

The deep subterranean environment as a potential model system in ecological, biogeographical and evolutionary research

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Abstract

One of the main challenges in ecology, biogeography and evolution is to understand and predict how species may respond to environmental changes. Here we focus on the deep subterranean environment, a system that minimizes most of the typical uncertainties of studies on epigeal (surface) environments. Caves are relatively homogeneous habitats with nearly constant environmental conditions and simplified biological communities, allowing to control for biotic interactions. Thus, this particular system could be considered a natural habitat whose environmental conditions are similar to what can be reproduced in a laboratory, being an ideal model system for ecological, biogeographical and evolutionary studies. Subterranean species may potentially be used to assess the capability to persist in situ in a global change scenario, as they cannot accommodate to drastic changing conditions by behavioural plasticity, microhabitat use or by migrating to distant, more suitable areas, something frequent in epigeal environments. In order to provide accurate predictions of the response of the subterranean biodiversity to climate change, we encourage evolutionary biologist, biogeographers and conservation biologist to work in this interesting ecosystem.

Keywords

caves, climate change, subterranean biodiversity, thermal tolerance, persistence capability

One of the main challenges in ecology, biogeography and evolution is to understand and predict how species may respond to environmental alterations, especially in the context of global change. If we aim to develop effective management strategies, accurate predictions of species response are mandatory. These predictions will be more accurate as we can obtain more reliable estimates of species dispersal ability, biotic interactions and species fundamental niche (and its geographical projection, understood as potential distribution; see Soberón et al. 2005).

Species fundamental niches can be defined as the multidimensional spaces of scenopoetic variables, typically measured at coarse spatial resolutions and over broad geographic extents (Peterson et al. 2011), and they are commonly inferred exclusively from the current climatic conditions of the localities in which the species are known to occur. In practical terms, this means that the simple presence of a species in a cell grid of a certain dimension is related to some average characteristics of this cell grid.

It is widely recognized that there are many sources of uncertainty (both conceptual and methodological) when relating species ecological niche to the environmental conditions of their distributions (Jiménez-Valverde et al. 2008). Thus, it is assumed that the variables affecting species performance and distribution are known, that species are found in their optimal climatic niches, and what is more important, that these environmental conditions are homogeneous through the spatial units used (usually grid cells), ignoring both temporal (daily and often even seasonal) and spatial (micro-habitat) heterogeneity (Hannah et al. 2014, Klečková et al. 2014, Rezende et al. 2014). Consequently, both behavioural and phenological accommodation to different environmental conditions (Parmesan 2006, Sunday et al. 2014) are frequently ignored, assuming that organisms have no control over the conditions to which they are exposed (see Charman-tier et al. 2008, Wong and Candolin 2015). Lastly, it is assumed that species occur at all locations where environmental conditions are favourable, likely overestimating dispersal capabilities and underestimating the influence of biotic interactions (Guisan and Thuiller 2005, Araújo and Luoto 2007, Jiménez-Valverde et al. 2008, Wiens et al. 2009).

Most of the research to date has been based on distributional data of vertebrate species (using grid cells at different spatial resolutions) from terrestrial ecosystems. However, all these assumptions should be questioned when we consider the great variety of environments that can co-occur in a spatial unit of typical dimensions (e.g. cells of 10×10 km), the importance of extreme or unusual rather than average conditions (Schoepf et al. 2015), the possibility to be exposed to different environmental conditions simply through behavioural adaptations and adjustments in microhabitat use (Visser and Both 2005), or the possibility of competitive exclusion of a species from an environmentally suitable area.

We would like here to bring attention to a system in which most of these uncertainties are minimised: the deep subterranean environment. Contrary to what happens

in epigeal (surface) environments, the range of variables affecting a species in this environment is very limited. The humidity in the deep parts of a cave is always near the saturation point and the temperature is relatively constant through the day and year, and what is more interesting, it can be easily (though approximately) estimated from the mean annual temperature of the surface (Jeannel 1926, Poulson and White 1969, Juberthie and Decu 1994, Culver and Pipan 2009). To obtain a numerical estimation of this relationship, even if very crude, we compiled records of temperature inside 59 caves (28 from the North-eastern Iberian Peninsula, Sánchez-Fernández et al. 2016, and 31 from the western Alps, Mammola et al. 2017) and compared these values with those obtained from raster with the Mean Annual Temperature of the surface at 0.08 degree spatial resolution cells from WORLDCLIM version 1.3 (<http://www.worldclim.org>; Hijmans et al. 2005). We found that the temperature of the cave can be estimated with an average error of 1.90 °C using as only predictor the Mean Annual Temperature of the surface ($r = 0.79$, $n = 59$; $p < 0.01$; see Figure 1).

Compared with epigeal habitats, most of the environmental conditions are also virtually homogeneous through all possible microhabitats within the deepest parts of a cave system, so small-scale spatial heterogeneity and the possibility of behavioural adjustments, phenotypic plasticity or adaptive evolution are limited. Mammola and Isaia (2016) studied the environmental niche of a subterranean spider (*Troglohyphantes vignai* Brignoli, 1971) during a year, concluding that although some minimal spatial climatic variation was detected, neither temporal nor spatial variation of the niche of this species was found through the year. Finally, caves harbour comparatively simple biological communities (Racovitza 1907, Culver and Pipan 2009, Cardoso 2012), which minimizes the additional complexity of biological interactions. Most highly specialized cave species have also a well-defined distribution, as they show low mobility and extremely narrow geographical ranges, which minimizes sampling uncertainties. In summary, and unlike in surface environments, here the real and accurate environmental conditions that species experience are known.

However, there are not only advantages in this study system. Subterranean species violate a key assumption especially relevant for biogeographical research: compared with epigeal species, they show low dispersal abilities (Rizzo et al. 2013, 2017). Thus, they cannot be expected to occupy most of their suitable habitat, which means that they are then not in equilibrium with climatic conditions (see Svenning and Skov 2005, Sánchez-Fernández et al. 2012). In other words, in addition to climate, other factors (such as biotic interactions or limited dispersal) are important shaping their distributions. This situation could compromise biogeographical studies, especially those that are exclusively supported by species distribution models used for epigeal fauna (but see Mammola et al. 2017, Mammola and Leroy in press). However, in some situations this drawback could be seen as an opportunity, as i) their low mobility could also favour more accurate estimates of the climatic conditions that species experience, allowing to include past climates to estimate species climatic niches, as current records can in most cases be considered to reflect ancient distributions (Sánchez-Fernández et al. 2016), and ii) as for most of these species dispersal to more suitable locations is not an option, the only pos-

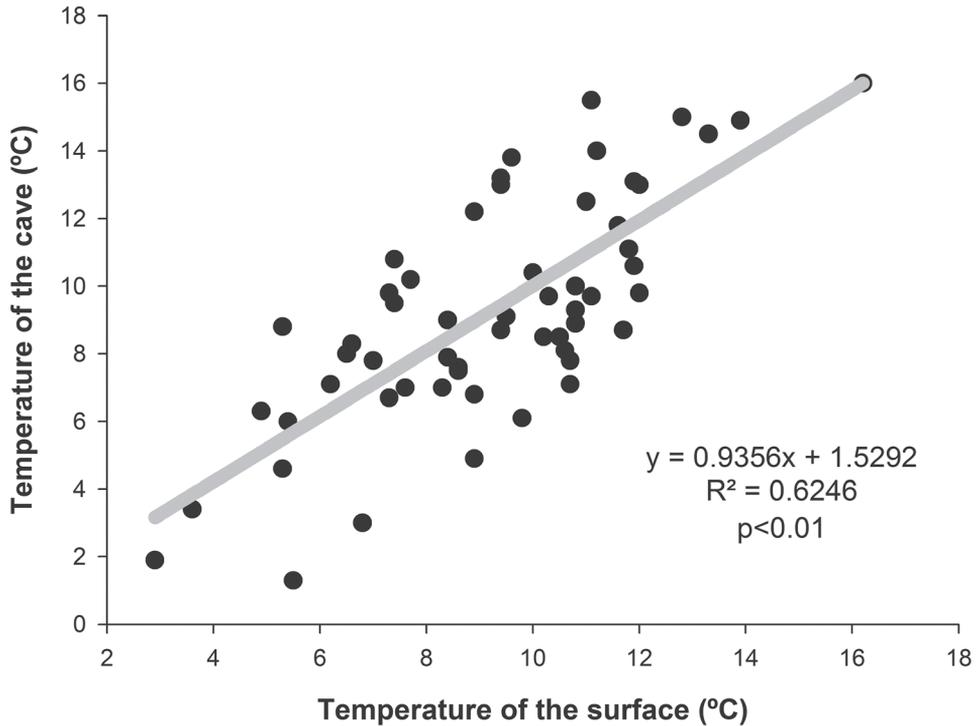


Figure 1. Relationship between the temperature inside the cave and the surface (Mean Annual Temperature (°C) of each pixel (0.08° cells).

sibility to cope with climate change is to persist in situ, so they can be used to estimate the capability of species to persist when facing climatic fluctuations. As an example, in a recent study Sánchez-Fernández et al. (2016) used the subterranean habitat to illustrate how traditional approaches to estimate species fundamental niche and potential distributions do not work for poor dispersing species. They make also a weak-up call on this issue, as these same methods have been applied (and are still being regularly applied) to many species for which dispersal ability or thermal tolerance are not known, but that they are assumed to disperse freely without any limit, and to be perfectly adapted to the temperatures they experience with their current distribution. We thus encourage biogeographers and conservation biologist to work in this interesting ecosystem in order to provide accurate predictions of the response of biodiversity to climate change.

Besides, other than to exemplify general principles, subterranean fauna is certainly of interest and value on its own, since it represents an often neglected but substantial part of our natural heritage. Although there is a general lack of knowledge of most subterranean groups worldwide, Culver and Holsinger (1992) estimated that there may be a total of 50,000 to 100,000 obligate subterranean species, with a high level of endemism (Gibert and Deharveng 2002). It is not surprising that biologists have long been fascinated by the peculiarities of typical subterranean organisms (e.g. Darwin 1859,

Racovitza 1907, Jeannel 1943), as they show morphological, physiological, and life-history adaptations reflecting severe environmental constraints which result in an invaluable resource for evolutionary studies (Juan et al. 2010, Rohner et al. 2013). Thus, it is worth to note the interest of subterranean biodiversity also from a conservationist perspective. However, and in sharp contrast to its relevance, in conservation programs subterranean biodiversity is usually either neglected or protection measures are recommended based on misconceptions on the subterranean environment and a most incomplete knowledge of the biology of their species.

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References

- Araújo MB, Luoto M (2007) The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology and Biogeography* 16: 743–753. <https://doi.org/10.1111/j.1466-8238.2007.00359.x>
- Cardoso P (2012) Diversity and community assembly patterns of epigeal vs. troglobiont spiders in the Iberian Peninsula. *International Journal of Speleology* 41: 83–94. <https://doi.org/10.5038/1827-806X.41.1.9>
- Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LE, Sheldon BC (2008) Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* 320: 800–803. <https://doi.org/10.1126/science.1157174>
- Culver DC, Pipan T (2009) *The biology of caves and other subterranean habitats*. Oxford University Press, Oxford.
- Culver DC, Holsinger JR (1992) How many species of troglobites are there? *National Speleological Society Bulletin* 54: 79–80.
- Darwin C (1859) *On the origin of species*. John Murray, London.
- Gibert J, Deharveng L (2002) Subterranean ecosystems: a truncated functional biodiversity. *BioScience* 52: 473–481. [https://doi.org/10.1641/0006-3568\(2002\)052\[0473:SEATFB\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2002)052[0473:SEATFB]2.0.CO;2)
- Guisan A, Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. *Ecology Letters* 8: 993–1009. <https://doi.org/10.1111/j.1461-0248.2005.00792.x>
- Hannah L, Flint L, Syphard A, Moritz MA, Buckley LB, McCullough IM (2014) Fine-grain Modeling of Species Response to Climate Change: Microrefugia, Holdouts and Stepping Stones. *Trends in Ecology and Evolution* 29: 390–397. <https://doi.org/10.1016/j.tree.2014.04.006>

- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978. <https://doi.org/10.1002/joc.1276>
- Jeannel R (1926) Faune cavernicole de la France avec une étude des conditions d'existence dans le domaine souterrain. *Encyclopédie Entomologique*, Paul Lechevalier, Paris.
- Jeannel R (1943) Les fossiles vivants des cavernes. Gallimard, Paris.
- Jiménez-Valverde A, Lobo JM, Hortal J (2008) Not as good as they seem: the importance of concepts in species distribution modelling. *Diversity and Distributions* 14: 885–890. <https://doi.org/10.1111/j.1472-4642.2008.00496.x>
- Juan C, Guzik MT, Jaume D, Cooper SJ (2010) Evolution in caves: Darwin's 'wrecks of ancient life' in the molecular era. *Molecular Ecology* 19: 3865–3880. <https://doi.org/10.1111/j.1365-294X.2010.04759.x>
- Juberthie C, Decu V (1994) Structures et diversité du domaine souterrain; particularités des habitats et adaptations des espèces. In: Juberthie C, Decu V (Eds) *Encyclopaedia Biospeologica* vol. 1. Société de Biospéologie, Moulis-Bucarest, 5–22.
- Klečková I, Konvička M, Klečka J (2014) Thermoregulation and microhabitat use in mountain butterflies of the genus *Erebia*: importance of fine-scale habitat heterogeneity. *Journal of Thermal Biology* 41: 50–58. <https://doi.org/10.1016/j.jtherbio.2014.02.002>
- Mammola S, Isaia M (2016) The ecological niche of a specialized subterranean spider. *Invertebrate Biology* 135: 20–30. <https://doi.org/10.1111/ivb.12113>
- Mammola S, Leroy B (in press) Applying species distribution models to caves and other subterranean habitats. *Ecography*. <https://doi.org/10.1111/ecog.03464>
- Mammola S, Goodacre SL, Isaia M (2017) Climate change may drive cave spiders to extinction. *Ecography* 40: 1–10.
- Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* 7: 637–669. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110100>
- Poulson TL, White WB (1969) The cave environment. *Science* 165: 971–981. <https://doi.org/10.1126/science.165.3897.971>
- Racovitza EG (1907) Essai sur les problèmes biospéologiques. *Archives de Zoologie Expérimentale et Générale* 4(6): 371–488.
- Rezende EL, Castañeda LE, Santos M (2014) Tolerance landscapes in thermal ecology. *Functional Ecology* 28: 799–809. <https://doi.org/10.1111/1365-2435.12268>
- Rizzo V, Sánchez-Fernández D, Alonso R, Pastor J, Ribera I (2017) Substratum karstificability, dispersal and genetic structure in a strictly subterranean beetle. *Journal of Biogeography* 44: 2527–2538. <https://doi.org/10.1111/jbi.13074>
- Rizzo V, Comas J, Fadrique F, Fresneda J, Ribera I (2013) Early Pliocene range expansion of a clade of subterranean Pyrenean beetles. *Journal of Biogeography* 40: 1861–1873. <https://doi.org/10.1111/jbi.12139>
- Rohner N, Jarosz DF, Kowalko JE, Yoshizawa MW, Jeffery R, Borowsky RL, Lindquist S, Tabin CJ (2013) Cryptic variation in morphological evolution: HSP90 as a capacitor for the adaptive loss of eyes in cavefish. *Science* 342: 1372–1375. <https://doi.org/10.1126/science.1240276>

- Sánchez-Fernández D, Lobo JM, Millán A, Ribera I (2012) Habitat type mediates time to equilibrium in the geographical distribution of Iberian diving beetles. *Global Ecology and Biogeography* 21: 988–997. <https://doi.org/10.1111/j.1466-8238.2011.00743.x>
- Sánchez-Fernández D, Rizzo V, Cieslak A, Faille A, Fresneda J, Ribera I (2016) Thermal niche estimators and the capability of poor dispersal species to cope with climate change. *Scientific Reports* 6: 23381. <https://doi.org/10.1038/srep23381>
- Schoepf V, Stat M, Falter JL, McCulloch MT (2015) Limits to the thermal tolerance of corals adapted to a highly fluctuating, naturally extreme temperature environment. *Scientific Reports* 5: 17639. <https://doi.org/10.1038/srep17639>
- Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino J, Huey RB (2014) Thermal-safety margins and the necessity of thermoregulatory behaviour across latitude and elevation. *Proceedings of the National Academy of Science USA* 111: 5610–5615. <https://doi.org/10.1073/pnas.1316145111>
- Svenning JC, Skov F (2004) Limited filling of the potential range in European tree species. *Ecology Letters* 7: 565–573. <https://doi.org/10.1111/j.1461-0248.2004.00614.x>
- Visser ME, Both C (2005) Shifts in phenology due to global climate change: The need for a yardstick. *Proceedings of the Royal Society B: Biological Sciences* 272: 2561–2569. <https://doi.org/10.1098/rspb.2005.3356>
- Wiens JA, Stralberg D, Jongsomjit D, Howell CA, Snyder MA (2009) Niches, models, and climate change: assessing the assumptions and uncertainties. *Proceedings of the National Academy of Science USA* 106: 19729–19736. <https://doi.org/10.1073/pnas.0901639106>
- Wong BBM, Candolin U (2015) Behavioral responses to changing environments. *Behavioral Ecology* 26: 665–673. <https://doi.org/10.1093/beheco/aru183>

First record of subterranean rissoidean gastropod assemblages in Southeast Asia (Mollusca, Gastropoda, Pomatiopsidae)

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Abstract

In February 2017 we investigated several caves and karstic springs in Laos for the presence of underground freshwater gastropod species. We report previously unrecorded freshwater gastropod assemblages in the largest cave in Laos, Tham Khon Dôn, and in the third largest cave, Pha Soung, in Khammouane Province, with single finds in Na Li Cave (Khammouane Province), an unnamed cave near Vieng Thong (Bolikhamsay Province) and a small karst spring near Phonsavan (Xianghouan Province). All 15 species recorded and described herein are new to science. Four species are assigned to the new genus *Pseudoiglica*: *P. pseudoiglica* **sp. n.**, *P. olsavskyi* **sp. n.**, *P. kameniari* **sp. n.**, and *P. phonsavanica* **sp. n.** Three species are assigned to the new genus *Thamkhondonia*: *T. moureti* **sp. n.**, *T. vacquiei* **sp. n.**, and *T. smidai* **sp. n.** Eight species are assigned to the genus *Tricula* Benson, 1843: *T. valenasi* **sp. n.**, *T. davisi* **sp. n.**, *T. spelaea* **sp. n.**, *T. lenahani* **sp. n.**, *T. reischuetzorum* **sp. n.**, *T. phasoungensis* **sp. n.**, *T. bannaensis* **sp. n.**, and *T. viengthongensis* **sp. n.**

Keywords

Troglobiont, Stygobiont, Spring, Cave, Laos, Khammouane, Bolikhamsay, Xianghouan

Introduction

The subterranean freshwater molluscan fauna of South and East Asia is virtually unknown. Bole and Velkovrh (1986) mentioned only one subterranean species from South Asia that was recorded in Sri Lanka (*Srilankiella horanae* Bole & Velkovrh, 1986, *nomen nudum* according Kabat and Hershler 1993) and 12 probable pomatiopsid taxa from Japan belonging to the genera *Akiyoshia* Kuroda & Habe, 1954, *Cochliopopsis* Mori, 1938 and *Moria* Kuroda & Habe, 1958. Despite the recent increased interest in South and East Asian terrestrial micromolluscs (Maassen 2008, Culver 2012, Páll-Gergely 2014, Páll-Gergely et al. 2015, Inkhavilay et al. 2016, Páll-Gergely et al. 2016, Páll-Gergely et al. 2017), our knowledge of the stygobiont gastropod fauna of the region has not advanced significantly since 1986. Despite the extraordinary high radiation of surface freshwater Pomatiopsidae Bourguignat, 1863, species of the tribe Triculini Annandale, 1924 in the Mekong tributary (Deshayes and Jullien 1876; Crosse and Fischer 1879; Annandale 1919; Brandt 1968, 1970, 1974; Davis 1979, Temcharoen 1971; Brandt and Temcharoen 1974; Strong et al. 2007), the troglobiont and stygobiont species of the area remained unknown. Studies of this extraordinary high diversity in the Mekong Basin of over 90 taxa was driven primarily by parasitological studies of the trematode *Schistosoma mekongi* Voge, Bruckner & Bruce, 1978, the main intermediate hosts of which are some of the local Triculini species such as *Neotricula aperta* (Temcharoen, 1971). The assumption that the high diversity of surface Triculini species could extend into the underground stygobiont habitat was confirmed in the present study.

Materials and methods

The material studied was collected in Laos in February 2017 from the localities shown in Figs 1 and 2. Various cave streams, outflows and karstic springs were sampled using microhabitat preference and sampling methods as described by Grego et al. (2017).

Samples (fine sand) were screened under a stereomicroscope. They were first screened wet for live animals. Then they were dried and screened again for dry shells that might have been overlooked during the wet screening. Frontal and lateral view images were taken with a digital camera and ImageJ scientific image analysing software was used to take measurements, with additional direct measurements obtained using an eyepiece micrometer.

We followed the shell morphology nomenclature according Davis et al. (1992) and Hershler and Ponder (1998).

Abbreviations

NHMUK Natural History Museum, London, UK

HNHM Hungarian Natural History Museum, Budapest, Hungary

OSUM Ohio State University Museum of Biological Diversity, Columbus, Ohio, USA

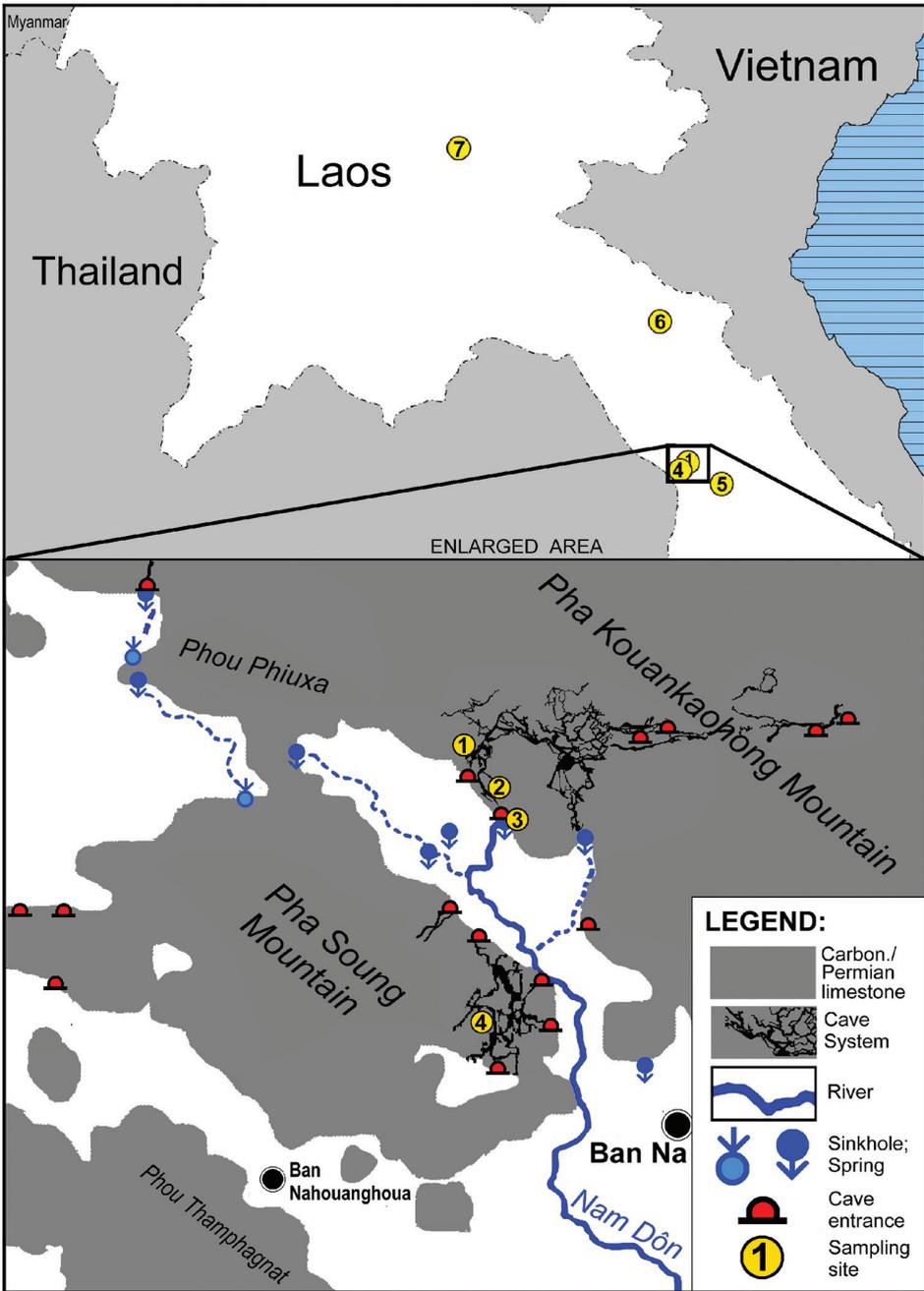


Figure 1. Map of sampling localities in Laos. **1–3** Khammouane: Tham Khon Dôn Cave **1** Earthquake Dome, Type locality (LT) of *Pseudoiglica pseudoiglica* gen. n., sp. n., *P. olsavskyi* n. sp., *P. kameniari* sp. n., *Thamkhondonia moureti* gen. n., sp. n., *T. vacquiei* sp. n., *T. snidai* sp. n., *Tricula valenasi* sp. n., *T. davisi* sp. n., *T. spelaea* sp. n., *T. lenabani* sp. n. and *T. bannaensis* sp. n. **2** Entrance passage **3** Source of Nam Dôn River **4** Khammouane: Tham Pha Soung Cave, Frog Lake, LT of *Tricula phasoungensis* sp. n. **5** Khammouane Cave Na Li, LT of *Tricula reischuetzorum* sp. n. **6** Bolikhamsay, 16 km W Vieng Thong, LT of *Tricula viengthongensis* sp. n. **7**, Xianghouan: Ban Nadom Village, LT of *Pseudoiglica phonsavanica* sp. n.

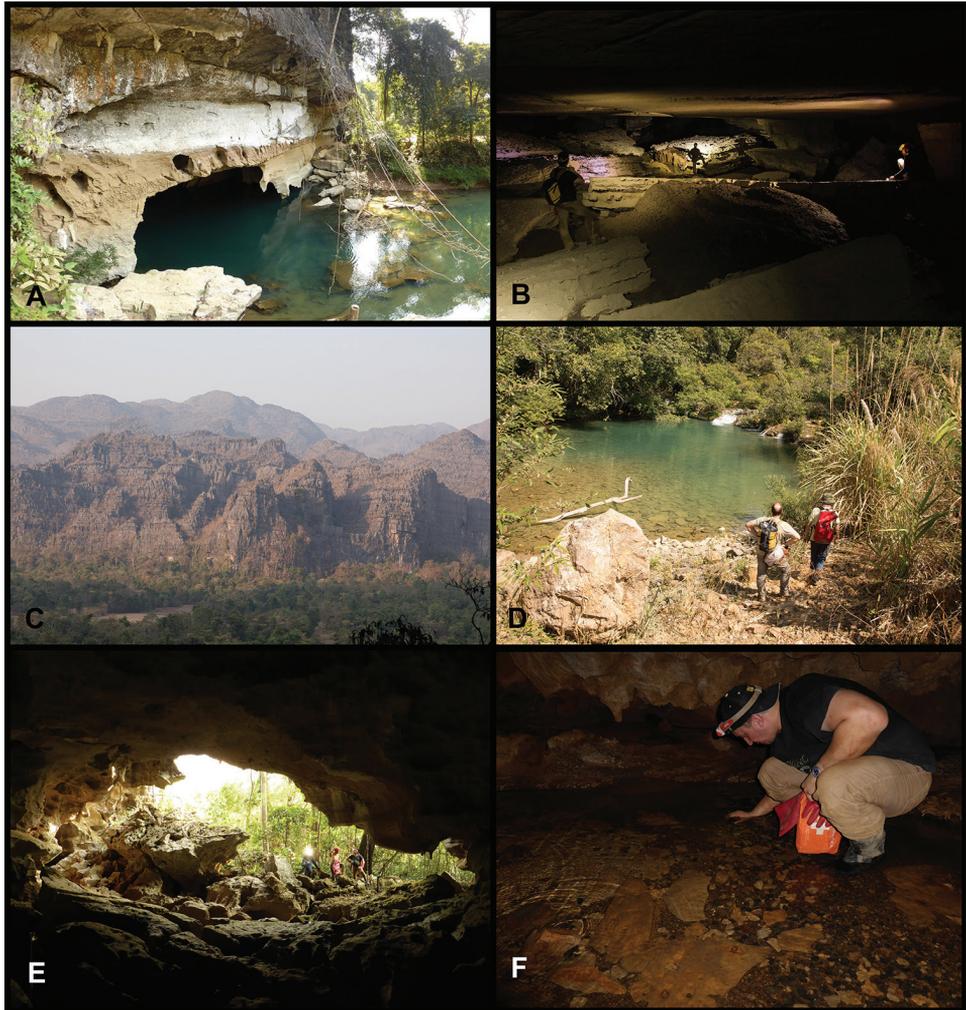


Figure 2. Photos of sites where subterranean gastropods were found. **A** Khammouane: main entrance of Tham Khon Dôn Cave with source of Nam Dôn River (2 in Fig. 1) **B** Earthquake Dome in cave Tham Khon Dôn (1 in Fig. 1) **C** Mount Pha Kouankaohong with entrance of Tham Khon Dôn cave at its foot **D** Bolikhamsay, travertine cascades below the LT of *Tricula viengthongensis* sp. n. (6 in Fig. 1) **E** Khammouane, one of the main entrances of Tham Pha Soung Cave **F** Tham Pha Soung Cave, sampling at Frog Lake (4 in Fig. 1). (Photos: Ondrej Kameniar, Mário Olšovský and Jozef Grego).

H	Shell height
W	Shell width
BH	Height of the body whorl
BW	Width of the body whorl
AH	Aperture height
AW	Aperture width
LT	Type locality
MY	Million years

Results

Many of the newly-recorded stygobiont Triculini have a convergent shell morphology superficially resembling to that of species of Moitessieriidae Bourguignat, 1863 and Hydrobiidae Troschel, 1857, known from other stygobiont habitats. All the present stygobiont specimens from the cave streams and spring outlets were empty shells collected during the dry season. As it is highly unlikely to find live specimens because of the inaccessibility of habitats during rainy seasons, the two new genera (*Pseudoiglica* gen. n. and *Thamkhondonia* gen. n.) were established based only on the shell morphology, without information on the soft parts and without molecular data. I assume, based on shell morphology that all the new species belong to the family Pomatiopsidae Bourguignat, 1863, tribe Triculini Annandale, 1924. The tribe is extremely diverse along the neighboring tributary of the Mekong River and represents the only known Gastropoda similar to the new taxa within the whole region.

Superfamily Truncatelloidea Gray, 1840

Family Pomatiopsidae Bourguignat, 1863

Tribe Triculini Annandale, 1924

Genus *Pseudoiglica* gen. n.

<http://zoobank.org/078DA479-6098-43ED-9357-D03815C8D782>

Diagnosis. The diagnostic features of the genus are the same as those of the type species, *Pseudoiglica pseudoiglica* sp. n. The elongated smooth shell shape with open umbilicus differs from all known genera of Pomatiopsidae in tributaries of the Mekong River.

Etymology. Named for the shell morphology, which is convergent with the subterranean moitessieriid genus *Iglica* Wagner, 1910 from the western Balkans.

Pseudoiglica pseudoiglica sp. n.

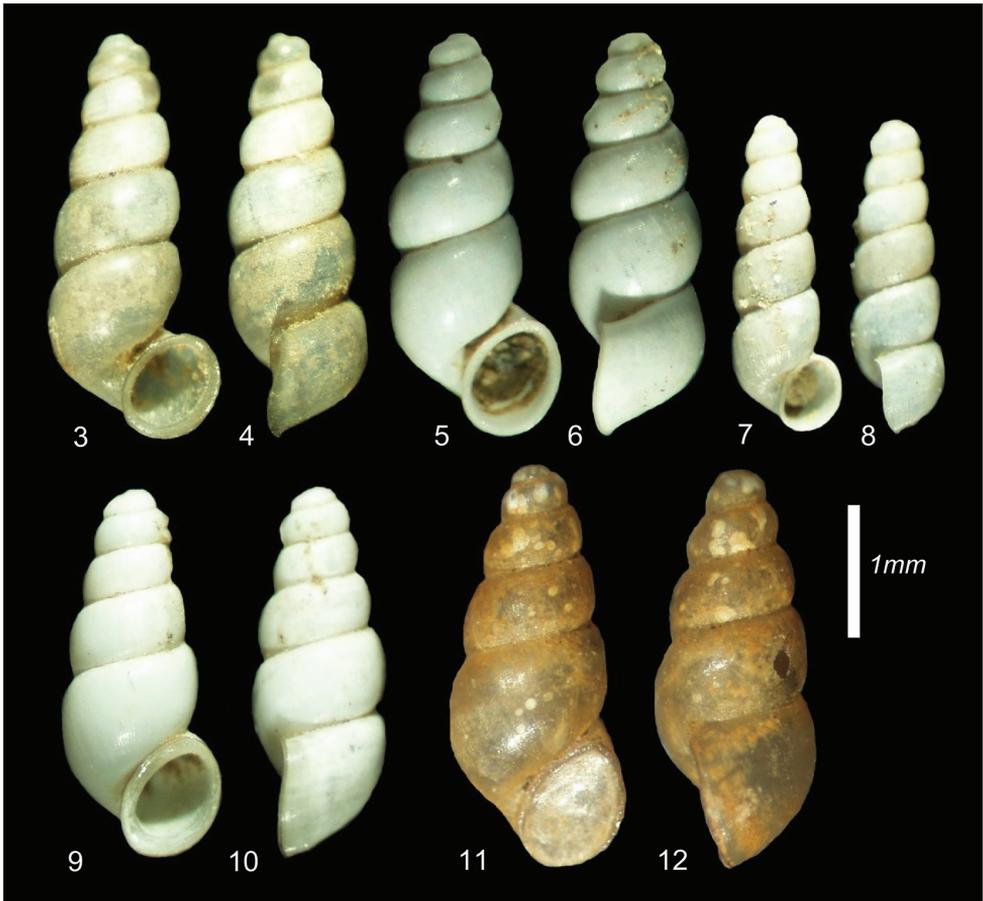
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Figures 3–6

Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Khon Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., Earthquake Dome 3 km from the south entrance, sand sediments on bank of cave river (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšovský leg. 11–12 February 2017 (NHMUK 20180001).

Paratypes: type locality (NHMUK 20180016 – 1 specimen; HNHM 102769 – 1 specimen; coll. Grego F0871 – 6 specimens); Laos, Khammouane Province, Tham Nam Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., temporary side rivulet sediment at entrance passage 1.5 km from the main entrance, dry sand on the cave floor; J. Grego leg. 11 February 2017 (coll. Grego F0863 – 1 specimen).



Figures 3–12. Representatives of the genus *Pseudoiglica* gen. n. **3–6** *Pseudoiglica pseudoiglica* sp. n. (**3–4** holotype NHMUK 20180001 **5–6** paratype 1 coll. Grego F0871) **7–8** *P. olsavskyi* sp. n. (holotype NHMUK 20180003) **9–10** *P. kameniari* sp. n. (holotype NHMUK 20180002) **11–12** *P. phonsavanica* sp. n. (holotype NHMUK 20180004).

Other material. Laos, Khammouane Province, 2 km WNW of Ban Na village, Pha Soung Cave, Frog Lake, 155 m a.s.l., J. Grego leg. 09 February 2017, 17°33.052'N; 104°52.410'E (coll. Grego F0888).

Measurements. Holotype: H 3.08 mm; W 1.32 mm; BW 0.95 mm; BH 1.50 mm; AH 0.81 mm; AW 0.75 mm; H/W 2.33; AH/AW 1.08; W/BW 1.39; H/BH 2.05; H/AH 3.80; W/AW 1.76. Paratype 1: H 3.05 mm; W 1.31 mm; BH 1.00 mm; BW 1.55 mm; AH 0.87 mm; AW 0.75 mm; H/W 2.33; AH/AW 1.16; W/BW 1.31; H/BH 1.97; H/AH 3.51; W/AW 1.76.

Diagnosis. This new species is similar to the syntopic *Pseudoiglica kameniari* sp. n., from which it differs by its more slender, elongated shell with a more prominent umbilicus and less elongated aperture situated further to the right of the columellar axis.

It differs from syntopic *P. olsavskyi* sp. n. by its markedly larger and more conical shell shape and proportionally larger aperture. *Pseudoiglica phonsavanica* sp. n. (Xianghouan Province) has more a robust shell with more prominent body whorl and a differently shaped aperture.

Description. The milky yellowish silky shell has six tumid convex whorls with a deep suture and a blunt apex. The surface is smooth and shiny. The shell is elongated, almost cylindrical, slightly tapering towards a blunt apex, the umbilicus is tiny, open. In frontal view, the lateral aperture protrudes against the rest of the teleoconch. The aperture is ovoid, separated from the body whorl by a gap. The peristome margin is blunt, equally thick all the way around and slightly reflexed outwards. The outer lip is sinuous in lateral view and slightly scooped forward at its lower end.

Etymology. See the etymology of the genus *Pseudoiglica* gen. n.

Distribution. Only known from the type locality and nearby sites in Tham Khon Dôn Cave as well as in the related source of the Nam Dôn River and from sediments in Tham Pha Soung Cave.

Ecology. Empty shells of the new species were extracted from the side stream sandy sediments of an underground river inside the cave Tham Khon Dôn about 3 km from the main entrance situated above the source of Nam Dôn River (Fig. 1A). The cave Tham Khon Dôn is situated under the massif of Mount Pha Kouankaohong (Fig. 2C) north of Ban Na Village. It represents the largest explored cave system in Laos, with a length of known passages of ca. 42 km. The cave is morphologically diverse with large domes and passages modeled by phreatic corrosion representing the cave multiple genetic horizons formed during the past 11 million years (since Late Miocene-Lower Pliocene) of its natural history possibly driven by a hydrothermal, H₂S speleogenesis (Mouret 2005). Despite the remarkable length of the cave system, its water passages are accessible only for a limited length near the entrance. The major part of the underground river comprises mostly unexplored submerged cave passages. The main sampling site was located at the bottom of Earthquake Dome (Fig. 2B), named after the sounds of the earthquake experienced here by the first explorers (Claude Mouret and Jean-Francois Vacquié pers. com.). The dome floor is covered by very large flat limestone slabs approx. 0.8–1.2 m thick fallen from the horizontally flat ceiling that reflects the horizontal beds of Carboniferous/Permian Khammouane Limestone. The fallen slabs fragmented the underground stream into several lakes and helped to create the sedimentation zones in which empty shells could be deposited during high water flows. A few shells were also found in the sand floor of the entrance passage approximately 1.5 km from the entrance close to the junction with a temporary side stream, as well as in the sand deposited directly at the cave entrance at the source of the Nam Dôn River (Fig. 1A). The character of the material deposited in the cave sediments and the freshwater shell assemblages suggested their autochthonous origin rather than the allochthonous influence of horizontal surface waters. The occurrence of tiny terrestrial gastropod shells of mainly soil and leaf litter dwelling families such as Vertiginidae Fitzinger, 1833 (*Hypselostoma* sp., *Angustopila*

sp., *Krobylos* sp., *Paraboysidia* sp.) and Diapheridae Panha & Naggs, 2010 (*Sinoennea* sp.) in the underground river sediments suggests a stronger influence of vertically circulating surface karst waters. The presence of the same species in the sediments of nearby Tham Pha Soung Cave indicates possible communication between the two caves through phreatic waters under Ban Na polje or during the rainy seasons. Surface Triculini species inhabiting the Nam Dôn River are larger, with a stronger periorostracum and different shell morphology. It appears that they are not penetrating deeply into the dark cave system, probably because of a lack of their main algal food. The new species probably inhabits the so far unexplored submerged cave passages of the Tham Khon Dôn system.

***Pseudoiglica olsavskyi* sp. n.**

<http://zoobank.org/3BB62BF8-DD8E-44F6-BD93-618321D7161F>

Figs 7–8

Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Khon Dôn Cave 17°33.82'N; 104°52.30'E, 161m a.s.l., Earthquake Dome 3 km from the south entrance, sand sediments on cave river bank (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšavský leg. 11–12 February 2017 (NHMUK 20180003). Paratypes: type locality (NHMUK 20180149 – 1 specimen; coll. Grego F0872 – 3 specimens); Laos, Khammouane Province, Tham Nam Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., temporary side rivulet sediment at entrance passage 1.5 km from the main entrance, dry sand on the cave floor; J. Grego leg. 11 February 2017 (coll. Grego F0864 – 3 specimens); Laos, Khammouane Province, 3 km NW of Ban Na Village, sand on the bottom of Nam Dôn River source at 149 m a.s.l.; J. Grego leg. 07 February 2017, 17°33.20'N; 104°52.38'E (coll. Grego F0854 – 4 specimens) (Fig. 2A).

Measurements. Holotype: H 2.45 mm; W 0.85 mm; BW 0.52 mm; BH 1.00 mm; AH 0.61 mm; AW 0.45 mm; H/W 2.88; AH/AW 1.36; W/BW 1.63; H/BH 2.45; H/AH 4.02; W/AW 1.89

Diagnosis. The tiny, elongated cylindrical shell of *P. olsavskyi* sp. n. with a proportionally very small aperture distinguishes this species from all other known members of the genus, which all have larger shells.

Description. The tiny milky shell has six flattened convex whorls with a deep suture and a blunt apex. The surface is smooth and shiny. The shell is cylindrically elongated, slightly narrowing towards the apex. Umbilicus is slit-like. In frontal view, the aperture protrudes laterally from the shell periphery outline. Aperture is ovoid, separated from the body whorl by a weak furrow. The peristome margin is sharp, equally thick all the way around and very slightly reflexed outwards. The outer lip is sinuous in its lateral profile and its lower end scooped backward.

Etymology. Named after my friend Mario Olšavský, a geologist and speleologist from Banská Bystrica, Slovakia, who actively participated in sample collection in the cave Tham Khon Dôn.

Distribution. Only known from the type locality and nearby sites in Tham Khon Dôn Cave as well as from the related source of Nam Dôn River

Ecology. The same as *Pseudoiglica pseudoiglica* sp. n.

***Pseudoiglica kameniari* sp. n.**

<http://zoobank.org/A529966B-65AD-404D-BCC6-552FF9832934>

Figures 9–10

Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Khon Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., Earthquake Dome 3 km from the south entrance, sand sediments on bank of cave river (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšovský leg. 11–12 February 2017 (NHMUK 20180002). Paratypes: type locality (NHMUK 20180017 – 1 specimen; HNHM 102770 – 1 specimen; OSUM 42383 – 1 specimen; coll. Grego F0873 – 11 specimens).

Measurements. Holotype: H 2.85 mm; W 1.21 mm; BW 0.85 mm; BH 1.50 mm; AH 0.95 mm; AW 0.75 mm; H/W 2.36; AH/AW 1.27; W/BW 1.42; H/BH 1.60; H/AH 3.00; W/AW 1.61.

Diagnosis. Similar to syntopic *Pseudoiglica pseudoiglica* sp. n., from which it differs by its smaller, more robust shell with a less prominent umbilicus and more elongated aperture situated more towards the columellar axis. It differs from the syntopic *P. olsavskyi* sp. n. by its larger and more conical shell shape and proportionally larger aperture. It differs from *P. phonsavanica* by its less robust shell, less prominent body whorl and the different shape of the aperture.

Description. The milky yellowish shell has five convex whorls with a deep suture and a blunt apex. The shell has a smooth and shiny surface and is elongated-conical. Umbilicus is slit-like. In frontal view, the aperture aligns with the shell periphery outline. Aperture is ovoid, attached to the body whorl by a very weak furrow. The peristome margin is a blunt callous, equally thick all the way around and slightly reflexed outwards. The outer lip is weakly sinuous in lateral profile.

Etymology. Named after Ondrej Kameniar, young speleologist and biologist, friend from Ľubochňa, Slovakia, who actively participated in our 2017 field trip to Laos.

Distribution. Only known from the type locality and nearby sites in Tham Khon Dôn Cave as well as from the related source of Nam Dôn River.

Ecology. The same as *Pseudoiglica pseudoiglica* sp. n.

***Pseudoiglica phonsavanica* sp. n.**

<http://zoobank.org/A659C91D-394C-42F4-9C29-8EE9FEFACB5F>

Figs 11–12

Type locality. Laos; Xianghouan Province, Ban Nadom Village, 18 km SE of Phonsavan, 3 km N of Ban Kaua cement factory at highway 1D (9 km ENE of Xiang

Khouang), small spring at eastern foot of limestone hill, 19°23.142'N; 103°17.630'E, 1196 m a.s.l., fine sand directly at spring zone.

Type material. Holotype: type locality: J. Grego leg. 22 February 2017 (NHMUK 20180004).

Measurements. Holotype: H 3.05 mm; W 1.30 mm; BW 1.00 mm; BH 1.71 mm; AH 0.93 mm; AW 0.79 mm; H/W 2.35; AH/AW 1.18; W/BW 1.30; H/BH 1.78; H/AH 3.28; W/AW 1.65.

Diagnosis. Similar to *Pseudoiglica kameniari* sp. n. (Khammouane Province), from which it differs by its more robust shell with a more prominent umbilicus and a proportionally smaller aperture. The robust shape differentiates the species from all other members of the genus.

Description. The light orange, silky shell has five tumid convex whorls with a weak suture and a blunt apex. The smooth shell surface is covered by sparse rusty incrustations. The shell is elongated conical, with a prominent body whorl. Umbilicus is slit-like. In frontal view, the aperture is aligned with the shell periphery outline. Aperture is ear-shaped, separated from the body whorl by a weak sulcus. The peristome margin is blunt, not reflexed and slightly callous internally. The labral lip has a straight profile in lateral view, scooped backward from the columellar axis. The elongate ellipsoidal spiral operculum is light yellowish corneous with submarginal nucleus.

Etymology. Named after the city of Phonsavan, Laos, capital of Xianghouan Province, which is the closest large city to the type locality.

Distribution. Only known from the type locality.

Ecology. The locality is a small karstic spring rising at the foot of a rounded cone-shaped limestone hill at the boundary between limestone beds and a sandy slate substrate just a few meters above the road. The spring is connected to a small waterworks to supply water to the nearby village Ban Nadom. The water supply seems to be permanent throughout all seasons.

Remarks. The body whorl of *P. phonsavanica* sp. n. is proportionally larger than that of all other species of the genus. The more teardrop-shaped aperture suggests that this geographically distant species could represent a new genus distinct from *Pseudoiglica* gen. n. Anatomical and molecular data are needed to confirm such a possible distinction.

Genus *Thamkhondonia* gen. n.

<http://zoobank.org/114E71B9-6D08-4028-A309-5EA7422D9CF5>

Diagnosis. The diagnostic features of the genus are the same as those of the type species, *Thamkhondonia moureti* sp. n. The elongated axially and radially sculptured shell with an ear-shaped aperture differs from that of any known genus of Pomatiopsidae from tributaries of the Mekong River. The shell shows some resemblance to those of species in the triculinid genus *Paraprosopthenia* Annandale, 1919 and the marine genus *Attenuata* Hedley, 1918, but differs from both by the characteristic shell sculpture consisting of spiral cords and axial ribs.

Etymology. Named after the type locality inside the Tham Khon Dôn Cave situated under the massif of Pha Kouankaohong near village Ban Na in Khammouane Province.

***Thamkhondonia moureti* sp. n.**

<http://zoobank.org/FC1E7A3C-3E20-4B42-BD32-669EB5A70E53>

Figs 13–14

Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Khon Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., Earthquake Dome 3 km from the south entrance, sand sediments on cave river banks (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšavský leg. 11–12 February 2017 (NHMUK 20180005). Paratypes: type locality (NHMUK 20180018 – 2 specimens; HNHM 102771 – 2 specimens; OSUM 42384 – 2 specimens; coll. Grego F0874 – 33 specimens); Laos, Khammouane Province, Tham Nam Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l, temporary side rivulet sediment at entrance passage 1.5 km from the main entrance, dry sand on the cave floor; J. Grego leg. 11 February 2017 (coll. Grego F0865 – 2specimens); Laos, Khammouane Province, 3 km NW of Ban Na Village, sand on the bottom of Nam Dôn River source at 149 m a.s.l.; J. Grego leg. 07 February 2017, 17°33.20'N; 104°52.38'E (coll. Grego F0855 – 1 specimen) (Fig. 2A).

Measurements. Holotype: H 4.48 mm; W 1.42 mm; BW 0.80 mm; BH 1.55 mm; AH 1.01 mm; AW 0.91 mm; H/W 3.15; AH/AW 1.11; W/BW 1.78 H/BH 2.89; H/AH 4.44; W/AW 1.56.

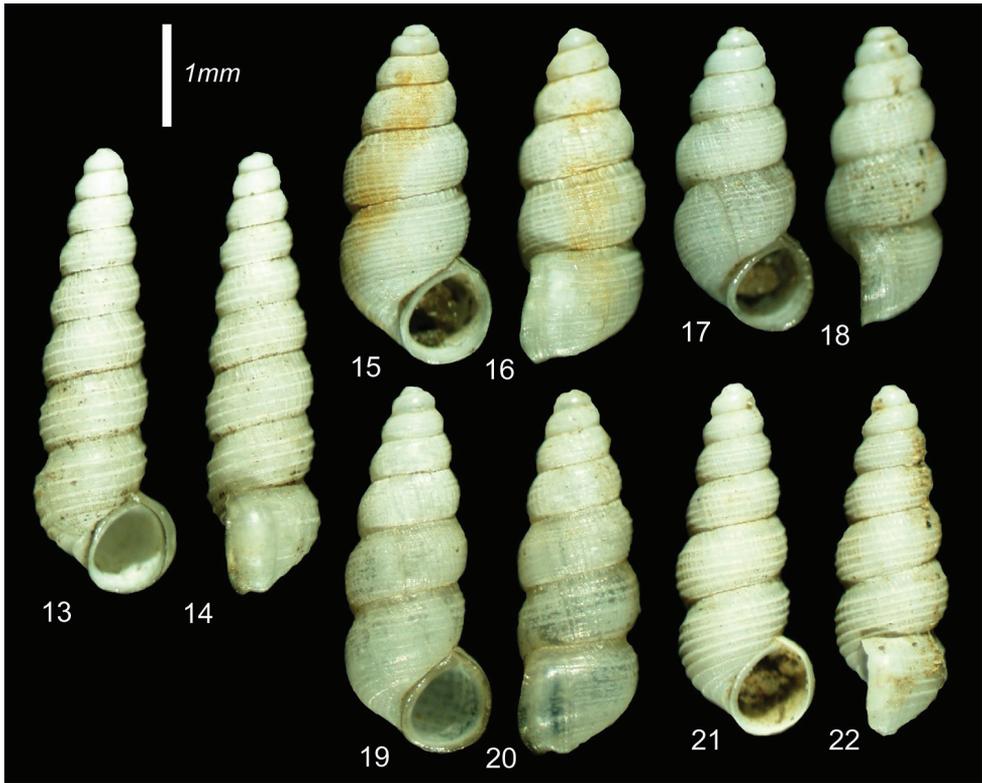
Diagnosis. Compared to the most closely related syntopic species, *Thamkhondonia vacquiei* sp. n. and *T. smidai* sp. n., *T. moureti* sp. n. has a much higher and more slender shell with much coarser spiral sculpture and a proportionally smaller aperture.

Description. The milky whitish, elongated turritiform shell has nine slightly convex whorls with a weak suture. The surface sculpture consists of 4–5 coarse spiral cords crossed by very fine axial ribs. The aperture is oval ear-shaped and extends beyond the shell periphery outline; the peristome is sharp and expands only at its columellar side. The lateral profile of the labral lip is straight and a characteristic axial varix is parallel to the labral lip. The umbilicus is closed.

Etymology. Named after my friend, a renowned French geologist and speleologist Claude Mouret (Magnac-Bourg, France), who in 2006 discovered and for the first time explored the Tham Khon Dôn cave system, the largest cave in Laos, and led annual expeditions to explore caves of Khammouane. Without his substantial help, the sampling of the type locality in cave Tham Khon Dôn would not have been possible.

Distribution. Only known from the type locality and nearby sites in Tham Khon Dôn Cave as well as in the related source of Nam Dôn River.

Ecology. The same as *Pseudoiglica pseudoiglica* sp. n.



Figures 13–22. Representatives of the genus *Thamkhondonia* gen. n. **13–14** *Thamkhondonia moureti* sp. n. (holotype NHMUK 20180005) **15–18** *T. vacquiei* sp. n. (**15–16** holotype NHMUK 20180006 **17–18** paratype 1 coll. Grego F0875) **19–22** *T. smidai* sp. n. (**19–20** holotype NHMUK 20180007 **21–22** paratype 1 coll. Grego F0876).

***Thamkhondonia vacquiei* sp. n.**

<http://zoobank.org/CF534C85-D526-43BF-95C0-02956FF6891D>

Figs 15–18

Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek; Tham Khon Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., Earthquake Dome 3 km from the south entrance, sand sediments on cave river banks (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšavský leg. 11–12 February 2017 (NHMUK 20180006). Paratypes: type locality (NHMUK 20180019 – 5 specimens; HNHM102772 – 5 specimens; OSUM 42385 – 5 specimens; coll. Grego F0875 – 274 specimens); Laos, Khammouane Province, 3 km NW of Ban Na Village, sand on the bottom of Nam Dôn river source at 149 m a.s.l.; J. Grego leg. 07 February 2017, 17°33.20'N; 104°52.38'E (coll. Grego F0856 – 1 specimen) (Fig. 2A).

Measurements. Holotype: H 3.35 mm; W 1.51 mm; BW 1.25 mm; BH 1.65 mm; AH 0.95 mm; AW 0.90 mm; H/W 2.22; AH/AW 1.06; W/BW 1.21; H/BH 2.03;

H/AH 3.53; W/AW 1.68; Paratype 1: H 3.00 mm; W 1.40 mm; BH 1.20 mm; BW 1.55 mm; AH 0.90 mm; AW 0.85 mm; H/W 2.14; AH/AW 1.06; W/BW 1.17; H/BH 1.94; H/AH 3.33; W/AW 1.65.

Diagnosis. *Thamkhondonia vacquiei* sp. n. differs from the two syntopic species *T. moureti* sp. n. and *T. smidai* sp. n. by its shorter and more robust shell shape with a proportionally larger aperture and by its much finer and more numerous spiral sculpture.

Description. The whitish translucent, conical shell has six convex whorls with a weak slightly wavy suture. The surface sculpture consists of 11–12 weak spiral cords crossed by fine axial ribs. The aperture is oval ear-shaped with an indication of a posterior canal that extends slightly beyond the shell periphery outline. The peristome is sharp and expands only on the columellar side. The lateral edge of the labral lip is sinuous and a weak axial varix is present parallel to the labral edge. The umbilicus is slit-like.

Etymology. Named after my friend Jean-Francois Vacqu   (Castelnau d'Estretfonds, France), a French speleologist who participated in the explorations of Tham Khon D  n Cave and supported our activities during the 2017 field trip.

Distribution. Only known from the type locality and nearby sites in Tham Khon D  n Cave as well as in the related source of Nam D  n River.

Ecology. The same as *Pseudoiglica pseudoiglica* sp. n.

***Thamkhondonia smidai* sp. n.**

<http://zoobank.org/62043A71-D01B-4BA3-8EE5-1C65D5B1EF62>

Figs 19–22

Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Khon D  n Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., Earthquake Dome 3 km from the south entrance, sand sediments at cave river bank (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšovsk   leg. 11–12 February 2017 (NHMUK 20180007). Paratypes: type locality (NHMUK 20180020 – 2 specimens; HNHM 102773 – 2 specimens; OSUM 42383 – 2 specimens; coll. Grego F0876 – 34 specimens); Laos, Khammouane Province, 3 km NW of Ban Na Village, sand on the bottom of Nam D  n river source at 149 m a.s.l.; J. Grego leg. 07 February 2017, 17°33.20'N; 104°52.38'E (coll. Grego F0902 – 2 specimens) (Fig. 2A).

Measurements. Holotype: H 3.62 mm; W 1.45 mm; BW 1.15 mm; BH 1.45 mm; AH 1.05 mm; AW 0.75 mm; H/W 2.50; AH/AW 1.15; W/BW 0.36; H/BH 2.16; H/AH 3.45; W/AW 1.59. Paratype 1: H 3.45 mm; W 1.38 mm; BH 1.10 mm; BW 1.50; AH 1.00 mm; AW 0.87 mm; H/W 2.50; AH/AW 1.15; W/BW 0.36; H/BH 2.18; H/AH 3.45; W/AW 1.59.

Diagnosis. *Thamkhondonia smidai* sp. n. differs from syntopic *T. moureti* sp. n. by its smaller shell with less coarse and more numerous spiral sculpture and from *T. vacquiei* sp. n. (syntopic) by its longer and more slender shell shape with coarser spiral cords.

Description. The whitish translucent, elongate shell has seven convex whorls with a weakly wavy suture. The shell surface sculptured by 5–6 spiral cords crossed by very

fine axial ribs. The oval ear-shaped aperture has a weak posterior canal and extends slightly beyond the shell periphery outline; the peristome is blunt and reflexed at the columellar side. The lateral edge of the labral lip is weakly sinuated and an axial varix is present parallel to the labral lip. The umbilicus is closed.

Etymology. Named after the famous Slovak speleologist Branislav Šmída, Bratislava, Slovakia, who actively participated in our 2017 biospeleology survey of Laos.

Distribution. Only known from the type locality and nearby sites in Tham Khon Dôn Cave as well as in the related source of Nam Dôn River.

Ecology. The same as *Pseudoiglica pseudoiglica* sp. n.

Remark. The shell morphology of *T. smidai* sp. n. is an intermediate between that of *T. moureti* sp. n. and *T. vacquiei* sp. n.

Genus *Tricula* Benson, 1843

Nore. Based on their shell morphology the species described below are provisionally placed in the genus *Tricula* until anatomical and molecular data can be obtained.

Tricula valenasi sp. n.

<http://zoobank.org/D6866F52-BB80-40F1-8B16-8F79C53CEAC0>

Figs 23–24

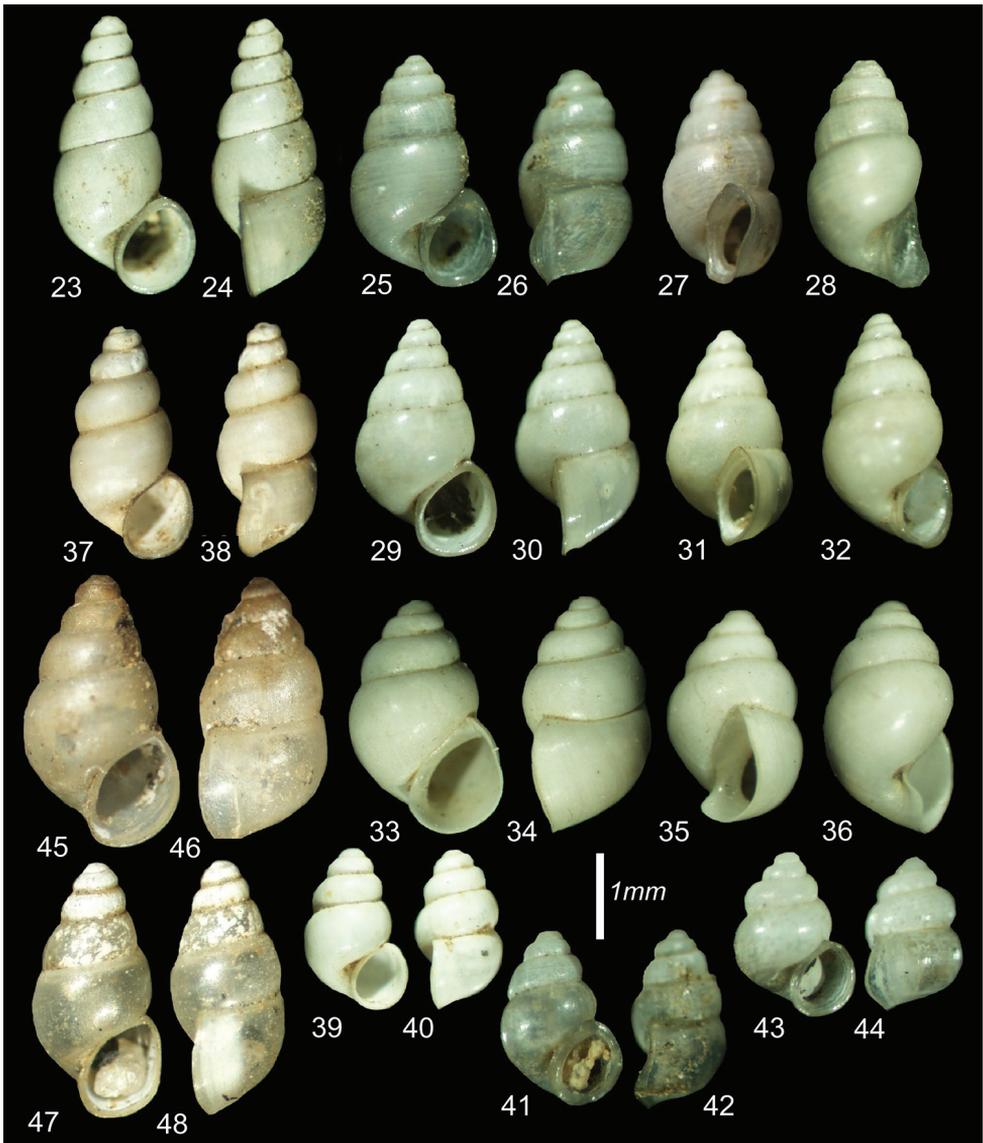
Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Khon Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., Earthquake Dome 3 km from the south entrance, sand sediments at cave river banks (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšavský leg. 11–12 February 2017 (NHMUK 20180008). Paratypes: type locality (coll. Grego F0877 – 3 specimens).

Measurements. Holotype: H 3.25 mm; W 1.65 mm; BW 1.05 mm; BH 1.25 mm; AH 1.15 mm; AW 0.95 mm; H/W 1.97; AH/AW 1.2; W/BW 1.57; H/BH 2.60; H/AH 2.83; W/AW 1.74.

Diagnosis. *Tricula valenasi* sp. n. is similar to syntopic *T. lenahani* sp. n., from which it differs by its elongate shell with a less open umbilicus and an aperture more prominent at the shell periphery outline. It differs significantly from syntopic *T. davisii* sp. n. by its longer, more elongated shell shape, its more inflated whorls and its proportionally smaller aperture with a straight columellar margin rather than the columellar sinuation characteristic of *T. davisii* sp. n. From *T. bollingi* Davis, 1968 it differs by the aperture shape, which extends beyond the shell periphery outline and by its more open umbilicus and more blunt apex. It can be distinguished from *T. burchi* Davis, 1968, by its more slender and more conical shape with a smaller aperture, a more prominent umbilicus and a less blunt apex.

Description. The whitish shell has five rounded convex whorls with a semi-deep suture and a blunt apex. The surface is smooth and shiny. The shell is narrow-coni-



Figures 23–48. Representatives of the genus *Tricula*, Benson, 1843. **23–24** *Tricula valenasi* sp. n. (holotype NHMUK 20180008) **25–28** *T. lenabani* sp. n. (holotype NHMUK 201800010) **29–32** *T. spelaea* sp. n. (holotype NHMUK 20180011) **33–36** *T. davisi* sp. n. (holotype NHMUK 20180009) **37–38** *T. reischuetzorum* sp. n. (holotype NHMUK 20180012) **39–42** *T. phasoungensis* sp. n. (**39–40** holotype NHMUK 20180013 **41–42** paratype 2 coll. Grego F0881) **43–44** *T. bannaensis* sp. n. (holotype NHMUK 20180014) **45–48** *T. viengthongensis* sp. n. (**45–46** holotype NHMUK 20180015 **47–48** paratype 1 coll. Grego F0904).

cal with prominent body whorl. Umbilicus is tiny opened. In frontal view, the outer part of the aperture protrudes from the shell periphery outline. Aperture is ovoid ear-shaped, separated from the body whorl by a very weak furrow and an adapical gap. The

peristome margin is blunt, slightly reflexed outwards. The outer lip is slightly callous and has a straight lateral profile.

Etymology. Named after my friend Liviu Valenas (Nuremberg, Germany), an avid speleologist born in Romania, who spent 10 years exploring the cave system Pha Soung, which, with its currently documented 20.4 km, is the third largest cave system in Laos.

Distribution. Only known from the type locality.

Ecology. The same as *Pseudoiglica pseudoiglica* sp. n.

***Tricula lenahani* sp. n.**

<http://zoobank.org/3FE20728-0E33-4D26-B964-0E38B9C93F06>

Figs 25–28

Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Khon Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., Earthquake Dome 3 km from the south entrance, sand sediments of cave river banks (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšavský leg. 11–12 February 2017 (NHMUK 201800010). Paratypes: type locality (NHMUK 20180021 – 5 specimens; HNHM 102774 – 5 specimens; OSUM 42391 – 5 specimens; coll. Grego F0879 – 103 specimens); Laos, Khammouane Province, Tham Nam Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., temporary side rivulet sediment at entrance passage 1.5 km from the main entrance, dry sand on the cave floor; J. Grego leg. 11 February 2017 (coll. Grego F0868 – 1 specimen); Laos, Khammouane Province, 3 km NW of Ban Na Village, sand on the bottom of Nam Dôn River source at 149 m a.s.l.; J. Grego leg. 07 February 2017. 17°33.20'N; 104°52.38'E (coll. Grego – 6 specimens) (Fig. 2A).

Measurements. Holotype: H 2.72 mm; W 1.71 mm; BW 0.96 mm; BH 1.70 mm; AH 1.15 mm; AW 0.93 mm; H/W 1.59; AH/AW 1.24; W/BW 1.78; H/BH 1.60 H/AH 2.37; W/AW 1.84.

Diagnosis. This species is similar to the syntopic *Tricula valenasi* sp. n., but differs from it by its more robust, shorter shell with a more open umbilicus as well as by its sinuated labral margin profile. It differs from syntopic *T. spelaea* sp. n. by its more inflated shell shape, and a different arrangement of the whorls, a larger umbilicus and a different shape of the aperture. It can be distinguished from syntopic *T. davisi* sp. n. by its more slender, less inflated shell and the shape of the columellar peristome. From *T. bollingi* Davis, 1968 it differs by its general shell shape and the position of the aperture, and by its more open umbilicus and blunter apex.

Description. The whitish, semi-translucent shell has five convex whorls with a deep suture. The surface is smooth with fine, whitish, inconsistent axial bands. The shell is ovate-conical with whorls smoothly tapering towards the apex. The aperture is ear-shaped; the peristome expands outwards especially at the columellar side. The lateral edge of the labral lip is characteristically sinuated, as well a weak sinuation is pre-

sent at apical inner peristome. The umbilicus is open, partly obscured by the reflected columellar margin.

Etymology. This species is named after my ever helpful friend Peter Lenahan, an avid caver from New York City, USA, for his great support during the field trip and for his indispensable help to Ban Na village by supporting construction of a new well and tap water supply for the villagers.

Distribution. Only known from the type locality and nearby sites in Tham Khon Dôn Cave as well as in the related source of Nam Dôn River.

Ecology. The same as *Pseudoiglica pseudoiglica* sp. n..

***Tricula davisi* sp. n.**

<http://zoobank.org/0BB6C9B6-08D5-411A-BCC8-9818E0BCF4EC>

Figs 29–32

Type locality. Laos; Khammouane Province; Ban Na village 20 km NNE of Thakhek; Tham Khon Dôn Cave, 161 m a.s.l.; 17°33.82'N; 104°52.30'E; Earthquake Dome 3 km from the south entrance, sand sediments at cave river banks (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšavský leg. 11–12 February 2017 (NHMUK 20180009). Paratypes: type locality (NHMUK 20180022 – 3 specimens; HNHM 102775 – 3 specimens; OSUM 42387 – 3 specimens; coll. Grego F0878 – 76 specimens); Laos, Khammouane Province, 3 km NW of Ban Na Village, sand on the bottom of Nam Dôn River source at 149 m a.s.l.; J. Grego leg. 07 February 2017, 17°33.20'N; 104°52.38'E (coll. Grego F0858 – 6 specimens) (Fig. 2A).

Measurements. Holotype: H 2.72 mm; W 1.81 mm; BW 1.23 mm; BH 1.88 mm; AH 1.5 mm; AW 1.05 mm; H/W 1.50; AH/AW 1.29; W/BW 1.47; H/BH 1.45; H/AH 2.01; W/AW 1.72.

Diagnosis. This new species is similar to syntopic *Tricula lenahani* sp. n., from which it differs by its more oval, inflated shell with more inflated whorls, a closed umbilicus and the shape of the aperture. The aperture of *T. davisi* sp. n. has a characteristic sinuation at the columellar peristome and a straight labral peristome, distinguishing it from *T. lenahani* sp. n., which has a sinuated labral peristome and a different columellar peristome, as well from the syntopic *T. spelaea* sp. n., the peristome of which lacks significant sinuation on both sides. From *T. bollingi* Davis, 1968 and *T. burchi* Davis, 1968 it differs by its shell and aperture shapes.

Description. The shell is rounded oval-conical with four slightly inflated whorls with elevated spire and a deeper suture. The surface is milky whitish and smooth with faint growth lines. The aperture is oval ear-shaped, the peristome slightly callous attached to the body whorl and expanding only towards the columella. The labral lip lateral profile is straight, while a characteristic deep sinuation is present at the apical inner lip. The last whorl is broadening towards the aperture and from lateral view is curved upward. The umbilicus is closed.

Etymology. Named after George M. Davis, (Washington D.C., USA) who contributed much to the molecular phylogeny and taxonomy of the Mekong River Pomatiopsidae.

Distribution. Only known from the type locality and nearby sites in Tham Khon Dôn Cave as well as from the related source of Nam Dôn River.

Ecology. The same as *Pseudoiglica pseudoiglica* sp. n.

***Tricula spelaea* sp. n.**

<http://zoobank.org/2665A01E-E4EA-4D7E-BEC2-A91FFF12085C>

Figs 33–36

Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Khon Dôn Cave 17°33.82'N; 104°52.30'E, 161 m a.s.l., Earthquake Dome 3 km from the south entrance, sand sediments on cave river bank (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšovský leg. 11–12 February 2017 (NHMUK 20180011). Paratypes: type locality (Grego F0880 – 3 specimens); Laos, Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Pha Soung Cave, 17°33.052'N; 104°52.410'E, 155 m a.s.l., sandy sediment on the bottom of Frog Lake at central part of the cave (coll. Grego F0886 – 2 specimens).

Measurements. Holotype: H 2.80 mm; W 1.65 mm; BW 1.05 mm; BH 1.80 mm; AH 1.10 mm; AW 0.95 mm; H/W 1.70; AH/AW 1.16; W/BW 1.57; H/BH 1.56; H/AH 2.55; W/AW 1.74.

Diagnosis. This new species is similar to syntopic *Tricula lenahani* sp. n., from which it differs by its more conical shell with less inflated whorls, a closed umbilicus and the different shape of the aperture. It differs significantly from syntopic *T. valenasi* sp. n. by its shorter, more inflated shell shape, more flattened whorls, straight lateral labral profile and closed umbilicus. From the *T. bollingi* Davis, 1968 it differs by its shell and aperture shape, position of the aperture and a more blunt apex. From *T. burchi* Davis, 1968, it differs by its more conical shape with a smaller aperture. The labral lateral profile of *T. spelaea* sp. n. is straight, and no sinuation is present on its columellar side.

Description. The shell shape is conical with five somewhat flattened but still convex whorls with a weak suture and blunt apex. The surface is milky whitish and smooth. The aperture is oval tear-shaped; the peristome slightly callous expanding outwards. The labral lip lateral profile is straight, and no sinuation is present at the apical inner lip. The umbilicus is closed.

Etymology. Named after its type locality inside the Cave Tham Khon Dôn in Khammouane.

Distribution. Only known from the type locality and nearby sites in Tham Khon Dôn Cave as well from the related source of Nam Dôn River.

Ecology. The same as *Pseudoiglica pseudoiglica* sp. n.

***Tricula reischuetzorum* sp. n.**

<http://zoobank.org/A23FADBE-1D6C-42A8-876D-0D0AF3B313B8>

Figs 37–38

Type locality. Laos; Khammouane Province, Cave Tham Na Li 8 km E of Thakhek on road AH131, bottom of cave river Nam Xiangliap, 17°27.20'N; 104°54.54'E.

Type material. Holotype: type locality: J. Grego leg. 16 February 2017 (NHMUK 20180012).

Measurements. Holotype: H 2.68 mm; W 1.29 mm; BW 0.86 mm; BH 1.61 mm; AH 0.93 mm; AW 0.79 mm; H/W 2.08; AH/AW 1.18; W/BW 1.50; H/BH 1.66; H/AH 2.88; W/AW 1.63.

Diagnosis. The shell is similar to that of *Tricula valenasi* sp. n. (Tham Khon Dôn Cave), from which it differs by being smaller, less elongate and with more inflated whorls, a more closed umbilicus and a more elongated aperture situated more towards the columella. It differs significantly from *T. lenahani* sp. n. (Tham Khon Dôn Cave) by its overall shell shape, the position of aperture and the closed umbilicus. From *T. bollingi* and *T. burchi* it differs by general shell and aperture shape and the position of the aperture.

Description. The milky white, narrow oval-conical shell with four convex whorls and deep suture has a blunt apex. The shell surface is smooth and shiny. The shell is narrow-conical. Aperture is ovoid elongated and its lower part slightly angled towards the columella. The aperture is attached to the body whorl by a weak furrow. The peristome margin is sharp, somewhat darker stained. The outer lip is slightly sinuated laterally. Umbilicus is closed.

Etymology. Named after active researchers of the Balkan stygobiont gastropod fauna, Peter L. and Alexander Reischütz (Horn, Austria), who brought our attention to the Na Li cave.

Distribution. Only known from the type locality.

Ecology. The shells were found in the sandy sediment inside the cave Tham Na Li close to the river outlet. The about 300m long cave passage was formed by the river Nam Xiangliap under the limestone hill, and thus the cave habitat has a direct contact with surface waters.

***Tricula phasoungensis* sp. n.**

<http://zoobank.org/FD3FC3E5-702E-4796-A928-9064C91A5E52>

Figs 39–42

Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Pha Soung Cave, 33.052'N; 104°52.410'E, 155 m a.s.l., sandy sediment on the bottom of Frog Lake at central part of the cave (Fig. 2F).

Type material. Holotype: type locality: J. Grego leg. 09 February 2017 (NHMUK 20180013).

Paratypes: type locality (Grego F0887 – 1 specimen); Laos, Khammouane Province; Ban Na village 20 km NNE of Thakhek; Tham Khon Dôn Cave, 17°33.82'N; 104°52.30'E, 161 m a.s.l., Earthquake Dome 3 km from the S entrance, sand sedi-

ments on cave river banks (NHMUK 20180023 – 3 specimens; HNHM 102776 – 3 specimens; OSUM 42388 – 3 specimens; coll. Grego F0881 – 60 specimens).

Measurements. Holotype: H 1.91 mm; W 1.20 mm; BW 0.70 mm; BH 1.25 mm; AH 0.82 mm; AW 0.65 mm; H/W 1.59; AH/AW 1.26; W/BW 1.71; H/BH 1.53; H/AH 2.33; W/AW 1.85; Paratype 2: H 2.05 mm; W 1.43 mm; BH 0.75 mm; BW 1.35 mm; AH 1.06 mm; AW 0.84 mm; H/W 1.43; AH/AW 1.26; W/BW 1.91; H/BH 1.52; H/AH 1.93; W/AW 1.70.

Diagnosis. With its small hydrobioid shell it is similar to *T. bannaensis* sp. n., but differs from it by its more slender shell, its less inflated whorls, its more closed umbilicus and the shape of the aperture. The similar but larger *T. lenahani* sp. n. differs by having less inflated whorls, different shell and aperture shapes and a narrower umbilicus. *Tricula reischuetzorum* sp. n. has a more elongate shell with more inflated whorls, a smaller umbilicus and a different shape of the aperture.

Description. The milky whitish shell has four convex whorls with a deep suture and a smooth, shiny surface. The bythinelloid-shaped shell is oval sub-conical with a blunt apex. The aperture is oval; the peristome is slightly callused and outwardly expanded and connected to the body whorl. The lateral edge of the labral lip is very slightly sinuated. The umbilicus is open and conspicuous.

Etymology. Named after the type locality inside the Pha Soung Cave system, which is 20.4 km long and the third longest cave in Laos.

Distribution. Only known from the type locality and nearby sites in Tham Khon Dôn Cave as well as in the related source of Nam Dôn River.

Ecology. Shells were found in the sandy sediment of a small and shallow “Frog Lake” (Fig. 2F) in the central part of the Pha Soung Cave system. The site is a shallow 20–30 cm deep pond at the lowest part of a cave meander passage, which holds the remains of water after seasonal water crossflow. Green-black streaked frogs *Rana chloranata* (Günther, 1876) washed from the surface inhabit the pond. The Pha Soung cave system (fig. 2E) is 20.4 km long and is situated under the massif of Mount Pha Soung near village Ban Na in SW Khammouane. The cave consists of several floors of old corrosive and phreatic passages developed during the past 11 MY of speleogenesis in the Khammouane limestone of Carboniferous/Permian age. During the rainy season it drains the Ban Na polje towards a closed flat bottom karstic depression in the southern part of the Pha Soung Mountain and then downwards to the Mekong basin. Many of the entrances act not only as sinkholes, but seasonally also as large springs, which indicate a connection to the deep karst phreatic zone also situated under the Ban Na polje.

***Tricula bannaensis* sp. n.**

<http://zoobank.org/C27849D1-8078-44EE-ADB7-BDDF40E06F18>

Figs 43–44

Type locality. Laos; Khammouane Province, Ban Na village 20 km NNE of Thakhek, Tham Khon Dôn Cave, 17°33.82'N; 104°52.30'E, 161 m a.s.l., Earthquake Dome 3 km from the south entrance, sand sediments on cave river banks (Fig. 2B).

Type material. Holotype: type locality: J. Grego and M. Olšovský leg. 11–12 February 2017 (NHMUK 20180014). Paratypes: type locality (NHMUK 20180024 – 5 specimens; HNHM 102777 – 5 specimens; OSUM 42389 – 5 specimens; Grego F0904 – 277 specimens).

Measurements. Holotype: H 1.90 mm; W 1.41 mm; BW 0.75 mm; BH 1.15 mm; AH 0.92 mm; AW 0.76 mm; H/W 1.35; AH/AW 1.21; W/BW 1.88; H/BH 1.65; H/AH 2.07; W/AW 1.86.

Diagnosis. The small hydrobioid shell is similar to that of *T. phasoungensis* sp. n., from which it differs by being more robust and with more inflated whorls, and by its larger umbilicus and larger aperture. *Tricula lenahani* sp. n. has a larger shell with less inflated whorls and a different shape of the aperture.

Description. The whitish, translucent shell has four inflated convex whorls with a deep suture. The shell is inflated ovoid-conical with an oval aperture and slightly outward reflexed margins. Its inner side is attached to the body whorl by a marginal callus. The labral lip is typically sinuated at its lateral profile. The umbilicus is open and conspicuous.

Etymology. Named after the village Ban Na, where the team enjoyed the hospitality of villagers in our base camp in the local Buddhist temple.

Distribution. Only known from the type locality and nearby sites in Tham Khon Dôn Cave as well as in the related source of Nam Dôn River.

Ecology. The same as *Pseudoiglica pseudoiglica* sp. n.

***Tricula viengthongensis* sp. n.**

<http://zoobank.org/4169E1A2-FE23-470A-9B52-D00F67AED790>

Figs 45–48

Type locality. Laos; Bolikhamsay Province, 16 km West of Vieng Thong, 500 m North of the road from Vieng Thong to Ban Samsok Noy (and Sôp Sang), unnamed cave with entrance above large karst spring with travertine cascades (Fig. 2D), 18°34.080'N; 104°31.79'E, sand sediments on the bottom of cave rivulet.

Type material. Holotype: type locality: J. Grego and B. Šmída leg. 19 February 2017 (NHMUK 20180015) Paratypes: , type locality (coll. Grego F0904 – 3 specimens).

Measurements. Holotype: H 2.64mm; W 1.64mm; BW 0.93mm; BH 1.71mm; AH 1.14mm; AW 1.00mm; H/W 1.6; AH/AW 1.14; W/BW 1.76; H/BH 1.54; H/AH 2.32; W/AW 1.64.

Diagnosis. The small conical shell is similar to that of *T. valenasi* sp. n. (Khammouane Province) from which it differs by its smaller size and its more blunt apex. From *T. lenahani* sp. n. (Khammouane Province) it differs by having a smaller shell with a weaker suture and a closed umbilicus. *Tricula phasoungensis* sp. n. and *T. bannaensis* sp. n. have a more robust and rounded shell with more convex whorls and an open umbilicus.

Description. The shell of *Tricula viengthongensis* sp. n. is whitish, semi-translucent with four slightly flattened convex whorls and a weak suture. The shape of the shell

is conical with a blunt apex and an oval ear shaped aperture. The marginal lips with a slight callosity are not reflexed and laterally have a straight profile without any sinuation. The umbilicus is closed.

Etymology. Named after the city of Vieng Thong (Bolikhamsay Province), the larger settlement closest to the type locality.

Distribution. Only known from the type locality.

Ecology. The unnamed cave is situated immediately above a large karstic spring with travertine cascades (fig. 2D), and drains a limestone ridge NW of the main water outlet. The cave entrance is a 15m vertical abyss continuing upstream the underground river to a siphon lake. The total length is approximately 400 m with several parallel draining passages. The specimens were collected from sandy sediment at the bottom of a cave stream about 40 m upstream from the entrance.

Discussion

The present study indicates that the underground freshwater gastropod species are more widely distributed in the Southeast Asian habitats than was hitherto supposed. The wide diversity of karstic and cave habitats in the region (Culver 2012) together with the extraordinary high diversity of the surface and troglobiont gastropod species (Culver 2012) indirectly predicted rich stygobiont malacocenoses. We believe the habitat preferences and the factors driving their diversity are the same as we assumed for the stygobiont habitats of the Balkans (Grego et al. 2017) and subsequent SE Asian studies could prove their presence also in the local non-karstic springs, wells and adjacent groundwater systems. Additionally, the natural history of the area had not been influenced by the dramatic climate changes over the Late Cenozoic and Holocene and this may have facilitated uninterrupted diversification through evolutionary adaptation towards a very rich subterranean diversity.

The shell shape evolutionary convergence between the studied SE Asian species and stygobiont species from other parts of the world is remarkable. This indirectly confirms our theory (Grego et al. 2017), that the slender elongated shape could be evolved in the species preferably inhabiting habitats with permanently higher water velocity within the small caverns of gravel and cracklings. The shell shape of *Iglica* Wagner, 1910, *Pseudoiglica* gen. n. and *Thamkhondonia* gen. n. species provides lower frontal hydrodynamic resistance by turning the shell in the flow direction avoiding the shear stress caused by turbulences and thus preventing dislodging from the substrate. Gastropods with globose shell would have a problem to stay attached at high water velocity as also the adaptive evolution of gastropod mucus adhesion to the substrate has some limitations. Small species living interstitially among smaller gravel or sand have still elongated but proportionally shorter shells (*Paladilhioopsis* Pavlović, 1913, *Thamkhondonia smidaï* sp. n., *Tricula reischuetzorum* sp. n.) to enable movement under the limitation by the smaller space. The species adapted to calmer waters within larger cavi-

ties such as cave lakes tend to have more globose shapes (*Dabriana* Radoman, 1974, *Horatia* Bourguignat, 1887, *Pontohoratia* Vinarsky et al., 2014, *Motsametia* Vinarsky et al., 2014, *Tricula* Benson, 1843). The species living in habitats with alternating water velocities (calm waters with frequent occasional floods and temporary very high water velocity) have developed their survival strategy by the globose, but more robust shell shape with more thick shell walls, additionally with broadening apertures (sometimes up to a limpet like shape). The expanded aperture has frequently folds and reflexions along the margin to help attachment of the animal on the rocky substrate during the interim periods with high water velocity. (*Plagigeyria* Tomlin, 1930, *Tricula davisi* sp. n., *Tricula lenahani* sp. n., *Pseudotricula eberhardi* Ponder, 1992)

As in the other well-known hotspots of stygobiont gastropod biodiversity (Pyrenees, Dinarides) (Darwall et al. 2014) the hidden, hardly accessible subterranean habitats only seldom allow researchers to obtain live specimens for anatomical and molecular studies (Glöer and Grego 2015). This hidden habitat was the main reason why the underground species had been overlooked for such a long time.

However, Laos as well as other SE Asian countries are facing the same ecological threats as most tropical countries worldwide: deforestation, drought, erosion and environmental pollution, together with artificial dam construction, which floods valleys and basins with stagnant water. These negative anthropogenic influences could negatively impact the more stable underground aquatic habitats and all of the sensitive stygobiont/troglobiont species could rapidly vanish prior to the scientists having a chance to fully understand their biology and role in the subterranean ecosystem.

Conclusions

This study confirmed the presence of underground freshwater gastropod species in Laos. Species inhabiting similar habitats were mainly known only from North and South America, North Africa, Europe and the Balkans through Turkey and the Caucasus to Central Asia (Bole and Velkovrh 1986, Kabat and Hershler 1993). The localities from Laos are situated between the single so far known locality in Sri Lanka (wells in the village of Pokonwita, south of Horana) and the few localities hitherto known throughout the Japanese Archipelago (Mori 1938; Kuroda and Habe 1957; Habe 1965; Kuroda 1963; Matsumoto 1976; Bole and Velkovrh 1986). The new finds partly fill the zoogeographical gap and also suggest their probable presence in a much wider area than hitherto supposed: from Naga Hills and Arunachal-Pradesh in India through Myanmar, Thailand, Laos and Vietnam to South China (YunNan and GuangXi) and likely northward to the central and east mainland of China and Korea. I hope this study will encourage all people involved in local biospeleological investigation throughout SE Asia to focus more towards the so far underestimated diversity of subterranean Mollusca and thus gather more knowledge about their biology, anatomy and phylogeny in the near future.

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References

- Annandale N (1919) The gastropod fauna of old lake beds in upper Burma. *Records of Geological Survey of India* 50: 209–240. [3 pls]
- Bole J, Velkovrh F (1986) Mollusca from continental subterranean aquatic habitats. In: Botosaneanu P (Ed.) *Stygofauna mundi*, Brill and Backhuys Publishers, Leiden, 177–208.
- Brandt RAM (1968) Descriptions of new non-marine mollusks from Asia. *Archiv für Molluskenkunde* 98: 213–289.
- Brandt RAM (1970) New freshwater gastropods from the Mekong. *Archiv für Molluskenkunde* 100: 183–205.
- Brandt RAM (1974) The non-marine aquatic Mollusca of Thailand. *Archiv für Molluskenkunde* 105(1–4): 423 pp. [30 pls]
- Brandt RAM, Temcharoen P (1974) The molluscan fauna of Mekong at the foci of schistosomiasis in south Laos and Cambodia. *Archiv für Molluskenkunde* 101: 111–140.
- Crosse H, Fischer P (1879) Mollusques fluviatiles, recueillis au Cambodge, par la Mission scientifique française de 1873. *Journal de Conchyliologie* 24: 313–342. [pls 10–11]
- Culver DC (2012) Mollusks. In: White WB, Culver DC (Eds) *Encyclopedia of Caves* (Second Edition). Academic Press, New York, 512–517. <https://doi.org/10.1016/B978-0-12-383832-2.00074-8>
- Darwall W, Carrizo S, Numa C, Barrios V, Freyhof J, Smith K (2014) Freshwater Key Biodiversity Areas in the Mediterranean Basin Hotspot. Informing species conservation and development planning in freshwater ecosystems. IUCN, Cambridge, UK and Malaga, Spain.

- Davis GM (1979) The origin and evolution of the gastropod family Pomatiopsidae, with emphasis on the Mekong River Triculinae. Academy of Natural Sciences of Philadelphia, Monograph 20: 1–120.
- Davis GM, Chen Cui-E, Wu Cchun, Kuang Tiee-Fu, Xing Xin-Guo, Li Li, Liu Wen-Jian, Yan Yu-Lun (1992) The Pomatiopsidae of Hunan, China (Gastropoda: Rissoacea). *Malacologia* 34(1-2): 143–342.
- Deshayes PG, Jullien J (1876) Mémoire sur les Mollusques nouveaux du Cambodge envoyés au museum par M.le doctéaur Jullien. *Bulletin des Nouvelles Archives du Museum* 10(1874): 115–162. [pls 5–9]
- Glöer P, Grego J (2015) New subterranean freshwater Molluscs from Bosnia and Hercegovina (Mollusca: Hydrobiidae). *Ecologica Montenegrina* 2(4): 307–314.
- Grego J, Glöer P, Eröss ZP, Fehér Z (2017) Six new subterranean freshwater gastropod species from northern Albania and some new records from Albania and Kosovo (Mollusca, Gastropoda, Moitessieriidae and Hydrobiidae). *Subterranean Biology* 23: 85–107. <https://doi.org/10.3897/subtbiol.23.14930>
- Habe T (1965) Descriptions of one new species and one new subspecies of freshwater Gastropods from Japan. *Venus* 23(4): 20–209.
- Hershler R, Ponder WF (1998) A review of Morphological Characters of Hydrobioid snails. *Smithsonian Contributions to Zoology* 600: 1–55. <https://doi.org/10.5479/si.00810282.600>
- Inkhavilay K, Sutcharit C, Tongkerd P, Panha S (2016) New species of micro snails from Laos (Pulmonata: Vertiginidae and Diapheridae). *Journal of Conchology* 42(4): 213–232.
- Kabat AR, Hershler R (1993) The prosobranch snail family Hydrobiidae (Gastropoda: Rissooidea): review of classification and supraspecific taxa. *Smithsonian Contributions to Zoology* 547: 1–94. <https://doi.org/10.5479/si.00810282.547>
- Kuroda T, Habe T (1957) Troglolobiontic aquatic snails from Japan. *Venus* 19: 183–196.
- Kuroda T (1963) A Catalogue of the non-marine Molluscs of Japan, including the Okinawa and Ogasawara Islands. A Congratulatory Publication on the 77th Birthday of Dr. Tokubei Kuroda, 7–71.
- Maassen WJM (2008) Remarks on a small collection of terrestrial molluscs from north-west Laos, with descriptions of three new species (Mollusca: Pulmonata: Streptaxidae, Vertiginidae). *Basteria* 72: 233–240.
- Matsumoto K (1976) An introduction on the Japanese groundwater animals with reference to their ecology and hygienic significance. *International Journal of Speleology B*: 141–155. <https://doi.org/10.5038/1827-806X.8.1.13>
- Mori S (1938) Molluscan fauna of the limestone caves Miyakozirna of the Ryukyu Islands. Description of two new molluscs *Cochliopopsis basiangulata* n.g. n.sp. and *Pisidium cavernicum* sp. n.. *Transactions of the Biogeographical Society of Japan* 3(1): 110–114.
- Mouret C (2005) Main 2001 to early 2005 results on the karst of Khammouane, central Laos: long caves, sloping caves, hollow stalagmites and others. In: *Proceeding of the 14-th International Congress of Speleology (Athens-Kalamos, 21–18 August 2005)* Paper No. Stuttgart, 143: 411–414.

- Páll-Gergely B (2014) Description of the second *Laotia* Saurin, 1953; a genus new to the fauna of Vietnam (Gastropoda: Cyclophoroidea). *Folia Malacologica* 22(4): 289–292. <https://doi.org/10.12657/folmal.022.025>
- Páll-Gergely B, Fehér Z, Hunyadi A, Asami T (2015) Revision of the genus *Pseudopomatias* and its relatives (Gastropoda: Cyclophoroidea: Pupinidae). *Zootaxa* 3937(1): 1–49. <https://doi.org/10.11646/zootaxa.3937.1.1>
- Páll-Gergely B, Muratov IV, Asami T (2016) The family Plectopylidae (Gastropoda, Pulmonata) in Laos with the description of two new genera and a new species, *ZooKeys* 592: 1–26. <https://doi.org/10.3897/zookeys.592.8118>
- Páll-Gergely B, Hunyadi A, Đõ ĐS, Naggs F, Asami T (2017) Revision of the Alycaeidae of China, Laos and Vietnam (Gastropoda: Cyclophoroidea) I: The genera *Dicharax* and *Metalycaeus*. *Zootaxa* 433(1): 1–124. <https://doi.org/10.11646/zootaxa.4331.1.1>
- Strong EE, Gargominy O, Ponder WF, Bouchet P (2007) Global diversity of gastropods (Gastropoda: Mollusca) in freshwater. *Hydrobiologia* 595: 149. <https://doi.org/10.1007/s10750-007-9012-6>
- Temcharoen P (1971) New aquatic mollusks from Laos. *Archiv für Molluskenkunde* 101: 91–109.

A morphological gap for Iberian *Zospeum* filled: *Zospeum percostulatum* sp. n. (Gastropoda, Eupulmonata, Carychiidae) a new species from Asturias (Spain)

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Abstract

Zospeum percostulatum sp. n. from Cueva de La Herrería (Llanes, Asturias) is described. It is characterized by a relatively large shell (1.4–1.8 mm height), conical, with ovate aperture, continuous peristome and thickened parietal callus; shell costulate except two first whorls; without any sort of inner formations. It is the first clearly costulate Iberian species, filling a morphological gap in the Iberian clade, and the largest species from the Cantabrian region, being the first species described from Asturias.

Keywords

Cave-dwelling species, troglobiont gastropod, microgastropod, shell variability, ecology

Resumen

Se describe *Zospeum percostulatum* sp. n. de la Cueva de La Herrería (Llanes, Asturias), caracterizada por una concha relativamente grande (1.4–1.8 mm de longitud), cónica, con abertura ovalada, peristoma continuo y callo parietal engrosado; superficie costulada excepto en las dos primeras vueltas y carente de cualquier rastro de formaciones internas. Es la primera especie ibérica nítidamente costulada, lo que llena un hueco morfológico del clado ibérico, y la de mayor tamaño de la región cantábrica, y es la primera especie descrita de Asturias.

Palabras clave

Especie cavernícola, gasterópodo troglobio, microgasterópodo, variabilidad conchológica, ecología

Introduction

The genus *Zospeum* Bourguignat, 1856 is the only troglobiont genus of land snails present in the Iberian Peninsula, and has also been for a long time the only troglobiont genus of the family Carychiidae until the recent description of the genus *Koreozospeum* (Jochum et al. 2015a). The geographical distribution comprises two disjunct areas: eastern Alps and Dinaric Alps from where about twenty species are known (Bole 1974; Pezzoli 1992; Slapnik and Ozimec 2004; Weigand 2013; Jochum et al. 2015b), and the Pyrenean-Cantabrian region from where six species have been described so far. The first Iberian species described was *Zospeum schaufussi* Frauenfeld, 1862, collected inside an unknown Spanish cave by L. Schaufuss and briefly described with 16 words (Frauenfeld 1862). Unaware of the occurrence of any *Zospeum* species in western Europe, Gittenberger (1973) attributed the novelty to *Zospeum bellesi* Gittenberger, 1973, from the Cueva de Aso (northern part of Huesca province). Realizing that *Zospeum* is more widely distributed than in the Pyrenees, Gittenberger (1980) revised new Iberian material and the type material of *Z. schaufussi*, revalidating the already known species and further describing *Zospeum suarezi* Gittenberger, 1980 from a cave in Puente Viesgo (Cantabria) and other Cantabrian caves. Gómez and Prieto (1985) described *Zospeum biscaiense*, an odd toothed species from Cueva de Otxas in Yurre (Bizkaia), and more recently Jochum et al. (2015c) have described *Zospeum vasconicum* Prieto, De Winter, Weigand, Gómez & Jochum, 2015 from several caves from Bizkaia and Gipuzkoa provinces, and *Zospeum zaldivarae* Prieto, De Winter, Weigand, Gómez & Jochum, 2015 from Cueva de Las Paúles in Berberana (Burgos). Meanwhile, Weigand et al. (2013) had already demonstrated the molecular divergence between those two species and four genetic lineages conchologically grouped under *Zospeum suarezi*, thus evidencing that the biodiversity in the Cantabrian region is still far from completion.

Although based on an incomplete species sampling, both Alpine-Dinaric and Cantabrian species groups of *Zospeum* have been recovered as monophyletic sister clades (Weigand et al. 2013), but no morphological synapomorphies have been found for any of them. With respect to the Pyrenean-Cantabrian clade, a wider morphospace in the Alpine-Dinaric clade is evident, as exposed by Kobelt (1898, pl. 218–219) or Bole (1974), with small to large (up to 2.5 mm height), globose to slender, round to widely auriculate aperture, edentate to heavily toothed, smooth to ribbed species. On the contrary, Iberian diversity is restricted to smaller (less than 2mm), smooth, edentate species, with the only exception of the dentate *Z. biscaiense*.

The finding of a population with sharply costulated and relatively large shells allow us to describe it as a new species, since all known Iberian species have smooth shells, and only in one of the Cantabrian species, *Z. zaldivarae*, shells reach 1.6 mm in height (Jochum et al. 2015c).

Material and methods

The material studied has been gathered up mainly by flotation of fully desiccated clay sediments collected at the foot or holes of the walls where white, empty shells, have been detected. Shredded sediment was sieved through a fine mesh, then submerged in water, so most part of intact shells, together with organic remains, passes to the flotsam due to the formation of a bubble air inside them. Live individuals have also been collected through direct search on concretinated cave walls covered by a wet film of percolated clay.

SEM photographs were obtained with a Quanta 200 microscope; the other photographs are combined images produced through the Heliconfocus v.6.7.1 software from series of photographs obtained with a Nikon DS5M camera mounted on a stereomicroscope Nikon SMZ-1500. Shell measurements were done using the image analysis software of the Nikon DS5M camera. The analysis of the measurements was done with the PAST software (Hammer 2017) and the distribution map with the DMAPW software (Morton 2004).

Abbreviations

Public institutions:

MNCN Museo Nacional de Ciencias Naturales, Madrid.

MHNS Museo de Historia Natural, Universidad de Santiago de Compostela, Santiago de C.

ZUPV Colección del Departamento de Zoología, Universidad del País Vasco, Bilbao.

Private collections:

CAA Colección Álvaro Alonso.

CSQS Colección Sergio Quiñonero-Salgado.

Systematics

Family Carychiidae Jeffreys, 1830

Genus *Zospeum* Bourguignat, 1856

Type species. *Carychium spelaeum* Rossmässler, 1839 (Bourguignat 1856, orig. des.)

Zospeum percostulatum sp. n.

<http://zoobank.org/9E01B0C5-FF3E-413C-B41C-6D2F07043AB9>

Type locality. Cueva de La Herrería (also known as Las Herrerías or La Mina) (43°23.98'N, 4°45.95'W, 30TUP5700006726, 45 m), La Pereda village, municipality

of Llanes (Asturias) (Fig. 1). The cave has a wide entrance, 2.5 m high, forming a deep shelter, continued by a short gallery on the left, about 20 m long, that leads to a series of small chambers, with height decreasing towards the end to less than 1 m. Most of the right side of the cave is labyrinthic and, because of its low height and the density of columns, some parts are almost impenetrable. Descending south-west from the smaller secondary entrance, there is a large (but quite low) diamond shaped chamber with four openings. To the south, this chamber leads to a long gallery with signs of sporadic hydraulic activity. To the west, protected by a fence, there is a chamber where one of the few samples of not figurative prehistoric painting in the north of Spain can be found, consisting of red grills made by parallel lines enclosed in a quadrangular contour, but lacking any signs of animal figuration (Jordà and Mallo 1972).

This cave was firstly named as Cueva de Bolao by Jeannel and Racovitza (1915) in their account of the exploration made in 1913 by the archaeologist H. Breuil. Cueva El Bolao was treated in some works as a nearby -but different- cave. Our explorations on the surroundings of La Herrería seem to confirm the suspicion of Bolívar (1923), who first pointed that El Bolao and La Herrería may be just two different names of a single cavity.

Type material. Holotype, a complete specimen (in etanol 96°) [MNCN 15.05/200017H, ex ZUPV-4885], 18.07.2017, Á. Alonso, C. Prieto, S. Quiñonero-Salgado, J. Ruiz-Cobo leg.

Paratypes: 16 adult shells and 5 complete specimens [ZUPV-4885]; 50 adult shells [ZUPV-4913]; 30 adult shells [CAA-0737-A]; 25 adult shells [CSQS]. Paratypes from the sample ZUPV-4914 will be housed also in MNCN [15.05/200017P], Naturhistorisches Museum Wien and Naturalis Biodiversity Center.

Other material. 40 adult shells [ZUPV-4863]: Cueva Collubina (43°23.94'N, 4°43.37'W, 30TUP6048806568, 45 m), San Roque del Acebal, municipality of Llanes (Asturias), 18.07.2017, Á. Alonso, C. Prieto, S. Quiñonero-Salgado, J. Ruiz-Cobo leg.

Diagnosis. Species characterized by a relatively large shell, if compared to other Spanish *Zospeum* species, raised spire, ribbed surface, ovate opening, continuous peristome and absence of apertural teeth or internal lamellae.

Description (Figs 2–4). Shell 1.4–1.8 mm in length, fragile, hyaline when fresh, elongate to conic-ovate in smaller shells, with spire formed by 5–5.75 whorls. Protoconch (Figs 2F, 3B–D) rounded, somewhat globose, apparently smooth, with a small core and a very wide first half whorl, with a smooth surface that extends for a little more than a whorl, reaching a diameter of 0.4 mm. At high magnification it can be seen that the shell surface is full of spirally aligned small depressions formed by dots or short lines (Fig. 3C–D).

Teleoconch has about four well convex whorls, slightly shouldered and separated by a deep suture. These whorls present a sculpture of prosocline, narrow, sharp ribs with a rounded profile, somewhat irregularly arranged and sometimes incomplete, without reaching the inferior suture; its number and robustness increases every whorl and in the last one it can be 50 or more (8–10/0.5 mm).

Last whorl is near 60 % of the shell height, a bit more in smaller shells, barely and progressively ascending towards the aperture, where the rear ribs are somewhat closer together. Aperture ovoid, somewhat oblique, with almost vertical columellar border,

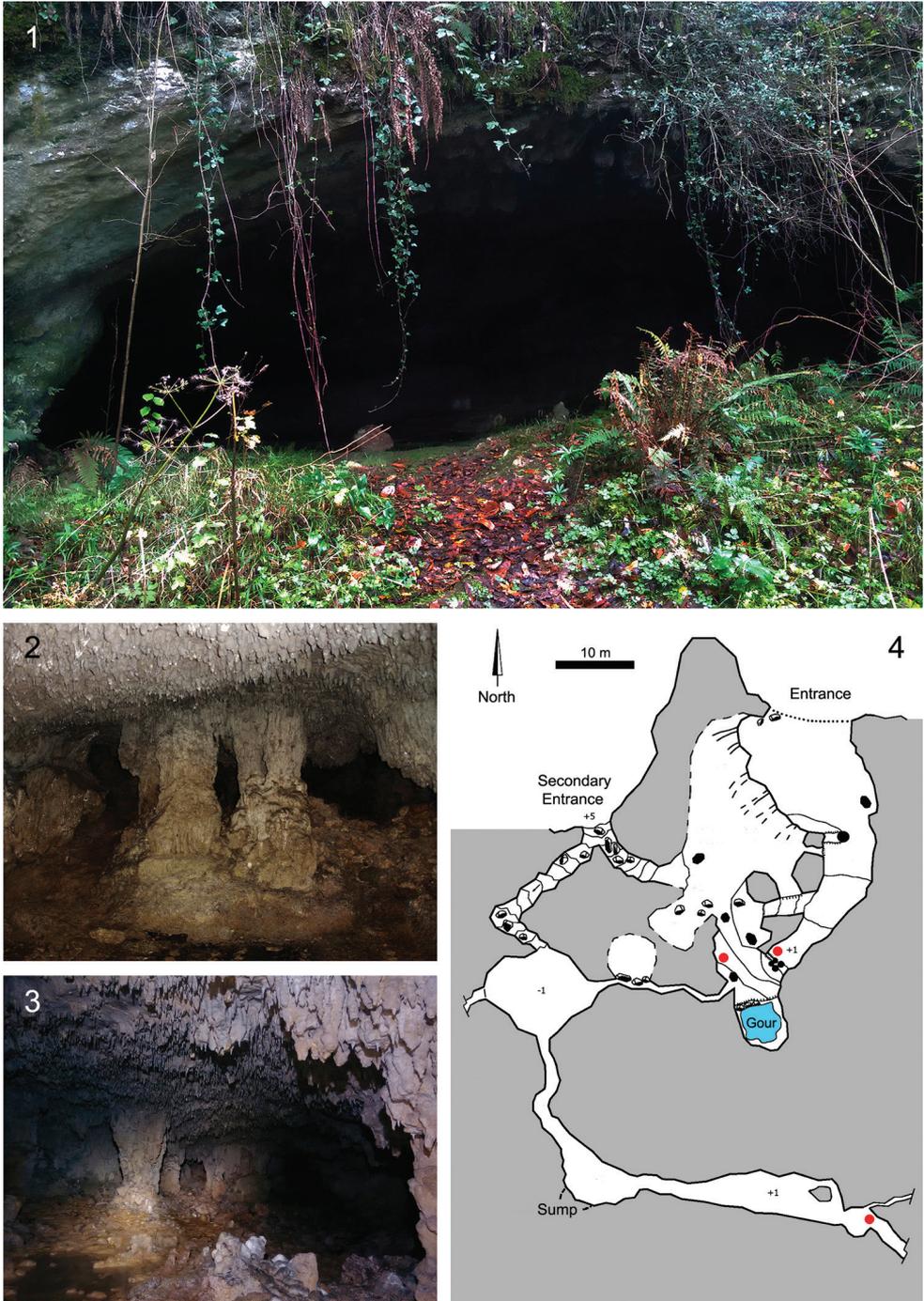


Figure 1. Cueva de La Herrería. Main entrance (1), two views of the *Zospeum* biotope (2, 3) and cave plan (4) (red dots: *Zospeum* sites). Photos and plan: S. Quiñonero-Salgado / A. Alonso.

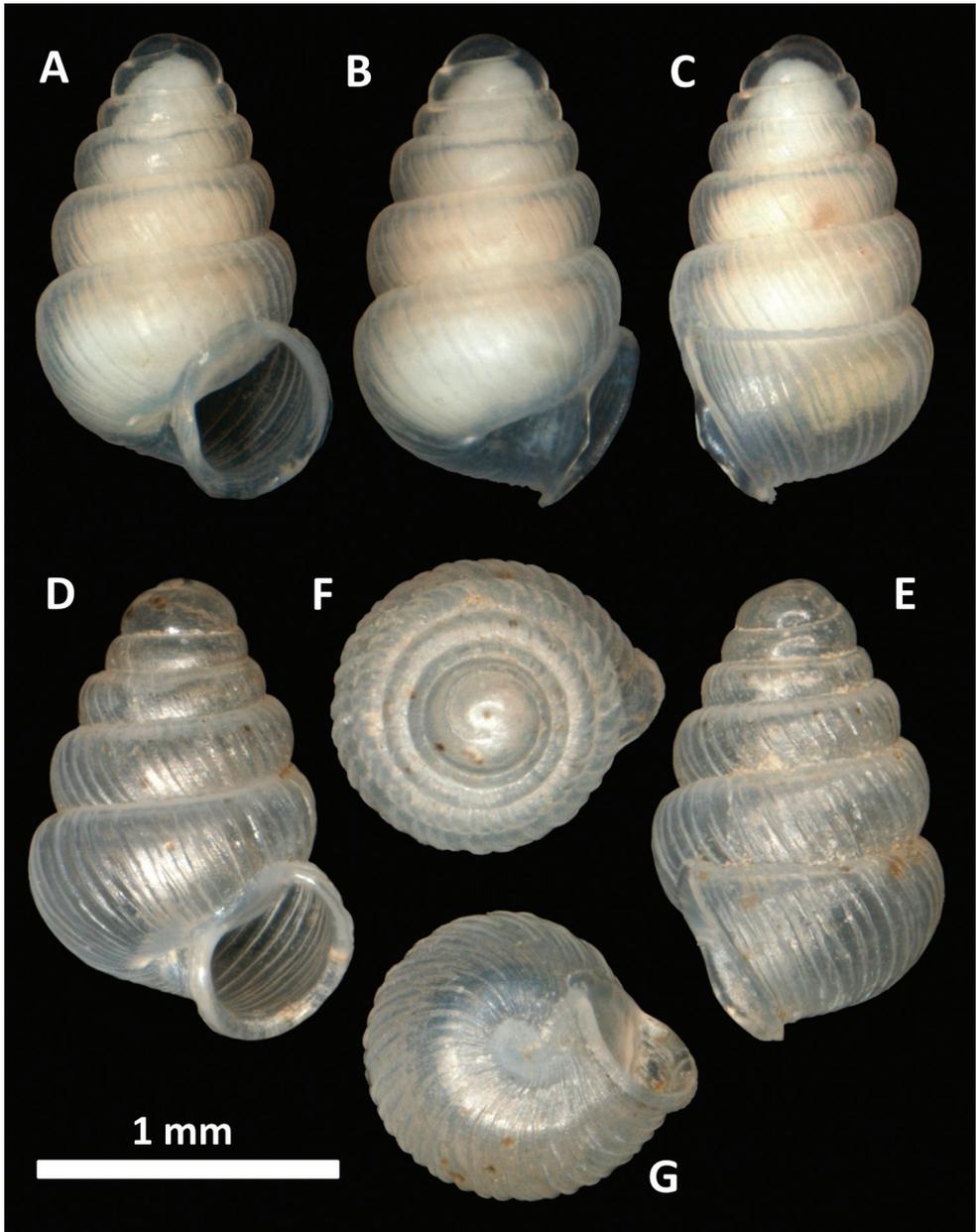


Figure 2. *Zospeum percostulatum* sp. n. Different views of the holotype (**A–C**) and a paratype 1.58 mm height (**D–G**). Note the inner whitish/yellowish mass in the holotype corresponds to their soft parts and the different orientation of the longer aperture axis between **A** and **D**.

rounded outer edge and parietal edge delineated by a parietal callus, thickened in more stylized shells. Peristome reflected, sometimes bilabiate, and thickened, especially in the columellar border. Last whorl without internal formations, with cylindrical colu-

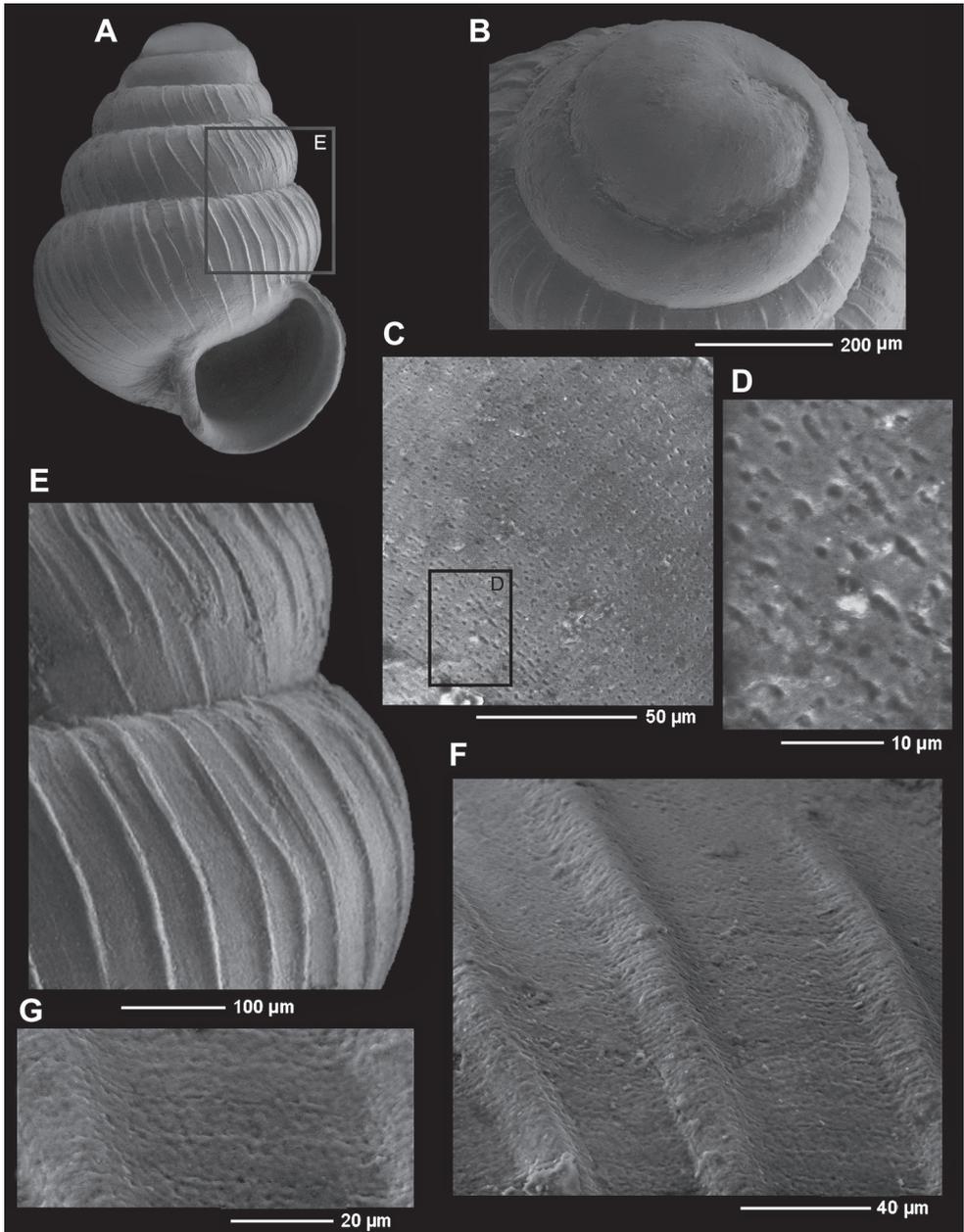


Figure 3. *Zospeum percostulatum* sp. n. Frontal view of a paratype (A, 1.42 mm height) [MHNS] and some partial views (B–G): B protoconch C microsculpture of the protoconch D detail selected in C E suture and costulae of the last whorl F shape of costulae G microsculpture of the teleoconch.

mella, 0.15 mm in diameter. Umbilicus shallow, with imperceptible umbilical groove. Shell surface seems smooth, but irregular longitudinal lines crossed with little marked spiral lines can be seen at high magnification (Fig. 3F–G).

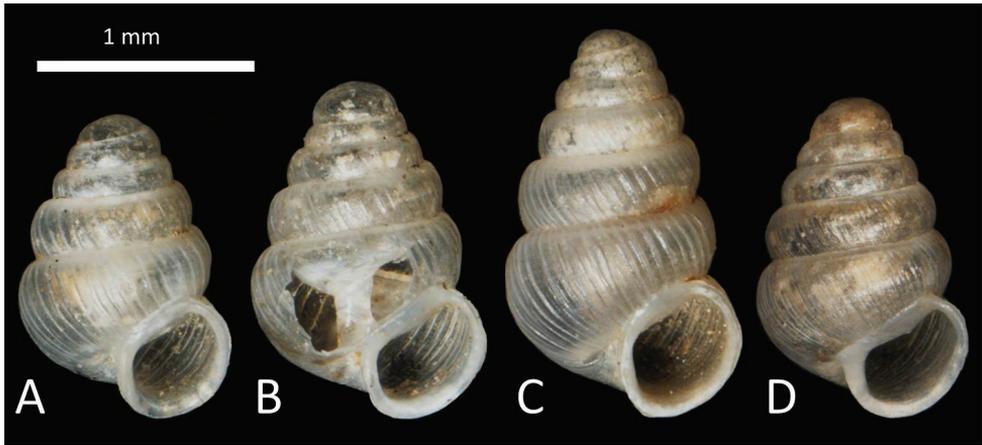


Figure 4. *Zospeum percostulatum* sp. n. Frontal views of three paratype shells (**A–C** 1.41, 1.80, 1.58 mm) and a shell from Cueva Collubina (**D** 1.49 mm) [ZUPV]. Note the robust, smooth columella through the window opened in the body whorl (**C**) and closer, smaller riblets in **D**.

Dimensions. The holotype shell measures 1.59 mm height and 1.00 mm width (Fig. 2A–C), and the body whorl reaches 58 % of the shell height. Morphometric data from the type locality and Cueva Collubina (Table 1, Fig. 5) indicate that both populations are somewhat distinct, being formed the later by somewhat smaller individuals with more closer riblets per whorl.

Etymology. The specific epithet refers to the ribbed surface of the shell in diminutive ('costulata') with the Latin prefix 'per-' (meaning 'very') to emphasize the best diagnostic character with regard to the remaining Iberian species.

Habitat. Strict troglobiont living on wet, concretationed cave walls covered by a clay film, although it can also be found on the ground, under concretion fragments. Some shells were collected at only 35 m away from the cave entrance. Empty shells are much more abundant than live individuals, which are much harder to find because their transparent shell and light cream color camouflages them with clay and concretions. Due the absence of decalcification in empty shells, these can last a long time (perhaps centuries) and accumulate by hundreds in a litter of clay sediments at the foot of cave walls. This species shares its biotope with a distinctly smaller *Zospeum* species belonging to a conchological *suaresi* species group (see Discussion) although a topographical differentiation cannot be discarded. More external sites for *Zospeum* in Cueva de la Herrería (Fig. 1) mostly provided *Z. percostulatum* sp. n., with only a few empty shells of *Z. cf. suaresi*, whereas the most internal site provided all found living snails of this last species, many individuals in close vicinity on corrugated patches of clay, as depicted by Jochum et al. (2012: Fig. 2), but only a few empty shells of the larger *Z. percostulatum* in a proportion of 10:1. A similar microtopographical distribution has been observed in other caves from the Basque region (C. Prieto, unpub. obs.) where the smaller species concentrates on clay patches and the larger species wander lonely on stalactites and concretationed walls.

Table 1. Conchological parameters of populations of *Zospeum percostulatum* sp. n. from the type locality and Cueva Collubina. SH, shell height; SW, shell width; BWH, body whorl height; AH, aperture height; AW, aperture width; WN, whorl number. Measurements in mm except WN.

		SH	SW	BWH	AH	AW	WN
Cueva de LA HERRERIA (n=32)	HOLOTYPE	1,594	1,000	0,920	0,606	0,541	5,550
	min	1,403	0,954	0,859	0,538	0,504	4,900
	max	1,803	1,123	1,006	0,637	0,621	5,750
	mean	1,574	1,040	0,926	0,595	0,555	5,394
	st.dev.	0,097	0,044	0,034	0,026	0,025	0,224
Cueva COLLUBINA (n=10)	min	1,340	0,909	0,798	0,478	0,520	5,150
	max	1,554	0,997	0,926	0,575	0,588	5,500
	mean	1,452	0,968	0,857	0,550	0,549	5,343
	st.dev.	0,069	0,025	0,044	0,037	0,023	0,140

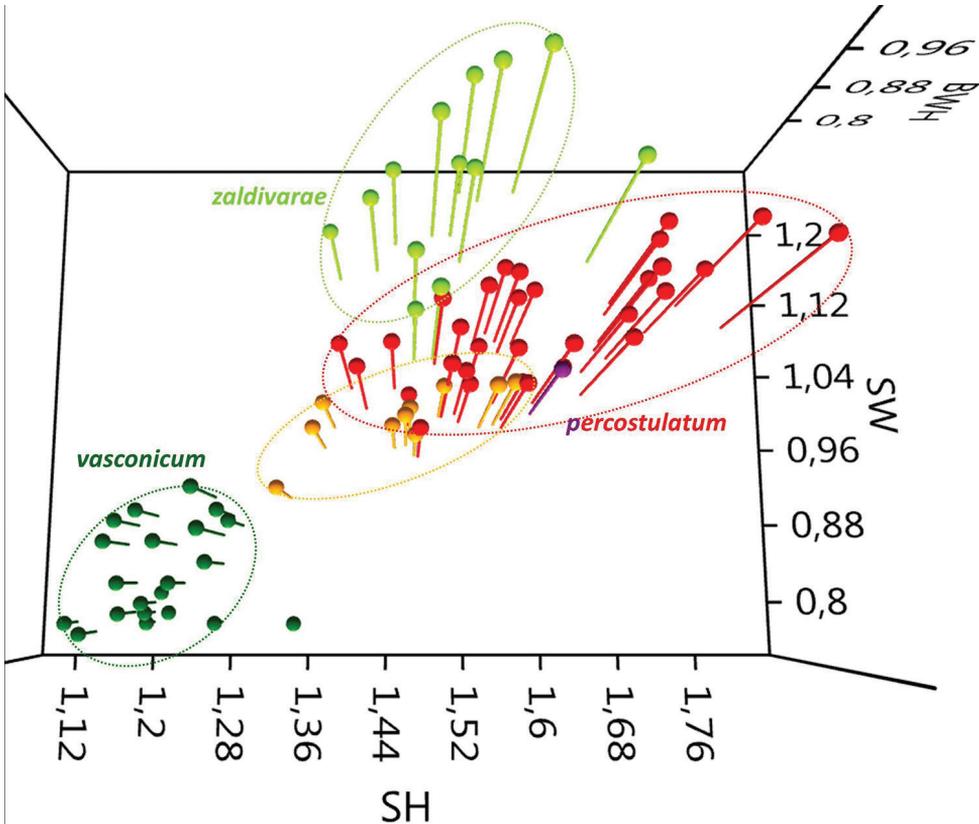


Figure 5. Tridimensional plot of main conchological parameters (SH, SW and BWH) of *Zospeum vasconicum*, *Z. zaldivarae* and *Z. percostulatum* sp. n. (data in Excel files of C. Prieto). Note the holotype (violet dot) and the smaller shells from Cueva Collubina (orange). Note also that a large shell of *Z. zaldivarae* (described in Prieto and Gómez 1985) and a slender, teratological shell of *Z. vasconicum* lie outside their circumscribing ellipses.

Regarding the biocoenosis of the cave, no other strict troglobiont gastropods (i.e. *Cryptazeca*) were found, and only some common species like *Oxychilus* sp. or *Elona quimperiana* (Blainville, 1821) seem to live inside. Other troglobiont species are the coleopterans *Laemostenus peleus* (Schaufuss, 1861), *Breuilia triangulum* (Sharp, 1872) and *Quaestus occidentalis* (Jeannel, 1911) (Jeannel and Racovitza 1915; Español 1954; Collado 1977). Jeannel and Racovitza (1915) recorded the finding of springtails, diplopods and isopods but apparently they were never published.

Distribution. *Zospeum percostulatum* sp. n. has been found in two caves 3.5 km far away from each other, placed in the lower part of northern foothills of Sierra de Cuera (Fig. 6). This sierra, a calcareous east-west formation of about 25 km in length and up to 1315 m high, not yet investigated for the genus *Zospeum*, is separated from the Picos de Europa by the Cares river, on whose northern foothills are placed the caves cited for *Z. suarezi* and *Z. schaufussi*. The distance between these caves and those inhabited by *Z. percostulatum* sp. n. is about 12–15 km.

Discussion

Shell morphology alone cannot be seen as sufficiently informative for recognizing microgastropod species of *Zospeum*, at least in certain species-groups, as was evidenced by Weigand et al. (2013) by identifying four genetic lineages having shells like *Z. suarezi* (but see e.g., Jochum et al. 2015b). Although more integrative evidence as anatomical or molecular data is desirable, the description of new clearly recognizable taxa cannot be hindered by arguing the absence of genetic data, which is the rule for most of marine or other subterranean micromolluscs, inclusively with the erection of supraspecific taxa (e.g., Jochum et al. 2015a). As *Zospeum percostulatum* sp. n. is the only one Iberian species with ribbed shell, the possibility that future genetic analyzes do not confirm their uniqueness is unlikely.

The two westernmost Iberian species, *Z. schaufussi* and *Z. suarezi*, have been recorded for Asturias in two nearby caves from Cabrales, both from Cueva de Inguanzo and the later also from Cueva de los Quesos (Gittenberger 1980). Strikingly, Gittenberger (1980) drew the unique published sketch for each species from shells from Cueva de Inguanzo, being in fact the conchological reference for both species. However, considering that the type locality of *Z. suarezi* is 70 km far eastward, that there are four (at least) cryptic species with similar conchological features (Weigand et al. 2013) and that the description of Frauenfeld (1862) for *Z. schaufussi* does not match with Gittenberger's drawing and description, the true identity of Asturian records is doubtful. Therefore, *Zospeum percostulatum* sp. n. can be considered the first unequivocal Asturian species.

Zospeum percostulatum sp. n. has been found syntopically with another *Zospeum* species having a smaller size, conical shell with well convex whorls, smooth surface, round aperture with continuous peristome and inner columellar lamella which pokes at the opening. These conchological features allow ascribing this second species to a species group, which would be integrated, at least, by the four genetic lineages evidenced by

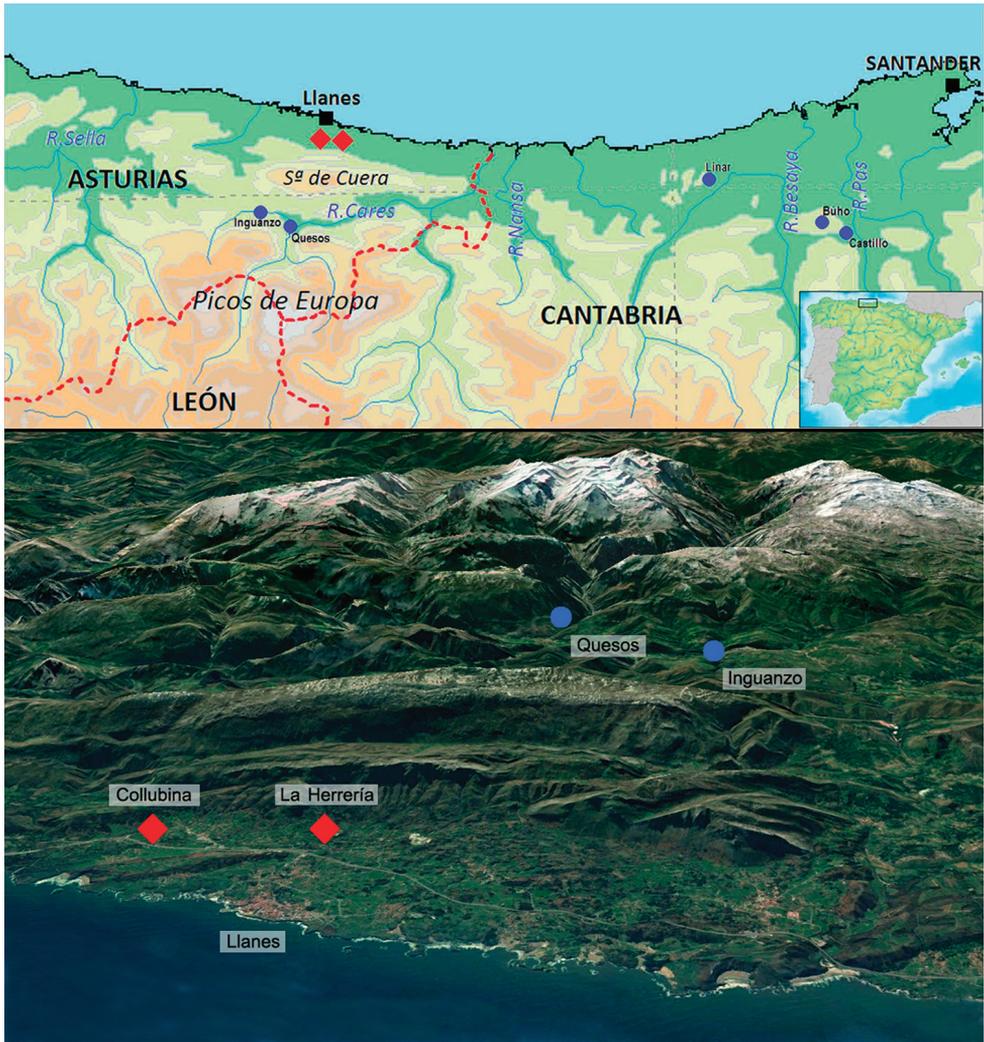


Figure 6. Map (above) and virtual aerial view (below, from the North) with mentioned caves for Asturian-Cantabrian *Zospeum* species: *Z. percostulatum* (♦); *Z. schaufussi* / *suarezi* (●). Map made with Dmap; aerial view of Digital Globe (screen shot of Google Earth).

Weigand et al. (2013) and cited as *Zospeum suarezi*. They recognized that the populations of Bosque (Inguanzo), Linar (La Busta), Las Paúles and Los Cuervos (Bizkaia) caves represent different species with a similar conchological morphology. This *suarezi* species group includes also another genetic lineage for populations from eastern Bizkaia and western Gipuzkoa caves, which were conchologically recognized as a new undescribed species by Altonaga et al. (1994) many years before its description as *Zospeum vasconicum* (Jochum et al. 2015c). For the moment, due to the complexity of this open question, which increases with nomenclatorial aspects related to *Z. schaufussi*, we do not address the issue of the identity of this companion species.

No graphical representation of the conchological morphospace occupied by *Zospeum* species has been yet published. As exposed before, Alpine-Dinaric taxa would constitute a monophyletic clade (Weigand et al. 2013) much more diverse than the Pyrenean-Cantabrian sister clade. Apart from tripling the number of known species, some of them have no conchological counterpart in the Iberian Peninsula, as *Z. spelaicum costatum* (Freyer, 1855), a large, strong ribbed species showing a carychioid facies, with well developed lamellae reaching the apertural plane, and also palatal teeth. On the contrary, *Z. biscaiense* with two apertural parietal lamellae, two palatal teeth and without inner lamellae (Gómez and Prieto 1985) has no counterpart in Alpine-Dinaric taxa.

Some *Zospeum* species of the Alpine-Dinaric region present a wide variability as shown by Bole (1974) or Pezzoli (1992), both in sculpture and development of aperture formations as well as in size and diameter, being obviously greater in species with wider geographic range. Thus, Pezzoli (1992) gives a size range for *Z. globosum* of 1.2–2.2 mm, even with some cavities in which two clearly distinguishable morphologies are present (eg. Grotta del Soglio: standard shape, 1.6–1.7 mm and turreted shell, 2–2.1 mm). Such wide variability has not been described for any Iberian species although Gittenberger (1980) cites a size of 1.3–1.9 mm for *Z. bellesi*.

The presence of conspicuous and prominent axial ribs constitutes a taxonomic character that until now has not been indicated for Iberian species, so *Z. percostulatum* sp. n. can be differentiated immediately from all of them. Furthermore, this new species fills one of the conchological gaps – the lack of ribbed species – between Alpine-Dinaric and Pyrenean-Cantabrian taxa. Occasionally, some shells of the known Iberian species have some stronger streaks, especially under the suture, taking the aspect of incomplete riblets but in *Z. percostulatum* sp. n. ribs form a constant and regular pattern. In addition, the new species has relatively large shells, reaching 1.8 mm length, in comparison to the other Cantabrian species, whose largest shells barely reach 1.45 mm height (Gittenberger 1980), making its recognition and discrimination easier.

In Cueva Collubina, 3.5 km eastward from the type locality, we have found shells attributable to *Z. percostulatum* sp. n., but they are somewhat smaller and wider, with more auriculate aperture, thinner parietal callus and finer and tighter ribs. Although they could be minor differences linked to geographical isolation, we consider that these individuals belong to the same species.

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References

- Altonaga K, Gómez BJ, Martín R, Prieto CE, Puente AI, Rallo A (1994) Estudio faunístico y biogeográfico de los Moluscos terrestres del norte de la Península Ibérica. Eusko Legebiltzarrak - Parlamento Vasco (Premio Xabier María de Munibe), Vitoria, 505 pp.
- Bole J (1974) Rod *Zospeum* Bourguignat 1856 (Gastropoda, Ellobiidae) v Jugoslaviji. Slovenska Akademija Znanosti in Umetnosti. Razprave 17(5): 1–43.
- Bolívar y Pieltain C (1923) Notas sobre sílfidos cavernícolas de España (Col. Bathysciinae). Boletín de la Real Sociedad Española de Historia Natural 23: 423–428.
- Bourguignat JR (1856) Aménités malacologiques. § LI. Du genre *Zospeum*. Revue et Magasin de Zoologie pure et appliquée (2)8: 499–516. https://doc.rero.ch/record/119551/files/mol_r_2_2.pdf
- Collado J (1977) Coleópteros cavernícolas de la región asturiana. Comunicacions del 6^o Simposium d'Espeleologia, Bioespeleologia, Terrassa: 55–63.
- Español F (1954) Lo que sabemos sobre los Bathysciinae de la región asturiana (Col. Catopidae). Speleon 5: 171–178.
- Frauenfeld GR von (1862) Ueber ein neues Höhlen-*Carychium* (*Zospeum* Brg.) und zwei neue fossile Paludinen. Verhandlungen der Zoologisch-botanischen Gesellschaft in Wien 12: 969–972.
- Gittenberger E (1973) Eine *Zospeum*-Art aus den Pyrenäen, *Zospeum bellesi* sp. nov. Basteria 37: 137–140.
- Gittenberger E (1980) Three notes on Iberian terrestrial gastropods. Zoologische Mededelingen 55(17) 201–213. <http://www.repository.naturalis.nl/document/149219>
- Gomez BJ, Prieto CE (1983) *Zospeum biscaiense* nov. sp. (Gastropoda, Ellobiidae) otro molusco troglóbico para la Península Ibérica. Speleon 26–27: 7–10.
- Hammer O (2017) PAST PAleontological STatistics Version 3.18. Reference Manual, 259 pp. <https://folk.uio.no/ohammer/past/past3manual.pdf>
- Jeannel R, Racovitza GM (1915) Biospeleologica XXXIII. Enumeration des grottes visitées 1911–1913 (Cinquième Série). Archives de Zoologie Expérimentale et Générale 53: 325–558.
- Jochum A, Weigand AM, Slapnik R, Valentinčič J, Prieto CE (2012) The microscopic ellobioid, *Zospeum* Bourguignat, 1856 (Pulmonata, Ellobioidea, Carychiidae) makes a big debut in Basque Country and the province of Burgos (Spain). MalaCo 8: 400–403. http://www.bik-f.de/files/publications/jochum_malaco_2012.pdf
- Jochum A, Prozorova L, Sharyi-ool M, Páll-Gergely B (2015a) A new member of troglobitic Carychiidae, *Koreozospeum nodongense* gen. et sp. n. (Gastropoda, Eupulmonata, Ellobioidea) is described from Korea. ZooKeys 517: 39. <https://doi.org/10.3897/zookeys.517.10154>
- Jochum A, Slapnik R, Klusmann-Kolb A, Páll-Gergely B, Kampschulte M, Martels G, Vrabec M, Nesselhauf C, Weigand AM (2015b) Groping through the black box of variability: An integrative taxonomic and nomenclatural re-evaluation of *Zospeum isselianum* Pollonera, 1887 and allied species using new imaging technology (Nano-CT, SEM), conchological, histological and molecular data (Ellobioidea, Carychiidae). Subterranean Biology 16: 123–165. <https://doi.org/10.3897/subtbiol.16.5758>
- Jochum A, Winter AJ de, Weigand AM, Gómez B, Prieto C (2015c) Two new species of *Zospeum* Bourguignat, 1856 from the Basque-Cantabrian Mountains, Northern Spain (Eupulmonata, Ellobioidea, Carychiidae). ZooKeys 483: 81–96. <https://doi.org/10.3897/zookeys.483.9167>

- Jordá F, Mallo M (1972) Las pinturas de la Cueva de Las Herrerías (Llanes, Asturias). Seminario de Prehistoria y Arqueología de la Universidad de Salamanca.
- Kobelt W (1898) In: Rossmässler EA (Ed.) *Iconographie der Land- und Süßwasser-Mollusken mit vorzüglicher Berücksichtigung der europäischen noch nicht abgebildeten Arten* (2) 8(1/2): 1–40. [pl. 211–220]
- Morton (2004) DMAP Distribution Mapping Software (Version 7.2c) <http://www.dmap.co.uk/>
- Pezzoli E (1992) Il genere *Zospeum* Bourguignat, 1856 in Italia (Gastropoda Pulmonata Basommatophora) censimento delle stazioni ad oggi segnalate. *Natura Bresciana* 27: 123–169.
- Prieto CE, Gómez BJ (1985) Primeros datos de *Zospeum* (Mollusca, Gastropoda, Ellobiidae) para la provincia de Burgos. *Actas de II Simposium Regional de Espeleología de la Federación Castellana Norte de Espeleología* (Burgos, 12–14 de octubre de 1984): 143–147.
- Slapnik R, Ozimec R (2004) Distribution of the genus *Zospeum* Bourguignat, 1856 (Gastropoda, Pulmonata, Ellobiidae) in Croatia. *Natura Croatica* 13(2): 115–135. <http://hrcak.srce.hr/10658>
- Weigand AM (2013) New *Zospeum* species (Gastropoda, Ellobioidea, Carychiidae) from 980 m depth in the Lukina Jama – Trojama cave system (Velebit Mts., Croatia). *Subterranean Biology* 11: 45–53. <https://doi.org/10.3897/subtbiol.11.5966>
- Weigand AM, Jochum A, Slapnik R, Schnitzler J, Zarza E, Klussmann-Kolb A (2013) Evolution of microgastropods (Ellobioidea, Carychiidae): integrating taxonomic, phylogenetic and evolutionary hypotheses. *BMC Evolutionary Biology* 13: 1–23. <https://doi.org/10.1186/1471-2148-13-18>

Is the Italian stream frog (*Rana italica* Dubois, 1987) an opportunistic exploiter of cave twilight zone?

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Abstract

Studies on frogs exploiting subterranean environments are extremely scarce, as these Amphibians are usually considered accidental in these environments. However, according to recent studies, some anurans actively select subterranean environments on the basis of specific environmental features, and thus are able to inhabit these environments throughout the year. We present the first study on the abundance and spatial use of the Italian stream frog, *Rana italica*, in subterranean environments. We monthly collected data from 66 cave sectors during a whole year (2013), recording > 120 detections of *R. italica*. Frogs were more frequently found close to the cave entrance, without significant differences between age classes or sexes. Adults generally were observed being higher up along cave walls compared to juveniles. Frogs abundance was higher in areas showing specific environmental features, such as warm temperature, low incident light and the presence of potential prey. *Rana italica* likely occupies subterranean areas characterized by a combination of microclimatic suitability and prey availability.

Keywords

biospeleology, Anuran, amphibian, cave biology, prey, microclimate, spatial use, food web

Introduction

Several amphibian species are known to regularly exploit subterranean environments but, besides numerous reports concerning several salamanders species (Niemiller and Miller 2007, Pierce et al. 2014, Manenti et al. 2017, Soares et al. 2017, Vörös et al. 2017), only a few species of frogs and toads (Anura) are known to exploit these environments (Fenolio et al. 2005, Köhler et al. 2010, Lunghi et al. 2014, Koller 2017). Frogs have been usually considered as an accidental presence in subterranean environments (Bressi and Dolce 1999), but recently many authors showed that some Anuran species can be quite common in these sites, selecting caves with specific environmental features (Köhler et al. 2010, Rosa and Penado 2013, Biswas 2014, Lunghi et al. 2014, Matavelli et al. 2015). One of these species is the Italian stream frog *Rana italica* Dubois, 1987, which is endemic of Italian Apennines (Lanza et al. 2006, Canestrelli et al. 2008, Buono et al. 2014, Sindaco and Grieco 2014). According to the known habitat requirements, *R. italica* is strongly bounded to freshwater environments and usually occurs in forested areas; in some circumstances, the species is also found in natural and artificial subterranean sites, where it seems to be able to breed (Lanza et al. 2006, Vanni and Nistri 2006). Although most of the previous observations of *R. italica* refer to individuals trapped in wells and vertical caves (Bressi and Dolce 1999), recent studies underlined that this frog is able to actively exploit subterranean environments during the whole year (Lunghi et al. 2017).

Subterranean environments are characterized by peculiar microclimatic features: in temperate areas, they generally have a relatively constant temperature, which roughly corresponds to the mean annual temperature at the surface. Furthermore, air humidity is generally very high, and incoming light is only present within the first meters after the cave entrance (Romero 2009, Lunghi et al. 2015). Close to the surface (cave entrance and twilight zone), the inner microclimate is mostly affected by external environmental conditions, and such peculiarity can promote the abundance of troglophile species (Manenti et al. 2015, Lunghi et al. 2017). The microclimate characterizing the shallow part of subterranean environments seems to be particularly suitable for *Rana italica* (Lunghi et al. 2014); therefore, the use of subterranean spaces during periods characterized by unsuitable (e.g., dry) surface conditions may be of key importance for population survival (Forrester et al. 2015, Lunghi et al. 2017). Beside a suitable microclimate, in subterranean environments *R. italica* probably finds shelter from predators (Vanni and Nistri 2006). Nevertheless, information on the biology of Italian stream frogs in subterranean environments, and on the relevance of these habitats for its conservation, is extremely limited. With this study we aim to provide the first assessment of *R. italica* in subterranean environments, mostly focusing on population abundance and habitat use.

Methods

Data collection

From January 2013 to December 2013, we monthly surveyed nine subterranean environments located in the north of Tuscan Apennines (between 43°53'17"N, 11°06'22"E and 44°03'48"N, 10°48'09"E), where the presence of *Rana italica* was already assessed by a previous study (Lunghi et al. 2014). All surveyed environments did not show a morphology hampering frogs to move freely between subterranean and outdoor areas. Three of them were World War II refuges, one was a drainage tunnel, while the remaining six were natural caves (Table 1). Subterranean environments were explored until the point in which speleological equipment was necessary, and only when environmental conditions did not pose a risk (e.g., flooding). All studied environments were divided into portions of 3-linear meters of length (hereafter sectors); this subdivision allows a good data collection for both biotic and abiotic features characterizing subterranean environments (Lunghi et al. 2015, Manenti et al. 2015, Lunghi et al. 2017). Within each sector we recorded air temperature and humidity (measured with a Lafayette TDP92 thermo-hygrometer; accuracy: 0.1 °C and 0.1%) and the average incident light (obtained by averaging max and min illuminance recorded with a Velleman DVM1300 light meter; minimum recordable light: 0.1 lux). We recorded the abundance of *Rana italica* within sectors using visual encounter surveys (VES; Crump and Scott 1994) and adopting a standardized survey method (7.5 min/sector) which limits potential effects of imperfect species detection (Banks-Leite et al. 2014, Lunghi et al. 2017). Using the same procedure (VES + standardized survey method), we assessed the presence of seven invertebrate species which potentially represent prey items for *R. italica*: one dipteran (*Limonia nubeculosa* Meigen, 1804), three spiders (*Meta menardi* (Latreille, 1804), *Tegenaria* Latreille, 1804 sp., *Mettellina merianae* (Scopoli, 1763)), one cricket (*Dolichopoda laetitiae* Minozzi, 1920) and two gastropods (*Chilostoma planospira* (Férussac, 1832), *Oxychilus draparnaudi* (Beck, 1837)). All considered species have stable populations in subterranean environments, even if they are not obligate cave species (Lunghi et al. 2017). We measured the snout-vent length (SVL) of all captured frogs (Fig. 1a); individuals with SVL \leq 30 mm were considered juveniles (Buono et al. 2014). Within adults, we recognized males on the basis of secondary sexual characters (nuptial pads). For each frog, we measured the distance from the cave entrance and the elevation from the cave ground floor.

Statistical analyses

We used Generalized Linear Mixed Models (GLMM) to assess whether abundance of *Rana italica* was related to both biotic and abiotic recorded parameters. As dependent variable we used the observed abundance of *R. italica*, which represents an index of frog abundance (Barke et al. 2017). Microclimatic features (temperature, humidity and illuminance), biotic features (presence/absence of the seven prey species), month

Table 1. Data of explored subterranean environments. For each site are shown: longitude, latitude and elevation, orientation of the main entrance, typology (natural (N), semi-natural (SN), artificial (A)), total development of the inner environment (Max area) and length explored in this study (linear meters), maximum number of observed frogs during a single survey, deepest observed frog (linear distance from cave entrance). *The length of last cave sector was only 1 m as a syphon blocked the passage. **This is a provisional data as the cave is still interested by speleological exploration. "X" means that for the artificial site no information is available for its total length.

Site	Longitude	Latitude	Elevation	Orientation	Typology	Max area (m)	Explored area (m)	Maximum number of observed frogs	Deepest observed frog (m)
Site1	11.21	43.87	227	S-E	N	12	12	2	9
Site2	11.13	43.91	286	N	N	15	10*	2	9.5
Site3	10.82	44.00	948	N	SN	12	12	8	12
Site4	10.82	44.00	853	N-E	SN	15	15	1	11.5
Site5	10.82	44.00	850	N-E	SN	21	21	1	10.45
Site6	10.85	44.04	744	N-E	A	X	18	2	12.8
Site7	11.16	43.91	699	N-E	N	6	6	2	5.9
Site8	11.15	43.91	715	N	N	52	42	2	20.5
Site9	11.15	43.97	492	N-W	N	78**	60	12	24.5

of survey and sector depth were used as independent variables; sector and cave identity were used as random factors.

We used Linear Mixed Models (LMM) to identify whether differences in spatial use of caves occur between age classes or sexes. To assess differences between adults and juveniles, we run two LMMs using age class and month of survey as independent variables, while both cave and sector identity were included as random factors. The distance of individuals from cave entrance, and the vertical position of frogs (i.e., height above the cave floor) were the dependent variables of the two LMMs. This procedure was repeated on adults only, replacing the independent variable age class with sex, in order to detect differences between adult males and females.

All analyses were performed in the R environment using packages lme4, lmerTest, nlme and unmarked (Fiske and Chandler 2011, Douglas et al. 2015, Kuznetsova et al. 2016, Pinheiro et al. 2016, R Core Team 2016).

Results

We performed a total of 765 surveys within 66 cave sectors (missing data due to unfavorable climatic condition represent 4.42%), recording 122 detections of *Rana italica* (60 females, 9 males, 38 juveniles and 15 unsexed individuals) (Table 1); on average (\pm Error Standard; ES), we observed 1.14 ± 0.21 individuals per survey (Fig. 2).

The abundance of *R. italica* was strongly related to the depth of the sector ($F_{1,98.28} = 30.40$, $P < 0.001$), air temperature ($F_{1,716.39} = 4.29$, $P = 0.038$), illuminance ($F_{1,143.82} = 8.01$, $P = 0.005$) and to the presence of two insect species, *Dolichopoda laetitiae* ($F_{1,645.63} = 7.71$,

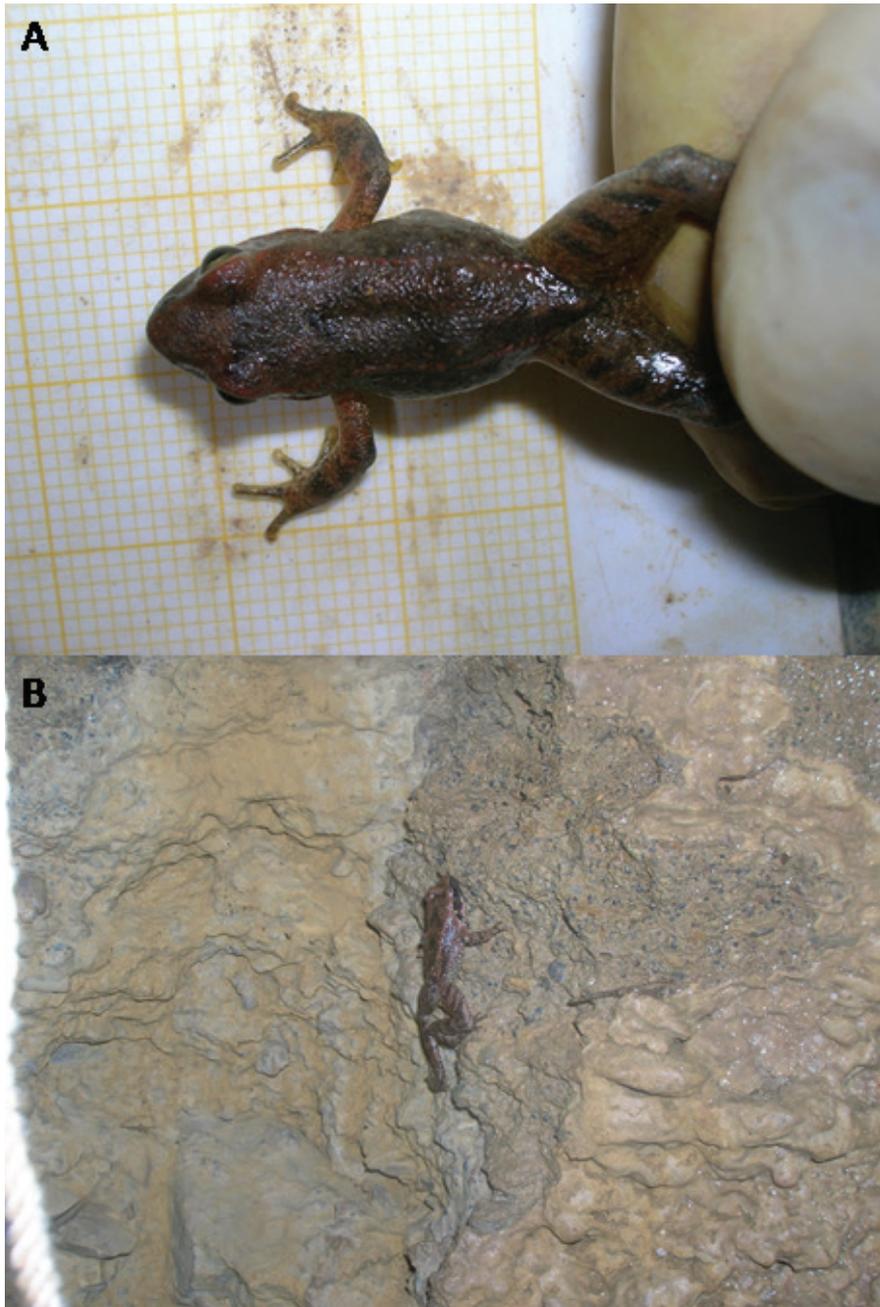


Figure 1. Two juveniles of *Rana italica*: a) during the measurement of SVL and b) climbing cave walls.

$P = 0.006$) and *Limonia nubeculosa* ($F_{1,730.17} = 5.50$, $P = 0.019$). Overall, frogs were more abundant in shallow sectors with less light and warmer temperature, and with the presence of both *D. laetitia* and *L. nubeculosa*. Furthermore, we detected significant differences of

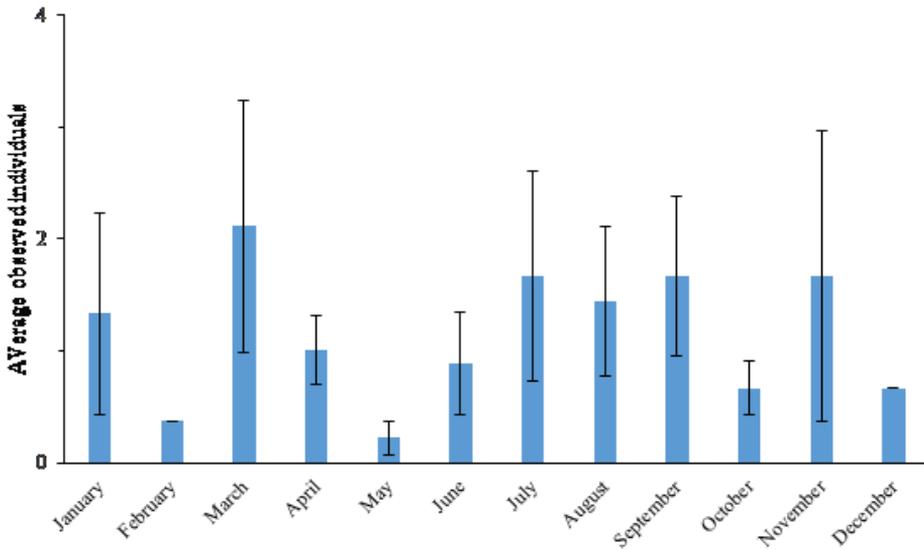


Figure 2. Average monthly observation (\pm ES) of *Rana italica* within studied caves.

frog abundance among months ($F_{11,704,58} = 2.46$, $P = 0.005$). March and November were the survey months in which we recorded the highest frog abundance (Fig. 2). All other considered variables were not significant ($P > 0.17$).

The distance from the cave entrance did not show significant differences between age classes, without differences among survey months (age class: $F_{1,67} = 0.80$, $P = 0.374$; month: $F_{9,67} = 1.04$, $P = 0.417$) (Fig. 3A), nor between sexes (sex: $F_{1,30} = 0.56$, $P = 0.46$; month: $F_{9,30} = 0.45$, $P = 0.894$) (Fig. 3B). The use of walls was significantly different between age classes ($F_{1,67} = 4.92$, $P = 0.03$) (Fig. 3C) without differences among survey months ($F_{9,67} = 1.86$, $P = 0.073$); adults generally occupied higher position compared to juveniles. Within adults, we did not detect differences between males and females (month: $F_{9,30} = 1.07$, $P = 0.413$; sex: $F_{1,30} = 0.01$, $P = 0.912$) (Fig. 3D).

Discussion

Rana italica was observed in subterranean environments throughout the year, even though the number of observations varied between months (Fig. 2). The abundance of *Rana italica* in subterranean environments was higher in areas close to the cave entrance, but deep enough to show almost no light. These particular areas correspond to the twilight zone (Culver and Pipan 2014), where external influence is moderate and abundance of non-strictly cave-dwelling species is the highest (Lunghi et al. 2015, Lunghi et al. 2017). The amount of incoming light represents an important factor for *R. italica*, as it influences both presence and abundance of the species in subterranean environments (Lunghi et al. 2017). Stream frogs are mostly nocturnal, and preference

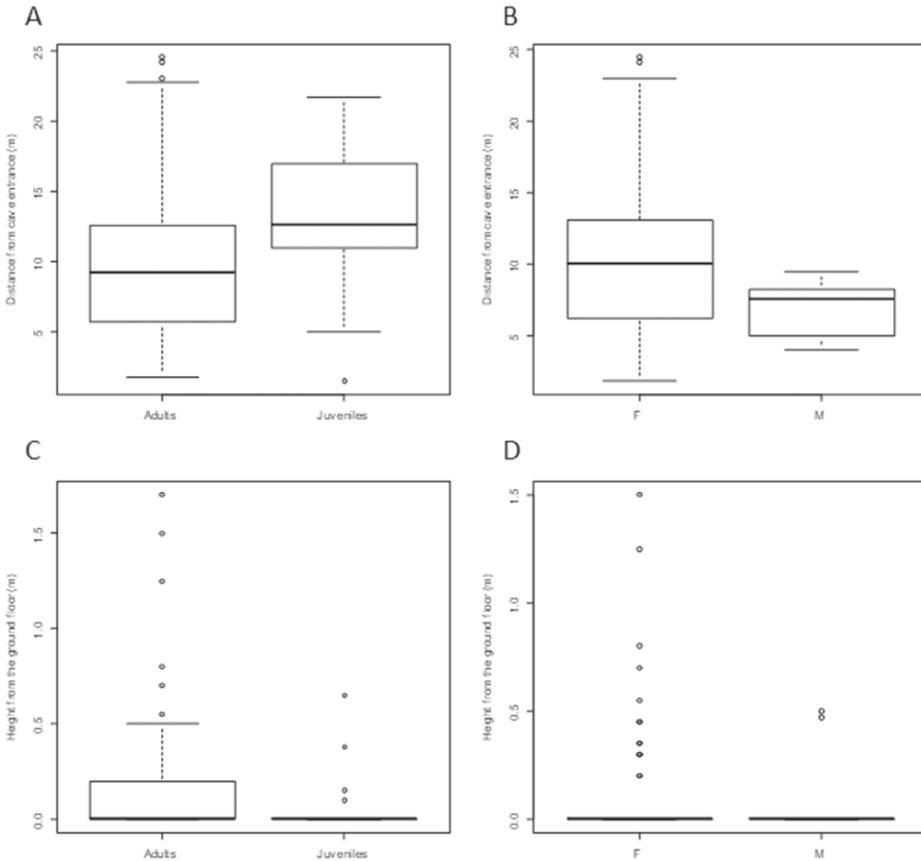


Figure 3. Boxplots indicating differences in the use of cave spaces. Difference between **A** age classes (Adults/ Juveniles) and **B** adult sexes (Females/Males) in the use of the subterranean surface area; differences between **C** age classes and **D** adult sexes in the use of cave walls. Diagonal bar inside the box represents the median.

for twilight areas might help them to avoid predators (Vanni and Nistri 2006); however, we cannot exclude that the selection of sectors without light may be also related to the avoidance of harsher climatic conditions (hot and dry), which are present close to the surface (Hetema et al. 2012, Everall et al. 2014). Temperature was an additional parameter strongly related to abundance of *R. italica*. Frogs were more abundant in relatively warm cave sectors. The temperature of cave sectors is the result of complex interactions between external temperature, mean annual temperature, and air circulation of karstic systems (Badino 2004, Badino 2010, Lunghi et al. 2015). In winter, temperature is highest far from the surface, thus these sectors represent ideal shelters during hibernation. Conversely, in summer the temperature is highest close to cave entrance where prey richness is highest (Manenti et al. 2015, Lunghi et al. 2017). Indeed, the abundance of frogs was positively related to the presence of invertebrates such as the cave cricket *Dolichopoda laetitiae* and the fly *Limonia nubeculosa*. Stream frogs are

generalist predators of small invertebrates, and these two insects are often abundant in subterranean environments, therefore they have a key role in sustaining subterranean trophic networks (de Pasquale et al. 1995, Lavoie et al. 2007, Manenti et al. 2015). Recent studies underlined that the occurrence of the cricket *D. laetitiae* is strongly related to inner cave temperature, as this cricket prefers cave areas with relatively warm temperature (Lunghi et al. 2015, Lunghi et al. 2017). No particular information is available on the seasonal occupancy of caves by *Limonia nubeculosa*. Therefore, the relationship between *R. italica* abundance and the occurrence of these species is likely to represent the result of trophic interactions.

As already observed for other amphibians present in subterranean environments, individuals of *R. italica* probably face a trade-off between the selection of suitable conditions, and the need of trophic resources. Thus, frogs occupy cave portions that are deep enough to have a suitable microclimate and lack of predators, but that are not too far from feeding areas (Ficetola et al. 2013, Lunghi et al. 2016). The abundance and the frequency of individuals found in subterranean environments suggest that further investigations should be performed on the trophic role and the impact that *R. italica* may have on the cave food web. In a study performed on the pickerel frog (*Rana palustris* (LeConte, 1825)) involving analyses with both stable isotopes and stomach contents, authors identified a significant predator activity of this species within the cave habitat (Fenolio et al. 2005). In our study, the correlation between *R. italica* and the two insect species suggests that some trophic relationships may occur, as it is possible that frogs are more abundant in cave sectors with the presence of potential prey. On the other hand, it is also possible that some unrecorded features (e.g., availability of resources) positively influence the abundance of multiple species, including both frogs and insects. Indeed, as recently underlined for aquatic subterranean sites, amphibian predators exploiting subterranean environments not only contribute to the top-down control of prey, but also provide an important subsidy in terms of energy for such environments (Barzaghi et al. 2017).

Frog distribution within caves was similar between age classes and sexes (Fig. 3A–B), suggesting that there is neither spatial segregation between age classes, nor between adult sexes. Actually, no information exists on a possible spatial segregation between individuals of *Rana italica*. In our study, the abundance and diversity of potential prey observed in subterranean environments likely promote the co-existence of different individuals. However, the use of the subterranean space by *R. italica* was not limited to the cave floor, as frogs often climbed walls (Figs 1B, 3C–D). This particular behavior, which is generally unusual for frogs, might be compared to observations reported for other two anuran species. In green frogs (*Pelophylax synklepton hispanicus* (Bonaparte, 1839)) some adults were observed preying on high tree branches (Mori et al. 2013). In artificial subterranean environments, the Iberian frog (*Rana iberica* Boulenger, 1879) has been observed climbing up the walls, probably looking for shelters (Rosa and Penado 2013). In our study, adults were observed more frequently being up higher along cave walls compared to juveniles (Fig. 3C); this divergence could be related to a different climbing ability and/or behavior between age classes.

Conclusions

This study represents the first analysis on the factors related to the abundance and the spatial use of *Rana italica* in subterranean environments. The obtained results enable us to hypothesize that subterranean abundance of *R. italica* is mostly related to foraging activities, thus likely make this species one of the top predators of the twilight zone. Our study underlines that the trophic role of terrestrial predators exploiting subterranean environments should be taken into account for the ecological comprehension of determinants which have promoted the colonization of caves. Nonetheless, subterranean environments may also be of key importance to maintain the viability of anuran populations in the light of the occurring global warming.

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References

- Badino G (2004) Cave temperatures and global climatic change. *International Journal of Speleology* 33: 103–114. <https://doi.org/10.5038/1827-806X.33.1.10>
- Badino G (2010) Underground meteorology – “what’s the weather underground?”. *Acta Carsologica* 39: 427–448. <https://doi.org/10.3986/ac.v39i3.74>
- Banks-Leite C, Pardini R, Boscolo D, Righetto Cassano C, Püttker T, Santos Barros C, Barlow J (2014) Assessing the utility of statistical adjustments for imperfect detection in tropical conservation science. *Journal of Applied Ecology* 51: 849–859. <https://doi.org/10.1111/1365-2664.12272>
- Barke RJ, Schofield MR, Link WA, Sauer JR (2017) On the reliability of N-mixture models for count data. *Biometrics* 1–9. <https://doi.org/10.1111/biom.12734>
- Barzaghi B, Ficetola GF, Pennati R, Manenti R (2017) Biphase predators provide biomass subsidies in small freshwater habitats: a case study of spring and cave pools. *Freshwater Biology* 62(9): 1637–1644. <https://doi.org/10.1111/fwb.12975>
- Biswas J (2014) Occurrence and distribution of cave dwelling frogs of peninsular India. *Ambient Science* 1(2): 17–25. <https://doi.org/10.21276/ambi.2014.01.2.rv02>
- Bressi N, Dolce S (1999) Osservazioni di anfibi e rettili in grotta. *Rivista di Idrobiologia* 38: 475–483.
- Buono V, Guarino FM, Vignoli L (2014) Maximum body size and age distribution in the Italian stream frog, *Rana italica* Dubois 1987 (Amphibia: Anura). *Acta Herpetologica* 9(2): 231–235. https://doi.org/10.13128/Acta_Herpetol-14209

- Canestrelli D, Cimmaruta R, Nascetti G (2008) Population genetic structure and diversity of the Apennine endemic stream frog, *Rana italica* – insights on the Pleistocene evolutionary history of the Italian peninsular biota. *Molecular Ecology* 17: 3856–3872. <https://doi.org/10.1111/j.1365-294X.2008.03870.x>
- Crump ML, Scott NJ (1994) Visual Encounter Surveys. In: Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS (Eds) *Measuring and monitoring biological diversity: standard methods for Amphibians*, Smithsonian Institution Press, Washington, 84–92.
- Culver DC, Pipan T (2014) *Shallow Subterranean Habitats: Ecology, Evolution, and Conservation*, Oxford University Press. New York, 288 pp. <https://doi.org/10.1093/acprof:oso/9780199646173.001.0001>
- de Pasquale L, Cesaroni D, di Russo C, Sbordoni V (1995) Trophic niche, age structure and seasonality in *Dolichopoda* cave crickets. *Ecography* 18: 217–224. <https://doi.org/10.1111/j.1600-0587.1995.tb00124.x>
- Douglas B, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models using lme4. *Journal of Statistical Software* 67(1): 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Everall N, Johnson MF, Wilby RL, Bennett CJ (2014) Detecting phenology change in the mayfly *Ephemera danica*: responses to spatial and temporal water temperature variations. *Ecological Entomology* 40(2): 95–105. <https://doi.org/10.1111/een.12164>
- Fenolio DB, Graening GO, Stout JF (2005) Seasonal movement patterns of pickerel frogs (*Rana palustris*) in an Ozark cave and trophic implications supported by stable isotope evidence. *Southwestern Naturalist* 50: 385–389. [https://doi.org/10.1894/0038-4909\(2005\)050\[0385:SMPOPF\]2.0.CO;2](https://doi.org/10.1894/0038-4909(2005)050[0385:SMPOPF]2.0.CO;2)
- Ficetola GF, Pennati R, Manenti R (2013) Spatial segregation among age classes in cave salamanders: habitat selection or social interactions? *Population Ecology* 55: 217–226. <https://doi.org/10.1007/s10144-012-0350-5>
- Fiske I, Chandler R (2011) unmarked: an R package for fitting hierarchical models of wildlife occurrence and abundance. *Journal of Statistical Software* 43(10): 1–23. <https://doi.org/10.18637/jss.v043.i10>
- Forrester TD, Casady DS, Wittmer HU (2015) Home sweet home: fitness consequences of site familiarity in female black-tailed deer. *Behavioral Ecology and Sociobiology* 69: 603–612. <https://doi.org/10.1007/s00265-014-1871-z>
- Hetema RS, Strauss WM, Fick LG, Maloney SK, Meyer LCR, Shobrak M, Fuller A, Mitchell D (2012) Activity re-assignment and microclimate selection of free-living Arabian oryx: responses that could minimise the effects of climate change on homeostasis? *Zoology* 115: 411–416. <https://doi.org/10.1016/j.zool.2012.04.005>
- Köhler J, Vences M, D'Cruze N, Glaw F (2010) Giant dwarfs: discovery of a radiation of large-bodied 'stump-toed frogs' from karstic cave environments of northern Madagascar. *Journal of Zoology* 282: 21–38. <https://doi.org/10.1111/j.1469-7998.2010.00708.x>
- Koller K (2017) Underground occurrences of three species of amphibians and reptiles with special emphasis on *Rana latastei* (Amphibia: Anura). *North-Western Journal of Zoology*, 13(1): 176–179.
- Kuznetsova A, Brockhoff B, Christensen HB (2016). lmerTest: Tests in Linear Mixed Effects Models – R package version 2.0–2.9. <http://www.r-project.org>

- Lanza B, Andreone F, Bologna MA, Corti C, Razzetti E (2006) Fauna d'Italia. Amphibia, Calderini. Bologna, 537 pp.
- Lavoie KH, Helf KL, Poulson TL (2007) The biology and ecology of North American cave crickets. *Journal of Cave and Karst Studies* 69: 114–134.
- Lunghi E, Manenti R, Canciani G, Scari G, Pennati R, Ficetola GF (2016) Thermal equilibrium and temperature differences among body regions in European plethodontid salamanders. *Journal of Thermal Biology* 60: 79–85 <https://doi.org/10.1016/j.jtherbio.2016.06.010>
- Lunghi E, Manenti R, Ficetola GF (2014) Do cave features affect underground habitat exploitation by non-troglobite species? *Acta Oecologica* 55: 29–35. <https://doi.org/10.1016/j.actao.2013.11.003>
- Lunghi E, Manenti R, Ficetola GF (2015) Seasonal variation in microhabitat of salamanders: environmental variation or shift of habitat selection? *PeerJ* 3: e1122. <https://doi.org/10.7717/peerj.1122>
- Lunghi E, Manenti R, Ficetola GF (2017) Cave features, seasonality and subterranean distribution of non-obligate cave dwellers. *PeerJ* 5: e3169. <https://doi.org/10.3986/ac.v44i1.649>
- Manenti R, Lunghi E, Ficetola GF (2015) Distribution of spiders in cave twilight zone depends on microclimatic features and trophic supply. *Invertebrate Biology* 134: 242–251. <https://doi.org/10.1111/ivb.12092>
- Manenti R, Lunghi E, Ficetola GF (2017) Cave exploitation by an usual epigeal species: a review on the current knowledge on fire salamander breeding in cave. *Biogeographia* 32: 31–46. <https://doi.org/10.21426/B632136017>
- Matavelli R, Martins Campos A, Neves Feio R, Lopes Ferreira R (2015) Occurrence of anurans in Brazilian caves. *Acta Carsologica* 44(1): 107–120.
- Mori E, Bruni G, Domeneghetti D, Menchetti M (2013) *Pelophylax synklepton hispanicus* (Bonaparte, 1839) on the branches of a tree. *Herpetology Notes* 5: 515–517.
- Niemiller ML, Miller BT (2007) Subterranean reproduction of the southern two-lined salamander (*Eurycea cirrigera*) from short mountain, Tennessee. *Herpetological Conservation and Biology* 2(2): 106–112.
- Pierce BA, Mcentire KD, Wall AAE (2014) Population size, movement, and reproduction of the Georgetown salamander, *Eurycea naufragia*. *Herpetological Conservation and Biology* 9(1): 137–145.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RC (2016) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–128, <http://CRAN.R-project.org/package=nlme>
- R Core Team (2016) R: a language and environment for statistical computing. <http://www.R-project.org/>
- Romero A (2009) Cave Biology: Life in Darkness (Ecology, Biodiversity and Conservation). Cambridge University Press, Cambridge, 306 pp.
- Rosa GM, Penado A (2013) *Rana iberica* (Boulenger, 1879) goes underground: subterranean habitat usage and new insights on natural history. *Subterranean Biology* 11: 15–29. <https://doi.org/10.3897/subtbiol.11.5170>
- Sindaco R, Grieco C (2014) Monitoring of *Salamandrina perspicillata* (Savi, 1821) and *Rana italica* Dubois, 1987, twenty years later (Amphibia: Salamandridae, Ranidae). *Alytes* 30: 27–32.

- Soares D, Adams R, Hammond S, Slay ME, Fenolio DB, Niemiller ML (2017) Evolution of coprophagy and nutrient absorption in a Cave Salamander. *Subterranean Biology* 24: 1–9. <https://doi.org/10.3897/subtbiol.24.15013>
- Vanni S, Nistri A (2006) Atlante degli Anfibi e dei Rettili della Toscana, Regione Toscana, Università degli Studi di Firenze, Museo di Storia Naturale, Sezione Zoologica “La Specola”. Firenze, 379 pp.
- Vörös J, Maarton O, Schmidt BR, Tünde Gál J, Jelić D (2017) Surveying Europe’s only cave-dwelling chordate species (*Proteus anguinus*) using environmental DNA. *PLoS ONE* 12(1): e0170945. <https://doi.org/10.1371/journal.pone.0170945>

Diversity of *Potamolithus* (Littorinimorpha, Truncatelloidea) in a high-diversity spot for troglobites in southeastern Brazil: role of habitat fragmentation in the origin of subterranean fauna, and conservation status

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Abstract

The Alto Ribeira karst area, southeastern Brazil, is a high-diversity area for troglobites. Three species of freshwater gastropods *Potamolithus* occur in the area: *P. ribeirensis*, only found in epigeal waters at the Iporanga and Ribeira rivers; *P. troglobius*, which is endemic to the Areias cave system; and *P. karsticus*, a troglomorphic species from Calcário Branco Cave and an epigeal stream nearby. We investigated their distribution based on shell morphology and internal anatomy of epigeal species, troglomorphic populations, and troglobitic species. Distribution patterns of *Potamolithus* were compared to those of other aquatic taxa from the region (such as crustaceans and fishes). Besides the three species already described for the region, we recorded 12 additional ones, for a total of 15 species/morphs (six troglobites, seven troglomorphs, and two epigeal). *Potamolithus* spp. are restricted to micro-basins and/or caves, showing small areas of distribution and probably a high degree of endemism. Geomorphology (irregular landscape, with limestone outcrops intercalated with insoluble rocks, which probably act as geographic barriers for cave populations), paleoclimatic evidence, and ecological/biological factors, such as the low degree of mobility of these gastropods (sedentary habit), explain the distributional patterns. We observed troglomorphisms such as reduction/absence of eyes and pigmentation (body and periostracum), and a coiled intestine. Apparently, there is no cause-and-effect between miniaturization and intestine coiling for *Potamolithus*, in contrast to observations for other cave snails. *Potamolithus* snails are threatened in the region due to water pollution, uncontrolled tourism, and overcollection.

Keywords

Caenogastropoda, Caves, Distribution, Neotropical region, *Potamolithus*

Introduction

Neeritimorpha gastropods, and mainly neogastropods, have been recorded in the subterranean (hypogean) realm (e.g., *Theodoxus subterrelictus* Schütt, 1963; *Georissa papuana* Bernasconi, 1995). Among neogastropods, truncatelloids comprise 97% of the troglobitic species (with source populations restricted to the hypogean environment; Trajano 2012) (Bole and Velkovrh 1986). Subterranean truncatelloids also include troglomorphic populations (subterranean source populations genetically connected with epigean populations of the same species by individuals commuting between these environments; Trajano 2012).

In general, troglobites may be distinguished by the presence of characteristics related to isolation in the hypogean environment, the so-called troglomorphisms. The most ubiquitous troglomorphisms are a reduction, or complete loss, of the eyes and melanic pigmentation, observed in many subterranean truncatelloids throughout the world (e.g., Boeters 1979). Other frequent specializations reported for troglobitic truncatelloids are miniaturization, and, as a consequence, the complex coiling of the intestine and a lack, or reduction, of the ctenidium and the semen receptacle (Hershler and Holsinger 1990). This miniaturization is proposed as an adaptive characteristic to environments with poor food supplies (see Sanders and Allen 1973, Hershler and Longley 1986), or as an ancestral characteristic present in epigean relatives (exaptation, *sensu* Arnold 1994).

Truncatelloids are very common in caves in Europe, North America, Africa, Japan, Australia, and New Zealand (Hershler and Longley 1986, Angelov 2000, Ponder et al. 2005); there are also records from the phreatic waters of the Toscana region, Italy (Bodon et al. 1996). In South America, a troglomorphic species, *Andespyrgus sketi* Hershler & Velkovrh, 1993, was reported from caves in Ecuador and Colombia, and several species of *Potamolithus* Pilsbry, 1896 have been found in caves from southeastern Brazil (Simone and Moracchioli 1994, Bichuette 1998).

The genus *Potamolithus* is characterized by a tiny, oval to rounded shell, with a prosocline, rounded to oval aperture; the shell whorls are arched and the last one is much larger than the others (*sensu* Wenz 1938). According to the World Register of Marine Species (WoRMS Editorial Board 2017), the genus comprises 18 species, which occur in the freshwaters of meridional South America, mainly in the Ribeira, Itajaí-açu, and Jacuhy Rivers in southern Brazil, and the Uruguay River, part of the Paraná and Río de la Plata drainage systems, with one species in Patagonia (de Lucía and Gutiérrez-Gregoric 2017a); nine of these species occur in Brazil. de Lucía and Gutiérrez-Gregoric (2017b) redescribed *Potamolithus supersulcatus* Pilsbry, 1896 for Argentina, and concluded that the genus possesses 31 valid species, which is in contrast to other databases (e.g., WoRMS Editorial Board 2017), reinforcing the problem of

inconsistent data for this group. For example, *Potamolithus* was traditionally classified, based on morphology, as Lithoglyphidae (Davis and Pons-da-Silva 1984) and as Hydrobiidae (e.g., Simone 2006). However, molecular studies indicated a position within the Tateidae (Wilke et al. 2013), a much smaller family with 32 genera, distributed in Australasia/Oceania and the Neotropics (Wilke et al. 2013, Haase and Zielske 2015). Considering the possibility of conflict with molecular phylogenies and morphology-based classifications, as recently shown for some Tateidae (Becker et al. 2016), we choose not to take any position regarding the familial status of *Potamolithus* until all the evidence corroborates one of these, or different, taxonomic hypotheses. Comparisons are made with other subterranean truncatelloids.

The Alto Ribeira karst area, in São Paulo State, southeast Brazil, is a high-diversity spot for troglobites (Trajano et al. 2016). So far, three species of *Potamolithus* have been described for this area: *Potamolithus ribeirensis* Pilsbry, 1911, only found in epigeal waters of the Iporanga and Ribeira rivers; *Potamolithus troglobius* Simone & Moracchioli, 1994, from the Areias cave system (the first troglobitic mollusk from Brazil); and *Potamolithus karsticus* Simone & Moracchioli, a troglomorphic species, from Calcário Branco cave and an epigeal stream nearby. Other Brazilian troglobitic aquatic gastropods include *Spiripockia punctata* Simone, 1994 (Truncatelloidea: Pomatiopsidae), a new genus and species described from Serra do Ramalho karst area, Bahia State, northeast Brazil, and an undetermined species, possibly also of the genus *Spiripockia* Simone, 2012, from Serra da Bodoquena karst area, Mato Grosso do Sul, southwest Brazil.

The paleoclimatic model (Barr 1968), based on alternating humid and dry phases of glacial cycles as periods of colonization of subterranean habitats followed by isolation and differentiation respectively, in the habitats in which troglobites originate, has been proposed for tropical areas, particularly Brazil, since the early 1990s (Trajano 1995, 2001, Trajano and Britski 1992, Bichuette et al. 2015). Geomorphological and paleontological evidences point to the existence of a corridor of open vegetation along the Rio Ribeira valley, southeastern Brazil, during the last glacial phase (Ab'Sáber 1981). This region is currently covered by Atlantic rainforest. Dating of speleothems from Santa Catarina, southern Brazil (Cruz et al. 2005) corroborates the hypothesis of dry glacial and humid interglacial phases in SE-S Brazil. Therefore, in the Alto Ribeira karst area, the interglacial phases would be times of colonization of caves and establishment of troglomorphic populations, alternating with glacial dry phases when forests would be substituted by open vegetation and the epigeal drainage would be disrupted, isolating those troglomorphic populations in the subterranean environment (Trajano and Britski 1992, Trajano 1995). This model was recently proposed by Fernandes et al. (2013) to explain the diversity of troglobitic anomuran crustaceans, genus *Aegla* Leach, 1820, in the Alto Ribeira.

Subterranean systems are threatened throughout the world and much-needed conservation policies depend, among other factors, on knowledge of the ecology, biology, and behavior of subterranean species. Because truncatelloids are small organisms with low mobility, but with species in different environments all over the world, there is great interest in their biogeography, comparative ecology, behavior, and physiology

(Davis et al. 1982). However, these studies depend on the recognition and delimitation of species, as accurately as possible with the available data, as a basis for systematics analysis and for proposals for phylogenetic hypotheses. The few existing studies on Brazilian freshwater truncatelloids have focused mainly on the description and distribution of a few species (Pilsbry 1911, Malek 1983, Pons-da-Silva 1993, Simone and Moracchioli 1994) and the ecology and behavior of cave *Potamolithus* (Bichuette and Trajano 1999, 2003).

As part of a broader investigation on truncatelloids from the Alto Ribeira karst area (Bichuette 1998), that included population ecology (Bichuette and Trajano 2003) and behavior (Bichuette and Trajano 1999, Bichuette and Menna-Barreto 2005), the main cave systems and associated epigeal (surface) streams were surveyed and a comparative taxonomic study, based on shell morphology and external and internal anatomy, was performed. We present the results of this study, as a contribution to knowledge of the diversity of subterranean aquatic gastropods and morphological specializations of troglobites, by comparing epigeal species, troglomorphic populations, and troglotic species. Furthermore, analyses of distribution patterns, exploring their causes/factors, were compared to other aquatic taxa, such as the anomuran *Aegla* crustacean (Decapoda).

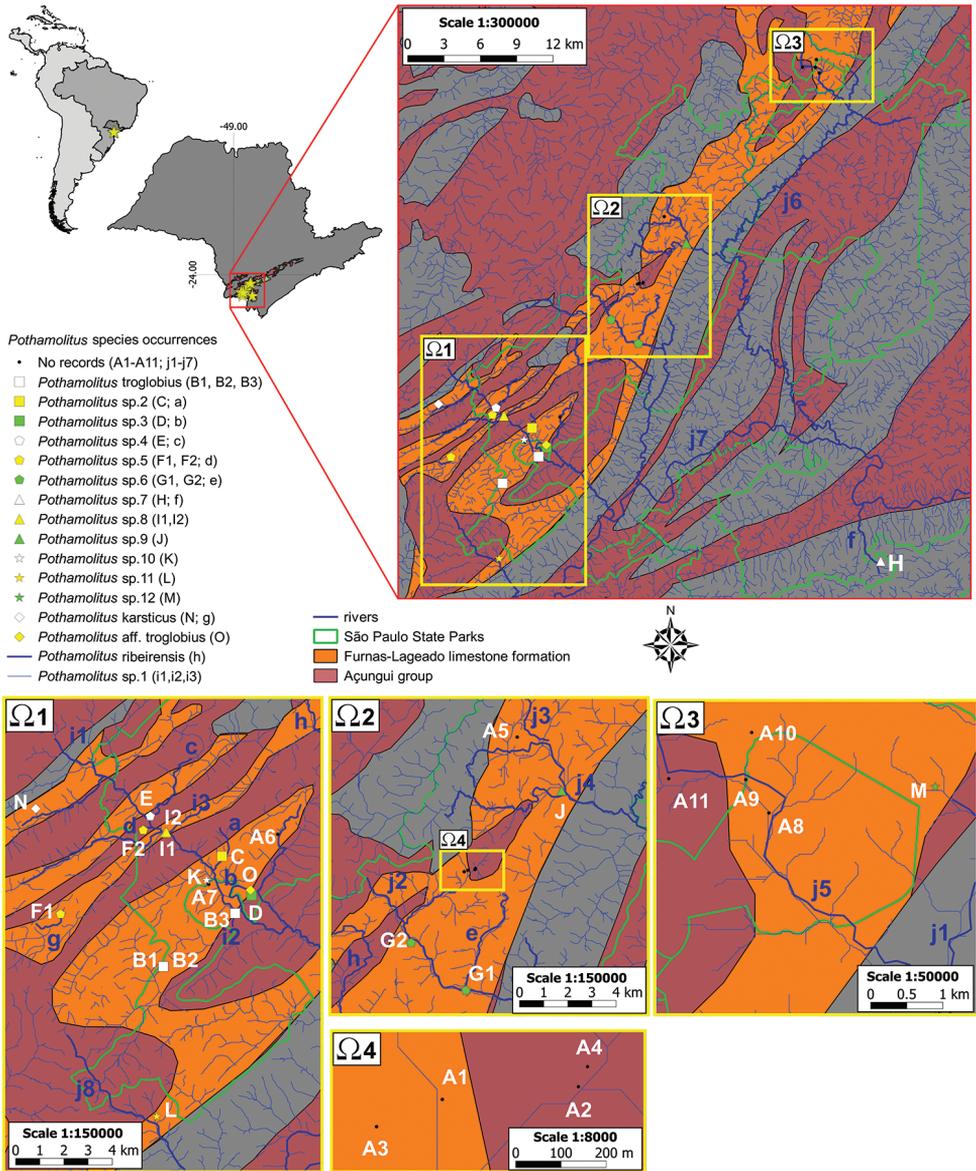
Materials and methods

Study area

The study area is situated in the Alto Ribeira karst area, São Paulo State, southeastern Brazil. It is geologically inserted in the Açungui Group, composed of Upper Precambrian metasedimentary rocks (Karmann and Sánchez 1979). Four NE-SW limestone outcrops extend over 20 km, with widths varying from 1 to 8 km, alternate with insoluble detrital rocks, such as phyllites (Trajano et al. 2016) (Figure 1).

The Alto Ribeira river valley is located in the transition between the Tropical Atlantic and Araucaria Forest domains (Ab'Sáber 1977) (Figure 2). The climate in the study area is type “Cfa”, which is defined as tropical wet to temperate humid without a dry season. Total precipitation is around 1,500 mm and precipitation in the driest and coolest month (July) is more than 30 mm. Temperatures are above 22 °C in the warmest month (January) and below 4 °C in the coolest month; mean annual temperature (as generally observed deep inside caves) is around 19–20 °C in the Parque Estadual Turístico do Alto Ribeira (PETAR), and 17–18 °C in the upper Parque Estadual Intervalles (PEI), where collections were carried out (Köeppen 1948). This area is mostly covered by perennial subtropical humid forest (Hueck 1972), with typical well-oxygenated headwater streams, with rocky beds and rapids alternating with some soft-bottomed pools, which cross the rock outcrops (Figure 2).

Faunistic surveys were conducted in epigeal and cave stream reaches in two continuous regions in the Alto Ribeira karst area, mostly protected by state parks: PETAR



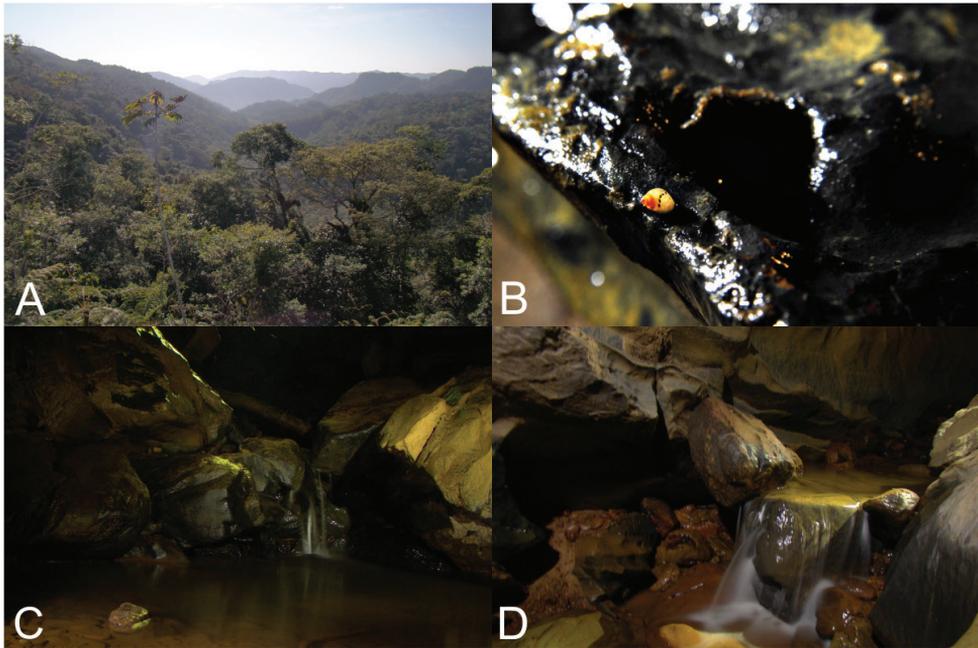


Figure 2. Landscape (Atlantic Rainforest) (A), *Potamolithus* sp. 5 in natural habitat, Santana cave (B) and subterranean streams (C, D) from Alto Ribeira karst area, Southeastern Brazil. Photographs: PP Rizzato (A, C, D), A Gambarini (B).

and PEI. Altitudes vary from 300 to 1,000 m in PETAR, which is crossed by four limestone outcrops. One of these outcrops bifurcates and extends toward the northeast, crossing PEI, where altitudes reach up to 1,200 m.

We visited 33 caves and 10 epigeal streams in PETAR and four caves and two epigeal streams in PEI (Figure 1). We also collected in the Tapagem Cave (a partially touristic cave, also known as Caverna do Diabo - Devil's Cave) and the epigeal stream that sinks into it, a relatively isolated karst area in the middle of the Rio Ribeira valley, protected by the Parque Estadual Caverna do Diabo (PECD). The survey areas were located in four basins, or micro-basins (Figure 1).

The caves (from northeast to southwest) are shown in Figure 1: PEI - Fendão, Paiva, Jane Mansfield, Minotauro, and Colorida; PETAR - Temimina II, Pescaria, Aranhas, Chapéu, Chapéu Mirim I, Chapéu Mirim II, Água Sumida, Casa de Pedra, Calcário Branco, Água Suja, Santana, Pérolas, Morro Preto, Couto, Córrego Seco, Alambari de Cima, Alambari de Baixo, Gurutuva, Ouro Grosso, Areias de Cima, Areias de Baixo, Resurgência das Areias de Água Quente, Betari de Baixo, and Jeremias; PECD - Tapagem.

Rivers, separated by micro-basins, are also shown in Figure 1: Pilões micro-basin - Pilões, Lageado, Pescaria, Temimina, and Bocaina; Iporanga micro-basin - Iporanga, Maximiano, and Espírito Santo; Betari micro-basin - Betari, Calcário Branco, Água Suja, Roncador, Morro Preto, Ouro Grosso, Alambari, and Água Quente; Ostras and Cutia de Cima micro-basins.

Methods

Systematic field trips to the Alto Ribeira karst area were carried out during 11 months between 1996 and 1997 (Bichuette and Trajano 2003); additional collections were done in 2008, 2009, 2010 and 2013 (four samplings, one per year). *Potamolithus* specimens were collected by hand after visual inspection of the substrate, mostly under boulders, pebbles, and other submerged objects, and by straining of thin substrates, such as sand and clay. Specimens were fixed in 70% ethanol and brought to the laboratory for morphological and taxonomic study. Identification at the genus level was confirmed with the collaboration of LRL Simone (Seção de Moluscos, Museu de Zoologia da Universidade de São Paulo, MZUSP).

For a preliminary separation of species/morphs, we considered the following characters in samples containing between 15 and 82 specimens: shape of intestine, observed by transparency in an abapertural view (slightly curved to left; slightly curved to right; markedly curved to left; two marked curves, a broad fold to left; a constricted fold to left) (Figure 3); pattern of body (mantle) pigmentation; presence *versus* absence of eyes; shell shape (fusiform; globose; fusiform-globose); shell dimensions (height – from the apex to the base, following the columellar axis, and width – the maximum measurement at a right angle to the columellar axis); and periostracum coloration observed in live individuals (dark brown, brown, pale yellow, white, translucent). Shell measurements were taken using digital calipers with 0.01 mm precision; we analyzed only adult individuals (smaller individuals that showed mature gonads – see Bichuette and Trajano 2003). We followed the anatomical nomenclature from Simone (2006).

To illustrate the shell opening, shape, and ornamentation, one or two individuals per population were studied under a scanning electron microscope (SEM) (Zeiss electron microscope) and through images acquired via LAS software (Leica Application Suite v3.7). Criteria for distinction between troglobites and troglophiles, according to the Schiner-Racovitza system, were used as in Trajano and Carvalho (2017). The absence of records in the epigeal environment allied to troglomorphic characters (such as translucent periostracum and absence of eyes) were considered for troglobite status; for troglophile status, we considered the occurrence of well-established (source) populations in both epigeal and hypogean environments, indicated by the presence of many individuals of all size/age classes deep inside caves throughout the annual cycle.

Examined material was deposited at the Museu de Zoologia, Universidade de São Paulo (MZUSP) and the Laboratório de Estudos Subterrâneos, Universidade Federal de São Carlos (LES).

Data analysis

To detect statistical differences among individuals from each OTU (Operational Taxonomic Unit - Sokal and Sneath 1963), the shell sizes (adults) were visually compared using boxplot graphs. A variance analysis (One Way ANOVA), followed by

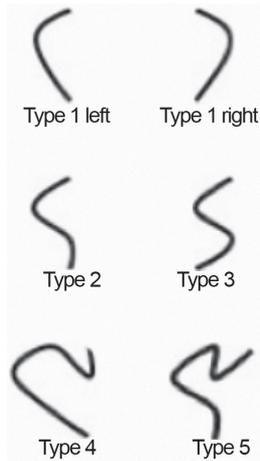


Figure 3. Schematic intestine forms observed in *Potamolithus* species from Alto Ribeira karst area, South-eastern Brazil. Abapertural view.

Dunn’s post-hoc test, was performed using the software PAST version 3.09 (Hammer et al. 2001). Before the analysis, data were tested in relation to normality and homogeneity of variances.

Species concept adopted

The concept of a species is one of the most complicated and debatable problems in biology. For several decades now, hundreds of publications have focused on this subject but no consensus has been achieved, and probably never will be (see Wheeler and Meier 2000, for a debate on the theme). Herein we adopted the Operational Taxonomic Unit (OTU; Sokal and Sneath 1963), which most taxonomists use in their daily task of recognizing, characterizing, and describing “species” and most inclusive clades. This is the first, and necessary, step for phylogenetic studies, as well as ecological studies for conservation, among others. Describing biodiversity is urgent for several reasons, and the OTU approach is not only a practical way to begin, but has a sound theoretical basis.

In our approach, OTUs are a spin-off from the evolutionary species (“An evolutionary species is an entity composed of organisms that maintains its identity from other such entities through time and over space and that has its own independent evolutionary fate and historical tendencies”), or species-as-lineage concepts (Wiley and Mayden 2000). According to these authors, if lineages are independent, at the empirical level we can expect to eventually discover differences among them. Such differences are expressed as character states indicating the lineages’ individuality (not to be confused with statements of similarity).

Therefore, the species as recognized and characterized below are lineages distinguished by sets of diagnostic traits (that may be apomorphic or plesiomorphic, which is not relevant in our approach).

Results

Potamolithus gastropods are widely distributed in the Alto Ribeira karst area, at least in São Paulo State, at altitudes lower than 800 m. In epigeal rivers, these animals occur under pebbles, boulders, or branches with relatively smooth surfaces. In the hypogean environment, they occur both under and over pebbles and rocky blocks with smooth surfaces.

After a collecting effort of ca. 60 days of fieldwork, besides the three species of *Potamolithus* previously described, we recorded 12 additional distinguished morphs (OTUs) (Table 1 and Figures 4 to 8). The occurrence of one species per cave (for most records) and the mosaic distribution of character states in the sampled populations (see below) support their treatment as separate morphs (OTUs), which we refer to as unnamed *Potamolithus* spp. We numbered the species in increasing order according to their status in the Schiner-Racovitza system (see Methods). Two of the species were only sampled in epigeal streams (referred to as epigeal), seven were from source populations in both epigeal and subterranean streams (troglophiles), and six were found exclusively in caves (troglobites), showing troglomorphisms. We could not find consistent differences separating the Alambari de Cima population from *P. troglobius*, and we, therefore, conservatively treated the former as *Potamolithus* aff. *troglobius*. Intraspecific variability in the presence *versus* absence of eyes, and pigmentation of the mantle and

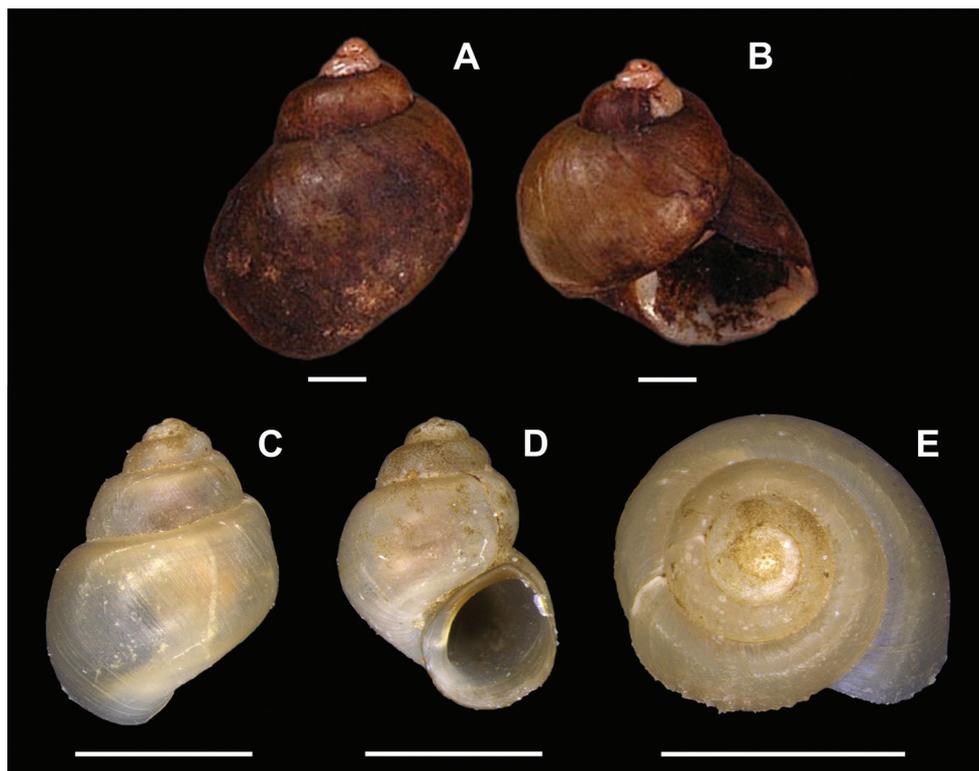


Figure 4. *Potamolithus ribeirensis* - **A** (dorsal view) **B** (apertural view); *Potamolithus* sp. 1 **C** (dorsal view) **D** (apertural view) **E** (apical view). Scale bars: 1 mm. (Photographs: LBR Fernandes).



Figure 5. *Potamolithus karsticus* - **A** (dorsal view) **B** (apertural view); *Potamolithus* sp. 2 **C** (dorsal view) **D** (apertural view) **E** (apical view); *Potamolithus* sp. 3 **F** (dorsal view) **G** (apertural view) **H** (apical view). Scale bars: 1mm. (Photographs: LBR Fernandes).

the periostracum, were observed for *Potamolithus* spp. 5 (troglophile), 8, and 9 (troglóbites) (Table 1, Figures 4–8).

Figures 9 and 10 show, respectively, the distributions of frequencies of shell height and width, comparing the epigean, troglóphilic, and troglóbite samples. SEM images showed an absence of ornamentation on both proto- and teleoconch.

The one-way ANOVA analyses revealed a significant difference between the shell sizes (height) of *Potamolithus* species ($F = 48.12$; $df_1 = 15$; $df_2 = 671$; $p = 8.211E-96$).

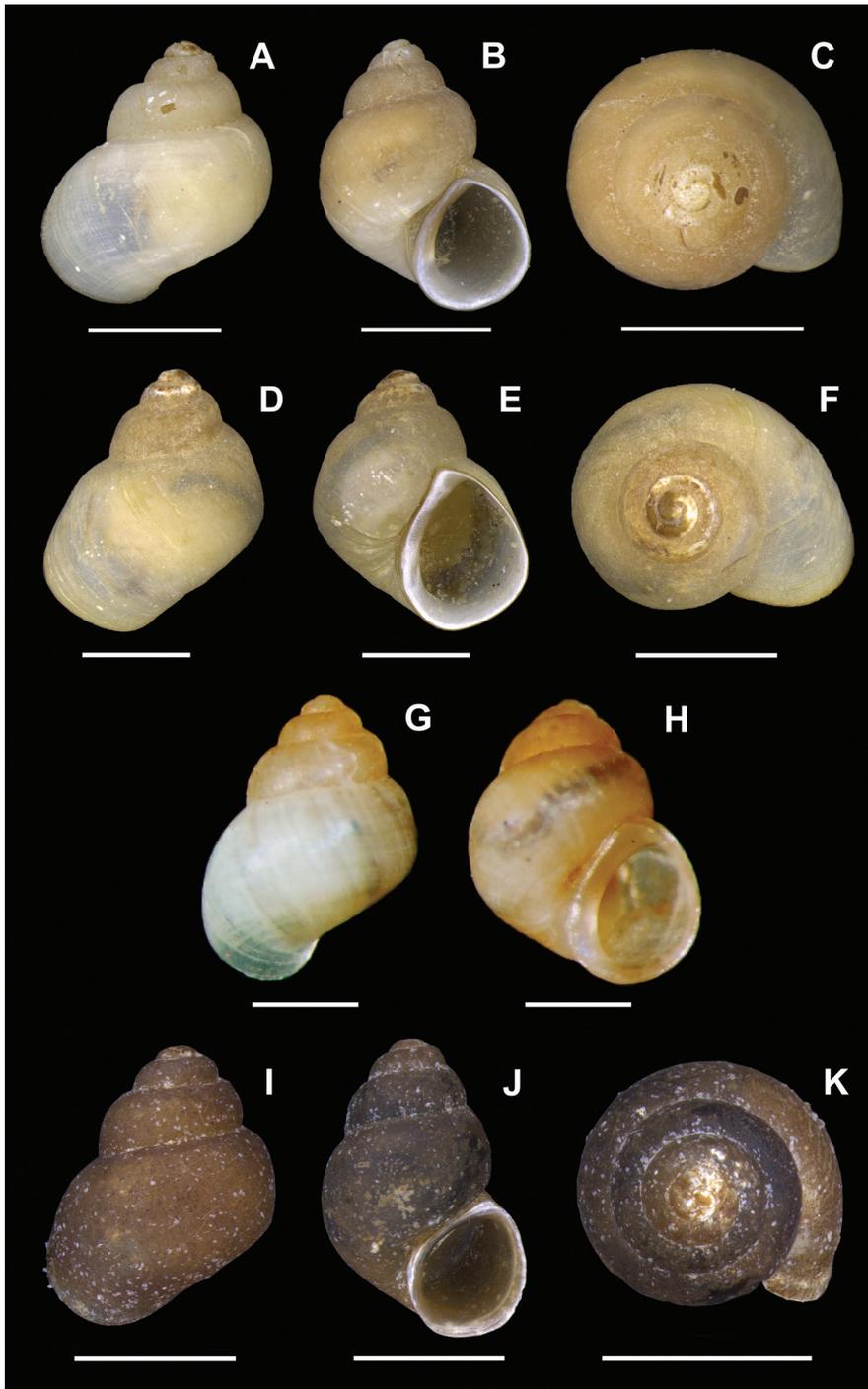


Figure 6. *Potamolithus* sp. 4 **A** (dorsal view) **B** (apertural view) **C** (apical view); *Potamolithus* sp. 5 **D** (dorsal view) **E** (apertural view) **F** (apical view); *Potamolithus* sp. 6 **G** (dorsal view) **H** (apertural view); *Potamolithus* sp. 7 **I** (dorsal view) **J** (apertural view) **K** (apical view). Scale bars: 1 mm. (Photographs: LBR Fernandes).

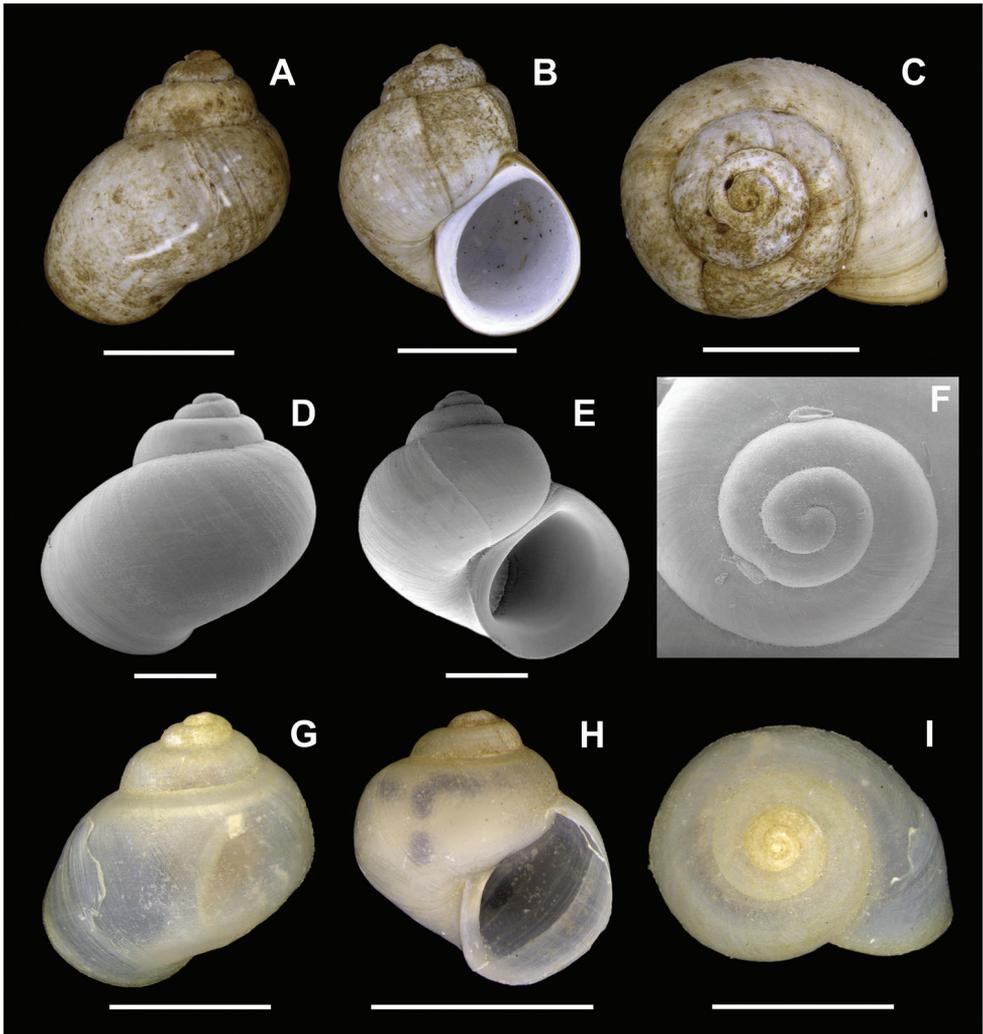


Figure 7. *Potamolithus troglobius* **A** (dorsal view) **B** (apertural view) **C** (apical view); *Potamolithus* aff. *troglobius* **D** (dorsal view) **E** (apertural view) **F** (apical view); *Potamolithus* sp. 8 **G** (dorsal view) **H** (apertural view) **I** (apical view). Scale bars: 1 mm. (Photographs: LBR Fernandes).

The post-hoc analyses (Dunn's test) (Table 2) showed that smaller sizes, especially in the troglobite from Colorida cave (*Potamolithus* sp. 12), were also observed in troglomorphic ones (e.g. *P. karsticus*, *Potamolithus* spp. 4 and 7) and in the epigean population from the Betari basin (*Potamolithus* sp. 1). On the other hand, the troglomorphic gastropods from Pescaria cave, *Potamolithus* sp. 9, reached larger sizes, only surpassed by the epigean *P. ribeirensis* (Figures 9 and 10, Table 2 for statistical results).

As mentioned, there is a mosaic of character states, with almost all species differing from the others by at least one state. The only exceptions were *P. troglobius* and the population from Alambari de Cima cave; for this reason, the latter was treated as

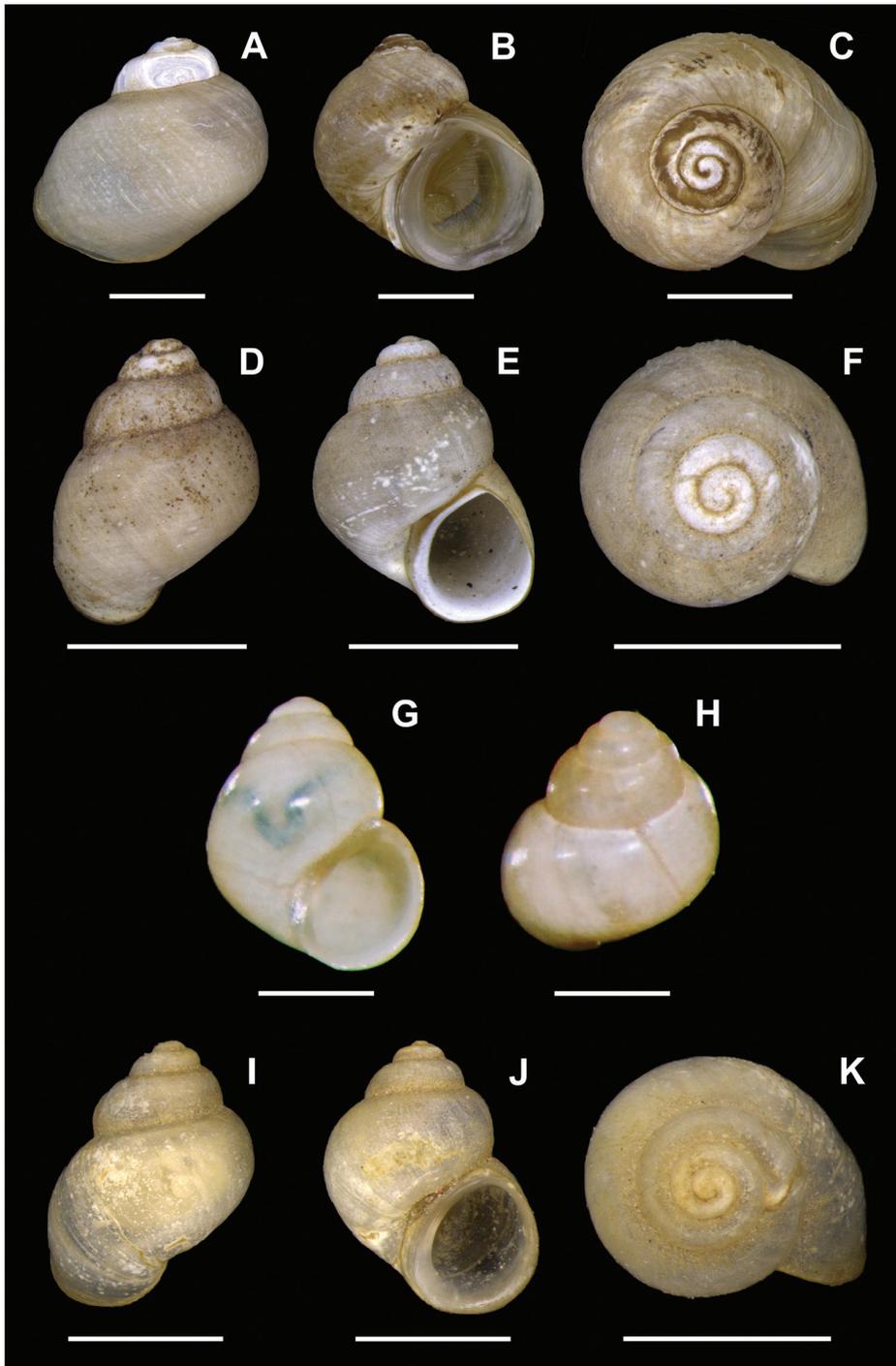


Figure 8. *Potamolithus* sp. 9 **A** (dorsal view) **B** (apertural view) **C** (apical view); *Potamolithus* sp. 10 **D** (dorsal view) **E** (apertural view) **F** (apical view); *Potamolithus* sp. 11 **G** (dorsal view) **H** (apertural view); *Potamolithus* sp. 12 **I** (dorsal view) **J** (apertural view) **K** (apical view). Scale bars: 1 mm. (Photographs: LBR Fernandes).

Table 1. *Potamolithus* spp. from Alto Ribeira karst area, São Paulo state, Southeastern Brazil; e, epigeic, tph, troglophile, tb, troglobite; N, number of examined specimens; max. height × width (mm), height × width of largest individual (larger shell height); + present, - absent; » rivers, W caves; hom., homogenous.

Taxa		Characters							Localities
N	Shell shape	Max. height × width (mm)	Periostracum	Eye	Body pigment	Intestine shape			
50	Globose	5.7×3.7	Dark brown	+	Black (hom.)	U-shaped (sensu Simone & Moracchioli, 1994)		≈ Iporanga	
44	Fusiform	2.8×2.2	Light brown	+	Black spots along the body	Type 2		≈ Betari, Água Quente, Morro Preto	
27	Fusiform	2.7×1.8	Dark brown	+	Black spots along the body	Type 1 - left		≈ Calcário Branco; W Calcário Branco	
43	Fusiform	2.8×1.7	Light brown	+	Depigmented	Type 1 - right		≈ Ouro Grosso; W Ouro Grosso (close to cave resurgence)	
46	Fusiform-globose	3.6×2.3	Light brown	+	Black tentacles	Type 2		≈ Alambari; W Alambari de Baixo	
36	Fusiform-globose	2.8×1.3	Pale yellow	+	Depigmented	Type 1 - left		≈ Água Suja; W Água Suja	
68	Fusiform-globose	2.8×1.7	Pale yellow/Translucent	+	Depigmented	Type 2		≈ Roncador; W Santana, Pérolas	
50	Fusiform	2.7×1.5	Light brown	+	Black spots between eyes	Type 1 - left		≈ Maximiano; W Casa de Pedra, Água Sumida	
15	Fusiform	2.0×1.0	Brown	+	Black stripes in the dorsal region	Type 1 - left		≈ Rio das Ostras; W Tapagem	
70	Globose	2.8×2.1	Translucent/White	-	Depigmented	Type 4		Ω Areias System	
82	Globose	3.2×2.3	Translucent/White	-	Depigmented	Type 4		Ω Alambari de Cima	
57	Globose	2.3×1.3	Translucent	+/-	Depigmented	Type 5		Ω Couto, Morro Preto	
35	Globose	3.9×2.9	White	+/-	Depigmented	Type 3		Ω Pescaria	
17	Fusiform	2.6×1.4	Translucent	-	Depigmented	Type 4		Ω Betari de Baixo	
30	Globose	2.4×1.7	Translucent	-	Depigmented	Type 1-left		Ω Jeremias	
17	Fusiform	1.8×1.1	Pale yellow	-	Depigmented	Type 2		Ω Colorida	

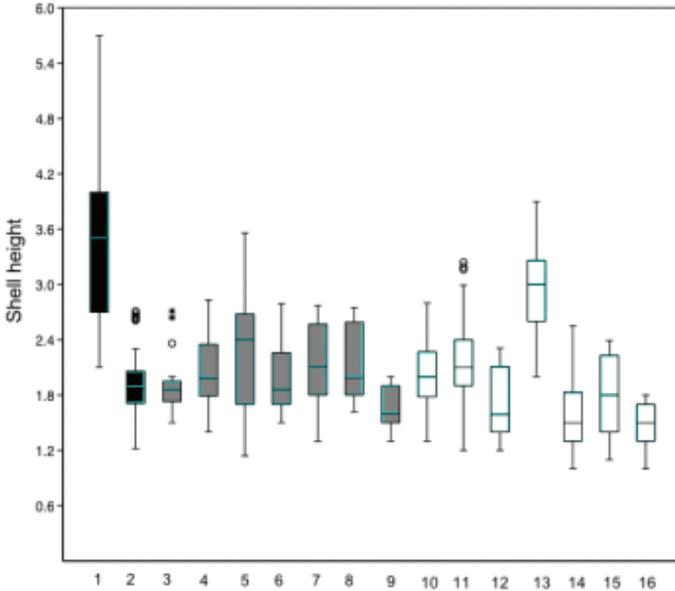


Figure 9. Boxplots showing shell heights on *Potamolithus* spp. Horizontal Bar, median; vertical bar, whiskers with minimal and maximum observations. **1** *P. ribeirensis* **2** *Potamolithus* sp. 1 **3** *P. karsticus* **4** *Potamolithus* sp. 2 **5** *Potamolithus* sp. 3 **6** *Potamolithus* sp. 4 **7** *Potamolithus* sp. 5 **8** *Potamolithus* sp. 6 **9** *Potamolithus* sp. 7 **10** *P. troglobius* **11** *Potamolithus* aff. *troglobius* **12** *Potamolithus* sp. 8 **13** *Potamolithus* sp. 9 **14** *Potamolithus* sp. 10 **15** *Potamolithus* sp. 11 **16** *Potamolithus* sp. 12. Black bars, epigeal species; gray bars, troglophilic species; white bars, troglobitic species; circles, outliers; *, extremes.

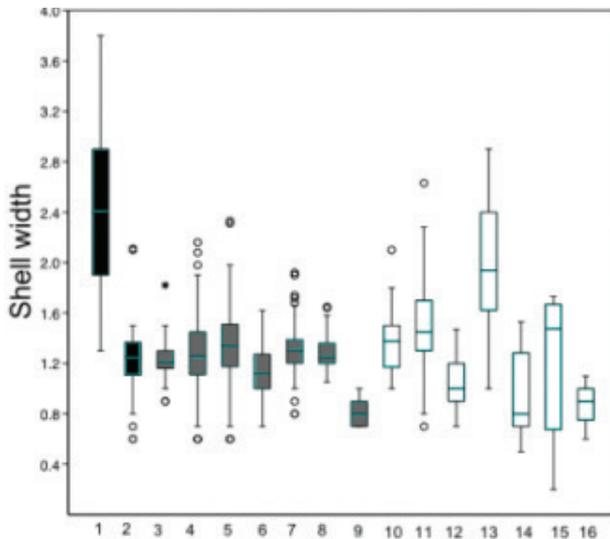


Figure 10. Boxplots showing shell widths on *Potamolithus* spp. Horizontal Bar, median; vertical bar, whiskers with minimal and maximum observations. **1** *P. ribeirensis* **2** *Potamolithus* sp. 1 **3** *P. karsticus* **4** *Potamolithus* sp. 2 **5** *Potamolithus* sp. 3 **6** *Potamolithus* sp. 4 **7** *Potamolithus* sp. 5 **8** *Potamolithus* sp. 6 **9** *Potamolithus* sp. 7 **10** *P. troglobius* **11** *Potamolithus* aff. *troglobius* **12** *Potamolithus* sp. 8 **13** *Potamolithus* sp. 9 **14** *Potamolithus* sp. 10 **15** *Potamolithus* sp. 11 **16** *Potamolithus* sp. 12. Black bars, epigeal species; gray bars, troglophilic species; white bars, troglobitic species; circles, outliers; *, extremes.

Potamolithus aff. *troglobius*. In addition, there were no cases of syntopy. Considering the shell surface, SEM images showed an absence of ornamentations for *Potamolithus* studied here.

Potamolithus snails are distributed in three separate tributary basins of the Upper Ribeira River: the Betari, the Iporanga, and the Pilões river basins. In the Betari basin, these gastropods were found in its six tributaries (Água Suja, Roncador, Morro Preto, Ouro Grosso, Alambari, and Água Quente), and in 15 out of 16 visited caves (the only exception being the Córrego Seco cave). In the Iporanga river basin, representatives of this genus were recorded in two (Iporanga and Maximiano) of the four visited tributaries, and in two of the five visited caves (Água Sumida and Casa de Pedra caves), which are crossed by the Maximiano river. In the Pilões river basin, *Potamolithus* gastropods were recorded in one out of two visited caves (Pescaria cave) and there were no records in the epigeal streams. Toward the northwest (Bocaina river basin, tributary of Pilões basin), *Potamolithus* specimens were recorded in a single cave (Colorida cave), among the four visited and their respective epigeal reaches. To the southeast, *Potamolithus* gastropods were found in an epigeal river (Rio das Ostras), and formed a troglomorphic population in Tapagem cave.

The only case of a troglomorphic *Potamolithus* occurring in caves from different systems was recorded in Couto and Morro Preto caves (*Potamolithus* sp. 8). However, these caves connect through their vadose zones and, during floods, there is an opportunity for dispersal between habitats.

Below we present an identification key to the *Potamolithus* from the Alto Ribeira karst area in São Paulo State:

- | | | |
|---|--|--|
| 1 | Eyes absent, if present then periostracum white or translucent..... | 2 |
| – | Eyes present | 7 |
| 2 | Periostracum white, intestine type 3..... | |
| | <i>Potamolithus</i> sp. 9 (Pescaria cave) (Figure 8A, B, C) | |
| – | Other combination of characters..... | 3 |
| 3 | Intestine type 2..... | <i>Potamolithus</i> sp. 12 (Colorida cave) (Figure 8I, J, K) |
| – | Other combination of characters..... | 4 |
| 4 | Intestine type 5 | |
| | <i>Potamolithus</i> sp. 8 (Morro Preto and Couto caves) (Figure 7G, H, I) | |
| – | Other combination of characters..... | 5 |
| 5 | Intestine type 1-left..... | <i>Potamolithus</i> sp. 11 (Jeremias cave) (Figure 8G, H) |
| – | Intestine type 4 | 6 |
| 6 | Shell globose..... | <i>P. troglobius</i> and <i>Potamolithus</i> aff. <i>troglobius</i> (Areias system and Alambari de Cima cave) (Figure 7A–F) |
| – | Shell fusiform..... | |
| | <i>Potamolithus</i> sp. 10 (Betari de Baixo cave) (Figure 8D, E, F) | |
| 7 | Shell globose, periostracum dark brown, intestine U-shaped..... | |
| | <i>P. ribeirensis</i> (Iporanga river) (Figure 4A, B) | |
| – | Other combination of characters..... | 8 |

8	Shell fusiform, periostracum dark brown and intestine type 1-left.....	
 <i>P. karsticus</i> (Calcário Branco cave) (Figure 5A, B)	
–	Other combination of characters.....	9
9	Shell fusiform	10
–	Shell fusiform-globose.....	13
10	Periostracum light brown, intestine type 2	Potamolithus sp. 1
	(Betari, Água Quente, and Morro Preto rivers) (Figure 4C, D, E)	
–	Other combination of characters.....	11
11	Mantle depigmented, intestine type 1-right.....	Potamolithus sp. 2
	(Ouro Grosso cave and epigeal drainage) (Figure 5C, D, E)	
–	Other combination of characters.....	12
12	Periostracum light brown, mantle with black spots between eyes.....	Potamolithus sp. 6
	(Maximiano river, Casa de Pedra, and Água Sumida caves) (Figure 6G, H)	
–	Periostracum brown, mantle with black stripes in the dorsal region	
 Potamolithus sp. 7 (Ostras river and Tapagem cave) (Figure 6I, J, K)	
13	Periostracum pale yellow, mantle depigmented, and intestine type 1-left.....	
	Potamolithus sp. 4 (Água Suja cave and epigeal drainage) (Figure 6A, B, C)	
–	Other combination of characters.....	14
14	Periostracum light brown, mantle with black tentacles....	Potamolithus sp. 3
	(Alambari de Baixo cave and epigeal drainage) (Figure 5F, G, H)	
–	Periostracum pale yellow/translucent, mantle depigmented.....	
	Potamolithus sp. 5 (Roncador river, Santana, and Pérolas caves) (Figure 6D, E, F)	

Discussion

Distribution, habitat preferences, and fragmentation: the origins of the diversity of *Potamolithus* gastropods in the Alto Ribeira karst area

With more than 60 troglobitic species, the Alto Ribeira is a high-diversity area for troglobites (*sensu* Trajano et al. 2016, Gallão and Bichuette 2018). This is among the highest genetic diversities recorded for Brazil, and is likely related to three factors: the high degree of habitat fragmentation, limited mobility leading to low capacity for dispersal, and habitat preferences.

Potamolithus gastropods prefer relatively shallow (0.6 m maximum depth) lentic locations, with clear waters, neutral to basic pH (values between 7 and 8), high conductivity (0.211 ms/cm), and relatively high temperatures (20 °C average) for Ribeira standards (Bichuette and Trajano 2003). These snails appear to be bioindicators of good quality water, since no specimen was observed in locations close to sewage discharges. Indeed, the spatial distribution of *Potamolithus* in the Ribeira Valley seems to be constrained by environmental conditions, since we did not record specimens in sites with strong currents, domestic pollution, at depths greater than 1 m, or devoid

of substrates for attachment (such as pebbles and boulders). The pH and conductivity values were high in the different caves/epigean rivers with *Potamolithus* records.

Solem and van Bruggen (1984) observed significant positive correlations between the species richness of freshwater gastropods and high values of pH, conductivity, hardness, and alkalinity on the one hand and, on the other, negative correlations with high levels of suspended particles. According to Macan (1949) and Dillon (2000), good conditions for the occurrence of operculate snails in freshwater are a large water volume, moderately warm and alkaline habitats, with little or no organic solids, and no pollution. Among truncatelloids from temperate regions, *Bithynia tentaculata* (Linnaeus, 1758) are extremely sensitive to low oxygen concentrations and avoid water with strong currents, showing a preference for backwaters (branches of rivers) with depths between 70 and 180 cm. Polluted waters, with low dissolved oxygen, are avoided by *Peringia ulvae* (Pennant, 1777) (Fretter and Graham 1962), another truncatelloid species. The troglobitic *Pseudotricula eberhardi* Ponder, 2002, from Tasmania, set up on smooth rocks that occur in small hypogean streams, preferring shallow sites (Ponder 1992).

The main tributaries of the Alto Ribeira basin in São Paulo State (left bank) cross limestone outcrops, forming several, semi-isolated micro-basins. Most of the rivers flowing across the limestone of the Alto Ribeira valley form cave systems, and almost all of them have their own troglomorphic, or troglobitic, populations (Trajano et al. 2016). Moreover, insoluble rocks, such as phyllites, are intercalated with limestone outcrops (Karmann and Sánchez 1979), which probably limit subterranean dispersal (Fernandes et al. 2013). Insoluble rocks represent barriers, which may be facilitating in the isolation and speciation process. Furthermore, the fact that there are no observed cases of syntopy, points to allopatric speciation events.

In addition to the hydrogeology, another factor that may contribute to the restricted distribution observed in *Potamolithus* in Alto Ribeira is the limited capacity for dispersal due to the small size and slow locomotion of these animals, favoring isolation in confined areas. Ponder (1994) observed a high degree of endemism in mollusks in Southeast Australia and argued that limited mobility and restriction to some habitats (in that case, springs and ponds) would allow for relatively rapid speciation. The same was observed in Tasmania when epigean and cave fauna were compared (Ponder et al. 2005).

The paucity of records at higher altitudes in the Ribeira Valley was probably due to the lower temperatures, since the other variables (pH, conductivity, dissolved oxygen) were within the ranges observed elsewhere and the waters were even more pristine (Bichuette and Trajano 2003). In the Intervalles karst area (PEI), despite the extensive collecting effort expended by E. Trajano and colleagues since the 1980s, *Potamolithus* specimens have been found in just one cave, and, in Espírito Santo-Caboclos area, located between PEI and Betari valley, these gastropods have been found in only two caves. The winter temperatures may drop to negative values in PEI, much lower than those observed in the Betari and Iporanga valleys located in PETAR (ca. 4 °C), which could be limiting the colonization of PEI by *Potamolithus* gastropods. It is possible that the PEI region represents the northern boundary of the genus distribution, at least in the Upper Ribeira Valley.

The mosaic distribution of character states observed encompasses not only the classic troglomorphic traits (presence *vs.* absence of eyes; body and periostracum pigmentation), but also apparently neutral characters under the hypogean selective regime (shell shape) and those with a less clear relationship with the subterranean way of life (body size and intestine shape - see below). This observation, allied to the high degree of habitat fragmentation, provides evidence for an independent origin of these species from one or more unknown epigeal ancestors living in some of the main tributaries of the Upper Ribeira River. These ancestral populations would have colonized the micro-basins upstream and possibly became isolated in epigeal headwaters during the dry phases of paleoclimatic cycles, originating in species such as *P. karsticus* and *Potamolithus* spp. 1 to 6). Further steps would be the colonization of subterranean habitats, with the establishment of troglomorphic populations (all of the above except for *Potamolithus* sp. 1), and isolation in the subterranean realm followed by troglomorphic speciation. In the case of *P. troglobius* and *Potamolithus* spp. 8 to 12, these originated from unknown epigeal ancestors; we could not relate any of these morphs to the epigeal and troglomorphic ones based on distribution and morphology.

Morphological traits and subterranean life

It is noteworthy that troglomorphic *Potamolithus* from the Alto Ribeira karst are not generally smaller than the troglomorphic and epigeal ones. For instance, the troglomorphic *Potamolithus* from Pescaria cave reached larger sizes than all the troglomorphs, as did the epigeal *Potamolithus* sp. 1. On the other hand, all *Potamolithus* spp. recorded in the study area, including the epigeal one from the Betari river basin and the troglomorphs from the Iporanga and Bocaina river basins, were smaller than *P. ribeirensis*, found exclusively in the Iporanga river. Simone and Moracchioli (1994) selected four epigeal *Potamolithus* species occurring near the Alto Ribeira, in addition to *P. ribeirensis*, for comparison with *P. karsticus* and *P. troglobius*, and all of them were larger than the latter. Therefore, even without a phylogeny, we feel confident in proposing that the small size of these species is due to miniaturization.

The present data corroborate Bichuette and Trajano (2003) in that the small size of *Potamolithus* gastropods in the Alto Ribeira would be a preadaptation (in the sense of exaptation) favoring the colonization of subterranean habitats and establishment of troglomorphic populations, and explains the wide distribution of these mollusks in the area.

Morphological data from *Potamolithus* spp. from the Alto Ribeira do not support the hypothesis of a strict correlation, or a single cause-and-effect relationship, between miniaturization and intestine coiling. The species reaching the largest size, the epigeal *P. ribeirensis*, presents a U-shaped intestine, and the second largest one, the troglomorphic *Potamolithus* sp. 9, from Pescaria cave, has a type-3 intestine, a coiling condition that is more advanced than that observed in the smaller troglomorphic *Potamolithus* spp. 11 (type 1) and 12 (type 2). Other factors besides body size may affect intestine shape, for instance selection for an increase in intestine length (increase in absorptive sur-

face) as an adaptation to increase the efficiency of nutrient intake. This hypothesis is corroborated by the observed tendency of troglobites to have more coiled intestines (types 3, 4, or 5, except the aforementioned cases, *versus* types 1 or 2 in the epigean and trogliphilic populations). In fact, a coiled condition of the intestine is also observed in deep-sea mollusks, and this is related to an increase in the area for nutrient absorption (Sanders and Allen 1973).

The relatively large size of *Potamolithus* sp. 9 may be a plesiomorphic trait, i.e., the retention of the non-miniaturized state present in a population that was extinct in the epigean habitat, or it may be the result of a secondary increase in size in a formerly miniaturized population (character reversal). Trajano and Bichuette (2016) hypothesized that the catfish *Ituglanis passensis* Fernández & Bichuette, 2002, from Passa Três cave in São Domingos karst area, Central Brazil, originated from a miniaturized population that vertically colonized the subterranean habitat through the epikarst (see Bichuette et al. 2015). As food input drastically increased—due to the opening of large conduits and collapse of the ceiling which then formed a typical lotic habitat with large amounts of organic matter carried into the cave—intraspecific competition without the constraints of food limitation would have selected larger sizes, resulting in a gigantic miniaturized species. There is no evidence of vertical colonization of caves by *Potamolithus* gastropods in the Alto Ribeira karst area; on the contrary, observations of epigean and trogliphilic populations indicate that these organisms colonize the hypogean environment mostly through cave sinkholes and resurgences. However, the Pescaria cave is ecologically similar to Passa Três cave, and the model proposed for *I. passensis* may also account for the large size of *Potamolithus* sp. 9. The type-3 intestine in the latter corroborates this hypothesis because it seems inconsistent with the present-day conditions; apparently the cave habitat is not food-limited, and the relatively large size of the shell does not require coiling. A proper test for the two hypotheses—large size as a plesiomorphic condition *versus* character reversal—would start with a character optimization in a phylogeny of *Potamolithus* gastropods including the species of interest. Unfortunately, none is available to date.

The degree of troglomorphism, usually relative to visual structures and dark pigmentation, has frequently been used to infer the phylogenetic age of troglobitic taxa (species or higher), i.e., its time of isolation in the subterranean environment (Poulson 1963, Wilkens 1982, Trajano 1995). Therefore, *Potamolithus* spp. 8 and 9, from Morro Preto and Couto caves in the Betari micro-basin, and from Pescaria Cave in the Pilões micro-basin respectively, were isolated in the hypogean habitat later than the other troglobitic species (they would be “younger troglobites”). However, as pointed by Trajano (2007), this reasoning assumes fairly constant rates of morphological differentiation among subterranean taxa, but mosaics of character states in closely related groups indicate that different mechanisms act at different rates in each population. In addition, differences in population sizes affect rates of divergence.

It is noteworthy that three of the seven *Potamolithus* spp. that form trogliphilic populations in the Alto Ribeira are characterized by a depigmented body (sp. 2 from

Ouro Grosso, 4 from the Água Suja, and sp. 5 from Roncador systems; the periostracum is also depigmented in spp. 4 and 5), indicating relaxed selection for dark pigmentation. On the other hand, all epigeal and troglomorphic populations have eyes, indicating a stabilizing selective pressure for maintenance of eyes in the epigeal habitat.

Conservation: threats to *Potamolithus* gastropods in the Alto Ribeira karst area and the problem of over-collection

Degradation of water quality resulting, for instance, from quarrying and mining activities, poorly controlled tourism, and deforestation, especially upstream of the subterranean systems, may severely impact cave dwelling *Potamolithus* populations, since they show a strong preference for clean, well-oxygenated waters. Eberhard (1992) described the negative impacts of mining activities on cave snails from Tasmania.

The Alto Ribeira karst area in São Paulo State is mostly situated within the limits of State Parks, which should warrant protection for the aquatic gastropods endemic to this region. Unfortunately, in practice, this is not the case because Brazilian law allows for the exclusion of areas for any extension or social or economic reasons considered strategic for the State. This has happened recently with the area including the Areias resurgence in the PETAR, the locality of a differentiating population of the blind catfish, *Pimelodella kronei* (Miranda Ribeiro, 1907), a troglomorphic catfish included on the IUCN Brazilian Red List (Endangered - EN category) (ICMBio 2016). Therefore, even protection by conservation units is not definitive. Moreover, the headwaters of several micro-basins (e.g., Pilões and Iporanga rivers) and the sources of the Betari river are not protected and are subject to pollution. Pollution by pesticides has been reported for the Betari river (Moraes et al. 2003)

Another important threat to the subterranean fauna is over-collection (Trajano et al. 2012). *Potamolithus troglobius* and *P. karsticus* are listed as Critically Endangered (CR) on the IUCN Brazilian Red List (ICMBio 2016) and collections must be controlled and responsible. *P. troglobius* is endemic to a single cave system (Areias) and it is an important component of the diet of *P. kronei*.

Regrettably, a loose ethical attitude of many modern researchers imbued by the “publish or perish” philosophy (Trajano 2017), allied with poor control of collections by the licensing authorities, have resulted in instances of over-collection. An illustrative example is the collection of a total of 3,325 specimens of *P. troglobius* from 12 sites during four sampling occasions using the Surber technique (Silva et al. 2015). This sampling technique is non-selective (with respect to benthic organisms) and disturbs the streambed in the area inside the sampling frame—where the rocky substrate must be cleaned and all fauna dislodged—and can result in significant impacts on the ecosystem when used without proper criteria; the huge number of gastropods collected (+ 3,000) indicates that this was the case in the Areias system. It is noteworthy that such collections were done for a study authorized by the Park authorities, in a cave

where another similarly authorized study—on the troglobitic catfish population using mark-recapture—was in progress for almost five years (Guil and Trajano 2013), in the context of one of the longest monitoring projects of a subterranean species worldwide, which started in the 1980s (Trajano 1991). Indeed, *P. troglobius* is part of the diet of the catfish *Pimelodella kronei*, the first troglobite described for Brazil collected in the late 1890s, and Areias system is the type-locality of *P. kronei*. There is no justification for such an enormous number of specimens to be collected. This cave has been studied biologically since the 1940s and was recognized as the first high-diversity spot for troglobites in 2005 (Trajano et al. 2016).

It is clear that any experimental design, and its associated sampling techniques, that result in the collection of such a huge number of individuals is a misconception, and a threat to be avoided. No scientific study, even focusing on a completely unknown species (not the case), would require such a sample size.

On the other hand, collections are indispensable because effective conservation policies are based on good biological data, and good biological data depend on careful examination of specimens by experts. There are no a priori rules regarding sample sizes, which depend not only on the objectives of the study but also on the taxon under consideration.

Only ethically oriented, experienced researchers are qualified to assess the need for collections and the scientifically acceptable minimum and maximum sample sizes. Therefore, the best way to ensure protection of biodiversity is to rely on the scientific community's self-regulation, initiatives such as the São Francisco Declaration on Research Assessment (DORA) as guidelines for refereeing processes for funding agencies, editorial policies for scientific journals, and selection of positions at universities and research centers, etc.

Conclusions

Potamolithus shows high morphological diversity in the Alto Ribeira karst area, and from 15 potential species, six are troglobitic ones, reinforcing the status of the region as a high-diversity spot for troglobites. Morphological data observed herein (and frequently related to isolation in subterranean environments) do not support the hypothesis of a strict correlation, or a single cause-and-effect relationship, between miniaturization and intestine coiling, as observed and stated for other subterranean aquatic gastropods. Relaxed selection for dark pigmentation in trogliphilic species and stabilizing selective pressure for maintenance of eyes in the epigeal and part of the trogliphilic populations explain the morphological characters in these populations. There is evidence that epigeal and trogliphilic populations of *Potamolithus* colonize the hypogean environment mostly through cave sinkholes and resurgences. Finally, these species are threatened mostly by over-collection procedures, pollution of subterranean waters, and uncontrolled tourism.

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References

- Ab'Sáber AN (1977) Os domínios morfoclimáticos na América do Sul: primeira aproximação. *Geomorfologia* 53: 1–23.
- Ab'Sáber AN (1981) Domínios morfoclimáticos atuais e quaternários na região dos cerrados. *Craton Intracraton* 14: 1–39
- Angelov A (2000) *Mollusca (Gastropoda et Bivalvia) aquae dulcis, catalogus Faunae Bulgaicae*. Pensoft & Backhuys Publ, Sofia, Leiden.
- Arnold EN (1994) Investigating the origins of performance advantage: adaptation, exaptation and lineage effects. In: Eggleton P, Vane-Wright RI (Eds) *Phylogenetics and Ecology*. Academic Press, London, 123–167. <https://doi.org/10.1086/282512>
- Barr TC (1968) Cave ecology and the evolution of troglobites. *Evolutionary Biology* 2: 35–102. https://doi.org/10.1007/978-1-4684-8094-8_2
- Becker M, Zielske S, Haase M (2016) Conflict of mitochondrial phylogeny and morphology-based classification in a pair of freshwater gastropods (Caenogastropoda, Truncatelloidea, Tateidae) from New Caledonia. *Zookeys* 603: 17–32. <https://doi.org/10.3897/zookeys.603.9144>
- Bichuette ME (1998) Distribuição e biologia de gastrópodes de água doce, gênero *Potamolithus*, no Vale do Alto Ribeira, São Paulo (Mollusca: Gastropoda: Hydrobiidae). Master's Degree thesis, Instituto de Biociências, Universidade de São Paulo.

- Bichuette ME, Menna-Barreto L (2005) Preliminary data on locomotor activity rhythms on epigeal and cave snails, genus *Potamolithus* (Hydrobiidae), from southeastern Brazil. *Subterranean Biology* 3(58): 43–48.
- Bichuette M, Trajano E (1999) Light reaction, spontaneous and feeding behaviour in epigeal and cave *Potamolithus* species from upper Ribeira Valley, Southeastern Brazil (Mollusca: Gastropoda: Hydrobiidae). *Mémoires de Biospéologie* (Cessou em 2001. Cont. ISSN 1768-1448 *Subterranean Biology*) 26: 1–6.
- Bichuette ME, Trajano E (2003) A population study of epigeal and subterranean *Potamolithus* snails from Southeast Brazil (Mollusca: Gastropoda: Hydrobiidae). *Hydrobiologia* 505: 107–117. <https://doi.org/10.1023/B:HYDR.0000007299.26220.b8>
- Bichuette ME, Rantin B, Hingst-Zaher E, Trajano E (2015) Geometric morphometrics throws light on evolution of the subterranean catfish *Rhamdiopsis krugi* (Teleostei: Siluriformes: Heptapteridae) in eastern Brazil. *Biological Journal of the Linnean Society* 114: 136–151. <https://doi.org/10.1111/bij.12405>
- Boeters HD (1979) Species concept of prosobranch freshwater molluscs in Western Europe, I. *Malacologia* 18: 57–60.
- Bodon M, Cianfanelli S, Talenti E (1996) Idrobiidi freatobi del bacino del fiume era in Toscana (Gastropoda: Prosobranchia: Hydrobiidae). *Bolletino Malacologico* 32(5–8): 95–120.
- Bole J, Velkovrh F (1986) Mollusca from continental subterranean aquatic habitats. In: Botosaneanu L (Ed.) *Stygofauna Mundi*, 177–208.
- Cruz FW Jr., Burns SJ, Karmann I, Sharp WD, Vuille M, Cardoso AO, Ferrari JA, Dias PLS, Viana-Júnior O (2005) Insolation-driven changes in atmospheric circulation over the past 116 ky in subtropical Brazil. *Nature* (London) 434: 63–66. <https://doi.org/10.1038/nature03365>
- Davis GM, Mazurkiewickz M, Mandracchia M (1982) *Spurwinkia*: Morphology, systematics and ecology of a new genus of North American marshland Hydrobiidae (Mollusca: Gastropoda). *Proceedings of the Academy of Natural Sciences of Philadelphia* 134: 143–177.
- Davis GM, Pons-da-Silva MC (1984) *Potamolithus*: morphology, convergence, and relationships among hydrobioid snails. *Malacologia* 25(1): 73–108.
- Dillon RT (2000) *The Ecology of Freshwater Molluscs*. University Press, Cambridge. <https://doi.org/10.1017/CBO9780511542008>
- Fernandes CS, Bueno, SLS, Bichuette ME (2013) Distribution of cave-dwelling *Aegla* spp. (Decapoda: Anomura: Aegliidae) from the Alto Ribeira karstic area in southeastern Brazil based on geomorphological evidence. *Journal of Crustacean Biology* 33: 567–575. <https://doi.org/10.1163/1937240X-00002159>
- Fretter V, Graham A (1962) *British prosobranch molluscs*. Ray Society, London.
- Eberhard S (1992) *The Effect of Stream Sedimentation on Population Densities of Hydrobiid Molluscs in Caves*. Unpublished report to Dept. Parks, Wildlife & Heritage, May 1992, 11 pp.
- Gallão JE, Bichuette ME (2018) Brazilian obligatory subterranean fauna and threats to the hypogean environment. *ZooKeys* 746: 1–23. <https://doi.org/10.3897/zookeys.746.15140>
- Guil AL, Trajano E (2013) Dinâmica populacional do bagre cego de Iporanga, *Pimelodella kronei*: 70 anos de estudo. *Revista da Biologia* 10: 34–39. <https://doi.org/10.7594/revbio.10.02.06>

- Haase M, Zielske S (2015) Five new cryptic freshwater gastropod species from New Caledonia (Caenogastropoda, Truncatelloidea, Tateidae). *ZooKeys* 523: 63–87. <https://doi.org/10.3897/zookeys.523.6066>
- Hammer O, Harper DAT, Ryan PD (2001) PAST, Paleontological Statistics software package for education and data analysis. *Paleontologia Eletrônica* 4: 9.
- Hershler R, Holsinger JR (1990) Zoogeography of North American hydrobiid cavesnails. *Stygologia* 5: 5–16.
- Hershler R, Longley G (1986) Phreatic hydrobiids (Gastropoda: Prosobranchia) from the Edwards (Balcones Fault Zone) aquifer region, South-Central Texas. *Malacologia* 27: 127–172.
- Hueck K (1972) *As florestas da América do Sul*. Editora Polígono, São Paulo.
- ICMBio (2016) Livro Vermelho da Fauna Brasileira Ameaçada de Extinção – Sumário Executivo. Ministério do Meio Ambiente, Brasília.
- Karmann I, Sánchez LE (1979) Distribuição das Rochas Carbonáticas e Províncias Espeleológicas do Brasil. *Espeleo-Tema* (São Paulo) 13: 105–168.
- Köeppen W (1948) *Climatología*. Fondo de Cultura Economica, Cidade do México.
- de Lucía M, Gutiérrez-Gregoric DE (2017a) The genus *Potamolithus* Pilsbry, 1896 (Gastropoda: Tateidae) on the Somuncurá Plateau, Patagonia, Argentina. *Molluscan Research* 37(3): 202–211. <https://doi.org/10.1080/13235818.2017.1279476>
- de Lucía M, Gutiérrez-Gregoric DE (2017b) Redescrición de *Potamolithus supersulcatus* Pilsbry, 1896 (Gastropoda, Tateidae) del Sur de la Cuenca del Plata. *Papéis Avulsos de Zoologia, Museu de Zoologia da Universidade de São Paulo* 57(17): 207–219. <https://doi.org/10.11606/0031-1049.2017.57.17>
- Macan TT (1949) A key to fresh- and brackish- water gastropods with notes on their ecology. Scientific Publication 13. Biological Association, Ambleside.
- Malek EA (1983) The South American hydrobioid genus *Idiopyrgus* Pilsbry, 1911. *Nautilus* 97(1): 16–20.
- Moraes R, Elfvendahl S, Kylin H, Molander S (2003) Pesticide residues in rivers of a Brazilian Rain Forest Reserve: assessing the potential concern for effects on freshwater biota and human health. *Ambio* 4: 258–263. <https://doi.org/10.1579/0044-7447-32.4.258>
- Pilsbry HA (1911) Non-marine Mollusca of Patagonia. In: Scott WB (Ed.) *Reports of the Princeton University Expedition to Patagonia - 1896–1899*, 3(2): 513–633.
- Ponder W (1992) A new genus and species of aquatic cave-living snail from Tasmania (Mollusca: Gastropoda: Hydrobiidae). *Papers and Proceedings of the Royal Society of Tasmania* 126: 23–28.
- Ponder W (1994) Australian freshwater Mollusca: conservation priorities and indicator species. *Memoirs of the Queensland Museum* 36(1): 191–196.
- Ponder WF, Clark SA, Ebehard S, Studdert JB (2005) A radiation of hydrobiid snails in the caves and streams at Precipitous Bluff, southwest Tasmania, Australia (Mollusca: Caenogastropoda: Rissooidea: Hydrobiidae s.l.). *Zootaxa* 1064: 1–66. <https://doi.org/10.11646/zootaxa.1074.1.1>
- Pons-da-Silva MC (1993) Dados morfológicos de *Heleobia parchappei* (Orbigny, 1835) (Prosobranchia, Hydrobiidae, Littoridininae). *Iheringia, Série Zoológica, Porto Alegre* 75: 81–87.
- Poulson TL (1963) Cave adaptation in Amblyopsid fishes. *American Midland Naturalist* 70: 257–290. <https://doi.org/10.2307/2423056>

- Sanders H, Allen JA (1973) Studies on deep-sea Protobranchia (Bivalvia); prologue and the Pristiglomidae. *Bulletin of the Museum of Comparative Zoology* 145: 237–262.
- Simone LRL, Moracchioli N (1994) Hydrobiidae (Gastropoda Hydrobioidea) from the Ribeira Valley, SE Brazil, with descriptions of two new cavernicolous species. *Journal of Molluscan Studies* 60: 445–459. <https://doi.org/10.1093/mollus/60.4.445>
- Silva GGR, Martins VM, Silva MS, Ferreira RL (2015) Distribuição especial e temporal de *Potamolithus troglobius* (Mollusca: Gastropoda: Hydrobiidae), no Sistema Areias, Iporanga. I Simposio Brasileiro de Biologia Subterrânea – Livro de Resumos. UFLA, Lavras, 22–22.
- Simone LRL (2006) Land and freshwater molluscs of Brazil: an illustrated inventory on the Brazilian Malacofauna, including neighbor regions of the South America respect to the terrestrial and freshwater ecosystems. FAPESP, EGB, São Paulo.
- Sokal RR, Sneath PHA (1963) Principles of Numerical Taxonomy, W.H. Freeman, San Francisco.
- Solem A, van Bruggen AC (1984) World-wide snail. Biogeographical studies on non-marine Mollusca. E J Brill, Leiden.
- Trajano E (1991) Populational ecology of *Pimelodella kronei*, troglobitic catfish from southeastern Brazil (Siluriformes, Pimelodidae). *Environmental Biology of Fishes* 30: 407–21. <https://doi.org/10.1007/BF02027984>
- Trajano E (1995) Evolution of tropical troglobites: Applicability of the model of Quaternary climatic fluctuations. *Mémoires de Biospéologie* 22: 203–209.
- Trajano E (2001) Mapping subterranean biodiversity in Brazilian karst areas. In: Culver DC, Deharveng L, Gibert J, Sasowsky ID (Eds) Karst Waters Institute Special Publications 6: 67–70.
- Trajano E (2007) The challenge of estimating the age of subterranean lineages: examples from Brazil. *Acta carsologica* 36: 191–198. <https://doi.org/10.3986/ac.v36i1.221>
- Trajano E (2012) Ecological classification of subterranean organisms. In: White WB, Culver DC (Eds) Encyclopedia of Caves. Academic Press, Waltham. <https://doi.org/10.1016/B978-0-12-383832-2.00035-9>
- Trajano E (2017) Bioética e Ciência: Natureza Biológica dos Humanos e Ciência no Século XXI. In: Hossne WS, Pessini L, Barchifontaine CP (Eds) Bioética no Século XXI Anseios, receios e devaneios. Edições Loyola, São Paulo: 41–63.
- Trajano E, Batalha MA, Bichuette ME (2012) Estudos ambientais em cavernas: os problemas da coleta, da identificação, da inclusão e dos índices. *Espeleo-Tema* (São Paulo) 23: 13–22.
- Trajano E, Bichuette ME (2016) Multiple-step vertical colonization of the subterranean environment- Brazilian troglobitic catfishes as case studies. In: The French and German National Chapters of the International Association of Hydrogeologists, orgs. 43rd IAH Congress – Abstract Book [abstract number 2521]. <http://60iah2016.org> [Accessed 25 July 2017]
- Trajano E, Britski HA (1992) *Pimelodella kronei* (Ribeiro, 1907) e seu sinônimo *Caecorhamdella brasiliensis* Borodin, 1927: morfologia externa, taxonomia e evolução (Teleostomi, Siluriformes). *Boletim de Zoologia*, São Paulo 12: 53–89. <https://doi.org/10.11606/issn.2526-3358.bolzoo.1988.122378>
- Trajano E, de Carvalho MR (2017) Towards a biologically meaningful classification of subterranean organisms: a critical analysis of the Schiner-Racovitza system from a historical perspective, difficulties of its application and implications for conservation. *Subterranean Biology* 22: 1–26. <https://doi.org/10.3897/subtbiol.22.9759>

- Trajano E, Gallão JE, Bichuette ME (2016) Spots of high diversity of troglobites in Brazil: the challenge of measuring subterranean diversity. *Biodiversity and Conservation* 25: 1805–1828. <https://doi.org/10.1007/s10531-016-1151-5>
- Wenz W (1938) *Gastropoda*. Gebriüder Borntraeger, Berlin.
- Wiley EO, Mayden RL (2000) The evolutionary species concept. In: Wheeler QD, Meier R (Eds) *Species concepts and phylogenetic systematics: A debate*. Columbia University Press, New York, 70–89.
- Wilke T, Haase M, Hershler R, Liu HP, Misof B, Ponder W (2013) Pushing short DNA fragments to the limit: Phylogenetic relationships of ‘hydrobioid’ gastropods (Caenogastropoda: Rissosoidea). *Molecular Phylogenetics and Evolution* 66: 715–736.
- Wilkens H (1982) Regressive evolution and phylogenetic age: the history of colonization of freshwaters of Yucatan by fish and crustacea. *Bulletin of the Texas Memorial Museum* 128: 237–243.
- WoRMS Editorial Board (2017) *World Register of Marine Species*. Available from <http://www.marinespecies.org> at VLIZ. [Accessed 2017-12-29] <https://doi.org/10.14284/170>

Supplementary material I

Morphometric data

Authors: Maria Elina Bichuette, Eleonora Trajano

Data type: measurement

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