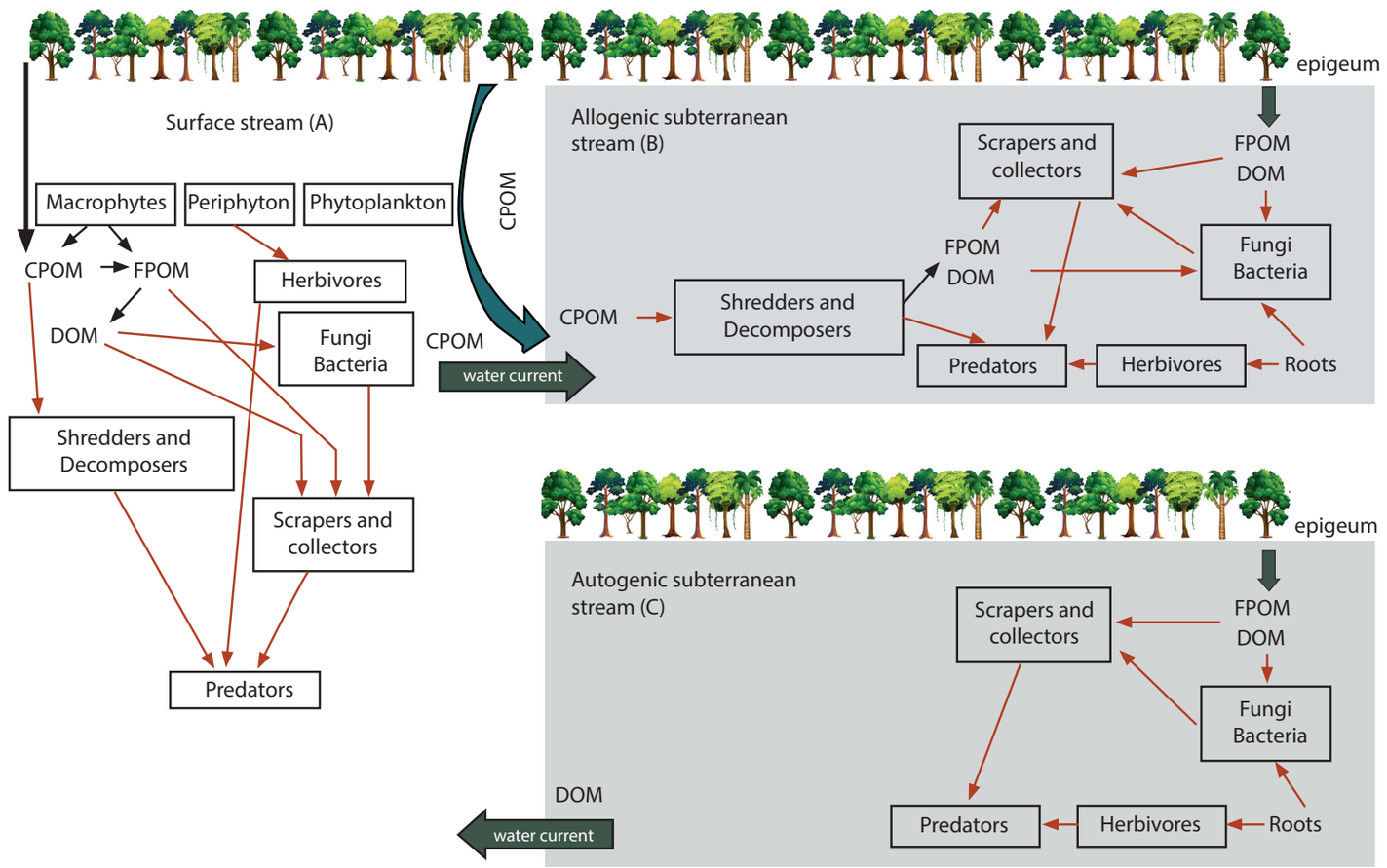


Figure 11. Representation of the flow of organic matter (detritus) in tropical cave streams in relation to surface streams. Input and/or production of organic matter (black arrows), energy flow (red arrows). Direction of water flow (wide blue arrows). Functional groups dependent on organic matter (decomposers, shredders, scrapers, collectors, herbivores, predators).

FPOM and CPOM (SIMON *et al.*, 2003).

The amount and types of vegetal detritus (fragments, large pieces of wood, roots, etc.) that penetrate caves with high connectivity with the epigean environment can be directly related to the productivity of the local epigean vegetation, the degree of seasonality, the volume of rainfall in the region, the extension of the catchment area of the basin and existing irregularities and topographic barriers (Figures 11 and 12E, F). However, flow volume presents a great destructive power for the deposits of organic resources. Consequently, a large stream entering a cave will carry a large amount of organic matter (CPOM and FPOM) to support invertebrates and, indirectly, the biofilm (Figures 11A, B and 12G), but will cause perturbation and destruction to the biofilm covering the substrate, as well as the resident fauna. However, small streams with a reduced volume of water or those that are autogenic will carry a smaller amount of organic matter, although they will be more spatially and temporally stable, causing less disturbance to associated communities (Figures 11C and 12C).

A 12-month study carried out by Souza-Silva *et al.* (2011) in Lapa do Ribeirão dos Porcos, in Mambaí, state of Goiás, Brazil, showed that detritus penetrates less in the dry season of the year when compared to the rainy season. However, the vegetation of limestone outcrops, which has a high proportion of deciduous species, provides a greater accumulation of leaves in litter during the dry season (BRINA 1998, 2020). These authors observed that detritus accumulated in the soil was carried in greater quantities into the cave in the rainy season than in the dry season. However, the authors discussed whether there is really a greater availability of these resources for invertebrates in

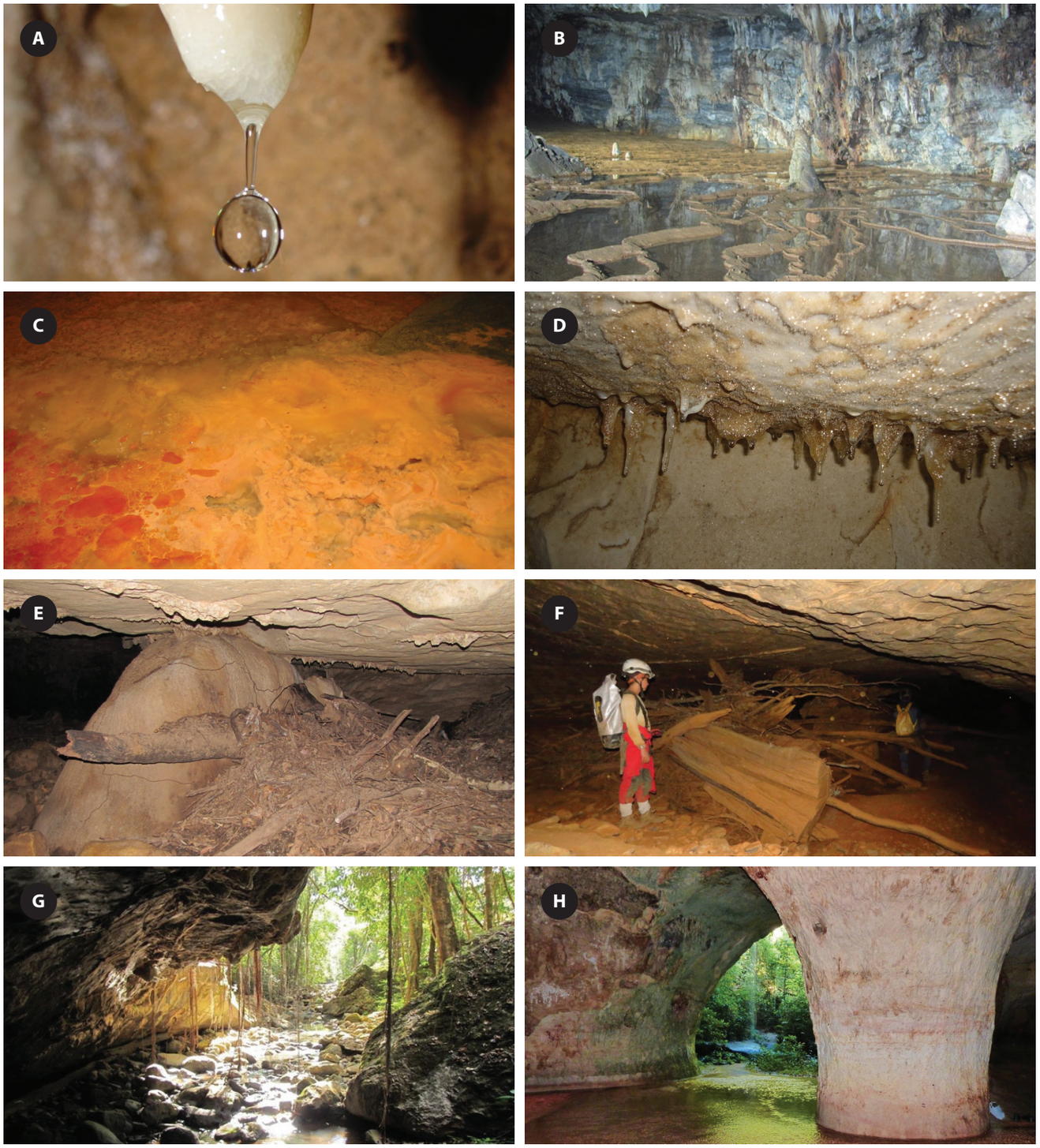


Figure 12. Movement of water in caves and different types of transported organic matter. **A)** Percolated water in speleothems; **B)** percolation water deposited in travertine reservoirs; **C)** heterotrophic microbial biofilm in an aquatic environment; **D)** ceiling-pendant microbial biofilm in a quartzite cave; **E and F)** coarse particulate organic vegetal matter deposited by an intermittent allogenic stream; **G)** perennial allogenic stream; **H)** perennial allogenic stream. Photos: A, C, D, E, G (Rodrigo Lopes Ferreira), B and H (Marconi Souza Silva), F (Gabrielle Soares Muniz Pacheco).

the rainy season because the greater input of vegetal detritus in the rainy season is accompanied by a greater intensity of "flood pulses", which remove and leach such organic resources out of the cave.

Thus, it is only in the dry season that detritus accumulates in hypogean stream sediment. Consequently, the colonization of these resources by decomposer fauna should occur more intensively during this period. In the rainy season, flood flows act as a disruptive force on the retention and processing of food resources, which can reoccur in periods subsequent to more intense flows. Thus, the frequency of flood flows affects the dynamics of transport, deposition and consumption of organic material in allogeneic streams in caves. In this way, perhaps only the amount of food resources brought by water to the interior of a cave is not the best indicator of its trophic status, but factors such as permanence time of resources and their consequent availability to organisms should also be considered. This permanence may, in turn, depend on the speed with which the detritus is consumed and the frequency of leaching to which it is subjected. Generally, the occurrence of exceptional flows modifies the bottom sediment of streams, due to strong transport energy, but after these flows, gradually, there may be a new phase of deposition of particles on the bottom. If such flows occur at a high frequency, there may be a delay or impediment to the return to the resource accumulation processes and, consequently, of the fauna that depends on it (JANINE GIBERT *et al.*, 1994; PACIOGLU *et al.*, 2019).

Detritus input via terrestrial routes

In addition to wind, gravity and water, organic detritus is transported to caves by numerous species of vertebrate and invertebrate, with bats being the most frequent. Some animals are residents, others are occasional cave visitors, and some are accidental (Figure 13). The occurrence of additional organic resources from owl regurgitates (egagropylae), insect feces (cockroaches and crickets), termite nests and anthill waste dumps may be uncommon in tropical caves, so their use by invertebrates is limited. These resources rarely function as trophic support for more complex food webs in tropical caves (FERREIRA, 2000; SOUZA-SILVA *et al.*, 2013). However, the amount and types of resources brought into caves differ among these groups of organisms.

In Brazil, bats and swifts, after foraging in surface environments, are the main producers of guano in caves (Figure 14). Swifts are insectivores and use caves as nocturnal shelters (Pichorim, 2002). There are few studies that report the occurrence of invertebrates in guano of Swifts, however, mites (Macrochelidae, Ascidae, Ereynetidae and Anoetidae), dipterans (Phoridae, Psychodidae, Chironomidae and Drosophilidae), coleopterans (Staphylinidae and Carabidae) and springtails (Hypogastruridae) have been found (Figure 14G).

The herbivorous rodents *Kerodon acrobata* and *K. rupestris*, known as rock mocós, are often associated with rocky environments, including caves in the Cerrado and Caatinga regions. These rodents usually produce large amounts of feces (Figure 14F), which are used by numerous invertebrates, such as parasitoid microhymenoptera, springtails, psocoptera, lepidopteran larvae, ants and mites, among others (FERREIRA; MARTINS, 1999).

Bats can be crepuscular or nocturnal, with a varied diet, offering different possibilities to cave fauna in relation to deposited guano (carnivore, hematophage, insectivore, nectarivore, frugivore and piscivore). However, hematophages, insectivores and frugivores seem to be the most frequent in Brazilian caves. Many bat species feed in the epigean environment at night and rest in caves during the day, often forming large colonies. Such large aggregations of bats (colonies) produce a considerable amount of guano in caves (PIMENTEL *et al.* 2022). A study carried out in Lapa do Ribeirão dos Porcos found that the amount of guano produced by different species of bats is greater in the rainy season, when the availability of food (insects, flowers, fruits) in surface ecosystems is likely to increase (Figure 15) (SOUZA-SILVA *et al.*, 2011).

The dynamics of trophic interactions between invertebrates in guano piles was well detailed by Ferreira & Martins (1999) in a review of studies on guano in Brazilian caves. The importance of bat guano as a high-quality energy source for cave invertebrates is demonstrated by the rich communities with complex trophic structures associated with this resource, especially in the tropics (FERREIRA; MARTINS, 1999; FERREIRA, 2019). In these substrates, invertebrates can directly consume guano or feed on microorganisms that grow on this resource. However, due to the ephemeral characteristics of guano, the communities associated with it can present extremely dynamic



Figure 13. Carcasses of **A, C, E)** vertebrates and of **B, D, F)** invertebrates in the interior of caves. Photos: A, B, C, E, F (Rodrigo Lopes Ferreira), D (Marconi Souza Silva).

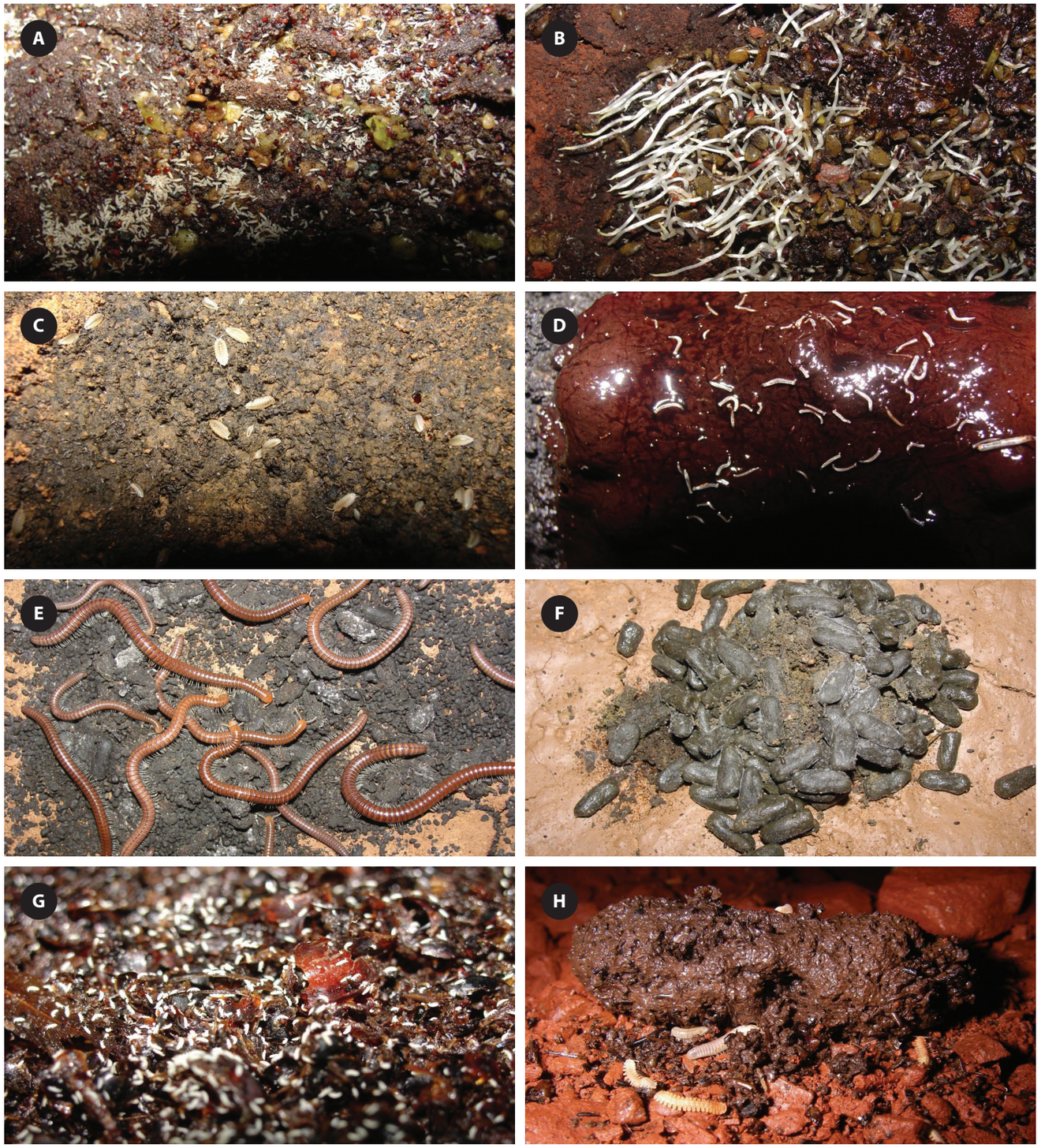


Figure 14. Feces of vertebrate animals in the interior of caves. **A, B)** Frugivore bat guano; **C, D, E)** hematophagous bat guano; **F)** mocó feces; **G)** swift guano; **H)** amphibian feces. Photos: A - G (Rodrigo Lopes Ferreira), H (Acervo Vale).

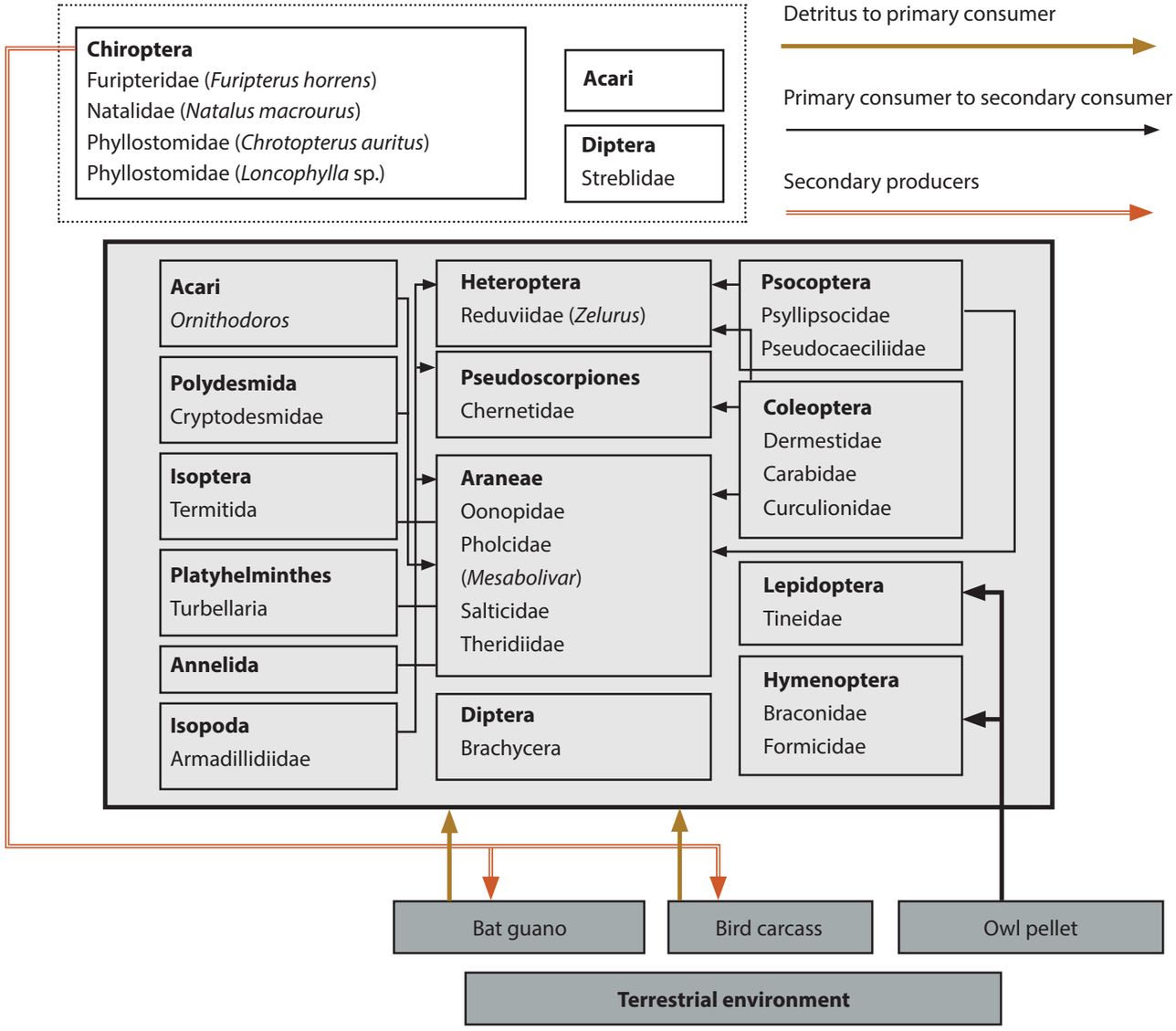


Figure 15. Importance of guano, bird carcasses and owl regurgitations as shelter and food resources for terrestrial invertebrate fauna in Lapa do Ribeirão dos Porcos, Mambaí, state of Goiás, Brazil. Arrows indicate habitat/resource as well as resource producers/vectors. Source: Souza-Silva *et al.* (2011).

structures, since the organic matter that is quickly consumed is not replaced in a homogeneous way. However, the presence of a colony of bats residing in a cave allows a constant replenishment of the organic matter that was consumed or mineralized, which makes guano behave differently in relation to traditional ephemeral resources (FERREIRA 2019; PIMENTEL, 2022). Despite this, numerous groups of invertebrates can be associated with guano from bats in tropical caves, including mites, spiders, pseudoscorpions, isopods, coleopterans, springtails, dipterans, crickets, bedbugs, ants, parasitoid wasps, termites, moth adults and larvae, neuropterans, psocopterans, fleas, thysanopterans and beetles (FERREIRA 2019).

Trophic budget evaluating

Although some aspects of trophic relationships are known

for subterranean environments, many ecological processes within these ecosystems are still not fully understood (FERREIRA, 2005; FERREIRA; MARTINS, 1999; FONG, 2019; SOUZA-SILVA *et al.*, 2011). It is clear that less emphasis has been given to the flow of nutrients in subterranean environments (especially carbon, phosphorus, nitrogen), principally in tropical regions (CULVER; PIPAN, 2009; SIMON *et al.*, 2007). Despite this, researchers of trophic dynamics in these environments agree that, instead of focusing on quantifying biomass, numbers of individuals, number of species and other unitary trophic components of the system, focus should be directed to flows and stocks of organic matter for a better understanding of energy flow. In addition, it is important to integrate an appropriate spatial scale of analysis and consider elements of the landscape, such as extension of drainage basins, reliefs, vegetation types, water courses and human uses (CULVER; PIPAN, 2009; JANINE GIBERT *et al.*, 1994).

Studies that evaluated the trophic dynamics in caves have revealed that the most appropriate scale to study energy flow with an ecosystem approach is the one that includes the relevant energy sources and for which the input and output balances of matter can be evaluated. Energy inputs should include not only autochthonous production in caves, but the importation, by water, of dissolved organic matter (DOM) and particulate organic matter (FPOM and CPOM) originating from surface vegetation or transported by wind, gravity and animals.

Conceptual models describing the circulation dynamics of dissolved and particulate organic matter have been proposed by some authors in temperate and tropical regions (CULVER; PIPAN, 2009). The pioneers were Janine Gibert *et al.* (1994), who compiled information and contextualized energetic dynamics of the three main subterranean aquatic compartments (porous, vadose, phreatic), showing the flow of dissolved and particulate inorganic substances (DIM and PIM) and of dissolved and particulate organic matter (DOM and POM), through food webs contained in these aquifers. The authors emphasized a lower complexity in food webs in subterranean water compared to surface streams.

Simon *et al.* (2007) presented a conceptual model of the movement of dissolved organic carbon in the "Organ Cave" caves in western Virginia, United States, and in the "Postojna-Planina Cave System (PPCS)" in Slovenia. To this end, they carried out a series of measurements of organic carbon in waters dripping from the epikarst, from sinkholes and from upwellings of allogeneic underground streams. They showed that particulate organic carbon (POC) and dissolved organic carbon (DOC) enter the system via localized flow through sinks and wells and diffuse flow through soils and epikarst. Most of the organic carbon enters through the epikarst in average concentrations comma, around 1 mg/L. Upon entry, organic carbon is processed and moved within hypogean habitats, before being lost through microbial respiration or leaching.

Souza-Silva *et al.* (2011) first described the dynamics of coarse particulate organic matter (CPOM) availability in tropical cave environments. Import, stock, consumption and export routes were quantified bimonthly over a year, generating an energy balance for terrestrial and aquatic environments of a limestone cave. The main routes were through water and bats (Figure 16). Despite the predominance of organic detritus imported by the stream, those deposited by bats in terrestrial environments proved to be more persistent and were stored longer in the cave. The influence of flood flows was decisive in the dynamics of entry and in the stock of organic matter. The highest stock rates occurred in the dry periods of the year and leaching and consumption in the rainy periods, showing high instability of the trophic balance, mainly in the rainy periods of the year.

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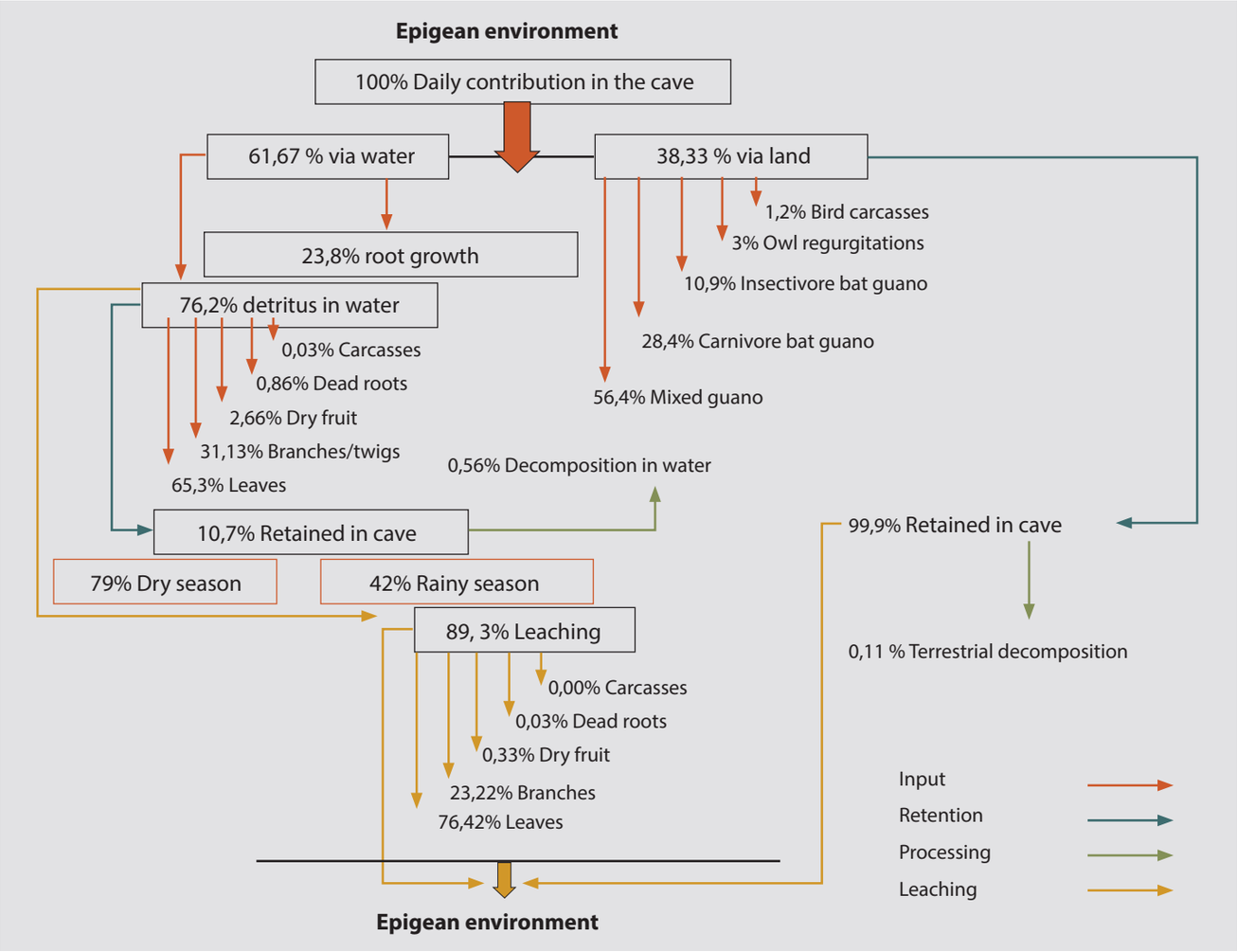


Figure 16. Dynamics of input, retention, processing and leaching of detritus in terrestrial and aquatic environments in Lapa do Córrego dos Porcos, Mambaí, state of Goiás, Brazil. Source: Modified from Souza-Silva (2003).

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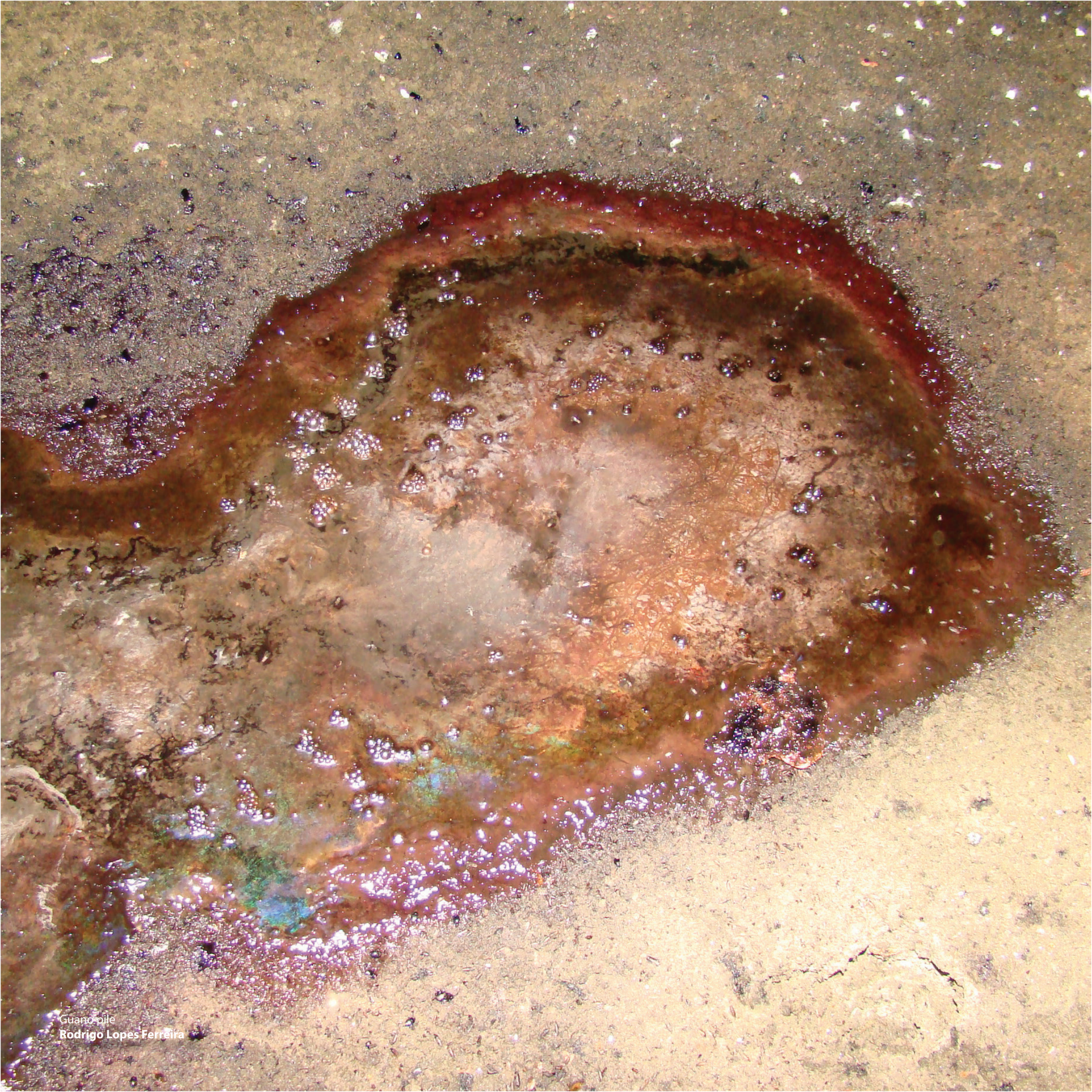
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Guano pile
Rodrigo Lopes Ferreira

3

Communities associated with guano

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Guano: general concepts and importance

Guano can be defined as any accumulation of feces produced by flying animals such as birds, bats and some insects. Guano deposits are usually found in places where colonies of these animals aggregate, such as islands (in the case of seabirds), buildings and caves (in the case of bats or insects). The name "guano" comes from the indigenous peoples of Chile and Peru, who used to call thick deposits of seabird droppings, which they use as fertilizer for their crops, "huanu" – from the Quechua "wanu" (CLARK; FOSTER, 2009). Later, the Spaniards adopted the term, starting to pronounce guano.

Among the various places where these deposits can be found, caves stand out, in which this type of substrate becomes differentiated. As caves are generally oligotrophic environments, guano deposits represent an important source of organic matter, often comprising the main trophic resource in these environments (DECU, 1986; FERREIRA; MARTINS, 1999; HARRIS, 1970). In permanently dry caves, guano may represent the most important source of organic matter, constituting the trophic base for many invertebrate communities. However, guano is also found in caves with watercourses, and can even be deposited directly into the water, becoming partially or fully submerged (Figure 1A).

The composition of guano can be quite varied, being directly determined by the diet of the species that produced it. In temperate regions, bats are almost exclusively insectivorous, as frugivorous and nectarivorous species cannot survive the rigors of winter, and the supply of fruit and flowers suitable for bats is much smaller at high latitudes. On the other hand, the feeding habits of bats in tropical regions are remarkable. There are bats that feed on fruits, insects, nectar, blood and other vertebrates (lizards, mammals, birds, frogs and fish), although some groups are found in caves more often than others. The most common guano deposits in Brazilian caves are produced by vampire bats (with a pasty consistency and reddish color when fresh – Figure 1B, turning black and often powdery when older), fruit bats (with small undigested seeds – Figure 1C) and insectivorous bats (containing small chitinous parts of insects or other arthropods – Figure 1D). In rarer cases, guano deposits produced by carnivorous (*Chrotopterus auritus* Peters, 1856 – Figure 1E), piscivorous (*Noctilio leporinus* (Linnaeus, 1758)) (Figure 1F) and nectarivorous (*Anoura* spp.) species can be observed. Guano can also be produced by birds, such as cave swifts in Brazil (*e.g.*, *Streptoprocne* spp.), species of the genus *Aerodramus* in Southeast Asia and northern Australia, and by guácharos or oilbirds (*Steatornis caripensis* Bonaparte, 1843) in some countries of Central and South Americas. Bird guano is similar to that produced by insectivorous (in the case of swifts) and/or frugivores (in the case of guácharos) bats, but always has the whitish uric acid pellets normally found in bird droppings (Figure 1G).

Guano-producing species defecate in the area just below their colonies, but this deposition ceases if they leave the area. Therefore, guano can be considered an ephemeral resource for cave organisms. Thus, the availability of guano, as a trophic resource in caves, depends on several factors, many of which are related to the biology of the species that produce it. In temperate regions, for example, bats hibernate or migrate during the winter, which causes an interruption in guano deposition during this season. In the tropics, however, bats or birds can produce guano throughout the year given the greater climate stability. Although Gnaspini and Trajano (2000) mention that many Brazilian bat populations are commonly nomadic, resulting in non-continuous deposition of guano in caves, this may not be the rule, as many caves harbor extremely persistent colonies that may remain in the same cave for years or decades. Such differences in the deposition and, consequently, in the availability of this resource structures the communities associated with guano in different ways.

Once a new colony of bats (or birds) is established in a cave and begins to produce a new deposit of guano, pioneer species begin a process of heterotrophic succession, characterized by changes in community structure and composition over time,

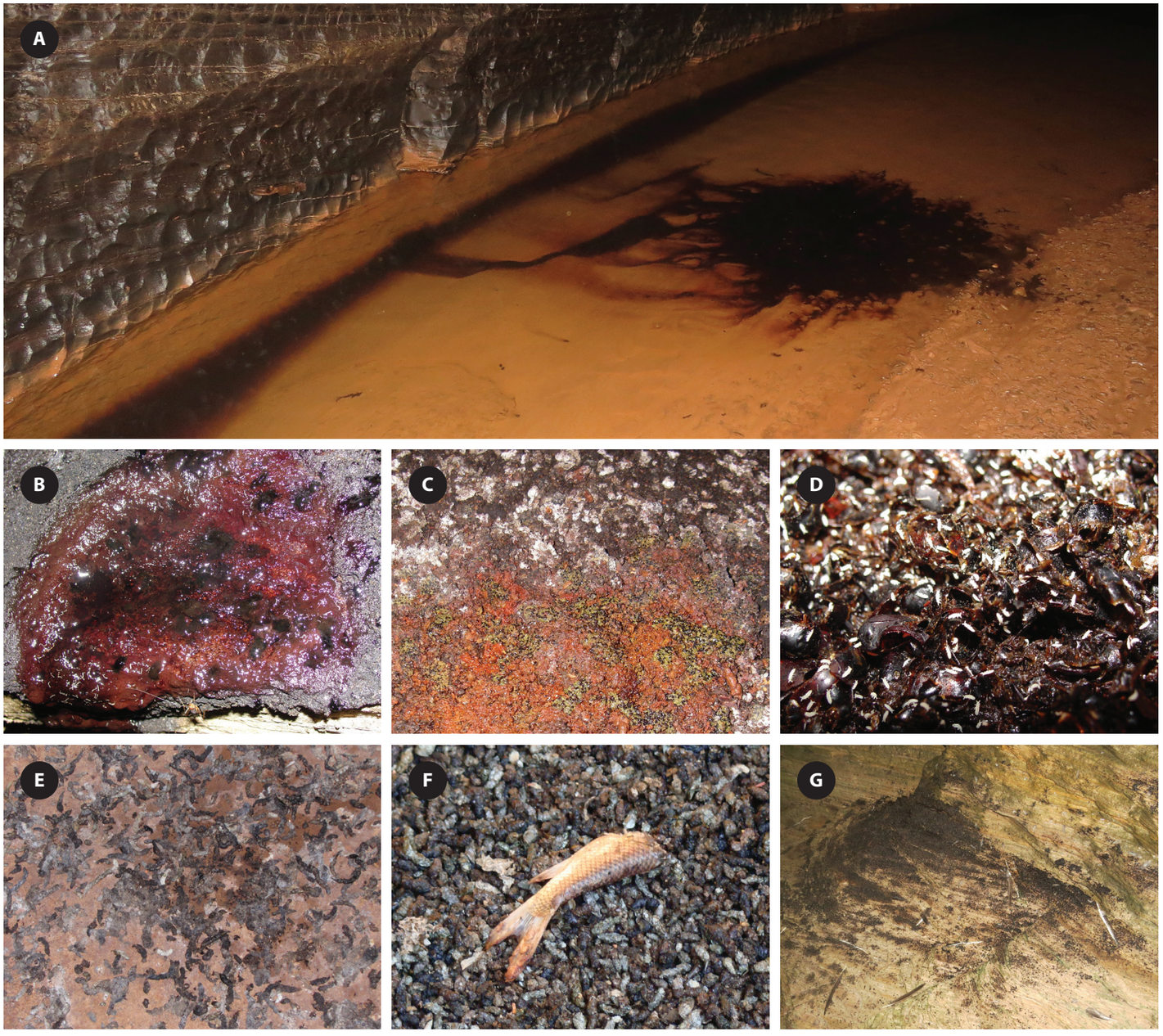


Figure 1. Different types of guano produced by animals with varying diets. **A)** Deposit of guano from hematophagous bats submerged in a subterranean stream; **B)** guano from hematophagous bats; **C)** guano from frugivorous bats; **D)** guano from insectivorous bats; **E)** guano from carnivorous bats; **F)** guano from piscivorous bats; **G)** guano from swifts. Photos: (Rodrigo L. Ferreira).

influenced by the physicochemical changes of the guano. Unless there is continued deposition of guano, succession will culminate in the demise of the community as a consequence of resource depletion. Thus, the ecological succession that occurs in guano can also be classified as degradative.

The first species to colonize new deposits (pioneer species) are often dipterans. Such organisms arrive at these resources attracted by the odor of the ammonia fermentation of guano. Protozoa and nematodes can also be found in newly deposited accumulations, as they may already be present in bat feces. After the establishment of detritivorous pioneer species, predatory species begin to be attracted (such as beetles of the families Histeridae and Staphylinidae). With the aging of the deposit, other detritivorous groups, such as mites and springtails, also become associated with this resource. Over time, larger predators (centipedes and spiders) come to colonize the deposits.

Bahia (2007) studied ecological succession in guano from bats in a cave in Brazil, following successional processes for a period of one year in an experiment using guano from insectivorous bats. Newly deposited guano was removed from a cave in the state of Tocantins, where it occurred in enormous abundance, and later sterilized and divided into 140 experimental guano deposits, which were separated into five different treatments and allocated in another cave located in the state of Minas Gerais. In addition to invertebrates, the fungi and bacteria associated with the colonization of this resource were also evaluated. An important finding of the work was that the dispersion of fungi and bacteria is closely related to invertebrates that transit between deposits. Furthermore, analysis of community similarity and structure over time evidenced three distinct successional phases: colonization, resource assimilation and species dispersion. The work demonstrated the concomitant occurrence of the three models of ecological succession (facilitation, tolerance and inhibition) proposed by Connell and Slatyer (1977). According to Bahia (2007), the succession model depends on the types of ecological relationships existing among the species that make up the succession at a given phase. In this way, some organisms, by modifying guano, facilitate colonization by certain species and make it difficult for others. In addition, as there is no strong intra- and interspecific competition between detritivores and fungivores, they could be tolerated more easily (BAHIA, 2007).

Sinusias can be defined as structural units of a large ecological community characterized by relative uniformity and, generally, constituting particular strata of a given community. Although this term is mostly used for plant communities, invertebrates associated with guano deposits can also be considered components of a synusia. The term chronosinusia, used by Decu (1986), refers to guano communities of temperate caves, which present particular dynamics and succession linked to seasonal guano deposition. The microclimate and nutritional quality of guano deposits that harbor chronosinusias vary according to the presence or absence of bat colonies. In this way, the invertebrate communities associated with them have a cyclical dynamic, established by the seasonal presence of guano-producing colonies (DECU, 1986).

Guano deposits can vary considerably in shape, size, and depth, depending on the number of individuals that make up the colony that produces them (Figure 2A and B). Bat colonies can range from a few specimens (for example, *C. auritus*, a carnivorous bat whose colonies rarely have more than six individuals) to 20 million, as is the case with "free-tailed bats" (*Tadarida brasiliensis* (I. Geoffroy, 1824)) in Bracken Cave, Texas, United States (ISKALI; ZHANG, 2015). Thus, deposits have very different sizes, ranging from small discrete deposits to huge deposits that cover entire cave galleries (Figure 2C and D). In addition to importing organic matter into caves, bats can have a major influence on the climate of these environments, especially those that harbor huge populations. When a huge congregation of bats settles in large chambers connected to other parts of a cave by narrow passages, the temperature tends to rise (intensified by bat body heat), sometimes reaching around 40ºC, forming what are known as "*hot caves*" (LADLE *et al*., 2012). However, not all caves that feature huge bat colonies are *hot caves*. The maintenance of high temperatures depends not only on the presence of bats, but also on the structural configuration of the cave itself. Generally, caves that feature huge congregations of bats are called "*bat caves*". However, the concept of a *bat cave* is arbitrary, as it is difficult to define what a "huge congregation" would be. Ferreira (2019) proposed a definition of *bat caves* as "those that harbor colonies of bats that modify, due to their presence, the entire trophic and climatic environment of the cave, regardless of the absolute number of bats". Therefore, even a small cave with not so large of a colony (perhaps hundreds or a few thousand individuals) could be

considered a *bat cave* if the presence of this colony completely alters (from a trophic and/or climatic point of view) most of the substrates of the cave.

It is important to note that guano may have a wide range of scientific and economic applications, some of which will be explored further. As an example, the composition of the bat assemblage of a given region can be determined by analyzing bat DNA obtained from guano. A DNA mini-barcode assay for the entire order Chiroptera was developed by Walker *et al*. (2016) based on a segment of the mitochondrial cytochrome C oxidase I (COI) gene. The primers developed by these authors were highly specific for bats, being able to exclude sequences from invertebrate species that inhabit the deposits. According to the authors, the method has several applications around the world, both for determining bat assemblages in a given area and for detecting the presence of threatened species or examining the impacts of diseases on vulnerable species.

Physicochemical properties of guano and their variation in space and time

Guano deposits can have extremely variable compositions depending on the diets of the animals that produce them, with special emphasis on tropical regions where there are bat species with very diverse diets. However, in addition to compositional



Figure 2. Guano deposits produced by bat colonies of different sizes. **A)** Small colony of hematophagous bats, *Desmodus rotundus* (É. Geoffroy, 1810); **B)** large colony of insectivorous bats, *Pteronotus gymnonotus* Natterer, 1843; **C)** small guano deposit produced by hematophagous bats; **D)** large deposit of guano produced by insectivorous bats. Photos: (Rodrigo L. Ferreira).

differences linked to the diet of guano producers, these deposits also undergo changes in their physicochemical and nutritional characteristics from the moment of their deposition. In this way, guano tends to be a much more variable substrate than other cave biotopes. Once deposited, guano tends to be alkaline and moist, becoming more acidic and drier over time (FERREIRA; MARTINS, 1999; HERRERA, 1995; MOULDS, 2004). In addition, organic content also tends to decrease with guano aging, since organic compounds are consumed by organisms associated with the deposits or mineralized by the action of microorganisms. Thus, one might think that guano age can be inferred by pH, moisture and organic content, since all these parameters tend to change over time. However, Ferreira *et al*. (2000) showed that these parameters do not always reflect the time since guano deposition since these deposits are "open systems", which can be affected by chemical (water percolation, flooding, etc.) and physical (mud/landslides, sedimentation etc.) processes that occur in caves. As an example, an old deposit (which would have a more acidic pH) present on a speleothem that receives percolation water in a limestone cave may have a more alkaline pH due to the carbonates brought by the water. In addition, such a deposit would also be moistened by the percolation water, causing it to have an elevated level of moisture content and pH, in contrast to what would be expected due to its age.

The physicochemical properties of guano also change spatially. The center of the surface of a small deposit tends to be the youngest part, and generally more alkaline and moist and with a higher organic matter content, when compared to the edges (Figure 3A). In the vertical plane, the surface tends to be richer in nutrients when compared to the deeper layers. In some cases where nutrients were evaluated, carbon content was significantly higher in the upper layers of the deposits, while nitrogen and phosphorus concentrations were not significantly different in relation to the depth of the guano (ISKALI; ZHANG, 2015). However, in larger deposits, the guano surface tends to be quite variable, especially due to non-uniform deposition of guano by the colony overhead. Large deposits are generally characterized as a mosaic of microhabitats, which allow the presence of various zoological communities at different successional stages. Although these deposits usually occupy large areas of the floor, in certain cases bats can produce stalagmite-like structures known as guanomites (Figure 3B). The communities associated with these structures have not yet been investigated in detail and are certainly deserving of specific studies.

It should be noted that the population size of different species associated with guano deposits tends to be proportional to the amount of guano available. However, if there is a high production of guano, it accumulates and is not fully consumed. Therefore, guano itself is not normally a limiting factor for the invertebrates that consume it, and numerical fluctuations in their populations may be more related to the quality of the guano than its quantity.

In guano deposits, several complex reactions (mainly of biological origin) occur, capable of releasing nitric, phosphoric and sulfuric acids (FORTI, 2001; ONAC; FORTI, 2011), which then react with carbonate rock or cave sediments to form more than 100 secondary minerals (ONAC, 2005, 2011) (Figure 3C). Some of the minerals related to guano, such as the organic compounds guanine and urea, are secreted directly from the guano itself. For example, phosphate minerals observed in a Romanian cave resulted from the interaction of acidic solutions derived from guano deposits with limestone, calcite speleothems and/or detrital sediments at various conditions of pH and relative humidity (TĂMAŞ *et al*., 2014). In addition, minerals derived from guano have already been observed in caves present in several European countries, such as Slovakia, France, Macedonia and Italy (AUDRA *et al*, 2019). In Brazil, speleothems with guano-derived compounds have been observed in iron caves in the Carajás region (ALBUQUERQUE *et al*., 2018).

Microbiota associated with guano

Although studies on the biology of guano deposits in caves have historically focused on invertebrates, a considerable number of studies on the microbiology of these deposits has been produced in recent years. Such articles have highlighted the importance of microorganisms for various processes related to the dynamics of deposits (some bacteria, for example, are involved in nitrification processes), as well as demonstrating the existence of incredibly rich microbial communities that these organic substrates can harbor (Figure 4).

The first work carried out in Brazil on microbiota associated with guano deposits was that of Ferreira *et al.* (2000b), who

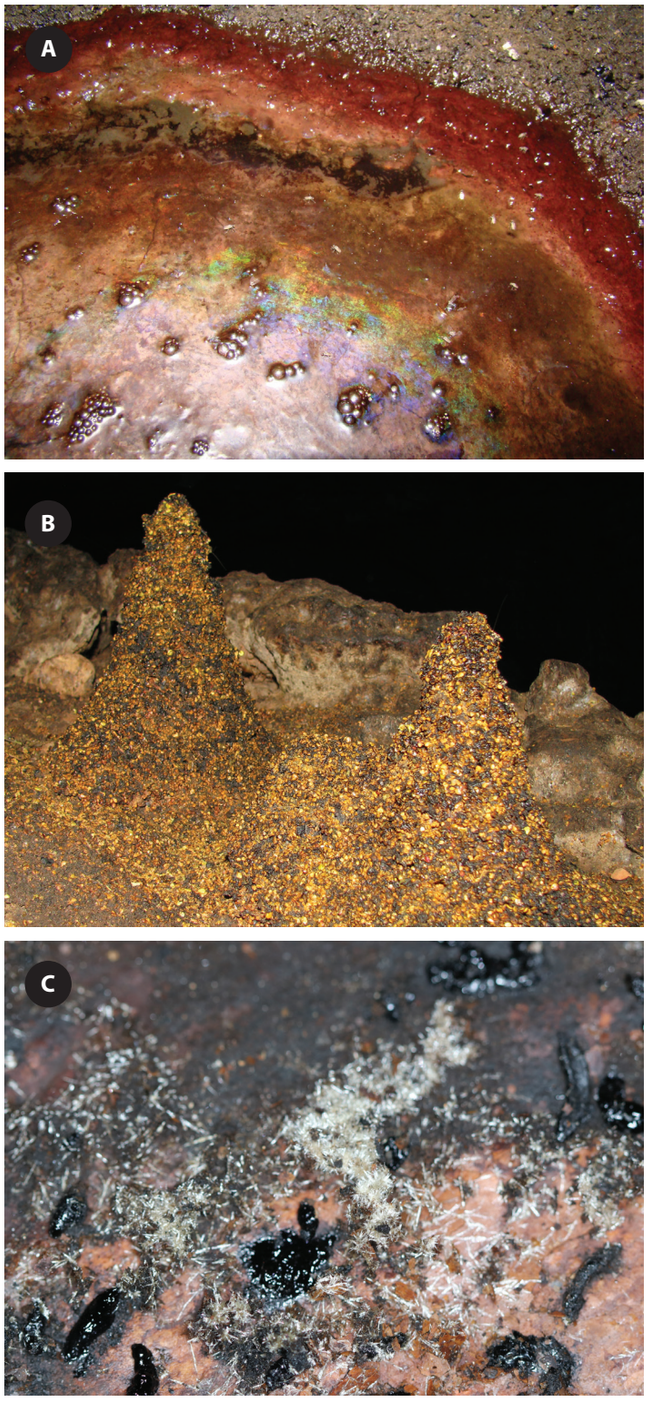


Figure 3. A) Deposits of guano produced by hematophagous bats, showing variation in guano conditions from the center of the deposit to the edges; **B)** guano "stalagmites" or "guanomites" produced by frugivorous bats in a cave in Bahia; **C)** minerals formed from guano in a cave in Bahia. Photos: (Rodrigo L. Ferreira).

studied the fungal community associated with guano in a cave in southeastern Brazil. The authors evaluated the influence of some physicochemical parameters of the deposits on the richness and abundance of these microorganisms. Fifty-three morphotypes were found, of which filamentous fungi (81% of the total richness) stood out when compared to yeasts. Furthermore, they found that larger deposits with higher organic content and alkaline pH generally harbor richer and more abundant fungal communities in the studied cave. The authors also found a negative relationship between the abundance of fungi and the richness of invertebrates associated with each deposit, suggesting that invertebrates are contributing to fungi reduction through the consumption of these microorganisms or by continually churning the surface of the guano, thus preventing the growth of many species.

Yeasts have also been shown to be extremely diverse in guano, and many new species await formal description. Sugita *et al*. (2005) carried out a survey of yeasts associated with bat guano in 20 limestone and volcanic caves in Japan. Of the hundreds of yeast colonies observed, nine species of *Trichosporon* were found in 15 caves, seven of which are potentially new to science. They found that species of *Trichosporon* are the most abundant yeast species in bat guano in Japan and that this organic substrate is a potentially rich source of undescribed yeast species.

Other microorganisms are also present in guano deposits. Chroňáková*a et al.* (2009) studied the Archaea community associated with bat guano deposits in Domica Cave (Slovakia) and found that non-thermophilic Crenarchaeota dominated this community (99% of clones belonged to this group). These authors found Crenarchaeota clones previously isolated from surface soils and deep underground acidic waters. Archaea communities were more diverse in the upper part of the deposits, which also had higher moisture, organic carbon and nitrogen content.

Different studies on bacteria associated with guano are revealing that they comprises an extremely important group in these deposits. As an example, the abundance of bacteria in the guano produced by the fruit bat *Rousettus leschenaultii* (Desmarest, 1820) at Robber Cave (Maharashtra, India) ranged from 1.71×10^{7} to 3.34×10^{10} CFU/g (colony-forming units /gram) (BANSKAR *et al.*, 2016). These authors also found 105 species belonging to 37 genera and four different bacterial phyla. De

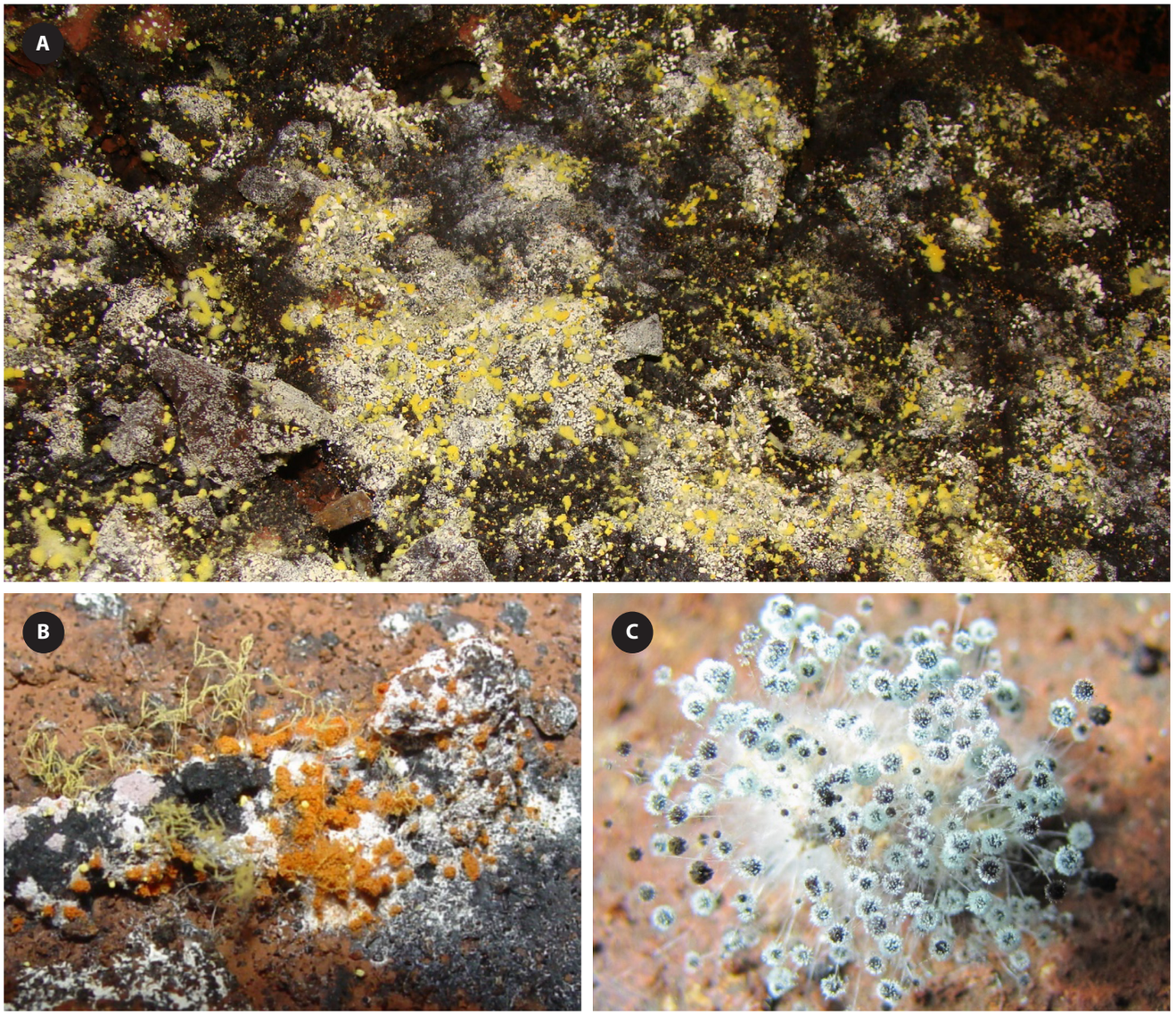


Figure 4. A) Guano deposit filled with colonies of microorganisms, especially filamentous fungi; **B)** different species of filamentous fungi growing in profusion along with bat guano; **C)** detail of a filamentous fungus growing on a guano *pellet*. Photos: (Rodrigo L. Ferreira).

Mandal *et al.* (2015), using next-generation Illumina sequencing, found 1,282 OTUs (operational taxonomic units) associated with guano deposits in the Pnahkyndeng Cave (northeast India), comprising 18 bacterial phyla. They found that Chloroflexi, Actinobacteria and Crenarchaeota dominated the guano bacterial community. In addition, most of the identified genera are involved in nitrogen cycling. On the other hand, significant parts of the OTUs were not adequetly identified, again indicating that new species may occur in guano.

Banskar *et al.* (2016) also pointed out some interesting aspects about the relationships between bacterial diversity and guano aging. These authors found that the decomposition process can lead to increased bacterial diversity. Important biogeochemical processes that determine the cycling of organic and inorganic compounds of guano are carried out by microorganisms that alter guano pH (releasing ammonia) and nutritional content. Such changes can favor the growth of several species of bacteria.

Finally, guano can also contain several pathogens, which

can pose risks to both bats and humans (VALDEZ *et al.*, 1999). According to Borda *et al.* (2014), caves with guano have a relatively high concentration of microorganisms in the air, some of which can pose a threat to animals including humans. In general, the physicochemical conditions observed in guano deposits, especially in large accumulations, are suitable for some pathogenic microorganisms, such as *Pseudogymnoascus destructans* Blehert *et al.*, 2008, which causes "white nose syndrome" in bats (MULEC). *et al.* 2013), or *Histoplasma capsulatum* (Darling, 1906), which causes histoplasmosis in humans (TAYLOR *et al*. 1962; LYON *et al.* 2012; MULEC *et al.* 2020).

Organisms associated with guano

The invertebrate communities associated with guano can be quite diverse, being composed of several taxa in different stages of development (Figure 5). Furthermore, the composition of these communities varies among different countries or continents. Although certain invertebrate orders are frequently observed regardless of the region where the cave is located, variation can occur at the family level. Such variation is clearer when organisms that are facultatively associated with deposits are considered.

Several aspects of the trophic structure and natural history of invertebrate communities associated with bat guano in caves were extensively discussed by Ferreira and Martins (1999). Subsequently, Moulds (2004) produced an extensive list of invertebrates present in guano deposits of Australian caves. Detritivorous organisms that directly consume guano or microorganisms that grow in the deposits form the basis of the food webs of these communities (Figure 6). Using examples of taxa that occur in Brazilian caves, there are mites, which occur virtually everywhere where guano is found (*e.g.*, Aphelacaridae, Uropodidae and most species of Oribatida). Populations of some species of mites in guano can be exceptionally large in some caves, such as in Australia, for example (33 million/m^2 - Harris 1973; Bellati 2001). Other detritivorous invertebrates include springtails (*e.g.*, Entomobryidae, Hypogastruridae, Arrhopalitidae), psocopterans (mainly Psyllipsocidae), beetles (*e.g.*, Dermestidae, Tenebrionidae, Leiodidae and Histeridae), moths (mainly larvae of Tineidae and Pyralidae), and dipteran larvae, preferably associated with fresh guano (*e.g.*, Drosophilidae, Phoridae and Milichiidae). Facultative detritivores can also be found, sometimes forming large populations, especially when other organic resources such as plant debris are scarce. Examples are isopods (*e.g.*, Armadillidae, Plathyarthridae and Styloniscidae), millipedes (primarily Spirostreptida), cockroaches, crickets (primarily Phalangopsidae) and moths (Lepismatidae). Other detritivores, such as earthworms, gastropods and harvestmen, are also occasionally found. It is important to mention that many invertebrates can also consume carcasses that may be present in guano deposits (Figure 7). Such

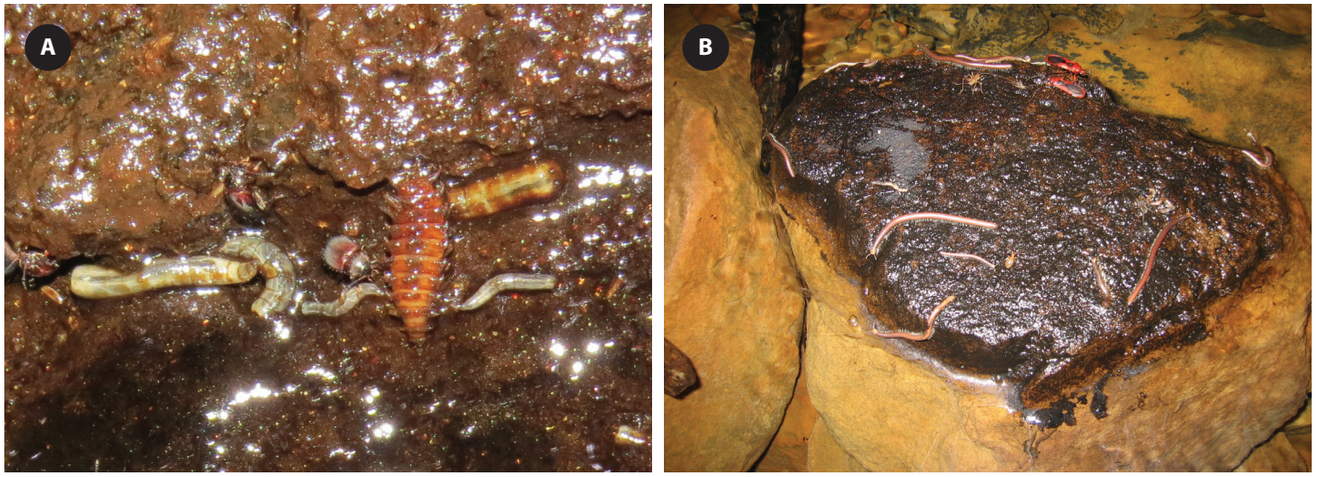


Figure 5. Communities associated with guano deposits. **A)** Guano deposit with evident Diptera and Coleoptera larvae (cave in Minas Gerais); **B)** guano deposit with evident millipedes, hemipterans and crickets (cave in São Paulo). Photos: A (Rodrigo L. Ferreira); B (Xavier Prous).

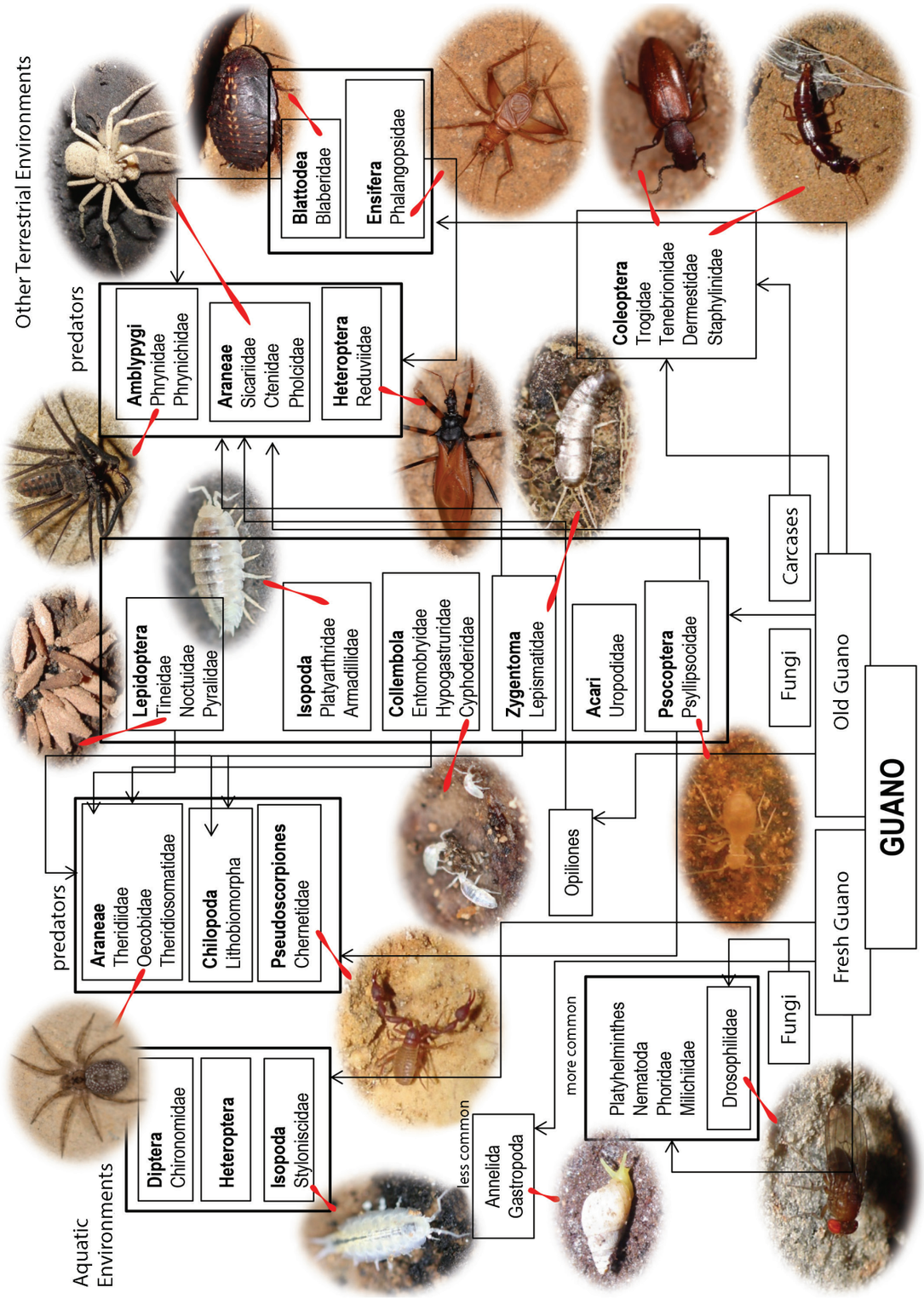


Figure 6. Hypothetical food web representing interactions that can be observed in guano deposits in the Neotropics. Photos: (Rodrigo L Ferreira).

carcasses (especially of bats) are relatively common, as these organisms end up dying in colonies and falling on deposits, thus offering an important additional resource for the fauna.

A wide variety of predators, in turn, consume the detritivorous species. Mites (*e.g.*, Laelapidae, Macrochelidae, Macronyssidae and Phytoseiidae), pseudoscorpions (mainly Chernetidae), spiders (*e.g.*, Theridiidae, Sicariidae and Oecobiidae) and insects (*e.g.*, Reduviidae) are the most common predators in guano-associated communities in Brazilian caves. Facultative predators, such as scorpions and whip spiders (Amblypygi), may also be present. It is important to note that many predators do not stay directly inside the guano deposits, but nearby (Figure 8). Some spiders (*e.g.*, Sicariidae, Theridiidae, and Oecobiidae) can prey not only on detritivores directly associated with guano, but also opportunistic species that occasionally visit guano for foraging (such as crickets).

Decu (1986) proposed three categories of organisms associated with guano, according to the degree of affinity with these substrates. According to this author, guanoxenes are those that can feed or reproduce in/on guano deposits but depend on other organic resources to complete their life cycle. Guanophiles can consume or reproduce in/on guano or other organic waste. Finally, guanobites comprise those species that exclusively inhabit guano deposits. Decu (1986) argued that a guanobitic species must also be troglobitic and therefore incapable of establishing populations in epigean environments. However, Gnaspini and Trajano (2000) proposed that a guanobitic species need not necessarily be troglobitic, arguing that some species, despite occurring in external environments, could be found exclusively in guano deposits when present in caves. This classification, however, has some restrictions that must be considered. First, many studies have shown that most invertebrates associated with guano deposits are extremely opportunistic (i.e., they can feed preferentially on guano if it is abundant but can feed on other organic resources if guano is scarce and vice versa) (FERREIRA *et al.*, 2007; FERREIRA; MARTINS, 1999). Thus, it is difficult to assume such strong affinities as those proposed by Decu (1986). Furthermore, given the ephemeral nature of guano deposits, it is extremely unlikely that some species can only survive if they consume or reproduce in guano deposits. Moulds (2004) found that many guanobitic and guanophilous species have unexpectedly wide distributions, sometimes spanning multiple climatic regions of Australia. Although the author has presented some hypotheses for these findings, widely-distributed guanobitic species also support the idea that species exclusively dependent on guano may not exist. Thus, the notion of a guanobitic species as strictly dependent on guano must be refuted; guanobitic species are simply those that preferentially feed on this resource.

Studying communities associated with guano deposits in Brazilian caves, Gnaspini-Netto (1989) proposed that certain invertebrate species may show preferences for different types of guano (insectivorous, frugivorous or hematophagous guano).



Figure 7. Organisms feeding on bat carcasses along with guano. A) Coleoptera (Tenebrionidae) and Cockroaches (Blaberidae) feeding on a bat carcass (cave in Tocantins, Brazil); B) a cricket (*Endecous bahiensis*) feeding on a bat carcass (cave in Bahia, Brazil). Photos: (Rodrigo L. Ferreira).

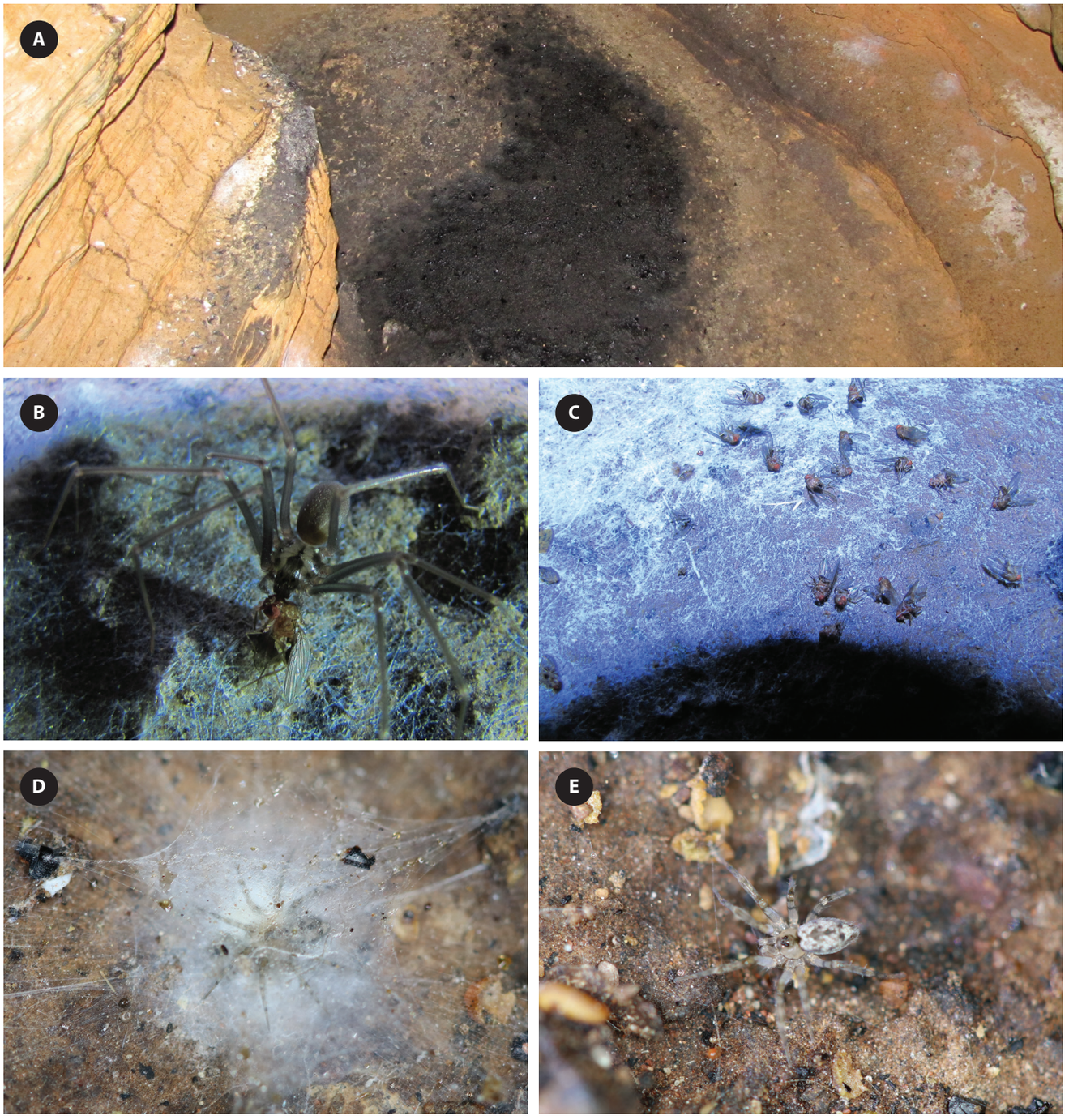


Figure 8. A) An old deposit of hematophagous bat guano in the conduit of a cave in Minas Gerais (Brazil), where it is possible to see the residential webs (white areas on the walls of the cave) of opportunistic brown spiders, *Loxosceles similis* (Moenkhaus, 1898), which can feed on both cave invertebrates and guano; **B)** brown spider (*L. similis*) feeding on a dipteran (*Drosophila* sp.) that visited a guano deposit; **C)** large amount of dipterans (*Drosophila* sp.) from guano captured in a single residential brown spider web; **D)** a spider, *Oecobius navus* Blackwall, 1859, under its residential web, often built over a guano deposit or on the periphery of deposits; **E)** a spider, *O. navus*, out of its web. Photos: (Rodrigo L. Ferreira).

According to this author, some families were found only in specific types of guano. However, Ferreira *et al*. (2007) demonstrated that the supposed preference of certain invertebrates to specific types of guano is not as strong as previously thought. For example, beetles of the family Dermestidae, considered by Gnaspini-Netto (1989) to be specifically associated with insectivorous bat guano, have been found in guano produced by fruit bats. Isopods and psocopterans, previously thought to be specific for guano of frugivorous bats, have been found in guano produced by hematophagous bats. Finally, pseudoscorpions and mites, considered specific for guano from insectivorous bats, were recorded in deposits produced by hematophagous bats (Ferreira *et al*. 2007). It is important to highlight that most of the invertebrates present in guano deposits are generalist detritivores, capable of consuming many types of organic resources (FERREIRA *et al*., 2000a; FERREIRA *et al*., 2007). Thus, patterns of association seem to be more strongly determined by the type and amount of resources available in each cave than by possible species preferences for specific types of guano.

It is important to mention that there is a general idea that troglobitic species avoid guano deposits, especially large or newly deposited ones. In large deposits, a few generalist species usually become dominant and can form large populations, as in some tropical caves where cockroaches, beetles or mites can have populations of tens of thousands to millions of individuals (in the case of mites) (Figure 9). However, there are cases where troglobitic species can directly consume guano, sometimes with their main populations being associated with this resource. These associations are more likely to occur when the guano is old, mixed with the ground or in small deposits present in more isolated areas of caves. Among the troglobitic species that have populations associated with guano (in both terrestrial and aquatic environments), are the troglobitic carabid *Coarazuphium formoso* Pellegrini & Ferreira, 2011, of northern Bahia, Brazil (Figure 10A), troglobitic silverfish of the genus *Anelpistina* of caves in Guatemala (Figure 10A). 10B), earthworms, planarians, isopods (*Namibianira arnhemensis* Kensley 1995 - Figure 10C) and amphipods (*Troglolеleupia gobabis* (Ruffo, 1951)) (Figure 10D) associated with submerged guano in caves in Namibia.

It is important to note that other potential resources can be transported along with guano, consisting of alternative food sources for various invertebrates. The best example is seeds that pass through the digestive tract of bats. In these cases, the seeds deposited in guano germinate, and the shoots remain viable as long as there are reserves in the cotyledons (Figure 11A). Seedlings that grow in association with guano deposits may provide additional resources for many cave invertebrates (Figure 11B).

Ecology of communities associated with guano

Several studies have already shown that communities associated with guano deposits are excellent models for ecological studies (FERREIRA *et al*., 2007; FERREIRA; MARTINS, 1998, 1999). First, physicochemical conditions (which are generally easy to assess) can be highly variable when compared among different deposits. Furthermore, although the communities tend to be simplified relative to communities at the surface, there is a large number of well-defined interactions among their components and with the general cave environment. Although guano communities have historically been considered "isolated" from the cave environment as a whole, studies have shown that diverse groups of cave invertebrates can associate and interact with guano communities (FERREIRA *et al*., 2000a).

Many physical and chemical characteristics of guano deposits can be decisive for the structuring of associated communities (FERREIRA *et al*., 2000a). Distance from the cave entrance, for example, can determine the richness of guano communities in some caves, although the effects of this parameter can vary among them. Some studies have shown that as the distance from the cave entrance increases, the richness of associated communities decreases (FERREIRA *et al*., 2007; FERREIRA; POMPEU, 1997), while others found no effect of the distance from the entrance on these communities (FERREIRA *et al*., 2000a). Proximity to the cave entrance can favor colonization by external invertebrates, and this relationship may influence the structure and composition of these guano communities (which seems more likely in smaller caves). In addition, the effect of inputs may also be related to the presence of supplementary organic matter that accumulates in these regions, providing additional food resources that may attract some invertebrate groups. On the other hand, when the predominant colonization is by troglophilic invertebrates (as in larger caves where guano

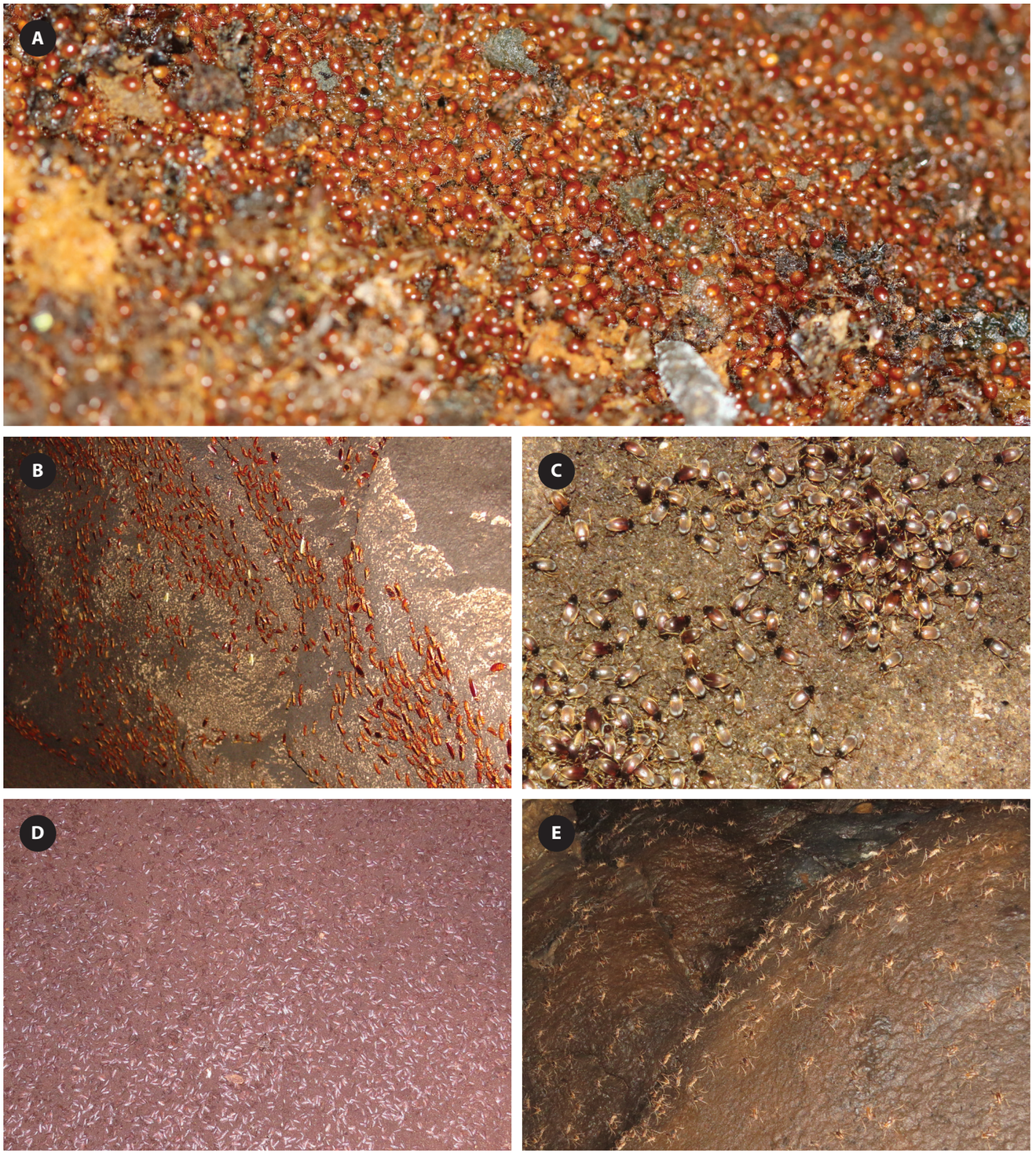


Figure 9. Large populations of invertebrates associated with bat guano in caves. **A)** Mites in a cave in northern Bahia (Brazil); **B)** cockroaches (*Periplaneta* sp.) in a cave in Sergipe (Brazil); **C)** beetles (Leiodidae) in a cave in Kenya; **D)** Lepidoptera larvae (Tineidae) in a cave in Kenya; **E)** crickets (*Diestrammena* sp.) in a cave in Japan. Photos: (Rodrigo L. Ferreira).

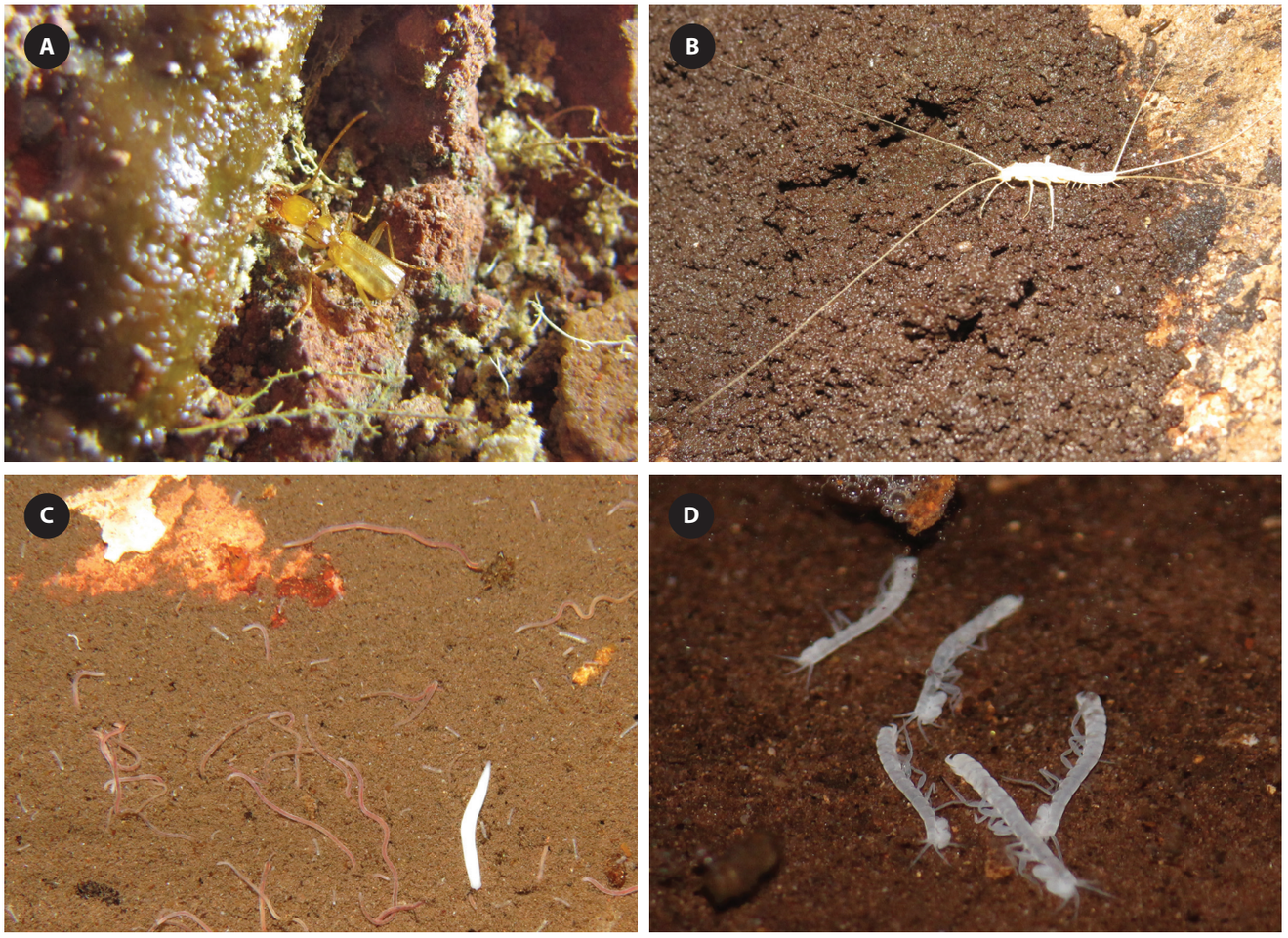


Figure 10. Troglobite species associated with guano deposits. **A)** *Coarazuphium formoso* (Coleoptera: Carabidae) feeding on a guano *pellet* in a cave in northern (Bahia); **B)** *Anelpistina* sp. (Zygentoma: Nicoletiidae) feeding on guano in a cave in Guatemala; **C)** annelids, planarians and isopods, *Namibianira arnhemensis* (Isopoda: Protojaniridae), associated with guano submerged in caves in Namibia, **D)** amphipods, *Trogloleleupia gobabis* (Amphipoda: Ingolfiellidae), also associated with guano submerged in caves in Namibia. Photos: (Rodrigo L. Ferreira).

may occur far from entrances), there may be no entrance effect (FERREIRA; MARTINS, 1998).

The relationship between the area of deposits and the richness of associated communities, on the other hand, seems to comprise a well-established pattern: richer communities are always associated with larger deposits (FERREIRA *et al*., 2000a; FERREIRA *et al*., 2007; FERREIRA; MARTINS, 1998). The number of individuals or species that use guano deposits tends to be proportional to their availability. However, with large deposits, guano accumulates without being consumed, since most organisms preferentially associate with the surface of guano. Therefore, fluctuations in population size seem to be more related to guano quality than quantity (Decu, 1986). Thus, larger deposits generally have more species due to the greater diversity of surface microhabitats and not due to the amount of available guano. This pattern, however, is not always observed for community diversity. In some Brazilian caves, for example, large populations of some species (such as moths and mites) reduce the evenness and, consequently, the diversity of these communities.

The shape of guano deposits appears to have nothing to do with community richness or diversity. Some authors (FERREIRA *et al*., 2000a; FERREIRA *et al*., 2007) have determined the shape of guano deposits using an index, the Margin Development Index – MDI (BARBOUR; BROWN, 1974; KENT; WONG, 1982),

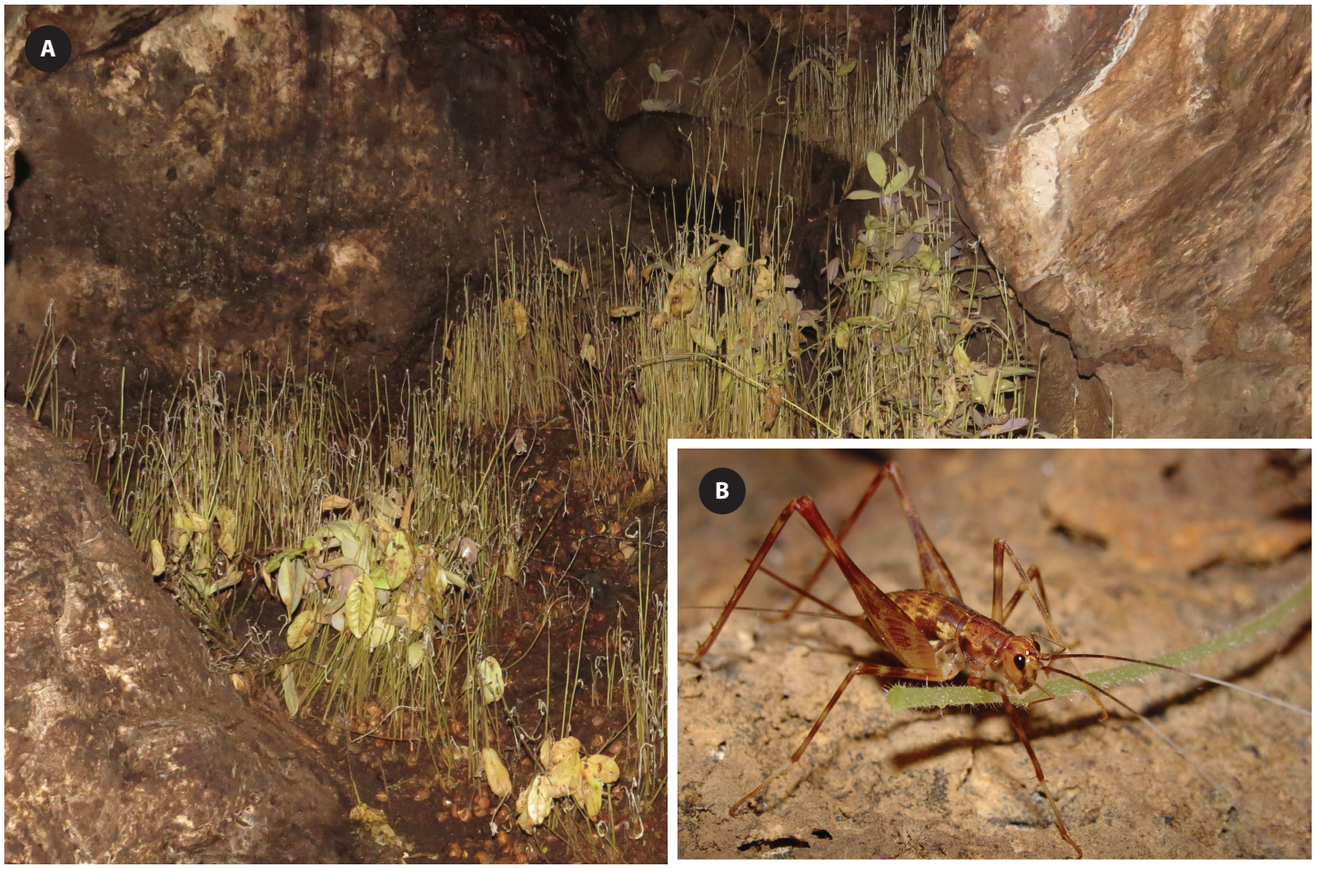


Figure 11. A) Seedlings growing in association with guano deposits in an Amazon cave; **B)** a cricket (*Eidmanacris* sp.) feeding on a sprout in a cave in Bahia (Brazil). Photos: (Rodrigo L. Ferreira).

and found no correlations among these parameters. This lack of correlation suggests that most species appear to locate guano by chemotaxis. If colonization occurred randomly, a positive correlation would be expected between MDI and richness and diversity, since more dendritic deposits would be found and colonized more easily than rounded deposits with similar area.

It is important to highlight that guano communities are also influenced by some physicochemical parameters, such as pH, moisture and organic content. In some Brazilian caves, the most species-rich deposits generally have a moderate pH. The relationship between pH and species richness of guano is probably due to a preference (by most species) for an optimal and intermediate pH value (FERREIRA *et al*., 2000a). However, if guano pH reflects the age of the deposits — which is not necessarily true as already discussed — fresh deposits with higher pH values may not have had enough time to be colonized, while older deposits with lower pH values may have been depleted by consumption or mineralization. However, Pellegrini and Ferreira (2013) found a positive relationship between neutral/moderate pH and diversity for guano communities in a cave in southeastern Brazil. High pH values may favor, in some cases, the dominance of a few species, which is reflected in low diversity.

The moisture content of guano can also influence the richness and diversity of associated communities (wetter deposits are generally richer and more diverse), although its effects are not fully elucidated (FERREIRA *et al*., 2007; PELLEGRINI; FERREIRA, 2013). Thus, drier deposits are likely to be restrictive to most organisms, although they may favor some species, whose populations may become large. Organic content also seems to favor invertebrates associated with guano, since, in general, more diverse communities are found in deposits with higher organic content (FERREIRA *et al*., 2000a; FERREIRA *et al*.,

2007). This relationship is expected, since deposits with greater amounts of organic matter have higher nutritional values, and so potentially attract more species.

Finally, few studies have evaluated the effects of nitrogen and phosphorus concentrations on guano communities. Herrera (1995) found that high concentrations of nitrogen and phosphorus in guano from guácharos (oilbirds) favored the existence of arthropod communities in a cave in Venezuela. In Brazil, Pellegrini and Ferreira (2013) found that phosphorus and nitrogen were related, respectively, to the richness and diversity of communities associated with guano. The authors found nitrogen content to be highest in areas where guano was freshest and most abundant, decreasing along the interface of guano replacement with adjacent cave soil.

Gilpin and Hanski (1991) were the first authors to propose the concept of metacommunity. According to them, a metacommunity represents a set of local communities from different areas connected by the dispersion of one or more of its components. Furthermore, a metacommunity presents the same dynamics as a metapopulation, being subject to local extinctions and recolonizations. Ferreira *et al*. (2007) argued that guano communities may represent metacommunities in Brazilian caves. According to the authors, the low abundance of some populations in old deposits increases local extinctions while possible colonization can occur in newer deposits. Furthermore, Ferreira *et al*. (2000a) suggested that larger deposits may act as sources of species likely to colonize smaller deposits. In Gruta da Lavoura, municipality of Matozinhos, Minas Gerais, these authors evaluated the similarity between communities of a large guano deposits and their surrounding smaller deposits. These authors found a negative correlation between similarity and distance from the source deposit, that is, deposits closer to the largest are more similar to it than are those that are further away.

Obviously, similarity does not necessarily indicate the flow of organisms between guano deposits. However, if we assume a small distance between deposits, coupled with the fact that in many Brazilian caves, especially those that are permanently dry, guano is the main resource, the hypothesis that similarity may indicate the transit of organisms between different deposits cannot be excluded. There are three main possibilities regarding the similarities observed among guano communities: i) high similarity could indicate frequent flow of individuals, suggesting the existence of a single community, whose component populations may use many deposits; ii) low similarity could indicate small, isolated communities, each one restricted to one or a few nearby deposits; and iii) moderate similarity (as observed in some Brazilian caves) may suggest a metacommunity dynamic. Regarding the last possibility, and as mentioned previously, guano is an ephemeral resource, consequently, when it ages, it loses quality as an organic supply. Therefore, the associated community starts to progressively migrate from the older deposits, seeking more suitable habitats. These migrant organisms can colonize other deposits to maintain their populations, which would produce moderate similarity among a given set of deposits. However, it is important to highlight that invertebrate movements between deposits remain largely unknown and certainly deserve further research (FERREIRA *et al*., 2007).

Impacts and conservation

As mentioned earlier, guano has a wide range of uses around the world, with its use as a fertilizer standing out. In many countries, such as Indonesia, Thailand, Cuba, Jamaica, Mexico and Brazil (in the past), people collect bat guano for agricultural purposes. Guano is widely used as a fertilizer, especially due to its high nitrogen content combined with the possible nematocidal effects it can have (KELEHER; SARA, 1996). In addition, organic matter, total carbon, total nitrogen and phosphate are usually present at high levels, so the incorporation of small amounts of bat guano into the soil increases agricultural production (SRIDHAR *et al*., 2006). In addition to its use as fertilizer, there is evidence that it is possible to improve the nutritional value of straw for ruminants by incorporating small amounts of bat guano, as shown in a study carried out in Tanzania (MLAY; SAGAMIKO, 2008).

Unfortunately, knowledge about the biology of many bat species is still limited, as it is for the communities associated with guano in caves. Uncertainty regarding property/mining rights for guano removal in caves in most countries has led to inappropriate and unsustainable practices, resulting in the loss of millions of bats and invertebrates associated with this resource (IUCN SSC, 2014). In many cases, guano exploitation can

destroy or modify bat shelters, thus altering the flow of energy in caves. In addition, this practice can pose a considerable risk to human health, since "guano miners" are exposed (to a greater or lesser degree) to pathogens transmitted by bats or their feces (WACHARAPLUESADEE *et al*., 2013).

The IUCN guidelines for minimizing the impacts of removing guano from caves (IUCN SSC, 2014) indicate some practices that can contribute strongly to reducing the aforementioned impacts. Such guidelines recommend: i) preventing extreme modifications to cave habitats during guano removal; ii) not obstructing cave entrances and not digging ditches to access guano deposits; iii) not modifying cave entrances (including opening artificial entrances) without consulting experts; and iv) minimizing the use of pesticides in places where they are likely to cause harm to bats, especially foraging areas.

Another possible impact on guano-associated communities is biological invasions, especially by ants, although this topic is still very little studied in caves throughout the world. Although ants are commonly found in caves, their association with these habitats remains up for debate. Some researchers believe that ants are accidental in caves, although some species are considered troglobites (*e.g*., *Leptogenys khammouanensis* Roncin & Deharveng, 2003, from Laos and *Aphaenogaster gamagumayaa* Naka & Maruyama, from Japan). However, there are reports of ants entering artificial galleries and collecting guano, as observed in Australia (Moulds, 2006). Although Moulds (2006) did not observe ants attacking arthropods associated with guano, this behavior has been recorded in Brazilian caves (Figure 12A). In the state of Bahia (Brazil), ants have been observed attacking larvae of moths of the family Tineidae, a common group usually associated with old guano deposits. The ants removed the larvae from their cocoons and attacked them. There are also records of guano itself being collected and transported by ants (Figure 12 B-E), in some cases this being the only organic resource visible in the cave (FERREIRA, 2019). Although this phenomenon seems rare, the potential risks of biological invasions cannot be ignored, especially given the strong changes undergone in various karst landscapes around the world.

Another phenomenon that has been observed in Brazilian caves is the replacement of guano produced by bats with different diets (frugivores, insectivores, piscivores, among others) by guano produced by hematophagous bats. In many regions of Brazil, native vegetation has been removed for the establishment of monocultures or pastures. In this way, food for many bats (insects, nectar, fruits, among others) has been drastically reduced in many areas, forcing bats to migrate in search of more suitable foraging areas or even becoming extinct locally. Furthermore, when native forest is replaced by pasture, the food supply for hematophagous bats increases (with the establishment of herds of cattle, goats and horses), leading to population growths for these species. Consequently, caves that were previously supplied with various types of guano began to receive only guano from hematophagous bats, which has lower energy value as a food resource (HUTCHINSON, 1950). Although there are no studies on the effects of this substitution on guano communities, it is plausible to assume that the reduced diversity of guano types results in simplification of the associated communities, leading to impoverishment of these communities in caves.

Finally, it is important to highlight that the greatest threat to this biodiversity is certainly the threat of extinction (local or global) of guano-producing animals. Therefore, to avoid impacts on communities associated with guano in caves, it is essential the populations of guano producers, as well as the external habitats with which they are related, are preserved.

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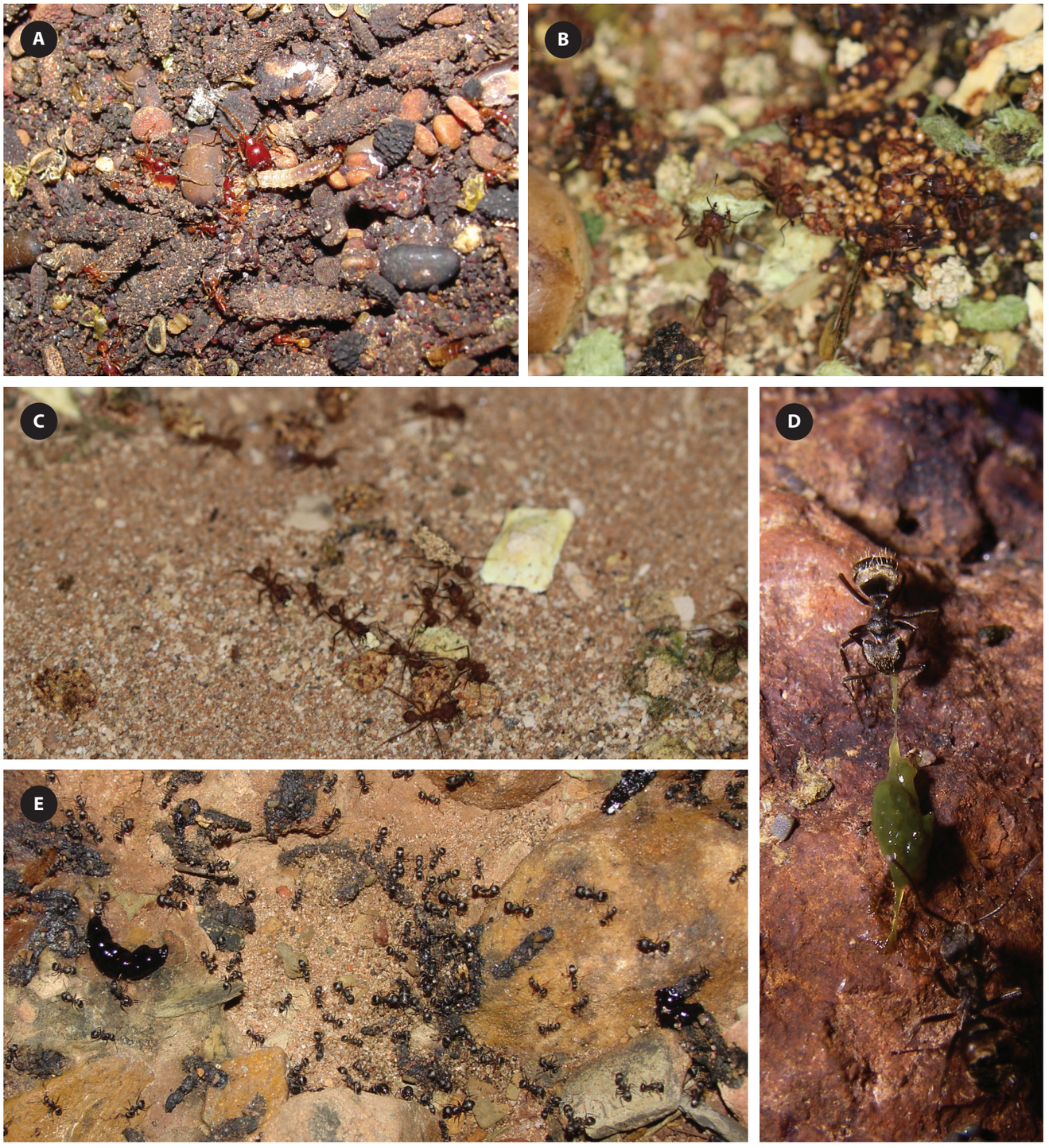


Figure 12. A) Ants feeding on moth larvae (Tineidae) in a cave in Bahia (Brazil); **B)** ants collecting guano from frugivorous bats in a cave in Bahia; **C)** ants transporting guano to the nest in a cave in Bahia; **D)** ant feeding directly on guano in a cave in Pará (Brazil); **E)** ants feeding on guano in a cave in Bahia. Photos: A-C, E (Rodrigo L. Ferreira); D (Marcus P. de Oliveira).

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Biofilme em canga ferruginosa
Robson de Almeida zampaulo

4

Diversity of Cave Microbiomes

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General introduction

Caves are part of the subterranean environment, and the process of their formation is directly related to their lithology (type of matrix rock). Caves in soluble rocks (limestone, dolomite, gypsum, halite) are present in karst landscapes and their formation usually occurs by chemical dissolution of the rock matrix, that is, by the action of carbonic acid (H_2CO_3) in contact with the rock. Karst represents about 15% to 20% of the Earth's surface, disregarding the areas covered by ice (FORD; WILLIAMS, 2007). However, we can find caves in other types of landscapes called pseudokarstic, which include lava tube caves, glacial caves and fumaroles, iron ore caves, coastal and marine caves and caves in sandstone, quartzite or basalt.

Cave environments can be divided into different zones, determined by the amount of incident natural light. Up to three zones can be distinguished in this environment: i) the entrance zone, where there is direct incidence of sunlight; ii) the twilight zone, where there is indirect incidence of sunlight; and iii) the aphotic zone, where the absence of light is constant. In some cases, such as in temperate environments, caves can be considered extreme oligotrophic environments with less than 0.5 mg/L of total organic carbon (BARTON; JURADO, 2007). The partial or total absence of light, together with the limitation of trophic resources, contribute to the uniqueness of these places, mainly from the biological point of view, which includes the absence of phototrophic organisms. These environmental conditions indirectly support evidence on the presence of typical characteristics of organisms that inhabit the subterranean environment, such as greater longevity and reduced metabolic rate, according to the resource limitation hypothesis defended by Huppop (2000). However, some caves can receive large inputs of organic matter from the surface through dripping water, penetrating roots, flooding from subterranean rivers or by the entry of animals from surface environments. Mainly in tropical regions, such as Brazil, caves can receive a greater contribution of organic carbon in relation to caves found in temperate regions (PAULA *et al.*, 2020).

A variety of microorganisms inhabit cave environments, namely: autotrophic bacteria, using inorganic compounds to obtain carbon and energy; heterotrophic bacteria, using organic compounds as a source of energy; filamentous fungi, acting on nutrient cycling; and viruses, protozoa and microinvertebrates (BENDIA *et al.*, 2020; MARQUES *et al.*, 2018; PAULA *et al.*, 2019; SIGALA-REGALADO *et al.*, 2011). Cave microbiology is a relatively young field, although the number of research groups and publications have increased in recent years (LEE *et al.*, 2012). Much of this work has focused on traditional limestone caves, with little microbial exploration having taken place in caves present in other lithologies. Although there is a consensus that subterranean environments have an active microbial community, knowledge about microbial diversity in caves, the factors that control the distribution patterns of cave microbiota and the role of microorganisms in the subterranean world is still incipient.

Therefore, this chapter presents a review of the current knowledge of microbial diversity and its ecological role in cave ecosystems. Knowing more about microorganisms in unique habitats, such as caves, offers the opportunity to expand our understanding of microbial diversity from a global perspective and the potential to gain new information about the energetic dynamics of cave communities, not to mention the enormous biotechnological potential that these microorganisms can offer to society.

What are microorganisms?

Microorganisms are microscopic living beings and can be unicellular or multicellular. The main organisms are archaea, bacteria, algae, fungi and protozoans. Many researchers do not include viruses and prions in the group of organisms because they are not considered living beings. However, the study of viruses and prions comprises one of the areas of microbiology with great importance not only for human health, but also for analyzing their biological relationships with other organisms. Microorganisms can also be divided into two groups according to their cellular structure: prokaryotes — those that do not

have a nucleus and membranous organelles; and eukaryotes — organisms that have a nucleus and membranous organelles (Brock *et al.*, 1994).

With the emergence of genomic analysis of organisms in the 1970s, a new division was proposed with three domains: *Archaea*, *Bacteria* and *Eukarya* (WOESE; FOX, 1977). This division organisms can be observed in the tree of life produced by Hug *et al.* (2016) from genetic sequences obtained from public databases (Figure 1). The tree of life clearly shows that microorganisms comprise most of the living things on Earth. However, many researchers estimate that about 99% of the Earth's microorganisms have yet to be identified and/or cultivated (STEEN *et al.*, 2019).

Ecological functions of microorganisms

Microorganisms play important roles in carbon and nutrient cycles, in animal (including human) and plant health, in agriculture and in the global food chain. Microorganisms live in all environments on Earth that are occupied by macroscopic organisms and are the only known forms of life found in environments considered extreme, such as hydrothermal vents at the bottom of the oceans. Microorganisms date back to the origin of life on Earth, at least 3.8 billion years ago, and are likely to exist far beyond any extinction events in the future (BROCK *et al.*, 1994).

ARCHAEA

Archaea and bacteria are prokaryotic, single-celled organisms, which can be very similar, but there are some important differences between the two groups. The structures of their cells have different components, especially in relation to the cell membrane, and the genetic material is completely different. Archaea can generate energy through different metabolic processes and perform unique ecological functions such as methane production. They are also known to inhabit environments considered extreme, such as habitats with high temperatures (above 100° Celsius), with high concentrations of salts, in the depths of the ocean or even places with high gamma or UV radiation, as well as frozen, acidic or alkaline environments, (JARREL *et al.*, 2011). The domain *Archaea* consists of two main subdivisions, *Crenarchaeota* and *Euryarchaeota*, and a smaller, older lineage, *Korarchaeota*. Other subdivisions have also been proposed, including *Nanoarchaeota* and *Thaumarchaeota* (HOWLAND, 2000).

BACTERIA

Bacteria can be classified into five groups according to their basic shapes: spherical (cocci), rod (bacilli), spiral (spirilla), curved flagellated rods (vibrios) or spirochetes (BROCK *et al.*, 1994). Most bacteria are not pathogenic to humans. Many bacterial species live in our body and help us stay healthy; there are approximately ten times more bacterial cells in our body than human cells. In addition, bacteria can be found on all types of substrates, where they play an active role in cycling nutrients such as carbon, nitrogen and sulfur. Bacteria can be classified as autotrophs or heterotrophs, according to how energy is obtained. Autotrophic bacteria carry out the fixation of carbon dioxide (CO_2) for energy. This process can be performed using light energy (photoautotrophs) or by the oxidation of nitrogen, sulfur, iron or other chemical elements (chemoautotrophs). On the other hand, heterotrophic bacteria obtain energy through the breakdown of organic compounds absorbed in the environment around them, such as the decomposition of organic matter. The process of decomposing organic matter is the release of nutrients back into the environment, which is one of the most important roles of bacteria. Another important ecological role played by bacteria is nitrogen fixation, the main way by which nitrogen becomes available to organisms. These bacteria convert nitrogen gas into nitrates or nitrites as part of their metabolism, the resulting products of which are released into the environment. Other denitrifying bacteria carry out this metabolism in the reverse direction, transforming nitrates into nitrogen gas or nitrous oxide (BROCK *et al.*, 1994). All these processes highlight the active role of bacteria in maintaining ecosystems.

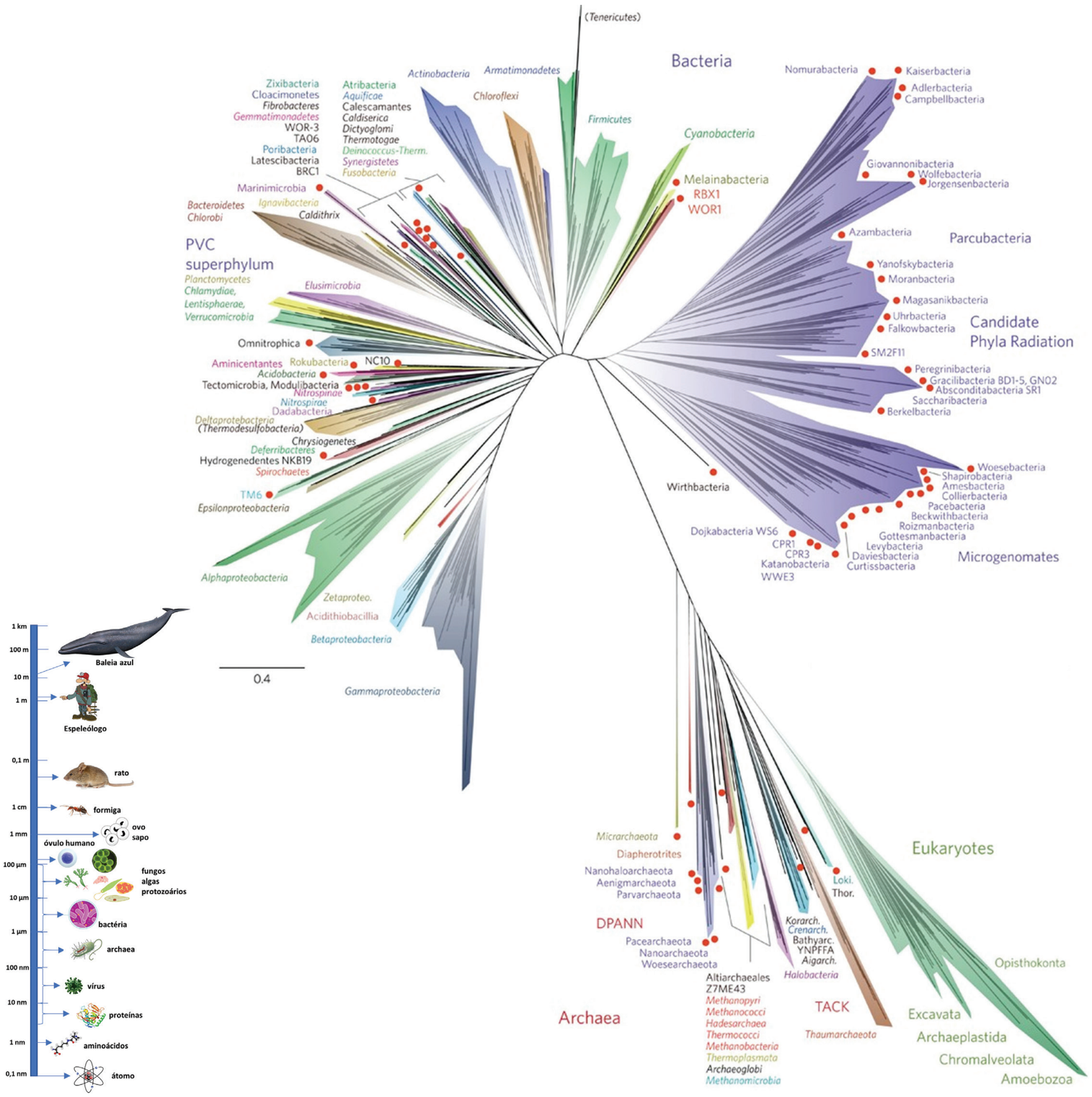


Figure 1. Representation of the tree of life proposed by Hugh *et al.* (2016) highlighting the domains *Archaea*, *Bacteria* and *Eukarya*. Groups marked with red dots represent organisms that have not yet been isolated/cultured. The diagram on the side shows a graphical representation on a numerical scale of the variety of sizes of micro and macroscopic organisms and structures.

ALGAE

The domain *Eukarya* contains microorganisms such as algae, fungi and protozoans. Most algae live in fresh or salt water (phytoplankton), while other groups can grow on rocks, soil or on vegetation, as long as there is sufficient moisture. A characteristic of algae is the presence of the chlorophyll pigment *Chla* (other types of chlorophyll such as Chl*b,* Chl*c,* Chl*d* and Chl*f* may also be present) contained in chloroplasts, which gives many algae their green color. However, some algae can be brown, yellow or red because, in addition to chlorophylls, they have other accessory pigments, such as carotenoids. Algae produce organic molecules from carbon dioxide (CO_2) and water through the process of photosynthesis, by which they capture energy from sunlight. Thus, algae need light energy to survive. Similar to land plants, algae are at the base of the food chain. In addition to fixing CO_2, algae produce oxygen as a by-product of photosynthesis, and produce more than half of the global net oxygen available for respiration by humans and other terrestrial animals (CHAPMAN, 2013).

PROTOZOANS

Protozoans, in general, are heterotrophic, unicellular, non-filamentous organisms and are found in aquatic ecosystems or environments with high humidity. Some protozoa, such as *Paramecium bursaria*, have developed symbiotic relationships with eukaryotic algae (FLEMMING *et al.*, 2020), while some species can be parasitic, including representatives of the group Apicomplexa (Chromalveolata) and trypanosomes (Euglenozoa). Protozoa play important roles in bacterial population control by regulating bacterial populations in the environment. These microorganisms also excrete nitrogen and phosphorus, in the form of ammonium and orthophosphate, as products of their metabolism. Studies have shown that the presence of protozoans in the soil can promote plant growth (WEIDNER *et al.*, 2017).

FUNGI

Fungi can be very complex unicellular or multicellular organisms. They are among the most widely distributed organisms on Earth and have are of great environmental and medical importance. Many fungi are free-living in soil or water, while others form parasitic or symbiotic relationships with plants or animals. Fungi are classified according to their life cycle, the presence or structure of their fruiting bodies, and the arrangement and type of spores (reproductive cells). The three main groups of fungi are: a) multicellular filamentous fungi; b) macroscopic filamentous fungi that form large fruiting bodies, known as mushrooms (the mushroom is just the part of the fungus that we see above ground, called the fruiting body); and c) unicellular fungi, also known as yeasts. Fungi, along with bacteria, play a key role in the decomposition of organic matter, especially in relation to more complex organic molecules such as lignin and cellulose, releasing carbon, nitrogen and phosphorus into the environment. Fungi are also used in our daily lives, such as in the manufacture of bread, wine, beer and other foods. Some are plant parasites that cause diseases, such as rusts, scabs or cankers, and a very small number of fungi cause diseases in animals including humans, such as skin diseases like athlete's foot, ringworm and thrush. Despite the pathogenic potential of some species, fungi have medical relevance due to the ability to produce a wide variety of antibiotics that revolutionized medical practice, such as penicillin. The phylogenetic classification of fungi divides the group into seven phyla (Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Glomeromycota, Microsporidia and Neocallimastigomycota), 10 subphyla, 35 classes, 12 subclasses and 129 orders (AINSWORTH, 2008).

Methods for studies of cave microbiota

Early studies of microbial communities in caves used cultivation-dependent techniques (see Figure 2D-E), which described the cave microbiota as a reflection of surface soil microbiology (CAUMARTIN, 1963; HESS, 1900; SCOTT, 1909). One of the limitations of cultivation-dependent techniques is the underestimation of biodiversity due to the inability to cultivate most microorganisms present in the environment (TORSVIK *et al.*, 2002). Therefore, the understanding of microbial life in caves was limited until the emergence of molecular techniques, with less than 40 articles published until 1997 (HERSHEY; BARTON, 2018).

With the advent of molecular techniques, researchers managed to overcome this problem, even though molecular methods also have their limitations. Angert *et al.* (1998) published the first study of cave microbiota using molecular methods. As a result, they observed the dominance of Epsilonproteobacteria bacterium in the Sulfur River Cave

Kentucky (USA), a bacterial group that, until then, was found only in hydrothermal vents in the oceans. The most commonly used methods are based on the analysis of molecular markers, which include more conserved regions of genetic material such as the small (16S rRNA) and large (23S rRNA) ribosome subunits, as well as functional genes, such as *soxB* (present in sulfur oxidizing microorganisms), *amoA* (present in ammonia oxidizing organisms) and *RuBisCO* (found in chemoautotrophic microorganisms) (HOLMES *et al.*, 2004). The genetic sequences of these genes are amplified from a *pool* of DNA extracted from environmental samples and then the product of this amplification is sequenced (Figure 2A) and/or analyzed by electrophoresis using "fingerprint" methods (Figure 2B), such as: DGGE (Denaturing Gradient Gel Electrophoresis), RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified DNA Polymorphism) and ARISA (Automated Ribosomal Intergenic Spacer Analysis) (WOOLEY *et al.*, 2010). Another frequently applied technique is FISH (Fluorescence In Situ Hybridization), which is based on the hybridization of nucleic acid with specific fluorescence-labeled oligonucleotides (Figure 2C), allowing the quantification and identification of microorganisms directly in the sample using an epifluorescence microscope (MOTER; GÖBEL, 2000). Incubation of microbial populations with the carbon isotope 13C followed by extraction of labeled DNA, also known as DNA-SIP (DNA stable-isotope probing), is an efficient method to detect which microorganisms actively assimilate CO_2 in a studied environment (CHEN *et al.*, 2009). Finally, it is possible to use methods based on RNA extraction and carry out studies related to gene transcription, which can determine which microorganisms present in an environmental sample are metabolically active. The use of various methods to assess microbial diversity allows a more complete view of its biodiversity, so that the results are complementary.

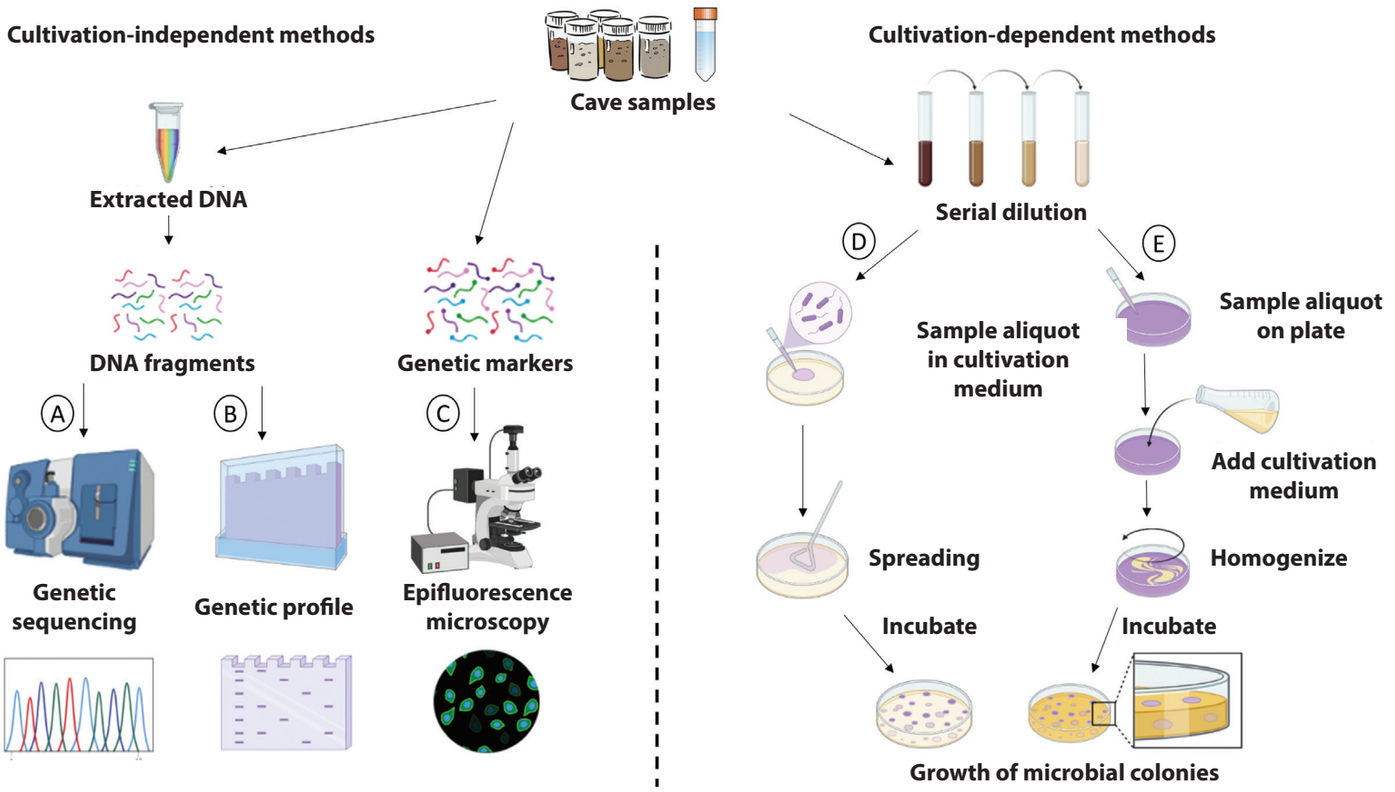


Figure 2. Main cultivation-dependent and independent methods used to study microbial communities in caves. Cultivation-independent methods include: **A)** genetic sequencing; **B)** genetic profile analysis ("fingerprint"); **C)** microscopy with genetic markers. The main cultivation-dependent methods include: **D)** spread plate; **E)** pour plate.

A set of methods allows a better understanding of which microorganisms are present at a study site and how they are functionally acting in the environment.

With the development of more accurate and low-cost methods, microbiological research in caves has advanced since the 1990s. In 1994, the Karst Geomicrobiology and Redox Geochemistry Conference had 59 works presented in the area and, in 2001, a special edition of Geomicrobiology Journal was published containing ten articles on the topic. In 2009, there was a special session on Microbiology and Geomicrobiology of Caves and Karst Environments at the International Congress of Speleology. The cumulative impact of these changes was a five-fold increase in the number of articles on cave microbiology between 1997 and 2012 (HERSHEY; BARTON, 2018). Currently, there are more than 400 articles that describe the structures of microbial communities related to different types of caves in the world (ENGEL, 2010; HERSHEY; BARTON, 2018; LEE *et al.*, 2012). The following section presents a review of the main published works on microbial biodiversity in caves around the world, with special attention to Brazilian caves.

Cave microbial diversity

BACTERIA

Several studies have presented the rich bacterial biodiversity present in caves. More detailed studies initially emerged after the 1990s, along with the development of molecular techniques, especially next-generation sequencing (NGS), which revealed a microbial world composed of thousands of hitherto unidentified taxa (SOGIN *et al.*, 2006). Many researchers have since been working with NGS to assess the main bacterial groups present in cave ecosystems, the results of which can be consulted in the National Center for Biotechnology Information (NCBI, USA) database (LEINONEN *et al.*, 2010).

The first study using NGS to assess bacterial communities in caves was carried out at Kartchner Caverns, United States (ORTIZ *et al.*, 2013). The researchers found 13 bacterial phyla previously identified in caves using cloning techniques. However, eight more phyla and 12 new candidate phyla were first observed in this cave, suggesting that the cave contained a rare microbial biosphere. In this same study, the researchers demonstrated that only 16% of the genetic sequences obtained were present both in the external and internal environments of the cave, being some of the first evidence of the uniqueness of microbial communities in cave environments. Some evidence suggests that a lower influence of the surface ecosystem on certain microbial communities in caves may be due to: I) the low percentage of cave microorganisms that share taxonomic identity with surface soil species; II) the different evolutionary adaptations observed in the genomes of microorganisms present in subterranean environments; and III) some bacterial groups associated with human activities are rapidly suppressed in the cave environment (JOHNSTON *et al.*, 2012; ORTIZ *et al.*, 2013). Therefore, the surface soil can influence the microbiota in these environments, but environmental and selective pressures are crucial to defining the structure of these communities in the subterranean environment. On the other hand, more recent studies have demonstrated the influence that the surface has on cave microbial communities as part of a source population that seeds the subterranean environment, mainly in long-term impacted areas (HERSHEY; BARTON, 2018; YUN *et al.*, 2016).

Diversity

Caves have, in general, 10 dominant bacterial phyla (Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Gemmatimonadetes, Nitrospirae, Planctomycetales, Proteobacteria and Verrucomicrobia), along with 14 other bacterial phyla present in the rare biosphere (Armatimonadetes, Chlorobi, Cyanobacteria, Elusimicrobia, Spirochetes, and candidate phyla BRC1, GN04, NC10, OP3, TM6, WS1, and WS3) (HERSHEY; BARTON, 2018). Datasets resulting from studies using NGS also show a significant proportion of sequences that cannot be classified using reference taxon databases such as Greengenes and SILVA (QUAST *et al.*, 2012). These sequences, that cannot be easily placed within the current taxonomic framework, are known as the black box of the microbial world and represent the unexplored diversity of this biodiversity. These unidentified genetic sequences tend to have a greater representation in caves than in other habitats, suggesting that the true microbial diversity in caves requires more attention and more detailed description (HERSHEY; BARTON, 2018; RINKE *et al.*, 2013). This structure and composition of bacterial communities appears to be robust for most studies carried out in caves, indicating that the main determinants of the structure of microbial communities in these habitats are consistent diversity

represents a central microbiome in the cave environment.

Bacterial diversity in Brazilian caves has been studied in a variety of substrates (Figure 3) (BENDIA *et al.*, 2020; FELESTRINO *et al.*, 2018; MARQUES *et al.*, 2018, 2019; PAULA, 2018; PARKER *et al.*, 2018). A survey carried out in two caves in Paripiranga, state of Bahia, detected a dominance of the phylum *Proteobacteria* (classes *Alphaproteobacteria* and *Gammaproteobacteria*) and *Firmicutes* (class *Bacilli*) in the drip water of speleothems. The family *Bacillaceae* was predominant in this environment, however, other families such as *Erythrobacteraceae, Burkholderiaceae, Pseudomonadaceae, Xanthomonadaceae* and *Phyllobacteriaceae* were also found at the site (MARQUES *et al.*, 2019). Previous studies carried out in temperate caves showed a dominance of the classes *Beta* and *Gammaproteobacteria* in drip water, a composition different from what was observed in Brazilian caves (YUN *et al.*, 2016). This difference can be explained by the fact that the caves studied in the temperate climate are located at higher altitudes and with low temperatures (about 10°C), unlike the environment in which the caves in northeastern Brazil are located, characterized by low altitudes and higher temperatures (about 25°C).

Bacterial communities from Monte Cristo cave in Diamantina, state of Minas Gerais, were studied using metagenomic analysis (BENDIA *et al.*, 2020), a technique that allows assessing a greater number of genetic sequences than NGS. The phyla *Actinobacteria* (21.3% of the bacterial community) and *Proteobacteria* (19.7%) were dominant in this cave. However, other less abundant phyla were also observed, such as *Acidobacteria* (8.2%), *Bacteroidetes* (6.5%), *Firmicutes* (6.5%), *Nitrospira* (6.5%), *Chloroflexi* (4.9%), *Patescibacteria* (4.9%), *Bipolaricaulota* (3.3%), *Dadabacteria* (1.6%), *Deinococcota* (1.6%), and *Methylomirabilota* (1.6%), while 6.5% of the sequences were associated with phyla not yet classified. This study demonstrates the enormous bacterial biodiversity present in Brazilian caves and highlights a biodiversity that has not even been described by science. The results also show the presence of bacteria from the family *Trueperaceae*, reported for the first time in a cave environment. This family is composed of chemoorganotrophic microorganisms known for their resistance to ionizing radiation. Some of the genes found in this bacterial family are related to DNA repair mechanisms, as a response to oxidative stress and food shortages. Although there is no incidence of UV light in the cave, these defense mechanisms can also act in conditions of high salinity or desiccation, conditions found in the Monte Cristo cave with high electrical conductivity due to the high concentration of salts.

Ecology

In recent years, iron ore caves in Brazil have attracted the attention of researchers around the world, especially those found in the Quadrilátero Ferrífero (Iron Quadrangle) region in the state of Minas Gerais. Caves in this region are inserted in rock of more recent origin (about 65 million years old) known as canga, which consists of weathered fragments of itabirite and hematite, cemented by a matrix of goethite, and which has high porosity, facilitating infiltration of water in its matrix (SCHUSTER *et al.*, 2012). The vegetation associated with canga is characterized by herbaceous-shrubby formations associated with rocky outcrops or generally shallow soils. The plants in this region seem to sprout from the rocks, however, they generally look for cracks and crevices in this rocky profile where water accumulates. Due to the shallow and acidic soil, together with high levels of manganese and iron and low concentrations of nutrients (mainly phosphorus), the roots of plants of this landscape penetrate the cracks in the rock and develop on the surface of the iron ore caves, also known as penetrating roots, and establish an ecological relationship with cave microorganisms.

A study carried out in a cave of this region evaluated the bacterial community associated with these penetrating roots. Using culture-dependent methods, the researchers isolated 23 bacteria, which showed production of the phytohormone indolylacetic acid (IAA), hydrogen cyanide, amylolytic and proteolytic enzymes and siderophores, as well as phosphate solubilization and antibiotic activity (FELESTRINO *et al.*, 2018). In this ecological relationship between plants and microorganisms, plants can provide a unique niche for the maintenance of various microbial communities inside of caves while, on the other hand, microorganisms help in the health and survival of plants (acting as growth promoters, fixing nitrogen, solubilizing phosphate, producing phytohormones or protecting the plant against other pathogens).

Another interesting example was observed in three caves in the Quadrilátero Ferrífero (Triângulos, Piedade and VL-02), where researchers noticed that bacterial communities, in addition to playing a fundamental ecological role, also played an active role in the formation of the caves (PARKER *et al.*, 2018). These caves showed a bacterial community

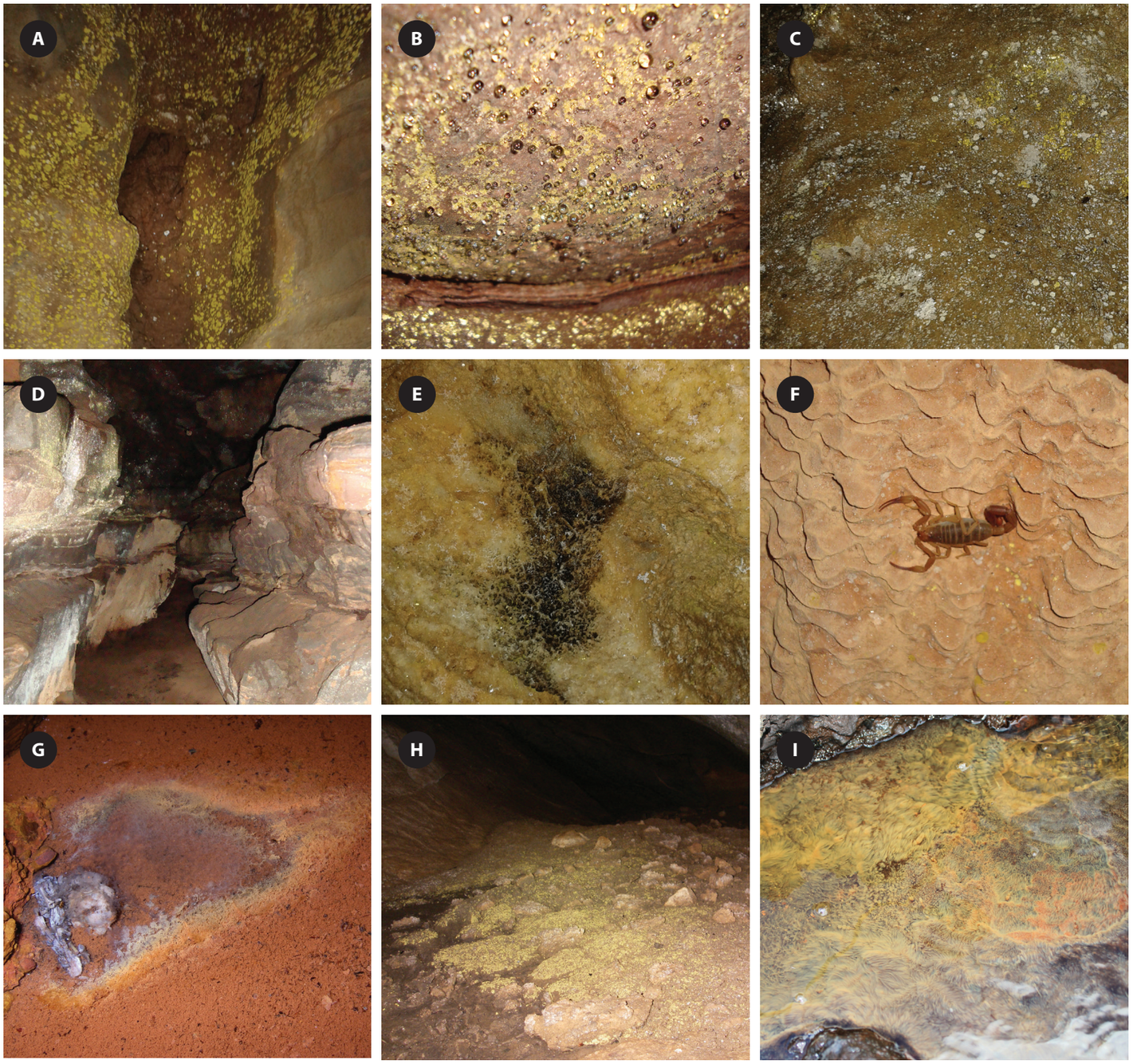


Figure 3. Bacterial colonies on rocky surface: **A)** Olhos D'agua cave, Itacarambi, Minas Gerais; **B)** Cave Russão, Posse, Goiás; **C)** Chýnov cave, Dolní Hořice, Czech Republic; **D)** Cave São Bernardo III, São Domingos, Goiás; **E)** Cave do Diabo, Eldorado, São Paulo; **F)** Cave Revolucionários, Posse, Goiás. Bacterial communities in **G)** Cave MJ_0005 sediment, Brumadinho, Minas Gerais; microbial biofilm **H)** in Cave A-30, Doresópolis, Minas Gerais; **I)** in ferruginous canga of Serra Sul, Canaã dos Carajás, Pará. Photos: A-F (Caio Cesar Pires de Paula); G-I (Robson de Almeida Zampaulo).

composition similar to that of limestone caves, with a predominance of *Alphaproteobacteria*, *Betaproteobacteria, Gammaproteobacteria*, *Chloroflexi, Planctomycetales, Bacteroidetes* and *Acidobacteria*. However, when analyzing some bacterial strains isolated from this environment, the researchers observed that the bacteria reduced Fe(III), resulting in the removal of soluble Fe(II) and, consequently, contributing to the formation of iron ore caves. Such bacteria became known as iron-reducing microorganisms (FeRMs). Different bacterial taxa of the groups *Actinobacteria, Proteobacteria, Nitrospirae* and *Firmicutes* have already been related to iron ore, acting mainly in the leaching of minerals such as iron oxides and sulfides under acidic conditions (HURTADO *et al.*, 2020). In this context, organic carbon stimulates the reduction and dissolution of Fe(III) and acts as an electron donor for the growth of FeRMs, which use Fe(III) oxides as electron acceptors during their development (PARKER *et al.* , 2013).

The communities of ammonia oxidizing bacteria (AOB) and nitrogen fixing bacteria were the focus of another study in a sandstone cave in Chapada da Diamantina (Gruta do Lapão). The AOB communities of this Brazilian cave were composed of *Nitrosomonas* and *Nitrospira*, while the nitrogen fixing communities were composed of the genera *Azospirillum, Bradyrhizobium, Pseudomonas* and *Anabaena* (MARQUES *et al.*, 2018). However, the structure of bacterial communities differed among cave zones, with greater diversity of AOB and nitrogen fixing bacteria at the entrance than in the deeper part of the cave. This greater diversity at the cave entrance is probably due to the greater energy demand of these metabolic pathways (ammonia oxidation and nitrogen fixation). The interior of the cave is an oligotrophic environment when compared to the external environment and the cave entrance. Therefore, the lower amount of microorganisms related to these ecological functions can be explained by the low metabolic activity of this community.

Different environmental factors can influence the structure and composition of microbial communities, whether physical, chemical or biological. In the aforementioned study at Gruta do Lapão, the researchers observed differences in the bacterial profile of two caves (Gruta do Lapão and Gruta Manoel Ioiô) of different lithologies (sandstone and limestone). In this case, the chemical composition of the caves can influence the establishment of some bacterial groups, as limestone caves have high concentrations of magnesium and calcium, while sandstone caves have high concentrations of silica and aluminum. Bacteria in karst sediments are more limited by carbon and phosphorus than by nitrogen, and so it can be assumed that sources of nitrogen in cave sediments may favorably enrich certain dominant taxa, thus decreasing the overall species richness of the bacterial community (CHEN *et al.*, 2019). Another study carried out in Heshang Cave, China, showed that total organic carbon and pH were the main environmental factors determining the structure of bacterial communities (YUN *et al.*, 2016). The researchers also did an excellent analysis of the changes in environmental conditions in relation to the increase in acid rain in the region and how this scenario affects the bacterial communities in the caves. The pH is an important factor in controlling the rate of decomposition of organic matter by the microbiota, and the alkaline state of karst soils and related habitats maintains a lower rate of decomposition of organic matter, a favorable condition for bacterial communities in caves. The acidification of the surface soil and the penetration of acidic fluids to greater depths within a karst system, due to the increase in the amount of precipitation and acid rain, can notably increase the decomposition of organic matter by microbial action. Therefore, the decrease in pH promotes an increase in the rate of decomposition by heterotrophic bacteria, favoring bacterial groups with greater decomposition activity and rapid growth. In a future scenario, this would result in a drastic reduction in the amount of carbon in this ecosystem and increase the release of CO2, interfering not only with subterranean biodiversity but also with the speleogenesis of the cave. This same cave in China allowed researchers to notice variation in bacterial communities among different seasons, where temperature was the main environmental factor that affected bacterial community structure throughout the study (YUN *et al.*, 2016). In summary, both studies demonstrated that climate change can drastically influence the ecological dynamics of bacterial communities in cave environments.

ARCHAEA

It was long believed that archaea were only present in extreme environments, but later studies have demonstrated their presence in a variety of habitats, including caves. Archaea can be aerobic, facultatively or strictly anaerobic, chemolithotrophic, chemoheterotrophic, acidophilic, thermophilic or halophilic (HORIKOSHI; GRANT, 1998). Little is known about the occurrence of archaea in subterranean systems. They were first described for a subterranean environment in Lechuguilla Cave, United States

(NORTHUP *et al.*, 2003). Since then, research based on molecular techniques has been undertaken to analyze the diversity of archaea in caves, such as in water samples from Movile Cave, Romania (CHEN *et al.*, 2009), in sediment of Wind Cave, United States (CHELIUS; MOORE, 2004) and in Grotta del Fiume, Italy, caves with extremely acidic substrates (pH 0–1) and which have about 40% of the microbial community of snottites composed of archaea of the genus *Ferroplasma*, organisms that prefer low pH and oxidize sulfur and iron (JARREL *et al.*, 2011; MACALADY *et al.*, 2007).

Diversity

Despite few published studies, archaea are also found in Brazilian caves. The archaea community at Gruta Manuel Ioiô is composed of the phyla *Crenarchaeota* and *Euryarchaeota*, which showed slower metabolism compared to other microorganisms, which leads to a later response to environmental changes (MARQUES *et al.*, 2017). However, this community also showed great resilience after an environmental change by returning to its natural state of composition. Archaea belonging to the phylum *Thaumarchaeota* were found in Monte Cristo Cave (BENDIA *et al.*, 2020), and research carried out in Terra Ronca I Cave (Goiás) showed for the first time the dominance of archaeans of the phylum *Haloarchaea* in a subterranean environment (PAULA, 2018). In general, *Thaumarchaeota*, *Crenarchaeota* and *Euryarchaeota* are the most recurrent phyla in cave environments. Many studies have highlighted the large percentage of genetic sequences obtained for the domain *Archaea* that cannot be identified in available databases, reaching up to 50% of taxa present in a microbial community (BORSODI *et al.*, 2018). These data show that databases need to be supplied with more genetic information about archaea, but due to the difficulties of cultivating these organisms in the laboratory, little is known about the metabolic pathways used by the group and few species are described in the scientific literature.

Ecology

Members of the group *Thaumarchaeota* are very small microorganisms (up to 100 times smaller than a bacterial cell), with slow growth rates and great resistance to energy stress, making them good competitors under extreme nutrient limitation. This may be one of the reasons for the high recurrence of this group of archaea in cave environments (BROCHIER-ARMANET *et al.*, 2012; HERSHEY; BARTON, 2018). These microorganisms play an important role in nitrification, carrying out the oxidation of nitrogen (NH_3 / NH_4^+) to generate energy. To compete with bacteria for available NH_3, members of *Thaumarchaeota* have high-affinity transport mechanisms, making them more adapted to the low level of nitrogen found in caves and providing them with an alternative growth strategy in an environment where competition for other resources is high (BROCHIER-ARMANEt *et al.*, 2012). In caves with extremely low pH, where all available NH_3 would be ionized into NH_4+, some members of *Thaumarchaeota* are able to use urea through intracellular ureases. Archaea are known to play an important role in the energetic dynamics of cave environments, actively participating in aerobic nitrification. Most of these organisms can also metabolize sulfur and are the only ones known to carry out processes of methanogenesis and anaerobic methane oxidation (CAVICCHIOLI, 2011), suggesting a syntropic association with other microorganisms and showing a more complex metabolic cooperation (DESAI *et al.*, 2013).

Studies have shown that archaea populations can be dominant in places where carbon and nitrogen concentrations are limited and phosphate, sulfate and ammonia concentrations are high. The subterranean environment has, in general, a greater abundance of archaea than other surface ecosystems. The percentage of archaea in the deepest regions of Lechuguilla Cave, United States, for example, reached 86%, a region of this subterranean system where the concentration of available nitrogen is more limiting (RAJI *et al.*, 2019). The Brazilian caves Gruta do Lapão (limestone cave) and Gruta Manoel Ioiô (sandstone cave) showed different community profiles of archaea between the different lithologies, with greater diversity in the sandstone cave and dominance of a few taxa in the limestone cave (MARQUES *et al.*, 2018). The greater richness in the sandstone cave is probably related to its acidic pH, which is commonly more suitable for this microbial group. The dominance of archaea under limiting nutritional conditions can be attributed to their adaptation to chronic energy stress, which may be a primary factor in differentiating bacterial ecology from archaea ecology (JARREL *et al.*, 2011).

FUNGI

Environmental characteristics of caves, such as low temperature variation and high relative humidity, are some of

the main factors that make this environment very favorable to fungal colonization (Figure 4). The viability of fungal spores for long periods of time also allows these organisms to survive in this nutrient-poor environment. Humboldt published the first study on the presence of fungi in caves in 1794, as described in Dobat (1967), and the first report of their ecological role in caves was made by Megušar (1914). More than 1,150 species of fungi, distributed among 550 genera, have been observed in caves around the world (ZHANG *et al.*, 2018). Most of the published studies on fungal communities in caves used isolation and cultivation methods (VANDERWOLF *et al.*, 2013), limiting the knowledge of the true diversity present in this environment, as well as the exploration of the ecological functions performed by these microorganisms. Most fungi are not cultivable in the laboratory, especially those that colonize rocks and have low metabolic activity (MAN *et al.*, 2018). Isolation strategies, such as the use of different culture media and incubation times and temperatures, can significantly affect the results of mycological studies in caves due to the different physiological requirements possessed by different fungal taxa (MAN *et al.*, 2015). Typically, more than 30 days are required for slow-growing fungi to form visible colonies due to their low metabolic rates or due to the low number of cells present in the substrate. An alternative is to use molecular methods to assess more broadly and deeply the entire mycological community. The use of molecular methods in Heshang Cave (China) made it possible to detect a greater diversity of fungi compared to culture-dependent methods used in a previous study, indicating that the mycological diversity of the cave had been underestimated until then (MAN *et al.*, 2015, 2018).

Diversity

Following the same worldwide pattern, in which studies of prokaryote communities in caves are more frequent, research on fungal communities in Brazilian caves are still scarce. Most of these studies used cultivation methods and observed taxa that are often present in caves in different regions of the globe (FONSECA *et al.*, 2019; MELO *et al.*, 2013; PAULA *et al.*, 2016; TAYLOR *et al.*, 2013, 2014). The Lapa Nova Cave (Minas Gerais), for example, presented 43 species of fungi distributed among 12 genera: *Aspergillus, Penicillium, Cladosporium, Eurotium, Fusarium, Mucor, Emericella, Gliocladium, Paecilomyces, Purpureocillium, Rhizopus* and *Trichoderma* (TAYLOR *et al.*, 2013). An interesting report from the caves of the Quadrilátero Ferrífero showed that the fungus *Sagenomella stratispora* may be related to iron oxidation, a process also observed in bacterial communities. While bacteria oxidize iron using it as an electron donor, fungi perform iron deposition as an extracellular detoxification process. Among the studies carried out in Brazilian caves, only one survey, carried out in the Parque Estadual de Terra Ronca (PeTER), has thus far used the NGS method to assess the diversity of fungi in caves in the region (PAULA, 2018). The PeTER caves had nine fungal phyla (Ascomycota, Basidiomycota, Chytridiomycota, Entomophthoromycota, Glomeromycota, Mortierellomycota, Mucoromycota, Olpidiomycota and Rozellomycota), with the most abundant taxa belonging to the classes Sordariomycetes, Eurothiomycetes, Dothideomycetes and Saccharomycetes. About 18% of the genetic sequences present in the fungal communities of these caves were not taxonomically identified in the UNITE database https://unite.ut.ee/. This high percentage of unidentified taxa in the cave environment highlights this ecosystem as a potential *hot spot* for new species of fungi, as observed by research carried out in Gruta do Catão (Bahia), where a fungus belonging to a possible new clade within the genus *Aspergillus* was isolated (PAULA *et al.*, 2019). In general, *Ascomycota* is the dominant phylum in caves, followed by Basidiomycota, Zygomycota, Chytridiomycota, Rozellomycota and Glomeromycota (MAN *et al.*, 2018). Ascomycota is typically the most abundant phylum in caves due to the group's more efficient strategies of colonizing nutrient-limited environments. Some genera of this phylum are frequently detected in caves, such as *Penicillium* and *Aspergillus*, as they are capable of degrading rocks and solubilizing minerals (STERFLINGER, 2000). The oligotrophic conditions of the subterranean environment do not favor fungi of the phylum Basidiomycota, which need substrates rich in organic matter, and Zygomycota, which demand more energy due to their high growth rate and spore production (MAN *et al.*, 2018).

Colonization

Fungal spores can be transported through caves by organisms, such as invertebrates, bats, rodents and humans, or environmental factors, such as any water flow within a cave (RAWAT *et al.*, 2017). Reproductive structures of fungi of the genus *Basidiobolus* were identified on the exoskeleton of centipedes (family Geophilidae) in caves in southeastern Brazil (FONSECA *et al.*, 2019). The fungus uses the surface of the centipede's exoskeleton as support for its asexual reproductive

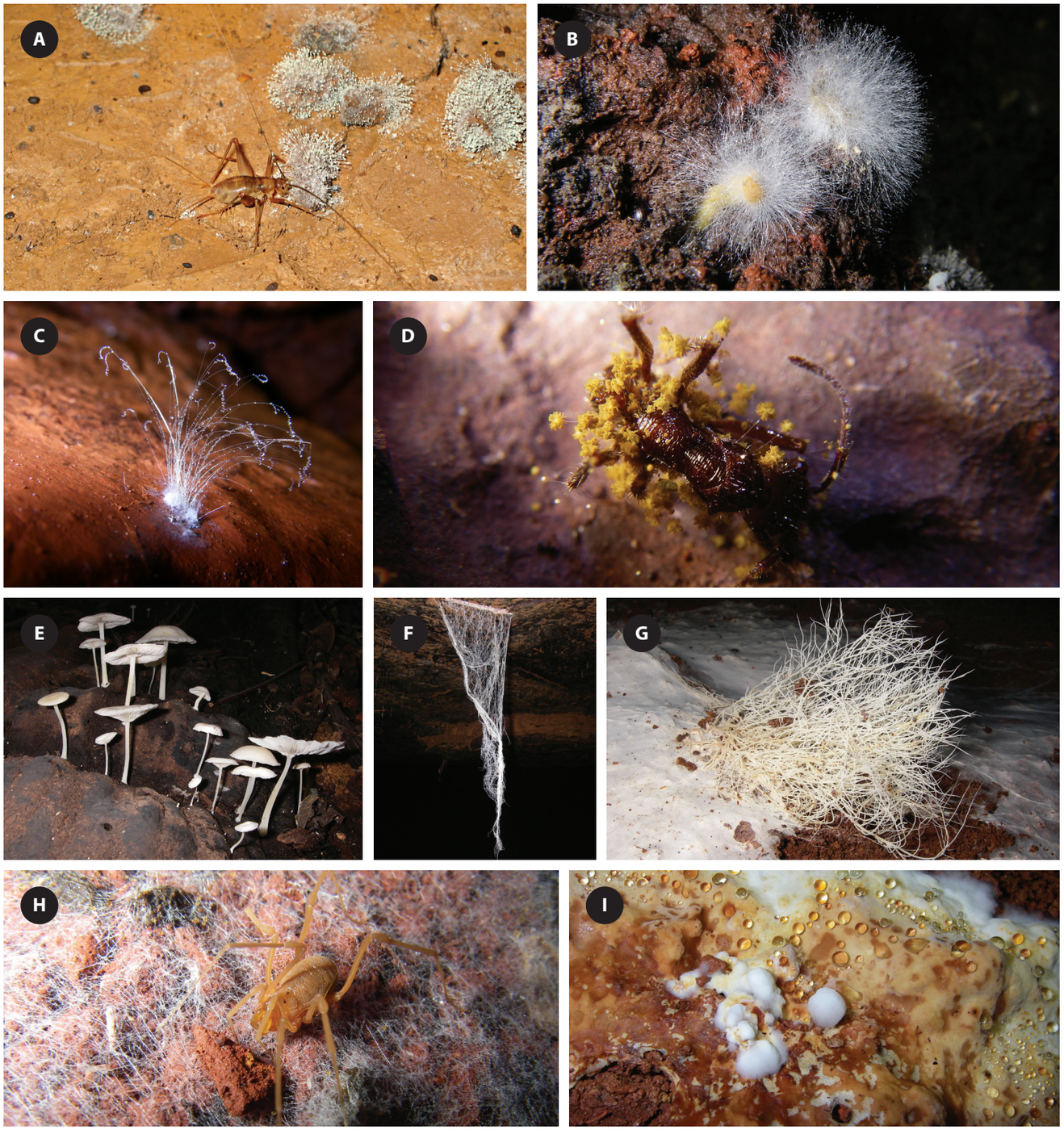


Figure 4. Fungi colonizing different substrates in: **A)** Caverna do Brega, Pains, Minas Gerais; **B)** Caverna MJ_0005, Brumadinho, Minas Gerais; **C)** Caverna CPMT_0016, Nova Lima, Minas Gerais; **D)** entomopathogenic fungus parasitizing arthropods (Pselaphinae) in Caverna GEM 1195, Parauapebas, Pará; **E)** Caverna 44L, Prudente de Morais, Minas Gerais; **F)** Caverna Morena, Cordisburgo, Minas Gerais; **G)** Caverna SERR_0014, Nova Lima, Minas Gerais; **H)** Caverna RM_0038, Nova Lima, Minas Gerais; **I)** Cavernas RF_0071, Barão de Cocais, Minas Gerais. Photos: A (Xavier Prous); B-I (Robson de Almeida Zampaulo).

structure and for the purpose of dispersing its spores as the animal moves through the environment. The air currents that enter and circulate inside caves can also be an important means of transporting spores from the external environment to the subterranean environment. Therefore, the study of cave structure and airflow is important for determining the origin of a cave's fungal community. Wang *et al.* (2010) reported greater fungal abundance and diversity in summer compared to winter in Mogao Grottoes, China, and Docampo *et al.* (2011) also observed a positive correlation between periods of rain and high concentrations of spores present in the air inside Nerja Cave, Spain. These studies highlight how external influences, such as seasonality and precipitation, can change the fungal diversity inside caves.

Ecology

Most cave fungi are decomposers or parasites, although they can also act in the formation of speleothems through the precipitation of secondary minerals (RAWAT *et al.*, 2017). Fungi are the main saprophytic organisms in cave ecosystems and play an important role in the food chain, contributing to the decomposition of organic matter and providing their biomass (spores and mycelia) as an essential food source for many arthropod communities, such as isopods and springtails, in addition to protozoa (VANDERWOLF *et al.*, 2013). On the other hand, parasitic fungi, such as *Isaria*, *Beauveria* and *Rhachomyces*, can use cave fauna as a source of nutrients. These entomopathogenic fungi, as they are known, were identified in the caves of the Parque Estadual de Terra Ronca and can parasitize various invertebrates such as crickets, harvestmen and spiders (PAULA, 2018). However, the pathogenic fungi found in caves that receive the most attention are *Histoplasma capsulatum* and *Pseudogymnoascus destructans*.

PATHOGENIC FUNGI

The fungus *Pseudogymnoascus destructans* is a bat pathogen that can be found in caves in eastern Canada, northeastern United States, and many European countries. Several species of bats can present "White Nose Syndrome" (for more details see http://www.whitenosesyndrome.org/), a disease caused by *P. destructans*, which is characterized by a change in behavior during the hibernation period and the presence of a white powdery substance on the nose and wing membranes (FRICK *et al.*, 2010). Later it was discovered that this white substance was actually spores of *P. destructans*, which can parasitize live bats, especially during the hibernation period, and use them as a source of nutrients (VOYRON *et al.*, 2011). Since 2006, the first time the disease was reported, this infection has killed about 6.7 million bats in North America and caused up to US$ 3.7 billion/year in losses in agricultural areas, as bats play an important role in pest control in this region (BOYLES *et al.*, 2011), making it one of the most devastating wildlife diseases (HERSHEY; BARTON, 2018). Despite the high mortality of bats in North America, no significant mortality or morbidity has been documented among European bats (TURNER *et al.*, 2011). Even though this fungus is detected in European caves, the absence of bat mortality in Europe may be due to environmental differences in the caves in this region, different host immune responses or other aspects of physiology and behavior or variation in the mycological community or associated fauna.

In turn, *Histoplasma capsulatum*, the causative agent of histoplasmosis, is frequently found in soil enriched with guano (feces) from bats or birds, both inside and outside caves, and is certainly the most studied fungus in cave environments. Histoplasmosis occurs worldwide but is considered endemic in Southeast Asia, India, Australia, Africa, and parts of South and North America (VANDERWOLF *et al.*, 2013). Although histoplasmosis can affect healthy people, the disease has often been linked to immunocompromised patients. The initial stage of the disease is usually asymptomatic, however, with rapid progression, more severe manifestations are common and, in many cases, lethal. Therefore, an accurate and rapid diagnosis is necessary to ensure an effective treatment (ROCHA-SILVA *et al.*, 2014). Infection occurs after exposure to *H. capsulatum* and inhalation of spores present in the environment. Inhaled spores initially settle in the alveoli of the lungs and mainly cause fever, cough, and fatigue. Once the fungus has established itself in the lung, the spores of the fungus transform into yeast cells, characterizing the dimorphism of this species. After the first report of histoplasmosis in Brazil in 1939, the number of cases has increased significantly, especially after the 1990s. The disease has been detected in all regions of the country, but only 14% of reported cases are related to activities carried out in caves (ALMEIDA *et al.*, 2019).

Other fungal species can be pathogenic under specific conditions. These fungi, known as opportunists, also affect immu-

nocompromised people. Opportunistic fungal infections have become increasingly frequent in recent decades, mainly due to the increasing number of immunosuppressive therapies and emerging diseases (PFALLER; DIEKEMA, 2004). The number of fungi causing opportunistic infections has also increased in recent decades, and the genera *Aspergillus*, *Penicillium*, *Paecilomyces*, *Purpureocillium*, *Fusarium* and *Candida* have been frequently reported as opportunistic pathogens. Some of these opportunistic fungi were found in the caves of the Parque Estadual de Terra Roca, such as the species *Aspergillus fumigatus*, the main etiological agent of aspergillosis (PAULA, 2018). To develop aspergillosis, a high load of spores of the fungus present in the environment must be inhaled. For healthy people, inhaled spores can be quickly eliminated by the immune system or only a mild allergy develops. However, for people in the risk group, inhalation of spores of this fungus can lead to a serious lung infection. Periodic monitoring of the amount of spores of these opportunistic fungi in caves is extremely important in order to prevent people from entering at times with high concentrations in the air, especially in drier periods. Without proper monitoring, the use of caves for tourist and scientific purposes can cause health problems for visitors, researchers and guides. It is important to emphasize that monitoring should be considered as a preventive measure that competent bodies should adopt to guarantee the health safety of people who intend to visit caves open to public visitation.

PROTOZOA

Protozoa are present in different cave substrates such as sediment, water puddles, rivers and streams, drip water, guano, etc. However, there are still few studies on the structure and composition of this group in cave environments. Protozoa can enter caves by any water flow or infiltration of the soil, by air currents or carried by cave fauna. They are heterotrophic microorganisms that participate in the process of decomposition of organic matter and may be associated with detritus banks. Some groups are bacterivorous and extremely important for controlling the bacterial population, while other protozoa are parasites of vertebrates and invertebrates that live in caves.

Diversity

Some records have highlighted the presence and diversity of these microorganisms in caves, such as *Paramecium multimicronucleatum* and *Spirostomum ambiguum* in Virginia, United States (HOLSINGER, 1966), or *Phacus* sp., *Paramecium* sp., *Halteria* sp., *Difflugia* sp. and *Peranema* sp. as free-living microorganisms in Mammoth Cave, Kentucky, United States (BARR, 1968). The protozoan community in Cueva de Los Riscos, Mexico, was found to consist of eight ciliates (*Aspidisca* sp., *Litonotus* sp., *Colpoda* sp., *Coleps hirtus*, *Paramecium caudatum*, *Cyclidium* sp., *Tetrahymena pyriformis* and *Vorticella* sp.), three flagellates (*Entosiphon* sp., *Distigma* sp., and *Peranema* sp.), an amoeba (*Vahlkampfia* sp.) and a heliozoan (*Actinophrys sol*) (SIGALA-REGALADO *et al.*, 2011).

The first report on the presence of protozoa in Brazilian caves detected the genus *Trypanosoma* parasitizing the bat *Phyllostomus hastatus* (BARBOSA *et al.*, 1973). Since then, some studies have reported the presence of these microorganisms in caves in Brazil (SILVA *et al.*, 2012; SIMÕES *et al.*, 2013), but no in-depth study has been carried out on the composition and dynamics of these communities. The lack of studies of protozoan communities in Brazilian caves leaves a knowledge gap regarding their biodiversity, functions and ecological relationships. It is extremely important to know this microbial group for a better understanding of the trophic relationships between micro- and macroorganisms. In addition, a better understanding of the ecological dynamics in caves involving microbial groups such as protozoa is essential for future management plans.

ALGAE

The gradual reduction of light in the cave environment promotes a zoning of phototrophic organisms from phanerophytes, pteridophytes and bryophytes in areas close to the cave entrance, to algae and cyanobacteria in the twilight zone (Figure 5). Microscopic phototrophs, especially algae, can colonize not only water bodies, such as rivers, streams, and puddles, but also rock surfaces in the entrance and twilight zones.

Diversity

The main groups of algae found in caves are *Chlorophyta* (green algae), *Chrysophyta, Bacillariophyta* (diatoms) and *Xanthophyta*, and some studies also report the presence of *Charophyta, Ochrophyta* and *Rhodophyta* (VAN VUUREN *et al.*, 2019). A zoning of algal groups along a cave is often notable, such as a greater diversity of diatoms relative to green algae in parts further away from the cave entrance, while the population of *Xan-*



Figure 5. Phototrophic community (algae, cyanobacteria and bryophytes) in the entrance and penumbra zone of the caves: **A)** Terra Ronca II, São Domingos, Goiás; **B)** Angelica, São Domingos, Goiás; **C) and F)** Caverna Santana, Iporanga, São Paulo; **D)** Caverna BRU_0041, São Gonçalo do Rio Below, Minas Gerais; **H)** Caverna do Diabo, Eldorado, São Paulo; **I)** Gruta do Sobradinho, São Thomé das Letras, Minas Gerais. Lampeflora is common in caves that have artificial lighting, as in: **E)** Caverna do Diabo, Eldorado, São Paulo; **G)** Lamprechtshöhle, Salzburg, Austria. Photos: A-C, E-I (Caio Cesar Pires de Paula); D (Robson de Almeida Zampaulo).

thophyta gradually decreases with distance from the entrance zone (VINOGRADOVA *et al.*, 2009). Studies carried out in Brazil report the presence of algae in cave areas with incidence of sunlight, colonizing the aquatic environment and rocky surfaces (PROUS *et al.*, 2015; SCHEFFLER *et al.*, 2019). Despite the reports cited above, only one published study presents a list of algae species — those found in Lagoa Misteriosa Cave, in the state of Mato Grosso do Sul (SCHEFFLER *et al.*, 2019). This submerged cave has an algal bloom during the dry season, predominantly composed of *Aulacoseira* sp., *Coelastrum* sp. and *Tetrastrum triangulare*. During the rainy season, with lower water turbidity and lower phytoplankton density, the predominance of *Aulacoseira* sp. remains, however, the genera *Peridinium* and *Oscillatoria* can also be found during this period. The predominance of *Aulacoseira* sp. in this community can be explained by its tolerance to high turbidity, as occurs in the dry season, and low alkalinity (pH between 7 and 8.5), conditions found in this cave. After a chronological series following algal bloom events in this cave, the researchers highlighted the main genera responsible for the increase in water turbidity, including *Aulacoseira, Tetrastrum, Peridinium, Oscillatoria, Coleastrum, Closterium* and *Euastrum*.

Human Interference

Caves are places with high heritage value due to their natural and cultural characteristics (prehistoric paintings and engravings). Many of these caves have already been modified in order to facilitate tourist activities and all changes cause disturbances to microclimatic parameters and preexisting biological communities. In caves equipped with artificial lighting, it is possible to observe the colonization of the illuminated areas by algae and cyanobacteria and, in many cases, bryophytes and ferns can also be found (Figure 5). This community, called lampenflora, is composed mainly of terrestrial aerophytic algae of rapid reproduction and has lower diversity than the phototrophic communities present at the entrance of the caves. The concentration of chlorophyll in areas colonized by lampenflora is almost double compared to areas colonized by algae in the entrance zone (MULEC *et al.*, 2008). Higher temperatures around artificial lighting points stimulate the rapid growth of algae, which can colonize these environments even with 1 mmol photon/m^2/s or less (ENGEL *et al.*, 2010). Some of these algae can also easily adopt photoheterotrophic or fully heterotrophic metabolic pathways, instead of photoautotrophic metabolism, due to the absence or insufficient amount of light. Lampenflora can be observed in several caves in Brazil that have an internal lighting system, such as Caverna do Diabo, in the state of São Paulo.

This invasive community, a consequence of human interference with the use of artificial lighting, generates many concerns not only from an ecological point of view, but also in relation to the preservation of cultural heritage. Some algae, as well as other microbial groups, are involved in the process of physical-chemical deterioration of the rock surface, such as the excretion of acids. At the same time, lampenflora can be a threat to prehistoric paintings, as the proliferation of algae and cyanobacteria can cover these historical records with a microbial biomass and/or cause these paintings to deteriorate in a process of depigmentation of the rocky surface. One of the best-known examples occurred in Lascaux Cave (Dordogne, France), which was closed to the public in 1963 due to the proliferation of photosynthetic microorganisms that threatened unique prehistoric paintings dating back 17,000 years. This algal and cyanobacterial *bloom* became known as the "green disease" and the phenomenon was attributed to the alteration of the cave environment due to excessive tourist exploitation inducing radical changes among the biotic and abiotic factors of this ecosystem (BORDERIE *et al.*, 2015).

Faced with the proliferation of lampenflora in some tourist caves, some physical and chemical methods were tested in order to remove these communities from the cave environment. Among the physical processes are the direct removal of green biomass and the use of high-pressure water. These methods did not prove to be effective, because with their application, the lampenflora biomass can be more easily dispersed throughout the cave and damage fragile structures of the speleothems (MULEC; KOSI, 2009). Some chemical agents have been proposed to eliminate lampenflora, such as the use of formaldehyde (CH_2O), herbicides such as simazine and atrazine, sodium hypochlorite (NaClO) and hydrogen peroxide (H_2O_2). Although these chemical agents are efficient in removing lampenflora, another problem is created due to contamination of the subterranean environment by chemical residues. The less aggressive and recommended method is the use of light-emitting diodes, known as LED lamps, which emit a wavelength of 595 nm, which corresponds to a portion of the spectrum of light that is less active for the photosynthetic process. The use of presence sensors on the walkways, in order to

control and reduce the lighting time, together with good management of limiting the capacity of visits to the caves, can also be more effective and less harmful means to seek to reduce the impacts caused by lampenflora in the cave environment (BORDERIE *et al.*, 2015).

Relevance of microorganisms in caves

In view of the above sections, the great microbial diversity in cave environments is remarkable. Studies of microbial communities in caves reveal great biodiversity, which, in a way, can be considered a paradox for the ecological principles of competitive exclusion, which determines that two species cannot coexist if they use the same source of nutrients, which in this case is limited. However, due to the limitation of nutrients, which are usually present as chemically complex compounds, the metabolic activities necessary for the growth of microorganisms require high amounts of energy (BARTON; JURADO, 2007). Instead of competing for nutrients, microorganisms can use a strategy to overcome this energy limitation, which is the formation of different types of biofilms (with different microbial groups), where cooperation between microorganisms or even mutualistic relationships occur. These interactions, for example, may explain the difficulty of cultivating cave microorganisms under laboratory conditions, since the growth of some strains depends on interspecific interactions.

The entire microbial community in caves is involved in the trophic network of this environment through autotrophic or heterotrophic processes. Two types of food chains can be found in the aphotic areas of caves: those based on detritivores (dependent on organic matter of animal or plant origin) and those based on chemoautotrophic bacteria. This type of trophic relationship contrasts with food chains of surface environments that are supported by photoautotrophic primary producers. This trophic network supported by photoautotrophs is present in cave areas with direct or indirect light, such as entrance and twilight zones. However, discovery of the chlorophylls Chl*f* and Chl*d* showed that algae and cyanobacteria (such as *Leptolyngbya*) can use infrared radiation (700–780 nm) to carry out photosynthesis in caves (BEHRENDT *et al.*, 2020). Surprisingly, the deepest parts of caves still have infrared radiation, a consequence of the reflectance of specific wavelengths by some components of the rocky surface, such as limestone. The concentration of Chl*f* and Chl*d* is favored in environments with low luminosity or without light and that still receive infrared radiation. This allows the coexistence of some microorganisms, such as endolithic algae and certain groups of cyanobacteria, in the same niche as detritivorous and fast-growing microbial groups. However, future research is needed to evaluate and discuss the role of these photoautotrophic microorganisms in the deeper regions of caves, their distribution patterns and their adaptive mechanisms to survive in these conditions.

Detritus-based food webs are a trophic relationship found in all cave environments. This trophic relationship allows recalcitrant organic matter, carried to the subterranean environment, to be decomposed into less complex organic compounds and made available to other organisms. At the same time, the microbial biomass that assimilates the compounds during the decomposition process is an important source of nutrients for springtails, isopods, millipedes and protozoa, among other organisms (PAULA *et al.*, 2020). In turn, caves with trophic networks based on chemoautotrophy are supported by microorganisms that acquire energy through the oxidation of inorganic compounds, including sulfide (ENGEL *et al.*, 2010), methane (BENDIA *et al.*, 2020), ammonium (MARQUES *et al.*, 2018), iron (PARKER *et al.*, 2018) and manganese (NORTHUP *et al.*, 2003). The use of metagenomic analysis at the Kartchner Caves identified more than 365,000 gene fragments in microbial populations and demonstrated a large portion of genes involved in the chemoautotrophic fixation of CO_2 (ORTIZ *et al.*, 2014). This energy obtained via chemoautotrophy is then used to transform carbon dioxide or bicarbonate into microbial biomass that in turn supports the ecosystem's primary productivity and sustains the cave's food web.

In addition to the trophic relationship between microorganisms and cave fauna, the microbial community plays a key role in nutrient cycling through the decomposition of detritus. A study carried out in the caves of Parque Estadual de Terra Ronca (Goiás) demonstrated how microbial communities directly influence the availability of nutrients. The reduction in microbial biomass results in greater mineralization of nutrients while an increase in microbial biomass indicates greater immobilization of nutrients (PAULA *et al.*, 2020). Therefore, the dynamics of microbial biomass in the cave environment can be a useful parameter to indicate the trophic condition of that ecosystem, that is, to monitor how limiting that environment is in relation to the

availability of nutrients.

Another important aspect is related to the formation of caves, or speleogenesis, which can occur through different processes, including the chemical dissolution of rocks. However, it is already known that microbial communities also play an important role in speleogenesis, either releasing some acids that act in the chemical dissolution process or reducing ores such as iron, as previously described about the caves of the Quadrilátero Ferrífero (PARKER *et al.*, 2018). By interacting with minerals, microorganisms play an important role in the formation of caves, participating in the formation of structures such as stalactites, stalagmites and accumulation of metallic oxides by microbial activity. Some microorganisms precipitate $CaCO_3$ on the surface of their cells, contributing to limestone formation. As previously described, Paleolithic paintings present in the subterranean environment are also subject to microbial colonization and activity, which also poses a threat to cave paintings in these environments. The depigmentation or deterioration of the rocky surface where these paintings are located are processes that damage these historical records. For this reason, many researchers focus their efforts on characterizing the natural microbial communities of these environments, in order to develop effective and efficient methods to preserve natural biodiversity and prevent colonization by invasive microorganisms, the main agents that threaten the preservation of such archaeological records.

The study of cave microbiota is also of great importance for the advancement of astrobiology in the search for life on other planets. The exploration of Mars, for example, is one of the most promising future prospects in the space area and the environmental conditions of some areas of Mars are very similar to the conditions of some caves, such as high pH, high concentrations of minerals and CO_2 and low concentrations of organic compounds. The discovery of caves on Mars opened new perspectives for the search for biosignatures as refuges of life on the red planet (LÉVEILLÉ; DATTA, 2010). Another discovery on the planet Venus reported a high concentration of phosphine in its atmosphere (GREAVES *et al.*, 2020). Phosphine can be produced by anaerobic microorganisms under extreme conditions, such as swamps and subterranean environments. Therefore, we may be able find microorganisms that have this metabolic pathway in our ecosystems. The temperature in the atmosphere of Venus is around 50°C, and so to explain a possible biological origin of phosphine on Venus, researchers have concentrated their search for phosphine-producing microorganisms in environments that have this temperature range. Other studies have demonstrated range of microorganisms in caves that have a unique metabolisms under extreme conditions. A study carried out in Monte Cristo Cave (Minas Gerais) showed that the anaerobic oxidation of methane was carried out by the microorganism *Methylomirabilis* sp. (BENDIA *et al.*, 2020). This discovery could be a promising model to look for signs of life on other planets, bringing together two important aspects that may be key elements for the presence of life outside Earth — methane consumption by microorganisms under anaerobic conditions and occurrence in the subterranean environment.

Finally, the subterranean environment has great unexplored biodiversity. Every year new species of microorganisms are described in cave environments, many of which have enormous biotechnological potential (ZADA *et al.*, 2021). The discovery of new microbial species in this environment makes it possible to search for new metabolic pathways and new by-products, such as enzymes, antibiotics and antioxidants, among other compounds. A new species of *Aspergillus* isolated in Gruta do Catão showed high cellulolytic activity with lower protein production, which demonstrates the greater efficiency of this enzymatic class (PAULA *et al.*, 2019). In caves of the Quadrilátero Ferrífero, microorganisms showed resistance to antifungal compounds and increased enzymatic activity of peroxidases and superoxide dismutase (TAYLOR *et al.*, 2017). Gallic acid production and antioxidant activity have also been reported for microorganisms isolated in Brazilian caves (TAVARES *et al.*, 2018), while the production of antimicrobial compounds by microorganisms in caves is frequently reported, mainly for bacteria of the *Actinobacteria* group (GHOSH). *et al.*, 2017).

Despite efforts in several areas of science to characterize microorganisms in the subterranean environment, Brazilian legislation does not cite in any of its regulations the use of microbial studies and research as a tool to assist in the monitoring, use and conservation of caves. Even in the face of several studies demonstrating the environmental and economic importance of microbial communities in caves, investment in research that covers the scope of subterranean microbiology has been scarce and neglected. If microorganisms in caves were perceived as key organisms for the maintenance and preservation of

the subterranean environment and fundamental for the maintenance of ecosystem services for society or were seen as a range of possibilities for new treatments of lethal diseases, or even as a possible source of enzymes and biomolecules for national industry, then governments, environmental agencies and society would be more willing to discuss the conservation and management of the microbial communities in these environments. The simplest way to start discussing the importance of microorganisms in cave ecosystems is to carry out more research on microbial diversity and its functional role, as well as discussing the impacts and protective measures for these communities in Brazilian caves. Only from this information will the complex role of microbial communities in caves be understood and how anthropogenic impacts on these communities and, consequently, on the functioning of the subterranean environment, will affect society.

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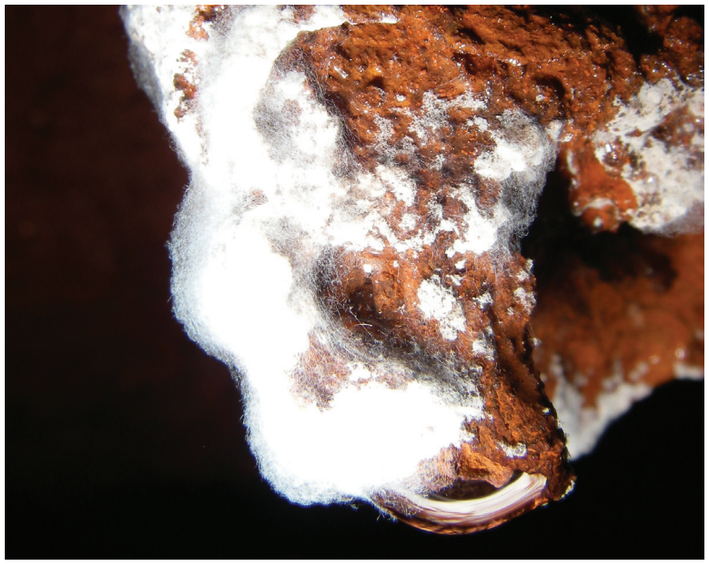
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5

Molecular tools for the study of cave fauna

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General introduction

The use of taxonomy for characterization of cave fauna is a difficult and inefficient process because it requires meticulous work with a scarcity of informative characters and a limited number of specialists, especially in the case of troglobitic invertebrates. On the other hand, recent advances in new technologies for genetic and genomic analyses bring significant progress to help characterize species. Molecular technologies allow the evaluation of specimens at different stages of development or sexes and even incomplete specimens deposited in scientific collections (STOEV *et al.*, 2013).

Molecular studies allow for assessing diversity at different levels of resolution or scope. At the first level are DNA barcodes, usually based on the genetic marker of the mitochondrial gene for the enzyme cytochrome oxidase 1 (COI or Cox1) (KRESS; ERICKSON, 2008) when the interest is to evaluate specimens of fauna genetically. Despite being widely used for the molecular characterization of fauna, DNA barcodes often do not have sufficient resolution for species separation (HEBERT *et al.*, 2004; SHNEER, 2009). Another essential factor to consider when working with DNA barcodes is the existence of reference databases. However, there is a global effort to generate libraries of genetic references, including all species worldwide. BOLD Systems (http://www.boldsystems.org/), one of the leading public databases for barcodes, currently contains over ten million references.

At the second level, greater depth can be achieved through complete sequencing of the mitochondrial genome, which can generate a more specific genetic signature (BAPTESTE *et al.*, 2002; LEE *et al.*, 2015). Finally, the maximum resolution is reached when sequencing the nuclear genome of a species (RUSSELLO *et al.*, 2020; WRIGHT *et al.*, 2020). Through these genomic data, species can be diagnosed with very high resolution, besides determining population structure in the environment and identifying markers linked to local adaptations (LATCH, 2020). Another aspect of using genetic information is adding quantitative data to a typically descriptive science that enables large-scale data analysis in a digitized way (EDMUNDS *et al.*, 2013). The digital treatment of information allows the integration of molecular and morphological data when captured digitally (i.e., integrative taxonomy), in addition to comparing groups of taxonomically related specimens (DAVIS *et al.*, 2016; RIEDEL *et al.*, 2013). Therefore, this chapter will discuss, the main molecular approaches for describing cave fauna will be discussed with illustrative examples.

Characterization of genetic diversity

A wide variety of components of the genetic material of eukaryotic organisms can be used to characterize the diversity and determine the identity of species, or even of other taxonomic entities, such as genera, families and orders, for example (LEMEY *et al.*, 2009; RENEKER). *et al.*, 2012; SEGATTO *et al.*, 2017). The main characteristic considered in determining which genomic region can be used for this purpose is the expected or observed level of variation. The most conserved regions, in order words, with less variability among individuals, are more informative for differentiating and grouping higher taxonomic levels (*e.g.*, genera and families), as in the case of histone-coding genes and *Ultra Conserved Elements* (UCEs). These regions have been widely used in studies that seek to understand the evolutionary relationships among species (ERIVES, 2017; ESPOSITO *et al.*, 2015; LANGILLE *et al.*, 2021; MORRIS *et al.*, 2020; RENEKER *et al.*, 2012; ZHANG; *et al.*, 2019). On the other hand, genomic regions that do not encode proteins, such as the *Internal Transcribed Spacers* (ITSs) ITS1 and ITS2 of the 45S ribosomal DNA (rDNA) (composed of the 18S, 5.8S and 28S rRNA genes), tend to present higher levels of polymorphism, making them informative for analyses among closely related species or even for population studies (ÁLVAREZ; WENDEL, 2003; COLEMAN; VACQUIER, 2002; FELINER; ROSSELLÓ, 2007; HEEG; WOLF, 2015).

Considering the mitochondrial genomes (mitogenomes) of animal species, protein-coding genes (PCGs) can also show considerable variability. In these taxa, variation in the size of mitogenomes is relatively minor in size, commonly presenting 13–17 kbp (Figure 1), with uses from characterizing genetic diversity

to evolutionary studies and phylogenetic analyses (IRISARRI *et al.*, 2020). Often, often only a small portion of mitogenomes have been used to genetically characterize species, which may be complete or partial sequences of PCGs or rDNA. Among the regions available in public DNA sequence banks, such as BOLD Systems and GenBank (www.ncbi.nlm.nih.gov/genbank), the cytochrome c oxidase enzyme subunit 1 gene (COX1 or COI) is the most abundantly found. Although the complete gene has

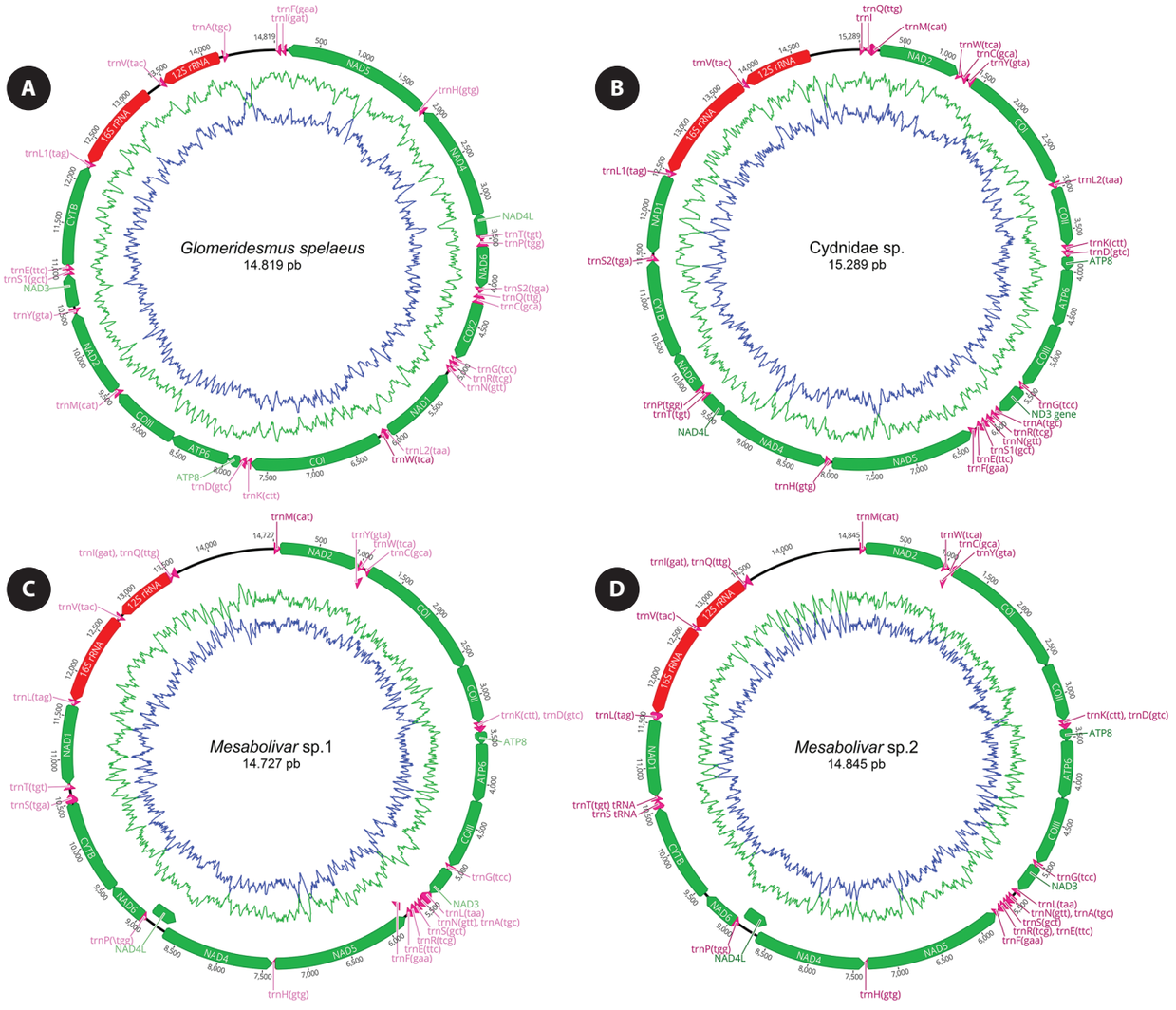


Figure 1. Circular structure of mitochondrial genomes (mitogenomas) of cave invertebrates from Serra dos Carajás, southeastern Pará. **A)** *Glomeridesmus spelaeus* (Myriapoda, Glomeridesmida, Glomeridesmidae); **B)** Cydnidae sp. (Insecta, Hemiptera); **C-D)** *Mesabolivar* sp.1 and sp.2 (Arachnida, Araneae, Pholcidae), respectively. The 13 protein-coding genes, two rRNA genes and 22 tRNA genes are represented in green, red and pink, respectively.

more than 1,500 bp, an internal region of approximately 700 bp is used as a DNA barcode (DNA barcode) to genetically characterize animal species (HEBERT *et al.*, 2003; RATNASINGHAM; HEBERT, 2013). In addition to COX1, animal mitogenomes are generally composed of 12 other PCGs (ATP6, ATP8, COX2, COX3, CYTB, NAD1, NAD2, NAD3, NAD4, NAD4L, NAD5 and NAD6), two rRNA genes (12S and 16S) and 22 transfer RNA (tRNA) genes (BOORE, 1999).

Although COX1 sequences are available for many taxonomic groups, the representation of animal diversity in genetic databases is still very low, especially for megadiverse regions such as tropical America. Of the more than 1,325,000 cataloged species of arthropods for the planet (www.gbif.org), only about 265,000 species (20%) have at least one specimen with a DNA sequence deposited in BOLD Systems. Of this total of species with DNA barcodes, approximately 4,200 (1.9%) occur in Brazil, representing only 0.6% of the total arthropod species (ca. 660,000) recorded for the country until January 2022 (http://www.boldsystems.org).

The scenario is even less favorable for species from relatively less-known ecosystems, such as Brazilian cave environments, which still lack genetic and phylogenetic diversity studies. Although only about 150 troglobitic species have been formally described for Brazil to date (GALLÃO; BICHUETTE, 2018), DNA sequences are available in BOLD Systems for only seven: two of Decapoda (*Aegla cavernicola* Türkay, 1972 and *A. leptochela* Bond-Buckup & Buckup, 1994), two of Opiliones (*Pachylospeleus strinatii* Šilhavý, 1974 and *Relictopiolus galadriel* Pérez-González, Monte & Bichuette, 2017), two of Scorpiones (*Troglorhopalurus lacrau* (Lourenco & Pinto-da-Rocha, 1997) and (*T. translucidus* Lourenço Baptista & Giupponi, 2004) and one of Glomeridesmide (*Glomeridesmus spelaeus* Iniesta & Wesener 2012). Thus, generating large-scale genetic references for cave invertebrates is essential to assist in the characterization of the large number of currently-recognized biological entities that have yet to be formally described (GALLÃO; BICHUETTE, 2018). In addition, this study can help to identify possible events of cryptic speciation and adaptive convergence in cave ecosystems, which are known to have excellent ecological stability (HOWARTH; MOLDOVAN, 2018). In the case of the leafhopper *Kinnapotiguara troglobia* (Hoch & Ferreira 2013) (Kinnaridae), for example, there is evidence of population isolation and cryptic speciation between caves located along the Apodi River basin in the state of Rio Grande do Norte (VASCONCELOS *et al.*, 2019). Such data were obtained in a study that was developed in partnership among Instituto Tecnológico Vale (ITV), Universidade Federal do Rio Grande do Norte (UFRN), Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and Universidade Federal de Lavras (UFLA).

In addition to DNA barcodes based on COX1 sequences, more robust genetic references have been made available for species associated with ferruginous caves in Serra dos Carajás in the southeast of the state of Pará (Eastern Amazon), such as the complete mitochondrial genome sequence of the troglobitic millipede *G. spelaeus* (Glomeridesmida) (NUNES *et al.*, 2020) (Figure 1A). Additionally, the genetic variability observed in mitochondrial genomes has helped in the identification and characterization of taxa not yet formally described, as in the case of two new species of spiders of the genus *Mesabolivar* (Pholcidae) (OLIVEIRA *et al.*, 2019) and a stink bug species of the family Cydnidae (DIAS *et al.*, 2019), all also from caves in Serra dos Carajás (Figure 1B-D).

Genomics

Cave ecosystems present a unique opportunity to study organisms existing in habitats and conditions perhaps analogous to those on our planet many millions of years ago. The particular adaptations and evolutionary processes that gave rise to extremophiles (i.e., those adapted to environments with extreme geochemical conditions), such as some cave organisms, allow us to examine questions related to the origin and early evolution of life on Earth and related applications (BONILLA-ROSSO *et al.*, 2012; CHRISTIN *et al.*, 2010; PROTAS *et al.*, 2011).

Many cave species are threatened with extinction. Factors that contribute to the risk of extinction of these animals include high endemism and rarity of some species, human interference, issues of water quantity and quality, urbanization, deforestation, floods, rainwater drainage, erosion and waste disposal (CARDOSO *et al.* al., 2021; CULVER; PIPAN, 2019; PELLEGRINI *et al.*, 2016).

Molecular tools, such as DNA sequencing, are important for the genetic inventory of cave fauna, in addition to the possibility of helping to address issues related to complexes of cryptic species that are widespread in these environments (NEIBER *et al.*, 2011). Likewise, morphological differences between the life stages of a cave-dwelling species are common, especially for

invertebrates and, in many cases, pose important challenges for taxonomic identification and classification. This is especially common with rare or poorly studied species.

Molecular tools generate vast amounts of genomic data that can be used to investigate rates of evolution, diversification and speciation in cave fauna, as well as allow the investigation of population structure and gene flow patterns at unprecedented resolution (LEYS *et al.*, 2003; MCCORMACK *et al.*, 2013). One method that can be applied to fine-scale phylogeography studies to evaluate species populations is restriction site-associated DNA sequencing (RAD-Seq). This methodology that allows the sequencing, identification and use of thousands of genetic markers, such as Single Nucleotide Polymorphisms (SNPs), distributed among hundreds or thousands of loci (MCCORMACK *et al.*, 2013). DNA sequencing associated with restriction sites reduces the complexity of the genome to be investigated by using restriction enzymes, which allows for less-costly genomic analyses without the need to have complete nuclear genome assemblies (TOONEN *et al.*, 2013). Verovnik *et al.*, (2004) also used molecular data from the mitogenome of the crustacean *Asellus aquaticus* (Linnaeus, 1758) (Isopoda), in combination with paleogeographic information, to reveal possible scenarios of the hydrological history of Dinaric karst in the Balkan Peninsula.

RNA sequencing provides essential biological information for evolutionary studies of cave biota. RNA-Seq refers to high-throughput sequencing of RNA from a specific tissue or organism at a given time (DE WIT *et al.*, 2012). The technique is based on reverse transcription of the extracted RNA to cDNA, followed by high-throughput sequencing by a *Next Generation Sequencing* (NGS) platform (e.g., Illumina and PacBio) and subsequent *de novo* assembly of the sequenced reads or alignment of the produced sequences on a reference genome (DE WIT *et al.*, 2012; ZHANG *et al.*, 2011). The resulting transcriptome assembly can then be characterized to identify transcripts expressed at that specific time in a tissue, organism and/or life stage (DE WIT *et al.*, 2012). Although it is purely descriptive, a characterized transcriptome provides a basis for constructing further so-called functional analyses. The characterized transcriptome set can be used as a reference and the original and additional sequenced reads — for other treatments, for example — can be aligned back together so that the set obtains quantitative data on gene expression and genetic variation. These data can be used in several applications, such as developing molecular markers and even the identification of events associated with speciation processes.

The combination of speleogenomics with speleotranscriptomics allows the strategies and mechanisms of adaptation to extreme environments to be elucidated, as well as the assessment of the prevalence of molecular convergence and the patterns it can follow in cave environments, where strong selective pressures can lead to homologous mechanisms of genetic adaptation in different taxa. Bats, for example, have been used as models for exploring omics in cave species and are targets of a worldwide consortium called Bat1K (http://bat1k.com).

Bat genomics

Understanding bat biology is important not only for understanding human health, but also for maintaining and improving the health of ecosystems. Bats contribute extensively to ecosystem functions, such as the regeneration of tropical forests, (KUNZ *et al.*, 2011). They play a unique role in dispersing pollen between plants and seeds over large distances, maintaining plant genetic diversity and assisting in the regeneration of forests after deforestation. They also act in insect control, feeding on many species considered pests and disease vectors (MAINE; BOYLES, 2015). Insectivorous bats are responsible for a savings of approximately one billion dollars a year for the corn production chain in the United States, just by preventing grain losses due to consumption by pests (MAINE; BOYLES, 2015). Bat genomes will help elucidate the genetic adaptations that allow bats to detect flowering plants and act as pollinators. Additionally, such data will provide basic genomic resources and tools for future population-level analysis to investigate and understand the relationships between their sometimes-threatened populations and the ecosystems on which they depend.

Therefore, the Bat1K consortium aims to is to sequence and assemble genomes at the chromosomal level and make data for all living species of bats publicly available. The actual number of species that can be envisioned is unknown. However, the estimate of Bat1K is approximately 1,300 species. The Bat1K genome consortium brings together expert bat biologists,

computer scientists, conservationists, geneticists, and individuals interested and committed to a better understanding of the evolutionary mechanisms behind the unique adaptations of bats.

Knowledge about the rare bat species *Furipterus horrens* (F. Cuvier, 1828), *Natalus macrourus* (Gervais, 1856) and *Lonchorhina aurita* Tomes, 1863 is the current focus of genomic studies at Instituto Tecnológico Vale (ITV; Figure 2). We at ITV seek to make a broad study of the complete nuclear genomes and transcriptomes of different tissues to understand their genetics and physiology, based on inferences from NGS sequencing data. Genomic sequencing work, in general, starts based on an estimate of nuclear DNA content and karyotyping of the species. However, especially for tropical species, these data are not always available. Table 1 indicates the genome size values available in the literature. The information is incomplete and unknown data will be approximated with data from other species until we can generate or capture new information from the literature.

Genomes assembled from short reads by NGS sequencing often lead to highly fragmented assemblies. In the case of the *Lonchorhina aurita,* for example, evaluation of the completeness of the assembly by the "single-copy BUSCO" (S) parameter captures only approximately 14% of the genes expected for the species. Complementarily, the transcriptomes of the three species were studied and a higher percentage of complete mammalian orthologous genes were recovered in the assemblies, when compared to published assemblies of transcriptomes for other bat species (LEE *et al.*, 2015; MORENO-SANTILLÁN *et al.*, 2019), indicating that the generated transcriptomes can be considered a good quality reference for the three bat species. A new version of the genomic studies of these species is underway, with greater sequencing depth and the use of technologies that allow the generation of long sequences that enable high-quality assembly. All assemblies will be made available in the public domain and must be part of the Bat1K consortium.

Although cave speleogenomic research has seen significant advances in recent decades, new and emerging genomic technologies have only begun to unravel the deepest mysteries of this dark world (FRIEDRICH, 2013; PÉREZ-MORENO *et al.*, 2016). Adopting these omics technologies will not only significantly expand the breadth of scientific questions that can be addressed, but also the depth to which they can be answered. Providing the knowledge and tools necessary to manage and conserve these intriguing and endangered habitats of unique biodiversity is of paramount importance for a broad understanding of cave fauna biology.

Table 1. Estimated size of bat genomes according to information available on the website http://genomesize.com/

	Natalus macrourus	***Furipterus horrens***	***Lonchorhina aurita***
Expected genome size (1C)	2.25 Gb (*Natalus* sp.)	Unknown	2.5 Gb
Diploid Number (2n)	Unknown	Unknown	32



Figure 2. Photos of bat specimens from which samples of biological material were obtained: **A)** *Lonchorhina aurita*; **B)** *Natalus macrourus;* **C)** *Furipterus horrens*. Photos: (Acervo Vale).

ENVIRONMENTAL DNA

Applying of approaches based on environmental DNA (eDNA; or metabarcoding) has become a key element in ecological studies (Figure 3). eDNA is a complex mixture of DNA fragments from different organisms, which may contain both intracellular and extracellular DNA, covering live and dead cells present in the sample. eDNA is all the DNA extracted directly from an environmental sample (soil, water, air, feces, roots, leaves, pollen and cellular tissues) without the need for prior isolation of a specific organism (THOMSEN; WILLERSLEV, 2015). TEcologists have widely used this approach to obtain taxonomic information from genetic data. Methods based on eDNA make it possible to detect and, in some cases, quantify the true occurrence and diversity of target species (rare, invasive, cryptic and threatened species) and multi-species targets (communities) on broad spatial and temporal scales (SIMMONS *et al.*, 2016; WANG *et al.*, 2019). Other advantages of eDNA sampling are simplicity, sensitivity and easy standardization, in addition to being independent of the life stage of the species to be detected. This method is also non-destructive and non-invasive and does not cause significant damage to the components of the sampled environment (ANTOGNAZZA *et al.*, 2019).

Technologies based on eDNA have gained space in monitoring and conservation of species, allowing complex biological questions to be answered quickly, efficiently and at a low cost beyond the necessary installed infrastructure (CAPORASO *et al.*, 2011; RUPPERT *et al.*, 2019). Conventional taxonomic identification methods are often limited by the need for more specialists on the taxonomic groups in question and the inherent difficulty in identifying and classifying small-sized organisms, rare species or deteriorated specimens. The eDNA approach has recently proved its enormous potential in biodiversity survey studies as it is not limited to analyzing a single specimen. This technique allows the simultaneous identification of multiple species based on NGS using universal molecular markers (HEBERT *et al.*, 2003; LYNGGAARD *et al.*, 2019).

In particular, the DNA metabarcoding technique has been

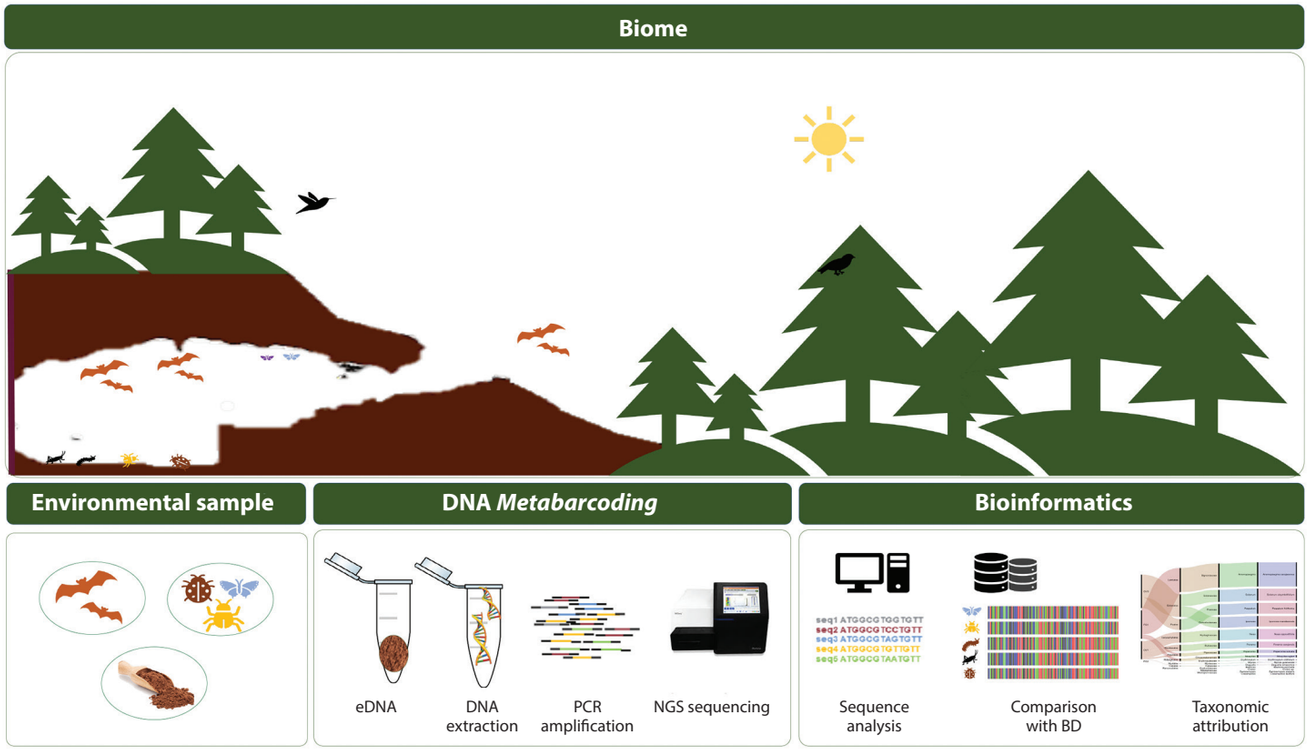


Figure 3. DNA metabarcoding approach, a technique that assesses species diversity from DNA extracted from environmental samples. The protocol follows the following steps: i) Collection of samples from the environment (soil, water, roots, guano, etc.); ii) DNA extraction - generation of multiple sequences from the amplification of molecular markers; and iii) NGS sequencing and bioinformatics analysis.

used in the detection of invasive, rare and cryptic species, in the reconstruction of plant and animal communities present in the past (EPP *et al.*, 2012; WANG *et al.*, 2019), in understanding relationships through the assessment of diets (AGUIAR *et al.*, 2021; GALAN *et al.*, 2018) and in studies of fauna (ANDERSEN *et al.*, 2012; COWART *et al.*, 2015) and flora (YOCCOZ *et al.*., 2012) in complex ecosystems.

For studies of caves and cave animals, the use of this approach has also been tested for the detection of aquatic animals including invertebrates and fish (BOYD *et al.*, 2020; DISTEFANO *et al.*, 2020; GORIČKI *et al.*, 2017; NIEMILLER *et al.*, 2017; NIEMILLER *et al.*, 2020; GORIČKI *et al.*, 2017; NIEMILLER *et al.* al., 2018; VÖRÖS *et al.*, 2017).

CAVE SUBSTRATE AND BAT GUANO AS A SOURCES OF eDNA

The successful application of DNA metabarcoding technology is directly associated with the availability of genetic references deposited in public databases, since DNA sequences obtained from environmental samples are compared with reference sequences previously made available in these databases (KING *et al.*, 2008). However, the diversity of taxa belonging to cave fauna in genetic databases still needs to be improved.

In order to deepen knowledge about the biodiversity of caves associated with ferruginous formations in Brazil, ITV has been implementing studies of faunal surveys of caves located in Serra dos Carajás (state of Pará), through the generation of genetic references and the application of DNA metabarcoding for monitoring species diversity. In this project, DNA traces are obtained from collected substrates (soil) to identify the groups of invertebrates that inhabit zones of differing luminosity incidence in the same cave or in different caves in the same area. DNA is extracted from environmental samples and amplified using specific molecular markers for invertebrate detection. Among the universal markers available, COX1 has been widely used for indirect eDNA detection of invertebrates (ELBRECHT *et al.*, 2019). Preliminary results showed that the ferruginous caves of Serra dos Carajás contain a wide diversity of invertebrates, with the detection of DNA mainly from members of the orders Acari, Araneae, Coleoptera, Collembola, Diptera and Hymenoptera. In terms of species, although the availability of genetic references is inconsistent due to the scarcity of records in databases, the technique allows identifying the diversity of faunal components and comparing areas or environments through diversity indices, thus responding to issues related to rarity and taxa sharing. These comparisons can be calculated using statistical indices such as the Chao1 richness index (which evaluates the total number of species observed in the community), the Shannon heterogeneity index (which evaluates the degree of variation of individuals of the species) and the Simpson dominance index (which reflects the probability that two individuals chosen at random in the community belong to the same species) (MORRIS *et al.*, 2014). Although, in many cases, it is not possible to identify taxa at more specific levels, such as species and genera, these parameters can show levels of similarity (or differentiation) between communities within or between evaluated caves, as well as the degree of rarity of certain organisms. These parameters are important for ecological studies and can help make decisions about environmental conservation and monitoring programs.

Studying the eating habits of bats is also extremely important for maintaining cave fauna. Bat feces (guano) constitutes an essential energy source for these ecosystems, since several organisms depend on this substrate to survive (FERREIRA, 2014; GNASPINI, 1992). Guano contains undigested food residues such as fruits, seeds and insects, for example, and serves both as a food source in caves (POMPANON *et al.*, 2012) and for seed dispersal in epigean environments. In addition to maintaining cave fauna, knowing the diet of bats is essential to understand their biology and plan conservation strategies (AIZPURUA *et al.*, 2018, GALAN *et al.*, 2018). The DNA metabarcoding approach makes it possible, for example, to estimate the diversity of insects and fruits ingested by bats through DNA present in guano robustly and accurately, since traditional morphological identification is practically impossible for these dietary components (AGUIAR *et al.*, 2021; BERRY *et al.*, 2017; GALAN *et al.*, 2018). Jordão (2019) applied the DNA metabarcoding technique to identify and quantify insects present in guano of bats from caves in the Caatinga, the Atlantic Forest and the Amazon. This was a pioneering work in Brazil, carried out in partnership between ITV and Universidade Federal de Pernambuco (UFPE), allowing the characterization of food items at different taxonomic levels (family, genus and species). In this work, DNA metabarcoding made it possible to correlate prey and predators at a specific level, showing that such an investigation strategy has enormous potential to contribute to the monitoring and managing of bat populations. In addition,

Aguiar *et al.* (2021) used the DNA metabarcoding technique to evaluate fecal DNA of insectivorous bats to investigate their diet in urban environments in the Distrito Federal. The authors found that the five insectivorous bats they studied consumed a total of 83 morphospecies of arthropods and, among them, most were characterized as agricultural pests. Thus, the importance of bats that inhabit the vicinity of urban areas and agricultural fields, as valuable natural controllers of insects harmful to human populations, is evident.

Conclusions

The impact of genetic technologies, especially genomic approaches, for advances in studies of cave fauna in Brazil, and in the world, is clear. Integrating morphological information with molecular data radically increases the degree of certainty in species identification, in addition to enabling several other ecological and functional studies. It is necessary, however, to advance in the construction of genetic references for cave biota, since this corresponds to a set of organisms that still need to be studied. Therefore, the incorporation of new molecular technologies into the biota description process will contribute to the understanding not only of species diversity, but also in relation to their distribution, genetic differentiation and gene flow between populations.

In this context, the transition of studies to the digital sphere is crucial. In addition to the possibility that, over time, the degradation of specimens in scientific collections may occur, the transport and loan of specimens is a slow process and only sometimes possible. The availability of genetic data also makes it possible to establish technologies for the robust study of environmental DNA, and this approach can contribute significantly to at least carrying out preliminary assessments of cave biotas more quickly.

Thus, it is important that the scientific community that studies cave fauna have access to genomic technologies and incorporate them into their studies so that advances in the description of cave fauna and in the development of environmental conservation and management strategies proceed with greater speed and scientific robustness

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INVERTE

BRATES



Callidosoma cassiculophylla
Lucas Mendes Rabelo

6

Acari

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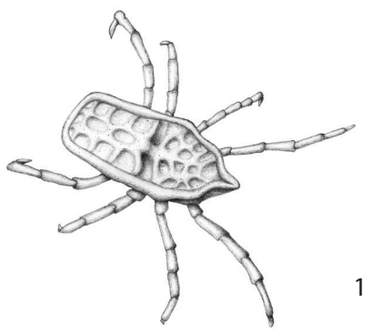
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General introduction

Mites are small arachnids that have shown great adaptive success and, in the course of their evolution, have developed morphological and behavioral specializations that have led to different modes of life, making them capable of colonizing virtually all known environments. They are easily found in very common places such as forest floors, among dust in human constructions, in nests, and on plants and other animals, in addition to habitats with extreme abiotic conditions, such as abyssal trenches (very deep portions of the ocean), permanently frozen regions, deserts, mountain tops and caves. The group includes free-living species — detritivores, phytophages, predators or those that feed on microorganisms — as well as parasites of vertebrates and invertebrates. Some species are relevant agricultural pests; disease vectors for humans, animals and plants; and even predators useful in the biological control of pests (KRANTZ, 2009; WALTER; PROCTOR, 2013).

Even though all these organisms are called mites, morphological analyses show little support for the subclass Acari as a monophyletic group, that is, sharing an exclusive common ancestor (SHULTZ, 2007). In these works, the two major groups of mites, Acariformes and Parasitiformes, are positioned differently in different hypotheses and may be more closely related to different orders of arachnids. Most studies that use molecular data, in turn, agree that Acariformes and Parasitiformes do not form a monophyletic group (ARRIBAS *et al.*, 2020; KLIMOV *et al.*, 2018; LOZANO-FERNANDEZ *et al.*, 2019; PEPATO *et al.*, 2019; PEPATO *et al.*, 2010; PEPATO; KLIMOV, 2015).

Regardless of their affinities, the two groups of mites diverged very early in Earth's geological history. Acariformes fossils appear in the Scottish Devonian (approximately 410 million years ago) (HIRST, 1923), whereas Parasitiformes appear only in the Cretaceous, in fossils contained in amber collected in the United States and Myanmar (approximately 100 million years ago) (DUNLOP *et al.*, 2020). However, three distantly related orders have already been recorded for the period: Opilioacarida, Mesostigmata and Ixodida (ticks) (DUNLOP; BERNARDI, 2014; OMID *et al.*, 2021; POINAR; BROWN, 2003). Molecular data tell a long story about the two major groups. Arribas *et al.* (2020), for example, dated the divergence of Acariformes to about 455 million years ago, between the Ordovician and Cambrian; and that of Parasitiformes between the Carboniferous and the Permian, about 310 million years ago. Thus, the latter group would have a long history of diversification not yet revealed by the fossil record.

The main evolutionary novelty (apomorphy) reputed for mites is the subdivision of the body into a portion specialized for taking-in food, the gnathosoma, formed by the chelicera, the epistome and the fused coxae of the palps the idiosome, containing most of the internal organs, eyes, and legs. Body segmentation is vestigial in groups that diverged early in evolutionary history and absent in those more derived. However, the morphological descriptions of mites also include other terms to refer to the parts of the body, nomenclatures that are not unified and differ considerably among the large groups, generating serious difficulties for those who begin to study them.

Although the monophyly of the group Acari is almost devoid of support from molecular and morphological evidence, the groups Acariformes and Parasitiformes, mentioned by Lindquist *et al.* (2009) as superorders, each have robust support from molecular data and a long list of distinctive morphological characters (DUNLOP; ALBERTI, 2007). Both are very diverse groups, with 12,384 species of Parasitiformes and 42,233 species of Acariformes being reported, according to the most recently published exhaustive surveys, for a total of 54,617 species of mites (BEAULIEU *et al.*, 2011; SCHATZ *et al.*, 2011; WALTER *et al.*, 2011). However, the total estimated diversity of Acari must be much higher, reaching between 500,000 and 1,000,000 species (WALTER; PROCTOR, 2013; ZHANG, 2011). The current number of known species is, of course, an underestimate, and although there is a great effort to resolve taxonomic gaps and describe the large extant diversity, the knowledge we have is uneven among countries, environments and groups sampled (LI; ZHANG, 2016; LIU *et al.*, 2013).

Parasitiformes are subdivided into four orders: Opilioacarida, Holothyrida, Ixodida (ticks) and Mesostigmata. The Mesostig-

mata include an impressive diversity of soil mites, parasites associated with arthropods and various vertebrates, in addition to other habits, and currently comprise more than 110 families (BEAULIEU *et al.*, 2011; WALTER, 2013). The Acariformes are more diverse and are divided into the orders Trombidiformes and Sarcoptiformes, with more than 150 and 110 families, respectively (SCHATZ *et al.*, 2011; WALTER *et al.*, 2011; ZHANG *et al.*, 2011). The Sarcoptiformes traditionally include basal Acariformes taxa, known as "Endeostigmata", which, however, are not supported by molecular analyses (KLIMOV *et al.*, 2018; PEPATO; KLIMOV, 2015); nonetheless, here we provisionally maintain the tradition of including these among the Sarcoptiformes.

Although mites are very diverse in the world, with over 50,000 described species, which represents almost half of all known species of Arachnida, they also are one of the most neglected groups of arachnids, since the effort to fill knowledge gaps regarding species (called the Linnean Shortfall, that is, the deficit regarding the description of species and record of their occurrences) occurs very unevenly across studied localities and taxonomic groups. Historically, for example, there has been less attention given to free-living species, while those of medical, veterinary and agricultural relevance have been studied more intensively (FLECHTMANN *et al.*, 2015; PALLINI *et al.*, 2007).

In cave environments, mites are also abundant and frequent in the most diverse lithologies, and can be found associated with bodies of water, soil, guano and organic matter deposits, carcasses and even directly attached to the bodies of animals of several groups of vertebrates and invertebrates. The vast diversity of habitats occupied by mites is reflected in the habits and behavior of the species observed in the hypogean environment, with species that consume microorganisms or are decomposers, predators of other small invertebrates or parasites of vertebrates and invertebrates (BERNARDI *et al.*, 2009; BRUCKNER, 1995; CORPUZ-RAROS; LIT, 2015; MAŚLAK; BARCZYK, 2011; PELLEGRINI; FERREIRA, 2013; SKUBAŁA *et al.*, 2013). Due to this immense diversity, any inference about the role and importance of mites in the ecology of subterranean communities is only possible after identifying the individuals found in this environment, at least to the category of family.

Main groups found in Brazilian caves

Bernardi *et al.* (2009) reviewed the mites obtained in 131 caves in 15 Brazilian states and the information present in the literature regarding occurrences of the group in caves in Brazil and recorded a total of 49 distinct mite families. To these data, we add the publications of the last 12 years and the occurrences present in the acarological collection of the Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais (UFMG/AC) and the Coleção de Invertebrados Subterrâneos de Lavras do Centro de Estudo em Biologia Subterrânea da Universidade Federal de Lavras (ISLA/UFLA). Thus, we report in this chapter a total of 126 mite families for the cave environment, with 77 new occurrences, especially associated with ferruginous geosystems in the states of Pará and Minas Gerais (Table 1 and Table 2). This survey is certainly not exhaustive and is far from being an accurate representation of subterranean mite biodiversity for Brazil, but it represents the largest dataset ever compiled for the country to date. Thus, below we will make a short introduction to each of the groups.

SUPERORDER PARASITIFORMES

Parasitiformes contain about 12 thousand described species of mites (BEAULIEU *et al.*, 2011). They can be separated from the Acariformes by having stigmas (openings of the tracheal system) in the idiosome (four dorsolateral pairs or one ventrolateral pair); legs with free and generally mobile coxae; tarsi with a peripodomeric fissure (which surrounds the entire tarsus) associated with sensory organs (lyrifissures) that divide the tarsus into basal and distal portions; and a set of dense setae at the extremity of the dorsal portion of the tarsus of the first anterior pair of legs, among them solenidia, setae that appear hollow under the microscope, as they are filled with cytoplasm, that has a chemoreceptor function. The four orders that make up Parasiti-

formes have been found in samples collected in Brazilian caves, although the order Holothyrida is restricted to the North Region of the country (Table 1).

Order Opilioacarida

Opilioacarida is a poorly diversified group of mites, with significantly lower species richness than other orders, such as Mesostigmata and Ixodida. They are free-living organisms, found in the soil and considered omnivorous, feeding mainly on plant and animal remains. They are relatively large compared to other mites, reaching up to 3 mm. The group is cosmopolitan, with records for 26 countries, and has about 50 known species classified in 13 genera and a single family (Opilioacaridae) (ARAÚJO *et al.*, 2020; BEAULIEU *et al.*, 2011; BERNARDI *et al.*, 2020; WALTER; HARVEY, 2009).

Species of Opilioacarida possess a leathery cuticle, usually with bluish-purple spots and stripes, and can be separated from other Parasitiformes by some unique characters, such as the presence of four pairs of dorsolateral spiracles in adults and tritonymphs; the presence of sternal verrucae between the coxae of the second pair of legs; and females with an eversible ovipositor. They also have characters considered to be plesiomorphic ("primitive"), such as terminal claws on the palps, the presence of two or three ocelli, tritosternum formed by unfused sternapophyses and structures associated with the gnathosoma (rutelae), which aid in the ingestion of solid material (KLOMPEN; VÁZQUEZ, 2015; VAN DER HAMMEN, 1976; WALTER; PROCTOR, 1998).

Records in Brazil include four genera (*Amazonacarus, Brasilacarus, Caribeacarus* and *Neocarus*) and 11 described species. These are distributed in caves formed in carbonate, magmatic, ferric and siliciclastic rocks in the states of AL, BA, MG, PA, PE, PI, RJ, RN and SP (ARAÚJO *et al.*, 2018; BERNARDI *et al.*, 2013; BERNARDI; BORGES-FILHO, 2018; VÁZQUEZ; ARAÚJO; FERES, 2014, 2015).

Order Holothyrida

The order Holothyrida comprises of three families with 27 species described in 13 genera (BEAULIEU *et al.*, 2011; LEHTINEN, 1991). They are relatively large (2–7 mm) carcass-consuming mites (WALTER; PROCTOR, 1998). They are brown in color and strongly sclerotized, with a single domed shield covering the entire back, which gave the group its name (Greek: *holo*, entire and *thyr*, shield). The peritremes (in the case of the Holothyrida and Mesostigmata, grooves with microtubercles associated with the stigmas, the openings of the tracheal respiratory system) are wide and short, with the stigmas above legs III, and may present a pair of dorsal structures called Thon's organs and a pair of lateral eyespots. The tritosternum (the process that projects under the gnathosoma, considered to be derived from the sternum of the third body segment, hence the name) may be present and flagelliform or absent. The hypostome possesses corniculi, generally pointed projections associated with that structure (WALTER, 2009). Species of Holothyrida are often confused with those of Mesostigmata due to their similarity between the peritremes and body shape. A conspicuous feature that separates them is the presence of several setae in the anal valves of Holothyrida, while these plates are either bare or have at most two setae in Mesostigmata.

The occurrence of Holothyrida in Brazilian caves is quite restricted, with only one species being recorded, *Diplothyrus schubarti* Lehtinen, 1999 (Holothyrida: Neothyridae), for ferruginous caves located in the southwestern portion of the state of Pará (BERNARDI *et al.*, 2011), in addition to individuals not yet completely identified for the same region, and which are deposited in the acarological collection of CCT-UFMG.

Order Ixodida

Ticks, a popular name used to refer to organisms of the order Ixodida, constitute a group with more than 800 species described worldwide (BEAULIEU *et al.*, 2011). It consists of mites that in some life stages are blood-sucking parasites of vertebrates. Species of this order are cosmopolitan and found mainly in tropical and subtropical regions. As they are often linked to the transmission of diseases, such as Rocky Mountain spotted fever and Lyme disease, among others, they are of great interest to research groups in the areas of Parasitology and Public Health (BOWMAN; NUTTALL, 2008).

A distinguishing feature of Ixodida is the modification of the hypostome into a specialized organ capable of puncturing the tissues that form the skin and attaching itself to the host with a variable number of rows of backward-facing teeth in its ventral portion. In addition, part of the sensory structures of tarsus I, characteristic of all Parasitiformes, is organized in the Haller's organ (BOWMAN; NUTTALL, 2008).

The order Ixodida contains three families, two of them — Ixodidae and Argasidae — present in Brazilian territory,

Table 1. Occurrence of mite families of the order Parasitiformes in caves in Brazilian states.

Order	Suborder	Family	State(s) of occurrence
Opilioacarida		Opilioacaridae	AL, BA, MG, PA, PE, PI, RJ, RN, SP
Holothyrida		Neothyridae	PA
Ixodida		Argasidae	AM, AL, BA, CE, ES, GO, MG, MT, MS, PA, PE, PI, PR, RN, RO, SE, SP, TO
		Ixodidae	AM, AL, BA, CE, ES, GO, MG, MT, MS, PA, PE, PI, PR, RN, RO, SE, SP, TO
Mesostigmata	Monogynaspida	Ameroseiidae	BA, MG, PA
		Ascidae	CE, BA, MG, PA, PR, RN, SP
		Blattisociidae	MG, PA
		Cillibidae	MG
		Dermanyssidae	MG, PA
		Diathrophallidae	MT
		Digamasellidae	MG
		Discourellidae	SP
		Dinychidae	MG
		Euthrachytidae	MG
		Eviphididae	MG, PA, SP
		Heterozerconidae	BA, MG, PA, RJ
		Ixodorhynchidae	BA, MG , SP
		Laelapidae	AL, BA, CE, ES, GO, MG, MT, MS, PA, PE, PR, RJ, RN, SE, SP, TO
		Macrochelidae	AL, BA, CE, ES, GO, MG, MT, PA, PE, PI, PR, RJ, RN, SE, SP, TO
		Macronyssidae	AL, BA, CE, ES, MG, MT, PA, PE, PI, RJ, RN, SE, SP, TO
		Melicharidae	MG, PA
		Ologamasidae	GO, MG, SP, PA
		Oplitidae	MG, PA
		Otopheidomenidae	BA, MG, PA, RN
		Pachylaelapidae	MG
		Parasitidae	MG, PA, PR, SP
		Phytoseiidae	MG, PA, PR, SP
		Podocinidae	ES, CE, MG, RJ, PA, SP
		Polyaspidae	SP
		Rhodacaridae	BA, MG, SP, PA
		Spinturnicidae	CE, MG, PA

Order	Suborder	Family	State(s) of occurrence
Mesostigmata	Monogynaspida	Spelaeorhynchidae	MG, PA
		Thinozerconidae	PR
		Trachytidae	MG
		Trematuridae	MG, PA
		Trachyuropodidae	MG, PA
		Uroactiniidae	MG
		Uropodidae	AM, BA, CE, MG, MT, PR, SP, TO,PA
		Veigaiidae	BA, ES, MG, PA, SP
		Ichthyostomatogasteridae	MG, RN
	Sejina	Reginacharlottiidae	MG, BA, PA, PE, PI, RN
		Sejidae	MG, PR, RN, SP
		Asternoseiidae	MG, PA
	Trigynaspida	Celaenopsidae	PR
		Davacaridae	MG
		Diplogyniidae	MG, PA
		Fedrizziidae	PR
		Heterochthoniidae	AM
		Neotenogyniidae	BA, MG, RJ, PA
		Pyrosejidae	MG
		Schizogyniidae	MG

are found both in epigean environments and in caves. The two families can be easily separated by the position of the gnathosoma: anterior and easily visible dorsally in Ixodidae; anteroventral and therefore hidden by the anterior part of the idiosoma in dorsal view in Argasidae. In addition, the position of the opening of the tracheas is variable: in post-larval stages of Ixodidae, stigmatic plates are found behind coxa IV, while in Argasidae they are found between coxae III and IV. Finally, the cuticle in Ixodidae includes a podonotal shield (dorsal shield at the height of the insertion of the legs) in females and nymphs, and a holonotal shield (covering the entire body) in males, structures absent in Argasidae (hence the popular name of soft ticks). Cuticle sclerotization in Ixodidae can also include a row of small plates on the posterior margin of the body, the festoons.

Some species of ticks of the genus *Antricola* (Argasidae) have been found, thus far, exclusively in caves, with populations that reach thousands of individuals, occurring mainly in hot caves known as "*hot or bat caves*", more frequent in the North and Northeast regions of Brazil (ESTRADA-PEÑA *et al.*, 2004; LABRUNA *et al.*, 2008). The larvae are exclusive parasites of bats and carry out only one large blood transfer and spend all their time associated with their hosts. The post-larval forms (nymphs and adults) present mouthparts with non-functional atrophied structures (reduced hypostome and without teeth), with a morphology incompatible with the parasitic habit. Thus, it is believed that the post-larval stages of species of *Antricola* can feed on guano, or other types of food that are associated with the substrate where they are found in the cave environment, which would represent a unique behavior among ticks (RIBEIRO *et al.*, 2012).

The geographic distribution of species of the order Ixodida in Brazilian caves covers almost the entire national territory. Frequently, new occurrences are found and new species described from the hypogean environment (e.g., DANTAS-TORRES *et al.*, 2009, 2012; HENRIQUE-SIMÕES *et al.*, 2012; NAVA *et al.*, 2010). Occurrences of subterranean species of Argasidae and Ixodidae currently cover all Brazilian states, except for Acre, Roraima, Maranhão and Paraíba, probably due to the lack of collections or publication of occurrences.

Order Mesostigmata

Mesostigmata is the most diverse order of Parasitiformes, containing about 90% of the described species of the group (BEAULIEU *et al.*, 2011). Most species are free-living predators, although there are parasitic, detritivorous, saprophagous, mycophagous and phytophagous species (LINDQUIST *et al.*, 2009; WALTER; PROCTOR, 2013).

The order Mesostigmata can be separated from other Parasitiformes by the presence of the stigma (opening of the tracheal system) between legs II and III, tritosternum usually present and with a distinct and single base, and anal valves with one or no pairs of setae. As mentioned, people trying to identify mites for the first time confuse some more sclerotized Mesostigmata with Holothyrida, which can easily be separated by the number of setae of the anal plates.

Mesostigmata includes some of the most common families of cave-associated predatory mites, being found roaming freely on the ground or among organic matter. Among these, the families Macrochelidae (genus *Macrocheles*), Laelapidae (genera *Stratiolaelaps, Gaeolaelaps, Cosmolaelaps* and *Androlaelaps*), Podocinidae (genus *Podocinum*) and Ascidae (*Asca*) are especially abundant. Other families, such as Uropodidae, are not as frequent but can reach populations of thousands of individuals in some caves where fresh guano from bats is found (PELLEGRINI; FERREIRA, 2013).

Among parasites, the families Macronyssidae, Spinturnicidae and Spelaeorhynchidae stand out. Since because they are associated with vertebrates, especially bats, they may be abundant and diverse inhabitants of caves. Some large-bodied species of Macronyssidae stay associated with their hosts during part of their life cycle (RADOVSKY, 1967; RADOVSKY, 2010) and can also be found walking on the ground and on cave walls (shelters), where they are easily collected. The families Spinturnicidae and Spelaeorhynchidae are obligatorily associated with bats; thus, in general, it is necessary to collect their hosts to find them (RUDNICK, 1960; CONFALONIERI, 1976).

SUPERORDER ACARIFORMES

A characteristic that marks the boundary between Parasitiformes and Acariformes is the presence of a particular substance in the setae, actinopilin, which gives them birefringence, that is, different refractive indices according to the direction of light incidence, a feature absent in Parasitiformes (DUNLOP; ALBERTI, 2007).

Many other characteristics, in addition to the presence of actinopilin, also contribute to differentiating Acariformes from Parasitiformes, although many species do not present all of them simultaneously: stigmas, when present, are anterior to coxa II; larvae have an osmoregulatory organ, the epimeral or Claparède organ, which is succeeded in nymphs and adults by paired structures in the genital area — the genital acetabulum — that fulfill the same function in these stages and that is multiple in freshwater forms and, sometimes, spread across the venter; and coxae fused to the venter; palp lacking an apotele, that is, without a pre-tarsus or a segment distal to the tarsus and which bears claws, as seen in Parasitiformes.

Some families classified in this clade are common cave dwellers. As it includes of many detritivorous species that feed on microorganisms, it is widely represented in decomposing organic matter, sometimes with populations of thousands of organisms especially associated with guano. The family Acaridae and others of the suborder Oribatida are quite common in these situations (PELLEGRINI; FERREIRA, 2013). Trombidiformes include predatory species that are found on the floor of caves. This group also contains parasitic species and the Parasitengona cohort, whose adults and nymphs are common on the floor of caves and whose larvae are parasites of arthropods and cave vertebrates. Due to their size and red, yellow or white color, they can be easily detected and collected, even with naked eyes.

Order Trombidiformes

Trombidiformes are the largest and most diverse order within Acari with more than 25,800 described species. It comprises of the suborders Sphaerolichida and Prostigmata, the former comprising only two families, while the latter contains 146 (ZHANG *et al.*, 2011).

Trombidiformes mites differ from those of Sarcoptiformes and Endeostigmata by lacking rutelae (structures associated with the ingestion of solid material) and by bearing a reduced number of setae on the opisthosoma (the portion of the idiosome posterior to the legs). Species of Trombidiformes have two (or rarely three) pairs of setae in the first row of this region of the body. This condition may be obscured by the multiplication of the setae of the idiosoma, or hypertrichia, as in the case of adults and nymphs of Parasitengona, or by the loss of most setae, as in the family Halacaridae.

Table 2. Occurrence of mite families of the order Acariformes in caves in Brazilian states.

Order	Suborder	Family	State(s) of occurrence
Sarcoptiformes	Endeostigmata	Alycidae	MG
		Grandjeanicidae	MG
	Oribatida	Acaridae	AL, BA, CE, MG, PR, SP, PA
		Aphelacaridae	MG
		Camisiidae	MG
		Cepheidae	PR, SP
		Ceratokalummidae	MG
		Chetochelacaridae	MG, PA
		Damaeidae	MG
		Epilohmanniidae	MG, PR
		Eremaeidae	PA
		Galumnidae	CE, MG, PR, SP, PA
		Glycyphagidae	BA, MG, PR, SP
		Haplochtoniidae	BA
		Haplozetidae	PR, MG
		Hermanniidae	PR
		Heterochthoniidae	MG
		Histiostomatidae	BA, CE, ES, GO, MG, PA, MG, PR, RN, SE, SP, TO
		Liacaridae	MG
		Lohmanniidae	PA
		Microzetidae	MG
		Mochlozetidae	MG
		Mycobatidae	MG
		Nanhermannidae	MG
		Oribatulidae	PR, SP
		Oppiidae	PR, SP, MG
		Parakalummidae	MG
		Pheroliodidae	MG
		Phitiracaridae	PR, MG
		Pyroglyphidae	PR
		Rosensteiniidae	CE, MG
		Sarcoptidae	MG
		Scheloribatidae	MG
		Sphaerochthoniidae	MG
		Stelechobatidae	MG
		Trhypochthoniidae	MG, PA

Order	Suborder	Family	State(s) of occurrence
		Adamystidae	MG
		Anystidae	AL, BA, ES, MG, PE, PA
		Bdellidae	BA, ES, MG, SP, PR, PA
		Caeculidae	MG
		Calyptostomatidae	MG
		Camerobiidae	MG
		Cheyletidae	BA, MG, PA, PR
		Chyzeriidae	MG
		Cunaxidae	BA, ES, GO, MG, PI, RJ, PA
		Ereynetidae	MG, SP
		Erythraeidae	BA, ES, MG, RJ, SP, PA
		Erythracaridae	BA, ES, GO, MG, MS, MT, CE, RJ, RN, PA, PE, SE, SP
		Eupodidae	MG, PA
		Eutrombidiidae	MG, PA
		Halacaridae	RN
		Hydrodromidae	MG
		Iolinidae	MG
		Johnstonianidae	MG, PA
		Labidostomatidae	BA, CE, ES, MG, PA
Trombidiformes	Prostigmata	Leeuwenhoekiidae	MG, PA
		Limnesiidae	MG
		Microtrombididae	MG
		Neothrombiidae	MG, SP
		Neotrombidiidae	MG
		Penthalodidae	MG, PA
		Podothrombiidae	MG
		Rhagidiidae	BA, ES, GO, MG, PA, SP, RJ
		Scutacaridae	MG, PA
		Smarididae	MG, PR, PA, SP
		Stigmaeidae	MG
		Tarsonemidae	MG, PA
		Teneriffiidae	BA, GO, MG, MS, MT, TO
		Tenuipalpidae	MG
		Tetranychidae	MG, PA
		Trombellidae	MG
		Trombiculidae	BA, CE, ES, MG, MT, PR, RJ, SP, PA
		Trombidiidae	BA, CE, ES, GO, MG, RJ, SP, PA
		Tydeidae	MG, PI, PA, PR
	Sphaerolichida	Sphaerolichidae	MG, PA

Frequent characteristics in Trombidiformes include non-chelated chelicerae, that is, a reduction of the fixed digit, thus losing the pincer format. In this case, they may have the shape of a sickle, stylus, hook, etc. The presence of palps with the tarsus opposing a tibial spine, producing a structure similar to a chela, known in acarological jargon as "tumb-claw complex" is also common.

An important part of the diversity of Trombidiformes is associated with plants, and the occurrence of families such as Tenuipalpidae and Tetranychidae inside caves, as shown in Table 2, is considered accidental or associated with entry zones of natural subterranean cavities. The occurrence of freshwater mites, such as Halacaridae or Hydrachnidia, is interesting, as it shows the occupation of subterranean freshwater niches. Small predators of the families Bdellidae, Cunaxidae, Erythracaridae and Rhagidiidae are especially

Table 3. Species described from specimens collected in caves in Brazilian states

Ordem	Família	Gênero / Espécie
Opilioacarida	Opilioacaridae	*Caribeacarus brasiliensis* Bernardi, Silva, Zacarias, Klompen & Ferreira, 2013
		Neocarus caipora Bernardi, Klompen & Ferreira, 2014
		Neocarus coronatus Araújo & Feres, 2018
		Neocarus jonasi Araújo & Duarte, 2021
		Neocarus potiguar Bernardi, Zacarias & Ferreira, 2012
		Neocarus proteus Bernardi, Klompen, Zacarias & Ferreira, 2013
		Neocarus simmonsi Bernardi, Zampaulo & Oliveira, 2020
		Neocarus spelaion Bernardi & Borges-Filho, 2018
Mesostigmata	Ameroseiidae	*Ameroseius mineiro* Narita, Bernardi & Moraes, 2013
	Macronyssidae	*Chiasmanyssus cavernicola* Gomes-Almeida & Pepato, 2021
	Reginacharlottiidae	*Reginacharlottia brasiliensis* Walter, 2013
	Trachytidae	*Uroseius subterraneus* Conceição & Pepato, 2021
Ixodida	Argasidae	*Antricola guglielmonei* Estrada-Peña, Barros-Battesti & Venzal, 2004
		Antricola delacruzi Estrada-Peña, Barros-Battesti & Venzal, 2004
		Antricola inexpectata Estrada-Peña, Barros-Battesti & Venzal, 2004
		Nothoaspis amazoniensis Nava, Venzal & Labruna, 2010
		Ornithodoros cavernicola Dantas-Torres, Venzal & Labruna, 2012
		Ornithodoros rondonienses Labruna, Tessini, Camargo, Brandão, Ribeiro & Estrada-Peña, 2008
	Ixodidae	*Ixodes paranaenses* Barros-Battesti, Arzua, Pichorim & Keirans, 2003
Trombidiformes	Bdellidae	*Cyta troglodyta* Hernandes, 2011
	Erythraeidae	*Callidosoma cassiculophylla* Bernardi, Wohltmani, Lorenzon & Ferreira, 2017
		Lasioerythraeus jessicae Costa, Klompen, Bernardi, Gonçalves, Ribeiro & Pepato, 2019
		Leptus (*Leptus*) *flechtmanni* Bassini-Silva, Jacinavicius & Barros-Battesti, 2020
		Leptus sidorchukae Costa, Klompen, Bernardi, Gonçalves, Ribeiro & Pepato, 2019
	Microtrombidiidae	*Eutrombidium carajas* Noei & Sundic, 2020
	Neotrombidiidae	*Birjandtrombella minae* Noei, Sundic & Bernardi, 2022
		Birjandtrombella pataxo Noei, Sundic & Bernardi, 2022
	Teneriffiidae	*Neoteneriffiola xerophila* Bernardi, Pellegrini & Ferreira, 2012

abundant in these environments. In addition to these, families of the Parasitengona group, such as Erythraeidae, Smarididae Leeuwenhokiidae and Trombiculidae, are also easily found in the hypogean environment. As mentioned earlier, these mites have a life cycle that includes a parasitic larva of vertebrates (such as bats) and arthropods (such as cave crickets and harvestmen), free-living nymphs and adult predators. Free-living forms are relatively large predators that are frequent on cave floors and in other subterranean environments.

Order Sarcoptiformes

Species of Sarcoptiformes, unlike those of Trombidiformes, are able to feed on solid material, and include detritus-consuming species, which makes them potentially relevant in soil trophic chains. The order is divided into two suborders: Endeostigmata and Oribatida. The first, however, can not be a group that shares an exclusive common ancestor with the second. Phylogenetic studies on the relationships of Endeostigmata are still scarce, but in recent works, its species appear divided between lineages external to Sarcoptiformes and Trombidiformes, and lineages at the base of Sarcoptiformes (KLIMOV *et al.*, 2018; PEPATO; KLIMOV, 2015). Oribatida are considered monophyletic and include the Astigmata group, mites with a great diversity of habits, such as parasites (*e.g.*, associated with bats), occurrence in organic matter deposits (*e.g.*, litter, guano and carcasses) and even as phoretic (associated with invertebrates and vertebrates) (OCONNOR, 1994, 2009).

Principal troglomorphisms

Moseley (2009) considers caves as ecotones, that is, areas of transition between the subsoil — including the system of small cavities, fissures and diaclases in the rock matrix where the caves are inserted — and the surface. In caves, there is a gradient of biotic and abiotic factors between the surface and the subsoil, which allows the occurrence of species from these two environments. Therefore, in caves, we can find epigean (surface dwellers), endogeic (deep soil dwellers, mineral horizon), and edaphic (soil dwellers) species, in addition to those that are restricted to the subterranean environment, i.e., troglobites (DUCARME *et al.*, 2004).

Commonly, there are three common approaches to categorize mite species found in subterranean environments and to classify which are effectively restricted to them.

DETAILED SURVEYS

Initially, one should consider carrying out detailed surveys of the diversity found in subterranean environments and make comparisons with the diversity of the epigean environment, paying attention to differences in community composition. Establishing the nature of the association between mites and caves requires a detailed description of the diversity of litter, soil and subsoil that surround caves (SKUBAŁA *et al.*, 2013). Unfortunately, there is little knowledge about the fauna present in caves in Brazil. Knowledge about epigean, edaphic and endogeic mite fauna is also very incipient, which makes it difficult or impossible to classify mites as to their association with the hypogean environment. In old-world countries, where the science of subterranean biology has a long history, some studies have provided a better understanding of the dependence and interactions between mites and cave environment. Noteworthy among these studies is Ducarme *et al.* (2004), who compared ecological relationships between the fauna of two caves and the mineral horizon of the soil of three forest sites in Belgium. By comparison, it becomes evident that the proportion of predators, phoretic mites and primitive oribatids was higher in the evaluated caves. Likewise, basal lineages of Oribatida and Mesostigmata, also predators, are among the most common mites in Brazilian caves (Tables 1 and 2). However, there are no studies of this nature for Brazil, which would be an important approach for the ecological and evolutionary classification of the species found in the country. Only with the characterization of species present in each of the compartments that make up the ecosystems from the soil to the deepest hypogean environment will enough information be acquired to enable the correct characterization and categorization of the mites that colonize, and are possibly restricted to, each of these habitats.

PHYLOGENY

A second approach to classifying mites regarding their ecological and evolutionary status is to use prior knowledge of both the phylogeny and the anatomy of the group to try to identify morphological changes in the species found in the cave environment that represent specific adaptations favoring the colonization and establishment of populations in the subterranean environment. For this, it is important to compare closely related species that are present in the epigean environment, in order

to identify the occurrence of troglomorphisms. However, the main difficulty in using troglomorphisms as a tool to identify troglobitic species is to differentiate them from edaphic species that also have these characteristics (*e.g.*, depigmentation and eye reduction). Cave and deep soil environments are similar in many ways, making it possible for many lineages to colonize caves directly from endogeic or edaphic environments (DUCARME *et al.*, 2004). Both are dark and damp environments. However, caves are spacious (empty) with, in general, greater climatic stability, while spaces in soil are restricted to interstices where thermal amplitude and humidity variation tend to be higher (DUCARME *et al.*, 2004; ZACHARDA, 1978, 1982, 1994; ZACHARDA *et al*., 2011). It should be borne in mind that features considered troglomorphisms for several groups of vertebrates and invertebrates (such as eye loss and lack of pigmentation) were already present in the ancestors of several soil and litter mite lineages before they occupied the cave environment. Thus, troglomorphisms must be evaluated in the context of the phylogeny of the group. For example, in a family of epigean mites where all specimens are pigmented and have eyes (*e.g.*, Bdellidae, Trombiculidae, Opilioacarida), eye loss and lack of pigmentation may in fact be troglomorphisms. To date, the best supported troglomorphisms in mites are related to size (longer bodies and especially appendages), which seems advantageous in the wider spaces of caves compared to soil interstices, in addition to changes in sensory organ size (increase in length). Modifications considered as effective troglomorphisms have already been observed in members of the families Rhodacaridae, Parasitidae, Veigaiidae, Eupodidae, Tydeoidea, Brachychthoniidae, Opilioacaridae and, especially, Rhagidiidae (DUCARME *et al.*, 2004; ZACHARDA, 1978, 1982, 1994; ZACHARDA *et al.*, 2011). Probable troglomorphisms related to sensory organs can be found in some species of Rhagidiidae restricted to caves, such as the Belgian species *Poecilophysis spelaea* (Wankel, 1861). Compared with the superficial species *Hammenia macrostella* Zacharda, 1980 and *Shibaia longisensilla* (Shiba, 1969), *P. spelaea* has an oblique ragidial organ (solenidia, that is, chemoreceptor setae that, instead of being erect, are horizontal and inserted in superficial grooves) and filiform and long trichobothria (mechanoreceptor setae specialized in detecting vibrations), while in superficial species the ragidial organ is parallel and the trichobothria are clavate. Furthermore, troglomorphic Rhagidiidae have significantly larger and more elongated chelicerae when compared to soil-dwelling species (DUCARME *et al.*, 2004; ZACHARDA, 1978, 1982, 1994; ZACHARDA *et al.*, 2011).

MOLECULAR TOOLS

Finally, molecular tools can be used to test whether the patterns observed for cave mites correspond to models of known troglobitic species (selection of characters related to the environment, genetic drift, opportunities for dispersal, etc.) and, thus, to infer the possibility of restriction of the studied species in relation to the cave environment. In some situations, troglobitic species have developed a strong dependence on specific conditions found inside caves, which restricts dispersal and cmay act as a reproductive barrier among populations (*e.g.*, RIESCH *et al.*, 2011). Thus, the distribution of truly cave-dwelling species (troglobites) may be restricted to a particular karst region, a continuous rocky massif or a single cave system (SBORDONI *et al*., 2012). Therefore, caves function as islands, that is, regions of suitable habitat for cave species isolated from each other by large areas of inhospitable habitat (surface) (MAMMOLA, 2019).

In general, two main processes are involved in the emergence of cave mite species. The first is habitat fragmentation, where multiple populations are isolated in distinct caves and can evolve separately. The other is the founder effect: new caves are colonized by the stochastic arrival of immigrants that can generate new populations (*e.g.*, phoretic mites or parasites traveling on their hosts) that can later be isolated and evolve separately. Both cases lead to total or partial isolation of populations, which generates high genetic structuring (isolation). Through phylogeography and population genetics, it is possible to measure the gene flow between populations of different caves and between caves and the epigean environment. In this way, it is possible to assess the existing genetic structure between populations and, thus, make inferences about their isolation and speciation in a cave or a system (SBOORDONI *et al*., 2012). This, of course, is a simplification of models found in nature. In situations of a continuous karst area, caves can be connected by micro and mesocavities, allowing constant communication between populations and making isolation between caves difficult. It is important to point out that each species and underground system is unique and it is necessary to treat each situation with a multidisciplinary approach. Even so, in many cases, the use of molecular tools makes it possible to study each situation in depth and detect the degree of isolation of populations,

leading to a better understanding of the origin and diversity of cave species (MAMMOLA, 2019).

However, studies meant to test the condition of endemism of subterranean species (troglobites) through analysis of their genetic structuring are not common, even though the use of molecular methods has elucidated old and complex problems concerning cave biota, such as the biogeographic origin of species (e.g., VON RINTELEN *et al.*., 2012; JURADO-RIVERA *et al.*, 2017), genetic structuring (*e.g.*, CACCONE; SBORDON, 2001; MELEG *et al.*, 2015) or even the processes of speciation that led to the formation of a cave population (*e.g.*, CACCONE; SBORDON, 2001; MELEG *et al.*, 2015; RIESCH *et al.*, 2011).

Troglobite species diversity described for Brazil

The taxonomic study of cave mites is still incipient in Brazil, with few research groups dedicated exclusively to publishing works in this area. Until a few years ago, most reports of mites in caves in the country were made in lists of species generated for work aimed at studying communities or ecological issues. Usually, these works report morphotypes identified to the category of family and are not subject to validation, as they generally do not present information on the destination (collections) of the analyzed material, nor do they report the biological collection where specimens were deposited.

Even with this precarious knowledge generated thus far, studies that reviewed the occurrences of mites in Brazilian caves indicate a great diversity (BERNARDI *et al.*, 2009; PINTO-DA-ROCHA, 1995). However, there is a lack of targeted collections, where appropriate methods are used, in addition to the participation of taxonomists capable of identifying and describing new species. Despite all the challenges, recently some research groups have been consolidating themselves in the specific study of cave mites and have started generating results that have contributed to knowledge about the biology of this group of animals and its relationship with the hypogean environment, in addition to taxonomic studies with descriptions of new species. These initiatives have increased not only the number of species records but also general knowledge about cave mite fauna (*e.g.*, ARAÚJO *et al.*, 2018; BERNARDI *et al.*, 2012; BERNARDI *et al.*, 2013a, b; BERNARDI *et al.*, 2013a, b; BERNARDI *et al.*, 2014; DANTAS-TORRES *et al.*, 2012; ESTRADA-PEÑA *et al.*, 2004; HERNANDES *et al.*, 2011; NARITA *et al.*, 2013; NOEI; ŠUNDIĆ, 2020). Tables 1 and 2 summarize the occurrence of families reported in the literature and those deposited in the above-mentioned taxonomic collections while Figures 1 and 2 present some taxa found in caves.

For some orders, such as Opilioacarida (a little diversified group) and Ixodida (a group that is well studied because of its medical importance), there are active researchers who have been constantly publishing biological studies and species descriptions (*e.g.*, ARAÚJO *et al.*, 2018; BERNARDI *et al.*, 2013a, b; BERNARDI *et al.*, 2014; DANTAS-TORRES *et al.*, 2012; ESTRADA-PEÑA *et al.*, 2004). Few families, in addition to these two orders, have been the subject of more detailed studies that have resulted in species descriptions. Currently, only 28 species of mites are known described from material collected in caves (*e.g.* BERNARDI *et al.*, 2017; BERNARDI *et al.*, 2012; HERNARDI *et al.*, 2011; NARITA *et al.*, 2013; NOEI; ŠUNDIĆ, 2020), only one of which has characteristics that indicate restriction to the subterranean environment (troglomorphisms), *Leptus sidorchukae* Costa, Klompen, Bernardi, Gonçalves, Ribeiro, Pepato 2019, which may be the only troglobitic species described thus far (COSTA *et al.*, 2019) (Table 3 and Figure 3).

Identification key

An identification key for cave mites of Brazil, up to the cohort category, is presented as supplementary material to this chapter and is hosted at https://acarologia.wordpress.com/chaves-de-identificacao-dos-acaros-coletados-em-cavernas-do-brasil/. The key is designed for use by researchers not specialized in cave mites and so presents many figures for the recognition of the structures used in the step-by-step identification. The on-line format allows the material to be constantly updated and improved, facilitating its use by interested parties. The website also provides access to a training video on the main large groups of mites and other information that can help a novice researcher or a curious student initiate their studies on acarology.

As the list of mites observed in the subterranean environment presented in this chapter is based on an incipient sampling effort, the elaboration of a taxonomic key including the families already registered for Brazilian caves would present serious limitations and could lead to mistakes by researchers unfamiliar with mite taxonomy. Thus, it is recommended that the researchers interested in cave mites also seek more comprehensive works.

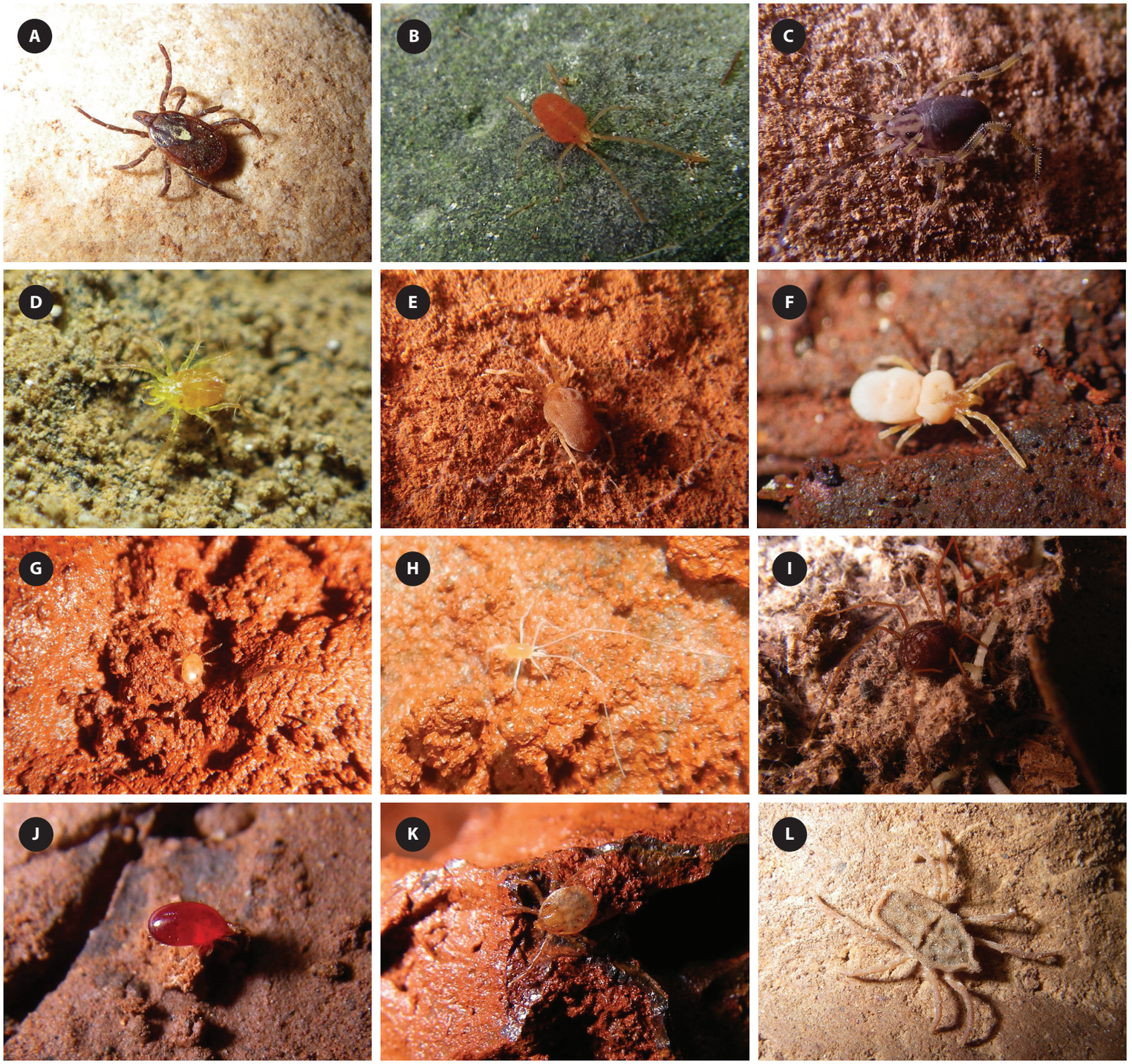


Figure 1. Specimens of mites found in Brazilian caves. **A)** *Amblyomma* sp. (Ixodida: Ixodidae); **B)** Smaridídae (Trombidiformes); **C)** *Neocarus* sp. (Opilioacarida: Opilioacaridae); **D)** *Neoteneriffiola xerophila* (Trombidiformes: Teneriffiidae); **E)** *Callidosoma cassiculophylla* (Erythraeidae: Trombidiformes); **F)** Trombiculoidea (Trombidiformes); **G)** Mesostigmata (Parasitiformes); **H)** *Linopodes* sp. (Eupodidae: Trombidiformes); **I)** Erythraeidae (Trombidiformes); **J)** Macronyssidae (Mesostigmata); **K)** *Amblyomma* sp. (Ixodida: Ixodidae); **L)** *Ornithodoros* sp. (Argasidae: Ixodida). Photos: A-H (Robson de A. Zampaulo), I-J (Matheus H. Simões), K-L (Lucas M. Rabelo).



Figure 2. Examples of mite specimens found associated with other invertebrates in Brazilian caves. **A-B)** *Eutrombidium carajás* Noei & Sundic 2020 (Trombidiformes: Eutrombidiidae) associated with cricket of the genus *Phalangopsis* sp. (Orthoptera: Phalangopsidae); **C)** Mites associated with *Heterophrynus longicornis* (Butler, 1873) (Amblypygi: Phrynidae); **D)** Details of mites associated with *H. longicornis*. Larger mites located in the central part of the prosoma: *Reginacharlottia* sp. (Mesostigmata: Reginacharlottiidae). Smaller mites located on the edge of the prosoma and chelicera: Chetochelacaridae (Sarcoptiformes). Photos (Robson de A. Zampaulo).

Main methods of collection and conservation

COLLECTION

One deficiency of studies carried out in Brazil is the limited use of collection methods, which biases mite sampling in caves in the country (personal observation), since many are limited to active searches without the aid of magnifying glasses or the use of specific traps. Detailed studies have applied numerous sampling methods (*e.g.*, SKUBALA *et al.*, 2013) and the compartmentalization of samples according to the different zones of the cave environment (*e.g.*, PROUS *et al.*, 2015). Caves also pose additional obstacles to the application of techniques commonly used on the surface, as they can be difficult to access, require extra care to minimize impacts on communities, and impose difficulties on researchers, such as obstacles, abysses and dangerous passages, beyond the obvious absence of light.

Free-living mites and those that are easily observed on the main cave substrates (floors, walls, speleothems, guano, organic matter, etc.) can be simply and efficiently collected by the method of active search of specimens, seen unaided with capture through the use of tweezers and brushes (PELLEGRINI; FERREIRA, 2012; SIMÕES *et al.*, 2014; WYNNE *et al.*, 2019; ZEPON; BICHUETTE, 2017). Obviously, the resulting sample is drastically biased towards

larger mites such as those of Ixodidae, Argasidae, Opilioacaridae, Macrochelidae, some Parasitengona and Oribatida. In this way, the sampling of small-bodied mites that live in the interstices of the substrate depends on the experience and visual acuity of the collector.

In this case, for efficient sampling of mites, it is essential to use extractors and traps. Drop traps, with or without bait (*e.g.*, carcasses, cheese, decaying fruit or feces from horses and cattle), are useful for collecting mites that actively move across surfaces. Soil mites can be removed by flotation followed by fractionation with hydrocarbons (such as commercial kerosene), which, in the authors' experience, yields diversified samples of very small mites and includes immobile stages, impossible to collect with other methods (KETHLEY, 1991). Aquatic mites can also be removed by flotation in the case of loose sediments. In this case, the sediment is placed in a bucket with water, which is vigorously shaken and then filtered through fine granulometric sieves (pore mesh size of 50 or 100 µm), with the mites suspended in the water being retained in the sieve mesh. If any submerged substrate is found, it can be washed with a strong jet of water directly over a fine granulometric sieve. To obtain hyporheic mites, water percolating from speleothems or deposited on the floor of caves can be pumped into sieves. In general, mites do not swim in the water column (*e.g.*, Halacaridae, Oribatida) or do so very little, preferentially staying around immersed substrates (*e.g.*, Hydrachnidia), such as trunks and litter. Surber nets may also be useful for removing substrate-associated aquatic mites.

In the laboratory, active methods of extracting mites from different substrates (soil, guano, litter, nests, among others) involve stimulation with light, heat and desiccation so that the mites move and fall into a container that may or may not contain a fluid preservative such as alcohol. The most commonly used extractor is the Berlese-Tulgreen funnel, which depends on the use of electrical energy, as it consists of a lamp that provides light and heat that stimulate the mites to move in the opposite direction until they fall into a reservoir (jar with preservative substance). An alternative is the Winkler apparatus, in which the mites move through the extractor as the moisture of the sample naturally reduces (MCSORLEY; WALTER, 1991; WALTER; KRANTZ, 2009). Finally, some ectoparasites abandon their hosts (Macronyssidae and Argasidae) and others associate with a host only during part of the life cycle (Parasitengona). Therefore, such mites can be found walking around freely and thus are obtainable with the aforementioned methods. However, the diversity of ectoparasitic mites can only be correctly estimated with the capture and inspection of hosts. Thus, different techniques are used to capture bats, rodents, lizards, anurans, arthropods or other invertebrates (*e.g.*, KUNZ; KURTA, 1988; REIS *et al.*, 2021; ALMEIDA *et al*., 1998). Whatever the case, knowledge of the biology of the groups to be studied is necessary, as well as time and effort in the field and laboratory for processing samples of hosts and their parasites (*e.g.*, FOWLER; COHEN, 1983; WHITAKER *et al.*, 1988).

CONSERVATION AND STUDY TECHNIQUES

Defining a conservation method requires awareness of the studies that will be carried out with the material. If mites are intended for taxonomy, conservation in 80–70% alcohol or the use of specific fixatives is preferred, such as Koenike's fluid, which has the advantage of maintaining the colors of mites of Hydrachnidia (WALTER; KRANTZ, 2009b). If specimens are intended for molecular biology studies, they must be fixed directly in ethyl alcohol (concentrations above 95%), kept under refrigeration (the colder, the better, with ideal temperatures ranging from -20 °C to -80 °C) and protected from light.

For DNA extraction, extraction kits sensitive enough for the minute amount of mite tissue can be used. Such kits include the QIAamp® DNA Micro Kit (Qiagen), indicated for mites with an idiosome of up to 1500 µm, and the Wizard® Genomic DNA Purification Kit (Promega), recommended for larger mites. DNA can be extracted following the manufacturer's protocol with the advantage that the mite exoskeleton can be recovered for further morphological study after digestion with proteinase K.

Specimens to be used for taxonomic work as voucher material after DNA extraction can be mounted in Hoyer's medium or glycerin gelatin (WALTER; KRANTZ, 2009b), while the DNA can be stored at -80 °C for long periods. One of the advantages of prior DNA extraction is the clearing of specimens through tissue digestion, which allows more details to be observed using light microscopy. Specimens that do not undergo DNA extraction need to be clarified, which can be done, depending on the mite group, in lactic acid, Nesbitt fluid or potassium hydroxide. It is necessary, however, to consult the literature for each group, as indications regarding reagents, concentrations and time for

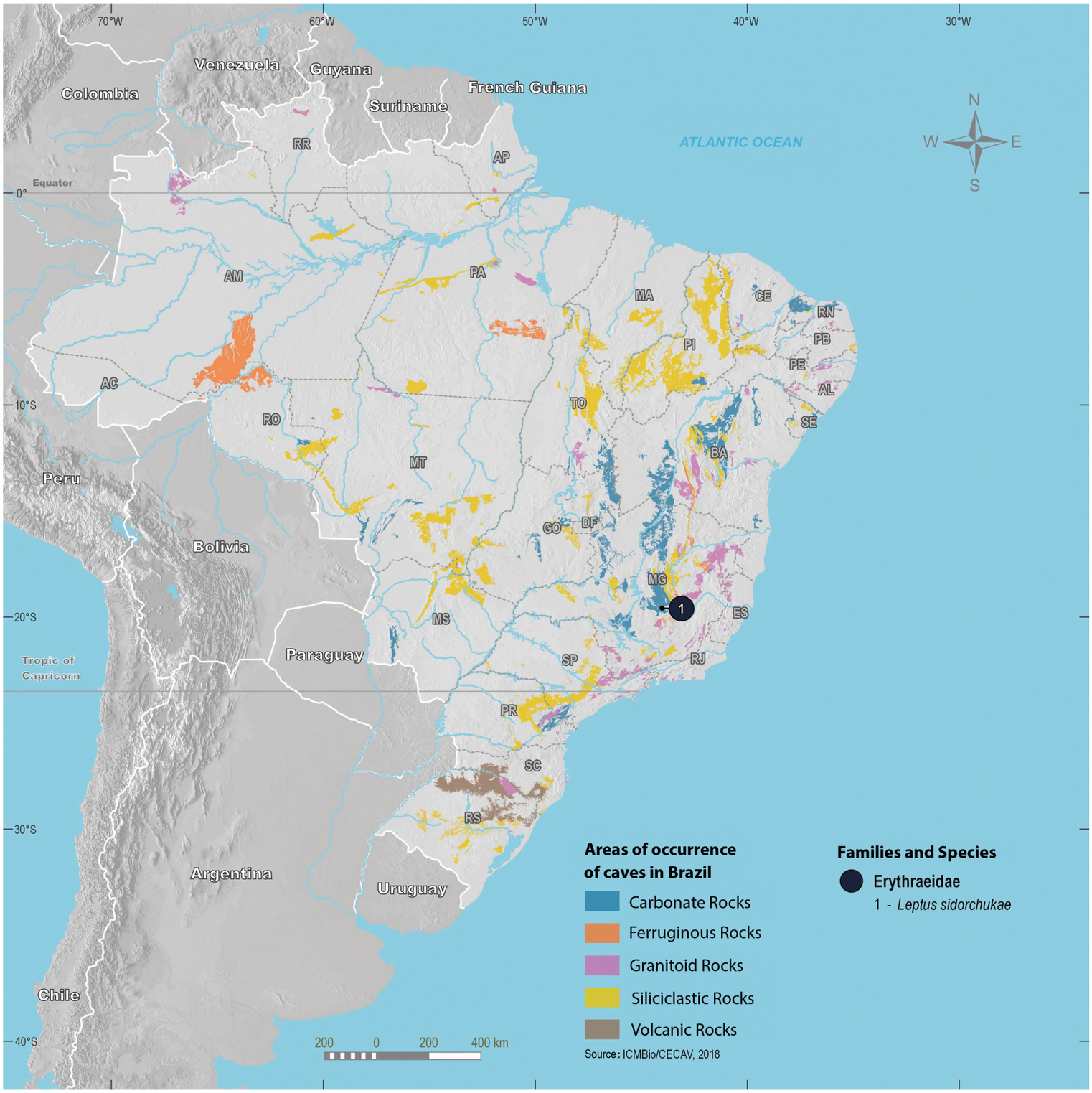


Figure 3. Map with the type locality of troglobitic mite species described for Brazil.

clearing vary among them.

The most common method for the morphological study of mites is the mounting of specimens and observation under optical microscopy. For this, collected mites are first clarified using substances such as lactic acid and Nesbitt, followed by mounting on slides using coverslips and Hoyer's medium. The slides are subsequently dried in an oven at 45–50 ºC for a period that can vary from 5 to 15 days. Observations of such mounts can be made using a common optical microscope, but the use of equipment that has mechanisms for phase contrast or differential interference contrast may be essential for the visualization of small structures and their most delicate shapes (NEUHAUS *et al.*, 2017; WALTER; KRANZ, 2009). Other forms of study can also be quite useful, such as the observation of specimens using electron microscopy (WERING *et al.*, 2000). There are several ways of preparing and observing in detail the morphology of mites. Therefore, seeking the method that is most appropriate and that fulfills the purposes of the work should always be an objective of a scholar in the area.

Main Brazilian collections

Currently, the mites collected in Brazilian caves are deposited in several research institutions. Among the most relevant collections, highlighted here are those that have the largest number of specimens and are curated, as well as those that have research groups dedicated to the biology of subterranean environments or that have research groups dedicated to acarology.

ISLA – Coleção de Invertebrados Subterrâneos de Lavras, Centro de Estudos em Biologia Subterrânea – CEBS, Universidade Federal de Lavras – UFLA, Lavras, Minas Gerais, Brasil. Lavras Collection of Subterranean Invertebrates, Center for Studies in Subterranean Biology – CEBS, Federal University of Lavras – UFLA, Lavras, Minas Gerais, Brazil.

LES – Laboratório de Estudos Subterrâneos, Universidade Federal de São Carlos (UFSCar), São Carlos, São Paulo, Brasil. Laboratory of Subterranean Studies, Federal University of São Carlos (UFSCar), São Carlos, São Paulo, Brazil.

MZUSP – Museu de Zoologia da USP – Universidade de São Paulo (USP), São Paulo, Brasil. USP Museum of Zoology – University of São Paulo (USP), São Paulo, Brazil.

These three collections concentrate a large number of mites from collections carried out in caves throughout Brazil and in some other regions of the world. This material originates from environmental consulting projects and scientific research. Despite their relevance, there has been, to date, no permanent acarologists curating the specimens deposited in these places.

Coleção Nacional de Carrapatos "Danilo Gonçalves Saraiva" – Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Universidade de São Paulo, São Paulo, Brasil. Faculty of Veterinary Medicine and Animal Science (FMVZ), University of São Paulo, São Paulo, Brazil.

Coleção Acarológica – Instituto Butantan, São Paulo, Brasil. Butantan Institute, São Paulo, Brazil.

Coleção de Acarologia – Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, São Paulo, Brasil. Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil.

Coleção de Acarologia do CCT-UFMG – UFMG_AC – Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brasil. UFMG_AC – Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.

These acarological collections have great national and international relevance, as they possess many type species (individual or set of specimens used by taxonomists during the description/designation of a new species for science) of deposited mites, with the curatorship of acarologists and active research groups. However, they are not specialized on cave mites, and materials from different types of ecosystems can be found.

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Robson de Almeida Zampaulo



Robson de Almeida Zampaulo



Rodrigo Lopes Ferreira



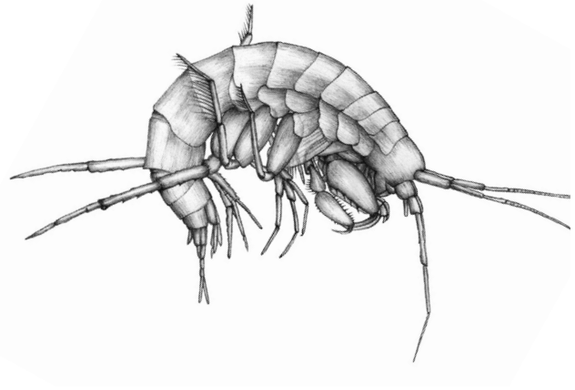


Spelaeogammarus sp.
Rodrigo Lopes Ferreira

7

Amphipoda

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General introduction

There are estimated 10,000 species of amphipods described worldwide (LOWRY; MYERS, 2017). The most recent catalog for Brazil records 303 species distributed among 142 genera and 56 families (SEREJO; SIQUEIRA, 2018). At least 156 freshwater species are currently known for the entire Neotropical region (LOWRY; MYERS, 2013; VÄINÖLÄ *et al.*, 2008), of which 35 occur in Brazil (BASTOS-PEREIRA; DE OLIVEIRA; FERREIRA, 2018; SEREJO; SIQUEIRA, 2018).

Amphipods are defined by having a laterally compressed body and the absence of a carapace, such that the seven thoracic segments (pereonites) are visible. The eyes, when present, are sessile. The thorax (pereon) is formed by seven segments and seven uniramous legs (pereopods), some with gills. The abdomen (pleon) has six segments and biramous appendages with the 4th to 6th (uropods) having one or two articles. Finally, they possess a telson as the last segment of the body (BARNARD; KARAMAN, 1991) (Figure 1).

Although the order Amphipoda is predominantly marine, species are also known for freshwater and terrestrial environments (SEREJO; SIQUEIRA, 2018). These crustaceans inhabit the most varied freshwater environments, such as streams, lakes and wetlands, both in epigean and hypogean compartments of the landscape (BASTOS-PEREIRA; BUENO, 2012; CARDOSO *et al.*, 2014; GONZÁLEZ; WATLING, 2003; RODRIGUES; BUENO; FERREIRA, 2012, 2014). Like many other taxonomic groups, amphipods can present morphological adaptations in response to the peculiar conditions of subterranean environments, such as the absence of light, for example, resulting in so-called troglomorphisms.

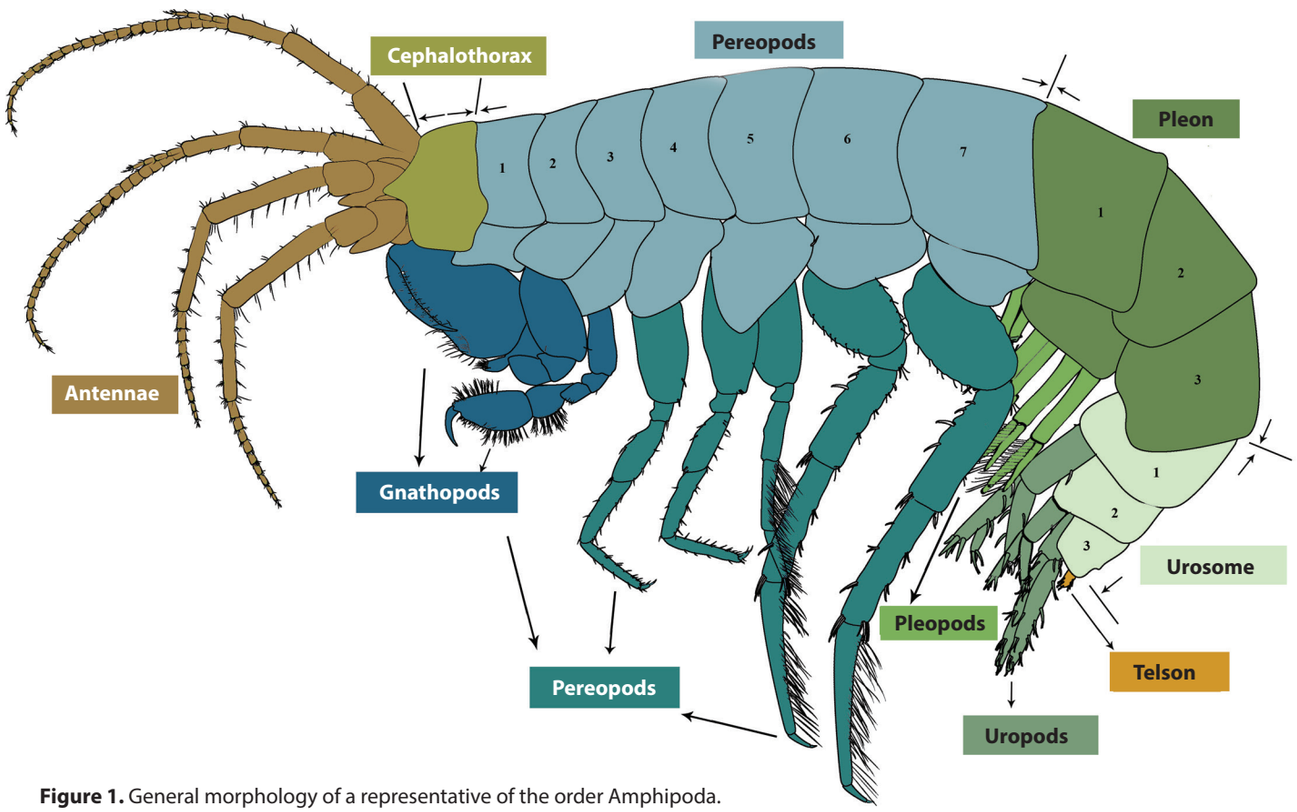


Figure 1. General morphology of a representative of the order Amphipoda.

Main troglomorphisms

Although there are generic troglomorphisms, that is, those widely disseminated in different taxonomic groups, the accurate identification of these morphological changes, resulting from isolation in subterranean habitats, can be quite challenging for certain taxa. Among these generic troglomorphisms are both regressive (such as the tendency for reduction of integumentary pigmentation, eye structures and wings, in the case of insects), and constructive characters (such as elongation of locomotor and sensory appendages) (CHRISTIANSEN, 2012; FRIEDRICH, 2019).

For decades, it was assumed that the degree of troglomorphism was associated with the amount of time of isolation in subterranean habitats, with more troglomorphic organisms being those belonging to lineages isolated for longer periods of time, as opposed to less troglomorphic, *a priori*, isolated for less time. An alternative to this hypothesis emerged in a study using amphipods as models (TRONTELJ; BLEJEC; FIŠER, 2012). From the analysis of 30 species of subterranean amphipods of the genus *Niphargus* Schiödte, 1849 (Niphargidae), using multivariate morphometry and phylogenetic reconstruction, the authors grouped the species into four ecomorphological classes associated with specific microhabitats in caves. Furthermore, these species had multiple independent phylogenetic origins (TRONTELJ; BLEJEC; FIŠER, 2012). Some morphological characteristics considered as generic specializations to the subterranean way of life (such as antennae length) were shown to be related to parameters of the microhabitats to which the species were associated, such as the speed of water flow. The authors concluded that there may be ecomorphological convergence of structures from pressures of different subterranean environments, so that morphological diversity is not necessarily the result of isolation time but may result from the partitioning of meso - and microscale niches (TRONTELJ; BLEJEC; FIŠER, 2012). Some Brazilian species of *Hyalella* S. I. Smith, 1874, for example, illustrate the pattern observed by these authors with species of larger body size being found in microhabitats with greater availability of space, such as lakes or water tables. Smaller species, on the other hand, are observed associated with epikarst (Figure 2). In other words, less troglomorphic organisms may have been isolated even longer than those that are more troglomorphic, with their morphology predominantly resulting from the pressures of the subterranean habitat in which they live. Extremely important from an evolutionary point of view, the results of this work changed the way troglomorphisms were understood. Thus, when interpreting troglomorphic characteristics, several parameters need to be considered, among them, isolation time, preferential microhabitat of the species and, mainly, the typical morphology observed in surface relatives, which is the morphological reference to establish the comparison between the levels of troglomorphisms observed.

Brazilian troglomorphic amphipods exhibit highly variable levels of troglomorphism. As previously mentioned, in Brazil, troglomorphic species of amphipods are known for five families, among which only one (Artesiidae) contains exclusively subterranean species; the others have representatives both in subterranean and surface habitats.

The family Artesiidae contains only two genera: *Artesia* Holsinger, 1980, and *Spelaeogammarus* da Silva Brum, 1975, the latter being found only in Brazil. As all species of the family are troglobites, indicating specific troglomorphisms is complex, given the inexistence of phylogenetically close taxa in epigean habitats (belonging to the same family). Thus, comparisons indicated here were performed only among the different levels of troglomorphism observed for the species of this family. Some species of *Spelaeogammarus* have very similar morphology (in terms of proportions) to species of *Artesia*. For example, the species *Artesia subterranea* Holsinger, 1980, which occurs in Texas (United States), has general proportions between body and appendages that are similar to that of the species *S. bahiensis* da Silva Brum, 1975, which demonstrates a certain homogeneity of troglomorphism among species belonging to this family. On the other hand, the species *S. titan* Senna, Andrade, Castelo-Branco & Ferreira,

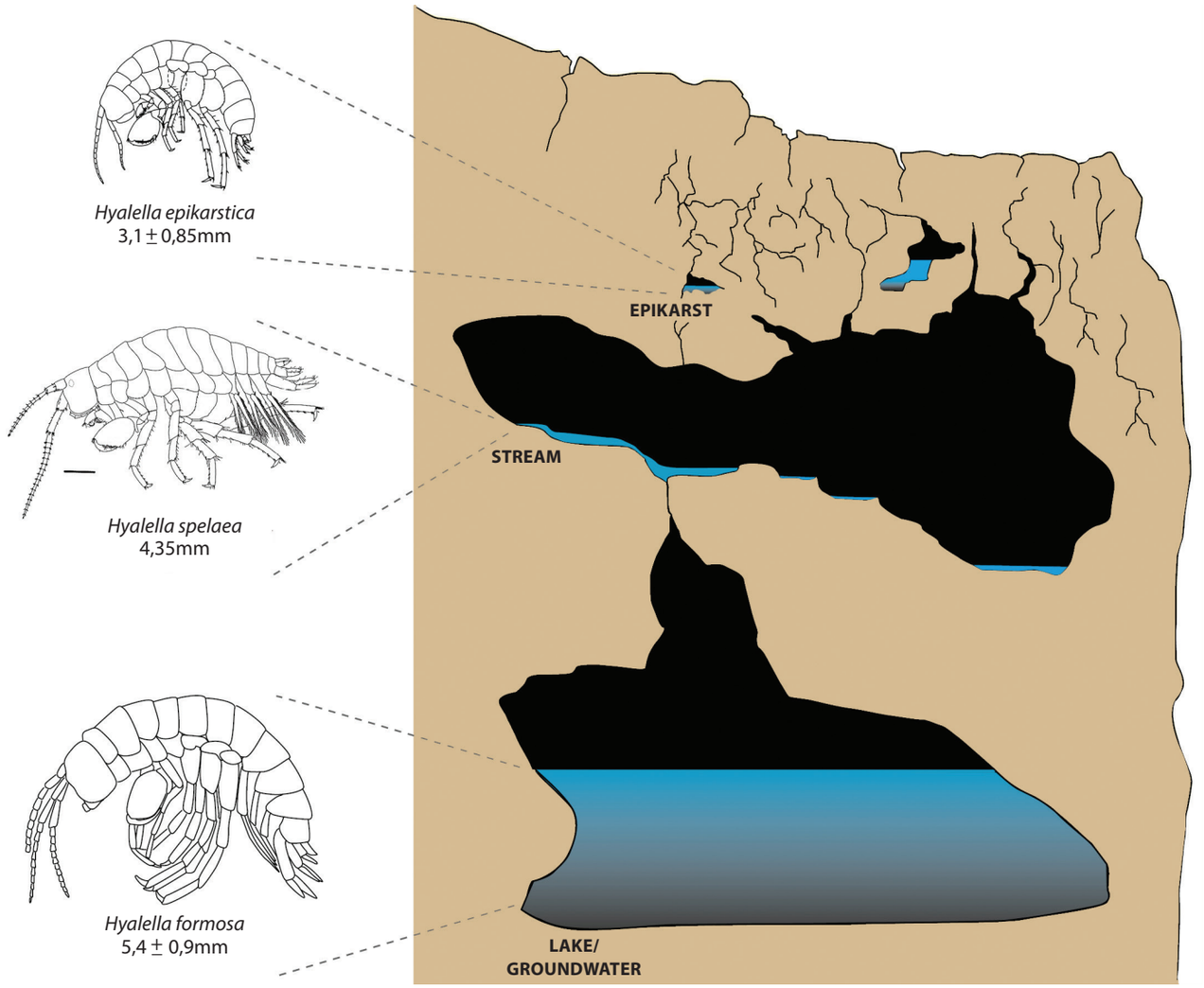


Figure 2. Relationship between body size of Brazilian subterranean amphipods and the type of microhabitats in which they live. Adapted from Trontelj *et al.*, (2012).

2014, stands out within the family due to a greater elongation of the appendages, as well as greater body size, characteristics often associated with a strictly subterranean way of life. Thus, this species can be considered the most troglomorphic of the family. However, the origin of these differences remains unknown, since all species, at least those belonging to the genus *Spelaeogammarus*, are associated with water tables, which are often configured inside caves as large lakes. Thus, specificities for distinct microhabitats do not seem to be determinant for the species of this genus. However, it should be noted that only phylogenetic and phylogeographic studies can elucidate this question.

The species *Megagidiella azul* Koenemann & Holsinger, 1999, of the family Bogidiellidae, in turn, has pronounced troglomorphisms. Several genera of this family are found in surface habitats, which allows for more assertive morphological comparisons (VÄINÖLLA *et al.*, 2008). *Megagidiella azul* has, in addition to considerably elongated appendages, an accentuated body size, which gave rise to its name. Its body size is probably related to the dimensions of the available habitat, as the species occurs in a huge subterranean lake (KOENEMANN; HOLSINGER, 1999). This species has several troglomorphic characteristics that can be compared with the artesiid *S. titan*, as they are considered highly troglomorphic animals.

The species *Potiberaba porakuara* Fišer, Zagmajster & Ferreira, 2013, is the only representative of the family Mesogammaridae in South America (FIŠER, ZAGMAJSTER; FERREIRA, 2013). This family has a very disjunct distribution, with species occurring in East India, South Korea, Japan, Alaska and Brazil. Of the six genera of this family, three have eyes (developed or vestigial), which allows comparisons. *Potiberaba porakuara* has several similarities with the species *Octopupilla felix* Tomikawa, 2007, found in Japan (TOMIKAWA *et al.*, 2007), such as a total fissure in the telson (although with different morphology) and less developed propodus in gnathopod 2 than in gnathopod 1. However, although *O. felix* still has vestigial eyes, its antennae are proportionately longer than those of *P. porakuara*. The species *Eoniphargus kojimai* (Uéno, 1955), also found in Japan, has morphology and proportions more comparable to *P. porakuara*. It is interesting to note that, while the Brazilian species is cave-dwelling (preferably inhabiting subterranean lakes), the other two mentioned above (found in Japan) inhabit sediment banks in rivers (hyporheic species). Thus, it is noted that, for this family, troglomorphic characteristics can be quite variable, with levels of regression (in the case of eyes) or amplification

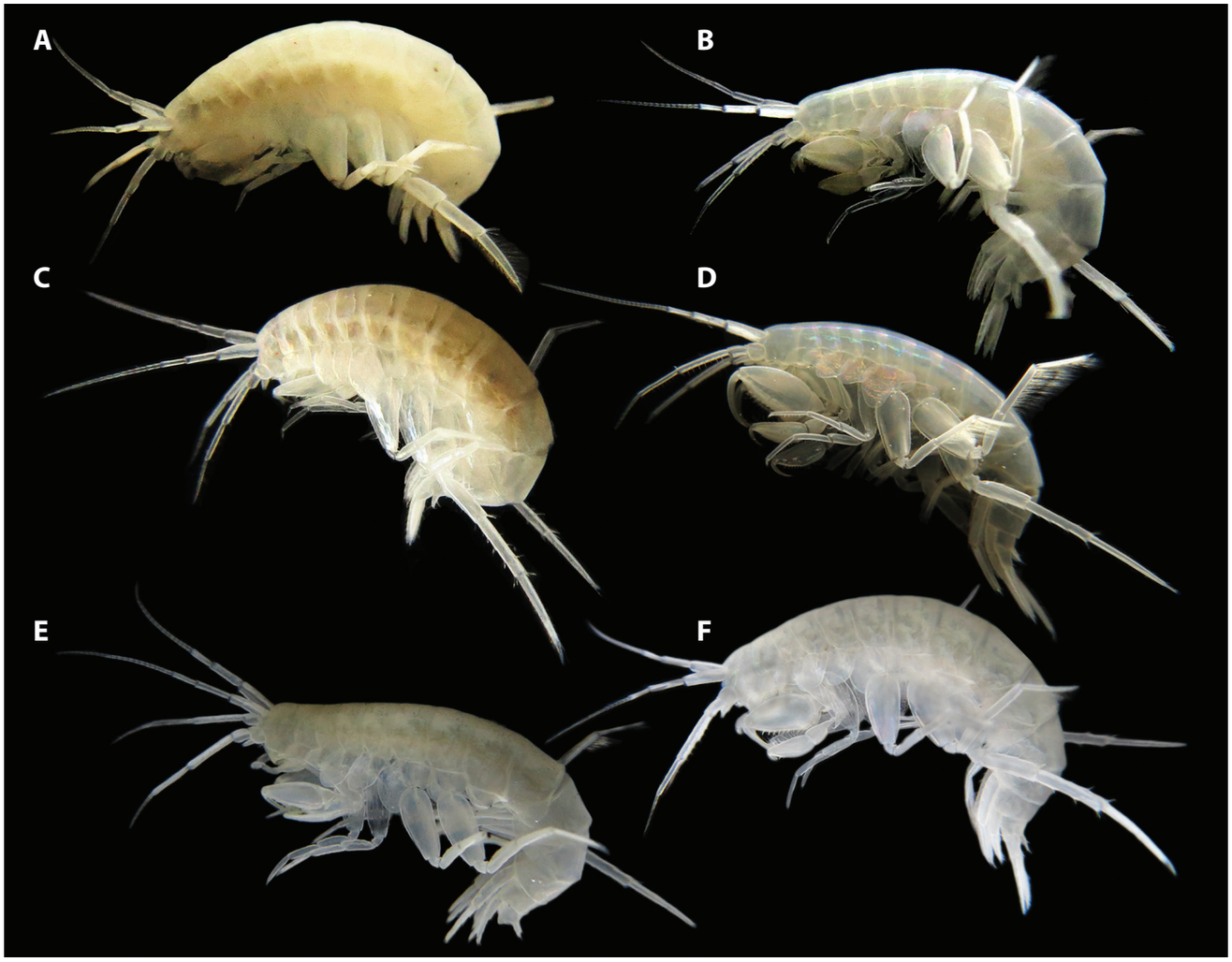


Figure 3. Specimens of Artesiidae photographed live. **A)** *Spelaeogammarus bahiensis*; **B)** *S. santanensis*; **C)** *S. spinilacertus*; **D)** *S. titan*; **E)** *S. sanctus*; **F)** *S. uai*. Phohtos: (Rodrigo L. Ferreira).

(in the case of appendages) that apparently do not show a direct relationship with the type of subterranean habitat preferably used.

The species *Seborgia potiguar* Fišer, Zagmajster & Ferreira, 2013, is also the only described representative of the family Seborgiidae for South America (FIŠER, ZAGMAJSTER; FERREIRA, 2013). Like Mesogammaridae, this family also has a disjunct distribution, with species occurring in the USA (Texas), Rennell Islands, Lealdade Islands, Vanuatu, Vietnam and Brazil. Among the eight species described for *Seborgia*, three have eyes, which allows comparisons with cave species. In general, the main troglomorphism observed for *S. potiguar* is anophthalmia. Its body size is extremely reduced (about 1.5 mm), as well as the proportions of the appendages, comparable to those observed in non-cave species. However, there are at least two new species for the genus known for the state of Rio Grande do Norte awaiting formal description. These species possess quite accentuated troglomorphisms, such as a considerably increased body size (one of the species measures about 5 mm, therefore considered "giant" for the family), as well as pronounced elongation of appendages.

Finally, the last family with troglomorphic species in Brazil is Hyalellidae. This family has the second highest number of troglobitic representatives in the country (six species, with Artesiidae containing seven), in addition to two troglomorphic species occurring in epigean environments. This can be explained by its great diversity in epigean habitats in South America as a whole. However, the troglobitic species of this family can be considered, in general, the least troglomorphic among Brazilian subterranean amphipods. Some have microphthalmia (*Hyalella spelaea* Bueno & Cardoso, 2011, and *H. veredae* Cardoso & Bueno, 2014), while the others are anophthalmic. However, other troglomorphisms, such as increased body size or elongation of appendages, are very little pronounced, even when present. In general, troglobitic species of *Hyalella* differ from epigean species basically by the reduction or absence of eyes, regardless of whether they occur in subterranean lakes (*H. formosa* Cardoso & Bueno, 2014), subterranean streams (*H. spelaea*), epikarstic environments (*H. epikarstica* Rodrigues, Bueno & Ferreira 2014) or even hypotelminorrheic environments (*H. imbya* Rodrigues & Bueno, 2012). Thus, as mentioned for species of the genus *Spelaeogammarus*, specificities for distinct microhabitats do not seem to define different levels of troglomorphism for the species of this genus, although phylogenetic and phylogeographic studies are essential to answer this question.

Rodrigues, Bueno and Ferreira (2014) indicate that troglobitic species of *Hyalella*, in general, show reduced body size; reduction or absence of eyes; reduction in gill size and number; elongation of antenna 1 and aesthetascs; changes in the maxilliped, such as increase in size and in number of setae; and pronounced reduction of the carpus of gnathopod 2. Other modifications were also identifiifed, such as a decrease in the amount or absence of serrated setae in the carpal lobe; pronounced elongation of propodus; and palm with an oblique inclination. There is also a deeper groove for the dactylus; absence of *comb-scales* in both gnathopods; elongation of pereopods 6 and 7; reduction in the size of uropod 3 and decrease in the number and abundance of setae on telson. However, some of these characteristics do not show such notable differences between troglobitic and epigean species. *Hyalella imbya* and *H. epikarstica*, for example, have as many setae on the maxilliped as does *H. castroi* González, Bond-Buckup & Araujo, 2006. Regarding gnathopod 2 of males, *H. troglofugia* Bastos-Pereira, Oliveira & Ferreira, 2018, has a small carpus, while that of *H. spelaea* is very elongated. On the other hand, differently from what is observed among epigean species, elongation of the first pair of antennae is a recurrent feature among strictly subterranean species of *Hyalella*, as well as a greater number of aesthetascs on the flagellum articles of this appendage and the reduction of the third pair of uropods. Thus, for the genus *Hyalella*, the following can be considered the main troglomorphisms: absence or reduction of eyes (despite the existence of anophthalmic epigean species e.g., *H. troglofugia*), elongation of antenna 1, increase in the number of aesthetascs and reduction of uropod 3.

Although most troglobitic amphipod species found in Brazil are microphthalmic or anophthalmic, there are also species with anophthalmic individuals that inhabit surface environments, such as *H. montana* Rodrigues, Senna, Quadra & Bueno, 2017, and *H. troglofugia* of Hyalellidae, and *Bogidiella neotropica* Ruffo, 1952, and *Marigidiella brasiliensis* (Siewing, 1953) of Bogidiellidae. Thus, the existence of eyeless species in epigean environments can raise important questions about the effective troglomorphisms observed in these organisms.

How could a given anophthalmic species maintain viable populations on the surface? At first this seems counter-intuitive, considering the importance of eyes and vision in epigean

habitats. In the case of *H. troglofugia*, its occurrence in epigean environments can be explained by the microhabitats this species uses (macrophyte roots or stretches with more intense water flow), which probably favor protection against predators (BASTOS-PEREIRA; DE OLIVEIRA; FERREIRA, 2018). Furthermore, according to the authors, some characteristics considered troglomorphisms do not necessarily limit the dispersion of subterranean species to adjacent external environments. Another example worth mentioning is the species *Bogidiella neotropica* (Ruffo, 1952), collected in the Amazon, very close to a spring. A single specimen was found exhibiting obvious troglomorphic characters. However, later specimens attributed to this species were recorded in Venezuela, in artesian wells (PLATVOET, 1983) and, more recently, in an iron cave in the Carajás region (Serra do Tarzan), state of Pará in northern Brazil. Excluding the holotype, all other records of the species occurred in subterranean habitats, in locations extremely distant from the type locality. In this case, it is important to consider the hypothesis that there are cryptic species (which are those that, although genetically distinct, are morphologically indistinguishable). Such species may present extremely similar morphology due to convergence, since even the holotype may have subterranean drainages as its main habitat, given the location and condition in which it was collected (RUFFO, 1952).

Finally, another emblematic question is whether the subterranean lifestyle is reversible. In an attempt to answer this question, Copilaş-Ciocianu *et al.* (2018) carried out a study focusing on two species of *Niphargus* that occur in surface waters and are widely distributed in central and eastern Europe: *N. hrabei* Karaman, 1932, and *N. valachicus* Dobreanu & Manolache, 1933. These authors aimed to determine whether the wide distribution of these species resulted from a secondary colonization of epigean environments (relatively well connected) and if this ecological change would have promoted the large-scale dispersion of these species. Their results indicated independent episodes of dispersion of these species in the Pleistocene and a relatively low, but significant, genetic differentiation between populations. Furthermore, they suggest that the ecological shift to surface environments had been facilitated by the ability of these species to tolerate hypoxic (low oxygen) environments, where rheophilic competitors of the family Gammaridae could not survive. In conclusion, the results of the study indicated that adaptation to subterranean waters is not a "no-return" evolutionary path and that troglomorphic species may occasionally recolonize and disperse widely across surface waters (Copilaş-Ciocianu *et al.*, 2018). This may possibly be the case for troglomorphic species found on the surface, such as *Hyalella troglofugia*.

Diversity of troglobitic species described for Brazil

The first record of a subterranean amphipod for Brazil dates from 1975, when Da Silva Brum described the first species of the genus *Spelaeogammarus* (*S. bahiensis*, which is troglobitic — strictly found in the subterranean environment) from a cave in Curaçá, in northern Bahia. At the end of the 1980s, the first anophthalmic species of *Hyalella* was described for Brazil, *H. caeca* (PEREIRA, 1989). In 1999, the presence of *Megagidiella azul*, another troglobitic species, was reported by Koenemann and Holsinger in cavities in the Bonito region in Mato Grosso do Sul. However, from the 2000s onwards the known diversity of amphipods for Brazil grew significantly.

Currently, troglomorphic amphipods belonging to five families are known for Brazil: Artesiidae, Bogidiellidae, Hyalellidae, Mesogammaridae and Seborgidae. Among these, Bogidiellidae is represented by three genera, while the other families are represented by only one each. Considering species occurring in caves, Hyalellidae and Artesiidae are the most representative families. The family Artesiidae contains only subterranean species (7), whereas Hyalellidae contains a single genus for which epigean species are also known. The genus *Hyalella* has six troglobitic species, in addition to two troglomorphic species occurring in epigean habitats (*H. montana* and *H. troglofugia*). In turn, Bogidiellidae is represented by the genera *Marigidiella, Megagidiella* and *Bogidiella*, all with only one species in Brazil. Finally, *Mesogammaridae* and *Seborgidae* are represented by only one genus and one species each. Below there is a list of troglomorphic amphipod species known for Brazil, with a bibliography containing mentions of each, as well as their current known distribution (Table 1).

Thus, 20 species of troglomorphic amphipods are currently known for Brazil, 17 of which have records for caves. Table 2 presents a summary of the diversity of these amphipods according to family, genus, species and habitat type. The distribution of species is shown in Figure 10.

Table 1 – List of troglobitic species and their updated records.

Family **Artesiidae** Holsinger, 1980

Genus ***Spelaeogammarus*** Da Silva Brum, 1975

1. ***Spelaeogammarus bahiensis*** Da Silva Brum, 1975 (Figure 3A)
Spelaeogammarus bahiensis (DA SILVA BRUM, 1975, p. 125), figs 1–17; (GNASPINI; TRAJANO, 1994, p. 554; HOLSINGER, 1981, p. 38; KOENEMANN; HOLSINGER, 2000, p. 118; PINTO-DA-ROCHA, 1995, p. 95; TRAJANO; SANCHÉZ, 1994, p. 535; WAKABARA; SEREJO, 1998, p. 564), figs 6d, h; 7f, g; (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.,* 2020, p. 857; SEREJO; SIQUEIRA, 2018, p. 58) (identification key).
Distribution: Loca d'água de Patamuté (Distrito de Patamuté, Curaçá, BA).
Obs.: In the original description, the name of the district where the cave is located was spelled incorrectly (Matamuté). The correct one is Distrito de Patamuté.

2. ***Spelaeogamarus santanensis*** Koenemann & Holsinger, 2000 (Figure 3B)
Spelaeogammarus santanensis (KOENEMANN; HOLSINGER, 2000, p. 113), figs 6a, e–g; 7a, b; 8c–e. (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.,* 2020, p. 858; SEREJO; SIQUEIRA, 2018, p. 59) (identification key).
Distribution: Gruta do Padre (Santana, BA).

3. ***Spelaeogammarus spinilacertus*** Koenemann & Holsinger, 2000 (Figure 3C)
Spelaeogammarus spinilacertus (KOENEMANN; HOLSINGER, 2000, p. 106), figs 1–5, 6c; (GALLÃO; BICHUETTE, 2018, p. 6; TRAJANO *et al.*, 2016, p. 1815), fig. 1c; (ROGERS *et al.,* 2020, p. 858; SEREJO; SIQUEIRA, 2018, p. 59) (identification key).
Distribution: Caverna Baixa do Salitre cave (type locality), Caverna do Jaburu, Gruta da Torrinha and Lapa Doce II (Figure 7A) (Iraquara, BA).

4. ***Spelaeogammarus trajanoae*** Koenemann & Holsinger, 2000
Spelaeogammarus trajanoae (KOENEMANN; HOLSINGER, 2000, p. 112), figs 6b, i; 7c, d; 8a, b; (ARAÚJO; PEIXOTO, 2014, p 28), fig. 4c; (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.*, 2020, p. 858; SEREJO; SIQUEIRA, 2018, p. 59; TRAJANO *et al.*, 2016, p. 1814) (identification key).
Distribution: Toca do Pitu (type locality), Toca do Convento, Toca do Carlito, Toca da Tiquara (Campo Formoso, BA), Toca da Jurema (Várzea Nova,BA) (Figure 7B) and Toca do Gonçalo (Umburanas, BA).

5. ***Spelaeogammarus titan*** Senna, Andrade, Castelo-Branco & Ferreira, 2014 (Figure 3D).
Spelaeogammarus titan (SENNA *et al.*, 2014, p. 56), figs 2–7; (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.*, 2020, p. 858; SEREJO; SIQUEIRA, 2018, p. 59) (identification key).
Distribution: Cave PEA–445 (Santa Maria da Vitória, BA) (Figure 7C).

6. ***Spelaeogammarus sanctus*** Bastos-Pereira & Ferreira, 2015 (Figure 3D).
Spelaeogammarus sanctus (BASTOS-PEREIRA; FERREIRA, 2015, p. 422), figs 4–8 (identification key); (BASTOS-PEREIRA; FERREIRA, 2017, p. 46; GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.*, 2020, p. 858; SEREJO; SIQUEIRA, 2018, p. 58) (identification key).
Distribution: Gruta dos Milagres (Bom Jesus da Lapa, BA).

7. ***Spelaeogammarus uai*** Bastos-Pereira & Ferreira, 2017 (Figure 3D)
Spelaeogammarus uai (BASTOS-PEREIRA; FERREIRA, 2017, p. 39), figs 2–5; (GALLÃO; BICHUETTE, 2018, p. 6; MONTE; BICHUETTE, 2020, p. 5; ROGERS *et al.*, 2020, p. 858; SEREJO; SIQUEIRA, 2018, p. 59) (identification key).
Distribution: Lapa D'Água do Zezé (Itacarambi, MG) (Figure 7D).

Family **Hyalellidae** Bulyčeva, 1957

Genus ***Hyalella*** Smith, 1874

1. ***Hyalella caeca*** Pereira, 1989 (Figure 4A)
   Hyalella caeca (PEREIRA, 1989, p. 49), figs 1–21; (GNASPINI; TRAJANO, 1994, p. 581; GONZÁLEZ *et al.*, 2006, p. 365; PINTO-DA-ROCHA, 1995, p. 96; TRAJANO; SANCHEZ, 1994, p. 535; WAKABARA; SEREJO, 1998, p. 569) (chave de identificação); (BASTOS-PEREIRA; BUENO, 2012, p. 59) (identification key); (DE LOS RÍOS-ESCALANTE *et al.*, 2012, p. 155), mapa; (MARRÓN-BECERRA *et al.*, 2014, p. 266; RODRIGUES *et al.*, 2012, p. 77) (identification key); (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.*, 2020, p. 852; SEREJO; SIQUEIRA, 2018, p. 105; TRAJANO *et al.*, 2016, p. 1817) (identification key).
   Distribution: Gruta Tobias debaixo (Iporanga, SP).

2. ***Hyalella spelaea*** Bueno & Cardoso, 2011
   Hyalella spelaea (CARDOSO *et al.*, 2011, p. 18), figs 1–28; (BASTOS-PEREIRA; BUENO, 2012, p. 59) (identification key); (DE LOS RÍOS-ESCALANTE *et al.*, 2012, p. 155; RODRIGUES *et al.*, 2012, p. 77), map; (MARRÓN-BECERRA *et al.*, 2014, p. 266; RODRIGUES *et al.*, 2012, p. 77) (identification key); (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.*, 2020, p. 853; SEREJO; SIQUEIRA, 2018, p. 109) (identification key).
   Distribution: Gruta da Toca (Itirapina, SP).

3. ***Hyalella imbya*** Rodrigues & Bueno, 2012
   Hyalella imbya (RODRIGUES *et al.*, 2012, p. 67), figs 1–6; (DE LOS RÍOS-ESCALANTE *et al.*, 2012, p. 155), map; (MARRÓN-BECERRA *et al.*, 2014, p. 266; RODRIGUES *et al.*, 2012, p. 77) (identification key); (SEREJO; SIQUEIRA, 2018, p. 107), fig. 4e; (ROGERS *et al.*, 2020, p. 853) (identification key).
   Distribution: flooded areas in the municipality of Roque Gonzales, RS.
   Obs. Hypotelminorrheic species. The authors indicate that this species may have become extinct as a result of changes in the landscape by human activities.

4. ***Hyalella epikarstica*** Rodrigues, Bueno & Ferreira 2014
   Hyalella epikarstica (RODRIGUES *et al.*, 2014, p. 201), figs 1–27 (identification key). (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.*, 2020, p. 853; SEREJO; SIQUEIRA, 2018, p. 106; TRAJANO *et al.*, 2016, p. 1817) (identification key).
   Distribution: Gruta Areias de Cima (Iporanga, SP) (Figure 5A).
   Obs. An epikarstic species that is also found in micro habitats inside the cave.

5. ***Hyalella formosa*** Cardoso & Bueno, 2014 (Figure 4B)
   Hyalella formosa (CARDOSO *et al.*, 2014, p. 362), figs 7–10 (identification key); (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.*, 2020, p. 853; SEREJO; SIQUEIRA, 2018, p. 106) (identification key).
   Distribution: Caverna das Andorinhas (Ponta Grossa, PR).

6. ***Hyalella veredae*** Cardoso & Bueno, 2014 (Figure 4C)
   Hyalella veredae (CARDOSO *et al.*, 2014, p. 354), figs 3–6 (identification key); (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.*, 2020, p. 853; SEREJO; SIQUEIRA, 2018, p. 109) (identification key).
   Distribution: Vereda da Palha cave (Presidente Olegário, MG) (Figure 5B).

7. ***Hyalella montana*** Rodrigues, Senna, Quadra & Bueno, 2017
   Hyalella montana (RODRIGUES *et al.*, 2017, p. 149), figs 2–6; (ROGERS *et al.*, 2020, p. 855) (identification key).
   Distribution: Parque Nacional de Itatiaia (Itamonte, MG).

8. ***Hyalella troglofugia*** Bastos-Pereira, Oliveira & Ferreira, 2018 (Figure 4D)
Hyalella troglofugia (BASTOS-PEREIRA *et al.*, 2018, p. 256), figs 2–6; (ROGERS *et al.*, 2020, p. 853) (identification key).
Distribution: Nova Lima, MG. Córrego da Fazenda, affluent of Rio das Velha.

Family **Bogidiellidae** Hertzog, 1936

Genus ***Megagidiella*** Koenemann & Holsinger, 1999

1. ***Megagidiella azul*** Koenemann & Holsinger, 1999 (Figure 8A)
Megagidiella azul (KOENEMANN; HOLSINGER, 1999, p. 573), figs 1–4; (CORDEIRO *et al.*, 2014, p. 5) (map); (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.*, 2020, p. 845; SEREJO; SIQUEIRA, 2018 (indication of the species probably belonging to Artesiidae), p. 60; TRAJANO *et al.*, 2016, p. 1817) (identification key)
Distribution: Gruta do Lago Azul (type locality) and Gruta do Mimoso (Bonito, MS); Abismo do Poço and Buraco da Abelha (Jardim, MS); Caverna do Clarão and Buraco do Bicho (Bodoquena, MS).

Genus ***Bogidiella*** Hertzog, 1933

1. ***Bogidiella neotropica*** Ruffo, 1952 (Figure 8B)
Bogidiella neotropica (RUFFO, 1952, p. 129), figs 1, 2; (GALLÃO; BICHUETTE, 2018, p. 6; HOLSINGER, 1981, p. 38; ROGERS *et al.*, 2020, p. 846; SEREJO; SIQUEIRA, 2018, p. 60; WAKABARA; SEREJO, 1998, p. 564) (identification key).
Distribution: 15 Km da foz do Rio Cupari no Rio Tapajós (Santarém, PA); Cave ST_0041 (Serra de Tarzan, Canaã dos Carajás, PA).

Genus ***Marigidiella*** Stock, 1981

1. ***Marigidiella brasiliensis*** (Siewing, 1953)
Bogidiella brasiliensis (SIEWING, 1953, p. 243), figs 24–26; (HOLSINGER, 1981, p. 38; WAKABARA; SEREJO, 1998, p. 563).
Marigidiella brasiliensis (KOENEMANN; HOLSINGER, 1999, p. 579; STOCK, 1981, p. 355) (map).
Distribution: Salvador, BA, and Ilhabela, SP.
Obs.: marine species found in coastal subterranean waters.

Family **Mesogammaridae** Bousfield, 1977

Genus ***Potiberaba*** Fišer, Zagmajster & Ferreira, 2013

1. ***Potiberaba porakuara*** Fišer, Zagmajster & Ferreira, 2013 (figures 6A and 8C)
Potiberaba porakuara (FIŠER *et al.*, 2013, p. 13), figs 2, 11–19; (GALLÃO; BICHUETTE, 2018, p. 6; ROGERS *et al.*, 2020, p. 838; SEREJO; SIQUEIRA, 2018, p. 95; TRAJANO *et al.*, 2016, p. 1817) (identification key).
Distribution: Três Lagos cave (Felipe Guerra, RN) (Figure 6B). Populations of this species have already been found in at least five other caves in which the water table can be accessed, which will be addressed in future works.

Family **Seborgiidae** Holsinger, 1980

Genus ***Seborgia*** (Fišer *et al.*, 2013).

1. ***Seborgia potiguar*** (Fišer *et al.*, 2013).
Seborgia potiguar (FIŠER *et al.*, 2013, p. 7), figs 3, 5–10; (ROGERS *et al.*, 2020, p. 838; SEREJO; SIQUEIRA, 2018, p. 23) (identification key).
Distribution: Caverna da Água (Governador Dix-Sept Rosado, RN).

Procedures and methods of collection and conservation

COLLECTION

The troglomorphic amphipods found in Brazilian caves to date are all aquatic, being found in streams, puddles, travertine reservoirs or water tables (Figures 5, 6 and 7). Such organisms may be associated with submerged roots, as observed for epigean species. When present in accessible places for manual collection, amphipods can be collected with the aid of nets and handled with brushes. In places of difficult access, or to increase the chances of capture, traps with attractive baits can be used, in which the organisms enter to feed and end up trapped. This has been the case adopted for collecting species of Mesogammaridae and Seborgidae in some caves that are difficult to access. Traps can be made with PVC pipe (minnow traps), the ends of which must be wrapped with a screen mesh of about 2 mm between nodes (Figure 9), in addition to using a bait, such as liver or beef. One of the most efficient baits for collections carried out in Rio Grande do Norte was butchered goat blood. The minnow traps must be kept submerged in water at the place of interest for 24 hours, being collected later (HUGHES; AHYONG, 2016).

PRESERVATION OF COLLECTED MATERIAL

Collected specimens are usually preserved in 70% ethanol, but it is recommended that, for molecular analysis, they are kept in 100% ethanol and immediately cooled in a thermal box containing ice until they can be properly placed in a freezer.

Table 2. Species of troglomorphic amphipods recorded for Brazil. (Ep): epigean, (Hp): hypogean, (Cv): with caves records, (Hypo): hypotelminorrheic.

Family	Genus	Species
Artesiidae	*Spelaeogammarus*	*Spelaeogammarus bahiensis* (Hp/Cv) *Spelaeogammarus santanensis* (Hp/Cv) *Spelaeogammarus spinilacertus* (Hp/Cv) *Spelaeogammarus trajanoae* (Hp/Cv) *Spelaeogammarus titan* (Hp/Cv) *Spelaeogammarus sanctus* (Hp/Cv) *Spelaeogammarus uai* (Hp/Cv)
Hyalellidae	*Hyalella*	*Hyalella caeca* (Hp/Cv) *Hyalella spelaea* (Hp/Cv) *Hyalella imbya* (Hp/Hipo) *Hyalella epikarstica* (Hp/Cv) *Hyalella veredae* (Hp/Cv) *Hyalella formosa* (Hp/Cv) *Hyalella troglofugia* (Ep) *Hyalella montana* (Ep)
Bogidiellidae	*Megagidiella* *Bogidiella*	*Megagidiella azul* (Hp/Cv) *Bogidiella neotropica* (Ep/Hp/Cv) *Marigidiella brasiliensis* (Ep)
Mesogammaridae	*Potiberaba*	*Potiberaba porakuara* (Hp/Cv)
Seborgidae	*Seborgia*	*Seborgia potiguar* (Hp/Cv)

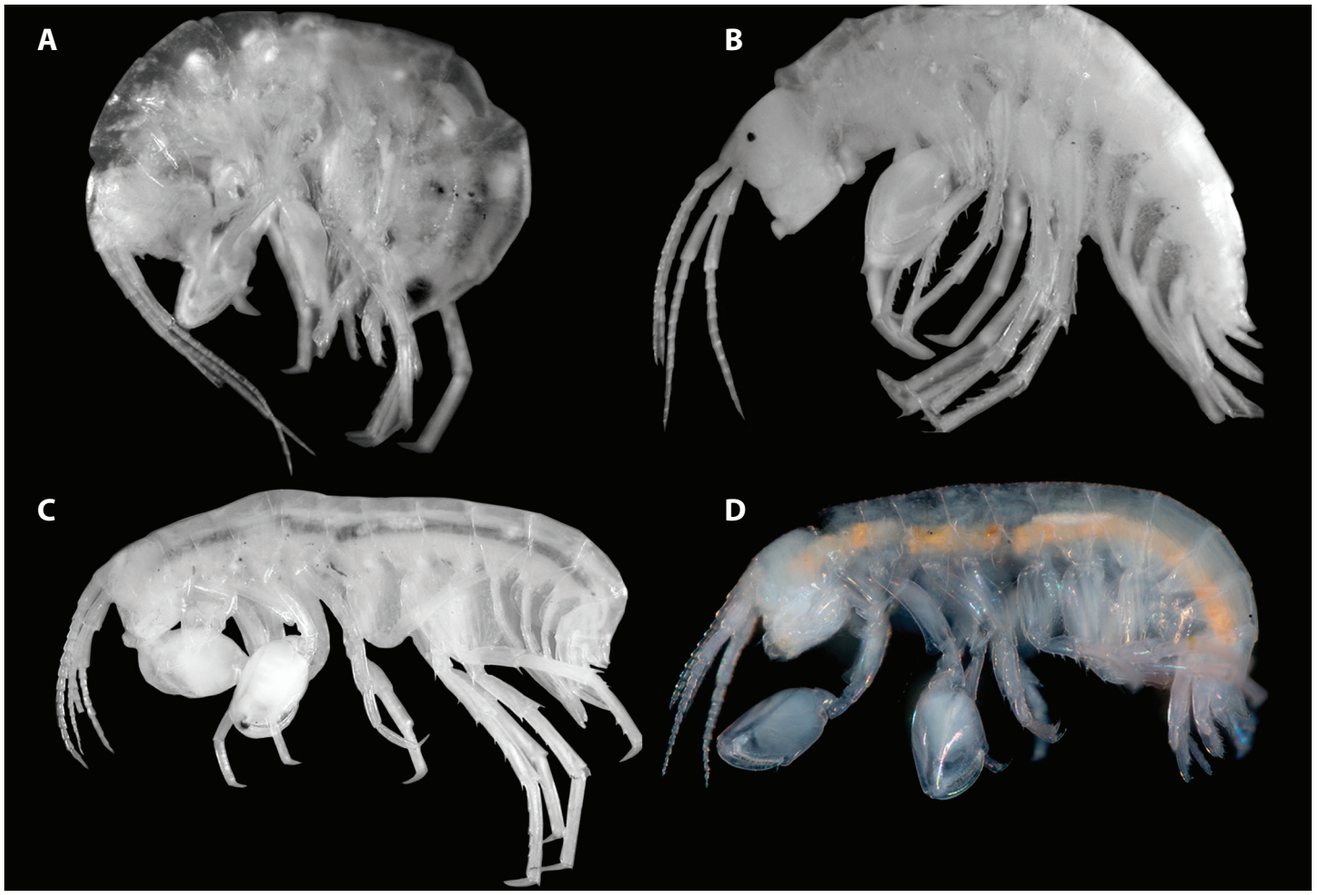


Figure 4. Specimens of Hyalellidae photographed under magnifying glass**. A)** *Hyalella caeca*; **B)** *H. formosa*; **C)** *H. veredae* and **D)** *H. troglofugia*. Scale: 0.5 mm. Photos: A-C (Giovanna M. Cardoso); D (Rafaela Bastos-Pereira).

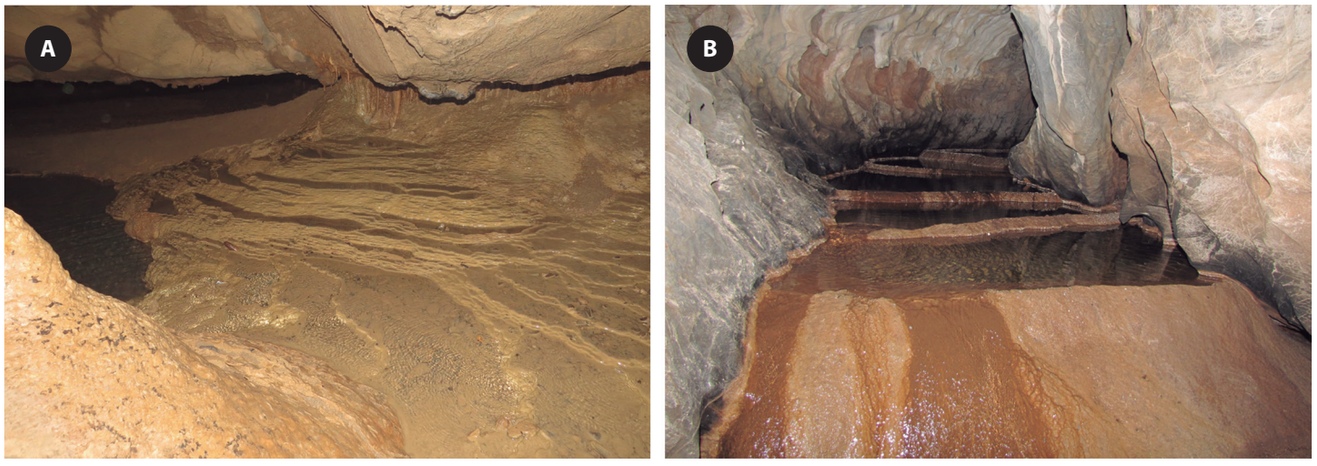


Figure 5. A) Areias de Cima cave (Iporanga, SP), location of collection of *Hyalella epikarstica*; **B)** Travertine reservoirs in Vereda da Palha cave (Presidente Olegário, MG) where specimens of *H. veredae* were collected. Photos: (Rodrigo L. Ferreira).

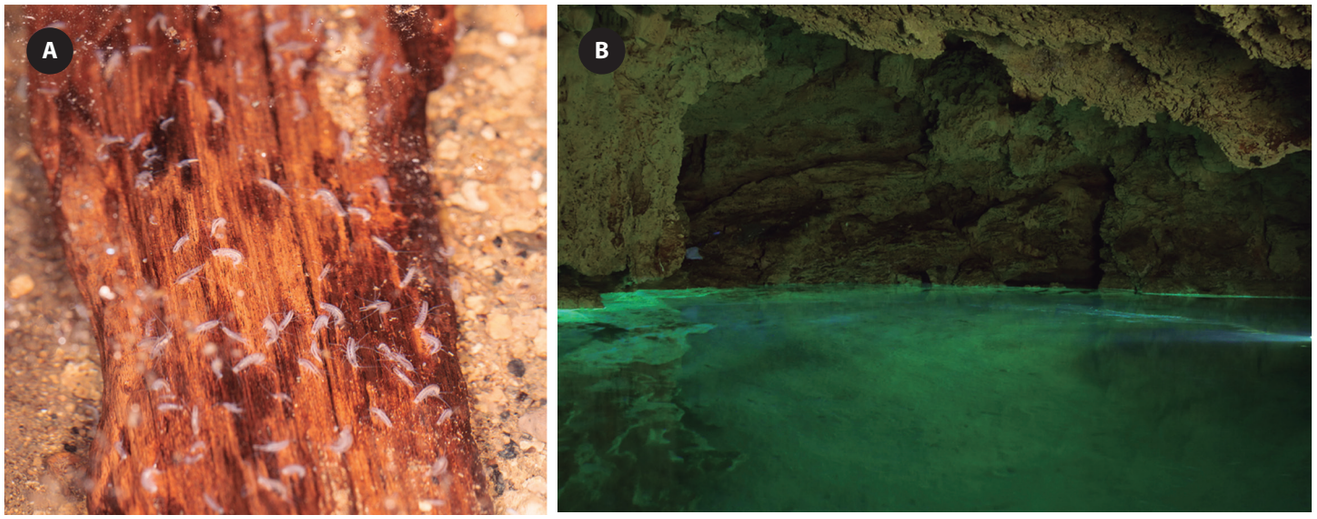


Figure 6. A) Live specimens of *Potiberaba porakuara*; **B)** Três Lagos cave, type locality of *P. porakuara*. Photos: A (Diego de M. Bento); B (Rodrigo L. Ferreira).



Figure 7. A) Lapa Doce II, one of the collection locations of *Spelaeogammarus spinilacertus* (Iraquara, BA); **B)** Toca da Jurema (Várzea Nova, BA) were specimens of *S. trajanoae* were collected; **C)** Lake in cave PEA-445 where specimens of *S. titan* were found (Santa Maria da Vitória, BA); **D)** Lake in which *S. uai* occurs in Lapa D'Água do Zezé cave (Itacarambi, MG). Photos: A (Marconi Souza-Silva); B-D (Rodrigo L. Ferreira).

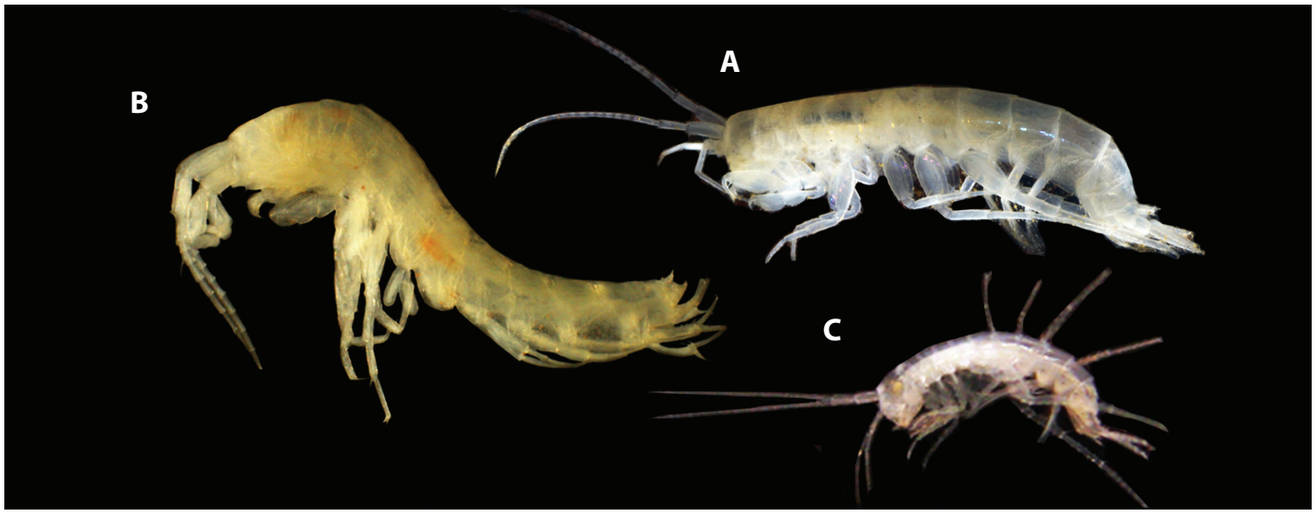


Figure 8. A) *Megagidiella azul* (Bogidiellidae); **B**) *Bogidiella neotropica* (Bogidiellidae), **C***) Potiberaba porakuara* (Mesogammaridae) Photos: A (Lívia Cordeiro e Paulo de Souza); B (BioEspeleo Consultoria Ambiental); C (Rodrigo L. Ferreira).

Although the maintenance of specimens in 100% ethanol is ideal for the preservation of genetic material, on which the success of molecular analysis depends, at this concentration ethanol makes the biological material more rigid, which can make it difficult to handle specimens at dissection for morphological analyses.



Figure 9. Trap made with PVC tube, screen and elastic used to collect Amphipoda in aquatic environments that are difficult to access. Photo: (Diego de M. Bento).

When kept in 70% ethanol, the material should be maintained in a place where there are no major thermal fluctuations nor sun exposure. The level of alcohol contained in the containers must be monitored periodically to avoid drying of the material, which makes future morphological analysis unfeasible.

SLIDE MOUNTING AND PREPARATION

The identification of species of Amphipoda usually requires the analysis of diagnostic morphological characteristics most often associated with the appendages, such as the gnathopods (more developed in males), palps of the mandible and telson shape. This analysis can involve observing and dissecting specimens under a stereomicroscope. The specimens can be handled with tweezers or brushes and dissected directly on a slide within medium (glycerin, glycerin gelatin, balm, Hoyer) (HOLANDA *et al.*, 2019; HUGHES; AHYONG, 2016). After positioning an entire specimen on a slide, its body length should be measured (anterior end of the head, close to the insertion of the first pair of antennae, to the posterior end of the telson). It is recommended that each portion of the body be dissected and preserved separately on separate coverslips (head, pereon and pleon). The formation of air bubbles must be avoided, taking care in handling the medium and the accommodation of the coverslip so that there is no extravasation and displacement of appendages. After positioning the coverslips, the slides are placed in an oven at 50 °C to dry (depending on the medium used, such as Hoyer and balm for example), and then sealed with varnish. Once dried and sealed, slides must be kept in a place free from humidity and sun exposure, and preferably with controlled temperature.



Figure 10. Map with the type localities of species of troglobitic amphipods described for Brazil.

Identification key

Figure 11 shows the main structures of taxonomic importance for the identification of amphipods. Below is a key to identify Neotropical Amphipoda families, which was adapted from Rogers *et al.* (2020). It is important to note that the key contains all the families known thus far for the Neotropical region. The purpose of this key presented in this book is to support studies in which amphipods found in the subterranean environment can be identified as to the taxonomic groups to which they belong, even if they have not yet been recorded for subterranean ecosystems in Brazil to date. For families that have a record of a single species for the Neotropical region, the species name is presented in the key, right after the family.

Five families are currently known (among those mentioned in the key) to occur in Brazilian subterranean environments. Since only one species is known for caves in Brazil for each of the families Mesogammaridae and Seborgidae, consultation of Fišer, Zagmajster and Ferreira (2013) is recommended for their identification. For Bogidiellidae, an identification key for the

NEOTROPICAL AMPHIPODA (adapted from Rogers *et al.*, 2020)

1. First antenna without accessory flagellum..... **2**
1′. First antenna with well-developed or miniscule accessory flagellum. **6**
2(1). Gnathopod 2 without sexual dimorphism..... **3**
2′. Gnathopod 2 with sexual dimorphism..... **4**
3(2). Coxa of pereopod 4 without postero-ventral lobe ..... **Hadziidae**
3′. Coxa of pereopod 4 with small or well-developed postero-ventral lobe ..... **Bogidiellidae** (key below)
4(2). Antenna 1 longer than peduncle of antenna 2 ..... **5**
4′. Antenna 1 shorter than peduncle of antenna 2..... **Talitridae**
5(4). Uropod 3 uniramus; mandible without palp; maxilliped palp, length of article two subequal to length of article one**:** .... ***Hyalella* (chave abaixo)**
5′. Uropod 3 uniramus or biramus; mandible with palp; maxilliped palp, length of article two > length of article one..... ..... **Corophiidae**
6(1). Length of accessory flagellum < length of three proximal articles of the main flagellum..... **...7**
6′. Length of accessory flagellum > length of three proximal articles of the main flagellum..... **14**
7(6). Uropod 3 uniramus..... **8**
7′. Uropod 3 biramus **10**
8(7). Coxal gills in segments 2–6 or 3–7 ..... **9**
8′. Coxal gills in segments 2–5..... **Seborgiidae: *Seborgia potiguar***
9(8). External gills present..... **Pseudingolfiellidae: *Pseudingolfiella chilensis***
9′. External gills absent..... **Metacrangonyctidae: *Metacrangonyx***
10(7). Eyes present..... **11**
10′. Eyes absent..... **13**
11(10). Uropod 3, endopod length subequal to exopod length..... **12**
11′. Uropod 3, endopod length < exopod length..... **Crangoweckeliidae**
12(11). Eyes poorly-developed..... **Paraleptamphopidae: *Rudolphia macrodactylus***
12′. Eyes rounded..... **Phreatogammaridae: *Ruffia patagonica***

13(10). Uropod 1 base without robust basofacial seta ..... **Falklandellidae**

13′. Uropod 1 peduncle with robust basofacial seta ..... **Hadziidae**

14(6). Antenna 2 calceoli present (at least in males) **15**

14′. Antenna 2 calceoli absent **16**

15(14). Coxal gills present in pereopods 2–6; uropod 3, endopod length 0.40x exopod length ..... **Gammaridae: *Gammarus minus***

15′. Coxal gills present in pereopods 2–7; uropod 3, endopod length 0.30x exopod length ..... **Anisogammaridae: *Eogammarus confervicolus***

16(14). Gnathopod 2 not reduced **17**

16′. Dactylus of gnathopod 2 reduced, attached to palm ..... **Mesogammaridae: *Potiberaba porakuara***

17(16). Mandibular molar with some type of grinding structure **18**

17′. Mandibular molar not grinding type **21**

18(17). Telson entire and notched **19**

18′. Telson cleft **20**

19(18). Length of pereopod 5 subequal to length of pereopod 6 ..... **Artesiidae *Spelaeogammarus*** (chave abaixo)

19′. Pereopod 5 shorter than pereopod 6 ..... **Bogidiellidae** (chave abaixo)

20(18). Length of antenna 1 subequal to length of antenna 2 ..... **Lysianassidae: *Ottenwalderia kymbalion***

20′. Antenna 1 longer than antenna 2 ..... **Maeridae: *Quadrivisio lutzi***

21(17). Telson entire and notched without robust setae ..... **Ingolfiellidae**

21′. Telson cleft with robust setae ..... **Pardaliscidae: *Spelaeonicippe provo***

NEOTROPICAL BOGIDIELLIDAE (adapted from Rogers *et al.*, 2020)

1. Pereonites 2–5 or 4–6 with large mid-sternal processes (= sternal humps) **2**

1′. Pereonites with mid-sternal processes **3**

2(1). Male with exopod of pleopod 2 without special seta inserted laterally in second article; peduncle of uropod 1 without hypertrophied face ..... ***Grossogidiella mauryi***

2′. Male: with exopod of pleopod 2 with modified distolateral seta in second article; peduncle of uropod 1 with modified hypertrophied robust facial seta ..... ***Patagongidiella***

3(1). Mandible with palp **4**

3′ Mandible without palp ..... ***Megagidiella azul***

4(3). Male: article 2 of exopod of pleopod 2 with unmodified setae **5**

4′. Male: exopod of pleopod 2 with flattened, concave seta with teeth, cilia or hamulus **9**

5(4). Palp of maxilla 1 with two articles; antenna 1 with accessory flagellum length ≥ length of first article of primary flagellum **6**

5′. Palp of maxilla 1 with one article; antenna 1 with accessory flagellum length ≥ length of first article of primary flagellum ..... ***Eobogidiella purmamarcensis***

6(5). Accessory flagellum of antenna 1 with two or three articles; uropod 1 sexually dimorphic, male exopod shortened and

modified and/or with raduliform setae on modified rami.....**7**

6′. Accessory flagellum of antenna 1 with two; uropod 1 generally without sexual dimorphism.....***Bogidiella***

7(6). Posterior margin of base of gnathopod 1 with three or five setae; accessory flagellum of antenna 1 with two articles.....**8**

7′. Posterior margin of base of gnathopod 1 with one seta.....***Arganogidiella***

8(7). Pereopod 7 sexually dimorphic (male enlarged carpus) .....***Orchestigidiella orchestipes***

8′ Pereopod 7 without sexual dimorphism .....***Guagidiella***

9(4). Telson length ≥ width; uropod 2 without sexual dimorphism **10**

9′Telson length < width; male usually with endpod of uropod 2 fused to basipodite or shortened **11**

10(9). Uropods 1–3 not sexually dimorphic.....**13**

10′. Uropod 1 sexually dimorphic, female rami macronate (male uropod normal) .....***Antillogidiella martini***

11(9). Pleopod 1 not sexually dimorphic, basipodite without extension on inner margin **12**

11′. Pleopod 1 sexually dimorphic, endopod with only one reduced article, exopod with three articles, male with first two articles dilated with modified setae com, second article with reduced and curved external setae with medial plumose part inserted at right angle before tip; medial margin of basipodite of pleopod 1 with ample internal growth .....
.....***Mesochthongidiella tucumanensis***

12(11). Male with endopod of uropod 2 fused with basipodite or very short endopod.....***Dycticogidiella***

12′. Male with uropodo 2 with subequal rami or slightly shortened endopod.....***Argentinogidiella***

13(10). Pleopod 1 not sexually dimorphic.....***Stygogidiella***

13′. Pleopodo 1 of male with differentiated seta (rigid, articulated, pointed and serrated).....***Mexigidiella***

TROGLOMORPHIC *HYALELLA* (HYALELLIDAE) OF BRAZIL

1. Male, internal ramus of uropod 1 without curved seta..... **2**

1′. Male, internal ramus of uropod 1 with curved seta **5**

2(1). Telson, apical setae absent ..... ***H. montana***

2′. Telson, apical setae present **3**

3(2). Telson, with two apical setae..... **4**

3′. Telson, with four apical setae ..... ***H. troglofugia***

4(1). Male, posterior margin of propodus of gnathopod 2 without *comb scales*.....***H. caeca***

4′. Male, posterior margin of propodus of gnathopod 2 with *comb scales*.....***H. spelaea***

5(1). Male, posterior margin of propodus of gnathopod 2 with *comb scales*.....***H. veredae***

5′. Male, posterior margin of propodus of gnathopod 2 without *comb scales*.....**6**

6(5). Internal face of propodus of gnathopod 1 with up to five pappose setae.....**7**

6′. Internal face of propodus of gnathopod 1 with more than five pappose setae .....***H. imbya***

7(6). Antenna 2 with up to eight articles on flagellum .....***H. epikarstica***

7′. Antenna 2 with more than eight articles on the flagellum .....***H. formosa***

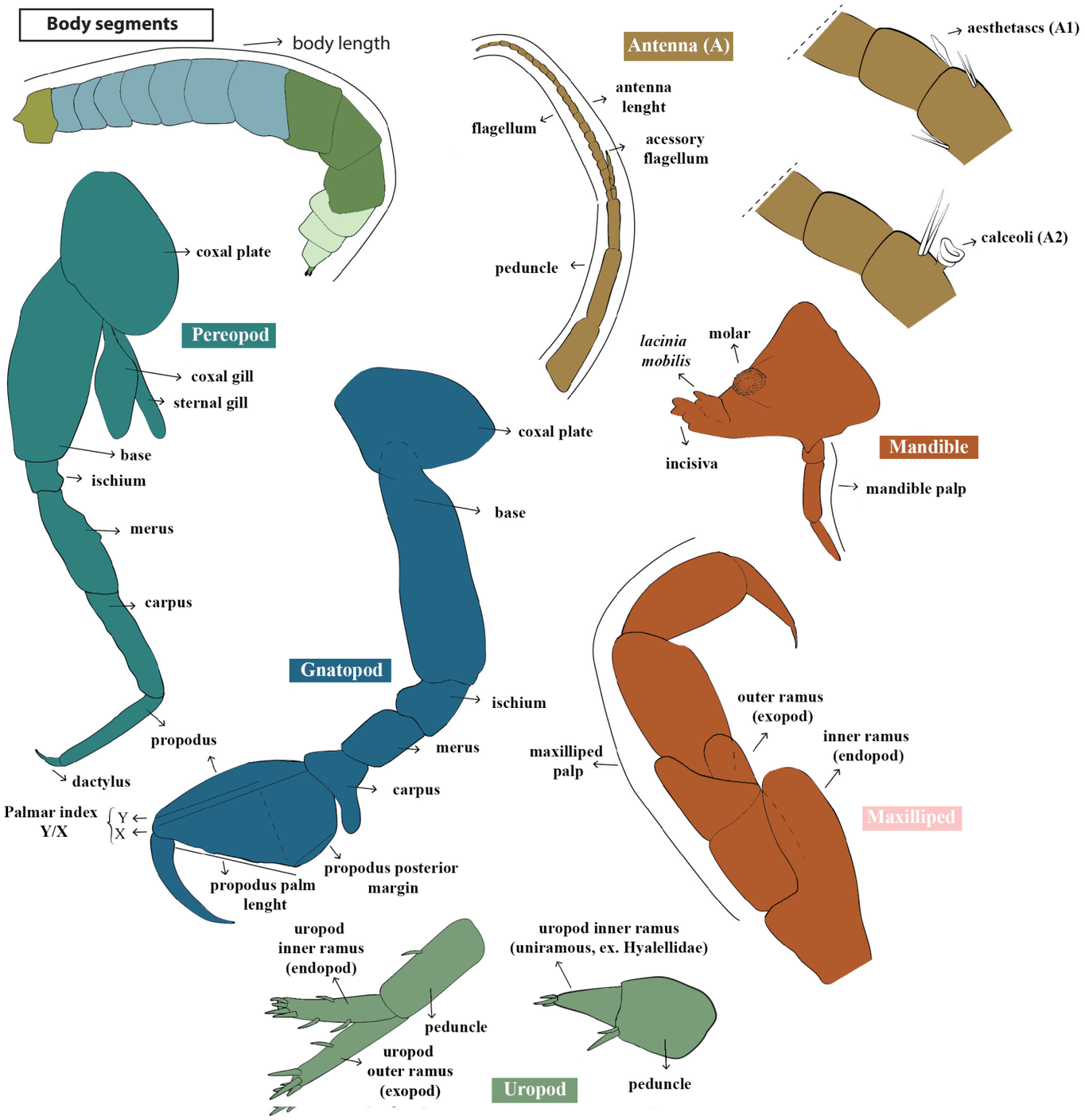


Figure 11. Main structures of taxonomic importance for amphipod identification.

Neotropical genera is presented. As *Megagidiella* is a monotypic genus, the characteristics for identifying the genus and *Megagidiella azul* can be consulted in Koenemann and Holsinger (1999). Since only one species of the genus *Bogidiella* occurs in Brazil, the description of the species (*Bogidiella neotropica*) can be consulted for identification (RUFFO, 1952). The family Hyalellidae, on the other hand, is composed only of the genus *Hyalella*, for which there is an identification key for the troglomorphic species of Brazil. For Artesiidae an identification key of the species of *Spelaeogammarus* is presented. It is important to emphasize that the identification of families, genera and species is fundamental and, whenever possible, should be confirmed based on the respective diagnoses, especially considering the large number of taxa not yet described that are commonly found associated with subterranean ecosystems in Brazil.

Main Brazilian collections

Most of the troglomorphic species of amphipods found in the country are deposited in national collections. Among them, the collection of the Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ) stands out for holding the most type specimens of troglobitic species in Brazil. This collection holds types of the species *Hyalella epikarstica, H. spelaea, H. imbya, Megagidiella azul, Spelaeogammarus spinilacertus* and *S. trajanoae*. Other type material are deposited in the Coleção de Invertebrados Subterrâneos da Universidade Federal de Lavras (ISLA/UFLA) (holotypes: *H. troglofugia, S. sanctus, S. titan* and *S. uai*; paratypes: *H. epikarstica, H. spelaea, H. imbya* and *H. veredae*, in addition to specimens of *Potiberaba porakuara* and *Seborgia potiguar*); at the Museu da Universidade de São Paulo (MZUSP) (holotypes: *H. veredae* and *H. formosa*); in the collection of the Universidade Federal do Rio Grande do Sul (UFRGS) (paratypes: *H. formosa*); and at the Museu da Universidade Federal da Bahia (MZUFBA) (paratype: *S. titan*).

Some collections abroad contain type material, such as the Collection of the Department of Biology of the University of Ljubljana (Slovenia) with material of *Potiberaba porakuara* and *Seborgia potiguar*; and the Museo Civico di Storia Naturale di Verona (Italy) with material of *Bogidiella neotropica*. In Brazil, Coleção de Invertebrados Subterrâneos de Lavras (UFLA) also contains representatives of these species deposited in its collection.

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Loxosceles troglobia
Lucas Mendes Rabelo

8

Araneae

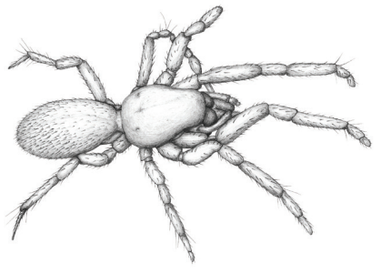
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General introduction

The order Araneae contains more than 50,000 described species worldwide (WORLD SPIDER CATALOG, 2022). The group is recognized for representing a significant part of the diversity in subterranean environments, more specifically in caves, when compared to other groups of invertebrates (TRAJANO; BICHUETTE, 2010).

Studies of cave spiders in Brazil can be considered recent. The first inventories in the country were only published in the 1970s, with works by Brignoli (1972) and Eickstedt (1975). These studies presented inventories of spiders collected in caves located in Iporanga, state of São Paulo. However, the first spider recorded in a cave was a white and blind member of the family Hahniidae, considered troglobitic, collected in 1968 by the Swiss speleologist Pierre Strinati at Gruta da Tapagem (currently Caverna do Diabo) in Eldorado, state of São Paulo (BRIGNOLI, 1972). Unfortunately, this primordial material of Brazilian fauna, as well as other species recorded at the time by Brignoli (1972), are now deposited in the Natural History Museum in Geneva, Switzerland.

The oldest records in Brazilian collections are from the 1970s, *Trechalea longitarsis* (C. L. Koch, 1847) (Trechaleidae), from Alambari Cave, collected in February 1970, and *Loxosceles adelaida* Gertsch, 1967 (Sicariidae), from Caverna de Santana (Caverna de Sant'Anna), collected in July 1972. Both are in the collection of Instituto Butantan and come from the Parque Estadual Turístico do Alto da Ribeira (PETAR), in Iporanga, state of São Paulo.

Several records of cave fauna were published for Brazil in the 1980s, in addition to some reports on Mygalomorphae. However, reports of this infraorder in caves can be considered incipient when compared to information about Araneomorphae and other arthropod groups. Trajano (1987) discussed mygalomorph fauna (family Theraphosidae) composition and stated that they were not uncommon in caves in the Speleological Province of Bambuí, especially in caves of the state of Bahia.

About two decades later came the first synopsis of the cave fauna of Brazil, published by Pinto-da-Rocha (1995), compiling data from studies published between 1907 and 1994. This extensive historical review presented the diversity of invertebrates from 284 natural subterranean cavities of eight Brazilian states, with records of 71 species from 34 spider families, without any highlights of troglobitic species. Several articles were subsequently published with new species of cave spiders in Brazil (BAPTISTA, 2003; BAPTISTA *et al.*, 2008; RHEIMS; BRESCOVIT, 2004). More recently, Trajano and Bichuette (2010) and Gallão and Bichuette (2018) synthesized this information, presenting a table with 15 species of Brazilian troglomorphic spiders, distributed among ten families. These works represent current knowledge, but there are numerous species yet to be described.

These articles exemplify the little knowledge about the taxonomy of spiders in caves in relation to other non-cave ecosystems. It is also notable that the first troglomorphic spider collected in Brazilian caves, *Speocera eleonorae* (Ochyroceratidae), was described only in the 21st century, by Baptista (2003). Since then, a number of other works with troglomorphic spiders have been published, highlighting the descriptions of *Tricongius ybyguara*, Gnaphosidae (RHEIMS; BRESCOVIT, 2004); *Metagonia diamantina*, Pholcidae (MACHADO *et al.*, 2011); *Brasilomma enigmatica*, Gnaphosidae (BRESCOVIT *et al.*, 2012*)*; *Tmesiphantes hypogeus*, Theraphosidae (BERTANI *et al.*, 2013); *Harmonicon cerberus*, Dipluridae (PEDROSO; BAPTISTA, 2014); *Carajas paraua*, *Tisentnops mineiro* and *T. onix*, Caponiidae (BRESCOVIT; SÁNCHEZ-RUIZ, 2016); *Paracymbiomma caecus* and *P. bocaina*, Gnaphosidae (RODRIGUES *et al.*, 2018); *Loxosceles troglobia*, Sicariidae (SOUZA; FERREIRA, 2018); *Tonton itabirito* and *T. matodentro*, Microstigmatidae (PASSANHA *et al.*, 2019); six species of the genus *Matta* (Tetrablemmidae; Brescovit and Cizauskas, 2019); *Indiani gaspar*, Gnaphosidae (RODRIGUES *et al.*, 2020); *Ochyrocera ritxoco, O. ritxoo, O. dorinha* and *O. rosinha*, Ochyroceratidae (BRESCOVIT *et al.*, 2021a; BRESCOVIT *et al.*, 2021b); and *Ctenus igatu*, Ctenidae (CIZAUSKAS *et al.*, 2022). These descriptions comprise 26 troglobitic species and represent a significant increase in knowledge of the Brazilian subterranean spider fauna (Table 1).

This period also witnessed an increase in the number of described non-troglomorphic species to 39 (BAPTISTA *et al.*, 2008; BRESCOVIT *et al.*, 2016; BRESCOVIT *et al.*, 2018; BRESCOVIT; CIZAUSKAS, 2018; FONSECA- FERREIRA *et al.*, 2017; GUADANUCCI *et al.*, 2016; PRETE *et al.*, 2016; SANTANNA; RODRIGUES, 2018; SANTANNA *et al.*, 2019), significantly expanding the diversity of the Brazilian spider fauna in cave environments.

We believe that this increase in records of cave species is due, in part, to the need for environmental licensing studies resulting from the publication of Federal Decree No. 6,640, of November 7, 2008. As a result, there was an expansion of speleobiological studies and a significant increase in the number of spiders deposited in the country's zoological collections. Currently, 13 years after the publication of this decree, there are thousands of samples, with more than 75,000 specimens of cave spiders deposited in the main arachnological collections in Brazil (CIZAUSKAS, 2017).

However, despite the high abundance of spiders found in cave ecosystems, associated with their behavioral characteristics, such as the construction of aerial webs that facilitate the location of specimens in this type of environment and, consequently, the collection of data for taxonomic, biological and ecological studies (CIZAUSKAS, 2017), basic knowledge about the diversity, distribution patterns, ecology and behavior, as well as level of level of endemism of these species in the subterranean environment is still very incipient (CARDOSO *et al.*, 2011; TRAJANO, 2012; TRAJANO; BESSI, 2017; TRAJANO; CARVALHO, 2017). The lack of information on the distribution and rarity of the vast majority of cave species represent important knowledge gaps for vulnerability assessment, as well as for the definition of conservation strategies, for subterranean species (CIZAUSKAS, 2017).

Main groups found In Brazilian caves

Spiders are among the most diverse and abundant predators in the subterranean environment, being found in edaphic horizons, on the surface subterranean environment (SSE), and in deep areas of the hypogean environment such as caves. The diversity of species currently known for Brazilian caves is still small (± 280 species) (CIZAUSKAS, 2017). However, their richness and abundance are considered high compared to most other groups of cave invertebrates (GALLÃO; BICHUETTE, 2018), and they are recognized as natural biological controllers in these environments (CIZAUSKAS, 2017).

The most diverse spider groups in Brazilian caves are represented by troglophilic species. Troglophiles are species with populations established in both epigean and hypogean environments, with individuals that can transit between, promoting gene flow between populations from different ecosystems (TRAJANO; CARVALHO, 2017). The most frequently observed spiders of this group are aerial web-building species, such as those of Pholcidae (e.g., *Mesabolivar* spp., *Metagonia* spp. – Figure 1A-B, 2G), Araneidae (e.g., *Alpaida* spp. – Figure 1C), Theridiosomatidae (e.g., *Plato* spp. – Figure 1D, *Cuacuba* spp.), Theridiidae (e.g., *Cryptachaea* spp.), and Scytodidae (*Scytodes* spp. – Figure 1E); species of wandering spiders usually found on the ground or on walls, such as of Sicariidae (e.g., Loxosceles spp. – Figure 1F) and Ctenidae (e.g., *Isoctenus* spp., *Enoploctenus* spp. – Figure 1G, H); funnel web building species like of Dipluridae (e.g., *Trechona* spp., *Diplura* spp. – 2K, L); and burrow building species such as of Rhytidicolidae (*Fufius* spp.), among others of the families Theraphosidae (e.g., *Dolichothele* spp., *Acanthoscurria* spp. – Figure 1J, L, *Theraphosa* spp., *Nhandu* spp.) and Microstigmatidae (*Tonton* spp.). These spiders are well represented in biologoical material collected in caves and deposited in collections.

On the other hand, some spider families have low diversity and are represented by only one or two species, such as Corinnidae (e.g., *Abapeba hoeferi* Bonaldo, 2000 – Figure 2A), Uloboridae (e.g., *Philoponella* spp.), Segestriidae (e.g., *Ariadna* spp. – Figure 2B), Filistatidae (e.g., *Misionella carajas* Brescovit *et al.*, 2016 – Figure 2C), Nesticidae (e.g., *Eidmannella pallida* (Emerton, 1875) – Figure 2D), Salticidae (e.g., *Soesilarishius* sp. 2H) and Mysmenidae (e.g., *Microdipoena* sp.). Among the known troglophilic species, it is worth mentioning the mygalomorphs *Trechona diamantina* Guadanucci *et al.* (2016), of the family Dipluridae, and *Guyruita metallophila* Fonseca-Ferreira *et al.*



Figure 1. A) *Mesabolivar togatus* (Keyserling, 1891) (Pholcidae); **B)** *Metagonia* sp. (Pholcidae); **C)** *Alpaida antonio* Levi, 1988 (Araneidae); **D)** *Plato novalima* Prete, Cizauskas & Brescovit, 2018 (Theridiosomatidae); **E)** *Scytodes eleonorae* Rheims & Brescovit, 2001 (Scytodidae); **F)** *Loxosceles similis* Moenkhaus, 1898 (Sicariidae); **G)** *Enoploctenus cyclothorax* (Bertkau, 1880) (Ctenidae); **H)** *Ctenus* sp. (Ctenidae); **I)** *Ochyrocera* sp. (Ochyroceratidae); **J)** *Acanthoscurria geniculata* (C. L. Koch, 1841) (Theraphosidae); **K)** Cyrtaucheniidae; **L)** *Acanthoscurria* sp. (Theraphosidae). Photos: A-I, K (Robson de A. Zampaulo); J (Igor Cizauskas); L (Lucas M. Rabelo).

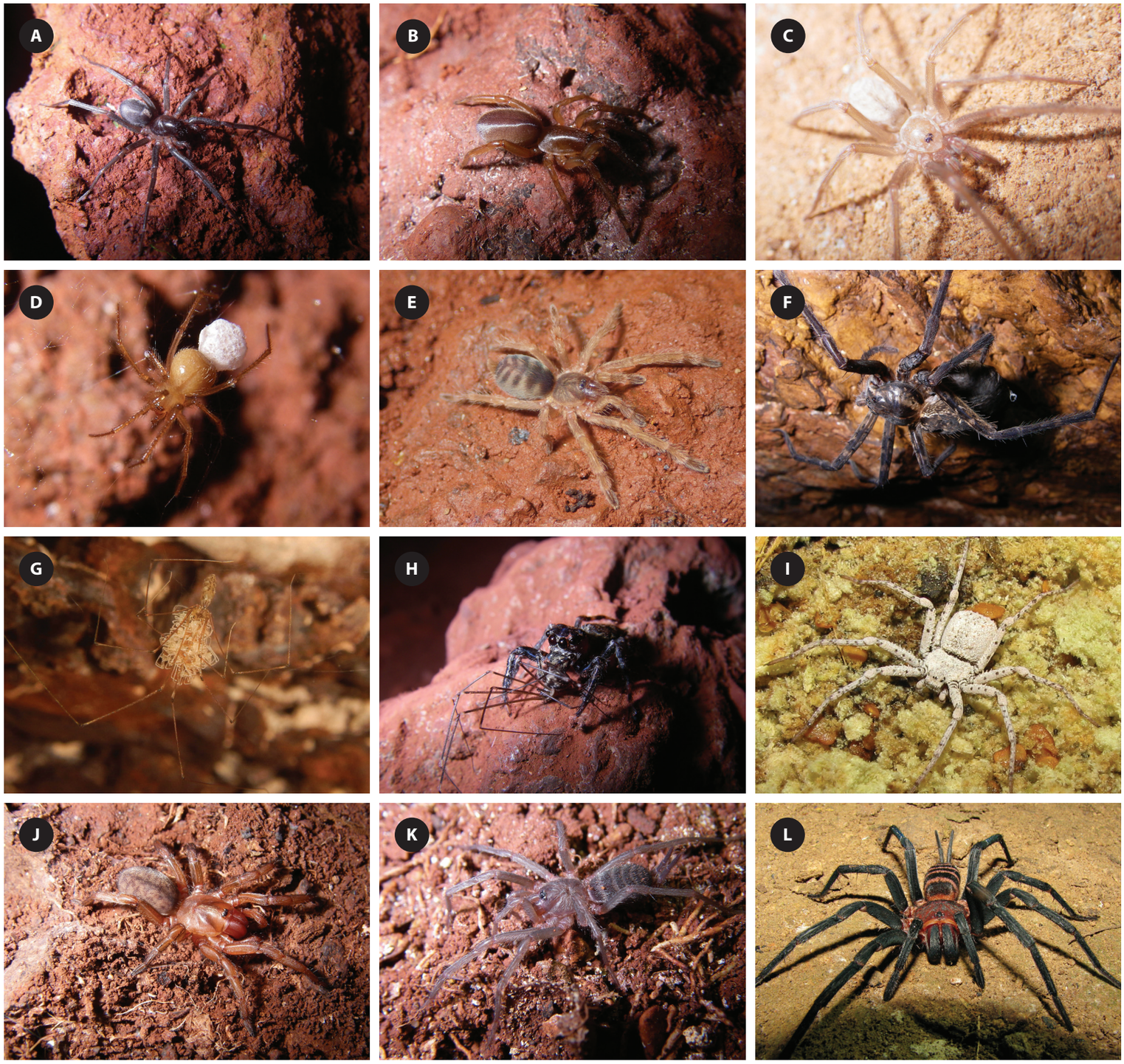


Figure 2. A) *Abapeba hoeferi* Bonaldo, 2000 (Corinnidae); **B)** *Ariadna* sp. (Segestriidae)*;* **C)** *Misionella carajas* Brescovit, Magalhães & Cizauskas, 2016 (Filistatidae); **D)** *Eidmannella pallida* (Emerton, 1875) (Nesticidae); **E)** *Guyruita metallophila* Fonseca-Ferreira, Zampaulo & Guadanucci, 2017 (Theraphosidae); **F)** *Trechaleoides keyserlingi* (F. O. Pickard-Cambridge, 1903) (Trechaleidae); **G)** Pholcidae sp.; **H)** *Soesilarishius* sp. (Salticidade); **I)** *Sicarius tropicus* (Mello-Leitao, 1936) (Sicariidae); **J)** Nemesiidae; **K)** *Linothele* sp. (Dipluridae); **L)** *Trechona* sp. (Dipluridae). Photos: A-B, D, F, H, J, K (Robson de A. Zampaulo); C, E (Lucas M. Rabelo); G (Matheus H. Simões); I, L (Xavier Prous).



Figure 3. A) *Carajas paraua* Brescovit & Sánchez-Ruiz, 2016 (Caponiidae); **B, K)** *Tisentnops mineiro* Brescovit & Sánchez-Ruiz, 2016 (Caponiidae); **C)** *Tonton itabirito* Passanha, Cizauskas & Brescovit, 2019 (Microstigmatidae); **D)** *Paracymbiomma caecus* Rodrigues, Cizauskas & Rheims, 2018 (Gnaphosidae); **E)** *Brasilomma* sp. (Gnaphosidae); **F)** *Metagonia* sp. (Pholcidae); **G)** *Loxosceles troglobia* Souza & Ferreira, 2018 (Sicariidae); **H)** *Ochyrocera dorinha* Brescovit *et al.*, 2021 (Ochyroceratidae); **I)** *Matta cambito* Brescovit & Cizauskas, 2019 (Tetrablemmidae); **J)** *Lygromma* sp. (Gnaphosidae); **L)** *Ctenus igatu* Polotow, Cizauskas & Brescovit, 2022. Photos: A-C, E-F, H-K (Robson de A. Zampaulo); D (Matheus H. Simões); G (Lucas M. Rabelo); L) Jonas E. Gallao.

(2017) (Figure 2E), of the family Theraphosidae, which have large populations and use caves as breeding sites (GUADANUCCI *et al.*, 2016; FONSECA-FERREIRA *et al.*, 2017).

In caves with sinkholes, waterfalls or water sources, spiders of the families Trechaleidae (e.g., *Trechaleoides* spp. – Figure 2F) and Pisauridae (e.g., *Thaumasia* spp.) are also frequently found. However, small spiders (< 1 cm), such as representatives of the families Oonopidae (e.g., *Neotrops* spp., *Stenoonops* spp., *Triaeris stenaspis* Simon, 1892), Ochyroceratidae (e.g., *Ochyrocera* spp., *Speocera* spp., *Theotima* spp.), Symphytognathidae (e.g., *Anapistula* spp.), Theridiidae (e.g., *Thymoites* spp.), Tetrablemmidae (e.g., *Matta* spp.) and Theridiosomatidae (*Plato* spp.), make up most of the biodiversity of spiders collected in caves in Brazil and most still lack specific taxonomic studies.

In addition to these groups, accidental spiders also occur in caves, which are specimens with epigean populations that enter caves passively or actively in search of temporary shelter, or carried by physical agents such as wind or water, and can only survive in caves temporarily (TRAJANO; CARVALHO, 2017). In general, these records are represented by species from the families Oxyopidae, Sparassidae, Lycosidae, Tetragnathidae, Selenopidae, Hersiliidae, Anyphaenidae, Thomisidae, Miturgiidae, Oecobiidae, Zoropsidae, Barychelidae, Idiopidae, Nemesiidae, Rhytidicolidae and Pycnothelidae. Examples of these families are usually found only in areas of the cave entrance, where environmental conditions are different and there is still incidence of epigean luminosity, as well as the establishment of vegetation.

In turn, troglobitic spiders (endemic species of subterranean habitats) represent a tiny portion of the diversity of species described for Brazil (± 0.8%) and a small portion of the species collected in caves in the country (± 9%). They are represented by 14 genera distributed among ten families: Caponiidae (Gen. *Carajas* and *Tisentnops* – Figure 3A, B), Ctenidae (*Ctenus* – Figure 3L), Dipluridae (*Harmonicon*), Gnaphosidae (*Tricongius*., *Brasilomma*, *Paracymbiomma* and *Indiani* – 3D-E), Ochyroceratidae (*Speocera* and *Ochyrocera* – 3H), Sicariidae (*Loxosceles* – 3G), Microstigmatidae (*Tonton* - 3C), Pholcidae (*Metagonia* – 3F), Tetrablemmidae (*Matta* - 3I) and Theraphosidae (*Tmesiphantes*).

Diversity, distribution and main troglomorphisms of troglobitic spiders

The term troglomorphism is used to distinguish a set of adaptations, especially morphological, for subterranean life (CHRISTIANSEN, 1962; TRAJANO; BICHUETTE, 2006). For subterranean spiders, these main morphological features include reduction or loss of cuticular pigments, ocular regression or loss, thinning of integument, elongation of appendages, trichobothria, and accentuated spinulation (ARNEDO *et al.*, 2007; DEELEMAN-REINHOLD, 1978; DEELEMAN-REINHOLD; DEELEMAN 1980; CIZAUSKAS *et al.*, 2021; COKENDOLPHER, 2004; GERTSCH; 1992; MAMMOLA; ISAIA, 2017; MARROQUÍN, 2014; MILLER, 2005; RODRIGUES *et al.*, 2018; PEDROSO; BAPTISTA, 2014; RŮŽIČKA, 1999; RŮŽIČKA *et al.*, 2011; RŮŽIČKA *et al.*, 2013).

In turn, physiological specializations refer to low metabolic rates, altered circadian rhythm, reduced fecundity, late maturity, slow development and changes in reproductive strategies (K strategies – larger eggs and greater quantity, in addition to less frequent reproductive cycles) (DEELEMAN-REINHOLD, 1978; DEELEMAN-REINHOLD; DEELEMAN, 1980; GERTSCH, 1992; HOWARTH, 1983; HÜPPOP, 2000; JUBERTHIE, 1985). As they are mostly scarce species and difficult to record, behavioral information for subterranean spiders is rare and represented, in large part, by occasional observations, usually related to parental care, such as females carrying ootheca, or reproductive behaviors (ALMEIDA-SILVA *et al.*, 2009; CARVER *et al.*, 2016; CHRISTIANSEN, 2012; DORAN *et al.*, 2001; LEDFORD, 2004).

The most frequently observed troglomorphisms of subterranean spiders known for Brazil are ocular reduction and reduced cuticular pigmentation. These morphological features are observed in species that colonize both the hypogean edaphic environment (soil) and deep subterranean environment (caves). However, families such as Gnaphosidae (e.g., *Paracymbiomma* sp.), Oonopidae (e.g., *Coxapopha* sp.), Symphytognathidae (*Anapistula* sp.), Microstigmatidae (*Tonton* sp.) and Hahniidae (e.g., *Harmiella* sp.) also have epigean species with ocular reduction and/or cuticular depigmentation in soil and leaflitter dwellers. An example of this condition is *Harmiella schiapelliae* Brignoli, 1979 (Hahniidae), a species with troglomorphic characteristics (body depigmentation, anophthalmia and few bristles in the eye area) described for litter environments in Nova Teutônia, state of Santa Catarina (BRIGNOLI, 1979).

The troglobitic spiders described so far are located in all Brazilian biomes, except in the Pampa of the state of Rio Grande do Sul. They are represented by 26 species, all with troglomorphic characteristics and distributed in different regions of Brazil. Table 1 presents the species diversity, the main troglomorphisms observed in each, the lithology, and biome(s) of occurrence. Figure 4 shows a map of the geographic

distribution of the troglobitic spider fauna described for Brazil, as well as the lithologies of the caves with which these spiders are associated.

Although expressive, when compared to other groups of invertebrates, the diversity of subterranean spiders currently described for the country represents only part of the knowledge of the cave fauna in Brazil. In addition to the species presented here, other troglomorphic species of the families Corinnidae, Ctenidae, Hahniidae, Nesticidae, Ochyroceratidae (*Ochyrocera* and *Theotima*), Oonopidae (*Stenoonops*, *Xyccarph*), Pholcidae (*Metagonia*) and Symphytognathidae (*Anapistula*), are already known from specimens deposited in biological collections but are not yet described. Indeed, several of these families, especially Ochyroceratidae, Oonopidae and Pholcidae, lack extensive taxonomic revisions.

Main collection methods

Terrestrial obligate subterranean species (troglobites) evolved under particular selective conditions, such as total darkness, low amounts of food (with exceptions) and high and constant relative humidity (GALLÃO; BICHUETTE, 2018; TRAJANO, 2012). Thus, the methodologies for collecting subterranean spiders are based on the principle of exploring the hypogean environment, adapting to the characteristics of the habitat and its limitations.

Unlike sampling in epigean environments, there is greater difficulty in installing traps in caves due to the intrinsic characteristics of the environment. Thus, active manual collection is the most used methodology for studying the biodiversity of cave spiders. In addition to low cost, active search allows the researcher to explore the entire environment within reach, both in aquatic and terrestrial environments (Figure 5A-B). Almost the entirety of the spider cave fauna deposited in scientific collections was collected by this method. Pitfall-traps (Figure 5C-D) are also used, especially for sampling wandering spiders, but are not efficient for collecting web-buidling specimens.

Methods for sampling soil (Figure 5E) and leaflitter (Figure 5F), include the Berlese Funnel and the Winkler Extractor (Figure 5F-H). Pitfall-traps installed in boreholes (Figure 5I) or directly into the soil are commonly used to sample the superficial subterranean environment (Figure 5J). However, for sampling rocky environments, vulcan-traps (Figure 5K) provide an alternative method, especially in caves where the floor is composed of the rock matrix itself or where it is not possible to excavate the soil due to the substrate having many loose rocks (blocks) or in cases where the sediment grains are thin (CIZAUSKAS; GIROTI, 2011).

These traps are essential for a better sampling of subterranean biodiversity because they are efficient and inexpensive. Likewise, sampling in epigean environments is important to support assessments of the ecological and evolutionary relationships of this biodiversity with the hypogean environment (endemism).

Table 1. List of the 26 troglobitic spider species described for Brazil, 2021. Troglomorphisms observed in each, abbreviations: (1) depigmentation, (2) ocular reduction, (3) absence of eyes, (4) elongation of appendages (legs and/or or pedipalps), (5) body reduction, (6) increased trichobothria. Biomes of occurrence: (Ama) Amazon Forest, (Atl) Atlantic Forest, (Pan) Pantanal, (Caa) Caatinga, (Cer) Cerrado. Lithology of the caves of occurrence: (Fe) iron rocks: (Ca) carbonate rocks, (Si) siliciclastic rocks.

FAMILY	N	SPECIES	Biome (State) / Lithology	Troglomorphisms
Caponiidae	1.	*Carajas paraua* Brescovit & Sánchez-Ruiz, 2016	Ama (PA) – Fe	3, 6
	2.	*Tisentnops mineiro* Brescovit & Sánchez-Ruiz, 2016	Cer (MG) – Fe	3, 6
	3.	*Tisentnops onix* Brescovit & Sánchez-Ruiz, 2016	Cer (MG) – Ca	2
Ctenidae	4.	*Ctenus igatu* Cizauskas *et al.*, 2021	Atl (BA) – Si	1, 2, 4, 5

Dipluridae	5.	*Harmonicon cerberus* Pedroso & Baptista, 2014	Ama (PA) – Fe	1, 2, 4
Gnaphosidae	6.	*Brasilomma enigmatica* Brescovit, Ferreira & Rheims, 2012	Cer (MG) – Fe, Si	1,3
	7.	*Indiani gaspar* Rodigues, Cizauskas & Lemos, 2020	Cer (MG) – Ca	1,2
	8.	*Paracymbiomma caecus* Rodrigues, Cizauskas & Rheims, 2018	Ama (PA) – Fe	1, 3, 4
	9.	*Paracymbiomma bocaina* Rodrigues, Cizauskas & Rheims, 2018	Ama (PA) – Fe	1,2
	10.	*Tricongius ybyguara* (Rheims & Brescovit, 2004)	Atl (MG) – Ca	1, 3, 4
Microstigmatidae	11.	*Tonton itabirito* Passanha, Cizauskas & Brescovit, 2019	Cer (MG) – Fe	1,3,5
	12.	*Tonton matodentro* Passanha, Cizauskas & Brescovit, 2019	Cer (MG) – Fe	1,2,5
Ochyroceratidae[1]	13.	*Speocera eleonorae* Baptista, 2003	Pan (MS) – Ca	1,2
	14.	*Ochyrocera ritxoco* Brescovit, Zampaulo & Cizauskas, 2021	Ama (PA) – Fe	1,2
	15.	*Ochyrocera ritxoo* Brescovit, Zampaulo & Cizauskas, 2021	Ama (PA) – Fe	1,2
	16.	*Ochyrocera dorinha* Brescovit *et al.*, 2021	Cer (MG) - Fe	1, 3, 6
	17.	*Ochyrocera rosinha* Brescovit *et al.*, 2021	Cer (MG) - Fe	1, 6,
Pholcidae	18.	*Metagonia diamantina* Machado, Ferreira & Brescovit, 2011	Atl (BA) – Ca	1,3
Sicariidae	19.	*Loxosceles troglobia* Souza & Ferreira, 2018	Caa (BA) – Ca	1, 2, 4
Tetrablemmidae	20.	*Matta cambito* Brescovit & Cizauskas, 2019	Cer (MG) – Fe	3
	21.	*Matta humhum* Brescovit & Cizauskas, 2019	Cer (MG) – Fe	3
	22.	*Matta humrrum* Brescovit & Cizauskas, 2019	Cer (MG) – Ca	3
	23.	*Matta nuusga* Brescovit & Cizauskas, 2019	Cer (MG) – Fe, Ca	2
	24.	*Matta pititinha* Brescovit & Cizauskas, 2019	Cer (MG) – Ca	3
	25.	*Matta teteia* Brescovit & Cizauskas, 2019	Cer (MG) – Ca	3
Theraphosidae	26.	*Tmesiphantes hypogeus* Bertani, Bichuette & Pedroso, 2013	Atl (BA) – Si	1, 2, 4

1 After the elaboration of this chapter, three new troglobitic species of the genus *Speocera* were described for the region of Carajás, state of Pará (BRESCOVIT *et al.* 2022).

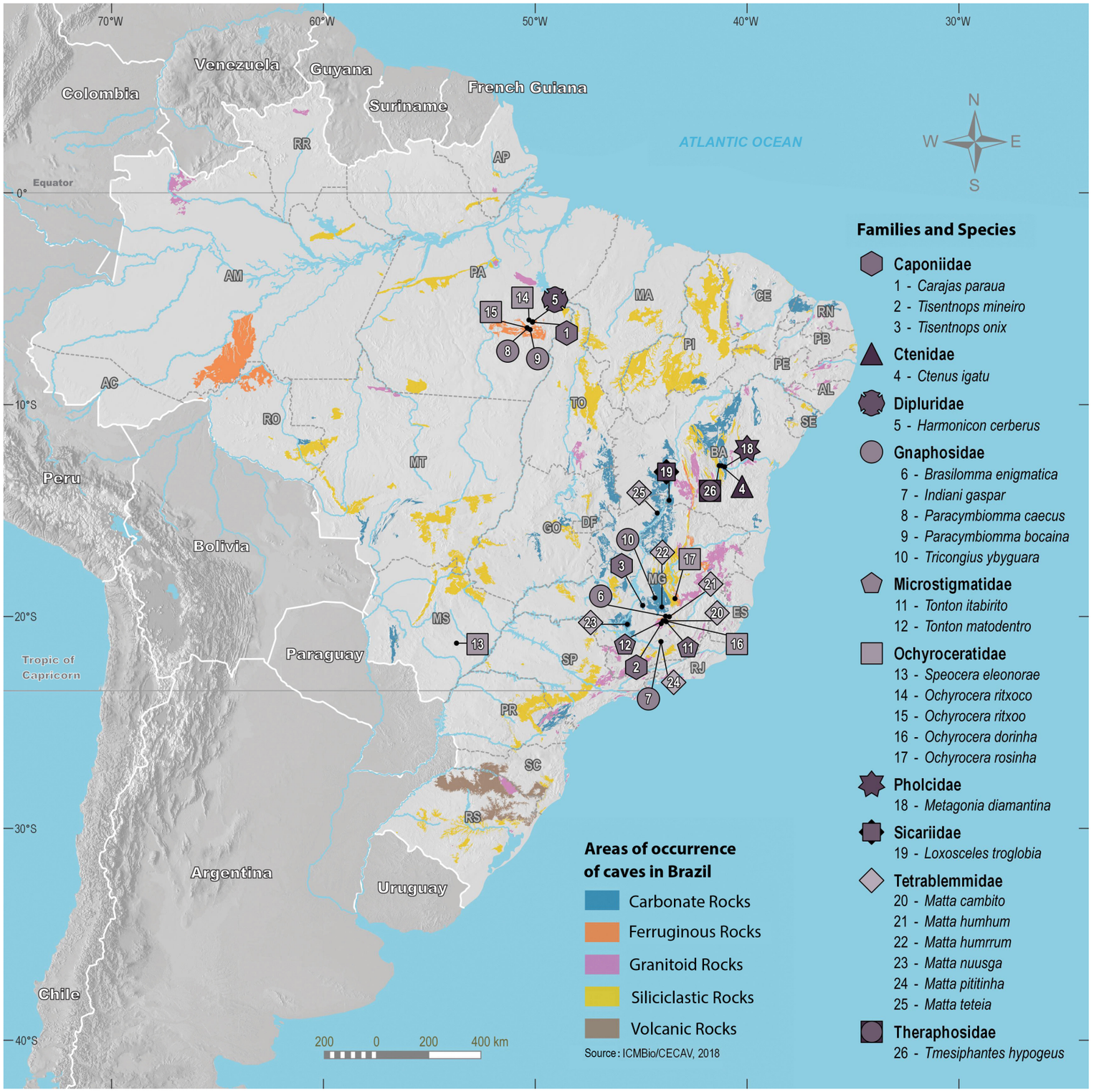


Figure 4. Map showing the type localities of the troglobitic spider species described for Brazil.



Figure 5. Methodologies for collecting spiders in subterranean and epigean environments. *Active manual collection in caves*: **A)** sampling soil; **B)** sampling on walls. *Vulcan-trap*; **C)** sampling in the Interior of caves; **D)** sampling in external rocky outcrops. Collection in the edaphic environment; **E)** Berlese sample collection; **F-H)** Winkler extractor sample collection. Collection in the subterranean surface medium; **I)** borehole sampling. Collection in the surface terrestrial environment; **J)** pitfall-trap (sampling of terrigenous environment); **K)** vulcan-trap (sampling of rocky environment). Photos: A (Renata de Andrade); B-E, G-K (Igor Cizauskas); F (Robson de A. Zampaulo).

Main Brazilian collections

Most zoological collections in Brazil contain cave spiders, and are very heterogeneous in terms of diversity and the number of specimens deposited. Of the numerous national collections, we highlight three that are dedicated to of this group. Two fo these colections are notorious for being exclusively dedicated to the cave fauna, one in São Carlos, state of São Paulo, and the other in Lavras, state of Minas Gerais. The Instituto Butantan has had, since 2011, a specific (individualized) collection of cave spiders separated from the regular collection of epigean spiders.

Instituto Butantan spider collection (Abbr: IBSP)

In 2011, after an agreement among Vale S.A., environmental consultants and researchers in the area, a procedure was adopted to keep spiders from caves separate from regular collections. This curation strategy was established to facilitate access to this material for research and to obtain information necessary for environmental licensing. Today the collection is curated by the researcher Antonio Domingos Brescovit.

The collection of cave spiders comprises more than 41,000 lots, totaling more than 63,100 listed specimens. Currently there are 269 named species of 64 families between troglobites and non-troglobites. The best represented families are Pholcidae (9,520 lots), Theridiidae (4,841 lots), Ochyroceratidae (4,857 lots) and Theridiosomatidae (4,303 lots). The material comes from 20 Brazilian states and the Federal District. However, 54% of this material was collected from areas of the Quadrilátero Ferrífero, in Minas Gerais, and almost 37% from the Carajás region, in the state of Pará.

Collection of the Universidade Federal de Lavras (Abbr: ISLA)

This collection contains only cave fauna, including invertebrates, such as insects, arachnids, myriapods, crustaceans, gastropods, and some groups of vertebrates. It is curated by the researcher Rodrigo Lopes Ferreira.

The spider material in this collection is represented by approximately 4,500 listed lots and almost twice as much material not yet listed. The material comes from almost all Brazilian states, with the exception of Acre, Rondônia, Roraima and Amazonas. In addition, there are also representatives of spiders from 14 countries, on almost all continents, serving as a reference cave fauna collection for comparisons by specialists.

Collection of the Laboratório de Estudos Subterrâneos, UFSCar (Abbr: LES)

This is another almost exclusive collection of representatives of the cave fauna of Brazil, with abundant material of arachnids, myriapods, insects and subterranean fishes. It is curated by the researcher Maria Elina Bichuette.

The spider collection has 3,616 lots currently listed, with 3,388 lots of Araneomorphae and only 121 lots of Mygalomorphae. The most represented families are Ctenidae (644 lots), Pholcidae (452 lots) and Theridiosomatidae (269 lots). The material comes from almost all Brazilian states, with greater representation from the Southeast and Northeast regions of the country.

Conservation of the subterranean spider fauna: diversity and sustainability

One of the challenges for the conservation of biodiversity in a sustainable way is to understand the limits that reconcile the use of natural resources and the protection of ecosystems for future generations. A consensus is that the more information about diversity and their relationships with the environment, the greater the ability to make decisions to mitigate impacts concerning the conservation of these ecosystems. In this way, studies of species diversity are fundamental and provide essential information for projects aimed at the sustainable use of natural ecosystems (NIESENBAUM, 2019).

One of the natural environments where the sustainable use of its resources is extremely controversial is the karst ecosystem (RABELO *et al.*, 2018), as it represents regions of great socioeconomic interest (TEIXEIRA; BEISIEGAL, 2006). On the other hand, they are also environments, with unique geological and biological characteristics, with unquestionable representation of biodiversity, especially of subterranean fauna (CULVER; PIPAN, 2009).

In Brazil, spiders are a relevant group for studies aimed at the conservation of subterranean biodiversity. They are abundant and diverse, found in most caves and considered good indicators of human interference. Subterranean species are particularly vulnerable to ddisturbance because their populations are directly influenced by the conservation status of the surrounding environment and the cave ecosystem itself (CULVER, 1986; CULVER; PIPAN, 2009).

Although subterranean spiders are an extremely relevant

group in these ecosystems, knowledge of this fauna in Brazil is still limited. Many priority karst areas for conservation and/or destined for the sustainable use of their natural resources lack ecological and taxonomic studies, or even basic inventories of biodiversity. In addition, a large portion of the spiders deposited in the main scientific collections depend on taxonomic revisions and species descriptions, fundamental knowledge in defining guidelines for the conservation of the subterranean arachnofauna in the country.

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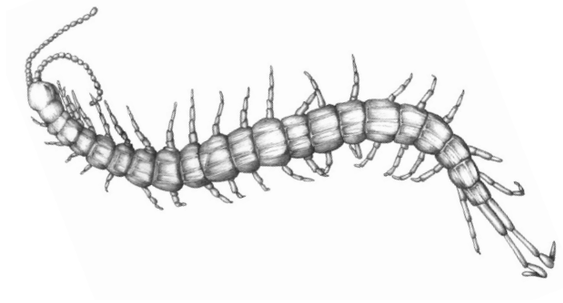


Sphendononema guildingii
Matheus Henrique Simões

9

Chilopoda

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General introduction

Centipedes or lacraias (Arthropoda, Myriapoda, Chilopoda) are predatory terrestrial arthropods found in different habitats on all continents except Antarctica (EDGECOMBE; GIRIBET, 2007). More than 3300 species are distributed in five extant orders (Scutigeromorpha, Lithobiomorpha, Craterostigmomorpha, Scolopendromorpha and Geophilomorpha) and an extinct order (Devonobiomorpha) (BONATO *et al.*, 2016). Centipedes are nocturnal and can be found in diverse microhabitats such as soil, decaying logs, litter, underground galleries and termite mounds, and are also adapted to widespread environments such as grasslands, deserts, beaches and caves (LEWIS, 1981). However, little is known about cave-dwelling centipedes, despite the fact that members of these taxa are commonly found in subterranean environments. There are records in the literature of about 78 species of centipedes globally that live exclusively in caves (troglobites). Most of them (56 species) are found on the European continent (CULVIER; SHEAR, 2012; FODDAI; MINELLI, 1999; MINELLI; NEGREA, 1994; STOEV *et al.*, 2015), followed by the Neotropical Region (12 species) (ÁZARA ; FERREIRA, 2013, 2014a, b; CHAGAS-JR; BICHUETTE, 2015; CHAGAS-Jr; SHELLEY, 2003; MATIC *et al.*, 1977; NUNES *et al.*, 2020; NEGREA *et al.*, 1973; SCHILEYKO, 2013) , Australia (five species) (EDGECOMBE, 2005, 2006; EDGECOMBE *et al.*, 2019), Africa (3 species) (EDGECOMBE *et al.*, 2020; MINELLI; NEGREA, 1994; STOEV *et al.*, 2010) and (one species each) China (QIAO *et al.*, 2021) and the United States of America (MINELLI; NEGREA, 1994).

The first records of centipedes in Brazil date back to the 1980s and were for specimens collected in limestone caves in the states of Goiás, Mato Grosso do Sul, Minas Gerais and São Paulo (CHAIMOWICZ, 1984; DESSEN *et al.*, 1980; GODOY, 1986; GNASPINI-NETTO; TRAJANO, 1991a, b; GNASPINI; TRAJANO, 1994; TRAJANO, 1987) and specimens collected in sandstone caves in the state of Pará (TRAJANO; MOREIRA, 1991). All these records were for unidentified specimens of the orders Scutigeromorpha, Lithobiomorpha, Scolopendromorpha and Geophilomorpha. Only in recent years have the centipede fauna of Brazilian caves been described and reports on obligatory cave organisms become available. In 2013, Ázara and Ferreira (2013) described the first troglobitic centipede species from South America (*Cryptops iporangensis*), found in Sistema Areais of Parque Estadual Turístico do Alto Ribeira (PETAR), in the state of São Paulo. In the following year, the same authors recorded three other troglobitic centipede species (ÁZARA; FERREIRA, 2014a-b), all of the order Scolopendromorpha, from limestone caves in Northeast Brazil (*Cryptops spelaeoraptor; Newportia potiguar; Newportia spelaea*). One year later, another troglobitic species of Scolopendromorpha (*Scolopocryptops troglocaudatus*) was described from sandstone caves in the Chapada Diamantina region, state of Bahia (CHAGAS-JR; BICHUETTE, 2015). In 2018, Chagas-Jr and Bichuette (2018) made the first compilation of all available data on centipedes reported from Brazilian caves. They analyzed information on more than 500 centipede specimens from approximately 274 caves of different lithologies in eleven Brazilian states. More recently, the first troglobitic species of the order Geophilomorpha (*Schendylops janelao*) from South America was described (NUNES *et al.*, 2020), found in a limestone cave in the state of Minas Gerais. Although six troglobitic centipede species are already known for Brazil, a distribution pattern for the species in the country and the conservation status of their cave habitats have not yet been determined.

Main groups found in Brazilian caves

Of the five living orders of centipedes known in the world, four — Scutigeromorpha, Lithobiomorpha, Scolopendromorpha and Geophilomorpha — have been reported from Brazilian caves, with no records for Craterostigmomorpha outside Tasmania and New Zealand. According to Chagas-Jr and Bichuette (2018), the most representative order in number of specimens found in Brazilian caves is Scolopendromorpha with 41%, followed by Geophilomorpha with 26%, Scutigeromorpha with 23% and Lithobiomorpha with 10%. These numbers represent a total of 10 families, 18 genera, 30 species and 47 morphospecies of centipedes recorded in Brazilian caves, equivalent to 21% of the centipede fauna known for the Brazilian territory.

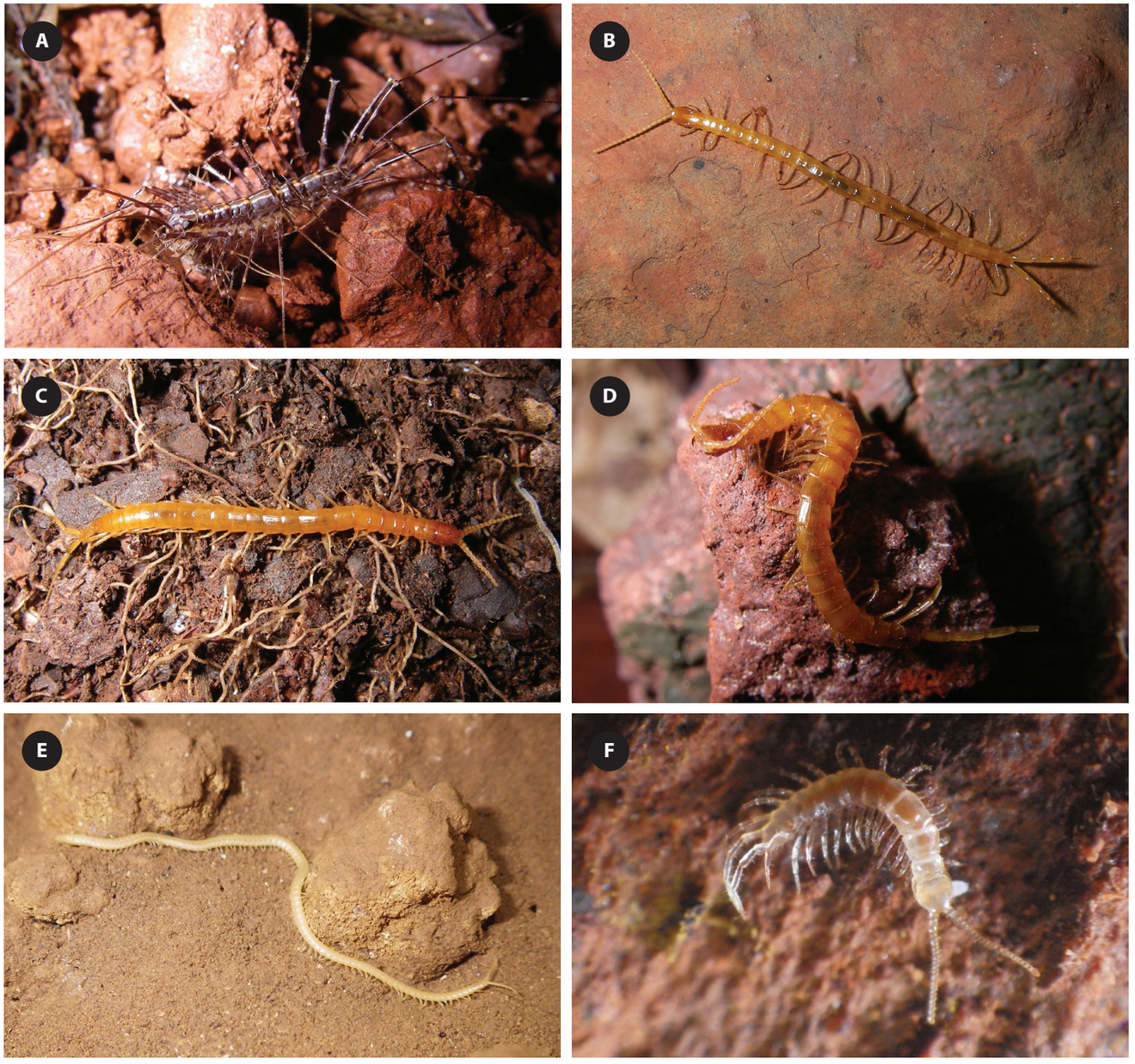


Figure 1. A) *Sphendononema guildingii* (Newport, 1845) (Scutigeromorpha), Cave SL_0074, Curionópolis (PA); **B)** *Cryptops* sp. (Scolopendromorpha), Cave MDIR_0028, São Gonçalo do Rio Abaixo (MG); **C)** *Newportia* sp. 1 (Scolopendromorpha), Cave SERR_0002, Nova Lima (MG); **D)** *Newportia* sp. 2 (Scolopendromorpha), Cave GEM1786, Parauapebas (PA); **E)** Geophilomorpha, Baixa Fortuna cave, Iuiu (BA); **F)** *Lamyctes* sp. (Lithobiomorpha), Santo Antônio do Fanado cave, Capelinha (MG). Photos: A-D (Robson de A. Zampaulo); E-F (Lucas M. Rabelo).

The most frequent species in Brazilian caves is *Sphendononema guildingii* (Newport, 1845) (Figure 1A), a common member of Scutigeromorpha, which is mainly found in iron ore and limestone caves, but also in sandstone and quartzite caves. Some genera of Scolopendromorpha, such as *Cryptops* (Figure 1B), *Scolopocryptops* and *Newportia* (Figures 1C-D), are also abundant and diverse groups in Brazilian caves. The other two orders, Lithobiomorpha and Geophilomorpha (1E) are mainly represented by the genera *Lamyctes* (Figure 1F) and *Schendylops*, respectively. Most centipede species reported in Brazilian caves are accidental visitors, with the exception of those of *Cryptops* and *Newportia,* genera which have species living exclusively in caves. These two genera, in addition to presenting four of the six troglobitic centipede species in Brazil, also have records of troglobitic species on other continents. The currently known troglobitic species of *Cryptops* have been described for Cuba, Spain, the Canary Islands, Italy, Romania, Australia and Botswana. The troglobitic species of *Newportia* have been described for Cuba, Puerto Rico and Mexico. Another genus with potential for troglobitic species diversity is *Scolopocryptops*; in addition to *Scolopocryptops troglocaudatus* Chagas-Jr & Bichuette 2015, there is also a troglobitic species in Venezuela and another in China. Therefore, these genera are apparently the most likely to be found exclusively in caves.

Main troglomorphisms

The main troglomorphisms found in centipedes of Brazil are associated with the length of the trunk segments and appendages (antennae and legs), pronounced depigmentation and reduced sclerotization of the cuticle (ÁZARA; FERREIRA, 2013, 2014a, b; CHAGAS-JR; BICHUETTE, 2015). In addition, troglomorphic centipedes may also have a high density of long bristles on the cephalic plate and on the first three antennal articles (ÁZARA; FERREIRA, 2014).

Diversity of troglobitic species described for Brazil

The diversity of troglobitic centipede species in Brazil can be considered low when compared to the number of species described for the country (153 species). Currently, five troglobitic species of the order Scolopendromorpha (from the genera *Cryptops, Newportia* and *Scolopocryptops*) and one species from the order Geophilomorpha (genus *Schendylops*) are known.

Of these six troglobitic species of Brazilian centipedes, two are considered endangered by the *International Union for Conservation of Nature* (IUCN), the institution responsible for the global red list that assesses the risk of species extinction: *Cryptops iporangensis* Ázara and Ferreira, 2013, classified as "Endangered", and *Cryptops spelaeoraptor* Ázara & Ferreira, 2014, classified as "Vulnerable". These conservation statuses place these species as the only threatened troglobitic centipede species in any category of extinction risk in the world.

Main methods of collection and conservation

There are two types of methods used for collecting centipedes: active and passive collecting. Both are widely used for other groups of terrestrial invertebrates found in caves and adapted according to each specificity. Active manual collecting is the most used and most efficient of all the collecting methods for cave centipedes. In addition, it has a low-cost and is a higher-quality type of collecting for the preservation of captured specimens. Active manual collection does not rely on complex and costly equipment. In general, active collecting involves capturing centipedes with the help of tweezers of different sizes and/or brushes or, in some situations, with one's own hands, depending on the size and danger of the organism. Immediately after capture, individuals are stored in containers of different sizes containing 70% or 100% alcohol, depending on the purpose of the study. These containers, in general, are 15 or 50 ml Falcon tubes or 2 ml Eppendorf microtubes. Manual active collecting of centipedes can follow a protocol based on time, exhaustion or even quadrants of 0.25 m^2 (BICHUETTE *et al.*, 2015; ZEPELINNI-FILHO *et al.*, 2003). Centipedes are collected in all potential microhabitats inside the cave, such as guano deposits, accumulated litter and branches, trunks, and spaces between rocks, soil and wall.

Passive collection can be accomplished through the use of the Winkler extractor and pitfall traps. In the case of the Winkler extractor, samples are taken from the litter or sediment and are stirred through sieves to remove excess material. The samples are subsequently placed in a coarse mesh fabric bag and placed in a mini-Winkler extractor for a period of time (FISHER, 1999). The material filtered by the extractor is housed in a container containing 70% alcohol. This method is most efficient for capturing small centipedes that are difficult to see during active

manual collection. Pitfall traps, on the other hand, use 500ml plastic containers, with a 90mm diameter mouth and a height of 128mm, buried in the ground up to the edge and filled with a fixative liquid that occupies 30% of the internal area. Depending on the purpose of the study, several containers can be buried in different locations within a cave, following an exposure-time protocol. Collecting with pitfall traps is often more laborious and ineffective for centipedes.

Table 1 - Updated list of described and registered troglobitic centipede species.

Scolopendromorpha

Cryptopidae

1. *Cryptops iporangensis* Ázara & Ferreira, 2013
Resurgence of Sistema Areias (24°33'45.55"S, 48°40'18.18"O), Iporanga, São Paulo.

2. *Cryptops spelaeoraptor* Ázara & Ferreira, 2014
Toca do Gonçalo cave (10º30'41"S, 40º53'39.8"O), Campo Formoso, Bahia.

Scolopocryptopidae

3. *Newportia spelaea* Ázara & Ferreira, 2014
Toca do Gonçalo cave (10º30'41"S, 40º53'39.8"W), Campo Formoso, Bahia.

4. *Newportia potiguar* Ázara & Ferreira, 2014
Gruta do Geraldo Gusso (5°33'4.6"S, 37°39'50.4"O) Apodi / Gruta da Carrapateira (5°33'38.2"S, 37°39'50.4"O) Felipe Guerra, Rio Grande do Norte

5. *Scolopocryptops troglocaudatus* Chagas-Jr & Bichuette, 2015
Gruna do Cantinho (12°51'49.90"S, 41°19'26.76"O), Igatu, Andaraí, Bahia.

Geophilomorpha

Schendylidae

6. *Schendylops janelao* Nunes, Chagas-Jr & Bichuette, 2019
Gruta do Janelão cave (15°06'S, 44°14'W), Januária, Minas Gerais.

Identification key

The identification key below was prepared from the list of species in the works by Chagas-Jr and Bichuette (2018) and Oliveira *et al.* (2019). Troglobite species are marked with an asterisk.

1 - Spiracles dorsal **2**
1´- Spiracles lateral **3**
2 – Antennae articles longer than wide ..... ***Sphendononema guildingii*** (Newport, 1845)
2´ - Antennae articles wider than long ..... ***Thereuoquima admirabilis*** Bücherl, 1949
3 - 21 or 23 pairs of legs **4**
3´ - More than 23 pairs of legs **31**
4 - Ocelli present in the cephalic plate **5**

4´ - Ocelli absent **14**

5 - With spiracle divided into a three-valved flap..... **6**

5´ - With oval spiracle without a three-valved flap..... **9**

6 - Cephalic plate with basilar plates..... ***Cormocephalus impressus*** Bücherl, 1942

6´ - Cephalic plate without basilar plates..... **7**

7 - Cephalic plate with longitudinal sutures diverging anteriorly ..... ***Scolopendra viridicornis*** Newport, 1844

7´ - Cephalic plate with incomplete median longitudinal suture **8**

8 - All legs without tarsal spurs..... ***Scolopendropsis thayeri*** Meinert, 1886

8´ - All legs with tarsal spurs..... ***Scolopendropsis bahiensis*** (Brandt, 1841)

9 – Seventh trunk segment with pair of spiracles ..... ***Rhysida brasiliensis*** Kraepelin, 1903

9´- Seventh trunk segment without pair of spiracles..... **10**

10 - Tergites without keels..... **11**

10´ - Tergites with keels..... **12**

11 - Posterior edge of coxopleura in males ending in short, rounded, pointed lobe with digitiform appendage at posterior edge of tergite 21..... ***Otostigmus caudatus*** Brölemann, 1902

11' - Posterior edge of coxopleura in males truncated and without digitiform appendage at posterior edge of tergite 21..... ***Otostigmus tibialis*** Brölemann, 1902

12 – Sternites with short and incomplete paramedian sutures..... ***Otostigmus tidius*** Chamberlin, 1914

12´ - Sternites with quasi-complete paramedian sutures..... **13**

13´ - Posterior margin of sternite 21 slightly concave..... ***Otostigmus muticus*** Karsch, 1888

13 - Posterior margin of sternite 21 straight..... ***Otostigmus amazonae*** Chamberlin, 1914

14 - 17 antennal articles and without coxopleural process..... **15**

14´- 17 antennal articles and with coxopleura process..... **19**

15 – All articles of last leg with teeth in the form of a saw ..... ***Cryptops spelaeoraptor**** Azara & Ferreira, 2013

15'- Tibia and tarsus 1 of last leg with teeth in the form of a saw **16**

16 – Tergites with anterior oblique sutures..... ***Cryptops hephaestus*** Azara & Ferreira, 2013

16' - Tergites without anterior oblique sutures..... **17**

17 – Ventral face of article 1 of antennae with inverted Y-shaped sutur ..... ***Cryptops iporangensis**** Ázara & Ferreira, 2013

17' - Ventral face of article 1 of antennae without inverted Y-shaped suture **18**

18 – 13–21 teeth in the form of a saw on tibia and 5–7 on tarsus 1 ..... ***Cryptops iheringi*** Brölemann, 1902

18' – 7–8 teeth in the form of a saw on tibia and 3–4 on tarsus 1..... ***Cryptops galatheae*** Meinert, 1886

19 – Pre-femur of last legs with one ventral spinal process..... **20**

19' - Pre-femur of last legs with more than one ventral spinal process..... **23**

20 – Seventh segment of trunk with spiracle..... ***Scolopocryptops miersii*** Newport, 184

20' - Seventh segment of trunk without spiracle..... **21**

21 – Forcipular coxosteral toothplates formed by two long, thick chitinous thickenings, which do not fuse with each other..... ***Scolopocryptops melanostoma*** Newport, 1845

21' - Forcipular coxosternal toothplates formed by two long, thick chitinous thickenings, which do not fuse with each other and are higher on the sides than in the middle **22**

22 – Length of last legs never exceeding half of total body length ... ***Scolopocryptops ferrugineus macrodon*** (Kraepelin, 1903)

22'- Length of last legs exceeding half of total body length ..... ***Scolopocryptops troglocaudatus*** * Chagas-Jr & Bichuette, 2015

23 - With dentiform plate **24**

23´- Without dentiform plate **26**

24 – Pre-femur of last legs with 4 large spinous processes..... ***Newportia nisargani*** (Chagas-Jr, 2011)

24'- Pre-femur das of last legs with 3 spinous processes..... **25**

25 – Femur last legs without spinous processes ..... ***Newportia balzanii*** Silvestri, 1895

25' – Femur of last legs with 1 or 2 ventral spinous processes..... ***Newportia simus*** (Chamberlin, 1915)

26 - Cephalic plate with transverse suture that crosses paramedian suture at posterior margin **27**

26´- Cephalic plate without transverse suture that crosses paramedian suture at posterior margin **28**

27 - Pre-femur of last pair of legs with 6–7 ventral spinous processes..... ***Newportia ernsti ernsti*** Pocock, 1891

27´ - Pre-femur of last pair of legs with 5 ventral spinous processes ..... ***Newportia ernsti fossulata*** Bücherl, 1942

28 – Tibia of locomotor legs with spurs **29**

28' - Tibia of locomotor legs without spurs..... ***Newportia phoreta*** Chamberlin 1950

29 – Pre-femur of last legs with 5–7 ventral spinous processes..... **30**

29 – Pre-femur of last legs with 4 ventral spinous processes..... ***Newportia potiguar*** * Ázara & Ferreira, 2014

30 – Tergites 5–21 median longitudinal keel..... ***Newportia stolli*** (Pocock, 1896)

30' – Tergites without median longitudinal keel..... ***Newportia spelaea*** * Ázara & Ferreira, 2014

31 – Antennae long and slender or long and moniliforme ..... ***Schendylops janelao*** * Nunes, Chagas-Jr & Bichuette 2018

31'- Antennae short, distally attneuated..... ***Orphaneus brevilabiatus*** (Newport, 1845)

Main collections in Brazil

The main collections of centipedes in Brazil are mainly concentrated in the Southeast Region of the country, in the states of São Paulo and Minas Gerais. The collections with cave centipedes in São Paulo are those of the Museu de Zoologia da Universidade de São Paulo (MZUSP), the Laboratório Especial de Coleções Zoológicas of Instituto Butantan (IBSP) and the Laboratório de Estudos Subterrâneos of the Universidade Federal de São Carlos (LES/UFSCar). Among the three collections, that of LES/UFSCar is the most significant, as it has kept specimens from caves from different regions of Brazil since 2006. In addition, it has specimens of centipedes collected in caves since the 1970s and 1980s, from the works of pioneers in Brazilian caves. These specimens were deposited at the Departamento de Zoologia of the Universidade de São Paulo and were donated to LES/UFSCar by Eleanor Trajan. The MZUSP collection is a traditional and faithful depository and so receives specimens collected from consulting projects or type specimens that have been described by researchers in subterranean biology. Despite being the largest in Brazil, the IBSP centipede collection does not have a collection of cave centipedes compatible with its magnitude. The collection recently received financial support from Vale S.A. to house specimens of myriapods collected in caves in the states of Pará and Minas Gerais, mainly from caves inserted in iron formations. However, most of the material has yet to be identified to the species level.

In Minas Gerais, there is also the Coleção de Invertebrados Subterrâneos de Lavras (ISLA), part of the Centro de Estudos em Biologia Subterrânea of the Universidade Federal de Lavras

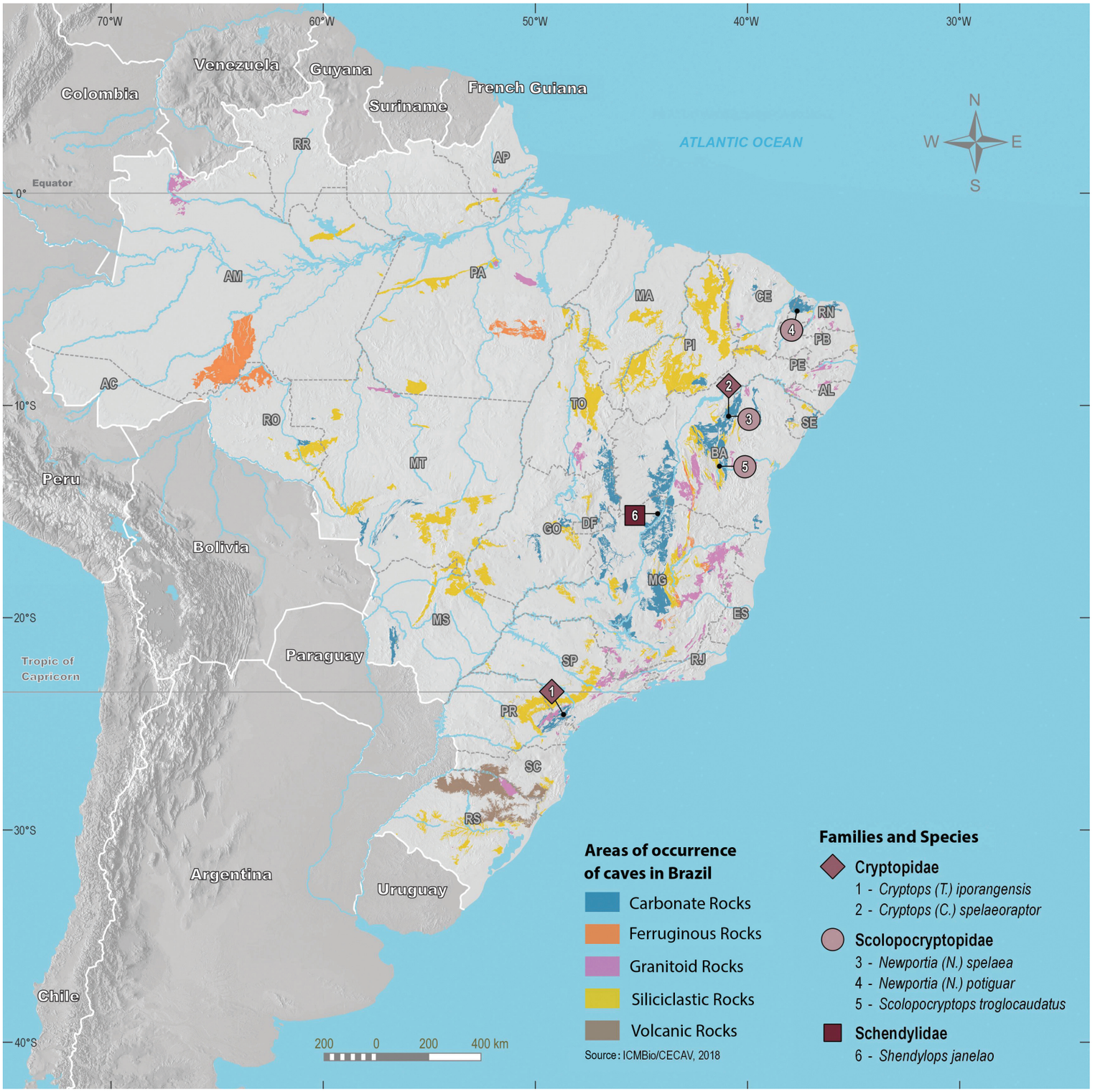


Figure 2. Map with the type localities of troglobitic centipede species described for Brazil.

(UFLA). It is one of the largest collections of cave invertebrates in Brazil and has centipede specimens from caves from different regions of the country, including type specimens of species described in recent years. There is also the Coleção Zoológica de Invertebrados (wet) of the Universidade Federal de Mato Grosso (CZUFMT) with a good collection of cave centipedes, mainly from the states of Pará and Minas Gerais, but also specimens of the regional epigean fauna.

Acknowledgements

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Robson de Almeida Zampaulo



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Coarazuphium tessai
Rodrigo Lopes Ferreira

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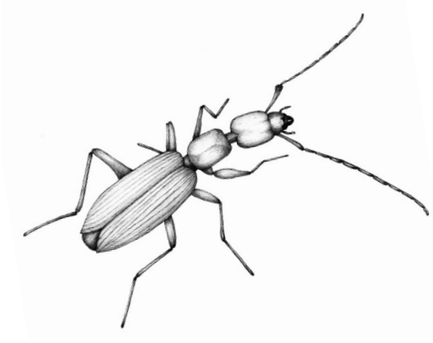
Coleoptera

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General introduction

Coleoptera, popularly known as beetles, is the most diverse group of organisms on Earth with more than 357,000 described species (BOUCHARD *et al.*, 2009). They occupy very different environments, including caves. Their evolutionary origin is terrestrial but over time there have been multiple and independent conquests of the aquatic environment (HUNT *et al.*, 2007). The repeated exchanges between aquatic and terrestrial environments expanded their occupied niches and, consequently, the diversity of the group. The subterranean environment, in turn, presents several potential biotopes to be colonized by beetle species, ranging from caves to small cracks and interstices in rock (RACOVIŢĂ, 1907). There are also subterranean aquatic environments, which comprise the levels of base (aquifers), subterranean lakes and rivers, impoundments (e.g., travertine) and saturated epikarst zones, among others.

The great diversity of epigean beetles is reflected in the cave environment, where they are among the insect groups with the most troglobitic representatives in the world (CASALE *et al.*, 1998; DECÚ; JUBERTHIE, 1998). Despite this, most Brazilian troglobitic beetles have only been described in the last few decades. This is because the subterranean fauna in Brazil has only recently been more intensively assessed as a result of legal requirements intrinsic to the licensing process in the country (GALLÃO; BICHUETTE, 2018).

Troglobitic beetle species have been described from caves distributed in all regions of Brazil, especially of three families — Carabidae, Dytiscidae and Staphylinidae. These families possess strong phylogenetic signal of genera that have troglobitic species described in Old World countries (HLAVÁČ *et al.*, 2017), reinforcing the need for investment in the collection and description of new species and the conservation of cave habitats.

There are currently 20 species of troglobitic beetles described for Brazil (Table 1). The family Dytiscidae contains a troglobitic species, *Copelatus cessaima* Caetano *et al.*, 2013 (Copelatinae), whose distribution is restricted to three ferruginous caves in the state of Pará. Four species of troglobitic Staphylinidae are currently recorded for Brazil, belonging to the genera *Metopiellus, Metopioxys* and *Oxarthrius* (Pselaphinae), the first two being genera with distributions restricted to the Neotropics. Carabidae is the beetle family with the greatest number of troglobitic species in Brazil. Fourteen species have been described thus far, 11 of which belong to the genus *Coarazuphium* (Zuphiini) (Figures 1A 2D-G). The first species of *Coarazuphium* Gnaspini, Vanin & Godoy, 1998, was described in 1990 and had been assigned to the genus *Parazuphium* (GODOY; VANIN, 1990). Eight years after later, the genus *Coarazuphium* was described with the designation of new species, all of them troglobites. Four new species of the genus are currently known and are in the process of being described, three from the state of Pará, associated with iron ore caves, and one from a limestone cave in the Serra do Ramalho region, state of Bahia. The only two troglobitic species of the carabid tribe Clivinini are *Schizogenius ocellatus*, Whitehead, 1972, and *Ardistomis ferreirai* Balkenohl, 2018. A third species of the tribe found in the cavernous "*hotspot*" Toca do Gonçalo was considered by Souza Silva and Ferreira (2016) as troglobitic; however, this species has not yet been described. Recently, a specimen of the tribe Perigonini was described as a new troglobitic species, *Perigona (Neoperigona) spelunca* Pellegrini, Ferreria & Vieira, 2022, which was found in caves associated with the karst region of Arcos, Pains and Doresópolis, state of Minas Gerais. The species represents the first of this genus in the world to be considered troglobitic (PELLEGRINI *et al.*, 2022b). Another carabid tribe, Bembidiini, also contains new species considered troglobites that are yet to be described (T.G. Pellegrini, personal communication). Recent descriptions and new species still formally unknown by the scientific community in Brazil reinforce the need to intensify taxonomic studies of carabid fauna.

It is important to note that, thus far, larval forms of Brazilian troglobitic beetle species remain unknown. Nonetheless, some researchers have already collected carabid larvae with characteristics that, apparently, could represent troglomorphisms of immature forms, including the absence of eyes and depigmentation (Figure 1B). Such larvae were

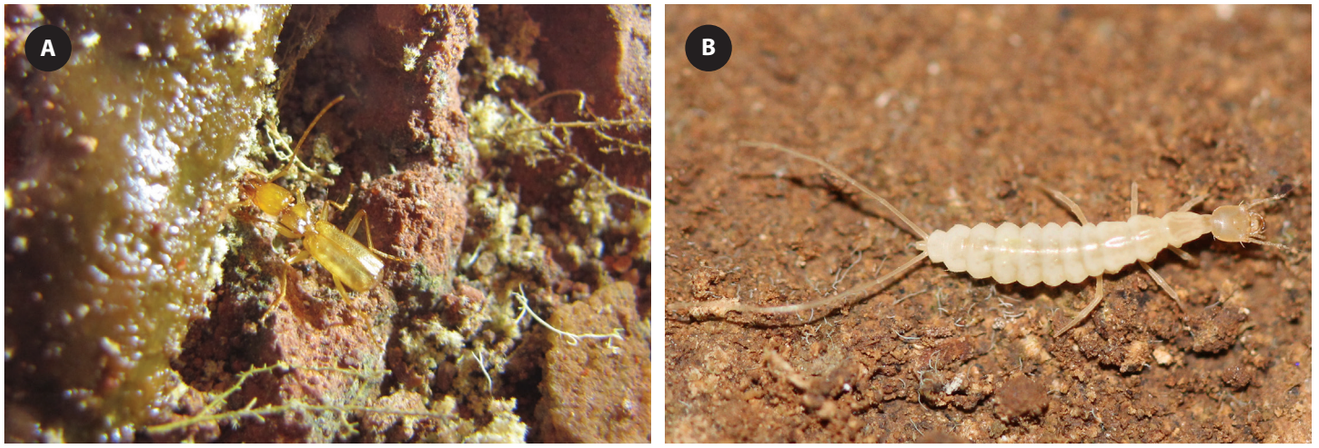


Figure 1. A) Live specimen of *Coarazuphium formoso* Pellegrini & Ferreira, 2011 found in the cave Toca da Barriguda in Campo Formoso, Bahia. Image courtesy of Rodrigo L. Ferreira. **B)** Live larval specimen of the family Carabidae with troglomorphic characteristics found in cave Gruta Paranoá, in Pains, Minas Gerais. Photos: (Marconi Souza-Silva).

found associated with caves where previously described species of *Coarazuphium* are known to occur or nearby such caves, supposedly attributing them to the genus (R.L. Ferreira, R.A. Zampaulo and M. Souza-Silva, personal communication). However, efforts spent on raising these larvae have not been successful to date, making it necessary to use molecular tools to demonstrate that the larvae in effect belong to the genus, with their morphological description being carried out later. Therefore, biological material needs to be fixed in 100% ethyl alcohol to preserve the genetic material of specimens. Furthermore, when more than one larva is found, part of the series should be preserved in 70% alcohol/KAA/Kahle Dietrich or in another liquid preservative, to avoid alterations to morphological characteristics (see item "Collection, curation and conservation methods" in this chapter). This example represents just one of the knowledge gaps in the study of beetles that needs to be addressed.

Principal troglomorphisms

Beetle species whose lineages are able to adapt to cave environments may undergo changes in their external morphology, with adaptive convergence in their morphological characters, called troglomorphisms (CHRISTIANSEN, 1962). Subterranean beetle species can be grouped according to the compartment with which they are associated, namely: **Edaphic Environment**, formed by interstitial spaces in the soil such as fissures and cracks and accessed, in general, by edaphobiont organisms (FERREIRA, 2019); **Superficial Subterranean Medium (SSM)**, also known as "*Milieu Souterrain Superficiel*" (MAMMOLA *et al.*, 2016; ORTUÑO *et al.*, 2013), is located at the interface between the soil and the rocky substrate already composed of larger cracks; **Epicarst**, which is characterized as the last extract before the cave, with rock discontinuities partially saturated with water (PIPAN; CULVER, 2005); and the **Macrocaves** themselves, also called caves. These groupings can result in different degrees of morphological adaptations, depending on the habitat they occupy.

EDAPHIC ENVIRONMENT (SOIL)

The edaphic environment is where it is possible to find a lot of decomposing organic matter, microorganisms (e.g., bacteria and fungi) and other small invertebrates (e.g., springtails and mites). Beetles of this habitat are not considered troglobites, but they can exhibit loss of pigmentation, extremely reduced to anophthalmic

eyes (e.g., *Geomitopsis, Polyderidius*), absence of the second pair of wings (e.g., Jubini, Polyderidius), reduced length of body appendages such as antennae and legs (e.g., Leptotyphlinae) and, in general, small body size. It is important to highlight that analyses based on external morphology without deep knowledge of the group, can lead to a misclassifications between troglobitic species and soil fauna (e.g., several Staphylinidae, such as Osoriinae, Pselaphinae, and some Carabidae, such as those of the tribe Anillini, etc.). This is especially true for species of the tribe Anillini, which are cosmopolitan organisms with very small body sizes. Their morphological characteristics pose a challenge to determining which species are truly troglobitic and which are edaphic (FAILLE, 2019).

SUPERFICIAL SUBTERRANEAN MEDIUM (SSM)

The SSM is characterized by a network of subterranean channels and fissures filled with air, developed amid multiple layers of rock fragments (ORTUÑO *et al.*, 2013). Among the characteristics of these environments, the most notable, and shared with caves, is the permanent absence of light and, when compared to surface environments, a lower thermal amplitude (PIPAN *et al.*, 2010). Organisms associated with this compartment are, in general, anophthalmic and depigmented (ORTUÑO *et al.*, 2014) and are also considered troglobitic organisms, sharing morphological characteristics common to inhabitants restricted to macrocaves (CULVER; PIPAN, 2008). The SSM can be used as a dispersal corridor between caves (JUBERTHIE *et al.*, 1980), but it also has its own troglobitic beetle fauna (CULVER; PIPAN, 2008; ORTUÑO *et al.*, 2014; RENDOŠ *et al.*, 2012; RENDOŠ *et al.*, 2012). Studies of the SSM are incipient in Brazil and have not yet revealed the presence of effectively subterranean organisms (MENDONÇA *et al.*, 2019), but it is speculated that some troglobitic beetle species preferentially inhabit this compartment, as is the case for *Metopiellus painensis* and *Coarazuphium spinifemur* (ASENJO *et al.*, 2017; PELLEGRINI; FERREIRA, 2017). Repeated sampling events were carried out in the type locality caves of both of these species but with low capture success. In addition, *C. spinifemur* has a reduced body size and reduced locomotor and sensory appendages compared to other troglobitic species of *Coarazuphium*, reinforcing the hypothesis that they inhabit small fissures and canaliculi in rock (PELLEGRINI; FERREIRA, 2017).

EPICARST

Most often inhabited by small crustaceans, especially copepods (PIPAN; CULVER, 2005; PIPAN *et al.*, 2018), beetles of the family Dytiscidae can also be found exclusively inhabiting the epikarst with characteristics in common with stygobites[1], such as the absence of eyes and depigmentation (CULVER *et al.*, 2019). There is also terrestrial fauna associated with empty spaces (not filled by water) of the epikarst, of which beetles are occasionally a part (CULVER; PIPAN, 2008; GIBERT, 1986; PAPI, 2016), which, in general, are also found in macrocaves. Like SSM organisms, terrestrial representatives of the epikarst show reduced or complete absence of ocular structures, in addition to depigmentation (ASSMANN, 2010). On the other hand, the fauna associated with this compartment tends to have smaller body sizes, although the size of the antennae does not differ from the occupants restricted to macrocaves (CULVER *et al.*, 2019). There is still no evidence of the existence of beetles associated with epikarst in Brazil. However, it is speculated that *Copelatus cessaima*, of the family Dytiscidae, is associated with this environment, since it is usually found in small intermittent pools from epikarst drips (FERREIRA *et al.*, 2018).

MACROCAVES

Macrocaves are larger spaces in rock that allow access to the subterranean environment for sampling. Troglobitic organisms in these spaces may, in general, show loss of color, often being confused with tenerals. They may also have a thinner cuticle, strongly reduced to absent eyes (e.g., *Metopioxys carajas, Coarazuphium cessaima, Schizogenius occelatus*), a strongly reduced to absent second pair of wings (membranous wings; e.g., Coarazuphium), and fusion of the internal side of elytra with the thorax. Other features that may be present include a strongly elongated body (especially the head and prothorax) and body appendages, such as antennae and legs (e.g., *Coarazuphium, Metopiellus*, etc.), and the presence of long sensory setae (ASENJO *et al.*, 2019a; FAILLE, 2019; FIŠER, 2019; PELLEGRINI; FERREIRA, 2017). The strong morphological adaptation of these organisms restricts their presence in subterranean environments, thus earning them recognition as troglobites.

1 estigóbio: organismo que habita águas subterrâneas (Romero, 2009).

SUBTERRANEAN AQUATIC ENVIRONMENT

Historically, there have been few studies in Brazil whose main focus was the aquatic cave environment (ZEPON *et al.*, 2019). Furthermore, compared to terrestrial species, there are few representatives of beetles known to science that have conquered and evolved into their stygobitic forms. Only one species described so far for this type of environment in Brazil, *Copelatus cessaima* (Dytiscidae), which has a discolored tegument, absence of compound eyes, and slightly elongated body appendages, antennae and legs (CAETANO *et al.*, 2013). It is worth mentioning here, as mentioned earlier, that this species is speculated to be associated with epikarst. This family is also the most diverse and well distributed beetle family in caves in the world, including hundreds of species recently discovered in Australia (JEAN *et al.*, 2014) and new undescribed species from Brazil.

List of troglobitic species described for Brazil and their updated records

The following is a complete list of Brazilian beetle species described as troglobites, organized by family, genus and species. The genera and species are listed alphabetically, and the name of each species has the author and year of publication, as well as its type locality. The distribution lists all reported localities for each species, together with the source of information (see Table 1).

Table 1 – List of troglobitic species described for Brazil and their updated records.

Family **Carabidae** (4 genera, 15 species)
Ardistomis Putzeys, 1846
1. *Ardistomis ferreirai* Balkenohl, Pellegrini & Zampaulo, 2018 (Figure 2A)
Type Locality: Brazil: Pará, Parauapebas, Flona de Carajás, Gruta GEM_1195 (6°06'33.02"S, 50°08'05.7"W).
Distribution: Brazil: Pará, Cave N5S_0021 (06°05'15.31"S, 50°07'33.52"W).
Reference: (BALKENOH *et al.*, 2018)

Schizogenius Putzeys, 1846
2. *Schizogenius* (*Schizogenius*) *ocellatus* Whitehead, 1972 (Figure 2B)
Type Locality: Brazil: São Paulo, Grutas das Areias (24°35'28"S, 48°43'23"W) of Sistema de Grutas das Areias in PETAR.
Distribution: Brazil: São Paulo, Caverna do Santana (24°31'51"S, 48°42'06"W).
Reference: (TRAJANO, 2007).

Perigona Castelnau, 1835
3. *Perigona (Neoperigona) spelunca* Pellegrini, Ferreira & Vieira, 2022 (Figure 2C)
Type Locality: Brazil: Minas Gerais, Pains, Gruta do Éden (20°23'5"S, 45°39'59"W).
Distribution: Brazil: Minas Gerais, Pains, Gruta do Zezinho Beraldo (20°21'25.04"S, 45°50'4.68"W); Gruta Santuário (20°25'11.61"S, 45°46'27.25"W); Gruta Cinderela (20°26'44"S, 45°36'22"W); Gruta da Fazenda Amargoso (20°23'53.63"S, 45°35'38.34"W); Gruta Serro Azul (20°22'31.97"S 45°38'48.35"W); Gruta do Brega (20°25'4.37"S, 45°46'20.16"W); Cave S1-GEC-035 (20°23'26.1"S, 45°35'54.6"W).
Reference: (PELLEGRINI *et al.*, 2022b).

Coarazuphium Gnaspini, Vanin & Godoy, 1998
4. *Coarazuphium amazonicus* Pellegrini & Ferreira, 2017 (Figure 2D)
Type Locality: Brazil: Pará, Parauapebas, Flona de Carajás, Cave N5SM2_0102 (6°07'17.52"S, 50°07'52.30"W).
Distribution: Brazil: Pará, Cave N5SM2_0093 (6°07'17.13"S, 50°07'55.95"W); Cave N5SM2_0089 (6°07'14.69"S, 50°07'43.74"W); Cave N5SM2_0046 (6°07'54.20"S, 50°08'06.47"W); Cave N5SM2_0102 (6°07'7.50"S, 50°07'52.33"W); Cave N5SM2_0057 (6°07'46.73"S, 50°08'05.10"W); Cave N5SM2_0021 (6°07'58.35"S, 50°07'51.70"W); Cave N5SM2_0089 (6°07'14.69"S, 50°07'43.74"W); Cave N5SM2_0037 (6°07'58.63"S, 50°08'04.98"W); Cave N5SM2_0057 (6°07'46.73"S,

50°08'05.10"W); Cave N5SM2_0037 (6°07'58.63"S, 50°08'04.98"W); Cave N5SM2_0078 (6°07'23.09"S, 50°07'48.70"W); Cave N5SM2_0086 (6°07'16.18"S, 50°07'47.22"W); Cave N5W_0003 (6°04'52.29"S, 50°08'03.86"W); Cave N4E_0092 (6°02'23.96"S, 50°09'32.50"W); Cave N4E_0010 (6°02'21.57"S, 50°09'39.67"W)..
Reference: (PELLEGRINI; FERREIRA, 2017).

5. *Coarazuphium auleri* Pellegrini & Vieira, 2021
Type Locality: Brazil: Mato Grosso, Nobres, Caverna Lagoa Azul (14°26"48.1"S 56°01'33.2"W).
Distribution: Known only for the type locality.

6. *Coarazuphium bambui* Pellegrini & Vieira, 2022
Type Locality: Brazil: Bahia, Feira da Lapa Municipality, Gruna Boca da Lapa cave (13°56'46"S, 44°11'12"W).
Distribution: Known only for the type locality.
Reference: (PELLEGRINI *et al.*, 2022a).

7. *Coarazuphium bezerra* Gnaspini, Vanin & Godoy, 1998
Type Locality: Brazil: Goiás, Caverna Lapa do Bezerra cave (13°32'51"S, 46°22'35"W).
Distribution: Known only for the type locality.

8. *Coarazuphium caatinga* Pellegrini & Ferreira, 2014
Type Locality: Brazil: Bahia, Campo Formoso, Toca do Gonçalo (10°30'41"S, 40°53'39,8"W). Note: Information taken from holotype labels.
Distribution: Brazil: Bahia, Toca de São Tomé cave (10°36'06"S, 40°55'57"W).
Reference: (PELLEGRINI; FERREIRA, 2014).

9. *Coarazuphium cessaima* Gnaspini, Vanin & Godoy, 1998 (Figure 2E)
Type Locality: Brazil: Bahia, Itaetê, Lapa do Bode cave (12°56'04"S, 41°03'55"W).
Distribution: Brazil: Bahia, Gruta Poço Encantado (12°56'42.36"S, 41°6'18.72"W).
References: (GNASPINI *et al.,* 1998; PELLEGRINI *et al.*, 2020b).

10. *Coarazuphium formoso* Pellegrini & Ferreira, 2011
TType Locality: Brazil: Bahia, Campo Formoso, Barriguda cave (10°8'28.48"S, 40°51'8.85"W). Note: Information taken from holotype labels.
Distribution: Brazil: Bahia, Toca do Calor de Cima (10°8'26"S, 40°50'55"W).
Reference: (PELLEGRINI; FERREIRA, 2011).

11. *Coarazuphium kayapo* Pellegrini, Ferreira & Vieira *in* PELLEGRINI *et al.*, 2022c
Type Locality: Brazil: Pará, Serra Sul de Carajás, Cave S11B-0177, (6°20'21.4"S, 50°30'21.0"W).
Distribution: Brazil: Pará, Cave S11D-0111 (6°23'48.1"S, 50°20'27.3"W).
Reference: (PELLEGRINI *et al.*, 2022c).

12. *Coarazuphium lundi* Pellegrini, Ferreira & Vieira *in* PELLEGRINI *et al.*, 2020b (Figure 5: PELLEGRINI *et al.*, 2020b)
Type Locality: Brazil: Minas Gerais, Prudente de Morais, Cave 06L (19°26'29.0"S, 44°6'26.9"W).
Distribution: Known only for the type locality.
Reference: (PELLEGRINI *et al.*, 2020b).

13. *Coarazuphium pains* Álvares & Ferreira, 2002 (Figure 2F)
    Type Locality: Brazil: Minas Gerais, Pains, Gruta Tabocas III cave (20°22'54"S, 45°44'34"W).
    Distribution: Brazil: Minas Gerais, Gruta Água Limpa III (20°27'4.60"S, 45°39'13.55"W), Gruta Zé Serafim I (20°24'48.64"S, 45°37'37.91"W), Caver S1- CRH-121 (20°19'43.34"S, 45°34'40.67"W).
    References: (ÁLVARES; FERREIRA, 2002; PELLEGRINI *et al.* 2020b).

14. *Coarazuphium ricardoi* Benà & Vanin, 2014 (Figure 2G)
    Type Locality: Brazil: Paraná, Doutor Ulysses, Gruta do Varzeão cave (24°37'49"S, 49°29'58"W).
    Distribution: Known only for the type locality.

15. *Coarazuphium spinifemur* Pellegrini & Ferreira, 2017
    Type Locality: Brazil: Pará, Parauapebas, Flona de Carajás, Caverna SL_0074 (5°57'58.04"S, 49°37'55.95"W).
    Distribution: Known only for the type locality.

16. *Coarazuphium tapiaguassu* Pellegrini & Ferreira, 2011
    Type Locality: Brazil: Pará, Parauapebas, Flona de Carajás, Cave SL_0030 (49°38'36.12"S, 5°58'19.11"W). Note: Information taken from holotype labels.
    Distribution: Brazil: Pará, Cave SL_0031 (5°58'20.30"S, 49°38'36.70"W); Caverna SL_0035 (5°58'32,75"S, 49°38'16,84"W).
    Reference: (PELLEGRINI; FERREIRA, 2011b).

17. *Coarazuphium tessai* (Godoy & Vanin, 1990)
    Type Locality: Brazil: Bahia, Santana, Padre cave (13°13'07"S, 44°03'53"W).
    Distribution: Known only for the type locality.

18. *Coarazuphium xikrin* Pellegrini, Ferreira & Vieira *in* PELLEGRINI *et al.*, 2022c
    Type Locality: Brazil: Pará, Serra dos Carajás, Cave N1N8/N1-0020 Flona Carajás, (6°01'57.7"S 50°16'18.6"W).
    Distribution: Brazil: Pará, Cave N1N8/N1-0022 (6°01'57"S, 50°16'18.6"W); Cave N1N8/N1-0008 (6°02'19.9"S, 50°16'13.1"W); Cave N1N8/N1-0073 (6°01'13.5"S, 50°17'17.4"W); Cave N1N8/N1-0037 (6°01'49.9"S, 50°16'27.7"W); Cave N1N8/N1-0016 (6°01'09.7"S, 50°16'40.9"W); Cave N1N8/N1-0168 (6°01'16.3"S, 50°18'05.1"W); Cave N1N8/N1-0240 (6°01'18.5"S, 50°16'26.1"W); Cave N1N8/N1-0101 (6°01'08.7"S, 50°16'46.2"W); Cave N1N8/N1-0037 (6°01'49.9"S, 50°16'27.7"W).
    Reference: (PELLEGRINI *et al.*, 2022c).

19. *Coarazuphium xingu* Pellegrini, Ferreira & Vieira *in* PELLEGRINI *et al.*, 2022c
    Type Locality: Brazil: Pará, São Félix do Xingu, Cave SFX-0057, (6°24'06.2"S, 51°54'08.1"W).
    Distribution: Known only for the type locality.
    Reference: (PELLEGRINI *et al.*, 2022c).

Family **Dytiscidae** (1 genus, 1 species)
Copelatus Erichson, 1832
20. *Copelatus cessaima* Caetano, Benà & Vanin, 2013 (Figure 2H)
    Type Locality: Brazil: Pará, Parauapebas, Floresta Nacional de Carajás, Gruta N4WS_0013 (6°4'21.79"S, 50°11'29.90"W).
    Distribution: Brazil: Pará, Gruta N4E_0022 (6°2'2.10"S, 50°10'3.74"W), Gruta N4E_0010, (6°2'20.16"S, 50°9'38.28"W).
    Reference: (CAETANO *et al.*, 2013).
Family **Staphylinidae** (3 genera, 4 species)

Metopiellus Raffray, 1908

21. *Metopiellus painensis* Asenjo, Ferreira & Zampaulo, 2017 (Figure 2I)
    Type Locality: Brazil: Minas Gerias, Pains, Loca dos Negros (20º26'7"S, 45º39'34"W).
    Distribution: Brazil: Minas Gerias, Grutas das Cerâmicas (20º24'16"S, 45º35'52"W).
    Reference: Asenjo *et al.* (2017).

Metopioxys Reitter, 1885

22. *Metopioxys carajas* Asenjo *in* (ASENJO *et al.*, 2019a) (Figure 2J)
    Type Locality: Brazil: Pará, FLONA Carajás, Gruta N4E_0010 (6°2'21"S, 50°9'39"W).
    Distribution: Brazil: Pará, Gruta N5S_0020 (6°5'16"S, 50°7'37"W), Gruta N5S_0010 (6°6'20"S, 50°7'53"W), Gruta N5S_0085 (6°5'13"S, 50°7'36"W), N4E_0021 (6°2'3"S, 50°9'37"W).
    Reference: (ASENJO *et al.*, 2019a).

Oxarthrius Reitter, 1883

23. *Oxarthrius aurora* Asenjo *in* Asenjo, Zampaulo & Ferreira, 2018 (Figure 2K)
    Type Locality: Brazil: Tocantins, Gruta Lage I (12°37'22.58"S, 46°26'6.05"W).
    Distribution: Brazil: Tocantins, Gruta das Rãs (12°41'19"S, 46°24'34"W).
    Reference: (ASENJO *et al.*, 2018).

24. *Oxarthrius inexpectatus* Asenjo *in* Asenjo, Zampaulo & Ferreira, 2018 (Figure 2L)
    Type Locality: Brazil: Minas Gerais, Cave RM_0033 (20°02'05"S, 43°59'40"W).
    Distribution: Known only for the type locality.
    Reference: (ASENJO *et al.*, 2018).

Identification key

The identification keys presented here are only for adult individuals of the troglobitic beetle species already described for Brazilian caves. It is noteworthy that there is practically no information on the larval stages of troglobitic species. If species are identified in locations other than the geographic distribution described in Table 1, they should be forwarded to reference collections for the group, since many of them lack data and this information is important for the definition of conservation strategies for Brazilian speleological heritage. Next, we provide a map with information on the diversity and distribution of troglobitic beetles in Brazil (Figure 3).

CARABIDAE

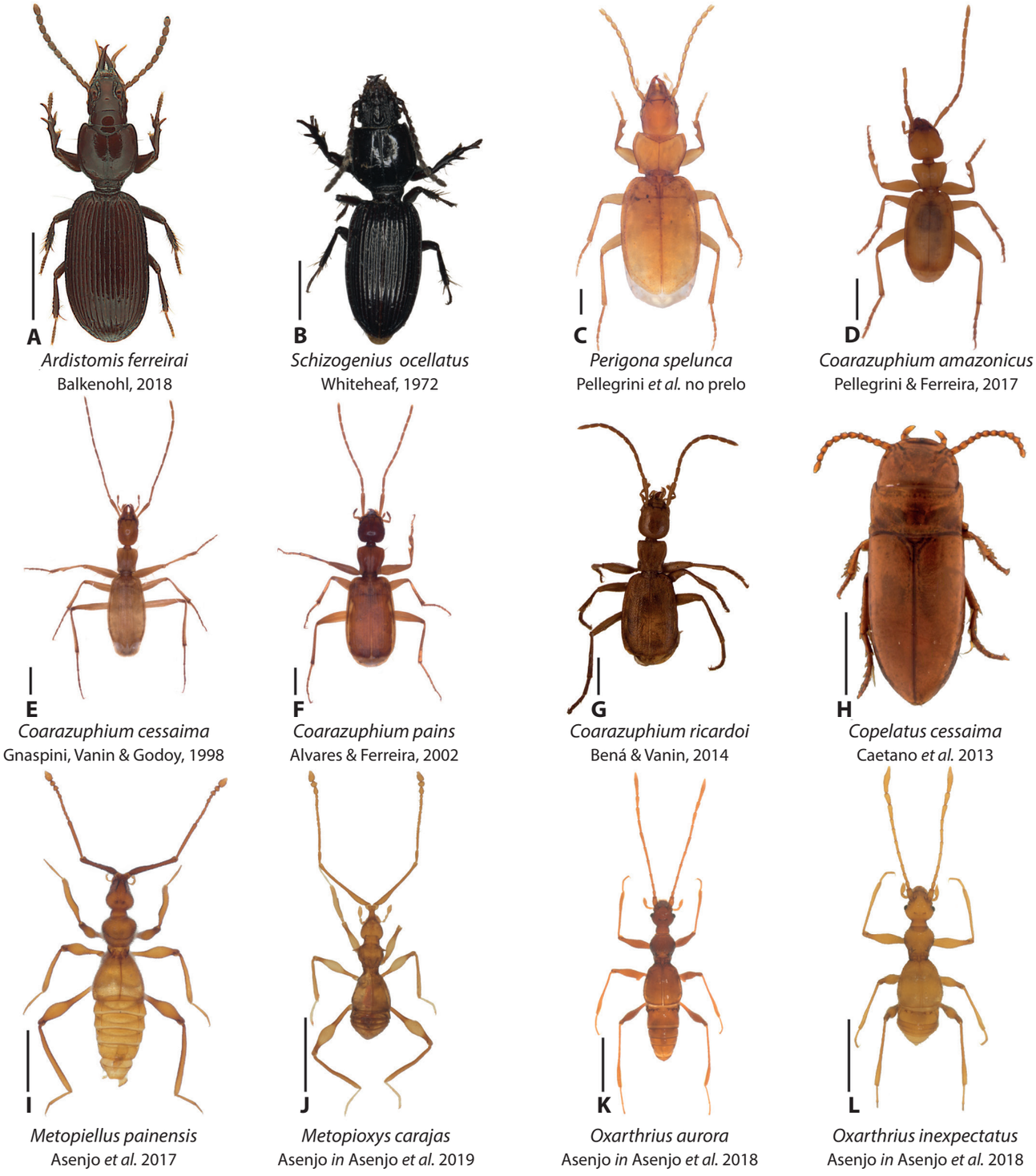
Carabids are popularly known by various names, such as bombardier beetles and tiger beetles. The family shows considerable variation in size, shape and color. It is the most diverse family that occurs in the subterranean environment, with the tribe Trechini being morphologically more modified with cave adaptations (FAILLE, 2019). The genus *Coarazuphium* is the group in Brazil that has the most evident morphological adaptations (GNASPINI; TRAJANO, 1994).

Diagnosis

Carabids have an elongated body with a dark brown to light brown color, yet some tribes have very colorful specimens, which may show iridescence. They range between 3.38 and 5.91 mm in length and possess filiform antennae. Although there are no works describing the larval instars of troglobitic species, the family as a whole has campodeiform larvae with well-developed legs and prominent mandibles.

Habitat

The described troglobitic species were always collected in aphotic regions of caves. Nothing is known yet about their feeding habits, except for a photographic record of *Coarazuphium formosus* feeding on fungi associated with bat guano *pellets* (Figure 1A) (PELLEGRINI; FERREIRA, 2011a).



Ardistomis ferreirai
Balkenohl, 2018

Schizogenius ocellatus
Whiteheaf, 1972

Perigona spelunca
Pellegrini *et al.* no prelo

Coarazuphium amazonicus
Pellegrini & Ferreira, 2017

Coarazuphium cessaima
Gnaspini, Vanin & Godoy, 1998

Coarazuphium pains
Alvares & Ferreira, 2002

Coarazuphium ricardoi
Benà & Vanin, 2014

Copelatus cessaima
Caetano *et al.* 2013

Metopiellus painensis
Asenjo *et al.* 2017

Metopioxys carajas
Asenjo *in* Asenjo *et al.* 2019

Oxarthrius aurora
Asenjo *in* Asenjo *et al.* 2018

Oxarthrius inexpectatus
Asenjo *in* Asenjo *et al.* 2018

Figure 2. A-L, Dorsal image of habitus. **A)** Modified from Balkenohl *et al.*, 2018; **B)** Image from T.G. Pellegrini; **C)** Image from T.G. Pellegrini; **D)** Modified from Pellegrini & Ferreira, 2014; **E)** Modified from Pellegrini *et al.*, 2020b; **F)** Modified from Pellegrini *et al.*, 2020b; **G)** Modified from Benà & Vanin, 2014; **H)** Modified from Caetano *et al.*, 2019; **I)** Modified from Asenjo *et al.*, 2017; **J)** Modified from Asenjo *et al.*, 2019; **K)** Modified from Asenjo *et al.*, 2018; **L)** Modified from Asenjo *et al.*, 2018. Scales: 1 millimeter.

CARABIDAE

1. Body weekly pedunculated, scutellum extending beyond peduncle separating base from elytra (Figures 2C-G); antennal insertion visible in dorsal view..... **2**

- Body distinctly pedunculated, scutellum entirely contained in peduncle (Figures 2A-B); antennal insertion not visible in dorsal view..... **18**

2(1). Antennal scape long, longer than second and third segments combined (Figure 2F) **3**

- Antennal scape shorter than second and third segments combined, eighth stria of elytra developed and expanded obliquely almost to apical angle (Figure 2C); metatrochanter long, nearly half metafemur leng th..... ***Perigona (Neoperigona) spelunca*** (Figure 2C)

3. Apical margins of elytra truncated, not sinuous (Figure 2A: PELLEGRINI; FERREIRA, 2011a) **4**

- Apical margins of elytra sinuous (Figure 1: GODOY; VANIN, 1990) or slightly sinuous (Figure 5: PELLEGRINI *et al.*, 2020b) ... **10**

4(3). Head with two pairs of setae, one anterior supraorbital (asos) and one post-ocular (pos) (Figure 5: PELLEGRINI; FERREIRA, 2017) **5**

- Dorsal portion of head with three or more pairs of setae; rarely two pairs. If only two pairs, there is no post-ocular setae (pos) **7**

5(4). Metafemur with spine on mid-portion of ventral surface (Figure 4: PELLEGRINI; FERREIRA, 2017); antennae short, about 0.68 times total body length..... ***Coarazuphium spinifemur***

- Metafemur without spine on ventral surface; antennae long, arriving at metafemur insertion..... **6**

6(5). Aedeagus very elongated and narrow, about 2.89 times left paramere length (Figures 2D-F: PELLEGRINI; FERREIRA, 2011b) ..... ***Coarazuphium tapiaguassu***

- Aedeagus smaller, about 2.60 times left paramere length (Figures 6C-E: PELLEGRINI; FERREIRA, 2017) ..... ***Coarazuphium amazonicum*** (Figure 2D)

7(4). Dorsal portion of head with two pairs of setae, one anterior supraobital (asos) and one posterior supraorbital (psos) (Figure 5: BENÁ; VANIN, 2014) ..... ***Coarazuphium ricardoi*** (Figure 2G)

- Dorsal portion of head with three or more pairs of setae, at least one pair of anterior supraorbital setae (asos), one pair of post-ocular setae (pos) and one pair posterior supra-orbital setae (psos) **8**

8. Dorsal portion of head with three pairs of setae (the three mentioned in step 7) ..... ***Coarazuphium auleri***

- Dorsal portion of head with three or more pairs of setae: with at least one pair of anterior supraorbital setae, one pair of post-ocular setae and one pair of posterior supraorbital setae **9**

9 – Dorsal portion of head with four seta insertions. In addition to asos, pos and psos, the head possesses a pair of posterior supernumerary setae (psus), located more anterior and laterally to posterior supra-orbital setae (psos) (Fig. 22: PELLEGRINI *et al.*, 2020b) ..... ***Coarazuphium pains*** (Figure 2F)

- In addition to asos, pos, psos and psus, the dorsal portion of the head possesses a pair of occipital setae located more posterior/medially than the posterior supernumerary setae (psus) (Figure 8: Pellegrini *et al.* 2022c) ..... ***Coarazuphium xikrin***

10(3). Dorsal portion of head with at least two pairs of setae, with anterior supraorbital setae (asos) and post-ocular setae (pos) always present **11**

- Dorsal portion of head with four or five pairs of setae, with a pair of anterior supraorbital setae (asos), a pair of post-ocular setae (pos), a pair of posterior supraorbital setae (psos) and a pair of occipital setae (ocs) always present **14**

11(10). Dorsal portion of head with only two pairs of setae, one pair of anterior supraorbital setae (asos) and one pair post-ocular setae (pos) (Figure 29: PELLEGRINI *et al.*, 2022c) ..... ***Coarazuphium xingu***

- Dorsal portion of head with three pairs of setae..... **12**

12(10). General body shape elongated (Figure 33: PELLEGRINI et al. 2020b), maximum elytra width close to half. Head elongated, width to length ratio 0.6. Apical sinuosity of elytra with tapered inner edges (Figure 33: PELLEGRINI et al., 2020b). Male genitalia: right paramere stilliform (Figure 10: GNASPINI et al., 1998), as long as left paramere ..... ***Coarazuphium cessaima*** (Figure 2E)

- Head subquadrangular, width to length ration about 0.95 ..... **13**

13(12)- Left paramere of male genitalia stilliform (Figure 2: GODOY; VANIN, 1990) ..... ***Coarazuphium tessai***

- Left paramere of male genitalia globose (Figure 11: PELLEGRINI *et al.*, 2022a) ..... ***Coarazuphium bambui***

14(10). Dorsal portion of head with two pairs of occipital setae (ocs) (Figure 2B: PELLEGRINI; FERREIRA, 2014) **15**

- Dorsal portion of head with one pair of occipital setae (ocs) **17**

15(14). Head showing pubescence (Figure 18: PELLEGRINI et al., 2022c), also with medium-sized seta anterior-lateral to posterior supernumerary setae (psus) ..... ***Coarazuphium kayapo***

- Head in dorsal view glabrous, except for fixed setae..... **16**

16(15). Elytra with slight apical sinuosity (Figure 12A & C: PELLEGRINI; FERREIRA, 2014) ..... ***Coarazuphium formoso***

- Elytra with more pronounced apical sinuosity, aspect of dorsal/posterior portion of head with two protuberances (Figures 12B & D: PELLEGRINI; FERREIRA, 2014) ..... ***Coarazuphium caatinga***

17(14). Dorsal portion of head with one pair of posterior supernumerary setae (psus) (Figure 6: PELLEGRINI *et al.*, 2020b) ..... ***Coarazuphium lundi***

- Dorsal portion of head without pair of posterior supernumerary setae (psus) ..... ***Coarazuphium bezerra***

18(1). Front without longitudinal carinae; mandibles elongated (Figure 2: BALKENOHL *et al.*, 2018); Geographic distribution: state of Pará ..... ***Ardistomis ferreirai*** (Figure 2A)

- Front with four or five pairs of longitudinal carinae; Geographic distribution: state of São Paulo ..... ***Schizogenius ocellatus*** (Figure 2B)

Diversity

There are 1,812 species of Carabidae in Brazil (ANICHTCHENKO *et al.*, 2021), of which 15 species were described as troglobites.

DYTISCIDAE

Dytiscidae constitutes one of the most important families of aquatic beetles, with bodies very well adapted for swimming (hydrodynamic) and flattened hind legs accompanied by long setae. The family is present in all regions of the world, but remains a little studied group in Brazil, especially in subterranean aquatic environments.

Diagnosis

Species of the family have an oval and dorsoventrally flattened body with a light brown color, are around 3.90 mm in length and possess filiform antennae. The hind legs are flat and adapted for swimming to varying degrees and the tarsi are five-segmented (5-5-5) with one or two claws. Although there are no works describing the larval instars of troglobitic species (FERREIRA; COSTA, 2017), larvae of the family, in general, have legs with well-developed bristles that aid in swimming and accentuated and falcate mandibles.

DYTISCIDAE

1. Scutellum visible with elytra closed (Figure 2H); metacoxal lines strongly approximated medially (Figures 2,4: Caetano *et al.*, 2013); metatarsal nails of similar length in both sexes. Compound eyes absent; mentum and gula fused (Figures 2,4: CAETANO *et al.*, 2013) .....***Copelatus cessaima*** (Figure 2H)

Habitat

Both larvae and adults of this family are generalist predators in aquatic environments and are present in all continental freshwater bodies (rivers, lakes, ponds, etc.). Species of the family have been collected in high altitude regions, as well as in water bodies inside caves.

Diversity

Dytiscidae is represented by 322 species in Brazil (ASENJO, 2022, unpublished data), with only one species, *Copelatus cessaima* from the region of Carajás, state of Pará, being described as a troglobite (CAETANO *et al.*, 2013).

STAPHYLINIDAE

Staphylinidae is one of the most diverse coleopteran families in the world. It presents a wide variety of colors and shapes with the vast majority of species being elongated with short elytra exposing part of the abdomen. Some species are popularly known as "potós" and can cause accidents of medium importance, with injuries to humans who may come into contact with them.

Diagnosis

All troglobitic species of Staphylinidae known for Brazil thus far belong to the subfamily Pselaphinae, although other subfamilies occur in caves (e.g., Scydemaninae and Osoriinae). Species of Pselaphinae are between 1.92 and 3.25 mm in length and have head, pronotum and elytra with deep fovea, short elytra, and tarsi with three segments (3-3-3) with a nail. Although there are no works describing the larval instars of troglobitic species (FERREIRA; COSTA, 2017), larvae of the family are, in general, active campodeiform-type predators with well-developed legs and articulated urogomphi present at the end of the abdomen.

Habitat

The described troglobitic species of the family were collected in the aphotic region of caves in the region of Carajás, state of Pará (*Metopioxys carajas*), in the state of Tocantins (*Oxarthrius aurora*), in the Pains region of the state of Minas Gerais (*Metopiellus painensis*) and in iron and carbonate caves in the Center-North of Minas Gerais (*Oxarthrius inexpectatus*). Nothing is yet known about the feeding habits of the described cave species.

Diversity

Staphylinidae has 2,829 described species/subspecies in Brazil (ASENJO *et al.*, 2019b), of which four species were described as troglobites, one from Tocantins, one from Pará and two from Minas Geais.

STAPHYLINIDAE

1. First antennomere short, less than a quarter of total antenna length (Figures 2K-L); compound eyes reduced, but with more than one ommatidia.....**2**

- First antennomere very long, almost half of total antenna length (Figures 2I-J); eyes composed of one ommatidia or absent.....**3**

2(1). Males with truncated spine on basal half of posterior margin of mesofemur (Figure 16: ASENJO *et al.*, 2018) ..... ***Oxarthrius aurora*** (Figure 2K)

- Males with long non-truncated spine on basal half of posterior margin of mesofemur (Figure 5: ASENJO *et al.*, 2018)..... ***Oxarthrius inexpectatus*** (Figure 2L)

3(1). Lateral region of pronotum without long spine (Figures 3-4: ASENJO *et al.*, 2017); eyes practically absent (one ommatidia) (Figure 3: ASENJO *et al.*, 2017); second antennomere (pedicel) long, approximately half of first antennomere (scape) length (Figure 5: ASENJO *et al.*, 2017)..... ***Metopiellus painensis*** (Figure 2I)

- Lateral region of pronotum with long spine (Figure 5: ASENJO *et al.*, 2019a); eyes absent (Figure 3: ASENJO *et al.*, 2019a); second antennomere (pedicel) short, less than a quarter of first antennomere (scape) length (Figure 4: ASENJO *et al.*, 2019a) ..... ***Metopioxys carajas*** (Figure 2J)

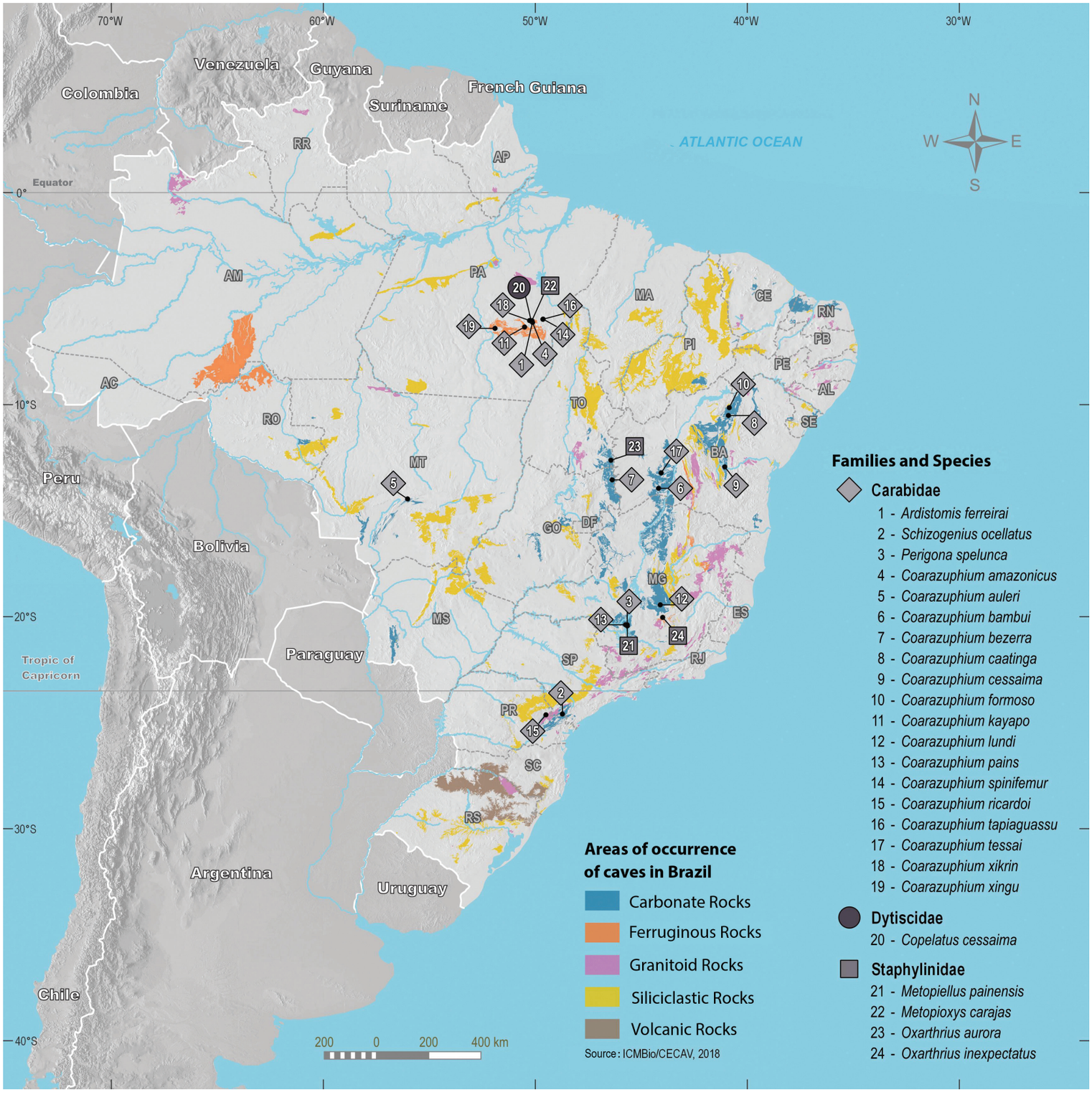


Figure 3. Map with type localities of troglobitic beetle species described for Brazil.

Collection, curation and conservation methods

Current advances in the knowledge of troglobitic beetle species is the result of efforts to train personnel and public and private investment for the collection and description of new species. The maintenance of biological collections in Brazil that have infrastructure for the conservation of type material is also a constant challenge, but one that has been mediated. The curatorship of collections requires long-term investments in personnel and infrastructure, so that the deposited specimens are maintained intact over the decades. Therefore, knowledge about biological diversity and the maintenance of collections can only be achieved with continuous research and funding, which is especially critical when dealing with rare species, such as most troglobitic species. The choice of institution for the deposition of troglobitic specimens must made with care so that they receive the appropriate curation, and it should be a reference collection with free access to researchers whenever necessary. The Brazilian troglobitic beetles described to date are deposited in reference collections in Brazil and throughout the world, which allows these specimens to contribute to knowledge about the subterranean habitat.

COLLECTION

The collection methods used for the group are proportional to beetle diversity (IANNUZZI *et al.*, 2021). However, devices applied in the surface environment to collect beetles are not necessarily the most recommended for the cave environment (WEINSTEIN; SLANEY, 1995). Troglobitic beetles are, for the most part, rare. The known distribution for many is only a single cave with small populations. These characteristics make the use of passive collection methods (i.e., "pitfall traps") dangerous, as there may be an oversampling of individuals, which can result in drastic reductions in the size of populations (HUNT; MILLAR, 2001). As a result, the use of standardized methods for collecting cave beetles, especially in ecological studies, becomes a challenge (IANNUZZI *et al.*, 2021).

TERRESTRIAL CAVE ENVIRONMENT

Active capture methods are the most widely used methods for capturing beetles in the terrestrial environment of Brazilian caves (e.g., FERNANDES *et al.*, 2016; PELLEGRINI; FERREIRA, 2012b; SOUZA SILVA *et al.*, 2011; TRAJANO; GNASPINI-NETO, 1990; ZEPON; BICHUETTE, 2017). The most commonly employed method is active visual search, which consists of searching for beetles in preferred biotopes, such as under logs and rocks, in litter and guano accumulations, and in damp places in caves (Snyder *et al.*, 2006). Once found, organisms are captured manually with the aid of tweezers, brushes or entomological aspirators. Two variations of this method are employed to standardize collection by effort for ecological studies — standardization by time and by area delimited in quadrants. The time technique consists of standardizing the sampling effort by time/area of collection (OLIVEIRA, 2014; WYNNE *et al.*, 2018). The quadrant technique has been more widely used in ecological studies and in fauna monitoring studies (BICHUETTE *et al.*, 2015; PELLEGRINI; FERREIRA, 2012b; SHARRATT *et al.*, 2000; TOBIN *et al.*, 2013; ZEPON; BICHUETTE, 2017). This involves delimiting squares or rectangles of standardized area in different places of the cave and, in these delimited areas, performing thorough searches for invertebrates (SHARRATT *et al.*, 2000). The use of the quadrant technique can result in greater beetle richness than the active search technique throughout the cave (BICHUETTE *et al.*, 2015). In large halls, or places that offer many shelters for invertebrates, and where active search is more difficult, the use of baits such as cheese or pieces of liver is an option (FAILLE *et al.*, 2014; HUNT; MILLAR, 2001). Such baits attract other cave invertebrates that end up attracting their predators, especially beetles of the family Carabidae. Once attracted, they can be captured manually (FAILLE *et al.*, 2014). It is worth mentioning that the use of baits should always be done with caution and away from entry regions, so as to prevent epigean species from being attracted, collected and attributed to the cave environment (CASTRO SOUZA *et al.*, 2019). The use of these active collection methods, in which the number of collected individuals is controlled, reduces the chances of oversampling of invertebrates (PAQUIN; PROVOST, 2010).

Given the sampling difficulties imposed by the cave environment, combined with the fact that most beetles considered strictly subterranean in Brazil belong to the family Carabidae, the use of "fall" traps (i.e., *pitfall*) seems to be a good alternative. *Pitfalls* consist of containers that remain buried in the ground for about five days, making these traps efficient in sampling arthropods that live on the ground, such as beetles (BROWN; MATTHEWS, 2016). However, as already mentioned,

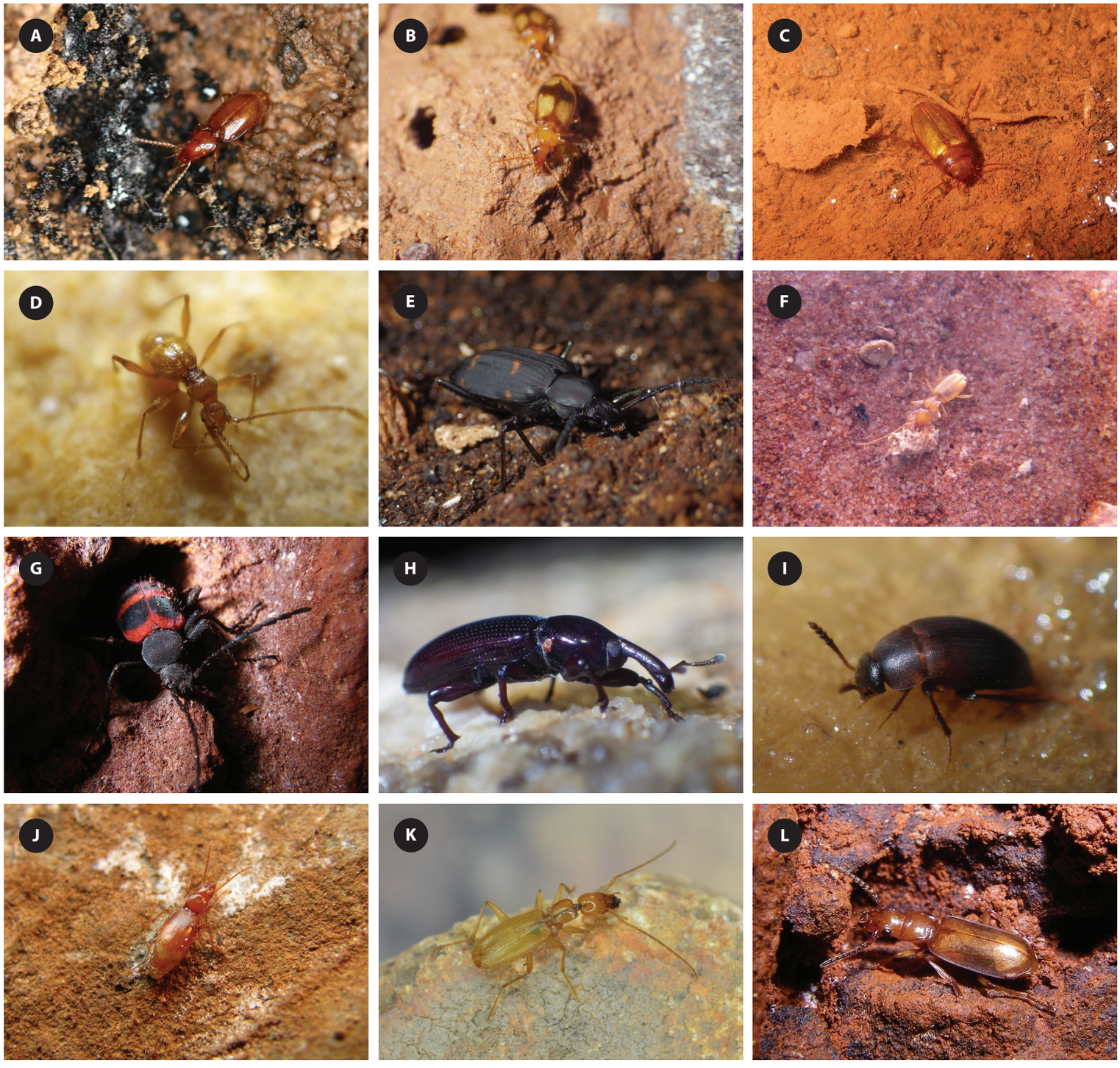


Figure 4. A-L, Examples of beetle families frequently found in Brazilian caves. **A)** *Perigona spelunca* (Carabidae); **B)** *Nemotarsus* sp. (Carabidae); **C)** Dytiscidae; **D)** *Oxarthrius* sp. (Staphylinidae); **E)** Tenebrionidae; **F)** *Coarazuphium formoso* (Carabidae); **G)** Tenebrionidae; **H)** Curculionidae; **I)** Subfamily Cholevinae (Leiodidae); **J)** *Perigona spelunca* (Carabidae); **K)** *Coarazuphium cessaima* (Carabidae); **L)** Tribe Ozaenini (Carabidae). Photos: A, J, F (Rodrigo L. Ferreira); B, D, E, F, H, I (Lucas M. Rabelo); C, G, L (Robson de A. Zampaulo); K (Leonardo S. Carvalho).

the use of *pitfalls* should be avoided in cave environments due to the risk of oversampling troglobitic species (HUNT; MILLAR, 2001; KOZEL *et al.*, 2017). Although *Winkler* extractors and *Berlese-Tullgren* funnels are not established methods for caves (BICHUETTE *et al.*, 2015), they are good alternatives when there is accumulation of organic matter, such as litter (OLIVEIRA, 2014) and guano (PELLEGRINI; FERREIRA, 2013). The extractors are efficient in sampling small-bodied and runner beetles (e.g., some Staphylinidae, Ptiliidae and Bembidiini). In both methods, part of the substrate is allocated to *Winkler* extractors or *Berlese-Tullgren* funnels. This feature of extractors makes it possible to restrict the sampled area, avoiding oversampling of sensitive individuals with low population densities.

SUPERFICIAL SUBTERRANEAN MEDIUM (SSM)

Access to organisms that inhabit SSM spaces can be accomplished by using shovels to dig the soil and actively seek out beetles with the aid of tweezers, brushes and suckers. It can also be passive, by which, after removing the soil, part of the substrate is placed in a container with water for flotation, and the organisms are captured as they float to the surface of the water. The substrate could also be placed in a sifter, whereby the organisms are released from the rock and are directed to a collection container. There are also devices specifically designed for SSM called "subterranean sampling devices" (SSDs). These devices comprise PVC pipes with multiple perforations on their sides for the fauna to access the inside of the trap where there are containers, baited or not, arranged in layers with a preservation liquid (ORTUNÕ *et al.*, 2019). The PVC pipe is buried in the ground and the perforations correspond to the multiple layers of the SSM. The PVC pipe is capped at the surface to avoid external contamination and to allow samples to be removed over time without the need to dig and cause a new disturbance with each sampling (LOPÉZ; OROMÍ, 2010; MAMMOLA *et al.*, 2016; PIPAN *et al.*, 2010).

EPICARST

The most conventional collection method is to sample drip points on the cavern ceiling, where funnels are placed that direct the water to a collection container. The container is a metal or plastic device, with openings on the sides, covered by a mesh that allows the passage of water without the organisms being able to escape (Figure 1 in CULVER *et al.*, 2019).

A second, and even more indirect, method of collection is through small puddles formed in the cave, coming from drips from the ceiling. Fauna is accessed with the aid of zooplankton nets or by means of a pump, in cases where the pool contains a small volume of water or has many fissures and speleothems (Figure 2 in CULVER *et al.*, 2019). The water is then poured into a container, where it is filtered, and the organisms are collected. This method is simpler and more immediate, but it loses efficiency in collecting stygobites (PIPAN *et al.*, 2010). It is worth mentioning that in both methods the water must be shaken vigorously so that the individuals associated with the substrate detach themselves, thus allowing their collection.

AQUATIC SUBTERRANEAN ENVIRONMENT

The method most commonly used in small puddles (i.e., travertine puddles) is active visual search with the aid of suckers (pipettes), an efficient method for collecting small and fast-swimming beetles (HUNT; MILLAR, 2001). For larger accumulations of water, such as lakes or when the water table is accessible in the cave environment, passive collection methods are more efficient. The use of plastic pots (which can be easily made with pet bottles containing attractive baits) is a good option when dealing with large reservoirs that are often difficult to access. This type of trap must remain in the environment for at least 24 hours. In lotic environments, methods more widely used in the epigean environment are also efficient in caves. Ecological studies have used passive collection methods that allow standardization of the sample area, such as the use of *Surber* nets by which species of Dytiscidae, Elmidae, Gyrinidae, Hydrophilidae and Scirtidae are commonly captured (MARTINS; FERREIRA, 2020; PELLEGRINI *et al.*, 2018; PELLEGRINI *et al.*, 2020a). The use of hand nets can also be a good option for active search since they are easy to transport and can be used in lotic and lentic environments.

PRESERVATION

The preservation of collected material must be done, as a priority, in liquid form. Ethanol at 70% is generally the most used, since at this concentration there is a balance between specimen conservation and water loss from the material by osmotic pressure (ALMEIDA *et al.*, 1998). When the number

of collected specimens is greater, part of the series should be allocated to absolute alcohol for molecular studies.

Immature specimens are ideally sacrificed in hot water for 1 to 5 minutes and then transferred to 70% ethanol or Kahle's solution, to avoid possible morphological changes. It should be noted that liquid preservation can change specimen color over time, especially in the case of larvae. Therefore, material should be photographed before this occurs, especially when it is suspected to be a troglobitic species.

If the number of specimens allows, it may be desirable to preserve part of them in a dry manner to facilitate their handling. However, this process should be done by specialists to avoid damage to the morphology of the animal. To assemble beetles, first check to see that they are dry, as in this case it may be necessary to place them in a humid chamber for softening, which is not necessary when handling fresh specimens. A humid chamber is a container with a wide opening and a lid, filled with a layer of wet sand mixed with naphthalene, on which a filter paper is placed where the insects to be softened will be arranged (ALMEIDA *et al.*, 1998). The chamber should then be left close to a heat source (e.g., a lamp) for the amount of time needed to rehydrate the specimen(s), which can vary from 12 to 24 hours or more, depending on specimen size.

For the assembly of troglobite beetles, due to the small size of the known species, direct pinning should be avoided, since perforation of the insect's body can damage its morphology. Instead, small and elongate triangles of heavyweight paper (e.g., 180g/m^2) can be used, with the tip bent and base pierced by an entomological pin, leaving about 1cm between the triangle and the head of the pin. The beetles are then glued to the bent end, preferably at the base of the right elytra, so that the animal is at a perpendicular angle to the pin, to facilitate its visualization and handling. The glue must be water soluble in case it is necessary to undo the assembly later.

It is important to remember that, whether using liquid (in a bottle) or dry (with a pin) preservation, there must always be a label of origin with collection data (e.g., cave, coordinates, municipality, collector, etc.). More information on mounting and labeling can be found in Rafael *et al.* (2012), among others.

Main collections

Entomological collections store immeasurable amounts of information about biological diversity that can eventually be used for the recognition of ecological and evolutionary patterns and processes. Access to specimens deposited in collections has become an increasingly promising way to produce scientific knowledge in several areas, using sophisticated techniques and tools. Microscopy and computed tomography contribute to the advancement of the morphological study of species. The digitization of biological collections and the creation of databases allow species distribution modeling techniques to contribute to the advancement of research related to biogeography and patterns of global changes (SHORT; DIKOW; MOREAU, 2017). These are just a few examples of the importance of maintaining and curating entomological collections. Especially in older collections, there remains access to specimens collected in past centuries by great naturalists, which highlights historical character and the preservation of national and international heritage (PAPVERO, 1994).

Collections of cave beetles are stored mainly in dry and wet methods, but can also be stored on slides, due to the small size of some specimens (TRIPLEHORN; JOHNSON, 2011). However, for the preservation of specimens, the wet method may be the best way of conditioning, especially for specimens of troglobitic species with small dimensions (< 5 mm) and soft bodies, as well as larvae and pupae. Material conditioned dry is mostly kept in a separate place from the material kept in liquid.

The largest collections of beetles are in entomological collections located in several countries and type specimens, mainly the primary types (holotype, lectotype, neotype and syntype), must be deposited in well-established collections with the technical capacity and infrastructure for specimen curation.

The first entomological collections in the world were established between the 15th and 16th centuries, based on material collected by European expeditions carried out by naturalists. *Systema Naturae*, published in 1735 by Carol von Linné, allowed the organization of species in natural history museums and the beginning of a basis for classifying insects (NISHIDA, 2009).

The main entomological collections in Brazil began to be established in the 18th century. However, much of the material collected in Brazil during this period was sent and deposited in foreign collections. Large collections of Coleoptera began to be organized in the early 19th century, with the establishment of the Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ), Instituto Oswaldo Cruz (IOC), Museu Paraense Emílio Goeldi

(MPEG) and the inclusion of the zoology section in Museu Paulista in 1894, now known as Museu de Zoologia da Universidade de São Paulo (MZUSP) (COSTA *et al.*, 2000). Currently, the collections in Brazil that possess the largest number of specimens of cave beetles are the collection of Invertebrados Subterrâneos (ISLA) of the Universidade Federal de Lavras (UFLA), state of Minas Gerais; the collection of Laboratório de Estudos Subterrâneos (LES) of the Universidade Federal de São Carlos (UFSCar), state of São Paulo; and the collection of Museu de Zoologia da USP (MZUSP), São Paulo.

NATIONAL

Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP).

The collection of Museu de Zoologia da USP (MZUSP) is one of the most important beetle collections in Brazil and in the Neotropical Region. It possesses about 40,000 specimens preserved in dry and wet manners, of which 2,300 are type specimens. The beetles collected in caves are conditioned in a wet manner. It is important to emphasize that the best represented and identified collection of Neotropical Carabidae is deposited at MZUSP.

Coleção de Invertebrados Subterrâneos de Lavras, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil (ISLA).

The Coleção de Invertebrados Subterrâneos is housed at the Centro de Estudos em Biologia Subterrânea (CEBS). It has a collection with several specimens from caves in Brazil and abroad. Highlighted among the specimens are type material of several species of troglobite beetles of the genera *Coarazuphium*, *Ardistomis*, *Perigona*, *Metopiellus, Oxarthrius* and *Metopioxys*.

Coleção do Laboratório de Estudos Subterrâneos, Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil (LES).

The Laboratório de Estudos Subterrâneos possesses a scientific collection of more than 17,000 specimens of diverse taxa, including invertebrates and vertebrates. Various representatives of troglobite coleopterans are deposited in LES, among which are specimens of *Coarazuphium* spp., already described and in the process of being described.

Centro de Coleções Taxonômicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil (CCT-UFMG).

The CCT-UFMG insect collection has more than 188,000 specimens, including type specimens. The collection is accessible to researchers for consultation and loan.

Setor de Entomologia da Coleção Zoológica da Universidade Federal de Mato Grosso, Departamento de Biologia e Zoologia, Cuiabá, Mato Grosso, Brazil (CEMT).

Since 2008, the Setor de Entomologia of the Coleção Zoológica of UFMT has been transformed into a collection of national and international relevance, with thousands of specimens representing more than 130 families of Coleoptera, kept dry (VAZ DE MELLO, 2017). The superfamily Scarabaeoidea is the best represented to date, with more than 6,000 species identified to the species level. More than 300,000 specimens also make up the collection, with representatives mainly from the families Staphylinidae, Carabidae, Histeridae, Tenebrionidae, Meloidae and Buprestidae.

Coleção Entomológica da Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil (CEUFLA).

The CEUFLA insect collection has more than 50,000 specimens of insects including cave beetles. The collection is fully accessible for consultation and loan.

INTERNATIONAL

Museum d'Histoire Naturelle, Geneva, Switzerland (MHNG).

The Coleoptera collection at Museu de História Natural de Geneva has specimens from all over the world. The best represented taxonomic groups are Staphylinoidea, Chrysomeloidea and Scarabaeoidea. This collection has 180,000 specimens of Pselaphinae (Staphylinidae)

University of Alberta, Strickland Museum, Edmonton, Alberta; Canada (UASM).

The Coleoptera collection of the **Strickland Museum** has great representation of insects of agricultural importance. Beetles of the family Carabidae are organized by zoogeographic

regions and not taxonomically, as collections are often organized. One of the services provided by this institution is species identification of specimens of Carabidae.

Section of Invertebrate Zoology, Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, United States (CMNH)

The Coleoptera collection of the **Carnegie Museum of Natural History** has specimens from different parts of the world. The collection includes several families of Coleoptera, including Carabidae and Staphylinidae. It also maintains the Thomas C. Barr Collection with carabid specimens from caves (Nearctic fauna).

Department of Entomology, National Museum of Natural History, Smithsonian Institute, Washington DC., United States (NMNH)

The collection of Coleoptera at the NMNH is one of the largest coleopteran collections in the world, with around 20,000 primary type specimens. Among the best represented families are Carabidae and Staphylinidae, with many specimens of Neotropical species.

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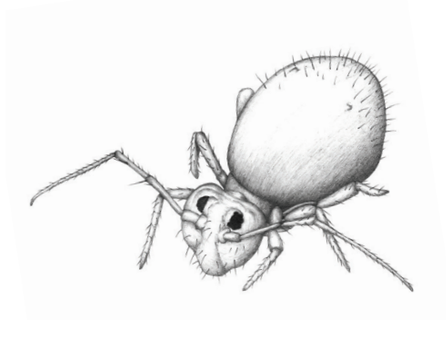
Sminthurinae sp.
Lucas Mendes Rabelo

11

Collembola

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General introduction

Class Collembola (Arthropoda, Hexapoda) is a basal taxon of Hexapoda comprising small terrestrial animals that usually measure between 0.1 mm and 2 mm in length (HOPKIN, 1997), without wings and with mouthparts (mandible and maxilla) inside a cephalic capsule (ZEPPELINI; BELLINI, 2004).

They are similar to insects in having three tagmata (head, thorax and abdomen), one pair of antennae and three pairs of thoracic legs but differ in the presence of three abdominal appendages: ventral tube (collophore), tenaculum and furcula (manubrium + tooth + mucro) (Figure 1). These appendages are considered synapomorphies of the taxon, although the tenaculum and furcula may be absent in some members of the class (CIPOLA *et al.*, 2018; HOPKIN, 1997).

All species of Collembola have a ventral tube (collophore) consisting of reversible vesicles derived from a pair of appendages on the first abdominal segment. This organ is extremely important for absorbing water and transporting Na+, K+, Cl-, H+ and NH4+ (KONOPOVÁ *et al.* 2019) and can also function as a sticky appendage allowing the animal to cling to slippery surfaces (HOPKIN 1997). In some species, the ventral vesicles can extend to more than twice the body length and can be used for self-stabilization after a jump (Brackenbury, 1990).

Collembolans, or springtails, are present and abundant in almost all terrestrial ecosystems, from polar regions to tropical forests and deserts. They live in a variety of habitats, such as in treetops, on the ground, in caves and even in marine habitats, in the intertidal zone and in surface water. In addition to being very abundant, they are interesting due to several peculiar phenomena: (1) their ability to survive sub-zero temperatures allows them to even colonize environments with cold climates, such as Antarctica, and they are active in snow in temperate climates; (2) synchronized reproduction in favorable conditions and climate sometimes leads to the formation of collembolan swarms, a striking phenomenon where often several million individuals aggregate at high densities on a small surface; and (3) are highly diverse in subterranean habitats around the world (LUKIĆ, 2019).

They generally feed on bacteria, fungi, algae and myxomycetes

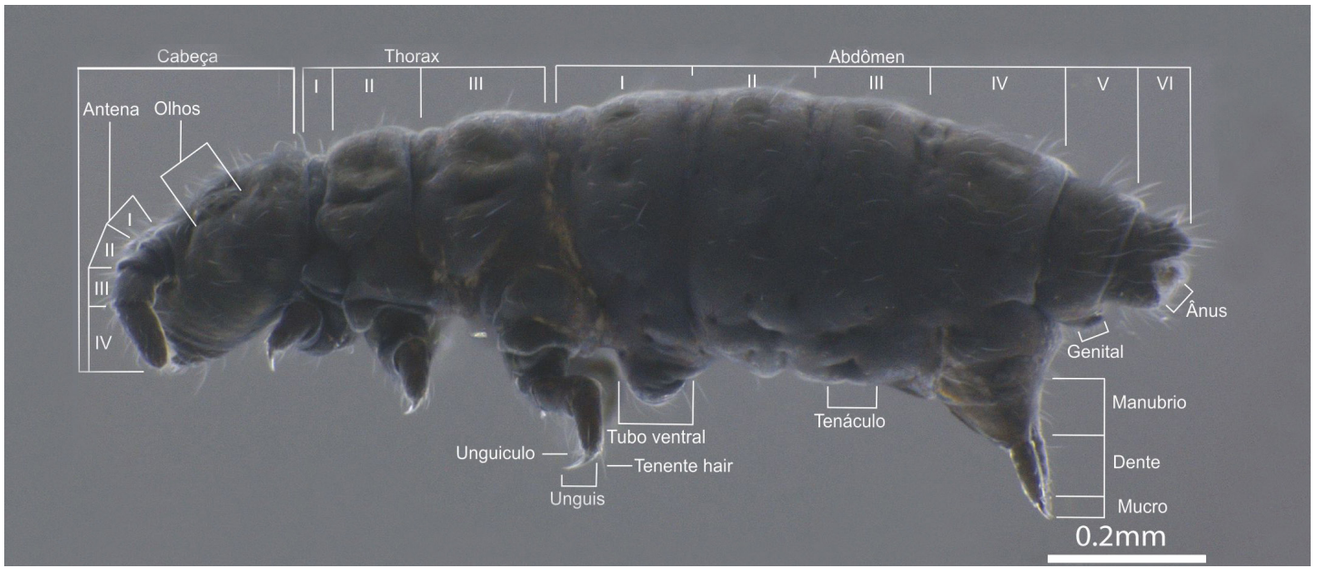


Figure 1. Collembolan external morphology. Modified from Cipola (2020).

and are numerically dominant hexapods, especially in soil and litter, where they play important roles in the decomposition of organic matter and in the food web. Collembolans are very abundant in subterranean habitats, being at the base of the food web. They grow on organic matter of these environments, including guano. They are present in different subterranean habitats, from "Shallow Subterranean Habitats" (SSHs) (CULVER; PIPAN, 2014), to deeper environments such as caves. Troglobitic species (eutroglophiles) are very common and abundant in several caves. In addition, some species that inhabit guano deposits may be restricted to this type of substrate, being classified as guanobia (GNASPINI; TRAJANO, 2000).

Main groups found in Brazilian caves

Collembola includes the oldest terrestrial invertebrates, whose fossil records are approximately 400 million years old (CHRISTIANSEN; PIKE, 2002). The class is divided into four orders and 33 families (Table 1): Poduromorpha Börner, 1913 (11 families); Neelipleona Massoud, 1971 (only one family); Entomobryomorpha Börner, 1913 (11 families) and Symphypleona Börner, 1901 (10 families).

There are currently about 9,000 described species of collembolans in the world (BELLINGER *et al.* 2020). However, this substantial species richness probably represents only a small portion of the true diversity of the class, which may exceed 50,000 species (CICCONARDI *et al.*, 2013; CIPOLA *et al.*, 2018; DEHARVENG *et al.*, 2008; HOPKIN, 2008; HOPKIN, 2013; CIPOLA *et al.*, 2018; 1997). A total of 444 species are recognized in Brazil, distributed in 117 genera and 20 families (ZEPPELINI *et al.*, 2020).

Collembolans represent one of the richest groups among all cave arthropods. Recurrent in these environments, they are one of the most successful groups in colonizing subterranean environments, with troglobitic species recorded in the most different karst regions of the world and an expressive diversification of groups and species. Table 1 shows the orders and families of Collembola existing in the world, of which five have troglobitic species described for Brazil.

Table 1. Orders and families of the class Collembola, according to Bellinger *et al.* (2020).

Order	Family
Poduromorpha Börner, 1913	Brachystomellidae Stach, 1949
	Gulgastruridae Lee & Thibaud, 1998
	Hypogastruridae Börner, 1906*
	Isotogastruridae Thibaud & Najt, 1992
	Neanuridae Börner, 1901
	Odontellidae Massoud, 1967
	Onychiuridae Lubbock, 1913
	Pachytullbergiidae Stach, 1954
	Paleotullbergiidae Stach, 1954
	Poduridae Latreille, 1804
	Tullbergiidae Bagnall, 1935
Neelipleona Massoud, 1971	Neelidae Folsom, 1896

Entomobryomorpha Börner, 1913	Actaletidae Börner, 1902
	Coenaletidae Bellinger, 1985
	Entomobryidae Schäffer, 1896*
	Isotomidae Schäffer, 1896
	Oncobryidae Christiansen, 2002
	Oncopoduridae Carl & Lebedinsky, 1905
	Orchesellidae Börner, 1906
	Paronellidae Börner, 1906*
	Praentomobryidae Christiansen, 2006
	Protentomobryidae Folsom, 1937
	Tomoceridae Schäffer, 1896
Symphypleona Börner, 1901	Arrhopalitidae Stach, 1956*
	Bourletiellidae Börner, 1913
	Collophoridae Bretfeld, 1999
	Dicyrtomidae Börner, 1906
	Katiannidae Börner, 1913
	Mackenziellidae Yosii, 1961
	Sminthuridae Lubbock, 1862*
	Sminthurididae Börner, 1906
	Spinothecidae Delamare Deboutteville, 1961
	Sturmiidae Bretfeld, 1994

* Families with troglobitic species registered for Brazil.

Main troglomorphisms

Troglomorphic traits are well documented among different taxa, but within Collembola they are exceptionally diverse (Table 2). Some characteristics are shared with other arthropods, while others are restricted to Collembola, among them the regression of the tibiotarsal *Tenent hair* and elongation of the mucro.

The morphology of the collembolan tarsal claw is probably one of the best studied adaptive troglomorphisms. In cave species, the unguis is usually elongated and thinner and the inner and lateral teeth are usually reduced or absent, while the basal pair of teeth is displaced more proximally or, in extreme cases, completely reduced (Figure 2B). This adaptation facilitates the locomotion of the species in very humid environments such as the interior of caves.

Table 2. Main troglomorphic characteristics of Collembola. Source: Deharveng (1988); Christiansen (2012); Lukic (2019).

Larger body size
Elongation of locomotor appendages
Elongation and subsegmentation of antennae
Specialization of sensory bristles (sensilla) on antennae and body
Basal displacement and regression of inner and lateral teeth of unguis
Elongation of empodial complex
Elongation of tracking bristle (tenent hair)
Elongation of dens and mucro
Reduction of eyes
Reduction of pigmentation

Almost all families of Collembola have significantly modified troglomorphic species in European caves (DEHARVENG; BEDOS, 2012), with notable examples of Entomobryidae, in addition to Southeast Asian with species of Paronellidae (DEHARVENG *et al.*, 2018; JANTARIT *et al.*, 2019). Reduced eyes and depigmentation, which are also observed in non-troglobitic collembolans (such as Anurophorinae and Cyphoderinae), are generally very frequent in troglobitic species, being the most common characteristics among Brazilian subterranean collembolans.

Diversity of troglobitic species

More than 400 troglobitic species of Collembola are known in the world (DEHARVENG; BEDOS, 2012) Collembolans generally dominate, in abundance, invertebrate communities in terrestrial cave environments: Entomobryidae and Paronellidae in tropical caves; Hypogastruridae, Onychiuridae and Entomobryidae in temperate caves.

Although the first record of Collembola in Brazil dates from the 19th century, *Seira musarum* Ridley, 1890, the first records of troglobitic collembolans were made only a century later by Yoshii (1988), with the description of two species, *Trogolaphysa aelleni* Yoshii, 1988 , from Gruta das Areias, in Parque Estadual Turístico do Alto Ribeira – PETAR in Iporanga (state of São Paulo) and *T. hauseri* Yoshii, 1988, from Gruta da Tapagem (Caverna do Diabo), in Parque Estadual de Jacupiranga (currently Parque Estadual Caverna do Diabo) in Eldorado (São Paulo). Four years later, the species *Acherontides eleonorae* Palacios-Vargas & Gnaspini-Netto, 1992 was described, a species that currently has records in six caves at PETAR. Such species were always observed associated with bat guano (PALACIOS-VARGAS; GNASPINI-NETTO, 1992). Subsequently, another 10 troglobitic species were described in Brazil (*Troglobius brasiliensis* Palacios-Vargas & Zeppelini, 1994; *Arrhopalites amorimi*, *A. gnaspinius, A. lawrencei* and *P. wallacei* Palacios-Vargas & Zeppelini, 1996; *P. papaveroi* Zeppelini & Palacios-Vargas, 1999; A. *alambariensis, A. botuveraensis, A. heteroculatus* and *A. paranaensis* Zeppelini, 2006). It is worth mentioning that, despite the type locality of *T. brasiliensis* being Caverna Limoeiro, in Medicilândia (state of Pará), in the North Region of Brazil, the paratypes come from a different locality in the southeast of the country (Gruta dos Paiva, Iporanga, Fazenda Intervales, São Paulo) far from the type locality. Some differences were observed between specimens from the two localities, as mentioned in the original description by Palacios-Vargas and Zeppelini (1995) and later by Zeppelini *et al.* (2014). This suggests that the holotype and paratypes may represent different species, therefore, a detailed review of the holotype of *T. brasiliensis* is needed (ZEPPELINI *et al.*, 2014).

All troglobitic collembolans described thus far are inhabitants of macrocaves in well-developed karst systems with a high degree of isolation from populations. However, recently, more detailed studies of surface cave fauna and SSHs in ferruginous areas have demonstrated that this type of environment also has the potential for the development of a high diversity of troglobitic species (BRITO *et al.*, 2019; CIPOLA *et al.*, 2020; ZEPPELINI *et al.*, 2014; ZEPPELINI *et al.*, 2018a, 2018b; ZEPPELINI; BRITO, 2014), representing a true "*hotspot*[1]" for the genera *Pseudosinella, Pararrhopalites* and *Arrhopalites* in the ferruginous areas of the state of Minas Gerais. Even so, knowledge about the diversity of troglobitic collembolans in Brazil can still be considered incipient.

So far, 29 troglobitic species have been described for the

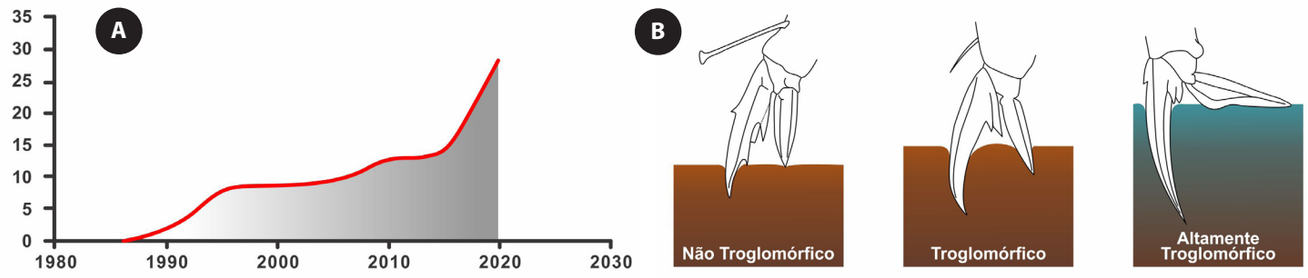


Figure 2. A) Species accumulation curve for troglobitic collembolans of Brazil; **B)** Morphological modification of the claw observed in the process of adaptation to the cave environment: i - weak penetration into the soil, typical of non-troglomorphic species; ii - increased penetration that occurs in troglomorphic species; iii - very thin and elongated claw that allows highly troglomorphic species to walk on a surface film of water. Modified from Christiansen (2012).

1 Term created by Myers (1988), refers to an area of ecological relevance with high endemism that is critically endangered.

Table 3. List of troglobitic species described for Brazil and their respective states of occurrence

Order	Family	Genus	N	Species	UF
Symphypleona	Arrhopalitidae	*Arrhopalites*	1	*A. alambariensis* Zeppelini, 2006*	SP
			2	*A. amorimi* Palacios-Vargas & Zeppelini, 1996*	SP
			3	*A. botuveraensis* Zeppelini, 2006*	SC
			4	*A. gnaspinii* Palacios-Vargas & Zeppelini, 1996*	SP
			5	*A. heteroculatus* Zeppelini, 2006*	SP
			6	*A. lawrencei* Palacios-Vargas & Zeppelini, 1996*	SP
			7	*A. paranaensis* Zeppelini, 2006*	PR
			8	*A. glabrofasciatus* Zeppelini, Brito & Lima 2018*	MG
			9	*A. mendonceae* Brito, Lima & Zeppelini, 2019	MG
	Sminthuridae[2]	*Pararrhopalites*	10	*P. papaveroi* (Zeppelini & Palacios-Vargas, 1999)*	MS
			11	*P. wallacei* (Palacios-Vargas & Zeppelini, 1996)*	SP
			12	*P. queirozi* Brito, Lima & Zeppelini, 2019	MG
			13	*P. sideroicus* Zeppelini & Brito, 2014*	MG
			14	*P. ubiquum* Zeppelini, Lima & Brito, 2018	MG
Arthropleona	Entomobryidae	*Pseudosinella*	15	*P. alfanjeunguiculata* Bellini, Cipola & Souza, 2020	MG
			16	*P. brumadinhoensis* Cipola, 2020	MG
			17	*P. cearensis* Oliveira, Brito & Cipola, 2020	CE
			18	*P. guanhaensis* Zeppelini, Brito & Lima, 2018	MG
			19	*P. keni* Cipola, 2020	MG
			20	*P. mitodentunguilata* Bellini, Cipola & Souza, 2020	MG
			21	*P. neriae* Bellini, Cipola, & Souza, 2020	MG
			22	*P. phyllunguiculata* Oliveira, Lima & Cipola, 2020	MG
			23	*P. prelabruscervata* Oliveira, Lima & Cipola, 2020	MG
			24	*P. serpentinensis* Cipola, 2020	MG
	Paronellidae	*Troglobius*	25	*T. brasiliensis* Palacios-Vargas & Zeppelini, 1995*	PA
			26	*T. ferroicus* Zeppelini, Silva, & Palacios-Vargas, 2014*	MG
		Trogolaphysa	27	*T. aelleni* Yoshii, 1988*	SP
			28	*T. hauseri* Yoshii, 1988*	SP
Poduromorpha	Hypogastruridae	*Acherontides*	29	*A. eleonorae* Palacios-Vargas & Gnaspini-Netto,1992*	MG

* Represents species that are in Livro Vermelho de Espécies Ameaçada de Extinção (ICMBio/MMA, 2018).

country (Table 3, Figure 2A and Figure 3), which corresponds to approximately 7% of all troglobitic collembolan species in the world. Among these species are representatives of five families (Arrhopalitidae, Entomobryidae, Hypogastruridae, Paronellidae and Sminthuridae) and six genera (*Arrhopalites, Pararrhopalites, Pseudosinella, Troglobius, Trogolaphysa* and *Acherontides*). Also, of these 29 species, 16 are listed in Livro Vermelho da Fauna Brasileira Ameaçada de Extinção (ICMBio/MMA 2018) (Table 3). The Brazilian state with the highest recorded number of troglobitic species of Collembola is Minas Gerais, with 16 species, followed by São Paulo, with eight, and the states of Ceará, Mato Grosso do Sul, Pará, Paraná and Santa Catarina, with only one species each (Figure 3). Despite a representative diversity in the world context, troglobitic collembolan diversity is not very expressive in some regions. In Europe, for example, there are more than 240 described species restricted to subterranean environments (CULVER; PIPAN, 2009).

In addition to described species, many others have been

2 A new genus and species of troglobitic collembolan (*Troglobentosminthurus luridus* Souza, Medeiros & Bellini, 2022) was described after writing this chapter.

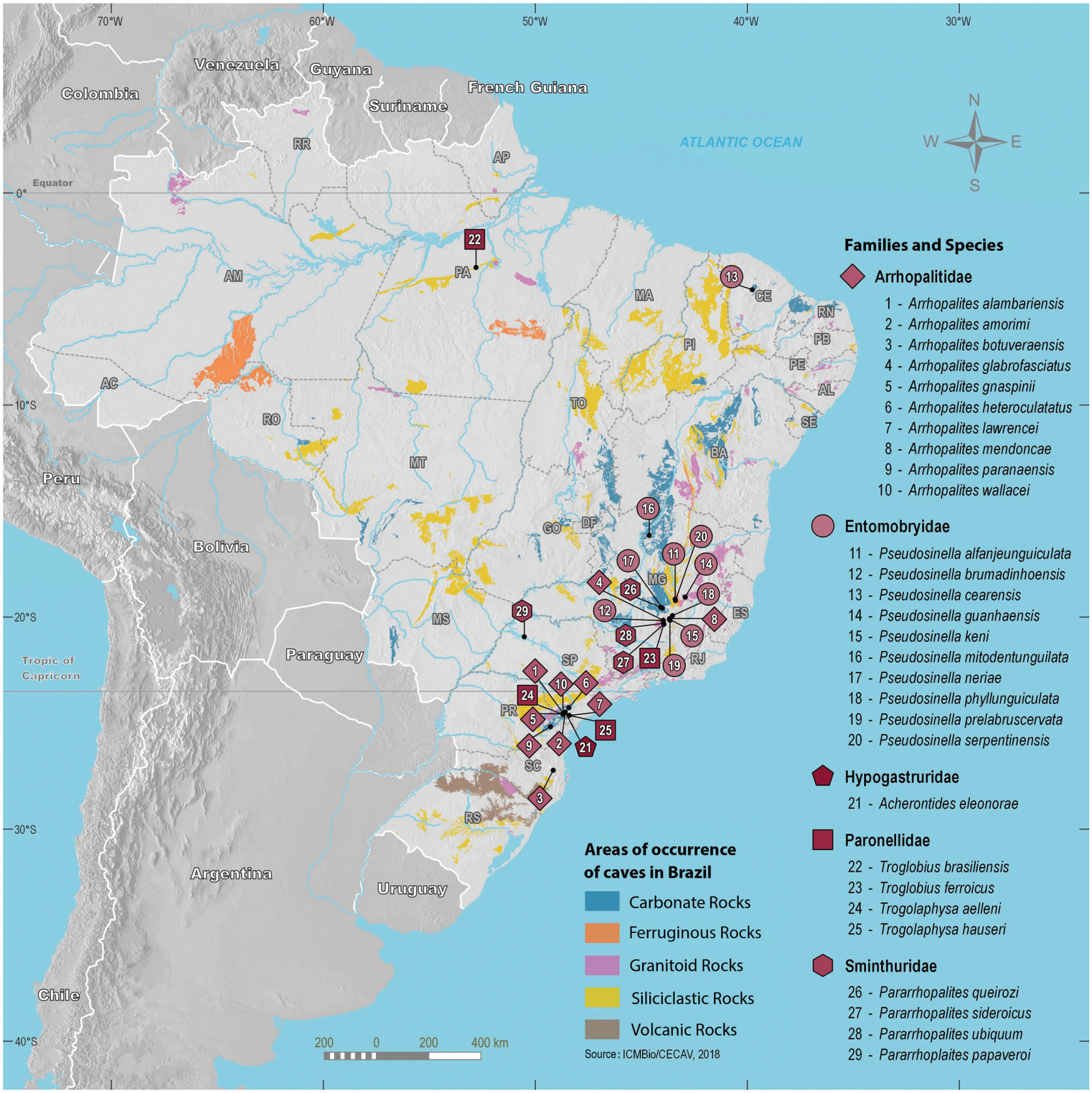


Figure 3. Map with type localities of the species of troglobitic collembolans described for Brazil.

collected in Brazil and await taxonomic description. This scenario highlights a knowledge gap regarding the diversity of cave collembolans in the country. However, when compared to Europe and North America, where caving is historically more developed, it is necessary to emphasize that studies on cave fauna in Brazil are recent, with the first works published in the early 1980s (DESSEN *et al.*, 1980), which are related to the composition and preliminary characterization of the fauna of these environments.

Despite recent advances, the species accumulation curve (Figure 2A) shows an exponential growth of new troglobitic species, indicating that the diversity of Brazilian troglobitic collembolans is still undersampled and does not represent an acceptable estimate of reality.

Main methods of collection and conservation

Several techniques can be used to capture collembolans and must be chosen considering the type of environment and study that is intended to be developed. Manual collection is usually one of the most used techniques in cave environments. This direct collection of specimens can be done with the aid of a fine brush moistened with alcohol, with the specimens being placed in glass dampened or plastic tubes. In addition to this technique, entomological aspirators, Berlese-Tullgren funnels and pitfall traps can also be used.

In the laboratory, in collections, collembolans can be placed in small Eppendorf-type glass or plastic tubes or on semi-permanent slides (which allow for later fixation). Between 70 and 95% ethanol is generally used in these cases. Either semi-permanent slides are used for morphological analysis under an optical microscope and are usually fixed in Royer's liquid after the specimens have been diaphanized and bleached.

Identification key

The following is an identification key for all troglobitic species of Collembola described thus far for Brazil:

1 – Body globose, thoracic and abdominal segments fused (Figure 4A-C) ..... **Symphypleona 2**

1'- Body elongate, segments clearly articulated **15**

2 - Metatrochanteral spine present (Figure 5A), metatrochanteral organ absent, furcular tooth Ie with posterior bristles, Ii normal, mps1–3 normal, anal valve always smooth (Figure 5B) without cuticular spines.....Sminthuridae... ***Pararrhopalites* 3**

2'- Metatrochanteral spine absent, metatrochanteral organ elongate, (Figure 5C), furcular tooth Ie with bristles, Ii spiniform (Figure 5D), mps 1–3 generally lamellate (Figure 5E), anal valve with cuticular spines ..... Arrhopalitidae..... ***Arrhopalites* 7**

3 – Eyes absent **4**

3'- Eyes present **6**

4 - Ant. IV with 10 subsegments, unguis I without internal tooth (Figure 5F), female subanal appendage acuminate, reduced in size (450µm)..... ***Pararrhopalites papaveroi***

4'- Ant. IV with 9 or 11 subsegments, unguis I with internal tooth (Figure 5G), size greater than 700 µm..... **5**

5 – Empodial tooth absent from unguiculi I–III; female subanal appendage filamentous in apical third, size ~1000µm ..... ***P. sideroicus***

5'- Empodial tooth present, female subanal appendage acuminate, size ~700–750µm ..... ***P. ubiquum***

6 - Ant. IV with 13 subsegments, ungues I–III with internal tooth, 10 bristles on anterior surface of furcular tooth, female subanal appendage unilaterally fringed..... ***P. queirozi***

6'- Ant. IV with 11 subsegments, ungues I–III without internal tooth, 1–12 bristles on anterior surface of furcular tooth, female subanal appendage acuminate..... ***P. wallacei***

7 – Eyes absent, smooth area without cuticular ornamentation in front of antennae (Figure 5H) ..... ***Arrhopalites glabrofasciatus***

7'- Eyes 1+1 or 2+2, continuous cuticular ornamentation in the antennal region **8**

8 - Eyes 2+2 ..... **9**

8'- Eyes 1+1 ..... **10**

9 - Eyes symmetrical, unguis I with tunic, ms1 of anal valve lamellate, body size ~1000µm ..... ***A. amorimi***

9'- Eyes asymmetrical, unguis I without tunic, ms1 of anal valve with enlarged base, body size ~520µm ..... ***A. heteroculatus***

10 - Ant. IV simple, unguis I with tunic..... ***A. alambariensis***

10'- Ant. IV subdivided, unguis I without tunic..... **11**

11 - Ant. IV with 5 subsegments..... **12**

11'- Ant. IV with 6 subsegments..... **14**

12 - Tunic absent, ms1 of anal valve smooth..... ***A. mendonceae***

12'- Tunic present, ms1 of anal valve lamellar **13**

13 - Unguis III with diminutive or absent inner tooth, unguiculus with apical filament, extending beyond unguis apex (Figure 5G) ..... ***A. botuveraensis***

13'- Unguis III with well-developed inner tooth, unguiculus without apical filament ..... ***A. gnaspinii***

14 - Bristle ms1 of anal valve with enlarged base ..... ***A. lawrencei***

14'- Bristle ms1 of anal valve lamellate ..... ***A. paranaensis***

15 - Prothorax present with bristles (Figure 5I), abdominal segments uniform in size..... Poduromorpha (Figure 4D—F)..... Hypogastruridae (Figure 4F) ..... ***Acherontides eleonorae***

15'- Prothorax reduced without bristles (Figure 5J), Abd. IV more than 2× length of Abd. III in midline of segments..... Entomobryomorpha (Figure 4G—L)..... **16**

16 - Furcular teeth smooth, mucro with more than 2 teeth (Figure 5K)..... **Paronellidae 17**

16'- Furcular teeth crenelate, mucro with 2 teeth (Figure 5L)..... Entomobryidae..... ***Pseudosinella* 20**

17 - Mucro elongated and serrated ..... ***Troglobius* 18**

17'- Mucro sub quadrate with 4–5 teeth ..... ***Trogolaphysa* 19**

18 - Unguis with two unpaired apical teeth, mucro with two serrated lamellae, total adult body length less than 2mm (~1.5mm), antennae 1.8× head length..... ***Troglobius brasiliensis***

18'- Unguis with one unpaired apical tooth, mucro with smooth inner lamella serrated outer lamella, total adult body length more than 2mm (~2.3mm), antennae 2.3× head length..... ***T. ferroicus***

19 - Eyes 2+2, unguis with one small unpaired apical tooth..... ***Trogolaphysa aelleni***

19'- Eyes 0+0, unguis elongated without ímpar unpaired apical tooth, paired basal tooth small ..... ***T. hauseri***

20 - Pre-labral (PL) bristles bifid (Figure 6A)..... **21**

20'- Pre-labral bristles simple (Figure 6B) **22**

21 - PL bristles not ramified, cephalic macrochaeta M2 present (Figure 6A), mesothorax with only microchaetae, macrochaetae absent ..... ***Pseudosienlla phyllunguiculata***

21'- PL bristles ramified, cephalic macrochaeta M2 absent (Figure 6C), mesothorax with one macrochaeta (p3)..... ***P. prelabruscervata***

22 - Posterior external lamellae of inguiculus (pe) with one large tooth (Figure 6D) **23**

22'- Posterior external lamellae of unguiculus (pe) acuminate (Figure 6E)..... **26**

23 - Head with macrochaetae Pa5 and A2 (Figure 7A), manubrium and base of furcular tooth with smooth bristles, tenent hair capitate..... ***P. guanhaensis***

23'- Head with microchaeta Pa5 (Figure 7B), all manubrium and tooth bristles ciliated, tenent hair variable **24**

24 - Mesothorax and metathorax with two macrochaetae each (Figure 7C), labral bristles smooth, tenent hair acuminate..... ***P. brumadinhoensis***

24'- Mesothorax without macrochaetae (Figure 7D)..... **25**

25 - Cephalic bristle M2 present with microchaetae, basal tooth of unguis with small subdivision ..... ***P.keni***

25'- Cephalic bristle M2 present with macrochaetae, basal tooth of unguis simple without division ..... ***P. serpentinensis***

26 - Mesothorax with macrochaetae, labral bristles a1–2 ramified..... **27**

26'- Mesothorax with only microchaetae, labral bristles simple **28**

27 - PL and labral p0–2 bristles finely ciliated, labral bristles multiciliated, Abd IV with macrochaeta B5..... ***P. cearensis***

27'- PL and labral p0–2 bristles smooth, labral bristles smooth or finally ciliated, Abd IV with three macrochaetae (B5–6 and C1) ..... ***P. mitodentunguilata***

28 - PL bristles smooth, unguis elongated, unguis without apical tooth, unguiculus elongated without empodial tooth ..... ***P. alfanjeunguiculata***

28'- PL bristles ciliated, unguis with unpaired apical tooth and paired basal tooth, unguiculus lanceolate with serrated external lamella..... ***P. neriae***

Main Brazilian collections

Five Brazilian biological collections are known to have more expressive numbers of specimens of Collembola: Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ), Instituto Nacional de Pesquisas da Amazônia (INPA), Museu de Zoologia da Universidade de São Paulo (MZUSP), Museu Paraense Emílio Goeldi and Coleção de Referência da Fauna de Solo da Universidade Estadual da Paraíba (CRFS-UEPB).

The first collection of Collembola in Brazil began with researcher Roger Hipolyte Pierre Arlé (1909-2001), in 1932, in the MNRJ collection. According to Maria Cleide de Mendonça, current curator of the Coleção de Collembola at the Museum, there were 4,000 lots of specimens during the management of Arlé. Subsequently, beginning in 1980, about 3,200 lots were added in during the management of Mendonça. The MNRJ includes a collection of 70 type species, making it one of the largest collections of Collembola in South America. Unfortunately, this entire collection was destroyed on the night of September 2, 2018, as a result of a major fire that affected the facilities of this important institution.

The Collembola collection at INPA began around 1970 and currently has type material for 58 species, including 48 holotypes and 50 paratypes (CIPOLA; ALMEIDA, 2018; RIBEIRO *et al.*, 2008).

O Museu Paraense Emílio Goeldi and MZUSP each have a large number of deposited samples, but little of the material has been analyzed by Collembola specialists.

Coleção de Referência de Fauna de Solo (CRFS) of the Universidade Estadual da Paraíba (CRFS-UEPB) is one of the most recent collections of Collembola in Brazil, having been founded in June 2013, with the aim of forming a reference base of material of species recorded in caves, making it the most relevant in the country regarding material from these ecosystems. In the future, CRFS intends to develop a data platform with virtual access and navigation, providing the identification of species of Collembola in the collection, as well as collection data and optical and electronic microscopy images. CRFS is made up of biological specimens from several countries including Brazil, Canada, Slovakia, Spain (Canary Islands), United States of America, Iran and Poland. This material comes from diverse ecosystems, such as seasonally dry tropical forests, tropical rainforests, Amazon rainforest, mangroves, coastal areas, oceanic islands, caves and Antarctica. In addition, CRFS has vouchers of fauna from the most important mining regions in Brazil, such as the Quadrilátero Ferrífero (Minas Gerais) and Carajás (Pará), in addition to different carbonate regions. The collection is organized in slides and alcohol, with more than 16 thousand slides dating from 1961, and just over 300 wet lots containing Collembola specimens organized in numbers of

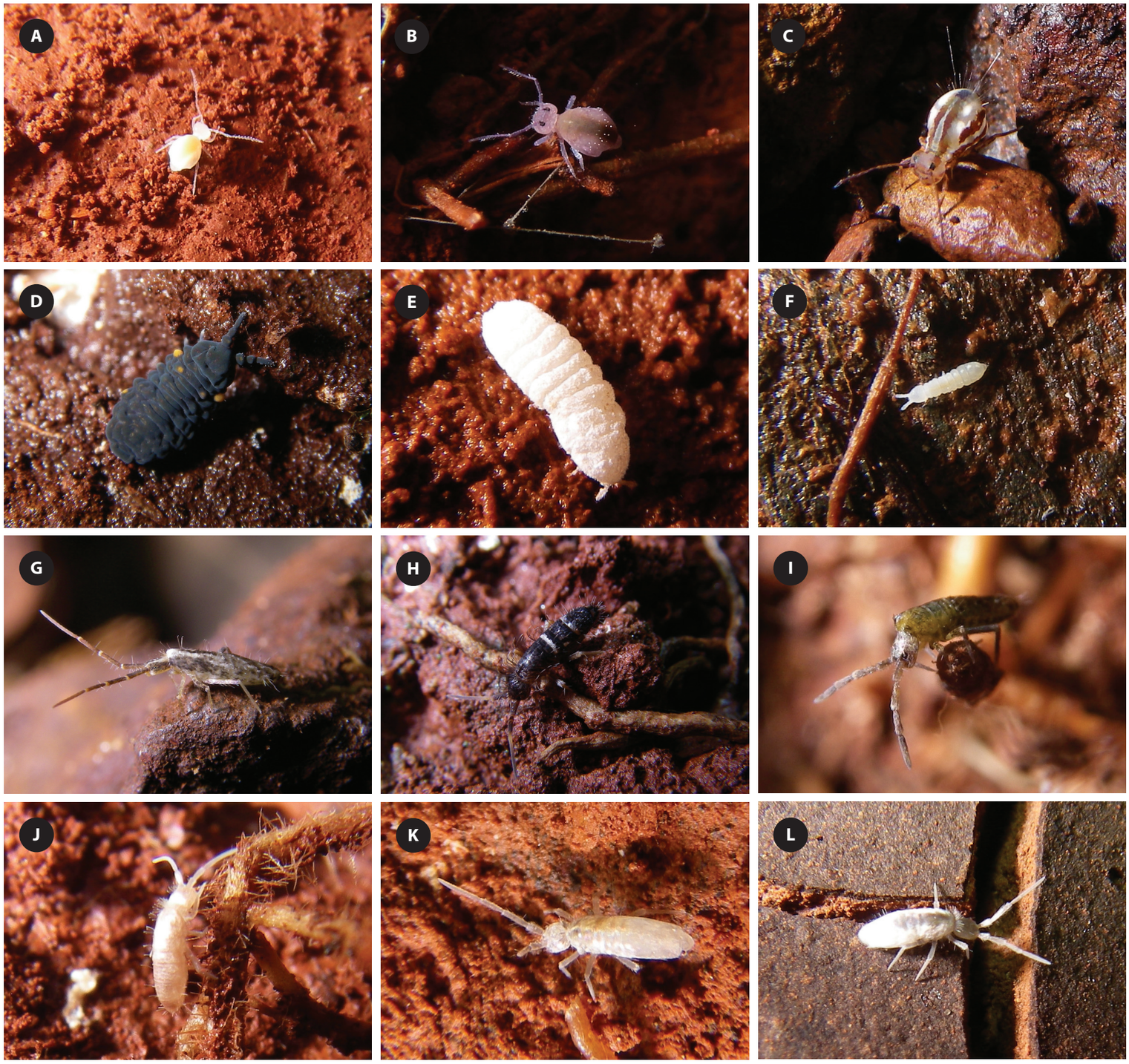


Figure 4. Species of Collembola photographed in caves. **A-C)** Symplypleona; **D-F)** Poduromorpha; **G-L)** Entomobryomorpha. Photos: (Robson de A. Zampaulo).

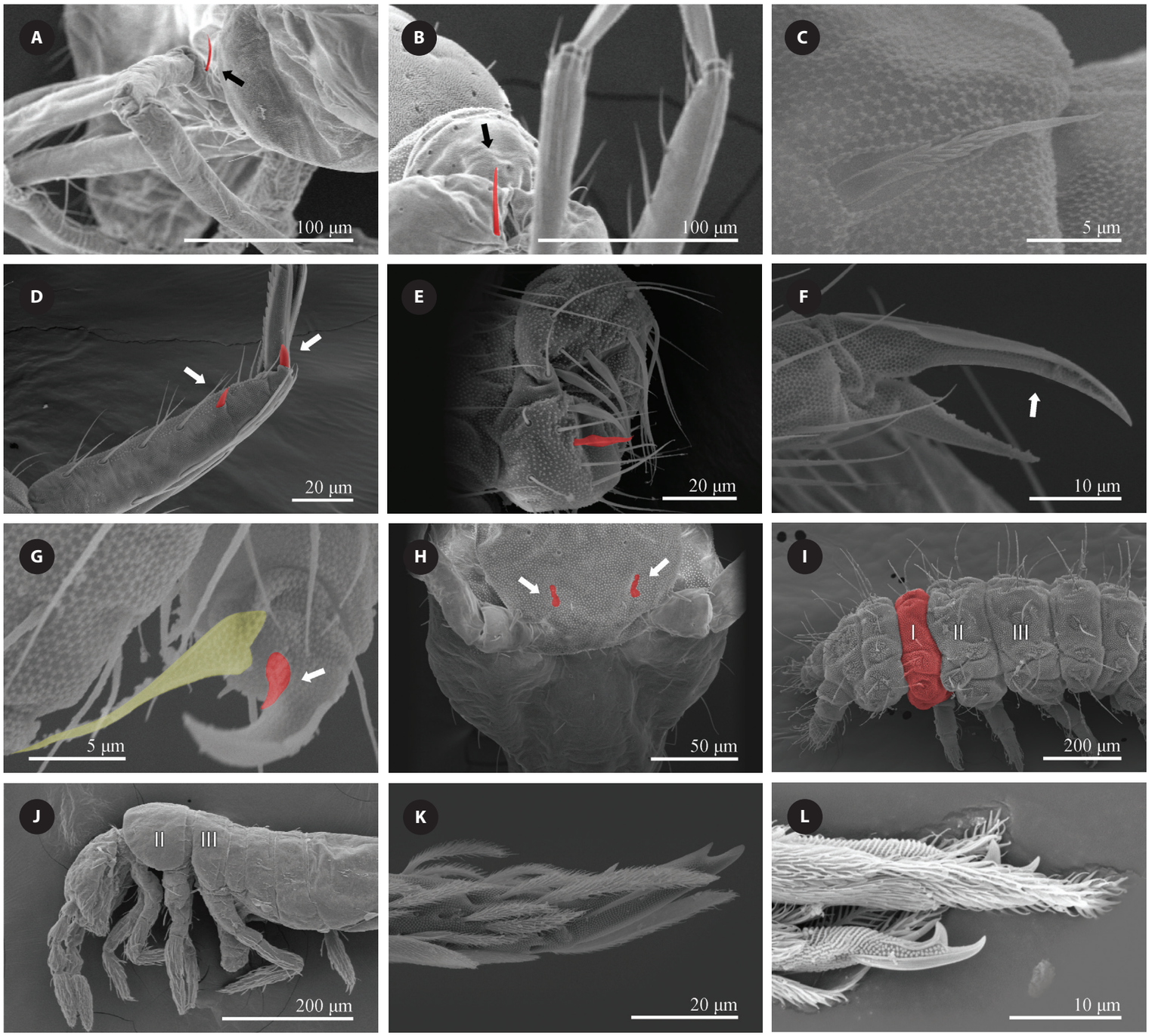


Figure 5. Morphological structurers of Collembola photographed by scanning electron microscopy (SEM). **A)** *Pararrhopalites* sp., metatrochanteral spine highlighted; **B)** *Pararrhopalites* sp., smooth anal valve highlighted; **C)** *Arrhopalites* sp., metatrochanteral organ; **D)** *Arrhopalites* sp., spiniform bristles of furcular tooth; **E)** *Arrhopalites* sp., lamellar bristles on abdomen VI; **F)** *Pararrhopalites* sp., arrow indicates unguis without internal tooth; **G)** *Arrhopalites* sp., in red, unguis with internal tooth and, in yellow, filamentous unguiculus surpassing unguis; **H)** *Arrhopalites* sp., highlighted head with smooth area without cuticular ornamentation in front of antennae; **I)** Poduromorpha, highlighted prothorax present with bristles; **J)** Entomobryomorpha, prothorax reduced with bristles and developed thorax II and III; **K)** Paronellidae, mucro with more than two teeth; **L)** Entomobryidae, mucro with two teeth.

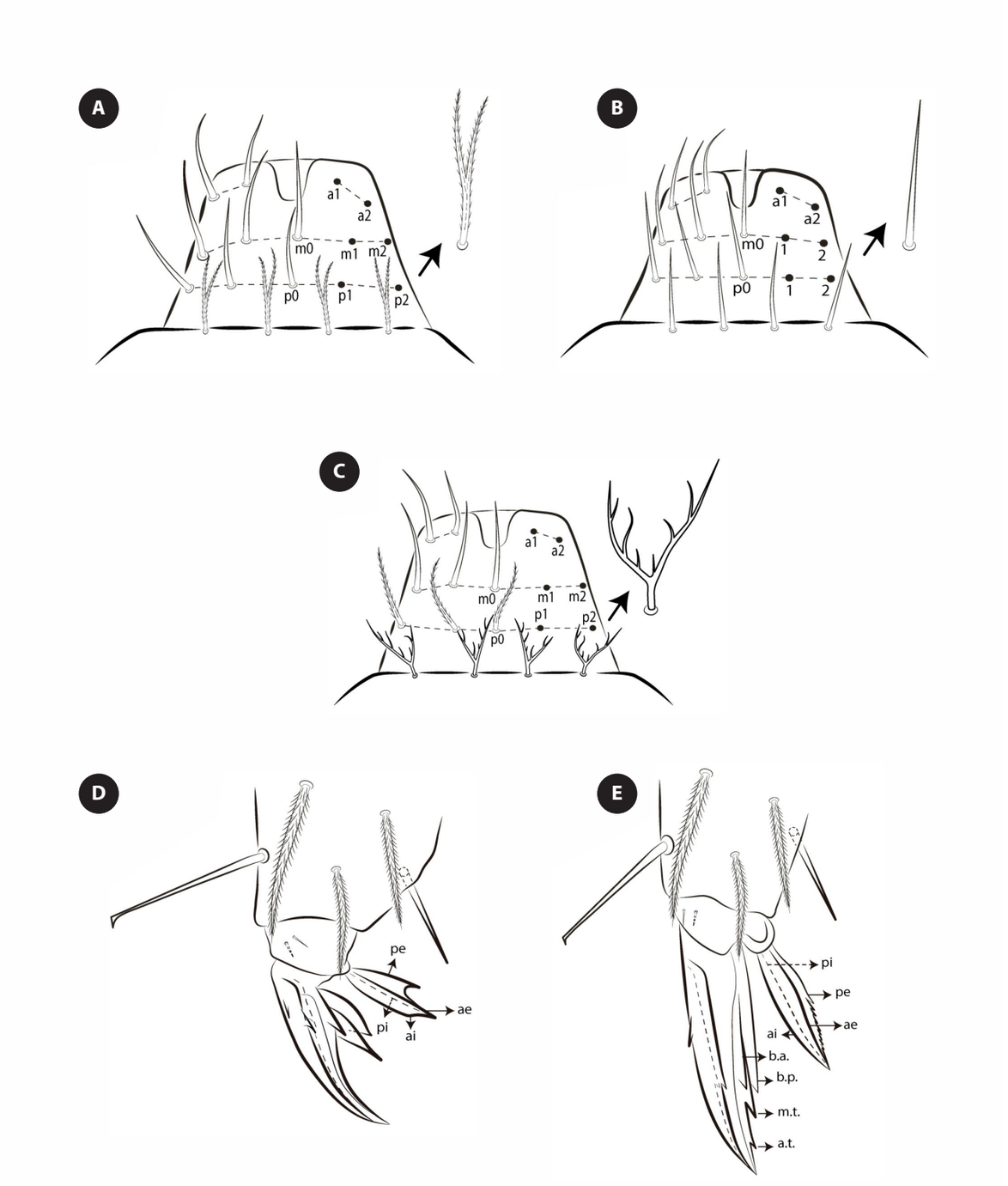


Figure 6. *Pseudosinella* sp., pre-labral and external lamellar bristles of unguiculus. **A)** *P. phyllunguiculata,* arrow indicating bifid pre-labral bristles; **B)** *P. paraensis,* arrow indicating simple pre-labral bristles; **C)** *P. prelabruscervata*, arrow indicating pre-labral bifid and ramified bristles; **D)** *P. guanhaensis,* unguiculus with one large tooth (pe); **E)** *P. cearensis,* large tooth absent. Figures modified from Cipola *et al.* (2020).

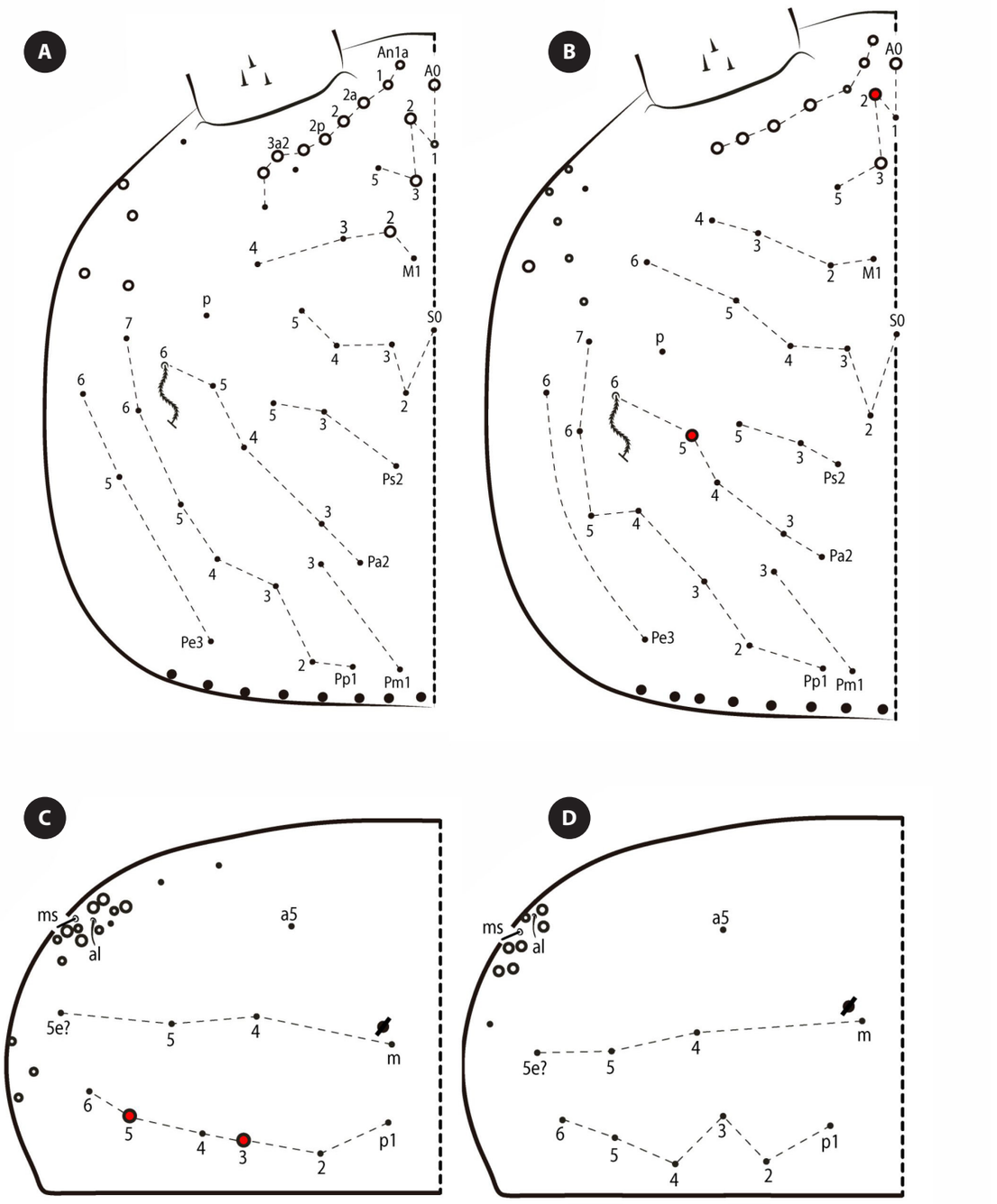


Figure 7. *Pseudosinella* sp., head and mesothorax with chaetotaxy. **A)** *P. guanhaensis*, highlighted macrochaetae Pa5 and A2 on head; **B)** *P. brumadinhoensis*, microchaeta Pa5; **C)** *P. brumadinhoensis,* highlighted macrochaetae p3 and p5 of mesothorax; **D)** *P. Keni*, macrochaetae absent. Figures modified from Cipola *et al.* (2020).

falls. CRFS currently has the largest collection of troglobitic collembolans in Brazil.

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Rodrigo Lopes Ferreira



Robson de Almeida Zampaulo



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Pseudonannolene lundi
Lucas Mendes Rabelo

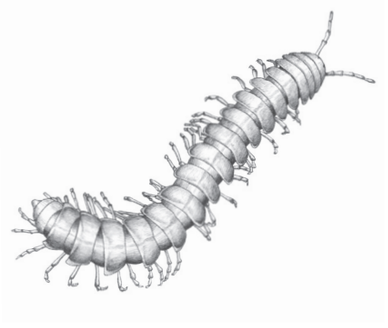
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Diplopoda

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General introduction

The class Diplopoda is composed of approximately 12,000 described species (ENGHOFF *et al.*, 2015). Although little explored, it ranks as the third largest group among terrestrial arthropods, behind only Insecta and Arachnida. Diplopods, or millipedes, are distributed on all continents, except Antarctica, occurring in tropical, subtropical and temperate forests and tundra, taiga and desert regions (GOLOVATCH; KIME, 2009). A significant richness of species occurs in karst outcrops and in macro- and micro-caves (ENGHOFF, 2015; GOLOVATCH; KIME, 2009; SHEAR, 1973). Millipedes have low vagility (i.e., ability to move), resulting a high degree of endemism in small areas of occurrence.

They are peaceful animals, characterized by slow movements and cryptic habits. Most species in the group can release secretions based on phenols or quinones, which provide protection from possible predators. These secretions, when in contact with human skin, can cause erythema, mild swelling or local itching, yet there are no known cases with any evolution to moderate or severe clinical conditions. Current studies are focused on the biotechnological potential of the group in relation to the secretion of quinones, such as hydro-benzoquinones (SHEAR, 2015). Millipedes occur in all strata of the soil, from surface structures (rocks and fallen tree trunks) to deeper layers of leaf litter, where they play important roles in the cycling of organic nutrients and litter fragmentation (DAVID, 2015).

Although there is no updated list of Brazilian species of this class, just over 600 species are currently recognized. These species are largely concentrated in the Southeast Region, mainly in the states of São Paulo and Rio de Janeiro (BOUZAN *et al.*, 2021), due to the greater volume of faunal surveys and taxonomic studies carried out by European researchers in the region at the beginning of the 20th century.

Morphological and molecular studies suggest that Diplopoda is the sister group to Pauropoda, a small class of myriapods, within the group Dignatha. Both classes, together with Symphyla, form a clade called Progoneata, which is the sister group of Chilopoda (FERNÁNDEZ *et al.*, 2018). More recent classifications divide the class Diplopoda into 16 orders, however, their evolutionary relationships are still under discussion (Figure 1).

Diplopods are identified by the presence of fused segments forming body rings (= diplosegments). In general, diplosegments (Figure 3A-B) correspond to those segments from the fourth or fifth body ring, while the first postcephalic rings are apodous or with only a pair of locomotor appendages (ENGHOFF *et al.*, 1993; KOCH, 2015). All rings, including the first called the *collum*, are subdivided into two regions called the prozone and the metazone. Comparatively, each ring is composed of a tergite, two pleurites and sternite associated with spiracular plates (ENGHOFF *et al.*, 1993, 2015; WESENER *et al.*, 2014). The gnathochilarium (Figure 3C-D) is composed of elements from the podomeres of the first maxilla (KOCH, 2015). The relationship of the first maxilla (and mandible), which is closely associated with eating habits, favors the grouping between Pauropoda and Diplopoda within Dignatha (FERNÁNDEZ *et al.*, 2018). As a basic architecture, the gnathochilarium is composed of cardo and stipes, delimiting the postmentum, mentum, promentum and lamella linguales. The composition and morphology of the gnathochilarium sclerites varies among orders, families or even between related genera (HOFFMAN, 1980). Phylogenetic studies have shown that the variation observed in the gnathochilaria of some groups may be the result of several independent events, in addition to the common ancestry of their taxa (INIESTA *et al.*, 2020).

The main characteristics used in species identification correspond to sexual structures. Males and females have paired genital openings at the base of the second pair of legs (KOCH, 2015). In males, the structure may be associated with the penis (INIESTA *et al.*, 2020) or gonapophysis (BOUZAN *et al.*, 2020), while in females, they correspond to the vulvae or scyphopods (Figure 3F).

For Diplopoda, sexual structures imply rapid and divergent evolution at the species level. At the center of this discussion about genital variation are the evolutionary mechanisms that determine its modifications, such as the lock and key (gonopods and vulvae), sexual selection by female choice and antagonistic co-evolution between males and females.

Gonopods (or gonopodia), legs modified in males for sexual function, are mostly used for species determinations. In juveniles, these structures still correspond to functional locomotor legs (ENGHOFF *et al.*, 1993) but as ecdysis occurs, they undergo mild to abrupt changes, until complete metamorphosis. Sperm mass is released by the male gonopore and transferred to the female through involucres (in Penicillata) or via gonopods (in Chilognata) during copulation. Secondary structures can also be associated with gonopods, such as peltogonopods (with accessory function) or paragonopods (modified anterior and/or posterior legs).

Gonopod morphology varies among groups. In Pentazonia, they correspond to the last pair of legs (so called telopods). In Helminthomorpha, they correspond to the eighth and/or ninth pair of legs. Gonopods are divided into two parts: the gonocoxa, the most proximal part, and the telopodite, which extracts seminal fluid from the gonopore and injects it into the female receptacle (KOCH, 2015). When the seminal canal is visible in the gonocoxa, it is called a cannula, while in the telopodite it corresponds to a seminal sulcus. The channel opens at the apex of the telopodite, the region being called the solenomere (Figure 3E). Secondary modifications are observed in the gonopod of numerous groups, such as macro-bristles, lamellae, tubercles and other secondary processes.

Millipede reproduction occurs through internal fertilization, accompanied by a mating process (or intertwining, as in Helminthomorpha) that can last from a few minutes to 48 hours. Fertilized eggs are spherical, with a waxy outer layer and nutrient reserves in the yolk. These are laid in numbers that vary from group to group, reaching up to 2,000 eggs for members

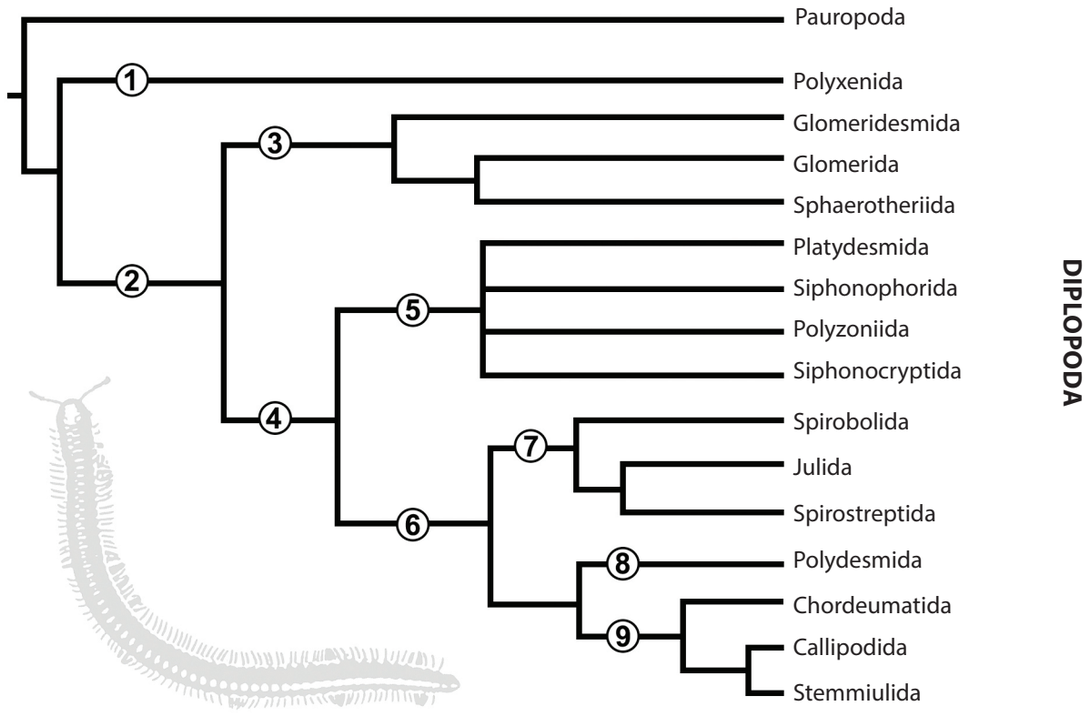


Figure 1. Relationships among the orders of Diplopoda (modified from SIERWALD; BOND, 2007). Clades: **1**, Penicillata; **2**, Chilognatha; **3**, Pentazonia; **4**, Helminthomorpha; **5**, Colobognatha; **6**, Eugnatha; **7**, Juliformia; **8**, Merochaeta; **9**, Nematophora.

of Merochaeta. For Juliformia, the eggs are individualized and arranged interspersed with litter or humus, while for the order Polydesmida they are protected under a chamber built of clay or mud by the female, from which the juvenile polydesmids, with a thinner, whitish cuticle, and reclusive habit, emerge (Figure 2E).

Variation in body architecture among different groups can be explained by differences in lifestyle habits (Figure 4). Species associated with tree bark have smaller bodies and less calcified exoskeletons (Penicillata) (Figure 2C); those found among the surface litter strata have more dorsoventrally flattened bodies (Merochaeta) (Figure 2D, 6F); those with burrowing habits have cylindrical bodies and compact rings (Juliformia) (Figure 2A); species adapted to drilling have vermiform bodies and modified gnathochilaria (Colobognatha) and, finally, those capable of conglomerating, forming an almost perfect sphere (Pentazonia) (GOLOVATCH; KIME, 2009).

Main groups found in Brazilian caves

Some millipede species have a compact and rigid exoskeleton, mainly due to the addition of calcium salts (KOCH, 2015). Millipedes are mostly nocturnal, preferring relatively humid environments, with availability of organic resources and relative calcium concentration. In view of this scenario, the relationship of the distribution and richness of the class with karst regions is evident (GOLOVATCH; KIME, 2009).

Much of the information available on cave millipedes is concentrated in studies in the Holarctic (CULVER; SHEAR, 2012). Currently known troglobitic species in the world belong to the orders Glomeridesmida, Glomerida, Polydesmida, Chordeumatida, Julida and Spirostreptida and are distributed mainly in the Dinaric Alps, the Pyrenees, North America, Mexico and, more recently, in Brazil and Southeast Asia. It is noteworthy that, although the description of troglobites is the focus of recent studies, many species are still unknown due to both sampling deficiency and the lack of specialized taxonomists. Questions have recently been raised about some cave species of Chordeumatida in North America, since examinations of genetic material suggest possible different species in morphologically identical populations (CULVER; SHEAR, 2012). The same scenario can be observed for troglobitic species in Brazil, mainly for the orders Glomeridesmida, Polydesmida and Spirostreptida.

Studies on the cave fauna of Diplopoda in Brazil began with descriptions made by the German researcher Otto Schubart in the mid-20th century. Important information has since been obtained by species listing studies. Particularly worthy of mentioning is the article published by Trajano *et al.*, (2000), which focused on the diversity of cave millipede species (troglobites and non-troglobites) of the country.

The diversity of species in caves in Brazil reflects the richness of the group in epigean environments. The most recurrent groups in the cave environment are the orders Polydesmida (Chelodesmidae, Cyrtodesmidae, Cryptodesmidae, Fuhrmannodesmidae, Oniscodesmidae, Paradoxosomatidae and Pyrgodesmidae), Spirobolida (Rhinocricidae) and Spirostreptida (Spirostreptidae and Pseudonannolenidae).

Other orders, although infrequent, can also be found in cave environments, such as Glomeridesmida, Polyxenida, Siphonophorida and Stemmiulida. Introduced species have also been observed, such as *Oxidus gracilis* (Koch, 1847) and *Orthomorpha coarctata* (Saussure, 1860) (Paradoxosomatidae). Species of the genera *Brasilodesmus* Brölemann, 1929, *Leptodesmus* de Saussure, 1859 (Chelodesmidae), *Pseudonannolene* Silvestri, 1895 (Pseudonannolenidae) and *Rhinocricus* Karsch, 1881 (Rhinocricidae) are commonly observed in caves in Brazil. In general, genera/species are determined using adult males for dissection of several sexual structures (not just gonopods) and for comparison with type series, given that many groups still lack taxonomic revisions. Therefore, the largest possible sample of males is highly recommended, especially for understanding morphological variation or polymorphism. For the vast majority of groups, it is not possible to accurately determine species, genera, tribes or even families from only females and juveniles.

The carbonate regions of the Bambuí and Açungui groups concentrate around 80% of millipede records in Brazil (Figure 5). Floresta Nacional de Carajás (FLONA de Carajás), and adjacent areas located in the southeast of the state of Pará, have been recently highlighted for the richness found (see item Diversity of troglobitic species described for Brazil). In addition, FLONA de Carajás also stands out for its concentration of troglobitic species or groups recorded solely for the region (INIESTA *et al.*, 2012; INIESTA; FERREIRA, 2013a).

Main troglomorphisms

Morphological characteristics of species that occupy the litter and subsurface soil layers favor their colonization of deeper portions of the subterranean environment (SHEAR, 1973).

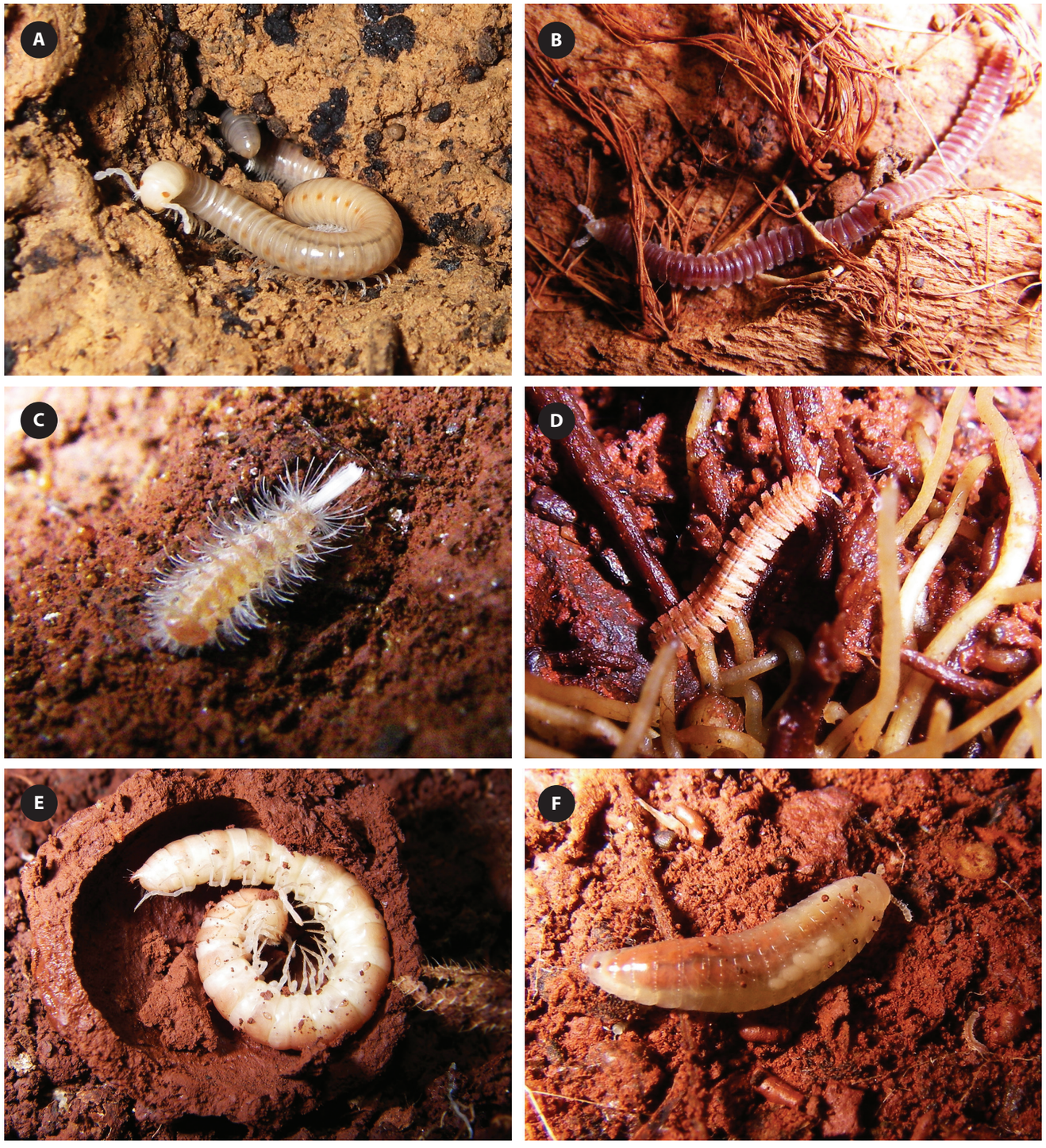


Figure 2. Specimens of different orders of Diplopoda found in caves of Brazil. **A)** Spirostreptida: *Pseudonannolene ambuatinga* (troglobitic species); **B)** Siphonophorida; **C)** Polyxenida; **D)** Polydesmida: Pyrgodesmidae; **E)** Polydesmida: Juvenile of Chelodesmidae; **F)** Glomeridesmida: *Glomeridesmus spelaeus* (troglobitic species). Photos: (Robson de A. Zampaulo).

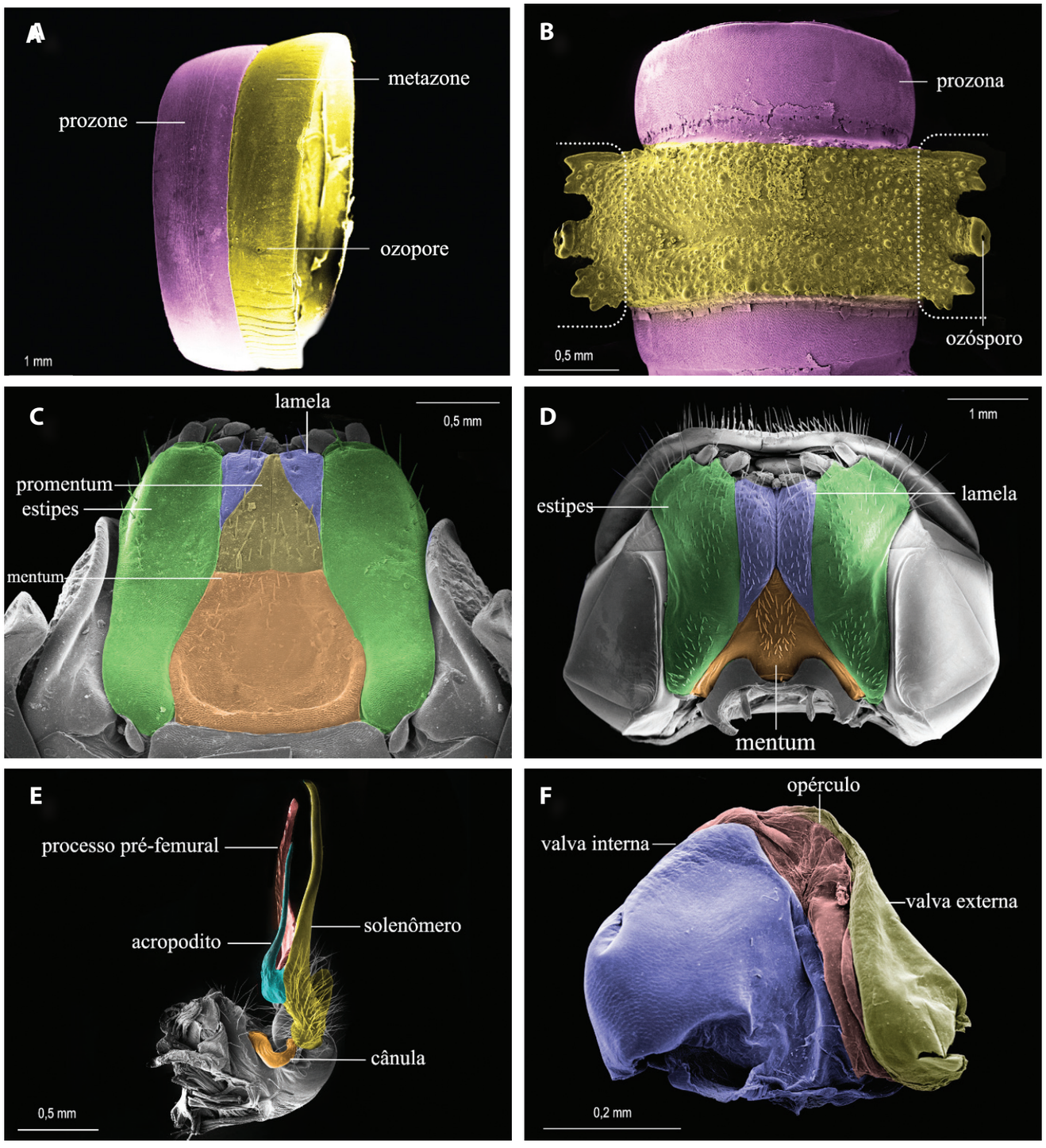


Figure 3. A) Body ring in Pseudonannolenidae, side view; **B)** Body ring in Chelodesmidae, dorsal view, dotted line refers to the paranotum; **C)** Gnathochilarium in Pseudonannolenidae, ventral view; **D)** Gnathochilarium in Chelodesmidae, ventral view; **E)** Gonopod of *Parastenonia carajas* Bouzan & Iniesta, 2020 (Chelodesmidae), in mesal view; **F)** Right vulva of *Pseudonannolene microzoporus* Mauries, 1987 (Pseudonannolenidae).

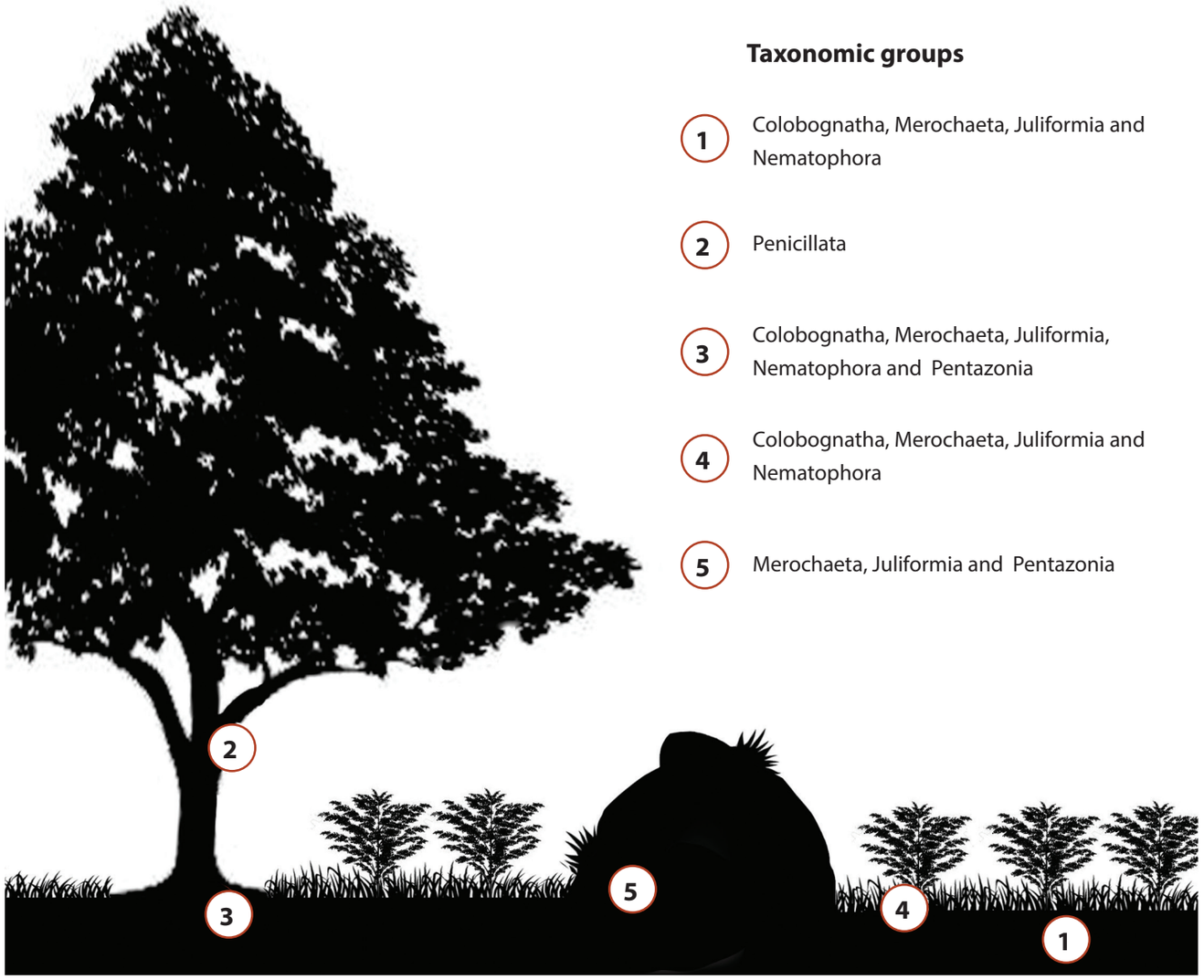


Figure 4. Representation of the relationship between places of occurrence and possible groups of Diplopoda to be observed. The numbers represent biotopes and their respective taxonomic groups.

However, such characteristics may overlap with those presented by species that exclusively inhabit the subterranean environment (troglobites), which makes the assertive delimitation of non-troglomorphic and troglomorphic/troglobitic species difficult (TRAJANO *et al.*, 2000). Thus, the delimitation of troglomorphic species becomes an extremely arduous task.

There is no well-defined pattern of troglomorphisms for members of Diplopoda. There are morphological variations of different structures that can show them for certain groups, but not for others, including groups with taxonomic proximity (SHEAR, 1973). Liu *et al.* (2017) highlighted that body size, depigmentation and elongation of femur and tarsus in legs have been evidenced for all troglobitic groups (species of Glomerida, Polydesmida, Chordeumatida and Spirostreptida). Characters rarely reported may also indicate restriction to the subterranean environment, such as changes in the epihypopharynx and spiracles (ENGHOFF *et al.*, 1997), sensilla of the antennae (INIESTA; FERREIRA, 2013a), sensory cones and tarsal claws (REBOLEIRA; ENGHOFF, 2018).

In general, the main troglomorphisms observed in millipedes are depigmentation (Figure 6A), reduction in cuticle thickness (in Chilognatha), reduction or absence of eyes for non-

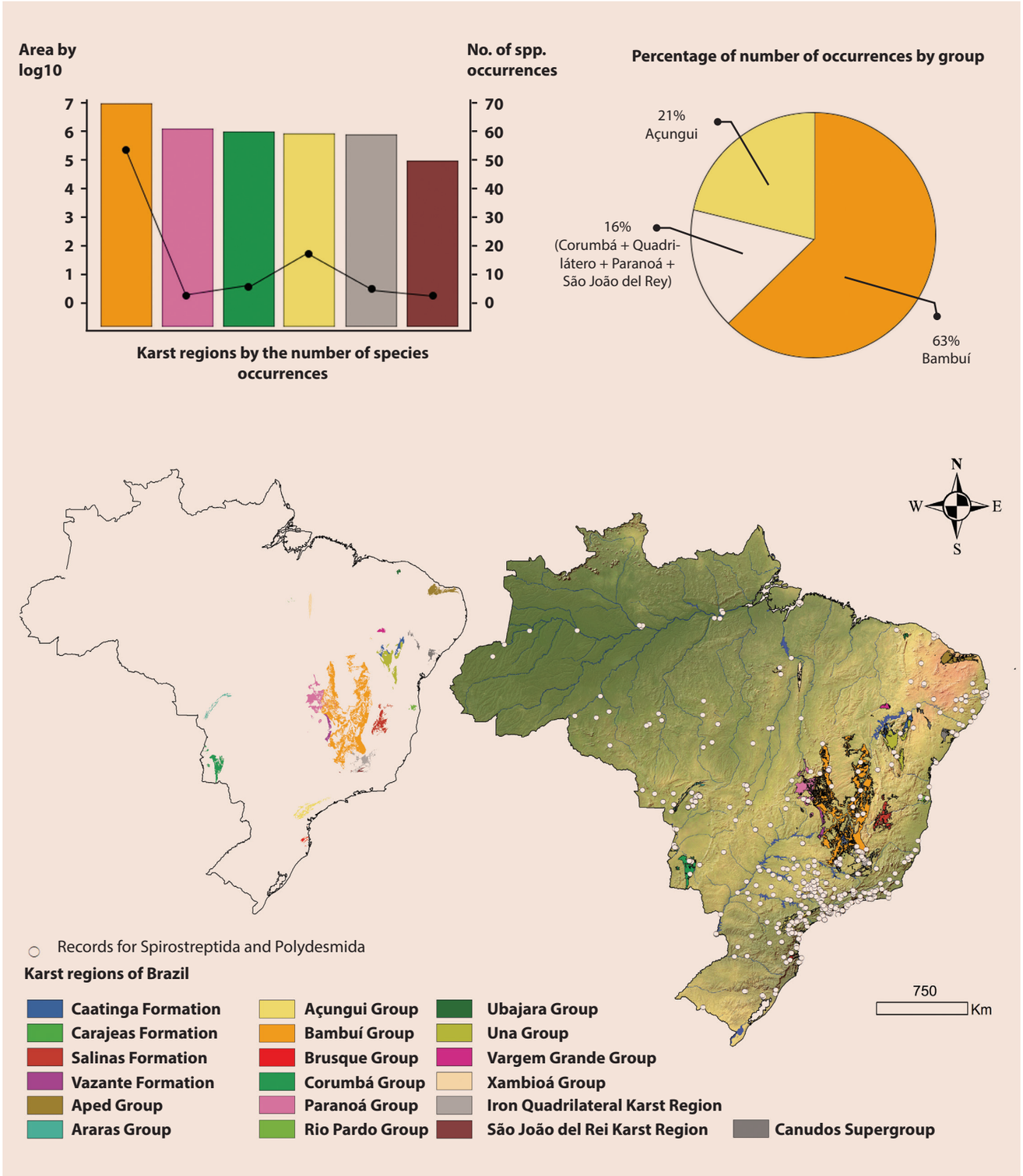


Figure 5. Map of distribution and occurrence percentages (updated to 2019) for the orders Spirostreptida and Polydesmida in karst regions of Brazil.

anophthalmic groups (Figure 6C), elongation of appendages and reduction (or increase) in body size, when compared to related epigean species (Figure 6A) (INIESTA *et al.*, 2012; INIESTA; FERREIRA, 2013a; TRAJANO *et al.*, 2000). It is important to highlight that, although such characteristics can be associated with restriction to the cave environment, the presence of, or variation in, these characteristics is not fully indicative of the troglobitic condition of these species. Therefore, more in-depth taxonomic study is highly recommended to confirm the status of species and avoid possible mistakes.

The most common errors in the determination of troglobitic species correspond to developmental stages (newly hatched juveniles and subadults). In Spirostreptida, for example, juveniles have a smaller number of ocelli that are loosely grouped (Figure 6C-D), due to successive processes of metamorphosis throughout their development. An important point is that the reduction of ocelli may not necessarily be indicative of troglomorphism, since adults of non-troglobitic species may also present them in smaller numbers (INIESTA *et al.*, 2020). In addition, juveniles may also have a less sclerified (newly hatched or recent ecdysis) (Figures 2E, 6B and 6E) and little pigmented cuticle, in addition to intensification of more cryptic habits to protect against possible predators.

Diversity of troglobite species described for Brazil

The first descriptions of invertebrate species restricted to caves in Brazil were the chelodesmid *Leodesmus yporangae* (Schubart, 1946) and the pyrgodesmid *Yporangiella stygius* Schubart, 1946, from the Alto do Ribeira region, São Paulo. Most troglobite millipede species are concentrated in carbonate caves in the Southeast Region and, more recently, in ferruginous caves in Floresta Nacional de Carajás (Pará). The status of restriction to the subterranean environment for the species *Strongylosomides troglobius* Golovatch, Bouzan & Gallo, 2022 (Chelodesmidae) and *Onciurosoma troglobium* Golovatch & Gallo, 2022 (Paradoxosomatidae) is currently under discussion. In general, eleven troglobitic species, of the orders Polydesmida, Glomeridesmidae and Spirostreptida, are recognized in Brazil (Figure 8):

Leodesmus yporangae (Schubart, 1946)
(Polydesmida, Chelodesmidae)

The original description of *L. yporangae* highlights the fragility of specimens, probably referring to the reduction in cuticle thickness associated with cave species. According to Mauriès and Geoffroy (2000), the species has different ecomorphotypes, depending on morphological variation of the gonopod and population isolation, without overlapping or intermediate forms throughout its known distribution. The species is restricted to a set of caves in Parque Estadual Turístico do Alto Ribeira (PETAR).

Yporangiella stygius Schubart, 1946
(Polydesmida, Pyrgodesmidae)

The genus *Yporangiella* was described to accommodate *Y. stygius*, with no other species being assigned to it since. The taxonomy of Pyrgodesmidae is quite disorganized and a deep taxonomic review is needed for Neotropical groups. The only indication of troglomorphism in the description of *Y. stygius* is relative body depigmentation (SCHUBART, 1946). Only the holotype is known thus far, and its sexual structures are fixed on laminae. Many records of cave pyrgodesmids may correspond to new species, but they remain without refined identification due to the lack of taxonomists in Brazil. The lack of specialists, the unresolved taxonomy and its reduced body size are factors that corroborate the limited knowledge about this species and other members of the family. The type locality of the species is Gruta do Monjolinho, Iporanga, Parque Estadual Turistico do Alto Ribeira (PETAR), São Paulo.

Peridontodesmella alba Schubart, 1957
(Polydesmida, Cryptodesmidae)

In the same way that studies related to Pyrgodesmidae are difficult, there is also a lack of specialists for Neotropical species of Cryptodesmidae. So far, only one male is known for *P. alba* and its sexual structures are fixed on microscope slides (SCHUBART, 1957). The remainder of the type series is composed of females and juveniles. The type locality of the species is Gruta de Bethary, Iporanga, Parque Estadual Turistico do Alto Ribeira (PETAR), São Paulo.

Phaneromerium cavernicolum
Golovatch & Wytwer, 2004
Phaneromerium troglopterygotum
Golovatch & Gallo, 2022
(Polydesmida, Fuhrmannodesmidae)

The genus *Phaneromerium* is composed of ten species distributed in the Andes, Central Amazon and Cerrado (GOLOVATCH; WYTWER, 2004). The description of *P. cavernicolum* and *P. troglopterygotum* extended the distribution of the genus to the Northeast Region of Brazil. According to the descriptions, the species are considered troglomorphic due to possessing a depigmented trunk, clearly more elongated and narrow appendages, long bristles on the tergites and a relatively larger body compared to congeneric species. However, the authors themselves warn that Neotropical species of Fuhrmannodesmidae (= Trichopolydesmidae), which live under leaf litter, are generally whitish and with elongated appendages and a relatively larger body compared to close groups. The type locality of *P. cavernicolum* is Gruta do Padre, Santana, Bahia, and that of *P. troglopterygotum* is Gruta Serra Verde, Coribe, Bahia.

Glomeridesmus spelaeus Iniesta & Wesener, 2012 (Glomeridesmida, Glomeridesmidae)

Glomeridesmus spelaeus is the only representative of the order Glomeridesmida in Brazil, although other possible new species (troglobites and non-troglobites) are reported. Due to the simplicity of the morphological characteristics of the telopods, molecular studies are recommended for future taxonomic studies, especially for the distinction of possible peripatric species. *Glomeridesmus spelaeus* (Figure 2F) and *G. sbordonii* Shear, 1973 (Cocona cave, Tabasco, Mexico) are the only troglobitic species known for the order, being characterized by increased body size, thin cuticle and depigmentation (INIESTA *et al.*, 2012; SHEAR, 1973). It is important to note that adult females are easily confused with males, as they have elongated ovipositors in the anterior region of the body. *Glomeridesmus spelaeus* is the only glomeridesmid species with a sequenced mitochondrial genome (NUNES *et al.*, 2020). The species is restricted to a set of ferruginous caves in Serra Leste, Curionópolis, Pará.

Dobrodesmus mirabilis Shear, Ferreira & Iniesta, 2016 (Polydesmida, Dobrodesmidea, Dobrodesmidae)

Dobrodesmus mirabilis is the only known species of the suborder Dobrodesmidea, characterized mainly by the morphology of the gonopod and spinets. The family Dobrodesmidae is identified by the unusual number of body rings: 39 + telson. The observable characteristics of the species give it prominence in a unique setting for evolutionary hypotheses in Polydesmida (for more details see SHEAR *et al.*, 2016). Only one adult male and numerous juveniles are known. The species only occurs in Gruta da Mangabeira, Ituaçu, Bahia, a destination for extensive pilgrimages (about 100,000 pilgrims have been reported in a short interval of visits to the cave), which has numerous alterations to its interior and surroundings (staircase, altar in cave entrance, lighting equipment, among others).

Pseudonannolene spelaea Iniesta & Ferreira 2013
Pseudonannolene ambuatinga Iniesta & Ferreira 2013
Pseudonannolene lundi Iniesta & Ferreira 2015
Pseudonannolene canastra Gallo & Bichuette, 2020 (Spirostreptida, Pseudonannolenidae)

Pseudonannolene is the richest genus of the order in Brazil, for both epigean and hypogean species (INIESTA *et al.*, 2020). *Pseudonannolene spelaea* is the only species of the genus occurring in the Amazon region, except for *P. rugosetta* Silvestri, 1897, whose record for Guyana is uncertain. The four species are characterized by depigmentation and smaller body sizes compared to epigean species (GALLO; BICHUETE, 2020; INIESTA; FERREIRA, 2013a, b, 2015). For the genus (extending in part to Pseudonannolenidae, Spirostreptidae and Rhinocricidae), it is important to note that juveniles are confused with possible troglobites (see item Main troglomorphisms) and that determination is quite difficult due to the existence of intermediate forms of the gonopods. *Pseudonannolene spelaea* is distributed in ferruginous caves in Floresta Nacional de Carajás, Pará; *P. ambuatinga* (Figure 2A) in caves in the Pains karst region; *P. lundi* is restricted to Lapa Sem Fim, Luislândia; and *P. canastra* from São Roque de Minas, the last three in the state of Minas Gerais.

Main methods of collection and conservation

Differences in terms of habitats reflect the great morphological diversity of Diplopoda (Figure 4). Certain orders (in certain cases families and genera) show preferences regarding different biotopes (VOIGTLÄNDER, 2011). In this sense, the more diversified the collection methodologies, regardless of

efficiency, the greater the probability of specific assemblages being sampled. In general, methods based on manual collection are recommended as the best to achieve the highest species richness (MEANS *et al.*, 2015) and, when associated with extraction in Berlese-Tüllgren funnels (SNYDER *et al.*, 2006) and pitfall or bait traps (CULVER; SHEAR, 2012; OLIVEIRA *et al.*, 2019; TUF *et al.*, 2015), present the best scenario for sampling diversity among groups.

Manual collections can be done with the aid of a rake (MEANS *et al.*, 2015), gardening shovel for excavation or just looking for specimens in specific microhabitats (under rocks, litter and tree bark). Manual collections are more common in caves, with greater attention to microhabitats and different zones (such as entrance, penumbra and aphotic zones). Certain groups (e.g., Pyrgodesmidae and Furhmannodesmidae) require wet brushes for capture, while others (e.g., Chelodesmidae and Rhinocricidae) can be caught with tweezers. For morphological studies, specimens can be fixed in the field in 70 to 80% ethanol with renewal of ethanol immediately at the end of the expedition, since the repugnant liquid released by specimens at the time of collection accelerates specimen putrefaction and DNA degradation. Ideally, each specimen should be individualized, fixed in ethanol in small plastic bags with the trunk supported by wooden rods to avoid body entanglement. This method will reduce the need to break the specimen more than is necessary to analyze certain structures. For molecular studies, the protocol is the same except for fixation, which in this case occurs with the use of 96 GL alcohol (or absolute) with subsequent renewal. Some optimizations can be made, such as, instead of fixing the whole specimen in 96 GL, one can detach only the head and the first five rings for molecular study, not reaching the first ozospore or the last pairs of legs on a single side of the body. In these cases, the rest of the body is fixed in dilute ethanol. Transcriptome, metabolome, and proteome studies should follow their usual protocols. For complementary studies, such as those of repugnant liquids, different protocols have been proposed such as the maintenance of live specimens or the use of specific fixatives (SHEAR, 2015).

Identification key

The following are adult identification keys for introduced or endemic groups in Brazil (adapted from HOFFMAN *et al.*, 1996). Due to taxonomic diversity, the key is divided between orders and families. Taxonomic refinements should directly consult respective revision works, when available.

ORDERS

1a. Body little sclerotized; tergites with tufts of bristles; one pair of long bristles at posterior extreme of body ..... **POLYXENIDA**

1b. Body rigid, in part by calcium impregnation; bristles, when present, not arranged in tufts; more than 13 body rings **2**

2a. Absence of ozopores; Tömösvary organ large and oval; collum small in relation to other rings; adult males with last pair of legs modified; adult females with long ovipositor..... **GLOMERIDESMIDA**

2b. Presence of ozopores; Tömösvary organ absent, or small when present; collum proportional to other rings; males with last pair of legs not modified, female gonopore at base of second pair of legs. **3**

3a. Anterior region of head conical, in form of a beak..... **4**

3b. Anterior region of head rounded..... **5**

4a. Anophthalmic; distal segments of antennae expanded; transverse section of body forming semicircle ..... **SIPHONOPHORIDA**

4b. Ocular field positioned laterally, variable ocelli formula (1+1, 2+2, 3+3, 4+4); body wider than thick; dorsal region convex ..... **POLYZONIIDA**

5a. Head with up to two pairs of ocelli; when two pairs are present, one is larger than the other; antennae elongated, composed of eight antennomeres plus antennal disc; legs composed of eight distinguishable podomeres ..... **STEMMIULIDA**

5b. Anophthalmic or with more than two pairs of ocelli; antennae and legs never reaching eight articles.....**6**

6a. Anophthalmic (Figure 7F); with lateral projections on tergites (= paranota) (Figure 3B). ..... **POLYDESMIDA**

6b. Ocular field with innumerous ocelli, above 4+4 (including juveniles and troglobites); absence of paranota; sclerites forming complete. ring.....**7**

7a. Head, in frontal view, with median suture that extends upward from labrum; antennae generally positioned close to head; gnathochilarium with broad mentum, reaching lateral margins of structure .....**SPIROBOLIDA**

7b. Median suture of head not extending upward from labrum; antennae with more articulated aspect in relation to head; gnathochilarium variable in form and composition. **8**

8a. Gnathochilarium with lateral pieces (= stipes) connected in the median line in their proximal portion, separated distally by a glabrous promentum .....**JULIDA**

8b. Gnathochilarium variable, but lateral pieces (= stipes) never in contact with each other (Figure 3C)..... **SPIROSTREPTIDA**

POLYDESMIDA

1a. Adults with body composed of 40 body rings.....**Dobrodesmidae**

1b. Adults with body composed of 18 to 22 body rings **2**

2a. Body generally able to curl over itself, but not forming a perfect sphere (Figure 7D); paranota of second ring laterally hypertrophied, notched on posterior margin; collum reduced.....**3**

2b. Body not modified for curling, at most capable of forming a spiral; paranota of anterior rings not augmented, collum not reduced.....**4**

3a. Antennomere 5 larger and wider than 6; head may present frontal projections; tergites microtuberculate, with shallow, transverse posterior sulci, generally with short bristles on surface ..... **Oniscodesmidae**

3b. Antenna of club aspect; tergites tuberculate; setous, generally with visible regions of bristle insertion; paranota of remaining rings ventrally directed.....**Cyrtodesmidae**

4a. Collum enlarged, covering much of the head in dorsal view.....**5**

4b. Collum not enlarged, proportional to subsequent rings **7**

5a. Body dorsoventrally flattened; posterior margin distinctly fringed; ozopores absent or very small, never styliform (= porosteles).....**Cryptodesmidae**

5b. Body not dorsoventrally flattened; paranota directed downwards, with small lobulated margins; ozopores positioned at the extremities of porosteles; tergites with series of transverse or longitudinal tubercles, those of median line often hypertrophied **6**

6a. Paranota little developed, conferring a cylindrical appearance to body; porosteles, when present, mushroom-shaped; gonopod coxa not globose.....**Haplodesmidae**

6b. Paranota size variable, but visible; body rarely cylindrical; tergites generally with tubercles positioned in three transverse or sparse rows (Figure 7E); porosteles, if present, cylindrical.....**Pyrgodesmidae**

7a. Collum never covering head (Figure 7F); paranota well developed, tergites dorsal and/or lateral projections; or paranota of most body rings reduced; *paranota of 2nd ring, with few exceptions, positioned lower than those of the 3rd ring and collum; transverse constriction in tergites, separating pro-metazone; opening of gonopods with medial constriction ....**Paradoxosomatidae**

7b. Paranota of 2nd and 3rd rings approximately at the same level, strongly projected posteriorly; opening of gonopods without medial constriction.....**8**

8a. Telson spatulate (Figure 7A).....**9**

8b. Telson conical (Figure 7B)..... **10**

9a. Paranota toothed; ozopores arranged in small disks on dorsal surface; prozone surface microgranulate; spiracles opening into modified area almost as large as coxal articulation cavity (Figure 7C).....**Platyrhacidae**

9b. Opening of ozopores on lateral margin of paranota; prozone surface smooth and polished; opening of spiracles small .....**Aphelidesmidae**

10a. Small species (adults less than 10 mm); pigmentation reduced, tergites with claviform bristles, generally in three transverse rows; antennae typically angled between antennomeres 4 and 5.....**Fuhrmannodesmidae**

10b. Adults generally larger than 10 mm; tergites without bristles, or when present, discrete. **11**

11a. Collum and telson reduced; pigmentation uniform or with grayish or brownish spots; distal podomeres with globose bristles; gonopod generally presenting medially fused coxa and/or telopodite; gonopod coxae small and internal relative to gonopod opening.....**Dalodesmidae**

11b. Collum large; body pigmented, vividly colored; paranota wide (Figure 3B); distal podomeres in males with distinct projections; coxae of gonopods never fused (Figure 3E) and exposed in relation to gonopod opening.....**Chelodesmidae**

SPIROSTREPTIDA

1a. Promentum of gnathochilarium always absent; adult males with only one pair of gonopods, divided into two longitudinal plates.....**Spirostreptidae**

1b. Promentum present (except Physiostreptinae); adult males with one or two pairs of gonopods and not divided into plates. **2**

2a. Tergites with longitudinal carenas.....**Cambalidae**

2b. Promentum, when present, with or without longitudinal or transverse sutures (Figure 3C); tergites without longitudinal carenas.....**Pseudonannolenidae**

Main collections in Brazil

A large portion of the millipede species in Brazil were described in the 20th century by the Frenchman H. W. Brolemann (1860-1933) and the German living in Brazil O. Schubart (1900-1962). Some of the type material is deposited in several scientific collections in the Americas and Europe (SIERWALD; REFT, 2004). Specifically, for those species described by Schubart, the material is deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP). The collection comprises material in permanent slides (in Canada Balsam) fixed in 70% ethanol. As important structures for identification are fixed, slight morphological variation may occur due to the assembly process itself or due to time (such as bubbles and folds), differentiating them from specimens being compared.

Several institutions in the Southeast Region stand out for their richness of cave species. The collection of millipedes linked to Instituto Butantan is highlighted by its taxonomic diversity of cave species, especially Spirostreptida and Polydesmida. The collections of Laboratório de Estudos Subterrâneos (LES) of Universidade Federal de São Carlos (UFSCar) and of Centro de Estudos em Biologia Subterrânea (CEBS) of the Universidade Federal de Lavras (UFLA) present large collections of species, including type series and material sampled in caves of different lithologies in the country.

Unlike the situation for other groups of invertebrates, millipedes of most Brazilian zoological collections still lack taxonomic refinement. This fact reflects the scarcity of taxonomists for the Neotropics (see HOFFMAN *et al.*, 1996) or even the difficulty of taxonomic resolution for different groups

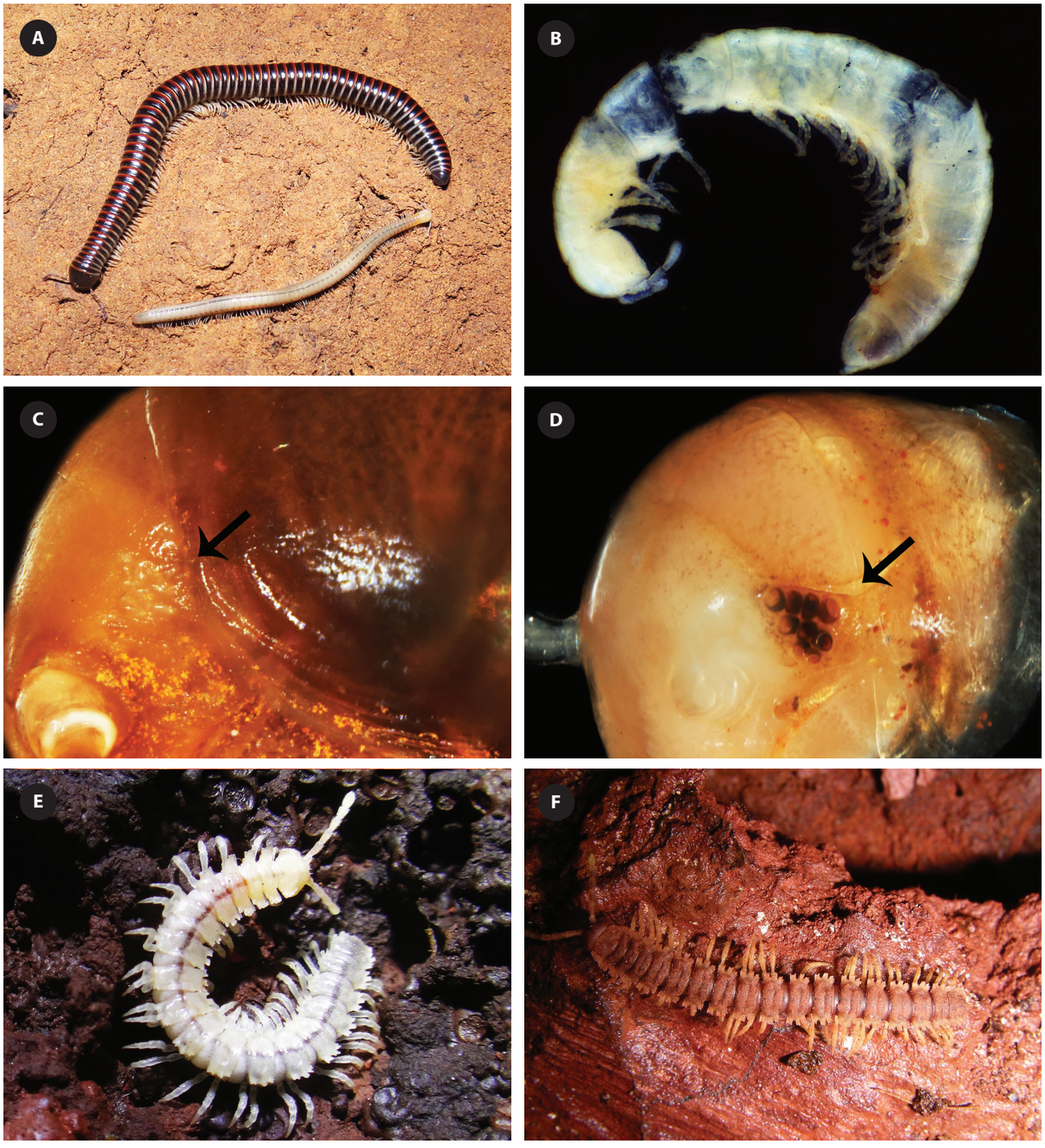


Figure 6. A) Troglophilic (pigmented) and troglobitic (depigmented) species de *Pseudonannolene*; **B)** Juvenile of Paradoxosomatidae; **C)** Head of troglobitic adult individual of *Pseudonannolene*. Arrow indicating ocular field; **D)** Head of non-troglobitic juvenile individual of *Pseudonannolene*. Arrow indicating ocular field (note the loosely grouped ocelli); **E-F)** Juvenile and adult of *Parastenonia carajas* Bouzan & Iniesta, 2020, a troglophilic species. Photos: A, C (Luiz Felipe Moretti Iniesta); B, D, E, F (Claudio Augusto Ribeiro de Souza).

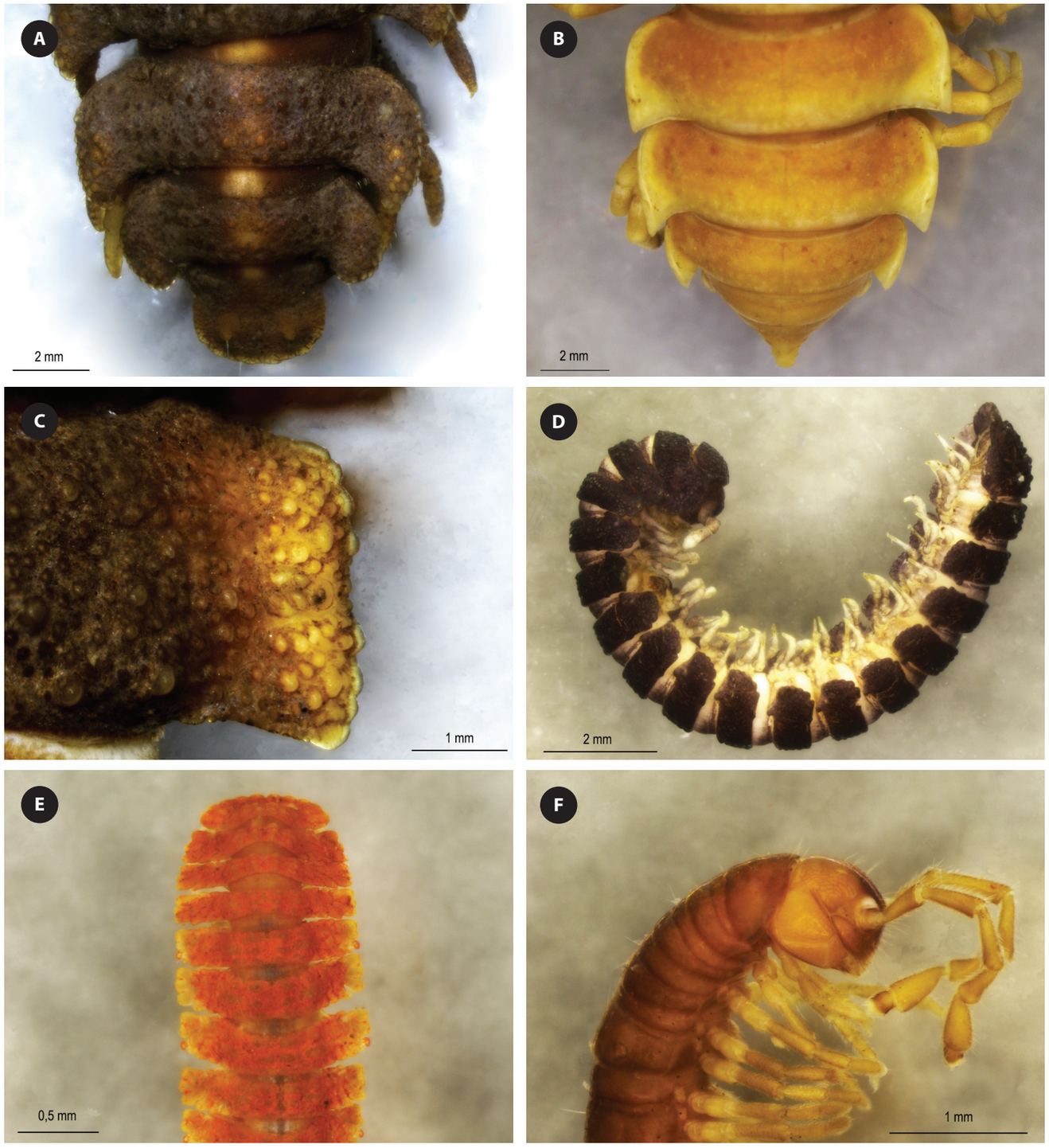


Figure 7. A) Telson of Platyrhacidae; **B)** Telson of Chelodesmidae; **C)** Paranota of Platyrhacidae; **D)** Body of Cyrtodesmidae; **E)** Anterior region of Pyrgodesmidae, in dorsal view; **F)** Anterior region of Paradoxosomatidae, in lateral view. Photos: Rodrigo Salvador Bouzan.



Figure 8: Map with type localities of troglobitic species of Diplopoda described for Brazil.

(e.g., Polydesmida and Spirostreptida). One of the few faunal surveys conducted in Brazil was that published by Brölemann (1909). Since then, only more regionalized studies have been carried out to understand species composition (GOLOVATCH *et al.*, 2005; HOFFMAN *et al.*, 1996). When considering the collections of species not necessarily sampled in caves, but with great relevance for taxonomic studies, zoological collections of the following stand out: Instituto Nacional de Pesquisas da Amazônia (INPA), Museu Paraense Emílio Goeldi (MPEG), Universidade Federal da Paraíba (UFPB), Universidade Federal do Mato Grosso (UFMT), Instituto Butantan, Museu de Zoologia da Universidade de São Paulo (MZUSP), Universidade do Vale do Rio dos Sinos (UNISINOS) and Museu de Ciências Naturais do Rio Grande do Sul (MCN).

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Robson de Almeida Zampaulo



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Phlebotominae
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13

Diptera: Psychodidae

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General introduction

The family Psychodidae (from the Greek *psyche*-butterfly; *oda*-stinging) of the order Diptera is a diverse, ubiquitous group with about 3,400 described species (CORDEIRO; WAGNER, 2018; CURLER *et al.*, 2019; GALATI, 2018). These insects are popularly known as psychodids and are included in six living subfamilies: Bruchomyiinae, Trichomyiinae and Horaiellinae, which have no medical or veterinary importance; Psychodinae, with rare cases of myiasis caused by *Clogmia albipunctata* (Williston, 1893); Sycoracinae, for which there are reports of individuals feeding on the blood of poikilothermic animals; and Phlebotominae, most females of which perform hematophagy on several vertebrates, including humans (YOUNG, 1979).

Except for Phlebotominae (sand flies), published information about species of Psychodidae that occur in natural subterranean cavities in Brazil is scarce (ALEXANDER, 1940; BRAVO *et al.*, 2008; BRAVO; BARATA, 2012). The few records of these species in these environments is mainly due to the lack of specific studies on the group, since its species are abundant and diverse in caves in other parts of the world (QUATE, 1962, 2000).

Sand flies have been the subject of several studies in caves, with a view to faunal surveys and the identification of ecological aspects (ALMEIDA *et al.*, 2019; ALVES *et al.*, 2011; BARATA *et al.*, 2008; BARATA; APOLINÁRIO, 2012; CAMPOS *et al.*, 2017, 2020; CARVALHO *et al.*, 2011b, 2013; CUTOLO *et al.*, 2008; GALATI *et al.*, 1997, 2003b, 2010a-b; OGAWA *et al.*, 2016). These studies have also contributed to the taxonomy of the group, enabling the description of new species for science (ALVES *et al.*, 2008; BARATA; APOLINÁRIO, 2012; CARVALHO *et al.*, 2010, 2011a; GALATI *et al.*, 1985; GALATI *et al.*, 2003b; LIMA, 1932; MARTINS *et al.*, 1957, 1962; VILELA *et al.*, 2015), including the description of a monospecific genus, *Edentomyia* Galati, Andrade-Filho, Silva & Falcão, 2003, whose type species is from caves and conserves the largest number of primitive character states from the subtribe Phlebotomini (GALATI *et al.*, 2003a, 2007). This particularity of records in natural subterranean cavities noted for the subfamily Phlebotominae is due to the investigation of parameters for assessing potential involvement of populations in the transmission of *Leishmania* spp., protozoa that cause leishmaniasis (DUTRA-RÊGO *et al.* 2022; YOUNG, 1979). On the other hand, it should be noted that little is known about the degree of dependence that most psychodid species have on the cave environment. This is because of the collection methods employed, which commonly involve artificial light as a source of attraction, and therefore capture insects from environments other than where the light focus was installed, including the epigean environment.

Trajano and Bichuette (2009) argue that the greatest difficulty for the progress of Brazilian biospeleology is the taxonomic impediment that affects a significant number of taxa. In general, several specimens from caves belonging to the orders Acari, Collembola and Diptera remain identified, mainly at higher taxonomic levels (family or higher). Still, according to the authors, even among those groups for which there are specialists, there is still many taxa of hypogean or even epigean occurrence to be identified and described, mainly in megadiverse tropical countries such as Brazil.

Animals from epigean environments, with typical nocturnal behavior, have a high probability of success in colonizing subterranean environments. These come from lineages that developed in humid environments and that have a cryptozoic habit, living or sheltering in environments with little or no light, such as crevices, under rocks, among fallen leaves on the ground or under trunks (TRAJANO; BICHUETTE, 2006). Such behavioral characteristics are typical of sand flies, mainly of their immature forms (FORATTINI, 1973). Caves, therefore, may represent natural breeding sites or daytime shelters for some species (FELICIANGELI, 2004) that can be found from the entrance to the deepest and completely aphotic zones of these environments (GALATI *et al.*, 2003b; ALVES *et al.*, 2011). Depending on their morphological conditions, caves may also present more stable climatic conditions when compared to epigean environments, with high relative humidity and milder temperatures. Such environmental conditions also favor colonization by psychodid dipterans.

Psycodids can, in this environment, maintain a dependent relationship with vertebrates, either using their feces (guano) as breeding grounds (ALVES *et al.*, 2011) or performing

hematophagy on them (COSTA *et al.*, 2021). Different substrates commonly observed inside caves could also serve as breeding grounds or refuges for winged forms of psychodids. Some species, like *Ed. piauiensis* and *Tonnoira distincta* Bravo, 2008, are indicators of environmental quality (FORATTINI, 1973). Therefore, the presence of these insects in caves may still represent a positive sign for their preservation.

There were no reports of troglobitic psychodids in Brazilian caves until 2010 (TRAJANO; BICHUETTE, 2009). This scenario changed with the description of *Deanemyia maruaga* (Alves, Freitas & Barret 2008), the only troglobitic species in Brazil, described from a sandstone cave in Presidente Figueiredo, Amazonas (ALVES *et al.*, 2008, 2011). Larvae of *Lutzomyia almerioi* Galati & Nunes 1999, a species described from Serra da Bodoquena, Mato Grosso do Sul, were obtained from a crevice inside a cave (GALATI *et al.*, 2006) and *Sciopemyia sordellii* (Shannon & Del Pont 1927) has been the most collected species in Brazilian caves (data from this chapter). However, these two species can also be found in epigean environments, with no evidence to classify them as troglobites (DUTRA-RÊGO *et al.,* 2022). In addition to these, two species of the subfamily Psychodinae, *T. distincta* and *T. robusta* Bravo, 2008, seem to maintain a more intimate relationship with natural subterranean cavities (BRAVO *et al.*, 2008) and although they are known to be detritivores, there are no data on the biology of these species in this type of environment.

Thus, the content of this chapter presents an extensive and detailed survey of the psychodid fauna associated with caves in Brazil, based on unpublished data, published data and the authors' experience. This chapter will also present the importance of records for this habitat and guidelines on how best to take advantage of collection data, in view of investments involved in the different types of study.

Main groups found in Brazilian caves

Among dipterologists (professionals who study insects of the order Diptera), phylogenetic hypotheses have inserted different groups in the infraorder Psychodomorpha. More recently, Wiegmann *et al.* (2011), based on data from multiple genes, hypothesized that Psychodomorpha comprises the families Blephariceridae, Tanyderidae and Psychodidae, with origin in the lower Jurassic or, probably, in the Triassic. However, based mainly on wing venation of living and fossil dipterans, Krzemiński and Krzemińska (2003) proposed including blepharicerids in the infraorder Blephariceridomorpha, being closer to Culicomorpha, Nymphomyiomorpha, Ptychopteromorpha and Tillyardomorpha in the suborder Neoneura. The suborder Diarchineura, then, would include the infraorders Psychodomorpha and Tanyderimorpha, with current representatives, along with three others (Grauvogeliomorpha, Hennigmatomorpha and Nadipteromorpha) with only fossil representatives. These authors considered that Psychodomorpha would comprise two families, Psychodidae and Phlebotomidae. Thus, sand flies would be classified in a different family from other psychodids. Previous proposals also considered elevating Phlebotominae to the category of family (ABONNENC; LEGER, 1976; ADLER; THEODOR, 1929; ROHDENDORF, 1964; WILLIAMS, 1993); however, most current classifications recognize Phlebotominae as a subfamily of Psychodidae.

In general, it should be noted that adult psychodids are small insects (up to 5 mm) with body and wings heavily covered with bristles (Figure 1 A-D); antennae formed by articulated flagellomeres, with hyaline sensory structures (ascoids); wings with 9 to 11 longitudinal veins reaching the wing edge; subcosta ending at costa or converging on R_1, before the middle of the wing; radial sector with three to five veins; R_1 reaching costa after the middle of the wing; and medial sector with four veins (GALATI, 2003; WAGNER; IBÁÑEZ-BERNAL, 2009). The family Psychodidae is composed of a fossil subfamily, Datziinae (STEBNER *et al.*, 2015), known only from Burmese amber (Myanmar) of the Upper Cretaceous (CURLER *et al.*, 2019) and six subfamilies with current and fossil representatives, which will be presented in sequence from least to most diverse.

HORAIELLINAE

Horaiellinae contains five current species included in the genus *Horaiella* Tonnoir, 1933, all with oriental distributions (CURLER;

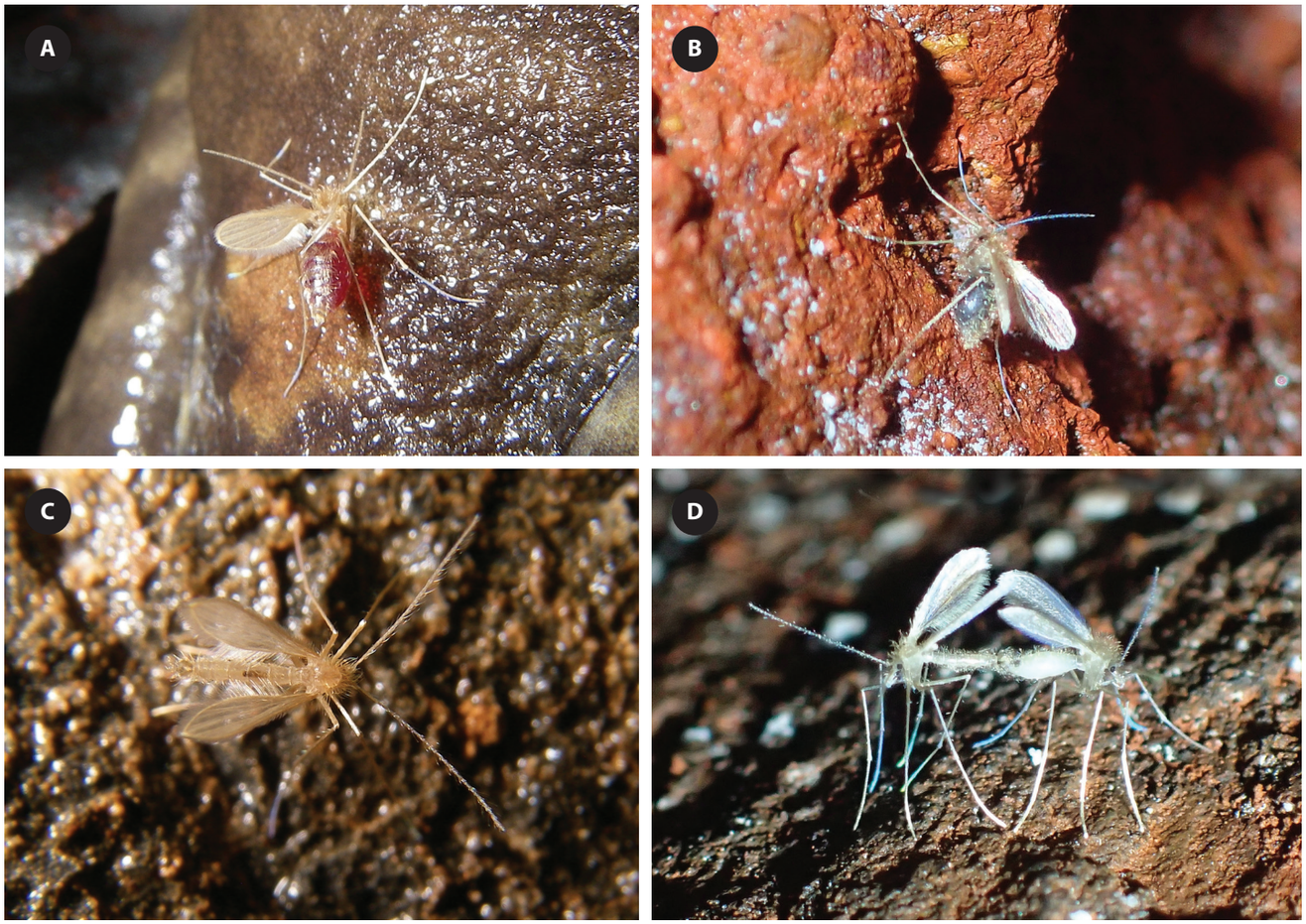


Figure 1. General aspects of psychodids. **A)** Female phlebotomine (subfamily Phlebotominae) with undigested blood, found in a cave in Serra do Rola Moça State Park, Nova Lima, Minas Gerais; **B)** female of *Sciopemyia* aff. *microps* (subfamily Phlebotominae) with partially digested blood, found in a natural ferruginous cavity in Serra do Gandarela, Rio Acima, Minas Gerais; **C)** male phlebotomine (subfamily Phlebotominae) found in a carbonate cave in the region of Pains, Minas Gerais; **D)** male (left) and female (right) specimens of *Sciopemyia* spp. (subfamily Phlebotominae) recorded copulating in a ferruginous cave in Serra do Gandarela, Rio Acima, Minas Gerais. Photos: A-C (Robson de A. Zampaulo); B-D (Júlio C.R. Costa).

PRIYADARSANA, 2015), and a fossil genus, *Protohoraiella* Curler, Krzemiński & Skibińska, 2019, with two described species from Burmese (Myanmar) amber of the Cretaceous (CURLER *et al.*, 2019). Specimens of Horaiellinae are rarely collected and the only two species with known immatures live on wet rocks that are constantly sprayed by mist from waterfalls (CURLER; COURTNEY, 2009). There is no record of any species in caves anywhere in the world.

SYCORACINAE

Sycoracinae contains 46 living species distributed in three genera (ROSSITO; PINHO, 2019): *Sycorax* Curtis, 1839, with 40 current species and five fossil species (AZAR; SALAMÉ, 2015); *Aposycorax* Duckhouse, 1972 with only one living species; and *Parasycorax* Duckhouse, 1972 with five living species and one fossil. The subfamily has a worldwide distribution. More recent descriptions include fossil genera *Xenosycorax* Azar & Salamé, 2015 from Turonian amber of the Upper Cretaceous, New Jersey, USA; *Palaeoparasycorax* Stebner *et al.*, 2015 from Myanmar amber of the Upper Cretaceous; and *Libanosycorax* Azar, Azar & Maksoud, 2018, from amber of the Lower Cretaceous of Lebanon. Very little is known about the behavior of the species of this subfamily. Some aquatic or wet soil larvae are known (CURLER *et al.* 2015) and adult females have been found performing hematophagy on

frogs (BRAVO; SALAZAR-VALENZUELA, 2009). *Sycorax malayensis* Quate, 1962 was described from Batu Cave, Kuala Lumpur, Malaysia, but was considered a trogloxene (QUATE, 1962). There is no record of any species in caves in the Americas.

BRUCHOMYIINAE

Bruchomyiinae contains 74 species, 53 current and 21 fossils, and occurs on all continents, except Antarctica. The subfamily is divided into 10 genera: *Nemopalpus* Macquart, 1838; *Palaeosycorax* Meunier, 1905; *Bruchomyia* Alexander, 1921; *Eutonnoiria* Alexander, 1940; *Boreofairchildia* Wagner & Stuckenberg, 2016; *Laurenceomyia* Wagner & Stuckenberg, 2016; *Notofairchildia* Wagner & Stuckenberg, 2016; *Hoffeinsodes* Wagner, 2017; *Palaeoglaesum* Wagner, 2017 and *Alexanderia* Wagner & Kvifte, 2018 (POLSEELA *et al.*, 2018; WAGNER; STUCKENBERG, 2016; WAGNER, 2017). *Notofairchildia spinosus* (Bravo & Barata, 2012) and *Bruchomyia mineira* Bravo & Barata, 2012 were described from specimens collected in caves in the state of Minas Gerais (Bravo and Barata 2012a, b) and *Bruchomyia brasiliensis* Alexander, 1940, in cave crevices in the municipality of Crato, Ceará (ALEXANDER, 1940). Only these three species are known to be linked to cave environments in Brazil.

TRICHOMYIINAE

Trichomyiinae comprises 215 species and has a ubiquitous distribution, except for Antarctica. It has four fossil genera — *Axenotrichomyia* Azar, Huang, Cai & Nel, 2015; *Eatonisca* Meunier, 1905; *Eotrichomyia* Nel, Meunier & De Plöeg, 2002; and *Xenotrichomyia* Azar, Mouawad & Salame, 2015 — and all current species are included in the genus *Trichomyia* Haliday in Curtis, 1839. *Trichomyia malaya* Quate, 1962 and *Trichomyia batu* Quate, 1962 occur in caves of Batu, Malaysia (QUATE, 1962). As for Sycoracinae, there is no record of any species of Trichomyiinae in caves in the Americas.

PHLEBOTOMINAE

Phlebotominae contains 1,029 species, 998 living and 31 fossil, distributed across all continents, except Antarctica. The Americas are home to 546 species, 529 living and 17 fossil (GALATI E.A.B 2022, personal communication). The systematics of Phlebotominae is a controversial topic. The most conservative classifications adopt six genera: *Phlebotomus* Rondani & Berté, 1840, *Sergentomyia* França & Parrot, 1920 and *Chinius* Leng, 1987, for the Eastern Hemisphere species, and *Brumptomyia* França & Parrot, 1921; *Lutzomyia* França, 1924 and *Warileya* Hertig, 1948 for species of the Americas (LEWIS *et al.* 1977). However, the division of sand flies into two tribes, Hertigiini and Phlebotomini, was proposed using a phylogenetic approach. The first with two subtribes — Hertigiina for the Americas and Idiophlebotomina for the Eastern Hemisphere — and the second with worldwide distribution, with five subtribes: Phlebotomina, Old World; Australophlebotomina, Australian region; Sergentomyiina, worldwide distribution; and Brumptomyiina, Lutzomyiina and Psychodopygina for the Americas. This classification proposed more than 31 genera, two of which are fossil and most of them of the Americas (GALATI, 1995, 2003). The genus *Edentomyia*, within the tribe Phlebotomini, was later described for the Americas as well; (GALATI *et al.*, 2003a), coming from a cave in the state of Piauí, however, without insertion in any subtribe. Four other fossils were later described for the Eastern Hemisphere (GALATI, 2018). Among the 23 genera, 19 subgenera, three species groups and 21 species series, in which American sand flies are classified, only four genera (*Warileya*, *Hertigia* Fairchild, 1949, *Oligodontomyia* Galati, 1995 and *Dampfomyia* Addis, 1945); three subgenera (*Coquillettimyia* Galati, 1995, *Dampfomyia* Addis, 1945, *Coromyia* Barretto, 1962); one species group (Delpozoi group) and five species series (Vexator, Chiapanensis, Pia, Verrucarum and Townsendi) do not have representatives in Brazil.

Only one troglobitic species of Amazonian origin is known from Brazil. Although Dutra-Rêgo *et al.* (2022) indicate other species of sand flies as obligate in cave environments, we believe that only *De. maruaga* has characteristics that allow its classification as such. Adult and immature specimens of *De. maruaga* were collected exclusively in the aphotic zone, using guano as substrate for their development. The immature stages of the species show morphological and physiological differences in relation to other sand fly species. The eggs of the species are gray in color, the upper pair of caudal bristles of the larvae are twice as long as the lower pair, and they have a long life cycle of 90 days. In addition, adult females do not feed on blood, are parthenogenetic and maintain a fat reserve in the abdomen (ALVES *et al.*, 2011). Such characteristics allowed the authors to conclude that *De. maruaga* is a troglobitic species. All other species found in caves so far can be classified as obligate and/or facultative (or accidental) troglophiles or trogloxenes, depending on the classification adopted and the findings of specific studies.

PSYCHODINAE

Psychodinae is the most numerous subfamily of Psychodidae, with 2,050 species of both fossil and current representatives, and has the widest worldwide distribution represented by more than 100 genera (PAPE *et al.*, 2011). Several tribes have been proposed (DUCKHOUSE, 1987; JEŽEK, 1983; VAILLANT, 1971), but none have been widely accepted. Phylogenetic study, based on mitochondrial DNA, of Palearctic Psychodinae found that several tribes proposed by Vaillant (1971) and Ježek (1983) constitute monophyletic groups (ESPÍNDOLA *et al.*, 2012). Bravo *et al.*, (2008) described two species of *Tonnoira* Enderein, 1937, from caves in Central Amazonia. Quate and Brown (2004) described *Tonnoira cavernicola* from a cave in Bolivia, where adults were collected at the cave entrance and in the external environment. Despite the absence of reports of other psychodines in Brazilian caves, there are records of several species of this subfamily in caves in other biogeographical regions (QUATE 1962, 2000), including synanthropic and ubiquitous species, such as *Clogmia albipunctata* and *Psychoda alternata* Say, 1825, or common and tropicopolitan (= ubiquitous in the tropics) species, such as *Psychoda savaiiensis* Edwards, 1828, which indicates the large gap in records in cave environments in Brazil.

Records of species of Psychodidae in Brazilian caves

The subfamily Phlebotominae currently has the largest number of records and most information available, thus we will emphasize it here in this topic. Data referring to the other subfamilies can be found in the other items of this chapter.

There are sampled caves with records of phlebotomines in at least one state in each region of Brazil. In the North Region, the state of Amazonas has three sampled caves (ALVES *et al.*, 2011); Rondônia, eight caves (OGAWA *et al.*, 2016); Tocantins, 11 caves (GALATI, E.A.B. 2022, personal communication; VILELA *et al.*, 2015) and Pará, 269 caves (GALATI, E.A.B. 2022, personal communication; TEODORO *et al.*, 2021). In the Southeast Region, Minas Gerais has 58 caves (ANDRADE FILHO *et al.*, 1999, 2004; BARATA *et al.*, 2008; BARATA; APOLINÁRIO, 2012; CARVALHO *et al.*, 2011a, b, 2013; CAMPOS *et al.*, 2020; LIMA, 1932; COSTA *et al.*, 2021; GALATI, E.A.B. personal communication; MARTINS *et al.*, 1957, 1962); Rio de Janeiro, one cave (ALVES *et al.*, 2020) and São Paulo, nine caves (GALATI *et al.*, 2010; GALATI, E.A.B. 2022, personal communication). In the Northeast Region, the state of Bahia has four caves (GALATI, E.A.B. 2022, personal communication) and Piauí, one cave (GALATI *et al.*, 2003a). In the Center-West Region, Mato Grosso do Sul has 14 caves (GALATI *et al.*, 1997, 2003b, ALMEIDA *et al.*, 2019), while In the South Region the state of Paraná has only one cave (GALATI, E.A.B. 2022, personal communication) (Figure 2).

According to the scientific literature review and field data of the authors of this chapter, these regions together have about 378 sampled caves distributed in 38 municipalities. Most of these works aimed at searching for invertebrates associated with these environments, without focusing on a single taxon or group, such as phlebotomines. This allowed many caves to be sampled, but the number of specimens collected is reduced (GALATI, E.A.B. 2022, personal communication). However, other temporally longitudinal studies sought to not only identify fauna, but also investigate the presence of species with the potential to transmit agents of leishmaniasis.

Thus far, 114 species of phlebotomines (including possible new species) have been found in natural subterranean caves, representing 39% of the 290 species found in Brazil. Similar data were found by Dutra-Rêgo *et al.* (2022). Only two genera — *Pressatia* Mangabeira, 1942 and *Viannamyia* Mangabeira, 1941 — that occur in Brazil have not been recorded in the cave environment. Distributions, according to subtribes and genera, are shown in Table 1.

Despite the expressive number of species identified in Brazilian caves, only for *De. maruaga*, as previously explained, is there evidence of it being a troglobite (ALVES *et al.*, 2011). However, *Ed. piauiensis, Pi. gruta* (Ryan, 1986) and unidentified specimens of *Deanemyia* (Gruta do Pitoco, Mato Grosso do Sul) have so far only been collected in caves. The species *Pi. gruta* and *Ed. piauiensis* were collected in 38% and 12% of 269 iron formation caves, respectively, in southeastern Pará, without the use of light traps. Only females of *Deanemyia* sp. were collected, mostly in the aphotic regions of Gruta do Pitoco (ALMEIDA *et al.*, 2019).

Troglophilous species comprise the majority of invertebrate taxa found in Brazilian caves (TRAJANO; BICHUETTE, 2009). Phlebotomine populations that colonize caves and which may also frequent the epigean environment, have been classified thusly (CAMPOS *et al.*, 2020; CARVALHO *et al.*, 2013; GALATI *et al.*, 2003b), despite collecting with light traps that can attract specimens that commonly live in the external environment. Among troglophile species, *Sc. sordellii* has the most dispersed populations in Brazilian caves. Populations of the species were collected from 80% of the 269 caves investigated in southeastern Pará; 100% of three caves investigated in Amazonas; 75% of

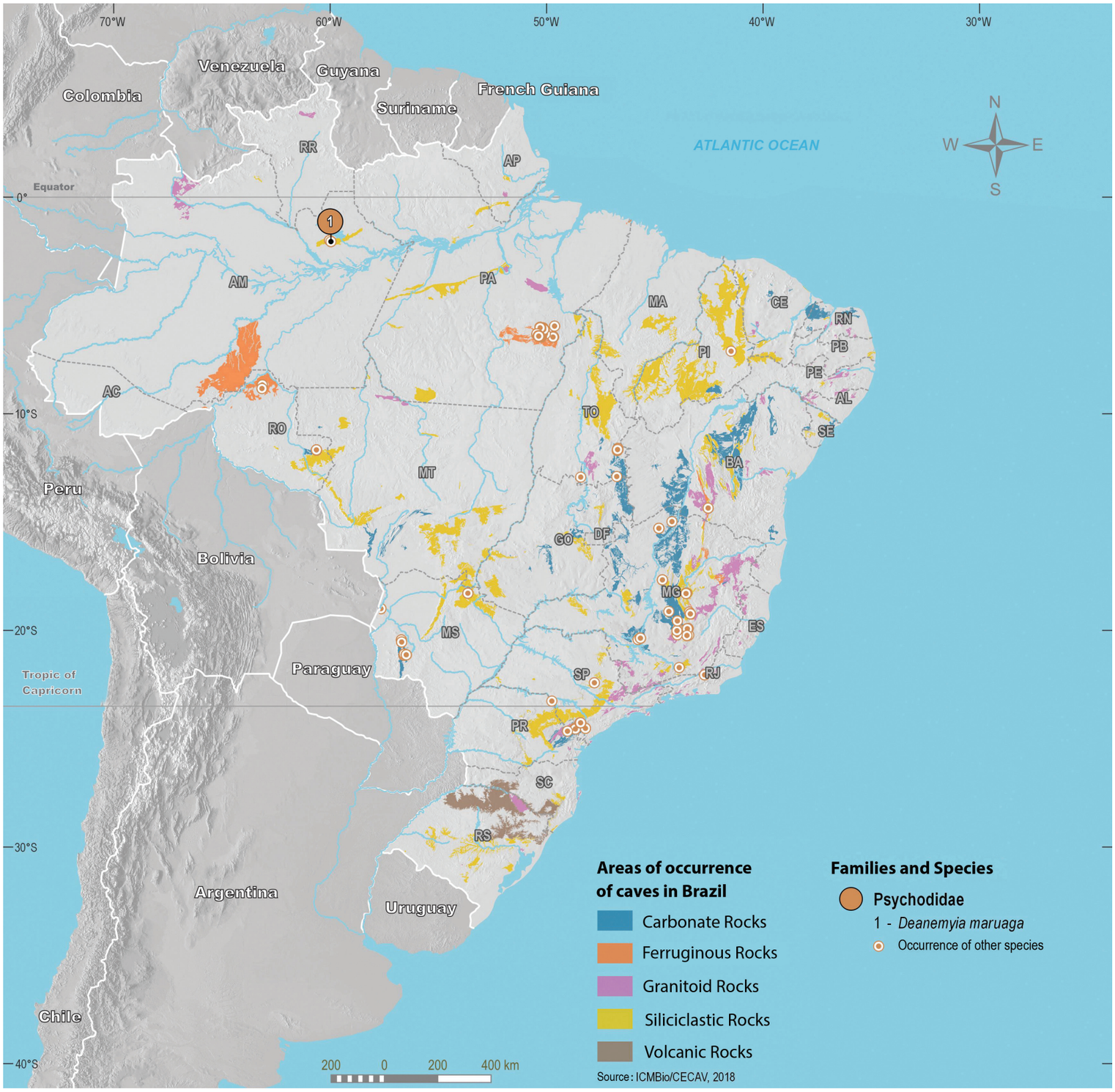


Figure 2. Map of Brazil with the locations of caves with records of psychodids (Diptera) identified to the taxonomic level of species and the locality of the only species described as a troglobite.

Table 1. Species of the subfamily Phlebotominae collected/captured in caves in Brazil, according to respective subtribe (in bold), according to the classification of Galati (2003a). The numbers after the genera refer to the number of species recorded in caves in relation to the number of species of the genus that occurs in Brazil (number in caves / number in Brazil).

Brumptomyiina

Brumptomyia (8/20)

Br. avellari, Br. brumpti, Br. carvalheiroi, Br. cunhai, Br. mesai, Br. nitzulescui, Br. pintoi, Br. troglodytes.

Lutzomyiina

Evandromyia (17/43)

Ev. bacula, Ev. brachyphala, Ev. carmelinoi, Ev. corumbaensis, Ev. cortelezzii, Ev. evandroi, Ev. georgii, Ev. inpai, Ev. lenti, Ev. monstruosa, Ev. sallesi, Ev. saulensis, Ev. sericea, Ev. spelunca, Ev. teratodes, Ev. termitophila, Ev. tupynambai.

Expapillata (1/2)

Ex. firmatoi.

Lutzomyia (11/32)

Lu. amarali, Lu. almerioi, Lu. cruzi, Lu. dispar, Lu. elizabethrangelae, Lu. forattinii, Lu. gaminarai, Lu. ischnacantha, Lu. ischyracantha, Lu. longipalpis, Lu. renei.

Migonemyia (1/6)

Mg. migonei.

Pintomyia (12/18)

Pi. christenseni, Pi. diamantinensis, Pi. fischeri, Pi. gruta, Pi. kuscheli, Pi. mamedei, Pi. misionensis, Pi. monticola, Pi. odax, Pi. pacae, Pi. pessoai, Pi. serrana.

Sciopemyia (5/7)

Sc. microps, Sc. pennyi, Sc. servulolimai, Sc. sordellii, Sciopemyia sp. (próxima a *Sc. microps*).

Trichopygomyia (2/10)

Tr. dasypodogeton, Tr. longispina.

Psychodopygina

Bichromomyia (2/5)

Bi. flaviscutellata, Bi. reducta.

Martinsmyia (5/8)

Mt. cipoensis, Mt. gasparviannai, Mt. minasensis, Ma. oliveirai, Mt. reginae.

Nyssomyia (9/15)

Ny. anduzei, Ny. antunesi, Ny. intermedia, Ny. neivai, Ny. richardwardi, Ny. shawi, Ny. umbratilis, Ny. whitmani, Ny. fraihai (previouly identified as Ny. yuilli yuilli).

Psathyromyia (13/32)

Pa. abonnenci, Pa. abunaensis, Pa. aragaoi, Pa. bigeniculata, Pa. brasiliensis, Pa. campograndensis, Pa. pifanoi (previouly identified as Pa. cuzquena), Pa. lanei, Pa. lutziana, Pa. pascalei, Pa. punctigeniculata, Pa. ribeirensis, Pa. scaffi.

Psychodopygus (6/28)

Ps. amazonensis, Ps. ayrozai, Ps. davisi, Ps. geniculatus, Ps. lloydi, Psychodopygus sp. (série Chagasi).

Trichophoromyia (4/26)

Th. auraensis, Th. brachipyga, Th. eurypyga, Th. octavioi, Th. ubiquitalis.

Sergentomyiina

Deanemyia (3/5)

De. maruaga, De. ramirezi, Deanemyia sp.

Micropygomyia (13/21)

Mi. acanthopharynx, Mi. echnatopharynx, Mi. micropyga, Mi. peresi, Mi. petari, Mi. pilosa, Mi. quinquefer, Mi. schreiberi, Mi. sp. (série Oswaldoi), *Mi.* (*Sauromyia*) sp., *Mi. trinidadensis, Mi. villelai, Mi. vonatzingeni.*

Subtribo *incertae sedis*

Edentomyia (1/1)

Ed. piauiensis.

eight in Rondônia; 18% of 11 in Tocantins; 85% of 14 in Mato Grosso do Sul; 46% of 58 in Minas Gerais; and 11% of nine in São Paulo. Species of the genus *Sciopemyia* Barretto, 1962 feed on cold-blooded animals in these environments (GALATI, E.A.B. 2022, personal communication), which was confirmed by Costa *et al.* (2021).

Among other species with populations classified as troglophilous, 20 predominate in cave fauna. The species *Pi. pacae* (Floch & Abonnenc, 1943) and *Evandromyia georgii* (Freitas & Barrett, 2002) stand out in Amazonas (ALVES *et al.*, 2011), as well as in Rondônia (OGAWA *et al.*, 2016). The species *Lu. almerioi, Lu. cruzi* (Mangabeira, 1938), *Lu. dispar* Martins & Silva, 1963, *Lu. forattinii* Galati *et al.*, 1985, *Lu. longipalpis* (Lutz & Neiva, 1912), *Ev. corumbaensis* (Galati, Nunes, Oshiro & Rego, 1989), *Mt. oliveirai* (Martins, Silva & Falcão, 1970), *Mi. peresi* (Mangabeira, 1942) and *Mi. quinquefer* (Dyar, 1929) stand out in Mato Grosso do Sul (GALATI *et al.*, 1997, 2003b, ALMEIDA *et al.*, 2019). In Minas Gerais, *Lu. cavernicola* (Costa Lima, 1932), *Lu. longipalpis, Lu. ischnacantha* Martins, Souza & Falcão, 1962, *Lu. ischyracantha* Martins, Falcão & Silva, 1962, *Lu. renei* (Martins, Falcão & Silva, 1957), *Mt. cipoensis* (Martins, Falcão & Silva, 1964), *Ev. edwardsi* (Mangabeira, 1941), *Ev. spelunca* Carvalho *et al.*, 2011, *Ev. tupynambai* (Mangabeira, 1942), *Pi. diamantinensis* (Barata *et al.*, 2012) and *Mt. oliveirai* have greater associations with caves (BARATA *et al.*, 2008; BARATA; APOLINÁRIO, 2012; CAMPOS *et al.*, 2017; CARVALHO *et al.*, 2013); while in the state of São Paulo, such species are *Ev. edwardsi* and *Lu. longipalpis* (GALATI *et al.*, 2010ab). Based on this evidence, one could infer that populations of the other species of these different genera may also fit the troglophilous condition.

The other genera and species shown in Table 1 are also believed to be able to live in caves, such as species of *Brumptomyia* and those of *Psathyromyia* Barretto, 1962. For the latter, this is especially true for those of the subgenus *Forattiniella* Vargas, 1978 [*Pa. abunaensis* (Martins, Falcão & Silva, 1965), *Pa. aragaoi* (Costa Lima, 1932), *Pa. brasiliensis* (Costa Lima, 1932), *Pa. campograndensis* Oliveira *et al.*, 2001, *Pa. lutziana* (Costa Lima, 1932) and *Pa. pascalei* (Coutinho & Barretto, 1940)], which commonly inhabit armadillo burrows. On the other hand, species of the genera *Expapillata* Galati, 1995, *Migonemyia* Galati, 1995, *Bichromomyia* Artemiev, 1991, *Nyssomyia* Barretto, 1962, *Psychodopygus* Mangabeira, 1941 and *Trichophoromyia* Barretto, 1962, whose immature forms develop in forest soil, would be expected to have accidental occurrence in caves, frequenting only the entrances.

Methods of collection, capture and conservation

Studies involving psychodids often do not adopt careful measures regarding the characterization of caves in relation to the presence of sunlight (photic, disphotic and aphotic zones) and regarding the ecological and evolutionary classification of the collected species (troglobites, troglophiles and trogloxenes). The luminosity reported in most studies is defined only by distance (meters) from the entrance (CARVALHO *et al.*, 2011a; OGAWA *et al.*, 2016; VILELA *et al.*, 2015). Thus, in this chapter, we chose to exclude from the analyses the terminologies used in characterizing the light zones of subterranean natural cavities and the evolutionary condition of the species. We believe that species that breed in an external environment (epigean) can be attracted, by the light of the collection traps, to the entrance (mouth) of caves or even to the aphotic zone in the hypogean environment, therefore, it is fundamental to control these variables with the applied collection methods.

The methods for collecting psychodids (Table 2, Figure 3) must consider the life cycle of these insects, which are

Table 2. Types and general aspects of traps that can be used to capture psychodids.

Trap (Author)	Operation	Source of attraction	Local	Objective	Specificity
Shannon (Shannon, 1939) [a]	Passive	Light/ Coloration/ Odor	P/ F	FS/ NI/ TS	General
Disney (Disney, 1966) [b]	Passive	Odor	F/ P	FS/ NI	Specific
CDC (Sudia & Chamberlain, 1962) [c]	Active	Light/ Coloration	I/ P/ F/ C	FS/ NI/ TS	General
New Jersey (Mulhern, 1942)	Active	Light	I/ P	FS/ TS	General
Falcão (Falcão, 1981)	Active	Light	I/ P/ F	FS/ NI/ TS	General
Damasceno (Damasceno, 1955)	Passive	Light/ Coloration	F	FS/ NI/ TS	Specific
Manual[d] or electric aspirator	Active/Passive	NA	I/ P/ F/ C	NI/ TS	General
Malaise (Malaise, 1937)	Passive	Coloration	F (open)	FS/ TS	General
Adhesive Card	Passive	Coloration	I/ P/ F	FS	General

I – intradomicile/ **P** – peridomicile/ **F** – forest area/ **C** – caves.
FS –fauna survey/ **NI** –natural infection (Phlebotominae) / **TS** – taxonomic studies.
Passive: without suction apparatus (e.g., fan).
Active: with suction apparatus.
NA: not applicable
[a]:modified, in black color, is very attractive to species of Phlebotominae that inhabit caves when installed close to them, (GALATI *et al.* 2001)
[b]: specific for Phlebotominae. [c]: already used to capture psychodids in cave environments. [d]: buccal aspirator is not recommended for use in caves.

holometabolous. Thus, all species included in the family go through the stages of egg, larva (four stages), pupa and winged adults. The immature forms are detritivorous and develop in different environments, including semiaquatic, terrestrial or aquatic. In the first case, they use bordering areas or else as a participant in the so-called threshold fauna, as happens with many Psychodinae (FORATTINI, 1973); the species of Bruchomyiinae, Trichomyiinae and Phlebotominae are terrestrial and those of Horaielinae, Sycoracinae and some of Psychodinae are exclusively aquatic (FORATTINI, 1973; CORDEIRO; WAGNER, 2018). The type of environment, temperature, humidity, oxygen level and decomposing organic matter, represented by plants, invertebrate animals such as terrestrial gastropods (VAILLANT, 1961) and animal excreta (ALENCAR *et al.*, 2011) or even the exoskeleton of dead insects, are basic requirements for the survival of immature forms and maintenance of the life cycle.

Studies with these groups generally look for adult forms, using different collection methods (Table 2, Figure 3), which will depend on the questions being addressed. Regardless of the objective of a proposed study, the choice of method is the first procedure to be adopted (MAROLI *et al.*, 1997).

It should be borne in mind that there is no standardized methodology for searching for psychodids in caves, even for adults. Light traps (Figures 3A, B and C), which can and have been used widely due to the ease of obtaining samples, should be used sparingly to avoid harming the population balance, bearing in mind that during periods of peak frequency in some caves, about 2,000 specimens of a single species can be collected in a single trap (GALATI, E.A.B. 2022, personal communication). In this way, the properly oriented use of these traps in the cave environment that allows the generation of information on diversity, seasonality, hourly rhythm and other parameters of ecological interest, must be encouraged.

The collection techniques of Malaise trap (interception) (Figure 3H) and adhesive card (Figure 3I), without interference from light focus, can be adapted for these purposes or even



Figure 3. Traps that can be used to capture psychodids. **A)** CDC trap (HP model) installed in a chicken coop; **B)** New Jersey trap installed in a peridomicile; **C)** Falcão trap installed in a peridomicile; **D)** Shannon trap installed in a forest area; **E)** Disney trap installed in a forest area; **F)** Damasceno trap installed in a forest area; **G)** active search in hole near a tree trunk; **H)** Malaise trap installed in a forest area; **I)** adhesive cart installed near rocks. Photos: A, D, I (Andrey J. Andrade); B (Cristian F. Souza); C (Wagner Fernandes); E (Renata A. Gama); F (Setor de Flebotomíneos, Laboratório de Diptera, Fiocruz/RJ); G (Heron Huerta); H (Lucas N. Perillo).

proposed new models for studies in caves. Buccal or electric aspirators present interference from the light focus of the individual collector (researcher) but allow the collection of insects on walls or the ground. However, the buccal aspirator (Figure 3G) is highly inadvisable in caves, due to the risk of ingesting pathogenic fungal spores and other particles. Nonetheless, it should be noted that collecting psychodids with manual or electric aspirators is not always easy when insects are perched on rocks, due to their use of claws and other adherent structures (GALATI, 2003b). An alternative to this methodology is the use of tubes, without aspiration, to directly capture insects at rest, including on vertebrates, as performed by Costa *et al.* (2021). Another method that can be used is human attractiveness, with due protection and authorization from specific ethics committees, by capturing insects when they land on body parts and, in the case of females, before they bite.

Immature forms of psychodids are not commonly collected, due to the difficulty in locating breeding sites (FORATTINI, 1973), which reflects the limited knowledge about them for the different described species. In caves, larvae of different instars may be associated with guano (ALVES *et al.*, 2011) or in small recesses (cavities) in cave walls (GALATI *et al.*, 2003b). Alves *et al.*, (2011) took standardized samples of 300 to 500g of guano from 12 collection points in the most distal portions of a cave. If collected sample is to be transported for later isolation of immature forms in the laboratory, the substrate must be kept in closed pots to maintain its moisture and prevent desiccation and death of immature forms.

Based on most of the studies carried out, regardless of the life stage collected, psychodids must be placed in tubes containing 70% alcohol until they are sent to institutions for identification to the level of genus or species, after clarification and permanent mounting on slides. Another possibility is to keep the insects dry (without 70% alcohol) and immobile (pressed between two pieces of soft paper, with a cotton layer on the bottom and another on top) inside the tube in which they will be packed to avoid breaking parts of the body (antennae, palps and legs), which are important for species identification.

It is recommended that specimens of the target group of this chapter that are collected by non-specialists be sent to laboratories of teaching and research institutions where specialized professionals are available, or be deposited in museums so that taxonomists of the group have access, thus expanding knowledge of this family in Brazilian caves.

Food source, natural breeding sites and behavior in natural subterranean caves

As previously highlighted, knowledge about the fauna of cave psychodids is incipient. However, considering some observations in the currently available literature, the use of these environments, mainly by members of the subfamily Phlebotominae, can be inferred based on the following evidence.

Caves have selective characteristics for the survival of several species, although they harbor a diverse fauna of vertebrates and invertebrates (TRAJANO; BICHUETTE, 2009). In general, the finding of some phlebotomine species in caves seems to be related to the presence of bats, rodents, birds, anurans, snakes and lizards (Figure 4), which can serve as a source of food (blood) for females (BARATA; APOLINÁRIO, 2012; COSTA *et al.*, 2021) or even as natural breeding grounds (ALVES *et al.*, 2011, COSTA, J.C.R. 2021, personal communication).

In addition, larvae could use decaying organic matter and some fungi as food. Therefore, vegetative material carried into caves, guano from colonies of bats, birds or even insects, as well as the excreta of other vertebrates, along with carcasses and invertebrate exoskeletons, could act as sources of food. Caves subject to flooding, either by flash floods or by increased levels of the water bodies with which the caves are associated, may experience an increase in population density, in the case of phlebotomines, from one to four months after the beginning of the rainy season cycle. It must also be considered that this period coincides with a rise in temperature, a factor that acts in accelerating the development of immature forms. Therefore, this reduction in the immature period and the increase in food availability could act in synergism, leading to an abrupt increase in the density of winged forms of phlebotomines (population explosion), as already observed in caves in the region of Corumbá, state of Mato Grosso do Sul (GALATI *et al.*, 1997).

Although little is known about the behavior of adult phlebotomines in caves, knowledge of the daily activity of these insects in this ecotope could clarify the period of the day with the greatest exposure of vertebrates, including humans, that live in or visit, respectively, these environments serving as food sources for females (CAMPOS *et al.*, 2017). In this sense, collections carried out by human attractiveness during 24 uninterrupted hours in front of the entrance to Gruta Pitangueiras in the municipality of Bonito (Mato Grosso do Sul), revealed peaks of attractiveness for *Lu. almerioi* in the morning and that this varied



Figure 4. Records of parasitism performed by specimens of the subfamily Phlebotominae in Brazilian caves. **A)** Specimens of *Sciopemyia sordellii* on a frog of the family Bufonidae in a cave located in Canaã dos Carajás, Pará; **B)** female of *Sciopemyia* sp. feeding on a frog of the species *Bokermannohyla martinsi* in a cave in Serra do Gandarela, Minas Gerais; **C)** specimens of phlebotomines on a frog of the genus *Rhinella* in an iron cave in Curionópolis, Pará; **D)** specimens of phlebotomines on a frog of the species *Pristimantis fenestratus* in an iron cave in Parauapebas, Pará; **E)** specimens of phlebotomines on an frog of the genus *Rhinella* in an iron cave in Curionópolis, Pará; **F)** specimens of phlebotomines near and on a lizard of the genus *Tropidurus* in a carbonate cave in Prudente de Morais, Minas Gerais; **G)** specimens of phlebotomines on a lizard of the species *Thecadactylus rapicauda* in an iron cave in Curionópolis, Pará. The yellow quadrant details female specimens with blood on the tail of a *T. rapicauda*; **H)** specimens of phlebotomines on a snake of the species *Epicrates assisi* (Jibóia-arco-íris-da-Caatinga/Caatinga Rainbow Boa) in a carbonate cave in the municipality of Jandaíra, Rio Grande do Norte. The yellow quadrant details specimens grouped and taking a blood meal; **I)** specimens of phlebotomines on an amphibian of the species *Rhaebo guttatus* in a cave in the region of Carajás, Pará, Brazil. Photos: A (Renata Andrade and collaborators); B (Júlio C.R. Costa); C, E-G, I (Robson de A. Zampaulo); D (Matheus H. Simões); H (Juan Carlos Vargas Mena).

according to the season of the year: in summer between 4 and 6h; in spring between 5 and 6h; in winter between 6 and 9h and in autumn between 9 and 10h, with females not being active or with little activity from 12 pm to 15h pm. In the municipality of Bodoquena, also in Mato Grosso do Sul, the period of greatest activity for this species in front of Gruta C.C.I was from 18 to 21h, indicating that local environmental variation may influence the behavior of *Lu. almerioi* populations (GALATI *et al.*, 2006). These studies reinforce the importance of external environmental conditions for the maintenance of phlebotomine communities in caves.

Some species are dominant in cave entrances and the diversity can differ from that of the external environment (GALATI; NUNES, 1999; GALATI *et al.*, 2003b, 2006; CARVALHO *et al.*, 2013), although, in general, the great majority of species recorded inside caves are also found in the surroundings, which suggests that they circulate in all these environments (CARVALHO *et al.*, 2013). On the other hand, some studies have shown that species richness may be slightly different from that of the external environment (CARVALHO *et al.*, 2013; GALATI; NUNES, 1999; GALATI *et al.*, 2003b, 2006). Thus, longitudinal studies are necessary to accurately confirm this information (CAMPOS *et al.*, 2017), in addition to active searches for these insects without the interference of artificial light (GALATI, E.A.B. 2022, personal communication, COSTA *et al.*, 2021). Longitudinal studies to investigate population seasonality require periods of two to three years, plus a greater range of caves in sampled areas, as they may vary due to exogenous factors (GALATI *et al.*, 2003b).

The use of collection and capture techniques that allow a more precise characterization of the distribution of phlebotomine populations by zones in relation to luminosity inside caves can lead to a better understanding of the biodiversity and ecological niches of the species and aid the planning of proposals for conservation. Campos *et al.* (2017) demonstrated that the rhythm of activity of phlebotomines differs among the interior, entrance and surroundings of cave areas due to differences in the presence of sunlight, which, according to the authors, would suppress the nocturnal flight activity of these insects. However, despite these investigations, little is known about phlebotomine distribution patterns (e.g., food source, habitat and breeding sites) in caves and their surroundings (CAMPOS *et al.*, 2020). Even less is known about other psychodids. Long-term academic studies, associated with consultation in environmental licensing processes, should be encouraged and disseminated.

According to Carvalho *et al.* (2013), caves could function as important geographic barriers and, despite the limitations and gaps in knowledge of the group, studies conducted in caves in Brazil in recent years have led to the description of new genera and species (ALVES *et al.*, 2011; CARVALHO *et al.*, 2011b, 2013; GALATI *et al.*, 2007; VILELA *et al.*, 2015), allowing bioecological assessments in these ecotopes (BARATA; APOLINÁRIO, 2012; CARVALHO *et al.*, 2013; COSTA *et al.*, 2021) and the detection of the presence of *Leishmania* DNA by molecular methods (CARVALHO *et al.*, 2017). Environmental degradation caused by anthropic actions is known to have a direct effect on the increase in cases of leishmaniasis worldwide (VALERO; URIARTE, 2020). However, this relationship remains unknown for caves. No studies have investigated in depth the presence of mammals as food sources for phlebotomines in caves and even less on how the different species, both vectors and potential hosts, would maintain the circulation of *Leishmania* in these environments. Research with this aim could actually lead to medium- and long-term predictions of the potential impacts of activities involving the removal of caves on the prevalence of these diseases in a given location.

Siller Jr. *et al.* (2021) published a case report of a patient from California who, after undertaking "adventure tourism" in a cave in Mexico, was diagnosed with *Leishmania mexicana* Biagi 1953, the species that causes cutaneous leishmaniasis. There has been no published scientific report of infection by any species of *Leishmania*, either among tourists or even speleologists, in caves of Brazil. However, informal reports of cases of cutaneous leishmaniasis among speleology practitioners are not rare (ZAMPAULO, R.A. 2022, personal communication). Rodents act as reservoirs for *Leishmania* and it is relatively common to observe broods of *Rhipidomys* inside ferruginous caves (COSTA, J.C.R. 2022, personal communication) and resident populations of mocós (*Kerodon rupestris*) at the entrances and surroundings of carbonate caves in northern Minas Gerais (COSTA, J.C.R. 2022, personal communication), Bahia, Goiás and Tocantins (ZAMPAULO, R.A. 2022, personal communication). Likewise, other mammals, such as "pacas" and bats, function as food sources for phlebotomines and as reservoirs of *Leishmania* (ALVES *et al.*, 2011, ROQUE; JANSEN, 2014), which are also reported in natural subterranean caves. Alternatively, phlebotomine fauna can be studied anticipating possible risks of infection, as reported for *Lu. dispar* in caves in Mato Grosso do Sul (ALMEIDA *et al.*, 2019) or for *Ny. antunesi* in caves in Rondônia (OGAWA *et al.*, 2016).

Thus, there is a need for more comprehensive and complete surveys regarding capture time (longitudinal studies) and the application of different methods (by light attraction, manual capture, collection by interception or adhesive cards), as well as better characterization of cave environments. Added to this, there is a need for investments in the training of professionals qualified to explore these environments and in the identification of species through taxonomic studies. The lack of knowledge about adequate ways of storing collected specimens and institutions where the identification of taxa can be carried out, as well as the limited availability of financial resources, can be bottlenecks in understanding the relationship between the family Psychodidae and Brazilian caves.

Identification key

There are no specific keys for identifying cave-associated fauna of Psychodidae. The key of Wagner and Ibánez-Bernal (2009) can be used for identification down to the subfamily level and that of Galati (2018) for the identification of adults of Phlebotominae. In addition, some basic bibliographies can be consulted for more general studies on the family, such as Forattini (1973), Rangel and Lainson (2003), and Conceição-Silva and Alves (2014).

Main Brazilian collections

The main Brazilian collections of non-phlebotomine psychodids are at the Museu de Zoologia da Universidade Estadual de Feira de Santana (MZFS), the Museu de Zoologia da Universidade de São Paulo (MZUSP), the entomological collections of the Instituto Nacional de Pesquisas da Amazônia (INPA) and of the Departamento de Zoologia da Universidade Federal do Paraná (DZUP). The collections of MZFS and MZUSP possess type material of the cave species *B. mineira* and *N. spinosus,* while INPA possesses that of *T. robusta* and *T. distincta*. Type specimens of *T. cavernicola* and *B. brasiliensis* are in foreign collections. For the subfamily Phlebotominae, more than 63% of the specimens collected in Brazil are deposited in reference collections in the country, such as those of the Faculdade de Saúde Pública da Universidade de São Paulo (FSP/USP), the Instituto Oswaldo Cruz (IOC), the Instituto René Rachou da Fundação Instituto Oswaldo Cruz (IRR/Fiocruz), the Instituto Evandro Chagas (IEC), the Insituto Butantan (IBUT) of MZUSP and INPA. Those that hold specimens collected and described from the cave environment are FSP/USP (*Lutzomyia almerioi*, *Lu. forattinii*, *Lu. elizabethrangelae* Vilela, Azevedo & Godoy, 2015, *Ed. piauiensis*, *Ev. corumbaensis* and *Micropygomyia petari* Galati, Marassá & Andrade 2003), IOC (*Lu. cavernicola*), IRR/Fiocruz (*Lu. renei*, *Lu. ischnacantha*, *Ev. spelunca*, *Martinsmyia reginae* Carvalho *et al.* 2010, *Micropygomyia acanthopharynx* (Martins, Falcão & Silva, 1962), *Mi. peresi*, *Pi. gruta* and *Pi. diamantinensis*) and INPA (*De. maruaga*). All these universities or research institutions are Brazilian centers of excellence for receiving arthropods, including psychodids, that may be collected for scientific purposes or associated with licensing.

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Robson de Almeida Zampaulo



Robson de Almeida Zampaulo



Darcy José dos Santos



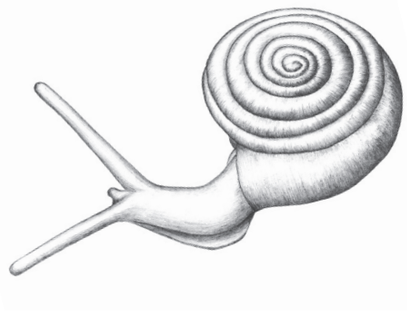


Leptinaria sp.
Lucas Mendes Rabelo

14

Gastropoda

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General introduction

The phylum Mollusca is the second most diverse group of living organisms, surpassed only by Arthropoda, insects in particular. Estimates range from 80,000 to 200,000 species, with predictions of over 1 million. Gastropoda (snails and slugs) is the most diverse class of Mollusca, comprising about 80% of the phylum, with representatives in all habitats, including freshwater and terrestrial environments. Mollusca has economic implications in many ways, ranging from food to disease transmission, to handicrafts, jewelry (pearls) and shell collecting. The shell is the most common protective structure among members of the phylum, and is the best known and most enduring, as it lasts far beyond the animal that manufactures it. The shell is produced by the outer layer of tissue called the mantle, which secretes calcium carbonate, responsible for hardness, in layers interspersed with conchiolin, a protein that provides elasticity and chemical resistance. A pearl, for example, has about 6% conchiolin. Molluscan taxonomy is very focused on shell characteristics, but internal details of anatomy and even DNA have been growing in importance and influencing taxonomic and phylogenetic studies.

Gastropods are relatively common findings in continental cave environments around the world, whereas freshwater bivalves are rarely observed. However, the first troglobitic bivalve species for the Americas was recently described: *Eupera troglobia* Simone & Ferreira (2022). Gastropods are usually at the base of ecological chains, mainly involved in the recycling of nutrients, especially calcium. In the continental environment (freshwater and terrestrial), most gastropods are herbivores or detritivores, although there are carnivorous taxa, such as the Streptaxidae, Scolodontidae and Spiraxidae, common land snails in Brazil. Of these, Scolodontidae is recurrent in caves.

Among the gastropods, several groups independently developed adaptations to the subterranean environment, as will be explained below. Therefore, this chapter clearly excludes animals from the epigean environment (e.g., SALVADOR *et al.*, 2017, 2021; SIMONE; CASATI, 2013). Gastropod shells and even live individuals are relatively common findings inside caves, but their presence is quite often just the result of leaching processes, which carry shells or whole animals into the cave. In this way, the records for many species are considered only as accidental.

For a species of snail to be considered adapted to the cave environment, some morphological (somatic) changes must have occurred that make it impossible to live outside this type of environment.

In general, troglobitic snails of Brazil can be divided into two groups: freshwater and terrestrial. Freshwater snails inhabit subterranean water bodies, sometimes in the most distal areas of these environments (deep within the cave and also within the water itself). Terrestrial snails usually inhabit the walls, sheltering in crevices and cracks, and on the floor, under blocks and associated with roots. Some species are also recurrent in areas without light (aphotic). The individuals of any of these groups are usually small, which, associated with the habit of hiding or even digging (fossorial), means that they are generally not seen by speleologists. Samples of cave snails that reach collections that have specialists are relatively rare and are generally collected as a by-product of some prospecting of sediment or of general microfauna. Thus, the currently described species of troglobitic or troglophilous gastropods represent only a small fraction of what actually exists in the country.

In general, caves are considered oligotrophic environments and organic matter penetrates these systems carried, continuously or temporarily, by physical and biological agents (CULVER, 1982; EDINGTON, 1984; FERREIRA; MARTINS, 1999; GNASPINI, 1989; HOWARTH, 1983). Among the physical agents, rivers, floods and different types of drainage that percolate through the roof or walls through existing openings or fractures, stand out (GIBERT *et al.*, 1994; JUBERTHIE; DEC 1994). Thus, the vast majority of cave snails are detritivores, which make up the base of the food chain and seem to be important in the cave ecosystem, at least as a source and recycler of proteins and micronutrients. In addition, some species associated with karst regions can be found inside caves (Figure 1), such as species

of the genera *Solaropsis* (Figure 1A), *Megalobulimus* (Figure 1B), *Happia* (Figures 1C, D), *Lamellaxis* (figures 1E, F), *Entodina* (Figure 1G) and *Leptinaria* (Figure 1H) (all images are of probable new species still under study), in addition to specimens of *Biomphalaria*, often found associated with karst lakes.

Main groups found in Brazilian caves

As previously explained, very little is known about the cave gastropod fauna of Brazil. Although large amounts of samples are deposited in scientific collections, the species still need to be studied and described (named). With this exception, the current panorama of the main taxa that have cave representatives is quite underestimated but, in general, includes species from the following groups.

In freshwater, members of the superfamily Truncatelloidea are almost invariably found, such as *Spiripockia* (Pomatiopsidae), *Heleobia* (Cochliopidae), *Potamolithus* (Tateidae) or *Littoridina* (Hydrobiidae), as well as of the superfamily Cyclophoroidea, such as *Habeastrum* (Diplommatinidae). All are tiny, between 2 and 3 mm, detritivorous snails, living in the bottom sediment, hidden near or under rocks.

In the terrestrial environment, almost all findings belong to the superorder Eupumonata, known as "pulmonados" (pulmonates), and to the order Stylommatophora, such as the genera *Lavajatus* (Subulinidae), *Habeas* (Urocoptidae) and *Gastrocopta* (Gastrocoptidae). They are detritivorous snails that, in general, occur near the entrance of caves, possibly dependent on allochthonous plant matter (Figure 2 and Table 1).

Main troglomorphisms

Species of the class Gastropoda, in general, can be divided mainly into two categories, troglobites and troglophiles, as follows:

TROGLOBITES

Troglobites are species restricted to the subterranean environment. In general, troglobitic gastropods have the following morphological characteristics (specializations):

1. Reduced size compared to congeners;
2. Absence or reduction of pigmentation, being generally translucent, white or light beige, both shells and soft parts.
3. Absence of, or at least significantly reduced, eyes, so that they are not detected in an external view of the active animal;
4. Shells generally with thin and translucent walls.

TROGLOPHILES

Troglophiles are species adapted to the subterranean environment (including caves) and capable of completing their life cycle in both epigean and hypogean environments. Thus, troglophilic snail species have some of the characteristics listed above, but not all. For example, the animal could be depigmented but possess eyes, or has no eyes but still has pigmentation. The combination of these features can, in general, be an important tool for determining those species that are unique to subterranean environments. However, it is considered important to compare their morphology with close epigean relatives, as well as perform external inventories (on the surface) to confirm the evolutionary ecological status of the species.

It is clear that this distinction is sometimes arbitrary, as some species or populations may still be in an intermediate phase of isolation, presenting clearly troglophilic populations, while others may be considered troglophilic, without, however, significant morphological modifications that allow the separation of species. In some cases, species of snails have been observed with individuals from the same population being fully morphologically adapted to the cave environment (troglobites), but with some scattered specimens still containing reduced eyes or only one eye (asymmetry). This demonstrates that the morphological characters are still being assimilated by the species, which apparently is still in the process of isolation in the subterranean environment

In general, the aforementioned adaptations to the cave

environment are basically due to the permanent absence of light, which generally excludes the need for species to have pigmentation and a visual guidance system (eyes). On the other hand, the reduction in size and thickness of the shell seem to be characters related to the scarcity of food in these environments.

Another characteristic of cave gastropods in Brazil is their high degree of endemism, that is, a given species is found in only a single cave or in a group of nearby caves (systems). In places rich in natural subterranean caves, such as Parque Estadual Turístico do Alto Ribeira (PETAR), in the state of São Paulo, which has a few hundred caves, there are endemic species to each cave and many are still being found and/or described.

Table 1. List of species of cave gastropods described for Brazil and their updated records.

Family **Diplommatinidae**
Genus ***Habeastrum*** Simone, 2019

1. ***Habeastrum omphalium*** Simone, 2019 (Figure 2B)
Classification: troglobite or troglophile, freshwater.
Location: Gruta Pitangueiras (21°06'37"S 56°34'52"W), Bonito, MS (endemic).
References: (SIMONE, 2019; SIMONE *et al.*, 2020).

2. ***Habeastrum parafusum*** Simone, 2019 (Figure 2A)
Classification: troglobite or troglophile, freshwater.
Location: Gruta Pitangueiras (21°06'37"S 56°34'52"W), Bonito, MS (endemic).
References: (SIMONE, 2019; SIMONE *et al.*, 2020).

3. ***Habeastrum strangei*** Simone, Cavallari & Salvador, 2020 (Figure 2C)
Classification: troglophile, freshwater.
Location: Lapa da Fazenda São Bernardo (18°16'37"S 46°06'46"W), Presidente Olegário, MG (type locality) and other caves of the central region of MG.
References: (SIMONE *et al.*, 2020).

Family **Tateidae**
Genus ***Potamolithus*** Pilsbry, 1896

1. ***Potamolithus karsticus*** Simone & Moracchioli, 1994 (Figure 2E)
Classification: troglophile, freshwater.
Location: Caverna Calcário Branco (24°30'20"S 48°44'25"W), PETAR, Apiaí, SP (endemic).
References: (SIMONE; MORACCHIOLI, 1994; SIMONE, 2006).

2. ***Potamolithus troglobius*** Simone & Moracchioli, 1994 (Figure 2D)
Classification: troglobite, freshwater.
Location: Caverna Areias, Rio Areias, PETAR, Iporanga, SP (endemic).
References: (SIMONE; MORACCHIOLI, 1994; SIMONE, 2006).

Family **Cochliopidae**
Genus ***Spiripockia*** Simone, 2012

1. ***Spiripockia punctata*** Simone, 2012 (Figure 2I)
Classification: troglobite, freshwater.
Location: Caverna Lapa dos Peixes (13°49'22"S 43°57'24"W), Rio São Francisco, Serra do Ramalho, BA (endemic).
References: (BIRCKOLZ *et al.,* 2016; SIMONE, 2012; SIMONE; SALVADOR, 2021).

2. ***Spiripockia umbraticola*** Simone & Salvador, 2021 (Figure 2G)
Classification: troglobite, freshwater.
Location: Gruna do Domingão (13°44'41"S 43°49'59"W), Serra do Ramalho, Carinhanha, BA (endemic).
References: (SIMONE; SALVADOR, 2021).

Family **Gastrocoptidae**
Genus ***Gastrocopta*** Wollaston, 1878

1. ***Gastrocopta sharae*** Salvador, Cavallari & Simone, 2017 (Figure 2H)
Classification: troglobite or troglophile, terrestrial.
Location: Gruta Revolucionários, Mambaí, GO (endemic).
References: (SALVADOR *et al.*, 2017).

Family **Subulinidae**
Genus ***Lavajatus*** Simone, 2018

1. ***Lavajatus moroi*** Simone, 2018 (Figure 2F)
Classification: troglophile, terrestrial.
Location: Caverna local (4°33'60.6"S 39°46'44.2"W), Santa Quitéria, CE (endemic).
References: (SIMONE, 2018).

Family **Urocoptidae**
Genus ***Habeas*** Simone, 2013

1. ***Habeas corpus*** Simone, 2013 (Figure 2J)
Classification: troglophile, terrestrial.
Location: Gruna Três Cobras (13°37'07.6"S 43°45'11.5"W), Serra do Ramalho, Carinhanha, BA (endemic).
References: (BIRCKOLZ *et al.,* 2016; SIMONE, 2013).

2. ***Habeas data*** Simone, 2013 (Figure 2L)
Classification: troglophile, terrestrial.
Location: Gruna do Cesário (13°31'06.1"S 43°38'26.2"W), Serra do Ramalho, Carinhanha, BA (endemic).
References: (BIRCKOLZ *et al.*, 2016; SIMONE, 2013).

3. ***Habeas priscus*** Simone, 2013 (Figure 2M)
Classification: troglophile or epigean, terrestrial.
Localização: Boqueirão do Maxixe (13°46'51.6"S 43°02'18.7"W), Central, BA (endemic).
References: (BIRCKOLZ *et al.,* 2016; SIMONE, 2013).

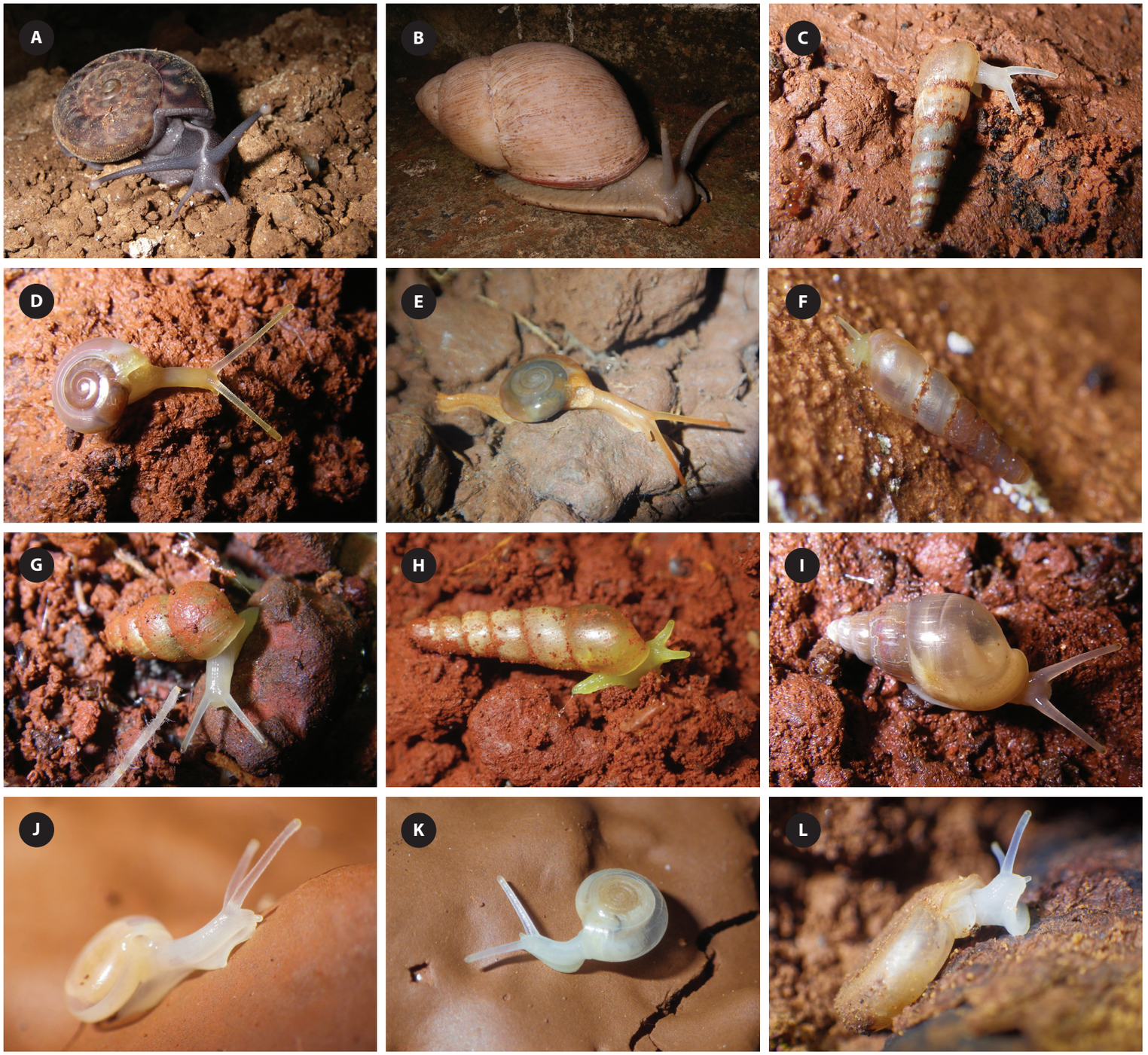


Figure 1. Specimens of Gastropoda found in Brazilian caves. **A)** *Solaropsis* sp. (Solaropsidae) - Carste de Lagoa Santa, MG; **B)** *Megalobulimus* sp. (Strophocheilidae) - Carste de Lagoa Santa, MG; **C)** *Lamellaxis* sp. (Subulinidae) - Curionópolis (PA); **D)** *Happia* sp. (Scolodontidae) Quadrilátero Ferrífero, MG; **E)** *Happia* sp. (Scolodontidae) - São Félix do Xingu (PA); **F)** *Lamellaxis* sp. (Subulinidae) - Montalvânia (MG); **G)** *Lamellaxis* sp. (Subulinidae) - Quadrilátero Ferrífero, MG; **H)** *Lamellaxis* sp. (Subulinidae) - Flona Carajás, PA; **I)** *Leptinaria* sp. (Subulinidae) - Curionópolis, PA; **J-K)** *Happia* sp. (Scolodontidae) - Carste de Iuiú, BA; **L)** *Entodina* sp. (Scolodontidae) - Serra do Cipó, MG. Shell length: A (~50 mm); B (~80 mm); C-L (~10-5 mm). Photos: A-D, G, I (Robson de A. Zampaulo); F, H, J-L (Lucas M. Rabelo); E (Matheus H. Simões).



Figure 2. Shells of Brazilian cave species described to date: **A)** *Habeastrum parafusum*, holotype MZSP 134301 (L 1.4 mm); **B)** *H. omphalium*, holotype MZSP 135583 (L 1.6 mm); **C)** *H. strangei*, holotype (MEV) MZSP 151626 (L 2.1 mm); **D)** *Potamolithus troglobius*, holotype MZSP 27947 (L 2 mm); **E)** *P. karsticus*, holotype MZSP 27943 (L 2 mm); **F)** *Lavajatus moroi*, holótipo MZSP 131060 (L 34.7 mm); **G)** *S. umbraticola*, holotype MZSP 151099 (L 5.2 mm); **H)** *Gastrocopta sharae*, holotype MZSP 122725 (L 1.9 mm); **I)** *Spiripockia punctata*, holotype MZSP 105000 (L 4.6 mm); **J)** *Habeas corpus*, holotype MZSP 110000 (L 10.3 mm); **K)** *H. data*, holotype MZSP 106810 (L 5.7 mm); **L)** *H. priscus*, holotype MZSP 103044 (L 4.6 mm).

Diversity of troglobitic species described for Brazil

Relatively few cave species of gastropods have been described thus far for the Brazilian territory, which are listed in Table 1. Of these, six species were considered potentially troglobitic (Figure 3). Although Salvador *et al.* (2022) list 18 exclusively cave-dwelling species (troglobitic), many of these species do not show troglomorphisms. However, it is certain that these species correspond to only a fraction of what actually exists. Much study is still needed for this list to reach a satisfactory and representative level of the malacofauna of this environment, thus it is extremely important that the material obtained by inventories in different regions of the country be destined to collections with specialists.

Table 1 shows that, so far, seven species of freshwater prosobranchs and five species of terrestrial pulmonates have been described for cave environments in Brazil that can be classified as troglobites or at least troglophiles. When there was doubt in this classification, the word "or" was introduced in the list, generally in the case of species only known by the shell.

Main methods of collection and conservation

The main method of collecting cave gastropods, and the most effective, is direct active search. In general, cave gastropods tend not to fall into traps and, due to their fragility, they do not resist scraping or dragging methods. In addition, for terrestrial organisms, it is effective to collect specimens associated with walls or under blocks, places that do not allow the installation of traps. It is generally necessary to use good lighting systems (flashlights), since many species are cryptic. Due to the fragility of the specimens, the ideal is not to collect them with tweezers or with fingers, but to use a delicate brush and push them into collection flasks.

In the case of freshwater animals, which live in the sediment, the direct visualization and collection technique is also the most common, but it is also possible to use sieves consistent with the size of the sediment and the animals. Sometimes, it is necessary to collect a portion of the sediment and sort it in the laboratory under a stereomicroscope (magnifying glass), separating the animals from the sediment with a brush.

Samples are ideally conserved in ethyl alcohol. An alcohol concentration of 96% can be used for 2 to 3 days, after which they are ideally transferred to 70% alcohol. Another important recommendation is to photograph animals that are still alive and active before fixing them, as the image can help determine external structures. For material to be deposited at the Museu de Zoologia da USP (MZUSP), for example, there is no need for any previous protocol, with sacrificing individuals directly in alcohol being sufficient. Some research centers require narcotization to relax specimens for further study. Another interesting action is to preserve a subsample in absolute alcohol to be kept in an ultrafreezer (when possible) or in a freezer, aiming at subsequent DNA sequencing.

Identification key

In general, dichotomous keys do not work very well for Gastropoda. For correct identification of groups and/or species, consultation with diagnoses is necessary, as well as detailed morphological studies or even molecular methods. Nonetheless, for those interested in starting to learn the taxonomy of the group, it is worth consulting Simone (2006) and Birckolz *et al.* (2016).

Main Brazilian collections

As seen in the list above, all references of studies focused on cave malacofauna had Simone as one of the authors. Naturally, all the material studied is deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP), especially the type specimens. Other large collections, such as the Museu Nacional da Universidade Federal do Rio de Janeiro (UFRJ) and the Universidade Federal de Juiz de Fora (UFJF), among others, which have a good sampling of non-marine molluscs, should also have cave malacofauna in their collections. However, such material is considered unpublished.

Acknowledgements

Most of the cave material available for study at MZUSP came from Maria Elina Bichuette of the Universidade Federal de São Carlos (UFSCar), who provided most of the discoveries in her research by the Laboratório de Estudos Subterrâneos (LES). Thanks go to Robson de Almeida Zampaulo, Lucas Mendes Rabelo and Matheus Henrique Simões for images of living specimens and help with the text.



Figure 3. Map with type localities of troglobitic species of Gastropoda described for Brazilian.

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Photos: Robson de Almeida Zampaulo









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Rodrigo Lopes Ferreira

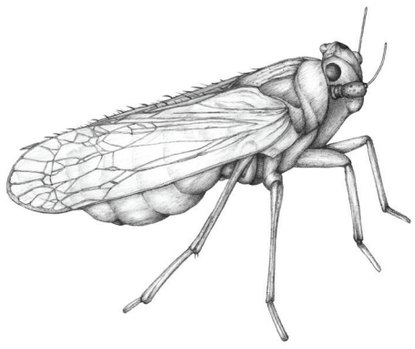
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Hemiptera: Auchenorrhyncha

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General introduction

The suborder Auchenorrhyncha was extracted from the old order Homoptera and, despite some conflicts, the latest proposed phylogenetic analyses (e.g., CRYAN; URBAN, 2012) suggest a single common ancestry (monophyly) for the group. Auchenorrhyncha is subdivided into two infraorders: Cicadomorpha and Fulgoromorpha. The latter is composed of a single extant superfamily (Fulgoroidea) and two extinct superfamilies (Coleoscytoidea, Surijokocixioidea) (BOURGOIN; SZWEDO, 2008), while the former is diverse and includes three superfamilies (Cercopoidea, Cicadoidea and Membracoidea) (DIETRICH, 2005). However, species capable of completing their entire life cycle in subterranean environments have never been reported for Cicadomorpha (HOCH, 2002). Therefore, in this chapter we will focus on Fulgoroidea, since it includes more than 60 obligate cave-dwelling species allocated in 27 genera and distributed in 22 different countries (Table 1). The species of Fulgoroidea capable of establishing viable populations and completing their entire life cycle in natural subterranean cavities are currently distributed in five families — Hypochthonellidae, Cixiidae, Delphacidae, Kinnaridae and Meenoplidae (HOCH, 2002) (Table 1).

Fulgoroids are commonly recognized as "planthoppers" due to their jumping habit, as also observed in crickets and grasshoppers. In Brazil, they are known as "cigarras" or "cigarrinhas". Several characters have been proposed to recognize fulgoroids and currently they can be identified mainly by the following: head with raised carinae separating the frons and gena; antennae below the compound eyes and antennae segmented into three parts — scape, pedicel and flagellum — with the pedicel being characteristic, larger than the scape, globular or cylindrical/elongated and with elevated sensilla; tegula at base of tegmina (in most taxa); and wings with pairs of anal veins forming a set of Y-shaped veins on the distal margin (in most taxa) (BAPTISTA, 2006; WILSON, 2005). The terminology and shape of the head and sensilla of the antennae were studied by Bourgoin (1985) and Bourgoin and Deiss (1994), respectively. Terminology of the veins and tegmina cells of Fulgoromorpha has been recently revised by Bourgoin *et al.* (2015). Bourgoin *et al.* (2015) also identified the main regions of the tegmina used in the description of Fulgoroidea (Figure 1C). Furthermore, they suggested that the position and number of cells are more relevant for identification than the number and position of the veins, and proposed the union of RP and MA veins — fork (RP+(MA)). Male genitalia have been studied by Fennah (1945a) and Bourgoin and Huang (1990), and female genitalia by Bourgoin (1993). O'Brien and Wilson (1985) and Carver *et al.* (1994) devoted themselves to the complete morphology of fulgoromorphs. A figure based on the most recent terminologies of structures of the head, thorax and tegmina associated with Fulgoroidea is provided in Figure 1.

Currently there is an exclusive database for Fulgoroidea, the *Fulgoromorpha Lists On the Web* (FLOW), where information is provided on the 35 families, 2,489 genera and 13,888 species (BOURGOIN, 2021). Some of these families have only been described from fossil records (e.g., Fulgoridiidae Handlirsch, 1939, Perforissidae Shcherbakov, 2007 and Neazoniidae Szwedo, 2007), most of which refer to the Cretaceous period (BOURGOIN, 2021; BOURGOIN; SZWEDO, 2008).

Baptista (2006) was the first to gather all articles with taxonomic information on Fulgoroidea for Brazil. The author demonstrated that 50% of the genera registered in the country also occur in other countries, although 75% of the registered species are endemic to Brazil. Based on articles published and indexed in Zoological Records, Baptista registered 627 species and 205 genera in 14 families distributed in Brazilian states and also accounted for the following for Brazil in the FLOW database: Acanaloniidae Amyot & Audinet-Serville, 1843, Achilidae Stål, 1866, Caliscelidae Emeljanov, 1999, Cixiidae Spinola, 1839, Delphacidae Leach, 1815, Derbidae Spinola, 1839, Dictyopharidae Spinola, 1839, Flatidae Spinola, 1839, Fulgoridae Latreille, 1807, Issidae Spinola, 1839, Kinnaridae Muir, 1925, Nogodinidae Melichar, 1807, Ricaniidae Amyot and Audinet-Serville, 1843, Tropiduchidae Stål, 1866 and Lalacidae † Hamilton, 1990 (BOURGOIN, 2021). Recently, Viegas (2019) recorded the occurrence of eight new species of the genus *Bebaiotes* Muir, 1923 (Achilixiidae) for the North Region of Brazil, reaching a total of 16 families of Fulgoroidea for the country.

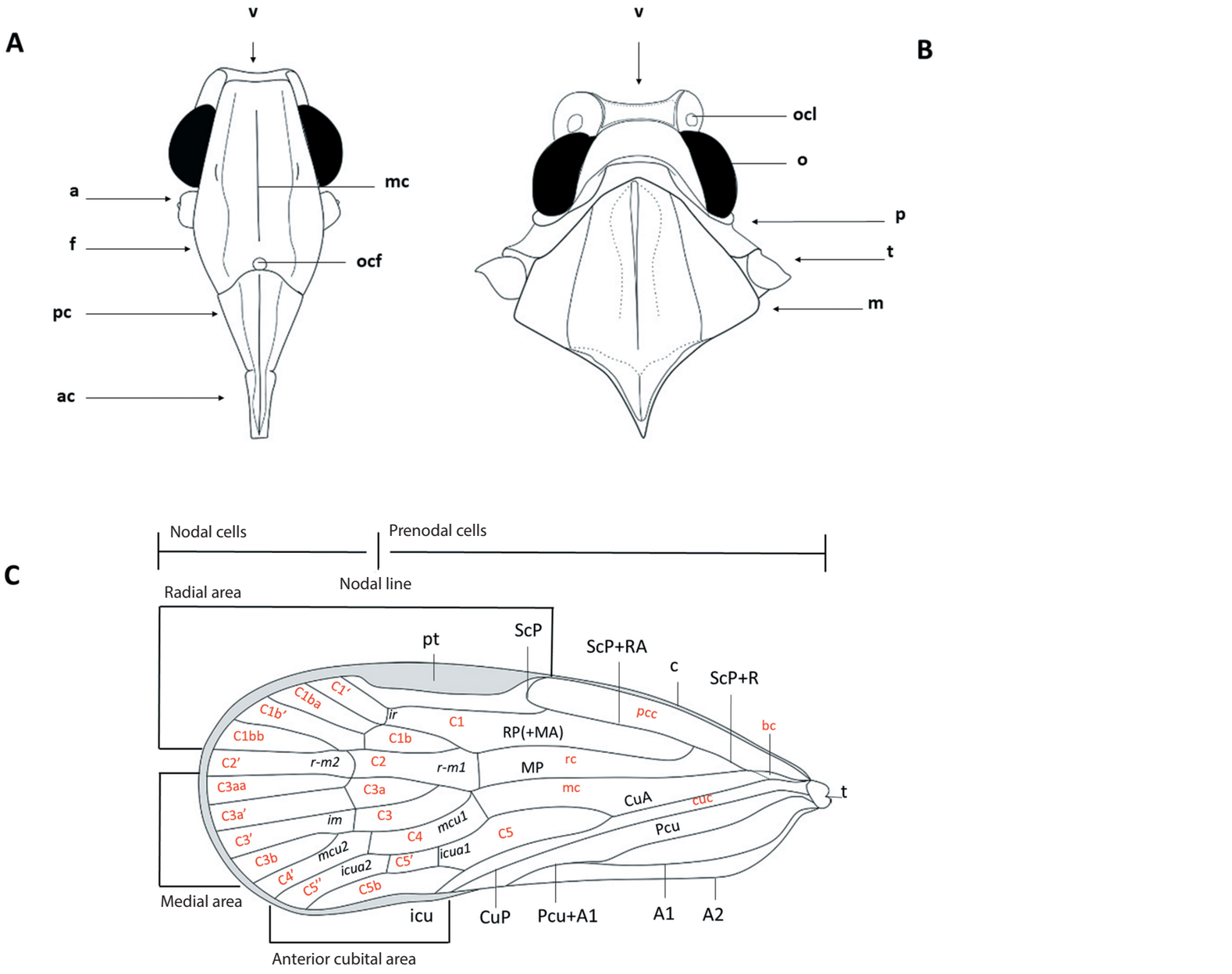


Figure 1. *Pintalia lateralis* Stål, 1862. **A)** Head in facial view, v = vertex; mc = median carina; a = antennae; f = frons; ocf = frontal ocellus (absent in some families); pc = postclypeus; ac = anteclypeus. **B)** Head in dorsal view, v = vertex; ocl = lateral ocellus; o = compound eye; p = pronotum; t = tegula; m = mesonotum. **C)** Cells in red and veins in black; t = tegula; bc = basal cell; ScP+R = posterior subcostal and radial veins united; c = costal margin; pcc = post-costal cell; ScP+RA = posterior subcostal and anterior radial veins united; pt = pterostigma; RP(+MA) = posterior radial and anterior medial veins united; MP = posterior medial vein; A1 and A2 = anal veins = vein in "y"; Pcu = post-cubital vein; CuP = posterior cubital vein; CuA = anterior cubital vein; icu = intercubital vein; rc = radial cell; mc = medial cell; cuc = cubital cell. Drawings: Júlio César do Carmo Vaz Santos.

Like the other members of Auchenorrhyncha, fulgoroids are hemimetabolous (CARVER *et al.*, 1994). Some species have a subterranean habit in the nymph stage while as adults, most of the time, they are associated with the green parts of plants (HOCH, 2002; WILSON, 2005). Due to their phytophagous (sap-sucking) feeding habits, consumption of angiosperms, gymnosperms, fungi, mosses and some pteridophytes has also been recorded for some species (O'BRIEN; WILSON, 1985; WILSON, 1994; WILSON, 2005). As for diet, species of planthopppers can be "monophagous", feeding on a single host species or genus; "oligophagous", feeding on up to two families of plants; or "polyphagous", feeding on plants from numerous families (NICKEL; REMANE, 2002; WILSON, 2005). Some families and species are economically important, such as Delphacidae - *Peregrinus maidis* Ashmead, 1890) (WILSON, 2005).

Although phytophagous and with a subterranean phase when immature (REMANE; HOCH, 1988), many species of

Table 1: Troglobitic genera of Fulgoroidea from around the world. Adapted from Hoch (2002) and Bourgoin (2021).

Family	Region	Genus	Reference
Hypochthonellidae	Zimbabwe	*Hypochthonella*	CHINA & FENNAH, 1952
Delphacidae	Nova Caledônia	*Notuchus*	FENNAH, 1980a; HOCH *et al.,* 2006
Kinnaridae	Mexico	*Oecidius*	FENNAH, 1973b
	Jamaica	*Oecidius*	FENNAH, 1980b
	Brasil	*Kinnapotiguara, Iuiuia*	HOCH & FERREIRA 2013, 2016
	Espanha	*Valenciolenda*	HOCH *et al.,* 2021
Meenoplidae	Australia	*Phaconeura*	FENNAH, 1973b; HOCH, 1990, HOCH, 1993
	Ilhas Canárias	*Meenoplus*	REMANE & HOCH, 1988; HOCH & ASCHE, 1993; HOCH *et al.,* 2012
	Samoa	*Suva*	HOCH & ASCHE, 1988
	Nova Caledônia	*Fennahsia*	HOCH, 1996
	Cabo Verde	*Nisia*	HOCH *et al.,* 1999
Cixiidae	Madagascar	*Typhlobrixia, Tsingya*	SYNAVE, 1953; HOCH, 2014; PERKINS *et al.,* 2015
	Mexico	*Cixius*	FENNAH, 1973b; HOCH, 1988
	Hawaii	*Oliarus*	FENNAH, 1973a; HOCH & HOWARTH, 1999
	Nova Zelândia	*Confuga*	FENNAH, 1975; SANTOS *et al.,* 2018
	Ilhas Canárias	*Cixius, Tachycixius*	REMANE & HOCH, 1988; HOCH & ASCHE, 1993
	Australia	*Solonaima, Undarana*	HOCH & HOWARTH, 1989a, 1989b
	Açores	*Cixius*	HOCH, 1991
	Argentina	*Notolathrus*	REMES LENICOV, 1992
	Galapagos	*Oliarus*	HOCH & IZQUIERDO, 1996
	Ilha da Reunião	*Brixia*	HOCH, 2003
	Italia	*Ibleocixius*	D'URSO & GRASSO, 2009; D'URSO & PUGLISI, 2019
	Indonésia	Celebenna	HOCH & WESSEL, 2011
	Brasil	*Ferricixius*	HOCH & FERREIRA, 2012
	Croácia	*Trirhacus*	HOCH, 2013
	Filipinas	*Bennaria*	HOCH, 2013
	Vietnã	*Sanghabenna*	HOCH & BOURGOIN, 2017
	[Baleares]		RACOVITZA, 1907 (unconfirmed data)
	[Nova Guiné]		Unpublished data

Fulgoroidea can remain in the nymphal habitat even after adulthood (HOWARTH, 2019). In this condition, nymphs and adults feed on roots of epigeal vegetation. Hoch (2002) indicated that this characteristic may be a possible ecological pre-adaptation that allows the colonization of different subterranean ecosystems, including caves. Currently, 62 species of Fulgoroidea from around the globe are considered troglobites. Among the families that harbor troglobitic species, Cixiidae stands out with the largest number of genera in this condition (16) (Table 1). Furthermore, all known troglobitic species belong to families that present a subterranean immature stage, suggesting that this feature is indeed an important evolutionary factor for the emergence of troglobitic species within Fulgoroidea.

The first report of a cavernicolous species of Fulgoroidea was that of Racovitza (1907), in the Balearic Islands (a Mediterranean archipelago close to the coast of Spain). Unfortunately, the specimens reported by Racovitza (1907) were not collected for further description. China and Fennah (1952) made the first official record of observed troglomorphisms in Fulgoroidea. The monotypic family Hypochthonellidae (CHINA; FENNAH, 1952) was erected to house a single soil species that feeds on tobacco roots in Zimbabwe. The first obligate cavernicolous species of Fulgoroidea, *Typhlobrixia namorokensis* Synave, 1953, was formally described and validated by Synave (1953) in Madagascar (HOCH, 2002), almost 50 years after the record of Racovitza (1907).

Historically, some authors have disputed the strictly subterranean *status* of some species of planthoppers, which was primarily based only on the morphological features they exhibited. As an example, based on morphology, Vandel (1965) and Howarth (1972) discussed the troglobitic *status* of the species *Typhlobrixia namorokensis* Synave, 1953. Recently, in a preliminary study, Perkins *et al.* (2015) analyzed vibrational communication ("singing"), adult morphology, as well as molecular data of *T. namorokensis* in Namoroka caves and concluded that, in fact, it is a troglobitic species. The use of singing is effective for determining troglobitic species of Fulgoroidea, since having enough food alone is insufficient for this existence. Other environmental and ecological conditions are needed to allow the evolution of the species in this environment (caves), including adaptations related to "singing" that allow communication between males and females, and even their reproduction, in the cave environment (PERKINS *et al.*, 2015). The authors also observed that the specimens only emitted vibrational signals when the humidity and temperature were high and low enough, respecitvely (ideal condition). In addition, the signal was simplified, as in other species of obligate cave-dwelling planthoppers (e.g., HOCH; WESSEL, 2006). Although preliminary, this study showed the importance of complementary behavioral studies as tools when listing species as endemic to subterranean environments.

Claridge (1985) and Claridge and Vrijer (1994) studied fulgoroid communication as a function of reproduction and speciation, suggesting that signals are efficient only over short distances and through a substrate or host plant. A field experiment carried out in a Hawaiian lava tube cave found that roots also proved to be an important tool for transmitting vibrational signals between individuals of *Oliarus* Stål, 1862, possibly being used for locating sexual partners in the interior of caves (HOCH; HOWARTH, 1993; HOCH, 2002). In addition, as previously mentioned, the main food resources that can be exploited by planthoppers in subterranean environments are roots from epigeal vegetation (which grow over caves) and possibly fungi and mosses. However, studies relating exclusively to the diet of cave-dwelling fulgoroids and dietary restrictions or preferences for roots of specific tree species were not found for Brazil.

Main groups found In Brazilian caves

Despite the history of biospeleological research in Brazil, it was only in the last decade that the first troglobitic fulgoroid was recorded for the country — *Ferricixius davidi* Hoch & Ferreira, 2012. Since then, two other species have been discovered in the country, *Kinnapotiguara troglobia* (Hoch & Ferreira, 2013) and *Iuiuia caeca* Hoch & Ferreira, 2016 (Figure 3), and Brazil became one of the three countries with the greatest diversity of genera of Fulgoroidea that are obligatorily cave-dwelling, alongside Australia and Spain (Canary Islands), all with at least three distinct genera (Table 1). Brazil is also the country with the greatest number of records of cave-dwelling genera of the family Kinnaridae in the world (2) (Table 1), even though this family is still little known in its territory, with only a single epigean species being described.

Although non-troglobitic fulgoroids are frequent in characterizations of cave fauna (e.g., FERREIRA *et al.*, 2009, 2010; SOUZA-SILVA; FERREIRA, 2009; SIMÕES *et al.*, 2013, 2015; SOUZA-SILVA *et al.*, 2011abc; PELLEGRINI *et al.*, 2016; PROUS *et al.*, 2004; ROCHA, 1996), works aimed at the description of troglophilous species (which can establish viable populations both inside and outside of caves) of this group are still scarce in Brazil. The Coleção de Invertebrados Subterrâneos de Lavras (ISLA), located at the Centro de Estudos em Biologia Subterrânea of

the Universidade Federal de Lavras (CEBS-UFLA) in Minas Gerais, currently has specimens of six families collected in caves in Brazil (Achilixiidae, Cixiidae, Kinnaridae, Derbidae, Delphacidae and Dictyopharidae) provisionally allocated in 19 genera (unpublished data). Among these, special attention should be paid to the family Cixiidae, due to its diversity of species, and to Achilliidae and Kinnaridae, due to the abundance of specimens sampled and the lack of taxonomic data on these families in the Brazilian territory.

CIXIIDAE Spinola, 1839

Cixiidae is the largest family of Fulgoroidea, having 2,583 species distributed in three subfamilies, 17 tribes, three subtribes and 247 genera (BOURGOIN, 2021). These taxa include still living and extinct representatives, with the oldest fossil referring to the Barremian (Cretaceous) [129.4 – 125 million years ago] (BOURGOIN, 2021). They occur across the globe (except polar areas), including Europe, Africa, Asia, Australasia, Pacific, North America and South America (BOURGOIN, 2021).

The taxonomy of Cixiidae has been further examined by Emeljanov (1989, 2002), Holzinger *et al.* (2002) and Ceotto and Bourgoin (2008). However, there are still issues to be clarified, such as its monophyly in relation to Delphacidae (ASCHE, 1987; BARTLETT, 2021; CEOTTO *et al.*, 2008; URBAN; CRYAN, 2007; URBAN *et al.*, 2010).

Although they are defined by plesiomorphic characters (characteristics shared with other families), cixiids can be recognized mainly by the median ocellus located on the frons, close to the frons-clypeus suture (character shared only with Kinnaridae); by the row of lateral spines (serrated) on the second posterior tarsomere; and by the generally long "orthopteroid"-type ovipositor (character shared only with Delphacidae). Due to the richness and abundance of recorded species, *Pintalia* Stål, 1862 (Figure 2 D-F) is possibly the most representative genus of Cixiidae found in caves in Brazil.

Pintalia is widely distributed throughout the country, with records in caves of 28 municipalities in Minas Gerais and Pará (material deposited at ISLA/CEBS-UFLA), in addition to the occurrence of the nine epigeal species described for Rio de Janeiro by Stål (1862) and another 10 described for the states of Pará, Rio de Janeiro and São Paulo, by Muir (1934a). The most recent diagnosis of the genus was elaborated by Kramer (1983), who indicated as diagnostic characters the shape and position of the carinae of the head, characters of the pronotum, the mesonotum, spines of the posterior tibias and the number of veins that reach the distal margin of the tegminae.

ACHILIXIIDAE Muir, 1923

The family Achilixiidae was proposed by Muir (1923b) to contain five species. The family currently comprises two genera, *Achilixius* (Old World) and *Bebaiotes* (New World), with only 24 species. Achilixiidae was allocated together with Achilidae and treated as two subfamilies by Emeljanov (1991) and, later, together with Cixiidae by Liang (1999, 2001). However, the phylogeny proposed by Urban and Cryan (2007) showed parity with Achilidae and the distancing of Cixiidae, which guarantees family treatment until further investigations are carried out. Treatment at the family level was reviewed by Fennah (1946) and Wilson (1989) and cataloged by Metcalf (1945) (BARLETT, 2021).

Achilixiids are easily recognized by the presence of one (gen. *Bebaiotes* – Figure 2 A-C) or two (gen. *Achilixius*) processes on the abdomen. Abdominal processes are also present in some cixiids (see: HOLZINGER; KUNZ, 2006), and so the monophyly of the family in relation to Cixiidae still generates uncertainty in some authors. Tegminae of Achilixiidae are strongly tectiform, transparent and markedly enlarged after the first MP bifurcation. The body is compressed (vs. depressed in Achilidae); the vertex is narrow laterally, mainly in *Bebaiotes*, where the lateral carinae are raised and continuous; and the antennae are simple and the ocelli distinct.

The genus *Bebaiotes* Muir, 1924 was initially proposed for only two species from Ecuador — *B. bucayensis* (type species) and *B. nigrigaster*. The genus currently contains 16 species, all epigeal, eight of which were recently recorded for the Amazon region in Brazil by Viegas (2019), where, until then, there had been no official record (GRAZIA *et al.*, 2012). For caves in the North Region of the country, the material deposited at ISLA/CEBS-UFLA draws attention due to a high abundance of individuals of two possible new species of the genus *Bebaiotes* obtained in caves in Tocantins and Pará.

KINNARIDAE Muir, 1925

Kinnaridae is a small family, with a total of 115 described species distributed in two subfamilies, six tribes and 25

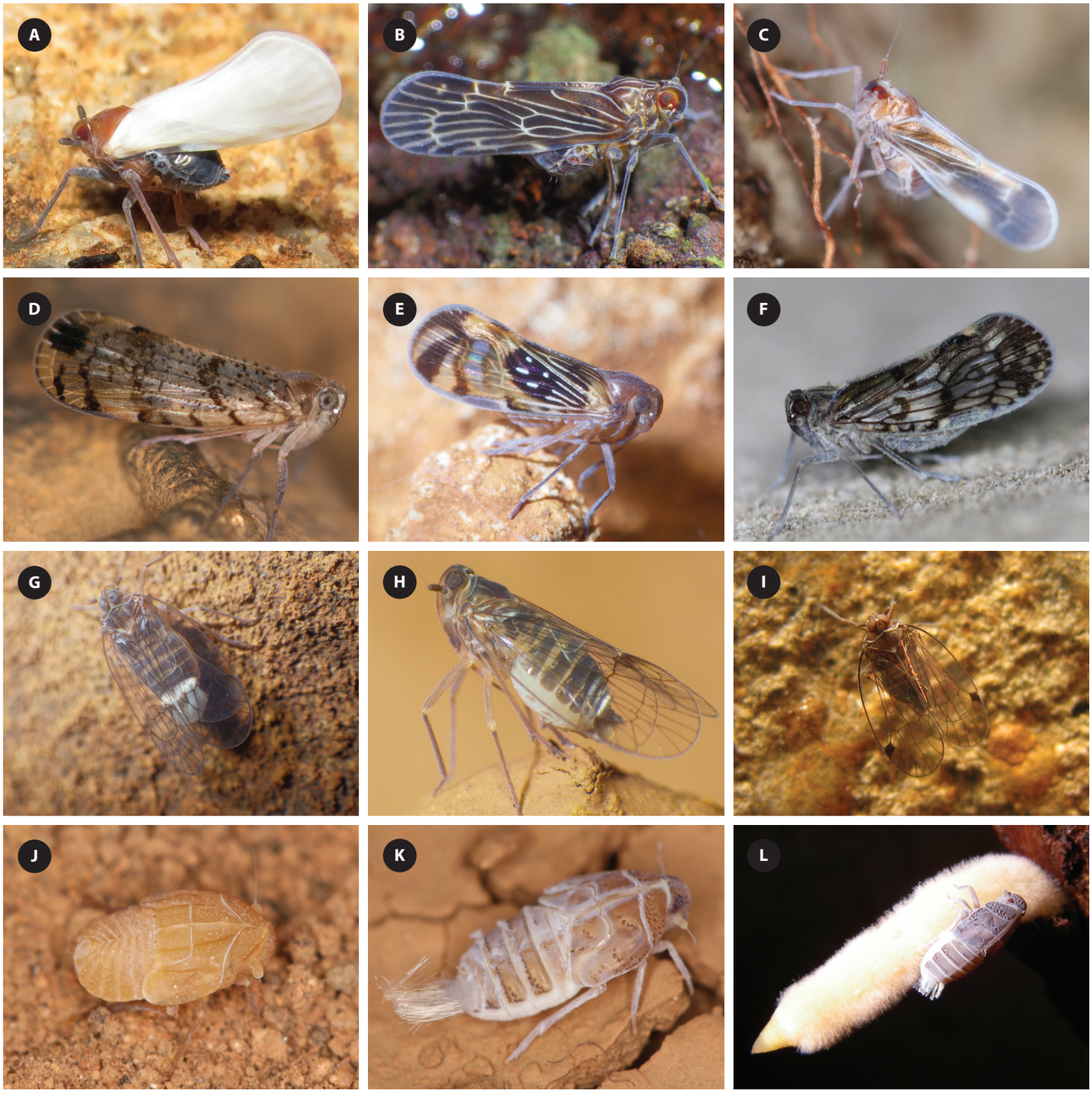


Figure 2. A-C) Species of Achilixiidae *Bebaiotes* sp. Muir, 1924; **D-F)** Species of Cixiidae *Pintalia* sp. Stål, 1862; **G-I)** Species of Kinnaridae sp. Muir, 1925; **J)** Kinnaridae sp. Muir, 1925 (nymph); **K-L)** Cixiidae sp. Spinola, 1839 (nymph). Photos: A, C, D, F-K (Rodrigo L. Ferreira); B (Marcus P. A. de Oliveira); E (Lucas M. Rabelo); L (Robson de A. Zampaulo).

genera (BOURGOIN, 2021). These taxa include living and extinct representatives, with the oldest record referring to the Oligocene [33.9 – 23.03 million years ago] (BOURGOIN, 2021). Many of these species are recorded for the New World, mainly for the Caribbean and North and Central America (HOCH; FERREIRA, 2013), in addition to three species for South America, two of which are troglobites.

Although very little is known about the biology and ecology of Kinnaridae, most known species are epigeal, exhibit well-developed compound eyes, vivid body and tegminae coloration, and have functional wings, being capable of flight (HOCH; FERREIRA, 2013).

Kinnarids can be confused with cixiids at first glance, since both can display lateral (serrated) spines on the second posterior tarsus and a median ocellus on the frons, close to the frons-clypeus suture (BARTLETT, 2021). However, female kinnarids have a smaller ovipositor and abdominal tergites (6–8) transformed into wax-producing plates and in the form of a "V" in some species (MUIR, 1925; LIANG, 2002). Still, the male genitalia essentially differs between families, most notably the aedeagus, which is generally small and bulbous in Kinnaridae (vs. elongated with spinal processes in Cixiidae).

Kinnarids are rarely reported in the literature for Brazil, with only one described epigean species, *Oeclidius paralellus* Muir, 1934, and two other troglobites, *Kinnapotiguara troglobia* (Hoch & Ferreira, 2013) and *Iuiuia caeca* Hoch & Ferreira, 2016. Among the material deposited in the collection of ISLA/CEBS/UFLA, are species of Kinnaridae with an epigeal habit (without apparent troglomorphisms), collected in caves in three Brazilian states (Minas Gerais, Bahia and Pará) (Figure 2 G-I). These species possibly belong to the genera *Southia* Kirkaldy, 1904, *Oeclidius* Van Duze, 1904 and *Iuiuia* Hoch & Ferreira, 2016, or even genera not yet described, the three of which draw attention due to the abundance and conditions of the caves where they are found.

Main troglomorphisms

There are currently three troglobitic species in Brazil: *Ferricixius davidi* Hoch & Ferreira, 2012, *Kinnapotiguara troglobia* (Hoch & Ferreira, 2013) and *Iuiuia caeca* Hoch & Ferreira, 2016 (Figure 3).

Although some behavioral and physiological factors can be interpreted as troglomorphisms (e.g., HOCH; HOWARTH, 1989ab; HOCH; WESSEL, 2006; HOWARTH, 1987), the vast majority of troglobitic planthoppers are described only on the basis of morphology. The morphological evolution of cave planthoppers in different parts of the world is characterized by reductive evolutionary trends of specific organs, suggesting a high degree of evolutionary parallelism among these species (WESSEL *et al.*, 2007). Although some taxa do not present all strongly-reduced troglomorphic characters, which makes it difficult to hypothesize the adaptation of some species to caves (see: Undarana Hoch & Howarth 1989), greater evidence can be obtained by the presence of specimens with intermediate troglomorphisms in the same area of study (adaptive shifts - HOWARTH, 2019) and relating estimates of genetic distance to evolutionary time (HOCH; HOWARTH, 1989a; NEI, 1972; NEI *et al.*, 1975).

Among the most common specializations in cave planthoppers are the reduction and/or loss of compound eyes, ocelli, tegminae, hindwings and body pigmentation (HOCH, 2002; HOCH *et al.*, 2021). The reduction of compound eyes was associated with a significant increase in vertex size (WESSEL *et al.*, 2007). The emergence of alternative specializations in non-troglomorphic characters may also occur. Modifications were observed in pre-tarsal claws of the hind tibiae of species of *Oliarus* and *Solonaima* in caves in Hawaii and Australia, respectively (HOCH, 2002). This character possibly confers greater adherence when walking on wet rocks inside the cave environment (HOCH, 2002; HOCH; HOWARTH, 1999; HOWARTH, 1983). Another modification observed in some species of troglobitic planthoppers is wax bristles on the tegminae, which possibly provide protection against disturbances, humidity and/or predators in the subterranean environment (HOCH, 2002; HOCH *et al.*, 2021; HOCH; FERREIRA, 2013).

Diversity of troglobitic species described for Brazil

Ferricixius davidi Hoch & Ferreira, 2012

Ferricixius davidi (Figure 4) is a troglobitic planthopper species of the family Cixiidae, with occurrence restricted to a single iron cave in Itabirito, Minas Gerais. It was the first species of troglobitic planthopper described for the country. It has a reduced size (ca. 3–4 mm in body length) and strongly troglomorphic morphology: compound eyes and ocelli absent, extremely short tegminae that almost do not reach the base

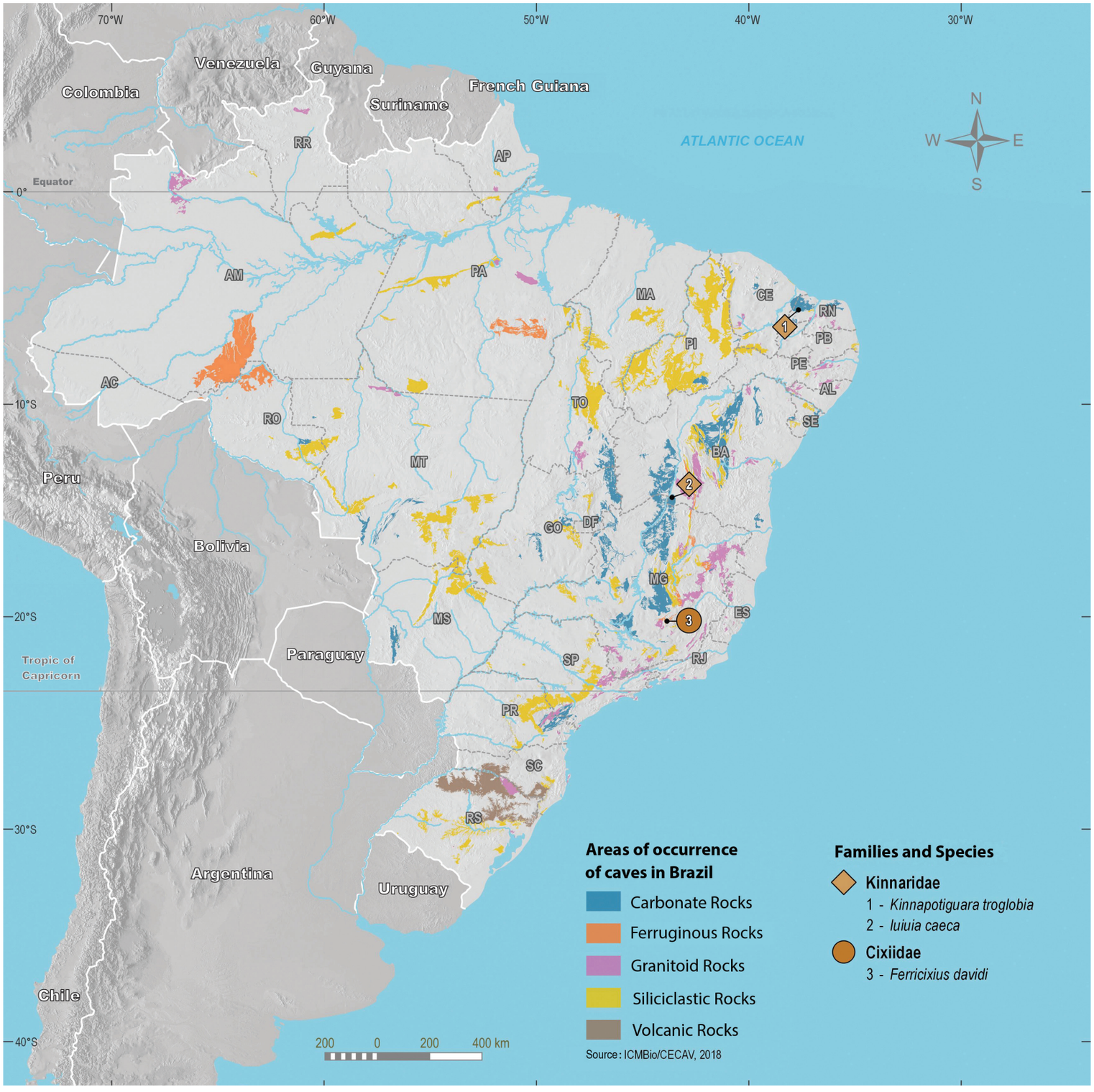


Figure 3. Map with the type localities of species of troglobitic hemipterans (Auchenorrhyncha) described for Brazil.

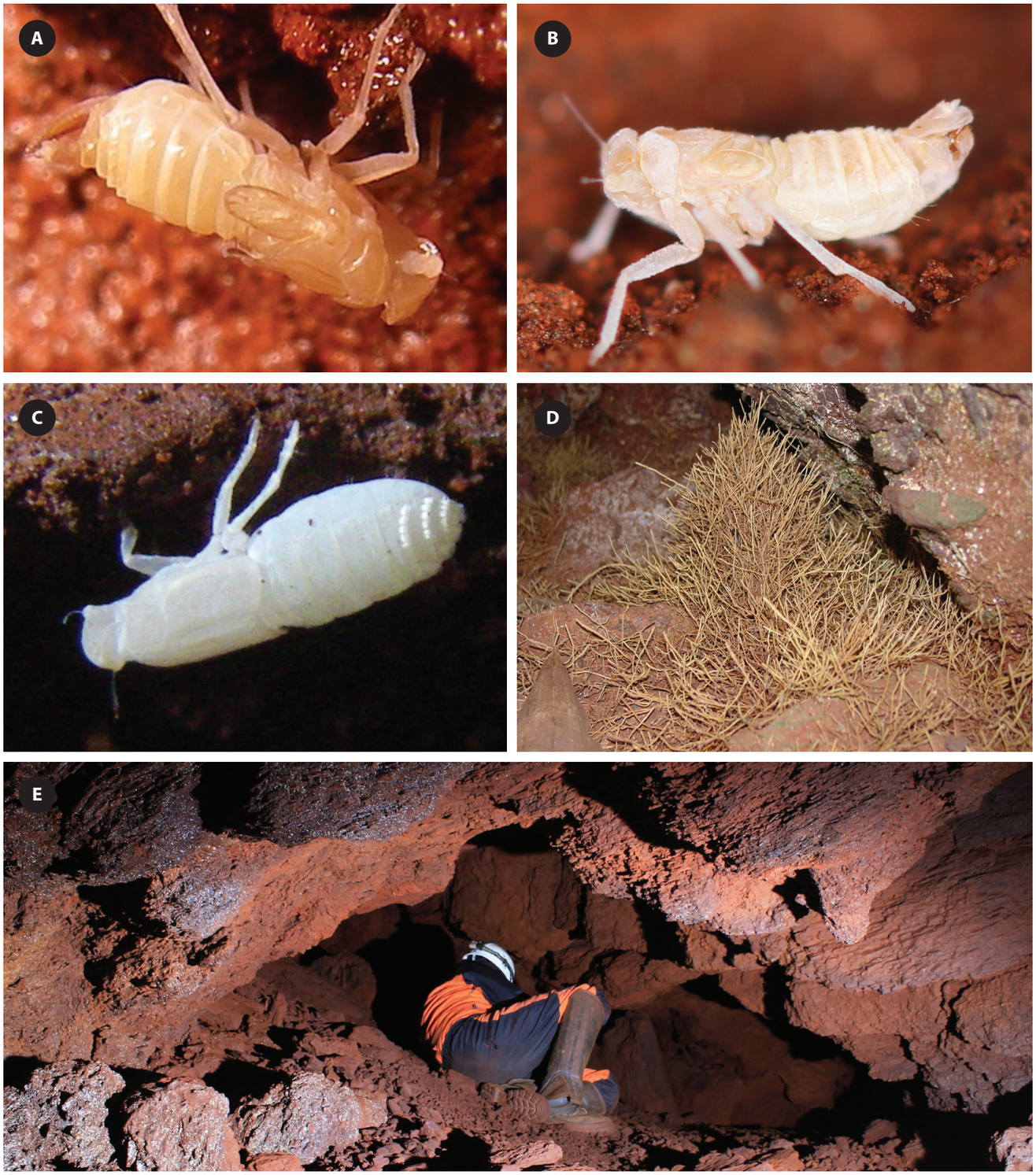


Figure 4. A) *Ferricixius davidi* Hoch & Ferreira, 2012. Female in dorsolateral view; **B)** Male in lateral view; **C)** Nymph in dorsolateral view; **D)** Roots in cave MP-008; **E)** Collection record by the method of active search in cave MP-008. Photos: A and C (Marcus P. A. de Oliveira); B and D (Rodrigo L. Ferreira); E (Robson de A. Zampaulo).

of the abdomen, vestigial wings, and extremely reduced body pigmentation. The species has no close relatives in the surface fauna, therefore being a relict species. However, in view of the high degree of lack of knowledge about the epigean fauna of Cixiidae in the Neotropical region, it cannot be excluded that close epigean relatives of *F. davidi* may still be discovered.

The cave where the specimens were collected (cave MP-008, UTM 23K 619771/7764742N) is located in the Quadrilátero Ferrífero (Iron Quadrangle), more specifically in the area of a mining project called "Mina do Pico", in allusion to the presence of Pico do Itabirito, a geographical and historical reference of the region. The caves associated with the iron formation in the Quadrilátero Ferrífero region are part of different lithotypes and may be associated with superficial ferruginous breccias (canga formation) or directly with iron ore (hematite and itabirite). Thousands of caves are known throughout the region and most of them were sampled during environmental licensing studies carried out in the last decade. However, this species has never been found in other caves, even those located very close to cave MP-008, which demonstrates its extreme rarity and endemism.

Cave MP-008 is considerably large for the iron formation (about 120 meters long), especially when compared to other caves in the region, which are generally less than 50 meters long. Furthermore, like other caves associated with this lithology, cave MP-008 is connected to a huge network of small spaces (channels), normally found in canga formation, which considerably increases the availability of habitats for subterranean fauna (FERREIRA, 2005; SOUZA-SILVA *et al.*, 2011a). However, even with this expressive connectivity to other subterranean habitats, the species is thus far known exclusively from cave MP-008.

The description of the species included a total of nine individuals, all of them collected between 2005 and 2012, both at the end of the dry period (September 2005) and at the end of the rainy season (March 2012). However, a greater number of specimens was observed in the rainy seasons, which suggests that they are more abundant in the macrocave during these periods, although additional studies are essential to assertively define the population dynamics of the species.

Cave MP-008 is located at an altitude of 1,550 meters, considerably above the heights of most caves in the Quadrilátero Ferrífero. The importance of altitude in the evolution of some troglobitic taxa has already been highlighted in previous studies (FERREIRA, 2005; PICKER; SAMWAYS, 1996; SOUZA-SILVA *et al.*, 2011a). Epigean environmental conditions at these altitudes may be more severe, due to greater temperature fluctuations (daily and annually) and more intense winds (causing faster desiccation), than observed at lower altitudes (FERREIRA, 2005). Such conditions may have favored the isolation of subterranean populations, contributing to the speciation of *F. davidi* and also to its (apparent) restriction to a single cave.

Cave MP-008 has many roots, which constitute the food resource of *F. davidi*. However, the diversity of surface vegetation in the area is high and it has not yet been determined whether the species has a specific host or a more diversified diet.

The region surrounding cave MP-008 has been significantly altered due to the expansion of mining activities (Figure 5). In view of the high degree of endemism of *F. davidi*, it is possible that conservation of the vegetation in the immediate surroundings of the cave may be sufficient for the maintenance of the species. However, it is essential that monitoring be carried out to verify the condition of this population, especially considering other impacts resulting from mining activities, such as the emission of particulates that can sediment inside the cave, modifying microhabitats and impairing root growth. In addition, the dismantling of rocks with the use of explosives or the movement of machines around the cave can, eventually, change the life cycle of the species, remembering that leafhoppers depend on acoustic signals (at different frequencies) for communication, especially for reproduction.

Kinnapotiguara troglobia (Hoch & Ferreira, 2013)

Kinnapotiguara troglobia is a species of troglobitic planthopper of the family Kinnaridae, occurring in several limestone caves located in the municipalities of Felipe Guerra and Governador Dix-Sept Rosado, in western Rio Grande do Norte State (BENTO *et al.*, 2021; HOCH; FERREIRA, 2013). It is a small kinnarid (ca. 3mm in body length) and strongly troglomorphic, with compound eyes and ocelli absent, reduced tegminae, vestigial wings and reduced body pigmentation (Figure 6).

According to the subdivision of Kinnaridae into the subfamilies Kinnarinae and Prosotropinae (FENNAH, 1945b), *K. troglobia* belongs to the former, which is characterized by the presence of wax-bearing plates on the sixth, seventh and eighth abdominal tergites of females (HOCH; FERREIRA, 2013).



Figure 5. Location of cave MP-008, municipality of Itabirito, Minas Gerais. Images obtained from Google Earth. **A)** 16 September 2010; **B)** 25 May 2011; **C)** 14 July 2013; **D)** 19 August 2020.

Although it presents some characteristics that would allow its inclusion in the genus *Oeclidius*, according to the key to New World genera proposed by Fennah (1980), some of the characters used are troglomorphisms (impossibility of flight, reduction of compound eyes and tegminae) and may be the product of convergent evolution. Thus, a new genus was established to accommodate the new species.

The genus *Kinnapotiguara* can be distinguished from all other genera of Kinnaridae by the unique combination of the following characters: bilaterally symmetrical male genitalia; genital segment on the caudal aspect rounded; anal segment on each side with two prominent processes; genital styles narrow proximally and distinct distally with three conspicuous processes; aedeagus bulbous at the base and compressed distally; and phallotrema exposed dorso-caudally (HOCH; FERREIRA, 2013).

As of now, the species has been recorded in 23 caves spread over an area of about 50 km^2 in western Rio Grande do Norte State, crossed by the main river in the region, the Apodi-Mossoró river, with 18 caves on the left bank (west) and five on the right bank (east) (Figure 7). Populations seem to be well established and individuals (males, females and nymphs) are observed throughout the year in caves that have areas that are always humid and with aggregations of roots. This, however, does not seem to be a pattern, since in some caves (mainly those that do not present great microclimatic stability) individuals are only observed in the rainy season and in others the abundance is greater in the dry season (BENTO *et al.*, 2016). Thus, it is possible that there is a vertical migration of the species from caves to other habitats (such as mesocavities not accessible to humans) and vice versa, according to environmental conditions (HOCH; FERREIRA, 2013).

The largest populations of *K. troglobia* were observed in Troglóbios cave (the type locality), in Felipe Guerra, and in Boca de Peixe cave, in GDS Rosado (25 and 17 individuals, respectively). Between one and seven individuals are usually observed in other caves, usually under rocks or walking on wet sediment, always associated with aggregations of roots (HOCH; FERREIRA, 2013). More recently, a large concentration of nymphs of different stages was observed in a small, rather shallow cave in the municipality of Felipe Guerra (BENTO, D.M., unpublished data).

With regard to diet and specificity of the plant-host association, ongoing studies (through DNA sequencing of root samples in the areas where individuals were found, and of

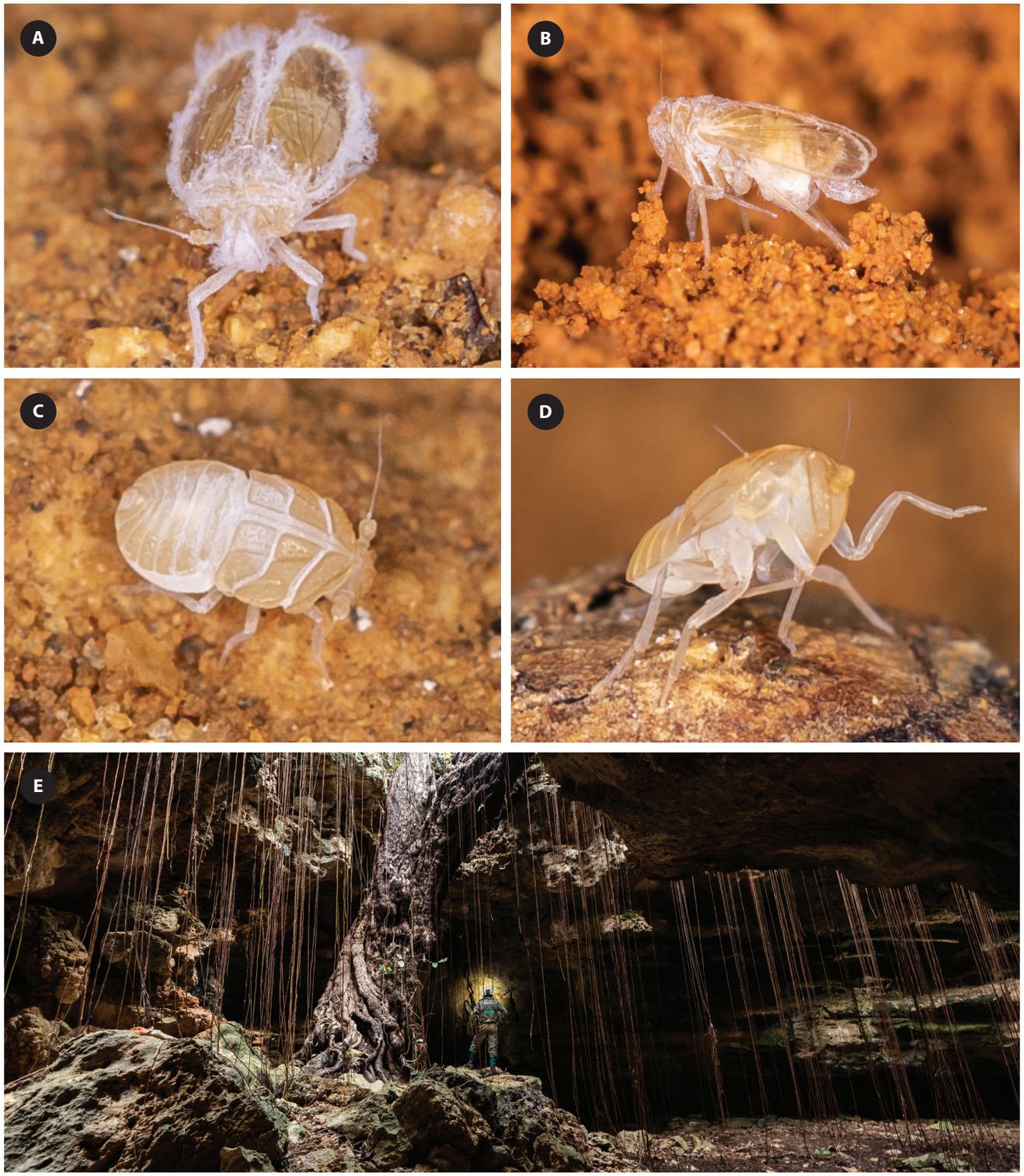


Figure 6. A) *Kinnapotiguara troglóbia* (Hoch & Ferreira, 2013). Male in frontodorsal view; **B)** Male in lateral view; **C)** Nymph in dorsal view; **D)** Nymph in frontolateral view; **E)** Caverna dos Crotes, Felipe Guerra, RN. Photo: (Diego de M. Bento).

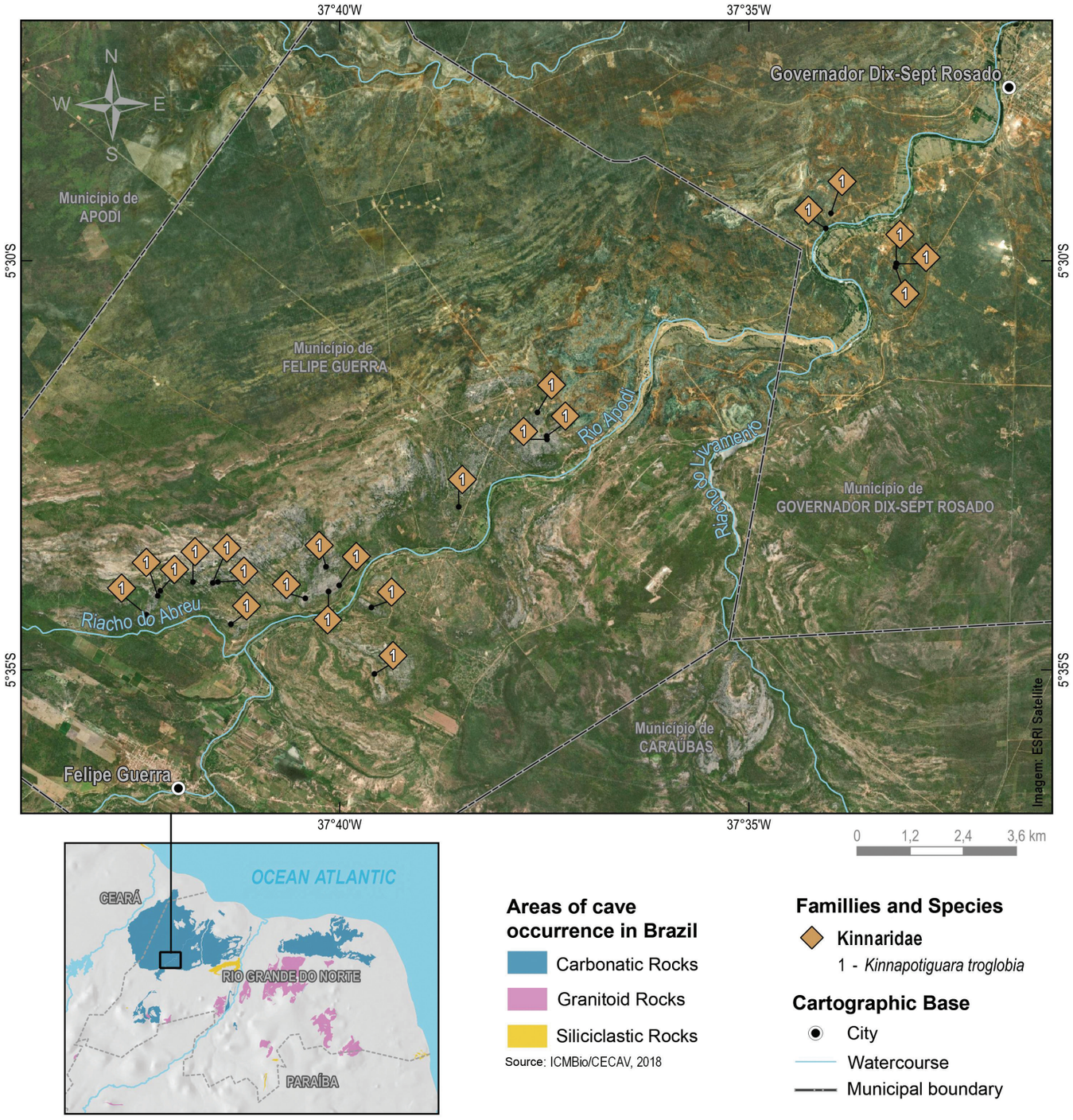


Figure 7. Distribution of *Kinnapotiguara troglobia* (Hoch & Ferreira, 2013).

leaves of plants in the vicinity of cave entrances) indicate that *K. troglobia* is probably a polyphagous species, feeding on sap from the roots of plants belonging to different families (BENTO, D.M., unpublished data).

Kinnapotiguara troglobia has a relatively wide distribution for a troglobitic species and there are populations in caves on both sides of the Apodi-Mossoró river (a possible biogeographical barrier). In view of this distribution, and the identification of slight variation in male genital morphology between specimens from different caves, Hoch and Ferreira (2013) suggested carrying out specific studies (genetic, for example) to assess whether these different populations of *K. troglobia* can represent, in fact, a complex of different species that are very similar morphologically.

In fact, ongoing molecular phylogeography studies indicate that *K. troglobia* may indeed be a species complex (BENTO, 2021). As speculated by Hoch and Ferreira (2013), the probable existence of a system of mesocavities should allow the migration of individuals between different caves, but gene flow is probably maintained only between populations of caves in the same limestone outcrop (BENTO, 2021).

Most caves where *K. troglobia* occurs are well preserved, and although some are used for tourist visitation, visitation is sporadic and of low intensity (CRUZ *et al.*, 2010; FERREIRA *et al.*, 2010; HOCH; FERREIRA, 2013). However, there are records of impacts related to limestone extraction and oil exploration in the known distribution area for the species (CRUZ *et al.*, 2010, FERREIRA *et al.*, 2010). In this context, the impacts related to the lime industry are particularly worrying, as the frequent use of native vegetation as fuel (firewood) for operating calcination kilns can indirectly affect the underground environment, and the extraction of limestone can lead to the destruction of epikarst and impacts on associated fauna, even up to the removal of caves (FERREIRA *et al.*, 2010, HOCH; FERREIRA, 2013).

Such impacts, although localized, may even lead to the extinction of genetically isolated lineages of *K. troglobia*, which may constitute new species. In this sense, it is advisable to establish a conservation unit to guarantee the preservation of caves in the western region of Rio Grande do Norte, which are home not only to *K. troglobia*, but also to several other species of troglobitic invertebrates, some of which are relicts (BENTO *et al.*, 2021; FERREIRA *et al.*, 2010; FIŠĚR *et al.*, 2013; HOCH; FERREIRA, 2013; SOUZA *et al.*, 2018).

Iuiuia caeca Hoch & Ferreira, 2016

Iuiuia caeca is a species of troglobitic planthopper also belonging to the family Kinnaridae, occurring in two caves located in the municipalities of Iuiú and Malhada, state of Bahia. It is a small kinnarid (ca. 2.8 mm in body length) and strongly troglomorphic, with compound eyes absent, reduced tegminae, vestigial wings, and reduced body pigmentation (Figure 8). *Iuiuia* can be distinguished from all other genera in the family by the unique combination of the following characters: vertex wide and short; male genitalia with genital segment on caudal face approximating the shape of an "8"; anal segment short, with a distinct wing-like compressed process ventrally on each side; genital styles slender and narrow, converging medially; aedeagus robust and tubular; and periandrium with two large lateral lobes.

The species was originally described from the Lapa do Baixão cave, located in the municipality of Iuiú, state of Bahia. The external vegetation of the area corresponds to the Caatinga formation. Lapa do Baixão has not been fully explored, as part of its galleries become submerged in the rainy season; however, the known passages extend for more than 500 meters. The only known entrance is relatively small, which determines great stability to the cave's atmosphere. Although the original description mentions that the species is apparently endemic to Lapa do Baixão, a second population was later found in the Tapera d'Água cave, located in the municipality of Malhada, also in Bahia. It is noteworthy that both caves, although located in different municipalities, belong to the karst set known as Serra de Iuiú, which represents an enormous continuous carbonate outcrop. Thus, it is plausible to assume that the species can still be found in other caves in the region (even if occasionally), especially in those located in the region between the two caves where populations were observed.

Both caves where the species occurs have many roots, which are, however, much more abundant in Lapa do Baixão (Figure 8-E). Unfortunately, it has not yet been possible to associate the roots with any species of epigean vegetation but considering the distance between the surface and the caves (approximately 30 meters), it seems likely that these roots belong to trees with pivoting root systems, capable of penetrating deeply into crevices in the ground and rock until they reach cave chambers. These roots are home to a variety of invertebrate species that feed especially on their decaying parts. However, many non-

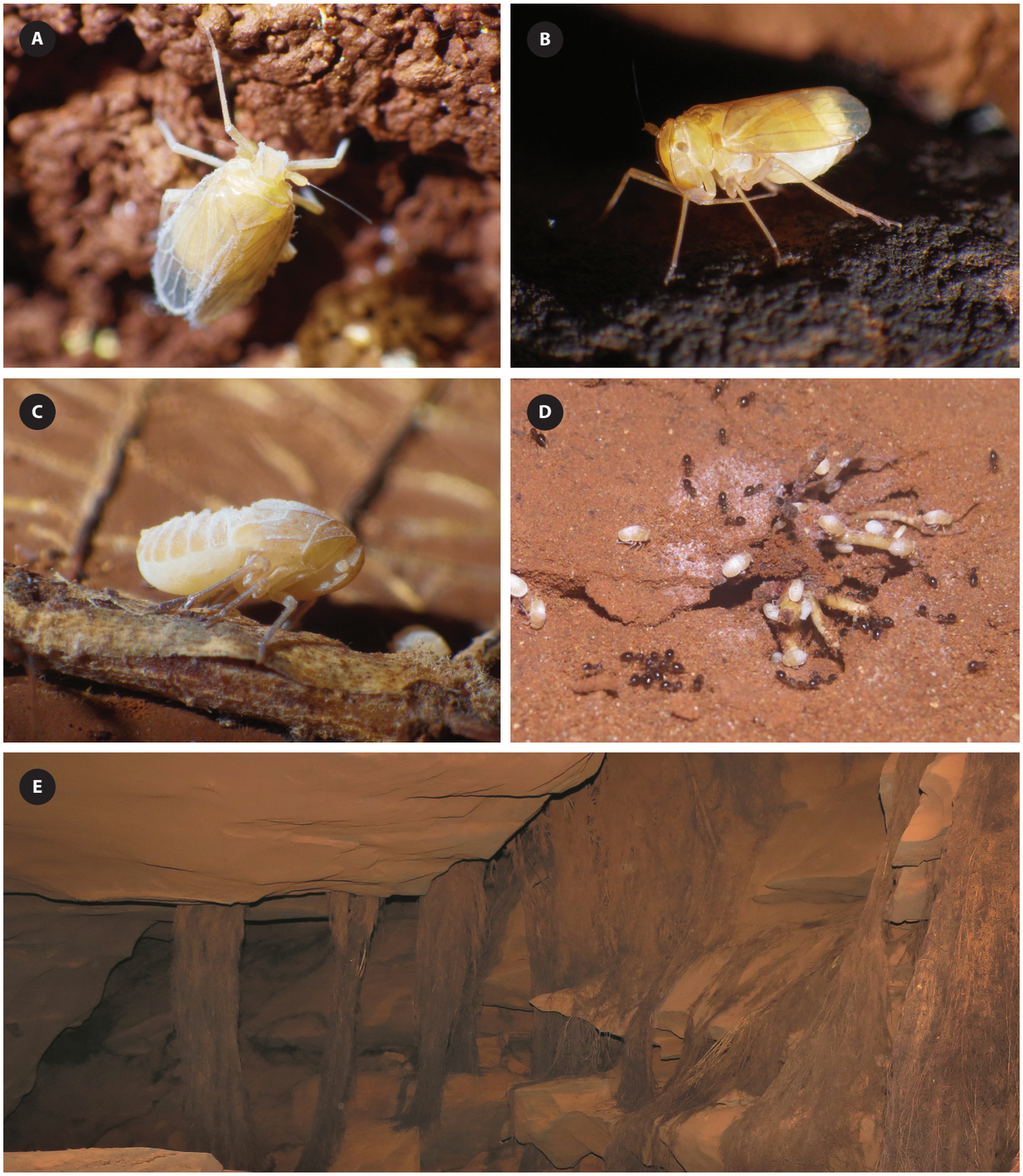


Figure 8. A) *Iuiuia caeca* Hoch & Ferreira, 2016. Female in dorsal view; **B)** Female in lateral view; **C)** Nymph in lateral; view **D)** Nymphs associated with roots; **E)** Interior of Lapa do Baixão cave. Photos: A, B and C (Lucas M. Rabelo); D and E (Rodrigo L. Ferreira).

troglomorphic cixiids (*Pintalia* spp.) were also observed feeding on roots, especially those located closer to the entrance (but also in aphotic zones). Specimens of *I. caeca* were preferentially observed in roots present in the deep zone of the caves, rarely having been observed in the same roots where the non-troglomorphic cixiids occur. Most of the specimens seen on a visit to these caves were nymphs, which are often concentrated near the ends of growing roots. Interestingly, they seem to coexist peacefully in these places with other non-troglobitic invertebrates, such as ants (Figure 8-D). Adult specimens are most commonly found on walls or speleothems, although they can also be seen close to roots. Potential predators include spiders (especially Ochyroceratidae), Amblypygi (*Charinus iuiu* Vasconcelos & Ferreira 2016) and a troglobitic species of pseudoscorpion (*Spelaeobochica iuiu* Ratton, Mahnert & Ferreira 2012).

The external area around the caves where the species occurs is considerably altered by human activities, such as agriculture and extensive cattle and goat raising. In addition, another major concern regarding the conservation of this species is that the area is being evaluated for its potential for limestone extraction. Thus, although mining activities do not yet occur in the distribution area of the species, it is likely that they will start in the near future, representing a potential risk for the caves where the species occurs. The Ferrovia de Integração Oeste-Leste (West-East Integration Railroad), which is being built to link the Brazilian coast to the interior of Bahia, will favor the flow of raw materials (mainly agricultural and mineral products), which will certainly contribute to the emergence of interest in exploiting the limestone of the region.

Given this context, it is recommended, as also suggested for *K. troglobia*, that a conservation unit be established to guarantee the preservation of caves in the Serra de Iuiú region, which is home not only to *I. caeca*, but also several other troglobitic invertebrates, many of which have not yet been described (HOCH; FERREIRA, 2016; RATTON *et al.*, 2012; SOUZA *et al.*, 2015).

Identification key

The taxonomy of Fulgoroidea in South America is still little explored, and for many genera there remains only the publication of the type series. The last author to review Brazilian Fulgoroidea was Baptista (2006), and although the review focused on Dyctiopharidae, the author provides an identification key for the families currently allocated to Fulgoroidea. The most abundant planthopper family found in caves, Cixiidae, was reviewed by Holzinger *et al.* (2002), who presented a list with the respective geographic regions of the genera allocated to the family until that time, in addition to having better explored the morphology of female genitalia. Currently, the FLOW (*Fulgoromorpha Lists On The Web*) Hemiptera database houses all information on Fulgoromorpha in the world, including Brazil. In the New World, most of the information gathered comes from the United States, and the website "*Planthoppers of North America*", from the University of Delaware, houses a large amount of information for Fulgoroidea, including keys and abstracts for many genera of families that also occur in Brazil. Unfortunately, there is no exclusive catalog or database for Fulgoroidea of Brazil and South America, although it is a very diverse and abundant group throughout the territory.

Here, we provide a provisional key to the main families and genera of Fulgoroidea that occur in caves in Brazil. The provisional status for this key is due to the vast material deposited in scientific collections that has not yet been properly examined. Therefore, the diversity is expected to be even greater.

FULGOROIDEA

1. Median ocellus on frons, tubercles and bristles usually present on tegminae. Ovipositor tubular and well developed generally surpassing the anal segment ..... **Cixiidae (2)**

1'. Tegmina veins without tubercles or bristles. Ovipositor very reduced. Abdomen distinct..... **(3)**

2. Habit highly troglomorphic. Eyes absent. Tegminae very reduced. Body depigmented. Posterior tibia with small lateral spines. Pygophore approximately trapezoidal in lateral view. Aedeagus tubular, with compressed ventral process arising approximately at midlength of aedeagal stalk, and ventral margin irregularly toothed ..... ***Ferricixius*** Hoch & Ferreira, 2012

2.1 Tegminae and eyes developed, not troglomorphic. Vertex trapezoidal with two compartments divided by a transverse apical carina. Tegminae generally with spots. Nodal cells approximately the same size and stacked side by side. Generally 12–13 veins reaching the distal margin. Posterior tibiae with lateral spines. Mesonotum flat in lateral view ..... ***Pintalia*** Stål, 1862

3. Tergites 6–8 in form of a "V" (more common in females). Tegminae totally hyaline. Up to 4 subapical cells .....**Kinnaridae (4)**

3'. Pronotum moderately large. Tegminae strongly tectiform, when resting above abdomen. Lateral margins of vertex and frons totally curved and continuous in lateral view. Third and fifth abdominal segments distinct .....**(5)**

4. Tegminae and eyes developed, not troglomorphic. Anterior margin of vertex distant from middle of eyes. Rostrum with apical segment longer than subapical segment. Transverse vein between M and Cu 1 at apex of basal cell. Aedeagus usually bulbous at base, and apex cylindrical..... .. ***Oeclidius*** Van Duze, 1914

4.1 Tegminae and eyes developed, not troglomorphic. Anterior margin of vertex slightly or not surpassing middle of compound eyes. Rostrum with apical segment shorter that subapical segment. Transverse vein between M and Cu 1 at apex of basal cell absent. Aedeagus concave at base/shell-shaped..... ***Southia*** Kirkaldy, 1904

4.2 Habit troglomorphic. Eyes absent. Body depigmented. Pygophore with lateral processes on caudal margin. Anal segment with two thin processes. Genital styles distinct with three processes. Aedeagus without lateral processes..... ..... ***Kinnapotiguara*** (Hoch & Ferreira, 2013)

4.3 Habit troglomorphic. Eyes absent. Body depigmented. Pygophore with two small and "wing-shaped" ventrolateral processes. Genital styles thin, curved and converging medially. Aedeagus with two lateral processes ..... ***Iuiuia*** Hoch & Ferreira, 2016

5. Body compressed. Abdomen generally wider than thorax. Basal segments of abdomen exhibit pairs of lateral processes with large, evident holes .... **Achilixiidae**

5.1 Eyes and wings developed. Vertex narrow with median carina absent. Pedicel elongated. Pair of lateral processes located on third abdominal segment ..... ***Bebaiotes*** Muir, 1924

Main Brazilian collections

Specimens for many species described for Brazil during the 18th and 19th centuries are deposited in collections in North America and Europe. The holdings of the Smithsonian National Museum of Natural History (NMNH), Triplehorn Insect Collection of Ohio State University (OSUC), Bishop Museum in Honolulu Hawaii (BPMNH), Naturhistoriska Riksmuseet (NHRS) and the Swedish Museum of Natural History may be useful for redescriptions and reviews of Neotropical species. Currently, ISLA/CEBS-UFLA also has specimens collected in various regions of the world, including of *Oliarius polyphemus* (Hawaii), *Tachycixius lavatubus* (Canary Islands), *Confuga persephone* (New Zealand), Meenoplidae (Kenya), and Cixiidae (Guatemala and Japan), in addition to many specimens of the main families that occur in Brazilian caves, namely Achilixiidae, Cixiidae and Kinnaridae.

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Zelurus zikani
Robson de Almeida Zampaulo

16

Hemiptera: Heteroptera

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General introduction

Heteroptera is a suborder of the order Hemiptera (Insecta) whose representatives are popularly called "percevejos" or "true bugs". Among insects with incomplete metamorphosis (hemimetabolous), it is the group with the greatest number of species. It is estimated that around 37,000 to 45,000 species of heteropterans have already been described (SCHAEFER; PANIZZI, 2000; SCHUH; WEIRAUCH, 2020) and approximately 25,000 have yet to be discovered (SCHAEFER; PANIZZI, 2000). Approximately 5,450 species of Heteroptera have been cataloged for Brazil (Catálogo Taxonômico da Fauna do Brasil – CTFB, available online: http://fauna.jbrj.gov.br/fauna/).

The etymology of Heteroptera, derived from the Greek terms hetero (= different) and pteron (= wing), alludes to the structure of the forewings, the hemelytra, which have a coriaceous anterior half and a membranous posterior half (GRAZIA; FERNANDES, 2012). Although the forewing is completely coriaceous or membranous in some species, most representatives of the suborder have the hemelytra type (COSTA LIMA, 1940). A prominent feature of the group shared with other Hemiptera is the structure of the mouthparts in the form of a segmented labium, which contains piercing-sucking mouthparts. These may have been one of the factors behind the evolutionary success of hemipterans in general (GRAZIA *et al.*, 2012). The presence of a connexivum, i.e., flattened lateral margins of the abdomen, standing out laterally to a greater or lesser extent (COSTA LIMA, 1940), is another peculiarity of most heteropterans that facilitates their recognition in relation to other insects.

Metamorphosis from egg to adult occurs as in other hemimetabolous insects. After emerging from the eggs, the immatures (nymphs), pass, in general, through five instars. Nymphs, although structurally similar to adults, have neither genitalia nor developed wings and can also differ from them in color. The wings are only functional and fully developed in adults, while in nymphs they develop while externally protected by alar thecae, which appear in the third nymphal stage and gradually increase in size until the last stage (COSTA LIMA, 1940; GRAZIA; FERNANDES, 2012).

Heteropterans are terrestrial, semi-aquatic or aquatic insects, ranging in length from 0.5 to 110 mm (GRAZIA; FERNANDES, 2012). They are mainly phytophagous, with several species of economic importance for causing damage to cultivated plants, or predatory, basically of other arthropods, while species of a few families are hematophagous, including Triatominae (Reduviidae) ("barbeiros" or "kissing bugs"), vectors of Chagas disease. Heteroptera is currently divided into seven infraorders — Enicocephalomorpha, Dipsocoromorpha, Gerromorpha, Nepomorpha, Leptodomorpha, Cimicomorpha and Pentatomomorpha (GRAZIA; FERNANDES, 2012; PANIZZI; GRAZIA, 2015; SCHUH; WEIRAUCH, 2020) — with 57 families occurring in Brazil (CTFB).

While no other major group of insects is considered to utilize such a wide variety of habitats as successfully as heteropterans (SCHUH; WEIRAUCH, 2020), including caves (e.g., KŁYS; LIS, 2013; PAPE, 2013; WYGODZINSKY, 1966), little has been recorded about their biology and ecology in this environment in Brazil (e.g., OLIVEIRA *et al.*, 2008; PINTO-DA-ROCHA, 1995; RESENDE *et al.*, 2016; TRAJANO, 2000). One of the few exceptions is Ferreira *et al.* (2016), which presents data on the diversity of *Zelurus* Hahn, 1826, recorded in caves in Brazil, as well as an assessment of the distribution of different species of this genus.

Here, we present a synthesis of current knowledge about the main families of Heteroptera recorded in Brazilian caves, including recent contributions. We hope that the knowledge presented here will serve as a starting point for all those interested in studying this group.

Main groups found in Brazilian caves

Terrestrial heteropterans

ANTHOCORIDAE

Anthocorids are very small (tiny), dark, flattened, oval or elongated insects. Although they are predators, they can feed on pollen and other plant parts (CARPINTERO, 2015). Schuh and

Weirauch (2020) presented an overview of the family worldwide, including a key to its seven tribes, whereas Carpintero (2002) published the first catalog dedicated to Neotropical species. Lattin (2000) reviewed the species that occur in managed ecosystems. Apparently, they are important in the biological control of pests in agroecosystems (BORROR *et al.*, 1989). There are about 100 species registered for Brazil (GRAZIA; FERNANDES, 2012). Pinto-da-Rocha (1995) and Loyola *et al.* (2007) reported them in caves.

CYDNIDAE

Cydnids are called percevejos-cavadores or digger bugs. They are ovoid in shape, small to medium in size and vary in color from black to brown. They have a flattened head and legs with strong, long spines; the tibiae and tarsi are modified in species with fossorial habits, which feed on roots. Some cydnids live above ground, in vegetation, feeding on seeds or plant tissues that fall (SCHAEFER, 2009; SCHWERTNER; NARDI, 2015; SCHUH; WEIRAUCH, 2020). Cydnids have a worldwide distribution, being well represented in tropical and temperate regions. The family includes over 750 described species in 93 genera and is divided into six subfamilies. Cydnids are poorly known for the Neotropics. Froeschner (1981) presented a key to the South American fauna of the subfamily Cydninae, the largest and most diverse of the family, with 90 genera and 300 species. Only data on unidentified cydnids has been presented in many articles about Brazilian caves, especially those found associated with guano from fruit bats (GNASPINI NETTO 1989; PELLEGRINI; FERREIRA, 2012; PINTO-DA-ROCHA, 1995; SIMÕES *et al.*, 2015; SOUZA-SILVA; FERREIRA, 2015; SOUZA *et al.*, 2020). In general, such species are abundant and found on the soil surface near roots and guano, as well as under blocks. Dias *et al.* (2018) sequenced the mitochondrial genome of a troglophilous species found in iron formation caves in the Carajás region.

DIPSOCORIDAE

The family Dipsocoridae comprises the genera *Alpagut* Kiyak, 1995 (Palearctic), *Cryptostemma* Herrich-Schäffer, 1835 (worldwide distributed) and *Pachycoleus* Fieber, 1860 (Palearctic), with about 30 described species. According to Štys (1995), *Pachycoleus*, which has often been considered a subgenus of Cryptostemma, is represented in the Neotropics by many undescribed species. Species are difficult to distinguish, in part due to poorly-illustrated original descriptions that lack details about important genital features (WEIRAUCH; FERNANDES, 2015); the exceptionally well-illustrated description by Wygodzinsky (1952) of *C. haywardi* Wygodzinsky, 1952 is an exception. Given this context, Pinto da Rocha (1995) and Cordeiro *et al.* (2014) reported the occurrence of the family in caves but did not advance in species identification.

ENICOCEPHALIDAE

This family includes most of the described genera and species of Enicocephalomorpha and most of the specimens deposited in collections. The species are small (from 2 to 15mm in length) and, in general, range in color from uniform yellow or brown to opaque black (with exceptions) (ŠTYS, 1995). Enicocephalidae comprises about 300 described species worldwide, which are classified into 47 genera and five subfamilies (ŠTYS, 1995, 2002). Štys (2008) indicated that at least 30 genera and hundreds of new species remain undescribed so far (FERNANDES; WEIRAUCH, 2015). Štys (1995) provided a key to the subfamilies of Enicocephalidae. The family has already been reported in caves by Bernardi *et al.* (2012), Pinto da Rocha (1995) and Silva *et al.* (2020), however, none of the specimens were identified to the species level.

LYGAEIDAE

Lygaeids range 1.2–12 mm in length and vary in shape and color, with patterns ranging from brown to black, in some cases exhibiting aposematic coloration. Baranowski and Slater (2005) published a key to the 10 subfamilies. A total of 120 species and 58 genera are registered for Brazil (GRAZIA; FERNANDES, 2012). In a survey of six caves in Parque Estadual do Sumidouro (Pedro Leopoldo, Minas Gerais), Iniesta *et al.* (2012) reported the family with a sample of six individuals.

REDUVIIDAE

Reduviidae is the second largest family of Heteroptera, with approximately 7,000 species described worldwide (MALDONADO, 1990; SCHUH; WEIRAUCH, 2020; WEIRAUCH *et al.*, 2014). Of this total, about 11% (~800) are cataloged for Brazil (CTFB). With the exception of hematophagous Triatominae, reduviids are considered predators of other arthropods. They are highly variable in size, body conformation and appendages, from some species of *Empicoris* Wollf, 1811 (Emesinae), with delicate appendages and reaching only 3 mm in total length, to several species with robust bodies, reaching 40 mm in length (SCHUH; WEIRAUCH, 2020; WYGODZINSKY, 1966). Diagnostic features for Reduviidae include: eyes usually large; ocelli usually present; labium usually curved and robust, but may be straight and/or thin, with three visible segments (except Hammacerinae with four); prosternum with stridulatory sulcus (stridulitrum); hemelytral membrane usually with two or three elongated cells; and spongy fossa present at the ventral end of the anterior and middle tibiae in many taxa (GIL-SANTANA *et al.*, 2015; SCHUH; WEIRAUCH, 2020). Among these characteristics, the stridulatory sulcus has been considered diagnostic of Reduviidae for a long time (e.g., COSTA LIMA, 1940), being particularly useful for non-specialists to separate them from heteropterans of other families (HRG-S, pers. obs.).

Currently, 24 subfamilies of Reduviidae are recognized as valid (SCHUH; WEIRAUCH, 2020), of which 20 are represented in the Neotropical region (GIL-SANTANA *et al.*, 2015) and 18 in Brazil (CTFB). The most recent keys for subfamilies of Reduviidae are those of Forero (2004), covering the Neotropical region; that of Grazia and Fernandes (2012) for Brazil; and those by Weirauch *et al.* (2014) and Schuh and Weirauch (2020), for those around the world. The last two, because of the accompanying illustrations, facilitate the understanding of the dichotomies. Consultation of Gil-Santana *et al.* (2015a) allows access to identification keys for the genera of several subfamilies or respective information, and to summaries about the taxonomy and biology of Neotropical reduviids. Maldonado (1990) is the general taxonomic catalog of Reduviidae, which covers the entire world fauna, while CTFB allows the consultation of updated faunal data for Brazil. Among the reduviids found in caves in Brazil, the largest number of species belong to the subfamilies Emesinae and Reduviinae. Representatives of Ectrichodiinae, Triatominae and Saicinae have also been recorded in Brazilian caves, as shown below:

Ectrichodiinae

Twenty-four genera are currently recognized for Ectrichodiinae in the Americas, including more than a hundred species (GIL-SANTANA *et al.*, 2015, 2020a; FORTHMAN; WEIRAUCH, 2017), among which 16 genera and just over 60 species have been registered for Brazil (CTFB). The genera found in Brazil can be identified by the key presented by Gil-Santana *et al.* (2015) or the most up-to-date version by Gil-Santana *et al.* (2020a). Representatives of Ectrichodiinae can be easily recognized by having a bifid scutellum (e.g., Figure 1B) and a completely or mostly glabrous integument (e.g., Figure 1A-C), with many species showing aposematic, brightly colored patterns (e.g., Figure 1A). The wings of females of many species are reduced in size (e.g., Figure 1A), to varying degrees, or absent. The scarce biological data suggest that species of Ectrichodiinae are specialized predators of millipedes (Gil-Santana *et al.*, 2015). The genera with the greatest number of species, including the most common ones, are *Brontostoma* Kirkaldy, 1904, *Pothea* Amyot & Serville, 1843 and *Rhiginia* Stål, 1859 (CTFB; COSTA LIMA, 1940a). Also belonging to these genera are the species already found in caves, whose record was made by the junior author (RAZ) and are presented here for the first time: *B. trux* (Stål, 1859) (Figure 1A; the female in this figure presents coloration with a very pronounced variation, yet we consider it to be of this species); another specimen of *B. trux* with a peculiar coloration also collected by RAZ; *P. furtadoi* Gil-Santana & Costa, 2005 (Figure 1B) and *R. atripennis* (Walker, 1873). It should be noted that all these species are primarily known from open environments (e.g., GIL-SANTANA *et al.*, 2013; GIL-SANTANA, 2014; HRG-S pers. obs.).

Emesinae

The elongated and relatively thin body and legs, together with the appearance of the raptorial forelegs, which resemble those of mantids ("louva-a-Deus" or "praying mantis"), characteristics presented by most emesines, make it easy to differentiate them from other reduviids (Figure 1D-L). It is also noteworthy that the emesines are one example of heteropterans with an entirely membranous forewing, without the structure of a hemelytron. Almost 90 species, included in 17 genera, have been recorded in Brazil (CTFB). Many species of Emesinae are occasionally or frequently found in caves (WYGODZINSKY, 1966), with the degree of adaptation and the frequency or exclusivity to this habitat being variable. *Mayemesa lapinhaensis*

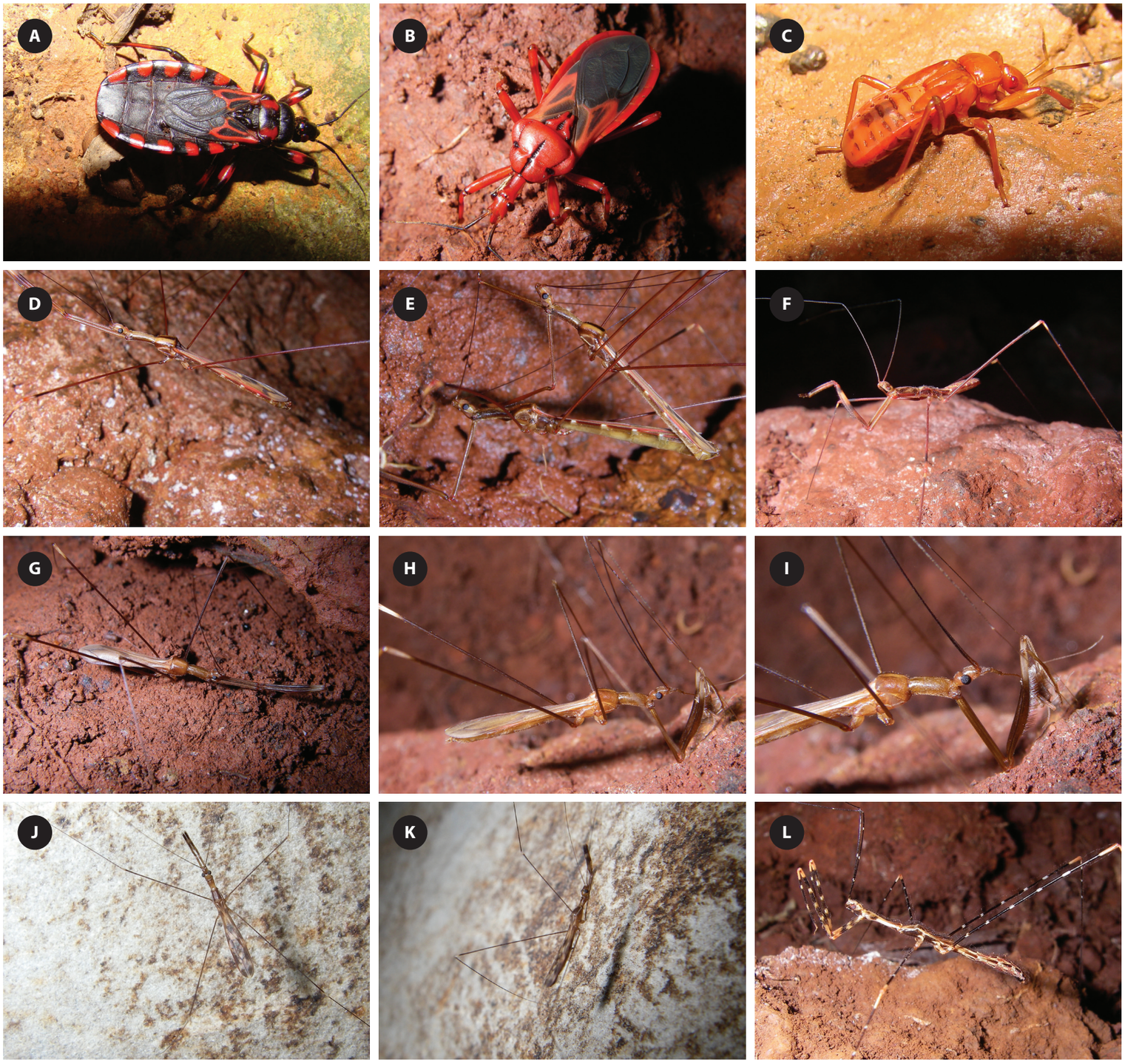


Figure 1. A) *Brontostoma* cf. *trux* (Stål, 1859), female; **B)** *Pothea furtadoi* Gil-Santana & Costa, 2005, female; **C)** nymph of unidentified species of Ectrichodiinae; **D-F)** *Phasmatocoris catarinae* Gil-Santana, 2015, **D)** adult, **E)** couple in copula, **F)** nymph; **G-I)** *Phasmatocoris galvaoi* Gil-Santana, 2015, **G)** adult, **H-I)** adult performing predation, I – detail; **J-K)** *Ploiaria* sp., adult at rest, **J)** dorsal view, **K)** dorsolateral view; **L)** Emesinae sp., lateral view. Photos: (Robson de A. Zampaulo).

(Wygodzinsky, 1950), for example, originally known only from caves, (WYGODZINSKY, 1966), was later found both in completely dissociated environments (GIL-SANTANA *et al.*, 1999) and again in caves (HRG-S, personal observation). An updated summary of the world literature, including a discussion of ecological and biological knowledge of emesines found or living in caves, was presented by Pape (2013). Species of *Dohrnemesa* Wygodzinsky, 1945, *Phasmatocoris* Breddin, 1904 (Figure 1D-I) and *Ploiaria* Scopoli, 1786 (Figure 1J-K) are frequent in Brazilian caves, with some species of the first two genera being described and, until now, known only from this environment, such as *Ph. catarinae* Gil-Santana, 2015 and *Ph. galvaoi* Gil-Santana, 2015 (e.g. GIL-SANTANA *et al.*, 2007; GIL-SANTANA 2015; GIL-SANTANA; FERREIRA, 2016, 2017), while there are at least two common, and possibly new, species of *Ploiaria* in caves awaiting identification (HRG-S, personal observation) (e.g., Figure 1J-K). In general, immatures of different stages of emesines can be found associated with litter on the floor of caves in entrance and dysphotic zones, where they probably find food in abundance. In turn, adults or last instars are generally more frequently observed along the walls and ceilings of caves, especially in predominantly dry areas. These reduviids are often recorded feeding on dipterans (e.g., Psychodidae), coleopteran larvae, spiders and immature crickets, among other small arthropods. Emesines can also be found associated with spiders of the genera *Loxosceles* Heineken & Lowe, 1832 and *Mesabolivar* González-Sponga, 1998, and may exhibit kleptoparasitic behavior (RESENDE *et al.*, 2016).

Reduviinae

Reduviinae includes nine genera that occur in Brazil, with about a hundred species (CTFB; GIL-SANTANA *et al.*, 2015), of which *Zelurus* is the most diverse, with almost 90 species recorded in the country. Ferreira *et al.* (2016) presented a synthesis of the numerous taxonomic references on species of *Zelurus*, which is one of the most ubiquitous cave taxa in Brazil. These authors presented a list of ten species of *Zelurus* recorded in Brazilian caves, among which *Z. diasi* (Costa Lima, 1940) (Figures 2A-B), *Z. sipolisi* (Fallou, 1889) (Figure 2C), *Z. zikani* (Costa Lima 1940) (Figures 2D-E) and *Z. festivus* (Stål, 1859) (Figure 2F) are illustrated in this chapter. We also add the occurrence of *Z. circumcinctus* (Hahn, 1825) and *Z. pintoi* (Costa Lima, 1940), updating the number of species recorded in Brazilian caves to 12.

It is worth noting that the known nymphs of this genus have cryptic coloration in relation to the substrate, often being covered by soil particles or even other environmental structures (e.g., lichens) (Figures 9A-D), which increases this camouflage. On the other hand, the adults seem to mimic wasps of the genus *Pepsis* Fabricius, 1804 (Hymenoptera: Pompilidae), both due to the color and shape of the body (HOGUE, 1993) and the movement they perform when flying (COSTA LIMA, 1940b). They are generalist predators, being nocturnal and quite aggressive (GROSSI *et al.*, 2012; SCHUH; WEIRAUCH, 2020).

In general, nymphs are found in large numbers in the entrance zone of predominantly dry caves, while adults are relatively rare in these environments. One of the plausible hypotheses is that caves function as sites for reproduction and the development of immature forms, since selective pressures different from those of the epigean environment (*e.g.*, lower predator abundance, lack of light and specific microclimatic conditions) could favor the reproductive success of countless species of this genus.

Adult forms of *Zelurus* sp. were recorded as predators of harvestmen (*Goniosoma* sp., Arachnida, Opiliones, Gonyleptidae) in almost all caves surveyed by Machado *et al.* (2003), while *Serracutisoma spelaeum* (Mello-Leitão, 1933; cited as *Goniosoma spelaeum*) was observed as prey of *Zelurus travassosi* (Costa Lima, 1940). This last species was considered the most important predator of the trogloxene *S. spelaeum* in the state of São Paulo and of the troglophile *Daguerreia inermis* Soares & Soares, 1947 (Opiliones, Gonyleptidae) in the state of Paraná (MACHADO *et al.*, 2003). The immature forms, on the other hand, have a large repertoire of prey, and can feed on crickets, coleopteran larvae, dipterans and spiders, among others.

Saicinae

There are seven genera and 22 species of Saicinae in Brazil (CTFB; GIL-SANTANA *et al.*, 2015, 2020b). Little is known about the biology of its representatives, which are usually collected by light attraction (SCHUH; WEIRAUCH, 2020). Biological data for the group in the Americas were summarized by Gil-Santana *et al.* (2010), while Gil-Santana *et al.*, (2020b), based on nymphs and adults found in a single cave in the state of Tocantins, described a new genus and species of Saicinae, *Quasitagalis* Gil-Santana, Oliveira & Zampaulo, 2020, and *Q. afonsoi* Gil-Santana, Oliveira & Zampaulo, 2020, recording for the first time a species of this subfamily as a cave dweller in the Americas.

Triatominae

Triatomines stand out among reduviids for feeding on vertebrate blood in all their life stages and for being vectors of the protozoan *Trypanosoma cruzi* (Chagas, 1909), the etiological agent of Chagas disease, a pathology that is still of significant public health importance in Latin America (MONTEIRO *et al.*, 2018). Several species of Triatominae have been collected or found in caves (LENT; WYGODZINSKY, 1979). The etymology of *Cavernicola* Barber, 1937, reflects the specialization for living in caves of the species for which the genus was described, *C. pilosa* Barber, 1937, which has a wide geographical distribution in South America and is found mainly in caves inhabited by bats (OLIVEIRA *et al.*, 2008; GALVÃO; GONÇALVES, 2014; GALVÃO, 2020). *Panstrongylus geniculatus* (Latreille, 1811) (Figures 2J-L) is one of the relatively common triatomine species and has the widest geographical distribution in South and Central America, where it is found in a variety of habitats, including caves with bat colonies (LENT; WYGODZINSKY, 1979) or caves in general (GALVÃO; GONÇALVES, 2014). Another species that occurs in Brazil and has already been recorded in caves is *Eratyrus mucronatus* Stål, 1859 (GALVÃO; GONÇALVES, 2014; GALVÃO, 2020).

Reduviids of other subfamilies

In addition to representatives of the subfamilies listed above, which are the most frequent, we have recorded the occurrence of other subfamilies of Reduviidae in caves (HRG-S personal observation), such as *Apiomerus* sp. (nymph) (Harpactorinae; Apiomerini) and *Vescia* sp. (Vesciinae) (Figure 2 I).

THYREOCORIDAE

Members of Thyreocoridae (= Corimelaenidae) are known as black bugs. The family is divided into Thyreocorinae and Corimelaeninae, the latter being represented in the Neotropical Region. They are relatively small and dark in color, with an oval-elongated body and a large scutellum (GRAZIA; FERNANDES, 2012). Galgupha Amyot & Serville, 1843 is the most diverse Neotropical genus, with 155 species in 15 subgenera. About 160 species of the family have been recorded in the Neotropics, but their biology is poorly understood. Black bugs are phytophagous, feeding on developing or mature flowers and fruits (MATESCO; GRAZIA, 2015). Despite occurring in many crops, they are considered minor pests in orchards and are not mentioned among heteropterans of economic importance (SCHAEFER; PANIZZI, 2000). Simões *et al.* (2015) found a species in caves in the state of Minas Gerais, while studying the how physical attributes of caves influence the structure and composition of cave invertebrate communities.

TINGIDAE

Tingids, known as "lace bugs", are delicate and small insects with a growth of the pronotum and forewings having a lacy appearance. The family is divided into three subfamilies (Tinginae, Cantacaderinae and Vianaidinae), all represented in the Neotropical Region (SCHUH *et al.*, 2006, GUIDOTI *et al.*, 2015). There are currently about 300 genera and 2,500 species in this family, with 70 genera and 600 species in the Neotropics. Few species have had their biology studied in this region, such as *Corythaica cyathicollis* (Costa, 1864) (KOGAN, 1960) and *Leptopharsa heveae* Drake & Poor, 1935 (Cividanes *et al.*, 2004), both of economic importance. There is only one record for caves, namely fifth-instar nymphs and males of T*haumamannia vanderdrifti* Van Doesburg, 1977, in caves in the iron formation of the Floresta Nacional de Carajás, in the municipality of Parauapebas, state of Pará (GUIDOTI *et al.*, 2014). Although there are other records for the family in the state of Minas Gerais (unpublished data), such specimens are considered relatively rare in Brazilian caves in general.

Aquatic and semiaquatic heteropterans

Aquatic and semiaquatic true bugs are much less diverse than their terrestrial relatives but correspond to almost 5,000 species distributed in the infraorders Leptopodomorpha (ca. 380), Gerromorpha (ca. 2,100) and Nepomorpha (ca. 2,300) (POLHEMUS; POLHEMUS, 2008). The leptopodomorphan fauna of Brazil is poorly known, with scarce records of only 10 species of the family Saldidae (CTFB), none of which were based on specimens collected in caves. Records in these environments in Brazil are also relatively rare for Gerromorpha and Nepomorpha, being more abundant in the case of the former. Pinto-da-Rocha (1995) summarized records from caves in Brazil for the families Gerridae, Veliidae (Gerromorpha), Belostomatidae and Naucoridae (Nepomorpha), but none identified to species.



Figure 2. A-B) *Zelurus diasi* (Costa Lima, 1940), adult, **A)** dorsal view, **B)** detail, lateral view; **C)** *Zelurus sipolisi* (Fallou, 1889), adult, frontal view; **D-E)** *Zelurus zikani* (Costa Lima, 1940), **D)** adult, dorsal view, **E)** couple in copula; **F)** *Zelurus festivus* (Stål, 1859); **G)** *Zelurus cf. flavipennis* (Mayr, 1865), dorsolateral view; **H)** *Zelurus* sp., dorsal view; **I)** *Vescia* sp., dorsal view; **J-L)** *Panstrongylus geniculatus* (Latreille, 1811), adults, **J)** dorsal view, **K)** lateral view, **L)** nymph, partially covered with soil detritus. Photos: (Robson de A. Zampaulo).

GERROMORPHA

Gerromorphans are semi-aquatic true bugs, most of which spend almost their entire active life on the surface or shores of freshwater bodies. Some species, however, are secondarily terrestrial or occupy coastal marine habitats, or even the open ocean (MOREIRA 2015). The infraorder is divided into eight families, of which Gerridae, Hebridae, Hydrometridae, Mesoveliidae and Veliidae are recorded from Brazil. A total of 244 species have been recorded from the country thus far, of which 15 have been reported in caves (CTFB).

Brachymetra albinervus (Amyot & Serville, 1843) (Gerridae: Charmatometrinae) (Figure 3A), a widely distributed species in the Neotropics, was recorded in Caverna dos Troncos, Parque Nacional Cavernas do Peruaçu, state of Minas Gerais (MONTE; BICHUETTE, 2020), and in Duto do Quebó, municipality of Nobres, state of Mato Grosso (MAGALHÃES *et al.*, 2021). The species is very common and abundant, and generally lives in sandy-bottomed streams, occasionally being found in and around caves. *Cylindrostethus palmaris* Drake & Harris, 1934 (Gerridae: Cylindrostethinae) (Figures 3B-C) has habitat preferences similar to those of *B. albinervus* and was recently recorded in Gruta do Catão, municipality of São Desidério, state of Bahia, and in the previously mentioned Duto do Quebó (MAGALHÃES *et al.*, 2021). It is the most common species of *Cylindrostethus* in South America and the only one that has winged adults, which may be related to its wide distribution

The family Hydrometridae includes two monotypic genera that were recently described based on troglobitic specimens. Both were tentatively allocated to the subfamily Hydromerinae based on the apical articulation between antennomeres I and II. *Cephalometra pallida* Polhemus & Ferreira, 2018 (Figure 7D) occurs in Gruta da Natividade, state of Tocantins, while *Spelaeometra gruta* Polhemus & Ferreira, 2018 (Figures 7B-C) was collected in four caves of the Parque Nacional Cavernas do Peruaçu (POLHEMUS; FERREIRA, 2018).

Mesovelia amoena Uhler, 1894 (Mesoveliidae: Mesoveliinae) (Figure 4A) occurs throughout most of the Americas and has even been introduced to Hawaii (DAMGAARD *et al.*, 2012). The species was recorded once in caves in Brazil, based on material from São Desidério, as well as the aforementioned *C. palmaris* (CORDEIRO; MOREIRA, 2015).

The greatest number of species of Gerromorpha recorded in caves in Brazil is in the family Veliidae. *Microvelia ayacuchana* Drake & Maldonado, 1952 (Veliidae: Microveliinae) (Figure 4B) was recorded based on material collected in pools in a cave in the municipality of Canaã dos Carajás, state of Pará (MOREIRA; CAMPOS, 2012). Individuals of this species generally live on the banks in pools beside waterfalls, running to land when disturbed. *Microvelia ioana* Drake & Hottes, 1952 (Figures 6E-F), endemic to the Atlantic Forest, was recorded in Gruta do Saco Grande, Florianópolis, state of Santa Catarina (MAGALHÃES *et al.*, 2021).

Rhagovelia kararao Floriano & Moreira, 2015 (Veliidae: Rhagoveliinae) (Figures 4C-D) was described based on material collected in two caves, one in Altamira and another in Vitória do Xingu, state of Pará, and has not been recorded again. Specimens of this genus are usually collected in open lotic environments with moderate to strong currents, but the above species was found in slow flowing waters in sandstone caves (FLORIANO; MOREIRA 2015). *Rhagovelia robusta* Gould, 1931 (Figures 3F-G) and *R. whitei* (Breddin, 1898) (Figures 6A-B), more widely distributed in south-central South America, were recorded in caves in the states of Mato Grosso and Minas Gerais (CORDEIRO; MOREIRA, 2015; MAGALHÃES *et al.*, 2021; TAYLOR; FERREIRA, 2012), but they are much more common in open environments. *Rhagovelia henryi* Polhemus, 1997 (Figures 3D-E), in turn, is found exclusively in the Atlantic Forest and, recently, was recorded for the first time in caves, based on material collected in the municipality of Iporanga, state of São Paulo (MAGALHÃES *et al.*, 2021).

Two species of the genus *Paravelia* Breddin, 1898 (Veliidae: Veliinae) have, thus far, only been found in caves. *Paravelia cunhai* Rodrigues & Moreira, 2016 (Figures 5A-B) was collected in this type of environment in the municipalities of Canaã dos Carajás and Vitória do Xingu, Pará (Rodrigues & Moreira 2016a), while *P. digitata* Rodrigues & Moreira, 2016 (Figures 6C -D) was collected in a series of caves in Bahia and Rio Grande do Norte (MAGALHÃES *et al.*, 2021; RODRIGUES; MOREIRA, 2016b). The morphology of these animals, however, does not indicate restriction to subterranean environments. *Paravelia polhemusi* Rodrigues, Moreira, Nieser, Chen & Melo, 2014 (Figure 5C) and *P. splendoris* (Drake & Harris, 1933) (Figure 5D), in turn, have already been recorded both in caves and in open environments in Brazil (DIAS-SILVA *et al.*, 2013; RODRIGUES *et al.*, 2014; RODRIGUES; MOREIRA, 2016b; RODRIGUES; ÁLVAREZ-ARANGO, 2019; FRANCO *et al.*, 2020).

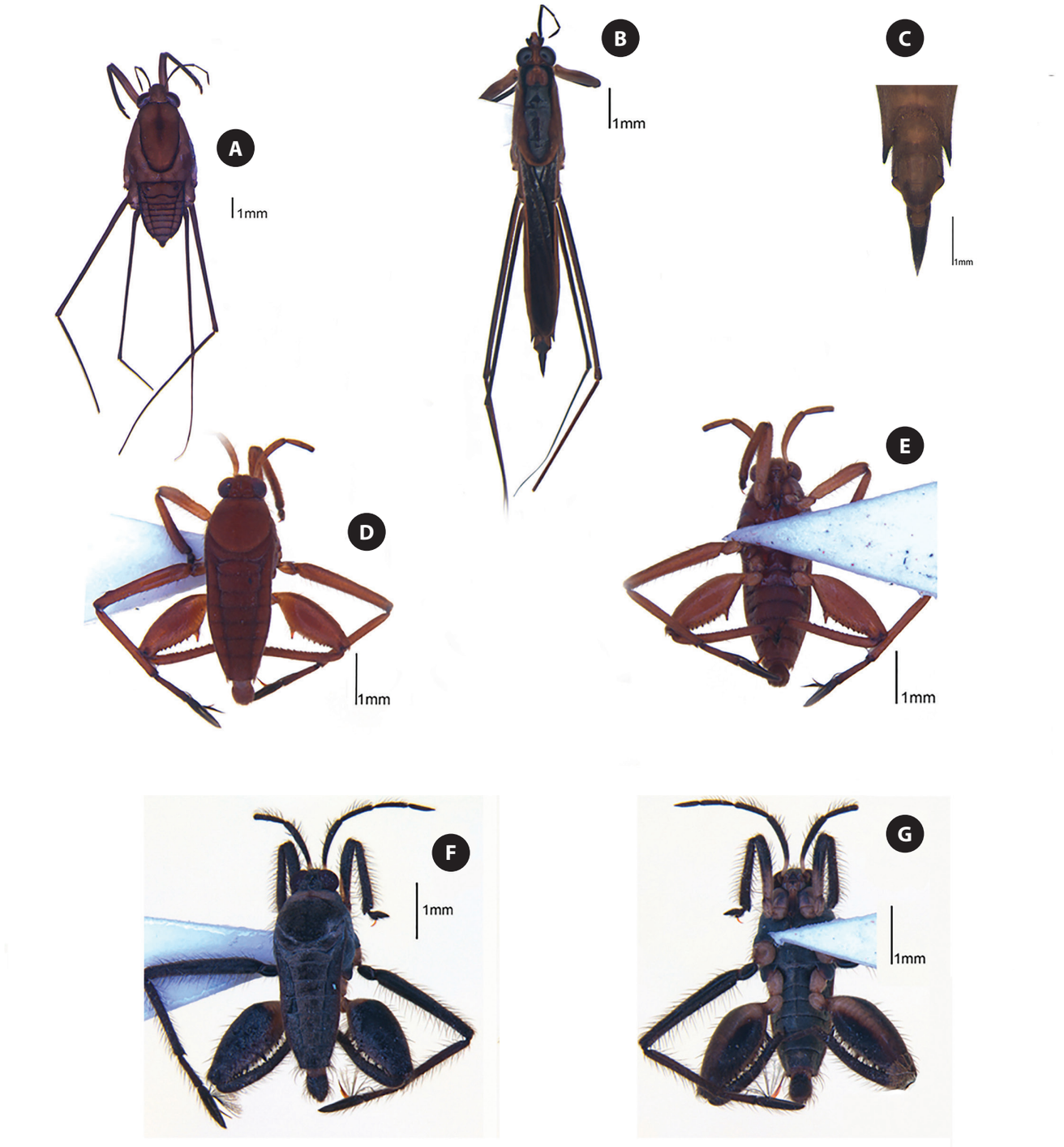


Figure 3. A) *Brachymetra albinervus*, apterous female, dorsal view; **B)** *Cylindrostethus palmaris*, macropterous male dorsal view; **C)** *Cylindrostethus palmaris*, macropterous male, abdomen apex, ventral view; **D)** *Rhagovelia henryi*, apterous male, dorsal view; **E)** *Rhagovelia henryi*, apterous male, ventral view; **F)** *Rhagovelia robusta*, apterous male, dorsal view; **G)** *Rhagovelia robusta*, apterous male, ventral view. Originally published in Magalhães *et al.,* (2021).

NEPOMORPHA

Nepomorphans, or truly aquatic true bugs, live submerged in freshwater bodies most of their lives, with representatives of two families being secondarily aerial (BARBOSA; RODRIGUES, 2015). Depending on the author, the infraorder is divided into 11 or 13 families, of which Belostomatidae, Corixidae (sensu lato), Gelastocoridae, Helotrephidae, Micronectidae, Naucoridae, Nepidae, Notonectidae, Ochteridae, Pleidae and Potamocoridae occur in Brazil. In all, 310 species have been recorded in the country (CTFB), but only three were reported based on material collected in caves.

Nerthra terrestris (Kevan, 1948) (Gelastocoridae: Nerthrinae) can be found on the banks of water bodies or away from them (NIESER, 1975). In Brazil, the species was recorded once at Abrigo da Gravura, municipality of Altamira, state of Pará (CORDEIRO; MOREIRA, 2015). *Maculambrysus stali* (La Rivers, 1962) and *Australambrysus teutonius* (La Rivers, 1951) (Naucoridae: Ambrysinae), in turn, are commonly collected in stony streams in open areas, with moderate to strong currents, but have already been recorded in caves in Pará and Minas Gerais, respectively (CORDEIRO; MOREIRA, 2015; TAYLOR; FERREIRA, 2012).

Main troglomorphisms

Among the species known from Brazil thus far (Figure 8), only two — *Cephalometra pallida* and *Spelaeometra gruta* (Gerromorpha: Hydrometridae) — present morphological adaptations that indicate some degree of restriction to subterranean environments, being considered effectively troglobites. Specializations, such as reduction of wings, depigmentation, reduction of ocular structures and elongation of body structures, were observed in these species.

Pape (2013) discusses that advanced troglomorphisms are apparently uncommon for Emesinae (Reduviidae), and so far only two troglobitic species of the genus Collartida Villiers, 1949 (C. anophthalma Espanol & Ribes, 1983 and C. tanausu Ribes, Oromi & Ribes, 1998), described from lava tubes of the Canary Islands, and a species of the genus Nesidiolestes Kirkaldy, 1902 (N. ana Gagné & Howarth, 1974), described from lava tube caves of Hawaii, have been considered troglobites. However, in this same work, the author mentions that, of the 44 species of Emesinae with records in caves, 24 are known only for this type of environment, which may indicate that some effectively subterranean species may not present the classic troglomorphisms for the majority of cave

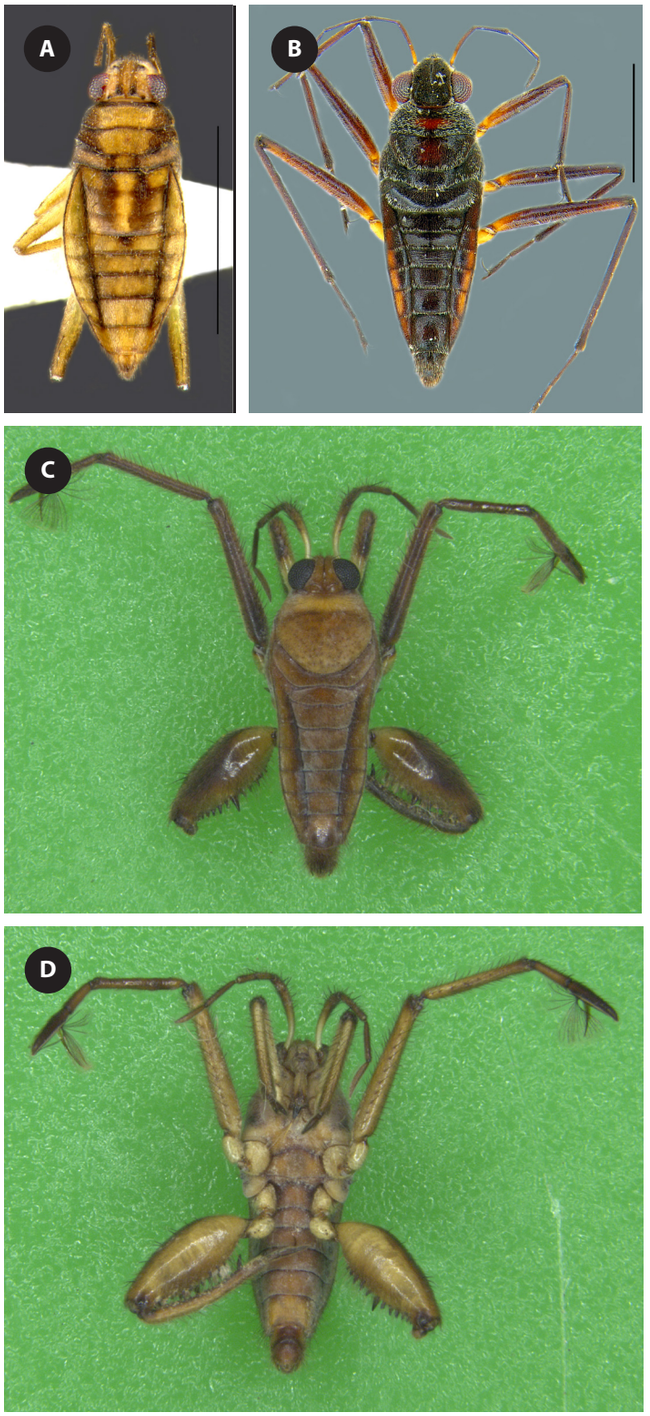


Figure 4. A) *Mesovelia amoena*, apterous female, dorsal view; **B)** *Microvelia ayacuchana*, apterous male, dorsal view; **C)** *Rhagovelia kararao*, apterous male, dorsal view; **D)** *Rhagovelia kararao*, apterous male, ventral view. Scale bars: 1 mm and only applies to figures A and B. Originally published in Rodrigues *et al.* (2021) (A, B) and Floriano & Moreira (2015) (C, D).

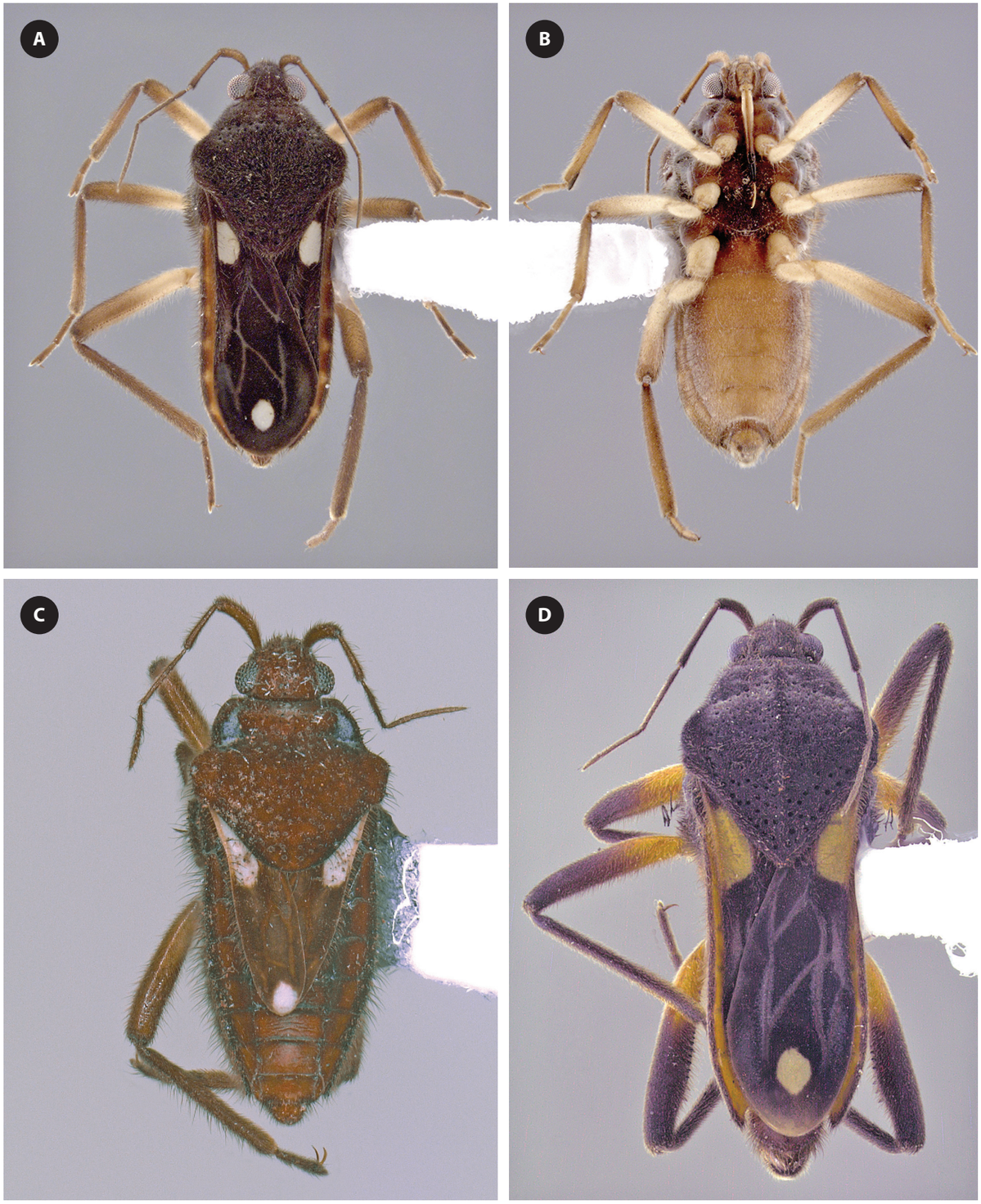


Figure 5. A) *Paravelia cunhai*, macropterous male, dorsal view; **B)** *Paravelia cunhai*, macropterous male, ventral view; **C)** *Paravelia splendoris*, brachypterous male, dorsal view; **D)** *Paravelia polhemusi*, macropterous male, dorsal view. Scale bar: 1 mm and only applies to figures A and B. Originally published in Rodrigues & Moreira (2016a) (A, B), Rodrigues *et al.* (2014) (C) and Rodrigues Álvarez-Arango (2019) (D).

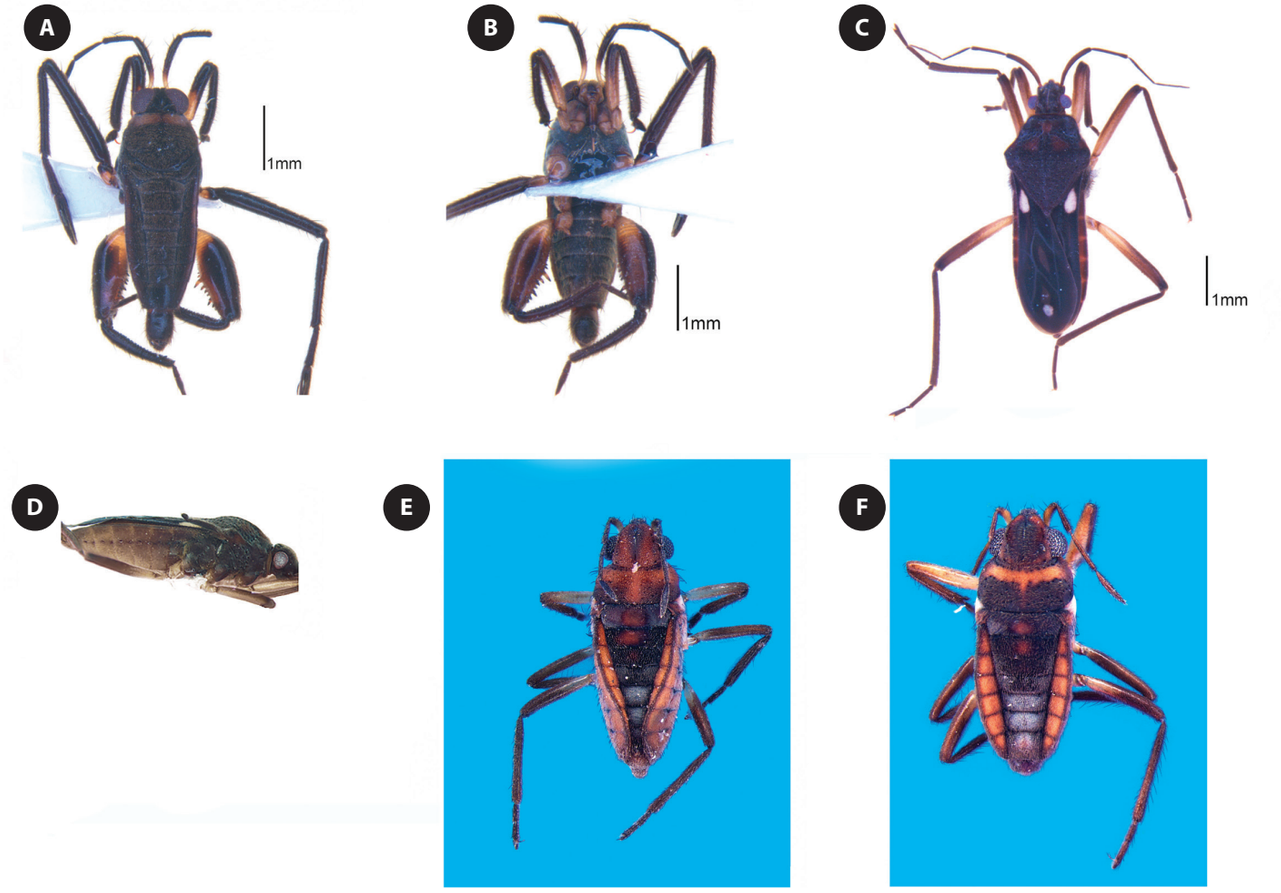


Figure 6. A) *Rhagovelia whitei*, apterous male, dorsal view; **B)** *Rhagovelia whitei*, apterous male, ventral view; **C)** *Paravelia digitata*, macropterous male, dorsal view; **D)** *Paravelia digitata*, macropterous male, lateral view; **E)** *Microvelia ioana*, apterous female, dorsal view; **F)** *Microvelia ioana*, apterous male, dorsal view. Originally published in Magalhães *et al.,* (2021).

groups, as observed by Wygodzinsky, (1966), Gil-Santana *et al.* (2007), Pape (2013) and Chłond *et al.* (2018). This condition may indicate an underestimation of the number of troglobitic species known for Brazil.

Main methods of collection and conservation

Collection methods for terrestrial heteropterans in epigean environments include the use of entomological umbrellas, light traps, baited traps, pitfall traps and extractors (Berlese, Berlese-Tüllgren and Winkler), in addition to manual collection on various substrates such as vegetation, soil, logs or under blocks, for example. As for other invertebrates, the most efficient and commonly used collection method in caves is the active search. Among the most ubiquitous groups, the reduviids of the genus Zelurus are often found associated with floor sediment during the young phase where, most of the time, they use this substrate to cover their bodies as a camouflage strategy (Figures 9A-D).

Adult specimens are much less frequent (compared to immature forms), being found associated with the walls and ceilings of caves. For the other groups, young emesines are recurrent among litter, but they can also be found associated with bare floors and cave walls. Specimens of the families Enicocephalidae and Tingidae are more frequent near the soil, litter and roots and under blocks. Specimens of the family Cydnidae are often found associated with guano deposits, especially those produced by fruit and hematophagous bats. The other families can be found in different substrates, especially in the entrance and penumbra zones.

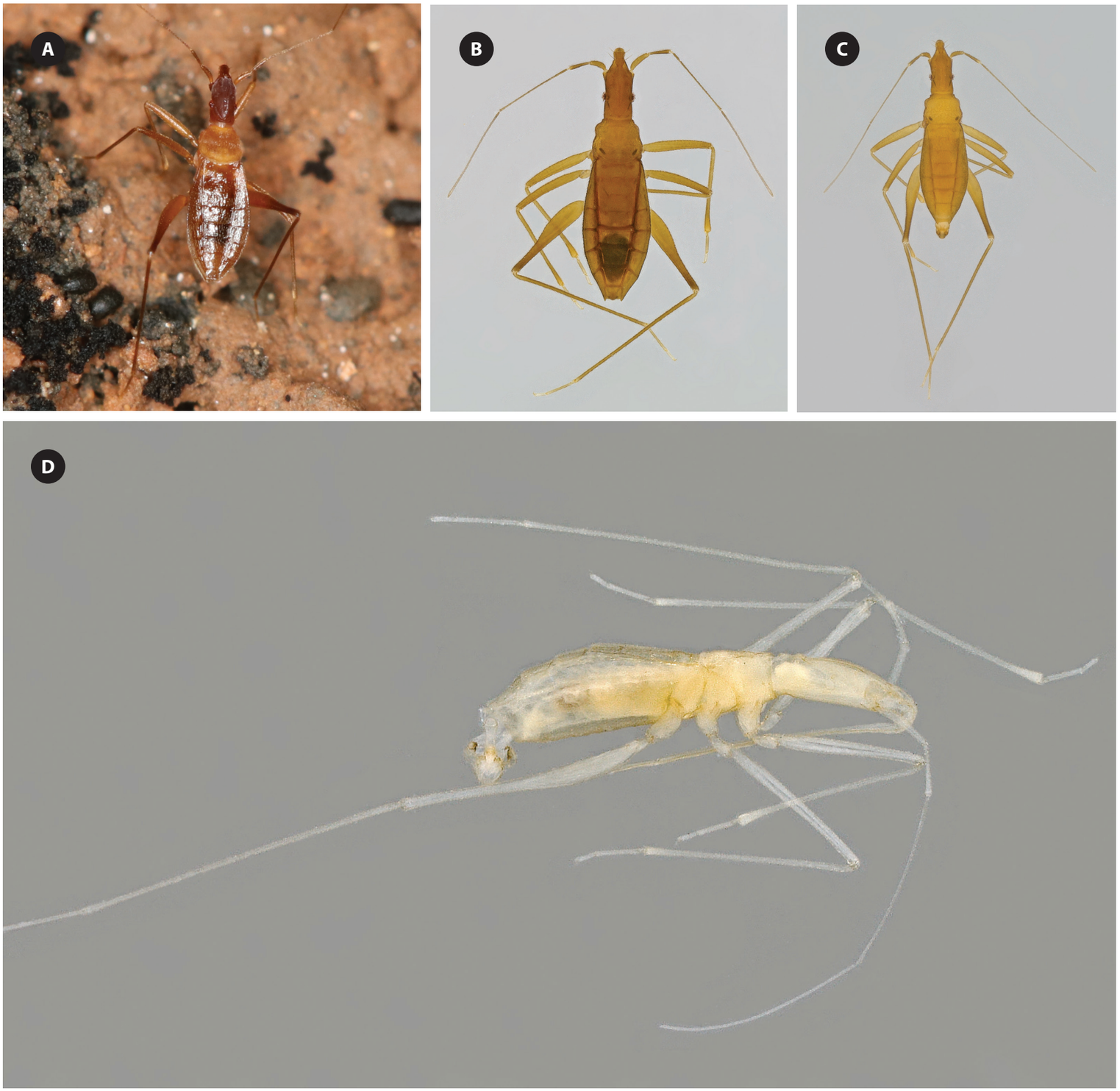


Figure 7. A) Live specimen of *Spelaeometra* sp.; **B)** *Spelaeometra gruta*, apterous female, dorsal view; **C)** *Spelaeometra gruta*, apterous male, dorsal view; **D)** *Cephalometra pallida*, apterous male, lateral view. Photos: A (Rodrigo L. Ferreira); B-C (Originally published in Polhemus & Ferreira, 2018).

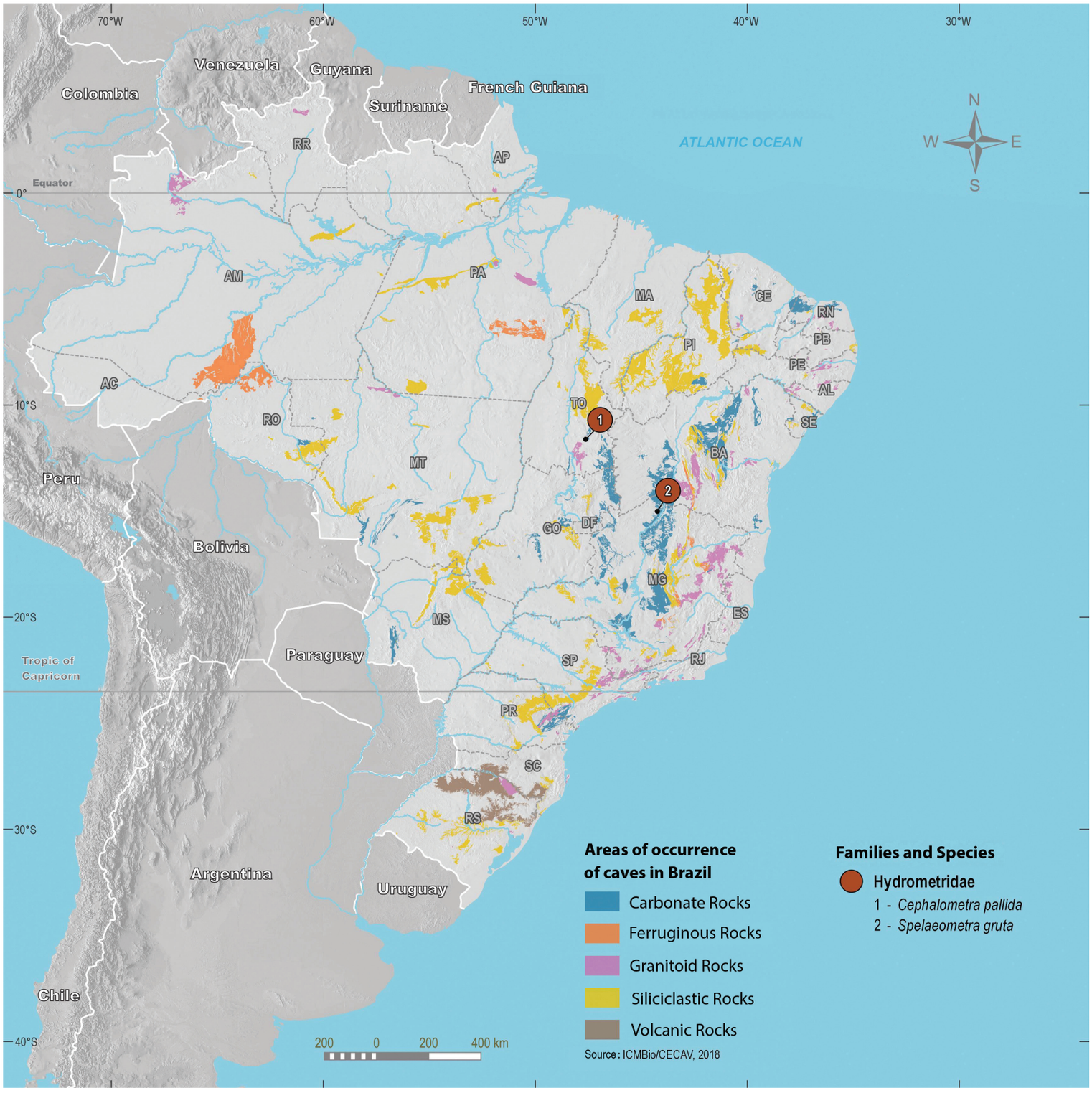


Figure 8. Map with type localities of troglobitic hemipteran species (Heteroptera) described for Brazil.

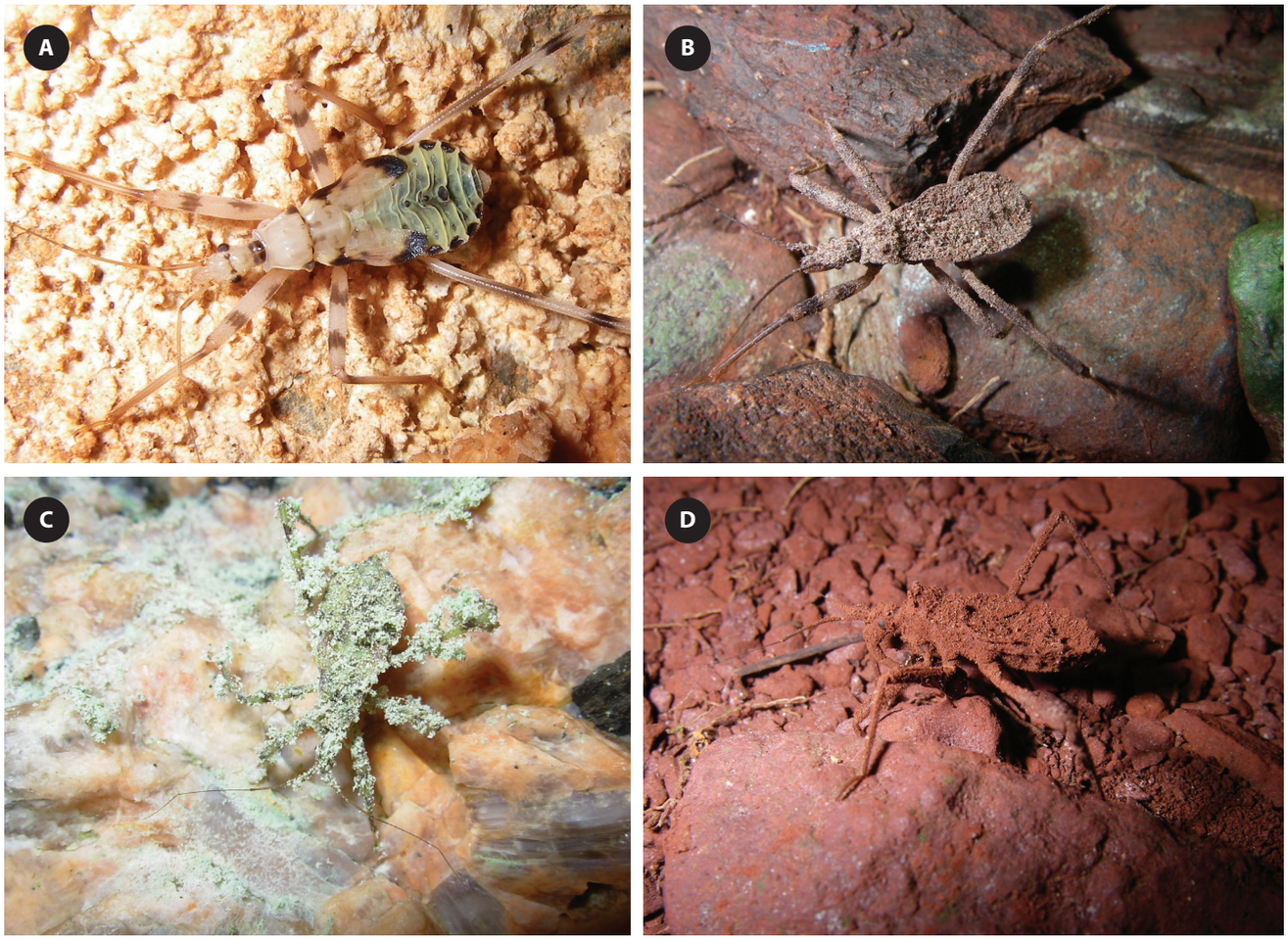


Figure 9. A-D. Nymphs of *Zelurus* sp.; **A)** Specimen without camouflage; **B)** Specimen camouflaged with sediment; **C)** Specimen camouflaged with lichens; **D)** Specimen camouflaged with sediment of an iron ore cave. Photos: (Robson de A. Zampaulo).

In relation to aquatic (Nepomorpha) and semi-aquatic (Gerromorpha) heteropterans (NIESER; MELO, 1997), in practice, the vast majority of accessible caves are dry, as they are currently in a senile condition, and therefore these groups are poorly represented in caves in general. However, these groups can be found in caves with the presence of upwellings, rivers, lakes (associated with the base level or not), travertine dams or any other type of water deposit (including puddles), whether perennial or intermittent. Individuals of Gerromorpha live on the surface film or banks of water bodies (SCHUH; SLATER, 1995), which facilitates their visual identification and sampling. Representatives of Nepomorpha, on the other hand, tend to have closer relationships with bottom substrates because they are benthic (HUNGERFORD, 1948), and so more specific methods must be employed.

In general, there are few works that have evaluated the diversity of aquatic invertebrates in caves in Brazil and that have used particular methods for sampling this faunal compartment, making these groups completely underestimated in the country (*e.g.*, PELLEGRINI *et al*., 2020; PELLEGRINI *et al.*, 2018; ZEPON *et al.*, 2019). Sampling in small puddles of water and travertine dams can be easily carried out with the aid of pipettes and small manual nets. However, in larger drainages or lakes, sampling should include the different submerged substrates, such as roots, sediments, gravel, rocks and organic matter deposited at the bottom of these water bodies. For this, sieves, nets, Surber-type collectors or "D" nets (rapiché), all with fine mesh, are recommended. In more specific studies, the removal of samples

of sediment and organic matter for processing in loco with the aid of plastic trays or later in the laboratory is recommended. A detailed description of the processing of these samples in the laboratory can be obtained in Silveira *et al.* (2004). Conservation of collected material should use 70% alcohol for general taxonomic studies and absolute alcohol and storage at -20ºC to avoid the degradation of the genetic material for molecular studies.

Main Brazilian collections

For Heteroptera in general, and particularly the families Miridae and Reduviidae, the collection with the largest number of types and specimens in Brazil was that of the Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ), until 02 September 2018 when it was completely destroyed in the great fire that hit this institution (ESCOBAR, 2018). Next, for Reduviidae, it is worth mentioning the collections on the campus of Instituto Oswaldo Cruz in Manguinhos, Rio de Janeiro. The Coleção de Triatomíneos of Instituto Oswaldo Cruz (CTIOC) is not only in the largest collection of triatomines in the world, but also includes numerous types and specimens of predatory Reduviidae, particularly species of *Zelurus* referred to in numerous articles by Herman Lent and Pedro (Petr) Wolfgang Wygodzinsky on this group (MALDONADO, 1990); while the Coleção Entomológica of the same institute (CEIOC), highlights the Reduviidae material studied by Ângelo Moreira da Costa Lima, separated into its own collection (Coleção Costa Lima), which includes specimens and types, recently cataloged by Almeida *et al.* (2014) and Rodrigues *et al.* (2017), and other specimens referred to in their articles, such as his review of *Zelurus* (as *Spiniger*) (COSTA LIMA, 1940b). Other collections with fewer type specimens, such as the collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP; recent catalog by CARRENHO *et al.*, 2020), or with less numerous collections but including types or specimens from caves, described more recently, such as the Coleção de Invertebrados Subterrâneos of the Universidade Federal de Lavras (ISLA/UFLA) Minas Gerais, also deserve to be mentioned.

For aquatic heteropterans from caves, most of the material currently collected is deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP), the Coleção de Invertebrados Subterrâneos of the Centros de Estudos em Biologia Subterrâneas of the Universidade Federal de Lavras (CEBS /UFLA), the Coleção Entomológica of the Instituto Oswaldo Cruz (IOC) and the Laboratório de Estudos Subterrâneos of the Universidade Federal de São Carlos (LES/UFSCar).

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Iuiuniscus iuiuensis
Rodrigo Lopes Ferreira

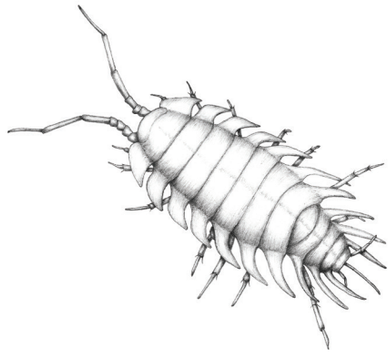
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Isopoda: Oniscidea

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General introduction

Terrestrial isopods, commonly known as "baratinhas de praia"[1], "tatuzinhos de jardim" (woodlice) or "tatu bolinha"[2] (pill bug), belong to the order Isopoda, suborder Oniscidea (ARAUJO, 1999a; WORMS, 2021). The group evolved from marine ancestors, probably during the Upper Carboniferous (ca. 236 Ma) and, due to morphological, physiological and behavioral modifications, represents the unique crustacean lineage completely adapted to the terrestrial habitats (BROLY *et al.*, 2013; HORNUNG, 2011; RICHARDSON; ARAUJO, 2015; TAITI, 2018). The group comprises about 4,000 species (CAMPOS-FILHO; TAITI, 2021), distributed in more than 500 genera and 38 families, occurring in the most varied terrestrial environments, including the coastal region, tropical forests, deserts, mountains (elevations of up to three thousand meters), urban environments and, of course, in caves (JAVIDKAR *et al.*, 2015; SCHMALFUSS, 2003; SFENTHOURAKIS; TAITI, 2015).

The monophyly of the suborder Oniscidea has been confirmed by several morphological and molecular studies (ERHARD, 1995, 1996, 1997; SCHMALFUSS, 1974, 1989; POORE, 2005; WÄGELE, 1989; WILSON, 2009). However, with the recent advancement of molecular methodologies, uncertain relationships exist regarding the presence of the family Ligiidae Leach, 1814 within Oniscidea, resulting in an emerging discussion about possible multiple invasions of the terrestrial environment (DIMITRIOU *et al.*, 2019). Thus far, the suborder has five recognized sections: Ligiidae, Tylida, Microcheta, Synocheta and Crinocheta, with the last accounting for more than 80% of the entire diversity of the suborder (SCHMALFUSS, 1989; SCHMIDT, 2002, 2008).

MORPHOLOGY

As representatives of the phylum Arthropoda, terrestrial isopods have a segmented body, a rigid cuticle and jointed appendages (BRUSCA *et al.*, 2016). In general, the body can be divided into three parts: (1) the **cephalothorax**, or cephalon, including two pairs of antennae, compound eyes (sometimes reduced or absent), and mouthparts composed of a pair of mandibles, maxillulae, maxillae and maxillipeds; (2) the **pereon**, or thorax, composed of seven segments, including one pair of pereopods per segment arranged ventrally; and (3) the **pleon**, or abdomen, divided into five segments, including one pair of biramous pleopods per segment disposed ventrally, the pleotelson and a pair of biramous uropods (Figure 1) (SUTTON, 1972). Figure 1 shows that these previously mentioned structures can be subdivided into subunits, or subarticulations, the nomenclature of which will be used from here on, as described below for a better understanding.

The cephalon (Figure 2), is formed by the fusion of a segment of the pereon and, for this reason, has a pair of maxillipeds and, sometimes, can present *noduli laterales* (described later). The cephalon is morphologically well known within Oniscidea (JACKSON, 1926, 1928) and presents important features used in the taxonomy of the group. In general terms, dorsally, it has a vertex[3], the compound eyes arranged laterally (they may be reduced or absent in endogenous and/or cave-dwelling species), the lateral lobes (better visualized in dorsal view), and the medial

1 The Portuguese term "baratinha de praia" (beach cockroach), sometimes also used for Oniscidea, generally refers to representatives of the family Ligiidae, which, for the most part, inhabit the rocky coastal environment. Brazilian representatives of the family belong to the genus *Ligia* Fabricius, 1798 (see Campos-Filho *et al.*, 2018) and have an interesting ecological-evolutionary strategy — the runner habit (see Schmalfuss, 1984). Therefore, when you (the reader) come across a small super-fast animal on the beach, you are probably observing terrestrial isopods of the genus *Ligia*. One of the recent productions of the comedy group *Porta dos Fundos*, entitled Reincarnation, mentions the group, even though it does not present representatives of Oniscidea (see video link available in the references). For the most assiduous, in the series *The Witcher*, hosted by Netflix, in the second episode of the second season, representatives of Oniscidea were discovered by the character *Geralt of Rivia*, in a moment in which he turns over a tree trunk (also an excellent way of collecting). Other Oniscidea videos can be found on YouTube by typing the word isopods into the platform's search engine.

2 The terms "tatuzinho de jardim" (woodlice) and "tatu bolinha" (pill bug) refer to representatives of the family Armadillidiidae, genus *Armadillidium* Brandt, 1831, commonly found in anthropized environments and with the ability to roll up into a perfect ball. Like the runner habit, this habit is also classified as an ecological-evolutionary strategy (see Schmalfuss, 1984).

3 The vertex may have tubercles and muscle insertions, which are clearly visible as depigmented areas.

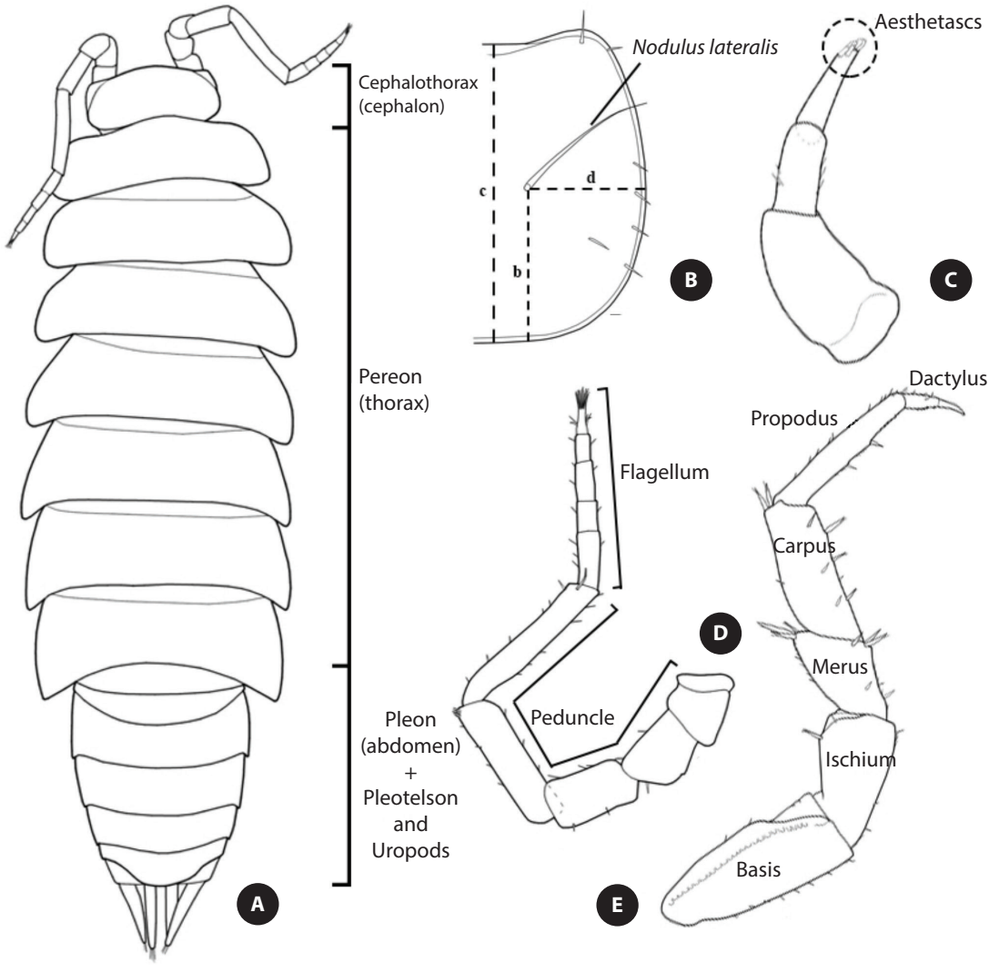


Figure 1. Body scheme of Oniscidea. **A)** *Xangoniscus* sp.; **B)** Example of pereonite with emphasis on the position of the *nodulus laterales* (Philosciidae); **C)** Antennula (*Xangoniscus* sp.); **D)** Antennae (*Xangoniscus* sp.); **E)** Pereopod 7 (Styloniscidae).

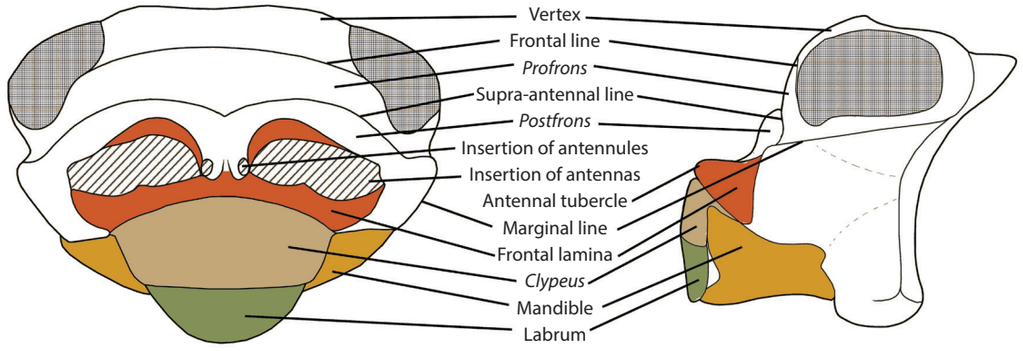


Figure 2. Morphological scheme of the head of Oniscidea. *Ligia oceanica* (Linnaeus, 1767), adapted from Jackson (1928).

lobe (sometimes can be well developed). Frontally, the *profrons*, the *postfrons*, the *clypeus* and the *labrum* can be visualized; still, there is the frontal line (*linea frontalis*), subdividing the *profrons* and the *postfrons*, and the supra-antennal line (*linea suprantennalis*), delimiting the upper portion of the *postfrons*[4]. In the *postfrons*, the two pairs of antennae are inserted (see also Figure 1A, C, D) and, medially, there may be a frontal lamina (*lamina frontalis*)[5] (VANDEL, 1943, 1952). Laterally, the head presents a marginal line, which delimits the lateral lobes (JACKSON, 1926). Ventrally, the head presents the mouthparts, composed of a pair of mandibles, maxillae, maxillulae and maxillipeds, which present important taxonomic information.

The pereon is formed by seven segments, the pereonites. Pereonites may have a smooth or tuberculated dorsal surface (in some cases the tubercles are reduced, giving a granulated appearance). On this surface there are two structures, which in general are very small and, often, overlooked by non-specialists — the scale-setae and the *noduli laterales* (HORNUNG, 2011 for full references; WOOD *et al.*, 2017). The scale-setae have distinct shapes, and may vary at family, genus, or species levels. In some representatives, such as species of the genera *Trichorhina* Budde-Lund, 1908 and *Metaprosekia* Leistikow, 2000, the pereonites may have a pilose appearance, which is nothing more than a high density of these setae. The *noduli laterales* (Figure 1) are sensory setae present in the epimera of pereonites 1–7 (lateral region of pereonites) and, in some groups, may be retained in the head (recalling that the head is formed by the fusion of one pereon segment), such as in *Pagana* Budde-Lund, 1908, family Trachelipodidae Strouhal, 1953. These setae, in general, are morphologically distinct from scale-setae, but in some groups they are not discernible, as in *Ischioscia* Verhoeff, 1928; in others, they are easily observed, as in *Androdeloscia* Leistikow, 1999; and in others, they are less noticeable, such as representatives of Armadillidae Brandt, 1831 or Scleropactidae Verhoeff, 1938, in which case, a "trained eye" will be necessary. The positioning of the *noduli* along the pereonites is of great taxonomic importance, as their measurements in relation to the lateral and posterior margins (measurements d/c and b/c, respectively) generate a graph of Cartesian coordinates (VANDEL, 1962), helping to recognize genera and, in some cases, species (see Figure 1B).

Still in the pereon, ventrally, there are seven pairs of locomotor appendages, the pereopods, which are composed of the following articles: basis, ischium, merus, carpus, propodus, and dactylus (Figure 1E). In general, the first and seventh pairs have the most taxonomic information, but in some cases, as in representatives of *Xangoniscus* Campos-Filho, Araujo & Taiti, 2014, all pereopods may have different characteristics. The carpus and propodus of pereopod 1 have the antennal cleaning apparatus (dense field of setae), which can be arranged transversely or longitudinally. The dactylus is formed by two claws, one thinner internally and the other more robust externally; however, in some groups the internal claw may be reduced or absent (SCHMIDT, 2002). Located between these claws is a sensory seta — the ungual seta — and dorsally to the external claw is another sensory seta — the dactylar seta or dactylar organ — which can vary distinctly between species of the same genus and is very useful in taxonomy. In more basal groups, or even in more derived groups, the sixth and seventh pairs of pereopods may have specific scales used to capture and transport water. In Oniscidea, these pereopods are also used for reproduction, when the male attaches to the female. In general, pereopods have important information for the taxonomy of Oniscidea, such as the shape of the articles, disposition and types of setae, arrangement of the antennae cleaning apparatus and presence of lobes, among others. These structures deserve special attention when observed.

Finally, the pleon (Figures 1 and 3), consists of five segments, the pleonites, a pair of uropods and the pleotelson, this last being the fusion of the last segment of the pleon and the telson (Figure 1A). The pleon has a reduced body plan in relation to the pereon, with pleonites 1 and 2 reduced and more closely inserted in the posterior portion of the seventh pereonite. Pleonites 3 to 5 have expanded lateral margins, and this expansion may vary. In representatives of runner or swimmer[7] body plan types, it is more common for pleonite epimera to be reduced, favoring running or swimming dynamics. In representatives with an

4 The presence and/or absence of frontal and supra-antennal lines, which are widely used in taxonomy, may vary according to the group of Oniscidea.

5 Like the frontal and supra-antennal lines, the frontal lamina can vary among representatives of Oniscidea.

6 These scales are also disposed ventrally close to the region of separation between the epimera of the pereonites and the oostegites (ventral plates), next to the base of insertion of the pereopods. They present a groove or line, which run transversely in all pereonites (see Hoese 1981).

7 Here it is worth mentioning that the troglobitic species *Iuiuniscus iuiuensis* Souza, Senna & Ferreira, 2015, despite being an amphibious representative, has extremely developed epimera of pereonites 1–7 and pleonites 3–5. However, this species has a builder-type ecological-evolutionary strategy, due to its ability to build shelters with cave sediment.

adherent, crawling or volvational body plan, it is more common to observe these epimera expanded, so that they fill the space between pereonite epimere 7 and the pleotelson. The uropods are biramous structures inserted laterally to the pleotelson and composed of a protopod (base) and two rami: the exopod and the endopod. According to the categories mentioned above, as well as the epimera of pleonites 3–5, the protopod can be found expanded, filling the space between the epimera of pleonite 5 and the pleotelson. The pleotelson, or just telson, may vary in shape according to the group (Figure 1A). Pleon structures are of great taxonomic importance for the recognition of families and/or genera.

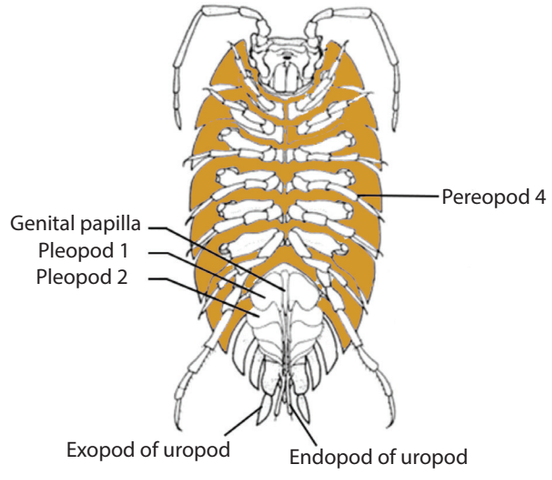


Figure 3. Body scheme of Oniscidea, ventral portion. *Oniscus asellus* Linnaeus, 1758, adapted from Sutton (1980).

The pleon, ventrally, has five pairs of biramous pleopods, consisting of an exopod and an endopod[8]. It should be noted that the first two pairs of the male, in addition to presenting reproductive modifications, carry extremely useful information for the taxonomy of the group. The first two endopod pairs are elongated and may have a complex structure. In Synocheta, the protopod, the exopod of pleopod 1 and the endopod of pleopod 2 of the male have different shapes according to the group. For example, species of *Xangoniscus* and *Pectenoniscus* Andersson, 1960 present the distal portion of the endopod of pleopod 2 of the male with complex structure. In Crinocheta, the protopod of pleopods 1 and 2 are reduced and the main features are retained in pleopod 1 and, in some cases, in endopod 2. For example, in representatives of *Benthana* Budde-Lund, 1908 and *Trichorhina* the endopod is considered simple, while in species of *Androdeloscia* and *Erophiloscia* Vandel, 1972, it can be extremely modified.

As mentioned earlier, Oniscidea conquered the terrestrial environment and became independent of an external source of water for embryonic development. Therefore, among the morphological modifications that made this evolutionary process possible, two important structures should be highlighted. The first is the emergence and development of pleopodal lungs, specialized structures for air breathing, which can vary from a simple respiratory area (reduction of the thickness of the exopod cuticle) to a branched structure that can reach close to the heart (FERRARA *et al.*, 1994, 1997; LEISTIKOW; ARAUJO, 2001; PAOLI *et al.*, 2002). The second structure is the marsupium or microaquarium, which promotes the development of offspring regardless of an external source of water (APPEL *et al.*, 2011; HOESE, 1984; HOESE; JANSSEN, 1989; WIESER, 1984).

REPRODUCTION

Copulation (Figure 4) occurs when the male positions himself obliquely or transversely on the female's back[10] (KIGHT, 2009; SUTTON, 1980). Contact takes place for a short period of time, thus reducing the risk of predation. Both this process and all reproductive physiological factors are complexly modulated by: 1) abiotic factors, such as temperature, photoperiod, humidity, latitude, etc.; 2) biotic factors, such as presence and interaction with males (increased vitellogenesis and receptivity), partner choice, sperm competition, presence of microorganisms, etc.; and 3) genetic factors (CAUBET *et al.*, 1998; KIGHT, 2009; LEFEBVRE; CAUBET, 2010; LEFEBVRE *et al.*, 2000; MOREAU *et al.*, 2001). The development of offspring is direct, that is, there are no larval stages as in other groups of crustaceans. Therefore, throughout the stages of development, they are born as "mancas"[11] (ARAUJO

8 In representatives of Synocheta, for example Styloniscidae, the protopod is developed and, in Tylidae, the pleopod 1 is absent.

9 The marsupium can be classified into two types; the amphibious or open type, found in representatives of Ligiidae Leach, 1814, and the terrestrial or closed type, found in more derived forms (see Hoese, 1984).

10 For a better visualization of the copulation process, a recent publication about the group provides two short videos that clearly demonstrate the positioning of the specimens (CAMPOS-FILHO *et al.*, 2022, see Supplementary Material).

11 Differentiated from juveniles by having a pereon composed of six segments, which will be composed of seven segments after the first or second molt.

et al., 2004; BRUM; ARAUJO, 2007; SOKOLOWICZ; ARAUJO, 2011; MONTESANTO, 2018; ZECCHIN; MONTESANTO, 2019), and become juveniles and then adults, with similar morphologies, all of these going through a molting stage, common in Arthropoda.

Morphologically, the male reproductive appendages, endopod of pleopods 1 and 2 (see Figure 3), are better known in Oniscidea, probably due to their use in taxonomy. The female genitalia, located on pereonite 5, close to the base of the respective pereopod, can only be observed during their reproductive period[12] (SUZUKI, 2005; SUZUKI; ZIEGLER, 2005), which has an important peculiarity — the presence of a spermatheca[13] (SUZUKI; ZIEGLER, 2005, 2014) to store spermatozoa. This storage, very important for vitellogenesis, can be seen as an evolutionary strategy for a possible absence of males in the population or in cases where males are unable to stimulate females properly (LEFEBVRE *et al.* 2019). During reproduction, females play a fundamental role in reproductive success and not only perform the functions of carrying, protecting and providing food[14] to the offspring, but also are responsible for choosing the male, the moment of copulation and pairing with the male, among other actions (LEFEBVRE *et al.* 2019).



Figure 4. *Xangoniscus santinhoi* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020 in copula. Photo: (Rodrigo L. Ferreira).

Parental care, common in other crustacean representatives, is rare in Oniscidea, with few representatives presenting this behavior. In general, isopods living in desert regions demonstrate some type of social behavior, including parental care. *Hemilepistus reaumurii* (Milne Edwards, 1840) (Agnaridae), the best known, also has peculiar behavioral characteristics, such as monogamy and a single reproductive event throughout life[15] (ACHOURI, 2012; SHACHAK *et al.*, 1979; THIEL, 2000). Other specific behaviors, which may be related to parental care, such as construction, maintenance and defense of galleries, diurnal or nocturnal habits and chemical recognition of offspring, are also known for *H. reaumurii* (SHACHAK *et al.*, 1979), as well as species of the genus *Porcellio* (Porcellionidae) inhabiting desert regions in Tunisia (MEDINI-BOUAZIZ, 2018; MEDINI-BOUAZIZ *et al.*, 2016) and *Schizidium tiberianum* Verhoeff, 1923 (Armadillidiidae) (WARBURG, 1994; WARBURG; COHEN, 1991). Parental care in *H. reaumurii* is performed by both adult individuals, while in *Porcellio albinus* Budde-Lund, 188516, only females guard the offspring (MEDINI-BOUAZIZ *et al.*, 2017; THIEL, 2000).

BEHAVIOR AND ECOLOGY

Among behavioral adaptations, two characteristics are worth mentioning that are strictly linked to aspects of morphology and physiology. The first involves the ability to roll up (volvation), which is exhibited in some families of the group, such as Armadillidae, Pudeoniscidae and Scleropactidae, where the morphological characteristics of the closing/locking mechanism are important in a taxonomic context (SCHMIDT, 2002, 2003). Furthermore, this ability is partly responsible for resistance to water loss (WIESER, 1963) and is also used as a defense strategy against predators (SCHMALFUSS, 1984). The second involves the gregarious habit (ALEE, 1926), which is stimulated by aggregation or reproduction pheromones, which reduces the exposed body surface sensitive to perspiration, thereby preventing water loss (BROLY *et al.*, 2014;

12 Here, well-trained eyes are also needed, both to recognize the pores and to identify whether the female is in the reproductive molt phase.

13 An interesting aspect, already mentioned by other researchers and/or colleagues who venture to raise isopods in aquariums, is that, sometimes, they do not understand how their aquarium became full of isopods when they placed only one individual. In response, we always asked what the individual's gender was.

14 It is worth remembering that in species without marsupial extension, the female does not feed when she is ovigerous, due to the narrowing of the alimentary duct and, in species with marsupial extension, the female feeds even when she is ovigerous. For a better understanding of this see Hoese (1984), Hoese and Janssen (1989) and Appel *et al.* (2011).

15 Monogamy and semelparity is also observed in *Hemilepistus klugii* (Brandt, 1833) and *Schizidium tiberianum* (see Warburg & Cohen, 1991; Warburg, 1994; Kashani *et al.*, 2011).

16 Species considered iteroparous.

SCHMALFUSS, 1998; TAKEDA, 1980, 1984). This habit also has additional benefits, such as reduced oxygen consumption, increased body growth and biotic stimuli for reproduction, among others (BROLY *et al.*, 2012, 2013, 2015).

It must be remembered that the process of becoming terrestrial involves a new diet. Soon, the diet of oniscids consisted mainly of decaying organic matter, such as leaves, wood, fungi and bacterial mats. Sometimes, oniscids can prey on other invertebrates and, occasionally, insect larvae (PAOLETTI; HASSAL, 1999; ZIMMER *et al.*, 1996, 2003; ZIMMER; TOPP, 2000, 2002). As these animals, together with other organisms, make up the edaphic macrofauna and are considered important detritivores related to the process of decomposition of organic matter, their dynamics and population density influence this process, possibly affecting the physical and chemical composition of the soil (QUADROS; ARAUJO, 2008; ZIMMER *et al.*, 2005; ZIMMER; TOPP, 2000).

As every animal is part of a trophic web at some level, oniscids can also serve as food for various animals, such as other arthropods (spiders, scorpions and insects) and vertebrates (amphibians, reptiles and insectivorous mammals) (ARAUJO, 1999a; PARIS, 1963; WARBURG *et al.*, 1984). However, when attacked, they show different defense strategies directly related to their body constitution (Figure 5), being classified as runners, clingers, creepers, spiny forms, volvational (rollers) or nonconformists, the last for species that do not fit into the previous categories (see SCHMALFUSS, 1984, for greater clarity about these categories). In addition to these, two other functional categories were proposed: jumpers (HASSALL *et al.*, 2006), recorded for a representative of *Burmoniscus* from Sabah, Malaysia, and swimmers (TAITI; XUE, 2012), mainly cave representatives secondarily adapted to the aquatic habitat of the subterranean environment.

Brazilian caves and Oniscidea

Brazil has a large number of natural subterranean cavities (caves) in its territory, exceeding 22,000 records in the official CECAV/ICMbio database (CANIE, 2020). These caves are formed of different types of rock, such as carbonate, siliciclastic, igneous and ferruginous. It is interesting to note that caves seem to be a very important refuge for isopods, as they are environments with high humidity and little temperature fluctuation, even in regions with semi-arid climates (FERNANDES *et al.*, 2019). This corroborates the idea that, possibly, terrestrial isopods represent the group with the largest number of subterranean taxa in Brazil, taking into account troglobitic and troglophile representatives. Several subterranean groups of Oniscidea appear to have preferences for specific microhabitats, such as fresh guano deposits and decomposed plant matter, or are opportunistic in their use of these resources (Figure 6).

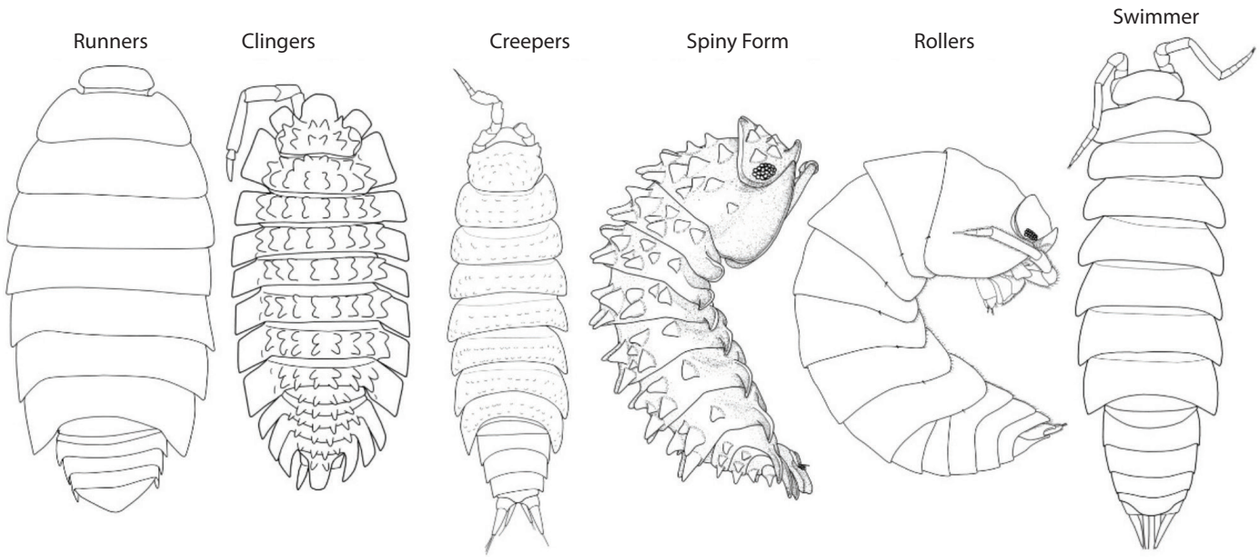


Figure 5. Ecomorphological categories of Oniscidea for the Brazilian cave environment, *sensu* SCHMALFUSS, (1984) and TAITI; XUE, (2012), including epigean species. From the left: *Benthana* sp. (Philosciidae), *Neotroponiscus* sp. (Bathytropidae), *Pectenoniscus* sp. (Styloniscidae), *Diploexochus echinatus* Brandt, 1833 (Armadillidae), *Circoniscus* sp. (Scleropactidae) and *Xangoniscus* sp. (Styloniscidae).



Figure 6. A) Maria E. Bichuette collecting at Gruta da Tapagem (Caverna do Diabo), Parque Estadual Caverna do Diabo, Eldorado, state of São Paulo; **B)** *Xangoniscus loboi* Campos-Filho, Gallão & Bichuette, 2022 inside a waterhole in Gruta da Pingueira II, Serra do Ramalho, state of Bahia; **C)** Entrance to an iron ore cave demonstrating the surrounding vegetation cover, Parauapebas, state of Pará; **D)** Collecting material together with decomposing organic matter; **E)** Dubioniscidae sp. in moist vegetation cover (moss) on the wall of a cave in the municipality of Altamira, state of Pará. Photos: A (Tamirez Zepon) B-E (Maria Elina Bichuette).

Diversity of troglobitic species

To date, 72 species of terrestrial isopods have been recorded for the subterranean environment in Brazil, 38 of which are classified as troglobites (Table 1, Figures 7 and 8); the other species are classified as troglophiles. Certainly, there are cases where species can be considered as possible trogloxenes or accidental. However, in general, there is a lack of information on occurrence outside of caves, that is, the absence of collections in the epigean or surface environment.

Generally, the main troglomorphic characteristics in Oniscidea are the absence of pigmentation and eyes, classic troglomorphisms in other diverse groups of invertebrates. Some groups exhibit elongation of the body and/or appendages (CAMPOS-FILHO *et al.*, 2014; FERNANDES *et al.*, 2016; REBOLEIRA *et al.*, 2015).

The following list includes all the species considered troglobitic until June 2022, and the map (Figure 8) shows the occurrences of these species. From the regions of occurrence of the species, it is clear that the distribution reflects the results of the collection efforts of the two most active laboratories focusing on studies of the subterranean fauna in Brazil: Laboratório de Estudos Subterrâneos da Universidade Federal de São Carlos (LES/UFSCar) and Centro

de Estudos em Biologia Subterrânea da Universidade Federal de Lavras (CEBS/UFLA). There are still many distribution gaps for faunal studies, which demand time for collections, human resources for sample processing and specialists for their recognition.

Most species occur outside conservation units and, mainly, in limestone lithology, with few records in sandstone and quartzite lithologies, which also demonstrates greater collection efforts in this type of lithology in Brazil. It is also worth highlighting the existence of hundreds to thousands of specimens collected in ferruginous caves, mainly from environmental consultancy studies (licensing). However, it can be said that for these specimens, studies are still in the beginning, as most have not been scientifically determined or even described. As observed for other taxa, taxonomic impediment is also the rule for Brazilian subterranean Oniscidea. It should also be noted that there are certainly dozens to hundreds of troglophile oniscids to be described, which will increase the representativeness of this group in Brazilian caves.

Figure 9 demonstrates some of the diversity of species of Oniscidea found in Brazilian caves.

Table 1. List of troglobitic species described for Brazil and their updated records.

Family **Styloniscidae** Vandel, 1952

Genus ***Chaimowiczia*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2021
1. ***Chaimowiczia obyobytyra*** Cardoso, Bastos-Pereira & Ferreira, 2022
Chaimowiczia obyobytyra Cardoso *et al.*, 2022a: 141, figs 1-5, 6C, D.
Amphibious species recorded only for Serra Verde cave, municipality of Coribe, state of Bahia (CARDOSO *et al.*, 2022a).

2. ***Chaimowiczia tatus*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2021
Chaimowiczia tatus Cardoso *et al.*, 2021: 48, figs 2-5.
Amphibious species recorded only for Gruta do Padre cave, municipality of Santana, state of Bahia (CARDOSO *et al.*, 2021).

3. ***Chaimowiczia uai*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2021
Chaimowiczia uai Cardoso *et al.*, 2021: 53, figs 6-9.
Amphibious species recorded only for Lapa d'água do Zezé cave, municipality of Itacarambi, state of Minas Gerais (CARDOSO *et al.*, 2021).

Genus ***Iuiuniscus*** Souza, Ferreira & Senna, 2015
4. ***Iuiuniscus iuiuensis*** Souza, Ferreira & Senna, 2015
Iuiuniscus iuiuensis Souza *et al.*, 2015: 6, figs. 1–3, 4D, E, 5, 6; Campos-Filho *et al.*, 2016: 2; Angarten *et al.*, 2017: 17; Bastos-Pereira *et al.*, 2017: 292; Campos-Filho *et al.*, 2017: 70; Cavalcanti, 2017: 50, Tab. 2; Campos-Filho *et al.*, 2018: 4; Gallão & Bichuette, 2018: 7, Tab. 1; Silva *et al.*, 2018: 55; Campos-Filho *et al.*, 2019: 423 e 425, figs 3-6, 17A; Fernandes *et al.* 2019: 1104, Tab. 1; Cardoso *et al.*, 2020a: 84.
Amphibious species recorded only for Lapa do Baixão cave, municipality of Iuiu, karst area of Serra do Iuiu, state of Bahia (SOUZA *et al.*, 2015).

Genus ***Pectenoniscus*** Andersson, 1960
5. ***Pectenoniscus carinhanhensis*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Pectenoniscus carinhanhensis Cardoso *et al.*, 2020b: 13, figs 11-13, 21, 22D.
Species recorded for the caves Gruna da Água Clara, Gruna dos Peixes II and Gruna dos Índios, all in the municipality of Carinhanha, karst area of Serra do Ramalho, state of Bahia (CARDOSO *et al.*, 2020b).

6. ***Pectenoniscus fervens*** Campos-Filho, Taiti & Bichuette, 2022
Pectenoniscus fervens Campos-Filho *et al.*, 2022a: 46, figs 1, 2C, D, 4D, E, 7,8.
Species recorded for Gruta Toca Coroa do Frade cave, municipality of Coronel José Dias, state of Piauí (CAMPOS-FILHO *et al.*, 2022a).

7. ***Pectenoniscus iuiuensis*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Pectenoniscus iuiuensis Cardoso *et al.,* 2020b: 10, figs 8-10, 21, 22C.
Species recorded for Baixa da Fortuna cave, municipality of Iuiu, karst area of Serra do Iuiu, state of Bahia (CARDOSO *et al.,* 2020b).

8. ***Pectenoniscus juveniliensis*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Pectenoniscus juveniliensis Cardoso *et al.*, 2020b: 6, figs 5-7, 21, 22B.
Species recorded for Gruta do Tabuleirinho cave, also known as Lapa Grande, municipality of Juvenília, state of Minas Gerais (CARDOSO *et al.,* 2020b).

9. ***Pectenoniscus liliae*** Campos-Filho, Bichuette & Taiti, 2019
Pectenoniscus liliae Campos-Filho *et al.,* 2019: 430, figs 8-10, 17; Cardoso *et al.,* 2020b: 2, fig. 21.
Species recorded for the caves Gruna do Chico Pernambuco and Gruna do Enfurnado, both in the municipality of Coribe, karst area of Serra do Ramalho, state of Bahia (CAMPOS-FILHO *et al.,* 2019).

10. ***Pectenoniscus montalvaniensis*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Pectenoniscus montalvaniensis Cardoso *et al.,* 2020b: 2, figs 1-4, 21, 22A.
Species recorded for Abrigo da Ema cave, municipality of Montalvânia, state of Minas Gerais (CARDOSO *et al.,* 2020b).

11. ***Pectenoniscus morrensis*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Pectenoniscus morrensis Cardoso *et al.,* 2020b: 17-20, 21, 22F.
Species recorded for Gruta dos Brejões cave, municipality of Morro do Chapéu, state of Bahia (CARDOSO *et al.,* 2020b).

12. ***Pectenoniscus pankaru*** Campos-Filho, Torres & Bichuette, 2022
Pectenoniscus pankaru Campos-Filho *et al.*, 2022a: 43, figs 1, 3E, 4A-C, 5, 6.
Species recorded for Gruna do Govi cave, municipality of Feira da Mata, state of Bahia (CAMPOS-FILHO *et al.*, 2022a).

13. ***Pectenoniscus santanensis*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Pectenoniscus santanensis Cardoso *et al.,* 2020b: 14-16, 21, 22E.
Species recorded for Gruta do Padre cave, municipality of Santana, state of Bahia (CARDOSO *et al.,* 2020b).

Genus ***Spelunconiscus**** Campos-Filho, Araujo & Taiti, 2014
14. ***Spelunconiscus castroi*** Campos-Filho, Araujo & Taiti, 2014
Spelunconiscus castroi Campos-Filho, Araujo & Taiti *in* Campos-Filho *et al.,* 2014: 368, figs. 5–8, 40; Campos-Filho *et al.,* 2016: 2; Angarten *et al.,* 2017: 17; Bastos-Pereira *et al.,* 2017: 292; Campos-Filho *et al.,* 2017: 70; Cavalcanti, 2017: 50, Tab. 2; Campos-Filho *et al.,* 2018: 4; Gallão & Bichuette, 2018: 7, Tab. 1; Silva *et al.,* 2018: 56; Campos-Filho *et al.,* 2019: 423.
Amphibious specie recorded for Eritrina cave, municipality of Matozinhos, state of Minas Gerais (CAMPOS-FILHO *et al.,* 2014).

Genus ***Xangoniscus*** Campos-Filho, Araujo & Taiti, 2014
15. ***Xangoniscus aganju*** Campos-Filho, Araujo & Taiti, 2014
Thailandoniscus sp. 2 Trajano & Bichuette, 2010: 10, Tab. 1; Cavalcanti, 2017: 50, Tab. 2.
Xangoniscus aganju Campos-Filho, Araujo & Taiti *in* Campos-Filho *et al.*, 2014: 373, figs. 9–13, 40.; Campos-Filho *et al.*, 2016: 2; Campos-Filho *et al.*, 2017: 70; Fernandes *et al.*, 2016: 7, Tab. 1; Trajano *et al.*, 2016: 1817; Angarten *et al.*, 2017: 17; Bastos-Pereira *et al.*, 2017: 292; Cavalcanti, 2017: 50, Tab. 2; Campos-Filho *et al.*, 2018: 5; Campos-Filho *et al.*, 2019: 423; Cardoso *et al.*, 2020a: 84, fig. 20.
Amphibious specie recorded for the caves Gruna do Chico Pernambuco, Gruna do Domingão and Gruna do Mandiaçu, all in the municipality of Carinhanha, karst area of Serra do Ramalho, state of Bahia (CAMPOS-FILHO *et al.,* 2014, 2019).

* After the writing of this chapter, a new troglodytic species of the genus *Spelunconiscus* was describe for teh region of Lagoa Santa, Minas Gerais (BASTOS-PEREIRA *et al*., 2022)

16. ***Xangoniscus ceci*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Xangoniscus ceci Cardoso *et al.*, 2020a: 93, figs 9-11, 19C, 20.
Amphibious specie recorded for Mãe Diná cave, municipality of São João da Ponte, state of Minas Gerais (CARDOSO *et al.*, 2020a).

17. ***Xangoniscus dagua*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Xangoniscus dagua Cardoso *et al.*, 2020a: 89, figs 5-8, 19B, 20.
Amphibious specie recorded for Lapa D'Águacave , Parque Estadual da Lapa Grande, municipality of Montes Claros, state of Minas Gerais (CARDOSO *et al.*, 2020a).

18. ***Xangoniscus ibiracatuensis*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Xangoniscus ibiracatuensis Cardoso *et al.*, 2020a: 97, figs 12-14, 19D, 20.
Amphibious specie recorded for Gruta São José III cave, municipality of Ibiracatu, state of Minas Gerais (CARDOSO *et al.*, 2020a).

19. ***Xangoniscus itacarambiensis*** Bastos-Pereira, Souza & Ferreira, 2017
Xangoniscus itacarambiensis Bastos-Pereira *et al.*, 2017: 292, figs. 1D-5; Campos-Filho *et al.*, 2017: 70; Campos-Filho *et al.*, 2018: 5; Gallão & Bichuette, 2018: 7, Tab. 1; Silva *et al.*, 2018: 56, Tabs. 1–4, figs. 2c, 3–9, 10a-d, f; Campos-Filho *et al.*, 2019: 423; Cardoso *et al.*, 2020a: 84, fig. 20.
Amphibious specie recorded for Gruta Olhos D'Água cave, municipality of Itacarambi, Parque Nacional Cavernas do Peruaçu, state of Minas Gerais (BASTOS-PEREIRA *et al.*, 2017).

20. ***Xangoniscus lapaensis*** Campos-Filho, Gallo & Bichuette, 2022
Xangoniscus lapaensis Campos-Filho, Gallo & Bichuette *in* Campos-Filho *et al.* 2022b: 4, figs 1, 2, 4-7.
Amphibious specie recorded for Gruna Boca da Lapa cave, municipality of Feira da Mata, karst area of Serra do Ramalho, state of Bahia (CAMPOS-FILHO *et al.* 2022b).

21. ***Xangoniscus loboi*** Campos-Filho, Gallão & Bichuette, 2022
Xangoniscus loboi Campos-Filho, Gallão & Bichuette *in* Campos-Filho *et al.* 2022b: 7, figs 1, 3, 8-11.
Amphibious specie recorded for Gruna da Pingueira II cave, municipality of Feira da Mata, karst area of Serra do Ramalho, state of Bahia (CAMPOS-FILHO *et al.* 2022b).

22. ***Xangoniscus lundi*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Xangoniscus lundi Cardoso *et al.*, 2020a: 85, figs 1-4, 19A, 20.
Endemic amphibious species recorded for Lapa Grande cave, Parque Estadual da Lapa Grande, municipality of Montes Claros, state of Minas Gerais (CARDOSO *et al.*, 2020a).

23. ***Xangoniscus odara*** Campos-Filho, Bichuette & Taiti, 2016
Xangoniscus odara Campos-Filho *et al.*, 2016: 2, figs. 2–7, 14; Angarten *et al.*, 2017: 17; Campos-Filho *et al.*, 2017: 70; Bastos-Pereira *et al.*, 2017: 292; Campos-Filho *et al.*, 2018: 5; Gallão & Bichuette, 2018: 7, Tab. 1; Silva *et al.*, 2018: 56; Campos-Filho *et al.*, 2019: 423; Cardoso *et al.*, 2020a: 84, fig. 20.
Amphibious specie recorded for Lapa do Cipó cave, Parque Nacional Cavernas do Peruaçu, municipality of Itacarambi, state of Minas Gerais (CAMPOS-FILHO *et al.*, 2016).

24. ***Xangoniscus santinhoi*** Cardoso, Bastos-Pereira, Souza & Ferreira, 2020
Xangoniscus santinhoi Cardoso *et al.* 2020a: 100, figs 15-18, 19E, 20.
Amphibious specie recorded for Lapa do Zezé cave and Lapa d'Água do João Ferreira, municipality of Itacarambi, state of Minas Gerais (CARDOSO *et al.*, 2020a).

Família **Philosciidae** Kinahan, 1857
Genus ***Alboscia*** Schultz, 1995
25. ***Alboscia jotajota*** Campos-Filho, Bichuette & Taiti, 2020
Alboscia jotajota Campos-Filho, Bichuette & Taiti *in* Campos-Filho *et al.*, 2020: 6, figs 2-4, 14.
Species recorded for the caves Gruta Minotauro, Parque Estadual de Intervales, and Ressurgência das Areias de Água Quente, Parque Estadual Turístico Alto do Ribeira, both in the municipality of Iporanga, state of São Paulo (CAMPOS-FILHO *et al.*, 2020).

Genus ***Benthana*** Budde-Lund, 1908
26. ***Benthana iporangensis*** Lima & Serejo, 1993
Benthana iporangensis Lima & Serejo, 1993: 490, figs. 1–4; Pinto-da-Rocha, 1995: 97; Souza-Kury, 1998: 659; Leistikow & Wägele, 1999: 14; Schmalfuss, 2003: 53; Campos-Filho *et al.*, 2015: figs. 30, 31, 32C; Pires *et al.*, 2015: 69, Tab. 1; Trajano *et al.*, 2016: 1819; Bastos-Pereira *et al.*, 2017: 292; Cavalcanti, 2017: 49, Tab. 2; Campos-Filho *et al.*, 2018: 10; Gallão & Bichuette, 2018: 7, Tab. 1; Campos-Filho *et al.*, 2019: 423; Campos-Filho *et al.*, 2020: 16, fig. 14.
Species recorded for the caves Gruta Detrás (Parque Estadual de Intervales), Ressurgência das Areias de Água Quente, Gruta do Couto and Gruta do Morro Preto (Parque Estadual Turístico Alto do Ribeira), all in the municipality of Iporanga, state of São Paulo (CAMPOS-FILHO *et al.*, 2020).

27. ***Benthana xiquinhoi*** Campos-Filho, Bichuette & Taiti, 2019
Benthana xiquinhoi Campos-Filho, Bichuette & Taiti *in* Campos-Filho *et al.*, 2019: 434, figs 11-13, 18D.
Species recorded for the caves Gruna Parede Vermelha, Gruna Veio de Aurélio and Gruna Lava Pé, all in Povoado de Igatu, in the municipality of Andaraí, within the limits of Parque Nacional da Chapada Diamantina, state of Bahia (CAMPOS-FILHO *et al.*, 2019).

Genus ***Metaprosekia*** Leistikow, 2000
28. ***Metaprosekia igatuensis*** Campos-Filho, Fernandes & Bichuette, 2020
Metaprosekia igatuensis Campos-Filho, Fernandes & Bichuette *in* Campos-Filho *et al.*, 2020: 18, figs 8-10, 14.
Species recorded for Gruna Rio dos Pombos cave, Povoado de Igatu, municipality of Andaraí, within the limits of Parque Nacional da Chapada Diamantina, state of Bahia (CAMPOS-FILHO *et al.*, 2020).

Family **Scleropactidae** Verhoeff, 1938

Genus ***Amazoniscus*** Lemos de Castro, 1967
29. ***Amazoniscus eleonorae*** Souza, Bezerra & de Araújo, 2006
Amazoniscus eleonorae Souza *et al.*, 2006: 37, figs. 1–20; Campos-Filho *et al.*, 2014: 361, fig. 40; Pires *et al.*, 2015: 69, Tab. 1; Campos-Filho *et al.*, 2016: 2; Angarten *et al.*, 2017: 17; Bastos-Pereira *et al.*, 2017: 292; Campos-Filho *et al.*, 2017: 70; Cavalcanti, 2017: 49, Tab. 2; Gallão & Bichuette, 2018: 7, Tab. 1; Campos-Filho *et al.*, 2018: 16; Campos-Filho *et al.*, 2019: 423.
Species recorded for the caves Pedra da Cachoeira, Planaltina and Limoeiro, all in the municipality of Altamira, state of Pará (SOUZA *et al.*, 2006).

30. ***Amazoniscus leistikowi*** Campos-Filho, Araujo & Taiti, 2014
Amazoniscus leistikowi Campos-Filho, Araujo & Taiti *in* Campos-Filho *et al.*, 2014: 391, figs. 23–25, 40; Campos-Filho *et al.*, 2016: 2; Angarten *et al.*, 2017: 17; Campos-Filho *et al.*, 2017c: 11; Campos-Filho *et al.*, 2017: 70; Cavalcanti, 2017: 49, Tab. 2; Campos-Filho *et al.*, 2018: 16; Gallão & Bichuette, 2018: 7, Tab. 1; Campos-Filho *et al.*, 2019: 423.
Species recorded for Abrigo do Sismógrafo cave, municipality of Altamira, state of Pará (CAMPOS-FILHO *et al.*, 2014).

31. ***Amazoniscus spica*** Campos-Filho, Aguiar & Taiti, 2020
Amazoniscus spica Campos-Filho, Aguiar & Taiti *in* Campos-Filho *et al.*, 2020: 23, figs 11-14.

Species recorded for the iron cave CAV-20, Jaguar, municipality of Parauapebas, state of Pará (CAMPOS-FILHO *et al.* 2020).
Genus ***Circoniscus*** Pearse, 1917
32. ***Circoniscus buckupi*** Campos-Filho & Araujo, 2011
Circoniscus buckupi Campos-Filho & Araujo, 2011b: 28, figs. 1–3, 7; Pires *et al.*, 2015: 69, Tab. 1; Campos-Filho *et al.*, 2016: 2; Angarten *et al.*, 2017: 17; Bastos-Pereira *et al.*, 2017: 292; Campos-Filho *et al.*, 2017: 70; Cavalcanti, 2017: 49, Tab. 2; Bueno *et al.*, 2018: 6, Tab. 2; Campos-Filho *et al.*, 2018: 17; Gallão & Bichuette, 2018: 7, Tab. 1; Campos-Filho *et al.*, 2019: 423.
Species recorded for various iron caves in the region of the municipality of Parauapebas, state of Pará (CAMPOS-FILHO; ARAUJO, 2011; CAMPOS-FILHO *et al.,* 2018).

33. ***Circoniscus carajasensis*** Campos-Filho & Araujo, 2011
Circoniscus carajasensis Campos-Filho & Araujo, 2011: 34, figs. 4–7; Campos-Filho *et al.*, 2014: 396, fig. 40; Pires *et al.*, 2015: 69, Tab. 1; Campos-Filho *et al.*, 2016: 2; Angarten *et al.*, 2017: 17; Bastos-Pereira *et al.*, 2017: 292; Campos-Filho *et al.*, 2017: 70; Cavalcanti, 2017: 49, Tab. 2; Campos-Filho *et al.*, 2018: 17; Gallão & Bichuette, 2018: 7, Tab. 1; Campos-Filho *et al.*, 2019: 423.
Species recorded for various iron caves in the region of the municipality of Canaã dos Carajás, state of Pará (CAMPOS-FILHO; ARAUJO, 2011; CAMPOS-FILHO *et al.,* 2018).

Family **Pudeoniscidae** Lemos de Castro, 1973

Genus ***Iansaoniscus*** Campos-Filho, Araujo & Taiti, 2017
34. ***Iansaoniscus georginae*** Campos-Filho, Araujo & Taiti, 2017
Iansaoniscus georginae Campos-Filho, Araujo & Taiti *in* Campos-Filho *et al.*, 2017: 78, figs.
6–8; Campos-Filho *et al.*, 2018b: 457; Campos-Filho *et al.*, 2018: 24; Gallão & Bichuette, 2018: 7, Tab. 1; Campos-Filho *et al.*, 2019: 423; Cardoso *et al.*, 2022b: 128, fig. 9.
Species recorded for Borboletas cave, municipality of Paripiranga, state of Bahia (CAMPOS-FILHO *et al.*, 2017).

35. ***Iansaoniscus iraquara*** Campos-Filho, Araujo & Taiti, 2017
Iansaoniscus iraquara Campos-Filho, Araujo & Taiti *in* Campos-Filho *et al.*, 2017: 74, figs. 3–5; Campos-Filho *et al.*, 2018: 24; Campos-Filho *et al.*, 2019: 423; Cardoso *et al.*, 2022b: 128, fig. 9.
Species recorded for Buraco do Cão cave (Bolo de Noiva), municipality of Iraquara, state of Bahia (CAMPOS-FILHO *et al.*, 2017).

36. ***Iansaoniscus leilae*** Cardoso, Bastos-Pereira & Ferreira, 2022
Iansaoniscus leilae Cardoso *et al.*, 2022: 129, figs 1-4.
Species recorded for Toca do Gonçalo and Toca da Tiquara, municipality of Campo Formoso, state of Bahia (CARDOSO *et al.*, 2022).

37. ***Iansaoniscus paulae*** Cardoso, Bastos-Pereira & Ferreira, 2022
Iansaoniscus paulae Cardoso *et al.*, 2022: 134, figs 5-8.
Species recorded for Lapa do Bode cave, municipality of Itaeté, state of Bahia (CARDOSO *et al.*, 2022).

Family **Armadillidae** Brandt, 1831

Genus ***Gabunillo*** Schmalfuss & Ferrara, 1983
38. ***Gabunillo aridicola*** Souza, Senna & Kury, 2010
Gabunillo aridicola Souza *et al.*, 2010: 2, figs. 1–9.
Species recorded for Gruta do Sobradinho cave, Sítio Pé Queimado, municipality of Aiuaba, state of Ceará (SOUZA *et al.* 2010).



Figure 7. A) *Chaimowiczia uai* Cardoso, Bastos-Pereira, Souza & Ferreira, 2021; **B)** *Iuiuniscus iuiuensis* Souza, Ferreira & Senna, 2015; **C)** *Pectenoniscus carinhanhensis* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020; **D)** *Spelunconiscus castroi* Campos-Filho, Araujo & Taiti, 2014; **E)** *Xangoniscus dagua* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020; **F)** *Metaprosekia igatuensis* Campos-Filho, Fernandes & Bichuette, 2020; **G)** *Amazoniscus spica* Campos-Filho, Aguiar & Taiti, 2020; **H)** *Circoniscus buckupi* Campos-Filho & Araujo, 2011; **I)** *Gabunillo* sp. Barra scale: 1 mm.

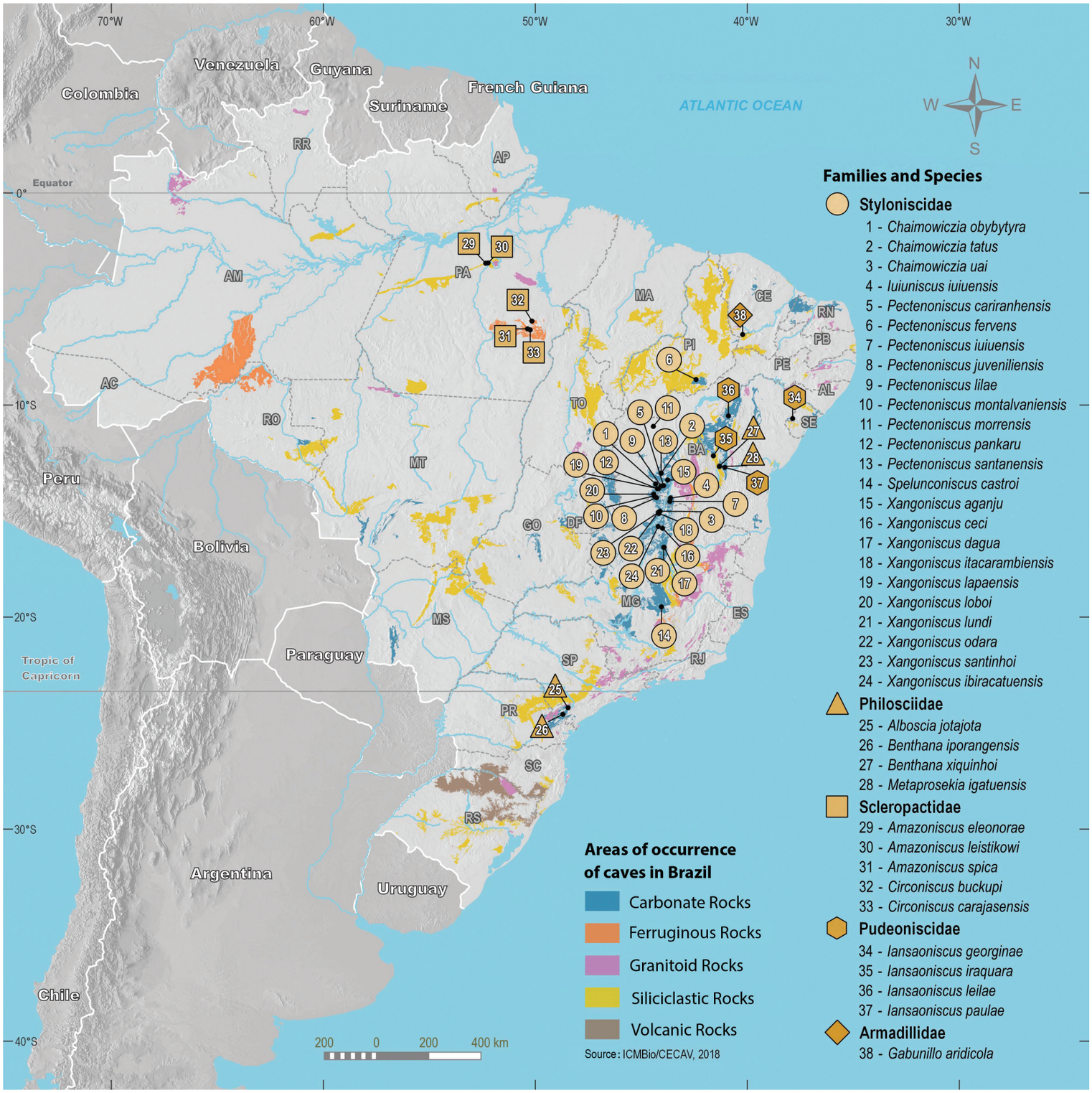


Figure 8. Map with the type localities of troglobitic isopod species described for Brazil.



Figure 9. A) *Iuiuniscus iuiuensis* Souza, Ferreira & Senna, 2015; **B)** *Pectenoniscus iuiuensis* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020; **C)** *Xangoniscus ibiracatuensis* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020; **D)** *Xangoniscus odara* Campos-Filho, Bichuette & Taiti, 2016; **E)** *Circoniscus* sp.; **F)** *Trichorhina* sp.; **G)** *Novamundoniscus* sp.; **H)** Armadillidae sp.; **I)** *Venezillo* sp.; **J)** Styloniscidae sp.; **K)** *Xangoniscus loboi* Campos-Filho, Gallão & Bichuette, 2022; **L)** Styloniscidae sp. Photos: A-C (Rodrigo L. Ferreira); D (Jonas E. Gallão); E, G (Robson de A. Zampaulo); F, H-J, L (Lucas M. Rabelo); K (Maria Elina Bichuette).

Main methods of collection and conservation

The collection of terrestrial isopods in the subterranean environment is mostly carried out actively and requires only a few materials, excluding basic cave incursion equipment (helmet, flashlight, overalls, ropes, etc.). The main requirement for collection is a "good eye" or a "trained eye" and a bit of stubbornness, considering that the subterranean populations of this group are sometimes reduced and, depending on the study to be carried out, the number of individuals may be insufficient. Highlighted among the main materials are thiner, elongated and/or short tweezers and brushes (both the most delicate and the most robust) for the direct capture of specimens (Figure 6). Another very useful instrument known in the entomological literature is the suction aspirator, which will prevent further damage to specimens and, if necessary, allows transportation alive to the laboratory for cultivation. However, if the cave has a high amount of organic matter, such as litter and/or logs, the use of a tray with a white or dark background it is recommended to provide contrast during collection and allow visualization of the entire invertebrate fauna. A form of collection not so common with Oniscidea, but which is very useful, is the use of fine mesh nets to collect amphibious species in travertines, pools or underground drainages existing inside caves, as it is a technique that reduces the possibility of damage to specimens.

For the conservation of collected specimens, the use of 70% ethanol and plastic microtubes with a capacity between 2 and 2.5 ml or 50 ml tubes is recommended, aiming at the individualization of fragile specimens. Alcoholic gradation will propitiate detailed morphological studies, as it is necessary for the solution to have a greater amount of water in order not to compromise the structures of the animals. When immersed in 95% or absolute ethanol, for molecular studies, the animals became more brittle, as the alcohol removes water from the specimens, making them more rigid and difficult to handle. For those less accustomed to the laboratory routine, a fundamental tip is to always label the collection vials using tracing paper and an ink pigmentation pen. The ink pigmentation will not become opaque or react in the presence of alcohol inside the tube, just write and wait a while for it to dry and insert into the vials.

If the microtubes are not closed correctly (not perceptible to the eye) and the alcohol evaporates, the specimens may dry out[17], and soon it will be necessary to use glycerin and a little patience, because in this case it will be necessary to wait a few days for the glycerin to penetrate the joints, thus allowing the handling of the material. From the moment the glycerin is added to the tubes, it should be the only preservation medium. If alcohol is added, even for observation under a stereomicroscope, the material will tend to become clear over time. If this occurs, in the case of troglobitic species, the work will become Herculean for the eyes, making it difficult to visualize the appendages through light microscopy, as only a tenuous external line of the structure will be visible. After the collection expedition, samples must be labeled again, the tubes changed and, depending on the amount of material, stored in a refrigerated environment (refrigerator) to prevent the alcohol from evaporating.

Finally, never forget a digital camera to record the environment around the cave, the microhabitats in which the animals occur in the cave and, if possible, photos and short videos of specimens *in situ*[18], detailing important behaviors.

Identification key

The identification keys for families and genera provided below attempt to cover the cave species discussed above. To facilitate future identifications, a key of the families registered so far is proposed. It should be remembered that the following keys are not necessarily dichotomous and may be composed of a set of characteristics. When possible, ecological features will be mentioned.

The families Philosciidae, Scleropactidae and Armadillidae could not be included in species keys due to their great morphological plasticity. These will have their taxonomic composition and distribution expanded as more localities are studied. Until now, considering the subterranean representatives of Brazil, Philosciidae has only two genera with troglobitic species, *Alboscia* and *Benthana*; the same is true for Scleropactidae, with *Amazoniscus* and *Circoniscus*; while for the family Armadillidae, only *Gabunillo* has a troglobitic representative. The other representatives of these families are considered troglophiles or without defined categories, or even accidental (CAMPOS-FILHO; ARAUJO, 2011; CAMPOS-FILHO *et al.*, 2015, 2018, 2019, 2020; FERNANDES *et al.*, 2019; SOUZA *et al.*, 2006, 2010).

17 This is discovered the moment more alcohol is added, and the specimens will float.

18 Currently, with the technology available, the most advanced cell phones can do this job without any worries.

Oniscidea

1 Animals without volvation capacity **2**

1' Animals with volvation capacity..... **3**

2 Creeper type body form (*sensu* Schmalfuss 1984) or swimmer (*sensu* Taiti & Xue, 2012), representatives terrestrial or amphibious, dorsal surface smooth or granulated, eyes absent (cave species), *noduli laterales* not discernable or absent, antennal flagellum composed of 3–7 articles, inner endite of maxillulae composed of 3 penicils, mandible with well-developed molar process and molar penicil absent ..... **Styloniscidae**

2' Creeper or runner type body form (*sensu* Schmalfuss, 1984), representatives exclusively terrestrial, dorsal surface smooth, eyes absent or composed of ommatidia, antennal flagellum composed of 3 articles, *noduli laterales* present, inner endite of maxillulae composed of 2 penicils, mandible with non-developed molar process and molar penicil present..... **Philosciidae**

3 Antennal flagellum composed of 2 articles, telson triangular and\or hourglass-shaped being as long as wide, exopods of pleopods with pleopodal lungs (*sensu* Paoli *et al.*, 2002) or respiratory areas (*sensu* Leistikow & Araujo, 2001..... **4**

3' Antennal flagellum composed of 3 articles, telson triangular and longer than wide, lateral margin of epimere of pereonite 1 presenting a sulcus (**not similar** to representatives of Eubelidae) for accommodation of antenna during volvation (sulcus reduced or absent in cave species), exopods of pleopods devoid of respiratory areas..... **Pudeoniscidae**

4 Head with vertex that may have deep sulcus (generally present in epigean species), dorsal surface may be smooth or slightly granulated or pilose (high density of scaly setae), lateral margin of epimere of pereonite 1 without sulcus for closing/locking pereonite 2 (accommodation of peronites during volvation), telson triangular with almost straight lateral margins (distal margin with rounded appearance), antenna apical organ long (similar in length to distal article of flagellum), exopods of pleopods 1 and 2 with respiratory areas (*sensu* Leistikow & Araujo, 2001)..... **Scleropactidae**

4' Head without deep sulcus(i), dorsal surface smooth or slightly tuberculated or strongly tuberculated (tubercles appear to possess long spines, *sensu* Schmalfuss 1984), epimeon of pereonite 1 lateral margin (sometimes reaching epimere 3) provided with sulcus for fitting pereonite 2 during volvation, telson triangular with convex lateral margins (distal margin with triangular appearance), or telson hourglass-shaped, antenna apical organ short, exopods of pleopods with pleopodal lungs (*sensu* Paoli *et al.*, 2002) ..... **Armadillidae**

Styloniscidae

1 Animals up to 3.5 mm in length, creeper type body form (*sensu* Schmalfuss 1984), exclusively terrestrial, dorsal surface granulated, male with exopods of pleopods 1 and 2 not covered by exopod of pleopod 3 ..... **2**

1' Animals ranging from 5 to 12 mm in length, swimmer type body form (*sensu* Taiti & Xue, 2012), amphibious, dorsal surface smooth, male with exopods of pleopods 1 and 2 not covered by exopod of pleopod 3..... **3**

2 Antennule with robust distal aesthetascs and distally fused or simple, outer endite of maxillulae composed of 5–7 simple teeth, mandible may possess a long seta visible under stereomicroscope (*sensu* Mulaik, 1960), exopod and endopod of uropod inserted at same level, endopod of pleopod 2 of male wide and with distal portion without complex structure..... ***Cylindroniscus***

2' Antennule with simple distal aesthetascs arranged in a comb shape, outer endite of maxillulae composed of 8–9 simple

teeth, exopod and endopod of uropod inserted at different levels, endopod of pleopod 2 of male wide and with distal portion presenting complex structure..... ***Pectenoniscus***

3 Epimera of pereonites 1–7 and pleonites 3–5 laterally expanded and tapering (epimera appearing spiny), species with building capacity (animals build shelters with cave substrate for protection) ..... ***Iuiuniscus***

3' Epimera of pereonites 1–7 and pleonites 3–5 unexpanded, species without building capacity.....**4**

4 Head with supra-antennal line bent downward in central portion (appearance as a U), epimera of pereonites 1–7 subrectangular, laterally expanded and spaced apart, antennal flagellum composed of 3 articles ..... ***Chaimowiczia***

4' Head with supra-antennal line strongly bent downward in central portion (appearance of a V), epimera of pereonites 1–7 not expanded and not spaced, antennal flagellum composed of more than 3 articles **5**

5 Ischium of pereopod 7 of male without spur-shaped ventral lobe, endopod of pleopod 2 of male with distal portion showing complex structure (twisted triangular lobe and transverse seta) ..... ***Xangoniscus***

5' Ischium of pereopod 7 of male with spur-shaped ventral lobe (see Bastos-Pereira *et al.*, 2022), endopod of pleopod 2 of male with tapering distal portion and without complex structure ..... ***Spelunconiscus***

Main Brazilian collections

Among the main collections of subterranean terrestrial isopods, including specimens from the epigean environment, the following can be highlighted:

Coleção de Carcinologia do Museu de Zoologia[19] (MZUSP), Universidade de São Paulo, São Paulo, under the curatorship of Marcos Domingos Siqueira Tavares. The MZUSP collection is a reference collection and faithful depositary, both for the deposit of types and non-type material, and where several cave and non-cave species can be found, which we had the opportunity to describe. The collection has a large amount of material from Eleonora Trajano[20], recognized as one of the references in the country for studies of subterranean fauna involving biodiversity and conservation.

Coleção de Crustáceos do Museu Nacional[21] (MNRJ), Universidade Federal do Rio de Janeiro, Rio de Janeiro, under the curatorship of Christiana Serejo. The MNRJ collection encompasses a large amount of material of Oniscidea, including specimens from the subterranean environment.

Coleção do Laboratório de Estudos Subterrâneos[22] (LES), Universidade Federal de São Carlos, São Carlos, São Paulo, under the curatorship of Maria Elina Bichuette. The LES collection strictly includes material from cave environments and their surroundings, containing material from almost all Brazilian karst areas, thus being one of the greatest references for the deposit of scientific material. It is noteworthy that the collection includes not only cave-dwelling terrestrial isopods, but also a wide faunal diversity of invertebrates and vertebrates and counts on the collaboration of national and international researchers and undergraduate and graduate students supervised there.

Coleção de Invertebrados Subterrâneos[23] (ISLA), Universidade Federal de Lavras, Minas Gerais, under the curatorship of Rodrigo Lopes Ferreira. The ISLA collection, like the LES collection, covers a wide range of material from various Brazilian karst areas and from different groups of invertebrates, and is also a collection for the deposit of scientific material. This collection also has the collaboration of undergraduate and graduate students supervised by Rodrigo L. Ferreira and with national and international researchers.

19 https://mz.usp.br/pt/laboratorios/carcinologia/

20 Eleonora Trajano worked at the Departamento de Zoologia of the Instituto de Biociências da Universidade de São Paulo from June 1981 to February 2015, and is currently retired. She continues to act as a reviewer for several journals and in the production of scientific articles and books, or book chapters, about the subterranean environment.

21 https://www.museunacional.ufrj.br/

22 http://www.lesbio.ufscar.br/

23 http://www.biologiasubterranea.com.br/pt/colecao/acervo/

Coleção de Invertebrados do Museu de História Natural de Capão da Imbuia[24], Curitiba, Paraná. This collection has more than 1,800 lots of crustacean representatives, including two species of *Trichorhina* from caves in the state of Paraná, published in 2015 (CAMPOS-FILHO *et al.*, 2015).

Coleção de Crustacea do Museu de Zoologia[25] **(UFBA),** Universidade Federal da Bahia, Salvador, Bahia, under the curatorship of Rodrigo Johnsson Tavares da Silva. Thus far, the collection holds type material referring to the description of *Iuiuniscus iuiuensis* (SOUZA *et al.*, 2015).

Coleção de Isópodes Terretres do Museu de História Natural de Florença (MZUF), Seção de Zoologia "La Specola"[26], Università degli Studi di Firenze, curated by Stefano Tahiti. The Oniscidea collection of MZUF holds the largest amount of terrestrial isopods in the world, including representatives from Brazilian cave environments.

24 https://mhnci.webnode.com/

25 http://www.mzufba.ufba.br/WEB/Cole%C3%A7%C3%B5es_Crustacea.html

26 https://www.sma.unifi.it/vp-245-la-specola.html

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Epiperipatus sp.
Rodrigo Lopes Ferreira

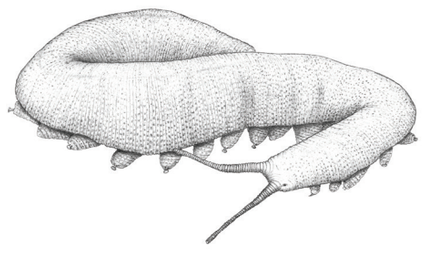
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Onychophora

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General introduction

The name Onychophora derives from the Greek *onycho*, "talon"; phora, "bearer", which means "the one who bears claws" (BRUSCA *et al.*, 2018). There is no common name in Portuguese for these animals, although biologists call them peripatus. However, it is known that in isolated locations in northern Brazil the name "embuá de chifre" is associated with onychophorans (MONGE-NÁJERA; MORERA-BRENES, 2015). The phylum is divided into two living families (Peripatidae and Peripatopsidae) and four fossil families (Helenodoridae, Succinipatopsidae, Tertiapatidae and Tertiapatoidea) (MAYER; DE SENA OLIVEIRA, 2013; OLIVEIRA *et al.*, 2012; POINAR, 2000). About 200 living species are currently known worldwide (BAKER *et al.*, 2021; OLIVEIRA *et al.*, 2012; COSTA *et al.*, 2018). Onychophorans have an extremely conservative morphology[1], which constitutes a complicating factor, especially in Peripatidae, for the taxonomic organization of their genera and species.

Peripatidae is currently organized into 10 genera and 74 species, while Peripatopsidae has 39 genera and 106 species (GIRIBET; EDGECOMBE, 2020). The Neotropical Peripatidae (Neopatida) accounts for the majority of onychophoran diversity in Central and South America, distributed from Mexico to southern Brazil. However, peripatopsids are also present, but limited to the Temperate Deciduous Forest biome in Chile. According to Bouvier (1905), there are two large and related groups of Neopatida: one group, called "Péripates andicoles", comprises all the species found along and on the western slope of the Andes Cordillera, which currently make up the genus *Oroperipatus* Cockerell, 1908; the other group, called "Péripates caraïbes", gathers species from the eastern slope of the Andes to the Antilles. This latter group includes minor or monotypic genera, such as *Heteroperipatus* Zilch, 1954; *Macroperipatus* Clark, 1913; *Mongeperipatus* Barquero, Vargas, Morera-Brenes, 2020; *Plicatoperipatus* Clark, 1913 and *Speleoperipatus* Peck, 1975; as well as the more diverse *Peripatus* Guilding, 1826 and *Epiperipatus* Clark, 1913.

In addition to being more diverse, *Epiperipatus* and *Peripatus* are also the genera with the most taxonomic problems, such as shared characteristics and high inter and intraspecific morphological variation. These are the most complicating factors, since no obvious diagnostic characters are found for delimiting the genera (BOUVIER, 1905; COSTA *et al.*, 2021; OLIVEIRA *et al.*, 2011; READ, 1988; SAMPAIO-COSTA *et al.*, 2009). Thus, the genera are ambiguously delimited based on the morphology of the dorsal papillae, as well as the count of crural papillae per pregenital leg (characteristic of only males), dorsal folds per segment, and scales of the main papillae. Even so, *Peripatus* and *Epiperipatus* have received new species in the last decade (COSTA *et al.*, 2018; MONGE-NÁJERA; MORERA-BRENES, 2015).

In this context, around 90% of peripatid diversity is found in the Neotropics and *Peripatus* and *Epiperipatus* have the widest distributions (COSTA *et al.*, 2021). The other 10% are distributed in Africa and Malaysia (COSTA; GIRIBET, 2016; OLIVEIRA, 2012). Furthermore, both genera overlap in Central America, but *Epiperipatus* stands out for having more species in South America, mainly in Brazil. *Epiperipatus* also draws attention for its wide distribution, occurring from Costa Rica (also occurring in the Antilles) down to South America, where it is distributed in Colombia, Venezuela, Guyana, Suriname, French Guiana and Brazil (BRUES, 1914; COSTA *et al.*, 2021; OLIVEIRA *et al.*, 2012). There are eight genera of Peripatidae in the Neotropical Region and the delimitation of all of them has always been unsatisfactory, due to: i) limited morphological information (it is a conservative group); ii) poor sampling of species; iii) descriptions based on ambiguous characters; and iv) few studies dedicated to the systematic review of the family or that contemplate a more comprehensive sampling of terminals.

These animals are invertebrate predators that capture their prey by means of a proteinaceous squirt expelled by a pair of organs located in the cephalic region, called oral papillae. In contact with the air, this mucus hardens and functions as a network of adhesive cords (CONCHA *et al.*, 2015) that serves both

1 The morphological delimitation of species is difficult because, in addition to a high degree of autapomorphic characters, the taxonomic characters of the phylum are generally ambiguous, mainly in Peripatidae.

for capture and for defense against invertebrate and vertebrate predators, which include reptiles, birds and snakes (HAMER *et al.*, 1997; MONGE-NÁJERA *et al.*, 1993).

They are usually nocturnal animals that occur mainly in tropical forests and live in humid and dark environments, where humidity and temperature conditions are relatively stable (NEWLANDS; RUHBERG, 1979). Thus, they are usually found buried in the ground, in leaf litter or under rotten trunks, under stones, in termite mounds and within bromeliad leaves and caves (CARVALHO, 1942; VASCONCELLOS *et al.*, 2006). Onychophorans are extremely dependent on humid environments, and so avoid the soil surface during periods of drought.

This evolutionarily-old group of invertebrates has scarce records in Brazil. They are generally found in low densities, with less than 100 specimens collected of each known species (except for Peripatus heloisae, of which Carvalho (1942) collected 628 specimens which were deposited at the Museu Nacional). In addition, they are highly endemic and typically restricted to their type locality. As a result, they are extremely susceptible to different types of environmental impacts, leading to the possibility of extinction. Consequently, our understanding of this group is limited. Of the 20 species described for Brazil, four are on the official list of endangered species in the country (MMA, 2022). As an example, given its importance, the Estação Ecológica do Tripuí in Ouro Preto, state of Minas Gerais, corresponds to the first, and one of the few, conservation units in the country created specifically for the protection of onychophorans in Brazil, in particular, *Epiperipatus acacioi* (MARCUS; MARCUS, 1955; MURIENNE *et al.*, 2014; OLIVEIRA *et al.*, 2014).

More recently, with the increase in inventories carried out in cave ecosystems in Brazil, a considerable amount of onychophorans have been recorded in these environments. Bearing in mind the existence of effectively troglobitic species in some parts of the world, as well as the increasingly frequent records of onychophorans in caves in Brazil, this chapter aims to contribute basic information about the biology and diversity of the group, as well as on its relevance and the importance of adequate conservation of biological material obtained in caves. New records of Onychophora are valuable for science, as this is an important phylum for understanding, for example, the evolution of Arthropoda. Therefore, the definition of strategies for the conservation of the group is considered a priority, with each record being extremely important for decision-making (NEW, 1995).

Main groups found in Brazilian caves

There are four genera and 20 species of onychophorans in Brazil (COSTA *et al.*, 2021), as listed below (Table 1) and represented in Figure 1. All described species are epigeal, although two yet to be described species with subterranean populations are known for caves in the Brazilian Center-West (CORDEIRO, 2008; L.C. MEDEIROS, 2022 – personal observation). However, it is worth mentioning that determining troglomorphisms in onychophorans can be a complex activity, since onychophorans only have primitive eyes and, in Brazil, species with the complete reduction of ocellar structures have not yet been found. Therefore, there are no species of onychophorans described as strictly cave-dwelling in Brazil (troglobites), however, it is occasionally possible to find isolated individuals exploring the entrance and interior of caves (Figure 2), this habit having been registered in the states of Minas Gerais, Mato Grosso, Mato Grosso do Sul, Goiás and Pará. Furthermore, there are troglobitic species described for other parts of the world, such as Jamaica and South Africa (PECK, 1975). A plausible explanation for these occurrences is due to the life habits of these animals since they are nocturnal and predators of soil fauna. In addition, as they are not able to control the loss of water to the environment, they need constantly humid environments, especially in arid regions or during periods of drought (dry season), with caves thusly being favorable subterranean ecosystems for their occurrence.

Next, we present a list of onychophoran species known from Brazil. However, some records raise doubts (indicated in the list with an asterisk) and have not undergone taxonomic revision at the time of publication of this book. Figure 3 shows some of the epigeal species already described for Brazil.

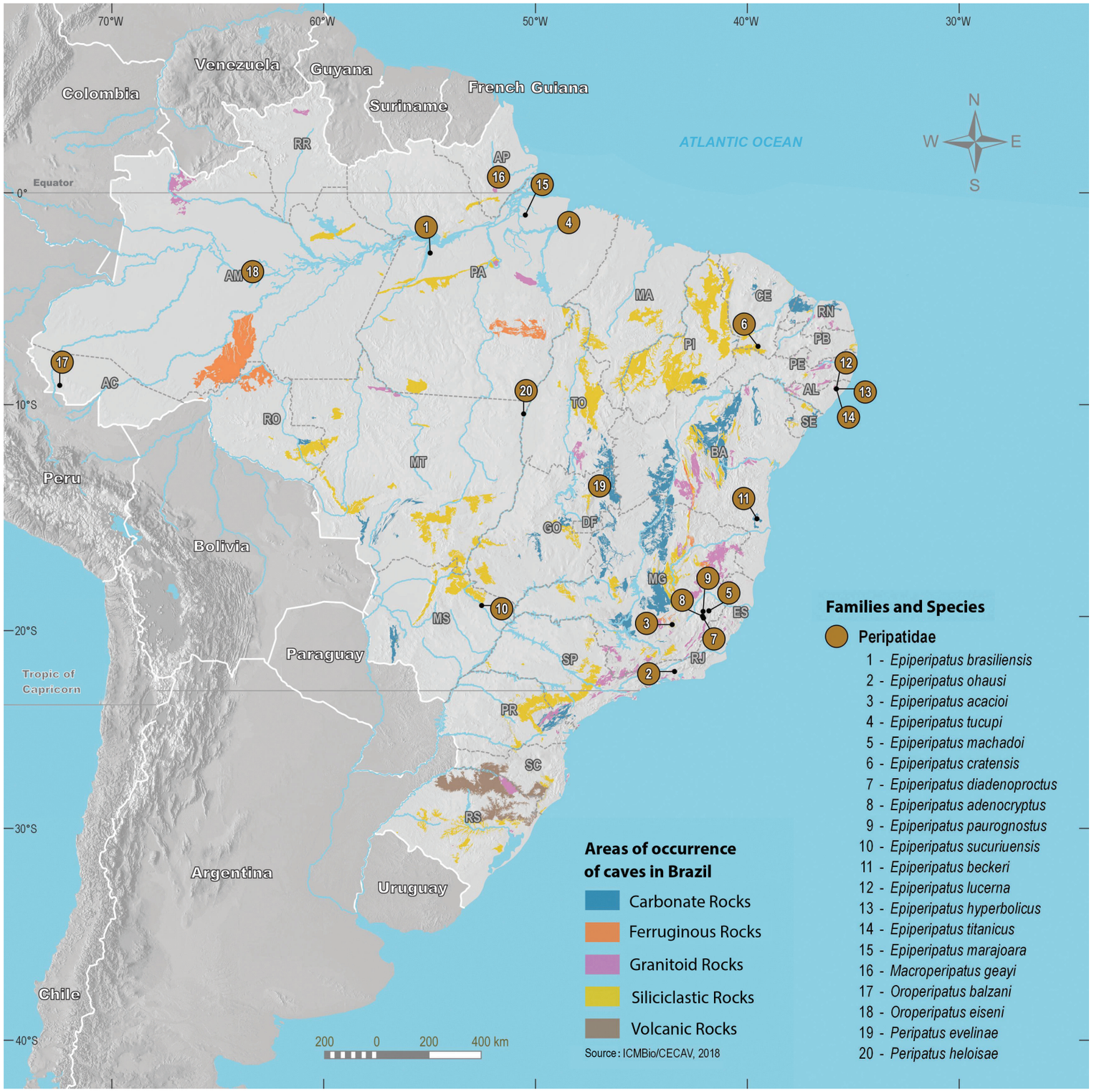


Figure 1. Map with the localities of species of Onychophora (non-troglobities) described for Brazil.

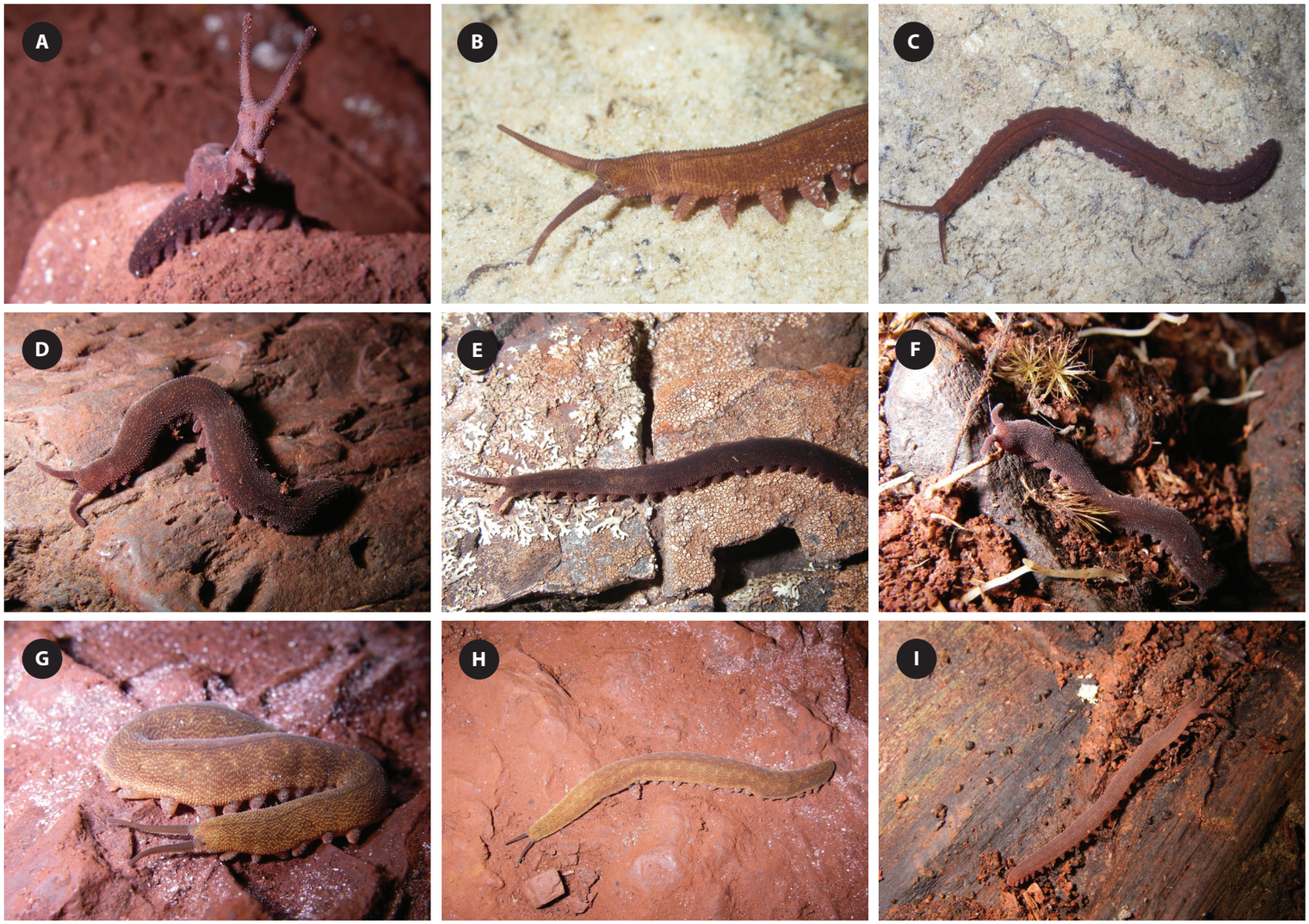


Figure 2. Records of unkown species of Onychophora in caves of the states of Minas Gerais and Pará. Photos: A, D-I (Robson de A. Zampaulo); B-C (Lucas M. Rabelo).

Table 1 – List of species described for Brazil.

Family **Peripatidae** Evans, 1901
Type species: *Peripatus juliformis* Guilding, 1826

Genus ***Epiperipatus*** Clark, 1913
1. ***Epiperipatus brasiliensis*** (Bouvier, 1899)
Distribution: Brazil: Para state: Santarém, Terra Rica (Km 67), Floresta Nacional (FLONA) do Tapajós.
General notes: *Epiperipatus brasiliensis* was the first species described for Brazil. The species has records of occurrence in the states of Pará, Maranhão and Ceará (SAMPAIO-COSTA *et al.* 2009: 556). However, recent studies suggest that the species is restricted to Santarém or nearby locations. (COSTA, 2016).

2. ***Epiperipatus ohausi*** (Bouvier, 1900)

Distribution: Brazil: Rio de Janeiro State: Petrópolis; Nova Iguaçu: Reserva Particular do Patrimônio Natural (Reserva Ambiental dos Petroleiros).

General notes: *Epiperipatus ohausi* was the second species to be described for Brazil.

3. ***Epiperipatus acacioi*** (Marcus & Marcus, 1955)

Distribution: Brazil: Minas Gerais State: Ouro Preto, Tripuí.

General notes: Estação Ecológica do Tripuí was created for the protection of the area of occurrence of the species.

4. ***Epiperipatus tucupi*** Froehlich, 1968

Distribution: Brazil: Pará State, no data on type locality.

General notes: The location of the species is imprecise and its identification tag contains the information "Pará".

5. ***Epiperipatus machadoi*** (Oliveira & Wieloch, 2005)

Distribution: Brazil: Minas Gerais State: Caratinga, Reserva Particular do Patrimônio Natural Feliciano Miguel Abdala.

General notes: The species is a good example of endemism among onychophorans, since until the present moment there are no records of *E. machadoi* beyond the extension of RPPN Feliciano Miguel Abdala.

6. ***Epiperipatus cratensis*** Brito *et al.*, 2010

Distribution: Brazil: Ceará State: Crato, APA da Chapada do Araripe/Floresta Nacional de Araripe-Apodi.

General notes: In 2016, the species underwent a taxonomic review in and an update of its distribution, which corroborated the endemism of the species in Chapada do Araripe.

7. ***Epiperipatus diadenoproctus*** Oliveira *et al.*, 2011

Distribution: Brazil: Minas Gerais State: Simonésia, Reserva Particular do Patrimônio Natural Mata do Sossego.

General notes: *Epiperipatus diadenoproctus* is one of the species considered cryptic, described for Brazil. Along with it, *E. adenocryptus* and *E. paurognostus* were delimited based on molecular data.

8. ***Epiperipatus adenocryptus*** Oliveira *et al.*, 2011

Distribution: Brazil: Minas Gerais State: Santa Bárbara do Leste, Córrego dos Ferreiras.

General notes: *Epiperipatus adenocryptus*, as well as *E. paurognostus* and *E. diadenoproctus*, were previously delimited based on molecular data.

9. ***Epiperipatus paurognostus*** Oliveira *et al.*, 2011

Distribution: Brazil: Minas Gerais State: Piedade de Caratinga, Mata do Eremitério.

General notes: *E. paurognostus*, as well as *E. adenocryptus* and *E. diadenoproctus*, were previously delimited based on molecular data.

10. ***Epiperipatus sucuriuensis*** (Oliveira, Lacorte, Weck-Heimann, Cordeiro, Wieloch & Mayer, 2015).

Distribution: Brazil: Mato Grosso do Sul State, Chapadão do Sul.

General notes: It is one of the most endangered species among the onychophorans known from Brazil. *Epiperipatus sucuriuensis* suffered a major impact with the creation of a small hydroelectric power plant on the Sucuriú River, reducing the distribution of this species to small fragments cut by that river.

11. ***Epiperipatus beckeri*** Costa, Chagas-Jr & Pinto-da-Rocha, 2018

Distribution: Brazil: Bahia State: Camacan, Reserva Particular do Patrimônio Natural Serra Bonita.

General notes: *Epiperipatus beckeri* is one of the most striking species of Brazilian onychophorans because of its lighter coloration — a more orange tone — differing from the darker tone observed in the other known species from Brazil.

12. ***Epiperipatus lucerna*** Costa, Chagas-Jr & Pinto-da-Rocha, 2018
Distribution: Brazil: Alagoas State, Murici, Estação Ecológica de Murici (Mata da Bananeira).
General notes: *Epiperipatus lucerna* is noteworthy for occurring in the same locality (in sympatry) as three other species in Alagoas, the E. E. de Murici. The species is considered less worrying in the assessment of endangered species, but the area where it is found is under constant pressure from human activities, especially agriculture.

13. ***Epiperipatus hyperbolicus*** Costa, Chagas-Jr & Pinto-da-Rocha, 2018
Distribution: Brazil: Alagoas State, Murici, Estação Ecológica de Murici (Mata da Bananeira).
General notes: *Epiperipatus hyperbolicus*, as with *E. lucerna*, was described for E.E. de Murici. Likewise, the species is considered less worrying in the assessment of endangered species, but the area where it is found is under constant pressure from human activities, especially agriculture.

14. ***Epiperipatus titanicus*** Costa, Chagas-Jr & Pinto-da-Rocha, 2018
Distribution: Brazil: Alagoas State, Murici, Estação Ecológica de Murici (Mata da Bananeira).
General notes: *Epiperipatus titanicus* completes the triad of species described for E.E. de Murici. As with the previous species, it is considered less worrying in the assessment of endangered species, but the area where it is found is under constant pressure from human activities, especially agriculture.

15. ***Epiperipatus marajoara*** Costa, Chagas-Jr & Pinto-da-Rocha, 2018
Distribution: Brazil: Pará State, Ilha do Marajó, Breves, Reserva de Extrativista Mapuá.
General notes: *Epiperipatus marajoara* was recognized in 2018 based on a study done through the combination of historical material and new records.

Genus *Macroperipatus* Clark, 1913

16.****Macroperipatus geayi*** (Bouvier, 1899)
Distribution: Brasil: Amapá State (region formerly known as "Contesté Franco-Brésilien").
General notes: *Macroperipatus geayi* is a dubious species that calls for urgent review.

Genus *Oroperipatus* Cockerell, 1908

17.****Oroperipatus balzani*** (Camerano, 1897)
Distribution: Bolívia: Coroico and Chulumani provinces; Brasil: Acre State: Marechal Thaumaturgo.
General notes: *Oroperipatus balzani* is a dubious species that calls for urgent review.

18.****Oroperipatus eiseni*** (Wheeler, 1898)
Distribution: Mexico: Nayarit: Tepic/Panama; Brasil: Amazonas State.
General notes: *Oroperipatus eiseni* is a dubious species that calls for urgent review.

Genus *Peripatus* Guilding, 1826

19.****Peripatus evelinae*** Marcus, 1937
Distribution: Brazil: Minas Gerais State/Goiás State, Nova Roma and Cana Brava.
General notes: *Peripatus evelinae* was described in 1937 and is the only work known about it, until now. It is a species that needs revision.

20.***Peripatus heloisae** Carvalho, 1941

Distribution: Brazil: Mato Grosso State: Santa Terezinha (formerly known as Barra do Tapirapé)/Tocantins: Miracema do Tocantins.

General notes: *Peripatus heloisae* is the most emblematic of the species known for Brazil, due to the sample size. Accompanying the ethnographic expedition of the Museu Nacional, Antenor Leitão de Carvalho had the rare chance of finding 628 specimens during a period of heavy rains and waterlogged soil in the region. However, little is known about this species since its description.



Figure 3. Species of Onychophora described for Brazil. **A)** *Epiperipatus brasiliensis;* **B)** *Epiperipatus cratensis;* **C)** *Epiperipatus titanicus;* **D)** *Epiperipatus acacioi;* **E)** *Epiperipatus beckeri;* **F)** *Peripatus heloisae;* **G)** *Epiperipatus machadoi;* **H)** *Epiperipatus marajoara;* **I)** *Epiperipatus titanicus*, ventral detail of head. Photos: A, B, H (Cristiano Sampaio Costa); C, I (Flávio Uemori Yamamoto); D (Davi Sampaio Costa); E (Amazonas Chagas Júnior); F (Rogério Bertani); G (Riccardo Mugnai).

Diversity of troglobitic species

Troglobites (troglobiontes) are organisms restricted to the subterranean environment (including caves) and, in general, can present different types of morphological, physiological and behavioral specializations. These organisms frequently present a reduction of ocular structures, depigmentation and elongation of appendages, adaptations denominated as troglomorphisms. In addition, they generally have a restricted geographical distribution, low population density, low tolerance to environmental fluctuations and a K-type reproductive strategy, which makes them potentially endangered due to changes in their environment (CULVER, 1982). Due to these characteristics, any troglobitic species is considered, at least, as vulnerable to extinction by the International Union for Conservation of Nature (IUCN).

There are only two truly troglomorphic species of onychophorans described so far in the world, *Peripatopsis alba* Lawrence, 1931 (Peripatopsidae) in South Africa and *Speleoperipatus spelaeus* Peck, 1975 (Peripatidae) (Peripatidae), in Jamaica (PECK, 1975). However, there are two other occurrences, one in the Galápagos (ESPINASA *et al.*, 2015) and one in Brazil, with two important populations recorded in caves in Mato Grosso do Sul (TRAJANO; BICHUETTE, 2010), which have yet to be described.

Among these, the species found in Gruta Dente de Cão presents a potentially troglomorphic morphology (Figure 4) being, therefore, the first record of a troglobitic species for Brazil (CORDEIRO, 2008). However, it is worth mentioning that the determination of troglomorphisms in onychophorans can be considered an intricate task, since eye reduction is recurrent among animals that live in the soil, as occurs among onychophorans, which have simple rhabdomeric eyes (GIRIBET; EDGECOMBE, 2020). Another recurrent troglomorphism in cave animals is the elongation of appendages. However, the legs of onychophorans are "retractable", and can therefore vary depending on the species, the size of the individual or even as a result of fixation methods. Lastly, depigmentation perhaps represents the most evident troglomorphism in this group. However, the described troglobitic species still lack a more detailed study about their evolutionary history and more molecular data. Therefore, evaluating the presence of morphological adaptations in Onychophora is an extremely difficult task.

Finally, the occurrence of troglomorphisms in onychophorans is, in general, very rare, and most records of these animals in caves are of opportunistic or even accidental cases. Most of these records represent isolated occurrences observed mainly in caves in the states of Minas Gerais and Pará and were found as a result of environmental licensing studies, especially for the exploration of ore in these states.

Main methods of collection and conservation

In the 18th century, it was very common to fix invertebrates with solutions based on denaturing agents, such as methanol, isopropyl alcohol, acetone and butanone, which preserved them (SEDGWICK, 1888; BOUVIER, 1905). This method, however, compromises the entire external morphology of soft-bodied animals (C.S. COSTA – personal observation). Currently, with the introduction of new analysis techniques, these fixation methods have been completely replaced or adapted for each type of material.

In addition, innovations, such as the use of scanning electron microscopy (SEM) and the sequencing of genetic material, provided an enormous leap in the knowledge of Onychophora. However, they forced taxonomists to develop techniques for preserving the external morphology of onychophorans. Fixing them directly in alcohol, as is done for most arthropods, distorts all the external characteristics of the specimens. This fixation method is recommended for molecular studies, for which specimens must be fixed in absolute alcohol. However, as will be seen in the next topic, another approach is suggested for morphological studies.

METHODOLOGY FOR FIXATION

Due to their soft body, the fixation and preservation of the external morphological characteristics of onychophorans require greater care, when compared to the fixation of arthropods. As little is known about onychophoran species, all information collected on specimens *in vivo* is valid. Therefore, it is suggested that, before fixation, the dorsal and ventral regions of specimens be photographed with the highest possible resolution and closeness. The ideal is to use cameras that have a macro mode or are equipped with a specific lens for macro photography and a circular flash. If possible, the distribution of papillae and the ventral region of the legs should be detailed



Figure 4. Species of *Epiperipatus* that occur in Gruta Dente de Cão, municipality of Bodoquena, Mato Grosso do Sul. Photos: (Lucas M. Rabelo).

(COSTA; GIRIBET, 2016; OLIVEIRA *et al.*, 2011; SAMPAIO-COSTA *et al.*, 2009). After photography, specimens must be anesthetized and then fixed. A suggested protocol follows.

Anesthesia

The methodology for anesthesia of onychophorans consists of exposure to ethyl acetate in a death chamber (MONGE-NÁJERA; MORERA-BRENES, 1994). For every 100 mg of animal weight, 5 mg of ethyl acetate is required, with anesthesia occurring by exposure to ethyl acetate vapor for about 80 seconds (MONGE-NÁJERA; MORERA-BRENES, 1994). Contact with ethyl acetate causes onychophorans to relax and helps reduce specimen contraction during fixation.

Fixation

Once anesthetized, the onychophoran specimen is fixed by placing it horizontally in a petri dish, where its contraction is controlled as it is submerged in 70% alcohol. It is recommended to leave the specimen in a horizontal position for at least 24 hours so that the integument gains sufficient rigidity to resist the pressure of gravity and, therefore, does not bend or contort during vertical storage.

Scanning electron microscopy

For scanning electron microscopy (SEM) analyses, the parts chosen for study (e.g., dorsal region of the integument, legs, jaws and antennae) should be pre-cleaned. Suggested cleaning involves rehydration in 10% by 10% and washing with a 3% PA chlorine solution diluted in distilled water in a Glass Kline Plate. Hard parts can be washed in ethylenediaminetetraacetic acid (EDTA). With sprays, the solution is stirred to suspend the particles of dirt and grease attached to the tissue of the washed items. The supernatant solution is removed with a micropipette and then distilled water is added back to the Glass Kline Plate. The water is then stirred with a Pasteur pipette, and the supernatant removed. The previous step must be repeated until the supernatant no longer contains suspended particles. After cleaning, dehydration should be carried out in the following alcohol series: 30%, 50%, 70%, 80%, 90% and 100%.

Critical point

All parts must go through drying in a critical point device.

Metallization

After drying in a critical point devise, the pieces for analysis are metallized. The Backscatter Electron Detector function of the SEM, if available, should be used during microscopy to reduce noise in the capture of electrons.

Identification key

Identification key for species of the genus *Epiperipatus* (except *E. sucuriuensis*) that occur in Brazil, originally published in Costa *et al.* (2018).

1 Only dorsal folds[2] complete..... **2**

1' Incomplete folds present **3**

2 Apical piece conical and symmetrical, 29 pairs of legs (males), 31–32 (females) ..... ***E. brasiliensis*** Bouvier, 1899

2' Apical piece conical and symmetrical, 30–33 pairs of legs (males) and 33–34 (females) ..... ***E. cratensis*** Brito *et al.*, 2010

2" Apical piece well-developed, spherical and asymmetrical (similar to *E. cratensis*), 34–35 pairs of legs (females) ..... ***E. tucupi*** Froehlich, 1968

3 Apical piece conical..... **4**

3' Apical piece spherical..... **5**

4 Apical piece conical, regular (normal size) **6**

4' Apical piece conical/cylindrical, reduced (similar to *E. edwardsii*) **7**

5 Apical piece spherical, regular 8

5' Apical piece spherical, robust (similar to *E. edwardsii*)..... ***E. hyperbolicus*** Costa *et al.*, 2018

6 Apical piece cylindrical and asymmetrical (COSTA *et al.*, 2018: Figure 8), two primary papillae separated by one to three accessory papillae (COSTA *et al.*, 2018: Figure 30), sulci within folds with accessory papillae, 27 pairs of legs (males) and 31 (females) ..... ***E. marajoara*** Costa *et al.*, 2018

6' Apical piece conical (sail-shaped, COSTA *et al.*, 2018: Figure 9), dorsal papillae aligned on top of dorsal folds (COSTA *et al.*, 2018: Figure 18), two main papillae isolated by one to five accessory papillae, sulcus between folds without accessory papillae (COSTA *et al.*, 2018: Figure 18), 27–29 pairs of legs (males) and 29–31 (females) ..... ***E. lucerna*** Costa *et al.*, 2018

7 Apical piece reduced, or hidden within base, symmetrical, two main papillae separated by one to three accessory papillae (COSTA *et al.*, 2018: Figure 14), in dorsal view background color reddish-brown (in life, COSTA *et al.*, 2018: Figure 13), dorsal papillae aligned on top of folds, 36–38 pairs of legs (males) and 36–39 (females) ..... ***E. titanicus*** Costa *et al.*, 2018

7' Apical piece reduced, dorsal papillae aligned on top of folds (COSTA *et al.*, 2018: Figure 25), primary papillae, when separated, have two to four accessory papillae between them (COSTA *et al.*, 2018: Figure 25), in dorsal view background color yellowish-pink (COSTA *et al.*, 2018: Figure 24), 28–30 pairs of legs (females) ..... ***E. beckeri*** Costa *et al.*, 2018

8 Peripheral accessory papillae often occur on flanks (COSTA *et al.*, 2018: Figure 30) **9**

8' Peripheral accessory papillae rarely present on flanks (OLIVEIRA *et al.*, 2011: Figure 4A) **10**

9 Dorsal color of brownish background, 27–29 pairs of legs (males) and 28–31 pairs of legs (females ..... ***E. machadoi*** Oliveira & Wieloch, 2005

9' Dorsal color of deep red background, 24–26 pairs of legs (males) and 26–29 (females) ..... ***E. acacioi*** Marcus & Marcus, 1955

10 Anal gland conspicuous (only in males) (OLIVEIRA *et al.*, 2011, Figure 5C)..... **11**

10' Anal gland imperceptible (only in males) (OLIVEIRA *et al.*, 2011, Figure 5E)..... **12**

11 Anal glands (only in males) are two well-developed pores (with light spots) on anterior plate of anal opening (OLIVEIRA *et al.*, 2011: Figure 5C), 26–28 pairs of legs (males) and 29–30 pairs of legs (females) ..... ***E. diadenoproctus*** Oliveira *et al.*, 2011

12 Anal gland imperceptible, bean-shaped, little visible (OLIVEIRA *et al.*, 2011: Figure 5D), 26 –27 pairs of legs (males) and 28–30 pairs of legs (females) with no overlap between sexes..... ***E. adenocryptus*** Oliveira *et al.*, 2011

12' Only primary papillae prominent (CHAGAS-JÚNIOR; COSTA 2014, Figure 6), primary papillae with robust apical piece (CHAGAS-JÚNIOR; COSTA 2014, figures 8, 9), 26–28 pairs of legs (males) and 27–29 (females)..... ***E. ohausi*** Bouvier, 1900

12" All dorsal papillae prominent (COSTA *et al.*, 2018: Figure 36), poorly developed anal gland opens in two pores on anterior plate of anal opening (OLIVEIRA *et al.*, 2011: Figure 5E), 26–27 pairs of legs (males) and 27–29 pairs of legs (females) ..... ***E. paurognostus*** Oliveira *et al.*, 2011

2 Counted in dorsal section between two legs. See Costa *et al.* (2018).

Main Brazilian collections

Among the largest collections in Brazil are the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ[3]), the Museu de Zoologia da Universidade de São Paulo (MZUSP) and the Museu da Universidade Federal de Minas Gerais (UFMG). However, there are other important collections in the country, such as those of the Universidade Federal de Mato Grosso (UFMT), the Universidade Federal Mato Grosso do Sul (UFMS), the Universidade Federal da Paraíba (UFPB) and the Museu Paraense Emílio Goeldi (MPEG), among others.

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3 The collection of onychophorans at the Museu Nacional had more than 900 specimens, which were lost — including types of *Epiperipatus hyperbolicus, E. lucerna, E. marajoara* and *E. titanicus* and *Peripatus heloisae* — in the tragic fire that occurred in 2018.

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landumoema cuca
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19

Opiliones

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General introduction

The order Opiliones constitutes the third largest group of arachnids in number of species, with approximately 6,600 described worldwide (KURY *et al.*, 2020), after only spiders (more than 48,000 species; WORLD SPIDER CATALOG, 2020) and mites and ticks (more than 50,000 species; CODDINGTON *et al.*, 2004). Known as harvestmen, they are especially diverse in tropical rainforest, such as the Amazon and the Atlantic Forest, but are little known by the general public due to the predominance of their cryptic and nocturnal habits. Although harvestmen are distributed in all Brazilian biomes (KURY, 2003), knowledge about the diversity and ecology of these organisms in the different biomes is heterogeneous, as studies have not historically been carried out with the same effort for all of them (BRAGAGNOLO; PINTO-DA-ROCHA, 2003; DESOUZA *et al.*, 2014).

The Atlantic Forest is the biome with the highest number of species of harvestmen per location (12–64) (PINTO-DA-ROCHA *et al.*, 2005), being the best sampled, while the Amazon registers 21 to 28 species per locality (PINTO-DA-ROCHA; BONALDO, 2006). The Caatinga and Cerrado biomes have a lower diversity of harvestmen, with less than 10 species per location (KURY *et al.*, 2010; PINTO-DA-ROCHA *et al.*, 2005). However, this uneven diversity may be the result of sampling biases (KURY, 2003; PINTO-DA-ROCHA; BONALDO, 2006).

Harvestmen of the Atlantic Forest are highly endemic, with endemicity rates reaching 97.5% (PINTO-DA-ROCHA *et al.*, 2005). Such rates are partially because of the reduced dispersal capacity of most of the order (especially the suborders Cyphophthalmi and Laniatores), when compared to other arthropods of the same size, and to their high philopatry (MACHADO *et al.*, 2000; MESTRE; PINTO-DA-ROCHA, 2004; WILLEMART; GNASPINI, 2004). Of the approximately 6,600 species of harvestmen described worldwide, approximately 600 occur only in the Atlantic Forest (PINTO-DA-ROCHA *et al.*, 2005). Their restricted geographical distributions, combined with their preferred habitats, such as preserved humid forests and caves, increases the extinction potential for many species of this group (BRAGAGNOLO *et al.*, 2007). On the other hand, the high endemicity of harvestmen in the Atlantic Forest makes them excellent models for biogeographical and ecological studies (DASILVA *et al.*, 2015; PINTO-DA-ROCHA *et al.*, 2005).

The living species are classified into four suborders, all monophyletic: Cyphophthalmi, Eupnoi, Dyspnoi and Laniatores. Many studies corroborate these groupings as natural groups, although their composition has recently been contested (GROH; GIRIBET, 2015). There is a recently-described fifth suborder, the fossil (extinct) Tetrophthalmi from the Paleozoic (GARWOOD *et al.*, 2014).

Within Laniatores, the superfamily Gonyleptoidea stands out as the most diverse and with the most morphologically showy representatives, with 14 families and 2,032 species (KURY *et al.*, 2020). It is also the group that concentrates the largest number of systematic revisions on Laniatores (PINTO-DA-ROCHA *et al.*, 2012), a factor of paramount importance for the correct identification of species and their inclusion in ecological and biogeographical studies. Within this superfamily, the family Gonyleptidae has the largest number of ecological, biogeographical and taxonomic studies in Brazil (ÁZARA *et al.*, 2013; DASILVA *et al.*, 2015; PINTO-DA-ROCHA *et al.*, 2012). In this context, the subfamily Goniosomatinae stands out as the best studied, considering ecological research in epigean and hypogean environments.

Parallel to the use of harvestmen for ecological, population and behavioral studies, research in caves has also contributed to the recording of them in subterranean environments. The first work listing cave fauna in Brazil to report harvestmen (at order level) was Dessen *et al.* (1980) and the first to report harvestmen of the superfamily Gonyleptoidea was Trajano (1986), who listed eight species distributed in 19 caves in the states of Goiás and São Paulo. Subsequently, other listings were made in which a growing number of different species was observed, as more caves were studied, such as: 10 species distributed in 15 caves in the states of Amazônia, Goiás, Mato Grosso and São Paulo (TRAJANO; GNASPINI, 1990); 17 species distributed in 43 caves in the states of Bahia, Goiás, Mato Grosso do Sul, Minas Gerais

and São Paulo (GNASPINI, TRAJANO, 1994); and 35 species in 114 caves in the states of Bahia, Goiás, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pará, Paraná and São Paulo (PINTO-DA-ROCHA, 1995). More than 20 years passed after the work of Pinto-da-Rocha (1995), which was based mainly on caves in São Paulo and a few in other states, until Ázara and Ferreira (2018) published a more up-to-date study. This listing is an extensive checklist of harvestmen of the superfamily Gonyleptoidea related with Brazilian caves, based on an analysis of the literature and scientific collections. They recorded harvestmen for 952 caves, 152 municipalities and 17 states in Brazil, indicating a substantial increase of more than 130 species and 800 caves compared to the work of Pinto-da-Rocha (1995). This increase in the number of records of cave species is related to the strong boost to carrying out inventories in Brazilian caves as a mandatory procedure for the environmental licensing process, in accordance with new speleological legislation, such as Federal Decree No. 6640 of 2008 (ÁZARA; FERREIRA, 2018; PILÓ *et al.*, 2015).

Caverna do Quarto Patamar, located in Serra de Paranapiacaba, state of São Paulo, was the cave with the greatest richness of harvestmen in the study of Ázara and Ferreira (2018), with seven species. On the other hand, 70% of the caves had records of only one species. This richness contrasts with other groups of arachnids, such as spiders, which are richer in these environments (INIESTA *et al.*, 2013; SILVA; FERREIRA, 2015). This probably reflects the greater richness of the order Araneae (ca. 3,013 species) in epigean environments (OLIVEIRA *et al.*, 2017) and, therefore, the greater number of species that may potentially colonize caves in other subterranean environments. The authors also recorded 155 species/morphospecies belonging to 61 genera, 12 subfamilies and seven families. In addition to the listing, the paper includes the distribution of these species in epigean (considering the described species) and hypogean environments, besides photographs of the male and female habitus for most species. In this way, this publication enables the recognition and preliminary identification of these harvestmen by specialists and the non-specialized public.

Ázara and Ferreira (2018) detected a high number of undescribed species, totaling 46 morphotypes, of which nine with potentially troglomorphic characteristics. In addition to the undescribed species, about 19% of the morphotypes were not identified to the species level, which demonstrates the underestimated richness of the group. Furthermore, from the taxonomic impediment point of view, there are groups that have not been revised using modern methods, including the study of male genitalia, and are still identified by inadequate descriptions by current standards. As examples, it is possible to cite some of the genera of Cosmetidae and Pachylinae that are artificial groups (groups formed by apparent similarity, such as "warm-blooded" animals, and not because they are descendents of an exclusive common ancestor, such as mammals), with descriptions are not very detailed, hampering the accurate identification of these taxa.

Harvestmen not only occupy natural hypogean environments, but also artificial ones such as geological research galleries. Azara *et al.* (2016) evaluated the assemblage of harvestmen in artificial caves in the state of Minas Gerais. Despite the anthropic genesis, this type of environment has characteristics similar to natural subterranean caves. The study recorded harvestmen in 52 of the 111 artificial caves inventoried, accounting for a total of 17 species associated with these environments, belonging to 10 genera, seven subfamilies and four families. As can be seen, surveys in natural caves or artificial galleries highlight the importance of these environments as habitats for harvestmen in Brazil.

According to Ázara and Ferreira (2018), it can also be concluded that the collections present a high amount of unpublished data, such as undescribed species and new records. This condition means that this information is not formally available in papers for the scientific community. Therefore, it becomes essential to intensify the processing, organization and analysis of harvestmen deposited in scientific collections, combined with the development of taxonomic studies and incentives to carry out faunal inventories. Thus, a better/greater understanding of Brazilian cave opilionofauna would become possible, especially in the face of increasing anthropic

disturbance, which often results in the loss of their external habitats or even in the suppression of caves, leading troglobitic species to extinction.

Main groups found In Brazilian caves

Brazil has a rich fauna of harvestmen associated with its caves, most of which are species of the superfamily Gonyleptoidea. There are only two species associated with the cave environment described so far in Brazil that do not belong to Gonyleptoidea: (1) *Canga renatae* DaSilva, Pinto-da-Rocha & Giribet, 2010 (Cyphophthalmi: Ogoveoidea: Neogoveidae), described from caves in the municipality of Parauapebas, in Serra dos Carajás, state of Pará, and (2) *Relictopiolus galadriel* Pérez-González *et al.*, 2017 (Laniatores: Zalmoxoidea; Kimulidae), described for Caverna Olhos D'água, in Parque Nacional Cavernas do Peruaçu, Itacarambi, state of Minas Gerais.

Gonyleptoidea houses the 16 species that were originally described from caves (ARAUJO-DA-SILVA *et al.*, 2020; ÁZARA; FERREIRA, 2018; MEDRANO; KURY, 2018; VILLARREAL *et al.* 2019), not considering troglobites, which will be discussed in another topic that will also include undescribed species with troglomorphisms. Since the publication of taxonomic and ecological works and the checklist proposed by Ázara and Ferreira (2018), there has been a considerable increase in species recorded from Brazilian caves, both from those species that already had records for these environments, and for species that had never been recorded in them previously (Figure 1) (ÁZARA; FERREIRA, 2018). Thus, there was an important evolution in the systematization of the cave opilionofauna of the superfamily Gonyleptoidea, allowing a glimpse of the current panorama of this group in these environments. Unfortunately, the lack of ecological, populational and behavioral studies makes it difficult to place some of these species in the modified Schinner-Racovitza ecological-evolutionary classification system (SKET, 2008).

Comments are provided below on the families, subfamilies and genera of Opiliones found in Brazilian caves, based on the analysis of the results of the literature. Non-gonyleptoid families that do not have systematized and published data on diversity and composition in cave environments will also be briefly addressed based on the analysis of material deposited in the Coleção de Invertebrados Subterrâneos de Lavras da Universidade Federal de Lavras (ISLA/UFLA) and the Museu de Zoologia da Universidade de São Paulo (MZUSP). The term species here includes those described and those not described, according to the list proposed by Ázara and Ferreira (2018).

It is noteworthy that, from the point of view of systematics, Opiliones inherited a classification system called the Roewerian system (ROEWER, 1923), which, despite its virtues (such as standardized descriptions and illustrations), was flawed for having adopted a limited number of characters to characterize groupings, such as genera and families, that is, it did not allow the inclusion of new characters. This system also disregarded variations within the same species, resulting in the creation of artificial groups or groups composed of only one species, which hampers the proposition of phylogenetic relationships among them (PINTO-DA-ROCHA *et al.*, 2012). This scenario began to change with greater intensity from the 1960s onwards (ŠILHAVÝ, 1961), with the study of male genitalia and the inclusion of more morphological characters. Subsequently, the use of computing to propose relationships among species (PINTO-DA-ROCHA, 1997), access to DNA sequences (whether excerpts from the genome or in its entirety) (BENAVIDES *et al.*, 2021; PINTO-DA-ROCHA *et al.*, 2014), and the advancement of morphological studies (KURY; VILLARREAL, 2015) led to major changes in proposing phylogenetic relationships and, consequently, in classification. It should be mentioned that the growing number of researchers that study this group has also contributed in this regard (HARA *et al.*, 2017). Thus, in the future, some of the diagnoses presented below can be expected to experience changes. This dynamism highlights the scientific nature of systematics, in which hypotheses of classification are replaced by others in the light of new evidence from the most diverse sources of data.

AGORISTENIDAE

The family Agoristenidae occurs in the Greater Antilles and northern South America and is organized into three subfamilies, totaling 76 species (KURY *et al.*, 2020). Agoristenids can be recognized by legs III and IV, which are comparatively thicker than legs I and II, in addition to having a typical penis, with a reduced distal part of the ventral plate and bifid and trifid macrosetae (KURY, 2012; PINTO-DA-ROCHA; KURY, 1973 apud PINTO-DA-ROCHA *et al.*, 2007). There is only one undescribed species registered for this family: *Avima* sp. 1 (ÁZARA; FERREIRA, 2018).

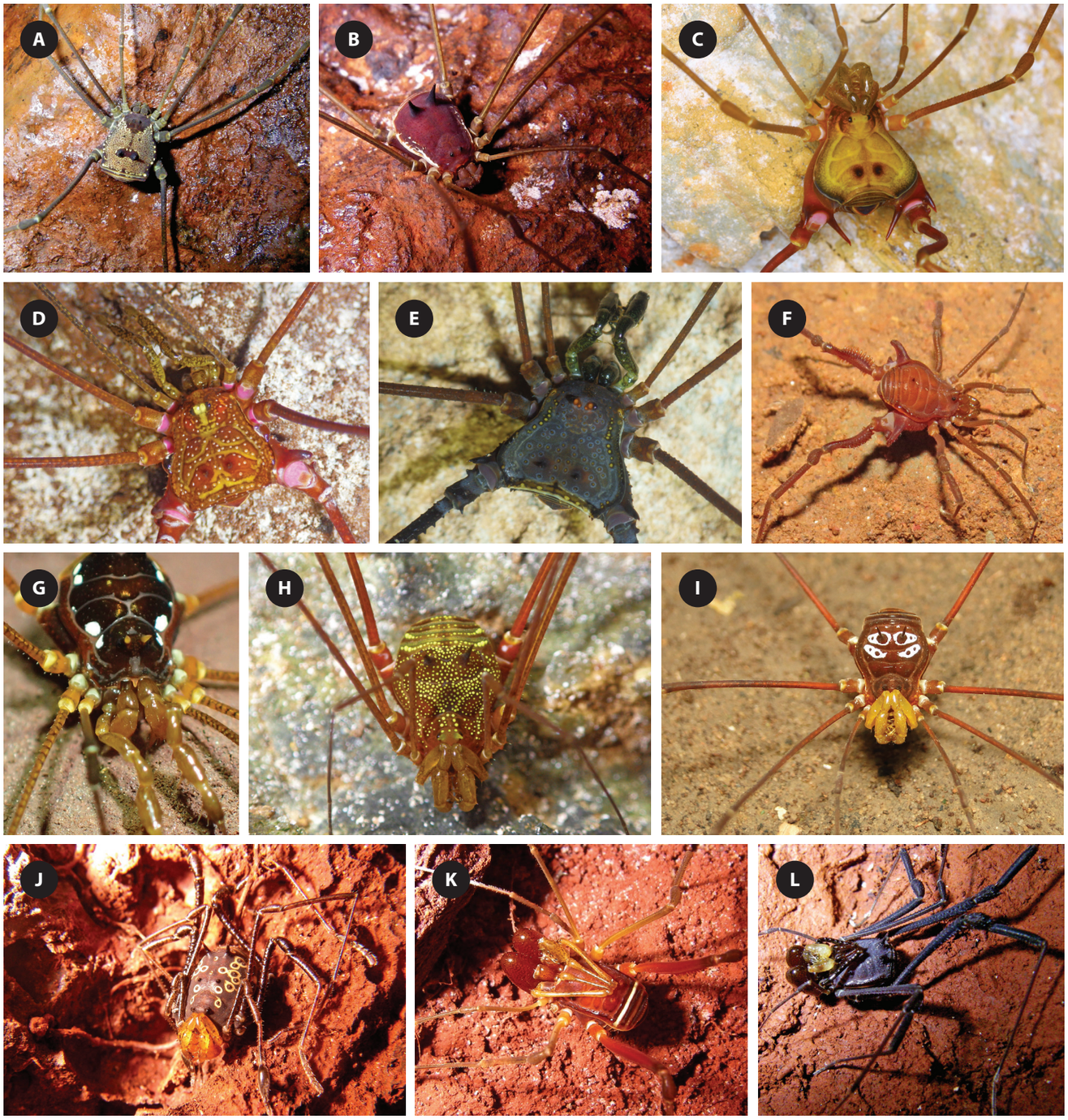


Figure 1. Examples of the life habitus of harvestment found in Brazilian caves. **A)** *Flirtea valida* (Roewer, 1928); **B)** *Gryne marginalis* (Perty, 1833); **C)** *Goniosoma vatrax* Koch, 1845; **D)** *Mitogoniella indistincta* Mello-Leitão, 1936; **E)** *Serracutisoma* sp.; **F)** *Eusarcus hastatus* Sørensen, 1884; **G)** *Ruschia maculata* (Soares, 1974); **H)** *Promitobates viridigranulatus* (Soares & Soares, 1946); **I)** *Paragoniosoma cachaceiro* Araujo-da-Silva, DeSouza & DaSilva, 2020; **J)** *Syncranaus cribrum* Roewer, 1913; **K)** *Obidosus laevis* (Sørensen, 1932); **L)** *Protimesius lucifer* Villarreal, Ázara & Kury, 2019. Photos: A, B, G, J–L (BioEspeleo Consultoria Ambiental), I (Leonardo S. Carvalho), D, E, F, H (Lucas M. Rabelo), C (Ubirajara Oliveira).

COSMETIDAE

Cosmetidae is the second richest family of Laniatores (KURY *et al.*, 2020) and easily recognized by the characteristic pedipalps (laterally flattened femur, spoon-shaped tibia and without conspicuous setae) (PINTO-DA-ROCHA; KURY, 1973 apud PINTO-DA-ROCHA *et al.*, 2007). However, it presents a major taxonomic impediment, since only about 8% of its species have been revised and analyzed in the light of new evidence, such as genital morphology. Major changes in cosmetid classification were proposed by Medrano *et al.* (2021), which should reduce this impediment in the future. The family occurs from southern North America to southern Brazil. It is the second largest family in number of species associated with the cave environment, with 23 species (Figure 1A, B), of which six have yet to be described (ÁZARA; FERREIRA, 2018).

CRYPTOGEOBIIDAE

The family Cryptogeobiidae was recently proposed and presents great morphological richness but with a typical penis (reduced distal part of ventral plate of the penis and massive proximal part bearing macrosetae), which is shared with Gerdesiidae. Typically, the family can be recognized by interlocking fasciolate processes on coxae IV and trochanters IV of males. The family comprises 52 species, occurring mainly in the Atlantic Forest (KURY, 2014). This is the third largest family in number of species associated with the cave environment, with 13 species (one troglobite), of which five are undescribed (ÁZARA; FERREIRA, 2018; KURY, 2014).

GERDESIIDAE

The family Gerdesiidade, recently described by Bragagnolo *et al.* (2015), is the sister group (that is, it is the most closely related family) to Cryptogeobiidae and is the smallest in number of species, with six total organized into two genera. It presents a disjunct distribution, with the genus *Gerdesius* in northern Brazil and *Gonycranaus* in the southeast. Of the latter, two species (*Gonycranaus pluto* and *Gonycranaus androgynus* Bragagnolo *et al.*, 2015) are associated with the cave environment, (*Gonycranaus pluto*) being classified as a troglobite.

GONYLEPTIDAE

Gonyleptidae is the richest family of Gonyleptoidea in number of species, with great morphological diversity, and is the most studied taxonomically, being composed of 17 subfamilies, 212 genera and 747 species (KURY *et al.*, 2020). Thus, it is to be expected that many of the species associated with caves in Brazil belong to this family. Sixty-one species are known to be associated with caves, of which approximately half are not described (29 species) and there are still five species that could not be identified to the species level (ÁZARA; FERREIRA, 2018). It should be noted that this family is also among the most studied in in relation to care, defensive behavior, chemical composition of defense secretion, aggregation, distribution and biogeography, among other aspects (PINTO-DA-ROCHA *et al.*, 2007). Considering that this family is well studied taxonomically, compared to the others, a brief description is included with subfamilies organized in descending order of the number of currently described non-troglobitic species (richness).

Pachylinae

Pachylinae is the most speciose subfamily of Gonyleptidae. It is polyphyletic (i.e., non-natural) (PINTO-DA-ROCHA *et al.*, 2014), composed of species that have four scutal areas and does not present the synapomorphies of other subfamilies. Due to its comprehensive diagnosis, it is expected to be the subfamily with the highest number of non-troglobitic species associated with caves: 38 species (Figure 1F) (ÁZARA; FERREIRA, 2018). Pachylinae will undergo major changes in the future, with probable dismemberment into smaller monophyletic units, as has already been happening, with propositions of subfamilies such as Roeweriinae (CARVALHO; KURY, 2018) and expansion of the concept of others, such as Gonyassamiinae (KURY *et al.*, 2020).

Goniosomatinae

Goniosomatinae is the most studied subfamily associated with cave environments in relation to ecology, population biology, physiology, behavior and biogeography (CHELINI *et al.*, 2011; DASILVA; GNASPINI, 2010; GNASPINI *et al.*, 2003; MACHADO *et al.*, 2003). Goniosomatinae includes conspicuous species that are easy to collect and maintain in captivity. It has been taxonomically revised and analyzed cladistically, allowing other studies to emerge, such as those mentioned above. It is the second largest group with non-troglobitic species associated with caves, with 18 species (Figure 1C–E) (ÁZARA; FERREIRA, 2018).

Mitobatinae

Species of Mitobatinae show sexual dimorphism by the elongation of the legs of the male (PINTO-DA-ROCHA *et al.*, 2014). They are often found in caves, with 16 known species (Figure 1G, H), making it the third most speciose subfamily.

Gonyleptinae

Gonlyleptinae is the second most speciose subfamily of Gonyleptidae with 127 species (KURY *et al.*, 2020). It is also polyphyletic and nested in clade K92 (PINTO-DA-ROCHA *et al.*, 2014). Its diagnosis is similar to that of Pachylinae, but with three scutal areas instead of four (PINTO-DA-ROCHA *et al.*, 2014). It is fourth in number of non-troglobitic species associated with caves, with 11 species (ÁZARA; FERREIRA, 2018).

Gonyassamiinae

Gonyassamiinae was recently reconceptualized in a catalog (KURY *et al.*, 2020), including several species previously included in Pachylinae. Its characterization is mainly based on the penis, highlighted by the stylus with a quadrangular and flattened distal region in lateral view (KURY *et al.*, 2020). It has three undescribed species associated with caves (ÁZARA; FERREIRA, 2018).

Roeweriinae

The recently described subfamily Roeweriinae (CARVALHO; KURY 2018) includes several species previously placed in the speciose genus *Discocyrtus* Holmberg, 1878. As *Discocyrtus* is revised, more genera exhibiting disctinct habitus have been included in the subfamily, increasing its morphological diversity. Currently, there are three species associated with caves, the most famous being *Khazaddum inerme* Soares & Soares, 1947, previously called *Daguerreia inermis* Soares & Soares, 1947 (KURY *et al.*, 2020).

Hernandariinae

With a confused taxonomic history, the characterization and identity of the subfamily Hernandariinae became clear after its revision (DASILVA; PINTO-DA-ROCHA, 2010). Many species in this group have bodies covered in debris. It has only one species associated with caves (ÁZARA; FERREIRA, 2018).

Tricommatinae

The subfamily Tricommatinae was properly characterized recently, having historically been associated with Cryptogeobiidae (KURY, 2014). Not a very rich group, it is composed of five species organized in three genera. It has an undescribed species associated with caves (ÁZARA; FERREIRA, 2018).

MANAOSBIIDAE

The family Manaosbiidae was recently proposed as a subfamily of Gonyleptidae according to analyses based on molecular data (PINTO-DA-ROCHA *et al.*, 2014). It occurs from Central America to northern/central Brazil. It presents a considerably conservative body morphology (very homogeneous), highlighted by the typical dorsal scutum, inflated spindle-shaped basitarsus I in males and the characteristic penis, without dorsal or ventral processes of the glans and elongated ventral plate (KURY, 1839 apud PINTO-DA-ROCHA *et al.*, 2007). There are two non-troglobitic manaosbiid species associated with caves (Figure 1J) (ÁZARA; FERREIRA, 2018).

STYGNIDAE

The family Stygnidae harbors some of the oldest species described from the Neotropics and was one of the first to be revised using modern methodologies, including cladistic analysis (PINTO-DA-ROCHA, 1997). The family comprises 110 species (KURY *et al.*, 2020), with several others having been described in the last decade, indicating that its richness is underestimated. It occurs in the North, Northeast and Central-West regions of Brazil and is easily recognized by the ocularium (= eye mound) divided into two parts and the sub-rectangular dorsal scutum, in addition to the typical penis. There are ten non-troglobitic species associated with caves (Figure 1K, L) (ÁZARA; FERREIRA, 2018).

Main troglomorphisms

The identification of troglomorphisms in Opiliones, as in other cave groups, involves issues such as identfying which attributes are truly associated with the condition of and restriction to the subterranean environment. The presence of attributes such as depigmentation and loss of eyes, is usually directly associated with these ecosystems, but other attributes, such as the elongation of appendages, must be more carefully considered. To identify whether a certain trait is troglomorphism or not, phylogenetically closely epigean groups should be analyzed (ideally, the closest related epigean species), to understand

which morphologies are intrinsic to the group and which are unrelated to the constraint of caves. It should also be noted that it is possible for a species to be troglobitic without showing evident troglomorphism. Therefore population ecology study is essential for proper ecological-evolutionary classification, crucial information in conservationist point of view.

Typically, the main troglomorphisms cited in the descriptions of the 14 Brazilian troglobitic species are: (i) varying degrees of reduction in eye size, from comparable to granules or tubercles to total absence; (ii) depigmentation to varying degrees, from slightly darkened forms to forms totally devoid of pigments (in this case, the eye, typically black, has the same color as the cuticle); (iii) lengthening of appendages (chelicerae, pedipalps and legs); and (iv) increase in tarsal counts.

Diversity of troglobitic species described for Brazil

Brazil currently has 14 described troglobitic species of harvestmen, mainly from the states of Minas Gerais and São Paulo (Figure 2, 3). The first to be described was *Spaeleoleptes spaeleus* H. Soares (1966), from Lapa Nova de Maquiné, municipality of Cordisburgo, Minas Gerais, which was placed in to Minuinae (Phalangodidae) and later transferred to Escadabiidae by Kury and Pérez-González (2007a). Although the original description does not indicate the species as restricted to the cave environment, the absence of eyes and the presence of numerous elongated setae on the appendages indicate restriction to the subterranean environment (troglobitic).

Šilhavý (1974) described *Pachylospeleus* to allocate the new species *Pachylospeleus strinatii* (Figure 2D), even erecting a subfamily (Pachylospeleinae) to house it. This species was described from Caverna das Areias, municipality of Iporanga, state of São Paulo. It was the first work describing a Brazilian species in which troglomorphisms were explained: reduction in eye size, depigmentation, elongation of appendages and an increase in the number of distitarsi I and II.

Pinto-da-Rocha (1996) described the third troglobitic species, *Iandumoema uai*, from Gruta Olhos D'água, municipality of Itacarambi, Minas Gerais. Do Monte *et al.* (2015) expanded the distribution of this species with a new record for Lapa do Cipó, which is 9.5 km northwest of the type locality. Currently, it is the only non-monotypic genus, with six species in total, with phylogenetic relationships based on cladistic analysis. *Iandumoema smeagol* was the fourth species of troglobitic harvestmen described and the third completely anophthalmic (PINTO-DA-ROCHA *et al.* 2015). The following species currently compose the genus: *I. cuca* ÁZARA *et al.*, 2020 (Figure 2A); *I. gollum* ÁZARA *et al.*, 2020; *I. setimapocu* HARA; PINTO-DA-ROCHA, 2008; *I. smeagol* PINTO-DA-ROCHA *et al.*, 2015 (Figure 2B); *I. stygia* ÁZARA *et al.*, 2020 (Figure 2C); and *I. uai* PINTO-DA-ROCHA, 1997 (ÁZARA *et al.*, 2020).

Kury and Pérez-González (2002) described *Giupponia chagasi* from Lapa do Boqueirão and Gruta do Zé Bastos, both in the municipality of Carinhanha, state of Bahia. This species stands out for being the second anophthalmic species described, besides possessing other troglomorphisms considered remarkable by the authors, such as high tarsal counts (leg II with 34–37 tarsomeres), elongated pedipalps and legs, and a high degree of depigmentation. The species was allocated to Gonyleptidae but was not included in any subfamily due to the lack of diagnostic features for the 15 subfamilies described up to that time.

Kury and Pérez-González (2008) described *Spinopilar moria* from the Morena cave (Figure 2E), in the municipality of Cordisburgo, Minas Gerais, allocating it to Tricommatinae. Later, Kury (2014) transferred the genus *Spinopilar* to the family Cryptogeobiidae, together with several other genera.

Kury (2008) described two new troglobitic species for Pachylinae: *Discocyrtus pedrosoi*, from Gruna do Brejo e Verruga cave, municipality of Andaraí, and *Eusarcus elinae*, from Caverna Pedra Furada, municipality of Iraquara, both in the state of Bahia.

Bragagnolo *et al.* (2015) described the troglobitic species *Gonycranaus pluto*, allocating it to Gerdesiidae, a family circumscribed in the same work. The species was described from Cave MP01B, municipality of Morro do Pilar, Minas Gerais, a region with a high number of ferruginous caves.

Pérez-González *et al.* (2017) described *Relictopiolus galadriel*, the first cave-dwelling species of the family Kimulidae. The species was described from Caverna Olhos D'água, Parque Nacional Cavernas do Peruaçu, municipality of Itacarambi, Minas Gerais. Thus, it is the second species of troglobitic harvestmen for this cave, something unusual to see.

Main methods of collection and conservation

Several sampling methods can be used to collect harvestmen in cave environments, but the only selective one for them is active manual collection. This method consists of actively



Figure 2. Examples of the life habitus of harvestment found in Brazilian caves. **A)** *Iandumoema cuca* Ázara, Hara & Ferreira, 2020; **B)** *Iandumoema smeagol* Pinto-da-Rocha, Fonseca-Ferreira & Bichuette, 2015; **C)** *Iandumoema stygia* Ázara, Hara & Ferreira, 2020; **D)** *Pachylospeleus strinatii* Šilhavý, 1974; **E)** *Spinopilar moria* Kury & Pérez-González, 2008. Photos: A–D (Lucas M. Rabelo), E (Robson de A. Zampaulo).

searching, prioritizing specific microhabitats according to the morphological, ecological and behavioral characteristics of the organisms of interest. In this way, special attention is given to searching under logs, under rocks, in crevices, among organic material and on wet rocky walls, among other places. With this collection method, it is important that the collector inspect/turn over rocks and dead logs, noting both the surface it was resting on and the bottom of the object itself, as specimens can be adhered to it. It is also recommended that, if possible, the substrate be inspected by turning it over, as with litter, or even digging to shallow depths, in the case of sand, soil or gravel. When found, harvestmen are collected manually with the aid of forceps or brushes and stored in containers with preservative liquid (alcohol diluted to 70% or absolute alcohol).

Capture by means of pitfall traps is also a frequently used method. Collecting is carried out with 500ml and 7cm in diameter plastic pots (measures may vary), which are buried in the ground until the rim, containing from 170 to 200ml (may vary) of preservative liquid with a drop of detergent to reduce surface tension. During displacement on the substrate, the harvestmen fall into the pots and become preserved in the liquid inside. Protective screen can be used over the traps to prevent dripping from overflowing the preservative liquid or diluting its content, thereby impairing chemical fixation. After a certain period of time (may vary according to climatic conditions in the epigean environment), the material collected by the traps is transferred to definitive preservative liquid (alcohol diluted to 70% or absolute), depending on the objectives of the research. It is important that the trap is not exposed for a period longer than seven days to avoid capturing an excessive number of animals, because besides altering the properties of the preservative liquid, it can lead to saturation and overflow of its contents, leading to material degradation.

Other methods can be adapted depending on the conditions of the cave environment. In environments with litter and abundant decomposing organic material, such as leaves, trunks and branches, leaf litter sieving can be employed. In this active



Figure 3. Map with the type localities of troglobitic species of harvestmen described for Brazil.

method, one attempts to collect the harvestmen that inhabit the leaf litter, sieving it through plastic meshes, with an opening of approximately 1cm. The objective is to separate and collect small and camouflaged species from the rest of the leaf litter. The obtained material is collected in a square of blackout-type canvas for further processing by visual search for harvestmen.

The Winkler extractor method can also be adapted for sampling small invertebrates in the litter. The method first consists of delimiting 1m^2 plots, the litter of which is sieved and collected in a concentrator. The resulting particulate matter is then placed for two days in a Winkler extractor, which consists of a plastic mesh bag, inside another white cloth bag, containing a bottle of alcohol tied at the bottom to collect the organisms.

There have been no studies evaluating the efficiency of collection methods for harvestmen in cave environments. However, several studies indicate that the active manual collection method is the most efficient for collecting harvestmen in forest environments, as it allows sampling a greater number of species and individuals (PORTO *et al.*, 2016; TOURINHO *et al.*, 2014, 2018). Thus, manual active collection should be prioritized in inventories in cave environments and, when possible, be complemented with the methods listed above, depending on the specific conditions of the subterranean habitat.

Identification key

The following key was developed to help identify families, based on known records for Brazilian caves.

1. Pedipalps subcylindrical, provided with conspicuous spines on the tibia and tarsus **2**

- Pedipalps with laterally flattened femur and spoon-shaped tibia, without conspicuous spines (easily visible to the naked eye) ..... **Cosmetidae**

2. Ocularium divided into two parts (eyes separate, not sharing common elevation)..... **3**

- Ocularium single **4**

3. Body shape subrectangular, or with dilated median region, distal part of ventral plate of penis developed, assuming subrectangular shape, glans without ventral process..... **Gonyleptidae (Tricommatinae)**

- Body shape subrectangular, distal part of ventral plate of penis little developed..... **Stygnidae**

4. Legs III–IV comparatively thicker than legs I–II; chelicerae teeth weak..... **Agoristenidae**

- Legs I–IV of comparable thickness; chelicerae teeth well-developed..... **5**

5. Tibiae I–II of males thickened, with notch or process..... **Escadabiidae**

- Tibiae I–II comparable to other legs **6**

6. Ocularium displaced to anterior margin of dorsal scutum; penis stylus protected by stragulum and ventral penis plate organized into rutrum and pergula..... **Zalmoxidae**

- Ocularium in a variable position in relation to the anterior margin of the dorsal scutum; penis stylus exposed and ventral plate of penis organized differently from Zalmoxidae **7**

7. Scutal area I undivided or slightly divided into right and left halves; distal part of ventral plate of penis reduced, forming a short tongue..... **8**

- Scutal area I divided into right and left halves; distal part of ventral plate of penis well-developed, forming a sub-rectangular plate..... **9**

8. Coxa IV of males with ventro-distal apophyses (generally fasciolate and hyaline) that interlock with ventro-retrolateral apophyses of trochanter IV..... **Cryptogeobiidae**

- Coxa IV without the apophyses of Cryptogeobiidae, or if present, with a distinct shape..... **Gerdesiidae**

9. Basitarsus I of males inflated and spindle-shaped; penis with rectangular and very elongated ventral plate; glans without ventral process..... **Manaosbiidae**

- Basitarsus I variable, usually not inflated; penis with ventral plate of variable shape, rarely very elongated; glans usually with ventral process ..... **Gonyleptidae**

Species described for Brazilian caves

There is a large number of harvestmen deposited in the collections of Coleção de Invertebrados Subterrâneos de Lavras da Universidade Federal de Lavras (ISLA/UFLA); Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ); and Museu de Zoologia da Universidade de São Paulo (MZUSP) belonging to the families Sclerosomatidae (Eupnoi), Zalmoxidae (Laniatores) and Escadabiidae (Laniatores) that have never been studied.

The family Escadabiidae was described from only one diagnosis (KURY, 2003) to allocate four genera and six species originally placed in Phalangodinae (Phalangodidae). Subsequently, it was properly characterized (KURY; PÉREZ in PINTO-DA-ROCHA; MACHADO; GIRIBET, 2007a), an occasion when the troglobitic species *Spaeleoleptes spaeleus* H. Soares, 1966 was formally transferred from Minuinae (Phalangodidae). Little has since been done taxonomically about the group, whose distribution is inferred to be from the coast of Northeast Brazil to the central region of Minas Gerais (KURY; PÉREZ in PINTO-DA-ROCHA; MACHADO; GIRIBET, 2007a), and so it is quite possible that its richness is underestimated. Identification of the family based only on external morphology is insufficient and leads to mistakes, which makes this group challenging when it is necessary to screen samples, for example. Thus, for the correct identification of representatives of this family, male genitalia must be examined. Despite some diagnostic characters, such as tibiae I and II and armature of free tergites (KURY; PÉREZ in PINTO-DA-ROCHA; MACHADO; GIRIBET, 2007a), the habitus of this group is easily confused with other phylogenetically distinct taxa, such as Tricommatinae (Gonyleptidae), Cryptogeobiidae and Zalmoxidae, among others.

Members of the family Zalmoxidae are small harvestmen with a disjunct distribution in the Neotropical, Australasian and Afrotropical and Indo-Malay regions (KURY; PÉREZ in PINTO-DA-ROCHA; MACHADO; GIRIBET, 2007a). It lacks taxonomic revisions, with a special focus on penile morphology, as suggested by Pérez-González (2011) to verify the validity of described genera and their respective monophyly. The fact that almost nothing is known beyond the name of the species in this group makes inferences regarding putative troglomorphisms very uncertain, since little is known about its populational, morphological and ecological aspects. To make matters worse, the tiny size makes dissection and morphological study difficult, especially of the penis. The morphotypes analyzed in the listed collections show apparent body depigmentation, a generally troglomorphic characteristic for several groups of already-described troglobitic harvestmen (ÁZARA; FERREIRA, 2018; ÁZARA *et al.*, 2020). However, as mentioned above, its classification is uncertain due to the possibility of being a characteristic that already occurs in epigean species.

The family Sclerosomatidae (Eupnoi) presents issues similar to the two families listed above, with a considerable taxonomic impediment due to the lack of specialists and, consequently, of specific studies, such as taxonomic revisions, phylogenetic analyses and descriptions of new taxa. A considerable number (over 100 lots) have already been inventoried for collections, but this material has never been properly studied.

Main collections in Brazil

Ázara and Ferreira (2018) compiled the records of the three main collections that have lots of harvestmen associated with caves (curator in parentheses): Coleção de Invertebrados Subterrâneos do Centro de Estudos em Biologia Subterrânea da Universidade Federal de Lavras (ISLA ; Rodrigo Lopes Ferreira), Coleção de Aracnídeos do Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ; Adriano Brilhante Kury) and Museu de Zoologia da Universidade de São Paulo (MZUSP; Ricardo Pinto-da-Rocha). The study counted 111 species for the ISLA collection, 65 for MZUSP and 29 for MNRJ. However, most of the Museu Nacional collection of harvestmen was destroyed by a fire in September 2018, leaving no lots of harvestmen collected in caves.

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Endecous troglobius
Lucas Mendes Rabelo

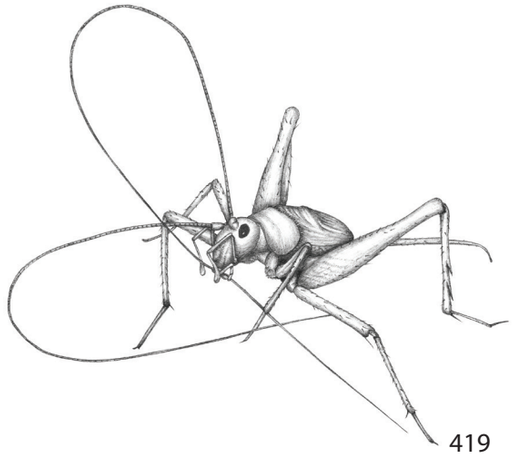
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Orthoptera

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General introduction

Brazilian cave fauna began to be known in the 1980s, after a series of faunal surveys (CHAIMOWICZ, 1984; DESSEN *et al.*, 1980; PINTO-DA-ROCHA, 1994; TRAJANO, 1992; TRAJANO; MOREIRA, 1991). Together with other invertebrates (beetles, spiders and opiliones) and vertebrates (fish and bats), these studies reported crickets as common components of this fauna (GNASPINI; TRAJANO, 1994).

Orthoptera is the most diverse order of Polyneoptera, with over 28,000 described species. They are cosmopolitan, inhabiting tropical and subtropical regions, and can be found at high altitudes and latitudes. Their populations are generally large, and the abundance of individuals makes them an important faunal component. Crickets (Orthoptera, suborder Ensifera) are the orthopterans classified in the superfamily Grylloidea, the second most diverse lineage in Ensifera. The other suborder, Caelifera, whose main representatives are locusts, is characterized by diurnal and phytophagous insects, being rare or absent from caves. Representatives of Ensifera are characterized by having long antennae; eardrums on the anterior tibias; a dorso-ventrally flattened body; anterior wings modified for the production and propagation of acoustic signals, only in male and adult individuals of the species that stridulate (apterous or brachypterous species are common); and, finally, tarsi with three segments. There are 5,900 known species distributed worldwide, with 257 in Brazil, classified into four families: Gryllidae, Mogoplistidae, Phalangopsidae and Trigonidiidae (CHINTAUAN-MARQUIER *et al.*, 2016; CIGLIANO *et al.*, 2022).

Since the 19th century, some authors have drafted definitions about animals that inhabit caves, often called trogloxenes, troglophiles and troglobites (BARR JR; HOLSINGER, 1985; HOLSINGER; CULVER, 1988; RACOVITZA, 1907; SCHINNER, 1854). The study of this classification extends until today with the most recent definition being that proposed by Trajano (2012), in which the author defines: (1) troglobites as exclusively subterranean source-populations, while sink populations can be found in epigean environments; (2) troglophiles as source populations in both environments (epigean and hypogean), with individuals regularly transiting between these habitats and promoting the introgression of selected genes into the subterranean population regime (and vice versa); and (3) trogloxenes as fractions of the source population in epigean habitats, but which use subterranean resources (in so-called obligate trogloxenes, all individuals are dependent on both subterranean and epigean resources).

Inventories and studies on the classification of cave fauna are common in Brazil (GNASPINI; TRAJANO, 1994; PINTO-DA-ROCHA, 1997; TRAJANO, 1987; TRAJANO; MOREIRA, 1991; TRAJANO; BICHUETTE, 2010). However, few works have studied the evolutionary biology or subterranean habits of crickets (*e.g.* ALEXANDER; OTTE, 1967; BOAKE, 1984; DAMBACH; LICHTENSTEIN, 1978; DESUTTER-GRANDCOLAS, 1993, 1997, 1997/1999; GNASPINI; HOENEN, 1999; GNASPINI; PELLEGATTI-FRANCO, 2002), and only recently were the first Brazilian records of troglobitic crickets published: *Endecous* (*Endecous*) *peruassuensis* Bolfarini, 2015, *E. (Pedroecous) apterus* Bolfarini & Dias, 2014, *E. (Pedroecous) troglobius* Castro-Souza, Pereira Junta & Lopes Ferreira, 2020 and *Erebonyx catacumbae* de Mello, 2021.

Main groups found in Brazilian caves

All known species of cave crickets in Brazil, so far, are classified in the family Phalangopsidae (Table 1). This family differs from other lineages of Grylloidea by having elongated appendages, especially the legs, giving a spidery appearance to its species. Phalangopsids, in general, also share a well-developed antennal scape and often a marbled color pattern, in various shades of brown. Phalangopsid crickets are usually brachypterous, with absent hindwings. Some species are apterous and a few are macropterous (fore and hind wings developed).

Phalangopsidae has about 1,100 described species and is the most diverse family of Grylloidea in the Neotropics, with 118 species registered for Brazil. Phalangopsids are mostly nocturnal crickets, inhabitants of leaf litter, various cavities (cracks, crevices in ravines, burrows and caves) or living trunks. Although the greatest diversity of this family is in the Neotropical region (CIGLIANO *et al.*, 2022), other representatives of the group,

which also have a cave habit, such as the genera *Phaeophilacris* Walker, 1871 and *Socotracris* Desutter-Grandcolas, 2012, can be found in Africa and the Middle East, respectively. So far, 34 species that inhabit caves have been recorded for Brazil, distributed in the genera: *Strinatia* Chopard, 1970 (one species), *Eidmanacris* Chopard, 1956 (six species), *Endecous* Saussure, 1878 (12 species), *Bambuina* de Mello, Horta & Bolfarini, 2013 (one species), *Phalangopsis* Serville, 1831 (eight species), *Erebonyx* de Mello, 2021 (one species) and Adelosgryllus Mesa & Zefa, 2004 (five species). Some species of these genera are shown in Figure 1.

Desutter-Grandcolas (1995) investigated habitat diversity, evaluating phalangopsid crickets of French Guiana. In this work, the author proposes the term "cavícolous" (cavicoly) when referring to troglophiles, which are characterized by inhabiting natural cavities in the soil, such as burrows, crevices in rocks, ravines and fallen logs, and can be found at the entrance of caves. Trogloxenes were not mentioned in the study, probably because

Table 1 – List of species described from Brazilian caves.

Phalangopsidae Blanchard, 1845

***Adelosgryllus* Mesa & Zefa, 2004**
A. lucifugus Sant'Ana Merlo, Castro-Souza, Pereira Junta & Lopes Ferreira, 2021
Adelosgryllus endogeus Merlo, Castro-Souza, Junta & Ferreira, 2022
Adelosgryllus ferratilis Merlo, Castro-Souza, Junta & Ferreira, 2022
Adelosgryllus megapterus Merlo, Castro-Souza, Junta & Ferreira, 2022
Adelosgryllus xambioa Merlo, Castro-Souza, Junta & Ferreira, 2022

***Bambuina* de Mello, Horta & Bolfarini, 2013**
B. bambui de Mello, Horta & Bolfarini, 2013

***Eidmanacris* Chopard, 1956**
E. alboannulata (Piza, 1960)
E. dissimilis Desutter-Grandcolas, 1995
E. larvaeformis Chopard, 1938
E. neomarmorata Campos, 2017
E. scopula Campos, 2017
E. speluncae (Mello-Leitão, 1937)

***Endecous* Saussure, 1878**
subgênero *Endecous* Saussure, 1878
E. (E.) alejomesai Zefa, 2010
E. (E.) betariensis de Mello & Pellegatti-Franco, 1998
E. (E.) cavernicolus Costa Lima, 1940
E. (E.) painensis Castro-Souza, Pereira Junta & Lopes Ferreira, 2020
E. (E.) peruassuensis Bolfarini, 2015
E. (E.) ubajarensis Zefa, 2014

subgênero *Notendecous* Gorochov, 2014
E. (N.) bahiensis Castro-Souza, Zefa & Lopes Ferreira, 2017
E. (N.) potiguar Castro-Souza, Zefa & Lopes Ferreira, 2017
E. (N.) onthophagus (Berg, 1891) *onthophagus*

subgênero *Pedroecous* Gorochov, 2014
E. (P.) apterus Bolfarini & Souza-Dias, 2014
E. (P.) didymus Castro-Souza, Zefa & Lopes Ferreira, 2020
E. (P.) troglobius Castro-Souza, Zefa & Lopes Ferreira, 2020

***Erebonyx* de Mello, 2021**
E. catacumbae de Mello, 2021

***Phalangopsis* Serville, 1831**
P. araguaia Pereira Junta, Castro-Souza & Lopes Ferreira, 2020
P. arenita Mews & Sperber, 2008
P. bauxitica Mews & Sperber, 2008
P. ferratilis Pereira Junta, Castro-Souza & Lopes Ferreira, 2020
P. kyju Pereira Junta, Castro-Souza & Lopes Ferreira, 2020
P. kysuia Pereira Junta, Castro-Souza & Lopes Ferreira, 2020
P. longipes Serville, 1831
P. quartzitica Pereira Junta, Castro-Souza & Lopes Ferreira, 2020

***Strinatia* Chopard, 1970**
S. brevipennis Chopard, 1970

there are no records of phalangopsid crickets in this category. In turn, troglobites were defined as crickets that exclusively live in caves. The phalangopsid taxa recorded in Brazilian caves follow:

Adelosgryllus

The genus *Adelosgryllus* has representatives in Brazilian and Peruvian territories, with eleven species currently described. Five species have been recorded in caves thus far: *Adelosgryllus lucifugus*, recorded in Morcegos and Onça Morta caves, both in the state of Piauí; *A. ferratilis*, found in caves of Serra Leste, state of Pará; *A. xambioa*, found in Explosão cave, state of Tocantins; *A. megapterus*, found in caves of Conceição do Mato Dentro, state of Minas Gerais, and *A. endogeu*s, found in Santuario cave, in Minas Gerais.

Bambuina

Bambuina is a monotypic genus with only the species, *Bambuina bambui* de Mello, Horta & Bolfarini, 2013, described from specimens collected in Gruta do Centenário in Mariana, state of Minas Gerais.

Eidmanacris

The genus *Eidmanacris* has 29 described species, of which three have been recorded only in Brazilian caves (*E. speluncae, E. scopula* and *E. neomarmorata*) and another three with records both inside and outside of caves (*E. alboannulata, E. dissimilis* and *E. larvaeformis*). This genus has a wide distribution throughout the Brazilian territory in the Atlantic Forest and Cerrado. There are also species described for Bolivia and Paraguay, in the Chiquitano and Chaco Forests, respectively. *Eidmanacris* does not occur in the Amazon Forest. Although the other species have not been recorded in caves, they are often found in small natural cavities, such as burrows of small mammals at ground level and in ravines close to rivers.

Endecous

Among the most representative in Brazilian caves, the genus *Endecous* is divided into three subgenera, *E. (Endecous), E. (Notendecous)*, and *E. (Pedroecous)*. It currently has 21 described species, distributed throughout Brazil and three other countries in South America: Argentina, Bolivia and Uruguay. They are found in several biomes, such as the Atlantic Forest, Caatinga, Cerrado, Chaco and Pampa. There are no records of *Endecous* in the Amazon Forest, with the caves in this region being colonized by crickets of the genus *Phalangopsis*. Twelve species have been recorded in caves in Brazil: *E.* (*Endecous*) *alejomesai*, *E.* (*Endecous*) *betariensis*, *E.* (*Endecous*) *cavernicolus*, *E.* (*Endecous*) *painensis*, *E.* (*Endecous*) *peruassuensis*, *E.* (*Endecous*) *ubajarensis*, *E.* (*Notendecous*) *bahiensis*, *E.* (*Notendecous*) *potiguar*, *E.* (*Notendecous*) *onthophagus*, *E.* (*Pedroecous*) *apterus*, *E.* (*Pedroecous*) *didymus* and *E.* (*Pedroecous*) *troglobius*.

Erebonyx

Erebonyx is a recently described new cave genus. It comprises only one species, *E. catacumbae*, which was recorded in a limestone cave in Campo Formoso, in the north of the state of Bahia. This cricket is considered troglobitic, with its most notable character being the absence of ommatidia in the eyes. Morphologically, Erebonyx resembles the genus *Adelosgryllus*, mentioned previously.

Phalangopsis

Found in a large number of caves in northern Brazil, the genus *Phalangopsis* comprises 11 species, eight of which have already been recorded in caves in Brazil: *P. araguaia, P. arerita, P. bauxitica, P. ferratilis, P. kyju, P. kysuia, P. longipes* and *P. quartizica*. This genus occurs in the Brazilian Amazon and French Guiana, with its distribution limited to the Amazon biome.

Strinatia

The genus *Strinatia* is found in regions of the Atlantic Forest and has only two described species, *S. brevipennis* and *S. teresopolis*. Only *S. brevipennis* has been recorded in caves, mainly in the south of the state of São Paulo and north of the state of Paraná.

Uvaroviella

Another genus of cricket found in caves in the Amazon and in transition regions with the Cerrado in the states of Pará and Tocantins is *Uvaroviella* Chopard, 1923, which, despite being known by the scientific community, does not yet have any officially published species. Species of this genus inhabit the epigean environment but are common in cave entrance and penumbra zones in the northern region of Brazil. The genus was not included in Table 1 because there are still no species described for Brazil.

Main troglomorphisms

Troglomorphism comprises a set of convergent characteristics found in organisms that live in subterranean,

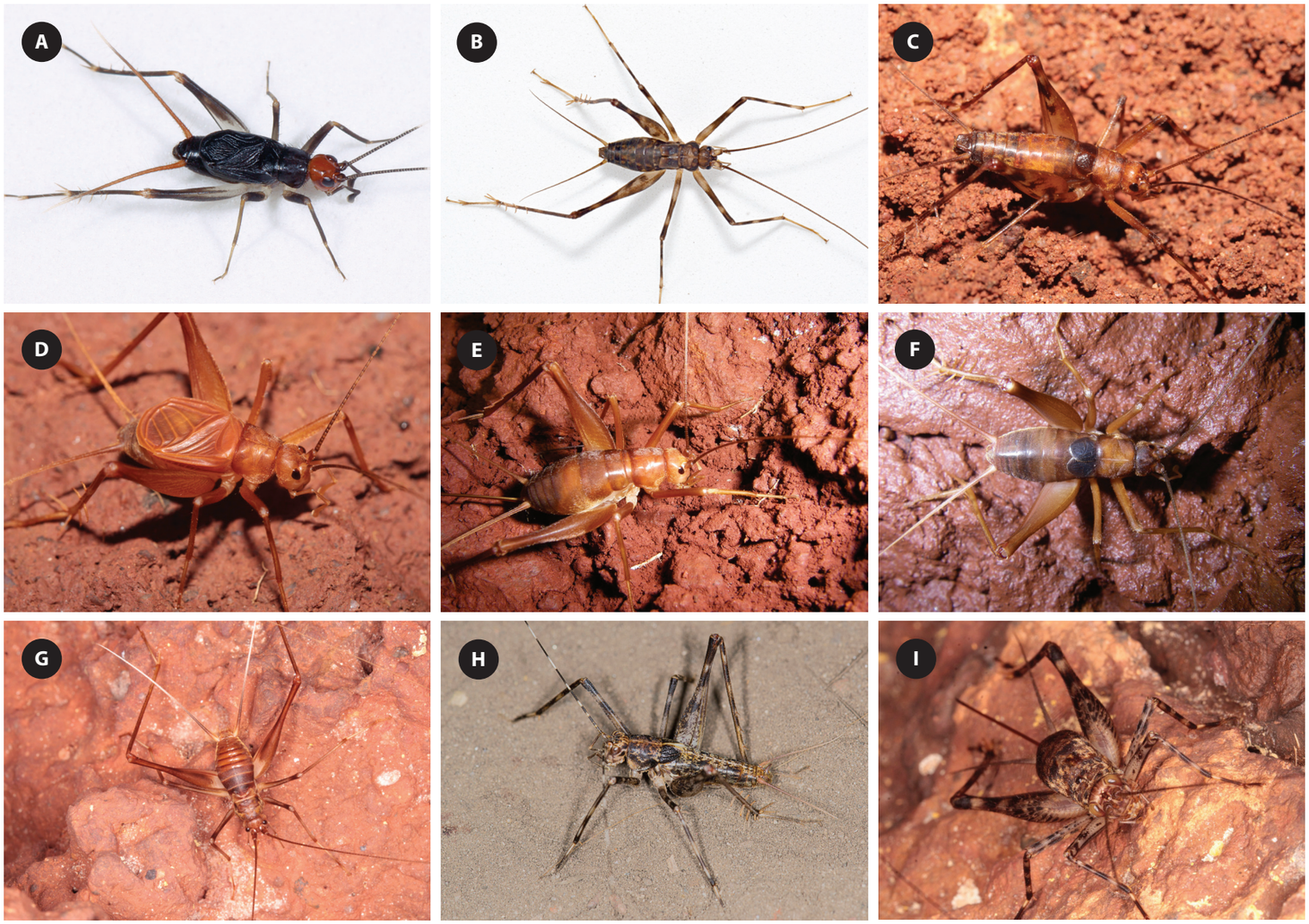


Figure 1. A) Male of *Adelosgryllus* sp.; **B)** male of *Bambuina* sp.; **C)** *Strinatia brevipennis,* species of brachypterous cricket found in caves in the Parque Estadual Turístico do Alto Ribeira (PETAR, SP); **D)** male of *Endecous painensis,* cricket with wings and functional stridulatory apparatus; **E)** apterous female of *Endecous* sp.; **F)** *Endecous* young male. Note the partially developed wings; **G)** male of *Phalangopsis longipes;* **H)** male of *Eidmanacris neomarmorata* on the floor of Casa de Pedra cave in Parque Nacional da Chapada dos Guimarães (MT); **I)** male of *Uvaroviella* sp. n. from caves of Canaã dos Carajás (PA). Photos: A, B, H (Lucas Denadai de Campos), C, D, G (Marcio Bolfarini), E, F, I (Robson de A. Zampaulo).

aphotic environments, presumably resulting from similar selective pressures (CHRISTIANSEN, 1962). These features are called troglomorphies, while the species that possess them are called troglomorphics (CULVER; PIPAN, 2019b). Troglomorphies often include a mixture of reductions and/or enlargements, such as loss of eyes and body pigmentation or overdevelopment of non-optic sensory structures and elongation of appendages (CULVER; PIPAN, 2019b). For many years, troglomorphism was thought to result from selective factors operating in caves and linked to changed patterns of nutrient availability and habitat type. However, as the absence of light is the only common factor in all these environments (CULVER *et al.*, 2010), these modifications may not be exclusively related to cave environments.

In an ecological-evolutionary classification, the obligatory residents of subterranean habitats are called troglobites. Curiously, not all organisms that live in subterranean environments are troglomorphs, while on the other hand, not

all organisms that present troglomorphic characteristics are troglobites (CULVER; PIPAN 2019b; KONEC *et al*., 2015; PIPAN; CULVER, 2012; SKET, 2008). The fact that troglomorphic features are present in species that live in non-cave environments, such as soil and litter, for example, indicates that their evolution is more complex than supposed (HEADS, 2010). Troglomorphisms are character states that suggest a possible relationship between the species and a hypogeal environment, and may not only be morphological, but also physiological or behavioral (BOLFARINI; BICHUETTE, 2015). They differentiate hypogean organisms from epigean species, but do not define the exclusive relationship of these species with the subterranean environment (JUBERTHIE; DEC, 1994). Therefore, the combination of the occurrence of a living (breeding) population within caves or other subterranean habitats with the absence of established populations in the epigean environment, hence with restricted distribution, is a better strategy to define a species as a troglobite without using a cladistic analysis. However, according to Desutter-Grandcolas (1997), the phylogenetic context is the most accurate procedure to define troglobites.

The troglomorphic conditions found in crickets in the Brazilian territory were first mentioned with the species *Endecous (Pedroecous) apterus* Bolfarini & Souza-Dias, 2014, for which the authors described some morphological characteristics considered as troglomorphisms, such as pale coloration and absence of the auditory eardrum and wings. The absence of wings is the most notable feature of this species and is unique among the 21 species of the genus. In 2015, another troglomorphism was described for the group, with the species *E. (Endecous) peruassuensis* Bolfarini, 2015. The authors described that certain observed morphological and physiological characteristics observed may be present in troglomorphic species, such as reduced size for the genus (miniaturization) and low mobility. Such behavior is easily observed in individuals who move slowly when touched by researchers. This slow reaction was also observed for the species *E. (Pedroecous) troglobius* (CASTRO-SOUZA *et al.* 2020), which also has another notable troglomorphic condition, the presence of depigmented ommatidia (Figure 2). Thus, the genus Endecous has three species with characters considered troglomorphic, some shared by more than one of them. Likewise, *E. (Pedroecous) troglobius* and *Erebonyx catacumbae* share having a large part of the ommatidia depigmented as a troglomorphism.

Studies involving evolutionary biology and troglomorphisms are still in their initial stages and are restricted to the recording and description of possible troglomorphies. Therefore, we reinforce the need for further studies on troglomorphic conditions in crickets, especially in light of cladistic analysis.



Figure 2. *Endecous (Pedroecous) troglobius*. Species with compound eyes with depigmented ommatidia. Photo: (Marcio Bolfarini).

Diversity of troglobitic species described for Brazil

The diversity of troglobitic crickets for Brazil is poorly studied and incipient. Only three species described for the genus *Endecous* and one for the genus *Erebonyx* are considered troglobites, namely: *Endecous (Endecous) peruassuensis, E. (Pedroecous) apterus, E. (Pedroecous) troglobius* and *Erebonyx catacumbae* (Figure 3). The distributions of these species are restricted to a few caves in the regions where they live. The first species to be considered troglobitic, *E. (P.) apterus*, was described only in 2014 and its distribution is restricted to three natural subterranean caves in the municipality of Ituaçu, state of Bahia. The species *E. (E.) peruassuensis* and *E. (P.) troglobius* are found, respectively, in the Olhos D'agua caves, in the region of Parque Nacional Cavernas do Peruaçu, in the municipality of Itacarambi, and in the Lapa Sem Fim cave, in the municipality of Luislândia, both in the state of Minas Gerais. *Erebonyx catacumbae* is the most recent discovery among Brazilian troglobitic crickets. Described in 2021, this species was recorded in a single cave, "Toca do Gonçalo", in the municipality of Campo Formoso, Bahia. Furthermore, recent records indicate that this cave was artificially drained (subterranean water exploitation) and that no more individuals of *Erebonyx catacumbae* were found inside it. Thus, this recently described species is probably already threatened with extinction (DE MELLO; FERREIRA, 2021).

CONSERVATION OF CAVERNICOLOUS CRICKETS

The cricket fauna, as well as Brazilian biodiversity as a whole, is constantly threatened by several factors, among which it is worth highlighting agriculture, soil pollution, urban expansion and energy production and mining activities (Instituto Chico Mendes de Conservação da Biodiversidade, 2018). Phalangopsidae is the only family with representatives evaluated in lists of endangered animals, highlighting cavernicolous crickets as the most endangered. When we surveyed references on the conservation status of Brazilian Orthoptera, we found a great lack of information on populations and knowledge gaps about the distribution of species, whether based on scientific data published in indexed journals or technical-scientific reports sent to the responsible environmental agencies. When considering crickets that inhabit caves, these data are even rarer, mainly regarding the assessment of the conservation status of the species and the environments where they live.

The most recent list of threatened animals for the state of São Paulo (2018) presents two species — *Strinatia brevipennis* and *Endecous (Endecous) betariensis* — of cavernicolous crickets with wide distributions in the Atlantic Forest of Parque Estadual Turístico do Alto Ribeira (PETAR), as being classified as "Vulnerable" (VU) for the state. A species is "Vulnerable" when the best available evidence indicates that it meets any of the predetermined criteria, be it population reduction, restricted geographic distribution, or even small declines or fluctuations in populations, and is therefore considered to be at a high risk of extinction in nature. We do have knowledge about the biology and distribution of the cave cricket *Strinatia brevipennis* in several caves in the region, which allowed us to start its evaluation. The species *Endecous (Endecous) betariensis* has a very similar distribution to that of *Strinatia brevipennis* and often exclusive populations within caves in the region. The species is well known and was studied in terms of distribution in surveys carried out in the 1980s and 1990s (PINTO-DA-ROCHA, 1995; TRAJANO, 1987; TRAJANO; GNASPINI-NETO, 1991). The evaluation and classification of these species as "Vulnerable" (VU) were based on their geographic distribution being restricted to some caves and the absence of records in the epigean environment, as well as the reduced size of their populations. Both suffer threats due to the intrinsic fragility of the subterranean environment in which they live and the increase in human activities, such as mining, raising livestock and tourism, which occur in the PETAR region.

More recently, in 2018, the new edition of the official list of animals at risk of extinction for the Brazilian territory continues reports produced in 2003, 2004, 2005 and 2008, and revises lists published by the Ministério do Meio Ambiente at the end of 2014, according to Ordinances nº 444 and 445 of the portfolio, and the 2008 Red Book. This list, which awaits official publication, contains two species of cavernicolous crickets considered troglobites.

- The species *Endecous* (*Pedroecous*) *apterus* is known from only three caves, in the municipality of Ituaçu, state of Bahia. Records of the species in the Mangabeira cave, in the same municipality, increased its distribution by 80km^2. Samplings in other caves in the municipality of Ituaçu and in the region of Chapada Diamantina did not locate the occurrence of the species. There is research for limestone exploration in the region of its occurrence and there is intense visitation in the Mangabeira cave, which has contributed to a significant decline in the quality of the habitat. Considering the locations, *E. (P.) apterus* was categorized as "Endangered" (EN) by criterion B1ab(iii).
- The other species evaluated and considered threatened is *Endecous* (*Endecous*) *peruassuensis*, known for only three caves — Olhos d'Água, Lapa do Cipó and Lapa D'Água João Ferreira — located in the municipality of Itacarambi, Minas Gerais, in Parque Nacional Cavernas do Peruaçu with "Extent of Occurrence" calculated at 30km^2. Sampling in other caves within this park did not locate the species. The resurgence of the Olhos d´Água cave is located outside the limits of the National Park, where a decrease in precipitation and an increase in the use of groundwater for agricultural purposes in the region has been observed. In this locality, there is also the construction of several dams upstream of the swallow hole and inside the cave itself, being used to store water for use during the dry season. These impacts change the water dynamics of this cave and the amount of food available for troglobite populations in general. Therefore, the species was categorized as "Endangered" (EN) by criterion B1ab(iii).

Measures for the conservation of species, even if still unknown on a regional scale, can be undertaken by the creation of protected areas (conservation units) of full protection or sustainable use, the latter allowing the use of natural resources and the conservation of nature, including its biodiversity. Actions like these, in addition to work that addresses systematics, inventories and proposals for environmental

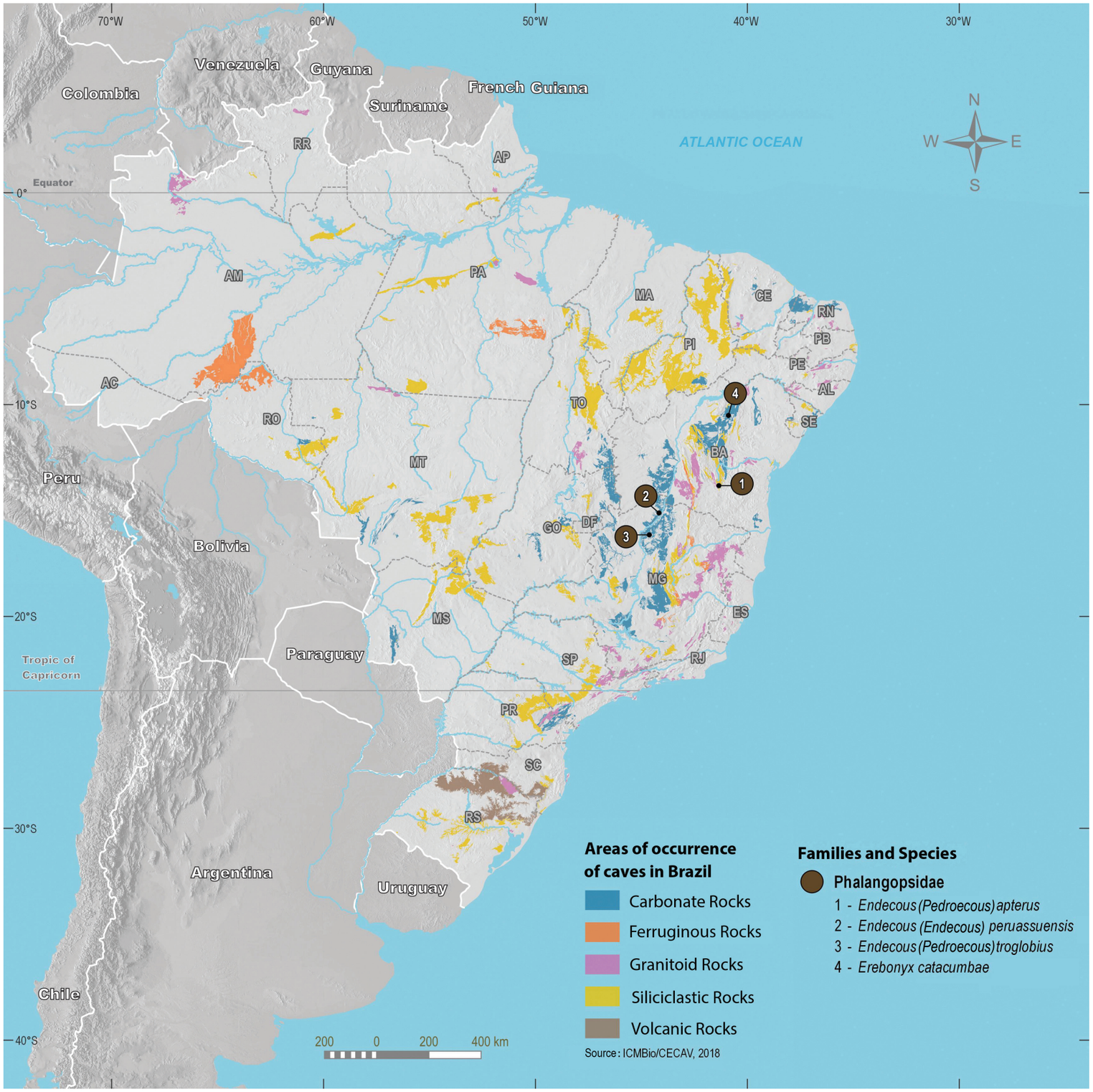


Figure 3. Map with type localities of troglobitic orthopteran species described for Brazil.

monitoring, should be considered a priority within conservation biology. In a practical way, funding is needed for more accurate and comprehensive studies, research focused on endangered fauna, as well as foreseeing the inclusion of species without formal descriptions (but which present unequivocal diagnoses and have voucher specimens deposited in scientific collections) in the official lists of endangered species. Success in conserving the remaining habitats and their biodiversity will increasingly depend on correct and urgent decisions, many of which conflict with economic and social interests. However, conserving biological diversity is an imminent need, in view of the benefits of ecosystem services for the economic, social and cultural development of humanity.

Main methods of collection and conservation

Studies of cavernicolous fauna, due to the intrinsic fragility presented by these ecosystems and their species, are extremely important and required by environmental licensing processes, with their results being decisive for the need for preservation or the possibility of suppression of each cave as a result of the installation of certain enterprises (highways, mining, hydroelectric plants, among others). Therefore, the choice of suitable sampling methods is crucial for obtaining good results. Below are the most commonly used methods for collecting crickets in subterranean environments:

DIRECT INTUITIVE SEARCH (DIS)

Direct intuitive searches specifically target a microhabitat and/or environmental zone to address a research question and/or increase the likelihood of maximizing the number of species detected. These microhabitats include flood debris, penetrating tree roots hanging from ceilings/walls, guano deposits, edges of dripping pools and ponds, muddy banks and animal and/or insect carcasses. These areas are prioritized as they are likely to support high diversity or may contain specific functional groups (WYNNE *et al.*, 2019). The main sampling method for terrestrial fauna in Brazil is manual collection by DIS, observing all possible habitats, without using any specific device (e.g., SOUZA-DIAS *et al.*, 2014; BOLFARINI; BICHUETTE; 2015). This methodology involves the search for the greatest possible diversity of environments found within natural subterranean cavities, prioritizing the points where organisms are frequently observed on the ground, wall and ceiling, where specimens can be viewed and captured manually, with the aid of tweezers, brushes and tubes. In this way, it is possible to measure the number of individuals collected and reduce the impacts that excessive removal (oversampling) of these individuals can generate on the ecosystem. This method is very efficient for collections involving taxonomic studies, however, little can be quantified or standardized in samples from irregular areas.

QUADRAT METHOD

The *quadrat* sampling method is a specialized type of visual inspection, whereby sample areas are delimited, optimizing the collection of subterranean fauna. Thus, this method allows the measurement of sampling effort since the exact sampled area is known. Thus, comparisons between subterranean systems with an ecological focus are plausible, as long as the assumptions of the collection methods are respected, namely: number of individuals counted or collected in each quadrant is recorded; the quadrat size is known; and the quadrat samples are representative of the study area as a whole (BICHUETTE *et al.*, 2015; KREBS, 1999). Sampling by the quadrat method has long been used in ecological studies, but mainly in plant ecology (GLEASON, 1920; WEAVER, 1918). This method consists of a square frame to delimit an area (quadrat) in which species are counted and/or collected (HENDERSON, 2009). The quadrats are distributed on each collection point (one or more collectors), the points are chosen and collection takes place for a certain amount of time. Thus, it is possible to relate the results obtained to the collection area, the sampling time and the number of individuals (N minutes in each base x N bases x N repetitions). Sampling sites can be qualitative, targeting known niches (e.g., guano, carcasses) or randomly drawn.

PITFALL TRAPS AND VULCAN TRAPS

Pitfall traps are also normally employed in caves and, although very efficient, they must be used with caution, as they can impact all terrestrial fauna due to oversampling of some taxonomic groups, such as collembolans, crickets and cockroaches (SHARRATT *et al.*, 2000). Traps are made from low-edged dishes or buried bottles/pots (on unconsolidated earth substrates) in order to create "artificial pools" for easy access by local fauna. They can be installed in places with and without

organic substrates (e.g., guano deposits), and with or without the use of bait. For consolidated substrates or where the cave floor is composed of a rock matrix, the solution for pitfall traps is "vulcan traps" (SPERBER *et al.*, 2021), which are volcano-shaped traps where the sides act as access to fauna, so they do not need to be buried. The traps must not exceed 48 hours of exposure, as they can accumulate dead animals and attract unwanted groups.

TRANSPORT AND PACKAGING

After manual or trap capture, specimens are placed in plastic tubes and data such as collection number, substrate and zoning, if any, are recorded. The tubes are previously filled with 70% alcohol for taxonomic collection or 100% for molecular studies, and labeled with information on location, date, cave name, lithology and collector. In the laboratory, specimens are kept in the same fixative and stored in closed cabinets.

Identification key

The key presented below includes all genera recorded in Brazilian caves so far, except Uvaroviella, which does not yet have formally described species for Brazil.

1 – Males with membranous tegmina, covering more than half the abdomen, or absent (*E. (P.) apterus*); when present, tegmina of males with regions modified for sound production and propagation; eardrums present (Figures 4A and 4D) **2**

- Males with membranous or coriaceous tegmina, covering only the metanotum or first abdominal tergite; regions involved in sound production and propagation reduced or absent, eardrums absent or present (Figures 4B and 4E)..... **4**

2 – Forewings triangular, abdominal tergites glandular, evidenced by long bristles aligned transversely, eardrums absent..... ***Erebonyx*** de Mello, 2021

- Forewings oval, abdominal tergites non-glandular, eardrums usually present..... **3**

3 - General body coloration dark brown to black, head light brown to reddish brown (Figure 4D); maxillary palps not distinctly elongated; tegmina usually covering only half of abdomen, harp with up to four veins; mirror divided by one or two slightly curved veins. Females with reduced wings..... ***Adelosgryllus*** Mesa & Zefa, 2004

- General body and head color generally light brown to reddish brown (Figure 4A), uniform; maxillary palps elongated; tegmina, when present, usually covering more than half of the abdomen, harp with five or more veins, mirror divided by one or two angulated veins. females apterus..... ***Endecous*** **Saussure, 1978**

4 - Apex of maxillary palp curved, not globose; tegmen reduced, membranous or coriaceous, apex not ventrally curved **5**

- Apex of maxillary palp globose; tegmen reduced, coriaceous, apex distinctly curved ventrally (Figure 4E)..... ***Bambuina*** de Mello, Horta & Bolfarini, 2013

5 - General color of body marbled in different shades of brown. Tegmina reduced, coriaceous, regions involved in acoustic communication absent. Male metanotum usually with structures and projections present; posterior femur expanded laterally, similar in size or shorter than body (Figure 4B)..... **6**

- Body color uniform overall. Membranous or coriaceous tegmina; if membranous, regions involved in acoustic communication reduced or absent. Male metanotum without structures or projections; posterior femur filiform or slightly laterally expanded, longer than the body (Figure 4F)..... ***Phalangopsis*** Serville, 1831

6 - General coloration marbled in shades of medium to dark brown (Figure 4B). Tegmina coriaceous, absent or present; if present, usually triangular or subtriangular (Figure 4C), apex usually glandular; supra-anal plate of males with latero-posterior projections in some species (Figure 4G) ..... ***Eidmanacris*** Chopard, 1956

- General coloration marbled yellowish-brown. Tegmina coriaceous, rounded, apex non-glandular (Figure 4H); supra-anal plate of males without latero-posterior projections..... ***Strinatia*** Chopard, 1970

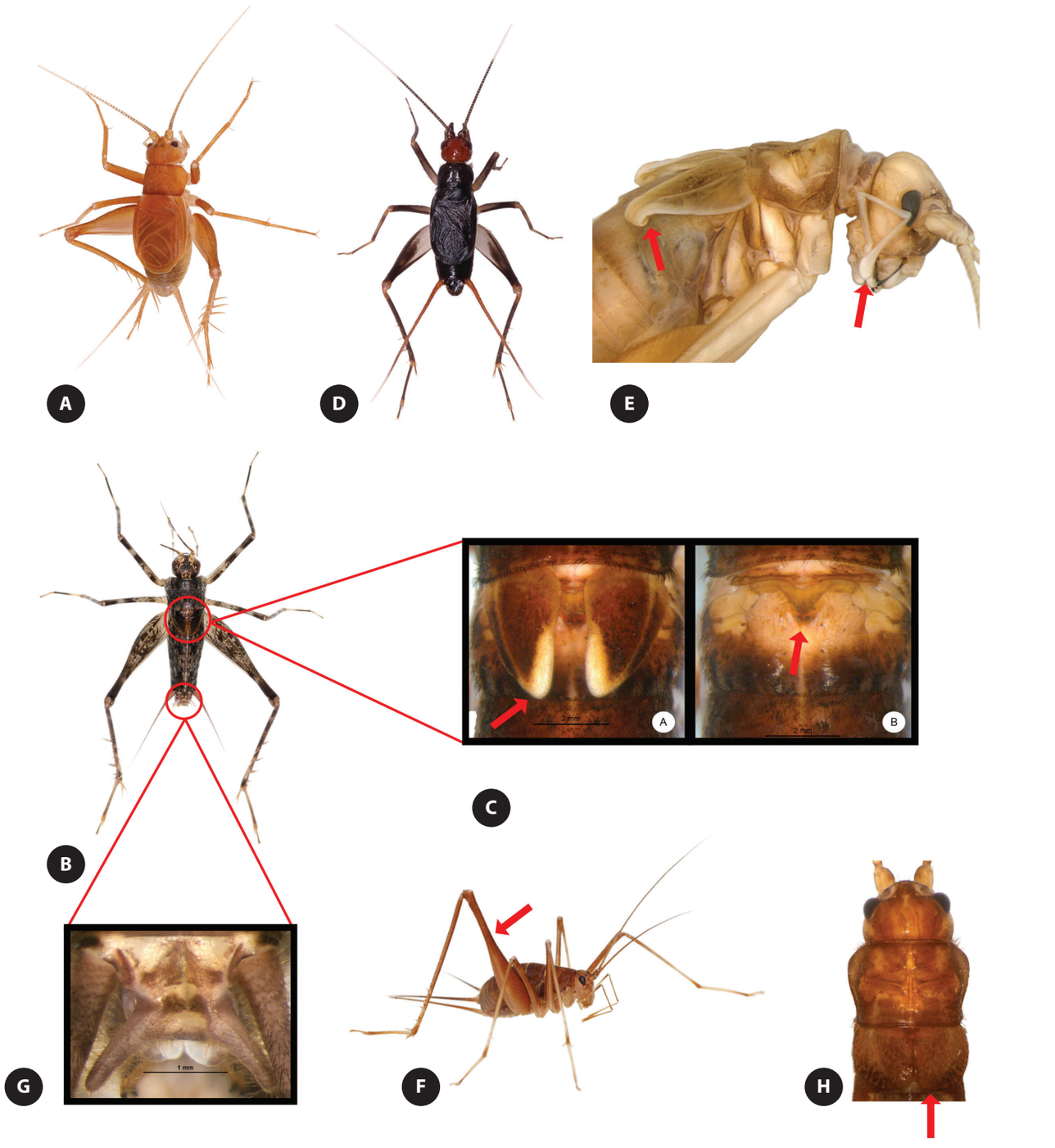


Figure 4. Characters mentioned in the identification key of the genera of Brazilian cavernicolous crickets. **A)** *Endecous* sp., male, dorsal habit; **B)** *Eidmanacris desutterae*, male, dorsal habit; **C)** *Eidmanacris*, male, metanotum in dorsal view, A-with as tegmina, B-without tegmina; **D)** *Adelosgryllus* sp., male, dorsal habit; **E)** *Bambuina bambui*, male in lateral view; **F)** *Phalangopsis* sp., female in lateral view; **G)** supra anal plate of *Eidmanacris neomarmorata*; **H)** *Strinatia brevipennis*, dorsal view of the head, pronotum and tegmina.

THE SPECIES OF THE GENERA MENTIONED ABOVE CAN BE FOUND IN THE FOLLOWING REFERENCES

Adelosgryllus

Despite eleven described species, this genus has never been revised and there is no identification key for its species.

Bambuina

There is no identification key as this is a monotypic genus; only *Bambuina bambui* is currently known. Genus and species descriptions can be found in De Melo *et al.* (2013):

DE MELLO, F. A. G.; HORTA, L.; BOLFARINI, M. P. *Bambuina bambui*: a new genus and species of cave cricket from Brazil (Orthoptera: Grylloidea: Phalangopsidae: Luzarinae). **Zootaxa**, v. 3599, n. 1, p. 87-93, 2013.

Endecous

There are two keys available for *Endecous*, but neither includes all species described for the genus. (CASTRO-SOUZA *et al.*, 2017; GOROCHOV, 2014).

Key for subgenera: GOROCHOV, A. V. Classification of the Phalangopsidae subfamily group, and new taxa from the subfamilies Phalangopsinae and Phaloriinae. **Zoosystematica Rossica**, v. 23, n. 1, p. 7-88, 2014.

Key for species of *Endecous* (*Notendecous*): CASTRO-SOUZA, R. A.; ZEFA, E.; FERREIRA, R. L. Two new species of cave crickets *Endecous* (*Notendecous*) Gororchov, 2014 (Orthoptera: Grylloidea: Phalangopsidae) from northeastern Brazil. **Zootaxa**, [*s.l.*], v. 4318, n. 3, p. 474-498, 2017.

Eidmanacris

This is the only taxonomically revised genus mentioned here. The identification key for the species does not include *Eidmanacris paramarmorata* because it is described through a female. Females have few taxonomically informative characters for identifying species within this genus (CAMPOS *et al.,* 2017).

Key for species: CAMPOS, L. D.; SOUZA-DIAS, P. G. B.; NIHEI, S. S. Taxonomic review of *Eidmanacris* Chopard, 1956 (Orthoptera: Grylloidea: Phalangopsid. **Zootaxa** [*s.l.*] ,v. 4321, n. 1, p. 1-93, 2017.

Erebonyx

There is no identification key as this is a monotypic genus; only *Erebonyx catacumbae* is currently known. Genus and species descriptions can be found in De Mello; Ferreira (2021):

DE MELLO, F. A. G.; FERREIRA, R. L. *Erebonyx* catacumbae, n. gen et sp.: a blind, troglobitic cricket from Brazil (Orthoptera, Grylloidea, Phalangopsidae). **Zootaxa**, [*s.l.*], v. 4975, n. 2, p. 343-356, 2021.

Phalangopsis

The genus *Phalangopsis* is composed, so far, of 11 species, being widely distributed throughout the Amazon region and found in caves and in the epigean environment (humid areas such as ravines, crevices and holes). In hypogean environments, their populations are relatively large when compared to other cavernicolous genera, with hundreds to thousands of individuals having been recorded in just one cave. Common notable features are their brachypterous condition and their long antennae. With more than four times the body size, the antennae of species of this genus are activated when there is air movement inside the caves. With circular movements of the antennae, these crickets use them to identify predators, for example (JUNTA *et al.*, 2020).

Key for species: JUNTA, V. G. P.; CASTRO-SOUZA, R. A. FERREIRA, R. L. Five new species of *Phalangopsis* Serville, 1831 (Orthoptera: Phalangopsidae) from Brazilian caves in the Amazon Forest. **Zootaxa**, [*s.l.*], v. 4859, n. 2, p. 151-194, 2020.

Strinatia

There is no identification key for the two currently described species, *Strinatia brevipennis* and *Strinatia teresopolis*.

Main Brazilian collections

The first zoological collections in Brazil began with the arrival of the Portuguese royal family and the creation of the Museu Real (today the Museu Nacional), in 1818. Still in the 19th century, other museums dedicated to zoological collections were created, such as the Museu Paulista (1895) whose zoological collection is now part of the Museu de Zoologia of the Universidade de São Paulo and the Museu Paraense (1871), today the Museu Paraense Emílio Goeldi.

Currently, the largest entomological collections in the country are under the custody of the following institutions: Universidade Federal do Paraná (Coleção Entomológica Padre Jesus Moure, DZUP), Museu de Zoologia da Universidade de São Paulo (MZUSP), Instituto Nacional de Pesquisas da Amazônia (INPA), Museu Paraense Emílio Goeldi (MPEG) and Museu Nacional do Rio de Janeiro (MNRJ). The entomological collection of MNRJ,

one of the largest collections in the country, was destroyed by the 2018 fire and is currently undergoing reconstruction. The MNRJ housed the largest Orthoptera collection in the country, with more than 28,000 mounted specimens and more than 10,000 incorporated into the collection awaiting processing ("backlog"), totaling around 40,000 lost specimens.

Scientific collections that have cave crickets listed in their systems are, mainly, the scientific collection of the Laboratório de Estudos Subterrâneos (LES) of the Universidade Federal de São Carlos (UFScar/São Carlos-SP), the collection of Invertebrados Subterrâneos de Lavras (ISLA) of the Universidade Federal de Lavras (UFLA/Lavras-MG), the collection of Orthoptera do Departamento de Zoologia of the Universidade Estadual Paulista (UNESP/Botucatu-SP), and the collection of Laboratório de Orthoptera of the Museu Nacional do Rio de Janeiro (MNRJ).

Although Brazil has its first zoological collections dating from the 17th century, the country still has few scientific collections in relation to the number of known, and to be described, species for its territory. The vastness of the animal world, with approximately more than one and a half million known species, restricts zoologists to one or a few fields of research interest, making it impossible to collect, preserve and study all animal groups. Therefore, each animal group or type of research requires particularities for the capture or collection of material and the preservation of and access to collections (PAPAVERO, 1994). Collections, of any kind, must follow a series of ethical norms and organizational procedures for accountability purposes (PAPAVERO,1994).

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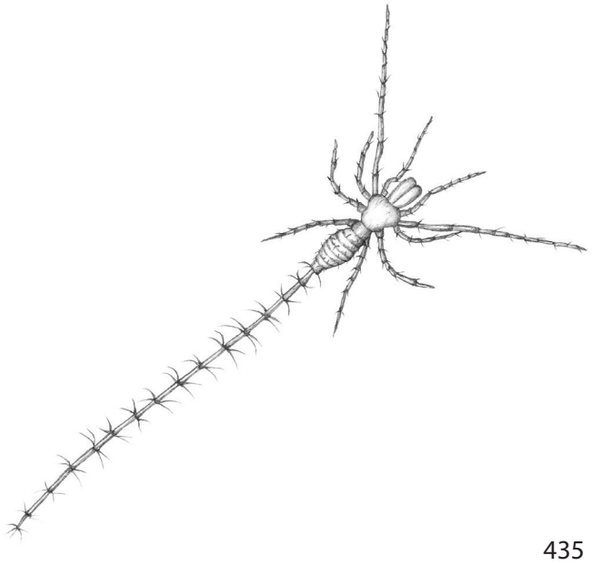


Eukoenenia sp.
Rodrigo Lopes Ferreira

21

Palpigradi

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General introduction

Palpigrades are depigmented and anophthalmic arachnids that have a characteristic flagellum at the end of the abdomen (Figures 1-2, 4). This structure, formed by several segments provided with sensory setae, tends to remain erect and move laterally while individuals walk on the substrate. Thus, it is believed that the flagellum is probably associated with the perception of the environment (CONDÉ, 1996; FERREIRA; SOUZA, 2012; FRANZ-GUESS; STARCK, 2020; MAYORAL; BARRANCO, 2002a). Palpigrades are also very small animals, with body sizes ranging from 0.65 to 2.8 mm (without the flagellum) (GIRIBET *et al.*, 2014; MAYORAL, 2015). Other striking morphological characteristics are their pincer-shaped chelicerae, leg-like pedipalp (helping with locomotion) and antenniform first pair of legs, which are longer than the others and have a sensory function (MAYORAL; BARRANCO, 2002a) (Figure 1).

Representatives of the order are distributed on all continents, except for Antarctica, and live in environments with stable temperature and high humidity, such as interstitial spaces in soil and caves. Edaphic species are distributed from the surface to more than one meter in depth and can be found close to the surface, sheltering under logs and rocks during the rainy seasons in tropical regions. There are also some species that were collected from the sand of beaches in Saudi Arabia, Congo, Madagascar and the Philippines. Most of the species distributed in temperate regions are restricted to subterranean environments, such as caves, and in the Superficial Subterranean Habitat (SSH), due to the severe environmental conditions observed in the external environment (CONDÉ, 1981, 1996, 1998; MAYORAL; BARRANCO, 2002a). More specifically, 85% of European species are troglobites (CHRISTIAN *et al.*, 2012). However, palpigrades are common not only in caves in temperate regions, being frequently recorded in tropical subterranean environments (MAMMOLA *et al.*, 2021; SOUZA; FERREIRA, 2020). Inside caves, they can be captured under stones, clay slabs, plant remains or floating on the surface of water accumulated in travertines. Troglobitic species can also be observed walking freely on the ground, on speleothems, on walls and on sediment banks (CHRISTIAN *et al.*, 2014; CONDÉ, 1996; SOUZA; FERREIRA, 2010; SOUZA *et al.*, 2020).

Palpigradi was the last order of Arachnida to be described, with the first species (*Eukoenenia mirabilis*) being published in 1885 by Grassi and Calandruccio. These arachnids are often reported in the literature as enigmatic since the number of known species is very small and many aspects of their biology remain unknown (e.g., BALLESTEROS *et al.*, 2019; GIRIBET *et al.*, 2014; HARVEY, 2002; SOUZA; FERREIRA, 2013). Therefore, it is considered one of the rarest and most neglected groups of terrestrial arthropods (GIRIBET *et al.*, 2014).

The lack of knowledge about these animals is mainly related to their small body size and cryptic habit, which often make the collection of specimens difficult. As they are arthropods that perform gas exchange through the integument, they are highly sensitive to microclimatic variation and very susceptible to desiccation. Therefore, it has not been possible to keep individuals in the laboratory for long periods to carry out detailed behavioral studies (MAYORAL; BARRANCO, 2002a). Another characteristic that hinders the study of the biology of palpigrades is the rarity of many species. Although relatively abundant populations of certain soil species are known (some of them sampled with the aid of soil microarthropod extractors), most species are represented only by the holotype or by a few specimens of the type series, not having been rediscovered after their original description (ADIS *et al.*, 1997; CHRISTIAN *et al.*, 2010, 2014; RUCKER, 1903). Troglobitic species especially tend to be extremely rare, as one or a few individuals are observed even when it is possible to make several visits to their habitat. In addition, they are also highly endemic, with distributions restricted to one or a few neighboring caves (CHRISTIAN *et al.*, 2014; MAYORAL, 2015; MAMMOLA *et al.*, 2021).

Only the edaphic species *E. florenciae* (Rucker, 1903) and *E. mirabilis* are considered exceptions within the order Palpigradi, since they managed to reach a very wide distribution, being recorded on several continents (MAMMOLA *et al.*, 2021). *Eukoenenia florenciae* is a parthenogenetic species that was

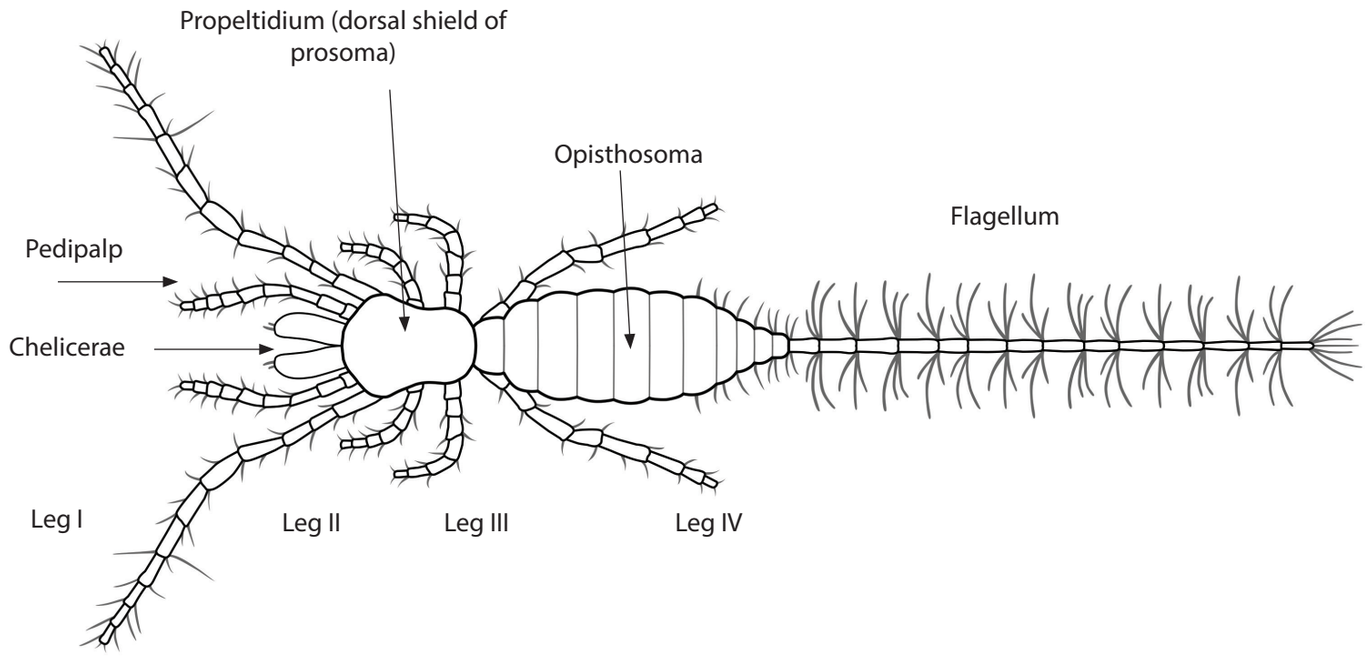


Figure 1. Schematic of a specimen of Palpigradi showing the morphology of the appendages and the general appearance of the body in dorsal view. Illustration: Marcos Brito.

first described for Texas, United States, and has subsequently been recorded in several locations around the world, including edaphic and hypogean environments in Brazil (CHRISTIAN; CHRISTOPHORYOVÁ, 2013; LEHMANN; FRIEDRICH, 2020; SOUZA; FERREIRA, 2020) (Figure 2A). In Mexico, there are two other species morphologically very similar to *E. florenciae* — *E. hanseni* (Silvestri, 1913) and *E. chilanga* Moreno, 2012 — but with bisexual populations, which indicates a possible origin in this region (CHRISTIAN; CHRISTOPHORYOVÁ, 2013; MAYORAL, 2015). *Eukoenenia mirabilis* was first described for Italy, having also been recorded in several other Mediterranean localities, some of them with bisexual populations. Also occurring in this region is the species *E. berlesei* (Silvestri, 1903), a species very similar to *E. mirabilis*. All other populations outside the Mediterranean region known so far are parthenogenetic, always represented only by females. This evidence indicates that *E. mirabilis* probably originated from some part of the Mediterranean and was later introduced to other locations, establishing populations in regions with a predominantly Mediterranean climate (HARVEY; ŠTÁHLAVSKÝ; THERON, 2006; MAYORAL, 2015). Although it has already been found in South America, more precisely in Chile, the species has not yet been recorded in Brazil. These two species were probably accidentally introduced in various regions of the world along with the soil of exotic plants, often being found in environments greatly altered by human action, including parks, cemeteries, botanical gardens and greenhouses (HARVEY; ŠTÁHLAVSKÝ; THERON, 2006; LEHMANN; FRIEDRICH, 2020).

As a consequence of the rarity and difficulty of collecting many specimens of Palpigradi, most of the articles published about these arachnids to date contain morphological and anatomical descriptions, with very little information about their biology. The few publications on the behavior, ecology and natural history of palpigrades are based on the maintenance of individuals for short periods of time in the laboratory (KOVAČ *et al.*, 2002), on casual

observations of cave species (BALESTRA *et al.*, 2019; FERREIRA *et al.*, 2019; FERREIRA *et al.* al., 2011; FERREIRA; SOUZA, 2012), on the study of the phenology and life history of four edaphic species (ADIS *et al.*, 1997; CHRISTIAN *et al.*, 2010; MORENO; FRANCKE, 2013) and on ecological analyses carried out with a database of species distribution around the world (MAMMOLA *et al.*, 2021). Kovač *et al.* (2002) managed to keep individuals of the troglobitic species *E. spelaea* (Peyerimhoff, 1902) in the laboratory for four weeks, but were unsuccessful in observing any aspect of their feeding or reproduction; only behaviors related to locomotion and cleaning of the appendages were described. Observations in the field resulted in the description of the locomotion and appendage cleaning repertoire of an individual of *E. maquinensis* Souza & Ferreira, 2010, observed in Gruta de Maquiné (Cordisburgo, Minas Gerais, Brazil) and in the record of an individual of *E. potiguar* Ferreira *et al.*, 2011 being preyed upon by a spider of the species *Metagonia potiguar* Ferreira *et al.*, 2011 in a Brazilian cave, including the entire prey recognition and capture process (FERREIRA *et al.*, 2011; FERREIRA; SOUZA, 2012. By compiling data on the global distribution of palpigrades, Mammola *et al.* (2021) showed that most species have a very restricted distribution (around 0.01 km^2). This distribution is explained primarily by climatic conditions and secondarily by the availability of habitat and nutrients. Despite the large gaps in current knowledge about the biogeography of the group pointed out by the authors, the results suggest that Europe and Brazil are important centers of diversification of troglobitic palpigrades and that edaphic species are distributed on a wider geographic scale, mainly in the Southern Hemisphere.

The feeding habits of these arachnids are also not very well known. Due to the lack of experimental data, it has historically been proposed that they are predators and that there is an external pre-digestion, as seen in spiders. This hypothesis is based on the morphology of the chelicerae and on the fact that the study of the contents of the digestive tract of these animals has not allowed the recognition of any element (CONDÉ, 1998; MAYORAL, 2015; MILLOT, 1942). So far, only two observations have been recorded that confirm that palpigrades are predators. One of them was a personal communication made by P. Weygoldt to Bruno Condé, in which he states that palpigrades kept in the laboratory captured small collembolans with the chelicerae (CONDÉ, 1998). Furthermore, a video about Collembola in caves produced by Marko Lukić, member of the Croatian Society of Biospeleology, shows a palpigrade capturing a small collembolan with its chelicerae and feeding on it in a cave in Croatia. However, this information is not formally disclosed in scientific papers, which makes it difficult to identify the cave and the palpigrade species, if it indeed has already been described. Only more recently, some more solid information has been published. Smrž *et al.* (2013) analyzed the contents of the digestive tract of specimens of *E. spelaea* collected in a cave in Slovakia and were able to identify cyanobacteria, as well as metabolic evidence of their digestion, suggesting that these microorganisms have served as a nutritional source for palpigrades in that cave. Balestra, Lana and Casale (2019) reported an individual of *E. strinatii* Condé, 1977 approaching a dead collembolan, piercing its abdomen with the aid of the chelicerae and sucking its internal contents while over the surface of a pool of water in a cave in the Italian Alps. Thus, as suggested by Mayoral (2015), the diet of these arachnids is probably variable and depends on the availability of resources present in the habitat in which they are found.

The only thing known regarding reproduction is that males produce a spermatophore, but there is no evidence related to its mode of transfer to females (CONDÉ, 1998; SMRŽ *et al.*, 2013). The eggs are not known and, so far, only the ovules of three species have been observed: two or three mature ovules measuring 100–120 µm of the edaphic species *E. mirabilis* and one or two mature ovules measuring 130–250 µm of the troglobitic species *E. austriaca* (Hansen, 1926) (CONDÉ, 1996). Franz-Guess and Starck (2020) reported the presence of six and twelve ovules (diameter ranging fom 15–60 µm), probably at different stages of development, in the ovaries of two females of the troglobitic species *E. spelaea*. As mentioned earlier, some species can reproduce by parthenogenesis since males are apparently absent in some populations and, even in many bisexual populations, the proportion of females is often much higher than that of males (CONDÉ, 1996).

The order Palpigradi is considered one of the micro-diverse orders of Arachnida, including, to date, 120 species (118 living and two fossil) distributed in two families: Eukoeneniidae Petrunkevitch, 1955 and Prokoeneniidae Condé, 1996. Eukoeneniidae includes the living genera *Allokoenenia* Silvestri, 1913 (three species), *Eukoenenia* Börner, 1901 (93 species), *Koeneniodes* Silvestri, 1913 (nine species) and *Leptokoenenia* Condé, 1965 (five species). The family Prokoeneniidae, in turn, includes only the genera *Prokoenenia* Börner, 1901 (seven species) and *Triadokoenenia* Condé, 1991 (monotypic) (BU *et al.*, 2019, 2021ab; HARVEY, 2003; SOUZA; FERREIRA, 2019, 2020, 2022; SOUZA *et al.*, 2020). The order has also received

little attention from a phylogenetic point of view due to the aforementioned sampling obstacles. The only work on the phylogeny of the group was published by Giribet *et al.* (2014). The phylogenetic hypothesis proposed by these authors was based on molecular data and included a single species of *Prokoenenia* and a few species of *Eukoenenia* due to the difficulty in obtaining sufficiently well-preserved specimens for the extraction of suitable genetic material for the analyses. The results, although inconclusive due to the small number of samples, indicate that Palpigradi and Eukoeneniidae are monophyletic and propose the division of Eukoeneniidae into four clades, among which three included species from more than one continent.

Main groups found in Brazilian caves

Only the family Eukoeneniidae has been found in Brazil so far, being formally represented by species of the genera *Eukoenenia*, *Leptokoenenia* and *Allokoenenia*, the latter two being little known. *Leptokoenenia* includes only five species: two found in ferruginous caves in the state of Pará (Brazil) (Figure 2B), two that inhabit coastal ecosystems in Congo and Saudi Arabia, and another found in the soil of a *Quercus suber* plantation (the tree from which cork is extracted) on the coast of Italy (CHRISTIAN, 2009; HARVEY, 2003; SOUZA; FERREIRA, 2013).

Allokoenenia is formally represented by only three species: *Allokoenenia afra* (Silvestri, 1913), an edaphic species from Guinea, on the African continent; and the Brazilian troglobitic species *A. canhembora* Souza & Ferreira, 2022, found in Toca do Gonçalo (Campo Formoso, state of Bahia); and *A. stygia* Souza & Ferreira, 2022, described for two ferruginous caves in Serra da Bocaina (Canaã dos Carajás, state of Pará) (SILVESTRI, 1913; SOUZA; FERREIRA, 2022) (Figure 2C).

The genus *Eukoenenia* is the most widely distributed and richest not only in Brazil, but in the world, harboring approximately 80% of the known species for the order, including edaphic and troglobitic species (SOUZA; FERREIRA, 2013) (Figures 2A, 5). Among the 23 species of Palpigradi native to Brazil known thus far, 19 belong to this genus. Therefore, of the genera of the family Eukoeneniidae, only *Koeneniodes* has not yet been found in Brazil, being distributed in Asia and Africa.

Main troglomorphisms

Approximately half of the described species of Palpigradi in the world have been found only in caves. Apart from the genus *Triadokoenenia*, known by a single species collected in the rainforest of northwest Madagascar, all other genera of Palpigradi have been recorded in caves (CONDÉ, 1998). Records of *Allokoenenia* in these ecosystems were a little doubtful, as Condé (1992) attributed two immature specimens found in caves in Thailand as possibly belonging to this genus but did not formally describe these species. Records of species of *Allokoenenia* in Brazilian caves (SOUZA; FERREIRA, 2022) confirm the presence of representatives of this genus in hypogean habitats. Nonetheless, as most known palpigrades belong to the genus *Eukoenenia*, including edaphic and cave-



Figure 2. Live specimens belonging to the three genera of Palpigradi registered in Brazil thus far. **A)** Individual of the species *Eukoenenia florenciae* found in Gruta da Lapinha, Lagoa Santa, Minas Gerais; **B)** individual of the species *Leptokoenenia pelada* found in Caverna SL 42, Curionópolis, Pará; **C)** individual belonging to a new species of the genus *Allokoenenia* found in a ferruginous cave located in the Floresta Nacional de Carajás, Parauapebas, Pará. Photos: A (Rodrigo L. Ferreira); B and C (Vale Collection).

dwelling species, it is possible to detect that some lineages acquired a series of characteristics associated with evolution in the subterranean environment. Therefore, troglomorphisms for Palpigradi are based on the morphological modifications presented by representatives of this genus (CONDÉ, 1998).

As all species of Palpigradi are naturally anophthalmic and depigmented, the main troglomorphisms in this group are increased body size, elongation of appendages and enhancement of sensory structures (CONDÉ, 1996, 1998; MAYORAL; BARRANCO, 2002a) (Figure 3).

In general, troglobitic palpigrades tend to be larger than those that are edaphic, with body lengths often ranging from 1.2 to 2.3 mm. However, this characteristic is not very reliable, since specimens may suffer some deformation after collection and preservation in alcohol (CONDÉ, 1998). In addition, there are some troglobitic species that, despite having other troglomorphisms, have a reduced body size. Examples include the only known specimen (an adult male) of *E. spelunca* Souza & Ferreira, 2011, with a body length of 720 µm, and the only registered adult male *of E. lundi* Souza & Ferreira, 2020, which measures 815 µm (SOUZA; FERREIRA, 2011b, 2020).

The optimization of sensory structures is manifested mainly by an increase in the number of blades that form the lateral organs, which are located in the anterolateral region of the prosoma (CONDÉ, 1996, 1998). Franz-Guess and Starck (2020) studied the ultrastructure of these organs in *E. spelaea* and concluded that these blades correspond to modified sensory setae that probably function as chemoreceptors and/or hygroreceptors. Soil species generally have lateral organs formed by 1 to 3 blades (a few species found exclusively in soil have 4 to 6), while troglobitic species have 3 to 18 blades (BU *et al.*, 2021a; CONDÉ, 1996, 1998) (Figure 3). The exceptions are *E. madeirae* Strinati & Condé, 1995, *E. gadorensis* Mayoral & Barranco, 2002, *E. amatei* Mayoral & Barranco, 2017 and *E. indalica* Mayoral & Barranco, 2017, which are considered adapted to the cave environment even with the lateral organ

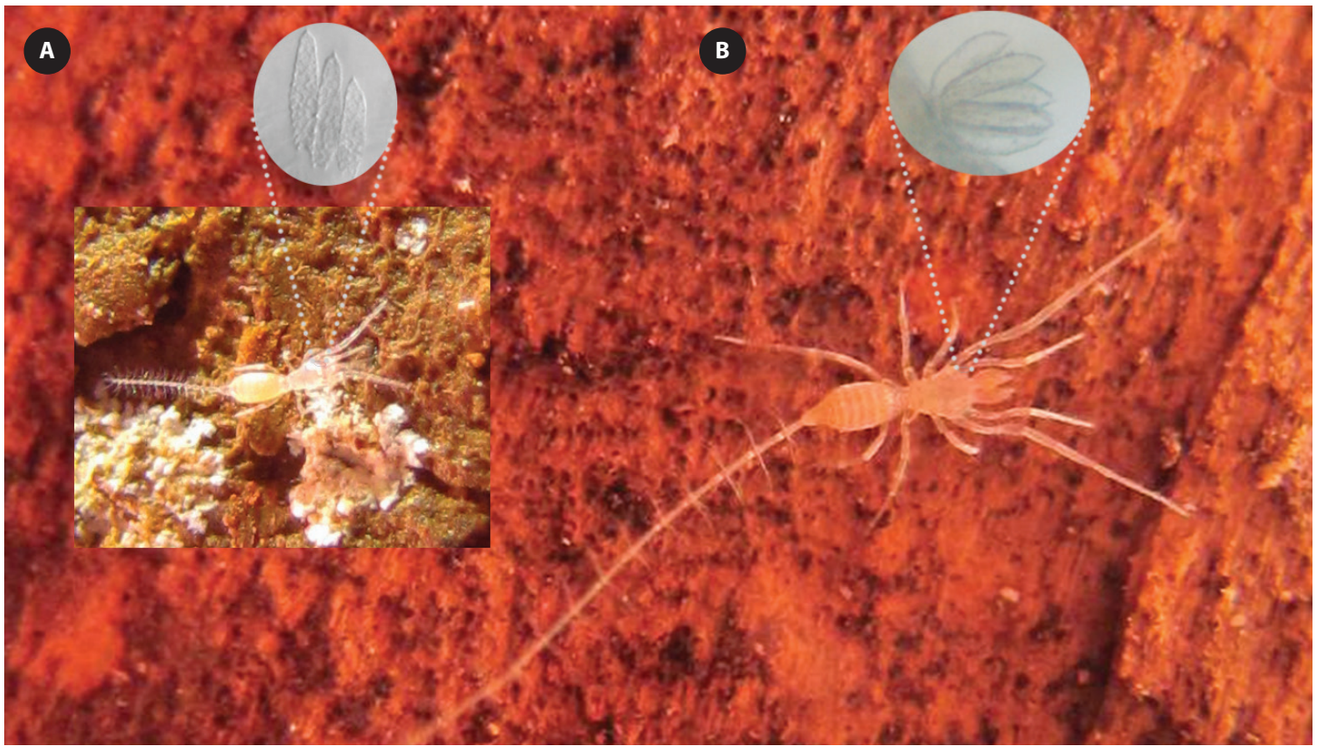


Figure 3. Live specimens of the edaphic species *Eukoenenia florenciae* **A)** and the troglobitic species *Eukoenenia maquinensis* **B)** at approximately the same scale, showing increased body size and elongation of appendages in the troglobitic species. The ellipses present in the anterolateral region of the prosoma of the specimens show the approximate location of lateral organs. The larger ellipses show in detail the blades that make up the lateral organs, which become more numerous in troglobitic species. Photos: Rodrigo L. Ferreira; the image of the lateral organs of *E. florenciae* was obtained from Christian and Christophoryová (2013).

formed by a single blade (MAYORAL; BARRANCO, 2002b, 2017a-b; STRINATI; CONDÉ, 1995).

Leg elongation is usually quantified by the ratio between the lengths of the basitarsus and tibia of leg IV (IVbta/ti) and between the lengths of the propeltidium (dorsal shield of the prosoma) and basitarsus of leg IV (B/IVbta). Specifically, the value of the IVbta/ti ratio is often between 0.9 and 1 in troglomorphic species and is less than 0.9 in edaphic species, while for the B/IVbta ratio the values are closer to 2 and 3, respectively (CONDÉ, 1996, 1998). It is important to emphasize here that the ratios for some species are intermediate between those established for edaphic and troglobitic palpigrades, which often makes it difficult to diagnose the ecological-evolutionary status of many species of this group

The flagellum of troglobitic species also tends to be more elongated compared to that of edaphic species (Figure 3). However, as this structure is very fragile, and is often lost during collection, the descriptions of many species do not include the flagellum, which makes more detailed comparisons difficult. The flagellum of troglobitic species is normally longer than the body, while that of soil species reaches at most the body length (CHRISTIAN *et al.*, 2010, 2012, 2014; CONDÉ, 1996; FERREIRA *et al.*, 2011; SOUZA; FERREIRA, 2010, 2012a, 2020). However, there are some exceptions, such as the troglobitic species *E. ibitipoca* Souza & Ferreira, 2019, whose flagellum is approximately the same length as the body, and *E. navi* Souza & Ferreira, 2018, whose flagellum is shorter than the body (SOUZA; FERREIRA, 2018, 2019a).

Eukoenenia naxos Condé, 1989, a troglobitic species from Greece, also has the tergal seta of the basitarsus of leg IV replaced by a trichobothrium. The presence of this sensorial seta on this article is considered a troglomorphism because all other species of *Eukoenenia* always present a total of seven trichobothria distributed on the articles of leg I (CONDÉ, 1989; MAYORAL; BARRANCO, 2002a).

As is the case for other groups of invertebrates, the most reliable way to determine whether a species of Palpigradi is troglobitic is through detailed knowledge of its distribution, which would allow detection of whether its populations are restricted to the cave environment. However, studies of soil fauna in Brazil are quite scarce, which often prevents knowing whether the species are also associated with soil habitats outside the caves. Given this scenario of scarce data on the distribution of many species, the presence or absence of troglomorphisms becomes an important indicator for determining their ecological-evolutionary status.

Diversity of troglobitic species described for Brazil

In Brazil, specifically, the order Palpigradi was represented by only two soil species until the year 2010: *Eukoenenia roquettei* (Mello–Leitão & Arlé, 1935) from Rio de Janeiro and *E. janetscheki* (CONDÉ, 1993), from the Amazon (CONDÉ, 1993; MELLO–LEITÃO; ARLÉ, 1935). In the last 12 years, 21 species have been described from specimens collected inside caves, showing that the diversity of the group is much greater than what was previously known. Thus, in addition to the previously mentioned species, Brazilian palpigrades are currently represented by another 17 species of the genus *Eukoenenia* distributed in the states of Minas Gerais, Espírito Santo, Goiás and Rio Grande do Norte; two belonging to the genus *Leptokoenenia* that occur in the state of Pará; and two of the genus *Allokoenenia* that are distributed in the states of Pará and Bahia (FERREIRA *et al.*, 2011; SOUZA; FERREIRA, 2010, 2011a, b, 2012a-b, 2013, 2016, 2018, 2019a-b, 2020, 2022 ; SOUZA *et al.*, 2020). Among these species described from cave specimens, only *E. ferratilis* Souza & Ferreira, 2011, *E. potiguar, L. pelada* Souza & Ferreira, 2013 and *L. thalassophobica* Souza & Ferreira, 2013 are considered edaphic because they do not show any troglomorphisms (FERREIRA *et al.*, 2011; SOUZA; FERREIRA, 2011a, 2013). The species *E. igrejinha* Souza & Ferreira, 2019 and *E. jequitai* Souza & Ferreira, 2020 present intermediate morphology between soil species and troglobitic species, requiring more detailed studies of the edaphic fauna of the region where they occur to determine whether they are restricted to caves or not (SOUZA; FERREIRA, 2019b, 2020). However, while there is no additional information on the distribution of these species, they will be considered as troglobites for conservation purposes. The other species present evident troglomorphisms and have been recorded only inside caves, and so are considered troglobites. Most of them are endemic to a single cave and were described based on one or a few individuals, and are therefore considered rare (SOUZA; FERREIRA, 2010, 2011b, 2012a, b, 2016, 2019a, 2020, 2022). Thus, Brazilian troglobitic palpigrades are currently represented by 17 species (Figure 5), with their distribution shown in Figure 6.

Table 1. List of troglobitic species described for Brazil.

Family: **Eukoeneniidae** (17 species)

Eukoenenia Börner, 1901

1. *Eukoenenia maquinensis* Souza & Ferreira, 2010 (Gruta de Maquiné, Cordisburgo, Minas Gerais) (Figure 5A)
2. *Eukoenenia spelunca* Souza & Ferreira, 2011 (Gruta Archimedes Passini, Vargem Alta, Espírito Santo).
3. *Eukoenenia sagarana* Souza & Ferreira, 2012 (Gruta da Morena, Cordisburgo, Minas Gerais) (Figure 5B).
4. *Eukoenenia virgemdalapa* Souza & Ferreira, 2012 (Lapa Nova, Vazante, Minas Gerais).
5. *Eukoenenia jequitinhonha* Souza & Ferreira, 2016 (Lapa do Córrego do Vieira, Caraí, Minas Gerais).
6. *Eukoenenia cavatica* Souza & Ferreira, 2016 (Gruta Cazanga, Arcos, Minas Gerais) (Figure 5F).
7. *Eukoenenia navi* Souza & Ferreira, 2018 (iron caves in the municipality of Barão de Cocais, Minas Gerais) (Figure 5D).
8. *Eukoenenia eywa* Souza & Ferreira, 2018 (iron caves in the municipalities of Caeté, Santa Bárbara, Rio Acima, Barão de Cocais and Nova Lima, Minas Gerais) (Figure 5E).
9. *Eukoenenia neytiri* Souza & Ferreira, 2018 (iron caves in the municipality of Mariana, Minas Gerais).
10. *Eukoenenia ibitipoca* Souza & Ferreira, 2019 (Gruta das Casas, Lima Duarte, Minas Gerais) (Figure 5C).
11. *Eukoenenia igrejinha* Souza & Ferreira, 2019 (Gruta da Igrejinha, Ouro Preto, Minas Gerais).
12. *Eukoenenia magna* Souza & Ferreira, 2020 (Lapa Grande and Lapa da Santa, Montes Claros, Minas Gerais) (Figure 5G).
13. *Eukoenenia lundi* Souza & Ferreira, 2020 (Lapa Sem Fim, Luislândia, Minas Gerais) (Figure 5H).
14. *Eukoenenia jequitai* Souza & Ferreira, 2020 (Lapa do Dim, Jequitaí, Minas Gerais)
15. *Eukoenenia audax* Souza *et al.*, 2020 (Gruta Cabeceira d'água, Nova Roma, Goiás) (Figure 5I).
16. *Allokoenenia canhembora* Souza & Ferreira, 2022 (Toca do Gonçalo, Campo Formoso, Bahia).
17. *Allokoenenia stygia* Souza & Ferreira, 2022 (Caves SB_0114 e SB_0112, Canaã dos Carajás, Pará).

Main methods of collection and conservation

As previously described, the order Palpigradi groups very small and fragile arachnids, which, consequently, require delicate handling and are a challenge to study. Their visual capture, with the aid of a fine brush, requires patience and skill since it is necessary to search in detail for potential habitats. Once exposed to the open air, in the case of individuals found under stones or clay slabs, they immediately begin to move quickly and erratically, and can disappear in any crack or porosity in the rock (CONDÉ, 1996, 1998). Even with these difficulties, the manual collection of these animals is the most frequent, mainly inside caves, and results in more complete and well-preserved specimens, including the flagellum. To do so, a very fine brush, slightly moistened with alcohol, should be used. It is recommended that, before specimen collection, excess alcohol be removed from the tip of the brush by passing it lightly over the edge of the collection tube, thus leaving it without any apparent drop. When capturing a specimen, the tip of the brush must be touched on its front or side to prevent the flagellum from contacting the bristles of the brush and being lost (Figure 4). It is also advisable to pack a single specimen per tube containing alcohol, as the flagellum often detaches from the body after collection. There are also reports in the literature of specimens captured with the aid of plankton nets while floating in pools of water inside caves (CONDÉ, 1998).

Edaphic species can also be captured by hand collection and individuals are normally found under stones partially buried in the soil, especially during the rainy season, in regions with marked seasonality. Other methods include direct screening of soil or litter samples in search of individuals or the separation of specimens from these substrates by means of flotation, as well as collection with the aid of extractors such as Berlese and

Kempson (ADIS *et al.*, 1997; BROZA *et al.*, 1993; BU *et al.*, 2019; CHRISTIAN *et al.*, 2010; CONDÉ, 1984, 1996, 1998; RUCKER, 1903).



Figure 4. Specimen of Palpigradi collected manually with the aid of a pin in a cave in Bosnia Herzegovina. Photo: Maja Zagmajster.

Individuals are normally stored in small vials containing alcohol (of variable concentration) or on microscopy slides (CONDÉ, 1988). It is preferable that specimens for morphological studies be conditioned in 70% alcohol. Specimens intended for molecular analysis must be preserved in absolute alcohol and stored in a freezer after collection. In the case of large populations, it is advisable to collect larger specimens in 70% alcohol, since they are more likely to be adults (which are essential for taxonomic work), and the smallest, which are more likely to be immatures, in absolute alcohol for future molecular studies. Mounting specimens on slides is usually required for more detailed morphological studies, such as the description of new species. Specimens are often clarified before mounting using solutions such as Nesbitt, Marc André I fluid, 50% lactic acid and Lactophenol. The most commonly used mounting medias are Hoyer (also known as Marc André II medium) or PVA (polyvinyl alcohol). Once slides are made, they must remain in an oven at 50° for approximately 20 days, for the mounting medium to dry, after which they are sealed (CONDÉ, 1988; MAYORAL; BARRANCO, 2017; MORENO; FRANCKE, 2013; SOUZA; FERREIRA, 2019a).

Identification key

The two families of Palpigradi can be distinguished by the presence of ventral sacs positioned just below the line of insertion of bristles in opisthosomal sternites IV-VI in representatives of the family Prokoeneniidae, structures which are absent in Eukoeneniidae (HARVEY, 2003).

The family Prokoeneniidae is represented by only two genera: *Prokoenenia* and *Triadokoenenia*. The latter is monotypic, being represented only by the species *T. millotorum* (Condé, 1950) from Madagascar, males of which have three pairs of fusules on the genitalia (CONDÉ, 1991); males of species of the genus *Prokoenenia* (and of the other species of Palpigradi) have only two pairs.

So far, there is only one key for identifying the families and genera of Palpigradi, proposed by Rowland and Sissom (1980). However, this key is quite outdated, as it includes an alleged fossil representative of Palpigradi (genus *Sternarthron*), which later studies revealed to be the nymphal stage of an insect (ENGEL *et al.*, 2016). Furthermore, it does not include the family Prokoeneniidae, which had not yet been proposed at the time. As the internal phylogenetic relationships of the order have not been the subject of more complete and detailed investigations, it is highly likely that some taxa represent paraphyletic groupings (BARRANCO; HARVEY, 2008). Thus, any attempt to update information of the key or previously proposed diagnoses would be unproductive at the moment, as the groups need to be revised. Therefore, we will only provide the main characteristics used to diagnose the representatives of each genus of Eukoeneniidae, adapting the information provided by Rowland and Sissom (1980).

EUKOENENIA

Ninth opisthosomal segment slightly narrower than eighth and about twice as wide as eleventh; pygidium narrowed posteriorly; flagellum longer than opisthosoma; each of the opisthosomal sternites IV and VI forms a protuberance (Figure 2A).

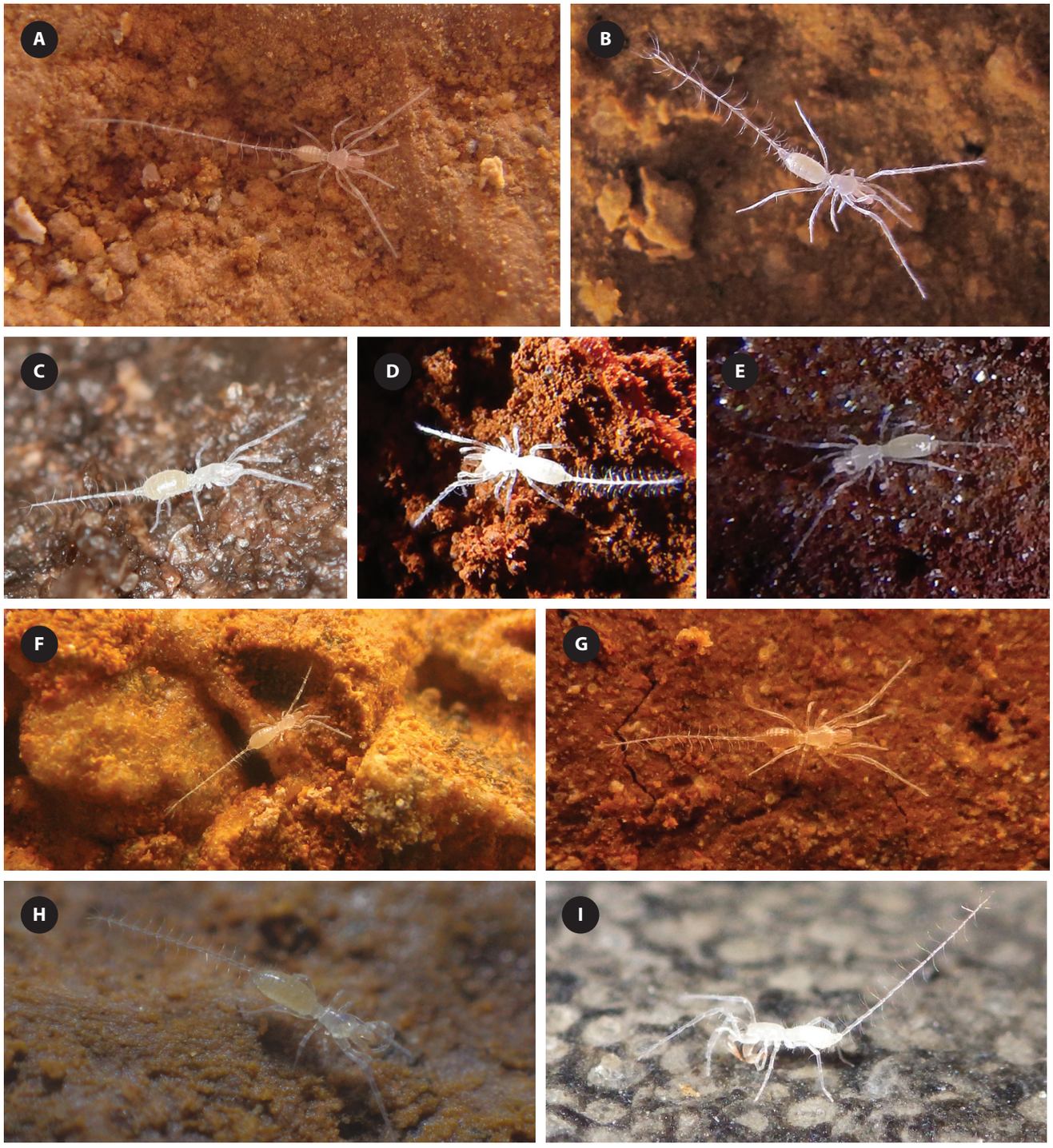


Figure 5. Live specimens of some troglobitic species of Palpigradi described for Brazil thus far. **A)** *Eukoenenia maquinensis*; **B)** *Eukoenenia sagarana*; **C)** *Eukoenenia ibitipoca*; **D)** *Eukoenenia navi*; **E)** *Eukoenenia eywa*; **F)** *Eukoenenia cavatica*; **G)** *Eukoenenia magna*; **H)** *Eukoenenia lundi*; **I)** *Eukoenenia audax*. Photos A, B, C, F, G and I (Rodrigo L. Ferreira); D and E (Matheus H. Simões); H (Lucas M. Rabelo).



Figure 6. Map of the type localities of troglobitic species of Palpigradi described for Brazil.

KOENENIODES

Ninth opisthosomal segment slightly narrower than eighth and about twice as wide as eleventh; pygidium narrowed posteriorly; flagellum longer than opisthosoma; opisthosomal sternites IV and VI form a single protuberance.

LEPTOKOENENIA

Ninth opisthosomal segment approximately the same width as eighth and slightly wider than eleventh; pygidium slightly narrowed posteriorly; flagellum shorter than opisthosoma (Figure 2B).

ALLOKOENENIA

Ninth and eleventh opisthosomal segments similar in width, approximately half the width of the eighth and wider than the tenth; pygidium not greatly narrowed posteriorly; flagellum shorter than opisthosoma and with moniliform segments (Figure 2C).

Main Brazilian collections

Most of the type material for Brazilian species, more precisely a total of 72 specimens to date, is deposited in the Coleção de Invertebrados Subterrâneos de Lavras (ISLA), associated with the Centro de Estudos em Biologia Subterrânea (Departamento de Ecologia e Conservação) of the Universidade Federal de Lavras (UFLA). This collection also holds several lots identified as *E. cf. florenciae*, a species probably introduced in the country, as mentioned above, as well as additional specimens of some Brazilian species and several specimens belonging to species not yet described. This material comes from collections carried out inside caves during the execution of subterranean biology research projects and of studies for environmental licensing in several Brazilian regions. This collection also houses specimens of Palpigradi from other countries, such as Guatemala, South Africa, Morocco, Romania, Slovakia and Italy. Due to the large volume of specimens belonging to various taxa, this institution houses one of the most important Palpigradi collections not only in Brazil, but in the world.

The other type-specimens of Brazilian species are deposited at the Instituto Butantan (IBSP; specimens of *E. ferratilis*, *E. maquinensis* and *E. audax*), the Museu de Zoologia da Universidade de São Paulo (MZUSP; specimens of *E. eywa* and *A. stygia*), the Instituto Nacional de Pesquisas da Amazônia (INPA) and the Museu de História Natural de Genebra (MHNG); these last two institutions house the type material of the species *E. janetscheki*. Type specimens of *E. roquettei*, the first species of Palpigradi described for Brazil, were deposited at the Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ). However, the specimens were lost even before the fire that destroyed part of the historical and scientific collection of this institution in 2018.

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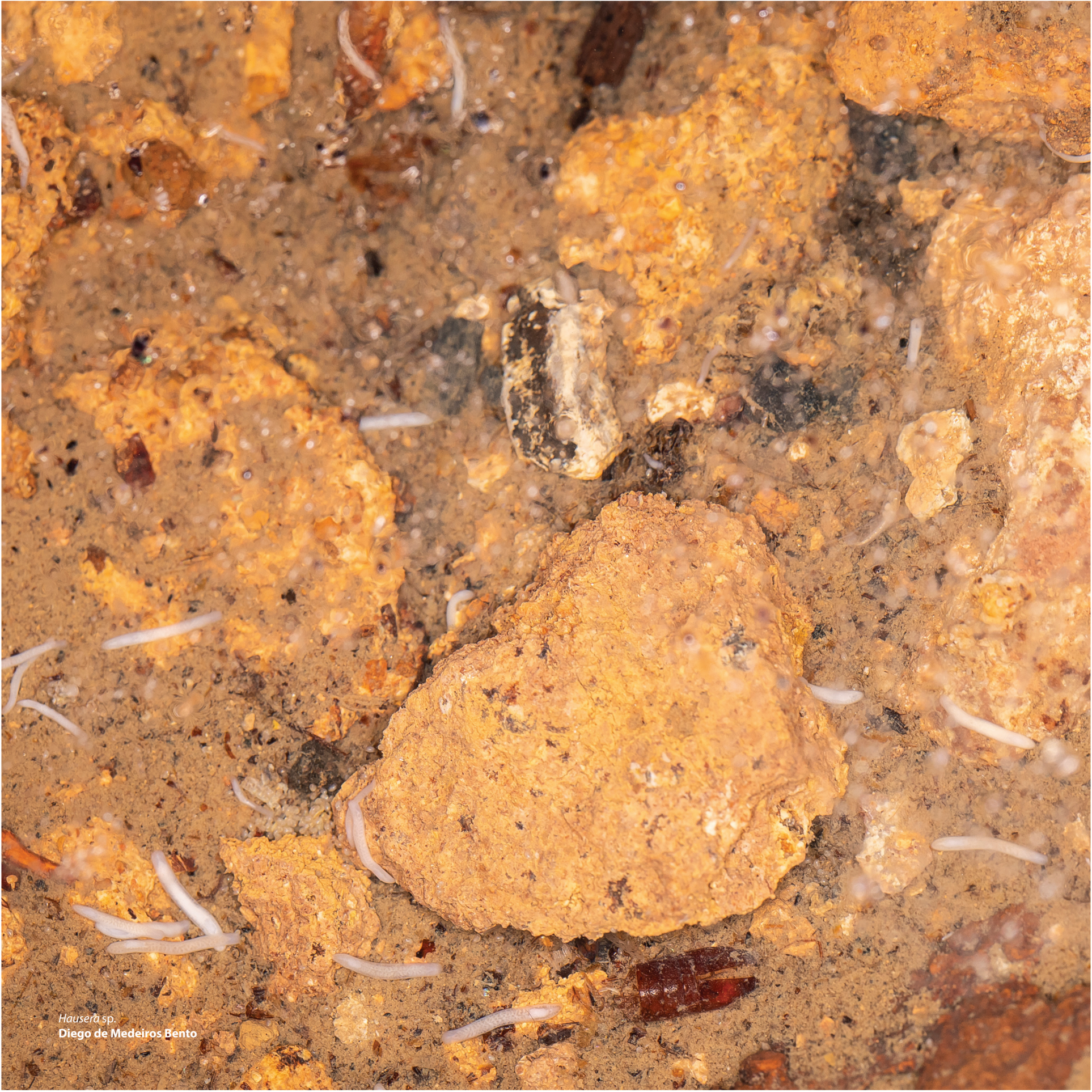
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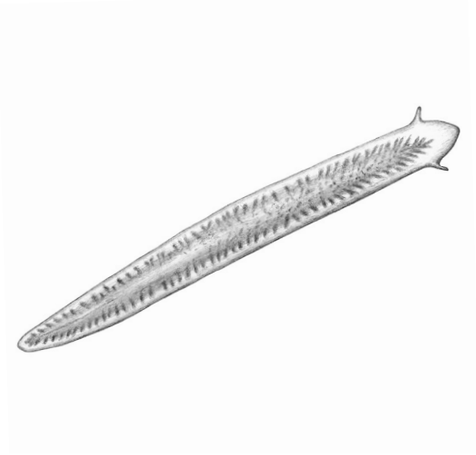
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22

Platyhelminthes

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General introduction

Platyhelminthes are acoelomate animals, mostly hermaphrodites, having a centralized and relatively simple nervous system, in addition to an incomplete digestive system (LEAL-ZANCHET; AMATO, 2016; MARTÍN-DURAN; EGGER, 2012). Until recently, they were considered the most basal group of Bilateria, being much studied to understand the evolutionary origins of bilateral symmetry. However, recent phylogenetic studies have grouped platyhelminths into the superphylum Lophotrochozoa, indicating the occurrence of a secondary simplification process in their body structure (DUNN *et al.*, 2008). They constitute one of the most diverse groups of animals, with about 29,000 described species (ZHANG, 2011), 75% of which are obligate parasites of vertebrates. Many species of platyhelminths are considered model organisms for studies of regeneration and embryonic development (MARTÍN-DURAN; EGGER, 2012; MITSI *et al.*, 2019).

The free-living platyhelminths are included in the group commonly known as "Turbellaria", a non-monophyletic group, subdivided into three main clades: Acoelomorpha, Catenulida and Rhabditophora. The latter includes the order Tricladida (NOREÑA *et al.*, 2019), the only one recorded in caves in the Neotropics. Triclads, commonly called planarians, can be easily identified by having the intestine divided into three branches, a characteristic that gives the order its name (Figures 1A–D). The order is subdivided into three clades: Maricola, Cavernicola and Continenticola. As indicated by the name, Maricola contains mostly marine animals; Cavernicola, organisms that inhabit limnic environments in caves; and Continenticola, specimens occurring in limnic and terrestrial continental environments. However, there are also representatives of Maricola and Continenticola that live in caves, just as there are representatives of Cavernicola that occur in epigean environments (LEAL-ZANCHET; MARQUES, 2018a, b; SLUYS; LAUMER, 2019; SOUZA *et al.*, 2018). The suborder Continenticola includes two superfamilies — Planarioidea and Geoplanoidea, — the latter being divided into two families: Dugesiidae, which includes limnic planarians, and Geoplanidae, which includes the land planarians (RIUTORT *et al.*, 2012; SLUYS *et al.*, 2009).

Limnic planarians reach up to 30 mm in body length and have a less conspicuous color pattern, ranging from brown to black (SLUYS; RIUTORT, 2018). Representatives of Cavernicola and Maricola are usually smaller than limnic representatives of Continenticola. The anterior end of the body may be triangular with the presence of lateral projections (auricles), truncated or more rounded (Figures 1A–D). The auricles are pointed in Dugesiidae or more rounded in Maricola (SLUYS, 1989, 1996; SLUYS *et al.*, 1997), while in Cavernicola the anterior end does not have lateral projections (Figure 1A). Limnic tricladids usually have a pair of ocelli at the anterior end of the body (Figure 1D). The ocelli of tricladids are generally composed of photoreceptors that indicate the intensity and direction of light present in the environment (LEAL-ZANCHET; AMATO, 2016).

Land planarians, on the other hand, are generally 20 to 150 mm long and their body has varied color patterns, which can combine bright and contrasting colors. The shape of the anterior end varies among subfamilies and genera, with many species having the anterior and posterior ends similarly shaped (Figures 2A–B). Land planarians may have a pair of ocelli at the anterior end of the body, but many species have multiple tiny ocelli distributed along the sides of the body (BRUSA *et al.*, 2020; SEITENFUS; LEAL-ZANCHET, 2004).

The mouth of triclads is located on the ventral surface, approximately in the middle third of the body, through which feeding and excretion occur. The three intestinal branches (one anterior and two posterior) contain many lateral branches and communicate with a muscular tubular organ, the pharynx (Figures 1B–C), used for sucking prey. The reproductive system is relatively complex and, in most species, has a main opening posterior to the mouth, the gonopore, through which copulation occurs, with mutual transfer of spermatozoa and internal fertilization (LEAL-ZANCHET; AMATO, 2016; LEAL-ZANCHET; BAPTISTA, 2009; SEITENFUS; LEAL-ZANCHET, 2004).

Planarians are carnivores and usually predators. Tricladids feed on small invertebrates that occur in their microhabitats, such as arachnids, isopods, annelids, gastropods, microcrustaceans and other turbellarian species (BOLL *et al.*, 2015; BOLL; LEAL-ZANCHET, 2016; BOLL; LEAL-ZANCHET, 2018; CSEH *et al.*, 2017; CUEVAS-CABALLÉ *et al.*, 2019; GEE; YOUNG,

1993; PRASNISKI; LEAL-ZANCHET, 2009). They are photophobic organisms, avoiding direct light, and do not have efficient water retention mechanisms. Limnic planarians are benthic organisms, found associated with the substrate in limnic or lentic environments. Land flatworms are very dependent on the humidity of their microhabitat for survival (FROEHLICH, 1955; KAWAGUTI, 1932; SLUYS, 2019; WINSOR, *et al.*, 1998). To avoid desiccation, they use some behavioral strategies, such as seeking shelter in damp places, such as litter, under stones and fallen logs, and are more active during the night, when the environment is more humid and the temperature mildest (ANTUNES *et al.*, 2012). Another important ecological characteristic of land planarians is their low capacity for locomotion, with a tendency for low dispersal capacity and high endemism (ÁLVAREZ-PRESAS *et al.*, 2011, 2014; FICK *et al.*, 2006). These characteristics combined make land planarians sensitive to environmental changes in their microhabitat, although there are some more tolerant species (ÁLVAREZ-PRESAS *et al.*, 2011, 2015).

Taxonomic identification of triclads relies on characteristics of external morphology, such as body shape, color pattern and eye arrangement, as well as anatomical and histological aspects of the body wall and digestive and reproductive systems. The main characteristics of taxonomic importance are sensory organ morphology, body musculature structure and development, and pharynx anatomy and musculature structure, as well as anatomical and histological aspects of the gonads, genital ducts and copulatory organs (SEITENFUS; LEAL- ZANCHET, 2004; SLUYS, 1989, 1996; SLUYS; RIUTORT, 2018).

Main groups found in Brazilian caves

Faunal inventories, for the purposes of environmental licensing and research activities, have indicated the occurrence of populations of limnic planarians established in Brazilian caves, while land planarians seem to use this environment occasionally. There are records of representatives of all three triclad suborders (Cavernicola, Continenticola and Maricola) in these ecosystems in different regions of the country (KAWAKATSU; FROEHLICH, 1992; LEAL-ZANCHET *et al.*, 2014; LEAL-ZANCHET; MARQUES, 2018a, b; SOUZA *et al.*, 2018).

The suborder Cavernicola has a single family, Dimarcusidae, and six genera of disjunct distribution, with species recorded for Brazil, Mexico, Tahiti and Malaysia (BENÍTEZ-ÁLVAREZ *et al.*, 2020; SLUYS, 1990). Most species occur in limnic environments. There are two monotypic genera of Dimarcusidae recorded in the country, one of them only in epigean environments (*Rhodax* Marcus, 1946) and the other only in hypogean environments (*Hausera* Leal-Zanchet & Souza, 2014). The type-species of *Hausera*, *H. hauseri* Leal-Zanchet & Souza, 2014 (Figure 1A), was recorded for Crotes cave in Felipe Guerra, state of Rio Grande do Norte, located in the Caatinga biome (LEAL-ZANCHET *et al.*, 2014).

The suborder Maricola is widely diverse, but relatively understudied, being composed of three superfamilies and six families (SLUYS, 1989). There are records of eight species for Brazil, seven occurring in coastal marine environments and one in a hypogean limnic environment (CARBAYO; FROEHLICH, 2008; SOUZA *et al.*, 2018). The genus *Sluysia* Leal-Zanchet, Souza & Riutort, 2018 was proposed for the latter, considered troglobitic. *Sluysia triapertura* Leal-Zanchet & Souza, 2018 (Figure 1B), of the family Uteriporidae, was recorded for the Três Lagos cave in Felipe Guerra, Rio Grande do Norte, located in the Caatinga biome (SOUZA *et al.*, 2018).

Within Continenticola, the family Dugesiidae has a wide distribution in limnic environments of the Neotropical Region, through representatives of the genus *Girardia* Ball, 1974. About 60 species are currently known for this genus, 19 of them with records in Brazil, of which 11 have been recorded in caves. Of these, four species are possibly troglophiles and another seven troglobites (HELLMANN, *et al.*, 2018; HELLMANN *et al.*, 2020; KAWAKATSU; FROEHLICH 1992; MORAIS *et al.*, 2021; SOUZA *et al.*, 2015, 2016). The first record of a species in a limnic environment in caves in the country, which was considered troglophilous and identified as *Girardia paramensis* Fuhrmann, 1914, was in the sandstone caves Pedra da Cachoeira, Planaltina and Limoeiro, in the region of Altamira, state of Pará, in the Amazon biome (KAWAKATSU; FROEHLICH, 1992). The first troglobitic species of

Dugesiidae recorded in the country, *Girardia multidiverticulata* Souza *et al.*, 2015 (Figure 1C), was found in a limestone cave in Serra da Bodoquena, state of Mato Grosso do Sul, in a Cerrado area (SOUZA *et al.*, 2015). Subsequently, a troglobitic species, *Girardia desiderensis* Souza & Leal-Zanchet, 2016 and a troglophilous species, *Girardia pierremartini* Souza & Leal-Zanchet, 2016, were described from caves of the Bambuí geomorphological group in, respectively, São Desidério, state of Bahia, in an area of Cerrado-Caatinga transition, and Presidente Olegário, state of Minas Gerais, in a Cerrado area (SOUZA *et al.*, 2016). Two syntopic troglobitic species, *Girardia arenicola* Hellmann & Leal-Zanchet, 2018 (Figure 1D) and *Girardia paucipunctata* Hellmann & Leal-Zanchet, 2018, were recorded for the Areias system, state of São Paulo, in an area of Atlantic Forest (HELLMANN *et al.*, 2018).

More recently, five other species of *Girardia*, three troglobites and two possible troglophiles, were described for the country (HELLMANN *et al.*, 2020; MORAIS *et al.*, 2021). The troglobitic species, *Girardia spelaea* Hellmann & Leal-Zanchet, 2020, was recorded in limestone caves of the Águas Claras system (Serra do Ramalho, Bahia), in an area of Cerrado-Caatinga transition. *Girardia ibitipoca* Hellmann & Leal-Zanchet, 2020, a troglophile, has been recorded only at its type-locality, a quartzite cave (Andrelândia geological group) located in the Parque Estadual de Ibitipoca, in an area of the Atlantic Forest. Similarly, the troglophilous species *Girardia asymmetrica* Hellmann & Leal-Zanchet, 2020 was recorded only at its type-locality, a limestone cave (Bambuí geological group), in Minas Gerais, in a Cerrado area (HELLMANN *et al.*, 2020). The troglobitic species *Girardia corumbataiensis* Morais & Leal-Zanchet, 2021 was recorded in a sandstone cave of the Botucatu Formation, located in the "Cuestas de Corumbataí" environmental protection area in São Paulo, in an area of Atlantic Forest-Cerrado transition. *Girardia noblesis* Morais & Leal-Zanchet, 2021, also troglobitic, was recorded only in a limestone cave at its type-locality, in the Araras Formation, Speleological Province of Alto Paraguai, in Araguaia, state of Mato Grosso, in a Cerrado area (MORAIS *et al.*, 2021).

Regarding the terrestrial representatives of Continenticola, the family Geoplanidae has a great diversity of species in the Neotropical Region, especially in Brazil, with Geoplaninae being the subfamily with the greatest diversity in the country with about 230 species, the majority registered for the Atlantic Forest biome in the South and Southeast regions (CARBAYO; FROEHLICH, 2008; NEGRETE *et al.*, 2019; OLIVEIRA *et al.*, 2020; ROSSI *et al.*, 2019; ROSSI *et al.*, 2020; SILVA; CARBAYO, 2020). Few species are known for the other biomes due to the scarcity of studies. For the Amazon biome, for example, there have been only two taxonomic studies with records of representatives of the group (AMARAL *et al.*, 2019b; FROEHLICH; FROEHLICH, 1972). There have been no studies for the Pampa and Pantanal biomes, leaving the diversity of land planarians in these regions as unknown (MARQUES, 2020).

There are records of five species of land planarians occurring in caves in the country, all belonging to the subfamily Geoplaninae. One of them belongs to the monotypic genus *Difroehlichia* Leal-Zanchet & Marques, 2018, one to the genus *Paraba* Carbayo *et al.*, 2013 and the remaining three to the genus *Pasipha* Ogren & Kawakatsu, 1990, with the last being the genus of Geoplaninae with the highest number of records in caves in Brazil (AMARAL *et al.*, 2019a, b; LEAL-ZANCHET; MARQUES, 2018a-b). Three species, *Difroehlichia elenae* Leal-Zanchet & Marques, 2018, *Pasipha ferrariaphilla* Leal-Zanchet & Marques, 2018 and *Pasipha tutameia* Amaral & Leal-Zanchet, 2019, were recorded in the Cerrado, the first two in ferruginous caves in Conceição do Mato Dentro and the third in a carbonate cave in Cordisburgo, both in Minas Gerais (AMARAL *et al.*, 2019b; LEAL-ZANCHET; MARQUES, 2018a-b). *Paraba pankaru* Amaral & Leal-Zanchet, 2019 (Figure 2A), occurring in a carbonate cave in Serra do Ramalho, Bahia, is the only land planarian species recorded for the Caatinga biome (AMARAL *et al.*, 2019a), while *Pasipha carajaensis* Amaral & Leal-Zanchet, 2019, occurs in a ferruginous cave in the Amazon biome, in the Province of Carajás, Curionópolis, Pará (AMARAL *et al.*, 2019b). In addition to these, there are still records of two other morphospecies, one belonging to the genus *Choeradoplana* Graff, 1896 and the other unidentified (BICHUETTE *et al.*, 2017; TRAJANO; GNASPINI-NETTO, 1990).

Despite recent advances in the study of cave planarians in Brazil, knowledge about the group is still incipient and the diversity of tricladid species recorded for hypogean environments is low. In addition to the 13 limnic species and five known land species, there is information from faunal surveys indicating the occurrence of 60 other species of tricladids, identified to the family or subfamily level, in caves in Brazil (AMARAL *et al.*, 2018; LEAL-ZANCHET *et al.*, 2018; MARQUES, 2017), most with records in ferruginous caves in the Amazon biome. The small number of specialist researchers for the group in the country and the fact that taxonomic works are, in general, time consuming, have meant that a high number of species are still under analysis for description.

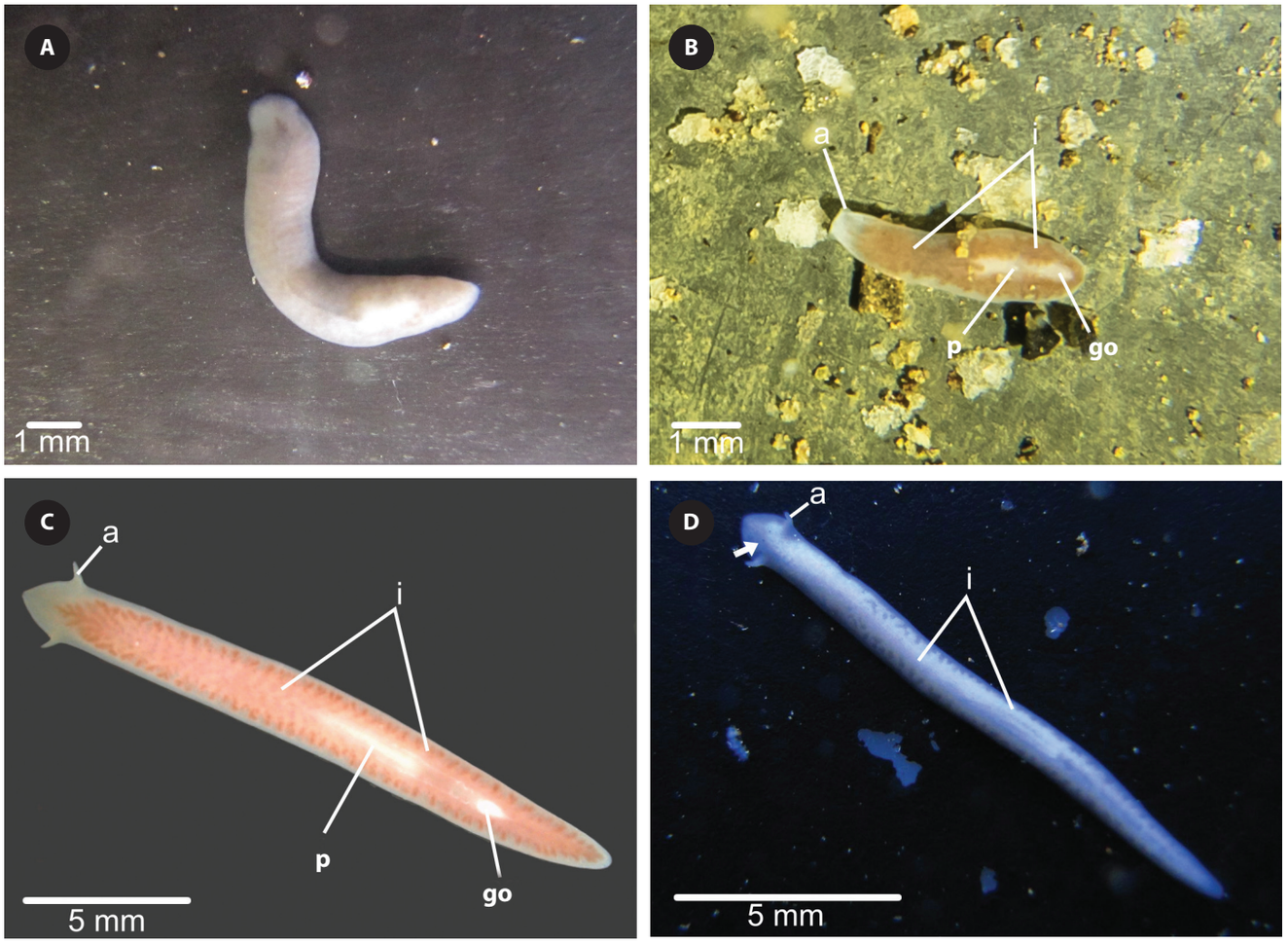


Figure 1. Dorsal view of troglobitic, limnic species of tricladids: **A)** *Hausera hauseri* (Cavernicola: Dimarcusidae); **B)** *Sluysia triapertura* (Maricola: Uteriporidae); **C)** *Girardia multidiverticulata* (Continenticola: Dugesiidae); **D)** *Girardia arenicola* (Continenticola: Dugesiidae). Anterior end to the left. a: auricles; p: pharynx; i: intestine; go: genital organs. Arrow: ocelli. Photos: A, D (Rodrigo L. Ferreira) modified from Leal-Zanchet *et al.* (2014) and Hellmann *et al.* (2018), respectively; B (Diego de M. Bento); C (Lívia Medeiros Cordeiro) modified from Souza *et al.* (2015).

Main troglomorphisms

Most of the limnic species recorded in caves in the country show troglomorphisms (Figures 1A–C), such as the absence of eyes and body pigmentation (Figure 1D) (HELLMANN *et al.*, 2018; HELLMANN *et al.*, 2020; LEAL-ZANCHET *et al.*, 2014; MORAIS *et al.*, 2021; SOUZA *et al.*, 2015, 2016). In addition, there is a reduction in the size of the ocelli in some species (HELLMANN *et al.*, 2018). There is also, compared to epigean species, a reduction in body size in four hypogean species of Dugesiidae, although there is no clear pattern (HELLMANN *et al.*, 2018; HELLMANN *et al.*, 2020; MORAIS *et al.*, 2021). The representatives of Cavernicola and Maricola occurring in caves are tiny (Figures 1A–B) but have a similar body size to that of epigean species of these suborders.

The occurrence of troglomorphisms is rare among land planarians recorded in caves. Only *Difroehlichia elenae* and *Paraba pankaru* (Figure 2A) have features that can be considered troglomorphic, i.e., hypertrophy of sensory margin and longer sensory pits, respectively (AMARAL *et al.*, 2019a; LEAL-ZANCHET; MARQUES, 2018a-b). Additionally, the type-locality of *P. pankaru* is an extremely dry region and the specimen was collected in the aphotic zone of the cave, possibly

indicating dependence on the hypogean environment. Although there are no records in the literature, it is known that other troglomorphisms may occur in land planarians, such as the absence of ocelli (MARQUES, 2017). Even so, continuous sampling of land triclads in karst areas is necessary to obtain information about the relationship of these organisms with the hypogean environment.

Diversity of troglobitic species described for Brazil

Considering ecological, morphological and distributional characteristics, the species of the suborders Cavernicola (*Hausera hauseri*) and Maricola (*Sluysia triapertura*) with records in hypogean environments and seven species of Continenticola (*Girardia* spp.) are considered troglobites. Thus, currently, there are records of nine species of troglobitic planarians for the country (Figure 4), corresponding to about 60% of the limnic species recorded in caves (HELLMANN *et al.*, 2018; HELLMANN *et al.*, 2020; LEAL- ZANCHET *et al.*, 2014; MORAIS *et al.*, 2021; SOUZA *et al.*, 2015, 2016, 2018).

Main methods of collection and conservation

The standard method for sampling planarians is by active search, looking in places of possible occurrence, in the substrate of limnic and terrestrial environments, usually in litter or under logs and stones in the latter. Limnic species can occur in lotic or lentic environments (Figures 3A–C), including small pools formed by seeping water. Inside caves, land flatworms should be searched for on the floor and walls. Planarians must be handled carefully, as they have a very sensitive epidermis and can be damaged if not handled correctly. A small brush can be used for the collection of aquatic planarians (Figures 3B, D), but a Pasteur pipette is better for capturing minute specimens. Land flatworms should be removed from the substrate carefully with a dampened soft-bristle brush. It is important that specimens are photographed in life during sampling, due to the presence of relevant characteristics of body shape and color pattern for their determination to the species level (Figures 1–2).

When it is necessary to preserve planarians at the sampling site, fixation in formalin solution is preferred for anatomical studies, and absolute ethanol for molecular studies. Efforts are currently being made to carry out taxonomic determination based on an integrative approach, combining morphological and molecular methods. If these fixatives are not available, specimens can be preserved directly in 70% ethyl alcohol.

Alternatively, whenever possible, and so that there is no loss of important data for taxonomic determination, individuals should be transported alive to the laboratory and fixed according to the appropriate protocol. To this end, limnic planarians must be placed in clean containers with water from the sampling site itself and can be stored in the same container per collection point. Land flatworms, on the other hand, must be stored in a container with soil and organic matter from the sampling site to preserve humidity. Each specimen shoud be stored in an individual container, as some species of land planarians prey upon others. Plastic or glass containers can be used, without the need for openings for air to enter, and it is important to close them well. Containers having collected individuals must be stored in a cooled thermal box.

In the laboratory, photos and measurements of living organisms should be taken. For DNA extraction and molecular analyses, some whole specimens or body fragments need to be preserved in 100% ethyl alcohol. Whole specimens are usually preserved in this way when dealing with limnic planarians, which are usually represented by several specimens in a population. In the case of land flatworms, often represented by a single specimen of each species in a sampling, a small fragment of the posterior region of the individual should be cut with the aid of a stylet.

Limnic specimens are usually anesthetized on an ice cube covered with moistened filter paper, on which the specimen is placed, and the fixative solution dripped onto its body. Land flatworms should be placed on a Petri dish, slightly moistened with water, to avoid dehydration and animal stress. Each specimen should be placed individually on the plate, allowing it to move for a few seconds. Once the animal is in a well-extended position, boiling water is poured over it, causing instant death and avoiding suffering and contraction of the organism. Fixation should take place preferably in a 10% formalin solution or another histological fixative, for about 24 to 48 hours, depending on specimen body size. After this period, individuals must be removed from the formalin and transferred to a container with 70% ethyl alcohol, to be preserved for analysis (WINSOR; SLUYS, 2018).



Figure 2. Dorsal view of terrestrial tricladid species recorded in hypogean environments: **A)** *Paraba pankaru* (Continenticola: Geoplaninae); **B)** *Notogynaphallia* sp. (Continenticola: Geoplaninae). Photos: Rodrigo L. Ferreira (A), modified from Amaral *et al.* (2019a); Giuly Gouvêa Iturralde (B). Anterior end to the left.

ECOLOGICAL ASPECTS AND DISITRIBUTION

The species *Hausera hauseri* and *Sluysia triapertura* were collected in the Jandaíra Formation, Potiguar basin. The Três Lagos cave, type-locality of *H. hauseri*, is a cavity of small dimensions (approximately 60 m of horizontal projection), largely flooded by a main lake. In the first samplings at the site, hundreds of specimens were observed associated with the substrate, under rock fragments or submerged guano (Figure 3D). During prolonged periods of drought, the main lake was found completely empty and a reduced number of specimens of *H. hauseri* was restricted to the deeper lake of the cave (LEAL-ZANCHET *et al.*, 2014). The Crotes cave, type-locality of *S. triapertura*, belongs to a complex of 70 caves called Lagedo do Rosário. The main gallery of Crotes cave has a linear projection of 237 m, featuring mainly dry substrate, with a perennial stream (10–15 cm deep) that drains water from the epikarst system (Figure 3A–B) (SOUZA *et al.*, 2018). There are also records of three other troglobitic species of *Hausera*, not yet formally described, for other caves of the Jandaíra Formation, where Três Lagos and Crotes are located, indicating that this region can be considered a diversity *hotspot* for limnic tricladids (BENÍTEZ-ÁLVAREZ *et al.*, 2020; LEAL-ZANCHET *et al.*, 2018).

Within Continenticola, the dugesiid species *Girardia multidiverticulata* was collected in a lake (10 m^2) at the bottom of the Buraco do Bicho cave, in Serra da Bodoquena, at a depth of 25 m in relation to the cave entrance. During sampling activities, the species was found to have a density of approximately 5 to 10 specimens per m^2 (SOUZA *et al.*, 2015). Specimens of *G. multidiverticulata* were kept in the laboratory for several years and reproduced sexually (Lívia Medeiros Cordeiro, personal comm).

Girardia desiderensis was collected in small pools (0.2 m deep), with light water flow, about 200 m from the entrance of Baixa Fria cave, in the karst area of São Desidério (SOUZA *et al.*, 2016).

Girardia arenicola and *G. paucipunctata* were collected in a set of small lakes formed by percolation water from the epikarst, about 600 m from the entrance of Areias de Cima cave, in the Areias system. Because they were syntopic, and were similar in external appearance, these planarians were initially considered to belong to the same species. Anatomical analyses in the laboratory indicated that they were two different species (HELLMANN *et al.*, 2018).

The type-locality of *Girardia spelaea* is in the Águas Claras system, with the largest number of specimens being found in a small stream of the cave Lapa dos Peixes II, located 100 meters from the main entrance of the cave. Some specimens, in fewer numbers, were also observed in lakes of the main conduit of the Lapa dos Peixes I cave (HELLMANN *et al.*, 2020).

The type-locality of *Girardia corumbataiensis*, Gruta da Toca cave, has a horizontal projection of about 370 m. Planarians showed high density in intermediate and aphotic zones of the cave, where an underground stream has low current. The type-locality of *G. noblesis* is a small cave, Gruta Olho D'Água, with a lake with a maximum depth of about 5 m. Specimens were



Figure 3. Samplings and some hypogean environments with records of limnic tricladids: **A-B)** Crotes cave, Felipe Guerra, RN; **C)** Areia de Cima cave, Iporanga, SP; **D)** Três Lagos cave, Felipe Guerra, RN. Photos: A, C (Rodrigo L. Ferreira); B, D (Diego de M. Bento).

abundant in the aphotic zone of the cave, on the surface of this lake, under precipitated calcite, as well as at the bottom of the lake under submerged guano (MORAIS *et al.*, 2021).

MORPHOLOGICAL CHARACTERISTICS

Similar to other troglobites of Cavernicola, *Hausera hauseri* is characterized by the absence of body pigmentation and ocelli, the same occurring with the maricolan *Sluysia triapertura*. Both have a truncated anterior end of the body but differ by the absence of lateral expansions in *H. hauseri* and the occurrence of a median protuberance and two rounded auricles in *S. triapertura*. The posterior end differs in that it is pointed in *H. hauseri* and rounded in *S. triapertura* (Figure 1A–B). Individuals of *H. hauseri* measure up to 7.5 mm in length, while those of *S. triapertura* have very small body sizes, reaching 4 mm in length (LEAL-ZANCHET *et al.*, 2014; SOUZA *et al.*, 2018).

Among troglobites of Continenticola, variation is observed in relation to the occurrence of body and ocelli pigmentation and body length in comparison to epigean species. Four species (*Girardia arenicola, G. multidiverticulata, G. noblesis* and *G. spelaea*) do not have body pigmentation, while another three (*G. corumbataiensis, G. desiderensis* and *G. paucipunctata*) have sparse pigmentation (HELLMANN *et al.*, 2018; HELLMANN *et al.*, 2020; MORAIS *et al.*, 2021; SOUZA *et al.*, 2015, 2016). Ocelli are absent in *G. desiderensis, G. multidiverticulata* (Figure 1C), *G. noblesis* and *G. spelaea* and are small in *G. arenicola* and *G.*



Figure 4. Map with type-localities of troglobitic species of flatworms described for Brazil.

paucipunctata. In contrast, the ocelli of *G. corumbataiensis* are of the usual size (HELLMANN *et al.*, 2018; HELLMANN *et al.*, 2020; MORAIS *et al.*, 2021; SOUZA *et al.*, 2015, 2016). As for body length, *G. arenicola, G. multidiverticulata* and *G. noblesis*, measuring 11 to 20 mm, have a similar length in comparison to epigean species of *Girardia* (SOUZA *et al.*, 2015, 2016). *Girardia arenicola, G. corumbataiensis, G. paucipunctata* and *G. spelaea*, measuring 5 to 8 mm, have reduced body length compared to epigean species of the genus (SOUZA *et al.*, 2015, 2016; HELLMANN *et al.*, 2018; HELLMANN *et al.*, 2020; MORAIS *et al.*, 2021).

Identification key

The identification of tricladids to the levels of genus and species is based on external aspects and anatomical and histological characteristics of the epidermis, body musculature and digestive and reproductive systems. However, to make this key more accessible, it has been prepared based on external characters only. This, however, also restricts its applicability, of anatomical and histological characteristics in addition to the characters of external morphology.

1. Occurs in aquatic environments. **2**
1′. Occurs in terrestrial environments. **9**
2. Head triangular in shape **3**
2′. Head truncated, eyes absent. **13**
3. Eyes present. **4**
3′. Eyes absent. **14**
4. Body pigmentation present **5**
4′. Body pigmentation absent ..... ***Girardia arenicola*** Hellmann & Leal-Zanchet, 2018*
5. Body pigmentation sparse **6**
5′. Body pigmentation abundant **17**
6. Eyes of usual size **7**
6′. Eyes of reduced size ..... ***Girardia paucipunctata*** Hellmann & Leal-Zanchet, 2018*
7. Body length less than 8 mm in mature individuals. **8**
7′. Body length greater than 8 mm in mature individuals ..... ***Girardia asymmetrica*** Hellmann & Leal-Zanchet, 2020
8. Body length greater than 5 mm in mature individuals ..... ***Girardia corumbataiensis*** Morais & Leal-Zanchet, 2021*
8′. Body length less than 5 mm in mature individuals ..... ***Girardia ibitipoca*** Hellmann & Leal-Zanchet, 2020
9. Dorsal color pattern homogeneous or nearly homogeneous **10**
9′. Dorsal color pattern presenting stripes **11**
10. Body flattened ..... ***Pasipha ferrariaphilla*** Leal-Zanchet & Marques, 2018
10′. Body subcylindrical in transverse section ..... ***Difroehlichia elenae*** Leal-Zanchet & Marques, 2018
11. Dorsum with longitudinal stripe **12**
11′. Dorsum with transverse stripe ..... ***Paraba pankaru*** Amaral & Leal-Zanchet, 2019
12. Dorsum light gray with yellowish median stripe ..... ***Pasipha carajaensis*** Amaral & Leal-Zanchet, 2019
12′. Dorsum brown with dark spots and light brown median stripe ..... ***Pasipha tutameia*** Amaral & Leal-Zanchet, 2019
13. Auricles present ..... ***Sluysia triapertura*** Leal-Zanchet & Souza, 2018*
13′. Auricles absent ..... ***Hausera hauseri*** Leal-Zanchet & Souza, 2014*
14. Body length less than 12 mm in mature individuals **16**

14'. Body length greater than 12 mm in mature individuals..... ***Girardia multidiverticulata*** Souza *et al.*, 2015*
15. Auricles medium in size; posterior end rounded.....**17**
15'. Auricles long and pointed; posterior end pointed..... ***Girardia spelaea*** Hellmann & Leal-Zanchet, 2020*
16. Dorsum whitish; without pigmentaiton..... ***Girardia nobresis*** Morais & Leal-Zanchet, 2021*
16'. Dorsum yellowish; with sparse, fine pigmentation..... ***Girardia desiderensis*** Souza & Leal-Zanchet, 2016*
17. Dorsum uniform dark brown, with lighter margins and pharyngeal region..... ***Girardia paramensis*** (Fuhrmann, 1914)
17'. Dorsum light brown, covered by several dark homogeneously-distributed spots .....
..... ***Girardia pierremartini*** Souza & Leal-Zanchet, 2016

Main Brazilian collections

The main scientific collections where tricladid type-material is deposited are the Coleção de Zoologia of the Universidade do Vale do Rio dos Sinos (MZU), in São Leopoldo, Rio Grande do Sul, and the Museu de Zoologia of the Universidade de São Paulo (MZUSP). In addition to specimens preserved in ethyl alcohol, the type-material of these organisms includes histological preparations, necessary for identification to the levels of genus and species.

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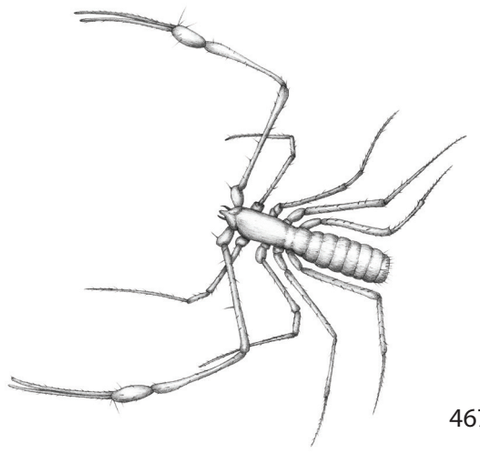


Spelaeobochica iuiu
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23

Pseudoscorpiones

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General introduction

Pseudoscorpiones is a group of small arachnids ranging from 0.5mm to 1cm in length. They superficially resemble scorpions with chelate pedipalps but lack a tail ending in a stinger (HARMS; DUNLOP, 2017). They are cryptobiotic organisms (habit of living hidden or burrowed) and generally live in micro spaces, but they can also be found among leaf litter or plant debris, under fallen logs or tree bark, and in rock crevices, insect and vertebrate nests and caves (HOFF, 1959; MORIKAWA, 1962; MUCHMORE, 1990; REDDELL, 2012; WEYGOLDT, 1969). Thus, most species spend most of their lives inside crevices and rarely appear on exposed soil (WEYGOLDT, 1969).

These animals are relatively common, although little known. This is due to their reduced size and cryptobiotic habit, combined with the existence of few specialists for the group in the world. Thus, the material contained in samples is often neglected (HARVEY, 2002; RODRIGUES; PEDROSO, 2018; WEYGOLDT, 1969).

Certain environmental factors are important for the survival of pseudoscorpions, such as high humidity and stable temperatures. Yet there are, there are species that live in arid conditions and even deserts, such as some species of Olpiidae and Cheliferidae that live under high temperatures that are essential for the completion of their life cycle. The relevance of conditions such as soil acidity and environmental salinity to the distribution of representatives of this group, however, is not known (WEYGOLDT, 1969).

Some species of this taxon present phoresy, a behavior that enables their dispersion — a pseudoscorpion attaches itself to a larger animal, invertebrate or vertebrate (WEYGOLDT, 1969), and moves with them. Aguiar and Bührnheim (1998) observed that 19 of the 64 species described for the Amazon region were associated with flying insects. Pseudoscorpions generally have a low intrinsic dispersal capacity (walking) and generally non-phoretic species tend to have limited distributions, such as some species found only in subterranean environments (e.g., *Pseudochthonius biseriatus* Mahnert, 2001; *Spelaeobochica muchmorei* Andrade & Mahnert, 2003, each restricted to a single underground system: Gruta Olhos d'água, in the state of Minas Gerais, and Sistema Areias, in the state of São Paulo, respectively) (COSGROVE *et al.*, 2016; GARDINI, 2013, 2014; HARRISON *et al.*, 2014; HARVEY *et al.*, 2016).

No phoretic pseudoscorpions have been found in Brazilian caves to date. However, there are records of phoresy for at least 10 families of pseudoscorpions (POINAR *et al.*, 1998), including dispersal by vertebrates such as birds (HARVEY *et al.*, 2015) and bats (FINLAYSON *et al.*, 2015), which, according to some authors (MOULDS *et al.*, 2007), can be an important means for the colonization of some subterranean environments.

A phylogeographical analysis of a species widely distributed in Europe, *Chernes hahnii* (Koch, 1839), suggests that phoresy in pseudoscorpions may be the most efficient way of large-scale geographic dispersal (OPATOVA; ŠŤÁHLAVSKÝ, 2018). Some authors (POINAR *et al.*, 1998) discuss the phoresy behavior of this taxon and indicate Diptera, Lepidoptera and Coleoptera as its main vectors. However, information is scarce as to the specificity in this relationship between the phoretic animal and its vector (ZEH; ZEH, 1997).

Work regarding the general biology of Pseudoscorpiones in Brazil includes studies of natural history (ANDRADE, 1999; LIRA *et al.*, 2020a; MOURA *et al.*, 2018; RIBEIRO *et al.*, 2018), population biology (ANDRADE, 2004) and feeding and social behavior (DEL-CLARO; TIZO-PEDROSO, 2009; TIZO-PEDROSO; DEL-CLARO, 2005, 2011, 2014, 2018), as well as studies more focused on distribution (AGUIAR; BÜHRNHEIM, 1998; AGUIAR *et al.*, 2006; LIRA *et al.*, 2014, 2020b; SANTOS *et al.*, 2005) and conservation of this fauna (SCHIMONSKY; BICHUETTE, 2019).

MORPHOLOGY

The body of pseudoscorpions is divided into the prosoma (anterior) and the opisthosoma (posterior) (Figure 1A). All six pairs of appendages of these organisms are inserted in the prosoma: bisegmented chelicera (Figure 1B), pedipalp ending in pincer structures (Figure 1C) and four pairs of locomotor legs (Figure 1A). Most pseudoscorpions have one or two pairs of eyes with simple lenses, and there are those that have ocular reduction or absence.

The opisthosoma is flattened dorsally and has 12 segments, 11 dorsal tergites and 11 ventral sternites, of which the first and second sternites are fused to form the genital plate. The twelfth tergite and sternite are fused to form a plate with an anal cone (Figure 1A). Between the tergites and sternites is a pleural membrane. Both the body and the appendages are covered with different types of bristles.

The chelicerae (Figure 1B) are positioned anteriorly and constitute the first pair of appendages. They are used for feeding and self-cleaning, forming the anterior part of the oral cavity. They are bisegmented, with one part consisting of the body of the chelicera plus the fixed finger, and the other with a movable finger opposite the fixed finger. They possess sensory structures (bristles and liriform organs).

The synapomorphic characters of pseudoscorpions, the interior and exterior serrulae (for self-cleaning) and the exit of the silk gland through the *galea*, are present in this appendage. In addition to these, they have a structure called the *rallum*, also used in self-cleaning and important in the taxonomy of the group (JUDSON, 2007).

The pedipalps constitute the second pair of appendages and have several functions, such as holding prey, defense and mating. These are formed by the coxa, trochanter, femur, tibia and tarsus. The tibia forms the hand of the chela and the fixed finger, and the tarsus gives rise to the movable finger (Figure 1C). There are sensory bristles (trichobothria) that are important for species identification. The fingers of the pedipalps have a row of teeth and some species also have accessory teeth. Some taxa may have openings for venom glands at the anterior end of the fingers of the chela (*e.g.*, Cheliferoidea), while others lack these glands (*e.g.*, Chthonioidea).

The legs, with only a locomotor function, have seven articles (coxa, trochanter, femur, patella, tibia, metatarsus and tarsus), however, they are reduced to six segments in some taxa (metatarsus and tarsus fused).

Regarding internal morphology, the genitalia are taxonomically important for some families. Some characteristics of females, such as the disposition of setae on the genital operculum and size shape of the diverticulum and cribriform plates, as well as their pores, are useful in defining groups and identifying genera and even species (LEGG, 2008). The use of spermatheca, in the groups that have them, can be useful for understanding the relationships among genera and species as well. This structure has proven useful for the taxonomy of Chernetidae, as well as in Atemnidae (MUCHMORE, 1974).

SYSTEMATICS

The order Pseudoscorpiones has about 4132 described species, distributed in 472 genera and 25 living families (HARVEY, 2013; BENAVIDES *et al.*, 2019; HARMS *et al.*, 2022). It presents great diversity among the orders of Arachnida, behind only Araneae, Acariformes, Parasitiformes and Opiliones (HARVEY, 2002; HARMS *et al.*, 2022).

The order has been recovered as the sister group of Solifugae in several phylogenetic analyses with morphological data (GIRIBET *et al.*, 2002; LEGG *et al.*, 2013; SHULTZ, 1990, 2007; WEYGOLDT; PAULUS, 1979; WHEELER; HAYASHI, 1998). However, in other recent analyses, including using conservative genes, Pseudoscorpiones is recovered as the sister group of Scorpiones (GARWOOD; DUNLOP, 2014; SHARMA *et al.*, 2014) (Figure 2). These differences in results may be because pseudoscorpions are a very old group and have a distant evolutionary history, which can create artifacts in analyses, depending on the methodology and dataset used.

Within Arachnida, the clade Arachnopulmonata was previously called Pulmonata but had its nomenclature changed (SHARMA *et al.*, 2014). This clade includes scorpions (Scorpiones) as the sister group to another clade called Tetrapulmonada (Araneae + Pedipalpi). These clades and their relationships are well supported by different analyses with morphological and molecular data, however, the relationship of Arachnopulmonata with apulmonates (other orders of arachnids that do not have lungs) is not resolved (GIRIBET, 2018) (Figure 3).

Although there is no consensus on the relationships among the other groups of Arachnida, the hypothesis of relationship for the group of pseudoscorpions appears to have been resolved recently (ONTANO *et al.*, 2021). A close

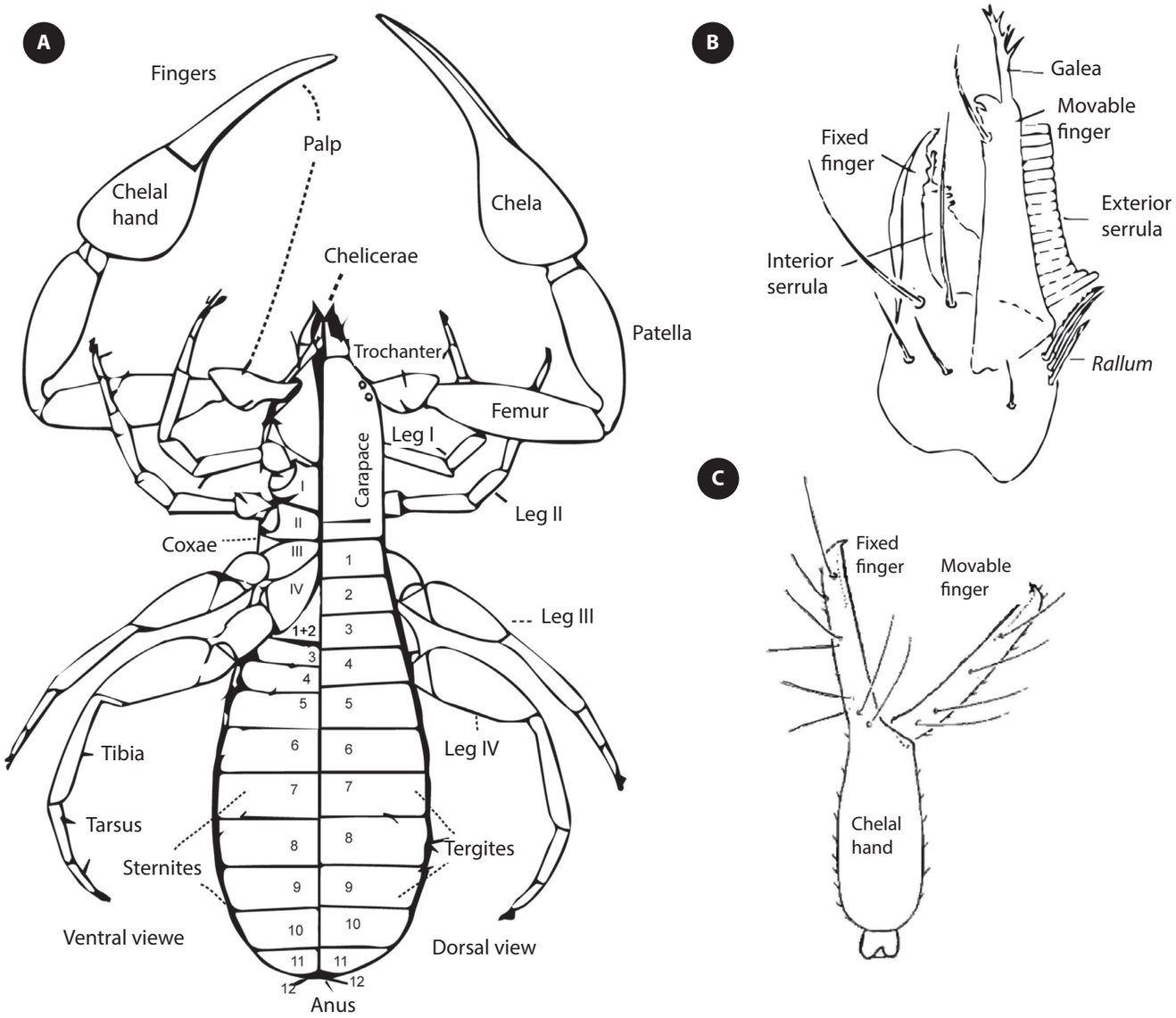


Figure 1. Schematic of the general buaplan of the external morphology of a pseudoscorpion. **A)** Entire body, ventral (right) and dorsal (right) views; **B)** Left chelicera in external lateral view; **C)** Chela of the right pedipalp in lateral view. Figures without scale, modified from CHAMBERLIN, 1931; HOFF, 1949).

relationship between Pseudoscorpiones and Solifugae (forming a clade called Hapclonemata) has been supported by different analyses (GIRIBET *et al.*, 2002; SHULTZ, 1990; VAN DER HAMMEN, 1989; WEYGOLDT; PAULUS, 1979; WHEELER; HAYASHI, 1998) based mainly on morphology. Within this relationship, Pseudoscorpiones is considered a monophyletic group supported by two synapomorphies (SHULTZ, 2007): (i) presence of silk glands, which discharge silk via the movable finger of the chelicera through a structure called the galea; and (ii) the presence of outer and inner serrulae on the fingers of the chelicerae.

With new sources of genetic data for pseudoscorpions, including complete genomic sequences, *Hox* genes and *miRNAs*, and through phylogenetic analyses, Ontano *et al.*(2021) proposed that Pseudoscorpiones is the sister group to Scorpiones, in a new clade called Panscorpiones (Figure 4). This

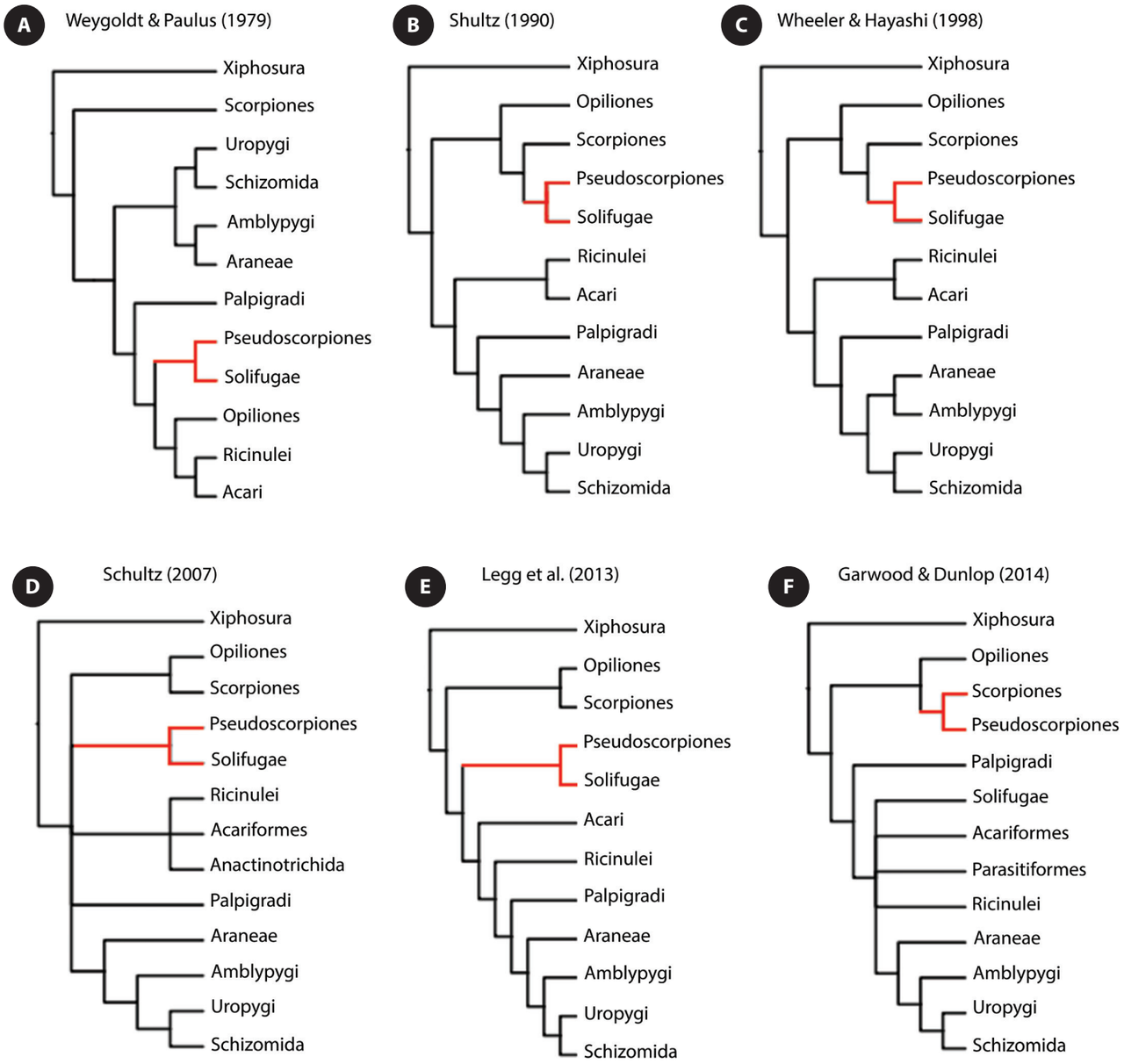


Figure 2. Hypotheses of the relationships among groups of arachnids based on morphological or morphological and molecular data. Most of the hypotheses **A-E)** place Pseudoscorpiones and Solifugae as sister groups, based on morphological data. One of the hypotheses **F)** places Pseudoscorpiones as the sister group to Scorpiones, based on morphological and molecular data. Figure modified from Giribet (2018).

clade is then the sister group to Tetrapulmonata and therefore pseudoscorpions are included in Arachnopulmonata, which has been redefined. The authors demonstrated that analyses of total genotype, duplications of gene families and paralogous gene trees specific to Arachnopulmonata, and mitochondrial RNA duplications, independently support the relationship between Pseudoscorpiones and Scorpiones (ONTANO *et al.*, 2021). Thus, the group of pseudoscorpions lost foliaceous lungs throughout its evolutionary history and developed trachea independently of other groups of Arachnida (ONTANO *et al.*, 2021).

The relationships for pseudoscorpion families have not yet been studied as closely as those of other arachnids (*e.g.*, spiders

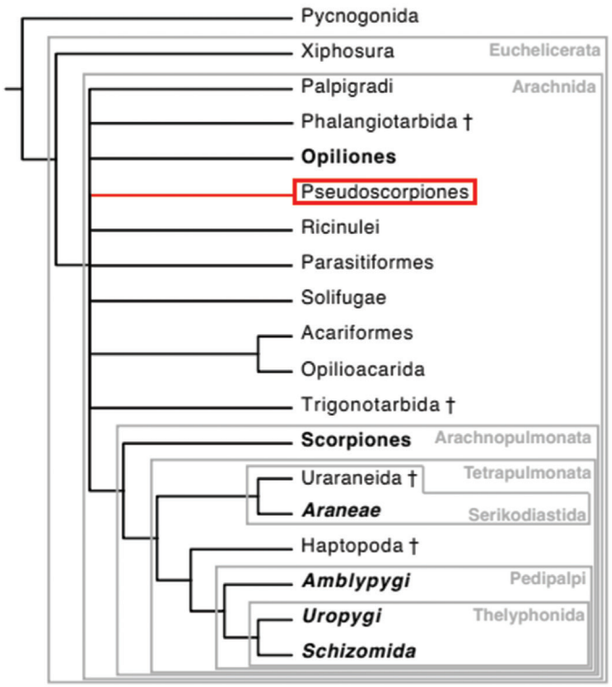


Figure 3. Hypothesis of the relationships among living and fossil arachnid orders. View of current classification with resolution in some taxa, not derived from any specific analysis. Figure modified from Giribet (2018).

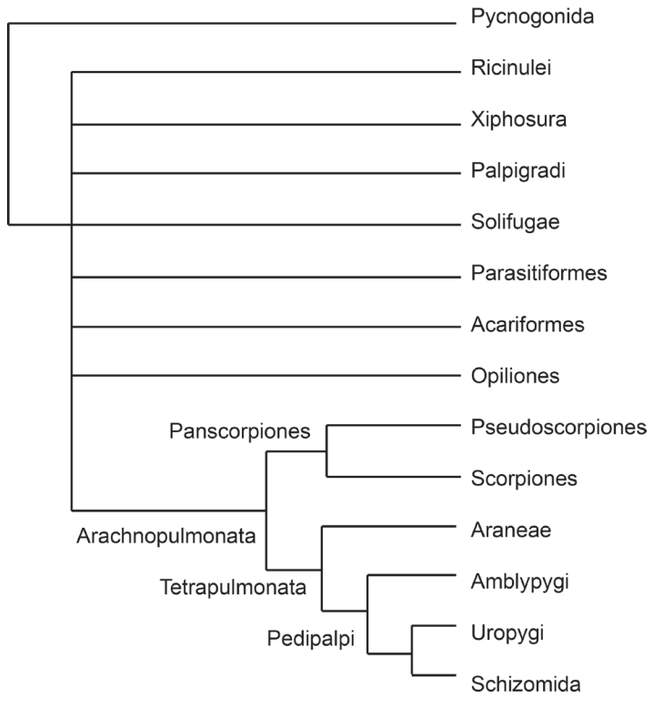


Figure 4. Hypothesis of the relationships among living arachnid orders. Most current classification with resolution in some taxa, derived from phylogenetic analysis. Orders Pseudoscorpiones and Scorpiones in the clade Panscorpiones, as a sister group to Arachnopulmonata. Figure modified from Ontano *et al.* (2021).

and/or opilions) (SHARMA; GIRIBET, 2014; WHEELER *et al.*, 2017). However, there have been several classifications proposed since 1879 (MURIENNE *et al.*, 2008).

An important contribution to the proposed classification is the first cladistic analysis for the order using morphological data of 126 characters and 24 taxa (HARVEY, 1992) (Figure 5). The classification has since undergone a few modifications (BENAVIDES *et al.*, 2019; MURIENNE *et al.*, 2008), but still presents inconclusive relationships (polytomies) for some clades. One of these polytomies is in the superfamily Cheliferoidea, among Chernetidae, Atemnidae and Cheliferidae; another is within Neobisioidea, among Neobisiidae, Syarinidae and Parahyidae.

Murienne *et al.* (2008) reconstructed a phylogeny for most pseudoscorpion groups using molecular data, which corroborated the monophyly of the order and most of the superfamilies (Feaelloidea, Chthonioidea, Cheiridioidea and Sternophoroidea), but not for others (Neobisioidea, Cheliferoidea and Garypoidea). Harms and Dunlop (2017) summarized these previous classifications and inserted data regarding the ages of the taxa, with fossil dating of pseudoscorpions (Figure 6). A recent study using transcriptome analysis (BENAVIDES *et al.*, 2019) brought to light a new phylogenetic classification, with changes at the level of family and above, and represents the most accepted evolutionary history to date (Figure 7).

Some proposals have been presented to elucidate kinship relationships and understand the phylogenetic positioning of some genera, with morphological (HARVEY, 1993, 1994; HARVEY; VOLSCHENK, 2007) or molecular (HARRISON *et al.*, 2014; HARVEY *et al.*, 2016; MOULDS *et al.*, 2007) data, however, no study considered both types of data in the same analysis. In this way, there are no resolutions for kinship relationships of different pseudoscorpion genera, especially the most diverse.

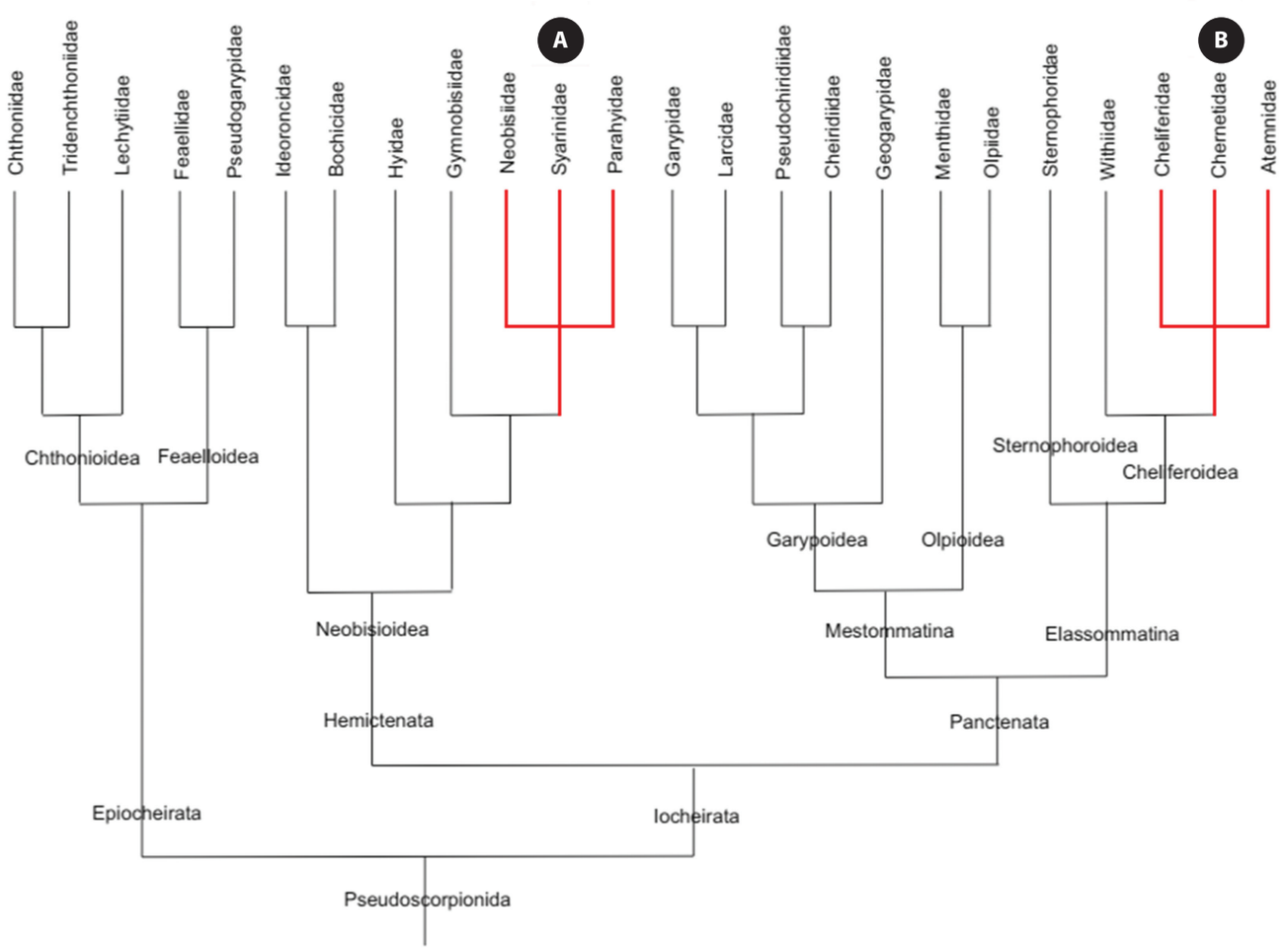


Figure 5. Cladogram of relationships among pseudoscorpion families recognized by Harvey (1992), based on parsimony analysis of 126 morphological characters. Highlighted in red are polytomies within: **A)** Neobisioidea (Neobisiidae + Syarinidae + Parahyidae) and **B)** Cheliferoidea (Cheliferidae + Chernetidae + Atemnidae). Figure modified from Murienne *et al.* (2008).

SEXUAL BEHAVIOR AND CARE OF OFFSPRING

Pseudoscorpions may or may not have sexual dimorphism. When present, males and females can be differentiated by observing the genitalia and/or the pedipalp. Males of some groups may have larger pedipalps with protuberances. These animals reproduce using spermatophores, which are structures for the transfer of sperm from the male to the female. Spermatophore transfer can occur in four different ways: (1) without mating, with females and males acting independently in time and space; (2) without mating, with the male active only in the presence of the partner, and with mating, with both sexes being active, (3) with constant body contact and performing a mating dance or (4) no constant body contact while performing a mating dance.

In the first case, spermatophores are produced by males and deposited in the environment randomly and independently of the female. However, a large waste of male gametes occurs with this method. It is carried out in a humid environment and, generally, by species with gregarious habits. In the second case, the presence of the female acts as a trigger for the production of spermatophores by males, and thus it is easier for the female to find them. In some cases, males can weave a type of web in a format that directs the female to the spermatophore (WEYGOLDT, 1969). When there is mating, there is practically no loss of spermatozoa

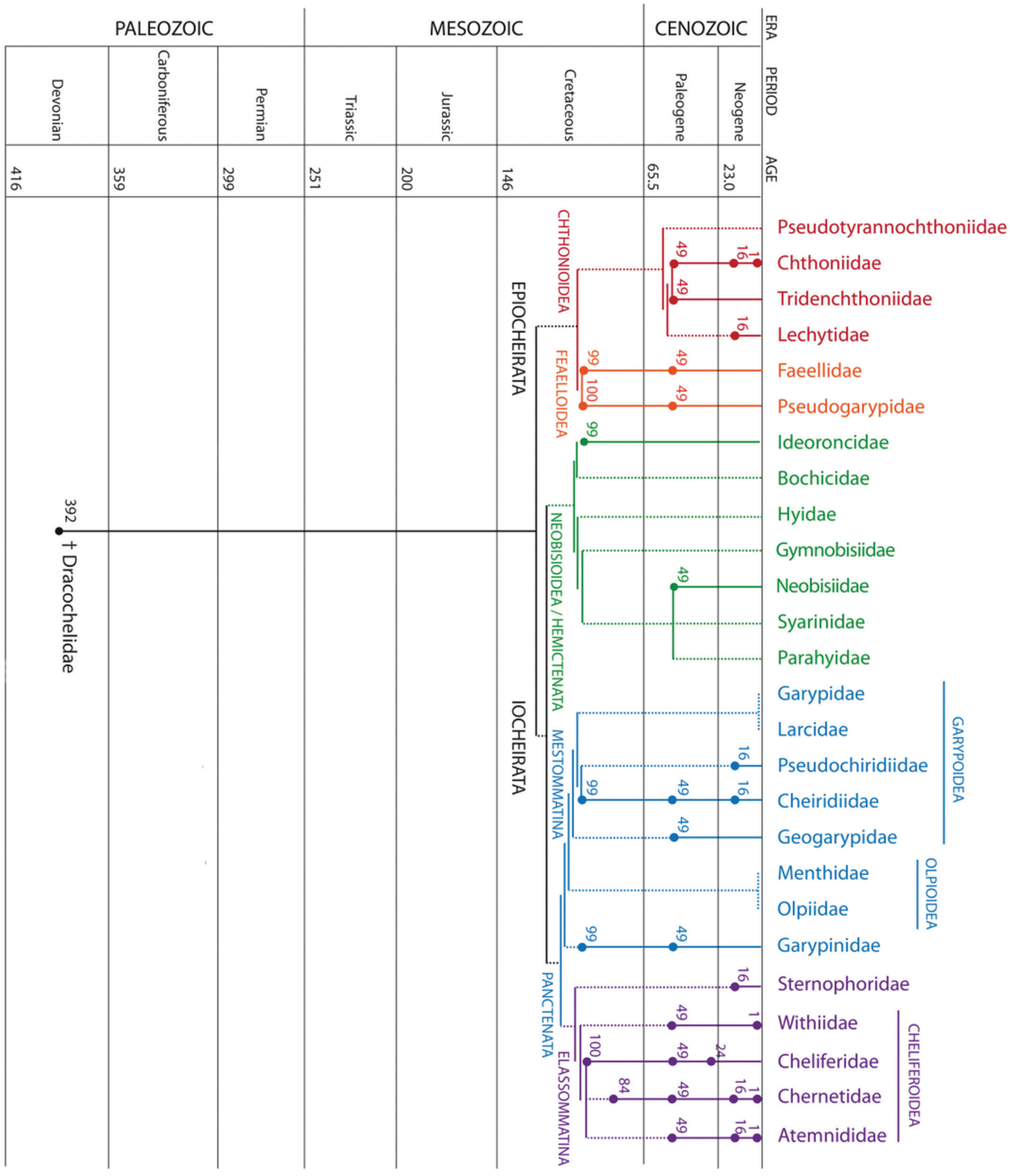


Figure 6. Phylogenetic tree with evolutionary time, redrawn from Harvey (1992) with modifications according to Murienne *et al.* (2008). Dotted lines indicate families with no fossil record and solid lines indicate families with a fossil record. Also provided are the estimated minimum ages (in millions of years) according to the fossil record. Figure taken and modified from Harms and Dunlop (2017).

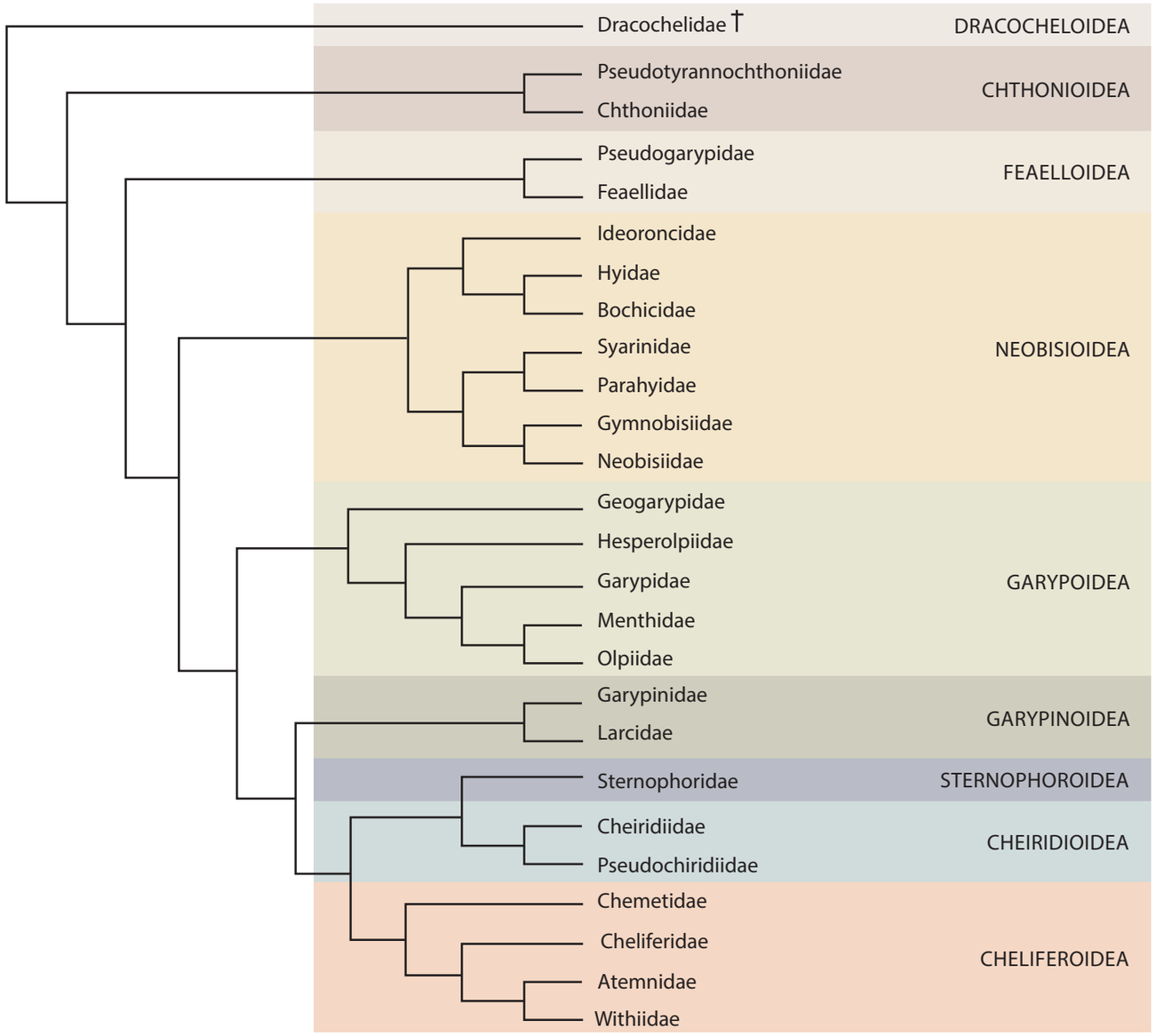


Figure 7. Phylogenetic tree taken and modified from Benavides *et al.* (2019) presenting a new classification regarding the level of family and superfamily.

in the transfer. Males and females act together and the placement of the spermatophore occurs only when the female is receptive and prepared to accept it (WEYGOLDT, 1969).

FOSSIL PSEUDOSCORPIONS

Fossils of pseudoscorpions are rare compared to the diversity of recent groups. Most of the fossils are preserved in amber in different regions of the world (Figure 8). The oldest record is dated to the Devonian and is preserved in shale, while most records are preserved in amber dating to the Cretaceous and Cenozoic (HARMS; DUNLOP, 2017).

Harms and Dunlop (2017) presented a synthesis of pseudoscorpion fossils in the world with 49 fossil species described in the literature, of which three are still extant. These authors present the oldest records of living families and place them on a phylogeny as an indication of the minimum age of most recognized lineages. They also discuss the ecological and taphonomic data for these animals, including records of phoresy also preserved in amber.



Figure 8. World map showing the locations of fossil deposits with records of pseudoscorpions. 1, Gilboa; 2, Lebanese amber; 3, Archingeay amber; 4, Alava amber; 5, Burmese Amber; 6, New Jersey amber; 7, Canadian amber; 8, Fushun (Chinese) amber; 9, Paris amber; 10, Baltic amber; 11, Rivne amber; 12 Romanian amber; 13, Bitterfeld amber; 14, Aix-en-Provence; 15, Dominican amber; 16, Chiapas (Mexican) amber; 17, Kauri (New Zealand) amber; 18, East African copal; 19, Madagascar copal; 20, Colombian copal. Taken from Harms and Dunlop (2017).

Pseudoscorpions of Brazil

Knowledge about pseudoscorpions in Brazil is fragmented and data on habitat, distribution and ecology are still incipient, especially for those that occur in caves (MAHNERT, 2001). However, a recent survey presents the distribution of these organisms at the genus level for caves in Brazil (SCHIMONSKY; BICHUETTE, 2019).

There are 184 described species of pseudoscorpions in Brazil, distributed in 15 families (Atemnidae, Bochicidae, Cheiridiidae, Cheliferidae, Chernetidae, Chthoniidae, Feaellidae, Garypinidae, Geogarypidae, Hesperolpiidae, Ideoroncidae, Olpiidae, Pseudochiridiidae, Syarinidae and Withiidae) (ASSIS *et al.*, 2021; BEDOYA-ROQUEME *et al.*, 2020; BICHUETTE, 2019; HARVEY, 2013; HARVEY *et al.*, 2016; SCHIMONSKY *et al.*, 2020). Thus, Brazil is the fifth most species rich country for pseudoscorpions, behind the United States of America (432), Spain (286), Italy (272) and Australia (214) (HARVEY, 2013). It is the richest country in the Neotropics, with Mexico in second place (173), followed by Chile (90) and Argentina (76) (HARMS *et al.*, 2022; HARVEY, 2013; WPC, 2022).

It should be noted that Brazil, a megadiverse country, has a relatively low richness of pseudoscorpions, when compared to other countries where the general diversity is not great, such as Spain and Italy, evidencing how little is known about the fauna of these organisms in Brazil and the great potential to be explored.

Most of the knowledge about Brazilian species is found in faunal inventories or descriptions of species in different parts of the country, although without any regional estimate. For small portions of the Brazilian Amazon region, however, there are estimates of the taxonomic diversity of this group. For example, about 40 species have been recorded in the Reserva Florestal Adolpho Ducke, near Manaus (MAHNERT, 1979); about 10 species have been recorded in the lower Amazon, close to Belém (MAHNERT, 1985); and about 20 species in the Urucu river region, in Coari (AGUIAR, 1999; AGUIAR; BÜHRNHEIM, 1998). About 75 species have been recorded for the entire Amazon

region, with an estimate that there are more than 150 species (ADIS; HARVEY, 2000). In addition to these, there are about 20 species that occur in caves throughout the national territory (MAHNERT, 2001).

The families with the greatest species richness in Brazil are Chernetidae (Figure 9A) with 69 described species (BEDOYA-ROQUEME *et al.*, 2022, WPC, 2022), followed by Chthoniidae (Figure 9E), with 34 described species (BENAVIDES *et al.*, 2019; HARVEY, 2013; WPC, 2022).

Pseudoscorpions in Brazilian caves

Populations of pseudoscorpions are often found in subterranean habitats around the world, with numerous troglobitic representatives (HEURTAULT, 1994; REDDELL, 2012). The families Chthoniidae, Neobisiidae and Bochicidae are representative in the subterranean (or hypogean) environment, with the greatest diversity of troglobitic species.

Most families of Pseudoscorpiones (Atemnidae, Bochicidae, Cheiridiidae, Cheliferidae, Chernetidae, Chthoniidae, Feaellidae, Garypidae, Geogarypidae, Hesperolpiidae, Ideoroncidae, Neobisiidae, Pseudochiridiidae, Olpiidae, Pseudogarypidae, Sternophoridae, Syarinidae, Tridenchthoniidae and Withiidae) have at least one subterranean representative (troglobitic or not), some with troglomorphic characteristics (HARVEY *et al.*, 2000; REDDELL, 2012). The main troglomorphic characteristics that pseudoscorpions can present are a reduction of eyes and cutaneous melanic pigmentation, an elongation of appendages and an increase in the number of trichobothria (CHAMBERLIN; MALCOLM, 1960).

These animals are rarely mentioned, mainly in taxonomic studies and faunal surveys in general (ANDRADE, 2004). In some cases, they have been cited in qualitative and quantitative studies on fauna associated with accumulations of guano in the hypogean environment (ANDRADE; GNASPINI, 2002; DECU; DECU, 1964; GNASPINI; TRAJANO, 2000; NEGREA; NEGREA, 1971; MARTIN, 1977).

The pseudoscorpions recorded in caves are troglobites or troglophiles. The categorization as trogloxenes is unlikely due to the small size of the organisms and restricted mobility, which would make it difficult to travel at regular intervals to the epigean environment.

In most cases, hypogean pseudoscorpions with typical troglomorphic character states (reduced/absent pigmentation and/or eyes, and elongated appendages) are considered troglobites; if these characteristics are not present, they are considered troglophiles (ANDRADE, 2004). However, the epigean fauna of pseudoscorpions in general is little known in some regions, such as in South America (MAHNERT; ADIS, 2002), which makes it difficult to compare epigean representatives to validate these characteristics and verify the ecological-evolutionary status of hypogean species (troglobites or troglophiles).

Subterranean populations of a total of 38 described species in 22 genera belonging to 11 families of Pseudoscorpiones (Atemnidae, Bochicidae, Cheiridiidae, Chernetidae, Chthoniidae, Geogarypidae, Hesperolpiidae, Ideoroncidae, Olpiidae, Pseudochiridiidae and Syarinidae), have been registered for Brazil (HARVEY *et al.*, 2016; MAHNERT, 2001; RATTON *et al.*, 2012; SCHIMONSKY; BICHUETTE, 2019; VIANA *et al.*, 2018; VIANA; FERREIRA, 2020; WPC, 2022), corresponding to about 17% of the total (180) described species (epigean and hypogean) for the country.

Pseudoscorpions can be found on different substrates in caves, such as rock, sediment banks or associated with accumulations of organic matter, according to the species or even the stage of development. Many have been frequently recorded on bat guano accumulations, with some species restricted to this type of substrate (e.g., *Maxchernes iporangae* Mahnert & Andrade, 1998) (GNASPINI-NETTO, 1989; GNASPINI; TRAJANO, 1994, 2000; MAHNERT, 2001). Few species have been studied in terms of population structure or feeding behavior (*e.g.*, *M. iporangae*), with a relatively large population (ANDRADE, 2004; ANDRADE; GNASPINI, 2002; GNASPINI; TRAJANO, 2000).

Some of the pseudoscorpions found in Brazilian caves were described by Mahnert (2001). In this work, the author considered *Pseudochthonius strinatii* (Chthoniidae) with the type locality of Caverna Areias de Cima, in Parque Estadual Turístico do Alto Ribeira (PETAR), but distributed in other caves in the state of São Paulo [Caverna Morro Preto and Caverna Santana, both also in PETAR, and Caverna Tapagem or Caverna do Diabo, in Parque Estadual Cavernas do Diabo (PECD)], in Minas Gerais (Buraco do Medo and Lapa Vermelha) and in Paraná (Gruta do Rocha), as well as *P. biseriatus*, with occurrence restricted to the Gruta Olhos d'Água in Minas Gerais; and *Ideoroncus cavicola* (Ideoroncidae), with distribution in Areias de Cima caves, in Gruta do Alambari de Baixo in PETAR and in Gruta de Lacinhas in Paraná, all of which are restricted to the subterranean environment (troglobites).

Additionally, Andrade and Mahnert (2003) described a species of Bochicidae, *Spelaeobochica muchmorei*, with pronounced troglomorphic characteristics, such as elongated appendages (Figure 9L). This species occurs in the Areias System,

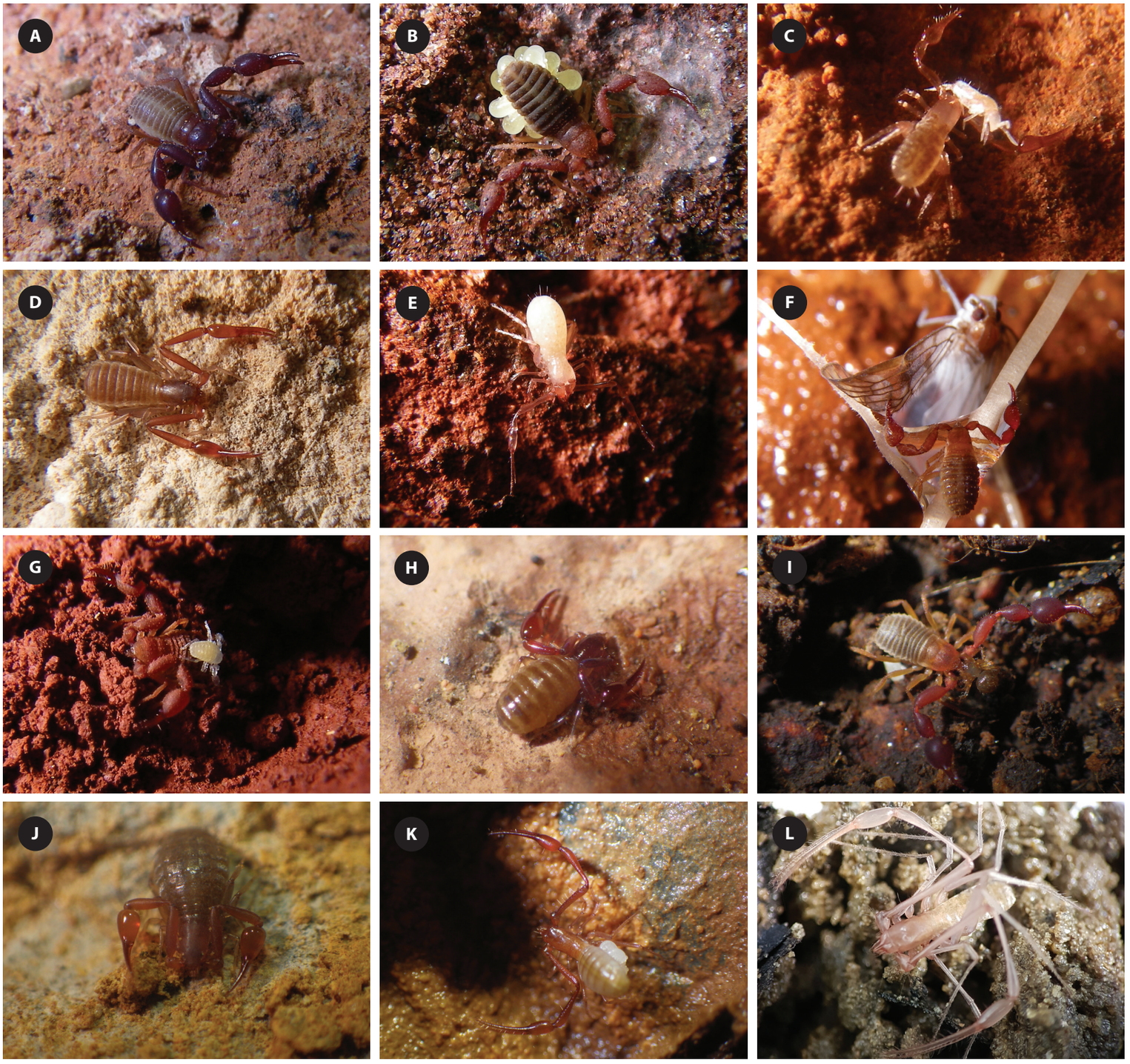


Figure 9. A) Chernetidae; **B)** female of Chernetidae, with egg sac; **C)** pseudoescorpion preying upon a collembolan; **D)** Olpiidae, genus *Progarypus*; **E)** Chthoniidae, genus *Pseudochthonius*; **F)** Chernetidae, holding a specimen of Homoptera (Cixiidae) between roots; **G)** adult of Chernetidae with protonymph; **H)** Pseudoescorpion; **I)** Chernetidae preying upon a spider; **J)** Olpiidae, genus *Progarypus*; **K)** Bochicidae, *Spelaeobochica iuiu*, troglobitic species, with egg sac; **L)** individual of Bochicidae, *Spelaeobochica muchmo*rei, troglobitic species. Photos A-G (Robson de A. Zampaulo); H-K (Lucas M. Rabelo); L (Diego Monteiro von Schimonsky).

in PETAR, in Caverna Areias de Cima and Caverna Ressurgência das Areias de Águas Quentes, and can be considered one of the most modified pseudoscorpions in the world, rivaling another bochicid described in Portugal (*Titanobochica magna* Zaragoza & Reboleira, 2010) (REBOLEIRA *et al.*, 2010).

In turn, *Maxchernes iporangae*, in addition to being a troglobite, is considered a guanobite, that is, it is restricted to patches of guano from bats in Gruta do Alambari de Baixo, in PETAR, and has a single record in Caverna Ressurgência das Areias de Águas Quentes (BICHUETTE *et al.*, 2018a; GNASPINI-NETTO, 1989).

Recently, three more species of the family Bochicidae were described for Brazil and considered exclusive to the subterranean environment: *S. iuiu* Ratton, Mahnert & Ferreira, 2012, occurring in Lapa do Baixão in Iuiu, Bahia; *S. goliath* Viana, Souza & Ferreira, 2018, occurring in Lapa do Baianinho in Ibiricatu, Minas Gerais; and *S. mahnerti* Viana & Ferreira, 2020 from Gruta Velha Nova, in Monjolos, Minas Gerais. *Spelaeochernes popeye* Schimonsky & Bichuette, 2019, of the family Chernetidae, is also considered a troglobite and has been described for several caves on the border of the states of Sergipe and Bahia. In addition to these, *Pseudochthonius ramalho* Assis, Schimonsky & Bichuette, 2021; *P. olegario* Schimonsky, 2022; *P. diamachi* Prado & Ferreira, 2023; *P. koinopoliteia* Prado & Ferreira, 2023 and *P. pali* Prado & Ferreira, 2023, of the family Chthoniidae, were recently described and considered troglobites.

Despite not presenting evident troglomorphisms, the genus *Spelaeochernes* Mahnert, 2001, is considered a troglobitic genus, according to the definition of the ecological-evolutionary classification (TRAJANO, 2012; TRAJANO; DE CARVALHO, 2017). Even with a wide distribution in Brazilian caves, no representatives or populations of this genus have yet been found outside the subterranean environment (BICHUETTE; SCHIMONSKY, 2018b).

Like *Spelaeochernes*, the bochicid species *Spelaeobochica allodentatus* Mahnert, 2001 also does not have evident troglomorphic characteristics, however, it is restricted to the cave environment, occurring in Gruta do Impossível, in Palmeiras, Bahia (BICHUTTE *et al.*, 2018a). Therefore, there are currently 23 species of troglobitic pseudoscorpions in Brazil (Table 1 and Figure 10).

It is also worth mentioning the record of a species of the family Pseudochiridiidae (*Pseudochiridium* aff. *insulae* Hoff, 1964) in a Brazilian cave, in the municipality of São Desidério, Bahia, which is the only record of the family Pseudochiridiidae for the South American continent (SCHIMONSKY *et al.*, 2014).

Main methods of collection and conservation

Pseudoscorpions can be sampled and collected in different ways, with or without the use of traps. They can be captured in pitfall traps, in leaf litter samples and extracted with the aid of Winkler extractors or Berlese-Tüllgreen funnels, or in soil samples using traps for edaphic fauna, for example. Without the use of traps, the collection method is by active search in habitats where these organisms may appear, such as under logs, tree bark, recesses in rock, in the middle of leaf litter, in guano or even exposed on rock in some caves. Thus, when sighting these organisms, manual collection is used with the aid of brushes so as not to damage the specimens. The use of tweezers is also routine; however, excessive pressure exerted on the instrument can damage samples and so they should be used with caution.

These animals can be maintained in the laboratory by using small plastic packages or Petri dishes, for example. It is necessary to keep the environment humid, with substrate preferably from the environment where the animal was collected. In addition, keeping the container closed prevents them from escaping. For food, small flies (*e.g., Drosophila*), termite nymphs and beetle larvae, for example, can be offered. Shortly after feeding, or not, the terrarium should be cleaned to prevent the growth of fungi, which can lead to the death of pseudoscorpions (ANDRADE, 1999; WEYGOLDT, 1969).

For preservation, specimens can be stored in 70% ethanol with the addition of 5% glycerin, which reduces the rigidity of the animal's joints after fixation. Thus, the animal can be studied with the aid of a stereomicroscope and/or optical microscope, after first clearing of the specimen to verify internal structures, such as spermatheca, for example. Clearing can be done with different methodologies that will reduce the sclerotization of the animal, such as the use of lactic acid. The time of exposure to the agents will depend on the degree of sclerotization and can vary from hours to days.

The animals can then be dismembered for better observation of structures and measurements. For this, a chelicera, a leg I and a leg IV and a pedipalp are removed, usually all on the same side of the body. These parts are placed on a microscope slide and covered with a coverslip resting on a 0.05mm or larger nylon thread so as not to crush the structures. In this way, it is possible to move them without having to reassemble the slide. After studying a specimen, it must be placed in small plastic or glass bottles, including its structures, preferably not loose but in a smaller bottle in 70% ethanol and duly labeled.

Table 1. Troglobitic species of pseudoscorpions registered for Brazil.

Family	N	Species	Type Locality	Municipality State	Coordinates WGS84
Chthoniidae	1.	*Pseudochthonius strinatii* Beier,1969	Caverna Areias de Cima	Iporanga SP	S24° 33' 26.9" W48° 40' 36.5"
	2.	*Pseudochthonius biseriatus* Mahnert, 2001	Gruta Olhos d'água	Januária MG	S15° 10' 48.1" W44° 12' 18.7"
	3.	*Pseudochthonius ramalho* Assis, Schimonsky & Bichuette, 2021	Gruna do Vandercir	Serra do Ramalho	S13°38'11.40" W43°50'5.10"
	4.	*Pseudochthonius olegario* Schimonsky, 2022	Lapa Zé de Sidnei	Presidente Olegário - MG	S18° 18' 05.6" W46° 05' 40.6"
Bochicidae	5.	*Spelaeobochica allodentatus* Mahnert, 2001	Gruta do Impossível	Palmerias BA	S12° 30' 53.3" W41° 34' 30.0"
	6.	*Spelaeobochica muchmorei* Andrade & Mahnert, 2003	Ressurgência das Areias de Águas Quentes	Iporanga SP	S24° 33' 26.9" W48° 40' 36.5"
	7.	*Spelaeobochica iuiu* Ratton, Mahnert & Ferreira, 2012	Lapa do Baixão	Iuiu BA	S14° 24' 44.4" W43° 33' 03.5"
	8.	*Spelaeobochica goliath* Viana, Souza & Ferreira, 2018	Lapa do Baianinho	Ibiracatu MG	S15°45'56.2" W44°14'02.9"
	9.	*Spelaeobochica mahnerti* Viana & Ferreira, 2020	Caverna Velha Nova	Monjolos - MG	S18°16'36.2" W44°06'07.9"
Ideoroncidae	10.	*Ideoroncus cavicola* Mahnert, 2001	Caverna Areias de Cima	Iporanga SP	S24° 33' 26.9" W48° 40' 36.5"
Chernetidae	11.	*Maxchernes iporangae* Mahnert & Andrade, 1998	Gruta Alambari deBaixo	Iporanga SP	S24° 33' 26.9" W48° 40' 36.5"
	12.	*Spelaeochernes altamirae* Mahnert, 2001	Caverna do Limoeiro	Medicilândia PA	S3° 26' 41.5" W52° 53' 24.7"
	13.	*Spelaeochernes armatus* Mahnert, 2001	Gruta do Botuverá	Botuverá SC	S27° 11' 53.6" W49° 04' 22.8"
	14.	*Spelaeochernes bahiensis* Mahnert, 2001	Poço Encantado	Itaetê BA	S12° 58' 58.9" W40° 58' 01.5"
	15.	*Spelaeochernes dentatus* Mahnert, 2001	Caverna Água Boa	Almirante Tamandaré PR	S25° 18' 29.5" W49° 17' 41.7"
	16.	*Spelaeochernes dubius* Mahnert, 2001	Gruta da Fazenda da Toca	Analândia SP	S22° 07' 36.9" W47° 39' 52.0"
	17.	*Spelaeochernes eleonorae* Mahnert, 2001	Caverna Sertãozinho de Baixo	Altinópolis SP	S21° 01' 31.5" W47° 22' 27.8"
	18.	*Spelaeochernes gracilipalpus* Mahnert, 2001	Gruta dos Paiva	Iporanga SP	S24° 33' 26.9" W48° 40' 36.5"
	19.	*Spelaeochernes pedroi* Mahnert, 2001	Gruta Milagrosa	Pau Brasil BA	S15° 27' 53.3" W39° 39' 07.0"
	20.	*Spelaeochernes popeye* Schimonsky & Bichuette, 2019	Caverna Toca da Raposa	Laranjeiras SE	S10° 48' 17.5" W37° 09' 59.3"

P. diamachi Prado & Ferreira, 2023, *P. koinopoliteia* Prado & Ferreira, 2023 and *P. pali* Prado & Ferreira, 2023 were described after the publication of this book in Portuguese.



Figure 10. Map with the type localities for troglobitic species of pseudoscorpions described for Brazil.

Identification key

Identification key for families that occur in Brazil (HARVEY, 1992; MAHNERT *et al.*, 1986).

1. Forelegs with one tarsal article, hindlegs with two; pedipalp chela fingers without venom apparatus; trichobothria *dx* present..... **2**

1´. Forelegs and hindlegs with same number of tarsal articles; presence of venom apparatus in at least one of the fingers of the pedipalp chela; absence of trichobothria *dx*..... **4**

2. Anterior margin of carapace with lobes; opisthosoma with lateral plates; pedipalps with raptorial form..... **Feaellidae**

2´. Anterior margin of carapace without lobes; opisthosoma without lateral plates; pedipalps non-raptorial **3**

3. *Rallum* with bristles arranged in a straight line, with curved penultimate bristle; trichobothria *eb* and *esb* on dorsum of hand of pedipalp chela..... **Lechytiidae**

3´. *Rallum* with bristles not arranged in a straight line, arising from an elliptical areola; trichobothria *eb* and *esb* on base of fixed finger..... **Chthoniidae**

4. Legs with two tarsal articles..... **5**

4'. Legs with one tarsal article..... **10**

5. Femur of leg IV shorter than patella, with diagonal suture between the two; carapace narrowing anteriorly (cucule), and anterior margin of carapace without epistome..... **6**

5'. Femur and patella of leg IV approximately the same length, with vertical suture between the two; carapace without formation of cuculo, and anterior margin of carapace with epistome **8**

6. Carapace subtriangular; opisthosoma suboval; absence of trichobothria on pedipalp femur; *rallum* with 1 bristle ..... **Geogarypidae**

6'. Carapace subrectangular; opisthosoma subrectangular; 1–2 trichobothria may occur on the pedipalp femur; *rallum* with more than one bristle..... **7**

7. Venom ducts short, do not go beyond trichobothria *et* ..... **Olpiidae**

7'. Venom ducts long..... **Hesperolpiidae**

8. Trichobothrium *t* lanceolate ..... **Syarinidae**

8'. Trichobothrium *t* acuminate..... **9**

9. One pair of eyes, greater number of trichobothria on chela, pedipalp femur without tubercle..... **Ideoroncidae**

9'. Eyes absent, normal number of trichobothria on chela, pedipalp femur with tubercle ..... **Bochicidae**

10. Carapace subtriangular; opisthosoma suboval..... **11**

10'. Carapace subrectangular; opisthosoma subrectangular **12**

11. Posterior region of carapace without circular depression; tergites chevron shaped; coxa IV very elongated posteriorly..... **Pseudochiridiidae**

11'. Posterior region of carapace with circular depression; tergites chevron shaped; coxa IV not elongated posteriorly..... **Cheiridiidae**

12. Femur/patella junction I and II perpendicular; sternites of males with discrete fields of sensory bristles ..... **Withiidae**

12'. Femur/patella junction I and II oblique; sternites of males without discrete fields of sensory bristles **13**

13. Poison apparatus present in both pedipalp fingers; males with coxal sacs and "ram's horn" organ..... **Cheliferidae**

13'. Poison apparatus present in only one of the pedipalp fingers; males without coxal sacs and without "ram's horn" organ..... **14**

14. Pedipalp fingers with at least one accessory tooth; poison apparatus only on movable finger .....**Chernetidae**
14'. Pedipalp fingers without accessory teeth; poison apparatus only on fixed finger..... **Atemnidae**

Main Brazilian collections

The main collections with specimens of pseudoscorpions of Brazil are those of the Museu de Zoologia da Universidade de São Paulo (MZUSP) and the Instituto Nacional de Pesquisas da Amazônia (INPA). An important collection was that of the Museu Nacional do Rio de Janeiro (MNRJ), which was unfortunately lost in a fire in 2018. The collection of Laboratório de Estudos Subterrâneos da Universidade Federal de São Carlos (LES/UFScar) is an important collection especially for subterranean fauna. Other collection related with subterranean fauna is Centro de Estudos em Biologia Subterrânea da Universidade Federal de Lavras (CEBS/UFLA).

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VERTE

BRATES



Epicrates cenchria
Thiago dos Santos

24

Amphibians and Reptiles

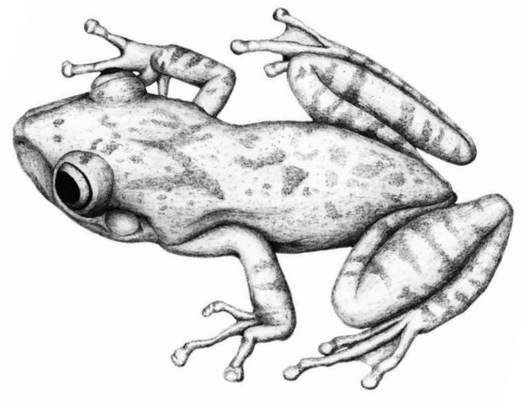
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General introduction

The initial records of the presence of herpetofauna in caves were made in Europe and North America, with the first reference dating back to the year 1689, when Baron Janez Vajkard Valvasor (also known as Johann Weikhard Freiherr von Valvasor) comments in his book "The Glory of the Duchy of Carniola", the existence of a "dragon" inhabiting underground lakes in Slovenia (TRAJANO; BICHUETTE, 2006). It was a salamander (*Proteus anguinus*), which was later described by Laurenti in 1768 and represents a milestone for subterranean biology, as it was the first endemic species of this environment to receive a formal description (TRAJANO; BICHUETTE, 2006). Later, in the 1950s, five species of frogs, three species of salamanders and two species of snakes were recorded in caves in the United States (BARR, 1953; BLAIR, 1951; BRODE, 1958; RAND, 1950).

Historically, salamanders, as they include troglobitic species, have been more reported and studied in caves than other amphibians and reptiles in general (BESHARSE; HOLSINGER, 1977; BRUCE, 1979; GORI£KI *et al.*, 2019; MILLER; NIEMILLER, 2008). Fossil records demonstrate that these animals already used caves during the Pleistocene (BLAIN *et al.*, 2008; CRUZ *et al.*, 2016). This behavior probably originated as a result of the use of caves as shelters/refuge during periods of climatic extremes (glaciations) representing, therefore, behavioral thermoregulation (DOWLING, 1956; FENOLIO *et al.*, 2005; RESETARITS JR., 1986). Carrying out studies that address the presence of amphibians and reptiles (in addition to salamanders) in caves has only recently become more frequent. Some of these studies involved only occasional collections or records, while others involved some type of monitoring and identification of troglobitic and troglophilous species (BISWAS, 2014; KÖHLER *et al.*, 2010; MATAVELLI *et al*., 2015; NIEMILLER; MILLER, 2009; SUWANNAPOOM *et al.*, 2018).

Herpetofauna and and the cave environment

Brazil currently has 1,188 species of amphibians and 848 species of reptiles (COSTA *et al*., 2021; SEGALLA *et al.*, 2021), one of the richest herpetofaunas and one of the most diverse speleological heritages in the world. However, the presence of herpetofauna in caves in the country has been neglected for decades (MATAVELLI *et al.*, 2015). The first reports emerged in the 1980s, in publications that addressed synopses of Brazilian cave fauna, generally restricted to the family or genus level, with few records at the species level (DESSEN *et al.*, 1980; PINTO-DA -ROCHA, 1995; TRAJANO, 1987; TRAJANO; GNASPINI-NETO, 1991). Such records, although initially important, occurred punctually and did not involve any type of monitoring or delve into the ecological and behavioral aspects of the use of the cave environment.

Historically, the lack of more consistent studies on the subject in Brazil helped, until recently, to spread the false impression that the occurrence of reptiles and amphibians in Brazilian caves happened, mainly, accidentally. This fact can be clearly observed in a large part of the synoptic studies of Brazilian cave fauna and, mainly, in the great majority of the environmental impact studies of recent decades that, when mentioning some record of the herpetofauna in caves, are limited to classification at higher taxonomic levels, without giving due importance. Although still rare in the scientific community, there are reports of certain behaviors practiced by different species in this type of environment, such as predation, vocalization and even signs of reproduction (DONATO *et al.*, 2012; GOUVEIA *et al*., 2009; LIMA *et al.*, 2012). Such records started to show that the search for the subterranean environment can be motivated and not merely accidental.

Some recent publications have revealed the diversity of anurans and reptiles in Brazilian caves. Matavelli *et al.* (2015) recorded 54 species of anuran amphibians in 223 caves sampled in eleven Brazilian states, three biomes and seven lithologies. Santos *et al.* (2022) sampled the herpetofauna of 269 caves located in six lithologies in four Brazilian states, with two seasonal collections (dry and rainy), obtaining records of 53 species, 34 of anuran amphibians and 19 of reptiles. However, a study carried out with the species *Bokermannohyla martinsi* Bokermann, 1964 (Figure 1A) was the first to conduct seasonal

monitoring of a herpetofauna taxon in a Brazilian subterranean environment, with information at the individual level regarding fidelity to the caves (ANDRADE *et al.*, 2021).

New studies have been developed in recent years focusing on the behavioral ecology of amphibians and reptiles in Brazilian caves, including systematic seasonal monitoring, seeking to show the importance of these environments for some species (ANDRADE *et al.*, 2021; BERNARDI *et al.*, 2017; CAMPOS *et al.*, 2017). However, this approach can still be considered incipient in Brazil, where information about the presence and relationship of herpetofauna with natural subterranean cavities needs to be better clarified. The available data still do not even allow the assertive classification of most species as troglophilous or trogloxenous, for example.

On the other hand, studies carried out outside Brazil, such as in Mexico, the United States, India and Italy, have shown that some frog species can be common in caves, exploiting them regularly or selecting them based on specific environmental characteristics (BISWAS, 2014; ESPINO DEL CASTILHO *et al.,* 2009; FENOLIO *et al.*, 2005; KÖHLER *et al.*, 2010; LUNGHI *et al.*, 2014). Likewise, although most reptiles are considered accidental, some species may seek refuge in caves during hot and dry periods (NIEMILLER; MILLER, 2009). Salamanders remain the only herpetofaunal group that has known troglobitic species (NIEMILLER *et al.*, 2016). However, several studies have shown that some species of reptiles and amphibians are trogloxenes or even troglophiles, which demonstrates different levels of dependence of these species in relation to the subterranean environment (ESPINO DEL CASTILHO *et al.,* 2009; FENOLIO *et al*., 2005; NIEMILLER *et al*., 2016; NIEMILLER; MILLER, 2009; RESETARITS JUNIOR, 1986; ROGOWITZ *et al.*, 2001; SUWANNAPOOM *et al.*, 2018).

Reduced predation rates, high humidity, relative environmental stability and food availability are some of the characteristics of caves that can attract different species (opportunistic or not) to exploit these environments (ANDRADE *et al.*, 2021; BISWAS, 2014). Thus, some species may use caves as food sources and places for shelter, hibernation, reproduction and protection against predators (ANDRADE *et al.*, 2021; BISWAS, 2014; BROWN, 1984; MATAVELLI *et al.*, 2015; TRAJANO; BICHUETTE, 2006).

However, an important component of the ecological niche of an ectothermic species (which includes all species of reptiles and amphibians) is the availability of environments where they can carry out behavioral thermoregulation (POUGH *et al.*, 2008). For amphibians, there is also dependence on environments with high humidity due to the need to maintain water homeostasis in the skin (POUGH *et al.*, 2008). Thus, caves serve as a refuge against unfavorable external environmental conditions (BISWAS, 2014) because they generally have high humidity and mild and stable temperatures in areas farther from the entrance (FERREIRA, 2005).

In temperate regions, caves can be used by frogs in summer to avoid dehydration caused by heat and low humidity (BARR, 1953; PRATHER; BRIGGLER, 2001), as well as to avoid mortality caused by temperature extremes in winter (RESETARITS JUNIOR, 1986; RESETARITS JUNIOR; ALDRIGER, 1988). In these regions, frogs are recorded in greater numbers in caves during autumn and winter, periods when external environmental conditions are very severe (FENOLIO *et al.*, 2005; LUNGHI *et al.*, 2014; RESETARIST JUNIOR, 1986). Such organisms hibernate or reduce activity during these periods (ROSA; PENADO, 2013). In arid and semi-arid regions, the colonization of caves by anurans is related to the search for milder temperatures and higher relative humidity than that observed in surface environments (BROWN, 1984; FELLERS *et al.*, 2010; GOUVEIA *et al.*, 2009; TRAJANO; BICHUETTE, 2006), allowing frogs to escape or mitigate the effects of adverse weather (ARZABE, 1999; BENTLEY, 1966).

The morphology of cave entrances seems to be another factor related to the colonization and composition of cave herpetofauna (BLAIN *et al.*, 2008). In sites with vertical entry, squamates (mainly lizards and geckos) prevail as representatives of the herpetofauna, while in sites with predominantly horizontal entry, frogs stand out (BLAIN *et al.*, 2008); but an attractive and favorable environment for the colonization of caves is not enough. Behavioral adjustments, phenotypic variations and

morphological and physiological adaptations are also necessary for the success of these species in the subterranean environment (BISWAS, 2009; 2010). In the case of frogs, the nocturnal habit and the presence of digital discs and interdigital membranes are some of the behavioral and morphological adaptations common to the most frequent species in caves (ANDRADE *et al.*, 2021; BISWAS, 2014; LURÍA-MANZANO; RAMÍREZ-BAUTISTA, 2017).

Exploitation of caves as breeding sites, although well reported among salamanders, seems uncommon and is recorded for a small number of species of anuran amphibians (FARAONE; VALVO, 2018). However, reproduction in caves, when recorded, suggests that these environments are effectively exploited by these species (FARAONE; VALVO, 2018). Reports of herpetofauna foraging in caves also demonstrate adaptation to the subterranean environment. In addition to participating in the food chain as predators (FENOLIO *et al.,* 2005) and, sporadically, as prey, these animals can also, in some cases, contribute energy to cave environments, which are generally poor in energy, with their feces and corpses (RESETARITS JUNIOR, 1986).

Although studies involving the presence of herpetofauna in caves have a way to evolve in several countries, the results obtained so far in recent years have led to an increase in knowledge about the biology and ecology of several species, including even the description of new species (CANEDO *et al.*, 2012; SUWANNAPOOM *et al.*, 2018). The records cited here show the importance and the need to properly assess the reasons for the true association of herpetofauna species with caves and the different specializations of these species to the subterranean environment. The advance of the destruction of external habitats means that caves can become important shelters for some species, which further justifies the need to better understand these associations. Obviously, effective conservation and management measures for caves depend on broader knowledge that includes all their diversity in general (FERREIRA *et al.*, 2015; GIBERT; DEHARVENG, 2002; SOUZA-SILVA *et al.*, 2011).

Therefore, in view of the increasing anthropization of natural environments and a greater demand for natural resources, knowledge about the biology of species can help in conservation actions for herpetofauna and speleological heritage, minimizing the impact on affected animal populations and the destruction of caves. Thus, in the next items we present an extensive review of available information on the occurrence, biology and ecology of reptiles and amphibians in Brazilian caves, while intending to stimulate new studies involving the herpetofauna present in this type of environment. It is important to point out that several species of reptiles and amphibians recorded in caves are endemic to a region, little known and/or threatened with extinction, conditions that in themselves should be taken into account for their conservation.

Main groups found in Brazilian caves

We present here a compilation of records acquired over a period of 22 years (1999 to 2021), including occasional data collections and more intense seasonal studies. The data represent a compilation of Andrade *et al.* (2021), Matavelli *et al.* (2015) and Santos et *al.* (2022), in addition to unpublished records of the authors of this chapter and contributions from other collaborators. Despite different sources, the methodology employed consisted, in all cases, of censuses by visual encounters via active search throughout the entire length of caves, including a thorough inspection of the floor, walls and, when possible, the ceiling, when these were accessible. Furthermore, all inventories were carried out during the day.

However, it is worth mentioning that in more recent studies, in addition to active searches, auditory transections (Audio Strip Transect - AST) were also used to investigate vocalization behaviors of anuran amphibians (SANTOS *et al.*, 2022). These methods complement each other (CRUMP; SCOTT JUNIOR, 1994; ZIMMERMAN, 1994) and aim at diagnosing patterns of relative abundance of species in a short period of time. The nomenclature adopted for amphibians follows the provisions of Frost (2020) and for reptiles those of UETZ (2020).

As a result, records were obtained in 534 caves in 13 states: Amazonas (1), Bahia (21), Ceará (9), Espírito Santo (4), Goiás (1), Pará (229), Minas Gerais (241), Mato Grosso (13), Rio Grande do Norte (6), Rio de Janeiro (1), São Paulo (1), Sergipe (5) and Tocantins (2). These caves are inserted in seven lithologies: iron ore (330), limestone (149), granite (18), quartzite (19), sandstone (12), conglomerate (5) and marble (1), and are located in four biomes: Amazon, Cerrado, Caatinga and Atlantic Forest, in addition to areas in the ecotone between Cerrado and Atlantic Forest. There is evident disparity in records by state and even by lithologies, showing gaps in knowledge.

Regarding exclusive records by biome, the Amazon had

25 species, 16 frogs and nine reptiles (four lizards and five snakes); the Cerrado had 17 species, 12 frogs and five reptiles (three lizards and two snakes); the Atlantic Forest had nine species, eight frogs and one snake; and the Caatinga had ten species, seven frogs, one caecilian and two snakes. The ecotone areas between Cerrado and Atlantic Forest had 50 species, with 31 species of frogs and 19 of reptiles (five lizards and 14 snakes).

It should be noted that, so far, no species of Urodela has been recorded in Brazilian caves, despite the occurrence of salamanders of the genus *Bolitoglossa* in the north of the country. Among reptiles, there are no published records of amphisbaenians, chelonians and crocodilians in Brazilian caves. However, Alves (2007) reported the occurrence of a species of crocodilian (*Paleosuchus* cf. *trigonatus*) (Figure 2F) and a chelonian (*Podocnemis* sp.) near the entrance region of a cave in the state of Amazonas. Although rare, there are records of the use of natural subterranean cavities as shelter for a population of *Crocodylus niloticus* Laurenti, 1768 in Africa (WILSON, 1987) and for chelonians in Tennessee, North America (NIEMILLER et al., 2016). In Brazil, there are records of jacarés (caimans) in caves in the northern region of the country, especially in environmental licensing studies, however, these data are not available in the scientific literature.

Regarding herpetofauna, 121 species have been recorded in Brazilian caves (Table 1), 83 species of amphibians (82 species of frogs and one species of Gymnophiona) and 38 species of Squamata (13 species of lizards and 25 species of snakes). For amphibians, 22 genera were found, distributed in 13 families: Aromobatidae, Brachycephalidae, Bufonidae, Craugastoridae, Cycloramphidae, Dendrobatidae, Hylidae, Hylodidade, Microhylidae, Phyllomedusidae, Pipidae, Leptodactylidae and Siphonopidae. For Squamata, 25 genera (nine of lizards and 16 of snakes) were registered, distributed in 10 families, five of which are lizards (Gekkonidae, Gymnophthalmidae, Phyllodactylidae, Teiidae and Tropiduridae) and five are snakes (Boidae, Colubridae, Dipsadidae, Viperidae and Elapidae) (Table 1).

The family Hylidae had the greatest richness of genera and species (six genera and 17 species), which is not surprising, since it is the most diverse family of anurans, with 373 species described so far for Brazil (SEGALLA *et al.*, 2021). In addition, it is worth mentioning that hylids have morphological and behavioral adaptations that may facilitate the occupation of caves due to their arboreal or semi-arboreal habits, thus occupying a greater amount of extracts and microenvironments. Next, Brachycephalidae (one genus and 12 species) and Bufonidae (two genera and 11 species) presented greater richness. In addition, it is worth mentioning that hylids have morphological and behavioral adaptations that may facilitate the occupation of caves due to their arboreal or semi-arboreal habits, thus occupying a greater amount of extracts and microenvironments. Next, Brachycephalidae (one genus and 12 species) and Bufonidae (two genera and 11 species) presented greater richness. In addition to these, a species of caecilian was found, being the only representative of the order Gymnophiona. Among squamates, the snake family Dipsadidae was the most representative, with nine genera and 12 species recorded.

The ecotone areas of the Cerrado/Atlantic Forest and the Amazon biome showed greater species richness, which is probably due to the fact that they have been more sampled in terms of the number of caves inventoried. Ecotone areas between Cerrado and Atlantic Forest had the highest number of species, with 36 frogs and 20 squamates (Table 1). The greater diversity of phytophysiognomies found in these areas, such as montane seasonal semi-deciduous forest, seasonal deciduous forest, campo rupestre, campo limpo, cerrado sensu stricto and cerradão, among others, may also favor the occurrence of species exclusive to the Atlantic Forest and Cerrado, as well as species common to both biomes.

Among the sampled lithologies, iron ore caves had the greatest richness of herpetofauna, probably because they are located in the Amazon (mainly in the state of Pará) and in the Quadrilátero Ferrífero (state of Minas Gerais), both being regions with high diversity of species, many of which are endemic to these biomes. Despite generally having a relatively reduced extension in relation to other lithologies (OLIVEIRA *et al.*, 2011), natural subterranean cavities in iron ore also present high climatic stability (FERREIRA, 2005), offering favorable conditions for the thermoregulation of these animals. In addition, since, in general, most species found in iron caves are positioned in areas close to the entrances (euphotic and dysphotic zones), the small size of these caves would not be a limiting factor for the use of these environments. Furthermore, the diversity of microenvironments peculiar to this lithology can stimulate the search for daytime shelters or foraging sites by many species of amphibians and reptiles (SANTOS *et al.*, 2022).

Table 1: Species of herpetofauna recorded in 530 sampled caves in seven lithologies and four biomes of Brazil. "Ecotone" refers to areas of transition between the Atlantic Forest and Cerrado. Information obtained from Andrade *et al.* (2021), Matavelli *et al.* (2015) and Santos *et al.* (2022).

Amphibia: Anura			
Family	**Species**	**Species**	**Biome**
Aromobatidae	*Allobates* gr. *marchesianus* (Melin, 1941)	Iron	Amazon
	Allobates sp.	Iron	Amazon
	Allobates sp.1	Iron	Amazon
	Allobates sp.2	Iron	Amazon
Brachycephalidae	*Ischnocnema guentheri* (Steindachner, 1864)	Iron	Ecotone
	Ischnocnema izecksohni (Caramaschi & Kisteumacher, 1989, 1988)	Limestone	Ecotone
	Ischnocnema juipoca (Sazima & Cardoso, 1978)	Iron, Limestone, Conglomerate, Quartzite	Ecotone
	Ischnocnema surda (Canedo, Pimenta, Leite & Caramaschi, 2010)	Limestone, Iron	Ecotone
	Ischnocnema sp.	Sandstone	Cerrado
	Ischnocnema sp. 1	Limestone	Cerrado
	Ischnocnema sp. 2	Granite	Atlantic Forest
	Ischnocnema sp. 3	Quartzite	Ecotone
	Ischnocnema sp. 4	Quartzite	Ecotone
	Ischnocnema sp. 5	Iron	Ecotone
	Ischnocnema sp. 6	Iron, Limestone	Ecotone
	Ischnocnema sp. 7	Limestone	Ecotone
Bufonidae	*Rhaebo guttatus* (Schneider, 1799)	Iron, Sandstone, Quartzite	Amazon
	Rhinella marina (Linnaeus, 1758)	Iron	Amazon
	Rhinella ornata (Spix, 1824)	Iron, Quartzite	Ecotone
	Rhinella rubescens (A. Lutz, 1925)	Iron, Quartzite	Ecotone
	Rhinella diptycha (Cope, 1862)	Limestone, Sandstone	Ecotone, Cerrado
	Rhinella crucifer (Wied-Neuwied, 1821)	Granite	Atlantic Forest
	Rhinella granulosa (Spix, 1824)	Limestone	Caatinga
	Rhinella aff. *magnussoni*	Iron	Amazon
	Rhinella jimi (Stevaux, 2002)	Limestone	Caatinga
	Rhinella sp.1	Limestone	Atlantic Forest
	Rhinella sp.2	Limestone	Atlantic Forest
Craugastoridae	*Haddadus binotatus* (Spix, 1824)	Quartzite	Ecotone
	Pristimantis fenestratus (Steindachner, 1864)	Conglomerate, Iron	Amazon
	Pristimantis sp.	Sandstone	Cerrado

	Thoropa miliaris (Spix, 1824)	Iron, Quartzite, Granite, Marble	Ecotone, Atlantic Forest
Cycloramphidae	*Thoropa megatympanum* Caramaschi & Sazima, 1984	Iron	Ecotone
	Proceratophrys boiei (Wied-Neuwied, 1824)	Iron	Atlantic Forest
	Proceratophrys sp.	Iron	Amazon
	Adelphobates galactonotus (Steindachner, 1864)	Iron	Amazon
Dendrobatidae	*Ameerega* aff. *berohoka*	Granite	Cerrado
	Ameerega flavopicta (A. Lutz, 1925)	Iron, Sandstone	Amazon
	Trachycephalus nigromaculatus Tschudi, 1838	Limestone	Ecotone
	Bokermannohyla alvarengai (Bokkermann, 1956)	Iron, Quartzite	Ecotone
	Bokermannohyla nanuzae (Bokkermann & Sazima, 1973)	Limestone, Iron	Ecotone
	Aplastodiscus cavicola (Cruz & Peixoto, 1985)	Limestone, Iron	Ecotone
	Bokermannohyla martinsi (Bokermann, 1964)	Limestone, Iron, Quartzite	Ecotone
	Bokermannohyla saxicola (Bokermann, 1964)	Iron	Ecotone
	Bokermannohyla sp.	Iron	Ecotone
	Bokermannohyla sp.1	Iron	Ecotone
	Scinax eurydice (Bokermann, 1968)	Limestone	Ecotone
Hylidae	*Scinax fuscovarius* (A. Lutz, 1925)	Sandstone, Conglomerate, Iron, Limestone, Granite, Quartzite	Caatinga, Ecotone
	Scinax perereca Pombal, Haddad & Kasahara, 1995	Iron, Limestone	Ecotone
	Scinax ruber (Laurenti, 1768)	Iron	Ecotone
	Scinax aff. *ruber*	Sandstone, Conglomerate	Cerrado, Ecotone
	Dendropsophus aff. *nanus*	Sandstone	Cerrado
	Boana albopunctata (Spix, 1824)	Limestone	Ecotone
	Boana aff. *boans*	Iron	Amazon
	Boana faber (Wied, 1821)	Quartzite	Ecotone
Hylodidae	*Hylodes otavioi* Sazima & Bokermann, 1983, 1982	Iron	Ecotone
	Hylodes sp.	Conglomerate	Atlantic Forest
Phyllomedusidae	*Phyllomedusa burmeisteri* Boulenger, 1882	Limestone	Atlantic Forest
Pipidae	*Pipa* aff. *pipa*	Iron	Amazon
Microhylidae	*Dermatonotus muelleri* (Boettger, 1885)	Limestone	Caatinga

Leptodactylidae	*Leptodactylus labyrinthicus* (Spix, 1824)	Conglomerate, Iron, Limestone	Ecotone, Cerrado
	Leptodactylus syphax Bokermann, 1969	Limestone, Iron	Amazon, Caatinga
	Leptodactylus macrosternum Miranda-Ribeiro, 1926	Limestone	Caatinga, Cerrado
	Leptodactylus mystacinus (Burmeister, 1861)	Iron, Limestone	Cerrado
	Leptodactylus pentadactylus (Laurenti, 1768)	Iron	Amazon
	Leptodactylus troglodytes A.Lutz, 1926	Limestone	Caatinga
	Leptodactylus vastus A.Lutz, 1930	Limestone	Cerrado
	Leptodactylus sp.	Limestone	Ecotone
	Leptodactylus sp. 1	Limestone	Caatinga
	Leptodactylus sp. 2	Iron	Amazon
	Leptodactylus sp. 3	Iron	Amazon
	Leptodactylus sp. 4	Limestone	Caatinga
	Physalaemus erythros (Caramaschi, Feio & Guimarães-Neto, 2003)	Iron	Ecotone
	Physalaemus cuvieri Fitzinger, 1826	Iron, Limestone	Ecotone, Caatinga
	Physalaemus nattereri (Steindachner, 1863)	Iron	Cerrado
	Physalaemus sp.	Sandstone	Cerrado
	Physalaemus sp. 1	Sandstone	Cerrado
	Physalaemus sp. 2	Limestone	Cerrado
	Physalaemus sp. 3	Iron	Ecotone
	Physalaemus sp. 4	Quartzite	Ecotone
	Physalaemus sp. 5	Conglomerate	Atlantic Forest
	Physalaemus sp. 6	Limestone	Caatinga
	Physalaemus sp. 7	Sandstone	Cerrado
Gymnophiona			
Siphonopidae	*Siphonops* sp.	Limestone	Caatinga

Note: The records identified only at the genus level do not necessarily correspond to new species but are due to the lack of the collection of specimens for identification to species.

Reptilia: Squamata			
Familia	**Espécie**		
Gekkonidae	*Hemidactylus mabouia* (Moreau de Jonnès, 1818)	Limestone	Cerrado, Ecotone
Gymnophthalmidae	*Potamites ecpleopus* (Cope, 1875)	Iron	Amazon
Phyllodactylidae	*Gymnodactylus darwinii* (Gray, 1845)	Limestone, Iron	Ecotone
	Phyllopezus pollicaris (Spix, 1825)	Sandstone	Cerrado
	Thecadactylus rapicauda (Houttuyn, 1782)	Iron	Amazon
Teiidae	*Salvator merianae* Duméril & Bibron, 1839	Limestone	Ecotone
	Ameiva ameiva (Linnaeus, 1758)	Iron	Ecotone
Tropiduridae	*Plica plica* (Linnaeus, 1758)	Iron	Amazon
	Tropidurus torquatus (Wied, 1820)	Limestone	Ecotone
	Tropidurus sp.	Limestone, Iron	Ecotone
	Tropidurus semitaeniatus (Spix, 1825)	Sandstone	Cerrado
	Tropidurus hygomi Reinhardt & Lutken, 1862	Sandstone	Cerrado
	Tropidurus oreadicus Rodrigues, 1987	Iron	Amazon
Boidae	*Epicrates crassus* Cope, 1862	Limestone	Ecotone
	Epicrates assisi Machado, 1945	Limestone	Cerrado
	Epicrates cenchria (Linnaeus, 1758)	Iron	Amazon
Colubridae	*Mastigodryas boddaerti* (Sentzen, 1796)	Iron	Amazon
	Clelia sp.	Limestone	Cerrado
	Spilotes pullatus (Linnaeus, 1758)	Iron/Limestone	Amazon, Cerrado
Dipsadidae	*Atractus pantostictus* Fernandes & Puorto, 1994	Limestone	Ecotone
	Dipsas mikanii (Schlegel, 1837)	Limestone	Ecotone
	Erythrolamprus aesculapii (Linnaeus, 1766)	Limestone	Ecotone
	Erythrolamprus poecilogyrus (Wied, 1824)	Limestone	Ecotone
	Erythrolamprus sp.	Iron	Ecotone
	Oxyrhopus guibei Hoge & Romano, 1978	Limestone	Ecotone
	Pseudablabes patagoniensis (Girard, 1858)	Limestone	Ecotone
	Xenopholis undulatus (Jensen, 1900)	Iron	Ecotone
	Pseudablabes patagoniensis (Lichtenstein, 1823)	Iron	Amazon
	Leptodeira annulata (Linnaeus, 1758)	Iron	Amazon
	Xenodon merremii (Wagler, 1824)	Iron	Ecotone
	Thamnodynastes strigatus (Günther, 1858)	Granite	Atlantic Forest
Viperidae	*Bothrops jararaca* (Wied, 1824)	Limestone	Ecotone
	Bothrops neuwiedi Wagler, 1824	Iron	Ecotone
	Bothrops moojeni Hoge, 1966	Limestone	Ecotone
	Bothrops erythromelas Amaral, 1923	Limestone	Caatinga
	Bothrops taeniatus Wagler, 1824	Iron	Amazon
	Crotalus durissus Linnaeus, 1758	Limestone	Ecotone
Elapidae	*Micrurus* sp.	Limestone	Caatinga



Figure 1. A) *Bokermannohyla martinsi* (Hylidae); **B)** *Bokermannohyla saxicola* (Hylidae); **C)** *Scinax fuscovarius* (Hylidae); **D)** *Thoropa miliaris* (Cycloramphidae); **E)** *Pristimantis fenestratus* (Craugastoridae); **F)** *Physalaemus cuvieri* (Leptodactylidae); **G)** *Leptodactylus labyrinthicus* (Leptodactylidae); **H)** *Rhinella diptycha* (Bufonidae); **I)** *Rhinella marina* (Bufonidae); **J)** *Ischnocnema juipoca* (Brachycephalidae); **K)** *Rhaebo gutattus* (Bufonidae); **L)** *Leptodactylus mystacinus* (Leptodactylidae). Photos A-F, J-K (Robson de A. Zampaulo); G-I, L (Thiago dos Santos).

ANURANS

Anurans recorded in Brazilian caves include both widely distributed species and endemic species of different Brazilian biomes. Some species show a recurrence in the cave environment, remaining there for long periods, especially during drier periods, demonstrating that they cannot be considered accidental. *Bokermannohyla martinsi*, a species endemic to the Quadrilátero Ferrífero region, uses caves throughout the year. However, it is present in greater numbers in the dry period, when external conditions are unfavorable, indicating that it is not an accidental species (ANDRADE *et al.*, 2021; CAMPOS *et al.*, 2017). In addition, the species prefers natural subterranean cavities with conditions of more stable temperature and humidity (ANDRADE *et al.*, 2021).

Scinax fuscovarius (A. Lutz, 1925) (Figure 1C) occurs in caves of various lithologies and states, has a wide geographic distribution and adapts well to different environments, including anthropized areas (CAFOFO-SILVA et al., 2009), even being found inside homes. The species can be very abundant in caves, demonstrating that it is very well adapted to this environment.

Thoropa miliaris (Spix, 1824) (Figure 1D) is another species commonly recorded in natural subterranean iron ore, quartzite and granitic cavities. The species occurs in rocky areas and has semi-terrestrial tadpoles associated with rocks, making it suitable even for reproduction in cave environments.

Pristimantis fenestratus (Steindachner, 1864) (Figure 1E) is a species with direct development, that is, without a larval stage, observed in ferruginous caves in the Amazon in high abundance in the dry period (BERNARDI *et al.*, 2017). Like *Pristimantis*, different species of the genus *Ischnocnema* (Figure 1J) are litter dwellers in forested areas. They also reproduce with terrestrial eggs and direct development (DUELLMAN; TRUEB, 1986; HADDAD; PRADO, 2005; POMBAL JUNIOR; HADDAD, 2007) and are frequently recorded in ferruginous and carbonate cavities. The independence from water bodies for reproduction for these genera with direct development can facilitate the continuous use of caves by such species (SANTOS *et al*., 2022), making the development of specific studies on their biology important.

Anuran reproduction inside caves has not yet been reported in Brazil, although there are indications. The presence of tadpoles in bodies of water inside natural subterranean cavities, although recorded, does not exclude the possibility of transport from eggs deposited upstream in an epigean environment. Therefore, more studies are needed.

Frog feeding has also been poorly documented inside Brazilian caves. *Leptodactylus vastus* A. Lutz, 1930 was recorded feeding on arthropods (cockroaches, crickets and spiders) in a cave in the state of Sergipe (FERREIRA *et al.*, 2009a). Records over a decade in a cave in Sergipe showed predation of bats (*Pteronotus personatus* Wagner, 1843) by two species of frogs, *Rhinella jimi* (Stevaux, 2002) and *L. vastus*, as an established foraging habit in their populations (GOUVEIA *et al.,* 2009). In general, these frogs gather at the narrow entrance to the cave (Caverna Casa de Pedra, municipality of Itabaiana) at dusk and the bats are preyed on when they go out to forage, as they collide with each other and fall to the ground (GOUVEIA *et al.*, 2009).

Detailed studies, however, are rare for most species, but could reveal the nature of their relationship with the cave environment. Abundant species in the places where they occur, such as species of the genera *Ischnocnema, Pristimantis* and *Scinax*, are considered ideal for ecological studies and monitoring in karst systems.

LIZARDS

There is great ecological variety, both among lizards and among snakes, recorded in caves, represented by the occurrence of terrestrial, arboreal, fossorial, and active or passive foraging species, in addition to those with specialized feeding and others considered as generalists. There are studies that suggest that some species of reptiles may use caves as places for shelter (BAUER *et al.*, 2002, 2003, 2010; ELLIS; PAUWELS, 2012) and others that suggest foraging as the main stimulus (SCHÄTTI, 1984).

Among the lizard species recorded in caves, *Thecadactylus rapicauda* (Houttuyn, 1782), *Hemidactylus mabouia* Moreau De Jonnès, 1818, *Phyllopezus pollicaris* (Spix, 1825) (Figure 2B) and *Gymnodactylus darwinii* (Gray, 1845) have common characteristics, such as nocturnal habits and the ability to climb rocks. *Thecadactylus rapicauda* (Gekkonidae) (Figure 2A) is commonly recorded in ferruginous caves in the north of the country (SANTOS *et al.*, 2022) and has been recurrent in this environment during the day, suggesting its use as a daytime shelter. Studies with lizards of the genus *Cyrtodactylus* (Gekkonidae) suggest that their nocturnal habit and the ability to climb walls favor the occupation of caves as a refuge. However, species of this genus are more recurrently associated with leaf litter than truly adapted



Figure 2. A) *Thecadactylus rapicauda* (Phyllodactylidae); **B)** *Phyllopezus pollicaris* (Phyllodactylidae); **C)** *Plica plica* (Tropiduridae); **D)** *Potamites ecpleopus* (Gymnophthalmidae); **E)** *Tropidurus* sp. (Tropiduridae); **F)** *Paleosuchus trigonatus* (Alligatoridae). Photos: A, D (Thiago dos Santos); B, F (Xavier Prous); C, E (Robson de A. Zampaulo).

to caves (ELLIS; PAUWELS, 2012). Nocturnal reptiles and passive foragers were found to be more recurrent in cave environments in general (SANTOS *et al.*, 2022).

Lizards can enter natural subterranean cavities to forage in search of invertebrates while snakes forage in search of small vertebrates, such as amphibians and small mammals (including bats and rodents) that can be visualized or perceived by smell. Studies in epigean environments have demonstrated that the diet compositions of *Hemidactylus mabouia* (exotic species) and *Phyllopezus pollicaris* showed a generalist feeding pattern, based mainly on insects (ALBUQUERQUE *et al.*, 2013). In turn, the most frequent prey in the diet of *P. pollicaris* were Coleoptera, Araneae and Homoptera, for *H. mabouia* they were Araneae, Coleoptera and Hemiptera as the most frequent (ALBUQUERQUE *et al.*, 2013). In this way, food supply can act as a stimulus for the use of caves by generalist species, even if they are not so adapted to the cave environment. The lizard *Lepidophyma smithii* Bocourt, 1876, for example, inhabits a cave system in Guerrero, Mexico. An analysis of its stomach contents showed that 91% of the dry weight was composed of a type of native fig that fell inside the cave (MAUTZ; LOPEZ-FORMENT, 1978).

SNAKES

According to the data analyzed, the number of snake species recorded inside natural subterranean cavities is greater than that of lizards. Chiropterophagy, the act of eating bats, is reported for some species of snakes (SCHÄTTI, 1984) and could motivate the use of caves as a foraging area.

In the New World tropics, the best-known bat predation records with the largest number of publications are for the family Boidae, particularly the species *Chilabothrus inornatus* (Reinhardt, 1843) in the Caribbean (DÁVALOS; ERIKSSON, 2004; KOENIG; SCHWARTZ, 2003; PRIOR; GIBSON, 1997; RODRIGUEZ-DURAN, 1996; RODRIGUEZ; REAGAN, 1984), with records of one or more individuals feeding on bats simultaneously at the entrance or close to the cave. In turn, the species *Pseudelaphe flavirufa* (Cope, 1867) is known as a constrictor of small prey, mainly mammals, but also birds, lizards (KÖHLER, 2003) and bats (SCHULZ, 1993), and also has records in caves in Mexico.

For Brazil, however, records of predation of bats by snakes in caves are still rare. Examination of the stomachs of 175 specimens of the snake *Bothrops pauloensis* Amaral, 1925 (Viperidae), a typical species of cerrado areas of central Brazil, revealed only one bat (without identification of the species) in one of the analyzed stomachs (VALDUJO *et al*., 2002). Outside the cave environment, four bat predation events were recorded by the snake species *Boa constrictor* Linnaeus, 1758 (Boidae), *Spilotes pullatus* Linnaeus, 1758 (Colubridae) and *Corallus hortulanus* Linnaeus, 1758 (Boidae) in different regions of the country (ESBERARD; VRCIBRADIC, 2007).

The most emblematic records for caves perhaps correspond to snakes of the genus *Epicrates* that feed on bats in caves in the North and Northeast regions of the country in different lithologies (DONATO et al., 2012, personal observations). Such snakes can forage close to the entrance zone of the caves, where they capture bats when they leave their shelter during the night, or even in the deepest zones, where they manage to hang on the walls and ceiling of the caves by means of their prehensile tail and capture bats in flight or resting in colonies.

It should be noted that the opportunistic predation of bats at cave entrances is not restricted to species of the family Boidae and has also been recorded for Colubridae (HAMMER; ARLETTAZ, 1998; HERREID, 1961). It seems that the main strategy employed by colubrids that look for food near or at cave entrances is to attack bats that are on the ground or clinging to shelter walls (HAMMER; ARLETTAZ, 1998; HERREID, 1961), but there are also reports of colubrids preying on bats when they leave their refuges (HUTSON *et al.*, 2001; LÓPEZ *et al.*, 2005).

In addition to this close relationship between snakes and bats, there are also species with specialized feeding such as, for example, *Oxyrhopus guibei* (Figure 3C) and *Erythrolamprus aesculapii* (Linnaeus, 1758) (which feed predominantly on snakes); *Atractus pantostictus* Fernandes & Puorto, 1993 (Figure 3G) (on invertebrates), *Dipsas mikanii* (Schlegel, 1837) (Figure 3K) (on molluscs) and *Xenodon merremii* (Wagler, 1824) (on anuran amphibians), to snakes with more generalist feeding habits, such as *Pseudablabes patagoniensis* (Girard, 1858), Philodryas olfersii Lichtenstein, 1823 and *Oxyrhopus guibei* Hoge & Romano, 1977, that can feed on mammals, reptiles, amphibians and birds. Among the mentioned species, the occurrence of *X. merremii* inside caves may be related to foraging opportunities, since only one individual of the species was recorded preying on a specimen of *Leptodactylus labyrinthicus* Spix, 1824 and several other species of anuran amphibians were recorded in the same caves where this snake was found. Snakes of the family Viperidae, in turn, can be attracted both by the odor of bat colonies and by other species of mammals that use caves as shelters.



Figure 3. A) *Epicrates cenchria* (Boidae); **B)** *Epicrates assisi* (Boidae); **C)** *Oxyrhopus guibei* (Dipsadidae); **D)** *Bothrops moojeni* (Viperidae); **E)** *Bothrops jararaca* (Viperidae); **F)** *Bothrops neuwiedi* (Viperidae); **G)** *Atractus pantostictus* (Dipsadidae); **H)** *Xenopholis undulatus (Dipsadidae); I) Crotalus durissus (Viperidae); J) Mastigodryas boddaerti (Colubridae); k) Dipsas mikanii (Dipsadidae); L) Leptodira annulata (Colubridae)*. Photos: A, B, H (Xavier Prous); C (Lucas M. Rabelo); D (Rodrigo L. Ferreira); E, F, I-K (Thiago dos Santos); G, L (Robson de A. Zampaulo).

Finally, the data presented here demonstrate the use of caves by a large number of species of amphibians and reptiles in Brazil, and many of these species show signs of non-accidental use and may even present potentially troglophilous or trogloxenous populations. These data make clear the need to understand the importance of cave environments for herpetofauna, as well as the importance of herpetofauna in the trophic webs of these environments. In view of the intense degree of threat to which many environments rich in caves are subject (*e.g.*, the Quadrilátero Ferrífero in Minas Gerais, under intense mining pressure, Jacobi *et al.*, 2007), it is urgent to invest in more specific studies on the herpetofauna associated with these ecosystems. Cave environments may constitute, for many species, refuges against climatic adversities, foraging environments and, who knows, maybe even places for reproduction. Until we better understand these relationships, we may inadvertently threaten populations, unique and unknown ecological relationships, as well as endemic or rare species.

Main methods of collection and conservation

Amphibians and reptiles are present in different types of environments because they have species with different habits (terrestrial, arboreal, saxicolous, fossorial and aquatic). Thus, the undertaking of herpetofaunistic studies in the epigean environment requires the use of different methods to collect quantitative and qualitative data. The most commonly used methods are active search, zoophony and interception and fall traps (*pitfall traps*).

The active search method can be used during daytime and nighttime. It basically consists of walking slowly along a grid of trails. A perpendicular distance of 5 meters is sampled to a height of 3 to 4 meters on each side of the trail. During this process, every type of environment is inspected: litter, decomposing trunks, trunk cavities, vegetation inside holes in the ground and other places that can serve as shelter for amphibians and reptiles.

Zoophony is exclusively used for anuran amphibians and involves making records through the vocalizations emitted by males in reproductive activity.

Interception and fall traps are considered effective for capturing terrestrial reptiles and amphibians, mainly species typical of leaf litter but also fossorial and semi-fossorial species (CECHIN; MARTINS, 2000) that leave their shelters to migrate or even to forage. "*Pitfall traps*" consist of buckets buried to ground level, installed in a Y or linear arrangement, connected by plastic canvas guide fences and wooden stakes. The guide fences are intended to intercept and lead the specimens to the buckets and, once the animal falls into the smooth-walled trap, it cannot get out.

There are a number of restrictions for the application of some of the aforementioned methods when carrying out herpetofaunistic studies in caves. In general, there is no way to effectively install interception and fall traps inside caves and, even if it were possible, changes in environmental conditions would be disastrous for the cave fauna. The absence of favorable environments for the reproductive activity of anurofauna is also a limiting factor for the use of zoophony. Thus, active search is the most appropriate method for recording amphibians and reptiles inside caves. There are other forms of trapping that could work, such as funnel traps. However, active search is more effective at data collection because it is not environment-selective, as can occur with the use of funnel traps, for example.

Another important aspect for the study of amphibians and reptiles in caves that must be addressed is capturing and collecting for species identification. In this case, capture is carried out only for the elucidation of the species in the sampling place and collection when identification in the laboratory is necessary. Many amphibians and reptiles can only be identified to species level by viewing certain morphological features. Some species of snakes, for example, differ from each other by characteristics of the ventral, subcaudal and cloacal scales, which requires the handling of specimens.

In environmental licensing studies conducted by biospeleology teams, the identification of amphibian and reptile species (as well as other groups of vertebrates) is normally carried out through the use of photographs. However, some factors, such as photo quality or the position of the specimen that makes it difficult to visualize parts of the body, among other factors, may impair taxonomic elucidation. Collecting, as well as standardization of the photographic record (which characters and body parts should be photographed) are tools that would greatly help with the identification of amphibian and reptile species that occur in cave environments.

Identification key

With more than 2,000 species of amphibians and reptiles in Brazil, a specific identification key is not feasible. However, some

works are important references that can help those who wish to enter this fantastic field of herpetology and biospeleology. In addition, for example, the Sagarana Lab - Laboratório de Herpetologia da Universidade Federal de Viçosa, Florestal campus, has as an initiative an interactive identification key that can be accessed through the electronic address: http://saglab.ufv.br/aqf/chaves/

Several illustrated guides to species of amphibians and reptiles are available in the specialized literature, many of which are regional in scale, such as: Anfíbios Anuros da Serra do Cipó; Guia de Lagartos da Reserva Adolpho Ducke - Amazon Central; Guia Ilustrado: A Herpetofauna das Caatingas e Areas de Altitude do Nordeste Brasileiro; Serpentes Peçonhentas e Acidentes Ofídicos no Brasil; Anfíbios do Rio Grande do Sul: Catálogo, Diagnoses, Distribuição, Iconografia; and Os Répteis da Região Costeira do Extremo Sul do Brasil. In this way, we suggest that those interested look for works by renowned researchers that are affiliated with research institutions.

Main Brazilian collections

Few works in caves have been carried out with systematic collections for deposition of specimens in scientific collections, however, some research entities are extremely important for receiving and consulting biological material, such as: Coleção Herpetológica of the Museu de Ciências Naturais of Pontifícia Universidade Católica de Minas Gerais (PUC Minas); Coleção Herpetológica of the Departamento de Zoologia of the Universidade Federal de Minas Gerais (UFMG); Coleção de Herpetologia of the Museu Paraense Emílio Goeldi; Coleção de Anfíbios e Répteis of the Museu Nacional da Universidade Federal do Rio de Janeiro (MNRJ) and Coleção Herpetológica Alphonse Richard Hoge of Instituto Butantan.

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Scytalopus petrophilus
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25

Birds

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General introduction

More than 10,420 species of birds are recognized worldwide (BIRDLIFE INTERNATIONAL, 2021), yet researchers consider this number to be underestimated (BARROWCLOUGH *et al.* 2016). Approximately 3,590 species occur in South America, of which 1,971 can be observed in the Brazilian territory (PACHECO *et al.,* 2021; LEPAGE, 2022), comprising 1,742 breeding residents or migrants, 126 non-breeding visitors and 103 vagrants (PACHECO *et al.*, 2021).

Birds show significant adaptive plasticity to different habitats, including natural subterranean cavities. The relationship between birds and caves may be once ancient, if we consider, for example, the fossil records collected in caves in Europe (BOCHENSKI *et al.*, 2018; DAVIS, 2002; ZELENKOV *et al.*, 2019), Asia (MEIJER, 2014; RECCHI; GOPHER, 2002), Africa (GOODMAN *et al.*, 2013; KRUGER; BADENHORST, 2018), North America (JAMES, 2020; STEADMAN, 2005), Central America (JIMÉNEZ; ORIHUELA, 2021; OLSON, 1982; SUÁREZ, 2020) and South America (NASCIMENTO; SILVEIRA, 2020; OLSON, 1985; TAMBUSSI; DEGRANGE, 2013). However, probably not all of the fossils found in caves belong to species that effectively used this habitat. Different processes, physical or biological, may have carried the material into caves, where fossilization took place (LUND, 1836, 1950a, 1950b).

The first fossil records of birds in caves in Brazil were reported in 1837 by the Danish naturalist Peter Wilhelm Lund when describing Lapa Nova de Maquiné (Gruta de Maquiné), located in the current municipality of Cordisburgo, state of Minas Gerais. In 1846, Lund published the work "Olhar sobre o mundo animal do Brasil", in which he unveiled a fact that puzzled European speleologists (HOLTEN; STERLL, 2011). He investigated the presence of countless bones of small animals in caves in Ouro Preto and Curvelo, in Minas Gerais, and found that they were the regurgitated pellets of barn owls (at the time *Strix perlata*, currently *Tyto furcata tuidara* (J.E. Gray, 1829)), which "inhabited" those caves (HOLTEN; STERLL, 2011). A review carried out by Nascimento and Silveira (2020) presents the enormous scientific contribution that Lund provided, both for paleornithology and for current ornithological science. The authors highlight the importance of most of the Quaternary records obtained by Lund in caves in Lagoa Santa, Minas Gerais, based on the taxonomic diversity and historical significance of these findings. In addition other paleornithological records and studies involving Brazilian caves have since been published (ALVARENGA, 2007; ALVARENGA; OLSON, 2004; GUERIN *et al.*, 2002).

Some birds are recognized worldwide as frequently associated with or dependent on cave ecosystems or their varied features. From Alaska to Southeast Asia, several species of Apodidae depend on caves and crevices in rock walls for roostingg and breeding (CHANTLER; DRIESSENS, 2000). In the Indo-Pacific region, species of the genera *Collocalia* and *Aerodramus* use caves for nesting, and the nests of the Edible-nest Swiftlet (*Aerodramus fuciphagus*) (Thunberg, 1812), which are made with saliva, are commercially harvested and used as a high value ingredient in oriental cuisine (LOOI; OMAR, 2016; MANCHI; SANKARAN, 2009; THORBURN, 2015).

Typical examples of the use of these environments as roosts and nesting sites in the Americas are: the Oilbird (*Steatornis caripensis* Humboldt, 1817), which occurs in Venezuela and Colombia (CÁRDENAS *et al.*, 2021; HERRERA, 2003; THOMAS, 1999), and at least four genera of Apodidae: Cypseloides, *Streptoprocne, Chaetura* and *Aeronautes* (BIANCALANA, 2014; CHANTLER, 1999; ESPÍN, 2019; PICHORIM, 2002; WHITACRE, 1989). In the United States and Canada, the Black Swift (*Cypseloides niger borealis*) (Kennerly, 1858) nests in cavities and caves in small canyons along the Rocky Mountains up to British Columbia, as well as in caves located in rock walls by the sea (LEGG, 1956). The species is a migratory visitor to the Brazilian Amazon, between October and April (BEASON *et al.*, 2012, BIANCALANA, R.N. 2021, personal communication), but there are still no records of its occurrence in caves in the country. In Mexico, the karst abyss mistakenly known as *Sótano de las Golondrinas* (swallows in Spanish) in San Luís Potosí, has one of the largest colonies of the White-naped Swift (*Streptoprocne semicollaris*) (DeSaussure, 1859) ever recorded in caves in the world (RAINES, 1968). Two other good examples of the association with these ecosystems are the

Biscutate Swift (*Streptoprocne biscutata*) (Sclater, 1866) in Brazil (PICHORIM, 2002) and the White-collared Swift (*Streptoprocne zonaris*) (Shaw, 1796) in Mexico, Argentina, Brazil and Cuba (BIANCALANA, 2014; ESPÍN, 2019; PASSEGGI, 2011; ROWLEY; ORR, 1965). In general, all this behavioral knowledge may have been facilitated by involving gregarious species that have the cave ecosystem as an essential habitat in their life cycle. However, there are several other non-colonial species that also associate with these environments, at least seasonally.

The recording of birds particularly in Brazilian caves intensified from the 1970s onwards, with reports of species belonging to the orders Galliformes, Columbiformes, Psittaciformes, Strigiformes, Apodiformes and Passeriformes by different ornithologists and speleologists (ANDRADE, 1982, 1983; ANDRADE *et al.*, 1983; BIANCALANA, 2014; CHAIMOWICZ, 1984; COELHO, 1977; GODOY, 1986; PICHORIM, 2002; TRAJANO, 1987; TRAJANO; BICHUETTE, 2006; TRAJANO; GNASPINI-NETTO, 1990). More recently, Sick (1997) provided a compilation of studies and reports of the presence of birds in Brazilian caves and demonstrated that most records occurred in carbonate and quartzite caves. However, with the advance of studies in caves formed in other lithotypes, especially ferruginous caves, the recording of several species and nests, and even new descriptions of reproductive behavior in these environments, has became common (CARSTE, 2013; DE ANDRADE *et al.*, 2020; MAURÍCIO *et al.*, 2013; OMENA JÚNIOR; MARTINS, 2007; PINTO *et al.*, 2018). Thus, it is necessary to have a better understanding of these species, their level of association and dependence on the cave environment and the importance, even if seasonal, of these environments.

Therefore, this chapter presents technical and scientific information regarding the type of use of natural subterranean cavities by species of Brazilian avifauna. We hope that this information will be useful to consulting professionals, licensing bodies, prosecutors, speleologists and ornithologists, by generating support that can guide them in the elaboration of their studies and in the analysis of environmental impacts on speleological heritage. We also hope to contribute to Brazilian ornithology by compiling already existing information and unpublished records for these environments, in addition to stimulating new studies in the subterranean environment. This could be a new and attractive field of research, possibly previously unknown to many ornithologists and other Brazilian researchers, not to mention the tourist potential for birdwatchers. Although the data that will be presented here do not represent the totality of species that use these habitats, the chapter serves as a first step towards providing a new look at the neglected avifauna associated with these unique environments.

Morphological and behavioral adaptations of birds to the cave environment

The Oilbird and certain species of Apodiformes (swifts) and Strigiformes (owls) present anatomical, physiological and behavioral adaptations directed to environments of rock walls, darkness or low light incidence (BRINKLØV *et al.*, 2013; MANE; MANCHI, 2019; MARTIN *et al.*, 2004; ROJAS *et al.*, 2004; SUTHERS; HECTOR, 1985). Like bats, these birds are classified as trogloxenes and contribute to the base of the food chain in cave ecosystems, as they produce food resources (guano and egagropylae[1]) for a wide variety of invertebrates (DEHARVENG; BEDOS, 2018).

The Oilbird is a gregarious South American bird that breeds and roosts in the darkness of caves, forming colonies of hundreds to thousands of individuals in countries such as Venezuela and Colombia (HERRERA, 2003; THOMAS, 1999). The species, which plays an important ecological role in Neotropical forests, leaves caves at night, and can travel kilometers away to feed, mainly on palm fruits, guided mainly by sight, with smell playing a secondary role (CÁRDENAS *et al.*, 2021; HOLLAND *et al.*, 2009; SNOW, 1961; STEVENSON *et al.*, 2021). The retina is dominated by small rods (photoreceptor cells) arranged in an inclined structure that is unique among all terrestrial

1 A mass of undigested matter from the food of birds that some species occasionally regurgitate. The composition is variable and may include insect exoskeletons, indigestible plant matter, bones, fur, feathers, beaks, claws and teeth.

vertebrates (FITE; ROSENFIELD-WESSELS, 1975; MARTIN *et al.*, 2004; MEYER, 1977). The density of these photoreceptor cells (rods) is the highest ever reported for any vertebrate eye (FITE; ROSENFIELD-WESSELS, 1975; MARTIN *et al.*, 2004; REYMOND, 1987). The cones occur in low numbers and the eyes are relatively small, with the pupils achieving the highest light-gathering capacity ever identified for a bird (MARTIN *et al.*, 2004). The absence of a *tapetum lucidum*[2] in the retina is also remarkable, since it is a common structure in animals with nocturnal habits, including birds of the order Caprimulgiformes (NICOL; ARNOTT, 1974). Although its visual sensitivity may be close to the maximum, its spatial visual resolution must be low, requiring the use of smell and echolocation in low light environments (MARTIN *et al.*, 2004). The large bristles, modified feathers present at the base of the beak, also have a sensory function.

Although the name Apodidae refers to the absence of feet, swifts possess this structure, which is anatomically adapted to cling to vertical surfaces such as rock walls and foliage, but not to thin branches or power cables (in urban areas) like other birds (BIANCALANA, R.N. 2021, personal communication; CHANTLER; DRIESSENS, 2000). The very sharp claws work as climbing boot crampons (BIANCALANA, R.N. 2021, personal communication). Species of the genus *Aerodramus* and the Oilbird have a very particular characteristic — the ability to spatially guide themselves through echolocation (BRINKLØV *et al.*, 2013). The basic principle is to the extract environmental information from the return of clicks produced by the bird, which echoes on the cave walls (BRINKLØV *et al.*, 2013). Unlike most bats, birds use frequencies audible to humans, emit short acoustic signals (clicks) and exhibit no change in sound frequency over time (BRINKLØV *et al.*, 2013). However, alterations in the repetition rates of these sounds were verified when these birds approach obstacles or their nests, when they make evasion maneuvers or when they enter or leave caves (COLES *et al.*, 1987; FULLARD *et al.*, 1993; KONISHI; KNUDSEN, 1979).

Species of the order Strigiformes, represented by owls, present a selection of evolutionary features that make them excellent nocturnal predators, such as a differentiated circadian rhythm, retinas adapted for better night vision, asymmetrical ears, facial discs to improve hearing and cryptic and soft plumages that allow silent flight (ESPÍNDOLA-HERNÁNDEZ *et al.*, 2020; LISNEY *et al.*, 2012; MENQ, 2017). The genes involved in sensory perception in owls show a signal of positive selection throughout the genome of these animals, contributing to their predominantly nocturnal and predatory habits. This includes genes involved in acoustic and light perception, photosensitivity, phototransduction, low-light vision, and the development of the retina and inner ear. Thus, owls have developed a unique DNA packaging mechanism in the retina that increases light channeling in photoreceptors (ESPÍNDOLA-HERNÁNDEZ *et al.*, 2020). Some species in the Americas use caves to feed, shelter and nest, as is the case of the Barn Owl (*T. furcata*) and the Great Horned Owl (*Bubo virginianus*) (ARAGÓN *et al.*, 2002; JESUS; OLIVEIRA, 2017; LAVARIEGA *et al.*, 2016; MANCINI *et al.*, 2018; SMITH, 1968; ZIGLER; YANCEY, 2019).

Although most birds are not fully adapted evolutionarily to effectively colonize, live-in or exploit this type of environment, in a different way many species find suitable microhabitats to spend periods of reproduction, feeding or even resting in the varied geomorphological features of karst areas.

In Illinois, Georgia and Tennessee, in the United States, it is common to find the tyrannid Eastern Phoebe (*Sayornis phoebe*) (Latham, 1790) nesting inside caves (NIEMILLER *et al.*, 2016; WEBB *et al.*, 1993; ZIGLER *et al.*, 2020), although this behavior is not exclusive to this environment (BEHELER *et al.*, 2003; WEEKS JUNIOR, 1978; WHITAKER, 1974). The nest is formed by mud and moss under rocks or cave walls, with high mate and nesting site fidelity (BEHELER *et al.*, 2003; WEBB *et al.*, 1993). Endemic to Australia, and also a passerine, the Rockwarbler (*Origma solitaria*) (Lewin, 1808) usually builds nests suspended and fixed to the ceilings and walls of sandstone caves (occasionally limestone or granite) near Sydney, New Wales region of the South (PROBETS *et al.*, 2019; SMITH; SMITH, 2012).

Some seabirds also use caves for breeding. There are records of the African Penguin (*Spheniscus demersus*) (Linnaeus, 1758) and cormorants of the genus *Phalacrocorax* breeding in cave colonies in the central Namibian desert, within Namib-Naukluft National Park, northwest of Mount Sylvia (BARTLETT *et al.*, 2003; SIMMONS; KEMPER, 2003). The Fiordland Penguin (*Eudyptes pachyrhynchus* Gray, GR, 1845) and the Balearic Shearwater (*Puffinus mauretanicus* Lowe, 1921) were found

2 A membrane positioned inside the eyeball (back of the eye) of certain vertebrate animals capable of reflecting light, through the retina, that enters the eyes, thus improving the animal›s vision in low light conditions.

nesting, respectively, in caves along the coast of Fiordland and the Stewart and South Islands, in New Zealand, and on the northwest coast of the island of Mallorca, Spain (GUILFORD *et al.*, 2012; MATTERN; LONG, 2017; MEIER *et al.*, 2015; WARHAM, 2008). The Humboldt Penguin (*Spheniscus humboldti* Meyen, 1834), a species native to South America, also uses marine caves along the coasts of Peru and northern Chile for reproduction (COKER, 1919; LÜTHI, 2011; PAREDES *et al.*, 2002; PAREDES; ZAVALAGA, 2001).

The interest in the cave environment for some species may be, initially, merely opportunistic. The falconid American Kestrel (*Falco sparverius* Linnaeus, 1758) has the habit of perching near or at the entrance of caves, waiting for bat colonies to leave, immediately before or during sunset (BIRD; PALMER, 1988; LENOBLE *et al.*, 2014). A similar behavior is performed by the falconid Bat Falcon (*Falco rufigularis*) (Daudin, 1800) (COSTA, J.C.R. 2021, personal communication). The fringillid Apapane (*Himatione sanguinea*) (Gmelin, JF, 1788) has its occurrence restricted to native humid forest habitats at altitudes above a thousand meters in some islands of Hawaii (FANCY; RALPH, 1997). A nectarivorous and insectivorous species, it has, among insects, lepidopterans as its main diet (BALDWIN, 1953). Despite being an unusual nesting site for the species, several nests have been recorded in lava tube caves in Hawaii, and their occurrences are associated with the presence of moth colonies in these environments (HOWARTH; STONE, 2020; VAN RIPER, 1973).

It is important to emphasize that the reproductive success of birds is directly linked to the quality of the habitat where the nest is built, since food resources can be found in greater abundance and quality in one given environment compared to another (HANSELL, 2000; JONES, 2001; WITTENBERGER, 1980). In addition, certain nesting sites can provide better safety conditions against forest fires, bad weather and/or predators, which is one of the most relevant factors for the loss of broods (CURTI *et al.*, 2014; LOPES; MARINI, 2005; NÓBREGA; PINHO, 2010; PINHO *et al.*, 2006; VASCONCELOS, 1996). In this context, caves tend to provide greater protection and climate stability in relation to the external environment, depending on their physical and structural particularities (FERREIRA, 2005; GOMES *et al.*, 2021; ROJAS-LIZARAZO, 2016).

Birds also show various specializations or behavioral adaptations related to both nest building and nesting site. Tapaculos (Rhinocryptidae), for example, build closed and well-elaborated nests inside caves or in other types of cavities (DE ANDRADE *et al.*, 2020; MCCULLOUGH *et al.*, 2019), whereas most psittacines and cathartids only lay eggs directly on the substrate of these environments (simple/naked nest) (SICK, 1997; SIMON; PACHECO, 2005). The site used to build a nest in natural cavities is also quite variable among species and may occur in crevices or openings present in rock walls, trees, ravines, termite mounds, soil or in caverns.

Following this behavioral principle, we believe that species typically associated with karst areas, adapted to building intra-cavitary nests for reproduction, can better exploit the cave environment more frequently and recurrently compared to other species. However, its use will depend on the local availability of shelters that are most commonly used by the species, and on its level of reproductive specialty or generality (shape, size, position of the nest in relation to the sun throughout the day; opening, width and texture of the cave; and nearby food items, for example).

Furthermore, caves are not exclusively aphotic and represent a transitional gradient between epigean and hypogean environments, with different levels of light intensity, temperature and humidity (GOMES *et al.*, 2021; PROUS *et al.*, 2004). Therefore, such conditions may be favorable for the sheltering, foraging and reproductive success of some species that have adaptations for constructing extra-cavitary nests that may be architected in these environments (LOPES *et al.*, 2020; PINTO *et al.*, 2018).

Main groups found In Brazilian caves

The data presented here comprise an extensive survey with more than 230 records of the occurrence of birds and nests in different types of natural subterranean cavities in Brazil. They were obtained through the review of scientific literature, books and studies for environmental licensing, in addition to unpublished data from authors and collaborators, records available on citizen science portals such as *Wikiaves* and *eBird*, and tourism. In total, 38 species were recorded, distributed in nine orders and 18 families. The Table 1 presents information such as popular and scientific names of species, threat status, types of lithologies and biomes where records were made, as well as some behavioral and reproductive aspects. Images and illustrations of some species are provided in figures 1 to 8, whose reproductive biology and singularities will be discussed below,

in addition to other species also recorded in caves developed at ground level, on walls or in other types of rock outcrops, such as the Cipo Cinclodes (*Cinclodes espinacensis* Freitas, Chaves, Costa, Santos & Rodrigues, 2012) (COSTA *et al.*, 2019) and the House Wren *Troglodytes musculus* Naumann, 1823 (PINTO *et al.*, 2018) (figures 5A-C , 8E).

APODIFORMES

Apodiformes — Apodidae (swifts) and Trochilidae (hummingbirds) — represent the most commonly recorded birds in caves in Brazil.

Apodidae (Figure 1 A-I)

In general, the following characteristics are common to Brazilian apodids of Cypseloidinae: i) reproductive season associated with the beginning of the rainy season; ii) construction of disk-shaped nests, fixed to platforms or small cavities in the walls of caves or in rock walls close to waterfalls; iii) nests made of moss, roots, mud and leaves; iv) reuse and renewal of nests (fidelity to breeding sites); v) laying of white eggs, with male and female acting in incubation; vi) birth of altricial[3] offspring, fed by regurgitated insect balls; and vii) less frequent return of parents to the nest during the day, as chicks develop (BIANCALANA, 2014, 2015a, 2015b; BIANCALANA *et al.*, 2012; CHANTLER; DRIESSENS, 2000; PICHORIM, 2002; WHITTAKER; WHITTAKER, 2008).

The White-chinned Swift (*Cypseloides cryptus* Zimmer, 1945) is considered an Amazonian species, with records for Brazil in the states of Acre and Amazonas (BIANCALANA; MAGALHÃES, 2016; BRITO *et al.*, 2015; WHITTAKER; WHITTAKER, 2008). It usually occurs alone or in small groups with other species of the genera *Cypseloides* or *Streptoprocne*. In contrast, the Great Dusky Swift (*Cypseloides senex*) (Temminck, 1826) occurs in all five regions of Brazil (SICK, 2001). Both species nest on rock walls or in caves near waterfalls and lay only a single egg in each reproductive cycle.

The Sooty Swift (*Cypseloides fumigatus*) (Streubel, 1848) has a wide but sparse distribution throughout South America (CHANTLER; DRIESSENS, 2000). Like the Great Dusky Swift, it occurs in Argentina, Bolivia, Brazil and Paraguay (BROOKS *et al.*, 1995; PEARMAN *et al.*, 2010). The species can be found in all five regions of Brazil (ALBANO; GIRÃO, 2008; BIANCALANA, 2015a; BIANCALANA *et al.*, 2012, CHANTLER; DRIESSENS, 2000; SICK, 2001; SILVEIRA *et al.*, 2005; VASCONCELOS *et al.*, 2006). It usually lays a single egg in intra-cavitary nests on rocky walls or caves near waterfalls or in gallery forests (BIANCALANA *et al.*, 2012; MAURÍCIO *et al.*, 2013; ZAMPAULO, R.A, 2021, personal communication).

In Brazil, there are three swifts of the genus *Streptoprocne*: Biscutate Swift (*S. biscutata*), Tepui Swift (*Streptoprocne phelpsi*) (Collins, 1972) and White-collared Swift (*S. zonaris*). The first has a restricted distribution in South America, in the region of Misiones in Argentina and from the state of Rio Grande do Sul to Minas Gerais in Brazil, in addition to populations in the states of Piauí and Rio Grande do Norte (SICK. 2001). The species is more easily found nesting in dry caves with nests in dark areas. Clutches vary between one and three eggs (PICHORIM, 2002). The Tepui Swift has a restricted distribution in the northern region of South America, being found in caves of the tepuis in Brazil, in the states of Amazonas and Roraima (BIANCALANA *et al.*, 2021). There are only two records of nests for this species, both in caves in Venezuela, one in the region of Cerro de la Neblina, in the state of Amazonas, and the other in the tepuis of the southeast of the state of Bolívar, on the border with Brazil (next to the state of Roraima), with few details about its reproductive biology (BIANCALANA *et al.*, 2021; WILLARD *et al.*, 1991). Finally, the White-collared Swift has a wide distribution throughout South America, the Caribbean, Central America and southern Mexico (CHANTLER; DRIESSENS, 2000). In Brazil, the species can be found from Rio Grande do Sul to Bahia and in the states of Mato Grosso, Pará, Tocantins, Amazonas and Roraima. It nests on rock walls near waterfalls and in caves, alone or in large colonies. In Saltos das Andorinhas e de Dardanelos, municipality of Aripuanã, northwest Mato Grosso, the species, together with *C. senex*, forms the largest colony of Apodidae known in Brazil, with approximately 1.5 million individuals (DE LUCA *et al.*, 2009). Nests in caves are often located in aphotic zones and usually have two eggs, but there are records of clutches with only one or three eggs (BIANCALANA, 2014; MAURÍCIO *et al.*, 2013).

3 Birds that are underdeveloped when they hatch, unable to move or feed themselves, requiring parental care for thermoregulation. They are nesting species, as they build a nest and remain there for some time. Chicks usually hatch blind and without feathers, with poorly developed thermoregulatory and sensory systems.

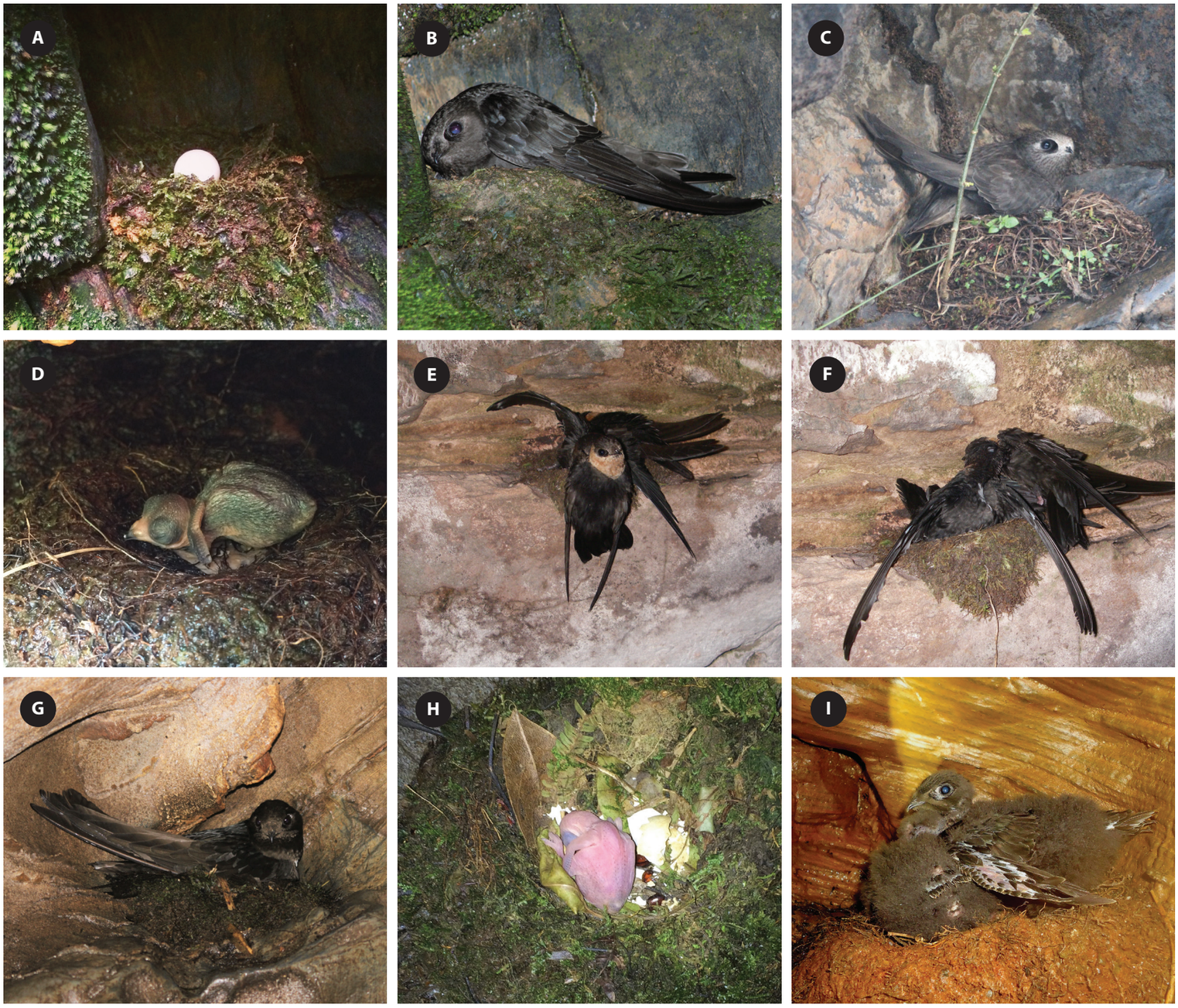


Figure 1. Species of Apodiformes commonly associated with karst environments in Brazil. **Apodidae: A-B)** nests with egg and adult of *Cypseloides fumigatus* in a cave in P.E. Intervales, SP; **C-D)** adult of *Cypseloides senex* in a nest in Cabeceira Grande, MG, near UHE Queimado, and nestling of the species in Cachoeira do Lajeado, Jalapão, TO; **E-F)** nest with specimens of *Streptoprocne phelpsi* in Tepuis cave, RR; **G-H)** nests with adult and nestling of *Streptoprocne zonaris* in a cave in P.E. Intervales, SP; **I)** nestlings of *Streptoprocne* sp. in the nest in a cave in Conceição do mato Dentro, MG. Photos: A-D, G-H (Renata N. Biancalana); E-F (Robson de A. Zampaulo); I (Thiago dos Santos).

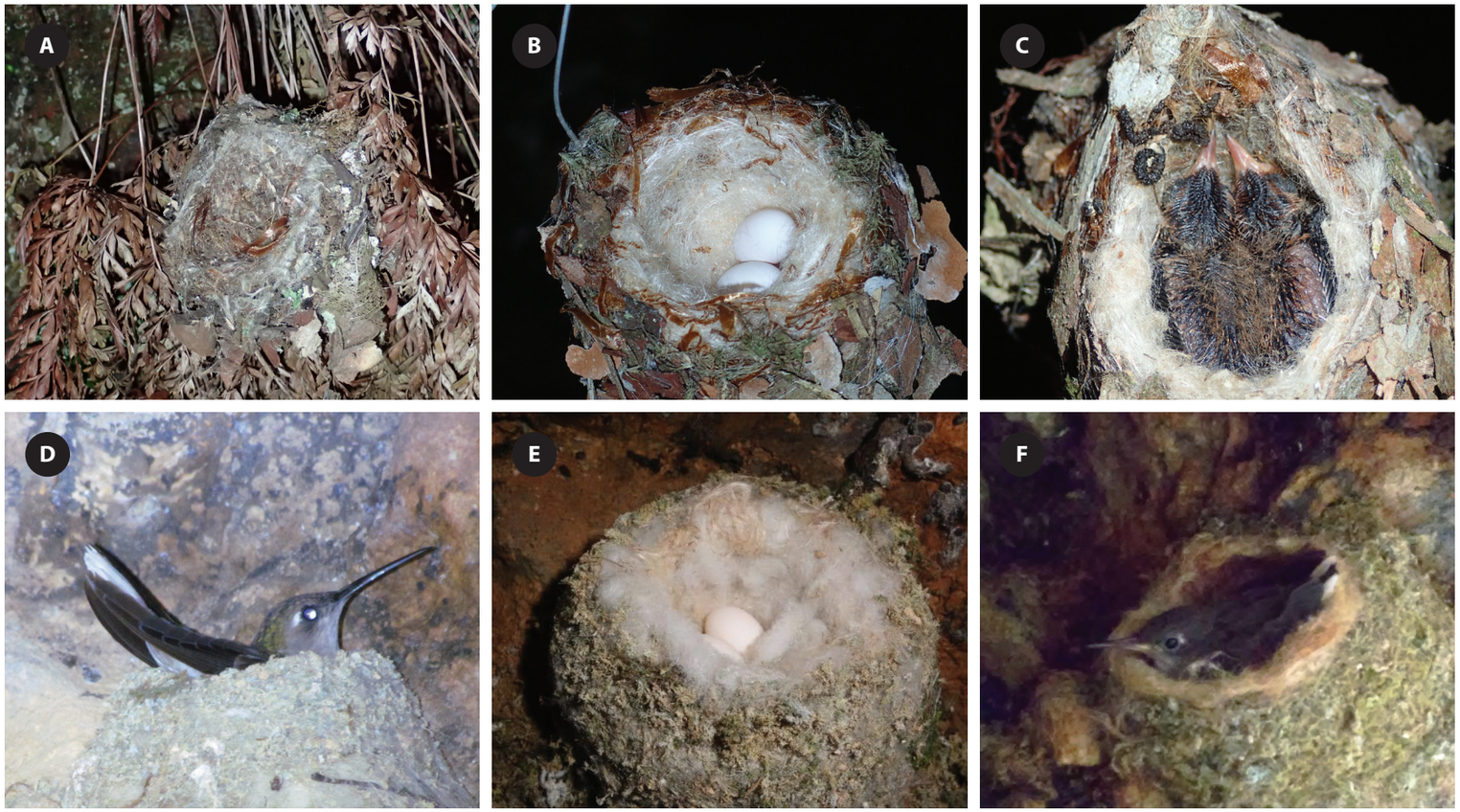


Figure 2. Species of Apodiformes commonly associated with karst environments in Brazil. **Trochilidae: A-C)** nests, inactive, with eggs and nestlings of *Chlorostilbon lucidus* in caves of P.N. da Serra do Gandarela, MG; **D-F)** nests with adult, eggs and nestlings of *Campylopterus calcirupicola* in caves of P.E. da Lapa Grande, Montes Claros, MG. Photos: A-C (Júlio C. R. Costa); D-F (Warley Miranda); E (Wagner Nogueira).

Trochilidae (figures 2 A-F, 8 B)

Trochilids, hummingbirds, are known to roost nocturnally on ceilings and walls of abandoned caves and mines in the high altitudes of the Peruvian Andes (Pearson 1953; Wolf *et al.*, 2020). The presence of nests, mainly of *Oreotrochilus estella* (d'Orbigny & Lafresnaye, 1838), and their reuse, also occur in these environments (PEARSON, 1953). Thermoregulation during torpor is common in trochilids, having been reported in several genera (JAMESON, 1981; PEARSON, 1953; WOLF *et al.*, 2020). Therefore, the search for caves and artificial cavities in regions of high altitudes is mainly due to two factors: the impossibility of returning from a state of torpor in negative temperatures, such as mountain environments, and the greater climatic stability of these ecosystems in relation to the external environment (FERREIRA, 2005; JAMESON, 1981; PEARSON, 1953; WOLF *et al.*, 2020).

In Brazil, especially in Minas Gerais, trochilid nests are commonly found near the entrance to caves, or even inside them in illuminated or dim areas (ARCADIS, 2014; CARSTE, 2013; LOPES *et al.*, 2020; PINTO *et al.*, 2018), being therefore more recurrent in small caves or caves with large openings. The Glittering-bellied Emerald (*Chlorostilbon lucidus*) (Shaw, 1812) and the Dry-forest Sabrewing (*Campylopterus calcirupicola* Lopes, Vasconcelos & Gonzaga, 2017) have already been recorded in these environments, the former with suspended nests associated with leaves, stems or plant roots present in ferruginous caves (COSTA, J.C.R. 2021, personal communication; PINTO *et al.*, 2018), and the latter with nests settled on rocks in limestone caves (LOPES *et al.*, 2020). The species lay two white, very small and elongated eggs (COSTA, J.C.R. 2021, personal communication; LOPES *et al.*, 2020). Unlike the Glittering-bellied Emerald, a much more common species, *C. calcirupicola* is a cryptic and recently described

species endemic to regions of seasonal deciduous forest in karst areas (limestone outcrops), present along the basins of the São Francisco and Paranã rivers (LOPES *et al.*, 2017).

CAPRIMULGIFORMES

Steatornithidae (Figure 8 C)

The Oilbird is the only representative of the family Steatornithidae. It occurs in Bolivia, Peru, Ecuador, Colombia, Panama, Costa Rica, Venezuela, Trinidad and Tobago, the Guianas and Northern Brazil (states of Amazon and Roraima) (GRANTSAU; PALO, 2010) at altitudes from seven to 3,500 meters (SICK, 1997). It was first recorded in Brazil just in 1998, from the finding and collection of the remains of a specimen in Palmari, Amazonas (WHITTAKER *et al.*, 2004). The distribution of colonies seems to be limited to the presence of caves and large crevices that are used exclusively for reproduction and daytime shelter (HERRERA, 2003; SICK, 1997; TELLO *et al.*, 2008), therefore it is considered an obligatory trogloxene. However, records in Brazil are occasional and restricted to the extreme north of the country, especially on Monte Roraima (GODOY *et al.*, 2021; GRANTSAU; PALO, 2010; URBANI, 1984; WHITTAKER *et al.*, 2004). In caves, the nest is built on rock ledges, with regurgitated lumps glued to the substrate with the bird's own feces, where two to four eggs are laid, which are incubated by the couple for 33 days (SICK, 1997). Chicks can remain in the nest for up to four months (SICK, 1997). The bird is one of the few species in the world that is frugivorous, both in the young (nesting) and adult stages, specializing on fatty and proteinaceous fruits (ROJAS-LIZARAZO, 2016; THOMAS *et al.*, 1993). Frugivory, which is exclusive in the young phase, helps with the growth and development of the young, as well as with their thermoregulatory processes (ROJAS-LIZARAZO, 2016; THOMAS *et al.*, 1993).

CATHARTIFORMES

Cathartidae (Figure 3 A-C)

The presence of cathartid eggs and nestlings in carbonate and iron-bearing caves is common, especially for the Black Vulture (*Coragyps atratus*) (Bechstein, 1793) (CARSTE, 2013; DONATO *et al.*, 2012; ZAMPAULO; SIMÕES, 2016). Although less frequent, there are also records of nesting of the Turkey Vulture (*Cathartes aura*) (Linnaeus, 1758) and the King Vulture (*Sarcoramphus papa*) (Linnaeus, 1758) in caves and rock walls in Brazil (CARDOSO, 2018; CARVALHO FILHO *et al.*, 2004; MAGELA, G. 2020, personal communication; SILVA; REGALADO, 1998). However, the only indication of recorded use of caves is often the slight evidence of excrement on prominent rocks at its entrance (COLES, 1944). Adult feathers or down can also be occasionally found inside caves (COLES, 1944). The absence of a standard or typical nest, it being formed only by a few sticks or solid waste deposited directly on the ground (simple/naked nest) (SIMON; PACHECO, 2005); the laying of large white eggs (King Vulture) or eggs speckled or spotted with brown (Black Vulture and Turkey Vulture); the long incubation period, which can be carried out by both sexes; and the birth of semi-altricial young[4] are reproductive characteristics common to the species of this family (ARÉVALO-AYALA; FUNES, 2019; CARVALHO FILHO *et al.*, 2004; COLES, 1944; COSTA, J. C. R. 2021, personal communication; LEAL, 2016; RABENOLD, 1986; SCHLEE, 1994).

FALCONIFORMES

Falconidae (Figure 3 D-F)

Most species of Falconiformes typically nest in tree hollows, between branches or in abandoned nests. However, some species may also use caves or other rock cavities for reproductive activity. In Brazil, the nesting of the Laughing Falcon (*Herpetotheres cachinnans*) (Linnaeus, 1758) is known in caves present in limestone walls in Minas Gerais and sandstone in Bahia (PACÍFICO; SILVEIRA, 2008; SPECHT *et al.*, 2008); of the Collared Forest-Falcon (*Micrastur semitorquatus*) (Vieillot, 1817) in carbonate caves in Minas Gerais, Paraná and Santa Catarina (PEREIRA, 2017; VALLEJOS *et al.*, 2008); and of the Bat Falcon (*Falco rufigularis*) in caves present in sandstone walls in Bahia (PACÍFICO; SILVEIRA, 2008).

PASSERIFORMES

Tyrannidae (figures 3 G-L, 7A)

Passerines are commonly recorded inside rock cavities, either in foraging activity or nesting behavior. There are records for two

4 Birds that are more developed when they hatch. The recently hatched chick has some independence to move around and feed but still depends on the adult. Young hatch with their eyes open and their hearing functions well, but they are unable to move on their own, feed themselves, or accompany the group on trips.

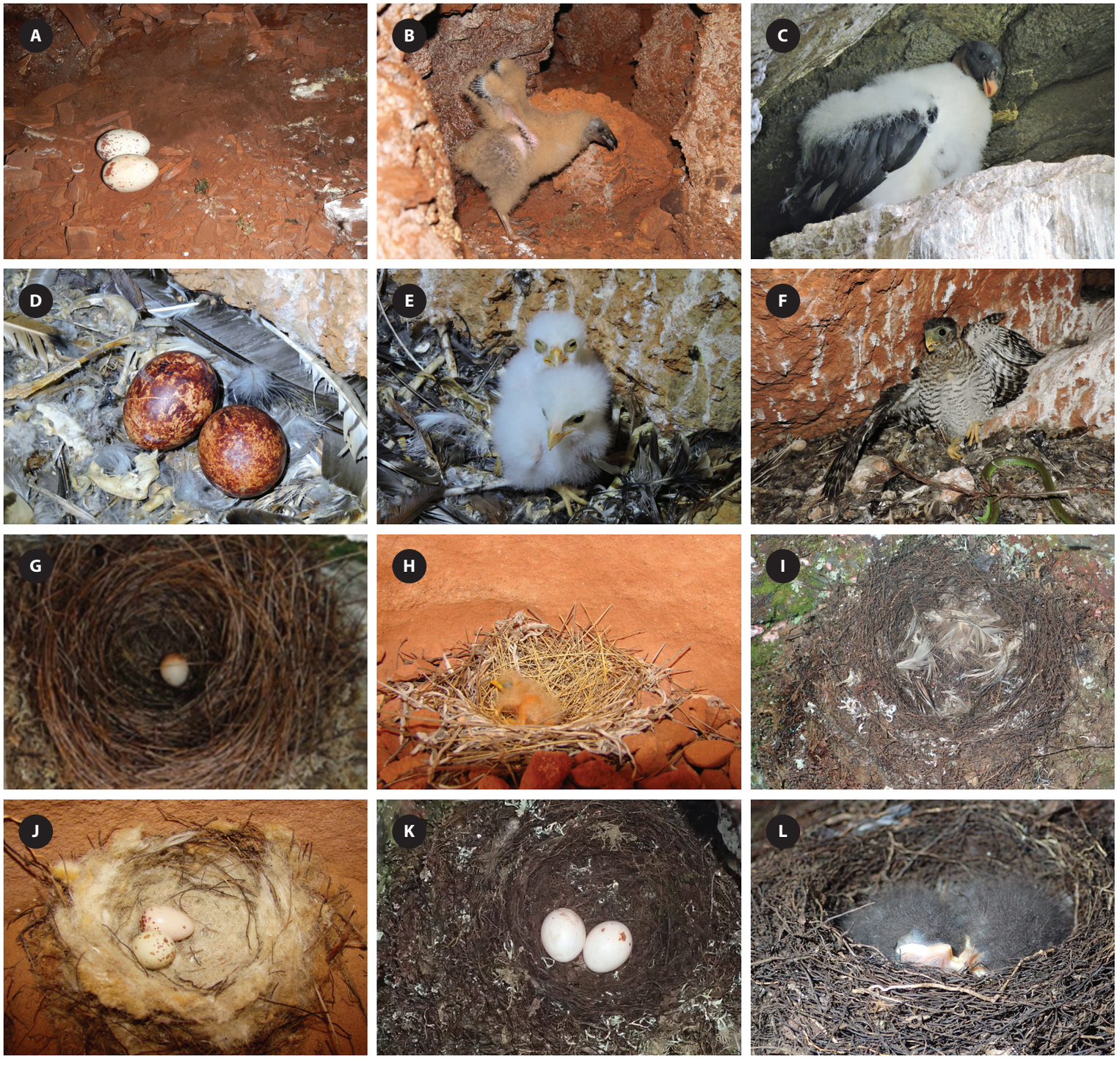


Figure 3. Records of birds and nests in caves in Brazil. **Cathartidae: A-B)** eggs and hatchling of *Coragyps atratus* in caves in MG; **C)** young *Sarcoramphus papa* perched in a cave at Curvelo, MG. **Falconidae**: **D-F)** nest with eggs, nestlings and young of *Micrastur semitorquatus* in a cave in Curvelo, MG. **Tyrannidae**: **G)** nest with *Hirundinea ferruginea* egg in a cave in Conceição do Mato Dentro, MG; **H)** nest with *H. ferruginea* chick on rock walls in the caatinga, BA; **I, K-L)** empty nests, with eggs and nestlings of *Knipolegus nigerrimus* in caves in Parque Nacional da Serra do Gandarela, MG; **J)** Nest with *K. nigerrimus* eggs on rock walls in the caatinga, BA. Photos: A-B (Matheus H. Simões); C-F (Geraldo Magela); G (Carste Consultores Associados Collection); H, J (Patrick I. Pina); I, K (Mauricio C. M. Andrade); L (Júlio C. R. Costa).

tyrannids in caves in Brazil: Cliff Flycatcher (*Hirundinea ferruginea belicosa*) (Vieillot, 1819) and the Velvety Black-tyrant (*Knipolegus nigerrimus*) (Vieillot, 1818). The former occurs in regions of rugged relief in the Northeast, Southeast and South regions of Brazil, associated with cliffs and rock outcrops (BORBA *et al.*, 2016; RIDGELY *et al.*, 2015; SIGRIST, 2014). The latter is endemic to the highlands of the South and Southeast regions, mountainous regions, inhabiting mainly rock areas with the presence of trees and scrub (FARNSWORTH *et al.*, 2020). Both species reproduce in spring and summer (FERREZ, 1992; PICHORIM *et al.*, 1996) and build open nests, in the form of shallow bowls, in caves, slabs, cavities and crevices of walls, cliffs or rock outcrops (FARNSWORTH *et al.*, 2020; PICHORIM *et al.*, 1996; SICK, 1997; SIGRIST, 2014; PINTO *et al.*, 2018).

The nest of the Cliff Flycatcher is usually lined with stones, followed by twigs and soft plant fibers that form its oological chamber[5]. It lays two to three white eggs with rust-colored spots. The species was recorded in tafone-type sandstone cavities in Rio Grande do Sul (BORBA *et al.*, 2016), in a cave in the state of Ceará (JÚNIOR, 2015), in a quartzite cave and a limestone wall cavity in Minas Gerais (CARSTE, 2014a; SPECHT *et al.*, 2008) and in sandstone/limestone cavities of rock walls in Bahia (PACÍFICO; SILVEIRA, 2008). In turn, the nest of the Velvety Black-tyrant is made up of roots and dry moss, with the incubation chamber devoid of any lining or with the presence of some feathers (COSTA, J.C.R. 2021, personal communication; PICHORIM *et al.*, 1996). It lays two to three smooth, white eggs with a few brown spots (COSTA, J.C.R. 2021, personal communication; PICHORIM,



Figure 4. Records of birds and nests in caves in Brazil. **Rhinocryptidae**: **A-C)** nests inactive, active and with adult of *Scytalopus petrophilus* in a cave in Parque Nacional da Serra do Gandarela, MG; **D)** female of *Merulaxis ater* foraging in a cave in P.E. Intervales, SP; **E)** male of *M. ater* foraging in a cave in P.E. Intervals, SP. **Thamnophilidae**: **F)** male of *Myrmelastes caurensis* foraging in the penumbra of a cave in Serra do Apiaú, Mucajaí, RR. Photos: A, B (Maurício C. M. Andrade); C (Júlio C. R. Costa); D (Elena M. Pattacini); E (Renato Paiva); F (Jorge Macedo).

5 Place in the nest built or used by birds with the aim of depositing their eggs and later rearing the chicks.

1996). Active and inactive nests of the species were recorded in several ferruginous caves in the Quadrilátero Ferrífero (Iron Quadrangle) in Minas Gerais (CARSTE, 2013, 2014b; PINTO *et al.*, 2018).

Rhinocryptidae (figures 4 A-E, 7B, 8A)

Rhinocryptids are considered primitive, photophobic birds, having short, rounded wings and long, strong legs, flying little and moving mainly by jumping (OLIVEIRA *et al.*, 2011). There are records of the Rock Tapaculo (*Scytalopus petrophilus* Whitney, Vasconcelos, Silveira & Pacheco, 2010) and the Slaty Bristlefront (*Merulaxis ater* Lesson, 1830) in Brazilian caves. The Rock Tapaculo is endemic to mountain environments in the states of Minas Gerais and São Paulo (SOUZA, 2013; WHITNEY *et al.*, 2010), at altitudes ranging between 750 and 2,100 meters (RIDGELY *et al.*, 2015). The species had its first and only nesting records occurring in ferruginous caves in Minas Gerais, with the reuse of nests or nesting sites also being observed, probably by the same couple, for consecutive breeding seasons (DE ANDRADE *et al.*, 2020; PINTO *et al.*, 2018). Due to its reproduction being known exclusively in these environments thus far, the species should be considered as an obligate trogloxene. The nest consists mainly of cryptogams, some grasses and passerine remiges[6]. This species lays two white colored eggs and both adults take care of the offspring (DE ANDRADE *et al.*, 2020). On the other hand, the entufado, an endemic species of the Atlantic Forest, occurs from the south of Bahia to the south of Santa Catarina (BERNARDO; THORNS, 2009; BIRDLIFE INTERNATIONAL, 2004; GRANTSAU; PALO, 2010), at altitudes from 100 to 1,800 meters (SIGRIST, 2014), in understory and edge of lowland and mountain forest (RIDGELY *et al.*, 2015). There are some records of specimens inside limestone caves in the state of São Paulo (OLMOS; SILVA, 2008; PAIVA, 2015; PATRIAL, 2015, 2018; PATTACINI, 2015). Although the presence of nests in these environments has already been confirmed (OLMOS; SILVA, 2008; PAIVA, R. 2021, personal communication), there is still no clear description of their format or composition. However, it is known that both sexes participate in its construction and that the nest has intra-cavitary characteristics (BERNARDO; THORNS, 2009; OLMOS; SILVA, 2008).

Cotingidae (figures 5 G-I, 8 G)

The Guianan Cock-of-the-rock (*Rupicola rupicola*) (Linnaeus, 1766) is one of the most beautiful birds of the American continent and, like its congener, Andean Cock-of-the-rock (*Rupicola peruvianus*) (Latham, 1790), takes the term rupicolous in its name because it is associated with rock environments, such as cliffs or outcrops. Although the species is not locally rare, there are few places where it can be found with certainty: Brazil (three sites), Guyana, Suriname (about 15 known sites) and Venezuela (KIRWAN; GREEN, 2011). The bird nests at the entrance to caves and in crevices or small cavities of walls with a dry and bare face, with the presence of humidity and penumbra, close to water bodies, which produces adequate temperature and microclimate for the stability and conservation of the nest (OMENA JÚNIOR; MARTINS, 2007). The nest is made with mud, fibers and vegetable resin (GILLIARD, 1962; MARTINS, 2007; OMENA JÚNIOR; SNOW, 1971), with the male having no participation in its construction nor in feeding or protecting the offspring (OMENA JÚNIOR; MARTINS, 2007). The species lays one to two white eggs with brown spots more concentrated at the poles, and has an incubation period of 27 to 28 days (OMENA JÚNIOR; MARTINS, 2007). In Brazil, the species is commonly recorded nesting in caves and on sandstone walls of Área de Proteção Ambiental Maroaga and Área de Proteção Ambiental Urubuí, Presidente Figueiredo, Amazonas (OMENA JÚNIOR; MARTINS, 2007), but there are also records for granitic caves of Serra do Apiaú, Mucajaí, Roraima (BICHINSKI, T. 2021, personal communication).

PROCELLARIIFORMES

Procellariidae (Figure 5 J-L)

Two seabird species nest in rock cavities on Brazilian oceanic islands (LOCATELLI, 2011; SOTO; FILIPPINI, 2003). Currently, nests of the Trindade Petrel (*Pterodroma arminjoniana*) (Giglioli & Salvadori, 1869), a migratory species, are only found on the island of Trindade, Espírito Santo (MANCINI *et al.*, 2016), and the Audubon's Shearwater (*Puffinus lherminieri* Lesson, 1839) on the islets of Morro do Leão and Morro da Viuvinha, in the Fernando de Noronha archipelago, Pernambuco (EFE; SERAFINI, 2018). Nesting in colonies; absence of nesting material; the laying of a single egg that is incubated by both sexes; the slow growth of the chicks; the irregular feeding frequency of the chicks, which is exclusively nocturnal; and fidelity to the nest, to the colony and to the partner, are reproductive characteristics common to

6 The large wing feathers that propel the bird in flight.



Figure 5. Records of birds and nests in caves in Brazil. **Troglodytidae**: **A-C)** nests inactive, with eggs and with nestlings of *Troglodytes musculus* in caves in Parque Nacional da Serra do Gandarela, MG. **Turdidae**: **D-E)** lateral and superficial aspects of the active nest of *Turdus leucomelas* in a cave in Parque Estadual da Serra do Rola-Moça, MG. **Thraupidae**: **F)** active nest with female and nestlings of *Tersina viridis*, in a cave in Conceição do Mato Dentro, MG. **Cotingidae**: **G-I)** active nests of *Rupicola rupicola*, with eggs, female in incubation activity and with young individual, in caves and walls of Presidente Figueiredo, AM. **Procellariidae**: **J-L)** nestling of *Pterodroma arminjoniana*, on the island of Trindade, Vitória, ES, and external and internal panorama of the nesting site of *Puffinus lherminieri* in the Archipelago of Fernando de Noronha, PE. Photos: A, D, E (Júlio C. R. Costa); B, C (Mauricio C. M. Andrade); F (Thiago Santos); G, I (Mario Fernandez); H (Natacha Hausner); J (Patricia Serafini); K, L (Andrei Ross).

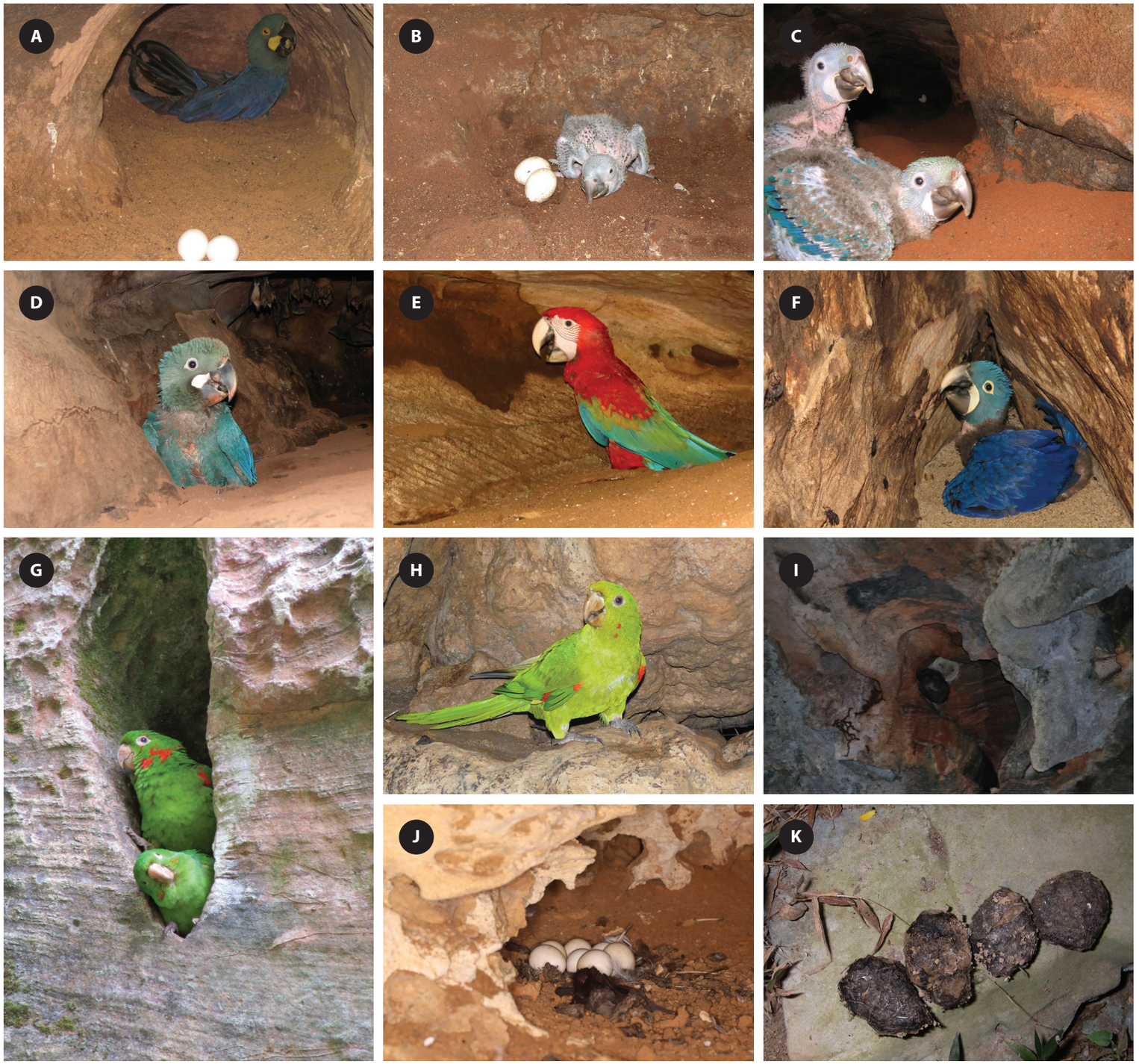


Figure 6. Records of birds, nests and traces in caves in Brazil. **Psittacidae**: **A-D)** cave-like cavities in rock walls of the caatinga, northeast of BA, with adult, eggs, nestlings and young of *Anodorhynchus leari*, in addition to the presence of bats in the background; **E-F)** young offspring of *Ara chloropterus* and *Anodorhynchus hyacinthinus* in cave-like cavities present on walls in TO; **G-H)** pair of the species of *Psittacara leucophthalmus* in a crevice of a cave in the municipality of Sacramento, MG, and adult of the species in a cave located in Monjolos, MG. **Tytonidae**: **I-K)** *Tyto furcata* adult recorded in a cave in Montes Claros, MG. Posture of *T. furcata* in a cave in the Parque Nacional da Furna Feia, RN, and typical owl pellet of the species recorded in a cave in the RPPN Santuario do Caraça, MG. Photos: A-D (Érica C. Pacífico); E-F (Neiva M.R. Guedes); G (Blog revolteio.com); H (Fabrini Pontello); I (Mauricio C.M. Andrade); J (Diego de M. Bento); K (Robson de A. Zampaulo).

both species (EFE, 2004; FONSECA-NETO, 2004; LUIGI *et al.*, 2009; SILVA; OLMOS, 2010).

PSITTACIFORMES

Psittacidae (figures 6 A-H, 8 D)

Psittacines, (parots, parakeets, macaws, among others) and columbids (pigeos and doves) that live close to caves or that nest in their crevices or openings, can benefit from the artificial lighting of tourist caves to reach originally aphotic regions (COSTA, J.C.R. 2021, personal communication). Consequently, these birds can carry potential zoonotic pathogens, such as the fungus *Histoplasma capsulatum* and the yeast *Cryptococcus neoformans*, to the deepest halls of caves with this type of use (DIAZ, 2018; LUGARINI *et al.*, 2008; REOLON *et al.*, 2004). The possible impact of such artificial apparatuses on cave fauna is still little known. However, it is already known that, according to the lighting system adopted, there may be changes in the temperature and humidity of these environments, in addition to favoring the growth of photosynthetic organisms in originally aphotic zones (BERNARDI *et al.*, 2011).

Some psittacines have adapted to nesting in deep cavities present in rock walls, mainly made up of sandstone/carbonate rocks in the caatinga and cerrado biomes, where in some situations there is the incidence of low light (GUEDES, N.M.R. 2020, personal communication; HART, 1992; IBAMA, 2006; PACÍFICO, 2011; PACÍFICO *et al.*, 2014). For the Lear's Macaw (*Anodorhynchus leari* Bonaparte, 1856), a species endemic to northeastern Bahia, reproductive activities are exclusive to this environment, and therefore there are no records of the species in hollow trees (IBAMA, 2006; PACÍFICO, 2011), as is the case for most psittacines. Its nesting area is restricted to the walls present at the Estação Biológica de Canudos and the Estação Ecológica do Raso da Catarina, Bahia (HART, 1992; PACÍFICO, 2011; PACÍFICO *et al.*, 2014). The natural cavities (including caves) where the species nests are, on average, 26.78 m above the ground and can be up to 18 meters deep (PACÍFICO, 2011) (Figure 7C-D). Several invertebrates can still be found within them, such as coleopterans, arachnids and native and invasive bees, in addition to other vertebrates, such as bats, rodents and lizards (PACÍFICO, E.C. 2021, personal communication; PACÍFICO, 2011; PACÍFICO *et al*., 2020). Other psittacines, such as the Blue-crowned Parakeet (*Thectocercus acuticaudatus*) (Vieillot, 1947) and the Turquoise-fronted Amazon (*Amazona aestiva*) (Linnaeus, 1758), as well as some cathartids and falconids, also use the caves in the walls of this region to nest (PACÍFICO; SICK, 1997; SILVEIRA, 2008).

The Red-and-green Macaw (*Ara chloropterus* Gray, 1859) is commonly recorded in caves of Parque Estadual Terra Ronca, state of Goiás, and in Chapada dos Guimarães, Mato Grosso. The species also occurs in the doline "Buraco das Araras", municipality of Jardim, Mato Grosso do Sul, where it nests in numerous sandstone cavities associated with this karst feature. The species lays two to three eggs (JUNIPER; PARR, 1998). The presence of young chicks of the Red-and-green Macaw and the Hyacinth Macaw (*Anodorhynchus hyacinthinus*) (Latham,1790) has already been observed in sandstone/calcareous meso- and macrocavities present in walls in Tocantins (GUEDES, N.M.R. 2020, personal communication). In the Pantanal and in the south of Pará, the species nests mainly in hollows of manduvis trees (*Sterculia apetala*), however, in the region of Gerais (among the states of Piauí, Maranhão, Tocantins and Bahia) openings of rock walls are the main places chosen by the species for laying and incubating the eggs (COLLAR, 1997; GUEDES, N.M.R. 2020, personal communication).

There are also records for the psittacines Scaly-headed Parrot (*Pionus maximiliani*) (Kuhl, 1820), White-eyed Parakeet (*Psittacara leucophthalmus*) (Statius Muller, 1776) and Pfrimer's Parakeet (*Pyrrhura pfrimeri* Miranda-Ribeiro, 1920) using caves in Brazil. The first two species use caves for reproduction or resting (BUCHMANN *et al.*, 2015; DIAS; CARVALHO, 1982; PONTELLO, 2009; SICK, 1997; ZANATTO, 2016) while the Pfrimer's Parakeet, an endangered species, uses areas next to limestone outcrops, near a cave, in São Domingos, state of Goiás for geophagy (DORNAS *et al.,* 2016). Such outcrops are considered important components of the habitat of this species. There are still no records or descriptions of the nests, but it is believed that they may be associated with cavities or crevices in the limestone massifs.

STRIGIFORMES

The occurrence of species of Strigiformes in caves is more often evidenced by the presence of their egagropylae on the floor than by visualization of individuals. As with Falconiformes, prey carcasses can be found near laying sites during the reproductive period. The species do not build nests but are able to use the nests of other large birds for laying or deposit their eggs directly

Table 1. Records of species of avifauna, with their respective behavioral and reproductive characteristics, carried out in natural cavities of different rocky lithotypes and biomes in Brazil and degree of threat according to the International Union for Conservation of Nature (IUCN).

Order (Family)	Species/ IUCN Category	Popular name (Sexual dichromatism)	Seasons of reproduction/ nesting*	Lithology	Biome	Use (Cavity type)	Philopatry to reproductive site	Parental care
Apodiformes (Apodidae)	*Cypseloides cryptus* Zimmer, 1945/ LC	White-chinned Swift (Absent)	SU, A, W	Silicicl, Gran	AM	Roost, Nesting (Cav)	Yes	Both
	Cypseloides fumigatus (Streubel, 1848)/ LC	Sooty Swift (Absent)	SP, SU	Silicicl, Gran	CE, AF	Roost, Nesting (Cav)	Yes	Both
	Cypseloides senex (Temminck, 1826)/ LC	Great Dusky Swift (Absent)	SP, SU	Silicicl	AM, CE, AF	Roost, Nesting (Cav)	Yes	Both
	Streptoprocne biscutata (Sclater,1866)/ LC	Biscutate Swift (Absent)	SP, SU	Silicicl, Carbon, Gran	CE, AF, CA	Roost, Nesting (Cav)	Yes	Both
	Streptoprocne phelpsi (Collins, 1972)/ LC	Tepui Swift (Absent)	W, SP	Silicicl	AM	Nesting (Cav)	UNK	UNK
	Streptoprocne zonaris (Shaw, 1796)/ LC	White-collared Swift (Absent)	SP, SU	Silicicl, Carbon	AM, CE, AF	Roost, Nesting (Cav)	Yes	Both
Apodiformes (Trochilidae)	*Chlorostilbon lucidus* (Shaw, 1812)/ LC	Glittering-bellied Emerald (Present)	SU, A, W	Ferru	CE, AF	Nesting (Cav)	Yes	Female
	Campylopterus calcirupicola Lopes, Vasconcelos & Gonzaga, 2017/ VU	Dry-forest Sabrewing (Absent)	SP, SU, A	Carbon	CE	Nesting (Cav)	Yes	Female
Caprimulgiformes (Steatornithidae)	*Steatornis caripensis* Humboldt, 1817/ LC	Oilbird (Absent)	SP, SU, A	Silicicl, Carbon	AM	Roost, Nesting (Cav)	Yes	Both
Cathartiformes (Cathartidae)	*Cathartes aura* (Linnaeus, 1758)/ LC	Turkey Vulture (Absent)	W, SP	Carbon	CA, CE, AF	Nesting (Cav)	Yes	Both
	Coragyps atratus (Bechstein, 1793)/ LC	Black Vulture (Absent)	W, SP	Carbon, Silicicl, Ferru	CA, CE, AF	Nesting (Cav)	UNK	Both
	Sarcoramphus papa (Linnaeus, 1758)/ LC	King Vulture (Absent)	SP, SU, A	Carbon, Silicicl	CA, CE, AF	Nesting (CavPar, Cav)	UNK	Both
Columbiformes (Columbidae)	*Leptotila verreauxi* (Bonaparte, 1855)/ LC	White-tipped Dove (Absent)	SP, SU, A	Carbon	AF	Nesting (Cav)	UNK	Both

Order (Family)	Species/ IUCN Category	Popular name (Sexual dichromatism)	Seasons of reproduction/ nesting*	Lithology	Biome	Use (Cavity type)	Philopatry to reproductive site	Parental care
Falconiformes (Falconidae)	*Falco rufigularis* (Daudin, 1800)/ LC	Bat Falcon (Absent)	W, SP	Carbon, Siliciclastic	CA, CE	Nesting (CavWal, Cav)	UNK	Both
	Herpetotheres cachinnans (Linnaeus, 1758)/ LC	Laughing Falcon (Absent)	W, SP	Carbon, Siliciclastic	CA, CE	Nesting (CavPar)	Yes	Both
	Micrastur semitorquatus (Vieillot, 1817)/ LC	Collared Forest-Falcon (Absent)	W, SP, SU	Carbon	AF	Nesting (Cav)	Yes	Both
Passeriformes (Tyrannidae)	*Hirundinea ferruginea* (Vieillot, 1819)/ LC	Cliff Flycatcher (Absent)	SP, SU	Carbon, Siliciclastic	CA	Nesting (Cav)	Yes	Both
	Knipolegus nigerrimus (Vieillot, 1818)/ LC	Velvety Black-tyrant (Present)	SP, SU	Ferru, Carbon, Siliciclastic	CE, AF, CA	Nesting (Cav)	Yes	Both
Passeriformes (Rhinocryptidae)	*Scytalopus petrophilus* Whitney, Vasconcelos, Silveira & Pacheco, 2010/ LC	Rock Tapaculo (Present)	SP, SU	Ferru	CE, AF	Nesting (Cav)	Yes	Both
	Merulaxis ater Lesson, 1830/ NT	Slaty Bristlefront (Present)	UNK	Carbon	AF	Roost, Nesting (Cav)	UNK	UNK
Passeriformes (Furnariidae)	*Cinclodes espinhacensis* Freitas, Chaves, Costa, Santos & Rodrigues, 2012/ EN	Cipo Cinclodes (Absent)	SP, SU	Siliciclastic	CE	Nesting (Cav) (Aflo)	Yes	Both
Passeriformes (Thamnophilidae)	*Myrmelastes caurensis* (Hellmayr, 1906)/ LC	Caura Antbird (Presente)	UNK	Gran	AM	Foraging (Cav)	UNK	UNK
Passeriformes (Troglodytidae)	*Troglodytes musculus* Naumann, 1823/ LC	House Wren (Absent)	SP	Ferru	CE, AF	Nesting (Cav)	Yes	Both
Passeriformes (Turdidae)	*Turdus leucomelas* (Vieillot, 1818)/ LC	Pale-breasted Thrush (Absent)	SP	Ferru	CE, AF	Nesting (Cav)	Yes	Both

Order (Family)	Species/ IUCN Category	Popular name (Sexual dichromatism)	Seasons of reproduction/ nesting*	Lithology	Biome	Use (Cavity type)	Philopatry to reproductive site	Parental care
Passeriformes (Thraupidae)	*Tersina viridis* (Illiger, 1811)/ LC	Swallow-tanager (Present)	W, SP	Ferru, Silicicl	CE, AF	Nesting (Cav)	UNK	Both
Passeriformes (Cotingidae)	*Rupicola rupicola* (Linnaeus, 1766)/ LC	Guianan Cock-of-the-rock (Present)	SP, SU, A	Silicicl, Gran	AM	Roost, Nesting (Cav)	Yes	Female
Procellariiformes (Procellariidae)	*Pterodroma arminjoniana* (Giglioli & Salvadori, 1869)/ VU	Trindade Petrel (Absent)	W, SP, SU, A	Gran	OI	Roost, Nesting (Cav)	Yes	Both
	Puffinus lherminieri Lesson, 1839/ NT	Audubon's Shearwater (Absent)	W, SP	Gran	OI	Roost, Nesting (Cav)	Yes	Both
Psittaciformes (Psittacidae)	*Amazona aestiva* (Linnaeus, 1758)/ NT	Turquoise-fronted Amazon (Absent)	W, SP, SU	Carbon, Silicicl	CE, CA	Roost, Nesting (CavWal)	Yes	Both
	Anodorhynchus leari Bonaparte, 1856/ EN	Lear's Macaw (Absent)	SP, SU, A	Carbon, Silicicl	CA	Roost, Nesting (CavWal)	Yes	Both
	Ara chloropterus Gray, 1859/ NT	Red-and-green Macaw (Absent)	W, SP, SU	Carbon, Silicicl	CE	Roost, Nesting (CavWal, Cav)	Yes	Both
	Anodorhynchus hyacinthinus (Latham,1790)/ VU	Hyacinth Macaw (Absent)	W, SP, SU, A	Carbon, Silicicl	CE	Roost, Nesting (CavWal)	Yes	Both
	Pionus maximiliani (Kuhl, 1820)/ NT	Scaly-headed Parrot (Absent)	W, SP, SU	Carbon	CE	Roost, Nesting (CavWal)	UNK	Both
	Thectocercus acuticaudatus (Vieillot, 1947)/ NT	Blue-crowned Parakeet (Absent)	W, SP, SU	Carbon, Silicicl	CA	Roost, Nesting (CavWal)	UNK	Both
	Psittacara leucophthalmus (Statius Muller, 1776)/ NT	White-eyed Parakeet (Absent)	W, SP, SU	Carbon, Silicicl	CE, AF	Roost, Nesting (CavWal, Cav)	Yes	Both
	Pyrrhura pfrimeri Miranda-Ribeiro, 1920/ EN	Pfrimer's Parakeet (Absent)	UNK	Carbon	CE	Roost, Geophagia (RocOut)	UNK	UNK

Order (Family)	Species/ IUCN Category	Popular name (Sexual dichromatism)	Seasons of reproduction/ nesting*	Lithology	Biome	Use (Cavity type)	Philopatry to reproductive site	Parental care
Strigiformes (Strigidae)	*Bubo virginianus* (Gmelin, 1788)/ LC	Great Horned owl (Absent)	W, SP, SU, A	Carbon, Siliciclastic	AF	Roost, Nesting (CavWal, Cav)	Yes	Both
Strigiformes (Tytonidae)	*Tyto furcata tuidara* (J.E. Gray, 1829)/ LC	Barn Owl (Absent)	A, W, SP, SU	Carbon, Siliciclastic	CE, CA	Roost, Nesting (CavWal, Cav)	Yes	Both

Legend: 1) – 2nd column: LC - Least Concern; NT - Near Threatened; VU - Vulnerable; EN - Endangered; CR - Critically/Endangered/ **2) – 4th column:** A - autumn; W - winter; SP - spring; SU - summer; UNK - unknown; * - The specific breeding seasons may vary between Brazilian regions / **3) – 5th column:** Siliciclastic - siliciclastic (sandstone and quartzite); Carbon - carbonaceous (limestone, marble and dolomite); Ferru - ferruginous (canga, itabirite, banded iron formation); Gran - granitic (granite, vulcanic)/ **4) – 6th column:** AF - Atlantic Forest; CE - Cerrado; CA - Caatinga; AM - Amazon; OI - Oceanic Insular/ **5) – 7th column:** (CavWal) - caves (meso and macro caves) developed in rock walls; (Cav) - caves in general with other forms of insertion in the landscape; (RocOut) - rocky outcrop/ **6) – 8th and 9th columns:** UNK - unknown. **NOTE: The philopatry confirmed here is for the species and not for the individual, as there was no marking methodology in most studies or records.**

on the surface of rock shelters in cavities or protrusions present in walls or canyons, as well as in caves on a layer formed by the items that make up their egagropylae (ARAGÓN *et al.*, 2002; MANCINI *et al.*, 2018; SMITH, 1968). Rodents and bats, animals considered trogloxenes, are part of the diet of these owls (ARAGÓN *et al.*, 2002; JESUS; OLIVEIRA, 2017; LAVARIEGA *et al.*, 2016; LOONEY, 1972; MANCINI *et al.*, 2018), which, together with nocturnal and crepuscular habits, favor their occurrence in or near these environments.

Strigidae

The Great Horned Owl is a Nearctic and Neotropical bird, distributed from Alaska to Nicaragua, and in much of South America, including Brazil (KÖNIG *et al.*, 2008; HOLT *et al.*, 2018; SICK, 1997). Its occurrence in natural subterranean cavities is more documented in North American countries (ARAGÓN *et al., 2002; S*MITH, 1968). There are few studies on the reproductive biology of the species in Brazil and those few did not involve subterranean environments (PATIU *et al.*, 2020; TOMAZZONI *et al.*, 2004). The jacurutu lays two to three eggs (sometimes up to six), all round and white (WIKIAVES, 2021a).

Tytonidae (figures 6 I-K, 8 F)

The suindara is cosmopolitan (absent only at the poles and large deserts) and occurs in open and semi-open habitats, with good tolerance of disturbed areas (ALIABADIAN *et al.*, 2016; BONVICINO; BEZERRA, 2003; ROCHA *et al.*, 2011). The species or its remains are commonly recorded in caves in various Brazilian regions (GODOY, 1986; JESUS; OLIVEIRA, 2017; MANCINI *et al.*, 2018). It usually lays four to seven eggs (WIKIAVES, 2021b).

Bioacoustics as a tool for evaluating anthropic impacts on birds

Bioacoustics is the science that studies the production, transmission, and reception of biological sounds and their relationship with the environment (BRADBURY; VEHRENCAMP, 2011). The recording of sounds produced by animals has been used for decades in the description and identification of species, as well as in ethology (BEER, 1971; MARLER, 2004; VIELLIARD, 1993). With the advancement of passive acoustic monitoring technologies, it is currently possible to record several vocalizations at the same time and monitor environments long-term, without the researcher remaining in the field (PIERETTI *et al.*, 2015).

The set of sounds emitted in an environment constitutes what is called an acoustic landscape, being composed of sounds produced by animals, plants (such as the movement of sap in conducting vessels), abiotic natural phenomena and humans, and their technologies. The recording of environmental sounds with passive monitoring recorders allows the investigation of biological sounds in a broad way, addressing ecological

questions at the level of individuals, species, populations, communities, or landscapes (FARINA, 2014; PIJANOWSKI *et al.*, 2011). This new technology provides the opportunity to analyze the relationships between anthropic, geophonic and biological sounds, and to assess the effects of environmental disturbances on natural communities (DUARTE *et al.*, 2015, SUGAI *et al.*, 2019).

Acoustic communication is essential for birds, as it is used for the selection of sexual partners, flight synchronization and coordination, territorial defense, parental care, foraging, and perception and emission of signals to avoid predators (MARLER, 2004). Several studies have shown that the increase in urbanization, and the consequent expansion of highways and industrial activities, has generated a loss of vegetation, in addition to increased levels of anthropic noise in natural areas (DEICHMANN *et al.*, 2017; DUARTE *et al.*, 2015; LAIOLO, 2010). The suppression of vegetation, in addition to extinguishing habitats, can change the environment in which birds are inserted by removing acoustic obstacles (EY; FISHER, 2009).

Bird vocalizations are the product of thousands of years of evolution that take into account, among other factors, the structural characteristics of the environment for better efficiency in signal transmission (NEMETH *et al.*, 2001). In general, calls emitted in environments with a denser and more complex plant structure degrade with distance much faster than do those emitted in open areas (MORTON, 1975). Therefore, this type of environment favors the transmission of lower frequency and longer wavelength vocalizations (SEBASTIÁN-GONZÁLEZ *et al.*, 2018). More open areas, on the other hand, generally favor the transmission of higher frequency vocalizations, due to the absence of obstacles (MORTON, 1975). Thus, changes in vegetational structure can alter animal acoustic signals, causing them to reach the receiver in a distorted way, which can generate behavioral and evolutionary consequences for populations (WONG *et al.*, 2007).

The anthropic noise generated by urbanization can mask the vocalizations of birds and cause negative impacts on their reproduction and survival (BARBER *et al.*, 2010; ROSA; KOPER, 2018). In general, species vary in their tolerance of noise pollution. In response to noise, some species may abandon their habitats, while others may alter their song structure and vocal behavior to communicate in noisy areas (SLABBEKOORN; RIPMEESTER, 2008). Examples of such alterations include increasing the amplitude of vocalizations; increasing or decreasing frequencies, song duration and number of notes; and changing the time of songs (BRUMM, 2004; DUARTE *et al.*, 2015; FULLER *et al.*, 2007; NEMETH; BRUMM, 2009; SLABBEKOORN; PEET, 2003). However, science still does not know the physiological costs of these behavioral responses and whether they are sufficient to compensate for noise and increase communication efficiency (ZOLLINGER *et al.*, 2011). In addition, the avoidance of areas and compensatory mechanisms to reduce the effects of noise can change the acoustic complexity of an already well-established community and decrease abundance, richness and diversity in noisy environments (BAYNE *et al.*, 2008; PROPPE *et al.*, 2013).

The evaluation, control, and monitoring of noise in areas inhabited by humans in Brazil are based on the resolution of the Conselho Nacional de Meio Ambiente (CONAMA 001/1990), which, in turn, considers the protocols of the Associação Brasileira de Normas Técnicas - NBR10151 (ABNT, 1987) and NBR 10152 (ABNT, 1990). Unfortunately, this legislation does not apply to the maximum noise levels allowed in areas inhabited by wild animals, such as urban parks and conservation units close to noisy human activities. Noise can negatively impact birds, especially those species that emit calls that occupy bands of lower frequency (< 2000 Hz) and are more subject to masking[7] (DUARTE *et al.*, 2015; WARREN *et al.*, 2006).

Therefore, the creation and use of protocols for monitoring the impact of noise on bird communication is essential to foster public policies that regulate the maximum levels of anthropic noise emissions allowed in natural areas. Bioacoustics, therefore, is an important tool in the preparation of licensing studies and the monitoring of environmental impacts. New technologies in passive acoustic monitoring, associated with records of bird behavior, can be important allies in analyzing the impacts of human activities on species that use caves close to mining projects, for example.

Birds in the context of Brazilian speleological environmental licensing

From a quick analysis of the other chapters related to other taxa that make up this book, it becomes clear to the reader that an enormous gap in knowledge exists related to the subject addressed here. In fact, studies are still incipient, but very

7 Occurs when noise is emitted at the same time and occupies the same frequency bands and the same intensity, or a greater intensity, than the animals' vocalizations, thus affecting the efficiency of communication.

promising and important both for Subterranean Biology and for Brazilian Ornithology. We believe that the dependency on the use of caves by birds may vary not only among species, but also among individuals, populations and locations, which would need to be better elucidated, especially in karst areas that are under strong anthropic pressure. Examples include the Quadrilátero Ferrífero, in Minas Gerais; the Maciço do Urucum, in Mato Grosso do Sul and the Serra dos Carajás, in Pará, the main iron-bearing areas of mineral exploration in Brazil (AULER; PILÓ, 2015; FREITAS, 2021). However, in addition to mining, other types of impacts occur in these environments or close to them, such as the construction of small hydroelectric power plants (SHPs), or large plants in biomes such as the Cerrado and the Amazon, in the region of the headwaters of the Tapajós River, Aripuanã River, Teles Pires River and Xingu River (ANEEL 2016; LEES *et al.*, 2016; STOPIGLIA; RAPOSO 2007). Thus, from the advance of impacts in these regions, the species that live and use these environments as fundamental or support areas for reproduction, such as those of Apodidae, for example, become equally vulnerable to the degradation of the caves and the environments that surround them.

In the classification of the degree of relevance of natural subterranean cavities (Article 2, of Decree MMA n.º 6.640/2008 and IN MMA n.º 2/2017) (BRASIL, 2008; BRASIL, 2017), the ecological or biological attribute most directly related to the presence of wild birds in these environments is their use as a "nesting place". As the current legislation only considers the verification of this type of use, any records of nesting behavior can be considered in the valuation of this attribute, regardless of whether it is recurrent or occasional in the cavity under analysis.

Mistakenly, most of the studies done in the context of environmental licensing consider the nests found in caves to be definitely inactive, solely due to the current characteristics of the nest or momentary absence of eggs, nestlings (young), or adults, without carrying out any subsequent follow-up. Many studies, in addition to not informing the builder species, also do not present photographic records of the types of nests found. Such problems occur for the following reasons: punctual sampling in the dry and wet seasons, that is, restricted to only two visits to cavities during the inventory/diagnosis stage; lack of knowledge about species and their nesting biology; lesser importance given to the occurrence of birds in this environment; and lack of consultation with specialist ornithologists to assist in the correct identification of the records obtained. Thus, sampling may simply not coincide with the exact reproductive period of the species, resulting in an underestimate of the use and possibly the reuse of momentarily inactive nests.

Another important fact is that the survey carried out for this chapter was able to identify species that frequently use natural subterranean cavities with less than five meters of linear development for reproduction, which are mostly considered as of low relevance in speleological studies (Article 12 of IN MMA No. 02/2017) (BRASIL, 2017). In this context, many features of shelter type and tafoni, despite being recurrent, may be being neglected in terms of their seasonal importance as a breeding site for birds. In addition, outcrops or rock walls and cliffs are of vital importance for the conservation of several species, which demands ecological knowledge and some caution in defining the use of these areas for the implementation of different types of projects (CARVALHO FILHO *et al.*, 2004).

The practice of better sampling and monitoring, aiming to know the natural history of the species, allows a better understanding of nesting sites, types of nests, and the level of fidelity to reproductive shelters. The results obtained may even help in the future to determine the area of influence and the degree of relevance of these environments. Studies involving specific nesting rates associated with knowledge of reproductive success in cave environments can be compared with reproductive data obtained in an external environment to determine the relative importance of caves for these species at a local or regional level, for example.

Finally, it is important to highlight that, in addition to the use of caves as a nesting site, there are other attributes in the legislation that may be related to the presence of certain species of birds in caves: "essential shelter for the preservation of genetically viable populations of animal species in risk of extinction, on official lists" (e.g., Lear's Macaw); "unique ecological interactions" (e.g., possible parasitism exclusive to the nestling stage of obligate trogloxenes) and "presence of established populations of species with an important ecological function" (e.g., reproductive colonies of swifts).

Methods of locating and accessing nests and registering specimens

The number of speleological ornithologists working in Brazil is extremely limited. However, the exponential growth in the number of birdwatchers and amateur ornithologists working in



Figure 7. Records of birds associated with caves in Brazil and details of cavities used by *Anodorhynchus leari* for nesting. **A)** Male of *Knipolegus nigerrimus* near the entrance of a cave in Parque Nacional da Serra do Gandarela, MG, used for nesting; **B)** adult of *Scytalopus petrophilus* leaving a cave in the Parque Nacional da Serra do Gandarela, MG, used for nesting; **C)** rappelling technique used to access nests of *Anodorhynchus leari* and other psittacines on walls of sandstone/carbonate rocks in the caatinga and cerrado biomes (external image of the wall showing several cavities and the researcher in access activity); **D)** *in loco* record of a wall cave, showing ceiling, floor and walls. Photos: A (Maurício C.M. Andrade); B (Júlio C.R. Costa); C (Patrick Pina); D (Érica C. Pacífico)

the country, associated with the creation and popularization of platforms for sharing sound and photographic records such as *eBird, WikiAves* and *Xeno-canto*, among others, has significantly contributed to Brazilian ornithological science. Rare species that are difficult to observe, new occurrences, and previously undocumented behaviors are being discovered solely through these records.

In addition to these contributors, speleologists in general, consultants and nature enthusiasts can also make significant contributions to scientific knowledge by sharing their records (photographs, sounds, and footage) of species and their nests in caves or other karst features, sometimes involving rare in situ observations. Therefore, appropriate ways and techniques of location, search and approximation are necessary to obtain the best records in these environments, such as those that follow:

1) Wear helmets, overalls or clothing in camouflage or neutral tones, such as beige, olive or gray, while avoiding colored clothing. **2)** If the cave has a significant opening, walk calmly along the access trail, avoiding making any noise and always look towards

8 Material used by birds to build their nests.

the entrance so that if any specimen is present, its escape can be detected and recorded. It is recommended that a camera or camcorder is already turned on and in hand. **3)** Conduct a silent search near the cave entrance, with a particular focus on locating possible nests attached to the walls and identifying traces. **4)** Uppon entering, crouch down and proceed quickly to actively search for nests, in silence, using flashlights for assistance. Check cracks, holes, or platforms (projections) on the ceilings and walls. Also, inspect the floor for nestlings or young individuals, as well as simple/naked or simple/platform nests (SIMON; PACHECO, 2005), and look for traces such as footprints, nesting material[8], insect fragments, bird carcasses, eggshells, feathers, feces, and owl egagropylae. Avoid close contact with bird feces, especially when concentrated or numerous, to prevent infections caused by enterobacteria and fungi with zoonotic potential, which can lead to diseases such as histoplasmosis, psittacosis, and cryptococcosis (BRAZ *et al.*, 2014; DIAZ, 2018; GONZÁLES -ACUÑA; LLANOS- SOTO, 2020; LUGARINI *et al.*, 2008; REOLON *et al.*, 2004). When locating a nest or specimen, maintain a safe distance from the animal, frame it properly, assess the need for artificial lighting, and record consecutively. **5)** Ensure the initial records by approaching slowly and progressively, paying attention to details. For your safety and the well-being of the bird, understand and respect its reproductive behavior, and be mindful of the limits of disturbance and approach. Avoid stressing the specimens and interrupting their reproductive cycle. **6)** If the bird leaves the nest, approach it to check for eggs, nestlings, or feces. When taking a photographic record, use a scale and observe the attachment and composition details. If the nest is closed, and the adult(s) have not been registered, remain crouched in the cave for at least another 10 minutes, preferably in a dark or dimly lit area away from the nest. Wait for the specimen(s) to return or depart before attempting another recording. **7)** In the absence of adults, verification of activity in closed nests can be quickly obtained by optical probe, coupled to a computer or cell phone (PESQUERO *et al.*, 2014; VELOSO *et al.*, 2018), or by a portable digital borescope. Efforts should be taken during this process to avoid touching eggs and/or nestlings and damaging the oological chamber.

The identification of adult birds that may be recorded can be done with the help of traditional image guides in the form of books or booklets (SIGRIST, 2014, 2015), or through smartphone applications that utilize artificial intelligence for specimen identification based on photographs. One example is the Merlin Bird ID application (The Cornell Lab of Ornithology), which is free and can be used offline. This application allows the user to download bird packages specific to a particular region or the entire national collection. It also provides options for identifying birds based on location, morphological features, and behavior.

For studies monitoring the use and/or reuse of nests and nesting success, once the cave has been located and the nest and species have been recorded, the playback of its specific song can be employed to attract it out of the cave. This minimizes reproductive stress and increases the possibility of new records. The use of camera traps is also recommended as a less stressful monitoring technique for birds. However, caution must be exercised in fixing the equipment to avoid compromising the nesting process and the structural integrity of the cave.

Among the techniques for locating and accessing bird nests in caves or natural crevices, such as walls, rocky cliffs, dolines, caves, cliffs, canyons, chasms, or rock faces (Figure 7C), notable methods include the use of mirror poles (OMENA JÚNIOR; MARTINS, 2007), rappelling (PACÍFICO et al., 2014), climbing (SPECHT et al., 2008), drones (MESQUITA et al. 2020) and even the Google Street View tool (OLEA; MATEO-TOMÁS, 2013).

All this variety of techniques and efforts aimed at behavioral or natural history observations of species that occur in karst areas can bring unprecedented or complementary information to the field of ornithology. The tapaculo-serrano, a recently described species (WHITNEY et al., 2010) and, therefore, with little known behavioral biology, had its first and only nesting records occurring in ferruginous caves of the Parque Nacional da Serra do Gandarela, in Minas Gerais (DE ANDRADE *et al.*, 2020; PINTO *et al.*, 2018).

Guidance on collecting nests and eggs and the main Brazilian ornithological collections

The collection and transport of specimens of wild fauna or their biological material (egagropylae, nests, eggs, bones, etc.) *in situ*, among other activities, with educational or scientific purposes, in the national territory, on the continental shelf, in the territorial sea and in the exclusive economic zone, are controlled and regulated by the Instituto Chico Mendes de Conservação da



Figure 8. Illustrations of some bird species associated with karst environments in Brazil. **Rhinocryptidae: A)** adult of *Scytalopus petrophilus*; **Trochilidae: B)** pair of *Chlorostilbon lucidus*; **Steatornithidae: C)** adult of *Steatornis caripensis*; **Psittacidae: D)** adult of *Pyrrhura pfrimeri*; **Furnariidae: E)** adult of *Cinclodes espinhacensis*; **Tytonidae: F)** adult of *Tyto furcata*; **Cotingidae: G)** pair of *Rupicola rupicola*. Artist: Leonardo Marujo.

Biodiversidade (ICMBio), through Portaria Nº 748, of September 19, 2022, which, among other objectives, establishes the rules for the use of the Sistema de Autorização e Informação em Biodiversidade (SISBio). The teacher or researcher interested in obtaining an authorization or a permanent license for collection must first be registered with SISBio. It is important to point out that a permanent license is not valid for sampling in caves.

On the other hand, collections, captures and transport carried out during the management stages (survey, monitoring, rescue and disposal) of wild fauna and arising from activities that involve environmental licensing, can be regulated and controlled by IBAMA (undertakings that involve possible impacts in more than one state jurisdiction), ICMBio (undertakings involving possible impacts on Unidades de Conservação Federais) and/or by the competent state environmental agency (undertakings that do not contemplate the mentioned situations). In possession of the proper authorizations, the teacher, researcher or environmental consultant must identify the specimens collected, and send them to specialists or resorting to scientific collections as necessary.

The collection and transport of eggs and nests are only recommended when these are not fully known to science. Therefore, records obtained in detail (photographs), associated with field observations, should be sent to specialist ornithologists for possible identification of the builder bird, avoiding unnecessary collections and possible impacts on the species and the cave environment. Once the scientific novelty is confirmed, it will be up to the specialist to provide all the guidelines regarding the need and procedures for collecting and preserving samples for description and deposition in a scientific collection. Before any collection is performed, disposable gloves should be used to check the measurements of the nest, after which eggs, preferably spoiled ones, can be carefully removed. The nest must always be removed from its base and/or laterality of insertion, preserving its shape, oological chamber and original constitution.

Currently, there are 59 ornithological collections in Brazil, of which approximately 85% are fully or partially digitized (FONTANA *et al.*, 2017). Most of these collections (60%), including eggs and nests, are located in the South and Southeast regions of the country, with specimens from the Atlantic Forest (ALEIXO; STRAUBE, 2007; FONTANA *et al.*, 2017). In addition to biological material, many collections also provide sound and visual records of numerous species, which can help identify taxa recorded in the field. One example is the audiovisual collection of the Museu de Zoologia "Adão José Cardoso", at the Universidade Estadual de Campinas, which is integrated with the Fonoteca Neotropical Jacques Vielliard (FNJV) and by collections of videos and photos. The five most valuable Brazilian ornithological collections are: Museu Paraense Emílio Goeldi; Museu de Zoologia of the Universidade de São Paulo (MZUSP); Museu de Ciências e Tecnologia of the Pontifícia Universidade Católica do Rio Grande do Sul; Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ); and the Instituto Nacional de Pesquisas da Amazônia (INPA) (FONTANA *et al.,* 2017).

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Mimon bennettii
Robson de Almeida Zampaulo

26

Bats

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General introduction

Bats are known to be the only mammals able to fly. They comprise the order Chiroptera, the second largest order of mammals, with more than 1,400 known species worldwide and 181 species, distributed in 68 genera and nine families, registered so far in Brazil (GARBINO *et al.*, 2020a).

With a remarkable diversity of shapes, morphological adaptations and eating habits that allow the use of the most varied niches (KALKO, 1997; PEDRO *et al.*, 1995), bats also developed the ability to emit high frequency sounds for echolocation. This ability helps with spatial location and movement in dark environments, in addition to being an important ally in the detection of food resources. Bats can be classified according to food preference as frugivores, insectivores, nectarivores, hematophages, piscivores and carnivores.

Bats can shelter in the most varied structures, which can be natural (tree hollows, leaves, caves, rocks, among others) or artificial (abandoned civil constructions, under roof tiles or in ceilings, bridges and manholes). Subterranean natural cavities, or simply caves, are also important shelters for bats around the world, functioning as refuges, providing protection and a relatively stable environment (KUNZ, 1982; KUNZ and LUMSDEN, 2003; TRAJANO, 1985). According to data updated for this chapter, about 45% of the known bat species of Brazil have been recorded in caves, which are used permanently or temporarily as daytime shelters or night roosts (*e.g.*, places of passage, food consumption or digestion). In temperate zones, caves are also used by some species for hibernation. In general, many caves support a high richness of bat species throughout the seasons, functioning as important resources for these animals, which promote and maintain important ecosystem services (FUREY; RACEY, 2016), such as pollination, dispersal of plant species and the control of insect populations (KUNZ; LUMSDEN, 2003; PIMENTEL *et al.*, 2022).

More stable environmental conditions inside caves have been considered important attributes involved in the selection of caves by bats (FUREY; RACEY, 2016; KUNZ, 1982), but it is known that the structure, diversity and distribution of caves, in addition to the availability of resources present in the surrounding landscapes, are also relevant factors for this selection (ARITA, 1996; GILBERT; DEHARVENG, 2002; KUNZ, 1982; KUNZ; LUMDSEN, 2003; TRAJANO, 1985). The choice of caves as shelters by bats may also be mediated by species-specific or even interspecific requirements (AVILA-FLORES; MEDELLÍN, 2004; BARROS *et al.*, 2020; RODRÍGUEZ-DURÁN; SOTO-CETENO, 2003; VARGAS -MENA *et al.*, 2020). However, data on the biology of cave bats are still scarce, especially those that may be correlated with limitations and vulnerabilities regarding the selection of habitats and shelters.

Light is a limiting factor for cave biota, given that in deeper zones (aphotic), primary production by photosynthetic organisms is nonexistent, which is restricted to the euphotic or penumbra zone. Thus, the availability of trophic resources necessary for the maintenance of cave communities is largely dependent on the input of allochthonous nutrients, carried in the form of dissolved or particulate organic matter, the latter consisting of leaf litter and animal remains and excrement (FERREIRA; MARTINS, 1999, 2009; POULSON, 1972; SILVA *et al.*, 2011). This is exactly where the importance of bats for these ecosystems exists. Classified as trogloxenes, they use caves as shelters, but need to emerge from them to forage and seek their own food. Bat feces, called guano, deposited on the floor of caves provides the organic matter that nourishes decomposers, establishing a food base for the complex trophic networks of cave ecosystems.

This situation makes the presence of bats indispensable for the existence of life in some caves where the main source of energy is guano, which is therefore of vital importance for many cave species that are often endemic to this type of environment (BAHIA; FERREIRA, 2005; FERREIRA *et al.*, 2000; FERREIRA; MARTINS, 1998; GNASPINI; TRAJANO, 2000).

Considering that caves can be excellent shelters for bats, some species are able to colonize this environment, forming

congregations that can range from a few to thousands of individuals. These caves that house stable and exceptionally sized populations are called "*bat caves*" (Figure 1). These colonies are extremely important for caves, as they are responsible for depositing large amounts of guano (Figure 1D), which supports other communities of cave organisms, making bats essential animals for maintaining the ecological balance in the subterranean environment (FERREIRA *et al.*, 2000, 2007; JAFFÉ *et al.*, 2018; PALMERIM; RODRIGUES, 1992; TRAJANO; BICHUETTE, 2006).

Finally, it is worth noting that knowledge about the biology of cave chiropterofauna species, and understanding the dynamics of subterranean ecosystems, as well as the relationship between bats and caves, are essential to necessary conservation and



Figure 1. A-B) Examples of congregations of bats of the family Mormoopidae (*Pteronotus* sp.) in bat caves located in the state of Pará; **C)** moment of the departure of bats to the epigean environment in the Floresta Nacional de Carajás, state of Pará; **D)** large deposit of guano in Caverna Furna Feia, Rio Grande do Norte. Photos: A (Daniele Pedrosa de Oliveira); B (Mariane Soares Ribeiro Pereira); C (Xavier Prous); D (Diego de M. Bento).

management actions, such as decision-making for the selection of priority areas for conservation.

Brief history of studies of cave bats in Brazil

One of the first studies on bats in caves in Brazil was carried out by Augusto Ruschi and published in 1952 in the Boletim do Museu de Biologia Mello Leitão. Ruschi carried out a survey of the fauna associated with three caves (Limoeiro, Monte Líbano and Itaúnas) located, respectively, in the municipalities of Castelo, Cachoeiro do Itapemirim and Conceição da Barra, in the state of Espírito Santo, bringing data on cohabitation, behavior and the home range of bats (RUSCHI, 1952).

In the 1980s, Dessen and collaborators published data on invertebrate and vertebrate cave fauna (including bats) of 28 caves in six different regions of Brazil (DESSEN *et al.*, 1980). From the 1980s onwards, the most intensive work on cave chiropterofauna in Brazil began, carried out by Eleonora Trajano in the complex of caves located in Vale do Alto Ribeira, state of São Paulo (TRAJANO, 1985, 1995, 1996; TRAJANO; GIMENEZ, 1998). Since then, studies developed by Trajano, her students and collaborators have contributed with unprecedented information on the biology and ecology of cave chiropterans (ARNONE, 2008; ROCHA; BICHUETTE, 2016; TRAJANO, 1995, 1996; TRAJANO; GIMENEZ, 1998).

More recently, Guimarães and Ferreira (2014) presented a very important compilation on cave bats in Brazil, considering their own records and those found in the literature. It is true that studies directed at cave bats are still incipient in Brazil, given that, according to those authors, only about 2% of the more than 12,000 known caves in the country at the time had information available on chiropterofauna. However, it is worth noting that more than 22,000 caves are currently known in the country (CECAV, 2021).

Therefore, in view of the existing knowledge gaps, this chapter intends to present the state of the art about bats that use caves in Brazil. The intention here is to present a compilation of information about bat species that use caves as shelter, in addition to presenting the various methodologies that can be applied in studies, methods for capturing, collecting and fixing specimens and information about Brazilian scientific collections.

Diversity of bats in Brazilian caves

The order Chiroptera is highly diverse in the Neotropics, with Brazil, according to available knowledge, being home to about 12% of the 1,400 known bat species on the planet (BERNARD *et al.*, 2011; TAVARES *et al.*, 2012). According to the Sociedade Brasileira para o Estudo de Quirópteros (SBEQ), the country has recorded the occurrence of 181 species of bats distributed in nine families and 68 genera (GARBINO *et al.*, 2020a).

Bibliographic and scientific collection reviews, and specific works on cave bat fauna, indicate that at least 80 species from all nine families and 50 genera have already been recorded in Brazilian caves (Table 1) (BARROS *et al.*, 2020; GUIMARÃES; FERREIRA, 2014; OLIVEIRA *et al.*, 2018; TAVARES *et al.*, 2012; TORRES; BICHUETTE, 2019), making up about 44% of the bat species that occur in the national territory. Some species found in Brazilian caves are illustrated in Figure 2.

The relationships of bats with their roosts range from obligate to opportunistic (KUNZ, 1982). Arita (1993) proposed that bats be classified according to the use they make of caves as: **preferably cavernicolous** - species for which caves are the main shelter; **usually cavernicolous** - species often found both in caves and other shelters; **occasionally cavernicolous** - species that have been recorded in caves, but have a preference for other shelters; and **non-cavernicolous** - species not recorded in caves.

However, in Brazil, the classification most used today is an adaptation of the aforementioned classification proposed by Guimarães and Ferreira (2014). The classification was modified by the authors from four to three categories: **essentially cavernicolous** - species that mostly live in caves; **opportunistically cavernicolous** - species that use caves opportunistically, but routinely use another type of shelter; and **non-cavernicolous** - species not recorded in caves or that prefer other shelters (including species sampled in only one cave).

More recently, Voss *et al.* (2016) proposed categories of guilds considering roosts for bats in central and western Amazonia and French Guiana, with bats that use caves being classified as "*rock- and/or cave-roosting species*".

Among the embalonurids recorded in natural subterranean cavities in Brazil, species of the genus *Peropteryx* are more associated with rocky environments, being commonly observed in caves, especially closer to the entrances (personal observation).

Table 1. List of species of bats with occurrence in Brazilian caves and reference where the record is located: 1. Tavares *et al.* (2012); 2. Guimarães and Ferreira (2014); 3. Oliveira *et al.* (2018); 4. Torres and Bichuette (2019); 5. Barros *et al.* (2020). Only species with confirmed occurrence for Brazil were considered in this survey (GARBINO *et al.*, 2020a).

Order Chiroptera (9 families, 50 genera, 80 species)		
Families and Subfamilies	**Species**	**References**
Emballonuridae Gervais, 1856 (3 genera, 5 species)		
Emballonurinae Gervais, 1856	*Peropteryx kappleri* Peters, 1867	1, 2, 3, 5
	Peropteryx macrotis (Wagner, 1843)	1, 2, 3, 5
	Rhynchonycteris naso (Wied, 1820)	3, 4
	Saccopteryx bilineata (Temminck, 1838)	2, 3
	Saccopteryx leptura (Schreber, 1774)	2, 5
Phyllostomidae Gray, 1825 (30 genera, 45 species)		
Micronycterinae Van Den Bussche, 1992	*Lampronycteris brachyotis* (Dobson, 1879)	1
	Micronycteris megalotis (Gray, 1842)	1, 2, 3
	Micronycteris microtis Miller, 1898	1
	Micronycteris minuta (Gervais, 1856)	2, 3, 5
Desmodontinae Wagner, 1840	*Desmodus rotundus* (É. Geoffroy, 1810)	2, 3, 4, 5
	Diaemus youngii (Jentnik, 1893)	2, 3, 5
	Diphylla ecaudata Spix, 1823	1, 2, 3, 4, 5
Lonchorhininae Gray, 1866	*Lonchorhina aurita* Tomes, 1863	1, 2, 3, 4, 5
Phyllostominae Gray, 1825	*Chrotopterus auritus* (Peters, 1856)	2, 3, 4, 5
	Gardnerycteris crenulatum (É. Geoffroy, 1803)	3
	Lophostoma brasiliense Peters, 1867	2
	Lophostoma silvicola d'Orbigny, 1836	3
	Macrophyllum macrophyllum (Schinz, 1821)	2, 3, 5
	Mimon bennettii (Gray, 1838)	2, 3, 4, 5
	Phylloderma stenops Peters, 1865	2, 3, 5
	Phyllostomus discolor Wagner, 1843	2, 3
	Phyllostomus elongatus (É. Geoffroy, 1810)	1, 2, 5
	Phyllostomus hastatus (Pallas, 1767)	2, 3, 5
	Phyllostomus latifolius (Thomas, 1901)	1
	Tonatia bidens (Spix, 1823)	2, 3, 4, 5
	Trachops cirrhosus (Spix, 1823)	1, 2, 3, 4, 5
	Vampyrum spectrum (Linnaeus, 1758)	3,5

Glossophaginae Bonaparte, 1845	*Anoura caudifer* (É. Geoffroy, 1818)	1, 2, 3, 4, 5
	Anoura geoffroyi Gray, 1838	1, 2, 3, 4
	Choeroniscus minor (Peters, 1868)	2, 3
	Glossophaga soricina (Pallas, 1766)	1, 2, 3, 4, 5
Lonchophyllinae Griffiths, 1982	*Hsunycteris thomasi* (Allen, 1904)	1
	Lionycteris spurrelli Thomas, 1913	1, 2, 3, 4, 5
	Lonchophylla bokermanni Sazima, Vizotto & Taddei, 1978	2
	Lonchophylla dekeyseri Taddei, Vizotto & Sazima, 1983*	2, 3, 4, 5
	Lonchophylla mordax Thomas, 1903	2, 3
Carolliinae Miller, 1924	*Carollia brevicauda* (Schinz, 1821)	1
	Carollia perspicillata (Linnaeus, 1758)	1, 2, 3, 4, 5
Glyphonycterinae Baker *et al.*, 2016	*Glyphonycteris behnii* (Peters, 1865)	2, 3
	Glyphonycteris sylvestris Thomas, 1896	2, 3
Stenodermatinae Gervais, 1856	*Artibeus fimbriatus* Gray, 1838	2, 3
	Artibeus lituratus (Olfers, 1818)	2, 3, 4
	Artibeus obscurus (Schinz, 1821)	2, 3
	Artibeus planirostris (Spix, 1823)	2, 3, 5
	Chiroderma doriae Thomas, 1891	2, 3, 4
	Platyrrhinus lineatus (É. Geoffroy, 1810)	2, 3, 4, 5
	Pygoderma bilabiatum (Wagner, 1843)	2, 3
	Sturnira lilium (É. Geoffroy, 1810)	2, 3, 4
	Sturnira tildae de la Torre, 1959	2, 3, 4
	Vampyressa pusilla (Wagner, 1843)	4
Mormoopidae Saussure, 1860 (1 genera, 3 species)		
	Pteronotus personatus (Wagner, 1843)	1, 3
	Pteronotus rubiginosus (Wagner, 1843)	1, 2, 3, 5
	Pteronotus gymnonotus (Wagner, 1843)	1, 2, 3
Noctilionidae Gray, 1821 (1 genera, 2 species)		
	Noctilio albiventris Desmarest, 1818	3
	Noctilio leporinus (Linnaeus, 1758)	2
Furipteridae Gray, 1866 (1 genera, 1 specie)		
	Furipterus horrens (Cuvier, 1828)*	1, 2, 3, 4, 5

Thyropteridae Miller, 1907 (1 genera, 1 specie)		
	Thyroptera tricolor (Spix, 1823)	2, 3
Natalidae Gray, 1866 (1 genera, 1 specie)		
	Natalus macrourus (Gervais, 1856)*	1, 2, 3, 4, 5
Molossidae Gervais, 1856 (7 genera, 10 species)		
Molossinae Gervais, 1856	*Cynomops abrasus* (Temminck, 1826)	3
	Eumops bonariensis (Peters, 1874)	3
	Eumops perotis (Schinz, 1821)	3
	Molossops temminckii (Burmeister, 1854)	2, 3, 5
	Molossus molossus Pallas, 1766	2, 3
	Molossus rufus É. Geoffroy, 1805	2
	Nyctinomops laticaudatus (É. Geoffroy, 1805)	2, 3, 5
	Nyctinomops macrotis (Gray, 1840)	2, 3
	Promops nasutus (Spix, 1823)	3
	Tadarida brasiliensis (I. Geoffroy, 1824)	3
Vespertilionidae Gray, 1821 (5 genera, 12 species)		
Vespertilioninae Gray, 1821	*Eptesicus brasiliensis* (Desmarest, 1819)	2, 3
	Eptesicus diminutus Osgood, 1915	3, 4
	Eptesicus furinalis (d'Orbigny & Gervais, 1847)	3
	Histiotus velatus (I. Geoffroy, 1824)	3
	Lasiurus ega (Gervais, 1856)	2, 3
	Lasiurus blossevillii ([Lesson, 1826])	3
	Rhogeessa hussoni Genoways e Baker, 1966	5
Myotinae Tate, 1942	*Myotis lavali* Moratelli, Peracchi, Dias & Oliveira, 2011	5
	Myotis levis (I. Geoffroy, 1824)	3
	Myotis nigricans (Schinz, 1821)	2, 3, 4
	Myotis riparius Handley, 1960	2, 3
	Myotis simus Thomas, 1901	4

* Species on the list of species threatened with extinction (BRASIL, 2022).



Figure 2. A) *Lonchorhina aurita;* **B)** *Lampronycteris brachyotis;* **C)** *Mimon bennettii;* **D)** *Platyrrhinus lineatus;* **E)** *Diphylla ecaudata;* **F)** *Desmodus rotundus;* **G)** *Carollia perspicillata;* **H)** *Anoura geoffroyi;* **I)** *Furipterus horrens;* **J)** *Pteronotus gymnonotus;* **K)** *Peropteryx macrotis;* **L)** *Natalus macrourus.* Photos: A-K (Robson de A. Zampaulo); L (Mariane S. R. Pereira).

In the case of Micronycterinae, *Lampronycteris brachyotis* (Dobson, 1879) and *Micronycteris microtis* Miller, 1898 are apparently more strongly associated with caves in the Carajás region (TAVARES *et al.*, 2012), and *M. microtis* seems to have the same pattern of association in the Southeast, in caves of iron regions in Minas Gerais (GOMES *et al.*, 2015).

Hematophagous bats, that is, species of the subfamily Desmodontinae, are often found associated with caves in Brazil, and the common vampire bat, *Desmodus rotundus* (É. Geoffroy, 1810), also frequently occupies artificial shelters or other types of natural shelters (GOMES; UIEDA, 2004).

Bats of the subfamily Phyllostominae use natural subterranean cavities in Brazil, such as *Chrotopterus auritus* (Peters, 1856), *Phylloderma stenops* Peters, 1865, *Phyllostomus hastatus* (Pallas, 1767), and *Tonatia bidens* (Spix, 1823), but most of them also use other types of shelters. The species of Phylostominae apparently most associated of the genus *Lophostoma* are, for example, known to be specialists in using termite mounds (termites) as shelters (ESQUIVEL *et al.*, 2020). The species of Phylostominae apparently most associated with caves are *Mimon bennettii* (Gray, 1838) and *Phyllostomus latifolius* (Thomas, 1901), the latter found exclusively in caves in the Carajás region (TAVARES *et al.*, 2012).

Among the predominantly nectarivorous bats, the glossophagines, those of the genus Anoura are more associated with caves, while *Glossophaga soricina* (Pallas, 1766), although quite common in caves, uses a wide spectrum of types of shelters (REIS *et al.* al., 2007; REIS *et al.*, 2017; WEBSTER, 1993). Among lonchophylines, species of the genera *Lionycteris* and *Lonchophylla* are more associated with caves, with the exception of *Lonchophylla mordax* Thomas, 1903, for which data are sparse, and *Xeronycteris vieirai* Gregorin & Ditchfield, 2005, endemic to the caatinga and has only been recorded so far in a cave in Rio Grande do Norte (CORDEIRO-SCHMIDT *et al.*, 2017).

All bats of the subfamilies Carollinae (predominantly frugivores), Glyphonycterinae (predominantly insectivorous leaf-cleaners) and Stenodermatinae (fruitivores), in addition to bats of the family Noctilionidae, use various types of shelters. However, those of Carollinae seem to use caves more frequently as shelters in some regions where they occur in greater concentration (TAVARES *et al.*, 2012).

Among members of the family Molossidae, only *Nyctinomops* seems to be more associated with caves, and populations of hundreds to thousands of individuals of the species *Nyctinomops laticaudatus* (É. Geoffroy, 1805) can be found in boulders of the Madeira River, western Amazonia (TAVARES *et al.*, 2017).

The family Natalidae, or funnel-eared bats, are represented in Brazil by the species *Natalus macrourus* (Gervais, 1856) and the furipterids by the species *Furipterus horrens* (Cuvier, 1828), both of which are strongly associated with rocky shelters in the country, including caves, with *F. horrens* being found in other types of non-rocky shelters in other locations outside Brazil (VOSS *et al.*, 2016). Other possible non-rock shelters are also mentioned in the literature for mormoopides but in Brazil species of *Pteronotus* are strongly associated with caves in the North and Northeast regions of the country.

Main methods used in the study of cave bats

Studies on bats that use caves can be carried out in different ways, using different methods, depending on the objectives.

To carry out such studies, as well as those involving the management of Brazilian wild fauna, it is first necessary to provide the correct documentation, including a request for authorizations for capture and collection issued by the competent environmental agencies: state secretaries of the environment, Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

It is very important that the professionals involved in the fieldwork are properly trained and qualified to capture and handle animals, as well as have knowledge of health protocols, human safety and animal ethics.

CAPTURE METHODS

One of the most widely used methods for bat inventories in the external environment is mist netting. The use of nets has also been applied to cave studies in different ways, especially at the entrance to these environments. However, it is important to point out that the use of capture traps in caves must comply with the provisions of the Resolução Conselho Federal de Biologia - CFBio 301/2012.

Mist nets can be found in specialized stores and come in different sizes and meshes. Some points to be considered when choosing a net are the size and shape of the cave entrance, adapting the net, whenever possible, to these variables. In addition to being a scientifically recognized method, the use

of nets allows standardization of the sampling effort employed (BIANCONI; STRAUBE, 2003).

Despite being a recommendable method for the vast majority of caves with small populations and complex morphologies (irregular ceilings, narrow conduits or low ceilings, labyrinths, among others), nets should be used with caution and consideration of disarmament, depending on the intensity of captures (ROCHA; BICHUETTE, 2016).

The use of nets for sampling in caves must be extremely parsimonious, since nets close to the exit of shelters increases the chance of many individuals being captured in a short period. Thus, before setting up a net near any cave, it must be visually inspected and, in cases where large populations are present, another methodology must be chosen. It is also important to point out that nets must be carefully monitored over short time intervals (in general, intervals of 20 minutes) to remove captured individuals and reduce the stress caused to animals.

On the other hand, armadilhas de harpa or *harp-traps*, which are still relatively little used in sampling bats in Brazilian caves, are a good alternative for caves occupied by large populations. A harp trap is composed of two parallel rods of rigid material, from which one or more rows of equidistant nylon threads depart (Figure 3). The bottom part of the trap is made up of a bag where the bats fall after colliding with the nylon threads (CONSTANTINE, 1958; KUNZ; KURTA, 1988; PALMERIM; RODRIGUES, 1993). This type of trap can be installed at the entrance of the cave, with captured individuals being removed manually and very quickly, reducing animal stress and damage to equipment (*e.g.*, mist nets). Its uninstallation is quick and can be considered a good alternative for collections in caves with large numbers of bats (ZORTÉA *et al.*, 2015).

Another trap widely used to capture bats in natural subterranean cavities is the hand net. This method can be quite efficient for experienced collectors and in caves where the morphology allows reaching the resting places of bats (PERACCHI; NOGUEIRA, 2010). However, cavities with high ceilings and with very heterogeneous morphology can influence visualization and, consequently, sampling efficiency (TRAJANO, 1985). The efficient use of this method depends on the researcher's skill with the equipment, which, in the case of inexperience, can cause, in addition to sample bias, injuries or even death of individuals due to contacting the animal with parts of the equipment.

In addition to capture methods, direct observation is very important for understanding the structure of colonies inside of a cave and the distribution of bats in relation to the environmental conditions of the ecosystem (BARROS *et al.*, 2020). This information can be useful for planning sampling and, in association with other methods, in research involving ecology and behavior. However, direct observation alone is not recommended for species identification and population estimates. Population censuses carried out using only this methodology are highly questionable and imprecise, and may underestimate or overestimate bat populations in caves. On the other hand, individual counting methods and population estimates based on recapture rates are very important for bat ecology work in natural subterranean cavities (ELLIOT *et al.*, 2005; OTÁLORA-ARDILA *et al.*, 2019; VARGAS-MENA *et al.*, 2020). More recently, thermal cameras have also been used for this purpose in caves with large bat populations such as bat caves (AMMERMAN *et al.*, 2009, OTÁLORA-ARDILA *et al.*, 2019).

It is important to point out that the methodology used must be chosen according to the research objectives and that the use of complementary methodologies is essential to obtain quality and reliable data (GUIMARÃES; FERREIRA, 2014; KUNZ *et al.*, 2009; TRAJANO, 1985). In many cases, as caves are located in areas of difficult access, with stretches of long walks or high financial cost to access them, such as in more remote regions of the country for example, opting for a simpler methodology may often seem ideal. However, one must consider that each collection is a unique opportunity and that optimizing sampling designs to carry out the best and most complete inventories, according to the proposed objectives and questions, can bring important results and save resources, as well as minimize stress caused to animals.

Finally, it is worth remembering that, regardless of the chosen method, in all cases when there is contact between bats and equipment, it is important to adopt a cleaning routine between episodes of field work. In this way, possible contamination and spread of diseases between bats from different regions can be prevented (SHELLEY *et al.*, 2013).

MARKING METHODS

Marking methods for individual identification of bats are necessary in some studies that aim to evaluate displacement,

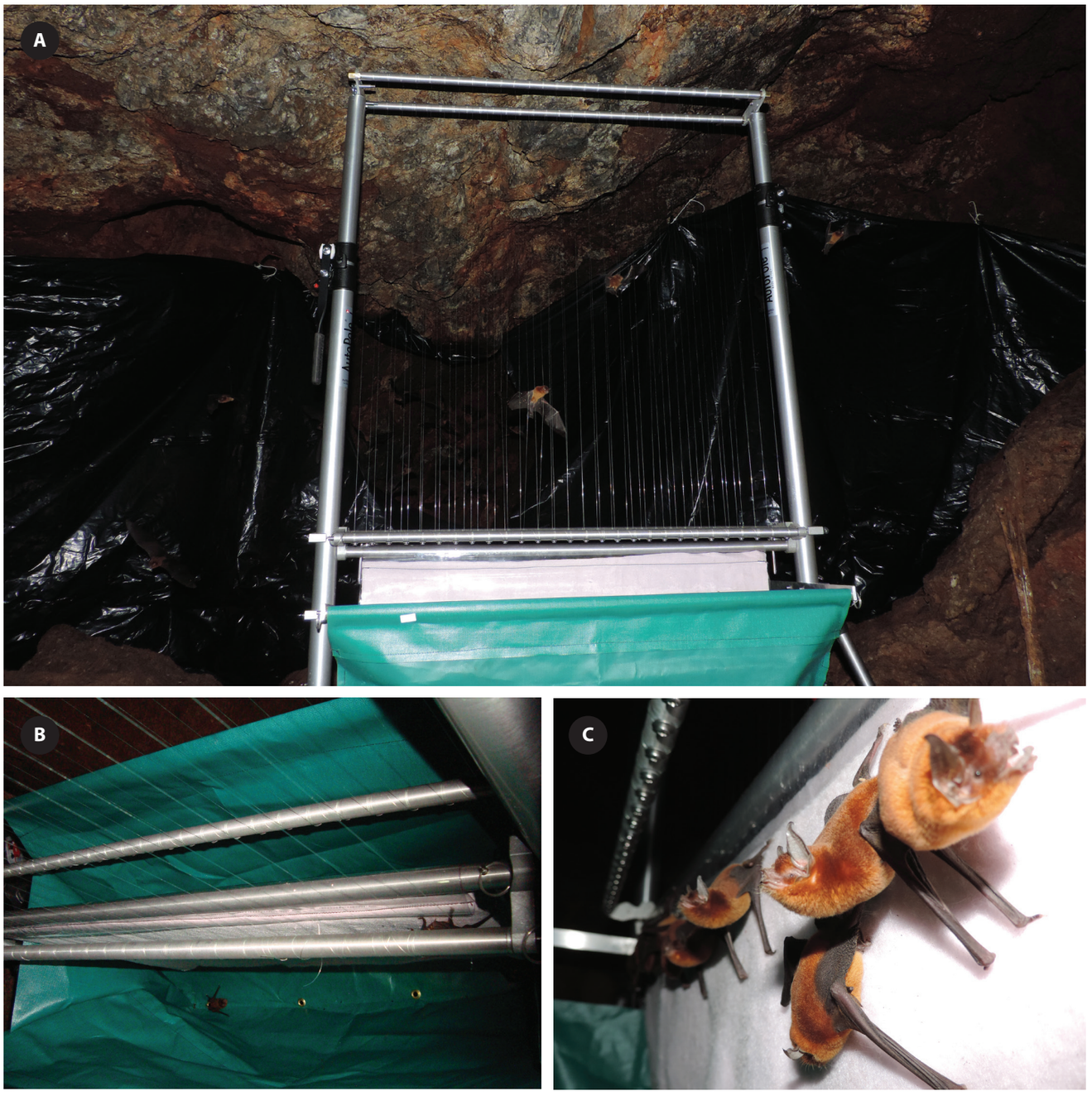


Figure 3. Armadilha de harpa or *harp trap*. Photos: Vale Collection.

rates of infestation and reinfestation by ectoparasites, population size and fidelity to shelters, among other aspects of bat biology (BARROS *et al.*, 2006; BERNARD; FENTON, 2003; BIANCONI *et al.*, 2004; LOURENÇO, 2011; OLIVEIRA, 2010; TRAJANO,1996).

Research carried out by Augusto Ruschi in the 1950s is possibly among the first to include the individual marking of bats captured in caves in Brazil. In this study (RUSCHI, 1952), aluminum washers placed on the forearms of captured individuals were used to acquire data to estimate areas of use of different colonies of bats in caves in the municipality of Santa

Teresa, state of Espírito Santo.

In addition, a few studies on bats in Brazilian caves have included tagging data (ARNONE, 2008; PEREIRA, 2018; REIS, 2018; ROSADA *et al.*, 2015; SANTANA, 2006; TRAJANO, 1985). The use of marking for consulting work related to environmental licensing varies according to the researcher and there is no standardization of marking method. However, the last five years has seen more frequent marking of bats sampled in speleological studies due to the request of technicians from licensing environmental agencies, or on the initiative of those responsible for the projects, since the results can be important to understanding population dynamics and the biology of species that roost in caves.

In general, there are several possible marking techniques, which should be chosen according to the objective and availability of resources for the study. Markings can be temporary (*e.g.*, use of hair dye or discoloration, hair cutting, painting nails or even making small holes in the patagium) or semi-permanent (rings on the forearm, collars with rings, microchips, among others) (ALLEN, 1921; BARROS *et al.*, 2012; KUNZ; WEISE, 2009; PACHECO, 2004; STEBBINGS, 2004; TRAPIDO; CROWE, 1946). The choice of marking method should be based on the characteristics of each species, always looking for the most appropriate method that also causes the least possible inconvenience to the marked individual. The use of an inappropriate marking technique or the failure to observe animal-specific issues (such as semi-permanent marking of young individuals or inattention to the morphological characteristics of each species) can compromise the development and other vital activities of bats. Consequently, the injuries caused (Figure 4) can result in permanent and disabling damage, with the potential to doom the individual to death (BARROS *et al.*, 2012; KUNZ; WEISE, 2009; STEBBINGS, 2004).

Unfortunately, Brazil still does not have databases or a national banding system that would unify and standardize markings carried out in the country, such as the Sistema Nacional de Anilhamento de Aves Silvestres (SNA) for birds, managed by the Centro Nacional de Pesquisa e Conservação de Aves Silvestres (CEMAV/ICMBio). The lack of standardization of the methods and codes used in banding and the centralization of data make it impossible to have a more comprehensive understanding of migration processes and the use of space and shelters by bats, limiting the generation of basic knowledge about displacements, lifespan and fidelity of roost (caves) use, among other aspects.

TELEMETRY

Telemetry is a tool used in studies of behavior and ecology that are important for the promotion of management and conservation actions for several animal species. The technique basically consists of fixing a radiofrequency transmitter or GPS (Global Positioning System) on the body of a given animal and later monitoring the signal emitted by means of antennas and receivers (fixed or mobile) or via satellite, during a period of time (JACOB; RUDRAN, 2003; MELLO, 2010; POWELL; MITCHELL, 2012).

Telemetry has been used in studies of Neotropical bats to identify shelters, movement patterns, space use and habitat preferences (AGUIAR *et al.*, 2014; BERNARD; FENTON, 2003; RIBEIRO *et al.*, 2019; TREVELIN *et al.*, 2013). This method can also be used in behavioral studies to help locate monitored animals.

BIOACOUSTICS

Bioacoustics is the branch of science that studies animal communication through sound (*e.g.*, how sounds are produced, emitted, received and used intra- or interspecifically). It has applications in ethological, physiological, biophysical, zoological and ecological studies. Acoustic signals are used by different animals in different situations and ways and in different contexts, such as defending territory, interacting with groups, attracting a sexual partner, searching for food or location, as is the case with bats (AHLÉN and BAAG, 1999; FENTON *et al.*, 1987; PEREIRA, 2011).

Assuming that bioacoustics uses information captured from the ultrasound emitted by the echolocation of bats, this tool presents itself as a non-invasive method with a good cost-benefit ratio. It also has the potential to help, alone or together with other methods, in increasing information on the richness, behavior and ecology of Neotropical bat species (ARIAS-AGUILAR *et al.*, 2018; HINTZE *et al.*, 2016).

Bioacoustic methods, despite being widely used in studies of bats in temperate countries (AHLÉN; BAAG, 1999; FENTON *et al.*, 1987), may still encounter some challenges in tropical regions (ARIAS-AGUILAR *et al.*, 2018; HINTZE *et al.*, 2016; WALTERS *et al.*, 2013). The great diversity of species, the lack of extensive knowledge about their acoustic profiles and the high similarity



Figure 4. Injuries observed as a result of the use of washers **(A and B)** and collar **(C and D)**. Photos: A-C (Aline da Silva Reis); D (Mariane S. R. Pereira).

found in the calls of some groups (especially phyllostomid bats) make up the main challenges of implementing bioacoustics in studies of Neotropical bats (ARIAS-AGUILAR *et al.*, 2018; HINTZE *et al.*, 2016; WALTERS *et al.*, 2013). Another important point that should be mentioned is that bioacoustic studies generate a large volume of information and the manual processing of data can be difficult or even unfeasible (HINTZE *et al.*, 2016). Currently, existing software for automatic identification of sounds emitted by bats are inefficient for studies in the Neotropical region, which means that the adoption of bioacoustics depends on trained people and great effort in data analysis, depending on the purpose of the study, such as for faunal surveys, for example (FRITSCH; BRUCKNER, 2014; HINTZE *et al.*, 2016; JENNINGS *et al.*, 2008; LEMEN *et al.*, 2015; MENON *et al.*, 2018, preprint; RUSSO; VOIGT, 2016; RYDELL *et al.*, 2017).

However, the potential contribution of bioacoustics to the advancement of studies on chiropterofauna is undeniable and its use should be encouraged, implemented and improved, with caution in its current use and, especially, in data analysis (HINTZE *et al.*, 2016). Thus, efforts should be directed towards the collection of robust and reliable bioacoustic information and the implementation of a systematized database, while automation of analysis also represents an important increment to facilitate and expand the use of this method (ARIAS-AGUILAR *et al.*, 2018; HINTZE *et al.*, 2016). In this way, a library of validated sounds is the key point to enabling the use of this method more regularly. Until this happens, the implementation of bioacoustics in diagnostic studies should only be done gradually and in addition to other methods.

COLLECTION AND CONSERVATION

The collection of animals is an essential practice for documenting studies, and is fundamental for research on evolution, systematics, and genetics, as well as for reference collections, voucher series documentation and/or identification confirmation (SOUSA; BARBOSA, 2020). In these cases, methods of euthanasia allowed by Brazilian environmental legislation, and previously described in the capture and collection license issued by the competent environmental agency, must be used.

According to Article 8 of Resolution No. 301 of the Conselho Federal de Biologia (CFBio), when it is necessary to collect a specimen, euthanasia must be performed to minimize suffering, using methods that produce rapid unconsciousness and subsequent death without evidence of pain or agony or using anesthetics in doses sufficient to produce painless loss of consciousness, followed by cardiorespiratory arrest. Article 7 of Resolution No. 1000, May 11, 2012, of the Conselho Federal de Medicina Veterinária (CFMV) instructs that euthanasia must be performed in a peaceful and adequate environment, respecting the behavior of each species. Therefore, whenever euthanasia is necessary, one should seek to know the legal precepts that govern the activity and act in line with current legislation and in accordance with ethical principles.

In cases of collection, specimens must be incorporated into zoological collections, in dry or wet manners, taking care to indicate, minimally, basic information about the specimen and collection site (*e.g.*, measurements of total length; ear, tail and foot lengths; weight; sex; reproductive condition; locality; collector; and geographic coordinates). It is highly recommended that tissue be collected immediately after the animal's death and prior to any fixation (to avoid degradation of genetic material). It is important to keep the material preserved in absolute alcohol and refrigerated, always following the pre-established methodology according to the approved project and linked to the capture and collection licenses and the rules of the receiving institution. The most commonly used form of conservation in Brazil is wet preservation, while it is also important to undertake efforts to prepare specimens in a dry manner (taxidermy). In the case of wet preservation, after tissue collection, the specimen is generally fixed in 10% formaldehyde and preserved in 70% alcohol (NAGORSEN; PETERSON, 1980; VIZOTTO; TADDEI, 1973).

Identification key

What mastozoologist who studies the order Chiroptera has not received a photo with the question: "What bat is this"? or the request: "Identify for me". So, we start this topic by answering: it is not easy and, most of the time, it is impossible to identify a bat species from a photo, in flight or just from a distance. In a mega-diverse country like Brazil, with 181 species of bats registered thus far (GARBINO *et al.*, 2020a), correct identifications can only be made from the analysis of morphological, genetic and, in some cases, bioacoustic characters, and only by trained professionals to ensure the correct determination of species.

Although external characters (*e.g.*, hair color and banding) are fundamental for the identification of some species, this situation is often not trivial, requiring analyses of other characters that can be observed in the skull and dentition, for example, even including morphometric measurements (*e.g.*, forearm size) and molecular characters.

There are some taxonomic keys available that can help in the recognition of important characters for the correct identification and registration of Brazilian bats, taking into account that it is necessary to be aware of limitations due to the scope of the observations used. It should also be kept in mind that many of these keys are constructed from local/regional material and therefore may not reflect global species variation, and also that keys often become obsolete after new species revisions, and so it is necessary to monitor for and study possible taxonomic rearrangements. Some keys focus on specific taxa, such as: Emballonuridae (JONES; HOOD, 1993), Molossidae (GREGORIN; TADDEI, 2002), *Cynomops* (MORAS *et al.*, 2018), *Diclidurus* (CEBALLOS; MEDELLÍN, 1988), *Sturnira* (GIANNINI; BARQUEZ, 2003), *Chiroderma* (GARBINO *et al.*, 2020a), *Micronycteris* (SIMMONS, 1997), *Lasiurus* (SHUMP Jr.; SHUMP, 1982), *Lonchorhina* (LASSIEUR; WILSON, 1989), *Lonchophylla* (MORATELLI; DIAS, 2015), *Hsunycteris* (VELAZCO *et al.*, 2017), *Mimon* (ORTEGA; ARITA, 1997), *Platyrrhinus* (FERRELL; WILSON, 1991), *Myotis* (MORATELLI, 2009) and *Eumops* (EGER, 1977). Other keys comprise several taxa from the same region, such as, for example, Rio Grande do Sul (SILVA; FALLAVENA, 1985), Londrina, (state of Paraná) (REIS *et al.*, 1993) and part of the central Amazon (LÒPEZ-BAUCELLS *et al.*, 2016); or even of Brazil (REIS *et al.*, 2017; VIZOTTO; TADDEI, 1973) and South America (GARDNER, 2008).

Main Brazilian collections

The history of the collection of biological specimens dates back to the beginning of the 19th century, since the implementation of the "cabinets of curiosities" of the European nobility, which became the forerunners of the largest collections and museums distributed around the world (ZAHER; YOUG, 2003). The first Brazilian zoological collection seems to have started in 1818, when Dom João VI founded the Casa dos Pássaros, which gave rise to the current Museu Nacional do Rio de Janeiro (MNRJ) (SEREJO, 2020; ZAHER; YOUG, 2003), the collection of which consists of more than half a million specimens, with 80,000 representatives of Brazilian mastofauna (SEREJO, 2020). Today, the Museu Nacional do Rio de Janeiro, the Museu Paraense Emílio Goeldi, created in 1866, and the Museu de Zoologia da Universidade de São (MZUSP), created in 1886, house the largest collections containing vouchers of the biodiversity of Brazil.

According to Vivo *et al.* (2014), there are five main types of zoological collections, namely: i) systematic; ii) research; iii) reference; iv) didactic; v) expository. Although each type has specific functions and uses, although not exclusive, all have equal importance in relation to science and scientific dissemination, and knowledge and preservation of biological diversity.

Brazil has dozens of collections, some of which are housed in important museums, such as the three largest ones mentioned here plus the Museu de História Natural Capão da Imbuia (Curitiba, Paraná), the Museu de Biologia Professor Mello Leitão (Santa Teresa, Espírito Santo) and the Instituto Nacional de Pesquisas da Amazônia (Manaus, Amazonas) (GREGORIN; PAVAN, 2018).

Gregorin and Pavan (2018) point out, for a conservative estimate, that there are about 135,000 bat specimens deposited in Brazilian collections, representing the 181 species that occur in the country. These authors researched 41 institutions and provided a list with location and contact data, which we present in an adapted form in Table 2. The vast majority of these institutions are open to visitation and/or the lending of material, in addition to providing access to digitalized data, from 80% of the collections (GREGORIN; PAVAN, 2018).

Collections can grow by both the contributions of researchers, through academic research projects, and through studies related to environmental licensing, as well as by donations and exchanges, among other avenues (VIVO *et al.*, 2014). Valuable information for studies of taxonomy, natural history, evolution, phylogeny and phylogeography, reproductive biology,

Table 2. List of institutions that have collections of bats in Brazil and their locations. Source: Adapted from GREGORIN and PAVAN, 2018.

Collection	City/State
Coleção de Morcegos, Laboratório de Biologia e Conservação de Morcegos, Universidade de Brasília (UnB)	Brasília, Distrito Federal
Laboratório de Biodiversidade Animal	Jataí, Goiás
Universidade Anhanguera-Uniderp	Campo Grande, Mato Grosso do Sul
Coleção Zoológica de Referência da Universidade Federal do Mato Grosso do Sul (UFMS)	Campo Grande, Mato Grosso do Sul
Coleção de Mamíferos da UNEMAT	Nova Xavantina, Mato Grosso
Coleção de Mamíferos da UFMT	Cuiabá, Mato Grosso
Coleção de Mamíferos do Instituto Nacional de Pesquisa na Amazônia	Manaus, Amazonas
Coleção Mastozoológica do Museu Paraense Emílio Goeldi	Belém, Pará
Coleção Referência da Mastofauna de Rondônia	Porto Velho, Rondônia
Coleção Mastozoológica da UFAC	Rio Branco, Acre
Zoneamento Ecológico e Econômico do Acre-Mamíferos	Rio Branco, Acre

Collection	City/State
Coleção de Mastozoologia do Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá	Macapá, Amapá
Coleção de Mamíferos do Museu de História Natural da UFAL	Maceió, Alagoas
Coleção de Mamíferos da Universidade Federal do Sergipe (UFS)	Aracaju, Sergipe
Coleção de Mamíferos da Universidade Federal da Paraíba (UFPB)	João Pessoa, Paraíba
Coleção de Mamíferos da Universidade Federal do Pernambuco (UFPE)	Recife, Pernambuco
Coleção de Mamíferos Alexandre Rodrigues Ferreira, Universidade Estadual de Santa Cruz (UESC)	Ilhéus, Bahia
Universidade Federal do Piauí Universidade Federal do Piauí (UFPI)	Teresina, Piauí
Coleção do Departamento de Zoologia da Universidade Federal do Paraná (UFPR)	Curitiba, Paraná
Coleção de Mamíferos do Museu de História Natural Capão da Imbuia	Curitiba, Paraná
Coleção de Mamíferos do Museu de Ciências e Tecnologia da Pontifícia Universidade Católica (PUC)	Porto Alegre, Rio Grande do Sul
Fundação Zoobotânica do Rio Grande do Sul	Porto Alegre, Rio Grande do Sul
Coleção Mastozoológica, Museu de Ciências Naturais da Universidade Federal do Rio Grande do Sul (UFRS)	Porto Alegre, Rio Grande do Sul
Coleção de Mamíferos do Museu de Zoologia da Universidade Estadual de Londrina	Londrina, Paraná
Fundação Universidade Regional Blumenau	Blumenau, Santa Catarina
Coleção de Mamíferos da Universidade Federal de Santa Catarina (UFSC)	Florianópolis, Santa Catarina
Coleção de Mamíferos da Universidade Federal do Espírito Santo (UFES)	Vitória, Espírito Santo
Coleção de Mastozoologia do Museu de Ciências Naturais da Pontifícia Universidade Católica (PUC)	Belo Horizonte, Minas Gerais
Museu de Zoologia João Moojen, Universidade Federal de Viçosa (UFV)	Viçosa, Minas Gerais
Coleção de Mamíferos da Universidae Federal de Lavras (UFLA)	Lavras, Minas Gerais
Mastozoologia, Departamento de Zoologia da Universidade Federal de Minas Gerais (UFMG)	Belo Horizonte, Minas Gerais
Coleção de Mamíferos, Museu de Biologia Professor Mello Leitão	Santa Teresa, Espírito Santo
Coleção de Mamíferos, Museu Nacional da Universidade Federal do Rio de Janeiro (UFRJ)	Rio de Janeiro, Rio de Janeiro
Laboratório de Diversidade de Morcegos, Universidade Federal Rural do Rio de Janeiro (UFRRJ)	Seropédica, Rio de Janeiro
Coleção ALP do Laboratório de Mastozoologia, Universidade Federal Rural do Rio de Janeiro (UFRRJ)	Seropédica, Rio de Janeiro
Laboratório de Chiroptera, Departamento de Zoologia e Botânica da Universidade Estadual Paulista (UNESP)	São José do Rio Preto, São Paulo
Laboratório de Chiroptera, Universidade Estadual Paulista (UNESP)	Araçatuba, São Paulo

Collection	City/State
Mastozoologia, Museu de Zoologia da Universidade de São Paulo (USP)	São Paulo, São Paulo
Coleção de Mamíferos, Museu de Zoologia da Universidade Estadual de Campinas (UNICAMP)	Campinas, São Paulo
Coleção do Laboratório de Mamíferos, Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ/USP)	Piracicaba, São Paulo

molecular biology and ecology, among many other possibilities, can be obtained from such biological collections.

Thus, Pavan and Gregorin (2019) present recommendations for the collection, preparation and preservation of specimens for scientific collections and tissue samples for molecular investigations, which must be observed to enable better scientific use of information about a specimen or sample. It is important to record data on the species and collection environment that is consistent and as complete as possible. This is because the loss of habitat resulting from anthropic actions is increasingly accentuated in the national territory, and potentially affects the characterization of original conditions a posteriori (PAVAN; GREGORIN, 2019).

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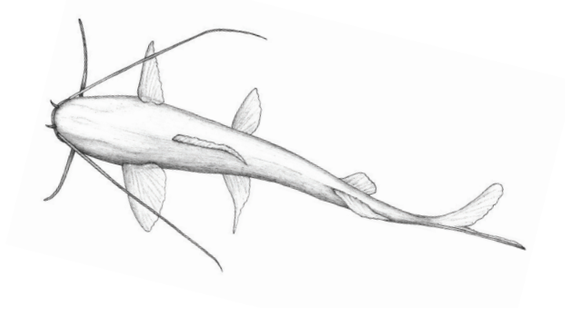


Rhamdiopsis sp.
Maria Elina Bichuette

27

Fishes

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General introduction

Brazil emerges as a country rich in natural subterranean cavities, with more than 22,000 officially registered caves (CECAV, 2021). In addition to caves, which develop in different types of rock or sediments (limestones, dolomites, sandstones, quartzites, granites, iron ores), there are other subterranean habitats with bodies of water in the form of: i) drainages (streams of base level that connect or not with the surface by sinks or upwellings); ii) water table outcrops (saturated zone) in flooded caves or in the form of puddles and lakes inside cavities and; iii) pockets of upper aquifers formed by water infiltration into the rock, defined as epikarst, which can form vadose tributaries. In some cases, groundwater does not surface in caves, but in alluvium near rivers, representing a phreatic horizon. Brazilian subterranean fish occupy this great diversity of habitats and horizons, with variable characteristics (BICHUETTE, 2021; TRAJANO; BICHUETTE, 2010).

More than half of living vertebrates are fish, about 32,000 species. They arose and began to radiate more than 500 million years ago and exhibit unrivaled diversity in their morphology, habits, ecology, physiology and behaviors. Fish include a vast array of surprising adaptations to nearly every aquatic environment on Earth, including subterranean habitats (NELSON, 2016). Brazil is a megadiverse country in terms of ichthyofaunistics, especially in freshwater environments, which include large rivers, streams and hypogean habitats. There are currently about 5,000 registered and valid species of fish in Brazilian freshwaters (FROESE; PAULY, 2020).

Brazilian subterranean ichthyofauna is composed of exclusively subterranean populations (troglobites and phreatobes) and facultative subterranean species (troglophiles). However, some individuals are found solitary in caves and close to the surface environment. Because of this they are considered to be of accidental occurrence.

The species that occur in these subterranean habitats belong to the group Ostariophysi, the ostariophysians. It is one of the most diverse groups of Teleostei with about 8,000 species, representing 28% of the known fish species in the world and 68% of freshwater species (NELSON, 2016). Ostariophysi is considered a monophyletic (natural) group with unique and shared characters among its representatives, such as the production of an alarm substance (also called the alarm reaction), specializations in the inner ear and anterior vertebrae known as the Weber complex, and specializations in the swim bladder. The group comprises five orders — Gonorhynchiformes, Cypriniformes, Characiformes, Gymnotiformes and Siluriformes — of which only three occur in the Neotropical region (Characiformes, Gymnotiformes and Siluriformes). Cypriniformes includes carp and the like; Gonorhynchiformes occurs mainly in the Indo-Pacific and Africa; Characiformes includes fish of the genera *Astynax*, *Leporinus* and *Hoplias*, among others, widely distributed in the Brazilian territory; Gymnotiformes is represented by electric fish, from the large "poraquês" (electric fish) of the Amazon Basin to the small "tuviras" and "sarapós" (*Gymnotus* sp.); and, finally, Siluriformes comprises bagres (catfish) and cascudos, the famous fish with leather and plates, such as "jundiás" and "jaús" (gilded catfish). In addition to these, two other groups of non-ostariophysian fish occur in caves and other Brazilian subterranean habitats: Cichliformes, represented by the family Cichlidae, including the "acarás" and the like (including "tilapias", which are exotic species to Brazil), and the order Synbranchiformes ("mussum" - marbled swamp eel), represented by the family Synbranchidae (genus *Synbrachus*). For these cases, there are only troglophile or possibly accidental representatives (BICHUETTE, 2021).

Troglophile fish populations are also particularly important, and Brazil also stands out in this category. However, the differentiation between troglophile and accidental fish in caves depends on population studies focusing on distribution and reproduction in this environment. Defining such populations is not a trivial task and few studies of an ecological nature have been conducted so far, with the majority being on the occurrence of taxa or aspects of biology, such as diet and reproduction (BICHUETTE; TRAJANO, 2003). Fish of the orders Siluriformes ("bagres" and "cascudos" - catfish) and Gymnotiformes poraquês, tuviras and sarapós have been the most recurrent in caves in the states of Bahia, Goiás, Minas Gerais and São Paulo (BICHUETTE; TRAJANO, 2003; RATTON *et al.*, 2018; SECUTTI; BICHUETTE, 2013; TRAJANO *et al.*, 2009). For Characiformes, records have also been

recurrent for populations of traíras of the family Erythrinidae, frequent in some caves that harbor populations of potential prey, in addition to several species of the family Characidae, popularly known as "lambari" and "piabas" - *Astyanax* sp. (BICHUETTE; TRAJANO, 2003).

Currently, there are records of 37 exclusively subterranean fish species for Brazil (among those described and recognized as new) distributed in six families and 13 genera. There is high taxonomic diversity occurring in hypogean habitats in five Brazilian river basins (BICHUETTE; GALLÃO, 2021; BICHUETTE, 2022, author's personal observation). The countries with the highest troglobitic fish richness are China (more than 79 species distributed in 10 genera) and Brazil (37 species distributed in 13 genera) (NIEMILLER *et al.*, 2019; BICHUETTE, 2022, unpublished data), which demonstrates that Brazil, in addition to being rich, can be considered more taxonomically diverse than China. This, together with the approximate number of 50 troglophile populations registered for the country (BICHUETTE; TRAJANO, 2003; RABELLO, 2021; RATTON *et al.*, 2018; SECUTTI; BICHUETTE, 2013; TRAJANO; BICHUETTE, 2010), highlights the urgent need to protect Brazilian subterranean aquatic habitats and the fish that live there.

Main groups found In hypogean habitats of Brazil

The taxonomic groups that occur in caves and other hypogean habitats in Brazil belong to Ostariophysi (troglobitic species and several troglophilous species); Cichliformes, represented by the family Cichlidae; and Synbranchiformes, represented by the family Synbranchidae (troglophilous species). It is interesting to note that there are also records of exotic species for some subterranean natural cavities, such as tilapia (Cichliformes) (RABELLO, 2021).

Occurrence patterns can be observed considering species already formally described and those already recognized as new. Among the troglobites, the catfish of the family Trichomycteridae emerge with 15 species of the genera *Trichomycterus, Ituglanis, Glaphyropoma* and *Copionodon*, which occur in different horizons, such as base level streams, epikarst, vadose tributaries and the saturated groundwater zone. Heptapterid bagres (catfish) represent the second richest group in groundwater (10 species of the genera *Rhamdia, Rhamdiopsis, Phenacorhamdia* and *Pimelodella*) and also occur in different horizons, from base level streams to flooded caves, or even pools fed by the hyporheic environment. This is also the case for the genus *Phreatobius*, with three subterranean species unique to the Amazon Basin. They occur in a unique environment, formed by water from aquifers, which emerges in the litter or in artificial wells dug in the ground. These environments are always marginal to drainages and are called the hyporheic zone by some authors (OHARA *et al.*, 2016). The catfish of the family Loricariidae are represented by three species, all of the genus *Ancistrus*, which occur in stretches of high current in streams at base level.

Two orders, Gymnotiformes and Characiformes, and a family of Siluriformes (Callichthyidae), have a single subterranean representative each. The electric fish of the order Gymnotiformes live in murky waters and/or are nocturnally active, orienting themselves by electroreception, useful characteristics for a subterranean way of life. The troglobitic species *Eigenmannia vicentespelaea* occurs in a single cave system in Brazil, in stretches of low current in base-level streams (BICHUETTE; TRAJANO, 2015). The only subterranean representative for the order Characiformes is the emblematic piaba, *Stygichthys typhlops*, whose family position is considered uncertain by some authors, and in other cases is included in the family Characidae. Placement in the family Characidae was adopted here for didactic purposes. The species is phreatic and highly specialized, inhabiting the water table in the region of the municipality of Jaíba in the north of the state of Minas Gerais (BICHUETTE, 2021; MOREIRA *et al.*, 2010). The only troglobitic species of Callichthyidae in South America, *Aspidoras mephisto*, occurs in three caves in the region of Posse, state of Goiás (TENCATT; BICHUETTE, 2017).

Table 1 provides a summary of the formally described troglobitic species and Figure 1 shows a map with their distribution.

The karst areas located in the northeast of the state of Goiás and included in the Upper Rio Tocantins basin are the richest in subterranean species (12 formally described species)

Table 1. Diversity of Brazilian subterranean fish, states of occurrence, types of rock in which they live (lithologies) and hydrographic basins.

Taxon	State/Lithology	Basin
Characiformes		
Characidae		
1. *Stygichthys typhlops* Brittan & Böhlke, 1965	Minas Gerais/Limestone	Middle Rio São Francisco
Gymnotiformes		
Sternopygidae		
2. *Eigenmannia vicentespelaea* Triques, 1996	Goiás/Limestone	Upper Rio Tocantins
Siluriformes		
Loricariidae		
3. *Ancistrus cryptophthalmus* Reis, 1987	Goiás/Limestone	Upper Rio Tocantins
4. *Ancistrus formoso* Sabino & Trajano, 1997	Mato Grosso do Sul/Limestone	Upper Rio Paraguai
Callichthyidae		
5. *Aspidoras mephisto* Tencatt & Bichuette, 2017	Goiás/Limestone	Upper Rio Tocantins
Trichomycteridae		
6. *Trichomycterus itacarambiensis* de Pinna & Trajano, 1996	Minas Gerais/Limestone	Middle Rio São Francisco
7. *Trichomycterus dali* Rizzato, Costa-Jr, Trajano & Bichuette, 2011	Mato Grosso do Sul/Limestone	Upper Rio Paraguai
8. *Trichomycterus rubbioli* Bichuette & Rizzato, 2012	Bahia/Limestone	Middle Rio São Francisco
9. *Ituglanis passensis* Fernández & Bichuette, 2002	Goiás/Limestone	Upper Rio Tocantins
10. *Ituglanis bambui* Bichuette & Trajano, 2004	Goiás/Limestone	Upper Rio Tocantins
11. *Ituglanis epikarsticus* Bichuette & Trajano, 2004	Goiás/Limestone	Upper Rio Tocantins
12. *Ituglanis ramiroi* Bichuette & Trajano, 2004	Goiás/Limestone	Upper Rio Tocantins
13. *Ituglanis mambai* Bichuette & Trajano, 2008	Goiás/Limestone	Upper Rio Tocantins
14. *Ituglanis boticario* Rizzato & Bichuette, 2014	Goiás/Limestone	Upper Rio Tocantins
15. *Glaphyropoma spinosum* Bichuette, de Pinna & Trajano, 2008	Bahia/Sandstone	Upper Rio Paraguaçu
Heptapteridae		
16. *Pimelodella kronei* (Ribeiro, 1907)	São Paulo/Limestone	Upper Rio Ribeira
17. *Pimelodella spelaea* Reis, Bichuette & Trajano, 2007	Goiás/Limestone	Upper Rio Tocantins
18. *Rhamdia enfurnada* Bichuette & Trajano, 2005	Bahia/Limestone	Middle Rio São Francisco
19. *Rhamdiopsis krugi* Bockmann & Castro, 2010	Bahia/Limestone	Upper Rio Paraguaçu
20. *Phreatobius cisternarum* Goeldi, 1905	Pará, Amapá, Amazonas/ Alluvial	Amazonas
21. *Phreatobius dracunculus* Shibatta, Muriel-Cunha & de Pinna, 2007	Rondônia/Alluvial	Amazonas
22. *Phreatobius sanguijuela* Fernández, Saucedo, Carvajal-Vallejos & Schaefer, 2007	Rondônia/Alluvial	Amazonas

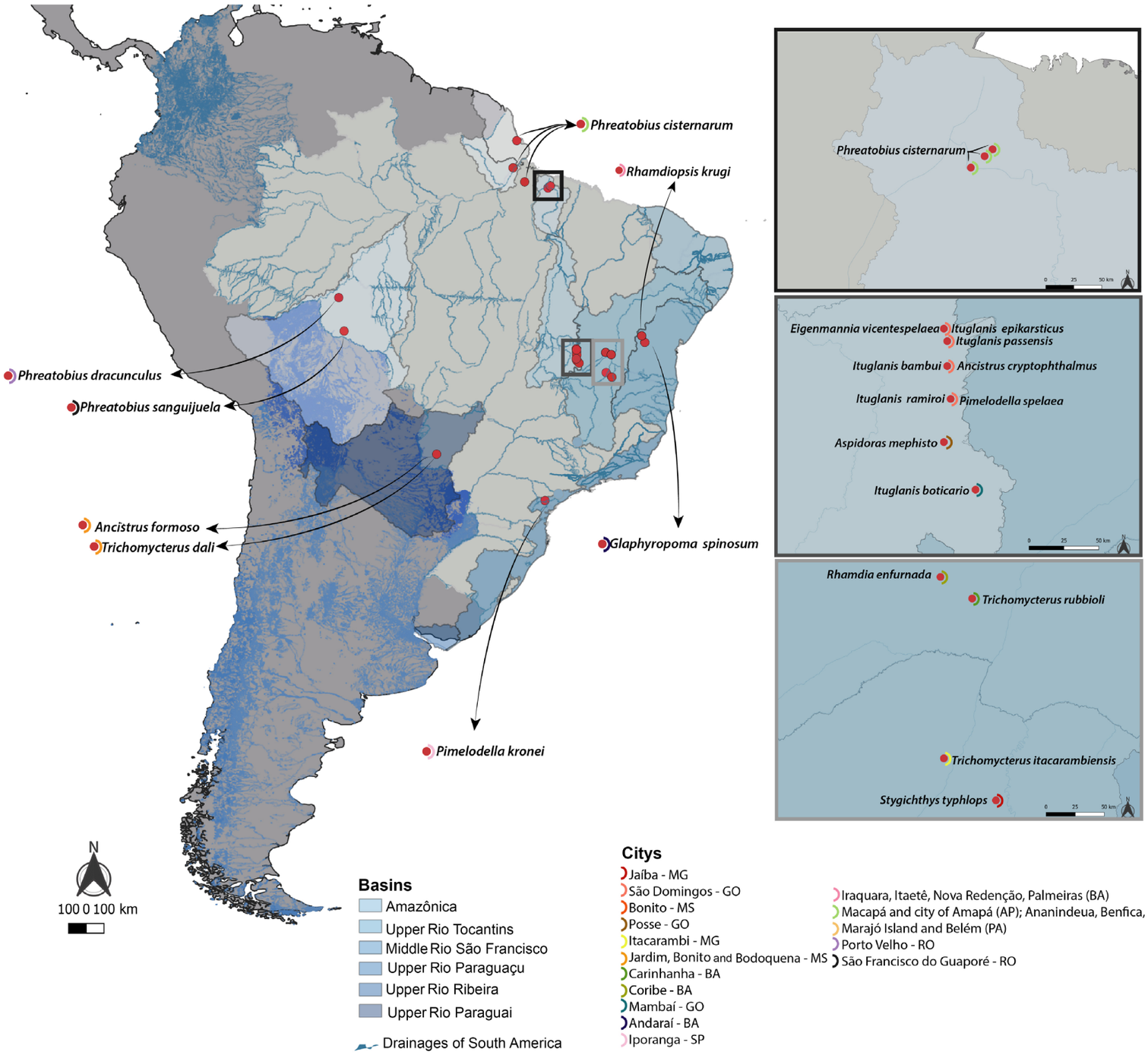


Figure 1. Distribution map of exclusively subterranean fish species in relation to the main river basins in Brazil. Authorship: L. de Assis.

(BICHUETTE; GALLÃO, 2021; BICHUETTE; TRAJANO, 2004, 2008; FERNÁNDEZ; BICHUETTE, 2002; REIS, 1987; RIZZATO; BICHUETTE, 2014; TENCATT; BICHUETTE, 2017; TRIQUES, 1996) and troglophiles (BICHUETTE, 2021; BICHUETTE; TRAJANO, 2003). However, the most specialized troglobites occur in semi-arid karstic areas located in Bahia (Upper Rio Paraguaçu basin and Middle Rio São Francisco basin). The Upper Rio Paraguay basin (in the state of Mato Grosso do Sul) is also particularly important, even if its richness is still low, as there are fish populations in several flooded caves in this region, which implies a potential area for high troglobite diversity (BICHUETTE; GALLÃO, 2021; CORDEIRO, 2014; RIZZATO *et al.*, 2011). Despite the occurrence of a single troglobitic species in the Upper Rio Ribeira basin, the blind catfish *Pimelodella kronei*, its discovery represents a historic milestone, as it corresponds to the first troglobite formally described for South America, in a region located in the southeast of the state of São Paulo.

Figure 2 presents some representatives of Brazilian subterranean fish with different degrees of troglomorphisms and specializations.

Troglophile fish populations are also particularly important, and Brazil also stands out for this category. Fish of the orders Siluriformes and Gymnotiformes have been the most recurrent in caves in the states of Bahia, Goiás, Minas Gerais and São Paulo (BICHUETTE; TRAJANO, 2003; RATTON *et al.*, 2018; SECUTTI; BICHUETTE, 2013; TRAJANO *et al.*, 2009). For Characiformes,



Figure 2. Brazilian subterranean fish. **A-C)** *Ancistrus cryptophthalmus*; **B)** *Stygichthys typhlops* in foraging behavior; **D)** *Rhamdia enfurnada;* **E)** *Phreatobius sanguijuela*. Photos: A-B (Maria E. Bichuette); D (Jonas E. Gallão); E (W. Ohara).

records of the family Erythrinidae have been more recurrent, in addition to several species of Characidae and cichlids of the order Cichliformes (BICHUETTE; TRAJANO 2003). Other groups have been quite common, such as the mussuns (Synbranchiformes, Synbranchidae) in some caves in central-north Brazil.

Main troglomorphisms

Subterranean fish have been used as a model for the study of morphological evolution, especially in the case of the regressive evolution of characters. For many years, regressive evolution was the predominant theme among studies on subterranean fish, many of them seeking to support different theories about the evolution of characters related to subterranean life (RIZZATO; BICHUETTE, 2016).

However, we must draw attention to the fact that subterranean organisms, in general, present not only the so-called regressive characteristics, but also a set of progressive characters, that is, they represent an increase or gain of characters or character states. As these are convergent character states, it is clear that the detection of troglomorphisms is not trivial and, in the case of fish, requires detailed anatomical study (externally and internally) associated with comparisons with phylogenetically close relatives.

One of the main characteristics of subterranean species in general is the reduction and even loss of eyes and cutaneous melanic pigmentation (Figure 3A), known as classic troglomorphisms. However, in many cases, these conditions show wide variation in populations. This variability seems to be the rule for Brazilian troglobitic fish, as observed for the Siluriformes families of Loricariidae, Trichomycteridae, Heptapteridae and Callichthyidae (genera *Ancistrus, Trichomycterus, Ituglanis, Glaphyropoma, Rhamdia, Pimelodella* and *Aspidoras*), in addition to marked variability in the electric fish *Eigenmannia vicentespelaea*. Of the 22 species described, only six have homogeneous populations in terms of reduced and/or completely absent eyes and pigmentation: the Brazilian blind characid *Stygichthys typhlops*, the catfish *Ancistrus formoso*, the catfish *Rhamdiopsis krugi* (Figure 3A) and the three species of *Phreatobius*. We will not deal here with cases of true albinism, which is rare in troglobitic fish, as well as in animals in general (BICHUETTE, 2003), with proven records for the species *Trichomycterus itacarambiensis* (TRAJANO; BICHUETTE, 2010).

In addition to classic troglomorphisms, it is essential to seek to understand the morphological specializations within each group under consideration. As taxonomic diversity is high for Brazil, comparative morphological studies between these fish and those phylogenetically close are fundamental for unequivocal proposals of troglomorphisms, particularly unconventional troglomorphisms. Many troglomorphisms are constructive and can be adaptive, that is, useful for a subterranean way of life, for example:

For catfish, there is a lot of discussion about the **lengthening of the barbels** (body projections that carry structures that perceive smells and vibrations and are involved in detecting food, avoiding obstacles and predators, or even sexual attraction) (Figure 4) as a constructive character that can have an adaptive value.

The **skin folds** observed along the dorsal region of the body of some bagres of the family Trichomycteridae may configure a troglomorphism (Figure 6). These folds can store fat that can be useful in an environment generally poor in food such as caves and other subterranean environments.

Another unconventional troglomorphism is the **enlargement of the pectoral fins** (paired, Figures 4 and 6), which may present a series of structures that perceive taste (gustatory) and provide a better exploration of the environment (observed for bagrinhos of the genus *Ituglanis*). This structure is also efficient in accurately detecting prey.

Finally, the **elongation of the body and snout** (Figure 6) is discussed as an adaptive troglomorphism, since the cephalic region and the trunk house a series of structures that perceive vibration in the water, the so-called neuromasts (superficial or in canals, Figure 5). Some studies have shown that the increase in the size of these neuromasts associated with their number and distribution, both in the head and along the body, should be considered a troglomorphism (SOARES; NIEMILLER, 2013). However, for some troglobitic fish, a reduction was observed in the canals and an increase only in the superficial neuromasts. Examples are the Brazilian blind characid *Stygichthys typhlops* and catfish of the genus *Ituglanis* (PASTANA *et al.*, 2020; RIZZATO; BICHUETTE, 2016).

Still, in addition to these troglomorphisms related to external morphology, detailed evidence must be sought regarding internal morphology (a prerequisite for the taxonomic description of species). Evidence includes: a) an increase in the size of the brain and one of its regions, the cerebellum; b) enlargement of structures of the inner ear (semicircular canals); c) an increase in the size of the swim bladder; d) a decrease in the number of vertebrae and ribs (vertebral column); and e) an

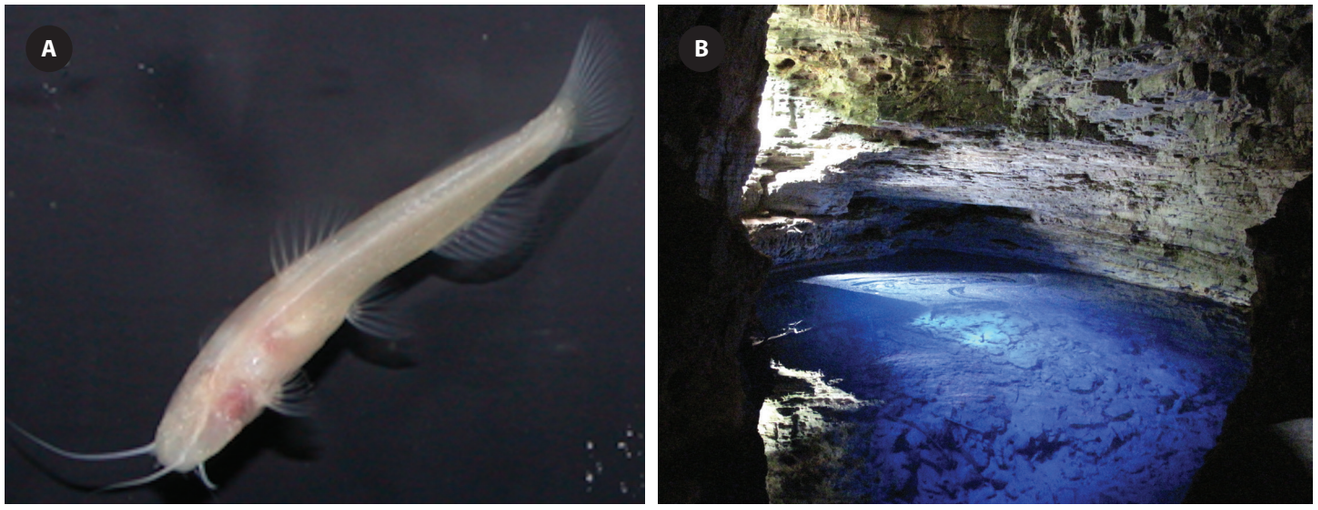


Figure 3. A) Catfish *Rhamdiopis krugi* of caves of Chapada Diamantina, Bahia. A homogeneously anophthalmic and depigmented species; B) Poço Encantado cave, Itaetê, Bahia, type locality of the species, groundwater. Photos (Maria E. Bichuette).

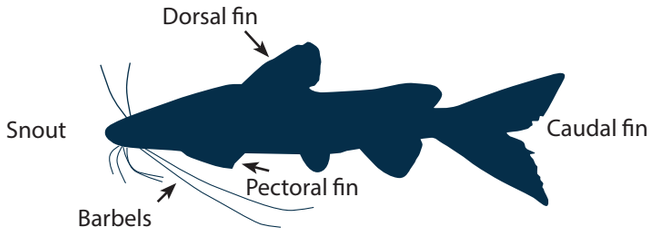


Figure 4. Schematic drawing showing some regions of the fish body that can present troglomorphisms. Left side view. Created with BioRender.com

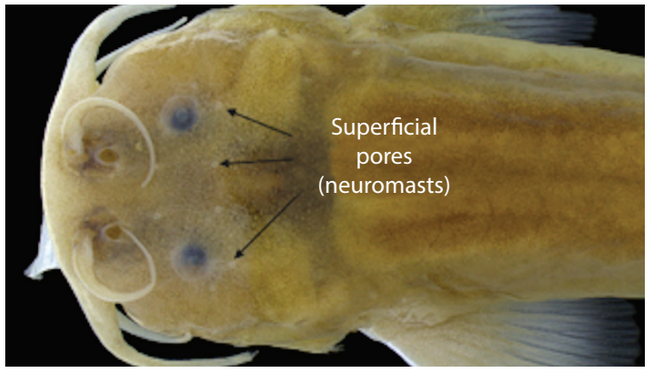


Figure 5. Superficial pores in the head region of a bagre (catfish) species of the family Trichomycteridae. Photo (Maria E. Bichuette).

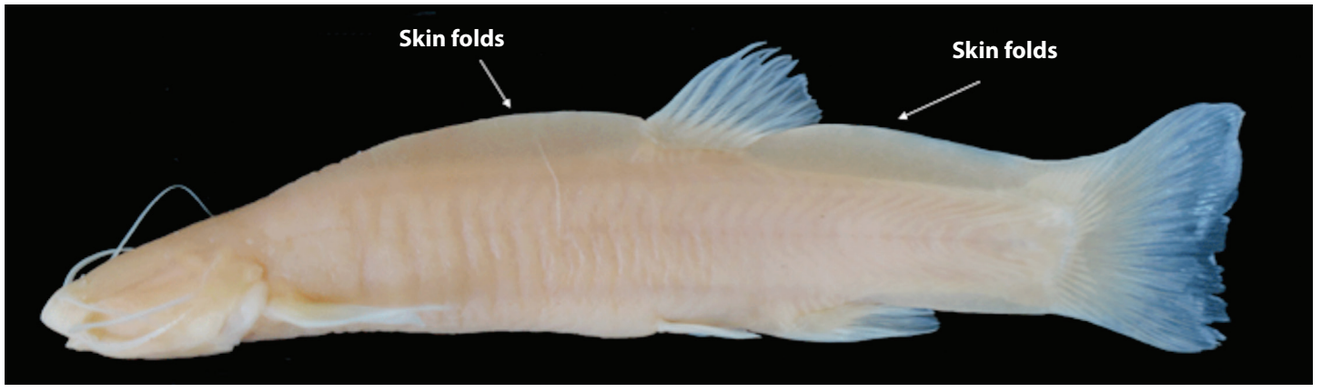


Figure 6. Dorsal skin folds and elongated snout observed in the bagrinho *Trichomycterus dali* from caves in Mato Grosso do Sul. Photo (Pedro P. Rizzato).

increase in the amount of adipose bodies (fat), among others (SOARES; NIEMILLER, 2013).

Diversity of troglobitic species

Currently, more than 220 valid species of subterranean fish are known in the world (NIEMILLER *et al.*, 2019). Of the 37 species known for Brazil, 22 have been formally described and are valid (see Table 1 and Figure 7) and 16 are included in the Lista de Fauna Ameaçada Brasileira (BICHUETTE; GALLÃO, 2021; GALLÃO; BICHUETTE, 2012, 2018). This list was approved by the Instituto Chico Mendes para Conservação da Biodiversidade (ICMBio) and published in 2014 in accordance with Ordinance MMA No. 148, of June 7, 2022 (BRASIL, 2022).

There is a great deficit, considering the discoveries and collections of these fish until the formal description, a common fact for several groups of animals. At the moment, 15 species are being described by researchers from the Laboratório de Estudos Subterrâneos (LES) of the Universidade Federal de São Carlos (UFSCar), the Universidade de Brasília (UnB) and other collaborative research groups. However, in most cases there is a need for comprehensive studies even for the accurate determination of the taxon, which demands time and investment in long-term projects with training of specialists in the area.

Species that are officially endangered include: nine in the category of "Vulnerable" (VU; *Ancistrus formoso, Eigenmannia vicentespelaea, Glaphyropoma spinosum, Ituglanis epikarsticus, I. passensis, I. ramiroi, Rhamdiopsis krugi, Trichomycterus dali* and *T. rubbioli*), five in the category of "Endangered" (EN; *Ancistrus cryptophthalmus, Ituglanis mambai, Pimelodella kronei, P. spelaea* and *Stygichthys typhlops*) and two in the category of "Critically Endangered" (CR; *Ituglanis bambui* and *Trichomycterus itacarambiensis*). One species is considered "Least Concern" (LC; *Rhamdia enfurnada*). Unfortunately, many of the fish not yet described by science are already endangered yet cannot appear on the official lists of endangered species until their formal description is completed.

Threats to subterranean fish populations are numerous, such as deforestation of riverbanks upstream of cave sinkholes and consequent siltation and reduction of food supply for fish populations; habitat alteration; alteration of the flow of rivers and streams due to the construction of hydroelectric plants and dams; pollution of aquifers by pesticides; overcollection of fish that have small and vulnerable populations; and tourism in caves without management plans implemented for visitation, among others (BICHUETTE; GALLÃO, 2021; GALLÃO; BICHUETTE, 2018).

The inclusion of species in endangered fauna lists is effective at protecting the place where they occur, however, and mainly in the specific case of caves, the surroundings and recharge areas must also be in the protection area. The spatial limits of species protection, in addition to encompassing the habitat, should also include the basins, microbasins and associated aquifers where they are inserted, since a large part of the food present in the subterranean environment is of allochthonous origin and there is a strong influence of impacts to the surface landscape on the subterranean biota (BICHUETTE; GALLÃO, 2021; BICHUETTE; TRAJANO, 2010). An important example is the northeast region of the state of Goiás, on the limits of the Parque Estadual de Terra Ronca (Peter), in the Upper Rio Tocantins basin. In this area, the rivers that cross the large cave systems originate in the veredas, which have suffered impacts due to the large soy and cotton plantations in their surroundings. As a consequence, river volumes have decreased, which can disturb and impact the local subterranean ichthyofauna, considered the richest in South America (BICHUETTE; TRAJANO, 2021). Finally, the inclusion of species without formal description in the lists of endangered fauna should be made more flexible, at least for those that present unequivocal diagnoses and have voucher specimens deposited in faithful scientific collections.

Main methods of collection and conservation

Conventional and classic methods of collecting fish in rivers, streams, creeks and lakes in surface environments are not necessarily applied in subterranean habitats. Subterranean fish populations are often small and fragile, so common sense should prevail when catching and collecting these animals. Therefore, many tools and methodologies used in ichthyofauna collections in surface rivers (Figure 8) can be used and/or adapted for use in subterranean water bodies. However, some equipment has been replaced to avoid overcollecting a particularly fragile fauna. Among them, it is worth highlighting electrofishing, trawling and gill nets, as well as cast nets.

Handnets, fish traps with suitable baits (dog food, chicken liver), fine mesh sieves (1 mm or less), umbrella traps for slower (and often deep) water wells and nets with long, adapted handles are the most frequently used for collections (Figure 8). The installation time for traps can vary from a few hours to

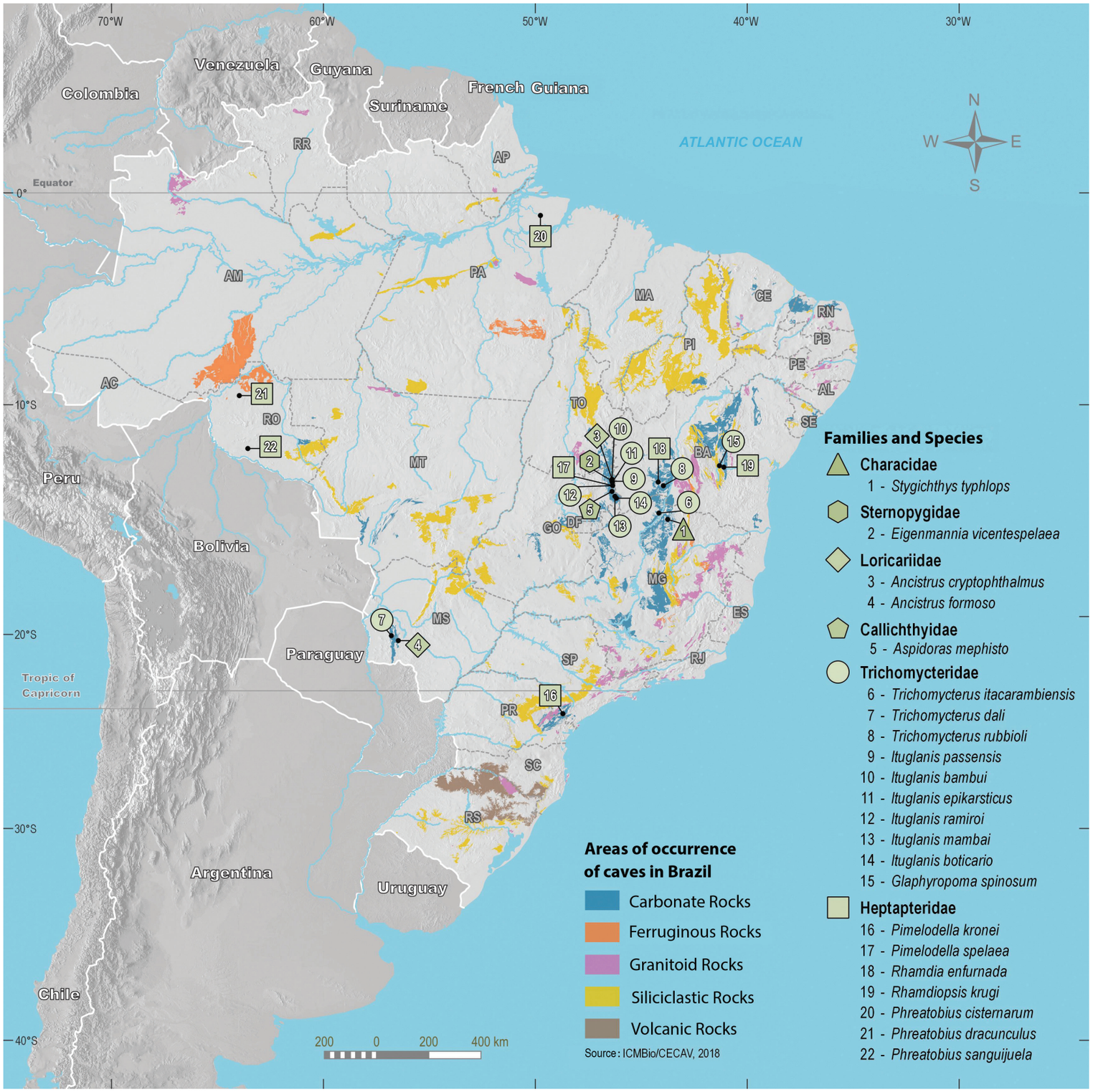


Figure 7. Map with localities of the troglobitic fish species described for Brazil.

overnight (24 hours), when there is the possibility of returning to the site at the same time of collection. It is important that this period does not extend beyond 24 hours, since several fish can enter the trap and cause injuries to each other.

For those fish that live in flooded caves or even where the water table appears, capture is only possible through advanced speleodiving techniques or the simple use of a breathing tube (*snorkel*) and a mask, known as the *snorkeling* technique (a technique also used in surface rivers and streams) (Figure 8). In these cases, conventional or even adapted hand nets with elongated bottoms are used. These must be attached to the collector's belt, as must the flasks and containers for storing live specimens, which will be properly anesthetized and fixed at the end of the dive.

Extreme care must be taken when handling collected specimens, both to avoid damaging the skin and important structures, and for animal welfare. Anesthesia is the rule after capturing fish, often using benzocaine diluted in a solution composed of water and 92.8% alcohol, clove oil or the imported anesthetic MS 222 (tricaine methane sulfonate). Both benzocaine and MS 222 are sold in Brazil and require that authorizations be obtained from Federal and Civil Police. After the region of the operculum (where the gills are located) completely stops beating (Figure 9), the fish can be transferred



Figure 8. Collection techniques for rivers and subterranean habitats. Created with BioRender.com

to fixative substances: formaldehyde diluted at 10% (buffered) or 100% PA alcohol for future molecular studies. Depending on the question of the work, knowledge of proper fixatives other than just formaldehyde and alcohol is required.

It is essential to consider that any material collected must be properly labeled (with data on origin, such as the name of the cave or locality, region, municipality, state, country, date of collection, collectors, information about the habitat and climate information, among other basic information) and transported with extreme care. Such material should be deposited in a scientific collections after its detailed study. Therefore, care and responsibility with the material must be the same as those conducted during sampling.

Identification keys

Constructing dichotomous keys for fish in general is not a trivial task, given the mega-diversity of the group, with more than 32,000 species among vertebrates, with many taxonomic and phylogenetic uncertainties. The taxonomic diversity observed among subterranean fish in Brazil makes this task unfeasible and there are no specific identification keys made for subterranean fish in general. We must consider that these are part of large taxonomic groups, many of them non-monophyletic (non-natural), which may invalidate the elaboration of identification keys. This difficulty in constructing keys is not just the merit of subterranean fish, and the comparison, which represents the evolution of knowledge, must be cautious.

However, the groups recorded in Brazilian hypogean habitats represent a small portion of South American ichthyofauna, making it possible to provide general diagnoses for families and genera and to help in work to determine taxa in hierarchies more (families) or less (genera) inclusive. In the case of species, diagnoses can be found in formal descriptions and may be composed of a combination of characters.

Table 2 presents general diagnoses for the orders that occur exclusively in Brazilian hypogean habitats: Siluriformes, Characiformes and Gymnotiformes.

Table 3 presents a comparative table for the families Sternopygidae, Characidae, Loricariidae, Callichthyidae, Trichomycteridae and Heptapteridae. Most of the diagnoses are composed of characters of internal morphology, requiring detailed anatomical study for accurate determination and, for many taxa, there is still confusion considering phylogenetic relationships and positioning.

Main Brazilian collections

Subterranean fish collected in diverse regions of Brazil are deposited in faithful collections in São Paulo (collection of the Laboratório de Ictiologia da Universidade de São Paulo, Ribeirão Preto - acronym LIRP; ichthyology collection of the Museu de Zoologia da Universidade de São Paulo - MZUSP), in Rio de Janeiro (ichthyology collection of the Museu Nacional, Universidade Federal do Rio de Janeiro - UFRJ), in Rio Grande do Sul (ichthyology collection of the Museu de Ciências e Tecnologia/PUCRS - MCP), in Mato Grosso do Sul (zoology reference collection of the Universidade Federal do Mato Grosso do Sul - ZUFMS) and in the north of the country (scientific collections of the Instituto Nacional de Pesquisas da Amazônia

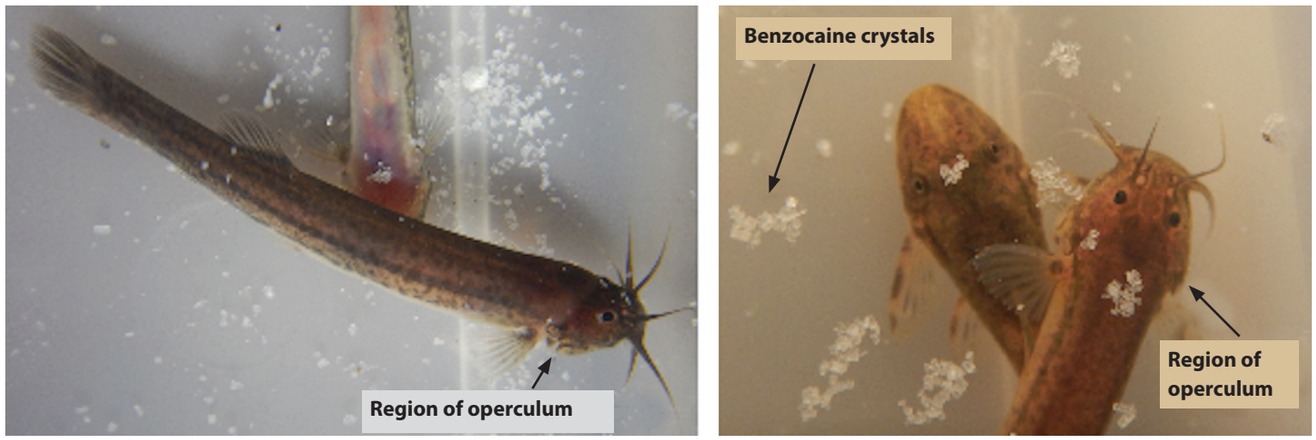
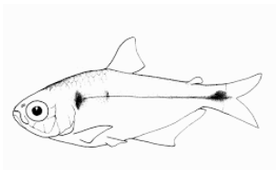
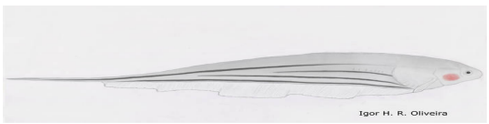
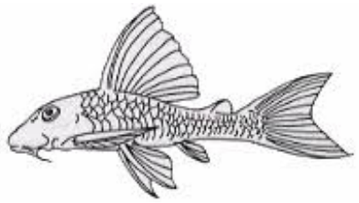
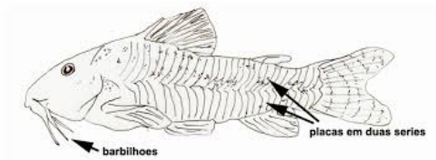


Figure 9. Fish undergoing anesthesia in a benzocaine solution for subsequent fixation. Photos: (Maria E. Bichuette).

Table 2. Diagnoses and main characteristics of the orders Siluriformes, Characiformes and Gymnotiformes.

Siluriformes	Absence of scales on body, covered by thick leather or dermal bony plates; presence of three pairs of barbels with sensory function.
Characiformes	Presence of adipose fin and generally well-developed scales; pelvic fins with 5–12 rays and short to moderately long anal fin (less than 45 rays); lateral line often curved and sometimes incomplete; absence of barbels.
Gymnotiformes	No maxillary teeth; sternopygidia; no gustatory papillae in the extra-oral tegument; tuberous-shaped high-frequency electroreceptor organs; operculum triangular; absence of pelvic fins; absence of dorsal or adipose fins; long anal fin, with more than 150 rays; anus located anterior to midbody length.

Table 3. Diagnoses and main characteristics of the families Characidae, Sternopygidae, Loricariidae, Callichthyidae, Trichomycteridae and Heptapteridae.

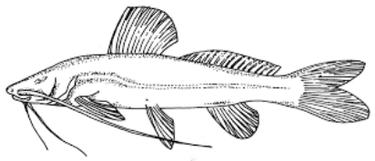
Family	Morphological characteristics
Characidae Latreille, 1825 	Generally small fish, showing a reduction in the lateral line system, in the complexity of the skeletal system, in the number of fin rays, in the number of scales and a decrease in the size of the eyes in relation to the length of the head.
Sternopygidae Cope, 1871 	Eyes developed; anterior nostrils located outside the margins of the mouth; origin of anal fin at isthmus; without urogenital papilla, caudal fin or dorsal fin; elongated body shape, with body highly laterally flattened and translucent.
Loricariidae Rafinesque, 1815 	Body embedded in a set of dermal plates; mouth in a ventral position in the form of a "suction cup"; mouth and teeth show strong adaptation to food type - algae, detritus and small invertebrates can be components of the diet.
Callichthyidae Bonaparte, 1838 	Body almost completely protected by bone armor made up of two longitudinal series of dermal plates; lateral line sensory canal reduced to 1–6 plates; presence of two longitudinal rows of bony plates between head and tail; small (20 mm) to medium sized fish, with exceptions.

Trichomycteridae Bleeker, 1858



Presence of opercular apparatus modified by a system of odontodes; absence of spines on pectoral and dorsal fins; dorsal-fin position beyond half standard length, posterior to pelvis; absence of adipose fin; presence of i+4 rays in pelvic fins; rictal barbel inserted in upper lip.

Heptapteridae Bockmann & Guazelli, 2003



Bare skin (no scales); simple cutaneous latero-sensory canal; small size, usually 20 cm or less in standard length; nostrils well apart and not bearing barbels; three pairs of barbels; well-developed adipose fin; caudal fin strongly forked, scalloped, rounded or lanceolate; gill membranes free, with gill openings unrestricted; orbital margin free or not; and first ray of dorsal and pectoral fins ranging from having sharp spines to completely flexible or mostly segmented.

in Manaus, Amazonas - acronym INPA and Museu Paraense Emílio Goeldi in Belém, Pará - MPEG) (Table 4). A good portion of these collections is computerized and inserted in the platform *species*Link (http://splink.cria.org.br/), where consultations and loan requests for scientific studies can be made. *species*Link is an information system that integrates primary data from scientific collections and is institutional.

There are other scientific collections in the country that also house cave fish, such as those at the Universidade Federal de Lavras (UFLA) and Universidade Federal de Minas Gerais (UFMG), both in the state of Minas Gerais, in addition to the collection of the Universidade Federal do Pará (UFPA), campus de Bragança, in the state of Pará.

The scientific collection (fish section) of the Laboratório de Estudos Subterrâneos da Universidade Federal de São Carlos (LESCI) began in 2006, with the hiring of researcher and collection curator Maria Elina Bichuette, and has become the main such collection in the country, housing troglobitic, troglophile and even accidental fish. Part of the material collected during research projects in different regions of Brazil is deposited in the collection and represents important voucher material, in addition to composing standard material. The material is also partitioned and deposited in traditional faithful depository institutions in Brazil, among them the Museu Nacional do Rio de Janeiro (MNRJ/UFRJ), the Museu de Zoologia da USP (MZUSP), the Laboratório de Ictiologia de Ribeirão Preto - USP (LIRP/USP), and the Museu de Ciências da PUC do Rio Grande do Sul (MCP/RS), among others.

Currently, at LESCI there is a significant collection of 2,190 lots of fish from caves and other subterranean habitats, the majority (two-thirds of the specimens) consisting of fish of the orders Siluriformes and Characiformes. The collection is legally supported by licenses previously issued by the Sistema de Autorização e Informação em Biodiversidade do Instituto Chico Mendes de Conservação da Biodiversidade (Sisbio/ICMBio) and state environmental agencies for the collection, capture and transport of fauna. Furthermore, the collection is registered with the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) and Sistema de Informação Sobre a Biodiversidade Brasileira (SiBBr, https://collectory.sibbr.gov.br/collectory/public/show/co369?lang=pt_BR), and is deposited in a database under the acronym LESCI. The material is available for consultation by other Brazilian researchers. Information about the collection is also available at the *website* http://www.lesbio.ufscar.br.

Table 4. Acronyms and links to some faithful scientific collections that house Brazilian subterranean fish in their collections.

MZUSP	https://mz.usp.br/pt/laboratorios/ictiologia/
MUSEU DE CIÊNCIAS E TECNOLOGIA | PUCRS	https://www.pucrs.br/mct/colecoes/ictiologia/
INPA INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA	https://portalcolecoes.inpa.gov.br/index.php/zoocol
MUSEU GOELDI	https://www.museu-goeldi.br/assuntos/colecoes/biologicas/colecoes-de-zoologica/colecao-de-ictiologia
UFMS FUNDAÇÃO UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL	https://inbio.ufms.br/zufms/
MUSEU NACIONAL UFRJ	https://ipt.sibbr.gov.br/mnrj/resource.do?r=mnrj_ictiologia&request_locale=pt
LABORATÓRIO DE ESTUDOS SUBTERRÂNEOS - UFSCar	LESCI http://www.lesbio.ufscar.br

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Caves.

Formed millennia ago, they have resisted the most diverse adversities while going through time. However, even though they are tough, they transform slowly, in an invisible dance with the elements.

And, in this silent choreography and in its magical permeability, they accept others who are attached to them.

Traces of our history were engraved on them. Records of a rich past life were added to them. A distant past was being accumulated, like sentences written in a book, eager to be read.

And, among everything that was incorporated, they were always open, even though hidden and silent, to life.

A life that, being so curious, chose to leave the light, in search of new worlds. And that, because of how well received it was, many times decided to stay there, even in the face of the hardships of darkness.

A life that found refuge in them, when the world above became uninhabitable. And that also began to transform.

This diverse, changeable, spectacular and unique life also choreographs, each in its own way, involving and connecting worlds, of light and shadow.

But, even often oblivious to the outside world, this life has never been so threatened.

We humans seem to have forgotten what was given to us over millennia by caves. Shelter, refuge, warmth and union have always been provided by them. But today, we focus only on the thick rock that covers them, forgetting all the magic dance that slowly continues to happen in their entrails.

Caves are a unique heritage of the Brazilian people.

They keep our past, the past of other species that lived here, unique geological formations and a diverse and spectacular fauna.

They (and many of their residents) provide us with essential ecosystem services.

And yet, they are being consumed at an unprecedented speed.

The knowledge, rescued through the reading of these books buried in time, is what contains the recipe for its continuity. This knowledge, more than ever, needs to be disseminated.

Only then can these spectacular environments, endowed with a unique life, be saved and continue their dance through time.

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