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First record of a freshwater cave sponge (Porifera, unknown gen. and sp.) in a cave inhabited by *Astyanax* cavefish in the Sierra de El Abra, San Luis Potosí, Mexico

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Abstract

The karstic cave, la Cueva de Los Sabinos, located in the Sierra de El Abra in the state of San Luis Potosí, Mexico, is mostly known for hosting a population of blind, depigmented *Astyanax mexicanus* cavefish. Herein, we report the discovery of a non-pigmented sponge (Porifera) in the final sump of this cave. No genus or species name could be attributed because we did not collect any specimen. Up to now, the sponge distribution seems restricted to a single pool in la Cueva de Los Sabinos, but further careful exploration of other pools of the cave as well as closely related cavities is warranted. To our knowledge, this observation constitutes the fourth report of a freshwater, white, cave-adapted sponge in the world and the first for Mexico and North America. It is also the eleventh troglobite species encountered in Los Sabinos. Our discovery confirms the exceptionally rich biodiversity of this cave ecosystem.

Resumen

La cueva kárstica de Los Sabinos, localizada en la Sierra de El Abra en el Estado de San Luis Potosí, México, es principalmente conocida por albergar una población de peces de cueva *Astyanax mexicanus*, ciegos y despigmentados. Aquí, reportamos el descubrimiento de una esponja despigmentada (Porifera) en el sifón final de esta cueva. No se pudo asignar ningún nombre de género o especie porque no se colectó ningún espécimen. Hasta ahora, la distribución de la esponja parece restringida a un único estanque de la cueva de Los Sabinos, pero será necesaria una exploración más detallada y cuidadosa de otros cuerpos de agua de la cueva, así como de las cavidades aledañas con posible conexión con esta cueva. Hasta donde sabemos, esta observación constituye el cuarto reporte de una esponja de agua dulce, no pigmentada y adaptada a cuevas en el mundo y la primera para México y Norteamérica. También es la undécima especie troglobia que se ha encontrado en la cueva de Los Sabinos. Nuestro descubrimiento confirma la excepcional riqueza en biodiversidad de este ecosistema cavernícola.

Keywords

Astyanax, karst, subterranean

Introduction

Sponges (Porifera) are very common organisms in marine environment with more than 9000 valid species from around the world (de Voogd et al. 2023). These metazoans are less known and studied in freshwater systems, yet they appear relatively common in different continental water habitats like rivers and lakes (Manconi and Pronzato 2008; Evans 2016), except in Antarctica. In the Neotropical region, Manconi and Pronzato (2008, 2015) reported 73 species in 26 genera of Spongillina (Class Demospongiae) in freshwater, among a total of 238 species around the world. Today, the number of species probably approximates 250, as new species are regularly discovered (Nicacio et al. 2011; Hernández and Barreat 2017; Carballo et al. 2018; Gómez 2011; Briceño et al. 2020; Carballo et al. 2021). New occurrences of previously described species are often reported (Rueda and Mesquita-Joanes 2016; Briceño et al. 2020). The taxonomic classification is rapidly evolving and complex (Sollas 1885; Manconi and Pronzato 2002; Cândido et al. 2010; Annesley and Henderson 2011).

In Mexico, nine species of freshwater sponges are listed in the World Porifera Database (<https://www.marinespecies.org/porifera/distribution.php?p=details&id=2224>) and in WoRMS (World Register of Marine Species). For most of them, occurrences are old in literature and need revision (Potts 1885, 1885b; Martinez 1940; Rioja 1940a, 1942; Penney and Racek 1968; Bushnell 1971; Poirrier 1982). In addition, four other species (not listed on WPDdatabase for presence in Mexico) could be present according to old literature as well (Ehrenberg 1841; Old 1936; Rioja 1940b, 1940c, 1953a, 1953b; Poirrier 1982). Moreover, three species have been described very recently (Carballo et al. 2018, 2021; Gómez et al. 2019). A complete discussion of the freshwater sponge species encountered in Mexico can be found in Gómez-López (2011).

There are, on the other hand, very few records of cave-adapted, strictly freshwater sponges throughout the world. Of note, marine or anchialine sponges can be found in sub-

terranean habitats, but most of them are not specific, cave-adapted animals. Strictly freshwater, troglobite, non-pigmented sponges are rarely found, maybe in part due to the difficulties in exploring and surveying these extreme underground habitats. Two cave sponges were described so far in Brazil: *Arinosaster patriciae* Volkmer-Ribeiro, Tavares-Frigo, Ribeiro & Bichuette, 2021 from the Arinos River basin, state of Mato Grosso and *Racekiela cavernicola* Volkmer-Ribeiro, Bichuette & de Sousa Machado, 2010 in a large cave system in the state of Bahia (Volkmer-Ribeiro et al. 2010, 2021). In Europe, the species *Eunapius subterraneus* (Sket & Velikonja, 1984) that includes two subspecies, *E. s. subterraneus* and *E. s. mollisparspanis*, was reported in Croatia (Gottstein Matočec et al. 2002; Bedek et al. 2008; Harcet et al. 2010; Jalžić et al. 2013). To our knowledge, these three species constitute the only records of sponges in karstic freshwater caves (Trajano and Bichuette 2010; Volkmer-Ribeiro et al. 2021), and no species has been described in Mexico (Reddell 1981).

Here, we document the discovery of a subterranean freshwater sponge in a cave inhabited by troglomorphic *Astyanax mexicanus* (De Filippi, 1853) in the state of San Luis Potosí, Mexico (Fig. 1A). The region is famous for the presence of at least 33 caves hosting troglomorphic populations of *Astyanax mexicanus/jordani* complex (Elliott 2018; Espinasa et al. 2018, 2020; Miranda-Gamoá et al. 2023). La Cueva de Los Sabinos, located in the Sierra de El Abra was first discovered in 1942 (Mitchell et al. 1977). It is a well-known cave that has been studied extensively by cavers and biologists (Elliott 2018). Sixty animal species belonging to diverse phyla have been inventoried, including nine troglobite species. However, no sponge has been recorded yet (Elliott 2018). According to Elliott (2018), the nine troglobite species in Los Sabinos are:

1. *Microdiaptomus cokeri* (Osorio-Tafall, 1942): Crustacea, Copepoda, Diaptomidae.
2. *Hobbsiella cirolanae* (Rioja, 1951): commensal ostracod crustacean, known from 15 caves in the Sierra de El Abra and Sierra de Guatemala.
3. *Speocirolana pelaezi* Bolívar & Pieltai, 1950: aquatic isopod crustacean.
4. *Pseudosinella strinatii* (Gisin, 1952): collembolan (springtail) known from nine caves in the Sierra de El Abra.
5. *Spherarmadillo cavernicola* Mulaik, 1960: pillbug (terrestrial isopod crustacean) from the El Abra and Sierra de Guatemala.
6. *Brackenridgia bridgesi* (Van Name, 1942): a sowbug (isopod crustacean) from the El Abra and Sierra de Guatemala.
7. *Hoplobunus boneti* (Goodnight & Goodnight, 1942): harvestman (Opiliones), known from 15 caves.
8. *Anelpistina (Neonicoletia) quinterensis* (Paclt, 1979): thysanuran or silverfish (Nicoletiidae, Zygentoma, Insecta), known from many caves in the Sierra de El Abra and Sierra de Guatemala
9. *Astyanax mexicanus/jordani*: Characiformes, Characidae cavefish, actually named *A. mexicanus/jordani* complex with *A. mexicanus* (De Filippi, 1853), *A. jordani* (Hubbs & Innes, 1936) from La Cueva Chica, *A. antrobius* (Alvarez, 1946) and *A. hubbsi* (Alvarez, 1947) from La Cueva de El Pachón and La Cueva de Los Sabinos. Thirty-three populations of *Astyanax* cavefish are currently known.

A tenth species should be included: *Spelaeomysis quinterensis* (Villalobos, 1951) (Crustacea, Mysida). While Elliott describes this species as present in Sótano del Arroyo and Cueva La Tinaja, but absent in Los Sabinos, we have found this mysid to be quite abundant in that cave, which is no surprise given that the three caves form a single hydrological system.

Materials and methods

We have visited la Cueva de Los Sabinos during five different field trips in 2013, 2017, 2019, 2022 and 2023. These expeditions were focused on *Astyanax* cavefish biology and ecology, under the auspices of permits delivered by SEMARNAT to POG and SR (02241/13; 05389/17; 1893/19; 03334/22). The 2023 visit to Los Sabinos was also under supervision of the CONANP and the local “ejido” Los Sabinos community. All our visits to this cave occurred during the dry season, in February and March (see figures for exact dates).

No samples were collected nor manipulated. Only photographs (aerial and underwater) were taken, using an Olympus TG-4 camera. All photographs shown below were taken in the Los Sabinos pool 2. This pool is a final sump of one branch of the cave, about 400 meters from and 62 meters below the entrance (Fig. 1B). The size of sponges was estimated by comparing to adult *Astyanax* cavefish, which are approximately 6 cm long in that pool (Blin et al. 2020).

In addition, the temperature of the water in Los Sabinos pool 1 and pool 2 was recorded in March 2011, March 2017, March 2018, March 2019 and February 2022 and 2023 with a Combo Hanna HI98129. Oxygen concentrations were measured with a Hanna oxymeter HI98193.

Results and discussion

La Cueva de Los Sabinos is located in the Sierra de El Abra, SLP, Mexico (Fig. 1A). It is inhabited by a population of *Astyanax* cavefish first described as *Anoptichthys hubbsi* (Alvarez 1947), now named *Astyanax mexicanus/jordani* species complex. Inside the cave, cavefish are distributed in two distinct pools (Fig. 1B). These two pools may sometimes merge in case of important increase in water level and cave flooding, but we never witnessed this during our trips.

In February 2022, white spots on the brown stones on the bottom of pool 2 caught our attention (Fig. 1B, photo inset). Their size varied from ~2 to ~12 cm and they were clearly distinguishable. The cave isopod *Speocirolana pelaezi* and the cavefish *Astyanax mexicanus/jordani* were observed very close to some sponge specimens, but no direct interactions were seen (Fig. 1B, thin brown arrows). In February 2023, high magnification pictures were obtained and confirmed that the macroscopic aspect of the organism corresponds to typical sponge anatomy. Typical specimens between ~2 and ~7 cm on a small rock are shown in Fig. 2. Small and larger holes were observed, probably

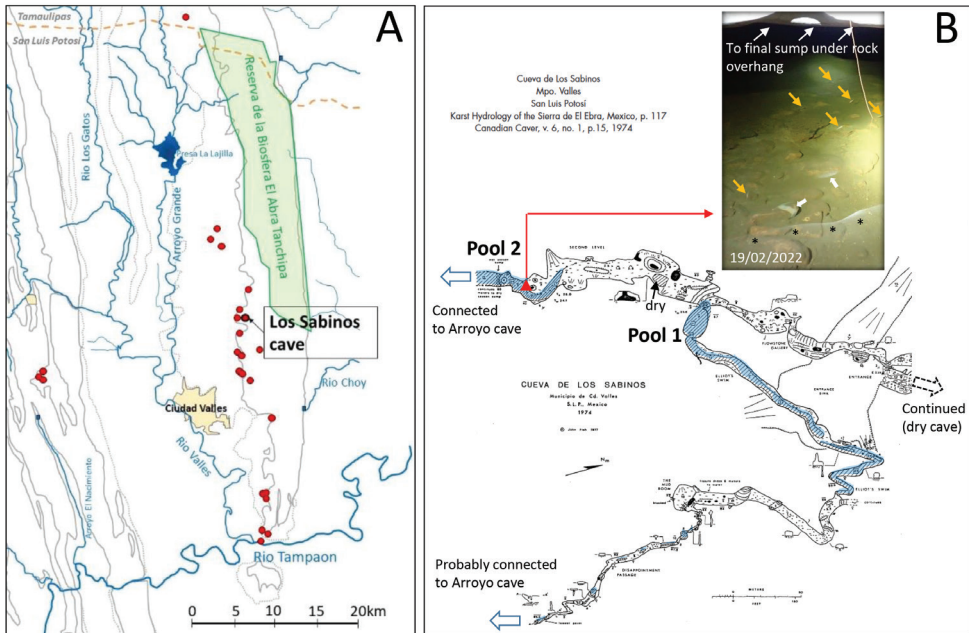


Figure 1. Maps of the geographical area and la Cueva de Los Sabinos **A** map of the Sierra de El Abra region in North East Mexico for localization of la Cueva de Los Sabinos. Red dots indicate cave locations where *Astyanax* cavefish populations are described. The Reserva de la Biosfera Sierra del Abra Tanchipa is highlighted in green **B** map of Los Sabinos topography. Pool 1 and pool 2 are identified. The red triangle points to the place where the sponges were observed in pool 2. Water bodies are colored in blue. There are more pools with the potential of also having sponges after “Elliott’s swim”. The large arrows show the probable connection with Sótano del Arroyo. The photo inset shows pool 2, where the first observations of the sponge were made in February 2022. Thick white arrows point to white sponges, brown arrows point to *Astyanax* cavefish populating the pond. Black asterisks indicate an artefactual line of reflection of the rocky overhang on the water surface. The small cord is an attachment for a temperature probe. Sponges are associated and clinging to the rocks scattered on the bottom of this pool.

corresponding to water intake pores and water release pores (osculum), respectively. Small spicules pointing out from the surface of the animals were also visible. We propose that these organisms are a *bona fide*, novel, non-pigmented, cave-adapted and freshwater sponge species. No genus or species name could be attributed because we did not collect samples for further anatomical or genetic analyses. However, according to macroscopic aspects, the species may belong to the genus *Racekiela* (Demospongiae, Spongillidae).

After this discovery, older pictures and underwater videos taken in Los Sabinos pool 2 during previous expeditions were re-scanned and re-examined for the presence of the sponge (Fig. 3). The earliest available evidence of the sponge presence in our photographic records dates from March 2013 (Fig. 3B). Large, recognizable non-pigmented sponges were repeatedly seen in an aerial video capture from March 2017 (Fig. 3A) and an underwater video from March 2019 (Fig. 3C). Thus, the presence of sponges in Los Sabinos pool 2 has been continual for at least 10 years (2013–2023), suggesting that they constitute an integral part of the underground community.

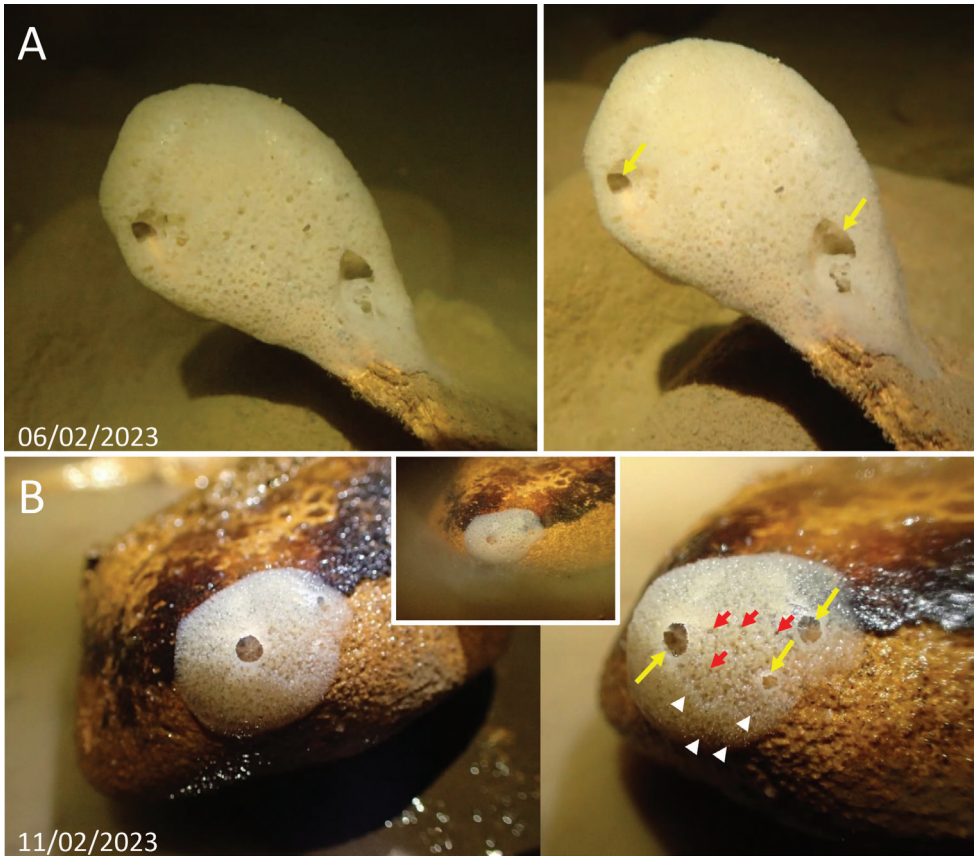


Figure 2. La Cueva de Los Sabinos pool 2, high magnification pictures, February 2023. Two specimens are shown (**A**, **B**). Arrows indicate water intake (red) and release (yellow) pores (osculum) as well as spicules (white). Dates of observation of the two specimens are indicated.

As of now, no sponge specimen has been observed in Los Sabinos pool 1. Yet, this pool needs to be further checked to confirm the absence of sponges. As mentioned above, the two pools are not connected during the dry season, but the two water bodies may join during exceptionally heavy rainy seasons. Interestingly, water parameters recorded in pool 1 and pool 2 were very different and may explain why the sponge is found only in the second pool (Table 1). The water temperature in the two pools differs by an average of 1.8 °C, with pool 2 always being warmer than pool 1 over the years. The minimum difference between the two pools was 1.6 °C, and the maximum difference was 2.2 °C. The dissolved oxygen concentrations were also repeatedly higher in pool 2 than in pool 1, in both February 2022 and February 2023. Studies have shown that water quality influences the distribution of sponge species (Evans 2016). Thus, the Los Sabinos sponge may have a temperature preference and/or oxygen preference that restricts its presence to pool 2, but further exploration is needed to support this preference.

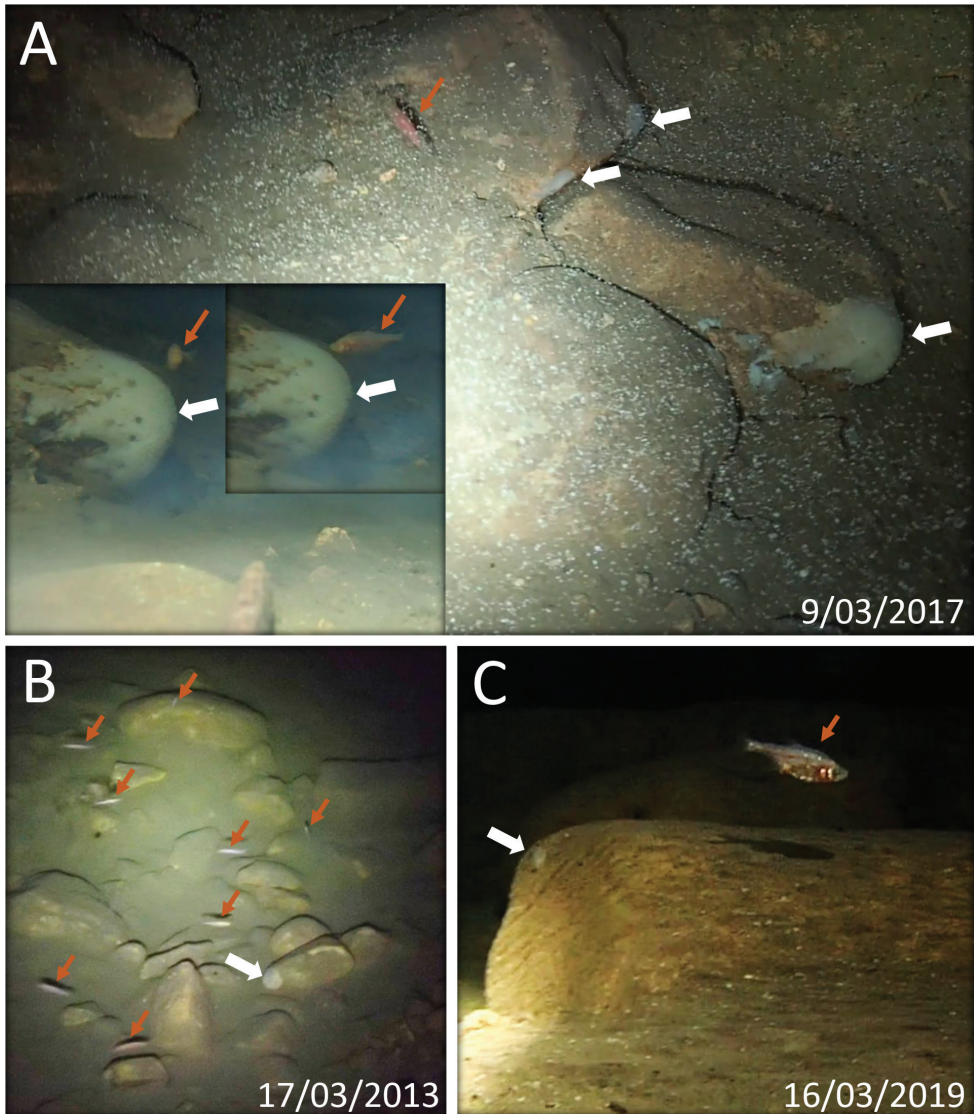


Figure 3. La Cueva de Los Sabinos pool 2. Photographs and videos showing the presence of sponges across multiple years **A–C** show photographs or snapshots from videos where sponges can be observed. Dates of observations are indicated. The continued presence of sponges throughout 2013 to 2023 suggests sponges are not accidental, but instead constitute an integral part of the underground community. Thick white arrows point to sponges, thin brown arrows point to *Astyanax* cavefish.

Another distinguishing factor for the possible exclusion of sponges from pool 1 is the substrate. The floor of pool 1 primarily consists of deep mud, where anchoring may be difficult for the sponges. While pool 2 also has large amounts of mud, sponges were systematically perched on top of the many rocks that can be found in this pool (Bell et al. 2015; Schönberg 2016).

Table 1. Physico-chemical parameters of the water in pool 1 and pool 2 are shown, at the indicated dates.

Date	O ₂ (mg/mL)/saturation (%)			Temperature (°C)			
	Feb. 2022	Feb. 2023	March 2017	Feb. 2018	March 2019	Feb. 2022	Feb. 2023
Pool 1 (no sponge)	3.66 / 42.6%	3.72 / 42.8%	22.8	22.4	22.6	22.3	22.5
Pool 2 (with sponge)	5.73 / 70.3%	5.7 / 69.2%	24.4	24.2	24.3	24.5	24.4
Difference	2.07	1.98	1.6	1.8	1.7	2.2	1.9

Conclusion and perspectives

Excluding anchialine and brackish caves related to the sea (Gómez and Calderón-Gutiérrez 2020), this is the first record of a strictly continental and freshwater cave sponge in Mexico and North America. To our knowledge, it is only the fourth species of true freshwater cave sponge in the world. It is also the eleventh troglobitic species of la Cueva de Los Sabinos. The discovery of this non-pigmented cave sponge was unexpected. This cave is very famous within the international research community interested in the *Astyanax* cavefish model, and tourists and cavers visit it as well. The cave was first explored in 1942 and, up to now, the presence of sponges has never been reported, even though sixty different animal species were described to live there or be present in this cave (Elliott 2018).

Further exploration needs to confirm the presence or absence of the cave sponge in the first pool of la Cueva de Los Sabinos and in the other small pools in continuity (see Fig. 1B). It will also be interesting to check for the presence of the sponge in the Sótano del Arroyo, which is geographically very close to Los Sabinos and might connect through the underground aquifer with the final sump of Los Sabinos pool 2, where the sponge was discovered. Perhaps the Sótano de La Roca and the Sótano de La Tinaja will have to be checked for the sponge presence as well, as they are also close to Los Sabinos and part of the same hydrologic system.

It will also be of utmost interest to provide a detailed morphological description and to perform DNA sequence analyses to describe this probable new species of subterranean freshwater cave sponge. For the moment, we were unable to characterize this sponge species further because we did not take any samples due to the lack of proper authorization from the Mexican authorities. Our SEMARNAT permit was specific for *A. mexicanus* and, thus, no collecting attempts were done. For future research, a proper species permit will be requested. Scientific work in la Cueva de Los Sabinos will also be conducted under authorization by CONANP. This cave is under strict protection and control by the Reserva de la Biosfera El Abra Tanchipa and by the council of the ejido (or village) of Los Sabinos. The discovery of a unique, novel cave-adapted and non-pigmented sponge species in Los Sabinos further illustrates the richness of the natural resources and biodiversity in the underground realm of this region of Mexico. Follow-up studies to understand its conservation status and potential need of protection will be established (Mammola et al. 2022). Long-term and precise monitoring studies are needed to better understand the ecology of this cave ecosystem.

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