

A comparison of morphology and web geometry between hypogean and epigean species of *Metellina* orb spiders (family Tetragnathidae)

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Abstract

Studies on the behaviour of subterranean animals are rare, mainly due to the problems with collecting data in these inaccessible habitats. Web-building cave spiders, however, leave a semi-permanent record of their foraging behaviour, which can relatively easily be recorded. In this study, we compare size, leg lengths and web characteristics between hypogean populations of *Metellina merianae* with its close wood-inhabiting relative *M. mengei*. We confirm previous observations that *M. merianae* does not show any obvious morphological and behavioural adaptations to a subterranean life-style, although individuals of the cave species were significantly larger and had webs with relatively fewer radii and capture spiral turns than *M. mengei*. We were, however, not able to determine if these findings indicate a transition towards behavioural adaptation to caves or if they are a result of behavioural flexibility in response to the different humidity and temperature between caves and woodland. Finally, we did not find any effect of cave characteristics on either the number of radii or the area of the *M. merianae* web.

Keywords

behavioural adaptations, orb webs, morphological adaptations, troglophile, leg length, *Metellina merianae*, *Metellina mengei*

Introduction

The unique adaptations to the dark and nutrient poor subterranean habitat have fascinated biologists since the dawn of speleobiology more than 200 years ago. Studies have discovered numerous examples of convergent evolution including physiological adaptations such as low metabolic rates and other mechanisms to survive long-term starvation (Hervant et al. 1997; Lipovsek et al. 2018), and morphological adaptations such as elongation of limbs, loss of eyes and pigments (Poulson and White 1969; Christiansen 2004; Culver and Pipan 2009; Mammola 2018). However, very few studies have looked into behavioural adaptations to the hypogean environment (but see: Juberthie-Jupeau 1988; Hoch and Howarth 1993; Parzefall 2005), likely because in-situ studies of animal behaviour are fraught with difficulties given the scarcity and inaccessibility of most cave organisms. Arachnids are promising candidate organisms for addressing this imbalance as they are one of the most numerous taxa in subterranean habitats, and display a wide range of interesting behaviours (Foelix 2011; Chapin and Hebets 2016; Mammola and Isaia 2017). Surprisingly given the lack of light and low abundance of flying insects, even orb spiders, whose two-dimensional webs are especially adapted to intercept and retain flying prey, are represented in the entrance and twilight zone of caves and mines with at least four genera in the family Tetragnathidae (*Meta*, *Metellina*, *Okileucauge* and *Orsinome*) (Eberhard 1990; Mammola and Isaia 2017; Hesselberg et al. 2019).

Orb spiders are often considered model organisms in the study of animal behaviour due to the ease with which quantitative measures of foraging behaviour can be obtained from photographs of the web, and due to their high behavioural flexibility (Zschokke and Herberstein 2005; Hesselberg 2015). Despite, mainly being found within the first 20 m of caves and not showing any troglomorphic adaptations, cave orb spiders in the genus *Meta* are one of the few cave organisms, where unique behavioural adaptations to subterranean life have been observed (Novak et al. 2010; Hesselberg et al. 2019). These include modified orb webs with missing frames and radii attaching directly to the substrate (Yoshida and Shinkai 1993; Simonsen and Hesselberg unpublished), possibly off-web foraging (Eckert and Moritz 1992; Smithers 2005a) and a life history which includes one or two instars spent outside of the subterranean environment (Smithers 2005b; Mammola and Isaia 2014).

Much less information is available on the other tetragnathid genera with troglophile members. *Okileucauge* and *Orsinome* are two small genera with currently 9 species all from China and Japan, and 16 species found mainly in Asia, respectively. We have almost no information on the ecology or behaviour of any of these species (World Spider Catalog 2019), although *Orsinome cavernicola* (Thorell, 1878) from Indonesia, an unidentified *Orsinome* from Tasmania and *Okileucauge geminuscavum* (Chen & Zhu, 2009) from China have been found in caves (Eberhard 1992; Chen and Zhu 2009). *Metellina* is a small genus currently containing 11 species with a cosmopolitan distribution (World Spider Catalog 2019), but it is in a state of flux with a considerable number of these 11 species added recently (Kallal and Hormiga 2018; Marusik and Larsen 2018). Not much is known on the ecology and behaviour of its members either, although some studies have

been conducted on mating and web-building behaviour in the common European species *M. segmentata* (Clerck, 1757) and *M. menzei* (Blackwall, 1870) (Prenter et al. 1994; Bridge et al. 2000; Tew and Hesselberg 2017, 2018). *Metellina* species are known to prefer dark and humid places to build their orb webs, although *M. menzei* can be found both in the dark interior, in clearings and at the edge of forests (Tew and Hesselberg 2017). At least two species, *M. merianae* (Scopoli, 1763) from Europe and the Middle East and *M. villiersi* (Denis, 1955) from Guinea are found in caves and classified as troglophiles (Hesselberg et al. 2019). While, no information is available on the biology of the latter, *M. merianae* have been the focus of a few studies. It is a relatively widespread species that is found in burrows, hollow trees and within the entrance and twilight zone of caves. Cave populations are known to co-exist with *Meta menardi* (Latreille, 1804) with limited spatial niche separation in that *M. merianae* are more likely to be found closer to the cave entrance, but with some temporal niche separation in that *M. merianae* show more pronounced difference in abundance throughout the year compared to *M. menardi* (Novak et al. 2010). Unlike, *Meta* orb spiders, *Metellina merianae* does not appear to show any behavioural adaptations to subterranean life as their orb webs resemble standard orb webs with frame threads and a large number of radii (Eckert and Moritz 1992; Novak et al. 2010), although this has not been systematically investigated. Here we aim to verify this claim by conducting a comparative study on overall size, relative leg length and detailed web geometry in the hypogean *M. merianae* and the epigean *M. menzei*.

Methods

Metellina merianae

Thirteen karst caves and abandoned mines with horizontal and easily accessible entrances were surveyed on Mallorca and Menorca, Balearic Islands, Spain during October and November 2018. Depending on the size, between 20 and 60 minutes were spent in the twilight zone visually searching for spider webs or lone spiders on cave floors, walls and ceilings. Out of the 13 caves (3 tiny, 4 small, 3 medium and 3 large), the following 5 harboured populations of *M. merianae*: Puig de Randa (large mine, UTM 31S 493175N, 4375097), Cova de Raviols (large cave, UTM 31S 458560, 4385672), Coves des Pillar (large cave, UTM 31S 464641, 4383561), Cova de Sa Trinxeta (medium cave, UTM 31S 464697, 4383578) and Cova de Sa Cometa des Morts (medium cave, UTM 31S 490691, 4408759). The size classifications used were: tiny (1–2 meters long and shed sized), small (2–5 meters long and room sized), medium (5–15 meters long) and large (longer than 15 meters).

Once the presence of *M. merianae* was confirmed, accessible webs built by juveniles or adult females within the twilight zone (here defined as 2–30 meters from the entrance) were measured with a ruler (to a precision of 0.1 cm) and the following data collected (which are standard measurements of field webs – see for example Hesselberg and Triana 2010): number of spirals above and below hub from which the average

number of spirals were calculated, the number of radii, horizontal and vertical diameter of the capture spiral, the diameter of the hub and free zone and the maximum distance from the web plane to either the cave wall or floor (depending on the orientation of the web). We used the ellipse-hub equation to calculate the area of the capture spiral ($\text{vertical diameter}/2 * \text{horizontal diameter}/2 * \pi - (\text{hub diameter}/2)^2$) (Herberstein and Tso 2000) as a measure of web area. We included web data from both juveniles and adult webs in order to obtain a decent sample size. This should not affect our comparisons with adult *M. mengei* webs (see below) since most characteristics of orb webs scale isometrically (Sensenig et al. 2011). The temperature at the site of the webs ranged from 11 °C to 14 °C and the humidity ranged from 72% to 93% rH.

The resident spider was collected in 90% ethanol and taken back to the laboratory, where the total length, the cephalothorax width, as a measure of spider size, and the combined patella-tibia length of leg I and III, as measurements of leg lengths, were measured with a digital calliper under a light microscope to a precision of 0.1 mm. The life stage of the spider was determined under the microscope as either juvenile (epigyne absent) or adult (epigyne present). In addition, distance from the cave entrance, temperature and humidity data was gathered at the location of the web as well as whether most of its attachment threads were connected to the floor, wall or ceiling. Finally, adult or subadult females without webs were collected directly from cave walls or occasionally from webs that were inaccessible for measurements in order to get additional spiders for the morphological measurements to ensure that only late instar juveniles and adult spiders were used.

Metellina mengei

Metellina mengei web and morphological data was collected in May and June 2018 from Wytham Woods, Oxfordshire, UK (UTM 30U 614887, 614887) as part of a larger comparative study of temperate tetragnathid spiders (Simonsen and Hesselberg, unpublished). 27 webs were surveyed within 2 m on either side of 10 randomly chosen 50 m transects. The temperature and the humidity in the vicinity of the webs ranged from 14 °C to 23 °C and from 55% to 86% rH. Only undamaged webs built by adult females were included in the survey and care was taken to sample webs from different height in the vegetation. The resident spiders were collected and brought to the laboratory, where they were killed in 70% ethanol before being measured. The same web characteristics and morphological data was gathered using similar methods as those described above for *M. merianae*.

Data analysis

To compare differences in overall morphology and web characteristics between the two species, we first performed a principal component analysis with the *prcomp()* function in R before using the *ggplot2* package (Wickham 2016) to generate ordination plots. To

control for differences in spider size, the following variables were used in linear mixed models (LMMs) as the response variable: relative patella-tibia length of leg I and III (patella tibia length divided by cephalothorax width), relative web area (web area divided by cephalothorax width squared), number of spiral turns per cm² web (average number of spiral turns divided by web area) and number of radii per cm² web (number of radii divided by web area) with species as predictor variable and location as a random effect. The LMMs were developed using the *lmer()* function from the *lme4* package (Bates et al. 2015). The two models were validated using diagnostic plots from the *mcp.fnc()* function from the *LMERConvenienceFunctions* package (Tremblay and Ransijn 2015). Relative web area and the number of capture spirals and radii per cm² web had to be log transformed to achieve normality. P values were found using the Type II Wald F-test.

In order to investigate the potential factors affecting the *M. merianae* webs, two LMMs were developed and validated as stated above with the number of radii and the area of the capture spiral as response variables, distance from the cave entrance, cephalothorax width, the location of the web (cave wall or floor), cave size (medium and large) and the number of radii (for the area of capture spiral model) as well as the interactions between cephalothorax width and the distance to the cave entrance and the location of the web as predictor variables and cave as a random effect. The models were reduced using the backwards elimination method based on P values using the Type II Wald F test. For all analyses, R (R Core Team 2018) was used with a significance level of 0.05. Figures were created with the *ggplot2* package (Wickham 2016).

Results

Comparisons of leg length and web characteristics between *Metellina merianae* and *M. mengei*

The differences in morphology and web parameters between *M. merianae* and *M. mengei* were visualised with a PCA ordination plots (Fig. 1), which showed that the two species differed substantially in size with the *M. merianae* about 1 ½ times larger than *M. mengei* (Fig. 1A; Table 1). The difference in web parameters were less pronounced and were almost entirely driven by differences in web area with *M. merianae* webs being more than twice as large as those of *M. mengei*, although they also showed much more variation in size (Fig. 1B; Table 1). Interestingly, despite their much larger webs, webs of *M. merianae* had slightly fewer spiral turns and radii than the webs of *M. mengei*.

However, to make meaningful comparisons between the hypogean *M. merianae* and the epigean *M. mengei*, we need to control for the difference in size (Table 1). The relative patella-tibia length (length divided by cephalothorax width) for leg I did not differ significantly between the two species (LMM: $F = 0.81$, $df = 1$, $P = 0.45$). However, *M. merianae* had a significantly longer relatively patella-tibia leg length for leg III than *M. mengei* (LMM: $F = 23.46$, $df = 1$, $P = 0.002$). The relative web area (here divided by cephalothorax width squared to keep it dimensionless) was not significantly

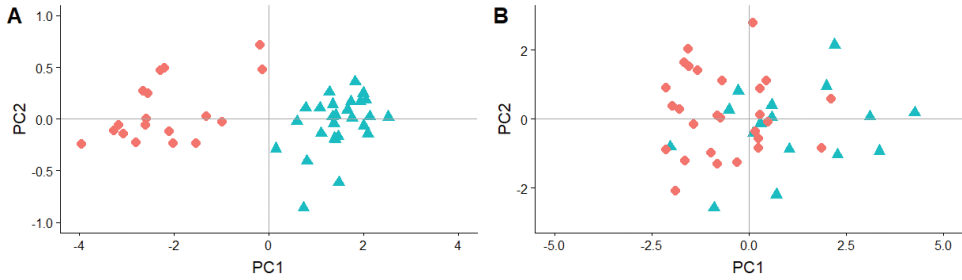


Figure 1. Principal component ordination plots of morphological and web parameters of *Metellina merianae* (red circle) and *Metellina menzei* (blue triangle). **A** Morphological variables (total length, cephalothorax width, patella-tibia length of leg I and leg III). The combined proportion of variance explained by PCA1 and PCA2 was 98%. **B** Web parameters (number of spirals, number of radii, vertical length of web, horizontal length of web and hub diameter). The combined proportion of variance explained by PCA1 and PCA2 was 78%.

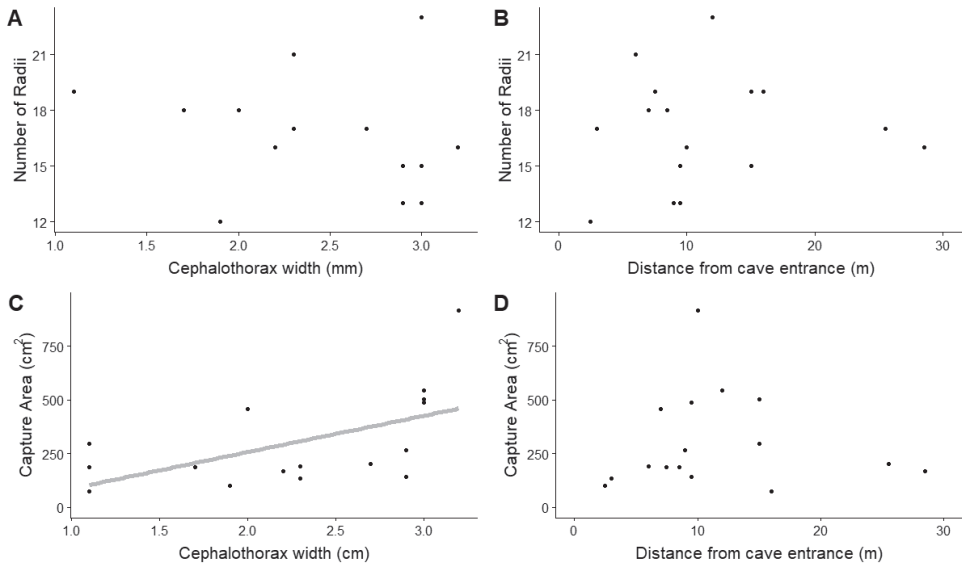


Figure 2. Area of webs of *Metellina merianae* in response to spider size and position in cave. **A** The number of radii in the web as a function of cephalothorax width of the resident spider. Not significant. **B** The number of radii in the web as a function of its distance from the cave entrance. Not significant. **C** The area of the capture spiral as a function of cephalothorax width. Significant. The grey line represents the equation ($y = 169x - 81$, $R^2 = 0.31$) arising from a simple linear regression. **D** The area of the capture spiral in the web as a function of its distance from the cave entrance. Not significant.

different between the two species (LMM: $F = 0.41$, $df = 1$, $P = 0.54$). However, both the number of spirals and the number of radii per cm^2 web area differed significantly with fewer in the webs of *M. merianae* (spiral number: LMM: $F = 24.3$, $df = 1$, $P = 0.002$; radii number: LMM: $F = 20.7$, $df = 1$, $P = 0.004$).

Table 1. Morphological traits and web characteristics of hypogean *Metellina merianae* spiders from caves in the Balearic Islands, Spain and epigean *Metellina menzei* from Wytham Woods, UK.

Variables	<i>Metellina merianae</i>	<i>Metellina menzei</i>
Morphological traits		
Sample size	18	27
Total length (mm)	7.1 ± 1.1	4.6 ± 0.5
Cephalothorax width (mm)	2.8 ± 0.5	1.6 ± 0.2
Patella-tibia length – leg I (mm)	5.8 ± 1.1	3.1 ± 0.3
Relative patella-tibia length – leg I ^a	2.05 ± 0.25	1.99 ± 0.14
Patella-tibia length – leg III (mm)	2.8 ± 0.6	1.3 ± 0.1
Relative patella-tibia length – leg III ^a	0.98 ± 0.13	0.81 ± 0.08
Web characteristics		
Sample size	16	27
Web area (cm ²)	96.7 ± 71.0	36.5 ± 15.7
Relative web area ^b	2113 ± 1992	1513 ± 652
Average number of spiral turns	13.0 ± 3.0	15.8 ± 4.1
Spiral turns per cm ² web area	0.21 ± 0.14	0.52 ± 0.26
Number of radii	16.9 ± 3.0	18.5 ± 3.0
Radii per cm ² web area	0.27 ± 0.18	0.62 ± 0.30

Mean ± Std. dev.; ^a Relative length is original length divided by cephalothorax width; ^b Relative web area is original web area divided by cephalothorax width squared

Factors affecting web characteristics in the hypogean *Metellina merianae*

Webs from 11 juvenile and 5 adult *M. merianae* were used in the analysis. The number of radii in the webs was not significantly influenced by the distance from the cave entrance (LMM: $F = 0.41$, $df = 1$, $P = 0.53$), whether webs were found on the wall or the floor of the cave (LMM: $F = 0.001$, $df = 1$, $P = 0.98$), the size of the cave (LMM: $F = 3.26$, $df = 2$, $P = 0.45$) or the size of the spider (LMM: $F = 0.43$, $df = 1$, $P = 0.53$) nor were any of the interactions tested found to be significant (data not shown). Similarly, the size of the web did not depend on the distance from the cave entrance (LMM: $F = 0.001$, $df = 1$, $P = 0.98$), whether webs were found on the wall or the floor of the cave (LMM: $F = 1.68$, $df = 1$, $P = 0.26$), the number of radii (LMM: $F = 0.17$, $df = 1$, $P = 0.68$), the size of the cave (LMM: $F = 0.70$, $df = 2$, $P = 0.81$) or any of the interactions tested (data not shown). However, the size of the capture area was positively related to the cephalothorax width of the resident spider (LMM: $F = 5.39$, $df = 1$, $P = 0.04$).

Discussion

The most obvious convergent adaptations to a subterranean life-style are the morphological adaptations including limb elongation, depigmentation and lack or reduction of eyes (Poulson and White 1969; Christensen 2004; Culver and Pipan 2009). Our results confirm the findings of previous studies (Eckert and Moritz 1992; Novak et al. 2010) that *Metellina merianae* do not show any clear morphological adaptations. Similarly, our results confirm that *M. merianae* build large, normal looking orb webs enclosed by a complete set of frame threads, and therefore do not show the clear behavioural adaptations reported in the sister genus *Meta* (Mammola and Isaia 2014; Hesselberg et al.

2019). However, our results indicate some differences in both morphological and web characteristics between *M. merianae* and its epigeal sister species *M. mengei*.

In terms of morphology, *M. merianae* were about 50% longer and had a 75% wider cephalothorax than *M. mengei*. There is some indication that Foster's rule for larger size for small animals on islands, may also hold for cave animals (Whittaker 1998; Trontejl et al. 2012), although see Mammola (2018) for a cautionary note on the cave-as-islands paradigm. For example, troglomorphic cave millipedes are significantly longer than epigeal species (Liu et al. 2017). However, *M. merianae* are less than half the size of the cave orb spiders in the genus *Meta* (Hesselberg et al. 2019). We furthermore found no difference in the relative length of the front legs between *M. merianae* and *M. mengei*, although the relative length of leg III was 20% longer in *M. merianae*. The first pair of legs in orb spiders is important during prey attack behaviour and for measuring distances during construction of the capture spiral (Robinson et al. 1969; Vollrath 1987), while leg III seemingly plays a much less significant role and is mainly used to grip the radii during capture spiral construction (Eberhard and Hesselberg 2012).

In terms of foraging behaviour, or web geometry, the webs of cave populations of *M. merianae* were superficially similar to those of the woodland *M. mengei* in that they both had a similar number of radii and spiral turns, although the area enclosed by the capture spiral was two and a half times larger in *M. merianae*. When controlling for the difference in spider size, the webs between the two species did not differ significantly in size. Interestingly, when controlling for web area, webs of *M. merianae* were larger spaced (fewer spiral turns) and had fewer radii than *M. mengei*. Thus, while *M. merianae* webs differed substantially from webs of the cave spider *Meta menardi* in that they were larger and had intact frame threads, they had relatively few radii and radii spiral turns similar to the webs of *Meta menardi* (Simonsen and Hesselberg unpublished). These modifications could be viewed as adaptations to the cave environment. The number of radii is an important determinant of prey stopping potential (Sensenig et al. 2012), suggesting that these webs are not adapted to capture heavy or fast flying prey. The larger distances between capture spiral turns, however, suggest that these webs are optimised to capture large, but weak prey (Sandoval 1994; Blackledge and Zevenbergen 2006). Both of these observations fit with the prey likely to be encountered in caves, where the majority of flying insects are relatively spindly and slow-flying gnats, moths and caddisflies (Smithers 2005a; Novak et al. 2010).

In this preliminary study on the drivers of web characteristics in *M. merianae* from caves, the size of the cave was not an important determinant. However, it is worth noting that *M. merianae* were only found in medium and large caves. They were not found in any of the 7 tiny and small caves visited as part of this study, possibly because the aggressive web-invading cellar spider *Pholcus phalangoides* (Füssli, 1775) were relatively abundant in the majority of these smaller caves (Jackson and Brassington 1987). Interestingly, the current study did not find any effect on the web characteristic of the distance from the cave entrance or whether webs were on the wall or floor, although it

is worth noting that the sample size in the current study was limited. Not surprisingly, we found that spider size positively influenced web area. Previous studies (Eckert and Moritz 1992; Novak et al. 2010) did not look at the structure of the web, but only recorded presence/absence. Eckert and Moritz (1992) report that *M. merianae* populations in Germany prefer cold and damp caves, although in the current study they were also found in relatively dry (down to 73% rH) and warm (up to 14 °C) caves on the Balearic Islands. Similar tolerance to high temperatures and drier caves were also found in a Slovenian population (Novak et al. 2010). It is possibly that preferences differ in different geographical locations, although clearly much more data is needed on how cave characteristics affect both the presence and the webs of *M. merianae*.

Conclusions

In conclusion, the present study finds evidence of some minor morphological differences between hypogean and epigean *Metellina* with the possibility that the larger size of *M. merianae* could be an adaptation to subterranean life. Similarly, minor differences in web geometry were evident with *M. merianae* webs having larger distances between spiral turns and fewer radii relative to web area than those of the epigean *M. menzei*. However, the present study is not able to determine if these differences are potential indications of a transition process towards adaptation to subterranean life or are instead the results of behavioural flexibility. Orb spiders are well known for displaying a significant degree of behavioural flexibility in the structure of their orb webs in response to a range of biotic and abiotic variables (Heiling and Herberstein 2000; Hesselberg 2015; Eberhard in press). The araneid *Araneus diadematus* (Clerck, 1758), for example, slightly reduces the number of radii and significantly increased the distance between capture spiral turns when going from building webs at 24 °C to 12 °C in a laboratory study (Vollrath et al. 1997). A similar mechanism could explain the differences we found between webs built in the colder caves compared to the warmer woodland. Humidity is also known to affect web geometry (Vollrath et al. 1997), and higher humidity such as found in caves could render capture threads more efficient at retaining prey (Boutry and Blackledge 2013), thus potentially requiring less capture silk as found in our study. Wind, although not measured in our study, also affects orb geometry. However, caves are likely to experience less windy conditions than in woodland, which should, in contrast to our findings, result in webs with more radii and capture spiral turns (Vollrath et al. 1997; Wu et al. 2013). Clearly more studies are needed under controlled laboratory conditions and on the web geometry of *M. merianae* from different cave and epigean environments, taking advantage of the fact that this species can also be found in dark and damp woodland, in order to investigate the intriguing possibility that this species is in the process of evolving behavioural adaptations to the hypogean environment mirroring the likely process that has taken place in the larger sister genus *Meta*.

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References

- Blackledge TA, Zevenbergen JM (2006) Mesh width influences prey retention in spider orb webs. *Ethology* 112: 1194–201. <https://doi.org/10.1111/j.1439-0310.2006.01277.x>
- Boutry C, Blackledge TA (2013) Wet webs work better: humidity, supercontraction and the performance of spider orb webs. *Journal of Experimental Biology* 216: 3606–3610. <https://doi.org/10.1242/jeb.084236>
- Bridge AP, Elwood RW, Dick JTA (2000) Imperfect assessment and limited information preclude optimal strategies in male-male fights in the orb-weaving spider *Metellina menzei*. *Proceeding of the Royal Society of London. Series B*. 267: 273–279. <https://doi.org/10.1098/rspb.2000.0997>
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Chapin KJ, Hebets EA (2016) The behavioral ecology of amblypygids. *Journal of Arachnology* 44: 1–15. <https://doi.org/10.1636/V15-62.1>
- Chen H, Zhu M (2009) One new species of triglophilous species of the genus *Okileucae* (Araneae: Tetragnathidae) from Guizhou, China. *Acta Arachnologica* 58: 23–25. <https://doi.org/10.2476/asjaa.58.23>
- Christiansen K (2004) Adaptation: morphological (external). In: Gunn J (Ed.) *Encyclopedia of Caves and Karst Science*. Fitzroy Dearborn, New York, 7–9.
- Culver DC, Pipan T (2009) *The biology of caves and other subterranean habitats*. Oxford University Press, Oxford.
- Eberhard SM (1992) *The invertebrate cave fauna of Tasmania: Ecology and Conservation*. MSc Thesis. University of Tasmania.
- Eberhard WG (1990) Function and phylogeny of spider webs. *Annual Review of Ecology and Systematics* 21: 341–372. <https://doi.org/10.1146/annurev.es.21.110190.002013>
- Eberhard WG (in press) *Spider Webs: Function, Behavior and Evolution*. University of Chicago Press, Chicago, IL.
- Eberhard WG, Hesselberg T (2012) Cues that spiders (Araneae: Araneidae, Tetragnathidae) use to build orbs: lapses in attention to one set of cues because of dissonance with others? *Ethology* 118: 610–620. <https://doi.org/10.1111/j.1439-0310.2012.02048.x>
- Eckert R, Moritz M (1992) *Meta menardi* (Latr.) und *Meta merianae*: Zur Lebensweise und Verbreitung der beiden häufigsten Spinnen in den Höhlen des Harzes, des Kyffhäusers, Thüringens und des Zittauer Gebirges. *Mitteilungen der Zoologisches Museum Berlin* 68: 345–350. <https://doi.org/10.1002/mmzn.19920680217>

- Foelix R (2011) Biology of Spiders. 3rd edn. Oxford University Press, Oxford.
- Heiling AM, Herberstein ME (2000) Interpretations of orb-web variability: a review of past and current ideas. *Ekológia* (Bratislava) 19: 97–106.
- Herberstein ME, Tso IM (2000) Evaluation of formulae to estimate the capture area of orb webs (Araneoidea, Araneae). *Journal of Arachnology* 28: 180–184. [https://doi.org/10.1636/0161-8202\(2000\)028\[0180:EOFTET\]2.0.CO;2](https://doi.org/10.1636/0161-8202(2000)028[0180:EOFTET]2.0.CO;2)
- Hervant F, Mathieu J, Barré H, Simon K, Pinon C (1997) Comparative study on the behavioural, ventilator, and respiratory responses of hypogean and epigean crustaceans to long-term starvation and subsequent feeding. *Comparative Biochemistry & Physiology* 118A: 1277–1283. [https://doi.org/10.1016/S0300-9629\(97\)00047-9](https://doi.org/10.1016/S0300-9629(97)00047-9)
- Hesselberg T (2015) Exploration behaviour and behavioural flexibility in orb-web spiders: a review. *Current Zoology* 61: 313–327. <https://doi.org/10.1093/czoolo/61.2.313>
- Hesselberg T, Simonsen D, Juan C (2019) Do cave orb spiders show unique behavioural adaptations to subterranean life? A review of the evidence. *Behaviour* 156, 969–996. <https://doi.org/10.1163/1568539X-00003564>
- Hesselberg T, Triana E (2010) The web of the acacia orb-spider *Eustala illicita* (Araneae: Araneidae) with notes on its natural history. *Journal of Arachnology* 38: 21–26. <https://doi.org/10.1636/HI09-59.1>
- Hoch H, Howarth FG (1993) Evolutionary dynamics of behavioural divergence among populations of the Hawaiian cave-dwelling planthopper *Oliarus polyphenus* (Homoptera: Fulgoroidea: Cixiidae). *Pacific Science* 47: 303–318.
- Jackson RR, Brassington RJ (1987) The biology of *Pholcus phalangioides* (Araneae, Pholcidae): predatory versatility, araneophagy and aggressive mimicry. *Journal of Zoology* 211: 227–238. <https://doi.org/10.1111/j.1469-7998.1987.tb01531.x>
- Juberthie-Jupeau L (1988) Mating behaviour and barriers to hybridization in the cave beetle of the *Sponomus delarouzei* complex (Coleoptera, Catopidae, Bathysciinae). *International Journal of Speleology* 17: 51–63. <https://doi.org/10.5038/1827-806X.17.1.3>
- Kallal RJ, Hormiga G (2018) An expanded molecular phylogeny of metaine spiders (Aranea, Tetragnathidae) with new description of new taxa from Taiwan and the Philippines. *Invertebrate Systematics* 32: 400–422. <https://doi.org/10.1071/IS17058>
- Lipovsek S, Leitinger G, Novak T, Janzekovic F, Gorgon S, Kaminska K, Rost-Roszkowska M (2018) Changes in the midgut cells in the European cave spider, *Meta menardi*, during starvation in spring and autumn. *Histochemistry and Cell Biology* 149: 245–260. <https://doi.org/10.1007/s00418-017-1623-z>
- Liu W, Golovatch S, Wesener T, Tian M (2017) Convergent Evolution of Unique Morphological Adaptations to a Subterranean Environment in Cave Millipedes (Diplopoda). *PLoS ONE* 12: e0170717. <https://doi.org/10.1371/journal.pone.0170717>
- Mammola S (2018) Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. *Ecography* 41: 1–21. <https://doi.org/10.1111/ecog.03905>
- Mammola S, Isaia M (2014) Niche differentiation in *Meta bourneti* and *M. menardi* (Aranea, Tetragnathidae) with notes on the life history. *International Journal of Speleology* 43: 343–353. <https://doi.org/10.5038/1827-806X.43.3.11>
- Mammola S, Isaia M (2017) Spiders in caves. *Proceeding of the Royal Society of London. Series B*. 284: 20170193. <https://doi.org/10.1098/rspb.2017.0193>

- Marusik YM, Larsen N (2018) A synopsis of African *Metellina* (Aranei, Tetragnathidae, Metainae) with description of a new species from South Africa. *Vestnik Zoologii* 52: 205–216. <https://doi.org/10.2478/vzoo-2018-0021>
- Novak T, Tkavc T, Kuntner M, Arnett AE, Delakorde SL, Perc M, Janzekovic F (2010) Niche partitioning in orbweaving spiders *Meta menardi* and *Metellina merianae* (Tetragnathidae). *Acta Oecologia* 36: 522–529. <https://doi.org/10.1016/j.actao.2010.07.005>
- Parzefall J (2005) Behavioral adaptations. In: Culver DC, White WB (Eds) *Encyclopedia of caves*. Elsevier Academic Press, Amsterdam, 51–56.
- Poulson TL, White WB (1969) The cave environment. *Science* 165: 971–981. <https://doi.org/10.1126/science.165.3897.971>
- Prenter J, Elwood RW, Colgan S (1994) The influence of prey size and female reproductive state on courtship of the autumn spider, *Metellina segmentata*: a field experiment. *Animal Behaviour* 47: 449–456. <https://doi.org/10.1006/anbe.1994.1059>
- R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/>
- Robinson MH, Mirsck M, Turner O (1969) The predatory behavior of some areneid species and the origin of immobilization wrapping. *Psyche* 70: 487–501. <https://doi.org/10.1155/1969/68029>
- Sandoval CP (1994) Plasticity in web design in the spider *Parawixia bistriata*: a response to variable prey type. *Functional Ecology* 8: 701–707. <https://doi.org/10.2307/2390229>
- Sensenig AT, Agnarsson A, Blackledge TA (2011) Adult spiders use tougher silk: ontogenetic changes in web architecture and silk biomechanics in the orb-weaver spider. *Journal of Zoology* 285: 28–38. <https://doi.org/10.1111/j.1469-7998.2011.00809.x>
- Sensenig AT, Lorentz KA, Kelly SP, Blackledge TA (2012) Spider orb webs rely on radial threads to absorb prey kinetic energy. *Journal of The Royal Society Interface* 9: 1880–1891. <https://doi.org/10.1098/rsif.2011.0851>
- Smithers P (2005a) The diet of the cave spider *Meta menardi* (Latreille 1804) (Araneae, Tetragnathidae). *Journal of Arachnology* 33: 243–246. <https://doi.org/10.1636/CT-05-2.1>
- Smithers P (2005b) The early life history and dispersal of the cave spider *Meta menardi* (Latreille, 1804) (Araneae: Tetragnathidae). *Bulletin of the British Arachnological Society* 13: 213–216. <https://doi.org/10.1636/CT-05-2.1>
- Tew N, Hesselberg T (2017) The effect of wind exposure on the web characteristics of a Tetragnathid orb spider. *Journal of Insect Behavior* 30: 273–286. <https://doi.org/10.1007/s10905-017-9618-0>
- Tew N, Hesselberg T (2018) Web asymmetry in the tetragnathid orb spider *Metellina mengei* (Blackwell, 1869) is determined by web inclination and web size. *Journal of Arachnology* 46: 370–372. <https://doi.org/10.1636/JOA-S-17-065.1>
- Trontelj P, Blejec A, Fiser C (2012) Ecomorphological convergence of cave communities. *Evolution* 66: 3852–3865. <https://doi.org/10.1111/j.1558-5646.2012.01734.x>
- Tremblay A, Ransijn J (2015) LMERConvenienceFunctions: Model Selection and Post-hoc Analysis for (G)LMER Models. R package version 2.10. <https://CRAN.R-project.org/package=LMERConvenienceFunctions>

- Vollrath F (1987) Altered geometry of webs in spiders with regenerated legs. *Nature* 328: 247–248. <https://doi.org/10.1038/328247a0>
- Vollrath F, Downes M, Krackow S (1997) Design variability in web geometry of an orb-weaving spider. *Physiology & Behavior* 62: 735–743. [https://doi.org/10.1016/S0031-9384\(97\)00186-8](https://doi.org/10.1016/S0031-9384(97)00186-8)
- Whittaker RJ (1998) *Island biogeography: ecology, evolution, and conservation*. Oxford University Press, Oxford, 73–75.
- Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York. https://doi.org/10.1007/978-3-319-24277-4_9
- World Spider Catalog (2019) *World Spider Catalog*. Version 20.0. Natural History Museum Bern. <http://wsc.nmbe.ch> [accessed on 30th of January 2019]
- Wu CC, Blamires SJ, Wu CL, Tso IM (2013) Wind induces variations in spider web geometry and sticky spiral droplet volume. *Journal of Experimental Biology* 216: 3342–3349. <https://doi.org/10.1242/jeb.083618>
- Yoshida M, Shinkai A (1993) Predatory behavior and web structure of *Meta menardi* (Araneae: Tetragnathidae). *Acta Arachnologica* 42: 21–25. <https://doi.org/10.2476/asjaa.42.21>
- Zschokke S, Herberstein ME (2005) Laboratory methods for maintaining and studying web-building spiders. *Journal of Arachnology* 33: 205–213. <https://doi.org/10.1636/CT04-72.1>

Cave millipedes of the United States. XV. *Coloradesmus* gen. nov. (Diplopoda, Polydesmida, Macrosternodesmidae), and four new species from caves in Colorado, USA

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Abstract

Coloradesmus, gen. nov., is established in the family Macrosternodesmidae based on *Speodesmus aquiliensis* Shear, 1984, **comb. nov.** and includes four new species: *Coloradesmus hopkinsae* **sp. nov.**, *C. manitou* **sp. nov.**, *C. beckleyi* **sp. nov.**, and *C. warneri* **sp. nov.** All are from high altitude limestone caves in Colorado, USA, and are likely troglobionts.

Keywords

Troglobionts, new genus, new species, new records, sphaerotrichomes

Introduction

The generic name *Speodesmus* Loomis, 1939 has long been used for a number of small to tiny, troglobiotic polydesmidan millipedes from Texas (Elliott 2004, Elliott and Reddell 2009), New Mexico, and Colorado (Shear 1984). However, more careful

study has shown that these widely scattered species are not all components of the same genus. Shear and Reddell (2017) revived the genus name *Speorthus* Chamberlin, 1952, for two species from southeastern New Mexico and the west Texas panhandle. They also reassigned all the central Texas species nominally belonging to *Speodesmus* and the two *Speorthus* species to the family Macrosternodesmidae and discussed at length the distinctions between that family and Polydesmidae.

Speodesmus aquiliensis Shear, 1984, from Fulford Cave in Eagle Co., Colorado, was briefly mentioned by Shear and Reddell (2017), but no new information was provided. However, for at least ten years, prompted by extensive new collections by DBS, WAS suspected that *S. aquiliensis* and some undescribed species from Colorado caves were not congeneric with *Speodesmus echinourus* Loomis, 1943, the type species of *Speodesmus*. Here *aquiliensis* and four new species are grouped in the new genus *Coloradesmus* Shear & Steinmann.

The central Texas species remaining in *Speodesmus* need restudy and revision. Based on the study of specimens and illustrations of gonopods, there appear to be two quite distinct groups of species, and those around *Speodesmus bicornourus* Causey, 1959 may require a new generic name.

Methods

Specimens were collected at sites around moist organic material inside the caves and field-preserved in 70–85% ethanol. Morphological studies were done using an Olympus SZH stereomicroscope and an Olympus BX50 compound microscope equipped with Nomarski optics. Gonopods were temporarily mounted on microscope slides in glycerine for detailed study up to 400X magnification. Measurements were taken using an ocular micrometer on the SZH (lengths of millipedes are highly variable because the body rings can be extended and telescoped to a considerable degree; for polydesmids, breadth of a midbody metazonite is a much better indicator of size) or relying on scale lines accompanying scanning electron micrographs. Drawings were made using an Olympus U-DA drawing tube fitted to the BX-50. Specimens were air-dried and mounted on 12.7 mm diameter aluminum scanning electron microscopy (SEM) stubs affixed with double-sided carbon conductive tape. These were sputter-coated with a 10 nm thickness layer of platinum and palladium metals using a Leica EM ACE600 high vacuum sputter coater. Scanning electron micrographs were taken with a FEI Quanta 600 FEG environmental SEM. Photographs and drawings were edited and refined using GIMP and plates were composed in InkScape.

Due to the ecological and archeological sensitivity of the caves visited for this study, coordinates for their locations are given only in general terms (minutes and seconds).

Aside from the holotype specimen of *C. aquiliensis*, all material referred to in this study has been deposited in the Denver Museum of Nature & Science, Denver, Colorado, USA.

Taxonomy

Family Macrosternodesmidae Brölemann, 1916

Macrosternodesmini Brölemann, 1916, p. 585.

Macrosternodesmidae Hoffman, 1980, p. 177.

The tribe was proposed by Brölemann (1916) under his “Strongylosomides” for the species *Macrosternodesmus palicolis* Brölemann, 1908, a minute, widespread synanthrope from western Europe. Hoffman (1980) raised the name to family status and listed the included genera. We think it very likely that *Chaetaspis* Bollman, 1887 (see Lewis 2002), is a senior synonym and that North America, the home of all other known *Chaetaspis* species, is the source of the synanthrope *M. palicolis*. This will be argued in detail in a forthcoming paper. In any case, the family name will remain Macrosternodesmidae under ICZN rules. For a detailed discussion of the validity of the family and its differences from, and relationship to, Polydesmidae, see Shear and Reddell (2017).

We are adopting, with slight modifications, the gonopod terminology used in Shear and Reddell (2017). Macrosternodesmine gonopods have the *prefemorite* (**pf** in Figures) densely to sparsely setose and transversely oriented to the *coxa* (**cx** in Figures). There is a prominent, sometimes bipartite *prefemoral process* (**pfp** in Figures) arising anterolaterally from the prefemorite; this process and the *acropodite* (**a** in Figures) of the gonopod are usually (but not always) separated from the prefemorite by a distinct *stem* (**s** in Figures), oriented at right angles to the prefemorite. The *acropodite* consists of a seminiferous branch on which the pore opens, often through a short or long solenite with tiny cuticular fingers or fimbriae surrounding the pore, and an *endomerite* (*sensu* Golovatch; **e** in Figures) arising near the pore or at the base of the seminiferous branch. Any portion of the acropodite distal to the pore is called the *distal zone* (**dz** in Figures). The course of the *seminal canal* (or prostatic canal; although much of the literature refers to this structure as a “groove” SEM pictures do not show it as such. It is a closed canal internal to the gonopod best seen by transmitted light microscopy. It is not clear if this canal carries sperm or merely a “prostatic” secretion. The mechanism of sperm transfer in polydesmidan millipedes remains unknown) begins mesally in the prefemorite fossa and crosses laterally to the acropodite without any loops or vesicles.

Two subfamilies are recognized: Macrosternodesminae are small, often unpigmented animals with setose metazonites and short antennae with rather bulbous distal segments, while Nearctodesminae Chamberlin & Hoffman 1950 are larger, have smooth red, pink or brown metazonites and long antennae with cylindrical distal segments. See Shear and Reddell (2017) for lists of genera belonging to each subfamily. Recently, Shear and Shelley (2019) added a new genus *Packardesmus* (type species *Polydesmus cavicola* Packard, 1877) to the subfamily Macrosternodesminae.

Macrosternodesmine species are usually 2.5–12 mm long, unpigmented and with narrow, toothed paranota. The collum and metazonites bear three rows of setae set

on more or less distinct tubercles, or densely scattered setae not arranged in rows and not on obvious tubercles; the setae may be long and acute or short and clavate. The epiproct (telson) is short-triangular with four spinnerets in a shallow depression. The legs of males have podomeres (coxae, prefemora and femora) that are enlarged, dorsally swollen, and set with sphaerotrichomes. Antennomere six is enlarged and longer than antennomere five. The majority of North American species in this subfamily have been collected exclusively in caves, mostly west of the Mississippi Valley.

Sphaerotrichomes

Sphaerotrichomes are unusual, enigmatic structures that occur on some or all podomeres of many species of polydesmoidean and dalodesmoidean millipedes (Fig. 4). Typically sphaerotrichomes are found only on the legs of males. They consist of a spherical base set in a socket, with a pointed projection, resembling a more typical seta, extending distally (toward the tip of the leg) from a sharp demarcation with the spherical base. Mesibov (2004) has documented four distinct types of sphaerotrichome from Australian dalodesmids. The shape of the spherical base varies from a more typical and widespread rounded, slightly pyramidal form to more nearly spherical, and the surface can be either smooth or with evenly spaced, concentric ridges, or with an apical dimple. The seta-like projection may be absent, short and acute, long and slightly curved at the tip, or with an expanded tip, according to the SEMs published by Mesibov (2004, figs 1A–D). Only a few species of North American polydesmids and macrosternodesmids have been examined for these structures using SEM. In the polydesmids, the enlarged anterior legs of the males are densely setose, and what may be vestigial sphaerotrichomes are scattered amongst the setae. In the macrosternodesmids, setae of the ventral leg surfaces are suppressed and sphaerotrichomes are large and prominent. There appears to be little variation, with distinctly rimmed sockets, subpyramidal, ridged bases and relatively short, acute setal projections. Mesibov (2004) has suggested that the distribution and form of sphaerotrichomes may be of phylogenetic importance, and we agree. A survey of these structures in a wide range of taxa would be extremely useful.

There have been no ultrastructural studies of sphaerotrichomes using transmission electron microscopy, and they are not mentioned in a recent review of millipede sense organs as such (Müller and Sombke 2015). Their function remains unknown, but since they are found only in males, some connection to sexual behavior may be inferred.

Coloradesmus gen. nov.

<http://zoobank.org/7D2A725E-B05E-4124-9744-6A2BE883EA0F>

Type species. *Speodesmus aquiliensis* Shear, 1984.

Diagnosis. Distinct from the similar *Pratherodesmus* Shear, 2009 in its much larger gonopod solenomere and endomerite, from *Tidesmus* Chamberlin, 1943, *Sequoiadesmus* Shear & Shelley, 2008 and *Nevadesmus* Shear, 2009 in having a simple,

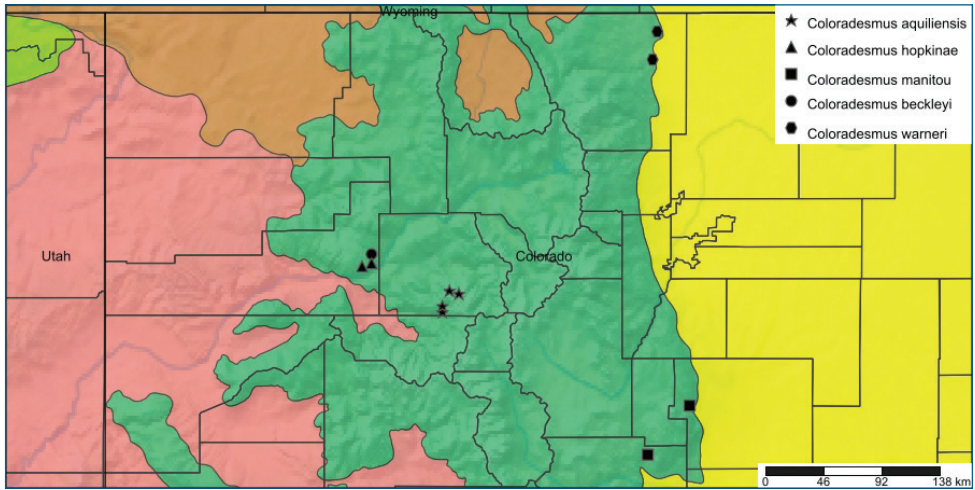
unbranched endomerite, or endomerite lacking. *Packardasmus* Shear & Shelley, 2019 has all gonopod branches clustered at the tip of an extended prefemoral stem.

Description. Small, probably troglobiotic macrosternodesmines 4.0–11.0 mm long, lacking pigment. Nineteen trunk rings (collum + 17 pedigerous rings + telson). Head sparsely to densely setose. Antennae (Fig. 22) relatively short, elbowed between antennomeres three and four, antennomere six much enlarged, with subapical accessory sensory organ. Order of length of antennomeres: $6 > 3 = 4 > 5 > 2 > 7 > 1 > 8$. Collum with three rows of eight setae (number may vary on more posterior rings); anterior row at anterior margin of collum, middle row may be dispersed, posterior row at posterior margin of collum. Collum setae on low tubercles or sockets more or less flush with surface. Subsequent rings with anterior row posterior to anterior margin of metazonite, but posterior row at posterior edge of metazonite, rows of 6–10 setae or setae becoming more scattered on more posterior rings. Metazonites with narrow paranota bearing three short, marginal teeth corresponding to setal rows, posteriolateral metazonite corners acute to projecting. Limbus minutely dentate. Ozopores laterodorsal, at posteriolateral corners, opening in a distinct pore callus. Pore formula 5, 7, 9, 10, 12, 13, 15–18. Penultimate ring with 10–18 scattered setae, telson with 8–10 scattered setae, epiproct process short, blunt, with four spinnerets set in shallow depression. Paraprocts and hypoproct with two setae. Dorsal setae post-collum on more prominent tubercles, setae themselves may be long, acute, or short, clavate. Males with all legs having dorsally swollen prefemora, femora also dorsally swollen, curved, both podomeres ventrally with many sphaerotrichomes; postfemora and tibiae normal, with few ventral sphaerotrichomes or sphaerotrichomes absent (Fig. 4). Gonopod socket rounded-cordate, often with anterior rim, midposterior portion extending slightly between ninth coxae. Gonopods joined by tough membrane anteriorly; small, more sclerotized strip represents sternal remnant, articulates mesally with coxae. Gonopod coxae not movable, tightly appressed in midline but not fused, anteriomesally excavate to receive telopodite. Telopodites movable only in plane parallel to body axis. Prefemorites transverse across posterior surface of coxae. Prefemoral process single, broad, prefemoral process and acropodite on short stem arising from prefemorate. Acropodite with long seminiferous branch, pore surrounded by minute cuticular extensions, these sometimes extending distad along concave mesal surface of distal zone. Distal zone long, tapering, acute, or short, blunt. Endomerite arising basally or midway on acropodite.

Etymology. From the state of Colorado, to which the genus appears endemic, and the common combining stem *-desmus* in the order.

Species included. In addition to the type species, the following new species: *beckleyi*, *warneri*, *hopkinsae* and *manitou*.

Distribution. See Map 1. Caves in central and northern Colorado, USA. Species distributions appear to be defined by major rivers and large canyons. Groaning Cave where *C. hopkinsae* occurs is across Deep Creek Canyon from La Sunder Cave where *C. beckleyi* occurs and this canyon is 2,000' deep. The Colorado River may define the limits of *C. aquiliensis* relative to *C. beckleyi* and *C. hopkinsae* because *C. aquiliensis* has only been collected south of the Colorado River, while the other two species were



Map I. Northwestern Colorado, showing the distribution of *Coloradasmus* species. Green area indicates Colorado Rocky Mountain Forest Ecoregion. Symbols may represent more than one locality as some caves are very close to one another. Stars, *C. aquiliensis*, triangles, *C. hopkinsae*, squares, *C. manitou*, circles, *C. beckleyi*, hexagon, *C. warneri*.

collected north of the river. It is likely that *Coloradasmus* will extend into Wyoming because *C. warneri* was collected 5 miles from the state line.

Notes. Two species groups are recognizable in this genus, distinguished primarily by size and the presence or absence of a distinct endomerite. *Coloradasmus aquiliensis*, *C. hopkinsae* and *C. manitou* are 0.6 mm or greater in width and from 5–11 mm long, depending on the contraction or extension of the body. *Coloradasmus beckleyi* and *C. warneri* are considerably smaller, about 0.4 mm wide and 4 mm long, placing them among the smallest of all millipedes. The former three species have distinct endomerites, while endomerites seem lacking in the latter two. Division of *Coloradasmus* may prove desirable in the future but for now we prefer to group all the species in a single genus.

All specimens of *Coloradasmus* were collected in the dark zones of limestone caves. The preferred habitat for the genus appears to be caves with moist organic materials including wood, scat and guano. Millipedes representing *Coloradasmus* were found under rocks, burrowing in cave soils, and on wet cave formations. Many of the caves where *Coloradasmus* occurs are remote and at high-altitudes with temperatures of 2–4 °C.

Establishing troglobiosis is difficult in macrosternodesmines due to the small size and depigmented appearance of nearly all species except those of *Tidesmus*. Eyelessness is not a marker of trogllobiotic adaptation in Polydesmida, since all known species of the order, some thousands, are eyeless. However, despite antennae and legs that seem not much elongated compared to those of litter-dwelling species of *Chaetaspis*, the species of *Coloradasmus* have a loose-jointed, elongated appearance as a whole that, along with the weak sclerotization of the rings, suggests a significant degree of adaptation.

As WAS has repeatedly emphasized in previous publications, collecting in caves has generally been more intense than surface collecting, especially in western parts of the United States. Further, suspect troglobionts tend to be very small and would only be

found outside caves by very careful sifting of forest litter at an appropriate season of the year, if they exist there. Many caves in the Rocky Mountains are at high altitudes or are situated in surroundings that would not be very conducive to the survival of small, delicate arthropods on the surface. The mesovoid space of small cavities from a few centimeters to meters underground has not been explored by collectors in western North America.

Key to species

- 1 Width of midbody metazonites 0.6 mm or greater; metazonital setae acute **2**
- Width of midbody metazonites 0.4 mm or less; metazonital setae blunt, subclavate.....**4**
- 2 Distal zone of gonopod short, blunt; endomerite distinctly elbowed..... *C. manitou*
- Distal zone of gonopod long, tapering, acute; endomerite very short or not elbowed**3**
- 3 Endomerite short, perhaps seemingly absent, but tightly appressed to prefemoral process; prefemoral process broadly spatulate, with lateral subapical tooth..... *C. hopkinsae*
- Endomerite long, obvious; prefemoral process scoop-like, without a tooth ... *C. aquiliensis* (Shear)
- 4 Prefemoral process with thin, pointed tip; distal zone bent laterally at right angle or less.....*C. beckleyi*
- Prefemoral process broad at tip; distal zone evenly continuing line of acropodite.....*C. warneri*

Coloradesmus aquiliensis (Shear, 1984), **comb. nov.**

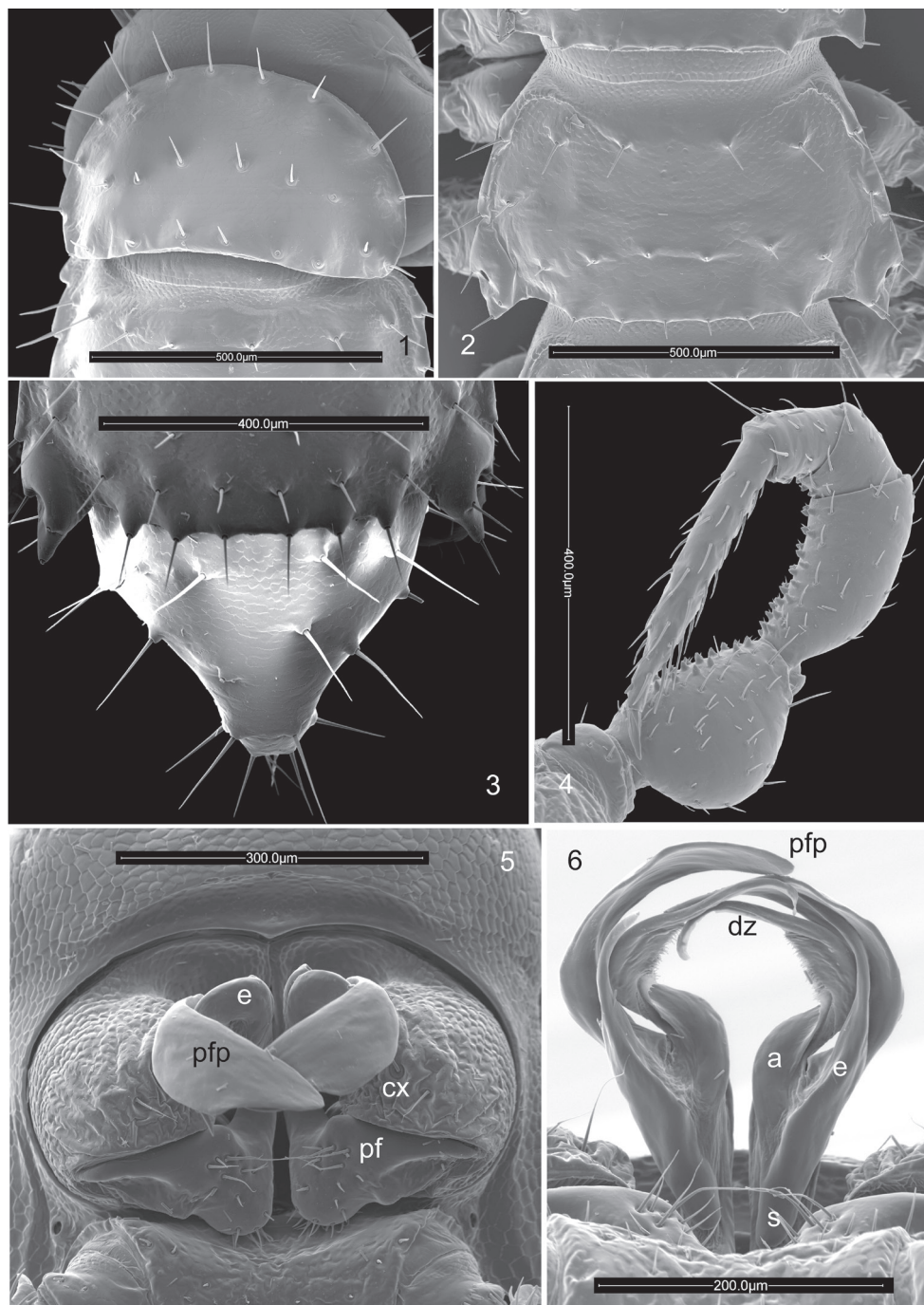
Figs 1–8

Speodesmus aquiliensis Shear, 1984, p. 96.

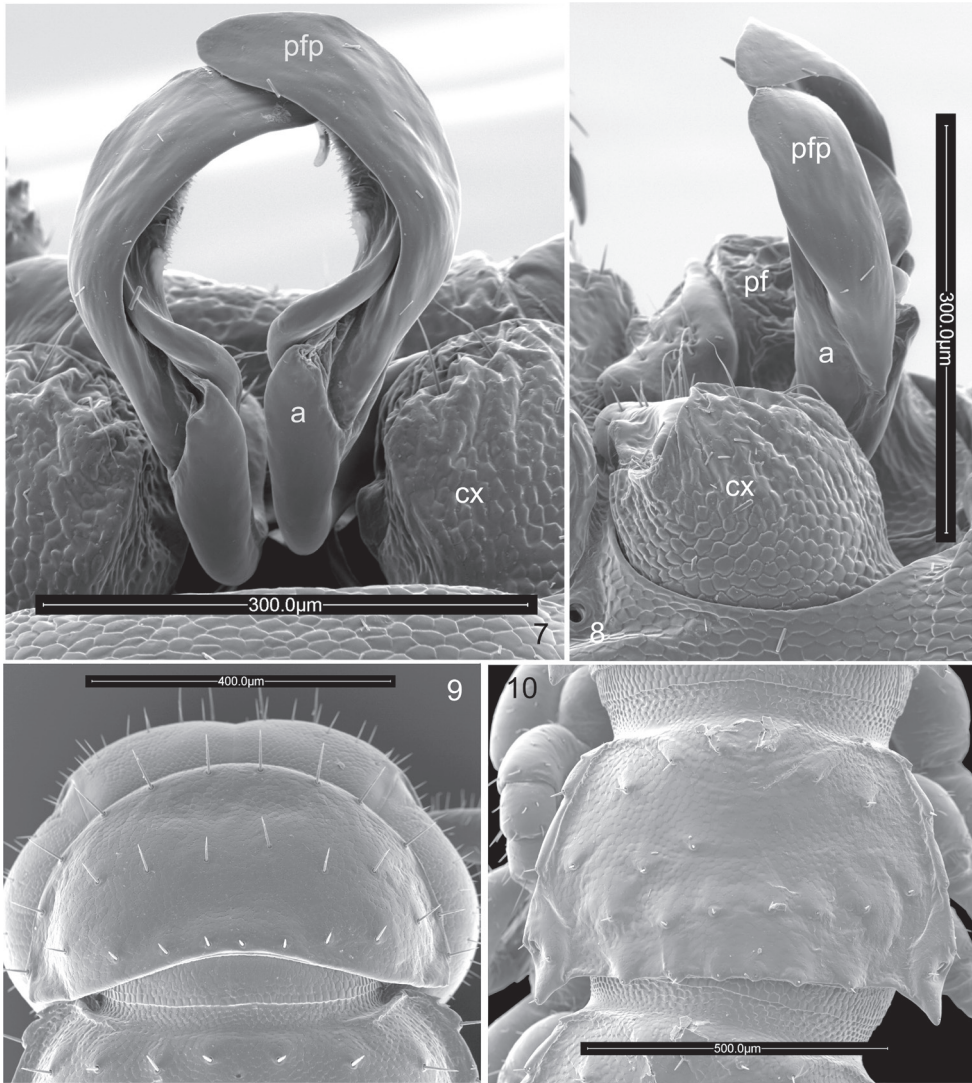
Types. Male holotype (American Museum of Natural History) from Fulford Cave, 21 mi SE of Eagle, Eagle Co.: Colorado, collected 6 July 1980 by J. R. Holsinger et al.

Diagnosis. A larger species of *Coloradesmus* distinguished by details of the gonopods; in *Coloradesmus hopkinsae* the prefemoral process is much broader and has a laterodistal tooth. *Coloradesmus manitou* differs from *C. aquiliensis* in the endomerite, which in *manitou* emerges from the acropodite at nearly a right angle, then turns sharply distad. *Coloradesmus beckleyi* and *C. warneri* are only half the length of the other three known species of the genus.

Corrections to original description. With the characters of the genus. Lengths of a series of specimens from Fulford Cave, the type locality, varied from 4.5–6.2 mm, the longer specimens strongly extended. Setae on collum, metazonites and telson long, acute. In the description of the gonopods, the prefemoral process is erroneously de-



Figures 1–6. *Coloradasmus aquiliensis* male. **1** Collum, dorsal view **2** midbody ring, dorsal view **3** telson, dorsal view **4** right leg 7, posterior view **5** gonopods, ventral view **6** gonopods, posterior view. See text for explanation of labels.



Figures 7–10. *Coloradesmus* species males. **7** *C. aquiliensis* gonopods, anterior view **8** *C. aquiliensis* gonopods, lateral view **9** *C. hopkinae* collum, dorsal view **10** *C. hopkinae* midbody ring, dorsal view. See text for explanation of labels.

scribed as bifid and the endomerite is shown in the drawings as attached to the prefemoral process, giving that impression.

Records. COLORADO: Eagle Co.: Fulford Cave, 10,000' asl, organic matter in packrat midden, 39°29'N, 106°33'W, 22 June 1999, 7 males, 13 females (topotypes); Devil's Den Cave, 11,500' (3506 m) asl, 35°F, under stones and logs in dark zone, 39°30'N, 106°37'W, 25 August 1999, 18 males, 7 females, juveniles; Herbie's Deli Cave, 9200' asl, under rocks in dark zone, 40°01'N, 105°40'W, 31 August 2003, mf; 15 August 2007, 2 males; Lime Creek Cave, 9200' (2804 m) asl, 40°00'N, 105°40'W, 5 September 2001, 2 males.

***Coloradasmus hopkinsae* Shear & Steinmann, sp. nov.**

<http://zoobank.org/12C578A0-7C71-4D06-8462-C558394F3046>

Figs 9–15

Types. Male holotype and male paratypes from Colorado, Garfield Co.: Bair Cave, 9800' asl, 39°38'N, 107°14'W, 200 ft from entrance, collected September 1998 and 19 June 1999 by D. Steinmann.

Diagnosis. Similar in somatic characters to both *C. aquiliensis* and *C. manitou*, but differing from both in the broader, more irregularly shaped prefemoral process of the gonopods, and in having a short endomerite tightly appressed to the prefemoral process.

Etymology. The species is named in honor of Kay Hopkins of the United States Forest Service for her contributions to cave conservation, cave research and cave preservation.

Description. Male holotype. Length about 7.0 mm, greatest width 0.66 mm. With the characters of the genus. Head sparsely setose. Basal joint of mandibles not greatly exceeding width of collum; front margin of collum evenly arcuate, posterior margin shallowly embayed (Fig. 9). Setae of collum and metazonites acute throughout (Figs 9–11), setal rows of metazonites sometimes interrupted, supplemental setae sometimes present (Fig. 10). Setal tubercles become more prominent posteriorly (*cf.* Figs 10, 11). Telson rounded, with two distinct rows of setae; epiproct blunt (Fig. 11). Gonopod socket occupies entire width of prozonite, subcordate, indenting ninth sternite (Fig. 12). Gonopods with robust acropodite (a, Fig. 13), ending in long, acute distal zone (dz, Figs 13, 14); seminal pore long, with many tiny finger-like cuticular projections (Fig. 15); endomerite (e, Fig. 13) short, tightly appressed to prefemoral process. Prefemoral process (pfp, Figs 13, 14), large, broad, scoop-shaped, with subapical lateral tooth, tip acute, slightly lobed.

Distribution. COLORADO: Garfield Co.: Groaning Cave, 39°42'N, 107°10'W, 9800' (2804 m) asl, 9 October 2008, D. Steinmann, male, females, juveniles.

***Coloradasmus manitou* Shear & Steinmann, sp. nov.**

<http://zoobank.org/0AF33302-F58F-4A31-ACAF-99DA75F6B27B>

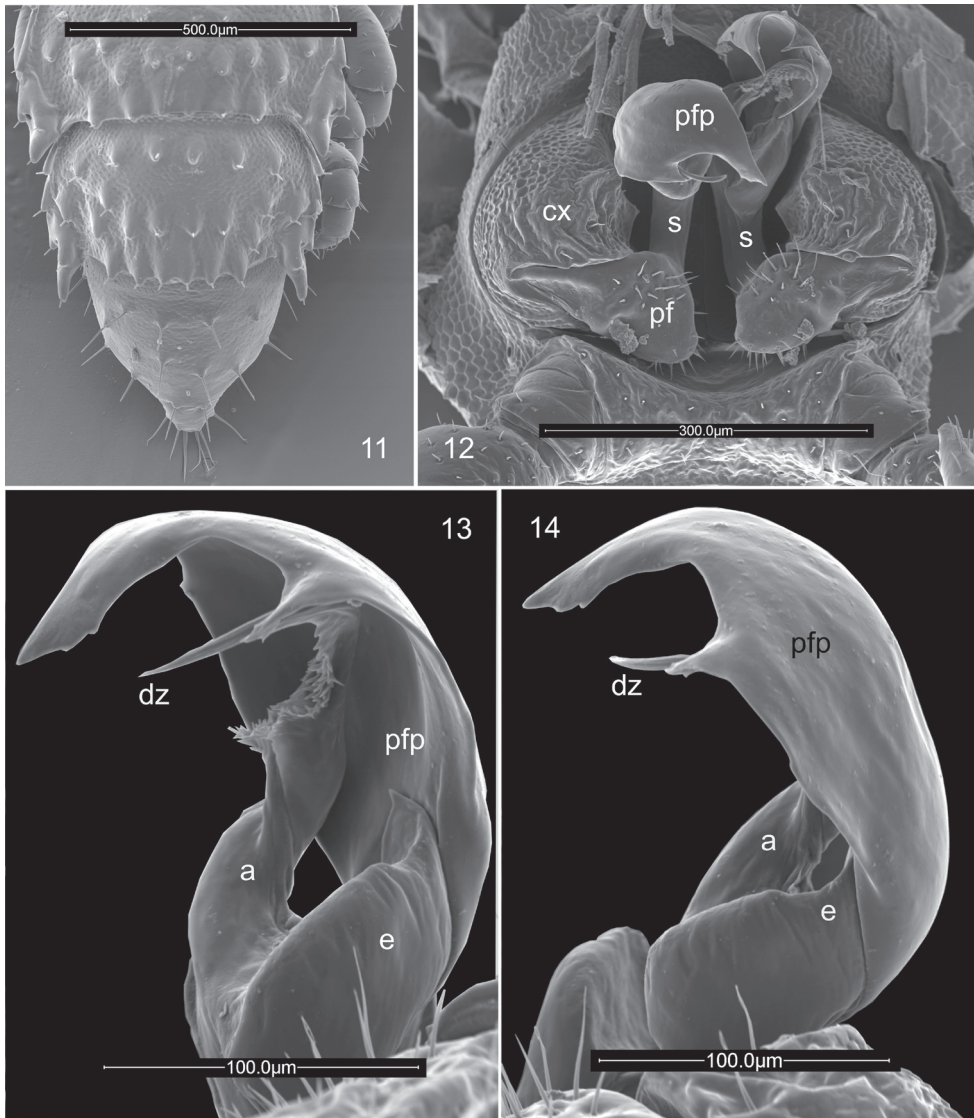
Figs 16–21

Types. Male holotype and many male and female paratypes from Manitou Cave, El Paso Co.: Colorado, 7000' asl, collected 3 August 1996, by D. Hubbard.

Diagnosis. A larger species, like *C. aquiliensis* and *C. hopkinsae*, but differing in that the metazonital setal tubercles are very low and often scattered across the metazonite, not in rows. The gonopod endomerite is stouter than in *C. aquiliensis* and emerges from the acropodite at almost a right angle, then turning distad; the distal zone is shorter and often curved. Unlike *C. hopkinsae*, the gonopod pore is compact with few cuticular projections.

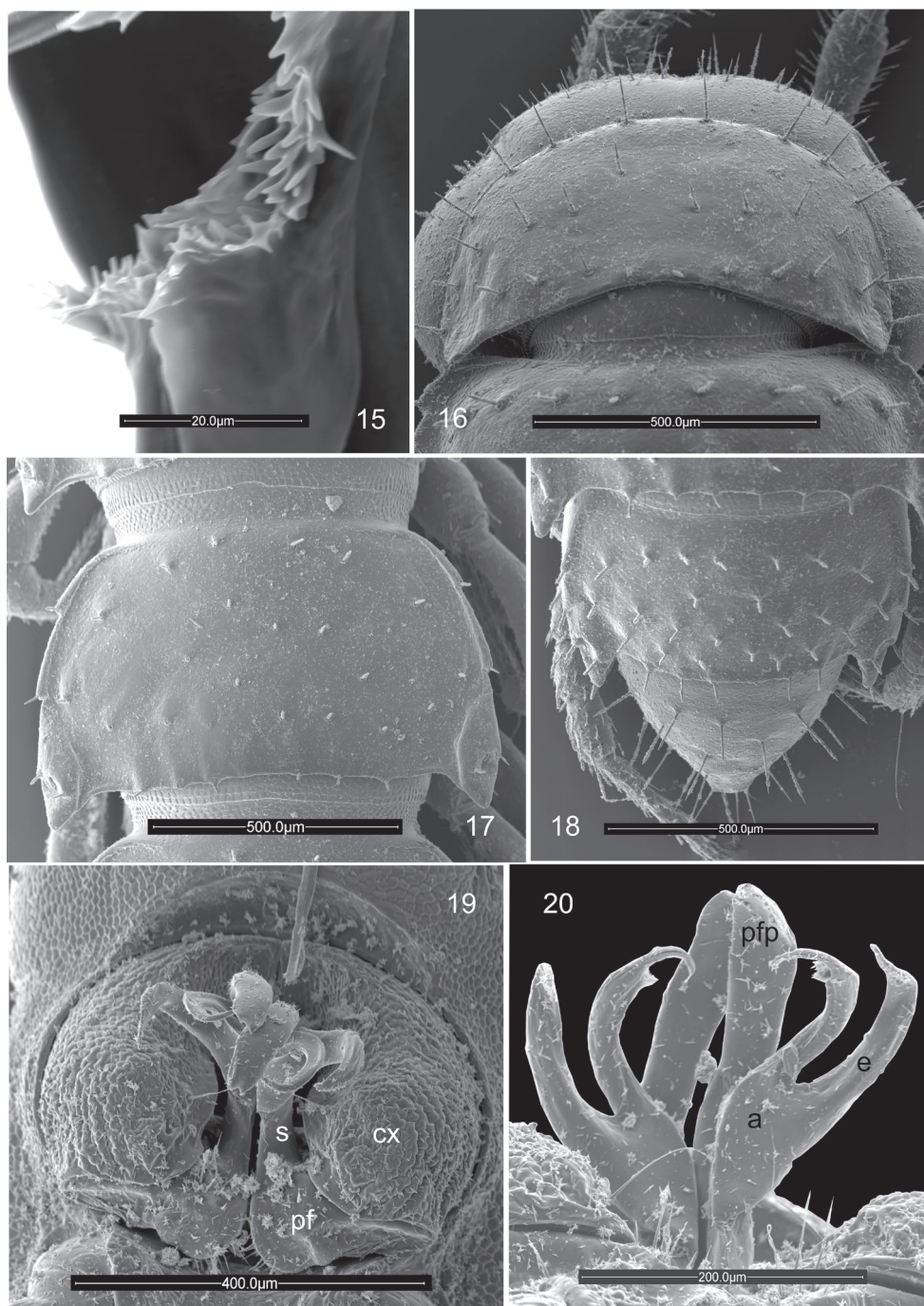
Etymology. The species epithet is a noun in apposition, after the type locality.

Description. Male paratype. Length 8.0–10.5 mm, depending on degree of extension, greatest width 0.9 mm. With the characters of the genus. Head densely se-



Figures 11–14. *Coloradesmus hopkinae* male. **11** Telson, dorsal view **12** gonopods, ventral view **13** right gonopod, mesal view **14** right gonopod, medioposterior view. See text for explanation of labels.

tose, basal joint of mandibles slightly exceeding width of collum (Fig. 16). Collum with three rows of long, acute setae (Fig. 16). Midbody metazonites nearly smooth, setal tubercles low to absent; setae becoming shorter posteriorly (Fig. 17), rows disrupted so that setae may appear scattered. Telson with 12–15 long, acute, scattered setae, rounded, epiproct hardly distinct. Gonopod socket occupying entire width of prozonite, nearly circular with strong projecting anterior margin, not indenting sternite nine (Fig. 19). Gonopods with subhemispherical coxae (cx, Fig. 19) immovable, completely filling socket; acropodite arises on distinct stem (s, Fig. 19); prefemoral process relatively narrow, margins even, not scoop-like (pfp, Figs 20, 21); acropodite



Figures 15–20. *Coloradoesmus* species males. **15** *C. hopkinae*, gonopod seminal pore **16–20** *C. manitou* **16** collum, dorsal view **17** midbody ring, dorsal view **18** telson, dorsal view **19** gonopods, ventral view **20** Gonopods, posterior view. See text for explanation of labels.

(a, Figs 20, 21) robust, distal zone (dz, Fig. 21) short, strongly curved; endomerite (e, Figs 20, 21) nearly as large, robust as acropodite, at wide angle to acropodite.

Female paratype. Closely similar to male in all nonsexual characters.

Distribution. COLORADO: Fremont Co.: Fly Cave, 7 August 1996, D. Hubbard, 2 males; Marble Cave, 7 August 1996, D. Hubbard, 7 males and 8 females (these two caves are nearly adjacent at 38°36'N, 105°13'6.500"W (1982 m) asl; El Paso Co.: Cave of the Winds, 7000' (2134 m) asl, 38°55'N, 104°55'W, 2 August 1996, D. Hubbard, 10 males, 6 females and 20 February 2007, David Steinmann, 6 males, 6 females; Dilation Cave, 7400' (2256 m) asl, 38°48'N, 104°52'W, 19 June 2009, D. Steinmann, male; Pedro's Cave, 6800' (2073 m) asl, 38°55'N, 104°55'W, 5 January 2008, D. Steinmann, male, females, juveniles.

***Coloradasmus beckleyi* Shear & Steinmann, sp. nov.**

<http://zoobank.org/9737DDFE-2F14-4F5A-96FF-8E47BB053387>

Figs 22–28

Types. Male holotype and male and female paratypes from La Sunder Cave, Garfield Co.: Colorado, 7500' (2287 m) asl, 39°42'N, 107°10'W, in organic matter and under cardboard along the first 1000' of passage, collected 31 July 1999 by D. Steinmann.

Diagnosis. A small species of *Coloradasmus*, as is the following species, but differing from *C. warneri* in details of the gonopods, as shown in Figs 28–29.

Etymology. The species epithet honors Steve Beckley, for his dedication to cave conservation and education as the owner of Glenwood Caverns in nearby Glenwood Springs.

Description. Male holotype. Length, about 4 mm, greatest width 0.4 mm. Head with short, sparse setae; basal joint of mandible exceeding width of collum (Fig. 22). Collum relatively short, posterior margin evenly, concavely curved (Fig. 23); collum setae short, clavate. Metazonites with somewhat suppressed but distinct paranota bearing obsolete teeth (Figs 22–25); setae distinctly clavate, short, set on prominent tubercles, rows even, uninterrupted. Telson (Fig. 25) with few setae, epiproct indistinct. Gonopods (Figs 26–28) with coxae rather oval, immovable; acropodite (a, Figs 26–28) robust, blocky, pore without cuticular fimbriae, endomerite evidently absent. Prefemoral process (pfp, Figs 26–28) exceeding acropodite, narrow, with distinct acute hook at tip.

Female paratype. Similar in all nonsexual characters to male.

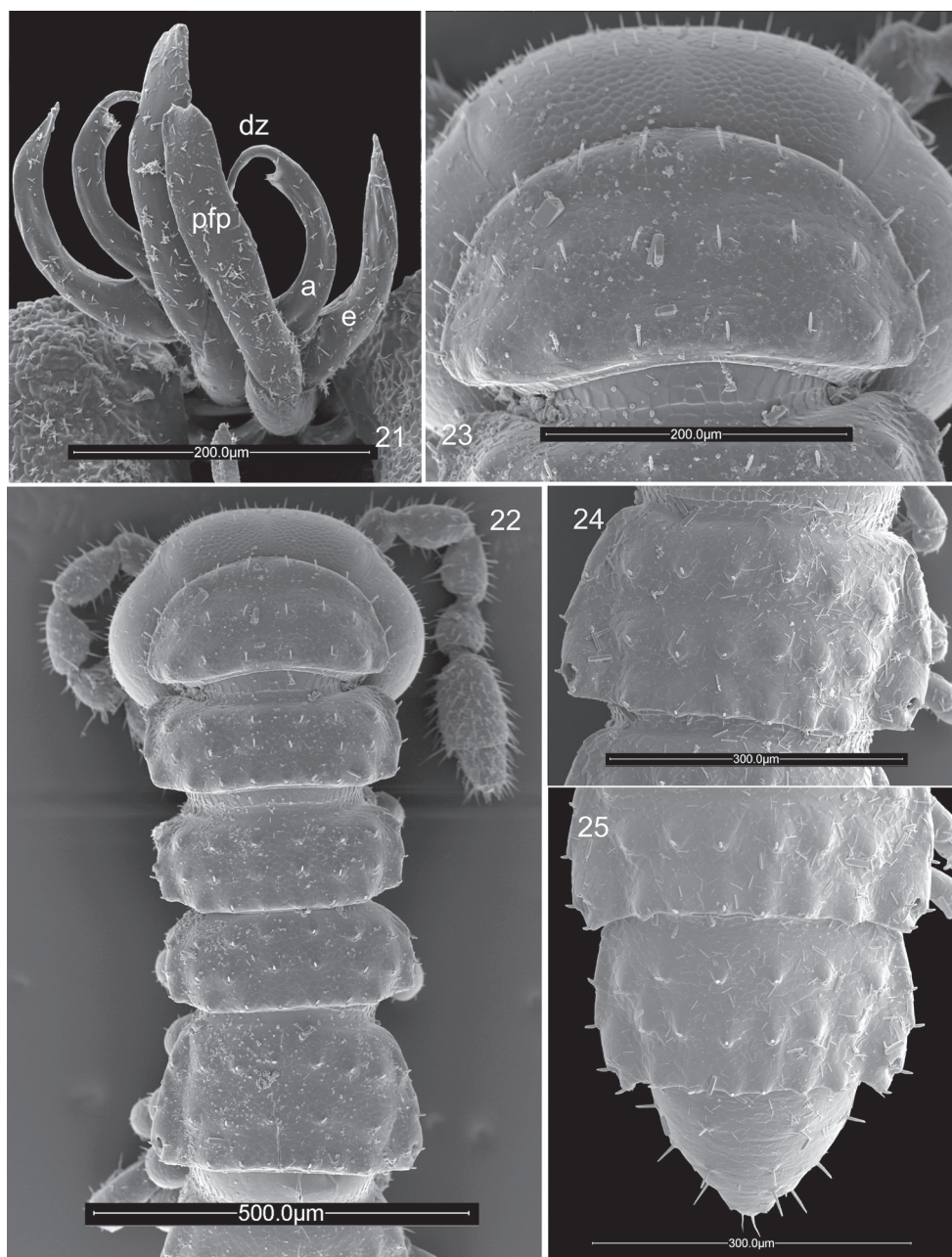
Distribution. Known only from the type locality.

***Coloradasmus warneri* Shear & Steinmann, sp. nov.**

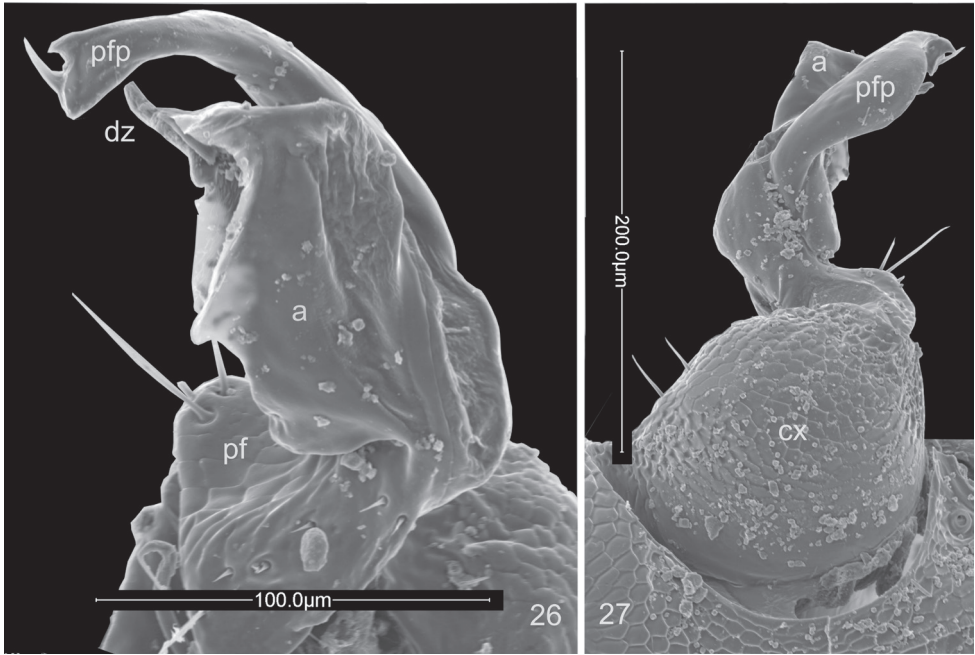
<http://zoobank.org/6D68C4EF-3755-4347-8D5E-F26CE56CE7EF>

Fig. 29

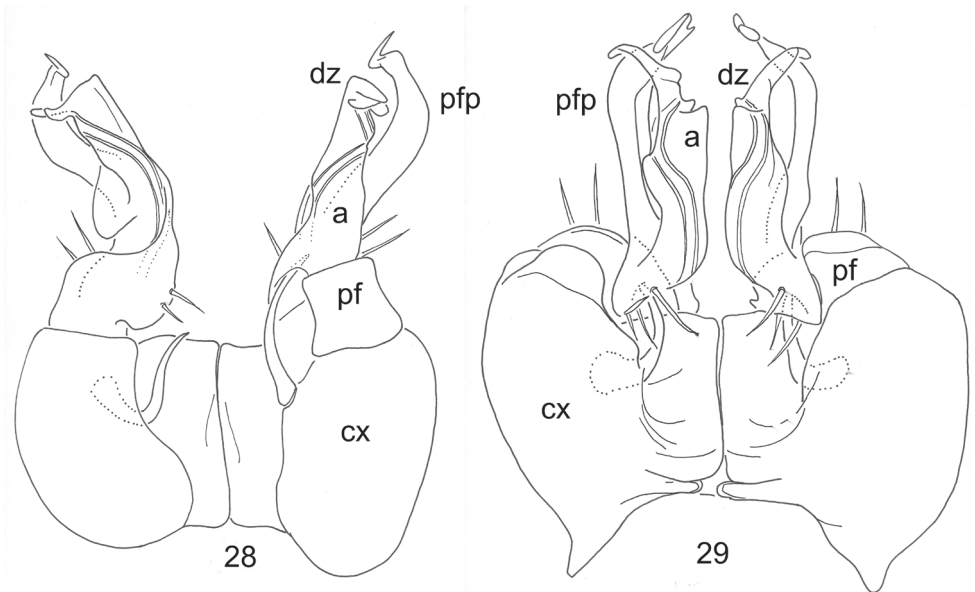
Types. Male holotype and female paratype from White Water Cave, 6100' (1860 m) asl, 40°54'N, 105°09'W, Larimer Co.: Colorado, collected 1 June 2007 by D. Steinmann.



Figures 21–25. *Coloradasmus* species males. **21** *C. manitou* gonopods, posterior view **22–24** *C. lasunda* male **22** head, collum and anterior four rings, dorsal view **23** collum, dorsal view **24** midbody ring, dorsal view **25** telson, dorsal view. See text for explanation of labels.



Figures 26, 27. *Coloradesmus lasunda* male. **26** Right gonopod, mesal view **27** right gonopod, lateral view. See text for explanation of labels.



Figures 28, 29. *Coloradesmus* species gonopods, posterior views. **28** *C. lasunda* **29** *C. warneri*. See text for explanation of labels.

Diagnosis. Very similar in nonsexual characters to the foregoing species, but differing in details of the gonopods. Compare Figs 28, 29.

Etymology. The species epithet honors Ed Warner, an active conservationist and donor to the Denver Museum of Nature & Science, for his dedication to nature and the environment.

Description. In size and in nonsexual characters, this species is nearly identical to *Coloradesmus beckleyi*. Gonopods (Fig. 29) with large subhemispherical coxae immovable in gonopod socket. Acropodite (a, Fig. 29) long, relatively slender, not blocky, slightly curved. Pore lacking cuticular filaments. Distal zone (dz, Fig. 29) curved laterad, tapering, not bent at an angle; prefemora process (pfp, Fig. 29) slender, tapering, gradually curved.

Female paratype: Similar in all nonsexual characters to male.

Distribution. COLORADO: Larimer Co.: White Water Cave, 3 November 2006, D. Steinmann, juveniles; Kremer's Cave, 40°45'N, 105°10'W, 5600' (1707 m) asl, dark zone, 12 August 2006, D. Steinmann, male; 29 March 2008, D. Steinmann, juveniles; Signature Cave, 40°75'N, 105°11'W, 6100' (1860 m) asl, 22 February 2011, D. Steinmann, male, juveniles; 22 October 2011, D. Steinmann, male, female, juveniles.

Notes. Females of a species of the European genus *Polydesmus* were collected along with the types in Whitewater Cave. All species of *Polydesmus* recorded from North America are anthropochoric.

Unassigned specimens

The following specimens appear to belong to *Coloradesmus* but could not be identified to species because of the absence of males. Future collecting in these caves should focus on obtaining male specimens. COLORADO: Eagle Co.: Hourglass Cave, 10000' (3050 m) asl, station 41/45, 1999, C. Mosch, female; Cattleguard Cave, 8000' (2439 m) asl, 4 September 2006, juveniles (2 vials). El Paso Co.: Swirling Mists Cave, 7000' (2134m) asl, , 5 January 2008, D. Steinmann, female.

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References

- Brölemann HW (1908) Description d'un genre nouveau et d'une espèce nouvelle de myriapodes de France. Bulletin de la Société Entomologique de France 1908(7): 94–96. <https://doi.org/10.5962/bhl.part.5606>
- Brölemann HW (1916) Essai de classification des polydesmides (Myriapodes). Annales de la Société Entomologique de France 84: 523–608. <https://doi.org/10.5962/bhl.part.24168>
- Causey NB (1959) Two new troglodytic millipedes from Texas. Proceedings of the Biological Society of Washington 72: 69–74.
- Chamberlin RV (1952) Three cave-dwelling millipedes. Entomological News 63: 10–12.
- Elliott WR (2004) *Speodesmus cave* millipedes. Four new species from central Texas (Diplopoda: Polydesmida: Polydesmidae). Texas Memorial Museum Speleological Monographs 6: 163–174.
- Elliott WR, Reddell JR (2009) New records of *Speodesmus* (Diplopoda: Polydesmida: Polydesmidae) from Fort Hood, Bell and Coryell Counties, Texas. Texas Memorial Museum Speleological Monographs 7: 147–150.
- Lewis JJ (2002) *Chaetaspis aleyorum*, a new species of millipede from Tumbling Creek Cave, Missouri, with a synopsis of the cavernicolous species of *Chaetaspis* (Diplopoda: Polydesmida). Myriapodologica 7: 101–111.
- Loomis HF (1939) The millipedes collected in Appalachian caves by Mr. Kenneth Dearolf. Bulletin of the Museum of Comparative Zoology 86: 165–193.
- Müller CHG, Sombke A (2015) 9 Diplopoda-sense organs. In: Treatise on Zoology – Anatomy, Taxonomy, Biology. The Myriapoda, vol 2, Minelli A, Ed., Brill, Leiden, 181–236. https://doi.org/10.1163/9789004188273_010
- Shear WA (1984) Cave millipedes of the United States. III. Two new species from the western states (Diplopoda: Polydesmida, Chordeumatida). Myriapodologica 1: 95–97.
- Shear WA, Reddell JR (2017) Cave millipedes of the United States. XIV. Revalidation of the genus *Speorthis* Chamberlin, 1952 (Diplopoda, Polydesmida, Macrosterodesmidae), with a description of a new species from Texas and remarks on the families Polydesmidae and Macrosterodesmidae in North America. Insecta Mundi, 0529, 1–13.
- Shear WA, Shelley RM (2007) The millipede genus *Tidesmus* Chamberlin, 1943 (Polydesmida, Macrosterodesmidae). Zootaxa 1656, 51–68. <https://doi.org/10.11646/zootaxa.1656.1.2>
- Shear WA, Shelley RM (2008) Cave millipeds of the United States. VI. *Sequoiadesmus krejcae*, n. sp., from Sequoia and Kings Canyon National Parks, California, USA (Diplopoda, Polydesmida, Polydesmidae, Macrosterodesmidae). Zootaxa 1693: 41–48. <https://doi.org/10.11646/zootaxa.1693.1.3>

- Shear WA, Shelley RM (2019) Solved after 140 years: the identity of the millipede *Polydesmus cavicola* Packard, 1877, and proposal of *Packardesmus* n. gen. (Diplopoda: Polydesmida: Macrosternodesmidae). *Zootaxa* 4559: 184–190. <https://doi.org/10.11646/zootaxa.4559.2.11>
- Shear WA, Taylor SJ, Wynne JJ, Krejca JK (2009) Cave millipeds of the United States. VIII. New genera and species of polydesmidan millipedes from caves in the southwestern states (Diplopoda, Polydesmida, Macrosternodesmidae). *Zootaxa* 2151: 47–65.

A new species of *Alpioniscus* (*Illyrionethes*) from the Dinaric Karst (Isopoda, Oniscidea, Trichoniscidae)

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Abstract

In the Dinaric Karst, *Alpioniscus* (*Illyrionethes*) is the taxon with the largest number of troglobiotic species (19), one of which is new and described here: *A. busljetai* **sp. nov.**, found in two caves in the coastal area of North Dalmatia. Both caves are threatened by human activities (IUCN threats 1.1, 9.1.1).

Keywords

Adriatic coast, cave fauna, new species, terrestrial isopods, Trichoniscinae, troglobiotic

Introduction

Alpioniscus Racovitza, 1908 is the most abundant and widespread terrestrial isopod genus in caves of the Dinaric Karst. It is represented by the subgenus *Illyrionethes* Verhoeff, 1927, with a range from Trieste in Italy to Durmitor in Montenegro (Schmalfuss 2003, Horvatić 2014). This subgenus is present also with six endogean, troglobiotic and stygobiotic species in Sardinia, Italy (Taiti et al. 2018). A recent integrative taxonomic study of *Illyrionethes* species from the Dinaric Karst revealed 19 troglobiotic species, one of which remained undescribed (Bedek et al. 2017, 2019, in press). They grouped into

three different lineages (*strasseri*-, *heroldi*- and *magnus*-lineage), with overlapping ranges. Nine species are restricted to coastal areas with limited distribution ranges: *A. christiani* (Potočnik, 1983) from the *strasseri*-lineage; *A. magnus* (Frankenberger, 1938), *A. lossinii* Bedek, Gottstein & Taiti, 2019, *A. drazinai* Bedek, Gottstein & Taiti, 2019, and *A. mandaliniae* Bedek, Gottstein & Taiti, 2019 from the *magnus*-lineage; *A. trogirensis* Buturović, 1955, *A. kratochvili* (Frankenberger, 1938), *A. haasi* (Verhoeff, 1931) and the new species described below from the *heroldi*-lineage.

Material and methods

Specimens were hand collected with tweezers, fixed and stored in 75% ethanol with glycerol or 96% ethanol. Several specimens were dissected and mounted for micro-preparations in Hoyer's medium (Anderson 1954). For identifications and illustrations in the description, the entire body and the following appendages were used: antennae, antennulae, buccal pieces, male pereopods 1 and 7, genital papilla and male pleopods 1 and 2. Specimens were examined under a Zeiss Stemi 2000-C, Zeiss Primo Star and Nikon Labophot microscopes. Micropreparations were photographed using Canon EOS 40D and EOS Utility software. Drawings were made from photographs. The examined material, description, etymology and remarks are given. The following numerical characters were counted: the number of (1) antennular aesthetascs, (2) antennal flagellum articles, (3) antennal flagellum articles bearing aesthetascs, and (4) setae on the male pereopod 1 carpus. The terminology used in species description is mainly based on Vandel (1960, 1962). The locality coordinates used the WGS84 datum. The map was drawn using ArcMap 10.1 software and related Shadow Relief layer. The IUCN threats are determined according to the Classification Schemes used in IUCN Red List assessments (IUCN 2012).

Repositories:

CBSSC Croatian Biospeleological Society Collection, Zagreb, Croatia
MZUF Museo di Storia Naturale dell'Università di Firenze, Sezione di Zoologia La Specola, Florence, Italy

Taxonomy

Family Trichoniscidae Sars, 1899

Subfamily Trichoniscinae Sars, 1899

Genus *Alpioniscus* Racovitza, 1908

Subgenus *Illyrionethes* Verhoeff, 1927

***Alpioniscus (Illyrionethes) busljetai* sp. nov.**

<http://zoobank.org/F48F1286-A661-4B19-AC7E-3B37FC333E53>

Figs 1–6

Alpioniscus sp. – Bregović et al. 2008: 109 [partim: Markova špilja].

Alpioniscus (Illyrionethes) sp. 3. – Bedek et al. in press: figs 3, 5.

Material examined. Holotype: ♂ Croatia, Starigrad Paklenica, Seline, Markova špilja (cave), 44°16.79'N, 15°28.63'E, 30.IX.2008, D. Hmura leg., CBSSC IT4252. **Paratypes:** 1 ♂, 1 ♀, 1 juv., same data as holotype, CBSSC IT2407; 1 ♀, 1 juv., ibid., 5.VI.2006, H. Bilandžija leg., CBSSC IT560; 2 ♂♂, 1 ♀, ibid., 5.VI.2006, M. Pavlek leg., CBSSC IT561; 2 ♂♂, 2 ♀♀, ibid., 1.V.2010, A. Kirin leg., CBSSC IT2235; 1 ♂ juv., ibid., 1.V.2010, M. Lukić leg., CBSSC IT3975; 1 ♂ juv., ibid., 18.XII.2012, A. Komerički leg., CBSSC IT2881; 1 ♀, ibid., 18.III.2013, K. Miculinić leg., CBSSC IT3974; 1 ♂, 1 ♀, 1 juv., ibid., 20.V.2018, P. Bregović leg., MZUF 9894; 3 ♂♂, 4 ♀♀, ibid., 20.V.2018, N. Kuharić leg., CBSSC IT4250; 2 ♀♀, ibid., 20.V.2018, N. Kuharić leg., CBSSC IT4398; 2 ♀♀, ibid., 20.V.2018, I. Jaklinović leg., CBSSC IT4251; 2 ♀♀, 1 juv., ibid., 20.V.2018, M. Čuček leg., CBSSC IT4399; 2 ♂♂, 1 ♂ juv., 2 ♀♀, Croatia, Starigrad Paklenica, Špecina špajza (cave), 44°17.10'N, 15°27.46'E, 29.VI.2013, A. Komerički leg., CBSSC IT3960; 2 ♀♀, ibid., 29.VI.2013, T. Dražina leg., CBSSC IT3961; 1 ♂, 1 ♀, ibid., 29.VI.2013, T. Dražina leg., CBSSC IT3962.



Figure 1. *Alpioniscus (Illyrionethes) busljetai* sp. nov. in situ in Markova špilja (by courtesy of Petra Kutleša). Scale bar: 1 mm.

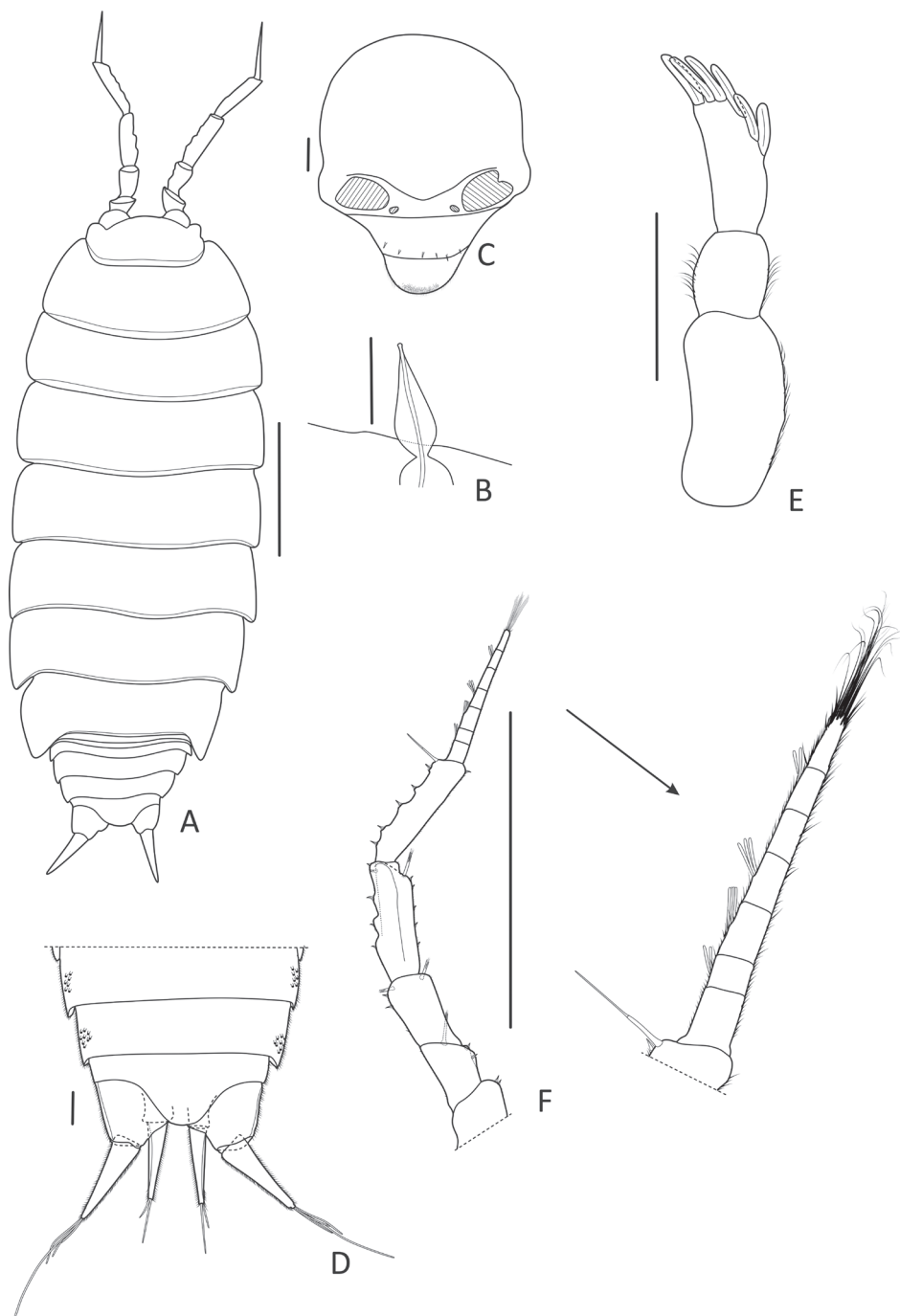


Figure 2. *Alphoniscus (Illyrionethes) busljetai* sp. nov. Paratype ♀ CBSSC IT2235 from Markova špilja **A** habitus in dorsal view. Paratype ♂ CBSSC IT2235 from Markova špilja **B** dorsal scale-seta **C** cephalon, dorsal **D** pleonites 4, 5, telson and uropods. Paratype ♂ CBSSC IT4250 from Markova špilja **E** antennula **F** antenna with enlargement of flagellum. Scale bars: 1 mm (**A**, **F**), 0.1 mm (**C–E**), 0.01 mm (**B**).

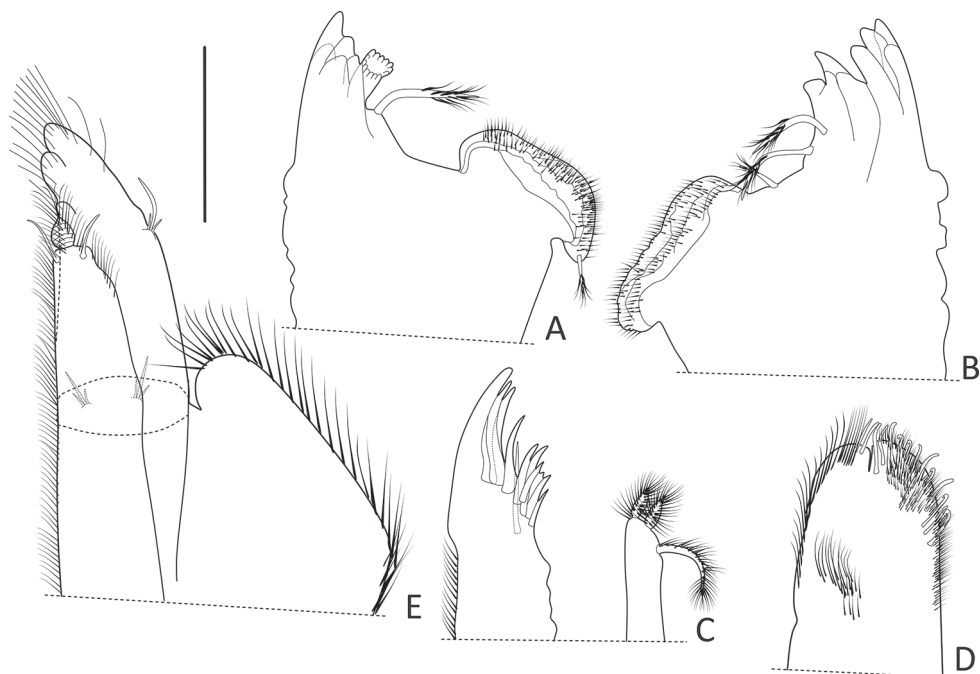


Figure 3. *Alpioniscus (Illyrionethes) busljetai* sp. nov. Paratype ♂ CBSSC IT2235 from Markova špilja **A** right mandible **B** left mandible **C** maxillula **D** maxilla **E** maxilliped. Scale bar: 0.1 mm.

Description. Maximum length: ♂, 4.4 mm; ♀, 6.0 mm. Colourless body, pereon with almost parallel sides, pleon narrower than pereon (Figs 1, 2A). Back smooth, with ridges near posterior margins of cephalon and pereonites, and some triangular scale-setae (Fig. 2B). Some gland pores on lateral margins of pleonites 4 and 5 (Fig. 2D). Eyes absent. Cephalon (Fig. 2C) with suprantennal line bent downwards; antennal lobes rounded. Posterior margin of pereonite 1 convex, of pereonites 2, 3 straight, and of pereonites 4–7 progressively more concave (Fig. 2A). Pleonites 3–5 with small posterior points visible in dorsal view (Fig. 2D). Distal part of telson with concave sides and broadly rounded apex (Fig. 2D). Antennula (Fig. 2E) of three articles, distal article flattened and bearing five to six aesthetascs. Antenna (Fig. 2F) with distal articles of peduncle granulated; flagellum of five to seven articles with one row of aesthetascs on two to four different articles, always on second and third. Mandibles (Fig. 3A, B) with one penicil in right and three in left; molar process with one penicil in right and none in left. Outer branch of maxillula (Fig. 3C) with 4+6 teeth, apically entire, and one slender stalk; inner branch with three penicils, outer and middle subequal, inner distinctly longer. Maxilla (Fig. 3D) with setose and bilobate apex, lobes subequal in width. Maxilliped (Fig. 3E) endite narrow, with large segmented apical penicil; palp distally with three rounded lobes, basal article with two small compound setae; basis with rounded outer lobe protruding posteriorly and covered with long setae on margin. Pereopods with large, bifid and setose dactylar seta (Fig. 4A). Uropod (Fig. 2D)

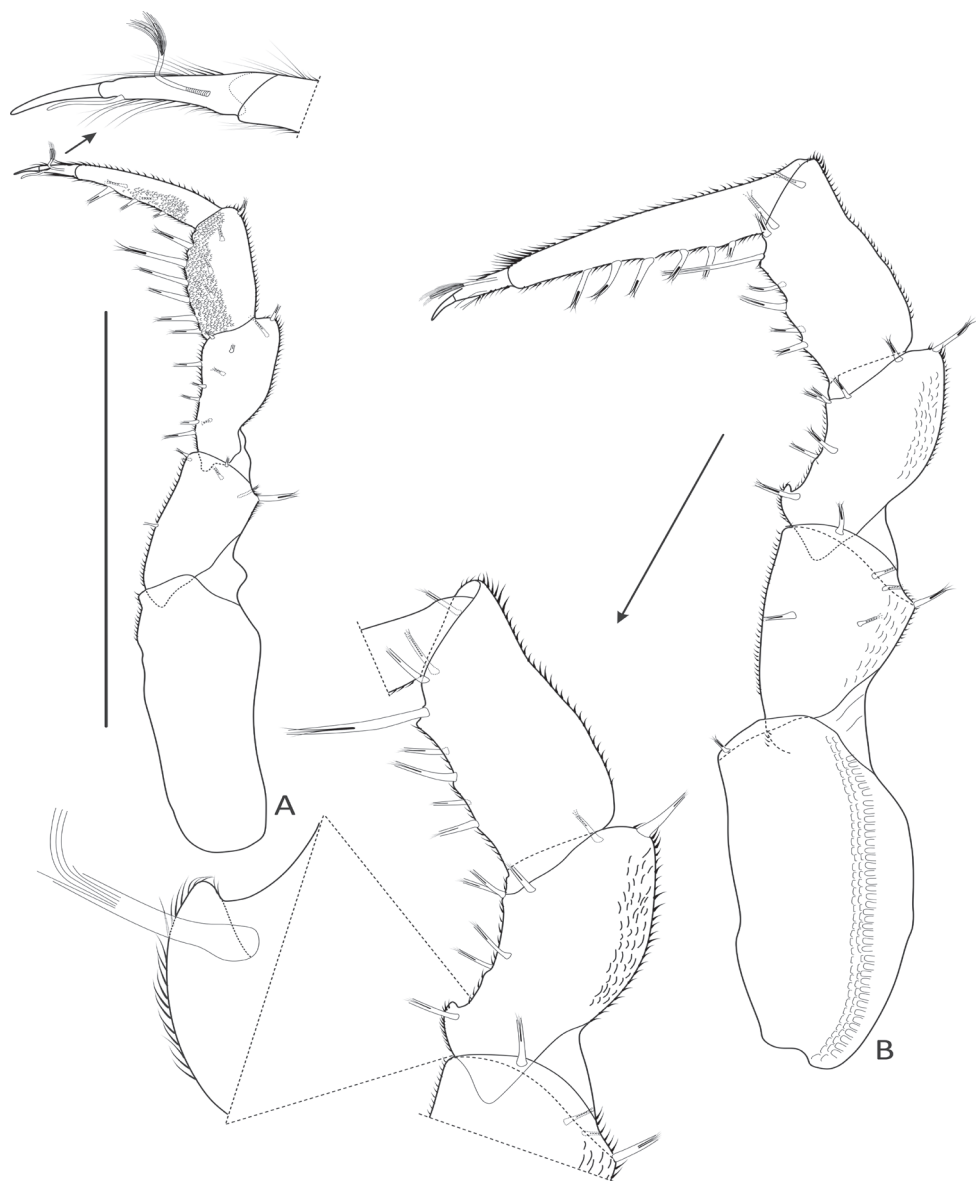


Figure 4. *Alpioniscus (Illyrionethes) busljetai* sp. nov. Paratype ♂ IT4250 from Markova špilja **A** pereopod 1 with enlargement of dactylus **B** pereopod 7 rostral view with enlargement of carpus and merus, and merus hook. Scale bars: 1 mm.

with protopod slightly grooved on outer margin; endopod distinctly shorter than exopod, proximally inserted.

Male. Pereopod 1 (Fig. 4A) carpus bearing four to six setae. Pereopod 1 and 2 with propodus and carpus bearing numerous short scales on rostral surface. Pereopods

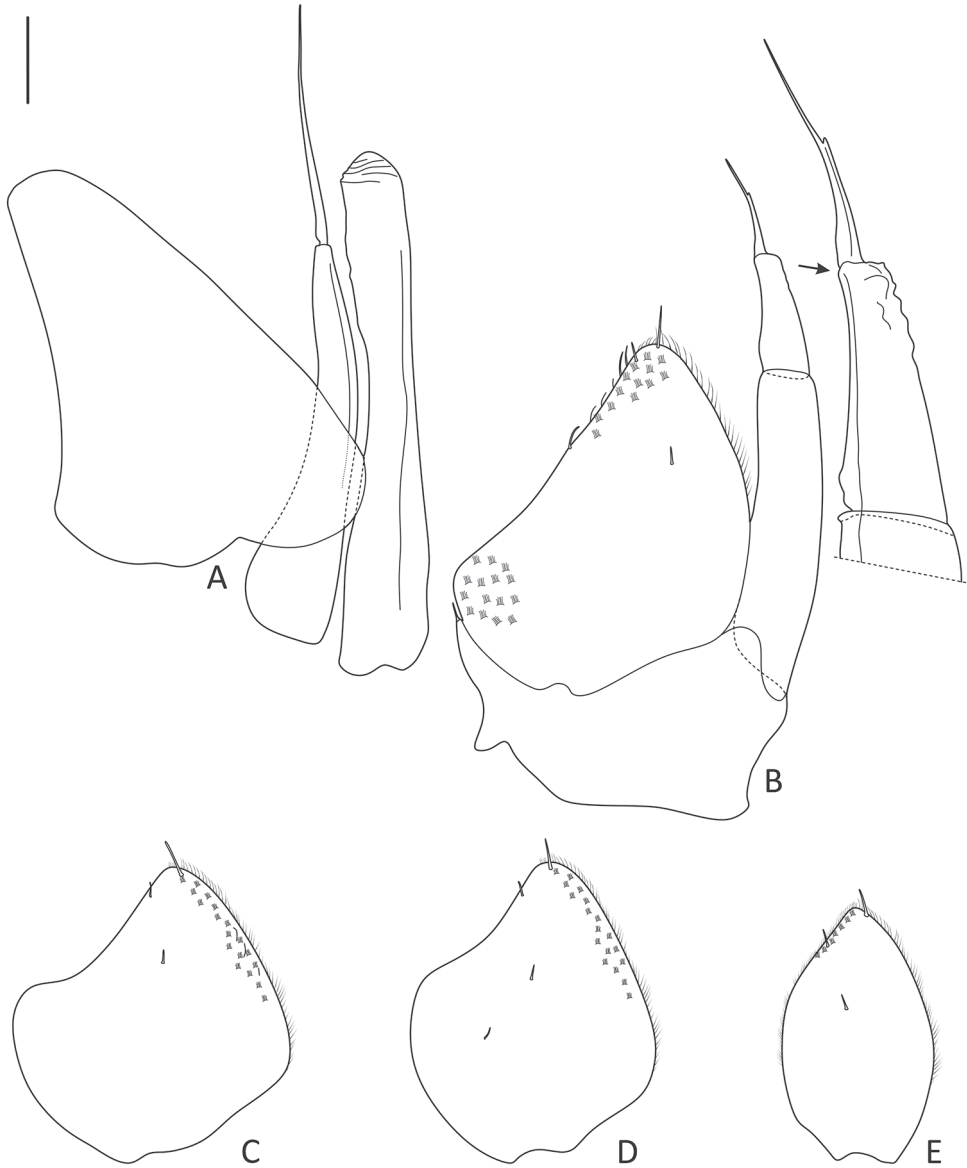


Figure 5. *Alpioniscus* (*Illyrionethes*) *busljetai* sp. nov. Paratype ♂ IT4250 from Markova špilja **A** genital papilla and pleopod 1 **B** pleopod 2 with enlargement of endopod tip. Paratype ♂ CBSSC IT2235 from Markova špilja **C** pleopod 3 exopod **D** pleopod 4 exopod **E** pleopod 5 exopod. Scale bar: 0.1 mm.

1–4 merus with sternal margin straight, pereopods 5, 6 merus with progressively more concave sternal margin and small lobe proximally. Pereopod 7 (Fig. 4B) ischium with straight sternal margin; merus with slightly concave sternal margin and small hook-shaped lobe in proximal part directed ventro-laterally and bearing one seta; carpus with

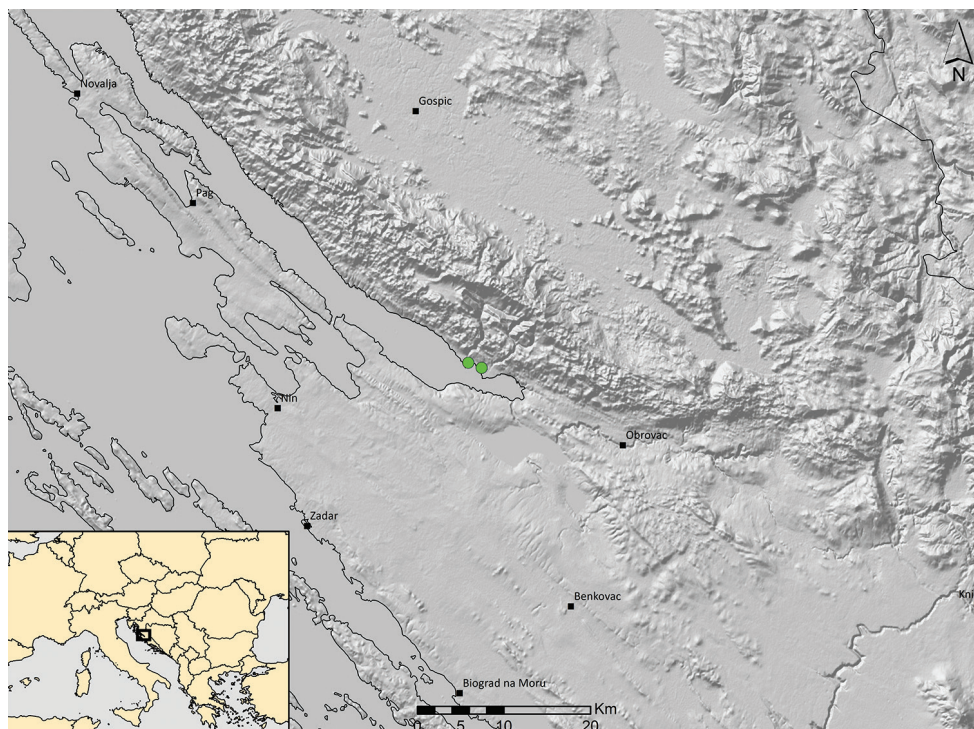


Figure 6. Distribution map of *Alphoniscus (Illyrionethes) busljetai* sp. nov.

straight sternal margin and shallow and long rounded tergal hump in proximal part. Genital papilla (Fig. 5A) with rounded apical part. Pleopod 1 (Fig. 5A) exopod with posterior apex broadly rounded, slightly concave outer margin, straight inner margin; endopod narrow with almost parallel sides, armed with long apical seta. Pleopod 2 (Fig. 5B) exopod triangular with concave outer margin; endopod of two articles, distinctly longer than exopod, posterior part narrower than anterior with strong bifid terminal seta. Pleopod 3–5 exopods as in Fig. 5C–E.

Etymology. The species is named after Dujo Bušljeta, the National park Paklenica ranger and Croatian Biospeleological Society field research guide within the Paklenica area.

Remarks. *Alphoniscus busljetai* sp. nov. differs from Dinaric *Illyrionethes* species by the shallow and long rounded tergal hump of the male pereopod 7 carpus, similar to the one present only in *A. trogirensis*. It differs from *A. trogirensis* in the shape of the male pleopod 1 exopod, with broadly rounded posterior apex and slightly concave outer margin (narrowly rounded posterior apex and sinuous outer margin in *A. trogirensis*). The shape of the male pleopod 1 exopod is similar to the one of *A. tuberculatus* (Frankenberger, 1939), from which it differs by the presence of the dorsal hump of the male pereopod 7 carpus and smooth habitus.

Discussion

Alpioniscus busljetai sp. nov. belongs to the *heroldi*-lineage according to the molecular analysis and to the slightly concave outer margin of the male pleopod 1 exopod, a character in common also with the species of the *magnus*-lineage (Bedeck et al. in press).

The majority of Dinaric *Illyrionethes* species are not particularly endangered (Ozimec et al. 2009). About half species are restricted to a relatively small, usually coastal, area, and five species are known from a single or just two caves. *Alpioniscus busljetai* sp. nov. is found in only two caves in the coastal area of the Velebit Mt., North Dalmatia. Both caves are located in a rural area, with strong potential for tourism growth. Markova špilja is a small, anchialine cave, in the vicinity of houses at the end of the village Seline. Further growth of the village represents the potential risk of a negative impact on the cave or even its destruction (IUCN threat 1.1). Špecina špajza is a small cave with a cave lake, placed among houses in the village Starigrad Paklenica. The human impact on the cave is already present, by housing waste water (IUCN threat 9.1.1) and because the entrance to the cave has been completely destroyed due to construction work (IUCN threat 1.1) (Ana Komerički and Tvrtko Dražina pers. com.).

All the narrow endemics of *Alpioniscus* in the Dinaric Karst are facing or already have been threatened by human activities due to the high urbanisation of the Adriatic coast. The cave fauna inventory and taxonomic analyses are still fundamental for the conservation of the cave biodiversity in the Dinaric Karst.

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References

- Anderson LE (1954) Hoyer's Solution as a Rapid Permanent Mounting Medium for Bryophytes. *The Bryologist* 57: 242. <https://doi.org/10.2307/3240091>
- Bedeck J, Gottstein S, Taiti S (2019) Taxonomy of *Alpioniscus* (*Illyrionethes*): *A. magnus* and three new species from the Dinaric Karst (Isopoda: Oniscidea: Trichoniscidae). *Zootaxa* 4657 (3): 483–502. <https://doi.org/10.11646/zootaxa.4657.3.4>

- Bedek J, Horvatić M, Karaman I (2017) A new trogllobiotic species, *Alpioniscus (Illyrionethes) iapodicus* n. sp. (Crustacea: Oniscidea: Trichoniscidae), from Lika region, Croatia. *Natura Croatica* 26: 205–214. <https://doi.org/10.20302/NC.2017.26.17>
- Bedek J, Taiti S, Bilandžija H, Ristori E, Baratti M (in press) Molecular and taxonomic analyses in trogllobiotic *Alpioniscus (Illyrionethes)* species from the Dinaric Karst (Isopoda: Trichoniscidae). *Zoological Journal of the Linnean Society*. <https://doi.org/10.1093/zoolinnean/zlz056>
- Bregović P, Crnčan P, Ćukušić A, Dražina T, Đud L, Hmura D (2008) Biospeleološka istraživanja NP Paklenica. In: Čolić L, Kapelj S (Eds) *Zbornik istraživačkih radova – Nacionalni park Paklenica 2008*. Udruga studenata biologije-BIUS, Zagreb, 95–117.
- Buturović A (1955) Isopodes nouveaux (Isop. terrestria) de Macédonie et Dalmatie. *Acta Musei Macedonici Scientiarum Naturalium* 3: 145–157.
- Frankenberger Z (1938) Sur les genres *Illyrionethes* Verh. et *Aegonethes*, g. n. (isopodes). *Archives de Zoologie expérimentale et générale* 80: 25–37.
- Frankenberger Z (1939) Über von Herrn Dr. J. Kratochvil in Jugoslawien gesammelte Höhlen-isopoden. II. *Zoologischer Anzeiger* 128: 98–106.
- Horvatić M (2014) Phyletic relations within Synocheta Legrand, 1946 section (Crustacea, Isopoda, Oniscidea) of the Balkan Peninsula. PhD Thesis, University of Novi Sad, Novi Sad.
- IUCN (2012) Threats classification scheme (version 3.2). <https://www.iucnredlist.org/technical-documents/classification-schemes/threats-classification-scheme> [June 18, 2019]
- Ozimec R, Bedek J, Gottstein S, Jalžić B, Slapnik R, Štamol V, Bilandžija H, Dražina T, Kletečki E, Komerički A, Lukić M, Pavlek M (2009) Crvena knjiga špiljske faune Hrvatske. [Red book of Croatian cave dwelling fauna]. Ozimec R, Katušić L (Eds) *Ministarstvo kulture. Državni zavod za zaštitu prirode, Zagreb*.
- Potočnik F (1983) *Alpioniscus (Illyrionethes) christiani* spec. nov., eine neue Trichoniscinae-Art (Isopoda terrestria) aus Jugoslawien. *Annalen des Naturhistorischen Museums in Wien* 84B: 389–395.
- Racovitza E (1908) Biospéologica. IX. Isopodes terrestres (seconde série). *Archives de Zoologie expérimentale et générale* 9: 239–415.
- Sars GO (1899) An account of the Crustacea of Norway with short descriptions and figures of all the species. Volume II. Isopoda. Bergen Museum, Bergen, 270 pp. [+pls. 1–104] <https://biodiversitylibrary.org/page/32172997>
- Schmalfuss H (2003) World catalog of terrestrial isopods (Isopoda: Oniscidea). *Stuttgarter Beiträge zur Naturkunde, Serie A*, 654: 1–341.
- Taiti S, Argano R, Marcia P, Scarpa F, Sanna D, Casu M (2018) The genus *Alpioniscus* Racovitza, 1908 in Sardinia: taxonomy and natural history (Isopoda, Oniscidea, Trichoniscidae). Hornung E, Taiti S, Szlavec K (Eds) *ZooKeys* 801: 229–263. <https://doi.org/10.3897/zookeys.801.24102>
- Vandel A (1960) Isopodes Terrestres (Première Partie). *Faune de France* 64: 1–416.
- Vandel A (1962) Isopodes Terrestres (Deuxième Partie). *Faune de France* 66: 417–931.
- Verhoeff KW (1927) *Illyrionethes* n. g. eine cavernicole Trichonisciden-Gattung. *Zoologischer Anzeiger* 72: 268–274.
- Verhoeff KW (1931) Zwei neue *Illyrionethes*-Arten aus Dalmatien. *Senckenbergiana* 13: 122–134.

Discovery of a new population of the federally endangered Alabama Cave Shrimp, *Palaemonias alabamae* Smalley, 1961, in northern Alabama

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Abstract

The Alabama Cave Shrimp *Palaemonias alabamae* Smalley, 1961 is a federally endangered cave shrimp endemic to just four cave systems within and near the greater Huntsville metropolitan area in Madison County, Alabama USA. It is one of two described atyid cave shrimp in the Interior Low Plateau karst region. Here we report the discovery of a new population of *P. alabamae* from the Fern Cave system in western Jackson County, Alabama. We observed four cave shrimp in August 2018 in an isolated pool in the base-level stream passage of the longest cave system in Alabama. Two cave shrimp were observed during a subsequent survey in July 2019: one in the same isolated pool and a second shrimp in a pool in the main stream passage. Morphological and genetic analyses confirm that this population is closely allied

with other populations in Madison County. This new population expands the known distribution of the species into a new county and watershed (Lower Paint Rock River). The potential exists to discover additional populations in Paint Rock River valley and other nearby regions.

Keywords

Atyidae, conservation, crustacean, Decapoda, distribution, range extension

Introduction

In the United States, over 1,350 troglobionts and stygobionts (i.e., terrestrial and aquatic cave-obligate species) have been described, and many additional taxa await formal description (Niemiller et al. 2019). However, our knowledge on the distribution, abundance, life history, and ecology of most subterranean biodiversity is grossly inadequate (Mammola et al. 2019). These biodiversity knowledge shortfalls are especially pronounced for invertebrate species (e.g., Cardoso et al. 2011; Niemiller et al. 2018), severely hampering our ability to accurately assess, manage, and conserve this unique fauna. For example, the Wallaeen shortfall refers to the lack of knowledge regarding the geographical distribution of species (Lomolino 2004). Our knowledge on species' distributions is intimately correlated with spatial and temporal variation in surveying effort (Hortal et al. 2008, 2015; Boakes et al. 2010). Such variation is widespread in subterranean environments where some regions, such as Mammoth Cave system in central Kentucky and cave systems in the Valley and Ridge of Virginia and West Virginia, have received considerably more attention from biospeleologists than many other regions (e.g., Holsinger et al. 1976, 2013; Holsinger and Culver 1988; Fong et al. 2007; Culver and Hobbs III 2017).

Most cave systems have not been adequately surveyed or studied, and many regions have been grossly underrepresented. For example, less than 7% of caves in Tennessee, the most cave-rich state in United States, have been surveyed for biological resources and even fewer have been repeatedly and comprehensively bioinventoried (Niemiller and Zigler 2013). Moreover, most troglobionts and stygobionts are endemic to a single site or known from very few (<5) cave systems (Christman et al. 2005; Deharveng et al. 2009; Niemiller and Zigler 2013) in a small geographical region (i.e., short-range endemism; Harvey 2002) and inherently assumed to be at higher extinction risk. However, uncertainly often exists whether such species are truly rare or if presumed rarity reflects inadequate surveying effort historically.

The Alabama Cave Shrimp, *Palaemonias alabamiae* Smalley, 1961, is a stygobitic atyid shrimp endemic to just four cave systems (six caves) in and around the greater Huntsville area in Madison County, Alabama, USA (Figure 1). It is one of two described stygobitic shrimp in the Interior Low Plateau karst region in the eastern United States; the other is the Kentucky Cave Shrimp, *P. ganteri* Hay, 1902, from the Mammoth Cave system in central Kentucky. *Palaemonias alabamiae* was officially listed as endangered under the authority of the Endangered Species Act of 1973 in 1988 (USFWS 1988) but critical habitat has not been designated to date. A recovery plan for

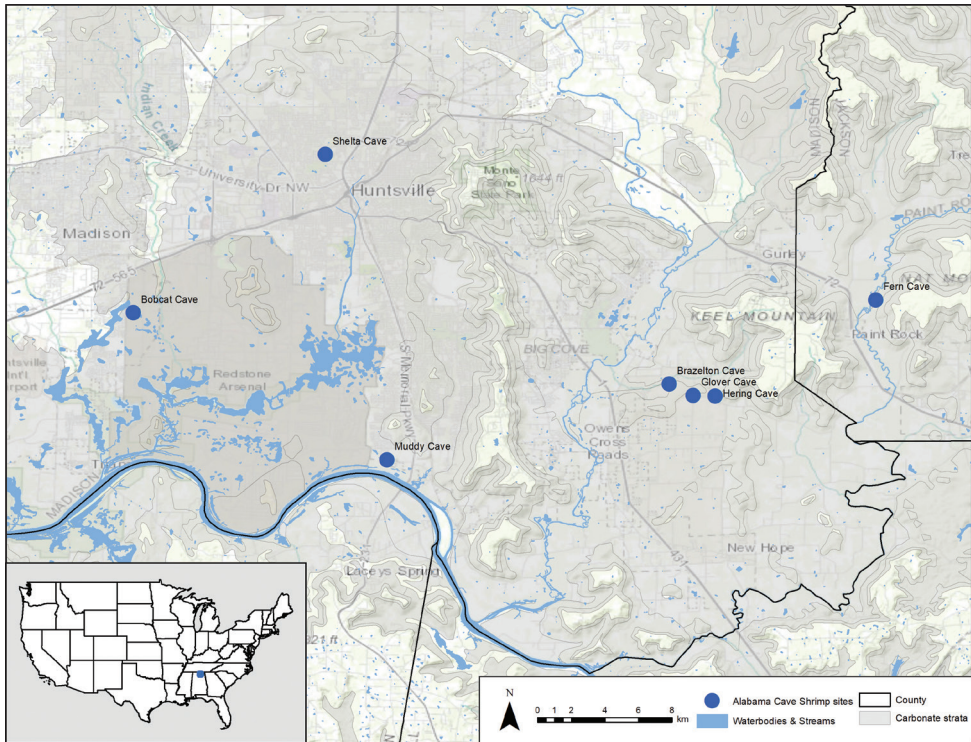


Figure 1. Distribution of the Alabama Cave Shrimp (*Palaemonias alabamae*) in Madison and Jackson counties, Alabama, USA. Carbonate strata are depicted in gray. Alabama Cave Shrimp sites are shown as blue dots.

the species was published in 1997 (USFWS 1997), and the last 5-year review was published in 2016 (USFWS 2016). *Palaemonias alabamae* is State Protected in Alabama under 22–2–98 (Invertebrate Species Regulation) and a Priority 1 Species (Highest Conservation Concern) under the State Wildlife Action Plan (Alabama Department of Conservation and Natural Resources 2015). This cave shrimp has been assessed as Endangered B1ab(iii) on the IUCN Red List (De Grave and Rogers 2013) and Imperiled-Vulnerable (G2G3) (Critically Imperiled [S1] in Alabama) by NatureServe (NatureServe 2019).

Palaemonias alabamae was first discovered at Shelta Cave (Alabama Cave Survey no. AMD4) in northwestern Huntsville, Madison County, Alabama by Thomas Poulson in 1958 and described by Smalley (1961). It was not until 1973 that *P. alabamae* were discovered at a second cave system when William Torode observed shrimp at Bobcat Cave (AMD1283) located 13.3 km southwest of Shelta Cave on Redstone Arsenal, a military installation operated by the U.S. Army (Cooper and Cooper 1974; Torode 1974). Another two decades passed before an additional population was discovered in the Hering-Glover-Brazelton cave system (Alabama Cave Survey nos. AMD6, AMD54, and AMD337, respectively) located 24.0 km southeast of Shelta Cave in southeastern Madison County (Rheams et al. 1992, 1994; McGregor et al. 1994). Finally, a fourth

population was discovered in November 2005, when a single cave shrimp was collected by Bernie Kuhajda and confirmed by DNA analysis from Muddy Cave (AMD1095) in southern Madison County (USFWS 2016). More recent surveys have confirmed that all but the Shelta Cave population are extant (McGregor et al. 2015; USFWS 2016; Niemiller et al., unpublished data). Cave shrimp have not been observed at Shelta Cave since 1973 despite periodic surveys since then (e.g., Lee 1987; Hobbs and Bagley 1989; Moser and Rheams 1992; Rheams et al. 1992; McGregor et al. 1994; Cooper and Cooper 2011; USFWS 2016; Niemiller et al. unpublished data).

Here, we report on the discovery of a population of *P. alabamiae* from the Fern Cave system (AJK597) located in the Paint Rock River watershed in western Jackson County, Alabama. This discovery represents the first new locality for this federally endangered cave shrimp in 14 years and just the fifth population discovered to date, extending the geographic range of *P. alabamiae* into a new hydrological basin. In addition, this new discovery suggests that this endangered species may be found in other cave systems in the Paint Rock watershed and perhaps further east along the Tennessee River.

Methods

Study site

The Fern Cave system is located along the eastern edge of the Paint Rock River Valley on Nat Mountain in western Jackson County between the cities of Huntsville and Scottsboro. It is the largest cave system in Alabama with over 25 km of passages developed primarily in the Mississippian-aged Bangor and Monteagle Limestone formations (Pinkley 2014). Seven entrances are known, four of which are located on the 199-acre Fern Cave National Wildlife Refuge. The other entrances are owned by the Southeastern Cave Conservancy, Inc. The largest winter colony of federally endangered Gray Bats *Myotis grisescens* (Howell, 1909) hibernate in portions of Fern Cave (Martin 2007).

Biological survey

As part of a two-year study implemented by the Inventory & Monitoring Branch of the U.S. Fish & Wildlife Service to biologically inventory the Fern Cave system comprehensively, four of us (MLN, BJ, JL, and NM) on 25 August 2018 entered the Davidson Entrance (aka Sump Entrance) located at the base of Nat Mountain at an elevation of ca. 187.5 m (615 ft). The Davidson Entrance directly accesses the “Bottom Cave” section of the Fern Cave system. For much of the year, this section of the cave is inaccessible or only accessible via cave diving due to high water levels. During our visit, water levels were low enough to permit entry through a low airspace swim and crawl for ca. 40 m leading to the main stream passage that can be traversed for several hundred meters to a sump. We searched aquatic habitats using time-constrained visual surveys

with headlamps and recorded all fauna observed. A cave shrimp was captured using a handheld dipnet in a shallow, isolated pool in the hands-and-knees crawl between the low-air-space pool near the entrance and the main stream passage. The retained specimen was photographed and then preserved in 100% ethanol for morphological and genetic analyses. We returned the following summer and conducted a second survey on 13 July 2019.

DNA extraction, PCR, and sequencing

We extracted genomic DNA from three pleopods using the Qiagen DNEasy Blood & Tissue Extraction Kit following the manufacturer's protocol. A 539-bp fragment of the mitochondrial 16S ribosomal DNA locus was amplified via PCR using primers *16Sar* and *16Sbr* (Simon et al. 1991). This marker exhibits significant genetic variation and has been used in past phylogenetic and population genetic studies of crustaceans (e.g., Zakšek et al. 2007; von Rintelen et al. 2012). In addition, 16S sequences are available on GenBank for *P. alabamae* and the undescribed species in Colbert and Lauderdale counties for comparison (GenBank accession nos. FN995378–FN995383). PCR amplicons were visualized via gel electrophoresis and then sent to Eurofins, Inc. (Louisville, Kentucky) for Sanger sequencing in both directions. The 16S sequence generated in this study was accessioned into GenBank (GenBank accession no. MN097157).

Phylogenetic analyses

Forward and reverse sequences were trimmed at the ends based on quality and assembled into contigs in ChromasPro v2.1.8 (Technelysium Pty Ltd, South Brisbane, Australia) then aligned using MUSCLE (Edgar 2004) in MEGA X version 10.0.5 (Kumar et al. 2018). We constructed a 16S gene genealogy using Bayesian analysis in MrBayes v3.2.7 (Ronquist and Huelsenbeck 2003). The best-fit model of nucleotide substitution was determined using corrected Akaike's Information Criterion (AICc) implemented in the R package *phangorn* v2.5.3 using the function 'modelTest' (Schliep 2011). Outgroup taxa included the atyid shrimp *Atyaephyra desmarestii* (Millet, 1831) (JX469087), *Dugastella marocana* Bouvier, 1912 (FN995363), and *D. valentina* (Ferrer Galdiano, 1924) (FN995365) (von Rintelen et al. 2012). Posterior probabilities were estimated using two independent runs, six Markov chains, and temperature profiles at the default setting of 0.2 for 10 million generations, sampling every 1,000 generations. Random trees were employed at the beginning of each Markov chain, and a molecular clock was not enforced. Stationarity was determined by examining the average standard deviations. We assumed stationarity was achieved if the average standard deviation was < 0.005. The first 25% of trees (5,000 trees) of each run were discarded as burn-in. The remaining trees from the stationarity distribution were sampled to generate a 50% majority-rule consensus tree.

Geographic range size determination

We calculated two measures of geographic range size for *P. alabamae* in the web-based program GeoCAT (Bachman et al. 2011; available at <http://geocat.kew.org/>): extent of occurrence (EOO, also referred to as range extent) and area of occupancy (AOO). Both metrics are used in IUCN Red List and NatureServe conservation assessments. EOO was calculated as a minimum convex hull, while a grid size of 2 km (4 km²) was used to estimate AOO (Faber-Langendoen et al. 2009; IUCN 2010).

Results and discussion

After wading and swimming through the low-air-space pool and passage near the Davidson entrance of the Fern Cave system for ca. 40m on 25 August 2018, we climbed onto a mudbank in a passage that leads to the main cave stream. We searched an isolated pool measuring ca. 5 m long, 2 m at its widest point, and water depth up to 25 cm, with mud/silt substrate and a few larger rocks. We encountered a substantial diversity of life in this pool, including a large cave shrimp (*Palaemonias* sp.). The shrimp was observed at the edge of the pool in 4 cm of water. This cave shrimp was captured and retained as a voucher by recommendation of USFWS personnel to confirm species identification (Figure 2). Although shallow, visibility throughout much of the pool was poor due to silt suspended in the water column. This section of the cave is flooded during most of the year. Aquatic life in this and other pools likely were stranded when water levels receded. We surveyed the remainder of aquatic habitat along the main stream passage until reaching the sump but did not encounter additional shrimp. On our exit of the cave, we stopped and searched again at this pool (Figure 3) and observed three additional cave shrimp ca. 12–18 mm total length measured from the rostrum to end of the uropod. These shrimp were photographed only and not captured.

Two cave shrimp were observed during a return trip the following summer on 13 July 2019. One shrimp was observed in the same isolated pool, which was slightly larger in surface area (ca. 6 m long and 2.5 m wide) due to higher water levels. A second shrimp was observed in a quiet pool ca. 0.4 m in depth with silt/sand substrate and scattered cobble in the cave stream. This pool is just downstream of the junction of the passage with the previously referenced isolated pool and the main cave stream.

We identified the specimen from Fern Cave morphologically as *Palaemonias alabamae* by the presence of degenerate eyes, lack of pigmentation, and a long rostrum with eight dorsal and one ventral spine. In the original description of *P. alabamae* by Smalley (1961), the merus of the third pereopod lacks a distal spine; however, this spine is present in the Fern Cave specimen. The female specimen measured 29.9 mm in total length (tip of rostrum to end of uropod). The specimen was cataloged into the Auburn University Museum of Natural History (AUM 45503). Molecular analysis confirmed that the Fern Cave population was closely related

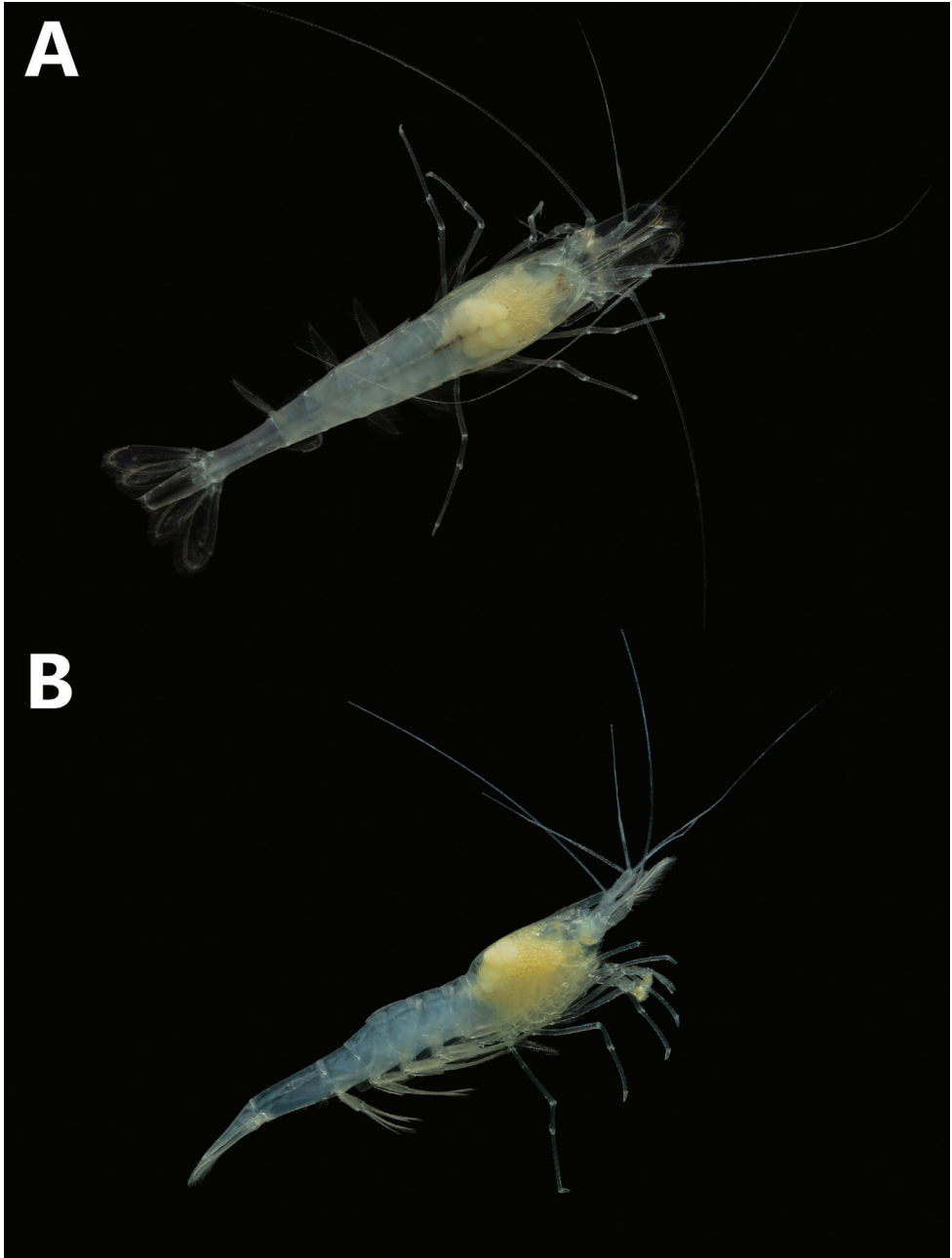


Figure 2. Photographs of the cave shrimp from Fern Cave, Jackson County, Alabama in life: dorsal (**A**) and lateral views (**B**).

to other populations of *P. alabamae* in Madison County (i.e., Hering and Bobcat caves), with 0.4–0.6% uncorrected sequence divergence at the 16S locus between Fern Cave and these two caves (Figure 4).



Figure 3. Joe Lamb and Bradley Jones searching for cave shrimp in an isolated pool near the Davidson Entrance to Fern Cave system on 25 August 2018.

Ecological associates

Palaemonias alabamiae co-occurs with several other species at Fern Cave, including Southern Cave Crayfish *Orconectes australis* Rhoades, 1941, *Caecidotea bicrenata* (Steeves, 1963), Southern Cavefish *Typhlichthys subterraneus* Girard, 1859, Cavespring Crayfish *Cambarus tenebrosus* Hay, 1902, and Banded Sculpin *Cottus carolinae* Gill, 1861. *Typhlichthys subterraneus* is thought to be a significant predator of *P. alabamiae* (Cooper and Cooper 2011) based on an observation in Shelta Cave in 1966 when a large *T. subterraneus* regurgitated an adult cave shrimp (Cooper 1974, 1975; Cooper and Cooper 1974). *Cottus carolinae* are seasonably abundant in many cave systems in the Interior Low Plateau and Appalachian Valley (Poly and Boucher 1996; Niemiller et al. 2006, 2016) and have been documented to feed on stygobitic prey (e.g., *Caecidotea* isopods in Mammoth Cave, Lisowski 1983), but no reports of predation on *P. alabamiae* are known. Another potential predator of cave shrimp is the Tennessee Cave Salamander *Gyrinophilus palleucus* McCrady, 1954, a large stygobitic, neotenic salamander endemic to the Interior Low Plateau karst region of central Tennessee, northern Alabama, and northwestern Georgia (Cooper 1968, Cooper and Cooper 1968; Miller and Niemiller 2008, 2012; Huntsman et al.

2011). *Gyrinophilus palleucus* are generalist predators that co-occur with *P. alabamiae* at Shelta and Bobcat caves (Cooper and Cooper 2011) but are yet to be reported from the Hering-Glover-Brazelton and Fern cave systems.

Distribution and range size

At the time of federal listing in 1988, *P. alabamiae* was known from two cave systems in Madison County (USFWS 1988): Shelta (type locality) and Bobcat caves (Table 1; Figure 1). Both caves are located in the Indian Creek watershed (USGS HUC10 0603000205) on the Tennessee River basin. Rheams et al. (1992, 1994) extended the distribution 24 km to the southeast into the Lower Flint River watershed (USGS HUC10 0603000204) when cave shrimp were discovered in October 1991 in Glover and Hering caves. Hering Cave is located at the base of Keel Mountain, an outlier of the Cumberland Plateau, in southeastern Madison County. The stream that issues from the spring entrance of Hering Cave flows on the surface for ca. 180-m into the upper entrance of Glover Cave during higher-flow conditions. Cave shrimp also were observed in nearby Brazelton Cave in November 1991 (Rheams et al. 1992, 1994) and later confirmed in November 1994 by McGregor et al. (1994). All three caves are hydrologically connected (Jones and Varnedoe 1968; Graham 1969; Rheams et al. 1992, 1994). Bernie Kuhajda discovered the fourth population at Muddy Cave located 18.4 km south of Shelta Cave in southern Madison County near the banks of the Tennessee River (USGS HUC10 0603000209) (USFWS 2016). Cave shrimp have not been observed from the type locality at Shelta Cave since 1973 (Cooper and Cooper 2011; USFWS 2016). We observed cave shrimp at Fern, Hering, and Bobcat caves in 2018–2019 (this study; Niemiller et al., unpublished data).

Our discovery at Fern Cave extends the distribution of *P. alabamiae* into the Lower Paint Rock River watershed (USGS HUC10 0603000202) and into western Jackson County, 9.7 km to the northeast of the nearest population (Hering-Glover-Brazelton cave system) (Figure 1). The Fern Cave system is developed in the carbonate strata along the western escarpment of Nat Mountain. Including the new population at Fern Cave, the EOO of *P. alabamiae* is expanded from 251.8 km² to 363.2 km² and AOO from 24 km² to 28 km². However, if Shelta Cave is presumed to be extirpated (Cooper and Cooper 2011; USFWS 2016), then EOO and AOO are estimated at 195.4 km² and 24 km², respectively. Although estimated range size is larger, no change in conservation status is recommended; *P. alabamiae* was last assessed as Endangered B1ab(iii) under IUCN Red List criteria (De Grave and Rogers 2013).

There are a few possible explanations as to why so few populations of *P. alabamiae* have been reported even though the species has been known to science for almost 60 years and is distributed within and adjacent to a major metropolitan area with extremely high recreational and scientific caving activity. First, *P. alabamiae* may in fact be quite rare with an extremely restricted distribution. Many troglobionts and stygobionts are considered short-range endemics (sensu Harvey 2002) with small ranges and re-

Table 1. Confirmed occurrences of *Palaemonias alabamiae* in Madison and Jackson counties, Alabama.

Cave	ACS no.	County	Geological formation	Watershed	First observed	Last observed
Shelta Cave	AMD4 ^a	Madison	Tuscumbia Limestone and Fort Payne Chert undifferentiated	Indian Creek	1958	1973
Bobcat Cave	AMD1283	Madison	Tuscumbia Limestone	Indian Creek	1973	2019
Hering Cave	AMD6 ^b	Madison	Monteagle Limestone	Lower Flint River	1991	2018
Glover Cave	AMD54 ^b	Madison	Monteagle Limestone	Lower Flint River	1991	1998
Brazelton Cave	AMD337 ^b	Madison	Monteagle Limestone	Lower Flint River	1991	1998
Muddy Cave	AMD1095	Madison	Tuscumbia Limestone	Tennessee River-Wheeler Lake	2005	2012
Fern Cave	AJK597	Jackson	Monteagle Limestone	Lower Paint Rock	2018	2019

^a type locality;

^b hydrologically connected.

ported from a single or few cave systems (Christman et al. 2005; Niemiller and Zigler 2013; Niemiller et al. 2017). In Alabama alone, several species have more restricted ranges than *P. alabamiae*, such as three cave crayfishes (Cooper and Cooper 1997a,b; Buhay and Crandall 2009), a cavefish (Cooper and Kuehne 1974), and several cave pseudoscorpions (Muchmore and Chamberlain 1995; Muchmore 1996) that are all single-site endemics.

Another hypothesis to explain the low number of *P. alabamiae* occurrences relates to detectability. Caves and groundwater ecosystems are extremely difficult for humans to access, survey, and study. Caves that are large enough to permit human entry and exploration as well as sinkholes, springs, and wells represent mere windows into a more expansive subterranean realm. And while many caves, springs, and other points of survey of subterranean biology exist within and near the distribution of *P. alabamiae* in northern Alabama, most of these sites have not been adequately sampled. An undescribed species of cave shrimp closely related to *P. alabamiae* is known from three caves to the west along the Tennessee River in Colbert and Lauderdale counties (Kuhajda and Mayden 2001; USFWS 2016; B. Kuhajda, pers. comm.). *Palaemonias alabamiae* exhibits 1.7–2.3% divergence from populations of this undescribed species (Figure 4). Caves in this region of the Highland Rim have not received the attention by cave biologists historically as cave systems to the east in Jackson, Madison, and Marshall counties.

Moreover, cave systems that have been repeatedly visited by biologists may harbor *P. alabamiae* populations that are not consistently observed each trip. For example, Muddy Cave had been visited on several occasions previously without reliable cave shrimp observations (Kuhajda 2004; USFWS 2016). Both Hering and Glover caves are well-known caves that had been visited periodically for decades by cave biologists before the species was discovered in the early 1990s (e.g., Rhoades 1941; Woods and Inger 1957; Hobbs and Barr 1960; Peck 1983, 1986). Dr. John Cooper, who studied *P. alabamiae* at Shelta Cave (Cooper 1975) and coauthored the most comprehensive paper on the ecology and life history of the species (Cooper and Cooper 2011), did not observe cave shrimp at Hering and Glover caves during the 1960s (Cooper and Cooper 2011; J. Cooper, pers. comm.).

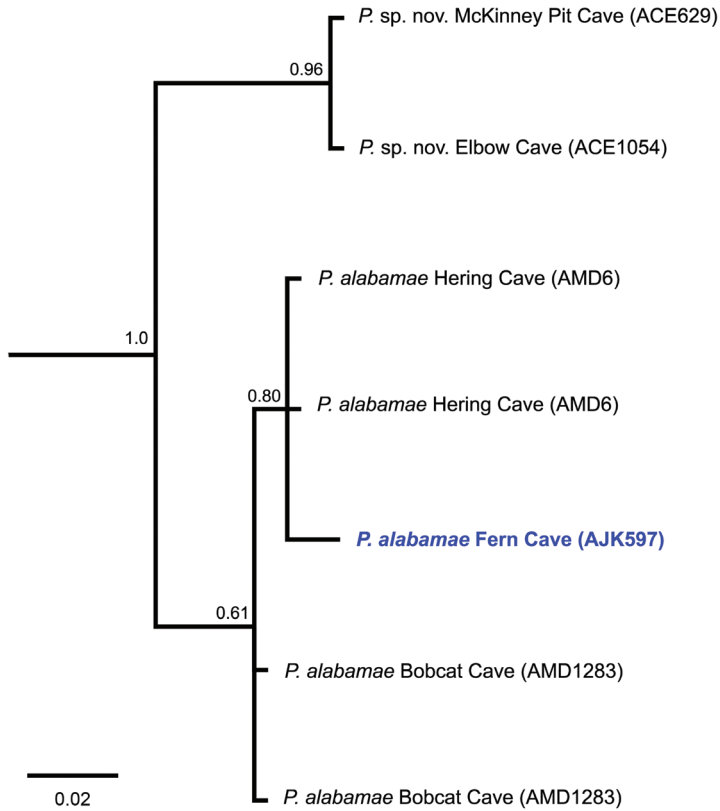


Figure 4. Bayesian phylogram showing the relationships among the new Fern Cave population and other populations of *P. alabamae* and *P. sp. nov.* in Alabama inferred from the mitochondrial 16S ribosomal RNA locus. Posterior probabilities are to the left of the corresponding node.

Low detection may be related to population dynamics or temporal variation of groundwater habitat extent, quality, and accessibility for surveys where *P. alabamae* occurs. Population dynamics of *P. alabamae* are unknown; however, some caves, such as Muddy Cave where very few shrimp have ever been observed, may represent sinks that are periodically extirpated but recolonized from currently unknown or inaccessible (to humans) source populations. Water levels in all caves where *P. alabamae* has been reported are highly variable. For example, aquatic habitat at Shelta Cave largely is reduced to shallow pools during summer and autumn but can fill to 6–8 m or more with higher precipitation during late winter and spring months restricting access to upper levels of the cave only (Cooper 1975; Hobbs and Bagley 1989). Likewise, passages where cave shrimp have been observed are inaccessible during high-water periods at Bobcat Cave, the Hering-Glover-Brazelton cave system, Muddy Cave, and Fern Cave. Consequently, almost all observations of cave shrimp have occurred during periods of low precipitation and low water levels (Cooper 1975; Rheams et al. 1992, 1994; McGregor et al. 1994, 2015; Cooper and Cooper 2011).

The dynamic hydrology of these cave systems has made it difficult to effectively assess and characterize *P. alabamiae* habitat. At most sites, cave shrimp are observed in silt or mud-bottomed, isolated pools with little to no flow that persist through dry seasons or remain once the water table has lowered after late winter-spring rainfall (Rheams et al. 1992, 1994; McGregor et al. 1994; Cooper and Cooper 2011). *Palaemonias alabamiae* also has been observed in shallow, lotic pools ranging from silt and mud to gravel and cobble substrate in the main stream at Hering Cave (Rheams et al. 1992, 1994; McGregor et al. 1994; Cooper and Cooper 2011; Niemiller et al., unpublished data). In Fern Cave, cave shrimp were observed in the isolated pool perched above the main stream but also in a shallow pool in the main stream. We searched several additional pools in the main cave stream similar in depth and substrate to those in Hering Cave but did not observe any additional cave shrimp. Based on observations of mud stains and debris on passage walls, water levels in the passage where cave shrimp were observed can fluctuate at least 3 m above the water levels observed during our 2018 and 2019 surveys. In those instances, the pool where cave shrimp were observed is hydrologically connected with the main stream. We anticipate additional cave shrimp will be observed in the main cave stream leading up to the sump and in isolated pools and main cave stream beyond the sump. Cave shrimp habitat beyond the sump is only accessible through cave diving the sump or by accessing “Bottom Cave” through highly technical, vertical passages from upper level entrances.

The discoveries of *P. alabamiae* at Muddy Cave in 2005 and at Fern Cave renew optimism that additional populations may be discovered in the future. The recovery plan for *P. alabamiae* identified two criteria for downlisting the species from endangered to threatened (USFWS 1997): identification and protection of reproductively viable populations in five groundwater basins (or aquifers) and demonstratable reproductive viability for all five populations over a 20-year period. Reproductively viable populations are known from just two cave systems (Bobcat and Hering-Glover-Brazelton) and potentially Fern Cave. The Shelta Cave population has been extirpated, and the status of the Muddy Cave population is unknown. However, several caves that are developed in the same geological formation with permanent or seasonal pools near the local water table exist in the Flint and Paint Rock river watersheds. Many of these caves have not been comprehensively surveyed for biological resources. We recommend a new survey initiative of caves that have not been surveyed in the past as well as revisiting Muddy and Fern caves to determine if these populations are reproductively viable.

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References

- Alabama Department of Conservation and Natural Resources (2015) Alabama's Wildlife Action Plan 2015-2025. Alabama Division of Wildlife and Freshwater Fisheries, Department of Conservation and Natural Resources, Montgomery, Alabama, USA, 473 pp.
- Bachman S, Moat J, Hill AW, de Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. *Zookeys* 150: 117–126. <https://doi.org/10.3897/zookeys.150.2109>
- Boakes EH, McGowan PJK, Fuller RA, Chang-qing D, Clark NE, O'Connor K, Mace GM (2010) Distorted views of biodiversity: spatial and temporal bias in species occurrence data. *PLOS Biology* 8: e1000385. <https://doi.org/10.1371/journal.pbio.1000385>
- Buhay JE, Crandall KA (2009) Taxonomic revision of cave crayfish in the genus *Cambarus*, subgenus *Aviticambarus* (Decapoda: Cambaridae) with descriptions of two new species, *C. speleocoopi* and *C. laconensis*, endemic to Alabama, USA. *Journal of Crustacean Biology* 29: 121–134. <https://doi.org/10.1651/08-3089.1>
- Cardoso P, Erwin TL, Borges PAV, New TR (2011) The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation* 144: 2647–2655. <https://doi.org/10.1016/j.biocon.2011.07.024>
- Christman MC, Culver DC, Madden M, White D (2005) Patterns of endemism of the eastern North American cave fauna. *Journal of Biogeography* 32: 1441–1452. <https://doi.org/10.1111/j.1365-2699.2005.01263.x>
- Cooper JE (1968) The salamander *Gyrinophilus palleucus* in Georgia, with notes on Alabama and Tennessee populations. *Journal of the Alabama Academy of Science* 39: 182–185.
- Cooper JE (1974) Biological studies in Shelta Cave, Alabama. *Proceedings of the 5th International Congress of Speleology, Stuttgart (1969), Bd. 4, B1/1–8.*
- Cooper JE (1975) Ecological and behavioral studies in Shelta Cave, Alabama, with emphasis on decapod crustaceans. PhD Thesis, University of Kentucky, Lexington, 364 pp.
- Cooper JE, Cooper MR (1968) Cave-associated herpetozoa II: salamanders of the genus *Gyrinophilus* in Alabama caves. *National Speleological Bulletin* 30: 19–24.
- Cooper JE, Cooper MR (1974) Distribution and ecology of troglobitic shrimp of the genus *Palaemonias* (Decapoda: Atyidae). *Association of Southeastern Biologist Bulletin* 21: 48 pp.
- Cooper JE, Cooper MR (1997a) A new species of troglobitic crayfish of the genus *Cambarus*, subgenus *Aviticambarus* (Decapoda: Cambaridae), endemic to White Spring Cave, Alabama. *Proceedings of the Biological Society of Washington* 110: 608–616.

- Cooper JE, Cooper MR (1997b) A new troglobitic crayfish of the genus *Orconectes*, subgenus *Orconectes* (Decapoda: Cambaridae), endemic to Shelta Cave, Huntsville, Alabama. *Journal of Caves and Karst Studies* 59: 119–127.
- Cooper JE, Cooper MR (2011) Observations on the biology of the endangered stygobiotic shrimp *Palaemonia alabamiae*, with notes on *P. ganteri* (Decapoda: Atyidae). *Subterranean Biology* 8: 9–20. <https://doi.org/10.3897/subtbiol.8.1226>
- Cooper JE, Kuehne RA (1974) *Speoplatyrhinus poulsoni*, a new genus and species of subterranean fish from Alabama. *Copeia* 1974: 486–493. <https://doi.org/10.2307/1442539>
- Culver DC, Hobbs III HH (2017) Biodiversity of Mammoth Cave. In: Hobbs III HH, Olsen RA, Winkler EG, Culver DC (Eds) *Mammoth Cave, Cave and Karst Systems of the World*. Springer, 227–234. https://doi.org/10.1007/978-3-319-53718-4_15
- De Grave S, Rogers C (2013) *Palaemonias alabamiae*. The IUCN Red List of Threatened Species 2013: e.T15887A788932. <https://doi.org/10.2305/IUCN.UK.2013-1.RLTS.T15887A788932.en> [25 March 2019]
- Deharveng L, Stoch F, Gibert J, Bedos A, Galassi D, Zgamaister M, Brancelj A, Camacho A, Fiers F, Martin P, Giani N, Magniez G, Marmonier P (2009) Groundwater biodiversity in Europe. *Freshwater Biology* 54: 709–726. <https://doi.org/10.1111/j.1365-2427.2008.01972.x>
- Edgar R (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acid Research* 32: 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Faber-Langendoen D, Master L, Nichols J, Snow K, Tomaino A, Bittman R, Hammerson G, Heide B, Ramsay L, Young B (2009) NatureServe conservation status assessments: methodology for assigning ranks. NatureServe, Arlington.
- Fong DW, Culver DC, Hobbs III HH, Pipan T (2007) *The invertebrate cave fauna of West Virginia*, 2nd edn. West Virginia Speleological Survey, 167 pp.
- Graham D (1969) Glover Sink Cave. In: Wilson J (Ed.) *Alabama caving '68–'69*. Special publication Huntsville Grotto, National Speleological Society, Huntsville, 10–11 [corrected].
- Harvey MS (2002) Short-range endemism among the Australian fauna: some examples from non-marine environments. *Invertebrate Systematics* 16: 555–570. <https://doi.org/10.1071/IS02009>
- Hobbs HH III, Bagley FM (1989) Shelta Cave management plan. Unpublished report prepared by Biological Subcommittee of the Shelta Cave Management Committee, Huntsville, 78 pp.
- Hobbs HH, Barr TC (1960) The origins and affinities of the troglobitic crayfishes of North America (Decapoda, Astacidae). I. The genus *Cambarus*. *The American Midland Naturalist* 64: 12–33. <https://doi.org/10.2307/2422890>
- Holsinger JR, Culver DC (1988) The invertebrate cave fauna of Virginia and a part of eastern Tennessee: zoogeography and ecology. *Brimleyana* 14: 1–162.
- Holsinger JR, Culver DC, Hubbard Jr DA, Orndorff WD, Hobson CS (2013) The invertebrate cave fauna of Virginia. *Banisteria* 42: 9–56.
- Holsinger JR, Baroody RA, Culver DC (1976) *The invertebrate cave fauna of West Virginia*. Bulletin 7. West Virginia Speleological Survey, 86 pp.

- Hortal J, de Bello F, Diniz-Filho JAF, Lewinsohn TM, Lobo JM, Ladle RJ (2015) Seven shortfalls that beset large-scale knowledge of biodiversity. *Annual Review of Ecology and Evolutionary Systematics* 46: 523–549. <https://doi.org/10.1146/annurev-ecolsys-112414-054400>
- Hortal J, Jimenez-Valverde A, Gomez JF, Lobo JM, Baselga A (2008) Historical bias in biodiversity inventories affects the observed environmental niche of the species. *Oikos* 117: 847–858. <https://doi.org/10.1111/j.0030-1299.2008.16434.x>
- Huntsman BM, Venarsky MP, Benstead JP, Hury AD (2011) Effects of organic matter availability on the life history and production of a top vertebrate predator (Plethodontidae: *Gyrinophilus palleucus*) in two cave streams. *Freshwater Biology* 56: 1746–1760. <https://doi.org/10.1111/j.1365-2427.2011.02609.x>
- IUCN (2010) Guidelines for using the IUCN Red List categories and criteria, version 8.1. Prepared by the Standards and Petitions Subcommittee in March 2010.
- Jones WB, Varnedoe WW (1968) Caves of Madison County, Alabama. Circular 52. Geological Society of Alabama, Division of Economic Geology, Tuscaloosa, 177 pp.
- Kuhajda BR (2004) Survey of biological resources in Muddy Cave, Madison County, Alabama. Unpublished report. Volkert 7 Associates, Inc., Mobile, 10 pp.
- Kuhajda BR, Mayden RL (2001) Status of the federally endangered Alabama cavefish, *Spelotyphlinus poulsoni* (Amblyopsidae), in Key Cave and surrounding caves, Alabama. *Environmental Biology of Fishes* 62: 215–222. <https://doi.org/10.1023/A:1011817023749>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Lee DM (1987) Shelta Cave survey. Proceedings of the 1987 annual convention, National Speleological Society, 34 pp.
- Lisowski EA (1983) Distribution, habitat, and behavior of the Kentucky cave shrimp *Palaemonias ganteri* Hay. *Journal of Crustacean Biology* 3: 88–92. <https://doi.org/10.2307/1547855>
- Lomolino MV (2004) Conservation biogeography. In: Lomolino MV, Heaney LR (Ed.) *Frontiers of Biogeography. New Directions in the Geography of Nature*, Sinauer, Sunderland, 293–296.
- Mammola S, Cardoso P, Culver DC, Deharveng L, Ferreira RL, Fiser C, Galassi DMP, Griebler C, Halse S, Humphreys WF, Isaia M, Malard F, Martinez A, Moldovan OT, Niemiller ML, Pavlek M, Rebolera ASPS, Souza-Silva M, Teeling EC, Wynne JJ, Zagamajster M (2019) Scientists’ warning on the conservation of subterranean ecosystems. *BioScience*: biz064. <https://doi.org/10.1093/biosci/biz064>
- Martin CO (2007) Assessment of the population status of the gray bat (*Myotis grisescens*). U.S. Army Corps of Engineers Report ERDC/EL TR-07-22. 106 pp.
- McGregor SW, O’Neil PE, Bearden RA (2015) Water quality and biological monitoring in Bobcat and Matthews caves, Redstone Arsenal, Alabama, 1990–2015. Open-file Report 1513, Geological Survey of Alabama, Ecosystems Investigations Program, Tuscaloosa, 42 pp.
- McGregor SW, Rheams KF, O’Neil PE, Moser PH, Blackwood R (1994) Geologic, hydrologic, and biologic investigations in Arrowwood, Bobcat, Matthews, and Shelta Caves and se-

- lected caves, Madison County, Alabama. Geological Survey of Alabama. Report prepared for the U.S. Fish and Wildlife Service, Jackson, 82 pp.
- Miller BT, Niemiller ML (2008) Distribution and relative abundance of Tennessee cave salamanders (*Gyrinophilus pallescens* and *Gyrinophilus gulolineatus*) with an emphasis on Tennessee populations. *Herpetological Conservation and Biology* 3: 1–20.
- Miller BT, Niemiller ML (2012) *Gyrinophilus pallescens*. *Catalogue of American Amphibians and Reptiles* 884.1.
- Moser PH, Rheams KF (1992) Hydrogeologic investigations of Shelta and Bobcat Caves and adjoining areas, Madison County, Alabama. Alabama Geological Survey, 86 pp. [unpublished report]
- Muchmore WB (1996) The genus *Tyrannochthonius* in the eastern United States (Pseudoscorpionida: Chthoniidae). Part II. More recently discovered species. *Insecta Mundi* 10: 153–168.
- Muchmore WB, Chamberlin JC (1995) The genus *Tyrannochthonius* in the eastern United States (Pseudoscorpionida: Chthoniidae). Part I. The historical taxa. *Insecta Mundi* 9: 249–257.
- NatureServe (2019) NatureServe explorer: an online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. <http://explorer.natureserve.org> [25 March 2019]
- Niemiller ML, Glorioso BM, Miller BT (2006) An eastern and subterranean maximum size record of the banded sculpin, *Cottus carolinae*, from Tennessee. *Journal of the Tennessee Academy of Science* 81: 41–43.
- Niemiller ML, Taylor SJ, Bichuette ME (2018) Conservation of cave fauna, with an emphasis on Europe and the Americas. In: Moldovan OT, Kovac L, Halse S (Eds) *Cave Ecology*. Springer, 451–478. https://doi.org/10.1007/978-3-319-98852-8_22
- Niemiller ML, Taylor SJ, Slay ME, Hobbs III HH (2019) Diversity patterns in the United States and Canada. In: Culver DC, White WB, Pipan T (Eds) *Encyclopedia of Caves*, 3rd edn. Elsevier, 163–176. <https://doi.org/10.1016/B978-0-12-814124-3.00018-2>
- Niemiller ML, Zigler KS (2013) Patterns of cave biodiversity and endemism in the Appalachians and Interior Plateau of Tennessee, USA. *PLoS ONE* 8: e64177. <https://doi.org/10.1371/journal.pone.0064177>
- Niemiller ML, Zigler KS, Ober KA, Carter ET, Engel AS, Moni G, Philips TK, Stephen CDR (2017) Rediscovery and conservation status of six short-range endemic *Pseudanophthalmus* cave beetles (Carabidae: Trechini). *Insect Conservation and Diversity* 10: 495–501. <https://doi.org/10.1111/icad.12263>
- Niemiller ML, Zigler KS, Stephen CDR, Carter ET, Paterson AT, Taylor SJ, Engel AS (2016) Vertebrate fauna in caves of eastern Tennessee within the Appalachians karst region, USA. *Journal of Cave and Karst Studies* 78: 1–24. <https://doi.org/10.4311/2015LSC0109>
- Peck SB (1983) Experimental hybridizations between populations of cavernicolous *Ptomaphagus* beetles (Coleoptera: Leiodidae: Cholevinae). *The Canadian Entomologist* 115: 445–452. <https://doi.org/10.4039/Ent115445-5>
- Peck SB (1986) Evolution of adult morphology and life-history characters in cavernicolous *Ptomaphagus* beetles. *Evolution* 40: 1021–1030. <https://doi.org/10.1111/j.1558-5646.1986.tb00569.x>
- Pinkley JE (2014) *Fern Cave, The Discovery, Exploration, and History of Alabama's Greatest Cave*. Blue Bat Books, Taft, 371 pp.

- Poly WJ, Boucher CE (1996) Nontroglobitic fishes in caves: Their abnormalities, ecological classification and importance: *American Midland Naturalist* 136: 187–198. <https://doi.org/10.2307/2426644>
- Rheams KF, Moser PH, McGregor SW (1992) Geologic, hydrologic, and biologic investigations in Arrowwood, Bobcat, Matthews, and Shelta Caves and selected caves, Madison County, Alabama. Geological Survey of Alabama. Report prepared for the U.S. Fish and Wildlife Service, Jackson, 262 pp.
- Rheams KF, Moser PH, McGregor SW (1994) Hydrogeologic and biologic factors related to the occurrence of the Alabama cave shrimp (*Palaemonias alabamiae*), Madison County, Alabama. Geological Survey of Alabama, Bulletin 161, Tuscaloosa, 147 pp.
- Rhoades R (1941) Notes on some crayfishes from Alabama caves, with the description of a new species and a new subspecies. *Proceedings of the United States National Museum* 91: 141–148. <https://doi.org/10.5479/si.00963801.91-3129.141>
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Schliep K (2011) phangorn: phylogenetic analysis in R. *Bioinformatics* 27: 592–593. <https://doi.org/10.1093/bioinformatics/btq706>
- Simon C, Franke A, Martin A (1991) The polymerase chain reaction: DNA extraction and amplification. In: Hewitt GM, Johnson AWB, Young JPW (Eds) *Molecular Techniques in Taxonomy*, Springer, 329–355. https://doi.org/10.1007/978-3-642-83962-7_22
- Smalley AE (1961) A new cave shrimp from southeastern United States (Decapoda, Atyidae). *Crustaceana* 3: 127–130. <https://doi.org/10.1163/156854061X00626>
- Torode B (1974) Bobcat Cave, Madison County, Alabama. *Huntsville Grotto Newsletter* 15: 62–63.
- U.S. Fish and Wildlife Service (USFWS) (1988) Endangered and threatened wildlife and plants; endangered status of the Alabama cave shrimp, *Palaemonias alabamiae*. *Federal Register* 53: 34696–34698.
- USFWS (1997) Recovery Plan for The Alabama cave shrimp (*Palaemonias alabamiae*). Atlanta, 59 pp.
- USFWS (2016) Alabama cave shrimp (*Palaemonias alabamiae*) 5-year review. Summary and evaluation. *Daphne*, 21 pp.
- Von Rintelen K, Page, TJ, Cai Y, Roe K, Stelbrink B, Kuhajda BR, Iliffe TM, Hughes J, von Rintelen T (2012) Drawn to the dark side: a molecular phylogeny of freshwater shrimps (Crustacea: Decapoda: Caridea: Atyidae) reveals frequent cave invasions and challenges current taxonomic hypotheses. *Molecular Phylogenetics and Evolution* 63: 82–96. <https://doi.org/10.1016/j.ympev.2011.12.015>
- Woods LP, Inger RF (1957) The cave, spring, and swamp fishes of the family Amblyopsidae of central and eastern United States. *American Midland Naturalist* 58: 232–256. <https://doi.org/10.2307/2422371>
- Zakšek V, Sket B, Trontelj P (2007) Phylogeny of the cave shrimp *Troglocaris*: evidence of a young connection between Balkans and Caucasus. *Molecular Phylogenetics and Evolution* 42: 223–235. <https://doi.org/10.1016/j.ympev.2006.07.009>

First record of *Amphoromorpha/Basidiobolus* fungus on centipedes (Geophilomorpha, Geophilidae) from Brazilian caves

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Abstract

We identified *Basidiobolus* fungi on geophilomorph centipedes (Chilopoda) from caves of Southeast Brazil. Twelve specimens of centipedes of the family Geophilidae were examined, and two of them carried the secondary capilliconidia of *Basidiobolus* on their exoskeleton. The fungus uses the surface of the exoskeleton as a support for the asexual reproductive structure. In this manner, the host is used for the purpose of dispersing its spores. This study expands current knowledge of the diversity of arthropods used as host for the fungus, and in particular for *Basidiobolus*, living in cave habitats.

Keywords

Cave habitat, fungus-host relationship, Chilopoda, capilliconidia, Brazil

Introduction

Fungi are abundant cave microorganisms owing to their high dispersion rate, spore survival, and colonization capacity (Wang et al. 2010, Paula et al. 2016). In 1914, Thaxter described two species of fungi of the genus *Amphoromorpha* that were similar to organisms observed by Racovitza (1907, 1908) in terrestrial isopods. For many years, the biology of the saclike thalli described for *Amphoromorpha* was unknown, but today we know that *Amphoromorpha* thalli are secondary capilliconidia of *Basidiobolus* Eidam, 1886, a genus classified in the family Basidiobolaceae (Blackwell and Malloch 1989).

Currently, three *Amphoromorpha* species are recognized, and studies (Blackwell and Malloch 1989) suggest that *Amphoromorpha* is junior synonymy of the genus *Basidiobolus* (Mycobank 2019). Thus, we use the term *Basidiobolus* in reference to the fungus found in this study.

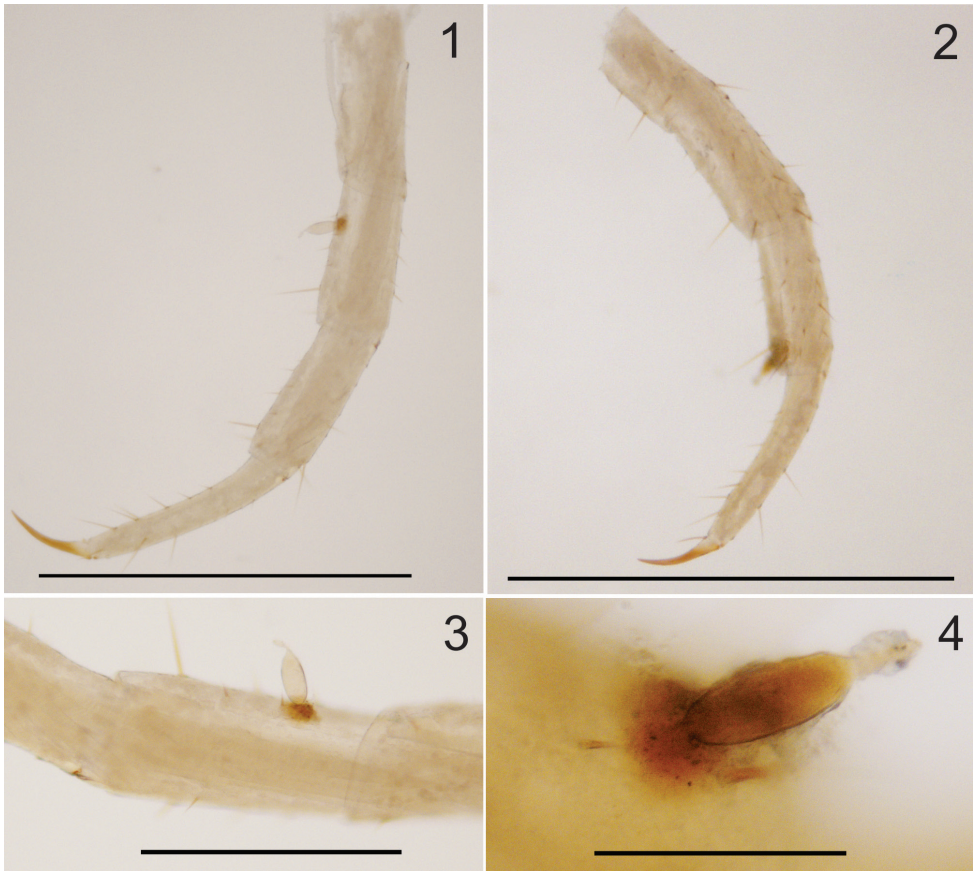
Secondary capilliconidia are asexual spores that transport through a long slender conidiophore by capillary action. During the reproductive stage, the conidiophore is evacuated from the cytoplasm, and the spore exhibits characteristics that enhance dispersal, such as an adhesive droplet at the distal end and a region of dehiscence from the conidiophore. This main feature of capilliconidia allows the spore to adhere to the surface of the arthropod exoskeleton (Blackwell and Malloch 1991, Weir and Blackwell 2005). Fungi of the genera *Amphoromorpha* and *Basidiobolus* are not classified parasites because, to our current knowledge, they do not produce haustoria, a specific structure that is used to penetrate the integument of the arthropod. Capilliconidia are phoretic spores, and they use the adhesion to arthropod integuments as a way of dispersing asexual spores.

Spore dispersion via arthropod vectors is a common propagation mechanism for many fungal species. The fungus-animal relationship is often beneficial for both partners. The arthropod transports the fungus to a new substrate, while the invertebrate is provided with a breeding ground (Basidiomycetes) or a nutrient source (Ascomycetes) (Schiestl et al. 2006). This dispersion mechanism may even be more effective than transport via wind or water as it depends on the locomotion abilities of invertebrates so that dispersion is directed to nutrient-rich environments (Lima 2012).

Our study presents the first record of a *Basidiobolus* fungus on centipedes of the order Geophilomorpha from caves in Southeast Region, Brazil.

Materials and methods

Twelve specimens of the family Geophilidae from the collection of the Coleção Zoológica do Laboratório de Estudos Subterrâneos (**LES**) of the Federal University of São Carlos (Brazil) were examined. The specimens were examined under a Leica EZ4 stereomicroscope and images were captured using a Leica M205C stereomicroscope and an Olympus BX51 photomicroscope (Wetzlar, Germany). The



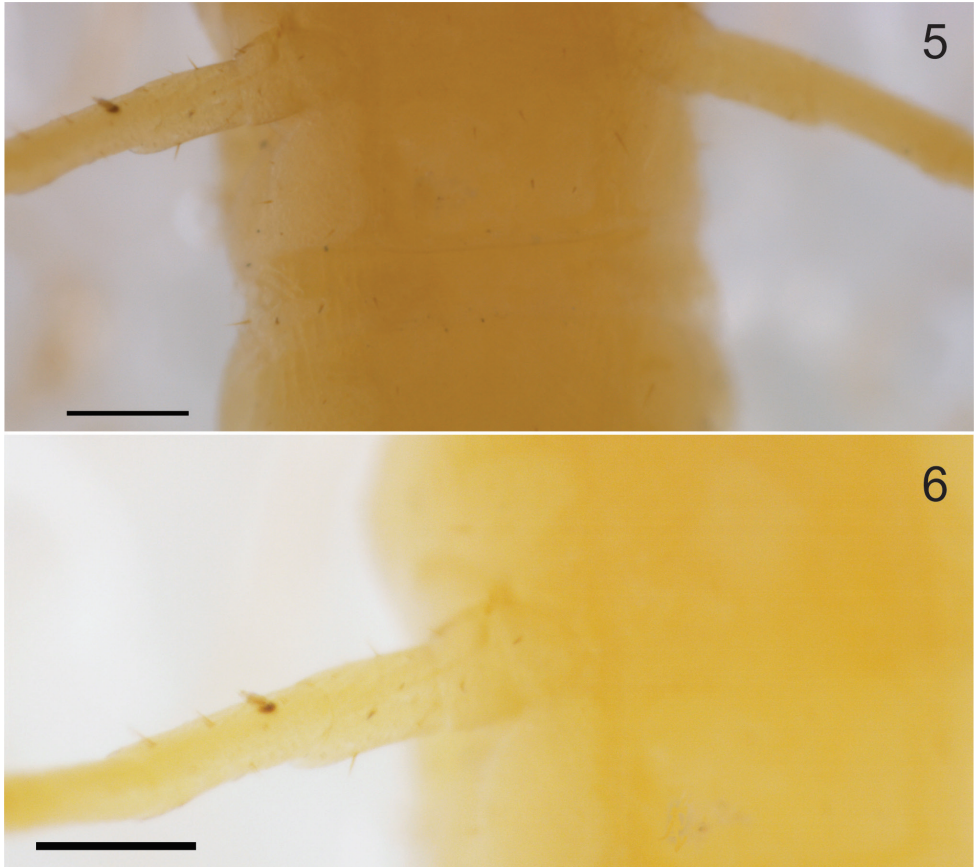
Figures 1–4. *Ribautia* sp. Brölemann, 1909 (Chilopoda: Geophilomorpha: Geophilidae) **1.** (LES 0016373) **1** Right leg 54 **2** Left leg 55 **3** Details of the fungus *Amphoromorpha/Basidiobolus* on right leg 54 **4** Details of the fungus *Amphoromorpha/Basidiobolus* on the left leg 55. Scale bars: 50.0 µm;

plates were made with the Corel DRAW X7 program (Corel Corporation, Ottawa, Canada) and length measurements were obtained in millimeters and microns using the ImageJ program.

Results

Secondary capilliconidia of *Basidiobolus* were found in the exoskeleton of two centipedes.

Two capilliconidia were observed on specimen LES 0016373, which was sampled in the Ressurgência das Areias Quentes Cave (24°33'53.0"S, 48°40'15.5"W), attached on right leg 54 and left leg 55. On right leg 54 (ventral view), the capilliconidia (14.1 µm length and 4.69 µm width) was fixed to the femur. On left leg 55 (ventral view) the capilliconidia (2.50 µm length and 0.69 µm width) was attached to the tibia (Figures 1–4).



Figures 5, 6. (LES 0010593) **5** Details of the fungus *Amphoromorpha basidiobolus* on right leg 52 **6** Details of the fungus *Amphoromorpha basidiobolus* on right leg 52. Scale bars: 200 μ m.

The specimen LES 0010593, which was sampled at Areias de Cima Cave (24°35'01.7"S, 48°42'01.7"W), had one capilliconidia (38.0 μ m length and 10.3 μ m width) attached to right leg 52 (ventral view) (Figures 5, 6).

Discussion

Secondary capilliconidia on the surface of the arthropod exoskeleton have been observed in several groups of arthropods, such as Collembola, Blattodea, Dermaptera, Hemiptera, Heteroptera, Coleoptera, Diptera, Isopoda, Diplopoda, Pseudoscorpiones, Araneae, and Acari (Blackwell and Malloch 1989, Christian 1990, Henriksen et al. 2017).

Jiang et al. (2017) demonstrated the presence of ectoparasitic fungi of the order Entomophthorales that were parasitizing the integument of two species of *Glyphilus*, *Glyphiulus latus* Jiang, Jing-Cai, Guo, Yu & Chen, 2017 and *Glyphiulus liangshanensis*

Jiang, Jing-Cai, Guo, Yu & Chen, 2017 (Spirostreptida, Cambalopsidae). These species were collected in caves in Sichuan Province, Southeast China (Jiang et al. 2017).

With regard to Myriapoda, Enghoff and Reboleira (2017) found amphoromorphs in several millipedes, including *Boreviulisoma barrocalense* Reboleira & Enghoff, 2013, *Acipes andalusius* Enghoff & Mauriès, 1999, an unidentified species of Spirostreptidae (Spirostreptida), and an unidentified species of Paradoxosomatidae (Polydesmida) from Australia. In this same study, the authors identified a possible “*Thaxteriola*” fungus that was attached to the antenna of *Pseudonannolene spelaea* Iniesta & Ferreira, 2013 (Spirostreptida, Pseudonannolenidae) that was sampled in a cave in the state of Pará, Brazil (Iniesta and Ferreira 2013).

For centipedes, Waldock and Lewis (2014) reported the occurrence of an unidentified structure, possibly a capilliconidia, attached to the right tarsungulum of the cryptopide *Paracryptops weberi* Pocock, 1891, a species belonging to the order Scolopendromorpha.

In contrast to that observed in ectoparasite fungi on the arthropod exoskeleton (e.g., Laboulbeniales), capilliconidia of the genus *Basidiobolus* do not have a specific adhesion site on the body of the animal (Blackwell and Malloch 1989). Secondary capilliconidia randomly adheres on the surface of the exoskeleton because the only prerogative for dispersal is an arthropod or another object (including a growing hypha) that touches the spore present on another organism, organic matter, soil, or rock surface (Christian 1990).

Therefore, capilliconidia of *Basidiobolus* can be observed anywhere on the body of an arthropod. The main feature of capilliconidia in the genus *Basidiobolus* is the production of an adhesive substance that becomes very resistant to mechanical friction after being adhered to a surface (Dykstra and Bradley-Kerr 1994). The fungus uses the surface of the exoskeleton only as a support for the asexual reproductive structure and uses the host for the sole purpose of dispersing its spores (Blackwell and Malloch 1989, Weir and Blackwell 2005), the fungus does not penetrate the integument of the host and consequently is believed not to negatively affect it.

This is the first record of capilliconidia associated with centipedes of the order Geophilomorpha. Reports involving the fungus *Basidiobolus* are still scarce in the literature and few studies allow for a discussion on the relationship between the fungus and the host arthropod, especially in cave environments. The present study shows that centipede geophilomorphs are also a type of arthropod used as a host by the fungus *Basidiobolus* for the purposes of spore dispersal. Studies such as this one allow for a better understanding of the diversity of organisms that are used by the fungus *Basidiobolus* and facilitate a more integrative discussion of the ecology and life cycle of this group of fungi.

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References

- Blackwell M, Malloch D (1989) Similarity of *Amphoromorpha* and secondary capilliconidia of *Basidiobolus*. *Mycologia* (81): 735–741. <https://doi.org/10.1080/00275514.1989.12025815>
- Blackwell M, Malloch D (1991) Life history and arthropod dispersal of a coprophilous *Stylopage*. *Mycologia* (83): 360–366. <https://doi.org/10.1080/00275514.1991.12026020>
- Christian E (1990) Ein nicht-myzelialer Pilz (*Amphoromorpha*) als Ektoparasit an Höhlen-Collembolen. *Carinthia II* (180): 373–380. https://www.zobodat.at/pdf/CAR_180_100_0373-0380.pdf
- Dykstra MJ, Bradley-Kerr B (1994) The adhesive droplet of capilliconidia of *Basidiobolus rana-rum* exhibits unique ultrastructural features. *Mycologia* (86): 336–342. <https://doi.org/10.1080/00275514.1994.12026418>
- Enghoff H, Reboleira AS (2017) Diversity of non-Laboulbenialean fungi on millipedes. *Studies in Fungi* 2(1): 130–137. <https://doi.org/10.5943/sif/2/1/15>
- Henriksen CB, Reboleira AS, Scharf N, Enghoff H (2017) First record of a *Basidiobolus/Amphoromorpha* fungus from a spider. *African Journal of Ecology*, 56(1): 153–156. <https://doi.org/10.1111/aje.12430>
- Iniesta LF, Ferreira RL (2013) The first troglobitic *Pseudonannolene* from Brazilian iron ore caves (Spirostreptida: Pseudonannolenidae). *Zootaxa*, 3669(1): 85–95. <https://doi.org/10.11646/zootaxa.3669.1.9>
- Lima JT (2012) Artrópodes como vectores de dispersão de espécies fúngicas – caso de estudo no Arquivo da Universidade de Coimbra. Dissertação (Biodiversidade e Biotecnologia Vegetal), Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Portugal, 74 pp. <http://hdl.handle.net/10316/24980> [Accessed 24/06/2019]
- Paula CCP, Montoya QV, Rodrigues A, Bichuette ME, Seleghim MHR (2016) Terrestrial filamentous fungi from Gruta do Catão (São Desidério, Bahia, Northeastern Brazil) show high levels of cellulose degradation. *Journal of Cave and Karst Studies* 78(3): 208–217. <https://doi.org/10.4311/2016MB0100>

- Racovitza EG (1907) Biospeologica IV: isopodes terrestres. Archives de Zoologie Expérimentale et Générale, 4(7): 145–225.
- Racovitza EG (1908) Biospeologica IX: isopodes terrestres. Archives de Zoologie Expérimentale et Générale, 4(9): 259–415.
- Schiestl FP, Steinebrunner F, Schulz C, Von Reuss S, Francke W, Weymuth C, Leuchtmann A (2006) Evolution of ‘pollinator’- attracting signals in fungi. Biology Letters (2): 401–404. <https://doi.org/10.1098/rsbl.2006.0479>
- Thaxter R (1914) On certain peculiar fungus-parasites of living insects. Botanical Gazette, (8): 235–253. <https://doi.org/10.1086/331399>
- Waldock JM, Lewis JGE (2014) Recent collections of centipedes from Christmas Island (Myriapoda: Chilopoda). Raffles Bulletin of Zoology (30): 71–80.
- Wang W, Ma X, Ma Y, Mao L, Wu F, Ma X, An L, Feng H (2010) Seasonal dynamics of airborne fungi in different caves of the Mogao Grottoes, Dunhuang, China. International Biodeterioration and Biodegradation 64: 461–466. <https://doi.org/10.1016/j.ibiod.2010.05.005>
- Weir A, Blackwell M (2005) Fungal biotrophic parasites of insects and other arthropods. In: Vega FE, Blackwell M (Eds) Insect-Fungal Associations: Ecology and Evolution. Oxford University Press, Oxford, 119–145.
- Xuan-Kong J, Jing-Cai LV, Xuan G, Zhi-Gang Y, Hui-Ming C (2017) Two new species of the millipede genus *Glyphiulus* Gervais, 1847 from Southwest China (Diplopoda: Spirostreptida: Cambalopsidae) Zootaxa 4323(2): 197–208. <https://doi.org/10.11646/zootaxa.4323.2.3>

Dark diversity in the dark: a new approach to subterranean conservation

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Abstract

When trying to predict biodiversity patterns, species absences in a community can be as informative as species presences. The concept of dark diversity considers geographical and ecological filters to set an expected species pool and to compare it with the observed species pool, through an index known as community completeness. Completeness shows no relationship with latitude, allowing the comparison of different communities and regions concerning community saturation. Here we propose the use of these methods to a better understanding of subterranean biodiversity patterns. We applied patterns of co-occurrence among phylogenetically related species to set the theoretical species pool and then compared it with the observed richness, using isopods as model taxon. Except for one cave, dark diversity was equal or higher than observed richness. Even though completeness was low in most cases, those caves with higher completeness represent a valuable sample of regional subterranean species pool and may act as a repository of diversity. Our study showed that the dark diversity approach is adaptable to studies of subterranean communities and may be coupled with other conservation tools towards more effective management decisions.

Keywords

cave, completeness, isopods, species pool

Introduction

Subterranean biodiversity patterns are marked by taxonomic and functional disharmony (*sensu* Cardoso 2012) and by high species turnover (Gibert and Deharveng 2002) when compared to the surface fauna in the same region. On the one side, taxonomic and functional disharmony occurs when species or functional groups are missing from the subterranean environment, because some taxa may be favoured to the detriment of other taxa during the occupation of caves (Cardoso 2012). On the other side, high species turnover reflects the smaller ranges of obligate cave fauna (Barr and Holsinger 1985; Gibert and Deharveng 2002). The processes underlying these patterns are generally attributed to regional factors, such as high habitat fragmentation and long term stability (Gibert and Deharveng 2002), and to local influences, such as resource and habitat availability, competition, and predation (Culver 1981; Christman and Culver 2001; Culver 2012; Resende and Bichuette 2016). Biodiversity, thus, bears an imprint of both influences (He et al. 2005) in such a way that species do not occur in all caves or habitat patches where their occurrences are expected (Culver and Pipan 2009; Culver 2012; Resende and Bichuette 2016). One way to understand the impact of these patterns on subterranean communities is by looking for those species whose presence was expected but are absent instead.

Observable patterns cannot always be completely explained by ecological mechanisms, because they reflect only part of the bigger picture (Lewis et al. 2017). Some species occur in the region, tolerate the conditions of the environment, but are not present in the focal area. The knowledge of this kind of absences can complement the understanding of ecological processes and increase the effectiveness of conservation strategies (Lewis et al. 2017). This concept is known as dark diversity (Pärtel et al. 2011). Understanding which species are in the dark diversity, why they are absent, and the consequences to community processes sheds light on several ecological processes still not completely understood or predicted by observed patterns of species distribution. Using dark diversity, one may better understand species distributions (Riibak et al. 2015), predict the restoration potential of degraded habitats, the likelihood of successful species invasions (Lewis et al. 2017) or even future distributions of threatened species under a climate change scenario (Tang et al. 2019). Possibilities are innumerable. For example, in subterranean communities we may question the implications of dark diversity in terms of community saturation and how this information can be applied to cave conservation.

Underlying the concept of dark diversity is the idea of how much of the species pool is realised within a community (Pärtel et al. 2013). With such information complementing other methods, ecologists may adapt informed and effective strategies for conservation and restoration (Lewis et al. 2017). Calculating the proportion of species absences (dark diversity) in relation to presences (observed richness) enables one to understand which part of the observed richness is natural variation in the regional pool size and which part is dark diversity (Pärtel et al. 2013; Pärtel 2014). An index known as community completeness (Pärtel et al. 2013) allows us to estimate this proportion

and it is calculated as the logistic expression between observed richness and dark diversity ($\log_e(\text{observed richness} / \text{dark diversity})$). Since species richness and dark diversity covary, community completeness shows no relationship with latitude, allowing the comparison of different communities and regions (Ronk et al. 2015).

Even though this concept of biodiversity has been encouraged and discussed since its definition by Pärtel et al. (2011), only a few studies effectively applied dark diversity to enhance the prediction power of ecological processes (e.g. Pärtel 2014; Riibak et al. 2015; Ronk et al. 2015; Tang et al. 2019) and to encourage its importance in supporting well informed management decisions (Lewis et al. 2017). Some controversy about the determination of species pool also has been raised (Mokany and Paine 2011), but methods already applied to the determination of suitable habitats, using Beals probability index based on co-occurrences (Beals 1984; Münzbergová and Herben 2004) or in Ellenberg indicator values proved applicable to dark diversity as well (Lewis et al. 2016). Despite this central role in understanding biodiversity and its importance in conservation and management, the concept of dark diversity has been applied only to plant communities, probably because of the availability of plant databases at several spatial scales. No study has applied dark diversity to subterranean communities to date. In the midst of this debate, we propose that the application of dark diversity to subterranean communities is feasible and interesting for conservation purposes.

We used an assemblage of terrestrial isopods (Oniscidea) as a model taxon for evaluating the dark diversity of caves. Isopods are a very diversified and widespread group not only in superficial but also in subterranean environments, particularly in caves, where they benefit from the milder conditions, high humidity, and the great variety of favourable substrates (Taiti 2003; Campos-Filho et al. 2014). Phylogenetically related taxa were used to set the species pool and to appropriately assess dark diversity, because only a few species of isopods co-occur inside caves (Fernandes et al. 2016). We expected subterranean communities to have high dark diversity and low completeness, because of their typically high beta diversity (Gibert and Deharveng 2000). Therefore, we aimed to compare how much of the habitat-specific regional isopod species pool was found within each local cave community. In addition, we advocate for the application of the dark diversity concept in ecological studies and monitoring of subterranean ecosystems to help guide conservation policies.

Methods

Study area

We collected terrestrial isopods from caves in the São Domingos karst area located in the state of Goiás, central Brazil (Figs 1, 2). The region is within the Morphoclimatic Domain of Cerrado (Ab'Saber 1977), predominantly with cerrado, and under tropical seasonal climate, with wet summers and dry winters (Köppen's "AW") (Alvares et al. 2014). A solid body of knowledge has been built about subterranean fauna from São

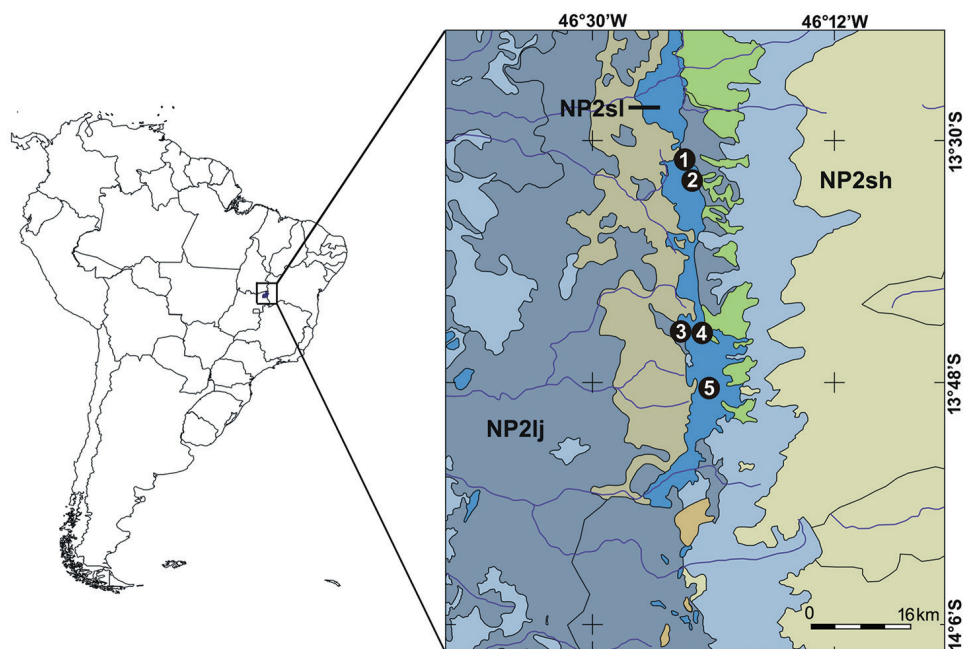


Figure 1. Caves studied in São Domingos karst area, state of Goiás, Brazil. **1.** Lapa do Angélica; **2.** Lapa do Bezerra; **3.** Lapa da Terra Ronca II; **4.** Lapa da Terra Ronca I (Terra Ronca System); **5.** Lapa São Bernardo. NP2lj = sequences of sedimentary rocks with low metamorphism; NP2sl = metalimestones intercalated with silty clay to sandy sediments; NP2sh = predominantly silty-clay sediments.

Domingos, because the region is explored since the onset of biospeleology in Brazil, in the early eighties (Dessen et al. 1980; Trajano and Gnaspini-Netto 1991, Pinto-da-Rocha 1995, Simões et al. 2013; Bichuette et al. 2019). Most published faunistic lists of subterranean fauna suffer from difficulties related to taxonomic identification and description of species (Trajano and Bichuette 2010), which prevented us from calibrating the species pool using previously published faunal lists.

Caves in São Domingos developed through dissolution of carbonate rocks by percolation of slightly acidic water. They were formed in Neoproterozoic metalimestones characteristic of the Bambuí Geomorphologic Unit, the largest set of rocks favourable to the formation of caves in Brazil (Karmann and Sánchez 1979). All caves are developed in a continuous outcrop of these metalimestones interspersed with dolomitic limestone and silty clay to sandy metasediments (Souza et al. 2004). Though subterranean connections among cave systems have not been determined as of yet, they are plausible considering the several interconnected spaces that are typically associated with this form of speleogenesis (Juberthie 2000) (Fig. 1). We elaborated the map of São Domingos using QGIS free software and shape files from Brazilian Geological Survey (CPRM 2015).

Even though São Domingos is protected by the boundaries of a State Conservation Unit (Terra Ronca State Park – PETER), the integrity of subterranean environments

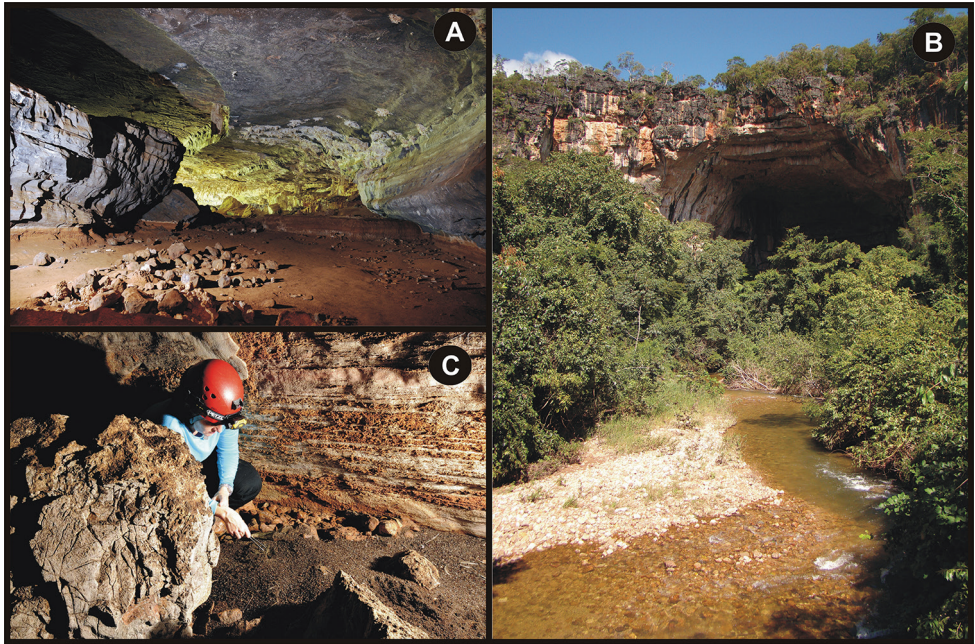


Figure 2. Representative photographs of sampled caves. **A** Lapa do Angélica (photo: A. Gambarini) **B** entrance of Lapa da Terra Ronca I (photo: ME Bichuette) **C** Guano pile inside Lapa do São Bernardo (photo: A. Gambarini).

is still at risk because portions of the headwaters of all subterranean rivers are unprotected or in close proximity to anthropogenic threats, such as soybean plantations and pastures. Therefore, the area is still affected by land ownership problems and by anthropic activities outside the park resulting in sedimentation and groundwater pollution (Simões et al. 2013).

Field sampling

We collected terrestrial isopods from early 2011 to late 2012 every three months in five caves and their surface vicinities distributed throughout São Domingos. All cave systems we visited were near each other, with the same level of environmental integrity at its environs and the same phytophysognomy (Cerrado). They are extensive caves, with large entrances and kilometers of extension. Resources are abundant, brought by floods and bats, and so is the availability of microhabitats. We searched for isopods in several types of microhabitats. Sampling effort was ca. 4 person-hours at each site, equally distributed inside (subterranean environment) and outside (surface environment) the caves. Because of the extension of the caves, we did not collect their full length, but searching was distributed equally at each zone (entrance, twilight, transition and aphotic). Inside the caves, the search consisted of turning over rocks, logs, and debris while

inspecting every organic substrate prone to harbour invertebrates. In addition to active visual encounter surveys, we removed soil at several locations outside of each cave but within 100 m of the cave entrance and sampled leaf litter, using Winkler extractors and Berlese funnels (Palacios-Vargas et al. 2013). We took approximately ten soil samples and collected leaf litter using a shovel or hands, near the locations of soil samples. We sampled around three bags with 20,000 cm³ of leaf litter each per cave and then, processed it in the traps while still in the field base. We deposited all isopods at the Federal University of São Carlos and sent some specimens of some taxa to taxonomists for species identification.

Estimating dark diversity and community completeness

To estimate the dark diversity (Pärtel et al. 2011) of the isopods, we defined the species pool as the set of species occurring in the study area that were potential colonisers of caves. Patterns of co-occurrence among phylogenetically related taxa were chosen to define the species pool. Accordingly, an absent species is part of the species pool if it typically co-occurs with observed species present in the community (Lewis et al. 2016). We considered the clade Mandibulata as monophyletic (Myriapoda, Crustacea, and Hexapoda), following the most recent advances in Arthropoda phylogenetic hypotheses (Giribet and Edgecombe 2012) and assumed these groups to have similar ecological requirements based on their shared evolutionary history (Webb 2000).

We applied the Beals Index of Sociological Favourability (Beals 1984), as modified by Münzbergová and Herben (2004) to exclude target species. This index estimates the probability of finding a species at a site regardless of whether the species actually occurs there. We organised the data in a composition matrix, with sites in columns, species in rows, and presences or absences in cells. Since no appropriate external database was available, we calculated Beals Index using only the data we sampled, with a total of 231 species and 1,083 individuals of Mandibulata. We used the function “beals” (De Cáceres and Legendre 2008), part of the R package Vegan (R Development Core Team 2013) to calculate the Beals Index.

To consider whether an unoccupied cave was habitable by a given species, we defined a threshold value for its suitability using the probability distribution of Beals values. To do so, we compared where in the cumulative frequency distribution of Beals values the unoccupied habitats ranked compared to occupied habitats. An unoccupied habitat with a Beals Index value below any occupied habitat would have 0% probability of being occupied, and so on. These percentiles of Beals Index can be directly interpreted as habitat suitability (Münzbergová and Herben 2004). The dark diversity corresponded to absent species of isopods with more than 50% of probability of occurring in the focal cave. Finally, we estimated the Community Completeness as the natural logarithm of observed richness divided by dark diversity ($\log(\text{observed richness} / \text{dark diversity})$), following the logistic expression of Pärtel et al. (2013).

Table 1. Estimates of dark diversity and community completeness of isopods inside caves from São Domingos. **OR** = observed richness; **DD**= dark diversity; **CC**= community completeness.

Cave	OR	DD	CC
Lapa do São Bernardo	1	4	-1386
Lapa do Angélica	4	2	0.693
Lapa do Bezerra	1	5	-1609
Lapa da Terra Ronca I	2	2	0
Lapa da Terra Ronca II	3	3	0

Results

By comparing the Beals Index distribution at both occupied and unoccupied habitats, we set the threshold value at 0.559, which was the median of the occupied habitats. Above this Beals Index value, an unoccupied habitat had 50% of probability of being occupied, representing suitable habitats for the presence of isopods already present in the species pool.

In the five caves we sampled, we found six species of isopods. On average, only two species inhabited each cave (mean $\pm$ s.d. = 2.2 ± 1.30), with the richest cave, Lapa do Angélica, having four species. Most species were in the dark diversity (3.2 ± 1.3), even when a given species was distributed in other nearby caves. Only Lapa do Angélica had positive value of completeness, with more species present than absent; the others had negative values, with more species in the dark diversity (Table 1).

Discussion

Cerrado is well preserved and is continually present outside all caves of São Domingos, allowing dispersal of the fauna via surface environments. Subterranean dispersal may also occur via the innumerable interconnected cracks and crevices typical of the karst, because the limestone outcrops in São Domingos are continuous (Bichuette and Trajano 2003). Despite this, isopods that were widespread on surface and caves nearby did not occur in all the caves where they potentially could.

Even if each cave is unique in its shape and resource inputs, almost all caves had dark diversity equal or higher than observed richness, with low completeness. From a conservation perspective, the caves with higher completeness may be a source of migratory individuals to other areas in the vicinity, which may suffer impacts and local extinctions (Lewis et al. 2017). Therefore, Lapa do Angélica, the only cave with positive value of completeness, has the higher regional relevance, acting as a potential repository of diversity to nearby caves.

Lapa do Angélica is an extensive cave with great input of organic matter during the rainy season. In its 14 km of horizontal projection, the cave is crossed by a river with almost 7 km of subterranean routes with stretches of rapids and several rainfalls resulting

in high amount of organic matter being imported and transported from adjacent communities (Auler et al. 2001; Bichuette and Trajano 2003). These conditions provide high diversity of microhabitats and food sources for the fauna, probably enabling the coexistence of the four species of isopods found there and minimising the number of species in the dark diversity. Lapa do Bezerra, on the other hand, was the least diverse in isopods and had the lowest completeness, despite its more than 8 km of horizontal projection traversed by almost 4 km of a river with high transport capacity accumulating great amounts of organic matter inside the cave (Bichuette and Trajano 2003). Lapa do Bezerra and Lapa do Angélica are part of the same cave system (Angélica-Bezerra cave system), possibly connected to each other and both with similar altimetric gradients (around 120 meters) (Guyot et al. 1996). It is possible that their proximity influenced the expected occurrences based on shared fauna and resulted in the lowest completeness of Lapa do Bezerra, just as the isopods in the dark diversity may be part of the fauna not discovered yet (hidden diversity *sensu* Pärtel 2014). Both Lapa do Bezerra and Lapa do São Bernardo caves have river beds with narrow margins, with narrower deposits of sediments and organic matter when compared to Lapa do Angélica (Auler et al. 2001). During the rainy season, organic matter is washed away from these microhabitats and the fauna can hide in inaccessible passages of the cave. On the one hand, the community can be saturated at lower richness, because of the lower input of organic matter. On the other, some species that are present may not have been sampled yet, because of their behavior facing the conditions of the environment. Consequently, both the hidden diversity and the completeness can be influenced by the shape of the caves.

Setting conservation priorities is essential, because funding sources are finite and several societal interests are involved (Díaz et al. 2011). Coupled with already existing conservation tools, dark diversity and completeness can be a helpful tool to aid and direct conservation efforts. Through an accurate assessing of completeness, researchers and managers can evaluate and monitor restoration of degraded habitats, avoid the risk of species invasion, and direct efforts to preserve a valuable sample of regional species pool (Lewis et al. 2017). This can be especially important in areas with caves and its irreplaceable and often vulnerable specialised fauna. Understanding the local species pools and how the species are distributed in caves within a region can help to adapt conservation efforts to preserve a representative sample of the subterranean fauna, from which at least part of lost species can be recouped. Our study showed that the dark diversity approach using patterns of co-occurrence among phylogenetically related taxa is adaptable to subterranean communities and may be the kickoff for both applied and theoretical studies aiming the better comprehension of ecological processes underlying patterns of subterranean biodiversity.

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References

- Ab'Saber AN (1977) Os domínios morfoclimáticos da América do Sul. *Geomorfologia* 52: 1–21.
- Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G (2014) Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22(6): 711–728. <https://doi.org/10.1127/0941-2948/2013/0507>
- Auler AS, Rubioli E, Brandi R (2001) As grandes cavernas do Brasil. Grupo Bambuí de Pesquisas Espeleológicas, Belo Horizonte.
- Barr TC, Holsinger JR (1985) Speciation in cave faunas. *Annual Review of Ecology and Systematics* 16: 313–337. <https://doi.org/10.1146/annurev.es.16.110185.001525>
- Bichuette ME, Trajano E (2003) Epigean and subterranean ichthyofauna from the São Domingos karst area. Upper Tocantins River basin. Central Brazil. *Journal of Fish Biology* 63(5): 1100–1121. <https://doi.org/10.1046/j.1095-8649.2003.00227.x>
- Bichuette ME, Simões LB, Zepón T, von Schimonsky DM, Gallão JE (2019) Richness and taxonomic distinctness of cave invertebrates from the northeastern state of Goiás, central Brazil: a vulnerable and singular area. *Subterranean Biology* 29: 1–33. <https://doi.org/10.3897/subtbiol.29.30418>
- Beals EW (1984) Bray-Curtis ordination: an effective strategy for analysis of multivariate ecological data. *Advanced Ecological Research* (14): 1–55. [https://doi.org/10.1016/S0065-2504\(08\)60168-3](https://doi.org/10.1016/S0065-2504(08)60168-3)
- Campos-Filho IS, Araujo PB, Bichuette ME, Trajano E, Taiti S (2014) Terrestrial isopods (Crustacea: Isopoda: Oniscidea) from Brazilian caves. *Zoological Journal of the Linnean Society – London* 172: 360–425. <https://doi.org/10.1111/zoj.12172>
- Cardoso P (2012) Diversity and community assembly patterns of epigean vs. troglobiont spiders in the Iberian Peninsula. *International Journal of Speleology* 41(1): 83–94. <https://doi.org/10.5038/1827-806X.41.1.9>

- Culver DC (1981) Some implications of competition for cave stream communities. *International Journal of Speleology* 11: 49–62. <https://doi.org/10.5038/1827-806X.11.1.6>
- Culver DC (2012) Species interactions. In: White WB, Culver DC (Eds) *Encyclopedia of Caves*. Elsevier Academic Press, Amsterdam, 743–748. <https://doi.org/10.1016/B978-0-12-383832-2.00109-2>
- Culver DC, Pipan T (2009) Superficial subterranean habitats – gateway to the subterranean realm? *Cave and Karst Science* 35(1): 5–12.
- Christman MC, Culver DC (2001) The relationship between cave biodiversity and available habitat. *Journal of Biogeography* 28(3): 367–380. <https://doi.org/10.1046/j.1365-2699.2001.00549.x>
- CPRM (2015) Serviço Geológico do Brasil (Geological Survey of Brazil). <http://geobank.sa.cprm.gov.br/> [accessed july 2015]
- De Cáceres M, Legendre P (2008) Beals smoothing revisited. *Oecologia* 156: 657–669. <https://doi.org/10.1007/s00442-008-1017-y>
- Dessen EMB, Eston VR, Silva MS, Temperini-Beck MT, Trajano E (1980) Levantamento preliminar da fauna de cavernas de algumas regiões do Brasil. *Ciência e Cultura* 32: 714–725.
- Díaz S, Quétier F, Cáceres DM, Trainor SF, Pérez-Harguindeguy N, Bret-Harte MS, Finegan B, Peña-Claros M, Poorter L (2011) Linking functional diversity and social actor strategies in a framework for interdisciplinary analysis of nature's benefit to society. *PNAS* 108(3): 896–902. <https://doi.org/10.1073/pnas.1017993108>
- Fernandes CS, Batalha MA, Bichuette ME (2016) Does the Cave Environment Reduce Functional Diversity? *PLoS ONE* 11(3): e0151958. <https://doi.org/10.1371/journal.pone.0151958>
- Gibert J, Deharveng L (2002) Subterranean Ecosystems: a Truncated Functional Biodiversity. *BioScience* 52(6): 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)
- Giribet G, Edgecombe GD (2012) Reevaluating the Arthropod Tree of Life. *Annual Review of Entomology* 57: 167–86. <https://doi.org/10.1146/annurev-ento-120710-100659>
- Guyot JL, Vendramini G, Resende L, Soares L, Pastorino VC (1996) *Expedições Espeleológicas Franco-Brasileiras*. Cecav, Brasília.
- He F, Gaston KJ, Connor EF, Srivastava DS (2005) The local–regional relationship: immigration, extinction, and scale. *Ecology* 86(2): 360–365. <https://doi.org/10.1890/04-1449>
- Juberthie C (2000) The diversity of the karstic and pseudokarstic hypogean habitats in the world. In: Wilkens H, Culver DC, Humphreys WF (Eds) *Ecosystems of the World 30. Subterranean Ecosystems*. Elsevier, Amsterdam, 17–39.
- Karmann I, Sánchez LE (1979) Distribuição das rochas carbonáticas e províncias espeleológicas do Brasil. *Espeleo-Tema* 13: 105–167.
- Lewis RJ, Szava-Kovats R, Pärtel M (2016) Estimating dark diversity and species pools: an empirical evaluation of two methods. *Methods in Ecology and Evolution* 7: 104–113. <https://doi.org/10.1111/2041-210X.12443>
- Lewis RJ, de Bello F, Bennett JA, Fibich P, Finerty GE, Gotzenberger L, Hiiesalu I, Kasari L, Lepš J, Májková M, Ondrej M, Riibak K, Ronk A, Rychtecká T, Vitová A, Pärtel M

- (2017) Applying the dark diversity concept to nature conservation. *Conservation Biology* 31(1): 40–47. <https://doi.org/10.1111/cobi.12723>
- Mokany K, Paini DR (2011) Dark diversity: adding the grey. *Trends in Ecology and Evolution* 26(6): 264. <https://doi.org/10.1016/j.tree.2011.03.009>
- Münzbergová Z, Herben T (2004) Identification of suitable unoccupied habitats in meta-population studies using co-occurrence of species. *Oikos* 105: 408–414. <https://doi.org/10.1111/j.0030-1299.2004.13017.x>
- Palacios-Vargas JG, Recamier BEM, Zepellini D (2013) Técnicas atuais para estudo de micro e mesoartrópodes de solo. EDUEPB, Campina Grande.
- Pärtel M (2014) Community ecology of absent species: hidden and dark diversity. *Journal of Vegetation Science* 25(5): 1154–1159. <https://doi.org/10.1111/jvs.12169>
- Pärtel M, Szava-Kovats R, Zobel M (2011) Dark diversity: shedding light on absent species. *Trends in Ecology and Evolution* 26(3): 124–127. <https://doi.org/10.1016/j.tree.2010.12.004>
- Pärtel M, Szava-Kovats R, Zobel M (2013) Community Completeness: linking local and dark diversity within the species pool concept. *Folia Geobotanica* 48: 307–317. <https://doi.org/10.1007/s12224-013-9169-x>
- Pinto-da-Rocha R (1995) Sinopse da fauna cavernícola do Brasil (1907–1994). *Papéis Avulsos de Zoologia* 39(6): 61–173.
- R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org> [accessed 2018]
- Resende LPA, Bichuette ME (2016) Sharing the space: coexistence among terrestrial predators in Neotropical caves. *Journal of Natural History* 50(33–34): 2107–2128. <https://doi.org/10.1080/00222933.2016.1193641>
- Riibak K, Reitalu T, Tamme R, Helm A, Gerhold P, Znamenskiy S, Bengtsson K, Rosén KE, Prentice HC, Pärtel M (2015) Dark diversity in dry calcareous grasslands is determined by dispersal ability and stress-tolerance. *Ecography* 37: 01–09. <https://doi.org/10.1111/ecog.01312>
- Ronk A, Szava-Kovats R, Pärtel M (2015) Applying the dark diversity concept to plants at the European scale. *Ecography* 38: 1–11. <https://doi.org/10.1111/ecog.01236>
- Simões LB, Ferreira TCS, Bichuette ME (2013) Aquatic biota of different karst habitats in epigeal and subterranean systems of Central Brazil – visibility versus relevance of taxa. *Subterranean Biology* 11: 55–74. <https://doi.org/10.3897/subtbiol.11.5981>
- Souza JD, Kosin M, Heineck CA, Lacerda Filho JV, Teixeira LR, Valente CR, Guimarães JT, Bento RV, Borges VP, Santos RA, Leite CA, Neves JP, Oliveira IWB, Carvalho LM, Pereira LHM, Paes VJC (2004) Folha SD. 23, Brasília. In: Schobbenhaus C, Gonçalves JH, Santos JOS, Abram MB, Leão Neto R, Matos GMM, Vidotti R, Ramos MAB, de Jesus JDA (Eds) Carta geológica do Brasil ao Milionésimo. Sistema de Informações Geográficas – SIG Programa Geologia do Brasil. CPRM, Brasília. CD-ROM. <http://www.cprm.gov.br/publique/Geologia/Geologia-Basica/Carta-Geologica-do-Brasil-ao-Milionesimo-298.html> [access July 2017]

- Taiti S (2003) Crustacea: Isopoda: Oniscidea (woodlice). In: Gunn J (Ed.) *Encyclopedia of Caves and Karst Science*. Taylor and Francis Group, London, 547–551.
- Tang L, Wang R, He KS, Shi C, Yang T, Huang Y, Zheng P, Shi F (2019) Throwing light on dark diversity of vascular plants in China: predicting the distribution of dark and threatened species under global climate change. *PeerJ* 7:e6731. <https://doi.org/10.7717/peerj.6731>
- Trajano E, Gnaspini-Netto P (1991) Composição da fauna cavernícola brasileira, com uma análise preliminar da distribuição dos táxons. *Revista Brasileira de Zoologia* 7(3): 383–407. <https://doi.org/10.1590/S0101-81751990000300017>
- Trajano E, Bichuette ME (2010) Diversity of Brazilian subterranean invertebrates, with a list of troglomorphic taxa. *Subterranean Biology* 7: 1–16.
- Webb CO (2000) Exploring the Phylogenetic Structure of Ecological Communities: An Example for Rain Forest Trees. *The American Naturalist* 156(2): 145–155. <https://doi.org/10.1086/303378>

Supplementary material I

Supplementary file

Authors: Camile Sorbo Fernandes, Marco A. Batalha, Maria Elina Bichuette

Data type: script

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The study of amphipods in rimstone pools of Akiyoshi-do Cave, Japan

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Abstract

Akiyoshi-do Cave is the largest show cave in Japan and has been recognised as a hotspot of cave animals due to their diversity in species. Human-induced alterations in the cave environment have been a significant concern catching the attention of tourists and managers. Previous studies indicated water quality alteration induced by tourism could affect the population densities of amphipods. However, no study went further than qualitative observation in terms of human impacts. This study targets two amphipods living in Akiyoshi-do Cave, *Pseudocrangonyx akatsukai* and *Gammarus nipponensis* and measures water characteristics in which they live. Results show that the population densities of the amphipods have decreased compared to the 1970s. Their living habitat has changed, probably induced by tourism.

Keywords

show cave, human impact, amphipod, water quality, protection

Introduction

In Japan, caves have a long history of being recognised as places where gods live, but when civilisation was developed, tourism use of caves began and now there are over 100 show caves in Japan (Zaizenji et al. 1993; Itoda and Goto 2018).

Akiyoshi-do Cave is famous as one of the largest show caves in the country, and in the last years the annual number of tourists reached about 500,000. The area has

been actively explored and researched since the early 19th century and the fields of research include geography, hydrology, biology, archaeology and humanities (Yamaguchi Caving Club 1992). Figure 1 shows the number of studies concerning Akiyoshi-do Cave made between the 1900s and the 2010s. Fifty-four of more than 120 studies targeted cave animals. They include: reports of new species, behavioural characteristics of bats, microbial community analysis, and others (Uemura 1941; Kuroda and Watanabe 1958; Miyoshi 1958; Okafuji 1958; Ueno S-I 1958a, b; Uchida and Kuramoto 1968; Hori et al. 2008). The Chimachida rimstone pools are one of the main tourist spots in the cave, rich in cave animals, and their water quality and biological diversity have been investigated by Uéno (1933) and Wakisaka et al. (1962).

A crustacean amphipod, *Pseudocrangonyx shikokunis* is a troglobite described in the first biological paper focusing on Akiyoshi-do Cave, which was collected from the Chimachida rimstone pools (Uéno 927). The amphipod was revealed to be a new species and was renamed *Pseudocrangonyx akatsukai* Tomikawa & Nakano, 2018. *P. akatsukai* contributed to the further understanding of the biogeographical history of *Pseudocrangonyx* in western Japan. The water quality of the Chimachida rimstone pools was measured in 1931 and 1932 and indicated a water temperature of 12.7–16.0 °C, pH of 7.7–8.0, and an alkalinity of 3.8–4.3 meq/ml (Uéno 1933). Calcium and magnesium concentrations of the pools were measured as 63.7 mg/l and 2.9 mg/l, respectively, in 1962 (Wakisaka et al. 1962). Another resident of the Chimachida rimstone pools is *Gammarus nipponensis* Uéno, 1940, which is a troglophile originating outside the cave. The population density fluctuations of *P. akatsukai* and *G. nipponensis*, in the Chimachida rimstone pools, were surveyed for 3 years and 9 months from April 1971 to January 1975. The results showed that their population densities exhibited seasonal changes: 1–13 ind/m² for *P. akatsukai* and 1–990 ind/m² for *G. nipponensis*. It was suggested that the amphipods were affected by the number of tourists, increasing in summer and decreasing in winter (Nakamura and Kuramoto 1978). However, it was

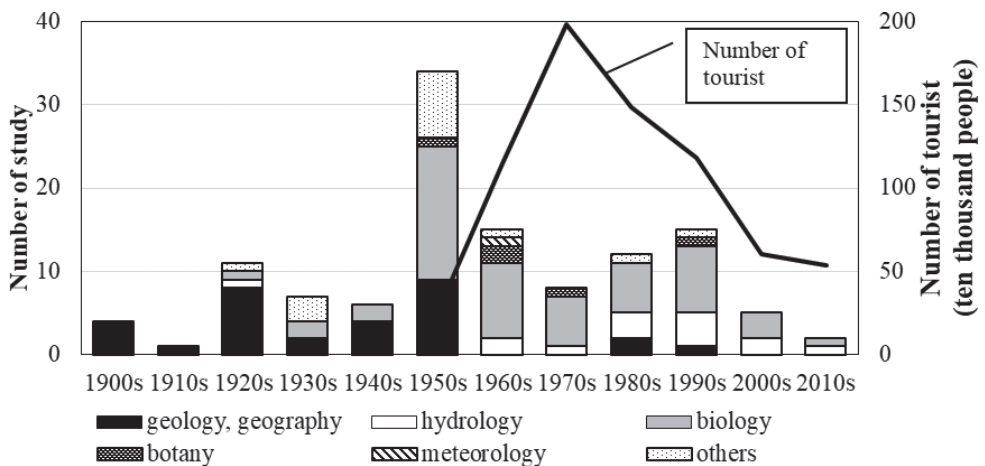


Figure 1. The number of existing studies and their breakdown of academic fields for each decade.

not mentioned in which pool, among the approximately 500 Chimachida rimstone pools, the water quality was measured by Uéno (1933) and Wakisaka et al. (1963). This means that the habitat of *P. akatsukai* and *G. nipponensis* lacks reliable water quality data. In addition, the most recent data on water quality is absent because water monitoring has not yet been installed. Even though the fluctuations of the population density of the two amphipod species depending on organic matter input was suggested by Nakamura and Kuramoto (1978), it remains unknown if and how the amphipods respond to water characteristics.

The objective of this study is to reveal the quality of the amphipods' habitat and to observe their population and distribution while taking tourism impacts into consideration.

Materials and methods

Study site

Akiyoshi-do Cave is a show cave located at 34°13.44'N, 131°18.14'E and roughly at 84 m above sea level (Figure 2). It is located in Akiyoshi-dai Plateau National Park, Yamaguchi Prefecture in Japan, and is known as one of the most visited show caves in the country due to its development, big stalactites and biodiversity of cave inhabitants. The cave is on the lists of Natural Monuments (since 1922), Special Natural Monuments (since 1955), Ramsar Convention Wetlands (since 2005) and Japan Geoparks (since 2015) and offers people learning experiences in a natural environment.

The Chimachida rimstone pools are one of the main tourist spots in Akiyoshi-do Cave located about 330 metres from the main entrance. They are composed of approximately 500 large and small pools arranged within a diameter of 20 m (Figure 3). The upper part is in contact with the tourist trail, has a gentle slope over the lower part and water eventually joins the cave river towards the main entrance, which is southwards. There is a wall with a film of flowing water on the opposite side across the tourist trail and the water is transported by a pipe under the tourist trail and discharged into the pools. In addition, pumped-up cave river water is continuously provided to the pools; this water supply started on 19 October 1971 with the aim of improving the water quality, in response to the fact that the pools' contamination had become noticeable. Spring water at the bottom of some pools is another source of water supply to the pools. Dripping water from the ceiling is another supply source but much smaller compared with the others.

Ten pools were selected from the Chimachida rimstone pools and named CH1 to CH10. Visual observations were made of the colour of the pool bottom, the particle size of sediments, the presence of human-related debris and other features, for qualitative evaluation of each pool on 16 November 2015. The water quality of pools and the population densities of the two amphipod species were measured/observed on 17 November 2015, 17 February, 17 May and 8 August 2016.

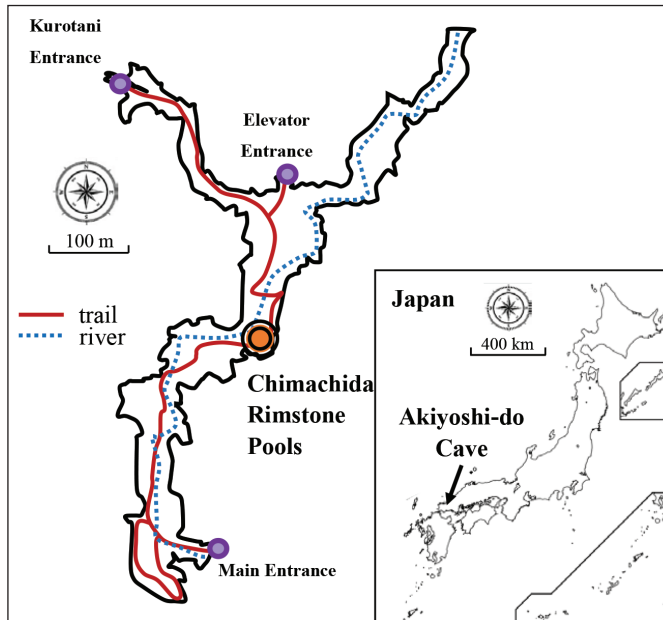


Figure 2. Location of the Akiyoshi-do Cave in Japan and of the surveyed pools inside the cave. The cave river flows to the south. The map of Akiyoshi-do Cave was drawn based on karusuto.com (<https://akiyoshido.karusuto.com/html/guide/>).

Nakamura and Kuramoto (1978) classified the pools into three types, based on the sediment of the pool bottom and the presence/absence of the amphipods. Type A pools were defined by the deposition of reddish-brown clay which suggested little inflow of organic matter. Type B pools were characterised by the deposition of black-brown clay, suggesting a large influx of organic matter. Type C pools were characterised by a larger amount of polluted black-brown clay deposition compared with type B pools. In this study, pools were classified in the same way, plus, the water source was considered. The small differences between the previous and our classification concerned the pools unselected in the previous study, the improved condition of the pool bottom, thus reclassifying some from type C to B, and worsened condition of the pool bottom, thus reclassifying some from A to C.

The 10 study pools can be classified into three major types, according to the type of water supplied to the pools, either water on the walls across the tourist trail or pumped-up cave river water. The first type, named type A pool, are pools that receive a larger amount of water from the wall, this being transported under the tourist trail and supplied to the pool, and this corresponds to CH1-5. Another type, named type B pool, are pools which receive a large amount of pumped cave river water, and this corresponds to CH6-9. Finally, there is a type C pool characterized by the absence of water exchange, and it corresponds to CH10. In addition, CH10 has a higher elevation of the pool bottom than adjacent pools and is isolated by a rimstone wall thicker than 5 cm.



Figure 3. The main entrance of Akiyoshi-do Cave (left) and the Chimachida Rimstone Pools (right).

The aquatic fauna in pools

P. akatsukai is a troglobite, with white body, reduced eyes, developed sense of touch, and is adapted to an oligotrophic environment (Uéno 1927; Tomikawa and Nakano 2018) (Figure 4). Many of these amphipods live in places where the flow of water is slow, such as pools and underground rivers. *G. nipponensis* is a troglophile that lives both inside and outside of caves, prefers organic matter more than *P. akatsukai* and has no preference for water flow speed (Figure 4).

Field observations and measurements

A 50 cm square quadrat was created in order to measure the population densities of *P. akatsukai* and *G. nipponensis*. The quadrat was placed in the pools and the number of amphipods was counted with the naked eye.

Water temperature, pH and electric conductivity were measured on site using a HORIBA handy meter (model SSS054, D-54). The quantity of dissolved oxygen was measured using a HORIBA handy meter (model SS054, D-55s).

Experiments in laboratory

Water samples were collected on site using two 250 ml water bottles per site, placing them in a cool box at below 10 °C and transported to the laboratory.

The alkalinity (C) was determined by titration method. Chemical oxygen demand (COD) (mg/l) was determined by the potassium permanganate titration method. Total nitrogen (TN) was measured by Merck's pack test. The total phosphorus content (TP) was also determined by Merck's pack test.

The number of all bacteria in the water sample, which is known as one of the indicators of water pollution, was determined. A test tube containing 9 ml of sample water was prepared and 1/10 of a formalin solution 1 ml was added and mixed well to fix bacteria in the sample. Bacteria are stained by adding DAPI (4', 6-diamidino-2-phe-



Figure 4. *Pseudocrangonyx akatsukai* (left) and *Gammarus nipponensis* (right).

nylindole) nucleic acid stain, water is filtered through an anopore inorganic membrane filter produced by Whatman plc and the total number of bacteria was counted using a fluorescence microscope.

The number of viable bacteria is also a biological index of water pollution, as well as the total number of bacteria. Undiluted sample water, 1/10 diluted sample water and 1/100 diluted sample water were placed in 1/10 Nutrient Broth, 1.5% agar medium and cultured for 3 days in an incubator set at 20 °C. Two plates were made for each dilution level. The experiment was performed on a clean bench environment. Bacterial colonies were marked on plates by dots with a pen until the cultivation period ended.

Statistical analysis

Canonical Correlation Analysis was performed with software R in order to clarify the correlation between each of the water quality parameter and the two amphipod species. Total nitrogen and total phosphorus as nutrients for the amphipods, and both total number of bacteria and total number of viable bacteria as indicators of water pollution, were used in the analysis.

Results

Physicochemical characteristics of pools

The results of macroscopic inspection of the pools are shown in Figure 5 and Table 1.

Table 1 shows contaminants and other characteristics of the three types of pools. In the type A pools, no contaminants such as green algae or hair, were observed or there

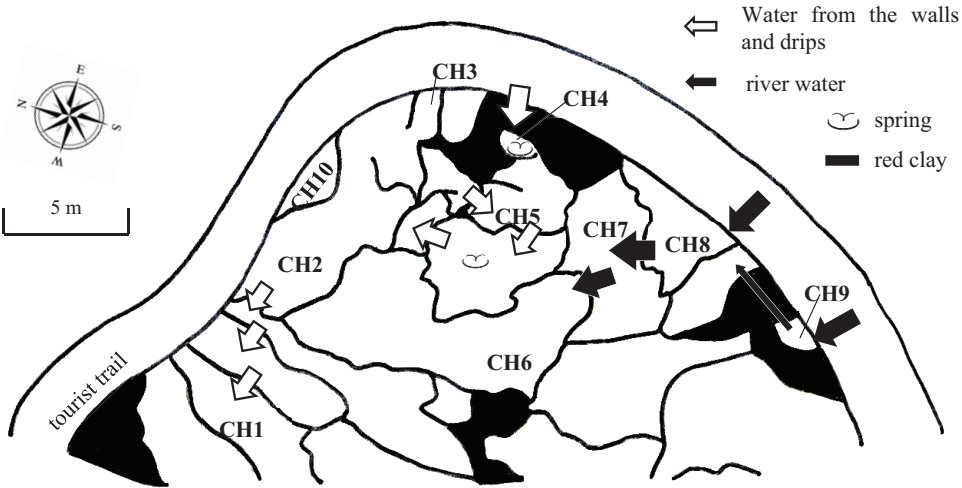


Figure 5. Pools with arrows pointing the flowing directions. Water from the walls and drips was supplied to CH4 and water flowed to CH2 and CH1 via CH5. Pumped cave river water was supplied to CH8 and CH9 and water flowed to CH6 and CH7.

Table 1. Results of macroscopic observation of the analysed pool in Akiyoshi-do Cave.

Name	Type	Water resource	Bottom color	Grain size	Contamination	Other features
CH1	A	transported upwelling water	ochre	sand, clay	–	–
CH2	A	transported upwelling water	ochre	gravel, sand, clay	algae, hair	–
CH3	A	transported upwelling water	blackish	sand, clay	algae, hair, plastic	–
CH4	A	transported upwelling water	reddish	clay, sand	–	considerable sand surrounding the spring
CH5	A	river water, spring water, transported upwelling water	entirely reddish, especially the surrounding of the spring	clay	–	–
CH6	B	river water, spring water	reddish ochre	clay	–	cave coral develops
CH7	B	river water	black	sand, clay, gravel	algae, hair, woodchip	a fresh water crab was observed
CH8	B	river water	deep black	clay	considerable algae, plastic	–
CH9	B	river water	deep black	clay, sand	big plastic chips, algae, cans scrap, lint	–
CH10	C	transported upwelling water	blackish ochre	grave, sand, clay	algae, hair, woodchip	isolated from surrounding pools

were in very small amount compared with the type B pools. By contrast, in the type B pools, contaminants, such as hair and plastic fragments, were particularly noticeable and abundant green algae had developed. On the other hand, in the type C pool, green algae development and contaminants, such as hair and wood chips, were more prominent than in type A and B pools.

Laboratory measurements

Table 2 shows the mean values of the water parameters across the Chimachida rimstone pools. The water temperature was slightly lower than 17 °C, the mean temperature of

the atmosphere in Akiyoshi-do Cave. The values of total nitrogen and total phosphorus of type A, B, C pools in each survey are also represented in Table 2. The mean values of total phosphorus were in increasing order in type A, type B and type C pool, of 0.05 mg/l, 0.11 mg/l and 0.17 mg/l, respectively. The mean values of chemical oxygen demand were 0.2 mg/l for both type A and type B pools, and 0.6 mg/l for the type C pool (Table 2). The type C pool showed the total number of bacteria of 1.3×10^6 Cells/ml and the total number of viable bacteria of 1.3×10^4 CFU/ml, both exceeding the mean values of the type A and B pools. The mean values of the total number of bacteria were 5.7×10^5 Cells/ml and 9.8×10^5 Cells/ml for type A and B pools, respectively. The mean values of the total number of viable bacteria were 3.7×10^3 Cells/ml and 1.1×10^4 Cells/ml for type A and B pools, respectively.

Population densities and distribution of amphipods

Table 3 shows the population densities of *P. akatsukai* and *G. nipponensis* of all surveys in this study. The population densities of both species varied from 0 to 10 ind/m². The population densities of *P. akatsukai* were 0–8 ind/m², 0–6 ind/m², and 0–1 ind/m² for type A, B, and C pools, respectively. The population densities of *G. nipponensis* were 0–10 ind/m², 0–4 ind/m², and 0–20 ind/m² in type A, B, and C pools, respectively. Table 4 and Figure 6–8 show the comparison between population densities of 1970s and present. The maximum population densities of *P. akatsukai* in type A pools in the 1970s was 12 ind/m², and that of the present was 12 ind/m². The maximum population densities of *G. nipponensis* in type A pools in the 1970s and the present were both 8 ind/m². The maximum population densities of *G. nipponensis* decreased from the 1970s to the present in type B and C pools: from 260 to 20 ind/m², from 935 to 10 ind/m², respectively.

Seasonal changes in population densities of amphipods

Number of both *P. akatsukai* and *G. nipponensis* increased in autumn, but decreased in winter (Table 3). In type B pools *P. akatsukai* increased in spring and summer, and *G. nipponensis* increased in autumn and winter (Table 3). In the type C pool, *P. akatsukai* was not observed, whereas *G. nipponensis* was observed in summer and autumn (Table 3).

Canonical Correlation Analysis

The analysis results are shown in Figure 9. *P. akatsukai* had a relatively strong negative correlation with total nitrogen. *G. nipponensis* had a positive correlation with the total phosphorus, the total number of bacteria and the total number of viable bacteria.

Table 2. Mean water characteristics of the 4 surveys (17th November 2015, 17th February 2016, 17th May 2016, and 8th August 2016) in the different pools of Akiyoshi-do Cave. PA: *Pseudocrangonyx akatsukai*, GN: *Gammarus nipponensis*, WT: water temperature, EC: electric conductivity, DO: dissolved oxygen, ALK: alkalinity, TN: total nitrogen, TP: total phosphorus, COD: chemical oxygen demand, TB: total number of bacteria, TVB: total number of viable bacteria.

Pool type	A					B					C		Mean total
	CH1	CH2	CH3	CH4	CH5	mean	CH6	CH7	CH8	CH9	mean	CH10	
WT (°C)	14.9	14.8	14.9	14.9	14.9	14.9	14.8	14.9	15	14.8	14.9	14.9	14.9
pH	7.6	7.6	7.6	7.7	7.8	7.7	7.8	7.8	7.9	7.9	7.9	7.8	7.8
EC (mS/m)	27.8	28.3	31	26.5	28.5	28.4	28.2	26.5	26.9	27.6	27.6	26.6	27.8
DO (mg/L)	8.7	7.7	7.3	8.3	8.1	8.0	8.8	7.3	8.2	8	8.1	7.4	8.0
ALK (meq/L)	3.0	2.9	3.1	2.6	2.8	2.9	2.8	2.7	2.6	2.7	2.7	2.8	2.8
TN (mg/L)	1.1	1.4	1.4	1.4	1.3	1.3	1.4	1.2	1.1	1.4	1.3	1.0	1.3
TP (mg/L)	0.10	0.02	0.08	0.04	0.03	0.05	0.13	0.19	0.05	0.08	0.11	0.17	0.1
COD (mg/L)	0.2	0.1	0.1	0.4	0.3	0.2	0.3	0.3	0.1	0.2	0.2	0.6	0.3
TB (Cells/mL)	2.3×10 ⁵	6.3×10 ⁵	6.4×10 ⁵	4.3×10 ⁵	9.1×10 ⁵	5.7×10 ⁵	5.8×10 ⁵	9.4×10 ⁵	6.9×10 ⁵	1.7×10 ⁶	9.8×10 ⁵	1.3×10 ⁶	8.1×10 ⁵
TVB (Cells/mL)	3.4×10 ³	3.3×10 ³	1.5×10 ³	2.7×10 ³	7.4×10 ³	3.7×10 ³	1.0×10 ⁴	9.1×10 ³	8.2×10 ³	1.7×10 ⁴	1.1×10 ⁴	1.3×10 ⁴	7.6×10 ³

Table 3. Population densities of *Pseudocrangonyx akatsukai* and *Gammarus nipponensis* for both different pool types and periods in Akiyoshi-do Cave.

		Nov-15		Feb-16		May-16		Aug-16	
		<i>P. akatsukai</i>	<i>G. nipponensis</i>	<i>P. akatsukai</i>	<i>G. nipponensis</i>	<i>P. akatsukai</i>	<i>G. nipponensis</i>	<i>P. akatsukai</i>	<i>G. nipponensis</i>
Type A pools	CH1	6	0	1	0	0	1	2	0
	CH2	8	8	2	2	2	1	4	0
	CH3	6	2	2	0	0	0	2	0
	CH4	0	0	4	0	10	0	6	0
	CH5	6	4	0	0	2	0	2	0
Type B pools	CH6	6	2	1	0	1	0	0	0
	CH7	0	4	0	1	1	0	2	0
	CH8	0	0	0	1	1	1	0	4
	CH9	0	20	1	1	0	4	0	8
Type C pools	CH10	0	10	0	0	0	0	0	6

Table 4. Mean population density of amphipods measured in 1970’s and during this study in Akiyoshi-do Cave. Numbers in the parentheses indicate the numbers of data taken.

Pool type	Amphipods	1971–1975 (45)			2015–2016 (4)		
		min	max	mean	min	max	mean
A	<i>Pseudocrangonyx akatsukai</i> (ind/m ²)	1	12	5.1	0	10	3.3
	<i>Gammarus nipponensis</i> (ind/m ²)	3	8	41.9	0	8	0.9
B	<i>Pseudocrangonyx akatsukai</i> (ind/m ²)	N.D.	N.D.	N.D.	0	6	0.8
	<i>Gammarus nipponensis</i> (ind/m ²)	18	269	112.6	0	20	2.9
C	<i>Pseudocrangonyx akatsukai</i> (ind/m ²)	N.D.	N.D.	N.D.	0	0	0.0
	<i>Gammarus nipponensis</i> (ind/m ²)	8	935	304.9	0	10	4.0

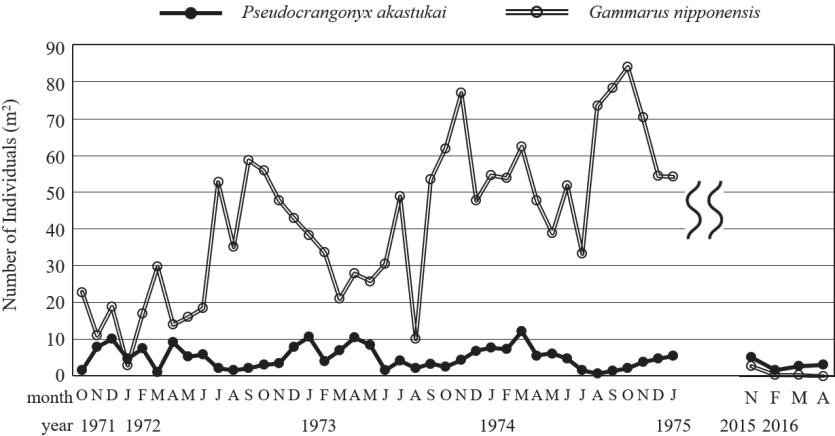


Figure 6. Fluctuation of the population densities of *Pseudocrangonyx akatsukai* and *Gammarus nipponensis* in the type A pools (1971–1975, 2015–2016).

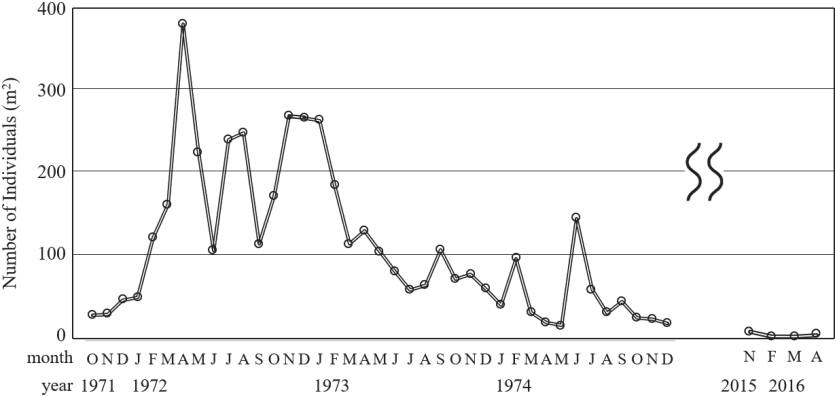


Figure 7. Fluctuation of the population density of *Gammarus nipponensis* in the type B pools (1971–1975, 2015–2016).

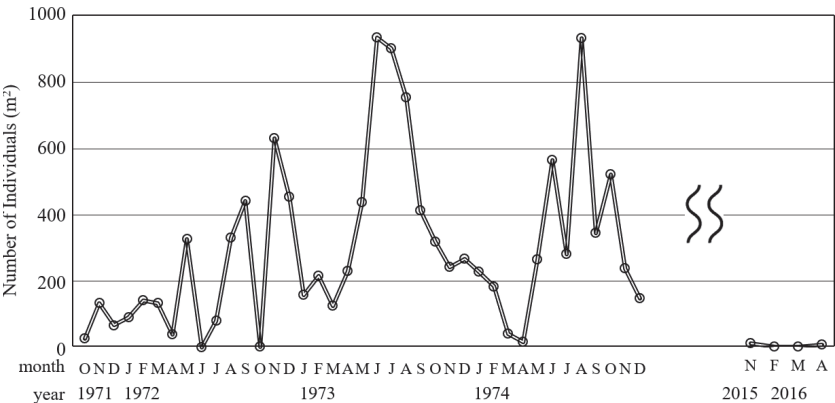


Figure 8. Fluctuation of the population density of *Gammarus nipponensis* in the type C pools (1971–1975, 2015–2016).

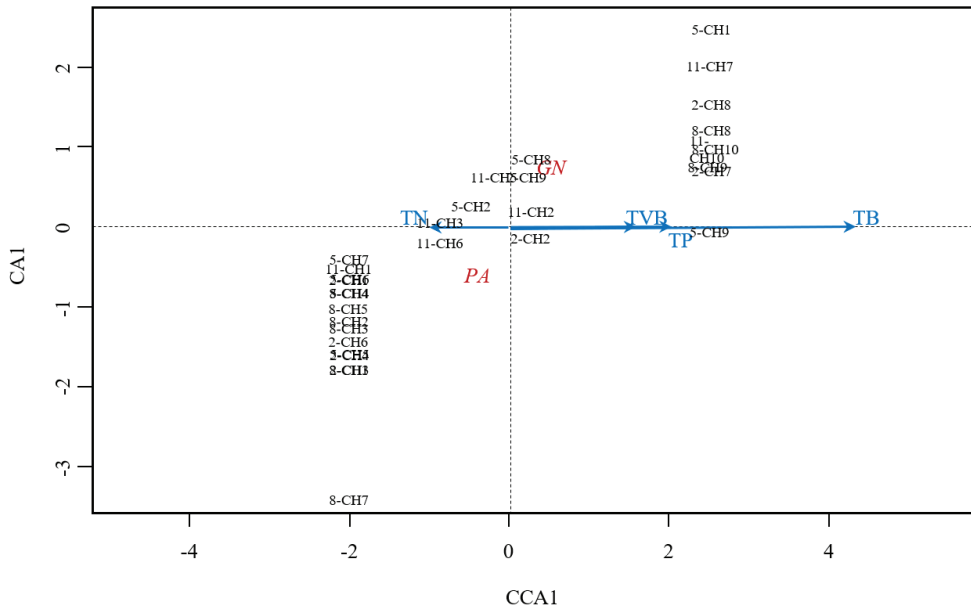


Figure 9. Result of CCA analysis on the amphipod abundances and water characteristics. TN: total nitrogen, TP: total phosphorus, TB: total number of bacteria, TVB: total number of viable bacteria. Other symbols mean pools surveyed in each season by “month-pool number”: for example, 5-CH1 corresponds to CH1 pool surveyed in May.

Discussion

Correlation between amphipods and water quality

The obtained correlations corroborate with the findings reported by Nakamura and Kuramoto (1978) that *P. akatsukai*, a troglobite, does not like the nutrient-rich environment (total nitrogen) and, hence, it was suggested that the increase in organic matter contamination caused by tourism could reduce their habitat availability. On the other hand, the population density of *G. nipponensis*, a troglophile, increased as the number of microorganisms and the amount of nutrient (total phosphorous) increased. However, it was observed that the population density of *G. nipponensis* decreases as water pollution progresses (Nakamura and Kuramoto 1978). It, thus, might indicate a threshold for the concentration of organic matter in the water in which *G. nipponensis* can live.

Seasonal changes in population densities of amphipods

The study of Nakamura and Kuramoto (1978) showed that the population density of *P. akatsukai* increased in winter and decreased in summer and that of *G. nipponensis* increased in summer and decreased in winter, in type A pools. The present study showed different results in type A and B pools.

Habitat ranges of amphipods – Comparison with the 1970s results

It was stated that *P. akatsukai* did not inhabit pools CH7-9 in the 1970s. In the four surveys conducted in this study, *P. akatsukai* was confirmed in those three pools. Pumped river water supply started in 1971, possibly contributing to the habitat expansion of *P. akatsukai*. On the other hand, in CH10, the type C pool, formerly classified as a type A pool, *P. akatsukai* was not found in the present survey. The CH10 pool is surrounded by a thick wall of 5 cm or more and water exchange was not confirmed. No other water supply exists for CH10. This pool is located at the top of Chimachida rimstone pools and is one of the pools with the highest frequency of contact with tourists. Therefore, relatively large amounts of tourist-derived organic matter are supplied and eutrophication progresses. This can be the reason why *P. akatsukai*, which is known not to inhabit eutrophic environments, retreated from this pool.

Population density of amphipods – Comparison with the 1970s results

The population density of both *P. akatsukai* and *G. nipponensis* in any type of pool decreased compared to the 1970s. In the absence of population density data prior to the 1970s makes it difficult to discuss the impact of tourism development in Akiyoshi-do Cave.

Akiyoshi-do Cave experienced the highest number of tourists in the 1970s and has shown a gradual decreasing trend since then to the present, but the population density of *P. akatsukai* and *G. nipponensis* became significantly smaller.

Impacts on the Chimachida rimstone pools

It is difficult to conclude that the source of organic matter contamination to the Chimachida rimstone pools is limited to cave tourism. The tourist activities on the surface where Akiyoshi-do Cave is developing, the so-called Akiyoshi-dai Plateau, also take part in environmental alteration of the water inside the cave. There is a museum, observation decks and shops located on the plateau within the range of the catchment area of Akiyoshi-do Cave. The sewage from these tourist facilities is transported to the basement through the underground sewer and treated. If sewage leaks out it can contaminate the Chimachida rimstone pools. In the 1990s, the sewer pipe was damaged, contaminating the upwelling water and creating obnoxious odours at another place not connected to the Chimachida rimstone pools in Akiyoshi-do Cave. Kuramoto and Nakamura (1995) and Kuramoto (1995) conducted a survey to find the influence the leaked sewage had on the fauna in Akiyoshi-do Cave. As a result, they found that many of the subterranean animals that were identified at the time of the survey by the Japan Cave Groundwater Research Association (1957) were observed, even in 1995. In addition, organic matter originating from animal manure can reach Akiyoshi-do Cave from poultry farms and an abandoned ranch in the catchment area of Akiyoshi-do

Cave. Kawano and Fujii (1985), Yoshimura and Inokura (1992) and Haikawa (2006) reported the water system of Akiyoshi-dai Plateau National Park. Their tracer experiments gave no details on the inflows that reach the pools in the Akiyoshi-do Cave.

Conclusion

This study focused on amphipods living in the Chimachida rimstone pools for the first time after 40 years and discovered that their habitat range and population density are different from the past. The habitat range of *P. akatsukai* expanded, while that of *G. nipponensis* narrowed. The population density of both *P. akatsukai* and *G. nipponensis* decreased. The seasonal variation in the population density of the amphipods was not confirmed in this study. As the observation period was of about 1 year which was shorter than the survey done in the 1970s, it needs more data to describe seasonal changes better. The habitat range and the population density of the amphipods should be monitored regularly. There is also need for hydrological investigations at the places suspected to be the origins of organic matter for the Chimachida rimstone pools, in the future.

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References

- Haikawa T (2006) The groundwater system of Akiyoshi-dai Plateau. Bulletin of the Akiyoshi-dai Museum of Natural History 41: 17–31.
- Hori M, Ishida M, Shimano S (2008) An attempt of analysis of soil microbial community of Limestone Caves in Akiyoshi-dai, Japan. Bulletin of the Akiyoshi-dai Museum of Natural History 44: 55–62.
- Itoda N, Goto S (2018) Questions of Caves: exploration, tourism, animals, looking into the unknown world. Seizando Shoten, 154 pp.
- Japan Groundwater Study Group (1957) Japan cave research briefings, 1. Akiyoshi region. Japan Groundwater Study Group.
- Kawano M, Fujii A (1985) The groundwater system of Kumozuhara area in the southwestern Akiyoshi-dai Plateau. Akiyoshi-dai Plateau Kumozuhara: Academic research report of the medium scale sightseeing recreation district: 20–33.
- Kuramoto T, Nakamura H (1995) Impacts of sewage inflow to subterranean animals in Akiyoshi-do Cave. Groundwater of Akiyoshi-do Cave: Emergency impact investigation report with sewage inflow, 24–28.

- Kuramoto T (1995) Process of the impact investigation on sewage inflow in Akiyoshi-do Cave. Groundwater of Akiyoshi-do Cave: Emergency impact investigation report with sewage inflow, 1–2.
- Kuroda T, Watanabe T (1958) Trogllobiontic aquatic snails from Japan. *Venus* 19(3, 4): 183–196.
- Miyoshi Y (1958) Contributions to the knowledge of Japanese myriopods: 25th Essay: On a new genus and a new kind of diplopods. *Zoological Magazine* 67(70): 297–298.
- Nakamura H, Kuramoto T (1978) Changes in population density of the subterranean amphipods at Chimachida Rimstone Pools in Akiyoshi-do Cave, with special reference to the influence of water pollution on amphipods. *Bulletin of the Akiyoshi-dai Museum of Natural History* 13: 55–62.
- Okafuji G (1958) Limestone caves and shellfish of Akiyoshi-dai Plateau. *Collecting and breeding* 20(11): 222–328.
- Tomikawa K, Nakano T (2018) Two new subterranean species of *Pseudocrangonyx* Akatsuka & Komai, 1922 (Amphipoda: Crangonyctoidea: Pseudocrangonyctidae), with an insight into groundwater faunal relationships in western Japan. *Journal of Crustacean Biology* 38(4): 460–474. <https://doi.org/10.1093/jcabi/ry031>
- Uchida T, Kuramoto T (1968) Modes of life in the colony of cave bats: with apicial remarks on dense mixed colony consisting of different species. *Mammalian Science* 8(2): 3–15.
- Uemura T (1941) Report on the new species of Theridiidae from Akiyoshi-do Cave. *Acta Arachinol* 6(2): 45–49.
- Ueno S-I (1958a) The cave beetles from Akiyoshidai karst and its vicinities. I. A new species of the Genus *Trechiana*. *Memoirs of the College of Science Kyoto Imperial University* B25(1): 39–48.
- Ueno S-I (1958b) The cave beetles from Akiyoshidai karst and its vicinities. II. *Uozumitrechus*. *Memoirs of the College of Science Kyoto Imperial University* B25(1): 49–61.
- Uéno M (1927) Notes on some subterranean and amphipods of Japan. *Memoirs of the College of Science Kyoto Imperial University* B3(3): 355–368.
- Uéno M (1933) The underground water and subterranean animals of Akiyoshi-dai Plateau. *The Japanese Journal of Limnology* 2(3): 91–95. <https://doi.org/10.3739/rikusui.2.91>
- Uéno M (1940) Some freshwater amphipods from Manchoukuo, Corea and Japan. *Bulletin of the Biogeographical Society of Japan* 10(4): 63–85.
- Wakisaka N, Kuramoto T, Morie T (1962) Hydrologic studies on the subterranean waters in Shûhōdō Cave. 1. On the hydrogen ion concentration and the hardness. *Bulletin of the Akiyoshi-dai Museum of Natural History* 2: 7–16.
- Yamaguchi Caving Club (1992) *Akiyoshi-dai and Limestone Cave Exploration*. Yamaguchi Caving Club, Yamaguchi Prefecture, 230 pp.
- Yoshimura K, Inokura Y (1992) Evolution of groundwater chemistry in the hydrological cycle of the Akiyoshi-dai Plateau limestone area. *Journal of Groundwater Hydrology* 34(3): 183–194. <https://doi.org/10.5917/jagh1987.34.183>
- Zaizenji I, Tsuchiya S, Yoshimura T, Ikeda Y (1993) *Mine Region Historical Stories*. Setouchi Bussan, Yamaguchi Prefecture.

Kut gen. nov., a new troglomorphic spider genus from Turkey (Araneae, Dysderidae)

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Abstract

A new genus of troglomorphic Dysderidae is described, based mainly on the morphology of copulatory organs. The new genus *Kut* gen. nov., with the type species *Harpactocrates troglophilus* Brignoli, 1978, also includes two recently discovered new species from the coastal Mediterranean Turkey: *Kut izmiricus* sp. nov. and *K. dimensis* sp. nov. All three species display troglomorphic traits, most distinct in *K. dimensis* sp. nov. Another genus-level trait is a characteristic simple type of bulb in males. Female copulatory organ shows similarity to the endemic Caucasian genus *Cryptoparachtes* Dunin, 1992 in paraspermatheca structure, whereas the male copulatory organ is unique among all known Dysderidae.

Keywords

Anatolia, cave-dwelling, endemic, eyeless spider, MSS, troglobite

Introduction

Dysderinae is one of the three subfamilies of the spider family Dysderidae, along with Harpacteinae and Rhodinae. Its members can be clearly distinguished by the unique morphology of sternum and labium joints, as well as by having claw tufts of setae on all tarsi and scopulae on posterior metatarsi (Deeleman-Reinhold and Deeleman 1988).

Subfamily Dysderinae is quite diverse and includes the following genera (number of species given in square brackets): *Cryptoparachtes* Dunin, 1992 [3 spp.]; *Dysdera* Latreille, 1804 [285 spp.]; *Dysderella* Dunin, 1992 [2 spp.]; *Dysderocrates* Deeleman-Reinhold & Deeleman, 1988 [8 spp.]; *Harpactocrates* Simon, 1914 [13 spp.], *Hygrocrates* Deeleman-Reinhold, 1988 [5 spp.], *Parachtes* Alicata, 1964 [13 spp.], *Stalitochara* Simon, 1913 [1 sp.] and *Tedia* Simon, 1882 [2 spp.] (WSC 2019).

Most of the genera of subfamily Dysderinae are endemic to the West Palearctic, with limited ranges. *Cryptoparachtes* and *Dysderella* are Caucasian endemics; *Hygrocrates* has a broader range from the Mediterranean Turkey and Caucasus; *Harpactocrates* and *Parachtes* are West European; *Dysderocrates* is East Mediterranean; and *Tedia* is known from Syria and Israel (WSC 2019). Only the genus *Dysdera* is widespread and mega species-rich, within a range covering the entire Mediterranean basin, all of Eurasia and the Macaronesian Archipelago; however, it includes many narrow endemics in the West Palearctic (Cardoso et al. 2008; Řezáč et al. 2008; WSC 2019).

Many species of Dysderidae are adapted to life in the darkness and display troglomorphic traits (Mammola et al. 2018). Even though most of troglobites belong to subfamilies Harpacteinae and Rhodinae, cave forms are known in Dysderinae as well (Arnedo and Ribera 1999). One of these species, *Harpactocrates troglophilus* Brignoli, 1978, has been described from the Zindan Cave in Mediterranean Turkey (Isparta Province).

Various authors doubted the generic placement of *H. troglophilus*, yet there have not been any attempts for a revision. In his original description, Brignoli (1978) wrote: “I am not entirely sure about the generic position of this strange species, similar in habitus with the *Harpactocrates*, but very different with the bulb (simpler)”. Absence of females was undoubtedly a reason for this hesitation. Later, in their seminal paper, Deeleman-Reinhold and Deeleman (1988) marked this species as “invisae inquirendae” (“not seen, doubtful”). Recently, Bidegaray-Batista et al. (2014) provided a molecular evidence that *H. troglophilus* belongs to a different lineage than monophyletic western Mediterranean *Harpactocrates*, which appeared to be “...more closely related to the eastern Mediterranean Dysderinae genera *Dysderocrates* and *Cryptoparachtes*...”.

This paper aims to contribute to the knowledge of the Dysderinae of Turkey by establishing a new genus based on troglomorphic *Harpactocrates troglophilus* and two other, new species recently discovered in Mediterranean Turkey.

Material and methods

Specimens were collected in different provinces of Turkey, either by hand collecting, pitfall, or MSS (Mesovoid Shallow Substratum) trapping and preserved in 70% ethanol. MSS trap design and application based on López and Oromí (2010) with several modifications to optimize sampling: 80 cm long PVC pipes of 11 cm diameter were used, with many small holes (5–7 mm) drilled along surface, except for 10 cm part at the bottom for preserving a plastic beaker, and a 40 cm part at the top to avoid sampling surface dwellers, enabling sampling between 40 to 70 cm of depth.

Digital images of the left palp and vulva were taken with a Leica DFC295 digital camera attached to a Leica S8AP0 stereomicroscope. Five to 30 photographs were taken in different focal planes and combined using COMBINE ZP image stacking software. Photographic images were edited using ADOBE PHOTOSHOP CS6 and CORELDRAW HOME & STUDENT SUITE X7 was used to create the plates. All measurements are in mm. Terminology for the body parts and copulatory organ structures follows Deeleman-Reinhold and Deeleman (1988) and Chatzaki and Arnedo (2006).

Abbreviations: **AL**, abdominal length; **CL**, carapace length; **CW_{max}**, maximum carapace width; **CW_{min}**, minimum carapace width; **AME**, anterior median eyes; **PLE**, posterior lateral eyes; **PME**, posterior median eyes; **AMEd**, diameter of anterior median eyes; **PLEd**, diameter of posterior lateral eyes; **PMEd**, diameter of posterior median eyes; **ChF**, length of cheliceral fang; **ChG**, length of cheliceral groove; **ChL**, total length of chelicera (lateral external view); **E** embolus; **T** tegulum; **Ms** midspermatheca; **Ps** paraspermatheca; **Ta**, tarsus; **Me**, metatarsus; **Ti**, tibia; **Pa**, patella; **Fe**, femur; **Tr**, trochanter; **C**, coxa; **D**, dorsal; **Pl**, prolateral; **Rl**, retrolateral; **V**, ventral.

Depositories: **AZMM**, Zoology Museum of Alaşehir Vocational School, Celal Bayar University, Manisa, Turkey; **ETZM**, Eskişehir Technical University Zoology Museum, Eskişehir, Turkey.

Taxonomy

Family Dysderidae C. L. Koch, 1837

Subfamily Dysderinae C. L. Koch, 1837

Diagnosis. Sternum edge at labium-sternum joint approximately 2.5–3 times longer than the edges at gnathocoxa-sternum joints. All tarsi with claw tufts.

A key to the genera of Dysderinae

- | | | |
|---|---|------------------------------------|
| 1 | Male | 2 |
| – | Female | 8 |
| 2 | Distal extensions of tegulum simple | 3 |
| – | Distal extensions of tegulum complex | 4 |
| 3 | Tegulum globular. Embolus long, sinuous. Conductor absent..... | |
| | Harpactocrates Simon, 1914 | |
| – | Tegulum pear-shaped. Embolus is a simple extension of tegulum | |
| | Kut gen. nov. (Figs 1D–F, 2D–F, 3D–F). | |
| 4 | Distal extensions divided with haematodocha | Dysdera Latreille, 1804 |
| – | Distal extensions without haematodocha division | 5 |
| 5 | Tegulum Z-shaped, with two apophyses | Cryptoparachtes Dunin, 1992 |
| – | Tegulum pear-shaped, straight or cylindrical..... | 6 |

6	Tegulum pear-shaped, with two apophyses in addition to hook-shaped embolus.....	<i>Hygrocrates</i> Deeleman-Reinhold, 1988
–	Tegulum cylindrical	7
7	Tip of tegulum with a broad, well-developed, chitinized apophysis.....	<i>Dysderocrates</i> Deeleman-Reinhold & Deeleman, 1988
–	Tip of tegulum with a chitinized but underdeveloped, small apophysis.....	<i>Parachtes</i> Alicata, 1964
8	Anterior spermatheca club-shaped	9
–	Anterior spermatheca not club-shaped	10
9	Dorsal arc well-developed. Posterior diverticulum distinct	<i>Kut</i> gen. nov. (Figs 1G–H, 2 G–H, 3 G–H)
–	Posterior diverticulum indistinct.....	<i>Cryptoparachtes</i> Dunin, 1992
10	Spermatheca with two distinct sections, distal and proximal	<i>Hygrocrates</i> Deeleman-Reinhold, 1988
–	Spermatheca without two distinct sections.....	11
11	Anterior diverticulum arc- or T-shaped	12
–	Anterior diverticulum simple	<i>Harpactocrates</i> Simon, 1914
12	Anterior diverticulum arc-shaped.....	13
–	Anterior diverticulum T-shaped	<i>Parachtes</i> Alicata, 1964
13	Anterior femora without numerous spines	<i>Dysdera</i> Latreille, 1804
–	Anterior femora with numerous spines	<i>Dysderocrates</i> Deeleman-Reinhold & Deeleman, 1988

***Kut* gen. nov.**

<http://zoobank.org/12362EEF-8192-4147-81F7-6170210ED5EA>

Type species. *Harpactocrates troglophilus* Brignoli, 1978.

Derivatio nominis. “*Kut*” is a Turkish word traceable to the old Turkic language with multiple meanings, such as “fortune (good or bad)”, “lifelong energy” or “vigour”, and more, depending on a dialect or a historical period. Gender: masculine.

Diagnosis. The new genus resembles genera *Dysderocrates*, *Harpactocrates* and *Hygrocrates* in the general morphology of the male palp; however, it can be identified by the following characters:

In male copulatory organ of *Kut* gen. nov., tegulum is pear-shaped and embolus is a simple extension of a tegulum. Embolus represents the only distal extension of tegulum, any other apophyses are absent, unlike in *Dysderocrates* and *Hygrocrates*.

In *Kut* gen. nov., female copulatory organ is unique among the majority of *Dysderinae* genera in having the anteriorly located, club-shaped spermathecae, bearing a resemblance to those in *Cryptoparachtes*. *Kut* gen. nov. differs from *Cryptoparachtes* by having a well-developed dorsal arc and a distinct posterior diverticulum, as well as by the male copulatory organ (see Dunin 1992: 42).

Anterior side of sternum (the side touching labium) is much more convex compared to other Dysderinae genera.

Description. Large-sized Dysderinae spiders. Somatic characters as in other Dysderidae. Detailed description see under *Kut troglophilus* (Brignoli, 1978) comb. nov.

***Kut troglophilus* (Brignoli, 1978) comb. nov.**

Figure 1

Harpactocrates troglophilus; Brignoli, 1978: 38, f. 4–5 (D♂).

H. troglophilus; Deeleman-Reinhold and Deeleman 1988: 250, f. 18 (♂, probably misplaced).

H. troglophilus; Le Peru 2011: 286, f. 473 (♂).

Material examined. 11 ♂♂, 9 ♀♀ (ETZM and AZMM), Turkey: Konya Province, Beyşehir District, Kurucaova Town (37°40.483'N, 31°22.700'E), pitfall trap, August–October 2012, leg. E.A. Yağmur & O. Tutar; 1 ♂, Konya Province (ETZM), Beyşehir District, Kurucaova Town (37°40.450'N, 31°22.633'E), MSS trap, 08 August–10 October 2018, leg. E.A. Yağmur.

Diagnosis. The simple structure of male palp in *Kut troglophilus* (Brignoli, 1978) is similar to that in some species of *Harpactocrates*, *Harpactea*, *Parachtes*, and *Stalagtia*. In *K. troglophilus* palp, the bulb gradually gets thinner and eventually gives rise to embolus, with a transition between tegulum and embolus rather indistinct, unlike in the other genera. Another unique feature of this genus is that the tip of the embolus is continuously bent posteriorly. In females, *K. troglophilus* vulva resembles that of the Caucasian genus *Cryptoparachtes*; however, in all three species of the latter (*C. adzhari-cus*, *C. charitonowi* and *C. fedotovi*), spermathecae are club-shaped. Paraspermatheca is distally spherical in all species of *Cryptoparachtes*, whereas in *K. troglophilus* paraspermathecae club shaped and more or less of equal length like the midspermatheca, unlike in three *Cryptoparachtes* species mentioned.

Measurements (♂/♀). **AL** 3.75–4.70/5.00–5.50; **CL** 3.50–4.48/3.50–4.00; **CW** 2.70–3.44/2.70–3.15; **AMEd** 0.14–0.18/0.12–0.16; **PLEd** 0.08–0.12/0.07–0.09; **PMEd** 0.08–0.11/0.07–0.12; **ChF** 0.95–1.25/0.96–1.05; **ChG** 0.55–0.80/0.60–0.70; **ChL** 1.60–2.10/1.60–1.75.

Description. **Carapace** hexagonal, reddish brown, with a smooth surface. Cephalic region darker than the thoracic region (Fig. 1A). Eyes reduced. Posterior eyes approximately of the same diameter and aligned. Posterior median eyes adjacent, as distant as their diameter to posterior lateral eyes. Chelicera robust, dark brown (Fig. 1B, C). Cheliceral groove with four teeth, two on pro- and two on retromargin. Among retromarginal teeth, proximal one smaller, distant one larger. Promarginal teeth approximately of the same size, proximal one in alignment with the proximal one of the retromargin.

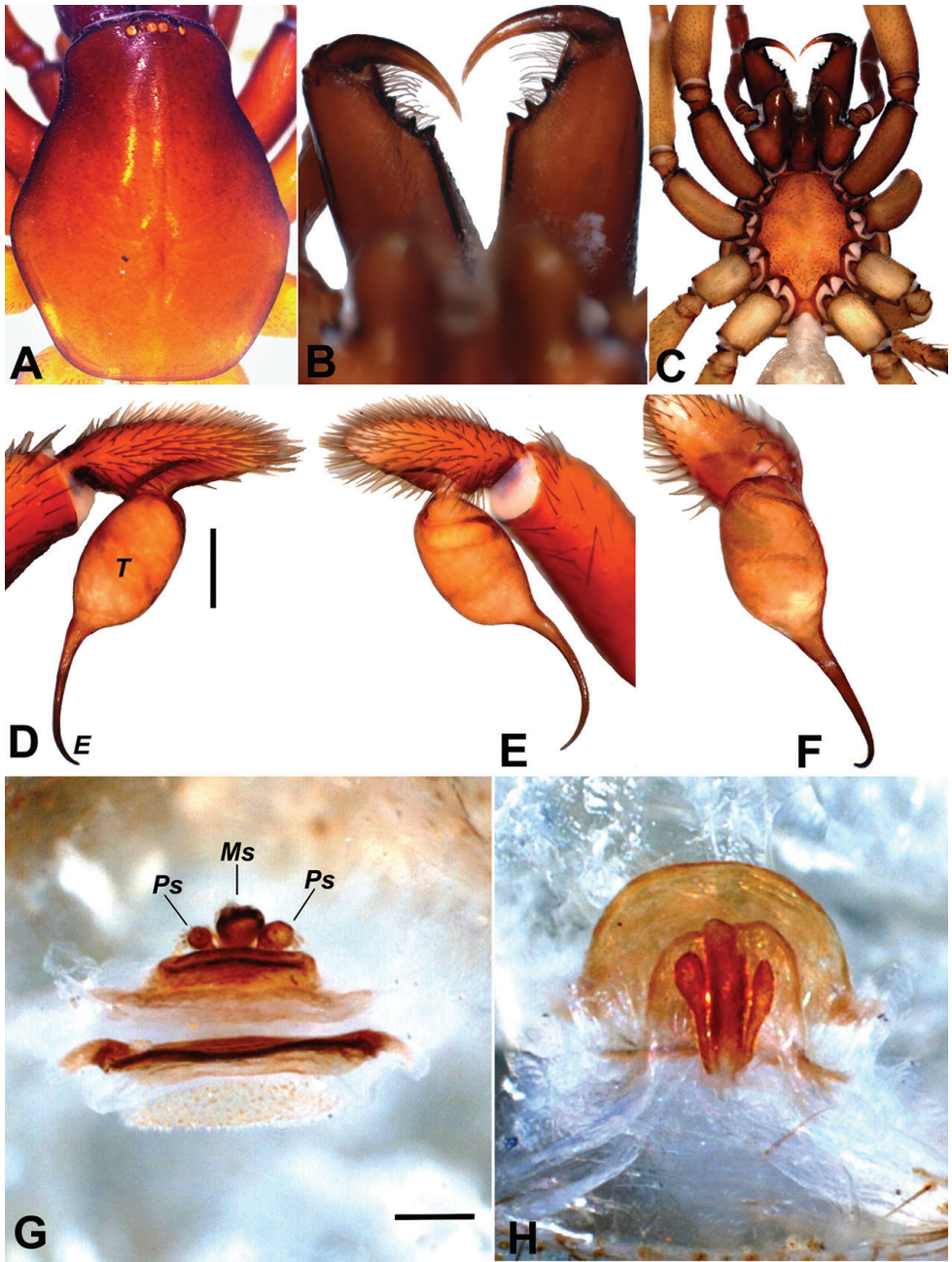


Figure 1. *Kut troglophilus* (Brignoli, 1978) comb. nov. **A** carapace **B** chelicerae **C** prosoma **D** male palp, retrolateral view **E** ditto, prolateral view **F** ditto, nearly retrolateral view **G** vulva, dorsal view **H** ditto, ventral view. Scale bars: 0.5 (**D**), 0.125 (**G**).

Labium and gnathocoxae brown. Anterior sides of gnathocoxae chitinized at borders and dark brown. Labium anteriorly with a V-shaped incision. Sternum yellowish brown. Anterior side of sternum (touching labium) slightly convex (Fig. 1C).

Table 1. Leg measurements of *Kut troglophilus* (Brignoli, 1978) comb. nov. (♂/♀).

Legs	I	II	III	IV
C	1.98/1.84	1.80/1.55	1.20/1.00	1.25/1.15
Tr	0.45/0.60	0.50/0.40	0.50/0.40	0.48/0.50
Fe	3.60/3.20	3.35/2.90	2.80/2.50	3.50/3.35
Pa	2.48/2.80	2.40/2.05	1.90/1.40	2.00/1.80
Ti	3.04/2.30	2.95/2.35	2.35/2.00	3.05/2.35
Me	3.04/1.85	3.00/2.25	2.75/2.55	3.50/3.35
Ta	0.95/0.65	0.80/0.70	0.80/0.80	0.90/0.80
Total	15.54/13.24	14.80/12.20	12.30/10.65	14.68/13.30

Table 2. Leg spination of *Kut troglophilus* (Brignoli, 1978) comb. nov.

Legs	I	II	III	IV
Fe	4–6 Pl	1, 1, 2–1, 2 Pl	2–3 D	3–5 Pl 3–5 D
Pa	0	0	1–2 Pl 1–3 Rl	1 Pl 1–2 Rl
Ti	0–1 Pl 0–1 V	0–1 Pl 1–2 V	3–6 Pl 3–7 Rl 1, 1, 2 V	3–6 Pl 3–6 Rl 1, 1, 2 V
Me	0	0	2–4 Pl 4–5 Rl 0–1, 1, 2 V	3–5 Pl 4–7 Rl 1, 1, 2 V

Legs yellowish, light brown. Leg coxa, trochanter, and femora darker in colour at joints. Femora and tibiae I and II prolaterally with spines; number of spines variable among individual spiders. Tibiae I and II with ventral spines. See Tables 1, 2 for details on leg measurements and spination. Abdomen long and slender, greyish cream in colour.

Palp (Fig. 1D–F). Palpal tarsus shorter than tibia, conical. Tarsus and tibia lighter in colour compared to other palp segments. Tegulum cylindrical; embolus originates from the anterior dorsal side of tegulum and is bent posteriorly as an arc. Tegulum and embolus distinctly different in colour.

Vulva (Fig. 1G, H). Midspermatheca club-shaped. Paraspermathecae pin-shaped and similar in size to midspermatheca. Spermathecae surrounded by a dome-shaped chitinized structure. Spermathecae posteriorly with two horizontally aligned bars of equal sizes; of these, one closer to spermathecae is less chitinized than the other.

Note. Brignoli (1978) described *Harpactocrates troglophilus* based on two adult males collected from the Zindan Cave (Isparta Province, Turkey). He also recorded (marked as “cf.”) additional juvenile specimens from three other localities (all from caves), at the close vicinity of the type locality. Our specimens of *Kut troglophilus* (Brignoli, 1978) comb. nov. were collected from one of these localities, Kurucaova Village (Konya Province), close to the İnönü Cave, by both pitfall and MSS traps. Sampling inside the cave yielded no Dysderidae except subadult *Dysderocrates*.

Kut izmiricus sp. nov.

<http://zoobank.org/AE2AA947-3925-4558-A384-685B2368000A>

Figure 2

Material examined. Holotype: 1 ♂ (ETZM), Turkey: İzmir Province, Kemalpaşa District, Vişneli Village, Nif Mountain (38°23.073'N, 27°21.614'E), MSS trap, 01 July–08 September 2015, leg. E.A. Yağmur, P. Mitov, S. Örgel & Ç. Altın. Paratypes: 1 ♀ (ETZM),

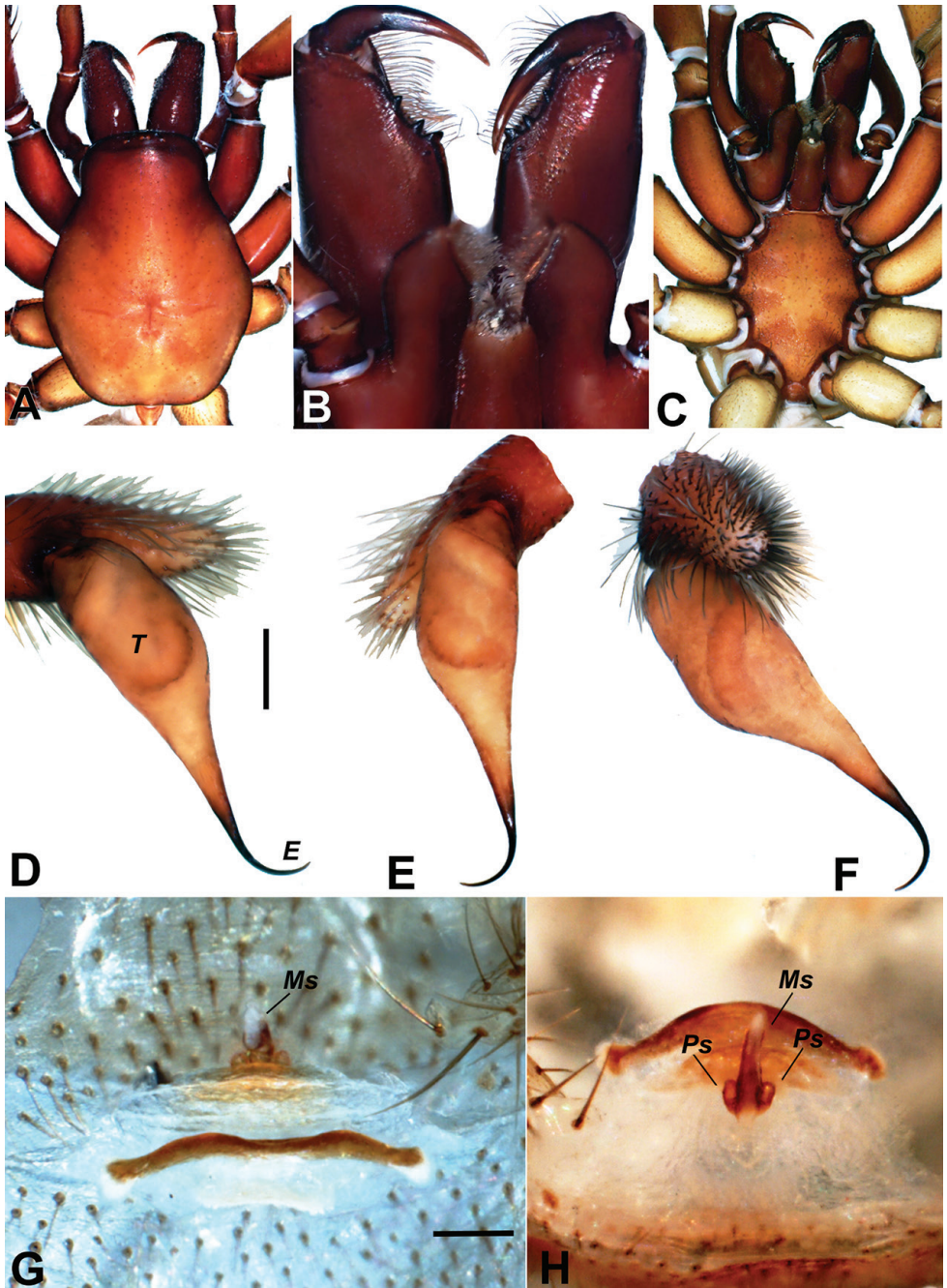


Figure 2. *Kut izmiricus* sp. nov. **A** carapace **B** chelicerae **C** prosoma **D** male palp, retrolateral view **E** ditto, prolateral view **F** ditto, nearly prolateral view **G** vulva, dorsal view **H** ditto, ventral view. Scale bars: 0.5 (**D**), 0.125 (**G**).

3 juveniles, 05 May–01 July 2015, leg. E.A. Yağmur, S. Örgel & S. Yaman, other data same as holotype; 3 subadults ♂♂, 07 September 2015–11 July 2016, leg. E.A. Yağmur, S. Örgel & S. Yaman, other data same as holotype; 1 ♂ (AZMM), 1 subadult ♀, 1 juvenile, 11 July–27 October 2016, leg. E.A. Yağmur, S. Örgel & S. Yaman, other data same as holotype; 1 ♂ (AZMM), 2 subadults ♀♀, 1 juvenile, 17 April–22 October 2017, leg. E.A. Yağmur, S. Örgel & S. Yaman, other data same as holotype; 3 subadults ♂♂ (AZMM), 09 June–20 October 2018, leg. E.A. Yağmur, S. Örgel & S. Yaman, other data same as holotype.

Diagnosis. Male palp of *Kut izmiricus* sp. nov. resembles those of *K. troglophilus* and *K. dimensis* sp. nov.; in *Kut izmiricus* sp. nov. the bulb is more slender and exceeds embolus in length, unlike in the other two species. Female of *Kut izmiricus* sp. nov. differs from others by having a longer midspematheca.

Derivatio nominis. The specific name is a toponym that refers to the type locality, İzmir Province.

Measurements (Holotype ♂ / Paratype ♀). AL 6.56/5.80; CL 6.00/5.00; CW 4.32/3.60; AMEd 0.14/0.11; PLEd 0.08/0.10; PMEd 0.05/0.08; ChF 1.45/1.15; ChG 1.00/0.90; ChL 3.10/2.25.

Description. *Carapace* reddish brown, with thick dark brown contours at the borders. Fovea distinct, longitudinal (Fig. 2A). Carapace surface with tiny dents; these dents with tiny, black setae inside. These setae are longer in the cephalic region, especially around eyes. Eyes tiny, reduced. Distance between anterior eyes approximately 2.5 times their diameter. Posterior eyes spherical, slightly concave. Chelicera, gnathocoxa, and labium dark brown. The new species resembles *K. troglophilus* by the other chelicer-al traits (Fig. 2B). Sternum brown, covered with setae, and with dark brown contours at the borders. Sternum with eight dark brown blotches, symmetrically reaching from sides toward center. Gnathocoxae with yellowish setae prolaterally at tips (Fig. 2B, C).

Table 3. Leg measurements of *Kut izmiricus* sp. n (Holotype ♂ / Paratype ♀).

Legs	I	II	III	IV
C	2.80/2.15	2.56/2.00	1.50/1.35	1.70/1.25
Tr	0.80/0.50	0.78/0.50	0.60/0.40	0.60/0.50
Fe	5.84/4.25	5.00/3.75	4.40/3.25	5.44/4.20
Pa	3.60/2.70	3.28/2.50	2.16/2.30	2.70/2.25
Ti	4.80/3.25	4.64/3.50	3.50/3.75	4.20/3.75
Me	4.40/3.00	4.32/3.25	4.30/3.50	5.70/4.25
Ta	1.04/0.90	1.04/0.75	0.80/0.85	1.12/1.00
Total	23.28/16.75	21.62/16.25	17.26/15.40	21.46/17.20

Table 4. Leg spination of *Kut izmiricus* sp. nov.

Legs	I	II	III	IV
Fe	11 Pl 5 RI	6 Pl 3 RI	1 D 4 Pl 3–5 RI	5 Pl 1 RI 5 D
Pa	0	0	1 Pl 2 RI	1 Pl 1 RI
Ti	1–2 Pl 0–1 RI 2 V	2–3 V	3 Pl 6 RI 1, 1, 2 V	6 Pl 6 RI 1, 1, 2 V
Me	0	0	1, 1 Pl 5 RI 1, 1, 2 V	4 Pl 5 RI 1, 2 V

Legs brown. Coxae I, II and III darker in colour compared to other leg segments. See Tables 3, 4 for details on leg measurements and spination. Abdomen slender, cylindrical, greyish cream in colour.

Palp (Figs 2D–F). Palpal tarsus shorter than tibia, conical. Tegulum exceeds embolus in length. First half of tegulum cylindrical, second half reversely conical. The anterior side of the tegulum smoothly straight. Embolus short and hook-shaped, bent posteriorly.

Vulva (Fig. 2G, H). Midspermatheca club shaped, very long, its tip transparent and slightly bent posteriorly. Paraspermathecae short, spherical. Posteriorly to spermathecae, two horizontally aligned bars; among these, one closer to spermathecae is shorter, distant one twice as long and more chitinized.

***Kut dimensis* sp. nov.**

<http://zoobank.org/F80CCEC0-AE94-4923-9D39-7DF2E3C72580>

Figs 3, 4

Material examined. Holotype: 1 ♂ (ETZM), Turkey: Antalya Province, Alanya District, Kestel Town, Dim Cave (36°32.405'N, 32°6.603'E), hand collecting from stalactites and stalagmites, 04 January 2013, leg. K.B. Kunt & M. Elverici. Paratypes: 3 ♂♂, 5 ♀♀, 4 juveniles (ETZM), 1 ♂, 1 ♀ (AZMM), same data as holotype.

Diagnosis. Male palp of *Kut dimensis* sp. nov. closely resembles that of *K. troglophilus* (Brignoli, 1978) comb. nov. *Kut dimensis* sp. nov. palp differs by having an embolus with tip sharply bent posteriorly, instead of gradually, and by having a more slender tegulum. Difference between *Kut dimensis* sp. nov. and *K. izmiricus* sp. nov. palps is more distinct, since in *K. izmiricus* sp. nov. tegulum/embolus ratio is higher



Figure 3. *Kut dimensis* sp. nov., female. *In situ* in the Dim Cave (photograph by M. Elverici).

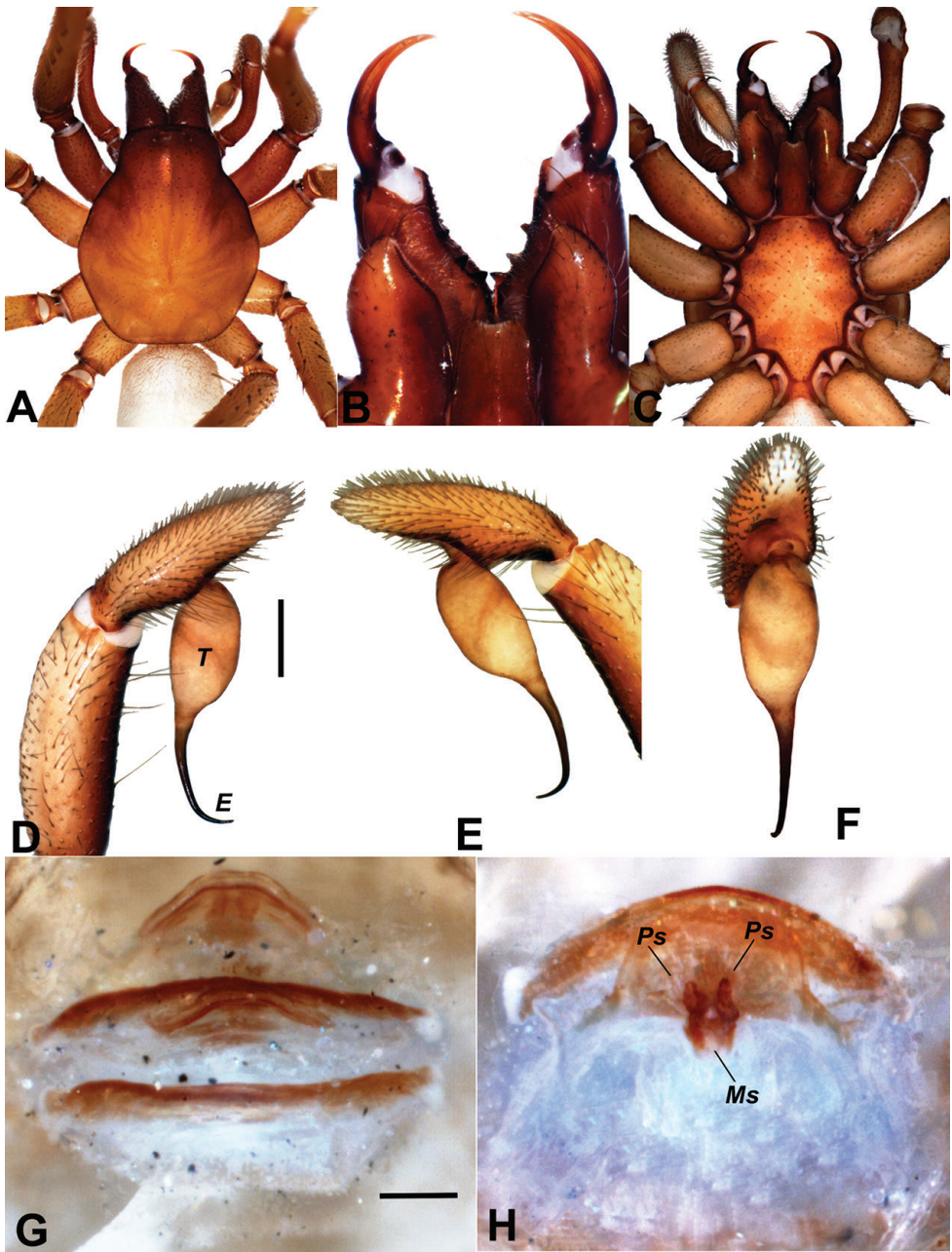


Figure 4. *Kut dimensis* sp. nov. **A** carapace **B** chelicerae **C** prosoma **D** male palp, retrolateral view **E** ditto, prolateral view **F** ditto, nearly anterior view **G** vulva, dorsal view **H** ditto, ventral view. Scale bars: 0.5 (**D**), 0.125 (**G**).

compared to other species of the genus. *K. dimensis* sp. nov. females differ from other species by having longer paraspermathecae compared to midspermathecae, which is the opposite in the other two species.

Derivatio nominis. The specific name is a toponym that refers to the type locality, Dim Cave located at Dim Valley in Alanya District.

Measurements (Holotype ♂ / Paratype ♀). AL 4.25/4.11; CL 4.40/4.25; CW 3.50/3.30; ChF 0.95/1.10; ChG 0.75/0.75; ChL 1.70/2.05.

Description. Somatic traits of *Kut dimensis* sp. nov. resemble the other two species in general. Eye reduction at an extreme level, eyes almost absent (Fig. 4A). Anterior and posteriolateral eyes barely visible only under stereomicroscope, in a state of indistinct spots. Legs more slender than in *K. troglophilus* (Brignoli, 1978) comb. nov. Anterior metatarsi with ventral spines. See Tables 5, 6 for details on leg measurements and spination.

Palp (Fig. 4G, H). Palpal tarsus shorter than tibia, conical. $\frac{3}{4}$ part of embolus continues straight after the separation from the tegulum, then bent with a sharp coil at the 4th quarter, shaped like an “L” towards posterior.

Vulva (Fig. 4G, H). Midspermatheca fingertip shaped, shorter than paraspermathecae. Paraspermathecae pin-shaped. Spermathecae surrounded by a dome-shaped chitinized structure. Spermathecae posteriorly with two lucent, strongly sclerotized, horizontally aligned bars.

Note. *Kut dimensis* sp. nov. is not the first troglomorphic species recorded from its type locality, the Dim Cave. López-Pancorbo et al. (2013) described a troglobiont spider *Kryptonesticus dimensis* (López-Pancorbo, Kunt & Ribera, 2013) (Nesticidae) from the same cave. A cave cricket *Troglophilus alanyaensis* Taylan et al., 2012 (Orthoptera, Rhaphidophoridae) is another endemic species known only from this cave.

The Dim Valley, the valley where the Dim Cave located, has been studied by our team extensively in the recent past. There have been new species of Dysderidae discovered and described like *Harpactea alanyana* Özkütük et al., 2015 and *Harpactea ballarini* Kunt et al., 2013. Furthermore, the locality has been extensively sampled by the first author, as it was covered in his MSc thesis, titled “Harpacteinae Fauna of Antalya

Table 5. Leg measurements of *Kut dimensis* sp. nov. (Holotype ♂ / Paratype ♀).

Legs	I	II	III	IV
C	2.00/1.90	1.75/1.75	1.14/1.10	1.50/1.50
Tr	0.55/0.35	0.35/0.40	0.30/0.45	0.50/0.50
Fe	4.00/4.00	4.30/3.75	3.75/3.50	5.00/4.85
Pa	2.75/2.50	2.80/2.50	1.75/1.75	2.40/2.30
Ti	4.00/3.30	3.60/3.05	3.00/2.00	4.50/4.25
Me	3.50/3.55	3.36/3.60	4.00/3.25	4.85/4.85
Ta	0.75/0.85	0.50/0.80	0.70/0.75	0.90/0.65
Total	17.55/16.45	16.66/15.85	14.64/12.80	19.65/18.90

Table 6. Leg spination of *Kut dimensis* sp. nov.

Legs	I	II	III	IV
Fe	6–9 Pl 3–4 RI	5–7 Pl 0–4 RI	0–3 D 3–8 Pl 4–5 RI	3–4 D 4–6 Pl 3–4 RI
Pa	0	0	1 Pl 3–4 RI 0–1 V	1 Pl 1 RI 0–1 V
Ti	1, 1 V	2–3 V	6 Pl 6 RI 1, 1, 2 V	5–7 Pl 5–6 RI 1, 1, 2 V
Me	1 V	1 V	3–5 Pl 5 RI 1, 1, 2 V	5–6 Pl 6 RI 0–1, 1, 2 V

Province". We provide these details to point out that *Kut dimensis* sp. nov. has not been found outside of the cave, in surface habitats, despite extensive sampling efforts, which is one of the reasons we consider this species a troglobiont.

Discussion

With the description of *Kut* gen. nov., the number of genera belonging to spider family Dysderidae is now 25. Even though there are other genera known exclusively from hypogean habitats such as caves (for example *Minotauria* Kulczyński, 1903 and *Folkia* Kratochvil, 1970 from Harpacteinae; *Stalita* Schiödte, 1847 from Rhodinae), *Kut* gen. nov. is the first genus of Dysderidae ever discovered, in which all known species display troglomorphic traits such as reduced eyes or hypogean foraging. *Kut troglophilus* (Brignoli, 1978) is currently the only species of the *Kut* gen. nov. with recorded surface activity; however, it was also recorded from hypogean habitats: in MSS with this study and the Zindan Cave (Brignoli 1978). The other two species were recorded either exclusively from caves (*Kut dimensis* sp. nov.) or from MSS (*Kut izmiricus* sp. nov.) (Fig. 5).

With this paper, we have resolved the discussion regarding the generic placement of *Kut troglophilus*. In our opinion, *Kut* gen. nov. has a unique and important position among Dysderidae due to its troglobitic and biogeographic affinities, coupled with the unique morphology of copulatory organs.

During our sampling efforts throughout the Mediterranean Turkey by using MSS traps, we have discovered further populations that might very likely belong to *Kut* gen. nov. as well. Moreover, we have collected subadult individuals from central parts of Anatolia with similar morphology, which may indicate a more or less continuous range of the genus throughout the Anatolia. We hope to reveal the specific identity of these



Figure 5. *Kut* gen. nov. distribution in Turkey.

populations, study their relationship to the known Aegean, Mediterranean, and Central Anatolian species, and the relationship between *Kut* gen. nov. and the Caucasian *Cryptoparachtes* in the near future. A high level of endemism of Dysderinae around Anatolia at both species and genus levels indicates that many more taxa are still waiting to be discovered.

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References

- Arnedo MA, Ribera C (1999) Radiation in the genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: The island of Tenerife. *Journal of Arachnology* 27: 604–662.
- Bidegaray-Batista L, Ferrández MÁ, Arnedo MA (2014) Winter is coming: Miocene and Quaternary climatic shifts shaped the diversification of Western-Mediterranean *Harpactocrates* (Araneae, Dysderidae) spiders. *Cladistics* 30(4): 428–446. <https://doi.org/10.1111/cla.12054>
- Brignoli PM (1978) Ragni di Turchia IV. Leptonetidae, Dysderidae ed Agelenidae nuovi o interessanti di grotte della Turchia meridionale (Araneae). *Quaderni di Speleologia, Circolo Speleologico Romano* 3: 37–54.
- Cardoso P, Borges PAV, Macías-Hernández N (2008) *Dysdera crocata* C.L. Koch, 1838. In: Silva L, Ojeda EL, Rodríguez-Luengo JL (Eds) *Invasive Terrestrial Flora and Fauna of Macaronesia: TOP 100 in Azores, Madeira and Canaries*. ARENA, Ponta Delgada, 415–417.
- Chatzaki M, Arnedo MA (2006). Taxonomic revision of the epigean representatives of the spider subfamily Harpactinae (Araneae: Dysderidae) on the island of Crete. *Zootaxa* 1169: 1–32. <https://doi.org/10.11646/zootaxa.1169.1.1>
- Deeleman-Reinhold CL, Deeleman PR (1988) Revision des Dysderinae (Araneae, Dysderidae), les espèces méditerranéennes occidentales exceptées. *Tijdschrift voor Entomologie* 131: 141–269.
- Dunin PM (1992) The spider family Dysderidae of the Caucasian fauna (Arachnida Aranei Haplogynae). *Arthropoda Selecta* 1(3): 35–76.

- Le Peru B (2011) The spiders of Europe, a synthesis of data (Vol. 1). Atypidae to Theridiidae. Mémoires de la Société Linnéenne de Lyon 2: 1–522.
- López H, Oromí P (2010) A pitfall trap for sampling the mesovoid shallow substratum (MSS) fauna. Speleobiology Notes 2: 7–11.
- López-Pancorbo A, Kunt KB, Blagoev G, Deltchev C, Ribera C (2013) *Nesticus dimensis* new species, a new troglobitic spider from Turkey (Araneae, Nesticidae), with comments on its phylogenetic relationships. Zootaxa 3721(2): 183–192. <https://doi.org/10.11646/zootaxa.3721.2.5>
- Mammola S, Cardoso P, Ribera C, Pavlek M, Isaia M (2018) A synthesis on cave-dwelling spiders in Europe. Zoological Systematics and Evolutionary Research 56: 301–316. <https://doi.org/10.1111/jzs.12201>
- Řezáč M, Král J, Pekár S (2008) The spider genus *Dysdera* (Araneae, Dysderidae) in Central Europe: Revision and natural history. Journal of Arachnology 35: 432–462. <https://doi.org/10.1636/H06-38.1>
- World Spider Catalog (2019) World Spider Catalog. Version 20.5. Natural History Museum Bern. <http://wsc.nmbe.ch> [accessed on 1.11.2019]

Siberian weasel *Mustela sibirica* Pallas, 1773 predatism on bats during winter period

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Abstract

This work presents the results of a three-year natural environment experiment in a cave in Barsukovskaya, Siberia), aimed at assessing the possible impact of mammals preying on a wintering group of bats. The average consumed biomass amount per year was about 2108 g and the estimated number of prey animals was 214, which is about 20% of the maximum number of animals observed. The biomass consumed poorly correlates with the number of animals in the cave. The proportion of the various species remaining in the excrement of predators is strongly determined by the number of these species in the accessible part of the cave. The amount of excrement indicates the regular predatism on bats and, therefore, the presence of specific behavioural adaptation in *Mustela sibirica*.

Keywords

cave, Siberia, Vespertilionidae, Altai-Sayan

Introduction

The majority of works devoted to predatism on bats note the random nature of this phenomenon (Ryberg 1947; Dwyer 1964; Gillette and Kimbrough 1970; Taylor 1964; Fenton and Fleming 1976; Sparks et al. 2000; Molinari et al. 2005). Only a few studies indicate the possibility of species specialisation for hunting chiropterans (Bauer 1956; Black et al. 1979; Estók et al. 2009) which leads to insignificant losses in the prey population. However, the cited works mention targeted hunting for birds.

Data on winter preying by mammals on bats are scarce (Tiunov and Yudin 1986; Dulitsky 2001; Khritankov and Shishikin 2001; Sparks et al. 2003) and do not provide a quantitative assessment of the predator impact, whereas groups of bats, wintering in underground and other shelters, are most vulnerable to mammals.

This work presents the results of a three-year natural environment experiment, aimed at assessing the possible impact of mammals preying on a wintering group of bats.

A case of bats predation by *Mustela sibirica* Pallas, 1773 is addressed. The area of siberian weasel is mainly in Asia. It inhabits mainly forest and forest-steppe spaces, often settling near rivers. The basis of the diet in natural landscapes are small mammals and birds.

Methods

To estimate the impact of predators on the wintering group of bats, observations were made in Barsukovskaya cave (54°22.15'N, 83°57.39'E) located in Altai-Sayan mountain country of the Salair ridge western spurs in the forest-steppe zone at an altitude of 181 m above sea level. The entrance to the cave is located on the southern slope which is covered with grassy, shrubby and woody vegetation (Figure 1). The river Ukrop is 50 m from the entrance while poplars, birch and bird cherry grow in the bottom area. Barsukovskaya cave is of karst origin and is a corridor-labyrinth type. The depth of the cave is 19 m, the length is 100 m. The average passage height is about 1.5 meters (minimum – 30 cm; maximum – 5 m), average stroke width of about 2 m (minimum – 60 cm; maximum – 12 m). On the walls and ceiling there are a large number of ledges and cracks.

The cave is located in the continental climate zone. The average annual air temperature is 0.2 °C. The duration of the cold period is 178, warm – 188, frost-free – 120 days. The annual rainfall is ≈ 425 mm. The air temperature in the cave does not fall below + 3 °C.

In September 2015, excrement from *Mustela sibirica* Pallas, 1773 was discovered in the cave. The species identification was carried out via excrement size and shape (Formozov 2018). The bottom of the cave was cleared of all excrement at that time. The cave examinations were carried out from December 2015 to December 2017, when excrement was collected and the number and species of the wintering animals were determined.

The collected excrement was kept in a dry-heat oven for 3 hours at a temperature of 120 °C then weighed to the limits of a centigram. To identify the content of excrement, it was saturated with water and studied in a Petri dish with a microscope. The identification of the species of prey animals was determined through the occurrence of their species-specific skulls parts (e.g. jaws or their parts, molars, tympanic bones or skull occipital parts).

To estimate the biomass consumed, a correction index showing the ratio between the consumed biomass of prey and undigested residues was used (Lockie 1961; Brzeziński and Marzec 2003). Due to the lack of experimental data on the index value for bats, values for small mammals (rodents, insectivores) of 22 were used (Lockie 1961). Having determined the occurrence of each object in the diet and, thereby, the share of its biomass from the total and dividing by the average mass of the prey in the winter, the number of consumed chiropteran individuals was obtained.



Figure 1. Landscape at the entrance to Barsukovskaya cave (author photo).

Table 1. Weather conditions during the research period.

Period	Average air temperature (°C)	Rainfall (mm)	Number of days with precipitation	Average snow depth (mm)	Maximum snow depth (mm)
07.09.2015–13.12.2015	-0.7	181	63	138	330
13.12.2015–20.12.2016	-5.5	294	137	348	630
20.12.2016–15.04.2017	-10.6	92	65	640	840
15.04.2017–01.12.2017	5.5	271	75	98	180

In order to determine the average mass of species, individuals that were in an active state during the cave survey were weighed. Before weighing the animals were placed in a rag bag, with previously established mass. The measurements were carried out with an electronic weight-scales Pesola MS500. After weighing the animals were placed back on the walls of the cave.

The air temperature at the surface, precipitation and the snow-cover height were analysed for the whole observation period (Table 1), with the exception of the summer months when bats were only found sporadically in the cave (Zhigalin et al. 2019). Weather conditions were obtained from the nearest weather station archive, located in the village of Maslyanino (54°21.15'N, 84°12.03'E).

The value of variables correlations was estimated with the Pearson coefficient.

Results

In total, the cave was examined 5 times during the observation period. The examinations detected 2313 individuals of 6 species: *Myotis petax* Hollister, 1912, *Myotis sibiricus* Kastschenko, 1905, *Myotis dasycneme* (Boie, 1825), *Eptesicus nilssonii* (Keyserling, Blasius, 1839), *Plecotus ognevi* Kishida, 1927 and *Murina hilgendorfi* Peters, 1880 (Table 2).

M. petax and *Mu. hilgendorfi* (Figure 2) dominate in the cave, other species being found sporadically and not annually. In autumn and winter periods, most animals stay in the inaccessible parts of the cave; up to 300 individuals were found in the accessible area during different examinations. An exception was the beginning of winter 2017, when 615 animals were observed in the cave. The maximum number of bats in this shelter was found in the spring, when there were up to 1000–1300 individuals.

When examining the cave, most of the animals were located in the cavities and cracks of the ceiling. The number of animals on the walls of the cave and in their cracks in different periods ranged from 15 to 23%. The minimum height from the ceiling where animals were located was 30 cm, the maximum – 5 m. Representatives of the *Myotis* and *Murina* genera, as a rule, are located in close proximity to each other, forming clusters of up to several dozen individuals. All individuals of *E. nilssonii* and *P. ognevi* held alone and located in the entrance part of the cave.

The average weight of the bat was calculated by weighing 348 *Myotis petax*, 8 *Myotis sibiricus* and 124 *Murina hilgendorfi* individuals. The study showed that the average mass of *M. petax* is about 9.0 g, *M. sibiricus* – 7.7 g and *Mu. hilgendorfi* – 11.01 g. The analysis of the weasel excrement showed that the remains belonged to three species of bats: *M. petax*, *M. sibiricus* and *Mu. hilgendorfi* (Table 3).

In the cave, the remains of an *M. petax* were discovered (Figure 3).



Figure 2. *Mu. hilgendorfi* (left) and *M. petax* (right) in Barsukovskaya cave (author photo).

Table 2. The number of bats in the cave on the days of the survey.

Species	07.09.2015	13.12.2015	20.12.16	15.04.2017	01.12.2017
<i>M. petax</i>	57	198	136	543	348
<i>M. sibiricus</i>	2	–	6	–	–
<i>M. dasycneme</i>	–	–	–	27	–
<i>M. hilgendorfi</i>	–	56	170	498	267
<i>E. nilssonii</i>	–	–	2	–	–
<i>P. ognevii</i>	–	–	3	–	–
Total	59	254	317	1068	615

Table 3. The occurrence of bats remains in *Mustela sibirica* coprological material, estimated biomass and the number of prey animals.

Period of accumulation of excrement	Species	Frequency of occurrence (%)	Calculated biomass (g)	Calculated number of eaten individuals
07.09.2015–13.12.2015	<i>M. petax</i>	63.8	589.5	65.5
	<i>Mu. hilgendorfi</i>	36.2	334.8	30.4
	Total	100	924.3	95.9
13.12.2015–20.12.2016	<i>M. petax</i>	47.7	566.7	62.9
	<i>Mu. hilgendorfi</i>	52.3	621.3	56.4
	Total	100	1188	119.3
20.12.2016–15.04.2017	<i>M. petax</i>	60.2	1123.9	124.9
	<i>Mu. hilgendorfi</i>	39.1	729.9	66.4
	<i>M. sibiricus</i>	0.7	13.1	0.93
	Total	100	1876	192.23
15.04.2017–01.12.2017	<i>M. petax</i>	52.1	389.2	43.2
	<i>Mu. hilgendorfi</i>	47.9	357.8	32.5
	Total	100	747	75.7

**Figure 3.** Remains of a bat discovered in a cave 15.04.2017 (author photo).

Discussion

During the cave survey, the *Mustela sibirica* excrement was found in all parts, including the deepest cavities. The excrement study showed that it consists fully of bats remains (wool, bones), which makes this case unique. In previous studies, the maximum proportion of bat remains in predator droppings was no more than 75% in sable *Martes zibellina* L., 1758, which hunts in the cave of the Stolby reserve (Khritankov and Shishikin 2001). The absence of the remains of other animals and plants in the studied material may indicate that the predator entering the cave was very hungry. It was also found that the biomass consumed poorly correlates with the number of animals in the cave ($r = 0.73$; $p > 0.05$).

The data shows that most of the prey are *M. petax*, which account for up to 63.8% of all remains. The proportion of the remains of *Mu. hilgendorfi* is up to 52.3%. The proportion of the remains of various species in the excrement is strongly determined by the number of these species in the accessible part of the cave ($r = 0.7$; $p < 0.05$). The proportional ratio of the species number and their remains in the excrement indicate the absence of species preferences in the predator.

The average consumed biomass amount per year is about 2108 g and the estimated number of prey animals is 214, which is about 20% of the maximum number of animals observed.

Correlation analysis revealed that the amount of consumed biomass is connected with the average snow cover height ($r = 0.99$; $p < 0.01$) and its maximum values ($r = 0.95$; $p < 0.05$). The reason for this, in our opinion, is that with an increase in snow cover, hunting for small mammals becomes more energy-consuming and less effective.

The siberian weasel hunting strategy for bats remains unclear. Apparently, *Mustela sibirica* hunts animals that are located closest to the floor of the cave, climbing via the cracks to reach the bats. This strategy has already been recorded in another location in Siberia (Khritankov and Shishikin 2001). Active hunting for awakened and flying bats is also possible, which is most typical for siberian weasel.

Conclusion

The amount of excrement indicates the regular predatism on bats and, therefore, the presence of specific behavioural adaptation in *Mustela sibirica*. The calculated values of the prey number indicate significant losses in the bat group due to the hunting of *Mustela sibirica*. Hunting for chiropterans, perhaps, is a more efficient way to forage with lower energy costs in the snowy period.

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References

- Bauer K (1956) Schleiereule (*Tyro alba* Scop.) als Fledermausjager. *Journal of Ornithology* 97: 335–340. <https://doi.org/10.1007/BF01973733>
- Black HL, Howard G, Stjernstedt R (1979) Observations on the feeding behavior of the Bat Hawk (*Macheiromphus alcinus*). *Biotropica* 11: 18–21. <https://doi.org/10.2307/2388165>
- Brzeziński M, Marzec M (2003) The origin, dispersal and distribution of the American mink *Mustela vison* in Poland. *Acta theriologica* 48: 505–514. <https://doi.org/10.1007/BF03192496>
- Dulitsky AI (2001) Biodiversity of Crimea. Mammals: history, condition, conservation, prospects. Simferopol, 208 pp. [In Russian]
- Dwyer PD (1964) Fox predation on cave-bats. *Australian Journal of Science* 26: 397–398.
- Estók P, Zsebók S, Siemers BM (2009) Great tits search for, capture, kill and eat hibernating bats. *Biology letters* 6: 59–62. <https://doi.org/10.1098/rsbl.2009.0611>
- Fenton MB, Fleming TH (1976) Ecological interactions between bats and nocturnal birds. *Biotropica* 6: 104–110. <https://doi.org/10.2307/2989629>
- Formozov AN (2018) Tracker Companion. Moscow, AST, 448 pp. [In Russian]
- Gillette DD, Kimbrough JD (1970) Chiropteran Mortality. In: Slaughter BH, Walton DW (Eds) *About Bats*. Dallas Southern Methodist University Press, Dallas, 262–281.
- Khritankov AM, Shishikin AS (2001) Natural enemies of bats in Central Siberia. *Proceedings of the Stolby State Reserve* 16: 95–102. [In Russian]
- Lockie JD (1961) The food of the pine Marten *martes* in west Ross-Shire, Scotland. *Journal of Zoology* 136: 187–195. <https://doi.org/10.1111/j.1469-7998.1961.tb06171.x>
- Molinari J, Gutiérrez EE, Ascensão AA, Nassar JM, Arends A, Marquez RJ (2005) Predation by giant centipedes, *Scolopendra gigantea*, on three species of bats in a Venezuelan cave. *Caribbean Journal of Science* 41: 340–346.
- Ryberg O (1947) *Studies on bats and bat parasites*. Lund, Sweden: Berlingska Boktryckeriet.
- Sparks DW, Roberts KJ, Jones C (2000) Vertebrate predators on bats in North America north of Mexico. In: Choate JR (Ed.) *Reflections of a naturalist: papers honoring Professor Eugene D. Fleharty*. Sternberg Museum of Natural History, Fort Hays State University, Hays, Kansas, 229–241.
- Sparks DW, Simmons MT, Gummer CL, Duchamp JE (2003) Disturbance of roosting bats by woodpeckers and raccoons. *Northeastern Naturalist* 10: 105–109. <https://doi.org/10.2307/3858677>
- Taylor WP (1964) Noteworthy predation on the guano bat. *Journal of Mammalogy* 45: 300–301. <https://doi.org/10.2307/1376997>
- Tiunov MP, Yudin VG (1986) Sable predation at bats wintering (Chiroptera). *Ecology* 5: 84–85. [In Russian]
- Zhigalin AV, Gadzhiev AA, Daudova MG, Salimkhanov NG, Shestopalov AM (2019) Ecology of chiroptera bats in Altai-Sayan region of Southern Siberia. *South of Russia: ecology, development* 14: 9–25. <https://doi.org/10.18470/1992-1098-2019-1-9-25> [In Russian]

