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Snapshot on Cave Microinvertebrates Assemblages along the Environmental Gradient of the Balkan Region

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ABSTRACT Caves are considered stable and unique environments, which are characterized by a permanent lack of light. The most characteristic feature of organisms inhabiting caves are adaptations to the subterranean environments, which are visible in an increasing morphological and physiological specialization. The distribution patterns of cave fauna are a consequence of repeated, independent invasions, isolation and adaptation to the cave environment. The main aim of our study was comparison of faunal assemblages along environmental gradient from forest, through entrance to the deep cave. Three caves in Serbia were selected for this study. The main factor influencing the extraordinary variety of the troglobiotic fauna of this region include continuity of continental phases in different areas of the Balkans and the lithology of karst. Each of the sampling sites was divided into three zones along environmental gradient: photic, disphotic and aphotic. Six freshwater (sediments) and 17 terrestrial (mosses, mixed soil/mosses) samples were collected. In the samples, we found representatives of Acari, Collembola, Copepoda, Insecta, Isopoda, Oligochaeta, Nematoda, Tardigrada and Rotifera. The highest number of taxa was in material collected from forest surrounding entrance to the cave. However, higher diversity index was found in the dark cave zone. Therefore, highest taxa richness of cave entrance suggest that the cave entrance corresponds to the ecotone. Microfauna assembladges differ between chosen caves and within designated zones. We provide the first study of changes in diversity trends along ecological gradient in subterranean ecosystems of Balkan Peninsula.

KEY WORDS Cave Fauna; Ecological Gradient; Balkans.

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INTRODUCTION

Caves are characterized by a permanent lack of light, stable temperatures far from entrances, high humidity and diminished oxygen availability (Culver, 1982). Obligate cave dwellers are often relatively easy to distinguish by their depigmentation, loss of eyes and hypertrophy of sensorial appendages (Culver, 1982). The environmental condition inside caves depends on the geological settings and influenced by the local and regional external climatic regime. Cave ecosystems directly depend upon the surrounding surface environment, where resources provided to the cave. That is mainly organic matter imported to caves by water, gravity and animal excreta or their dead bodies (Sket et al., 2004; Sket, 2008).

Study about hypogean fauna in relations to cave depth in Krubera-Voronja (Western Caucasus) by Sendra & Reboleira (2012) demonstrates that the differences in the composition and structure of the invertebrate communities in caves are not only the product of biogeographical variations (Sendra & Reboleira, 2012). Ferreira & Pompeu (1997) found a clear reduction of species richness and diversity with increasing distance from the entrance in Taboa cave (Brazil) (Ferreira & Pompeu, 1997). The results of Ferreira et al. (2000) showed the opposite pattern. The pick in richness and diversity was found at 80 m from the cave entrances, and they suspect its connection to the existence of water percolation during the rainy seasons in this area. Meleg et al. (2011) was investigating the diversity of some caves in the regional context of the Romanian Western Carpathians, but in turn, only on the aquatic communities (Oligochaeta, Nematoda, Cyclopoida, Harpacticoida, Ostracoda, Collembola, Acari, Amphipoda, Isopoda, Gastropoda and Insecta larvae).

This area is one of the most important European regions in studies on cave biodiversity. The main factor influencing the extraordinary variety of the troglobitic fauna of this Balkans region includes continuity of continental phases in different areas of the Balkans, the lithology of karst and presence of refugium during the last glaciation. A great number of endemic and relict cave animals of diverse groups inhabits the Serbian karst: Oligochaeta, Hirudinea, Cnidaria, Turbellaria, Gastropoda and various representatives of arthropods (Ćurčić & Decu, 2008). Until now studies on Serbian cave biodiversity reveal that the insects are the most numerous arthropods in caves and pits of the Kamena Gora (southwestern part of Serbia) and that trogloxene species of arthropods are the most distributed in caves and pits, whereas troglophile and troglobiotic are rare (Pavicevic et al., 2012). However, fauna of this region still remains enigmatic and require more attention in interpreting the biogeographical pattern of subterranean life.

Although important, studies on the environmental gradients and shifts in species composition along forests, cave entrance and deep caves are poorly recognized. Thus, this work addresses this important ecological question and the aim of this work is to determine the gradient of biodiversity of invertebrate's community from the entrance of the cave to its depth.

MATERIAL AND METHODS

Study area

The territory of the Balkan Peninsula (including Serbia) represents one of the three major glacial refugees in Europe (Antić et al., 2013). The Republic of Serbia occupies the central and northern parts of the Balkan Peninsula and lies at the convergence of three large geotectonic units (three main mountain systems): the Rhodopian mass in the middle, the Carpatho-Balkan mountain system in the east and the Dinaric mountain system in the west (Fig. 1).

As a study area we have choosen three Serbian caves from three different mountain system builted form carbonate rocks (predominantly limestone with occurrence of dolomites) of Mesozoic age. Petnička cave belongs to the Dinaric mountain system, while from Catpatho-Balkan region we chose one hydrologically active Grza cave and not active - Izviđačka.

Cave in the valley of the Suvaja river ("Izviđačka cave") is located in the village of Strmosten, Despotovac municipality, eastern Serbia (N 44°04' 21.91" E 21°38'27.26"). The altitude of the cave entrance is 559 meters. The cave is located in the forest, far from any significant human presence or activity. The observed forest community is domi-

Figure 1. Examined caves on the Serbia map (distributed under a CC-BY 2.0 license, modified).

nated by *Fagus* L. with occasional *Carpinus betulus* L. The cave is understudied, but few millipedes species are known from this cave (Antić et al., 2013).

Grza cave is located near the Izvor village, Paraćin municipality, central Serbia (43°54’01.2”N 21°39’11.1”E.). The altitude of the cave entrance is 442 meters. Grza cave is located in a valley of a Grza river (relatively small, mountain river). The wellhead of the river is about 20–30 meters below the cave entrance, however, the water that flows in the cave is hydrologically connected to the river itself. Due to the relative vicinity of the city of Paraćin, the general area is frequently visited. The inside of the cave is unaffected by human presence. The measured passages are just under 400 meters long. The entrance itself is in the forest community dominated by *Carpinus betulus*, surrounded by relic forests of *Fagus* and *Quercus* L. species. In the cave, there has not been any faunistical or ecological research carried out so far.

Petnička cave, near the Petnica village, Valjevo municipality, western Serbia (44°14’42.5”N 19°56’ 08.8”E). The altitude of the entrance is 199 meters. Due to the relative vicinity of the city of Valjevo and the Petnica Research Center, the cave is frequently visited. The measured passages are a bit less than 600 meters long. It is in the forest community dominated by *Carpinus betulus* with occasional *Quercus petraea*. On the other hand, there is a Banja river running out of the cave, so the cave entrance itself has very little foliage cover, therefore a significant amount of direct sunlight. The cave is very well known and studied, but not in terms of faunistical research (Pipan & Brancelj, 2001; Brancelj, 2002; Mitić, 2002; Pipan & Brancelj, 2004; Moldovan et al., 2007; Lazarević, 2008; Pipan et al., 2008; Simić, 2008; Antić et al., 2021). From Petnička cave is known several centipedes species (Mitić & Tomić, 2002).

Sample collection and processing

Sampling design and division of caves into zones were chosen to show changes in environmental conditions. The forest surrounding entrance of the cave represents the photic zone with full access to the sunlight, variable temperatures and diverse vegetation, the entrance is the disphotic zone with limited sunlight and more stable temperature, and the deep cave is the aphotic zone with stable abiotic conditions with no access to the sunlight. Samples were collected in July–August 2018 and January 2019 from each of the zones: photic (zone A), disphotic (zone B), aphotic (zone C). From Grza cave, four sediments samples were collected from cave’s depth (III zone), one sample of moss and crust from cave entrance (II zone) and from forest six bryophytes samples (I zone) (Fig. 2). From Petnička cave four bryophyte samples from I zone,

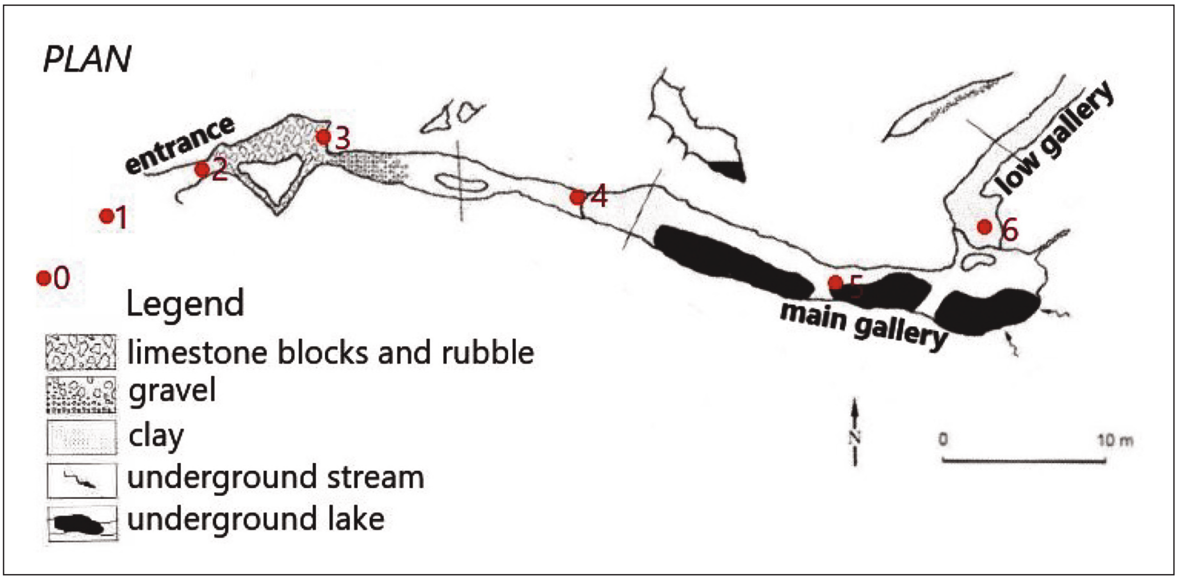

Figure 2. Grza cave map and site positions (modified from Đurović 1998).

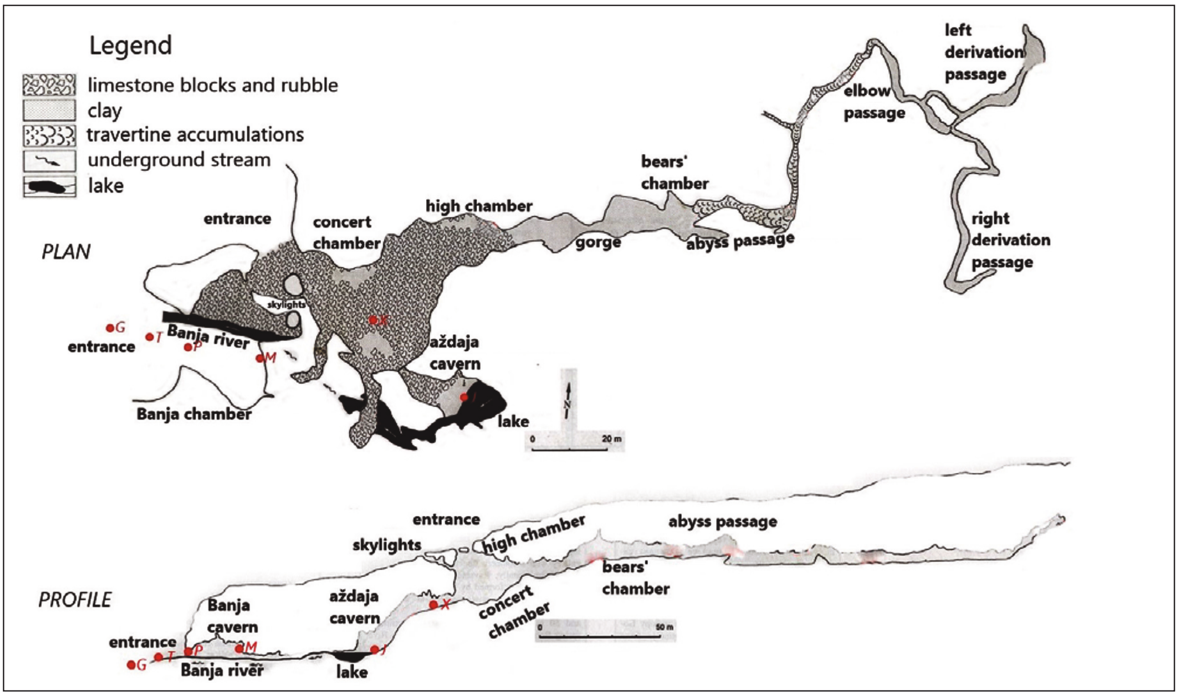

Figure 3. Petnička cave map and site positions (modified from Đurović, 1998).

one sample of moss and crust from II zone and one sediments sample from III zone were collected (Fig. 3). From Izviđačka cave two bryophyte sample (I zone), one of bryophyte and crust (II zone) and one sediment sample (III zone) were collected. Inside the cave, bryophytes or other plants were absent. Sediments samples were gathered by pipette. Biogenic crust and bryophytes were collected by hand from the rock or cave walls. Every sample was preserved in 96% ethanol. A total of 23 samples were collected. After collection, bryophytes were stored in envelopes, which allowed them to dry up slowly, while sediments were stored in vials with alcohol. Bryophytes were examined for microinvertebrates using standard methods of Dastych 1980: animals were isolated by placing the material in a beaker filled with 200 ml of H2O. After *ca*. six hours, bryophytes were vigorously shaken and comminuted within the beaker. The material was separated optically in the beaker and the supernatant containing invertebrates inhabiting bryophytes was stirred and poured into a 250 ml cylinder. After sedimentation *ca*. 50 ml of precipitate was stirred, poured into glass Petri dishes, examined, and counted under a stereomicroscope. The same procedure was applied to sediments but without soaking of material in water (Dastych, 1980).

Statistical analyses

The organisms were determined to different superior taxa, based on ecological and morphological similarity. For the statistical analysis a higher taxonomic level was used. Since the amount of collected samples was small and differences in abundance of taxa were highly different between zones, we used square transformation of data matrix. We test significance of variability in abundance and composition of the taxonomic groups among zones using ANOSIM multiple comparison test in *RStudio* v.1.2.5042. For graphically representing variation between taxa and two variables - caves and zones - we used Redundancy Analyses plot. We also calculated Simpson diversity index and richness for each zone.

RESULTS

In this study, we analyzed samples collected in three zones reflecting different environmental conditions (light availability, organic matter and temperature). All found taxa are for the first time reported for Izviđačka, Grza and Petnička caves. However, those cosmopolitan groups of animals are typical inhabitants of subterranean ecosystems, with wide tolerance to different, sometimes extreme environmental conditions (Ćurčić & Decu, 2008). From all sampling sites in total 3916 specimens belonging to 9 higher level taxa were isolated. Representatives of Acari, Collembola, Copepoda, Insecta, Isopoda, Nematoda, Oligochaeta, Rotifera and Tardigrada were found in samples. The samples from the first zone were taken from the closest surroundings of the cave, often from the rock and walls at the entrance however exposed on the direct sunlight, in contrast to the samples from the second zone. Entrance to the caves characterized by mixed conditions. Cave walls and rocks from which moss and biogenic crust was collected, are only partially and periodically exposed to sunlight and wind. However, in biologically less active conditions of the depths of the cave, it was only possible to collect sediments samples. Comparing diversity and abundance of organisms from all zones cannot be equivocal. The α-diversity in each sample also was calculated by Simpson Diversity index is 0.252 – 0.820 and for A zone the mean value was 0.467, B – 0.484, C – 0.536. Richness of zone A was – 3.5, B – 4.6, C – 3.6. Each studied cave was located near the river, which, as other research indicates, positively influences the species richness of caves. In addition, similar geological structure and surrounding vegetation composition of caves offer qualitatively similar ecological resources, which can imply in the presence of the same type of organisms of the interior cave. Five taxa appeared in the sediments samples from the deepest cave zone: Nematoda, Oligochaeta, Collembola, Acari and Copepoda. Nematodes, earthworms and mites inhabit all designated zones. ANOSIM multiple comparison statistics show significant variation between forest surrounding cave and deep cave part (*p-value* 0.0074) (Fig. 4). They are cosmopolitan groups of animals and typical inhabitants of subterranean ecosystems, with wide tolerance to different, sometimes extreme environmental conditions (Ćurčić & Decu, 2008).

Results of ANOSIM multiple comparison test show significant dissimilarity of invertebrate's composition between zones and caves as well. However, only first and third zones are significantly dissimilar in taxa assemblages. On Fig. 5, we can see that Grza cave differ from two others; Oligochaeta and Acari mainly differ between zone A and zone C. It seems that the most important variance

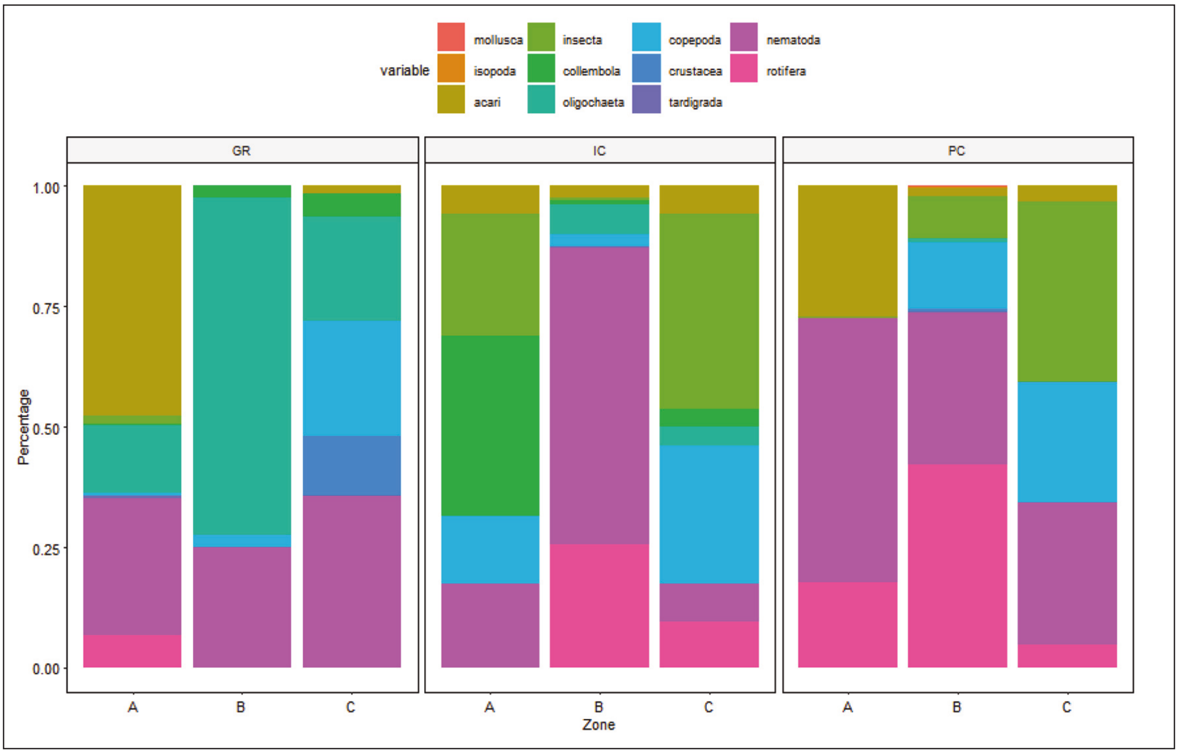

Figure 4. Comparison of taxa abundancy variability differentiating zones and caves. Zone A – forest surrounding entrance cave, zone B – entrance to the cave, and zone C – depth of the cave. GR – Grza cave, PC – Petnička, IC – Izviđačka.

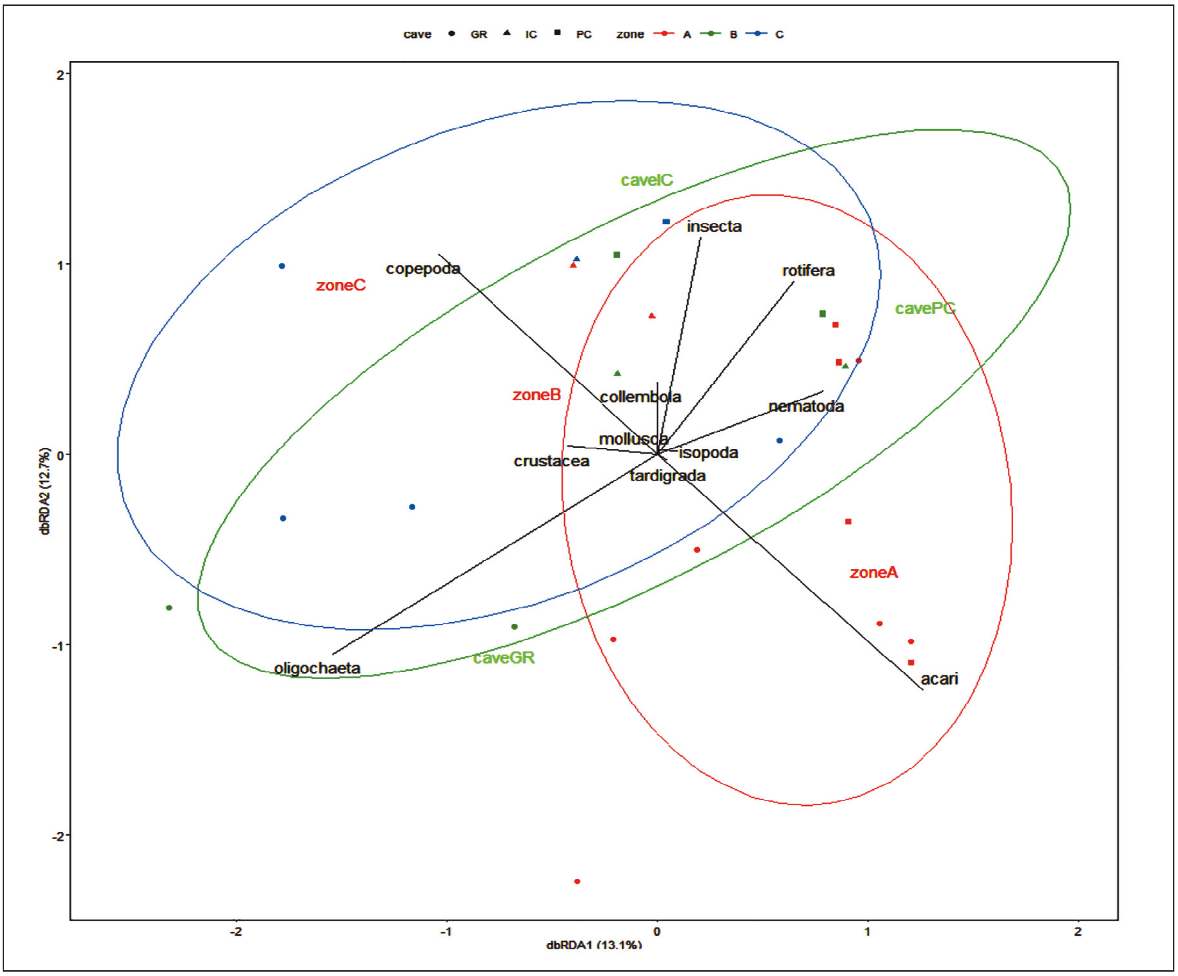

Figure 2. The distribution of plant species by families.

between mentioned zones explains Oligochaeta and Acari presence and abundance. Grza cave differ particularly from Petnička and Izviđačka caves, maybe due to the fact that this cave is frequently visited by tourists, has different organic material flow.

DISCUSSION

In this study, we analyzed samples collected in three zones from three caves reflecting different environment. The highest number of taxa was in material collected from forest surrounding entrance to the cave. However, Simpson diversity index is higher in zone C and there is increasing diversity trend from forest to deep cave. Therefore, the highest taxa richness of zone B may suggest that the cave entrance is a transition zone between two ecosystems. Mixed environmental conditions, increased humidity and periodic darkening are alluring for microfauna and characterize this ecotone (Prous et al., 2015). Diversity index result agree with previous research of Ferreira et al. (2000), however, richness trend is similar to Ferreira and Pompeu (1997).

Due to the endemicity of the troglobiotic species, it is important to analyze not only the entire assemblages of organisms but individual species populations. On the example of the only special determination we conducted of Tardigrada, it has been observed that *Paramacrobiotus* and *Mesobiotus* sustain the conditions prevailing at the beginning of the cave in contrast to *Isohypsibius* (only appears in the forest part). In the light of the growing number of publication on cryptic species, these analysis requires not only a morphological approach but also molecular. It is especially cautious to be in the Balkans, where very high pseudocrypticity of species was observed in different groups (Grabowski et al., 2017, Kociołek et al., 2017).

CONCLUSIONS

Assemblages of cavernicolous organisms are characterized by relatively low biodiversity and density, but very high level of endemism of species. This is one of the most important reasons for the value of conservation and detailed studies of caves. Caves have relatively stable conditions. However, this ecosystem is dependent on matter from the cave's surroundings and is vulnerable to climate change. Uncontrolled tourism also has a negative effect, because it provides an excess of organic matter, which disturbs the circulation of energy and matter in caves. Heterogeneity of microinvertebrates assemblages in examined caves zones show promising trends for further research. Research on ecological dependencies, nutrient networks and gradients of diversity in caves in different regions enriches our knowledge and contributes to creation of a cave protection and exploitation plan.

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