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Yearly microbial cycle of human exposed surfaces in show caves

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Abstract

The human impact upon the subterranean microbiomes is not only a peril to the cave environment but might also affect future visitors. We focused on the changes that humans induced on the surfaces they came in direct or indirect contact with inside two intensely visited Romanian show caves, by means of commercially available microbial rapid test kits and molecular identification.

Overall culturable bacteria abundance in the caves maintained high levels year-round while *Enterobacteriaceae*, coliform bacteria and *Escherichia coli* levels peaked during the touristic season, reaching levels that could pose a threat to the health of the visitors. Culturable fungi abundance usually peaked in the spring, remained at a high level in the summer and started to slowly decrease towards the winter months. Differences were observed between the direct and indirect exposed surfaces, as the later had lower overall levels of bacteria and fungi, with increased *Enterobacteriaceae* loads. Most of the taxa identified are known biodeteriorants of subterranean surfaces and were previously associated with human altered caves. A *Dothideomycete* sp. previously unknown to the cave environments was detected.

This was the first study to analyse the dynamics of the microbial communities of delicate subterranean surfaces in show caves through the use of commercially available test kits. We revealed that exposed surfaces in show caves, in direct or indirect contact with tourists, are host to high concentrations of cultivable microbes. The touristic activity was shown to influence the abundance and dynamics of the microbial communities inhabiting surfaces of show caves.

Keywords

subterranean, bacteria, fungi, 16S 18S rRNA, Romania, Rida Count, tourists, *Enterobacteriaceae*, *E. coli*, coliform bacteria

Introduction

Humans willingly and unwillingly alter the biomes they visit or inhabit, modifying the microbiology of these sites, including caves (Saiz-Jimenez et al. 2011). The human induced changes in cave microbiomes are sometimes obvious as stains, colorations or microbial mats appear on speleothems, cave rocks and walls (Saiz-Jimenez et al. 2011). While some of the microbes carried by tourists into caves are affecting the natural environment through degradation of the host rock (Northup and Lavoie 2001), others, such as opportunistic pathogens, might affect future visitors, particularly immunodeficient persons (Jurado et al. 2010a).

Pristine subterranean systems self-regulate and adapt to what is usually an environment scarce in resources, lacks light and has limited exchange with the outside world (Hobbs III et al. 2017). Although limited interaction between caves and humans has little effect on the microbiology of caves (Fraschetti et al. 2001), once it becomes a true show cave widely open to the public the situation changes drastically (Fernandez-Cortes et al. 2011). The extensive human impact begins with the physical alterations that involve blasting, constructions of walkways and installing various utilities (Van Beynen and Townsend 2005). As soon as the cave starts to be prepared for mass visitation, biological and atmospheric changes occur and can range from migration of microscopic species to nontouristic parts of the cave or local extinctions, to foul odours emanating from pools or muds, stains on speleothems, relicts or cave art (Lavoie and Northup 2006; Fernandez-Cortes et al. 2011; Saiz-Jimenez et al. 2011; Hobbs III et al. 2017).

At first, the microbial indicators of human impact in caves were considered *Bacillus* sp., *Escherichia coli*, and *Staphylococcus aureus*, and were regarded as 'Human Indicator Bacteria', based on studies by Lavoie and Northup (2006) in Lechuguilla Cave (USA). The degree of human impact is reflected in a decrease of diversity, as determined by Ikner et al. (2007) in a study of the Kartchner Caverns (USA). Investigations, performed due to the deterioration of the Palaeolithic paintings of Lascaux Cave (France), revealed that the cave was a reservoir of potential pathogenic protozoa and bacteria such as *Ralstonia*, *Pseudomonas*, *Legionella*, *Achromobacter*, *Bordetella*, *Shigella* or *E. coli*, previously linked to outbreaks related to air-conditioning systems and cooling towers in community hospitals and public buildings (Bastian et al. 2009). The use of designated 'indicator organisms' such as *Enterobacteriaceae* (Peters et al. 2018), *E. coli* (Anderson et al. 2005) or coliform bacteria (Shakoor et al. 2018) can detect human induced disturbances, as well as faecal associated contamination (Lavoie and Northup 2006). It was found that bacterial abundance varies greatly at different sampling times, as shown by Wang et al. (2010; 2012) in four different sites in the Mogao Grottoes (China). The increase in number of visitors lead to an increase of the bacterial abundance in all of the accessible sampled sites.

A number of studies have examined the diversity of surface-associated communities in bathrooms, as these are potential harbourers of pathogens. Skin-associated bacteria dominate on surfaces that are routinely touched by hands, although 19 bacterial phyla were identified with most sequences belonging to *Actinobacteria*, *Bacteroidetes*,

Firmicutes and *Proteobacteria* (Flores et al. 2011). Analyses of the contamination of high-touched surfaces in hospitals showed that average bacterial load was 1.32×10^4 bacteria per cm^2, almost half of the samples were culture-positive and some included multidrug resistant organisms (Costa et al. 2019). Confocal laser scanning microscopy demonstrated live bacteria on 76.7% of culture-negative samples while biofilm was present on all surfaces subjected to microscopy (Costa et al. 2019).

We focused on the microbial changes on surfaces humans came in direct or indirect contact with inside show caves. The surface microbiota was sampled on stalagmites that tourists come in direct contact with by touching and on cave bear (*Ursus spelaeus*) skeletons that are on display in the caves. The skeletons are indirectly exposed as they are behind a protective railing and cannot be touched. We monitored monthly changes in the culturable bacterial and fungal abundances focusing on constantly exposed surfaces from the visited sectors, as these are subject to high external allochthonous input. For the microbial monitoring itself we used commercially available Rida Count (R-Biopharm, Germany) rapid test kits. These test kits are based on the principle of using a specific chromogenic detection system imbedded in the standard nutrient media used for cultivating microorganisms. During growth, microbial colonies form and, due to the presence of specific enzymes, they will have distinctive colours. Second and third generation microbes were grown *ex-situ* on specific media for the purpose of ribosomal ribonucleic acid (rRNA) extraction and identification. Along the microbiological changes we monitored the cave climate. Such a monitoring program was not yet used for the subterranean surfaces, as previous studies used longer or unequal intervals between samplings, single samplings, or monitored few microbial groups and parameters. While environmental parameters influence the microbial communities, human presence is also known to alter such communities. We aimed to establish how this influence is identifiable upon the culturable bacterial and fungal abundances of surfaces exposed to tourists inside two Romanian show caves. We analysed the variability of these abundances over a yearly cycle and between surfaces that were directly and indirectly exposed to humans.

Materials and methods

Study sites and sampling locations

The microbiological monitoring of total aerobic bacteria, yeasts and moulds and indicator organisms (*Enterobacteriaceae*, *E. coli* and coliform bacteria) took place from March 2015 to March-April 2016, in two of the most visited caves in Romania, Peștera Urșilor de la Chișcău (Ursilor) and Peștera Muierilor (Muierilor) (Figure 1). The caves were sampled monthly to obtain an overview of the microbial changes that occur over a yearly cycle. All monitoring sites were exposed to tourists yearlong. Two of the monitoring sites were on stalagmites that were in direct contact with tourists (e.g. touched by hands). The other two monitoring sites were on the skeletons and were not in direct contact with tourists.

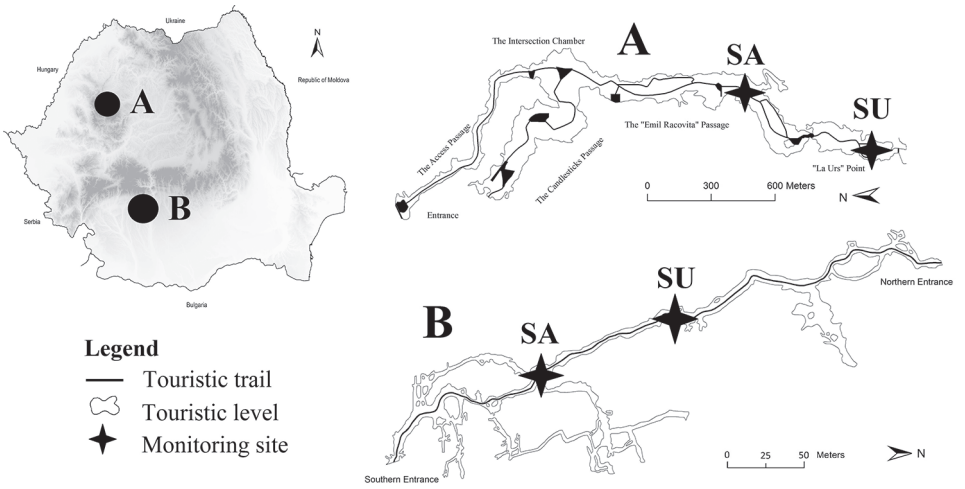

Figure 1. Monitoring sites; Location of the studied caves in Romania with the cave maps and monitoring sites in Ursilor (**A** modified after Rusu and Racoviţă 1981) and Muierilor (**B** modified after map by Grigore, Fofirică, Dăscălescu and Iliescu, unpublished).

Environmental parameters (temperature, airflow, relative humidity, CO_2 and particles per cubic centimetre of air) in the monitoring areas were also measured.

Ursilor is located in the Bihor Mountains (46°33'14"N, 22°34'10"E, Apuseni Mountains, Western Romania,) (Figure 1). The cave is formed in Late Jurassic (Tithonian) recrystallized limestones and had been sealed until it was discovered in 1975 during blasting in a limestone quarry. The cave entrance is housed in a large building constructed into the hill that hosts the cave. The trail is mostly made out of concrete, with one footbridge made of wood planks on a metal structure. The trail has a length of ~500m, with a metal handrail being present through most of the length of the trail. Some stalagmites are part of this handrail. One of these stalagmites (SA), along with the *U. spelaeus* skeleton that is on display (SU), were sampled monthly. The SA monitoring site was at a height of 1.2m from the trail (Figure 1A). A dark coloration was present on the touched part of the stalagmite. The SU monitoring site, was situated at the end of the tourist trail (Figure 1A), at a distance of 1.8m from the inner side of the trail and was displayed on the cave floor. The display area was littered with coins and presented lampenflora whose growth was promoted by the display lights.

Muierilor is located in the southern part of the Parâng Mountains (45°11'13"N, 23°45'9"E, Southern Carpathians) (Figure 1). The cave developed within a stripe of massive limestones of Late Jurassic on at least four levels, of which only the upper level is open to the public. The trail is made out of concrete, has sectors that are dug into the flour of the cave and has no handrail throughout most of its length. The monitoring site for the touched speleothem is around halfway through the touristic path (SA). The site was at a height of 1.2m, on a stalagmite where the pathway was 2m wide and the tourists used this speleothem as a handrail. A dark coloration was present on the touched part of the stalagmite. The monitoring site represented by the *U. spelaeus*

skeleton (SU), displayed upright on a metal stand, was at a height of 1.2m and at a distance of 1.8m from the tourist trail. The display area presented lampenflora whose growth was promoted by the display lights.

Microbial sampling and laboratory protocols

Sampling was performed using Rida Count (R-Biopharm, Germany) test sheets for total aerobic heterotroph bacteria, yeasts and moulds and indicator organisms (*Enterobacteriaceae*, *E. coli* and coliform bacteria). At each of the sampling sites, each test sheet was moistened and thus activated by adding 1 ml of sterile physiological saline solution (0.9% NaCl), just before contact with the sampled surface. After *in-situ* inoculation, the plates were transported at ambient cave temperature in a cooler bag. In the laboratory, the plates were placed in CulturaR Mini incubators (Almedica, Switzerland) at 37 °C for total bacteria and indicator organisms plates. Yeasts and moulds plates were incubated at 25 °C, according to the manufacturer's instructions. Incubation was between 24 and 72h with additional periods suggested by both the manufacturer and previous studies for cave microbes, as they have a slower rate of growth on selected media (Mulec et al. 2012b; 2012a; Mulec et al. 2012c; Bercea et al. 2018). The plates were analysed at 24 h intervals, the results being expressed in the total readings after five days. A five day incubation period was found to be better suited for cave studies as some colony forming units (CFU) can be identified only at the final analysis (Bercea et al. 2018). The counts were expressed as CFU/100cm^2.

Molecular analyses, statistical analyses and environmental parameters

The most visible and well-grown CFU were transferred from Rida Count plates to R2A agar PPM CS100 (VWR Chemicals, USA) for bacteria growth and to Czapek Yeast Extract Agar (Atlas 2010) for yeasts and moulds growth. After incubation, rRNA was extracted from the resulted colonies. Extraction was performed with Chelex 100 (Bio-Rad, USA) 5% solution in TE buffer (Walsh et al. 1991). When this method was not successful, we performed rRNA extraction with Isolate II Genomic DNA Kit (Bioline, UK) and followed the manufacturer's instructions. Total rRNA concentration was measured using a BioDrop DUO Spectrophotometer (BioDrop, UK). After extraction the rRNA was stored at -20 °C until further use. Two bacterial specific primers, targeting the 16S rRNA gene, were used for screening the bacterial diversity: 27F (5'-AGA GTT TGA TCC TGG CTCAG-3') and 1492R (5'-ACG GHT ACC TTG TTA CGA CTT-3') (McGenity et al. 1998). For yeasts and moulds analysis primer set FR1 (5'-AIC-CAT-TCA-ATC-GGT-AIT-3') and FF390 (5'-CGA-TAA-CGA-ACG-AGA-CCT-3') of 18S rRNA were used. For bacterial samples, an amplification program with initial denaturation for 3min at 95 °C, followed by 35 cycles of 95 °C for 30s, 55 °C for 30s, 72 °C for 90min and final elongation at 72 °C for 10min, was

used. For yeasts and moulds the program was initial denaturation at 97 °C for 3min, 35 cycles 95 °C for 60s, 49 °C for 25s, 72 °C for 35s and final elongation at 72 °C for 5min. The polymerase chain reaction (PCR) was performed with a Techne TC-512 gradient thermal cycler (Bibby Scientific, UK). PCR products were purified with FavorPrep™GEL/PCR Purification Kit (Favorgen Biotech Corp., Taiwan) following the manufacturer's instructions. rRNA was sequenced by Sanger method (Sanger and Coulson 1975; Sanger et al. 1977) at a commercial company (Macrogen Europe, The Netherlands). The sequences obtained were compared to 16S and 18S rRNA gene sequences deposited in NCBI BioSystems Database (GenBank) using BLAST algorithm (Geer et al. 2010; Sayers et al. 2019a; Sayers et al. 2019b). Sequences have been deposited in GenBank. Statistical analyses were performed with SSS 2019 One-Way Repeated Measures ANOVA Calculator (Stangroom 2019).

The following parameters were measured *in-situ* at each sampling site: temperature and airflow with a PCE-423 Hot Wire Anemometer (PCE Instruments, UK); relative humidity with a Hygropalm 3 Digital Humidity Meter (RoTronic, Switzerland); CO_2 values with a Vaisala Measurement Indicator MI70 equipped with a Vaisala Carbon Dioxide Probe GMP70 (Vaisala, Finland); particles per cubic centimetre of air with a Condensation Particle Counter 3007 (TSI, USA). The tourist traffic flow was continuously monitored with a Long-Range IR Beam Indoor People Counters with internal data logger (Chamber Electronics, Scotland). Over the course of the study, the average temperature and CO_2 in Ursilor was overall slightly higher than in Muierilor, while particles per cubic centimetre of air was higher in Muierilor. Relative humidity and airflow were stable during the study period for all the sites. The average number of visitors in the years preceding the study were estimated by the cave managers at around 130,000 and 120,000 for Ursilor and Muierilor, respectively. The pattern of visitation during the study period was similar for the two caves, with numbers rising from spring up to the beginning of autumn, followed by a steep drop for the winter season (Bercea et al. 2018). Muierilor had bat populations in both the touristic and non-touristic sectors that congregated in the winter and also summer colonies. This could have contributed to an increase in the CFU of Muierilor as bats introduce low, but not negligible, concentrations of microbes on their fur (Borda et al. 2014).

Results

Microbiota

On the surfaces of Ursilor and Muierilor, the total bacterial communities presented high numbers year-round. *Enterobacteriaceae*, coliform bacteria and *E. coli* peaked during the touristic season. An exception was represented by the *U. spelaeus* skeleton on display in Muierilor, were *Enterobacteriaceae* were in high numbers year-round. Yeasts and moulds usually peaked in the spring, remained at a high level in the summer, and slowly decreased towards the winter months. The overall total bacterial counts (~87000 CFU) and yeasts and moulds (~83000 CFU) were at close levels to each other. Overall

Enterobacteriaceae (~12000 CFU) were far behind, whilst coliform bacteria (~3600 CFU) and *E. coli* (~1100 CFU) were even lower.

On the monitored stalagmite in Ursilor, yeasts and moulds values peaked in the spring (>6500 CFU) and slowly declined towards the winter period (~200 CFU) (Figure 2A). Total bacterial levels were relatively constant from spring to autumn (~1500 CFU), dropping only for the winter (~200 CFU). *Enterobacteriaceae*, coliform bacteria and *E. coli* all peaked in the summer season, reaching an all high in July (~3500 CFU), with smaller peaks in May and June. The highest levels of yeasts and moulds, recorded during the study period in Ursilor, were registered in May (>6500 CFU) (Figure 2A). On the similar surface in Muierilor, yeasts and moulds values peaked in the spring (~5800 CFU) and autumn periods (~6500 CFU), with slightly lower values for the summer season. Total bacteria followed an almost identical pattern, although the summer period values did not drop as much as the fungal ones. *Enterobacteriaceae*, coliform bacteria and *E. coli* all peaked in the summer season, reaching an all high in June (~1800 CFU) and a smaller peak in August (~900 CFU). Coliform bacteria also peaked during the winter season (~500 CFU). The highest level of yeasts and moulds CFU, recorded during the study period in all sites, was registered in September in Muierilor (6559 CFU), while that of total bacteria peaked in April in Muierilor (7155 CFU) (Figure 2B).

On the exposed and not touched *U. spelaeus* skeleton on display in Ursilor, yeasts and moulds followed a similar trend as on the touched surface, only with lower levels, peaking in the spring period (~2400 CFU) and then slowly declining. As in the case of yeasts and moulds, total bacteria followed a similar trend, with lower and relatively constant levels from spring to autumn, dropping for the winter (Figure 3A). *Enterobacteriaceae*, coliform bacteria and *E. coli* all peaked in the summer season, reaching an all high in July (~600 CFU), with a smaller peak June. On the *U. spelaeus* skeleton on display in Muierilor, the yeasts and moulds followed a similar trend as on the touched surface, only with lower levels, peaking in the spring (~3400 CFU) and registering lower values for the summer and autumn seasons. Total bacteria and *Enterobacteriaceae* started to rise in the spring, peaking in the autumn (~4300 and ~1100 CFU, respectively) and then declining in the winter. Coliform bacteria and *E. coli* peaked in the spring season, with smaller peaks in the summer (Figure 3B).

Differences were observed between the overall yeasts and moulds loads of the sampling sites on touched stalagmites and the *U. spelaeus* skeletons on display, as the overall counts for the first reached ~55,000 CFU, whilst for the later it was an order of magnitude lower, at only ~4,300 CFU. A smaller difference was observed for total bacteria, as the overall load reached ~50,000 CFU on stalagmites and ~37,000 CFU on skeletons. There were no bacteria cultivated in Ursilor cave from March to April 2015. *Enterobacteriaceae* had a higher count on the *U. spelaeus* skeletons on display, especially on the one in Muierilor. Coliform bacteria and *E. coli* followed a similar trend as yeasts and moulds and total bacteria. As the differences between the caves go, yeasts and moulds had slightly higher numbers in Ursilor than in Muierilor, while for total bacteria it was the opposite. An almost double number of *Enterobacteriaceae* were present in Muierilor compared to Ursilor, while coliform bacteria and *E. coli* were only slightly higher than in Ursilor (Figures 2, 3).

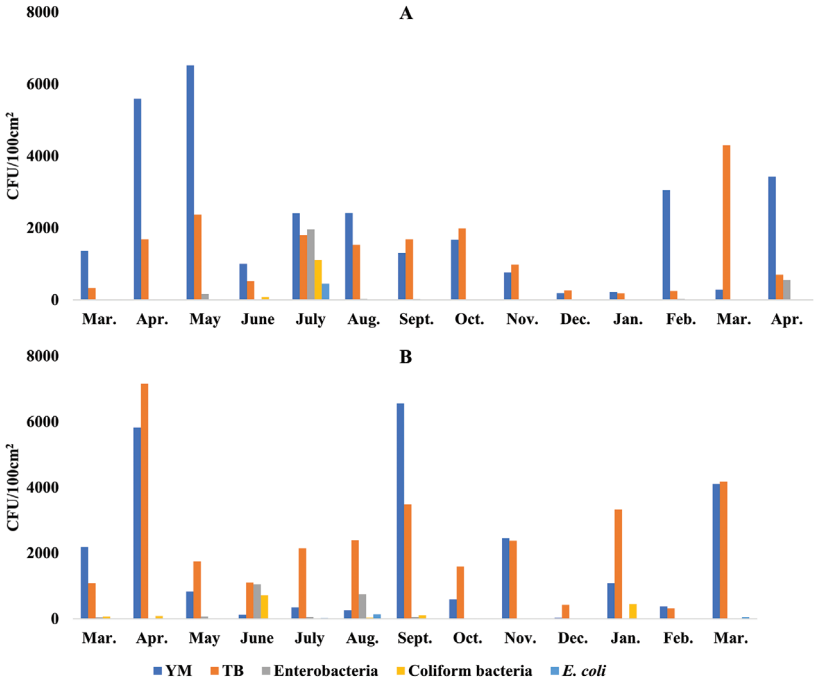

Figure 2. Distribution of culturable bacterial and fungal abundances on touched stalagmites from 2015 to 2016 in Ursilor (**A**) and Muierilor (**B**). TB, total aerobic bacteria; YM, yeasts and moulds; *E. coli*, *Escherichia coli*.

The one-way analysis of variance performed for each site resulted in p-values < .00001 for all but one site (the skeleton site from Muierilor, p .000236) for a significance value of p .01. The F-ratio value for the stalagmite site in Ursilor was F = 12.46052 (4/65 numerator/denominator degrees of freedom) and F = 21.52592 (4/65) for the skeleton site, while for the stalagmite site in Muierilor it was F = 11.84504 (4/60) and F = 6.56377 (4/65) for the skeleton site.

Sequence diversity

Identification of bacteria by 16S rRNA gene sequencing revealed the presence of *Mortierella* sp. (closest GenBank match, KT964847.1; identity, 100%; length of matched segment, 272 base pairs [bp]) in Ursilor SU site and of *Staphylococcus* sp. (closest GenBank match, KX262673.1; identity, 100%; length of matched segment, 899 bp) in Muierilor SU site. The yeasts and moulds group identified by 18S rRNA gene sequencing was represented in the SU site of Muierilor by *Dothideomycete* sp. (closest GenBank match, AY275186.1; identity, 100%; length of matched segment, 309 bp) and *Penicillium* sp. (closest GenBank match, KU350746.1; identity, 99%; length of matched segment, 277 bp) while *Apiotrichum* (*Trichosporon*) sp. (closest GenBank match, KF036718.1; identity, 99%; length of matched segment, 263 bp) was found in the SA site.

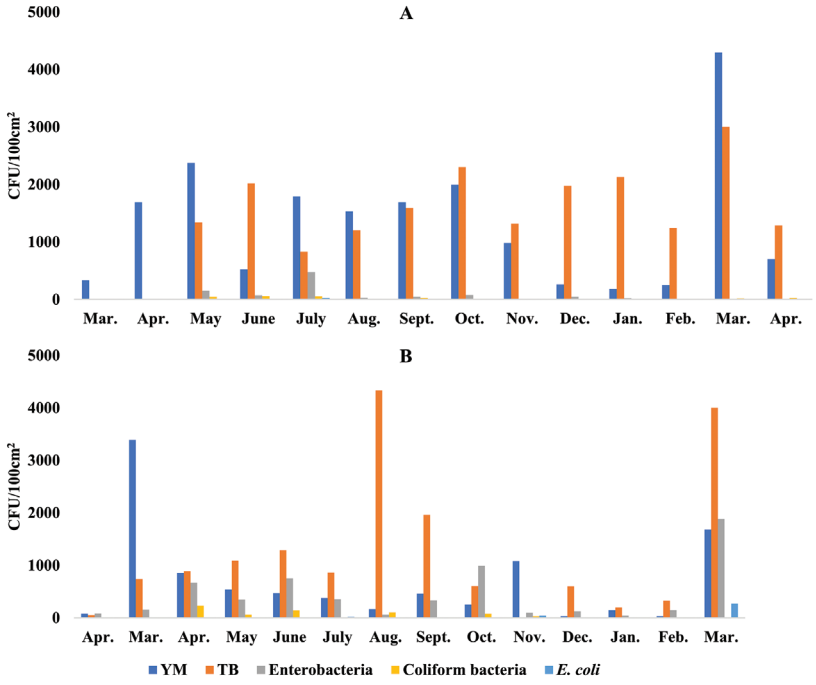

Figure 3. Distribution of culturable bacterial and fungal abundances on the *Ursus spelaeus* skeletons on display from 2015 to 2016 in Ursilor (**A**) and Muierilor (**B**). TB, total aerobic bacteria; YM, yeasts and moulds; *E. coli*, *Escherichia coli*.

Environmental parameters

As previously described for these caves, the airflow was the most stable environmental parameter measured, relative humidity was above 94% inside the caves and the increase in the number of tourists was synchronous with an increase in the levels of CO_2 and temperature inside the caves during spring and especially during summer months (Bercea et al. 2018). Average particulates levels were much lower in Ursilor compared to Muierilor (Table 1).

Table 1. Mean values of environmental parameters over the course of the study period. SD, Standard deviation; PT/CC, particles per cubic centimetre of air.

Site	Mean value ± SD				
	Temperature (°C)	Relative humidity (%)	Airflow (m/s)	CO_2 (ppm)	PT/CC
Ursilor					
Overall	11.33 ± 0.63	94.69 ± 2.44	1.40 ± 0.67	3057.14 ± 1834.94	469.86 ± 543.98
Skeleton	11.29 ± 0.68	94.66 ± 2.42	1.28 ± 0.07	3050.71 ± 1811.37	425.50 ± 493.01
Stalagmite	11.37 ± 0.56	94.71 ± 2.46	1.52 ± 0.93	3063.57 ± 1858.19	514.21 ± 587.24
Muierilor					
Overall	10.04 ± 1.33	95.19 ± 2.47	1.22 ± 0.11	620.37 ± 293.6	5175.63 ± 6176.29
Skeleton	10.18 ± 1.08	95.40 ± 2.58	1.24 ± 0.12	632.86 ± 299.94	5508.57 ± 6732.27
Stalagmite	9.89 ± 1.54	94.96 ± 2.33	1.20 ± 0.09	606.92 ± 286.02	4817.08 ± 5492.69

Discussion

The increase in total bacteria and yeasts and moulds CFU on the touched surfaces of Ursilor and Muierilor mirrors the visitation pattern of these caves and of other Romanian show caves. The number of CFU start to rise in the early spring, maintaining elevated levels up to the end of touristic season. Peaks of indicator organisms were recorded mainly during the summer, concomitant with high tourist influx in the caves. Despite the reduced number of caves that this study involved and based on the coinciding visitation pattern observed for Romanian show caves (Bercea et al. 2018), there is a probability that the surfaces of most Romanian show caves could harbour similar evolving microbiomes, subject to human influence. The lower level of particulates in Ursilor compared to Muierilor could be attributed to the fact that Ursilor has air lock style entrances.

Although *Dothideomycete* have high numbers of known inhabitants of caves, including known biodeteriorants that slowly erode the surfaces they inhabit (Mammola et al. 2017), the one identified via GenBank was not previously found in caves, marking a step forward in mapping the environments that this class inhabits and possibly a novel species for the cave environment. *Staphylococcus* sp. are also known biodeteriorants of the surfaces they inhabit in some caves (De Leo et al. 2012) while also being classified as human pathogens, correlated with speleological exploration and visitation (Boga et al. 2007). The genus is known to inhabit frequently touched subterranean objects and surfaces and was previously found in the studied show caves (Borda et al. 2014; Bercea et al. 2018). *Mortierella* is a known inhabitor of altered mycobiotas of show caves (Jurado et al. 2010b) and was previously found in Romanian caves (Epure et al. 2014). *Apiotrichum* (*Trichosporon*) have previously been correlated with fungal outbreaks in show caves and can lead to the establishment of biofilms on subterranean surfaces (Jurado et al. 2010a; Jurado et al. 2010b; Urzì et al. 2010). *Penicillium* are a common occurrence on various surfaces from the altered mycobiotas of show caves as a result of tourism (Urzì et al. 2010; Mammola et al. 2017).

Preventing the pollution of caves and cleaning them of what has already been inflicted upon these fragile habitats are easy-to-do actions with the higher purpose of quality management, whilst also implementing a proper land management for the surrounding areas, thus limit the allochthonous input from several sources (Watson 1997; Rechtschaffen 1998). A remediation program for a subterranean environment cannot be perceived only from the mindset of cleaning frequently touched surfaces, it has to be an overall policy that ameliorates the accumulated human disturbance.

Traditionally, microbial sampling is a burdensome task in caves as it involves tools that are fragile and need delicate handling for proper operation, like agar-based media in Petri dishes fabricated out of glass or rigid polymeric materials. Recent studies analysed and proved the potential of commercially available, ready to use test sheets (Mulec et al. 2012b; 2012a; Mulec et al. 2012c; Borda et al. 2014; Moldovan et al. 2015; Bercea et al. 2018). The advantages of such media compared to a Petri dish are, among others: the smaller size that allows scientists to carry a significantly larger number of media; toughness due to elasticity, as it can withstand repeated impacts that would shatter any

glass, or for that matter, rigid polymeric material Petri dishes; and the easy-to-handle characteristics. The robustness and universality of such test sheets for quantification of total aerobic bacteria, yeasts and moulds and indicator organisms (*Enterobacteriaceae*, *E. coli* and coliform bacteria) was previously tested for surfaces, waters and the air of caves, thus proving the viability of such methods (Mulec et al. 2012b; 2012a; Mulec et al. 2012c; Borda et al. 2014; Moldovan et al. 2015; Bercea et al. 2018). As selective plate media were used to assess the bacterial and fungal community, we must also mention this as a shortcoming, as these media were not specifically designed for use in caves.

Although some test sheets might be selective to some taxa, including the ones used in this study, their use, never the less, should be involved in future monitoring programs of show caves, and, if faced with high CFU levels, the deployment of molecular methods should be a top priority. When faced with dangerously high microbial loads, the first action by cave management should be to limit or even deny the access of visitors to the afflicted areas or to the whole cave. As proper cave management goes, the issue of surveying a site before opening it to the public should imply, among many others aspects, the microbial monitoring over at least a yearly cycle on a monthly basis and maintaining the said monitoring program through the operational life cycle of the show cave, albeit at lower frequencies, such as bimonthly or seasonally. As far as the type of selective media that should be used for sampling, aspects such as robustness, ease of use in the cave environments and affordability must be taken into consideration.

This was the first study to analyse the dynamics of the microbial communities of delicate subterranean surfaces and those of *U. spelaeus* skeletons displayed in show caves through the use of commercially available test kits. We revealed that exposed surfaces in show caves, in direct or indirect contact with tourists, host high concentrations of cultivable microbes. The presence of indicator organisms was confirmed through most of the year and in higher numbers on the surfaces with which tourist come in direct contact constantly. The touristic activity was shown to influence the abundance and dynamics of the microbial communities inhabiting surfaces within show caves.

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How is the anchialine fauna distributed within a cave? A study of the Ox Bel Ha System, Yucatan Peninsula, Mexico

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Abstract

A study describing the diversity and distribution pattern of the stygobitic fauna in the Ox Bel Ha anchialine cave system adjacent to the Caribbean coast of the Yucatan Peninsula, Mexico is presented. A total of 15 species of crustaceans were collected in three surveys at four points situated along a 10.2 km transect perpendicular to the coast line. A freshwater mass dominated throughout the transect with a halocline that appeared progressively deeper, from 10 to 18 m, with increasing distance from the coast. All the recorded species, except for one, occurred throughout the transect with no defined pattern. Abundance and species richness did not vary significantly with distance from the coast, whereas diversity (H') peaked in the second sampling site at 3.17 km from the coast. As expected, most of the organisms occurred only in the freshwater layer, except for the remipede *Xibalbanus tulumensis* (Yager, 1987) that was found always at or below the halocline, and five other species that were found above and below the halocline. In the horizontal scale, species composition and occurrence mixed without a defined pattern, both, for sampling dates and sites. The results show that the analyzed fauna is distributed throughout the 10.2 km transect without showing any defined horizontal zonation pointing to a high connectivity among all sections. Due to the high connectivity within the caves in the area, it is expected that significant variation in species composition and distribution will be found at a larger regional scale.

Keywords

Diversity, abundance, vertical distribution, connectivity

Introduction

The anchialine cave systems of the world have been intensely studied during the last three decades since their discovery and the improvement of diving techniques to explore them (Iliffe 2018, Iliffe and Alvarez 2018). The vast majority of studies so far conducted with anchialine fauna are of a taxonomic nature, often with descriptions of new species, or presenting new distributional records (Alvarez and Ojeda 2018, Iliffe and Alvarez 2018, Alvarez et al. 2015, 2019). Although basic ecological questions are of great importance, few studies have been published regarding the population dynamics of key species, trophic interactions and seasonal variations in diversity and abundance in these environments (Pohlman et al. 1997, Havird et al. 2015, Brankovits et al. 2017). Two main reasons have hindered the progress of this type of studies: first, the very specialized diving techniques needed to gather samples and data from the flooded cave environments (Iliffe 2018) and second, the poor knowledge about the total diversity of these systems reflected in the constant finding of new species (e.g., Alvarez et al. 2017, Suárez-Morales et al. 2017).

The anchialine caves of the Yucatan Peninsula (YP) along the Caribbean coast of Mexico between the City of Cancun and the town of Tulum (Fig. 1) are exceptionally large, some of them exceeding 250 km of passageways (QRSS 2018). These caves have developed in a 10–15 km wide band adjacent to the coast, of Pleistocene origin, at an average depth of 15 m (Smart et al. 2006). The caves around the town of Tulum exhibit extensive horizontal branching, are connected to the sea by defined conduits and become deeper away from the coastline.

While several studies have explored the distribution of anchialine fauna at a regional scale (Kornicker and Iliffe 1998, Alvarez and Iliffe 2008) and others have described the bathymetric distribution of the fauna and the microbial communities (Humphreys 1999, Gonzalez et al. 2011), few studies have looked at the within cave or cave system distribution. A first example is the study by Martínez-García et al. (2009) who described the diversity and distribution of anchialine fauna in the Corona lava tube in Lanzarote, Canary Islands, recognizing three main sections with different habitats and a consequent zonation of species. A second example is the study by Calderón-Gutiérrez et al. (2018) who described how density of organisms and species richness varied in four different anchialine caves in Cozumel Island, Quintana Roo, Mexico. In the latter study, the main objective was to register the diversity of organisms found in different caves, thus the differences encountered were associated to the type of cave rather than to the variety of habitats found within each cave. Part of the explanation for the absence of this type of studies is the fragmented and isolated nature of most anchialine habitats, such as sinkholes (cenotes), blueholes and coastal caves, which are studied as independent localities.

The anchialine caves of the YP are unique in terms of their extension and in the relative ease with which they can be accessed through cenotes. Although the explorations made by divers suggest that there is a high connectivity throughout most of the caves' passageways, no studies have been conducted to describe if the anchialine fauna is distributed uniformly within large caves or if there is a zonation or differential oc-

cupation of defined habitats or sections. In this study we analyzed the spatio-temporal variation of the anchialine fauna along a 10.2 km transect (distance on the surface) in the Ox Bel Ha (OBH) anchialine cave, one of the largest underwater caves on earth (QRSS 2018). The study was designed to determine what species were present in the cave, and to test if there is a pattern of differential use of the cave´s sections or if the connectivity along the passageways would allow a uniform species distribution. The working hypothesis was that diversity and abundance of anchialine organisms would decrease with increasing distance from the coast as tidal influence would fade away and the salt water layer below the halocline would be found at increasing depths.

Materials and methods

Field work

A transect was established along the northern branch of the OBH system, south of the town of Tulum, Quintana Roo, Mexico (Fig. 1). This branch runs perpendicular to the coastline, within it the penetration of marine water creates a gradient that is detected in the increasing depth of the halocline with distance from the coastline. Four cenotes were used to access the cave: Tábano (20°10'8"N, 87°27'22.7"W), Odyssey (20°10'26"N, 87°28'14.6"W), Muknal (20°11'19"N, 87°29'24.3"W) and Bang (20°12'38"N, 87°30'4.1"W); situated 0.86, 3.17, 6.74 and 10.17 km from the coastline, respectively. Three of the four cenotes are similar in size, with exposed pools ranging from 6 to 10 m in diameter, surrounded by dense vegetation; cenote Odyssey develops under a semicircular crack producing a half moon shape twilight zone. In three cenotes, the twilight zone gives way through restrictions to the cave zone, whereas in cenote Bang the single pool gradually becomes the cave at around 12 m depth. All faunal samplings were made in the cave area in complete darkness.

Three surveys were conducted in February, August and December, 2013. A group of three divers performed several tasks in each survey. First, one diver profiled the water column for temperature, salinity, dissolved oxygen concentration, pH and conductivity with a Hydrolab DataSonde 5X probe in the area where the biological sampling was going to be conducted; a second diver obtained samples from the water column above and below the halocline with two separate plankton nets with a 500 μm mesh; and the third diver captured individual organisms with glass vials recording the depth at which the sample was taken.

All samplings, in the four cenotes and adjacent cave in the three sampling dates, were conducted by three divers who performed the same tasks. The divers followed the same route inside the caves in every survey. Air supply was the same for all dives which lasted between 90 and 100 min, so that sampling effort was as similar among surveys as possible. Although neither fish nor copepods were considered in our sampling, the endemic blind brotula, *Typhliasina pearsei* (Hubbs, 1938), was observed in all four cenotes. All specimens were collected under the scientific collector's license issued to FA (FAUT 0104) by the Mexican environmental authority (SEMARNAT).

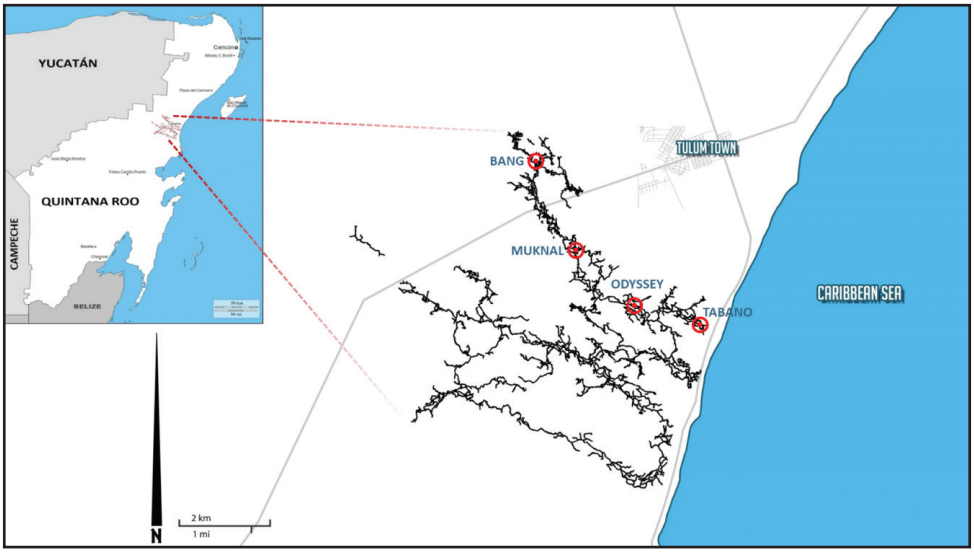

Figure 1. Map of the Ox Bel Ha anchialine cave system near the town of Tulum. Quintana Roo, Mexico. The four cenotes used to access the cave were: Tábano, Odyssey, Muknal and Bang.

Sample processing

All samples were preserved in 80% EtOH and all organisms identified to species. Appropriate taxonomic keys were used to identify the collected organisms: amphipods (Bowman 1977, 1987, Bowman et al. 1984, Holsinger 1990), atyid shrimps (Alvarez et al. 2005, Botello et al. 2013), hippolytid shrimps (Escobar-Briones et al. 1997), isopods (Botosaneanu and Iliffe 1997), ostracods (Kornicker and Iliffe 1998), palaemonid shrimps (Botello and Alvarez 2006, 2010), thermosbaenaceans (Bowman and Iliffe 1988), mysids (Kallmeyer and Carpenter 1996) and remipedes (Yager 1987, Olesen et al. 2017).

Data analysis

To test for differences in the distribution of organisms along the transect abundance data were tested for normality with a Kolmogorov-Smirnov test, and a Cochrane test of homocedasticity. One-way analysis of variance was used to compare abundances by site and by sampling date. In order to describe the number of species and their abundances Shannon's diversity index was obtained by site (Shannon and Weaver 1949). Hutcheson t-tests were used to compare the diversity indices of each sampling site (Magurran 1988).

Relationships among the faunal assemblages of each cenote were examined via a similarity matrix constructed using the Jaccard's coefficient. Jaccard's similarity is, in this framework, a measure of the degree of overlap between sample points in terms of species occurrence and composition. The resulting similarity matrix was used for both cluster analysis (UPGMA) and non-metric multidimensional scaling (nMDS), as has

been suggested by several authors (Field et al. 1982, Clarke and Warwick 2001, Legendre and Legendre 2012). To perform the above procedures we used the package "Vegan" in the statistical software R 3.1.3 (R Development Core Team 2018). To analyze the effect of environmental variables on species abundance by cenote, we run a multivariate analysis of variance ("Adonis" routine within "Vegan" package in the statistical software R 3.1.3; R Development Core Team (2018)) using distance from the coast, pH and halocline depth; we did not use salinity, dissolved oxygen concentration and temperature in this analysis because their continuous variation through the water column.

Results

Water column profiles

In cenote Tábano salinity ranged from 2 to 32 psu, with a halocline present at a depth of 11–12 m; for cenote Odyssey the values were: 0.6 to 35 psu and a halocline between 13–14 m; for cenote Muknal 0.5 to 33 psu, and a halocline between 14–15 m; and for cenote Bang 1 to 34.5 psu, and a halocline at 18–20 m depth (Fig. 3). Throughout the sampling period temperature fluctuated between 25.5 and 26.9 °C in the freshwater layer, and between 25.8 and 27.2 °C in the saline water layer; pH fluctuated between 6.9 to 7.7, and from 6.9 to 7.9, in the freshwater and saline water layers, respectively (Table 1). The dissolved oxygen concentration (incomplete data due to equipment failure in the February sampling) showed more variation than the other parameters. In August the freshwater layer along the transect had between 0.21 and 0.78 mg/l of O_2,

Table 1. Water quality parameters recorded in the three samplings (February, August and December, 2013) in the four sites of the Ox Bel Ha system, Quintana Roo, Mexico: Tábano, Odyssey, Muknal, Bang. Columns correspond to: depth of the halocline in m, salinity in psu, temperature (°C), pH and dissolved oxygen concentration in mg/l ($[O_2]$). Dashes indicate readings not taken, "NR" stands for not recorded when the halocline was deeper than the deepest surveyed area.

		Salinity		Temperature		pH		$[O_2]$	
	Halocline	Above Halocline	Below Halocline	Above Halocline	Below Halocline	Above Halocline	Below Halocline	Above Halocline	Below Halocline
February									
Tabano	10–12	4.34	30.4	26.9	27.2	6.9	7.1	–	–
Odyssey	12–13	4	32.1	25.7	26.7	6.8	7.3	–	–
Muknal	13	–	–	–	–	–	–	–	–
Bang	18–19	2.2	32.7	25.5	25.8	6.9	7.4	–	–
August									
Tabano	10–11.7	4.34	27.9	26.1	26.4	7.7	7.8	0.21	0.32
Odyssey	12–12.5	5.1	33	25.8	26.7	7.7	7.8	0.38	1.5
Muknal	15–16.5	2.99	33	25.6	26.3	7.6	7.9	0.78	1.95
Bang	18–20.9	2.2	32.8	25.5	25.8	7.7	7.8	0.69	2.1
December									
Tabano	12	4	NR	26	NR	6.72	NR	5.6	NR
Odyssey	13–14	4.9	31.9	25.6	26.4	6.7	6.9	0.44	2.42
Muknal	16–18	4.3	31.5	25.8	26.2	6.7	6.9	0.31	2.1
Bang	18–20	2.71	26.9	25.8	NR	6.5	NR	0.15	NR

while the saline layer had 0.32 to 2.1 mg/l; in December Tábano had 5.6 mg/l in the freshwater layer due to recent rains while the rest of the transect had 0.15 to 0.44 mg/l of O_2. No halocline was found at the maximum depth (12 m) in Tábano in December due to recent rains. The general trend is one in which the halocline becomes deeper with distance from the coast, temperature and dissolved oxygen concentration are higher in the marine water mass below the halocline, and pH shows a small variation (Table 1).

Anchialine fauna

A total of 368 organisms were collected, 123 in cenote Tábano, 93 in Odyssey, 88 in Muknal, and 64 in Bang. Although total abundance decreases with increasing distance from the coast, mean abundance did not differ significantly along the transect (Anova, $F_{[3, 11]} = 0.8763$, $p = 0.5039$; Fig. 2A). The total number of organisms by survey were:

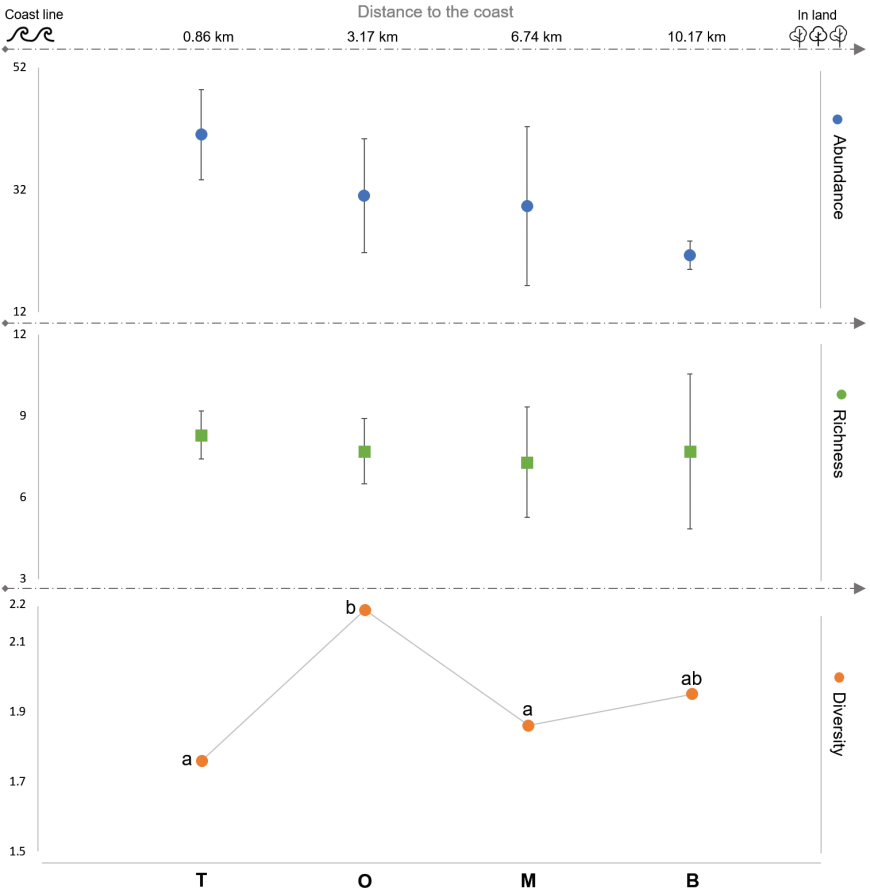

Figure 2. Ecological parameters of the anchialine fauna in the Ox Bel Ha anchialine cave system along a transect **A** mean ± one standard error of the abundance **B** mean ± one standard error of the species richness **C** Shannon's diversity index. The sites are: T, Tábano; O, Odyssey; M, Muknal; B, Bang.

103 in February, 162 in August, and 103 in December; no significant differences were found among the mean number of organisms per survey through the year (Anova, $F_{[2, 11]} = 1.3294$, $p = 0.3438$). A total of 15 species were collected, the most abundant one was the thermosbaenacean *Tulumella unidens* Bowman and Iliffe, 1988, with 81 organisms, followed by *Typhlatya mitchelli* Hobbs and Hobbs, 1976, with 79. The single rare species that was represented by one individual was the hippolytid shrimp *Calliasmata nohochi* Escobar-Briones, Camacho and Alcocer, 1997, found in cenote Muknal. The rest of the fauna is composed by another 12 species of crustaceans belonging to 10 different families (data available as supplementary material). The recorded diversity is about one fourth of all the anchialine fauna recorded so far from the YP (Alvarez et al. 2015, Olesen et al. 2017).

The distribution of species within the transect showed a slight variation, cenote Tábano had a total of 9 species, Odyssey 12, and Muknal and Bang 11; the mean species richness found did not differ among cenotes (Anova, $F_{[3, 11]} = 0.25$, $p = 0.8587$; Fig. 2B); the number of species differed significantly per survey, with December having significantly less species than February and August (Anova, $F_{[2, 11]} = 6.4883$, $p = 0.0316$). The only species present in all three surveys and in all four cenotes was *T. mitchelli* (Table 2). Five species (*Creaseria morleyi* (Creaser, 1936), *Creaseriella anops* (Creaser, 1936), *Tulumella unidens*, *Tuluweckelia cernua* Holsinger, 1990, *Typhlatya mitchelli*) occurred along the whole transect in all four cenotes. This group of species represents the most common anchialine species in the YP, occurring from the Ring of Cenotes around the City of Merida in the State of Yucatan to the Caribbean Cave Area along the coast of the State of Quintana Roo (Alvarez and Iliffe 2008). Another five species (*Mayaweckelia cenoticola* Holsinger, 1977, *Stygiomysis holthuisi* (Gordon, 1958), *Typhlatya dzilamensis* Alvarez, Iliffe and Villalobos, 2005, *T. pearsei* Creaser, 1936, *Xibalbanus tulumensis*) occurred in three of the four cenotes without any defined

Table 2. Occurrence of species by site (Tábano, Odyssey, Muknal, Bang) and sampling (F = February, A = August, D = December) in the Ox Bel Ha system, Quintana Roo, Mexico. In blue species that occurred in the four sites, in green species that occurred in three of the four sites, in yellow species that occurred in two of the four sites and in red the single species that occurred in just one site.

	Tabano			Odyssey			Muknal			Bang		
	F	A	D	F	A	D	F	A	D	F	A	D
Typhlatya mitchelli	•	•	•	•	•	•	•	•	•	•	•	•
Tulumella unidens	•	•	•		•	•	•	•	•	•	•	•
Tuluweckelia cernua	•	•			•	•		•		•	•	
Creaseriella anops	•			•	•		•	•	•	•	•	
Creaseria morleyi		•		•	•		•	•		•		•
Typhlatya dzilamensis		•	•	•	•	•	•	•				
Typhlatya pearsei	•	•	•	•		•				•		
Stygiomysis holthuisi	•	•			•					•		
Xibalbanus tulumensis					•			•			•	
Mayaweckelia cenoticola		•				•					•	
Antromysis cenotensis				•	•		•	•				
Humphreysella mexicana							•				•	•
Stygiomysis cokei	•	•						•				
Metacirolana mayana					•	•					•	
Calliasmata nohochi								•				

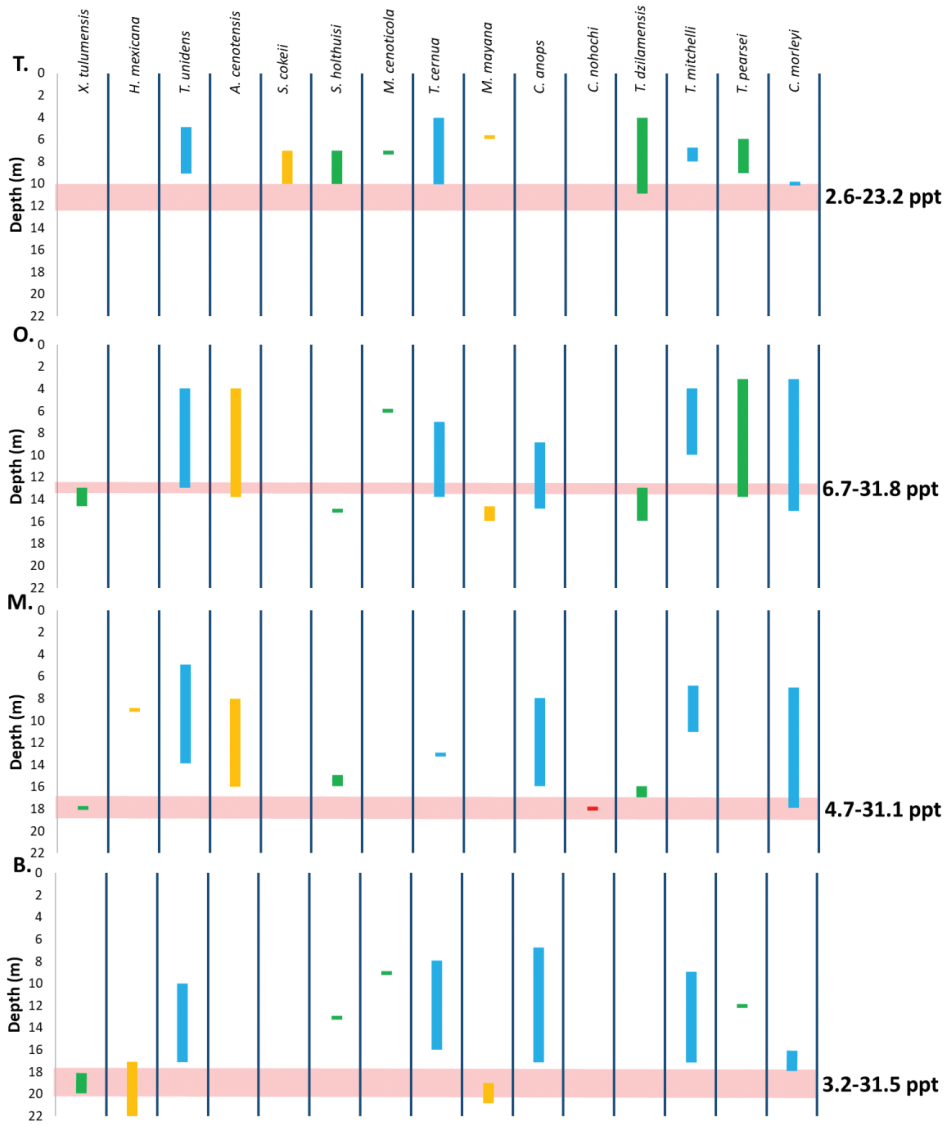

Figure 3. Distribution of organisms by species in relation to depth in the four cenotes studied in the Ox Bel Ha cave: A, cenote Tábano; B, cenote Odyssey; C, cenote Muknal; and D, cenote Bang. The red band depicts the halocline, its width represents the thickness of the interface.

pattern, four species (*Antromysis cenotensis* Creaser, 1936, *Humphreysella mexicana* (Kornicker & Iliffe, 1989), *Metacirolana mayana* (Bowman, 1987), *Stygiomysis cokei* Kallmeyer & Carpenter, 1996) were found in two cenotes and only the hippolytid shrimp *Calliasmata nohochi* was found in one cenote in one survey (Table 2).

Shannon's diversity index (H) varied from 1.765 in Tabano to 2.195 in Odyssey, with Muknal and Bang having intermediate values (Fig. 2C). Odyssey diversity index

Table 3. Results of the multivariate analysis of variance ("Adonis" routine in the statistical software package R 3.1.3; R Development Core Team (2018)) of species abundance using distance from the coast (DC), pH and halocline depth (Halocline).

	Df	Sums. Of Sqs.	Mean. Sqs.	F. Model	R^2	Pr(>F)
DC	1	0.34744	0.34744	1.48583	0.13756	0.2079
pH	1	0.37364	0.37364	1.59785	0.14793	0.1089
Halocline	1	0.32141	0.32141	1.37451	0.12726	0.2772
DC: pH	1	0.12133	0.12133	0.51888	0.04804	0.8218
DC: Halocline	1	0.23741	0.23741	1.01526	0.094	0.4356
pH: Halocline	1	0.11867	0.11867	0.50748	0.04698	0.8416
DC: pH:Halocline	1	0.07044	0.07044	0.30125	0.02789	0.9604
Residuals	4	0.93535	0.23384		0.37033	
Total	11	2.52568			1	

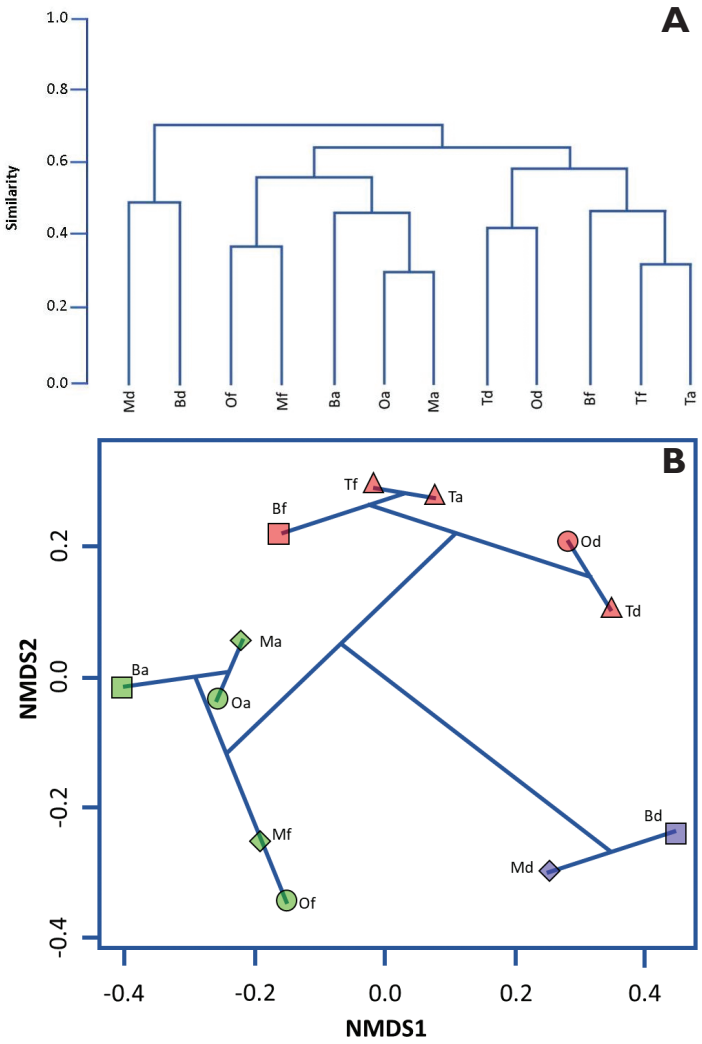

Figure 4.A Dendrogram and **B** non-metrical multidimensional scaling (nMDS) ordination plot, both resulting from the similarity matrix based on Jaccard's similarity index.

was similar to Bang, but significantly different from Tábano and Muknal (Hutcheson T-test, Fig. 2C). Out of the 15 species recorded, the only one restricted to the salt water layer was the remipede *Xibalbanus tulumensis*, always found below the halocline in the three internal cenotes (Fig. 3). The remaining 14 species occurred mainly in the freshwater/brackish layer, however, five of them had occasional occurrences below the halocline (Fig. 3). None of the environmental variables used in the multivariate analysis of variance showed a significant effect on species abundance per site; pH,which was the most relevant variable, explained only 15% of the total variance (Table 3).

The analysis of similarity shows three groups in which all four cenotes and the three surveys are mixed (Fig. 4A, B). One group is formed by the samples of Muknal and Bang in December, while the other two groups cannot be defined clearly. The obtained pattern may indicate that there is no marked zonation within the cave in terms of species composition.

Discussion

The obtained results on the distribution and abundance of anchialine fauna along a 10.2 km transect in the OBH cave, suggest the existence of a high connectivity throughout the conduits that prevent a horizontal zonation. The hydrology of this area along the Caribbean coast of the YP has been studied showing the presence of a dynamic "subterranean estuary" where saline water penetrates inland below a freshwater lens and where groundwater discharge can be considerably high (Beddows 2004). Our temperature and salinity measurements along the transect are consistent with what would be expected according to the ground water circulation model of Beddows et al. (2007), in which near the coast salinity above the halocline is higher and the temperature difference between water layers is larger, and where with increasing distance from the coast salinity decreases above the halocline and the temperature of the two water masses tends to be the same (Table 1). These data evidence a hydrological continuum that is preserved by the circulation occurring through the large underground conduits (cave passageways) in the area.

The hydrological connectivity amongst caves within the anchialine system in this area of the YP allows species to distribute throughout the conduits without a defined pattern. Our results show that most of the recorded species can occur in any section of the studied cave system. The limitation for the species that are restricted to the high salinity water mass, such as the remipede *X. tulumensis*, in this particular branch of the OBH would be the absence of a marine layer due to the shallow conduits. The conduits develop at a depth of 12 to 14 m, but with an irregular bathymetry large sections of cave could be above the level of the halocline. In contrast, the freshwater species can exlpoit a much larger area.

The number of anchialine species recorded in this study (15) represents about 27% of the known diversity for the anchialine caves of the YP (Alvarez and Iliffe 2008, Alvarez et al. 2015, Olesen et al. 2017). The assemblage of species found in the OBH system includes both, common and widely distributed throughout the YP (e.g.,

Creaseriella anops, *Creaseria morleyi*, *Typhlatya mitchelli*) and some rare ones which are present in this area only (e.g., *Xibalbanus tulumensis*, *Calliasmata nohochi*).

While species composition along the transect did not show any defined pattern, abundance of organisms shows a slight, not significant, trend to decrease with increasing distance from the coast. The amount of nutrients could be increasing towards the coast where organic matter that enters via cenotes or percolation from the rainforest floor accumulates due to the circulation of freshwater (Beddows et al. 2007); however, near the coast is also where human impact is occurring more intensely (Hernández-Terrones et al. 2011). Alternatively, natural cycling of carbon and nutrients takes place in conserved areas where decomposition on the rainforest floor is connected to the water mass contained in the caves (Brankovits et al. 2017). In any case, precise data on the amount of available nutrients throughout a transect as the one studied here are lacking.

The obtained results suggest that isolation within a cave along the Caribbean coast of the YP would not be significant to allow for population differentiation. The high degree of connectivity represents a continuum, at least in the freshwater/brackish water layer above the halocline, allowing for free movement of organisms. Thus, the scale of variation among populations that would be expected would correspond to a regional scale throughout the YP. In fact, several species endemic to this part of the YP (such as the remipede *Xibalbanus tulumensis*, and the amphipod *Tuluweckelia cernua*) are common in the whole Caribbean cave area that runs from Puerto Morelos to Tulum, in the state Quintana Roo.

Conclusion

The sampling along the transect in the OBH cave shows that the anchialine fauna is distributed throughout the flooded cave without a defined pattern. The hydrological data obtained is consistent with a high connectivity system typical of the cavernous area in this part of the Caribbean coast of Mexico. As expected most of the anchialine fauna found are freshwater species, with only a few restricted to the high salinity water mass.

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RESEARCH ARTICLE

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Distribution of Oribatida (Acari) along a depth gradient in forested scree slopes

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Abstract

Mesovoid shallow substratum (MSS) of scree slopes constitutes a transition habitat between the soil and the network of voids in the vadose zone of a bedrock massif. In the present study, the vertical distribution of oribatid communities along a depth of 95 cm was studied at five forested MSS sites in the Western Carpathians, Slovakia. The sites differed in type of bedrock, topography and gradient of the microclimate and nutrients content. In all, 909 specimens were captured in subterranean traps exposed for one year. Most Oribatida represented edaphic forms, and their presence in the depth profile of the screes was accidental. *Pantelozetes cavatica* (Kunst, 1962) was the only species closely linked to deep subterranean environments found in the deeper part of the single limestone site studied. Species richness and the activity of oribatids along the scree profile at the sites clearly reflected the content of organic carbon in the soil substratum. The communities had very low numbers of individuals and low species richness at three sites with soil pH < 7 and organic carbon content in the upper soil layer ≤ 10%. However, they differed markedly in internal temperature dynamics. The other two sites, with a slightly alkaline soil pH and a higher carbon content, showed distinctly higher activity and a relatively uniform pattern of oribatid distribution across the depth profile. The soil pH and organic carbon content in the topsoil layer were substantial factors that determined the Oribatida diversity and vertical distribution in the forested screes.

Keywords

Environmental factors, mesovoid shallow substratum, oribatid mites, subterranean environment, vertical distribution

Introduction

A shallow subterranean habitat represents an environment differing from deep caves by its close contact with the upper layers of soil (under 10 metres) and thus better access to nutrients (Růžička 1999; Moseley 2009; Culver and Pipan 2014). Colluvial 'mesovoid shallow substratum' (MSS) is a type of this aphotic habitat consisting of a network of cracks (voids) among rock fragments at the bottom of stony walls in steep mountain slopes (Juberthie et al. 1980; Juberthie 2000; Mammola et al. 2016). Such stone accumulations, also known as screes, stony debris or talus deposits, are often a frequent component of the relief in European mountains and form 'island habitats' (Růžička 1990; Růžička 1999; Barranco et al. 2013). Many authors have highlighted the presence of hypogean and epigean fauna in MSS but also specialized species that clearly prefer this environment (e.g., Růžička et al. 1995, 2012; Gers 1998; Giachino and Vailati 2010; Culver and Pipan 2014; Rendoš et al. 2016b).

The majority of the mite fauna in caves and subterranean habitats consists of Mesostigmata and Oribatida (e.g., Skubała et al. 2013; Jiménez-Valverde et al. 2015). Oribatid mites have a great potential to colonize MSS habitats due to their small body size and high density that they can reach in the forest soils, e.g. up to 200,000 ind./m^2 in boreal forests (Maraun and Scheu 2000). However, studies on Oribatida from subterranean environments are very rare, and only a few of them deal with the group in mesovoid shallow substratum. The first investigation of oribatid communities in MSS carried out by Arillo et al. (1994) in the Canary Islands led to the discovery of two new-to-science species. Much later, Skubała et al. (2013) detected that among Belgian speleofauna most oribatids were typical inhabitants of the upper layer of forest soil, and representatives of small oribatids, characteristic of deeper soil layers (e.g. Oppiidae), were rarely found. Recently, Nae and Băncilă (2017) described oribatid communities of an MSS site in the Romanian Carpathians, where common forest soil or tree-bark dwellers predominated. Similarly, Jiménez-Valverde et al. (2015) recorded three epigeic species of Oribatida when performing a study in Spain of subterranean fauna of bare scree slopes sparsely covered by the soil.

The present study was focused on Oribatida communities inhabiting the vertical gradient of five forested scree slopes in the Western Carpathians of Slovakia. Based on current knowledge, we assumed that the communities would differ noticeably along the environmental and nutrient gradients, and the differences in their structure would be closely related to changes in the environmental parameters of these screes.

The aims of the present study were: (1) to describe the distribution of oribatid communities along a vertical gradient at five scree sites in the Western Carpathians varying in topography and type of parent rock, (2) to clarify the presence of subterranean forms at these scree sites, and (3) to detect the response of the communities to environmental factors (internal scree temperature, soil pH and organic carbon content).

Material and methods

Study sites

Oribatida were sampled at five sites situated in different geomorphological units of the Western Carpathians in Central Europe (Fig. 1). The detailed site characteristics are provided in Table 1.

- Ardovská jaskyňa Cave (AJ) – a cave in the Slovak Karst near the village of Ardovo (south-eastern Slovakia). A southern scree slope with cornel-oak forest covered with soil and leaf litter and rocks with mosses, situated near the cave entrance. The scree profile: leaf litter and humus (0–15 cm), an organo-mineral layer with admixtures of small rocks and spaces partially filled with soil (15–75 cm) and a deeper scree layer formed by large rocks (75–100 cm).
- Belinské skaly Rocks (BS) – rocky locality in the Cerová vrchovina Highlands, near the village of Belina (south-eastern Slovakia). The site is a south-west exposed, carbon-poor volcanic scree slope with oak-hornbeam forest. The scree profile: leaf litter and humus (0–5 cm), an organo-mineral layer with a mixture of small basalt rocks and mineralized soil (5–70 cm) and a scree of small stones with spaces filled with soil (70–110 cm).
- Drienčanský kras Karst (DK) – a small karst area in the Revúcka vrchovina Highlands, near the village of Španie Pole (south-eastern Slovakia). The site is formed by a limestone ridge and a steep north-facing scree slope with beech-hornbeam forest lying a few metres below the entrance of the Špaňopolská jaskyňa Cave. The scree profile: leaf litter and humus (0–5 cm), an organo-mineral layer and soil with tiny stones (5–70 cm) and a scree of bigger rocks partially clogged with soil (70–110 cm).
- Malý Ružínok Valley (MR) – a karst valley in the Čierna hora Mountains, near the village of Malá Lodina (eastern Slovakia). A massive limestone cliff with several short caves and a north-exposed scree slope with linden-maple forest at its base are the typical features of this site. The scree profile: leaf litter and humus (0–15 cm), an organo-mineral layer (15–45 cm) and a clearly separated scree of bigger stones with spaces partially filled up with soil (45–110 cm).
- Silická ľadnica Cave (SL) – a cave with the permanent floor ice at the Silická Plateau in the Slovak Karst, near the village of Silica (south-eastern Slovakia). A west-facing scree slope with hornbeam-maple-linden-oak forest is located in the sinkhole near the cave entrance with a dense herbal cover. The scree profile: leaf litter and humus (0–10 cm), an organo-mineral layer with well developed rhizosphere and spaces substantially filled with soil (10–35 cm) and an extremely stony scree (35–110 cm).

Sampling and species identification

At each site, a few metres below the top of the scree slope, a pit approximately 200 × 40 cm in area and over 100 cm deep was excavated, and the soil of the particular

Figure 1. Localities of samplings. 1 – Ardovská jaskyňa Cave, 2 – Belinské skaly Rocks, 3 – Drienčanský kras Karst, 4 – Malý Ružínok Valley, 5 – Silická ľadnica Cave.

layers was carefully separated. Three subterranean traps, after Schlick-Steiner and Steiner (2000), were vertically placed into the pit, 50 cm away from one another. The pit was subsequently backfilled with the dug-out soil and stones in the original order of the layers. The subterranean trap consisted of a plastic cylinder (length 110 cm, diameter 10.5 cm) with openings (diameter 0.8 cm) drilled around at 10 horizontal levels (5, 15, 25, 35, 45, 55, 65, 75, 85 and 95 cm), and a demountable system of 10 plastic cups (volume 500 ml), connected by a central iron rod and nuts. The buried cylinder served as a casing, allowing the insertion of cups filled with a 4% formaldehyde solution. The position of the cups inside the cylinder corresponded to the openings on the cylinder perimeter. The top of the cylinder was tightly closed with a plastic cap (Fig. 2). The subterranean traps at the Malý Ružínok Valley were exposed from October 2008 to October 2009 (366 days). The traps at the Belinské skaly Rocks and Drienčanský kras Karst were exposed from October 2012 to October 2013 (370 days) and at the Ardovská jaskyňa and Silická ľadnica caves from October 2014 to October 2015 (361 days). To remove the captured specimens, the system of plastic cups was pulled out of the buried cylinder, and the individual cups were dismounted. The contents of the cups were then poured into plastic bottles and transported to the laboratory for analysis. Oribatida were separated from the samples, mounted on temporary slides with lactic acid and identified using a Leica DM1000 light microscope and identification keys (Kunst 1971; Pavlitshenko 1994; Weigmann 2006).

At each sampled site, microclimatic and chemical parameters were collected from depths of 5, 35, 65 and 95 cm. The temperature was measured continually over the sampling period at 4-hour intervals using iButton DS1921G data-loggers mounted on the walls of the plastic cups. To determine the soil chemical parameters (pH and

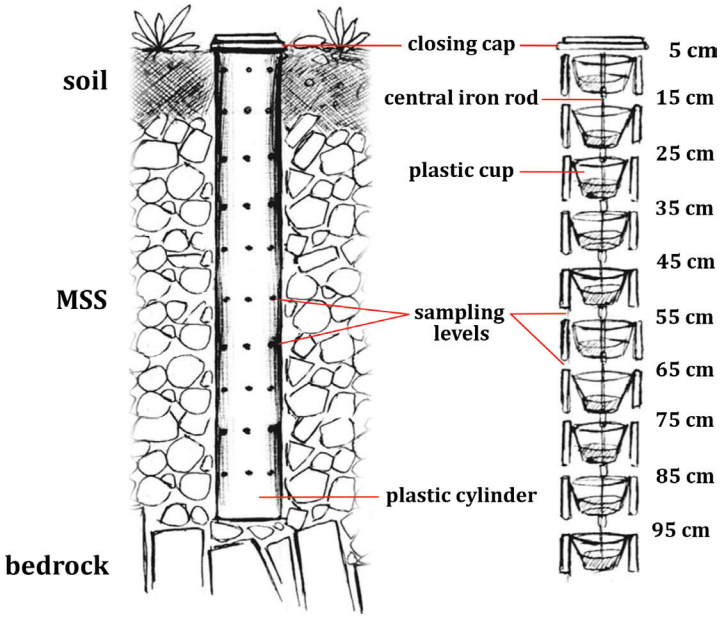

Figure 2. Subterranean trap design in MSS (modified after Mammola et al. 2016).

organic carbon content), soil samples were taken once during the excavation of pits, with a total of 20 samples analysed (5 sites × 4 depths). Samples were hand-mixed, and coarse particles, such as stones and vegetation remnants, were removed. In the laboratory, the samples were air-dried for several weeks and subsequently sieved (mesh size 2 mm). Soil pH was measured potentiometrically in a 1:5 soil:deionised water suspension, and organic carbon content was analysed using the dynamic combustion method (Carter and Gregorich 2008).

Statistical analysis

The Sørensen (qualitative) similarity index (Chao et al. 2006) and the Bray-Curtis (quantitative) similarity index (Magurran 2004) were calculated for each site to emphasize the community similarities along the depth profile.

Results

Site temperature regime and characteristics

The sites were defined by similar patterns of temperature regime. More variable temperature fluctuations were noted on the soil surface horizons (0–35 cm). Deeper in the

soil (55–95 cm), the fluctuations were slighter but synchronized with the climate dynamics near the surface (Fig. 3 and Table 1). The dependence between yearly temperature averages and soil depth showed various results at individual sites but a temperature was more stable with increasing depth (Table 1). The average internal temperature decreased gradually along the depth gradient at Ardovská jaskyňa and Silická ľadnica Caves, unlike the Belinské skaly Rocks, Driencanský kras Karst, and Malý Ružínok Valley sites, where a temperature increase was observed (Table 1). The Belinské skaly Rocks site had slightly acidic soil, whereas the other four sites had slightly alkaline to neutral soil pH. The carbon content basically showed a decreasing trend with increasing depth, but it differed considerably between the individual scree sites (Table 1).

The dependence between monthly temperature averages and soil depth showed various results at individual sites but a temperature was more stable with increasing depth (Table 1).

Table 1. Topographic, microclimatic and soil-chemical characteristics of the study sites (soil type classification after the FAO system). T – average yearly temperature and standard deviation (monthly measures), pH_{H2O} – soil acidity, C_{org} – organic carbon content.

	Site				
	Ardovská jaskyňa Cave	**Belinské skaly Rocks**	**Driencanský kras Karst**	**Malý Ružínok Valley**	**Silická ľadnica Cave**
Coordinates [DDM]	48°31.3'N, 20°25.2'E	48°13.3'N, 19°51.8'E	48°31.7'N, 20°07.1'E	48°50.5'N, 21°06.6'E	48°33.0'N, 20°30.2'E
Altitude [m a.s.l.]	317	460	315	530	455
Slope [°]	20–25	20	35	15	20
Exposition	SW	SW	N	NE	W
Bedrock	limestone	basalt	limestone	limestone	limestone
Soil type	rendzina	calcaric cambisol	rendzina	rendzina	rendzina
Forest composition	cornel-oak	oak-hornbeam	beech-hornbeam	linden-maple	hornbeam with *Acer campestre*, *Tilia* sp., *Quercus* sp.
T [°C]					
0 cm	10.2 ± 6.6	8.9 ± 7.4	–	8.1 ± 6.0	–
15 cm	9.9 ± 5.4	9.5 ± 6.6	8.0 ± 5.7	8.7 ± 5.2	8.0 ± 5.3
35 cm	10.3 ± 4.8	9.7 ± 5.6	8.2 ± 5.2	8.8 ± 4.9	7.8 ± 4.8
55 cm	9.6 ± 4.4	9.1 ± 4.9	8.2 ± 4.8	9.2 ± 4.6	7.7 ± 4.1
75 cm	9.9 ± 4.1	9.7 ± 4.6	8.5 ± 4.4	–	7.6 ± 3.9
95 cm	9.8 ± 3.7	9.6 ± 4.1	8.3 ± 4.1	9.3 ± 4.0	6.6 ± 3.6
pH_{H2O}					
5 cm	7.3	5.0	6.6	7.7	6.7
35 cm	7.9	5.6	8.1	8.2	7.4
65 cm	8.1	6.3	8.2	8.2	7.8
95 cm	8.3	6.4	8.3	8.3	7.9
C_{org} [%]					
5 cm	12.2	3.2	7.3	15.5	10.0
35 cm	1.8	0.8	3.6	9.2	8.1
65 cm	2.2	0.8	2.4	9.6	4.0
95 cm	2.3	0.5	1.7	8.8	3.7

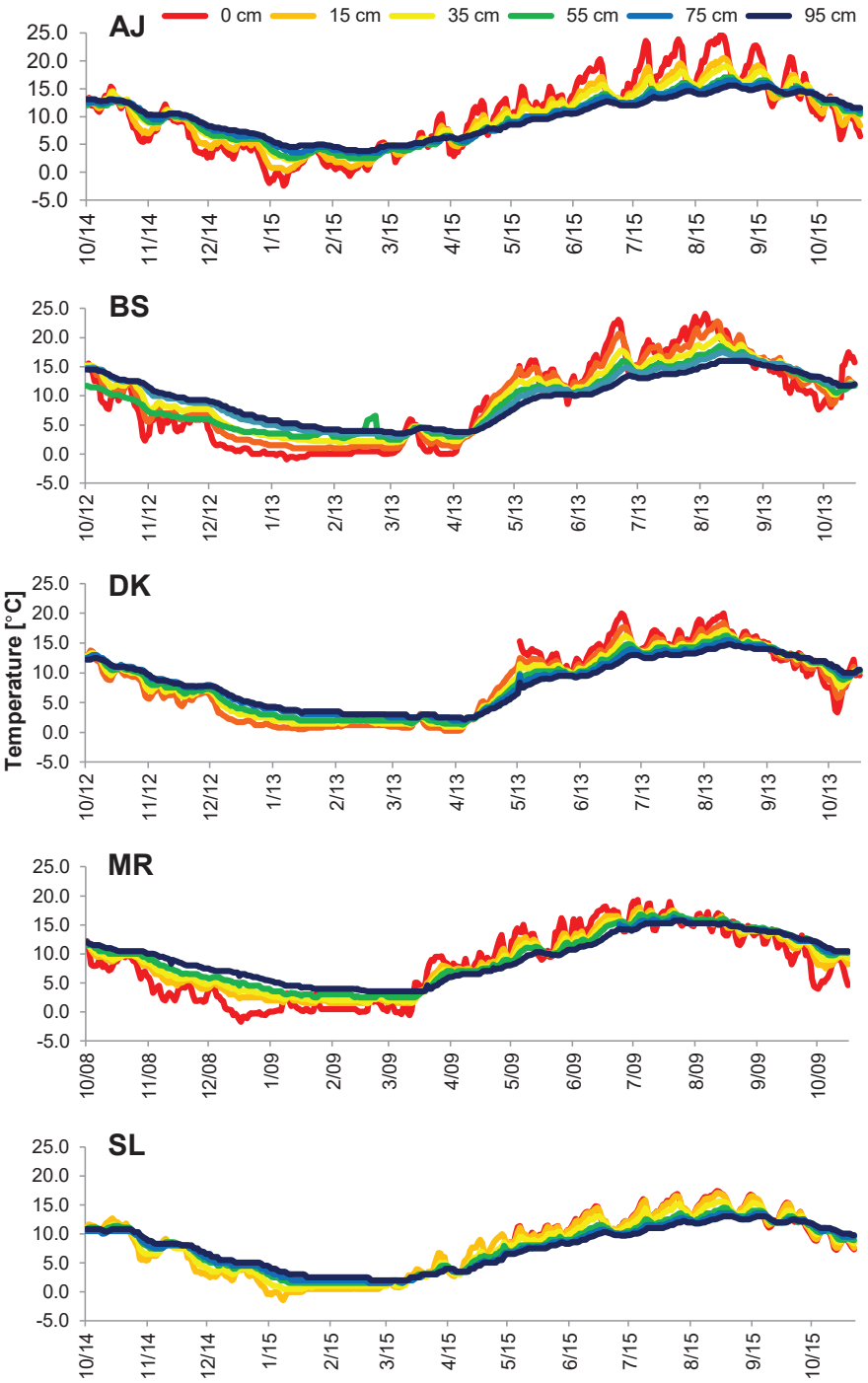

Figure 3. Monthly temperature fluctuations along the depth gradient of the investigated screes. Abbreviations: AJ – Ardovská jaskyňa Cave, BS – Belinské skaly Rocks, DK – Driečanský kras Karst, MR – Malý Ružínok Valley, SL – Silická ľadnica Cave.

Diversity and vertical distribution of Oribatida

In total, 909 individuals of oribatid mites belonging to 58 species were recorded at the study sites. The number of oribatids captured per trap ranged from 0 to 122, and the species richness from 0 to 37 (Appendix 1). We found very low oribatid numbers at all sites, except the Malý Ružínok Valley (MR), where 80% of all individuals were recorded. At the Ardovská jaskyňa Cave (AJ), the number of specimens captured was six-times less than at MR. The total number of individuals found at other localities was also very low, varying from 10 to 24 per study period (12 months). Total species richness was directly related to the number of individuals. The MR site showed the highest species richness, followed by AJ (Table 1, Appendix 1). Species richness at the other scree sites was very low.

The vertical distribution of oribatid mites reflected the distribution of organic carbon content in the soil along the vertical scree slope. The highest activity of oribatids was recorded in the upper soil layers and rapidly decreased with scree depth (Appendix1). The same pattern was registered in the number of species. At the Belinské skaly Rocks, Drienčanský kras Karst and Silická ľadnica Cave, almost all individuals were recorded at a depth of 5 cm, i.e. below the soil surface. The AJ site was characterized by a gradual decrease in activity from 15 to 55 cm and a slight increase at a depth of 85 cm. The species richness was constantly low from 15 cm downwards. At Belinské skaly Rocks (BS) and Silická ľadnica Cave (SL), Oribatida occurred only until the depth of 25 cm, and 45 cm respectively. In contrast, the Drienčanský kras Karst (DK) site revealed an activity gap from 25 to 45 cm depth. At MR, the highest proportions of individuals (90.1%) and species richness (90.2%) were recorded at 5 cm depth. The decrease in both species richness and activity was very marked between 5 and 15 cm, and from 25 cm downwards both community parameters had lower values, similarly as at the AJ site (Fig. 4; Appendix 1).

Three species – *Ceratoppia bipilis* (Hermann, 1804), *Scheloribates pallidulus* (C. L. Koch, 1841) and *Xenillus tegeocranus* (Hermann, 1804) – were trapped at three sites (AJ, DK and MR) and the genus *Phthiracarus* sp. was collected at four sites (AJ, DK, MR and SL). The most abundant species, *Chamobates voigtsi* (Oudemans, 1902), occurred only at the MR site. Based on the Starý's paper (2006), two new species were found for Slovakia: *Banksinoma lanceolata* (Michael, 1885) and *Oribatula amblyptera* Berlese, 1916. Both species were recorded only at the MR site, co-occurring with the eutroglophile *Pantelozetes cavatica* (Kunst, 1962).

Sites BS, DK and SL showed only few records of oribatid individuals. At the AJ site, the oribatid community was the most stratified across the depth gradient (Table 2). At this habitat, the highest degree of Bray-Curtis (quantitative) similarity was recorded between the depths of 15–25 cm (0.74) and 35–45 cm (0.75), respectively. The lowest quantitative similarity was observed between the depths of 5 cm and 75 or 95 cm (0.00 for both pairs). Additionally, the Sørensen (qualitative) similarity index confirmed that the depth of 5 cm was relatively dissimilar in species composition from the rest of the vertical profile. In contrast, the MR site showed no remarkable similarity pattern of the community among the particular depth layers.

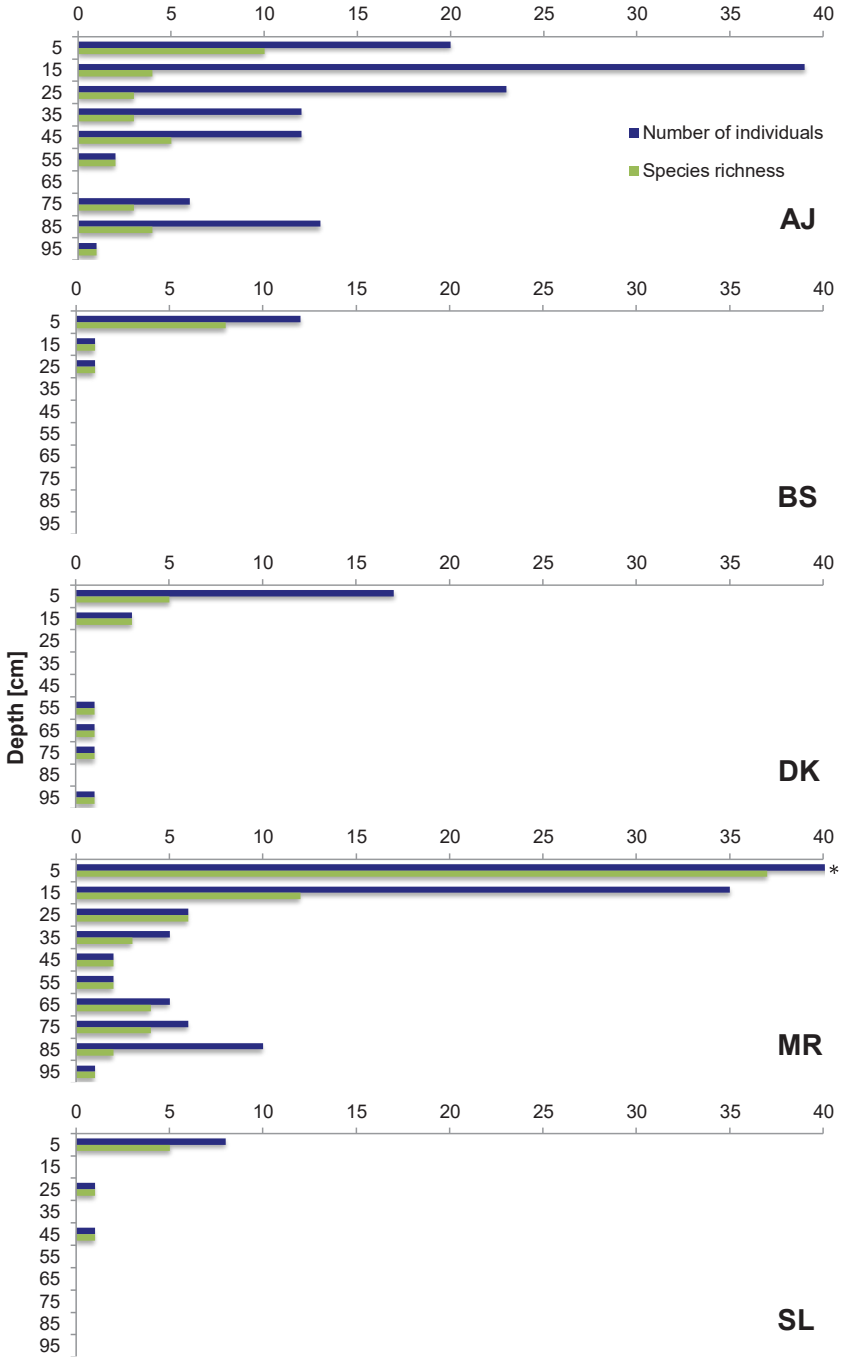

Figure 4. Distribution of Oribatida along the vertical profile of the screes expressed as a total number of trapped individuals and species richness. Abbreviations: AJ – Ardovská jaskyňa Cave, BS – Belinské skaly Rocks, DK – Drienčanský kras Karst, MR – Malý Ružínok Valley, SL – Silická ľadnica Cave, *Number of individuals = 661.

Table 2. Similarity of oribatid communities along the depth gradient at five scree slope sites. Above the diagonal: the Bray-Curtis index, below the diagonal: the Sørensen incidence-based index. Index values > 0.50 are indicated in bold. Value 1.00 indicates identical communities.

Ardovská jaskyňa Cave										
Depth	**5 cm**	**15 cm**	**25 cm**	**35 cm**	**45 cm**	**55 cm**	**65 cm**	**75 cm**	**85 cm**	**95 cm**
5 cm		0.34	0.37	**0.56**	0.44	0.18	0.00	0.15	0.48	0.10
15 cm	0.43		**0.74**	0.47	0.35	0.05	0.00	0.13	0.38	0.05
25 cm	0.31	**0.86**		**0.51**	0.40	0.08	0.00	0.07	**0.56**	0.08
35 cm	0.31	**0.86**	**1.00**		**0.75**	0.14	0.00	0.33	**0.64**	0.15
45 cm	0.27	**0.67**	**0.75**	**0.75**		0.14	0.00	0.44	0.48	0.15
55 cm	0.33	0.33	0.40	**0.40**	0.29		0.00	0.00	0.13	**0.67**
65 cm	–	–	–	–	–	–		0.00	0.00	0.00
75 cm	0.15	0.29	0.33	0.33	**0.50**	0.00	–		0.00	0.00
85 cm	0.29	**0.50**	**0.57**	**0.57**	0.44	0.33	–	0.29		0.14
95 cm	0.18	0.40	**0.50**	**0.50**	0.33	**0.67**	–	0.00	0.40	
Belinské skaly Rocks										
Depth	**5 cm**	**15 cm**	**25 cm**	**35 cm**	**45 cm**	**55 cm**	**65 cm**	**75 cm**	**85 cm**	**95 cm**
5 cm		0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15 cm	0.22		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25 cm	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00
35 cm	–	–	–		–	–	–	–	–	–
45 cm	–	–	–	–		–	–	–	–	–
55 cm	–	–	–	–	–		–	–	–	–
65 cm	–	–	–	–	–	–		–	–	–
75 cm	–	–	–	–	–	–	–		–	–
85 cm	–	–	–	–	–	–	–	–		–
95 cm	–	–	–	–	–	–	–	–	–	
Drienčanský kras Karst										
Depth	**5 cm**	**15 cm**	**25 cm**	**35 cm**	**45 cm**	**55 cm**	**65 cm**	**75 cm**	**85 cm**	**95 cm**
5 cm		0.10	0.00	0.00	0.00	0.11	0.11	0.11	0.00	0.00
15 cm	0.25		0.00	0.00	0.00	**0.50**	**0.50**	**0.50**	0.00	0.00
25 cm	–	–		–	–	0.00	0.00	0.00	–	0.00
35 cm	–	–	–		–	0.00	0.00	0.00	–	0.00
45 cm	–	–	–	–		0.00	0.00	0.00	–	0.00
55 cm	0.29	**0.50**	–	–	–		0.00	0.00	0.00	0.00
65 cm	0.33	0.00	–	–	–	0.00		0.00	0.00	0.00
75 cm	0.33	0.00	–	–	–	0.00	0.00		0.00	0.00
85 cm	–	–	–	–	–	–	–	–		0.00
95 cm	0.00	0.00	–	–	–	0.00	0.00	0.00	–	
Malý Ružínok Valley										
Depth	**5 cm**	**15 cm**	**25 cm**	**35 cm**	**45 cm**	**55 cm**	**65 cm**	**75 cm**	**85 cm**	**95 cm**
5 cm		0.10	0.01	0.01	0.00	0.01	0.01	0.02	0.03	0.00
15 cm	0.49		0.10	0.10	0.05	0.11	0.00	0.25	0.18	0.00
25 cm	0.19	0.22		0.20	0.00	0.29	0.20	0.20	0.13	0.33
35 cm	0.10	0.27	0.22		**0.57**	0.00	0.00	0.20	0.13	0.33
45 cm	0.05	0.14	0.00	**0.80**		0.00	0.00	0.29	0.17	**0.67**
55 cm	0.10	0.29	0.25	0.00	0.00		0.00	0.29	0.00	0.00
65 cm	0.15	0.00	0.40	0.00	0.00	0.00		0.00	0.00	0.00
75 cm	0.20	**0.50**	0.20	0.29	0.33	0.33	0.00		0.00	0.00
85 cm	0.05	0.14	0.00	0.40	**0.50**	0.00	0.00	0.33		0.00
95 cm	0.00	0.00	0.00	**0.50**	**0.67**	0.00	0.00	0.00	0.00	

Silická ľadnica Cave										
Depth	**5 cm**	**15 cm**	**25 cm**	**35 cm**	**45 cm**	**55 cm**	**65 cm**	**75 cm**	**85 cm**	**95 cm**
5 cm		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15 cm	–		0.00	–	0.00	–	–	–	–	–
25 cm	0.00	–		0.00	0.00	0.00	0.00	0.00	0.00	0.00
35 cm	–	–	–		0.00	–	–	–	–	–
45 cm	0.00	–	0.00	–		0.00	0.00	0.00	0.00	0.00
55 cm	–	–	–	–	–		–	–	–	–
65 cm	–	–	–	–	–	–		–	–	–
75 cm	–	–	–	–	–	–	–		–	–
85 cm	–	–	–	–	–	–	–	–		–
95 cm	–	–	–	–	–	–	–	–	–	

Discussion

Worldwide, there are only a few studies on Acari occupying shallow subterranean environments (Borges 1993, Arillo et al. 1994; Růžička and Zacharda 1994; Schlick-Steiner and Steiner 2000; Zacharda et al. 2005). Within Europe, previously published studies on Oribatida in MSS have been carried out in France, Romania and Spain (Cassagne et al. 2008; Sendra et al. 2014; Jiménez-Valverde et al. 2015; Nae and Ivan 2015; Nae and Băncilă 2017). However, they used a different type of pitfall traps, which were monitored for shorter period than one year. In Slovakia, several studies on Arthropoda from MSS habitats have been published (Rendoš et al. 2012, 2014, 2016a, 2016b; Mock et al. 2015; Rudy et al. 2018; Jureková et al. 2019), but the present study is the first one on Oribatida. Here we provide data on oribatid communities inhabiting 1m-deep profile of screes in 10 cm stratification gained during a one-year period at five sites differing in microclimate, soil parameters and bedrock.

Shallow subterranean habitats are highly dynamic in terms of environmental factors (Culver and Pipan 2010). In the present study, differences in environmental characteristics along a depth gradient have been observed mainly in temperature and organic carbon content, but also in soil pH. It is known that temperature can regulate oribatid communities directly or through its effect on moisture conditions, and moreover, it may become a factor essential for the existence of certain species (Jiménez-Valverde et al. 2015). A mean temperature along the depth profile of screes tends to decline and only small temperature fluctuations through the year are noticed in the deeper levels of these habitats (e.g., Pipan et al 2011; Rendoš et al. 2012, 2016b; Mammola et al. 2016; Jureková et al. 2019). This is in conflict with our findings, since an increase of mean temperature towards deeper horizons appeared at the three study sites (BS, DK, MR), although the general trend of temperature fluctuation during the year was the same at all sites. Rendoš et al. (2016b) noted a decrease of mean temperature along the depth profile for these three sites only during a half-year period (May – October), unlike to our one year study period (October – October). Therefore, the length of study period notably influence the interpretation

of temperature regime across the depth profile. Besides that, the two study slopes with a very similar temperature regime, i.e. BS and MR, with the range of temperature along a depth profile from 8.1 °C to 9.7 °C, visibly contrasted in Oribatida activity. Thus, internal scree temperature was not the factor that could substantially affect the oribatid numbers. Since the two most abundant sites had a very similar temperature regime, we may state that temperature was an important though not a main factor limiting the number of oribatid individuals. Another environmental factor that probably influences oribatid mite communities indirectly is soil pH, partly associated with the type of bedrock. The AJ and MR sites, with a high organic carbon content and slightly alkaline topsoil, clearly showed higher oribatid activities and vertical stratification of communities along the scree profile compared to the other sites. The litter quality affects the densities of soil macro-decomposers (Scheu et al. 2003; Salamon et al. 2005; Eisenhauer 2010) and activity and the biomass of soil microorganisms (Bååth and Anderson 2003; Dequiedt et al. 2011), which serve as a main food source for Oribatida (Behan-Pelletier 1999). Rapid limitation of food sources and open spaces along the vertical gradient presumably led to a more or less sudden decline of Oribatida activity and species diversity at individual screes. The total number of individuals and species richness of oribatids trapped inside the forested screes distinctly reflected the amount of organic residue along the vertical profile of the given site. Consequently, the microclimate, organic carbon content and soil pH in the topsoil layer seem to be crucial factors that determined oribatid diversity and distribution within a depth profile of the studied screes.

All sites with limestone bedrock were situated near caves, and we expected to find cave dwelling species in the subterranean traps. The eutroglophilous *Pantelozetes cavatica*, often found in Slovak caves in association with bat guano (Ľuptáčik and Miko 2003), was recorded only at the MR site. Since we found this species to inhabit deeper layers (35, 45 and 95 cm), it is probable that this species is also able to migrate between MSS habitats and caves under suitable environmental conditions. Furthermore, the eurytopic species *Dissorhina ornata* (Oudemans, 1900), known to inhabit karst caves in Slovakia (Ľuptáčik and Miko 2003), was registered only at the basalt Belinské skaly Rocks site in the upper soil layer.

The dominant species differed between the particular sites, but the occurrence of the majority of them was limited to the surface soil layer (5–15 cm). Several species (*Ceratoppia bipilis*, *Oppiella subpectinata* (Oudemans, 1900), *Oribatella calcarata* (C. L. Koch, 1835), *Xenillus tegeocranus*) showed the potential to colonize deeper scree horizons from the surface. *Chamobates voigtsi*, defined as a psychrophilous species preferring acid forest soils, although it is able to tolerate a wider range of factors (Miko 1986; Starý 2003), was the most abundant species at the study sites.

Compared to data on Collembola (Rendoš et al. 2016b; Jureková et al. 2019) from the same study sites, oribatid mites showed considerably lower activity. The use of subterranean pitfall traps seems to be an adequate method for comprehensive studies on Collembola or other more mobile soil invertebrates (Rendoš et al. 2016b; Jureková et al. 2019). However, it is not an effective sampling technique for Oribatida in the

MSS environment, since it estimates the abundance of the given arthropod taxon as a function of its activity during the sampling period and population density in the habitat (Brown and Matthews 2016).

Conclusions

The present study was the first attempt to cover the diversity and distribution of soil oribatid mites along a depth profile of forested scree slopes in the Western Carpathians. Communities of Oribatida with relatively poor abundance and species richness were found to dwell at the studied MSS sites. The exception was the Malý Ružínok Valley site with suitable microclimate conditions, where more abundant oribatid communities had a clear vertical stratification. Edaphic species were limited especially to the topsoil layers rich in organic carbon. The unique status of the MSS habitat is supported by the recording of the rare specialized eutroglophilous species, *Pantelozetes cavatica*. The presence of this species relatively close to the scree surface underlines a function of MSS habitats as corridors for migration of subterranean oribatids towards the soil and surface dwellers to deeper subterranean spaces. Among the soil parameters measured, organic carbon content in the soil and soil pH were the governing factors affecting the diversity and vertical distribution of oribatid mites in the forested screes.

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Appendix 1

List of Oribatida species collected in the vertical gradient of the study screes.

Table 3.

Species	Ardovská jaskyňa Cave											Belinské skaly Rocks											Driečanský kras Karst											Malý Ružínok Valley											Silická ľadnica Cave										
Vertical gradient [cm]	5	15	25	35	45	55	65	75	85	95	Σ	5	15	25	35	45	55	65	75	85	95	Σ	5	15	25	35	45	55	65	75	85	95	Σ	5	15	25	35	45	55	65	75	85	95	Σ	5	15	25	35	45	55	65	75	85	95	Σ
Achipteria coleoptrata (Linné, 1758)	3	1	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–
Achipteria nitens (Nicolet, 1855)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	2
Adoristes ovatus (C. L. Koch, 1839)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–
Banksinoma lanceolata (Michael, 1885)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–
Camisia spinifer (C. L. Koch, 1835)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	1	–	–	–	2	–	–	–	5	–	–	–	–	–	–	–	–	–	–
Carabodes femoralis (Nicolet, 1855)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–
Carabodes labyrinthicus (Michael, 1879)	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–
Carabodes ornatus Storkan, 1925	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–
Carabodes rugosior Berlese, 1916	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Carabodes tenuis Forsslund, 1953	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10	1	–	–	–	–	–	–	–	–	11	–	–	–	–	–	–	–	–	–	–
Ceratoppia bipilis (Hermann, 1804)	2	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	1	–	–	5	6	–	–	–	–	–	1	–	–	–	7	–	–	–	–	–	–	–	–	–	–
Ceratoppia quadridentata (Haller, 1882)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	1	–	–	–	–	–	–	–	–	8	–	–	–	–	–	–	–	–	–	–
Ceratozetes peritus Grandjean, 1951	–	–	–	–	–	–	–	2	2	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–
Chamobates pusillus (Berlese, 1895)	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	1	–	–	–	4	–	–	–	–	–	–	–	–	–	–
Chamobates spinosus Sellnick, 1928	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–	–	–	–	–	–	–	–
Chamobates voigtsi (Oudemans, 1902)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	335	12	1	1	–	–	–	–	–	–	349	–	–	–	–	–	–	–	–	–	–
Damaeus gracilipes (Kulczynski, 1902)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–
Damaeus riparius Nicolet, 1855	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9	–	–	–	–	–	–	–	–	–	9	–	–	–	–	–	–	–	–	–	–
Disorhina ornata (Oudemans, 1900)	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Species	Ardovská jaskyňa Cave												Belinské skaly Rocks												Driencanský kras Karst												Malý Ružínok Valley												Silická ľadnica Cave											
Vertical gradient [cm]	5	15	25	35	45	55	65	75	85	95	Σ		5	15	25	35	45	55	65	75	85	95	Σ		5	15	25	35	45	55	65	75	85	95	Σ		5	15	25	35	45	55	65	75	85	95	Σ		5	15	25	35	45	55	65	75	85	95	Σ	
Eueremaeus oblongus (C. L. Koch, 1835)	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		1	–	–	–	–	–	–	–	–	–	1		–	–	–	–	–	–	–	–	–	–	–	
Eueremaeus silvestris (Forsslund, 1956)	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		1	–	–	–	–	–	–	–	–	–	1		–	–	–	–	–	–	–	–	–	–	–	
Eupelops hirtus (Berlese, 1916)	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		3	1	–	–	–	–	–	–	–	–	4		–	–	–	–	–	–	–	–	–	–	–	
Eupelops plicatus (C. L. Koch, 1835)	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		7	–	1	–	–	–	–	–	–	–	8		–	–	–	–	–	–	–	–	–	–	–	
Euphthiracarus cribrarius (Berlese, 1904)	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		2	–	–	–	–	–	–	–	–	–	2		–	–	–	–	–	–	–	–	–	–	–	
Euzetes globulus (Nicolet, 1855)	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		1	–	–	–	–	–	–	–	–	–	1		–	–	–	–	–	–	–	–	–	–	–	
Fosseremus laciniatus (Berlese, 1905)	1	–	–	–	–	1	–	–	–	–	2		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	
Galumna lanceata (Oudemans, 1900)	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		6	–	–	–	–	–	–	–	–	–	6		–	–	–	–	–	–	–	–	–	–	–	
Gustavia microcephala (Nicolet, 1855)	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		1	–	–	–	–	–	–	–	–	–	1		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	
Heminothrus targionii (Berlese, 1885)	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		2	–	–	–	–	–	–	–	–	–	2		–	–	–	–	–	–	–	–	–	–	–	
Hermanniella dolosa Grandjean, 1931	–	–	–	–	2	–	–	–	–	–	2		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	
Liacarus subterraneus (C. L. Koch, 1841)	–	3	2	1	1	–	–	–	1	–	8		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	1	–	–	–	–	–	1	
Metabelba pulverosa Strenzke, 1953	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		6	–	–	–	–	–	–	–	–	–	6		–	–	–	–	–	–	–	–	–	–	–	
Multioppia laniseta Moritz, 1966	–	–	–	–	–	–	–	–	–	–	–		–	–	1	–	–	–	–	–	–	–	1		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	
Oppia denticulata (R. & G. Canestrini, 1882)	–	–	–	–	–	–	–	–	–	–	–		1	–	–	–	–	–	–	–	–	–	1		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	
Oppiella (Oppiella) nova (Oudemans, 1902)	–	–	–	–	–	–	–	–	–	–	–		1	–	–	–	–	–	–	–	–	–	1		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	
Oppiella (Rhinoppia) obsoleta (Paoli, 1908)	–	–	–	–	–	–	–	–	–	–	–		1	–	–	–	–	–	–	–	–	–	1		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	
Oppiella (R) subpectinata (Oudemans, 1900)	7	28	20	7	5	1	–	–	9	1	78		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	
Oribatella calcarata (C. L. Koch, 1835)	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		21	4	–	1	1	–	–	3	9	–	39		1	–	–	–	–	–	–	–	–	–	1	
Oribatula amblyptera Berlese, 1916	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		8	–	–	–	–	–	–	–	–	–	8		–	–	–	–	–	–	–	–	–	–	–	
Oribatula tibialis (Nicolet, 1855)	–	–	–	–	–	–	–	–	–	–	–		3	–	–	–	–	–	–	–	–	–	3		–	–	–	–	–	–	–	–	–	–	–		45	1	–	–	–	–	1	–	–	–	47		–	–	–	–	–	–	–	–	–	–	–	

Species	Ardovská jaskyňa Cave											Belinské skaly Rocks											Drienčanský kras Karst											Malý Ružínok Valley											Silická ľadnica Cave										
Vertical gradient [cm]	5	15	25	35	45	55	65	75	85	95	Σ	5	15	25	35	45	55	65	75	85	95	Σ	5	15	25	35	45	55	65	75	85	95	Σ	5	15	25	35	45	55	65	75	85	95	Σ	5	15	25	35	45	55	65	75	85	95	Σ
Pantelozetes cavatica (Kunst, 1962)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	1	–	–	–	–	1	5	–	–	–	–	–	–	–	–	–	–	–
Phthiracarus spp.	–	–	–	–	1	–	–	1	–	–	2	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	1	–	–	–	5	11	–	–	–	–	–	–	–	–	–	11	1	–	–	–	–	–	–	–	–	–	1
Pilogalumna tenuiclava (Berlese, 1908)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	20	3	–	–	–	1	–	–	–	–	24	–	–	1	–	–	–	–	–	–	–	1
Platynothrus peltifer (C. L. Koch, 1839)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	22	1	1	–	–	1	–	1	–	–	26	2	–	–	–	–	–	–	–	–	–	2
Poroliodes farinosus (C. L. Koch, 1840)	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Punctoribates punctum (C. L. Koch, 1839)	–	–	–	–	–	–	–	–	–	–	–	2	1	–	–	–	–	–	–	–	–	3	7	–	–	–	–	–	–	–	–	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Scheloribates (Hemileius) initialis (Berlese, 1908)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–
Scheloribates laevigatus (C. L. Koch, 1835)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–	–	–	–	–	–
Scheloribates latipes (C. L. Koch, 1844)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Scheloribates pallidulus (C. L. Koch, 1841)	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–
Scheloribates quintus Wunderle, Beck & Woas, 1990	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	42	4	–	–	–	–	–	–	–	–	46	–	–	–	–	–	–	–	–	–	–	–
Spatiodamaeus fageti (Bulanova-Zachvatkina, 1957)	1	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Steganacarus (Tropacarus) carinatus (C. L. Koch, 1841)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	2
Tectocepheus velatus sarekensis Trägardh, 1910	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–
Tritegeus bisulcatus Grandjean, 1953	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–
Xenillus tegeocranus (Hermann, 1804)	2	7	1	4	3	–	–	3	–	–	20	–	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	1	–	–	–	–	3	8	4	–	–	–	–	–	1	–	–	13	–	–	–	–	–	–	–	–	–	–	–
Zetorchestes falzonii Coggi, 1898	1	–	–	–	–	–	–	–	1	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	61	2	–	–	–	–	–	–	–	–	63	–	–	–	–	–	–	–	–	–	–	–
Zygoribatula exilis (Nicolet, 1855)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–
Number of individuals	20	39	23	12	12	2	0	6	13	1	128	12	1	1	0	0	0	0	0	0	0	14	17	3	0	0	0	1	1	1	0	1	24	661	35	6	5	2	2	5	6	10	1	733	8	0	1	0	1	0	0	0	0	0	10
Species richness	10	4	3	3	5	2	0	3	4	1	14	8	1	1	0	0	0	0	0	0	0	9	5	3	0	0	0	1	1	1	0	1	8	37	12	6	3	2	2	4	4	2	1	41	5	0	1	0	1	0	0	0	0	0	7

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Keywords
karst, groundwater, stygofauna, Asellidae, Crangonyctidae, Kenkiidae, Cirolanidae

It has come to our attention that in Table 2, four records of *Cirolanides* sp. were mistakenly labeled as having been catalogued in the University of Texas Insect Collections (UTIC), when in fact they are catalogued in the Aquifer Biology Collection at the Edwards Aquifer Research and Data Center at Texas State University, San Marcos, Texas. All other information about the specimens is correct.

The CORRECT Table is as follows:

Table 2. Voucher specimens. Complete listing of all specimens collected. UTIC = University of Texas Insect Collection. ABC = Aquifer Biology Collection at the Edwards Aquifer Research and Data Center at Texas State University. Collector initials are as follows: TJD = Thomas J. Devitt; BDN = Bradley D. Nissen; MSS = Mark S. Sanders; NFB = Nathan F. Bendik; AGG = Andy G. Gluesenkamp; RG = Randy Gibson; DAC = Dee Ann Chamberlain; PS = Peter Sprouse. N = Specimens collected. † = new county record. * = specimen accessioned at San Marcos US Fish and Wildlife Service Fish Hatchery.

Taxon	Sites	N	Date	Collectors	Catalog #
Caecidotea reddelli	Hays Co.: Roy Creek, Red's Spring†	4	16 Sep 2016	TJD	UTIC 92016
	Travis Co.: Zilker Park, Eliza Spring	1	1 Apr 1999	DAC	UTIC 93008
	Travis Co.: Barton Creek Habitat Preserve, Sweetwater Spring 4	4	10 Apr 2017	TJD, BDN	UTIC 92021
		3	17 Apr 2017	TJD, BDN	UTIC 92020
	Travis Co.: Barton Creek Habitat Preserve, Sweetwater Spring 1	3	17 Apr 2017	TJD, BDN	UTIC 92019
		1	1 May 2017	TJD, BDN	UTIC 92018
	Travis Co.: Old San Antonio District Park, Old San Antonio Spring	2	19 Jan 2018	TJD, BDN	UTIC 93014
Cirolanides sp.	Travis Co.: Blowing Sink Cave†	1	14 Oct 2010	MSS	UTIC 91874
	Travis Co.: City of Austin WQPL, Blowing Sink Tract, State Well No. 5850411	1	4 Dec 2017	BDN	ABC 000047
	Hays Co.: City of Austin WQPL, Hays County Ranch Tract, State Well No. 5849939†	2	1 Sep 2010	NFB, AGG	UTIC 91879
		3	12 Nov 2010	NFB, AGG	UTIC 91876
		1	3 Dec 2010	NFB, AGG	UTIC 91877
		3	14 Jan 2011	NFB, AGG	UTIC 91875
		2	27 Jan 2011	NFB, AGG	UTIC 91880
		2	21 Oct 2016	AGG, TJD, BDN	UTIC 92014
		1	15 Nov 2016	BDN	UTIC 92013
		3	6 Apr 2017	BDN	ABC 000045
		2	20 Apr 2017	BDN	ABC 000046
		1	5 Jan 2018	BDN	ABC 000048
Crangonyx nr. *pseudogracilis*	Hays Co.: Old San Antonio District Park Spring	2	31 Jan 2018	BDN	Cp31012018*
	Travis Co.: Treadwell Spring	3	21 June 2016	PS	UTIC 91369
Sphalloplana mohri	Travis Co.: Cold Spring	1	24 Feb 2011	RG	SM-Sm24022011*
Stygobromus balconis	Travis Co.: City of Austin WQPL, Ed's Crossing Tract, State Well No. 58499SH	1	6 Apr 2017	BDN	UTIC 92024
	Travis Co.: Barton Creek Habitat Preserve, Sweetwater Spring 4	4	10 Apr 2017	BDN	UTIC 92025
Stygobromus bifurcatus	Travis Co.: Zilker Park, Eliza Spring	1	29 Aug 2016	DAC	UTIC 92030
		1	5 Mar 2017	DAC	UTIC 93011
	Travis Co.: Barton Creek Habitat Preserve, Sweetwater Spring 4	8	17 Apr 2017	TJD, BDN	UTIC 92026
	Hays Co.: Onion Creek, Ben McCulloch Spring	1	31 Jan 2017	TJD	UTIC 92029
	Blanco Co.: Bamberger Ranch Spring	1	21 Jun 2017	TJD, BDN	UTIC 92028

Taxon	Sites	N	Date	Collectors	Catalog #
Stygobromus russelli	Blanco Co.: Bamberger Ranch Spring	1	22 Mar 2018	TJD, BDN	UTIC 93016
	Travis Co.: Zilker Park, Eliza Spring	1	19 Nov 2015	DAC	UTIC 92033
	Travis Co.: Barton Creek Wilderness Park, Barton Creek Greenbelt tract, State Well No. 5842820	3	3 Dec 2010	NFB, AGG	UTIC 91888
		2	27 Jan 2011	NFB, AGG	UTIC 91882
		1	8 Mar 2011	NFB, AGG	UTIC 91883
	Travis Co.: City of Austin WQPL, Ed's Crossing Tract, State Well No. 58499SH	2	8 Mar 2011	NFB, AGG	UTIC 91886
		2	4 Dec 2017	BDN	UTIC 93012
		1	3 Jan 2018	BDN	UTIC 93013
	Travis Co.: City of Austin WQPL, Blowing Sink Tract, State Well No. 5850411	1	30 Mar 2018	BDN	UTIC 93017
	Hays Co.: Onion Creek, Ben McCulloch Spring	1	31 Jan 2017	TJD	UTIC 92029
		3	21 Mar 2017	TJD, BDN	UTIC 92031
		5	3 May 2017	TJD, BDN	UTIC 92039
	Hays Co.: City of Austin WQPL, Sky Ranch Tract, State Well No. 5857507	1	1 Sep 2010	NFB, AGG	UTIC 91889
		3	3 Dec 2010	NFB, AGG	UTIC 91885
		8	14 Jan 2011	NFB, AGG	UTIC 91887
		1	8 Mar 2011	NFB, AGG	UTIC 91884
	Hays Co.: Onion Creek, Bello Spring	2	18 Apr 2017	TJD, BDN	UTIC 92035
	Hays Co.: Onion Creek, Emerald Spring	1	13 Jan 2017	TJD, BDN	UTIC 92040
		2	18 Apr 2017	TJD, BDN	UTIC 92032
	Hays Co.: Roy Creek, Red's Spring	2	23 Apr 2017	TJD, BDN	UTIC 92038
		1	25 Jun 2017	TJD, BDN	UTIC 92037