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A cave with remarkably high subterranean diversity in Africa and its significance for biodiversity conservation

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Abstract

Aziza cave, which is also known as kef Aziza or Tazouguert cave, represents an important and large karstic system that consists of more than 3.5 km of surveyed galleries, standing as the fifth most extensive cave system in Morocco and one of the ten largest in North Africa. This study unveils Aziza cave as an important spot of subterranean diversity in Africa. Here, we provide the first checklist of subterranean fauna in this cave, with 26 taxa, comprising 22 troglobiotic and 4 stygobiotic species. Of this total, eight species still require further confirmation of their status. The richest taxa include Coleoptera (5 species), Araneae (4 species), Entomobryomorpha (3 species), and Isopoda (2 species). However, it is noteworthy that only around 34.6% of the cave-restricted species found in the cave have been formally described to date. Additionally, the biodiversity of large system areas remains to be discovered as these areas need to be further explored. Furthermore, this paper highlights the broader conservation challenges faced by subterranean habitats in Morocco, particularly considering human-induced impacts on these remarkable ecosystems. We aim to draw attention to the crucial ecological role of subterranean environments and their extraordinary biological diversity. By doing so, we aim to inspire increased research and conservation initiatives, not just in this area but across Africa.

Keywords

Aziza, cave conservation, cave diversity, Noth-Africa, protection strategies

Introduction

Subterranean ecosystems play a significant role, serving as crucial freshwater reservoirs on a global scale (Goldscheider et al. 2020). These areas are also home to unique species, some with potential biotechnological applications and others that play important roles in maintaining a healthy environment. Despite their importance, these habitats remain poorly explored in many regions worldwide (Pipan et al. 2020; Sánchez-Fernández et al. 2021; Canedoli et al. 2022). Concealed beneath the surface, subterranean habitats harbor an extraordinary diversity of species that have evolved unique adaptations to survive in darkness, facing challenges posed by oligotrophic conditions and high, constant moisture (Kováč 2018; Pipan et al. 2020). While much remains unknown about these environments, recent studies have revealed caves and other subterranean habitats that are recognized as hotspots of subterranean biodiversity (Culver and Sket 2000; Pipan et al. 2020; Iannella et al. 2021; Ferreira et al. 2023; Pipan and Culver 2024).

Several regions worldwide are renowned for hosting hotspots of subterranean biodiversity (HSB), as evidenced in various studies (Pipan et al. 2020; Culver et al. 2021; Huang et al. 2021; Iliffe et al. 2021; Souza-Silva et al. 2021; Ferreira et al. 2023; Pipan and Culver 2024). Although there is some criticism concerning the cutoff criteria to define a subterranean hotspot (Ferreira et al. 2023), the initial concept proposed by Culver and Sket (2000) encompasses caves or other subterranean habitats that harbor 20 or more cave-restricted species.

Among the most well-known is the Dinaric Karst in Balkan Peninsula, extending through several Balkan countries, which provides a habitat for a rich diversity of cave-dwelling species (Sket et al. 2004). Subterranean hotspot locations have been identified across all continents except Africa and Antarctica. These hotspots span latitudes from 25°S to 45°N, including sites within the tropics, specifically within the seasonal tropics. The Southern Hemisphere contains significantly fewer sites, with none located farther south than 25°S. A concentration of hotspots is observed between 40° and 50°N latitudes, corresponding to the previously described ridge of high cave biodiversity in Europe (Culver et al. 2006; Zagmajster et al. 2018; Pipan and Culver 2024). Additionally, hotspots are nearly absent around the equator (Souza-Silva and Ferreira 2016; Souza-Silva et al. 2021; Ferreira et al. 2023; Pipan and Culver 2024).

However, given the arbitrary cutoff number for defining HSB, it is crucial to identify sites or caves with a remarkable diversity of cave-restricted species, even if their count does not reach the minimal cutoffs. This is important for two reasons: (i) the concept of "high diversity" is often context-dependent, varying with the area where a cave is located, and (ii) new species can be discovered even in well-studied caves, as the subterranean realm extends beyond the accessible macrocaverns. Therefore, identifying and emphasizing caves with a high diversity of cave-restricted fauna holds significant potential for conservation efforts and the establishment of specific protection policies. The specialized adaptations and limited distributions of several subterranean species render them highly susceptible to habitat loss and degradation. As such, the identification and conservation of these hotspots play a pivotal role in ensuring the long-term survival of this exceptional, significant, and fascinating fauna (Niemiller et al. 2018; Pipan et al. 2020; Sánchez-Fernández et al. 2021; Ferreira et al. 2022).

Some areas in Africa have been studied by speleologists (Juberthie and Decu 1994), and a short survey of highly biodiverse cave regions in Africa can be found in Deharveng et al. (2024). Italian zoologists researched Somalia, while Sjöstedt, Alluaud, and Jeannel explored caves in East Africa (Kenya and Zanzibar). In Congo, Heuts and Leleup explored caves, and Decary, Millot, and Paulian conducted extensive studies in the large caves of Madagascar. Hiernaux and Villiers collected fauna from sandstone and laterite caves in Guinea. Additionally, in North Africa, Peyerimhoff studied subterranean fauna in Algeria, and Strinati and Aellen examined cave fauna in the Taza mountains of Morocco, among others. Furthermore, Leleup worked on a comprehensive review of cave fauna in tropical Africa (Jeannel 1926; Strinati and Aellen 1959; Juberthie and Decu 1994; Vandel 2013). Despite these efforts, significant gaps remain, and African subterranean ecosystems have received little attention from researchers in recent decades. This has made Africa one of the least studied continents regarding cave-restricted species and their associated habitats, likely due to insufficient sampling and the rarity of favorable habitats (Deharveng and Bedos 2018). Despite this lack of attention, previous studies have revealed a diverse array of subterranean species, many of which are found nowhere else on Earth (Hamer and Brendonck 1997; Messana 2004; Messouli 2012; Boutin et al. 2001; Messouli and Boutin 2001; Kayo et al. 2002; Ferreira et al. 2020; Monticelli-Cardoso et al. 2021). The predominant focus of historical research has been on species descriptions, with only a limited number of recent publications delving into cave ecology, community, and conservation (Togouet et al. 2009; Kayo et al. 2012; Vandel et al. 2013; Deharveng and Bedos 2018; Ferreira et al. 2020; Du Preez et al. 2023). These remarkable species are uniquely adapted to thrive in challenging conditions, rendering them particularly intriguing subjects for researchers studying evolutionary biology and biogeography (Trontelj et al. 2012; Culver and Pipan 2019; Pipan et al. 2020; Sánchez-Fernández et al. 2021).

This paper highlights the occurrence of a cave with a remarkable subterranean diversity in Africa, known as the Aziza cave (also referred to as Tazouguert cave and locally as Kef Aziza), located in Morocco. Our study analyzes the cave's distinctive geological and hydrological characteristics and the diverse species inhabiting this unique ecosystem. Additionally, we address the conservation challenges that some subterranean habitats in Morocco face, particularly concerning human-induced impacts on subterranean species. By highlighting these ecosystems and their exceptional biodiversity, we aim to inspire more research and conservation efforts in this region and across Africa.

Materials and methods

Study area

The Aziza cave is located in the Tazzouguert Plateau, within the administrative boundaries of the Oued Naam commune near Boudnib town, in the province of Er-Rachidia, within the Drâa Tafilalt region of eastern Morocco (Fig. 1A, B). This cave is in the Moroccan Eastern High Atlas, approximately 80 kilometers from Errachidia town. The area has a population exceeding 92,000 residents (HCP 2019).

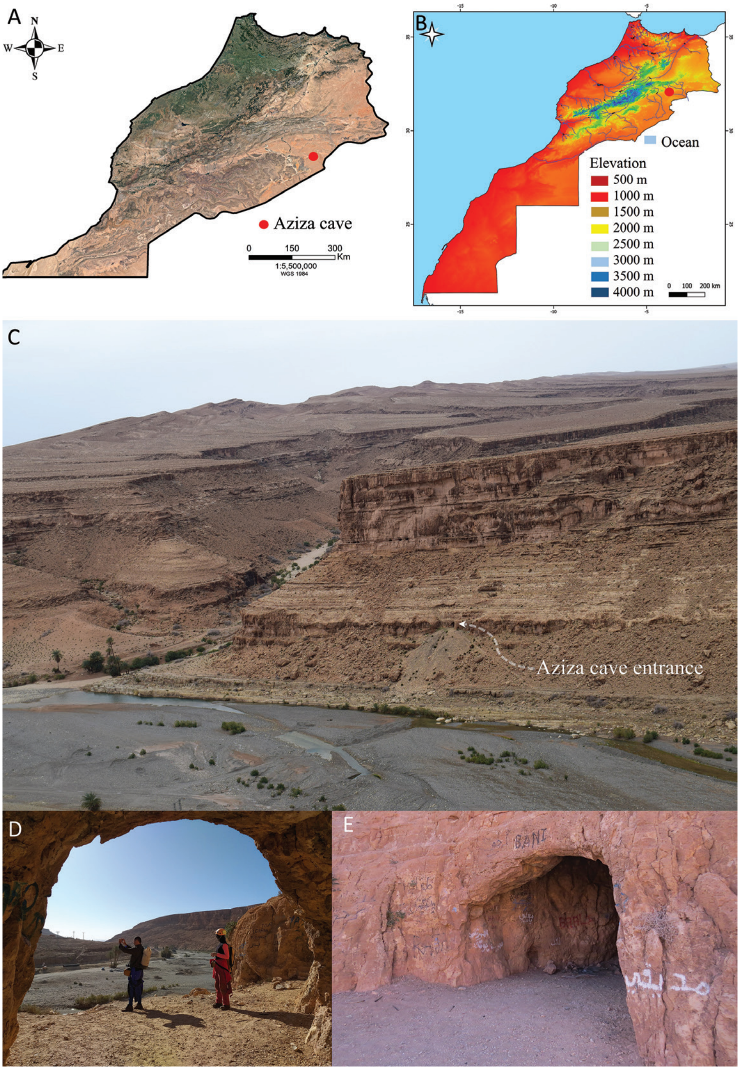

Figure 1. The location of Aziza cave in Morocco Sahara Desert on a landscape map (**A**) and an altimetric map (**B**). A landscape view of the surrounding area of Aziza Cave (**C**). View from the inside to outside at the entrance (**D**), outside view of the entrance (**E**).

The Aziza cave is situated in a pre-Saharan zone of Morocco (Karmaoui et al. 2022), where approximately 37% of the land area in the province is covered by a stony desert landscape known as 'Hamadas'. This landscape features an arid environment with hard soil, rock slabs, plateaus, and minimal sand (Fig. 1C). The distribution of rainfall in the Errachidia province is irregular both in time and space, ranging from over 250 mm in the elevated regions, such as the High Atlas, to approximately 130 mm near Errachidia city and dropping to less than 75 mm in the desert areas of the Tafilalet plain (HCP 2018). These significant variations result in hyper-arid climatic conditions within the Tafilalet Basin (Herzog et al. 2021). The temperatures in this region range from -5 °C to 40 °C (Ben Salem et al. 2011).

The area is part of the Guir's Hamada, a plateau spanning approximately 1000 square kilometers. This plateau is primarily composed of sedimentary layers of Cenomanian-Turonian limestone, consisting of bioclastic limestones with bryozoans and stromatoporidae, followed by marl limestones containing bioclastic ammonites, lamellibranches, echinoderms, gastropods, and bryozoans (Ettachfini et al. 2020).

The Cretaceous sedimentary basin in the region comprises three overlapping exploitable aquifers. Below is an impermeable substratum of Cenomanian Marl (Infracenom), while Senonian sandstones and clay sands are above. Between these layers exists the Turonian, which serves as the primary aquifer in the basin. The thickness of this reservoir varies between 20 and 100 meters. Fissures and karstification of the limestone formations contribute to the formation of several springs, such as Mouy (Qm p 80 l/s), Tamazirt (Qm p 135 l/s), Meski (Qm p 167 l/s), and others, along with the underground network of the Aziza cave, which is classified as hypogenic (El Ouali et al. 1999; Audra 2017).

The province's economy relies heavily on agriculture, making it highly dependent on water resources. Moreover, the region faces several challenges, including soil and water salinization, desertification, and silting. These challenges exacerbate the region's vulnerability, underscoring the urgent need for sustainable strategies to mitigate their impacts (Karmaoui et al. 2022). Addressing these issues is crucial to safeguarding the region's agricultural productivity and ensuring the well-being of its inhabitants.

Network galleries of Aziza cave

Aziza Cave (32.0146°N, -3.4717°W) is situated in the Eastern High Atlas, approximately 80 kilometers from Errachidia city towards Boudnib, at an elevation of 1059 m above sea level (Benani et al. 2022). The cave is positioned 30 meters above the riverbed on the right bank of the Guir River, within the Turonian limestone formation (Fig. 1C). It boasts two entrances: a horizontal one measuring 2 × 3 m, visible from the road on the river's opposite side above a scree cone. A few meters away, there is a second entrance, which is vertical (Fig. 1C–E). It is important to note that the cave initially had only a single entrance (the vertical one), and the horizontal entrance was artificially excavated.

The gallery of the cave gently descends, featuring some meanders and detours while maintaining a consistent SE-NW orientation. The first part of the cave features remarkable and expansive dimensions. After about 450 m from the entrance, one reaches the Guano Room (large cavities in Fig. 2), the largest chamber of the cave, reaching up to 30 m in height. This chamber is home to a colony of hundreds of bats, and on the ground, there are big guano piles, some of which are partially fossilized in ancient areas. Over the subsequent 400 m, the corridor alternates between narrowing and widening, displaying a higher sinuosity coefficient than the first section, resembling a meandering form. The galleries are then continuously divided into two distinct passages that join as tributaries (Branch work cave) (Fig. 2).

Aziza cave speleogenesis

The location of Aziza Cave near the Guir River suggests that it might have served as an insurgence of the river during periods when the water level was higher than the current cave entrance. However, a study conducted by a Croatian team in 2003 proposed an alternative hypothesis, suggesting that the cave's formation occurred in two distinct stages. The first stage involved a phreatic genesis underground, which was ancient and led to fossilization. During this phase, the slope of the cave might have been lowered due to the erosion caused by the Oued Guir, possibly affecting a part of the cave system. Subsequently, a second phase occurred, which is more recent and primarily affected the deeper parts of the cave through vadose processes. This second phase is

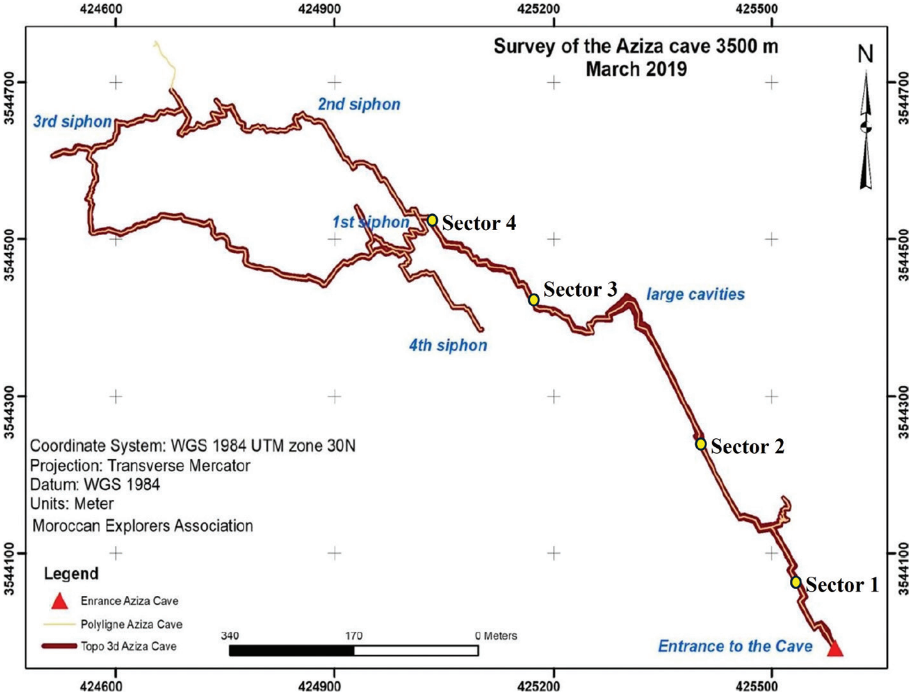

Figure 2. Topography of the AZIZA Cave 2019-2020 (Bennani et al. 2022) showing the conduits extension and siphons and sample units (sectors and quadrants) distributions.

not directly linked to the genesis of the main cave system. Instead, it is believed to have evolved in drier climates with well-developed speleogenetic mechanisms, possibly influenced by different lithotypes and favored by widespread absorption on heavily cracked plains of the hamada (Buzio et al. 2003).

The presence of bats has also significantly influenced the cave's morphology. Biogenic corrosion, caused by the secretions of these animals, has had a profound impact on the condition of the cave walls and roofs. The mineralized urine droplets directly attack the surrounding limestone, leading to corrosion over time. Moreover, the combination of ammonia from urine and CO_2 from bats' respiration produces carbonic acid through condensation, further extending the corrosion process. As a result of these physicochemical processes, the main gallery now exhibits various unique features, including ceilings with bell holes, hemispherical domes, and smooth walls, which have erased the original morphologies of the cave (Fig. 3A, B). This chain of erosion processes, driven by bats, their secretions, and the heat they generate, has led to a late enlargement of the cave and the modification of its geometry and characteristics (Dandurand et al. 2019).

Explorations, topography, and research

The first known explorations of Aziza Cave date back to 1925, followed by another expedition in 1948 (Benani et al. 2022). The Moroccan Caving Society conducted the initial topographical survey of the cave in 1953, resulting in a network development of approximately 1540 meters (up to the first two siphons) with basic estimates of the gallery's height and width (Buzio et al. 2003).

1970 a Spanish expedition explored only 1000 meters of the cave (Buzio et al. 2003). Between 1972 and 1979, the cave was partially explored and surveyed, extending the network to around 1500 meters as part of a project funded by the Keimer Foundation in Basel (Buzio et al. 2003). In 1982–1983, various Croatian cave groups discovered a new cave section, adding approximately 960 meters to its total length (Bolanic et al. 1983). In 2003, Italian speleologists conducted a new topographical survey of Aziza Cave, expanding the network to an impressive length of 3500 meters (Buzio et al. 2003). Then, in 2019, a Moroccan team utilized advanced technologies, including Disto X and 3D laser representation, to conduct a topographic survey, revealing a network length of over 4000 meters (Benani et al. 2022) and making it the fifth-largest cave system in Morocco, and one of the 10th largest in North Africa (MET 1981; Nehili and Naouadir 2021; Benani et al. 2022).

Aziza cave has been the subject of several biospeological expeditions over the years. The first survey was conducted in 1968 by the Atlas Expedition of "Equip de Recerques Espeleològiques from the Centre Excursionista de Catalunya" (Canals and Viñas 1969; Español 1969). In 1977, a new Atlas expedition was carried out by the "Grup Mediterrania de Barcelona and the Secció d'Investigacions i Recerques Espeleològiques of the Unió Excursionista de Catalunya" (Lagar 1978). In April 1990, an entomological expedition was organized by Catalan members of the

Figure 3. The interior of Aziza cave features stunning galleries with bell holes, hemispherical domes, and smooth walls labeled as **A, B, C, E**. Additionally, there are water ponds (**D**).

"Asociación Europea de Coleopterología" (Ribera 1983). Then, in 1997, the cave was visited by the Catalan entomologist Carles Hernando (Barranco and Mayoral 2007). This was followed by an Atlas expedition organized by the Catalan association BIOSP in 2001 (Stüben 2009).

Literature review on the cave fauna

The data on the Aziza cave fauna was gathered by conducting a comprehensive literature search, focusing on relevant keywords such as "African cave biodiversity," "Aziza," "Tazouguert," "cave fauna," "troglobitic fauna," "stygobitic fauna," "groundwater," "pollution," and "North Africa." We selected the most pertinent databases that contained information related to cave biodiversity.

Cave climate, organic resources, and physical traits

To investigate the spatial variation of temperature and humidity within the cave, we used temperature and humidity data loggers (accuracy ± 1 °C for temperature and ± 5% for relative humidity). Starting from the cave entrance, we positioned the instrument on the cave floor and recorded data for 15 minutes (Souza-Silva et al. 2021). Our measurements were taken at eight distinct distances within the cave: 35 meters, 85 meters, 140 meters, 165 meters, 500 meters, and 800 meters from the entrance. In Addition, two temperature measurements were conducted near siphons 1 and 2. We closely monitored the readings until the temperature and humidity levels reached a stable state inside the cave at each point. We also conducted an in-situ examination of the organic resources on the walls and floor of Aziza Cave to gain insights into potential food sources for the fauna. However, we did not quantitatively measure the number of organic resources, their accumulation, access pathways, or decomposition rates. Consequently, our trophic characterization was limited to a qualitative evaluation. Finally, we examined the substrate characteristics of the cave floor to describe the microhabitats available for the fauna.

Sampling cave invertebrates

to create a comprehensive documentation of invertebrate species to the cave environment, we used various search methods to thoroughly investigate the different microhabitats within the cave (Souza-Silva et al. 2021). Sampling was conducted during multiple cave visits, specifically in 2002, 2003, 2020, and 2022. The survey of invertebrates was conducted utilizing tweezers and brushes using different methods and sampling techniques following the procedures described by Wynne et al. (2019): 1-hand sampling with Direct Intuitive Search (DIS), visual search, and opportunistic collecting. 2-Pitfall trapping. 3-Aquatic substrate sampling. 4-Attractions using bait. Besides this, 12 quadrats (measuring one m^2) and four sectors (measuring 10 × 3 m) were used to search for small invertebrates within microhabitats during our visit in 2020 (Fig. 2), with the participation of three collectors in the sampling process, and sampling efforts continued until all invertebrates were accounted for. This approach led to a significant increase in the number of cave-restricted species documented in the cave. However, it is important to note that methods involving invertebrate extraction from substrates, such as Berlese-Tullgren funnels, were not employed.

Determination of troglobionts and stygobionts

The terms stygobionts and troglobionts encompass species that live in caves and various shallow subterranean and above-ground habitats. While most subterranean species typically display troglomorphy, including reduced eyes and pigment, increased size, elongated appendages, and extra-optic sensory structures, certain troglobionts may exhibit limited or no troglomorphy due to factors such as habitat volume, twilight exposure, isolation age, genetic variability, and others. This variability suggests some species may qualify as eutroglophiles (Deharveng et al. 2024). Consequently, uncertain cases have been considered potential troglobionts for terminological consistency, pending further detailed studies to clarify their categorization.

Species restricted to subterranean environments, such as troglobionts and stygobionts, cannot complete their life cycles in aboveground habitats (Sket 2008). One way to identify these subterranean-adapted species is by recognizing specific morphological traits, known as troglomorphisms, commonly observed in troglobitic and stygobitic fauna (Sket 2008; Culver and Pipan 2019). These adaptations include reduced eyes, pigmentation, and hypertrophy of nonvisual sensory structures and locomotor appendages. Such traits provide evidence of the species' adaptation and isolation in subterranean habitats. Many species are easily recognizable as soil species, lacking pigmentation and eyes due to their specialization for life in topsoil and leaf litter. We classified species as "potential troglobionts" if they are exclusively known from Aziza cave and exhibit troglomorphic traits, lacking pigmentation and eyes. However, some species do not display specific adaptations to subterranean life (features inconsistent across all troglobiont species e.g., *Scaurus tingitanus gimeli*). Nevertheless, they were only found within the cave, despite several samplings in external areas and the considerable size of this species. These species were never observed near the entrances, either inside or outside, or at other locations in North Africa more broadly (Chavanon et al. 2015). It is important to note that many specimens of the recently discovered species have not been properly identified and are only classified at higher taxonomic levels. While this lack of taxonomic precision is a limitation, many of these specimens likely represent new taxa. Therefore, as in other instances where species-level identification is not feasible for all taxa (Clark et al. 2021; Deharveng et al. 2021; Ferreira and Souza-Silva 2023; Ferreira et al. 2023), we are presenting the taxa at the highest possible taxonomic level achieved.

While troglomorphisms offer valuable insights into the potential status of species, their analysis must consider the contexts of the external ecosystems surrounding caves (Ferreira and Souza-Silva 2023). For example, suppose a species showing complete depigmentation, blindness, and a reduced cuticle is found in a cave in an arid region. In that case, these morphological traits strongly indicate potential troglobionts. In the surrounding epigean environments, such organisms rarely encounter suitable microhabitats for survival. Hence, considering the epigean desert surrounding the Aziza cave (with temperatures reaching up to 40 °C and air moisture measured on October 6, 2022, of approximately 22.6%), it is unlikely that troglomorphic species could maintain viable populations on the surface. In contrast, the temperature inside the cave is around 23 °C (±1.5), with an air moisture level of about 92.2%. Therefore, troglomorphic traits not only played a crucial role in identifying these species as troglobitic but also the external conditions, which were highly restrictive, imposing physiological constraints and preventing their occurrence in external habitats. All these factors collectively lead us to attribute troglobiont characteristics to these new species.

Human uses and alterations

Based on our recent cave explorations and a review of existing literature, we have assessed and categorized human interactions with modifications to the cave and its surrounding areas. Our evaluation, guided by the framework established by Souza-Silva et al. (2015), provides a detailed qualitative account of these impacts. Additionally, one of our researchers, S. Moutaouakil, has visited the cave and its vicinity to identify potential human-induced changes.

Results

Cave's climate, microhabitats and organic resources

The cave displayed distinct conditions within its inner regions. Close to the entrance, the air was noticeably dry (up to 80 m – 62.4% moisture content), while deeper inside (after 100–150 m), it became increasingly humid (reaching 98.2% moisture content after 800–850 m), resulting in a diverse and heterogeneous cave ecosystem concerning humidity levels. The average air temperature inside the cave hovered around 23 °C (±1.5), with inner portions maintaining an average air moisture of about 80% (±18). The cave floor displays organic and inorganic materials such as bat feces, plant debris (from torches used by visitors), and various types of clastic sediments, ranging from large rocks to gravel, sand, silt, mud, and hardpan. This diverse mix of materials allows for different microhabitats with varying characteristics as you move from the cave entrance to its deeper sections. Notably, the substrate composition near the entrance showed more diversity, while it became less varied in the deeper parts of the cave.

The primary sources of nutrients for terrestrial and aquatic fauna within the cave are guano and bat carcasses. Additionally, plant debris is scattered throughout the cave, displaying various degrees of decomposition (Fig. 6A–C). This plant debris appears to have been brought into the cave by sporadic human visitors from external environments. While not serving as a regular food source, the cave-restricted millipede *Jeekelosoma abadi* was observed feeding on both the plant debris (Fig. 6C) and guano pellets (Fig. 6D), demonstrating the generalist diet of this species, which may also be the case of several other cave-restricted detritivores found in this cave.

Figure 4. Long-tailed bat *Rhinopoma hardwickei* (**A**), and other bats (**B, C**) were observed inside Aziza cave.

Bat assemblages and abundance in Aziza cave

In May 2002, the cave hosted several colonies of *Rhinolophus euryale* Blasius, 1853, and *Myotis punicus* Felten, Spitzenberger & Storch, 1977. These bats were mostly found scattered across the ceiling of the large chamber, approximately 450 meters from the cave's entrance (Fig. 4). However, in April 2003, the colonies were significantly smaller, accounting for about one-third of the population observed in 2002. By the winter of 2003, the number of bats further declined, with only approximately two hundred individuals observed, including *Myotis punicus*, *Rhinolopus euryale*, and *Rhinopoma hardwickei* Gray, 1831. The latter species, with a tail almost as long as its body, is generally found in small groups of up to 10 individuals or even solitary (Buzio et al. 2003).

The discovery of some bat corpses enabled species identification through bone measurements, revealing the presence of a fourth species: *Miniopterus schreibersii* (Kuhl, 1817). In 2014, the Aziza cave was designated as the type locality for a new species: *Miniopterus maghrebensis* Puechmaille, Allegrini, Benda, Bilgin, Ibañez & Juste, 2014 (Puechmaille et al. 2014). This discovery increased the diversity of bats in this cave, bringing the total number to five species.

Cave-restricted fauna

To date, 26 troglobitic and stygobitic species have been documented within Aziza cave, comprising 22 troglobiotic and 4 stygobiotic species. Of this total, eight species still require further confirmation of their status; thus, at least 18 are cave-restricted. These species are distributed across several taxonomic groups: Arachnida (7 species), Insecta (6 species), Crustacea (4 species), Collembola (4 species), Chilopoda (2 species), Gastropoda (2 species), and Diplopoda (1 species) (Table 1). The richest taxa include Coleoptera (5 species), Araneae (4 species), Entomobryomorpha (3 species), and Isopoda (2 species). The remaining taxa, such as Bathynellacea, Copepoda, Eupumonata, Geophilomorpha, Sternorrhyncha, Neotaenioglossa, Palpigradi, Polydesmida, Pseudoscorpiones, Scolopendromorpha, and Symphypleona, are represented by one species each (Fig. 5).

Notably, only around one-third (34.6%) of the cave-restricted species found in the cave have been formally described to date. These described species include *Dysdera caeca* Ribera, 1993, *Lepthyphantes fadriquei* Barrientos, 2020, *Eukoenenia maroccana* Barranco & Mayoral, 2007, *Platyderus insignitus presaharensis* Lagar Mascaró, 1978, *Torneuma troglodytis* Stüben, 2009, *Apteranillus ruei* Español, 1969 (Perreau and Faille 2012), *Scaurus tingitanus gimeli* Peyerimhoff, 1948, *Jeekelosoma abadi* Mauriès, 1985, *Magnezia gardei* Magniez, 1978.

Regarding the aquatic fauna, Aziza Cave has revealed the presence of four stygobiont species: the isopod *Magniezia gardei*, a hydrobiid gastropod, a copepod species,

Table 1. The Aziza cave in Er-Rachidia, Morocco, is home to a diverse array of terrestrial and aquatic obligate cave-dwelling invertebrates. Unidentified (un). TB -Troglobite; SB - Stygobite; TB? - Potential troglobionts (further studies needed for confirmation).

Taxon	Taxon	Family name	Species/morphotypes	Status
Arachnida	Acari	Parasitengona	Parasitengona sp. 1	TB?
	Araneae	Hahniidae	Hahniidae	TB
		Dysderidae	*Dysdera* sp.	TB
			Dysdera caeca Ribera, 1993	TB
		Linyphiidae	*Lepthyphantes fadriquei* Barrientos, 2020	TB
	Palpigradi	Eukoeneniidae	*Eukoenenia maroccana* Barranco an Mayoral, 2007	TB
	Pseudoscorpiones	Chthoniidae	Chthoniidae sp.	TB?
Collembola	Symphypleona	Arrhopalitidae	*Arrhopalites* sp.	TB?
	Entomobryomorpha	un	Entomobryidae sp. 1	TB?
		un	Entomobryidae sp. 2	TB?
		un	Isotomidae sp. 1	TB?
Insecta	Coleoptera	Carabidae	*Platyderus insignitus presaharensis* Lagar Mascaró, 1978	TB
		Curculionidae	*Torneuma troglodytis* Stüben, 2009	TB
		Pselaphinae	*Tychobythinus* sp.	TB?
		Staphylinidae	*Apteranillus ruei* Español, 1969	TB
		Tenebrionidae	*Scaurus tingitanus gimeli* Peyerimhoff, 1948	TB
	Sternorrhyncha	Kinnaridae	Kinnaridae sp.	TB
Diplopoda	Polydesmida	Paradoxosomatidae	*Jeekelosoma abadi* Mauriès, 1985	TB
Chilopoda	Scolopendromorpha	Cryptopidae	*Cryptops (T.) aff. numidicus aelleni* Manfredi, 1956	TB?
	Geophilomorpha	un	Geophilomorpha sp.	TB?
Crustacea	Bathynellacea	Bathynellaceae	Bathynellaceae sp.	SB
	Isopoda	Ollibrinidae	*Castellanethes* sp.	TB
	Asellota	Stenasellidae	*Magnezia gardei* Magniez, 1978	SB
	Copepoda	un	Copepoda sp.	SB
Gastropoda	Eupumonata	un	Eupulmonata sp.	TB?
	Neotaenioglossa	Hydrobiidae	Hydrobiidae sp.	SB

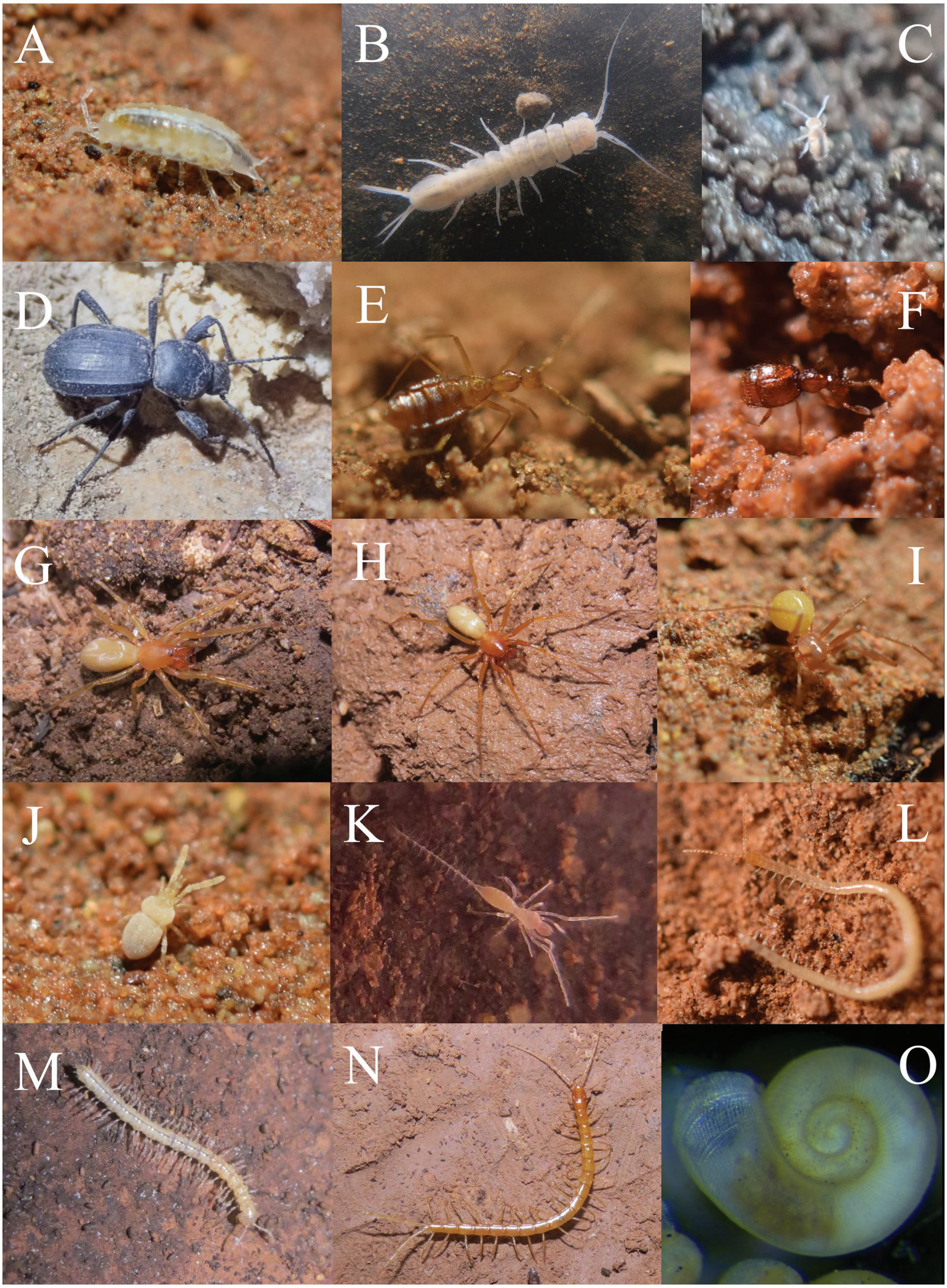

Figure 5. Some of the species restricted to the Aziza cave, Morocco. *Castellanethes* sp. 1 (**A**), *Magnezia gardei* (**B**), *Arrhopalites* sp. 3 (**C**), *Scaurus tingitanus gimeli* (**D**), *Apteranillus ruei* (**E**), *Tychobythinus* sp. (**F**), *Dysdera* sp. 1 (**G**), *Dysdera caeca* (**H**), *Lepthyphantes fadriquei* (**I**), Parasitengona sp. 1 (**J**), *Eukoenenia maroccana* (**K**), Geophilomorpha sp. 1 (**L**), *Jeekelosoma abadi* (**M**), *Cryptops* (*Trigonocryptops*) aff. *numidicus aelleni* (**N**), Eupumonata sp. 24 (**O**).

Figure 6. In Aziza Cave, terrestrial invertebrates rely on plant debris (**A, B, C**) and small guano pellets (**D**) as sources of nutrients. Specimens of *J. abadi* forage on plant debris for sustenance, indicated by red circles in section **C**. Additionally, the red arrow in section **D** highlights the presence of *J. abadi* specimens foraging on guano pellets.

and a Bathynellacea species. The first two species are found in a small, clayey substrate puddle 4 meters in length and 1 meter in depth, located in the left branch 1000 meters from the entrance (Figs 2, 3D) (water temperature: 22.44 °C; conductivity: 506 µS/cm). The copepod and Bathynellacea were found in a large lake on the right branch after the second siphon (Fig. 2) (water temperature: 22.5 °C; conductivity: 348 µS/cm).

Magniezia gardei was first described from Aziza Cave but has a relatively broad distribution, having been found in several wells in the Errachidia and Zagora regions (Ait Boughrous et al. 2007; Boudellah et al. 2022). The stygobiotic gastropod is a new species of a new genus currently under description (M. Ghamizi, pers. comm.).

The terrestrial fauna in Aziza Cave exhibits remarkable diversity, comprising 22 troglobitic and/or troglomorphic species (Table 1). Species classified as troglomorphic exhibit adaptations and characteristics indicative of specialization in subterranean environments. For example, the spider Hahniidae displays pale coloration and is eyeless, while the pseudoscorpion Chthoniidae has a pale-yellow color and lacks eyes or eye spots. *Scaurus tingitanus gimeli*, with its elongated legs and antennae, differs from the typical form found in epigean populations in northern Morocco (Labrique 1995; Vinolas and Maso 2005).

Regarding their sampling areas, Hahniidae sp. were observed 400 meters from the entrance, while the Coleoptera *Scaurus tingitanus gimeli* is distributed between 200 meters and 800 meters. Because the species is only known from caves and appears absent on the surface (assuming adequate sampling in the area), it is justifiable to categorize it as a troglobiont, regardless of the Labrique (1995) statement, which considered the species as troglophile, even assuming its restriction to the cave environment.

A solitary individual of Chthoniidae sp. was captured 600 meters from the entrance, near the remains of a mammal (sheep or goat bones were observed).

Several species are endemic to this cave, including the Diplopoda *Jeekelosoma abadi*, the Araneae *Dysdera caeca* and *Lepthyphantes fadriquei*, and the Palpigradi *Eukoenenia maroccana*. These species are distributed in the explored part of the cave beyond the guano room, a large cavity depicted in Fig. 2. Additionally, the Coleoptera *Apteranillus ruei* and *Torneuma troglodytis* were captured in narrow galleries after 800 meters from the entrance. The coleopteran *Platyderus insignitus presaharensis* was 200 meters from the entrance (Espanol 1969; Mascaro 1978; Barranco and Mayoral 2007; Stüben 2009; Barrientos 2020).

The coleopteran Pselaphinae *Tychobythinus* sp., the Sternorrhyncha Kinnaridae sp., and the Geophilomorpha sp. were collected in clayey habitats with humidity levels close to saturation (over 98%) after 800 meters from the entrance. The species of Kinnaridae was collected near the third siphon.

Human use and alterations

In addition to its proximity to a town (Boudnib, 19.7 km), the cave is situated near a paved road (Fig. 7A), making it easily accessible to visitors. The cave entrance currently lacks a gate, allowing unrestricted access. In addition, there are ongoing activities in the riverbed directly in front of the cave entrance, involving the use of tractors to extract gravel (Fig. 7A, B).

The initial section of the cave, easily accessible to visitors, has unfortunately been heavily impacted by graffiti and trampling. As one approaches the entrance, litter and shattered rocks, along with fragments of glass and plastic, can be observed (Fig. 7C). Various drawings and writings have marred the once pristine white walls of the cave, created using mud, paint, or charcoal (Fig. 7D–G). Moreover, remnants of ash, burned pieces of wood, and fragments of broken glass can be found inside the cave. The local inhabitants, possibly fearing respiratory diseases caused by guano (such as histoplasmosis), may have attempted to remove the animals by using numerous wind torches placed in the walls around the large chamber (Moutaouakil S. Personal communication). These activities potentially threaten the cave environment and its delicate ecosystem.

Figure 7. Human activities have significantly impacted the Aziza caves and their surrounding area. The construction of roads and using tractors near the riverbed have affected the caves directly, particularly entrances (**A, B**). Additionally, the caves have been marred by the deposition of garbage at entrance **C** and graffiti along the cave walls at positions **D, E, F, G**.

Discussion

The Aziza Cave stands out as a significant subterranean habitat, presenting 18 troglobiotic and stygobiotic species, plus eight taxa that may also represent cave-restricted species, making it the richest cave regarding troglofauna and stygofauna in Africa. The second richest cave is the Wynberg Cave System, located in the mountains of Cape Town, South Africa, which hosts 19 cave-restricted species (Ferreira et al. 2021). It is worth mentioning that although the Wynberg Cave System has not been officially proposed as a subterranean biodiversity hotspot, it indeed represents a hotspot in the African continent, based on criteria proposed by Souza-Silva et al. (2015) and Ferreira et al. (2023). According to these authors, the Wynberg Cave System should be considered a hotspot of subterranean biodiversity due to its significant richness of cave-restricted species, considering both its siliciclastic lithology, which typically harbors fewer species of this category, and the increasing anthropogenic impacts it faces. Therefore, both Aziza and the caves from the Wynberg Cave System deserve the utmost attention in terms of protection and conservation policies.

Subterranean biodiversity of Aziza cave

The high number of cave-restricted species in Aziza Cave can be attributed to a combination of factors. Firstly, its location in the Sahara Desert gives this cave a unique setting, distinguishing it from the arid and harsh surface environment. The high and constant humidity conditions within the cave starkly contrast with the arid surroundings. The species richness may also be linked to historical climatic changes in the region where the cave is situated (Jeannel 1943; Magniez 1978; Huges et al. 2018).

Furthermore, the substantial size of the cave, stretching approximately 4 kilometers, provides ample space for the development of several microhabitats, which can support various distinct invertebrate taxa. Lastly, the presence of subterranean water bodies further enhances the diversity of terrestrial and aquatic habitats available for colonization by the fauna. It is important to mention that a high richness of cave-restricted species is often associated with large subterranean spaces, high productivity, and/or isolated water bodies separate from the surface (Culver and Pipan 2009). These factors collectively contribute to the remarkable biodiversity observed in Aziza Cave.

The distribution and degree of adaptation of most cave-dwelling invertebrates within caves are often more influenced by the physical environment of the cave rather than by food resources or cave geology (Novack et al. 2012; Pacheco et al. 2020; Nicolosi 2021; Souza-Silva et al. 2021; Furtado-Oliveira et al. 2022). Obligate cave-dwelling invertebrates typically have their distribution determined by humidity conditions, thriving in places with stagnant air that is saturated with water vapor. However, many species also venture into the transition zone, either in search of food or inadvertently (Howarth 1988; Humphrey 1990; Wilkens et al. 2000; Souza-Silva and Ferreira 2016; Souza-Silva et al. 2021; Souza and Ferreira 2022).

Sampling effort and the "lack" of Subterranean Biodiversity Hotspots in Africa

The apparent scarcity of Subterranean Biodiversity Hotspots in Africa is a noteworthy issue. Despite the continent's vast expanse and its potential for hosting unique subterranean ecosystems, there has been limited exploration and documentation of these habitats. This dearth of attention to subterranean biodiversity has led to significant knowledge gaps, impeding our understanding of the richness and ecological importance of these ecosystems in most parts of the continent.

While the biodiversity of Aziza Cave has been extensively documented, it is important to highlight that the current fauna list is likely incomplete. There are still unexplored areas within the cave that necessitate comprehensive biological inventories. Furthermore, many microhabitats have not been adequately sampled, as specific techniques for sampling smaller invertebrates in terrestrial and aquatic habitats were not employed, as mentioned in the methodology.

We must not assume that the species richness of a subterranean habitat is fully known, which is why new explorations are often necessary (Souza-Silva and Ferreira 2016; Culver et al. 2021; Ferreira and Souza-Silva 2023). Sampling subterranean environments poses challenges due to the inaccessibility of fissures, interstitial habitats, and caves (Culver and Pipan 2009; Trontelj et al. 2012; Ortunõ et al. 2013; Mammola et al. 2021; Ferreira et al. 2023). It is important to conduct multiple collections to accurately document the diverse range of life in subterranean habitats. However, for comparative studies, rapid assessment methods can be used with success (Simões et al. 2015; Souza-Silva et al. 2015), if a standardized sampling approach is employed. In situations like these, caves with many troglobiotic species are expected to stand out even with minimal collection efforts (Souza-Silva and Ferreira 2016). The historical absence of biodiversity hotspots in Africa's subterranean regions can be attributed to the lack of comprehensive studies on this continent. Additionally, the absence of standardized sampling methods may have hindered the identification of areas requiring conservation attention (Souza-Silva and Ferreira 2016; Culver et al. 2021; Ferreira et al. 2023).

On the other hand, according to Culver et al. (2021), the latitudinal distribution of subterranean biodiversity hotspots exhibits a bimodal pattern. Most of these sites are predominantly situated in temperate zones, typically between 40 and 50 degrees north or south of the equator. Particularly for the northern hemisphere, this latitude corresponds to the region traditionally regarded as having the highest richness of subterranean species, a pattern often attributed to the effects of repeated Pleistocene glaciations (Jeannel 1943). A second region is represented by subtropical and sub-temperate areas, approximately 20 and 30 degrees north and south of the equator. These locations mainly consist of lava tubes and wells connected to chemoautotrophic zones. Interestingly, none of these sites are in arid tropics, suggesting that food availability and maintaining high moisture content may also be significant factors influencing species richness (Culver et al. 2021).

Conservation and protection of Aziza cave fauna

Conserving the fauna of Aziza Cave presents a significant challenge, as it is impacted not only by local factors but also by local and global climate issues (Akdim 2015; Boudellah et al. 2022; Karmaoui et al. 2022).

Aziza Cave is situated within the UNESCO Biosphere Reserve, Oasis du Tafilalet (Ramsar site no. 1483), a site of significant biological and ecological importance. Oasis du Tafilalet is in Errachidia, Goulmima, Sahara SE Morocco (31°17'N, 004°15'W), covering an area of approximately 1,370 km^2. The site encompasses a series of oases, serving as the reservoir for one of Morocco's oldest dams, Hassan Ad-Dakhil, which contains small rivers, irrigation channels, and lagoon areas. It serves as an essential wintering ground for migratory birds and is home to notable populations of Rüppell's pipistrelle bat (*Pipistrellus rueppelli*). Agriculture is a prominent activity in the region, with Alfalfa, cereals, henna, date palms, and fruit trees being the primary crops. Sheep farming is also prevalent.

However, the management of dam water releases downstream has resulted in some channels having water only during specific times of the year, further exacerbated by excessive water abstraction for agriculture and human consumption, along with the increased frequency of droughts in recent decades. Additionally, soil salinization has become a problem in various areas due to high evaporation rates (Ait Boughrous et al. 2007; Messouli et al. 2008; https://rsis.ramsar.org/ris/1483). These chemical differences in water dripping from epikarst can affect certain communities' persistence (e.g., copepods) (Pipan 2004; Pipan et al. 2006; Culver and Pipan 2010).

Subterranean habitats, such as caves, are relatively unexplored environments with limited attention due to their challenging accessibility (Mammola et al. 2021). Cave environments' extreme isolation and distinct conditions render many species rare and vulnerable. Numerous obligate cave-dwelling species are deemed threatened or endangered at regional or global scales, primarily due to their confinement to small geographical areas (Culver and Pipan 2019).

When Culver and Sket (2000) classified subterranean biodiversity hotspots, they overlooked the degree of threat these habitats faced, neglecting the hotspot model proposed by Myers et al. (2000) for surface environments. Areas susceptible to economic activities undergo rapid landscape transformations, and in many cases, these changes are irreversible. Previously well-conserved landscapes can be swiftly altered into grazing areas or devastated by mining activities (Souza-Silva et al. 2015; Souza-Silva et al. 2021; Ferreira et al. 2023).

In this context, relying solely on the richness of troglobiotic species may not accurately reflect the "health" of a specific subterranean system, as this will depend on the type of impact it has endured (Souza-Silva et al. 2015; Ferreira et al. 2023). Therefore, the degree of impact to which a cave is subjected should be incorporated into this concept (Ferreira et al. 2023), especially considering that conservation policies often prioritize investments. Regrettably, numerous caves are being damaged or experiencing adverse impacts due to unsustainable tourism, overcrowding, and acts of vandalism, jeopardizing their integrity and ecological value (Culver et al. 2021; Nanni et al. 2023).

Numerous conservation efforts have been undertaken worldwide to safeguard subterranean habitats, their fauna, and the ecosystem services they provide. A comprehensive global assessment, drawing from the opinions of over 150 experts, has identified that legislation, public policies, landscape protection and management, and environmental education constitute the most crucial conservation measures (Nanni et al. 2023). In the context of Aziza Cave, implementing these conservation measures is imperative to ensure the protection of its endemic cave-dwelling invertebrates and the preservation of its unique ecosystem services.

Conservation based on establishing legally protected areas and their proper management has been widely recognized as one of the most effective strategies to combat biodiversity loss in various ecosystems (Maxwell et al. 2020). Protecting large areas has proven effective, particularly in cases where little is known about the true extent of the habitat to be preserved and the ecology of the species present, as often seen in subterranean ecosystems (Nanni et al. 2023). However, the effectiveness of these strategies is not absolute, and all sites continue to face threats to some degree. In addition, there is a tangible risk of conflicts if legislation regarding subterranean environments is imposed, as demonstrated by the challenges faced protecting karsts in the Philippines (Urich et al. 2001). Therefore, it is not feasible to rely on a single model to reduce threats and protect subterranean biodiversity spots. Each situation must be carefully considered individually, especially during threat assessments. The threat levels serve as the fundamental criteria for devising appropriate protective measures. However, it is essential to recognize that all global subterranean biodiversity hotspots are worth protecting and serve as valuable sources of regional and national heritage information (Culver et al. 2021). In Morocco, we posit that engaging the local population, when they are cognizant of the significance of these ecosystems and integrated into the implemented projects, will be a crucial foundation for the long-term protection of the cave. The law on Protected Areas (22/07) can be employed to safeguard these sites through a participatory approach.

Promoting awareness campaigns, adopting responsible practices, and embracing sustainable approaches must be the focal point of our conservation endeavors, particularly in the case of Aziza Cave, which harbors such diversity of cave-dwelling invertebrates. Cultivating deep respect for these fragile environments among the local population and implementing effective conservation strategies are paramount. Through these endeavors, we can ensure the enduring survival and safeguarding of these subterranean treasures for the benefit of future generations (Souza-Silva et al. 2019; Gavish-Regev et al. 2023).

Finally, addressing these challenges requires concerted efforts to increase sampling and exploration activities in African caves and other subterranean environments. By focusing on areas with high potential for cave-restricted species, researchers can contribute to identifying and designating new Subterranean Biodiversity Hotspots on the continent. These designated hotspots will serve as focal points for conservation and research initiatives, allowing us to protect better and comprehend the unique life forms that thrive in these underground realms.

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New vouchered and taxonomically verified records of cave-dwelling populations of catfishes of the genus *Rhamdia* (Siluriformes, Heptapteridae) from Costa Rica

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Abstract

Dedicated ichthyological surveys in two karstic caves in Costa Rica resulted in the discovery of hypogean populations from three epigean species of catfishes of the genus *Rhamdia*. The taxonomic identity of these populations was initially determined based on morphological traits and subsequently corroborated with comparative DNA sequence data in a phylogenetic framework. Individuals from all hypogean populations documented herein exhibit only partial troglomorphism, characterized by only moderate (vs. complete) integumentary depigmentation without extreme eye reduction/loss. A similar pattern of incomplete troglomorphism at the individual level has been observed in other cave-dwelling species/populations of Middle American *Rhamdia*, and tentatively attributed to gene flow with and/or incipient speciation from epigean lineages. Since most hypogean forms of *Rhamdia* derive from/are part of a larger clade of primarily *R. laticauda*, our discovery of cave-dwelling populations assignable to *R. nicaraguensis* and *R. guatemalensis* is noteworthy, particularly in the case of the former, which represents the first taxonomically verified record of a cave-dwelling population of this epigean species. Among our findings is the remarkable discovery of hypogean populations from two different species of *Rhamdia* (*R. laticauda* and *R. nicaraguensis*) inhabiting the same cave (Gabinarraca). This finding is particularly significant because it represents the first time that cave-dwelling populations from different species of *Rhamdia* are reported to be living in syntopy. Continued discovery of cave-dwelling populations during targeted ichthyological surveys reinforces the notion that our understanding of the diversity of hypogean *Rhamdia* is incomplete and that sustained exploration and taxonomically sound documentation work are paramount to advancing knowledge about the diversity and evolution of these group of Neotropical catfishes.

Keywords
Cavefishes, hypogean, stygobionts, stygofauna, troglomorphism

Introduction

With 27 currently valid species distributed throughout most of the tropical Americas, catfishes of the genus *Rhamdia* Bleeker 1858 are a group of moderately diverse Neotropical freshwater fishes noteworthy, among other things, because of their tendency to colonize subterranean waters and maintain resident hypogean populations (Hubbs 1938; Silfvergrip 1996; Perdices et al. 2002; Bichuette and Trajano 2005; Hernández et al. 2015; Arroyave and De La Cruz Fernández 2021a, 2021b; Buenavad-González et al. 2023; Fricke et al. 2024). In fact, several hypogean populations of *Rhamdia* have been described as distinct species, recognized as different from each other and from any of their epigean counterparts (Miller 1984; Wilkens 1993; Weber and Wilkens 1998; Weber et al. 2003; DoNascimiento et al. 2004; Bichuette and Trajano 2005), although recent research has called into question the taxonomic validity of some of these cave-adapted species (Arroyave and De La Cruz Fernández 2021b).

Despite its widespread distribution, the bulk of the subterranean diversity of the genus *Rhamdia* has been primarily described from karstic regions in Mexico. Mexican cave *Rhamdia* includes four of the six currently valid troglobitic species in the genus plus at least 10 cave-dwelling populations taxonomically assigned, for the most part, to the epigean species *R. laticauda* (Kner 1858) (Arroyave and De La Cruz Fernández 2021a, 2021b, Buenavad-González et al. 2023). Recent efforts dedicated to investigating the taxonomic diversity and evolution of Middle American species of *Rhamdia* have shown that the existence of cave-dwelling populations is more geographically widespread than previously thought and that exploration and dedicated ichthyological surveys of underground karstic caves and their associated aquatic environments is likely to result in the discovery of novel subterranean populations, further supporting the idea that cave colonization in the group is widespread (Arroyave and De La Cruz Fernández 2021a, Buenavad-González et al. 2023). Discovery and documentation of novel hypogean populations not only entails the uncovering of new biodiversity, but also brings forth additional comparative material for future research on the systematics and biogeography of the genus *Rhamdia*. This material (specimens and tissue samples) is key to shed light on the evolution of cave colonization and troglomorphism, intriguing topics that can be studied from both morphological and genetic perspectives.

Whereas recent studies have uncovered and documented the existence of previously unknown cave-dwelling populations of *Rhamdia* in Mexico (Arroyave and De La Cruz Fernández 2021a, Buenavad-González et al. 2023), similar efforts appear to be lacking for the remainder of the distribution of the genus. Costa Rica, a small yet megadiverse country in southern Central America (Avalos 2018), is a part of a natural bridge between North and South America and home to three epigean species of *Rhamdia*: *R. guatemalensis* (Günther 1864), *R. laticauda*, and *R. nicaraguensis* (Günther 1864) (Fricke et al. 2024). Despite harboring several species of *Rhamdia* and having over 250 documented caves (Ulloa et al. 2011), accounts of cave-dwelling populations of *Rhamdia* from Costa Rica are rare, mostly reported by speleologists and mainly supported by anecdotal evidence (Strinati et al. 1987; Debeljak 1988; Juberthie and Strinati 1994; Sandí 2012; Deleva et al. 2023), sometimes including photographs, but never taxonomically verified voucher specimens catalogued in ichthyological collections. Lack of vouchered evidence when documenting hypogean fish fauna—and all biodiversity for that matter—is less than ideal because accurate taxonomic identifications often rely on the direct, close, and careful examination of morphological traits (Rocha et al. 2014; Ceríaco et al. 2016; Nachman et al. 2023). In the case of populations potentially representing undescribed species, the need of voucher specimens is even more pressing. Furthermore, vouchers, when tissued prior to formalin fixation, allow for further taxonomic verification using molecular data, which can also be used in a comparative framework to investigate an assortment of evolutionary questions that require genetic markers.

Historical accounts of hypogean *Rhamdia* from Costa Rica include reported sightings of cave-dwelling forms of *R. guatemalensis* (five sites), *R. nicaraguensis* (one site), and *Rhamdia* sp. (four sites) (Deleva et al. 2023). None of these, however, constitute taxonomically authoritative/verified records (i.e., supported by voucher specimens—not just observations—from which morphology- and/or DNA-based taxonomic identifications have been conducted), thus rendering them potentially unreliable (Rocha et al. 2014; Ceríaco et al. 2016; Nachman et al. 2023). These reports, nonetheless, offer an important baseline to plan and conduct targeted ichthyological surveys aimed at determining the existence and taxonomic identity of any residing cavefish populations.

In a first effort towards properly and accurately documenting the taxonomic diversity of cave *Rhamdia* in Costa Rica, we conducted dedicated ichthyological surveys in two karstic caves with anecdotal reports on the presence of these catfishes: Corredores and Gabinarraca (or Cavernas de Venado) (Fig. 1). This study presents our findings, focusing on the taxonomic nature and phylogenetic placement of these populations, as inferred from morphological and molecular comparative data.

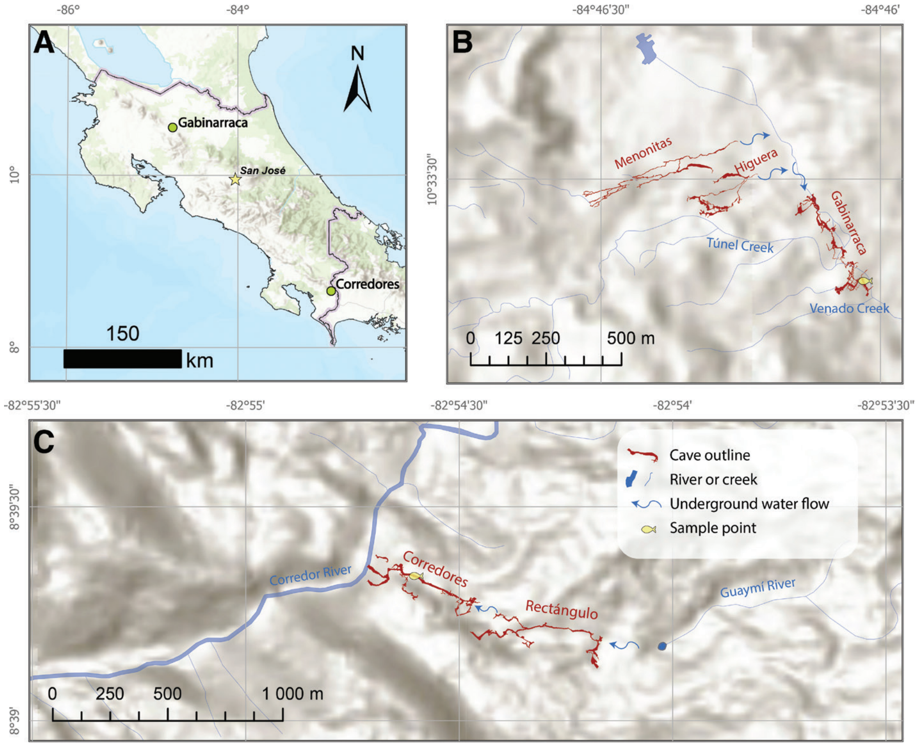

Figure 1. Location of the surveyed caves **A** map of Costa Rica displaying the location of Gabinarraca and Corredores caves (green dots) at the country-level scale (borders with Nicaragua to the north and Panama to the south highlighted in light purple). Maps displaying the location of the surveyed caves at the local scale, including geomorphological and hydrological features: **B** Gabinarraca and **C** Corredores.

Methods

Area of study

Gabinarraca and Corredores caves are located in the Costa Rican provinces of Alajuela and Puntarenas, respectively (Fig. 1A). Each cave constitutes the terminal segment of a karst system comprised of multiple interconnected caves with a perennial underground river (Fig. 1B, C). Gabinarraca cave is on the Caribbean versant of northwestern Costa Rica (although very near the continental water divide), c. 11 km northwest of the Arenal volcano. It is part of the Venado karstic system, which developed from Miocene limestone from the Venado Formation, and comprises three main caves: Menonitas (1620 m in length), Higuera (954 m in length), and Gabinarraca (2351 m in length), totaling almost 5 km of underground passages (Ulloa et al. 2021) (Fig. 1B). Not only is Gabinarraca cave the longest of the Venado karstic system caves, but it is also the best known, since it has been a tourist attraction for over two decades (Ulloa et al. 2021) (Fig. 2A, B). Corredores cave, on the other hand, is located on the Pacific versant of southern Costa Rica, very close to the border with Panama, c. 3.5 km east of Ciudad Neily (Fig. 1A). It is part of the Cerro Corredores karst system, which extends over a

Figure 2. Photographs displaying physical features of the surveyed caves and associated rivers/streams **A** Gabinarraca cave entrance, showing the outflow of water into Quebrada El Túnel **B** inside Gabinarraca cave while electrofishing **C** Quebrada El Túnel close to the entrance of Gabinarraca cave **D** Corredores cave entrance **E** Río Corredor as seen from the cave entrance higher up **F** Río Corredor riverbed at a small canyon on the way to the cave access point.

length of c. 3 km carrying water from the Guaymí River that infiltrates from the east through a sinkhole and flows underground northwest through several caves, including La Bruja (221 m), El Rectángulo (1411 m), and Corredores (1624 m), before emerging from the aquifer and flowing into the Corredor River (Peacock and Hempel 1993) (Figs 1C, 2D–F). The karst in the area develops in Eocene limestones associated with the Fila de Cal Formation (Chesnel and Rodríguez 2021).

Specimen sampling and preservation

Both sampled caves are wet (containing active watercourses but not fully flooded or submerged) and mostly horizontal, and therefore did not require specialized vertical caving techniques for surveying and sampling. Specimen sampling was accomplished using a combination of baited minnow traps and electrofishing. Inside the surveyed caves, we collected fishes along the main longitudinal axis of the cave up to the point of maximum penetration, which was approximately a few hundred meters in both caves. In addition to the caves, we collected specimens of *Rhamdia* from epigean populations from streams and rivers mainly near Gabinarraca cave (Río Frío basin), including the very stream flowing out of the cave (Quebrada El Túnel) (Figs 2C, 3). Because of logistical issues, we were not able to secure samples of epigean *Rhamdia* from the Río Corredor, the river most closely associated to the Corredores cave. After capture, we euthanized the fishes using the anesthetic tricaine mesylate (MS-222) and then took tissue samples (fin clips) for the genetic component of our study. Tissues were preserved in 96% ethanol and eventually frozen at −20 °C. After tissuing, we fixed voucher specimens using a 10% formalin solution. Back in the lab, we washed formalin-fixed specimens and then gradually transferred them to 70% ethanol for long-term storage in the Colección Ictiológica del Museo de Zoología de la Universidad de Costa Rica (UCR), where they were catalogued and deposited (Table 1). Fishes were handled in accordance with recommended guidelines for the use of fishes in research (Jenkins et al. 2014). Specimens were collected under permit R-SINAC-SE-DT-PI-029-2023 issued by the Sistema Nacional de Áreas de Conservación (SINAC). Access permit to genetic resources (CBio-54-2022-#359) was extended by the Comisión Institucional de Biodiversidad of the UCR. Taxonomic identification of preserved specimens based on external morphology was conducted using relevant published keys and systematic revisions (Silfvergrip 1996; Bussing 1998; Hernández et al. 2015).

Figure 3. Collecting sites of epigean populations of *Rhamdia* sampled in this study **1** Quebrada El Túnel (at Gabinarraca cave entrance) **2** Quebrada El Túnel (further downstream from Gabinarraca cave entrance) **3** Río Pataste **4** Río Arenal **5** Quebrada Palma, and **6** Quebrada Altamira. All sites are within the Alajuela province, Atlantic versant. Black outline in map corresponds to the continental water divide.

Table 1. Sampling localities with their respective species, catalog/vouchers numbers, and GenBank accession numbers of sequences generated in this study and used in phylogenetic analyses. *at Gabinarraca cave entrance; **further downstream from Gabinarraca cave entrance.

Habitat	Locality	Coordinates	Municipality	Province	Basin	Versant	Species	Catalog (n)	Tissued vouchers	DNA voucher	GenBank Accession	
											COI	CYB
Hypogean	Gabinarraca cave	10°33'17"N, 84°46'01"W	Venado	Alajuela	Río Frío	Atlantic	*Rhamdia laticauda*	UCR 3323-01 (6)	JA1913-17	JA1913	PQ451515	PQ458956
										JA1914	PQ451516	PQ458957
										JA1915	PQ451517	PQ458958
										JA1916	PQ451492	PQ458959
							Rhamdia nicaraguensis	UCR 3323-02 (13)	JA1902-12	JA1902	PQ451502	PQ458966
										JA1903	n/a	PQ458967
										JA1904	PQ451503	PQ458968
										JA1905	PQ451504	PQ458969
										JA1906	PQ451505	PQ458970
										JA1907	PQ451506	PQ458971
										JA1908	PQ451507	PQ458972
										JA1909	PQ451508	PQ458973
										JA1910	PQ451509	PQ458974
										JA1911	PQ451497	PQ458975
										JA1912	PQ451510	PQ458976
	Corredores cave	08°39'23"N, 82°54'44"W	Ciudad Neily	Puntarenas	Río Coto	Pacific	*Rhamdia guatemalensis*	UCR 3330-01 (8)	JA2068-74	JA2068	PQ451499	PQ458950
										JA2070	n/a	PQ458951
										JA2071	PQ451500	PQ458952
										JA2072	PQ451501	PQ458953
										JA2073	n/a	PQ458954
										JA2074	n/a	n/a
Epigean	Quebrada El Túnel*	10°33'17"N, 84°46'01"W	Venado	Alajuela	Río Frío	Atlantic	*Rhamdia laticauda*	UCR 3324-01 (3)	JA1921-23	JA1921	PQ451518	PQ458960
										JA1922	PQ451493	PQ458961
										JA1923	PQ451519	PQ458962
							Rhamdia nicaraguensis	UCR 3324-02 (3)	JA1918-20	JA1918	PQ451511	PQ458977
										JA1919	PQ451512	PQ458978
										JA1920	PQ451513	PQ458979
	Quebrada El Túnel**	10°33'17"N, 84°45'20"W	Venado	Alajuela	Río Frío	Atlantic	*Rhamdia laticauda*	UCR 3325-01 (7)	JA1924-30	JA1927	PQ451520	n/a
										JA1928	PQ451494	PQ458963
							Rhamdia nicaraguensis	UCR 3325-02 (2)	JA1931-32	n/a	n/a	n/a

Habitat	Locality	Coordinates	Municipality	Province	Basin	Versant	Species	Catalog (n)	Tissued vouchers	DNA voucher	GenBank Accession	
											COI	CYB
Epigean	Río Arenal	10°30'09"N, 84°35'38"W	Venado	Alajuela	Río San Carlos	Atlantic	*Rhamdia laticauda*	UCR 3326-01 (8)	JA1952-59	JA1955	PQ451495	PQ458964
							Rhamdia nicaraguensis	UCR 3326-02 (2)	JA1960-61	n/a	n/a	n/a
	Río Pataste	10°34'07"N, 84°40'02"W	Venado	Alajuela	Río Frío	Atlantic	*Rhamdia laticauda*	UCR 3327-01 (4)	JA1943-46	JA1946	PQ451521	n/a
							Rhamdia nicaraguensis	UCR 3327-02 (7)	JA1936-42	JA1939	n/a	PQ458980
										JA1940	PQ451514	n/a
	Quebrada Altamira	10°44'01"N, 85°03'34"W	Bijagua	Alajuela	Río Zapote	Atlantic	*Rhamdia laticauda*	UCR 3328-01 (15)	JA1804-11	JA1804	PQ451496	PQ458955
	Quebrada Palma	10°29'54"N, 84°41'19"W	Palma	Alajuela	Río San Carlos	Atlantic	*Rhamdia nicaraguensis*	UCR 3329-01 (5)	JA1734-38	JA1734	PQ451498	PQ458965
							Rhamdia laticauda	UCR 3329-02 (5)	JA1729-33	n/a	n/a	n/a

Comparative data generation and analysis

To document patterns of phenotypic and genetic variation, and to shed light on the taxonomic nature of the cave-dwelling populations of *Rhamdia* reported herein, we generated meristic and morphometric data from hypogean samples as well as DNA sequence data from both hypogean and epigean samples. Morphometric measurements and meristic counts follow previous taxonomic studies of *Rhamdia* (Silfvergrip 1996; Hernández et al. 2015; Buenavad-González et al. 2023). All measurements were taken on the left side of specimens using a Mitutoyo digital caliper (precision = 0.1 mm; accuracy = ± 0.02 mm). Comparative genetic data consisted of partial fragments of the mitochondrial protein-coding genes cytochrome c oxidase subunit I (COI) and cytochrome b (CYTB). We generated COI and CYTB data from representatives of all cave-dwelling populations documented herein, as well as from samples of epigean *Rhamdia* collected during the fieldwork component of this study and relevant for comparative purposes (Table 1). To increase our precision in estimating the phylogenetic placement of the Costa Rican cave-dwelling populations with respect to the entire Middle American clade of the genus, we broadened the taxonomic and geographic coverage of samples used for phylogenetic analyses by including additional COI and CYTB sequences of *Rhamdia* retrieved from GenBank (www.ncbi.nlm.nih.gov/Genbank) and from unpublished data previously generated by the lead author (JA). To generate DNA sequence data, we first extracted total genomic DNA from fresh tissue samples using the Qiagen DNeasy Tissue Extraction Kit, following the manufacturer's protocol. We amplified and sequenced COI and CYTB following the procedures (primers and PCR thermal profiles) employed in recent molecular phylogenetic studies of the genus that used the same markers (Arroyave and De La Cruz Fernández 2021a, 2021b, Buenavad-González et al. 2023). DNA extraction, amplification, and sequencing were carried out at Laboratorio de Secuenciación Genómica de la Biodiversidad y de la Salud (Instituto de Biología, UNAM) and Pritzker Molecular Laboratory at the Field Museum of Natural History (FMNH). Contig assemblage, sequence editing, multiple sequence alignment—using MUSCLE (Edgar, 2004)— and the calculation of uncorrected genetic distances (*p*-distances) were performed in Geneious Prime 2024.0.7 (https://www.geneious.com). The resulting COI and CYTB matrices consisted of 116 and 126 ingroup (trans-Andean/Middle American *Rhamdia*) terminals, including representatives from 9 and 10 of the 11 valid species in the clade, respectively. In addition to the individual gene alignments, we analyzed a concatenated alignment—assembled with the software 2matrix (Salinas and Little 2014)—taxonomically limited to 59 ingroup terminals (with voucher specimens) from nine species for which both COI and CYTB data were available. In all cases, the cis-Andean species *Rhamdia quelen* was used as the outgroup and root, based on previous phylogenetic research that supports the reciprocal monophyly between cis- and trans-Andean clades of *Rhamdia* (Perdices et al. 2002; Hernández et al. 2015; Arroyave and De La Cruz Fernández 2021b). For each alignment, statistical selection of the best-fit model of nucleotide substitution was implemented with the software jModelTest2 (v. 2.1.10) (Darriba et al. 2012) under the following likelihood settings:

number of substitution schemes = 3; base frequencies = +F; rate variation = +I and +G with nCat = 4; base tree for likelihood calculations = ML optimized; and base tree search = NNI, effectively evaluating 24 alternative models. We inferred a phylogenetic tree for each individual gene matrix as well as for the concatenated alignment using the software RAxML-NG (v. 1.2.1) (Kozlov et al. 2019) under their respective best-fit models of molecular evolution. Clade support was estimated using the bootstrap character resampling method (Felsenstein 1981) based on 1000 pseudoreplicates.

Results

Our sampling efforts in the surveyed caves (Corredores and Gabinarraca) resulted in the discovery of three cave-dwelling populations of *Rhamdia*, identified as members of the species *R. guatemalensis* (Corredores cave; n = 8) (Figs 4, 7), *R. laticauda* (Gabinarraca cave; n = 6) (Figs 5, 7), and *R. nicaraguensis* (Gabinarraca cave; n = 13) (Figs 6, 7). Furthermore, we collected individuals from epigean populations of both *R. laticauda* and *R. nicaraguensis*, from six and five localities, respectively (Table 1). Taxonomic identifications were initially based on external morphology and further corroborated by comparative molecular data in a phylogenetic context (Figs 8–10). All hypogean populations displayed partial loss of body pigmentation, resulting in a yellowish coloration in life, which contrasts with the dark, melanic phenotype typical of epigean forms (Fig. 6D). Notably, none of the hypogean populations exhibited a pattern of eye reduction/loss, except for a single specimen of *R. guatemalensis* from the Corredores cave which displayed a slight and asymmetric eye degeneration and reduction (Fig. 4A).

Morphological and meristic data from the specimens collected at the surveyed caves are presented in Tables 2, 3. Inter- and intraspecific variation in morphological traits of taxonomic importance (i.e., those used to distinguish among the three species of *Rhamdia* present in Costa Rica) such as pectoral spine serration, interdorsal space, length of barbels, and head length, conformed with the expectations of our species designations.

GenBank accession numbers corresponding to the DNA sequence data generated in this study (COI and CYTB) are presented in Table 1. Regardless of marker/matrix, the inferred phylogenies (Figs 8–10) unambiguously place the Costa Rican cave-dwelling populations of *Rhamdia* documented herein (colored in red) well nested within the *R. guatemalensis* clade (in the case of samples from the Corredores cave) and the "*R. laticauda*-group" clade (Weber and Wilkens 1998) (in the case of samples from the Gabinarraca cave). Although these phylogenies consistently recover *R. laticauda* deeply paraphyletic, samples from Gabinarraca morphologically identified as *R. laticauda* were resolved within a well-supported clade consisting exclusively of Costa Rican samples of this species, both hypogean and epigean (Figs 8–10). Similarly, samples from Gabinarraca cave identified morphologically as *R. nicaraguensis* nested within a moderately to well-supported clade consisting exclusively of *R. nicaraguensis* samples, including epigean ones. Notably, within the "*R. laticauda*-group" clade, *R. nicaraguensis* was consistently recovered as monophyletic (Figs 8–10).

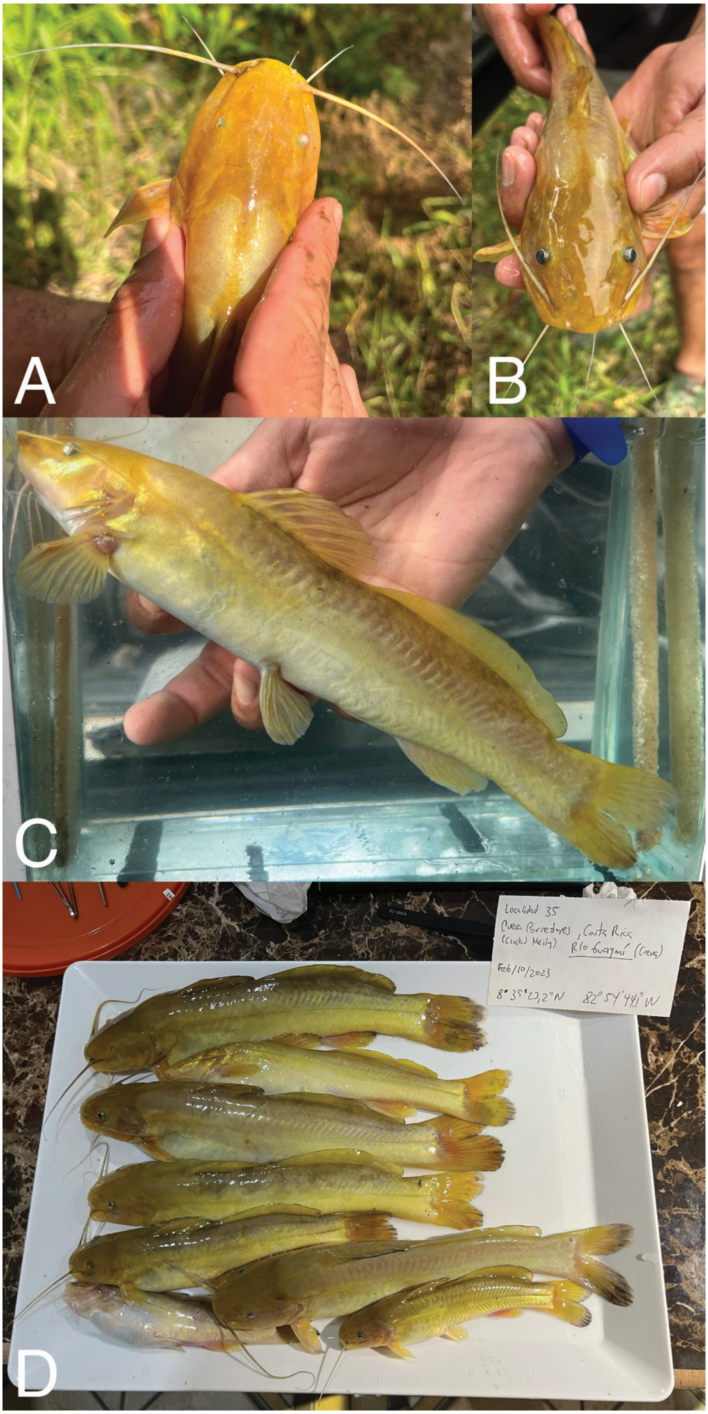

Figure 4. Photographs of hypogean *Rhamdia guatemalensis* from Corredores cave **A, B** dorsal views of head and anterior part of body in life after capture **C** lateral view of live specimen in aquarium **D** totality of specimens collected (n = 8), immediately postmortem and before tissuing and preservation.

Table 2. Meristic comparative data from samples of hypogean populations of *Rhamdia* spp. from Gabinarraca and Corredores caves. Meristic traits abbreviations as follows: PFR = pectoral-fin rays, PvFR = pelvic-fin rays, DFR = dorsal-fin rays, ARF = anal-fin rays, uCFR = upper caudal-fin rays, and lCFR = lower caudal-fin rays. Caudal-fin rays numbers (x,y,z) correspond to unsegmented (x), unbranched segmented (y), and branched segmented (z) rays.

Cave	Species	Catalog	Voucher	PFR	PvFR	DFR	AFR	uCFR	lCFR
Gabinarraca	*Rhamdia laticauda*	UCR 3323-01	3323-01-A	I-9	6	I-6	9	3,2,8	3,2,9
			JA1906	I-9	7	I-6	9	3,2,9	3,2,9
			JA1902	I-9	7	I-5	10	3,2,9	3,2,8
			3323-01-B	I-9	7	I-6	9	3,2,8	3,2,10
	Rhamdia nicaraguensis	UCR 3323-02	JA1903	I-9	7	I-5	9	3,2,6	3,2,8
			JA1904	I-9	7	I-6	10	3,2,9	3,2,10
			JA1905	I-9	7	I-6	9	3,2,8	3,2,9
			JA1907	I-9	7	I-6	9	3,2,9	3,2,9
			JA1908	I-9	8	I-6	9	3,2,8	3,2,8
			JA1909	I-9	7	I-6	9	3,2,9	3,2,9
			JA1910	I-9	7	I-6	9	3,2,9	3,2,9
			JA1911	I-9	7	I-6	9	3,2,7	3,2,9
			JA1912	I-9	7	I-6	9	3,2,9	3,2,8
Corredores	*Rhamdia guatemalensis*	UCR 3330-01	3330-01-A	I-7	6	I-6	9	3,2,9	3,2,10
			JA2068	I-8	7	I-6	9	3,2,9	3,2,10
			JA2069	I-9	7	I-6	9	3,2,9	3,2,10
			JA2070	I-9	6	I-6	8	3,2,8	3,2,9
			JA2071	I-8	7	I-6	8	3,2,9	3,2,9
			JA2072	I-7	6	I-7	8	3,2,9	3,2,7
			JA2073	I-9	7	I-6	9	3,2,9	3,2,9
			JA2074	I-9	7	I-6	9	3,2,8	3,2,9

Discussion

In the most recent and comprehensive review of Costa Rican cave-dwelling fauna, Deleva et al. (2023) reported on the presence of hypogean populations of *R. guatemalensis* in the Corredores and Bananal cave systems, as well as in other adjacent caves. Our results confirm the presence of *Rhamdia* catfishes in the Corredores cave (Figs 4, 7–10). The existence of hypogean populations of *R. guatemalensis* in other caves from southern Costa Rica—such as Bananal—has yet to be verified; however, these may seem likely given our Corredores cave findings and previous reports from the region (anecdotal and otherwise). The existence of cave-dwelling populations of *R. guatemalensis* in Costa Rica is noteworthy considering that most hypogean populations of *Rhamdia* that have been documented so far are derivatives of *R. laticauda*, either as cave-adapted populations or as species-level lineages within the "*R. laticauda*-group" clade (Arroyave and De La Cruz Fernández 2021a, 2021b, Buenavad-González et al. 2023). To our knowledge, the Corredores cave population effectively constitutes the fourth taxonomically verified record of a hypogean population of *R. guatemalensis*; the first three being from Mexican caves in the states of Tabasco (Grutas de Coconá) and Chiapas (Los Bordos and El Encanto caves) (Buenavad-González et al. 2023).

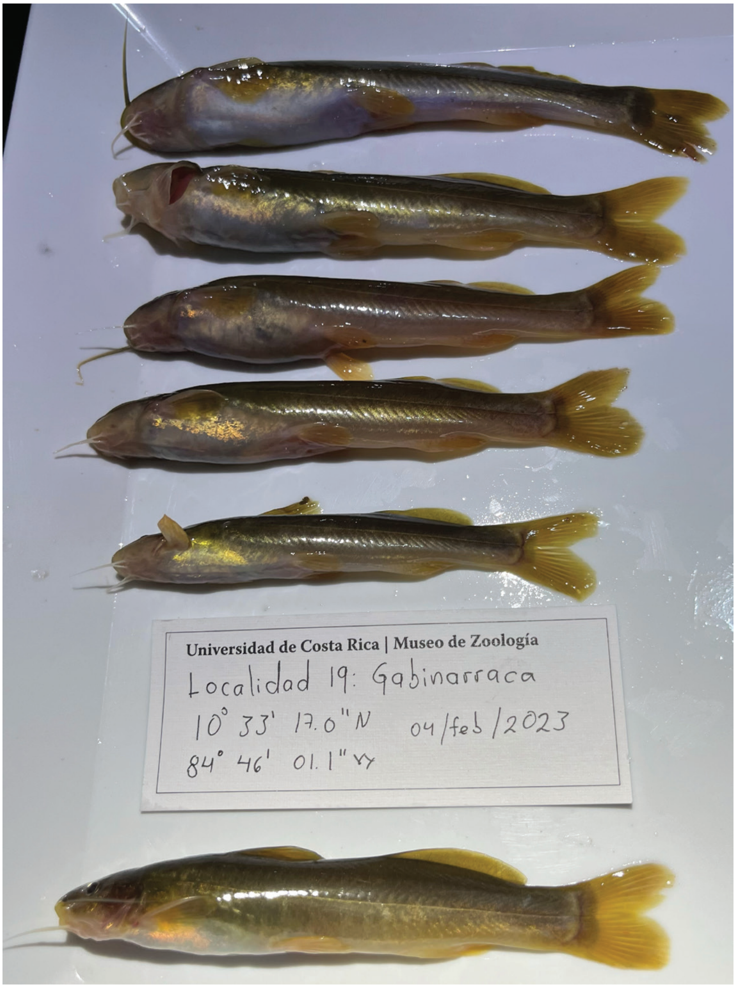

Figure 5. Photograph of hypogean *Rhamdia laticauda* from Gabinarraca cave. Totality of specimens collected (n = 6), immediately postmortem and before tissuing and preservation.

Despite the Corredores cave population not exhibiting full troglomorphism (due to the presence of eyes), it appears to be the most depigmented of all four *R. guatemalensis* hypogean populations documented to date (Fig. 4).

Remarkably, among our findings is the discovery of syntopic hypogean populations from two different *Rhamdia* species (*R. laticauda* and *R. nicaraguensis*) inhabiting the Gabinarraca cave system. This finding is noteworthy because it represents the first taxonomically verified record of a cave-dwelling population of *R. nicaraguensis*. Furthermore, the population of *R. laticauda* from Gabinarraca cave constitutes the latest addition to the extensive list of hypogean records for this epigean species—which includes five populations from the karstic Sierra de Zongolica in the state of Veracruz (Arroyave and De La Cruz Fernández 2021a) and two populations from karstic caves

Table 3. Morphometric comparative data from samples of hypogean populations of *Rhamdia* spp. from Gabinarraca and Corredores caves. Measurements abbreviations as follows: SL = Standard Length, HL = Head Length, HL = Head Length, BW = Body Width, DFH = Dorsal Fin Height, DSH = Dorsal-fin Spine Height, AFL = Anal Fin Length, AdFL = Adipose Fin Length, PFL = Pectoral Fin Length, PSL = Pectoral-fin Spine Length, PvFL = Pelvic Fin Length, ISL = Interdorsal Space Length, CPL = Caudal Peduncle Length, CPD = Caudal Peduncle Depth, IOW = Interorbital Width, ORB = Orbital Diameter, SNT = Snout Length, MBL = Maxillary Barbel Length, MdBL = Mandibular Barbel Length, MeBL = Mental Barbel Length.

	Gabinarraca Cave						**Corredores Cave**		
	***Rhamdia laticauda* (n = 4)**			***Rhamdia nicaraguensis* (n = 9)**			***Rhamdia guatemalensis* (n = 8)**		
	Range	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**
				mm					
SL	119.94–198.00	150.45	34.85	107.16–171.00	132.82	21.23	124.41–237.00	193.43	45.70
HL	29.75–43.29	34.45	6.04	24.80–40.43	31.32	5.50	28.60–52.92	43.10	10.27
				% SL					
HL	21.46–24.99	23.14	1.74	22.64–24.64	23.53	0.67	20.49–22.99	22.30	0.78
BW	16.35–17.92	17.19	0.64	15.48–18.14	16.56	0.76	18.00–18.91	18.48	0.32
DFH	13.25–14.75	14.00	0.64	10.17–14.88	13.34	1.28	12.48–15.64	13.93	1.03
DSH	5.32–6.20	5.81	0.37	4.19–5.05	4.55	0.29	5.98–6.72	6.35	0.28
AFL	17.29–18.80	17.95	0.63	18.32–21.42	20.15	1.05	17.61–19.60	18.59	0.60
AdFL	40.80–42.41	41.93	0.75	39.90–45.58	43.84	1.99	36.54–38.39	37.74	0.57
PFL	10.49–13.89	12.22	1.50	12.45–13.79	13.15	0.45	12.16–13.89	12.95	0.57
PSL	6.67–8.58	7.45	0.89	7.02–8.89	7.88	0.62	7.30–9.24	8.41	0.62
PvFL	9.90–12.61	11.32	1.40	11.43–12.94	12.08	0.47	11.01–13.12	11.63	0.83
ISL	4.61–4.78	4.69	0.08	1.46–2.43	1.67	0.30	5.27–7.75	6.67	0.85
CPL	16.35–18.94	17.47	1.22	17.79–18.96	18.24	0.41	17.90–20.23	18.48	0.75
CPD	9.95–10.35	10.11	0.17	9.78–11.12	10.30	0.40	9.71–11.36	10.83	0.55
				% HL					
IOW	36.13–42.67	38.94	2.72	35.02–39.45	37.13	1.46	40.05–46.97	43.71	2.14
ORB	17.39–20.10	18.65	1.23	15.94–20.24	17.98	1.44	9.01–16.09	12.07	2.16
SNT	37.75–41.49	39.48	1.60	35.54–41.75	38.15	1.77	32.72–41.85	36.82	2.73
MBL	100.11–117.27	111.31	7.64	88.97–136.11	121.53	14.67	124.49–228.91	158.01	33.37
MdBL	47.62–54.66	50.70	3.01	54.28–74.97	60.26	5.95	62.72–90.31	72.73	9.92
MeBL	27.66–36.79	32.88	3.97	32.69–46.50	35.71	4.21	41.76–55.44	47.45	5.06

in the state of Chiapas (Buenavad-González et al. 2023)—further supporting the notion that *R. laticauda* is the quintessential cave colonizer species in the genus. Lastly, and perhaps more interestingly, this is the first time that cave-dwelling populations from different species of *Rhamdia* are reported to be living in syntopy. Coexistence of multiple species of hypogean fishes in the same cave is not widespread but neither uncommon; around 40% of obligate cavefish species co-occur with other such species, although very rarely totaling more than two syntopic species per cave (Trajano 2001). Approximately half of the known instances of syntopy in cavefishes involve species relatively distant phylogenetically, while the other half involve species from the same family although mostly from different genera (Niemiller and Soares 2015). Apart from the case of *Rhamdia* in the Gabinarraca cave reported herein, the only other known instances of intrageneric syntopy in cavefishes are restricted to viviparous brotulas of the genus *Lucifuga* Poey 1858 in two Cuban caves, with three and two syntopic species,

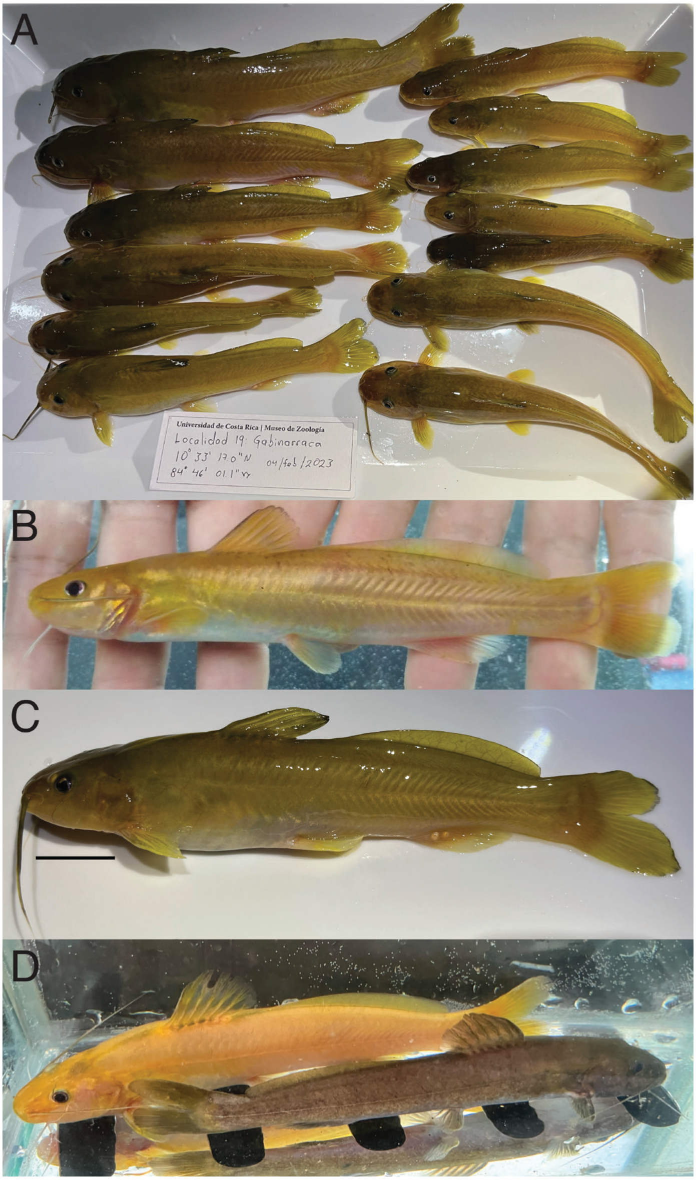

Figure 6. Photographs of hypogean *Rhamdia nicaraguensis* from Gabinarraca cave **A** totality of specimens collected (n = 13), immediately postmortem and before tissuing and preservation **B** lateral view of live specimen in aquarium **C** lateral view of specimen immediately postmortem. Metacercariae (two) of the parasitic fluke *Clinostomum* sp. are visible at the base of the anal fin **D** live specimens (in aquarium) of hypogean (Gabinarraca, yellow) and epigean (Quebrada El Túnel, dark brown) *R. nicaraguensis*. Scale bar: 1 cm.

respectively (Trajano 2001). It is generally assumed that coexistence in syntopy requires some type of resource (mainly food and space) partitioning so as to minimize competition (Pianka 1973), and this is especially true of closely related and morphologically

Figure 7. Photographs of preserved representatives of the populations of cave-dwelling *Rhamdia* from Costa Rica reported in this study **A** *R. guatemalensis* from Corredores cave (UCR 3330-01; 230 mm TL) **B** *R. nicaraguensis* from Gabinarraca cave (UCR 3323-02; 165 mm TL), and **C** *R. laticauda* from Gabinarraca cave (UCR 3323-01; 158 mm TL). Scale bar: 1 cm.

similar species co-occurring in hypogean habitats that are generally oligotrophic and with simplified food webs (Niemiller and Soares 2015). In some well-documented cases, habitat partitioning and/or differences in diet between syntopic cavefish species have been posited as the evolutionary and ecological mechanisms allowing coexistence with minimum competition (Trajano 2001). This pattern of ecological differentiation and resource partitioning in cavefishes, however, has only been demonstrated in some instances of syntopy involving relatively phylogenetically distant species (from different genera and families). In cases of closely related and morphologically conserved syntopic cavefish species such as *Lucifuga* spp. from Cuban caves, the mechanisms enabling local coexistence are not fully understood, although it appears that competitive-driven shifts in diet and habitat preferences have yet to evolve (Trajano 2001). With hypogean populations of two species of *Rhamdia* (*R. laticauda* and *R. nicaraguensis*)

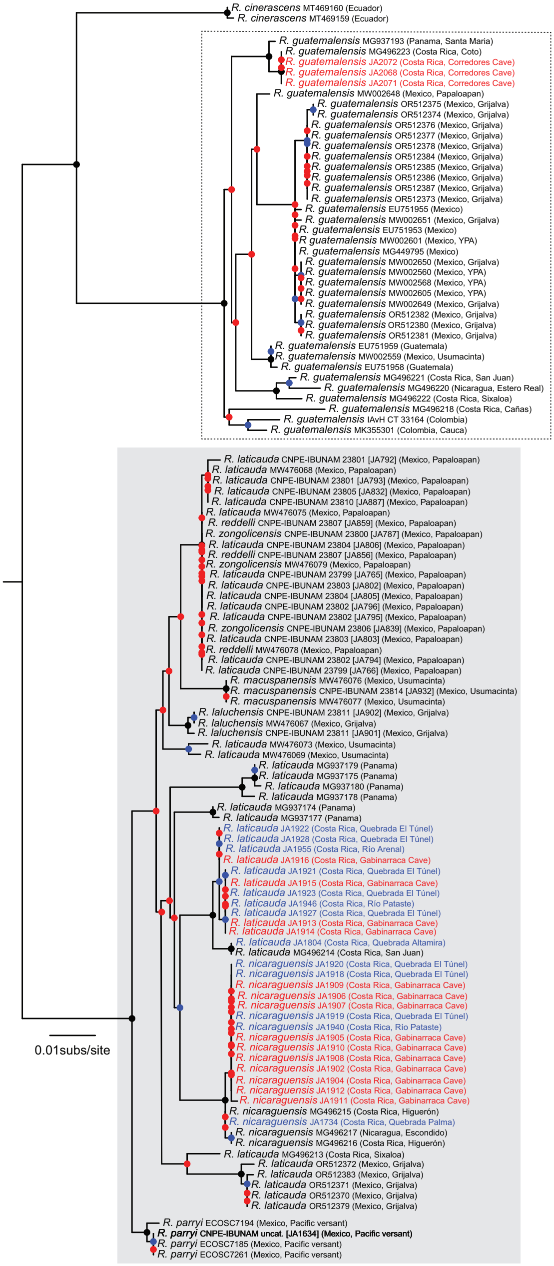

Figure 8. Phylogenetic relationships of Middle American *Rhamdia* inferred from comparative COI data. Colored circles on nodes indicate degree of clade support as determined by bootstrap values: black > 95, 95 ≥ blue ≥ 75, red < 75. Terminal names as follow: Species name + GenBank accession or catalog/voucher (for new/unpublished sequences) + country, basin (in parenthesis). Terminals corresponding to samples from populations of Costa Rican hypogean *Rhamdia* (documented herein) in red. Terminals corresponding to samples from Costa Rican epigean *Rhamdia* collected in this study in blue. Outgroup taxon (*Rhamdia quelen*) not shown. The dashed rectangle indicates the *R. guatemalensis* clade, whereas the light gray rectangle indicates the "*R. laticauda*-group" clade, inclusive of *R. nicaraguensis*, *R. parryi*, and the four Mexican stygobitic species.

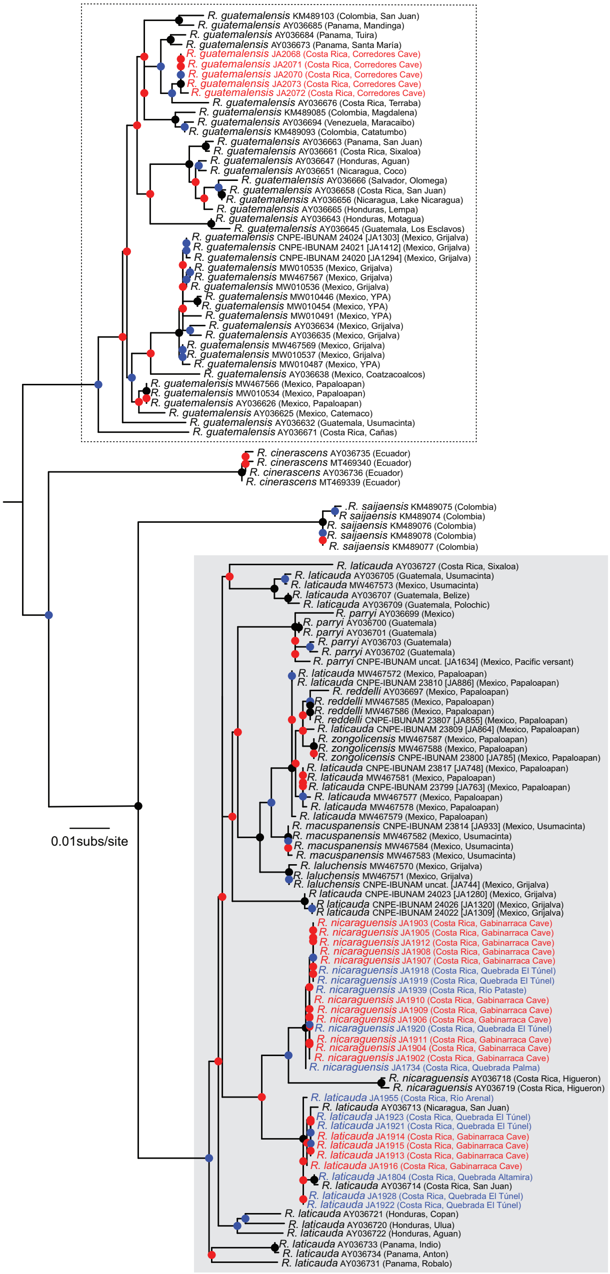

Figure 9. Phylogenetic relationships of Middle American *Rhamdia* inferred from comparative CYTB data. Colored circles on nodes indicate degree of clade support as determined by bootstrap values: black > 95, 95 ≥ blue ≥ 75, red < 75. Terminal names as follow: Species name + GenBank accession or catalog/voucher (for new/unpublished sequences) + country, basin (in parenthesis). Terminals corresponding to samples from populations of Costa Rican hypogean *Rhamdia* (documented herein) in red. Terminals corresponding to samples from Costa Rican epigean *Rhamdia* collected in this study in blue. Outgroup taxon (*Rhamdia quelen*) not shown. The dashed rectangle indicates the *R. guatemalensis* clade, whereas the light gray rectangle indicates the "*R. laticauda*-group" clade, inclusive of *R. nicaraguensis*, *R. parryi*, and the four Mexican stygobitic species.

living in syntopy, Gabinarraca cave in Costa Rica effectively constitutes a new model to investigate the processes allowing the local coexistence of closely related, morphologically similar, and seemingly ecologically equivalent species/populations of cavefishes. Future research aimed at generating and analyzing detailed data on diet and microhabitat occupation will be necessary to start shedding light on the ecological mechanisms allowing syntopy of *Rhamdia* catfishes in this cave system.

Given the ample taxonomic and geographic coverage, the resulting phylogenies (Figs 8–10) offer an extensive panorama of interspecific relationships within the Middle American *Rhamdia* clade and of phylogeographic structure within wide-ranging species such as *R. guatemalensis* and *R. laticauda*. As such, our phylogenetic results corroborate the relentless paraphyly of *R. laticauda* (with respect to *R. nicaraguensis*, *R. parryi*, and the Mexican stygobitic species) (Perdices et al. 2002; Arroyave and De La Cruz Fernández 2021b) and support the taxonomic distinctiveness (from *R. laticauda*) and monophyletic status of both *R. nicaraguensis* (Silfvergrip 1996; Bussing 1998) and *R. parryi* (Miller 2005), although uncertainty regarding their phylogenetic placement within the larger "*R. laticauda*-group" clade remains (Perdices et al. 2002; Arroyave and De La Cruz Fernández 2021b; Buenavad-González et al. 2023).

Phylogeographic structure in *R. guatemalensis* and *R. laticauda* appears generally consistent with a latitudinal gradient and the expectations from catchment hydrology (Perdices et al. 2002; Hernández et al. 2015; Arroyave et al. 2021). However, within these species-level clades, most internodes are short and poorly supported, rendering their phylogeographic histories unclear. The morphology-based species designations of the cave-dwelling *Rhamdia* populations reported herein were in all cases corroborated with molecular data in a phylogenetic framework (Figs 8–10). As expected from a biogeographic perspective, besides being resolved within their respective species-level clade, these hypogean populations always associated most closely with the geographically/hydrologically closest epigean populations. Samples from the Corredores cave (near the border with Panama) are more closely related to epigean populations of *R. guatemalensis* from Costa Rica and Panamá. Likewise, hypogean *Rhamdia* from Gabinarraca cave (*R. laticauda* and *R. nicaraguensis*) are more closely related—and practically genetically identical (i.e., *p*-distances < 0.3%)—to epigean samples of their respective species from the same basin (Río Frío) (Figs 8–10).

This pattern, also documented for numerous cave-dwelling populations of *Rhamdia* catfishes in southern Mexico (Arroyave and De La Cruz Fernández 2021a, 2021b, Buenavad-González et al. 2023), coupled with the observed relatively incipient troglomorphism (partial depigmentation but without eye loss), suggests recent cave colonization events and/or continued gene flow between epigean and hypogean populations. Further research into this subject, ideally based on genome-wide comparative data, would be required to properly test hypoteses of gene flow and the timing of lineage divergences and cave colonizations in *Rhamdia*. Similarly, further research is needed to shed light on basic yet poorly known aspects about the ecology of cave *Rhamdia*, such as diet and demography. Although we currently lack data about their trophic ecology and population dynamics, we assume that these hypogean populations are resident, and hypothesize that their incipient troglomorphism has evolved as a result of living

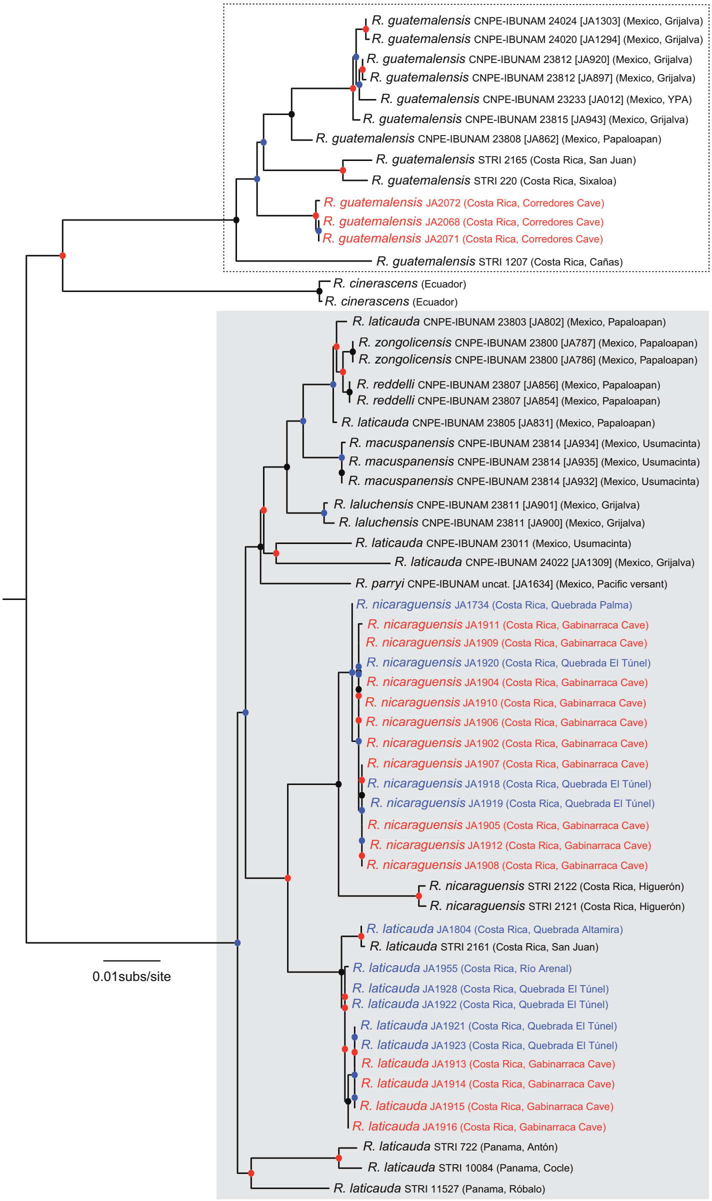

Figure 10. Phylogenetic relationships of Middle American *Rhamdia* inferred from comparative COI + CYTB data (concatenated). Colored circles on nodes indicate degree of clade support as determined by bootstrap values: black > 95, 95 ≥ blue ≥ 75, red < 75. Terminal names as follow: Species name + GenBank accession or catalog/voucher (for new/unpublished sequences) + country, basin (in parenthesis). Terminals corresponding to samples from populations of Costa Rican hypogean *Rhamdia* (documented herein) in red. Terminals corresponding to samples from Costa Rican epigean *Rhamdia* collected in this study in blue. Outgroup taxon (*Rhamdia quelen*) not shown. The dashed rectangle indicates the *R. guatemalensis* clade, whereas the light gray rectangle indicates the "*R. laticauda*-group" clade, inclusive of *R. nicaraguensis*, *R. parryi*, and the four Mexican stygobitic species.

in subterranean environments, a hypothesis subject to future testing. The results from recent population genomics studies conducted on other cavefish complexes such as *Astyanax mexicanus* (Garduño-Sánchez et al. 2023) and *Typhlichthys subterraneus* (Hart et al. 2024) suggest that analyses of genome-wide data in a phylogeographic framework are a promising avenue for investigating modes of cave-adapted evolution in other fishes with cave-dwelling populations such as *Rhamdia*.

In conclusion, this study adds to a body of work showcasing the continued discovery of cave-dwelling populations of *Rhamdia* catfishes during targeted ichthyological surveys in karstic regions of Middle America (Arroyave and De La Cruz Fernández 2021a, Buenavad-González et al. 2023), and as such, it reinforces the notion that our understanding of the diversity of hypogean *Rhamdia* is only partial and that sustained exploration and taxonomically sound documentation work are paramount to advancing knowledge about the diversity and evolution of these group of Neotropical catfishes. We hope that by uncovering and taxonomically documenting cavefish diversity present in Costa Rica and its karstic systems, this study opens the gates for future research on the subject in the region.

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A modified trap design for sampling subterranean habitats for central Texas *Eurycea* salamanders

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Abstract

In this paper, we describe modifications to a sampling technique for surface, stream-dwelling salamanders for use in subterranean settings. Leaf litter bags are an effective and commonly used trap for salamanders, and their construction purposefully allows animals to move freely in and out of the trap. However, this presents a problem in subterranean deployment because retrieving the trap over long vertical distances, such as well sampling, allows time and space for the animals to escape. To overcome this challenge, we enclosed a leaf litter bag in a suspended net system contained by a lanyard to sample a 3-meter deep well. Our trap modifications resulted in the live capture of adult and immature federally threatened Salado Salamanders (*Eurycea chisholmensis*) from the well in addition to aquatic invertebrates. This represents a novel trapping technique within a habitat system for which stygofauna sampling options are limited.

Keywords

Active trap, Amphibian sampling, Karst Biology, Leaf litter trap, Salado Salamander, Stygofauna, Threatened Species

Introduction

Knowledge of stygofauna and their ecology is inevitably less available than that of epigean fauna because the subterranean habitats stygofauna occupy are less accessible for sampling (Hahn 2002; Larned 2012; Hose et al. 2017). Conducting manual biological surveys (i.e., hand collection) may be a preferred way to collect stygofauna, but this requires a point of access to the subterranean environment in the form of geologic features such as caves or sinkholes. The occurrence of these geologic features is often rare (Larned 2012), especially features large enough to accommodate human passage.

Subterranean sampling and trapping techniques have been developed to survey areas inaccessible to humans. Commonly employed methods for sampling stygofauna have been restricted to the hyporheic zone using water well or bore sampling, as well as the deployment of traps (Larned 2012). Hyporheic zone sampling often involves the extraction of interstitial water through methods such as hyporheic pumping, which allows researchers to collect organisms residing in the water-sediment interface. For deeper groundwater environments, bottle traps or funnel traps are commonly employed. These are typically deployed in wells or at cave entrances to capture fauna inhabiting these otherwise inaccessible zones. While these methods primarily target invertebrates, such as crustaceans and other small aquatic organisms (Benedict 1896; Fenolio et al. 2015, 2017; Külköylüoğlu et al. 2017a, 2017b, 2017c), targeted sampling strategies are essential for vertebrates when they do occur in these environments.

Despite extensive research on invertebrate sampling, methods for effectively sampling subterranean vertebrates remain less established. Although the use of bottle and funnel traps to sample for stream-dwelling (i.e., epigean) salamanders is also well documented (e.g., Richter 1995; Mushet et al. 1997; Fronzuto and Verrell 2000; Wilson and Dorcas 2003; Nowakowski and Maerz 2009), their effectiveness for subterranean vertebrates is limited and not well documented (McDermid et al. 2015). Despite their utility, bottle traps and funnel traps have drawbacks. Because these traps restrain captured animals, they must be checked frequently to prevent mortality, a critical consideration when researching endangered and threatened species (Wilson and Gibbons 2010).

A popular technique for sampling surface (i.e., epigean) stream-dwelling salamanders is the use of leaf litter bags (Pauley and Little 1998; Jung et al. 2000; Waldron et al. 2003; Edwards et al. 2016). Leaf litter bags attract and concentrate, rather than trap, fauna by providing favorable structure objects, and animals are able to freely move in and out of the bag (Willson and Gibbons). This makes leaf litter bags a favorable technique when deploying traps for long periods because there is less risk of accidental death from target fauna becoming truly trapped and not being able to leave the trap (Waldron et al. 2003). This is especially important for listed endangered or threatened species, where the health and well-being of the animals takes precedence.

In surface waters, researchers check leaf litter bags by quickly sliding a tray or sieve under the bag to catch any target fauna that may fall through the mesh netting (Willson and Gibbons 2010). However, this presents a problem in subterranean deployment because traps often need to be retrieved vertically through the water column (or air), allowing ample time for fauna to escape. Here, we present a technique to remedy this problem to allow the effective use of leaf litter bags in subterranean settings.

The study species

The target of this sampling methodology was Salado Salamanders (*Eurycea chisholmensis*), a fully aquatic groundwater obligate salamander. Salado Salamanders are the northernmost species within a radiation of central Texas *Eurycea* salamanders and are endemic to groundwater habitats north of the San Gabriel River and south of Salado Creek in Williamson and Bell counties, Texas. (US Fish and Wildlife Service 2014; Devitt et al. 2019). Because of anthropogenic pressures and concerns of reduced water quantity and quality within the Edwards/Trinity aquifer system that contains this radiation of distinct species, *E. chisholmensis* along with several other species in this radiation have been listed under the Federal Endangered Species Act or are being prepared for future proposed listings (e.g., U.S. Fish and Wildlife Service 2013, 2014).

Methods

Study area

Cobbs Spring is ephemeral and discharges from the northern segment of the Edwards Aquifer in the Berry Creek watershed and is located on private property within Williamson County, Texas, USA. The soil and associated geology near the spring consists of silty and clayey slope alluvium underlain by Edwards limestone. Pecan (*Carya illinoinensis*) is the dominant overstory vegetation surrounding the spring. Within the spring run, the Edwards limestone is weathered entirely exposing the topmost Comanche bedrock.

Early settlers of this property installed an approximately 3 m deep hand-dug well (Fig. 1) located about 100 m downstream of the spring outlet and 50 m west of the main channel of the spring run. We observe this well gaining and losing water in correspondence with discharge volume in the spring itself, and thus we assume it is fed by the same source of groundwater. We visited Cobbs Spring monthly as part of an ongoing capture-mark-recapture study of *Eurycea* salamanders at select Williamson County sites (Cambrian 2020, 2021). Salamanders are previously known to occur within the well (U.S. Fish and Wildlife Service 2014), and we opportunistically observed salamanders in the well over the course of population monitoring.

Trap design

We modify a commonly used and documented trapping technique for stream-dwelling salamanders (Jung et al. 2000; Waldron et al. 2003; Edwards et al. 2016; Peirson et al. 2016) for use in subterranean aquatic applications. Although the factors influencing stygofauna occurrence are not well studied, some research indicates that the most important factors are habitat structure and the supply of organic matter (Korbel and Hose 2015; Ercoli et al. 2019). The method we describe herein adds a potential foraging environment to a sparse environment. This may attract salamanders either for its

Figure 1. Hand cut limestone well under a pecan tree. The well near Cobbs Spring is a relatively shallow well made of limestone blocks that were cut by hand. Situated under a pecan tree, the well is uncovered, allowing leaves and organic matter to naturally fall inside.

use as habitat structure or by first attracting salamander prey species with food in the form of organic leaf matter, which in turn attract salamanders.

Our goal when designing this trap method was to create a leaf litter bag that could be lowered into a well within a mesh bag (Figs 2, 3), which when lowered to the bottom of the well would lay flat allowing salamanders access to the leaf litter trap (Fig. 4), but upon extraction enclose around the leaf litter trap (Fig. 5), ensuring capture of all animals harbored within. Leaf litter bags are typically fashioned from polypropylene mesh netting designed for landscaping; leaf litter is collected and contained within the poly netting. Although the netting is typically used to exclude deer or birds, salamanders are able to enter and leave the ‘trap’ freely because the 2.5 cm wide squares that make the netting are large enough for several salamander species to pass through. We constructed a leaf litter bag approximately 30 cm × 30 cm × 45 cm in size using “Vigoro Polypropylene Deer Block Netting, UV Treated” (Model # NMVDB07100 Store SKU #295769 purchased from Home Depot) which has 1 inch (2.54 cm) apertures, and cable ties. The leaf litter used was collected at the site and composed mostly of Pecan, which also naturally occurs inside the well from the surrounding canopy. We used native leaf litter collected at the site because a change to the quality or quantity of litter entering caves has the potential to disrupt the structure and function of cave communities (Hillis et al. 2008). Additionally, Pecan leaves are larger than the 2.5 cm aperture of the deer block netting allowing them to be contained satisfactorily. The bottom of the trap was made from a “Leslie’s Pro grade 18 inch Leaf Rake” (Sku:

Figure 2. Leaf litter bag and modified pool net. A leaf litter bag is held above a modified pool bag, demonstrating the trap used for collecting and examining aquatic salamanders.

82627 LPM #: 82627; manufacturer location - HQ - Phoenix Arizona) brand pool skimmer. This device features a fine mesh whose apertures are smaller than we would expect any *Eurycea* salamander, at any life stage, to be able to escape through. The pool skimmer was deconstructed and only the mesh bag was used to fashion the trap. A wire coat hanger was modified into a circle and mounted onto the rim of the pool skimmer bag with cable ties. In order to lower the trap into the well, 30lb test monofilament line was tied to four areas on the rim of the wire coat hanger circle. With the leaf litter bag inside the pool skimmer bag, the trap was lowered to the bottom of the well, adjusting the position so that the pool skimmer bag laid flat along the bottom of the well.

The trap was checked four hours after first deployment (Fig. 6), it was then redeployed and rechecked three times, each one month apart coinciding with our capture-

Figure 3. Lowering the Leaf Litter Trap into the Well. The image shows the trap being lowered into the well using the lanyard. The leaf litter bag, contained within the pool net, is carefully guided down to the bottom of the well, positioning it to lay flat and become accessible to salamanders. This setup ensures a smooth descent and accurate placement of the trap in the well.

Figure 4. Leaf litter bag with no lanyard tension. Illustrating how the leaf litter bag is fully accessible to salamanders when the pool net lies flat against the bottom of the well, with no tension on the lanyard. This design ensures that the leaf litter bag provides unobstructed access to the trap contents, allowing salamanders to enter and leave freely.

Figure 5. Leaf litter bag with lanyard tension. Demonstrating the trap in its closed position with tension applied to the lanyard, causing the pool net to enclose the leaf litter bag. This configuration securely captures the salamanders and prevents their escape, ensuring all animals within are retained during extraction.

Figure 6. First captured *Eurycea chisholmensis* with this trap. The first two federally listed *E. chisholmensis* salamanders captured using the modified leaf litter trap. One is an adult, and the other is a juvenile, showcasing the trap's effectiveness in capturing different life stages. Photos of these salamanders were taken to be able to identify them as recaptured in successive surveys using the Wild ID software.

mark-recapture study. We retrieved the trap by hoisting it out of the well by the 30lb test monofilament line. Each time the trap was checked, water from the nearby spring was collected and then poured over the leaf litter bag as it was gently shaken to allow any invertebrates and salamanders to fall out of the leaf litter bag and into the pool skimmer bag. While processing the invertebrates and salamanders that ended up in the pool skimmer bag, we placed the leaf litter bag over a sieve (Adcock et al. 2022) in case any more invertebrates or salamanders remained in the leaf litter bag after the initial washing and shaking. Salamanders were categorized as juvenile if their total length was approximately <25 mm based on a visual estimate (Pierce et al. 2010) and also checked for gravidity (i.e., the presence of oocytes within the translucent venter). Salamanders were then photographed against a 5 mm background to be later measured for total length and snout-trunk length (STL) and to identify individuals (Bolger et al. 2012; Bendik et al. 2013). Salamanders were then replaced into the pool skimmer bag along with the leaf litter trap and returned to the well, as the trap system was redeployed. Representative specimens of each captured invertebrate were also vouchered, and excess captures were released back into the well, following the same protocol as with the salamanders. Recaptured salamanders were identified from dorsal photos based on their unique chromatophore patterns using the free open-source photo recognition software ‘Wild ID’, a tool designed to identify individuals based on unique patterns from photos instead of using a physical tag on the animal (Bolger et al. 2012). Salamanders were measured from photos using the free open-source software ‘Image J’, using the 5 mm grid background as a reference.

Results

A total of 18 salamanders were captured, representing 12 unique individuals (determined through capture mark recapture methods) (Table 1). Multiple salamanders were captured during every sampling event, and we never collected an injured or dead salamander (Table 2). We additionally captured four species of invertebrates representing three Classes (Insecta, Clitellata, and Gastropoda), as well as Rio Grande Leopard Frog tadpoles (*Lithobates berlandieri*).

Discussion

Stygofauna are understudied, and many species within this group are of conservation concern due to their cryptic nature (Hahn 2002; Simões et al. 2013). These species are often granted protected status, such as threatened or endangered, under the Endangered Species Act, necessitating research and data collection (Doremus and Pagel 2008). Consequently, there is a critical need to develop and refine survey techniques to provide reliable sampling options for practitioners working to conserve and manage these vulnerable species.

Although this water well is shallow and open to the surface, we expect the methods we describe above to be applicable to a variety of sampling requirements and unique situations. The successful capture of salamanders within this well, given its proximity

Table 1. Salado Salamander (*Eurycea chisholmensis*) measurement and gravidity data.

Date	Salamander Unique ID	Total Length (mm)	STL (mm)	Gravid
31 Oct 2019	C0432	24.1	14.6	No
	C0433	47.1	25.9	No
20 Nov 2019	C0434	32.4	18.3	No
	C0435	33.7	18.6	No
	C0436	43.2	23.0	No
19 Dec 2019	C0432	24.9	15.8	No
	C0454	43.6	25.7	No
	C0435	33.6	19.3	No
27 Jan 2020	C0435	34.7	19.0	No
	C0470	40.6	22.5	No
	C0471	30.3	17.7	No
24 Feb 2020	C0493	32.6	19.2	No
	C0494	31.5	20.0	No
	C0495	34.0	21.0	No
	C0470	41.2	23.9	No
	C0471	31.6	20.4	No
	C0496	42.2	24.5	No
	C0432	28.4	17.7	No

Table 2. Salado Salamander (*Eurycea chisholmensis*) capture results using the modified leaf litter trap.

Date	Deployment Time	Total Adult Captures	Total Juvenile Captures	Recaptured Individuals
31 Oct 2019	4 hours	1	1	–
20 Nov 2019	20 days	3	0	0
19 Dec 2019	29 days	2	1	2
27 Jan 2020	39 days	3	0	1
24 Feb 2020	28 days	7	0	3

to the known occupied spring, demonstrates the use of subterranean habitat by this species within the karst subterranean habitat that underlies the uplands.

The recapture of 6 unique individual salamanders over five sampling events without mortalities demonstrates that salamanders can enter and leave the trap safely. This trap was designed specifically for this well, but it could potentially be used as a sampling technique for other wells, caves, and hard-to-reach places in occupied springs. This methodology may also have utility to inventory invertebrates that utilize leaf litter, and in federally mandated occurrence surveys for this group of salamanders (USFWS 2021). With some modifications, we expect that this trap can be deployed in various deep-water settings, caves, solution cavities, and wells of different sizes and depths. Additionally, the leaf litter bag could be replaced by other artificial structures meant to attract fauna, such as mop heads, which are commonly used to sample both groundwater salamanders and invertebrates (e.g., Gibson et al. 2008; Devitt and Nissen 2018). There are additional species within the genus *Eurycea* that are federally listed and continue to be understudied, especially in subterranean habitats. This sampling method holds potential for effective use in studying the majority of this genus within the Edwards/Trinity aquifer system, given the shared life history strategies observed among most central Texas *Eurycea* species.

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The distribution of bat species across the underground sites of Georgia

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Abstract

In Georgia, investigations of bat species using caves have been conducted since the beginning of the 20th century. However, robust knowledge is still largely unavailable, as only about 15% of the roughly 2,000 underground sites have been surveyed for bats. The primary objective of this article was to document the use of underground habitats by various bat species. To achieve this, we consolidated field observations with existing data from literature and assessed potential threats to these underground habitats, specifically focusing on caves that harbor significant bat colonies. In this study, we considered underground sites across Georgia where at least one bat species has been documented. In total, twenty out of the thirty bat species recorded in Georgia were found in these underground sites. We characterize the spatial distribution, elevational range, usage of underground habitat, and colony size for these species. These twenty species belong to three families and ten genera. Three of these species have conservation status on the Georgian Red List, and one species is on the Red List of Threatened Species, according to the International Union for Conservation of Nature (IUCN). We also report the spatial and elevational distribution of bat species richness and frequency of records in order to rectify conservation priorities in this important biodiversity hotspot.

Keywords

Caucasus, Chiroptera, Diversity, Distribution, underground

Introduction

Caves and other underground (also called subterranean) habitats, including man-made ones, are important roosting sites for many bat species. Underground habitats can be used by different bat species for regular roosting, reproduction, and/or hibernation and play a significant role in their life cycle (Dietz and Kiefer 2016). However, underground habitats are also under increasing anthropogenic pressure (such as touristic impact, habitat modification, light pollution etc.) that pose a threat to bat populations (Mitchell-Jones et al. 2007; Nanni et al. 2023). Thus, understanding spatial distribution of underground habitats and threats to them is essential for identifying urgent risks and developing comprehensive conservation strategies that include legal protection and community-based initiatives to safeguard these vital roosting sites.

The country of Georgia, as a part of the Caucasus and Irano-Anatolian biodiversity hotspots, is rich with habitat and landscape diversity (Mittermeier et al. 2011; Habel et al. 2019), including subterranean habitats. However, the study of Georgia's subterranean environment and its biodiversity, including bats, is still in its infancy (Mumladze et al. 2020). The first attempts to investigate the bat fauna of Georgia were initiated in the middle of the 19 century (e.g Nordmann 1840; Kolenati 1860) However, this research was sparse, and the sporadic data about bat species diversity and distribution were scattered in publications mainly in Russian and Georgian (e.g. Satunin 1896, 1903, 1908, 1912, 1913, 1915; Chkhikvishvili 1926; Ognev 1928; Kuzyakin 1950; Papava 1949, 1953, 1960; Janashvili 1953, 1963; Perov 1980, 1983). Unsurprisingly, publications related to bats in caves and other underground habitats were even rarer, with large time periods between publications (Fig. 1). By the 1980s, 13 bat species had been identified in Georgia from 71 subterranean sites.

More systematic studies of Georgian bats started at the end of the twentieth century. These included general publications on Georgian bats, which also provided information on bats in caves and other underground sites (Benda 2011; Bukhnikashvili 2004: Bukhnikashvili et al. 2004, 2009; Gazaryan 2005; Ghazaryan et al. 2006; Ivanitsky 2010, 2015, 2017; Smirnov et all 2016; Yavruyan et al. 2008; Natradze et al. 2023) albeit to a limited extent. The only sources specifically targeting bats in caves were limited to individual caves (Ivanitsky 2002; Imnadze et al. 2020).

These later works include 115 locations in Georgia but did not record any new species. In addition to these published reports, we collected bat data from more than 300 surveys over a 20-year period at 44 additional underground locations. While some of the data concerning Georgia's bat diversity and distribution were previously documented in the literature (Natradze et al. 2023). We now present findings in underground habitats for the first time in a readily accessible format. However, it is important to highlight that the availability of information on bat diversity and distribution in Georgia's subterranean environment remains severely limited, as most of our data was not published prior this study.

To provide a comprehensive overview of the diversity and distribution of bat species in Georgia's underground habitats, we compiled and analyzed all available data on

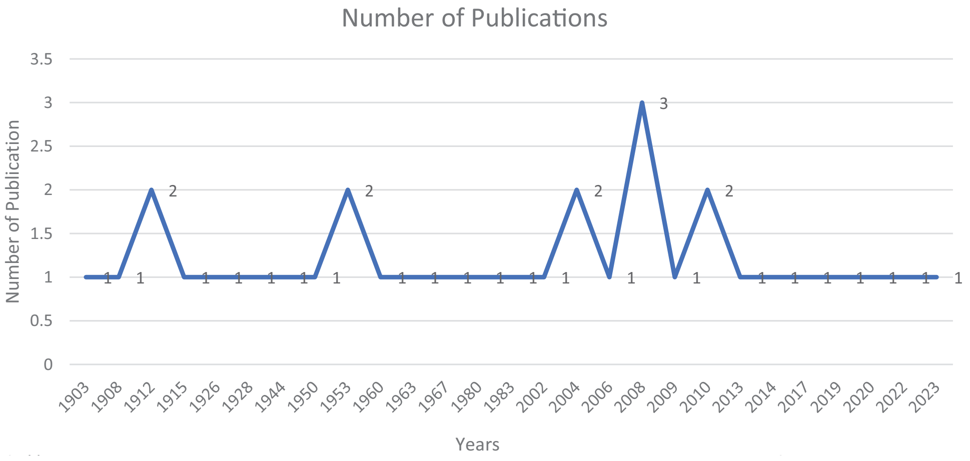

Figure 1. Number of publications with data on bats in underground habitats of Georgia, arranged by year.

bat records from these environments across the country. We characterized the spatial distribution, elevational range, and habitat use of the 20 bat species observed. This enhanced understanding of bat species distribution in Georgia is critical for conservation prioritization, particularly in light of the threats posed by anthropogenic pressures in this important biodiversity hotspot.

Materials and methods

Literature review

We gathered all known publications on Georgian bats, most of which are not available in English and/or through electronic databases of the scientific literature. Most of these publications are held in the scientific libraries of Georgian and Russian research institutions. We used the snowball survey method, where we began with a set of known references, including peer-reviewed, white, and gray literature, and traced back from there all additional references mentioned in this known set. Our known set began with materials from influential Georgian bat biologists prior to the 1970s. We included additional materials from a complete review of cataloged resources in our institute's academic library and other academic libraries within Georgia, as well as Georgian museums with bat collections. We visited relevant academic libraries and museums in Russia (e.g., the collection of the Zoological Institute of the Russian Academy of Science [Saint Petersburg]; the Zoological Museum of Moscow State University) and searched for references to bats in the Caucasus region. We also searched the European databases (GBIF, EUROBATS underground habitats database) and a compendium of Soviet publications (Chkhikvishvili 1926; Ognev 1928; Bobrinsky at al. 1944; Kuzyakin 1950; Janashvili 1953; Papava 1953, 1960; Janashvili 1963; Kipiani at al. 1966; Matsaberidze and Khotenovskii 1967; Perov 1980, 1983), for references to bats in the Caucasus region. Finally, we made use of our network of colleagues in the Caucasus region, Russia, and Europe to check our list of references for any additions. From the literature search, we recorded all reported instances of bats and included species, data, location, altitude, and coordinates. While exact coordinates for observations were often unavailable in older literature (i.e., prior to 1999), we georeferenced most of the sampling locations based on the names of the underground habitats.

Field data collection

Our surveys of caves and other subterranean sites were conducted from 1999 to 2024 during all seasons, resulting in more than 300 surveys across the country. In this paper, we report on 115 underground sites where bats were recorded. For each survey, we surveyed the underground sites for species presence. Additionally, during the swarming period, we mist-netted bats in front of the underground sites. Nets were constantly patrolled, and bats were removed immediately from the net and placed in bags where they remained calm while awaiting processing. We collected morphological measurements for species identification, according to Kuzyakin (1950) and Dietz (2016). After taking measurements, bats were released at the site of their capture. Bats were netted and handled under agreements #2722/01, 2302/01, R/057-21 issued by the Ministry of Environmental Protection and Agriculture of Georgia.

The sampling area covered all the major subterranean environments of Georgia. Natural caves are found mainly in karstic outcrops that cover 6.4% of the total area of Georgia (4,475 km^2). These caves are mostly found in western part of the country in the peripheral zone of the southern slope of the Caucasus and on the hilly zones north of the Colchis lowland (Ukleba 1981). In eastern Georgia, karstic outcrops are not fully developed and are present as limestone deposits where a few small grottos and crevices have formed. Overall, there are more than 1500 known karst and conglomerate caves in Georgia (Asanidze et al. 2017).

We also surveyed man-made subterranean sites. In western Georgia, these are mainly mines. The mine surveys were somewhat limited within the Chiatura municipality where abandoned manganese mines are unsafe to enter. In eastern Georgia, there are mainly man-made caves covered with built-in walls and small grottoes. These man-made caves are predominantly in the gorges of the Mtkvari (Kura), Iori, Khrami, Algeti and Mashavera rivers. They are formed in sandstone, while in other places they are made by widening volcanic caverns.

When bats were observed in colonies, we employed several methods to draw the rough estimate of the colony size. Due to difficulties associated with colony size estimates for bats (Kunz et al. 2009), for small and fully visible groups, we conducted direct counts of all individuals. In larger colonies, (where possible) individuals were counted for representative subgroups (i.e., subgroups delimited using the square light), and the total colony size was then estimated by multiplying the subgroup count by the estimated number of individuals. If the direct counts within subgroups were not possible (due to distance or danger of harassment) or for very large aggregations, sometimes consisting of multiple groups, we used photographic methods. When groups were too large to capture in a single image, multiple photographs were taken, and individuals were counted from the images.

Data analysis

In order to understand the association of each species with the subterranean habitat, we classified the lifestyle of bats into six categories based on literature descriptions and/or our field observations: (i) exclusively cave dwelling species, i.e. species that roost in caves only; (ii) mainly cave dwelling species, i.e. species that roost in caves, but can also use other types of underground habitats; (iii) partially cave dwelling species, i.e. species that mainly use trees as summer roosts but use caves and other subterranean habitats for wintering, mating or swarming; (iv) forest species, i.e. species that roost on trees but can use underground habitats for mating or cave entrances and crevices in the entrances of caves for temporary roosting; (v) species living in different types of crevices e.g. buildings, rocks, entrances of caves, joints of bridges; and (vi) species with no shelter preference, i.e. species with the widest spectrum of sheltering preferences.

We summarized and visualized the data using custom R code (R Core Team 2022). Location data were masked or aggregated to protect the location information of bats that may be endangered or harassed. We aggregated the data using a 50 × 50 km cell size as this size optimized the density of information for biologically meaningful visualization and was neither too dense nor too sparse.

Assessment of main threats to underground roosts

In addition to documenting the presence of bats, our surveys assessed potential threats to underground roosts at selected sites with colonies. We identified threats such as i) excessive disturbance, ii) destruction, alteration, or change in the use of habitat, and iii) light pollution, as highlighted in the studies by Mann et al. (2002), Mitchell-Jones et al. (2007), Voigt et al. (2018) and Kyheröinen et al. (2019). We quantified each threat on a scale from 1 to 3, where 1 represented low threat, 2 indicated medium threat, and 3 denoted high threat. Simultaneously, we evaluated the accessibility of each cave, assigning levels where 1 indicated hard access, 2 signified moderate access, and 3 denoted easy access. By calculating an average score for each site, combining threat and accessibility levels, we developed a comprehensive quantifier that enhanced our ability to assess the vulnerability of each site. This systematic quantification of both threats and accessibility transformed qualitative assessments into quantifiable data, crucial for comparing conditions across sites. This methodology enabled us to identify more vulnerable caves due to greater threats or easier access, guiding the development of precise conservation strategies and management practices. These assessments are pivotal in protecting these vital roosting areas, underscoring our commitment to converting subjective assessments into objective data for targeted conservation efforts.

Results

Species composition

Papers published from 1912 to 2010 recorded 13 bat species from 71 underground sites. Since 2010, we have added 44 underground sites and recorded a total of 20 species. Of these 20 species, one (*Rhinolophus blasii*) was recorded for the first time in the country; twelve species overlapped previously published reports, while the presence of previously recorded *R. mehelyi* could not be confirmed. In addition, six species that usually did not use underground habitats were unexpectedly recorded. Thus, at the time of writing, there were 331 records of 20 bat species from 115 sites in Georgia (Annex 1. Table 1).

During our surveys from 1999–2024, some bat species were recorded multiple times (Fig. 2). The most common species observed were *Rhinolophus ferrumequinum*, followed by *R. hipposideros*, *Miniopterus schreibersii*, *Myotis blythii*, and *R. euryale*. The species *R. ferrumequinum*, *M. blythii*, *R. euryale*, and *M. schreibersii* persist in significant numbers in specific cave habitats.

The most commonly encountered species included six primarily cave-dwelling species: *Rhinolophus ferrumequinum*, *R. hipposideros*, *R. euryale*, *Myotis blythii*, *M. emarginatus*, and *M. schreibersii*. Additionally, seven partially cave-dwelling species were recorded: *M. mystacinus*, *M. brandtii*, *M. daubentonii*, *M. nattereri*, *Barbastella barbastellus*, *Plecotus auritus*, and *P. macrobullaris*. Only one species, *R. blasii*, was found exclusively in caves (Fig. 3). We also recorded two forest species, *Myotis bechsteinii*, and *Nyctalus leisleri*; three species living in various types of crevices, *Hypsugo savii*, *Eptesicus serotinus*, and *Vespertilio murinus*. Finally, we recorded one species, *Pipistrellus pipistrellus*, which showed no clear roosting preference.

The results of species counts and records using the grid with 50 × 50 km cell size, which are illustrated in Suppl. material 1: map S1, provide insight into the distribution of these species across the surveyed areas. Counting species richness using a bar plot with 300-meter bands showed that the highest species diversity occurred in the 0–300 and 1200–1500 meters above sea level (m.a.s.l) elevation range, followed by the 600–900 m.a.s.l elevation range (Figs 4, 5). Spatial distribution maps for species are provided in Suppl. material 1: maps S2–S10.

Table 1. Threat Assessment Scores for Selected Bat Roosting Sites in Georgia.

#	Roost	Extensive disturbance	Destruction, maintenance or change of use	Light pollution	Accessibility	Average scores
1	Ghliana	3	3	2	3	2.75
2	Melouri	1	1	1	2	1.25
3	Samrtskhle Klde	2	3	1	2	1.75
4	Vardigora	1	1	1	3	1.5
5	Sakishore	1	1	1	2	1.25
6	Becho	1	1	1	2	1.25
7	Matkhoji Cave	2	1	1	2	1.5

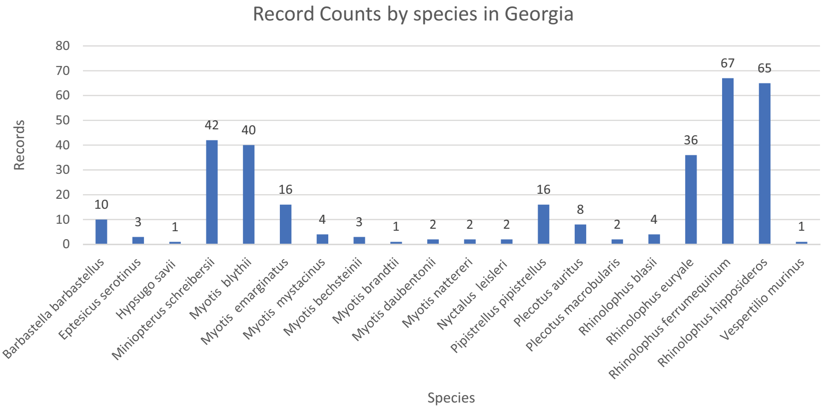

Figure 2. Number of records of Bat species in subterranean habitats.

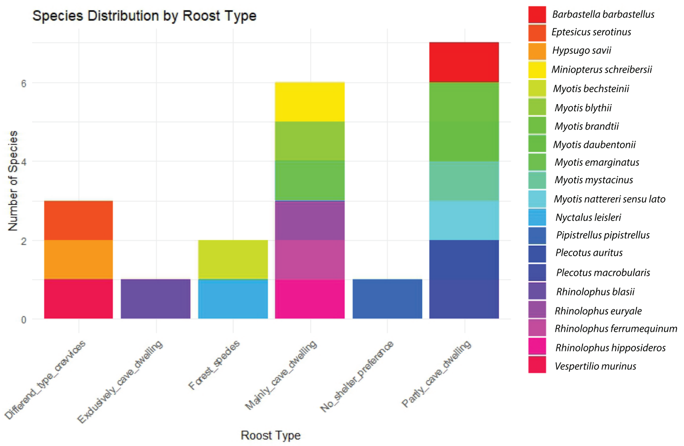

Figure 3. Cave associated species diversity in Georgia arranged according to habitat use.

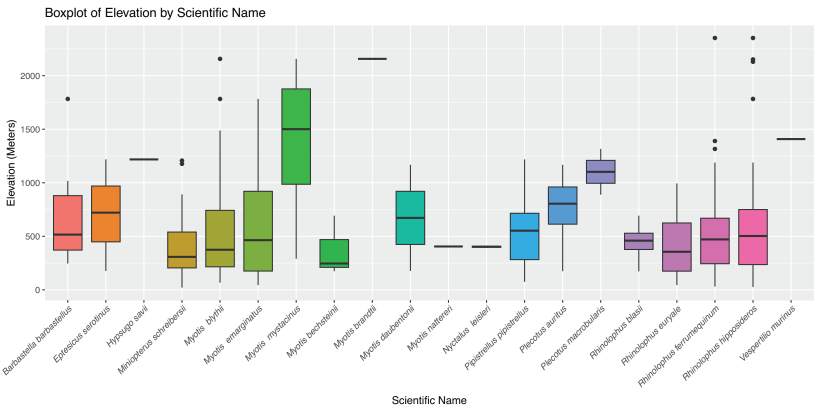

Figure 4. Species records by elevation.

Figure 5. Species richness by 300 meters bins.

The summary of threats affecting bat caves shows that Ghliana Cave and its colony are the most threatened, while the rest of the caves with colonies are moderately threatened by human disturbance (Table 1).

Discussion

We consistently recorded three (*Rhinolophus ferrumequinum*, *Myotis blythii*, *Miniopterus schreibersii*) species in the literature prior to 1999, as well as in our own surveys from 1999 to 2024, suggesting that the species richness has remained constant over time. We found that most of these species in underground environments are located in colonies of up to 1000 individuals. Our analysis highlights that the highest richness and diversity of bats in underground habitats of Georgia are localized in western Georgia, particularly within karstic outcrops. Notably, the highlighted grid areas emerged as locations with the highest species richness and number of records, underscoring their critical importance for bat conservation.

The most frequently encountered bat species, *Rhinolophus ferrumequinum*, *R. hipposideros*, *R. euryale*, *Myotis blythii*, and *Miniopterus schreibersii*, were assigned to the category of 'mainly cave dwelling species.' From these five species, *R. euryale* is included in the Red List of Georgia with the category Vulnerable (VU), and *M. schreibersii* is also designated VU on the IUCN Red List. All five of these species are included in the list of Emerald Network priority species (https://rm.coe.int/1680746afc), highlighting these locations as critical microhabitats for their survival. *R. blasii*, a species that roosts exclusively in caves (Dietz and Kiefer 2016), was recorded in Georgia for the first time in 2006, and since then it has been found in three other caves (Suppl. material 1: map S5). Notably, during our extensive surveys, we did not record *Rhinolophus mehelyi*, which should be considered as 'mainly cave-dwelling species', and was last recorded in 1964 (Matsaberidze, Khotenovskii 1967). Although we have not encountered this species, the possibility that it still occurs in the country cannot be conclusively ruled out, highlighting the need for continuous monitoring of these habitats.

In one of Georgia's largest nursery colonies, Ghliana Cave, a mixed maternity colony is present, with the number of species varying by year and season. The cave is annually inhabited by *Myotis blythii* and *Miniopterus schreibersii*. Additionally, in different years and seasons, it provides a roost site for *Rhinolophus ferrumequinum* and *R. euryale*. In some years, *M. emarginatus* has also joined this mixed colony. The population of the maternity colony fluctuates annually, ranging from 4,000 to 8,000 individuals (Campester 2019), reaching its peak in late July to early August.

Another of Georgia's most numerous nursery colonies is in the Samertskle Klde Cave, which also reaches 8,000 individuals or more in certain years, primarily consisting of *Myotis blythii* and *Miniopterus schreibersii.* Besides these nursery colonies, Sakishore Cave hosts the largest known wintering colony, with more than 1,000 *M. schreibersii.*

Based on our observations, various levels of threats affect underground habitats in Georgia. The results of the assessment enable us to identify specific risks and tailor conservation approaches to meet the unique needs of each site, thereby ensuring the protection of the most crucial habitats. The divergent threat levels observed at these key locations necessitate targeted management practices for the sustained protection of these essential bat populations. Our findings underscore the immediate need for customized conservation efforts for high-risk caves, such as Ghliana, and call for diligent monitoring due to their greater accessibility and moderate threat levels. The variation in threat severity across these sites highlights the necessity for specific protective actions and emphasizes the importance of continuous monitoring and adaptive management strategies.

Despite recent anthropogenic pressures, the outlook for bat biodiversity conservation in Georgia is encouraging: no species have been lost, and some species of concern remain locally abundant. However, of the important karstic areas that host bat diversity hotpots, only 18 caves or cave complexes are legally protected. These include the Imereti Caves Protected Area with protection status of several caves as natural monuments. Additionally, only two caves are listed as important by the Emerald Network (Emerald Network 2020), while two caves with Georgian protection status and two man-made caves are included in the database of the Agreement on the Conservation of Populations of European Bats (EUROBATS). Of the protected caves, only the Ghliana and Melouri caves host large bat colonies. From these, the Ghliana cave has the highest endangered score based on our estimates (Table 1) despite the fact that Georgian Ministry of Nature Protection and Agriculture developed a conservation action plan for Ghliana Cave in 2019 (Campester 2019).

Based on the existence of well-developed karstic areas, high frequency of encounter, high richness, and high diversity, we consider the Imereti, Samegrelo, and Apkhazeti regions of Georgia as the most important areas for bat conservation. Access in these regions is difficult due to the absence of roads and the high altitudes of some caves, which ensures some sort of de facto protection. However, such protection is not sufficient for conservational purposes, and only protection of underground sites with large colonies is not sufficient. Protective measures should also include surrounding habitats that are used by bat colonies as commuting routes and foraging grounds. We recommend that more conservation and research should be undertaken in the Imereti and Samegrelo regions to ensure bat protection. Also, we urge the establishment of conservation status for four more caves: Samertskhle Klde, Vardigora, Becho, and Sakishore. By protecting these caves, Georgia will contribute to the improvement of conservation status of *Rhinolophus euryale*, *Myotis blythii*, and *Miniopterus schreibersii.* While the current status of Georgian bat biodiversity is encouraging, more needs to be done to ensure protection in the face of anthropogenic threats. Long term monitoring of bat species in underground sites and analysis from a biodiversity perspective enhances our understanding of their natural history and distribution, thereby helping to prioritize conservation efforts.

Acknowledgements

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Supplementary material 1

Additional information

Authors: Ioseb Natradze, Alexander Bukhnikashvili, Suren Gazaryan, Giorgi Sheklashvili, Levan Mumladze

Data type: pdf

Explanation note: **map S1.** The number of records and species found in each quadrat of Georgia. **maps S2–S10.** Species distribution Maps of records in underground habitats.

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RESEARCH ARTICLE

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Describing to preserve – three new species of *Xangoniscus* (Oniscidea, Styloniscidae) of unprotected caves in dry areas from Bahia state, northeastern Brazil

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Abstract

Three new troglobitic amphibious species of *Xangoniscus* (Styloniscidae) have been described from limestone caves of the Bambui Group semiarid ecosystems (Chacoan subregion) in the state of Bahia: *Xangoniscus antiquus* **sp. nov.**, *X. chaimowiczi* **sp. nov.**, and *X. jonasi* **sp. nov.** Natural history information is provided for *X. chaimowiczi* **sp. nov.** and *X. jonasi* **sp. nov.** Considering the differences between the known species of *Xangoniscus* and those described in this study, we redefined the diagnostic characteristics of the genus. Moreover, the distribution of *Xangoniscus* is discussed. The species described in this study, along with those previously reported, serve as fundamental tools for decision-making processes aimed at conserving Brazil's speleological natural heritage.

Keywords

Amphibious Oniscidea, Cerrado, Caatinga, troglobitic, woodlice

Introduction

Terrestrial isopods (Oniscidea) are among the most diverse groups within the order Isopoda, comprising more than 4,000 species across 38 or 39 families in more than 500 genera (Javidkar et al. 2015; Sfenthourakis and Taiti 2015; Dimitriou et al. 2019). These organisms have adapted to a wide range of terrestrial environments, from tropical forests to deserts, and from the supralittoral zone to mountain forests (Schmalfuss 2003; López-Orozco et al. 2022). Additionally, a significant number of species inhabit caves, and from an ecological-evolutionary perspective, they are classified as either troglophilous (facultative cave dwellers) or troglobitic (restricted to cave habitats) (Taiti 2004; Taiti and Gruber 2008; Bedek et al. 2011; Taiti and Xue 2012; Tabacaru and Giurginca 2013; Reboleira et al. 2015; Campos-Filho et al. 2014, 2023a, 2023b).

In Brazil, more than 50% of terrestrial isopod species are found in caves, highlighting the significant potential of this subterranean habitat (Campos-Filho et al. 2014, 2015, 2016, 2018, 2019, 2020, 2022a, 2022b, 2022c, 2023a, 2023b; Souza et al. 2015; Cardoso et al. 2020a, 2020b, 2021, 2023; Cardoso and Ferreira 2023a, 2023b; López-Orozco et al. 2024a, 2024b). To date, over 230 species have been identified, of which more than 40 are considered troglobitics, belonging to the families Armadillidae, Philosciidae, Pudeoniscidae, Scleropactidae, and Styloniscidae (Campos-Filho et al. 2014, 2019, 2020, 2022a, 2022b, 2022c, 2023a, 2023b; Bastos-Pereira et al. 2017, 2022; Cardoso et al. 2020a, 2020b, 2021, 2023; Cardoso and Ferreira 2023a, 2023b; López-Orozco et al. 2024a, 2024b).

Within the Oniscidea, the family Styloniscidae comprises 128 species in 17 genera, exhibiting a broad distribution and inhabiting a wide range of terrestrial environments, including caves (Schmalfuss 2003; Boyko et al. 2024). Of these genera, nine have been recorded in Brazil: *Chaimowiczia* Cardoso, Bastos-Pereira, Souza & Ferreira, 2021, *Clavigeroniscus* Arcangeli, 1930, *Cordioniscus* Gräeve, 1914, *Cylindroniscus* Arcangeli, 1929, *Iuiuniscus* Souza, Ferreira & Senna, 2015, *Pectenoniscus* Andersson, 1960, *Spelunconiscus* Campos-Filho, Araujo & Taiti, 2014, *Styloniscus* Dana, 1852, and *Xangoniscus* Campos-Filho, Araujo & Taiti, 2014. This represents over 50% of the global generic diversity within the family.

In the present work, three new species of *Xangoniscus* are described from caves in the state of Bahia, northeastern Brazil. We also discuss aspects of the morphology, distribution, and conservation of the species in the genus.

Material and methods

Study area

The specimens were collected from three limestone caves of the Bambui Geomorphological Group located in the southwest of the state of Bahia, between the Caatinga and Cerrado (savannah-like) biomes (Fig. 1). This area is characterised by a dry tropical

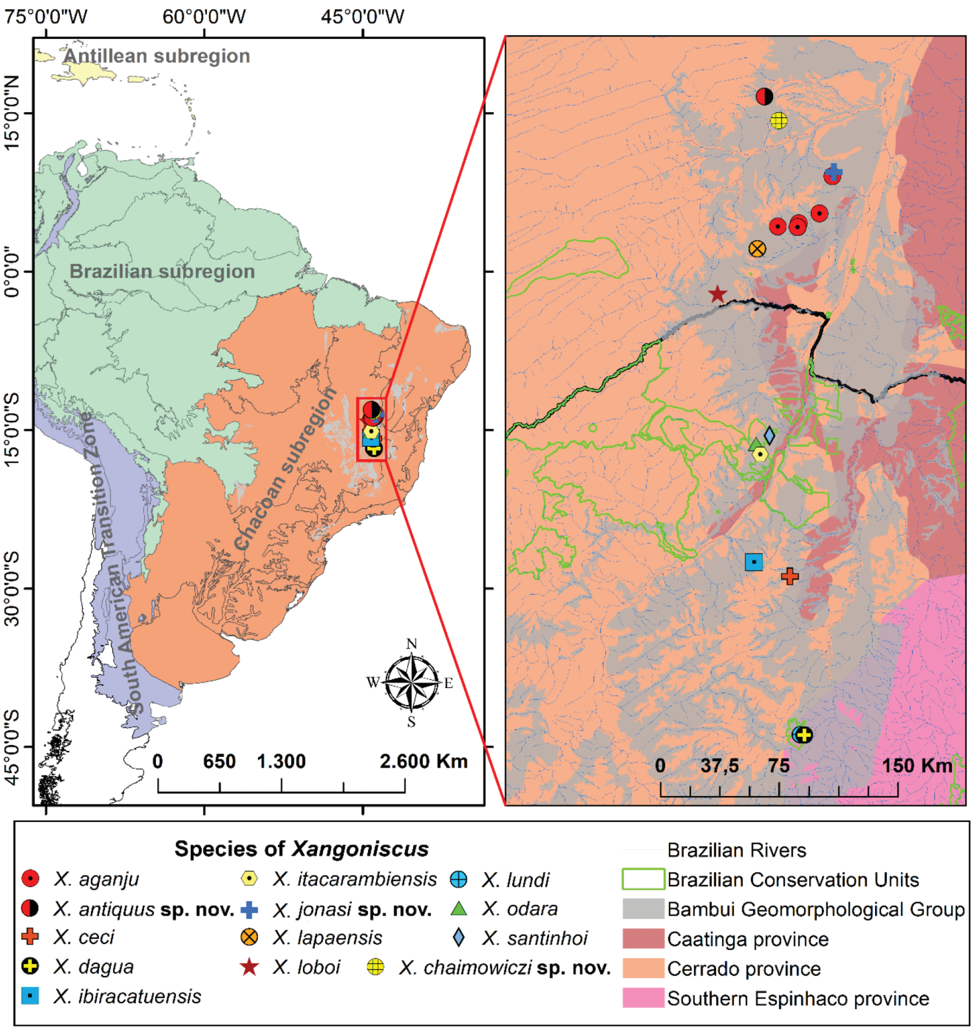

Figure 1. Map of the study area and distribution of the *Xangoniscus* species.

climate (Aw) with an average annual rainfall of up to 640 mm (Alvares et al. 2013; Beck et al. 2018, 2020).

The Gruna da Serra Solta cave (Fig. 2), is located in the Serra do Ramalho karst area, considered a hotspot of subterranean biodiversity (Trajano et al. 2016); however, there are no legal protection zones for these systems, which are threatened due to deforestation for the establishment of crops, extraction of wood, plus potential mining projects (Gallão and Bichuette 2018). To the north of the Serra do Ramalho, there is the Gruta do Padre and Gruta da Represa caves (Fig. 1). Gruta do Padre is located between the municipalities of Santana and Santa Maria da Victoria and represents one of the

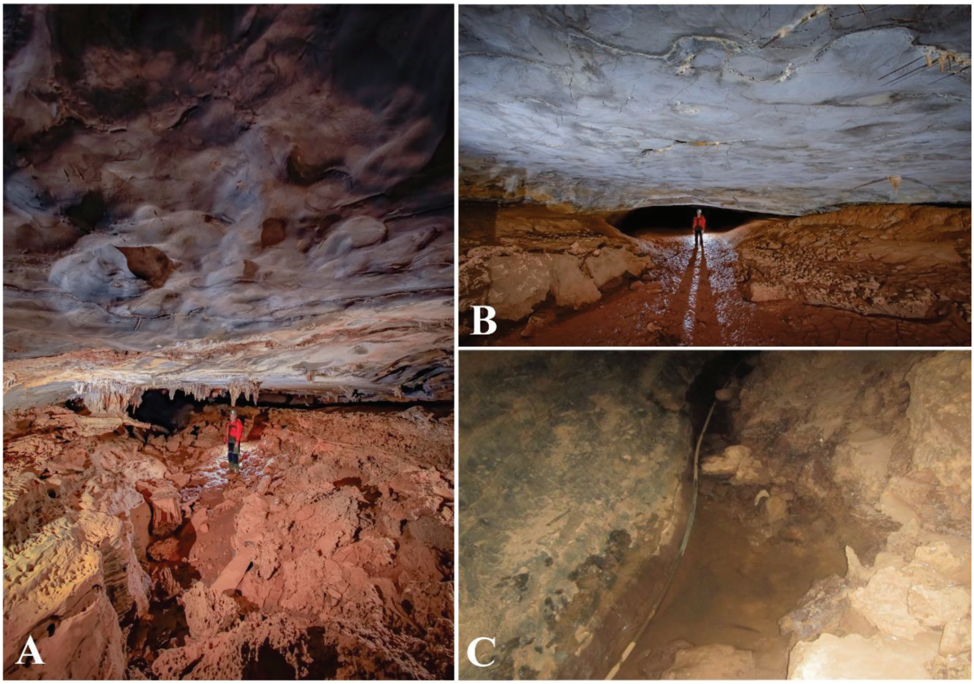

Figure 2. Gruna da Serra Solta **A, B** gallerys of the Gruna da Serra Solta **C** hose for extracting water inside the cave. Photo: Alexandre Lobo.

largest caves in Brazil (16.4 km). This cave has two known entrances, is characterised by its dry environment, and has been the scene of multiple speleological explorations (Auler and Rubbioli 2019).

Specimen preparations and taxonomy

Specimens were collected by hand and stored in 75% ethanol, and identifications were based on morphological characteristics using micropreparations in Hoyer's medium (Anderson 1954). For each new species, the type material, diagnosis, description, etymology, distribution, and remarks are provided. The subterranean classification followed Trajano and Carvalho (2017). Descriptions and morphological terms follow Campos-Filho et al. (2014). The images of each species were obtained with a stereomicroscope Nikon SMZ800N with an adapted Prime Life Science camera and edited using GIMP (v. 2.8). Illustrations were made with the aid of a camera lucida mounted on a Zeiss Stemi SV6 stereomicroscope and Leica DMLS microscope. The final illustrations were prepared using GIMP (v. 2.8), using the method proposed by Montesanto (2015, 2016).

The material examined is deposited in the Collection of the Laboratório de Estudos Subterrâneos (LES, curator: M. E. Bichuette), Universidade Federal de São Carlos, São Carlos, Brazil, and in the collection of the Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP, curator: Marcos Tavares).

Results

Systematic account

Suborder Oniscidea Latreille, 1802
Family Styloniscidae Vandel, 1952

Genus *Xangoniscus* Campos-Filho, Araujo & Taiti, 2014

Type species. *Xangoniscus aganju* Campos-Filho, Araujo & Taiti, 2014 by original designation and monotypy.

Xangoniscus chaimowiczi López-Orozco, Borja-Arrieta & Campos-Filho, sp. nov.

https://zoobank.org/72A41F39-A8EF-4961-8BA8-B5E52EE0ADE3
Figs 1, 3–6

Type material. ***Holotype*** • 1 male (MZUSP 29529), Bahia, Santana, Gruta do Padre cave, -13.216325, -44.065194, Sala dos Anfipodes, July 1987, leg. F. Chaimowicz. ***Paratypes*** • 1 male (parts in slides), 1 male (MZUSP 29529), same data as holotype • 2 males (MZUSP 29429), same data as holotype • 1 male (MZUSP 29565), sedimentos do conduto das plaquetas (no rio), same data as holotype • 4 males (MZUSP 29540), rio dos Travertinos, same data as holotype • 2 males, 1 female (MZUSP 29421), Conduto Biógeo, same data as holotype.

Description. Maximum body length: male 10.2 mm, female 6.5 mm. Body outline as in Figs 3A, B, 4A. Colourless (Fig. 3). Dorsal surface smooth, with scattered fringed scale setae (Figs 3A, B, 4A, B). Cephalon (Figs 3C, 4C) with large quadrangular antennary lobes; vertex with slightly lateral depression to fit antennae when extended backward, profrons with V-shaped suprantennal line, not surpassing antennule insertion; eyes absent. Pereonite 1 epimera with distal corners developed frontwards, posterior corners right-angled; pereonites 2–7 epimera gradually directed backward posterior corners progressively more acute (Figs 3A, B, 4A); pleon narrower than pereon; pleonites 3–5 epimera posterior point not developed (Figs 3D, 4A). Telson with concave sides and rounded apex (Fig. 4D). Antennula (Fig. 4E) composed of three articles, proximal and distal articles subequal in length, second article shortest, and distal article with 12 long aesthetascs. Antenna (Fig. 4F) short, not surpassing pereonite 2 when extended backward; flagellum shorter than fifth article of peduncle, with three articles subequal in length. Left mandible (Fig. 4G) with two penicils; right mandible (Fig. 4H) with one penicil. Maxillula (Fig. 4I) inner endite with three penicils; outer endite with 5+5 teeth, apically simple, and two plumose stalks. Maxilla (Fig. 4J) with setose and bilobate apex, outer lobe smaller. Maxilliped (Fig. 4K) basis enlarged on distal portion bearing fringe of fine setae; first article of palp with two tiny setae, distal articles with three tufts of setae; endite rectangular, outer and medial margins setose, apically with two triangular teeth and large rounded penicil. Uropod (Fig. 4D) branches short and inserted at same level, exopod slightly longer than endopod. Pereopods 1–7

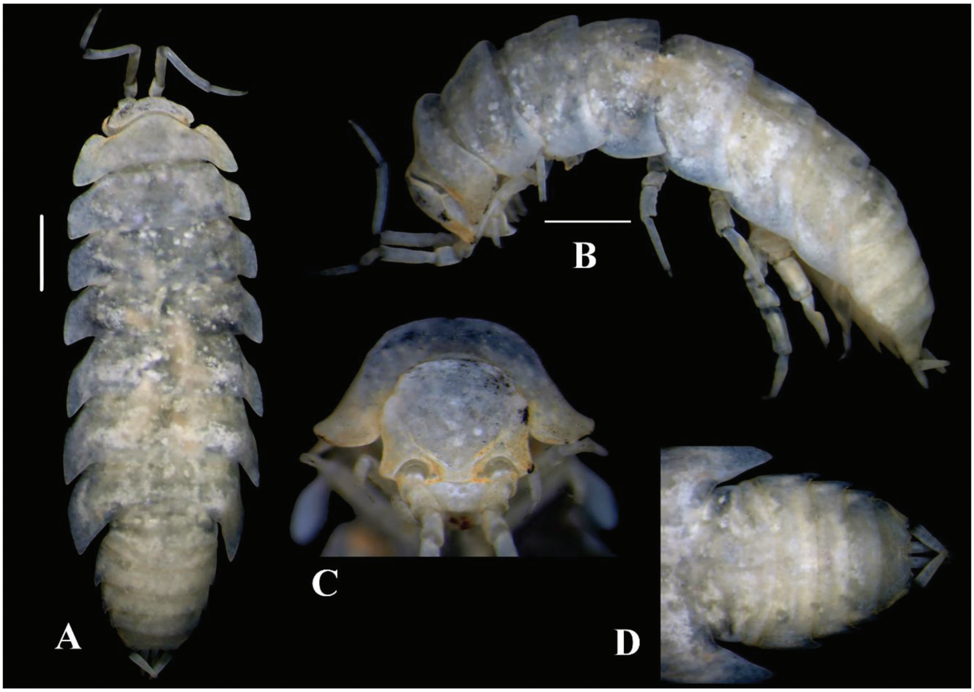

Figure 3. *Xangoniscus chaimowiczi* López-Orozco, Borja-Arrieta & Campos-Filho, sp. nov. Male **A** habitus, dorsal view **B** habitus, lateral view **C** cephalon and pereonite 1, frontal view **D** pereonite 7, pleon and telson, dorsal view. Scale bars: 1 mm.

(Fig. 5A–G) gradually elongated, with merus, carpus, and propodus bearing sparse setae; dactylus of one claw bearing many setae on outer margin.

Male. Pereopods 1–6 (Fig. 5A–F) merus with proximal portion bearing fringed scales and thin setae on sternal margin. Pereopod 1 (Fig. 5A) carpus with large antennal grooming brush. Pereopod 5 (Fig. 5E) carpus with slightly lobe on proximal sternal margin. Pereopod 6 (Fig. 5F) ischium enlarged with flattened sternal part; carpus enlarged, rostral portion bearing dense field of tiny lobules; propodus with central part enlarged, with longitudinal furrow in ventral view, and very tiny setae in dorsal. Pereopod 7 (Fig. 5G) without distinct modifications. Genital papilla (Fig. 6A) lanceolate. Pleopod 1 (Fig. 6A) exopod subtriangular, longer than wide, inner and outer margins bearing fringe of fine setae; endopod longer than exopod, with narrow basal article and flagelliform distal article; basipod distal margin elongate and acute apex, longer than exopod, with fine and long setae. Pleopod 2 (Fig. 6B) exopod trapezoidal, distal margin slightly convex; endopod of two articles, distal article about three times as long as proximal, with distal margin subquadrangular and apex rounded, with transversal process in V-like on apex in ventral view. Pleopod 3 (Fig. 6C) exopod triangular, longer than wide, covering pleopods 1 and 2, fringed with short setae. Pleopod 4 and 5 (Fig. 6D, E) exopods trapezoidal, wider than long, with margins bearing several short setae.

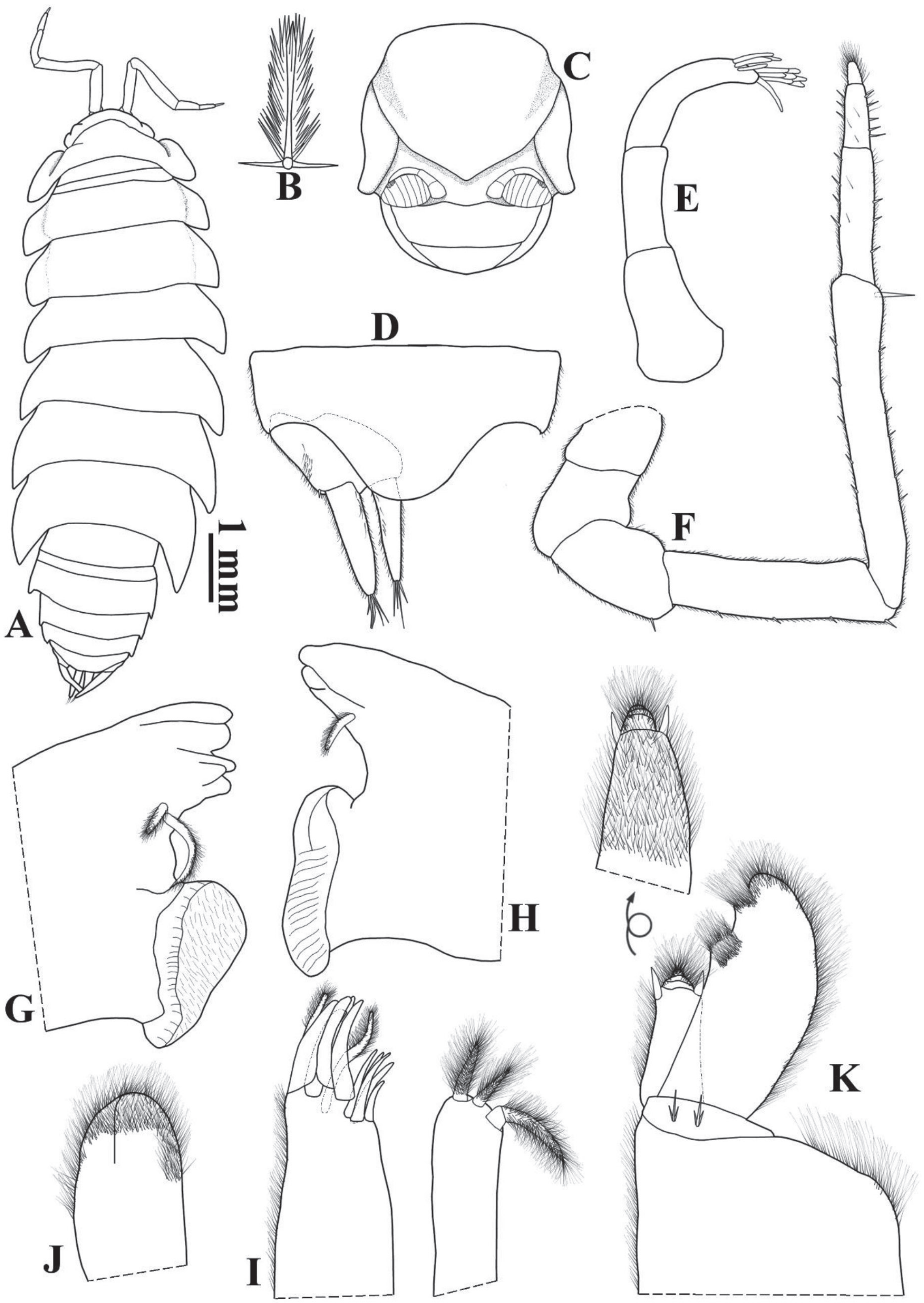

Figure 4. *Xangoniscus chaimowiczi* López-Orozco, Borja-Arrieta & Campos-Filho, sp. nov. Male **A** habitus, dorsal view **B** dorsal scale-seta **C** cephalon, frontal view **D** telson and uropod **E** antennula **F** antenna **G** left mandible **H** right mandible **I** maxillula **J** maxilla **K** maxilliped, arrow illustrating the endite in caudal view.

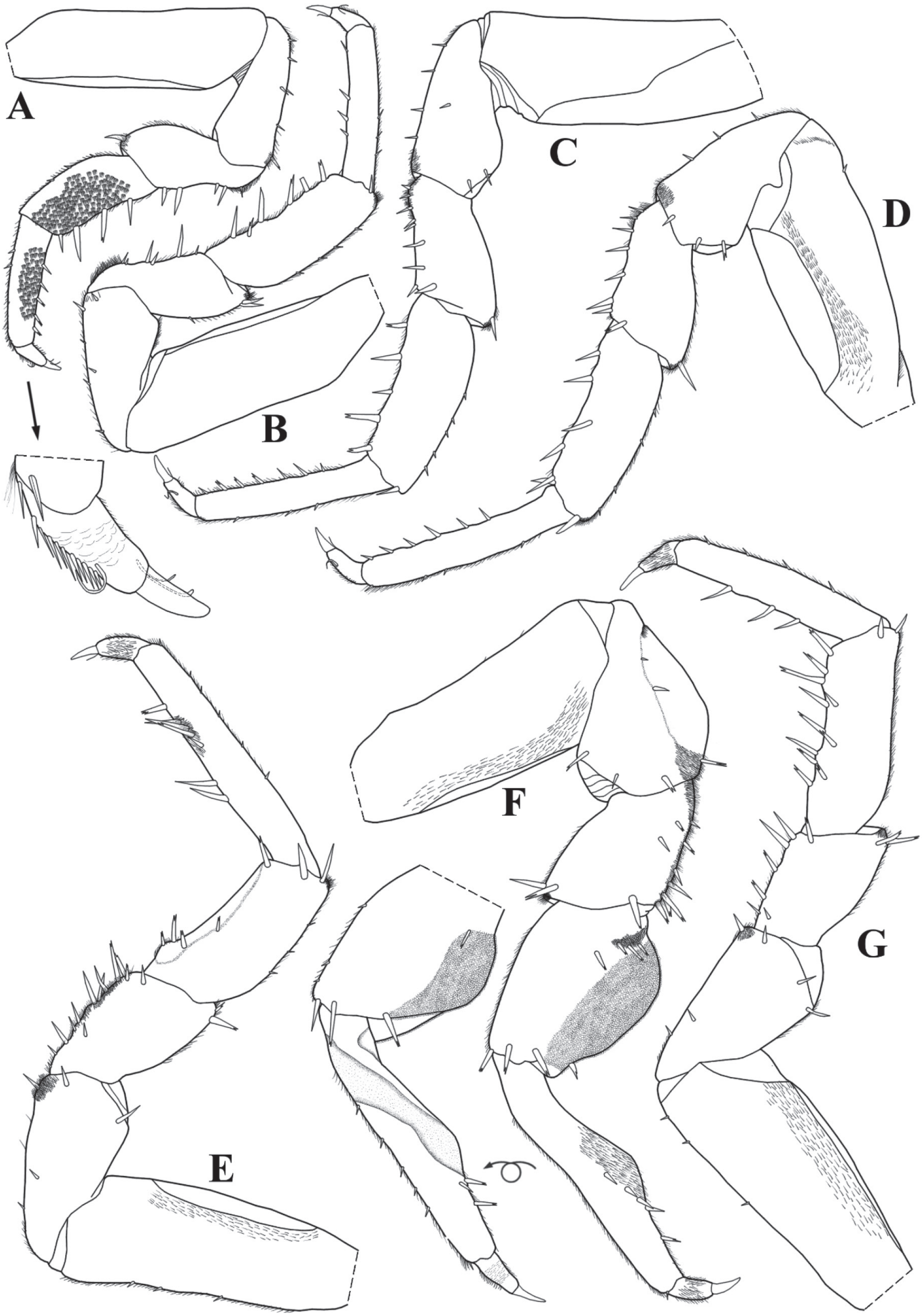

Figure 5. *Xangoniscus chaimowiczi* López-Orozco, Borja-Arrieta & Campos-Filho, sp. nov. Male **A** pereopod 1 **B** pereopod 2 **C** pereopod 3 **D** pereopod 4 **E** pereopod 5 **F** pereopod 6 **G** pereopod 7.

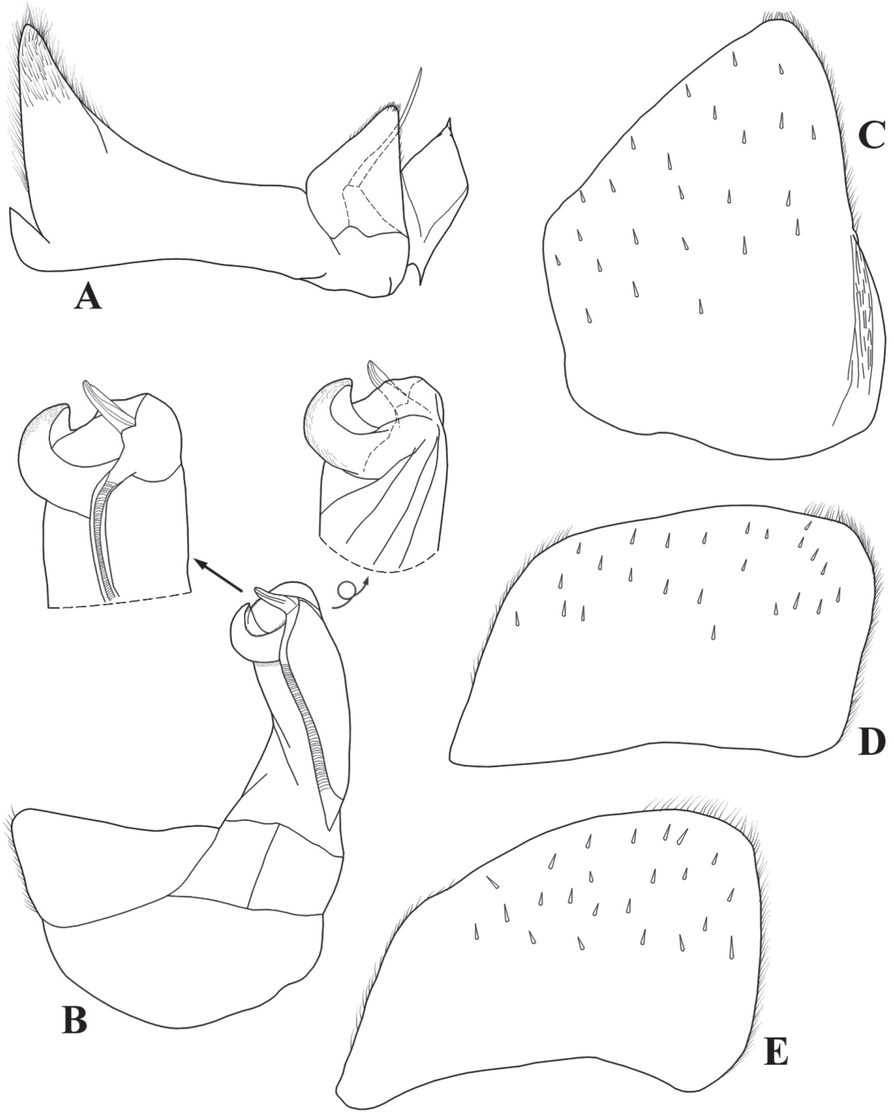

Figure 6. *Xangoniscus chaimowiczi* López-Orozco, Borja-Arrieta & Campos-Filho, sp. nov. Male **A** pleopod 1 and genital papilla **B** pleopod 2 **C** pleopod 3 exopod **D** pleopod 4 exopod **E** pleopod 5 exopod.

Etymology. The new species is named after Dr. Flavio Chaimowicz, for his important contributions to the knowledge of the Brazilian speleology, who collected the specimens.

Distribution. Presently known only from Gruta do Padre cave in the state of Bahia, northeastern Brazil (Fig. 1).

Remarks. The genus *Xangoniscus* includes 10 amphibious troglobitic species, with a distribution restricted to caves of the Bambui Geomorphological Group between the northeast and southeast regions of Brazil in the states of Bahia and Minas Gerais. Currently, the genus is well defined morphologically, and the main characteristic is the complex shape of the apex of the endopod of pleopod 2 in males. *Xangoniscus chaimowiczi* sp. nov. differs from all other species of the genus in having the antennula with twelve long aesthetascs (*vs*. two in *X. aganju* Campos-Filho, Araujo & Taiti, 2014, *X. lapaensis* Campos-Filho, Gallo & Bichuette, 2022, and *X. odara* Campos-Filho, Bichuette & Taiti, 2016, three in *X. ibiracatuensis* Bastos-Pereira, Souza & Ferreira, 2017, *X. lundi* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020, and *X. santinhoi* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020, four in *X. dagua* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020, four or five in *X. ceci* Cardoso, Bastos-Pereira, Souza & Ferreira, 2020, five in *X. loboi* Campos-Filho, Gallão & Bichuette, 2022, six in *X. itacarambiensis* Bastos-Pereira, Souza & Ferreira, 2017), pereopod 5 carpus with tiny lobe in proximal margin (*vs*. absent in all species), pereopod 6 carpus enlarged (*vs*. not enlarged in all species), and pereopod 6 propodus with longitudinal furrow (*vs*. absent in all species) (Campos-Filho et al. 2015, 2022b; Bastos-Pereira et al. 2017; Cardoso et al. 2020).

Natural history. *Xangoniscus chaimowiczi* sp. nov. inhabits various areas of the Gruta do Padre cave. Specimens were collected from the river, specifically within the conducto das plaquetas, where they were found living in the sediment. It also inhabits travertine ponds inside the cave. This latter behaviour has been documented in several other *Xangoniscus* species (Cardoso et al. 2020; Campos-Filho et al. 2022b).

Xangoniscus jonasi **López-Orozco, Bichuette & Campos-Filho, sp. nov.**

https://zoobank.org/018645EB-FC2A-4CEA-B1B3-9EC60D23F2BA
Figs 1, 7–10

Type material. ***Holotype*** • 1 male (parts in slides) (LES 0030097), Bahia, Carinhanha, Gruna da Serra Solta cave, -13.510573, -43.75207, 24 October 2023, leg. JE Gallão.

Description. Maximum body length: male 6.5 mm. Body outline as in Figs 7A, B, 8A. Colourless (Fig. 7). Dorsal surface smooth, with scattered fringed scale setae (Figs 7A–E, 8B). Cephalon (Figs 7C, D, 8C) with large quadrangular antennary lobes; vertex with slightly lateral depression to fit antennae when extended backwards, profrons with V-shaped suprantennal line, not surpassing antennule insertion; eyes absent. Pereonite 1 epimera with distal corners not developed frontwards, posterior corners right-angled; pereonites 2–7 epimera gradually directed backwards, posterior corners progressively more acute; pleon narrower than pereon; pleonites 3–5 epimera posterior point not developed (Figs 7A, B, D, 8A). Telson with concave sides and rounded apex (Fig. 8D). Antennula (Fig. 8E) composed of three articles, proximal and distal articles subequal in length, second article short, distal article with eight very long aesthetascs. Antenna (Fig. 8F) short, not surpassing pereonite 3 when extended backwards; flagellum of equal length of fifth article of peduncle, composed of three articles subequal

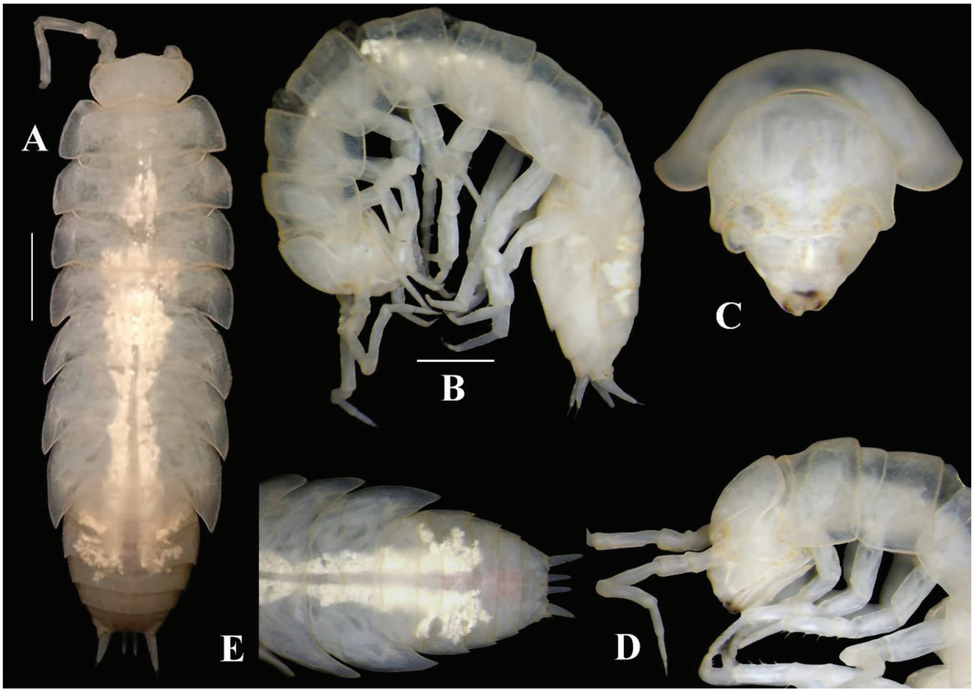

Figure 7. *Xangoniscus jonasi* López-Orozco, Bichuette & Campos-Filho, sp. nov. Male **A** habitus, dorsal view **B** habitus, lateral view **C** cephalon and pereonite 1, frontal view **D** cephalon and pereonites 1–2, lateral view **E** pereonite 7, pleon and telson, dorsal view. Scale bar: 1 mm.

in length. Left mandible (Fig. 8G) with two penicils; right mandible (Fig. 8H) with lacinia mobilis and one penicil. Maxillula (Fig. 8I) inner endite with three penicils; outer endite with 5+5 teeth, apically simple, and two plumose stalks. Maxilla (Fig. 8J) with setose and bilobate apex, outer lobe smaller. Maxilliped (Fig. 8K) basis enlarged on distal portion bearing fringe of fine setae; first article of palp with two tiny setae, distal articles with three tufts of setae; endite rectangular, outer and medial margins setose, apically with two triangular teeth and large rounded penicil. Uropod (Fig. 8D) branches short, endopod inserted proximally, exopod and endopod equal in length. Pereopods 1–7 (Fig. 9A–G) gradually elongated, with merus, carpus, and propodus bearing sparse setae; dactylus of one claw bearing many setae on inner and outer margins.

Male. Pereopod 1 (Fig. 9A) carpus with large antennal grooming brush. Pereopod 2–4 (Fig. 9B–D) merus with scales on sternal margin. Pereopods 5 and 7 (Fig. 9E, G) without distinct modifications. Pereopod 6 (Fig. 9F) carpus slightly grooved on sternal margin, propodus slightly enlarged on median portion. Genital papilla (Fig. 10B) lanceolate. Pleopod 1 (Fig. 10A) exopod trapezoidal, longer than wide, distal margin slightly convex, inner and outer margins bearing fringe of fine setae; endopod longer than exopod, with narrow basal article and flagelliform distal article; basipod distal margin elongate, longer than exopod, with semicircular apex, with fine and long setae.

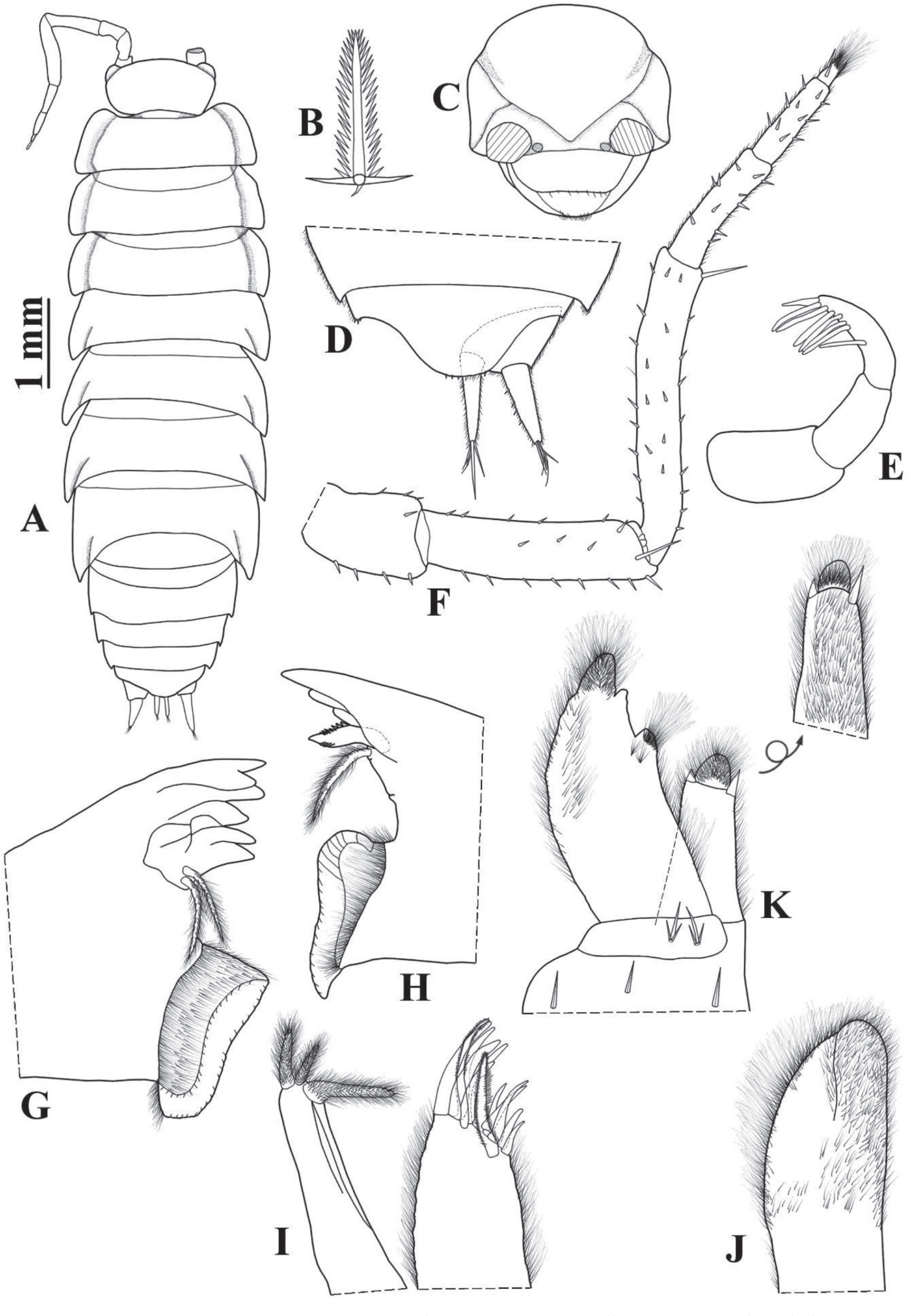

Figure 8. *Xangoniscus jonasi* López-Orozco, Bichuette & Campos-Filho, sp. nov. Male **A** habitus, dorsal view **B** dorsal scale-seta **C** cephalon, frontal view **D** telson and uropod **E** antennula **F** antenna **G** left mandible **H** right mandible **I** maxillula **J** maxilla **K** maxilliped, arrow illustrating the endite in caudal view.

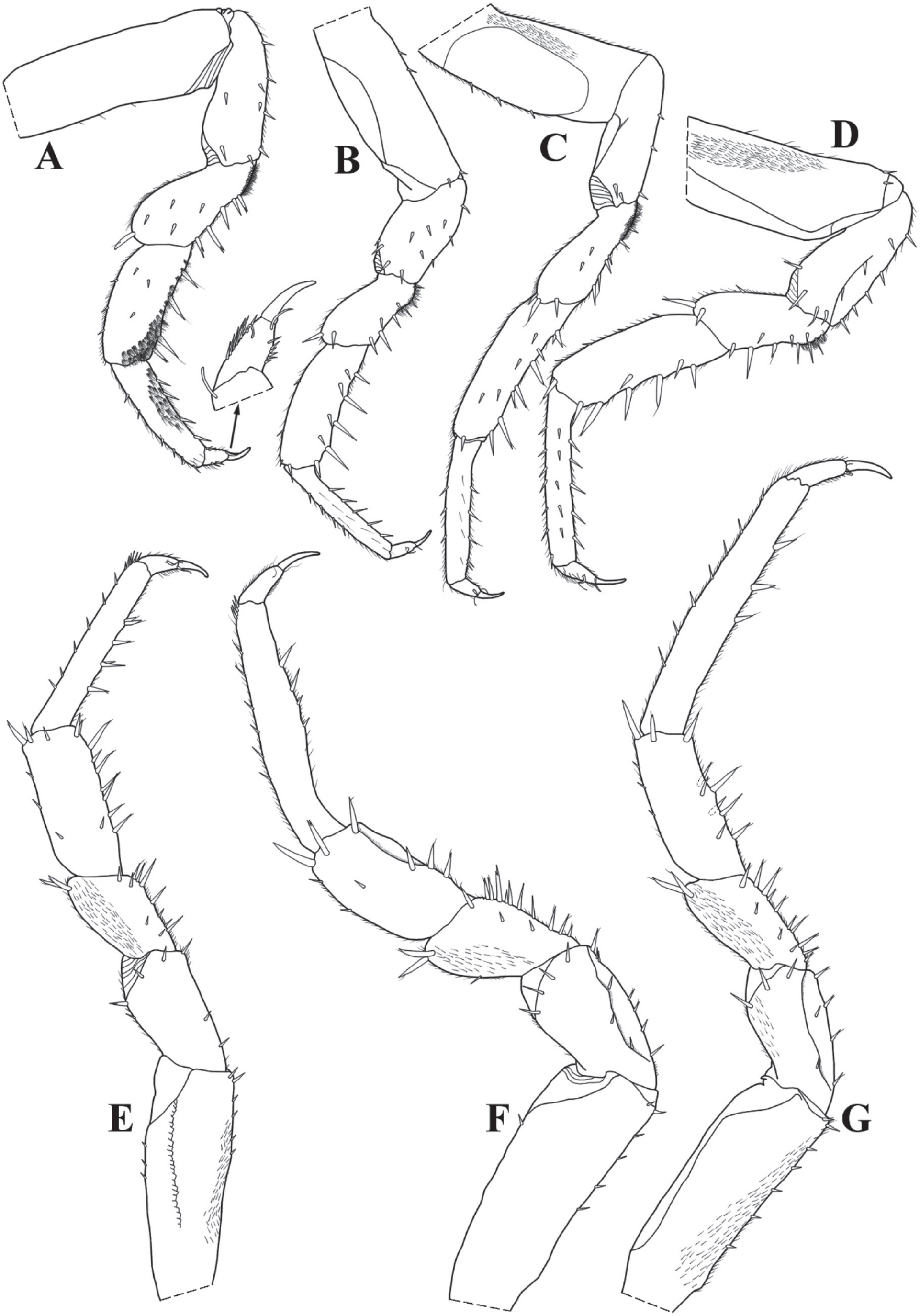

Figure 9. *Xangoniscus jonasi* López-Orozco, Bichuette & Campos-Filho, sp. nov. Male **A** pereopod 1 **B** pereopod 2 **C** pereopod 3 **D** pereopod 4 **E** pereopod 5 **F** pereopod 6 **G** pereopod 7.

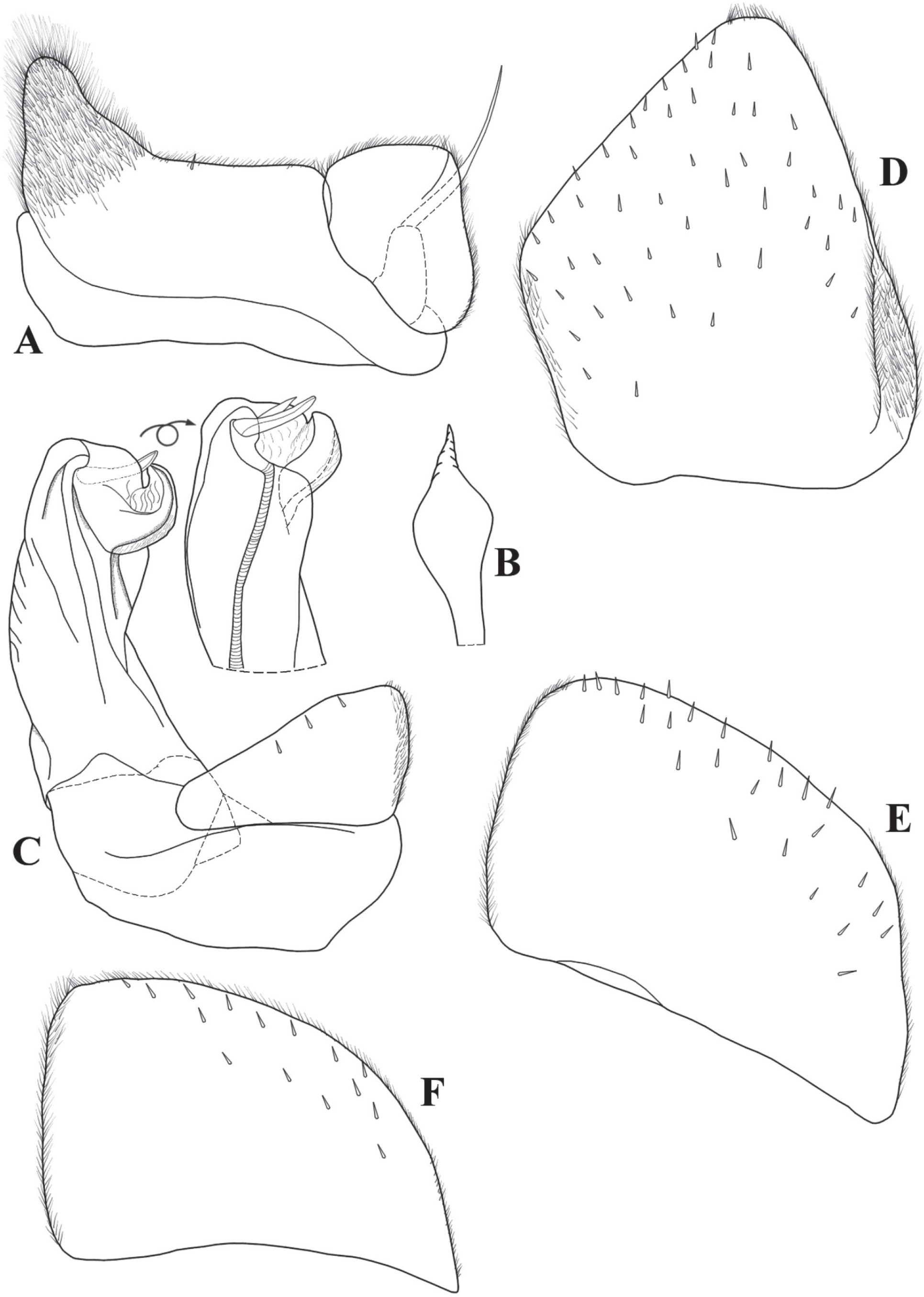

Figure 10. *Xangoniscus jonasi* López-Orozco, Bichuette & Campos-Filho, sp. nov. Male **A** pleopod 1 **B** genital papilla **C** pleopod 2 **D** pleopod 3 exopod **E** pleopod 4 exopod **F** pleopod 5 exopod.

Pleopod 2 (Fig. 10C) exopod trapezoidal, distal margin slightly convex bearing three setae; endopod of two articles, distal article about three times as long as proximal, with distal margin subquadrangular and apex rounded, with transversal process in V-like on apex in ventral view. Pleopod 3 (Fig. 10D) exopod triangular, longer than wide, covering pleopods 1 and 2, fringed with short setae. Pleopod 4 and 5 (Fig. 10E, F) exopods trapezoidal, wider than long, with margins bearing several short setae.

Etymology. The new species is named after Jonas Kahnwald, the protagonist of the "Dark" series, who explores caves to travel through time and space. The epithet "*jonasi*" is a tribute to the isolation and sense of separation from time and space that caves represent, evoking the central theme of "Dark". The species reflects extreme adaptation to dark depths, just as Jonas adapts to the complexities and paradoxes of time.

Distribution. Presently known only from Gruna da Serra Solta cave in the Serra do Ramalho karst area, state of Bahia, northeastern Brazil (Fig. 1).

Remarks. *Xangoniscus jonasi* sp. nov. differs from all other species of the genus in having the antennula with eight long aesthetascs (*vs*. two in *X. aganju*, *X. lapaensis*, and *X. odara*, three in *X. ibiracatuensis*, *X. lundi* and *X. santinhoi*, four in *X. dagua*, four or five in *X. ceci*, five in *X. loboi*, six in *X. itacarambiensis*, twelve in *X. chaimowiczi* sp. nov.), pleopod 1 basipod distal margin semicircular apex, longer than exopod (*vs*. simples and shorter than exopod in *X. lundi*, *X. dagua*, *X. ceci*, *X. ibiracatuensis*, *X. itacarambiensis*, *X. loboi*, *X. odara*, *X. santinhoi*, acute and longer than exopod in *X. chaimowiczi* sp. nov., *X. lapaensis* and *X. aganju*).

Natural history. The species showed low abundance (only one individual caught along the cave). The only individual of *X. jonasi* sp. nov. captured was found in the aphotic zone on a very wet, muddy substrate, under the cracks formed by the stream current, just a few meters from a pond (Fig. 1C).

Xangoniscus antiquus **López-Orozco, Carpio-Díaz & Campos-Filho, sp. nov.**

https://zoobank.org/A6A94235-318F-4C1B-9429-3B16B83EF0AB
Figs 1, 11–14

Type material. ***Holotype*** • 1 male (MZUSP 29513), Bahia, Canápolis, Gruta da Represa cave, -13.08105, -44.145703, February 1986, leg. A. Auler. ***Paratypes*** • 1 male (parts in slides) (MZUSP 29513), same data as holotype.

Description. Maximum body length: male 7.8 mm. Body outline as in Figs 11A, B, 12A. Colourless (Fig. 11). Dorsal surface smooth, with scattered fringed scale setae (Figs 11, 12B). Cephalon (Figs 11C, 12C) with large quadrangular antennary lobes; vertex with slightly lateral depression to fit antennae when extended backwards, profrons with V-shaped suprantennal line, not surpassing antennule insertion; eyes absent. Pereonite 1 epimera with distal corners developed frontwards, posterior corners right-angled; pereonites 2–7 epimera gradually directed backwards, posterior corners

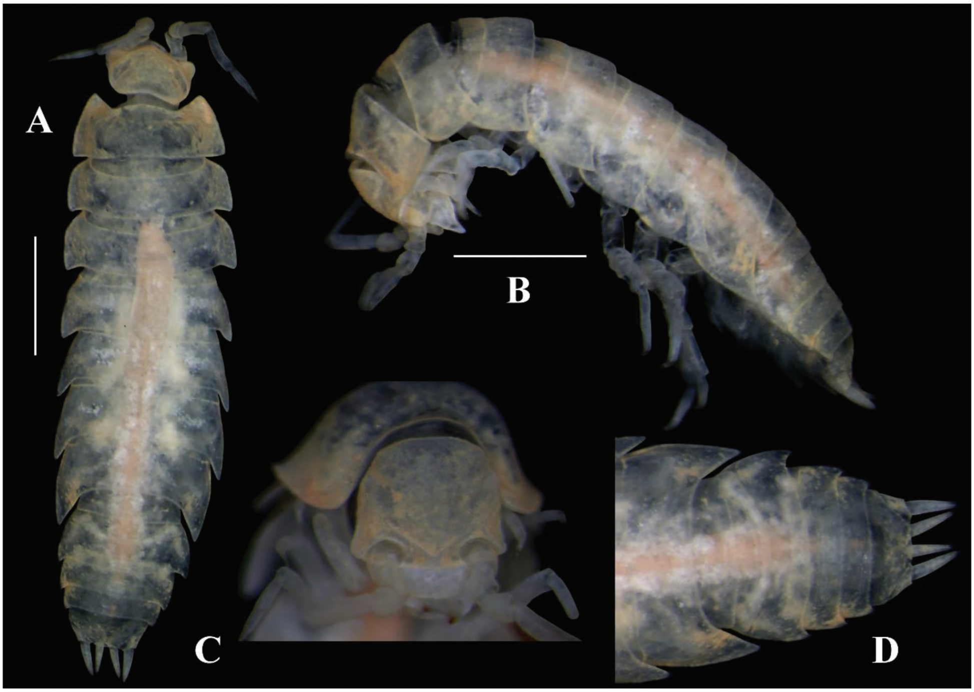

Figure 11. *Xangoniscus antiquus* López-Orozco, Carpio-Díaz & Campos-Filho, sp. nov. Male **A** habitus, dorsal view **B** habitus, lateral view **C** cephalon and pereonite 1, frontal view **D** pereonite 7, pleon and telson, dorsal view. Scale bar: 1 mm.

progressively more acute; pleon narrower than pereon; pleonites 3–5 epimera posterior point developed (Figs 11A, B, 12A). Telson with concave sides and an almost straight apex (Fig. 12D). Antennula (Fig. 12E) composed of three articles, proximal and distal articles subequal in length, second article short, distal article with eight very long aesthetascs. Antenna (Fig. 12F) short, not surpassing pereonite 2 when extended backwards; flagellum shorter than of fifth article of peduncle, composed of three articles subequal in length. Left mandible (Fig. 12G) with two penicils; right mandible (Fig. 12H) with lacinia mobilis and one penicil. Maxillula (Fig. 12I) inner endite with three penicils; outer endite with 5+5 teeth, apically simple, and two plumose stalks. Maxilla (Fig. 12J) with setose and bilobate apex, outer lobe smaller. Maxilliped (Fig. 12K) basis enlarged on distal portion bearing fringe of fine setae; first article of palp with two tiny setae, distal articles with three tufts of setae; endite rectangular, outer and medial margins setose, apically with two triangular teeth and large rounded penicil. Uropod (Fig. 12D) branches short and inserted at same level, exopod slightly longer than endopod. Pereopods 1–7 (Fig. 13A–G) gradually elongated, with merus, carpus, and propodus bearing sparse setae; dactylus of one claw bearing many setae on outer margin.

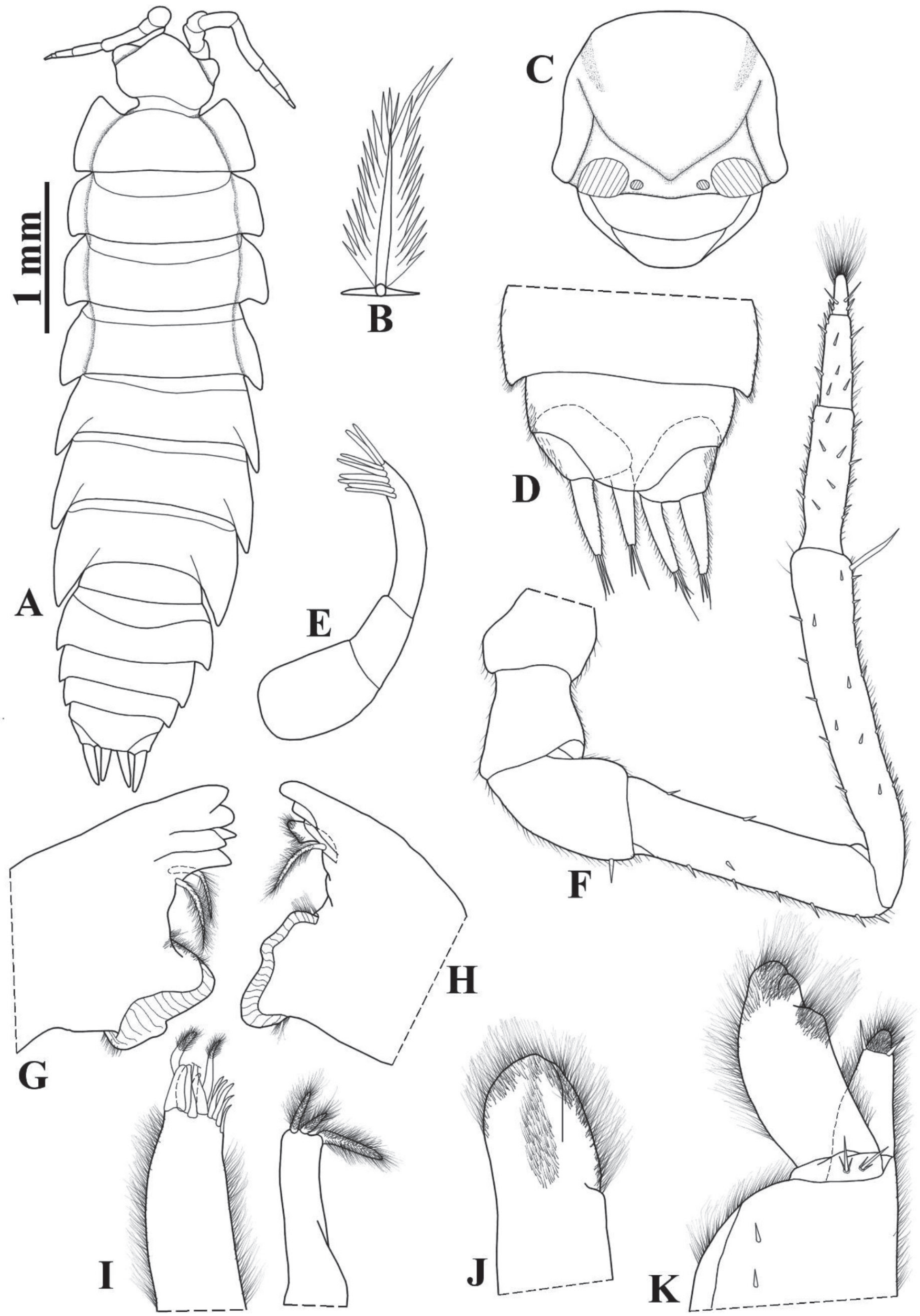

Figure 12. *Xangoniscus antiquus* López-Orozco, Carpio-Díaz & Campos-Filho, sp. nov. Male **A** habitus, dorsal view **B** dorsal scale-seta **C** cephalon, frontal view **D** telson and uropod **E** antennula **F** antenna **G** left mandible **H** right mandible **I** maxillula **J** maxilla **K** maxilliped, arrow illustrating the endite in caudal view.

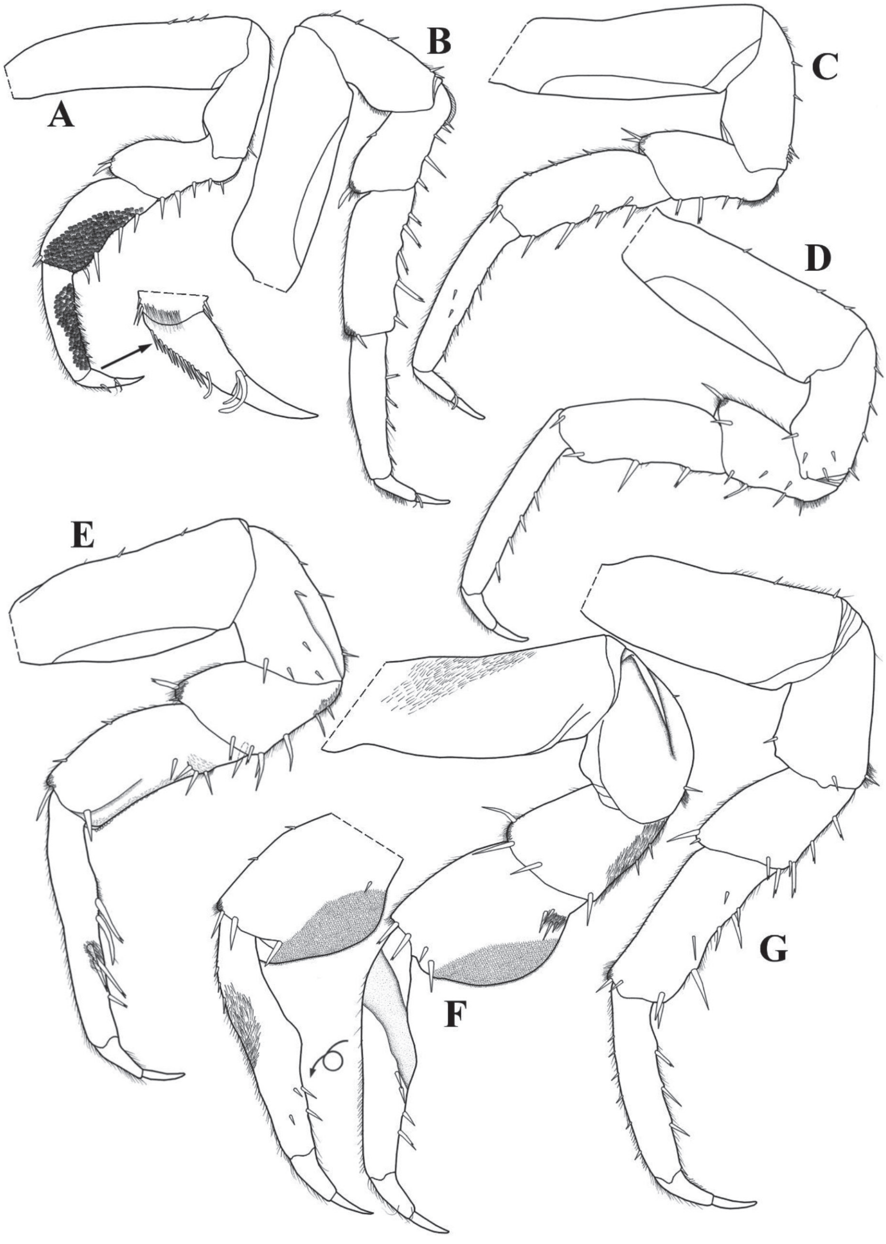

Figure 13. *Xangoniscus antiquus* López-Orozco, Carpio-Díaz & Campos-Filho, sp. nov. Male **A** pereopod 1 **B** pereopod 2 **C** pereopod 3 **D** pereopod 4 **E** pereopod 5 **F** pereopod 6 **G** pereopod 7.

Male. Pereopods 1–6 (Fig. 5A–F) merus with proximal portion bearing fringed scales and thin setae on sternal margin. Pereopod 1 (Fig. 13A) carpus with large antennal grooming brush. Pereopod 5 (Fig. 13E) carpus with small lobe on proximal sternal margin. Pereopod 6 (Fig. 13F) ischium enlarged with flattened sternal part;

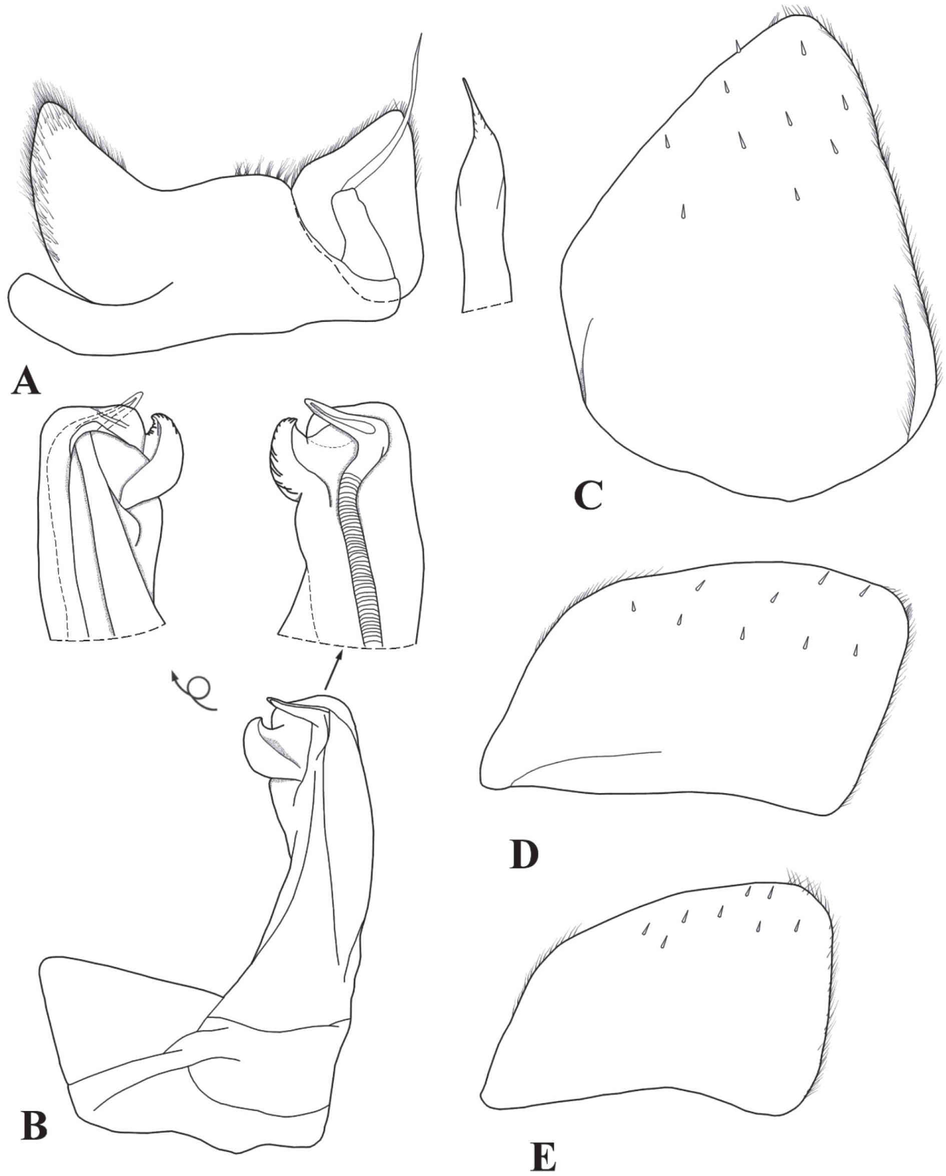

Figure 14. *Xangoniscus antiquus* López-Orozco, Carpio-Díaz & Campos-Filho, sp. nov. Male **A** pleopod 1 **B** genital papilla **C** pleopod 2 **D** pleopod 3 exopod **E** pleopod 4 exopod **F** pleopod 5 exopod.

carpus enlarged, rostral portion bearing dense field of tiny lobules; propodus with central part enlarged, with longitudinal furrow in ventral view, and field of short setae on dorsal part. Pereopod 7 (Fig. 13G) without distinct modifications. Genital papilla (Fig. 14A) lanceolate. Pleopod 1 (Fig. 14A) exopod subtriangular, longer than wide, inner and outer margins bearing fringe of fine setae; endopod longer than exopod, with narrow basal article and flagelliform distal article; basipod distal margin elongate and semicircular apex, slightly longer than exopod, with fine and long setae. Pleopod 2 (Fig. 14B) exopod trapezoidal, distal margin almost straight; endopod of two articles, distal article about three times as long as proximal, with distal margin subquadrangular and apex rounded, with transversal process in V-like on apex in ventral view. Pleopod 3 (Fig. 14C) exopod triangular, longer than wide, covering pleopods 1 and 2, fringed with short setae. Pleopod 4 and 5 (Fig. 14D, E) exopods trapezoidal, wider than long, with margins bearing several short setae.

Etymology. Latin, *antiquus* = old. The new species name refers to the long period of time that the samples remained preserved until their description. Perhaps this material represents the first specimens of *Xangoniscus* collected in Brazil.

Distribution. Presently known only from Gruta da Represa cave, in the state of Bahia, northeastern Brazil (Fig. 1).

Remarks. *Xangoniscus antiquus* sp. nov. resembles *X. chaimowiczi* sp. nov. in the modifications on male pereopods and number of articles on flagellum of antenna; however, it differs in the number of aesthetascs on antennula (eight *vs.* twelve in *X. chaimowiczi* sp. nov.), telson apex (almost straight *vs.* rounded apex in *X. chaimowiczi* sp. nov.), and basipod distal margin (semicircular apex, slightly longer than exopod *vs.* acute apex, longer than exopod in *X. chaimowiczi* sp. nov.).

Discussion

The genus *Xangoniscus* was erected by Campos-Filho et al. (2014) to include the troglobitic amphibious species *X. aganju* from Gruna do Mandiaçu cave, Carinhanha, state of Bahia. The following characters were proposed as diagnostic for the genus: pleonites 3–5 with well-developed epimera, with visible posterior points; antennule with long apical aesthetascs; antenna with flagellum of three articles; male pleopod 1 exopod shorter than endopod. Subsequent studies, including the species described here (see also Campos-Filho et al. 2015, 2022b; Bastos-Pereira et al. 2017; Cardoso et al. 2020), *X. antiquus* sp. nov., *X. ceci*, *X. dagua*, *X. itacarambiensis*, *X. ibiracatuensis*, *X. jonasi* sp. nov., *X. loboi*, *X. lundi*, *X. odara*, *X. santinhoi*, and *X. chaimowiczi* sp. nov. do not present the epimera of pleonites 3–5 developed; the aesthetascs of the distal article of the antennula are short in *X. dagua*, *X. ceci*, *X. itacarambiensis*, and *X. lundi*; and the number of articles in the flagellum of the antenna varies between four and six across different species. In addition, the description of *X. ceci* mentioned that the male pleopod 1 endopod is equal in length to the exopod; the illustrations showed that the endopod is shorter than the exopod (see fig. 10E in Cardoso et al. 2020). Considering the above, the mentioned characters must be modified as follows: pleonites 3–5 epimera well developed or short with small posterior points directed backward; antennule with long or short apical aesthetascs; antenna with flagellum of three to six articles; male pleopod I exopod longer or shorter than endopod. To date, two morphological characteristics can be defined as synapomorphies of *Xangoniscus*: cephalon with a transversal groove on the vertex along the frontal margin and the complex apex of the male pleopod 2 endopod (Campos-Filho et al. 2014). Moreover, a taxonomically important characteristic is the shape and size of the distal margin of the basipodite of male pleopod 1, which allows differentiation between some species.

Species of the genus *Xangoniscus* have been recorded in travertine pools and streams formed by infiltration of water, inhabiting microhabitats composed of rocky substrates, silty sediment, sand, and decomposing plant material (Campos-Filho et al. 2015, 2022b; Bastos-Pereira et al. 2017; Cardoso et al. 2020). *Xangoniscus chaimowiczi* sp. nov. and *Xangoniscus jonasi* sp. nov. follow this pattern and occupy similar microhabitats in the caves they inhabit. However, the specific microhabitat of *Xangoniscus antiquus* sp. nov. in Gruta da Represa cave remains unknown. Therefore, further research into these subterranean environments is needed to better understand the ecology and habits of the species that live there.

Regarding the current distribution of the genus *Xangoniscus*, it is restricted to limestone caves of the Bambui Geomorphological Group located within semiarid environments of the Chacoan subregion, in the biogeographic provinces of Cerrado, Caatinga, and Southern Espinhaco (sensu Morrone et al. 2014, 2022), in the states of Bahia and Minas Gerais (Fig. 1). This distribution suggests that in caves with water or tributaries of the São Francisco River basin within this geomorphological group, there is a high potential for the occurrence of species of this genus. However, macroecological studies are needed to elucidate the geographic patterns of this genus, along with increased sampling efforts in other caves in the area. The records of *X. antiquus* sp. nov. and *X. chaimowiczi* sp. nov. extend the known distribution of this genus to the northern part of this geological formation. To date, only *X. aganju* has been reported from multiple caves (Fig. 1); however, as noted by Campos-Filho et al. (2019), molecular studies could uncover potential cryptic diversity within the species.

Among the three species described here, *Xangoniscus* now includes 13 troglobitic amphibious species, making it the second most species-rich genus of Styloniscidae in Brazil. Among these species, only five are found in national conservation units in Minas Gerais (*X. dagua*, *X. itacarambiensis*, *X. lundi*, *X. odara*, and *X. santinhoi*) (Fig. 1); nevertheless, they are threatened by uncontrolled tourism and the expansion of agricultural activities (Gallão and Bichuette 2018). Special attention should be directed toward the remaining species, particularly in the state of Bahia, where there are no conservation areas dedicated to the protection and management of speleological resources in the studied caves. Areas such as Serra do Ramalho, which is considered a hotspot of subterranean biodiversity (Trajano et al. 2016), should be prioritised for proposals aimed at creating new legally protected areas. Several caves are affected by water extraction, a situation that has been documented in various studies (Gallão and Bichuette 2018; Cardoso et al. 2022), including at Gruna da Serra Solta (Fig. 2C). This activity could negatively affect populations of species with amphibious habits. Regarding the Gruta do Padre cave, Auler and Rubbioli (2019) noted that it has a good conservation status. Since the Tatus II project, an experiment conducted in 1987 where a group of speleologists stayed for 21 days inside the Gruta do Padre cave, the cave has only been sporadically visited by researchers, and its resources remain unexploited (Chaimowicz 1987). With the description of *X. chaimowiczi* sp. nov., we increased the number of troglobitic species that inhabit Gruta do Padre cave to six, the second species of Oniscidea described from this locality: *Chaimowiczia tatus* Cardoso, Bastos-Pereira, Souza & Ferreira, 2021 (Oniscidea), *Coarazuphium tessai* (Godoy & Vanin, 1990) (Coleoptera), *Spelaeogammarus santanensis* Koenemann & Holsinger, 2000 (Amphipoda), *Phaneromerium cavernicolum* Golovatch & Wytwer, 2004 (Polydesmida) (Godoy and Vanin 1990; Koenemann and Holsinger 2000; Golovatch and Wytwer 2004; Cardoso et al. 2021).

Acknowledgements

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Remarkably low genetic diversity in the widespread cave spider *Phanetta subterranea* (Araneae, Linyphiidae)

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Abstract

Most cave-obligate species (troglobionts) have small ranges due to limited dispersal ability and the isolated nature of cave habitats. The troglobiontic linyphiid spider *Phanetta subterranea* (Emerton, 1875), the only member of its genus, is a notable exception to this pattern; it has been reported from more counties and caves than any other troglobiont in North America. As many troglobionts exhibit significant genetic differentiation between populations over even small geographic distances, it has been hypothesized that *Phanetta* may comprise multiple, genetically distinct lineages. To test this hypothesis, we examined genetic diversity in *Phanetta* across its range at the mitochondrial cytochrome c oxidase subunit I gene for 47 individuals from 40 caves, distributed across seven states and 37 counties. We found limited genetic differentiation across the species' range with haplotypes shared by individuals collected up to 600 km apart. Intraspecific nucleotide diversity was 0.006 +/- 0.005 (mean +/- SD), and the maximum genetic p-distance observed between any two individuals was 0.022. These values are within the typical range observed for other spider species. Thus, we found no evidence of cryptic genetic diversity in *Phanetta*. Our observation of low genetic diversity across such a broad distribution raises the question of how these troglobiontic spiders have managed to disperse so widely.

Keywords
Appalachians, genetic diversity, Interior Low Plateau, Linyphiidae, *Phanetta subterranea*

Introduction

Caves are populated by a diverse community of organisms, with more than 1,300 cave-obligate species (i.e., troglobionts) known from the United States alone (Niemiller et al. 2019). Because caves provide 'islands' of habitat for cave-limited species, and because troglobionts typically have limited ability to disperse through surface habitats, most troglobionts have small, restricted distributions, and many are restricted to a single or few geographically clustered cave systems. For example, 31% (218/710) of troglobionts in the Appalachians and Interior Low Plateau karst regions in the eastern United States are known from a single cave, with many other species limited to just a handful of nearby caves (Christman et al. 2016). Only a select few species have even moderately broad ranges, with just nine troglobionts (three arachnids, three hexapods, and three crustaceans) reported from more than 30 counties (Christman and Culver 2001).

Spiders are a significant component of cave biodiversity, with more than 100 troglobiontic spiders known from the United States (Niemiller et al. 2019) and ~1,000 troglobiont spiders described worldwide (Mammola et al. 2017). The best studied cave spiders in the eastern United States are from the genus *Nesticus*, which has diversified into three dozen cave and surface species across the southern Appalachians (Hedin and Milne 2023). As is often the case for troglobionts, cave-limited *Nesticus* species are characterized by small ranges (three species are known from just a single cave, and many others from just a handful of caves) (Hedin and Milne 2023). In cases where a *Nesticus* species is known from multiple caves, they often exhibit high genetic divergence between caves, even over short distances (Hedin 1997; Snowman et al. 2010; Balogh et al. 2020; Zigler and Milne 2022; Hedin and Milne 2023).

The linyphiid spider *Phanetta subterranea* (Emerton, 1875) (Fig. 1), the only member of its genus, is a small (1.5–2 mm in total length) troglobiont. They are found in multiple cave habitats, from near entrances to deep cave zones, and are often quite common (Poulson, 1977, 1981). They are thought to feed on springtails (Poulson, 1977, 1981). *Phanetta* exhibit variation in the degree of eye formation; most individuals have eyes, but in some cases eyes are nearly absent (Millidge, 1984). *Phanetta* can grow from hatching to full size in about four months and have a lifespan of about one year (Poulson 1981). Clutch size ranges from three to 16 eggs that are ~0.6 mm in diameter, and a single spider can lay multiple clutches within a year (Poulson 1975). Its range extends across two karst regions (the Interior Low Plateau and the Appalachians (Niemiller et al. 2019)) spanning a dozen states, and the species is known from more counties and caves

Figure 1. Subterranean Sheetweb Spider (*Phanetta subterranea*). Photo by Matthew L. Niemiller**.**

than any other troglobiont in North America (Christman and Culver 2001; Niemiller et al. 2013; Christman et al. 2016). Although widespread and common in caves of the eastern United States, *Phanetta* has never been reported from surface habitats.

Despite its remarkably broad range, nothing is known about genetic diversity in this species. It has been suggested that modern taxonomic study would result in the splitting of *Phanetta* into multiple species (Christman and Culver 2001). This scenario was observed in the Southern Cavefish (*Typhlichthys subterraneus*) species complex, which is known from the southern Interior Low Plateau, southern Appalachians, and Ozarks karst regions. Genetic analysis of *T. subterraneus* revealed nine genetically distinct lineages, including the identification of *T. eigenmanni* as a distinct species, and efforts to delineate and describe other lineages as distinct species are underway (Niemiller et al. 2012; Niemiller et al. 2013; Hart et al. 2023). Similar results have been reported for various troglobionts in other parts of the world (e.g., Lefébure et al. 2006; Zhang and Li 2014), including the cave beetle *Darlingtonea kentuckensis* from eastern Kentucky (Boyd et al. 2020).

In this study we investigated potential cryptic diversity in *Phanetta* across its broad distribution through genetic analysis of the mitochondrial cytochrome c oxidase subunit I gene (*COI*), a marker commonly employed in the study of genetic diversity in invertebrates. We sought to estimate genetic diversity and explore genetic structure within this spider while addressing the question of whether *Phanetta* represents a complex of morphologically similar but genetically distinct lineages, or a single genetic lineage connected through gene flow over broader spatial scales.

Methods

Geographic analysis

We surveyed the literature to compile a list of all known *Phanetta subterranea* occurrences. Resources consulted included Culver et al. (2000), Christman et al. (2016), and Zigler et al. (2020), as well as unpublished records from various cave biologists. We mapped the range of *Phanetta*, and our sampling sites (Fig. 2), using ArcGIS Online (https://www.arcgis.com/index.html). We calculated the range extent/extent of occurrence (EOO) for *Phanetta* using GeoCAT (https://geocat.iucnredlist.org/editor). Range extent/EOO is the area of a minimum convex polygon which contains all the sites of occurrence (Bachman et al. 2011).

Sampling

Phanetta were collected by hand between 1998–2023 from 40 caves in 37 counties across seven states (Alabama, Georgia, Illinois, Indiana, Kentucky, Tennessee, and Virginia) (Table 1) and two karst regions (the Interior Low Plateau and the Appalachians)

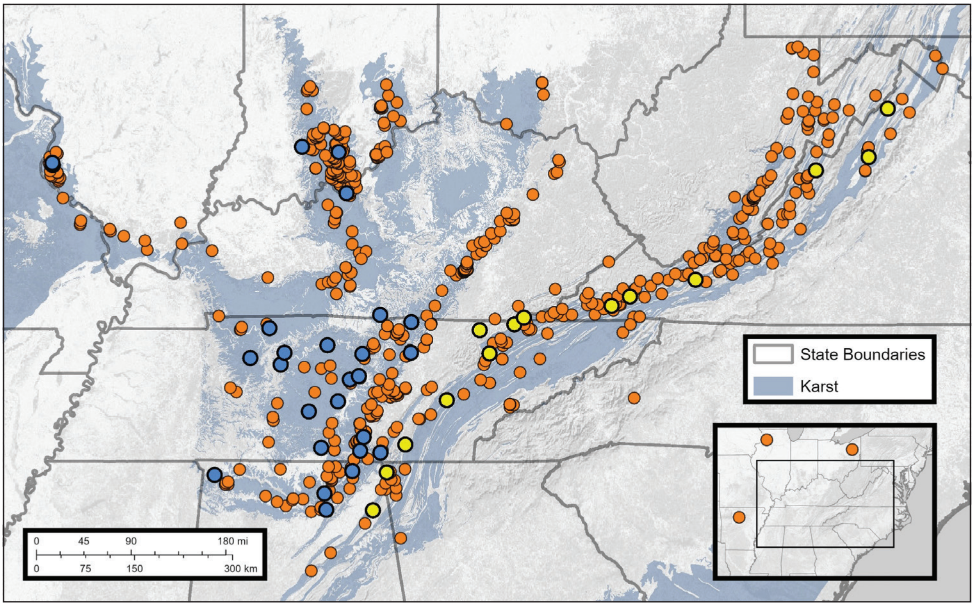

Figure 2. Range and sampling map. The distribution of *Phanetta subterranea* in the eastern United States. State boundaries are indicated by grey lines and karst terrain as blue-grey shading. Sites where *Phanetta* has been reported are indicated by orange points. Sites sampled in this study in the Interior Low Plateau karst region are indicated by blue points, and sites sampled in the Appalachians karst region are indicated by yellow points. This map includes ~600 georeferenced *Phanetta* sites. The inset indicates the extent of the main map, and includes three additional *Phanetta* sites (one in northeast Ohio, one in northwest Illinois, and one in central Arkansas), each more than 200 km from any other known *Phanetta* site, that are not visible on the main map.

(Fig. 2). We aimed to sample as broadly as possible, so generally limited our sampling to one cave per county. Specimens were preserved in 95% ethanol and stored at -20 °C until DNA extraction. Individuals were identified to species under the microscope; mature *Phanetta* females are easily identified by their distinctive epigynum (Emerton, 1875). In most cases, one spider per cave was sequenced; however, we sequenced two spiders from seven different caves. Collections were permitted by a variety of agencies (see Acknowledgements). Voucher specimens from this study are accessioned at the Auburn University Museum of Natural History.

Table 1. Sample sites for *Phanetta subterranea*.

State	County	Cave
Alabama	Colbert	Georgetown Cave
Alabama	DeKalb	Manitou Cave
Alabama	Jackson	Pseudo Lava Cave B
Alabama	Madison	Hering Cave
Alabama	Marshall	MacHardin Cave
Georgia	Dade	Howards Waterfall Cave
Illinois	Monroe	Danes Cave
Illinois	Monroe	Icebox Cave
Indiana	Dubois	Vowell Cave
Indiana	Harrison	Big Mouth Cave
Indiana	Washington	Twin Oaks Pit
Kentucky	Monroe	cave near Hestand, KY
Tennessee	Bedford	Fountain Cave
Tennessee	Campbell	New Mammoth Cave
Tennessee	Campbell	Norris Dam Cave
Tennessee	Cannon	Sycamore Creek
Tennessee	Claiborne	Obie Mill Cave
Tennessee	Coffee	Jernigan Cave
Tennessee	Davidson	Bull Run Cave
Tennessee	Davidson	Newsom Branch Cave
Tennessee	DeKalb	Indian Grave Point Cave
Tennessee	Dickson	Sinuous Stream Cave
Tennessee	Franklin	Tom Pack Cave
Tennessee	Grundy	Crystal Cave
Tennessee	Hamilton	Levi Cave
Tennessee	Lincoln	Kelso Saltpeter Cave
Tennessee	Marion	Pryor Cave Spring
Tennessee	Meigs	Sensabaugh Cave
Tennessee	Montgomery	Durham Cave
Tennessee	Overton	Mill Hollow Cave
Tennessee	Pickett	Frog Cave
Tennessee	Smith	New Salem Cave No. 1
Tennessee	Wilson	Spring Cave
Virginia	Bland	Repass Saltpeter Cave
Virginia	Highland	Five Springs Cave
Virginia	Lee	Grassy Springs Cave
Virginia	Rockingham	Massanutten Cave
Virginia	Russell	Bundys Cave No. 2
Virginia	Scott	Jesse Branch Cave
Virginia	Shenandoah	Flemmings Cave

Molecular techniques

We extracted DNA from specimens using the DNeasy Blood and Tissue Kit (Qiagen; Cat. No. 69504). We followed the manufacturer's protocol for extractions from whole or partial spiders. Polymerase chain reactions (PCRs) were prepared using the DNA extractions as template, GoTaq G2 Green Master Mix (Promega; Cat. No. M7822), dH_2O, and primers. Two different primer sets were employed to amplify a 651 base pair fragment of the mitochondrial *COI* locus. We initially used the primers HCO2198+M13F and LCO1490+M13R (modified from Folmer et al. (1994)), but we subsequently developed primers (PsHCO+M13F and PsLCO+M13R) that were more effective for amplifying *Phanetta* (Table 2). The PCR protocol was initial denaturation for 5 minutes at 95 °C, then 35 cycles of 15 seconds of denaturation at 95 °C, 30 seconds of primer annealing at 45 °C, and 60 seconds of extension at 72 °C. PCR products were visualized on 1% agarose gels. Successful PCRs were prepared for sequencing by treatment with Antarctic Phosphatase (New England Biolabs, Cat. No. M0289) and Exonuclease I (New England Biolabs, Cat. No. M0293). Samples were then sequenced on both strands using M13F and M13R primers on an Applied Biosystems 3730×l DNA Analyzer at the Keck DNA Sequencing Core of the Yale University School of Medicine (New Haven, CT).

Genetic analysis

We trimmed, assembled, edited, and aligned *COI* sequences using Geneious Prime (v. 2022.1.1). All sequences were submitted to GenBank (accession nos. PP815877–PP815923). We used MEGA11 (Tamura et al. 2021) to calculate genetic distances between sequences. P-distance, the genetic distance measure used here, is the proportion of nucleotides that differ between any two sequences. We used POPART (Leigh and Bryant 2015) to build a median joining tree (Bandelt et al. 1999) from the *COI* sequences. We looked for a pattern of isolation by distance by comparing linear geographic distance between sites and *COI* p-distance between individuals from those sites.

Results

Phanetta is known from 669 caves across 12 states and 155 counties (Fig. 2). When calculating the species range extent, we excluded three sites (one in northeast Ohio, one in northwest Illinois, and one in central Arkansas) because each was more than 200 km

Table 2. Primer names and sequences. Primers used to amplify a 651 bp fragment of the mitochondrial cytochrome oxidase I gene in *Phanetta subterranea*.

Primer name	Sequence (5'-3')	Reference
HCO2198+M13F	TGTAAAACGACGGCCAGTCGGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
LCO1490+M13R	CAGGAAACAGCTATGACCTAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
PsHCO+M13F	GTAAAACGACGGCCAGTACAAATCATAAAGATATTGGAAGTTTG	This study
PsLCO+M13R	CAGGAAACAGCTATGACCTTCAGGGTGACCAAAAAATCAAAATAA	This study

from any other known *Phanetta* site, raising the possibility of identification errors, or vagrancy. Even after excluding those sites, the species' EOO was 412,223 km^2 (Table 3).

We sequenced 47 *Phanetta* individuals from 40 caves across seven states and 37 counties (Fig. 2, Table 1). Full-length (651 bp) sequences were obtained from all individuals, and no indels or stop codons were observed. Genetic distances between *Phanetta* samples were low. Nucleotide diversity (π) in *Phanetta* was 0.006 ± 0.005 (mean ± SD), with a minimum pairwise p-distance of 0.000 and a maximum pairwise p-distance of 0.022 (Table 3). Twenty-one haplotypes were observed, and seven of these were shared, ranging in frequency from two to 14 individuals. The most common haplotype was present in *Phanetta* from Indiana, Illinois, Tennessee, and Virginia.

In seven cases, we sampled two individuals from the same cave. In six of those cases, the two individuals had identical *COI* sequences, and in the seventh case there was a single nucleotide difference between the two individual sequences. We found a positive correlation between the genetic distance between *Phanetta* individuals and the linear geographic distance between their sample sites (d.f. = 779, $R^2 = 0.32$, F = 373.7, significance $F < 0.0001$), indicating a pattern of isolation by distance, although the correlation was not particularly strong, and identical haplotypes were identified from sites as far as 600 km apart.

As one of the few troglobionts that is widespread across two major karst regions – the Appalachians and the Interior Low Plateau (Niemiller et al. 2019) (Fig. 2, Table 3) – *Phanetta* provided an opportunity to explore the effect of differing geologic history on genetic diversity within a single species. Haplotype diversity (*h*) was similar for the two karst regions ($h_{\text{Appalachians}} = 0.892$, $h_{\text{Interior Low Plateau}} = 0.841$) (Table 3). However, nucleotide diversity in *Phanetta* from the Appalachians ($\pi_{\text{Appalachians}} = 0.009 \pm 0.006$) was greater than in *Phanetta* from the Interior Low Plateau ($\pi_{\text{Interior Low Plateau}} = 0.003 \pm 0.004$) (Table 3). This pattern can be visualized in the haplotype network (Fig. 3) where haplotypes from the Interior Low Plateau are quite similar, mostly differing by just one or a handful of nucleotide differences. In contrast, haplotypes from the Appalachians typically differed from one another by multiple nucleotide differences (Fig. 3). Only one haplotype was shared by individuals from the Interior Low Plateau and the Ap-

Table 3. Distribution of and genetic diversity in *Phanetta* across karst regions. Range extent of *Phanetta* in the Interior Low Plateau and the Appalachians karst regions, and combined across the two regions, calculated as extent of occupancy (EOO). Measures of genetic diversity were calculated from all pairwise comparisons between individuals within the specified region. Based on cytochrome oxidase I sequences.

	Karst region		Combined
	Interior Low Plateau	Appalachians	
Range extent (EOO)	214,418 km^2	140,669 km^2	412,223 km^2
# of georeferenced sites	392	206	598
# of individuals sequenced	31	16	47
# of haplotypes	13	9	21
Haplotype diversity (*h*)	0.841	0.892	0.878
# of segregating sites	17	20	32
Nucleotide diversity (π) (+/- SD)	0.003 (+/- 0.004)	0.009 (+/- 0.006)	0.006 (+/- 0.005)
Maximum pairwise p-distance	0.015	0.020	0.022

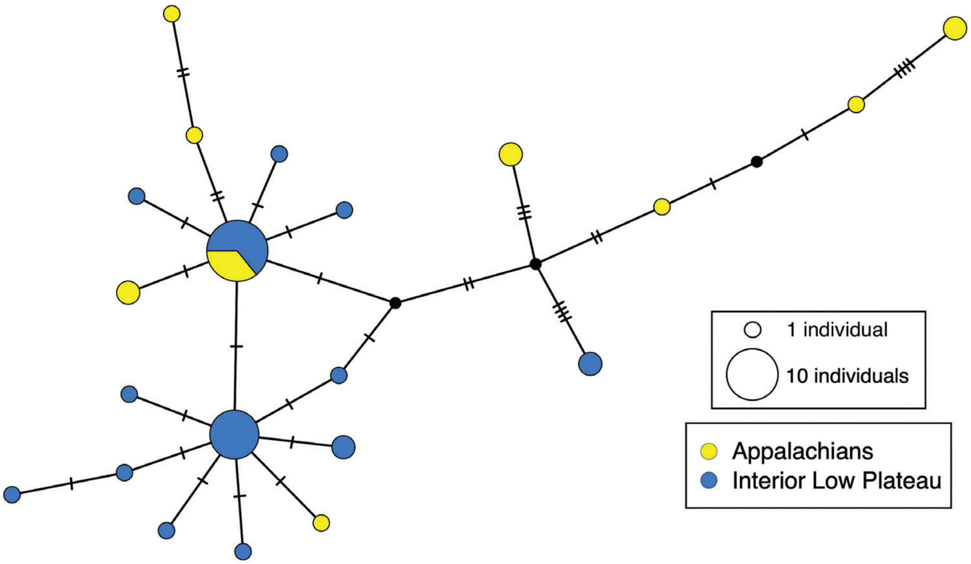

Figure 3. Median joining haplotype network for all *Phanetta* sequences. Haplotypes are indicated by circles and nucleotide differences between haplotypes are indicated by hash marks. Haplotypes are colored by karst region of origin as in Figure 2. Circle size indicates the number of individuals sharing a haplotype. The multicolored circle indicates the single haplotype shared by individuals from the Interior Low Plateau and individuals from the Appalachians.

palachians (Fig. 3). The higher genetic divergence observed in the Appalachians may be related to the great linear distance (~850 km) across which samples were collected (Fig. 2), although *Phanetta* does range across a greater area in the Interior Low Plateau (Table 3).

Overall, we observed remarkably low genetic variation across the broad range of *Phanetta*, with individuals from the Interior Low Plateau being particularly genetically uniform. *Phanetta* from the Appalachians exhibited slight genetic divergence from those from the Interior Low Plateau, and were also relatively more divergent from each other, but the overall genetic distance between any two *Phanetta* individuals was low. There was no evidence of cryptic genetic diversity within *Phanetta*.

Discussion

Phanetta subterranea is known from more caves and more counties than any other North American troglobiont. We aimed to determine whether *Phanetta* comprised a complex of genetically distinct lineages, or if it was genetically uniform across its range. After sampling 47 *Phanetta* individuals from 37 counties across seven states in the eastern United States, we found no evidence of cryptic genetic diversity. Genetic distances between sites were low, and haplotypes were shared across significant geographic distances (up to 600 km). *Phanetta* from the Appalachians exhibited slight genetic differentiation from individuals from the Interior Low Plateau, as well as more genetic variation from each other (Fig. 3, Table 3). The higher nucleotide diversity observed in Appalachian *Phanetta* (Table 3) may be due to the highly faulted and fractured karst of the Appalachians causing greater isolation between *Phanetta* populations, whereas the lower nucleotide diversity observed in Interior Low Plateau *Phanetta* (Table 3) may reflect the more contiguous horizontal carbonate layers of this karst region, which could foster population connectivity.

We can compare our results to other spider species and to other troglobiont spiders from the eastern United States. A review of DNA barcoding efforts in spiders (Čandek and Kuntner 2015), using the same genetic marker (*COI*) that we employed in our study, provides a broad comparison. Summarizing results for 162 species, Čandek and Kuntner (2015) reported a mean intraspecific nucleotide diversity of 0.009, slightly higher than the 0.006 that we observed in *Phanetta.* Further, Domènech et al. (2022) used *COI* sequences to study genetic diversity in 371 spider species across a similarly-sized geographic region in Spain. They found a mean maximum intraspecific distance of 0.021, which is similar to the maximum intraspecific distance of 0.022 we observed for *Phanetta*. Clearly, the amount of genetic diversity we observed in *Phanetta* is not out of the ordinary range for a spider species.

In contrast, the *Phanetta* results are quite different from those observed in other troglobiont spiders for which genetic data are available. *Nesticus* spiders of the southern Appalachians exhibit high species diversity across a region smaller than the range extent of *Phanetta*, with many species having very small ranges (Hedin and Milne 2023). Multiple species of *Nesticus* are often found in close proximity, sometimes at sites just a few kilometers apart (Zigler and Milne 2022; Hedin and Milne 2023). Previous studies found considerable genetic diversity within species, even when those species ranges are very small. For example, Zigler and Milne (2022) reported *COI* genetic distances of 0.026 (in *N. cressleri*) and 0.031 (in *N. lula*) for cave populations less than 10 kilometers apart. As an additional example, *Nesticus barri* is known from around 60 caves on the southern Cumberland Plateau in Tennessee and Alabama. Genetic analysis of *N. barri* from a dozen caves found no haplotypes shared by individuals that were more than 12 km apart, and genetic distances (also for the *COI* locus) between individuals from different caves were as high as 0.045 (Snowman et al. 2010). These patterns strongly contrast with *Phanetta*, where haplotypes were shared by individuals as far as 600 km apart, and the maximum genetic distance (across a vastly larger geographic range) between individuals was 0.022.

Phanetta has never been reported from surface habitats, not even in a study of sinkholes within the range of the species (Lewis et al. 2020), and we have shown that populations across its range are genetically uniform. This raises the question as to how *Phanetta* has managed to colonize so many caves across such a broad area. We offer two, potentially complementary, hypotheses. First, as a tiny spider, it may be moving, undetected, through subterranean passageways such as caves and the interstitial spaces in shallow subterranean habitats (SSH) (Culver and Pipan 2019), including the epikarst and the “milieu souterrain superficiel” (MSS), a layer of fractured rock beneath an insulating soil layer (reviewed in Mammola et al. 2016). In deeper cave habitats, troglobiont spiders have been shown to traverse through historical cave connections (Marsh et al. 2023). However, the distances traveled by historical *Phanetta* populations to form its current distributions are magnitudes larger than that studied by Marsh et al. (2023) and it is unknown if subterranean dispersal could fully explain its current range. Spiders have been collected within the MSS, especially in Europe (e.g., Růžička 1990, 1996; Růžička and Thaler 2002). However, studies on spiders from the MSS in North America are non-existent (Mammola et al. 2016).

A second possibility is that *Phanetta* disperses via ballooning, where spiders use their silken threads to be carried by the wind from one place to another (Greenstone et al. 1987). Studies of the diversity of ballooning spiders in the United States and Europe indicate that members of the family Linyphiidae are the spiders most commonly observed (Dean and Sterling 1985; Plagens 1986; Greenstone et al. 1987; Blandenier 2009; Blandenier et al. 2014), so it is not unreasonable to suggest *Phanetta*, a linyphiid spider, may also disperse in that way. If this is occurring, ballooning would probably have to be paired with at least some subsequent surface movement of individuals post-landing, as caves and cave entrances are relatively rare on the surface. Ballooning, which would allow the spiders to disperse across great distances, could explain the species' broad range, and the sharing of *COI* haplotypes between individuals collected as far as 600 km apart. However, the fact that *Phanetta* have never been observed on the surface weighs against the likelihood of ballooning as a method of dispersal.

This study could be extended in several ways. Further sampling of *Phanetta* from eastern Kentucky and from West Virginia would be valuable. We were unable to acquire samples from those areas. We also suggest searching for *Phanetta* from the three peripheral populations (Fig. 2) that we omitted from our estimation of range extent, as confirming or dismissing those observations would clarify the true range of the species. Two other linyphiid species – *Porrhomma cavernicola* and *Anthrobia monmouthia* – are wide-ranging troglobionts in eastern North America whose ranges overlap with *Phanetta* (Miller 2005a, 2005b). While neither is as common nor as wide-ranging as *Phanetta*, both are found across multiple states and karst regions, and genetic analyses of these species would provide an interesting comparison to the patterns we observed in *Phanetta*.

We also recommend exploring the possibility of ballooning in *Phanetta*. It might be possible to search directly for ballooning in *Phanetta* by setting aerial traps at the entrance of caves known to host *Phanetta*, aiming to catch any spiders leaving the cave by ballooning. Although some research on ballooning has been conducted in the United States (e.g., Dean and Sterling 1985; Plagens, 1986; Greenstone et al. 1987), none of these studies were done within the range of *Phanetta*. As a result, it remains unclear whether *Phanetta*, like many linyphiid species, disperses by ballooning. In addition, study of SSH within the range of *Phanetta* could clarify whether *Phanetta* are present in these habitats. In combination, studies of ballooning and SSH could support or reject our hypotheses for how *Phanetta* spread across such a large range.

In summary, we reject the suggestion that *Phanetta subterranea* contains cryptic genetic diversity and represents multiple species. Rather, it is a single, genetically uniform, species that has dispersed broadly across the caves of eastern North America. How it has managed to do this remains a mystery.

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