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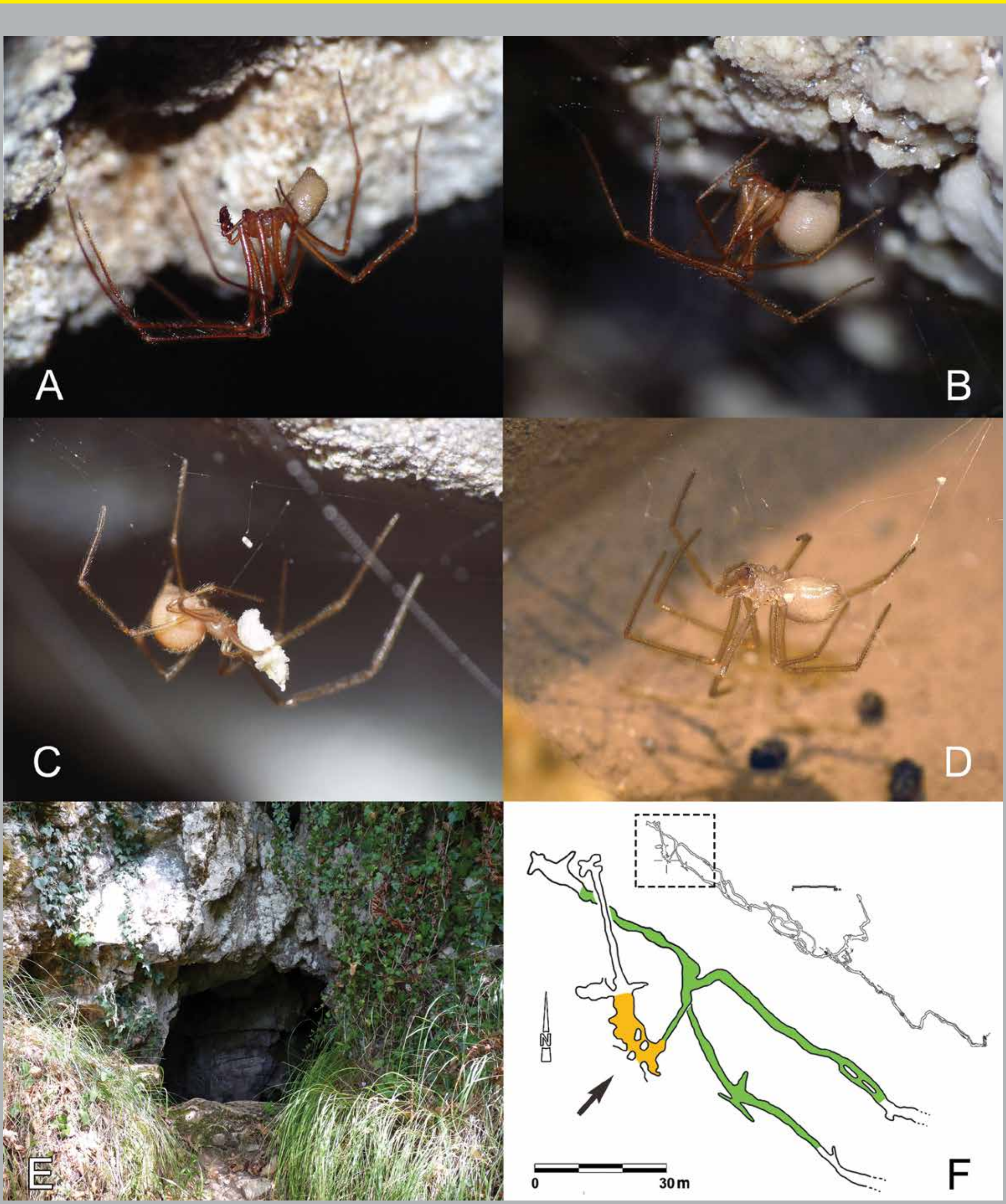

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Front cover: Habitus and type locality of *Domitius culsu* sp. nov. See Ballarin in this issue.

Nataša Nikolić, Nikola Zarubica, Bojan Gavrilović, Dragana Predojević, Ivana Trbojević, Gordana Subakov Simić and Slađana Popović. Lampenflora and the entrance biofilm in two show caves: comparison of microbial community, environmental, and biofilm parameters. *Journal of Cave and Karst Studies*, v. 82, no. 2, p. 69-81. DOI:10.4311/2018EX0124

LAMPENFLORA AND THE ENTRANCE BIOFILM IN TWO SHOW CAVES: COMPARISON OF MICROBIAL COMMUNITY, ENVIRONMENTAL, AND BIOFILM PARAMETERS

Nataša Nikolić[1,C], Nikola Zarubica[1], Bojan Gavrilović[3], Dragana Predojević[1], Ivana Trbojević[1], Gordana Subakov Simić[1], and Slađana Popović[2]

Abstract

Phototrophic microorganisms from two caves in Serbia (Podpeć and Stopić) were examined. Samples were taken from the entrances where natural light was present, as well as from the inside caves near artificial light (lampenflora community). *Cyanobacteria*, *Chlorophyta*, *Bacillariophyta* and *Xanthophyta* were documented, with 51 taxa in total. The highest number of taxa recorded in the *Cyanobacteria* were coccoid cyanobacteria; *Gleocapsa* and *Chroococcus* were dominant. According to the redundancy analysis (RDA), *Cyanobacteria* were dominant at cave entrances while other groups (*Chlorophyta*, *Bacillariophyta* and *Xanthophyta*) were documented in lampenflora samples. Temperature, relative humidity, and light intensity were measured, as well as chlorophyll *a* concentrations and biofilm parameters (water, organic and inorganic matter content). Ecological parameters did not show significant variation, while light intensity depended on the position of sampling sites. RDA showed that the water content was higher in biofilm samples from cave entrances, while levels of inorganic matter were increased in lampenflora samples. The concentration of chlorophyll *a* did not show significant correlations with any of the measured ecological or biofilm parameters. Although the ecological parameters inside the cave did not show significant variation, they should be monitored because of the potential influence on the development of the lampenflora community that has a negative aesthetic impact on cave formations.

Introduction

Despite their extreme conditions, caves are unusual ecosystems inhabited by unique organisms. On the inside of caves, light intensity (LI) decreases as we go further away from the entrance, while the temperature (T) and air humidity show little or no variation, but the cave's entrance is under the influence of temperature and humidity from the outside environment (Hajdu, 1977; Vinogradova et al. 1998; Pedersen, 2000). In isolated and extreme environments, such as caves, specific organisms from different groups can be found (bacteria, cyanobacteria, algae, fungi, mosses, lichens, invertebrates, and vertebrates) (Mulec et al., 2008; Mulec and Kosi, 2009; Cerwik-Marcinkovska, 2013). Due to their natural beauty, caves are often open to the public, which can lead to the disturbance of the stable conditions in these habitats. The installation of artificial light in caves is the main reason for cave substrate colonization by phototrophic organisms. The lights change the values of the temperature and air humidity, which affects the rock surface the phototrophs' development (Mulec and Kosi, 2009). This phototrophic community is called *lampenflora* or lamp flora and includes many organisms, such as bacteria, cyanobacteria, algae, mosses, fungi and lichens (Dobat, 1998; Mazina and Severin, 2007). Lampenflora may include “r-selected species” and fast-growing species often capable of tolerating lower temperature (T), relative humidity (RH) and nutrient input (Aleya, 1991; Borderieet al., 2014). Biochemical deterioration (also known as biocorrosion) of the substratum is caused by the metabolic processes of the microorganisms (Macedo et al., 2009). During the respiration process, microorganisms release $CO_{2,}$ which when mixed with the surrounding water, produces the carbonic acid that causes the biodeterioration of cave structures (Aleya, 1991; Macedo et al., 2009; Borderie et al., 2014). According to Jurado et al. (2010) communities of these organisms can include potentially toxic or pathogenic microorganisms that represent a potential danger for animals and humans, and species whose physiology is still unknown.

The study of the diversity and growth control of lampenflora communities in tourist caves seems particularly important. In the Republic of Serbia, the pioneering study was performed by Popović et al. (2015a, b; 2016a, b, c; 2017a, b). Similar research endeavors in the region were conducted in the Republic of Slovenia (Klemenčič and Vrhovšek, 2005; Mulec and Kosi, 2008, 2009; Mulec et al., 2008, 2012) the Republic of Croatia (Ercegović 1925, 1932; Golubić, 1967) and the Republic of Macedonia (Tofilovska et al., 2014).

The aim of this study was to investigate the diversity of cyanobacteria and algae in two tourist caves, Podpeć and Stopić, in Serbia, and to compare the microbial communities from cave entrances with those deeper inside the caves

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from typical lampenflora. Additionally, environmental parameters were measured. Moreover, primary production and biofilm parameters (water, organic and inorganic matter content) were measured and compared between entrance biofilms and lampenflora.

Materials and Methods

Sampling Sites

Podpeć Cave is located in Western Serbia, in the village of Potpeće, 186 km from Belgrade (43°47'45.1"N 19°56'00.8"E). The cave entrance has a horseshoe shape 50 m height with a 22 m wide arch. The width at the base is 12 m. This cave was formed by the Petnica River as it sunk in Drežnička Valley and flowed through 5 km long underground streams. According to Cvijić (1914a), limestone rocks in this area were formed in the Middle Trias and are characterized by whitish colors, cracked porosity, and a mosaic structure (Marković, 1957). The cave has two levels of passages, older – the Upper Cave, and younger – the Lower Cave. The portion of the cave that is developed for visitors, with paths and artificial light, is 555 m long.

Stopić Cave is located on the northeastern side of Zlatibor Mountain, 250 km from Belgrade (43°42'12.0"N 19°51'12.4"E). The cave entrance is located on the right side of the Prištavica River, 711 mabove sea level. It is 35 m wide and 18 m high.The explored part of the cave is 2000 m long and in some places the ceiling is 50 m high. The limestone rocks were formed in the Middle Trias and are over 100 m thick (Cvijić, 1914b). The unique characteristics of the cave are rimstone pools formed by deposited limestone and an underground waterfall called “The Source of Life” formed by Trnavski stream that flows through the cave (Lazarević, 2012).The portion of the cave developed for tourists is several hundred meters long, and 1658 m of the cave system has been explored, so far. Locations of Stopić and Podpeć caves are presented in Figure 1.

For algological analyses, five sampling sites in Podpeć Cave were chosen. One sampling site was at the entrance (P5) and four sampling sites were chosen inside the cave (P1, P2, P3,P4). Six sampling sites were chosen in Stopić Cave, three inside (S1, S2, S3) and three at the entrance to the cave (S4, S5, S6). Sampling sites of the two investigated caves are presented in Figures 2 and 3. The sampling sites are shown in Figures 4 and 5. Samples were collected in July 2016.

Environmental Parameters

Environmental parameters were measured *in situ* using the DMV 1300 Luxmeter (Vellemarn, Belgium) for Light Intensity, and Temperature humidity meter (Extech, USA) for those parameters. These parameters were measured five times for each site and the mean values were calculated.

Biofilm Analyses

The samples for measuring the biofilm content (water content (WC), organic matter (OM) and inorganic matter (IM), and chlorophyll *a* (Chl *a*) extraction were scraped from stone substrata using a round metal moldcovering asurface area of 3.14 cm^2.

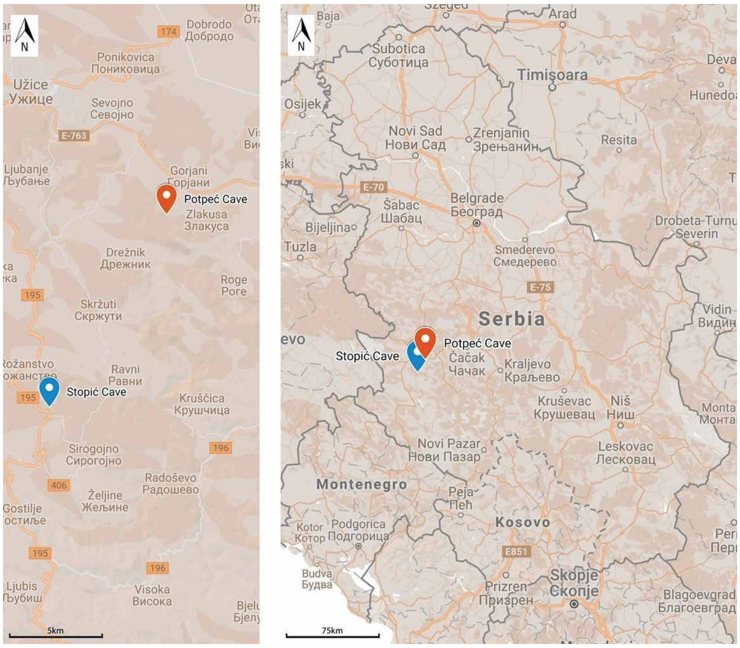

Figure 1. Maps of the two investigated caves: Stopić and Podpeć. Sources: Google Maps, http://www.clipart-library.com.

The WC, OM and IM content was calculated as the difference between the biofilm weight before and after drying at 105 °C and after ashing at 550 °C (Popović et al., 2017a). The WC was measured as the difference between the fresh and dried weight. OM was calculated as the weight difference between the dried and the ashed biofilm, while IM content was equal to the weight of the remains after the biofilm was ashed. All three parameters were expressed in two ways, as a quantity per surface area (mg/cm^2) and as a percentage of each constituent in a biofilm sample. The concentration of Chl *a* was determined by spectrophotometry using a formula described in Popović et al. (2015a) and expressed as µg/cm^2.

Qualitative analyses of algae were performed by using the non-destructive adhesive tape method (Urzi and de Leo, 2001) and by scraping the biofilm with a sterilized scalpel. The samples were fixed with a drop of glycerol and observed on a Zeiss Axio- Imager M1 light microscope with Axio Vision 4.8 software.

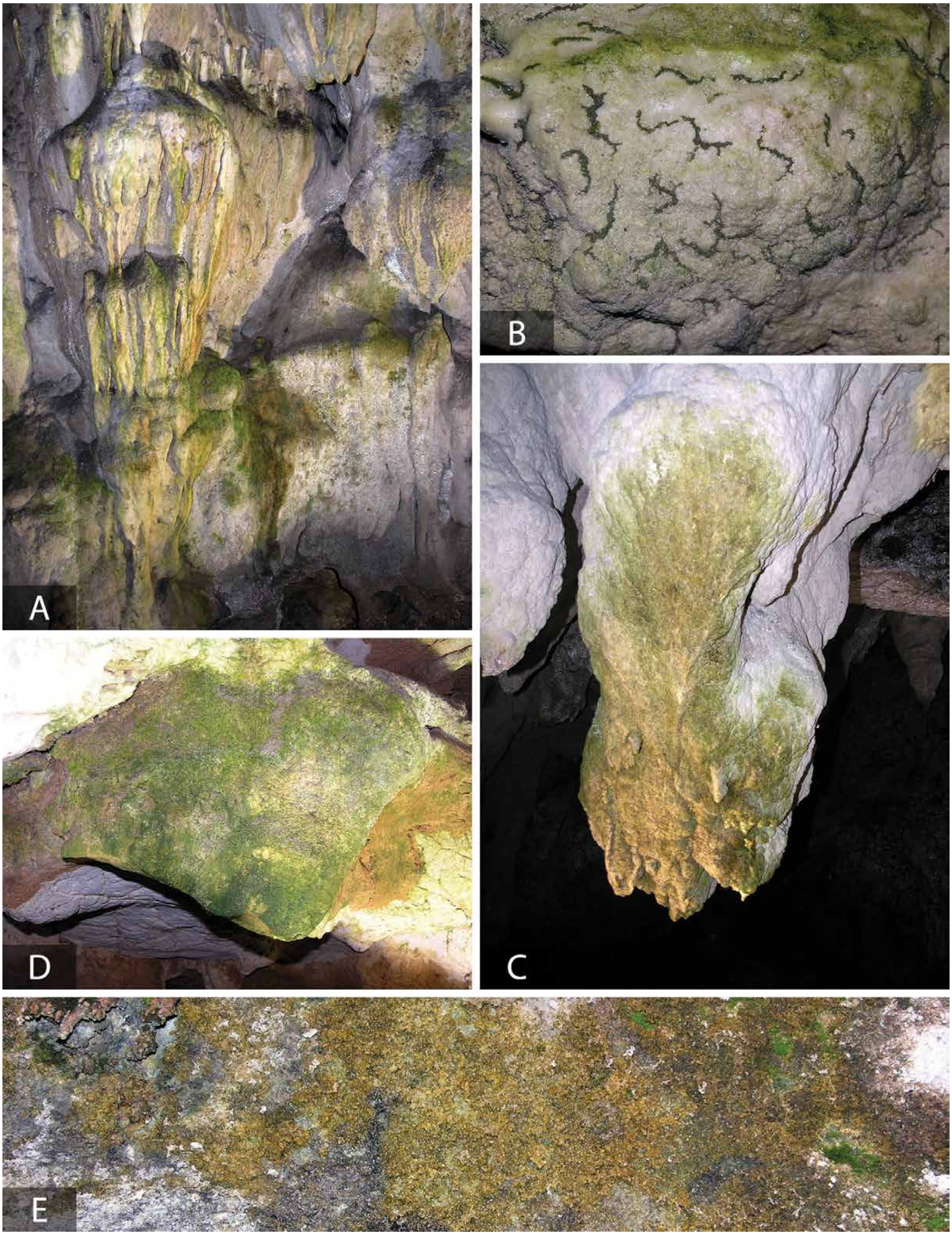

Figure 2. Podpeć cave, sampling sites: A–D – inside the cave: A – site P1; B – site P2; C – site P4; D – site P3; E – entrance of the cave, site P5.

Cyanobacteria and algae were identified using standard identification keys (Ettl, 1978; Komárek and Fott, 1983; Ettl and Gärtner, 1988; Komárek and Anagnostidis, 1998, 2005; John et al., 2003; Hoffman et al. 2013; Komárek, 2013).

Statistical Analyses

The redundancy analysis (RDA) was performed using the program CANOCO for Windows, Version 5.0 (Ter Braak and Šmilauer, 2012). The presence/absence of all recorded taxa was imported into the program and each taxon was assigned to its larger taxonomic group: *Cyanobacteria* (divided into *Chroococcales*, *Oscillatoriales* and *Nostocales*), *Chlorophyta*, *Bacillariophyta* and *Xanthophyta*. These six larger taxonomic groups were used instead of the individual genera and species that were identified. RDAwas performed to demonstrate the preference of each groupinone of two communities: lampenflora and at the cave entrances. The nominal variables, lampenflora and cave entrance, were used as explanatory variables. The WC and content of (OM) and (IM) in biofilms (expressed as percentages) were included as supplementary variables.

XLSTAT addition in Excel was used to calculatethe correlation between recorded physical parameters (T, RH, LI), Chl *a* and biofilm parameters (WC, OM and IM, expressed as µg/cm^2).

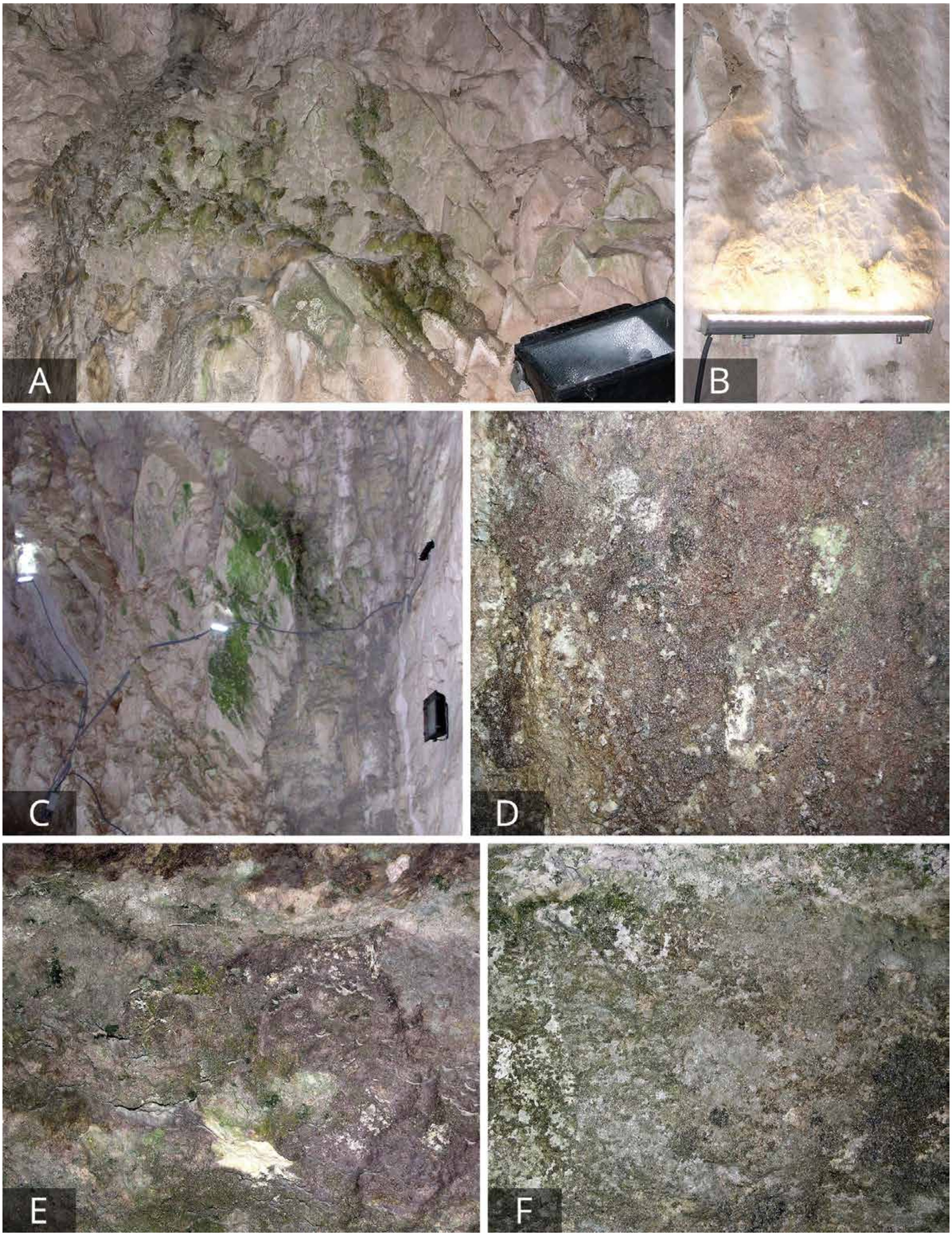

Figure 3. Stopić Cave, sampling sites: A–C – inside the cave: A – site S1; B – site S2; C – site S3; D–F – entrance of the cave: D – site S4; E – site S5; F – site S6.

Results

The ecological parameters measured in the Podpeć (P1, P2, P3 and P4 inside the cave, P5 at the entrance) and Stopić caves (S1, S2, S3 inside the cave, S4, S5, S6 at the entrance of the cave) are presented in Figure 6. For both caves, the ecological parameters (T and RH) showed a certain degree of variation, but only LI values showed notable differences among sampling sites, as well as between caves. The highest temperature was measured at P5 (22 ºC), while the lowest was at P2 (15.3 ºC) in Podpeć Cave, while in Stopić Cave the highest temperature was at S2 (19.9 ºC) and the lowest was at S3 (16.3ºC). RH varied from the lowest value of 56 % at S2 to the highest value of 78 % at sampling sites S5 and S6 in Stopić Cave. The highest value of relative humidity in Podpeć Cave was at the point P5 (85%), while the lowest was at P1 (58%). Between the sites inside Podpeć Cave, T varied up to 5 °C, RH varied up to 12 %, while between the sites inside Stopić Cave, T varied up to 3.6 °C while RH varied up to 19 %. Outside sampling points in Podpeć Cave had higher T and RH than inside the cave, while at the entrance of Stopić Cave, values of the T and RH were similar to those measured inside the cave. The lowest value of LI was 4 lux at sampling site S3 and the highest value was 3100 Lux at sampling site S2 in Stopić Cave. In Podpeć Cave, the highest value of LI was 840 Lux at point P4, while the lowest value of 265 Lux was at sampling site P2 .

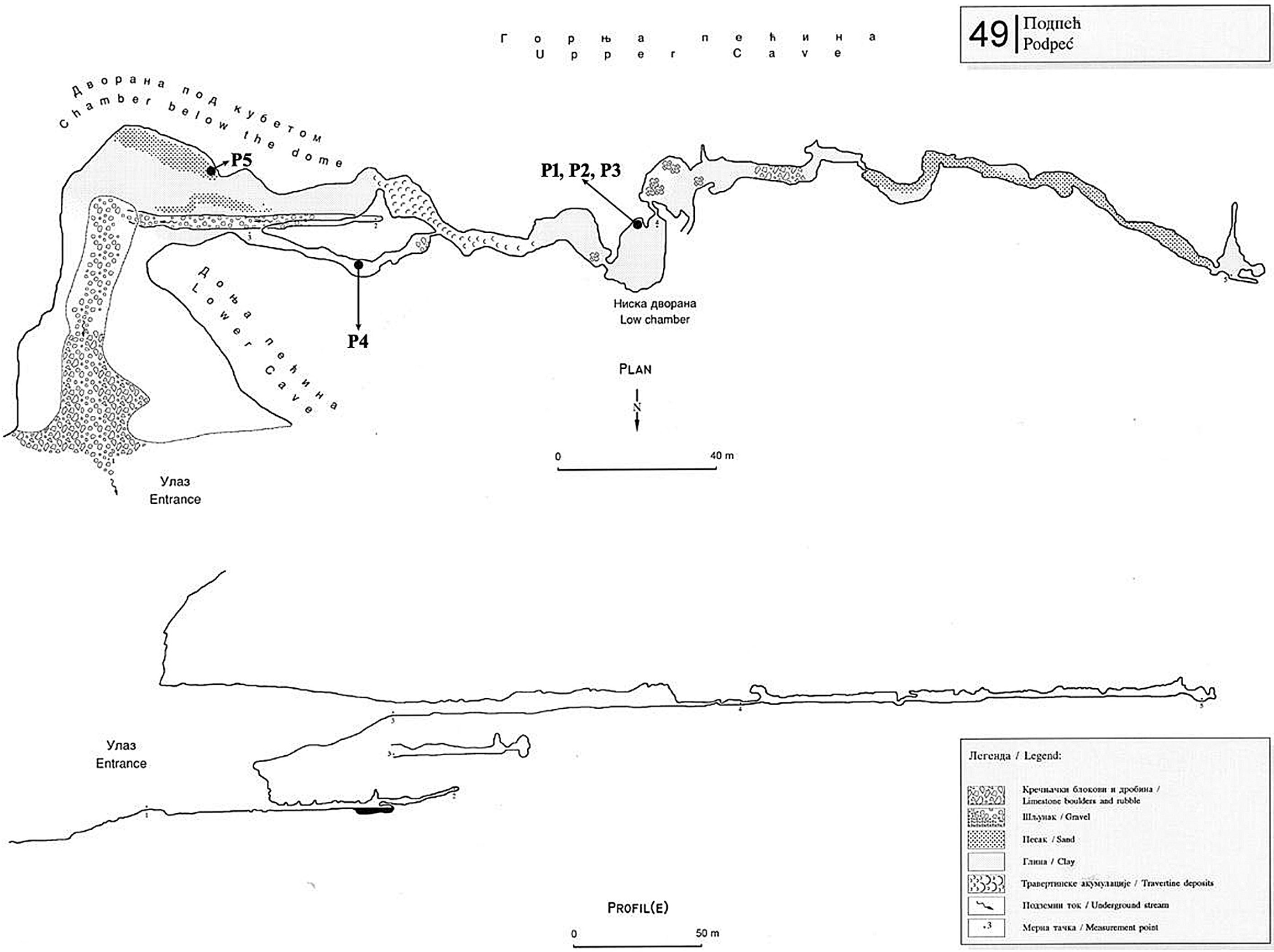

Figure 4. Map of the Podpeć Cave, sampling sites; P1−P4 – sampling sites inside the cave, P5 – sampling site at the entrance. Source: Speleological atlas of Serbia(1998).

Biofilm parameters (WC, OM and IM) expressed as mg/cm^2 depended on the position of the sampling site (Fig.7a). In Podpeć Cave, the (WC) was lowest at sampling site P4 and highest at P5. The OM content was also highest at P5 and very low at P1 and P2. P2 was also characterized by the lowest content of IM, while the highest value for this parameter in this cave was at sampling sites P3 and P5. In Stopić Cave, WC was highest at the site positioned at the cave entrance (S6) and lowest at S1 and S2 (lampenflora samples). The OM content was the highest at S6 and lowest at sampling sites S2 and S3. The IM content had the lowest measured value at sampling site S1 and the highest at site S5.

Figure 7b presents WC, OM and IM expressed in percentages for all biofilm samples. In Podpeć Cave, the highest WC percentage was found in the biofilms from sampling sites P2 and P5, while the lowest was found at sampling site P4. The highest percentage of OM was also recorded at P5 and the lowest at sampling site P1. On the other hand, IM had the highest value at sampling site P1, while the lowest was at P5 in Podpeć Cave. In Stopić Cave, the highest value of WC and IM was measured at sampling sites S6 and S3, respectively, while OM was highest at point S6. The lowest values of WC, OM and IM in Stopić Cave were found at sampling sites S2, S5 and S6, respectively.

The lowest concentration of Chl *a* expressed as μg/cm^2 was documented at sampling sites S3 and S1 in Stopić Cave and P2 in Podpeć Cave, while the highest values of Chl *a* were at P3 and S6 (Fig.7a).

Correlations between T, RH, LI, Chl *a*, WC, OM and IM were performedusing Pearson's coefficient (Table 1). It appears that Chl *a* does not show significant correlation, positive or negative, with any of the listed parameters. T and RH were significantly positively correlated with both WC and OM.

In total, 51 taxa were documented from the two caves (Table 2). The highest number of taxa belonged to *Cyanobacteria* (44) while the remaining taxa belonged to *Bacillariophyta* (4), *Chlorophyta* (2) and *Xanthophyta* (1). Considering the caves separately, 39 taxa were recorded in the*Cyanobacteria* division in Stopić Cave and 22 taxa were found in

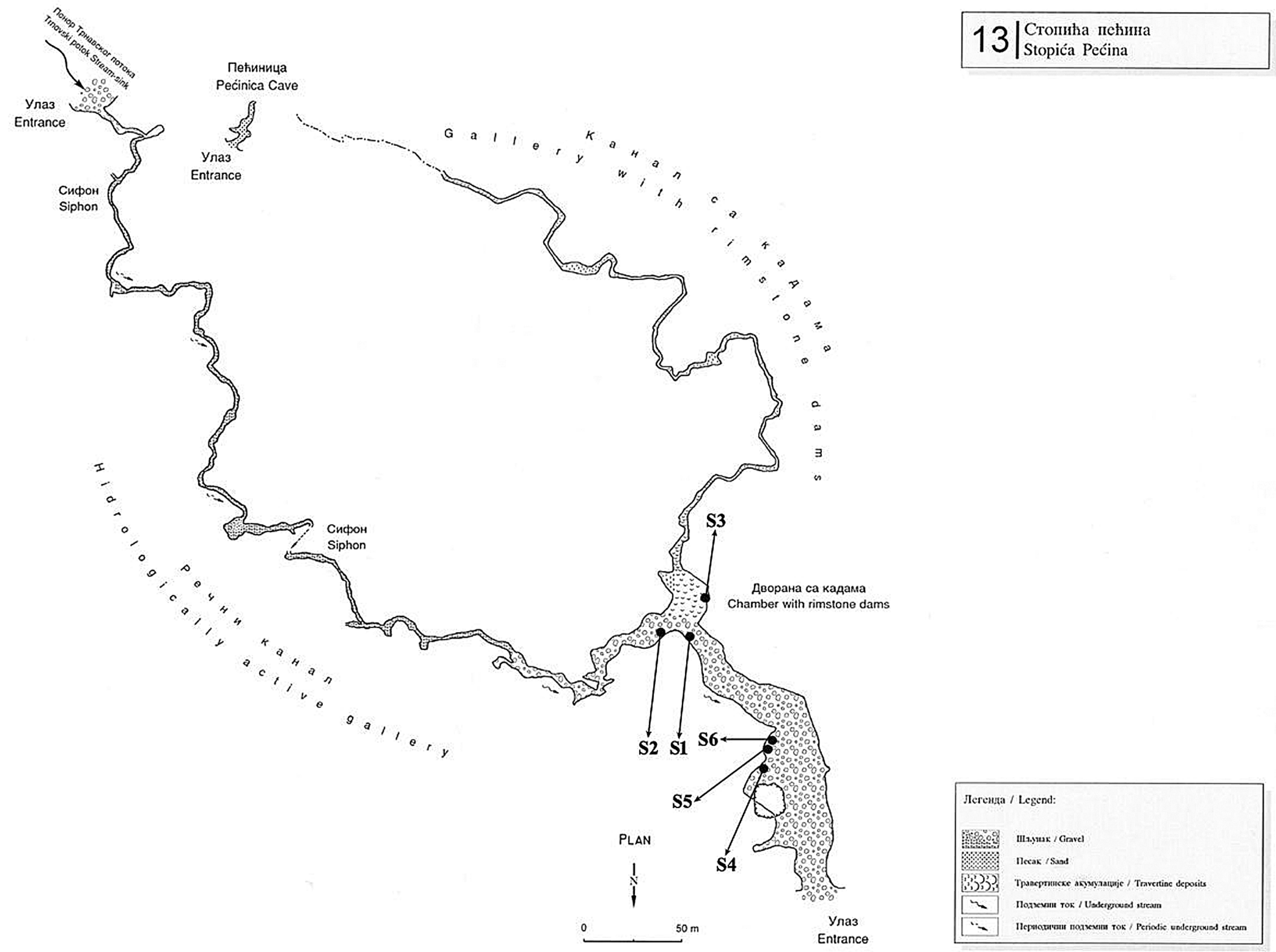

Figure 5. Map of Stopić Cave, sampling sites; S1−S3 – sampling sites inside of cave, S4−S6 – sampling sites at the entrance. Source: Speleological atlas of Serbia (1998).

Podpeć Cave. Stopić Cave was characterized by the presence of all four recorded *Bacillariophyta* taxa, while only one was documented in Podpeć Cave. Representatives of *Chlorophyta* and *Xanthophyta* were recorded in both caves. The most diverse cyanobacterial group was *Chroococcales* in both caves, where taxa of the genus *Gleocapsa* and *Chroococcus* dominate.The other two cyanobacterial groups, *Oscillatoriales* and *Nostocales*, were more numerous in Stopić Cave than in Podpeć Cave. *Asterocapsa* spp., *Chrococcus ercegovicii*, *Leptolyngbia foveolarum*, *Leptolyngbia* sp1, *Leptolyngbia* sp2, *Nostoc punctiforme* and an unknown taxon that belonged to *Xanthophyta* were present in all three sampling sites at the entrance of Stopić Cave, while inside the cave, the green algae cf. *Chlorella* sp. was dominant. *Leptolyngbia foveolarum* was the only cyanobacterial taxon found inside Stopić Cave. In Podpeć Cave, the green algae cf. *Chlorella* sp. and *Humidophila* sp. were found at the majority of the sampling sites inside the cave. At the cave entrance, *Cyanobacteria* were dominant. *Gleocapsa atrata* and *Leptolyngbia foveolarum* were the only taxa found inside Podpeć Cave. Two representatives of *Chlorophyta* have been recorded (*Chlorella* sp. and *Trochiscia* sp.) at every sampling site inside the caves (Table 2).

RDA analysis included nominal variables, lampenflora and communities at cave entrances, as the explanatory variables, and algal groups (*Cyanobacteria– Chroococcales*, *Oscillatoriales* and *Nostocales*, *Chlorophyta*, *Bacillariophyta* and *Xanthophyta*) as response data (Fig.8). The first RDA axis explained 58.8 % of the variability in our data. Nominal variable, Lampenflora, was placed on the left side of the ordination diagram (R = −0.9492) and the nominal variable, Cave entrance,on the right (R = 0.9492). The first axis represents the variation in microorganism assemblages between the two nominal variables. *Bacillariophyta* and *Chlorophyta*, as well as *Xanthophyta*, were dominant in lampenflora samples, while all three cyanobacterial groups were mostly documented in the biofilm samples taken at cave entrances. Supplementary variables show that the levels of IM in biofilms were higher in lampenflora samples, while the WC was higher in the biofilm samples at cave entrances (Fig.8).

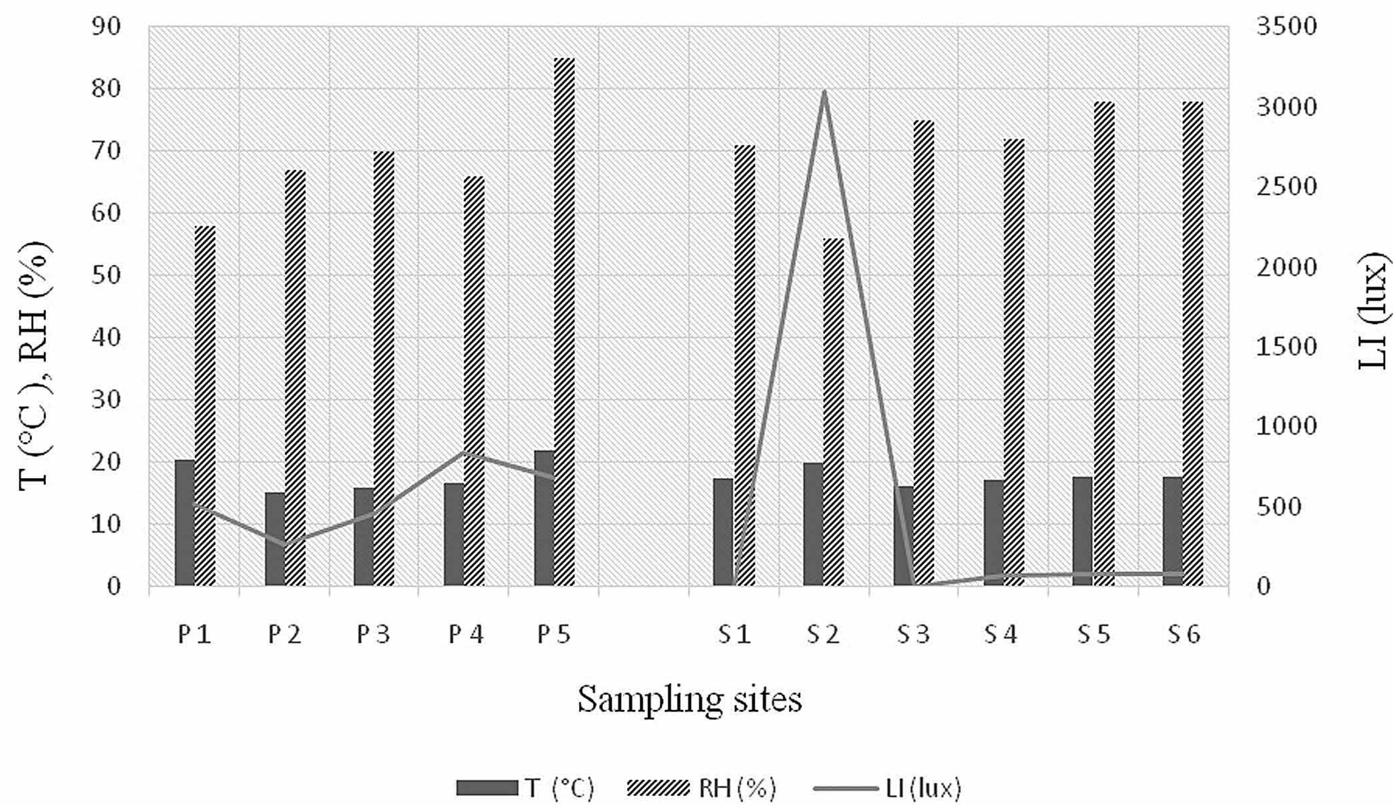

Figure 6. Values of ecological parameters: temperature (T in °C), relative humidity (RH in %), light intesity (LI in Lux) at sampling sites from Podpeć (P1–P5) and Stopić (S1–S6) caves.

Discussion

At the entrance of caves, the influence of the outside climate (T fluctuation, LI, water regime, and UV radiation) is evident, especially when T and RH are considered (Pentecost and Whitton, 2012). In Podpeć Cave, T and RH values differed at P5 compared to the rest of the sampling sites. At this site, located at the cave entrance, the highest value of T was measured, which coincides with the results by Popović et al. (2015a) and Cennamo et al. (2012). It should also be mentioned that the season in which the sampling was conducted also played a role. At the entrance of Stopić Cave, T and RH did not vary much from site to site and their values were similar to those measured inside the cave, probably due to the morphology of the cave and the big entrance zone which is influenced more by external conditions compared to Podpeć Cave. The LI at the entrance varied among sampling sites of both caves and depended on many factors, such as the size of the cave entrance, the presence/absence of vegetation, and exposure of the sampling sites. (Popović et al, 2017a).

According to Czerwik-Marcinkowska and Mrozińska (2011), T and RH are relatively stable inside caves; the temperature inside the caves of Central Europe ranges between 5 °C and 8 °C, while RH is between 85 % and 95 %. However, other authors mentioned (Smith and Olson, 2007; Mulec and Kosi, 2009) that the introduction and installation of artificial light (especially warm light) and the presence of cave tourists, can have a negative impact on the microclimate and can influence changes in T and RH. According to the information provided by guides in caves, the T in Stopić Cave vary from 9.5 °C to 18 °C and the lowest RH measured was 87 % and which becomes higher depending on the season. In Podpeć Cave, T values were between 9 °C and 10.2 °C, and had RH values of 94 % and higher.

Our samples were collected in the summer; however, in both caves, the measured T was higher, while RH was lower, due to the proximity of the sampling sites to artificial light sources, especially in Podpeć Cave. The type of lamps used differs between the caves: at the time of sampling, Podpeć Cave had lamps that emitted warmer light (these lamps have been changed since) compared to those in Stopić Cave, where LED lights had been installed. Cigna and Burri (2000) state that unsuitable lamps can lead to changes in environmental parameters, (e.g., Castellana Caves, South Italy where T increased from 15 °C to 25 °C while RH decreased from 95 % to 100 % to 55 % to 60% near the light source). It is interesting to note that point S2 was found to have lower RH despite the presence of LED lamps, probably because the sampling point is very close to the light (very high LI was measured compared to other sampling sites). As seen from Table 1, increases in T and RH lead to increases in WC and OM (a significant positive correlation was observed) and higher values of WC and OM mean that better developed biofilms are present.

Many groups of microorganisms can grow in the extreme oligotrophic conditions of cave environments (Czerwik-Marcinkowska, 2013), however, some recent studies suggest that the level of trophicity can be increased by anthropogenic factors or presence of animals (Trinh et al., 2018). The OM introduced by animals, humans, or brought by intermittent and seeping water, significantly contributes to the development of microorganisms in locations near artificial lights (Mazina and Maximov, 2009). Furthermore, throughout the cave, tourists can introduce and spread the spores and cysts of different microorganisms that remain dormant until the appearance of suitable conditions for their development, as documented by Mulec and Kosi (2008), Czerwik-Marcinkowska et al. (2015), and Meyer et al. (2017). Cyanobacteria and algae have developed mechanisms of protection from various adverse environmental conditions (Pentecost and Whitton, 2012). The development of cyanobacteria and algae depends primarily on light, but also on T and RH; all are considered the most important factors for their growth (Martinčič et al., 1981; Chang and Chang-Schneider, 1991). The microbial assemblage at the cave entrance and in lampenflora samples usually differs as a result of living in two different zones, one characterized by the presence of artificial light and nearly stable conditions, and the other influenced by the outside climate, daylight and factors that are more variable.

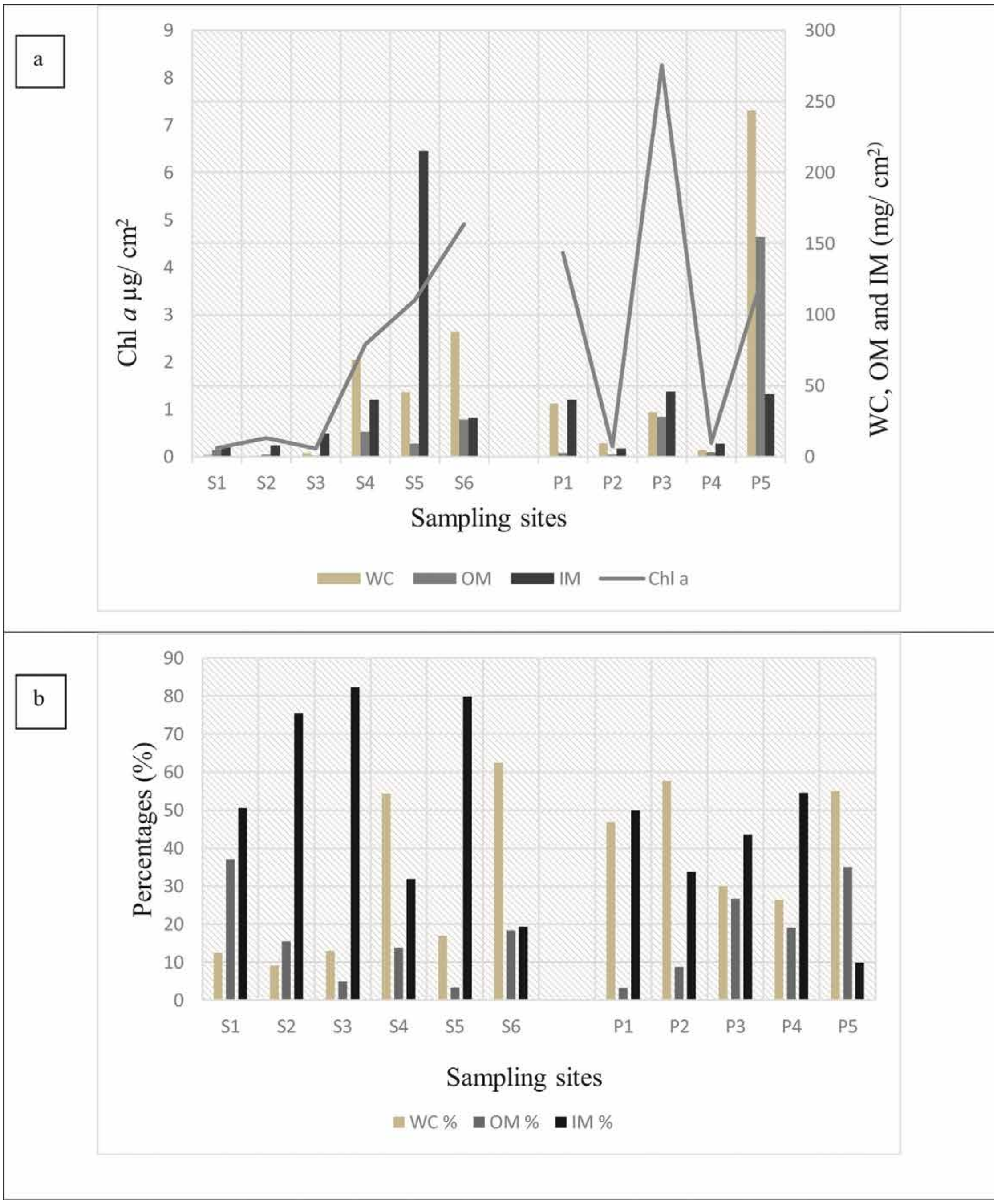

Figure 7. A – The concentration of chlorophyll *a* (Chl *a*) expressed as µg/ cm^2, water content (WC) and organic/inorganic matter (OM/IM) expressed as mg/ cm^2 from Podpeć (P1–P5) and Stopić (S1–S6) caves. B – Water content (WC), organic and inorganic matter (OM/IM) presented as percentages from Stopić (S1–S6) and Podpeć (P1–P5) caves.

Differences in the diversity and assemblages of aerophytic cyanobacteria and algae in this study are obvious when samples from the cave entrance and lampenflora are compared. The diversity of phototrophic microorganisms was higher at cave entrances, where *Cyanobacteria* were dominant, and lower inside the caves. Moreover, in Figure 8, consid-

Table 1. Correlations between T, RH, LI, Chl, WC, OM and IM using Pearson coefficient.

Variables	T	RH	LI	Chl *a*	WC	OM	IM
T	**1**	0.008	0.423	0.125	**0.635**	**0.607**	0.067
RH	0.008	**1**	−0.589	0.230	**0.664**	**0.629**	0.362
LI	0.423	−0.589	**1**	−0.198	−0.112	−0.016	−0.235
Chl *a*	0.125	0.230	−0.198	**1**	0.376	0.318	0.313
WC	**0.635**	**0.664**	−0.112	0.376	**1**	**0.956**	0.153
OM	**0.607**	**0.629**	−0.016	0.318	**0.956**	**1**	0.047
IM	0.067	0.362	−0.235	0.313	0.153	0.047	**1**

Note: Values in bold are different from 0 with a significance level alpha = 0.05.

ering the number of recorded taxa, *Cyanobacteria* were dominant at the cave entrances compared to algal groups. It should be noted that besides different environmental parameters, presence of seeping water and cave morphology, many microclimatic parameters can play a role. Accordingly, we cannot be certain which factors contribute to the much higher diversity in Stopić Cave. In the lampenflora samples collected inside the caves, diversity was low, but two genera of green algae (*Chlorella* sp., *Trohiscia* sp.) were quite abundant and the green algae cf. *Chlorella* sp. was always found in biofilms. In tourist caves near artificial light, a lower diversity of cyanobacteria and algae is commonly observed near artificial lights when compared to cave entrances, green algae often being the first colonizers of stone substrata (Mulec, 2008) and dominant components (Mulec and Kosi, 2008, Czerwik-Marcinkowska et al., 2015, and Meyer et al., 2017). Frequently, these green algae assemblages include fast growing and r-selective species (Albertano, 2012; Borderie et al., 2014, Czerwik-Marcinkowska et al., 2015) such as representatives of the genus *Chlorella.* This algae is reported in many caves worldwide and is considered to be a big problem for cave conservators. The stable microclimate inside the cave and suitable conditions under artificial lighting compared to the fast changing conditions at the entrance, as well as the absence of other extreme conditions, can promote the development of green algae and diatoms, since these groups prefer a more stable environment for their growth (Mulec, 2008; Borderie et al., 2014). Dripping water with suspended nutrients can also have a positive effect on algal growth, especially on the representatives of *Bacillariophyta* (Vinogradova et al., 2009; Piano et al., 2015). Recorded *Bacillariophyta* taxa in this study are considered a typical cosmopolitan and most frequent genera in caves (Czerwik-Marcinkovska and Mrozińska, 2011; Falasco et al., 2014). Inside Podpeć Cave, only *Humidophila* sp. was registered, a genus that is distributed globally and most commonly on the wet limestone walls in caves (Lowe et al., 2014, 2017). Even though we did not record seeping water at sampling sites at the time of sampling, it does not mean that seeping water is not present during certain periods of the year. There are also cases in which cyanobacteria prevail in lampenflora communities, but according to Mulec et al. (2008), it can happen in biofilms that have been growing undisturbed for some time. On the other hand, cyanobacteria are frequently dominant in the biofilms from cave entrances, since they are capable of enduring more extreme conditions than green algae and diatoms (Pentecost and Whiton, 2012).

Primary production at all sampling sites was assessed by measuring the Chl *a* concentration. Chl *a* concentration is usually correlated with the degree of biofilm development (Popović et al., 2015a; 2017a). In Stopić Cave, the highest concentration of this parameter was determined at sampling site S6, which was characterized by a thick biofilm with higher WC and OM. The correlation of Chl *a* with these two parameters in general had slightly positive values, but were not significant.

Water is the most significant factor influencing the development and growth of the phototrophic community on surfaces exposed to air (Pentecost and Whitton, 2012). Moisture originates from different sources: precipitation, humidity, or groundwater seepage, and its level can be highly variable, so higher RH can contribute to better developed phototrophs reflected through higher WC and OM (Table 1). Well -hydrated biofilms contained more viable and active cells than the ones that were water deficient or temporarily dry, which is probably the reason why Chl *a* was usually higher in such samples. The rest of the sampling sites at Stopić Cave, especially the sites near artificial light, had lower Chl *a* and OM concentrations, as expected, because the lampenflora were poorly developed in this cave and existing biofilms represented the remains of the old lampenflora that had developed during the previous year and before the cave reconstruction and the installation of new and better artificial LED lighting. In Podpeć Cave the concentration of Chl *a* varied and had high values at the sampling site at the cave entrance, but also at the two sampling sites near artificial light (sites P1 and P3) where lampenflora were quite well developed (Fig. 2). Compared to Stopić Cave, lampenflora in Podpeć Cave, especially at P1 and P3, were more developed and characterized with biofilm where, among cyanobacteria, algae and many organic and inorganic particles, mosses were also present (*Amblystegium serpens* was dominant and *Tortella tortuosa* was recorded sporadically).

Biofilms from cave entrances and from the internal cave environment were also different in terms of WC, OM and IM. High WC in biofilm samples from cave entrances, which is especially evident from sampling site P5 (Fig. 8), was the

Table 2. Cyanobacterial and algal taxa from Podpeć (P1- P8) and Stopić (S1- S6) caves.

	Stopić Cave						Podpeć Cave				
	Inside the Cave			Cave Entrance			Inside the Cave				Cave Entrance
Taxa/Samples	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**P1**	**P2**	**P3**	**P5**	**P8**
Cyanobacteria											
Chroococcales											
Aphanocapsa cf. *planctonica* (G.M.Smith) Komárek & Anagnostidis						+					
Aphanocapsa muscicola (Meneghini) Wille				+	+						
Aphanocapsa rivularis (Carmichael) Rabenhorst						+					+
Aphanocapsa sp. Nägeli						+					
Aphanothece caldariorum P.G. Richter					+						+
Aphanothece saxicola Nägeli					+						
Asterocapsa spp. H.-J.Chu				+	+	+					+
Chondrocystis dermochroa (Nägeli) Komárek & Anagnostidis				+	+						+
Chroococcus cf. *spelaeus* Ercegovic						+					+
Chroococcus ercegovicii Komárek & Anagnostidis				+	+	+					+
Chroococcus pallidus Nägeli											
Chroococcus turgidus (Kützing) Nägeli					+	+					
Chroococcus varius A. Braun				+							
Chroococcus sp. Nägeli				+	+	+					+
Cyanothece aeruginosa (Nägeli) Komárek					+						+
Eucapsis sp. F.E. Clements & H.L. Shantz				+	+	+					+
Gloeocapsa alpina Nägeli				+							+
Gloeocapsa atrata Kützing					+	+		+			+
Gloeocapsa cf. *granosa* (Berkeley) Kützing				+							+
Gloeocapsa compacta Kützing				+							+
Gloeocapsa nigrescens Nägeli				+							+
Gloeocapsa punctate Nägeli				+		+					
Gloeocapsa violacea Kützing											
Gloeocapsa sp. Kützing						+					
Gloeothece cf. *incerta* Skuja				+							
Oscillatoriales											
Leptolyngbya foveolarum (Gomont) Anagnostidis & Komárek	+			+	+	+		+			+
Leptolyngbya henningsi(Lemmermann) Anagnostidis					+	+					
Leptolyngbya valderiana (Gomont) Anagnostidis & Komárek					+	+					
Leptolyngbya sp.1 Anagnostidis & Komárek				+	+	+					+
Leptolyngbya sp. Anagnostidis & Komárek				+	+	+					+
Phormidium cf. *ambiguum* Gomont											+
Phormidium corium Gomont ex Gomont						+					
Phormidium sp. Kützing ex Gomont											+
Porphyrosiphon fuscus Gomont ex Frémy					+						
Nostocales											
Nostoc commune Vaucher ex Bornet & Flahault											+
Nostoc punctiforme Hariot				+	+	+					+
Nostoc sp. Paracelsus					+	+					
Scytonema cf. *bruneum* Schmidle				+							
Scytonema drilosiphon Elenkin & V.I. Polyansky					+						
Scytonema hofmannii C. Agardh ex Bornet & Flahault						+					
Scytonema mirabile Wolle				+							
Scytonema subtile K. Möbius				+	+						
Scytonema sp. Agardh ex Bornet et Flahault				+							+
Tolypothrix tenuis Kützing ex Bornet & Flahault						+					
Chlorophyta											
Chlorella sp. Beyerinck	+	+	+				+	+	+	+	
Trochiscia sp. Kützing	+		+	+		+				+	
Bacillariophyta											
Hantzschia sp. Grunow											
Humidophila sp. (Lange-Bertalot & Werum) R.L. Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová		+									
Orthoseira spp. Thwaites	+	+	+								
Pinnularia sp. Ehrenberg						+	+				
Xanthophyta											
Xanthophyta unknown	+			+	+	+	+		+		

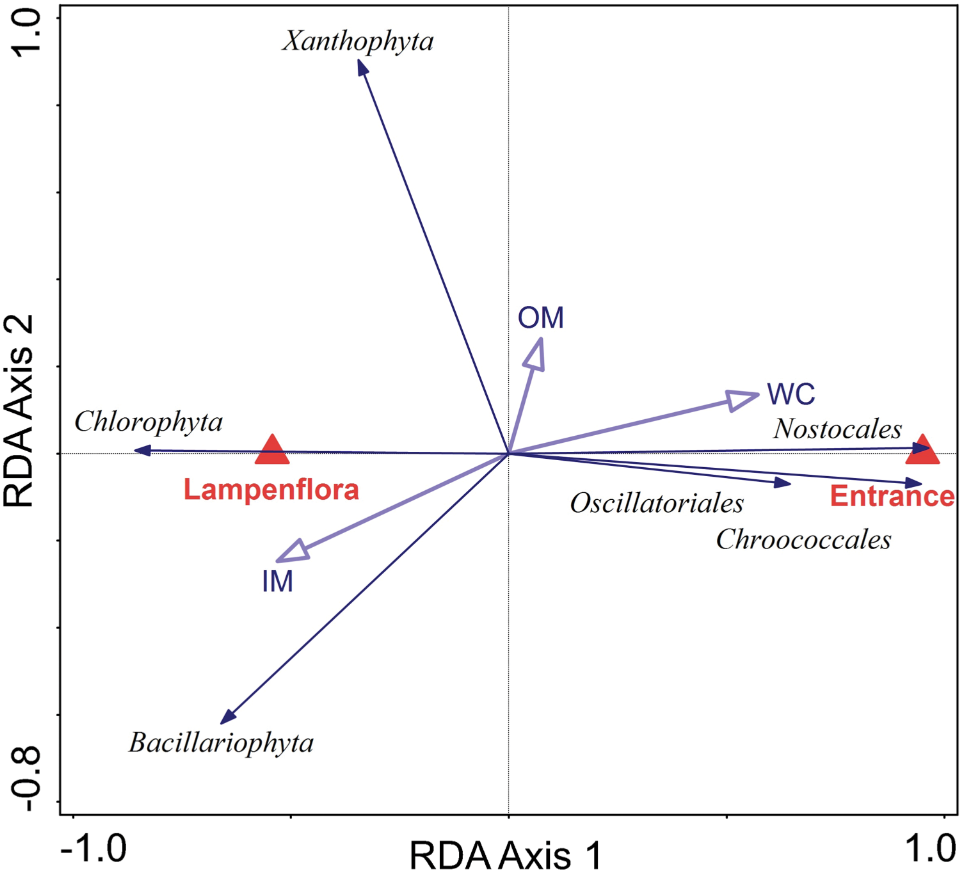

Figure 8. RDA analysis showing the relationship between explanatory variable, lampenflora and communities at cave entrances, and responce variables (*Cyanobacteria−Chroococcales*, *Oscillatoriales* and *Nostocales*), *Chlorophyta*, *Bacillariophyta* and *Xanthophyta*). Included supplemenatary variables are: content of organic (OM) and inorganic matter (IM) and water content (WC).

result of the presence and dominance of cyanobacteria. Cyanobacterial extracellular polysaccharides (EPS) play an important role in stress tolerance (Chug and Mathur, 2013) and thanks to these polysaccharides, cyanobacteria are not so vulnerable to variations in climatic conditions. One of their main roles is that they can retain water and enable cyanobacteria to better survive drought conditions, facilitating survival in an aerophytic habitat (Pentecost and Whitton, 2012; Chug and Mathur, 2013; Li et al., 2013).IM was higher in almost all lampenflora samples.

Lampenflora often cause the deterioration of stone substrata and cave formations. Lampenflora were especially very well-developed in Podpeć Cave, and at some sites, deteriorated parts of the stone base were mixed with biofilm components, which influenced IM content. The process of substrate deterioration in cave environments is of special concern, especially in caves with numerous attractive speleothems. The metabolic activities of microorganisms not only leads to undesired change in cave formations, but they can also disturb the habits of native organisms (Piano et al., 2015). The removal of lampenflora is achieved through various mechanical or chemical treatments. However, all such actions should be practicable and with minimal impact to the cave environment and organisms (Trih et al., 2018).

Conclusions

Cyanobacteria and algae were examined from biofilm samples taken from the Podpeć and Stopić caves. *Cyanobacteria*, *Chlorophyta*, *Bacillariophyta* and *Xanthophyta* were recorded, with the highest diversity found in the coccoid cyanobacteria. *Cyanobacteria* were dominant at cave entrances, while green algae were prominent elements of caves' lampenflora. Cf. *Chlorella* sp. was recorded in every lampenflora sample. Ecological parameters did not vary significantly, except for the LI that was dependent on the different aspects of cave entrances (i.e., their size) and sampling sites. Biofilm parameters (water content, content of organic and inorganic matter) varied greatly between samples collected near entrances and inside the caves. Chlorophyll *a* did not show clear correlations with any of the other measured parameters. The metabolic activity of green algae, which usually compromise part of the lampenflora, causes biodeterioration of the stone substratum and can lead to the irreversible damage of cave structures. Further investigations are necessary, since the knowledge on cave biofilms on the Balkan Peninsula is limited.

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References

Albertano, P., 2012, Cyanobacterial biofilms in monuments and caves, *in*: Whitton B.A., ed., Ecology of Cyanobacteria II: Their Diversity in Space and Time: London, Springer, p. 317–343. https://doi.org/10.1007/978-94-007-3855-3_11.

Aleya, L., 1991, The concept of ecological succession applied to an eutrophic lake through the seasonal coupling of diversity index and several parameters: Archiv für Hydrobiologie, v. 120, p. 327–343.

Borderie, F., Tête, N., Cailhol, D., Alaoui-Sehmer, L., Bousta, F., Rieffel, D., Aleya, L., and Alaoui-Sossé, B., 2014, Factors driving epilithic algal colonization in show caves and new insights into combating biofilm development with UV-C treatments: Science of the Total Environment, v. 48, p. 43–52. https://doi.org/10.1016/j.scitotenv.2014.03.043.

Cennamo, P., Marzano, C., Ciniglia, C., Pinto, G., Cappelletti, P., Caputo, P., and Pollio, A., 2012, A survey of the algal flora of anthropogenic caves of Campi Flegrei (Naples, Italy) archeological district: Journal of Cave and Karst Studies, v. 74 no. 3, p. 243–250. https://doi.org/10.4311/2011JCKS0194.

Chang, T.P., and Chang-Schneider, H., 1991, Algen in vier süddeutschen Höhlen(Algae in four southern German caves): Berichte der Bayer Bot Gesell, no. 62, p. 221−229. https://doi.org/10.1007/978-3-662-13231-9_14.

Chug, R., and Mathur, S., 2013, Extracellular polymeric substances from Cyanobacteria: Characteristics, isolation and biotechnological applications — A review: International Journal of Advances in Engineering, Science and Technology (IJAEST), v.3, no.2, p. 49-53. ISSN : 2249−913X.

Cigna, A.A., and Burri, E., 2000, Development, management and economy of show caves: International Journal of Speleology, v. 29, p. 1−27. https://doi.org/10.5038/1827-806X.29.1.1.

Cvijić, J., ed., 1914a, Pećina u Potpeći (promatranja iz 1913.) Cave in Potpec (observation from 1913.): Glasnik Srpskog geografskog društva, sv. 3−4, Beograd.

Cvijić, J., ed., 1914b, Stopića pećina u Rožanstvu i ponor u Trnavi (Stopić Cave in village Rožanstvo and abyss in Trnava): Glasnik Srpskog geografskog društva, sv. 3 i 4, Beograd.

Czerwik-Marcinkowska, J., and Mrozińska, T., 2011, Algae and cyanobacteria in caves of the Polish jura: Polish Botanical Journal, v. 56, no. 2, p. 203−243.

Czerwik-Marcinkowska, J., 2013, Observations on aerophytic cyanobacteria and algae from ten caves in the Ojców National Park: Acta Agrobotanica, v. 66, no. 1, p. 39−52. https://doi.org/10.5586/aa.2013.005.

Czerwik-Marcinkowska, J., Wojciechowska, A., and Massalski, A., 2015, Biodiversity of limestone caves: aggregations of aerophytic algae and cyanobacteria in relation to site factors: Polish Journal of Ecology, v. 63, p. 481–499.

Dobat, K., 1998, Flore de la lumiére artificiélle (lampenflora-maladieverte) [Flora of artificial light (lampenflora – green desease)], *in* Juberthie, C., and Decu, V., eds., Encyclopaedia Biospeleologica, Tome 2, Société de Biospéologie: Moulis-Bucarest, p. 1325–1335.

Djurović, P., ed., 1998, Speleoloski atlas Srbije (Speleological atlas of Serbia): Serbian Academy of Science and Art, Belgrade. 290p.

Ercegović, A., 1925, La végétation des lithophytes sur les calcaires et les dolomites en Croatie (The lithophyte vegetations on limestones and dolomites in Croatia): Acta Bot Croat, v. 1, p. 64–114.

Ercegović, A., 1932, Études écologiques et sociologiques des Cyanophycées lithophytes de la côteYougoslave de l'Adriatique (Ecological and sociological studies of lithophytes cyanophyceae on the Yugoslavian Adriatic coast): Bulletin of the International Academy of Yugoslavia, Science Beaux Arts v. 26, p. 33–56.

Ettl, H., 1978, Xanthophyceae. 1. Teil. *in*: Ettl, H., Gerloff, J., and Heynig, H., eds., Süßwasserflora von Mitteleuropa (Freshwater flora from Central Europe), 3: Gustav Fischer Verlag, Jena.

Ettl, H., and Gärtner, G., 1988, Chlorophyta II, Tetrasporales, Chlorococcales, Gloeodendrales. *in*: Ettl, H., Gerloff, J., Heynig, H., and Mollenhauer, D. eds., Süßwasserflora von Mitteleuropa (Freshwater flora from Central Europe), 10: Gustav Fischer Verlag, Stuttgart, New York.

Falasco, E., Ector, L., Isaia, M., Wetzel, C., E., Hoffman, L., and Bona, F., 2014, Diatom flora in subterranean ecosystems: a review: International Journal of Speleology, v. 43, n.3, p. 231−251. https://doi.org/10.5038/1827-806X.43.3.1.

Golubić, S., 1967, Algen vegetation der Felsen. Eine Öko-logische Algen studien im Dinarischen Karstgebiet (Algae vegetation of the rocks. An ecological algae study in the Dinaric karst area), *in*: Elster, H.J., and Ohle, W., eds, Die Biennenge wässer Schweitzerbart'sche Verlagsbuchhandlung ,Stuttgart, v. 22, p. 1–183.

Hajdu, L., 1977, The flora of Hungarian caves, Karsztés Barlang, Special Issue, Budapest.

Hofmann, G., Werum, M., and Lange-Bertalot, H., 2013, Diatomeen im Süßwasser— Benthos von Mitteleuropa. Bestimmungs flora Kieselalgenfür die ökologische Praxis (Freshwater diatoms — benthos from Central Europe. Determination diatoms flora for ecological practice): Königstein, Koeltz Scientific Books. 908 p.

Jimenez, S.C., 2012, Microbiological and environmental issues in caves: World Journal of Microbiology and Biotechnology, v. 28, p. 2453–2464. https://doi.org/10.1007/s11274-012-1070-x.

John, D.M., Whitton, B.A., and Brook, A.J., eds., 2003, The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae: UK, Cambridge University Press. 702 p.

Jurado, V., Laiz, L., Rodriguez-Nava, V., Boiron, P., Hermosin, B., Sanchez-Moral, S., and Saiz-Jimenez, C., 2010, Pathogenic and opportunistic microorganisms in caves: International Journal of Speleology, v. 39, p. 15–24. https://doi.org/10.5038/1827-806X.39.1.2.

Klemenćič, A.K., and Vrhovšek, D., 2005, Algal flora of Krška Jama Cave, Slovenia: Acta Musei Nationalis Pragae, Series B, Historia Naturalis, v. 61, no. 1–2, p. 77–80.

Komárek, J., and Fott, B., 1983, Chlorophyceae (Grünalgen). Ordnung: Chlorococcales. Das Phytoplankton děs Süßwassers, Systematik und Biologie (Freshwater phytoplankton, systematics and biology), *in* Elster, H.J., and Ohle, W., eds., Die Binnengewässer XVI, 7 (1): Stuttgart, Germany, Schweizerbart'scheVerlagsbuchhandlung, 1044 p.

Komárek, J., and Anagnostidis, K., 1998, Cyanoprokaryota 1. Teil/1st Part: Chroococcales, *in* Ettl, H., Gärtner, G., Heynig, H., and Mollenhauer, D., eds., Süßwasserflora von Mitteleuropa (Freshwater Flora From Central Europe) 19/1: Jena-Stuttgart-Lübeck-Ulm, Gustav Fischer, 548 p.

Komárek, J., and Anagnostidis, K., 2005, Cyanoprokaryota 2. Teil: Oscillatoriales, *in* Ettl, H., Gärtner, G., Heynig, H., and Mollenhauer, D., eds., Süßwasser flora von Mitteleuropa (Freshwater Flora From Central Europe), 19/2: Berlin, Spektrum Akademischer Verlag, 759 p.

Komárek, J., ed., 2013, Süßwasserflora von Mitteleuropa Bd 19/3 (Freshwater flora from Central Europe): Cyanoprokaryota 3: Heterocystous genera: Heidelberg, Springer Spektrum, 1130 p. https://doi.org/10.1007/978-3-8274-2737-3.

Lazarević, R., ed., 2012, Stopića pećina, Zlatibor, II dopunjeno izdanje(Stopić Cave, Zlatibor, 2^nd^ edition): Turistička Organizacija Zlatibor, p.94.

Li, P., Harding, E.S., and Liu, Z., 2013, Cyanobacterial exopolysaccharides: Their nature and potentional biotechnological applications: Biotechnology and Genetic Engineering Reviews, v. 18, no. 1, p. 375−404. https://doi.org/10.1080/02648725.2001.10648020.

Lowe R.L., Kociolek J.P., Johansen J.R., Van de Vijver, B., Lange-Bertalot, H. and Kopalová, K., 2014 , Humidophila gen. nov., a new genus for a clade of diatoms (Bacillariophyta) formerly within the genus Diadesmis: species from Hawai`i, including one new species: Diatom Research, v.29, p. 351−360. https://doi.org/10.1080/0269249X.2014.889039.

Lowe, R.L., Kociolek, J.P., You, Q., Wang, Q., and Stepanek, J., 2017, Diversity of the diatom genus *Humidophila* in karst areas of Guizhou, China: Phytotaxa, v. 305, no. 4, p. 269−284. https://doi.org/10.11646/phytotaxa.305.4.3.

Macedo, F.M., Miller, Z.A., Dionísio, A., and Saiz-Jimenez, C., 2009, Biodiversity of cyanobacteria and green algae on monuments in the Mediterranean Basin: an overview: Microbiology, v. 155, p. 3476−3490. https://doi.org/10.1099/mic.0.032508-0.

Marković, B., ed., 1957, Opšti pregled geološko-tektonskih osobina terena u dolini Velikog Rzava kod Drežnika (zapadnaSrbija) (General overview of the geological-tectonic features of terrain in VelikiRzav valley near Dreznik (West Serbia)): Zbornik radova Geološkog instituta, Jovan Žujović“, knj. IX, Beograd.

Martinčić, A., Vrhovšek, D., and Batič, F., 1981, Flora v jamah z umetno osvetlitvijo (Flora in caves with artificial lighning): Biološki Vestnik, v. 29, p. 27–56.

Mazina, S.E., and Severin, A.V., 2007, Development of a method for rehabilitation of anthropogenically transformed underground ecosystems: The example of the New Athos Cave: Journal of Ecological Chemistry, v. 16, no. 3, p. 175–181.

Mazina, S.E., and Maximov, V.N., 2009, Photosynthetic organism communities of the Akhshtyrskaya Excursion Cave: Moscow University Biological Sciences Bulletin, v. 66, no. 1, p. 37–41. https://doi.org/10.3103/S009639251101007X.

Meyer, E., Seale, L.D., Permar, B., and McClary, A., 2017, The effect of chemical treatments on lampenflora and a Collembola indicator species at a popular tour cave in California, USA: Environmental Management, v. 59, no. 6, p. 1034−1042. https://doi.org/10.1007/s00267-017-0842-3.

Mulec J. 2008, Microorganisms in hypogeon: examples from slovenian karst caves: Acta Carsologica, v. 37, no. 1, p. 153−160.

Mulec, J., and Kosi, G. 2008, Algae in the aerophytic habitat of Račiške ponikve Cave (Slovenia): Natura Sloveniae, v. 10, no. 1, p. 39–49.

Mulec, J., Kosi, G., and Vrhovšek, D., 2008, Characterization of cave aerophytic algal communities and effects of irradiance levels on production of pigments: Journal of Cave and Karst Studies, v. 70, no. 1, p. 3–12.

Mulec, J., and Kosi, G., 2009, Lampenflora algae and methods of growth control: Journal of Cave and Karst Studies, v. 71, no. 2, p. 109–115.

Mulec, J., Krištůfek, V., and Chroňáková, A., 2012, Comparative microbial sampling from eutrophic caves in Slovenia and Slovakia using RIDA ® COUNT RIDACOUNT test kits: International Journal of Speleology, v. 41, no. 1, p. 1–8. https://doi.org/10.5038/1827-806X.41.1.1.

Pedersen, K., 2000, Exploration of deep intraterrestrial microbial life: current perspective. Mini Review: FEMS Microbiology Letters, v. 185, p. 9–16. https://doi.org/10.1111/j.1574-6968.2000.tb09033.x.

Pentecost, A., and Whitton, B.A., 2012, Subaerial Cyanobacteria, *in*: Whitton B.A. ed., Ecology of Cyanobacteria II: Their Diversity in Space and Time: London, Springer, p. 291−315. https://doi.org/10.1007/978-94-007-3855-3_10.

Piano, E., Bona, F., Falasco, A., La Morgia, V., Bađino, G., and Isaia, M., 2015, Environmental drivers of phototrophic biofilms in an Alpine show cave (SW – Italian Alps): Science of the Total Environment, v. 536, p. 1007−1018. https://doi.org/10.1016/j.scitotenv.2015.05.089.

Popović, S., Subakov Simić, G., Stupar, M., Unković, N., Predojević, D., Jovanović, J., and Ljaljević Grbić, M., 2015a, Cyanobacteria, algae and microfungi present in biofilm from Božana Cave (Serbia): International Journal of Speleology, v. 44, no. 2, p. 141–149. http://dx.doi.org/10.5038/1827-806X.44.2.4.

Popović, S., Subakov Simić, G., Stupar, M., Unković, N., Predojević, D., Blagojević, A. and Ljaljević Grbić M., 2015b, Cyanobacteria, algae and microfungi from Degurić cave, west Serbia: Abstract presented at 2015, 6th Balkan Botanical Congress, University Campus Rijeka, Croatia, p. 100, 14−18 September.

Popović, S., Jovanović, J., Predojević, D., Trbojević, I., Blagojević, A., Jakovljević, O., and Subakov Simić, G., 2016a, Cyanobacteria and algae from biofilms: the comparison of phototrophic microorganism community from cave entrance and lampenflora - Lazareva cave, Serbia: Abstract presented at 2016, 5th Congress of Ecologists of Macedonia, Macedonia (Ohrid) p.125, 19−22 October.

Popović, S., SubakovSimić, G., Korać, A., Golić, I., and Komarek, J., 2016b, Nephrococcus serbicus, a new coccoid cyanobacterial species from Božana Cave, Serbia: Phytotaxa, v. 289, no.2, p. 135−146. https://doi.org/10.11646/phytotaxa.289.2.3.

Popović, S., Jovanović, J., Predojević, D., Trbojević, I., Blagojević, A., and Subakov Simić, G., 2016c, Phototrophic microorganisms in biofilm samples from Vernjika Cave, Serbia: Abstract EGU2016-16064 presented at 2016 EGU General Assembly, Vienna, Austria. Geophysical Research Abstracts, v. 18, 17−22 April.

Popović, S., Subakov Simić, G., Stupar, M., Unković, N., Krunić, O., Savić, N., and Ljaljević Grbić, M., 2017a, Cave biofilms: characterization of phototrophic cyanobacteria and algae and chemotrophic fungi from three caves in Serbia: Journal of Cave and Karst Studies, v. 79, no. 1, p. 10–23. https://doi.org/10.4311/2016MB0124.

Popović, S., Jovanović, J., Blagojević, A., Trbojević, I., Predojević, D., Nikolić, N., Vidović, M., and Subakov Simić, G., 2017b, Diversity of epilithic and endolithic cyanobacteria and green algae at the entrance of two caves in Serbia: Abstract ISSN 0031-8884 presented at 2017, 11th International Phycological Congress, Szczecin, Poland, Phycologia, Congress Abstracts, v. 56, no.4, Supplement, p. 150−151. 13–19 August.

Smith, T., and Olson, R., 2007, A taxonomic survey of Lamp Flora (Algae and Cyanobacteria) in electrically lit passages within Mammoth Cave National Park, Kentucky: International Journal of Speleology, v. 36, no. 2, p. 105−114. https://doi.org/10.5038/1827-806X.36.2.6.

Ter Braak, C.J.F., and Šmilauer, P., 2012, Canoco reference manual and user’s guide: software for ordination, version 5.0. Microcomputer Power, Ithaca, USA.

Tofilovska, S., Wetzel, E.C., Ector, L., and Levkov, Z., 2014, Observation on Achnanthes Bory sensu stricto (Bacillariophycae) from subaerial habitats in Macedonia and comparation with the type of material of A. coarctata (Brebisson ex W.Smith) Grunow, A. coarctata var. sinaensis Hustedt and A. intermedia Kutizing: Fottea,Olomouc, v. 14, no. 1, p. 15−42. https://doi.org/10.5507/fot.2014.002.

Trinh, D.A., Trinh, Q.H., Tran, N., Guinea, J.G., and Mattey, D., 2018, Eco-friendly remediation of lampenflora on speleothems in tropical karst caves: Journal of Cave and Karst Studies, v. 80, no. 1, p. 1−12 https://doi.org/10.4311/2017ES0101.

Urzi, C., and De Leo, F., 2001, Sampling with adhesive tape strips: an easy and rapid method to monitor microbial colonization on monument surfaces: Journal of Microbiological Methods, v. 44, p. 1–11. https://doi.org/10.1016/S0167-7012(00)00227-X.

Vinogradova, O.N., Kovalenko, O.V., Wasser, S.P., Nevo, E., and Weinstein-Evron, M., 1998, Species diversity gradient to darkness stress in bluegreen algae/cyanobacteria: a microscale test in a prehistoric cave, Mount Carmel, Israel: Isreali Journal of Plant Science. v. 46, p. 229–238. https://doi.org/10.1080/07929978.1998.10676732.

Vinogradova, O.N., Nevo, E., and Wasser, S.P., 2009, Algae of the Sefunim Cave (Israel): species, diversity affected by light, humidity and rock stresses: International Journal of Algae, v. 11, p. 99–116. https://doi.org/10.1615/InterJAlgae.v11.i2.10. www. clipart-library.com.

Francesco Ballarin. *Domitius Culsu* sp. nov. (Araneae, Nesticidae), a new troglobiont spider from Italy with notes on Italian nesticids of the genus *Domitius* Ribera, 2018. *Journal of Cave and Karst Studies*, v. 82, no. 2, p. 82-94. DOI:10.4311/2019LSC0103

DOMITIUS CULSU SP. NOV. (ARANEAE, NESTICIDAE), A NEW TROGLOBIONT SPIDER FROM ITALY WITH NOTES ON ITALIAN NESTICIDS OF THE GENUS *DOMITIUS* RIBERA, 2018

Francesco Ballarin[1, 2]

Abstract

Seven species from the spider family Nesticidae are currently known for the Italian fauna. Three Italian nesticids belong to the newly-established genus *Domitius* Ribera, 2018. All these species show a restricted distribution along the Apennine mountain chain and deep adaptation to cave life. Herein, a fourth species, *D. culsu* sp. nov. from a single cave in Northern Apennines is described. Detailed illustrations and diagnosis of the new species are provided. Molecular and morphological analysis of both sexes of *D. culsu* sp. nov. supports the validity of the new species and its close relationship with the other *Domitius* species from the same geographical area. A close affinity with the species distributed in the Iberian Peninsula is also observed. The potential susceptibility of *D. culsu* sp. nov. to external disturbance, and its extremely limited distribution, makes this spider of interest for conservation.

INTRODUCTION

Nesticidae Simon, 1894 is a small family of spiders with a worldwide distribution. Currently, 278 species and 16 genera are recognized (World Spider Catalog, 2020). At temperate latitudes, nesticids mostly occur in dark, damp environments such as caves, often showing high levels of endemism. Previously, the majority of nesticid species were included in the genus *Nesticus* Thorell, 1869. *Nesticus* has recently been partially revised (Lin et al., 2016; Pavlek and Ribera, 2017; Ribera, 2018) and several of its European species moved to different genera (e.g. *Typhlonesticus* Kulczyński, 1914, *Kryptonesticus* Pavlek and Ribera, 2017, *Domitius* Ribera, 2018).

Eight nesticid species belonging to five genera are currently known in Italy: *Domitius menozzii* (di Caporiacco, 1934), *D. sbordonii* (Brignoli, 1979), *D. speluncarum* (Pavesi, 1873), *Eidmannella pallida* (Emerton, 1875), *Kryptonesticus eremita* (Simon, 1880), *Nesticus cellulanus* (Clerck, 1757), *Typhlonesticus idriacus* (Roewer, 1931), and *T. morisii* (Brignoli, 1975) (Pantini and Isaia, 2019). The Italian *Domitius* species are all considered troglobionts (Mammola and Isaia, 2017) showing extreme adaptations to the subterranean environment (e.g. reduction of eyes, depigmentation, and elongation of legs). Such spiders are characterized by a limited distribution, and are endemic to a small number of caves in Northern (*D. menozzii*, *D. speluncarum*) or Central (*D. sbordonii*) Apennines mountains (Brignoli, 1979, Ribera, 2018). Initially, the taxonomy and geographic range of *D. menozzii* and *D. speluncarum* were uncertain: the two species were often mistaken for each other, or considered as subspecies or a synonymy of *K. eremita* (see Brignoli, 1971 202–205, in Italian). Dresco (1966) and Brignoli (1971) revised the taxonomy of *D. menozzii* and *D. speluncarum*, pinpointing their differences. Nevertheless, there is still occasional confusion regarding their taxonomic status: for example, *D. speluncarum* is sometimes wrongly attributed to the Dinaric Alps (Pavlek and Ribera, 2017).

Individuals of *D. speluncarum* and *D. menozzii* were found by the author while collecting in caves in Liguria and Toscana Regions (Italy). Specimens from one cave appeared to show distinct morphological differences. A more detailed examination of genitalia revealed that those specimens represented a new species. In this paper, the new species is described. The morphological differences between the Italian *Domitius* species are explored in detail, and the precise geographical distribution of the genus in the Italian peninsula is illustrated. To better establish the systematic position of the species, a phylogenetic tree of the genus *Domitius*, in relation with the other main European nesticid genera, is carried out.

MATERIAL AND METHODS

Taxonomy

Fresh specimens were hand-collected in caves and fixed in 96 % ethanol for molecular and morphological analysis. Photographs and measurements of the samples were taken at the Museo Civico di Storia Naturale of Verona, Italy, using a Leica DFC450 digital camera mounted on a Leica M165C steremicroscope. A Leiz Diaplan microscope was used to photograph the vulvae. Images were subsequently combined using Helicon Focus 6 image stacking software. The left palps of males were photographed. Epigynes were dissected using a sharp needle and boiled for a few minutes in a 20 % KOH solution to show the vulval internal structures. Leg measurements are given as following: total length (femur, patella,

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tibia, metatarsus, tarsus). All measurements are reported in millimeters. Unless otherwise specified, type descriptions are based on wet specimens in ethanol. Specimens used in this study are stored in the collections of the Museo Civico di Storia Naturale of Verona (MSNV) and Museo Civico di Storia Naturale “E. Caffi” of Bergamo (MSNB). The following abbreviations are used in the text and figures: AM = anterior median eyes; AL = anterior lateral eyes; C = conductor complex; Cd = copulatory duct; Co = copulatory opening; Da 1-3 = dorsal apophyses of the paracymbium; Di = distal apophysis of the paracymbium; E = embolus; Id = insemination duct; Ma = median apophysis; Ms = median septum; P = paracymbium; Pc 1-3 = processes of the conductor complex; PM = posterior median eyes; PL = posterior lateral eyes; S = spermatheca; St = subtegulum; T = tegulum; Va = ventral apophysis of the paracymbium; Vp = vulval pocket.

Breeding

To increase the available number of adult specimens and to obtain information about the life-cycle of the species for further research, juveniles of *D. culsu* sp. nov. at different growth stages were collected and raised in captivity in the basement of the Museo Civico di Storia Naturale of Verona. Each specimen was kept in a box (size 10 × 5 × 3 cm) made of plaster, with a glass lid and a layer of cave mud on the bottom. All boxes were placed in a large plastic tray with a transparent plexiglass lid and a layer of plaster on the bottom. To maintain the correct degree of humidity, the bottom of the tray was moistened with water every two weeks. Specimens were frequently checked and fed with fruit flies or mosquitoes approximately once or twice per month.

Molecular Analysis

Sequences of *Domitius* species and other European nesticids were obtained from freshly collected specimens or acquired from the GenBank online database (GenBank, 2018). Since preliminary phylogenetic analysis of the family suggests *Gaucelmus* is a sister clade to all Nesticidae *sensu stricto* (Ballarin and Li, in prep.), *Gaucelmus augustinus* Keyserling, 1884 from North America was set as an outgroup to root the tree. Extraction of DNA, and PCR amplification, were performed in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (IZCAS). All fresh specimens used for the molecular analysis were identified at species level using morphology, before storing at −20 °C at IZCAS. For each species, total genomic DNA was extracted from two legs of an adult specimen using a TIANamp Genomic DNA Kit (TIANGEN) under the standard protocol suggested by the manufacturer. The PCR amplifications were performed with an Eppendorf thermal cycler (Hamburg, Germany) using a final volume of 25µL. Purified PCR products were sequenced in both directions using an ABI 377 automatic sequencer (Applied Biosystems, Foster City, CA, USA) with a BigDye terminator cycle sequencing ready reaction kit. Partial fragments of the mitochondrial genes cytochrome c oxidase subunit I (COI) and 16S rRNA (16S) and the nuclear gene Histone H3 (H3) were selectively amplified following protocols and primers as indicated in Ballarin and Li (2018). Raw sequences were aligned using the online version of MAFFT v.7.0 (Katoh and Standley, 2013) under the algorithms G-INS-i for COI and H3 and Q-INS-i for 16S. Aligned sequences were subsequently visually inspected for mismatching and edited with BioEdit v.7.2.5 (Hall, 1999). A Maximum Likelihood (ML) analysis under a GTRGAMMA nucleotide substitution model was performed using the online version of RAxML v.8.2.0 (Stamatakis, 2014) on CIPRES Science Gateway V. 3.3 (Miller et al. 2010, available at: https://www.phylo.org/). One thousand replicates of rapid bootstrap were performed twice, using an individual gene partition scheme. Uncorrected pairwise-distance genetic divergences between the species was performed using MEGA v.7.0.14 (Tamura et al., 2013). The list of species used in the analysis and related GenBank accession numbers of the sequences are reported in Table 1.

RESULTS

Taxonomy

Class Arachnida Cuvier, 1812
Order Araneae Clerck, 1757
Family Nesticidae Simon, 1894
Genus *Domitius* Ribera, 2018
***Domitius culsu* Ballarin sp. nov.**
Figures 1A–G, 2A–E, 3A–D.
Nesticus speluncarum Brignoli, 1979: 214 (misidentification)

Type material. Holotype ♂. ITALY, ***Toscana***: Garfagnana area, Lucca Province, Coreglia Antelminelli Municipality, Tana delle Fate di Coreglia Antelminelli cave, 141/T/LU, 260m a.s.l., 44.046336°N 10.523525°E, 21.VIII.2018, leg. F. Ballarin and D. Avesani (MSNV). **Paratypes**. Same locality as the holotype, 1♀, 04.IX.1967, leg. A. Vigna Taglianti (MSNV) (Brignoli 1979, sub *Nesticus speluncarum*); 1♀, 15.VIII.2015, leg. F. Ballarin and M. Gaiga (MSNV); 4♀♀, 24.VI.2017, (3♀♀ collected as juveniles and raised in captivity, adults: 20.VIII.2017, 15.IX.2017 and 28.VI.2018 respectively), leg. F. Ballarin and R. Ballarin (MSNV); 4♀♀, 21.VIII.2018, leg. F. Ballarin and D. Avesani (MSNV, MSNB).

Table 1. Species, GenBank accession numbers, and locality of the specimens used in the phylogenetic analysis.

Species	Code	COI	16S	H3	Locality
Domitius baeticus	Dbae	MF693114	MF693118	MF693106	Cueva del Castillo. Siles, Jaén. Spain
Domitius luquei	Dluq	MF693112	EU746439	MF693104	Cueva de la Picona, San Pedro de Carmona, Cabuérniga, Cantabria, Spain
Domitius lusitanicus	Dlus	MF693113	EU746429	MF693105	Algar de Marradinhas II, Concelho de Alcanena, Portugal
Domitius menozzii	D213	MK860151[a]	MK860133[a]	MK860142[a]	Tanna da Suja, Prati di Bavari, Liguria, Italy
***Domitius culsu* sp. nov.**	**D555**	MK860152[a]	MK860134[a]	MK860143[a]	Tana delle Fate di Coreglia Antelminelli, Coreglia Antelminelli, Toscana, Italy
Domitius sbordonii	Dsbo	MF693110	MF693116	MF693102	Tana degli orchetti, Supino, Lazio, Italy
Domitius speluncarum	D557	MK860153[a]	MK860135[a]	MK860144[a]	Tana di Magnano, Canigiano, Lucca, Toscana, Italy
Nesticus cellulanus	N214	MK860154[a]	MK860136[a]	MK860145[a]	Cave of Koufovouno, Didimoticho, Thrace, Greece
Kryptonesticus dimensis	K566	MK860155[a]	MK860137[a]	MK860146[a]	Dim cave, Antalya, Turkey
Kryptonesticus eremita	K211	MK860156[a]	MK860138[a]	MK860147[a]	Grotta di Ponte Subiolo, Mori, Veneto, Italy
Carpathonesticus fodinarum	C162	MK860157[a]	MK860139[a]	MK860148[a]	Small cave along the river, Sighistel, Bihor, Romania
Carpathonesitcus lotriensis	C166	MK860158[a]	MK860140[a]	MK860149[a]	Humid and shadowed cliff near Lazaret village, Sibiu, Romania
Typhlonesticus obcaecatus	Tobc	KF939309	EU746437	MF693109	Cueva del Molino de Aso, Boltana, Huesca, Spain
Typhlonesticus idriacus	T167	MG201050	MG200521	MG201227	Grotta Pre Oreak, Nimis, Udine, Friuli Venezia Giulia, Italy
Typhlonesticus morisii	Tmor	KF939311	KF939308	···	Sotterranei del Forte di Vernante, Vernante, Cuneo, Italy
Gaucelmus augustinus	G601	MK860159[a]	MK860141[a]	MK860150[a]	Climax cave, Bainbridge, Georgia, USA

[a] New Sequences.

Examined comparative material. *Domitius speluncarum* (Pavesi, 1873): ITALY: *Liguria*: 1♂, 1♀, (topotypes), La Spezia Province, Grotta Bocca Lupara cave, 74/Li/SP, 120m a.s.l., 05.III.1969, leg. P.M. Brignoli (MSNV) (Brignoli, 1971); ***Toscana***: 1♀, Lucca Province, Garfagnana area, Villa Collemandina Municipality, Canigiano village, Tana di Magnano cave, 162/T/LU, 653m a.s.l., 44.177285°N, 10.38803°E, 03.XI.1967, leg. A. Vigna Taglianti (MSNV) (Brignoli, 1971), 1♀, 01.VIII.1975, leg. P. Magrini (MSNV), 4♀♀, 14.VIII.2015, leg. F. Ballarin and M. Gaiga (MSNV); 1♀, Fornovolasco (not reported in the label but very likely from Grotta del Vento cave), 700m a.s.l., 16.VI.1970, leg. O. Osella (MSNV) (Brignoli, 1971); 1♀, Grotta del Buggine cave, 166/T/Lu, 315m a.s.l., 07.X.1967, leg. G. Castellini (MSNV) (Brignoli, 1971); 1♀, Massa-Carrara, Buca della Freddana cave, 230/T/Ms, 550m a.s.l., 05.VI.1977, leg. C. Bonzano (MSNV) (Brignoli, 1985); 1♀, Buca del Bacile cave, 226/T/Ms, 10.III.1975, leg. unknown (MSNV) (Brignoli, 1985).

Domitius menozzii* (di Caporiacco, 1934): ITALY, *Liguria, 1♂, 4♀♀, north-east of Genova town, Tanna da Vulpe cave, 264/Li/GE, 23.XI.1969, leg. A. Vigna Taglianti (MSNV) (Brignoli, 1971); 2♂♂, 2♀♀, Creto, Tanna de Fate cave, 17/Li/GE, 30.X.1971, leg. G. Gardini (MSNV); 2♀♀ (topotypes), Prati di Bavari locality, Tanna da Suja cave, 5/Li/GE, 582m a.s.l., 44.422894°N, 09.035239°E, 30.V.2013, leg. F. Ballarin, A. Trotta, G. Gardini, and S. Zoia.

Domitius sbordonii* (Brignoli, 1979): ITALY, *Lazio, 1♂ (holotype), Frosinone Province, Supino, Valle Serena, Grotta della Croce cave, 01.IX.1977 leg. V. Sbordoni (MSNV) (Brignoli, 1979); 1♀ (paratype), 08.II.1976, leg. V. Sbordoni (MSNV) (Brignoli, 1979).

Kryptonesticus eremita* (Simon, 1880): ITALY, *Liguria: 2♂♂ (sub. *Nesticus menozzii*), Creto, Tanna de Fate cave, 17/Li/GE, 30.X.1971, leg. G. Gardini (MSNV); ***Emilia-Romagna:*** 2♀♀ Ravenna Province, Riolo Terme, Borgo Rivola, Grotta del Re Tiberio cave, 36/Er/RE. 19.II.1951, Leg. Denis (MSNV) (Zangheri, 1966, sub. *Nesticus speluncarum*); ***Toscana***: 1♀ Garfagnana area, Lucca Province, Coreglia Antelminelli Municipality, Tana delle Fate di Coreglia Antelminelli cave, 141/T/LU, 260m a.s.l., 44.046336°N, 10.523525°E, 15.VIII.2015, leg. F. Ballarin and M. Gaiga (MSNV); 3♀♀, 24.VI.2017, Leg. F. Ballarin and R. Ballarin (MSNV-MSNVRAr/m 0007); 1♀, 21.VIII.2018, leg. F. Ballarin and D. Avesani (MSNV); ***Campania:*** 1♀, Avellino Province, Bagnoli Irpino, Mt. Piacentini, Grotta Giovannino cave, 16.VI.1956, leg. S.Ruffo (MSNV) (Kritscher, 1958, sub. *Nesticus speluncarum*).

Etymology

The name of the new species is derived from the Etruscan goddess Culsu who, according to the Etruscan mythology, ruled the cave-like entrance of the underworld. Noun in apposition.

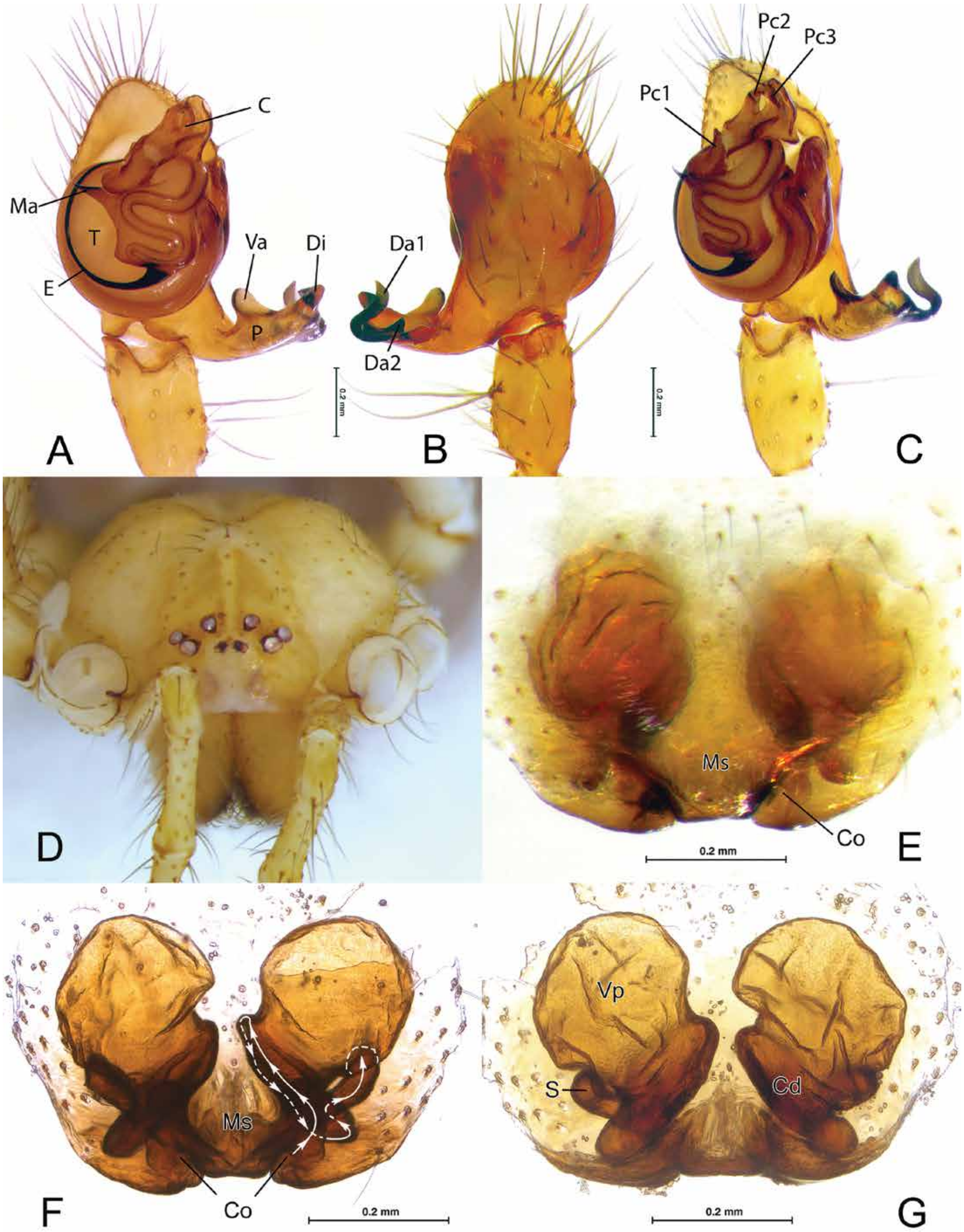

Figure 1. *Nesticus culs*u sp. nov. Male palp: A. ventral view; B. dorsal view; C. retrolateral view; D. female, cephalic region showing the eye pattern; E. epigyne, ventral view; F. epigyne after clearing, ventral view, the schematic course of internal ducts is outlined with a white line; G. vulva after clearing, dorsal view.

Diagnosis

Species closely related to *D. speluncarum* and *D. menozzii*. Males of *D. culsu* sp. nov. can be separated from males of all other Italian species of the genus *Domitius* by the different shape of the apophyses of the paracymbium (Figs. 1A–C, 2B,C vs. Fig. 4A–I). *D. culsu* **sp. nov** shows a robust, S-shaped dorsal apophysis 1 in contrast with a large, flat and axe-like Da 1 in *D. speluncarum* (Figs. 1A–C, 2B, C vs. Fig. 4A–C); a short and stumpy Da 1 in *D. menozzii* (Figs. 1A–C, 2B, C vs. Fig. 4D–F) or a long and thread-like Da 1 in *D. sbordonii* (Figs. 1A–C, 2B, C vs. Fig. 4G–I). Additionally, males of *D. culsu* sp. nov. have a well-developed, triangular median apophysis, absent in males of the other three species (Figs. 1A, 2A vs. Fig. 4A, D, G).

Female *D. culsu* sp. nov. can be easily distinguished from female *D. speluncarum* by the narrower, trapezoid-shaped median septum with slanting edges, in contrast with the larger, lobate Ms with rounded edges in *D. speluncarum* (Figs. 1E, 2D vs. Fig. 5A). Female *D. culsu* sp. nov. are separated from female *D. morisii* by the absence of a bulge on the Ms (clearly visible in *D. morisii*, Figs. 1E, 2D vs. Fig. 5C). Additionally, they can be distinguished by the different shape of copulatory ducts when the vulva is observed dorsally: with a rather uniform diameter in *D. culsu* sp. nov. and bearing a large, flattened middle trait in *D. morisii* (Figs. 1G, 2E vs. Fig. 5D). Female *D. culsu* sp. nov. are easily separated from those of *D. sbordonii* by the trapezoid-shaped Ms with slanting edges, in contrast with the squared Ms with vertical edges in *D. sbordonii* (Figs. 1E, 2D vs. Fig. 5E). They can further be distinguished by the different position of spermathecae, located in the lower half of the vulva and below the vulval pockets in *D. culsu* sp. nov., in contrast with S located in the upper half of vulva and above Vp in *D. sbordonii* (Figs. 1G, 2E vs. Fig. 5F).

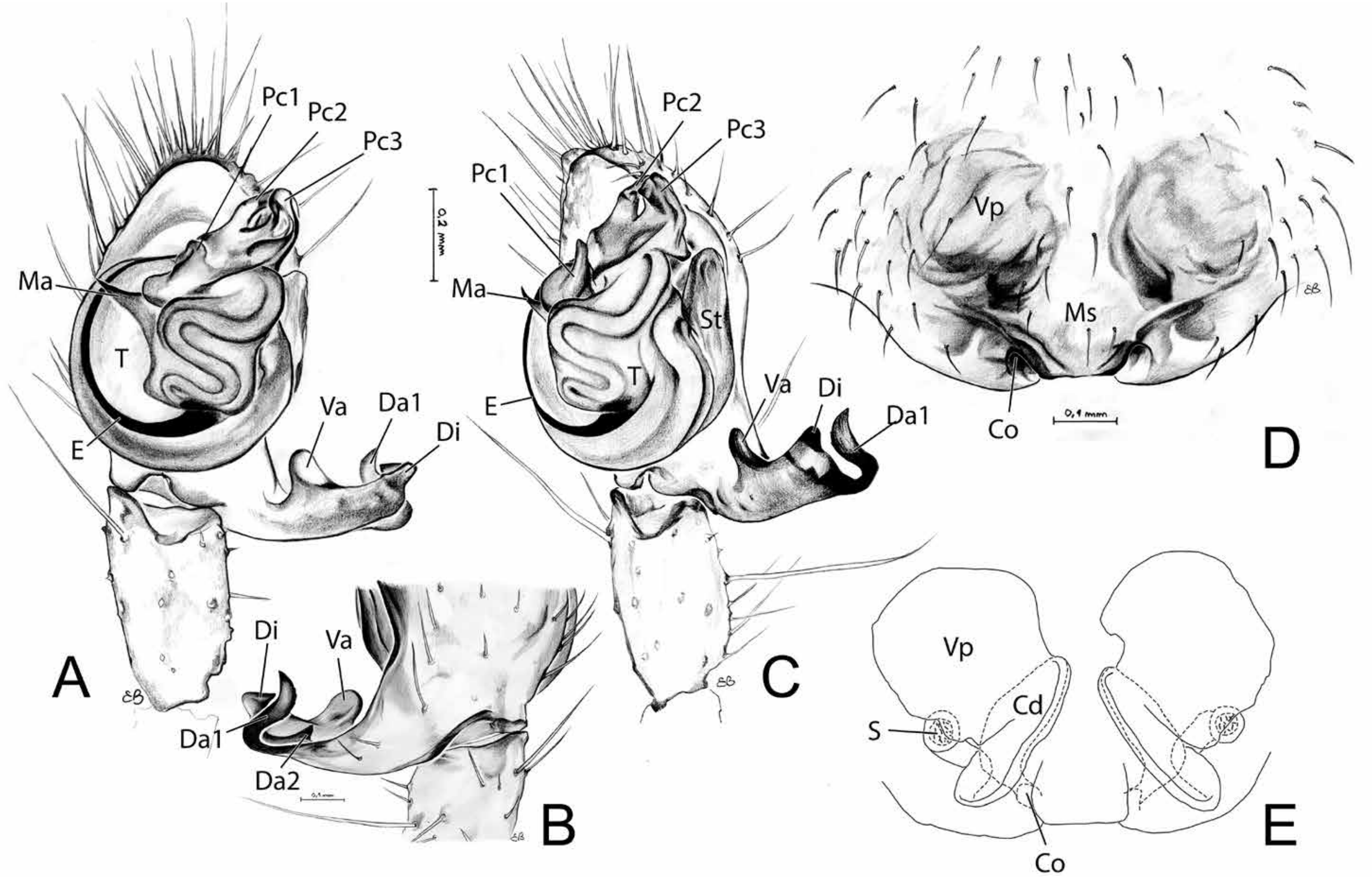

Figure 2. Genitalia of *Nesticus culsu* sp. nov. Male palp: A. ventral view; B. dorsal view of the paracymbium; C. retrolateral view; D. female epigyne, ventral view; E vulva, dorsal view.

Description. Male (holotype). Total length 4.19. Carapace: 1.81 long, 1.56 wide.

Habitus as in Fig. 3A. Carapace uniformly pale yellow with some sparse setae (more reddish while alive, see Fig. 3A). Cephalic region not clearly differentiated from the rest of carapace. Eyes reduced, AM missing, reduced to black maculae. Eye diameters: AM -, AL 0.079, PM 0.080, and PL 0.078. Thoracic grooves and fovea distinct. Mouthparts and sternum uniformly colored as the carapace. Promargin of chelicera with three teeth approximately of the same size, retromargin with several small denticles. Legs uniformly light yellowish. Legs measurements as follows: **I** 17.40 (4.89, 0.90, 4.78, 4.90, 1.93), **II** 14.03 (3.92, 0.80, 3.67, 3.76, 1.88), **III** 10.35 (3.19, 0.69, 2.53, 2.79, 1.15), **IV** 13.47 (4.34, 0.84, 3.53, 3.49, 1.27). Leg formula: **I**, **II**, **IV**, **III**. Opisthosoma gray-yellowish colored (lighter than carapace while alive, see Fig. 3A), covered with long hairs.

Palp as in Figs. 1A–C, 2A-C. Cymbium oval, covered with short, sparse setae, with a tuft of longer hairs in the prolateral distal area near the tip. Embolus filamentous, slender in the terminal part. Conductor complex with three distinct processes: Cp 1-3. Cp 1 stocky and roughly triangularly shaped, Cp 2 and Cp 3 located at the distal part of the bulb and diagonally protruding (approx. 2 o'clock seeing the left palp ventrally), their tips curved towards each other (Figs. 1A, C, 2A, C). Median apophysis well-developed, shaped as a long, sharp triangle, heading prolaterally (Figs. 1A, 2A). Paracymbium large with well-developed, sclerotized dorsal, distal and ventral processes. Two dorsal apophyses, Da 1-2: Da 1 robust and long, ending sharply, S-shaped when the palp is observed dorsally, Da 2 stocky, triangularly-shaped. Distal apophysis triangularly shaped. Ventral apophysis lobate, dorso-ventrally flattened and heading toward the cymbium (Figs. 1A–C, 2A-C).

Female (based on 4 paratypes). Total length 3.65–5.27. Carapace: 1.71–1.98 long, 1.54–1.64 wide.

Habitus as in Fig. 3B, C. Carapace uniformly yellowish with some sparse setae (often more reddish while alive, see Fig. 3B). Cephalic region not clearly differentiated from the rest of the prosoma. Eyes reduced, AM strongly reduced and barely visible, reduced to small, dark maculae in some specimens. Eye diameters: AM (when present): 0.032, AL: 0.087, PM: 0.078, and PL: 0.77. Thoracic grooves and fovea distinct. Mouthparts and sternum uniformly colored as in the carapace. Teeth of chelicera as in the male. Legs uniformly light yellowish. Leg measurements as follows: **I** 17.07 (4.38, 1.01, 4.95, 4.90, 1.83), **II** 13.69 (4.04, 0.92, 3.57, 3.64, 1.52), **III** 10.46 (3.43, 0.80, 2.50, 2.56, 1.17), **IV** 13.89 (4.64, 0.92, 3.59, 3.41,

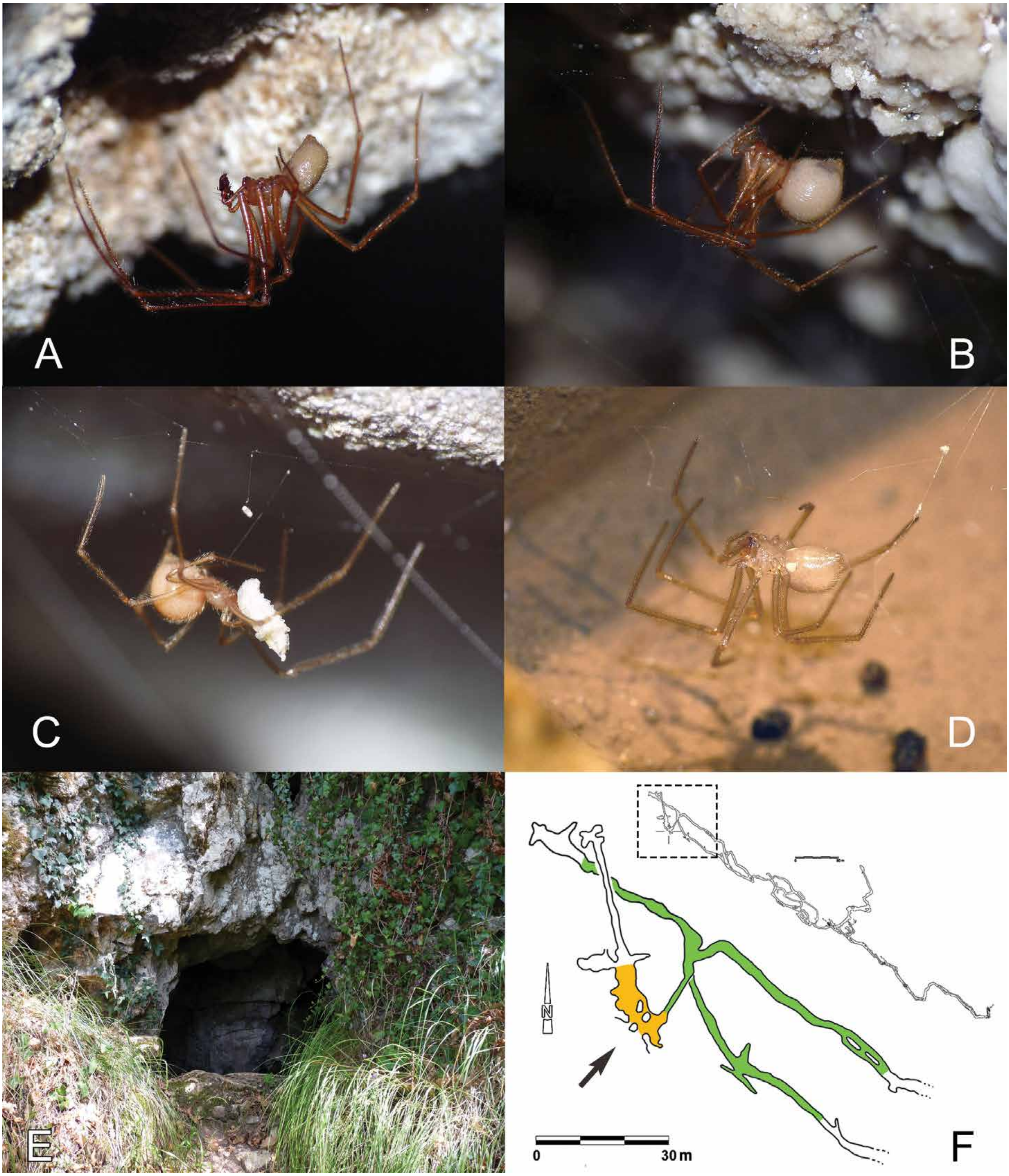

Figure 3. Habitus and type locality of *Domitius culsu* sp. nov. A. Habitus of male; B. habitus of female; C. female with prey; D. juvenile in captivity; E. entrance of Tana delle Fate di Coreglia Antelminelli cave; F. map of the cave and detail of the entrance, showing the spatial distribution of the two co-existing nesticid species living inside: green = *D. culsu* sp. nov., orange = *Kryptonesticus eremita*, arrow = entrance of the cave.

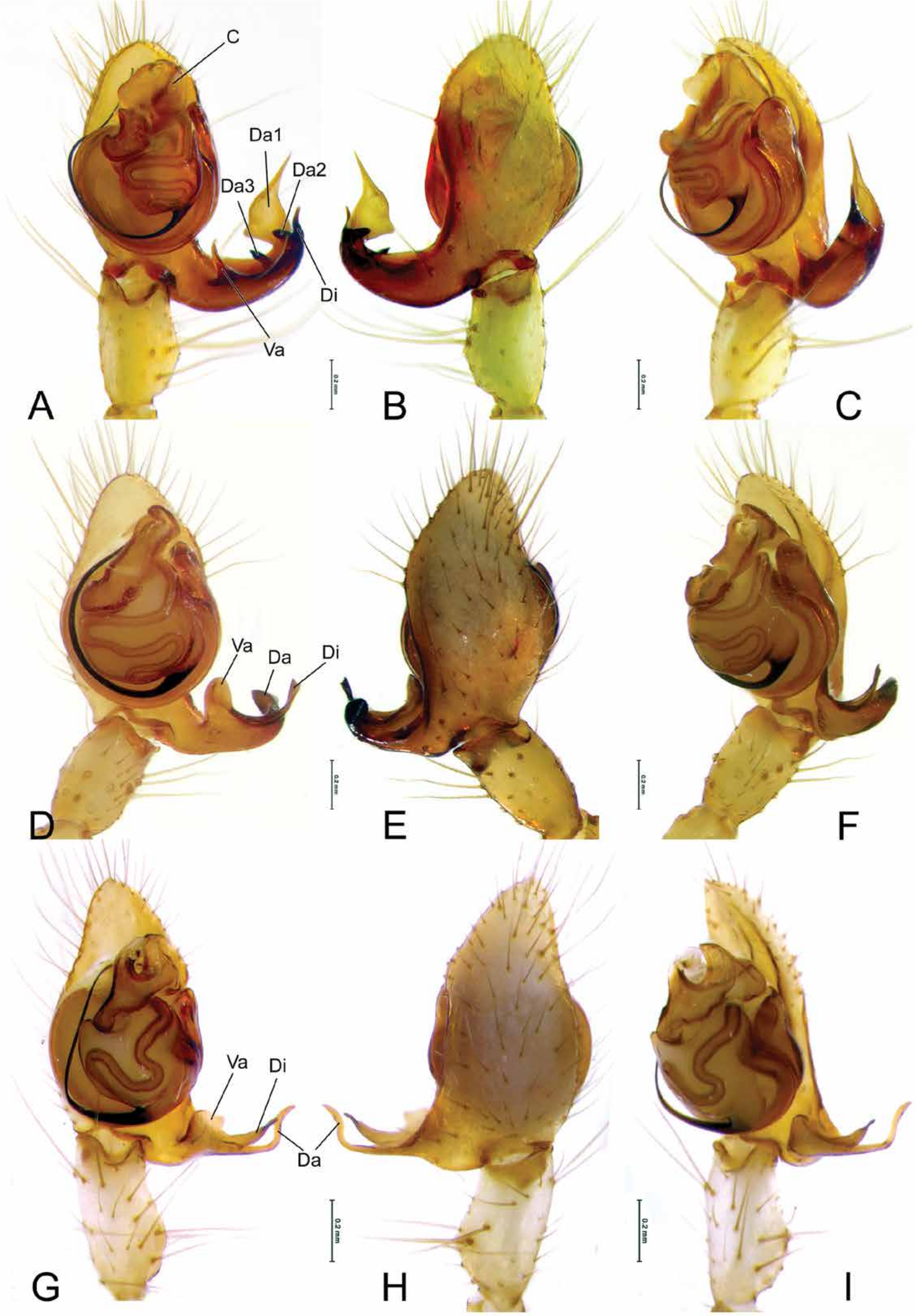

Figure 4. Male palps of the Italian *Domitius* species for comparison. *D. speluncarum* male palp: A. ventral view; B. dorsal view; C. retrolateral view; *D. menozzii* male palp: D. ventral view; E. dorsal view; F. retrolateral view; *D. sbordonii* male palp: G. ventral view; H. dorsal view; I. retrolateral view.

1.33). Leg formula: I, IV, II, III. Opisthosoma yellowish-gray (often lighter colored than carapace while alive, see Fig. 3B), covered with long hairs.

Epigyne as in Figs. 1E, F, 2D, E. Median septum short, not protruding, shaped as an inverted trapezoid with a narrower base. Vulval pockets and copulatory ducts externally visible by transparence trough the tegument. Copulatory openings at the lower, lateral side of median septum. Vulva as in Figs. 1G, 2E. Spermathecae small and round, located in the lower-half of the vulva, below vulval pockets and being partially covered by them. Vulval pockets wide and rounded, sac-shaped, located above spermathecae. Copulatory ducts with a wider diameter in the ventral trait and narrower in the dorsal trait, rolling up around the lower part of vulval pockets and reaching spermathecae with some turns (Figs. 1F, 2E). Insemination ducts beginning from the lower part of spermathecae and following the same course of copulatory ducts.

Distribution

Italy, endemic to the northern Apennines. Known only from the type locality; Tana delle Fate di Coreglia Antelminelli cave (Fig. 6).

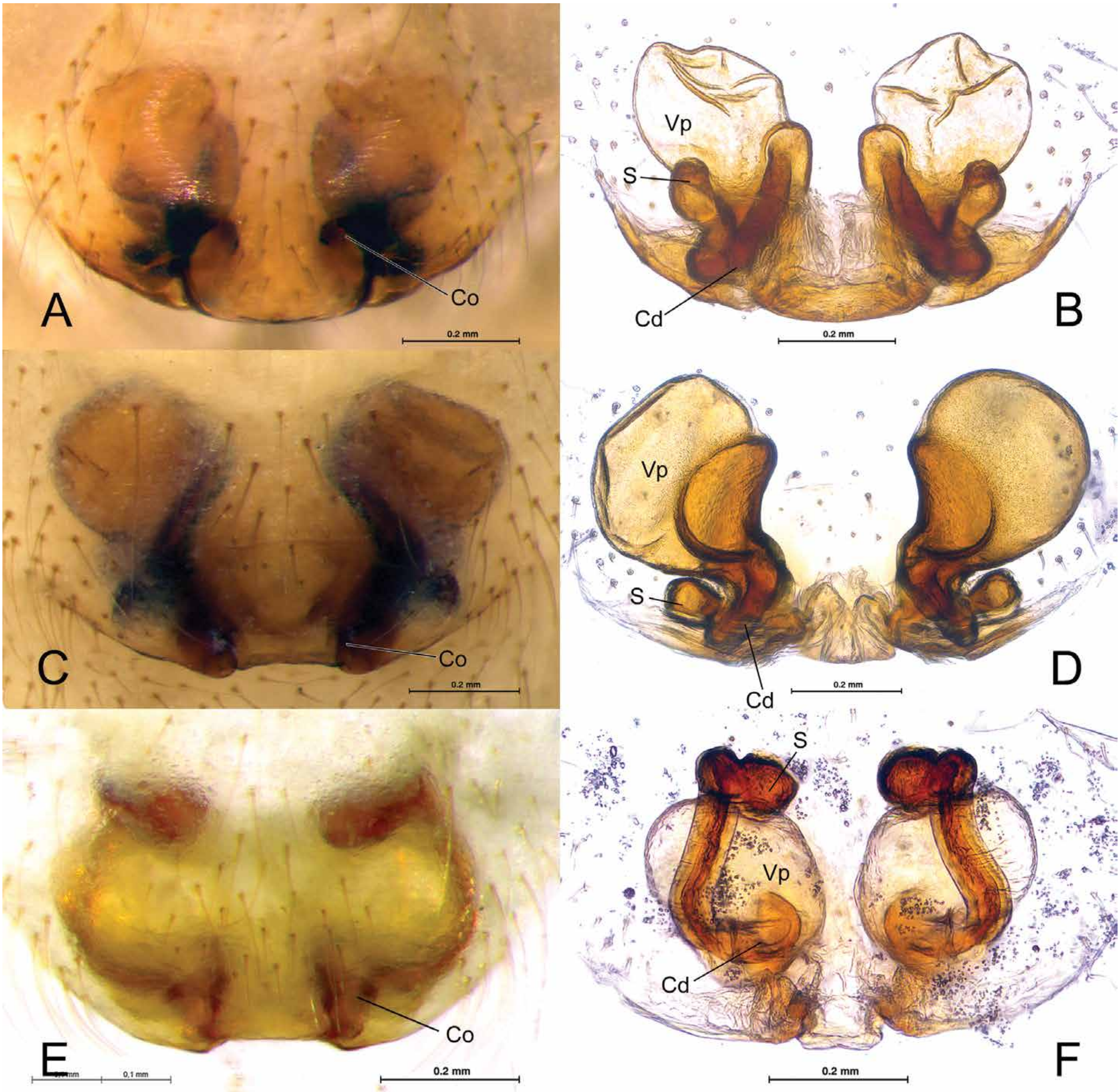

Figure 5. Female epigyne of the Italian *Domitius* species for comparison. *D. speluncarum*: A. epigyne, ventral view; B. vulva, dorsal view; *D. menozzii*: C. epigyne, ventral view; D. vulva, dorsal view; *D. sbordonii*: E. epigyne, ventral view; F. vulva.

Biospeleological and ecological notes

The entrance of Tana delle Fate di Coreglia Antelminelli cave (Italian National Caves Registry number: 141/T/LU; Fig. 3E) opens in the left bank of the narrow valley of Segone Creek in the Province of Lucca (Toscana region) at an elevation of 260 m a.s.l. The cave occurs in the limestone of the Maiolica formation (lower Tithonian–lower Aptian, ~150–120 Ma), which is particularly rich in flint nodules. After an initial steep slope (approximately 10 m deep), the cave continues with a long and sub-horizontal spatial development and a general NW–SE orientation (Fig. 3F). It branches with several, sub-circular tunnels as a result of ancient groundwater flows. The cave has an estimated total extension of 1100 m, although the deeper segments are still unexplored, as they are either filled with water or ending with sumps. The inner section is generally humid, with mud often covering the bottoms of the tunnels. The cave hosts a rich subterranean fauna including some endemic or locally protected species, e.g. the carnivorous land snail *Oxychilus* sp. (Gastropoda, Oxychilidae), the cave cricket *Dolichopoda laetitiae* Minozzi, 1920 (Orthoptera, Rhaphidophoridae), the

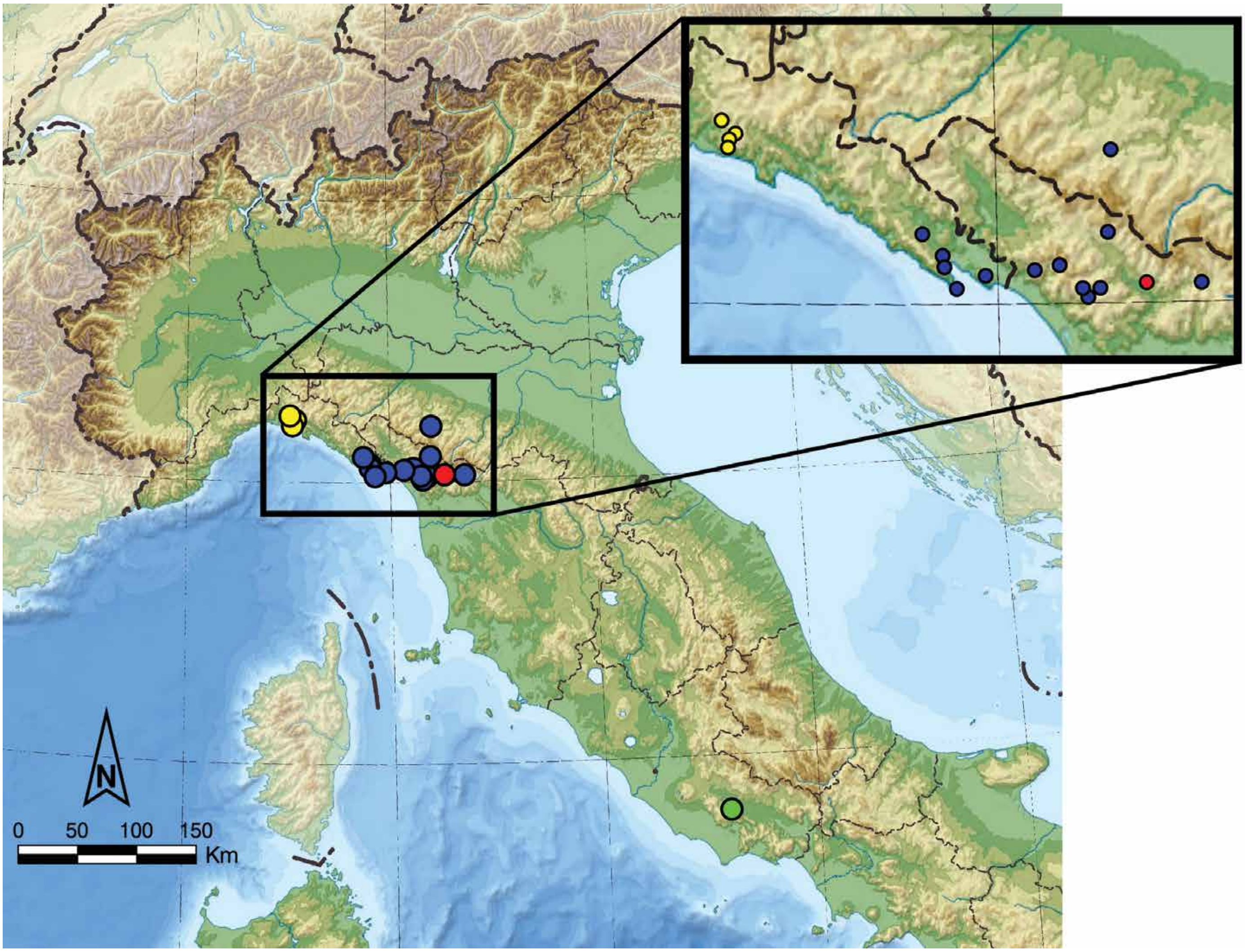

Figure 6. Distribution of the genus *Domitius* in Italy. Yellow dots = *D. menozzii*, blue dots = *D. speluncarum*; red dot = *D. culsu* sp. nov.; green dot = *D. sbordonii*.

blind subterranean beetle *Duvalius apuanus lanzai* Straneo, 1943 (Coleoptera, Trechinae), the Italian cave salamander *Speleomantes italicus* (Dunn, 1923) (Amphibia, Plethodontidae), and three species of bats: the greater horseshoe bat *Rhinolophus ferrumequinum* (Schreber, 1774), the lesser horseshoe bat *Rhinolophus hipposideros* (Bechstein, 1800) (Chiroptera, Rhinolophidae), and the common bent-wing bat *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera, Miniopteridae). Other animals known from the cave from the literature (Lanza, 1961) or directly observed by the author include: *Octodrilus complanatus* (Dugès, 1828), *O. hemiandrus (*Cognetti, 1901)*, O. transpadanus* (Rosa, 1884), and *Aporrectodea rosea* (Savigny, 1826) (Anellida, Lumbricidae); *Chaetophiloscia cellaria* (Dollfus, 1884) (Isopoda, Philosciidae), *Androniscus dentiger* Verhoeff, 1908, and *Spelaeonethes mancinii* (Brian, 1913) (Isopoda, Trichoniscidae); *Euscorpius carpathicus* (Linnaeus, 1767) (Scorpiones, Euscorpiidae); *Trogulus* sp. (Opiliones, Trogulidae), *Ischyropsalis adamii* Canestrini, 1873 (Opiliones, Ischyropsalididae); *Lithobius tylopus* Latzel, 1882 (Chilopoda, Lithobiidae), *Gryllomorpha dalmatina* (Ocskay, 1832) (Orthoptera, Gryllidae), *Hypaena* sp. (Lepidoptera, Noctuidae), *Stenophylax permistus* McLachlan, 1895 (Tricoptera, Limnephilidae), and a large population of limoniid crane flies (Diptera, Limoniidae). Near the entrance and in the early section of the cave, numerous spiders were also observed: *Amaurobius ferox* (Walckenaer, 1830), *A. pesarinii* Ballarin and Pantini, 2017 (Amaurobiidae), *Kryptonesticus eremita* (Simon, 1880) (Nesticidae), *Meta menardi* (Latreille, 1804), *Metellina merianae* (Scopoli, 1763) (Tetragnathidae), *Pholcus phalangioides* (Fuesslin, 1775) (Pholcidae), and *Tegenaria* sp. (Agelenidae). The new species was found in the initial segments of the cave, but at some distance from the entrance (Fig. 3F). During summer, when the cave was visited, adults, subadults, and juveniles of *D. culsu* sp. nov. were observed together, with a substantially higher number of adults and subadults during the month of August. Most of the juveniles collected in the cave and bred in captivity became adults after 2–3 months of captivity, while it took approximately one year for the youngest specimens to reach sexual maturity.

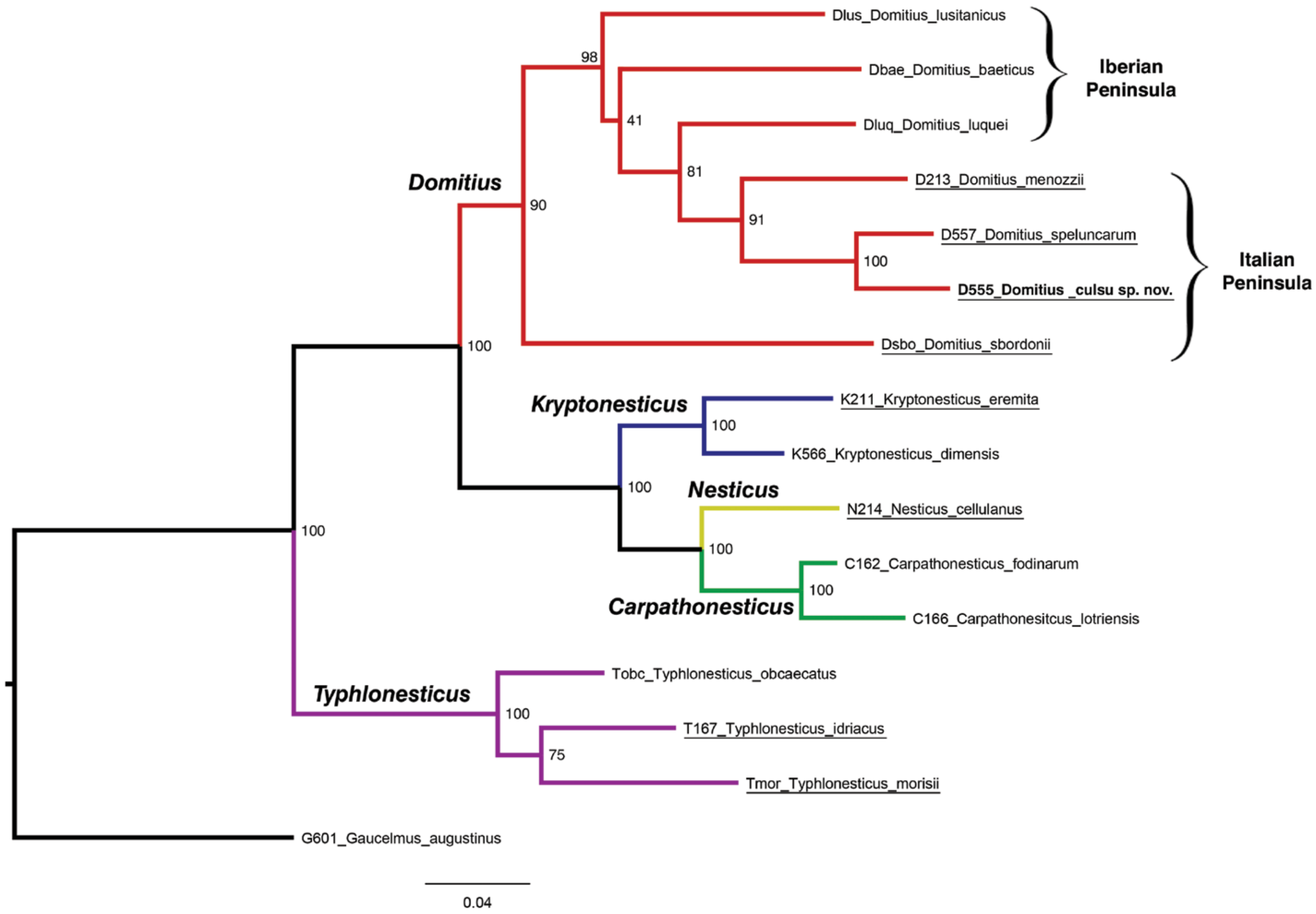

Figure 7. Phylogenetic tree of the main European nesticid genera inferred using ML in RAxML. Different colored branches reflect different genera: violet = *Typhlonesticus*, green = *Carpathonesticus*, yellow = *Nesticus*, blue = *Kryptonesticus*, red = *Domitius*. The newly-described species is highlighted in bold. Species distributed in Italy are underlined. Code before each species refers to the origin of the data, see Table 1. Branch lengths are scaled in relation to the number of substitutions per site; numbers at nodes denote bootstrap support according to ML.

Two different species of nesticid spiders, *K. eremita* and *D. culsu* sp. nov., were collected together in the Tana delle Fate di Coreglia Antelminelli cave. These species cover a different spatial distribution within the cave (Fig. 3F), coexisting without overlapping despite occupying approximately the same ecological niche. Cohabitant nesticids, in particular involving *D. menozzii* or *D. speluncarum* together with *K. eremita*, have been previously observed in several occasions in Italian caves, and sometimes collected at short distances from each other (Brignoli, 1971). However, no clear species overlap are reported within the same cave. Such distinct spatial partition can be explained by the different grade of adaptation to the hypogean environment showed by these arachnids. In fact, *K. eremita* appears to be a less specialized cave-dweller, lacking extreme morphological adaptations to subterranean life. Therefore, it mostly occurs near the entrance of caves or inside artificial tunnels, including, occasionally, shadowed epigean habitats with constant temperature and high relative humidity (Brignoli, 1971 and personal observations by the author). On the other hand, all *Domitius* species show a greater degree of adaptation to the subterranean habitat, as suggested by reduction of the eyes and body depigmentation. Such strong adaptation allows *Domitius* to occupy deeper segments of the caves, thus avoiding direct competition with *K. eremita*.

Conservation Notes

Since caves are a unique and delicate ecosystem, they are highly susceptible to external disturbance (Culver and Pipan, 2009). Its visible entrance and sub-horizontal extension makes Tana delle Fate di Coreglia Antelminelli cave easily accessible to visitors even with limited experience in speleology, and the cave is often used for training purposes by local speleological clubs. Although not threatened, *D. culsu* sp. nov. should be considered potentially at risk in case of frequent and long-lasting human disturbance due to its strict habitat requirements, its reduced population, and its extremely limited distribution, which appears to be confined to a single cave. Therefore, the new species is a good candidate for species conservation, deserving a place in the list of locally protected species.

Phylogenetic Analysis

A total of 16 nesticid species were used in this study, including representatives of the main nesticid genera present in Europe and all the species distributed in the Italian peninsula. Taxon sampling comprised the wide majority of *Domitius* species. Only *D. murgis* (Ribera and De Mas, 2003) from Spain was excluded from the analysis due to the absence of available sequences and fresh samples. The final dataset was formed by 1975 pair bases (bp) distributed as; COI = 1197 bp, 16S = 469 bp, and H3 = 309 bp. The resulting phylogenetic tree is illustrated in Figure 7, and the uncorrected pairwise distance between the species is reported in Table 2. The European nesticids cluster into five different clades corresponding to the main genera *Carpathonesticus*, *Domitius*, *Kryptonesticus*, *Nesticus*, and *Typhlonesticus*, each of them highly supported (bootstrap support value = 100%). Each lineage represents a different and well-defined evolutionary line. These results concur with the outcomes of recent morphological and phylogenetic studies on the family Nesticidae (Pavlek and Ribera, 2017; Ribera, 2018; Ballarin and Li, in prep.), supporting the validity of the newly-established genera. According to these results, *Domitius* represents the sister lineage of the monophyletic clade formed by the genera *Carpathonesticus*, *Kryptonesticus*, and *Nesticus*, with which it shares a common ancestor. The analysis supports *Typhlonesticus* as a basal clade within the European Nesticidae, as also suggested by recent molecular studies (Ballarin and Li, 2018; Ribera, 2018). Within *Domitius*, *D. culsu* sp. nov. shows a closer affinity with the species from the same geographic area; particularly *D. speluncarum*, but also *D. menozzi*. Its position at the far end of the phylogenetic tree of the genus suggests a more recent origin in comparison with the other congeneric species.

All the *Domitius* species distributed in the Northern Apennines share a close affinity with species from the Iberian Peninsula. Such close relations also reflected in genital morphology. For instance, all these species share a similar position of spermathecae, located in the lower-half of the vulva, and below the vulval pockets (see Figs. 1G, 5B, D and Figs. 4A–E in Ribera, 2018). On the other hand, *D. sbordonii* from the Central Apennines appears to be morphologically and genetically separated from all the other species of the genus, including those from Northern Apennines. The difference is highlighted in the peculiar shape of the vulva, being the only *Domitius* species showing simple-coiled internal ducts and spermathecae located in the upper-half of the vulva, over the vulval pockets (see Fig. 5F vs. Figs. 1G, 5B, D and Figs. 4A–E in Ribera, 2018). Upper-positioned spermathechae are also present in several other European nesticid genera such as *Carpathonesticus* (*sensu stricto*)*, Krypthonesticus*, and *Nesticus* (*sensu stricto*). Based on these results

Table 2. Uncorrected genetic p-distance of the COI partial sequence of the nesticid species discussed in the text. The newly described species is in bold.

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Dbae_Domitius_baeticus															
2	Dluq_Domitius_luquei	0.142														
3	Dlus_Domitius_lusitanicus	0.135	0.135													
4	D213_Domitius_menozzii	0.140	0.146	0.119												
5	D555_Domitius_culsu sp. nov.	0.167	0.144	0.133	0.121											
6	Dsbo_Domitius_sbordonii	0.181	0.176	0.167	0.181	0.185										
7	D557_Domitius_ speluncarum	0.162	0.144	0.121	0.121	0.071	0.190									
8	N214_Nesticus_cellulanus	0.172	0.174	0.151	0.190	0.181	0.176	0.172								
9	K566_Kryptonesticus_ dimensis	0.156	0.151	0.135	0.144	0.165	0.172	0.146	0.121							
10	K211_Kryptonesticus_ eremita	0.149	0.156	0.142	0.156	0.169	0.169	0.162	0.117	0.078						
11	C162_Carpathonesticus_ fodinarum	0.169	0.153	0.153	0.176	0.178	0.172	0.167	0.085	0.089	0.108					
12	C166_Carpathonesitcus_ lotriensis	0.144	0.160	0.146	0.162	0.190	0.176	0.190	0.112	0.094	0.101	0.069				
13	Tobc_Typhlonesticus_ obcaecatus	0.197	0.178	0.167	0.165	0.178	0.176	0.181	0.149	0.156	0.158	0.140	0.142			
14	T167_Typhlonesticus_ idriacus	0.181	0.174	0.167	0.183	0.192	0.197	0.185	0.142	0.156	0.165	0.149	0.156	0.096		
15	Tmor_Typhlonesticus_ morisii	0.174	0.174	0.174	0.181	0.199	0.208	0.181	0.183	0.160	0.183	0.176	0.174	0.117	0.124	
16	G601_Gaucelmus_ augustinus	0.229	0.229	0.227	0.222	0.245	0.243	0.236	0.252	0.211	0.238	0.240	0.233	0.249	0.254	0.247

it is possible to speculate that *D. sbordonii* represents a basal element within the genus *Domitius*, possibly still carrying the ancestral characters of the older forebear of the European nesticids.

Conclusions

All Italian species of the genus *Domitius* appear to be highly adapted to a permanent life in the subterranean environment, showing eye reduction and lack of body pigmentation. They further present a localized distribution, with distinct genetic and morphological differences between the species living in the Northern and Central areas of the Apennines. At the same time a close affinity with the species distributed in the Iberian Peninsula is observed. Such features, together with a high genetic p-distance among the species (Table 2), suggests a potentially complex evolutionary history of the genus *Domitius* that still needs to be properly explored (see also Ribera, 2018).

Because of their apparent similarities in habitus and female genitalia, *D. culsu* **sp. nov** has previously been mistaken for *D. speluncarum* and ignored as a distinct species by previous arachnologists. A detailed molecular and morphological analysis of both sexes of *D. culsu* **sp. nov** carried out in this work supports the validity of the new species and its close relationship with the other *Domitius* species from the same geographical area. Its potential susceptibility to external disturbance, and extremely limited distribution, makes *D. culsu* **sp. nov** of interest for conservation.

Finding a new nesticid species in Italy further suggests that our knowledge on the diversity of the family Nesticidae in Southern Europe is still far from complete. Further collections along the Italian peninsula will probably lead to the discovery of other highly-specialized nesticid species allowing a deeper and more precise understanding of the spider cave fauna in Italy and in the Mediterranean area.

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References

Ballarin, F., and Li, S., 2018, Diversification in tropics and subtropics following the mid-Miocene climate change: A case study of the spider genus *Nesticella*: Global Change Biology, v. 24, p. 577–591, doi: 10.1111/gcb.13958.

Brignoli, P.M., 1971, Note su ragni cavernicoli italiani (Araneae): Fragmenta Entomologica, p. 121–229.

Brignoli, P.M., 1979, Ragni d'Italia XXXI. Specie cavernicole nuove o interessanti (Araneae):. Quaderni del Museo di Speleologia "V. Rivera", v. 5, no. 10, p. 1–48.

Brignoli, P.M., 1985, Aggiunte e correzioni al "Catalogo dei ragni cavernicoli italiani": Memorie del Museo Civico di Storia Naturale di Verona, serie 2, v. 4, p. 51–64.

Culver D.C., and Pipan T., 2009, The Biology of Caves and Other Subterranean Habitats: Oxford, UK: Oxford University Press, p. 256.

Dresco, E., 1966, Étude de quelques espèces d'araignées du genre *Nesticus* (fam. Nesticidae): Annales de Spéléologie, v. 21, p. 795–813.

GenBank, 2018, GenBank sequence database: Available at: https://www.ncbi.nlm.nih.gov/genbank/, [accessed August, 2018].

Hall, T., 1999, BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT: Nucleic Acids Symposium Series, v. 41, p. 95–98.

Katoh, K., and Standley, D.M., 2013, MAFFT multiple sequence alignment software version 7: improvements in performance and usability: Molecular Biology and Evolution, v. 30, p. 772–780, https://doi.org/10.1093/molbev/mst010.

Kritscher, E., 1958, Araneen aus den Picentinischen Bergen: Memorie del Museo Civico di Storia Naturale di Verona, v. 6, p. 313–320.

Lanza, B, 1961, La fauna cavernicola della Toscana: Rassegna Speleologica Italiana, v. 13, no. 2, p. 23–51.

Lin, Y.C., Ballarin, F. and Li, S.Q., 2016, A survey of the spider family Nesticidae (Arachnida, Araneae) in Asia and Madagascar, with the description of forty-three new species: ZooKeys v. 627, p. 1–168. https://doi.org/10.3897/zookeys.627.8629

Mammola, S., and Isaia, M., 2017, Spiders in caves: Proceedings of the Royal Society B: Biological Sciences, v. 284: 20170193, https://doi.org/10.1098/rspb.2017.0193.

Miller, M.A., Pfeiffer, W., and Schwartz, T., 2010, Creating the CIPRES Science Gateway for inference of large phylogenetic trees: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, p. 1–8, https://doi.org/10.1109/GCE.2010.5676129.

Pantini P. & Isaia M. 2019. Araneae.it: the online Catalog of Italian spiders with addenda on other Arachnid Orders occurring in Italy (Arachnida: Araneae, Opiliones, Palpigradi, Pseudoscorpionida, Scorpiones, Solifugae). Fragmenta Entomologica 51 (2): 127-152. Available on line at www.araneae.it [accessed February 2020]. https://doi.org/10.4081/fe.2019.374.

Pavlek, M., and Ribera, C. 2017, *Kryptonesticus deelemanae* gen. et sp. nov. (Araneae, Nesticidae), with notes on the Mediterranean cave species: European Journal of Taxonomy, 262, p. 1-27, https://doi.org/10.5852/ejt.2017.262.

Ribera, C., 2018, A new genus of nesticid spiders from western European Peninsulas (Araneae, Nesticidae): Zootaxa, v. 4407, p. 229–240, https://doi.org/10.11646/zootaxa.4407.2.4.

Stamatakis, A., 2014, RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies: Bioinformatics, v. 30, p. 1312–1313, https://doi.org/10.1093/bioinformatics/btu033.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S., 2013, MEGA6: Molecular Evolutionary Genetics Analysis version 6.0: Molecular Biology and Evolution, v. 30, p. 2725–2729, https://doi.org/10.1093/molbev/mst197,

World Spider Catalog, 2020, World Spider Catalog. Version 21.0: Natural History Museum Bern, available at: http://wsc.nmbe.ch, [accessed February, 2020], doi: 10.24436/2.

Zangheri, P., 1966, Repertorio sistematico e topografico della flora e fauna vivente e fossile della Romagna, Tomo II - Regno Animale. Museo Civico di Storia Naturale di Verona: Memorie Fuori Serie n°1, Stamperia Valdonega, Verona, p. 854.

L. M. Kondratyeva, O. S. Shadrina, Z. N. Litvinenko, and E. M. Golubeva. Biogeochemical investigations of the speleothem moonmilk in the karst Proschalnaya Cave (Far East, Russia). *Journal of Cave and Karst Studies*, v. 82, no. 2, p. 95-105. DOI:10.4311/2019MB0106

BIOGEOCHEMICAL INVESTIGATIONS OF THE SPELEOTHEM MOONMILK IN THE KARST PROSCHALNAYA CAVE (FAR EAST, RUSSIA)

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Abstract

Results of investigations of natural waters (drip and fracture) and speleothem moonmilk from the karst Proschalnaya Cave (Russia, Far East) are reported. Concentrations of Fe and Mn in drip water were highest in spring, while the concentration of Mn was lowest in the fracture water, which may be due to the nature of infiltration of water through different channels after spring snowmelt and autumn rains. Molecular genetics investigation of the moonmilk mass revealed the presence of iron bacteria of the genera *Rhodoferax* and *Geothrix*. The visually plastic and homogeneous mass of moonmilk was shown to be highly heterogeneous, containing various microstructures. Tubular microstructures had a richer elemental composition (C, O, Ca, Fe, Mn, Si, Al, and S), in comparison with claviform formations (C, O, Ca, and Na). Binding matrix in the composition of moonmilk is represented by reticular structures similar to nanofibers. The results of this research conducted in a monsoon climate may be interesting for speleologists working with karst caves in other climatic conditions.

Introduction

Microbes present in the specific habitats of aquifers and pore space of rocks play an important role in the processes occurring in the water-rock contact zone (Perry et al., 2004). Various organic compounds and microorganisms that are capable of colonizing the surface of rocks enter karst caves from the ground biotopes with infiltration waters (Chelius et al., 2009). However, the cave microbial composition varies by the types and configuration of caves (Barton et al., 2004; Velikonja et al., 2014) and depends on the sampling location (Ghosh et al., 2017).

As the results of analysis of microbial communities sampled from the walls of caves located in Spain, Czech Republic, and Slovenia, Porca et al., (2012) proposed the hypothesis that the colonization of caves with microorganisms occurred through water infiltration from the overlying rock and soil. The heterogeneity and main mechanism of microbial diversity in caves are well-connected with surface environments (Wu et al., 2015). Microbial exopolysaccharides, alginate acids, siderophores, and other chelating compounds act as important factors determining the colonization and dissolution rate of mineral rocks (Perry et al., 2004; Ercole et al., 2007; Kuhn et al., 2014).

Alternatively, microbial cells have been shown to act as centers for precipitation and crystallization of many elements (Barton and Northup, 2007). Microorganisms are also capable of altering the mineral composition and solubility of carbonates, as well as crystal size and morphology, as demonstrated by the large, poorly soluble $CaCO_3$ crystals formed in the presence of *Bacillus pasteurii* (Mitchell and Ferris, 2006).

Dissolution of carbonate minerals and morphogenesis of karst cavities may be partially explained by bacterial activity (Hill and Forti, 2007). Infiltration, flood water, and airflow also introduce microbes into caves, where they can begin to influence the structure of the microbial community of caves. As weather parameters and water conditions change, the introduced microbial pool may also change strongly. As in surface environments, microorganisms act as active and passive promoters of redox reactions in the sedimentary processes in caves (dissolution, redeposition, secondary formation of minerals with participation of microorganisms) (Fornós et al., 2014).

The investigation of karst caves is presently carried out in several fields, including speleology, geology, and ecology. Research varies from large-scale analyses of landforms and processes involved in the formation of karst landscapes, to speleothems, including stalactites and stalagmites, to microscopic investigation of sinter formations. Among speleothems, moonmilk is a formation of high interest (Borsato et al., 2000; Cacchio et al., 2014). Moonmilk, one of the most common types of carbonate deposits (speleothems) formed in caves, has long been known as a habitat for microorganisms that are thought to be responsible for the origin of these commonly white and soft secondary calcite deposits (Reitschuler et al., 2016). Various forms of moonmilk deposition have been described, including encrustations, films, thick layers, deposits, and veins in clay. The metabolic activity of complex microbial communities can play an important role in the formation of moonmilk (Portillo and Gonzales, 2011).

The presence of microorganisms in moonmilk formations has been observed in caves around the world, from the tropics to high latitudes. It has been found that microbes participate in formation of the white and soft secondary calcite (calcium carbonate) deposits that can coat the walls, floors, and ceilings of caves. In this biologically-driven process,

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upper surface layers are actively formed, while the deeper and older parts become progressively dehydrated, encrusted, and inactive (Canaveras et al., 2006).

Moonmilk is primarily water by mass (60–90 %). In this geochemical environment, microbial cells can act as centers of precipitation and crystallization for many elements (Barton and Northup, 2007). As 90 % of Earth's biomass resides in the subsurface, and many of those environments are exposed to constantly cold conditions (below 5 °C), basic research on exotic habitats such as moonmilk through cultivation of microorganisms and geochemical analyses is important for understanding potentially widespread processes (Rodrigues and Tiedje, 2008). Low-temperature biotopes are successfully colonized by cold-adapted organisms, which include a large range of representatives from all three domains: Bacteria, Archaea, and Eukarya. As a result, psychrophiles are the most abundant in terms of biomass, diversity, and distribution (Struvay and Feller, 2012). The ability of psychrophilic microorganisms to grow at temperatures below 5 °C can be associated with their successful adaptation to the natural habitat. It is known that the microbial activity of psychrophiles and growth yield at low temperatures is higher than the growth rate at what is normally considered the optimal growth temperature (Margesin, 2009).

Microorganisms utilize several metabolic strategies to survive in the cave environment, such as synthesis of new organic matter from inorganic carbon (chemolithoautotrophy) and decomposition of organic matter (heterotrophy) (Chen et al., 2009). These processes, or its byproducts, can play a role in the transformation of rock through dissolution or formation of minerals (Lefevre et al., 2016).

Based on past studies conducted in the Snezhnaya Cave (Abkhazia) (Kondratyeva et al., 2016), it was hypothesized that the elemental composition of groundwater and the structure of microbial communities play a key role in determining the elemental composition of the moonmilk. Our research is devoted to the study of the elemental composition of groundwater and moonmilk, as well as the activity of microorganisms in the Proschalnaya Cave (Far East, Russia).

Investigation of moonmilk from Proschalnaya Cave was conducted in two stages: (1) microbiological research (molecular genetic techniques, isolation of cultured bacterial strains, determination of their physiological and biochemical activity) and (2) the analysis of nanostructures in the moonmilk mass by scanning electron microscopy with determination of their elemental composition.

The main objective of our research was to determine environmental factors that characterized pecularities of biofilm from moonmilk in a large karst cave on the Far East of Russia. For the first time interdisciplinary studies including physicochemical, microbiological, molecular genetic methods, and scanning electron microscopy of moonmilk from the Proshalnaya Cave were conducted. The results of research on biofilm and rock interactions in a monsoonal climate can be interesting for speleologists working with karst caves in other climatic conditions.

Materials and Methods

Sampling site

Proschalnaya Cave is on the eastern slope of the Sagdi-Selanka River valley (Amur River Basin) in the Khabarovsk region, Far East, Russia (47º18'32.7" N; 136º29'56.3" E) (Fig. 1). The climate of this region is moderately continental with signs of monsoonal: humid summers with frequent rains and winter with little snow. A monsoonal climate is characterized by a sharp contrast in the amount of precipitation over the year seasons and stability of the wind direction for one season with a sharp wind variation in the opposite direction during changing seasons. The cave is remote from settlements, not visited by tourists, and accessible for speleologists only. The cave does not have access for animals due to the complex labyrinths and deep depth.

The cave is a labyrinth with a total length of approximately 6 km, multiple levels, and a large number of halls, galleries, and grottos. There is a watercourse and many sources of drip and fracture water inside the cave; the walls and ceilings are covered with various speleothems, including moonmilk (Fig. 2). The chemical composition of the karst waters of the Russian Far East are primarily hydrocarbonate-calcium , and more rarely, chloride-hydrocarbonate-calcium with an average degree of mineralization (5−15 g/L) (Bersenyov, 1989). Surface waters of the Sagdi-Selanka River and groundwaters of Proschalnaya Cave are characterized by an increased content of Ca ions (82–86 % mg-Eq) and a very low concentration of Mg ions (9–12 % mg-Eq) (Shesterkin, 1983). Hydrochemical studies of natural waters in the Proschalnaya Cave have not been carried out in recent years.

Sampling characterization

In May 2015, 2016, and 2017 and in November 2015, water samples of different origins were taken from the cave (watercourse, drip, and fracture water) and from the Sagdi-Selanka River (surface water) according to the standards of sampling in hydrochemistry and microbiology (Gerhardt, 1983; Kuznetsov and Dubinina, 1989). In the study area, the average amount of precipitation during the month was: 105 mm in May 2015; 136 mm in May 2016, and 48 mm in May 2017. In November 2015, precipitation was minimum, 9.9 mm. In the cave air temperature was 1–4 °C.

Figure. 1. Geographical location of the Proschalnaya Cave in the Amur River Basin, Khabarovsk region (Far East, Russia).

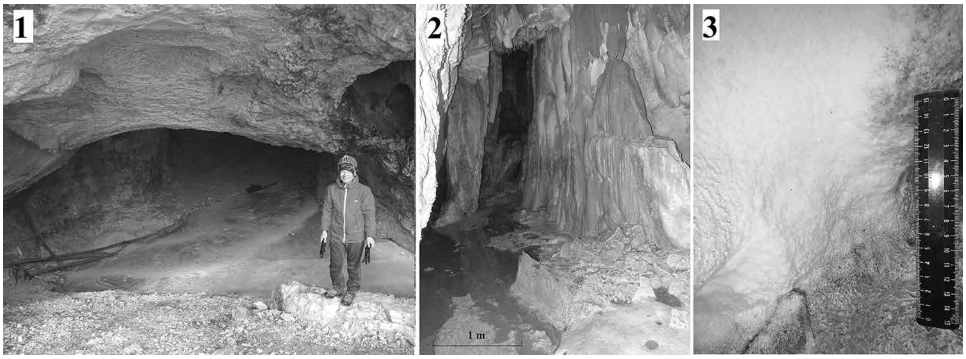

Figure. 2. Proschalnaya Cave (Russia): (1) Entrance to the cave, (2) Gallery in the Albatross system, (3) moonmilk on the wall (M2 — thick curdy ivory-white mass).

In the Marble room, samples of moonmilk deposits of different consistencies were aseptically collected in sterile tubes: M1—thin slimy white mass and M2—thick curdy ivory-white mass were taken from the walls; M3—dry white mass was taken from the surface of broken rocks. Samples were transported to the laboratory in coolers at 4 °C.

Determination of the elemental composition in samples of natural waters and moonmilk was carried out with use of the Total Quant ICP-MS method, PerkinElmer (USA), in accordance with standard methods (Federation Regulation, 2011).

Microbial studies

For the inoculum, 100 mg of wet mass of moonmilk was dispersed in 10 mL of sterile physiological saline; dilution was performed in 100-fold and 0.1 mL of suspension was used for spread-plating on the agar culture media. The abundance of heterotrophic and Fe-metabolizing cultivated bacteria (CFU/mL) in natural waters and in moonmilk was determined on the 7th day after cultivation on solid nutrient media with use of spread plates at 23 °C: SAA (starch ammonium agar) (Gerhardt, 1983); FPA (fish peptone agar) and FPA diluted 10 times (Kuznetsov and Dubinina, 1989); and Vinogradsky medium (Egorov, 1995). For the cultivation of Fe-metabolizing bacteria, Bromfield agar medium was used (Namsaraev et al., 2006).

Diagnostic system (SPA Microgen, Moscow, Russia) with color indicators and various carbon sources (carbohydrates, polyhydric alcohols, and amino acids) was used to determine the nutritional range of strains isolated from different types of water. The growth activity of the strains was evaluated by the color change of the dissolved substrate on the 7th day after cultivation at 23 °C. Amylase activity was determined on SAA after treating the colonies with Lugol's solution according to the diameter of the starch hydrolysis zones.

qPCR analysis

Microbial investigation of moonmilk samples (M1 and M2) were carried out with use of quantitative PCR (qPCR) analysis according to standard procedures (Kubista et al., 2006). DNA was extracted using a GeneMATRIX Soil DNA Purification Kit (Roboklon, Berlin, Germany). The total number of eubacterial DNA copies and the DNA copies of bacteria of the genera *Rhodoferax* and *Geothrix* were determined with use of specialized primers which were offered by Prof. U. Szewzyk (Technische Universität Berlin): Eubacteria (Uni338F_RC ACT CCT ACG GGA GGC AGC, Uni907R CCG TCA ATT CMT TTG AGT TT); *Rhodoferax ferrireducens* group (RdoR_RC GAC CTG CAT TTG TGA CTG YA, Uni907R CCG TCA ATT CMT TTG AGT TT), *Geothrix* (Gx. 193F_ GAC CTT CGG CTG GGA TGC TG, Gx. 448R_ AGT CGT GCC ACC TTC GT) (Braun et al., 2016). Quantitative PCR was performed with an RG-6000-5 Plex real-time DNA cycler (Rotor-Gene 6000). Non-DNA-containing samples were used as negative controls to ensure the accuracy of the qPCR. Cycles of qPCR characterized: 40 cycles for Eubacteria (Initial denaturation 95 °C, 2 min; Denaturation 95 °C, 20 s; Annealing 60.4 °C 30 s; Extension 72 °C, 1 min); 40 cycles for *Rhodoferax ferrireducens* (Initial denaturation 95 °C, 2 min; Denaturation 95 °C, 20 s; Annealing 58 °C, 30 s; Extension 72 °C, 1 min); 45 cycles for *Geothrix fermentans* (Initial denaturation 95 °C, 3 min; Denaturation 95 °C 20 s; Annealing 58 °C, 20 s; Extension 72 °C, 30 s). Quantification was performed using standard curves obtained from the amplification profiles of known concentrations of the respective standard. A melt curve analysis (55–99 °C) was performed at the end of PCR cycles to confirm specificity of primer annealing. The parameters for the calibration curves were $R^2 > 0.99$, efficiency from 92 % to 98 %.

Scanning electron microscopy

Textural and microstructural characterization of moonmilk was performed using a VEGA 3 LMH TESCAN scanning electron microscope (Czech Republic). The samples were prepared by air drying, and Pt coating. Then the moonmilk samples were placed on a conductive carbon tape, mounted on 12 mm diameter aluminum stubs that were then placed in the microscope chamber; magnification was up to 15,000×. Energy dispersive spectrometer X-max 80 with Aztec™ microanalysis system (Oxford Instruments, UK) was used for the elemental composition analysis of moonmilk. X-Max 80 provides a range of detected elements from boron to uranium with elements detection interval from 0.1–100 wt. %. The general process for sample preparation and scanning electron microscopy were carried out at the Khabarovsk Innovation and Analytical Center for Collective Use at the Institute of Tectonics and Geophysics, Far East Branch, Russian Academy of Science.

Results and Discussion

Elemental composition of water of different origins

The content of Ca in drip and fracture water in the Proschalnaya Cave is dependent on the amount of precipitation. The maximum amount of Ca in drip and fracture water was recorded in May 2016 (Fig. 3) at maximum amount of precipitation (136 mm/month). High Ca content is associated with dissolution of calcium carbonates contained in rocks in interaction with natural waters, especially when the acidity of the water increases in the presence of organic matter (OM) and microbial metabolic activities in the overlying soils.

We have shown (Kondratyeva et al., 2016) that *in vitro* the process of dissolution of $CaCO_3$ crystals was accelerated in the presence of nitrogen-containing OM. Microorganisms capable of synthesizing a polymer matrix played a determinative role. The formation of abundant slimy biofilms that formed on the surface of $CaCO_3$ crystals contributed to their dissolution (Fig. 4). During cultivation of moonmilk suspensions on agar nutrient media, we often observed growth of slimy colonies of heterotrophic bacteria capable of consuming different sources of carbon. The polymer matrix produced by these bacteria may be an active accumulator of other elements forming the moonmilk mass. It is known that over 99 % of microorganisms on Earth live within matrix consisting of a mixture of polymeric compounds (extracellular polymeric substance: EPS), which makes up the intercellular space of microbial aggregates and forms the structure and architecture of the biofilm matrix (Flemming, 2016).

Fe and Mn measurements for drip water were highest in the spring period. Enrichment of water with iron occurs as a result of leaching and dissolution of ferruginous minerals and rocks. Among the geochemical factors, ferric oxide was correlated with increased microbial diversity in the cave sediments (De Mandal et al., 2017). It should be noted seasonal asynchrony in the content of manganese in the drip and fracture water in the Proschalnaya Cave. In November 2015, the content of Mn in the fracture water was much higher than in the drip water and watercourses. In the spring of 2016, the concentration of manganese in the fracture water was lower than in other water samples, which may be due to the infiltration of water through different channels after spring snowmelt and autumn rains. Organic substances play an important role in determining the intensity of microbiological processes at the biogeochemical barrier of water-rock, which would also affect the content of dissolved forms of iron and manganese (Ferris, 2005).

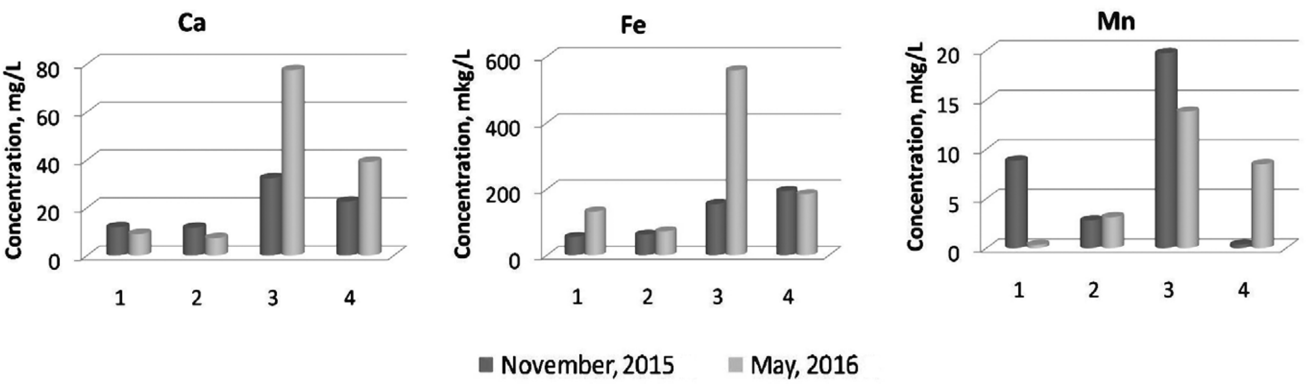

Figure. 3. The content of calcium, iron, and manganese in natural waters of different origins (November 2015; May 2016): (1) watercourse from the Proschalnaya Cave; (2) surface water from Sagdi-Selanka River; (3) drip water; (4) fracture water.

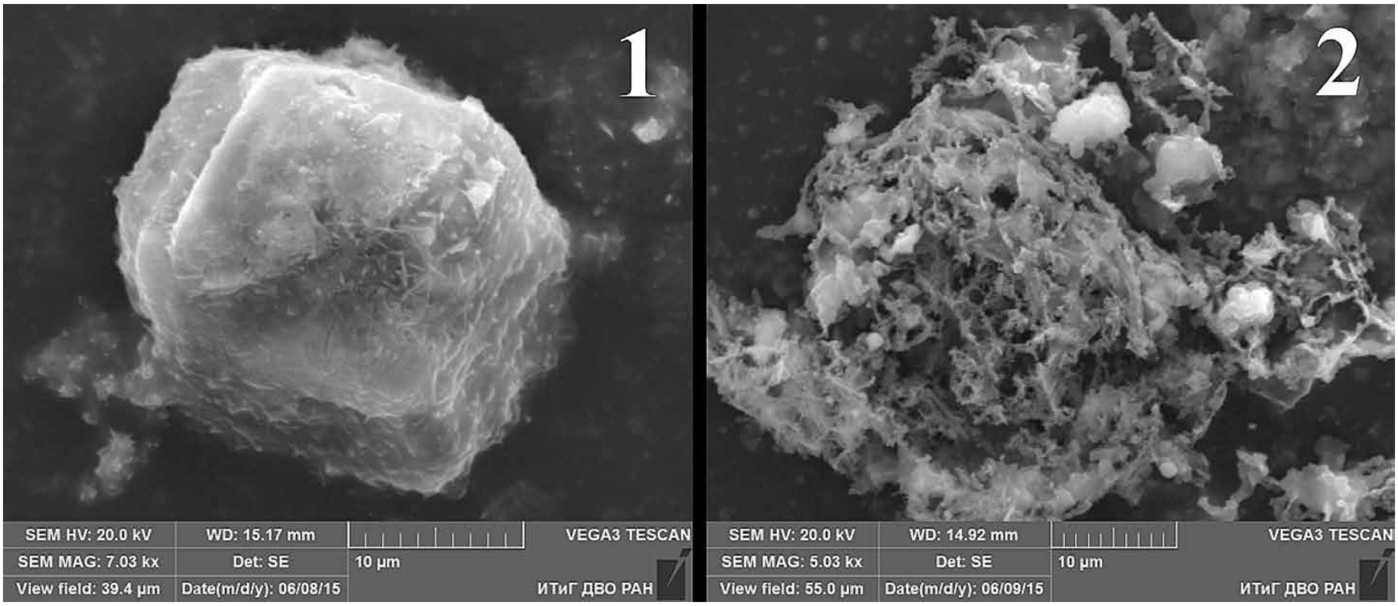

Figure. 4. SEM image of different stages of $CaCO_3$ crystal dissolution: (1) stage of bacterial cells adhesion and the formation of EPS on the surface of the $CaCO_3$ crystal; (2) final stage of complete dissolution of the crystal; formed biofilms retain the shape of the crystal in space.

Microbiological studies of water

In samples of water from the watercourse in the Proschalnaya Cave, regardless of the season, the predominant microorganisms preferred low concentrations of OM. Nitrifying and ferromanganese bacteria were also present in these samples. The most abundant microorganisms (6.28 × 10^3 CFU/mL) were in the cave watercourse in the spring, likely due to the increased transport of easily oxidized OM from the soil during snowmelt. During this time, the number of microorganisms present in the samples of surface water from Sagdi-Selanka River was lower due to increased flow velocity and volume. Autumn sampling from the cave watercourse and river surface water revealed decreased numbers of all physiological groups of cultivated microorganisms.

Fifty strains were isolated from water samples on different media (FPA; FPA diluted 10 times; Vinogradsky media). Using cultural and morphological characteristics, ten strains with active growth on agar media were selected for the study of biochemical activity. Strains isolated from the surface water of Sagdi-Selanka River and fracture water were most active and capable of utilizing the monosaccharides β-galactose, glucose, mannose, arabinose, arginine, ornithine, and mannitol as a source of carbon (Table 1). Strains of bacteria isolated from the river surface water and watercourse in the cave recycled the disaccharides lactose and sucrose, associated with the enzyme carbohydrase, which is responsible for the hydrolysis of di-, tri-, and polysaccharides. This enzyme also plays a role in regulating equilibrium between different forms of inorganic carbon, including bicarbonate, which is involved in the precipitation of calcium in nature (Müller et al., 2014).

Some strains isolated from fracture water and one representative of drip water utilized various amino acids (arginine, lysine, and ornithine) as a carbon source. Strains from the cave watercourse and fracture water actively utilized alco-

Table 1. Carbon utilization by the strains isolated from surface water of Sagdi-Selanka River and different water sources from the Proschalnaya Cave (May, 2017).

	Bacterial Strain per Water Source									
	Surface water from Sagdi-Selanka River			Watercourse in Cave		Drip Water in Cave			Fracture Water in Cave	
Source of Carbon	B 44	B 45	B 46	B 19	B 21	B 25	B 26	B 32	B 38	B 42
Glucose	+	+	+	+	+	+	−	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+
Lactose	+	−	+	+	−	+	+	+	+	−
Sucrose	+	−	−	+	−	+	−	−	+	−
Arginine	+	+	+	+	+	+	+	+	+	+
Lysine	−	−	−	−	−	+	−	−	+	+
Ornithine	+	+	+	+	+	+	+	+	+	+
Inositol	+	−	+	+	+	+	−	+	+	+
Mannitol	+	−	+	+	+	−	+	+	+	+
Sorbitol	−	−	+	+	+	+	+	+	+	+
β−galactose	+	+	−	−	−	−	−	−	−	+
Sodium malonate	+	−	+	+	+	+	+	+	+	+
Sodium citrate	+	−	+	+	+	+	+	+	+	+
Urea	−	−	+	−	+	+	−	+	−	+

Note: "+" is a positive reaction, "–" is a negative reaction.

hols (inositol, sorbitol, and mannitol). Most strains were also capable of using citrates as a source of carbon. Overall, strains isolated from fracture water had the most flexible carbon requirements.

Microbiological studies of the moonmilk

The abundance of cultivated bacteria within moonmilk varies strongly depending on its consistency (Table 2). In all samples, heterotrophic microorganisms dominated, consuming high concentrations of nitrogen-containing organic

Table 2. Structures of the microbial communities of moonmilk of different consistency from the Proschalnaya Cave.

		Abundance of microorganisms, CFU/g × 1000		
Media	Colony Morphotype	Sample No. M1 (thin slimy white mass)	Sample No. M2 (thick curdy ivory-white mass)	Sample No. M3 (dry white mass)
FPA	PR	111 ± 10.5	142 ± 16.8	15 ± 3.9
	PO	41 ± 6.4	5 ± 2.2	3 ± 0.7
	Y	74 ± 8.6	3 ± 0.7	···
	Total	226 ± 25.5	150 ± 12.7	18 ± 4.6
FPA:10	ST	74 ± 8.6	···	···
	OA	32 ± 5.6	···	5 ± 2.2
	B	2 ± 0.4	···	···
	G	···	6 ± 1.4	22 ± 4.7
	MM	···	86 ± 9.3	···
	Total	108 ± 14.6	92 ± 10.7	27 ± 6.9
SAA	OS	70 ± 8.4	···	n/a
	OSp	22 ± 4.7	52 ± 5.2	n/a
	Total	92 ± 13.1	52 ± 5.2	n/a

Note: Colony Morphotype: PR: Pale-yellow, rugose; PO: Pale-yellow, oily; Y: yellow; ST: slimy, translucent; OA: opaline, asterial; B: brown; G: grey; OS: opaline, slimy; OO: opaline, oily; OSp: opaline, spot, "···" no colonies of this morphotype, "n/a" not available.

substances (NOS) and differing slightly in the dominant morphotype of the colonies. Moreover, in a thin layer of curdy mass the abundance of different groups was higher than in a thicker layer of moonmilk. During cultivation on SAA containing starch as a carbon source, the abundance of bacteria was low in three samples. Periodically, violet-colored colonies, growing on Bromfield media containing $Fe(OH)_3$, were isolated from moonmilk. Such differences can be associated with different stages of the formation of biofilms from moonmilk and physico-chemical conditions at the sampling sites.

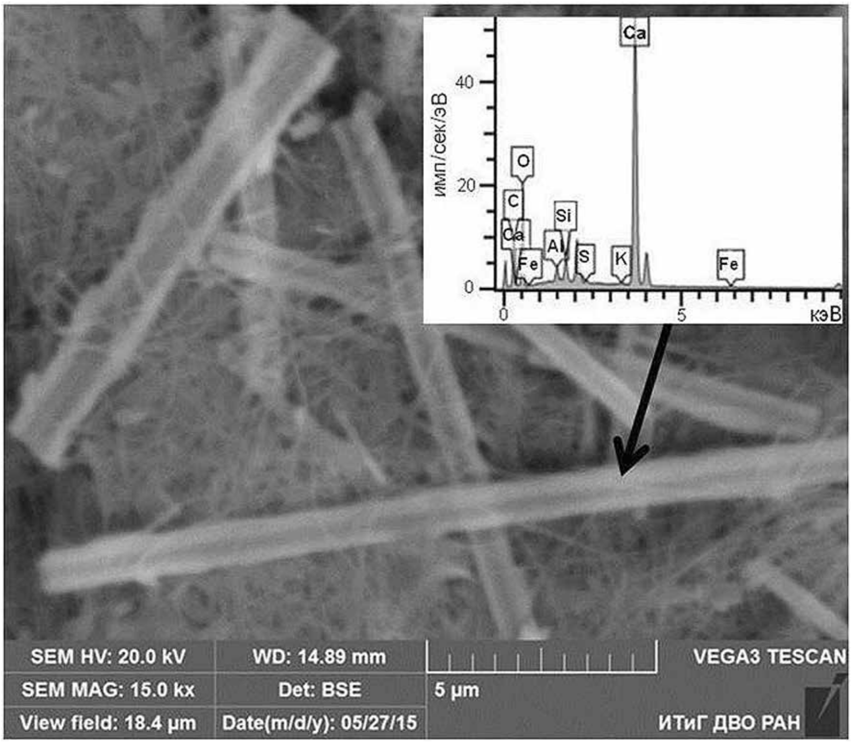

Figure. 5. SEM image and elemental composition of tubular microstructures in the composition of moonmilk, immersed in a mesh matrix. Magnification: 15,000x.

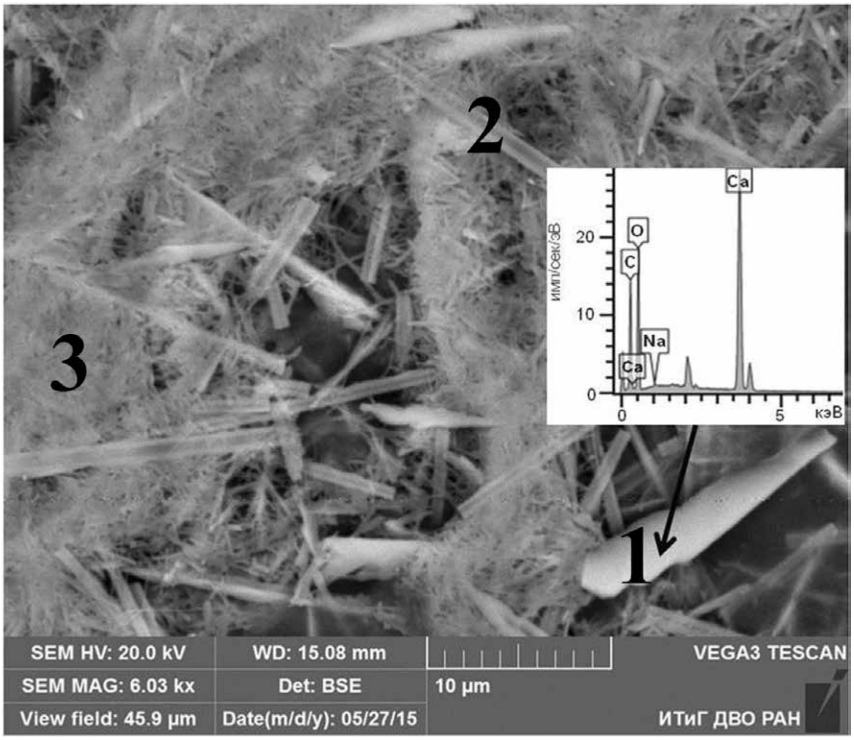

Figure. 6. SEM images of microtructures of moonmilk speleothem in the Proschalnaya Cave: (1) claviform, (2) tubular microstructures, and (3) nanofibres. Magnification: 6000x.

Minimal diversity of colony morphotypes and low abundance were recorded in the sample of dense slimy moonmilk. There is evidence that the structure of the microbial community strongly affects the intensity of $CaCO_3$ deposition and the composition of moonmilk (Cirigliano et al., 2018). High concentrations of calcium carbonate are able to precipitate in the slimy matrix and inhibit the development of bacteria. The physiological adaptation of bacteria to toxic Ca^{2+} ions occurs by calcification in Ca^{2+}-rich cave environments. Such activity creates the initial crystal nucleation sites that contribute to the formation of secondary $CaCO_3$ deposits within caves (Banks et al., 2010).

On the basis of cultural-morphological characteristics and a proposed scheme for identification of bacteria of the genus *Bacillus* (Vasiliev, 2013) with use of a series of tests (growth on citrate, arabinose, xylose, mannitol, urea, raffinose; catalase activity, and H_2S secretion). Among the twenty strains isolated from moonmilk in the Proschalnaya Cave, two strains were identified as *Bacillus*. It can be assumed the surface waters that drain the soil and karst rocks can act as the main source determining the composition of moonmilk. *Bacillus* are capable of producing polymeric slime and act as catalysts for the biogenic mineralization and weathering of rocks (Ercole et al., 2007). *Bacillus* can act as typical soil chemo-organotrophic bacteria that occur in freshwater, participate in the nitrogen cycle, and can reduce iron (Garcia et al., 2016). These bacteria are the centers of crystal formation, affect the morphology of crystals, the solubility of carbonates (Mitchel and Ferris, 2006), and take an active part in induced calcium carbonate precipitation (Achal and Pan, 2014).

Molecular investigations of moonmilk sampled from the Proschalnaya Cave revealed the presence of iron bacteria of the genera *Rhodoferax* and *Geothrix* (Table 3) that are commonly found in iron-containing groundwater. Members of

Table 3. Molecular genetics (qPCR) analysis of moonmilk, sampled from the Proschalnaya cave.

Sample Description	Total number of eubacterial DNA gene copies/g	Number of the *Rhodofera*× DNA gene copies/g	Number of the *Geothri*× DNA gene copies/g
M1: thin slimy white mass	1.17×10^9	5.64×10^6	3.25×10^5
M2: thick curdy ivory-white mass	1.08×10^9	1.44×10^6	3.76×10^5

Table 4. Elemental composition of nanostructures included in the composition of moonmilk in the Proschalnaya Cave.

Elements	Weight Percent		
	Tubular Nanostructure	Claviform Nanostructure	Nanofibres
C	19 ± 1	22 ± 2	41.5± 1.5
O	59 ± 1	65 ± 3	64 ± 2
Ca	17.5 ± 2.5	9.5 ± 3.5	12.5 ± 2.5
Na	···	0.325 ± 0.175	0.91 ± 0.41
Fe	0.22 ± 0.04	···	···
Mn	0.105 ± 0.005	···	···
Si	0.87 ± 0.17	···	0.135 ± 0.055
Al	0.63 ± 0.1	···	···
S	0.06 ± 0.01	···	0.505 ± 0.245

the genus *Rhodoferax* are psychrotolerant facultative anaerobes that often use $Fe(OH)_3$ as an electron acceptor (Finnerant et al., 2003). *Geothrix fermentans* is found within the Fe (III) reduction zone of subsurface environments. Such iron bacteria have been shown to attach to the surface of mineral particles by the production of adhesive biopolymer (Nevin and Lovley, 2002). We assume that *Rhodoferax* and *Geothrix* acting as primary colonizers, initiate the first stage of biofilm formation and create conditions favorable for the growth of other heterotrophic bacteria in moonmilk.

Bacteria capable of oxidizing iron and manganese have been repeatedly found in cave sediments. The presence of *Flavobacterium* spp. in the Iron Curtain Cave indicates that it might potentially participate in iron oxidation (Ghosh et al., 2017). *Flavobacterium* spp. was previously reported in abundance in ferromanganese deposits from the caves of the Upper Tennessee River Basin, along with other bacteria indicating that this bacterium contributed to Mn (II) oxidation (Carmichael et al., 2013).

Calcium salts promoting aggregation of bacterial cells and formation of slimy polymers can accelerate the formation of biofilms and their interaction with rocks (Das et al., 2014). In many cases, microorganisms and their extracellular polymeric substances act as effective centers for the formation of new structures that can lead to passive incrustation of biofilms (Flemming, 2016) and affect the structure of the speleothem (Sallstedt et al., 2014). The production of carbonic anhydrase, the enzyme regulating the equilibrium of inorganic carbon forms such as bicarbonate, can play the key role in the mechanism of biomineralization (Smith and Ferry, 2000; Müller et al., 2014).

Microstructure and elemental composition of the moonmilk from the Proschalnaya Cave

While speleologists, geologists, and microbiologists have different views on moonmilk genesis, modern research techniques have revealed an important role of biogenic factors in development of a number of sinter formations. Scanning electron microscopy (SEM) of moonmilk from the Grotta Nera Cave (Italy) revealed fibrous formations with calcites identified by X-ray refractometry (Cacchio et al., 2014). An array of elements were detected in the moonmilk, including Ca, Mg, Al, P, Si, S, Mn, K, and Fe. The proportion of CaO was as high as 60.87 % in some samples, while the portion of oxides such as MgO and Al_2O_3 never exceeded 1 %.

SEM imaging of moonmilk from the Proshchalnaya Cave showed the presence of morphologically distinct microstructures with different elemental composition. Tubular structures in the composition of moonmilk (Fig. 5) distinguished themselves by a rich chemical content. Except for the basic elements indicative of their carbonate genesis (C, O, and Ca), in tubular structures Al, Si, and Fe were also present. In one of the loci, impurities of magnesium and sulfur were observed. Al and Si oxides are often found as impurities in dolomite ($CaMg(CO_3)_2$), which is represented as inclusions in calcite and as part of fine-grained sediments, including in moonmilk (Hill and Forti, 2007). Similar tubular structures called nanofibres were found in caves and relate to secondary calcites (Bindschedler et al., 2014).

Calcium content in different samples of moonmilk from Snezhnaya Cave (Russia, Western Caucasus) indicated differences in nanostructures (Kondratyeva et al., 2016). The highest calcium content (up to 61.54 % by weight) was observed in the cubic crystal microstructures. In this locus, the contents of carbon and magnesium oxides were 33.79 %

and 3.26 % by weight, respectively. The highest level of carbon oxides (58.62 % to 82.73 % by weight) characterized the biofilm microstructures. These microstructures also had elevated levels of magnesium oxides (up to 16 % by weight). Detailed scanning of the images of moonmilk from Snezhnaya Cave moonmilk revealed specific microstructures resembling stacks of thin lamellars. Elemental composition of these plates was characterized by relatively low calcium content (0.1–0.14 % by weight) and considerably high magnesium content (14.65–22.6 % by weight).

In a Belgian Cave (Collembola Cave), abundant, randomly-oriented, single-crystal rods, and polycrystalline calcite fibers were present in the structure of moonmilk (Maciejewska et al., 2017). The tubular microstructures in moonmilk from the Proschalnaya Cave had high similarity with these microstructures. Also SEM images of the microstructures in moonmilk from the Proschalnaya Cave were similar to calcitic nanofibres, needle fibre calcite, tubular- and filament-like structures in other scientific literature (Shankar and Achyuthan, 2007; Maciejewska et al., 2015). However, the tubular nanostructures found by us are not similar to the reticulated filaments described earlier (Melim et al., 2008).

Various mechanisms for the formation of nanostructures are proposed: physicochemical processes, such as the deposition of salts on the cell surface or the deposition of calcite crystals on organic matrices; and calcination of fungal mycelium or actinobacteria (Bindschedler et al., 2014; Maciejewska et al., 2015). *Proteobacteria, Acidobacteria,* and *Actinobacteria* were the most common phyla in strong association with the needle calcite in moonmilk (Cirigliano et al., 2018).

Needle calcite is a common secondary speleothem (Cailleau et al., 2009). The presence of calcitic nanofibers and needle calcite in secondary $CaCO_3$ sediments can be used to characterize the paleoclimate and assess the ecological situation (Shankar and Achyuthan, 2007). Their ratio can indicate the alternation of arid and semi-arid climatic conditions, although both forms of calcite can also occur in a humid climate (Bindschedler et al., 2012).

For the first time in the mass of moonmilk in the Proschalnaya Cave we discovered claviform nanostructures (Fig. 6). In comparison with tubular structures, they have limited elemental composition (Table 4). The dominant components in these structures are carbon, oxygen, and calcium, but the calcium content in claviform nanostructure is lower than in tubular structures and nanofibers.

In some crystals of ancient calcites, needle structures composed of aragonite ($CaCO_3$) are found. It is assumed that, depending on the environmental conditions, sequential precipitation of calcite-aragonite-calcite can occur. The formation of aragonite in speleothems is associated with a high Mg/Ca ratio in the drip water, as Mg is an inhibitor of calcite growth (Wassenburg et al., 2012). In the nanotubes and claviform microstructures we observed that Mg was extremely rare. We assumed that during the formation of the moonmilk mass against the background of a decrease in the amount of rainfall, precipitation of calcites without magnesium occurred, in spite of its presence in groundwater.

Conclusions

The nature of the interaction of groundwater and surface water varies greater under the influence of the biochemical activity of microorganisms. Due to the movement of waters and the biochemical activity of microorganisms, the most intensive dissolution of the bedrock occurs in the spring, resulting in an increase in the calcium content where fracture and drip water interact with rock. Based on our studies, we assume that the formation of moonmilk in Proschalnaya Cave largely depends on the rate of entry of organic substances and the ratio of elements accumulating in bacterial polymers. Consequently, the movement of surface water and groundwater in the area of Proschalnaya Cave drive the biogeochemical processes important for the formation of moonmilk, and the origin of the water can dictate the mineral composition of the speleothems.

Climatic conditions are an important factor affecting the speed and stages of the formation of the biomass of moonmilk. Microorganisms are producers of polymeric compounds; they act as first settlers in the initial stages of the formation of the biofilm from moonmilk, and then accumulate other elements, forming biominerals. The biospheric approach to the moonmilk speleothem study is based on interdisciplinary research that relies on macro processes (geological and geochemical) and micro processes occurring at the scale of microbial cells and biofilms. Calcite transformation and geomorphology of karst caves are changed due to the specific formation of biofilms. Moonmilk provides clear evidence of the role of biofilms in transformation of rocks in underground ecosystems.

Acknowledgments

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References

Achal, V. and Pan X., 2014, Influence of calcium sources on microbially induced calcium carbonate precipitation by *Bacillus sp.* CR2: Journal of Applied Biochemistry and Biotechnology, v. 173, no. 1, p. 307–314. https://doi.org/10.1007/s12010-014-0842-1

Banks, E.D., Taylor, N.M., Gulley, J., Lubbers, B.R., Giarrizzo, J.G., Bullen, H.A., Hoehler, T.M., and Barton, H.A., 2010, Bacterial calcium carbonate precipitation in cave environments: a function of calcium homeostasis: Geomicrobiology Journal, v. 27, p. 444–454. https://doi.org/10.1080/01490450903485136

Barton, H.A., Taylor, M.R., and Pace, N.R., 2004, Molecular phylogenetic analysis of a bacterial community in an oligotrophic cave environment: Geomicrobiology Journal, v. 21, p. 11–20. https://doi.org/10.1080/01490450490253428

Barton, H.A. and Northup, D.E., 2007, Geomicrobiology in cave environments: past, current and future perspectives: Journal of Cave and Karst Studies, v. 69, p. 163–178.

Bersenyov, Yu.I., 1989, Karst of the Far East: Moscow, Nauka, 172 p. (*in Russian*)

Bindschedler, S., Cailleau, G., Braissant, O., Millière, L., Job, D., and Verrecchia, E.P., 2014, Unravelling the enigmatic origin of calcitic nanofibres in soils and caves: purely physicochemical or biogenic processes?: Biogeosciences, v. 11, p. 2809–2825. https://doi.org/10.5194/bg-11-2809-2014

Bindschedler, S., Millière, L., Cailleau, G., Job, D., and Verrecchia, E.P., 2012, An ultrastructural approach to analogies between fungal structures and needle fibre calcite: Geomicrobiology Journal, v. 29, p. 301–313. https://doi.org/10.1080/01490451.2011.558565

Borsato, A., Frisia, S., Jones, B., and Van der Borg, K., 2000, Calcite Moonmilk: crystal morphology and environment of formation in caves in the Italian Alps: International Journal of Sediment Research, v. 70, p. 1179–1190. https://doi.org/10.1306/032300701171

Braun, B., Schröder, J., Knecht, H., and Szewzyk, U., 2016, Unraveling the microbial community of a cold groundwater catchment system: Water Research, v. 107, p. 113–126. https://doi.org/10.1016/j.watres.2016.10.040

Cacchio, P., Ferrini, G., Ercole, C., Del Gallo, M., and Lepidi, A., 2014, Biogenicity and characterization of moonmilk in the Grotta Nera (Majella National Park, Abruzzi, central Italy): Journal of Cave and Karst Studies, v. 76, p. 88–103. https://doi.org/10.4311/2012mb0275

Cailleau, G., Verrecchia, E.P., Braissant, O., and Emmanuel, L., 2009, The biogenic origin of needle fibre calcite: Sedimentology, v. 56, p. 1858–1875. https://doi.org/10.1111/j.1365-3091.2009.01060.x

Canaveras, J.C., Cuezva, S., Sanchez-Moral, S., Lario, J., Laiz, L., Gonzalez, J.M., and Saiz-Jimenez, C., 2006, On the origin of fiber calcite crystals in moonmilk deposits: Naturwissenschaften, v. 93, p. 27–32. https://doi.org/10.1007/s00114-005-0052-3

Carmichael, M.J., Carmichael, S.K., Santelli, C.M., Strom, A., and Brauer, S.L., 2013, Mn(II)-oxidizing bacteria are abundant and environmentally relevant members of ferromanganese deposits in caves of the upper Tennessee river basin: Geomicrobiology Journal, v. 30, p. 779–800. https://doi.org/10.1080/01490451.2013.769651

Chelius, M.K., Beresford, G., Horton, H., Quirk, M., Selby, G., Simpson, R.T., Horrocks, R., and Moore, J.C., 2009, Impacts of alterations of organic inputs on the bacterial community within the sediments of Wind Cave, South Dakota, USA: International Journal of Speleology, v. 38, p. 1–10. https://doi.org/10.5038/1827-806x.38.1.1

Chen, Y., Wu, L., Boden, R., Hillebrand, A., Kumaresan, D., and Moussard, H., 2009, Life without light: microbial diversity and evidence of sulfur- and ammoniumbased chemolithotrophy in Movile cave: Journal of the International Society for Microbial Ecology, v.3, no. 9, p. 1093–1104. https://doi.org/10.1038/ismej.2009.57

Cirigliano, A., Tomassetti, M.C., Di Pietro, M., Mura, F., Maneschi, M.L., Gentili, M.D., Cardazzo, B., Arrighi, C., Mazzoni, C., Negri, R., and Rinaldi, T., 2018, Calcite moonmilk of microbial origin in the Etruscan Tomba degli Scudi in Tarquinia: Italy Scientific Reports, v. 8, no. 1, p. 1–10. https://doi.org/10.1038/s41598-018-34134-y

Das, T., Sehar, S., Koop, L., Wong, Y.K., and Ahmed, S., 2014, Influence of calcium in extracellular DNA mediated bacterial aggregation and biofilm formation: Plos One, v. 9, no. 3, p. 1–11. https://doi.org/10.1371/journal.pone.0091935

De Mandal, S., Chatterjee, R., and Kumar, N.S., 2017, Dominant bacterial phyla in caves and their predicted functional roles in C and N cycle: BMC Microbiology, v. 17, no. 1, p. 1–9. https://doi.org/10.1186/s12866-017-1002-x

Egorov, N.S., 1995, Microbiology Practice Guidance: Moscow, MGU, 224 p. (*in Russian*)

Ercole, C., Cacchio, P., Botta, A.L., Centi, V., and Lepidi, A., 2007, Bacterially induced mineralization of calcium carbonate: the role of exopolysaccharides and capsular polysaccharides: Microscopy and Microanalysis, v. 13, p. 42–50. https://doi.org/10.1017/s1431927607070122

Federation Regulation F 14.1: 2: 4.143-98, 2011 ed., Quantitative chemical analysis of water. Method for measuring the mass concentration of aluminum, barium, boron, iron, potassium, calcium, cobalt, magnesium, manganese, copper, sodium, nickel, strontium, titanium, chromium and zinc in drinking, natural and waste waters by ICP spectrometry method, FR.1.31.2013.13911; https://www.russiangost.com/p-275435-fr131201313911.aspx

Ferris, F.G., 2005, Biogeochemical properties of bacteriogenic iron oxides: Geomicrobiology Journal, v. 22, no. 3–4, p. 79–85. https://doi.org/10.1080/01490450590945861

Finnerant, K.T., Johnsen, C.V., and Lovley, D.R., 2003, *Rhodoferax ferrireducens* sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe (III): International Journal of Systematic and Evolutionary Microbiology, v. 53, p. 669–673. https://doi.org/10.1099/ijs.0.02298-0

Flemming, H.C., 2016, EPS—then and now: Microorganisms, v. 4, no. 4, 41 p. https://doi.org/10.3390/microorganisms4040041

Fornós, J.J., Ginés, J., Gràcia, F., Merino, A., Gómez-Pujol, L., and Bover, P., 2014, Cave deposits and sedimentary processes in Cova des Pas de Vallgornera (Mallorca, Western Mediterranean): International Journal of Speleology, v. 43, no. 2, p. 159–174. http://dx.doi.org/10.5038/1827-806X.43.2.5

Garcia, G.M., Márquez, G.M.A., and Moreno, H.C.X., 2016, Characterization of bacterial diversity associated with calcareous deposits and drip-waters, and isolation of calcifying bacteria from two Colombian mines: Microbiological Research, v. 182, p. 21–30. https://doi.org/10.1016/j.micres.2015.09.006

Gerhardt, F., 1983, General Bacteriology Methods: Moscow, Mir, 536 p. (*in Russian*)

Ghosh, S., Paine, E., Wall, R., Kam, G., Lauriente, T., Sangarmangkang, P.C., Derrick Horne, D., and Cheeptham, N., 2017, *In situ* cultured bacterial diversity from iron Curtain Cave, Chilliwack, British Columbia, Canada: Diversity, v. 9 , no. 3, p. 1–15. https://doi.org/10.3390/d9030036

Hill, C.A. and Forti, P., 2007, Cave mineralogy and the NSS: past, present, future: Journal of Cave and Karst Studies, v. 69, p. 35–45.

Kondratyeva, L.M., Polevskaya, O.S., Litvinenko, Z.N., Golubeva, E.M., and Konovalova, N.S., 2016, Role of the microbial community in formation of speleothem (moonmilk) in the Snezhnaya karst cave (Abkhazia): Microbiology (Moscow), v. 85, no. 5, p. 629–637. https://doi.org/10.1134/s002626171605009x

Kubista, M., Andrade, J.M., Bengtsson, M., Forootan, A., Jonake, J., Lind, K., Sindelka, R., Sjoback, R., Sjogreen, B., Strombom, L., Stahlberg, A., and Zoric, N., 2006, The real-time polymerase chain reaction: Molecular Aspects of Medicine, v. 27, p. 95–125. https://doi.org/10.1016/j.mam.2005.12.007

Kuhn, K.M., DuBois, J.L., and Maurice, P.A., 2014, Aerobic microbial Fe acquisition from ferrihydrite nanoparticles: effects of crystalline order, siderophores, and alginate: Environmental Science and Technology, v. 48, no. 15, p. 8664–8670. https://doi.org/10.1021/es501308b

Kuznetsov, S.I. and Dubinina, G.A., 1989, Methods for the Study of Aquatic Microorganisms: Moscow, Nauka, 288p. (*in Russian*)

Lefevre, E., Bossa, N., Wiesner, M.R, and Gunsch, C.K., 2016, A review of the environmental implications of *in situ* remediation by nanoscale zero valent iron (nZVI): behavior, transport and impacts on microbial communities: Science of the Total Environment, v. 565, p. 889–901. https://doi.org/10.1016/j.scitotenv.2016.02.003

Maciejewska, M., Adam, D., Naômé, A., Martinet, L., Tenconi, E., Całusińska, M., Delfosse, P.,

Müller, W.E., Schlossmacher, U., Schröder, H.C., Lieberwirth, I., Glasser, G., Korzhev, M., Neufurth, M., and Wang, X., 2017, Enzyme-accelerated and structure-guided crystallization of calcium carbonate: role of the carbonic anhydrase in the homologous system: Acta Biomaterialia, v. 10, no.1, p. 450–462. https://doi.org/10.3389/fmicb.2017.01181

Maciejewska, M., Pessi, I.S., Arguelles-Arias, A., Noirfalise, P., Luis, G., Ongena, M., Barton, H., Carnol, M., and Rigali, S., 2015, *Streptomyces lunaelactis* sp. nov*.,* a novel ferroverdin A-producing Streptomyces species isolated from a moonmilk speleothem: Antonie van Leeuwenhoek, v. 107, no. 2, p. 519–531. https://doi.org/10.1007/s10482-014-0348-4

Margesin, R., 2009, Effect of temperature on growth parameters of psychrophilic bacteria and yeasts: Extremophiles, v. 13, no. 2, p. 257–262. https://doi.10.1007/s00792-008-0213-3

Melim, L. A., Northup, D. E., Spilde, M. N., Jones, B., Boston, P. J., and Bixby, R. J, 2008, Reticulated filaments in cave pool speleothems: microbe or mineral?: Journal of Cave and Karst Studies, v. 70, p. 135–141. https://doi.org/10.7939/R3J38KZ7V

Mitchell, A.C. and Ferris F.G., 2006, The influence of *Bacillus pasteuriion* the nucleation and growth of calcium carbonate: Geomicrobiology Journal, v. 23, p. 213–226. https://doi.org/10.1080/01490450600724233

Müller, W.E., Schlossmacher, U., Schröder, H.C., Lieberwirth, I., Glasser, G., Korzhev, M., Neufurth, M., and Wang, X., 2014, Enzyme-accelerated and structure-guided crystallization of calcium carbonate: role of the carbonic anhydrase in the homologous system: Acta Biomaterialia, v. 10, no. 1, p. 450–462. https://doi.org/10.1016/j.actbio.2013.08.025

Namsaraev, B.B., Barkhutova, D.D., Khakhinov, V.V., 2006, Field workshop on aquatic microbiology and hydrochemistry: Methodical manual/ Ed. M. B. Vainshtein: Moscow–Ulan-Ude, Publishing House of the Buryat State University, 68 p.

Nevin, K.P. and Lovley, D.R., 2002, Mechanisms for accessing insoluble Fe (III) oxide during dissimilatory Fe (III) reduction by *Geothrix fermentans*: Applied Environmental Microbiology, v. 68, no. 5, p. 2294–2299. https://doi.org/10.1128/aem.68.5.2294-2299.2002

Perry, T.D., Duckworth, O.W., McNamara, C.J., Martin, S.T., and Mitchell, R., 2004, Effects of the biologically produced polymer alginic acid on macroscopic and microscopic calcite dissolution rates: Environmental Science and Technology, v. 38, p. 3040–3046. https://doi.org/10.1021/es035299a

Porca, E., Jurado, V., Zgur-Bertok, D., Saiz-Jimenez, C., and Pasic, L., 2012, Comparative analysis of yellow microbial communities growing on the walls of geographically distinct caves indicates a common core of microorganisms involved in their formation: FEMS Microbiology Ecology, v. 81, p. 255–266. https://doi.org/10.1111/j.1574-6941.2012.01383.x

Portillo, M.C. and Gonzales, J.M., 2011, Moonmilk deposits originate from specific bacterial communities in Altamira Cave (Spain): Microbial Ecology, v. 61, p. 182–189. https://doi.org/10.1007/s00248-010-9731-5

Reitschuler, C., Spötl, C., Hofmann, K., Wagner, A.O., and Illmer, P., 2016, Archaeal distribution in moonmilk deposits from Alpine caves and their ecophysiological potential: Microbial Ecology, v. 71, no. 3, p. 686–699. https://doi.org/10.1007/s00248-015-0727-z

Rodrigues, D.F. and Tiedje, J.M., 2008, Coping with our cold planet: Applied Environmental Microbiology, v. 74, p. 1677–1686. https://doi.10.1128/AEM.02000-07

Sallstedt, T., Ivarsson, M., Lundberg, J., Sjöberg, R., and Vidal Romaní, J.R., 2014, Speleothem and biofilm formation in a granite/dolerite cave, Northern Sweden: International Journal of Speleology, v. 43, no. 3, p. 305–313. https://doi.org/10.5038/1827-806x.43.3.7

Shankar, N. and Achyuthan, H., 2007, Genesis of calcic and petrocalcic horizons from Coimbatore, Tamil Nadu: micromorphology and geochemical studies: Quaternary International, v. 175, p. 140–154. https://doi.org/10.1016/j.quaint.2007.05.017

Shesterkin V.P., 1983, Chemical Content of Groundwater and Ice From Proschalnaya Cave. Genesis of groundwater: Irkutsk, Institute of the Earth's Crust, 140 p. (*in Russian*)

Smith, K.S. and Ferry, J.G., 2000, Prokaryotic carbonic anhydrases: FEMS Microbiology Reviews, v. 24, p. 335–366. https://doi.org/10.1016/s0168-6445(00)00030-9

Struvay, **C**. and Feller, G., 2012, Optimization to low temperature activity in psychrophilic enzymes: International Journal of Molecular Sciences, v.13, no. 9, p. 11643–11665. https://doi.org/10.3390/ijms130911643

Vasiliev, D.A., Kaldyrkaev, A.I., Feoktistova, N.A., and Aleshkin, A.V., 2013, Identification of Bacillus cereus Bacteria Based on Their Phenotypic Characteristics: Uliyanovsk, Nauchnoe izdanie, 98 p. (*in Russian*)

Velikonja, B.H., Tkavc, R., and Paši´c, L., 2014, Diversity of cultivable bacteria involved in the formation of macroscopic microbial colonies (cave silver) on the walls of a cave in Slovenia: International Journal of Speleology, v. 43, p. 45–56. https://doi.org/10.5038/1827-806x.43.1.5

Wassenburg, J.A., Immenhauser, A., Richter, D.K., Jochum, K.P., Fietzke, J., Deininger, M., Goos, M., Scholz, D., and Sabaoui, A., 2012, Climate and cave control on Pleistocene/Holocene calcite-to-aragonite transitions in speleothems from Morocco: elemental and isotopic evidence: Geochimica et Cosmochimica Acta, v. 92, p. 23–47. https://doi.org/10.1016/j.gca.2012.06.002

Wu, Y., Tan, L., Liu, W., Wang, B., Wang, J., Cai, Y., and Lin, X., 2015, Profiling bacterial diversity in a limestone cave of the western loess plateau of China: Frontiers in Microbiology, v. 6, no. 244, p. 1–10. https://doi.org/10.3389/fmicb.2015.00244

Nihal Doğruöz-Güngör, Begüm Çandıroğlu, and Gülşen Altuğ. Enzyme profiles and antimicrobial activities of bacteria isolated from the Kadiini Cave, Alanya, Turkey. *Journal of Cave and Karst Studies*, v. 82, no. 2, p. 106-115. DOI:10.4311/2019MB0106

ENZYME PROFILES AND ANTIMICROBIAL ACTIVITIES OF BACTERIA ISOLATED FROM THE KADIINI CAVE, ALANYA, TURKEY

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Abstract

Cave ecosystems are exposed to specific environmental conditions and offer unique opportunities for bacteriological studies. In this study, the Kadıini Cave located in the southeastern district of Antalya, Turkey, was investigated to document the levels of heterotrophic bacteria, bacterial metabolic avtivity, and cultivable bacterial diversity to determine bacterial enzyme profiles and antimicrobial activities. Aerobic heterotrophic bacteria were quantified using spread plates. Bacterial metabolic activity was investigated using DAPI staining, and the metabolical responses of the isolates against substrates were tested using VITEK 2 Compact 30 automated micro identification system. The phylogenic diversity of fourty-five bacterial isolates was examined by 16S rRNA gene sequencing analyses. Bacterial communities were dominated by members of Firmicutes (86 %), Proteobacteria (12 %) and Actinobacteria (2 %). The most abundant genera were *Bacillus, Staphylococcus* and *Pseudomonas*. The majority of the cave isolates displayed positive proteolytic enzyme activities. Frequency of the antibacterial activity of the isolates was 15.5 % against standard strains of *Bacillus subtilis, Staphylococcus epidermidis*, *S.aureus,* and methicillin-resistant *S.aureus*. The findings obtained from this study contributed data on bacteriological composition, frequency of antibacterial activity, and enzymatic abilities regarding possible biotechnological uses of the bacteria isolated from cave ecosytems.

Introduction

Caves are among the extreme environments in the world due to the low and generally stable temperature, minimum light, low nutrients, and high humidity (Palmer 1991; Tomczyk-Żak and Zielenkiewicz 2015). There are many cave types around the world and many different classifications have been used for these geological forms (Northup and Lavoie 2001; Engel 2011). Karstic caves are the most common, formed through geomorphological and microbiological processes (Engel 2010; Tisato et al., 2015; Bontognali et al., 2016). One of the critical subjects in cave research is the adaptation of cave microorganisms to extreme conditions. Previous studies have shown many different microorganisms in caves and identified them from water bodies, on rocky surfaces, in guanos, and on sediments (Herzog Velikonja et al., 2014; Tomczyk-Żak and Zielenkiewicz 2015). Organic materials introduced by people, dripping water, floods, and animals, especially bats, create an environment that allows heterotrophic bacteria to grow in caves (Borda et al., 2014). Microorganisms may enter caves through different processes. Water, wind or air conditions may facilitate their transportation, or sometimes animals can carry microorganisms into caves (Romero 2009). Other transportation paths of microorganisms are created by humans, resulting in changes to native microbial communities. Difficult environmental conditions and low nutrients in cave environments create competition among microorganisms, which can produce antibiotics against each other in these environments (Bhullar et al., 2012). This natural process offers unique opportunities for biotechnological applications and possible uses of new bioactive substances, including new antibiotics.

Appliations of enzymes in techology is a rapidly-developing field, and is increasingly dependent on microbial enzymes. Microbial enzymes are more stable and are produced at a faster rate in greater amounts, making them the preferred source of enzymes. These enzymes take an important role in the diagnosis, treatment, industrial applications, biochemical tests and monitoring of various diseases. Moreover, diverse peptidases with particular biochemical properties have been identified from studies of microbial diversity of bacteria and fungi. This has provided a broad range of peptidase applications, particularly in the field of microbial biochemistry. For this reason, it is important to identify bacteriological community structure and metabolic characteristics in caves ecosytems.

The study has three main goals:

1. to investigate the levels of culturable aerobic heterotrophic bacteria (HPC), cultivable bacterial composition, and the frequency of metabolically-active bacteria in a cave ecosystem;
2. to detect the frequency of antimicrobial activities of the cave isolates against selected bacteria;
3. to evaluate biotechnological potential of the cave isolates regarding metabolic definition, biochemical reactions, and enzyme profiles.

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Materials and Methods

Cave background and sampling site

Kadıini Cave, which is closed to tourist activities, is located in Obaköy, a village 3.8 km away from Alanya, a southeastern district of Antalya, Turkey (Fig. 1). Kadıini Cave is a horizontal cave with a length of 2027 m and a depth of 45 m (Turkey Cave Database). The first research in the Kadıini Cave was conducted in 1957. During the research, human skeletons and archaeological finds such as cups, hearths, whorls and stone tools were found. The human skeletons are from 6000 years ago and showed that people used the cave as a living/shelter space. The entrance hall of the Kadıini Cave is a gallery that is rich in terms of stalactites and stalagmites. It was a place of settlement during the Upper Paleolithic and the Early Bronze Age (Yılmazusta and Yakup Ipekoğlu 2019). Members of the Akdeniz University Cave Research Club (AKÜMAK) began investigating the Kadıini Cave in 2008 and delving into the unexplored cave sections. In 2014, the Anatolian Speleology Association (ASPEG) started to support these studies. During sampling it was observed that the source of organic substances is guano and the water seeping through the walls. 650 bats of which 400 *Rousettus aegyptiacus*, 200 *Rhinolophus blasii* and 50 *Miniopterus schreibersii* were identified in the project entitled "Identification and Protection of Important Bat Caves in Turkey" that was carried out in 2012 in the Kadıini Cave (Coraman et al., 2012). There is also an underground stream at the end of the cave.

Sample collection

Water and soil samples were collected under aseptic conditions in the two different sites from the dark zone of the Kadıini Cave (Fig. 1). The samples were maintained at 4 °C and transported within 24 hours to the laboratory.

Variable environmental parameters of the sampling area

The water samples collected from the cave were measured *in situ* in terms of temperature, pH, dissolved oxygen, conductivity, total dissolved solids (TDS) and salinity using a portable multiparameter tool (a Hach Lange HQ40D multimeter). Air temperature and humidity at the two investigation sites were also measured by a portable temperature/humidity meter. The Mann-Whitney U test was used to determine statistically significant changes between measured environmental parameters of the two sample regions. All analyzes were performed using SPSS for Windows Version IBM 21 and $P < 0.05$ was considered statistically significant.

Bacteriological analyses

For the analyses of bacteria, 200 mL water samples were concentrated with a 0.22 µm pore size polyamide filter and then the filters were re-suspended in 20 mL sterile tap water using a homogenizator (IUL Instruments) for 2 min. For soil samples, one gram was weighed and homogenized in flasks containing 9 mL of sterile saline water. Serial dilutions of water and soil (10^{-1} to 10^{-7}) were then prepared with sterile saline water and were used as an inoculum for the isolation and enumeration of HPC counts. To estimate the number of aerobic heterotrophic bacteria, triplicate 100 µL volumes of each dilution (10^{-1} to 10^{-7}) were spread onto R2A (OXOID) agar plates. These plates were aerobically incubated at 28°C for 7 days. After the incubation, for each water and soil samples, the dilution that

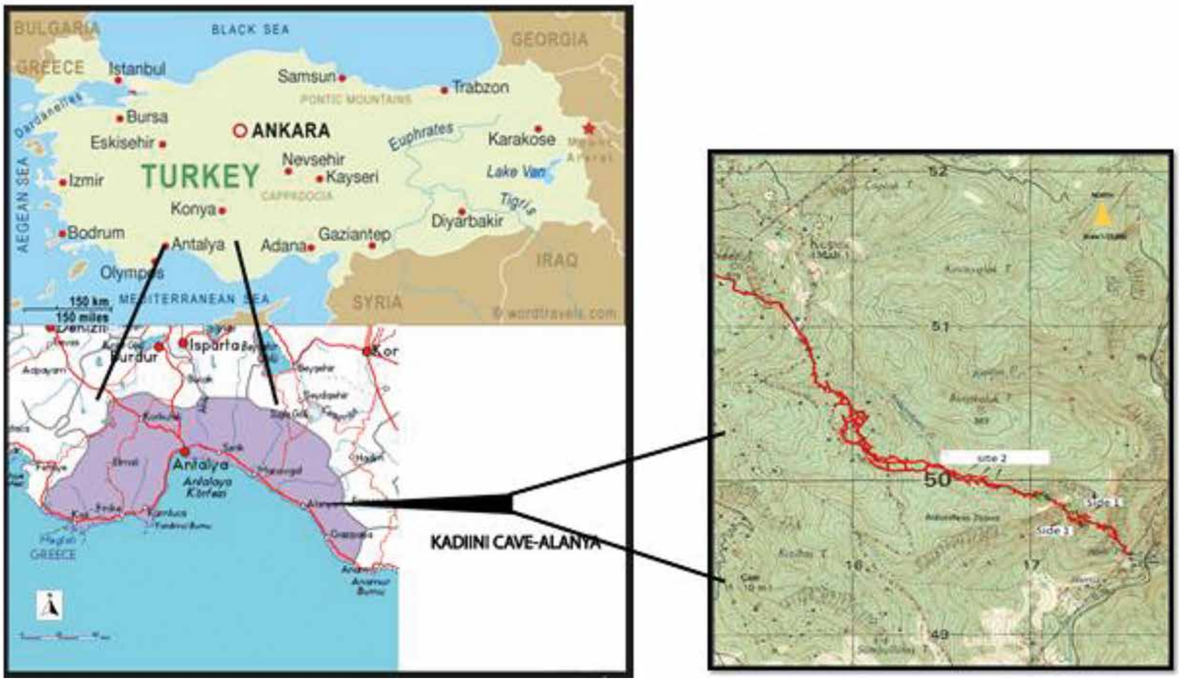

Figure 1. Map of the Kadıini cave, Alanya, Antalya-Turkey.

was counted contained between 30 and 300 colonies and expressed as the log of the total number of colony forming units (CFU) (Reasoner and Geldrich 1985).

Different colonies that grew on R2A plates were selected and stored at −86 °C for subsequent testing of the isolates. To determine the direct viable counts of actively respiring bacteria and the total number of the bacteria cells, the redox dye, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) and the DNA-binding fluorochrome, 4´,6-diamidino-2-phenylindole (DAPI) were used (Schwartz et al., 2003). CTC was used together with DAPI to distinguish between the metabolically active cells and the dead cells. The waters and soil suspensions (900 µl) were incubated with the aliquots of a 50 mM CTC redox dye solution to a final concentration of 5 mM in the dark at 28°C for 4 h. After the CTC incubation, 1.0 µg /mL DAPI solution was added to the samples and incubated for 1 h in the dark at 28°C. After incubation, the samples were filtered by a vacuum filtration onto black 0.2 µm pore size polycarbonate filters. The membrane was placed on a glass slide and examined under the oil immersion in a Nikon 80i microscope which was equipped with appropriate filters for CTC and DAPI. The number of bacteria was calculated from the counts of 20 randomly selected microscopic fields (at 1000×). For all the bacterial counting, an eyepiece with a calibrated graticule was used. The estimation of the number of cells in each sample was calculated using (Dogruoz Güngör and Sanli Yurudu 2015)

$$N = \frac{Sn}{CV} D,$$

where N is the number of microorganisms per milliliter, S is the real area of filtration, n is the average number of microorganisms per field of vision, C is the real area of microscopic range, V is the volume of filtered sample, and D is the sample dilution.

16S rDNA amplification

For the identification of isolates, their genomic DNA was isolated by using a bacterial DNA isolation kit (GeneAll Biotechnology, Seoul, Korea). The isolated DNA was amplified by 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) universal primers. The reaction was performed in a volume of 50 µL, comprising 20 nM each primer, 10 ng of cDNA, 2.5 U of Taq DNA polymerase (Bioline, UK), in a single block thermal cycler (Bio-rad, California, USA). The cycle conditions were at 95°C for 1 min initial DNA denaturation, followed by 35 cycles consisting of 15 s denaturation at 95°C, 15 s annealing at 55°C, and 10 s extension at 72°C. The PCR products were sequenced by the Sanger sorting method. Sequences were read by the ABI 3130 Sequencer (Applied Biosystems). Sequences and the top BLAST hit in NCBI were edited. The16S rDNA sequences were deposited in GenBank (NCBI) under the accession numbers MK491005-MK491049.

Biochemical characterization and enzyme profiles of the isolates

To determine biochemical characterization and enzyme profiles of bacterial isolates, a Gram stain, a catalase test, and an oxidase test were conducted. Then they were identified by using GN (Gram-negative fermenting and non-fermenting bacilli), GP (Gram-positive cocci and non-spore-forming bacilli) and BCL (Gram-positive spore-forming bacilli) cards in the automated micro identification system, specialized for environmental samples with industrial software, VITEK 2 Compact 30 (bioMerieux, France). The identification cards are predicated upon the established biochemical methods and recently developed substrates. The calculations are conducted on raw data and compared to thresholds to designate reactions for each test (Pincus 2005).

Evaluation of antimicrobial activity

By using inhibition zone technique, antimicrobial activity of the isolated bacteria were tested against bacterial strains, including pahogens [*S.epidermidis* (ATCC 12228), *B.subtilis* (ATCC 6633), *S.aureus* (ATCC 6538), *P.aeruginosa* (ATCC 9027), *E.coli* (ATCC 8739), methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC 33591) and vancomycin-resistant enterococci (VRE) (ATCC 51299)]. Each cave isolate was suspended in saline solution to obtain a concentration of 3×10^8 CFU/mL, then 0.1 mL of the suspensions were spread onto Muller Hinton Agar plates with a Drigalski spatula. Suspensions of the standard bacteria were prepared in saline solution with a final concentration of 1.5×10^8 CFU/mL then spread (0.1 mL) on the surface of Muller Hinton Agar plates using a Drigalski spatula. A small part (6 × 6 mm) of each cave isolate growth was cut and placed on the surface of the inoculated standard strains plates with a nichrome wire loop.

A small part of sterile Muller Hinton agar (6 × 6 mm) was placed on to surface of the inoculated standard strains plate as negative control. Disc of standard antibacterial agents erythromycin (15 µg) (OXOID discs, UK), vancomycin (30 µg) (OXOID discs, UK) , neomycin (10 µg) (OXOID discs, UK) , gentamicin (10 µg) (OXOID discs, UK) and tetracycline (10 µg) (OXOID discs, UK) were used as positive control. All the plates were incubated at 37°C, for 18-24 hours. After incubation, the antimicrobial activity was evaluated by measuring the inhibition zone diameter. Each test was performed twice and the average of the results was taken (Cotuk et al., 2005).

Results

Physicochemical Analysis

The water and soil samples taken from the two designated sites of the dark zone of the Kadıini Cave were analyzed. The air temperature measurements of the samples collected from Site 1 and Site 2 of the cave were 18.9°C and 17.5°C and the air humidity values were 92% and 86%. The environmental parameters of the water samples in the Kadıini Cave are shown in Table 1. Conductivity, TDS and salinity values of the Site 1 were statistically higher than the Site 2 ($p < 0.05$).

Table 1. The Physico-Chemical parameters of the water samples from Kadıini Cave in December 2014.

Parameters	Site 1	Site 2
Water temperature (°C)	17.8	17.4
pH	7.87	8.82
Conductivity (µS/cm)	442	294
TDS (mg/L)	211.5	140.3

Enumeration, isolation, and identification of culturable bacteria from Kadıini Cave

The levels of heterotrophic aerobic bacteria from Site 1 and Site 2 from the dark zone of the Kadıini Cave are given in Table 2. The total (live + dead) and the live bacteria count (log cell/mL, log cell/g) of the water and soil samples are shown in Table 2. By using DAPI-CTC staining, we showed that the total viable bacterial count is higher than the cultured bacterial count.

Table 2. Total (live + dead), live bacteria count (log cell/mL, log cell/g), viability (%) and total culturable aerobic heterotrophic bacteria of water and soil samples from Kadıini Cave in December 2014.

Bacteria Measures	Site 1 Water	Site 1 Soil	Site 2 Water	Site 2 Soil
Total bacteria count[a]	10	10.6	7.8	11
Live bacteria count[b]	9.8	10	7.6	10.5
Viability (%)	41.7	21	37.5	26.3
Culturable aerobic heterotrophic bacteria (log CFU)	2.5 ± 0.03	6 ± 0.2	3.2 ± 0.6	6.9 ± 0.01

[a] Total bacteria' counts determined by DAPI + CTC staining.
[b] Live bacteria' counts determined by CTC staining.

Fourty-five bacteria were isolated from the water and soil samples collected from Kadıini Cave. After amplification of the 16S rRNA gene for each isolate, three phylogenetic groups: *Firmicutes* (86%), *Proteobacteria* (12%) and *Actinobacteria* (2%) were recorded. The composition of cultivable heterotrophic aerobic bacteria, the names and distribution percentage of the identified genera are shown in Table 3. After phylogenetic analysis, a strong domination of Gram-positive aerobic heterotrophic bacteria was established (89%), belonging to four genera: *Bacillus, Viridibacillus, Staphylococcus,* and *Brevibacterium*. Gram-negative isolates (11 %) were represented by two genera: *Pseudomonas* and *Paracoccus* (Table 4).

Biochemical characterization and enzyme profiles of the isolates

Biochemical characterization and enzyme profiles of the bacilli (BCL and spore-forming) (Fig. 2), Gram positive (Fig. 3), and Gram negative (Fig. 4) isolates are determined as a result of the analyses conducted by using the automated micro identification system VITEK 2 Compact 30 (bioMerieux, France). Seventy-eight percent of all isolates have a positive reaction for production of the TyrA enzyme. The percentage of presence of PRY enzyme is found to be high in BCL/spores and GN strains. In addition, the percentage of ProA enzymes is higher in GN and GP strains. The positive reaction percentages of all isolates (BCL, GP, and GN) against the tested substrates are displayed in Figures 2−4.

Antimicrobial activity of the isolates

The cave bacterial isolates were screened to understand their antimicrobial activities against *S. epidermidis* (ATCC 12228), *B.subtilis* (ATCC 6633), *S.aureus* (ATCC 6538), *P.aeruginosa* (ATCC 9027), *E.coli* (ATCC 8739), MRSA (ATCC 33591), and VRE (ATCC 51299) strains by the agar plug diffusion method. The commercial antibacterial agents were used against all tested standard strains (Table 5). In this study, seven bacteria, isolated from the water and soil samples, displayed antimicrobial activity (15.5 %) against the control bacteria.

Discussion

This cave is unexplored from the microbiological point of view, making it interesting to study the bacterial diversity for possible industrial applications. In this study, the total viable bacteria counts were recorded to be higher than the culturable bacteria counts in the samples collected from Kadıini Cave. It was documented that many species of bacteria en-

Table 3. The composition of cultivable heterotrophic aerobic bacteria and percentage distribution of the identified genus.

Phylum	Class	Genus	%
Firmicutes	*Bacilli*	*Bacillus*	67
		Viridibacillus	2
		Staphylococcus	18
Actinobacteria	*Actinobacteria*	*Brevibacterium*	2
Proteobacteria	*Alphaproteobacteria*	*Paracoccus*	2
	Gammaproteobacteria	*Pseudomonas*	9

Table 4. Closest match of the bacterial isolates based on 16S rRNA gene phylogeny analysis.

Isolation ID (accession #)	Nearest relativea/ Bacterial division	Accession # (nearest relativea)	% Similarity
1 (MK491005)	*Bacillus cereus*	MH041184	99
4 (MK491021)	*Bacillus cereus*	KY316431	99
5 (MK491027)	*Bacillus cereus*	KX941839	99
16 (MK491045)	*Bacillus cereus*	MG563677	99
28 (MK491024)	*Bacillus cereus*	MG563677	98
38 (MK491036)	*Bacillus cereus*	MG563677	98
6 (MK491032)	*Bacillus cereus*	MG563677	99
20 (MK491023)	*Bacillus pumilus*	KC182057	98
30 (MK491035)	*Bacillus pumilus*	KF641848	98
31 (MK491041)	*Bacillus pumilus*	HG799995	98
39 (MK491042)	*Bacillus pumilus*	KF641848	99
42 (MK491014)	*Bacillus pumilus*	KF641848	99
46 (MK491037)	*Bacillus pumilus*	KF641848	98
47 (MK491043)	*Bacillus pumilus*	KC182057	99
50 (MK491015)	*Bacillus pumilus*	KY127313	98
37 (MK491030)	*Bacillus pumilus*	KY127313	98
11 (MK491016)	*Bacillus niacini*	KT720235	97
14 (MK491033)	*Bacillus litoralis*	KU983814	98
2 (MK491010)	*Bacillus thuringiensis*	LC146715	99
22 (MK491034)	*Bacillus toyonensis*	KY649418	98
33 (MK491008)	*Bacillus amyloliquefaciens*	CP018902	98
24 (MK491046)	*Bacillus weihenstephanensis*	KF831381	99
48 (MK491049)	*Bacillus mycoides*	MH169305	99
32 (MK491047)	*Bacillus subtilis*	EU883786	99
13 (MK491028)	*Viridibacillus arvi*	KU894793	99
12 (MK491022)	*Bacillus sp.*	MG548383	99
7 (MK491038)	*Bacillus sp.*	MH628022	99
10 (MK491011)	*Bacillus sp.*	FJ348046	97
21 (MK491029)	*Bacillus sp.*	KM108632	97
34 (MK491013)	*Bacillus sp.*	KT316413	99
25 (MK491007)	*Bacillus sp.*	MH698798	98

Table 4. (Continued).

Isolation ID (accession #)	Nearest relativea/ Bacterial division	Accession # (nearest relativea)	% Similarity
35 (MK491019)	*Staphylococcus warneri*	HG799993	99
36 (MK491025)	*Staphylococcus warneri*	KX453876	96
17 (MK491006)	*Staphylococcus warneri*	KX349994	99
15 (MK491039)	*Staphylococcus pasteuri*	KU922389	99
19 (MK491017)	*Staphylococcus pasteuri*	KU922319	98
43 (MK491020)	*Staphylococcus epidermidis*	KX926554	98
40 (MK491048)	*Staphylococcus epidermidis*	KX349995	99
8 (MK491044)	*Staphylococcus sp*	EU177793	99
27 (MK491018)	*Pseudomonas plecoglossicida*	MF716680	97
44 (MK491026)	*Pseudomonas sp.*	CP015992	97
45 (MK491031)	*Pseudomonas sp.*	KX301316	98
49 (MK491009)	*Pseudomonas sp*	KX301316	99
26 (MK491012)	*Paracoccus mutanolyticus*	CP030239	98
23 (MK491040)	*Brevibacterium frigoritolerans*	KU922165	99

Based upon a Blast search of the NCBI database.

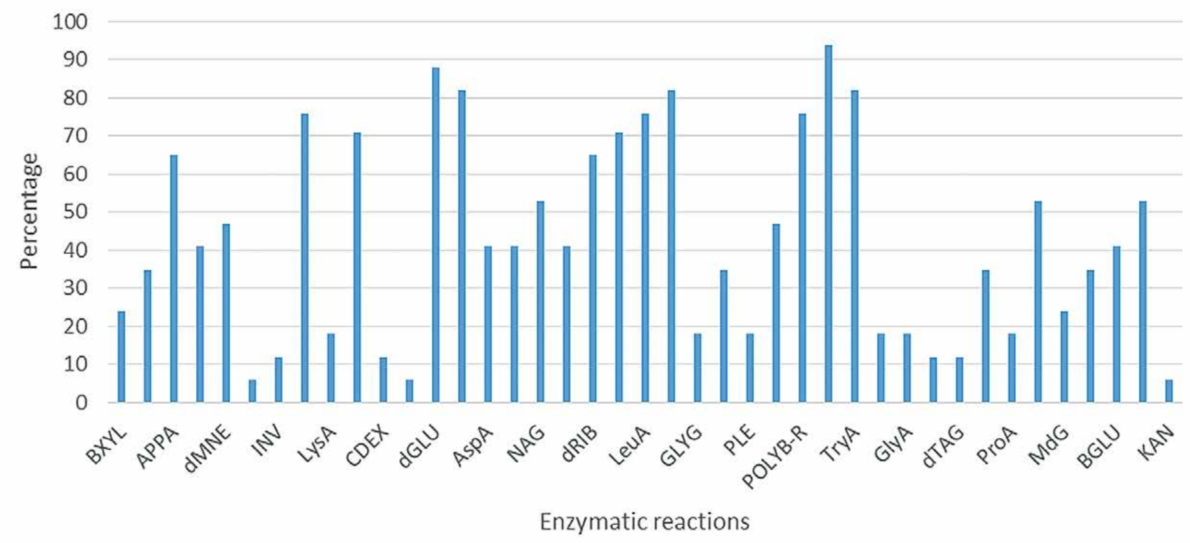

Figure 2. Biochemical characterization and enzyme profiles of the Gram-positive spore-forming bacilli (BCL) isolated from Kadıini Cave.

ter the viable but nonculturable (VBNC) phase under negative environmental conditions (Trevors 2011). Bacterial communities are often exposed to available nutrient constraint, temperature, salinity, osmotic stress, and variable oxygen saturation and enter the VBNC phase under these conditions. The VBNC phase is also described as the genetically programmed physiological response of bacterial cells that are fighting to survive under environmental stress (Besnard et al., 2002).

Conductivity is the capacity of water to conduct an electric current. At the same time it is an indirect measurement of salinity and total dissolved solids (TDS) content (Al Dahaan et al., 2016). In our study, it was determined that the conductivity, TDS and salinity values of Site 1 were statistically higher than Site 2 ($p < 0.05$). Conductivity, TDS and salinity are strongly related to the aquifer rock geochemistry (Bakalowicz 1994). These results may indicate that Site 2 is fed by a water source.

In our study, the microbial communities were dominated by the members of *Firmicutes* (85%), followed by *Proteobacteria* (13%) and *Actinobacteria* (2%). These phyla are encountered in various microbiological studies conducted through culture-based or molecular techniques (Barton 2015). *Firmicutes* are frequently encountered under extreme conditions;

furthermore, they are among the prokaryotes, which are most resistant against dessication and nutrient stress (Slepecky and Hemphill 1992).

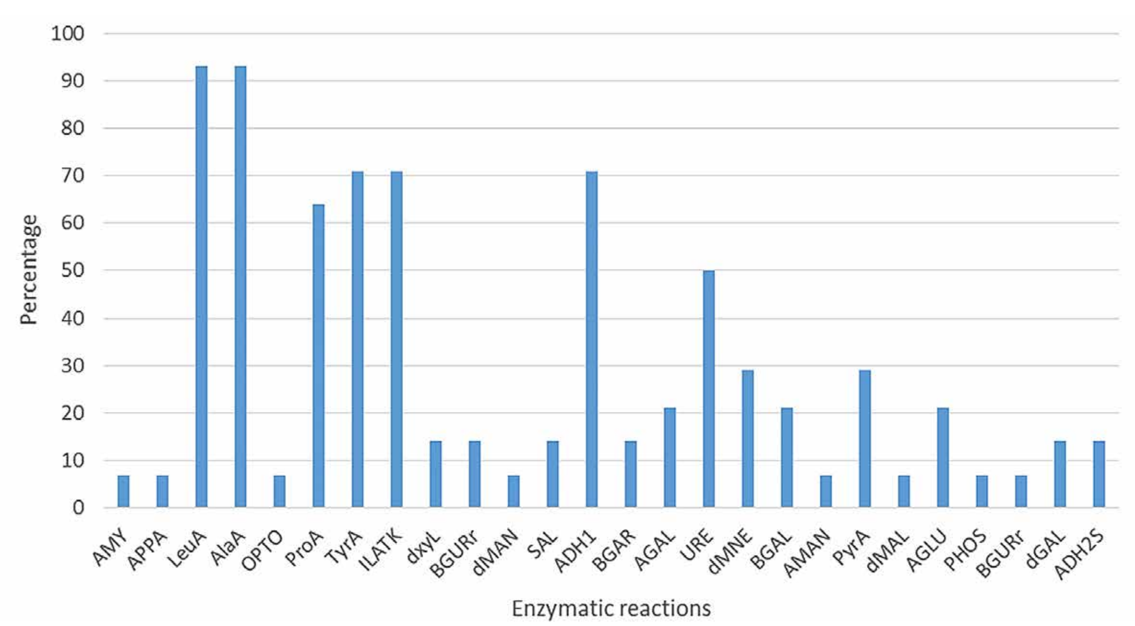

Figure 3. Biochemical characterization and enzyme profiles of the Gram-positive cocci and non-spore-forming bacilli (GP) isolated from Kadıini Cave.

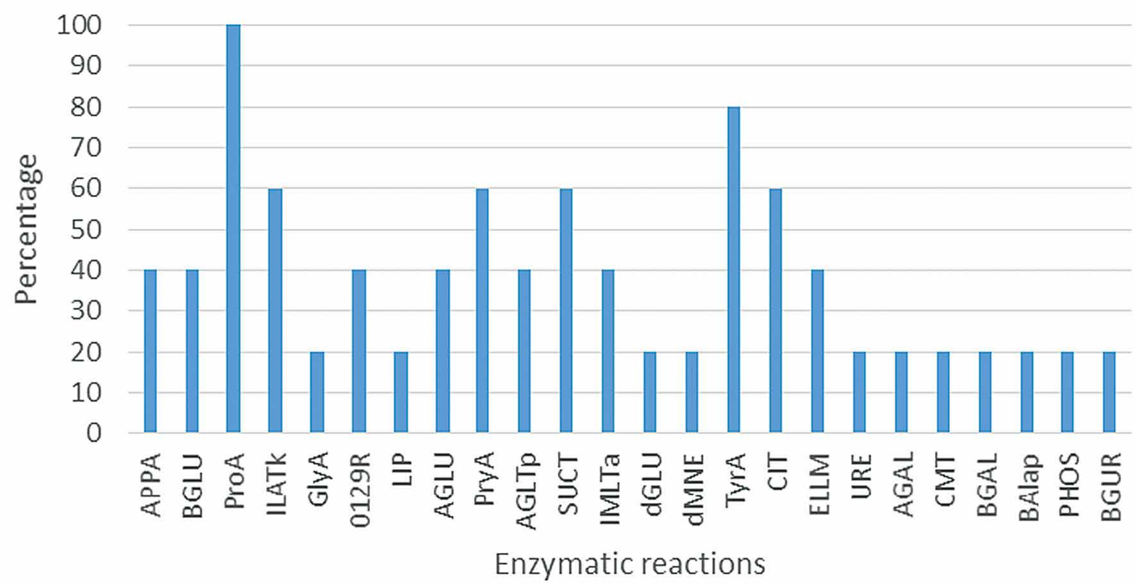

Figure 4. Biochemical characterization and enzyme profiles of the Gram-negative fermenting and non-fermenting bacilli (GN) isolated from Kadıini Cave.

In general, bacteria detected in caves are mainly represented by *Bacillus, Streptomyces, Kocuria, Pseudomonas, Microbacterium, Sphingomonas and Staphylococcus* (Cheeptham et al., 2013; Herzog Velikonja et al., 2014). Although all caves contain common bacterial communities, bacterial diversity in each cave ecosytem is unique. Microbial diversity can be influenced by the variable environmental factors such as pH, air flow, sampling area heterogeneity, the spatial variability of microbial population, sediment moisture content, and organic matter input in type and location (Portillo et al., 2009). In the present study, while *Bacillus* were the most common genera represented by nine species, *Staphylococcus* follow it as the second most common genera with three species. The most common species belonging to *Bacillaceae* family was *Bacillus pumilus,* followed by *Bacillus cereus.* Biochemical and biomolecular analyses showed that most calcifying strains are members of the genus *Bacillus* sp. and *Pseudomonas* sp. (Shirakawa et al., 2011, Banerjee and Joshi 2016). *S.epidermidis, S. pasteuri* and *S.warneri* isolated from Kadıini Cave belong to typical human and animal microbiota like *S.aureus*, these species are considered pathogens (Lavoie and Northup 2005; Mulec et al., 2017). These studies show that these bacteria are ubiquitous and have been previously identified in karstic caves (Herzog Velikonja et al., 2014; Ma et al., 2015; Banerjee and Joshi 2016; Iţcuş et al., 2016).

The types of microbial extracellular enzyme activities imply the kinds of nutrients present in that environment. Khizhnyak et al. (2003) reported that cultures from Siberian caves produced a low-level of amylolytic enzymes compared to other enzyms, which explains the abscence of natural sources of starch in these environment. In our study, it has been determined that 78 % of all isolates could break the aril-amid bonds in tyrosine. Additionally, all Gram positive

Table 5. The inhibition zones (mm) of standard bacterial strains against experimental isolates and some antibiotics. (-) no inhibition.

Isolates (ID)	Bacterial inhibition zones (mm)						
	S.epidermidis	***B.subtilis***	***S.aureus***	***P.aeruginosa***	***E.coli***	***MRSA***	***VRE***
***Brevibacterium frigoritolerans* (23)**	8	12	-	-	-	-	-
***Bacillus thuringiensis* (2)**	10	12	-	-	-	-	-
***Bacillus weihenstephanensis* (24)**	12	14	-	-	-	-	-
***Bacillus cereus* (28)**	-	8	-	-	-	-	-
***Bacillus cereus* (1)**	14	14	-	-	-	-	-
***Bacillus sp.* (12)**	-	10	-	-	-	-	-
***Pseudomonas sp.* (44)**	-	-	16	-	-	10	-
Erythromycin	34(S)	34(S)	18(I)	-	12	-	-
Vancomycin	40(S)	26(S)	16(R)	-	-	9(R)	28(S)
Neomycin	18 (I)	24(S)	-	10(R)	10(R)	14(R)	-
Gentamicin	24 (S)	32(S)	16(S)	22(S)	22(S)	20(S)	-
Tetracyclin	28(S)	32(S)	12 (R)	10(R)	20(S)	-	14(R)

S = sensitive, I = intermediate, R = resistant.

isolates displayed positive arylamidases reaction that were specific for leucine and alanine at varying rates between 76% and 93%. According to our results of the L-proline-arylamidase test, all of Gram-negative bacteria and 64% of Gram-positive bacteria were positive.

A range of enzymatic activities was found in bacteria from the Kadıini Cave. Most of these enzymes have proteolytic activity, of which 96% of BCL isolates, 96% of GP isolates, and 100% of GN isolates produce at least one enzyme required for protein metabolism. Tomova et al. (2013) reported that 87% of Magura Cave isolates have proteolytic activity. Tyrosine residues formed by the breakdown of tyrosine by arylamidase and proline residues formed by the breakdown of L-Proline by arylamidase have very important metabolic functions for the cell in bacteria. They are effective in cell metabolic activities and formation of secondary metabolites (Patterson et al., 1963; Westley et al., 1967; Levit 1981; Kohl et al., 1988; Shibasaki et al., 1999; Nagata et al., 2003; Curtis et al., 2004; Whitmore and Lamont 2012). Because of their possession of different proteolytic enzymes, they can survive in environments such as caves that are poor in nutrients. Glycosidases are the most commonly found enzyme group after proteolytic enzymes in cave isolates. Our study has shown a positive proteolytic enzymes activity of the bacilli isolates, as well as the ones regarding carbonhytrate catabolism.

Antimicrobial components have a central part in mankind's struggle against infections. Nevertheless, because of their various resistance mechanisms, native or acquired, many antibiotics are losing their function every year (Kmietowicz, 2017). Nowdays, the existance of microorganisms with different enzymatic and antimicrobial effects has been proven in extreme ecosystems and researchers have started to focus on caves as one extreme environment (Lavoie, 2015; Man et al., 2015). Certain microorganisms isolated from cave ecosytems have been proven to display antimicrobial activity (Cheeptham et al., 2013; Tomova et al., 2013; Klusaite et al., 2016). Our results showed that 15.5% of the isolates have antimicrobial activity against *B.subtilis* and *S.epidermidis* and the others ineffective. In addition, isolate number 7 is effective against *S.aureus* and methicillin-resistant *S.aureus*. Yücel and Yamaç (2010) investigated 19 karstic caves in Turkey. They reported that the antibiotic extract that they obtained from these caves had bactericidal effect on the model resistant strains in lower concentrations than the antibiotic streptomycin. An important part of these secondary metabolites are potent antibiotics, which has made *Bacillus* one of the major antibiotic-producing organisms exploited by the pharmaceutical industry (Ghosh et al., 2017).

Conclusions

The bacteria isolated from Kadıini Cave displayed a potential related for use not only products but also vegetative forms in biotechnological applications such as biodegredation, enzyme production, antimicrobial or antitumoral drugs, and bacterial self-healing concrete (Jariyal et al., 2015; Kanmani et al., 2015; Zhang et al., 2015; Lee and Park, 2018). In addition, research conducted in 2018 have used many bacterial species isolated from the Kadıini Cave against phytopathogens and entomopathogen (Durairaj et al., 2018, Karungu et al., 2018).

In our study of the bacterial communities of Kadiini Cave, the antimicrobial activity and the biotechnological use potentials were investigated. The study results showed that the most commonly detected bacterial genus in cave ecosytem was *Bacillus* (*Firmicutes*). Although there were differences in the bacterial species detected in Kadıini Cave, our results indicated that *Firmicutes*, *Proteobacteria* and *Actinobacteria* are the most common group with biochemical peculiarities of the isolated bacteria against tested substrates. There is the biotechnological potentiality of these strains for further studies and industrial applications. The results obtained in our study contributed to understanding the heterotrophic aerobic culturable bacteria profile of karstic Kadıini Cave ecosytems regarding a source of industrially important enzymes and antimicrobial compounds.

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References

Al Dahaan, S., Al-Ansari, N., Knutsson, S., 2016, Influence of groundwater hypothetical salts on electrical conductivity total dissolved solids: Engineering, v. 8, no.11, p. 823−830. https://doi.org/10.4236/eng.2016.811074.

Bakalowicz, M., 1994, Water Geochemistry: Water Quality and Dynamics, *in* Gibert, J., Danielopol, D.L., and Stanford, J.A., eds., Groundwater Ecology: Academic Pres, p. 97−127.

Banerjee, S., and Joshi, S., 2016, Culturable bacteria associated with the caves of Meghalaya in India contribute to speleogenesis: Journal of Cave and Karst Studies, v. 78, p. 144−157. https://doi.org/10.4311/2015mb0131.

Barton, H.A., 2015, Starving artists: Bacterial oligotrophic heterotrophy in caves: *in* Engel. A.S., and DeGruyter, W, eds., Life in Extreme Environments: Microbial Life of Cave Systems, Berlin, Germany, p. 79−95.

Besnard, V., Federighi, M., Declerq, E., Jugiau, F., and Cappelier, J.M., 2002, Environmental and physico-chemical factors induce VBNC state in Listeria monocytogenes: Veterinary Research, v. 33, p. 359−370. https://doi.org/10.1051/vetres:2002022.

Bontognali, T.R.R., D'Angeli, I.M., Tisato, N., Vasconcelos, C., Bernasconi, S.M., Gonzales E.R.G., and De Waele, J., 2016, Mushroom speleothems: stromatolites that formed in the absence of phototrops: Frontiers of Earth Science, v. 4, no. 49, p.1−8. https://doi.org/10.3389/feart.2016.00049.

Borda, D.R., Năstase-Bucur, R.M., Spînu, M., Uricariu, R., and Mulec, J., 2014, Aerosolized microbes from organic rich materials: Case study of bat guano from caves in Romania: Journal of Cave and Karst Studies, v. 76, p. 114–126. https://doi.org/10.4311/2013MB0116. https://doi.org/10.4311/2013MB0116.

Bhullar, K., Waglechner, N., Pawlowski, A., Koteva, K., Banks, E.D., Johnston, M.D., Barton H.A., Wright, G.D., 2012, Antibiotic resistance is prevalent in an isolated cave microbiome: PloS One, v. 7, no. 4, p. 1−11. https://doi:10.1371/journal .pone.0034953.

Cheeptham, N., Sadoway, T., Rule, D., Watson, K., Moote, P., Soliman, L.C., Azad, N., Donkor, K. K., and Horne, D., 2013, Cure from the cave: volcanic cave actinomycetes and their potential in drug discovery: International Journal of Speleology, v .42, p. 35−47. http://dx.doi.org/10.5038/1827-806X.42.1.5.

Coraman and Celik, 2012, Identification and Protection of Important Bat Caves in Turkey: Project final report, Project ID: 0534710.

Cotuk, A., Doğruöz, N., Zeybek, Z., Kimiran-Erdem A., and İlhan Sungur, E., 2005, The effects of *Pseudomonas* and *Aeromonas* strains on *Legionella pneumophila* growth: Annals of Microbiology, v. 55, p. 219−224.

Curtis, J., Shearer, G., and Kohl, D.H., 2004, Bacteroid proline catabolism affects N2 fixation rate of drought-stressed soybeans: Plant physiology, v. 136, no. 2, p. 3313−3318. https://doi/10.1104/pp.104.044024.

Doğruöz Güngör, N., and Sanli Yurudu, N.O., 2015, Evaluation of bacterial resistance to chloramine T biocide and effects on rifampicin antibiotic susceptibility as a consequence of biocide usage in Cooling System Biofilm including *Legionella pneumophila*: The Battle Against Microbial Pathogens, Basic Science, Technological Advances and Educational Programs, p.923−929

Durairaj, K., Velmurugan, P., Park, J.H., Chang, W.S., Park, Y.J., Senthilkumar, P., Choi, K.M., Lee, J.H., and Oh, B.T., 2018, An investigation of biocontrol activity *Pseudomonas* and *Bacillus* strains against *Panax* ginseng root rot fungal phytopathogens: Biological Control, v. 125, p. 138−146. https://doi.org/10.1016/j.biocontrol.2018.05.021.

Engel, A.S., 2010, Microbial diversity of cave ecosystem, *in* Barton, L.L, Mandi, M., Loy, and A. eds, Geomicrobiology, Molecular and Environmental Perspective: Springer Science and Business Media BV, p. 219−238. https://doi.org/10.1007/978-90-481-9204-5.

Engel, A.S., 2011, Karst Ecosystems, *in* Reitner, J., and Thiel, V. eds, Encyclopedia of Geobiology, Encyclopedia of Earth Sciences Series: Springer, Dordrecht, p. 521−531. https://doi.org/10.1007/978-1-4020-9212-1_125.

Ghosh, S., Kuisiene, N., and Cheeptham, N., 2017, The cave microbiome as a source for drug discovery: Reality or pipe dream?: Biochemical Pharmacology, v. 134, p. 18−34. https://doi.org/10.1016/j.bcp.2016.11.018.

Herzog Velikonja, B., Tkavc, R., and Pašić, L., 2014, Diversity of cultivable bacteria involved in the formation of macroscopic microbial colonies (cave silver) on the walls of a cave in Slovenia: International Journal of Speleology, v. 43, p. 45−56. https://doi.org/10.5038/1827-806x.43.1.5.

Iţcuş, C., Pascu, M.D., Brad, T., Perşoiu, A., and Purcarea, C., 2016, Diversity of cultured bacteria from the perennial ice block of Scarisoara Ice Cave, Romania: International Journal of Speleology, v. 45, p. 89−100. https://doi.org/10.5038/1827-806x.45.1.1948.

Jariyal, M., Gupta, V.K., Mandal, K., and Jindal, V., 2015, *Brevibacterium frigoritolerans* as a novel organism for the bioremediation of phorate: Bulletin of Environmental Contamination and Toxicology, v. 95, p. 680−686. https://doi.org/10.1007/s00128-015-1617-2.

Kanmani, P., Kumaresan, K., and Aravind, J., 2015, Utilization of coconut oil mill waste as a substrate for optimized lipase production, oil biodegradation and enzyme purification studies in *Staphylococcus pasteuri*: Electronic Journal of Biotechnology, v. 18, p. 20−28. https://doi.org/10.1016/j.ejbt.2014.11.003.

Karungu, S., Huang, D., Atoni, E., Waruhiu, C., Agwanda, B., Hu, X., and Yuan, Z., 2018, Isolation, identification, and evaluation of mosquito entomopathogenic *Bacillus* species and related genera from randomly selected sites in Kenya: African Journal of Microbiology Research, v. 12, p. 290−299. https://doi.org/10.5897/ajmr2018.8824.

Khizhnyak S.V., Tausheva I.V., Berezikova A.A., Nesterenko E.V, and Rogozin D.Y., 2003, Psychrophilic and psychrotolerant heterotrophic microorganisms of middle Siberian karst cavities: Russian Journal of Ecology, v. 34, p. 231−235.

Klusaite, A., Vickackaite, V., Vaitkeviciene, B., Karnickaite, R., Bukelskis, D., Kieraite-Aleksandrova, I., and Kuisiene, N., 2016, Characterization of antimicrobial activity of culturable bacteria isolated from Krubera-Voronja Cave: International Journal of Speleology, v. 45, p. 8. https://doi.org/10.5038/1827-806x.45.3.1978.

Kmietowicz, Z., 2017, Few novel antibiotics in the pipeline, WHO warns, BMJ: British Medical Journal (Online), BMJ 2017: 358: j4337. https://doi.org/10.1136/bmj.j4339.

Kohl, D.H., Schubert, K.R., Carter, M.B., Hagedorn, C.H., and Shearer, G., 1988, Proline metabolism in N2-fixing root nodules: energy transfer and regulation of purine synthesis: Proceedings of the National Academy of Sciences, v. 85, no. 7, p. 2036−2040. https://doi.org/10.1073/pnas.85.7.2036.

Lavoie, K.H., 2015, A grand, gloomy, and peculiar place: Microbiology in the Mammoth Cave Region: Life in Extreme Environments: Microbial Life of Cave Systems: Engel, AS, Ed, De Gruyter, Berlin, Germany, p. 47–78. https://doi.org/10.1515/9783110339888-005.

Lavoie, K.H., and Northup, D.E., 2005, Bacteria as indicators of human impact in caves: National Cave and Karst Management Symposium, p. 40−47.

Lee, Y. S., and Park, W., 2018, Current challenges and future directions for bacterial self-healing concrete: Applied Microbiology and Biotechnology, v. 102, p. 3059−3070. https://doi.org/10.1007/s00253-018-8830-y.

Levitt, M., 1981, Effect of proline residues on protein folding: Journal of molecular biology, v. 145, no. 1, p. 251−263. https://doi.org/10.1016/0022-2836(81)90342-9.

Ma, Y., Zhang, H., Du, Y., Tian, T., Xiang, T., Liu X., Wu, F., An, L., Wang, W., 2015, The community distribution of bacteria and fungi on ancient wall paintings of the Mogao Grottoes: Scientific Reports, v. 5, p. 7752. https://doi.org/10.1038/srep07752.

Man, B., Wang, H., Xiang, X., Wang, R., Yun, Y., and Gong, L., 2015, Phylogenetic diversity of culturable fungi in the Heshang Cave, central China: Frontiers in Microbiology, v. 6, no. 1158, p. 1−11. https://doi.org/10.3389/fmicb.2015.01158.

Mulec, J., Oarga-Mulec, A., Šturm, S., Tomazin, R., Matos, T., 2017, Spacio-temporal distribution and tourist impact on airborne bacteria in a cave (Škocjan Caves, Slovenia): *Diversity*, v .9, no. 28, p. 1−14. https://doi.org/10.3390/d9030028.

Nagata, K., Nagata, Y., Sato, T., Fujino, M.A., Nakajima, K., and Tamura, T., 2003, L-Serine, D-and L-proline and alanine as respiratory substrates of Helicobacter pylori: correlation between in vitro and in vivo amino acid levels: Microbiology, v. 149, no. 8, p. 2023−2030. https://doi.org/10.1099/mic.0.26203-0.

Northup, D.E., and Lavoie, K.H., 2001, Geomicrobiology of caves: a review: Geomicrobiology Journal, v. 18, p. 199−222. https://doi.org/10.1080/01490450152467750.

Palmer, A.N., 1991, Origin and morphology of limestone caves: Geological Society of America Bulletin, v. 103, p. 1−21. https://doi.org/10.1130/0016-7606(1991)103<0001:oamolc>2.3.co;2.

Patterson, E.K., Hsiao, S.H., and Keppel, A., 1963, Studies on Dipeptidases and Aminopeptidases I. distinction between leucine aminopeptidase and enzymes that hydrolyze l-leucyl-β-naphthylamide: Journal of Biological Chemistry, v. 238, no. 11, p. 3611−3620.

Pincus, D.H., 2005, Microbial Identification Using the BioMerieux Vitek® 2 System, *in* Encyclopedia of Rapid Microbiological Methods, Miller, M.J., ed., Parenteral Drug Association & Davis Healthcare International Publishing, Moore, OK, 2005; v. II, p. 1−32.

Portillo, M.C., Saiz-Jimenez, C., and Gonzalez, J.M., 2009, Molecular characterization of total and metabolically active bacterial communities of ‘white colonization’ in the Altamira Cave, Spain: Research in Microbiology, v. 160, p. 41−47. https://doi.org/10.1016/j.resmic.2008.10.002.

Reasoner, D.J., and Geldreich, E.E., 1985, A new medium for the enumeration and subculture of bacteria from potable water: Applied Environmental Microbiology, v. 49, p. 1−7.

Romero, A., 2009, Cave Biology: Life İn Darkness, Cambridge University Press, p. 159−181. https://doi.org/10.1017/CBO9780511596841.

Schwartz, T., Hoffmann, S., Obst, U., 2003, Formation of natural biofilms during chlorine dioxide and u.v. disinfection in a public drinking water distribution system: Journal of Applied Microbiology, v. 95, p. 591–601. https://doi:10.1046/j.1365-2672.2003.02019.x.

Shibasaki, T., Mori, H., Chiba, S., and Ozaki, A., 1999, Microbial proline 4-hydroxylase screening and gene cloning: Applied Environmental Microbiology, v. 65, no. 9, p. 4028−4031. https://doi.org/0099-2240/99/$04.0010.

Shirakawa, M.A., Cincotto, M.A., Atencio, D., Gaylarde, C.C., and John, V.M., 2011, Effect of culture medium on biocalcification by *Pseudomonas putida*, *Lysinibacillus sphaericus* and *Bacillus subtilis*: Brazilian Journal of Microbiology, v. 42, p. 499−507. https://doi.org/10.1590/s1517-83822011000200014.

Slepecky, R.A., and Hemphill, H.E., 1992, The genus *Bacillus*—nonmedical: The Prokaryotes, 2nd ed., Springer Verlag, NY; v. III, p 1663. https://doi.org/10.1007/0-387-30744-3_16.

Tisato, N., Torriani, S.F.F., Monteux, S., Sauro, F., De Waele, J., Tavagna, M.L., D’Angeli I.M., Chailloux, D., Renda, M., Eglinton, T.I., and Bontognali, T.R.R., 2015, Microbial mediation of complex subterranean mineral structures: Scientific Reports, v. 5, p. 15525. https://doi.org/10.1038/srep15525.

Tomczyk-Żak, K., and Zielenkiewicz, U., 2015, Microbial diversity in caves: Geomicrobiology Journal, v. 33, p. 20−38. https://doi.org/10.1080/01490451.2014.1003341.

Tomova, I., Lazarkevich, I., Tomova, A., Kambourova, M., and Vasileva-Tonkova, E., 2013, Diversity and biosynthetic potential of culturable aerobic heterotrophic bacteria isolated from Magura Cave, Bulgaria: International Journal of Speleology, v. 42, p. 65−76. https://doi.org/10.5038/1827-806x.42.1.8.

Turkey Cave Database. https://taban.magara.org [accessed: August 15, 2019].

Trevors, J.T., 2011, Viable but non-culturable (VBNC) bacteria: gene expression in planktonic and biofilm cells: Journal of microbiological methods, v. 86, no. 2, p. 266−273. https://doi.org/10.1016/j.mimet.2011.04.018.

Westley, J.W., Anderson, P.J., Close, V.A., Halpern, B., and Lederberg, E.M., 1967, Aminopeptidase profiles of various bacteria: Applied Environmental Microbiology, v.15, no. 4, p. 822−825.

Whitmore, S.E., and Lamont, R.J., 2012, Tyrosine phosphorylation and bacterial virulence: International journal of oral science, v. 4, 1−6. https://doi/10.1038/ijos.2012.6.

Yılmazusta, N.D., Yakutipekoğlu, H., 2019, An Anthropological Assessment About Burial Practices of Kadıini Cave People (Alanya/Antalya): The Journal of Mediterranean Civilisations Research Institute (MCRI) Cedrus VII, p. 41−58. https://doi.org/10.13113/CEDRUS.201902.

Yücel, S., and Yamaç, M., 2010, Selection of *Streptomyces* isolates from Turkish karstic caves against antibiotic resistant microorganisms: Pakistan Journal of Pharmacological Science, v. 23, no. 1, p. 1−6.

Zhang, L., Liu, M., Jamil, S., Han, R., Xu, G., and Ni, Y., 2015, PEGylation and pharmacological characterization of a potential anti-tumor drug, an engineered arginine deiminase originated from *Pseudomonas plecoglossicida*: Cancer Letters, v. 357, p. 346−354. https://doi.org/10.1016/j.canlet.2014.11.042.

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ANTHROPOGENIC IMPACTS ON THE BACTERIAL PROFILE OF YARIK SINKHOLE IN ANTALYA, TURKEY

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Abstract

Yarık Sinkhole is a vertical cave with a length of 1378 m and depth of 533 m. Its location was marked by The Anatolian Speleology Association in 2011, and expedition entries started in 2014. Yarık Sinkhole became the 12th deepest cave of Turkey. The aim of this study was to determine the bacterial profile of the Yarık Sinkhole using next generation metagenomic sequencing and to investigate whether the bacterial profile of the cave is affected by the activities of people living in this region. This study is important as the samples were collected during the first entrance of the exploration of the cave (up to 300 m). The samples were collected from depths of −80 m, −120 m and −300 m. A total of 33 OTUs (Operational Taxonomic Unit) contained 4 bacteria phyla. Only *Firmicutes, Proteobacteria* and *Actinobacteria* phyla comprised a mean abundance of more than 1% in each sample. However, 18 different species have been detected in the Yarık Sinkhole. The most predominant species are *Acinetobacter lwoffii*, *Methylobacterium tardum,* and *Propionibacterium acnes*. Although the sampling was done during the first exploration of the Yarık Sinkhole, the fact that the majority of bacteria found in the cave are human-associated, suggest serious impacts from people living near this cave from runoff with human and animal waste and trash.

Introduction

Caves are a subsurface habitat that are not explored as much with regard to biodiversity and community composition because of some environmental and geographical limitations. Natural caves generally involve an ecosystem that has a high humidity, limited nutritional sources, and a usually stable temperature, which are stable characteristics of a natural laboratory in terms of bacteria and their metabolic processes. Such environments are habitats only to those microorganisms that are specialized for the conditions in question. Therefore, natural caves are recognized as extreme environments (Palmer, 1991; Northup and Lavoie, 2001; Tomczyk-Żak and Zielenkiewicz, 2016). Microbial communities in caves are often highly variable dependent on the microenvironment. The range of bacterial diversity and composition are determined to be related to the geochemistry of host rocks (Barton et al., 2007). Nutrients also contribute to cave microbial diversity. Organic matter and microorganisms could be carried in to caves by air currents, seepage water, floods, seasonality, and animal/human activities (Shabarova et al., 2013).

The most appropriate way to unearth the bacterial diversity of a cave is to perform sampling at the time of exploration of the cave. Thus, the original microbiological diversity of the cave can be more realistically discovered by sampling before contamination associated with the cavers. However, the conditions may vary depending on the location and surroundings of the caves in terms of human impacts. Numerous cave microbiology studies have been carried out in the world. Since each cave is unique, these studies do not lose their importance (Busquets et al., 2014; De Mandal et al., 2014; Herzog Velikonja et al., 2014; Kieraite-Aleksandrova et al., 2015; Riquelme et al., 2015; Leuko et al., 2017).

It is estimated on the basis of the studies conducted in karstic areas that there are approximately 40,000 caves in Turkey (General Directorate of Mineral Research and Exploration). Cave microbiology studies from Turkish cave include: Yücel and Yamaç (2007), investigated the antimicrobial activity of *Streptomyces* spp. isolates from 19 different caves. In addition, characterization and definition of bacteria contributing to the formation of dripstone in Yıldızkaya cave systems in Erzurum have been studied by Barış (2009). Gulecal-Pektas and Temel (2017) studied the bacterial diversity and taxonomic composition of the Oylat Cave in Bursa and the Kaklık Cave in Denizli with poor oxygen, high temperature, and sulfur conditions. Even though the number of studies on microbiology in the caves of Turkey has been increasing, these studies are insufficient when the estimated number of caves is taken into account.

The culture technique is not sufficient on its own for determining the microbiological diversity of a cave. The rate of culturable bacteria in environments that contain complex microorganisms is only 0.1−1% of the total number due to their specific nutritional requirements (Torsvik and Øvreas, 2002). With the application of molecular methods, it was revealed that nutrient-poor caves had a surprisingly rich bacterial diversity. Metagenomics is a technique to access far more microbial diversity directly from environmental samples. Next-generation sequencing is cost-effective and provi-

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des more detailed taxonomic profiles between samples to be determined (Nelson el al., 2014). It has been shown that some microorganisms isolated by using culture methods were not detected by the molecular methods, and vice versa, which it has demonstrated that cultivation methods remain critical in microbial diversity studies (Donachie et al. 2007).

The Yarık Sinkhole is one of the caves which differ by its location in environmental conditions. During the summer months, the presence of people who are living there, as well as those who practice animal husbandry (approximately 30−50 people and 400 animals) are noticed. There are also tiny siphons where waters join the cave at both the main entrance of the Yarık Sinkhole and at different points deeper into the cave. In this study, we collected soil samples from three different points of the first-explored part of the cave, to both examine the bacteria profile, and, by means of the next-generation sequencing method, showed the impact of the anthropogenic effects on the cave's bacterial diversity.

Site Description

Geology

Yarık Sinkhole (GPS coordinate: UTM 448068.47 E 4036006.77 N) is located on the Sivastı Plateau that is centered 30 km north of Gazipaşa (Fig. 1) and is named with a specially-assigned geological sequence as the Sivastı formation. This formation, which is 2000-meters high, is one of the parts of the Taşeli Plateau.The study area, thought to be from the earliest Triassic age, has a complex structure with various orogenic movements such as Hershey and Alpen that undergo bending and fracturing. There are stratified schists and limestones at the Sivastı formation. Due to the different physical characteristics of the lithology, the schists are more curled and the limestones are more broken (Ulu, 1983).

A cross-sectional view of the cave map placed on the topography is presented in Figure 2. Yarık Sinkhole has a total length of 1378 m and 533 m depth. The first entrance to the cave was explored up to a depth of 300 m (Fig. 3). In 2016, the cave discovery reached a depth of 533 m. Yarık Sinkhole became the 12th deepest cave of Turkey. The entrance to the cave has a wide mouth created by fracture hence its name; Yarık in Turkish means fracture (Fig.4). The watershed of the Yarık Sinkhole is a closed valley where the main rock is limestone with little sediment on the basin of the valley. There is no vegetation except for some trees planted by villagers for shade.

Despite a wide opening, there are occasional narrow passages in the cave and a rapid downward descent is characteristic of this cave. When the cross section map of the cave is viewed after the bench, downward declination slowly gives away to horizontal passages with small ponds and lakes in them. The resulting bench size ranges from 5 m to 40 m on average. These benches generally formed as a result of active faults and fractures developed over time. The Yarık Sinkhole ceiling is high generally, but some of the narrow passages are difficult to pass, especially in the case of a flood when these passages will be totally blocked. Unlike most vertical caves, in the Yarık Sinkhole speleothems such as flowstones and cave pearls are found in the horizontal portion.

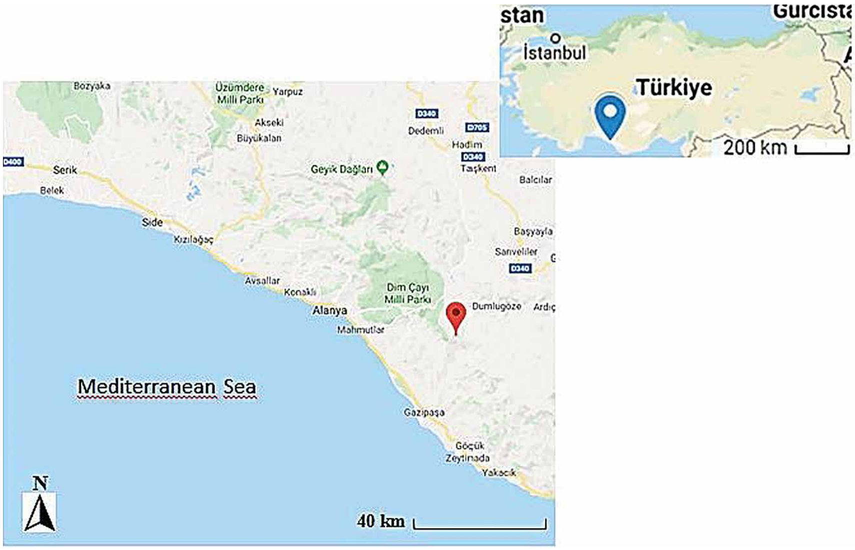

Figure 1. Yarık sinkhole location source [Google (Googlemaps, 2019)].

However, there are not any attempts to enter the entrance of the Yarık Sinkhole by the villagers as it will be fatal since the entrance is an 80-meter shaft. Water only flows during the melting of ice in the spring. The cave camp area is 50-meters away from the entrance in a small pasture surrounded with seasonal settlements. The area is filled up with ice and snow in winter and in spring time meltwater is siphoned through a small pit with sediment at the bottom. The waste of livestock also goes along with the water.

The impact of population can be explained by the settlers in that valley. Additional side galleries within Yarık Sinkhole carry water from other watershed areas where there are additional people. Even though the impact cannot be measured, it is evident by the garbage that we have found deep in the cave which can not come only from the main entrance.

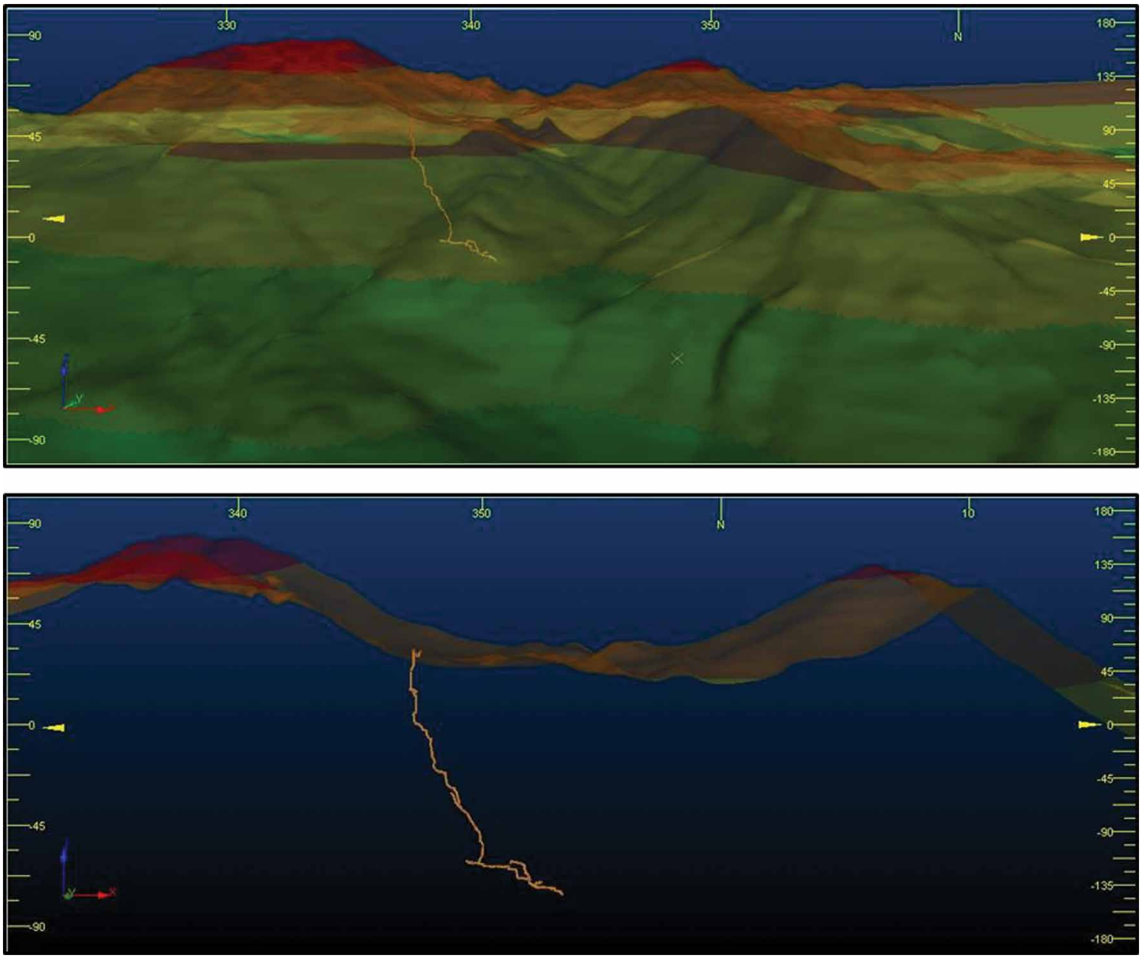

Figure 2. Cross-section view of topography (a) and Yarık Sinkhole's map (b) from The Anatolian Speleology Association(ASPEG).

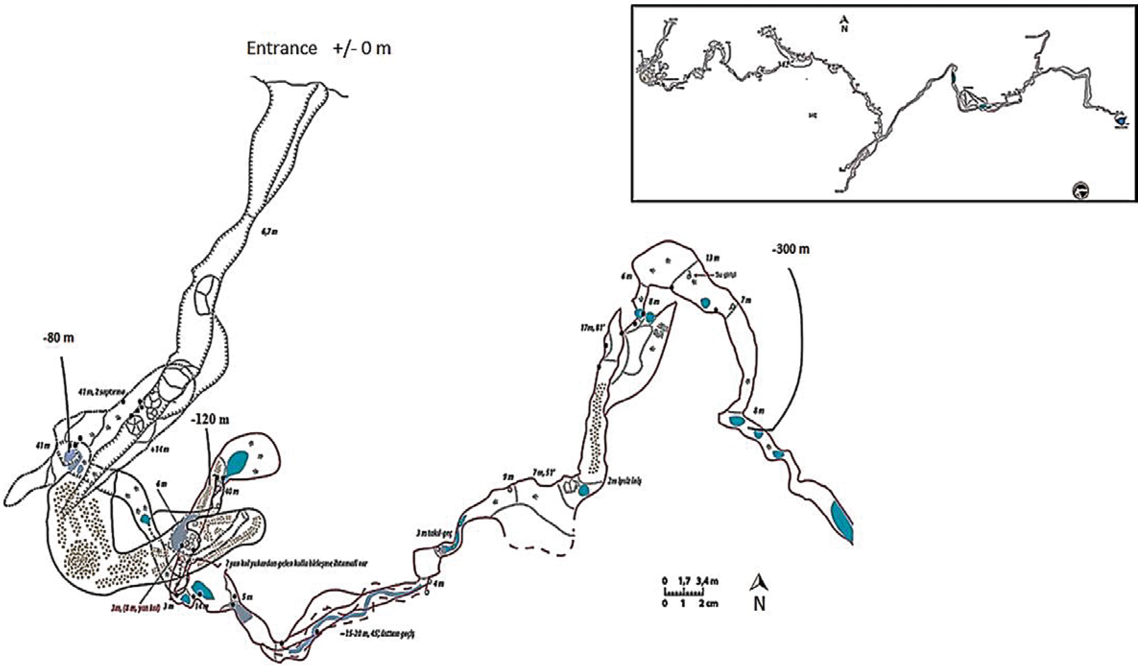

Figure 3. Plan and profile of Yarık Sinkhole and sampling points from the Anatolian Speleology Association (ASPEG).

MATERIALS AND METHODS

Sampling and Physical–chemical analysis

The soil samples were taken from depths of −80 m, −120 m in June 2014 and −300 m in August 2014, in parallel with the exploration of the cave. Approximately 10 g of soil was collected aseptically for microbial analysis from each sampling site in the cave. The samples were maintained at 4 °C and transported within 24 hours to the laboratory. The temperature and humidity of the sampling area were measured by a portable Temperature/ Humidity Meter (TFA 31.1O28).

The water sample was taken in a pool from Yarık Sinkhole (−100 m depth) for hydrochemical characteristics in June 2014. The chemical analysis (HCO_3^-, F^-, Ca^{2+}, Cl^-, Mg^{2+}, K^+, Na^+, and SO_4^{2-}) of the water sample were carried out according to different standard methods (Table 1) (APHA, 1992).

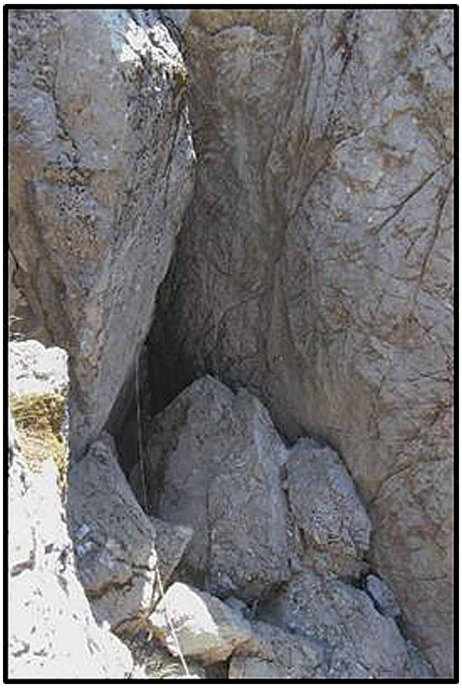

Figure 4. Yarık Sinkhole Entrance (about 10-meters long and 5-meters wide).

DNA extraction

DNA was extracted from 0.3 g (wet weight) of soil by using the Fast DNA Spin Kit for soil from Q-Biogene (Heidelberg, Germany) according to the manufacturer's instructions.

16S rRNA Metagenomic Sequencing Library Preparation and Sequencing

The microbial diversity at three depths was evaluated by using the Illumina MiSeq next generation sequencing approach (Novogene). The protocol includes the primer pair sequences for the V3 and V4 region of the 16S rRNA that create a single amplicon of approximately 460 bp (Klindworth et al., 2013). The protocol also includes overhang adapter sequences that must be appended to the primer pair sequences for compatibility with Illumina index and sequencing adapters. Illumina adapter overhang nucleotide sequences-16S rRNA specific sequences were 5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3' for the forward primer and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC-3' for the reverse primer. The first PCR was performed using BiospeedyTM Proof Reading DNA Polymerase 2x Reaction Mix (Bioeksen Ltd Co., Turkey) and 200 nm of each primer. The following program was performed on Biorad CFX Connect Instrument (Bio-Rad Laboratories, U.S.A.): 95°C for 3 minutes; 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds; 72°C for 5 minutes. The PCR product was run on an agarose gel to verify the size (~550 bp) and purified using BiospeedyTM PCR Product Purification Kit (Bioeksen Ltd. Co., Turkey).

The dual indices and Illumina sequencing adapters were attached to the purified first PCR products via the second PCR that was run using the Nextera XT Index Kit (Illumina Inc., USA) and the following program: 95°C for 3 minutes; 8 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds; 72°C for 5 minutes. The PCR products were purified using BiospeedyTM PCR Product Purification Kit (Bioeksen Ltd. Co., Turkey). The final library was run on a Bioanalyzer DNA 1000 chip to verify the size (~630 bp). The final library was diluted using 10 mM Tris pH 8.5 to 4 nM and the 5 µL aliquots were mixed for pooling the libraries. In preparation for cluster generation and sequencing, pooled libraries were denatured with NaOH, diluted with hybridization buffer (HT1), and then heat denatured before MiSeq sequencing. Illumina MiSeq v3 reagent kits were used for the runs. Each run included a minimum of 5% PhiX to serve as an internal control.

Bioinformatic Analysis

The raw sequence data (concatenated forward and reverse sequence reads) were cleaned, reduced, and analyzed using Mothur Version 1.36.1. First, the barcode and the primer sequences were trimmed and then unique sequences were identified. The trimmed unique sequences were aligned to the SILVA rRNA database sequences using blastn algorithm (Pruesse et al., 2007). Before this the SILVA database sequences were trimmed to include only the V3-V4 region. The overhangs at both ends were removed via filtering the sequences and the redundancy check was carried out. For further de-nosing, the sequences were pre-clustered. The chimeras were eliminated using the implanted code UCHIME (Edgar et al., 2011). The sequences were classified by using Bayesian classifier implanted in Mothur. The reference and taxonomy files were adopted from the SILVA database (Pruesse et al., 2007). After Operational Taxonomic Unit (OTU) picking and their taxonomic assignment using the SILVA rDNA database, the OTUs were binned into phylotypes.

Sequence Accession Numbers

The raw sequencing data generated in this study were deposited into the NCBI database under accession numbers SRP123547 in the NCBI Sequence Read Archive, with the following accession numbers: SRS2657311, SRS2657312, and SRS2657313.

Results

Physical–chemical environment

The chemical characteristic of the water sample is presented in Table 1. The concentration in Ca^{2+} was found to exceed that of Mg^{+}, whereby the predominant anion was determined to be HCO_3^{-}. The mean air temperature and humidity inside the Yarık Sinkhole were 12.4°C and 94% respectively.

Table 1. Parameters and test methods for chemical analysis of the Yarık Sinkhole water.

Parameter	Unit	Yarık Sinkhole Water Sample	Test Method	Reference
HCO_3^-	mg/L	124	SM 2320 B	APHA (1992)
F^-	mg/L	0.25	SM 4500-F D	APHA (1992)
Ca^{2+}	mg/L	35.7	EPA 200.7	APHA (1992)
Cl^-	mg/L	5	SM 4500 Cl^- B	APHA (1992)
Mg^{2+}	mg/L	7.22	EPA 200.7	APHA (1992)
Na+	mg/L	1.96	EPA 200.7	APHA (1992)
K+	mg/L	0.74	EPA 200.7	APHA (1992)
SO_4^{2-}	mg/L	15.5	SM 4500 SO_4^{-2} E	APHA (1992)
pH	···	7.42	TS EN ISO 10523	TSE (1999)
Conductivity	μS/cm	222	TS 9748 EN 27888	TSE (1996)

Bacterial taxonomy and distribution

Taxonomic assignment of 130,703 sequencing reads (Phred score ≥30; Mean read length >300 bp) from Yarık Sinkhole was obtained by targeting the V3 and V4 region of the bacterial 16S rRNA gene. A total of 33 OTUs (based on 97% cutoff) comprising 4 bacterial phyla were found. The variation of the fractions of the phyla according to the depths was shown in (Table 2). Only *Firmicutes, Proteobacteria,* and *Actinobacteria* phyla had a mean abundance of more than 1% in each sample holding more than 98% of the total sequence reads. The bacterial phylum with the highest presence percentage is *Proteobacteria* (average 80%) at all the depths. It is followed by *Actinobacteria* (average 16%). While the rate of presence of *Firmucutes* is more than 5% at −300 m, it is smaller than 1% at other depths. *Bacteroidetes* were found at less than 1% at other depths (Table 2).

At the class level, *Gammaproteobacteria* (average 17%) was present at all the depths. *Alphaproteobacteria* (average 24%), *Actinobacteria* (average 17%), *Bacilli* (average 2%) and *Bacteroidia* (average 0.3%) were also observed at all the depths, except for −80 m and −300 m, where *Betaproteobacteria* were absent. In total, 18 species were identified at 3 different depths. Only 9 of these bacteria were found to constitute 5% or more. The frequency of *Acinetobacter lwoffii*, *Methylobacterium tardum,* and *Propionibacterium acnes* were the ones with the highest presence. Other species were determined to be in lower percentages (Table 2).

Table 2. Relative abundance (%) of the detected species and phyla.

Phyla and Species	Sampling Depth, m		
	−80	−120	−300
Firmicutes (phylum)	0.552	0.848	5.550[a]
Bacteroidetes (phylum)	0.005	0.006	0.918
Actinobacteria (phylum)	0.807	10.264[a]	39.484[a]
Proteobacteria (phylum)	98.636[a]	88.882[a]	54.048[a]
Alphaproteobacteria (class)	6.513[a]	16.552[a]	49.186[a]
Gammaproteobacteria (class)	92.124[a]	72.255[a]	4.861[a]
Betaproteobacteria (class)	0.000	0.075	0.000
Methylobacterium tardum	6.505[a]	16.511[a]	49.179[a]
Propionibacterium acnes	0.743	10.225[a]	38.144[a]
Acinetobacter lwoffii	91.621[a]	72.238[a]	4.852[a]
Bacillus thermoamylovorans	0.039	0.036	4.212[a]
Streptococcus sanguinis	0.508	0.002	1.125[a]
Porphyromonas spp.	0.005	0.006	0.918
Rothia mucilaginosa	0.015	0.015	0.908
Rhodococcus spp.	0.002	0.024	0.271
Aeribacillus geobacillus pallidus	0.000	0.002	0.203
Micrococcus luteus	0.000	0.000	0.159
Acinetobacter johnsonii	0.503	0.017	0.009
Bacillus spp.	0.000	0.000	0.007
Methylobacterium fujisawaense	0.002	0.015	0.005
Staphylococcus pasteuri	0.005	0.807	0.002
Sulfitobacter sp.	0.000	0.024	0.002
Solirubrobacter spp.	0.047	0.000	0.002
Simonsiella muelleri	0.000	0.075	0.000
Methylobacterium radiotolerans	0.005	0.002	0.000

[a] Taxonomic group with an abundance higher than 1%.

Discussion

Caves are special due to their formation processes and their chemical nature, are also unique in terms of microbial diversity. When exposed to human activity, caves lose their microbial richness (Lavoie and Northup, 2005; Ikner et al., 2007; Chelius et al., 2009). For this reason, studies carried out by taking samples especially during first entry and from isolated environments aim to better understand the microbiota. For cave microbiology studies, it can be said that horizontal caves are preferred because of ease of sampling compared to vertical caves. For similar reasons, the cave microbiology studies in Turkey have focused on the horizontal caves (Gulecal-Pektas, 2016; Gulecal-Pektas and Temel, 2015; Gulecal-Pektas and Temel, 2017; Candıroğlu, 2018).

This study is the first microbiological research conducted in a vertical cave in Turkey with sampling during the first discovery of the cave. Anthropogenic impact is expected to be unlikely for the microorganism profile in the samples taken at the time of the initial exploration of vertical caves. These previously cited reasons made our study original and also important for its intended purpose. However, the area surrounding the Yarik Sinkhole area and entrance is cluttered with summer houses of villagers that live on the lower levels of the mountains. During periods of late April to late September, villagers migrate to these settlements and use the surroundings for pasture of their livestock of cows, sheep, and especially goats. These settlements do not have infrasturcture for toiletry and waste water. All of the liquid waste goes into opened pits and from there seeps to the underground. They use the entrance of Yarik Sinkhole as garbage disposal which we had to intervene, warning them to stop throwing rubbish that includes baby diapers to rubber cycle tires, etc. There are several siphons connected to the sink from such areas that suggest anthropogenic impacts on the Yarık Sinkhole. Our study aimed to evaluate the bacterial diversity of the Yarik Sinkhole and to show possible anthropogenic impacts on diversity.

The Yarık Sinkhole resembles most cave systems with its high level of humidity and stable air temperature (Riquelme et al., 2015; Lavoie et al., 2017; Leuko et al., 2017). Similar to our findings, Leuko et al., (2017) reported a higher Ca^{2+} concentration than that of Mg^{+} as a result of the chemical analysis of the water samples from the Su Betu limestone cave in Sardinia, Italy, and detected HCO_3^{-} as the predominant anion. Reasearchers conclued that the predominancy of HCO_3^{-} found in a cave indicates a calcium-bicarbonate type cave. On the other hand, the high level of SO_4^{2-} detected in the water sample from the Yarık Sinkhole may indicate an oxidation of the sulphur minerals contained in the rocks in contact with the water. pH, presence of nutrients, light, oxygen, sulphur, and compounds of other metals all affect the growth and structure of microbial communities in a humid cave. A change in those conditions can cause the differences in the composition of species (Engel et al., 2010; Jones and Bennett, 2014).

In the curent study, the phyla and their percentages present at −80 m, −120 m and −300 m depths of Yarık Sinkhole were determined with next generation sequencing (NGS). We detected *Proteobacteria, Actinobacteria, Firmicutes,* and *Bacteroidetes* phyla. The most predominant two phyla have been found to be *Proteobacteria* (average 80%) and *Actinobacteria* (average 16%). The dominant groups in caves vary by the characteristics of caves (Lee et al., 2012). The major phyla found as a result of metagenomic analysis of soil samples taken from 5 caves in the Ozark Cave system are *Proteobacteria* (27.7%), *Acidobacteria* (17.3%), *Actinobacteria* (12.2%), *Firmicutes* (8.2%), *Chloroflexi* (8.1%), *Bacteroidetes* (8%), and *Nitrospirae* (6%) (Oliveira et al., 2017). In another study, the most dominant phyla determined as a result of metagenomic analysis of sediment samples of 3 caves in Mizoram (India) were *Actinobacteria* (35.9%), *Chloroflexi* (13.9%), *Planctomycetes* (13.7%), *Acidobacteria* (11.44%), and *Proteobacteria* (6.6%) (De Mandal et al., 2014). Members of the *Proteobacteria* and *Actinobacteria* phyla, dominant in both our study and many other studies, are well-adapted to growth with limited nutrients (Jurado et al., 2010; Lee et al., 2012; Barton, 2015; Wiseschart et al., 2018).

Despite the undeniable dominance of *Proteobacteria* in cave ecosystems, the representation of the *Proteobacteria* classes varies in different environments. At the class level, *Gammaproteobacteria* was present at all the depths, as were *Alphaproteobacteria, Actinobacteria, Bacilli* and *Bacteroidia*. *Betaproteobacteria* was detected only at −120 m depth. *Proteobacteria* is a cosmopolitan bacterial group that is common and abundant (Laiz et al.,1999; Zhaou et al., 2007). Members of the *Proteobacteria* phylum have the abilities of utilizing different organic compounds, the fixation of atmospheric carbon, and nitrogen transformation (Tomczyk-Zak and Zielenkiewicz, 2016). It is also suggested that dominance of *Proteobacteria* is a result of the increasing organic input caused by cave visitors (Ikner et al., 2007). In the current study among the *Proteobacteria*, 92%, 72%, and 5% were recognized in the class of *Gammaproteobacteria*, 6.5%, 16.5% and 49% as *Alphaproteobacteria* at −80 m, −120 m and −300 m depths respectively. Similarly, it was determined that there were plenty of *Gammaproteobacteria* in soil samples taken from the Mangoo-Pee cave. 23% of the *Proteobacteria* found by 43% in soil sample of the Blowing Spring Cave was determined as *Gammaproteobacteria*, 19% as *Alphaproteobacteria,* and 1% as *Betaproteobacteria* (Barron et al., 2010).

Another dominant group, *Actinobacteria,* is known for being able to develop in environments containing limited nutrients, to degrade different humic material, and to dissolve phosphate and calcium carbonate (Ball et al., 1989; Dari et al., 1995; Laiz et al., 1999). It was reported that this phylum existed in cave walls, soil, sediment, and on speleothem surfaces, and it was suggested that it might have considerably contributed to the formation of the cave structures and the biomineralization in the cave ecosystem (Cuezva et al., 2012; Ortiz et al., 2013; Tomczyk-Zak and Zielenkiewicz, 2016).

Even though microorganism diversity differs by the method used (culture-depend or culture- independed), by the sampling area, and by the sample type (soil, cave wall, speleothem surface, etc.), the core phyla reported in previous cave studies are *Proteobacteria* and *Actinobacteria* (Groth et al., 1999; Tomczyk-Żak and Zielenkiewicz, 2016). Besides these, the presence frequency of the *Firmicutes, Acidobacteria, Bacteroidetes, Chloroflexi,* and *Planctomycetes* phyla in caves is at a considerable rate (Youssef and Elshahed, 2008; Jurado et al., 2010; Lee et al., 2012; Barton, 2015; Wiseschart et al., 2018).

The evaluation of the NGS results of our study shows low diversity (only 18 species were identified through metagenomics) compared to other studies. The ingress of the waters contaminated by human/animal wastes into the Yarık Sinkhole can be one of the reasons. The microorganisms and organic substances that enter the cave from outside via contamination might negatively influence the cave's ecosystem leading in turn to the irreversible loss of its native biodiversity (Ikner et al., 2007; Chelius et al., 2009).

On the other hand, in the study conducted by Yasir (2018), 13 strains were identified by the culture method and a few genera, including *Bacillus, Microbacterium, Pseudomonas,* and *Psychrobacter*, were determined by the pyrosequencing analysis. However, in the pyrosequencing data *Carnobacterium, Exiguobacterium, Paucisalibacillus* and *Fictibacillus* were not detected. In addition, studies have shown that low abundance bacteria can be captured by culture methods (Lagier et al., 2012; Shade et al., 2012; Stefani et al., 2015). For this reason, the bacterial diversity of the environment should be determined more accurately by combining the culture methods with series based studies such as 16S rRNA gene analysis and metagenomics. Although there are some commonalities among the groups detected by using culture and molecular techniques, microorganism groups obtained through molecular results are richer since they also contain nonculturable groups as well. The results change in accordance with the characteristics of each cave (Engel et al., 2010; Jurado et al., 2010; Jones et al., 2012; Lee et al., 2012; Barton, 2015). However, when results are evaluated on the basis of species, variations in bacterial diversity of each cave become more obvious.

In the present study, *Methylobacterium, Acinetobacter, Propionibacterium,* and *Bacillus* were found more than other genera. It is known that these bacteria can utilize a wide variety of carbon sources and play an important role in calcification (Hiraishi et al., 1995; Cacchio et al., 2004; Portillo et al., 2008; Busquets et al., 2014). *Propionibacterium acnes and Acinetobacter lwoffii* can really get an advantage *in vivo* from polyphosphate as an energy reserve and they may use it during periods of starvation or unfavorable conditions (Van Groenestijn et al., 1987; Chen, 1999). *A. lwoffii, P. acnes,* and *Streprococcus sanguinis* that were found at 1% or higher in at least one of the sampled depths cause diseases such as bacteremia, pulmonary infections, meningitis, sepsis, pneumonia, bacterial endocarditis, and periodontal diseases (Doughari et al., 2011; Baker et al., 2018; Achermann et al., 2014). Also *A. lwoffii* is a normal flora of the oropharynx and the skin in approximately 25% of healthy individuals (Regalado et al., 2009). According to research conducted by the Human Microbiome Project the bacteria of the genera Consortium *Lactobacillus, Propionibacterium, Streptococcus, Bacteroides, Corynebacterium, Staphylococcus, Moraxella, Haemophilus, Prevotella,* and *Veillonella* are of human origin (Huttenhower et al. 2012). Leuko et al., (2017) detected a high level of *P. acnes* in their study conducted in the Su Bentu limestone cave in Sardinia, Italy, and associated that result with human contamination. To our knowledge, all of the bacteria that were found in the samples collected from Yarık Sinkhole were previously found in caves, except for *Sulfitobacter* (Busquets et al., 2014; De Mandal et al., 2014; Herzog Velikonja et al., 2014; Kieraite-Aleksandrova et al., 2015; Riquelme et al., 2015; Leuko et al., 2017).

The achievement of sampling in parallel to the discovery of the Yarık Sinkhole separates this study from other cave microbiology studies to a significant extent. Analysis of the samples showed that the bacterial diversity is limited and the detected bacteria are generally originated from humans. These results show that anthropogenic activities around a vertical cave such as the Yarık Sinkhole cause contamination of the cave.

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References

Achermann, Y., Goldstein, E.J., Coenye, T., and Shirtliff M.E., 2014, *Propionibacterium acnes*: from commensal to opportunistic biofilm-associated implant pathogen: Clinical Microbiology Reviews, v. 27, no. 3, p. 419−440. https://doi.org/ 10.1128/CMR.00092-13.

APHA, 1992, Standard Methods for Examination Water and Wastewater, 17th ed. Am. Publ. Hlth Assoc.,Washington, D.C.

Baker, S.P., Nulton, T.J., and Kitten, T., 2018, Genomic, phenotypic, and virulence analysis of Streptococcus sanguinis oral and infective endocarditis isolates: Infection and Immunity, v. 87, no. 1, p. 1−18. https://doi.org/ 10.1128/IAI.00703-18.

Ball, A.S., Betts, W.B., and Mccarthy, A.J., 1989, Degradation of lignin-related compounds by actinomycetes: Applied and Environmental Microbiology, v. 55, p. 1642−1644.

Barron, S.K., Murdock, C.A., Blair, B.G., Meade, M.E., and Barger, T.W., 2010, Analysis of bacterial diversity in soils from Blowing Spring Cave (Lauderdale County, AL): The Journal of the Alabama Academy of Science, v. 81, p. 1−10.

Barton, H.A., 2015, Starving artists: Bacterial oligotrophic heterotrophy in caves: *in* Life in Extreme Environments: Microbial Life of Cave Systems, Ed. A. Engel, Walter DeGruyter, Berlin, Germany, p. 79−95.

Barton, H.A., Taylor, N.M., Kreate, M.P., Springer, A.C., Oehrle, S.A., and Bertog, J.L., 2007, The impact of host rock geochemistry on bacterial community structure in oligotrophic cave environments: International Journal of Speleology, v. 36, p. 93−104. http://dx.doi.org/10.5038/1827-806X.36.2.5.

Busquets, A., Fornós, J.J., Zafra, F., Lalucat, J., and Merino, A., 2014, Microbial communities in a coastal cave: Cova des Pas de Vallgornera (Mallorca, Western Mediterranean): International Journal of Speleology, v. 43, p, 205−216. https://doi.org/ 10.5038/1827-806X.43.2.8.

Cacchio, P., Contento, R., Ercole, C., Cappuccio, G., Martinez, M.P., and Lepidi, A., 2004, Involvement of microorganisms in the formation of carbonate speleothems in the Cervo Cave (L'Aquila-Italy): Geomicrobiology Journal, v. 21, no. 8, p. 497−509. https://doi.org/10.1080/01490450490888109.

Candiroglu, B., 2018, Antimicrobial component productivity and antibiotic resistance of bacteria isolated from a cave [M.S. thesis]: Istanbul, Istanbul University. https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Chelius, M.K., Beresford, G., Horton, H., Quirk, M., Selby, G., Simpson, R.T., Horrocks, R., and Moore, J.C., 2009, Impacts of alterations of organic inputs on the bacterial community within the sediments of Wind Cave, South Dakota, USA: International Journal of Speleology, v. 38, p. 1−10. http://dx.doi.org/10.5038/1827-806X.38.1.1.

Chen, K.Y., 1999, Study of polyphosphate metabolism in intact cells by 31-P nuclear magnetic resonance spectroscopy. *in* Inorganic Polyphosphates, Ed. Schröder, H.C., Müller, W.E.G, Springer, Berlin, Heidelberg. p. 253−273. https://doi.org/10.1007/978-3-642-58444-2_13.

Cuezva, S., Fernandez-Cortes, A., Porca, E., Pašić, L., Jurado, V., Hernandez-Marine, M., Serrano-Ortiz, P., Hermosin, B., Cañaveras, J.C., Sanchez-Moral, S., and Saiz-Jimenez C., 2012, The biogeochemical role of actinobacteria in Altamira cave, Spain: FEMS Microbiology Ecology, v.81, no.1, p. 281−290. https://doi.org/10.1111/j.1574-6941.2012.01391.x.

Dari, K., Bechet, M., and Blondeau, R., 1995, Isolation of soil *Streptomyces* strains capable of degrading humic acids and analysis of their peroxidase-activity: FEMS Microbiology Ecology, v. 16, p. 115−121. https://doi.org/10.1016/0168-6496(94)00075-8.

De Mandal, S., Sanga, Z., and Nachimuthu, S.K., 2014, Metagenomic analysis of bacterial community composition among the cave sediments of Indo-Burman biodiversity hot spotregion: PeerJ Preprints, 2: e631v1. https://doi.org/10.7287/peerj.preprints.631v1.

Donachie, S.P., Foster, J. S., and Brown, M.V., 2007, Culture clash: challenging the dogma of microbial diversity: The ISME Journal, v.1, no.2, p. 97−99. https://doi.org/10.1038/ismej.2007.22

Doughari, H.J., Ndakidemi, P.A., Human, I.S., and Benade S., 2011, The ecology, biology and pathogenesis of *Acinetobacter* spp.: an overview: Microbes and Environments, v. 26, no. 2, p**.** 101−112. https://doi.org/10.1264/jsme2.me10179.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R., 2011, UCHIME improves sensitivity and speed of chimera detection: Bioinformatics, v. 27, no. 16, p. 2194−2200. https://doi.org/10.1093/bioinformatics/btr381.

Engel, A.S., Meisinger, D.B., Porter, M.L., Payn, R.A., Schmid, M., Stern, L.A., Schleifer, K.H., and Lee N.M., 2010, Linking phylogenetic and functional diversity to nutrient spiraling in microbial mats from Lower Kane Cave (USA): The ISME journal, v. 4, no. 1, p. 98−110. https://doi.org/ 10.1038/ismej.2009.91.

General Directorate of Mineral Research and Exploration. http://www.mta.gov.tr/ v3.0/ arastirmalar/ magara-envanteri [accessed: July 28, 2017].

Groth, I., Vettermann, R., Schuetze, B., Schumann, P., and Saiz-Jiménez, C., 1999, *Actinomycetes* in karstic caves of Northern Spain (Altamira and Tito Bustillo): Journal of Microbiological Methods, v. 36, p. 115−122.

Gulecal-Pektas, Y., 2016, Bacterial diversity and composition in Oylat Cave (Turkey) with combined sanger/pyrosequencing approach: Polish Journal of Microbiology, v. 65, no. 1, p**.** 69−75. https://doi.org/ 10.5604/17331331.1197277.

Gulecal-Pektas, Y., and Temel, M., 2015, Determination of microbial diversity in moonmilk deposits with next generation sequencing: Fresenius Environmental Bulletin, v. 24, no. 3, p. 825−832.

Gulecal-Pektas, Y., and Temel, M., 2017, A window to the subsurface: microbial diversity in hot Ssprings of a sulfidic cave (Kaklik, Turkey): Geomicrobiology Journal, v. 34, no. 4, p. 374−384. https://doi.org/10.1080/01490451.2016.1204374.

Herzog Velikonja, B., Tkavc, R., and Pašić, L., 2014, Diversity of cultivable bacteria involved in the formation of macroscopic microbial colonies (cave silver) on the walls of a cave in Slovenia: International Journal of Speleology, v. 43, p. 45−56. https://doi.org/10.5038/1827-806x.43.1.5.

Hiraishi, A., Furuhata, K., Matsumoto, A., Koike, K.A., Fukuyama, M., and Tabuchi, K., 1995, Phenotypic and genetic diversity of chlorine resistant *Methylobacterium* strains isolated from various environments: Applied and Environmental Microbiology, v. 61, p. 2099−2107.

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J.H., Chinwalla, A.T., Creasy, H.H., Earl, A.M., FitzGerald, M.G., Fulton, R.S., Giglio, M.G., Hallsworth-Pepin, K., Lobos, E.A., Madupu, R., Magrini, V., Martin, J.C., Mitreva, M., Muzny, D.M., Sodergren, E.J., Versalovic, J., Wollam, A.M., Worley, K.C., Wortman, J.R., Young, S.K., Zeng, Q., Aagaard, K.M., Abolude, O.O., Allen-Vercoe, E., Alm, E.J., Alvarado, L., Andersen, G.L., Anderson, S., Appelbaum, E., Arachchi, H.M., Armitage, G., Arze, C.A., Ayvaz, T., Baker, C.C., Begg, L., Belachew, T., Bhonagiri, V., Bihan, M., Blaser, M.J., Bloom, T., Bonazzi, V., Brooks, J.P., Buck, G.A., Buhay, C.J., Busam, D.A., Campbell, J.L., Canon, S.R., Cantarel, B.L., Chain, P.S.G., Chen, I.A., Chen, L., Chhibba, S., Chu, K., Ciulla, D.M., Clemente, J.C., Clifton, S.W., Conlan, S., Crabtree, J., Cutting, M.A., Davidovics, N.J., Davis, C.C., DeSantis, T.Z., Deal, C., Delehaunty, K.D., Dewhirst, F.E., Deych, E., Ding, Y., Dooling, D.J., Dugan, S.P., Dunne, W.M., Durkin, A.S., Edgar, R.C., Erlich, R.L., Farmer, C.N., Farrell, R.M., Faust, K., Feldgarden, M., Felix, V.M., Fisher, S., Fodor, A.A., Forney, L.J., Foster, L., Francesco, V.D., Friedman, J., Friedrich, D.C., Fronick, C.C., Fulton, L.L., Gao, H., Garcia, N., Giannoukos, G., Giblin, C., Giovanni, M.Y., Goldberg, J.M., Goll, J., Gonzalez, A., Griggs, A., Gujja, S., Haake, S.K., Haas, B.J., Hamilton, H.A., Harris, E.L., Hepburn, T.A., Herter, B., Hoffmann, D.E., Holder, M.E., Howarth, C., Huang, K.H., Huse, S.M., Izard, J., Jansson, J.K., Jiang, H., Jordan, C., Joshi, V., Katancik, J.A., Keitel, W.A., Kelley, S.T., Kells, C., King, N.B., Knights, D., Kong, H.H., Koren, O., Koren, S., Kota, K.C., Kovar, C.L., Kyrpides, N.C., La Rosa, P.S., Lee, S.L., Lemon, K.P., Lennon, N., Lewis, C.M., Lewis, L., Ley, R.E., Li, K., Liolios, K., Liu, B., Liu, Y., Lo, C.C., Lozupone, C.A., Lunsford, R.D., Madden, T., Mahurkar, A.A., Mannon, P.J., Mardis, E.R., Markowitz, V.M., Mavromatis, K., McCorrison, J.M., McDonald, D., McEwen, J., McGuire, A.L., McInnes, P., Mehta, T., Mihindukulasuriya, K.A., Miller, J.R., Minx, P.J., Newsham, I., Nusbaum, C., O'Laughlin, M., Orvis, J., Pagani, I., Palaniappan, K., Patel, S.M., Pearson, M., Peterson, J., Podar, M., Pohl, C., Pollard, K.S., Pop, M., Priest, M.E., Proctor, L.M., Qin, X., Raes, J., Ravel, J., Reid, J.G., Rho, M., Rhodes, R., Riehle, K.P., Rivera, M.C., Rodriguez-Mueller, B., Rogers, Y.H., Ross, M.C., Russ, C., Sanka, R.K., Sankar, P., Sathirapongsasuti, J.F., Schloss, J.A., Schloss, P.D., Schmidt, T.M., Scholz, M., Schriml, L., Schubert, A.M., Segata, N., Segre, J.A., Shannon, W.D., Sharp, R.R., Sharpton, T.J., Shenoy, N.,. Sheth, N.U., Simone, G.A., Singh, I., Smillie, C.S., Sobel, J.D., Sommer, D.D., Spicer, P., Sutton, G.G., Sykes, S.M., Tabbaa, D.G., Thiagarajan, M., Tomlinson, C.M., Torralba, M., Treangen, T.J., Truty, R.M., Vishnivetskaya, T.A., Walker, J., Wang, L., Wang, Z., Ward, D.V., Warren, W., Watson, M.A., Wellington, C., Wetterstrand, K.A., White, J.R., Wilczek-Boney, K., Wu, Y., Wylie, K.M., Wylie, T., Yandava, C., Ye, L., Ye, Y., Yooseph, S., Youmans, B.P., Zhang, L., Zhou, Y., Zhu, Y., Zoloth, L., Zucker, J.D., Birren, B.W., Gibbs, R.A., Highlander, S.K., Methé, B.A., Nelson, K.E., Petrosino, J.F., Weinstock, G.M., Richard K. Wilson,R.K. and White, O., 2012, Structure, function and diversity of the healthy human microbiome: Nature, v. 486, no.7402, p. 207−214. https://doi.org/10.1038/nature11234.

Ikner, L.A., Toomey, R.S., Nolan, G., Neilson, J.W., Pryor, B.M., and Maier, R.M., 2007, Culturable microbial diversity and the impact of tourism in Kartchner Caverns, Arizona: Microbial Ecology, v. 53, p. 30−42. https://doi.org/ 10.1007/s00248-006-9135-8.

Jones, A.A., and Bennett, P.C., 2014, Mineral microniches control the diversity of subsurface microbial populations: Geomicrobiology Journal, v. 31, no. 3, p. 246−261. https://doi.org/10.1080/01490451.2013.809174.

Jones, D.S., Albrecht, H.L., Dawson, K.S., Schaperdoth, I., Freeman, K.H., Pi Y., Pearson, A., and Macalady, J.L., 2012, Community genomic analysis of an extremely acidophilic sulfur-oxidizing biofilm: The ISME Journal, v. 6, p. 158−170. https://doi.org/ 10.1038/ismej.2011.75.

Jurado, V., Laiz, L., Rodriguez-Nava, V., Boiron, P., Hermosin, H., Sanchez-Moral S., and Saiz-Jimenez, C., 2010, Pathogenic and opportunistic microorganisms in caves: International Journal of Speleology, v.39, p. 15−24. https://doi.org/ 10.5038/1827-806X.39.1.2.

Kieraite-Aleksandrova, I., Aleksandrovas, V., and Kuisiene, N., 2015, Down into the Earth: microbial diversity of the deepest cave of the world: Biologia, v. 70, no. 8, p. 989−1002. https://doi.org/ 10.1515/biolog-2015-0127.

Klindworth, A., Pruesse, E., Schweer, T., Peplles, J., Quast, C., Horn, M., and Glöckner, F.O., 2013, Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies: Nucleic Acids Research, v. 41, no. 1, p. 1−11. https://doi.org/10.1093/nar/gks808.

Laiz, L., Groth, I., Gonzalez, I., and Saiz-Jimenez, C., 1999, Microbiological study of the dripping waters in Altamira Cave (Santillana del Mar, Spain): Journal of Microbiological Methods, v. 36, p. 129−138.

Lagier, J.C., Armougom, F., Million, M., Hugon, P., Pagnier, I., Robert, C., Bittar, F., Fournous, G., Gimenez, G., Maraninchi, M., Trape, J.F., Koonin, E.V., La Scola, B., and Raoult, D., 2012, Microbial culturomics: paradigm shift in the human gut microbiome study: Clinical Microbiology and Infection, v. 18, no. 12, p.1185−1193. https://doi.org/10.1111/1469-0691.12023.

Lavoie, K.H., Winter, A.S., Read, K.J., Hughes, E.M., Spilde, M.N., and Northup, D.E., 2017, Comparison of bacterial communities from lava cave microbial mats to overlying surface soils from Lava Beds National Monument, USA: PloS One, v. 12, no. 12, e0169339. doi:10.1371/journal.pone.0169339.

Lee, I.T., Liu, J.Y., Lin, C.H., Oyama, K.I., Chen, C.Y., and Chen, C.H., 2012, Ionospheric plasma caves under the equatorial ionization anomaly: Journal of Geophysical Research, v. 117, no. A11309, p. 1−9. https://doi.org/10.1029/2012JA017868.

Leuko, S., Koskinen, K., Sanna, L., D'Angeli, I.M., De Waele, J., Marcia, P., Moissl-Eichinger, C., and Rettberg, P., 2017, The influence of human exploration on the microbial community structure and ammonia oxidizing potential of the Su Bentu limestone cave in Sardinia, Italy: PLoS One, v. 12, no. 7, p. 1−22. https://doi.org/10.1371/journal.pone.0180700.

Nelson, M.C., Morrison, H.G., Benjamino, J., Grim, S.L., and Graf, J., 2014, Analysis, optimization and verification of illumina-generated 16S rRNA gene amplicon surveys: PLoS One, v. 9, no. 4-e94249, p. 1−14. https://doi.org/10.1371/journal.pone.0094249.

Northup, D.E., and Lavoie, K.H., 2001, Geomicrobiology of caves: a review: Geomicrobiology Journal, v. 18, p. 199−222. https://doi.org/10.1080/01490450152467750.

Oliveira, C., Gunderman, L., Coles, C.A., Lochmann, J., Parks, M., Ballard, E., Glazko, G., Rahmatallah, Y., Tackett, A.J., and Thomas, D.J., 2017, 16S rRNA gene-based metagenomic analysis of Ozark Cave bacteria: diversity, v. 9, no. 3, p. 1−16. https://doi.org/10.3390/d9030031.

Ortiz, M., Legatzki, A., Neilson, J.W., Fryslie, B., Nelson, W.M., Wing, R.A., Soderlund, C.A., Pryor, B.M., and Maier, R.M., 2014, Making a living while starving in the dark: metagenomic insights into the energy dynamics of a carbonate cave: The ISME Journal, v. 8, no. 2, p. 478−491. https://doi.org/10.1038/ismej.2013.159.

Palmer, A.N., 1991, Origin and morphology of limestone caves: Geological Society of America Bulletin, v. 103, p. 1−21. https://doi.org/10.1130/0016-7606(1991)103<0001:OAMOLC>2.3.CO;2

Portillo, M.C., Gonzalez, J.M., and Saiz-Jimenez, C., 2008, Metabolicallyactive microbial communities of yellow and grey colonizations on the walls of Altamira Cave, Spain: Journal of Applied Microbiology, v. 104, p. 681−691. https://doi.org/ 10.1111/j.1365-2672.2007.03594.x.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glöckner, F.O., 2007, SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB: Nucleic Acids Research, v. 35, no. 21, p. 7188−7196. https://doi.org/10.1093/nar/gkm864.

Regalado, N.G., Martin, G., and Antony, S.J., 2009, *Acinetobacter lwoffii*: bacteremia associated with acute gastroenteritis: Travel Medicine and Infectious Disease, v. 7, no. 5, p. 316−317. https://doi.org/10.1016/j.tmaid.2009.06.001.

Riquelme, C., Marshall Hathaway, J.J., Enes Dapkevicius, M.L.N., Miller, A.Z., Kooser, A., Northup, D.E., Jurado, V., Fernandez, O., Saiz-Jimenez, C., and Cheeptham N., 2015, Actinobacterial diversity in volcanic caves and associated geomicrobiological interactions: Frontiers in Microbiology, v. 6, no. 1342, p. 1−16. https://doi.org/ 10.3389/fmicb.2015.01342.

Shabarova, T., Widmer, F., and Pernthaler, J., 2013, Mass effects meet species sorting: transformations of microbial assemblages in epiphreatic subsurface karst water pools: Environmental Microbiology, v. 15, no. 9, p. 2476−2488. https://doi.org/10.1111/1462-2920.12124.

Shade, A., Hogan, C.S., Klimowicz, A.K., Linske, M., McManus, P.S., and Handelsman, J., 2012, Culturing captures members of the soil rare biosphere: Environmental Microbiology, v. 14, no. 9, p. 2247−2252. https://doi.org/ 10.1111/j.1462-2920.2012.02817.x

Stefani, F.O., Bell, T.H., Marchand, C., Ivan, E., El Yassimi, A., St-Arnaud, M., and Hijri, M., 2015, Culture-dependent and-independent methods capture different microbial community fractions in hydrocarbon-contaminated soils: PLoS One, v. 10, no. 6-e0128272, p. 1−16. https://doi.org/10.1371/journal.pone.0128272.

Tomczyk-Żak, K., and Zielenkiewicz, U., 2016, Microbial diversity in caves: Geomicrobiology Journal, v. 33, p. 20−38. https://doi.org/10.1080/01490451.2014.1003341.

Torsvik, V., and Øvreås, L., 2002, Microbial diversity and function in soil: from genes to ecosystems: Current Opinion in Microbiology, v. 5, no. 3, p. 240−245.

TSE., 1996 TS 9748 EN 27888: Water quality—determination of electrical conductivity: Turkish Standard Institution, Ankara

TSE., 1999, TS 3263 ISO 10523: Water quality—determination of pH: Turkish Standard Institution, Ankara

Ulu, Ü., 1983, Sugözü-Gazipaşa (Antalya) Alanının Jeoloji İncelemesi: Jeoloji Mühendisliği Dergisi, v. 7, no. 1, p. 3−10.

Van Groenestijn, J.W., Deinema, M.H., and Zehnder, A.J., 1987, ATP production from polyphosphate in *Acinetobacter* strain 210A: Archives of Microbiology, v. 148, no. 1, p. 14−19. https://doi.org/10.1007/bf00429640.

Wiseschart, A., Mhuanthong, W., Thongkam, P., Tangphatsornruang, S., Chantasingh, D., and Pootanakit, K., 2018, Bacterial diversity and phylogenetic analysis of type II polyketide synthase gene from Manao-Pee Cave, Thailand: Geomicrobiology Journal, v. 35, no. 6, p. 518−527. https://doi.org/10.1080/01490451.2017.1411993.

Yasir, M., 2018, Analysis of bacterial communities and characterization of antimicrobial strains from cave microbiota: Brazilian Journal of Microbiology, v. 49, no. 2, p. 248−257. https://doi.org/10.1016/j.bjm.2017.08.005.

Youssef, N.H., and Elshahed, M.S., 2008, Diversity rankings among bacterial lineages in soil: The ISME Journal, v. 3, p. 305−313. https://doi.org/10.1038/ismej.2008.106.

Zhou, J., Gu, Y., Zou, C., and Mo, M., 2007, Phylogenetic diversity of bacteria in an earth-cave in Guizhou Province, Southwest of China: Journal of Microbiology, v. 45, p. 105−112.

Kirk S. Zigler, Matthew L. Niemiller, Charles D.R. Stephen, Breanne N. Ayala, Marc A. Milne, Nicholas S. Gladstone, Annette S. Engel, John B. Jensen, Carlos D. Camp, James C. Ozier, and Alan Cressler. Biodiversity from caves and other subterranean habitats of Georgia, USA. *Journal of Cave and Karst Studies*, v. 82, no. 2, p. 125-167. DOI:10.4311/2019LSC0125

BIODIVERSITY FROM CAVES AND OTHER SUBTERRANEAN HABITATS OF GEORGIA, USA

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Abstract

We provide an annotated checklist of species recorded from caves and other subterranean habitats in the state of Georgia, USA. We report 281 species (228 invertebrates and 53 vertebrates), including 51 troglobionts (cave-obligate species), from more than 150 sites (caves, springs, and wells). Endemism is high; of the troglobionts, 17 (33 % of those known from the state) are endemic to Georgia and seven (14 %) are known from a single cave. We identified three biogeographic clusters of troglobionts. Two clusters are located in the northwestern part of the state, west of Lookout Mountain in Lookout Valley and east of Lookout Mountain in the Valley and Ridge. In addition, there is a group of troglobionts found only in the southwestern corner of the state and associated with the Upper Floridan Aquifer. At least two dozen potentially undescribed species have been collected from caves; clarifying the taxonomic status of these organisms would improve our understanding of cave biodiversity in the state. Conservation concerns related to species found in Georgia caves are significant, with fourteen species (including 13 vertebrates) considered “High Priority Species” under the Georgia State Wildlife Action Plan, many of these species have additional state or federal protections. In addition, 17 invertebrate troglobionts (33 % of those known in the state) are considered “Critically Imperiled” by NatureServe. Several biologically important caves are not protected, these are an important conservation concern. However, remarkably, around one third of all caves in the state are on protected lands, including seven of the eight caves known to host ten or more troglobionts.

Introduction

Caves and karst in Georgia are limited to two geologically distinct and disconnected regions in the northwestern and the southwestern corners of the state (Culver et al., 2003; Hobbs, 2012). In northwestern Georgia, caves occur in the Appalachian Valley and Ridge physiographic province and along the escarpments of Sand Mountain and Lookout Mountain of the Cumberland Plateau. Caves in the northwest formed in carbonate sedimentary rock units that date to the Paleozoic era (i.e., Cambrian to Mississippian periods, from 400 to 350 million years old). These units have been folded and faulted during mountain building episodes associated with the southern Appalachian Mountains. In Georgia, Lookout Mountain divides the caves of Appalachian Valley and Ridge into two distinct groups – those west of Lookout Mountain in Lookout Valley, and those east of Lookout Mountain. In southwestern Georgia, caves are known from the Dougherty Plain, also known as the Lime Sink region of the Coastal Plain province, in Eocene- to Oligocene-aged (about 25 million years old) carbonate rocks that lie above the underlying Upper Floridan Aquifer. In total, 670 caves have been documented in Georgia (Georgia Speleological Survey, 2018). Twenty-six caves are more than 1 km in length, and four caves are more than 5 km long (Georgia Speleological Survey, 2018). The highest cave density occurs in the northwestern part of the state, with 247 and 186 caves known from Walker and Dade counties, respectively. No other county has more than 40 documented caves (Georgia Speleological Survey, 2018).

The first review of subterranean biodiversity in Georgia reported 130 species of invertebrates from 29 caves (Holsinger and Peck, 1971). Twenty-seven of those caves were in northwestern Georgia, and two caves were in the Gulf Coastal Plain. Franz et al. (1994) reviewed cave biodiversity in Florida, including records for a handful of sites in southwestern Georgia. A second major review of cave biodiversity in Georgia (Reeves et al., 2000) identified 173 invertebrate taxa from 47 caves. As more caves have been biologically investigated, the number of troglobionts (cave-obligate species) known from Georgia has increased from 24 to 27 (Holsinger and Peck, 1971) to 50 (Niemiller et al., 2019).

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Since the Reeves et al. (2000) review, numerous studies have added to our knowledge of subterranean biodiversity in Georgia. Biological surveys of Georgia caves have continued (Buhlmann et al., 2001; Reeves and McCreadie, 2001; Campbell et al., 2011, 2012; Jensen and Ozier, unpublished). Phylogeographic studies and taxonomic revisions have included taxa from Georgia caves (e.g., Niemiller et al., 2008, 2011; Shear, 2010; Ledford et al., 2011; Leray et al., 2019). Additional studies have reported behavioral information for species found in caves (Camp and Jensen, 2007; Disney and Campbell, 2011; Carver et al., 2016), and others have improved our understanding of the distribution of specific troglobionts in Georgia, such as the Southern Cavefish (*Typhlichthys subterraneus*; Niemiller et al., 2016) and the Dougherty Plain Cave Crayfish (*Cambarus cryptodytes*; Fenolio et al., 2017). This wealth of new information, as well as the results of our work in caves and wells in Georgia, encouraged us to review biodiversity of caves and other subterranean habitats across the state.

Methods

We conducted faunal bioinventories of caves and wells in nine counties of Georgia on more than 350 visits between 2000 and 2019. Many of these caves had never been bioinventoried. Bioinventories primarily consisted of visual encounter surveys for cave life in terrestrial, riparian, and aquatic habitats. Searched areas of caves included entrance areas starting at the drip line, accessible walls and ceilings, ledges, mud banks, rimstone pools, streams, and talus slopes. These surveys systematically traversed the cave, from the entrance to the farthest extent of the cave explorable by the research team. Search effort included examining and overturning rocks, detritus, organic debris, and other cover, as well as searching through stream cobble. At some sites we supplemented visual encounter surveys with baited traps and bulk samples of organic debris (including leaf litter, guano, and rodent nests) that were brought to the laboratory and placed on Berlese-Tullgren funnels to extract invertebrates.

We only field-identified common, more tractable invertebrate species. In all other cases, we collected invertebrate specimens and identified them in the laboratory using available taxonomic literature. We outsourced identification to experts for taxa with which we had insufficient taxonomic knowledge. For birds and mammals, we field-identified taxa by direct observation of individuals by sight or sound without capture or through taxonomically reliable indirect observations, such as visual identification of mammal scat or footprints left in mud. Where possible, we took voucher photographs of birds and mammals. For amphibians, fishes, and reptiles, we made a concerted effort to capture each observed individual to confirm its identification and obtain a voucher photograph with the specimen in hand. For some vertebrates, we collected tissue samples and voucher specimens. Depending on the extent of the cave system, surveys were done by two to five surveyors, with a search effort of two to 12 person-hours per cave visit.

We searched for additional records of Georgia subterranean fauna in the scientific literature, biodiversity databases, unpublished government reports, unpublished technical reports, unpublished specimen identification catalogs of taxonomists, and museum accession records. Scientific literature sources included peer-reviewed journals, books, proceedings, theses, and dissertations. We also reviewed caving organization newsletters. Biodiversity database sources we directly queried included the Georgia Department of Natural Resources biodiversity database and the Bat Population Data Project (https://my.usgs.gov/bpd/). We queried all records for Animalia from the Global Biodiversity Information Facility (GBIF, https://gbif.org), a data aggregator of specimen databases and museum collections (including VertNet, http://www.vertnet.org). Our GBIF search comprised 214,566 unique records from 272 datasets hosted in 20 countries of preserved animal specimens from a geographic polygon containing Georgia (http://www.GBIF.org, 2019). We parsed the downloaded data through iterative searches for all taxa having specimen collections from subterranean features (e.g., caves, mines, springs, and wells) (Supplementary Text S1), georeferenced each record to confirm its location within the state, and reviewed each record to confirm that it was found in a subterranean feature, as opposed to on the surface in the vicinity of a subterranean feature.

Cave data—including descriptions, locations, and maps—are maintained by the Georgia Speleological Survey (GSS; http://gss.io.caves.org/). For each cave we report the cave name and alphanumeric code (‘cave number’) in current use by GSS. Associating a record from the literature with a cave in the GSS database was generally straightforward, even in cases where a single cave has been referred to by more than one name in the past. In cases where we could not confidently identify the cave associated with an occurrence record, we included these data in the list of records (Supplementary Table S2) but excluded them from georeferencing. Due to the sensitivity of cave data, we refer to caves only by their cave number, cave name, and county. We recommend readers contact GSS or the corresponding author for information on particular cave systems. Locality and name data for springs in Georgia are in the public domain and maintained in a searchable database (USGS, 2019).

The annotated list includes the scientific name, authority, ecological classification, common name, and conservation status for each species. Taxonomic nomenclature primarily followed the Integrated Taxonomic Information System (https://itis.gov/), supplemented by taxon-specific sources such as the World Spider Catalog (https://wsc.nmbe.ch/), Bellinger et al. (1996-2019), and Harvey (1990, 2013). Where available we included common names. Ecological classifications of subterranean organisms (cavernicoles) have been proposed by several authors (e.g., Barr, 1968; Sket, 2008; Culver and Pipan, 2009). Following Niemiller et al. (2016), we used terminology from Barr (1968) with clarification from Sket (2008) and Culver and Pipan (2009) to indicate species found in terrestrial (troglo-) versus aquatic (stygo-) habitats. Four primary ecological categories were used: troglobiont (TB) or stygobiont (SB) (synonyms: troglobite or stygobite, respectively), troglophile (TP) or stygophile (SP) (synonym: eutroglophile), trogloxene or stygoxene (TX or SX) (synonym: subtroglophile), and accidental (AC) (synonym: trogloxene, *sensu* Sket, 2008). We also used two secondary ecological categories: edaphic (ED) for soil-dwelling animals not typically considered cavernicoles, and symbiont (SY) for commensals and parasites. Troglobionts and stygobionts are obligate cavernicoles that typically exhibit morphological, physiological, and behavioral adaptations for living in subterranean habitats and that have few or no records from surface habitats. Troglophiles and stygophiles frequent subterranean habitats and can complete their life cycles within caves but also may occur in surface habitats. Trogloxenes and stygoxenes use subterranean habitats seasonally, or for only a portion of their life cycles, but also rely significantly on surface habitats. Accidentals are species found in caves only by accident, such as by falling into a pit or being washed into a cave during a flood. When available, we relied on ecological categories assigned to taxa by earlier authors (e.g., Holsinger and Peck, 1971; Reeves et al., 2000; Buhlmann, 2001; Niemiller et al., 2016). With many species, these categories have necessarily been subjectively inferred by previous authors due to lacking or nonexistent natural history data, which is especially true with invertebrates. We altered categories in cases where it was justified by new ecological or morphological data.

When available, the conservation status of each species, based on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (http://www.iucnredlist.org/ [accessed January 12, 2019]) and NatureServe (http://www.natureserve.org/ [accessed January 12, 2019]), is included to provide a better understanding of the distribution and biogeography of subterranean organisms in Georgia, and to aid in the future conservation and management of this unique fauna. The status of a species according to the U.S. list of threatened and endangered species under the Endangered Species Act is included (http://www.fws.gov/endangered), as well as if a species is included on the list of rare animals in Georgia (https://georgiabiodiversity.org/natels/element_lists.html). Seven IUCN (International Union for the Conservation of Nature, 2012) Red List categories are recognized on a continuum of increasing extinction risk: Least Concern, Near Threatened, Vulnerable, Endangered, Critically Endangered, Extinct in the Wild, and Extinct. Critically Endangered, Endangered, and Vulnerable are considered Threatened categories. NatureServe conservation status ranks are based on a one to five scale, from most to least at risk of extinction (Faber-Langendoen et al., 2012): 1 (Critically Imperiled), 2 (Imperiled), 3 (Vulnerable), 4 (Apparently Secure), and 5 (Secure). Two additional ranks associated with extinction exist: H (Possibly Extinct) and X (Presumed Extinct). Status ranks are assessed at three geographic scales: global (G1–5), national (N1–5), and state (S1–5). At the global scale, a Questionable rank qualifier (Q) can be used to denote uncertainty in the conservation status rank (e.g., G2Q). Taxa not ranked at the Global or State levels were noted as “GNR” or “SNR”, respectively. Ranks at the global and state scales are given in the text when available, and in Tables 1, 2, and S3.

Results

Our annotated list includes records from 142 georeferenced sites in Georgia (121 caves and 21 wells or springs) and several non-georeferenced sites, totaling 281 described species (228 invertebrates and 53 vertebrates). Of these, 51 are troglobionts. In addition to the many new records we report here, we also provide a summary of all confirmed records of subterranean faunal biodiversity in Georgia. Our summary includes the first review of vertebrates in caves in Georgia and new occurrence records for many invertebrate species. We also highlight potentially new, as yet undescribed species that have been reported in literature or that we collected. With these data, we discuss conservation issues related to cave biodiversity in Georgia. Note that, in this paper, we limit our discussion to fauna (i.e., Kingdom Animalia); data on cellular slime molds and fungi from Georgia caves are presented in Reeves et al. (2000).

The Annotated List summarizes Tables S2 and S3 and, for many species, adds additional commentary on ecology, distribution, and systematics. The source for each record reported in the Annotated List is indicated in Table S2. With a few exceptions, we omitted records not identified to the genus or species level from the annotated list, although those records are included in Table S2. In cases where two or more studies reported a particular genus from a cave, but not all studies identified those specimens to the species level, we only included the more specific record in the Annotated List. Not all records could be identified to species level. Those at coarser taxonomic resolution were due to lack of available taxonomic expertise, lack of specimens of required maturity or sex necessary for identification, or, in some cases, may represent undescribed species. Further commentary related to many of these taxa can be found in Holsinger and Peck (1971), Reeves et al. (2000), and Buhlmann (2001). New records reported in the literature for the first time are indicated with an asterisk.

Annotated List of Fauna from Caves and other Subterranean Habitats of Georgia

Phylum Annelida
Class Clitellata
Order Branchiobdellida
Family Branchiobdellidae
Localities: Dade Co.: Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62); Washington Co.: Tennile Caves (GWS20); Decatur Co.: Climax Cave (GDC36).
Comments: These were collected as ectoparasites on crayfish (Holt, 1973; Reeves and Reynolds, 1999).

Order Opisthopora
Family Lumbricidae
Genus *Aporrectodea*
***Aporrectodea trapezoides* (Dugés, 1828) (ED) Southern Worm**
Localities: Dade Co.: Howards Waterfall Cave (GDD34), Morrison Cave (GDD86); Walker Co.: Horseshoe Cave (GWK12).
Comments: This species has been reported from caves in Georgia, Illinois, Missouri, and North Carolina (Peck and Lewis, 1978; Reynolds, 1994; Reeves and Reynolds, 1999; Reeves et al., 2000).

***Aporrectodea* sp. (ED) An Earthworm**
Localities: Walker Co.: Spooky Cave (GWK494).
Comments: This may be *A. trapezoides* or another species.

Genus *Bimastos*
***Bimastos tumidus* (Eisen, 1874) (ED) An Earthworm**
Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11).
Comments: This species was collected on woody debris by Reeves and Reynolds (1999). It has also been reported from caves in Alabama, Tennessee, and Virginia (Peck, 1989; Reynolds, 1994).

***Bimastos zeteki* (Smith & Gittins, 1915) (ED) An Earthworm**
Localities: Dade Co.: Cemetery Pit (GDD64).
Comments: This species was found in the soil at the bottom of the entrance pit at Cemetery Cave (Reeves and Reynolds, 1999).

Genus *Dendrobaena*
***Dendrobaena octaedra* (Savigny, 1826) (ED) Octagonal-tail Worm**
Localities: Dade Co.: Cemetery Pit (GDD64).
Comments: This species has been reported from caves in Georgia and North Carolina (Reynolds, 1994; Reeves and Reynolds, 1999).

Genus *Dendrodrilus*
***Dendrodrilus rubidus* (Savigny, 1826) (TP) European Barkworm**
Localities: Chattooga Co.: Parkers Cave (GKH119)*; Dade Co.: Boxcar Cave (GDD69)*, Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62); Decatur Co.: Climax Cave (GDC36); Gordon Co.: Rusty Cable Cave (GGO297)*; Grady Co.: Maloys Waterfall Cave (GGR27)*; Walker Co.: Goat Cave (GWK184), Horseshoe Cave (GWK12).
Comments: This species has also been reported from several caves in Georgia, Illinois, Missouri, North Carolina, Tennessee, and New Brunswick, Canada (McAlpine and Reynolds, 1977; Peck and Lewis, 1978; Reynolds, 1994; Reeves and Reynolds, 1999; Reeves et al., 2000).

Genus *Lumbricus*
***Lumbricus rubellus* Hoffmeister, 1843 (ED) Nightcrawler**
Localities: Bartow Co.: Anthonys Cave (GBT175); Dade Co.: Howards Waterfall Cave (GDD34); Walker Co.: Horseshoe Cave (GWK12).
Comments: This species has been reported from caves in Georgia, Illinois, and North Carolina (Peck and Lewis, 1978; Reynolds, 1994; Reeves et al., 2000).

Genus *Octolasion*
***Octolasion tyrtaeum* (Savigny, 1826) (ED) Woodland White Worm**
Localities: Dade Co.: Johnsons Crook Cave (GDD17).
Comments: This species has been reported from caves in Georgia, Illinois, North Carolina, and Tennessee (Holsinger and Peck, 1971; Peck and Lewis, 1978; Reynolds, 1994; Reeves, 2000; Lewis, 2005).

Family Megascolecidae
Genus *Amynthas*
***Amynthas minimus* (Horst, 1893) (ED) An Earthworm**
Localities: Dade Co.: Howards Waterfall Cave (GDD34).
Comments: This species was collected in soil with woody debris below a drip pool (Reeves and Reynolds, 1999).

Family Sparganophilidae
Genus *Sparganophilus*
***Sparganophilus tamesis* Benham, 1892 (SX/AC) An Aquatic Worm**
Localities: Dade Co.: Boxcar Cave (GDD69)*.
Comments: This species is common in mud sediments next to streams.

Order Tubificida
Family Naididae
Genus *Arcteonais*
***Arcteonais lomondi* Martin, 1907 (SP) An Aquatic Worm**
Localities: Bartow Co.: Anthonys Cave (GBT175).
Comments: This aquatic worm was collected from mammal feces in a drip pool (Reeves et al., 2000).

Phylum Arthropoda
Subphylum Chelicerata
Class Arachnida
Order Araneae
Family Agelenidae
Genus *Coras*
***Coras* cf. *juvenilis* (Keyserling, 1881) (TX?) A Funnel Weaver Spider**
Localities: Walker Co.: Fricks Cave (GWK14).
Comments: Questionable identification; large southern range expansion if validated.

***Coras* sp. (TX?) A Funnel Weaver Spider**
Localities: Dade Co.: Byers Cave (GDD66).

Family Araneidae
Genus *Araniella*
***Araniella* sp. (TX/AC) An Orbweaver Spider**
Localities: Walker Co.: Harrisburg Cave (GWK85).

Genus *Tegenaria*
***Tegenaria domestica* (Clerck, 1757) (TP/TX) Barn Funnel Weaver**
Localities: Walker Co.: Hickman Gulf Cave.

Genus *Wadotes*
***Wadotes* cf. *calcaratus* (Keyserling, 1887) (AC) A Hacklemesh Weaver Spider**
Localities: Dade Co.: Johnsons Crook Cave (GDD17).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: Questionable identification; large southern range expansion if true.

***Wadotes saturnus* Bennett, 1987 (TX?) A Hacklemesh Weaver Spider**
Localities: Dade Co.: Sittons Cave (GDD9)*.

Family Antrodiaetidae
Genus *Antrodiaetus*
***Antrodiaetus unicolor* (Hentz, 1842) (TP) Folding-Door Spider**
Localities: Dade Co.: Howards Waterfall Cave (GDD34); Walker Co.: Fricks Cave (GWK14).

Family Clubionidae
Genus *Elaver*
***Elaver excepta* (L. Koch, 1866) (TP) Spiny Sac Spider**
Localities: Chattooga Co.: Parkers Cave (GKH119)*; Dade Co.: Upper Valley Cave (GDD135).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Family Ctenidae
Genus *Anahita*
***Anahita punctulata* (Hentz, 1844) (AC) Southeastern Wandering Spider**

Localities: Dade Co.: Hurricane Cave (GDD62)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G4 (SNR in Georgia).

Family Cybaeidae
Genus *Calymmaria*
***Calymmaria persica* (Hentz, 1847) (TP/TX) A Dwarf Sheet Spider**

Localities: Chattooga Co.: Parkers Cave (GKH119); Dade Co.: Byers Cave (GDD66), Rusty's Cave (GDD70); Walker Co.: Bible Springs Cave (GWK74), Four Kings Cave (GWK77)*, Mountain Cove Farm Cave No. 1 (GWK73).

***Calymmaria* sp. (TP/TX) A Dwarf Sheet Spider**

Localities: Dade Co.: Boxcar Cave (GDD69)*; Walker Co.: Bee Rock Cave (GWK123)*, Nash Waterfall Cave (GWK72).
Comments: This may be *C. persica* or another species.

Family Desidae
Genus *Metaltella*
***Metaltella simoni* (Keyserling, 1878) (AC) Hacklemesh Weaver**

Localities: Grady Co.: Glory Hole Cave (GGR56)*.
Comments: This species is native to South America and introduced into the United States.

Family Hahniidae
Genus *Cicurina*
***Cicurina arcuata* Keyserling, 1887 (TP/AC) Curved Meshweaver**

Localities: Floyd Co.: Cave Springs Cave (GFL18).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

***Cicurina pallida* Keyserling, 1887 (TP/TX) Pallid Funnel-web Spider**

Localities: Randolph Co.: Griers Cave (GRA40).
Comments: Questionable identification; large southern range expansion if true.

***Cicurina* sp. (TP/TX) A Meshweaver Spider**

Localities: Dade Co.: Byers Cave (GDD66).

Family Halonoproctidae
Genus *Cyclocosmia*
***Cyclocosmia truncata* (Hentz, 1841) (ED) Ravine Trapdoor Spider**

Localities: Walker Co.: Missing Evan Well Cave (GWK488)*.

Family Hypochilidae
Genus *Hypochilus*
***Hypochilus thorelli* Marx, 1888 (TX) Thorell's Lampshade-web Spider**

Localities: Dade Co.: Boxcar Cave (GDD69), Byers Cave (GDD66), Sittons Cave (GDD9).
Conservation status: IUCN: Not Evaluated; NatureServe: G4 (SNR in Georgia).
Comments: These spiders can be found near cave entrances where they build webs on rocky substrates. The species ranges from northeast Alabama to southeastern Kentucky (Hedin, 2001).

Family Leptonetidae
Genus *Appaleptoneta*
***Appaleptoneta fiskei* (Gertsch, 1974) (TB)**

Localities: Walker Co.: Harrisburg Cave (GWK85), Pettijohns Cave (GWK29).
Comments: Endemic to Georgia and known only from these sites in Walker County (Ledford et al., 2011).

Genus *Ozarkia*
***Ozarkia georgia* (Gertsch, 1974) (TB)**

Localities: Dade Co.: Byers Cave (GDD66), Kilpatrick Cave (GDD67), Rusty's Cave (GDD70).
Comments: Endemic to Georgia and known only from these sites in Dade County (Ledford et al., 2011).

Family Linyphiidae
Genus *Anibontes*
***Anibontes* sp. (TX/AC) A Sheetweb Spider**

Localities: Chattooga Co.: Parkers Cave (GKH119)*.

Genus *Anthrobia*
***Anthrobia* sp. (TP/TX) A Sheetweb Spider**

Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11).

Genus *Bathyphantes*
***Bathyphantes pallidus* (Banks, 1892) (TX) Pale Sheetweb Weaver**

Localities: Dade Co.: Howards Waterfall Cave (GDD34)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Genus *Centromerus*
***Centromerus denticulatus* (Emerton, 1909) (TP) Toothy Spurred Sheetweaver**

Localities: Walker Co.: Horseshoe Cave (GWK12).
Comments: This record from Holsinger and Peck (1971) is likely incorrect.

Centromerus latidens (Emerton, 1882) (TX) Elephant Spurred Sheetweaver

Localities: Bartow Co.: Davis Farm Cave (GBT222)*; Chattooga Co.: Parkers Cave (GKH119)*; Grady Co.: Maloys Waterfall Cave (GGR27)*; Polk Co.: White River Cave (GPO7)*; Walker Co.: Screech Owl Cave (GWK205)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Genus *Mermessus*
***Mermessus maculatus* (Banks, 1892) (TP) Spotted Harvester Money Spider**

Localities: Bartow Co.: Busch Cave (GBT611), Davis Farm Cave (GBT222)*; Decatur Co.: Climax Cave (GDC36); Grady Co.: Maloys Waterfall Cave (GGR27).

Genus *Neriene*
***Neriene radiata* (Walckenaer, 1841) (AC) Filmy Dome Spider**

Localities: Gordon Co.: Jack Crider Cave (GGO298)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Genus *Phanetta*
***Phanetta subterranea* (Emerton, 1875) (TB) Subterranean Sheetweb Spider**

Localities: Dade Co.: Byers Cave (GDD66), Caboose Cave (GDD475)*, Howards Waterfall Cave (GDD34), Johnsons Crook Cave (GDD17), Morrison Cave (GDD86), Sittons Cave (GDD9); Floyd Co.: Cave Springs Cave (GFL18); Walker Co.: Cave Spring Cave (GWK94), Fricks Cave (GWK14), Harrisburg Cave (GWK85), Mouldy Bat Pit (GWK257)*, Pigeon Cave (GWK57).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: Widespread and common in caves across the Appalachians and Interior Low Plateaus (Miller, 2005). This species is known from more counties than any other troglobiont in eastern North America (Christman and Culver, 2001).

Genus *Porrhomma*
***Porrhomma cavernicola* (Keyserling, 1886) (TB) Appalachian Cave Spider**

Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: Widespread in caves of the southern Appalachians, extending to Indiana and Illinois. It is more common in caves in West Virginia and Virginia, and only occasionally encountered in caves in Tennessee, Alabama, and Georgia (Miller, 2005).

Family Lycosidae
Genus *Pirata*
***Pirata alachuus* Gertsch & Wallace, 1935 (AC) A Pirate Wolf Spider**

Localities: Dade Co.: Wild Bills Dakota Cave (GDD596)*.

***Pirata* sp. (AC) A Pirate Wolf Spider**
Localities: Walker Co.: Horseshoe Cave (GWK12).

Genus *Piratula*
***Piratula insularis* Emerton, 1885 (AC) Lonely Wolf Spider**
Localities: Grady Co.: Maloys Waterfall Cave (GGR27).

Family Mysmenidae
Genus *Maymena*
***Maymena ambita* (Barrows, 1940) (TP) Minute Cave Spider**
Localities: Walker Co.: Ellisons Cave (GWK51), Horseshoe Cave (GWK12).

Family Nesticidae
Genus *Eidmannella*
***Eidmannella pallida* (Emerton, 1875) (TP) Pallid Cobweb Spider**
Localities: Catoosa Co.: Crane Cave (GCZ80)*; Chattooga Co.: Subligna Cave (GKH145)*; Dade Co.: Howards Waterfall Cave (GDD34); Decatur Co.: Climax Cave (GDC36); Floyd Co.: Cave Springs Cave (GFL18); Grady Co.: Maloys Waterfall Cave (GGR27)*; Randolph Co.: Griers Cave (GRA40).
Comments: This troglophilic species is widespread in North America, Central America, and the Caribbean (Gertsch, 1984).

Genus *Gaucelmus*
***Gaucelmus augustinus* Keyserling, 1884 (TP) A Cave Cobweb Spider**
Localities: Decatur Co.: Climax Cave (GDC36); Houston Co.: Limerock Cave; Washington Co.: Tennile Caves (GWS20).
Conservation status: IUCN: Not Evaluated; NatureServe: G3G4 (SNR in Georgia).
Comments: This species is a troglophile and is common in caves from Florida to Texas, through Central America to Panama, and parts of the Caribbean (Gertsch, 1984).

Genus *Nesticus*
***Nesticus georgia* Gertsch, 1984 (TB) Georgia Cave Spider**
Localities: Dade Co.: Case Cavern (GDD1), Sittons Cave (GDD9), unnamed cave near Trenton.
Conservation status: IUCN: Not Evaluated; NatureServe: G1G2 (SNR in Georgia).
Comments: This eyeless spider is endemic to Georgia and is a member of the southern Appalachian radiation of *Nesticus* that includes numerous troglobiotic species (Gertsch, 1984; Hedin, 1997). Some information regarding feeding and reproduction has been reported (Reeves, 1999; Carver et al., 2016).

***Nesticus* sp. (TB/TP) A Cave Cobweb Spider**
Localities: Dade Co.: Rusty's Cave (GDD70)*; Walker Co.: Anderson Spring Cave (GWK46), Fingerhole Cave (GWK259)*, Matthews Sink (GWK133)*, Mouldy Bat Pit (GWK257)*, Pigeon Cave (GWK57), Bee Rock Cave (GWK123)*, Lula Falls Cave (GWK617)*.
Comments: These records represent at least two undescribed species. Records from Pigeon Mountain (Anderson Spring Cave, Matthews Sink and Pigeon Cave) are an undescribed eyeless species. Additional records from Pigeon Mountain (Fingerhole Cave and Mouldy Bat Pit) may also correspond to this species. The specimens from Lookout Mountain (Lula Falls Cave) have eyes and likely represent a second undescribed species. The affinity of the Rusty's Cave record is unclear.

Family Pholcidae
Genus *Pholcus*
***Pholcus dade* Huber, 2011 (TP) A Cellar Spider**
Localities: Dade Co.: Byers Cave (GDD66), Sittons Cave (GDD9); Walker Co.: Fricks Cave (GWK14), Spooky Cave (GWK494).
Comments: Huber (2011) notes the Byers Cave specimen is tentatively assigned to this species.

***Pholcus lanieri* Huber, 2011 (TP) Lanier's Cellar Spider**
Localities: Dade Co.: Hurricane Cave (GDD62).
Comments: Known only from Hurricane Cave, the type locality (Huber, 2011).

***Pholcus* sp. (TP/TX) A Cellar Spider**
Localities: Bartow Co.: Ladds Lime Cave (GBT384-GBT389); Catoosa Co.: Chapmans Cave (GCZ25)*; Dade Co.: Little Nicka Cave (GDD121)*, SSS Cave (GDD229)*; Floyd Co.: Cave Springs Cave (GFL18); Walker Co.: Zahnd Cave (GWK641)*.
Comments: Huber (2011) describes several new *Pholcus* species from Georgia.

Family Salticidae
Genus *Maevia*
***Maevia inclemens* (Walckenaer, 1837) (AC) Dimorphic Jumper**
Localities: Walker Co.: Hickman Gulf Cave.

Family Tetragnathidae
Genus *Meta*
***Meta ovalis* (Gertsch, 1933) (TP) Cave Orbweaver**
Localities: Dade Co.: Byers Cave (GDD66), Caboose Cave (GDD475)*, Goat Cave (GWK184), Howards Waterfall Cave (GDD34), Morrison Cave (GDD86), Sittons Cave (GDD9); Walker Co.: Fingerhole Cave (GWK259)*, Four Kings Cave (GWK77)*, Fricks Cave (GWK14), Harrisburg Cave (GWK85), Mountain Cove Farm Cave No. 1 (GWK73), Nash Waterfall Cave (GWK72), Pigeon Cave (GWK57), Rocky Cave (GWK496)*, Spooky Cave (GWK494).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This species is widely distributed and found in many caves in the central and eastern United States.

Family Theridiidae
Genus *Achaearanea*
***Achaearanea* sp. (?) A Cobweb Spider**
Localities: Dade Co.: Byers Cave (GDD66).

Genus *Cryptachaea*
***Cryptachaea porteri* (Banks, 1896) (TX) A Cobweb Spider**
Localities: Dade Co.: Hooker Cave (GDD90)*; Walker Co.: Fricks Cave (GWK14).

Genus *Parasteatoda*
***Parasteatoda tepidariorum* (Koch, 1841) (TP) Common House Spider**
Localities: Bartow Co.: Ladds Lime Cave (GBT384-GBT389); Catoosa Co.: Chapmans Cave (GCZ25)*; Dade Co.: Sittons Cave (GDD9); Gordon Co.: Roberts Cave (GGO147); Polk Co.: White River Cave (GPO7); Walker Co.: Bible Springs Cave (GWK74).

***Parasteatoda* sp. (TP/TX) A Tangle Web Spider**
Localities: Bartow Co.: Anthonys Cave (GBT175), Davis Farm Cave (GBT222)*; Walker Co.: Lofton Cave (GWK281)*.
Comments: This may be *P. tepidariorum* or another species.

Family Theridiosomatidae
Genus *Theridiosoma*
***Theridiosoma gemmosum* (Koch, 1877) (TX) Common Eastern Ray Spider**
Localities: Walker Co.: Horseshoe Cave (GWK12).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Family Zoropsidae
Genus *Liocranoides*
***Liocranoides gertschi* Platnick, 1999 (TP) Gertsch's Two-clawed Cave Spider**
Localities: Dade Co.: Byers Cave (GDD66), Hurricane Cave (GDD62), Sittons Cave (GDD9); Walker Co.: Horseshoe Cave (GWK12).
Comments: The range of *L. gertschi* extends to northern Alabama (Platnick, 1999). Yancey et al. (2018) described egg sacs for *Liocranoides* from Tennessee.

Liocranoides unicolor Keyserling, 1881 (TB) A Two-clawed Cave Spider

Localities: Chattooga Co.: Parkers Cave (GKH119); Dade Co.: Byers Cave (GDD66), Morrison Cave (GDD86); Walker Co.: Bible Springs Cave (GWK74), Hickman Gulf Cave, Horseshoe Cave (GWK12), Mountain Cove Farm Cave No. 1 (GWK73).

Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Comments: Platnick (1999) indicated that *L. unicolor* ranges no further south than central Tennessee; these records may correspond to *L. gertschi* or an undescribed species.

Liocranoides sp. (TB/TP) A Two-clawed Cave Spider

Localities: Bartow Co.: Davis Farm Cave (GBT222)*; Chattooga Co.: Parkers Cave (GKH119)*; Dade Co.: Case Cavern (GDD1)*, Hooker Cave (GDD90)*, Howards Waterfall Cave (GDD34)*, Kirchmeyer Cave (GDD196)*, Rusty's Cave (GDD70)*, SSS Cave (GDD229)*; Walker Co.: Anderson Spring Cave (GWK46), Ellisons Cave (GWK51)*, Fricks Cave (GWK14)*, Lofton Cave (GWK281)*, Pettijohns Cave (GWK29), Smartt Farm Cave (GWK124)*.

Comments: These records may be *L. gertschi* or an undescribed species.

Order Opiliones
Family Phalangodidae
Genus _Bishopella_
Bishopella laciniosa (Crosby and Bishop, 1924) (TP) Bishop's Harvestman

Localities: Bartow Co.: Busch Cave (GBT611); Catoosa Co.: Crane Cave (GCZ80)*; Chattooga Co.: Blowing Springs Cave (GKH54), Parkers Cave (GKH119)*, Scoggins II Cave (GKH405)*, Subligna Cave (GKH145)*; Dade Co.: Byers Cave (GDD66), Caboose Cave (GDD475)*, Hooker Cave (GDD90)*, Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62), Kirchmeyer Cave (GDD196)*, Limestone Caverns (GDD140)*, Lower Valley Cave (GDD136)*, Rusty's Cave (GDD70)*, Sittons Cave (GDD9); Floyd Co.: Cave Springs Cave (GFL18); Gordon Co.: Plainville Cave (GGO83)*; Polk Co.: White River Cave (GPO7); Walker Co.: Anderson Spring Cave (GWK46), Bee Rock Cave (GWK123)*, Bible Springs Cave (GWK74), Ellisons Cave (GWK51)*, Fricks Cave (GWK14)*, Harrisburg Cave (GWK85), Horseshoe Cave (GWK12), LittleJohn Cave (GWK280)*, Pettijohns Cave (GWK29), Smartt Farm Cave (GWK124)*.

Comments: This species is known from surface and cave habitats across the southern Appalachians (Hedin and Thomas, 2010).

Bishopella sp. (TP/TX)

Localities: Bartow Co.: Anthonys Cave (GBT175); Dade Co.: Case Cavern (GDD1), Sittons Cave (GDD9); Walker Co.: Ellisons Cave (GWK51), Nash Waterfall Cave (GWK72), Pigeon Cave (GWK57).

Comments: These records may be *B. laciniosa* or an undescribed species.

Genus _Crosbyella_
Crosbyella spinturnix (Crosby and Bishop, 1924) (TP) A Harvestman

Localities: Decatur Co.: Climax Cave (GDC36); Gordon Co.: Rusty Cable Cave (GGO297)*; Grady Co.: Glory Hole Cave (GGR56)*, Maloys Waterfall Cave (GGR27).

Comments: This troglophile has been reported from caves in Alabama, Arkansas, Florida, and Georgia (Crosby and Bishop, 1924; Goodnight and Goodnight, 1942; Peck, 1970; Holsinger and Peck, 1971; Peck, 1989; Graening et al., 2011).

Family Sabaconidae
Genus _Sabacon_
Sabacon sp. (TP/TX) A Harvestman

Localities: Walker Co.: Goat Cave (GWK184).

Comments: This record may represent an undescribed species or one of two described species: *S. cavicolens* or *S. jonesi*. *Sabacon cavicolens* primarily occurs in rocky and forested cool surface habitat across the central and northeastern USA and in southeastern Canada (Koponen, 1995; Shear, 1975), but has also been reported from caves across its distribution, with confirmed records from Ontario, Canada (Peck, 1988), Arkansas (Shear, 1975; Peck and Peck, 1982), and Tennessee (Niemiller et al., unpublished data). *Sabacon jonesi* is known only from one cave in Madison County, Alabama (Goodnight and Goodnight, 1942). If our record from Goat Cave represents either of the previously described species, then it will represent a range extension and new state record.

Family Sclerosomatidae
Genus _Leiobunum_
Leiobunum sp. (TX) A Harvestman

Localities: Bartow Co.: Anthonys Cave (GBT175).

Comments: Several species of *Leiobunum* are known to use subterranean features to seek shelter. They often aggregate in large clusters of individuals (>100), either as overwintering populations, or presumably to seek daytime shelter during hot dry summer weather (e.g., Holmberg et al., 1984). Aggregations of *Leiobunum* are typically only found in shallow karst features or in the transition or entrance zones of caves. This clustering behavior has yet to be reported from a Georgia cave. Reeves et al. (2000) reported a single immature specimen collected from Anthonys Cave in May 1999, but did not note whether an aggregation of individuals was observed.

Order Pseudoscorpiones
Family Chernetidae
Genus _Hesperochernes_
Hesperochernes mirabilis (Banks, 1895) (TB) Southeastern Cave Pseudoscorpion

Localities: Catoosa Co.: Chickamauga Cave (GCZ106)*, Crane Cave (GCZ80); Chattooga Co.: Parker Cave (GKH119), Scoggins II Cave (GKH405)*; Dade Co.: Howards Waterfall Cave (GDD34), Johnsons Crook Cave No. 2 (GDD19), Kirchmeyer Cave (GDD196)*, Morrison Cave (GDD86), Morrison Spring Cave (GDD110), SSS Cave (GDD 229)*; Murray Co.: Major Pullims Cave (GMA3)*; Walker Co.: Battlefield Cave Spring (GWK203), Fricks Cave (GWK14), Hickman Gulf Cave (GWK204), Mountain Cove Farm Cave No. 1 (GWK73), Pigeon Cave (GWK57)*.

Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Comments: This species is distributed widely in northern Georgia caves. It is typically associated with bat guano, active rodent nests, and scat. Holsinger and Peck's (1971) record from Johnsons Crook Cave (GDD17) was in error and repeated by Reeves et al. (2000); this record was based on specimen WM1347.01 in W.B. Muchmore's collection, with original collection label "Johnson Crook Cave #2, 4.5 mi NE Rising Fawn" (Muchmore, unpublished data). A bioinventory of Johnsons Crook Cave (GDD17) on 25 June 2016 did not recover this species or find its typical habitat. The record from Battlefield Cave Spring (GWK203) was previously reported as "Cave Spring" and "Cave Spring Cave" (Holsinger and Peck, 1971; Reeves et al., 2000). Holsinger and Peck's (1971) records for *Pseudozaona* sp. are here relegated to this species; *Chelifer mirabilis* Banks, 1895 was transferred by Hoff (1946) to *Pseudozaona* and then by Muchmore (1974) to *Hesperochernes*. Records in W.B. Muchmore's catalog (unpublished data) identified as "*Hesperochernes* sp." are here placed in *H. mirabilis* on the basis of ongoing work with this genus (Stephen, unpublished data). The last known collection in Georgia was in 2015 (this study); previously, the last published record from the state was collected in 1998 (Muchmore, unpublished data; Reeves et al., 2000).

Family Chthoniidae
Genus _Aphrastochthonius_
Aphrastochthonius sp. (?) A Pseudoscorpion

Localities: Dade Co.: Byers Cave (GDD66), Longs Rock Wall Cave (GDD101)*.

Comments: These records appear to represent undescribed species (Stephen, unpublished data). No described species of this genus are known to occur in Georgia.

Genus _Apochthonius_
Apochthonius minor Muchmore, 1976 (TX?) A Pseudoscorpion

Localities: Chattooga Co.: Parker Cave (GKH119); Dade Co.: Morrison Cave (GDD86).

Conservation status: IUCN: Not Evaluated; NatureServe: G1 (SNR in Georgia).

Comments: This species is only definitively known from organic debris in entrance zones of one cave and one karst feature in Georgia. A series of females and nymphs (catalog number WM8548.01) that W.B. Muchmore (unpublished data) tentatively identified as "*Apochthonius minor*?" was reported by Lewis (2005) as *A. minor* from a cave in Van Buren County, Tennessee; this record may be *A. minor* or a species not yet described. All confirmed occurrences of this species are from the type series in Parker Cave and Morrison Cave that were collected in summer 1967 (Muchmore, 1976, unpublished data).

Apochthonius sp. (TX?) A Pseudoscorpion

Localities: Chattooga Co.: Parker Cave (GKH119).

Comments: From the same 1967 collections in the entrance of Parker Cave that recovered types for *A. minor*, Muchmore (1976, unpublished data) also identified two larger adult specimens to the genus *Apochthonius* (catalog numbers WM1270.01 and WM1275.01). These may represent undescribed species.

Genus _Chthonius_

Chthonius sp. (?) A Pseudoscorpion

Localities: Walker Co.: Horseshoe Cave (GWK12); Dade Co.: Howards Waterfall Cave (GDD34).

Comments: Of this globally distributed, diverse genus (264 species in Harvey (2013)), in eastern North America four species are known, of which two are native: *C. paludis* and *C. virginicus*. These records were reported by Reeves et al. (2000) from collections made in 1998, and are the only published occurrences of *Chthonius* in Georgia. They also appear to represent the first observations of this genus from a North American cave (Harvey, 2013; GBIF.org, 2019; Muchmore, unpublished data). Reeves et al. (2000) reported the records as *C. paludis* from Horseshoe Cave and *C. virginicus* from Howards Waterfall Cave. Both were identified by W.B. Muchmore, but in his catalog (unpublished data) he gave only tentative specific identifications. The Horseshoe Cave "*C. paludis*?" identification was based on a single female (catalog number WM8265.01) that Muchmore noted to be abnormally slender for this species. The "*C. virginicus*?" identification from Howards Waterfall Cave was based on a single nymph (catalog number WM8267.01). If these tentative identifications are correct, then each would represent large range extensions, new records of both species in Georgia, and new records of both species from caves.

Genus _Kleptochthonius_

Kleptochthonius magnus Muchmore, 1966 (TB) A Cave Pseudoscorpion

Localities: Walker Co.: Mountain Cove Farm Cave No. 1 (GWK73).

Conservation status: IUCN: Not Evaluated; NatureServe: G1 (SNR in Georgia).

Comments: This species is known from four caves located in southeastern Tennessee, northeastern Alabama, and northwestern Georgia. The type locality is in Tennessee. It is a small, pale species, with two pairs of eyes. The last known collection in Georgia was in 1967 (Holsinger and Peck, 1971; Muchmore, unpublished data).

Kleptochthonius sp. (?) A Pseudoscorpion

Localities: Walker Co.: Mountain Cove Farm Cave No. 1 (GWK73)*; Walker Co.: Rumble Rock Canyon Cave (GWK627)*.

Comments: Subterranean species of *Kleptochthonius* tend to have geographically constrained distributions, sometimes restricted to a single cave. Each of these records may represent an undescribed species.

Genus _Mundochthonius_

Mundochthonius sp. (?) A Pseudoscorpion

Localities: Chattooga Co.: Parker Cave (GKH119)*.

Comments: In North America, there are nine *Mundochthonius* species, of which three occur in caves (Harvey, 1990, 2013). This is the first record of *Mundochthonius* in Georgia. Our sampling efforts across northwestern and southwestern Georgia in caves and on the surface (sampling leaf litter, deadwood, and under live tree bark) did not recover *Mundochthonius* (Stephen, unpublished data). In June 1967, along with *Hesperochernes* pseudoscorpions and *Miktoniscus* isopods, several specimens of *Mundochthonius* were collected from a Berlese extraction of debris near the entrance of Parker Cave. These were identified by W.B. Muchmore (catalog number WM2367.01, unpublished data). These specimens may represent range extensions of surface species or undescribed subterranean diversity.

Family Neobisiidae

Genus _Lissocreagris_

Lissocreagris subatlantica (Chamberlin, 1962) (TX) A Pseudoscorpion

Localities: Chattooga Co.: Parker Cave (GKH119).

Conservation status: IUCN: Not Evaluated; NatureServe: G2G4 (SNR in Georgia).

Comments: This species is known from five collection events, of which four are from within caves or in the entrance area of a cave. In Georgia it was collected from organic debris in the entrance of Parker Cave (Muchmore, 1969). It is a small, pale species, with two pairs of reduced eyes (Chamberlin, 1962). The last known collection in Georgia was in 1967 (Muchmore 1969, unpublished data).

Lissocreagris sp. (TB/TP) A Pseudoscorpion

Localities: Walker Co.: Pettijohns Cave (GWK29)*.

Comments: This appears to represent an undescribed species. It was collected by S. Peck and A. Fiske in a pitfall trap active 10-21 June 1967, identified by W.B. Muchmore (unpublished data) as "*Lissocreagris* n. sp.", and listed as *Microcreagris* sp. by Holsinger and Peck (1971). In his catalog, Muchmore (unpublished data) originally identified the genus to *Microcreagris* and later changed this to *Lissocreagris* after this genus was erected by Ćurčić (see comments for *Microcreagris*). In his catalog, Muchmore briefly notes that the single adult female (catalog number WM1311.01) was small and eyeless.

Genus _Microcreagris_

Microcreagris (sensu lato) sp. (TP/TX) A Pseudoscorpion

Localities: Walker Co.: Mountain Cove Farm Cave No. 1 (GWK73)*.

Comments: Ćurčić (1981, 1984, 1989) and Muchmore and Cokendolpher (1995) transferred all but one species of subterranean North American *Microcreagris* into several genera erected by Ćurčić. Holsinger and Peck (1971) listed two records of unidentified *Microcreagris*, from Pettijohns Cave (GWK29) and Johnsons Crook Cave (GDD17), commenting "A single female of this undetermined species was collected". Their pseudoscorpion identifications were done by W.B. Muchmore; in his catalog (unpublished data), he identified a single female "*Lissocreagris* n. sp." collected from Pettijohns Cave in 1967 by S. Peck and A. Fiske (see comments for *Lissocreagris*). There is no mention in Muchmore's catalog of *Microcreagris* (or the replacement genera erected by Ćurčić, 1989) from Johnson Crook Cave. The new record from Mountain Cove Farm Cave No. 1 consisted of an isolated pedipalp (catalog number WM2990.02) collected from the stomach contents of a *Eurycea lucifuga* found in the dark zone of the cave. Muchmore tentatively identified it to *Microcreagris*.

Microcreagris (sensu lato) sp. A Pseudoscorpion

Localities: Dade Co.: Hooker Cave (GDD90)*.

Genus _Minicreagris_

Minicreagris pumila (Muchmore, 1969) (TX) A Pseudoscorpion

Localities: Chattooga Co.: Parker Cave (GKH119).

Comments: This species is known from the entrance zone of one cave and one surface locality in Alabama, and from organic debris in the entrance zone of Parker Cave (Muchmore, 1969). It was incorrectly listed as "*Lissocreagris pumila*" by Peck (1989). The species is small and pale, and has one pair of reduced eyes (Muchmore, 1969). The last known collection in Georgia was in 1967 (Holsinger and Peck, 1971; Muchmore, unpublished data). In transferring *Microcreagris pumila* into *Minicreagris*, Ćurčić (1989) misquoted Muchmore (1969) by listing an epigean Tennessee locality: the species is only known from Alabama and Georgia (Muchmore, 1969, unpublished data).

Genus _Novobisium_

Novobisium carolinense (Banks, 1895) (AC) A Pseudoscorpion

Localities: Dade Co.: Johnson Crook Cave (GDD17)*.

Comments: This record represents a range extension and the first report of this species from a cave. The species is widely distributed in the southeastern USA, where it is typically found in leaf litter. The only Georgia records were collected from the bottom of the pit entrance of Johnson Crook Cave in 2016 (this study). A trap set in the sink outside of Johnson Crook Cave in 1967 also collected an unidentified species of *Novobisium* (Muchmore, unpublished data).

Order Ixodida
Family Argasidae
Genus *Ornithodoros*
***Ornithodoros kelleyi* (Cooley and Kohls, 1941) (SY) A Bat Tick**

Localities: Decatur Co.: Climax Cave (GDC36).
Comments: This bat tick was collected in guano piles by Reeves et al. (2000). The likely host was *Myotis austroriparius*.

Family Ixodidae
Genus *Dermacentor*
***Dermacentor variabilis* (Say, 1821) (SY) American Dog Tick**

Localities: Dade Co.: Howards Waterfall Cave (GDD34).
Comments: This tick is a common ectoparasite of mammals.

Genus *Ixodes*
***Ixodes cookei* Packard 1869 (SY) American Castor Bean Tick**

Localities: Walker Co.: Rocky Cave (GWK496).
Comments: This tick is a common ectoparasite of birds and mammals, including humans.

Order Mesostigmata
Family Laelapidae
Genus *Laelaspis*
***Laelaspis* sp. (TX/AC) A Mite**

Localities: Dade Co.: Case Cavern (GDD1); Walker Co.: Pettijohns Cave (GWK29).

Family Macrochelidae
Genus *Macrocheles*
***Macrocheles* sp. (TX/AC) A Mite**

Localities: Walker Co.: Fricks Cave (GWK14).
Comments: This mite was common on *Myotis grisescens* guano at Fricks Cave (Reeves et al., 2000).

Family Veigaiidae
Genus *Veigaia*
***Veigaia* sp. (TX/AC) A Mite**

Localities: Walker Co.: Nash Waterfall Pit (GWK360).
Comments: Reeves et al. (2000) collected a single specimen in 1995.

Order Sarcoptiformes
Family Acaridae
Genus *Troglocoptes*
***Troglocoptes* sp. (TX/AC) A Mite**

Localities: Walker Co.: Fricks Cave (GWK14).
Comments: Reeves et al. (2000) reported this potentially undescribed mite from *Myotis grisescens* guano.

Order Trombidiformes
Family Rhagidiidae
Genus *Rhagidia*
***Rhagidia* sp. (TB?/TP) A Mite**

Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11); Dade Co.: Byers Cave (GDD66), Morrison Cave (GDD86); Walker Co.: Bible Springs Cave (GWK74), Pettijohns Cave (GWK29).
Comments: These records were reported by Holsinger and Peck (1971).

Family Trombiculidae
Genus *Euschoengastia*
***Euschoengastia pipistrelli* Brennan, 1947 (SY) A Chigger**

Localities: Dade Co.: Howards Waterfall Cave (GDD34); Walker Co.: Fricks Cave (GWK14).
Comments: This species is an ectoparasite of *Perimyotis subflavus*.

Genus *Leptotrombidium*
***Leptotrombidium myotis* (Ewing, 1829) (SY) A Chigger**

Localities: Bartow Co.: Anthonys Cave (GBT175).
Comments: This species is an ectoparasite and was reported feeding on *Perimyotis subflavus* by Reeves et al. (2000).

Subphylum Crustacea
Class Branchiopoda
Order Diplostraca
Family Daphniidae
Genus *Daphnia*
***Daphnia* sp. (TX/AC) A Common Water Flea**

Localities: Bartow Co.: Anthonys Cave (GBT175).
Comments: Reeves et al. (2000) collected a single specimen from a drip pool.

Class Malacostraca
Superorder Peracarida
Order Amphipoda
Family Crangonyctidae
Genus *Crangonyx*
***Crangonyx antennatus* Cope and Packard, 1881 (SB) Appalachian Valley Cave Amphipod**

Localities: Catoosa Co.: Crane Cave (GCZ80)*; Chattooga Co.: Chelsea Gulf Cave (GKH54); Dade Co.: Byers Cave (GDD66), Cemetery Pit (GDD64), Chambliss Cave (GDD321), Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62)*, Johnsons Crook Cave (GDD17)*, Rusty's Cave (GDD70), Sittons Cave (GDD9), SSS Cave (GDD229)*, Upper Valley Cave (GDD135); Floyd Co.: Cave Springs Cave (GFL18); Walker Co.: Anderson Spring Cave (GWK46), Fricks Cave (GWK14), Gila Monster Cave (GWK379)*, Harrisburg Cave (GWK85), Horseshoe Cave (GWK12), Mountain Cove Farm Cave No. 1 (GWK73), Pettijohns Cave (GWK29), Spooky Cave (GWK494).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This is a widespread stygobiotic species whose range extends through the Valley and Ridge from southwestern Virginia into northeastern Alabama. It is common in cave streams and pools (Zhang and Holsinger, 2003).

***Crangonyx consimilis* Zhang and Holsinger, 2003 (SX) An Amphipod**

Localities: Dade Co.: Howards Waterfall Cave (GDD34)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G4 (SNR in Georgia).
Comments: This record likely is a misidentification, as this species is not otherwise recorded east of the Cumberland Plateau (Zhang and Holsinger, 2003).

Genus *Stygobromus*
***Stygobromus ackerlyi* Holsinger, 1978 (SB) Ackerly's Cave Amphipod**

Localities: Bartow Co.: Chert Chasm (GBT340); Floyd Co.: Cave Springs Cave (GFL18); Polk Co.: White River Cave (GPO7).
Conservation status: IUCN: Not Evaluated; NatureServe: G1G2 (SNR in Georgia).
Comments: This stygobite is endemic to Georgia and known only from these sites in the Coosa River drainage.

***Stygobromus dicksoni* Holsinger, 1978 (SB) A Cave Amphipod**

Localities: Chattooga Co.: Chelsea Gulf Cave (GKH54); Dade Co.: Byers Cave (GDD66), Cemetery Pit (GDD64), Howards Waterfall Cave (GDD34), Johnsons Crook Cave (GDD17)*, Rusty's Cave (GDD70); Walker Co.: Pettijohns Cave (GWK29).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: The range of this stygobite extends into adjacent northeastern Alabama and southern Tennessee (Holsinger, 1978).

***Stygobromus doughertyensis*, Cannizzaro and Sawicki, 2019 (SB) Dougherty Plain Cave Amphipod**

Localities: Dougherty County.: Radium Springs (GDG39).
Comments: This species is also known from Jackson Co., Florida.

***Stygobromus grandis* Holsinger, 1978 (SB) Parkers Cave Amphipod**

Localities: Chattooga Co.: Parkers Cave (GKH119).
Conservation status: IUCN: Not Evaluated; NatureServe: G1 (SU in Georgia).
Comments: This stygobite is endemic to Georgia and known only from Parkers Cave (Holsinger, 1978).

***Stygobromus minutus* Holsinger, 1978 (SB) Pettijohns Cave Amphipod**

Localities: Walker Co.: Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29).
Conservation status: IUCN: Not Evaluated; NatureServe: G2G3 (SU in Georgia).

Comments: This stygobite is endemic to Georgia and known only from these sites on Pigeon Mountain (Holsinger, 1978).

***Stygobromus* sp. (SB) A Cave Amphipod**
Localities: Dade Co.: Boxcar Cave (GDD69), Caboose Cave (GDD475).
Comments: Reeves et al. (2000) suggest these records represent an undescribed species.

Order Isopoda
Family Armadilliidae
Genus *Armadillidium*
***Armadillidium vulgare* (Latreille, 1804) (TX) Common Pill-bug**
Localities: Walker Co.: Horseshoe Cave (GWK12).
Comments: This common surface species was introduced from Europe and is now widespread in North America.

Family Asellidae
Genus *Caecidotea*
***Caecidotea cyrtorhynchus* (Fleming and Steeves, 1972) (SB) A Cave Isopod**
Localities: Walker Co.: Anderson Spring Cave (GWK46), Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29).
Conservation status: IUCN: Not Evaluated; NatureServe: G1 (SU in Georgia).
Comments: This stygobite is endemic to Georgia and known only from sites on Pigeon Mountain. The type locality is Pettijohns Cave.

***Caecidotea hobbsi* (Maloney, 1939) (SB) Hobbs Cave Isopod**
Localities: DeKalb Co.: Spring on Walter Chandler Estate at Emory University.
Conservation status: IUCN: Not Evaluated; NatureServe: G2G3 (SNR in Georgia).
Comments: This stygobite is only reported from one site in Georgia. It is more commonly known from Florida (Steeves, 1964). This record may be in error, and likely represents *C. putea* instead (J. Lewis, pers. comm).

***Caecidotea nickajackensis* Packard, 1881 (SB) Nickajack Cave Isopod**
Localities: Dade Co.: Johnsons Crook Cave (GDD17)*.
Conservation status: IUCN: Not Evaluated; NatureServe: GH (SNR in Georgia).
Comments: This species was presumed extinct after the flooding of Nickajack Cave in Marion County, Tennessee in the 1960s (Lewis, 2009) but was rediscovered in two caves near the junction of Tennessee, Alabama, and Georgia (Coleman and Zigler, 2015). This is the first record of the species in Georgia.

***Caecidotea putea* Lewis, 2009 (SB) Econfina Springs Cave Isopod**
Localities: Cobb Co.: road cut spring, Kennesaw; Thomas Co.: Wells at Experimental Station, Metcalf.
Conservation status: IUCN: Not Evaluated; NatureServe: G1G2 (SNR in Georgia).
Comments: This stygobite is known from only three widely-dispersed sites, including one in Washington County, Florida (Lewis, 2009).

***Caecidotea richardsonae* Hay, 1901 (SB) Tennessee Valley Cave Isopod**
Localities: Bartow Co.: seep 1 mi NE of Adairsville; Chattooga Co.: Blowing Springs Cave (GKH54), Chelsea Gulf Cave (GKH54); Dade Co.: Byers Cave (GDD66), Cemetery Pit (GDD64)*, Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62)*, Johnsons Crook Cave (GDD17), Lower Valley Cave (GDD136)*, Rusty's Cave (GDD70), Sittons Cave (GDD9)*, SSS Cave (GDD229)*; Floyd Co.: Cave Springs Cave (GFL18); Walker Co.: Blowing Springs Cave No. 1 (GWK41), Horseshoe Cave (GWK12), Mountain Cove Farm Cave No. 1 (GWK73).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: Widespread species whose range extends through the Valley and Ridge from southwest Virginia to northeast Alabama (Lewis, 2009). Common in cave streams and pools.

***Caecidotea* sp. (SB) A Cave Isopod**
Localities: Catoosa Co.: Chapmans Cave (GCZ25)*; Dade Co.: Longs Rock Wall Cave (GDD101)*; Mitchell Co.: USGS Well 11J012; Walker Co.: Ellisons Cave (GWK51).
Comments: The record from a well in Mitchell County was a female in the *hobbsi* species group (Fenolio et al. 2017).

Genus *Lirceus*
***Lirceus* sp. (SP/SX) An Isopod**
Localities: Chattooga Co.: Blowing Springs Cave (GKH54); Dade Co.: Howards Waterfall Cave (GDD34)*; Walker Co.: Nash Waterfall Cave (GWK72).
Comments: These records are eyed, pigmented specimens. They are a species from the *L. hargeri* group that also occurs in Tennessee and Virginia (J. Lewis, pers. comm.).

Family Cylisticidae
Genus *Cylisticus*
***Cylisticus convexus* (De Geer, 1778) (TX) Curly Woodlouse**
Localities: Bartow Co.: Anthonys Cave (GBT175); Dade Co.: Howards Waterfall Cave (GDD34), Morrison Cave (GDD86); Floyd Co.: Cave Springs Cave (GFL18); Walker Co.: Bible Springs Cave (GWK74), Cave Spring Cave, Horseshoe Cave (GWK12).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This species is common and known from caves ranging from Indiana to Virginia and Texas (Schultz, 1970).

Family Ligiidae
Genus *Ligidium*
***Ligidium elrodii* (Packard, 1873) (TX) A Woodlouse**
Localities: Dade Co.: Limestone Caverns (GDD140)*; Walker Co.: Ellisons Cave (GWK51), Pigeon Cave (GWK57); Chattooga Co.: Chelsea Gulf Cave (GKH54).
Conservation status: IUCN: Not Evaluated; NatureServe: G4G5 (SNR in Georgia).
Comments: This species is widespread in eastern North America. A subspecies (*Ligidium elrodii chattoogaensis*) was described from Chelsea Gulf Cave by Schultz (1970).

Family Trichoniscidae
Genus *Amerigoniscus*
***Amerigoniscus curvatus* Vandel, 1978 (TB) A Terrestrial Cave Isopod**
Localities: Walker Co.: Horseshoe Cave (GWK12).
Conservation status: IUCN: Not Evaluated; NatureServe: G1 (SU in Georgia).
Comments: This troglobite is endemic to Georgia and known only from this site.

***Amerigoniscus georgiensis* Vandel, 1978 (TB) Georgia Cave Isopod**
Localities: Walker Co.: Pettijohns Cave (GWK29).
Conservation status: IUCN: Not Evaluated; NatureServe: G1 (SU in Georgia).
Comments: This troglobite is endemic to Georgia and known only from this site.

***Amerigoniscus proximus* Vandel, 1978 (TB) A Terrestrial Cave Isopod**
Localities: Chattooga Co.: Chelsea Gulf Cave (GKH54); Dade Co.: Byers Cave (GDD66).
Conservation status: IUCN: Not Evaluated; NatureServe: G1G2 (SNR in Georgia).
Comments: This troglobite is endemic to Georgia and known only from these sites.

***Amerigoniscus* sp. (TB) A Terrestrial Cave Isopod**
Localities: Dade Co.: Case Cavern (GDD1), Johnsons Crook Cave (GDD17)*, Sittons Cave (GDD9). Walker Co.: Bible Springs Cave (GWK74), Mountain Cove Farm Cave No. 1 (GWK73).
Comments: These records may represent one of the described *Amerigoniscus* from Georgia or an undescribed species.

Genus *Miktoniscus*
***Miktoniscus* sp. (TB/TP) A Terrestrial Isopod**

Localities: Bartow Co.: Anthonys Cave (GBT175); Chattooga Co.: Blowing Springs Cave (GKH54), Parkers Cave (GKH119); Dade Co.: Howards Waterfall Cave (GDD34), Sittons Cave (GDD9); Decatur Co.: Climax Cave (GDC36); Grady Co.: Maloys Waterfall Cave (GGR27)*; Randolph Co.: Griers Cave (GRA40); Walker Co.: Horseshoe Cave (GWK12), Pigeon Cave (GWK57), Spooky Cave (GWK494).

Comments: Several of these records are from Reeves et al. (2000), who considered them to be troglobites and possibly an undescribed species.

Superorder Eucarida
Order Decapoda
Family Cambaridae
Genus *Cambarus*
***Cambarus bartonii* (Fabricius, 1798) (SP) Appalachian Brook Crayfish**

Localities: Dade Co.: Hurricane Cave (GDD62)*, Twin Snakes Cave (GDD140).

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This species is common in caves in the Appalachian Valley and Ridge (Fong et al., 2012).

***Cambarus cryptodytes* Hobbs, 1941 (SB) Dougherty Plain Cave Crayfish**

Localities: Baker Co.: Double Springs, USGS Well 10H009, USGS Well 12K014; Calhoun Co.: Chickasawhatchee Swamp WMA Well #18, Chickasawhatchee Swamp WMA Well #6, Chickasawhatchee Swamp WMA Well #7; Calhoun Co.: USGS Well 10K005; Decatur Co.: Climax Cave (GDC36), USGS Well 09F520; Dougherty Co.: Albany Field Well #8, Chameleon Springs, Radium Springs (GDG39), USGS Well 13L012; Early Co.: USGS Well 08K001; Miller Co.: USGS Well 08G001; Mitchell Co.: USGS Well 10G313; Seminole Co.: USGS Well 06F001.

Conservation status: IUCN: Least Concern; NatureServe: G2G3 (S2 in Georgia); listed as Threatened and considered a Species of Greatest Conservation Need in Georgia.

Comments: Fenolio et al. (2017) reported nine new records from wells in eight counties in southwestern Georgia. This stygobite also occurs into adjacent northwestern Florida (Hobbs et al., 1977; Franz et al., 1994; Fenolio et al., 2017).

***Cambarus latimanus* (Le Conte, 1856) (TP/TX) Variable Crayfish**

Localities: Chattooga Co.: Blowing Springs Cave (GKH54); Dade Co.: Byers Cave (GDD66), Hurricane Cave (GDD62); Walker Co.: Mountain Cove Farm Cave No. 1 (GWK73).

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This is a widely distributed species that is occasionally reported from caves.

***Cambarus striatus* Hay, 1902 (SP) Ambiguous Crayfish**

Localities: Chattooga Co.: Blowing Springs Cave (GKH54); Walker Co.: Bible Springs Cave (GWK74), Horseshoe Cave (GWK12).

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This is a widely distributed species that is occasionally reported from caves.

***Cambarus tenebrosus* Hay, 1902 (SP) Cavespring Crayfish**

Localities: Dade Co.: Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62).

Conservation status: IUCN: Least Concern; NatureServe: G5 (SNR in Georgia).

Comments: Reeves et al. (2000) reported this stygophile from these two Georgia caves. This species is common in caves in the Interior Low Plateau and occurs in the extreme northwestern part of the state (Niemiller et al., 2013).

***Cambarus* sp. (SP) A Crayfish**

Localities: Dade Co.: Longs Rock Wall Cave (GDD101)*, Sittons Cave (GDD9); Walker Co.: Anderson Spring Cave (GWK46)*, Ellisons Cave (GWK51), Fricks Cave (GWK14)*, Pigeon Cave (GWK57)*, Roger Branch Cave (GWK204)*.

Comments: The records likely represent one or more of the species listed above.

Class Maxillopoda
Order Cyclopoida
Family Cyclopidae
Genus *Acanthocyclops*
***Acanthocyclops robustus* (Sars, 1863) (SP) A Copepod**

Localities: Bartow Co.: Anthonys Cave (GBT175).

Comments: This species is common in surface waters but has been collected from caves in Georgia, Indiana, Illinois, Kentucky, and Tennessee (Lewis and Reid, 2007).

***Acanthocyclops vernalis* (Fischer, 1853) (SP) A Copepod**

Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11).

Comments: This species is also known from cave and surface sites in Indiana, Illinois, Kentucky, and Tennessee (Lewis and Reid, 2007).

Genus *Eucyclops*
***Eucyclops conrowae* Reid, 1992 (SX) A Copepod**

Localities: Washington Co.: Tennile Caves (GWS20).

Comments: This species is common in surface habitats (Lewis and Reid, 2007).

***Eucyclops elegans* (Herrick, 1884) (SX) A Copepod**

Localities: Bartow Co.: Anthonys Cave (GBT175).

Comments: This primarily surface species has been collected in caves in Georgia, Indiana, Kentucky, and Tennessee (Lewis and Reid, 2007).

Genus *Macrocyclops*
***Macrocyclops albidus* (Jurine, 1820) (SP) A Copepod**

Localities: Bartow Co.: Anthonys Cave (GBT175).

Comments: This species is also known from caves in Illinois, Indiana, Kentucky, and Tennessee (Lewis and Reid, 2007).

Genus *Megacyclops*
***Megacyclops donnaldsoni* (Chappuis, 1929) (SB) Donnaldson's Cave Copepod**

Localities: Dade Co.: Cemetery Pit (GDD64).

Conservation status: IUCN: Not Evaluated; NatureServe: G3G4 (SNR in Georgia).

Comments: This species was first described from Donnaldson Cave in Lawrence Co., Indiana, but has been collected from caves in Kentucky and Tennessee and is considered a strict troglobiont (Lewis and Reid, 2007).

Order Harpacticoida
Family Canthocamptidae
Genus *Attheyella*
***Attheyella illinoisensis* (Forbes, 1882) (SX/AC) A Copepod**

Localities: Walker Co.: Goat Cave (GWK184); Washington Co.: Tennile Caves (GWS20).

Comments: This species is also known from caves in Indiana (Lewis and Reid, 2007).

***Attheyella nordenskioldi* (Lilljeborg, 1902) (AC) A Copepod**

Localities: Dade Co.: Howards Waterfall Cave (GDD34); Walker Co.: Horseshoe Cave (GWK12).

Comments: This species is also known from springs and caves in Illinois and Indiana (Lewis and Reid, 2007).

***Attheyella pilosa* Chappuis, 1929 (SX) A Copepod**

Localities: Chattooga Co.: Blowing Springs Cave (GKH54)

Comments: This species is also known from springs and caves in Indiana and Kentucky (Lewis and Reid, 2007).

Genus *Elaphoidella*
***Elaphoidella bidens* (Schmeil, 1894) (AC) A Copepod**

Localities: Washington Co.: Tennile Caves (GWS20).

Class Ostracoda
Order Podocopida
Family Candonidae
Genus *Pseudocandona*
***Pseudocandona* sp. (SY) An Ostracod**

Localities: Dade Co.: Rusty's Cave (GDD70).

Comments: Reeves et al. (2000) collected two specimens in the cave stream at Rusty's Cave.

Family Cyprididae
Genus *Potamocypris*
***Potamocypris* sp. (SY) An Ostracod**
Localities: Walker Co.: Horseshoe Cave (GWK12).
Comments: This record was reported as *Potamocypris* cf. *fulva* by Reeves et al. (2000).

Family Entocytheridae
Genus *Uncinocythere*
***Uncinocythere warreni* Hobbs and Walton, 1968 (SB/SY) A Cave Ostracod**
Localities: Decatur Co.: Climax Cave (GDC36).
Conservation status: IUCN: Not Evaluated; NatureServe: G1 (SU in Georgia).
Comments: This species is endemic to Georgia and known only from Climax Cave where it is a commensal on *Cambarus cryptodytes* (Hobbs and Walton, 1968; Hart and Hart, 1974).

Subphylum Hexapoda
Order Collembola
Family Arrhopalitidae
Genus *Arrhopalites*
***Arrhopalites pygmaeus* (Wankel, 1860) (TP) A Springtail**
Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11).
Comments: This widely distributed springtail is known from caves in several states in the eastern United States (Bellinger et al. 1996-2019; Christiansen, 1960, 1966; Christiansen and Bellinger, 1981; Peck, 1995; Lewis, 2005).

***Arrhopalites* sp. (TP) A Springtail**
Localities: Walker Co.: Mountain Cove Farm Cave No. 1 (GWK73).
Comments: This may be *A. pygmaeus* or another species.

Family Entomobryidae
Genus *Lepidocyrtus*
***Lepidocyrtus* sp. (TP) A Slender Springtail**
Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11).
Comments: Reeves et al. (2000) reported this springtail from a drip pool. The status of *Lepidocyrtus* is uncertain and this report may in the future be attributed to a species of *Lepidosira* (Bellinger et al. 1996-2019).

Genus *Pseudosinella*
***Pseudosinella christianseni* Salmon, 1964 (TB) Christiansen's Cave Springtail**
Localities: Dade Co.: Case Cavern (GDD1), Cemetery Pit (GDD64), Chambliss Cave (GDD321), Ha-Ha Cave (GDD256), Howards Waterfall Cave (GDD34), Sittons Cave (GDD9), Upper Valley Cave (GDD135); Walker Co.: Anderson Spring Cave (GWK46), Ellisons Cave (GWK51), Fricks Cave (GWK14), Goat Cave (GWK184), Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29), Pigeon Cave (GWK57), Spooky Cave (GWK494).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This troglobite is eyeless and white without any trace of pigment (Christiansen and Bellinger, 1998). Its range extends across middle Tennessee to Kentucky and into northeastern Alabama and northwestern Georgia.

***Pseudosinella georgia* Christiansen and Bellinger, 1998 (TP) Georgia Cave Springtail**
Localities: Walker Co.: Ellisons Cave (GWK51), Fricks Cave (GWK14), Nash Waterfall Cave (GWK72), Pigeon Cave (GWK57).
Comments: This species is endemic to Georgia and known from only four sites (all caves or pits) but has eyes and scattered pigment across head and body (Christiansen and Bellinger, 1998), so it has been considered a troglophile and not a troglobiont.

***Pseudosinella hirsuta* (Delamare Deboutteville, 1949) (TB) Hirsute Cave Springtail**
Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11); Chattooga Co.: Blowing Springs Cave (GKH54), Chelsea Gulf Cave (GKH54); Dade Co.: Howards Waterfall Cave (GDD34), Johnsons Crook Cave (GDD17), Morrison Cave (GDD86), Running Water Cave (GDD120), Rusty's Cave (GDD70), Sittons Cave (GDD9); Polk Co.: Deatons Cave (GPO5); Walker Co.: Bible Springs Cave (GWK74), Harrisburg Cave (GWK85), Horseshoe Cave (GWK12), Mountain Cove Farm Cave No. 1 (GWK73), Mountain Cove Farm Cave No. 2 (GWK74), Pettijohns Cave (GWK29).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This troglobite is usually white and lacks eyes, although some variation is known (Christiansen and Bellinger, 1998). It is widespread in caves across Kentucky, Tennessee, Alabama, and into northwestern Georgia (Christman and Culver, 2001).

***Pseudosinella pecki* Christiansen and Bellinger, 1980 (TB) Peck's Cave Springtail**
Localities: Decatur Co.: Climax Cave (GDC36); Randolph Co.: Griers Cave (GRA40).
Conservation status: IUCN: Not Evaluated; NatureServe: G2G3 (SNR in Georgia).
Comments: This troglobite is eyeless and lacks any trace of pigment. The type locality is in Jackson County, Florida, but it is known from a handful of other caves in Georgia, Alabama, and Tennessee (Christiansen and Bellinger, 1998).

***Pseudosinella spinosa* (Delamare Deboutteville, 1949) (TB) Spiny Cave Springtail**
Localities: Dade Co.: Chapman Cave.
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This troglobite is the largest Nearctic *Pseudosinella*. It lacks eyes and pigment (Christiansen and Bellinger, 1998). It is known from just one cave in Georgia but ranges across middle Tennessee and northeastern Alabama.

***Pseudosinella* sp. (TB) A Cave Springtail**
Localities: Chattooga Co.: Blowing Springs Cave (GKH54); Dade Co.: Byers Cave (GDD66).
Comments: These record are likely one of the species listed above. The Blowing Springs Cave record was from GBIF (2019).

Family Isotomidae
Genus *Folsomia*
***Folsomia candida* Willem, 1902 (TP) White Springtail**
Localities: Walker Co.: Pettijohns Cave (GWK29).
Comments: This springtail is a widely distributed troglophile.

Family Neelidae
Genus *Neelus*
***Neelus murinus* Folsom, 1896 (TP) A Springtail**
Localities: Dade Co.: Howards Waterfall Cave (GDD34).
Comments: Reeves et al. (2000) collected this springtail from organic debris. It is also known form northwestern Europe (Bellinger et al., 1996-2019).

Family Tomoceridae
Genus *Pogonognathellus*
***Pogonognathellus bidentatus* Folsom, 1913 (TP) Two-toothed Springtail**
Localities: Chattooga Co.: Parkers Cave (GKH119); Dade Co.: Byers Cave (GDD66), Case Cavern (GDD1), Morrison Cave (GDD86); Walker Co.: Bible Springs Cave (GWK74), Ellisons Cave (GWK51), Nash Waterfall Cave (GWK72), Pigeon Cave (GWK57).
Comments: This troglophilic springtail is common in caves in the eastern United States (Christiansen, 1964).

***Pogonognathellus dubius* Christiansen, 1964 (TP) A Springtail**
Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11).
Comments: The taxonomic status of this species is unclear (Felderhoff et al., 2010), but this springtail has been reported from several caves across North America (Christiansen, 1964).

Pogonognathellus flavescens **Tullberg, 1871 (TP) A Springtail**
Localities: Dade Co.: Johnsons Crook Cave (GDD17); Walker Co.: Cave Springs Cave, Horseshoe Cave (GWK12).
Conservation status: IUCN: Not Evaluated; NatureServe: G5? (SNR in Georgia).
Comments: The taxonomic status of this species is unclear (Felderhoff et al., 2010). It is another widely distributed springtail commonly encountered in caves in North America (Christiansen, 1964).

Family Tullbergiidae
Genus *Tullbergia*
Tullbergia iowensis **(Mills, 1932) (TP) A Springtail**
Localities: Dade Co.: Howards Waterfall Cave (GDD34).
Comments: Reeves et al. (2000) collected this springtail from organic debris.

Order Diplura
Family Campodeidae
Genus *Litocampa*
Litocampa cookei **(Packard, 1871) (TB) Cooke's Cave Dipluran**
Localities: Dade Co.: Howards Waterfall Cave (GDD34)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Family Campodeidae
Localities: Bartow Co.: Chert Chasm (GBT340)*; Chattooga Co.: Blowing Springs Cave (GKH54), Subligna Cave (GKH145)*; Dade Co.: Bible Springs Cave (GWK74), Byers Cave (GDD66), Howards Waterfall Cave (GDD34)*, Johnsons Crook Cave No. 2 (GDD17), Limestone Caverns (GDD140)*, Longs Rock Wall Cave (GDD101)*, Morrison Cave (GDD86), Mountain Cove Farm Cave No. 1 (GWK73); Floyd Co.: Airport Cave (GFL189)*; Walker Co.: Anderson Spring Cave (GWK46)*, Cave Springs Cave, Ellisons Cave (GWK51), Fingerhole Cave (GWK259)*, Fricks Cave (GWK14), Goat Cave (GWK184)*, Lofton Cave (GWK281)*, Pettijohns Cave (GWK29)*, Pigeon Cave (GWK57)*, Spooky Cave (GWK494).
Comments: Campodeid diplurans are common in Georgia caves but poorly known. These records likely represent multiple undescribed species. Many records are likely in the genus *Litocampa*.

Family Japygidae
Localities: Chattooga Co.: Blowing Springs Cave (GKH54); Walker Co.: Mountain Cove Farm Cave No. 1 (GWK73).
Comments: This is a poorly-known group with occasional records from caves in the eastern United States.

Class Insecta
Order Coleoptera
Family Cantharidae
Genus *Cantharis*
Cantharis **sp. (TX) A Soldier Beetle**
Localities: Chattooga Co.: Parkers Cave (GKH119); Dade Co.: Quarry Cave; Gordon Co.: Rusty Cable Cave (GGO297)*; Walker Co.: Harrisburg Cave (GWK85), Horseshoe Cave (GWK12), Mt. Cove Farm Cave (GWK73), Pettijohns Cave (GWK29).

Family Carabidae
Genus *Anillinus*
Anillinus **sp. (TB?/ED) A Cave Ground Beetle**
Localities: Dade Co.: Hurricane Cave (GDD62), Morrison Cave (GDD86).
Comments: These small, eyeless carabid beetles occur in deep forest leaf litter and in soil. However, troglobites have been reported from several caves in the eastern United States (Sokolov et al., 2004).

Genus *Atranus*
Atranus pubescens **(Dejean, 1828) (TP) A Ground Beetle**
Localities: Dade Co.: Upper Valley Cave (GDD135); Decatur Co.: Climax Cave (GDC36); Walker Co.: Bible Springs Cave (GWK74).

Genus *Bembidion*
Bembidion lacunarium **(Zimmermann, 1869) (TP) A Ground Beetle**
Localities: Dade Co.: Howards Waterfall Cave (GDD34), Upper Valley Cave (GDD135).

Genus *Elaphropus*
Elaphropus ferrugineus **(Dejean, 1831) (TP) A Ground Beetle**
Localities: Decatur Co.: Climax Cave (GDC36).

Elaphropus **sp. (TX/AC) A Ground Beetle**
Localities: Grady Co.: Glory Hole Cave (GGR56)*.

Genus *Harpalus*
Harpalus pensylvanicus **(De Geer, 1774) (AC) Pennsylvania Dingy Ground Beetle**
Localities: Bartow Co.: Busch Cave (GBT611).

Harpalus **sp. (TX/AC) A Ground Beetle**
Localities: Dade Co.: Morrison Cave (GDD86); Walker Co.: Bible Springs Cave (GWK74).

Genus *Platynus*
Platynus parmarginatus **Hamilton, 1893 (AC) A Ground Beetle**
Localities: Walker Co.: Spooky Cave (GWK494).

Genus *Pseudanophthalmus*
Pseudanophthalmus digitus **Valentine, 1932 (TB) A Cave Beetle**
Localities: Dade Co.: Byers Cave (GDD66), Cemetery Pit (GDD64), Johnsons Crook Cave (GDD17).
Conservation status: IUCN: Not Evaluated; NatureServe: G1G2 (SNR in Georgia).
Comments: This troglobite is also known from Hamilton Co., Tennessee and is a member of the *hirsutus* species group (Barr, 1981, 2004).

Pseudanophthalmus fastigatus **Barr, 1981 (TB) Tapered Cave Beetle**
Localities: Walker Co.: Horseshoe Cave (GWK12).
Conservation status: IUCN: Not Evaluated; NatureServe: G1 (S1? in Georgia).
Comments: This species is only known from the type locality east of Lookout Mountain. It is a member of the *engelhardti* species group (Barr, 1981, 2004).

Pseudanophthalmus fulleri **Valentine, 1932 (TB) Fuller's Cave Beetle**
Localities: Dade Co.: Boxcar Cave (GDD69)*, Byers Cave (GDD66), Caboose Cave (GDD475)*, Cemetery Pit (GDD64), Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62)*, Johnsons Crook Cave (GDD17), Lower Valley Cave (GDD136)*, Morrison Cave (GDD86), Sittons Cave (GDD9), SSS Cave (GDD229)*, Upper Valley Cave (GDD135).
Conservation status: IUCN: Not Evaluated; NatureServe: G2G3 (SNR in Georgia).
Comments: This species is a member of the *engelhardti* species group (Barr, 1981, 2004).

Pseudanophthalmus georgiae **Barr, 1981 (TB) Georgian Cave Beetle**
Localities: Chattooga Co.: Chelsea Gulf Cave (GKH54); Walker Co.: Ellisons Cave (GWK51), Mountain Cove Farm Cave No. 1 (GWK73), Pettijohns Cave (GWK29).
Conservation status: IUCN: Not Evaluated; NatureServe: G1G2 (S1? in Georgia).
Comments: This troglobite is a Georgia endemic and a member of the *alabamae* species group (Barr, 1981, 2004).

Pseudanophthalmus **sp. (TB)**
Localities: Chattooga Co.: Parkers Cave (GKH119)*; Walker Co.: Four Kings Cave (GWK77)*.
Comments: The Parkers Cave record was reported as *P. fulleri*, but as all other records for this species are west of Lookout Mountain, this record likely represents *P. georgiae* or *P. fastigatus* instead. The Four Kings Cave record likely represents *P. georgiae*.

Genus *Pterostichus*
Pterostichus relictus **(Newman, 1838) (TX) A Ground Beetle**
Localities: Dade Co.: Upper Valley Cave (GDD135).

Genus *Rhadine*
***Rhadine caudata* LeConte, 1863 (TP) A Ground Beetle**
Localities: Dade Co.: Longs Rock Wall Cave (GDD101)*, Rusty's Cave (GDD70)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G3 (SNR in Georgia).

***Rhadine larvalis* LeConte, 1846 (TP) A Ground Beetle**
Localities: Dade Co.: Byers Cave (GDD66).

Genus *Sphaeroderus*
***Sphaeroderus stenostomus* (Weber, 1801) (TX) A Ground Beetle**
Localities: Dade Co.: Howards Waterfall Cave (GDD34).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Genus *Tachys*
***Tachys* sp. (TX) A Ground Beetle**
Localities: Grady Co.: Maloys Waterfall Cave (GGR27).

Family Histeridae
Genus *Margarinotus*
***Margarinotus egregius* (Casey, 1916) (AC) A Clown Beetle**
Localities: Walker Co.: Spooky Cave (GWK494).

Family Leiodidae
Genus *Catops*
***Catops gratiosus* (Blanchard, 1915) (TP/TX) Round Fungus Beetle**
Localities: Chattooga Co.: Parkers Cave (GKH119); Dade Co.: Johnsons Crook Cave (GDD17), Morrison Cave (GDD86), Johnsons Crook Cave No. 2 (GDD19); Walker Co.: Horseshoe Cave (GWK12), Mountain Cove Farm Cave No. 1 (GWK73).

Genus *Nemadus*
***Nemadus hornii* Hatch, 1933 (TP/TX) A Carrion Beetle**
Localities: Dade Co.: Johnsons Crook Cave (GDD17), Johnsons Crook Cave No. 2 (GDD19); Decatur Co.: Climax Cave (GDC36); Walker Co.: Rocky Cave (GWK496).
Conservation status: IUCN: Not Evaluated; NatureServe: G1 (SNR in Georgia).

***Nemadus* sp. (TP/TX) A Carrion Beetle**
Localities: Dade Co.: Morrison Cave (GDD86); Walker Co.: Cave Springs Cave, Horseshoe Cave (GWK12), Mountain Cove Farm Cave No. 1 (GWK73).

Genus *Prionochaeta*
***Prionochaeta opaca* (Say, 1825) (TP/TX) A Carrion Beetle**
Localities: Chattooga Co.: Blowing Springs Cave (GKH54); Walker Co.: Bible Springs Cave (GWK74), Cave Springs Cave, Horseshoe Cave (GWK12), Mountain Cove Farm Cave No. 1 (GWK73).
Conservation status: IUCN: Not Evaluated; NatureServe: G4 (SNR in Georgia).

Genus *Ptomaphagus*
***Ptomaphagus cavernicola* Schwarz, 1898 (TP) A Fungus Beetle**
Localities: Decatur Co.: Climax Cave (GDC36); Grady Co.: Maloys Waterfall Cave (GGR27).
Conservation status: IUCN: Not Evaluated; NatureServe: G4 (SNR in Georgia).
Comments: This species has well-developed eyes and functional flight wings. It ranges from Mexico to Texas, the Ozarks, and the southeastern United States. It has been collected in forests and caves in southwestern Georgia (Peck, 1973, 1982).

***Ptomaphagus fiskei* Peck, 1973 (TB) A Cave Fungus Beetle**
Localities: Walker Co.: Anderson Spring Cave (GWK46), Ellisons Cave (GWK51), Fingerhole Cave (GWK259)*, Kinda Pretty Cave (GWK258)*, Missing Evan Well Cave (GWK488)*, Mountain Cove Farm Cave No. 1 (GWK73), Mountain Cove Farm Cave No. 2 (GWK74), Pettijohns Cave (GWK29), Pigeon Cave (GWK57), Spooky Too Cave (GWK496).
Conservation status: IUCN: Not Evaluated; NatureServe: G1G2 (SNR in Georgia).
Comments: This troglobite is endemic to Georgia. It has greatly reduced eyes and lacks flight wings. It is the only troglobitic *Ptomaphagus* known from east of Lookout Mountain in Georgia and is limited to caves along Lookout Mountain and Pigeon Mountain in Walker County (Peck, 1973; Leray et al., 2019).

***Ptomaphagus whiteselli* Barr, 1963 (TB) A Cave Fungus Beetle**
Localities: Dade Co.: Byers Cave (GDD66), Case Cavern (GDD1), Cemetery Pit (GDD64), Hurricane Cave (GDD62), Limestone Caverns (GDD140), Morrison Cave (GDD86), Rusty's Cave (GDD70), Sittons Cave (GDD9).
Conservation status: IUCN: Not Evaluated; NatureServe: G2G3 (SNR in Georgia).
Comments: This species has greatly reduced eyes and lacks flight wings. It is limited to caves in Lookout Valley in Dade County and adjacent DeKalb Co., Alabama (Peck, 1973; Leray et al., 2019).

***Ptomaphagus* sp. (TB) A Cave Fungus Beetle**
Localities: Dade Co.: Johnsons Crook Cave No. 2 (GDD19), Morrison Cave (GDD86); Walker Co.: Smartt Farm Cave (GWK124)*.
Comments: The records from Dade Co. are likely *P. whiteselli*; the record from Walker Co. is likely *P. fiskei*.

Genus *Sciodrepoides*
***Sciodrepoides terminans* (LeConte, 1850) (TX/AC) A Fungus Beetle**
Localities: Walker Co.: Cave Springs Cave.
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Family Staphylinidae
Genus *Atheta*
***Atheta annexa* Casey, 1910 (TP) A Rove Beetle**
Localities: Bartow Co.: Yarbrough Cave (GBT30); Dade Co.: Morrison Cave (GDD86); Decatur Co.: Climax Cave (GDC36); Grady Co.: Maloys Waterfall Cave (GGR27); Walker Co.: Chickamagua Cave Spring Cave, Horseshoe Cave (GWK12), Mountain Cove Cave (GDD64).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

***Atheta klagesi* Bernhauer, 1909 (TP) A Rove Beetle**
Localities: Dade Co.: Byers Cave (GDD66), Howards Waterfall Cave (GDD34).

***Atheta lucifuga* Klimaszewski and Peck, 1986 (TP) Light Shunning Rove Beetle**
Localities: Walker Co.: Mountain Cove Cave (GDD64).
Conservation status: IUCN: Not Evaluated; NatureServe: G4 (SNR in Georgia).

***Atheta troglophila* Klimaszewski and Peck, 1986 (TP) A Rove Beetle**
Localities: Dade Co.: Byers Cave (GDD66), Howards Waterfall Cave (GDD34); Walker Co.: Mountain Cove Cave (GDD64).
Conservation status: IUCN: Not Evaluated; NatureServe: G4 (SNR in Georgia).

***Atheta* sp. (TP) A Rove Beetle**
Localities: Chattooga Co.: Blowing Springs Cave (GKH54), Parkers Cave (GKH119); Dade Co.: Byers Cave (GDD66), Johnsons Crook Cave (GDD17), Morrison Cave (GDD86); Walker Co.: Bible Springs Cave (GWK74), Cave Springs Cave, Horseshoe Cave (GWK12), Mountain Cove Farm Cave No. 1 (GWK73), Pettijohns Cave (GWK29).

Genus *Batriasymmodes*
***Batriasymmodes spelaeus* (Park, 1951) (TB) A Cave Ant-loving Beetle**
Localities: Chattooga Co.: Blowing Springs Cave (GKH54), Chelsea Gulf Cave (GKH54); Dade Co.: Kirchmeyer Cave (GDD196)*; Walker Co.: Mountain Cove Farm Cave No. 2 (GWK74).
Conservation status: IUCN: Not Evaluated; NatureServe: G3G4 (SNR in Georgia).
Comments: This species is also known from caves in northeastern Alabama and central and eastern Tennessee. Although eyed, it was considered a troglobiont by Park (1960) and others, but a troglophile by Holsinger and Peck (1971).

***Batriasymmodes* sp. (TB/TP) An Ant-loving Beetle**
Localities: Dade Co.: Howards Waterfall Cave (GDD34).

Genus *Batrisodes*
***Batrisodes lineaticollis* (Aubé, 1833) (TP) An Ant-loving Beetle**
Localities: Decatur Co.: Climax Cave (GDC36).
Comments: This species is widely distributed in eastern North America.

***Batrisodes* sp. (TP/TX) An Ant-loving Beetle**
Localities: Dade Co.: Howards Waterfall Cave (GDD34)*, Limestone Caverns (GDD140)*; Walker Co.: Pigeon Cave (GWK57).

Genus *Creophilus*
***Creophilus maxillosus* (Linnaeus, 1758) (TP) Hairy Rove Beetle**
Localities: Dade Co.: Howards Waterfall Cave (GDD34).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Genus *Geodromicus*
***Geodromicus brunneus* (Say, 1823) (TX/AC) A Rove Beetle**
Localities: Walker Co.: Bible Springs Cave (GWK74), Mountain Cove Farm Cave No. 1 (GWK73).

Genus *Lesteva*
***Lesteva pallipes* LeConte, 1863 (TP) A Rove Beetle**
Localities: Bartow Co.: Chert Chasm (GBT340); Chattooga Co.: Blowing Springs Cave (GKH54); Dade Co.: Hurricane Cave (GDD62)l Walker Co.: Bible Springs Cave (GWK74), Mountain Cove Farm Cave No. 1 (GWK73), Rocky Cave (GWK496).

***Lesteva* sp. (TX) A Rove Beetle**
Localities: Dade Co.: Byers Cave (GDD66).

Genus *Oxypoda*
***Oxypoda* sp. (TX/AC) A Rove Beetle**
Localities: Bartow Co.: Yarbrough Cave (GBT30); Dade Co.: Byers Cave (GDD66).

Genus *Philonthus*
***Philonthus cyanipennis* (Fabricius, 1792) (AC) A Rove Beetle**
Localities: Walker Co.: Pettijohns Cave (GWK29).

***Philonthus* sp. (AC) A Rove Beetle**
Localities: Grady Co.: Maloys Waterfall Cave (GGR27); Walker Co.: Bible Springs Cave (GWK74).

Genus *Quedius*
***Quedius erythrogaster* Mannerheim, 1852 (TP) A Rove Beetle**
Localities: Dade Co.: Morrison Cave (GDD86); Walker Co.: Harrisburg Cave (GWK85), Hickman Gulf Cave, Pettijohns Cave (GWK29).

***Quedius fulgidus* (Fabricius, 1793) (TP) A Rove Beetle**
Localities: Polk Co.: White River Cave (GPO7).

***Quedius* sp. (TP) A Rove Beetle**
Localities: Dade Co.: Howards Waterfall Cave (GDD34).

Genus *Sepedophilus*
***Sepedophilus littoreus* (Linnaeus, 1758) (TP) A Rove Beetle**
Localities: Walker Co.: Horseshoe Cave (GWK12).

Genus *Speleochus*
***Speleochus* sp. (TB) A Cave Rove Beetle**
Localities: Dade Co.: Limestone Caverns (GDD140)*; Walker Co.: Pigeon Cave (GWK57).

Genus *Subterrochus*
***Subterrochus* sp. (TB) A Cave Rove Beetle**
Localities: Walker Co.: Mountain Cove Farm Cave No. 1 (GWK73).

Genus *Tachinus*
***Tachinus fimbriatus* Gravenhorst, 1802 (TX/AC) A Rove Beetle**
Localities: Walker Co.: Pettijohns Cave (GWK29).

Genus *Xenota*
***Xenota* sp. (TP/TX) A Rove Beetle**
Localities: Dade Co.: Deans Pit (GDD273), Johnsons Crook Cave (GDD17); Walker Co.: Horseshoe Cave (GWK12), Pettijohns Cave (GWK29).

Family Trogidae
Genus *Trox*
***Trox aequalis* Say, 1832 (TX) A Hide Beetle**
Localities: Walker Co.: Fricks Cave (GWK14).

Order Diptera
Family Calliphoridae
Genus *Calliphora*
***Calliphora vicina* Robineau-Desvoidy, 1830 (TX) Blue Blow Fly**
Localities: Dade Co.: Deans Pit (GDD273), Howards Waterfall Cave (GDD34); Walker Co.: Horseshoe Cave (GWK12).

***Calliphora vomitoria* (Linnaeus, 1758) (TX) Blue Bottle Fly**
Localities: Dade Co.: Byers Cave (GDD66), Howards Waterfall Cave (GDD34); Walker Co.: Harrisburg Cave (GWK85).

Genus *Lucilia*
***Lucilia* sp. (TX/AC) A Blow Fly**
Localities: Dade Co.: Howards Waterfall Cave (GDD34).

Family Cecidomyiidae
Genus *Bremia*
***Bremia* sp. (TX/AC) A Gall Midge**
Localities: Dade Co.: Sittons Cave (GDD9).

Family Chironomidae
Genus *Chironomus*
***Chironomus decorus* Johannsen, 1905 (AC) A Non-biting Midge**
Localities: Washington Co.: Tennile Caves (GWS20).

Genus *Procladius*
***Procladius bellus* (Loew, 1866) (TX) A Midge**
Localities: Bartow Co.: Busch Cave (GBT611).

Genus *Tanytarsus*
***Tanytarsus* sp. (TX) A Non-biting Midge**
Localities: Bartow Co.: Busch Cave (GBT611).
Comments: This record was identified as *Tanytarsus* nr. *recurvatus* by Reeves et al. (2000).

Family Culicidae
Genus *Anopheles*
***Anopheles punctipennis* (Say, 1823) (TX) Spot-winged Malaria Mosquito**
Localities: Dade Co.: Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62); Walker Co.: Fricks Cave (GWK14), Horseshoe Cave (GWK12).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Genus *Culex*
***Culex territans* Walker, 1856 (TX) Northern Frog-biting Mosquito**
Localities: Walker Co.: Horseshoe Cave (GWK12).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

***Culex* sp. (TX) A Mosquito**
Localities: Dade Co.: SSS Cave (GDD229)*.

Family Dolichopodidae
Genus *Lianculus*
***Liancalus genualis* Loew, 1861 (TX) A Long-legged Fly**
Localities: Bartow Co.: Yarbrough Cave (GBT30).

Genus *Neurigonella*
***Neurigonella sombrea* (Harmston and Knowlton, 1945) (TX/AC) A Long-legged Fly**
Localities: Dade Co.: Upper Valley Cave (GDD135).

Family Drosophilidae
Genus *Drosophila*
***Drosophila* sp. (TX/AC) A Fruit Fly**

Localities: Dade Co.: Howards Waterfall Cave (GDD34).

Family Heleomyzidae
Genus *Amoebaleria*
***Amoebaleria defessa* (Osten-Sacken, 1877) (TX) A Sun Fly**

Localities: Bartow Co.: Busch Cave (GBT611), Kingston Saltpeter Cave (GBT11); Catoosa Co.: Crane Cave (GCZ80)*; Chattooga Co.: Parkers Cave (GKH119)*, Scoggins II Cave (GKH405)*; Dade Co.: Byers Cave (GDD66), Caboose Cave (GDD475)*, Cemetery Pit (GDD64), Hooker Cave (GDD90)*, Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62), Johnsons Crook Cave (GDD17)*, Kirchmeyer Cave (GDD196)*, Limestone Caverns (GDD140)*, Longs Rock Wall Cave (GDD101)*, Lower Valley Cave (GDD136)*, Morrison Cave (GDD86), Running Water Cave (GDD120), Rusty's Cave (GDD70), Sittons Cave (GDD9), SSS Cave (GDD229)*, Upper Valley Cave (GDD135), Wild Bills Dakota Cave (GDD596)*; Floyd Co.: Airport Cave (GFL189)*, Cave Springs Cave (GFL18)*; Polk Co.: White River Cave (GPO7)*; Walker Co.: Anderson Spring Cave (GWK46), Bee Rock Cave (GWK123)*, Ellisons Cave (GWK51), Harrisburg Cave (GWK85), Horseshoe Cave (GWK12), LittleJohn Cave (GWK280)*, Mountain Cove Farm Cave No. 1 (GWK73), Pettijohns Cave (GWK29), Screech Owl Cave (GWK205)*, Smartt Farm Cave (GWK124)*.

Comments: This sunfly is common in caves of the eastern United States (e.g., Peck, 1995; Reeves et al., 2000; Lewis, 2005).

Genus *Heleomyza*
***Heleomyza brachypterna* (Loew, 1873) (TX) A Sun Fly**

Localities: Walker Co.: Harrisburg Cave (GWK85), Mountain Cove Farm Cave No. 1 (GWK73).

Genus *Oecothea*
***Oecothea specus* (Aldrich, 1897) (TX) A Sun Fly**

Localities: Bartow Co.: Busch Cave (GBT611), Kingston Saltpeter Cave (GBT11); Catoosa Co.: Chapmans Cave (GCZ25)*; Chattooga Co.: Parkers Cave (GKH119)*, Scoggins II Cave (GKH405)*; Dade Co.: Hooker Cave (GDD90)*, Howards Waterfall Cave (GDD34)*, Johnsons Crook Cave (GDD17), Limestone Caverns (GDD140)*, Longs Rock Wall Cave (GDD101)*, Sittons Cave (GDD9), SSS Cave (GDD229)*, Wild Bills Dakota Cave (GDD596)*; Gordon Co.: Jack Crider Cave (GGO298)*; Polk Co.: White River Cave (GPO7); Walker Co.: Bible Springs Cave (GWK74), Cave Springs Cave, Horseshoe Cave (GWK12), LittleJohn Cave (GWK280)*, Lofton Cave (GWK281)*, Mountain Cove Farm Cave No. 1 (GWK73)*, Mountain Cove Farm Cave No. 2 (GWK74), Smartt Farm Cave (GWK124)*.

Comments: Like *Amoebaleria defessa*, this species is also common in caves of the eastern United States (e.g., Peck, 1995; Reeves et al., 2000; Lewis, 2005).

Family Muscidae
Genus *Chaetogenia*
***Chaetogenia* sp. (TX/AC) A House Fly**

Localities: Bartow Co.: Yarbrough Cave (GBT30).

Genus *Muscina*
***Muscina prolapsa* (Harris, 1780) (TX) A House Fly**

Localities: Dade Co.: Sittons Cave (GDD9); Walker Co.: Horseshoe Cave (GWK12).

Family Mycetophilidae
Genus *Leia*
***Leia* sp. (TP/TX) A Fungus Gnat**

Localities: Dade Co.: Byers Cave (GDD66), Howards Waterfall Cave (GDD34).

Genus *Rymosa*
***Rymosa* sp. (TP/TX) A Fungus Gnat**

Localities: Dade Co.: Sittons Cave (GDD9).

Family Phoridae
Genus *Megaselia*
***Megaselia breviterga* (Lundback, 1921) (TX) A Scuttle Fly**

Localities: Dade Co.: Byers Cave (GDD66), Deans Pit (GDD273), Howards Waterfall Cave (GDD34), Rock Shelter Pit (GDD209); Walker Co.: Harrisburg Cave (GWK85), Horseshoe Cave (GWK12), Missing Evan Well Cave (GWK488)*, Pettijohns Cave (GWK29).

Comments: This species was collected in large numbers at baited traps near cave entrances in northwestern Georgia (Campbell et al., 2011; Disney and Campbell, 2011). Disney and Campbell (2011) indicate *M. spelunciphila* is a synonym for *M. breviterga*.

***Megaselia cavernicola* (Brues, 1906) (TP) Cave Scuttle Fly**

Localities: Dade Co.: Byers Cave (GDD66), Howards Waterfall Cave (GDD34), Johnsons Crook Cave (GDD17), Johnsons Crook Cave No. 2 (GDD19); Gordon Co.: Rusty Cable Cave (GGO297)*; Walker Co.: Anderson Spring Cave (GWK46), Harrisburg Cave (GWK85), Horseshoe Cave (GWK12), Pettijohns Cave (GWK29).

Comments: This species was collected in large numbers at baited traps in caves in northwestern Georgia (Campbell et al., 2011; Disney and Campbell, 2011). When comparing *M. breviterga*, *M. cavernicola*, and *M. taylori*, Disney and Campbell (2011) noted that *M. cavernicola* was more common further from cave entrances than the other two species.

***Megaselia taylori* Disney, 2010 (TX) A Scuttle Fly**

Localities: Dade Co.: Byers Cave (GDD66), Howards Waterfall Cave (GDD34); Walker Co.: Harrisburg Cave (GWK85), Pettijohns Cave (GWK29).

Comments: This species was collected in large numbers at baited traps near cave entrances in northwestern Georgia (Campbell et al., 2011; Disney and Campbell, 2011).

***Megaselia* sp. (TP/TX) A Scuttle Fly**

Localities: Bartow Co.: Yarbrough Cave (GBT30); Chattooga Co.: Blowing Springs Cave (GKH54), Parkers Cave (GKH119); Dade Co.: Morrison Cave (GDD86); Polk Co.: White River Cave (GPO7); Walker Co.: Cave Springs Cave, Mountain Cove Farm Cave No. 1 (GWK73).

Genus *Puliciphora*
***Puliciphora virginiensis* Malloch, 1912 (TP) A Scuttle Fly**

Localities: Walker Co.: Horseshoe Cave (GWK12).

Family Psychodidae
Genus *Psychoda*
***Psychoda pusilla* Tonnoir, 1922 (TP) A Moth Fly**

Localities: Walker Co.: Horseshoe Cave (GWK12).

***Psychoda reevesi* Quate, 2000 (TP) Reeves' Moth Fly**

Localities: Dade Co.: Johnsons Crook Cave No. 2 (GDD19).

***Psychoda* sp. (TP) A Moth Fly**

Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11); Dade Co.: Byers Cave (GDD66), Howards Waterfall Cave (GDD34); Walker Co.: Harrisburg Cave (GWK85), Pettijohns Cave (GWK29).

Family Sciaridae
Genus *Bradysia*
***Bradysia forficulata* (Bezzi, 1914) (TP/TX) A Fungus Gnat**

Localities: Dade Co.: Johnsons Crook Cave (GDD17)*, Johnsons Crook Cave No. 2 (GDD19).

***Bradysia* sp. (TP/TX) A Fungus Gnat**

Localities: Dade Co.: Howards Waterfall Cave (GDD34); Walker Co.: Harrisburg Cave (GWK85).

Genus *Corynoptera*
***Corynoptera* sp. (TP/TX) A Fungus Gnat**

Localities: Chattooga Co.: Parkers Cave (GKH119)*; Dade Co.: Upper Valley Cave (GDD135); Walker Co.: Horseshoe Cave (GWK12).

Genus *Lycoriella*
***Lycoriella* sp. (TP/TX) A Fungus Gnat**

Localities: Bartow Co.: Anthonys Cave (GBT175); Dade Co.: Deans Pit (GDD273), Johnsons Crook Cave No. 2 (GDD19); Walker Co.: Horseshoe Cave (GWK12), Pettijohns Cave (GWK29).

Genus *Sciara*
***Sciara* sp. (TP/TX) A Fungus Gnat**

Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11); Dade Co.: Byers Cave (GDD66), Johnsons Crook Cave (GDD17); Polk Co.: White River Cave (GPO7); Walker Co.: Mountain Cove Farm Cave No. 1 (GWK73).

Family Simuliidae
Genus *Prosimulium*
***Prosimulium saltus* Stone and Jamnback, 1955 (TX) A Black Fly**
Localities: Dade Co.: Johnsons Crook Cave No. 2 (GDD19).

Genus *Simulium*
***Simulium parnassum* Malloch, 1914 (TX) Dark Black Fly**
Localities: Dade Co.: Johnsons Crook Cave No. 2 (GDD19).

Family Sphaeroceridae
Genus *Leptocera*
***Leptocera caenosa* (Rondani, 1880) (TP) A Lesser Dung Fly**
Localities: Dade Co.: Howards Waterfall Cave (GDD34)*, Johnsons Crook Cave No. 2 (GDD19); Walker Co.: Fricks Cave (GWK14)*, Pettijohns Cave (GWK29).

***Leptocera* sp. (TP/TX) A Lesser Dung Fly**
Localities: Chattooga Co.: Blowing Springs Cave (GKH54); Dade Co.: Byers Cave (GDD66), Johnsons Crook Cave (GDD17); Walker Co.: Bible Springs Cave (GWK74), Mountain Cove Farm Cave No. 1 (GWK73).

Genus *Spelobia*
***Spelobia tenebrarum* (Aldrich, 1897) (TB) Cave Dung Fly**
Localities: Chattooga Co.: Chelsea Gulf Cave (GKH54); Dade Co.: Howards Waterfall Cave (GDD34), Johnsons Crook Cave (GDD17), Limestone Caverns (GDD140)*, Rising Fawn Exit Cave (GDD397), SSS Cave (GDD229)*, Wild Bills Dakota Cave (GDD596)*; Walker Co.: Horseshoe Cave (GWK12)*, Mountain Cove Farm Cave No. 1 (GWK73), Mountain Cove Farm Cave No. 2 (GWK74)*, Pettijohns Cave (GWK29).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This dung fly is common on scat in caves across the southern Appalachians and Interior Low Plateau. Eyes are present but reduced in size relative to surface species of *Spelobia* (Marshall and Peck, 1985a, 1985b).

Family Syrphidae
Genus *Copestylum*
***Copestylum vesicularium* (Curran, 1947) (TX/AC) Irridescent Bromeliad Fly**
Localities: Grady Co.: Maloys Waterfall Cave (GGR27).

Family Tipulidae
Genus *Dolichopeza*
***Dolichopeza tridenticulata* Alexander, 1931 (TX) A Crane Fly**
Localities: Dade Co.: Sittons Cave (GDD9).

***Dolichopeza walleyi* (Johnson, 1931) (TX) A Crane Fly**
Localities: Bartow Co.: Anthonys Cave (GBT175).

Genus *Tipula*
***Tipula abdominalis* (Say, 1823) (TX) Giant Crane Fly**
Localities: Walker Co.: Ellisons Cave (GWK51).

Family Trichoceridae
Genus *Trichocera*
***Trichocera fattigiana* Alexander, 1952 (TX) A Winter Crane Fly**
Localities: Dade Co.: Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62); Walker Co.: Anderson Spring Cave (GWK46).

***Trichocera* sp. (TX) A Winter Crane Fly**
Localities: Walker Co.: Horseshoe Cave (GWK12).

Order Hemiptera
Family Cicadidae
Genus *Magicicada*
***Magicicada* sp. (AC) A Periodical Cicada**
Localities: Walker Co.: Horseshoe Cave (GWK12).
Comments: This genus is common in the southeastern USA, where it is edaphic as a nymph; this record represents a surface species.

Family Veliidae
Genus *Microvelia*
***Microvelia americana* (Uhler, 1884) (AC) A Water Strider**
Localities: Dade Co.: Howards Waterfall Cave (GDD34).

Order Hymenoptera
Family Braconidae
Genus *Aspilota*
***Aspilota* sp. (TX/AC) A Parasitoid Wasp**
Localities: Bartow Co.: Yarbrough Cave (GBT30); Dade Co.: Byers Cave (GDD66), Deans Pit (GDD273), Howards Waterfall Cave (GDD34), Johnsons Crook Cave (GDD17), Rock Shelter Pit (GDD209), Sittons Cave (GDD9), Upper Valley Cave (GDD135); Walker Co.: Horseshoe Cave (GWK12), Mountain Cove Farm Cave No. 1 (GWK73).

Family Formicidae
Genus *Myrmecina*
***Myrmecina americana* Emery, 1895 (TX) American Little Ant**
Localities: Dade Co.: Johnsons Crook Cave No. 2 (GDD19).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Order Lepidoptera
Family Erebidae
Genus *Scoliopteryx*
***Scoliopteryx libatrix* (Linnaeus, 1758) (TX) Herald Moth**
Localities: Dade Co.: Howards Waterfall Cave (GDD34), Johnsons Crook Cave (GDD17), Johnsons Crook Cave No. 2 (GDD19); Walker Co.: Anderson Spring Cave (GWK46)*, Horseshoe Cave (GWK12).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This moth commonly overwinters in caves in the eastern United States.

Family Noctuidae
Genus *Lophoterges*
***Lophoterges* sp. (TX/AC) An Owlet Moth**
Localities: Walker Co.: Fricks Cave (GWK14)*.

Order Megaloptera
Family Corydalidae
Genus *Corydalus*
***Corydalus cornutus* ((Linnaeus, 1758) (AC) Eastern Dobsonfly**
Localities: DeKalb Co.: Nice Gneiss Cave (GDK329)*.

Order Odonata
Family Cordulegastridae
Genus *Cordulegaster*
***Cordulegaster* sp. (AC) A Goldenring Dragonfly**
Localities: Washington Co.: Tennile Caves (GWS20).

Family Gomphidae
Genus *Progomphus*
***Progomphus obscurus* (Rambur, 1842) (AC) Common Sanddragon**
Localities: Washington Co.: Tennile Caves (GWS20).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Order Orthoptera
Family Gryllidae
Genus *Eunemobius*
***Eunemobius* sp. (TX/AC) A Ground Cricket**
Localities: Chattooga Co.: Subligna Cave (GKH145)*.

Family Rhaphidophoridae
Genus *Ceuthophilus*
***Ceuthophilus ensifer* Packer, 1881 (TX) A Camel Cricket**
Localities: Dade Co.: Byers Cave (GDD66), Howards Waterfall Cave (GDD34), Johnsons Crook Cave (GDD17), Morrison Cave (GDD86), Morrison Spring Cave (GDD110).
Comments: This camel cricket is a forest species that has been collected in a few caves (Hubbell, 1936; Lewis, 2005). Within Georgia, it is apparently limited to Dade County.

***Ceuthophilus gracilipes* (Haldeman, 1850) (TX) Slender-legged Camel Cricket**

Localities: Bartow Co.: Chert Chasm (GBT340)*, Davis Farm Cave (GBT222)*, Yarbrough Cave (GBT30); Dade Co.: Boxcar Cave (GDD69)*, Byers Cave (GDD66), Caboose Cave (GDD475)*, Case Cavern (GDD1), Hooker Cave (GDD90)*, Morrison Cave (GDD86), Morrison Spring Cave (GDD110), Sittons Cave (GDD9), Wild Bills Dakota Cave (GDD596)*; Gordon Co.: Jack Crider Cave (GGO298)*, Roberts Cave (GGO147), Rusty Cable Cave (GGO297)*, Steep Cave (GGO326)*; Walker Co.: Anderson Spring Cave (GWK46), Bible Springs Cave (GWK74), Ellisons Cave (GWK51), Fingerhole Cave (GWK259)*, Fricks Cave (GWK14), LittleJohn Cave (GWK280)*, Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29), Pigeon Cave (GWK57), Rocky Cave (GWK496)*, Smartt Farm Cave (GWK124)*.

Comments: This camel cricket is a forest species that enters caves. It ranges from New York to Florida (Hubbell, 1936).

***Ceuthophilus* sp. (TX) A Cave Cricket**

Localities: Walker Co.: Mountain Cove Farm Cave No. 2 (GWK74)*.

Comments: This record may be *C. ensifer* or *C. gracilipes*.

Genus *Diestrammena*

***Diestrammena asynamora* Adelung, 1902 (TX) Greenhouse Camel Cricket**

Localities: Catoosa Co.: Chapmans Cave (GCZ25)*.

Comments: This species was introduced from Asia and recently reported to be common in and around homes in the eastern United States (Epps et al., 2014). This is the first report of the species in a cave in North America. Chapmans Cave is <100 m from homes in a housing development, which may explain the presence of these crickets in the cave. Lavoie et al. (2019) reported an unknown cricket species with affinities to *Diestrammena* from a cave in Pennsylvania, which highlights the need for monitoring of cricket populations to identify the spread of exotic species into cave habitats.

Genus *Euhadenoecus*

***Euhadenoecus puteanus* (Scudder, 1877) (TX) Puteanus Camel Cricket**

Localities: Bartow Co.: Davis Farm Cave (GBT222)*; Dade Co.: Boxcar Cave (GDD69)*, Byers Cave (GDD66), Caboose Cave (GDD475)*, Case Cavern (GDD1), Hooker Cave (GDD90)*, Howards Waterfall Cave (GDD34), Johnsons Crook Cave (GDD17), Morrison Spring Cave (GDD110), Sittons Cave (GDD9), SSS Cave (GDD229)*, Wild Bills Dakota Cave (GDD596)*; Gordon Co.: Jack Crider Cave (GGO298)*, Roberts Cave (GGO147), Rusty Cable Cave (GGO297)*; Polk Co.: White River Cave (GPO7); Walker Co.: Anderson Spring Cave (GWK46), Bible Springs Cave (GWK74), Cherokee Cave (GWK94), Ellisons Cave (GWK51), Fingerhole Cave (GWK259)*, Fricks Cave (GWK14), Mountain Cove Farm Cave No. 1 (GWK73), Pigeon Cave (GWK57).

Comments: This camel cricket is widespread across the Appalachians and portions of the Interior Low Plateau. It is a surface species that enters caves but generally does not penetrate to deep cave environments (Hubbell and Norton, 1978).

Order Psocodea
Family Liposcelididae
Genus *Liposcelis*
***Liposcelis decolor* (Pearman, 1925) (TP) A Booklouse**

Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11); Walker Co.: Ellisons Cave (GWK51).

Family Psyllipsocidae
Genus *Psyllipsocus*
***Psyllipsocus ramburii* Selys-Longchamps, 1872 (TP) A Barklouse**

Localities: Bartow Co.: Yarbrough Cave (GBT30)*; Walker Co.: Cave Springs Cave, Harrisburg Cave (GWK85).

Order Siphonaptera
Family Hystrichopsyllidae
Genus *Ctenophthalmus*
***Ctenophthalmus pseudagyrtes* Baker, 1904 (SY) A Flea**

Localities: Walker Co.: Pettijohns Cave (GWK29).

Order Trichoptera
Family Hydropsychidae
Genus *Diplectrona*
***Diplectrona marianae* Reeves, 1999 (TX) A Caddisfly**

Localities: Dade Co.: Johnsons Crook Cave No. 2 (GDD19).

Conservation status: IUCN: Not Evaluated; NatureServe: G1 (SNR in Georgia).

Comments: This species was described in Reeves and Paysen (1999); it is endemic to Georgia and known only from the type locality, which was reported with the alternate name "Newsome Gap Spring Cave" (Reeves and Paysen 1999).

Order Zygentoma
Family Nicoletiidae
Genus *Nicoletia*
***Nicoletia* sp. (ED) A Silverfish**

Localities: Walker Co.: Horseshoe Cave (GWK12).

Comments: Holsinger and Peck (1971) suggested this may be an undescribed edaphic species.

Subphylum Myriapoda
Class Chilopoda
Order Geophilomorpha
Family Geophilidae
Genus *Arenophilus*
***Arenophilus bipuncticeps* Wood, 1862 (TX/AC) Northern Short-clawed Centipede**

Localities: Chattooga Co.: Blowing Springs Cave (GKH54).

Order Lithobiomorpha
Family Lithobiidae
Genus *Lithobius*
***Lithobius atkinsoni* Bollman, 1887 (TP) A Centipede**

Localities: Randolph Co.: Griers Cave (GRA40).

Genus *Neolithobius*
***Neolithobius voracior* Chamberlin, 1912 (TP) A Centipede**

Localities: Decatur Co.: Climax Cave (GDC36).

Genus *Paitobius*
***Paitobius* sp. (TX/AC) A Centipede**

Localities: Dade Co.: Morrison Spring Cave (GDD110).

Genus *Pampibius*
***Pampibius* sp. (TX/AC) A Centipede**

Localities: Walker Co.: Cave Springs Cave.

Genus *Typhlobius*
***Typhlobius caecus* Bollman, 1888 (TX/AC) A Centipede**

Localities: Walker Co.: Fricks Cave (GWK14).

Order Scolopendromorpha
Family Cryptopidae
Genus *Scolopocryptops*
***Scolopocryptops sexspinosus* (Say, 1821) (TX/AC) A Centipede**

Localities: Dade Co.: Johnsons Crook Cave (GDD17), Morrison Cave (GDD86); Walker Co.: Pettijohns Cave (GWK29).

Class Diplopoda
Order Callipodida
Family Abacionidae
Genus *Abacion*
***Abacion magnum* (Loomis, 1943) (TX) A Crested Millipede**

Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11); Davis Farm Cave (GBT222)*; Chattooga Co.: Blowing Springs Cave (GKH54); Dade Co.: Byers Cave (GDD66); Polk Co.: White River Cave (GPO7).

Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Comments: The record from Kingston Saltpeter Cave was reported as *A. lactarium* (Chamberlin, 1946), a species known from the Piedmont and Coastal Plain of the eastern United States, and likely represents a misidentification (Holsinger and Peck, 1971). The record from Davis Farm Cave, also known as Crystal Cave, was also reported as *A. lactarium* in GBIF (2019). We presume this record to be a misidentification of *A. magnum*.

Order Chordeumatida
Family Cleidogonidae
Genus *Pseudotremia*
***Pseudotremia aeacus* Shear, 1972 (TB) A Cave Millipede**

Localities: Dade Co.: Byers Cave (GDD66), Hurricane Cave (GDD62); Walker Co.: Pigeon Cave (GWK57).

Conservation status: IUCN: Not Evaluated; NatureServe: G1G2 (SNR in Georgia).

Comments: With the exception of the record from Pigeon Cave (in Walker County, east of Lookout Mountain), all known sites are west of Lookout Mountain in Dade County (plus one unpublished record from adjacent DeKalb County, Alabama). The Pigeon Cave record may have been confused with *P. eburnea*, which is known from Pigeon Mountain.

***Pseudotremia eburnea* Loomis, 1939 (TB) A Cave Millipede**

Localities: Dade Co.: Byers Cave (GDD66), Case Cavern (GDD1), Cemetery Pit (GDD64), Cricket Cave, Howards Waterfall Cave (GDD34), Hurricane Cave (GDD62), Johnsons Crook Cave (GDD17), SSS Cave (GDD229)*, Upper Valley Cave (GDD135). Walker Co.: Ellisons Cave (GWK51), Fingerhole Cave (GWK259)*, Hickman Gulf Cave, Mountain Cove Farm Cave No. 1 (GWK73), Pettijohns Cave (GWK29), Spooky Cave (GWK494).

Conservation status: IUCN: Not Evaluated; NatureServe: G2G4 (SNR in Georgia).

Comments: Most records are from caves on the escarpments of Lookout Mountain in Walker and Dade counties. Two additional records (Nickajack Cave in Marion Co., Tennessee and Davidson Cave in Marshall Co., Alabama) are further west along the Tennessee River.

***Pseudotremia fracta* Chamberlin, 1951 (TP) A Millipede**

Localities: Walker Co.: Bee Rock Cave (GWK123)*.

Comments: This species is known from surface and cave sites in eastern Tennessee and western North Carolina (Hoffman, 1981). This is the first record of the species in Georgia.

***Pseudotremia* sp. (TB/TP) A Millipede**

Localities: Dade Co.: Howards Waterfall Cave (GDD34)*, Hooker Cave (GDD90)*, Morrison Cave (GDD86), Morrison Spring Cave (GDD110), Running Water Cave (GDD120), Sittons Cave (GDD9); Walker Co.: Bible Springs Cave (GWK74), Harrisburg Cave (GWK85), Nash Waterfall Cave (GWK72), Pigeon Cave (GWK57)*.

Comments: These records include at least two undescribed species. Specimens from Howards Waterfall Cave are an undescribed species, and those from Hooker Cave represent a second undescribed species (W. Shear, pers. comm.). Buhlmann (2001) mentions other possibly undescribed populations of *Pseudotremia*.

Family Striariidae
Genus *Striaria*
***Striaria* sp. (TX) A Millipede**

Localities: Chattooga Co.: Parkers Cave (GKH119).

Comments: Troglobitic species in the genus are known but none from Georgia.

Family Trichopetalidae
Genus *Scoterpes*
***Scoterpes austrinus* Loomis, 1946 (TB) A Cave Millipede**

Localities: Bartow Co.: Busch Cave (GBT611); Chattooga Co.: Chelsea Gulf Cave (GKH54); Dade Co.: Cemetery Pit (GDD64), Johnsons Crook Cave (GDD17), Morrison Cave (GDD86), Sittons Cave (GDD9), Upper Valley Cave (GDD135); Walker Co.: Anderson Spring Cave (GWK46), Ellisons Cave (GWK51), Goat Cave (GWK184), Harrisburg Cave (GWK85), Horseshoe Cave (GWK12), Mountain Cove Farm Cave No. 1 (GWK73), Mountain Cove Farm Cave No. 2 (GWK74), Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29), Spooky Cave (GPO5).

Conservation status: IUCN: Not Evaluated; NatureServe: G3G4 (SNR in Georgia).

Comments: This troglobitic species is known from sites east and west of Lookout Mountain in northwestern Georgia and adjacent regions of northeastern Alabama (Shear, 2010). The record from Busch Cave (Bartow County) likely represents *S. nudus*, which Shear (2010) raised to species status after the record was reported by Reeves et al. (2000).

***Scoterpes nudus* Chamberlin, 1946 (TB) A Cave Millipede**

Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11); Polk Co.: Deatons Cave (GPO5), White River Cave (GPO7).

Conservation status: IUCN: Not Evaluated; NatureServe: G3G4T1T2 (SNR in Georgia).

Comments: The troglobitic species is endemic to Georgia. It is geographically isolated from all other *Scoterpes* species and is known from three caves in the Etowah River Valley of Bartow and Polk counties (Shear, 2010). A record of *S. austrinus* from Busch Cave (Bartow Co.) is likely *S. nudus*.

***Scoterpes willreevesi* Shear, 2010 (TB) Reeves' Cave Millipede**

Localities: Dade Co.: Byers Cave (GDD66), Cemetery Pit (GDD64).

Comments: This species is known from a few sites in Dade County, Georgia, and adjacent DeKalb County, Alabama (Shear, 2010).

***Scoterpes* sp. (TB) A Cave Millipede**

Localities: Chattooga Co.: Scoggins II Cave (GKH405)*; Dade Co.: Case Cavern (GDD1), Howards Waterfall Cave (GDD34), Longs Rock Wall Cave (GDD101)*, "Saw Mill Cave, Rising Fawn"; Walker Co.: Bee Rock Cave (GWK123)*, Fricks Cave (GWK14), Pigeon Cave (GWK57), Smartt Farm Cave (GWK124)*.

Comments: These records represent females or juveniles that could not be identified to species.

Order Julida
Family Blaniulidae
Genus *Blaniulus*
***Blaniulus guttulatus* (Fabricius, 1798) (ED) Spotted Snake Millipede**

Localities: Dade Co.: Howards Waterfall Cave (GDD34), Morrison Cave (GDD86).

Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Comments: This is a soil-inhabiting species that was introduced from Europe. The name of this species is problematic as there is an unresolved homonymy with the species *Julus guttulatus* Bosc, 1792, which has also been placed in *Blaniulus*.

Family Zosteractinidae
Genus *Ameractis*
***Ameractis satis* Causey, 1959 (TB) A Cave Millipede**

Localities: Dade Co.: Morrison Cave (GDD86).

Conservation status: IUCN: Not Evaluated; NatureServe: G2G4 (SNR in Georgia).

Comments: Holsinger and Peck (1971) reported this troglobiont from Georgia, but no new collections have been reported since then.

Order Platydesmida
Family Andrognathidae
Genus *Andrognathus*
***Andrognathus corticarius* Cope, 1869 (TX) Cope's Noodle Millipede**

Localities: Floyd Co.: Cave Springs Cave (GFL18).

Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Order Polydesmida
Family Paradoxomatidae
Genus *Oxidus*
***Oxidus gracilis* (Koch, 1847) (TP) Greenhouse Millipede**

Localities: Bartow Co.: Ladds Lime Cave (GBT384-GBT389); Catoosa Co.: Chapmans Cave (GCZ25)*, Crane Cave (GCZ80)*; Chattooga Co.: Scoggins II Cave (GKH405)*, Subligna Cave (GKH145)*; Dade Co.: Hooker Cave (GDD90)*, Howards Waterfall Cave (GDD34), Limestone Caverns (GDD140)*, Wild Bills Dakota Cave (GDD596)*; Decatur Co.: Climax Cave (GDC36); Floyd Co.: Cave Springs Cave (GFL18); Grady Co.: Maloys Waterfall Cave (GGR27); Polk Co.: White River Cave (GPO7)*; Washington Co.: Tennile Caves (GWS20); Walker Co.: LitteJohn Cave (GWK280)*, Mountain Cove Farm Cave No. 2 (GWK74)*, Smartt Farm Cave (GWK124)*.

Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Comments: This is an exotic species native to Japan and is now commonly encountered in caves.

Family Polydesmidae
Genus *Polydesmus*
***Polydesmus* sp. (TX/AC) A Flatback Millipede**
Localities: Dade Co.: Creek Bed Cave.

Family Xystodesmidae
Genus *Cherokia*
***Cherokia georgiana* (Bollman, 1889) (AC) Georgia Flat-backed Millipede**
Localities: Walker Co.: Pigeon Cave (GWK57).
Conservation status: IUCN: Not Evaluated; NatureServe: G4 (SNR in Georgia).

Order Spirostreptida
Family Cambalidae
Genus *Cambala*
***Cambala annulata* (Say, 1821) (TP) A Millipede**
Localities: Bartow Co.: Anthonys Cave (GBT175); Chattooga Co.: Scoggins II Cave (GKH405)*, Subligna Cave (GKH145)*; Dade Co.: Hurricane Cave (GDD62), Longs Rock Wall Cave (GDD101)*, Rusty's Cave (GDD70); Randolph Co.: Griers Cave (GRA40).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This species ranges from Pennsylvania and Indiana to Florida and is commonly encountered in caves (Shelley, 1979).

***Cambala hubrichti* Hoffman, 1958 (TP) A Millipede**
Localities: Catoosa Co.: Chickamauga Cave (GCZ106)*; Grady Co.: Maloys Waterfall Cave (GGR27)*; Walker Co.: Fricks Cave (GWK14), Spooky Cave (GWK494).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This species is also known from caves in North Carolina (Hertl, 1981). The record from Grady County, Georgia represents a range extension (Shelley, 1979).

***Cambala minor* Bollman, 1888 (TP) A Millipede**
Localities: Chattooga Co.: Parkers Cave (GKH119); Dade Co.: Morrison Cave (GDD86); Walker Co.: Horseshoe Cave (GWK12), Pettijohns Cave (GWK29).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This species is known from surface and cave collections across the Tennessee Valley, the Ozarks, and adjacent regions (Shelley, 1979).

***Cambala ochra* Chamberlin, 1942 (TP) A Millipede**
Localities: Bartow Co.: Chert Chasm (GBT340); Walker Co.: Horseshoe Cave (GWK12), Rocky Cave (GWK496).
Comments: This species is known from surface and cave records across the Tennessee River Valley and adjacent regions (Shelley, 1979).

***Cambala* sp. (TP) A Millipede**
Localities: Catoosa Co.: Chapmans Cave (GCZ25)*; Chattooga Co.: Blowing Springs Cave (GKH54); Dade Co.: Limestone Caverns (GDD140)*; Floyd Co.: Airport Cave (GFL189)*, Cave Springs Cave (GFL18); Walker Co.: Anderson Spring Cave (GWK46)*, Mountain Cove Farm Cave No. 2 (GWK74)*; Polk Co.: Deatons Cave (GPO5), White River Cave (GPO7).
Comments: Most of these records represent juveniles that likely are one of the four species listed above.

Class Symphyla
Family Scutigerellidae
Genus *Scutigerella*
***Scutigerella* sp. (ED) A Garden Centipede**
Localities: Dade Co.: Johnsons Crook Cave (GDD17), Sittons Cave (GDD9); Walker Co.: Harrisburg Cave (GWK85).
Comments: These soil-inhabiting arthropods are not well-represented from caves.

Phylum Nematomorpha
Order Gordioidea
Family Gordiidae
Genus *Gordius*
***Gordius* sp. (SY) A Horsehair Worm**
Localities: Polk Co.: White River Cave (GPO7).
Comments: Horsehair worms are common parasites of cave crickets (Studier et al., 1991).

Phylum Nemertea
Class Enopla
Order Hoplonemertea
Family Tetrastemmatidae
Genus *Prostoma*
***Prostoma* sp. (SX/AC?) A Ribbon Worm**
Localities: Dade Co.: Howards Waterfall Cave (GDD34).
Comments: The occurrence from Howards Waterfall Cave was reported as *Prostoma* cf. *graecense* by Reeves et al. (2000).

Phylum Mollusca
Class Gastropoda
Order Basommatophora
Family Physidae
Genus *Physella*
***Physella gyrina* (Say, 1821) (TX) Tadpole Physa**
Localities: Washington Co.: Tennile Caves (GWS20)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Order Neotaenioglossa
Family Pleuroceridae
Genus *Elimia*
***Elimia proxima* (Say, 1825) (TX) Sprite Elimia**
Localities: Washington Co.: Tennile Caves (GWS20)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (SNR in Georgia).

Order Stylommatophora
Family Gastrodontidae
Genus *Gastrodonta*
***Gastrodonta interna* (Say, 1822) (TX) Brown Bellytooth**
Localities: Walker Co.: Blowing Springs Cave (GWK41).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This species is usually found in damp leaf litter and among woody detritus (Hubricht, 1985). It is known from several caves in Tennessee (Lewis, 2005).

Genus *Ventridens*
***Ventridens gularis* (Say, 1822) (TX) Throaty Dome**
Localities: Walker Co.: Horseshoe Cave (GWK12).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This species is found in a variety of habitats, from floodplains and damp hillsides to limestone outcrops. It has been reported from several caves (Lewis, 2005).

***Ventridens ligera* (Say, 1821) (AC) Globose Dome**
Localities: Dade Co.: Kirchmeyer Cave (GDD196)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This species is a habitat generalist, though often found in successional forest habitat and in disturbed areas (Hubricht, 1985; Dourson, 2010).

***Ventridens* sp. (TX/AC) A Dome Snail**
Localities: Walker Co.: Horseshoe Cave (GWK12).

Genus *Zonitoides*

***Zonitoides arboreus* (Say, 1816) (TP/TX) Quick Gloss**

Localities: Dade Co.: Longs Rock Wall Cave (GDD101)*; Decatur Co.: Climax Cave (GDC36); Grady Co.: Maloys Waterfall Cave (GGR27); Walker Co.: Blowing Springs Cave (GWK41), Pettijohns Cave (GWK29).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This species is one of the most common and widespread land snails in North America. It is found in a variety of habitats, including several caves (Hubricht, 1964, 1985; Lewis, 2005)

Family Helicodiscidae

Genus *Helicodiscus*

***Helicodiscus barri* Hubricht, 1962 (TB) Raccoon Coil**

Localities: Walker Co.: Smartt Farm Cave (GWK124)*; Chattooga Co.: Parkers Cave (GKH119).
Conservation status: IUCN: Least Concern (Gladstone et al., 2018); NatureServe: G3 (SNR in Georgia).
Comments: This troglobiont is often found on woody detritus in damp cave environments (Hubricht, 1962, 1964, 1985; Gladstone et al., 2018). It is distributed throughout the Valley and Ridge and Interior Low Plateau. A single surface locality has been reported, but all other occurrences are from caves. Molecular analyses suggest this species might represent a cryptic species complex (Gladstone et al., 2019).

***Helicodiscus inermis* Baker, 1929 (TX) Oldfield Coil**

Localities: Polk Co.: White River Cave (GPO7); Walker Co.: Blowing Springs Cave (GWK41).
Comments: This calciphilic species is often found around rocky outcrops and limestone-rich environments (Hubricht, 1985; Dourson, 2010)

***Helicodiscus notius* Hubricht, 1962 (TX) Tight Coil**

Localities: Grady Co.: Maloys Waterfall Cave (GGR27)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G5Q (SNR in Georgia).
Comments: This calciphilic species is often found around rocky outcrops and limestone-rich environments (Hubricht, 1985; Dourson, 2010). It has been reported from several caves (Hubricht, 1964; Lewis, 2005).

***Helicodiscus parallelus* (Say, 1817) (TX) Compound Coil**

Localities: Decatur Co.: Climax Cave (GDC36).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This calciphilic species is often found around rocky outcrops and limestone-rich environments (Hubricht, 1985; Dourson, 2010). It has been reported from several caves (Hubricht, 1964; Lewis, 2005).

Family Oxychilidae

Genus *Glyphyalinia*

***Glyphyalinia cryptomphala* (Clapp, 1915) (TX) Thin Glyph**

Localities: Chattooga Co.: Parkers Cave (GKH119)*; Dade Co.: Upper Valley Cave (GDD135).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This common forest snail is often found in damp leaf litter or along weedy forests (Hubricht, 1985). It is associated with limestone-rich environments (Dourson, 2010).

***Glyphyalinia indentata* (Say, 1823) (TX) Carved Glyph**

Localities: Walker Co.: Harrisburg Cave (GWK85).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This common forest snail is often found in damp leaf litter or along weedy forests (Hubricht, 1985). It is associated with limestone-rich environments (Dourson, 2010) and known from several caves (Lewis, 2005).

***Glyphyalinia praecox* (Baker, 1930) (TX) Brilliant Glyph**

Localities: Bartow Co.: Anthonys Cave (GBT175).
Conservation status: IUCN: Not Evaluated; NatureServe: G4 (SNR in Georgia).
Comments: This common forest snail is often found in damp leaf litter or along weedy forests (Hubricht, 1985). It is associated with limestone-rich environments (Dourson, 2010) and known from several caves (Lewis, 2005).

***Glyphyalinia rhoadsi* (Pilsbry, 1899) (TX) Sculpted Glyph**

Localities: Washington Co.: Tennile Caves (GWS20).
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This common forest snail is often found in damp leaf litter or along weedy forests (Hubricht, 1985). It is associated with limestone-rich environments (Dourson, 2010) and known from several caves (Lewis, 2005).

***Glyphyalinia sculptilis* (Bland, 1858) (TX) Suborb Glyph**

Localities: Bartow Co.: Busch Cave (GBT611); Chattooga Co.: Parkers Cave (GKH119)*; Walker Co.: Bible Springs Cave (GWK74), Rocky Cave (GWK496), Spooky Cave (GWK494).
Conservation status: IUCN: Not Evaluated; NatureServe: G4 (SNR in Georgia).
Comments: This common forest snail is often found in damp leaf litter or along weedy forests (Hubricht, 1985). It is associated with limestone-rich environments (Dourson, 2010) and known from several caves (Lewis, 2005).

***Glyphyalinia specus* Hubricht, 1965 (TB) Hollow Glyph**

Localities: Chattooga Co.: Parkers Cave (GKH119); Dade Co.: Morrison Cave (GDD86); Walker Co.: Cave Springs Cave, Cherokee Cave (GWK94), Mount Cove Farm Cave, Pettijohns Cave (GWK29).
Conservation status: IUCN: Least Concern (Gladstone et al. 2018); NatureServe: G3 (SNR in Georgia).
Comments: This is a wide-ranging troglobiont found in dry leaf litter and on cave walls. Its distribution is suggestive of greater occurrence throughout Valley and Ridge (Gladstone et al., 2018).

***Glyphyalinia wheatleyi* (Bland, 1883) (TP/TX) Bright Glyph**

Localities: Floyd Co.: Cave Springs Cave (GFL18)*; Grady Co.: Maloys Waterfall Cave (GGR27)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This common forest snail is often found in damp leaf litter or along weedy forests (Hubricht, 1985). It is associated with limestone-rich environments (Dourson, 2010) and known from several caves (Lewis, 2005).

Family Philomycidae

Genus *Pallifera*

***Pallifera* sp. (TX/AC) A Mantleslug**

Localities: Dade Co.: Johnsons Crook Cave (GDD17)*.

Family Polygyridae

Genus *Inflectarius*

***Inflectarius rugeli* (Shuttleworth, 1852) (TX) Deep-tooth Shagreen**

Localities: Chattooga Co.: Parkers Cave (GKH119)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).
Comments: This species is primarily found in leaf litter, under logs, or in shaded mesic forest habitat, but can also be found in caves (Niemiller et al., unpublished).

Genus *Mesodon*

***Mesodon* sp. (TX/AC) A Globe Snail**

Localities: Walker Co.: Anderson Spring Cave (GWK46).
Comments: Most *Mesodon* species can be found in forest habitats, under logs or in dense leaf litter. Some species (e.g., *M. appressus*, *M. edentatus*, *M. sargentianus*) are considered calciphiles, and are common near cave entrances (Hubricht, 1985; Niemiller et al., unpublished).

Genus *Patera*

***Patera appressa* (Say, 1821) (TP/TX) Flat Bladetooth**

Localities: Dade Co.: Hooker Cave (GDD90)*.
Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Comments: This species is associated with rocky outcrops, forest ravines, disturbed habitats along roadsides, and limestone-rich environments. It is a common constituent of cave environments, though not limited to subterranean habitat (Hubricht, 1964, 1985; Lewis, 2005; Dourson, 2010).

***Patera perigrapta* (Pilsbry, 1894) (TP/TX) Engraved Bladetooth**

Localities: Chattooga Co.: Parkers Cave (GKH119); Dade Co.: Byers Cave (GDD66).

Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Comments: This species is associated with rocky outcrops, forest ravines, disturbed habitats along roadsides, and limestone-rich environments. It also is a common constituent of cave environments, though not limited to subterranean habitat (Hubricht, 1964, 1985; Lewis, 2005; Dourson, 2010).

Genus *Triodopsis*
***Triodopsis* sp. (TX/AC) A Threetooth Snail**

Localities: Dade Co.: Wild Bills Dakota Cave (GDD596)*.

Comments: *Triodopsis* snails occupy a diverse array of habitats, including mesic forest leaf litter, rock outcrops, and urban areas (Hubricht, 1985). This genus has also been reported from several caves (Niemiller et al., unpublished).

Family Pristilomatidae
Genus *Hawaiia*
***Hawaiia minuscula* (Binney, 1841) (TX) Minute Gem**

Localities: Dade Co.: Howards Waterfall Cave (GDD34).

Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Comments: This species is a habitat generalist, though often found in disturbed habitats, such as greenhouses and gardens (Hubricht, 1985; Dourson, 2010). It has been reported from several caves (Lewis, 2005; Niemiller et al., unpublished)

Family Strobilopsidae
Genus *Strobilops*
***Strobilops texasianus* Pilsbry and Ferriss, 1906 (AC) Southern Pinecone**

Localities: Decatur Co.: Climax Cave (GDC36)*.

Conservation status: IUCN: Not Evaluated; NatureServe: G5 (SNR in Georgia).

Comments: This species is associated with leaf litter and woody detritus forested habitat (Hubricht, 1985). This is the first record from a cave.

Phylum Platyhelminthes
Class Trepaxonemata
Order Neoophora
Family Kenkiidae
Genus *Sphalloplana*
***Sphalloplana georgiana* Hyman, 1954 (SB) Georgia Cave Planarian**

Localities: Dade Co.: Howards Waterfall Cave (GDD34).

Conservation status: IUCN: Not Evaluated; NatureServe: G1 (SNR in Georgia).

Comments: This species is known only from the type locality at Howards Waterfall Cave (Hyman, 1954; Kenk, 1977)

***Sphalloplana* sp. (SB) A Cave Planarian**

Localities: Dade Co.: Hurricane Cave (GDD62); Walker Co.: Anderson Spring Cave (GWK46), Pettijohns Cave (GWK29).

Comments: These records may represent other sites for *S. georgiana* or possibly undescribed species.

Phylum Chordata
Class Actinopterygii
Order Percopsiformes
Family Amblyopsidae
Genus *Typhlichthys*
***Typhlichthys subterraneus* Girard, 1859 (SB) Southern Cavefish**

Localities: Catoosa Co.: Crane Cave (GCZ80); Dade Co.: Case Cavern (GDD1), Limestone Caverns (GDD140), Longs Rock Wall Cave (GDD101), Sittons Cave (GDD9).

Conservation status: IUCN: Vulnerable; NatureServe: G4 (S1 in Georgia); listed as Endangered and considered a Species of Greatest Conservation Need in Georgia.

Comments: The record from Crane Cave is the first occurrence of this species from the Appalachians karst region (Niemiller et al., 2016). *Typhlichthys subterraneus* is a cryptic species complex (Niemiller et al., 2012), and populations from Georgia along with a few populations in Marion Co., Tennessee, are likely a distinct species.

Order Scorpaeniformes
Family Cottidae
Genus *Cottus*
***Cottus bairdii* Girard, 1850 (SP) Mottled Sculpin**

Localities: Walker Co.: Fricks Cave (GWK14).

Conservation status: IUCN: Least Concern; NatureServe: G5 (S4 in Georgia).

Comments: This is species is thought to be common in caves (Dearolf, 1956; Poly, 2001), and cave records exists from the TAG region (Buhlmann, 2001; Huntsman et al., 2011; Venarsky et al., 2012). Some records of *C. carolinae* may actually represent this species, as both species are very similar morphologically.

***Cottus carolinae* (Gill, 1861) (SP) Banded Sculpin**

Localities: Dade Co.: Longs Rock Wall Cave (GDD101)*; Walker Co.: Fricks Cave (GWK14)*, Mountain Cove Farm Cave No. 2 (GWK74), Roger Branch Cave (GWK204)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S4 in Georgia).

Comments: This species is the most commonly reported fish in caves of the Interior Plateau and Appalachians karst regions (e.g., Cope and Packard, 1881; Dearolf, 1956; Poly and Boucher, 1996; Niemiller et al., 2006, 2016). Several populations are thought to live year-round in caves, with some exhibiting some degree of troglomorphy (Espinasa and Jeffery, 2003; Espinasa et al., 2013).

***Cottus* sp. (SP) A Sculpin**

Localities: Catoosa Co.: Crane Cave (GCZ80)*; Walker Co.: Horseshoe Cave (GWK12)*.

Comments: These records may be *C. bairdii* or *C. carolinae*.

Order Siluriformes
Family Ictaluridae
Genus *Ameiurus*
***Ameiurus nebulosus* (Lesueur, 1819) (SX/AC) Brown Bullhead**

Localities: Walker Co.: Horseshoe Cave (GWK12).

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This species has been reported previously from caves in Florida and West Virginia (Franz et al., 1994; Hale and Streever, 1994; Pruitt, 1995; Poly and Boucher, 1996; Poly, 2001).

Class Amphibia
Order Anura
Family Bufonidae
Genus *Anaxyrus*
***Anaxyrus fowleri* (Hinckley, 1882) (AC) Fowler's Toad**

Localities: Dade Co.: Case Caverns (GDD1)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This toad has been reported infrequently from caves in eastern Tennessee (Dodd et al., 2001; Niemiller et al., 2016).

***Anaxyrus terrestris* (Bonnaterre, 1789) (AC) Southern Toad**

Localities: Burke Co.: Utleys Cave*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: Pleistocene remains of this species have been found in a cave in Citrus Co., Florida (Holman, 1958).

Family Hylidae
Genus *Hyla*
***Hyla chrysoscelis* Cope, 1880 (TX/AC) Cope's Gray Treefrog**
Localities: Walker Co.: Pettijohns Cave (GWK29)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).
Comments: This treefrog may use caves for shelter during periods of drought, although most records are thought to be accidental occurrences. It has been reported from a few caves in Alabama and Tennessee (Lewis, 2005; Godwin, 2008; Niemiller and Miller, 2009).

***Hyla gratiosa* LeConte, 1856 (AC) Barking Treefrog**
Localities: Walker Co.: Drag Fold Cave (GWK79)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Genus *Pseudacris*
***Pseudacris crucifer* (Wied-Neuwied, 1838) (TX/AC) Spring Peeper**
Localities: Dade Co.: Boxcar Cave (GDD69)*; Walker Co.: Pettijohn Cave (GWK29)*, Screech Owl Cave (GWK205)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).
Comments: This frog has been reported infrequently from caves (Black, 1971; Prather and Briggler, 2001; Godwin, 2008; Niemiller and Miller, 2009; Niemiller et al., 2016). It may seek refuge in caves during prolonged drought (Prather and Briggler, 2001).

***Pseudacris feriarum* (Baird, 1854) (AC) Upland Chorus Frog**
Localities: Grady Co.: Waterfall Cave (GGR27)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).
Comments: This frog has been reported infrequently from caves (Black, 1971; Osbourn, 2005; Godwin, 2008; Niemiller and Miller, 2009; Niemiller et al., 2016).

Family Ranidae
Genus *Rana*
***Rana catesbeiana* Shaw, 1802 (TX) American Bullfrog**
Localities: Dade Co.: Boxcar Cave (GDD69)*, Rusty's Cave (GDD70)*; Walker Co.: Anderson Spring Cave (GWK46)*, Pettijohns Cave (GWK29)*; Washington Co.: Tennile Caves (GWS20)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).
Comments: This species is occasionally reported from caves with substantial aquatic habitat, particularly near entrances and the twilight zone (Barr, 1953; Niemiller and Miller, 2009; Niemiller et al., 2016).

***Rana clamitans* Latreille, 1801 (TX) Green Frog**
Localities: Dade Co.: Longs Rock Wall Cave (GDD101)*; Decatur Co.: Climax Cave (GDC36)*; Grady Co.: Waterfall Cave (GGR27)*; Walker Co.: Anderson Spring Cave (GWK46), Ellisons Cave (GWK51), Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29), Screech Owl Cave (GWK205)*; Washington Co.: Tennile Caves (GWS20)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).
Comments: This species is occasionally reported from caves (Barr, 1953; Buhlmann, 2001; Dodd et al., 2001; Camp and Jensen, 2007; Niemiller and Miller, 2009; Niemiller et al., 2016).

***Rana palustris* (LeConte, 1825) (TX) Pickerel Frog**
Localities: Dade Co.: Hurricane Cave (GDD62)*, Longs Rock Wall Cave (GDD101)*, Sittons Cave (GDD9), Trenton Waterfall Cave; Walker Co.: Anderson Spring Cave (GWK46), Ellisons Cave (GWK51), Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29)*, Pigeon Cave (GWK57), Roger Branch Cave (GWK204)*, Screech Owl Cave (GWK205)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S4 in Georgia).
Comments: This species is regularly reported from caves near entrances and in the twilight zone (Cliburn and Middleton, 1983; Buhlmann, 2001; Camp and Jensen, 2007; Niemiller and Miller, 2009; Niemiller et al., 2016). The record for "Trenton Waterfall Cave" likely represents Howards Waterfall Cave.

***Rana sphenocephala* Cope, 1886 (TX/AC) Southern Leopard Frog**
Localities: Grady Co.: Maloys Waterfall Cave (GGR27)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).
Comments: Unlike other ranid frogs, this species is encountered infrequently in caves, but cave records exist from Tennessee (Lewis, 2005; Niemiller and Miller, 2009).

Order Caudata
Family Ambystomatidae
Genus *Ambystoma*
***Ambystoma tigrinum* (Green, 1825) (AC) Eastern Tiger Salamander**
Localities: Walker Co.: Drag Fold Cave (GWK79)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S3S4 in Georgia); considered a Species of Greatest Conservation Need in Georgia.
Comments: Although this mole salamander spends much of its life underground in burrows, it is not associated with caves and karst.

Family Plethodontidae
Genus *Aneides*
***Aneides aeneus* (Cope and Packard, 1881) (TX) Green Salamander**
Localities: Dade Co.: Byers Cave (GDD66)*, Case Caverns (GDD1)*, Howards Waterfall Cave (GDD34)*, Sittons Cave (GDD9)*; Walker Co.: Fricks Cave (GWK14), Lula Falls Cave (GWK617)*, Lula Falls Talus Cave*, Nash Waterfall Cave (GWK72).
Conservation status: IUCN: Near Threatened; NatureServe: G3G4 (S3 in Georgia); listed as Rare and considered a Species of Greatest Conservation Need in Georgia.
Comments: This species is occasionally reported around entrances of caves along the escarpments of the Cumberland Plateau, including Lookout Mountain and Pigeon Mountain. The type locality is "near the mouth" of Nickajack Cave in Marion Co., Tennessee.

Genus *Desmognathus*
***Desmognathus conanti* Rossman, 1958 (AC) Spotted Dusky Salamander**
Localities: Dade Co.: Hurricane Cave (GDD62)*; Walker Co.: Anderson Spring Cave (GWK46), Ellisons Cave (GWK51), Mountain Cove Farm Cave No. 2 (GWK74), Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29), Pigeon Cave (GWK57); Washington Co.: Tennile Caves (GWS20)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).
Comments: This species has been reported infrequently in and around entrances of spring entrances and is rarely observed in the dark zone (Himes et al., 2004; Niemiller and Miller, 2009; Niemiller et al., 2016).

***Desmognathus ocoee* Nicholls, 1949 (AC) Ocoee Salamander**
Localities: Habersham Co.: La Guarida del Diablo*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Genus *Eurycea*

***Eurycea cirrigera* (Gre0en, 1831) (TX) Southern Two-lined Salamander**
Localities: Decatur Co.: Climax Cave (GDC36)*; Polk Co.: White River Cave (GPO7)*; Walker Co.: Anderson Spring Cave (GWK46), Nash Waterfall Cave (GWK72)*, Pigeon Cave (GWK57); Washington Co.: Tennile Caves (GWS20).
Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).
Comments: This species has been reported infrequently from caves (Himes et al., 2004; Lewis, 2005; Camp and Jensen, 2007; Niemiller and Miller, 2009; Niemiller et al., 2016), although a population from Cannon County, Tennessee, has been documented breeding in a cave (Niemiller and Miller, 2007).

***Eurycea guttolineata* (Holbrook, 1838) (TX) Three-lined Salamander**
Localities: DeKalb Co.: Nice Gneiss Cave (GDK329)*; Washington Co.: Tennile Caves (GWS20)*.
Conservation status: IUCN: Least Concern; NatureServe: G5 (S4S5 in Georgia).
Comments: This species also has been reported from caves in Alabama and Mississippi (Cooper and Cooper, 1968; Himes et al., 2004).

***Eurycea longicauda* (Green, 1818) (TP/TX) Long-tailed Salamander**

Localities: Chattooga Co.: Scoggins II Cave (GKH405)*, Subligna Cave (GKH145)*; Dade Co.: Hurricane Cave (GDD62)*, Longs Creekside Cave (GDD45)*, Lookout Mountain Spring Cave*, Sittons Cave (GDD9); Walker Co.: Anderson Spring Cave (GWK46), Bible Springs Cave (GWK74)*, Fricks Cave (GWK14), Pettijohns Cave (GWK29), Pigeon Cave (GWK57), "small cave in rock quarry along Georgia Highway 136; 1.65 Highway miles West of Cooper Heights."

Conservation status: IUCN: Least Concern; NatureServe: G5 (S4 in Georgia).

Comments: This species is regularly observed in caves in the Appalachians and Interior Plateau karst regions (Buhlmann, 2001; Dodd et al., 2001; Lewis, 2005; Osbourn, 2005; Taylor and Mays, 2006; Camp and Jensen, 2007; Niemiller and Miller, 2009; Niemiller et al., 2016), although not as frequently as *E. lucifuga*.

***Eurycea lucifuga* Rafinesque, 1822 (TP) Cave Salamander**

Localities: Bartow Co.: Anthonys Cave (GBT175)*, Chert Chasm (GBT340)*; Catoosa Co.: Chapmans Cave (GCZ25)*, Crane Cave (GCZ80)*; Chattooga Co.: Blowing Spring Cave (GKH54)*, Parkers Cave (GKH119)*, Scoggins II Cave (GKH405)*; Dade Co.: Boxcar Cave (GDD69)*, Caboose Cave (GDD475)*, Case Cavern (GDD1), Cemetery Pit (GDD64)*, Chambliss Cave (GDD321), Hooker Cave (GDD90)*, Jeff's Hole Cave (GDD400)*, Johnsons Crook Cave (GDD17)*, Limestone Caverns (GDD140)*, Longs Rock Wall Cave (GDD101)*, Lower Valley Cave (GDD136)*, Morrison Cave (GDD86)*, Sittons Cave (GDD9), SSS Cave (GDD229)*, Trenton caves, Upper Valley Cave (GDD135)*, Wild Bills Dakota Cave (GDD596)*; Floyd Co.: Airport Cave (GFL189)*; Gordon Co.: caves near junction of Hwy.411 and Hwy.156 7.0 mi. N of Fairmount*, Ford Roberts Cave (GGO147)*; Murray Co.: Fincher Bluff Cave (GMA291)*; Polk Co.: Wise Cave (GPO6)*; Walker Co.: Anderson Spring Cave (GWK46), Bee Rock Cave (GWK123)*, Bible Spring Cave (GWK74)*, Cave Spring Cave*, Ellisons Cave (GWK51), Fingerhole Cave (GWK259)*, Goat Cave (GWK184)*, Harrisburg Cave (GWK85), Horseshoe Cave (GWK12)*, Kinda Pretty Cave (GWK258)*, LittleJohn Cave (GWK280)*, Missing Evan Well Cave (GWK488)*, Mouldy Bat Pit (GWK257)*, Mountain Cove Farm Cave No. 1 (GWK73), Mountain Cove Farm Cave No. 2 (GWK74), Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29), Pigeon Cave (GWK57), Roger Branch Cave (GWK204)*, Screech Owl Cave (GWK205), Smartt Farm Cave (GWK124)*, Spooky Cave (GWK494)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S4 in Georgia).

Comments: This is the most commonly observed salamander in caves in the Appalachians and Interior Plateau karst regions (Hutchinson, 1966; Buhlmann, 2001; Lewis, 2005; Niemiller and Miller, 2009; Camp and Jensen, 2015; Niemiller et al., 2016).

***Eurycea wallacei* (Carr, 1939) (SB) Georgia Blind Salamander**

Localities: Decatur Co.: Climax Cave (GDC36); Dougherty Co.: Deep well in Albany, Radium Springs (GDG39).

Conservation status: IUCN: Vulnerable; NatureServe: G2 (S1 in Georgia); listed as Threatened and considered a Species of Greatest Conservation Need in Georgia.

Comments: The type locality for this neotenic stygobite is a well near Albany in Dougherty County. It is found in subterranean waters of the Upper Floridan Aquifer in the Dougherty Plain of southeastern Georgia and adjacent northwestern Florida. *Eurycea wallacei* has been reported from seven sites, but only confirmed from Climax Cave in Decatur County and Radium Springs along the Flint River in Dougherty County (Means, 2005; Fenolio et al., 2013). There is an unconfirmed report from a spring cave in Baker County (Ben Martinez, pers. comm.).

Genus *Gyrinophilus*

***Gyrinophilus palleucus* McCrady, 1954 (SB) Tennessee Cave Salamander**

Localities: Walker Co.: Fricks Cave (GWK14), Harrisburg Cave (GWK85).

Conservation status: IUCN: Vulnerable; NatureServe: G2G3 (S1 in Georgia); listed as Threatened and considered a Species of Greatest Conservation Need in Georgia.

Comments: Although wide-ranging throughout south-central Tennessee and northern Alabama (Godwin, 2000; Miller and Niemiller, 2008, 2012), this neotenic stygobite is known from only two caves in Georgia (Buhlmann, 2001; Godwin, 2008; Miller and Niemiller, 2012).

***Gyrinophilus porphyriticus* (Green, 1827) (TP) Spring Salamander**

Localities: Dade Co.: Boxcar Cave (GDD69)*, Byers Cave (GDD66), Howards Waterfall Cave (GDD34)*, Hurricane Cave (GDD62)*, Johnsons Crook Cave (GDD17), Limestone Caverns (GDD140)*, Longs Rock Wall Cave (GDD101)*, Sittons Cave (GDD9), SSS Cave (GDD229)*, Wild Bills Dakota Cave (GDD596)*; Walker Co.: Anderson Spring Cave (GWK46), Ellisons Cave (GWK51), Gila Monster Cave (GWK379)*, Harrisburg Cave (GWK85), Mountain Cove Farm Cave No. 1 (GWK73), Mountain Cove Farm Cave No. 2 (GWK74), Nash Waterfall Cave (GWK72)*, Pettijohns Cave (GWK29), Pigeon Cave (GWK57), Spooky Cave (GWK494)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S4 in Georgia).

Comments: This species is common in caves throughout its range (Brandon, 1966; Cooper and Cooper, 1968; Miller and Niemiller, 2008), including several caves in Georgia (Buhlmann, 2001; Camp and Jensen, 2007).

Genus *Plethodon*

***Plethodon glutinosus* (Green, 1818) (TP) Northern Slimy Salamander**

Localities: Bartow Co.: Busch Cave (GBT611)*; Catoosa Co.: Crane Cave (GCZ80)*; Chattooga Co.: Parkers Cave (GKH119)*, Scoggins II Cave (GKH405)*; Dade Co.: Case Cavern (GDD1), Cemetery Pit (GDD64)*, Chambliss Cave (GDD321), Daniel Cave, Hooker Cave (GDD90)*, Howards Waterfall Cave (GDD34)*, Hurricane Cave (GDD62)*, Johnsons Crook Cave (GDD17)*, Limestone Caverns (GDD140)*, Longs Creekside Cave (GDD45)*, Longs Rock Wall Cave (GDD101)*, Lower Valley Cave (GDD136)*, Morrison Spring Cave (GDD110)*, Sittons Cave (GDD9), SSS Cave (GDD229)*, Upper Valley Cave (GDD135)*, Wild Bills Dakota Cave (GDD596)*; Floyd Co.: Airport Cave (GFL189)*, Cave Springs Cave (GFL18)*, "Bear Bone Cave" (probably Silver Creek Cave) (GFL173)*; Polk Co.: White River Cave (GPO7)*; Walker Co.: Anderson Spring Cave (GWK46), Bible Spring Cave (GWK74)*, Ellisons Cave (GWK51), Fingerhole Cave (GWK259)*, Gila Monster Cave (GWK379)*, Horseshoe Cave (GWK12)*, Kinda Pretty Cave (GWK258)*, LittleJohn Cave (GWK280)*, Lofton Cave (GWK281)*, Missing Evan Well Cave (GWK488)*, Mountain Cove Farm Cave No. 2 (GWK74), Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29), Pigeon Cave (GWK57), Roger Branch Cave (GWK204)*, Screech Owl Cave (GWK205), Slimy Slot Cave (GWK529)*, Smartt Farm Cave (GWK124)*, Spooky Cave (GWK494)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This species is a common inhabitant of caves throughout its range (Dodd et al., 2001; Lewis, 2005; Godwin, 2008; Niemiller and Miller, 2009; Niemiller et al., 2016), including Georgia (Buhlmann, 2001; Camp and Jensen, 2007).

***Plethodon petraeus* Wynn et al., 1988 (TP/TX) Pigeon Mountain Salamander**

Localities: Walker Co.: Harrisburg Cave (GWK85), Nash Waterfall Cave (GWK72)*, Pettijohns Cave (GWK29), Screech Owl Cave (GWK205).

Conservation status: IUCN: Vulnerable; NatureServe: G2 (S2 in Georgia); listed as Rare and considered a Species of Greatest Conservation Need in Georgia.

Comments: This species is endemic to Georgia, specifically on the eastern slope of Pigeon Mountain in Walker County. Although primarily associated with rock outcrops and exposures in hardwood forest, *P. petraeus* can be found around the entrances of some caves (Wynn et al., 1988; Camp and Jensen, 2007).

***Plethodon serratus* Grobman, 1944 (TX) Southern Red-backed Salamander**

Localities: Walker Co.: Anderson Spring Cave (GWK46), Fingerhole Cave (GWK259)*, Mouldy Bat Pit (GWK257)*, Pettijohns Cave (GWK29).

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: Unlike some other *Plethodon* salamanders, this species has rarely been reported from caves (Buhlmann, 2001).

Plethodon ventralis **Highton, 1997 (TP/TX) Zigzag Salamander complex**

Localities: Dade Co.: Caboose Cave (GDD475)*, Case Caverns (GDD1)*, Daniel Cave, Hurricane Cave (GDD62)*, Howards Waterfall Cave (GDD34)*, Morrison Cave (GDD86)*, Rusty's Cave (GDD70)*, Sittons Cave (GDD9); Walker Co.: Anderson Spring Cave (GWK46)*, Harrisburg Cave (GWK85), Hogjowl Cave*, Horseshoe Cave (GWK12)*, Pettijohns Cave (GWK29), Screech Owl Cave (GWK205).

Conservation status: *Plethodon ventralis* - IUCN: Least Concern; NatureServe: G4 (S4 in Georgia); *P. dorsalis* - IUCN: Least Concern; NatureServe: G5 (SNR in Georgia);

Comments: *Plethodon dorsalis* and *P. ventralis* are closely related and difficult to distinguish morphologically. Some authors treat all populations in Georgia as *P. ventralis* (e.g., Camp, 2008); however, the contact zones between these two species have not been adequately delineated. Regardless, this complex is encountered regularly in caves (Buhlmann, 2001; Lewis, 2005; Camp and Jensen, 2007; Godwin, 2008; Niemiller and Miller, 2009).

Genus *Pseudotriton*

Pseudotriton ruber **(Sonnini de Manoncourt and Latreille, 1801) (TP) Red Salamander**

Localities: Dade Co.: Rusty's Cave (GDD70); Walker Co.: Anderson Spring Cave (GWK46), Ellisons Cave (GWK51), Fricks Cave (GWK14)*, Harrisburg Cave (GWK85), Mountain Cove Farm Cave No. 2 (GWK74), Pigeon Cave (GWK57), Roger Branch Cave (GWK204)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This species is found frequently in the twilight zone and entrances of spring caves throughout the Interior Plateau and Appalachians karst regions (Buhlmann, 2001; Osbourn, 2005; Camp and Jensen, 2007; Miller et al., 2008; Niemiller and Miller, 2009; Niemiller et al., 2016). Reproduction in the dark zone of cave streams has been documented (Miller and Niemiller, 2005; Miller et al., 2008), including at Anderson Spring Cave in Walker County (Niemiller et al., 2006).

Family Salamandridae
Genus *Notophthalmus*
Notophthalmus viridescens **(Rafinesque, 1820) (AC) Eastern Newt**

Localities: Dade Co.: Lower Valley Cave (GDD136)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: Additional records of this species exist from caves in Alabama and Tennessee (Godwin, 2008; Niemiller and Miller, 2009).

Class Aves
Order Accipitriformes
Family Cathartidae
Genus *Cathartes*
Cathartes aura **(Linnaeus, 1758) (TX/AC) Turkey Vulture**

Localities: Floyd Co.: Airport Cave (GFL189)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This species nests on occasion at the entrances and within the twilight zones of caves (Coles, 1944; Lewis, 2005; Niemiller et al., 2016).

Order Passeriformes
Family Tyrannidae
Genus *Sayornis*
Sayornis phoebe **(Latham, 1790) (TX) Eastern Phoebe**

Localities: Catoosa Co.: Chapmans Cave (GCZ25)*; Chattooga Co.: Subligna Cave (GKH145)*; Dade Co.: Sittons Cave (GDD9)*; Walker Co.: Mountain Cove Farm Cave #2 (GWK74)*, Anderson Springs Cave (GWK46)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This species commonly nests in the entrances and twilight zones of caves in the TAG region (Lewis, 2005; Godwin, 2008; Niemiller et al., 2013, 2016).

Class Mammalia
Order Carnivora
Family Mustelidae
Genus *Neovision*
Neovision vison **(Schreber, 1777) (AC) American Mink**

Localities: Walker Co.: Roger Branch Cave (GWK204)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This mustelid also has been observed near cave entrances infrequently in Tennessee (MLN, personal observation).

Family Procyonidae
Genus *Procyon*
Procyon lotor **(Linnaeus, 1758) (TX) Raccoon**

Localities: Catoosa Co.: Chapmans Cave (GCZ25)*, Crane Cave (GCZ80)*; Chattooga Co.: Subligna Cave (GKH145)*; Dade Co.: Chambliss Cave (GDD321), Ha-ha Cave (GDD256), Trenton Bone Cave (GDD16)*; Lapp Hole; Floyd Co.: Cave Springs Cave (GFL18)*; Walker Co.: Bee Rock Cave (GWK123)*, Fricks Cave (GWK14)*, Horseshoe Cave (GWK12)*, Smartt Farm Cave (GWK124)*, Spooky Cave (GWK494)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: Evidence of this species (tracks and scat) is common in caves throughout the central and eastern United States.

Order Chiroptera
Family Vespertilionidae
Corynorhinus rafinesquii **(Lesson, 1827) (TX) Rafinesque's Big-eared Bat**

Localities: Rabun Co.: Bascoms Cave.

Conservation status: IUCN: Least Concern; NatureServe: G3G4 (S3 in Georgia); listed as Rare and considered a Species of Greatest Conservation Need in Georgia.

Comments: This bat is considered rare in Georgia and has only been documented at one cave in the state.

Genus *Eptesicus*
Eptesicus fuscus **(Palisot de Beauvois, 1796) (TX) Big Brown Bat**

Localities: Floyd Co.: Osborn Cave (GFL220)*; Polk Co.: Deatons Cave (GPO5), White River Cave (GPO7); Walker Co.: Fricks Cave (GWK14)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This species is observed frequently in TAG caves (Holliday, 2012; Flock, 2013, 2014; Niemiller et al., 2016), particularly in winter, but few records are known from caves in Georgia.

Genus *Lasiurus*
Lasiurus borealis **Müller, 1776 (AC) Eastern Red Bat**

Localities: Dade Co.: Byers Cave (GDD66); Polk Co.: Deatons Cave (GPO5).

Conservation status: IUCN: Least Concern; NatureServe: G3G4 (S5 in Georgia).

Comments: This forest-dwelling bat has been reported from caves infrequently (Mohr, 1952; Myers, 1960; Niemiller et al., 2016).

Genus *Myotis*
Myotis austroriparius **(Rhoads, 1897) (TX) Southeastern Myotis**

Localities: Decatur Co.: Climax Cave (GDC36); Grady Co.: Maloys Waterfall Cave (GGR27); Lee Co.: Chokee Cave (GLE575); Washington Co.: Sandersville Cave (GWS399).

Conservation status: IUCN: Least Concern; NatureServe: G4 (S3 in Georgia); considered a Species of Greatest Conservation Need in Georgia.

Comments: This is the most common *Myotis* species in caves of southwestern Georgia.

Myotis grisescens **Howell, 1909 (TX) Gray Bat**

Localities: Catoosa Co.: Chickamauga Cave (GCZ106); Chattooga Co.: Welcome Hill Cave (GKH163), Lowry Cave (GKH206); Dade Co.: Sittons Cave (GDD9); Polk Co.: Deatons Cave (GPO5), White River Cave (GPO7); Walker Co.: Anderson Spring Cave (GWK46), Fricks Cave (GWK14).

Conservation status: IUCN: vulnerable; NatureServe: G4 (S1 in Georgia); listed as Endangered under the U.S. Endangered Species Act; listed as Endangered and considered a Species of Greatest Conservation Need in Georgia.

Comments: In summer, this federally endangered bat is known to roost in just three caves in Chattooga, Walker, and Catoosa counties (Martin, 2007), although several additional occurrences exist. Unlike several *Myotis* species, *M. grisescens* does not appear to be nearly as susceptible to White-nose Syndrome.

Myotis leibii **(Audubon and Bachman, 1842) (TX) Eastern Small-footed Bat**

Localities: Dade Co.: Case Cavern (GDD1), Howards Waterfall Cave (GDD34); Union Co.: cave near Young Harris.

Conservation status: IUCN: Endangered; NatureServe: G4 (S1 in Georgia).

Comments: This small bat is rarely observed in Georgia caves.

Myotis lucifugus **(LeConte, 1831) (TX) Little Brown Bat**

Localities: Bartow Co.: Kingston Saltpeter Cave (GBT11); Dade Co.: Byers Cave (GDD66), Case Cavern (GDD1), Howards Waterfall Cave (GDD34), Sittons Cave (GDD9); Polk Co.: Deatons Cave (GPO5), White River Cave (GPO7); Walker Co.: Anderson Spring Cave (GWK46), Ellisons Cave (GWK51), Fricks Cave (GWK14)*.

Conservation status: IUCN: Endangered; NatureServe: G3 (S3 in Georgia); considered a Species of Greatest Conservation Need in Georgia.

Comments: This bat is encountered infrequently in Georgia caves during winter. Populations have sustained declines throughout its wide distribution in North America due to White-nose Syndrome. This species has not been observed during recent winter cave hibernacula surveys in Georgia (Morris and Ferrall, 2018).

Myotis septentrionalis **(Trovessart, 1897) (TX) Northern Long-eared Bat**

Localities: Bartow Co.: Davis Farm Cave (GBT222)*, Kingston Saltpeter Cave (GBT11); Dade Co.: Byers Cave (GDD66), Case Cavern (GDD1), Johnsons Crook Cave (GDD17)*, Sittons Cave (GDD9); Pickens Co.: Long Swamp Creek Cave; Polk Co.: Deatons Cave (GPO5), White River Cave (GPO7), Rabun Co.: Black Diamond Tunnel Cave; Walker Co.: Anderson Spring Cave (GWK46), Kinda Pretty Cave (GWK258)*, Nash Waterfall Cave (GWK72)*.

Conservation status: IUCN: Near Threatened; NatureServe: G1G2 (S1S3 in Georgia); listed as Threatened under the U.S. Endangered Species Act; listed as Threatened and considered a Species of Greatest Conservation Need in Georgia.

Comments: This species can be found in low numbers in Georgia caves during winter. However, it is one of the bat species most impacted by White-nose Syndrome. It is now listed as Threatened under the U.S. Endangered Species Act as of 2015. This species has not been observed during recent winter cave hibernacula surveys in Georgia (Morris and Ferrall, 2018).

Myotis sodalis **Miller and Allen, 1928 (TX) Indiana Bat**

Localities: Chattooga Co.: Lowry Cave (GKH206); Dade Co.: Case Cavern (GDD1), Cave 4 mi W of Trenton, Sittons Cave (GDD9).

Conservation status: IUCN: Near Threatened; NatureServe: G2 (S1 in Georgia); listed as Endangered under the U.S. Endangered Species Act; listed as Endangered and considered a Species of Greatest Conservation Need in Georgia.

Comments: This bat is not commonly encountered in Georgia caves. Case Cavern and Sittons Cave are Priority 4 sites for this federally endangered species.

Myotis **sp. (TX) A Bat**

Localities: Chattooga Co.: Subligna Cave (GKH145)*.

Comments: This record is probably *M. grisescens* but identification could not be confirmed.

Genus *Perimyotis*

Perimyotis subflavus **(Cuvier, 1832) (TX) Tri-Colored Bat**

Localities: Bartow Co.: Alfords Cave, Anthonys Cave (GBT175)*, Chert Chasm (GBT340)*, Jolley Cave (GBT187), Kingston Saltpeter Cave (GBT11), Ladds Lime Cave (GBT384 to GBT389); Bleckley Co.: Whistling Cave/Taylor Cave (GBL460/GBL461); Catoosa Co.: Chapmans Cave (GCZ25), Chickamauga Cave (GCZ106)*, Welcome Hill Cave (GKH163), Lowry Cave (GKH206), Parkers Cave (GKH119), Smiths Cave, Subligna Cave (GKH145), Trion Dam Cave (GKH158); Dade Co.: Trenton Bone Cave (GDD16), Alabama-Georgia Cave (GDD511), Boxcar Cave (GDD69)*, Byers Cave (GDD66), Caboose Cave (GDD475)*, Case Cavern (GDD1), Cave 4 mi W of Trenton, Cemetery Pit (GDD64)*, Chambliss Cave (GDD321), Dead Horse Cave (GDD111), Gypsy Cave (GDD32), Ha-ha Cave (GDD256), Howards Waterfall Cave (GDD34)*, Hurricane Cave (GDD62)*, Johnsons Crook Cave (GDD17)*, Lapp Hole, Longs Rock Wall Cave (GDD101)*, Lower Valley Cave (GDD136)*, Rising Fawn Exit Cave (GDD397), Rusty's Cave (GDD70)*, Sittons Cave (GDD9), SSS Cave (GDD229)*, Upper Valley Cave (GDD135)*; Decatur Co.: "Bainbridge in Powell Hill Cave", Climax Cave (GDC36)*; Floyd Co.: Cave Spring Cave (GFL18), Osborn Cave (GFL220), Spout Springs Cave (GFL150); Gordon Co.: Rusty Cable Cave (GGO297)*; Grady Co.: Biscuits and Gravy Cave (GGR602), Glory Hole (GGR56)*, Maloys Waterfall Cave (GGR27)*, Long Swamp Creek Cave; Polk Co.: Deatons Cave (GPO5), White River Cave (GPO7)*; Randolph Co.: Griers Cave (GRA40); Union Co.: "Young Harris Bat Caves" (GUN28, GUN391 & GUN392); Walker Co.: Allen Springs Cave (GWK318), Anderson Spring Cave (GWK46), Dry Creek, Ellisons Cave (GWK51), Fricks Cave (GWK14), Goat Cave (GWK184)*, Harris Cave, Horseshoe Cave (GWK12)*, Pettijohns Cave (GWK29), Pigeon Cave (GWK57), Roger Branch Cave (GWK204)*, Shook Cave (GWK190), Spooky Cave (GWK494)*; Randolph Co.: J C Jones Cave (GRA207)*; Whitfield Co.: Ketchums Cave (GWT13).

Conservation status: IUCN: Vulerable; NatureServe: G2G3 (S2 in Georgia); considered a Species of Greatest Conservation Need in Georgia.

Comments: This species is the most common bat observed in Georgia caves during winter where it can be found hibernating individually or in small clusters on cave walls and ceilings. Like several *Myotis* species, *P. subflavus* is susceptible to White-nose Syndrome and population declines have been noted for several Georgia caves based on recent winter cave hibernacula surveys (Morris and Ferrall, 2018).

Order Didelphimorphia

Family Didelphidae

Genus *Didelphis*

Didelphis virginiana **Kerr, 1792 (AC) Virginia Opossum**

Localities: Walker Co.: Rocky Cave (GWK496)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S1 in Georgia).

Comments: Opossums have been reported from a few caves in the eastern United States (Dearolf, 1956; Cliburn and Middleton, 1983; Holler et al., in review).

Order Rodentia

Family Castoridae

Genus *Castor*

Castor canadensis **Kuhl, 1820 (TX) American Beaver**

Localities: Walker Co.: Mountain Cove Farm Cave #2 (GWK74)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: A collection of beaver-chewn branches was present in the cave. Beavers are known to build lodges inside the entrances of stream caves (e.g., Gore and Baker, 1989; Niemiller et al., 2016).

Family Cricetidae

Genus *Neotoma*

Neotoma floridana **(Ord, 1818) (TX) Eastern Woodrat**

Localities: Dade Co.: Afterbirth Cave (GDD153)*, Caboose Cave (GDD475)*, Case Cavern (GDD1), Jeff's Hole Cave (GDD400)*, Limestone Caverns (GDD140)*, Lower Valley Cave (GDD136)*, Sittons Cave (GDD9), SSS Cave (GDD229)*; Walker Co.: Anderson Spring Cave (GWK46), Bee Rock Cave (GWK123)*, Ellisons Cave (GWK51), Fingerhole Cave (GWK259)*, Fricks Cave (GWK14), Horseshoe Cave (GWK12)*, Kinda Pretty Cave (GWK258)*, Mouldy Bat Pit (GWK257)*, Mountain Cove Farm Cave #2 (GWK74)*, Nash Waterfall Cave (GWK72), Pettijohns Cave (GWK29), Pigeon Cave (GWK57), Rocky Cave (GWK496)*, Spooky Cave (GWK494)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: Both *Neotoma floridana* and *N. magister* (Allegheny Woodrat) occur in Georgia, although the contact zone in Georgia is unclear. Here we treat all records as *N. floridana*, but note that some occurrences may represent *N. magister*. Several records are based on indirect evidence of inhabitation, such as the presence of latrines, caches, and nests.

Class Reptilia
Order Squamata
Family Colubridae
Genus *Carphophis*
***Carphophis amoenus* (Say, 1825) (AC) Eastern Worm Snake**

Localities: Walker Co.: Flowing Stone Cave (GWK524)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: This accidental also has been reported from caves and mines in West Virginia (Pauley, 1993; Osbourn, 2005).

Genus *Cemophora*
***Cemophora coccinea* (Blumenbach, 1788) (AC) Scarlet Snake**

Localities: Greene Co.: Parrott Cave*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S4S5 in Georgia).

Comments: To our knowledge, this is the first report of this species from a cave.

Genus *Diadophis*
***Diadophis punctatus* (Linnaeus, 1766) (AC) Ringneck Snake**

Localities: Walker Co.: Screech Owl Cave (GWK205)*, Kinda Pretty Cave (GWK258).

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Georgia).

Comments: Records from caves likely represent individuals washed into caves during flood events or that fall into pits (e.g., Osbourn, 2005; Niemiller et al., 2016).

Genus *Nerodia*
***Nerodia sipedon* (Linnaeus, 1758) (AC) Northern Water Snake**

Localities: Walker Co.: Anderson Springs Cave (GWK46)*.

Comments: This species also has been reported from caves in West Virginia (Osbourn, 2005).

Order Testudines
Family Emydidae
Genus *Terrapene*
***Terrapene carolina* (Linnaeus, 1758) (AC) Eastern Box Turtle**

Localities: Dade Co.: Boxcar Cave (GDD69).

Conservation status: IUCN: Vulnerable; NatureServe: G5 (S5 in Georgia).

Comments: This species commonly falls into pits or washes into caves during flood events (e.g., Osbourn, 2005; Niemiller et al., 2016).

Discussion

Summary of biological records

Our review of biodiversity in Georgia caves and other subterranean habitats identified 281 species, including 228 invertebrates and 53 vertebrates (Table S3) represented by more than 1200 occurrence records. There are ~350 additional records of taxa that have not been identified to species (Table S2). Of the taxa identified to species, five phyla (Annelida, Arthropoda, Chordata, Mollusca, and Platyhelminthes) are represented. The arthropods are the most diverse group documented with 195 species, including 50 arachnids, 34 crustaceans, and 78 insects. Fifty-one cave-obligate species (34 troglobites and 17 stygobites) have been reported from Georgia, and as many as a dozen additional troglobionts have been mentioned in the literature but are undescribed.

The biodiversity of a few caves has been studied using baits and repeated visits (e.g., Reeves and McCreadie, 2001; Campbell et al., 2011, 2012; Disney and Campbell, 2011). The application of such approaches in Howards Waterfall Cave (Dade County, with 88 total records), Horseshoe Cave (Walker County, 68 records), Pettijohns Cave (Walker County, 64 records), and Byers Cave (Dade County, 61 records) have provided the broadest picture of Georgia cave biodiversity.

Vertebrates

Fifty-three species of vertebrates have been reported from Georgia caves, including four fishes, 27 amphibians, five reptiles, two birds, and 15 mammals (Table 1). Mammal diversity was predominantly bats, with ten species. Most vertebrates reported from Georgia caves are accidental or occasional visitors, but several species of salamanders are common in caves, such as *Eurycea lucifuga* and *Gyrinophilus porphyriticus*, as are several cave-roosting bats and woodrats. Three vertebrate species are considered cave-obligates: the cavefish *Typhlichthys subterranneus* and the salamanders *Eurycea wallacei* and *Gyrinophilus palleucus*. All three species are known from few (≤5) verified occurrences in the state, but also occur in adjacent states. The salamander *Plethodon petraeus* is endemic to Pigeon Mountain in Walker County. All the other vertebrates have been reported from caves in other states (Lewis, 2005; Godwin, 2008; Niemiller et al., 2016).

Bats

Ten species of bats have been reported from caves across Georgia, and the Tri-Colored Bat (*Perimyotis subflavus*) is known from more caves (67) in more counties (16) than any other animal in the state (Table 1). However, most bats are known from few caves, and cave-dwelling bats, in general, are of great conservation interest. Seven are “High Priority Species” in the current State Wildlife Action Plan (Georgia Department of Natural Resources; 2015) (Table 1). In addition, Rafinesque’s Big-eared Bat (*Corynorhinus rafinesquii*) is designated “Rare” by Georgia, the Northern Long-eared Bat (*Myotis septentrionalis*) is listed as “Threatened” under the U.S. Endangered Species Act and by the state of Georgia, and the Gray Bat (*Myotis grisescens*) and the Indiana Bat (*Myotis sodalis*) are both listed as “Endangered” under the U.S. Endangered Species Act and by the state of Georgia. Critical population centers for bats in Georgia include Fricks Cave in Walker County, which hosts a bachelor colony of Gray Bats during the summer, and Climax Cave in Decatur County, a major site for Southeastern Myotis (*Myotis austroriparius*). Fricks Cave is owned by the Southeastern Cave Conservancy, Inc. and is generally closed to visitation (currently it is open just one day a year during the winter).

Table 1. Ecological classification, conservation status, and number of Georgia caves and counties from which vertebrate species have been documented. .

Species	Ecological classification	IUCN Red List[a]	NatureServe status	Government status[b]	Caves/ Wells[c]	Counties[c]	Georgia endemic?
Fishes							
Ameiurus nebulosus	SX/AC	LC	G5, S5		1	1	
Cottus bairdii	SP	LC	G5, S4		1	1	
Cottus carolinae	SP	LC	G5, S4		4	2	
Typhlichthys subterraneus	SB	VU	G4, S1	SE, SGCN	5	2	
Amphibians (Frogs and Toads)							
Anaxyrus fowleri	AC	LC	G5, S5		1	1	
Anaxyrus terrestris	AC	LC	G5, S5		1	1	
Hyla chrysoscelis	TX/AC	LC	G5, S5		1	1	
Hyla gratiosa	AC	LC	G5, S5		1	1	
Rana catesbeiana	TX	LC	G5, S5		5	3	
Rana clamitans	TX	LC	G5, S5		9	5	
Rana palustris	TX	LC	G5, S4		11	2	
Rana sphenocephala	TX/AC	LC	G5, S5		1	1	
Pseudacris crucifer	AC	LC	G5, S5		3	2	
Pseudacris feriarum	AC	LC	G5, S5		1	1	
Amphibians (Salamanders)							
Ambystoma tigrinum	AC	LC	G5, S3S4	SGCN	1	1	
Aneides aeneus	TX	NT	G3G4, S3	SR, SGCN	8	2	
Desmognathus conanti	AC	LC	G5, S5		8	3	
Desmognathus ocoee	AC	LC	G5, S5		1	1	
Eurycea cirrigera	TX	LC	G5, S5		6	4	
Eurycea guttolineata	TX	LC	G5, S4S5		2	2	
Eurycea longicauda	TP/TX	LC	G5, S4		11	3	
Eurycea lucifuga	TP	LC	G5, S4		52	9	
Eurycea wallacei	SB	VU	G2, S1	ST, SGCN	3	2	
Gyrinophilus palleucus	SB	VU	G2G3, S1	ST, SGCN	2	1	
Gyrinophilus porphyriticus	TP	LC	G5, S4		20	2	
Notophthalmus viridescens	AC	LC	G5, S5		1	1	
Plethodon glutinosus	TP	LC	G5, S5		44	7	
Plethodon petraeus	TP/TX	VU	G2, S2	SR, SGCN	4	1	Yes
Plethodon serratus	TX	LC	G5, S5		4	1	
Plethodon ventralis	TP/TX	LC	G4, S4		14	2	
Pseudotriton ruber	TP	LC	G5, S5		8	2	
Reptiles (Snakes)							
Carphophis amoenus	AC	LC	G5, S5		1	1	
Cemophora coccinea	AC	LC	G5, S4S5		1	1	
Diadophis punctatus	AC	LC	G5, S5		2	1	
Nerodia sipedon	AC	LC	G5, S5		1	1	
Reptiles (Turtles)							
Terrapene carolina	AC	VU	G5, S5		1	1	
Birds							
Cathartes aura	TX/AC	LC	G5, S5		1	1	
Sayornis phoebe	TX	LC	G5, S5		4	4	
Mammals (Bats)							
Corynorhinus rafinesquii	TX	LC	G3G4, S3	SR, SGCN	1	1	
Eptesicus fuscus	TX	LC	G5, S5		4	3	

Table 1. (Continued).

Species	Ecological classification	IUCN Red List[a]	NatureServe status	Government status[b]	Caves/ Wells[c]	Counties[c]	Georgia endemic?
Lasiurus borealis	AC	LC	G3G4, S5		2	2	
Myotis austroriparius	TX	LC	G4, S3	SGCN	4	4	
Myotis grisescens	TX	VU	G4, S1	FE, SE, SGCN	8	5	
Myotis leibii	TX	EN	G4, S2		3	2	
Myotis lucifugus	TX	EN	G3, S1	SGCN	10	4	
Myotis septentrionalis	TX	NT	G1G2, S2S1	FT, ST, SGCN	13	6	
Myotis sodalis	TX	NT	G2, S1	FE, SE, SGCN	4	2	
Perimyotis subflavus	TX	VU	G2G3, S2	SGCN	67	16	
Mammals (non-Bats)							
Castor canadensis	TX	LC	G5, S5		1	1	
Didelphis virginiana	AC	LC	G5, S5		1	1	
Neovision vison	AC	LC	G5, S5		1	1	
Neotoma floridana	TX	LC	G5, S5		22	2	
Procyon lotor	TX	LC	G5, S5		13	5	

[a] IUCN Red List: LC = Least Concern, VU = Vulnerable, NT = Near Threatened, EN = Endangered

[b] Government Status: FE = Federally Endangered, FT = Federally Threatened, SE = State Endangered, ST = State Threatened, SR = State Rare, SGCN = Species of Greatest Conservation Need (= State Wildlife Action Plan High Priority Species)

[c] Caves/Wells and Counties refer to sites in Georgia only

Ecological classifications include: TB = Troglobiont, SB = Stygobiont, TP = Troglophile, SP = Stygophile, TX = Trogloxene, SX = Stygoxene, and AC = Accidental. IUCN Red List categories include: LC = Least Concern, VU = Vulnerable, NT = Near Threatened, EN = Endangered. Government status categories include: FE = Federally Endangered, FT = Federally Threatened, SE = State Endangered, ST = State Threatened, SR = State Rare, SGCN = Species of Greatest Conservation Need (= State Wildlife Action Plan High Priority Species)

Table 2. Ecological classification, conservation status, and number of Georgia caves and counties from which troglobionts have been documented. Abbreviations are the same as in Table 1, with the addition of SY = Symbiont.

Species	Ecological classification	IUCN Red List[a]	NatureServe status	Government status[b]	Caves/ Wells[c]	Counties[c]	Georgia endemic?
Arachnids (pseudoscorpions)							
Apochthonius minor	TB		G1, SNR		2	2	Yes
Hesperochernes mirabilis	TB		G5, SNR		16	5	
Kleptochthonius magnus	TB		G1, SNR		2	2	
Arachnids (spiders)							
Appaleptoneta fiskei	TB		GNR, SNR		2	1	Yes
Liocranoides unicolor	TB		G5, SNR		7	3	
Nesticus georgia	TB		G1G2, SNR		3	1	Yes
Ozarkia georgia	TB		GNR, SNR		3	1	Yes
Phanetta subterranea	TB		G5, SNR		12	3	
Porrhomma cavernicola	TB		G5, SNR		1	1	
Crustaceans (amphipods)							
Crangonyx antennatus	SB		G5, SNR		22	5	
Stygobromus ackerlyi	SB		G1G2, SNR		3	3	Yes
Stygobromus dicksoni	SB		G5, SNR		7	3	
Stygobromus doughertyensis	SB		GNR, SNR		1	1	
Stygobromus grandis	SB		G1, SU		1	1	Yes
Stygobromus minutus	SB		G2G3, SU		2	1	Yes
Crustaceans (copepods)							
Megacyclops donnaldsoni	SB		G3G4, SNR		1	1	
Crustaceans (crayfish)							
Cambarus cryptodytes	SB	LC	G2G3, S2	ST, SGCN	17	8	
Crustaceans (isopods)							
Amerigoniscus curvatus	TB		G1, SU		1	1	Yes
Amerigoniscus georgiensis	TB		G1, SU		1	1	Yes
Amerigoniscus proximus	TB		G1G2, SNR		2	2	Yes
Caecidotea cyrtorhynchus	SB		G1, SU		3	1	Yes
Caecidotea hobbsi	SB		G2G3, SNR		1	1	

Table 2. (Continued).

Species	Ecological classification	IUCN Red List[a]	NatureServe status	Government status[b]	Caves/ Wells[c]	Counties[c]	Georgia endemic?
Crustaceans (isopods)							
Caecidotea nickajackensis	SB		GH, SNR		1	1	
Caecidotea putea	SB		G1G2, SNR		2	2	
Caecidotea richardsonae	SB		G5, SNR		16	5	
Crustaceans (ostracods)							
Uncinocythere warreni	SB/SY		G1, SU		1	1	Yes
Diplurans							
Litocampa cookei	TB		G5, SNR		1	1	
Insects (beetles)							
Batriasymmodes spelaeus	TB/TP		G3G4, SNR		4	3	
Pseudanophthalmus digitus	TB		G1G2, SNR		3	1	
Pseudanophthalmus fastigatus	TB		G1, S1?		1	1	Yes
Pseudanophthalmus fulleri	TB		G2G3, SNR		12	1	
Pseudanophthalmus georgiae	TB		G1G2, S1?		4	2	Yes
Ptomaphagus fiskei	TB		G1G2, SNR		10	1	Yes
Ptomaphagus whiteselli	TB		G2G3, SNR		8	1	
Insects (flies)							
Spelobia tenebrarum	TB		G5, SNR		12	3	
Springtails							
Pseudosinella christianseni	TB		G5, SNR		15	2	
Pseudosinella hirsuta	TB		G5, SNR		15	5	
Pseudosinella pecki	TB		G2G3, SNR		2	2	
Pseudosinella spinosa	TB		G5, SNR		1	1	
Myriapods (millipedes)							
Ameractis satis	TB/TP		G2G4, SNR		1	1	
Pseudotremia aeacus	TB		G1G2, SNR		2	1	
Pseudotremia eburnea	TB		G2G4, SNR		15	2	
Scoterpes austrinus	TB		G3G4, SNR		16	3	
Scoterpes nudus	TB		G3G4T1T2, SNR		3	2	Yes
Scoterpes willreevesi	TB		GNR, SNR		2	1	
Snails							
Glyphyalinia specus	TB	LC[d]	G3, SNR		6	3	
Helicodiscus barri	TB	LC[d]	G3, SNR		2	2	
Flatworms							
Sphalloplana georgiana	SB		G1, SNR		1	1	Yes
Vertebrates (fish, salamanders)							
Eurycea wallacei	SB	VU	G2, S1	ST, SGCN	3	2	
Gyrinophilus palleucus	SB	VU	G2G3, S1	ST, SGCN	2	1	
Typhlichthys subterraneus	SB	VU	G4, S1	ST, SGCN	5	2	

[a] IUCN Red List: LC = Least Concern, VU = Vulnerable

[b] Government Status: SE = State Endangered, ST = State Threatened, SGCN = Species of Greatest Conservation Need (= State Wildlife Action Plan High Priority Species)

[c] A count of the number of occurrences. Caves/Wells and Counties refer to sites in Georgia only

[d] After Gladstone et al. 2018

Many bat populations across eastern North America are in decline as a result of White-nose Syndrome (WNS), which is caused by the fungus *Pseudogymnoascus destructans* (Blehert et al., 2009). First confirmed in northwestern Georgia during the winter of 2012–2013, WNS is now known from many counties in north Georgia (https://www.whitenosesyndrome.org/spreadmap). Over the past decade, WNS has impacted some cave-dwelling bats more than others; Tri-Colored Bats, Northern Long-eared Bats, Indiana Bats, and Little Brown Bats (*Myotis lucifugus*) have suffered steep population declines, whereas Gray Bats and Big Brown Bats (*Eptesicus fuscus*) have not (Francl et al., 2012; Campbell, 2017; Morris and Ferrall, 2018). Declines in bat populations, especially over such rapid timescales, will undoubtedly af-

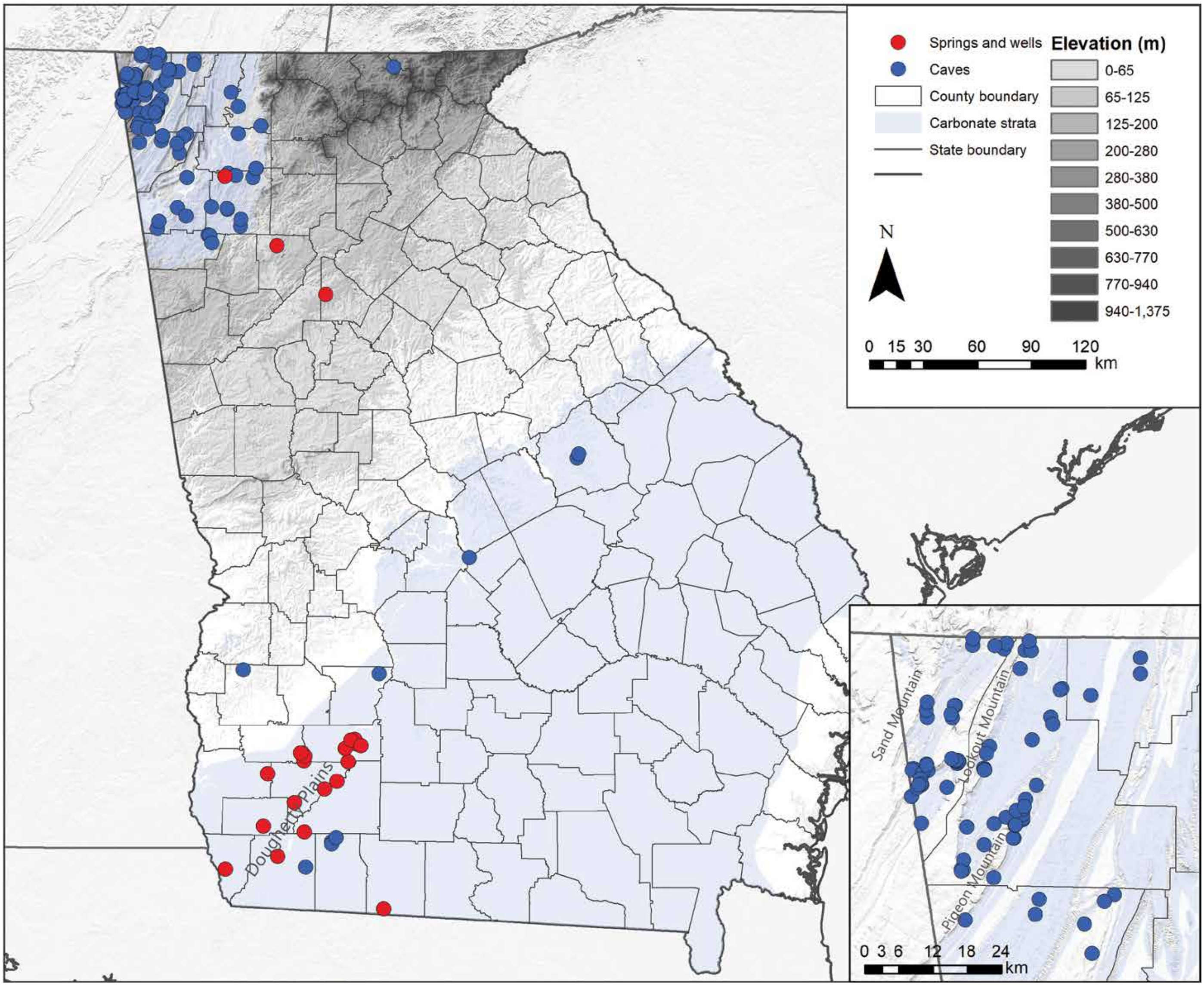

Figure 1. Distribution of the 142 georeferenced subterranean sites with biological records in Georgia. Caves are indicated by blue points, springs and wells by red points. Carbonate (karst) strata are indicated by light blue shading. An enlarged view of northwest Georgia is provided in the inset at the lower right. Geographic features mentioned in the paper are labeled. Cave locality data are from the Georgia Speleological Survey.

fect cave ecosystems because cave-dwelling bats are an important link between surface resources and cave habitats.

Troglobionts

Fifty-one species reported from caves in Georgia are considered troglobionts (34 terrestrial and 17 aquatic). Troglobitic diversity includes four phyla (Arthropoda, Mollusca, Platyhelminthes, and Chordata). Troglobitic species richness in Georgia is dominated by arthropods (45 species), distributed across the major arthropod subgroups of crustaceans (17 species), hexapods (13), arachnids (9), and myriapods (6). Two snails, one flatworm, and three vertebrates compose the remainder of the described troglobiotic fauna in the state (Table 2).

Biogeography

Nearly half of the Georgia's troglobionts are found in one of three geographically and hydrogeologically distinct areas: (1) west of Lookout Mountain in Lookout Valley; (2) east of Lookout Mountain, and (3) in the Dougherty Plains (Fig. 1). The taxa composing each group have ranges that do not overlap with members of the other groups.

Lookout Valley, primarily in Dade County, Georgia, is located west of Lookout Mountain and east of Sand Mountain (Fig. 1). This area is the southernmost extension of the Cumberland Plateau. Lookout Valley extends beyond Dade County to the north into Hamilton County, Tennessee, and to the south into DeKalb County, Alabama. At least seven troglobionts appear to be limited to Lookout Valley – the millipedes *Scoterpes willreevesi* and *Pseudotremia aeacus*, the

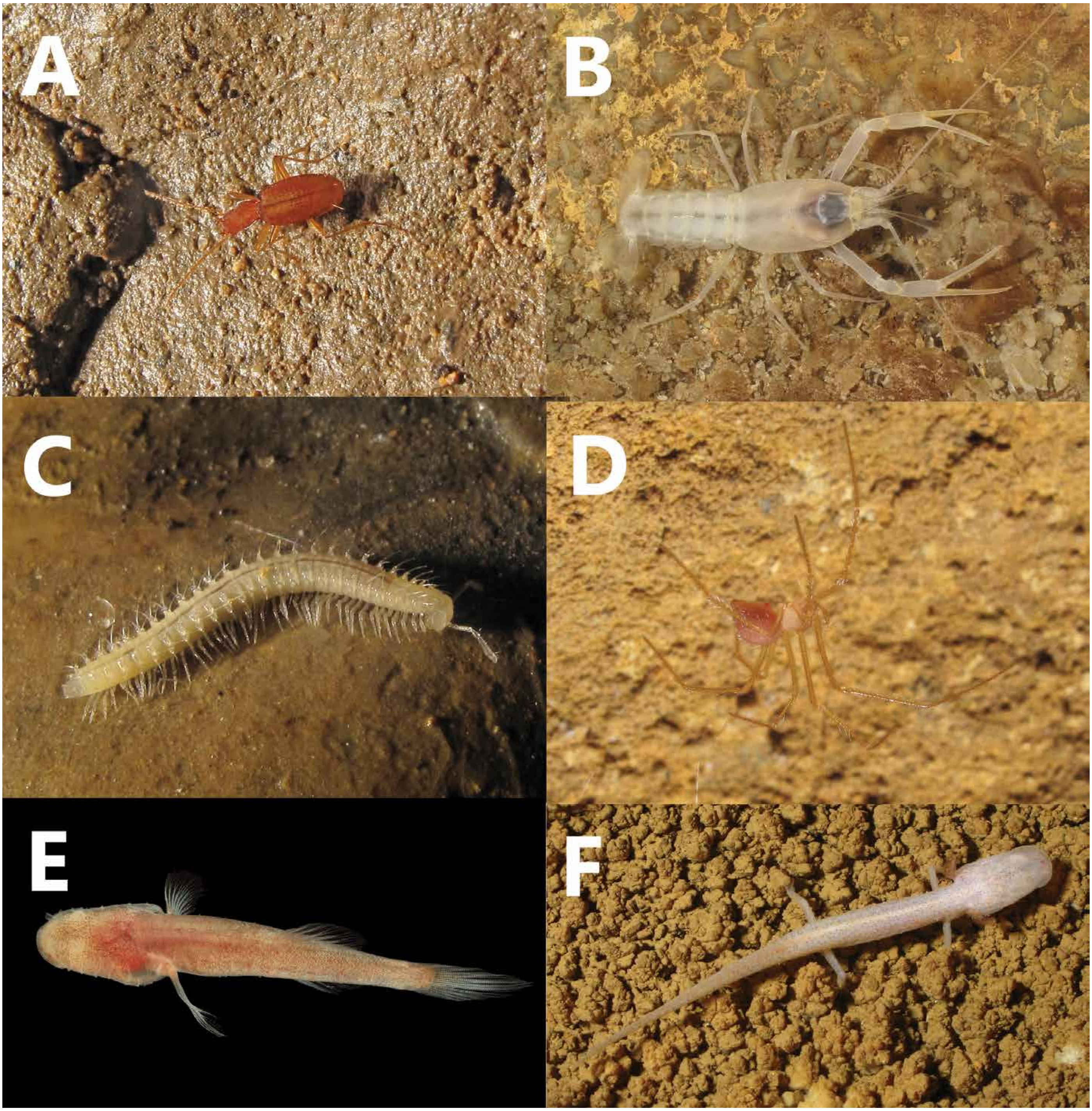

Figure 2. Representative troglobiotic fauna from Georgia caves: A) *Pseudanophthalmus* sp. from Four Kings Cave, Walker County; B) *Cambarus cryptodytes* from Climax Cave, Decatur County; C) *Scoterpes nudus* from White River Cave, Polk County; D) *Nesticus georgia* from Case Cave, Dade County; E) *Typhlichthys subterraneus* from Crane Cave, Catoosa County; and F) *Eurycea wallacei* from Salamander Cave, Jackson County, Florida. Photographs by Alan Cressler.

beetles *Ptomaphagus whiteselli*, *Pseudanophthalmus digitus*, and *Pseudanophthalmus fulleri*, and the spiders *Nesticus georgia* and *Ozarkia georgia*. The single-cave endemic flatworm *Sphalloplana georgiana* is another species known only from Lookout Valley, but unidentified *Sphalloplana* have been reported from Walker County, which may indicate a wider range for the species.

A second biogeographic group is located east of Lookout Mountain in Walker and Chattooga counties (Fig. 1). This group includes the beetles *Ptomaphagus fiskei* and *Pseudanophthalmus georgia*, the isopod *Caecidotea cyrtorhynchus*, the amphipod *Stygobromus minutus*, and the spider *Appaleptoneta fiskei*. In addition, four single-cave endemic

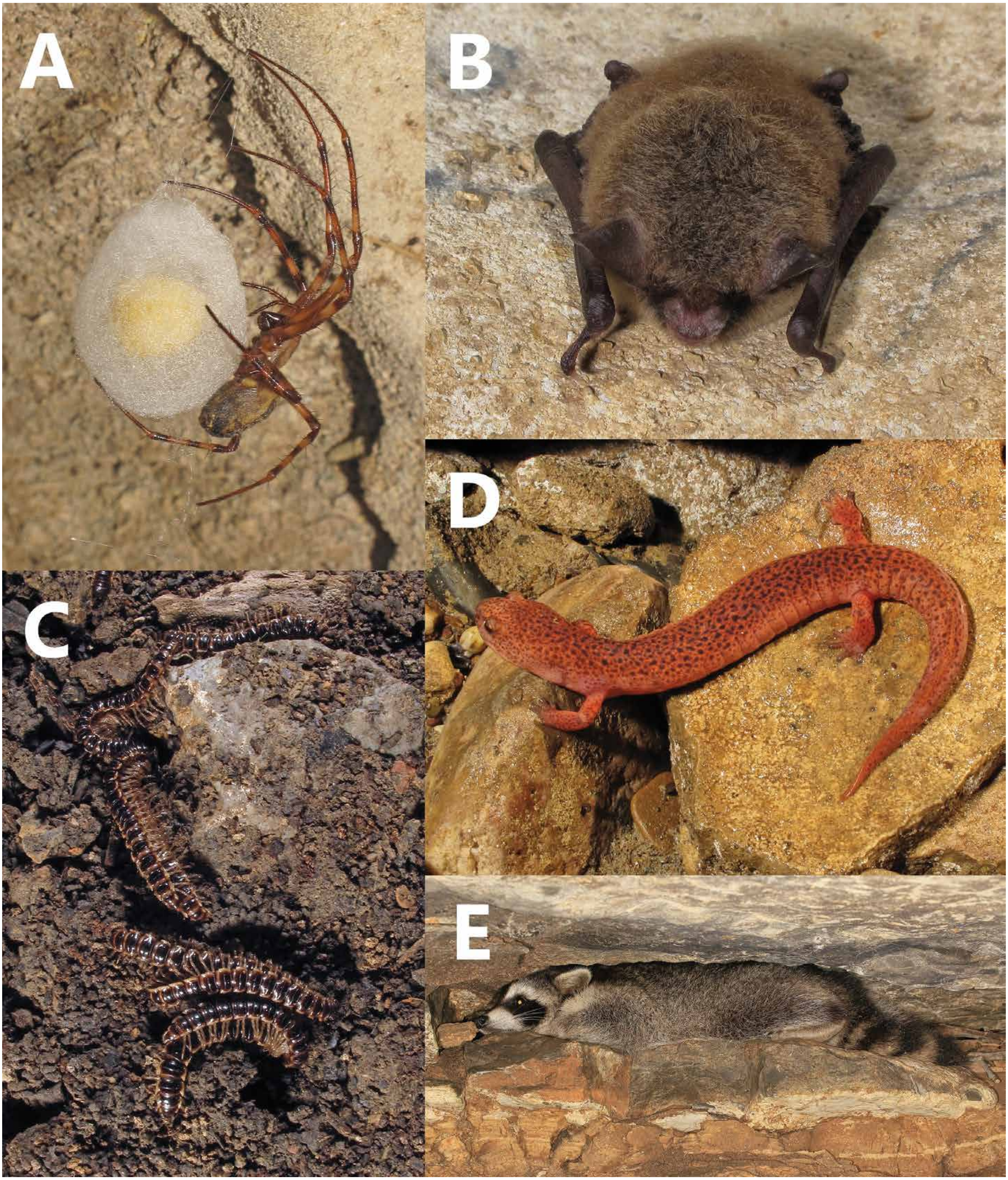

Figure 3. Representative non-troglobiotic fauna from Georgia caves: A) female *Meta ovalis* with egg case from Four Kings Cave, Walker County; B) *Myotis lucifugus* from Fricks Cave, Walker County; C) *Oxidus gracilis* from White River Cave, Polk County; D) *Pseudotriton ruber* from Fricks Cave, Walker County; and E) *Procyon lotor* from Trenton Bone Cave, Dade County. Photographs by Alan Cressler.

species are found in this region: the beetle *Pseudanophthalmus fastigatus*, the amphipod *Stygobromus grandis*, and the isopods *Amerigoniscus curvatus* and *A. georgiensis*. The troglophilic springtail *Pseudosinella georgia* is also known only from this area. Within this group, there is a cluster of troglobionts limited to Pigeon Mountain – *C. cyrtorhynchus*, *S. minutus*, *A. fiskei,* and an undescribed *Nesticus* species. The cave-associated Pigeon Mountain Salamander (*Plethodon petraeus*) is also limited to Pigeon Mountain. Slightly further south, the millipede *Scoterpes nudus* and the amphipod *Stygobromus ackerlyi* are limited to caves in Bartow, Floyd, and Polk counties.

Lastly, several stygobitic taxa are limited to the Floridan aquifer system of the Dougherty Plains in southwestern Georgia near the Georgia-Alabama-Florida state junction (Fig. 1). The Dougherty Plains Cave Crayfish (*Cambarus cryptodytes*) is known from seven counties in Georgia (Fenolio et al., 2017), with a range that extends into Florida. The Georgia Blind Salamander (*Eurycea wallacei*) shares a similar range (Fenolio et al., 2013), as does the amphipod *Stygobromus doughertyensis* (Cannizaro et al., 2019). The single-cave endemic ostracod *Uncinocythere warreni* is known only from its host *C. cryptodytes.*

The remaining half of Georgia's troglobionts are found in one or more of the biogeographic clusters described above, and some have ranges that include much of the southern Appalachians and Interior Low Plateaus. Within these species, at least three patterns are present: (1) two stygobionts, the amphipod *Crangonyx antennatus* and the isopod *Caecidotea richardonae*, are common throughout the Appalachian Valley and Ridge, ranging from southern Virginia into Alabama; (2) two vertebrates, the Tennessee Cave Salamander (*Gyrinophilus palleucus*) and the Southern Cavefish (*Typhlichthys subterraneus*), are widespread west of the Cumberland Plateau (Niemiller et al., 2008; 2012) and appear to have spread east via the ancestral Tennessee River and associated drainage network into one or a few sites in northwestern Georgia (e.g., Niemiller et al., 2016); and, (3) a few of species are widespread in caves across the southern Appalachians and the Interior Low Plateaus, such as the spiders *Phanetta subterranea* and *Porrhomma cavernicola*, the fly *Spelobia tenebrarum*, the pseudoscorpion *Hesperochernes mirabilis*, and the springtail *Pseudosinella hirsuta* (Christman and Culver, 2001). Some of these taxa may represent cryptic species complexes of morphologically similar, yet genetically distinct, lineages. Cryptic diversity is a common discovery from phylogeographic studies of subterranean organisms (e.g., Bradford et al., 2010; Niemiller et al., 2012; Katz et al., 2018). The remaining troglobionts in Georgia do not fit into the patterns described above. Species from poorly known groups, like pseudoscorpions and springtails, compose many of the remaining taxa. For some species, a lack of records prevents any confident description of their distribution within Georgia and beyond.

Endemism

Troglobionts typically exhibit high rates of endemism (Christman et al., 2005), and we noted this pattern in the Georgia fauna. Seventeen of the 51 (33 %) troglobionts known from Georgia are endemic to the state (Table 2). Thirteen of these species (26 %) are limited to a single county, and six species (12 %) are known from a single cave. These single-cave endemics are the beetle *Pseudanophthalmus fastigatus* (Horseshoe Cave, Walker County), the flatworm *Sphalloplana georgiana* (Howards Waterfall Cave, Dade County), the isopods *Amerigoniscus curvatus* (Horseshoe Cave, Walker County) and *A. georgiensis* (Pettijohns Cave, Walker County), the amphipod *Stygobromus grandis* (Parkers Cave, Chattooga County), and the ostracod *Uncinocythere warreni* (Climax Cave, Decatur County). Several other cave-associated non-troglobionts, such as the Pigeon Mountain Salamander, the springtail *Pseudosinella georgia*, the caddisfly *Diplectrona marianae*, and the spiders *Pholcus dade* and *Pholcus lanieri*, are also endemic to Georgia and have highly restricted ranges.

Hotspots of troglobionts

Troglobionts are not uniformly distributed across Georgia. Of the 670 caves known in the state, only 22 (3.2 %) are known to host five or more troglobionts, with 11 of those caves being in Dade County, nine in Walker County, and two in Chattooga County. Eight caves support ten or more troglobionts, these are: Pettijohns Cave (14 troglobionts), Byers Cave (13), Johnsons Crook Cave (12), Mountain Cove Farm Cave No. 1 (11), Howards Waterfall Cave (11), Cemetery Pit (11), Morrisons Cave (10), and Sittons Cave (10). Of these caves, six are in Dade County and two (Pettijohns Cave and Mountain Cove Farm Cave No. 1) are in Walker County. Maximum troglobiont diversity per cave is not as high in Georgia as in Tennessee, which has 24 troglobionts known from the Wonder/Crystal Cave system in Grundy County, or Alabama, where 24 troglobionts are known from Shelta Cave in Madison County. However, both Pettijohns Cave and Byers Cave would rank in the top ten caves in the state of Tennessee in terms of total troglobionts (Niemiller and Zigler, 2013).

Conservation considerations

The cave fauna of Georgia is diverse and includes numerous species of conservation concern, as well as many species with highly restricted ranges. This review provides background for conservation efforts related to cave biodiversity in Georgia.

Species of conservation concern and threats to subterranean ecosystems. Many of the species found in Georgia caves are at an elevated risk of extinction because of their extremely small ranges. Of the troglobionts, 17 (33 %) are ranked "G1—Critically Imperiled" under NatureServe criteria, and the four troglobionts not ranked by NatureServe would likely be considered "G1" as well (Table 2). All of these species are considered short-range cave endemics (e.g., Niemiller et al., 2017), known from just a few sites within a limited geographic area. In addition, the Southern Cavefish, the Tennessee Cave Salamander, and the Dougherty Plains Cave Crayfish are ranked "S1—Critically Imperiled" in Georgia (Table 2). Although all three species are more wide-ranging in adjacent states, they are intrinsically vulnerable to extinction, as are most troglobionts (Culver et al., 2006; Culver and Pipan, 2009; Niemiller et al., 2018).

Cave communities can be impacted by modification of the surface landscape around caves and cave entrances, by water pollution that enters or moves through caves, or by human disturbance of cave habitats and populations. In the longer term, climate change may impact caves due to changing temperature and precipitation patterns, and indirectly by any changes in forest cover that result. In addition, the emerging infectious disease WNS has been present in Georgia for less than a decade. It appears to be affecting bat populations, in particular those of the Tri-Colored Bat, the most commonly encountered cave bat in Georgia (Morris and Ferrall, 2018). It will be some time before we reach a new steady state for bat population densities and distributions.

Caves on protected lands. Our Annotated List shows that, after half a century of work, there is a good deal known about cave biodiversity in Georgia. There are biological records from 18 % (121 of 670) of Georgia caves, a higher frequency than reported for Tennessee, where 7 % of caves have records of troglobionts (Niemiller and Zigler, 2013). In addition, a remarkably large proportion of the caves in Georgia are on protected lands. According to the records of the Georgia Speleological Survey, 165 caves are on property owned by federal, state, or local government. Government landholdings with significant numbers of caves include Chickamauga and Chattanooga National Military Park, Crockford-Pigeon Mountain Wildlife Management Area, and Cloudland Canyon State Park. At least 60 other caves are located on property owned or managed by land trusts, the Southeastern Cave Conservancy, Inc., or the National Speleological Society. In combination, around one third of all caves in Georgia are located on protected lands. Notably, many caves of particular biological importance are protected. For instance, of the eight caves known to host the ten or more troglobionts, seven are on protected lands. Several of these caves are well known (e.g., Pettijohns Cave, Howards Waterfall Cave), and receive regular visitation, which may be detrimental to cave communities.

Cave biodiversity knowledge shortfalls. Although much is known about cave biodiversity in Georgia, significant knowledge gaps remain, similar to subterranean biodiversity globally (Niemiller et al., 2018; Ficetola et al., 2019 ; Mammola et al., 2019). Although state-level conservation assessments for vertebrates are almost universally complete, such assessments are almost completely lacking for invertebrates (Tables 1, 2, and S3). Of the 49 invertebrate troglobionts known in Georgia, only one, the Dougherty Plains Cave Crayfish, has a state ("S") ranking under the NatureServe system, and only six have been ranked using IUCN Red List criteria (Table 2). As many of these invertebrates have highly restricted distributions, state-level conservation assessments are particularly valuable. Most of the species have global ("G") rankings (Table 2), which should facilitate developing state rankings for the species. As models for how this could be done, recently published conservation assessments for *Bactrurus* cave amphipods (Niemiller and Taylor, 2016) and cave snails of the Interior Low Plateau and Appalachians karst regions (Gladstone et al., 2018) implemented both NatureServe and IUCN Red List assessment criteria, while Hutchins (2018) evaluated the conservation status of Texas groundwater invertebrates using the NatureServe methodology.

For most troglobionts in Georgia, we lack information about population sizes, population trends, and species distributions (i.e., the Prestonian and Wallacean shortfalls; Lomolino, 2004, Cardoso et al., 2011). Of the 17 troglobionts endemic to Georgia, only one is known from more than four sites (Table 2). In many cases, species are known from just one or a few collections, which limits our ability to assess population trends or persistence. As a specific example, the single-cave endemic beetle *Pseudanophthalmus fastigatus* was described from just two specimens collected in 1967 from Horseshoe Cave in Walker County. This species has not been collected since, and recent work in the cave (Reeves and McCreadie, 2001; this study) did not rediscover this population. Recent work on other *Pseudanophthalmus* species in Tennessee has shown that focused efforts often confirm the presence of long-lost populations and uncover new populations (Niemiller et al., 2017). Similar efforts are warranted for the many poorly-known troglobionts in Georgia.

More than two dozen undescribed species have been collected in caves in Georgia (Table 3). About half of these taxa are likely troglobionts, indicating a significant proportion of troglobiotic diversity in Georgia has not yet been described (i.e., the Linnaean shortfall; Brown and Lomolino, 1998). As discussed by Culver et al. (2013), not all of these taxa may turn out to be new species once they have been examined by taxonomic experts, but it is likely that many of them will be formally described. These taxa are dispersed across the major groups of arthropods and across the major karst regions of Georgia. As far as is currently known, many of these taxa could be single-cave endemics, which makes them conservation concerns. A full understanding of Georgia cave biodiversity will require the taxonomic evaluation of these taxa.

Table 3. Undescribed species reported from caves in Georgia.

Taxon	Cave(s)	Comments	References
Arachnids (harvestmen)			
Phalangodidae: *Bishopella*	Dade County: Howards Waterfall Cave	Described as "potentially troglobitic."	Reeves et al. (2000)
Arachnids (mites)			
Rhagidiidae: *hagidia*	Bartow County: Kingston Saltpeter Cave; Dade County: Byers Cave, Morrison Cave; Walker County: Bible Springs Cave, Pettijohns Cave		Holsinger and Peck (1971)
Arachnids (pseudoscorpions)			
Chthoniidae: *Aphrastochthonius*	Dade County: Byers Cave, Longs Rock Wall		Campbell et al. (2012); this study
Chthoniidae: *Apochthonius*	Chattooga County: Parker Cave	One large female from entrance zone.	this study
Chthoniidae: *Chthonius*	Walker County: Howards Waterfall Cave, Horseshoe Cave	Two undescribed species represented.	this study
Chthoniidae: *Kleptochthonius*	Walker County: Rumble Rock Canyon Cave		this study
Chthoniidae: *Mundochthonius*	Chattooga County: Parker Cave	Many collected from entrance zone.	Muchmore unpublished; this study
Chthoniidae	Walker County: Mt. Cove Farm Cave	From gut of *Eurycea lucifuga* found in dark zone. Partial specimen of an adult male. "Potentially new" because he did not place it in a genus.	Muchmore unpublished
Neobisiidae: *Lissocreagris*	Walker County: Pettijohn Cave	Small, eyeless.	Holsinger and Peck (1971); Muchmore unpublished; this study
Neobisiidae: *Microcreagris* (sensu lato)	Dade County: Johnson Crook Cave	Potentially an error, not in Muchmore's material. Most Nearctic *Microcreagris* were transferred to other genera by Ćurčić (1981, 1984, 1989).	Holsinger and Peck (1971); Muchmore unpublished; this study
Neobisiidae: *Microcreagris* (sensu lato)	Dade County: Hooker Cave		this study
Arachnids (spiders)			
Linyphiidae: *Anibontes*	Chattooga County: Parkers Cave		this study
Nesticidae: *Nesticus* n. sp. 1	Walker County: Anderson Spring Cave, Matthew Sink, Pigeon Cave (also possibly Mouldy Bat Pit and Fingerhole Cave)	All sites on Pigeon Mountain. Eyeless.	Buhlmann (2001); Jensen and Ozier; this study
Nesticidae: *Nesticus* n. sp. 2	Walker County: Lula Falls Cave	Eyed.	this study
Pholcidae: *Pholcus*	Bartow County: Ladds Lime Cave; Dade County: Hurricane Cave, Sittons Cave; Walker County: Fricks Cave, Spooky Cave	Described as "several undescribed species of *Pholcus*."	Reeves et al. (2000)
Crustaceans (amphipods)			
Crangonyctidae: *Stygobromus*	Dade County: Boxcar Cave, Caboose Cave		Jensen and Ozier

Table 3. (Continued).

Taxon	Cave(s)	Comments	References
Crustaceans (isopods)			
Trichoniscidae: *Miktoniscus*	Bartow County: Anthonys Cave; Chattooga County: Blowing Springs Cave, Parker Cave; Dade County: Howards Waterfall Cave, Sittons Cave; Decatur County: Climax Cave; Randolph County: Griers Cave; Walker County: Horseshoe Cave, Spooky Cave	May represent *M. alabamensis* or undescribed species.	Holsinger and Peck (1971); Muchmore unpublished; Reeves et al. (2000)
Diplurans			
Campodeidae	Bartow, Chattooga, Dade, Floyd, Walker Counties: 26 total sites	These records likely represent multiple undescribed species.	Holsinger and Peck (1971), Reeves et al. (2000), Buhlmann (2001), this study
Insects (beetles)			
Staphylinidae: *Speleochus*	Walker County: Pigeon Cave		Buhlmann (2001)
Staphylinidae: *Subterrochus*	Walker County: Mountain Cove Farm Cave		Holsinger and Peck (1971)
Insects (flies)			
Sciaridae: *Lycoriella*	Bartow County: Anthonys Cave; Dade County: Deans Pit, Newsome Gap Cave; Walker County: Pettijohns Cave, Horseshoe Cave	Described as “cavernicolous”	Reeves et al. (2000)
Insects (silverfish)			
Nicoletiidae: *Nicoletia*	Walker County: Horseshoe Cave		Holsinger and Peck (1971)
Myriapods (centipedes)			
Lithobiidae: *Pampibius*	Walker County: Cave Spring Cave		Holsinger and Peck (1971)
Myriapods (millipedes)			
Cleidogonidae: *Pseudotremia* n. sp. 1	Dade County: Howards Waterfall Cave	Identified by W. Shear	this study
Cleidogonidae: *Pseudotremia* n. sp. 2	Dade County: Hooker Cave	Also collected in adjacent Hamilton Co., TN. Identified by W. Shear	this study

Recommendations and Conclusions

Many opportunities to improve our understanding of cave biodiversity in Georgia exist, including addressing the knowledge shortfalls by (1) conducting state-level conservation assessments of cave invertebrates, (2) focusing efforts to increase our knowledge on the ecology and life history of poorly-known and highly endemic troglobionts, and (3) supporting further study of the many undescribed taxa that have been reported. In addition, conservation resources could be focused on caves of biological interest. It is an important observation that many of the most biodiverse caves in Georgia are already on protected lands. Managing these sites for cave biodiversity is particularly important. However, there also are a handful of caves on private lands with important biological diversity that are worthy of further study and protection. Climax Cave in Decatur County is one of the longest caves in the state, and it supports populations of the Georgia Blind Salamander and the Dougherty Plain Cave Crayfish, which are both High Priority Species under the State Wildlife Action Plan, one single-cave endemic species, and is a significant Southeastern Myotis site. Horseshoe Cave in Walker County has the second-most biological records for any cave in the state and supports eight troglobionts, including two single-cave endemics and two potentially undescribed species. The Chelsea Gulf/Blowing Spring Cave system in Chattooga County hosts eight troglobionts, more than any other cave in Chattooga County. Parkers

Cave in Chattooga County supports six troglobionts including one single-cave endemic, as well as three potentially undescribed species. Morrisons Cave in Dade County supports ten troglobionts. Crane Cave in Catoosa County supports the only known Appalachian Valley and Ridge population of the Southern Cavefish. Further protection of any of these sites would greatly support cave biodiversity in Georgia.

Although much attention is given to troglobionts and cave-roosting bats, caves and other subterranean ecosystems contain important habitats for many other non-troglobitic species for reproduction, hibernation, shelter, and other aspects of their life histories. For example, caves are important habitats for many plethodontid salamanders (Niemiller and Miller, 2009; Goricki et al., 2012), including several species in Georgia that use caves for shelter and reproduction (e.g., Buhlmann, 2001; Niemiller et al., 2006; Camp and Jensen, 2007). The importance of caves for other non-troglobitic taxa, particularly invertebrates, has not been well-studied and should be a priority of future research.

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Literature Cited

Barr, T.C., Jr., 1953, Notes on the occurrence of ranid frogs in caves: Copeia, v. 1953, p. 60–61. doi.org/10.2307/1440250.

Barr, T.C., Jr., 1968, Cave ecology and the evolution of troglobites: in Dobzhansky, T., Hecht, M.K., and Steere, W.C., eds., Evolutionary Biology, vol. 2: New York, Appleton-Century-crofts, p. 35–102.

Barr, T.C., Jr., 1981, *Pseudanophthalmus* from Appalachian caves (Coleoptera: Carabidae): The engelhardti complex: Brimleyana, v. 5, p. 37–94.

Barr, T.C., 2004, A classification and checklist of the genus *Pseudanophthalmus* Jeannel (Coleoptera: Carabidae: Trechinae): Virginia Museum of Natural History Special Publications, No. 11, p. 1–52.

Bellinger, P.F., Christiansen, K.A., and Janssens, F., 1996-2019, Checklist of the Collembola of the World; https://www.collembola.org/index.html (accessed August 2019).

Black, J., 1971, The cave life of Oklahoma. A preliminary study (excluding Chiroptera): Oklahoma Underground, v. 4, p. 2–53.

Blehert, D.S., Hicks, A.C., Behr, M., Meteyer, C.U., Berlowski-Zier, B.M., Buckles, E.L., Coleman, J.T., Darling, S.R., Gargas, A., Niver, R. and Okoniewski, J.C., 2009, Bat white-nose syndrome: an emerging fungal pathogen?: Science, v. 323, p. 227. doi:10.1126/science.1163874.

Bradford, T., Adams, M., Humphreys, W.F., Austin, A.D. and Cooper, S.J.B., 2010, DNA barcoding of stygofauna uncovers cryptic amphipod diversity in a calcrete aquifer in Western Australia's arid zone: Molecular Ecology Resources, v. 10, p. 41–50. doi:10.1111/j.1755-0998.2009.02706.x.

Brandon, R.A., 1966, Systematics of the Salamander Genus *Gyrinophilus*: Urbana, University of Illinois Press, Illinois Biological Monographs No. 35, p. 1–82. doi.org/10.5962/bhl.title.50088.

Brown, J.H., and Lomolino, M.V., 1998, Biogeography: Sunderland, Massachusetts: Sinauer Press.

Buhlmann, K.A., 2001, A biological inventory of eight caves in northwestern Georgia with conservation implications: Journal of Cave and Karst Studies, v. 63, p. 91–98.

Camp, C.D., 2008, Southern Zigzag Salamander, *Plethodon ventralis*, in Jensen, J.B., Camp, C.D., Gibbons W., and Elliott, M.J. eds., Amphibians and Reptiles of Georgia, University of Georgia Press, p. 227–229.

Camp, C.D., and Jensen, J.B., 2015, *Eurycea lucifuga* predation: Herpetological Review, v. 46, p. 609.

Camp, C.D., and Jensen, J.B., 2007, Use of twilight zones of caves by plethodontid salamanders: Copeia, v. 2007, p. 594–604.

Campbell, J.W., Woods, M., Ball, H.L., Pirkle, R.S., Carey, V. and Ray, C.H., 2011, Terrestrial macroinvertebrates captured with a baited ramp-pitfall trap from five limestone caves in North Alabama and Georgia (USA) and their association with soil organic matter: Journal of Natural History, v. 45, p. 2645–2659. doi:10.1080/00222933.2011.597884.

Campbell, J.W., Delong, B.E., Carey, V. and Ray, C., 2012, Survey of terrestrial invertebrate species from Byers Cave; Dade County, Georgia: Georgia Journal of Science, v. 70, p. 172–178.

Campbell, J., 2017, Tennessee Winter Bat Population and White-nose Syndrome Monitoring Report for 2016–2017: Tennessee Wildlife Resources Agency, https://www.tn.gov/content/dam/tn/twra/documents/WNS-2017-Annual-Monitoring-Report.pdf

Cannizzaro, A.G., Balding, D., Stine, M. and Sawicki, T.R., 2019, A new syntopic species of *Stygobromus* Cope, 1872 (Amphipoda: Crangonyctidae) from groundwaters in Georgia and Florida, USA, with notes on *S. floridanus* Holsinger & Sawicki, 2016: Journal of Crustacean Biology, v. 39, p. 407–418. doi:10.1093/jcbiol/ruz034.

Cardoso, P., Erwin, T.L., Borges, P.A. and New, T.R., 2011, The seven impediments in invertebrate conservation and how to overcome them: Biological Conservation, v.144, p. 2647–2655. doi:10.1016/j.biocon.2011.07.024.

Carver, L.M., Perlaky, P., Cressler, A. and Zigler, K.S., 2016, Reproductive seasonality in *Nesticus* (Araneae: Nesticidae) cave spiders: PloS one, v. 11, e0156751. doi:10.1371/journal.pone.0156751.

Chamberlin, J.C., 1962, New and little-known false scorpions, principally from caves, belonging to the families Chthoniidae and Neobisiidae (Arachnida, Chelonethida): Bulletin of the American Museum of Natural History, v. 123, p. 299–352.

Chamberlin, R.V., 1946, On some millipeds of Georgia. Entomological News, v. 57, p. 149–152.

Christiansen, K., 1960, A preliminary survey of the knowledge of North American cave Collembola: The American Midland Naturalist, v. 64, p. 39–44. doi.org/10.2307/2422892.

Christiansen, K., 1964, A revision of the nearctic members of the genus *Tomocerus*: Revue d'Ecologie et de Biologie du Sol., v. 1, p. 639–678.

Christiansen, K., 1966, The genus *Arrhopalites* (Collembola: Sminthuridae) in the United States and Canada: International Journal of Speleology, v. 2, p. 5. doi.org/10.5038/1827-806X.2.1.5.

Christiansen, K.A., and Bellinger, P.F., 1981, The Collembola of North America north of the Rio Grande. A taxonomic analysis. Part 4. Families Neelidae and Sminthuridae: Grinnell, Iowa, Grinnell College, v. 4, 279 p.

Christiansen, K., and Bellinger, P., 1998, The Collembola of North America North of the Rio Grande: Grinnell College. Grinnell, Iowa, 1322 p.

Christman, M.C., and Culver, D.C., 2001, The relationship between cave biodiversity and available habitat: Journal of Biogeography, v. 28, p. 367–380. doi:10.1046/j.1365-2699.2001.00549.x. doi:10.1046/j.1365-2699.2001.00549.x.

Christman, M.C., Culver, D.C., Madden, M.K. and White, D., 2005, Patterns of endemism of the eastern North American cave fauna: Journal of Biogeography, v. 32, p.1441–1452. doi:10.1111/j.1365-2699.2005.01263.x.

Cliburn, J.W., and Middleton, A.L., Jr., 1983, The vertebrate cave fauna of Mississippi caves: National Speleological Society Bulletin, v. 45, p. 45–48.

Coleman, W.T. and Zigler, K.S., 2015, The rediscovery of *Caecidotea nickajackensis* in Tennessee and Alabama: Speleobiology Notes, v. 7, p.10–13.

Coles, V., 1944, Nesting of the turkey vulture in Ohio caves: The Auk, v. 61, p. 219–228. doi.org/10.2307/4079365.

Cooper, J.E. and Cooper, M.R., 1968, Cave-associated herpetozoa II: salamanders of the genus *Gyrinophilus* in Alabama caves: Bulletin of the National Speleological Society, v. 30, p. 19–24.

Cope, E.D., and Packard, A.S., Jr., 1881, The fauna of the Nickajack Cave: The American Naturalist, v. 15, p. 877–882. doi.org/10.1086/272948.

Crosby, C.R., Bishop, S.C., 1924, Notes on the Opiliones of the southeastern United States with descriptions of new species. J. Mitchell Soc., v. 40, p. 8–26.

Culver, D.C., Christman, M. C., Elliott, W. R., Hobbs, H. H., and Reddell, J. R., 2003, The North American obligate cave fauna: regional patterns. Biodiversity & Conservation, v. 12, p. 441–468. doi:10.1023/A:1022425908017.

Culver, D.C., Deharveng, L., Bedos, A., Lewis, J.J., Madden, M., Reddell, J.R., Sket, B., Trontelj, P., and White, D., 2006, The mid-latitude biodiversity ridge in terrestrial cave fauna: Ecography, v. 29, p. 120–128. doi:10.1111/j.2005.0906-7590.04435.x.

Culver, D.C., and Pipan T., 2009, The Biology of Caves and Other Subterranean Habitats: Oxford University Press, 254 p.

Culver, D.C., Trontelj, P., Zagmajster, M., and Pipan, T., 2013, Paving the way for standardized and comparable subterranean biodiversity studies: Subterranean Biology, v. 10, p. 43–50. doi:10.3897/subtbiol.10.4759.

Ćurčić, B.P.M., 1981, A revision of some North American pseudoscorpions (Neobisiidae, Pseudoscorpiones): Bulletin du Muséum d'Histoire Naturelle Belgrade Série B, v. 36, p. 101–107.

Ćurčić, B.P.M., 1984, A revision of some North American species of *Microcreagris* Balzan, 1892 (Arachnida: Pseudoscorpiones: Neobisiidae): Bulletin of the British Arachnological Society, v. 6, p. 149–166.

Ćurčić, B.P.M., 1989, Further revision of some North American false scorpions originally assigned to *Microcreagris* Balzan (Pseudoscorpiones, Neobisiidae): Journal of Arachnology, v. 17, p. 351–362.

Dearolf, K., 1956, Survey of North American cave vertebrates: Proceedings of the Pennsylvania Academy of Science, v. 30, p. 201–210.

Disney, R.H. and Campbell, J., 2011, Scuttle flies (Diptera: Phoridae) from caves in Alabama and Georgia, USA: Subterranean Biology, v. 8, p. 65–67. doi:10.3897/subtbiol.8.1233.

Dodd, C.K. Jr., Griffey, M.L., and Corser, J.D., 2001, The cave associated amphibians of Great Smoky Mountains National Park: Review and monitoring: Journal of the Elisha Mitchell Scientific Society, v. 117, p. 139–149.

Dourson, D.C., 2010, Kentucky's Land Snails and Their Ecological Communities. Bakersville, North Carolina: Goatslug Publications, United States.

Epps, M.J., Menninger, H.L., LaSala, N. and Dunn, R.R., 2014, Too big to be noticed: cryptic invasion of Asian camel crickets in North American houses: PeerJ, v. 2, e523. doi:10.7717/peerj.523.

Espinasa, L., and Jeffery, W.R., 2003, A troglomorphic sculpin (Pisces: Cottidae) population: Geography, morphology and conservation status: Journal of Cave and Karst Studies, v. 65, p. 93–100.

Espinasa, L., Mendyk, A., Schaffer, E., and Cahill, A., 2013, The second northernmost cave-adapted fish in the world? Groundwork on the Tytoona Cave sculpin population: Northeastern Naturalist, v. 20, p. 185–196. doi.org/10.1656/045.020.0115.

Faber-Langendoen, D., Nichols, J., Master, L., Snow, K., Tomaino, A., Bittman, R., Hammerson, G., Heidel, B., Ramsay, L., Teucher, A., and Young, B., 2012, NatureServe Conservation Status Assessments: Methodology for Assigning Ranks, revised edition: Arlington, Virginia, NatureServe, 44 p.

Felderhoff, K.L., Bernard, E.C., and Moulton, J.K., 2010, Survey of Pogonognathellus Borner (Collembola: Tomoceridae) in the Southern Appalachians based on morphological and molecular data: Annals of the Entomological Society of America, v. 103, p. 472–491. doi:10.1603/AN09105.

Fenolio, D.B., Niemiller, M.L., Gluesenkamp, A.G., McKee, A.M. and Taylor, S.J., 2017, New Distributional Records of the Stygobitic Crayfish *Cambarus cryptodytes* (Decapoda: Cambaridae) in the Floridan Aquifer System of Southwestern Georgia: Southeastern Naturalist, v. 16, p.163–182. doi:10.1656/058.016.0205.

Fenolio DB, Niemiller ML, Levy M, & Martinez B., 2013, Conservation status of the Georgia blind salamander (Eurycea wallacei) from the Floridan Aquifer of Florida and Georgia: Reptiles and Amphibians, v. 20, p. 97 -111. Ficetola, G.F., Canedoli, C. and Stoch, F., 2019, The Racovitzan impediment and the hidden biodiversity of unexplored environments: Conservation Biology, v. 33, p. 214–216. doi.org/10.1111/cobi.13179.

Flock, B., 2013, 2013 Tennessee Bat Population Monitoring and White Nose Syndrome: Nashville, Tennessee Wildlife Resources Agency, TWRA Wildlife Technical Report 13-22, 17 p.

Flock, B., 2014, 2014 Bat Population Monitoring and White Nose Syndrome Surveillance: Nashville, Tennessee Wildlife Resources Agency, TWRA Wildlife Technical Report 14-07, 15 p.

Fong, D.W., Porter, M.L., and Slay, M.E., 2012, Cave Life of the Virginias: A Field Guide to Commonly Encountered Species: Huntsville, Alabama, Biology Section of the National Speleological Society. 41 p.

Francl, K.E., Ford, W.M., Sparks, D.W. and Brack Jr, V., 2012, Capture and reproductive trends in summer bat communities in West Virginia: assessing the impact of white-nose syndrome: Journal of Fish and Wildlife Management, v. 3, p. 33–42. doi:10.3996/062011-JFWM-039.

Franz, R., Bauer, J., and Morris, T., 1994, Review of biologically significant caves and their faunas in Florida and south Georgia: Brimleyana, v. 20, p. 1–109.

GBIF.org (26 June 2019) GBIF Occurrence Download https://doi.org/10.15468/dl.k9t2s8

Georgia Department of Natural Resources, Wildlife Resources Division, 2015, State Wildlife Action Plan, https://georgiawildlife.com/sites/default/files/wrd/pdf/swap/SWAP2015MainReport_92015.pdf

Georgia Speleological Survey, 2016, http://gss.io.caves.org/GSSWebsite/Home.html

Gertsch, W.J., 1984, The spider family Nesticidae (Araneae) in North America, Central America, and the West Indies: Texas Memorial Museum Bulletin, v. 31, p. 1–91.

Gladstone, N.S., Carter, E.T., McKinney, M.L., and Niemiller, M.L., 2018, Status and Distribution of the Cave-Obligate Land Snails in the Appalachians and Interior Low Plateau of the Eastern United States: American Malacological Bulletin, v. 36, p. 62–79. doi:10.4003/006.036.0107.

Gladstone, N.S., Niemiller, M.L., Pieper, E.B., Dooley, K.E., and McKinney, M.L., 2019, Morphometrics and phylogeography of the cave-obligate land snail *Helicodiscus barri* (Gastropoda, Stylommatophora, Helicodiscidae): Subterranean Biology, v. 30, p.1–32. doi:10.3897/subtbiol.30.35321.

Godwin, J.C., 2000, Reassessment of the historical and search for new localities of the Tennessee Cave Salamander (*Gyrinophilus palleucus*) in Alabama. Unpublished Report, Alabama Department of Conservation and Natural Resources, Montgomery.

Godwin, J.C., 2008, Biological Inventory of the Cave and Karst Systems of The Nature Conservancy's Sharp-Bingham Mountain Preserve: Auburn, Alabama, Alabama Natural Heritage Program, 40 p.

Goodnight, C.J. and Goodnight, M.L., 1942, New Phalangodidae (Phalangida) from the United States: American Museum Novitates, No. 1188.

Gore, J.A., and Baker, W.W., 1989, Beavers residing in caves in northern Florida: Journal of Mammalogy, v. 70, p. 677–678. doi:10.2307/1381451.

Goricki, S., Niemiller, M.L., and Fenolio, D.B., 2012, Salamanders, in White, W.B. and Culver, D.C. eds., Encyclopedia of Caves, 2nd Edition, Waltham, Massachusetts, Elsevier, p. 665–676. doi.org/10.1016/B978-0-12-383832-2.00098-0.

Graening, G.O., Fenolio, D.B., and Slay, M.E., 2011, Cave Life of Oklahoma and Arkansas: Exploration and Conservation of Subterranean Biodiversity: University of Oklahoma Press.

Hale, J.A. and Streever, W.J., 1994, Cave fauna distribution within fully-flooded cave systems in Florida: Journal of Freshwater Ecology: v. 9, p. 171–174. doi.org/10.1080/02705060.1994.9664883.

Hart, D.G., and Hart, Jr., C.W., 1974, The ostracod family Entocytheridae: Academy of Natural Sciences of Philadelphia, v. 18, p. 1–239.

Harvey, M.S., 1990, Catalogue of the Pseudoscorpionida: Manchester, United Kingdom, Manchester University Press, 726 p.

Harvey, M.S., 2013, Pseudoscorpions of the World, version 3.0. Western Australian Museum, Perth, http://museum.wa.gov.au/catalogues-beta/pseudoscorpions (accessed June 2019).

Hedin, M.C., 1997, Speciational history in a diverse clade of habitat-specialized spiders (Araneae: Nesticidae: *Nesticus*): inferences from geographic-based sampling: Evolution, v. 51, p.1929–1945. doi:10.1111/j.1558-5646.1997.tb05115.x.

Hedin, M.C., 2001, Molecular insights into species phylogeny, biogeography, and morphological stasis in the ancient spider genus *Hypochilus* (Araneae: Hypochilidae): Molecular Phylogenetics and Evolution, v. 18, p. 238–251. doi:10.1006/mpev.2000.0882.

Hedin, M., and Thomas, S.M., 2010, Molecular systematics of eastern North American Phalangodidae (Arachnida: Opiliones: Laniatores), demonstrating convergent morphological evolution in caves: Molecular Phylogenetics and Evolution, v. 54, p.107–121. doi:10.1016/j.ympev.2009.08.020.

Hertl, P.T., 1981, A preliminary inventory survey and management plan for the Bat Cave complex, Bat Cave, North Carolina. Unpublished report for the North Carolina Nature Conservancy.

Himes, J., Beckett, D.C., and Trousdale, A.W., 2004, Survey of salamanders in Mississippi limestone caves: Southeastern Naturalist, v. 3, p. 241–248. doi:10.1656/1528-7092(2004)003[0241:SOSIML]2.0.CO;2.

Hobbs Jr, H.H., Hobbs III, H.H. and Daniel, M.A., 1977, A review of the troglobitic decapod crustaceans of the Americas: Smithsonian Contributions to Zoology, v. 244, p. 1–182. doi:10.5479/si.00810282.244.

Hobbs Jr, H.H. and Walton, M., 1968, New entocytherid ostracods from the southern United States: Proceedings of the Academy of Natural Sciences of Philadelphia, v. 120, p. 237–252.

Hobbs III, H.H., 2012, Diversity patterns in the United States. In: White, W.B., Culver, D.C., editors. Encyclopedia of Caves, 2nd edition. Amsterdam: Academic Press. p. 251–264. doi:10.1016/B978-0-12-383832-2.00033-5.

Holler Jr., C., Mays, J.D., and Niemiller, M.L., in press, The fauna of caves and other subterranean habitats of North Carolina, USA: Journal of Cave and Karst Studies.

Holliday, C., 2012, 2012 White-Nose Syndrome Disease Surveillance and Bat Population Monitoring Report. A Report of the Tennessee WNS Response Cooperators: Tennessee Chapter of The Nature Conservancy, 10 p.

Holmberg, R.G., Angerilli, N.P.D., LaCasse L.J., 1984, Overwintering aggregations of *Leiobunum paessleri* in caves and mines (Arachnida, Opiliones): Journal of Arachnology, v. 12, p. 195–204.

Hoff, C.C.,1946, A study of the type collections of some pseudoscorpions originally described by Nathan Banks: Journal of the Washington Academy of Sciences, v. 36, p. 195–205.

Hoffman, R.L., 1981, On the taxonomic status, distribution, and subspecies of the milliped *Pseudotremia fracta* Chamberlin (Chordeumatida, Cleidogonidae): Brimleyana, v. 5, p.135–144.

Holman, J.A. 1958, The Pleistocene herpetofauna of Sabertooth Cave, Citrus County, Florida. Copeia, v. 4, p. 276–280. doi:10.2307/1439958.

Holsinger, J.R., 1978, Systematics of the subterranean amphipod genus *Stygobromus* (Crangonyctidae): Part II. Species of the eastern United States: Smithsonian Contributions to Zoology, v. 266, p. 1–144. doi:10.5479/si.00810282.266.

Holsinger, J.R. and Peck, S.B., 1971, The invertebrate cave fauna of Georgia: National Speleological Society Bulletin, v. 33, p. 23–44.

Holt, P.C., 1973, A summary of the branchiobdellid (Annelida: Clitellata) fauna of Mesoamerica: Smithsonian Contributions to Zoology, no. 142, p. 1–40. doi:10.5479/si.00810282.142.

Hubbell, T.H., 1936, A monographic revision of the genus *Ceuthophilus* (Orthoptera, Gryllacrididae, Rhaphidophorinae): University of Floridal Publications, Biological Science Series, v. 2, p. 1–551.

Hubbell, T.H. and Norton, R.M., 1978, The systematics and biology of the cave-crickets of the North American tribe Hadenoecini (Orthoptera: Saltatoria: Ensifera: Rhaphidophoridae: Dolichopodinae): Miscellaneous Publications, Museum of Zoology, University of Michigan, v. 156, p. 1–124.

Huber, B.A., 2011, Revision and cladistics analysis of *Pholcus* and closely related taxa (Araneae, Pholcidae): Bonner Zoologische Monographien, v. 58, p. 1–509.

Hubricht, L., 1962, New species of *Helicodiscus* from the eastern United States: The Nautilus, 75, p. 102–107. doi.org/10.5962/bhl.part.25951.

Hubricht, L., 1964, The land snails of Georgia: Sterkiana, v. 16, p. 5–10.

Hubricht, L., 1985, The distributions of the native land mollusks of the eastern United States: Fieldiana, v. 24, p. 1–191. doi:10.5962/bhl.title.3329.

Huntsman, B.M., Venarsky, M.P., Benstead, J.P., and Huryn, A.D., 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, v. 56, p. 1746–1760. doi.org/10.1111/j.1365-2427.2011.02609.x.

Hutchins, B.T., 2018, The conservation status of Texas groundwater invertebrates: Biodiversity and Conservation, v. 27, p. 475–501. doi:10.1007/s10531-017-1447-0.

Hutchinson, V.H., 1966, *Eurycea lucifuga*: Catalogue of American Amphibians and Reptiles, 24, p. 1–2.

Hyman, L., 1954, North American Triclad Turbellaria, XIII: Three new cave planarians: Proceedings of the United States National Museum, v. 103, p. 563–573. doi.org/10.5479/si.00963801.103-3333.563.

International Union for the Conservation of Nature, 2012, IUCN Red List Categories and Criteria: Version 3.1. Second edition: Gland, Switzerland, and Cambridge, UK, IUCN, 32 p.

Katz, A.D., Taylor, S.J. and Davis, M.A., 2018, At the confluence of vicariance and dispersal: Phylogeography of cavernicolous springtails (Collembola: Arrhopalitidae, Tomoceridae) codistributed across a geologically complex karst landscape in Illinois and Missouri: Ecology and Evolution, v. 8, p.10306–10325. doi:10.1002/ece3.4507.

Kenk, R., 1977, Freshwater Triclads (Turbellaria) of North America, IX, the Genus *Sphalloplana*: Smithsonian Contributions to Zoology, No. 246. doi.org/10.5479/si.00810282.246.

Koponen, S., 1995, Quelques opilions (Arachnida) de la Province de Québec: Fabreries, v. 20, p. 1-5.

Lavoie, K., Bordi, J., Elwess, N, Soroka, D., and Burgess, M., 2019, Colonization of a newly cleaned cave by a camel cricket: Asian invasive or native?: Speleobiology Notes, v. 10, p. 1–8.

Ledford, J., Paquin, P., Cokendolpher, J., Campbell, J. and Griswold, C., 2011, Systematics of the spider genus *Neoleptoneta* Brignoli, 1972 (Araneae: Leptonetidae) with a discussion of the morphology and relationships for the North American Leptonetidae: Invertebrate Systematics, v. 25, p. 334–388. doi:10.1071/IS11014.

Leray, V.L., Caravas, J., Friedrich, M. and Zigler, K.S., 2019, Mitochondrial sequence data indicate "Vicariance by Erosion" as a mechanism of species diversification in North American *Ptomaphagus* (Coleoptera, Leiodidae, Cholevinae) cave beetles: Subterranean Biology, v. 29, p. 35–57. doi:10.3897/subtbiol.29.31377.

Lewis, J.J., 2005, Bioinventory of Caves of the Cumberland Escarpment Area of Tennessee. Final Report to Tennessee Wildlife Resources Agency & The Nature Conservancy of Tennessee: Lewis & Associates, 158 p.

Lewis, J.J., 2009, On the identity of *Caecidotea nickajackensis* (Crustacea: Isopoda: Asellidae): Proceedings of the Biological Society of Washington, v. 122, p. 215–224. doi:10.2988/08-47.1.

Lewis, J.J. and Reid, J.W., 2007, Patterns and processes of groundwater invasion by copepods in the interior low plateaus of the United States: Acta Carsologica, v. 36, p. 279–289. doi:10.3986/ac.v36i2.197.

Lomolino, M.V., 2004, In: M.V. Lomolino and L.R. Heaney (Eds.), Conservation biogeography. Frontiers of biogeography: New directions in the geography of nature: Sunderland, Massachusetts: Sinauer Associates, p. 293–296.

Mammola, S, Cardoso, P., Culver, D.C., Deharveng, L., Ferreira, R.L., Fiser, C., Galassi, D.M.P., Griebler, C., Halse, S., Humphreys, W.F., Isaia, M., Malard, F., Martinez, A., Moldovan, O.T., Niemiller, M.L., Pavlek, M., Reboleira, A.S.P.S., Souza-Silva, M., Teeling, E.C., Wynne, J.J., and Zagmajster, M., 2019, Scientists' warning on the conservation of subterranean ecosystems: BioScience, v. 69, p. 641 -650.

Marshall, S.A. and Peck, S.B., 1985a, The origin and relationships of *Spelobia tenebrarum* (Aldrich), a troglobitic, eastern North American, sphaerocerid fly: The Canadian Entomologist, v. 117, p. 1013–1015. doi:10.4039/Ent1171013-8.

Marshall, S.A. and Peck, S.B., 1985b, Distribution of cave-dwelling Sphaeroceridae (Diptera) of eastern North America: Proceedings of the Entomological Society of Ontario, v. 115, p. 37–41.

Martin, C.O., 2007, Assessment of the Population Status of the Gray Bat (*Myotis grisescens*): Army Endangered Species Research Lab, No. ERDC/EL-TR-07-22, p. 1–97.

Means, D.B, 2005, *Haideotriton wallacei* Carr, 1939. Georgia blind salamander: in M. Lannoo, ed. Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley, California, p. 779–780.

Miller, B.T. and Niemiller, M.L., 2005, *Pseudotriton ruber* (red salamander). Reproduction: Herpetological Review, v. 36, p. 429.

Miller, B.T., and Niemiller, M.L., 2008, Distribution and relative abundance of Tennessee Cave Salamanders (*Gyrinophilus palleucus* and *Gyrinophilus gulolineatus*) with an emphasis on Tennessee populations: Herpetological Conservation and Biology, v. 3, p. 1–20.

Miller, B.T. and Niemiller, M.L., 2012, *Gyrinophilus palleucus*: Catalogue of American Amphibians and Reptiles, 884, p. 1–7.

Miller, B.T., Niemiller, M.L., and Reynolds, R.G., 2008, Observations on egg-laying behavior and interactions among attending female Red Salamanders (*Pseudotriton ruber*) with comments on the use of caves by this species: Herpetological Conservation and Biology, v. 3, p. 203–210.

Miller, J.A., 2005, A redescription of *Porrhomma cavernicola* Keyserling (Araneae, Linyphiidae) with notes on Appalachian troglobites: Journal of Arachnology, v. 33, p. 426–439. doi:10.1636/04-51.1.

Mohr, C.E., 1952, A cave record for the red bat, *Lasiurus b. borealis*: Bulletin of the National Speleological Society, v. 14, p. 62–63.

Morris, T., and Ferrall, E., 2018, 2018 White-nose Syndrome Season Summary: https://view.publitas.com/georgia-department-of-natural-resources/white-nose-syndrome-in-georgia-2018-report/

Muchmore, W.B., 1969, New species and records of cavernicolous pseudoscorpions of the genus *Microcreagris* (Arachnida, Chelonethida, Neobisiidae, Ideobisiinae): American Museum Novitates, no. 2392, p. 1–21.

Muchmore, W.B., 1974, Clarification of the genera *Hesperochernes* and *Dinocheirus* (Pseudoscorpionida, Chernetidae): Journal of Arachnology, v. 2, p. 25–36.

Muchmore, W.B., 1976, New species of *Apochthonius*, mainly from caves in central and eastern United States (Pseudoscorpionida, Chthoniidae): Proceedings of the Biological Society of Washington, v. 89, p. 67–80.

Muchmore, W.B., and Cokendolpher, J.C., 1995, Generic placement of the Empire Cave Pseudoscorpion, *Microcreagris imperialis* (Neobisiidae), a potentially endangered cave arachnid: Journal of Arachnology, v. 23, p. 171–176.

Myers, R.F., 1960, *Lasiurus* from Missouri caves: Journal of Mammalogy, v. 41, p. 114–117. doi.org/10.2307/1376526.

Niemiller M.L., Bichuette, E., and Taylor, S.J., 2018, Conservation of cave fauna in Europe and the Americas. P. 451–478 in: Ecological Studies: Cave Ecology (Moldovan, O.T., Kovac. L., and Halse, S., eds). Springer. doi:10.1007/978-3-319-98852-8_22.

Niemiller, M.L., Fenolio, D.B., and Zigler, K.S., 2012, The obligate cave fauna of Georgia: Bulletin of the Georgia Speleological Society, p. 6–12.

Niemiller, M.L., Fitzpatrick, B.M., and Miller, B.T., 2008, Recent divergence with gene flow in Tennessee cave salamanders (Plethodontidae: *Gyrinophilus*) inferred from gene genealogies: Molecular Ecology, v. 17, p. 2258–2275. doi:10.1111/j.1365-294X.2008.03750.x.

Niemiller, M.L. and Miller, B.T., 2007, Subterranean reproduction of the southern two-lined salamander (*Eurycea cirrigera*) from Short Mountain, Tennessee: Herpetological Conservation and Biology, v. 2, p. 106–112.

Niemiller, M.L., Near, T.J. and Fitzpatrick, B.M., 2012, Delimiting species using multilocus data: diagnosing cryptic diversity in the southern cavefish, *Typhlichthys subterraneus* (Teleostei: Amblyopsidae): Evolution, v. 66, p. 846–866. doi:10.1111/j.1558-5646.2011.01480.x.

Niemiller, M.L., Taylor, S.J., Slay, M.E., and Hobbs III, H.H., 2019, Biodiversity in the United States and Canada. In: Encyclopedia of Caves, 3rd edition (Culver, D.C., White, W.B., and Pipan, T., eds.): Academic Press, p. 163–177. doi:10.1016/B978-0-12-814124-3.00018-2.

Niemiller, M.L., and Zigler, K.S., 2013, Patterns of cave biodiversity and endemism in the Appalachians and Interior Plateau of Tennessee, USA: PLOS ONE, v. 8, art. e64177, 17 p. doi:10.1371/journal. pone.0064177. doi:10.1371/journal.pone.0064177.

Niemiller, M.L., Zigler, K.S., and Fenolio, D.B., 2013, Cave Life of TAG: A Guide to Commonly Encountered Species in Tennessee, Alabama and Georgia: Huntsville, Alabama, Biology Section of the National Speleological Society, 45 p.

Niemiller, M.L., Zigler, K.S., Hart, P.B., Kuhajda, B.R., Armbruster, J.W., Ayala, B.N. and Engel, A.S., 2016, First definitive record of a stygobiotic fish (Percopsiformes, Amblyopsidae, *Typhlichthys*) from the Appalachians karst region in the eastern United States: Subterranean Biology, v. 20, p.39–50. doi:10.3897/subtbiol.20.9693.

Niemiller, M.L., Zigler, K.S., Ober, K.A., Carter, E.T., Engel, A.S., Moni, G., Philips, T.K. and Stephen, C.D., 2017, Rediscovery and conservation status of six short-range endemic *Pseudanophthalmus* cave beetles (Carabidae: Trechini): Insect Conservation and Diversity, v. 10, p. 495–501. doi:10.1111/icad.12263.

Niemiller, M.L., Zigler, K.S., Stephen, C.D., Carter, E.T., Paterson, A.T., Taylor, S.J. and Engel, A.S., 2016, Vertebrate fauna in caves of eastern Tennessee within the Appalachians karst region, USA: Journal of Cave and Karst Studies, v. 78, p. 1–24. doi:10.4311/2015LSC0109.

Osbourn, M.S., 2005, The natural history, distribution, and phenotypic variation of cave-dwelling spring salamanders, *Gyrinophilus* sp. Cope (Plethodontidae), in West Virginia [Master's thesis]: Marshall University, 207 p.

Park, O., 1960, Cavernicolous pselaphid beetles of the United States: American Midland Naturalist, v. 64, p. 66–104. doi:10.2307/2422894.

Pauley, T.K., 1993, Report of the Upland Vertebrates in the New River Gorge National River. Volumes 1–3 (1989–1992). US Department of the Interior, National Park Service.

Peck, S.B., 1973, Systematic revision and the evolutionary biology of the *Ptomaphagus* (*Adelops*) beetles of North America (Coleoptera; Leiodidae: Catopinae), with emphasis on cave-inhabiting species: Bulletin of the Museum of Comparative Zoology, v. 145, p. 29–162.

Peck, S.B., 1982, Occurrence of *Ptomaphagus cavernicola* in forests in Florida and Georgia (Coleoptera; Leiodidae; Cholevinae): Florida Entomologist, v. 65, p. 378–379. doi:10.2307/3494315.

Peck, S.B., 1988, A review of the cave fauna of Canada, and the composition and ecology of the invertebrate fauna of caves and mines in Ontario: Canadian Journal of Zoology, v. 66, p. 1197–1213. doi:10.1139/z88-176.

Peck, S.B., 1970, The terrestrial arthropod fauna of Florida caves: Florida Entomologist, v. 53, p. 203–207. doi.org/10.2307/3493189.

Peck, S.B., 1989, The cave fauna of Alabama: Part I. The terrestrial invertebrates (excluding insects): National Speleological Society Bulletin, v. 51, p. 11–33.

Peck, S.B., 1995, The cave fauna of Alabama. Part II: The insects: National Speleological Society Bulletin, v. 57, p. 1–19.

Peck, S.B. and Lewis, J.J., 1978, Zoogeography and evolution of the subterranean invertebrate faunas of Illinois and southeastern Missouri: National Speleological Society Bulletin, v. 40, p. 39–63.

Peck, S.B. and Peck, J.H., 1982, Invertebrate fauna of Devils Den, a sandstone cave in northwestern Arkansas: Journal of the Arkansas Academy of Science, v. 36, p. 46–48.

Platnick, N.I., 1999, A revision of the Appalachian spider genus *Liocranoides* (Araneae, Tengellidae): American Museum Novitates, no. 3285, p. 1–13.

Poly, W.J., 2001, Nontroglobitic fishes in Bruffey-Hills Creek Cave, West Virginia, and other caves worldwide: Environmental Biology of Fishes, v. 62, p. 73–83. doi:10.1023/A:1011889613271.

Poly, W.J., and Boucher, C.E., 1996, Nontroglobitic fishes in caves: Their abnormalities, ecological classification and importance: The American Midland Naturalist, v. 136, p. 187–198. doi:10.2307/2426644.

Prather, J.W., and Briggler, J.T., 2001, Use of small caves by anurans during a drought period in the Arkansas Ozarks: Journal of Herpetology, v. 35, p. 675–678. doi:10.2307/1565910.

Pruitt, B.C., Jr., 1995, New records of fishes within Florida caves: National Speleological Society Bulletin, v. 57, p. 20–23.

Reeves W., 1999, Cave-dwelling Nesticidae (Araneae) in the southeastern United States: New distribution records and notes on their bionomics: Insecta Mundi, v. 13: p. 93–94.

Reeves, W.K., Jensen, J.B. and Ozier, J.C., 2000, New faunal and fungal records from caves in Georgia, USA: Journal of Cave and Karst Studies, v. 62, p. 169–179.

Reeves, W.K. and McCreadie, J.W., 2001, Population ecology of cavernicoles associated with carrion in caves of Georgia, USA: Journal of Entomological Science, v. 36, p. 305–311. doi:10.18474/0749-8004-36.3.305.

Reeves, W.K. and Paysen, E.S., 1999, Black flies (Diptera: Simuliidae) and a new species of caddisfly (Trichoptera: Hydropsychidae) in a northwestern Georgia cave stream. Entomological News, v. 110, p. 253–259.

Reeves, W.K. and Reynolds, J.W., 1999, New records of cave-dwelling earthworms (Oligochaeta: Lumbricidae, Megascolecidae, and Naididae) and other annelids (Aeolosomatida, Branchiobdellida, and Hirudinea) in the southeastern United States, with notes on their ecology: Megadrilogica, v. 7, p. 65–71.

Reynolds, J.W., 1994, Earthworms of North Carolina (Oligochaeta: Acanthodrilidae, Komarekionidae, Lumbricidae, Megascolecidae, Ocnerodrilidae and Sparganophilidae): Megadrilogica, v. 5, p. 53–72.

Schultz, G.A., 1970, Descriptions of new subspecies of *Ligidium elrodii* (Packard) comb. nov. with notes on other isopod crustaceans from caves in North America (Oniscoidea). American Midland Naturalist, v. 84, p. 36–45. doi:10.2307/2423724.

Shear, W.A., 1975, The opilionid genera *Sabacon* and *Tomicomerus* in America (Opiliones, Troguloidea, Ischyropsalidae): Journal of Arachnology, v. 3, p. 5–29.

Shear, W.A., 2010, The milliped family Trichopetalidae, Part 2: The genera *Trichopetalum*, *Zygonopus* and *Scoterpes* (Diplopoda: Chordeumatida, Cleidogonoidea): Zootaxa, v. 2385, p. 1–62. doi:10.11646/zootaxa.2385.1.1.

Shelley, R.M., 1979, A synopsis of the milliped genus *Cambala*, with a description of *C. minor* Bollman (Spirostreptida: Cambalidae): Proc. Biol. Soc. Washington, v. 92, p. 551–571.

Sket, B., 2008, Can we agree on ecological classifications of subterranean animals?: Journal of Natural History, v. 42, n. 21-22, p. 1549–1563. doi:10.1080/00222930801995762.

Sokolov, I.M., Carlton, C., and Cornell, J.F., 2004, Review of *Anillinus*, with descriptions of 17 new species and a key to soil and litter species (Coleoptera: Carabidae: Trechinae: Bembidiini): The Coleopterists Bulletin, v. 58, p. 185–233. doi.org/10.1649/611.

Steeves III, H.R., 1964, The troglobitic asellids of the United States: The hobbsi group: The American Midland Naturalist, v. 71, p. 445–451. doi:10.2307/2423303.

Studier, E.H., Lavoie, K.H., and Chandler, C.M., 1991, Biology of cave crickets, *Hadenoecus subterraneus*, and camel crickets, *Ceuthophilus stygius* (Rhaphidophoridae); Parasitism by hairworms: Journal of the Helminthological Society of Washington, v. 58, p. 248–250.

Taylor, S.J. and Niemiller, M.L., 2016, Biogeography and conservation assessment of *Bactrurus* groundwater amphipods (Crangonyctidae) in the central and eastern United States: Subterranean Biology, v. 17, p. 1–29. doi.org/10.3897/subtbiol.17.7298.

USGS, United States Geological Survey, 2019, U.S. Board on Geographic Names, Domestic Names, https://www.usgs.gov/core-science-systems/ngp/board-on-geographic-names/domestic-names.

Venarsky, M.P., Huryn, A.D., and Benstead, J.P., 2012, Re-examining extreme longevity of the cave crayfish *Orconectes australis* using new mark–recapture data: a lesson on the limitations of iterative size-at-age models: Freshwater Biology, v. 57, p. 1471–1481. doi.org/10.1111/j.1365-2427.2012.02812.x.

Wynn, A.H., Highton, R. and Jacobs, J.F., 1988, A new species of rock-crevice dwelling *Plethodon* from Pigeon Mountain, Georgia: Herpetologica, v. 44, p. 135–143.

Yancey, M.E., Mann, N.C., Milne, M.A. and Zigler, K.S., 2018, Egg sacs of *Liocranoides* Keyserling, 1881 (Araneae: Zoropsidae) cave spiders: Journal of Arachnology, v. 46, p. 553–556. doi:10.1636/JoA-S-17-074.1.

Zhang, J., and Holsinger, J.R., 2003, Systematics of the freshwater amphipod genus *Crangonyx* (Crangonyctidae) in North America: Virginia Museum of Natural History, Memoir, no. 6, p. 1–274.

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Volume 82 Number 2 June 2020

CONTENTS

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