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Richness and taxonomic distinctness of cave invertebrates from the northeastern state of Goiás, central Brazil: a vulnerable and singular area

Maria Elina Bichuette[1], Luiza Bertelli Simões[2], Tamires Zepon[1],
Diego Monteiro von Schimonsky[1], Jonas Eduardo Gallão[1]

1 *Laboratório de Estudos Subterrâneos, Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, Via Washington Luís, km 235, 13565-905, Caixa Postal 676, São Carlos, SP, Brazil* **2** *Universidade Estadual Paulista, Rio Claro, Brazil*

Corresponding author: *Maria Elina Bichuette* (lina.cave@gmail.com)

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Abstract

The karst area of the northeastern state of Goiás comprises two main municipalities: São Domingos and Posse. São Domingos is inside the limits of a Full Protection Conservation Unit known as Parque Estadual de Terra Ronca (PETeR), where a high number of caves occurs, some of them surpassing 10 km in length. Despite their protection by law, uncontrolled tourism has been threatening the integrity of the unique and fragile cave systems of São Domingos. On the other hand, the caves of Posse are much less visited, with its subterranean fauna poorly unknown and are strongly threatened by the urban areas and mining activities in the vicinity. We conducted six systematic surveys of invertebrates in 12 caves, seven of which are located in São Domingos and five in Posse, between 2010 and 2012. Immediately outside the caves, we sampled several microhabitats for comparison. We estimated richness, abundance, and taxonomic distinctness of the communities. We recorded 1,941 individuals of 344 morphospecies. Caves from São Domingos showed a higher species richness and were more taxonomic distinct than caves from Posse. Most morphospecies were considered troglophiles (188). We also found one trogloxene and one troglobite, as well as nine troglomorphic taxa that possibly are troglobitic as well. Both regions have markedly singular caves regarding its subterranean fauna, with high values of taxonomic distinctness. However, the richer caves were not necessarily the most taxonomic distinct. Conservation measures are especially necessary in the region of Posse, where caves are not legally protected and are within an area of significant urbanization. Cave entrances of Posse are near deforested vicinities, in some cases with domestic wastes and cement plants nearby.

Keywords
caves, conservation, phylogenetic diversity, subterranean fauna, São Domingos, Posse, Goiás

Introduction

The subterranean fauna consists of organisms able to survive in peculiar conditions of the environment, as the permanent darkness, the high relative humidity of the air and stable temperatures at the deepest zones (Barr 1968). These inherent features may act as an environmental filter (see Fernandes et al. 2016 to opposite view) in such a way that only part of the epigean taxa is capable to colonize and establish hypogean populations. So, the organisms found inside caves can be sorted in three ecological-evolutionary categories, considering its degree of specialization and dependence of surface environments: trogloxenes, troglophiles and troglobites.

Because of its unique conditions and the high degree of endemism, subterranean environments are fragile and sensitive to environmental changes, especially those regarding anthropic activities (Culver 1986, Gibert and Deharveng 2002, Trajano and Bichuette 2006). Among the main human interference in Brazilian caves, we may stress mining, construction of hydroelectric dams, deforestation for agriculture and uncontrolled tourism (Ferreira et al. 2014, Gallão and Bichuette 2018).

Brazil has about 17,000 registered caves (CECAV 2018). Some heterogeneity exists in the knowledge about the subterranean fauna regarding different Brazilian karst and non-karst areas. Some are well known, as the Vale do Ribeira (southeastern) (e.g., Trajano 1991, Bichuette and Trajano 2003, Rodrigues et al. 2014) and Chapada Diamantina (eastern-northeastern) (Gregorin and Mendes 1999, Bockmann and Castro 2010, Gallão and Bichuette 2015). Others, however, have its knowledge limited to preliminary data about species richness and abundance, which was obtained on a few sampling occasions (e.g. Altamira, northern) (Trajano and Moreira 2001). Besides this, only a few approaches covered long-term samplings, with replicas enough to contemplate more than one annual cycle (e.g. Trajano 1991, Gallão and Bichuette 2015, Resende and Bichuette 2016, Zepon and Bichuette 2017). Most often in tropical caves, the seasonal oscillation is of great importance in the dynamics of the subterranean environments (Trajano 2000).

Some studies have shown that phylogenetic diversity, a measure of species relatedness considering the phylogenetic relationship among species, are more sensitive to detecting responses of communities to environmental changes (Cianciaruso et al. 2009). This is because traditional diversity indexes have limited prediction about ecosystems functioning. According to Faith (1992), the emphasis on conservation regards preserving as much of this hierarchical variation as possible, no matter what the taxonomic identity involved. In this sense, a phylogenetic diversity index has been shown more efficient to proposals of conservation strategies than traditional indexes (Cianciaruso et al. 2009) and was used also for conservation purposes comparing taxonomic distinctness with α indexes (Gallão and Bichuette 2015).

In the present study, we sampled subterranean invertebrates at the karst area of the northeastern Goiás state, central Brazil, encompassing the municipalities of São Domingos and Posse. We estimated species richness and abundance by each cave and by each region, as well as by the karst area. Finally, we compared the fauna of these caves regarding geographical position and taxonomic distinctness of the communities. We expected that phylogenetic component of diversity would indicate those most singular caves in the region better than traditional indexes of diversity. Our purpose was to improve the knowledge about components of biological diversity in the region and, through a better knowledge of the ecological-evolutionary processes underlying these communities, provide best arguments for better conservation decisions.

Methods

Study Area

The karst area at northeastern Goiás represents one of the regional expressions of Bambuí geomorphological Unit, the largest set of limestone in Brazil, comprising approximately 105,200 km^2 (Auler et al. 2001). Surficial rivers belonging to the Paranã basin (Alto Tocantins) permeate the limestone after draining an extensive sandstone area, forming large cave systems (Karmann and Setúbal 1984, Auler and Farrant 1996).

Part of São Domingos region is inside the limits of a Conservation Unit, the Parque Estadual de Terra Ronca (PETeR, Figure 1), which was created in 1989, but still with many land ownership conflicts and inspection problems, resulting in timber harvesting and trampling by cattle near the headwaters. Additionally, it is aggravating the fact that the headwaters of the main rivers that flow through the cave systems are outside the limits of the Conservation Unit (Gallão and Bichuette 2012). São Domingos possess complex systems of surficial and subterranean drainage, with high potential of organic matter carriage and accumulation of debris in some caves. Based upon this, these caves present high richness of subterranean fauna, whether terrestrial or aquatic (Pinto-da-Rocha 1995, Rheims and Pelegatti-Franco 2003, Majer et al. 2003, Simões et al. 2013). Seven troglobitic species of fishes have been recorded so far, configuring a high diversity for this group (Bichuette 2003, Bichuette and Trajano 2003).

Posse, which represents the southernmost part of the same limestone outcrop, is 200 km from PETeR (Figure 1). In there, the systems and subterranean drainage are not as well developed as they are in São Domingos. Not all caves from Posse in this study are mapped yet, or their maps are still being produced; the Russão cave, which is currently being mapped, have an estimated development of, at least, 4 km (E.C. Igual pers. comm.).

Both regions are inside the limits of Cerrado Domain (Ab'Saber 1977), with a dry season between March and September, which may extend until October (Nimer 1979). Rainfall pattern is marked by floods in the rainiest periods (October to March), which bring large amounts of organic matter inside the caves.

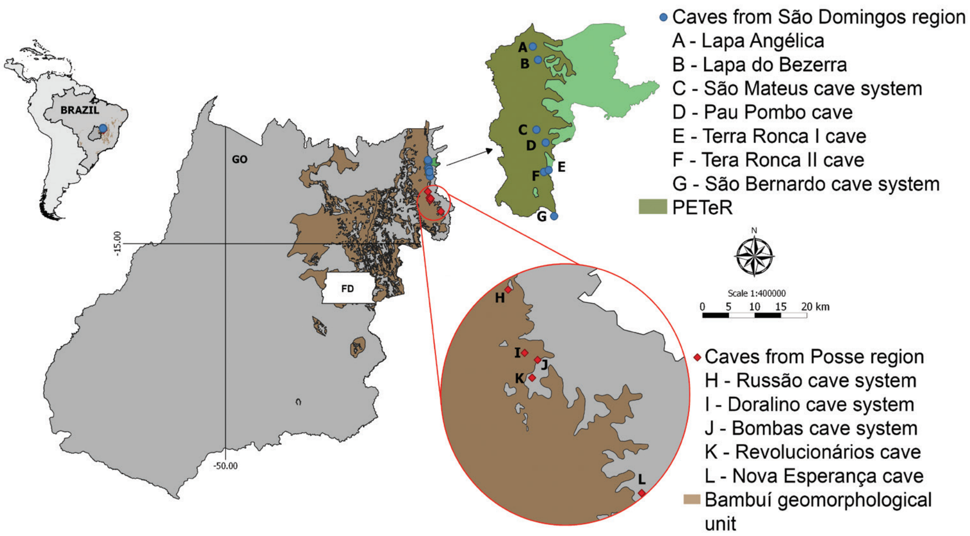

Figure 1. Map showing sampled caves from the São Domingos karst area (blue circles) within Terra Ronca State Park (PETeR, green area) and from the Posse karst area (red diamonds). FD, Federal District.

Sampling

In São Domingos, we sampled the following caves / cave systems: Lapa Angélica cave, Lapa do Bezerra cave, Pau Pombo cave, São Bernardo cave system, São Mateus cave system, Terra Ronca I cave and Terra Ronca II cave. In Posse, we sampled Doralino cave system, Nova Esperança cave, Bombas cave system, Russão cave system, and Revolucionários cave (Figure 1).

We visited the study area on six occasions, three during the raining season (April 2010, April 2011 and February 2012) and three during the dry season (October 2011, June and October 2012). Caves varied with respect to the number of visits: two visits, one during the dry season and other during the raining season in Lapa do Bezerra, São Bernardo and Terra Ronca I, two during the raining season in São Mateus, two during the dry season in Terra Ronca II, one during the dry season in Pau Pombo and Revolucionários, and one during the raining season in Bombas, Doralino, Nova Esperança and Russão. Lapa Angélica was the only cave monitored on all occasions.

We sampled terrestrial substrates and streams mainly by active search in all potential microhabitats, including piles of leaf litter, trunks, guano deposits, under rocks, among others, where specimens were collected with tweezers and brushes and immediately euthanized and preserved in 70% alcohol. In two caves, Lapa Angélica and Terra Ronca II, we also used 0.25 m^2 quadrats (Bichuette et al. 2015). For comparative purposes, i.e., to define ecological-evolutionary categories of taxa, we also sampled in surface habitats near the caves (ca 50 to 100 m from entrance). The collections were performed during night and daytime by active search in leaf litter piles, tree trunks, and fallen trunks, and by transects (10 m of length). We also removed soil by excavation method for more accurate evaluation at the laboratory, and we used Winkler extractor to collect fauna associated with leaf litter.

We identified collected specimens to the least inclusive taxonomic level possible (Operational Taxonomic Units / OTUs) using specific literature as a guide (Borror and DeLong 1969, Adis 2002, Brescovit et al. 2002, Zeppelini and Bellini 2004, Rafael et al. 2012). Part of the material was sent to taxonomic specialists for confirmation and more accurate identification. In cases when identification to the species level could not be obtained, we used higher-level hierarchies and classified specimens into morphotypes and, thus, they are reliable taxonomic categories. Immature individuals that could not be clearly attributed to adults already collected were excluded from all analyses.

Data analysis

All taxa we found were listed for each cave and study area (Appendix 1). Additionally, we defined ecological-evolutionary categories of the better-known taxa following the proposal of Trajano (2012) comparing with the epigean fauna, personal knowledge, and literature data. We also generated histograms of species richness and abundance towards a better understanding of the faunistic composition by region as well as by the Goiás karst area.

We determined the taxonomic distinctness of each cave using the index of phylogenetic diversity known as taxonomic distinctness (TD, Δ^*) (Warwick and Clarke 1995), using the package Vegan (Oksanen et al. 2018) in the software R (version 3.4.0) (R Core Team 2017). This index compares the phylogenetic relationship in a community considering each locality (in our case, the caves). Thus, caves with closely phylogenetic species show lower TD in relation to caves with species phylogenetic distinct. Higher TD values show singularity of an environment. Index of TD is calculated as the number of estimated nodes to connect two different species in a community, while the randomization estimates the confidence interval. This index is applicable to our data because it does not depend on the number of samples or abundance. Then, we did a simple linear regression in the software R (version 3.4.0) (R Core Team 2017) correlating richness and TD values for each area, in order to verify if these data are related.

Results

We collected a total of 1,941 individuals of 344 morphospecies belonging to 128 families, 37 orders and ten classes (Appendix 1) in both regions: 287 morphospecies and 1,554 individuals were from São Domingos and 122 morphospecies and 387 individuals were from Posse. Whether we consider São Domingos and Posse singly or combined as a larger karst area, insects and arachnids were the richest and more abundant taxa (Figure 2–4).

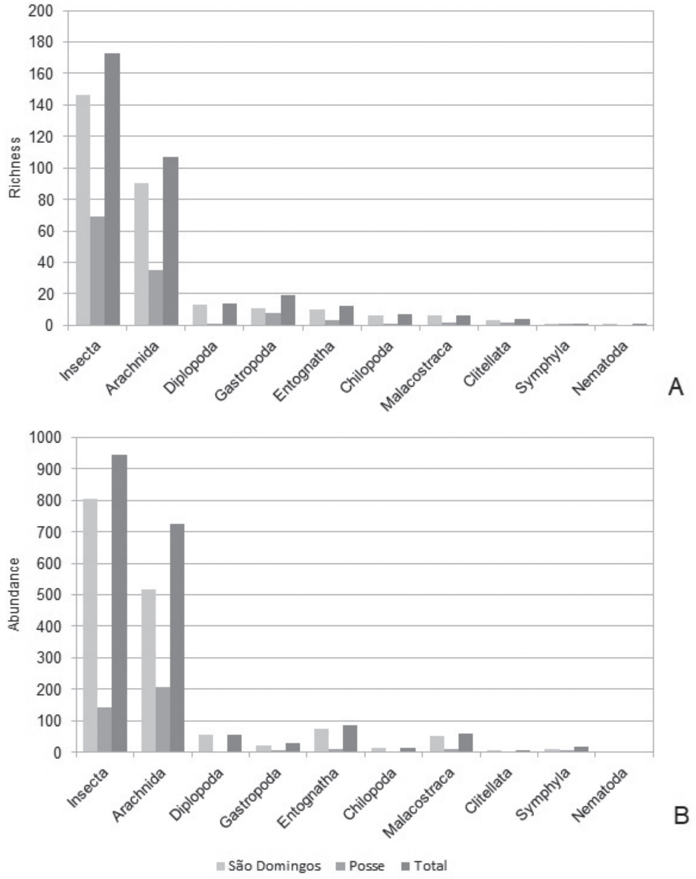

Figure 2. A Richness and **B** abundance of subterranean terrestrial invertebrate (by Class) from the São Domingos and Posse karst areas, state of Goiás, and from both regions combined.

Of the 344 morphospecies, we classified one as a trogloxene (TX) (Araneae: Ctenidae: *Enoploctenus* sp.), 188 as troglophiles (TF), one as a troglobite (TB) (Pseudoscorpiones: Chernetidae: *Spelaeochernes* sp.), nine as troglomorphic and, being such, possibly troglobitic (TM/TB?) and 48 as accidentals (AC). Due to lack of data, we considered the classification of 97 morphospecies as uncertain concerning their ecological-evolutionary relationship with caves.

In São Domingos, Lapa Angélica was the richest and most abundant cave, with 203 morphospecies and 865 individuals, followed by Terra Ronca II cave with 88 morphospecies and 274 individuals. Pau Pombo cave was the least rich and abundant with 11 morphospecies and 22 individuals (Table 1). In Posse, Revolucionários cave was the richest and the most abundant, with 43 morphospecies and 112 individuals, followed by Doralino cave system with 40 morphospecies and 95 individuals. The least rich and abundant was Nova Esperança cave with 17 morphospecies and 42 individuals.

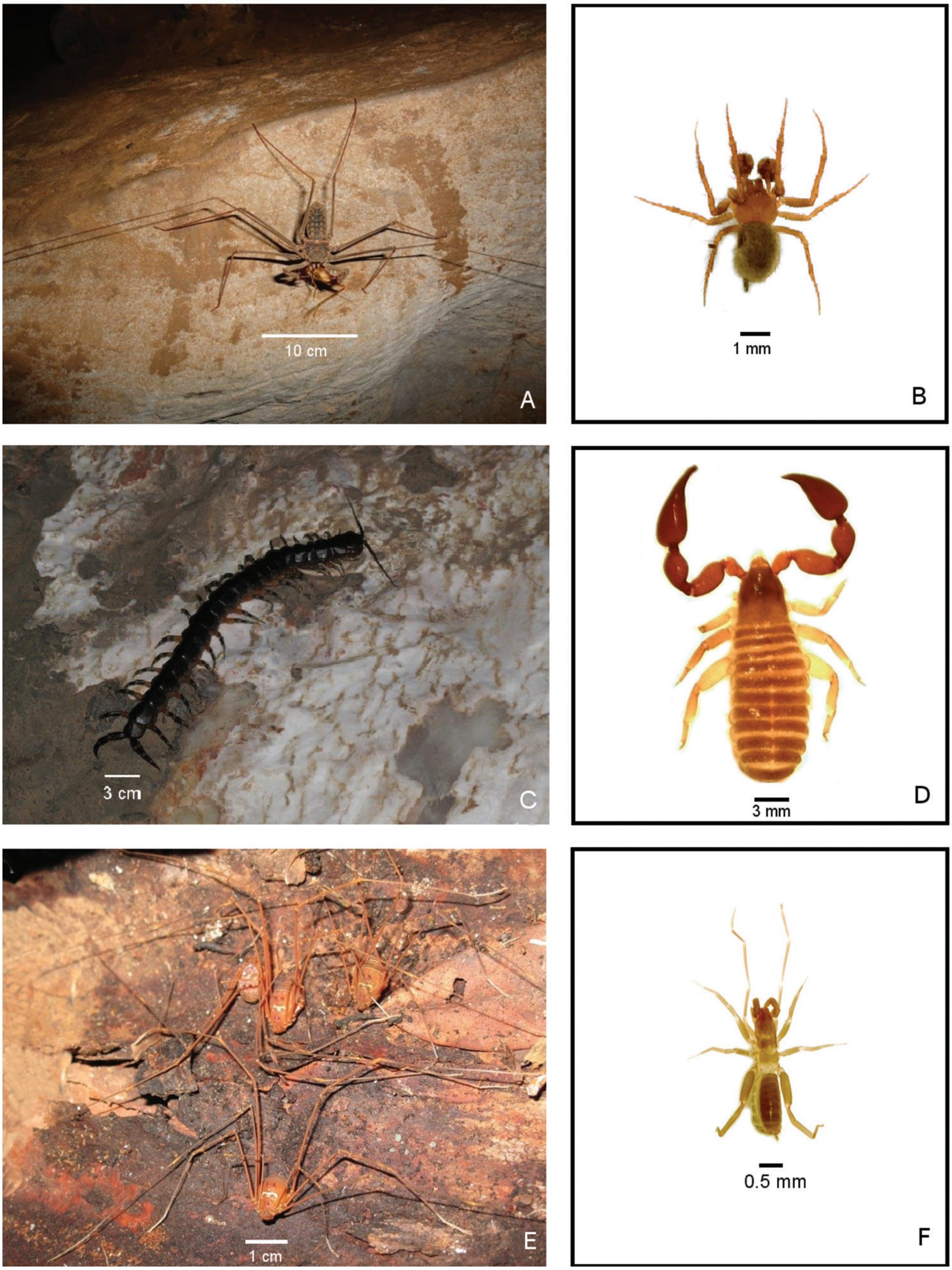

Figure 3. Fauna from caves of northeastern Goiás, central Brazil: **A** *Heterophrynus longicornis* (Amblypygi) preying a cricket *Endecous* sp. (Orthoptera: Phalangopsidae) **B** *Nesticodes rufipes* (Araneae: Theridiidae) **C** *Scolopendra viridicornis* (Chilopoda: Scolopendromorpha) **D** Chernetidae (Pseudoscorpiones) **E** *Flirtea batman* (Opiliones: Cosmetidae) **F** *Stenochrus portoricensis* (Schizomida: Hubbardiidae).

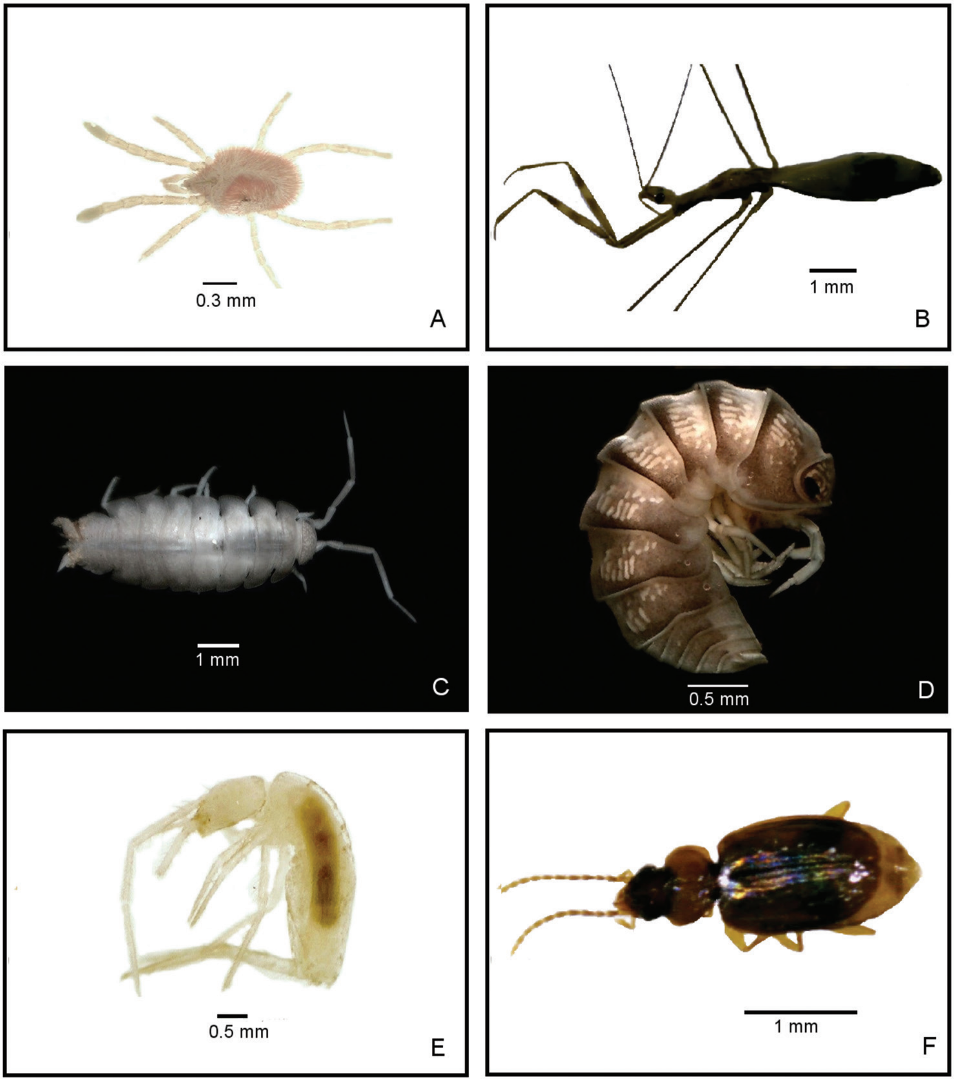

Figure 4. Fauna from caves of northeastern Goiás, central Brazil: **A** Mesostigmata (Acari) **B** Emesinae (Hemiptera: Reduviidae) **C** *Venezillo congener* (Isopoda: Armadillidae) **D** Dubioniscidae (Isopoda) **E** *Trogolaphysa* (Collembola: Paronellidae) **F** *Paratachys* sp. (Coleoptera: Carabidae).

For São Domingos, the expected TD (Δ+) value was 72.517, and the cave with the highest TD was São Bernardo (Δ+= 73.566), followed by Terra Ronca I (Δ+=72.110), Lapa Angélica (Δ+= 71.966), Lapa do Bezerra (Δ+= 71.760), São Mateus (Δ+=71.541), Terra Ronca II (Δ+= 69.669) and Pau Pombo (Δ+= 69.237) (Figure 5A, Table 1). Values of TD close to expected as shown herein revealed that caves from São Domingos share many taxa (morphospecies). One exception is São Bernardo, which shows higher TD in relation to other caves, above the expected value, which means that São Bernardo is the

Table 1. Species richness, individual abundance, and taxonomic distinctness (TD) of caves from northeastern Goiás karst area (São Domingos and Posse). Legend: SBer= São Bernardo cave system, Ang= Lapa Angélica, Bez= Lapa do Bezerra, SMat= São Mateus cave system, TR_I= Terra Ronca I cave, TR_II= Terra Ronca II cave, PPom= Pau Pombo cave, Rus= Russão cave system, Bom= Bombas cave system, Dor= Doralino cave system, NEsp= Nova Esperança cave, Rev= Revolucionários cave.

	Caves	Species Richness	Individual Abundance	TD
São Domingo	SBer	28	72	73.566
	Ang	203	865	71.966
	Bez	52	179	71.760
	SMat	37	66	71.541
	TR I	42	76	72.110
	TR II	88	274	69.669
	Ppom	11	22	69.237
Posse	Rus	40	90	67.847
	Bom	24	48	73.006
	Dor	40	95	67.955
	NEsp	17	42	64.680
	Revo	43	112	78.649

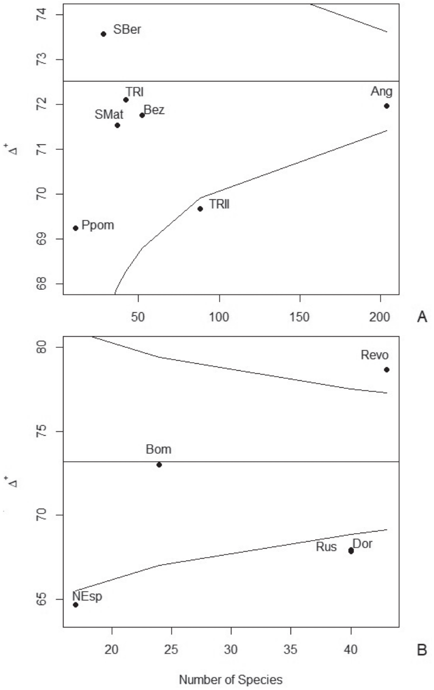

Figure 5. Taxonomic distinctness (TD) for caves from **A** São Domingos and **B** Posse karst areas. Horinzontal line presents the expected TD for the region and funnel graph means 95% confidence limits. Legend: SBer= São Bernardo cave system, Ang= Lapa Angélica, Bez= Lapa do Bezerra, SMat= São Mateus cave system, TR_I= Terra Ronca I cave, TR_II= Terra Ronca II cave, PPom= Pau Pombo cave, Rus= Russão cave system, Bom= Bombas cave system, Dor= Doralino cave system, NEsp= Nova Esperança cave, Rev= Revolucionários cave.

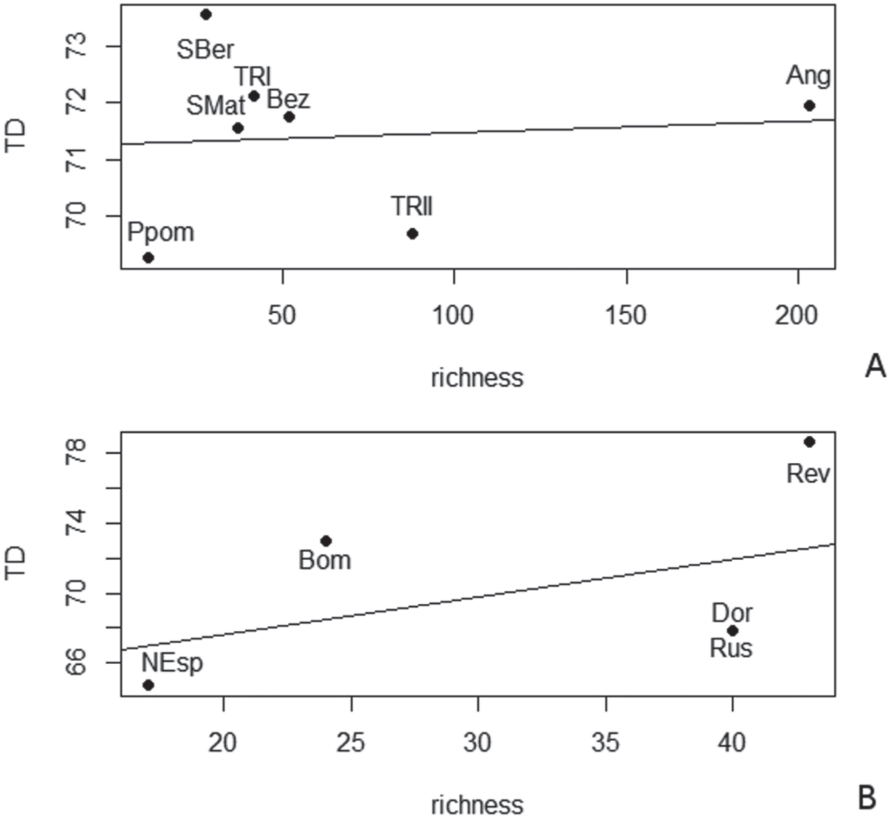

Figure 6. Simple linear regression between richness and Taxonomic distinctness (TD) values of caves from **A** São Domingos and **B** Posse karst areas. Legend: SBer= São Bernardo cave system, Ang= Lapa Angélica, Bez= Lapa do Bezerra, SMat= São Mateus cave system, TR_I= Terra Ronca I cave, TR_II= Terra Ronca II cave, PPom= Pau Pombo cave, Rus= Russão cave system, Bom= Bombas cave system, Dor= Doralino cave system, NEsp= Nova Esperança cave, Rev= Revolucionários cave.

most singular cave in São Domingos. In this cave some morphospecies were unique: one isopod (Dubioniscidae sp. 1), one spider (*Mysmena* sp. 1), one millipede (Pseudonannolenidae sp. 1), one dipteran (Mycetophilidae sp. 1), one Nematoda (sp.1) (Appendix 1).

For Posse, the expected TD (Δ+) value was 73.206, and the cave with the highest TD was Revolucionários (Δ+=78.649), followed by Bombas system (Δ+= 73.006), Russão (Δ+= 67.847), Doralino system (Δ+= 67.955) and Nova Esperança (Δ+= 64.680) (Figure 5B, Table 1). Contrasting to observed to São Domingos, most TD values for Posse caves were far from expected, with some caves showing TDs much lower than expected (Russão, Doralino and Nova Esperança caves), sharing many taxa (morphospecies). Bombas cave presented a TD value near the expected, showing a singularity in relation to the others and Revolucionários cave showed the highest TD value, being the most singular cave in the region (unique taxa/morphospecies). In this cave some morphospecies were unique: one centipede (*Newportia* (*Tidops*) *balzanii*), one pseudoscorpiones (Chthoniidae sp. 1), two isopods (Platyarthridae sp. 1 and Dubioniscidae sp. 2), one orthopteran (*Endecous* sp. 1), one opilionid (Pachylinae sp. 2), one scorpion (*Tityus* sp.), one collembolan (*Trogolaphysa* sp. 3), one coleopteran (*Tachys* sp. 1) and eight gastropods (Appendix 1).

According to simple linear regression, the richness and TD values are not correlated in both areas: São Domingos ($r = 0.001972$, $r^2 = 0.007384$, $F = 0.03719$, $p = 0.8547$) and Posse ($r = 0.2147$, $r^2 = 0.2044$, $F = 0.7708$, $p = 0.4446$), *i.e.*, the richest caves not necessarily present the higher TD value, as observed in the São Domingos area (Figure 6A, B).

Discussion

Our data show high species richness for both the São Domingos and Posse karst areas, which has been also observed for other Brazilian karst areas, such as Chapada Diamantina with c.a. 160 morphospecies (Gallão and Bichuette 2015) and Presidente Olegário with 382 morphospecies (Zepon and Bichuette 2017). Many species that we found during this study were often reported from other limestone caves whether in the same karst area or in other Brazilian karst areas (Trajano 1987, Pinto-da-Rocha 1995, Zepon and Bichuette 2017). As expected for Brazilian caves, most species we found are troglophiles (Trajano and Bichuette 2010).

In São Domingos karst area, Lapa Angélica cave was the richest and the most abundant. Although we sampled this cave on more occasions, its high richness is likely due to the high input of nutrients brought by the extense rivers crossing the cave systems and, consequently, to the high amount of dissolved organic carbon in subterranean terrestrial substrates (62.2 $mgC.L^{-1}$); the same was observed for Terra Ronca II cave (111.12 $mgC.L^{-1}$) (Paula 2018), which, in addition, has skylights along its length through which more organic matter may enter the system (Bichuette et al. 2017). In relation to Pau Pombo cave, the low richness and abundance values may be explained by the limited habitat we searched compared to other caves, as well as this is a typicall dry cave.

In Posse karst area, the higher species richness and abundance we found inside the cave Revolucionários is also attributable to the drainage traversing its length, as well as the high humidity of the terrestrial substrates and the high amount of trophic resources, composed mainly of leaf litter, frugivorous and hematophagous bat guano (Gnaspini-Neto 1992, Poulson and Lavoie 2000). Low diversity and abundance at Nova Esperança can be attributed to low amount of organic matter found inside the cave, mainly leaf litter and guano (ME Bichuette pers. obs). It is a small cave, crossed by a river and with few terrestrial habitats available for fauna, most of which contain rocky or clay substrates. There are several skylights at the ceiling and its epigean environs are impacted by the presence of cattle and pastures.

São Bernardo (São Domingos region) and Revolucionários (Posse region) were the most singular caves in Goiás karst area. Gallão and Bichuette (2015) also observed a high singularity for two caves from Bahia state, northeastern Brazil (Gruna Parede Vermelha and Morro de Alvo caves). Not necessarily the richest caves had higher TD. In other words, the richest caves hadn't the highest singularities in their faunistic compositions. For instance, Lapa Angélica, the richest cave of São Domingos, had a value of TD lower than the estimated mean for the region. This is related to the great number of morphospecies shared with other caves in the region. Our results reinforce that is inadequate to consider only the observed local diversity as the sole criterion for relevance attribution of an area, as this index by itself is not effective to compare different environments, regions, nor taxa (Pärtel et al. 2011). Numerical values of alpha diversity do not reflect the obvious singularities of the subterranean habitats, which is the main justification for its conservation (Trajano et al. 2012).

Lapa Angélica is also one of the most visited caves from PETeR and, on some occasions, visitors traverse its entire length. There has been no effective control of the number and frequency of tourist groups in the region since 2000 (ME Bichuette pers. obs.). We noted that there is only a few sparsely guano spots along the cave, probably reduced as a consequence of anthropic impacts (ground trampling and noise pollution, which drives off the bats). Disturbance diminishes the environmental heterogeneity and, consequently, reduces the offer of microhabitats and resources to the local community, as observed in Lapa Angélica in relation to bat guano along years (M.E. Bichuette pers. obs.). Environmental impacts (contamination) also cause reduction of taxonomic distinctness in marine species communities, while richness remained constant (Warwick and Clarke 1998).

We observed that some of the other richest caves which do not have high taxonomic distinctness, possibly because of anthropic impacts in the region. Taxonomic distinctness in the region may still be related to the trophic diversity of the community, in such a way that when there is a reduction in the number of guilds, TD decays (Clarke and Warwick 1999). The type of habitat affects trophic diversity. Consequently, more impacted areas are also more subject to a decrease in taxonomic distinctness.

Terra Ronca II cave, even with the second higher species richness, presented the lowest TD. The stretch of the cave we sampled has basically the same substratum type, which is predominantly composed of banks of sand and pebbles deposited at riverside. Despite its high species richness, the species are not taxonomically very distinct from each other (e.g., several morphospecies are from the beetle families Carabidae and Staphylinidae) when compared to other subterranean communities of the same region. In Posse, the richest cave (Revolucionários) also had the highest TD. Its high faunistic singularity is related to the presence of species and genera belonging to different families. Nova Esperança presented several common taxa and, therefore, low faunistic singularity.

Conservation remarks

São Domingos is remarkable for its high richness in troglobitic species of fishes that exhibit various degrees of specialization to subterranean life, including one species of Loricariidae catfish, four Trichomycteridae catfishes, one Heptapteridae catfish and one Sternopygidae electric fish (Reis 1987, Triques 1996, Fernández and Bichuette 2002, Bichuette and Trajano 2004, Trajano et al. 2004). Terrestrial invertebrates, such as Ctenidae, Scytodidae, and Symphytognathidae spiders, are also ubiquitous (Rheims and Pellegatti-Franco 2001). In Posse, only two troglobitic taxa are described to date: two catfishes of the families Callichthyidae and Trichomycteridae (Bichuette and Trajano 2008, Tencatt and Bichuette 2017).

If we excluded the troglophilic taxa and considered only troglobitic terrestrial invertebrates, species diversity recorded in northeastern Goiás would be considered poor when compared to other Brazilian regions, as has been demonstrated in this and in previous studies (Trajano and Bichuette 2010, Trajano et al. 2016). This poor troglobitic diversity can be related to the fact that this area comprises a relatively stable paleoclimatic zone (Barr 1973, Moore 1964). Under this model of climate fluctuations, areas where drastic climate fluctuations occurred in the past had greater troglobitic diversification, as can be found in the subterranean fauna in the northern Hemisphere (Barr 1968, Peck 1980). The most specialized Brazilian troglobites are found in caves of semiarid regions of Bahia state, as Chapada Diamantina and Campo Formoso (Trajano 1995).

As we corroborated in our study, a high degree of singularity is usually present in caves, even among those, which are geographically near other caves and inserted in the same rocky massif. To preserve as many singular caves as possible is urgent, because the ideal of environmental compensation is not applicable to these unique environments.

Even the caves from São Domingos are inside a legally protected area (PETeR), the tourist flow is intense and, several times, there is no adequate inspection. Thus, intense visitation may be affecting the subterranean communities we studied. Besides, part of the headwaters of rivers that crosses caves of São Domingos is outside the State Park limits. There, extensive cattle farming and monocultures are causing silting and pollution of rivers, bringing noxious materials to the caves and impacting the community (Simões et al. 2013). In February 2013, intense sedimentation occurred at the headwater of the São Vicente River (São Domingos), as a consequence of intensive agriculture upstream of the spring. In view of the above, it is difficult to preserve natural areas, even when legal reserve areas and parks are delimited. In Posse, the surroundings outside the caves are deforested and replaced by pastures and urban areas, while the rivers are being discharged from domestic sewage. Worryingly, Brazilian environmental laws do not legally protect the caves suffering these impacts.

In conclusion, caves of São Domingos and Posse karst areas exhibited a high taxonomic distinctness for terrestrial fauna, influenced by the high proportion of troglophilic, accidental and undetermined taxa. Our data show the relevance to considerer not only troglobitic taxa. Besides non-troglobionts, both areas showed a high diversity of troglobitic fish (Bichuette and Trajano 2003) and shelters highly important for bats (Bichuette et al. 2018). Anthropogenic impacts cause reduction of environmental heterogeneity and trophic diversity, and, consequently, diminishes faunistic singularity. Besides, these karst areas have high faunistic diversity when compared with other Brazilian regions. Therefore, protective measures should be taken, since the region is not totally preserved, even in the São Domingos area, where the surroundings of the headspring of rivers that enter on the caves (responsible for carrying food resources and increasing heterogeneity of habitats), are still outside the protection limits of the Park.

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Appendix I

Individual abundance of cave invertebrates in São Domingos and Posse karst areas, state of Goiás, central Brazil, and their status according to relationship with the subterranean environment. Legend: Caves: SBer= São Bernardo cave system , Ang= Lapa Angélica, Bez= Lapa do Bezerra, SMat= São Mateus cave system, TR_I= Terra Ronca I cave, TR_II= Terra Ronca II cave, PPom= Pau Pombo cave, Rus= Russão cave system, Bom= Bombas cave system, Dor= Doralino cave system, NEsp= Nova Esperança cave, Rev= Revolucionários cave. Status: TX= trogloxene, TF=troglophile, TB= troglobite, TM/TB?= troglomorphic/ possibly troglobitic, AC= accidental, ?= undetermined classification.

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
F. Arthropoda														
C. Arachnida														
Sc. Acari														
O. Ixodida														
Fa. Ixodidae														
Sf. Amblyomminae	*Amblyomma* sp. 1	?		1										
Fa. Argasidae	sp. 1	?												1
O. Mesostigmata	sp. 1	?								1				
	sp. 2	TF		1										
	sp. 3	?										1		
	sp. 4	?												6
	sp. 5	TF		1										
	sp. 6	?					2							
Fa. Laelapidae	sp. 1	?										1		
	sp. 2	?		1		1								
Fa. Tetranychidae	sp. 1	?		3										
O. Oribatida														
Fa. Galumnidae	sp. 1	?		1										
O. Amblypygi														F
Fa. Phrynidae	*Heterophrynus longicornis* (Butler, 1873)	TF		1										
O. Araneae														
So. Araneomorphae														
Fa. Anapidae	sp. 1	AC					2							

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Fa. Araneidae	*Alpaida* sp. 1	TF		9		1		1		2		8	1	1
	Eustala sp. 1	TF										1		
	Micrathena sp. 1	TF									2			
	Ocrepeira sp. 1	TF		1										
	Pronous sp. 1	TF		4										
Fa. Caponiidae	*Nops* sp. 1	TF		2			1							
Fa. Corinnidae	sp. 1	TF		2	4									
Fa. Ctenidae	*Enoploctenus* sp. 1	TX		4										
	Isoctenus sp. 1	TF		1		2	1	11	2	2	1	3	2	5
	Isoctenus sp.2	TF			3									6
Fa. Filistatidae	sp. 1	TF		1										
Fa. Linyphiidae	*Agyneta* sp. 1	TF		8	1	1								1
Fa. Lycosidae	sp. 1	AC			2									
	sp. 2	AC				1		1						
	Allocosa sp. 1	AC		2										
Sfa. Mysmenidae	sp. 1	?		3										
	Mysmena sp. 1	?	2											
Fa. Nesticidae	*Nesticus* sp. 1	TF								4		1		
Fa. Oonopidae	sp. 1	TF		5										
	sp. 2	TF		3										
	sp. 3	TF						2						
	Triaeris stenaspsis Simon, 1891	TF		2				1						
Fa. Palpimanidae	*Fernandezina* sp. 1	AC						1						
Fa. Pisauridae	sp. 1	TF										1		
Fa. Pholcidae														
Sfa. Ninetinae	sp. 1	TF	1	22										
	Ibotyporanga sp. 1	TF						1						
Sfa. Pholcinae	*Leptopholcus* sp. 1	TF										1		
Sfa. Modisiminae	*Mesobolivar* sp. 1	TF	7	10	27	1	1	7		4	5	13	8	
Fa. Salticidae	sp. 1	AC		1	3									
	sp. 2	AC						1						
	sp. 3	AC				1								

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Fa. Scytodidae	*Scytodes* sp. 1	TF		16	1		2	3						
	Scytodes sp. 2	TF		1										
	Scytodes aff. *eleonorae* Rheims & Brescovit, 2001	TF		2										
Fa. Segestriidae	sp. 1	TF		1										
Fa. Selenopidae	sp. 1	TF			1									
Fa. Sicariidae	*Loxosceles* sp. 1	TF		29	3	1	4	5						13
	Loxosceles sp. 2	TF		1					4					
	Loxosceles sp. 3	TF		11							3		6	
	Sicarius sp. 1	TF					1							
Fa. Symphytognathidae	*Anapistula* sp. 1	TF		1										
Fa. Tetragnathidae	sp. 1	TF	1	1					1					
	Crysometa sp. 1	?										1		
	Glenognatha sp. 1	?					5							
	Leucage sp. 1	TF		1			3							
	Leucage sp. 2	TF						1						
Fa. Theridiidae	sp. 1	TF		3		5								
	sp. 2	TF	1	1										
	sp. 3	TF		1	4									
	sp. 4	TF		1										
	sp. 5	TF		1										
	sp. 6	TF					1	6						
	Achaearanea sp. 1	TF	2		3									
	Chrysso sp. 1	TF		1										
	Latrodectus geometricus Koch 1841	TF			1									
	Nesticodes rufipes (Lucas, 1846)	TF		6							5			
	Theridion sp. 1	TF		5	1			2		3		6	3	
Fa. Theridiosomatidae	*Plato* sp. 1	TF	3	22	7			1	3				5	2
	Plato sp. 2	TF		15				2		2		6		
	Plato sp. 3	TF		2				4				1		
	Steatoda sp. 1	TF					1							
Fa. Thomisiidae	sp. 1	?		1										

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Fa. Trechaleidae	sp. 1	TF			1	1								
Fa. Uloboridae	sp. 1	TF					1							
Fa. Zodaridae	sp. 1	TF		3				1						
So. Mygalomorphae														
Fa. Dipluridae	sp. 1	?												2
	Ischnothele annulata Tullgren, 1905	?			1									
Fa. Idiopidae	sp. 1	TF		1										
Fa. Theraphosidae	sp. 1	?		2										
O. Opiliones														
So. Laniatores														
Fa. Phalangodidae						1								
Sfa. Phalangodinae	gen. n. sp. n.	TF												
Fa. Cosmetidae														
Sfa. Cosmetinae	*Flirtea batman* (Pinto-da-Rocha & Yamaguti, 2013)	TF	1	14	12	1	2	5	1		3	4		
	sp. 1	TF		1										
Fa. Gonyleptidae														
Sfa. Pachylinae	sp. 1	TF				1								
	sp. 2	TF												5
	Eusarcus sp. 1	TF		7	6	4	1	2		4		5		7
Fa. Tricommatidae														
Sfa. Tricommatinae	sp. 1	TF		1										
Fa. Stygnidae														
Sfa. Stygninae	sp. 1	TF		1										
So. Eupnoi														
Fa. Sclerosomatidae														
Sfa. Gagrellinae	sp. 1	TF		10	1		2	1	1	1		2		6
	sp. 2	TF								3		1		
O. Pseudoscorpiones														
Fa. Chthoniidae	sp. 1	TF	1	1	2		1							1
Fa. Atemnidae														
Sfa. Atemninae	*Paratemnoides* sp. 1	TF		6	1									

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Fa. Cheiridiidae														
Sfa. Cheiridiinae	*Cheiridium* sp. 1	TF		1										
Fa. Cheliferidae	sp. 1	TF				3				1				
Fa. Chernetidae	sp. 1	TF			3									
	sp. 2	TF										1		
Fa. Chernetinae														
T. Chernetini	*Spelaeochernes* sp. 1	TB				1	1					3		4
Fa. Geogarypidae	*Geogarypus* sp. 1	TF		1										
Fa. Olpiidae	sp. 1	TF					1	1						
	sp. 2	TF								16				
	sp. 3	TF	3	1										
Sfa. Hesperolpiinae	*Progarypus* sp. 1	TF		4										
O. Schizomida														
Fa. Hubbardiidae														
Sfa.Hubbardiinae														
T. Hubbardiinii	*Stenochrus portoricensis* Chamberlin, 1922	TF					3	8						
O. Scorpiones														
Fa. Buthidae														
Sfa. Buthinae														
T. Buthinii	*Tityus* sp. 1	TF												1
C. Chilopoda														
O. Geophilomorpha														
Fa. Ballophilidae	*Ityphilus* sp. 1	?					1							
Fa. Schendylidae	*Schendylops* sp. 1	?		1										
	Schendylops sp. 2	?						1						
O. Lithobiomorpha														
Fa. Henicopidae	*Lamyctes* sp. 1	?		1										
O. Scolopendromorpha														
Fa. Scolopocryptopidae														
Sfa. Newportiinae	*Newportia (Tidops) balzanii* (Silvestri, 1895)	?												1

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Fa. Scolopendridae														
Sfa. Scolopendrinae	*Scolopendra viridicornis* Newport, 1844	?			1									
O. Scutigeromorpha														
Sfa. Pselliodidae	*Sphendononema guildingii* (Newport, 1845)	TF		5	2	1		1						
C. Diplopoda														
O. Polydesmida	sp. 1	TM/TB?					3	1						
	sp. 2	?									1			
	sp. 3	?			1									
O. Spirostreptida														
Fa. Pseudonannolenidae	*Pseudonannolene* aff. *imbirensis* Fontanetti, 1996	TF		1		2								
	sp. 1	TM/TB?	3											
	sp. 2	TF			5	1								
	sp. 3	TF			4		1	9	1					
	sp. 4	TM/TB?			3	1								
	sp. 5	TF	1			2		2						
	sp. 6	TF			4			1						
	sp. 7	?						3						
	sp. 8	?							1					
Fa. Spirostreptidae	sp. 1	TF		2			1	1						
O. Siphonophorida	sp. 1	?			1									
C. Symphyla														
Fa. Scutigereliidae	*Hanseniella* sp. 1	TF		8				2		3		1		2
C. Entognatha														
O. Diplura	sp. 1	TF		3		1								
O. Collembola														
So. Entomobryomorpha														
Fa. Lepidocyrtidae														
Sfa. Cyphoderinae	*Cyphoderus* sp. 1	TM/TB?		19			1							

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Fa. Entomobryidae	sp. 1	TF	1			2		1		1				
	sp. 2	TF		11	6	1	1	4						
	sp. 3	?		1										
	sp. 4	?						2						
Sfa. Entomobryinae	*Entomobrya* sp. 1	?		1										
Fa. Paronellidae	sp. 1	TF		1		6								
	sp. 2	?		1										
	Trogolaphysa sp. 1	TM/TB?									4			
	Trogolaphysa sp. 2	TM/TB?	1	7	4									
	Trogolaphysa sp. 3	TF												5
C. Insecta														
O. Blattaria														
Fa. Blaberidae	sp. 1	TF		5										
Fa. Blattellidae	sp. 1	TF	1	48	3	4	2	4		1	3	3	1	1
	sp. 2	TF	7	7	2	3				1				
	sp. 3	TF		6										
Fa. Corydiidae	sp. 1	AC		3										
O. Coleoptera														
So. Adephaga														
Fa. Carabidae	sp. 1	?		2					1					
Sfa. Harpalinae														
T. Pentagonicini	*Pentagonicina* sp. 1	TF		3			1	1		2				
T. Platynini														
SubT Platynina	*Platynus* sp. 1	AC		1										
T. Pterostichini	sp. 1	TF						1			2			
SubT. Agonina	sp. 1	TF		29			1	21		1				
	sp. 2	AC		1										
SubT. Lozandrina	*Loxandrus* sp. 1	?		3										
Sfa.Trechinae														
T. Bembidiini														

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse			
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Subt. Tachyina	*Paratachys* sp. 1	TF		48	1			19		4	1			1
	Paratachys sp. 2	?		1				1						
	Pericompsus sp. 1	TF		2			1	4		4	1			
	Polyderis sp. 1	?		1				1						
	Tachys sp. 1	TF		1				2						1
T. Zuphiini	sp. 1	TF					2							
Fa. Dytiscidae	sp. 1	?		1										
So. Myxophaga														
Fa. Hydroscaphidae	sp. 1	?								1				
So. Polyphaga														
Fa. Cantharidae	sp. 1	AC		2										
	sp. 2	AC		1										
	sp. 3	AC		1										
Fa. Chrysomelidae	sp. 1	AC		2										
	sp. 2	AC		1				1						
Fa. Curculionidae	sp. 1	AC		1										
	sp. 2	AC						1						
Fa. Dryopidae	sp. 1	AC		1										
Fa. Elmidae	*Macrelmis* sp. 1	TF		9										
	Macrelmis sp. 2	TF		1										
	Hexacylloepus sp. 1	TF		1										
Fa. Lampyridae	sp. 1	AC		2	4	2						1		
Fa. Ptiliidae	*Micridium* sp. 1	TF								1				
Fa. Ptilodactylidae	sp. 1	TF										1		
	sp. 2	TF		3	1			1						
	sp. 3	?												1
Fa. Scarabaeidae	*Canthon* sp. 1	AC						1						
Fa. Scydmaenidae	sp. 1	?						1						

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Fa. Staphylinidae	sp. 1	TF		19				20				2		
	sp. 2	?				1		5						
	sp. 3	TF		1	1	1		3						
	sp. 4	AC				1								
	sp. 5	AC		1										
	sp. 6	AC		1										
	sp. 7	TF		2				7						
	sp. 8	AC						2						
	sp. 9	?					1	4						
	sp. 10	AC						1						
Fa. Aleocharinae	sp. 1	?		2				1						
Sfa. Osoriinae	*Holotrochus* sp. 1	?		1										
Sfa. Oxytelinae	sp. 1	TF		18				18			2			
	sp. 2	TF		2	1									
Sfa.Paederinae	sp. 1	TF						1		1				
	sp. 2	?		2				1						1
	Homeotarsus sp. 1	?								3				
	Homeotarsus sp. 2	?												2
	Homeotarsus sp. 3	?			2									1
Sfa. Pselaphinae	sp. 1	TM/TB?			1	1								
Sfa. Staphylininae														
T. Staphilinini	sp. 1	TF		2										
	sp. 2	?						1						
T. Xantholinini	sp. 1	?		1										
Sfa. Steninae	*Stenus* sp. 1	TF		2										
	Stenus sp. 2	?					1							
Sfa. Tachyporinae	sp. 1	?						1		1				
	sp. 2	TF		2										
	sp. 3	TF		1										
Sfa. Tenebrionidae	sp. 1	TF	3				1							
	sp. 2	TF												1

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
O. Diptera														
So. Brachycera														
Io. Muscomorpha/ Acalyptratae	sp. 1	?										2		
	sp. 2	?										2		
	sp. 3	?		1								1		
F. Drosophilidae	*Drosophila* sp. 1	TF	6					1				2		1
	Drosophila sp. 2	TF		2										
	Drosophila sp. 3	TF									1			
Fa. Muscidae	sp. 1	TF		2										
Fa. Phoridae	sp. 1	TF					1					1		
	sp. 2	TF						1						
	sp. 3	TF		1										
So. Nematocera														
Io. Bibionomorpha														
Fa. Cecidomyiidae	sp. 1	TF	2	2		1								
	sp. 2	TF	1			3		2						
	sp. 3	?		1										
Io. Culicomorpha														
Fa. Ceratopogonidae	sp. 1	?		2			1				3			
	sp. 2	?		1										
Fa. Chironomidae	sp. 1	?		3									2	
	sp. 2	?		6				1				1		
	sp. 3	?		6										
Fa. Culicidae	sp. 1	?									1			
	sp. 2	?						1				2		
Io. Tipulomorpha														
Fa. Limoniidae	sp. 1	?											1	
	sp. 2	?											1	
Io. Bibionomorpha														
Fa. Mycetophilidae	sp. 1	?	1											
	sp. 2	?		9										
	sp. 3	?		3										

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Io. Psychodomorpha														
Fa. Psychodidae	sp. 1	?		1										
Sfa. Phlebotominae	sp. 1	TF		7	15					4		1		
Io. Bibionomorpha														
Fa. Sciaridae	sp. 1	TF		6										
	sp. 2	TF		2							1			
	sp. 3	TF		1				1						
	sp. 4	TF		8										
Io. Culicomorpha														
Fa. Simulidae	sp. 1	?								1				
Io. Tipulomorpha														
Fa. Tipulidae	sp. 1	AC											1	
O. Ephemeroptera														
Fa. Baetidae	*Cloeodes* sp. 1	TF								1				
Fa. Leptophlebiidae	sp. 1	TF	2	12			8			1				
	Miroculis sp. 1	TF		3									1	
O. Hemiptera														
So. Auchenorrhyncha														
Fa. Cicadellidae	sp. 1	AC		2										
	sp. 2	AC		1										
Fa. Cixiidae	sp. 1	TF		6						1				
So. Heteroptera														
Fa. Belostomatidae	sp. 1	TF								1				
Fa. Gelastocoridae	sp. 1	TF		2				2						
	sp. 2	AC		1										
Fa. Notonectidae	sp. 1	AC			1									
Fa. Hydrometridae	sp. 1	?						2						
Fa. Coreidae														
Sfa. Coreinae	*Zicca* sp. 1	AC		1										
Lygaeidae	sp. 1	TF		1				7			2			
	sp. 2	?						1						
	sp. 3	?						1						

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Fa. Reduviidae	sp. 1	TF		8						1				
	sp. 2	TF	2	1	1									
	sp. 3	TF				1								
	sp. 4	TF		1										
Sfa. Ectrichodiinae	*Pothea furtadoi* Gil-Santana & Costa, 2005	AC								1				
Sfa. Emesinae	sp. 1	TF		3						1		3		
	sp. 2	TF		4				1						
	sp. 3	TF		1										
Sfa. Reduviinae	sp. 1	?		1						1				
	Zelurus sp. 1	TF		13	3			2					3	
Sfa. Harpactocorinae	sp. 1	AC		1										
Fa. Veliidae	sp. 1	TF		2										
O. Hymenoptera														
Fa. Formicidae														
Sfa. Cerapachyinae	*Acanthostichus kirbyi* Emery, 1895	AC		3										
Sfa. Ecitoninae	*Labidus coecus* (Latreille, 1802)	AC		1										
Sfa. Formicinae	*Nylanderia* sp. 1	AC												1
Sfa. Myrmicinae	*Pheidole* sp. 1	TF		1										
	Pheidole sp. 2	TF		6								3		
	Pheidole sp. 3	TF								2				
	Pheidole sp. 4	TF		16										
	Pheidole sp. 5	TF											1	
	Solenopsis sp. 1	TF		3										
	Wasmannia auropunctata (Roger, 1863)	AC								3				
Sfa. Ponerinae	*Hypoponera* sp. 1	TF		1										
	Hypoponera sp. 2	TF		1				11						
	Hypoponera sp. 3	TF												2
	Hypoponera sp. 4	TF		2										
	Leptogenys sp. 1	AC		1										

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Fa. Vespidae	sp. 1	AC		1										
SF.Ichneumonoidea	sp. 1	AC		1										
O. Isoptera														
Fa. Termitidae														
Sfa. Nasutitermitinae	sp. 1	TF		11				1				1		2
O. Lepidoptera														
Fa. Noctuidae	sp. 1	TF		3						1		4		
	sp. 2	TF										1		
Fa. Tineidae	sp. 1	TF	2	7				2	4		1			
O. Megaloptera														
Fa. Megalopteridae	sp. 1	?			2									
O. Neuroptera														
Fa. Myrmeleontidae	sp. 1	AC		1										
O. Odonata	sp. 1	AC						1						
O. Orthoptera														
So. Ensifera														
Fa. Phalangopsidae	sp. 1	TF		6	3	3	1							1
	sp. 2	TF		1		2								
Sfa. Luzarinae	*Endecous* sp. 1	TF	1	1	5			3						7
	Endecous sp. n. 1	TF		15					3	3		1		
	Endecous sp. n. 2	TF									2			
	Eidmanacris sp. 1	TF		3						1		1	3	3
Fa. Trigonidiidae														
Sfa. Trigonidiinae	*Cyrtoxipha* sp. 1	AC			1									
O. Plecoptera														
Fa. Perlidae	sp. 1	TF			1		1							
	sp. 2	TF		4										
Fa. Perlodidae	sp. 1	TF		3										
O. Psocoptera	sp. 1	TF		4								1	2	
	sp. 2	TF		8										
	sp. 3	TF		2										

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
O. Thysanoptera														
Fa. Lepismatidae	sp. 1	AC		2				1						
	sp. 2	AC		4				1						
	sp. 3	AC		1										
O. Trichoptera														
Fa. Calamoceratidae	sp. 1	TF									1			
Fa. Hydropsychidae	*Leptonema* sp. 1	TF		2									1	
Fa. Hydropsychidae	*Smicridae* sp. 1	TF		7				2						
Fa. Philopotamidae	*Chimarra* sp. 1	TF	1	8				3						
Fa. Odontoceridae	sp. 1	TF									1			
C. Malacostraca														
O. Isopoda														
Fa. Armadillidae	*Venezillo congener* (Budde-Lund, 1904)	TF		11	11									
Fa. Dubioniscidae	sp. 1	TM/TB?	14											
	sp. 2	TF					1	2						4
	sp. 3	TF					8	1						
Fa. Platyarthridae	sp. 1	TF						1						5
Fa. Styloniscidae	sp. 1	TM/TB?		2										
F. Mollusca														
C. Gastropoda														
O. Pulmonata														
So. Stylommatophora	sp. 1	?		1										
	sp. 2	?		2										
	sp. 3	?		4										
	sp. 4	?		1										
	sp. 5	?		5										
	sp. 6	?		2										
	sp. 7	?		1										
	sp. 8	?		1										
	sp. 9	?		2										
	sp. 10	?		1										
	sp. 11	?					1							

Taxa	Gen. sp./ morphotype	Status	Caves - São Domingos							Caves - Posse				
			SBer	Ang	Bez	SMat	TR I	TR II	Ppom	Rus	Bom	Dor	NEsp	Revo
Fa. Gastrocoptidae	*Gastrocopta sharae* Salvador, Cavallari & Simone, 2017	?												1
Fa. Charopidae	*Radiodiscus* sp. 1	?												1
Fa. Ferussaciidae	*Cecilioides consobrina* (d'Orbigny, 1842)	?												1
Fa. Scolodontidae	*Entodina jekylli* Baker, 1913	?												1
	Prohappia besckei (Dunker, 1847)	?												1
Fa. Systrophiidae	*Happia glaberrima* Thiele, 1927	?												1
Fa. Subulinidae	*Leptinaria concentrica* (Reeve, 1849)	?												1
Fa. Valloniidae	*Pupisoma dioscoricola* (Adams, 1845)	?												1
F. Annelida														
C. Clitellata														
O. Haplotaxida														
Fa. Glossoscolecidae	*Pontoscolex corethrurus* (Müller, 1857)	TF		1	1									
Fa. Megascolecidae	*Dichogaster saliens* (Beddard, 1893)	?		1							1			
	Dichogaster gracilis (Michaelsen, 1892)	TF									1			
Sc. Hirudinea	sp. 1	AC						2						
F. Nematoda	sp. 1	?	1											

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RESEARCH ARTICLE

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Mitochondrial sequence data indicate "Vicariance by Erosion" as a mechanism of species diversification in North American *Ptomaphagus* (Coleoptera, Leiodidae, Cholevinae) cave beetles

Vincent L. Leray[1], Jason Caravas[3], Markus Friedrich[2,3], Kirk S. Zigler[1]

1 *Department of Biology, The University of the South, 735 University Avenue, Sewanee, TN 37383, USA*
2 *Department of Biological Sciences, Wayne State University, 5047 Gullen Mall, Detroit, MI 48202, USA*
3 *Department of Anatomy and Cell Biology, Wayne State University, School of Medicine, 540 East Canfield Avenue, Detroit, MI 48201, USA*

Corresponding author: *Kirk S. Zigler* (kzigler@sewanee.edu)

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Abstract

Small carrion beetles (Coleoptera: Leiodidae: Cholevinae) are members of cave communities around the world and important models for understanding the colonization of caves, adaptation to cave life, and the diversification of cave-adapted lineages. We developed a molecular phylogeny to examine the diversification of the *hirtus*-group of the small carrion beetle genus *Ptomaphagus*. The *hirtus*-group has no surface-dwelling members; it consists of 19 short-range endemic cave- and soil-dwelling species in the central and southeastern United States of America. Taxonomic, phylogenetic and biogeographic data were previously interpreted to suggest the *hirtus*-group diversified within the past 350,000 years through a series of cave colonization and speciation events related to Pleistocene climate fluctuations. However, our time-calibrated molecular phylogeny resulting from the analysis of 2,300 nucleotides from five genes across three mitochondrial regions (*cox1*, *cytb*, *rrnL-trnL-nad1*) for all members of the clade paints a different picture. We identify three stages of diversification in the *hirtus*-group: (1) ~10 million years ago (mya), the lineage that develops into *P. shapardi*, a soil-dwelling species from the Ozarks, diverged from the lineage that gives rise to the 18 cave-obligate members of the group; (2) between 8.5 mya and 6 mya, seven geographically distinct lineages diverged across Kentucky, Tennessee, Alabama and Georgia; six of these lineages represent a single species today, whereas (3) the 'South Cumberlands' lineage in Tennessee and Alabama diversified into 12 species over the past ~6 my. While the events triggering diversification during the first two stages remain to be determined, the distributions, phylogenetic relationships and divergence times in the South Cumberlands lineage are consistent with populations being isolated by vicariant events as the southern Cumberland Plateau eroded and fragmented over millions of years.

Keywords

Cumberland Plateau, speciation, microphthalmy, troglobiont, biodiversity hotspot

Introduction

Lacking light and typically low in energy resources, caves represent a challenging environment to adapt to. In spite of these challenges, subterranean habitats harbor communities of taxonomically diverse species assemblages. The small carrion beetles (Coleoptera: Leiodidae: Cholevinae) are a significant component of cave biodiversity in temperate regions, having colonized caves on numerous occasions worldwide (Peck 1973, Ribera et al. 2010, Fresneda et al. 2011). Cave-adapted species that have reduced eyes (microphthalmy) or are eyeless (anophthalmy) have evolved many times in the family (Fig. 1) (e.g. Peck 1973, Fresneda et al. 2011, Peck and Wynne 2013).

As common and speciose cave inhabitants, leiodid beetles can provide insights into the colonization of caves, adaptation to cave life, and the diversification of cave-adapted lineages. Recent molecular work on a Palearctic radiation of subterranean leiodids (Leiodidae: Cholevinae: Leptodirini) provided insight into the timing of cave colonization, life history evolution, and diversification in the group (Ribera et al. 2010, Fresneda et al. 2011, Rizzo et al. 2013, Cieslak et al. 2014, Njunjić et al. 2018). Using a molecular clock calibrated by the tectonic separation of the Corso-Sardinian plate, Ribera et al. (2010) showed this was an ancient invasion of cave habitats, with the earliest subterranean lineages diverging in the Oligocene, around 30 million years ago (mya). They also traced subsequent evolution within the group, uncovering continued life-history evolution and diversification post-cave invasion (Cieslak et al. 2014).

Studies of leiodid beetles have also provided insight into the molecular changes associated with cave adaptation. The first transcriptome study on a cave species, the Nearctic leiodid *Ptomaphagus hirtus* (Leiodidae: Cholevinae: Ptomaphagini), revealed the conservation and expression of all genes known to be specifically required for phototransduction despite an extreme reduction of the visual system (Fig. 1). This observation was complemented by light-dark choice tests, which uncovered a strong negative photoresponse in *P. hirtus* (Friedrich et al. 2011). In addition, the conservation of circadian clock gene expression and the loss of expression of several genes in the ommochrome eye pigmentation pathway were observed (Friedrich et al. 2011).

The genus *Ptomaphagus* (subgenus *Adelops*) has been described as the most ecologically versatile group of New World Leiodidae (Peck 1973). Peck (1973) divided

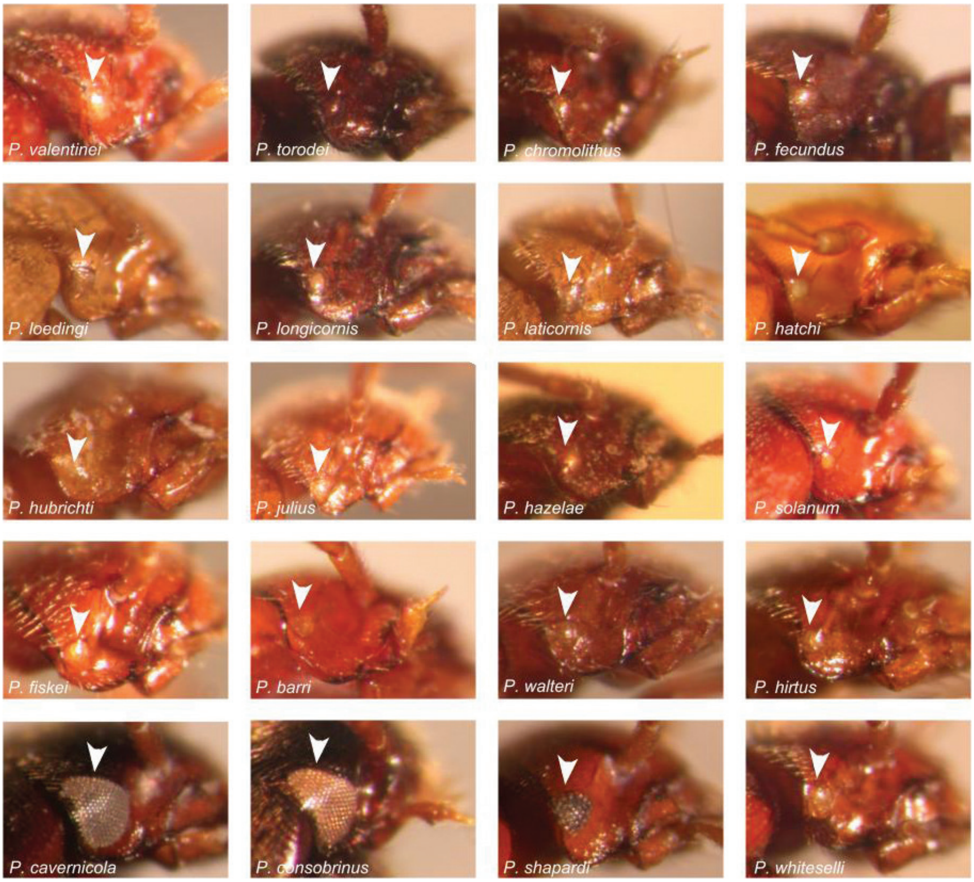

Figure 1. Eye morphologies in *Ptomaphagus*. Lateral view of head capsule and compound eye or eyelets (arrowheads) of *Ptomaphagus* species discussed in this paper. *Ptomaphagus cavernicola* and *P. consobrinus* are macrophthalmic and were used as outgroups in this study. *Ptomaphagus shapardi*, the only soil-dwelling species in the *hirtus*-group, has reduced eyes and is considered microphthalmic. The other 17 members of the *hirtus*-group are extremely microphthalmic.

Adelops into the *hirtus*-, *consobrinus*- and *cavernicola*-groups. Most members of the *consobrinus*- and *cavernicola*-groups are fully eyed (macrophthalmic), winged, and have large distribution ranges. Various lineages within all three groups are either facultative cave dwellers (eutroglophiles) or obligatory cave dwellers (troglobionts) (Sket 2008, Peck and Newton 2017). The trend toward cave-dwelling is most pronounced in the 19 species of the *hirtus*-group. Distributed across the central and southeastern United States of America, the *hirtus*-group is the largest Nearctic clade of cave-adapted Leiodidae (Peck 1973, 1984, 1986). With the exception of one soil-dwelling eutroglophile, all *hirtus*-group species are troglobionts, exhibiting extreme reduction and reorganization of the ancestral compound eye state to miniaturized camera-type eyelets (microphthalmy) and loss of wings (Fig. 1) (Peck 1973).

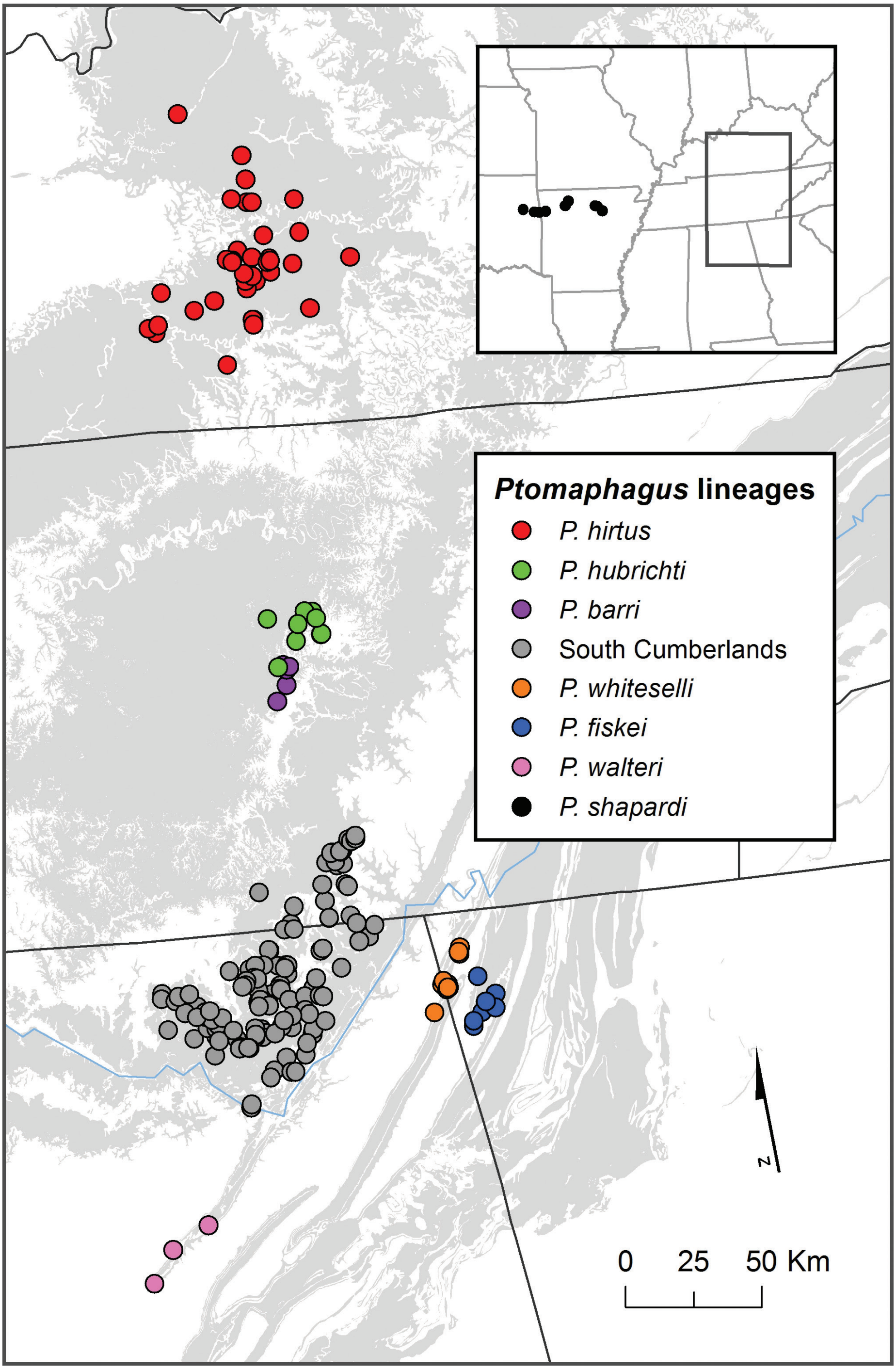

Figure 2A. Distribution of *hirtus*-group species. All known sites for *hirtus*-group species in Kentucky, Tennessee, Alabama, and Georgia. *P. shapardi* sites in Oklahoma and Arkansas are indicated in upper right inset map. A dozen species from the southern Cumberland Plateau in Tennessee and Alabama are combined.

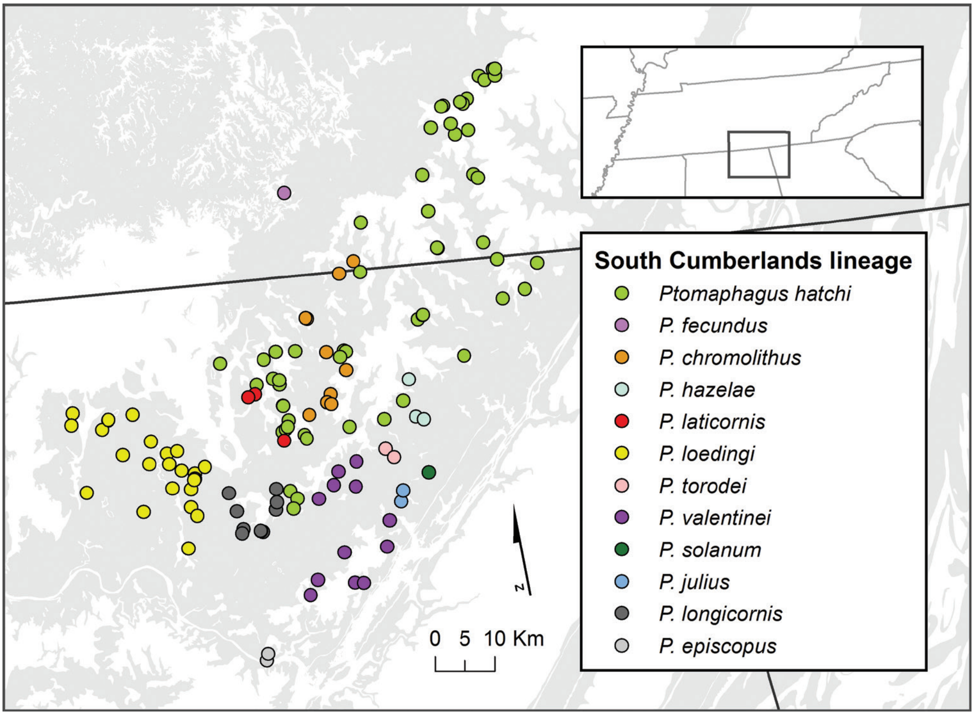

Figure 2B. Distribution of *hirtus*-group species. All known sites for members of the South Cumberlands lineage in Tennessee and Alabama. Exposed karst is shown in gray.

Members of the *hirtus*-group are found in four ecoregions (Omernik 1987): the Ridge and Valley, the Southwestern Appalachians, the Interior Plateau, and the Ozarks. *Ptomaphagus shapardi*, the soil-dwelling eutroglophile, is found in Arkansas and Oklahoma. The other species are troglobionts. *Ptomaphagus hirtus* is found in the Mammoth Cave region of central Kentucky, two species (*P. barri* and *P. hubrichti*) are found in central Tennessee, two species (*P. fiskei* and *P. whiteselli*) are found in northwest Georgia and one species (*P. walteri*) is known from the southern end of the Sequatchie Valley in Alabama. The other twelve *hirtus*-group species are clustered in the southern Cumberland Plateau of south-central Tennessee and northeast Alabama (Fig. 2A, B; Peck 1973).

As is typical for cave species, all *hirtus*-group species have small ranges. With distribution ranges <10,000 km^2, all 18 cave-obligate *hirtus*-group species are short-range endemics (Harvey 2002). Indeed, many are extreme short range endemics (Niemiller et al. 2017) with species ranges <100 km^2. Half of the *hirtus*-group species are known from five or fewer caves (Peck 1973).

Using morphological characters, Peck (1973, 1984) developed a phylogenetic framework of *Adelops* and *hirtus*-group diversification but left many relationships unresolved. Based on a lack of sympatry between *hirtus*-group species and the presence of microphthalmic eyes (as opposed to anophthalmy), Peck hypothesized that the *hirtus*-group invaded caves and diversified recently. In a version of the Climatic

Relict Hypothesis (reviewed in Culver and Pipan 2009), he suggested that glacial-interglacial cycles of the Pleistocene led to the isolation and diversification of most *hirtus*-group species over the last 350,000 years (Peck 1973, Peck 1984). Peck (1984) further hypothesized that *Ptomaphagus* populations retreated to cave- or cave-like habitats during warm and dry interglacial periods, eventually becoming cave-limited and, as a consequence, ultimately reproductively isolated. Following Peck (1984), the *hirtus*-group has been noted as an example of the Climatic Relict Hypothesis in the literature (e.g. Culver and Pipan 2009). The timing of Peck's (1984) scenario for the diversification of the *hirtus*-group (over the past ~350,000 years during the middle and late Pleistocene) differs markedly from divergence times subsequently estimated by molecular clock dating approaches for the European cave-dwelling Leiodidae, where most congeners diverged in the Pliocene or Miocene, as long as 15 million of years ago (Ribera et al. 2010).

Confronted with these divergent models for the timing of diversification in palearctic vs. nearctic cave-dwelling Leiodidae we investigated the diversification of the *hirtus*-group with molecular data. We aimed to (1) develop a molecular phylogeny for the group; (2) estimate the timing and pattern of diversification in the *hirtus*-group, and (3) gain insight into how these cave beetles diversified across distinct ecoregions and in the southern Cumberland Plateau.

Materials and methods

Specimens

Representatives of all 19 species of the *hirtus*-group were collected from 2012 to 2014. The Tennessee Wildlife Resources Agency permitted work in Tennessee (permit #1605). The Georgia Department of Natural Resources permitted work in Georgia (permit #8934). The National Park Service permitted collection of *P. hirtus* from Mammoth Cave National Park (permit #MACA-2013-SCI-0008). *P. shapardi* specimens from Oklahoma were collected by Matthew Niemiller. Two outgroup species representing the other main lineages in the subgenus *Adelops* (*P. cavernicola* and *P. consobrinus*) were collected in Florida. All beetles were collected by hand, typically with an aspirator or moist brush, and stored in 95% ethanol at -20°C. Seven species were collected from their type locality and several other species were collected from sites <1 km from their type locality. Sampling localities and species names are detailed in Table 1.

Molecular methods

We amplified and sequenced three regions of the mitochondrial genome (*cox1*, *cytb*, *rrnL-trnL-nad1*) totaling on average 2300 bp. These regions were previously used in

Table 1. *Ptomaphagus* specimens, sampling locations and Genbank accession numbers.

Species	Specimen	Locality	*cox1*	*cytb*	*rrnL-nad1*
P. cavernicola Schwarz, 1898	KSZ13-127	USA: Warrens Cave, Alachua County, Florida	KT167442	KT167490	KT167394
P. consobrinus (LeConte, 1853)	KSZ13-128	USA: Tallahassee, Florida	KT167443	KT167491	KT167395
P. barri Peck, 1973	TCN35_1	USA: Gunters Cave, Cannon County, Tennessee	KT167444	KT167492	KT167396
	TCN37_1	USA: Pleasant Ridge Cave, Cannon County, Tennessee	KT167445	KT167493	KT167397
	TCN37_2	USA: Pleasant Ridge Cave, Cannon County, Tennessee	KT167446	KT167494	KT167398
	TCN78_1	USA: Frog Hole Cave, Cannon County, Tennessee	KT167447	KT167495	KT167399
P. chromolithus Peck, 1984	AJK601_1	USA: Dub Green Cave, Jackson County, Alabama	KT167448	KT167496	KT167400
	AJK601_2	USA: Dub Green Cave, Jackson County, Alabama	KT167449	KT167497	KT167401
P. episcopus Peck, 1973	AMS3278_1	USA: Bloody Head Cave, Marshall County, Alabama	KT167450	KT167498	KT167402
P. fecundus Barr, 1963	TFR2_2	USA: Caney Hollow Cave, Franklin County, Tennessee	KT167451	KT167499	KT167403
P. fiskei Peck, 1973	GWK57_1	USA: Pigeon Cave, Walker County, Georgia	KT167452	KT167500	KT167404
	GWK57_2	USA: Pigeon Cave, Walker County, Georgia	KT167453	KT167501	KT167405
P. hatchi Jeannel, 1933	AJK289_1	USA: Kyles Cave, Jackson County, Alabama	KT167454	KT167502	KT167406
	AJK289_2	USA: Kyles Cave, Jackson County, Alabama	KT167455	KT167503	KT167407
	AJK826_1	USA: Roadside Cave, Jackson County, Alabama	KT167456	KT167504	KT167408
	AJK826_2	USA: Roadside Cave, Jackson County, Alabama	KT167457	KT167505	KT167409
	TFR423_6	USA: Grapevine Cave, Franklin County, Tennessee	KT167458	KT167506	KT167410
	TFR423_7	USA: Grapevine Cave, Franklin County, Tennessee	KT167459	KT167507	KT167411
	TGD10_1	USA: Crystal Cave, Grundy County, Tennessee	KT167460	KT167508	KT167412
	TGD10_2	USA: Crystal Cave, Grundy County, Tennessee	KT167461	KT167509	KT167413
P. hazelae Peck, 1973	AJK459_1	USA: Geiger Cave, Jackson County, Alabama	KT167462	KT167510	KT167414
P. hirtus (Tellkampf, 1844)	KWH_1	USA: White Cave, Edmonson County, Kentucky	KT167463	KT167511	KT167415
	KWH_2	USA: White Cave, Edmonson County, Kentucky	KT167464	KT167512	KT167416
P. hubrichti Barr, 1958	TCN26_1	USA: Tenpenny Cave, Cannon County, Tennessee	KT167465	KT167513	KT167417
	TCN26_2	USA: Tenpenny Cave, Cannon County, Tennessee	KT167466	KT167514	KT167418
	TDK8_1	USA: Cripps Mill Cave, DeKalb County, Tennessee	KT167467	KT167515	KT167419
	TDK8_2	USA: Cripps Mill Cave, DeKalb County, Tennessee	KT167468	KT167516	KT167420
P. julius Peck, 1973	AJK974_1	USA: House of Happiness Cave, Jackson County, Alabama	KT167469	KT167517	KT167421
	AJK974_2	USA: House of Happiness Cave, Jackson County, Alabama	KT167470	KT167518	KT167422
P. laticornis Jeannel, 1949	AJK290_1	USA: Rousseau Entrance to Gary Self Pit, Jackson County, Alabama	KT167471	KT167519	KT167423
	AJK290_2	USA: Rousseau Entrance to Gary Self Pit, Jackson County, Alabama	KT167472	KT167520	KT167424
P. loedingi (Hatch, 1933)	AMD120_3	USA: Cold Spring Cave, Madison County, Alabama	KT167473	KT167521	KT167425
	AMD120_4	USA: Cold Spring Cave, Madison County, Alabama	KT167474	KT167522	KT167426
	AMD60_2	USA: Cave Spring Cave, Madison County, Alabama	KT167475	KT167523	KT167427
	AMD60_3	USA: Cave Spring Cave, Madison County, Alabama	KT167476	KT167524	KT167428
P. longicornis Jeannel, 1949	AJK310_1	USA: Crossings Cave, Jackson County, Alabama	KT167477	KT167525	KT167429
	AJK310_2	USA: Crossings Cave, Jackson County, Alabama	KT167478	KT167526	KT167430
	AMD6_1	USA: Hering Cave, Madison County, Alabama	KT167479	KT167527	KT167431

Species	Specimen	Locality	*cox1*	*cytb*	*rrnL-nad1*
P. shapardi Sanderson, 1939	KSZ13-137	USA: Wady Cave #86, Adair County, Oklahoma	KT167480	KT167528	KT167432
P. solanum Peck, 1973	AJK166_1	USA: Sheldon's Cave, Jackson County, Alabama	KT167481	KT167529	KT167433
P. torodei Peck, 1984	AJK1068_1	USA: Two Way Cave, Jackson County, Alabama	KT167482	KT167530	KT167434
	AJK1068_2	USA: Two Way Cave, Jackson County, Alabama	KT167483	KT167531	KT167435
P. valentinei Jeannel, 1949	AJK174_1	USA: Schiffman Cave, Jackson County, Alabama	KT167484	KT167532	KT167436
	AJK174_2	USA: Schiffman Cave, Jackson County, Alabama	KT167485	KT167533	KT167437
P. walteri Peck, 1973	ABA355_1	USA: Bryant Cave, Blount County, Alabama	KT167486	KT167534	KT167438
	ABA355_2	USA: Bryant Cave, Blount County, Alabama	KT167487	KT167535	KT167439
P. whiteselli Barr, 1963	GDD66_1	USA: Byers Cave, Dade County, Georgia	KT167488	KT167536	KT167440
	GDD66_2	USA: Byers Cave, Dade County, Georgia	KT167489	KT167537	KT167441

studies of palearctic Leiodidae (Ribera et al. 2010, Fresneda et al. 2011, Rizzo et al. 2013, Cieslak et al. 2014). DNA extractions and PCR amplifications followed standard protocols (Dixon and Zigler 2011). The primers used were based on Ribera et al. (2010) but modified based on transcriptome sequence information available from *Ptomaphagus hirtus* (Friedrich et al. 2011) (Table 2). Both strands of successful PCRs were sequenced on an ABI3730. Sequences were aligned and edited in Sequencher (v. 4.9, GeneCodes Corp).

We sequenced the three gene regions from 48 individuals from 29 populations, including all 19 species of the *hirtus*-group and two outgroups (*P. cavernicola* and *P. consobrinus*) representing the other *Adelops* species-groups (Peck 1973). In 19 cases, we sequenced two individuals from the same cave. For five species, we sequenced individuals from more than one cave. All sequences have been submitted to Genbank (KT167394-KT167537; Table 1).

Table 2. Primers used, in 5' to 3' orientation. Primers were based on Ribera et al. (2010) but modified based on sequences available from *Ptomaphagus hirtus* (Friedrich et al. 2011).

Gene Region	Primer	Orientation	Sequence
cox1	hatchi.COIfor	Forward	CTGGTGGTGGGGATCCAATTC
	hirtus.COIfor	Forward	CAGGAGGTGGAGATCCTATTC
	hatchi.COIrev	Reverse	GCTTAAATTCATTGCACTAATCTGC
	hatchi.COIrev2	Reverse	TAAATTCATTGCACTAATCTGCCAT
cytB	CB3	Forward	GAGGAGCTACAGTTATTACAAA
	CB4	Reverse	AATAAAAAATATCATTCTGGTTGAAT
rrnL-trnL-nad1	16SaR	Forward	CGCCTGTTTAWCAAAAACAT
	16SaNew	Forward	CTTAAGTCTAATCTGCCCAATG
	16Sc	Forward	GATTGCGACCTCGATGTTGGA
	nad1	Reverse	ATTAGAATTTGAAGATCAACCTG
	16Sb	Reverse	CCGATTTAAACTCAGATCATGT
	nadnew	Reverse	ATTTCATAAGAAATAGTTTGAGC

Molecular tree estimation

The lack of indels or stop codons in the protein-coding *cox1*, *cytb*, and *nad1* regions allowed for unambiguous multiple sequence alignment across all sites. The ribosomal and transfer RNA coding *rrnL-trnL* sequences were aligned using MAFFT (Katoh et al. 2005). Similar alignments were obtained using MUSCLE (Edgar 2004) and CLUSTAL (Larkin et al. 2007). Bayesian analysis was conducted with MrBayes v.3.2.2 (Ronquist and Huelsenbeck 2003), using four partitions (*cox1*, *cytb*, *rrnL-trnL*, *nad1*). Evolutionary models were estimated prior to the analysis with jModelTest v.2.1 (Posada 2008). GTR+I+Γ was the preferred model for all partitions except *nad1* (TIM1+I+Γ) by AIC. MrBayes ran for 60 million generations using default values and the GTR+I+Γ model for each partition, saving every 6,000th tree. 10% of values were discarded as burnin. Similar results were obtained using codon-position partitions for the three protein coding regions and from a two partition approach (protein coding and non-protein coding sequences); both yielded similar or identical topologies with the only differences occurring at poorly-supported nodes.

Maximum likelihood searches were conducted using RAxML (Stamatakis 2014) with a GTR+Γ model used for each of the four partitions. Branch support was evaluated by rapid bootstrapping over 1,000 replicates (Suppl. material 3). Maximum likelihood analyses yielded similar topologies and node support to the Bayesian analyses.

Divergence time estimation

Divergence time estimates were generated with BEAST v1.8.1 (Drummond et al. 2012) using a total chain length of 60,000,000 generations with sampling every 1,000 generations. A Yule type speciation model with a wide normal distribution of tree height (treeModel.rootHeight = normal, initial: 15, Mean 15, Stdev 6, 95% of distribution between 5.495 mya and 24.89 mya) was used to model the tree. (We obtained similar results for tree height when using a uniform prior distribution of tree height between 5-25 mya.) The four data partitions shared a lognormal relaxed clock model with a substitution rate of 0.01 substitutions/site/my (stddev 0.002) (Rizzo et al. 2013). Each partition was assigned its own GTR+I+Γ substitution model. Tree topology was constrained to require monophyletic clades recovered with greater than 0.90 posterior probability in both MrBayes trees to appear in the final tree. We confirmed the convergence of parameter estimates by examination of three simultaneous runs using Tracer v1.5. Resulting tree files were summarized using TreeAnnotator v1.8.1, discarding 25% of samples as burnin and visualized using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Phylogenetic relationships and molecular clock estimates of diversification in the *hirtus*-group based on mitochondrial DNA sequences

Adopting the approach of Ribera et al. (2010), we produced a molecular phylogeny for the *hirtus*-group based on sequences from five genes across three mitochondrial regions, applying the molecular clock developed for the same genes in this subfamily of beetles (Leiodidae: Cholevinae) to establish divergence times for the phylogeny. Using that approach, the *hirtus*-group is estimated to have diverged from the *consobrinus*- and *cavernicola*-groups around 15 mya (Fig. 3). Within the *hirtus*-group we observed three stages of diversification. First, we found strong support for the soil-dwelling and geographically separated *P. shapardi* as sister species to the 18 cave-obligate species of the *hirtus*-group, with the two lineages diverging ~10 mya (Fig. 3). This divides the sole member of the *hirtus*-group from the Ozarks from the rest of the species in the southeastern United States (Fig. 2A). In the second stage of diversification, seven geographically distinct lineages originated between 8.5 and 6 mya (Fig. 3) across Kentucky, Tennessee, Alabama and Georgia, spreading *hirtus*-group members across several ecoregions (Fig. 2A). We were unable to resolve the branching order of these lineages with high confidence (Fig. 3). Six of these lineages are currently represented by a single species - *P. barri*, *P. fiskei*, *P. hirtus*, *P. hubrichti*, *P. walteri* and *P. whiteselli* (Figs 2A, 3). The seventh lineage contains all the species from the southern Cumberland Plateau (Fig. 2B), which form a well-supported clade (Fig. 3). In the third stage of diversification, the South Cumberlands lineage expanded from one to 12 species beginning around 6 mya. The branching order in the South Cumberlands clade is well-resolved (Fig. 3).

Ptomaphagus hatchi is polyphyletic

Most specimens sampled for a single presumed species formed monophyletic groups in our molecular phylogeny. This was not the case, however, for the *P. hatchi* specimens sampled from four different sites. Our molecular phylogenetic analysis reveals that *P. hatchi* constitutes a polyphyletic clade with respect to six species (*P. chromolithus*, *P. episcopus*, *P. fecundus*, *P. laticornis*, *P. torodei* and *P. valentinei*) with which it has overlapping or adjacent distribution ranges on the southern Cumberland Plateau (Figs 2, 3). One well supported clade joins several populations of *P. hatchi* with *P. episcopus*, *P. fecundus* and *P. chromolithus* (Fig. 3). A second well supported clade joins *P. hatchi* with *P. laticornis* (Fig. 3). We further noted that the intraspecific (between cave) divergence between *P. hatchi* populations was high, ranging from 1.2 to 5.1% P-distance for the *cox1* gene (Suppl. material 2). Intraspecific (between cave) distances for populations of other *Ptomaphagus* species are all < 2.0% (Suppl. material 2). In several cases, the intraspecific *P. hatchi* divergence is greater than the interspecific divergence observed between other *Ptomaphagus* species.

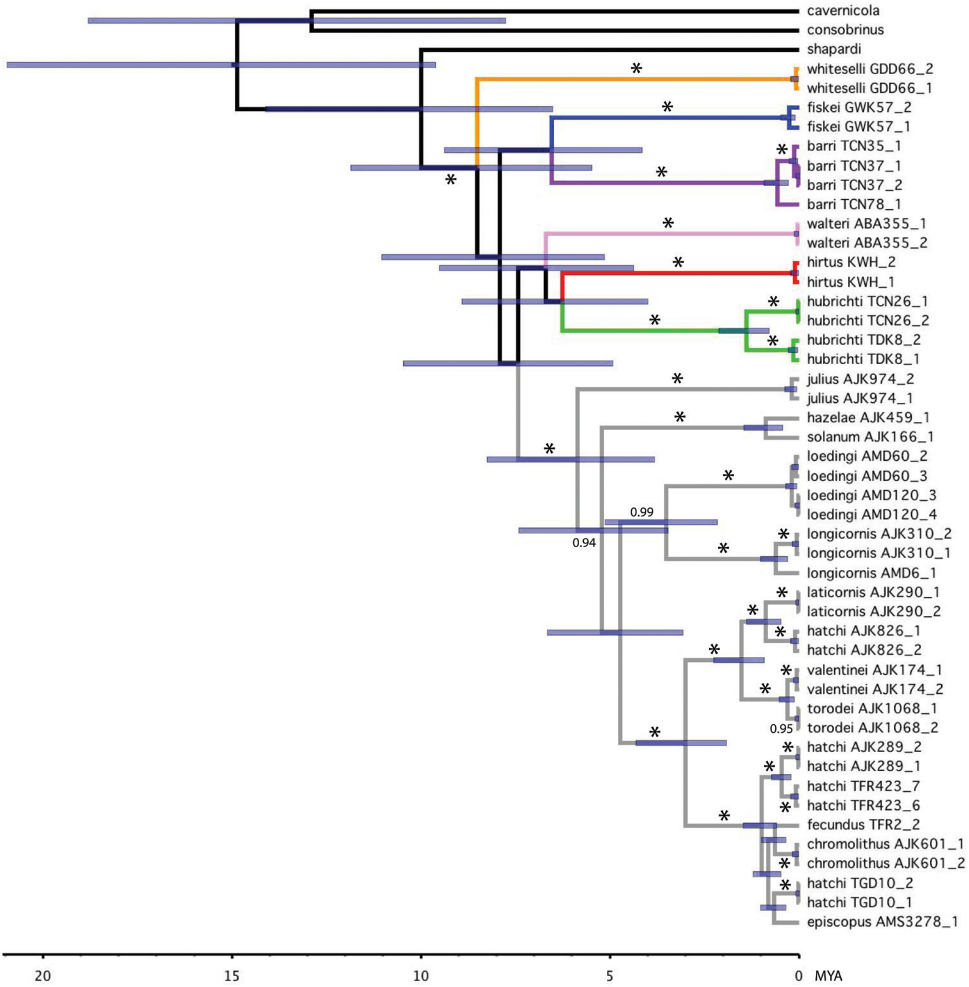

Figure 3. Ultrametric tree for the *hirtus*-group. Bayesian tree estimated from combined partial mitochondrial sequence data. Branches supported by posterior probability >0.90 are labeled with values or, for branches with posterior probability of 1.0, an asterisk. Blue bars indicate 95% confidence intervals of estimated ages for the nodes. Taxa are labeled with species name and specimen identifier (Table 1). Scale at bottom indicates divergence times in millions of years as estimated by BEAST (Drummond et al. 2012). Branch colors correspond to those in Figure 2A.

Intraspecific molecular variation

To assess genetic diversification within and between lineages, we also surveyed intraspecific variation at population (within cave) and species (between cave) levels. In 19 cases (representing 14 species), we sequenced two individuals from the same cave. Variation between individuals from the same cave was low, with a mean *cox1* p-distance of 0.15% (N = 19, range = 0.00 - 0.58%) (Suppl. material 1). In all 19 cases individuals from the same cave were each other's closest relatives (Fig. 3).

For five species, we sampled animals from multiple caves. Specifically, we sampled *P. hubrichti*, *P. loedingi* and *P. longicornis* from two caves, *P. barri* from three caves, and *P. hatchi* from four caves. Intraspecific variation between caves had a mean *cox1* p-distance of 2.1% for all pairwise comparisons (N = 12, range = 0.2 - 5.1%) (Suppl. material 2). Intraspecific variation between caves across the 2300 bp of all five sampled loci was slightly lower (mean p-distance = 1.7%), consistent with the previous observation that the *cox1* region evolves faster than the other mitochondrial regions in this group (Ribera et al. 2010) (Suppl. material 2).

Discussion

The *hirtus*-group diversified in three stages

Our molecular phylogeny provides a time-calibrated picture of the diversification of the *hirtus*-group, shedding new light on the origins of an important component of cave biodiversity in North America. The integration of molecular and biogeographic data now suggests that the *hirtus*-group diversified in three stages. The first stage, occurring mid-Miocene ~10 mya, separated the sole extant eutroglophile in the *hirtus*-group, *P. shapardi*, located in the central United States from the 18 exclusively troglobiotic species located in the southeastern United States (Figs 2A, 3). The second stage of diversification spawned seven lineages across the southeastern United States during the late Miocene, 8.5-6 mya (Figs 2A, 3). In the third stage, further fine scale diversification occurred in the southern Cumberland Plateau region during the Pliocene and Pleistocene over the past six million years (Figs 2B, 3).

How many times have *Ptomaphagus* invaded cave habitats?

During the second stage of *hirtus*-group diversification, the lineages that diversified into the 18 cave-obligate members of the *hirtus*-group were established in five distinct geographic regions (Fig. 2A): the Mammoth Cave region of Kentucky, central Tennessee, northwest Georgia, the southern end of the Sequatchie Valley in Alabama, and the southern Cumberland Plateau in Tennessee and Alabama. Although further investigation of the deeper nodes of the *hirtus*-group phylogeny is still warranted, it appears that the two species in central Tennessee (*P. barri* and *P. hubrichti*) do not form a monophyletic group, nor do the two species in northwest Georgia (*P. fiskei* and *P. whiteselli*). This suggests that distinct *hirtus*-group lineages invaded underground habitats as many as seven times. Any alternative explanation requires one or more episodes of up to 75 km long-distance dispersal across significant non-karst terrain separating current species distributions. We do not favor long-distance dispersal as an explanation for current species distributions as the dispersal ability of *hirtus*-group members appears to be quite limited. All species are wingless and small, and none have been collected in surface habitats. Even short-distance migrations appear unlikely, as two species have never been collected in the same cave, which is particularly notable in the case of the cave- and species-rich southern Cumberland Plateau. In many cases, uninhabited cave habitats are present within just one or a few kilometers of known *hirtus*-group populations (Peck 1973), indicating extremely low vagility across the *hirtus*-group.

Further consistent with minimal dispersal ability is the fact that the southern Cumberland Plateau lineage diversified into twelve species over the past 6 million years and no members of this lineage appear to have migrated from the southern Cumberland Plateau. Notwithstanding the strong circumstantial evidence, long-distance migration cannot be completely discounted as an explanation for some aspects of the species distributions we see today. For instance, long-distance dispersal has been proposed for one group of troglobiotic leiodids in the palearctic realm (*Troglocharinus* of Spain; Rizzo et al. 2013). Rizzo et al. (2013) proposed these beetles expanded their range in the early Pliocene from the central Pyrenees across a significant non-karst region to coastal karst habitats 60–70 km away, via stepping-stone migration across the surface during a permissive climate period, followed by subsequent isolation of the two lineages (Rizzo et al. 2013).

The limited dispersal capacity of troglomorphic (microphthalmic and wingless) *Ptomaphagus* species suggests that troglomorphy developed multiple times through convergent evolution in this group. This scenario and its implications could be further scrutinized at the molecular level. Previous transcriptome analyses on *P. hirtus*, for example, identified several genes in the ommochrome eye pigmentation pathway that are no longer expressed in *P. hirtus*, consistent with the lack of pigment granules in the highly reduced eyelets of this species (Friedrich et al. 2011). Similarly reduced and non-pigmented eyelets are found in all other cave-adapted species of the *hirtus*-group. A single cave colonization event during early *hirtus*-group evolution would predict shared lack-of-function mutations underlying the regression of eyes and eye pigmentation across the *hirtus*-group. Multiple independent cave colonization events, in contrast, would be reflected by a lack of shared lack-of-function mutations in eye pigmentation genes. This approach, which can be extended to other cave adaptive traits, will ultimately require genomic analyses to determine whether identical mutations are present in geographically distinct populations (consistent with a single cave invasion and the evolution of troglomorphy followed by long-distance dispersal) or whether different mutations are present in different lineages (consistent with multiple cave invasions followed by the convergent evolution of troglomorphy). Consistent with independent cave invasions and evidence for the feasibility of such an approach, distinct mutations have been observed in the pigmentation gene *cinnabar* and in the opsin genes of subterranean diving beetles (Leys et al. 2005, Tierney et al. 2015) and in *rhodopsin* genes of cavefish (Niemiller et al. 2013) in geographically distinct populations of those groups.

A new model of *hirtus*-group diversification: Vicariance by erosion

The Climatic Relict Hypothesis suggests that a species' initial colonization of caves occurred when it sought refuge from environmental stressors in the surface environment (reviewed in Culver and Pipan 2009). The Climatic Relict Hypothesis is often proposed for troglobiotic taxa lacking close relatives on the surface, with those relatives presumably extinct as a result of the changing surface conditions that forced the proto-troglobiotic taxa underground. The Climatic Relict Hypothesis can be supported by evidence that the cave species originated or diversified during a period of climate change. A convincing case for the Climatic Relict Hypothesis was developed by Leys et al. (2003) for Australian diving beetles where numerous independent invasions of isolated calcrete aquifers occurred. Leys et al. (2003) used a molecular clock to show that the timing of these invasions correlated with increasing aridity moving across the region from north to south during the Pliocene. In North America, the Climatic Relict Hypothesis has often been suggested as a driver of cave colonization related to climate change during late Pleistocene glaciation events (Culver and Pipan 2009). Peck (1973, 1984) offered the *hirtus*-group as a possible example of cave colonization via the Climatic Relict Hypothesis. To explain the species diversity of the *hirtus*-group, Peck (1973, 1984) suggested that the first *Ptomaphagus* to invade cave habitats subsequently dispersed to nearby caves during recent Pleistocene interglacial periods via cool and moist habitats such as leaf litter, talus or moss mats, after which the species became more completely cave adapted and isolated.

Our time-calibrated molecular phylogeny rejects the hypothesis of middle to late Pleistocene diversification in the *hirtus*-group. We found the timing of most speciation events in the *hirtus*-group to be an order of magnitude greater than Peck hypothesized (Peck 1973, 1984). Our divergence time estimates are similar to those derived from molecular data in other troglobiotic groups (e.g., Leys et al. 2003, Faille et al. 2010, Ribera et al. 2010, Derkarabetian et al. 2010, Niemiller et al. 2012). The divergence times estimated by these studies indicate that the species in these groups are much older than the recent Pleistocene. With most diversification of the *hirtus*-group occurring in the Miocene and Pliocene, we can rule out the Climatic Relict Hypothesis related to Pleistocene glaciations as a general rule for the *hirtus*-group.

Now recognizing that the third stage of *hirtus*-group diversification, the radiation in the southern Cumberland Plateau, began ~6 mya, we hypothesize that cave-adapted *Ptomaphagus* populations distributed throughout the region were isolated by vicariant events as the southern Cumberland Plateau eroded and fragmented over millions of years. Barr and Holsinger (1985) suggest gene flow between cave systems can be reduced via "simple erosion that divides a karst area into isolated segments by cutting down into underlying noncavernous strata." This mechanism is particularly applicable to terrestrial troglobionts, whose populations are unlikely to maintain connectivity via deep groundwater connections, as might be the case in aquatic troglobionts (e.g., Fenolio et al. 2017). The southern Cumberland Plateau is greatly dissected, with numerous isolated and peripheral mountains and ridges, and *hirtus*-group species are frequently limited to a single isolated ridge. For example, *P. longicornis* is limited to caves on Keel Mountain (in Madison and Jackson Counties, Alabama), *P. julius* is limited to caves on July Mountain (Jackson County, Alabama), and *P. solanum* is limited to Tater Knob (Jackson County, Alabama). Each of these ridges is an isolated remnant of the Cumberland Plateau (Fig. 4). Further consistent with the vicariance by erosion model (Fig. 5), the earliest lineages to diverge in the South Cumberlands clade (*P. julius*, *P. hazelae* + *P. solanum*, *P. loedingi* and *P. longicornis*) are found in peripheral and isolated ridges at the edge of the southern Cumberland Plateau, whereas later-diverging lineages are concentrated in the more intact central region of the southern Cumberland Plateau (Figs 3, 4).

Although we lack a clear picture of the timing and pattern of erosion and fragmentation of the southern Cumberland Plateau, it is clear that the ~6 my over which the South Cumberlands lineage diversified is sufficient for significant erosion and cave development to have occurred. In support of this, on the western edge of the Cumberland Plateau in middle Tennessee, extensive stream incision, erosion and cave development occurred over a similar period of time. In this region, the oldest caves (now located 60–90 m above current river level) were estimated to be 3.5–5.7 my old based on the dating of radioactive cave sediments (Sasowsky et al. 1995, Anthony and Granger 2004, 2007). Assuming a similar degree of erosion in the southern Cumberland Plateau, it is reasonable to assume that the *hirtus*-group has been heavily impacted by fragmentation, resulting in cave population isolation events over the past 6 my.

Questions arising from the present study

Understanding the lack of *hirtus*-group species diversity in the Mammoth Cave region

The *hirtus*-group is widespread in the Mammoth Cave region of Kentucky and in the southern Cumberland Plateau of Tennessee and Alabama (Fig. 2). Measured as extent of occupancy (Bachman et al. 2011), the range extent of *hirtus*-group species in the two regions is similar: 3,952 km^2 in the Mammoth Cave region and 4,151 km^2 in the southern Cumberland Plateau. It is striking that, across these similar ranges, only one species (*P. hirtus*) is present in the Mammoth Cave region, whereas 12 species are present in the southern Cumberland Plateau. As discussed above, the diversification in the southern Cumberland Plateau may be related to more frequent occurrence of vicariance by erosion, in contrast to the more continuous karst of the Interior Plateaus in the Mammoth Cave region. Indeed, such patterns have been proposed in the past (e.g., Barr and Holsinger 1985 and references therein).

One prediction from this scenario is that *P. hirtus* evolved less genetic diversity across its range than we observed across the South Cumberlands lineage. Alternatively, *P. hirtus* may represent a collection of cryptic species, as has been observed in numerous cave lineages (e.g., Trontelj et al. 2009, Derkarabetian et al. 2010, García-Machado et al. 2011, Hedin 2015, Zhang and Li 2013). Our analysis presented here sampled only a single *P. hirtus*

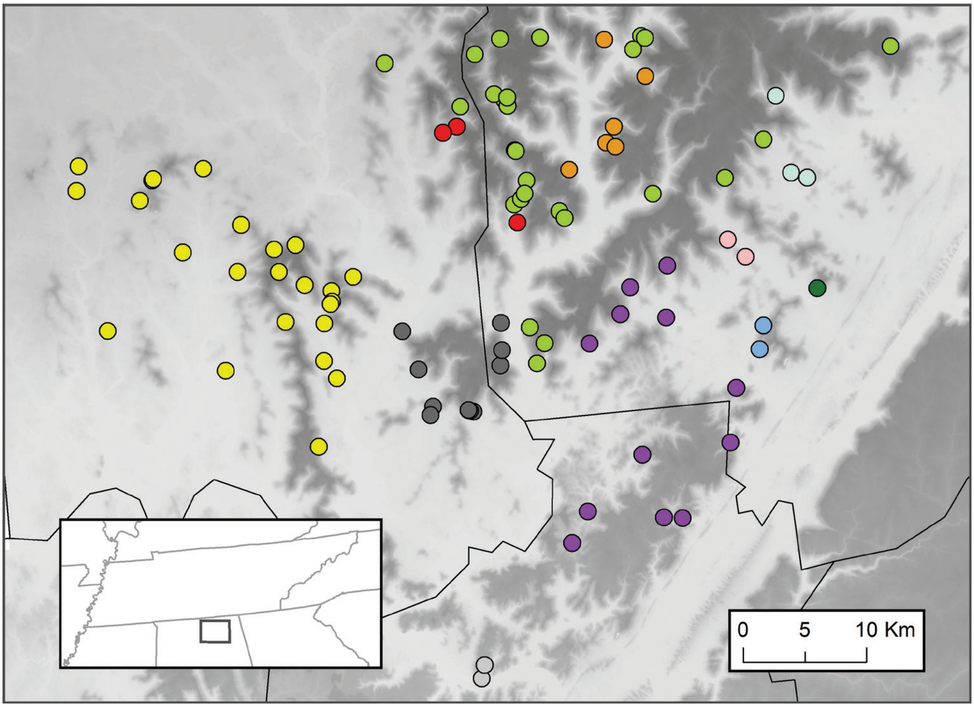

Figure 4. Distribution of *Ptomaphagus* species on the southern Cumberland Plateau, overlaid on a digital elevation model. Higher elevations (to 500 m) are indicated by darker shades, lower elevations (to 180 m) by lighter shades. *Ptomaphagus* species diverging early in the South Cumberlands lineage are limited to isolated ridges and mountains on the fringes of the plateau. These species are *P. loedingi* (yellow), *P. longicornis* (dark gray), *P. julius* (blue), *P. solanum* (dark green) and *P. hazelae* (light blue). The colors used here correspond to those in Figure 2B.

population (Table 1). Thus, further phylogeographic and population genetic studies of *P. hirtus* are warranted to clarify whether current species definitions are consistent with the genetic diversity present in the region. Such studies are further recommended given the evidence of cryptic species in what has previously been considered a single taxon, *P. hatchi*.

Molecular evidence that *Ptomaphagus hatchi* is polyphyletic

With the exception of *P. hirtus*, *P. hatchi* has the largest range extent of any *hirtus*-group species. *P. hatchi* is also the only member of the *hirtus*-group with a range that overlaps those of other species in the group. We found that the current species definition of *P. hatchi* is polyphyletic with respect to six species (*P. chromolithus*, *P. episcopus*, *P. fecundus*, *P. laticornis*, *P. torodei* and *P. valentinei*) from the southern Cumberland Plateau lineage (Figs 2, 3). Peck's (1983, 1984) study of species boundaries within *P. hatchi* relied on distinctions in the female spermatheca (Peck 1984) and evidence of

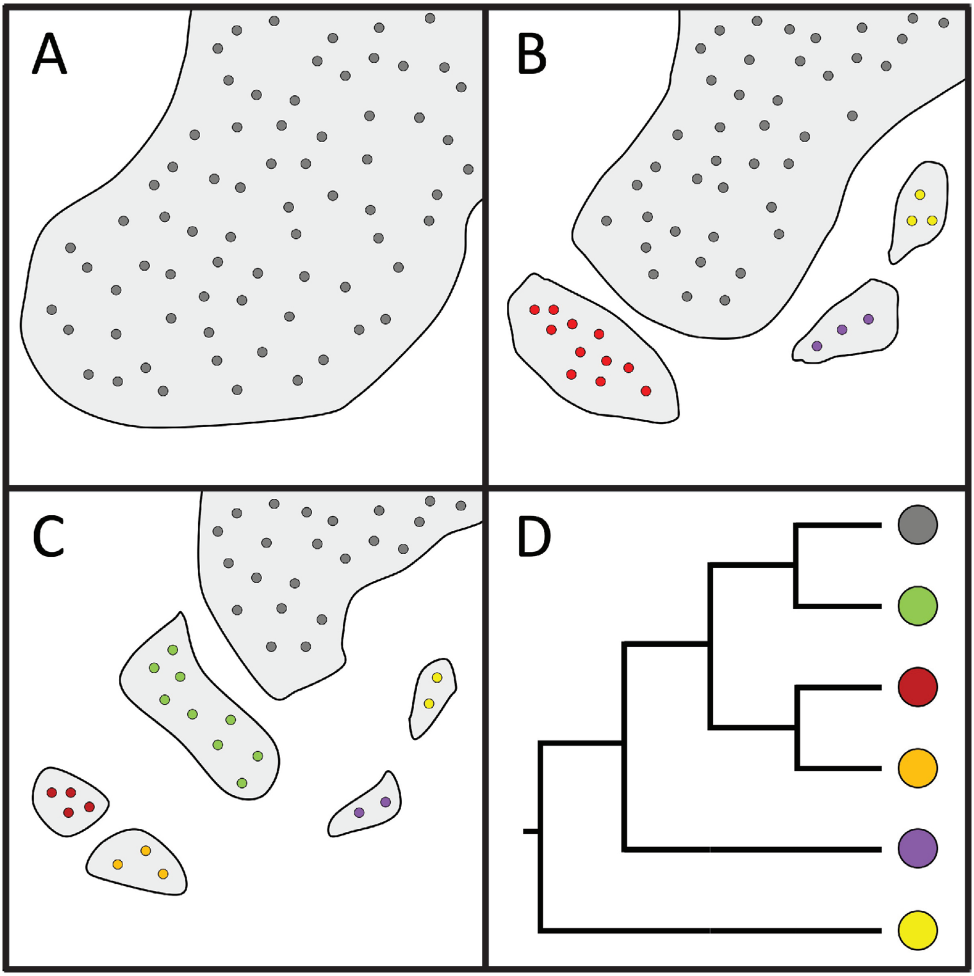

Figure 5. Biogeographic and phylogenetic expectations for a 'vicariance by erosion' scenario as hypothesized for the southern Cumberland Plateau. **A–C** Karst (gray) erodes and fragments over time, leading to the isolation and divergence of cave populations (colored circles) in the remaining patches of karst **D** A phylogeny consistent with the vicariance by erosion process, with taxa that diverge early distributed at the periphery of the eroding region.

reproductive isolation in various interpopulational crosses (Peck 1983). In the most recent taxonomic revision, Peck (1984) restricted *P. hatchi* to populations with 'form I' spermathecae and described *P. laticornis* (with 'form II' spermathecae) and *P. chromolithus* (with 'form III' spermathecae). He also described *P. torodei* as a close relative of *P. valentinei* and raised *P. fecundus* to full species status (Peck 1984).

Significantly, our mitochondrial sequence divergence data do not align with these distinctions. This could be due to a more dynamic variability of spermatheca form than previously envisioned. Alternatively, 'form I' spermathecae may represent an ancestral state that has been retained in some but not all descendant lineages (now called *P. hatchi*), leading to the erroneous support for a polyphyletic taxon based on the shared similarity of a plesiomorphic character state, i.e. symplesiomorphy. It is also possible that hybridization and introgression have confused the molecular phylogenetic picture of these lineages as we only analysed mitochondrial DNA. This, however, seems unlikely as two species of *Ptomaphagus* (or two spermathecal forms) have never been reported from the same cave (Peck 1984). At present, the available molecular data are too limited to recommend taxonomic revision, but further molecular analysis of these taxa across their distributions has now become essential to gain a reliable understanding of the *hirtus*-group diversification.

"Vicariance by erosion" and the development of a cave biodiversity hotspot on the southern Cumberland Plateau

Exhibiting high levels of taxonomic diversity and endemism, the southern Cumberland Plateau is a hotspot for cave biodiversity. This region has been compared to other centers of cave biodiversity such as the Dinaric karst of Slovenia, the French Pyrenees and the Mammoth Cave region (Culver et al. 2006). This biodiversity peaks in a six-county region in northeast Alabama and south-central Tennessee. Totaling ~10,000 sq km, this region has more than 4400 known caves that support more than 150 troglobionts (Culver et al. 2000, Niemiller and Zigler 2013). Members of the *hirtus*-group compose nearly 10% of the troglobionts present in the southern Cumberland Plateau.

With a few exceptions, we lack time-calibrated molecular phylogenies for troglobionts from the southern Cumberland Plateau. This has limited our understanding of the development of biodiversity in this cave biodiversity hotspot. Important exceptions exist for several aquatic taxa including crustaceans, fish and salamanders (Buhay and Crandall 2005, and comment in Trontelj 2007, Niemiller et al. 2008, Niemiller et al. 2012). These studies indicate diversification during the Pleistocene for all of these taxonomically diverse groups. Here, we provide the first time-calibrated study of a diverse terrestrial troglobiont group from the region. We found that the diversification of the South Cumberlands lineage of the *hirtus*-group extended across ~6 my, beginning near the end of the Miocene and continuing through the Pliocene and Pleistocene (Fig. 3). Thus, it appears troglobionts have accumulated in the southern Cumberland Plateau since at least the late Miocene.

We therefore suggest that vicariance by erosion played a generally significant role in the accumulation of troglobiotic species in this biodiversity hotspot. Better models of the fragmentation of the southern Cumberland Plateau and the development of time-calibrated phylogenies for other species-rich terrestrial troglobiotic taxa from the region (such as pseudoscorpions and millipedes) would allow further evaluation of "vicariance by erosion" model (Fig. 5) for the southern Cumberland Plateau cave biodiversity hotspot.

Acknowledgments

We thank Kurt Helf, Matthew Niemiller, Stewart Peck, Rick Toomey and Maja Zagmajster for assistance with collections, Stewart Peck for assistance with photographic documentation of collected specimens, and two reviewers for helpful comments. This project was supported by the University of the South and the Shearwater Foundation.

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Supplementary material 1

Table S1. Mean *cox1* P-distances between specimens from the same cave

Authors: Vincent L. Leray, Jason Caravas, Markus Friedrich, Kirk S. Zigler

Data type: statistical data

Link: https://doi.org/10.3897/subtbiol.29.31377.suppl1

Supplementary material 2

Table S2. Mean *cox1* and five gene (*cox1*, *cytb*, *rrnL-trnL-nad1*) P-distances between conspecific individuals from different caves

Authors: Vincent L. Leray, Jason Caravas, Markus Friedrich, Kirk S. Zigler

Data type: statistical data

Link: https://doi.org/10.3897/subtbiol.29.31377.suppl2

Supplementary material 3

Figure S1. Maximum likelihood majority rule consensus bootstrap tree

Authors: Vincent L. Leray, Jason Caravas, Markus Friedrich, Kirk S. Zigler

Data type: phylogenetic data

Explanation note: Maximum likelihood tree estimated from combined partial mitochondrial sequence data. Bootstrap values (from 1000 replicates) are indicated above branches. Taxa are labeled with species name and specimen identifier (Table 1).

Link: https://doi.org/10.3897/subtbiol.29.31377.suppl3

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RESEARCH ARTICLE

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The cave-dwelling dipluran (Diplura, Campodeidae) on the edge of the Last Glacial Maximum in Vancouver Island caves, North America (Canada)

Alberto Sendra[1,2], Craig Wagnell[3]

1 *Grupo de Investigación de Biología del Suelo y de los Ecosistemas Subterráneos, Departamento de Ciencias de la Vida, Facultad de Biología, Ciencias Ambientales y Química, Universidad de Alcalá, E-28871 – Alcalá de Henares, Madrid, Spain* **2** *Servei de Patrimoni Històric, Ajuntament de Valencia, E-46008 – Valencia, Spain* **3** *CICC "Central Island Caving Club" 4850 Benjamin Rd. Port Alberni, B.C., V9Y-8X8, Canada*

Corresponding author: *Alberto Sendra* (alberto.sendra@uv.es)

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Abstract

A new cave-dwelling dipluran of the North American endemic genus *Haplocampa* is described, coming from a couple of caves excavated in a small limestone karstic area near Port Alberni, Vancouver Island (British Columbia, Canada). To *Haplocampa* belong five soil-dwelling species. L. M. Ferguson cited no less than eight more species living in soil and cave habitats in several US states but without producing any formal descriptions. *Haplocampa*, in spite of its large lateral crests on the unequal claws, has clear taxonomical features as a Campodeinae and is closely related with the cave-dwelling *Pacificampa* and *Eumesocampa* genera, due to sharing similar macrosetae body distribution and absence or reduction of the lateral process. The new proposed species, *Haplocampa wagnelli* Sendra, **sp. n.**, is rather interesting for its troglomorphic features: antennae with 32 antennomeres; olfactory chemoreceptors, each a multiperforated, folded-spiral structure; and numerous gouge sensilla. In addition, it is one of the northernmost troglomorphic species to have colonised – presumably recently – an area occupied by the Late Wisconsinian North America ice sheet during the Last Glacial Maximum. Furthermore, the close affinities between *Haplocampa*, *Pacificampa* (from caves in the extreme east of continental Asia and the southern Japanese Islands), *Metriocampa* (from the east of Asia and North America) and *Eumesocampa* (endemic to North America) suggest probable dispersal events over the Bering Land Bridge.

Keywords

Haplocampa wagnelli, cave fauna, troglomorphic, biogeography, glaciation

Introduction

Although subterranean diplurans were already known from North America since the 19th century (Packard 1871), few species have been described (Condé 1949; Condé and Bareth 1996; Ferguson 1996; Sendra et al. 2016; Wygodzinsky 1944) and many remain undescribed despite a vast sampling effort. *Haplocampa* genus is a clear example. *Haplocampa* is endemic to North America (Silvestri 1911); it was first discovered in Shasta Springs, California, with its type species *Haplocampa wheeleri* Silvestri, 1911. Later, the same entomologist (Silvestri 1933) described three new species: *Haplocampa rugglesi* Silvestri, 1933; *Haplocampa chapmani* Silvestri, 1933; and *Haplocampa drakei* Silvestri, 1933. One more species of this genus could be included if we consider the form *Haplocampa* cf. *chapmani* by Condé and Geeraert (1962). These five species live in soil habitats and are distributed in Montana, Oregon and Washington in the USA and Alberta in Canada. During the 1980s and 1990s, a large sampling effort was carried out in 281 caves in California alone (Elliott et al. 2017) revealing 3 or more species of *Haplocampa*, and Ferguson (1981, 1992) reported on 7 or 8 species of *Haplocampa* from 21 lava tubes mainly in the Pacific Northwest. Ferguson (2009) also reported on his identification of 9 new species of *Haplocampa* from the caves of Colorado, bringing the total number of identified species of *Haplocampa* from caves to 26. Nevertheless, no formal description was made until now and many *Haplocampa* specimens remain undescribed in American museums and invertebrate collections (Graening et al. 2014). In 1996, the Central Island Caving Club started wandering the steep forested slopes of Vancouver Island. Since that time, the Club has mapped over 20 new caves, including what is now the most popular recreational cave on Vancouver Island. Thanks to the Club's great work, the explorations of a group of cavers from Vancouver Island (British Columbia, Canada) have given us the opportunity to describe the next new species of *Haplocampa* after more than half a century.

Materials and methods

Sampling methods

Fauna collections were carried out in two sites of Fossli Slot #2 and one site from Kiku Pot (see below the description of caves). Sampling was focused on regions where visible signs of chewed-up organic matter could be seen. Collections were made manually using #00 brushes and kept in small vials with 90% ethanol. Once the specimens were spotted, they were dabbed with a brush moistened with ethanol and immediately placed into vials, labelled and sealed for transport.

Material processing and identification

The specimens were washed using distilled water and were put between slides and glass coverslips to be examined under a phase-contrast optical microscope (Leica DMLS) using Marc André II solution. The illustrations were made with a drawing tube and the measurements were taken with an ocular micrometre. For measuring the body length, the specimens were mounted 'in toto' and were measured from the base of the frontal process distal macrochaetae to the abdomen's supra-anal valve. Two paratypes were coated with palladium-gold used for scanning electronic microscopic photography (Hitachi S-4100) and measurement of the sensilla.

The morphological descriptions and abbreviations used in this article follow Condé (1956). We use gouge sensilla for the concavo-convexly shaped sensilla located on the antennae and described by Bareth and Condé (1981), the function of which is still unknown, and rosette gland formations for the epicuticular glands described in several Campodeinae species (Bareth and Juberthie-Jupeau 1996).

Results

Haplocampa wagnelli Sendra, sp. n.

http://zoobank.org/C4B52929-5AB3-4FB6-9B7F-451BA52A7B81
Figs 1–27; Tables 1, 2; Suppl. material 1

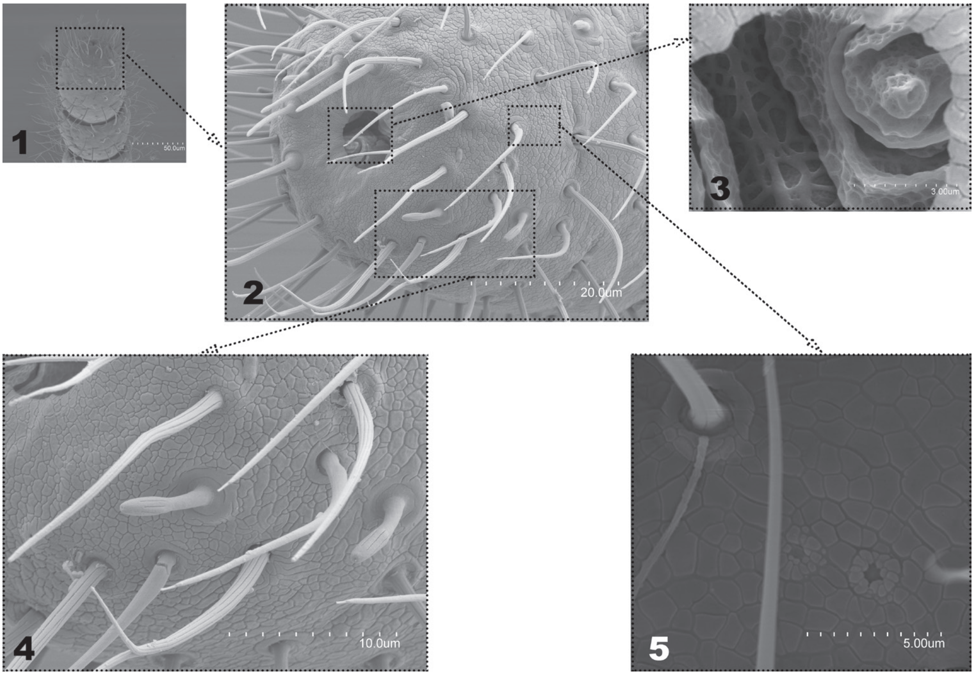
Etymology. This species is dedicated to the co-author of this article, a caver who has dedicated many years sampling and exploring in Vancouver Island caves.

Type material. Female holotype labeled ♀01 from Kiku Pot Cave, Port Alberni, Vancouver Island, Canada, 5th August 2018, C. Wagnell leg. (SEHU); 1 ♂ labeled ♂01, paratypes from Fossli Slots Caves, Port Alberni, Vancouver Island, Canada and 4 females labeled ♀02–♀05 from Fossli Slots Caves, Vancouver Island Canada, 15th July 2018, C. Wagnell leg. All type material mounted in Marc André solution. Deposited in AS collection.

Other studied material. Four specimens from Fossli Slots Caves, 8th June 2018, C. Wagnell leg as type material mounted in separated aluminum stages and coated with palladium-gold. Deposited AS collection.

Description. Body length 4.4 mm (male) and 3.4–6.0 mm (females). Epicuticle smooth under optical microscope but reticulated in high magnifications in round polygonal structures variable in size (Figs 4, 14–15); *rosette* gland formations present along the body (Figs 5, 16); body with abundant short and smooth clothing setae.

Moliniform antennae. Every intact antenna in the six type specimens has 32 antennomeres; from 0.5 to 0.7 times longer than body in larger and smaller adults, respectively. First antennomere four times shorter than second antennomere, and apical antennomere 1.3 longer than wide (Fig. 1); the other antennomeres, as long as wide (Fig. 7).

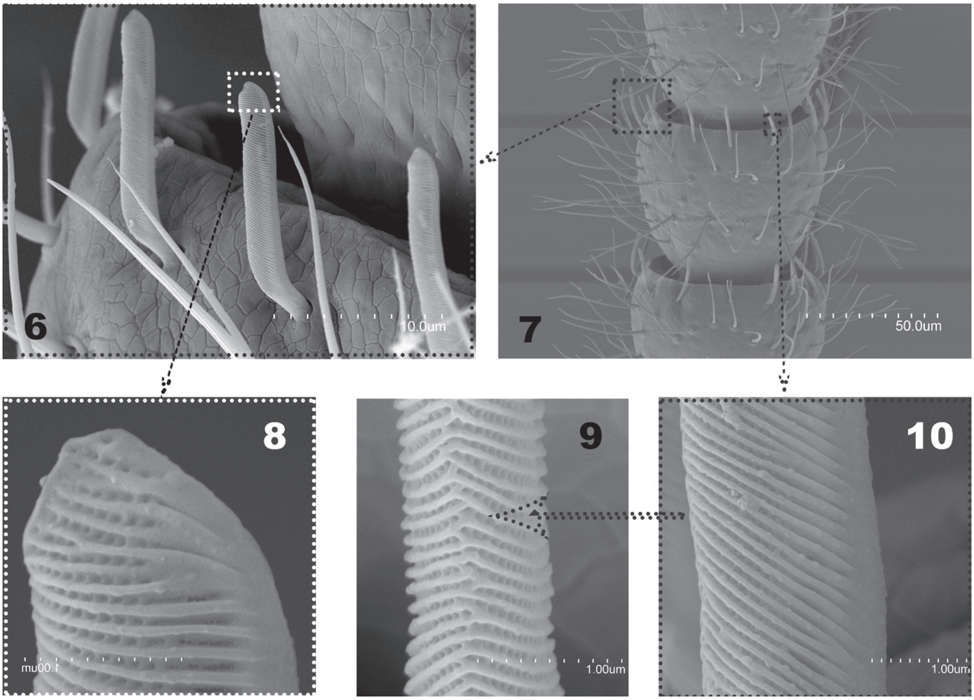


Figures 1–5. *Haplocampa wagnelli* Sendra, sp. n. **1** last and penultimate antennomere **2** olfactory chemoreceptors within the cupuliform organ **3** detail of olfactory chemoreceptor, paratype **4** coniform sensilla on the last antennomeres **5** two *rosette* sensilla in the last antennomere.

Cupuliform organ occupying 1/3 of the total length of the apical antennomere with about five complex olfactory chemoreceptors, each one a multiperforated–folded spiral structure, all tightly packed in the narrow open space of the cupuliform organ (Figs 2, 3). Distal and central antennomeres with a sensorial equipment: one whorl of bifurcated macrosetae, two to three whorls of untidy setae , and a single distal whorl of 10–13 short and thick *gouge* sensilla of 14–16 µm long 2–3 very short grooved closed–bud form sensilla 6–8 µm long; this sensorial equipment is also present in the apical antennomere (Figs 4, 6–10). Proximal antennomeres with typical trichobothria plus a thick and long sensillum on the third antennomere, located in ventral position, although the ventral *c* and *d* macrosetae are not differentiated.

Head subtrapezoidal with slightly protuberant lateral posterior angles (Fig. 11). Plain frontal process with the slightly differenced frontal smooth macrosetae (Fig. 12). From the three macrosetae along each side of the line of insertion of antennomere and *x* setae, *a* macrosetae is not differentiated and *i/p/x* have 36/57/23 relative lengths, all smooth (Fig. 12). Suboval labial palps with latero-external long thick sensillum, with two guard setae, up to 8 setae on anterior border and up to 80 neuroglandular setae.

Thoracic macrosetae distribution (Fig. 13): pronotum with 1+1 *ma*, 1+1 *la,* 1+1 *lp;* mesonotum with 1+1 *ma*, 1+1 *la*, 2+2 $lp_{2, 3}$; metanotum with 1+1 *ma*, 1+1 lp_2 macrosetae. All macrosetae relatively well developed, long with thin long barbs; marginal setae slightly longer and thicker than clothing setae and also smooth. Legs very slightly elon-



Figures 6–10. *Haplocampa wagnelli* Sendra, sp. n. **6** distal gouge sensilla whorl in a medial antennomere **7** medial antennomere **8** detail of the ending portion of a gouge sensillum **9** detail of external side of a gouge sensillum **10** detail of lateral side of a gouge sensillum.

gated, metathoracic legs reaching the VIII abdominal segment in smaller adults and the VI in larger ones. Femur and tibia similar in length but tarsus clearly shorter and thicker (tibia/tarsus ratio 0.6 to 0.7) (Table 1). Femur I–III with one dorsal macrosetae well differentiated with a few thin long barbs in its distal half. Calcars with a few thin, long barbs throughout. Tibia I–III with two or three ventral macrosetae bifurcated in the apex (Fig. 17). Three or two dorsal, lateral and sometimes ventral tarsal setae similar to clothing setae but much longer (Fig. 18). Unequal claws (posterior claw 1.5 longer than anterior) with large lateral crests and no lateral process; ventral side of the claws noticeably ridged and covered by a micro-granulation surface; a spiny protrusion is visible in the basal portion of both claws (Figs 18–21); posterior claw with a large backward overhang (Figs 18, 19).

Distribution of abdominal macrosetae on urotergites (Fig. 22): 1+1 *ma* on I-III; 1+1 *ma*, 1+1 lp_2 on IV; 1+1 *ma*, 1+1 *la* and 2+2 $lp_{2,3}$ on V-VII: 1+1 *ma* and 3+3 $lp_{1,2,3}$ on VIII; 1+1 *ma* (slightly backwards) and 5+5 $lp_{1,2,3,4,5}$ on IX abdominal segment. All tergal abdominal macrosetae long and well differentiated with thin barbs along the half to third distal.

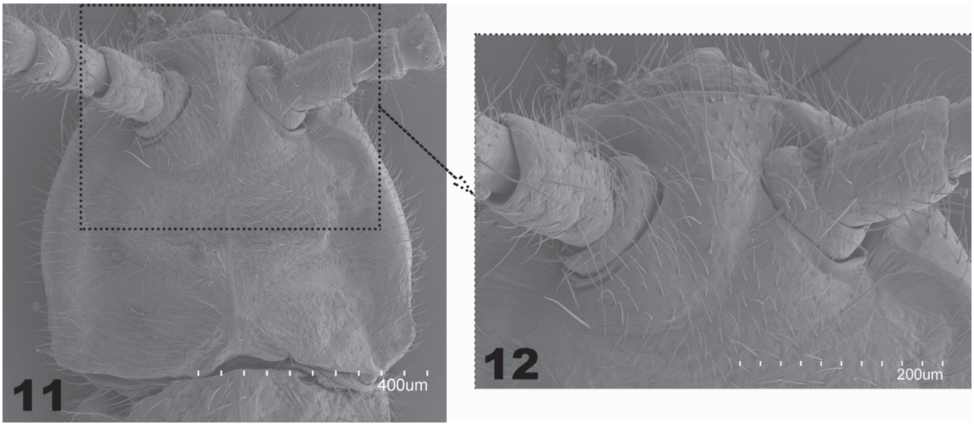
Urosternite I with 6+6 macrosetae (Figs 23–24); II to VII with 4+4 macrosetae; VIII with 1+1 macrosetae; short to middle size urosternal macrosetae, bi or trifurcated or with long barbs. Short styli with smooth short apical setae with two long teeth;

Table 1. *Haplocampa wagnelli* Sendra, sp. n., length of the body, antennae and metathoracic leg including their segments, the cerci (units in mm), and the number of antennomeres.

Specimen	Body length	Antennomeres	Antennae length	Segment length of a metathoracic leg						Total metathoracic length
				Coxa	Trochanter	Femur	Tibia	Tarsus	Pretarsus	
Paratype, ♀04	3.40	32	2.50	0.21	0.18	0.45	0.41	0.27	0.10	1.62
Paratype, ♀05	3.75	32	2.35	0.20	0.12	0.43	0.40	0.28	0.85	1.47
Paratype, ♂01	4.40	32	2.52	0.21	0.12	0.48	0.46	0.28	0.10	1.65
Paratype, ♀02	4.90	32	2.65	0.22	0.18	0.51	0.48	0.29	0.11	1.78
Paratype, ♀03	5.05	32	3.05	0.25	0.22	0.60	0.55	0.35	0.10	2.07
Holotype, ♀01	6.02	32	3.20	0.21	0.17	048	0.46	0.28	0.11	2.15

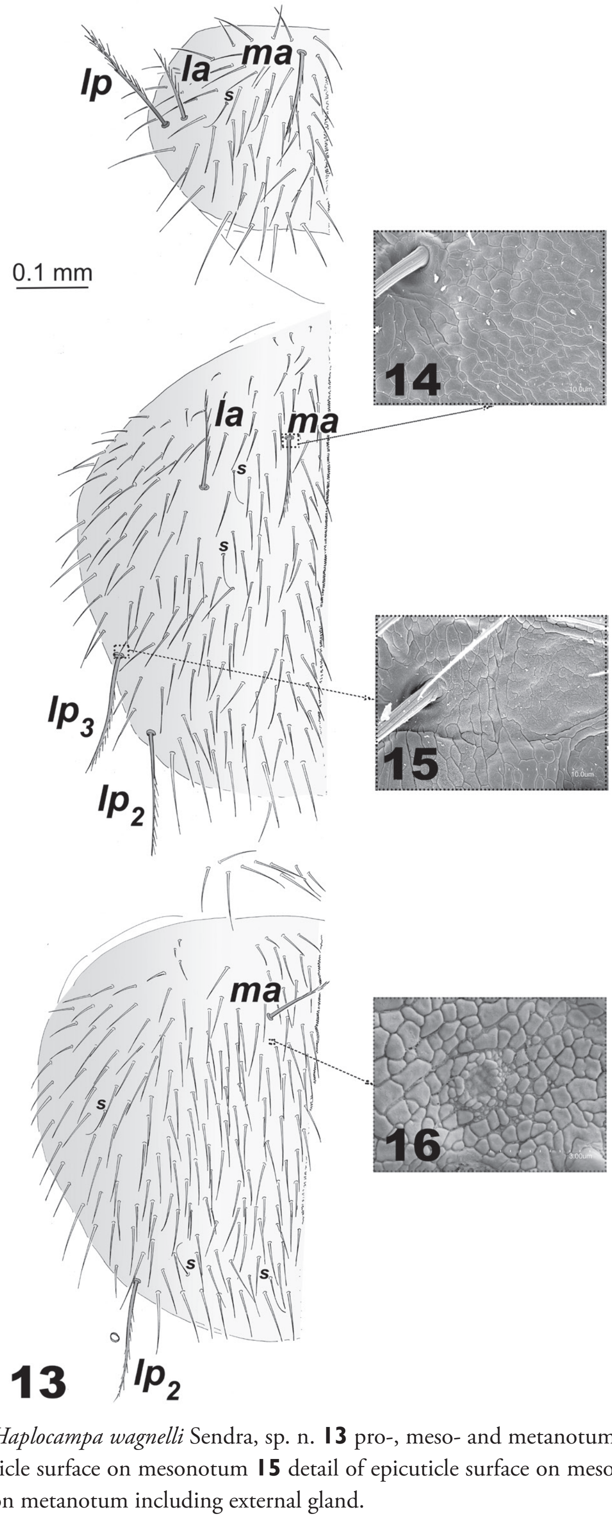
Table 2. *Haplocampa wagnelli* Sendra, sp. n., length of cercal articles and total length (units in mm) including number of articles of each cercus.

Specimen, body length	Cerci, Articles length							
	Base (secondary article)	1st	2nd	3rd	4th	5th	6th	Total length
Paratype ♀04, 3.40 mm	0.78 (7)	0.22	0.25	0.28	0.36	0.38	-	2.26
Paratype ♀05, 3.75 mm	0.70 (8)	0.22	0.30	0.38	0.40	0.42	-	2.42
Paratype ♂01, 4.4 mm	0.35 (3)	0.18	0.25	0.28	0.30	0.40	0.42	2.18
Paratype ♀02, 4.9 mm	0.62 (4)	0.21	0.24	0.26	0.31	0.39	0.44	2.48
Paratype ♀03, 5.05	0.92 (7)	0.25	0.28	0.30	0.32	0.38	0.35	2.70
Holotype ♀01, 6.02 mm	0.88 (6)	0.25	0.30	0.32	0.35	0.38	0.38	2.85

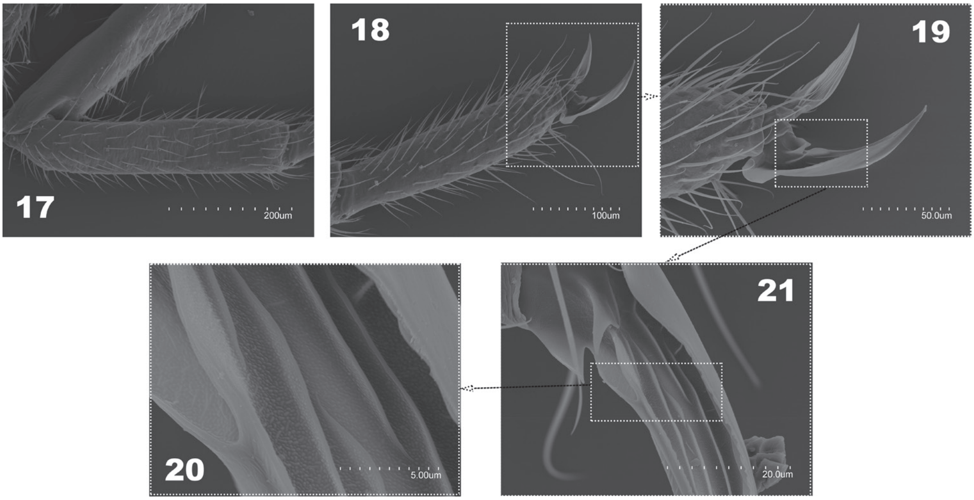


Figures 11, 12. *Haplocampa wagnelli* Sendra, sp. n. **11** dorsal side of the head **12** frontal process.

bifurcated subapical and ventromedial setae (Fig. 25). Cerci half shorter than the body length, from 0.64× in the smaller adults to 0.47× in the holotype; so, the length of the cerci increases disproportionally with the body size. As well, the length of the articles increases from proximal to distal. Basal article is divided into three to eight secondary articles, followed by five to six primary articles bearing from two to five whorls of



Figures 13–16. *Haplocampa wagnelli* Sendra, sp. n. **13** pro-, meso- and metanotum, left side, holotype **14** detail of epicuticle surface on mesonotum **15** detail of epicuticle surface on mesonotum **16** detail of epicuticle surface on metanotum including external gland.



Figures 17–21. *Haplocampa wagnelli* Sendra, sp. n. **17** distal portion of femur and tibia from a metathoracic leg **18** tarsus **19** end of the tarsus and telotarsus **20** detail of posterior claw, lateral side **21** detail of posterior claw, lateral side.

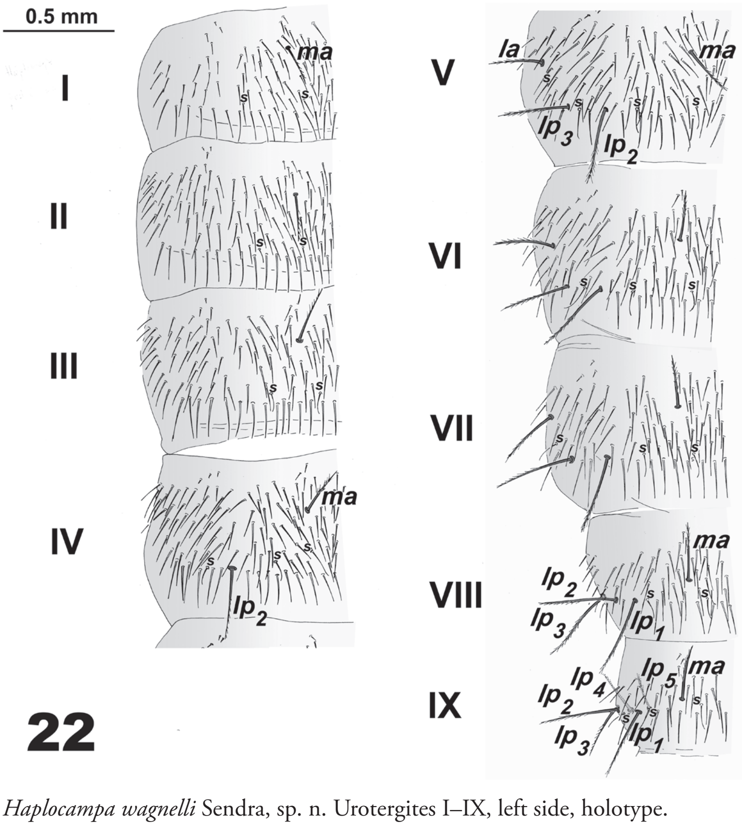


Figure 22. *Haplocampa wagnelli* Sendra, sp. n. Urotergites I–IX, left side, holotype.

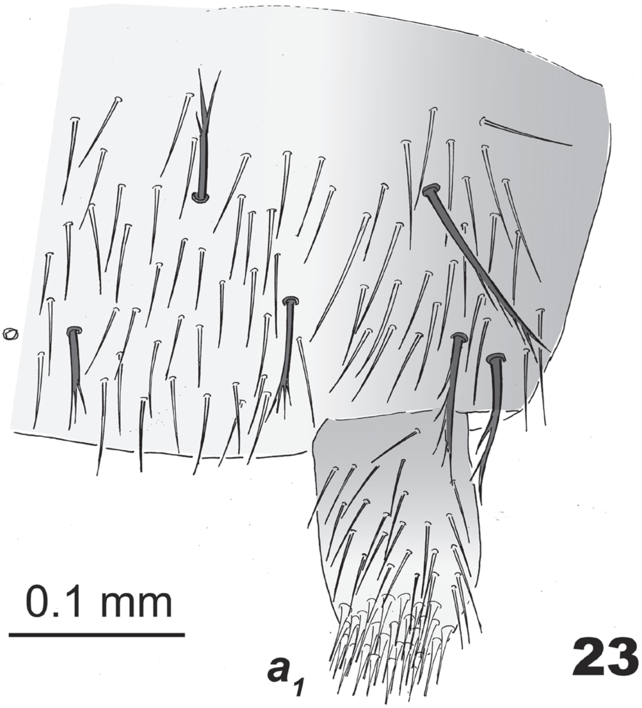


Figure 23. *Haplocampa wagnelli* Sendra, sp. n. First female urosternite, ♀ paratype, 6 mm.

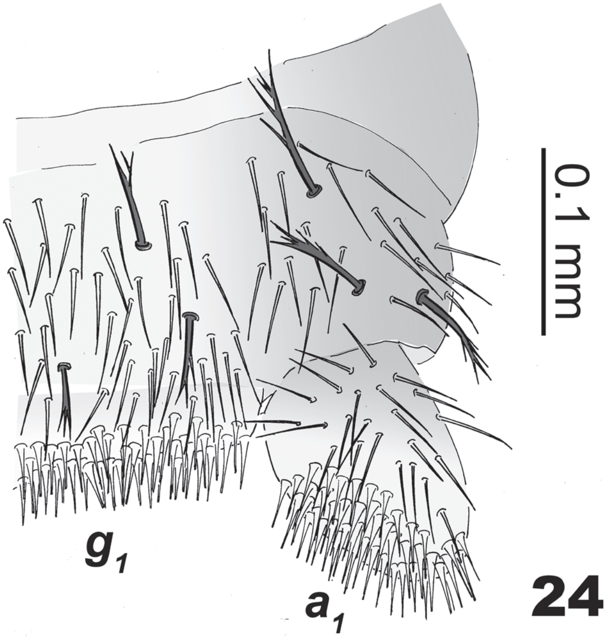


Figure 24. *Haplocampa wagnelli* Sendra, sp. n. First male urosternite, ♂ paratype, 4.4 mm.

Figure 25. *Haplocampa wagnelli* Sendra, sp. n. Left stylus of the sixth urosternite.

bifurcated macrosetae, and three to six smooth setae including the distal setae whorl in each primary article (Table 2; Figs 26, 27).

Female urosternite I with short subcylindrical appendages, each bearing up to 30 a_1-glandular setae in a distal field (Fig. 23).

Male urosternite I with short moderately thick subcylindrical appendages, each bearing about 50 a_1-glandular setae in a large field; up to setae covered the with two to three rows the posterior part of the first urosternite (Fig. 24).

Remarks. The most visible features are the slightly troglomorphic characteristics of *H. wagnelli* sp. n., as shown by its antennae with 32 antennomeres, a record within the genus *Haplocampa* but a moderate antennomere number in a troglomorphic campodeid; and the five complex olfactory chemoreceptors, each one a multiperforated, folded-spiral structure However, any comparison with other *Haplocampa* species is currently impossible since these sensilla have not been described in any other species of the genus. The closest species to *H. wagnelli* sp. n. is *H. rugglesi* from Mount Rainier (Washington, USA), with equal macrosetae distribution on nota and similar on urotergites. Nevertheless, some taxonomic features are unique to *H. wagnelli* sp. n., such as medial anterior macrosetae on urotergites and three tibial ventral macrosetae. Many taxonomical details remain incomparable what is known described species, due to the lack of high magnifications.

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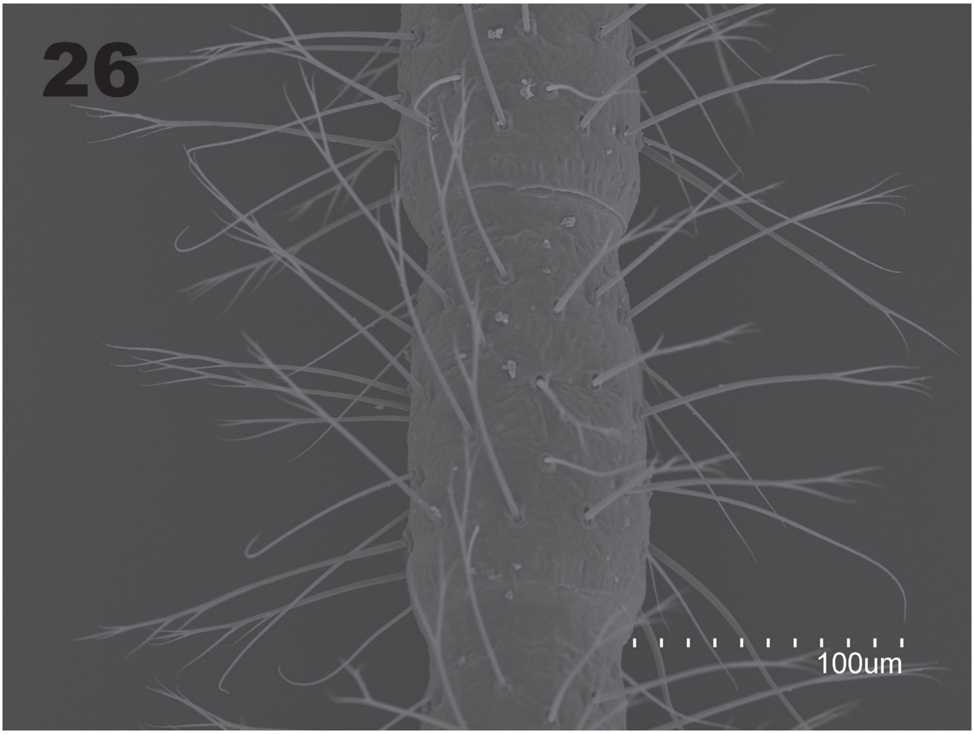

Figure 26. *Haplocampa wagnelli* Sendra, sp. n. First primary cercal article, lateral side.

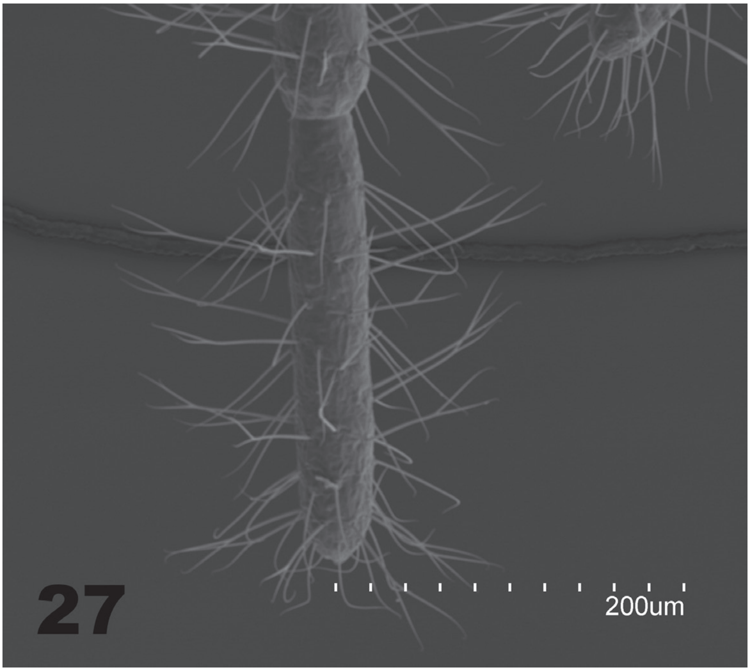

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Figure 27. *Haplocampa wagnelli* Sendra, sp. n. Last primary cercal article.

Discussion

An attempt at *Haplocampa* redescription with a key to species

Silvestri (1911) described the genus, and Paclt (1957) redescribed it. By following these taxonomical criteria and adding a few others, it is worth attempting a new description of *Haplocampa*:

Antennae with moniliform antennomeres and short cerci with a few primary articles. Subtrapezoidal head slightly enlarged in the posterior lateral side. Medial anterior (1+1), lateral anterior (1+1) and lateral posterior (1+1) macrosetae on pronotum; medial anterior (1+1), lateral anterior (1+1) and lateral posterior (2+2) on mesonotum; and medial anterior (1+1) and lateral posterior (1+1-2+2) on metanotum. Dorso-femoral macrosetae one. One to three tibial-ventral macrosetae. Tarsus short and enlarged. Unequal claws with large lateral crests and no lateral process; basal portion of both claws with a visible spiny protrusion; posterior claw with a large backward overhang. Medial anterior or medial posterior urotergal macrosetae present; with or without lateral anterior urotergal macrosetae; and, lateral posterior urotergal macrosetae 0, 1 (lp_2) or 2 ($lp_{2,3}$) on urotergites V to VII. Urosternite I with 6+6 macrosetae; urosternites II to VII with 4+4 macrosetae; urosternite VIII with 1+1. Plain stylus with smooth or a few tiny barbs on stylus setae. Male with glandular a_1 and g_1-setae. Female with glandular a_1-setae.

Haplocampa species taxonomical key

1 Lateral posterior macrosetae 2+2 on metanotum; without lateral anterior macrosetae on urotergites; antennae with 24–26 antennomeres .... ***Haplocampa drakei***
– Lateral posterior macrosetae 1+1 on metanotum; with lateral anterior macrosetae 1+1 on urotergites **2**
2 Without posterior macrosetae on first and second urotergites; antennae with 20 antennomeres .... ***Haplocampa chapmani***
– Medial anterior or medial posterior macrosetae 1+1 on first and second urotergites **3**
3 Medial posterior macrosetae 1+1 on first and second urotergites; two tibial ventral macrosetae .... ***Haplocampa* cf. *chapmani***
– Medial anterior macrosetae 1+1 on first and second urotergite **4**
4 Medial anterior macrosetae 1+1 on third to seventh urotergites; two or three tibial ventral macrosetae; antennae with 32 antennomeres .... ***Haplocampa wagnelli* sp. n.**
– Medial anterior macrosetae only on first urotergites; one tibial ventral macrosetae; antennae up to 21 antennomeres **5**
5 Lateral posterior macrosetae 1+1 on third urotergite; antennae with 19–21 antennomeres .... ***Haplocampa wheeleri***
– Without lateral posterior macrosetae on third urotergite; antennae with 21 antennomeres .... ***Haplocampa rugglesi***

Description of type localities (caves)

The caves are all located near the town of Port Alberni, Vancouver Island, British Columbia, Canada (Figs 28–33). The limestone area covers roughly 2400 hectares, and within that area lay over 20 hidden caves, as well as many resurgences, dry sinks, small pits and fissures. Only a short drive and an easy hike are required to reach most caves, providing great opportunities to turn this area into an outstanding caving recreational site if properly managed.

Fossli slot caves

Fossli #1 (L63.5 D25): swallet entrance with active surface stream. First sightings of *H. wagnelli* sp. n. on 5th April 2005.

Fossli #2 (L67.5 D26.5): small slot entrance filled with rotten wood. Upper samples collected 15th July 2018, 30 m from the entrance in a moist bed of sandy organic debris. Lower samples collected 6th August 2018, 60 m from the entrance in a moist bed of sandy organic debris 2 m above sump. *H. wagnelli* sp. n. mud workings visible at both sites.

Kiku Pot cave, (L1489 D92): Breakdown entrance with active stream passage throughout system. *H. wagnelli* sp. n. first sightings August 2017 while surveying. Collection taken 5th August 2018, at bottom waterfall just below where water disappears approximately 150 m from the entrance on a medium. A medium sized bed of sandy organic material with scattered woody debris. *H. wagnelli* sp. n. workings were visible over 75%. Area floods during high water. Many mud workings visible on muddy shelves higher in passage. At the time of collection, an adult fungus gnat was noticed hopping around.

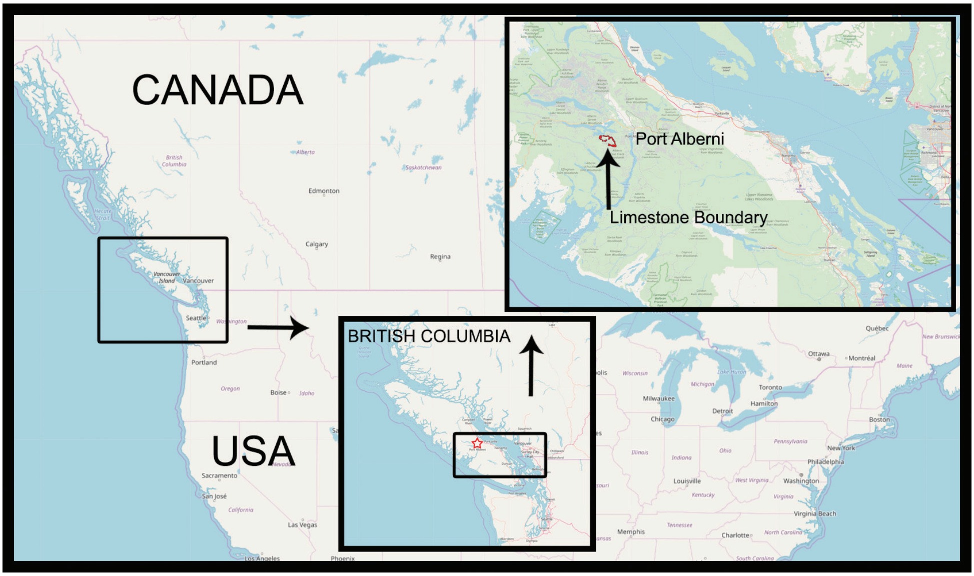
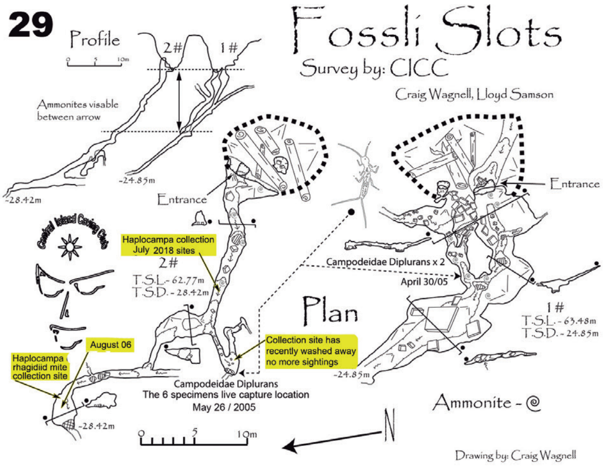
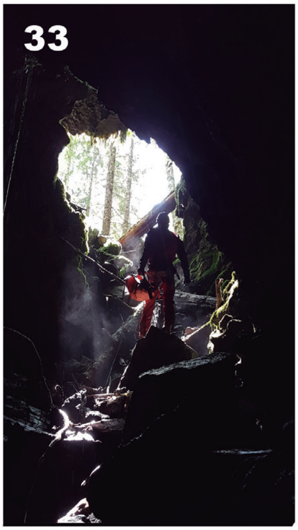
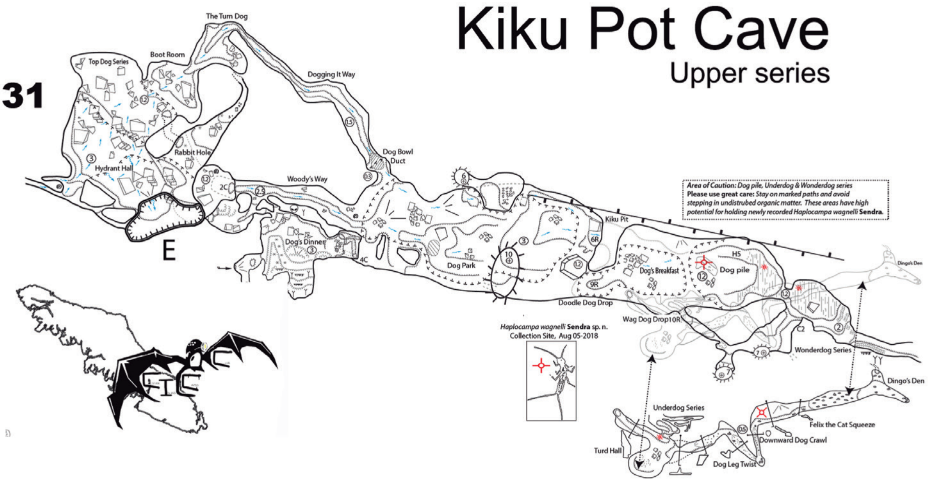


Figure 28. Map of western of North-America, highlighting in red the limestone area near Port Alberni (Vancouver Island, Canada) where Fossli Slots and Kiku Pots caves are located.







Figures 29–33. 29 Map of Fossli Slots caves with spots where *Haplocampa wagnelli* sp. n. was seen **30** entrance of Fossli Slot #2 cave **31** Kiku Pot cave with spots where *Haplocampa wagnelli* sp. n. was seen **32** entrance of Kiku Pot cave with Felix Ossigi-Bonanno and Craig Wagnell after the success finding **33** entrance of Kiku Pot cave viewed from inside the cave.

Cave Convervation Vancouver Island

Vancouver Island is known to have over 1600 caves, with more mapped and explored caves than the rest of Canada combined. Some caves reach over 10 km, and some host unique geological, palaeontological, archaeological and biological features. Vancouver Island caves are mostly active caves, with streams and rivers running through them most of the year. The caves help the streams to maintain constant water temperatures year round and a proper pH, which increases water quality for fish and wildlife. So far, little has been done to protect the caves from poor logging, mining and recreational practices. All caves west of the Port Alberni area are easily accessed by the public and none so far have protection or proper management. Unfortunately, some already have seen misuse. More needs to be done in the future if we want to protect this special resource.

Phylogenetic affinities of the genus *Haplocampa* with notable biospeleological and biogeographical comments

In *Haplocampa* species, their unequal claws have large lateral crests, as do many genera of Plusiocampinae. Applying the right criteria, Paclt (1957) included *Haplocampa* within Campodeinae. In fact, many taxonomical features of *Haplocampa* are held by Campodeinae, such as the distribution and number of pronotum macrosetae, with at most 3+3 medial anterior, lateral anterior and lateral posterior macrosetae. Other notable features of *Haplocampa* are shared with many Campodeinae genera, namely, epicuticle with rosette gland formations; plain frontal process; number and distribution of urotergal macrosetae with medial macrosetae and a maximum of three pairs of lateral posterior macrosetae; simple stylus setae; and number and distribution of urosternal macrosetae, with up to eight pairs of macrosetae on first the urosternite, up to five pairs of macrosetae on the second to seventh urosternites and one pair on the eighth.

In all likelihood, *Pacificampa* Chevrizov, 1978 is the most closely related to *Haplocampa,* given the strong similarities in the number and distribution of the macrosetae body and the absence of lateral pretarsus processes, with one important difference: the lack of lateral crests in *Pacificampa* (Chevrizov 1978; Sendra et al. 2018). The same differential feature is shown by other related genera of *Haplocampa*, in *Metriocampa* Silvestri, 1912 and *Eumesocampa* Silvestri, 1933. All these four genera can be found in the East of Asia and in North America, on both sides of the northern Pacific Ocean, which suggests dispersal events over the Bering Land Bridge. This hypothetical palaeobiogeographical distribution was suggested by Ferguson (1997) for *Eumesocampa, Haplocampa, Pacificampa* and *Plutocampa* Chevrizov, 1978. Another perhaps more parsimonious hypothesis could be that they had an ancient Laurasian distribution and their current distribution reflects a vicariance event after the breacking of the Bering Bridge (Loris Galli pers. suggestion).

Haplocampa and *Pacificampa* share ecological similarities, and phylogenetic, too. Both are present in subterranean ecosystems. In the case of *Pacificampa*, all of the five known species have been found in caves and can be considered troglobites. Three of these species can be found in the north of the Korean Peninsula (Chevrizov 1978; Ferguson 1997) and two were recently described in two southern Japanese Islands (Sendra et al. 2018). In *Haplocampa*, the five described species have been found within the soil at high-altitude localities. However, Ferguson (1981) suggested no less than eight undescribed species of *Haplocampa* from caves located in the Ozarks karstic region at the centre of North America and in the small volcanic and karstic areas of the Pacific coast. Ferguson (1981) remarked that in the case of *Haplocampa*, *Eumesocampa* and *Tricampa* Silvestri, 1933, 'All three have epigean members at high latitudes or high altitudes in the western mountains, and cavernicolous members at lower latitudes and altitudes'. Nevertheless, *Haplocampa wagnelli* sp. n. is an exception to Ferguson's comment (1981).

H. wagnelli sp. n. is a slight troglomorphic species and several features show this. Among these features are the very slight elongation of antennae, 0.5× to 0.7× the body length, with moniliform antennomeres, and the cerci 0.5× to 0.6× the body length. In troglomorphic campodeids, the length of antennae can double that of the body, with always more than 30 antennomeres, and the cerci can be two or three times the body length; there is also an increase in the number of the cercal articles. Furthermore, troglobitic campodeids have elongated legs, reaching the end of the abdomen and a slim body. All of these features are trademarks of highly adapted subterranean campodeid species (Sendra et al. 2017). However, in *H. wagnelli* sp. n., its legs reach the VI to VIII abdominal segment and the body is not slender. But the trogloniont condition of *H. wagnelli* is supported by the presence of 32 antennomeres, five complex, multiperforated, folded-spiral sensilla, and a high number (14–16) of thick gouge sensilla. These are solid evidences for subterranean adaptations. For these reasons, *H. wagnelli* sp. n. can be considered to be a slightly troglobiont species adapted to live in caves and other subterranean spaces, but it could probably also be found in soil habitats. This is the case for the soil-dwelling species *H. drakei*, with 26 antennomeres, found nearby in Banff National Park, Alberta, Canada (Silvestri 1933).

H. wagnelli sp. n. may represent one of the most northerly cave-dwelling adapted dipluran species being found at 49° latitude north. The most northerly cave-dwelling species is *Litocampa hubarti* Bareth, 1999, found in Grotte Lyell (Liège, Belgium) at 50° latitude north, and like *H. wagnelli* sp. n., it shows humble morphological subterranean-adapted features (Bareth 1999). Both species are geographically in the limits of the Last Glacial Maximum. Furthermore, in the case of *H. wagnelli* sp. n., its current distributional area was under the Cordilleran Ice Sheet the Late Wisconsinian North American ice sheet complex, during the Last Glacial Maximum 18 ka BP (Dyke 2004). Several hundred years later the retreat of the glacial ice occurred, and as a consequence, the subterranean habitat colonisation of Vancouver Island was possible.

Acknowledgements

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Supplementary material 1

A video shot by Felix Ossig-Bonanno (2017) from Fossli Slots Caves

Authors: Alberto Sendra, Craig Wagnell

Data type: multimedia

Link: https://doi.org/10.3897/subtbiol.29.31467.suppl1

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First record of subterranean freshwater gastropods (Mollusca, Gastropoda, Cochliopidae) from the cenotes of Yucatán state

Jozef Grego[1], Dorottya Angyal[2,3], Luis Arturo Liévano Beltrán[3]

1 *Horná Mičiná, SK-97401 Banská Bystrica, Slovakia* **2** *Department of Zoology, Hungarian Natural History Museum, H-1083, Baross utca 13, Budapest, Hungary* **3** *Faculty of Sciences, Academic Unit of Yucatán, National Autonomous University of México, Puerto de abrigo S/N, C.P. 97356, Sisal, Yucatán, México*

Corresponding author: *Jozef Grego* (jozef.grego@gmail.com)

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Abstract

The biospeleological investigations of several cenotes in the eastern region of Yucatán state, Mexico, during January 2018 yielded, among other invertebrates, two new truncatelloid gastropod species described herein as *Mexicenotica xochii* **gen. n. et sp. n.** and *Pyrgophorus thompsoni* **sp. n.** Both species represent the first record of stygobiont gastropod species from the cenotes of Yucatán indicating the high biodiversity potential of the studied area.

Keywords

Mexico, cave, phreatic, stygobiont, *Pyrgophorus*

Introduction

The North American stygobiont or phreatic Gastropoda, represented by 15 genera, are mainly known from the caves and springs in the eastern part of United States (23 species) (Herschler and Holsinger 1990; Culver 2012) from Kentucky to Texas and Florida. The stygobiont Mollusca of Mexico are still poorly studied. In Mexico, Bole and Velkovrh (1986) recorded three stygobiont genera (*Coahuilix* O. Taylor, 1966, *Emmericiella* Pilsbry, 1909 and *Pterides* Pilsbry, 1909) with six species, and two stygophile genera (*Potamopyrgus* Stimpson, 1865 = *Pyrgophorus* Ancey, 1888 and *Cochliopina* J. Morrison, 1946) with two gastropod species. A more recent inventory (Czaja et al. 2017) includes five recent genera: *Paludiscala* O. Taylor, 1966; *Coahuilix* O. Taylor, 1966; *Phreatoceras* Hershler & Longley, 1987; *Emmericiella* Pilsbry, 1906 and *Pterides* Pilsbry, 1909 with nine recent and two subfossil Holocene stygobiont species recorded. All the hitherto known Mexican stygobiont gastropod records come from Cuatro Ciénegas basin (Hershler 1985) with caves near, Coahuila Viesca (Czaja et al. 2017) and from San Luis de Potosí (Pilsbry 1939). Despite the enormous volume of the Yucatán cenote aquifer, no systematic study of local stygobiont Mollusca had been done so far, except the anchialine cenote Crustacea, located south of Puerto Morelos, Quintana Roo, Mexico with a record of a gastropod *Teinostoma brankovitsi* Rubio et al., 2016 from family Tornidae Sacco, 1896. Far fewer records are available for the subterranean gastropods of South America, limited to *Andesipyrgus sketi* Hershler & Velkovrh, 1993 from Colombia and Ecuador with *Potamotithus troglobius* Simone & Moracchioli, 1994 and *Spiripockia punctata* Simone, 2012 from Brazil. Most of the New World is still lacking information related to the presence of stygobiont Mollusca.

Material and methods

The studied material was collected during a SCUBA-dive biospeleological field trip during 2–6 January 2018 (Figs 1, 2.) Sediment samples were collected in marked 50 ml BD Falcon tubes from different depths of the cenote. Samples were stored in ice after the dives and were fixed in 70% ethanol in the laboratory in UNAM-UMDI Sisal, where minute invertebrates were selected under a stereomicroscope. Gastropod samples (empty shells) were labelled and dried for further analysis in Hungary and Slovakia. Afterwards, the dry samples were sorted again under an Olympus SZ-11 stereo microscope. Frontal, ventral and lateral view images of the shells were made by a Nikon SMZ25 microscope with Nikon D200 camera and an AF-S Micro NIKKOR 60 mm lens at Vienna Natural History Museum (**NHMW**), Austria.

Abbreviations

HNHM	Hungarian Natural History Museum, Budapest
NHMW	Naturhistorische Museum Wien, Austria
UNAM	National Autonomous University of México, Faculty of Sciences, Academic Unit of Yucatán, Yucatán, Mexico
H	Shell height
W	Shell width
WB	Width of the body whorl
AH	Aperture height
AW	Aperture width

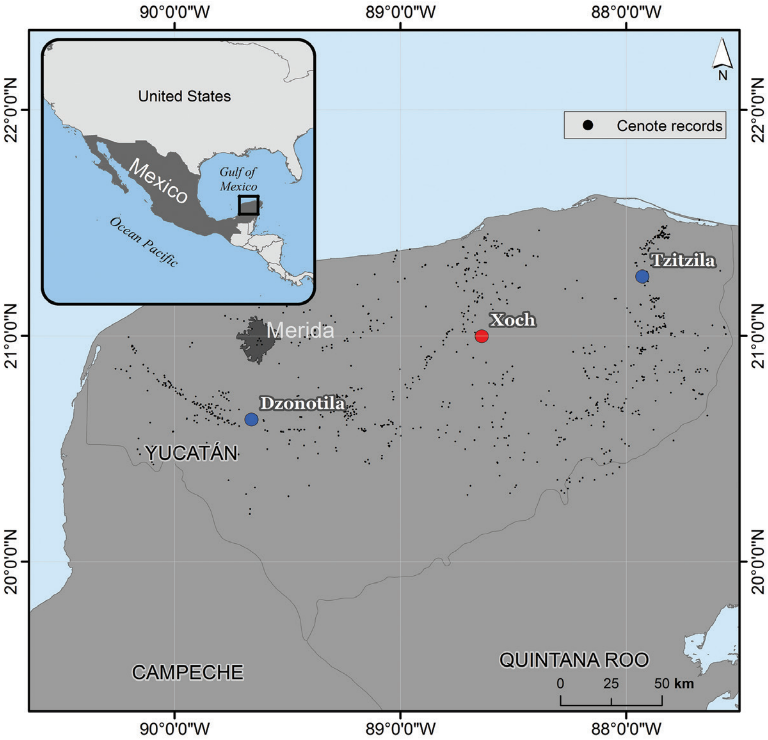


Figure 1. Map of the cenote distribution in the Yucatán state, Mexico with studied localities. The type locality of *Mexicenotica xochii* n. gen. sp. n. and *Pyrgophorus thompsoni* sp. n. in Cenote Xoch marked in red. Blue color indicates cenotes cited in this article as localities for *Pyrgophorus coronatus*.

Figure 2. Images of cenote Xoch, the type locality of *Mexicenotica xochii* n. gen. sp. n. and *Pyrgophorus thompsoni* sp. n.

Results

The scarce shell material obtained by sampling at a depth of 46 m in cenote Xoch near the municipality of Cenotillo contained two subterranean species not resembling hitherto known stygobiont species from Coahuila and San Luis de Potosí and described herein as new to the science.

Superfamily *Truncatelloidea* J. E. Gray, 1840

Family *Cochliopidae* Tryon, 1866

Genus *Mexicenotica* gen. n.

http://zoobank.org/419F28D8-1962-4495-8B5A-670DF596CDA0

Diagnosis. The diagnostic features of the genus are the same as those of the type species *Mexicenotica xochii* sp. n. The minute size with tear shaped shell and expanded oval aperture with a fragile flaring corrugated margin distinguish the new genus from any other related genera of Cochliopidae or Pomatiopsidae.

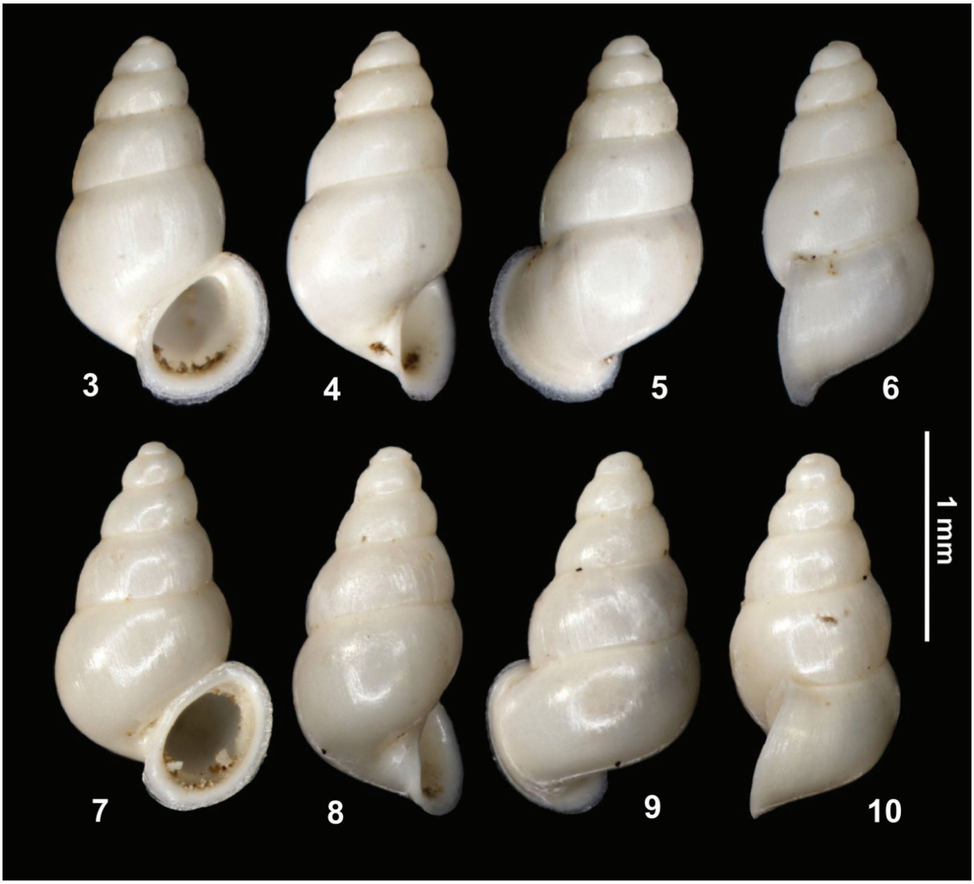
Etymology. Named after the country of origin and cenotes, the specific vertical sinkhole karstic formations of Yucatán´s plateau, whose phreatic waters and aquifers host the new genus.

Mexicenotica xochii sp. n.

http://zoobank.org/909D9B86-182F-4125-B1B7-0263604AE82D
Figures 3–10

Diagnosis. The shell shape of the new species closely resembles subterranean species belonging to family Pomatiopsidae from Laos (*Tricula valenasi* Grego, 2018 and *Tricula spelea* Grego, 2018), and the fine fragile faintly corrugated aperture margin is reminiscent of some cave dwelling specimens of pomatiopsid *Spiripockia punctata* (Simone 2012) from Brazil. However, the geographical distance and absence of pomatiopsid species hitherto known from the Yucatán region rather suggest the resemblance in the shell shape of a cochliopid species. From the geographically close Cochliopidae species, the closest resemblance in the shell shape is found in the Mesoamerican genus *Aroapyrgus* H.B. Baker, 1931 (*Aroapyrgus pasionensis* (Goodrich & Van der Schalie, 1937) from Alta Verapaz, Guatemala), but the new species differs by its more elongated conical shape, with a less prominent body whorl and a proportionally smaller aperture. Furthermore, the aperture of *M. xochii* sp. n. is expanded and finely marginated by a tiny, corrugated structure, while *A. pasionensis* has a rather smooth peristome.

Type locality. Mexico, Yucatán state, Cenotillo Municipality, Cenote Xoch, at 46m deep by SCUBA dive, inside cave sediments, 20,997565°N; 87,936659°W.



Figures 3–10. 3–6: *Mexicenotica xochii* sp. n. 46 m deep from Cenote Xoch, Cenotillo, Yucatán, Mexico (holotype, HNHM 104156) **7–10:** *M. xochii* sp. n. (paratype, coll. JG).

Type material. Holotype, Type locality: leg. Angyal and Liévano, 5 Jan. 2017. (HNHM 104156). Paratypes, same data (coll. Grego 1 specimen); type locality, leg. Angyal and Liévano.

Measurements. Holotype: H 1.84 mm; W 1.05 mm; WB 0.85 mm; AH 0.74, AW 0.56 (holotype). Figs 3–6. Paratype: H 1.82 mm; W 1.08 mm; WB 0.87 mm; AH 0.74, AW 0.56 (holotype). Figs 7–10.

Etymology. Derived from the type locality in Cenote Xoch, Cenotillo municipality, Yucatán state, Mexico.

Description. The snow-white elongate-conical shell with five rounded slightly convex whorls with a weak suture and a blunt apex. The surface smooth and shiny with very faint, almost invisible, transverse growth lines. Aperture elongate oval, ear-shaped, adapically, separated from the body whorl by a weak furrow. The peristome margin expanded, and its reflexed outline bordered by a slightly corrugated, thin and fragile collar. The columellar lip very slightly wavy in its lateral plane, the outer lip straight. Umbilicus closed and obscured.

Habitat. The cenote Xoch is a deep vertical cavern (Fig. 2.) with abundant sediments of plant debris found up to its depth at more than 70m. The cave system has one shallower and a deeper horizontal cave passage at 21m and more than 50m, respectively. The water temperature is 25°C. The organic material deposited in the cenote has two main resources: the epigean decaying dead plants and the belt of indigenous live green algae alongside the cavern walls from the water surface down to 11m depth. The sample with empty shells was taken during dive into 53m depth and at 400m horizontal cave passage. It was taken from a thick organic sediment deposited at 46 m depth. Inside the same sample, specimens of minute bivalvians of cf. *Pisidium* sp. and a few specimens of ostracods were also found. During the research dive, individuals of stygobiont crustaceans, such as *Antromysis cenotensis* Creaser, 1936, *Creaseriella anops* (Creaser, 1936), and *Typhlatya* sp. were also collected at the free water column of the cavern. Trichoptera and Plecoptera larvae, as well as ostracods and water mites were collected in the green algae layer from the cave walls.

Distribution. Only known from the type locality. Within the type locality the new species was found together with the *Pyrgophorus thompsoni* sp. n., and cf. *Pisidium* sp.

Remarks. Due to absence of molecular and anatomical data, the proposed positioning of the new genus in the family Cochliopidae is only provisional, based on the closest resemblance to the geographically closest relatives, but the overall shell shape with flaring margin shows some resemblance also to the Southeast Asian and Brazilian members of the family Pomatiopsidae, which have no close geographical analogue in the region. The new species rather represent by its shape an evolutionary resemblance of a cochliopid species induced by the similar environment to that of the Pomatiopsidae.

Genus *Pyrgophorus* Ancey, 1888

Pyrgophorus thompsoni sp. n.

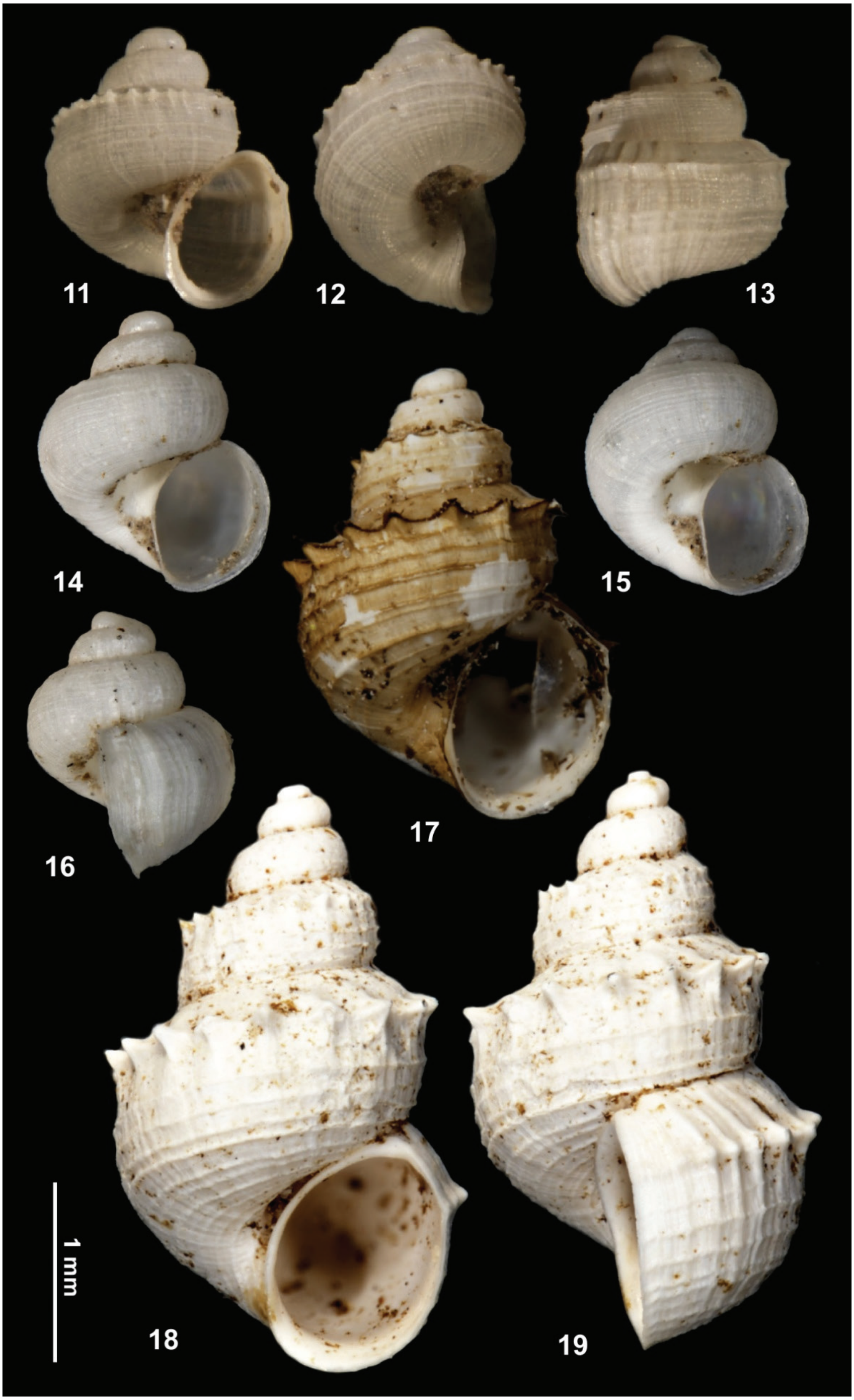
http://zoobank.org/D324A453-1294-48AB-AC2E-6DC359763FF7
Figures 11–16

Diagnosis. Compared to the most closely related *Pyrgophorus coronatus* (L. Pfeiffer, 1840), found in other cenotes and surface waters of Yucatán (Figs 17–19), the new species differs by its smaller and more pyramidal shell shape with blunter apex and finer surface sculpture, whitish periorostracum, flat protoconch and less numerous whorls, more opened umbilicus, smaller and more numerous nodules and different shape of the aperture with a characteristic sinuation at its columellar edge.

Type locality. Mexico, Yucatán state, Cenotillo Municipality, Cenote Xoch, at 46 m deep by SCUBA, buried in cave sediments, 20,997565°N; 87,936659°W.

Type material. Holotype, Type locality: leg. Angyal and Liévano, 5 Jan. 2017. (HNHM 104157). Paratypes, same data (coll. Grego 1 specimen); type locality, leg. Angyal and Liévano.

Measurements. Holotype: H 1.58 mm; W 1.56 mm; WB 1.08 mm; AH 0.90, AW 0.70 (holotype). Figs 11–13. Paratype: H 1.56 mm; W 1.30 mm; WB 1.06 mm; AH 0.84, AW 0.66 (holotype). Figs 14–16.



Figures 11–19. 11–13: *Pyrgophorus thompsoni* sp. n. 46 m deep from Cenote Xoch, Cenotillo, Yucatán, Mexico (holotype HNHM 104157) **14–16:** *P. thompsoni* sp. n. (paratype) **17–19:** *Pyrgophorus coronatus* (L. Pfeiffer, 1840) 8 m deep in Cenote Tzitzila, Dzonot Aké, Yucatán, Mexico (**17**) 27 m deep from Cenote Dzontila, Mucuyché, Yucatán, Mexico (**18–19**).

Etymology. Named after renowned malacologist and good friend Fred Thompson from University of Florida, Gainesville, who contributed much to the knowledge of Cochliopidae of US and Mexico and compiled the first checklist of terrestrial and freshwater gastropods of Mexico and Central America (Thompson 2008).

Description. The milky whitish, translucent conical shell with 3 inflated convex whorls with a deep suture, blunt apex, and flat protoconch. The first teleoconch whorls rapidly expanding, forming a characteristic depressed, sometimes umbilicated appearance. The surface finely lirated, crossed by very fine axial ribs. The spiral lirated sculpture gradually coarsened towards the upper suture of the body whorl, on which the prominent carina-like spiral rib with fine regular conical spines. In some specimens only nodules or only faint knobs present (paratype 1). The body whorl broadened near the aperture. The aperture axially elongated and oval; the columellar peristome reflexed and characteristically sinuated. Umbilicus broad and open.

Habitat. See *Mexicenotica xochii* sp. n.

Distribution. Only known from the type locality. Within the type locality the new species was found together with the *Mexicenotica xochii* sp. n. and cf. *Pisidium* sp.

Discussion

The empty shells of both new taxa were found in organic sediments in the vertical cavern at a depth of 46 m below the water surface. The almost stagnant freshwater at the sampling point would exclude longer transportation of empty shells and accumulation in thanatocoenosis. As the total volume of the phreatic caves under cenote Xoch is huge, additionally the phreatic cave walls surface with bottom floor comprise complicated forms of cave morphology increasing the surface of possible habitats, it is very hard to estimate the exact spot of the true habitat of both minute species within the spread of the cave system. Most likely the new species inhabits the rocky cave surfaces of the cave, feeding on the chemolithotrophic bacterial mats, likely on the blotches with rusty brown, orange or black incrusts layers of oxidation residuals after chemolithotrophic process or on any cave sediments covered by similar layers. We cannot exclude, that the main food could be also exogenous organic plant debris originating on the surface. Nevertheless, to find the site with live specimens of both new species will be rather challenging and more likely depending on luck than knowledge of the habitat.

Conclusions

The first findings of subterranean freshwater gastropod species in the cenote Xoch also indicates the possible larger distribution and diversity of stygobiont gastropods within the remarkable cavernous karstic aquifer of Yucatán. The several hundred kilometers-long cave systems inside the Yucatán carbonate plateau suggest the potential for existence of a very high, so far unexplored, molluscan biodiversity. Due to large cave systems accessible only by SCUBA diving, frequently with complicated logistic of material transport over very long distances, the research of minute cave animals has thus far been nearly impossible. We hope this study will motivate future researches to focus on the study of tiny cave invertebrates inhabiting the freshwater saturated cave labyrinths of the Yucatán Peninsula.

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RESEARCH ARTICLE

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New genus, two new species and new records of subterranean freshwater snails (Caenogastropoda; Cochliopidae and Lithoglyphidae) from Coahuila and Durango, Northern Mexico

Alexander Czaja[1], Gabriel Fernando Cardoza-Martínez[1],
Iris Gabriela Meza-Sánchez[1], José Luis Estrada-Rodríguez[1], Jorge Saenz-Mata[1],
Jorge Luis Becerra-López[1], Ulises Romero-Méndez[1],
Josué Raymundo Estrada-Arellano[1], Miguel Ángel Garza-Martínez[1],
José Antonio Dávila Paulín[2]

1 *Faculty of Biological Sciences, Juárez University of the State of Durango, 35010 Gómez Palacio, Durango, Mexico* **2** *National Commission of Protected Natural Areas (Comisión Nacional de Áreas Naturales Protegidas) CONANP, Área de Protección de los Recursos Naturales Cuenca Alimentadora del Distrino Nacional de Riego 004 Don Martín, Sabinas, Coahuila, Mexico*

Corresponding author: *Gabriel Fernando Cardoza-Martínez* (gfcm2115@gmail.com)

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Abstract

This paper describes a new genus, two new species and new records of subterranean gastropods from the Sabinas and Álamos River, Coahuila, and the Nazas River, Durango, in northern Mexico. *Phreatomascogos gregoi* **gen. n. et sp. n.** from Don Martín Basin, Coahuila, is described based on shells and opercula that show some morphological similarities with shells of *Phreatodrobia* Hershler & Longley, 1986 (Lithoglyphidae), which is a subterranean genus from neighboring area in Texas, United States. Conchologically, the new genus can be distinguished from *Phreatodrobia* and all other subterranean genera by a unique combination of characteristic shell morphology and opercula apomorphies. *Balconorbis sabinasense* **sp. n.** (Cochliopidae) is the second species of this genus, which was previously known only from caves and associated subterranean habitats in Texas. The new record of *Coahuilix parrasense*, Czaja, Estrada-Rodríguez, Romero-Méndez, Ávila-Rodríguez, Meza-Sánchez & Covich, 2017 (Cochliopidae) from Durango and

Coahuila is the first record of extant member of this genus out of its hitherto known habitat in the Cuatro Ciénegas basin, Coahuila. These records are remarkable because *C. parrasense* had been described recently as a fossil species. Shell morphologies of the new subterranean snails could be interpreted as possible evolutional adaptations to different hydrodynamic and other specific conditions in their habitat.

Keywords

Gastropods, phreatic, North America, systematics, interstitial habitat, shell adaptations

Introduction

Compared to worldwide biodiversity hotspots as the Balkans (Sket, 2012), the North American subterranean snail fauna is not very rich in known species, including only 39 species of 17 genera (Hershler and Longley 1990; Czaja et al. 2017a; Grego et al. 2019). Seven of these genera occur in Mexico: *Paludiscala* Taylor, 1966, *Phreatoceras* Hershler & Longley, 1987, *Coahuilix* Taylor, 1966, in Cuatro Ciénegas Valley, Coahuila, *Emmericiella* Pilsbry, 1909, *Pterides* Pilsbry, 1909 in the state of San Luis Potosí, *Pyrgophorus* Ancey, 1888 and the recently described genus *Mexicenotica* Grego, Angyal and Liévano-Beltrán, 2019 in Yucatán. The first two genera and *Mexicenotica* are monotypic, *Coahuilix* and *Emmericiella* have two, *Pterides* has three and *Pyrgophorus* has one extant subterranean species. Almost all of them are stygobiont (obligate subterranean) and only a few forms are stygophiles and they also occur in epigean habitats (surface-dwelling species). Very little is known regarding fossil subterranean snails of North America. From Mexico, so far only five species of the genera *Coahuilix*, *Paludiscala* and *Phreatoceras* have been reported from Coahuila (Czaja et al. 2014a, 2017a).

The aim of the present study is to describe the new subterranean snails from Coahuila and Durango, compare similarities and differences to their known relatives from Cuatro Ciénegas and Texas and to briefly discuss their shell adaptations. Like many of the worldwide subterranean snails, also the present new *Balconorbis* species was described based exclusively on shell morphology, in the case of *Phreatomascogos* gen. n., on shell and operculum morphology. Therefore, especially the family assignation of the new genus is tentative until living specimens will be obtained for anatomical and molecular studies. The present investigations form part of a broader study of fossil and extant land and freshwater molluscs from North Mexico, which began in 2013 and includes research on systematics, ecology and evolution (Czaja et al. 2014a, 2014b; Czaja and Estrada-Rodríguez 2015; Czaja et al. 2015, 2017a, 2017b, 2017c).

Material and methods

The studied subterranean snails were collected during July and November 2018 in nine sites along the Sabinas/Álamos River, Don Martín Basin, Coahuila and in one site on the Nazas River, northeastern Durango, Mexico (Fig. 1, Table 1). Fine sand and gravel

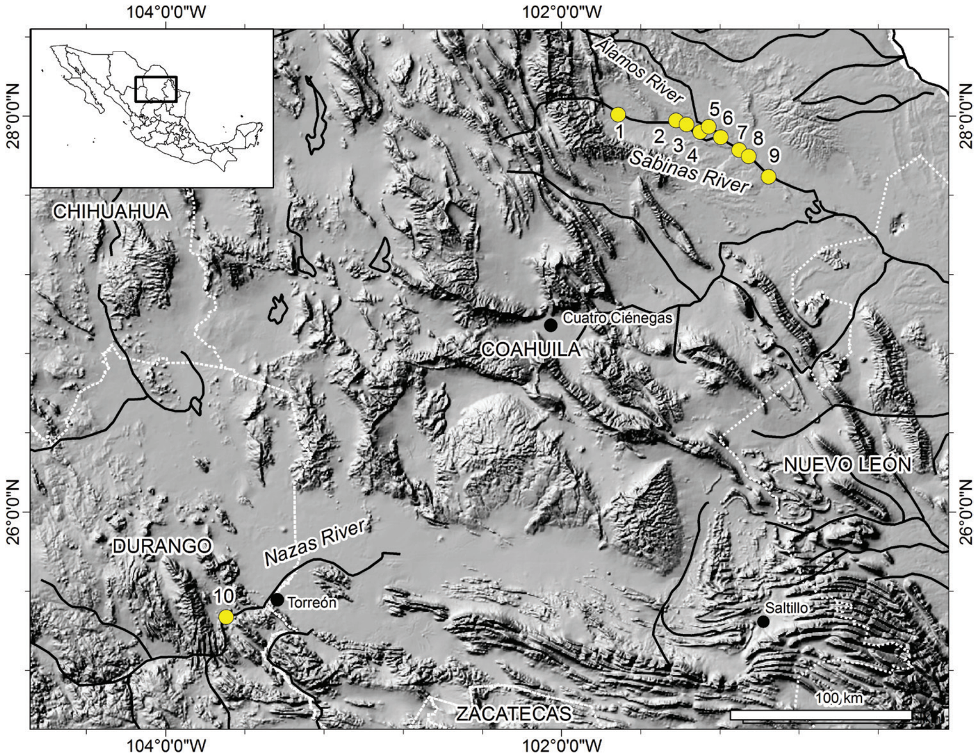


Figure 1. Map of the study area with localization of the sampling sites along the Álamos and Sabinas River in Coahuila and Nazas River in Durango. Sampling sites as in Table 1.

sediment samples of 1.5 kg each were taken from river shores on sites of both localities which were posteriorly screened through two sieves with a mesh size of 0.5 mm and 0.3 mm. Some specimens (especially their apertures) were cleaned of sediments using hydrogen peroxide. For the morphological analysis the shells and opercula were photographed and measured with a Zeiss AxioCamERc 5s camera attached to a Zeiss Stemi 2000-C microscope. Some specimens, especially their protoconchs, were examined in the C.I.D.T. Laboratory of the Peñoles Company in Torreon, Coahuila, using a high performance TESCAN MIRA3 scanning electron microscope (SEM).

We used following shell morphometric dataset (excluding ratios): total number of whorls, shell height, shell width, aperture height, and aperture width. The mean, standard deviation and sample size are given in text (shell measurements). Shell whorls were counted according to the method of Pilsbry (1939).

The studied material is deposited at the Malacological Collection of the Faculty of Biological Science of the Juarez State University of Durango and at the National Collection of Molluscs, National Autonomous University of Mexico (CNMO).

Abbreviations used: **WN**, total number of whorls; **SH**, shell height; **SW**, shell width; **AH**, aperture height; **AW**, aperture width; **HBW**, height of body whorl; **UJMC** = University Juárez Malacological Collection.

Table 1. Sampling localities with geographical coordinates, altitudes and occurrences of *Phreatomascogos gregoi* gen. n. et sp. n., *Balconorbis sabinasense* sp. n. and *Coahuilix parrasense* Czaja, Estrada-Rodríguez, Romero-Méndez, Ávila-Rodríguez, Meza-Sánchez & Covich, 2017 in the study area.

Locality	*P. gregoi*	*C. sabinasense*	*C. parrasense*	Coordinates	
1. Sabinas River, Ejido Nacimiento de los Mascogos II	X			101°42'46''W	28°00'25''N
2. Sabinas River, Ejido Santa María	X	X		101°25'16''W	27°58'37''N
3. Sabinas River, Ejido Sauceda del Naranjo	X			101°22'04''W	27°57'24''N
4. Sabinas River, Ejido San Juan de Sabinas				101°17'54''W	27°55'06''N
5. Álamos River, Ejido Paso del Coyote	X	X		101°15'19''W	27°56'45''N
6. Sabinas River, Las Adjuntas (Rancho San Carlos)	X	X		101°11'41''W	27°53'33''N
7. Sabinas River, Sabinas (Agua Prieta)	X	X		101°06'06''W	27°49'38''N
8. Sabinas River, Las Cazuelas				101°03'14''W	24°47'42''N
9. Sabinas River, La Vega	X	X		100°57'10''W	27°41'35''N
10. Nazas River, Los Cuates (Durango)			X	103°42'13''W	25°27'56''N

Systematics

Superfamily Truncatelloidea Gray, 1840
Family Lithoglyphidae? Tryon, 1866

Genus *Phreatomascogos* Czaja & Estrada-Rodríguez, gen. n.

http://zoobank.org/9A21F18D-6903-4835-B473-35FC7BDA3C1A

Type species: *Phreatomascogos gregoi* sp. n. by present designation.

Diagnosis. Shell small, valvatiform to low trochoid; transparent to whitish; teleoconch with one or two prominent, sometimes almost winged keels; umbilicus almost completely covered (bordered) by a basal keel-like structure; operculum near circular, paucispiral, strongly campanulate.

Differential diagnosis. The characteristic combination of mentioned three shell features (bordered umbilicus, prominent keels and strong campanulate operculum) separate the new genus clearly from shells of all other subterranean genera. Similar in size and general form are only shells of some members of *Phreatodrobia* Hershler and Longley from Texas, a genus which includes exclusively subterranean species. Nevertheless, none species of this genus possess keeled shells with a bordered umbilicus, smooth protoconch and such trochoid elevated opercula. The monotypic, recently erected (based on shell morphology) genus *Novalis* Quiñonero-Salgado & Rolán, 2017 (Hydrobiidae) from Spain resembles in some details the material from Coahuila (Quiñonero-Salgado and Rolán 2017). However, beside of the great geographic disjunction, shells of this European species are not keeled and have a different basal structure which do not cover the umbilicus.

Etymology. The genus name derives from the word *phreatos* (referring to the subterranean habitat) and from *Mascogos*, an afrodescendant ethnic group of Coahuila which escaped the threat of slavery in the United States and lives since 1852 in the Sabinas/Álamos River area.

Phreatomascogos gregoi Czaja & Estrada-Rodríguez, sp. n.

http://zoobank.org/78A129EE-EA6F-4CEF-B44F-204405955CE5
Figures 2–11

Type locality. MEXICO, Coahuila state, Don Martín Basin, Álamos River, Ejido Paso del Coyote (101°15'19"W, 27°56'45"N, 369 m a.s.l.) (Fig. 1).

Types. Holotype (Figs 2, 3), UJMC 400, from type locality, leg. Y. A. Sanchez-Montañez, 11/vii/2018. Paratypes, UJMC 401, CNMO 7900, from same lot, 16 dry shells.

Etymology. Named after Dr. Jozef Grego, a well renowned Slovakian malacologist and specialist of subterranean snails.

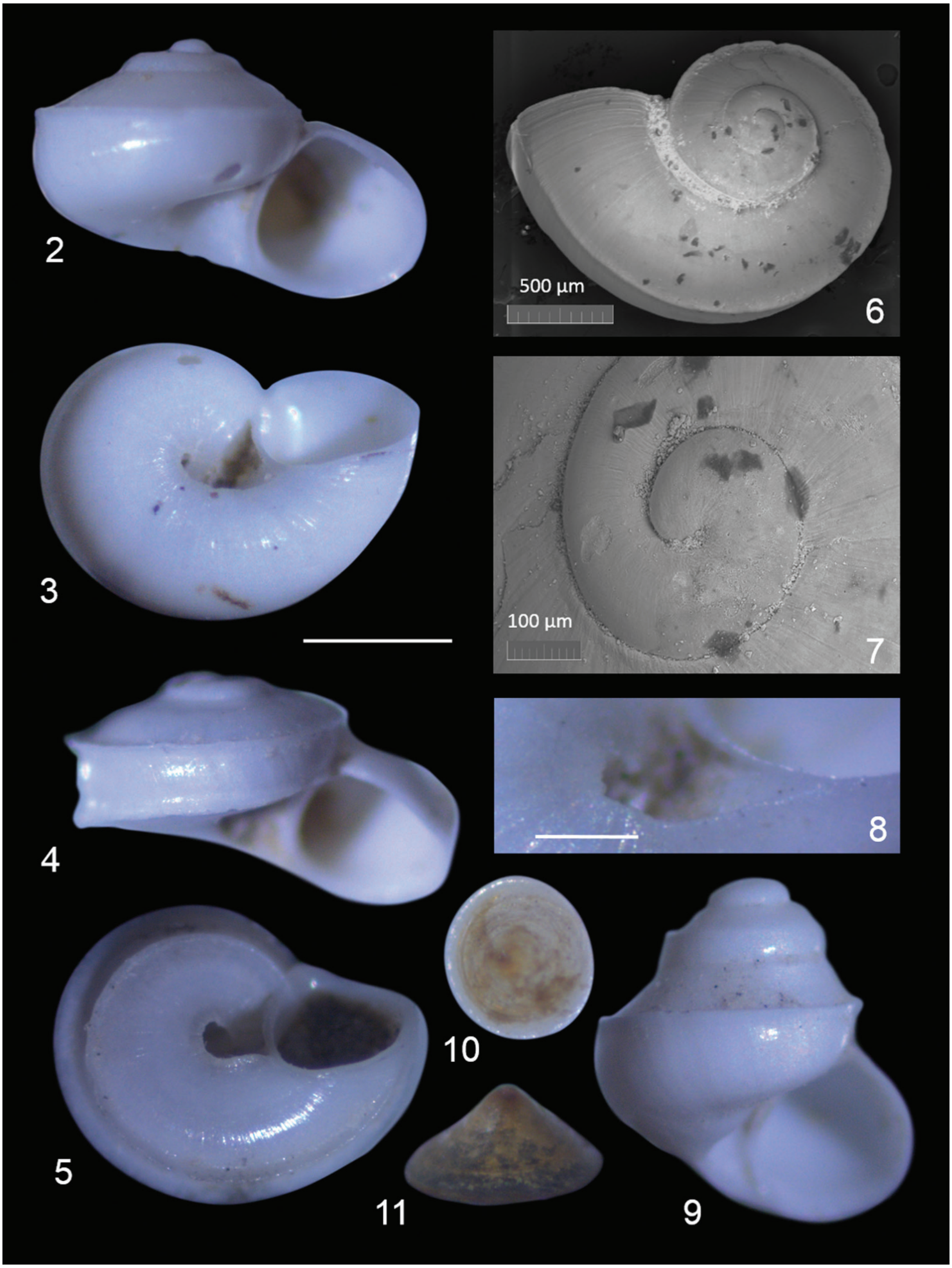
Referred material. COAHUILA. Don Martín Basin, Sabinas/Álamos River, UJMC 402, Ejido Nacimiento de los Mascogos II, Y. A. Sanchez-Montañez, 03/vii/2018. UJMC 403, Ejido Santa María, Y. A. Sanchez-Montañez, 03/vii/2018. UJMC 404, Ejido Sauceda del Naranjo, Y. A. Sanchez-Montañez, 08/vii/2018. UJMC 405, Las Adjuntas (Rancho San Carlos), Y. A. Sanchez-Montañez, 08/vii/2018. UJMC 406, Sabinas (Agua Prieta), Y. A. Sanchez-Montañez, 08/vii/2018. UJMC 407, La Vega, Y. A. Sanchez-Montañez, 08/vii/2018.

Diagnosis. The colorless, translucent to whitish shells are very small, valvatiform to low trochoid, umbilicate, with 3¾ or fewer rounded whorls; aperture near-circular, adnate to the bodywhorl; operculum paucispiral, strongly campanulate; teleoconch with bodywhorl which frequently has one or two, in some specimens winged, keels; umbilicus almost completely covered by a basal keel-like structure.

Description. Shell small, valvatiform, varying in shape from (mostly) flat-trochoid to (rarely) low conical, height 0.65–0.99 mm (1 trochoid shell of 1.42 mm considerably higher, Fig. 9), width 1.22–1.54 mm; umbilicus almost completely covered by a basal keel of the bodywhorl (Fig. 8); protoconch smooth (Fig. 7); teleoconch with 3¼ or fewer rounded whorls with less prominent axial growth lines (Fig. 6), the border between protoconch and teleoconch approximately after 1.5 whorls, not well distinct (Fig. 7); teleoconch whorls occasionally nearly smooth, frequently with one or two, in some specimens winged, spiral keels (carinae) on the last two whorls, keels on the central and basal part of the bodywhorl, central keel usually more prominent (Figs 2, 4, 5), whorls strongly convex with deep sutures; the aperture is ovate and in almost all specimens angled adapically (Figs 2, 4), inclined 20–30° to the coiling axis; peristome thin, inner lip partly fused to the penultimate whorl; operculum (from five shells obtained by shell cleaning) extremely thin, 0.48–0.51 mm in diameter, near-circular, campanulate (Fig. 11), placed deeply behind the aperture, strongly, light amber colored, paucispiral with sub-central nucleus, nuclear region darker, with 2.5 whorls (Fig. 10).

Shell measurements (mean ± standard deviation in parentheses; $n = 16$): SH 0.84 (0.09) mm, SW 1.36 (0.08) mm, AH 0.57 (0.07) mm, AW 0.55 (0.06) mm, WN 3.05 (0.19) whorls; HBW 0.72 (0.10) mm. Paratypes from the type locality.

Measurements of Holotype. SH 0.86 mm; SW 1.38 mm; AH 0.52 mm; AW 0.56 mm, WN 3.00 whorls; HBW 0.73 mm.



Figures 2–11. Shells and opercula of *Phreatomascogos gregoi* gen. n. et sp. n. **2, 3** holotype, UJMC 400 **4, 5** paratype 1, UJMC 401 **6, 7** paratype 2, UJMC 401a, shell apex with protoconch **8** umbilicus almost completely covered by a basal keel, UJMC 401b **9** paratype 3, UJMC 401d, conical specimen **10, 11** opercula **11** operculum showing the strongly campanulate shape. Scale bars: 0.5 mm (**2–5**, **9–11**); 0.3 mm (**8**).

Habitat. Stygobiotic. Shells of *Phreatomascogos gregoi* n. sp. were collected on the shore of the river in sandy and clayed sediments with many gravels on the bottom. We suppose, the new species likely inhabits the interstitial waters within the water saturated underground gravel layer of the hyporheic zone (see below). Although the habitat of *Phreatomascogos* lays within one of the eight federal protected zones APRN (=Mexican Protected Natural Resource Areas), their habitat may be threatened (especially the sites Ejido Sauceda del Naranjo and Ejido San Juan de Sabinas) by local coal mining and agriculture.

Distribution. *Phreatomascogos gregoi* sp. n. lives likely sympatrically with *B. sabinasensis* sp. n. (see below) in same subterranean, interstitial habitat. The species appears to be endemic to the Sabinas/Álamos River, Don Martín Basin, between the upper basin and *Venustiano Carranza* dam.

Remarks. The typical strong keels, the almost completely covered umbilicus structure and the strongly campanulate operculum are the most evident characteristics which differentiated the shell morphology of *Phreatomascogos gregoi* sp. n. from the shells of all other described subterranean gastropods. A North American subterranean species conchologically most closely related to our material is *Phreatodrobia nugax* Hershler & Longley, 1986 from South-Central Texas. This species shows similarities in size and general shell shape and in some details of the operculum structure (low conical shape). Nevertheless, they clearly differ by lack of the three mentioned shell features of *Phreatomascogos gregoi* sp. n. We considered especially the extreme campanulate operculum and the covered umbilicus of *Phreatomascogos gregoi* sp. n. as important apomorphic features that justify the erection of a new genus most likely within the Lithoglyphinae family. The mentioned conchological similarities between both *Phreatomascogos* from Coahuila and *Phreatodrobia* from Texas is the main reason of assigning the new genus to the family Lithoglyphinae (and not to the Cochliopidae). However, the resemblance in the shell shape could be also result of an evolutional convergence. There should be also considered the below mentioned biogeographic considerations which indicate a phyletic affinity of both genera gathering within the same family. The Edwards-Trinity-Aquifer region, that hosts all eight *Phreatodrobia* species, is a transboundary aquifer Zone which shares the water resources of the states of Texas and Coahuila. The Sabinas River karst region, where *Phreatomascogos gregoi* sp. n. occurs, belong to this zone and is hydrogeologically the southwestern extension of this huge aquifer (Boghici 2004; Sanchez et al. 2018). Biogeographically, both regions in Texas and Coahuila form a unit (province) where many subterranean forms radiated from a common epigean (?) ancestor (see also Hershler and Holsinger 1990). However, anatomic and molecular genetic data are needed to prove the hypothetical close relationship between both Lithoglyphidae genera.

Family Cochliopidae Tryon, 1866

Genus *Balconorbis* Hershler & Longley, 1986

Type species. *Balconorbis uvaldensis* Hershler & Longley, 1986

Balconorbis sabinasense Czaja, Cardoza-Martínez & Estrada-Rodríguez, sp. n.

http://zoobank.org/BAEA864F-38F1-4251-854E-D6AAD57D1B86
Figures 12–18

Type locality. MEXICO, Coahuila state, Don Martín Basin, Sabinas River, Las Adjuntas (Rancho San Carlos): 101°11'41"W, 27°53'33"N, 354 m a.s.l. (Fig. 1).

Type material. Holotype (Figs 12–14), UJMC 410, from type locality, leg. Cecilio Arreola Chapa, 30/xi/2018. Paratypes, UJMC 411, CNMO 7901, from the same lot, 11 specimens.

Etymology. Derived from the name of Sabinas River, Coahuila, where the type locality is situated.

Referred material. COAHUILA. Don Martín Basin, Sabinas River, UJMC 412, Ejido Santa María, Y. A. Sanchez-Montañez, 03/vii/2018. UJMC 413, Ejido Paso del Coyote, Cecilio Arreola Chapa, 30/xi/2018. UJMC 414, Sabinas (Agua Prieta), Y. A. Sanchez-Montañez, 08/vii/2018. UJMC 415, La Vega, Y. A. Sanchez-Montañez, 08/vii/2018.

Diagnosis. Shell minute, less than 2 mm in diameter (width), planispiral, apertural plane not or only slightly inclined, with 3¾ or less whorls, bodywhorl keeled, with spiral lines and axial growth lines, protoconch smooth.

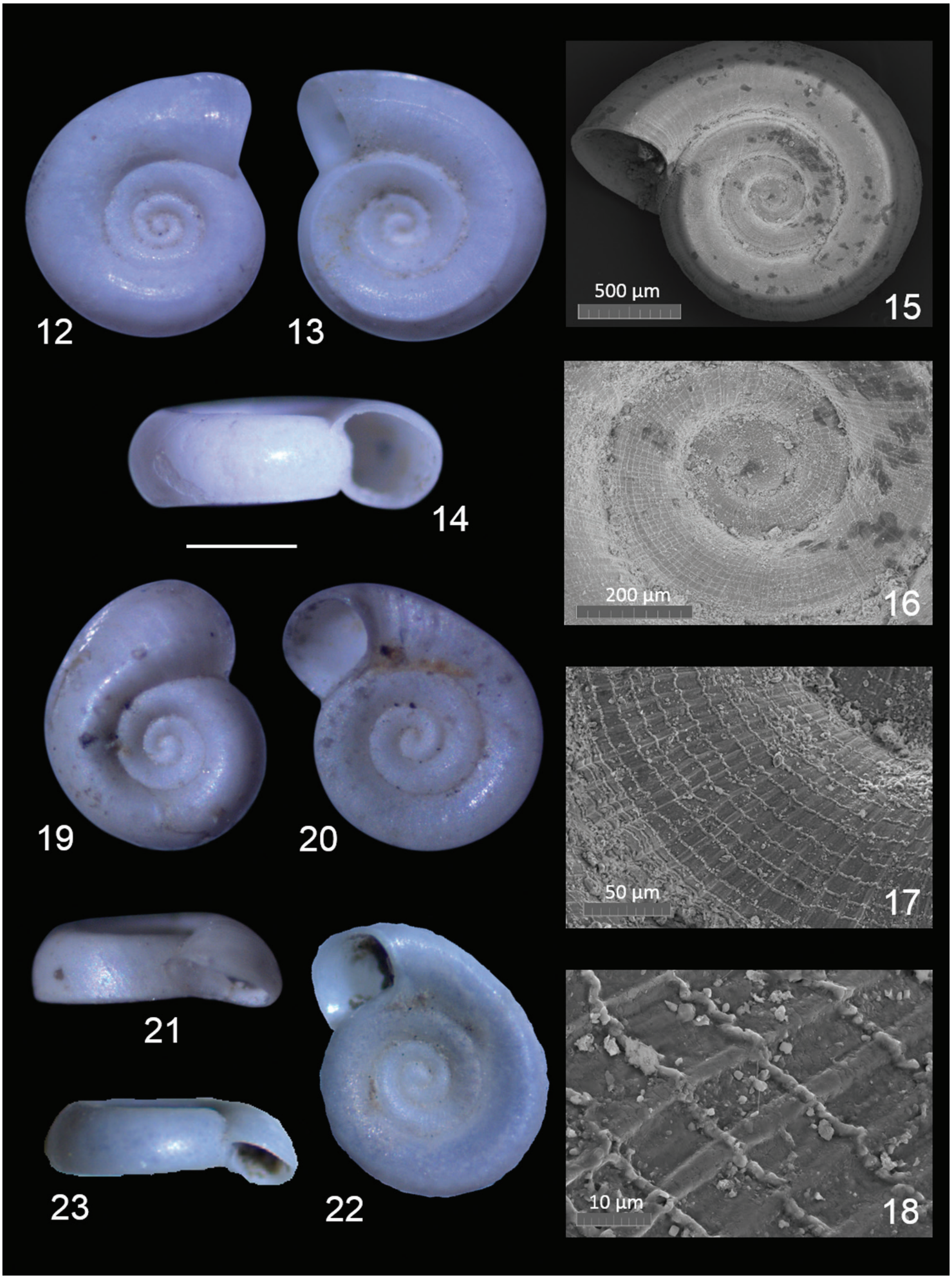
Differential diagnosis. According to Hershler and Longley (1986), the characteristic spiral structure (Figs 16–18) of the shells distinguish the genus *Balconorbis* clearly from other subterranean littoridinines. Shells of the new species differs from *B. uvaldensis* by larger shells (1.51–1.81 mm by *B. sabinasense* sp. n. versus 0.90–1.22 mm by *B. uvaldensis*) with weaker suture, more whorls, smooth to slightly pitted protoconch without spiral lines, and characteristic keels on the bodywhorl. Similar planispiral shells of the genus *Phreatodrobia* (*P. micra* Hershler & Longley, 1986, *P. plana* Hershler & Longley, 1986 and *P. rotunda* Hershler & Longley, 1986) are considerable smaller, lack the keels on the bodywhorl and do not have the characteristic regularly-spaced spiral lines and axial growth lines.

Description. Shell minute, planispiral, width 1.51–1.81 mm, height 0.42–0.61 mm, with 3¼–3¾ whorls; protoconch smooth to slightly pitted (Fig. 16), hidden in ventral view, has 1¼ whorls, first teleoconch whorl with strong and elevated axial growth lines which cross the spiral lines producing a square pattern (Figs 17, 18), about 80 elevated spiral lines are present on the bodywhorl, bodywhorl with one or two keels, one keel usually stronger; aperture rounded to ovate, apertural plane only slightly inclined from the columellar axis (Fig. 13); inner peristome is fused to the penultimate whorl, outer lip strong and advanced relative to the remaining peristome; shells transparent or whitish.

Shell measurements (mean ± standard deviation in parentheses; *n* = 11): SH 0.53 (0.07) mm, SW 1.68 (0.10) mm, AH 0.59 (0.05) mm, AW 0.49 (0.08) mm, WN 3.5 (0.19), aperture plane inclination relative to shell axis = 22°–31°. Paratypes from the type locality.

Measurements of holotype. SH 0.55 mm, SW 1.58 mm, AH 0.59 mm, AW 0.58 mm, HBW 0.50 mm, WN 3½, aperture plane inclination relative to shell axis = 25°.

Habitat. Similar to *Phreatomascogos gregoi* sp. n.



Figures 12–23. Shells of *Balconorbis sabinasense* sp. n. and *Coahuilix parrasense* Czaja, Estrada-Rodríguez, Romero-Méndez, Ávila-Rodríguez, Meza-Sánchez & Covich, 2017. **12–14** *B. sabinasense* n. sp., holotype, UJMC 410. **15–18** Paratype 1, UJMC 411. **19–21** *C. parrasense*, UJMC 418, from Nazas River, Durango. **22, 23** *C. parrasense*, fossil specimen from Parras de la Fuente, Coahuila, holotype, UJMC-320, from Czaja et al. 2017c, Fig. 4A, C. Scale bar: 0.5 mm (**12–14**, **19–23**).

Distribution. Known from the type locality and various sites among the Sabinas River between the localities Ejido Santa María and La Vega. Within the type locality and most other sites the new species occurs sympatrically to *Phreatomascogos gregoi* sp. n.

Remarks. Almost all the members of Cochliopidae family with planispiral shells are subterranean forms. Freshwater gastropods with similar shell characteristics can be found only in the genus *Balconorbis* which is monotypic with *B. uvaldensis* in the Edwards (Balcones Fault Zone) Aquifer in Uvalde County, Texas, United State. Our new species resembles *B. uvaldensis* but differs from this by having larger shells, smooth protoconch, one more whorl and a bodywhorl with one or two more or less strong keels and by much closer, compressed set of the whorls resulting in weaker suture and larger columellar peristome. The later feature is unique in the genus *Balconorbis* and could indicate the possible position of the new species in a new geographically isolated genus. These conchological differences confirm that the new species is morphologically diagnosable and distinct to shells of the snails from Texas. It is the first record of the genus *Balconorbis* in Mexico.

***Coahuilix* cf. *parrasense* Czaja, Estrada-Rodríguez, Romero-Méndez, Ávila-Rodríguez, Meza-Sánchez & Covich, 2017**

Figures 19–21

Material. COAHUILA, Don Martín Basin, Sabinas River, Las Adjuntas (Rancho San Carlos), UJMC 416, 417: 101°11'41"W, 27°53'33"N, 354 m a.s.l., 2 shells. DURANGO, Leon Guzmán District, Nazas River, 1 km E of the Bridge *Los Cuates,* UJMC 418, 103°42'13"W, 25°27'56"N, 1.158 m a.s.l., 1 shell (Figs 19–21).

Description. See Czaja et al. 2017a: 230.

Shell measurements. Shell 1 from Coahuila (UJMC 416): SH 0.39 mm, SW 1.44 mm, AH 0.52 mm, AW 0.36 mm, aperture plane inclination relative to shell axis = 58°. Shell 2 from Coahuila (UJMC 417): SH 0.41 mm, SW 1.43 mm, AH 0.46 mm, AW 0.38 mm, aperture plane inclination relative to shell axis = 63°. Shell from Durango (UJMC 418): SH 0.31 mm, SW 1.21 mm, AH 0.45 mm, AW 0.32 mm, aperture plane inclination relative to shell axis = 64°.

Remarks. The three empty shells from Coahuila and Durango resembles in all details the shells of *C. parrasense*, a sub-fossil species described recently from a dried-up stream (arroyo) near the town Molino, Parras de la Fuente, Coahuila by Czaja et al. (2017a) (Figs 22, 23). The only difference is the lack of the tooth-like bulges behind the peristome by the present shells so they can be placed only tentatively to this species. The strong inclination of the apertural plane in relation to the axis which reaches almost 60° (Figs 21, 23), is the main difference to the extant *C. landyei* Hershler, 1985 from Cuatro Ciénegas. *Coahuilix parrasense* seems to occur in Sabinas River sympatrically with *B. sabinasense* sp. n.

Discussion

Shell convergences

Because of convergences, the description of hydrobioid snails based only on shell characters can be misleading (see discussion in Hershler and Longley 1986 and Falniowski 2018). However, worldwide most of the extant subterranean snails were (and are) described only from empty shells because in many cases living populations were not accessible or available (Georgiev 2013; Grego et al. 2017; Quiñonero-Salgado and Rolán 2017; Hofman et al. 2018). According to Glöer et al. (2015), even empty shells of many phreatic forms are only occasionally washed out to the surface. Like many others subterranean species, also the genera *Coahuilix*, *Phreatoceras* and *Paludiscala* from Coahuila were first described based on empty shells (Taylor 1966) and it often took more than two decades and intensive searches before living specimens could be found and anatomically described (Hershler 1985). In these three mentioned cases and in many others recent descriptions (e.g., Georgiev and Glöer 2015; Glöer et al. 2015; Grego et al. 2017, Grego 2018; Quiñonero-Salgado and Rolán 2017), shell characteristics (including shell wall structure) were sufficient distinguishable for the generic differentiation, underscoring the usefulness of shells for taxonomic purposes. In the case of the described new genus and species from Coahuila, it was certainly helpful that related forms are still living near the area and their shells (and opercula) could be used for comparative diagnosis.

Shell morphology

Grego et al. (2017) emphasizes that subterranean truncatelloids are often referred as cave-dwellers but they are not restricted only to caves and caves-waters. Some of them occur also in the groundwater saturated zone such as interstitial spaces within the coarser sand and gravel sediments in the hyporheic zone of water streams. Our shells come from such river sediments and we are strongly convinced that *P. gregoi* sp. n., *B. sabinasensis* sp. n., and *C. parrasense* live in such interstitial waters. There are no indications for long transport on the shells and the very thin shells (and opercula) are almost all undamaged. The interstitial and cave habitats are cross-connected via the gravel of the alluvial sediments, and it is also likely the interstitial gastropod species could inhabit cave habitats and many of the recognized cave populations could communicate via existing interstitial habitats. However, some species have already adapted their shell morphology to cave habitats and thus have lost their capability to communicate through the interstitial channels.

These conditions of subterranean (interstitial) habitat might also explains the special morphologic adaptations of the shell. According to the hydrodynamic model of

Grego et al. (2017 and pers. com.), flat shells with strongly inclined apertures could be attached flat to the surface and reduce considerably the frontal hydrodynamic resistance area and could be therefore favored by selection (better resistance to stronger and turbulent water flow). The same applies also to the animal mobility within the (interstitial) sediment cavities, where a more inclined shell aperture position could be advantageous by creating lower resistance in the crevices during the moving. The shape also indicates the habitat in larger interstitial cavities (with cavities several fold larger than the shell dimeter) where the stronger water stream could appear at least periodically. Such extremely inclined aperture shows the stygobiont *C. parrasense* from Sabinas and Nazas River (Figs 19–21), while *C. landyei* and *C. hubbsi* Taylor, 1966 that live in the springs, lack such feature.

Also, the singular covered umbilicus and the general variable form from near planispiral to trochoid of *Phreatomascogos gregoi* sp. n. shells might be explained by the same hydrodynamic model of Grego et al. (2017 and pers. com.). A small, or better, a covered umbilicus surely avoids more efficiently the strong buoyancy forces which act on the shell redirecting the water flow. On the other hand, a large and open umbilicus can be disadvantageous because it regularly traps sand grains and sediment particles and thus makes so the snail locomotion difficult or even impossible. Our material also points to a positive correlation between the general shell form of *P. gregoi* sp. n. and (interstitial) water velocity, according to the mentioned hydrodynamic model. But whether the less conical, valvatiform shells (Figs 2, 4) could occur in sites with relatively higher velocities (and large interstitial cavities and larger gravel granularity) of interstitial water and the more trochoid shells (Fig. 9) in areas of lower flow rates (with smaller interstitial cavities and smaller gravel granosity) will be difficult to prove due to the cryptic subterranean environment conditions. However, the hypothesis must be statistically substantiated and further investigations with systematic collections in river sites of different flow rates and more specimens for statistical analysis could prove (or disprove) this hydrodynamic model for interstitial environments.

Conclusion

The above described new genus and species of subterranean snails from Coahuila and the recently reported findings from Yucatán (Grego et al. 2019) demonstrate that there is a great potential for discovering more stygobiont species in these large unexplored karst regions in Mexico. We hope that our efforts will also provide motivation for other researchers to investigate the diversity of phreatic gastropods in Mexico.

Acknowledgments

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Jozef Grego[1], Dorottya Angyal[2], Luis Arturo Liévano Beltrán[3]

[1] Horná Mičiná, SK-97401 Banská Bystrica, Slovakia **2** *Department of Zoology, Hungarian Natural History Museum, H-1083, Baross utca 13, Budapest, Hungary* **3** *Faculty of Sciences, Academic Unit of Yucatán, National Autonomous University of México, Puerto de Abrigo S/N, C.P. 97356, Sisal, Yucatán, México*

Corresponding author: *Jozef Grego* (jozef.grego@gmail.com)

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In our paper about stygobiont gastropod species from Yucatán State Mexico (Grego et al. 2019) we reported two new species provisionally placed in the family Cochliopidae: *Mexicenotica xochii* Grego, Angyal & Beltrán, 2019 and *Pyrgophorus thompsoni* Grego, Angyal & Beltrán, 2019. However, shortly after its publication, we realised the name *Pyrgophorus thompsoni* had been already used by Wesselingh 2006 † to name a fossil Miocene species from Indiana, Loreto, Peru (*Pyrgophorus thompsoni* Wesselingh, 2006† (Wesselingh 2006: 124–125, figs 156–159)). Therefore our previously published name is invalid and cannot be further used. To remove the homonymy we propose the new name *Pyrgophorus cenoticus* Grego, Angyal and Beltrán, 2019 to substitute the name *Pyrgophorus thompsoni* Grego, Angyal & Bertrán, 2019.

Etymology: Named after the cenotes of Yucatán, the main habitat of the new species"

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