

Cave-dwelling pholcid spiders (Araneae, Pholcidae): a review

Bernhard A. Huber¹

¹ *Alexander Koenig Research Museum of Zoology, Adenauerallee 160, 53113 Bonn, Germany*

Corresponding author: *Bernhard A. Huber* (b.huber@leibniz-zfmk.de)

Academic editor: *O. Moldovan* | Received 4 May 2018 | Accepted 29 May 2018 | Published 6 June 2018

<http://zoobank.org/E3AD5959-82BF-4FF3-95C3-C4E7B1A01D97>

Citation: Huber BA (2018) Cave-dwelling pholcid spiders (Araneae, Pholcidae): a review. *Subterranean Biology* 26: 1–18. <https://doi.org/10.3897/subtbiol.26.26430>

Abstract

Pholcidae are ubiquitous spiders in tropical and subtropical caves around the globe, yet very little is known about cave-dwelling pholcids beyond what is provided in taxonomic descriptions and faunistic papers. This paper provides a review based on a literature survey and unpublished information, while pointing out potential biases and promising future projects. A total of 473 native (i.e. non-introduced) species of Pholcidae have been collected in about 1000 caves. The large majority of cave-dwelling pholcids are not troglomorphic; a list of 86 troglomorphic species is provided, including 21 eyeless species and 21 species with strongly reduced eyes. Most troglomorphic pholcids are representatives of only two genera: *Anopsicus* Chamberlin & Ivie, 1938 and *Metagonia* Simon, 1893. Mexico is by far the richest country in terms of troglomorphic pholcids, followed by several islands and mainland SE Asia. The apparent dominance of Mexico may partly be due to collectors' and taxonomists' biases. Most caves harbor only one pholcid species, but 91 caves harbor two and more species (up to five species). Most troglomorphic pholcids belong to two subfamilies (Modisiminae, Pholcinae), very few belong to Smeringopinae and Arteminae, none to Ninetinae. This is in agreement with the recent finding that within Pholcidae, microhabitat changes in general are concentrated in Modisiminae and Pholcinae.

Keywords

Spider, Araneae, Pholcidae, troglomorphism, subterranean

Introduction

The colonization of subterranean habitats has occurred many times independently in many groups of animals (White and Culver 2012). This change of habitat has often resulted in more or less conspicuous and convergent modifications, or troglomorphisms (Hüppop 2012, Christiansen 2012, Protas and Jeffery 2012, Liu et al. 2017). Similar selective regimes have resulted in similar modifications in distantly related taxa, making cave animals an ideal model system to study fundamental evolutionary mechanisms. Interestingly, however, many widespread patterns continue to be contentious or even mysterious. For example, many recent debates focus on the mechanism responsible for regressive evolution such as the reduction and loss of eyes (Culver and Wilkens 2000, Christiansen 2012, Klaus et al. 2013, Krishnan and Rohner 2016). An old but still unsettled question concerns the apparent higher diversity of troglomorphic animals in temperate rather than in tropical regions (Howarth 1980, Culver and Sket 2000, Deharveng and Bedos 2012). And quite mysterious is the fact that only certain groups in any major taxon have entered caves and adapted to subterranean life while close relatives present in the same region have not (e.g., 91% of cave beetles are members of only two out of 166 beetle families; Moldovan 2012; see also Christiansen 2012).

Spider research has the potential to contribute significantly to these debates. Spiders are in several ways preadapted to life in caves. Many are nocturnal; most rely on mechanical and chemical rather than on visual stimuli; many groups are diverse in habitats that share certain characteristics with caves such as damp sheltered spaces or leaf litter; many spiders have a low energy demand and endure long periods of starvation; and most are generalist feeders (Foelix 2011, Mammola and Isaia 2017). As a result, spiders are among the most common inhabitants of terrestrial caves all over the world. According to Mammola and Isaia (2017) “around 1000 troglobiomorphic spiders have been classified as troglobionts”. However, a recent overview of the Brazilian obligatory subterranean fauna found that over 50% of troglobionts available in collections remain undescribed (Gallão and Bichuette 2018), suggesting that the actual number is much higher. Whatever the species numbers, compared to some other major groups like isopods, amphipods, carabid and leiodid beetles, salamanders, and fish, subterranean spiders have rarely been studied in much detail or propagated as model organisms for particular questions concerning evolution in subterranean environments (for exceptions, see Mammola and Isaia 2017; Mammola et al. 2017).

Pholcidae, one of the major spider families in terms of described species (World Spider Catalog 2018), epitomize this situation. On one hand, representatives of Pholcidae are among the most common spiders in tropical and subtropical caves around the globe. Very few studies exist that quantify this dominance but a recent study by Souza Silva and Ferreira (2015) on the invertebrates in 15 caves in Espírito Santo (Brazil) may well reflect a global pattern: Pholcidae was the spider family represented in the highest number of caves (13), followed by Theridiidae and Ctenidae (11 each) and Oonopidae (9). On the other hand, almost nothing is known about cave-dwelling pholcids beyond what is provided in taxonomic descriptions and faunistic papers. The present paper is an effort to provide a first step in filling this gap by (1) summarizing

the known information; (2) highlighting some emerging systematic and biogeographic patterns; and (3) proposing a list of easily accessible and promising open questions that will hopefully motivate more specific research.

Material and methods

The information summarized herein is largely taken from the taxonomic literature. In addition, data on 37 undescribed cave-dwelling species (eight of them troglomorphic) available in collections were added. The resulting table included all records of all species that have been reported from caves, i.e. also the epigeal records of species that occasionally enter caves ('accidentals') or that are commonly found both outside and within caves. Excluded were ten synanthropic species, all of which are occasionally found in caves, in most cases presumably as a result of human introduction. The resulting table thus included only 'native', i.e. non-introduced species; it had 3100 entries. Table 1 is an excerpt from this table, listing only the species classified as troglomorphic.

Species were classified as troglomorphic (i.e. showing features associated with cave life) versus non-troglomorphic in order to avoid the unprovable designation of a species as troglobiont (obligatory cave dweller; as opposed to troglophile). In many cases, the classification of a species as troglomorphic or non-troglomorphic was unambiguous (eyes reduced or absent; very pale coloration compared to epigeal relatives), but in several cases a clear distinction was not possible, especially when "long legs" and/or the loss of pigment were the only reported troglomorphisms. Some of the species in Table 1 are thus listed based on subjective assessments (by previous researchers or by me) rather than on a quantification of troglomorphy.

A further source of noise in the data comes from the definition of cave. I largely use the anthropocentric definition of caves as "a natural opening in the Earth, large enough to admit a human being, and which some human beings choose to call a cave" (White 1988). This is probably very close to, or congruent with, the definition implied in the taxonomic literature.

Most published records do not provide coordinates, and even some published coordinates are obviously wrong. This introduces a further source of error, even though I made an effort to georeference as many caves as possible and to verify published coordinates. For this I used a variety of online databases as well as Google Earth and direct information from collectors.

Results

Spiders

A total of 473 native pholcid species (including 37 undescribed species) have been found in caves. This represents approximately 25% of the species currently known to exist (i.e. 1662 described species + ~300 undescribed species available in collections).

Table 1. Troglomorphic Pholcidae. The references refer to the descriptions of the troglomorphies.

Species	Troglomorphy	Reference
<i>Anopsicus bolivari</i> (Gertsch, 1971)	Long legs	Gertsch 1982
<i>Anopsicus clarus</i> Gertsch, 1982	Pale, eyeless, long legs	Gertsch 1982
<i>Anopsicus cubanus</i> Gertsch, 1982	Eyeless, long legs	Gertsch 1982
<i>Anopsicus exiguus</i> (Gertsch, 1971)	White, long legs	Gertsch 1982
<i>Anopsicus grubbsi</i> Gertsch, 1982	Small eyes	Gertsch 1982
<i>Anopsicus gruta</i> (Gertsch, 1971)	Pale, eyes essentially obsolete, scarcely visible as pale vestiges, long legs	Gertsch 1971, 1982
<i>Anopsicus jarmila</i> Gertsch, 1982	Pale, long legs, essentially eyeless, with trivial vestiges of eyes	Gertsch 1982
<i>Anopsicus limpidus</i> Gertsch, 1982	Pale, long legs, eyes rudimentary, small, white	Gertsch 1982
<i>Anopsicus lucidus</i> Gertsch, 1982	Pale, long legs, eyes evanescent, essentially obsolete, without pigment	Gertsch 1982
<i>Anopsicus mckenziei</i> Gertsch, 1982	Long legs	Gertsch 1982
<i>Anopsicus mirabilis</i> Gertsch, 1982	Pale, long legs, eyes evanescent, reduced to inconspicuous, unpigmented vestiges	Gertsch 1982
<i>Anopsicus mitchelli</i> (Gertsch, 1971)	Small eyes, long legs	Gertsch 1971, 1982
<i>Anopsicus nebulosus</i> Gertsch, 1982	Pale, essentially eyeless, trivial vestiges of eyes, long legs	Gertsch 1982
<i>Anopsicus niveus</i> Gertsch, 1982	Pale, eyeless, long thin legs	Gertsch 1982
<i>Anopsicus pearsei</i> Chamberlin & Ivie, 1938	Essentially eyeless, widely spaced corneal vestiges	Gertsch 1982
<i>Anopsicus pecki</i> Gertsch, 1982	Pale, long thin legs, eyes small	Gertsch 1982
<i>Anopsicus quatuoroculus</i> Gertsch, 1982	Posterior lateral eyes missing	Gertsch 1982
<i>Anopsicus reddelli</i> Gertsch, 1982	Pale, eyes small	Gertsch 1982
<i>Anopsicus silvai</i> Gertsch, 1982	Long legs	Gertsch 1982
<i>Anopsicus soileauae</i> Gertsch, 1982	Pale, long legs	Gertsch 1982
<i>Anopsicus speophilus</i> (Chamberlin & Ivie, 1938)	Pale, eyes small	Gertsch 1982
<i>Anopsicus troglodytus</i> (Gertsch, 1971)	Small eyes, long legs	Gertsch 1971, 1982
<i>Anopsicus vinnulus</i> Gertsch, 1982	Long legs, eyes evanescent, without pigment, essentially obsolete	Gertsch 1982
<i>Artema</i> sp. n. "Om42" †	Pale, small eyes	Unpublished
<i>Aymaria floreana</i> (Gertsch & Peck, 1992)	Essentially eyeless	Gertsch and Peck 1992
<i>Aymaria jarmila</i> (Gertsch & Peck, 1992)	Essentially eyeless, without external indication of lost eyes or with small white vestiges	Gertsch and Peck 1992
<i>Belisana bantham</i> Huber, 2005	Only two eyes, pale	Huber 2005a
<i>Belisana khanensis</i> Yao & Li, 2013	Eyes absent	Yao and Li 2013
<i>Belisana pisinna</i> Yao, Pham & Li, 2015	Pale, small eyes	Yao et al. 2015
<i>Buitinga?</i> sp. n. "Reun1" ‡	Pale, eyeless	Unpublished
<i>Ciboneya antraia</i> Huber & Pérez, 2001	Pale, long legs	Huber and Pérez 2001
<i>Corysoccnemis clara</i> Gertsch, 1971	Pale, long legs	Gertsch 1973
<i>Hoplopholcus</i> sp. n. "Tur21" §	Pale	Unpublished
<i>Khonata jaegeri</i> Huber, 2005	Pale, small eyes	Huber 2005b
<i>Metagonia atoyacae</i> Gertsch, 1971	Pale, eyeless	Gertsch 1971
<i>Metagonia bellavista</i> Gertsch & Peck, 1992	Whitish, eyeless	Gertsch and Peck 1992
<i>Metagonia</i> sp. n. "Br15-153" †	Pale, very small eyes without pigment	Unpublished
<i>Metagonia chiquita</i> Gertsch, 1977	Eyeless	Gertsch 1977
<i>Metagonia debrasi</i> Pérez & Huber, 1999	Eyeless	Pérez and Huber 1999
<i>Metagonia diamantina</i> Machado, Ferreira & Brescovit, 2011	Eyeless	Machado et al. 2011

Species	Trogloomorphy	Reference
<i>Metagonia iviei</i> Gertsch, 1977	Pale, long legs	Gertsch 1977
<i>Metagonia jamaica</i> Gertsch, 1986	Eyeless	Gertsch 1986
<i>Metagonia jarmila</i> Gertsch, 1973	Pale, eyes obsolete or of moderate size, without pigment	Gertsch 1973
<i>Metagonia joya</i> Gertsch, 1986	Eyeless	Gertsch 1986
<i>Metagonia lepida</i> Gertsch, 1986	Eyeless	Gertsch 1986
<i>Metagonia luisa</i> Gertsch, 1986	Eyeless	Gertsch 1986
<i>Metagonia martha</i> Gertsch, 1973	Pale, rudimentary eyes without pigment	Gertsch 1973
<i>Metagonia maya</i> Chamberlin & Ivie, 1938	Whitish, long-legged, evanescent eyes without pigment	Gertsch 1977
<i>Metagonia oxtalja</i> Gertsch, 1986	Eyeless	Gertsch 1986
<i>Metagonia pachona</i> Gertsch, 1971	Pale, very small eyes	Gertsch 1971
<i>Metagonia potiguar</i> Ferreira et al., 2011	Pale, small eyes	Ferreira et al. 2011
<i>Metagonia puebla</i> Gertsch, 1986	Essentially eyeless, eyes present as small whitish vestiges	Gertsch 1986
<i>Metagonia pura</i> Gertsch, 1971	Pale, eyeless	Gertsch 1971
<i>Metagonia reederi</i> Gertsch & Peck, 1992	Whitish, eyeless	Gertsch and Peck 1992
<i>Metagonia tinaja</i> Gertsch, 1971	Pallid coloration, small eyes	Gertsch 1971
<i>Metagonia tlamaya</i> Gertsch, 1971	Pale, eyeless	Gertsch 1971
<i>Metagonia torete</i> Gertsch, 1977	Evanescent eyes, long legs	Gertsch 1977
<i>Metagonia yucataana</i> Chamberlin & Ivie, 1938	Pale, very long legs	Gertsch 1977
<i>Ossinissa justoi</i> (Wunderlich, 1992)	Eyes very small	Dimitrov and Ribera 2005
<i>Paramicromerys megaceros</i> (Millot, 1946)	Almost entirely unpigmented, very long legs, small eyes	Millot 1946
<i>Pholcus arcuatilis</i> Yao & Li, 2013	Small eyes, pale	Yao and Li 2013
<i>Pholcus baldiosensis</i> Wunderlich, 1992	Pale, reduced eyes	Wunderlich 1992
<i>Pholcus caecus</i> Yao, Pham & Li, 2015	Pale, "eyes absent" [vestiges visible in figures]	Yao et al. 2015
<i>Pholcus corniger</i> Dimitrov & Ribera, 2006	"Total reduction of eyes" [vestiges visible in figure]	Dimitrov and Ribera 2005
<i>Pholcus diopsis</i> Simon, 1901	Pale, small eyes	Huber 2011
<i>Pholcus dongxue</i> Yao & Li, 2017	Pale, small eyes	Dong et al. 2017
<i>Pholcus</i> sp. n. "G044" †	Pale, small eyes	Unpublished
<i>Psilochorus concinnus</i> Gertsch, 1973	Long legs	Gertsch 1973
<i>Psilochorus delicatus</i> Gertsch, 1971	Pale	Gertsch 1971
<i>Psilochorus diablo</i> Gertsch, 1971	Pale, eyes reduced in size	Gertsch 1971
<i>Psilochorus fishi</i> Gertsch, 1971	Pale	Gertsch 1971
<i>Psilochorus murphyi</i> Gertsch, 1973	Long legs	Gertsch 1973
<i>Psilochorus russelli</i> Gertsch, 1971	Pale	Gertsch 1971
<i>Psilochorus tellezi</i> Gertsch, 1971	Long legs	Gertsch 1971
<i>Spermophora falcata</i> Yao & Li, 2013	Very small vestigial eyes	Yao and Li 2013
<i>Spermophora</i> sp. n. "Deele466" ‡	Eyeless	Unpublished
<i>Spermophorides anophthalma</i> Wunderlich, 1999	Pale, eyes with strongly reduced lenses	Wunderlich 1999
<i>Spermophorides flava</i> Wunderlich, 1992	Pale, small eyes	Wunderlich 1992
<i>Spermophorides fuertecavensis</i> Wunderlich, 1992	Pale, small eyes	Wunderlich 1992
<i>Spermophorides reventoni</i> Wunderlich, 1999	Long legs, small eyes	Wunderlich 1992
<i>Stygopholcus absoloni</i> (Kulczynski, 1914)	Paler than epigeic <i>S. photophilus</i>	Unpublished

Species	Trogloomorphy	Reference
<i>Stygopholcus</i> sp. n. “Bal3” ^{††}	Paler than epigeal <i>S. photophilus</i>	Unpublished
<i>Uthina</i> sp. n. “Ind67” ^{‡‡}	Long legs, small eyes	Unpublished
<i>Zatavua andrei</i> (Millot, 1946)	Almost unpigmented	Millot 1946
<i>Zatavua ankaranae</i> (Millot, 1946)	Almost unpigmented	Millot 1946
<i>Zatavua impudica</i> (Millot, 1946)	Almost unpigmented	Millot 1946

[†] *Artema* sp. n. “Om42”: Oman, Ad Dakhiliyah, Al Ghubrat cave (ZFMK).

[‡] *Buitinga?* sp. n. “Reun1”: Réunion, St. Paul, Grotte de la Tortue (ZFMK).

[§] *Hoplopholcus* sp. n. “Tur21”: Turkey, Mersin Prov., Aydıncık District, Aynalgöl (Gilindere) Mağarası (ZFMK).

^{||} *Metagonia* sp. n. “Br15-153”: Brazil, Rio Grande do Norte, near Baraúna, Caverna dos Macacos (ZFMK).

[¶] *Pholcus* sp. n. “G044”: Cape Verde, Fogo, Cueva de Gancho (ZFMK).

[#] *Spermophora* sp. n. “Deele466”: Indonesia, West Papua, Irian Jaya, Gua Nomokendik, Nambuktep Kokas (RMNH).

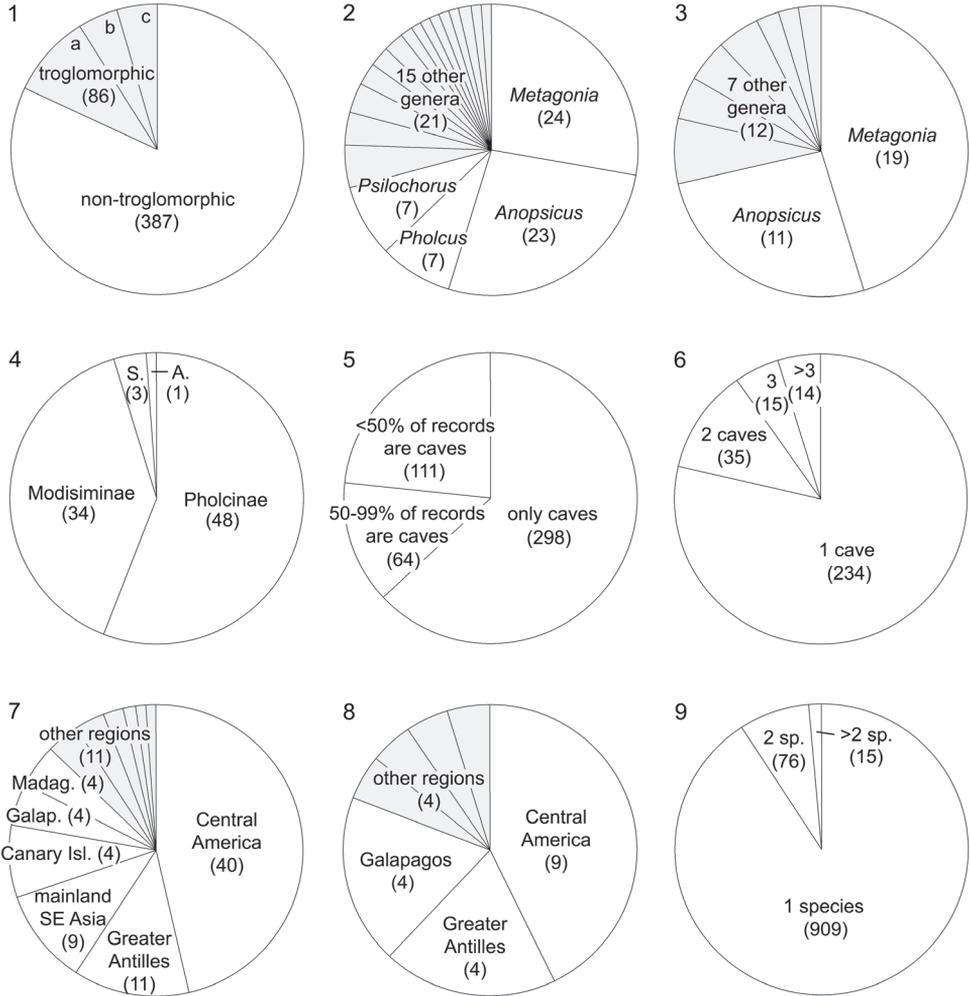
^{††} *Stygopholcus* sp. n. “Bal3”: Species limits among cave-dwelling *Stygopholcus* are unclear. A revision is in preparation, and I currently recognize two species: *S. absoloni* (a putative senior synonym of *S. skotophilus*; mainly in Montenegro) and the unnamed species “Bal3” (mainly in southern Croatia and Bosnia and Herzegovina).

^{‡‡} *Uthina* sp. n. “Ind67”: Indonesia, Sulawesi, South Sulawesi, Bantimurung, Gua Mimpi (ZFMK).

Of these, 86 are here considered to be troglomorphic (Fig. 1; Table 1). Troglomorphic species occur in 20 genera (of currently 77 genera), but a majority of them are representatives of just two genera: *Metagonia* Simon, 1893 and *Anopsicus* Chamberlin & Ivie, 1938 (Fig. 2). Together with three further genera (*Pholcus* Walckenaer, 1805; *Psilochorus* Simon, 1893; *Spermophorides* Wunderlich, 1992), they contain ~75% of all troglomorphic pholcid species. This bias is even more extreme when considering only the 42 ‘strongly’ troglomorphic species, i.e. those without eyes or with reduced eyes: 30 (71%) are in the genera *Metagonia* and *Anopsicus* (Fig. 3). Most troglomorphic pholcid species are in Pholcinae and Modisiminae (Fig. 4); four weakly troglomorphic species are in Smeringopinae and Arteminae; none in Ninetinae.

Of the 473 pholcid species known from caves, 298 are known from caves only, i.e. have so far not been collected from epigeal habitats; 64 species have the majority of records (i.e. 50–99%) from caves; 111 species have less than 50% of their records from caves (the ten synanthropic pholcid species would fall in this category too) (Fig. 5). Of the 298 species known from caves only, the large majority (234, i.e. 79%) are known from a single cave (Fig. 6). Among the other 64 species are some that appear fairly widely distributed, including six troglomorphic species that are known from 5–11 caves each. Two of these troglomorphic species occur in several caves but these caves are all close together (*Metagonia potiguar* Ferreira et al., 2011: five caves within 10 km; *Aymaria jarmila* (Gertsch & Peck, 1992): six caves within 15 km); the other four species cover large to very large areas (*Metagonia tinaja* Gertsch, 1971: nine caves within 50 km; *Anopsicus quatoculus* Gertsch, 1982: seven caves within 65 km; *Anopsicus pearsei* Chamberlin & Ivie, 1938: five caves within 190 km; *Metagonia maya* Chamberlin & Ivie, 1938: 11 caves within 260 km).

Few cave pholcids seem to be relicts, based on the apparent absence of epigeal close relatives (cf. Grandcolas et al. 2014). Examples include (1) the four species of



Figures 1–9. Main patterns in cave-dwelling pholcid spiders. Numbers in parentheses are usually species numbers (1–8), but cave numbers in (9). **1** Troglomorphic and non-troglomorphic cave-dwelling pholcids; a, eyes normal; b, eyes reduced in size and/or pigment; c, eyes absent **2** Generic assignment of the 86 troglomorphic pholcid species **3** Generic assignment of the 42 ‘strongly’ troglomorphic species **4** Subfamily assignment of the 86 troglomorphic species; S., Smeringopinae; A., Arteminae **5** Known habitat of 473 cave-dwelling pholcids **6** Number of caves known to be inhabited by 298 species known from caves only **7** Geographic distribution of the 86 troglomorphic species **8** Geographic distribution of the 21 eyeless species **9** Number of species found in each of 1000 caves.

Metagonia on Cuba, Jamaica, and the Galapagos Islands, all of which are eyeless and have no epigeal relatives on these islands (Gertsch 1986, Gertsch and Peck 1992, Pérez and Huber 1999); (2) an undescribed eyeless species on Réunion Island, possibly member of the East African genus *Buitinga* Huber, 2003 and with no known congener on Réunion (B.A. Huber, unpublished data); (3) the strongly troglomorphic *Ossinissa*

justoi (Wunderlich, 1992) on El Hierro (Canary Islands), only representative of the genus, possibly related to two other troglomorphic species on Tenerife currently in *Pholcus* and not closely related to epigeal *Pholcus* in the Canary Islands (Dimitrov and Ribera 2005, Huber 2011: 367).

Distribution

The most obvious and striking geographic pattern is the apparent dominance of Mexico. Of the 86 troglomorphic species, 39 (i.e. 45%) occur in Mexico, followed by Jamaica and the Canary Islands (7 species each), Galapagos Islands, Cuba, Madagascar, and Laos (4 species each). A more biologically meaningful comparison between regions rather than countries gives the same picture (Fig. 7): 40 species in Central America (following the United Nations geoscheme; <https://unstats.un.org/unsd/methodology/m49/>), followed by the Greater Antilles (11 species) and mainland SE Asia (9 species). The same pattern is observed when considering only the most strongly troglomorphic (i.e. eyeless) species: nine of 21 species (i.e. 43%) occur in Central America (all of them in Mexico) (Fig. 8).

A second striking pattern is the prevalence of islands. Apart from Mexico, most troglomorphic species occur on islands: discounting the 40 Central American troglomorphs, 30 of the remaining 46 troglomorphic species (i.e. 65%) occur on islands. This apparent bias is even more pronounced when considering only the most strongly troglomorphic (i.e. eyeless) species: ten occur on islands, only two eyeless species are neither Mexican nor from an island (*Metagonia diamantina* Machado et al., 2011 from Brazil and *Belisana khanensis* Yao & Li, 2013 from Laos).

Europe (incl. Turkey) appears like a hotspot in Fig. 10, but this refers to caves inhabited by pholcids (see below) and not to species diversity of troglomorphic pholcid spiders. Only three troglomorphic pholcids are known from Europe: two putative species of *Stygopholcus* on the Balkan Peninsula and one species of *Hoplopholcus* in Turkey (Table 1).

Caves and alpha-diversity

Native species of Pholcidae have been collected from 1000 caves, most of which are located in three geographic regions: Central America and the Caribbean; Dinaric Alps and eastern Mediterranean; and mainland SE Asia including southern China (Fig. 10). In the large majority of these caves (909, i.e. 91%) a single representative of Pholcidae has been found (Fig. 9); 76 caves contained two species; only 15 caves were found to contain more than two species. Of the 91 caves with more than one species, 36 (i.e. 40%) occur in Mexico, followed by Puerto Rico (6 caves), Cuba (5 caves), Brazil, Hispaniola, Crete, and Thailand (4 caves each). The highest known diversity has been reported from Puong Cave, Ba Be National Park, Vietnam (5 species), followed by Cueva de la Capilla, Tamaulipas, Mexico (4 species) and Cueva Aguas Buenas, Puerto Rico (4 species).

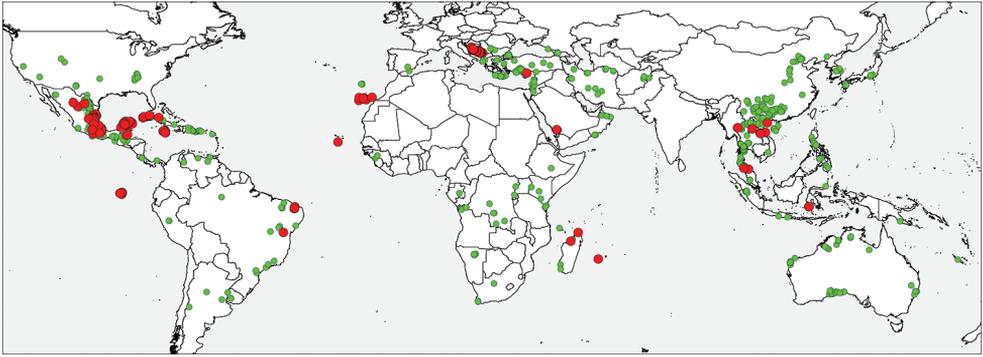


Figure 10. Geographic distribution of 1000 caves in which native pholcid spider species have been found. Green: non-troglophic; red: troglomorphic.

Discussion

Uneven systematic distribution

One of the most prevalent patterns in subterranean biology is that the large majority of troglomorphic animals belong to a relatively small number of major taxa (e.g., Moldovan 2012, Christiansen 2012). Unsurprisingly, this pattern is also found in Pholcidae. Five of the currently recognized 77 extant genera contain 75% of the troglomorphic species. To some degree, this might simply result from certain genera being present in cave-rich regions: the genera *Anopsicus* and *Metagonia* (that contain the majority of troglomorphic pholcid species) happen to be present in cave-rich countries like Mexico, Jamaica, and Cuba. Genera endemic to, for example, South America may have fewer troglomorphs simply because South America's carbonate outcrop area is much smaller than that of North America including Central America and the Caribbean (Ford and Williams 2007). However, the uneven systematic distribution remains when considering only cave-rich areas. Mexico, for example, is home to several species-rich pholcid genera in addition to *Anopsicus* and *Metagonia*, yet they contain very few troglomorphic species: while 38 of 92 Mexican species in *Anopsicus*, *Metagonia*, and *Psilochorus*, are troglomorphic (i.e. 41%), only three of the 76 species in the remaining seven native Mexican genera are troglomorphic (i.e. 4%).

Part of an answer for this mysterious pattern may come from a recent study on diversification patterns within the family (J. Eberle, D. Dimitrov, A. Valdez-Mondragón, B.A. Huber, unpublished data). Shifts among different microhabitats such as leaf-litter, large sheltered spaces, and undersides of life leaves (i.e. green leaves in the vegetation as opposed to dead leaves in the leaf litter) were not homogeneously distributed among the family but concentrated in two of the five subfamilies: Pholcinae and Modisiminae. This evolutionary ecological flexibility or 'evolvability' was thought to be among the main drivers of species diversification in those two subfamilies. It may also partly account for the uneven systematic distribution of troglomorphisms, where most troglomorphic pholcid species are in Pholcinae and

Modisiminae (Fig. 4). This might explain why certain species-rich genera that are frequently found in caves, such as *Physocyclus* in Mexico or *Trichocyclus* Simon, 1908 in Australia have not evolved troglomorphic species: both belong to Arteminae. On the other hand, it does still not explain the few troglomorphs in some genera of Modisiminae and Pholcinae in cave-rich regions, such as *Modisimus* Simon, 1893 and *Ixchela* Huber, 2000 in Mexico, or *Belisana* Thorell, 1898, *Khorata* Huber, 2005, and *Pholcus* in SE Asia.

Uneven geographic distribution

A second common and equally poorly understood phenomenon is that widespread taxa have troglomorphic representatives in certain geographic regions only (e.g., Deharveng et al. 2012). Pholcidae also follow this trend, with a remarkable concentration of troglomorphic taxa in Mexico, followed by mainland SE Asia and some islands, and very few troglomorphic species in mainland South America and mainland Africa, and none in Australia. In analogy to the argument above, this might simply result from the fact that certain taxa that are particularly preadapted to subterranean life happen to be present in certain regions and not in others. For example, *Anopsicus* has many troglomorphic species and happens to be restricted to Central America and the Greater Antilles. The case of *Metagonia* seems to contradict this idea. *Metagonia* is widely distributed from Argentina to the U.S.A., with similar numbers of species available from South America (incl. the Galapagos Islands) (58) and North America (incl. Central America and the Caribbean) (57). Troglomorphic species in *Metagonia*, however, are very unevenly distributed: only five species in South America versus 19 species in North America.

Several factors are likely to contribute to the apparent uneven geographic distribution: (1) The uneven distribution of carbonate outcrop (see above); it has been argued before that the best predictor of subterranean species diversity is the availability of habitat expressed by the number of caves in a region (Christman and Culver 2001, Culver et al. 2003). (2) The lower tendency for high energy caves to contain troglomorphic species (see *Tropical versus temperate caves* below). (3) Research biases. The latter also affects the uneven systematic distribution above, but is difficult to quantify. It includes collectors' as well as taxonomists' biases. India, for example, does not have a single record of a cave-dwelling pholcid, but this may simply result from the fact that India is among the most poorly studied countries regarding pholcid spiders in general. Mexican pholcids, on the other hand, have for many decades attracted the focused attention of collectors, and a single taxonomist (Willis J. Gertsch) has devoted a number of large papers to Mexican pholcids, with particular attention to cave-dwelling species (Gertsch 1971, 1973, 1977, 1982, 1986). Without Gertsch, Mexico would currently count only six troglomorphic pholcid species instead of 39. Some countries rich in troglomorphic pholcids may simply not have had the lucky combination of skilled and enthusiastic collectors and a dedicated taxonomist.

Surprising and difficult to explain is the over-representation of troglomorphic species on islands. This does not seem to be a widespread pattern in subterranean animals; there is no good reason to assume that collectors and taxonomists have been biased towards working on island faunas; and the ages of the islands vary widely from a few million years (Galapagos, Canaries, Réunion) to >100 million years (Greater Antilles, Cape Verde, Madagascar).

Tropical versus temperate caves

Pholcidae have most of their species diversity in the tropics and subtropics and thus do not contribute directly to the debate about the apparent higher number of troglomorphic species in temperate rather than in tropical regions. The historical bias of speleology and taxonomy towards European and North American cave faunas is uncontested (cf. Moldovan 2012, Deharveng and Bedos 2012, Gallão and Bichuette 2018). Nevertheless, for certain taxa such as most insects the trend towards a relatively low diversity in tropical caves does not seem to disappear with a more comprehensive study of tropical faunas. On the other hand, spiders and arachnids in general, may not follow this pattern but, on the contrary, occupy in the tropics the predatory positions that are in temperate caves held by beetles and other arthropods (Reddell 2012). However, a comprehensive review of subterranean arachnids or just spiders has apparently never been attempted.

Outlook and promising projects

Subterranean pholcid diversity is still poorly known in most parts of the world and it will clearly need massive long-time efforts by collectors and taxonomists to substantially change this. This section focuses instead on a few particular questions that seem relatively easy to answer within a limited period of time and with reasonable effort.

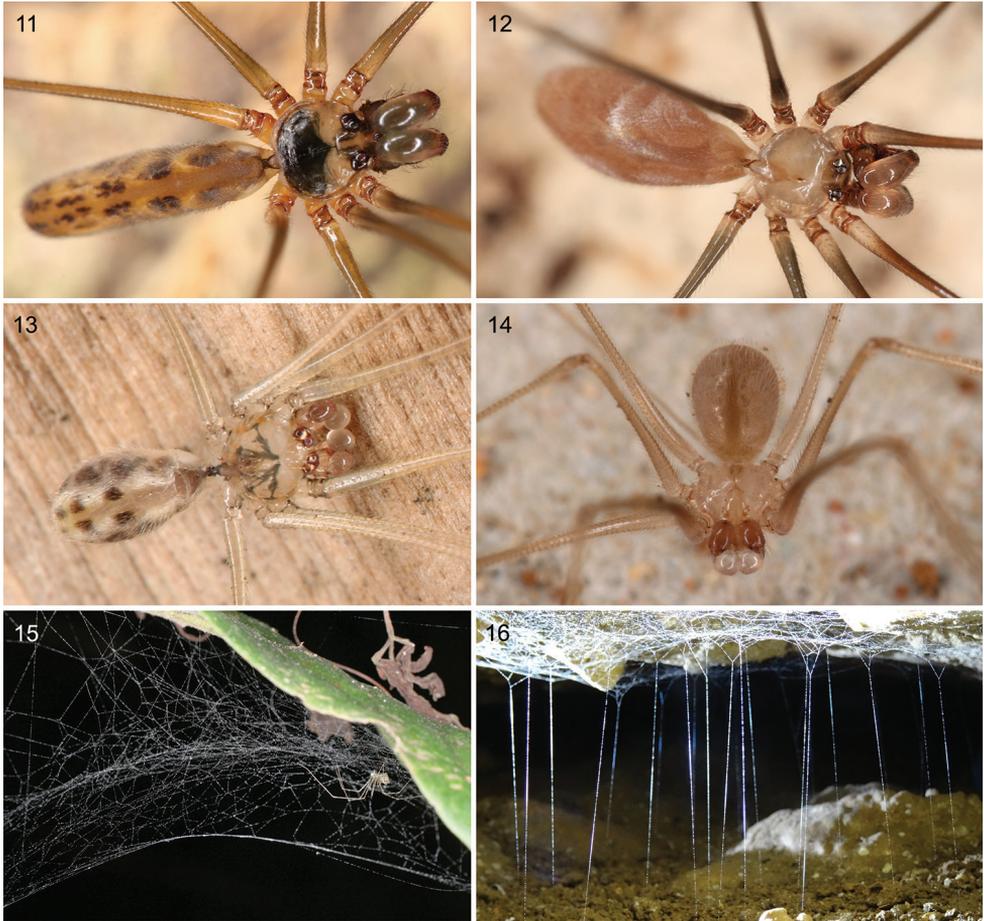
Troglomorphisms. There is basically no information in the literature about troglomorphisms in pholcid spiders beyond simple qualitative remarks about those troglomorphisms that are easily observed (Figs 11–14): eye reduction, pigment loss, and leg elongation. Theory predicts that several additional traits may be affected. For example, egg size is expected to increase while egg number is expected to decrease; the concentration of sensilla is expected to increase; excitement (reaction to disturbance) is expected to decrease; and longevity and body size are expected to increase (Culver 1982, Hüppop 2012). For several species the classification as troglomorphic or not is quite ambiguous, and this introduces noise in the analyses. A quantification of troglomorphisms would clarify limits and probably reveal interesting patterns. For example, a simple plot of relative leg lengths (tibia 1/carapace width) in 55 species of *Anopsicus* (taken from Gertsch 1982) shows a slight increase in leg length in species found only in caves compared to epigeal species, but a strong increase in species with reduced

eyes or without eyes. Individual taxa offer additional possibilities to study particular troglomorphisms. For example, the South American *Mesabolivar aurantiacus* (Mello-Leitão, 1930) is sexually dimorphic in coloration, with males (in particular large males) having the third femora bright red rather than dark brown. In a recently discovered cave population in the Brazilian state Amazonas, the male third femora showed little or no difference to other (and female) femora (Huber 2018). The significance of this sexual dimorphism remains unknown. Another potential troglomorphism concerns web structure. Food shortage is considered a major selective pressure responsible for troglomorphisms (Hüppop 2012, Poulson 2012), and prey-capturing devices such as spider webs should in theory be affected. This has never been studied in any pholcid (nor, to my knowledge, in any spider), but the web of a recently discovered Brazilian *Metagonia* species with evanescent eyes was indeed very different from ‘usual’ webs of *Metagonia* and other pholcids (B.A. Huber, unpublished data): instead of representing a sheet with tumble lines (suitable for intercepting flying prey) it included an exceptionally high number of gum-foot lines (suitable for catching walking prey; cf. Japyassú and Macagnan 2004) (Figs 15–16).

Habitats of apparently cave-restricted species. Of the 473 pholcid species found in caves, 218 have only been found in caves yet do not show any obvious troglomorphisms. In theory, many of them might indeed be specialized to the cave entrance ecotone, but the more plausible hypothesis is that the actual habitat of most of these species is in fact the shallow subterranean habitat (cf. Deeleman-Reinhold and Deeleman 1980, Culver and Pipan 2009). Good candidates to test this hypothesis are (1) non-troglomorphic species with numerous cave-records but no records so far from outside caves (e.g., *Modisimus boneti* Gertsch, 1971; *Metagonia blanda* Gertsch, 1973; *Hoplopholcus trakyaensis* Demircan & Topçu, 2017; *Uthina sai yokensis* Yao & Li, 2016; *Ixchela pecki* (Gertsch, 1971); and *Modisimus mitchelli* Gertsch, 1971); and (2) troglomorphic species that are known from numerous caves each (see examples above).

The genus Anopsicus. In Central America and the Caribbean, the genus *Anopsicus* offers an ideal opportunity to study a large radiation that includes both epigeal and hypogean species. Judging from the small ranges of epigeal species, it seems likely that hypogean species have evolved repeatedly from epigeal ancestors, but a phylogeny of the genus is not available.

The genus Aymaria. On the Galapagos Islands, four nominal species of *Aymaria* Huber, 2000 are currently recognized, two of them epigeal (on most or all islands), two hypogean and eyeless (on Santa Cruz and Floreana). The genitalia of all four ‘species’ appear essentially indistinguishable, and I have argued previously that only two species might be present, one epigeal, one hypogean (Huber 2000). In the meantime, several species in a range of major taxa are known to include epigeal and troglomorphic hypogean populations (e.g., Verovnik et al. 2004, Fong 2012, Gross 2012), and interspecific variation in troglomorphic traits has also been found in spiders (Shear 1978; further references in Mammola and Isaia 2017). As a consequence, the null hypothesis should be that only a single species of *Aymaria* occurs on the Galapagos Islands. Beyond species limits, *Aymaria* on Galapagos offers the opportunity to study



Figures 11–16. Examples of non-troglophoric and troglomorphic pholcid spiders and their webs. **11–12** Two undescribed species of *Uthina* from Bali and Sulawesi; note smaller eyes and paler color in cave-dwelling species (**12**). **13–14** Two undescribed species of *Metagonia* from Brazil (Pernambuco, Rio Grande do Norte); note evanescent eyes and pale coloration in cave-dwelling species (**14**). **15–16** ‘Typical’ pholcid web of epigeal *Metagonia bonaldoa* **15** domed sheet with tumble lines above sheet; Brazil, Santa Catarina), and unusual web of cave-dwelling *Metagonia potiguar* **16** sheet attached to rock surface and many vertical gum-foot lines; Brazil, Rio Grande do Norte). Photos BAH.

a case where strong troglomorphy has evolved relatively rapidly, as judged from the age of the islands (~ 1 – 2.5 My for Santa Cruz and Floreana) and the apparent lack of genitalic differences.

Sympatric species. In 91 caves, two or more species of Pholcidae were found to coexist, but beyond anecdotal observations there is almost nothing known about interspecific competition and niche partitioning. The only quantitative data describe spatial segregation in *Micropholcus piau* Huber et al., 2014 and *Mesabolivar spinulosus* (Mello-Leitão, 1939) in a cave in Brazil (by L.S. Carvalho, reported in Huber et al.

2014). Several alternatives to spatial segregation are plausible and have been reported in other spiders (Arnedo et al. 2007). Of particular interest are caves with multiple species of the same genus (e.g. Hanging Gardens Cave in California with three species of *Psilochorus*; Puong Cave in Vietnam with three species of *Khorata*).

Conclusions

Pholcid spiders are common inhabitants of tropical and subtropical caves, and many species in several genera have evolved strong troglomorphisms. Despite of some ‘noise’ in the data (e.g., uncertainty in classifying some species as troglomorphic or not; uncertainty of exact geographic coordinates of some caves; uncertainty in taxonomic status of some ‘species’) a few general conclusions can be drawn:

- A total of 473 native pholcid species have been found in caves. This means that about 25% of the species currently known to exist are either occasionally or exclusively found in caves. Most cave-dwelling pholcids are not troglomorphic and thus presumably not obligate cave-dwellers but ‘troglophiles’.
- About 86 species of troglomorphic pholcid species have been found, including 21 eyeless species and 21 species with strongly reduced eyes. Troglomorphic pholcids exist in 20 of currently 77 extant genera, but *Anopsicus* and *Metagonia* alone include almost half of the troglomorphic species.
- Mexico is by far the richest country in terms of troglomorphic pholcids, followed by several islands (Greater Antilles, Galapagos, Canaries, Réunion, Cape Verde, Madagascar, Sulawesi, New Guinea) and mainland SE Asia. The apparent dominance of Mexico may partly be due to collectors’ and taxonomists’ biases.
- Native pholcid spiders have been found in 1000 caves. In most of these caves, only one pholcid species has been found, but two and more species (up to five) have been found in 91 caves.
- Most troglomorphic pholcids belong to two subfamilies (Pholcinae, Modisiminae), very few belong to Smeringopinae and Arteminae, and none to Ninetinae. This is in agreement with the recent finding that within Pholcidae, microhabitat changes in general are concentrated in Modisiminae and Pholcinae.

Acknowledgements

I am grateful to colleagues for help with georeferencing localities: L.S. Carvalho, P. Oromí, M. Pavlek, P. Sprouse, and A. Váldez-Mondragón. I thank C. Hamilton and an anonymous reviewer for their constructive criticism and for their help in improving the manuscript. Financial support was provided by the German Research Foundation (DFG, project HU 980/11-1).

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A new species of spider (Araneae, Linyphiidae, *Islandiana*) from a southern Indiana cave

Marc A. Milne¹, Elizabeth Wells²

¹ Department of Biology, University of Indianapolis, Indianapolis, IN, USA 46227 ² 4932 Red Horizon Blvd, Indianapolis, IN, USA 46221

Corresponding author: Marc A. Milne (milnem@uindy.edu)

Academic editor: O. Moldovan | Received 8 April 2018 | Accepted 2 June 2018 | Published 12 June 2018

<http://zoobank.org/69DA1C98-31EC-45ED-8C6B-F89FA7C7DEC8>

Citation: Milne MA, Wells E (2018) A new species of spider (Araneae, Linyphiidae, *Islandiana*) from a southern Indiana cave. Subterranean Biology 26: 19–26. <https://doi.org/10.3897/subtbiol.26.25605>

Abstract

The genus *Islandiana* (Araneae, Linyphiidae) was erected by Braendegaard in 1932 and is comprised of 14 species, most of which are native to North America. Herein we add a 15th species, *Islandiana lewisi* sp. n., from southern Indiana, USA. This species resembles both *I. flavoides* Ivie, 1965 and *I. cavealis* Ivie, 1965, the latter of which is geographically-close.

Keywords

Erigoninae, Midwest, subterranean, underground, karst, sheet-web spider

Introduction

The linyphiid genus, *Islandiana* Braendegaard, 1932 possesses 14 species (World Spider Catalog 2017), most of which are in the United States. However, two species in the genus, *I. falsifica* Keyserling, 1886 and *I. cristata* Eskov, 1987, have Holarctic distributions and another, *I. princeps* Braendegaard, 1932, can be found in North America as well as Greenland and Iceland. The remaining species, 11 out of the 14, are restricted to the US and Canada (World Spider Catalog 2017). Most of the species in *Islandiana* may be found under rocks near the ground in forests, fields, mountains, beaches, and a

variety of other habitats in their known range (Ivie 1965). Four species are known only from caves in the US (Ivie 1965). The genus consisted of only two species, *I. falsifica* and *I. princeps*, upon its creation by Braendegaard (1932), but had two species added to it by Kaston (1948; *I. flaveola* Banks, 1892 and *I. longisetosa* Emerton, 1882). A revision of the genus was conducted by Ivie (1965) that added nine new species. A single species from Russia, Alaska, and Canada was later added by Eskov (1987).

A re-examination of new distribution records listed for the state of Indiana by Milne et al. (2016) revealed a misidentification of "*I. cavealis*." These specimens – collected from Stygian River Cave in southern Indiana were found not to be *I. cavealis*, but an undescribed species within the same genus. Herein we describe this species.

Methods

All measurements are in millimeters. Measurements were taken electronically using a Leica M165C stereoscope, Leica DMC2900 attached digital camera, and associated Leica Application Suite software (LAS Ver. 4.9.0 [Build: 129], Leica Microsystems, Switzerland) at the University of Indianapolis. Photographs were taken with specimens placed in glass dishes containing white sand and ethanol. Carapace width was measured at the widest part of the carapace. Specimens were prepared for SEM photography by dehydration using ethanol solutions that graduated from 70% to 100% over 5-minute intervals. Specimens were then placed in Hexamethyldisilazane (HMDS; 99+%; Alfa Aesar, Heysham, England) for 5 minutes before being removed and placed on filter paper to dry for 5 minutes. Specimens were then mounted on SEM stubs using 12 mm Pelco Tabs (TED PELLA, Inc. - carbon conductive tabs), sputter coated in gold (Cressington Scientific Instruments, Model No. 108) and photographed using a JEOL JCM-6000 NeoScope benchtop scanning electron microscope. The holotype male and paratypes of females and males were deposited at The Field Museum of Natural History (FMNH) in Chicago, IL. *Islandiana cavealis*, *I. flavoides*, and *I. speophila* were examined on loan from The American Museum of Natural History.

Taxonomy

Islandiana lewisi sp. n.

<http://zoobank.org/0BF2BC6C-9956-43E9-A863-76D196B211F8>

Figs 1–4

Islandiana cavealis (Ivie, 1965): Milne et al. 2016: 80 (male, female, misidentification).

Type-specimen. Holotype male and paratype males and females (FMNH, Cat. No. #3260642), in 90% alcohol, with genitalia in separate microvial. Label: "O'Bannon Woods State Park, Stygian River Cave, N38°10', W086°18', (parking lot). *Islandiana lewisi*."

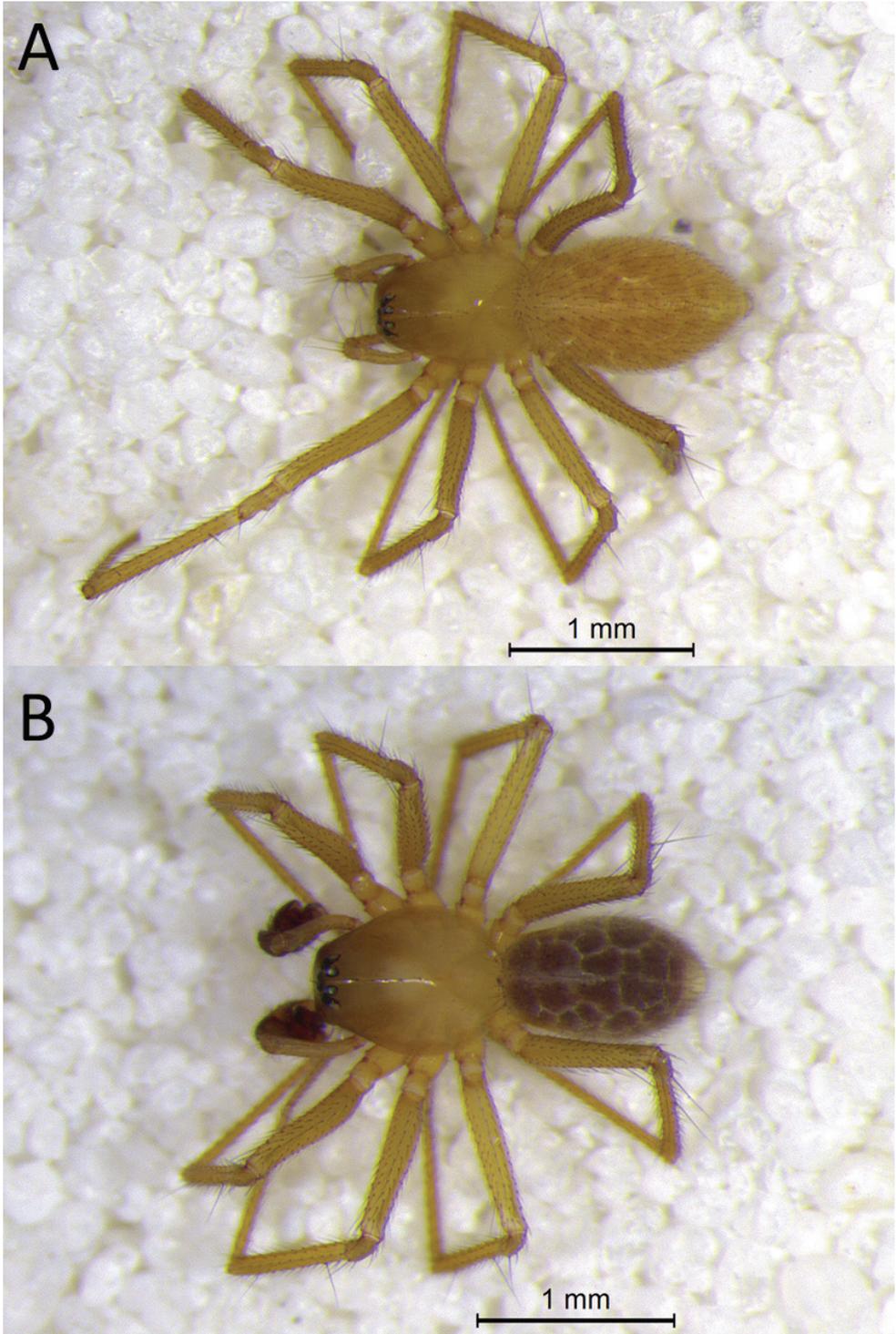


Figure 1. Habitus of *Islandiana lewisi* sp. n. **A** female **B** male.

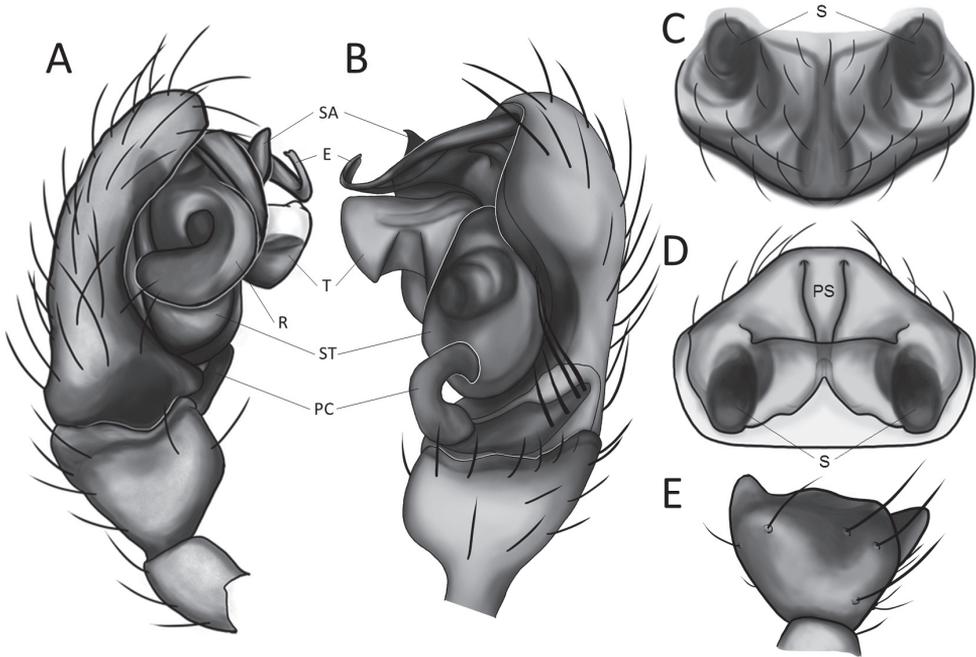


Figure 2. Illustrations of *Islandiana lewisi* sp. n. structures. **A** Left palp of male, medial view **B** Left palp of male, lateral view **C** Epigynum, ventral view **D** Epigynum, flipped – dorsal view **E** Tibial apophysis of left palp of male. E = Embolus; R = radix; SA = suprategular apophysis; ST = subtegulum; T = tegulum; PC = paracymbium; S = spermathecae; PS = posterior sclerite.

Specimen collected by M. Milne on 23 October 2016. Type-locality: USA, Indiana: Harrison County, Harrison-Crawford State Forest, Stygian River Cave (N38°10', W86°18'; exact coordinates hidden due to the ecological sensitivity of the type locality).

Examined comparative material. *Islandiana cavealis*: USA: Kentucky: Fayette County, Picadome Cave (N37°, W84°) 1 female (AMNH_IZC 00328222); collected by C. Krekeler and J. Rittmann on 1 July 1957. *Islandiana speophila*: USA: West Virginia: Pendleton County, Trout Cave (N38°, W79°) 1 female (AMNH_IZC 00328223); collected by L.G. Conrad on 4 March 1961. *Islandiana flavoides*: USA: New York: Orient (N41°, W72°) 1 male (AMNH_IZC 00328224); collected by C.R. Crosby and S.C. Bishop on 21 June 1934.

Etymology. The species name is a patronym in honor of our friend and colleague Dr Julian J. Lewis, a cave and karst specialist, a leading expert in North American isopod taxonomy, and the original collector of the species.

Diagnosis. This species is most similar to *I. flavoides* Ivie, 1965, a species native to New York, USA and keys out to this species using the key provided by Ivie (1965). However, the males of *I. lewisi* sp. n. possesses a sinuous embolus not present in *I. flavoides* (Figs 2A, 3 vs. fig. 27 in Ivie 1965). Moreover, the two enlarged setae on the paracymbium are thinner in *I. lewisi* sp. n. (Fig. 2B, 3C vs. fig. 27 in Ivie 1965) than in

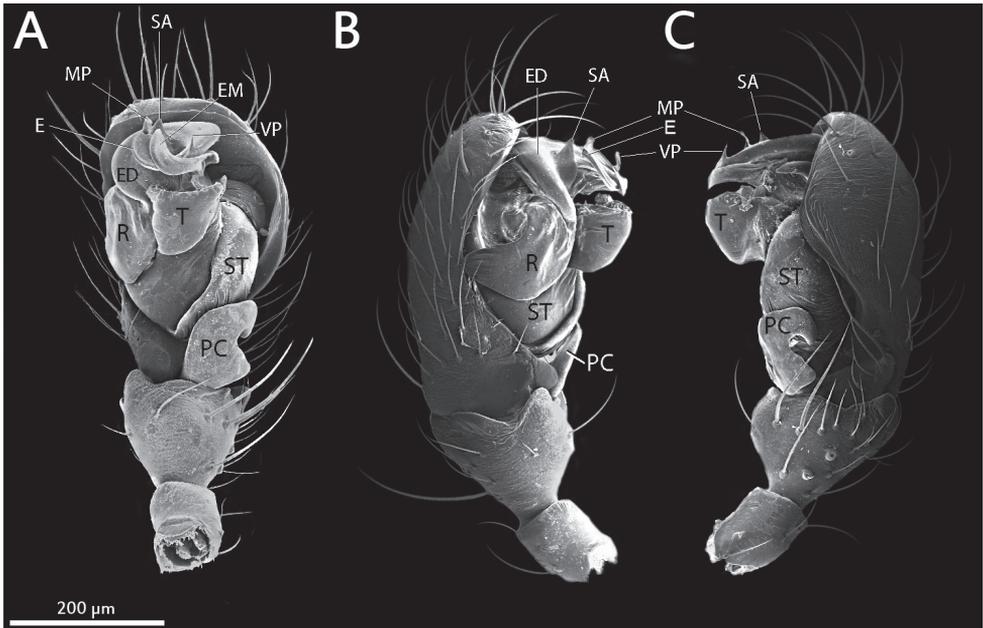


Figure 3. SEM micrographs of the left palp of *Islandiana lewisi* sp. n. **A** Ventral **B** Medial **C** Lateral. E = Embolus; ED = Embolic division; EM = Embolic membrane; MP = medial projection of ED; VP = ventral projection of ED; R = radix of ED; SA = supratregular apophysis; ST = subtegulum; T = tegulum; PC = paracymbium.

I. flavoides, and the pointed tibial apophysis is rounded in this new species (Fig. 2E vs. fig. 28 in Ivie 1965) compared to *I. flavoides*. The males and females of *I. lewisi* sp. n. are also larger (mean of 2 mm for the male and 2.2 mm for the female vs. 1.5 mm male and 1.8 mm female in *I. flavoides*). The female also differs from *I. flavoides* females in that the epigynum of the new species has a slightly longer scape, lacks the large circular posterior plate, and possesses ovoid spermathecae compared to the circular spermathecae in *I. flavoides* (Figs 2C–D, and 4). The female epigynum also resembles that of *I. cavealis*, but the *I. lewisi* sp. n. epigynum is more flattened posteriorly and the spermathecae are set more anteriorly than in *I. cavealis* (Figs 2C–D, and 4 vs. figs 50–51 in Ivie 1965).

Description. Male. (N = 2) Carapace 0.88–0.94 long, 0.69–0.75 wide. Total length 1.90–2.00. Carapace concolorous dusky yellow to tan with a small amount of black surrounding each eye. Five short setae along median line of carapace from fovea leading up to eyes, other setae between posterior median eyes and other eyes leading back to fovea. Abdomen light gray (Fig. 1B). Posterior median eyes about half the size of other eyes. Chelicerae dusky yellow with seven teeth on promargin and five denticles on retromargin. Chelicerae with mastidion low-set near teeth. Stridulatory file present with 14 stridulae. Maxillae concolorous and yellow with labium also dusky yellow. Sternum dusky yellow. Spination is as follows (only surfaces bearing spines listed): femora: I, II, III, IV v0-0-2; patellae: I, II, III, IV d0-0-1; tibiae: I, II, III d1-0-1; IV d1-0-0.

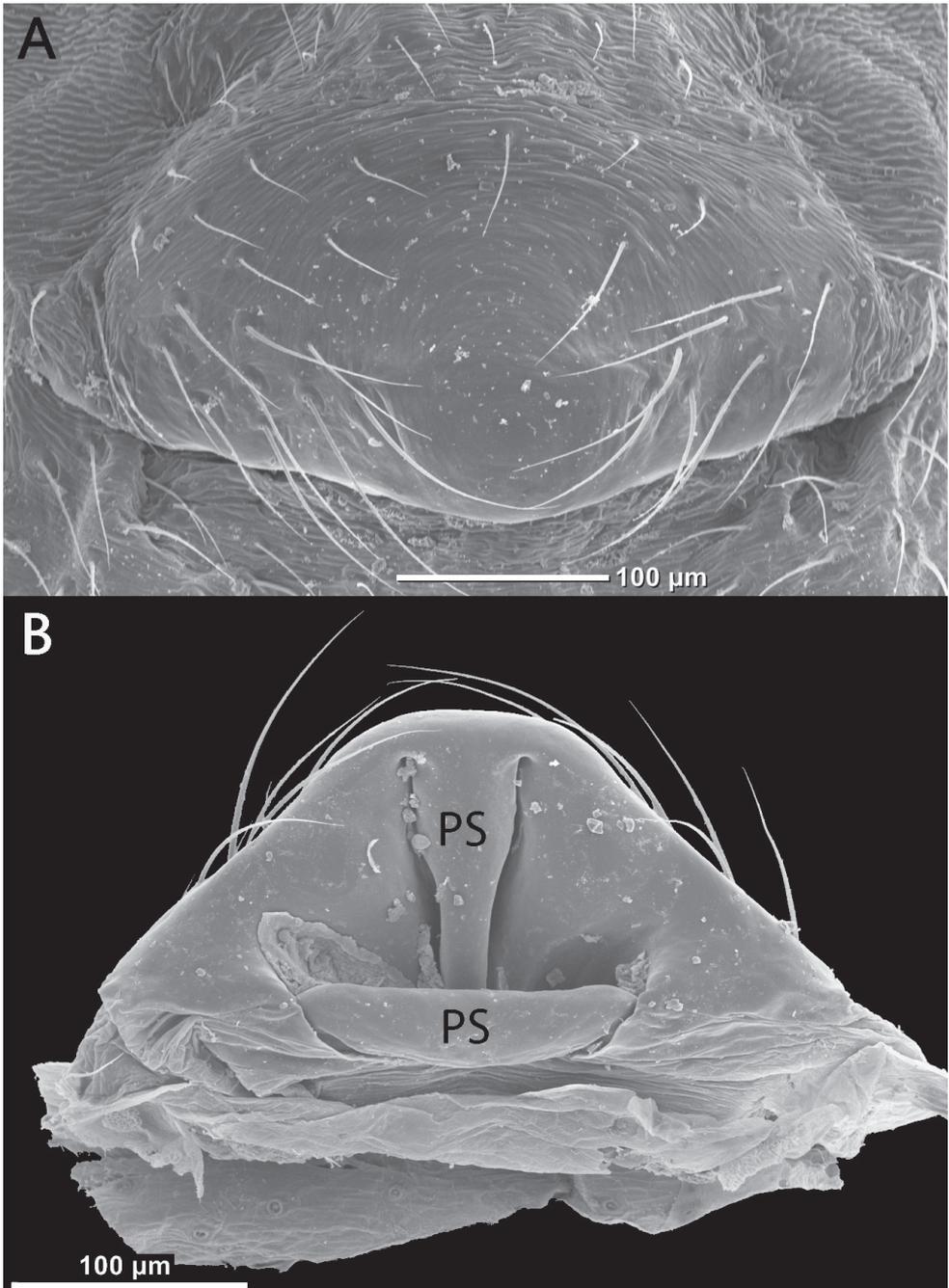


Figure 4. SEM micrographs of the epigynum of *Islandiana lewisi* sp. n. **A** Epigynum, ventral view **B** Epigynum, flipped – dorsal view. PS = posterior sclerite.

Mature male palp with tibial apophysis rather short and stout (Fig. 2E). Paracymbium notched, curved in a thick “C”-shape, and with two long thin setae and two short thin setae emanating from a deep section of the structure (Figs 2B, 3C). Large tegulum above subtegulum that extends out past subtegulum. Embolus within embolic membrane curves around in full 360° loop near anterior end of cymbium. Suprategular apophysis projects anteriorly within the center of the embolic loop. Ventral and medial processes project anteriorly from embolic division (Fig. 3).

Female. (N = 4) Carapace 0.88–1.00 long, 0.69–0.79 wide. Total length 2.1–2.3. Carapace coloration and setae pattern same as male. Abdomen light yellowish to tan (Fig. 1A). Chelicerae dusky yellow with six teeth on promargin and five denticles on retromargin. Chelicerae lacking mastidion. Stridulatory file present with 16–18 stridulae. Maxillae, labium, and sternum coloration same as in male. Spination same as in male.

Epigynum similar to *I. cavealis*. Epigynum protruding ventrally at distal portion; conical shape, posterior sclerite wider distally with flattened triangular shape proximally (Figs 2C–D, 4). Triangular shape of sclerite difficult to see using light microscopy.

Distribution. Known only from the type locality.

Habitat. Stygian River Cave is short and consists of a room filled with large, wet, muddy rocks and boulders. Because of the short length of the cave, the biota is likely heavily influenced by low winter humidity. The cave sits at the confluence of the Blue and Ohio rivers and at least part of the cave is regularly underwater when these rivers rise. These specimens were largely found in webs in between the large boulders within the largest room of the cave. The type locality was only visited once and were therefore only collected once, on October 23rd, 2016.

Acknowledgements

We would like to thank Julian J. Lewis for showing us the cave and acquiring permits, Don Buckle for taxonomic help, Nina Sandlin for the counting of stridulae, Chris Schmidt and Kevin Gribbins for SEM help, Louis Sorkin and Lorenzo Prendini for AMNH specimen loans, and two anonymous reviewers who made improvements to prior versions of this manuscript. Cave access permitted through IDNR approval received on January 26th, 2016.

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First record of the family Stenasellidae (Crustacea, Isopoda) in Iran with the description of a new cave-dwelling species

Yaser Bakhshi¹, Saber Sadeghi¹, Giuseppe Messina²

1 Department of Biology, Shiraz University, Shiraz, Iran **2** Institute for the Study of Ecosystems, UOS Firenze, Italy

Corresponding author: *Saber Sadeghi* (ssadeghi@shirazu.ac.ir)

Academic editor: *O. Moldovan* | Received 18 April 2018 | Accepted 5 June 2018 | Published 20 June 2018

<http://zoobank.org/58A21349-0B0C-4938-A76D-F4D2C1187C08>

Citation: Bakhshi Y, Sadeghi S, Messina G (2018) First record of the family Stenasellidae (Crustacea, Isopoda) in Iran with the description of a new cave-dwelling species. *Subterranean Biology* 26: 27–38. <https://doi.org/10.3897/subtbiol.26.25950>

Abstract

A new cave isopod species of the genus *Stenasellus* Dollfus, 1897 (Stenasellidae) from Iran is reported and described. The new species, *Stenasellus tashanensis* sp. n. was found in Tashan Cave (Khuzestan province, southwest Iran). A complete description and drawings of the new species are provided. This new species is morphologically close to *S. vermeuleni* Magniez & Stock, 2000 from Oman.

Keywords

Isopods, *Stenasellus*, Stygobiont, Cavernicolous

Introduction

With approximately 1.65 million km², Iran is a vast country in the Western Palearctic. Iran hosts a high variety of habitats including more than 2000 caves, mostly located in the Zagros Range (Raeisi et al. 2012). This mountain chain is rich in caves and has been the object of numerous speleological explorations dating back to the 1970's (Juberthie et al. 2001).

In the last two decades, several studies have been conducted regarding the fauna of Iranian caves (e.g. Karaman 1998; Casale and Quéinnec 2001; Sadeghi et al. 2014;

Esmacili-Rineh et al. 2015, 2017; Malek Hosseini et al. 2016). Nonetheless, the subterranean fauna of Iran is still poorly known. Several species of oligochaetes, crustaceans (Copepoda, Isopoda, Amphipoda), hydracarids, insects, fish and amphibians, have been collected and described from Iranian caves (Juberthie et al. 2001; Esmacili-Rineh et al. 2017; Moradmand 2017).

The family Stenasellidae, with more than 70 described species, is limited to subterranean aquatic habitats including karstic or phreatic springs (Wilson 2008). The first specimens of this family were discovered in the subterranean waters from SW France (Viré 1896). Later, Dollfus (1897) examined the specimens and described them as *Stenasellus virei* Dollfus, 1897. The genus *Stenasellus* Dollfus, 1897 has a wide distribution in the Old World from the Far East (Borneo, Cambodia, Sumatra, Thailand) to Central Asia (Turkmenistan), Arabian Peninsula (Oman, Socotra), East Africa (Kenya and Somalia), and southern Europe (Iberian Peninsula, southern France, Corsica and Sardinia) (cf. Magniez and Stock 2000).

In 1999, in a paper dealing with four new *Stenasellus* species from Oman, Magniez and Stock predicted the presence of Stenasellid isopods in Iran: "...we might ask if they (Stenasellidae) are present in intermediate geographical regions. Yemen, southwestern Oman and southern Iran could harbour this fauna...". In fact, by describing *Stenasellus tashanensis* sp. n. from Iran in the present study, their prediction has been confirmed and the antiquity and wide Tethyan distribution of the genus *Stenasellus* as stated by Magniez (2005, 2008), have been ratified. We collected some Stygobiont isopods from Tashan Cave (Figure 1), in southwest of Iran during a field trip on January 1, 2016, which on closer examination appeared to belong to a new species of the genus *Stenasellus*.

Material and methods

All the specimens (two males and three females) were collected using a small hand net in Tashan cave, located 12 km north of Behbahan city in Khuzestan Province (southwest Iran). Tashan cave is located on the western side of the Zagros Mountains (Figure 1) in Neozoic limestone. Tashan Cave is about 150 m long, with two stagnant water pools which are connected to each other by small channels. The depth of the first pool, where the specimens were found, was about 1 m in the deepest part. The second pool was deeper, about 4–5 m in the deepest part. No specimen was found in the second one. The bottom of both pools is represented by greyish-white fine sediments. The pools water had the following physico-chemical characteristics: TDS = 350 mg/l, Salinity = 0.35%, Conductivity = 717 μ S/cm, pH = 7.8, Temperature = 25.6 °C.

The collected specimens were preserved in ethanol 96%. One male and one female were dissected and microscopic slides of their body parts were prepared using Euparal mounting medium. To study the specimens, a stereomicroscope (Zeiss Stemi SV 11 Apo) and a compound microscope (Olympus BX 50) were used. For the chaetotaxy, Watling (1989) was followed.

The type specimens of *Stenasellus* species described from East Africa deposited at Zoological Museum of University of Firenze, Italy (MZP) were compared with our specimens by the third author, but since the authors did not have access to all the type material of the congeneric species, to compare them with the species in the present study, data (including descriptions and illustrations) from the literature (Birstein and Starostin 1949; Magniez and Stock 2000) were also used for this procedure.

Results

Order Isopoda Latreille, 1817

Suborder Asellota Latreille, 1802

Family Stenasellidae Dudich, 1924

Genus *Stenasellus* Dollfus, 1897

Stenasellus tashanensis Bakhshi & Messana, sp. n.

<http://zoobank.org/049C8EB6-2F82-4459-9200-9FCB206D4895>

Holotype. Male. 19 mm, Tashan Cave, Behbahan, Khuzestan, Iran 30°51'57.00"N, 50°10'30.09"E. 16.I.2016 leg. Y. Bakhshi (ZM-CBSU Iso 3421); Paratypes 1 male 19 mm (on three slides), 3 females (1 specimen (18, 15 mm) on two slides) same data as that of holotype (ZM-CBSU Iso 3422), maximum observed length of females is 18,15 mm, maximum observed length of males is 19 mm. The type specimens were deposited at the Zoological Museum of Shiraz University, Collection of the Biology Department (ZM-CBSU).

Diagnosis. *Stenasellus tashanensis* can be differentiated from all of the other congeneric species by the following characteristics: the shape of male pleopods I and II, 19 strong spines on dactyl and 19 strong dentate spines on propus of male peraeopod I, three sternal spines on dactylus of peraeopod 7, and seven toothed coupling hooks on endite the maxilliped which is the largest number found in any *Stenasellus* species.

Description (complementary to iconography) (Figure 2A). A relatively large *Stenasellus* with subparallel margins, maximum observed length (in males) 19 mm, maximum observed width (fourth segment) 3.8 mm. Head wider than long, with concave anterior margin, two typical round spots (otoliths of Bellonci's organs) on anterior lateral angles. Endopodites of pleopods III–V bifid. Uropods/pleotelson length ratio 0.625.

Antenna I (Figure 2B): short, less than half the length of antenna II, not reaching posterior margin of first pereonite; second article of peduncle with three plumose setae on its distal part; flagellum with up to 19 articles, with two aestetascas on distal external angle of each of the last 12 segments.

Antenna II (Figure 2C): long, reaching pereonite V; peduncle with six segments; squama on third article of peduncle (Figure 2C') sub-rounded, equipped with two pointed long setae of different sizes, flagellum with up to 55 articles.

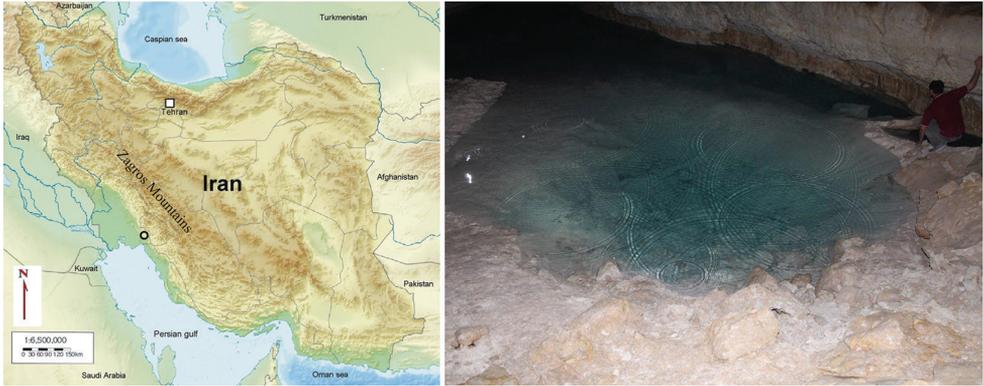


Figure 1. Left: Map of Iran, showing the location of Tashan Cave (black circle); Right: the type locality in Tashan Cave, with *Stenasellus tashanensis* sp. n.

Left mandible (Figure 2H): pars incisiva four-dentate; lacinia mobilis four-dentate; 18–20 lifting spines (12 spinulate); pars molaris with several setae.

Right mandible (Figure 2G): pars incisiva four-dentate; 29–30 lifting spines (19 spinulate); pars molaris with several setae.

Mandibular palp (Figure 2I): tri-articulate; first article with two setae on distal margin, one of them five times longer than the other, second article with several setae including a group of soft short ones and another group of combed long ones, third article with several spines of different sizes, the five distal ones significantly longer than the others.

First maxilla (Figure 2E): exite with 12 dentate combed setae and 8 plumose ones, endite with 3 strong and 2 thinner setose setae.

Second maxilla (Figure 2F): external exite with 10 and median exite with 11 falcate, combed setae; internal exite with 25 denticulate chaetae.

Maxilliped (Figure 2D): with 6 to 7-toothed coupling hooks on endite and 13 plumose setae on distal margin, 18 glabrous setae on the outer surface; palp five-segmented, first article with a single simple seta on the distal inner angle.

Peraeopod I (Figure 3A): strong, subchaelate; with 19 strong setae on dactyl and 19 strong denticulate setae and several simple setae of different sizes on propus.

Peraeopods II–VII (Figure 3B–G): progressively larger; basis with 9 to 12 long plumose setae; propus and carpus with plumose seta on distal tergal angles; Peraeopods 2–4 also with pectinate seta in same position; dactylus with strong medial spines, three in peraeopod 7 and two in peraeopods 2–6.

Genital papilla (Figure 4H): subcylindrical, passing anterior margin of second pleonite.

Male Pleopod I (Figure 4A): basipodite with one coupling hook; Exopodite with about 33 setae, the eighteen internal of which plumose.

Female pleopod II (Figure 4F): subtriangular; with a strongly convex outer margin, five setae on the sternal surface and ten plumose setae on the distal margin.

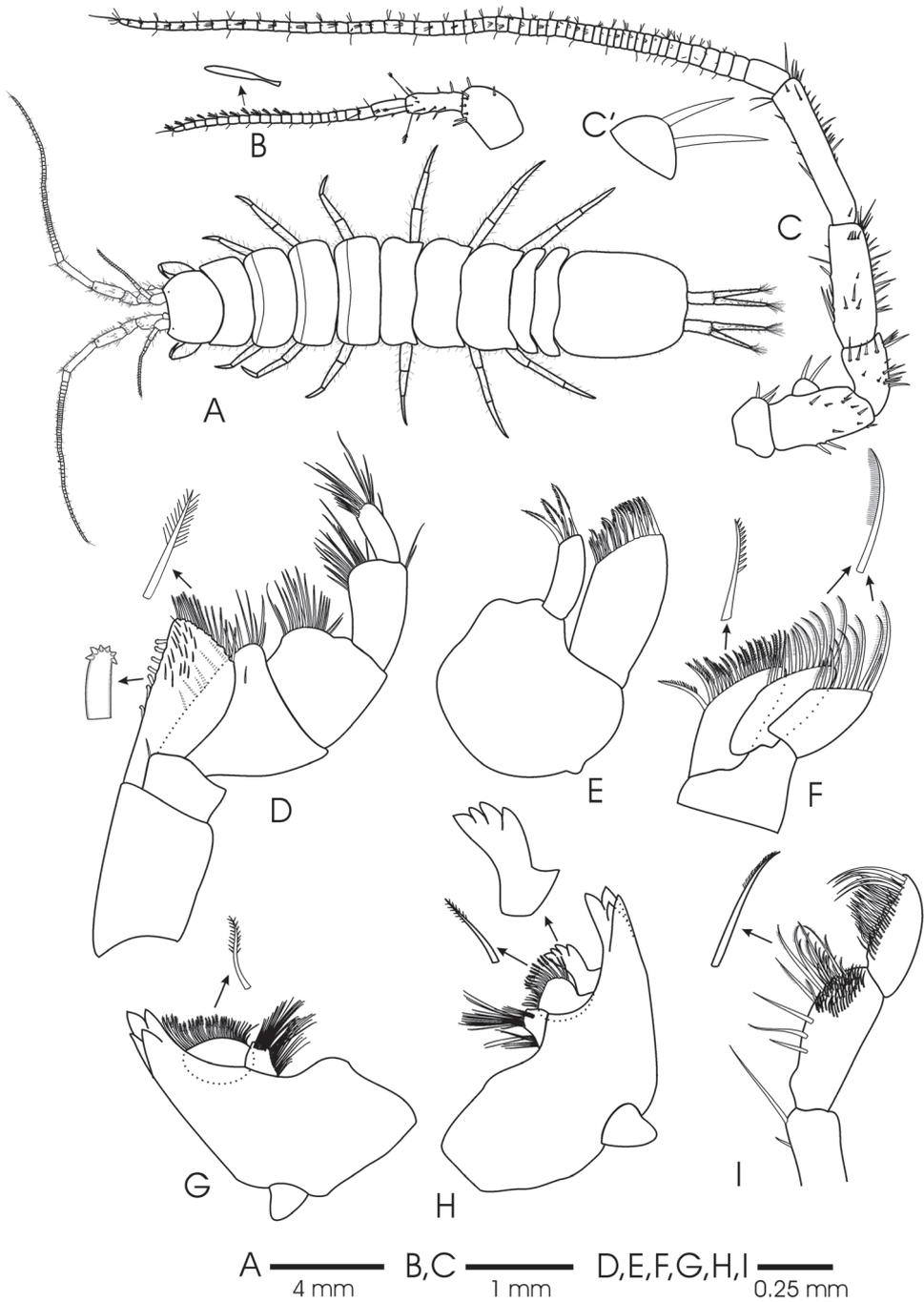


Figure 2. *Stenasellus tasbanensis* sp. n. male. **A** habitus (dorsal view) **B** antenna I **C** antenna II **C'** squama of antenna II **D** maxilliped **E** maxilla **F** maxilla II **G** right mandible **H** left mandible **I** mandibular palp.

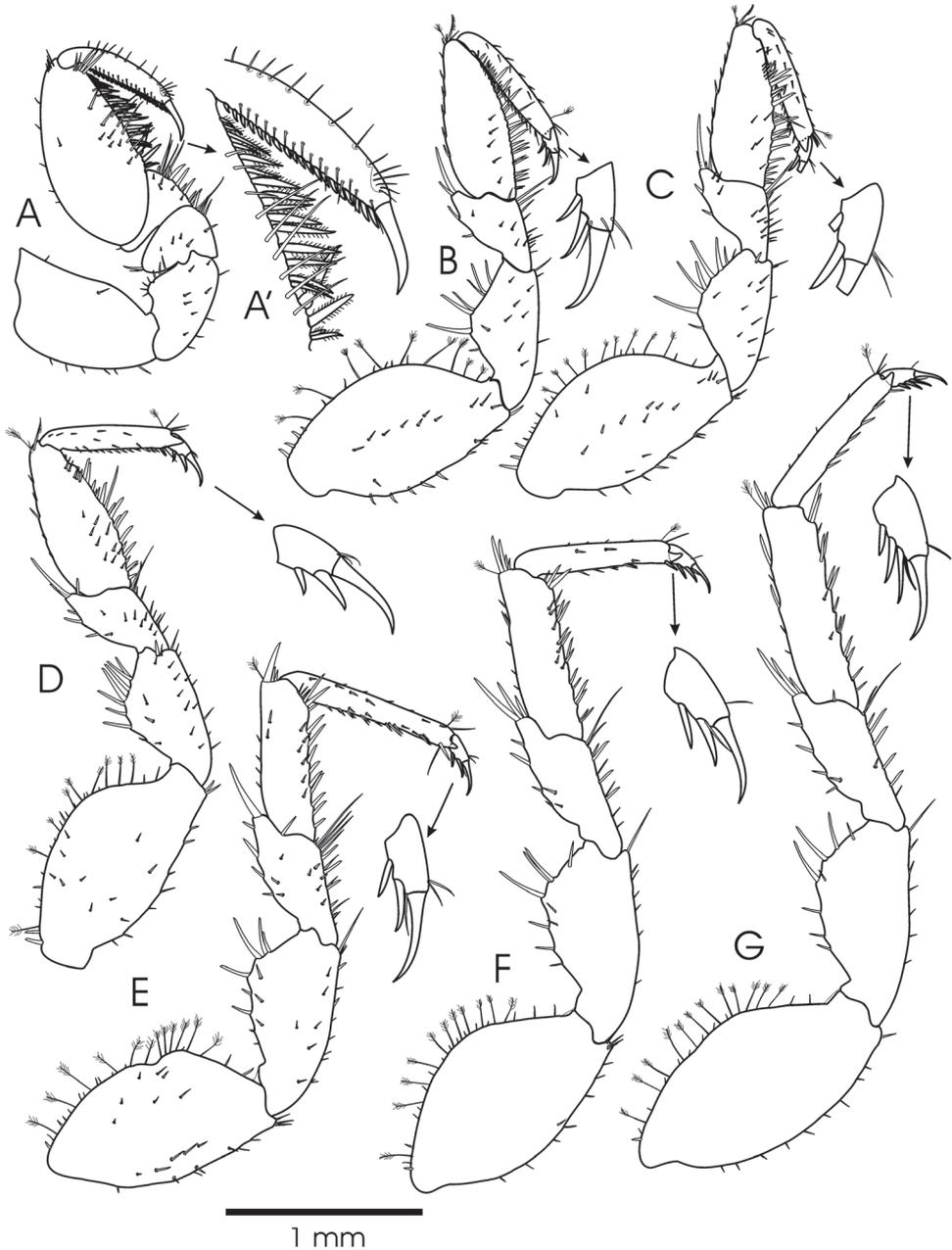


Figure 3. *Stenasellus tashanensis* sp. n. male. **A–G** peraeopods I–VII **A'** setation of propus and dactylus of first peraeopod.

Male pleopod II (Figure 4B): sympodite sub-rectangular; significantly longer than wide with subparallel external and internal margins, sternal surface with three paramedial setae; proximal article of exopodite with four glabrous setae, distal article subelliptical relatively elongated (longer than wide) with more than 40 plumose setae along the

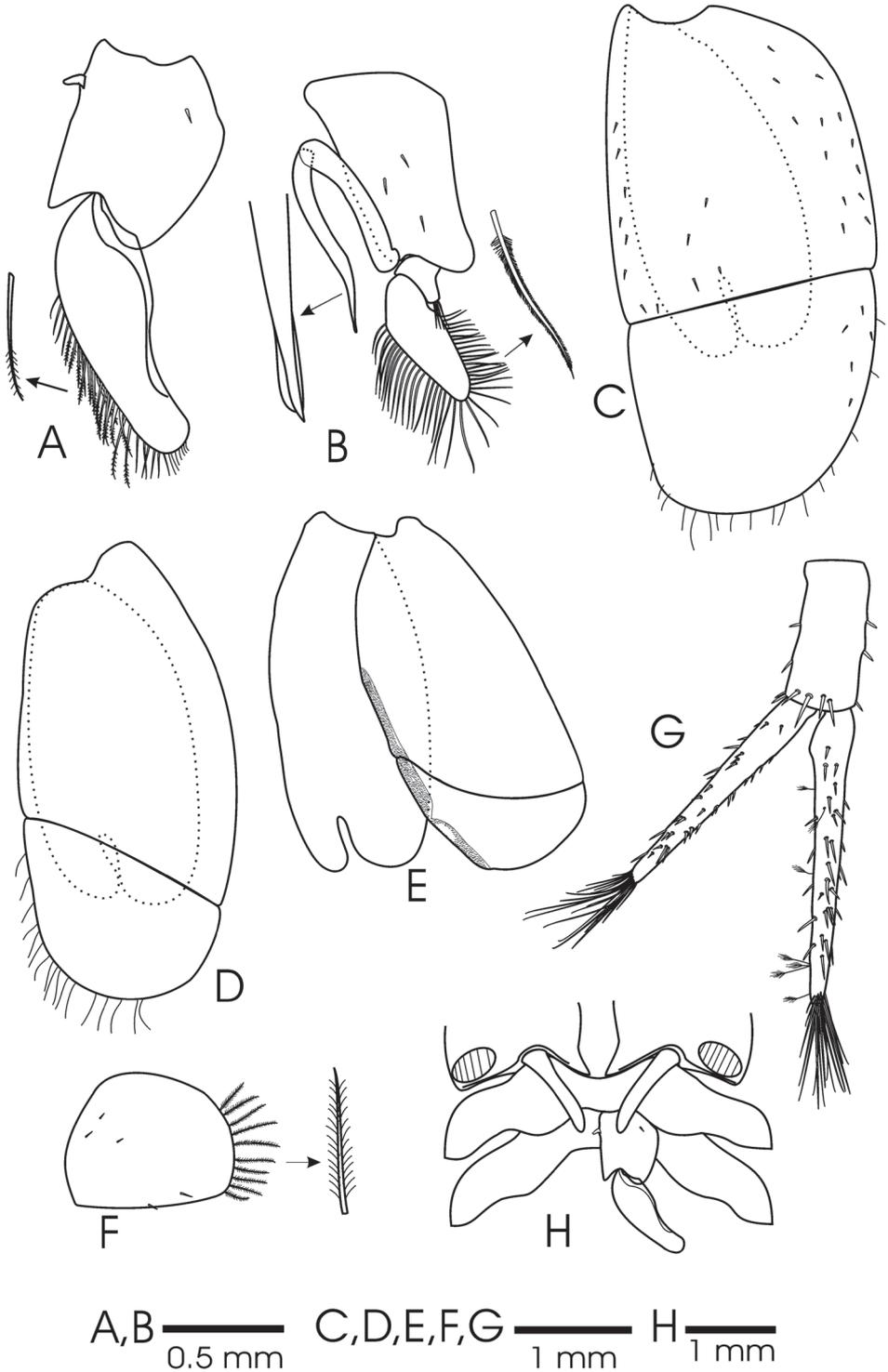


Figure 4. *Stenasellus tashanensis* sp. n. **A-E** pleopods I-V of male **F** pleopod II of female **G** uropod **H** genital papillae on seventh sternite.

margin; distal article of endopodite as long as proximal article and with accommodating groove, gutter-like in its caudal part.

Pleopods III–V (Figure 4C–E): exopodite with transverse oblique suture, sub-medial in the third and subterminal in the fourth and fifth pleopods; endopodite bilobate in all three pleopods.

Uropods (Figure 4G): exopodite slightly smaller than endopodite which bears seven plumose setae on proximal, medial, and distal parts.

Etymology. Topotypic, referring to the type locality, Tashan Cave.

Discussion

Several species of the genus *Stenasellus* were recorded in countries adjacent to Iran: *S. asiaticus* Birstein & Starostin, 1949 was recorded from Turkmenistan while four species including *S. vermeuleni* Magniez & Stock, 2000, *S. messanai* Magniez & Stock, 2000, *S. henryi* Magniez & Stock, 2000, and *S. grafi* Magniez & Stock, 2000 were recorded from Oman. One species was also described from Socotra Island (Messana pers. comm.) and seven species are present in East Africa (Messana 1990, 2001).

Hence, as Magniez and Stock (1999) stated “This possibility concerns particularly southern Iran. We must not forget that, as late as 18,000 BP, the Persian Gulf was reduced, offering a continuity of groundwater between the Oman and Iranian aquifers”, the presence of *Stenasellus tashanensis* n. sp. in Iran, strengthens the paleogeographic reconstructions dealing with this genus present in the karst and groundwater environments from the Far East to East Africa and Europe. Magniez and Stock (1999) showed a strict morphological similarity among the Far East, the Middle East, and the East African species, a result that may also be true for this new Iranian species.

S. tashanensis can be recognized from other *Stenasellus* species described from countries adjacent to Iran, by the significant differences including body size and the morphology of body appendages (Figures 5–7).

Maximum body length in *S. tashanensis* is 19 mm (vs up to 11 mm in *S. vermeuleni*, up to 8 mm in *S. messanai*, 3–4.5 mm in *S. henryi*, less than 2.5 mm in *S. grafi* and about 11.5 mm in *S. asiaticus*). The main morphological differences between *S. tashanensis* and other congeners from countries adjacent to Iran, are shown in Figures 5–7.

The general morphology of the *S. tashanensis* resembles that of *S. vermeuleni*, although there are significant differences in general and detailed characteristics such as the length of both antennae I and II, the number of strong spines on dactyls of the first paeopod and the shape of the first and second male pleopods. The new species can be recognized from *S. vermeuleni* by the detailed following characteristics: In *S. tashanensis* the exopodite of male pleopod I is flatter than in *S. vermeuleni*, where the exopodite is strongly twisted on its longitudinal axis (Figure 5); in male pleopod II, sympodite is sub-rectangular with almost parallel margins (vs sub-pentagonal with convex external margin and concave internal margin in *S. vermeuleni*), proximal article of exopodite bears 4 setae (vs 2–3 in *S. vermeuleni*), distal article is sub-elliptical and bears 40–42 setae (vs subtriangular with 30–35 setae in *S. vermeuleni*), the length of proximal article of endopodite is

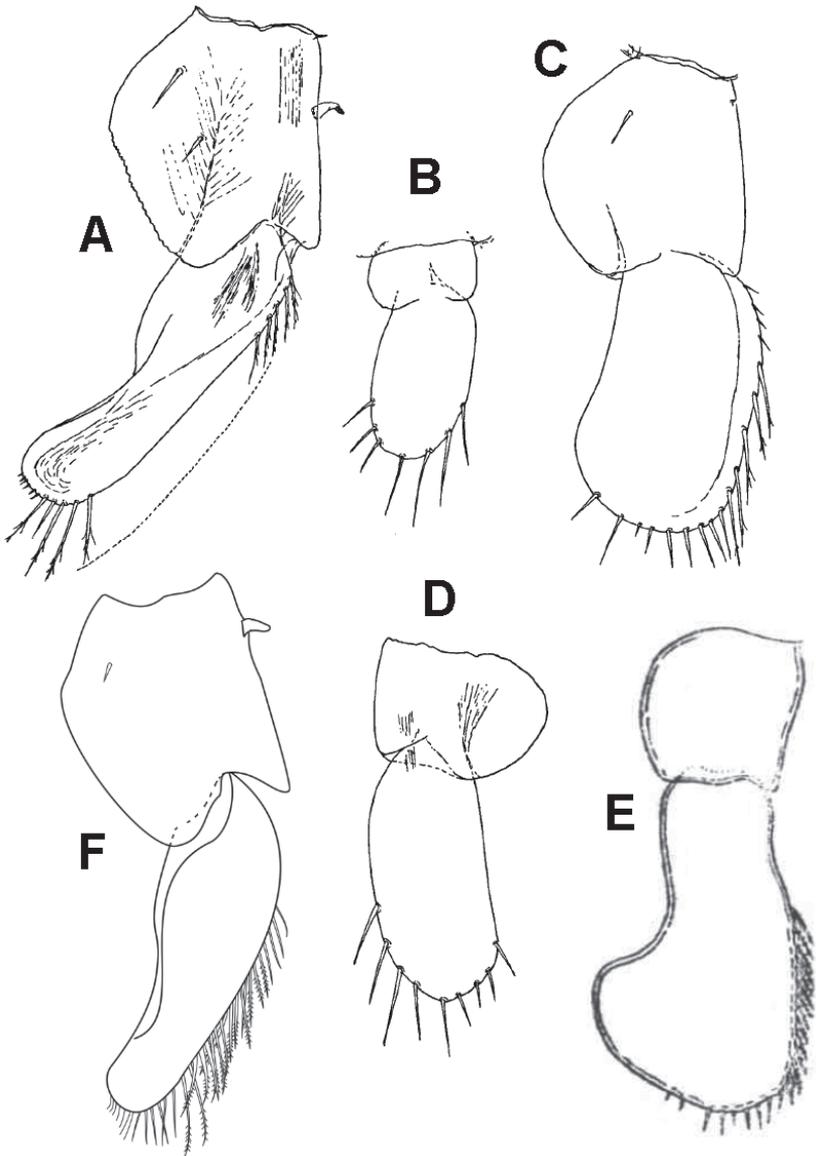


Figure 5. Male pleopod I of *Stenasellus vermeuleni* (A) *S. grafi* (B) *S. messanai* (C) *S. henryi* (D) *S. asiaticus* (E) and *S. tashanensis* sp. n. (F). (Figures A–D from Magniez and Stock 2000; Figure E from Birstein and Starostin 1949).

ca. 0.75 length of sympodite (vs almost as long as sympodite in *S. vermeuleni*), the length of distal article of endopodite is ca. 0.9 length of sympodite (vs 1.29 times longer than sympodite in *S. vermeuleni*); The second pleopod of female is sub-circular (vs subtriangular in *S. vermeuleni*) (Figure 6); Squama of antenna II bears two strong setae (vs 3 in *S. vermeuleni*) (Figure 7). The chaetotaxy of the mandibular lobe is strongly different in the mandibles of the two species. In *S. tashanensis* left mandible has 18–20 lifting spines

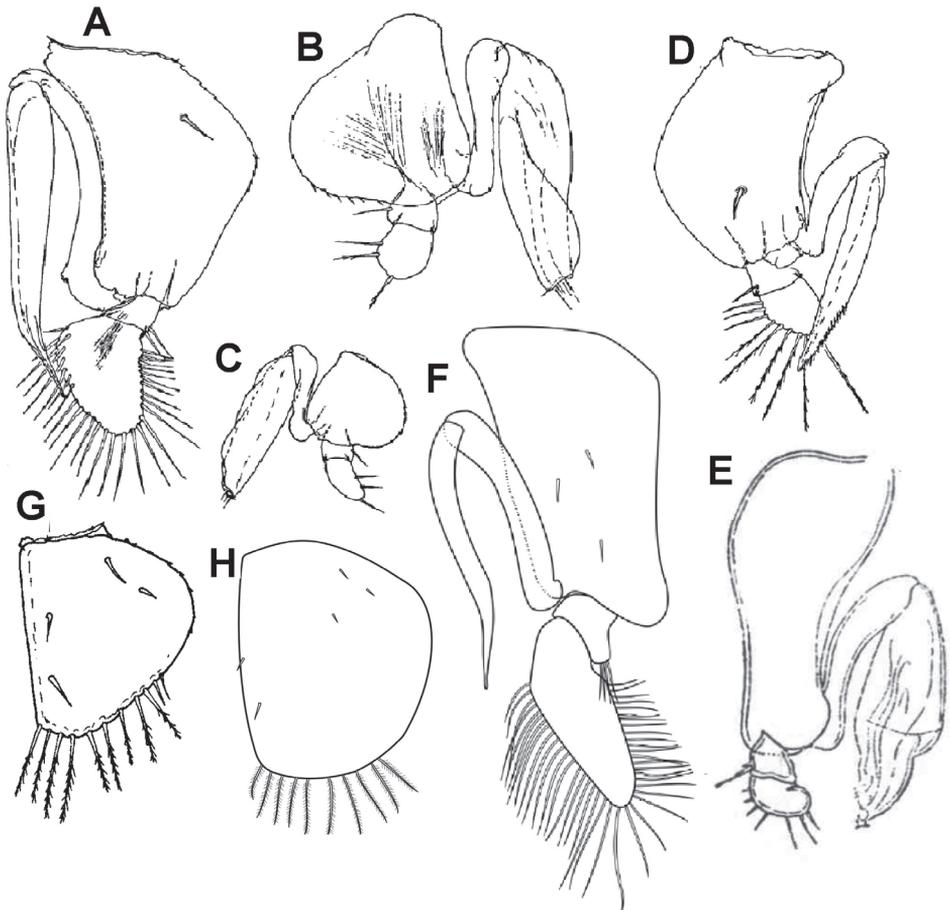


Figure 6. Male pleopod II of *Stenasellus vermeuleni* (A) *S. henryi* (B) *S. grafi* (C) *S. messanai* (D) *S. asiaticus* (E) and *S. tashanensis* sp. n. (F). (Figures A–D from Magniez and Stock 2000; Figure E from Birstein and Starostin 1949).

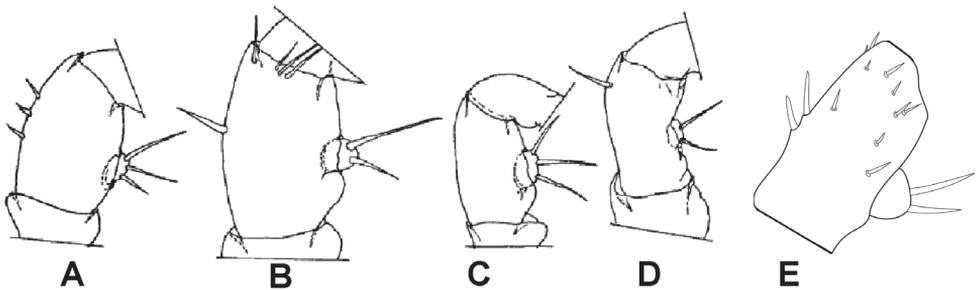


Figure 7. Squama of antenna II of *Stenasellus vermeuleni* (A) *S. messanai* (B) *S. henryi* (C) *S. grafi* (D) and *S. tashanensis* sp. n. (E). (Figures A–D from Magniez and Stock 2000).

(vs 12 in *S. vermeuleni*) and right mandible has 29–30 lifting spines (vs 13–14 in *S. vermeuleni*). Peraeopod I of *S. tashanensis* bears 19 strong denticulate setae on its propus (vs 11–13 in *S. vermeuleni*) and 19 strong setae on its dactylus (vs 12 in *S. vermeuleni*).

Probably molecular genetic analysis could lead to a better comprehension of the relationship of the various taxa present in the region. Several molecular studies exist on peri-Mediterranean and American stenasellids (Messana et al. 1995; Ketmaier et al. 1997, 2003; Baratti et al. 2003; Morvan et al. 2013). We know of no molecular studies of East African or Oriental stenasellids. Future studies will hopefully clarify the phylogenetic relationships within this group of these ancient stygobionts.

Acknowledgements

The authors thank Shiraz University for providing financial support. We would like to thank Prof Marian Glenn (Seton Hall University) for reviewing the English language of the manuscript. Our thanks are also due to Kelly L. Merrin (Australian Museum) for sending us some useful publications. We also thank Oana Moldovan, Julian Lewis and an anonymous referee, for their critical revision of the paper.

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A new cave locality for *Astyanax* cavefish in Sierra de El Abra, Mexico

Luis Espinasa¹, Laurent Legendre², Julien Fumey³,
Maryline Blin⁴, Sylvie Rétaux⁴, Monika Espinasa⁵

1 School of Science, Marist College, 3399 North Rd, Poughkeepsie, New York 12601, USA **2** UMS AMAGEN, CNRS, INRA, Université Paris-Saclay, Gif-sur-Yvette, France **3** Évolution, Génomes, Comportement, Écologie. CNRS, IRD, Univ Paris-Sud. Université Paris-Saclay, 91198 Gif-sur-Yvette, France **4** Equipe Développement Evolution du Cerveau Antérieur, Paris-Saclay Institute of Neuroscience, CNRS and University Paris-Sud and Paris-Saclay, 91198 Gif-sur-Yvette, France **5** Department of Mathematics, Science, Biology, Engineering and Technology, SUNY Ulster, Stone Ridge, USA

Corresponding author: Luis Espinasa (Luis.espinasa@marist.edu)

Academic editor: O. Moldovan | Received 13 May 2018 | Accepted 14 June 2018 | Published 4 July 2018

<http://zoobank.org/29CA8372-4D22-4625-8759-990B8C273E79>

Citation: Espinasa L, Legendre L, Fumey J, Blin M, Rétaux S, Espinasa M (2018) A new cave locality for *Astyanax* cavefish in Sierra de El Abra, Mexico. *Subterranean Biology* 26: 39–53. <https://doi.org/10.3897/subtbiol.26.26643>

Abstract

The characiform fish *Astyanax mexicanus* comes in two forms, a surface-dwelling morph which lives in the rivers of North and Central America and a blind, depigmented cave-dwelling morph which inhabits caves in Mexico. In recent years, this species has arguably become among the most influential model system for the study of evolutionary development and genomics in cave biology. While recent articles have analysed in great detail *Astyanax* genetics, development, physiology, phylogeny and behaviour, there have been comparatively few recent studies concerning its ecology and in particular its biogeography. Mitchell et al. (1977) reported the species inhabiting 29 caves in the Sierra de El Abra region. Despite the elapsing of over 40 years and the latest surge of interest in the model, not a single new cave locality had been described for the species. We describe here a new and 30th cave locality, Chiquitita Cave, inhabited by troglomorphic *A. mexicanus*. Their morphology, eye histology, 16S rRNA DNA sequence, and smelling capabilities are analysed. This cave represents the southernmost extension for the cave morph's habitat within the Sierra de El Abra. Its name, Chiquitita Cave (Tiny Cave), was chosen in reference to a potential hydrologic connection to “Chica Cave” (small cave), which is among the most studied populations of *Astyanax*.

Keywords

Astyanax, Chica Cave, Sierra de El Abra, troglomorphy, Troglöbite

Introduction

The blind Mexican tetra *Astyanax mexicanus* De Filippi 1853 has become the main contributor in the understanding of the genetic and developmental controls of troglomorphic features. It is also ranked among the influential model systems in evolutionary developmental (EvoDevo) biology (Jeffery 2001; Jeffery 2012). The cavefish have a conspecific surface-dwelling morph which lives in nearby surface streams throughout most of México. Both the eyed, pigmented surface morph and the eyeless, depigmented cave morph remain inter-fertile, making the species well-suited for experimental manipulations (Jeffery 2012; Elipot et al. 2014).

To date, 29 cave localities have been described for *A. mexicanus* (Mitchell et al. 1977), all of which occur within the El Abra region, in Northern Mexico. Two more cave localities, Granadas and La Joya, have been described for the closely related species of *Astyanax aeneus* in Guerrero, Southern Mexico (Espinasa et al. 2001; Kopp et al. 2017).

The cave morph was first discovered in Chica Cave by Salvador Coronado in 1936 (Hubbs and Innes 1936). The New York Aquarium expedition of Breder and associates in 1940 produced a map of the cave, a description of the environment, and the peculiarities of the population that inhabits this cave. A small entrance (1.5 by 3.5 m) is found at the base of a stream bed that is dry throughout the year except during particularly large storms in the rainy season. This stream bed has no surface fish. The cave is 591 m long and in a step-wise fashion the passage continuously pitches downward through a series of couple meter deep pits. Four main pools are encountered (Pool I-IV), with the lowest pool IV, the final sump, lying at 19 m below the entrance level (Mitchell et al. 1977; Elliott 2015).

The Chica Cave population is a highly varied one, being comprised of everything from typical eyeless, depigmented cave fish to typical surface fish. Since depigmented fish with eyes and pigmented fish without eyes can be found, introgression has been suggested (Avisé and Selander 1972). Interestingly, pool I, which is the one closest to the entrance, has the highest proportion of troglomorphic fish, while pool IV, which is the deepest and furthest from the entrance has the largest number of epigeomorphic fish. Mitchell et al. (1977) have argued that surface fish enter the cave not through its higher entrance, which is a dry stream bed during large parts of the year, but that instead surface fish swim in from a spring adjoining Tampaón river, reaching the lower and deeper pool IV of Chica cave. The final sump and Tampaón river are only 972 m apart. Pool IV's final sump is at an elevation of 31 m above sea level. Tampaón river is at 28.5 m. Thus the terminal pool is only 2.5 meters higher than the surface river. Further support for a surface connection is that these authors have found cichlid fish in the deeper pools, which are clearly surface river fish that must have swam in.

The Chica Cave population was included in the first genetic study conducted on cave *Astyanax* (Avisé and Selander 1972). They used isoenzymes. From this study a hypothesis to explain the mixed nature of the population was born. They observed that

the Esterase 2d haplotype was fixed (Pachón, Sabinos) or in high frequencies (Chica) in cave populations and absent from surface populations and the haplotype Glutamate Oxalate Transaminase 2d was fixed (Sabinos) or in high frequencies (Chica) in cave population and essentially absent from surface populations (the exception were only 2 individuals out of 257 assessed ie. 0.78%). When they divided the Chica individuals into eyeless, sunken eye and eyed individuals, from most troglomorphic to most epigeomorphic, they found a clear correlation between frequencies of homozygous individuals for the cave allele, heterozygous and homozygous for the surface allele, according to morphology. They concluded that “individuals from surface populations have recently entered the cave and are interbreeding with eyeless individuals” (Avisé and Selander 1972), confirming with genetic evidence that surface fish probably regularly enter the Chica cave.

Mitchell et al. (1977) discussed the most likely point of entrance of fish from Tampaón river into the Cueva Chica system. They argued for a trio of small, closely associated, “tinajas” (water-filled depressions) located in the eastern part of El Pujal town and about 225 m north of Tampaón river. From these tinajas a rather prominent arroyo enters the nearby channel of Tampaón river. At high flood stage, the Tampaón river fills this stream to the tinajas, which like the final sump of Chica are only about 3 m above the Tampaón river.

The purpose of this paper is to describe a new cave population of troglomorphic fish discovered near these tinajas as well as the cave environment they inhabit. Of relevance is that since 1977, this has been the first new *Astyanax* cave locality discovered in the Sierra de El Abra.

Methods

The initial purpose of this study was to observe the *Astyanax* fish population at the tinajas described by Mitchell et al. (1977) as the potential resurgence of Chica caves in hopes of finding hybrid fish. While at the town of El Pujal, local inhabitants pointed out the presence of a well just 45 m away from the tinajas. From this well, water is extracted for use by the town dwellers. Upon inspection, it appeared that the well was not an artificially excavated well but instead was a natural 5.7 m deep pit that reached a large pool of water. A pipe had been installed and water is extracted via a pump located inside the adjoining work house. Twenty-five meters from this well and in the direction of the tinajas, a small hole was found among the roots of a large tree. This hole led to a 4 m long chamber, which reached water level. From it a poecilid, an epigeomorphic *Astyanax* and two troglomorphic *Astyanax* fish were collected. The following day, the pit of the well was descended with the help of a cable ladder. The pool was examined with the help of a scuba diving lamp, mask and snorkel. A school of about 20 epigeomorphic *Astyanax* was seen as well as some troglomorphic *Astyanax*. Four more specimens were collected. All specimens were in the twilight area.

Cave

Topographic map of main chamber of the well and the small cave under the tree was made with tape and a Suunto compass and clinometer on 3/23/16. Croquis map of a side gallery of the well was made on 5/26/16 when water level was significantly lower and allowed for exploration. Although the less than 20 m of passage between the well and the small cave remain unconnected by human exploration, it is suggested they form part of a single system and thus the well and the small cave are referred here both as “Chiquitita Cave” (Fig. 1). Chiquitita cave is within the town of El Pujal, San Luis Potosi, Mexico, N21°50.890' W98°56.194' and 43 m above sea level (Fig. 2). Water pH on 3/23/16 was 7.37, conductivity 489 μ S, and temperature 26.2 °C.

Specimens

A poecilid, an epigeomorphic juvenile *Astyanax* and two juvenile (2.7 and 3 cm standard length) troglomorphic *Astyanax* fish were collected from the small cave under the tree on 3/20/16. Four adult (3.48 cm standard length, 0.96 gr; 5.60 cm, 4.82 gr; 6.08 cm, 4.63 gr; 6.68 cm, 6.49 gr in fixed fish) troglomorphic fish were collected from the well on 3/23/16. Juvenile specimens were measured and sacrificed in the field immediately after collection and deposited in 100% ethanol. Adults were taken alive to the laboratory where behavioral experiments were conducted the following day. Specimens were then measured, weighed, euthanized and deposited in formalin 10%. In two specimens the skull cap was opened to examine brain morphology. Collecting permit # SGPA/DGVS/02438/16 from Secretaría del Medio Ambiente y Recursos Naturales, México, was issued to Patricia Ornelas García. Photographs were taken on the field with a Canon EOS100 camera.

Histology

Optic capsule was extracted with the help of a scalpel and dissection needles under a dissection microscope, embedded in paraffin, and cut into 5- μ m sections using a microtome. Staining was done with hematoxylin-eosin as in Espinasa et al. (2001).

Olfaction

Four adult troglomorphic Chiquitita fish were deposited in a fish tank of 50 × 25 × 10 cm (total volume=12.5 L) with cave water and acclimatized for 24 hrs. Two 50-ml syringes were attached to opposite ends of the tank and connected to medical solution administration tubing containing a Luer stopper to control solution flow (Baxter, Thetford, UK). On one syringe the following protocol was performed: 50 ml of a solution

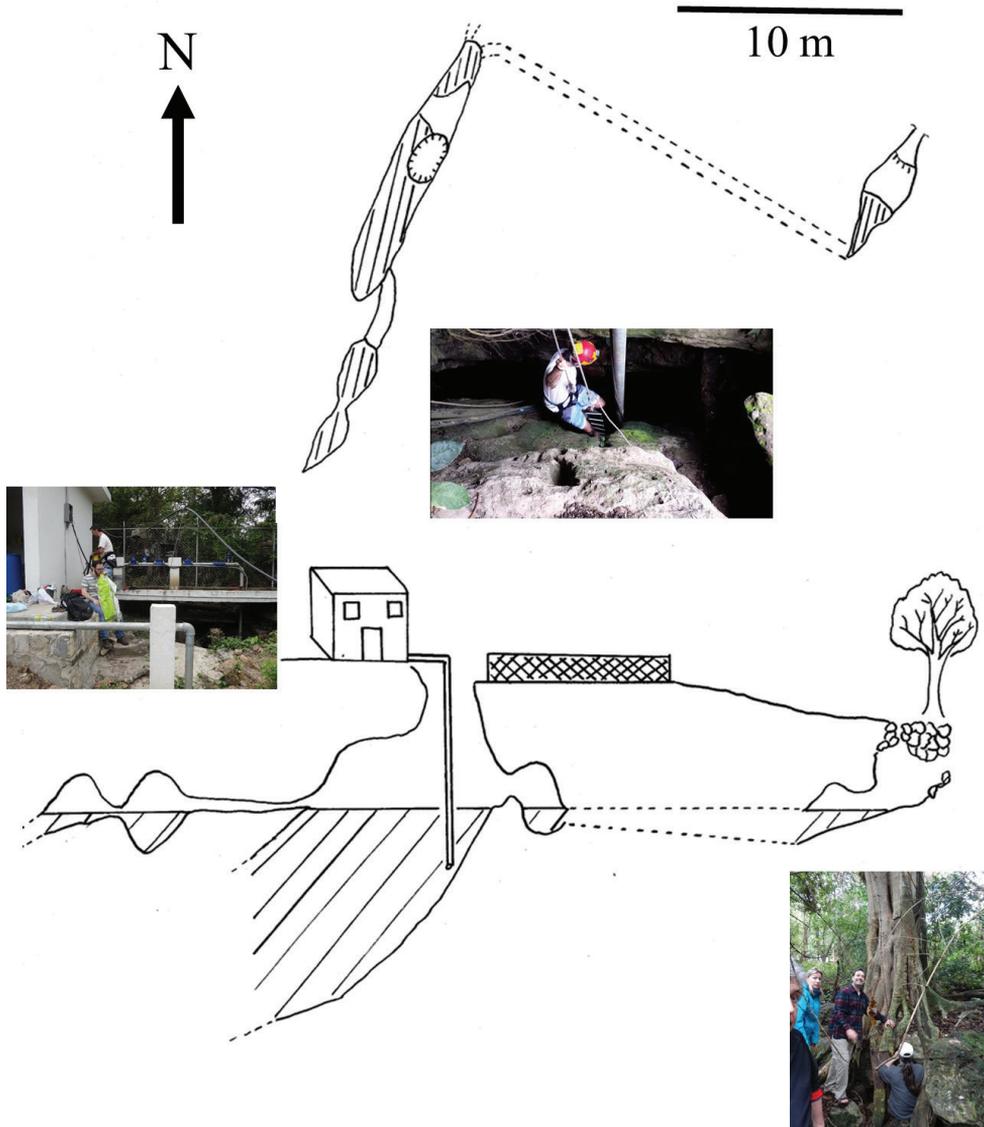


Figure 1. Chiquitita Cave map. The accessible and explored cave system is composed of a pit from which locals pump water out and a small chamber under the roots of a tree. Photographs from left to right are: **1** The pump facility with the pipe going into the pit **2** Descending into the pit **3** Entrance to the small chamber under the tree.

with 10^{-12} M of Alanine over a 2 min period was allowed to perfuse into the tank. Then 3 min were allowed to pass without adding anything. Then 50 ml of a solution 10^{-11} M was added over a 2 min followed by the 3 min of rest. This was repeated for solutions 10^{-10} and 10^{-6} M. On the other syringe, water was allowed to perfuse at the same rate and times. Solution flow from the experimental and control syringes was initiated si-

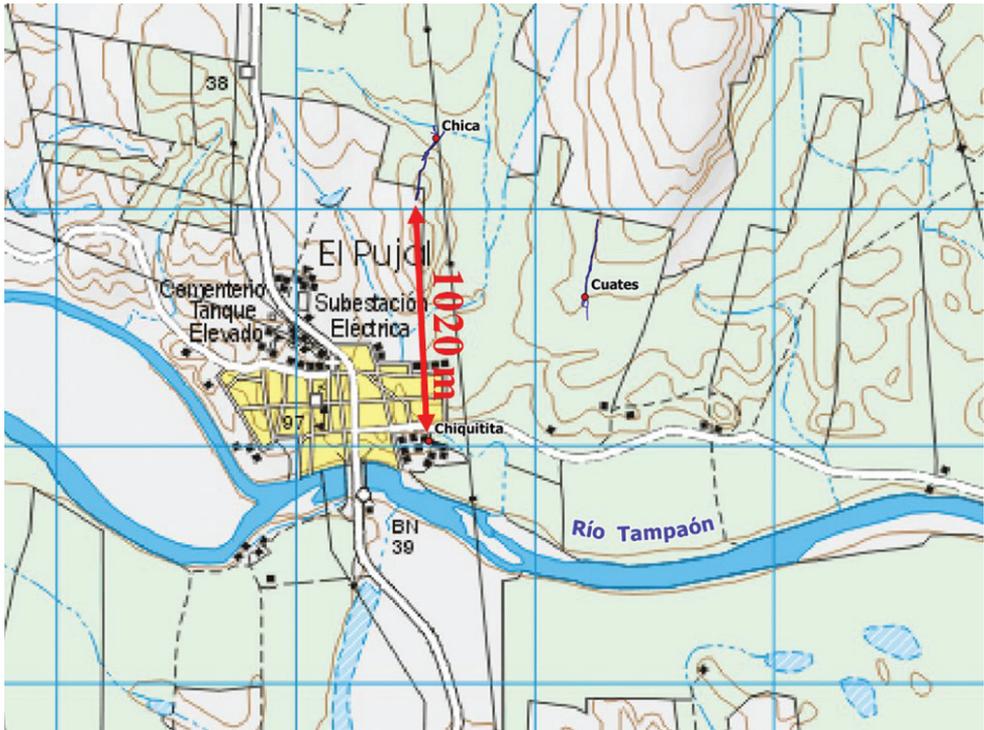


Figure 2. Topographic map of El Pujal area, in the southern-most Sierra de El Abra. Overlaid is the line topography of Chica, Cuates and Chiquitita Caves.

multaneously, and the experiment was filmed from the side using a Sony DCR–SR200 Handycam camcorder. Cavefish typically swim parallel to the ground and following one wall of the fish tank until the end of the tank, where they turn either on a 180 degree turn or a 90 degree to follow the next side of the tank. Feeding behaviour is characterized by sharp 360 degree turns around the food source, swimming in circles, or by biting the ground or food source at an angle of about 45° to the ground. For this test, number of sharp, 360 degree turns and bites in the area adjoining the tubing where water (control) or alanine (experimental) was being perfused were counted. Likewise number of turns or bites were counted on the experimental tube area during the rest periods when nothing was perfused. A Mann-Whitney test was performed to compare among conditions.

Fat deposits

Abdominal dissections were performed in the six Chiquitita Cave individuals with the help of a scalpel, micro-scissors and dissection needles. A flap of skin of the left side of the body, between the base of the opercula and the anal fin was raised so as to expose

the abdominal contents. An image of the abdominal cavity is recorded. From this image the portion of the abdominal lining with fat deposits is measured and compared to the total area of the abdomen so as to provide the percentage of abdominal lining covered with fat deposits. For comparison, 5 individuals from Boquillas river and 4 from Rascon surface fish were analysed. A Mann-Whitney test was then performed.

DNA sequencing

Previous mitochondrial DNA analyses has shown the presence of two broadly different clades that have been dubbed as lineages A and B or new and old by some authors (Gross 2012). For this study the mitochondrial 16S rRNA was sequenced. Four Chiquitita Cave individuals were analysed. For comparison, DNA was also sequenced from individuals from other locations. Specimens from localities with reported lineage “A” specimens included 2 Comandante surface river, 2 Molino Cave, 2 Caballo Moro Cave, 2 Pachón Cave and 1 Chica cave. Specimens from localities with lineage “B” included 4 Rascon surface stream, 2 Tamasopo surface stream, 2 Sabinos Cave, 4 Tinaja Cave, and 1 Curva cave. Genomic DNA samples were obtained following standard methods for DNA purification using Qiagen’s DNeasy® Tissue Kit, by digesting a fin clip of the individual in the lysis buffer. Markers were amplified and sequenced as a single fragment using the 16Sar (CGCCTGTTTATCAAAAACAT) and 16Sb (CTC-CGGTTTGAAGTACAGATCA) primer pair for 16S rRNA (Edgecombe et al. 2002). Amplification was carried out in a 50 µl volume reaction, with QIAGEN Multiplex PCR Kit. The PCR program consisted of an initial denaturing step at 94 °C for 60 sec, 35 amplification cycles (94 °C for 15 sec, 49 °C for 15 sec, 72 °C for 15 sec), and a final step at 72 °C for 6 min in a GeneAmp® PCR System 9700 (Perkin Elmer). PCR amplified samples were purified with the QIAquick PCR purification kit and directly sequenced by SeqWright Genomic Services. Chromatograms obtained from the automated sequencer were read and contigs made using the sequence editing software Sequencher™ 3.0. All external primers were excluded from the analyses. BLAST was used to identify GenBank sequences that resemble the specimens. Sequences were aligned with ClustalW2.

Results

Chiquitita cave, barely 200 m from Tampaón River, is the southern-most cave in the Sierra de El Abra (Fig. 2). The vertical pit and adjoining small cave are a resurgence during the rainy season as evidenced by the stream bed coming out of these caves, which was dry at the time of exploration. Water level in the cave was estimated to be 37 m above sea level. Tampaón River is reported to be 28.5 m by Mitchell et al. (1977). During the rainy season, the about 10 m vertical difference should be a trivial barrier for aquatic organisms. Evidence of this was the presence of typical surface poecilids and

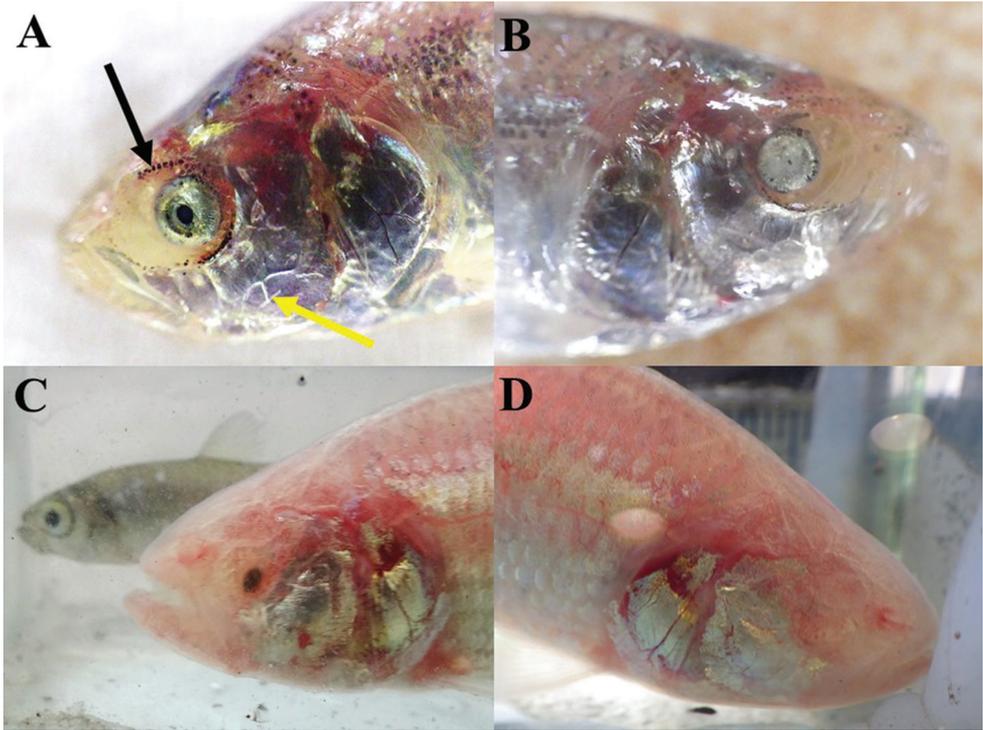


Figure 3. High variability in the eye and pigmentation level within the population inhabiting Chiquitita Cave. **A** eye size reduced **B** pupil closed **C** in the foreground a troglomorphic fish with reduced and embedded eyes and in the background a pigmented fish with large eyes **D** eyes and pigment mostly absent. Black arrow highlights pigmented cells in some troglomorphic fish and yellow arrow highlights fragmentation of the suborbital bone III.

a school of surface *Astyanax*. That this cave is part of a very large underground water conduit system is suggested by the fact that water is being continuously pumped out for human use without depleting its water.

Apart from the epigeal organisms, Chiquitita Cave is inhabited by mysid troglomorphic shrimps, presumably *Speleomysis quinterensis*, and a population of troglomorphic *Astyanax*. Fish were highly depigmented and were easily differentiated from the surface congeners by their characteristic pinkish-white coloration. There was high variability in the eye, with individuals having phenotypes such as small eye size (Fig. 3A), closed pupil (Fig. 3B), embedded eye (Fig. 3C), and eye mostly absent (Fig. 3D). Introgression between the surface morph and the cave morph is suggested by the presence of individuals that are highly depigmented, but with eyes (Fig. 4B) or individuals with pigment and reduced eyes (Fig. 3A–B).

When the eye capsule was histologically examined in the fish with the smallest eye remnant, it was noted that there was no lens. Retinal layers were disorganized and present only in small sections of the eye capsule (Fig. 5). With such level of eye degeneration, it

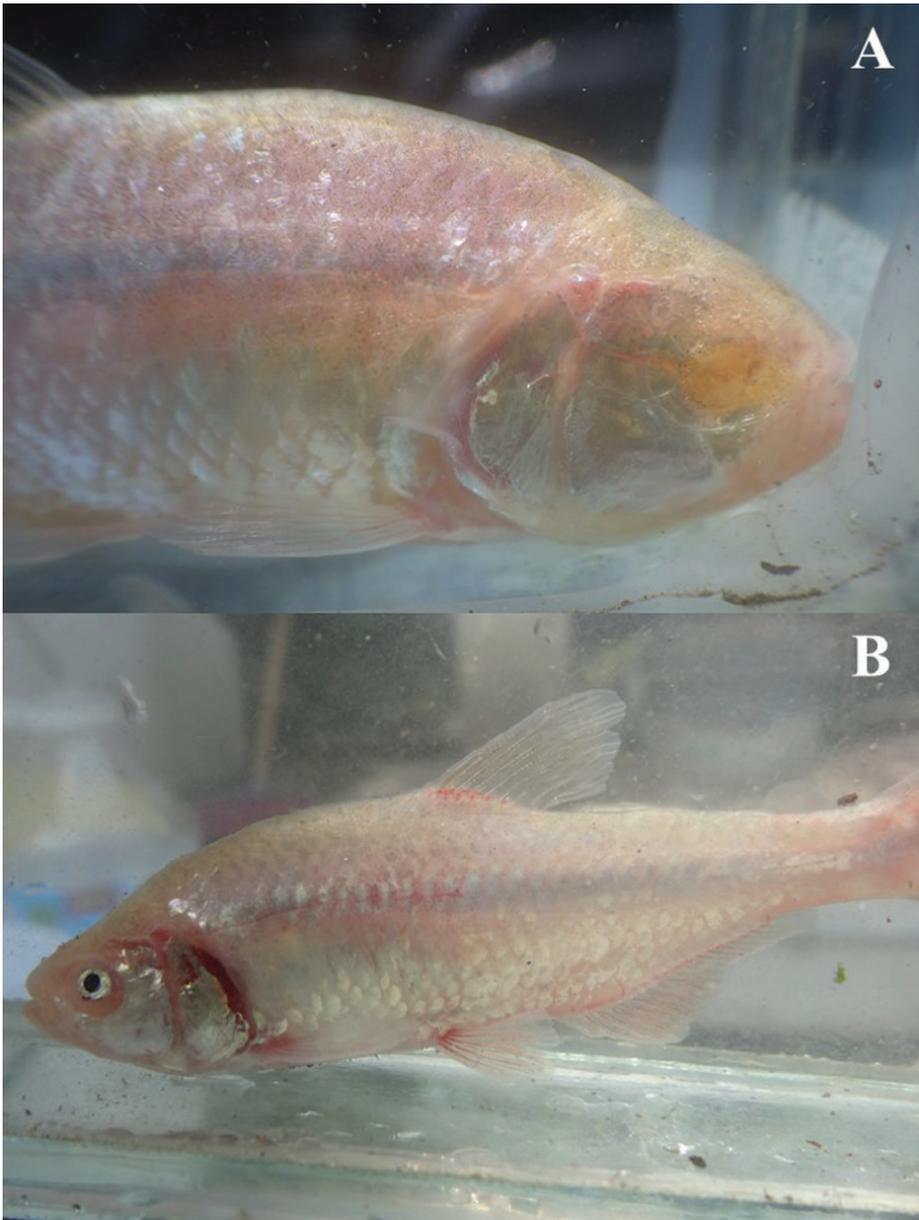


Figure 4. Variability in the correlation between eye and pigment may suggest introgression between the surface morph and the cave morph as evidenced by the presence of individuals that are highly depigmented, and without eyes (**A**) or individuals that are also highly depigmented but with eyes (**B**). For the other combinations of eye and pigment see Figure 3.

is likely that these fish are effectively blind. Depigmentation in at least some of the individuals is not due to an albinism mutation because some remnants of the eyes are black (Fig. 5B) and skin has some small level of pigmentation.

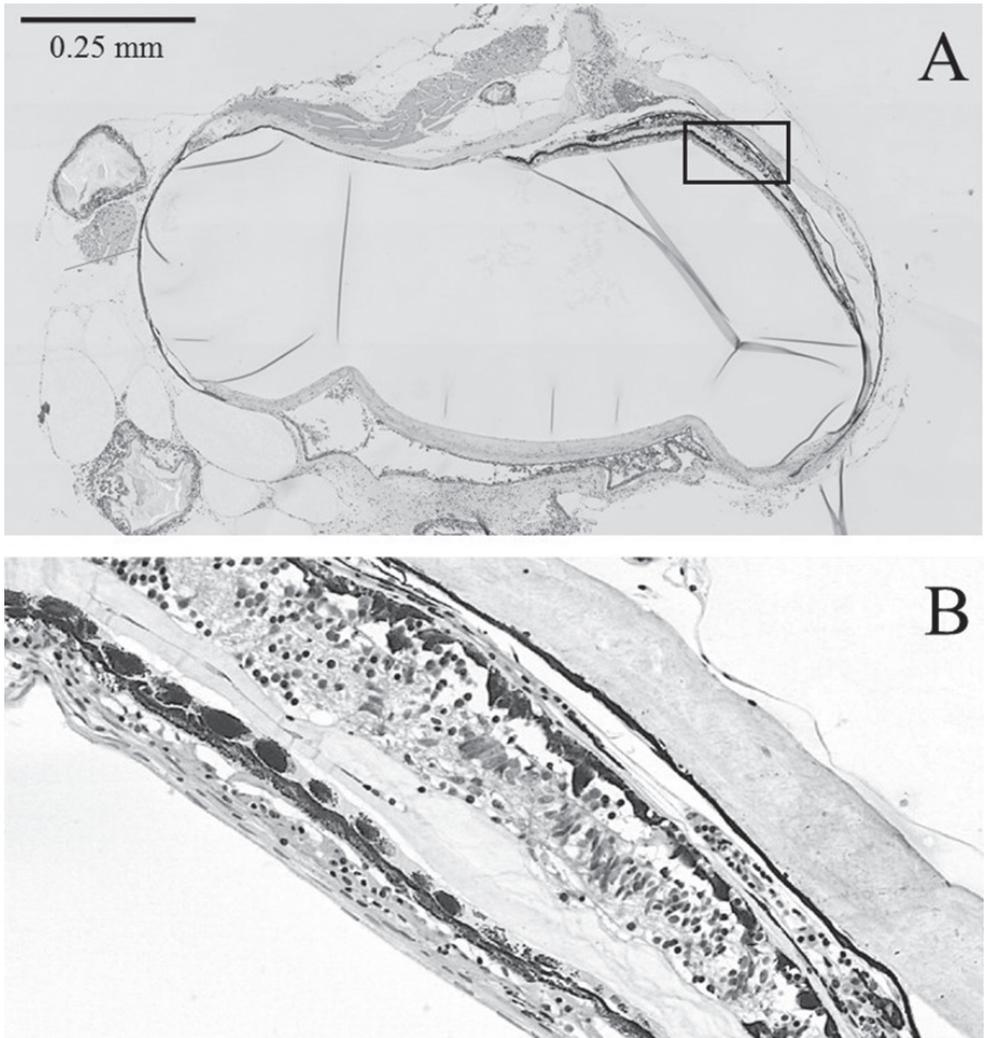


Figure 5. Eye histology in one of the fish with most degenerated eyes. **A** eye capsule. Notice the absence of lens. **B** retina. Notice the high disorganization of vestigial layers, which are for the most part unrecognizable when compared to surface fish retinal layers.

The maximum length of the optic lobes was small (1.57 and 1.75 mm) in comparison to their prosencephalon (2.26 and 2.46 mm), thus in the Chiquitita cave specimens, optic lobes were only 70 and 71% the length of the prosencephalon. Based on Espinasa et al. (2001), epigeomorphic *Astyanax* with this length of optic lobes are proportionally larger, being 90% the length of their prosencephalon. Such reduction of the optic lobes is in accordance with reports for troglomorphic *Astyanax* (Wilkins 1988).

These Chiquitita cavefish have other troglomorphic characters previously described for *Astyanax*. For example, they have natural bone fragmentation of the suborbital bones (Gross et al 2016), as highlighted by the yellow arrow in Figure 3A. Likewise

it has been reported that *Astyanax* cavefish have an enhanced ability for fat storage (Hueppop, 1989). In the troglomorphic individuals of Chiquitita Cave, increased subdermal fat reserves was also observed ($P=0.021$). In the Chiquitita Cave specimens, an average of 32.8% (± 19.1 StDev) of the lining of the abdomen is covered by fat deposits, while in surface fish it is 19.9% (± 14.4 StDev).

Chiquitita cavefish also appear to have enhanced olfaction capabilities. During perfusion at 10^{-12} M of Alanine, 2 turns and 0 bites were recorded on the experimental side. At 10^{-11} there were 6 turns and 2 bites. At 10^{-10} there were 7 turns and one bite, and at 10^{-6} there were 9 turns and 7 bites. During those same periods in the control side, where water was being perfused, there were 1, 0, 2 and 3 turns, with not a single bite. In the experimental side, during the rest period when nothing was being perfused there were 0, 1, 1, and 2 turns, with not a single bite. The total number of turns (15) in the area of the tubing perfusing Alanine concentrations equal or lower than 10^{-10} was significantly higher ($P=0.0047$) than the number of turns (3) in the control tubing perfusing water, or in the side of the experimental tubing (2) during resting time when nothing was being perfused ($P=0.0016$). This supports that feeding behaviour was being induced by smell at perfused concentrations equal or lower than 10^{-10} M of Alanine. Hinaux et al. (2016), using a different set-up from ours (one-month-old juveniles of about 5-6 mm in 9 cm wide \times 13 cm long tanks containing 150mL) showed that both surface and cavefish had a strong reaction at perfused concentrations of 10^{-5} M of Alanine, but there was a threshold at 10^{-6} M when surface fish stopped reacting. In their set-up cavefish still had strong reactions even at concentrations of 10^{-10} M. While experimental conditions are different in this study from Hinaux (adult vs juveniles, in 12.5L vs 150mL), data supports that Chiquitita cavefish may be responsive to perfused odorants at low concentrations levels. Remarkably, only sharks have been reported to present such sensitivity to amino acids with, for example, 10^{-11} M alanine eliciting electro-olfactogram responses in the hammerhead shark (Tricas et al. 2009).

DNA amplification of the mitochondrial 16S rRNA produced a 572 bp sequence. Results showed that three Chiquitita specimens had identical haplotype to the surface and cave populations belonging to the “A” lineage, to which the Chica Cave, Pachón Cave, and local surface *Astyanax* belong (Fig. 6). The fourth Chiquitita cave individual differed by a single base. When compared against the “B” lineage surface fish from Rascon and Tamasopo, Chiquitita specimens differed by 2-3 bp. When compared against the “B” lineage of cave fish from Sabinos, Tinaja and Curva, they differed by 5-6 bp (red arrows on Fig. 6). It is thus supported that Chiquitita specimens have a mitochondrial DNA most closely related to the “A” lineage, with some specimens being identical to individuals from Chica cave.

Discussion

The first cave to be described with a troglomorphic population of *Astyanax mexicanus* was Chica cave. Since then, cave *Astyanax* has arguably become among the most influential

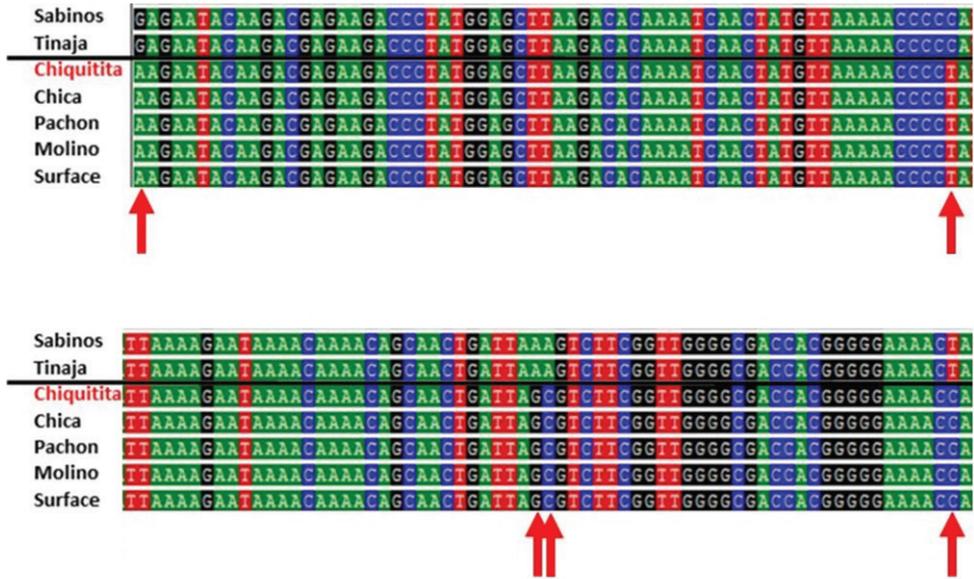


Figure 6. Fragment of the mitochondrial 16S rRNA. Individuals from Chiquitita Cave have identical sequence to members of the “A” lineage (Chica cave, Pachón cave, Molino cave and Rio Comandante surface river). Members of the “B” lineage (Sabinos cave and Tinaja cave) have 5-6 bp disagreements in this fragment, indicated by red arrows.

model system for the study of evolutionary development and genomics in cave biology. A total of 29 caves are now known to be inhabited by these cavefish (Mitchell et al. 1977). Chica population remains one of the most cited among them. The Chica population is special in that it is composed of a mixed population of troglomorphic and epigeomorphic individuals, which may be hybridizing with each other. Somehow counterintuitively, the most epigeomorphic individuals are not found near the entrance, but instead they are in the deeper pools and in particular at the final sump. Since this final sump is almost at the same altitude as the nearby Tampaón surface river, it has been proposed that surface fish are entering at a spring near Tampaón and are then dispersing through the continuous underwater conduits that reach Chica’s final sump (Mitchell et al. 1977). The more troglomorphic individuals are in perched pools, partially isolated vertically from the surface fish.

Mitchell et al. (1977) had proposed that the point of entrance of these surface fish was at some tinajas in the town of El Pujal, just 200 m from Tampaón River. When trying to confirm this hypothesis, Chiquitita Cave was discovered just 45 m from these tinajas. Chiquitita cave is only 1020 m at 357°(from true N) to the sump in Cueva Chica (Fig. 2). Chiquitita Cave is a resurgence active during the rainy season and part of an apparently very extensive system of underwater passages of which a minimal part can be explored without the use of cave scuba diving techniques. Surface fish probably have easy access to this resurgence during the rainy season as there are minimal vertical barriers between this cave and the high level of Tampaon River. Evidence for this is the

presence of a large school of surface *Astyanax* inside the cave as well as other surface species swimming in the penumbra.

Chiquitita Cave is also host to a population of troglomorphic fish. These fish showed variability in their eye development and pigmentation level like in the Chica cave, consistent with introgression. Nonetheless, the most troglomorphic fish were essentially devoid of pigment and eyes and presented other troglomorphic features such as fractured suborbital bones, increased subdermal fat reserves, and enhanced olfaction capabilities. In essence, some fish at Chiquitita Cave were essentially as troglomorphic as the most cave adapted individuals in Chica Cave or other El Abra populations.

Population genetics analyses performed on the *Astyanax mexicanus* cave and surface system have demonstrated the co-existence in the El Abra region of two mitochondrial haplotypes, A and B, initially defined after the ND2 gene sequence (Dowling et al. 2002; Strecker et al. 2003, 2004; Ornelas-Garcia et al. 2008). These mitochondrial DNA data have been used to propose evolving scenarios on the relatedness of *Astyanax* cave and surface populations in the region (Fumey et al. 2018). Here, we used 16S rRNA mitochondrial sequence analysis to start documenting the genetic lineage of the newly discovered Chiquitita cavefish population. We found that this mitochondrial DNA marker establishes Chiquitita as belonging to the “A” lineage, together with Molino, Caballo Moro, Pachón, and Chica cavefish, as well as the local surface fish streams. This result is consistent with the possibility that there could be continuous passages and therefore exchanges between the two very close caves of Chica and Chiquitita. Hence, the Chiquitita Cave troglomorphic population may essentially be seen as an extension of the Chica population. The distance of about 972 m between the two caves, at essentially the same altitude, should not pose an effective barrier to prevent individuals from dispersing between them. Likewise surface fish entering during the rainy season at the spring of Chiquitita Cave may be able to disperse easily towards the final sump of Chica Cave (see Fig. 2).

An important ecological difference between the two caves is that while Chica Cave has a large bat colony that provides enormous amounts of bat guano and carcasses as a food source, Chiquitita Cave must be more challenging. There seems to be minimal input of food sources in comparison, especially beyond the twilight where no more surface insects may fly in. This may be specially challenging for non-cave adapted surface fish.

Possible impacts and threats to this new population are mainly human-derived. This cave has a pipe from which water is extracted for local consumption. Nonetheless the cave appears to be connected to a large aquifer and despite constant usage, the water table appears to recharge readily. Pachon Cave, the most studied *Astyanax* cave population, also has a pipe for water extraction and the population has remained large. Being connected to a large aquifer probably translates into this pipe only potentially having a very local effect to the proportionally few individuals in the area. Of more concern is that the cave is located under the town of El Pujal and any chemical or toxic waste may find its way into the local aquifer.

Conclusions

Despite the latest surge of interest in the *Astyanax* model, not a single new cave locality had been described for *Astyanax* cavefish in El Abra region over the last 40 years. We describe here a new cave locality, Chiquitita Cave, inhabited by troglomorphic *A. mexicanus*. Chiquitita Cave is a resurgence found at the southern-most edge of the Sierra de El Abra, very close to Tropaón River. While some of its individuals are apparently fully troglomorphic in nature, it is a mixed population with surface individuals probably hybridizing with the troglomorphic population. It is also likely that there are continuous underwater passages between Chica and Chiquitita cave, thus potentially allowing for both troglomorphic and surface fish to migrate between both localities.

Acknowledgments

We would like to thank Patricia Ornelas-García who obtained a collective collection permit. María Elina Bichuette and an anonymous reviewer for their comments on the manuscript. Thanks to all group members who participated to the March 2016 field trip: D. Casane, L. Devos, C. Hyacinthe, S. Père, K. Pottin, E. Queinnec, V. Simon. This study was supported by Marist College and its School of Science (to LE), an ANR grant [BLINDTEST] and a FRM grant [Equipe FRM] (to SR), and a collaborative exchange program [Ecos-Nord] to SR and Patricia Ornelas-García.

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A redescription of the poorly known cave millipede *Skleroprotopus membranipedalis* Zhang, 1985 (Diplopoda, Julida, Mongoliulidae), with an overview of the genus *Skleroprotopus* Attems, 1901

Boyan Vagalinski¹, Kaibaryer Meng², Darina Bachvarova³, Pavel Stoev⁴

1 Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113, Sofia, Bulgaria **2** Institute of Zoology, Chinese Academy of Sciences, Beijing, China **3** Konstantin Preslavsky University of Shumen, 115 Universitetska Str., Shumen, Bulgaria **4** National Museum of Natural History, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria and Pensoft Publishers

Corresponding author: *Boyan Vagalinski* (boyan_vagalinski@excite.com)

Academic editor: *O. Moldovan* | Received 27 April 2018 | Accepted 3 July 2018 | Published 12 July 2018

<http://zoobank.org/2713914F-77CD-4C93-BD9F-7D91C2F5CE3B>

Citation: Vagalinski B, Meng K, Bachvarova D, Stoev P (2018) A redescription of the poorly known cave millipede *Skleroprotopus membranipedalis* Zhang, 1985 (Diplopoda, Julida, Mongoliulidae), with an overview of the genus *Skleroprotopus* Attems, 1901. *Subterranean Biology* 26: 55–66. <https://doi.org/10.3897/subtbiol.26.26225>

Abstract

We redescribe the poorly known Chinese millipede *Skleroprotopus membranipedalis* Zhang, 1985 recorded from Shi-Hua (Stone Flower) Cave, Fangshan County, Beijing. The species' original description is in Chinese in an obscure outlet which significantly hampers its recognition from its congeners. Here, based on newly collected material, we provide the first scanning electron micrographs of important taxonomic traits. In addition to its type locality, we report the species also from Yun-Shui (Cloud Water) Cave, situated in the same county, some 18 km away. We propose the genus *Senbutudoiusulus* Miyosi, 1957 to be a junior subjective synonym of *Skleroprotopus* Attems, 1901, **syn. n.**, and introduce the following new combination: *Skleroprotopus platypodus* (Miyosi, 1957), **comb. n.** (former *Senbutudoiusulus*).

Keywords

China, new record, new synonymy, troglobiomorphism

Introduction

The eastern Asian julidan family Mongoliulidae is currently known to comprise 8 genera and 36 species, as recently reviewed by Enghoff et al. (2017). Twenty of these species are described under *Skleroprotopus* Attems, 1901 making it by far the largest genus within the family, with a distribution range including northeastern China, the Russian Far East (Primorskiy Province), the Korean Peninsula, and Japan (see Fig. 1). Many *Skleroprotopus* species are supposed local endemics known only from their original descriptions. This is indeed the case with *S. membranipedalis* Zhang, 1985 described from Shi-Hua (Stone Flower) Cave, Fangshan County, Beijing, the original description of which is in Chinese, with rather crude line drawings which hamper its recognition. The species is of a particular interest as it stands out from its congeners with its troglomorphic habitus.

We here provide an emended description of *S. membranipedalis* based on both recently collected topotypic material and specimens from the Cloud Water Cave (new record), and present a brief overview of the genus *Skleroprotopus*.

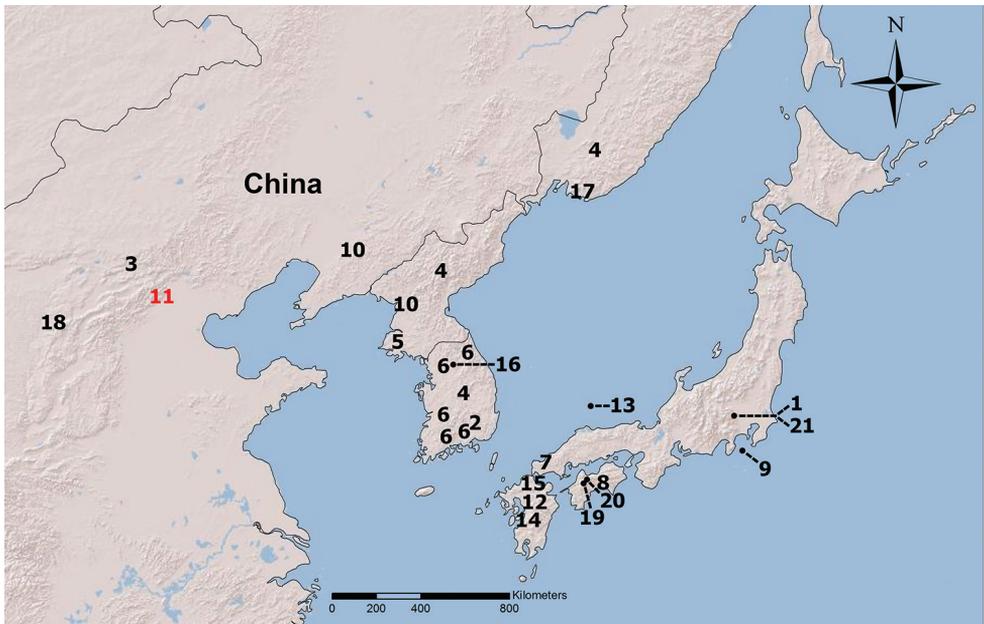


Figure 1. Summarized distribution map of species of *Skleroprotopus*. **Numbers:** **1** *Skleroprotopus chichibuenensis* Shinohara, 1960 **2** *S. chollus* Mikhaljova & Korsós 2003 **3** *S. confucius* Attems, 1901 **4** *S. coreanus* (Pocock, 1895) **5** *S. costatus* Mikhaljova & Korsós, 2003 **6** *S. hakui* Takakuwa, 1940 **7** *S. ikedai* Takakuwa, 1941 **8** *S. inferus* Verhoeff, 1939 **9** *S. insularum* Verhoeff, 1939 **10** *S. laticoxalis* Takakuwa, 1942 **11** *S. membranipedalis* Zhang, 1985 **12** *S. montanus* Takakuwa, 1942 **13** *S. okiensis* Takakuwa, 1941 **14** *S. osedoensis* Miyosi, 1957 **15** *S. platypodus* (Miyosi, 1957) **16** *S. ramuliferus* Lim & Mikhaljova, 2000 **17** *S. schmidti* Golovatch, 1979 **18** *S. serratus* Takakuwa & Takashima, 1949 **19** *S. sidegatakedensis* Miyosi 1957 **20** *S. simplex* Takakuwa, 1941 **21** *S. tori* Takakuwa, 1940.

Description of the sampling sites

Shi-Hua (Stone Flower) Cave

Shi-Hua Cave, also known as Qian-Zhen Cave and Shi-Fo Cave, is located on the south bank of Da-Shi River in Fang-Shan Mountain, Fang-Shan World Geological park, approximately 50 km southwest of Beijing. It is the largest limestone cave in northern China, and the one with the richest deposits of secondary carbonate sediments. The cave is locally important in both touristic and scientific aspects. It is part of the Shi-Hua Karst system formed by several caves, namely Ji-Mao, Yin-Hu, Shi-Hua, Qing-Feng and Kong-Shui, connected by an underground river (Lü et al. 2010).

Shi-Hua is a multilayer limestone cave consisting of 7 levels interconnected by numerous passages and chambers, measuring a total length of about 5640 m. The cave's highest point measured from the entrance is 14 m and the lowest is 158 m (Liu et al. 2015). Its stratum originates from sea deposits formed 400 MYA (Lü 2007). The actual formation of the cave started in the Neogene (23MYA), with the dissolving of the carbonatite (Lü et al. 2010).

Yun-Shui (Cloud Water) Cave

Yun-Shui Cave is also situated in Fang-Shan World Geological park, 75 km from Beijing and some 18 km in a straight line from Shi-Hua Cave. Yun-Shui comprises several halls with a total length of about 610 m. Despite the proximity to Shi-Hua, Yun-Shui, along with the caves San-Qing, Long-Xian-Gong and Xian-Xia, is part of a different karst system – the Tanghsien Karst system located on the northern bank of Ju-Ma River. The two systems – Tanghsien and Shi-Hua – are not connected by underground rivers or terrestrial channels.

The stratum of Yun-Shui was developed from epicontinental sea deposits 1000 MYA, and, similar to Shi-Hua, the cave itself was shaped during the Neogene (Lü 2007). The stalactites of Yun-Shui were formed after the Medio-Pleistocene (Lü 2007, Lü et al. 2010).

Material and methods

The material was collected in November 2013 by Pavel Stoev, Christo Deltchev and Shuqiang Li. It is deposited in the Myriapod collection of the National Museum of Natural History, Sofia (NMNHS). The scanning electron micrographs were obtained with the aid of a JEOL JSM-5510 at the Faculty of Chemistry, Sofia University. The examined body parts were mounted on cover glasses equipped with a sticky tape, and sputter-coated with gold-palladium. The states of the taxonomic characters presented in Table 1 were extracted from the original descriptions and drawings of the corresponding species, and from the few subsequent taxonomic treatments of certain species.

Taxonomic part

Genus *Skleroprotopus* Attems, 1901

***Mongoliulus* Pocock, 1903**

***Paraprotopus* Verhoeff, 1939**

***Nesoprotopus* Verhoeff, 1939**

***Senbutudoiulus* Miyosi, 1957 syn. n.**

Diagnosis. A genus of Mongoliulidae, most similar to the genus *Ansiulus* Takakuwa, both sharing a number of characters such as: ozopores in all body-rings, an anterior gonopod with an unsegmented telopodite (except for *S. schmidti*) and a slightly to considerably higher coxite carrying a well-developed flagellum, and a bifid (except for *S. simplex*) posterior gonopod consisting of a slender solenomerital process and a broader, shield-like, apically setose, caudal process. Differs from *Ansiulus* mostly by the complete absence of telopodites on posterior gonopods and by the strongly reduced telopodites of male leg-pair 7 (except for *S. sidegatakedensis*).

***Skleroprotopus membranipedalis* Zhang, 1985**

Skleroprotopus membranipedalis Zhang, 1985: 154–156, figs 1–8.

Material. 3 males, 7 females & 16 juv., China, Beijing, Fangshan Distr., Shi-Hua (Stone Flower/ Stone Buddha) Cave (type locality), 39°47'36.7"N, 115°56'32.1"E, 12.XI.2013, P. Stoev, Ch. Delchev & S. Li leg. (NMNHS); 7 females & 14 juv., same Distr., Mt. Shangfang, Yun-Shui (Cloud Water) Cave, the touristic part, 39°40'29"N, 115°48'35"E, 3.XI.2013, under stones, creeping on walls, humid clay, some 100 m from the entrance, same collectors (NMNHS).

Diagnosis. Differs from congeners mostly by the flattened, blade-like, basolateral process on the caudal face of the anterior gonopod, and by the completely pigmentless ocelli.

Redescription. Measurements: males 49–51 mm in length, 2.5–2.6 mm in height at mid-body, body ring formula $62+(1-2)+T$; females 49–60 mm, 2.4–2.8 mm, and $(61-67)+(1-2)+T$, respectively.

Colouration in life (Fig. 2): light brown-beige, head whitish; metazonites with darker, narrow, concentric bands, passing just behind the ozopores; repugnatorial glands reddish-brown in living specimens, becoming brown-greyish in ethanol.

General morphology: Adults with 25–40 very small and completely pigmentless ocelli arranged in a narrow triangular field; eye rows unclear. Vertigial setae and pits absent; 4 supralabral and 22–28 labral setae. Antennae (Fig. 3) slender, 1.4–1.5 times as long as head; antennomere 3 longest, ca. twice as long as 6; 2, 4 and 5 subequal in length; 5 and 6 insignificantly thicker than the previous three, their distal margins with a dense whorl of minute sensilla basiconica. Mandibles with 7 pectinate lamellae. Gnathochilar-



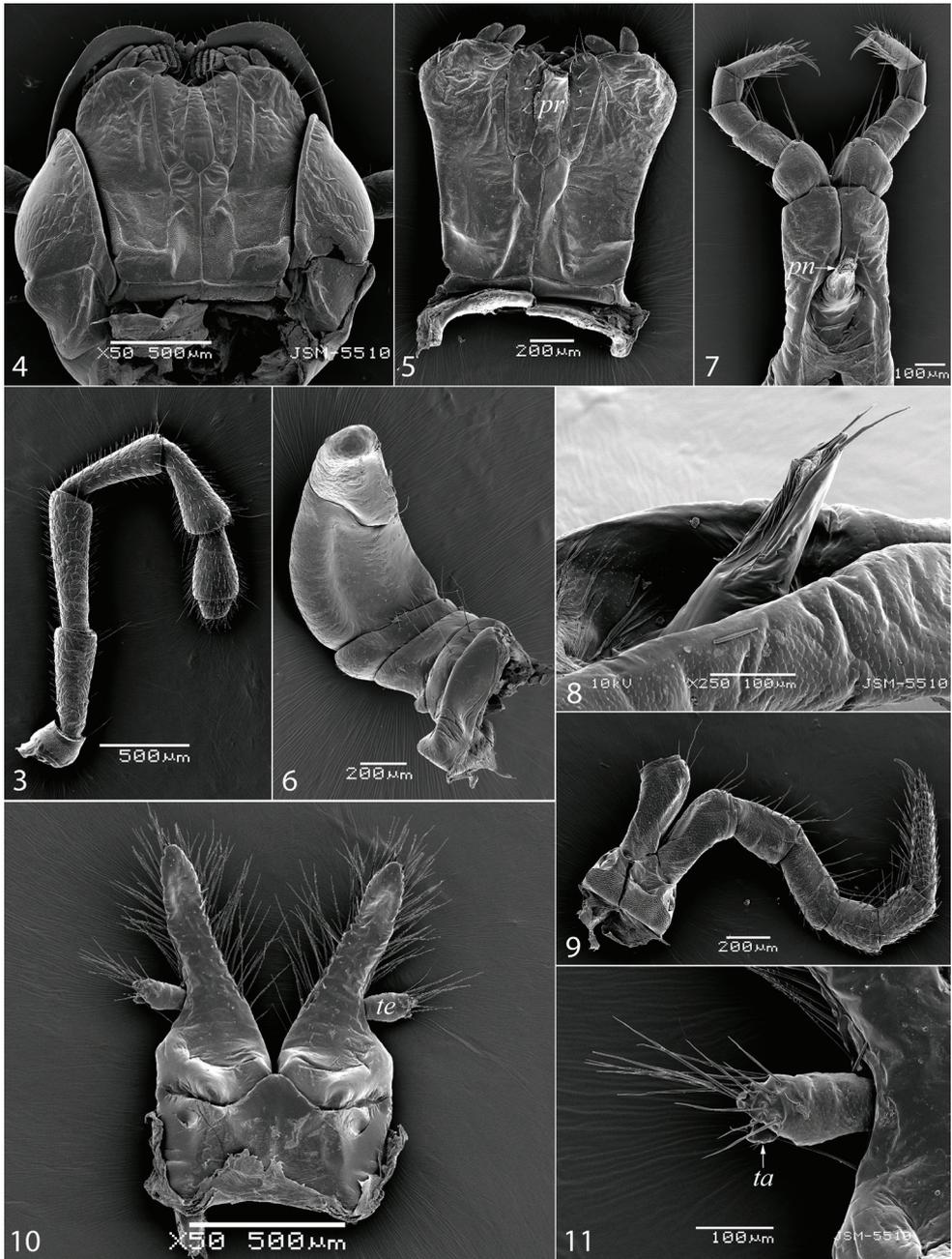
Figure 2. Living specimens of *Skleroprotopus membranipedalis* in Shi-Hua Cave.

ium (Figs 4, 5) with only three apical setae on each stipes and with 7–8 setae on each lamella lingualis arranged in a L-shaped row; promentum of an elongated pentagonal shape, just slightly longer than lamellae linguales, completely separating them.

Collum with ca. 10 shallow striae on each side. Body rings considerably vaulted, this becoming increasingly pronounced towards telson. Prozonites with several shallow, somewhat undulating, transverse striae encircling them (striae being more pronounced dorsally), and dense, short and shallow, longitudinal striation on dorsum near the pro-metazonital suture. Metazonites with rather sparse and shallow (deeper ventrally) longitudinal striae, these disappearing above the ozopore level; without setae on hind margins. Ozopores relatively small, set far behind the pro-metazonital suture, at ca. metazonital mid-length.

Epiproct very short and blunt in both sexes, marginally with one to several setae. Hypoproct broad and short, nearly semi-elliptic, edentate, tightly fitting under the paraprocts; its margin slightly more strongly vaulted in males. Paraprocts with only 2–3 setae each situated near the caudal margins. Walking legs slender; tarsi of mid-body legs 2–2.3 times longer than tibiae, and 3.3–3.7 times longer than the apical claw.

Male sexual characters: Male mandibular stipites enlarged, ventrally incised, forming two nearly equal, subconical lobes. Promentum (*pr* in Fig. 5) of gnathochilarium distally swollen. Leg-pair 1 (Fig. 6) massive, six-segmented, strongly compressed dorsoventrally, reaching up to distal margin of gnathochilarium, without any remnant of an apical claw; podomere 5 very large, strongly arched dorsad, approx. as long as the basal one; podomeres 2–4 each with several long setae in a transverse row, 5 and 6 medially with numerous minute setae. Leg-pair 2 (Fig. 7) with massive, robust coxae, fused to



Figures 3–11. *Skleroprotopus membranipedalis*, external morphology: **3** male antenna **4** female head, ventral view **5** male gnathochilarium, ventral view **6** left male leg 1, latero-dorsal view **7** male leg-pair 2 with penis, caudal view **8** penis *in situ*, lateral view **9** left male leg 3, caudal view **10** male leg-pair 7, caudal view **11** telopodite of male leg 7, caudal view. **Symbols:** *pn*: penis, *ta*: tarsal remnant with apical claw, *te*: telopodite.

each other, basally forming a deep sinus for the penis. Penis (Fig. 8 & *pn* in Fig. 7) elongated, membranous, dorso-medially with an oval groove, and with two long apical setae. All walking legs (Fig. 9) without adhesive pads or other modifications. Pleurotergite 7 ventrally forming small, rounded protrusions originating entirely from the metazonite, directed mesad. Leg-pair 7 (Fig. 10) modified to elongated, non-segmented, leaf-like coxites, proximally with rather small, non-segmented telopodites (Fig. 11 & *te* in Fig. 10), the latter with a minute subapical remnant of a tarsus (*ta*) bearing a claw.

Gonopods (Figs 12–16): *in situ* jutting out of the gonopodal sinus, distal parts of posterior gonopods partly concealed by the anterior gonopods laterally.

Anterior gonopod (Figs 12, 13) with an elongated coxite, gradually narrowing towards a rounded apex, with several setae medially on caudal face; coxite bearing a large, flattened axe blade-like process (*b*), emerging subbasally from its lateral margin, directed caudo-distad, and a well-developed telopodite (*te*), almost half as long as the coxite, non-segmented, apically setose, laterobasally with a minute remnant of a second podomere (*r*). Flagellum (*f*) ca. as long as the coxite, distally densely microsetose, giving it a brush-like appearance; tip not branched.

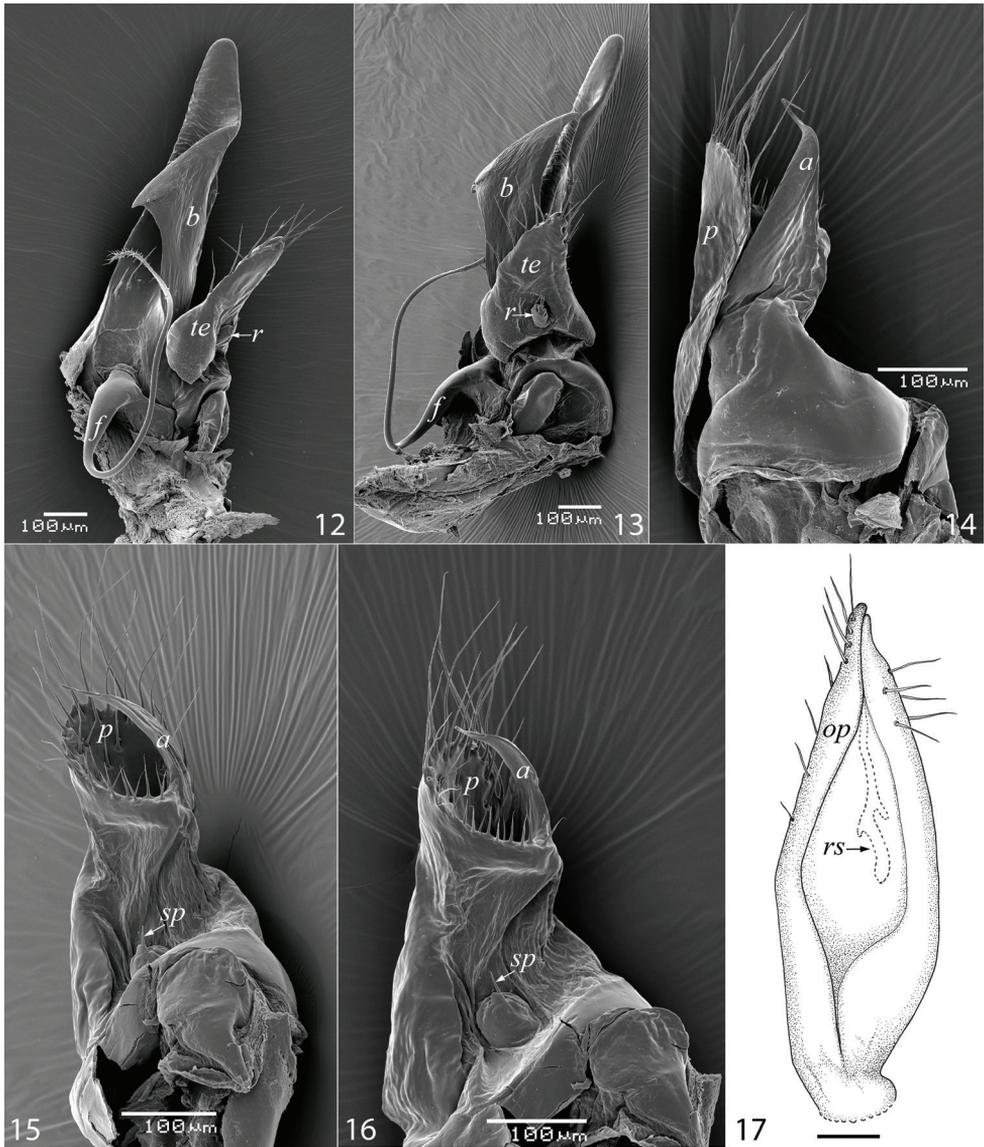
Posterior gonopod (Figs 14–16) stout, with a massive base, distally divided in two parts: a broad, shield-like posterior process (*p*), marginally and disto-frontally bearing long setae; and a simple, unipartite anterior process (*a*), gradually narrowing towards a fine, somewhat bent, filiform apex; mesobasal hump bearing a short spine (*sp*).

Female sexual characters: Leg-pair 1 somewhat thicker and shorter, leg-pair 2 also shorter, but not thicker than the following legs. Vulva (Fig. 17) elongated, subconical; operculum (*op*) slightly higher than bursa, both rather sparsely setose; receptaculum seminis (*rs*) rather small compared to overall size of vulva, in shape of a simple tube, with some very short, pocket-like branches.

Remarks. With its light coloration, slender legs and antennae, and pigmentless ocelli *S. membranipedalis* seems to be the most troglobiomorphic species within the genus (see e.g. Liu et al. 2017). Of the remaining six species occurring in caves, namely *S. ikedai*, *S. inferus*, *S. osedoensis*, *S. platypodus*, *S. sidegatakedensis*, and *S. toriii*, only *S. sidegatakedensis* and *S. ikedai* display similarly light-colored bodies, and all possess blackish ocelli, except for *S. ikedai* and *S. platypodus*, for which this feature is unknown.

However, the presence of ocelli in *S. membranipedalis* suggests a still ongoing adaptation towards troglobiism, meaning that the species has entered the underground relatively recently, possibly in the Pleistocene, in response to the increasingly cooler and drier climate and the gradual replacement of forests by grasslands in the temperate zones – the so called “climatic relict hypothesis” as a model of subterranean colonization (Culver and Pipan 2009). This makes sense also in the light of the rather young geological age of the caves Shi-Hua and Yun-Shui (see introduction part).

Apart from its troglobiomorphic alterations, *S. membranipedalis* is morphologically most similar to *S. coreanus* and *S. serratus*, with whom it shares the lack of claws in the male first legs, the elongated coxites of male 7th legs, the apically non-branched fla-



Figures 12–17. *Skleroprotopus membranipedalis*, gonopods and vulva: **12** left anterior gonopods, caudal, slightly lateral view **13** left anterior gonopod, lateral view **14** left posterior gonopod, lateral view **15** right posterior gonopod, antero-mesal view **16** same, mesal view **17** left vulva, lateral view. **Symbols:** *a*: anterior process, *b*: axe blade-like process, *f*: flagellum, *op*: operculum, *p*: posterior process, *r*: remnant of a podomere; *rs*: receptaculum seminis, *sp*: basal spine, *te*: telopodite. Scale bar (**17**): 0.2 mm.

gellum, and the well-divided posterior gonopod. On the other hand, its six-segmented male first legs suggest a proximity to *S. hakui*, but this is in contradiction with the apically bifurcated flagellum in the latter species.

Discussion

Miyosi (1957a) erected the genus *Senbutudoiusulus* with a sole species *S. platypodus* Miyosi, 1957, based on several characters distinguishing it from the morphologically similar *Skleroprotopus*, in particular: the strongly flattened male leg-pair 1, being almost equally broad until the penultimate podomere, and the slightly flattened leg-pair 2; mandibles with more than six pectinate lamellae, and gnathochilarium with an oval promontum. However, none of these characters can reliably define a genus for the following reasons:

1. Considering the great interspecific variations of the male leg-pair 1 in *Skleroprotopus*, including important features such as the number of podomeres or presence vs. absence of apical claws, their shape observed in the sole species of *Senbutudoiusulus* – *S. platypodus* Miyosi, 1957 – is not sufficiently unusual to justify a separate genus.
2. With the exception of *S. montanus*, the number of the mandibular pectinate lamellae exceeds 6 also in those species of *Skleroprotopus* for which this character is known, namely in *S. confucius* (the type species), *S. membranipedalis*, *S. schmidti*, *S. hakui*, and *S. toriii*, all showing 7 lamellae.
3. A similar oblong oval shape of the gnathochilarium is also observed in members of *Skleroprotopus*, such as *S. coreanus*.
4. The slightly unusual shape of the male leg-pair 2 is too weak for a main generic diagnostic character.

It is also noteworthy that the opisthomerite in *Senbutudoiusulus platypodus* is conspicuously similar to that in *Skleroprotopus osedoensis* Miyosi, 1957, described from a cave in the same Japanese prefecture as the former species (Miyosi 1957a). The similarity and possible synonymy of *Senbutudoiusulus* with *Skleroprotopus* was already suggested by Golovatch (1979), although not formalized.

Thus, we here propose a new synonymy: *Senbutudoiusulus* Miyosi, 1957 = *Skleroprotopus* Attems, 1901, syn. n., and a new combination: *Skleroprotopus platypodus* (Miyosi, 1957), comb. n.

Of the remaining mongoliulid genera, *Ansiulus* Takakuwa, 1940 is conspicuously similar to *Skleroprotopus*, and is a possible synonym of the latter, as already pointed out by Mikhaljova (2004). However, the most important diagnostic character of *Ansiulus*, namely the presence of a telopodital remnant at the base of the opisthomerite (Takakuwa 1940b), is so far unknown in members of *Skleroprotopus*, and seemingly distinguishes the genus well. Even though, some uncertainties about the exact species composition of *Skleroprotopus* still remain, mostly concerning the allocation of *S. sidegatakedensis*. The male 7th legs in this species have a remarkably similar conformation to that shown by males of *Ansiulus aberrans* Mikhaljova & Korsós, 2003. Moreover, the unknown posterior gonopods in the former species could possibly possess telopodital remnants – the other character distinguishing *Ansiulus* from *Skleroprotopus*.

Table 1. Main diagnostic characters of species of *Skleroprotopus*. Symbols: * - divided apically; ** - completely undivided.

	Number of articles of male leg-pair 1	Claws on male leg-pair 1	Number of articles of telopodites of male leg-pair 7	Coxites of male leg-pair 7	Promerital telopodites	Flagellum apically	Opisthomerite deeply divided into 2 branches	Sources
<i>S. chichi-buensis</i>	5	absent	3	massive, stout	~ 1/2 as long as coxites	bifurcated	yes	Shinohara 1960
<i>S. chollus</i>	5	absent	1	very short and stout	~ 1/2 as long as coxites	bifurcated	yes	Mikhailjova and Korsós 2003
<i>S. confucius</i>	5	absent	3	rather slender	~ 2/3 as long as coxites	bifurcated	yes	Attems 1901
<i>S. coreanus</i>	5	absent	3	long and slender	~ 3/4 as long as coxites	non-branched	yes	Golovatch 1979; Mikhailjova 2004
<i>S. costatus</i>	5	absent	1	rather slender	~ 2/3 as long as coxites	non-branched	no*	Mikhailjova and Korsós 2003
<i>S. bakui</i>	6	absent	1–2	long and slender	~ 3/4 as long as coxites	bifurcated	yes	Takakuwa 1940a
<i>S. ikedai</i>	7	absent	2	somewhat elongated	~ 2/3 as long as coxites	bifurcated	yes	Takakuwa 1941
<i>S. inferus</i>	5	present	2	massive, stout	~ 2/3 as long as coxites	bifurcated	yes	Verhoeff 1939
<i>S. insularum</i>	3	absent	1	massive, stout	~ 2/3 as long as coxites	?	?	Verhoeff 1939
<i>S. laticoxalis</i>	5	absent	3	very short and stout	~ 1/2 as long as coxites	bifurcated	yes	Takakuwa 1942
<i>S. membranipedalis</i>	6	absent	2, the 2 nd one minute	long and slender	~ 1/2 as long as coxites	non-branched	yes	Zhang 1985; pers. obs.
<i>S. montanus</i>	5	absent	3	rather short and stout	almost as long as coxites	?	yes	Takakuwa 1942
<i>S. okiensis</i>	5	absent	2	massive, stout	~ 2/3 as long as coxites	bifurcated	yes	Takakuwa 1941
<i>S. osedoensis</i>	5	absent	1	short and stout	~ 1/4 as long as coxites	bifurcated	yes	Miyosi 1957a
<i>S. platypodus</i>	5	absent	2	short and stout	~ 1/4 as long as coxites	bifurcated	yes	Miyosi 1957a
<i>S. ramuliferus</i>	5	absent	2	massive, somewhat elongated	~ 2/3 as long as coxites	non-branched	yes	Lim and Mikhailjova 2001; Mikhailjova and Korsós 2003
<i>S. schmidti</i>	5	present	2	somewhat elongated	~ 3/4 as long as coxites	non-branched	yes	Golovatch 1979
<i>S. serratus</i>	5	absent	1–3	long and slender	almost as long as coxites	non-branched	yes	Takakuwa and Takashima 1949
<i>S. sidegatake-densis</i>	5	present	2, both very large	short and stout	~ 1/2 as long as coxites	bifurcated	?	Miyosi 1957b
<i>S. simplex</i>	5	absent	2, the 2 nd one minute	somewhat elongated	~ 2/3 as long as coxites	non-branched	no**	Takakuwa 1941
<i>S. toriii</i>	5	absent	2, both very short	massive, rather short	~ 2/3 as long as coxites	bifurcated	yes	Takakuwa 1940a

In general, the distribution of the main taxonomic characters within *Skleroprotopus* is mosaic-like, as it can be traced in Table 1. The presence of morphologically isolated species, like *S. insularum* having 3-segmented male first legs, *S. schmidti* showing segmented promerital telopodites, and *S. sidegatakedensis* with conspicuously large telopodites of male legs 7, presents a further obstacle for a possible intrageneric division. Such has been applied only by Verhoeff (1939) who recognized three subgenera: *Skleroprotopus* s. str., *Paraprotopus* Verhoeff and *Nesoprotopus* Verhoeff, the latter two monotypic. And while *Nesoprotopus* deserves a special attention in respect to the outstanding morphology of its sole species, *S. insularum*, the males of which have 3-segmented legs 1, *Paraprotopus* can hardly be used today, as the two characters that distinguish it from the nominotypical subgenus – a bifurcated vs. an unipartite flagellum and male 2nd coxae fused vs. these being divided, respectively – have their states rather randomly distributed among species of the genus. Besides this, certain species (e.g. *S. hakui* and *S. toriii*) show only partial fusion of the coxae which reduces further the diagnostic value of this character.

Acknowledgements

PS is grateful to Shuqiang Li for hosting him at the Institute of Zoology, CAS in November 2013, and for arranging the field trips to the caves Shi-Hua and Yun-Shui. The study is part of the project “Speciation and conservation of cave invertebrate animals”, financially supported by the bilateral exchange program of the Chinese and Bulgarian academies of sciences. The study was partly funded by the University of Shumen, project RD-08-167/09.02.2018.

Part of the results of this study were presented at the 17th International Congress of Myriapodology in Krabi, Thailand, 23–26 July 2017.

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First report of the invasive alien species *Caenoplana coerulea* Moseley, 1877 (Platyhelminthes, Tricladida, Geoplanidae) in the subterranean environment of the Canary Islands

Daniel Suárez^{1,2}, Sonia Martín³, Manuel Naranjo³

1 Departamento de Biología Animal, Edafología y Geología. Facultad de Ciencias, Universidad de La Laguna, 38206, La Laguna, Spain **2** Island Ecology and Evolution Research Group. Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), 38206, La Laguna, Spain **3** Sociedad Entomológica Canaria Melansis, C/ Guaydil 3-1A, 35016, Las Palmas de Gran Canaria, Spain

Corresponding author: Daniel Suárez (danielsura94@gmail.com)

Academic editor: O.T. Moldovan | Received 17 April 2018 | Accepted 20 July 2018 | Published 6 August 2018

<http://zoobank.org/06607AA3-340B-496C-B5E8-CF1FAB7D0D20>

Citation: Suárez D, Martín S, Naranjo M (2018) First report of the invasive alien species *Caenoplana coerulea* Moseley, 1877 (Platyhelminthes, Tricladida, Geoplanidae) in the subterranean environment of the Canary Islands. *Subterranean Biology* 26: 67–74. <https://doi.org/10.3897/subtbiol.26.25921>

Abstract

The blue land planarian *Caenoplana coerulea* Moseley, 1877 is reported for the first time in the hypogean environment. Seven individuals of *C. coerulea* were collected in the most humid branch of an abandoned water mine in Gran Canaria (Canary Islands). Due to its character of generalist predator, it should be considered a threat for the endemic subterranean fauna.

Keywords

Caenoplana coerulea, invasive alien species, top predator, water mine, hypogean

Introduction

The endemic diversity of terrestrial planarians (Platyhelminthes, Tricladida, Geoplanidae) in oceanic islands is considerably scarce; they have limited powers of dispersal because they cannot survive long periods of immersion in water (Winsor et al. 2004).

Due to human-induced activities though, several species have been introduced to oceanic islands worldwide. They are top predators and, in some instances, are able to impact on native invertebrate populations (Boll and Leal-Zanchet 2016). This is the case for species such as *Bipalium kewense* Moseley, 1878 (introduced to Azores, Cape Verde, Fiji, Hawaii, Madeira, Réunion and St. Helena), *Platydemus manokwari* de Beauchamp, 1963 (introduced to Hawaii and Mariana Islands), *Rhynchodemus sylvaticus* (Leidy, 1851) (introduced to Azores) or *Kontikia bulbosa* Sluys, 1983 (introduced to Canary Islands and Madeira) (Winsor et al. 2004).

In the Canary Islands the blue land planarian *Caenoplana coerulea* Moseley, 1877 has been discovered recently in the islands of Gran Canaria and Tenerife although, to date, no record had been yet published (data from the Canarian Government). *Caenoplana coerulea* is an eastern Australian species that lives in areas with high humidity (Luis-Negrete et al. 2011). It has been introduced to gardens and agricultural sectors of New Zealand, Norfolk Island (Australia), the United States, Argentina, the United Kingdom, France, Spain and Menorca Island (Spain) (Breugelmans et al. 2012, Luis-Negrete et al. 2011, Sánchez García 2014), where it is considered an invasive species due its nature as a generalist predator. *Caenoplana coerulea* has been reported preying on several arthropods such as woodlouses, earwigs, millipedes, fly larvae and beetles (Álvarez-Presas et al. 2014).

Methods

Between September 2017 and March 2018, we surveyed the hypogean fauna of an abandoned water mine called “La Federica”, in the east of Gran Canaria (27.9829°N; -15.4647°W, ca. 345 m) (Figure 1A), focussing on the arthropod fauna. The mine is located inside a thermo-sclerophyllous woodland, dominated by *Pistacia lentiscus* L. and *Olea cerasiformis* Rivas-Mart. & del Arco, in a ravine of the Lomo Magullo protected landscape, less than 200 m far from rural areas (Naranjo et al. 2018). It is also 8 km far from the coast, 9 km far from the airport and up to 15 km far from the nearest commercial port. The closest source of water is a pond 440 m away. The cavity sits in an area with basaltic rocks and alluvial sediments, of Pleistocene and Holocene origination (Carracedo 2011).

The temperature, relative humidity and carbon dioxide inside the cave were measured with a Xintest HT-2000 Datalogger, and oxygen was measured with an Uygao ua6070b Datalogger. The captured specimens of *C. coerulea* were preserved in 70% ethanol. Specimens were observed under a binocular lens for external morphological characterization. In addition, to confirm the identification, one specimen was sequenced. DNA was extracted using QIAGEN DNeasy Blood and Tissue Kit. COI was amplified using LCO1490/HCO2980 primers (Folmer et al. 1994). PCR amplification profile was 40 cycles of 30 s at 94 °C, 35 s at 46 °C and 45 s at 72 °C, with an initial denaturation step of 2 min at 94 °C and a final extension step of 5 min at 72 °C. The amplification reaction was performed in 25 µL volume, using 1 µL of DNA

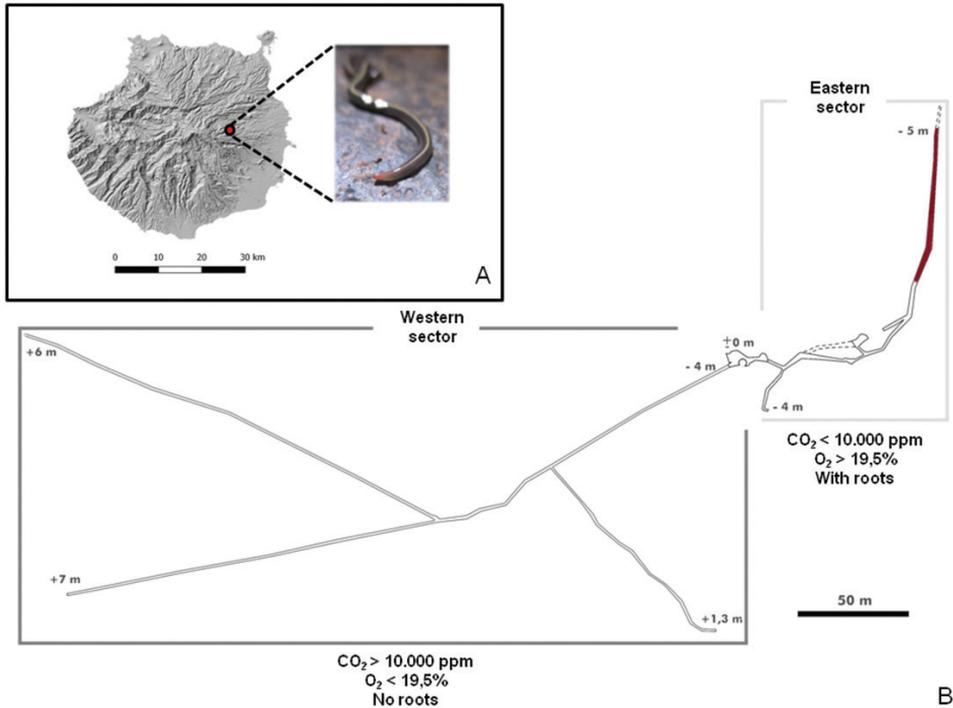


Figure 1. **A** location of “La Federica” mine (red dot) within Gran Canaria (Canary Islands) **B** topography of the mine. *C. coerulea* individuals were observed in the red shaded area.

and 0.1 μL of Taq polymerase (BioTaq). The reaction product was Sanger sequenced and the obtained sequence was blasted against the GenBank database for a proper taxonomic identification. The material is deposited in the Sociedad Entomológica Canaria Melansis collection.

Results

Nineteen individuals of *C. coerulea* were observed during the surveys and seven of them were collected and stored in ethanol (Table 1). Specimens exhibited a dark blue colour dorsally, with a median yellow stripe, while the ventral region was light blue. They also showed an orangish cephalic region where the eyes are arranged in a single row. We did not observe any special character related to the troglobiont syndrome such as eye reduction or loss of pigmentation. A 610 bp fragment was successfully amplified and sequenced (GenBank accession MH644583). It was 99.41% identical to the sequence KJ659650, which correspond to a *Caenoplana coerulea* specimen collected in a nursery of Bordils (Catalonia, Spain) (Álvarez-Presas et al. 2014).

The mine has two principal sectors; the western one showed high levels of CO_2 ($>10,000$ ppm) and a low O_2 concentration ($<19.5\%$), while the eastern one had CO_2

Table 1. Number of specimens of *C. coerulea* observed and collected during the survey of “La Federica” mine.

Date	Number of specimens observed (collected)	Temperature of the mine (°C)	Relative humidity of the mine (%)
9-IX-2017	6 (1)	21.2	86
13-X-2017	5 (0)	21.0	84
4-XI-2017	2 (2)	20.9	86.5
8-XII-2017	2 (2)	22.7	74
16-XII-2017	3 (2)	21.8	83
3-III-2018	1 (0)	Not measured	Not measured

and O₂ levels similar to those on the surface. The specimens in the eastern sector were seen under rocks and around roots in the most humid branch of the mine (Figure 1B). The mean temperature of this branch was $21.5 \pm 0.7^\circ$ C and the mean relative humidity was $82.7 \pm 5.1\%$.

Among the endemic potential prey of *C. coerulea* in the mine there is a millipede of the genus *Dolichoilus* Verhoeff, 1900 as well as two undescribed weevil species of the genera *Oromia* Alonso-Zarazaga, 1987 and *Laparocerus* Schoenherr, 1834. Those weevil are, to date, exclusive to this mine. During the surveys, the exoskeletal remains of a *Laparocerus* adult was found under a rock close to an individual of *C. coerulea*, indicating that it may have been consumed by the planarian. Also, another *C. coerulea* individual was photographed consuming a woodlouse (Figure 2) while wrapping itself around the woodlouse.

Discussion

The external morphology fitted with the description given by Álvarez-Presas et al. (2014) and Breugelmanns et al. (2012), thus suggesting that our individuals belong to the species *C. coerulea*. This is supported by the genetic data, which confirms that our specimens belong to *C. coerulea*. This is the first report for *C. coerulea* in a hypogean environment worldwide. It is possible that the established population here reported may come from some of the rural settlements surrounding the mine, as this species is passively dispersed by the transport of plant pots. Gardens usually harbour microclimatic conditions with high moisture, allowing this species to survive (Sánchez García 2014). The subterranean environment also displays high levels of relative humidity due to the infiltration of surface water and the low evaporation rate (Naranjo et al. 2009).

The mine “La Federica” is located in an area with high potential for subterranean fauna (Naranjo et al. 2014), and it is the artificial cavity of the Canary Islands with the highest richness of troglobionts. Among invertebrate we only detected arthropod species, apart from *C. coerulea* (see Table 2). The sector of the mine with the presence of *C. coerulea* is at 7–10 m beneath the surface, which makes it plausible for this species

Table 2. Check-list of the subterranean fauna in the mine “La Federica” (Gran Canaria, Canary Islands). Abbreviations: Y – Yes; N – No; ? – Doubtful.

Class/Order	Species	Troglobiont	Endemism	Potential prey
Class Arachnida				
Order Schizomida				
	<i>Stenochrus portorricensis</i> Chamberlin, 1922	Y	N	N
Order Araneae				
	<i>Tegenaria pagana</i> C.L. Koch, 1840	N	N	N
	<i>Dysdera</i> n. sp. 1	Y	Y	N
	<i>Dysdera</i> n. sp. 2	Y	Y	N
	<i>Scotophaeus</i> n. sp.	Y	Y	N
	<i>Setaphis gomeræ</i> (Schmidt, 1981)	N	Y	N
	<i>Troglohyphantes roquensis</i> Barrientos & Fernández-Pérez, 2018	Y	Y	N
	<i>Eidmanella pallida</i> (Emerton, 1875)	N	N	N
	<i>Pholcus ornatus</i> Bösenberg, 1895	N	Y	N
Order Pseudoscorpiones				
	<i>Microcreagrina cavicola</i> Mahnert, 1993	Y	Y	N
Class Chilopoda				
Order Lithobiomorpha				
	<i>Lithobius</i> sp.	N	N	N
Class Diploda				
Order Julida				
	<i>Dolichojuulus</i> cf. <i>longungis</i> Enghoff, 2012	Y	Y	Y
Order Polyxenida				
	<i>Polyxenus</i> sp.	N	N	Y
Class Malacostraca				
Order Isopoda				
	<i>Porcellio</i> sp.	N	N	Y
Class Insecta				
Order Zygentoma				
	<i>Canariletia holosterna</i> Molero, Gaju, López, Oromí & Bach, 2014	Y	Y	?
Order Blattodea				
	<i>Symploce microphthalma</i> Izquierdo & Medina, 1992	Y	Y	N
Order Hemiptera				
	<i>Meenoplous roddenberryi</i> Hoch & Naranjo, 2012	Y	Y	?
	<i>Collartida</i> n. sp.	Y	Y	?
Order Diptera				
	<i>Phlebotomus</i> sp.	N	N	Y
	<i>Megaselia</i> sp.	N	N	Y
Order Coleoptera				
	<i>Calathus angularis</i> Brullé, 1839	N	Y	?
	<i>Parazuphium</i> n. sp.	Y	Y	?
	<i>Oromia</i> n. sp.	Y	Y	Y
	<i>Laparocerus</i> n. sp.	Y	Y	Y



Figure 2. Individual of *C. coerulea* preying on an isopod (red circle).

to disperse through the soil pores. It is likely that the uppermost caves are more prone to invasion by *C. coerulea* than those having a greater depth of soil overlying them. The presence of *C. coerulea* in the mine was restricted to an area of high humidity and levels of oxygen and carbon dioxide similar to the surface, whereas in the branch with foul air it was never detected. This indicates that incursion by *C. coerulea* may be mediated by the subterranean atmospheric conditions, and that regions most similar to ambient surface conditions have a higher risk of invasion. The presence of roots may also play a role in the distribution of *C. coerulea* because the diversity and abundance of rhizophagous potential prey is correlated with the presence of roots in the mine. Mateos et al. (2013) described that during the attack of *C. coerulea*, it stands next to its prey and disposes of its pharynx to feed on it. However, in the case here reported the individual was wrapping the woodlouse while it was consuming it.

Introduced invertebrates are not very frequent in subterranean environments on the Canary Islands (Oromí and Martín 1992). In contrast, these environments harbour a great diversity of endemic troglobionts, with more than 150 described species. In fact, the Canaries are the most diverse volcanic region of the world in terms of their adapted subterranean species, and they are considered a hot-spot for hypogean biodiversity (Pérez-Delgado et al. 2016). Because of the aggressively predatory nature of *C. coerulea*, it is therefore important to monitor its occurrence and dispersion in the Canary Islands, not just in gardens but also in the subterranean environment, where a high proportion of endemic species can be threatened by this planarian.

Acknowledgements

This work has been supported by the project “Fauna subterránea del Barranco de los Cernícalos” funded by the Cabildo de Gran Canaria. We are very grateful to Octavio Fernández (GE-Tebexcorade-La Palma) for elaborating the topography of the mine as well as to Pedro Oromí (Universidad de La Laguna) for revising the manuscript. Brent Emerson and Heriberto López (IPNA-CSIC) provided assessment during the laboratory phase. We are also grateful to the referees Marta Álvarez-Presas and Kathryn Hall for their constructive suggestions that improved the quality of the manuscript.

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Phylogeographical convergence between *Astyanax* cavefish and mysid shrimps in the Sierra de El Abra, Mexico

Joseph Kopp¹, Shristhi Avasthi¹, Luis Espinasa¹

¹ School of Science, Marist College, 3399 North Rd, Poughkeepsie, New York 12601, USA

Corresponding author: Luis Espinasa (luis.espinasa@marist.edu)

Academic editor: O. Moldovan | Received 31 May 2018 | Accepted 25 July 2018 | Published 14 August 2018

<http://zoobank.org/84D5AECA-0F2A-4155-9000-C8F5817E7C7D>

Citation: Kopp J, Avasthi S, Espinasa L (2018) Phylogeographical convergence between *Astyanax* cavefish and mysid shrimps in the Sierra de El Abra, Mexico. *Subterranean Biology* 26: 75–84. <https://doi.org/10.3897/subtbiol.26.27097>

Abstract

The Sierra de El Abra is a long (120 km) and narrow (10 km) karstic area in northeastern Mexico. Some studies have suggested independent evolutionary histories for the multiple populations of blind cavefish *Astyanax mexicanus* that inhabit this mountain range, despite the hydrological connections that may exist across the Sierra. Barriers between caves could have prevented stygobitic populations to migrate across caves, creating evolutionary significant units localized in discrete biogeographical areas of the Sierra de El Abra. The goal of the present study was to evaluate if there is a correspondence in phylogeographical patterns between *Astyanax* cavefish and the stygobitic mysid shrimp *Spelaeomysis quinterensis*. *Astyanax* mtDNA and mysid histone H3 DNA sequences showed that in both species, cave populations in central El Abra, such as Tinaja cave, are broadly different from other cave populations. This phylogeographical convergence supports the notion that the central Sierra de El Abra is a biogeographical zone with effective barriers for either cave to cave or surface to cave gene flow, which have modulated the evolutionary history across species of its aquatic stygobitic community.

Keywords

Sierra de El Abra, cavefish, *Spelaeomysis quinterensis*, *Astyanax mexicanus*, convergent evolution, phylogeography

Introduction

The teleost *Astyanax mexicanus* has become one of the most influential models for studying regressive evolution and cave adapted organisms. The species consists of several eyeless, depigmented cave-dwelling forms and eyed, pigmented surface-dwelling forms. Since the forms remain interfertile, this allows exploration of the molecular, genetic, and developmental mechanisms of adaptation to the cave environment (Jeffery 2012). The surface-dwelling forms inhabit a high variety of hydrological systems (i.e. lakes, rivers, lagoons, cenotes, etc.) throughout Mesoamerica region, while the stygobitic forms inhabit a series of subterranean systems in the Sierra de El Abra, Sierra de Guatemala, and Micos area, in Northeast Mexico. The Sierra de El Abra, where the largest abundance of caves with cavefish populations are located, (i.e. 21 populations currently discovered), is a long (120 km) and narrow (10 km) limestone ridge. Despite its success as a model organism for study in the field of evolutionary development, there is still considerable controversy regarding its phylogeography (e.g. Ornelas-García et al. 2008; Bradic et al. 2012, Strecker et al. 2012). Central to this discussion has been the question of how many times have the surface populations independently colonized the cave environment and how much underground dispersal has occurred to establish the current cave populations. A plethora of publications have accumulated over time with terms such as phylogenetically old/new populations, lineages A/B, phylogenetically old/new clusters, and old/new epigeal stocks, with individual cavefish populations assigned contradictorily to one or to another set (Gross 2012).

Initial genetic studies with isoenzymes (Avice and Selander 1972) and RAPDs (Espinas and Borowsky 2001) supported that all of the Sierra de El Abra cave populations were monophyletic, suggestive of high underground dispersal amongst caves. However, when mtDNA was first sequenced (Dowling et al. 2002), results showed a drastically different scenario. A phylogeographical discordance was evident, where the populations were not necessarily most closely related to their nearest geographical neighbors. The northernmost cave in Sierra de El Abra (Pachón cave) and the southernmost cave (Chica cave) shared an identical haplotype, while populations from caves in the center (Curva, Sabinos and Tinaja) shared a broadly different group of haplotypes. Aforementioned authors labeled them as lineage A and lineage B respectively. This lineage B of centrally located cave populations is very different from the northernmost and southernmost cave populations, being more closely related to *Astyanax aeneus* from Costa Rica than to lineage A (Dowling et al. 2002). Authors suggested that lineage A and lineage B colonized independently the cave environment. Later studies including a larger data set with mitochondrial loci (e.g. 16S rDNA, *Cox1*, *Cytb* in Ornelas-García et al. 2008) recovered a similar pattern.

Recent studies evaluating mtDNA, nuclear and genomic data in cave and surface populations of *Astyanax* have shown a discordance between nuclear and mitochondrial phyletic patterns (e.g. Strecker et al. 2011; Coghill et al. 2014, Ornelas-García and Pedraza-Lara 2015). While mtDNA shows central caves such as Tinaja cave populations as very distant and of paraphyletic origin to northern and southern caves populations like Pachón and Chica, nuclear DNA can show the central cave populations as closely related to the other El Abra cave populations at the exclusion of surface populations.

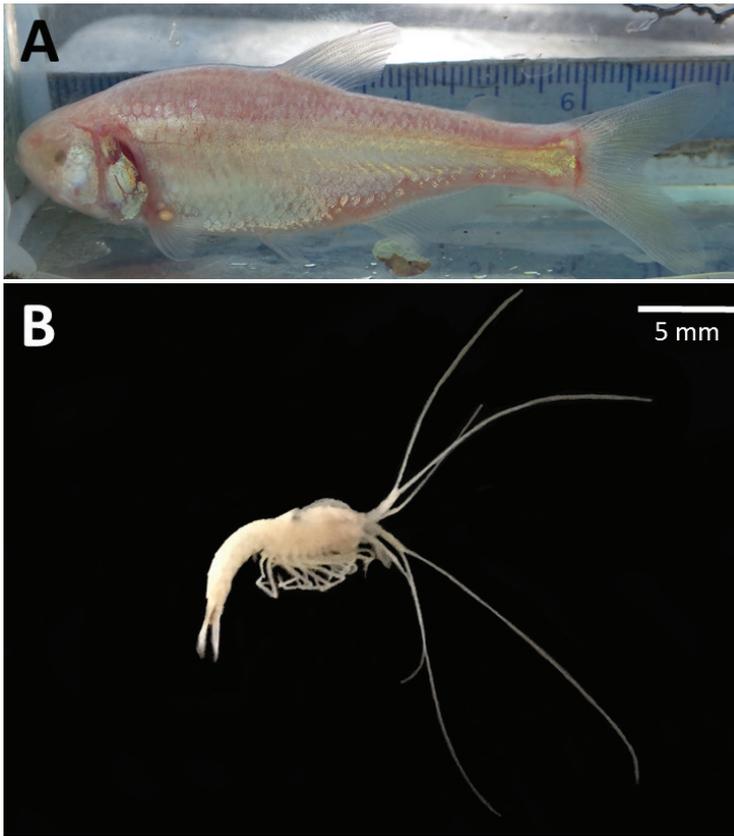


Figure 1. **A** *Astyanax mexicanus* from Chiquitita cave **B** The mysid shrimp, *Spelaeomysis quinterensis*, also from Chiquitita cave. Both stygobitic organisms have overlapping biogeographic ranges throughout the El Abra karstic area, in northeastern Mexico.

A possible solution to this discordance comes from admixture analyses, which indicated that there has been recent gene flow between distinct mitochondrial clades with mitochondrial “capture” that occurred between some caves (e.g. Pachón and Chica) to neighboring surface populations (Gross 2012).

Yoshizawa et al. (2012) proposed that the disparity in nuclear and mtDNA in cavefish populations that have hybridized with surface fish can be explained by paternal inheritance of Vibration Attraction Behavior (VAB). The results suggest that parental genetic effects in adaptive behaviors may be an important factor in biasing mtDNA inheritance in natural populations that are subject to introgression. Another hypothesis examined here is that there may be barriers for cave-to-cave or surface-to-cave gene flow that created evolutionary significant units localized in discrete biogeographical areas of the Sierra de El Abra. These barriers may affect the phylogeographic patterns of all the aquatic communities inhabiting this network of underground rivers. We have thus studied the phylogeny of another aquatic organism whose size and biogeographical range overlaps with *Astyanax*, the mysid shrimp *Spelaeomysis quinterensis* (Fig. 1B).

Materials and methods

Samples of *Astyanax* cavefish (Fig. 2) were collected from the following localities and they are ordered from north to south; Molino (N=2), Caballo Moro (N=2), Pachón (N=2), Sabinos (N=2), Tinaja (N=3), Curva (N=1), Chica (N=1), and Chiquitita (N=4). “Chiquitita” cave should not be confused with “Chica” cave. Chiquitita is the most recently discovered cave with a cavefish population (Espinasa et al. 2018), found south of Chica cave, in the town of El Pujal, at the southernmost tip of Sierra de El Abra.

Samples were also collected from a surface locality inhabited by Lineage A *Astyanax*; Río Comandante (N=2), and from two surface localities inhabited by Lineage B *Astyanax*; Rascón (N=2) and Tamasopo (N=1). Collecting permit # SGPA/DGVS/02438/16 from Secretaría del Medio Ambiente y Recursos Naturales, México, was issued to Patricia Ornelas García.

Samples of *S. quinterensis* were collected from four different cave localities (Fig. 2). From north to south, Caballo Moro (N=1) in the Sierra de Guatemala, and Pachón (N=2), Tinaja (N=3), and Chiquitita (N=1) from the Sierra de El Abra. Localities where N=1 was due to the fact that no more specimens could be found in these difficult to access caves, as is often the case in cave studies. In localities where N>1, Bp differences with populations was zero (See results), suggesting diversity within population was very low and thus supporting that mayor conclusions were not affected by sample size.

Genomic DNA was extracted using Qiagen’s DNEasy® Tissue Kit by digesting a fin clip or a leg in lysis buffer. For *Astyanax* samples, all markers were amplified and sequenced as a single fragment using the 16Sar (5’ CGCCTGTTTATCAAAAACAT 3’) and 16sb (5’ CTCCGGTTTGAACCTCAGATCA 3’) primer pair for mitochondrial 16S rRNA, following standard protocols (Espinasa et al. 2007). Since this particular primer pair is inefficient for amplifying crustaceans in general and since the goal of this study was only to get phylogeny of both groups without regard to having different molecular clocks among markers, *S. quinterensis* were amplified instead with the following primer pairs: H3aF (5’ ATGGCTCGTACCAAGCAGACVGC 3’) and H3aR (5’ ATATCCTTRGGCATRATRGTGAC 3’) for histone H3 (Espinasa et al. 2007). Amplification was carried out in a 50 µl volume reaction, with Qiagen’s Multiplex PCR kit. The PCR program for both markers consisted of an initial denaturing step at 94 °C for 60 sec, followed by 35 amplification cycles (94 °C for 15 sec, 49 °C for 15 sec, 72 °C for 15 sec), and a final step at 72 °C for 6 min in a GeneAmp® PCR System 9700 (Perkin Elmer). PCR products were then subsequently cleaned with Qiagen’s QIAquick PCR Purification Kit and sent to SeqWeight for direct sequencing. Chromatograms obtained from the automated sequencing were read and contigs made using the sequence editing software Sequencher™ 3.0. External primers were excluded from the analyses. Sequence identity was confirmed through BLAST analyses. Sequences were aligned and phylogeny trees obtained with ClustalW2 and base pair differences were counted with Sequencher™ 3.0.

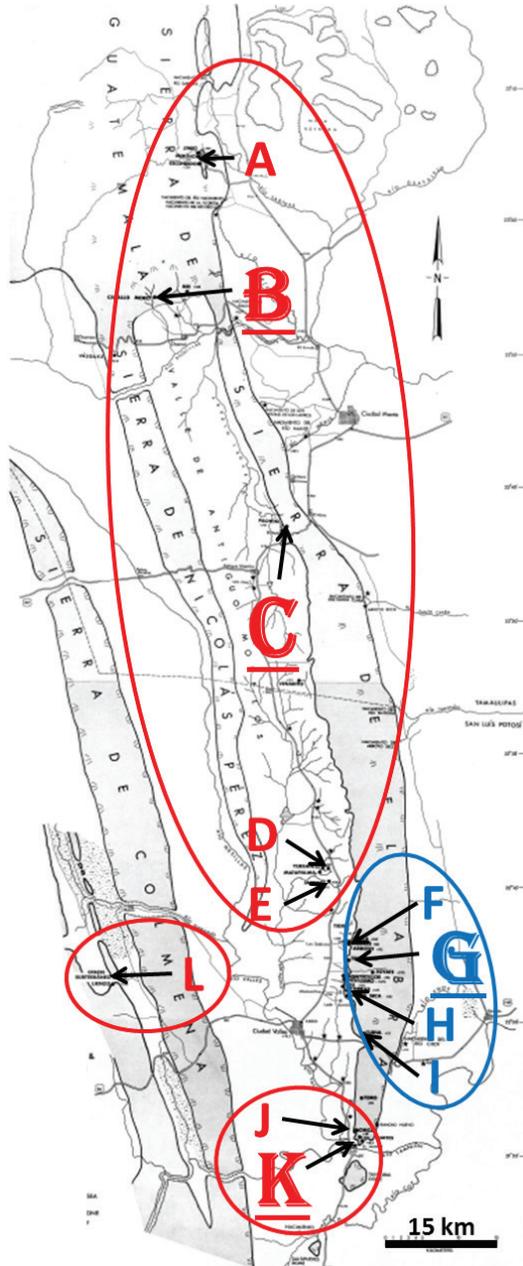


Figure 2. Cave localities of *A. mexicanus* whose mitochondrial DNA has been analyzed. With larger font and underlined are localities where *S. quinterensis* were also collected. In red are caves harboring lineage A and in blue those with lineage B for both mtDNA in *Astyanax* and histone 3 for *S. quinterensis*. Notice that lineage B is restricted to a small biogeographical zone, circled in blue. **A** Molino **B** Caballo Moro **C** Pachón **D** Yerbaniz **E** Japones **F** Sabinos **G** Tinaja **H** Piedras **I** Curva **J** Chica **K** Chiquitita **L** Rio Subterráneo. (Figure modified from Mitchell et al. 1977).

Results

All fragments for the mitochondrial 16S rRNA of *Astyanax* were 572 bp long. There were no indels when aligning the sequences. Specimens from Molino, Caballo Moro, Pachón, Chica, and Chiquitita caves and from the surface locality of Río Comandante all had identical sequences, except one out of four specimens from Chiquitita cave that differed by 1 bp (0.17%). The consensus sequence was also identical to GenBank sequence (AP011982.1) of “*Astyanax mexicanus* mitochondrial DNA, almost complete genome”. Since the localities of Molino, Pachón, Chica and Río Comandante are previously known to harbor lineage A mtDNA (Gross 2012), the newly assessed cave populations of Caballo Moro, and Chiquitita caves are determined to also have lineage A mtDNA.

The cave populations of Sabinos, Tinaja and Curva had identical sequences. Likewise the surface populations of Rascón and Tamasopo were identical. These five localities are known to harbor fish from the Lineage B (Gross 2012). Our surface sequences of lineage B differ from the Lineage A sequences by 2 bp (0.34%) at the 572 bp fragment positions of 296(A/G) and 297(A/C) respectively. Additionally, our cave sequences of lineage B differ from the Lineage A sequences by 5 bp (0.87%) at the 572 bp fragment positions of 198(G/A), 262(C/T), 296(A/G), 297(A/G), and 329(T/C) respectively.

Regarding the mysid shrimp, the H3 fragment from all seven specimens were 328 bp long (GenBank # MH422492–MH422494). There was no need for insertions or deletions to align the sequences. In the localities where more than one specimens was sequenced (Pachón N=2 and Tinaja N=3), no variability within populations was found and their sequences were identical. Two clades or lineages were found. The first lineage included specimens from Caballo Moro, Pachón and Chiquitita. The second lineage was made of Tinaja (Fig. 3). Similarity of sequences among populations did not follow geographical proximity between caves (Figs 3–4). Specimens from the northernmost (Pachón) and the southernmost (Chiquitita) portions of Sierra de El Abra were identical. The northernmost specimen, from Sierra de Guatemala, differed from these two Sierra de El Abra populations by 7 bp (2.1%). Specimens from central Sierra de El Abra (Tinaja) were the most distinct, differing by 31–36 bp (9.4–10.9%) from all of the aforementioned localities. The Tinaja lineage is close to 5 times more different than Sierra de Guatemala populations are to Sierra de El Abra. It is unexpected to find that within the Sierra de El Abra, Tinaja cave is so drastically divergent to the other populations from the same region. In particular because this population is located geographically in between two populations that had identical sequences, Pachón and Chiquitita (Fig. 2).

Discussion

To our knowledge, a total of 12 out of the 30 caves known to harbor cavefish in the El Abra region have had their mtDNA sequenced thus far, with the inclusion of the new

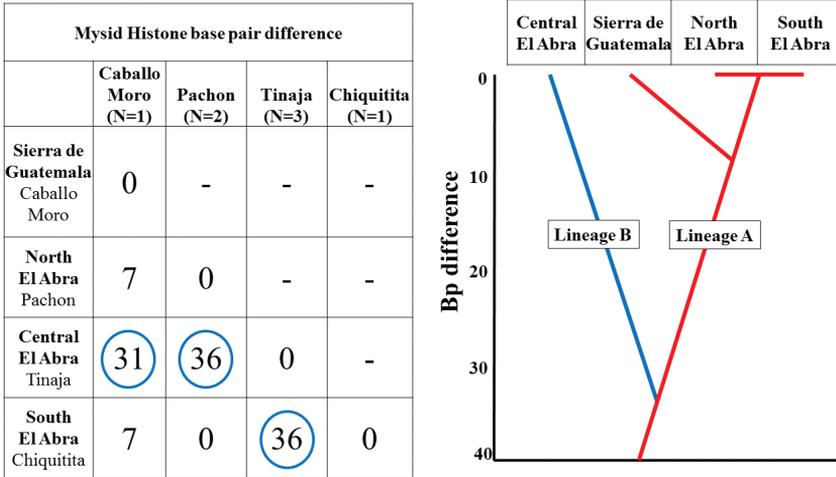


Figure 3. A, Base pair differences of histone 3 sequences between mysid shrimps. Specimens from central Sierra de El Abra (Lineage B) are markedly different from all other populations (Lineage A).

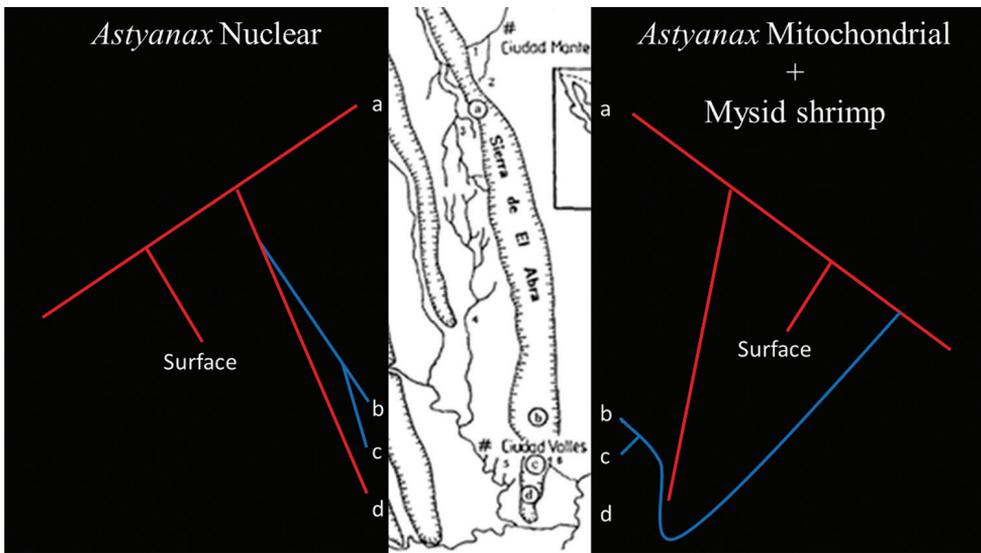


Figure 4. Pylogeographical convergence between mysid shrimps in the Sierra de El Abra and the mtDNA of *Astyanax cavefish* (right). Both aquatic species harbor the evolutionary signature of a phylogeographical discordance, where genetic markers of populations in central Sierra de El Abra are extremely distinct from the rest of the populations. Nuclear tree (left) based on the consensus of isoenzymes, RAPDs, microsatellite, and genomic sequences. a) Pachón as representative of northern populations. b-c) Sabinos and Tinaja as representative of central populations. d) Chica and Chiquitita as representative of southern populations.

Astyanax populations of this study. From this wealth of data, a pattern seems to emerge (Figs 2, 4). Cave populations with Lineage B mtDNA (Sabinos, Tinaja, Piedras and Curva) are restricted to an area in central Sierra de El Abra. This region is localized be-

tween 15 km north of Ciudad Valles to directly east of the city. The northern (Pachón, Yerbaniz and Japonés) and southern (Chica and Chiquitita) portions of Sierra de El Abra, Sierra de Guatemala (Molino and Caballo Moro), and Micos (Río Subterráneo) areas are all inhabited by cavefish with Lineage A mtDNA.

Results obtained from the stygobitic shrimp suggest that mysids in central Sierra de El Abra (Tinaja) derive also from a separate lineage different from the rest of the Sierra de El Abra (Pachón and Chiquitita caves) and Sierra de Guatemala (Caballo Moro) populations. This implies that the aquatic mysid shrimps had at least two separate evolutionary histories, or lineages, which are linked to distinct geographical areas within the Sierra de El Abra. The phylogeography of the mysids lineages is in agreement and overlaps with the mitochondrial lineages of *Astyanax* cavefish (Fig. 4). This phylogeographical convergence supports that some caves in the central area of Sierra de El Abra, where Tinaja cave is located, are within a biogeographical zone that has modulated to a certain extent the evolutionary histories across multiple aquatic stygobitic species. Probably, this has kept them partially isolated from gene flow from other El Abra zones or from surface populations.

Since this pattern has now been shown to be similar in two distinct taxa of aquatic organisms, *Astyanax* and mysid shrimps, it is proposed that independent colonization and/or underground barriers have created a separate biogeographical zones that promotes independent evolutionary histories across aquatic communities. Alternatively, some caves in this central zone are less connected to surface systems and therefore less prompt to be colonized or introgressed by surface populations. If surface gene flow is more difficult in the central area (i.e. Tinaja), this isolation could lead to population differentiation as observed in both mitochondrial *Astyanax* and in mysid shrimps. A caveat of this hypothesis is that it would require mysid shrimps to currently have a surface morph or a surface ancestor that has or had access to only northern and southern caves in recent times. *Spelaeomysis quintereensis* is a highly troglomorphic species that is unlikely to survive on the surface, and unlike *Astyanax*, no surface morph has been described. This study opens the necessity to further investigate the sister group of this species to better understand the evolutionary history of this group and their adaptation to the caves environment. Furthermore, any proposed barriers should not be considered as completely impermeable and effective to eliminate all gene flow. This is evidenced in nuclear sequences in *Astyanax* which show a different pattern from mitochondrial data, corroborating that at least some gene flow either from within the caves or from surface to caves exist (Ornelas-García and Pedraza-Lara, 2015).

Barriers for dispersal for aquatic and terrestrial organisms in the El Abra caves appear to be different. Espinasa et al. (2014) sequenced the 16S rRNA of *Anelpistina quintereensis* (Nicoletiidae, Zygentoma, Insecta), a highly troglomorphic nicoletioid silverfish insect that inhabits from southern Sierra de El Abra to Sierra de Guatemala. Using a calibrated molecular clock, their data supports that this species migrated underground to reach both mountain ranges within less than 12,000 years, implying relatively easy underground migration, with no isolating evolutionary barrier for the

central Sierra de El Abra, in terrestrial cave populations. So it would appear that at least for land troglobites, their phylogeography is different from aquatic species.

In conclusion, phylogeographic results obtained from the mysid Shrimp, *Speleomysis quinterensis* mimic the results of mitochondrial studies in *Astyanax*. This suggests that the geographic distribution of mitochondrial lineages in *Astyanax* is neither stochastic, nor exclusively explained by linkage to paternally inherited characters on distinct populations. Instead it supports that Sierra de El Abra has distinct biogeographic areas, with partial barriers that affect evolutionary histories creating evolutionary significant units for all members across different species of the aquatic cave community.

Acknowledgments

We would like to thank Sylvie Retaux, Emily Collins, Jenna Robinson, Jennifer Rutkowski, and Christian Schroeder who helped with specimen collection and or analysis. Patricia Ornelas-Garcia for reviewing the manuscript. Sequencing was performed with the help of students of the Spring 2017 BIOL320 Genetics course at Marist College. Partial support for the project was granted by the School of Science at Marist College and VPAA grants.

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