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Interstitial fauna of the Sava River in Eastern Slovenia

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Abstract. Interstitial water that occupies the pore spaces within unconsolidated sediments is a unique habitat, inhabited by surface as well as exclusively subterranean species (stygobionts). The best studied of all interstitial habitats is the hyporheic zone, an interface between surface water and groundwater (phreatic zone) environments. The Sava River in central Slovenia (i.e., at the Ljubljana alluvial plain) readily qualifies as one of the global hotspots of interstitial biodiversity, while data from other river sections are lacking. In 2015, we sampled two gravel bars on the final Slovenian section of the river (Eastern Slovenia), and collected nine samples per site using the Bou-Rouch pumping method. At »Čatež ob Savi« and »Obrežje« sites, at least 26 invertebrate species with 14 stygobionts and 25 invertebrate species with 13 stygobionts were identified, respectively. Altogether, 33 invertebrate taxa with 16 stygobionts were recorded, which increased the previously known interstitial stygobiotic richness of the Sava River in Slovenia by eight species (to 37 species). Three species of the stygobiotic amphipod genus *Niphargus* were recorded in Slovenia for the first time, one of which is even a new species to science. We discuss the novel results in the context of current national conservation practices.

Key words: interrstitial fauna, hyporheic, stygobionts, endemic species, Bou-Rouch pumping method, Sava River, Slovenia

Izvleček. Intersticijska favna reke Save v vzhodni Sloveniji – Intersticijska voda – voda med zrni nesprijetega sedimenta, je edinstven življenjski prostor tako za površinske kot tudi izključno podzemne vrste (stigobionte). Najbolje raziskan med plitvimi intersticijskimi habitati je hiporeik, prehodni pas med površinsko vodo in globoko podtalnico (freatikom). Odsek reke Save na območju Ljubljanskega polja (osrednja Slovenija) sodi med svetovne vroče točke intersticijske vrstne pestrosti, medtem ko podatkov iz drugih odsekov reke skoraj ni. Leta 2015 smo z metodo Bou-Rouch pridobili po devet vzorcev iz vsakega od dveh prodišč v končnem odseku Save (vzhodna Slovenija). Na prodiščih »Čatež ob Savi« in »Obrežje« smo zabeležili najmanj 26 oz. 25 nevretenčarskih vrst, od tega 14 oz. 13 stigobiotskih. Skupno število najdenih taksonov, t.j. 33 nevretenčarskih, od tega 16 stigobiotskih vrst, je zvišalo pestrost savskih stigobiontov za osem vrst (na 37 vrst). Tri vrste iz rodu slepih postranic (*Niphargus*) smo v Sloveniji zabeležili prvič, od tega je ena nova za znanost. V prispevku izsledke obravnavamo v luči trenutne naravovarstvene prakse.

Ključne besede: intersticijska favna, hiporeik, stigobionti, endemiti, Bou-Rouch metoda črpanja, Sava, Slovenija

Introduction

Water in voids between grains of unconsolidated sediments constitutes a unique subterranean habitat, the so-called aquatic interstitial (Angelier 1962, Danielopol 1976, Culver and Pipan 2014). The best studied of all shallow interstitial habitats is the hyporheic zone beneath and alongside the stream and river beds (Orghidan 1955): »a temporally and spatially dynamic saturated transition zone between surface- and groundwater bodies that derives its specific physical (e.g., water temperature) and biogeochemical (e.g., steep chemical gradients) characteristics from mixing of surface- and groundwater to provide a dynamic habitat and potential refugia for obligate and facultative species« (Krause et al. 2011). Its exact limit towards permanent groundwater (phreatic water) is difficult to define, due to its variability in space and time (Fraser and Williams 1998). The importance of the hyporheic zone as an essential component of river and stream ecosystems was widely recognized by the 1980s (Hancock et al. 2005), but major research gaps remain (Boulton et al. 2010).

This dynamic lotic ecotone is inhabited by a diverse array of surface (i.e., benthic) species that temporarily inhabit the substrate, and by specialized subterranean (i.e., aquatic troglobiotic or stygobiotic) species, which rarely if ever occur in the surficial water channel (Gibert et al. 1994, Mori and Brancelj 2011). The somewhat reduced dispersal capacity of stygobionts in comparison to their surface counterparts usually results in small distribution ranges of the former (Trontelj et al. 2009, Zagmajster et al. 2014). Many stygobionts are narrowly distributed, which makes them especially prone to extinction (Sket 1999). Dependence and interconnection of hyporheic and permanent groundwater (i.e., phreatic water) with surface water, combined with limited self-purification capability of aquatic interstitial habitat, is the reason for the negative effects of above-ground human threats on interstitial aquifers and their specialized and sensitive fauna.

One of the most interesting European rivers according to richness of the interstitial fauna is the Sava River (hereinafter referred to as the Sava). With its 219 km stretch from the spring to the Croatian border, it is the longest river in Slovenia. It starts as an alpine river, which then flows through the sub-Alpine hills in central Slovenia, to the plain in the eastern part of the country, where it gradually changes into a typical lowland river. After running through the Posavina region (Croatia and Bosnia and Herzegovina), it discharges into the Danube River in Serbia. The hyporheic and phreatic interstitial fauna along its 990 km long course was mainly studied in the mid-twentieth century. Most of the faunistic studies were carried out around Zagreb (Croatia) and Ljubljana (Slovenia) (Karaman 1954, 1983, Meštrov 1957, 1960, 1961, Meštrov et al. 1978, 1983, Sket & Velkovrh 1981, Rogulj et al. 1994). Nearly three decades have passed before the study focusing on microcrustacean assemblages (i.e., Copepoda, Ostracoda and Cladocera) in the Sava gravel bar near Ljubljana was carried out (Mori et al. 2012).

Based on literature review and some unpublished records, as many as 29 stygobiotic species were known from the interstitial habitats of the Sava in Slovenia till the end of 2014 (SubBioDB 2014). Although this confirmed its ranking among the global hotspots of interstitial fauna biodiversity (Sket & Velkovrh 1981), current data are almost exclusively based on samples collected from the alluvial plain north of Ljubljana (i.e., Ljubljansko polje aquifer,

central Slovenia). The lack of new studies and data from other sections of the river and its tributaries is hindering any attempt to evaluate the comparative importance of different river sections for interstitial species and their importance for conservation.

In order to fill this gap, a study of the interstitial fauna in the Slovenian lowermost section of the Sava (Eastern Slovenia) was conducted in 2015, which enabled the provision of invertebrate species list for this section. We paid special attention to stygobionts. Here, we discuss their contribution to the biodiversity of the Sava interstitial fauna in general. Also, we comment on the conservation status of the sampled taxa and the current national habitat management.

Materials and Methods

The two gravel bars are situated on the right bank of the lowland Sava, in the eastern part of Slovenia (Fig. 1). The site »Obrežje« (45°50'56'' N, 15°42'24'' E) is located immediately along the national border with Croatia. The site »Čatež ob Savi« (45°53'37'' N, 15°37'55'' E) is situated about 8 km upstream from the gravel bar »Obrežje«, north from the thermal spa »Terme Čatež« near Čatež (Fig. 1).

We sampled the hyporheic zone using the Bou-Rouch pumping method (Bou & Rouch 1967). At both sites, sampling was done at two depths in the river: 30–60 cm and 60–90 cm, while in the exposed gravel bar next to the river, only sampling at 60–90 cm was carried out. We repeated the sampling procedure three times at each site, within a distance of approx. 10 m along the gravel bar, resulting in nine samples per gravel bar. For each sample, we pumped 30 litres of a mixture of water, sediments and particulate organic matter. In the first 10 litres of collected mixture, the *in situ* dissolved oxygen, oxygen saturation, temperature, conductivity, and pH were measured with a portable multimeter CyberScan 600 (Eutech Instruments). The mixtures were then filtered to collect invertebrates, using hand nets of 0.5 mm and 0.1 mm mesh size. Invertebrates from the two hand nets were preserved separately in 96% ethanol and stored in the collection of the Department of Biology (Biotechnical Faculty, University of Ljubljana).

In the laboratory, only invertebrates from the 0.5 mm mesh size fraction were sorted and either morphologically or molecularly identified to the lowest taxonomic level possible. To present the interstitial diversity of each gravel bar, we pooled the data from all nine samples. Some taxa from the 0.5 mm mesh size fraction (e.g. Oligochaeta and Chironomidae) and all invertebrates from the 0.1 mm mesh size fraction remained unidentified due to the lack of taxonomists or/and methodological obstacles (e.g., immaturity, small body size and the need to implement molecular approaches).

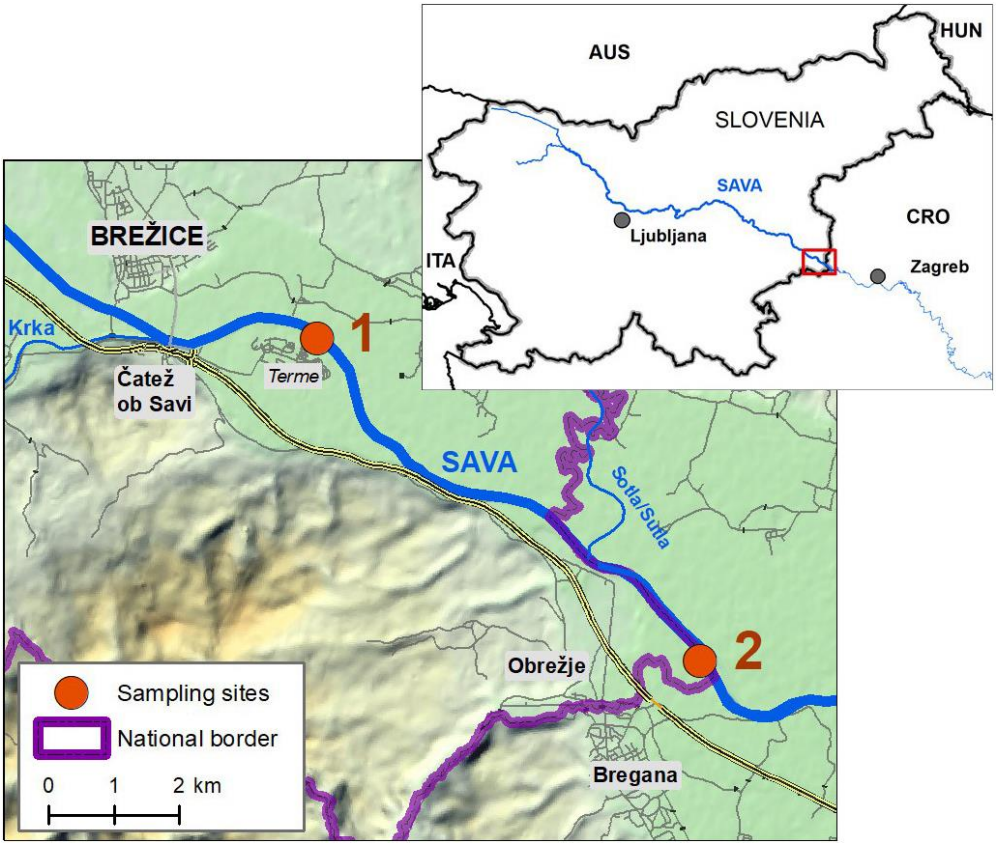

Figure 1. Map of the lower reach of the Sava River in Slovenia. The two sampling sites for interstitial fauna »Čatež ob Savi« (1) and »Obrežje« (2) are marked with red dots.
Slika 1. Zemljevid območja vzorčenja intersticijske favne na prodiščih Save v Sloveniji. Označeni sta mesti vzorčenja »Čatež ob Savi« (1) in »Obrežje« (2).

Results and Discussion

Observations on the species richness and species conservation status

Using the same sampling procedure at both gravel bars enabled comparisons between the sites, even though with some prudence (Malard et al. 2002). The species richness at »Čatež ob Savi« and »Obrežje« gravel bars is almost the same: 14 and 13 stygobiotic species out of 26 and 25 sampled taxa were identified, respectively (Tab. 1). As some invertebrates remained unidentified (discussed above in Materials and methods), these numbers represent the minimum number of taxa.

Table 1. List of invertebrates from gravel bars of the Sava River at Čatež ob Savi and Obrežje. The national (RS; Ur. l. RS 2002) and IUCN (IR; IUCN 2019) Red list statuses are given: LC = least concern, K = not evaluated, R = rare, V and VU = vulnerable. The names of taxa the identification of which might be questionable are marked »cf.« (similar to). The underlined names indicate the first confirmation of the species presence in interstitial habitats of the Sava River. Annotations: D – species included in the Decree on protected wild animal species (Ur. l. RS 2004a), En – Slovenian endemic species, juv. – juvenile, s – only shells found, T – species with the type locality in Slovenia, »*« – stygobiont, »+« – present, »!« – conservation status candidate.

Tabela 1. Seznam nevretenčarjev s prodišč reke Save v Čatežu ob Savi in Obrežju. Navedena sta nacionalni (RS; Ur. l. RS 2002) in IUCN (IR; IUCN 2019) status ogroženosti; LC = najmanj ogrožen, K = neocenjen, R = redek, V in VU = ranljiv. Vprašljiva identifikacija je označena s »cf.« (podoben kot). Podčrtana imena označujejo prvo najdbo v savskem intersticiju. Oznake: D – vrsta iz Uredbe o zavarovanih prosto živečih živalskih vrstah (Ur. l. RS 2004a), En – slovenski endemit, juv. – mladi, s – prazne hišice, T – vrste s tipsko lokacijo v Sloveniji, »*« – stigobiont, »+« – prisoten, »!« – kandidat za pridobitev statusa ogroženosti.

Higher taxa	Family	Genus/species	Čatež	Obrežje	RS/IR	Remarks
CNIDARIA:	Hydridae:	*Hydra* sp.		+		
TURBELLARIA:	Dendrocoelidae:	*Dendrocoelum* sp.	+			
GASTROPODA:	Acroloxidae:	*Acroloxus lacustris*		s	V/VU	
	Planorbidae:	*Gyraulus* cf. *albus*		s		
	Hydrobiidae:	*Iglica gracilis**	+	+	V/VU	T
		Bythinella cf. *austriaca*	+		-/LC	
		*Hauffenia media**	+		V/VU	
	Valvatidae:	*Valvata cristata*	s		K/-	
OLIGOCHAETA			+	+		
ACARINA:	Hygrobatidae:	*Hygrobates* sp.	+			
CRUSTACEA:						
Copepoda:	Cyclopidae:	*Acanthocyclops venustus**	+	+		
		*Diacyclops slovenicus**	+	+		
Ostracoda:	Candonidae:	*Typhlocypris cavicola**	+	+	R/VU	T, En
Amphipoda:	Niphargidae:	*Niphargus serbicus**	+	+		
		*Niphargus longidactylus**	+	+		
		Niphargus cf. *kenki**	+	+		T
		*Niphargus minor**		+		NT
		*Niphargus parapupetta**		+	!	first record
		*Niphargus labacensis**	+	+		T
		*Niphargus multipennatus**	+	+		NT
		*Niphargus lattingerae**	+		!	first record
		Niphargus sp. nov.*	+		!	new
	Gammaridae:	*Gammarus* sp. (juv.)	+	+		
	Crangonictidae:	*Synurella* cf. *ambulans*	+	+		
	Bogidiellidae:	*Bogidiella albertimagni**	+	+		
Isopoda:	Asellidae:	*Proasellus deminutus deminutus**	+	+		T
HEXAPODA (juv.):						
Diptera	Chironomidae		+	+		
	Ceratopogonidae		+			
Ephemeroptera:	Ephemerellidae:	*Ephemerella ignita*		+		
	Baetidae:	*Baetis fuscatus*	+	+		
	Potamanthidae:	*Potamanthus luteus*	+	+	R/-	
Coleoptera:	Elmidae:	*Macronychus quadrituberculatus*	+	+		D
Trichoptera:	Glossosomatidae:	*Glossosoma boltoni*		+		
	Number of all taxa / stygobiotic taxa		**26/14**	**25/13**		

Most stygobiotic species are snails (Gastropoda) and crustaceans (Crustacea), the latter especially from the amphipod genus *Niphargus* (9 species). Five of the collected species are listed in the Slovenian Red list of endangered plant and animal species (Ur. l. RS 2002) or/and in the IUCN Red list of threatened species (IUCN 2019; Tab. 1). Two of them are surface species: a mayfly nymph *Potamanthus luteus* (Linnaeus, 1767) and a snail *Acroloxus lacustris* (Linnaeus, 1758). Actual presence of the latter species at »Obrežje« could not have been confirmed as only empty shells were found. The other three species are stygobiotic, two snails: *Iglica gracilis* (Clessin, 1882) (Fig. 2A) and *Hauffenia media* Bole, 1961 (Fig. 2B) and one ostracod crustacean, *Typhlocypris cavicola* (Klie, 1935) (Fig. 2C). *Iglica gracilis*, sampled at both gravel bars (Hofman et al. 2018), is endemic to Slovenia where it is known from caves and springs in the basin of the Krka River to the junction with the Sava (De Mattia 2007). *Hauffenia media*, sampled at »Čatež ob Savi« (Rysiewska et al. 2017), is restricted to southern and southeastern Slovenia (i.e. caves and springs in the Dolenjska Region and Bela krajina) and Croatia (known from a single locality at the border with Slovenia) (Bole 1992). *Typhlocypris cavicola*, sampled at both gravel bars, has long been known only from the cave Krška jama (Klie 1935). During the last 17 years, however, it has been recorded from several locations in the Dinaric Karst of Slovenia, mostly from caves, springs and hyporheic zones (Mori and Brancelj 2011, Mori et al. 2011, Mori and Meisch 2012). In both the Slovenian and IUCN Red lists, *T. cavicola* is still listed under currently invalid name *Pseudocandona cavicola* (Klie, 1935).

At least three more species would be eligible for the national and global conservation assessment:

- two stygobiotic amphipods, *Niphargus parapupetta* G. Karaman, 1984 (Fig. 2D) and *N. lattingerae* G. Karaman, 1983, were recorded in Slovenia for the first time. They should be considered endangered under the Slovenian Red list (Ur. l. RS 2002), as is the case in *N. valachicus* Dobreanu & Manolache, 1933, with similar distribution range. Specifically, they all have extensive range in other countries, while in Slovenia they are narrowly distributed.
- a new stygobiotic *Niphargus* species was found at the »Čatež ob Savi« gravel bar. In addition to morphology, the uniqueness of this extremely small amphipod was confirmed by DNA analysis and its description is in preparation. It should be listed in one of the highest levels of threats under both Red lists.

Of all the species collected, only a small riffle beetle *Macronychus quadrituberculatus* is included in the Decree on protected wild animal species (Ur. l. RS 2004a, Appendices 1 and 2), which protects the species and its habitat.

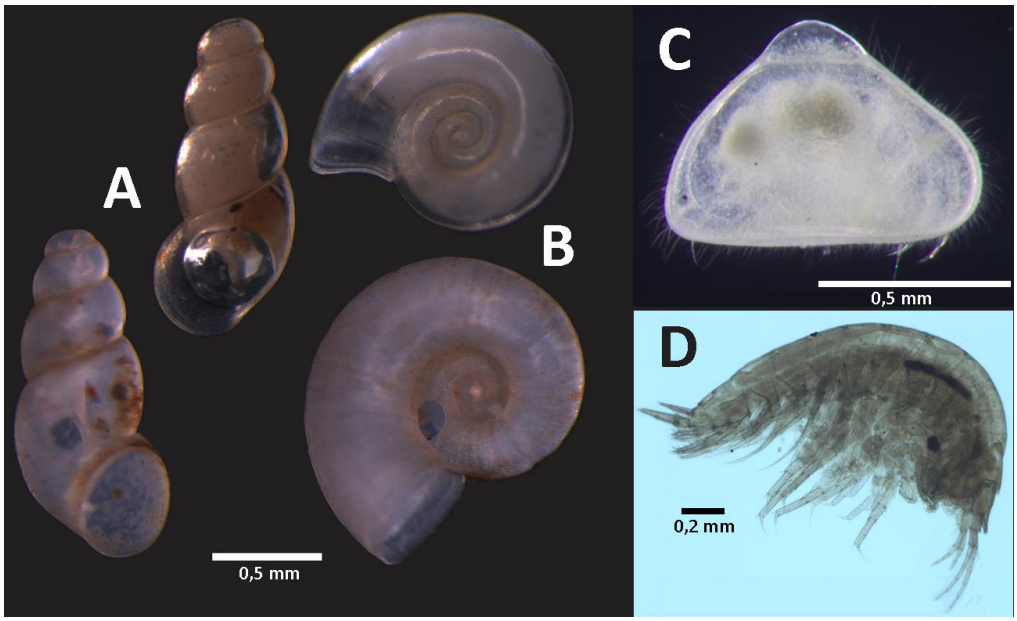

Figure 2. Interstitial stygobionts: snails (A) *Iglica gracilis* and (B) *Hauffenia media* and crustaceans (C) ostracod *Typhlocypris cavicola* and (D) amphipod *Niphargus parapupetta* (photos A, B, D: S. Prevorčnik, photo C: N. Mori).
Slika 2. Intersticijski stigobionti: polža (A) *Iglica gracilis* in (B) *Hauffenia media* ter raka (C) dvoklopnik *Typhlocypris cavicola* in (D) slepa postranica *Niphargus parapupetta* (fotografije A, B, D: S. Prevorčnik, fotografija C: N. Mori).

The need for further studies and conservation of the interstitial fauna

Although the number of taxa collected during sampling of two gravel bars is not final due to incomplete species identification, our preliminary results clearly indicate that the lower section of the Sava River in Slovenia hosts a high number of stygobiotic taxa as well. The data obtained from this study increased the known diversity of the interstitial stygobiotic fauna of the Slovenian Sava from at least 29 species known in 2014 (SubBioDB 2014) to at least 37 species. The identification of yet unidentified taxa is likely to increase the number.

The lack of studies over the last few decades, coupled with the new discoveries of our short-term and narrowly localized survey, unambiguously indicate the need for intensive and targeted sampling and exploration of understudied fauna of interstitial habitats in general. Unfortunately, this fauna often remains »invisible« in procedures related to assessing the potential impacts of various construction plans on the environment. In Slovenia, the interstitial fauna is not protected *per se*. Its protection often comes as a side effect of protecting the habitat for some other reason. For instance, gravel bars achieve some conservation concern as important habitats of protected terrestrial (mainly birds) and surface aquatic species (mainly fish) (Ur. l. EU 1992, 2010). Also measures established to safeguard groundwater as the source of drinking water have a positive effect on the hyporheic and phreatic as a habitat (Ur. l. RS 2009). But, these measures only cover aspects of chemical quality and quantity,

omitting other aspects relevant to stygobiotic inhabitants. Meanwhile, the high potential for endemism in these species, which would provide their direct protection according to Article 14 of the national Nature Conservation Act (Ur. l. RS 2004b), remains unduly overlooked. This article explicitly states that no actions are allowed that would result in endangering or extinction of any animal or plant species. Active protection of interstitial species under the Slovenian Red List of Endangered Animal and Plant Species (Ur. l. RS 2002) is generally deficient, due to the outdated list. The update would likely include several additional interstitial species.

The interstitial fauna is theoretically protected also via binding international legislative frameworks. For example, the Convention on Biological Diversity ratified also by Slovenia (Ur. l. RS 1996), reminds decision-makers that natural resources are not infinite and sets out a philosophy of their sustainable use. The EU Groundwater Directive (OJ EU 2006) contains a direct reference to interstitial habitats as ecosystems. The same is stated in EU Water Framework Directive (Ur. l. EU 2000). Both directives recognise the importance of groundwater also in relation to the »groundwater dependent« surface ecosystems (aquatic or terrestrial). Nevertheless, they only state the need for chemical and quantitative monitoring, ignoring biota.

It is well known that any human-induced watercourse degradation (e.g., building of dams, removal of gravel, etc.), as well as changing the natural water level regimes (e.g., due to over-exploitation of interstitial groundwater), can have significant and irreversible negative effects on interstitial fauna (Culver and Pipan 2014). Given that interstitial habitats and their fauna are severely understudied, with a great potential for new discoveries and high endemicity, they are undisputed candidates for further studies, as well as for the implementation of appropriate conservation measures.

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