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Study of subterranean biodiversity of the upper Neretva River catchment in Bosnia and Herzegovina

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Abstract. The Dinarides in the Western Balkans host a globally exceptional subterranean biodiversity, but still areas remain with little or no data on subterranean species. In this work, we present the study of subterranean fauna in the upper Neretva River catchment (Eastern Bosnia and Herzegovina), which included the first systematic exploration of the interstitial habitats in the country. During the five-day »Neretva Science Week« in the summer of 2022, we sampled six gravel bars, six springs and one cave. We gathered 268 records (taxon-locality-date) of 116 taxa, with 35 records referring to 27 terrestrial taxa. Nine terrestrial taxa were sampled in a cave, while 19 were found in aquatic samples, mostly from springs. 27 taxa were identified as obligate subterranean (troglobiotic) species, one terrestrial beetle from the cave, and 26 aquatic species, belonging to water mites, snails, and crustaceans. Eight of the aquatic troglobionts present the potential for new species to science. Of the latter, four species were distinguished based on morphology: two species of snails from the families Hydrobiidae and Moitessieriidae, one representative of Ostracoda and one Copepoda. Individuals of the aquatic troglobiotic isopods and amphipods were analysed molecularly, and based on molecular differences, two new species within the genus *Proasellus* and two within the genus *Niphargus* were proposed. Further work on all these taxa is needed for the formal descriptions of the new species to science. Despite the short study period, we have shown that the area is rich in endemic groundwater fauna. The hydroelectric power plant constructions planned in the region threaten groundwater communities by altering subterranean habitats and their connectivity. Further studies are needed to properly highlight the great diversity of the subterranean habitats of the Neretva River catchment and its wider region, and to include this knowledge in conservation strategies.

Key words: troglobiont, *Proasellus*, *Niphargus*, *Kerkia, Paladilhiopsis, Typhlocypris*, *Bryocamptus*, water mites, conservation, hyporheic

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Izvleček. Raziskava podzemne biodiverzitete zgornjega porečja reke Neretve v Bosni in Hercegovini – Čeprav je bogastvo podzemne biodiverzitete Dinaridov na zahodnem Balkanu izjemno v svetovnem merilu, tu še vedno najdemo območja z malo ali nič podatki o podzemnih vrstah. V tem delu predstavljamo študijo podzemne favne v zgornjem porečju reke Neretve (vzhodna Bosna in Hercegovina), ki je tudi prva sistematična raziskava intersticialnih habitatov v tej državi. V okviru dogodka »Neretva Science Week« poleti 2022 smo v petih dneh vzorčili šest prodišč, šest izvirov in eno jamo. Zbrali smo 268 podatkov (takson-lokacija-datum) o 116 taksonih, od tega 35 podatkov o 27 kopenskih taksonih. Devet podzemnih taksonov je bilo najdenih v jami, 19 pa v vodnih vzorcih, večinoma iz izvirov. Identificiranih je bilo 27 izključno podzemnih (troglobiotskih) vrst, od tega en kopenski hrošč iz jame in 26 vodnih vrst, ki pripadajo pršicam, polžem in rakom. Osem vodnih troglobiontov predstavlja potencialno nove vrste za znanost. Od slednjih smo morfološko razločili štiri: dve vrsti polžev iz družin Hydrobiidae in Moitessieriidae, eno vrsto dvoklopnikov (Ostracoda) in eno vrsto ceponožcev (Copepoda). Molekularno smo analizirali osebke vodnih troglobiotskih enakonožcev in postranic ter na podlagi molekularnih razlik identificirali dve novi vrsti v rodu *Proasellus* in dve v rodu *Niphargus.* Do opisov novih vrst za znanost bodo potrebne dodatne raziskave teh taksonov. Kljub kratkemu obdobju raziskave so smo pokazali, da je območje bogato z endemično podzemno vodno favno. Gradnje hidroelektrarn, ki so načrtovane v regiji, ogrožajo živalstvo podzemnih voda, tako s spreminjanjem podzemnih habitatov kot tudi njihove povezljivosti. Priporočamo nadaljnje študije, ki bodo lahko dodatno potrdile visoko vrstno pestrost podzemnih habitatov v porečju reke Neretve in širši regiji, to znanje pa je treba vključili v varstvene strategije.

Ključne besede: troglobiont, *Proasellus*, *Niphargus*, *Kerkia, Paladilhiopsis, Typhlocypris*, *Bryocamptus*, vodne pršice, varstvo, hiporeik

Apstrakt. Studija podzemnog biodiverziteta gornjeg toka rijeke Neretve u Bosni i Hercegovini – Iako je bogatstvo podzemnog biodiverziteta Dinarida na zapadnom Balkanu izuzetno na globalnom nivou, još uvijek postoje područja sa malo ili nimalo podataka o podzemnim vrstama. U ovom radu predstavljamo proučavanje podzemne faune u slivu rijeke Neretve (istočni dio Bosne i Hercegovine), koje je uključivalo prvo sistematsko istraživanje intersticijalnih staništa u zemlji. Tokom petodnevne »Sedmice nauke na Neretvi« u ljeto 2022. godine uzorkovali smo: šest lokacija sa šljunkom, šest izvora i jednu pećinu. Prikupili smo 268 zapisa (takson-lokalitet-datum) od 116 taksona, sa 35 zapisa koji se odnose na 27 kopnenih taksona. Devet kopnenih taksona uzorkovano je u pećini, dok je 19 pronađeno u vodenim uzorcima, uglavnom iz izvora. Identificirano je 27 taksona kao obavezne podzemne (troglobiotske) vrste, jedan tvrdokrilac iz pećine i 26 vodenih vrsta, koje pripadaju vodenim grinjama, puževima i rakovima. Osam vodenih troglobionta predstavljaju potencijalne nove vrste za nauku, od kojih, četiri vrste su izdvojene na osnovu morfologije: dvije vrste puževa iz porodica Hydrobiidae i Moitessieriidae, jedan predstavnik Ostracoda i jedan Copepoda. Molekularno su analizirane jedinke vodenih troglobiotičkih izopoda i amfipoda, a na osnovu molekularnih razlika predložene su dvije nove vrste u okviru roda *Proasellus* i dvije unutar roda *Niphargus*. Potreban je dalji rad na svim ovim taksonima do formalnih opisa novih vrsta za nauku. Unatoč kratkom periodu istraživanja, pokazali smo da je područje bogato endemskom faunom podzemnih voda. Planirana izgradnja hidroelektrana u regionu ugrožavaju zajednice podzemnih voda mijenjajući podzemna staništa i njihovu povezanost. Potrebna su dalja istraživanja, kako bi se na pravi način istakla velika raznovrsnost podzemnih staništa sliva rijeke Neretve i šireg regiona te da bi se ovo znanje uključilo u strategije očuvanja.

Ključne riječi: troglobiont, *Proasellus*, *Niphargus*, *Kerkia*, *Paladilhiopsis*, *Typhlocypris*, *Bryocamptus*, vodene grinje, očuvanje, hiporeično stanište

Introduction

The Dinarides are a mountainous karst ridge in the Western Balkans, with globally exceptional subterranean biodiversity (Sket 2012). Besides being a global hotspot in the number of subterranean species, it is also the home to some of the world's unique subterranean taxa, e.g. cave tube worm (*Marifugia cavatica* Absolon & Hrabe, 1930), cave hydrozoan (*Velkovrhia enigmatica* Matjašič & Sket, 1971), cave mussels (*Congeria* spp.), and the world's largest subterranean amphibian, the olm (*Proteus anguinus* Laurenti, 1768) (Sket 2012). Previous studies have identified regional hotspots of subterranean biodiversity but also knowledge gaps in the distribution of subterranean species (Zagmajster et al. 2008; Bregović et al. 2019; Borko et al. 2022). Both are characteristics of Bosnia and Herzegovina (BIH), extending over the middle and southeastern parts of the Dinarides. Whilst its southern parts have long been recognised as subterranean biodiversity hotspots (Culver et al. 2009; Zagmajster et al. 2014), there are parts of the country with few or even no data on subterranean species. The upper part of the Neretva River in eastern BIH, the focus of the studies conducted during the »Neretva Science Week« in the summer 2022, was also considered an overlooked area.

Studies of subterranean fauna typically focus on the sampling of caves and to a lesser extent on other subterranean habitats (Culver & Pipan 2014, 2019). Yet, subterranean species are not limited to caves, nor karst areas, but also occur outside consolidated geological substrata (Culver & Pipan 2014). An example of such are interstitial habitats, defined as a complex of dry or water-filled voids between sediment particles of different sizes (Culver & Pipan 2014). Even though many subterranean species have been described from the aquatic interstitial habitats of the Western Balkans, they received far less research attention than karst habitats, even within the Dinarides (SubBioDB 2023).

Prior to the present study in summer 2022, there were only a few records of subterranean species from the upper Neretva River (SubBioDB 2023). Most of the records refer to terrestrial species, especially Coleoptera. For example, the cave Velika Đeverđela near Ulog is a type locality of the subterranean beetle subspecies, *Anthroherpon ganglbaueri distinguendum* (Müller, 1913) (Giachino & Vailati 2005). Data on aquatic subterranean species were scarce (SubBioDB 2023), with two localities of *Niphargus borkanus* (Karaman S., 1960) described from a spring near Boračko jezero (Karaman 2014) and two endemic snail species, *Plagigeyeria konjicensis* Grego, 2020 and *P. ljutaensis* Grego, 2020, described from springs in Ljuta canyon close to Konjic (Grego 2020). The upper part of the Neretva River presents an interesting area for subterranean species, due to its pristine nature, presence of many gravel bars and karst hills surrounding the valley. Improved knowledge of the subterranean fauna of this area is especially important as there are many hydropower plants planned for the upper part of Neretva River, which will greatly affect the currently well-preserved area. Negative effects must also be expected for the down- stream sections, as they disturb the natural seasonal flow regime, and, in the worst case, create unexpected flow peaks (hydro-peaking). Hydropower constructions will affect the subterranean realm, by changing the natural subterranean water courses, their connections and water table levels, and will consequently negatively affect subterranean habitats (Fišer et al. 2022).

In this contribution, we present the results of the survey on the subterranean fauna conducted during the »Neretva Science Week« in the summer of 2022. We sampled various subterranean habitats, but specifically focused on the interstitial waters of the upper Neretva River, investigated here for the first time.

Materials and methods

Sampling area and sampling sites

The study was conducted in the upper Neretva River catchment, in the area extending from Krupac to Konjic (Fig. 1). We sampled river interstitial habitats on the accessible gravel bars near the river, but also springs, when found, and one cave. We determined the geographical position of each sampled locality using a Garmin handheld GPS or areal maps accessed via Google Maps web map service (http:\\maps.google.com).

The selection of sampling localities was carried out differently for the different habitat types. We selected gravel bars based on the »main« or »additional« sampling sites suggested by the organisers of the Neretva Science Week. We checked these places using areal images, as provided by the aforementioned web map service, and searched for accessible gravel bars (those being large enough to permit sampling; and being easily accessed by vehicles). Thereby, we identified five localities distributed along the upper stretch of the Neretva River for our work (Fig. 1). In addition, we searched for springs in the upper-most part of the focal area (following the upstream path along the Krupac tributary) but also sampled any spring we discovered during the fieldwork. We visited a cave thanks to the help of local inhabitants of the town of Ulog who guided us to the cave entrance.

Fieldwork

River interstitial habitats

We used the Bou-Rouch method (Bou & Rouch 1967) to sample interstitial waters at all gravel bars except one (Tab. 1). This sampling device consists of a hollow metal pipe with a pointed end, with holes of about 0.5 cm diameter distributed within about 30 cm length at its lower end. The pipe was pushed into the gravel to the desired depth by using a mallet, after which a hand pump was set on its upper part (Fig. 2A). Pumping creates a negative pressure pulls the interstitial water along with sediments and animals, into to the pipe and through the pump into a collection bucket. We sampled at multiple points at each gravel bar, to account for the potential variation amongst sampling points within a single gravel bar. At each locality, we identified at least three sampling points at the river bank with a distance of approx. 5 – 10 m between neighbouring points. We positioned the Bou-Rouch pipe at two depths – so that the section with holes reached depths between 30 – 60 cm, and between 60 – 90 cm. From each depth, we sampled at least 30 litres of water with sediment and organisms and then filtered these through hand water nets of two different mesh sizes – 0.5 mm for the coarse fraction, and 0.1 mm for the fine fraction. We sampled from two depths at three points along each gravel bar whenever possible. This protocol could not be completed at two gravel bars, due to equipment and weather problems (Tab. 1). At two localities, we sampled at more points on the gravel bar to collect more individuals from microcrustacean groups. We report cumulative findings of different taxa per gravel bar, without considering their abundance or differences in number of sampled points per locality.

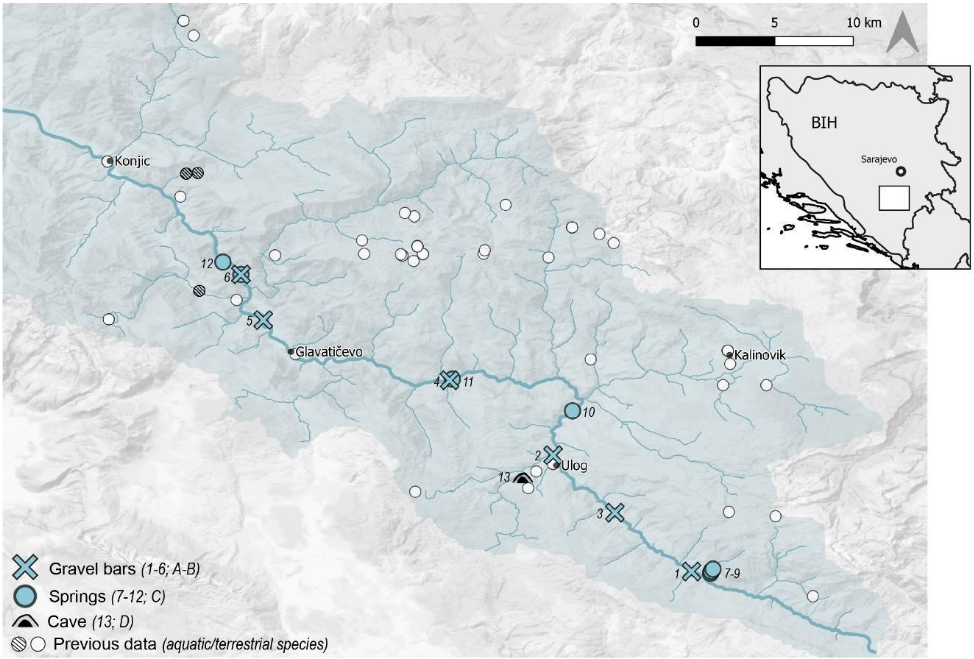

Figure 1. Map of studied subterranean localities in the upper catchment of Neretva River, combining those surveyed during »Neretva Science Week 2022« and those for which data existed before this study (SubBioDB 2023). The numbers on the map correspond to those in Tab. 1. The blue shaded area on the map represents the upper Neretva River catchment (HydroBASINS 1.0, level 08; Linke et al. 2019). The embedded map shows the position of the study area within Bosnia and Herzegovina. Photographs below show the sampling methods used during the study: A: Bou-Rouch method, B: Karaman-Chappuis method, C: sampling of a spring using a hand water net, D: sampling of invertebrates using visual inspection and direct collection. Photo: M. Zagmajster (A, B), E. Premate (C, D).

Slika 1. Zemljevid z lokacijami raziskanih podzemnih habitatov v zgornjem porečju reke Neretve, kjer so združeno prikazane lokalitete, raziskane med tednom »Neretva Science Week 2022«, in tiste, za katere so bili podatki zbrani že pred to študijo (SubBioDB 2023). Številke na karti se ujemajo s tistimi v tab. 1. Modro obarvano območje ponazarja porečje gornje Neretve (HydroBASINS 1.0, nivo 08; Linke et al. 2019). Vstavljena karta prikazuje položaj območja raziskave znotraj Bosne in Hercegovine. Na fotografijah spodaj so prikazane metode vzorčenja med raziskavo: A – Bou-Rouch-metoda; B – Karaman-Chappuis-metoda; C – vzorčenje izvira z vodno mrežo; D – vzorčenje nevretenčarjev z opazovanjem in neposrednim pobiranjem. Foto: M. Zagmajster (A, B), E. Premate (C, D).

Slika 1. Karta proučavanih podzemnih lokaliteta u gornjem slivu rijeke Neretve, kombinujući one istražene tokom »Sedmice nauke na Neretvi 2022.« i one za koje su postojali podaci prije ove studije (SubBioDB, 2023). Brojevi na karti odgovaraju onima u Tab. 1. Plavo osjenčano područje na karti predstavlja sliv gornje Neretve (HydroBASINS 1.0, nivo 08; Linke et al. 2019). Ugrađena mapa pokazuje položaj istraživanog područja unutar Bosne i Hercegovine. Fotografije ispod prikazuju metode uzorkovanja koje su korištene tokom istraživanja: A: Bou-Rouch metoda, B: Karaman-Chappuis metoda, C: uzorkovanje izvora korištenjem ručne mreže za vodu, D: uzorkovanje beskičmenjaka vizualnim pregledom i direktnim prikupljanjem. Foto: M. Zagmajster (A, B), E. Premate (C, D).

Figure 2. Presentation of sampling methods used during the study of subterranean fauna in the upper catchment of Neretva River. A: Bou-Rouch method, B: Karaman-Chappuis method, C: sampling of a spring using a hand water net, D: sampling of invertebrates using visual inspection and direct collection. Photo: M. Zagmajster (A, B), E. Premate (C, D).

Slika 2. Prikaz metod vzorčenja med raziskavo podzemne fauna v zgornjem porečju reke Neretve: A – Bou-Rouch-metoda; B – Karaman-Chappuis-metoda; C – vzorčenje izvira z vodno mrežo; D – vzorčenje nevretenčarjev z opazovanjem in neposrednim pobiranjem. Foto: M. Zagmajster (A, B), E. Premate (C,D).

Slika 2. Prikaz metoda uzorkovanja podzemne faune u gornjem slivu rijeke Neretve, koje su korištene tokom istraživanja: A: Bou-Rouch metoda, B: Karaman-Chappuis metoda, C: uzorkovanje izvora korištenjem ručne mreže za vodu, D: uzorkovanje beskičmenjaka vizualnim pregledom i direktnim prikupljanjem. Foto: M. Zagmajster (A, B), E. Premate (C,D).

At one gravel bar, we used the Karaman-Chappuis method to sample interstitial fauna (Fig. 2B; Karaman 1935; Chappuis 1942). We dug a hole in the gravel with a diameter of about one metre and a depth of about half a metre. Once the hole became filled with water, we sampled it using a hand water net with mesh size 0.5 mm, catching the animals that were flushed into the hole by the flow of water from the surrounding interstitial habitats. Smaller invertebrates were likely overlooked due to the mesh size.

Whilst in the field, whenever bigger animals were noticed by sight in the collected samples, they were directly collected with forceps and then stored in 96% ethanol. The remaining material was stored in large containers and also preserved with 96% ethanol.

Springs and caves

We sampled the springs using a kick-sampling method and collected the animals using hand water nets with 0.5 mm mesh size, again being aware of the possible loss of smaller invertebrates due to mesh size (Fig. 2C). The sampled springs were rheocrenes: places of groundwater discharge in the form of running water forming a channel or a spring brook. We visually checked the under sides of stones, and in some cases, collected samples of sand for later inspection.

To explore the cave, we used standard speleological equipment including caving harnesses and ropes. We only sampled the animals in the cave by visual inspection of different microhabitats: cave walls, ceiling, floor, puddles, beneath the stones, and near organic remains (Fig. 2D). Whenever an animal was found, it was carefully collected and stored in a vial with 96% ethanol.

Abiotic parameters

At each locality, we measured the abiotic parameters in the surface river water, and in 10 litres of each sampling point immediately after pumping, using the Eutech multimeter instrument CyberScan CD 650 (Eutech Instruments Pte Ltd.). We measured water temperature (°C), dissolved oxygen concentration (mg/l) and saturation (%), pH and conductivity (μS/cm). Measuring dissolved oxygen in water withdrawn with the Bou-Rouch pump might have slightly overestimated the oxygen content of sediment because of oxygenation introduced by the pump itself. Our previous experiences and tests indicated small deviations between the two when water was gently pumped to minimise mixing with the air. Measurements were carried out immediately after pumping and at the bottom of the bucket. Abiotic parameters were not measured in springs and caves, but at some springs we measured water temperature using a hand thermometer.

Sample processing

We sorted the samples in the laboratory using a stereomicroscope (10 – 40 x magnifications) and classified the animals into higher taxonomic groups. When the samples contained a lot of sediment with particles, we only analysed a subsample (of about 1/5 to 1/3 of the whole sample).

We sent specimens of various taxonomic groups to the relevant experts, but due to a large amount of material, identifications of some groups were not finished in time for the preparation of this publication. Most specimens were identified based on morphological characters; which have certain limitations in species identification. This approach enabled the identification of potential new species, but for the final description, further work, including molecular analyses, is needed. The coauthors of this contribution checked the following taxa: JG – Mollusca, MJ & VP – Acarina, NM – Ostracoda, AB – Copepoda, FM & CD – aquatic Isopoda, EP, BR& ŠB – Amphipoda, MB – aquatic Coleoptera. The remaining groups were identified by Miloš Vittori (terrestrial Isopoda), Peter Trontelj (Hirudinea) and Cene Fišer (Amphipoda). The identification of Neuroptera was carried out by Susanne Randolf, respective results are presented separately (see Randolf et al., this issue).

Two taxa, for which molecular analyses were conducted due to limitations in morphological identifications, were subterranean macrocrustaceans from the orders Amphipoda and Isopoda. In these cases, the individuals were identified using molecular markers. For DNA-isolation and COI-amplification in amphipods, we followed the protocol described in Borko et al. (2022). We used the COI LCO-HCO barcoding marker, following the procedure in Delić et al. (2017). We compared the COI sequences to those available in GenBank and our own DNA sequence collection (SubBioDB 2023) according to Borko et al. (2022). We calculated uncorrected p-distance and inferred the maximum likelihood phylogeny with IQ-TREE (Minh et al. 2020). Molecular analyses of asellid isopods (Isopoda, Asellidae) followed the standard procedure described in Morvan et al. (2013). Specimens were identified as belonging to new species based on their genetic divergence with other species, as evaluated from the COI gene. Following Lefébure et al. (2006), two clades diverging by more than 0.16 substitution per site, as measured by patristic distances, have a strong probability (ca. 0.99) of belonging to different species.

In the overview of taxa, we indicated the taxa that presumably live their whole life cycle in subterranean habitats as obligate subterranean (troglobiotic) taxa.

Results

We surveyed six gravel bars, six springs, and one cave (Tab. 1). The entire sampling protocol using the Bou-Rouch method, including sample collection at two depths and at three spots, was only carried out at three of the five gravel bars. At all the gravel bars, physical-chemical parameters of the interstitial water differed from those of the surface water, i.e. temperatures were slightly higher, pH was slightly lower, and dissolved oxygen concentrations were lower in the interstitial water (Tab. 2). Electric conductivity was highest just below Glavatičevo village (Loc. 5, Tab. 1).

Table 1. The list of localities sampled in the upper Neretva River catchment in summer 2022. Names in brackets and italic font refer to pre-determined localities by the »Neretva Science Week« team, coordinates are in decimal degrees (WGS84). Abbreviations refer to: S - shallow (30-60 cm) and D - deep (60-90 cm) interstitial layers.
Tabela 1. Seznam lokalitet na območju zgornjega porečja reke Neretve, kjer smo vzorčili poleti 2022. Imena v oklepajih in poševnem tisku se nanašajo na predhodno določene lokalitete s strani ekipe dogodka »Neretva Science Week«, koordinate so v decimalnih stopinjah (WGS84). Okrajšave pomenijo: S – plitki (30-60 cm) in D – globoki (60-90 cm) sloji intersticiala.
Tabela 1. Spisak uzorkovanih lokaliteta u slivu gornjeg toka Neretve u ljeto 2022. godine. Imena u zagradama odnose se na unaprijed određene lokalitete od strane tima »Sedmice nauke na Neretvi«, koordinate su u decimalnim stepenima (WGS84). Skraćenice se odnose na: S - plitki (30-60 cm) i D - duboki (60-90 cm) međuprostorni slojevi.

No.	Locality	Coordinates	Date	Methods	Number of samples
1	Neretva gravel bar, near the farm downstream from confluence Krupac-Neretva (*Krupac Confluence (1), main locality*)	18.41654, 43.33251	29.6.2022	Bou-Rouch method	3 x S 5 x D
2	Neretva gravel bar, at the swimming beach downstream from Ulog (*Swimming beach (5), main locality*)	18.308137, 43.423636	30.6.2022	Bou-Rouch method	3 x S 3 x D
3	Neretva gravel bar at Cerova (*Cerova (3), main locality*)	18.35621, 43.37887	1.7.2022	Bou-Rouch method	3 x S 5 x D
4	Neretva gravel bar upstream from Brijestov bridge (*Brijestov bridge (8), main locality*)	18.227032, 43.482262	2.7.2022	Bou-Rouch method	3 x S 2 x D
5	Neretva gravel bar below Tajoraft (*Tajoraft (10), additional locality*)	18.081052, 43.529472	3.7.2022	Bou-Rouch method	2 x S 1 x D
6	Spring in the gravel on the right bank of Neretva (rafting lunch location)	18.063583, 43.565139	3.7.2022	Karaman – Chappuis method	1
7	Spring 1 above Krupac confluence	18.43152, 43.33118	29.6.2022	Water net, direct collecting	1
8	Spring 2 above Krupac confluence	18.43196, 43.33272	29.6.2022	Water net, direct collecting	1
9	Spring 3 above Krupac confluence	18.43337, 43.33436	29.6.2022	Water net, direct collecting	1
10	Spring near the dirt road to Neretva at Nedavić	18.323332, 43.458369	2.7.2022	Direct collecting	1
11	Neretva tributary springs, left bank of Neretva, 200 m upstream from Brijestov most	18.22869, 43.482222	2.7.2022	Water net, direct collecting	1
12	Spring in the Neretva canyon, left bank (rafting)	18.049440, 43.574848 (+/-200m)	3.7.2022	Water net	1
13	Velika Đeverđela	18.284287 43.405874	30.6.2022	Direct collecting	1

Table 2. The abiotic parameters and number of taxa collected at each locality (Loc. no. refers to Tab. 1). Average value, standard deviation and number of measurements (N = number of samples; see Tab. 1) are given for each abiotic parameter. Measurements of the surface river water samples are given for the first five localities (R), while S indicates the groundwater measurements. At some springs, abiotic parameters are not reported, as they were not measured. The last four columns contain: all - the number of all taxa, aq – the number of aquatic taxa, tgb – the number of troglobiotic taxa, nsp - the number of potentially new species for science. See Tab. 3 for details on taxa.

Tabela 2. Abiotski parametri in število taksonov, nabranih na vsaki lokaliteti (Loc. no. se nanaša na oznake v tab. 1). Za vsak abiotski parameter so podane povprečne vrednosti, standardna deviacija in število meritev (N = število vzorcev; glej tab. 1). Meritve površinske vode reke (R) so podane za prvih pet lokalitet, medtem ko S označuje meritve podzemnih voda. Na nekaj izvirih nismo merili abiotskih parametrov, zato jih v tabeli ni. Zadnji štirje stolpci podajajo: all – število vseh taksonov, aq b- število vodnih taksonov, tgb – število troglobiotskih taksonov, nsp – število potencialnih novih vrst za znanost. Glej tab. 3 za podrobnost o taksonih.

Tabela 2. Abiotički parametri i broj taksona prikupljenih na svakom lokalitetu (lok. br. se odnosi na tab. 1). Prosječna vrijednost, standardna devijacija i broj mjerenja (N = broj uzoraka; vidjeti tab. 1) dati su za svaki abiotički parametar. Mjerenja površinske riječne vode data su na prvih pet lokaliteta (R), dok S označava mjerenja podzemnih voda. Na nekim izvorima abiotički parametri nisu prijavljeni, jer nisu mjereni. Posljednje četiri kolone sadrže: all - broj svih taksona, aq - broj vodenih taksona, tgb - broj troglobiotičkih svojti, nsp - broj potencijalno novih vrsta za nauku. Vidi tab. 3. za detalje o taksonima.

Loc. No.	Depth	N	T (°C)	pH	O_2 (%)	O_2 (mg/L)	Cond. (µS)	all	aq	tgb	nsp
1	S	8	17.1 (± 0.8)	8.2 (±0.1)	84.7 (± 5.3)	7.4 (±0.4)	253.6 (± 6.6)	34	33	8	3
	R	1	16.9	8.34	102.2	9.18	246.4				
2	S	6	18.8 (± 0.9)	7.7 (±0.2)	68.0 (±10.1)	5.8 (±1.0)	308,2 (± 5.8)	43	39	14	4
	R	1	17.8	7.8	86.9	7.97	298				
3	S	8	19.2 (±1.2)	7.8 (±0.1)	80.5 (± 8.6)	6.8 (±0.6)	287.3 (±3.4)	33	31	10	3
	R	1	16.8	7 8.	94.1	8.59	286.9				
4	S	5	17.2 (±1.7)	7.9 (±0.0)	82.7 (±8.7)	7.8 (±0.8)	276.5 (±3.1)	34	32	13	4
	R	1	15.4	8.28	105.3	10.27	277.5				
5	S	3	16.8 (± 0.6)	7.9 (±0.3)	59.0 (±10.2)	5.7 (± 0.9)	371.2 (± 31.6)	23	22	4	1
	R	1	12.5	8.03	91.7	10.38	322.9				
6	S	1	/	/	/	/	/	15	13	2	1
7	S	1	7.5	7.3	48.1	4.52	437.7	1	1	0	0
8	S	1	7.8	7.49	82	8.78	409.6	7	7	0	0
9	S	1	7	7.73	92.7	9.97	241.9	29	16	1	0
10	S	1	/	/	/	/	/	2	2	1	0
11	S	1	/	/	/	/	/	19	19	2	0
12	S	1	/	/	/	/	/	19	19	2	0
13	/	1	/	/	/	/	/	9	0	1	0

Altogether, 268 records (taxon-locality-date) of 116 taxa were recorded from the 13 localities (Tab. 3), with 35 records referring to 27 terrestrial taxa. While we sampled terrestrial taxa only in the cave, 19 were found in aquatic samples, mostly from springs. Overall, 27 obligate subterranean (troglobiotic) species were identified, one terrestrial beetle from the cave, and 26 aquatic species, belonging to water mites, snails and crustaceans (Tab. 3).

The highest richness of aquatic taxa was recorded in the Neretva gravel bar at the swimming beach near Ulog (43 taxa, Loc. 2, Tab. 1), followed by the gravel bar downstream of the Krupac-Neretva confluence (34 taxa, Loc. 1, Tab. 2) and the gravel bar upstream of Brijestov bridge (34 taxa, Loc. 4, Tab. 1) as well as the gravel bar near Cerova (33 taxa, Loc. 3, Tab. 2). The gravel bars at Ulog and Brijestov bridge harboured the highest number of troglobionts, followed by the other two aforementioned gravel bars (Tab. 2). There were up to four potentially new species per gravel bar – with at least one present at each of them.

We recorded much lower species diversity at springs. Some localities had many terrestrial taxa that were accidentally present in the aquatic samples (Tabs. 2,3). Troglobiotic taxa were found at four springs (Tab. 2).

Table 3. The list of taxa found in subterranean habitats of the upper Neretva River catchment between Krupac and Konjic in the summer of 2022. Acronyms refer to: T – troglobiont, L – larval state (only in insects), N.SP. – potentially new species for science, ter – terrestrial taxon, B – bone remains. The numbers of localities, given in the last three columns, refer to Tab. 1.

Tabela 3. Seznam taksonov, najdenih v podzemnih habitatih porečja zgornje rege Neretve med krajema Krupac in Konjic poleti 2022. Okrajšave pomenijo: T – troglobiont, L – ličinka (le pri žuželkah), N. SP. – potencialno nova vrsta za znanost, ter – kopenski takson, B – kostni ostanki. Številke lokalitet, podane v zadnjih treh stolpcih, se nanašajo na Tab. 1.

Tabela 3. Spisak taksona pronađenih u podzemnim staništima gornjeg sliva rijeke Neretve između Krupca i Konjica u ljeto 2022. godine. Akronimi se odnose na: T – troglobiont, L – stanje larve (samo kod insekata), N.SP. – potencijalno nova vrsta za nauku, ter – kopneni takson, B – ostaci kostiju. Brojevi lokaliteta, dati u posljednje tri kolone, odnose se na Tab. 1.

Group	Family	Taxon	Gravel bars	Springs	Cave
Nematoda		Nematoda	1, 2, 3, 4, 5, 6	9, 11, 12	
Turbellaria		Turbellaria		9	
Oligochaeta		Oligochaeta	1, 2, 3, 4, 5, 6	11, 12	
Hirudinea	Erpobdellidae	*Dina* cf. *dinarica*		8	
Gastropoda	Hydrobiidae	*Belgrandiella* sp. T	1, 3, 5	9, 11, 12	
		Bythinella sp.	2, 3, 4, 5	9, 10	
		Islamia sp. T	4	11, 12	
		Kerkia sp. T	2, 4		
		Kerkia sp.nov. T - N.SP.	4		
	Moitessieriidae	*Paladilhiopsis* sp.nov. T - N.SP.	2, 3, 4, 5		
		Paladilhiopsis sp. T	5, 6	10	
	Planorbidae	*Ancylus fluviatilis* O. F. Müller, 1774	5, 6	11, 12	
		Ancylus sp.	5	11	
	Lymnaeidae	*Galba truncatula* (O. F. Müller, 1774)	5		
		Radix sp.		12	
	Valloniidae	*Vallonia* sp. ter	2		
	Ellobiidae	*Carychium tridentatum* (Risso, 1826) ter	2		
		Carychium sp. ter	2, 4	9	
	Pristilomatidae	*Vitrea* sp. ter	5, 6	9	
	Zonitidae	*Aegopis* sp. ter		9	
	Punctidae	*Punctum pygmaeum* (Draparnaud, 1801) ter		9	

Group	**Family**	**Taxon**	**Gravel bars**	**Springs**	**Cave**
	Gastrodontidae	*Aegopinella* sp.	5		
		Zonitoides nitidus (O. F. Müller, 1774) [ter]		9	
	Discidae	*Discus perspectivus* (Megerle von Mühlfeld, 1816) [ter]	6		
	Cochlostomatidae	*Cochlostoma* sp. [ter]		9	
	Pupillidae	*Pupilla* sp. [ter]	3		
	Hygromiidae	*Monacha* sp. [ter]	3		
	Oxychilidae	*Oxychilus* sp. [ter]	4		
Bivalvia	Sphaeriidae	*Pisidium* sp.	1, 5		
Diplopoda		Diplopoda [ter]		9	
Symphyla		Symphyla [ter]			13
Acarina	Aturidae	*Aturus crinitus* Thor, 1902	3		
		Erebaxonopsis brevipes Motas & Tanasachi, 1947 [T]	2		
		Paraxonopsis inferorum Motas & Tanasachi, 1947 [T]	1, 2		
		Paraxonopsis vietsi (Motas & Tanasachi, 1947) [T]	2		
	Frontipodopsidae	*Frontipodopsis reticulatifrons* Szalay, 1945 [T]	2, 3, 4		
	Halacaridae	*Parasoldanellonyx typhlops* Viets, 1933 [T]	4		
		Parasoldanellonyx sp.	4		
	Hungarohydracari-dae	*Hungarohydracarus subterraneus* Szalay, 1943 [T]	2, 3		
	Hydryphantidae	*Partnunia* sp.		8	
	Hygrobatidae	*Atractides latipes* Szalay, 1935	3		
		Atractides pumilus Szalay, 1946 [T]	1, 2, 3, 4		
		Atractides sp.	1	9	
	Lethaxonidae	*Lethaxona pygmaea* Viets, 1932 [T]	2		
	Momoniidae	*Stygomomonia latipes* Szalay, 1934 [T]	4		
	Sperchontidae	*Sperchon glandulosus* Koenike, 1886		9	
	Torrenticolidae	*Torrenticola anomala* Koch, 1837	2, 3		
		Torrenticola jeanneli Motas & Tanasachi, 1947 [T]	3		
		Torrenticola tenuirostris Viets, 1936	3		
		Torrenticola sp.	1, 2		
	Oribatida fam.	Oribatida [ter]	1, 2	9	
	Acarina fam.	Acarina g.sp.	1, 2, 3, 4		
		Acarina g.sp. [ter]			13
Aranea	Aranea fam.	Aranea g.sp. [ter]			13
Opiliones	Phalangiidae	Phalangiidae g.sp. [ter]			13

Group	Family	Taxon	Gravel bars	Springs	Cave
Ostracoda	Cyprididae	Cyprididae	3		
		Cavernocypris subterranean (Wolf, 1920)	1		
		Cypridopsis sp.	4		
		Potamocypris sp.	4, 5		
	Candonidae	Candoninae	2, 3, 4, 5, 6		
		Candona candida (O.F.Müller, 1776)	4		
		Neglecandona sp.	1, 2, 4	8, 9	
		Pseudocandona albicans (Brady, 1864)	2, 4		
		Typhlocypris sp. T - N.SP.	4		
Copepoda	Cyclopoida fam.	Cyclopoida	1		
	Cyclopidae	*Diacyclops antrincola* Kiefer, 1968 T	4		
		Diacyclops cf. *antrincola* T	4		
		Diacyclops clandestinus (Yeatman, 1964) T	1, 2, 3, 4, 5		
		Eucyclops serrulatus (Fischer, 1851)	2		
		Megacyclops viridis (Jurine, 1820)	1, 2, 3, 4	11, 12	
	Harpacticoida fam.	Harpacticoida	5		
	Canthocamptidae	*Attheyella crassa* (G.O. Sars, 1863)	2, 3		
		Attheyella wierzejskii (Mrazek, 1893)	1, 2, 4	9	
		Bryocamptus cf. *macedonicus* T - N.SP.	1, 3		
		Bryocamptus pygmaeus (Sars G. O., 1863)	1		
		Bryocamptus typhlops (Mrazek, 1893) T	1, 2, 3		
		Bryocamptus zschokkei (Schmeil, 1893)	2		
		Bryocamptus sp.	1, 3		
		Elaphoidella elaphoides (Chappuis, 1923)	4		
		Moraria poppei (Mrazek, 1893)	1, 3		
Amphipoda	Niphargidae	*Niphargus* sp. T – MOTU 1 - N.SP.	1, 2, 4		
		Niphargus sp. T – MOTU 2 - N.SP.	2		
	Crangonyctidae	*Synurella* sp.	1, 2, 4, 5		
	Gammaridae	*Gammarus* sp.		7, 8, 9, 11, 12	
Isopoda	Asellidae	*Proasellus* sp. T	2	11, 12	
		Proasellus n.sp. *- MOTU 1* T *(P. anophthalmus clade)* - N.SP.	1, 2, 3		

Group	Family	Taxon	Gravel bars	Springs	Cave
	Asellidae	*Proasellus* n.sp. - *MOTU 2* [T] *(P. anophthalmus clade)* - N.SP.	6		
	Ligiidae	*Ligidium germanicum* Verhoeff, 1901 [ter]		9	
	Trichoniscidae	*Trichoniscus* sp. [ter]		9	
		Hyloniscus sp. [ter]		9	
		Trichoniscidae g.sp. [ter]			13
Collembola		Collembola [ter]		9	13
Diptera		Diptera (L)	1, 2, 3, 4, 5, 6		
	Chironomidae	Chironomidae (L)	1, 2, 3, 4, 5, 6	9, 11, 12	
Plecoptera		Plecoptera (L)	1, 2, 3, 4, 5, 6	9, 11, 12	
Ephemero-ptera		Ephemeroptera (L)	1, 2, 3, 4, 5, 6	8, 9, 11, 12	
Trichoptera		Trichoptera (L)	1, 2, 3, 5, 6	8, 9, 12	
Neuroptera	Nevrorthidae	*Nevrorthus apatelios* H. Aspöck, U. Aspöck & Hölzel, 1977 (L)	3		
Coleoptera	Coleoptera	Coleoptera (L)	1		
	Scirtidae	*Hydrocyphon* sp. (L)	1, 2		
		Elodes sp. (L)		8	
	Hydraenidae	*Hydraena* cf. *bosnica*		9	
	Elmidae	*Elmis bosnica* (Zaitzev, 1908)		9, 11, 12	
		Elmis sp. (L)		9, 11, 12	
		Esolus cf. *angustatus* (L)	6	11, 12	
		Esolus cf. *parallelepipedus* (L)		11, 12	
		Esolus sp. (L)	1, 2, 3, 5, 6		
		Limnius sp. (L)	1, 2		
		Limnius sp. 1 (L)		11, 12	
		Limnius sp. 2 (L)		11, 12	
		Stenelmis cf. *puberula* (L)	1, 2		
	Staphylinidae	Staphylinidae [ter]		9	
	Leiodidae	*Anthroherpon ganglaueri distinguendum* (Müller, 1913) [ter, T]			13
	Carabidae	cf. *Laemostenus* sp.			13
Pisces		Pisces		11	
Mammalia	Rhinolophidae	*Rhinolophus hipposideros* Bechstein, 1800 (B)			13

Molecular identifications of *Niphargus* sp. and *Proasellus* sp.

Representatives of the genus *Niphargus* sp. were found at three gravel bars (Tab. 1). Individuals from all localities were molecularly analysed, which revealed two different MOTUs (molecular operational taxonomic units). At three localities (Locs. 1, 2, and 4) we found a total of four individuals (voucher codes NG679, NG680, NG681 and NG682, SubBioDB 2023) that belong to the same MOTU 1 (Tab. 3). According to the COI marker, they form a closely related clade with less than 2% uncorrected p-distance, and with 9% uncorrected p- distance to the closest sister clade. In addition, we found one individual of a different MOTU (MOTU 2) at locality 2, which also has the potential to be a new species (voucher code: NG678, SubBioDB 2023). According to the COI results, it is highly divergent from all other *Niphargus* species, with more than a 15% uncorrected p-distance.

Representatives of the genus *Proasellus* sp. were found at three gravel bars (Locs. 1, 2 and 3) and at one gravel bar with the spring (Loc. 6). All specimens collected at the three gravel bars belonged to the same MOTU 1, which is most likely a species new to science. One spring located in the gravel on the right bank of Neretva (Loc. 6, rafting lunch location) contained different MOTU 2, which was also a species new to science (Tab. 3). According to the COI gene, the two MOTUs were highly divergent from all other known species of *Proasellus*, with divergence higher than 16%, as measured with patristic distances.

Discussion

In this study, we conducted the first systematic survey of subterranean fauna in the upper Neretva River. Despite the limited time frame, we gathered valuable new information on subterranean species in the upper Neretva River. Our efforts not only expanded the knowledge on the distribution of known subterranean species but also led to the discovery of species previously unknown to science.

To confirm the existence of these new species and prepare their formal descriptions, further in-depth studies combining molecular, morphological, and distribution data are needed. Molecular analyses conducted in the genera *Proasellus* and *Niphargus* revealed strong indication of new species to science. The comparative analyses of distances to other *Niphargus* species (data taken from Borko et al. 2022) have shown that the MOTUs found during this study are at 9% to 15% uncorrected p-distances to other known species. The MOTU 1, found at three localities, belong to the same MOTU as the MOTU we sampled in a spring near Gacko, south of this study's localities, in March 2021 (SubBioDB 2023, unpublished data). According to the COI marker, they form closely related clades with less than 2% uncorrected p-distance. The distance to other analysed *Niphargus* species is > 9%. Although we lack molecular data for a few species from BIH, they differed in morphology from the ones we sampled. Therefore, we can conclude that these four individuals from Neretva, as well as an individual from a locality near Gacko, belong to one new species of *Niphargus*. It is nested within the south Dinaric clade, together with *N. brevicuspis* and *N. factor* (Borko et al. 2022). The second MOTU and potentially new species of *Niphargus* was collected at the gravel bar near Ulog, in the same area as the previous one. According to the COI analysis, it is highly divergent from all other *Niphargus* species, with even more than a 15% uncorrected p-distance, and falls into the Pontic clade of primarily interstitial species (Borko et al. 2022).

Improving the knowledge on the subterranean fauna of this area is not only an interesting research question per se but is also important from a conservation point of view. Typically, subterranean species are highly endemic, with small distribution ranges, many being known from only single locality (Bregović et al. 2019). Groundwater biota of interstitial and surrounding aquatic habitats are an important part of the river ecosystem, being involved in both nutrient cycling and bioturbation (Hose et al. 2022). Groundwater species also significantly contribute to the overall biodiversity of freshwater ecosystems (Boulton et al. 2008), especially in subterranean diversity hotspots such as the Dinarides.

When limited knowledge on the general biodiversity is confronted with high developmental and economic interests, this makes the Neretva River region very vulnerable to destruction. Due to the lack of data on the existence of endemic species, it is essential to conduct baseline studies gathering both fundamental distribution and ecological data within the upper Neretva River. This is also important for subterranean habitats, which can be heavily affected by the planned hydropower plants in multiple ways (Fišer et al. 2022). The hydropower plant construction can irreversibly destroy the interstitial habitats during its construction. Additionally, following the formation of the reservoir behind the dam, groundwater habitats can be affected by colmation (i. e. filling the voids between gravel with fine sediments) and fragmentation of the previously connected habitats along the river valley. Dams can have a negative impact on the invertebrate communities living beneath them by causing disturbances in the water flow and temperature, leading to a decreased quantity and diversity of invertebrates (Dolédec et al. 2021). This latter study also showed that overall, there are fewer and less diverse invertebrate communities below the dam, favouring species with higher resistance and lower food specialisation and sensitivity to pollution.

The upper parts of the Neretva River are currently preserved in an almost intact state, surrounded by vast forests, and without large dams on the river. The similarity in taxonomic richness of interstitial communities along the studied part of the Neretva River indicates the intact nature of the river, which indicates the longitudinal connectivity of the interstitial habitats and connectivity with surrounding karstic subterranean habitats. It has been shown that karstic subterranean environments are connected through hydrological pathways (Bonacci et al. 2009), and that interstitial habitats are a highway or a migration corridor for subterranean fauna (Ward & Palmer 1994). The upper Neretva still provides such connectivity and migration possibilities, which could be severely affected by damming and hydropower plant construction.

These first results originating from a single field sampling campaign indicate that the upper Neretva area needs further, more extensive research. However, groundwater fauna is still often neglected in freshwater biodiversity studies. We encourage the inclusion of groundwater components in future studies of river biodiversity, especially in understudied areas that are at the same time threatened by destructive hydropower and other construction projects.

Povzetek

Dinaridi so kraško območje na Zahodnem Balkanu z globalno izjemno podzemno biotsko raznovrstnostjo (Sket 2012). A nekatera območja so slabo raziskana (Zagmajster et al. 2008; Bregović et al. 2019; Borko et al. 2022) in med taka spada tudi zgornji del porečja reke Neretve v vzhodni Bosni in Hercegovini. To območje je še zelo dobro ohranjeno, tu je veliko prodišč ob reki Neretvi in njenih pritokih ter kraških gričev, ki obkrožajo dolino. Pred našo študijo je bilo od tod le nekaj podatkov o podzemnih vrstah (SubBioDB 2023).

Raziskava je potekala na območju med krajema Krupac in Konjic (Sl. 1). Vzorčili smo rečni intersticij na dostopnih prodiščih ob reki, a tudi izvire in eno jamo. Z metodo Bou-Rouch (Bou & Rouch 1967) smo vzorčili intersticijske vode na vseh razen na enem prodišču (Tab. 1), kjer smo uporabili metodo Karaman-Chappuis (Karaman 1935; Chappuis 1942). Izvire smo vzorčili z metodo »kick-sampling« in živali zbirali z ročnimi vodnimi mrežami. V jami smo živali iskali s pregledovanjem različnih mikrohabitatov. Abiotske parametre smo merili v površinski rečni vodi in v 10 litrih načrpane podzemne vode na vseh prodiščih razen enem. Na nekaj izvirih smo izmerili temperaturo vode z ročnim termometrom.

Skupaj smo pregledali šest prodišč, šest izvirov in eno jamo. Zbrali smo 268 podatkov (takson-lokacija-datum) o 116 taksonih, od tega 35 podatkov za 27 kopenskih (Tab. 3). Medtem ko smo kopenske taksone vzorčili samo v jami, smo jih 19 našli v vodnih vzorcih, večinoma iz izvirov. Skupno smo zabeležili 27 izključno podzemnih (troglobiotskih) vrst, enega kopenskega hrošča v jami in 26 vodnih vrst, ki pripadajo pršicam, polžem in rakom (Tab. 3).

Največje bogastvo vodnih taksonov je bilo zabeleženo na prodišču Neretve na kopališču pri Ulogu (43 taksonov), sledita prodišče dolvodno od sotočja Krupac-Neretva (34 taksonov) in prodišče gorvodno od Brijestovega mostu (34 taksonov) ter prodišče pri Cerovi (33 taksonov). Največ troglobiontov je bilo v prodiščih pri Ulogu in Brijestovem mostu.

Osem vodnih troglobiontov kaže na potencialne nove vrste za znanost. Od slednjih smo morfološko ločili štiri vrste: dve vrsti polžev iz družin Hydrobiidae in Moitessieriidae, enega predstavnika Ostracoda in enega Copepoda. Na podlagi molekularnih razlik smo identificirali po dve novi vrsti v rodu *Proasellus* (Isopoda) in rodu *Niphargus* (Amphipoda).

Podzemne vrste so zelo endemične, imajo majhna območja razširjenostmi, mnoge so znane le iz po ene lokalitete, kar jih dela zelo ranljive (Bregović et al. 2019). Dokazano je, da so kraška podzemna okolja povezana s hidrološkimi povezavami (Bonacci et al. 2009), intersticialni habitati pa predstavljajo selitvene koridorje za podzemno favno (Ward & Palmer 1994). Zgornja Neretva še vedno omogoča takšno povezljivost in možnosti selitev, ki pa bi jih lahko ogrozile načrtovane zajezitve zaradi gradenj hidroelektrarn. Problematika podzemnih voda bi morala biti del študij biotske raznovrstnosti rek, zlasti na zelo ohranjenih, a premalo raziskanih območjih, ki jih ogrožajo načrtovane hidroelektrarne in druge večje konstrukcije.

Sažetak

Dinaridi su kraško područje na zapadnom Balkanu sa globalno izuzetnim podzemnim biodiverzitetom (Sket 2012). Međutim, neka područja su slabo istražena (Zagmajster et al. 2008; Bregović et al. 2019; Borko et al. 2022), a među njima je i gornji dio sliva rijeke Neretve u istočnom dijelu Bosne i Hercegovine. Ovo područje je još uvijek vrlo dobro očuvano, ima mnogo pješčanih sprudova duž rijeke Neretve i njenih pritoka i kraških brda koja okružuju dolinu. Prije našeg istraživanja, postojalo je samo nekoliko podataka o podzemnim vrstama sa ovog terena (SubBioDB 2023).

Istraživanja su obavljena na području između Krupca i Konjica (Sl. 1). Uzorkovali smo riječni intersticij na pristupačnim dijelovima riječnog korita, izvore i jednu pećinu. Intersticijalne vode uzorkovane su Bou-Rouch metodom (Bou & Rouch 1967) u svim šljunčanim sprudovima osim u jednom (Tab. 1), gdje je korištena Karaman-Chappuis metoda (Karaman 1935; Chappuis 1942). Izvori su uzorkovani metodom »kick-sampling«, a životinje su sakupljene ručnim mrežama za vodu. Tražili smo životinje u pećini ispitujući različita mikrostaništa. Abiotički parametri su izmjereni u površinskoj riječnoj vodi i u 10 litara ispumpane podzemne vode na svim sprudovima osim u jednoj. Na nekoliko izvora mjerili smo temperaturu vode ručnim termometrom.

Ukupno smo ispitali: šest šljunčanih sprudova, šest izvora i jednu pećinu. Prikupili smo 268 podataka (takson-lokacija-datum) za 116 taksona, od čega 35 podataka za 27 kopnenih (Tab. 3). Kopneni taksoni uzorkovani su samo u pećini, 19 ih je pronađeno u uzorcima vode, uglavnom iz izvora. Ukupno smo evidentirali: 27 isključivo podzemnih (troglobiotičkih) vrsta, jednog tvrdokrilca u pećini i 26 vodenih vrsta grinja, puževa i rakova (Tab. 3).

Najveće bogatstvo vodenih taksona zabilježeno je na ušću Neretve i plaži kod Uloga (43 taksona), zatim ušće nizvodno od ušća Krupac u Neretvu (34 taksona), ušće uzvodno od Brijestovog mosta (34 taksona) i ušće kod Cerove (33 taksona). Najviše troglobionta bilo je u šljunčanim sprudovima kod Uloga i Brijestovog mosta.

Osam vodenih troglobionta predstavljaju potencijalne nove vrste za nauku, od kojih su morfološki izdvojene četiri vrste: dvije vrste puževa iz porodica Hydrobiidae i Moitessieriidae, jedan predstavnik Ostracoda i jedan predstavnik Copepoda. Na osnovu molekularnih razlika identifikovali smo po dvije nove vrste u rodu *Proasellus* (Isopoda) i rodu *Niphargus* (Amphipoda).

Podzemne vrste su visokoendemične, imaju mala područja rasprostranjenja, mnoge su poznate samo sa jednog lokaliteta, što ih čini veoma ranjivim (Bregović et al. 2019). Pokazalo se, da su podzemne sredine u kršu povezane hidrološkim vezama (Bonaci et al. 2009), a intersticijska staništa predstavljaju migracione koridore za podzemnu faunu (Ward & Palmer 1994). Gornja Neretva još uvijek pruža takvu povezanost i mogućnosti migracije, koje bi mogle biti ugrožene planiranim branama zbog izgradnje hidroelektrana. Problem podzemnih voda trebao bi biti dio proučavanja biodiverziteta rijeka, posebno u dobro očuvanim, ali nedovoljno istraženim područjima ugroženim planiranim hidroelektranama i drugim velikim projektima.

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