Univerza v Ljubljani
Biotehniška fakulteta

NACIONALNI INŠTITUT ZA BIOLOGIJO

NATURA SLOVENIAE

Revija za terensko biologijo • Journal of Field Biology

Letnik • Volume 23 Številka • Number 1

Ljubljana
2021

NATURA SLOVENIAE

Revija za terensko biologijo • Journal of Field Biology

Izdajata • Published jointly by

Biotehniška fakulteta, Univerza v Ljubljani
Jamnikarjeva 101, SI-1000 Ljubljana
Tel.: (0)1 320 30 00; Telefax: (0)1 256 57 82
http://www.bf.uni-lj.si

Nacionalni inštitut za biologijo
Večna pot 111, SI-1000 Ljubljana
Tel.: (0)59 232 700; Telefax: (0)1 2412 980
http://www.nib.si

http://www.bf.uni-lj.si/bi/NATURA-SLOVENIAE/index.php

Naslov uredništva • Address of the Editorial Office
NATURA SLOVENIAE, Večna pot 111, SI-1111 Ljubljana, Slovenija

Izvlečki prispevkov so zavedeni v zbirkah **ASFA, AGRIS, Biological Abstracts, Biosis Previews, COBISS** in **Zoological Records**

ISSN: 1580-0814 **UDK: 57/59(051)=863=20**

Lektorji • Language Editors
za angleščino (for English): Henrik Ciglič
za slovenščino (for Slovene): Henrik Ciglič

Oblikovanje naslovnice • Layout
Daša Simčič akad. slikarka, Atelje T

Natisnjeno • Printed in
2021

Tisk • Print
Miha Košenina s.p., Brezovica pri Ljubljani

Naklada • Circulation
300 izvodov/copies

Sofinancira • Cofinanced by
Javna agencija za raziskovalno dejavnost RS/Slovenian Research Agency

Kazalo vsebine

ZNANSTVENA ČLANKA / SCIENTIFIC PAPERS

TERENSKE NOTICE / FIELD NOTES

NATURA SLOVENIAE 23(1): 5-20
SCIENTIFIC PAPER

Prejeto / Received: 20. 11. 2020
Sprejeto / Accepted: 16. 6. 2021

Harpacticoid assemblages (Copepoda: Harpacticoida) in the hyporheic zone of four streams in central Slovenia

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Abstract. Harpacticoids are an important component of meiofaunal assemblages in hyporheic zone. The goal of this study was to investigate distribution patterns of interstitial harpacticoid assemblages from four pre-Alpine streams originating in the Dinaric Karst and flowing into the Ljubljanica River. The sampling was conducted in 2002 at 12 locations distributed at a distance of approximately 1 km along each stream including tributaries, at a depth of 30–60 cm in the wetted channel (three sites per location) and depths from 65 to 160 cm on the stream banks (one site per location) using a Bou-Rouch pump. Concurrently, the interstitial water's physical and chemical parameters were measured at two sites within each location (streambed, streambank). A total of 24 harpacticoid species were found, 12 of which were stygobionts (i.e., species living exclusively in groundwaters). Among them, two previously unknown species for science were found. Harpacticoid assemblage composition, with the exception of those from the Iška stream, did not differ significantly between the streams, indicating interconnectivity of the interstitial milieu. Sediment structure, amounts of particulate organic matter, conductivity and redox conditions seemed to have certain impacts, indicating the importance of hydrological and geological settings for harpacticoid assemblages.

Key words: microcrustacea, species-environment relationship, biodiversity, distribution, groundwater

Izvleček. Združbe harpaktikoidov (Copepoda: Harpacticoida) v hiporeiku štirih rečic v osrednji Sloveniji – Cilj študije je bil raziskati vzorce porazdelitve intersticijskih združb harpaktikoidov v štirih rečicah, ki izvirajo v Dinarskem krasu in se izlivajo v reko Ljubljanico. Vzorčenje je bilo opravljeno leta 2002 na 12 lokacijah vzdolž vsake rečice s pritoki v globini 30–60 cm v omočeni rečni strugi (3 mesta) in v globinah od 65 do 160 cm na rečnih bregovih (1 mesto) s pomočjo Bou-Roucheve črpalke. Izmerili smo tudi fizikalne in kemijske lastnosti intersticialne vode v dveh vzorcih na vsaki lokaciji (omočena struga, rečni breg). Skupno smo našli 24 vrst harpaktikoidov, od tega 12 stigobiontov (tj. vrst, ki živijo izključno v podzemni vodi). Najdeni sta bili dve novi vrsti za znanost. Sestava združb harpaktikoidov, razen tistih v Iški, se med rečicami ni statistično značilno razlikovala, kar kaže na medsebojno povezanost intersticijskega življenjskega okolja. Kljub temu rezultati kažejo, da so sestava sedimentov, količine organskih delcev, prevodnost in redoks razmere tisti, ki najbolj vplivajo na sestavo združbe, kar kaže na pomembnost hidroloških in geoloških značilnosti v porečjih na sestavo združb harpaktikoidov v hiporeiku.

Ključne besede: nižji raki, odnosi med vrstami in okoljem, biodiverziteta, porazdelitev, podzemne vode

Introduction

Alluvial sediments with interstices saturated by water and located below wetted riverbed and extending laterally, the so-called »hyporheic zone«, form a transitional zone of active exchange of water, dissolved and particulate organic matter, and organisms between the river and the adjacent phreatic groundwaters (Orghidan 1955, Pennak & Ward 1986, Boulton et al. 2010, Orghidan 2010). Here, the invertebrate community is composed of surface benthic and stygobiotic species (i.e., species living exclusively in groundwaters), their ratio depending on hydrogeomorphological conditions in the hyporheic zone (Dole-Olivier & Marmonier 1992, Mori et al. 2012). In the areas where downwelling occurs, the sediments are well-oxygenated and primarily harbour organisms of benthic origin. With increasing residence time, below or lateral to the riverbed, hyporheic water becomes less oxygenated, biogeochemical processes become reductive, and the hyporheic fauna becomes dominated by stygobionts (Gibert et al. 1994, Mori & Brancelj 2011). Most common found invertebrates in the hyporheic zone are Nematoda, Oligochaeta, Gastropoda, Acarina, Crustacea (Copepoda, Ostracoda, Amphipoda, Isopoda) and insect larvae (Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera) originating from benthic habitats (Mori et al. 2011, 2012, Mathers et al. 2017, Prevorčnik et al. 2019).

Biological investigation of interstitial habitats in alluvial sediments has a long tradition in Europe (e.g. Karaman 1935, Angelier 1953, Schwoerbel 1961, Danielopol 1976) and some in North America (e.g. Pennak & Ward 1986). However, there have been fewer studies on fauna in alluvial aquifers in Slovenia. Meštrov (1960) and Meštrov with his colleagues (1983) investigated interstitial communities living in different groundwater habitats of the Sava River in southern Slovenia and Croatia. This author found many species new to this region and a high number of stygobionts. In the Ljubljansko Polje alluvial plain, hydrologically connected with the Sava River, Sket & Velkovrh (1981) found highly diverse groundwater fauna compared with other zoogeographic zones of the region. During the last 20 years, several new species for science were found in Slovenia during the studies of samples from boreholes (depths from 5 to over 100 meters) (Brancelj 2000, Brancelj et al. 2011, 2016). Investigation of the Bača and Sava Rivers hyporheic zones revealed that presence of stygobiotic species depends on the intensity of hyporheic hydrological connectivity with surface water (Mori & Brancelj 2011, Mori et al. 2011, 2012). The most recent study of interstitial habitats found several geographically restricted species and newly discovered species in the Sava River hyporheic zone (Prevorčnik et al. 2019). Additionally, negative impacts of hydropower impoundments and urban land use for stygobionts inhabiting the hyporheic zone were demonstrated by Mori et al. (2020).

Free-living freshwater harpacticoids are an abundant component of the benthic and interstitial fauna (Galassi 2001, Galassi et al. 2009). Globally, around 640 harpacticoid species out of 1,120 freshwater species are known to be stygobionts (Boxshall & Defaye 2008, Galassi et al. 2009). A recent study of data on harpacticoids in Europe's groundwaters resulted in 408 stygobiotic species and subspecies (distributed in 7 families and 42 genera) (Iannella et al. 2020). Many such stygobionts occur in restricted geographical areas (strict endemics), while others, such as the harpacticoid *Elaphoidella elaphoides* (Chappuis, 1924) and the cyclopoid *Graeteriella unisetigera* (Graeter, 1908), seem to be more widely distributed (Galassi et al. 2009). In most cases, surface (i.e., epigean) and stygobiotic harpacticoid species occur together in the hyporheic zone, with the ratio depending on species-specific requirements and local environmental conditions (Rouch & Danielopol 1997). Due to their high abundance and species richness in hyporheic zone, their sensitivity to environmental conditions, and distinctive ratios between surface and stygobiotic species in interstitial assemblages, the harpacticoids are a good model to investigate the biodiversity patterns in the hyporheic zones.

The goal of this study was to investigate the harpacticoid biodiversity in the hyporheic zone of four small streams that are hydrologically interconnected with karst groundwaters from the Dinaric Karst biogeographical region. We explored spatial distribution patterns and analysed environmental parameters that potentially determine the assemblage composition. Moreover, this study compares the hyporheic harpacticoid fauna of streams/rivers of central Slovenia with those from different geographical areas of the country.

Methods

Study area

The streams studied are located in the central part of Slovenia, south and southwest of Ljubljana (Fig. 1). They are 4^{th} order watercourses with relatively small catchment areas, extending between 1,100 and 300 m a. s. l., with moderate discharge, determined by the pluvio-nival regime (Tab. 1). The catchments of three streams – Želimeljščica, Iška, and Borovniščica – extend over the karst/dolomite area of the Krim and Mokrec massifs, south of Ljubljana. The catchment of the Podlipščica stream extends into the Rovte Hills, where metamorphic rocks (schists) prevail. At 310 m elevation, all of the streams enter an impermeable zone, which is the silt bottom of a lake, formed after the last glaciation; the area was a peat bog in Roman times. Between the 17^{th} and 19^{th} centuries, most of the peat was removed and used to heat the city of Ljubljana (Pavšič 2008).

Table 1. The main characteristics of the four streams, Želimeljščica, Iška, Borovniščica and Podlipščica, located in the central part of Slovenia. The data on discharges are provided by the Slovenian Environmental Agency.
Tabela 1. Značilnosti štirih rečic Želimeljščice, Iške, Borovniščice in Podlipščice. Podatki o pretokih so pridobljeni na Agenciji za okolje RS.

Stream	Želimeljščica	Iška	Borovniščica	Podlipščica
Catchment size (km^2)	99	151	92	74
Min. catchment altitude (m a.s.l.)	285	279	282	283
Max. catchment altitude (m a.s.l.)	1050	1100	990	885
Stream length (km)	15.4	31.3	16.7	13.3
Stream mean discharge (m^3 s^{-1})	0.9	1.4	1.4	0.7

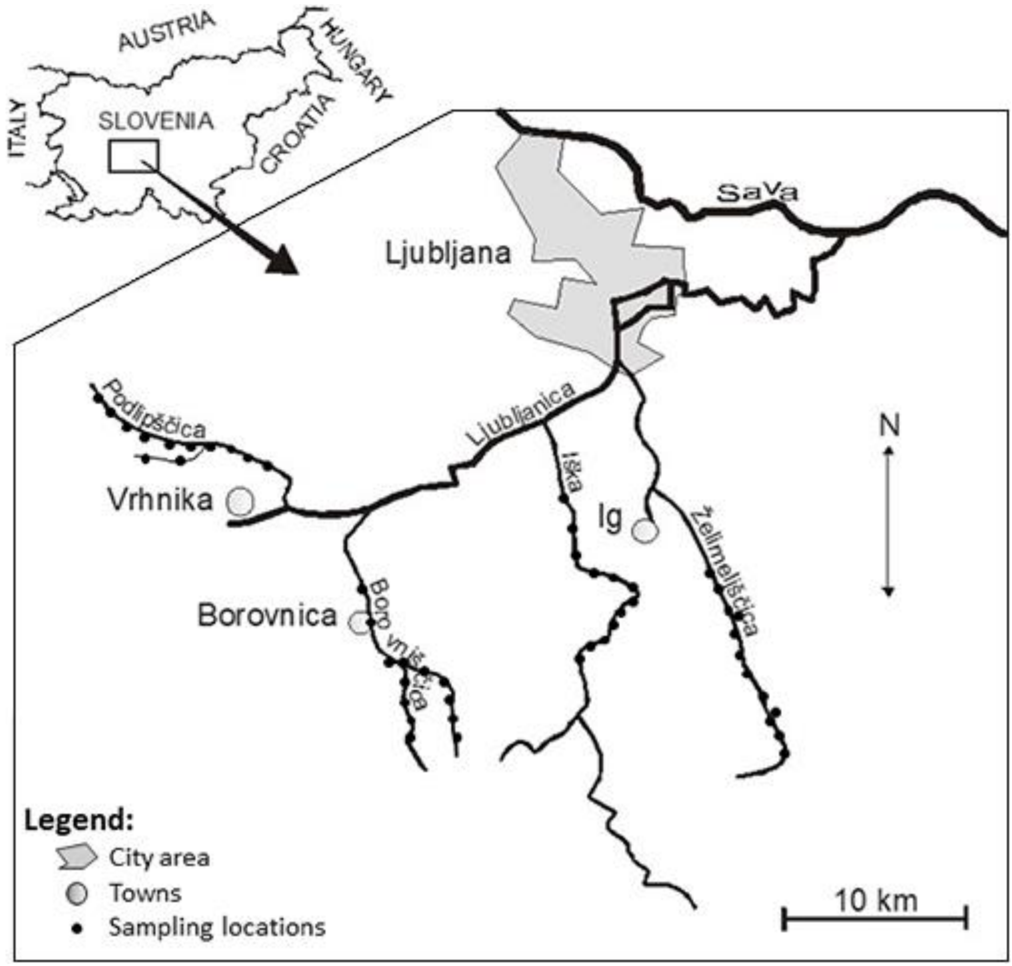

Figure 1. Map of the study area with indicated sampling locations of harpacticoids on the four streams in central Slovenia.
Slika 1. Karta območja raziskave z označenimi lokalitetami vzorčenja harpaktikoidov v štirih rečicah v osrednji Sloveniji.

The Iška and Borovniščica streams have the highest discharge, and their sediment deposits form an important porous aquifer south of Ljubljana and are far more extensive than deposits from the Podlipščica and Želimeljščica (Habič 1996). The Podlipščica valley has the highest anthropic impact due to intensive agriculture (crop production) and stream channel modifications. In the lower stretches, the Iška is subject to continuous exploitation of streambed sediments and water, the later for the drinking water supply of the city of Ljubljana. The Borovniščica is also intensively channelized and influenced by agricultural activities (cattle, crop production) in its catchment area.

Fieldwork

Twelve sampling locations were selected in the four streams' upper and middle reaches (= 48 locations) before they enter the impermeable zone in their downstream sections (Fig. 1). The measurements and sample collection were carried out from May to August 2002. The sampling campaign was part of the European project PASCALIS (Protocols for the ASsessment and Conservation of Aquatic Life in the Subsurface), where the same methodology was implemented in six European regions (Belgium, France – two regions, Italy, Slovenia, Spain). Four replicates (=sites) were selected at each location to encompass the variability in spatial distribution of harpacticoids at a reach scale. Three replicates were obtained from the wetted stream channel, where upwelling of advected channel water or groundwater was expected to occur. This was usually at the downstream end of a riffle, gravel bar, channel step, or meander bed. We measured the vertical hydraulic gradients using T-bar to identify sampling spots with positive hydraulic gradients (i.e., upwelling areas: Malard et al. 2002). Such spots had been established in previous studies as areas with predominance of stygobiotic species (Gibert et al. 1994).

Samples were taken from a depth interval of 30–60 cm below the streambed with a Bou-Rouch pump (Bou & Rouch 1967). A steel pipe (diameter of 5 cm), with a 30 cm long perforated zone (diameter of holes = 1 cm), was hammered into gravel in the hyporheic zone to the selected depth. An additional site was selected on the streambank, at a distance of 1 to 10 m from the wetted channel. Samples were then extracted from depths between 65 and 160 cm, depending on the thickness of the gravel layer above water level in the channel and the water level of the saturated zone.

Samples extracted by a Bou-Rouch pump, 10 L in volume, were composed of a mixture of water, sediment and organisms. Afterwards, samples were filtered through a 100 μm mesh net. Invertebrates and organic matter were then transferred into plastic bottles and preserved with 4% formaldehyde solution. The remaining inorganic sediment (particles > 100 μm) was collected separately in the plastic bottle and carried to the laboratory for further analyses. At each sampling site, the time required to pump 10 L of water was measured as a proxy for measurement of hydraulic conductivity.

After fauna samples collection, an oxygen probe (WTW, Multiline P4, Oxi 320) and a conductivity probe (WTW, Tetracon 325) were inserted into the pipe at two sampling sites - one at the wetted channel and one at the stream bank. In addition, one-litre sample of water from each sampling site was collected for chemical analysis in the laboratory. Samples were stored in a cold box, in the dark, at temperature of ~ 4°C during transportation to the laboratory, where they were stored at 4°C in a refrigerator.

Laboratory work

At the laboratory, we conducted analyses within 48 hours after sampling. Chemical parameters pH, nitrate, total nitrogen and total phosphorus were measured in each water sample following standard methods (APHA 1998). The amount of particulate organic matter was determined by loss on ignition (LOI). After retrieving the fauna, the remaining particulate organic matter was put into a ceramic vessel and oven-dried (24 h, 105°C). The dried organic matter was weighed, combusted at 520°C for 2 h, put in a desiccator for 48 h, and re-weighed. The amount of particulate organic matter (POM) was expressed as mg LOI per 1 litre of water sample. The amount of mobile sediment grains (particle size 0.1–5 mm) extracted by pumping of 10 L of water was measured and expressed as ml l^{-1}. Invertebrates were sorted from the samples under a stereomicroscope at 40-x magnification and preserved in 70% ethanol. Harpacticoids were counted and identified to the species level following the key provided by Janetzky et al. (1996), the updated nomenclature follows Walter & Boxshall 2021.

Data analysis

We compiled the list of harpacticoid species, indicated either as stygobiotic or epigean species, and presented their mean abundances in different stream systems. Prior to the further analyses, we merged the data from the different spatial replicates (4 sites) of the same location. We analysed spatial variability of harpacticoid assemblages using non-metric multidimensional scaling (nMDS) on a zero-adjusted Bray-Curtis dissimilarity matrix. The correlation coefficients of nine environmental variables with nMDS axes were calculated and presented as vectors in the nMDS ordination diagram. Species were overlayed on the nMDS ordination plane using their Spearman's correlation coefficients with nMDS axes. The significant differences between streams were tested using one-way ANOSIM analysis. The analyses were carried out using computer program PAST version 3.06 (Hammer et al. 2001).

Results

Environmental characteristics

The streams included in this study differed in catchment size, length and mean annual discharge, which is reflected in the physical and chemical characteristics of hyporheic water (Tab. 2). The hyporheic zone of the Iška is well oxygenated, water conductivity is the lowest, and POM and mobile sediment amounts are substantially lower than in the other three streams, indicating high hydraulic conductivity in the hyporheic sediments. Similarly, the POM and mobile sediments are extremely low in the Borovniščica, but conductivity is higher and oxygen concentrations lower than in the Iška hyporheic water. In comparison, the oxygen saturation in the Želimeljščica hyporheic zone is close to 50% and in the Podlipščica even lower (44.7%), with POM and mobile sediment reaching three to four times higher values than in the Iška and Borovniščica. This indicates a higher input of organic matter and nutrients in the hyporheic zone, and longer retention time of water (i.e., lower hydraulic conductivity) which leads to elevated trophic conditions.

Table 2. Physical and chemical characteristics of hyporheic water of the four streams, Želimeljščica, Iška, Borovniščica and Podlipščica, located in the central part of Slovenia, measured during PASCALIS project (May to Avgust 2002). Mean values and standard deviation for 96 sites are presented (4 streams x 12 sites x 2 sites).
Tabela 2. Fizikalne in kemijske lastnosti intersticialne vode štirih rečic, Želimeljščice, Iške, Borovniščice in Podlipščice iz osrednje Slovenije, merjeno v času projekta PASCALIS (maj do avgust 2002). Predstavljene so srednje vrednosti in standardne deviacije za 96 mest (4 rečice x 12 lokacij x 2 replikata).

	Želimeljščica	**Iška**	**Borovniščica**	**Podlipščica**
Temperature (°C)	11.3 ± 1.3	15.2 ± 2.6	15.3 ± 2.1	15.4 ± 1.3
Conductivity (µS cm^{-1})	522 ± 108	410 ± 23	486 ± 87	466 ± 87
pH	7.8 ± 0.3	8.2 ± 0.2	8.1 ± 0.3	8.0 ± 0.2
Oxygen (mg L^{-1})	5.6 ± 3.0	8.0 ± 1.5	6.0 ± 2.6	4.5 ± 2.5
Oxygen saturation (%)	52.8 ± 28.6	83.3 ± 15.3	61.8 ± 28.4	44.7 ± 27.4
N_{tot} (mg L^{-1})	2.6 ± 0.6	2.6 ± 0.3	2.8 ± 0.4	2.6 ± 0.7
P_{tot} (mg L^{-1})	48.2 ± 30.7	53.6 ± 13.6	61.9 ± 26.8	29.7 ± 21.9
Nitrate (mg L^{-1})	3.0 ± 2.4	3.5 ± 0.5	3.8 ± 1.6	2.7 ± 1.9
Particulate organic matter (mg DW L^{-1})	109.9 ± 112.5	27.1 ± 35.4	57.4 ± 61.1	108.6 ± 131.4
Mobile sediments (ml L^{-1})	22.3 ± 18.8	8.7 ± 11.4	6.1 ± 7.6	21.8 ± 23.0

Harpacticoid assemblages

In total, 24 harpacticoid species were collected in the hyporheic zone of the four streams (Tab. 3). Half of them, 12 species, were stygobionts. The most abundant species found in the samples were the epigean species *Bryocamptus dacicus* (Chappuis, 1923), *Pilocamptus pilosus* (Douwe, 1910) and *Bryocamptus zschokkei* (Schmeil, 1893). The rarest species, a mixture of epigean and stygobitic representatives, showed on average fewer than 10 collected specimens. In the individual samples, these species were *Attheyella wierzejskii* (Mrazék, 1893), *Elaphoidella gracilis* (Sars, G.O., 1863), *Moraria poppei* (Mrazék, 1893), *Moraria varica* (Graeter, 1911) (epigean species), and Ameridae gen. sp., *Elaphoidella jeanneli* (Chappuis, 1928), *Nitocrella* sp. and *Italicocaris* cf. *italica* (Chappuis, 1953) (stygobionts). Widespread species, defined as species occurring in the hyporheic zone of all four streams, were *Attheyella crassa* (Sars, G.O., 1853), *B. dacicus*, *B. pygmeus* (Sars, G.O., 1853), *B. typhlops* (Mrazék, 1893), *B. zschokkei*, *P. pilosus*, *Pesceus schmeili* (Mrazék, 1893), *Horstkurtcaris nolli alpina* (Kiefer, 1960) and *Parastenocaris gertrudae* Kiefer, 1968 (Tab. 3). All of these, except *B. typhlops* and representatives of family Parastenocarididae, belong to epigean/ubiquitous species.

The total number of species found in the hyporheic zone of the Želimeljščica and Iška was 16, while 15 species were found at the Borovniščica. Half of the collected species (8 species) were stygobionts. In the Podlipščica, 12 harpacticoid species were collected, but only four of them were stygobionts. Here, despite the lowest species richness, the abundances were among the highest (averaging 68 specimens in 10 L sample). Conversely, in the oligotrophic hyporheic zone of the Iška, the abundances were much lower compared to those from the other three streams (averaging 20 specimens in 10 L sample).

Table 3. List of harpacticoid species (Crustacea: Copepoda: Harpacticoida) and their mean abundances and overall species richness (number of individuals and species 10 L^{-1}) (±SD) in the hyporheic zone of the four streams in central Slovenia as found during PASCALIS project in summer 2002 (N=48, 12 locations x 4 replicates). * – stygobiont.

Tabela 3. Seznam vrst harpaktikoidov (Crustacea: Copepoda: Harpacticoida) in srednje vrednosti številčnosti in vrstne pestrosti v hiporeiku štirih rečic najdenih tekom projekta PASCALIS v poletni sezoni leta 2002 (število osebkov/vrst 10 L^{-1}) (N=48). * – stigobiont.

	Želimeljščica	**Iška**	**Borovniščica**	**Podlipščica**
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Ameridae gen. sp. *	3.0 ± 0.0			
Attheyella crassa (G.O. Sars, 1863)	6.5 ± 6.4	2.0 ± 0.0	1.0 ± 0.0	2.0 ± 1.4
Attheyella wierzejskii (Mrazek, 1893)	2.0 ± 0.0			
Bryocamptus minutus (Claus, 1863)				15.0 ± 0.0
Bryocamptus dacicus (Chappuis, 1923)	55.6 ± 85.1	15.8 ± 22.9	24.7 ± 44.3	59.3 ± 109.6
Bryocamptus pygmaeus (Sars, 1863)	1.5 ± 0.6	1.7 ± 0.6	1.0 ± 0.0	2.0 ± 0.0
Bryocamptus typhlops (Mrazek, 1893) *	3.5 ± 0.7	2.0 ± 0.0	1.0 ± 0.0	15.5 ± 20.5
Bryocamptus zschokkei (Schmeil. 1893)	16.4 ± 19.3	7.5 ± 7.8	4.1 ± 3.8	63.3 ± 78.4
Bryocamptus cf. *pyrenaicus* *	3.1 ± 1.5	5.0 ± 0.0		
Pilocamptus pilosus (Van Douwe, 1910)	45.0 ± 71.0	7.2 ± 6.0	28.9 ± 41.6	29.1 ± 34.7
Elaphoidella charon Chappuis, 1936 *		24.3 ± 42.5	5.1 ± 4.5	
Elaphoidella elaphoides (Chappuis, 1924) *	28.8 ± 38.1		14.1 ± 11.5	19.0 ± 18.7
Elaphoidella gracilis (Sars, 1863)			1.0 ±	3.0 ± 1.4
Elaphoidella jeanneli Chappuis 1928 *			1.5 ± 0.7	
Elaphoidella millennii Brancelj, 2009 *		5.2 ± 4.6	8.5 ± 9.2	
Epactophanes richardi Mrazek, 1893				8.5 ± 10.6
Moraria poppei (Mrazek, 1893)	1.0 ± 0.0	1.5 ± 0.7	2.0 ± 1.4	
Moraria varica (Graeter, 1910)		1.0 ± 0.0		
Nitocrella hirta Chappuis, 1923 *	6.8 ± 9.2	5.3 ± 3.8		
Nitocrella sp. *	1.0 ± 0.0			
Pesceus schmeili (Mrazek, 1893)	8.7 ± 15.6	13.5 ± 26.0	6.1 ± 6.1	
Italicocaris cf. *italica* *		1.0 ± 0.0		
Parastenocaris gertrudae Kiefer, 1968 *	2.6 ± 3.0	1.9 ± 1.1	7.0 ± 11.3	5.5 ± 6.4
Horstkurtcaris nolli alpina Kiefer, 1960 *	16.1 ± 14.5	2.7 ± 2.7	3.4 ± 2.8	8.0 ± 0.0
Overall mean abundance (specimen 10 L^{-1})	**72.6 ± 128.1**	**20.0 ± 33.7**	**46.4 ± 71.3**	**68.1 ± 124.6**
Overall mean species richness	**3.9 ± 2.4**	**2.9 ± 1.6**	**3.4 ± 1.8**	**2.1 ± 1.2**
Total number of species / stygobionts	**16 / 8**	**16 / 8**	**15 / 7**	**12 / 4**

The ANOSIM analysis indicated significant differences between the Iška and other three streams ($p<0.05$), most probably due to substantially lower harpacticoid abundances and low species richness. The nMDS ordination of sampling sites indicates a gradient in POM along first axis and a gradient in conductivity, POM, oxygen and pH along the second axis (Fig. 2). The first axis separates the Iška from the Podlipščica sites. Ordination of harpacticoid species obtained by overlaying the species on the space defined by the two nMDS axes on the basis of their correlation with the axes calculated using the Spearman's coefficient, revealed that two species showed preferences for the Želimeljščica and Podlipščica (*P. pilosus*, *B. dacicus*) and two (*Elaphoidella millenni* Brancelj, 2009, *Elaphoidella charon* Chappuis, 1936) for the Iška (Fig. 3).

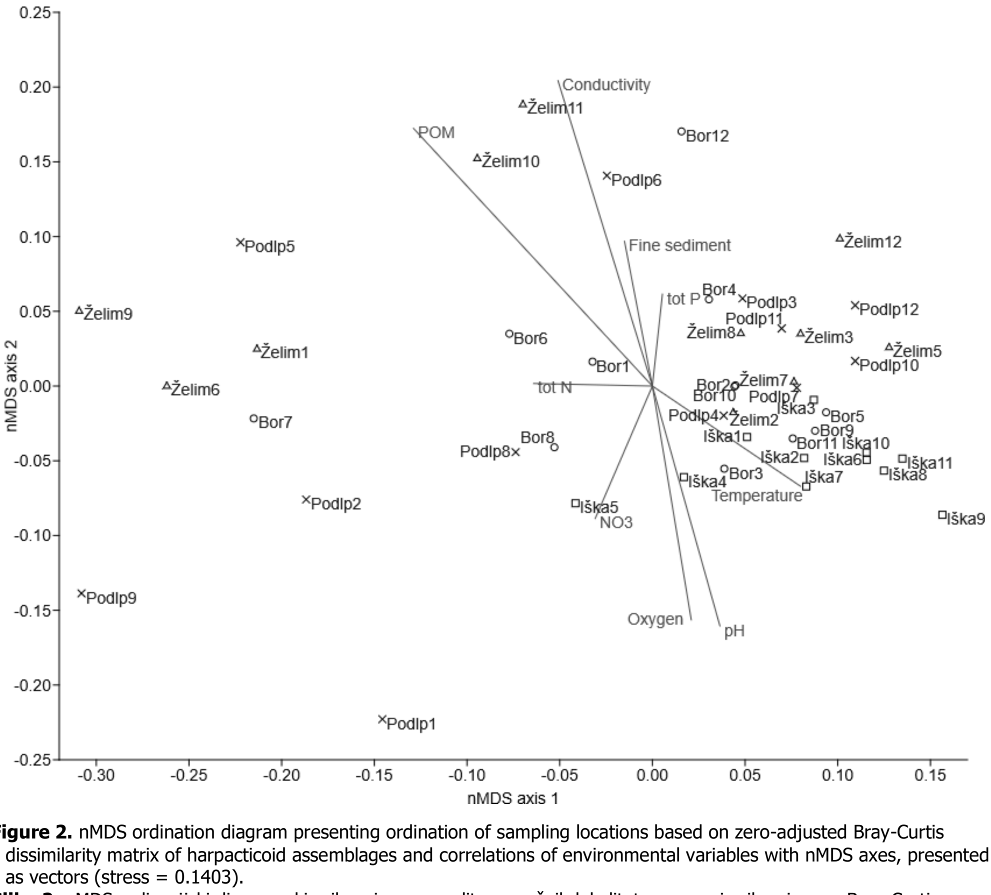

Figure 2. nMDS ordination diagram presenting ordination of sampling locations based on zero-adjusted Bray-Curtis dissimilarity matrix of harpacticoid assemblages and correlations of environmental variables with nMDS axes, presented as vectors (stress = 0.1403).

Slika 2. nMDS ordinacijski diagram, ki prikazuje razporeditev vzorčnih lokalitet na osnovi prilagojenega Bray-Curtisovega indeksa različnosti združb harpaktikoidov in korelacije okoljskih spremenljivk z nMDS osmi, prikazanih kot vektorji (stress = 0.1403).

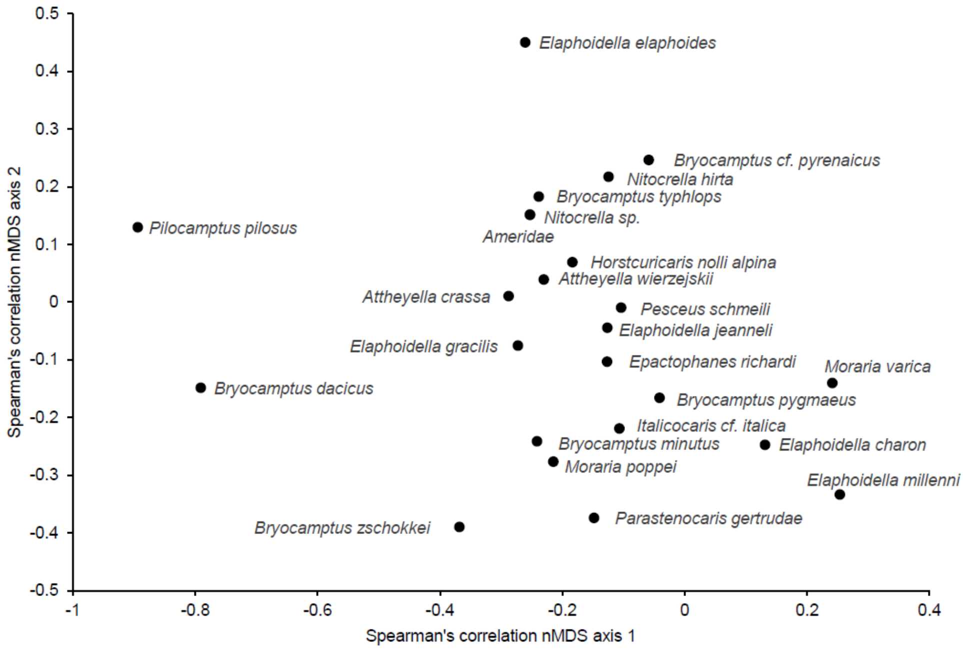

Figure 3. Ordination of harpacticoid species obtained by overlaying the species on the plane defined by the two nMDS axes on the basis of their correlation with the axes calculated using the Spearman's coefficient.
Slika 3. Razporeditev vrst harpaktikoidov v prostoru, definiranem z dvema nMDS osema na osnovi izračuna Spearmanovega koeficienta korelacije med abundancami vrst in vrednostmi osi.

Discussion

In the hyporheic zone of the four streams studied, a total of 24 harpacticoid species were found during a single sampling period. Six species of them belong to the genus *Bryocamptus*, five to *Elaphoidella*, and three to the family Parastenocarididae. Twelve species are stygobionts, and two are new species for science. In this study, the species *Nitocrella hirta* Chappuis, 1924, was found in Slovenia for the second time. Before this study, it had been reported only once, at the phreatic zone of the Sava River (Sket & Velkovrh 1981). A 2-year study of the hyporheic zone of the Sava River near Ljubljana in 2012, reported 12 harpacticoid species. Out of them, five of the species were stygobionts, with *Bryocamptus* as the most frequent genus; one representative of *Elaphoidella* and Parastenocarididae each, were also found (Mori et al. 2012). In both study areas, the commonest taxa found were six epigean species (i.e., *Bryocamptus dacicus*, *B. zschokkei*, *P. pilosus*, *P. schmeili*, *Attheyella crassa*, *Epactophanes richardi* Mrazék, 1893), and one stygobiont (*Elaphoidella elaphoides*). Two thirds of harpacticoid species present in the Sava River hyporheic zone were found also in this study.

In 1996, a total number of 44 harpacticoid species were known in Slovenia (Brancelj 1996). In 2001, 49 species and subspecies of harpacticoids were reported for Slovenia, 23 of them being stygobionts (Pipan & Brancelj 2001). From 2006 to present, additional four new species, previously unknown to the scientific world, have been found in Slovenian caves and interstitial habitats, as described by Brancelj (2006, 2009, 2011). Currently, there is a total of 53 harpacticoid species known to exist in Slovenia; among them, nearly half were found in the hyporheic zone of the four streams analysed in this study. Species composition of the studied stream assemblages was quite similar to that found in the Dinaric Karst region in southern Slovenia, with one third of species present in the Dinaric Karst region also present at the hyporheic zone of the studied streams. Additionally, almost half of the species from the studied hyporheic zone are also known from caves in the Dinaric Karst region (Brancelj 1986, Pipan & Brancelj 2003). Species found in common within different regions were primarily the epigean species that typically have wide distribution patterns, such as *B. dacicus*, while the common stygobionts present were rarer and found less frequently. Among the latter, *B. typhlops*, *Bryocamptus pyrenaicus* (Chappuis, 1923), and *H. nolli alpina* were found in both Dinaric Karst caves and hyporheic zone of the studied streams. A possible reason is the hydrological connectivity of the Dinaric Karst with the study area. This is possibly due to the complexity of the Ljubljanica River surface and subsurface flow. This flow starts in the south of Slovenia and flows over the Dinaric Karst region, passes few karst caves, and flows as a surface river into the Sava River near Ljubljana (Bonacci 2015). This hydrological connectivity, combined with a broad ecological tolerance of these species and their high ability to disperse, could explain the observed distributional patterns (Galassi et al. 2009). Another possible explanation, especially for stygobionts could be that current species encompasses complex of cryptic species (Lefébure et al. 2007).

The most frequently occurring and also the most abundant species found were the stygophiles *B. dacicus*, *B. zschokkei*, and *P. pilosus. Bryocamptus dacicus,* which have the Balkan distribution, is widespread in Slovenia. Its preferred habitat is groundwater (caves and interstitial groundwater), but it was found also in springs and in the benthos of alpine lakes (Jersabek et al. 2001). *Bryocamptus zschokkei* and *P. pilosus* are widespread and found in a variety of habitats (Gaviria 1998, Rundle et al. 2002), while the *Elaphoidella charon – jeanelli – millennii* complex is endemic to the Slovenian part of the Dinaric Karst region (Mori & Brancelj 2008, Brancelj 2009). *Attheyella wierzeyskii and Bryocamptus minutus* are widely distributed epigean species in Europe (Janetzky et al. 1996, Gaviria 1998). In Slovenia, *M. varica* has been, until now only found in the percolating water of karst caves, as it is a rare species that is limited in its distribution (Brancelj 1986, Pipan & Brancelj 2003). Nevertheless, it is well represented in groundwater of central Europe, as reported from seven localities of south-eastern Germany (Gaviria & Defaye 2017) and springs in Austria (Löffler & Neuhuber 1970, Gaviria 1998).

Species richness of harpacticoids was similar in the hyporheic zones of the Želimeljščica, Iška and Borovniščica, where oxygen concentrations were above 5 mg l^{-1}, but lower in the Podlipščica, where average oxygen concentrations were 4.5 mg l^{-1}. In opposite, abundances were low at the Iška and Borovniščica and high in the Podlipščica and Želimeljščica hyporheic zones. In the latter two streams, the sediments were finer and POM content higher. The causal factors for the composition and density of interstitial fauna are not always clear, in part because of the interplay between sediment pore size and interstitial water velocity. Another factor is the distribution of key environmental variables, such as temperature, oxygen and particulate organic matter (POM) (Strayer et al. 1997). Rouch (1988) demonstrated a functional relationship between permeability, concentration of oxygen, and the distribution of interstitial fauna. Harpacticoids were abundant and dominated over surface species in areas of high permeability and high oxygen saturation (>75%). In contrast, in areas of lower permeability and lower oxygen saturation, harpacticoids were low in abundance and stygobionts were the dominating species. Strayer et al. (1997) found that interstitial harpacticoids were found most frequently with the combination of high levels of dissolved oxygen and coarse-grained sediments, which have high hydraulic conductivity. Thus, according to both latter authors, interstitial harpacticoids are most abundant at sites with high levels of both oxygen and POM. In this study, our results indicated that with decreased permeability, the increased mobile sediment amounts and accumulation of POM in the interstitial spaces resulted in higher harpacticoid abundances, while higher oxygen concentrations lead to higher species richness.

The nMDS analysis indicated small differences in species composition among sites and streams due to strong dominance of the few abundant species *B. dacicus* and *P. pilosus*. Only the sites from the Iška significantly differed from others, primarily due to the low harpacticoid abundances. Qualitatively, the harpacticoid assemblages of the Iška and Borovniška were the most similar. The alluvial fans of both streams are extensive and interconnected (Mencej 1981). Their hyporheic zones have similar characteristics in regard to geomorphology, hydrology, and water chemistry. However, it seems that anthropic pressures (gravel extraction, channelization, water abstraction for drinking water and agriculture) and coarse-grained sediments with low POM amounts are not favourable for the abundance of harpacticoids. This conclusion is in accordance with Strayer et al. (1997), who stressed that the combined parameters that drive the hyporheic communities are hydrology, geomorphology, disturbance history of specific sites and biological interactions combined with temperature, seasonal changes and interannual variation.

Povzetek

Biološke in ekološke raziskave podzemnih voda rečnih (aluvialnih) vodonosnikov so v Sloveniji, v primerjavi z Evropo in Severno Ameriko, precej manj intenzivne, saj je večji del bioloških raziskav v Sloveniji že od začetka usmerjen predvsem na kraške podzemne vode. Cilj te raziskave je bil raziskati pojavljanje harpaktikoidov (Crustacea: Copepoda: Harpacticoida = raki: ceponožci: harpaktikoidi) v hiporeku štirih manjših rečic, ki izvirajo na območju Dinarske biogeografske regije in se stekajo v Ljubljanico. Harpaktikoidi so, poleg ciklopidov, med najbolj številčnimi in vrstno pestrimi predstavniki vodnih nevretenčarjev, ki naseljujejo hiporeik in globje plasti podtalnice. Raziskali smo tudi možne okoljske parametre, ki vplivajo na sestavo združb harpaktikoidov. Harpaktikoide smo vzorčili na 12 mestih vzdolž toka štirih rečic (Želimeljščica, Iška, Borovniščica, Podlipščica), in sicer v sezoni najnižjih letnih pretokov (maj-avgust) v letu 2002. Vzorčili smo v omočeni strugi, 30–60 cm globoko pod rečnim dnom in na bregu rek, v globinah od 65 do 160 cm. Na vsakem vzorčevalnem mestu smo, s pomočjo Bou-Roucheve črpalke, odvzeli vzorce vode, ki smo jih prefiltrirali prek 0.1 mm mrežice in tako prestregli harpaktikoide v načrpani vodi. Merili smo tudi fizikalno-kemijske lastnosti vode v hiporeiku in prisotnost anorganskih hranil, organskega drobirja v načrpani vodi ter ocenili stopnjo zamuljevanja medzrnskih prostorčkov. Določili smo 24 vrst harpaktikoidov, od tega 12 vrst, ki živijo izključno v podzemni vodi (tj., stigobionti). Najdeni sta bili dve novi vrsti za znanost. Združbo večinoma sestavljajo splošno razširjene vrste, pogoste v Sloveniji in tudi v Evropi. Razlike med štirimi rečicami so bile predvsem v številčnosti osebkov in v manjši meri v bogastvu vrst in njihovi sestavi.

Acknowledgements

The research was supported by the Slovenian Research Agency (P1-0255 & Young Researcher Programme). The study was partly funded by the EU project PASCALIS (EVK2-CT-2001-00121, 2002-2004). The authors would like to thank Gregor Bračko, Milijan Šiško and Andreja Jerebic for their assistance in the field and technical support in the laboratory.

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NATURA SLOVENIAE 23(1): 21-33
SCIENTIFIC PAPER

Prejeto / Received: 4. 5. 2021
Sprejeto / Accepted: 9. 8. 2021

Local host ant usage of scarce large blue *Phengaris teleius* and dusky large blue *P. nausithous* (Lepidoptera: Lycaenidae) at Goričko Nature Park (NE Slovenia)

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Abstract. In our contribution we report on the local host ants of two threatened and protected butterfly species, the scarce large blue (*Phengaris teleius*) and the dusky large blue (*P. nausithous*) at Goričko (NE Slovenia), which hosts one of the largest and the most important metapopulation for both species of large blues in Slovenia. Larvae of both species are developing in *Myrmica* ant nests and different ant species may be used as larval hosts in different parts of the species range. During our study, seven species of potential host ants of the genus *Myrmica* were found at 12 selected sampling sites. A total of 142 ant nests were examined for the presence of *Phengaris* larvae. Larvae of *P. teleius* were found in 25 nests of three ant species: *M. scabrinodis*, *M. rubra* and *M. gallienii*, while larvae of *P. nausithous* were found in 9 nests, all of *M. rubra*. The results of the present study provide the first insight into the local host ants of the two *Phengaris* species in Slovenia and could serve as an important source for active conservation of both species in Slovenia.

Key words: *Phengaris,* Slovenia, host ants, Goričko, *Myrmica*

Izvleček. Gostiteljske vrste mravelj strašničinega mravljiščarja *Phengaris teleius* in temnega mravljiščarja *P. nausithous* (Lepidoptera: Lycaenidae) v Krajinskem parku Goričko (SV Slovenija) – V prispevku predstavljamo rezultate prve raziskave gostiteljskih vrst mravelj dveh ogroženih in zavarovanih vrst dnevnih metuljev, strašničinega (*Phengaris teleius*) in temnega mravljiščarja (*P. nausithous*) v Sloveniji. Mravljišča in potencialne gostiteljske vrste mravelj smo vzorčili na Goričkem (SV Slovenija), kjer je središče razširjenosti obeh vrst v Sloveniji. Na dvanajstih lokacijah smo našli sedem vrst mravelj iz rodu *Myrmica*, ki so potencialne gostiteljske vrste gosenic mravljiščarjev. Prisotnost gosenic mravljiščarjev smo preverili v 142 mravljiščih. V 25 mravljiščih vrst *Myrmica scabrinodis*, *M. rubra* in *M. gallienii* smo našli gosenice strašničinega mravljiščarja, v devetih mravljiščih, vsa vrste *M. rubra*, pa gosenice temnega mravljiščarja. Rezultati raziskave dajejo prvi vpogled v gostiteljske vrste mravelj strašničinega in temnega mravljiščarja v Sloveniji in bodo lahko rabili kot pomembno izhodišče pri aktivnem ohranjanju teh dveh ogroženih vrst metuljev.

Ključne besede: mravljiščarji, *Phengaris*, Slovenija, gostiteljske mravlje, Goričko, *Myrmica*

Introduction

Butterflies of the genus *Phengaris* Doherty, 1891 (syn. *Maculinea* van Ecke, 1915) are among the most studied insects in Europe, mainly due to their highly specific myrmecophilous life cycle, vulnerability and endangerment (e.g. Settele et al. 2005). All four European *Phengaris* species, i.e. *P. arion* (Linnaeus, 1758), *P. alcon* (Denis & Schiffermüller, 1775), *P. teleius* (Bergsträsser, 1779) and *P. nausithous* (Bergsträsser, 1779), live in Slovenia (Verovnik et al. 2012). The scarce large blue (*P. teleius*) and the dusky large blue (*P. nausithous*) occur sympatricaly in northeastern Slovenia, where they often live syntopically. The range of the *P. nausithous* in Slovenia is limited to the northeastern part of the country, while the range of the *P. teleius* also extends to the western parts. Both species live on extensively used humid meadows that are among the most threatened habitats, mainly due to agricultural intensification, fragmentation and abandonment of traditional management (e.g. van Swaay et al. 2012). As such, they have become flagship species for nature conservation and both are protected by the Habitats' Directive (Annex II, IV) as a key instrument for biodiversity conservation in Europe (OJ EC 1992).

Although both species use the same host plant, the great burnet (*Sanguisorba officinalis* L.), and share some general characteristics of their habitat, there are differences in their ecology, including lifecycle, microhabitat preferences and use of host ants (e.g. Nowicki et al. 2005). The females lay their eggs only on the *S. officinalis* and the early instar of larvae feed on host plant's flowers and seeds. When the larvae reach the fourth instar, they leave the host plant. On the ground, they have to be adopted by ants of the genus *Myrmica* Latreille, 1804, which take the larvae into the ant nest. There they predate on ant brood and/or mimic ant larvae and are fed by ant workers (Elmes et al. 1992). They overwinter (one or two winters) in ant nests and pupate in late spring (Thomas et al. 1998).

Pioneering work on the adoption by *Myrmica* ants had suggested that each *Phengaris* species parasitizes a specific *Myrmica* species (Thomas et al. 1989). Later, it was shown that larvae can be adopted by different *Myrmica* species (the so-called primary and secondary host: Elmes et al. 1998) and host species and colony size have an important impact on larval survival (Witek et al. 2010). However, several recent studies raise doubts on a high host ant specialization of *Phengaris* species across Europe (e. g. Pech et al. 2007, Tartally et al. 2019). The most recent and comprehensive survey across Europe (Tartally et al. 2019) showed that each *Phengaris* species is predominantly specialised to a single, basically the most abundant *Myrmica* species found on *Phengaris* site. However, there is a geographic mosaic as a difference; populations of the same *Phengaris* species, sometimes in close proximity, often use different host ant species.

Most data on the host specificity of *Phengaris* were obtained in Central and Eastern Europe, especially in Poland, Hungary and Romania (e.g. Stankiewicz et al. 2005, Tartally & Varga 2008, Witek et al. 2008, Pech & Sedlachek 2016). The term host specificity usually refers to the ability of *Phengaris* butterflies to develop within the nests of particular host ant species. To quantify specificity, ideally the number of *Phengaris* larvae adopted by each *Myrmica* ant species, should be known, as well as the number that survive in ant nests and develop into adults (Thomas et al. 2005). Although the level of specialisation and the character of the *Phengaris*–*Myrmica* host system is now better understood, local data on hosts are still needed, and are useful for site management and species conservation at the local scale. Knowledge of host ant specificity is essential for the conservation of these two butterfly species.

Although the host ant specificity of *Phengaris* species (especially for *P. teleius* and *P. nausithous*) is relatively well known in Europe, there are no published data on this topic for Slovenia. Only a basic survey of *Myrmica* ants in the habitat of *P. teleius* and *P. nausithous* was conducted in the central area of Slovenske Gorice in 2003 and 2004 (Zakšek 2004). The study revealed that *M. scabrinodis* and *M. rubra* are the commonest species in the vicinity of *S. officinalis*, although none of the ant nests were opened and examined for the presence of *Phengaris* larvae. To fill this gap, we studied host ant specificity of *P. teleius* and *P. nausithous* in the Goričko region and the pattern of their local variability. Our results are of high importance for establishing site-specific management of the habitat of the two threatened butterfly species in the region.

Materials and methods

Study area and study sites

Goričko is a predominantly rural hilly countryside in NE Slovenia and most of it is included at Goričko Nature Park and the Goričko Natura 2000 site (SI3000221) designated to maintain a favourable conservation status of *P. teleius* and *P. nausithous* and other selected species and habitat types (Ur. l. RS 2004). Humid meadows in the valleys are home to the largest populations of *P. teleius* and *P. nausithous* in Slovenia (Zakšek et al. 2005). Twelve sampling sites were selected in the eastern part of Goričko NP (Fig. 1), where the population density of both species is the highest (Zakšek et al. 2012). All sampling sites hosted *P. teleius*, while *P. nausithous* was present at ten of the selected sites (see Tab. 1 for details).

Table 1. Detailed information on study sites in the Goričko region, NE Slovenia, together with occurrence data of *Phengaris teleius* and/or *P. nausithous* prior to the sampling in 2011.
Tabela 1. Podrobnejše informacije vzorčnih lokacijah na Goričkem skupaj s podatki o prisotnosti strašničinega (*Phengaris teleius*) in/ali temnega mravljiščarja (*P. nausithous*), zabeleženih pred vzorčenjem v letu 2011.

Study site code	**Locality**	**Coordinates (WGS84)**		**Presence of *Phengaris* species**
		Lat (°N)	**Long (°E)**	
NER1	Ženavlje, meadow at Koritiški potok, 600 m NW from hamlet Vreja	46.844218	16.180123	*P. teleius, P. nausithous*
NER2	Neradnovci, meadow 460 m NE from village Ženavlje, on the left bank of Koritiški potok	46.838818	16.185550	*P. teleius, P. nausithous*
ČEP	Čepinci, meadow 440 m SW from hamlet Smodin Breg	46.840992	16.215897	*P. teleius, P. nausithous*
STAN	Gornji Petrovci, meadow 320 m SE from hamlet Džešarni	46.804415	16.205508	*P. teleius, P. nausithous*
KUŠ	Kuštanovci, meadow at Kmetov potok 200 m NE from homestead Dirdin	46.779013	16.190457	*P. teleius, P. nausithous*
DOL	Dolenci, meadow 550 NE from hamlet Šoštarne Grabe	46.852637	16.271801	*P. teleius, P. nausithous*
ŠAL	Šalovci, meadow at Krplivniški potok SE from hamlet Vrvji Breg	46.804811	16.280853	*P. teleius*
IVAN1	Ivanjševci, meadows between hamlets Kotov Kraj in Balaškin Kraj	46.766287	16.292941	*P. teleius, P. nausithous*
IVAN2	Ivanjševci, meadow 400 m NE from hamlet Kotov Kraj	46.763510	16.296618	*P. teleius, P. nausithous*
MOT1	Motvarjevci, meadow NE from house Motvarjevci 73	46.706049	16.351857	*P. teleius, P. nausithous*
MOT2	Motvarjevci, left bank of the stream Kobilje affluent at Motvarjevci	46.704889	16.353332	*P. teleius, P. nausithous*
KOB	Kobilje, meadow 640 m SW from gravel pit at the western part of Kobilje village	46.689622	16.375921	*P. teleius*

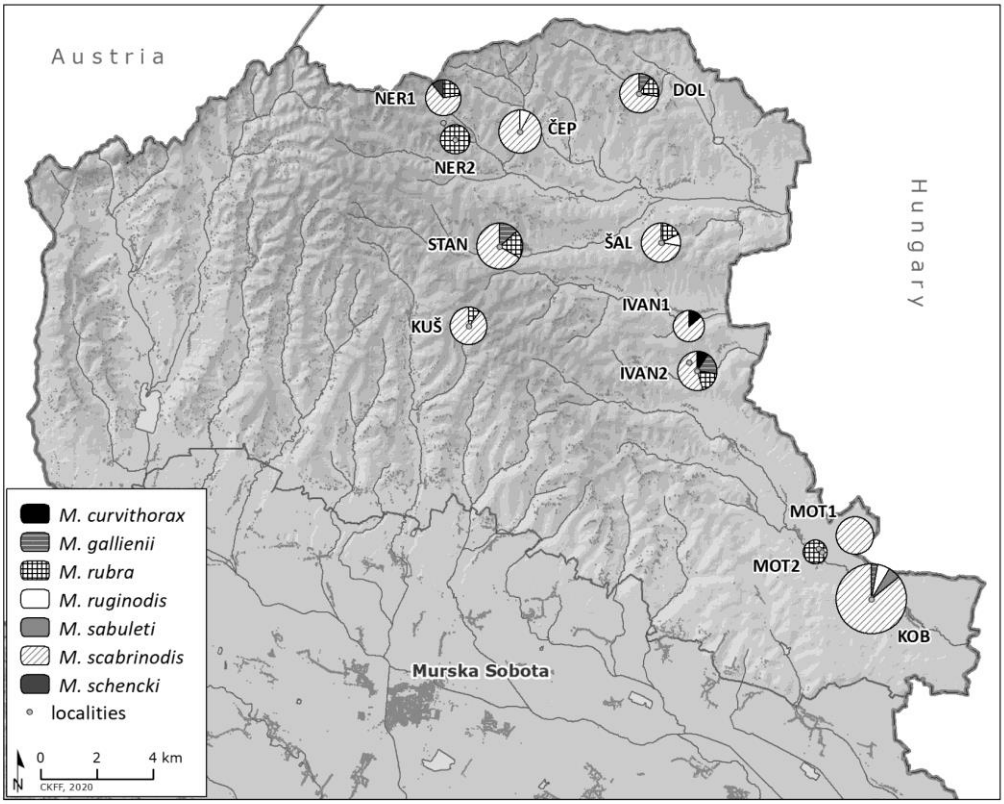

Figure 1. Map of Goričko Nature Park (dark line represents its southern border) showing the position of the sampling sites of our study in 2011 and 2012. The proportion of *Myrmica* species ant nests at each sampling site is shown with pie charts. The size of the circle corresponds to the number of examined *Myrmica* ant nests (see Tab. 3 for details). The small circles »localities« represents accurate location of sampling site, as the position of pie charts is moved to avoid their overlap.

Slika 1. Zemljevid Krajinskega parka Goričko (temna linija ponazarja njegovo južno mejo) z lokacijami vzorčnih mest v tej raziskavi v letih 2011 in 2012. Delež mravljišč mravelj iz rodu *Myrmica* na vsakem vzorčnem mestu je prikazan s tortnim grafikonom. Velikost kroga je sorazmerna številu pregledanih mravljišč mravelj iz rodu *Myrmica.* Majhne točke lokalitet prikazujejo natančne lokacije vzorčnih lokacij (v primerih prekrivanja so prikazi deležev mravljišč nekoliko premaknjeni).

Field work

Field sampling was carried out in 2011 and 2012, from mid-May to mid-June when *Phengaris* larvae were at the pre-pupal stage. At selected sites, we searched for *Myrmica* ant nests within one-metre radius of *S. officinalis*, which is likely within a foraging distance of *Myrmica* ants (Elmes et al. 1998). All *Myrmica* ant nests found were marked and carefully excavated. The nest material was placed on a white sheet, where the nests were opened to check for the presence of *Phengaris* larvae. After excavation, the nest material and vegetation were restored to their original condition as much as possible. Five to ten ant workers were collected from each nest and preserved in 70% ethanol for later identification. In addition, a random sample of foraging

Myrmica ant species was collected on sampling sites when searching for ant nests using the hand collection method. *Myrmica* species were identified according to Seifert (2018) and Radchenko & Elmes (2010). *Phengaris* larvae were identified at the site using a 20× magnifier lens and determination key by Śliwińska et al. (2006) and afterward returned to the nests.

Results

Ant species on the study sites

A total of 142 *Myrmica* nests were surveyed revealing presence of seven species at twelve sites hosting *P. teleius* and/or *P. nausithous* in the eastern Goričko NP: *M. curvithorax* Bondroit, 1920, *M. gallienii* Bondroit, 1920, *M. rubra* (Linnaeus, 1758), *M. ruginodis* Nylander, 1846, *M. sabuleti*, Meinert, 1861, *M. scabrinodis* Nylander, 1846 and *M. schencki* Viereck, 1903 (Tab. 2). The total number of *Myrmica* nests found on each locality varied from 4 at MOT 2 to 35 at KOB (Tab. 3). *Myrmica scabrinodis* was the commonest species of the genus found on ten sites with 105 recorded nests. It was followed by *M. rubra*, found on eight sites (22 nests), and *M. gallienii*, found on four sites (6 nests) (Tab 3). On all sites where it occurred, *M. scabrinodis* had the highest proportion of the total nests found (Fig. 1).

Table 2. All *Myrmica* ant species recorded on selected sampling sites at Goričko Nature Park during this study in years 2011 and 2012. For codes of sampling sites see Tab. 1. In species marked with asterisk only individual ant workers were recorded on the study site (no nests were found and excavated).

Tabela 2. Vse vrste mravelj iz rodu *Myrmica* najdene na izbranih vzorčnih mestih v Krajinskem parku Goričko tekom te raziskave v letih 2011 in 2012. Oznake vzorčnih mest so enake kot v Tab. 1. Pri vrstah označenih z zvezdico so bile med vzorčenjem najdene samo posamezne delavke na vzorčnem mestu.

Sampling site	*Myrmica* species found
NER1	*M. rubra*, *M. scabrinodis*, *M. schencki*
NER2	*M. rubra*
ČEP	*M. ruginodis*, *M. scabrinodis*
STAN	*M. gallienii*, *M. rubra*, *M. scabrinodis*
KUŠ	*M. rubra*, *M. scabrinodis*
DOL	*M. gallienii*, *M. rubra*, *M. scabrinodis*
ŠAL	*M. rubra*, *M. ruginodis*, *M. scabrinodis*
IVAN1	*M. curvithorax*, *M. scabrinodis*, *M. schencki**
IVAN2	*M. curvithorax*, *M. gallienii*, *M. rubra*, *M. sabuleti**, *M. scabrinodis*, *M. schencki**
MOT1	*M. sabuleti**, *M. scabrinodis*
MOT2	*M. rubra*
KOB	*M. gallienii*, *M. rubra**, *M. ruginodis*, *M. sabuleti*, *M. scabrinodis*

Table 3. Number of *Myrmica* ant nests and number of *Phengaris teleius* and *P. nausithous* larvae found in nests at each sampling site in Goričko Nature Park during our study in 2011 and 2012. For codes of sampling sites see Tab. 1.
Tabela 3. Število mravljišč rodu *Myrmica* in število najdenih gosenic strašničinega (*Phengaris teleius*) in/ali temnega mravljiščarja (*P. nausithous*) na posameznih vzorčnih mestih v Krajinskem parku Goričko tekom te raziskave v letih 2011 in 2012. Oznake vzorčnih mest so enake kot v Tab. 1.

Sampling site	*Myrmica* species	No. of nests	No. of nests with *P. teleius*	No. of *P. teleius* larvae	No. of nests with *P. nausithous*	No. of *P. nausithous* larvae
NER 1	*M. scabrinodis*	6	1	1	0	0
	M. rubra	2	0	0	0	0
	M. schencki	1	0	0	0	0
NER 2	*M. rubra*	6	3	6	5	57
ČEP	*M. scabrinodis*	12	2	2	0	0
	M. ruginodis	1	0	0	0	0
STAN	*M. scabrinodis*	10	3	10	0	0
	M. rubra	3	1	2	0	0
	M. gallienii	2	0	0	0	0
KUŠ	*M. scabrinodis*	9	2	2	0	0
	M. rubra	1	1	1	0	0
DOL	*M. scabrinodis*	8	2	3	0	0
	M. rubra	2	2	4	2	4
	M. gallienii	1	1	1	0	0
ŠAL 1	*M. scabrinodis*	8	0	0	0	0
	M. rubra	2	0	0	0	0
	M. ruginodis	1	0	0	0	0
IVAN 1	*M. scabrinodis*	6	1	2	0	0
	M. curvithorax	1	0	0	0	0
IVAN 2	*M. scabrinodis*	6	0	0	0	0
	M. rubra	2	0	0	0	0
	M. gallienii	2	0	0	0	0
	M. curvithorax	1	0	0	0	0
MOT 1	*M. scabrinodis*	10	4	10	0	0
MOT 2	*M. rubra*	4	1	11	2	26
KOB	*M. scabrinodis*	30	1	1	0	0
	M. gallienii	1	0	0	0	0
	M. ruginodis	2	0	0	0	0
	M. sabuleti	2	0	0	0	0
Total		**142**	**25**	**56**	**9**	**87**

Host ants

P. teleius larvae were found in 25 *Myrmica* ant nests and *P. nausithous* in 9 nests (Tab. 3). Altogether, 56 larvae of *P. teleius* and 87 larvae of *P. nausithous* were found (Tab. 3). *P. nausithous* larvae were found exclusively in *M. rubra* nests, while *P. teleius* larvae were recorded in ant nests of three *Myrmica* species: *M. scabrinodis*, *M. rubra* and *M. gallienii*. The highest proportion of all infested nests with *P. teleius* was in *M. scabrinodis* (64%), while the proportion of infestation with *P. teleius* per investigated ant nest and species was the highest in *M. rubra.* A different species of host ants were found on three locations. The co-occurrence of both butterfly species larvae in the same ant nest was observed in six ant nests, in all cases *M. rubra* was a host species.

In general, *P. teleius* was recorded in a larger number of ant nests compared to *P. nausithous*, while *P. nausithous* larvae were found in a larger number per ant nest. The median value of *P. teleius* larvae found per ant nest is 1, while the median value of *P. nausithous* larvae number found per ant nest is 3. In three *M. rubra* nests, larger numbers of larvae (more than 20) per nest were found. The largest number of larvae in a single nest was counted in *M. rubra* nest in the locality MOT2 – a total of 35 larvae, 11 larvae of *P. teleius* and 24 larvae of *P. nausithous*. In the locality NER2, 26 and 22 larvae of *P. nausithous* were found, respectively, accompanied by a single *P. teleius* larva in each nest. Altogether, more than half of all larvae of both species were found in the three *M. rubra* nests mentioned above.

Discussion

During our study, a total of seven species of *Myrmica* ants were recorded in meadows with *P. teleius* and/or *P. nausithous* at Goričko Nature Park, which is half of all *Myrmica* ant species known for Slovenia (Bračko 2007).

The fact that 19.7% of all *Myrmica* nests examined contained *Phengaris* larvae and most of these contained only a few larvae, follows the pattern of ant nests infestation on *Phengaris* sites across Europe (e.g. Witek et al. 2010). As ant nests vary greatly in their susceptibility to larval parasitism, typical *Phengaris* sites include many uninfested nests, several with moderate infestation, and a few with high infestation (Tartally et al. 2008). According to our results, we can consider *M. rubra* as the primary and most important ant host of *P. nausithous* at Goričko. A high specificity in relationship of *P. nausithous* with *M. rubra* has also been reported across Europe (Witek et al. 2008, 2010), including western Hungary, which is closest to our Goričko study region (Tartally & Varga 2005, Tartally et al. 2019). For *P. teleius*, *M. rubra* and *M. scabrinodis* can be considered as primary hosts at Goričko. *P. teleius* has the most diverse hosts among European *Phengaris* species and often uses the locally most abundant *Myrmica* species (e.g. Tartally & Varga 2008, Witek et al. 2010, Tartally et al. 2019). This is in line with our observations, as the two primary host *Myrmica* species were also the most abundant in our study area, and with observations at the Őrség National Park in Hungary (Tartally & Varga 2008). Among the other five *Myrmica* species recorded, we found only one larva of *P. teleius* in an ant nest of *M. gallienii*. Larvae of *P. teleius* were found in nests of *M. gallienii* also in Poland (Stankiewicz & Sielezniew 2002).

Although the number of *M. scabrinodis* nests discovered was much higher than that of *M. rubra* (105 vs. 22), the percentage of nests parasitized by *Phengaris* was higher in *M. rubra* (41% vs. 24%), as was the total number of larvae detected (111 in *M. rubra* vs. 31 in *M. scabrinodis*). Three nests of *M. rubra* contained more than 20 *Phengaris* larvae. Moreover, co-occurrence of both *Phengaris* species larvae was detected exclusively in *M. rubra* nests. We found six cases of co-occurrence out of total 8 or 9 nests found infected by *P. teleius* or *P. nausithous,* respectively. The large number of larvae and co-occurrence of the two *Phengaris* species in *M. rubra* nests was previously reported by Tartally & Varga from western Hungary (2005). In their study, they found a *M. rubra* colony with 36 larvae of both *Phengaris* species, which is comparable to our colony from MOT2 (35 larvae). Since *M. rubra* forms the most populous colonies of all Central European *Myrmica* species (Seifert 2018), the highest numbers of parasitizing larvae in their colonies is not surprising. Such large colonies have a higher carrying capacity to host and survive a large number of *Phengaris* larvae. Although *M. scabrinodis* nests were parasitized in lower percentage and contained lower total number of larvae, this species still represents an important host for *Phengaris* larvae at Goričko, mostly as it is the most frequent ant species in *Phengaris* sites. It is particularly important host for *P. teleius*, which as mentioned above, often uses locally most abundant *Myrmica* species.

Conservation implications

The nest of *Myrmica rubra* hosting the largest number of *Phengaris* larvae was found at Motvarjevci (MOT2), along an abandoned road verge. This finding indicates and confirms the importance of road verges and grassland margins as larval habitat for *P. teleius* and, in particular, for *P. nausithous* (e.g. Wynhoff et al. 2011). *Myrmica rubra,* as the main host ant of *P. nausithous*, is known to be less thermophilic than *M. scabrinodis,* preferring sheltered and more overgrown habitat for its nests (Wynhoff et al. 2011). According to Seifert (2018), *M. rubra* gains optimum in mesophilic to moist conditions, and is absent only from most xerothermic and sparsely-vegetated habitats. It is often the only ant in very high-grassy lowland meadows and tall herb communities. Therefore, it is important to maintain parts of more dense vegetation with the larval host plant along meadow margins, which could be beneficial for *M. rubra* and both target butterfly species (see also Tartally & Varga 2005). *Myrmica scabrinodis* is a hygrophilous to moderately thermophilic species and reaches highest densities in meadows or ecotones with moderate height of grasses (Seifert 2018). Each host ant species has its own niche and, according to current knowledge, the two *Myrmica* species require different mowing regimes (Wynhoff et al. 2011), which should be taken into consideration in practical management, especially on smaller, isolated sites where both ant species occur together. *Myrmica scabrinodis* seems to benefit from mowing in early June, while *M. rubra* benefits from mowing in late autumn which should be combined with some annually unmown parts/verges that are alternated among years to prevent overgrowing especially by invasive plants (Wynhoff et al. 2011). The general recommendation for habitat management should thus consider mosaic mowing offering many different microhabitat opportunities. However, at least in the last decade, the habitat and populations of both species in NE Slovenia have been declining (Zakšek et al. 2020), so moving towards more active conservation of both species is essential for their long term survival. With basic knowledge on their larval ecology in hand, potential restoration of new habitats or focusing on a conservation of the most important parts of the larval habitat of both species will enable a first step to halt their decline. It should be emphasised that there is no active habitat restoration possible without knowledge and monitoring of ant communities (e.g. Wynhoff et al. 2017).

Povzetek

Strašničin (*Phengaris teleius*) in temni mravljiščar (*P. nausithous*) sta ozko specializirani vrsti dnevnih metuljev. Gosenice obeh vrst se razvijajo v mravljiščih mravelj iz rodu *Myrmica*, izbira gostiteljskih vrst mravelj pa se lahko geografsko (npr. v različnih delih Evrope) ali lokalno razlikuje. Njun razvoj je vezan na zdravilno strašnico (*Sanguisorba officinalis*), na katero samice odlagajo jajčeca in kjer se razvijajo prvi stadiji gosenic. Po posvojitvi, od četrtega larvalnega stadija naprej, se gosenice obeh vrst razvijajo v mravljiščih mravelj iz rodu *Myrmica*. Tam se prehranjujejo na dva načina: plenijo zarod mravelj ali pa jih hranijo mravlje kot svoj zarod (t.i. kukavičji način prehranjevanja); ali pa uporabljajo kombinacijo obojega. Zaradi specifičnega razvojnega cikla in življenja na mokrotnih travnikih sta vrsti tako v Sloveniji kot drugod v Evropi ogroženi in tudi zavarovani. V letih 2011 in 2012 smo na Goričkem v severovzhodni Sloveniji ugotavljali, katere so gostiteljske vrste mravelj strašničinega in temnega mravljiščarja. To je prva raziskava gostiteljskih vrst mravelj v Sloveniji. Gostiteljske mravlje smo vzorčili na dvanajstih lokacijah na Goričkem, ki je eno največjih in najpomembnejših območij razširjenosti za obe vrsti mravljiščarjev v Sloveniji.

Na območju raziskave smo zabeležili sedem vrst mravelj iz rodu *Myrmica*, kar je polovica vseh znanih vrst tega rodu v Sloveniji. Prisotnost gosenic mravljiščarjev smo preverjali v 142 mravljiščih mravelj iz rodu *Myrmica*. Gosenice strašničinega mravljiščarja smo našli v mravljiščih treh različnih vrst mravelj, *M. scabrinodis*, *M. rubra* in *M. gallienii*, medtem ko so bile gosenice temnega mravljiščarja najdene izključno v mravljiščih *M. rubra*. Skupaj smo gosenice strašničinega mravljiščarja našli v 25 mravljiščih, gosenice temnega pa v devetih mravljiščih. Kljub manjšemu številu mravljišč je bilo skupno število najdenih gosenic temnega mravljiščarja višje, saj so bile gosenice te vrste v mravljiščih številnejše. Razlogov za to je lahko več: *M. rubra* je znana po svojih velikih kolonijah in kot take lahko gostijo večje število gosenic; temni mravljiščar v nasprotju s strašničinim, ki se hrani izključno plenilsko, uporablja obe strategiji prehranjevanja. Zanimivo je, da je bilo najvišje število najdenih gosenic v enem mravljišču *M. rubra* kar 35, zastopane pa so bile gosenice tako strašničinega kot temnega mravljiščarja. O tako visokem številu gosenic v enem mravljišču so v literaturi podatki tudi iz sosednje zahodne Madžarske.

Rezultati naše raziskave dajejo prvi vpogled v gostiteljske vrste mravelj za strašničinega in temnega mravljiščarja v Sloveniji. Ugotavljamo, da je temni mravljiščar tudi v Sloveniji glede izbire gostiteljskih mravlje ozko specifičen, medtem ko je za strašničinega mravljiščarja značilna večja plastičnost glede gostiteljskih vrst mravelj. Glede na to, da se ohranitveno stanje obeh vrst v Sloveniji slabša, bodo rezultati lahko pomembna osnova pri aktivnem ohranjanju teh dveh ogroženih vrst mravljiščarjev v Sloveniji.

Acknowledgements

The study was part of the project »Krajina v harmoniji« (Teritorialno sodelovanje, SLO-HU: 2007-2013). We are grateful to Primož Presetnik (CKFF) for his help during field work, to Kristjan Malačič (Goričko NP) for communicating with the land owners and to Ali Šalamun (CKFF) for preparation of the map. Nest disturbance was in accordance with the licence (35603-3/2010-4) issued by the Slovenian Environment Agency of the Ministry of Environment and Spatial Planning of the Republic of Slovenia to CKFF. The study was partially supported by the Slovenian Research Agency (Program P1-0184).

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NATURA SLOVENIAE 23(1): 35-36
FIELD NOTE

Prejeto / Received: 5. 1. 2021
Sprejeto / Accepted: 23. 7. 2021

First record of the species *Bithynia zeta* Glöer & Pešić, 2007 (Gastropoda: Hydrobiidae) in Bosnia and Herzegovina

Prvi podatek o vrsti *Bithynia zeta* Glöer & Pešić, 2007 (Gastropoda: Hydrobiidae) v Bosni in Hercegovini

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The genus *Bithynia* Leach, 1818 is widespread in the Western Palearctic, with its species particularly rich in the regions of the Balkans and Asia Minor (Glöer 2019). The species occurring in the southwestern Balkans exhibit very small distribution ranges (Glöer et al. 2007). In Bosnia and Herzegovina, three species have been known so far according to Karaman (2006): *Bithynia tentaculata* (Linnaeus, 1758), endemic species *Bithynia mostarensis* Möllendorff, 1873, and *Bithynia leachii* (Sheppard, 1823). Only the first two are listed for the country also by Bank & Neubert (2017) and Glöer (2019). Here we report on discovery of a new species for the country, *Bithynia zeta* Glöer & Pešić, 2007.

The species *B. zeta* was first described from Lake Skadar in southern Montenegro and has eventually been found also at many sites along the lake: Tanki Rt, Malo Blato, Vranjina, Karuč spring and Gornje Vrelo spring in Bar (Glöer & Pešić 2007). It has a distinctive anatomy of the penis and shape of the shell, and it also shows a clear DNA distinction from the species *B. cetinensis* and *B. tentaculata* (Pešić et al. 2019).

B. zeta has recently also been found in Bosnia and Herzegovina, about 70 km away from the north westernmost locality in Montenegro (Glöer & Pešić 2007). Samples of spring snails were taken from three springs along the Trebišnjica River (springs Vruljak 1, »Gorički Studenac« and Vruljak 2, near the Gorica settlement near Trebinje) on 18 July 2020. Vruljak 2 spring (coordinates N 42°42'38.00" E 18°22'33.60"), which represents one of the entrances to the cave bearing the same name, is inhabited by the species *Emmericia ventricosa* Brusina, 1870, and *B. zeta* specimens were collected from the stones there. Species determination was performed based on shell morphology. Vruljak 2 spring is in direct connection with the cave Vruljak 1, forming a cave system known for its large population of the olms (*Proteus anguinus*) (Lewarne et al. 2010, Lewarne 2018).

These samples were collected during a field trip within the framework of the student workshop »Strengthening research capacity«, which was part of the project »Distribution, population and threat status for biodiversity of freshwater snails of the family *Hydrobiidae*«, a project led by the Center for Karst and Speleology from Sarajevo and financed by the Critical Ecosystem Partnership Fund. The discovery of *B. zeta* in Bosnia and Herzegovina contributes to a better knowledge of the biogeography of this species, formerly known only from a small region in Montenegro. But, the discovery also shows the potential for new discoveries of aquatic, especially spring snails in Bosnia and Herzegovina.

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NATURA SLOVENIAE 23(1): 37-40
FIELD NOTE

Prejeto / Received: 11. 1. 2021
Sprejeto / Accepted: 17. 6. 2021

Unconfirmed presence of the territorial golden jackal *Canis aureus* and grey wolf *Canis lupus* groups in the Poljanska Sora river valley and Škofjeloško hribovje hills in July 2020

Nepotrjeno pojavljanje teritorialnih skupin zlatega šakala *Canis aureus* in volka *Canis lupus* v Poljanski dolini in Škofjeloškem hribovju julija 2020

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The grey wolf (*Canis lupus*) is one of the world's most widely distributed mammals and the most studied large carnivores (Ripple et al. 2014). In Slovenia, wolves were nearly extirpated in the 20th century, but have made a strong recovery in the last 10 years (Potočnik et al. 2010, Bartol et al. 2020). In 2019, newly established territorial packs of wolves were recorded in the pre-Alpine and Alpine regions of Slovenia for the first time after more than 100 years of absence (Jonozovič 2003, Bartol et al. 2020), though wolves have been present regularly and reproducing in the adjacent Dinaric plateau Trnovski Gozd since 1995 (Turk 2006).

On the other hand, the Golden jackal (*Canis aureus*) (hereinafter referred to as the jackal) started increasing its distribution and abundance during the late 20th and early 21th centuries and is considered widespread in Slovenia (Throuwburst et al. 2015, Potočnik et al. 2019). Hard data on territorial jackals in the pre-Alpine and Alpine regions of Slovenia are scarce, although jackals are present in some alpine valleys (Mihelič & Krofel 2012, Potočnik et al. 2019). The current distribution of the two canid species in Slovenia is largely segregated, with wolves mainly (but not exclusively) occurring in forested and hilly landscapes further from human settlements, while jackals are most widespread in fragmented agricultural-forested lowlands near human settlements (Krofel et al. 2017, Potočnik et al. 2019).

Due to the lack of finances needed for further systematic surveillance of the two canids in the 2020's (Potočnik et al. 2010, Bartol et al. 2020), our goal was to check the status of the two species in the pre-Alpine area, specifically in the Poljanska Sora river valley and Škofjeloško – Cerkljansko hribovje hills.

Standardized acoustic surveys were used for detecting both territorial groups of jackal and grey wolf, considering the differences in the spatial scale at which each survey needed to be performed for target species (Fig. 1). For the jackal survey, we followed slightly modified protocol described in Potočnik et al. (2019) using recordings of jackal vocalization. The survey was performed at night on 18 and 20 July 2020, surveying nine locations (4 and 5 locations per night on the first and second nights, respectively) along the Poljanska Sora river. The survey points were placed between 1 and 4 km apart, each covering approximately 3–4 km^2 in forested, agricultural, or semi-urban habitats (Fig. 1). The survey points were denser than suggested in Potočnik et al. 2019 due to the very diverse terrain in the narrow Alpine valley. The micro location for acoustic survey was determined on-site with regard to human settlements, pastures, vegetation, road activities and overall topology of the terrain. We used playback of territorial group call, and calling pattern with intermediate listening times as in other jackal acoustic survey studies (Giannatos et al. 2005, Krofel 2008). For the grey wolf survey, we performed howling surveys in 6 squares of a standard 3×3 km grid (Hartington & Mech 1982, Potočnik et al. 2010) (Fig. 1), covering 3 locations per night (23 and 24 July 2020). Owing to the limited time and manpower, we could not cover all points in the area but chose the most suitable points instead, based on the national wolf monitoring data (Bartol et al. 2020), where we predicted the species' most probable presence. Also, we did not repeat the survey for three consecutive nights due bad weather conditions. Sudden weather changes also terminated howling session at survey point No. 6 (Fig. 1).

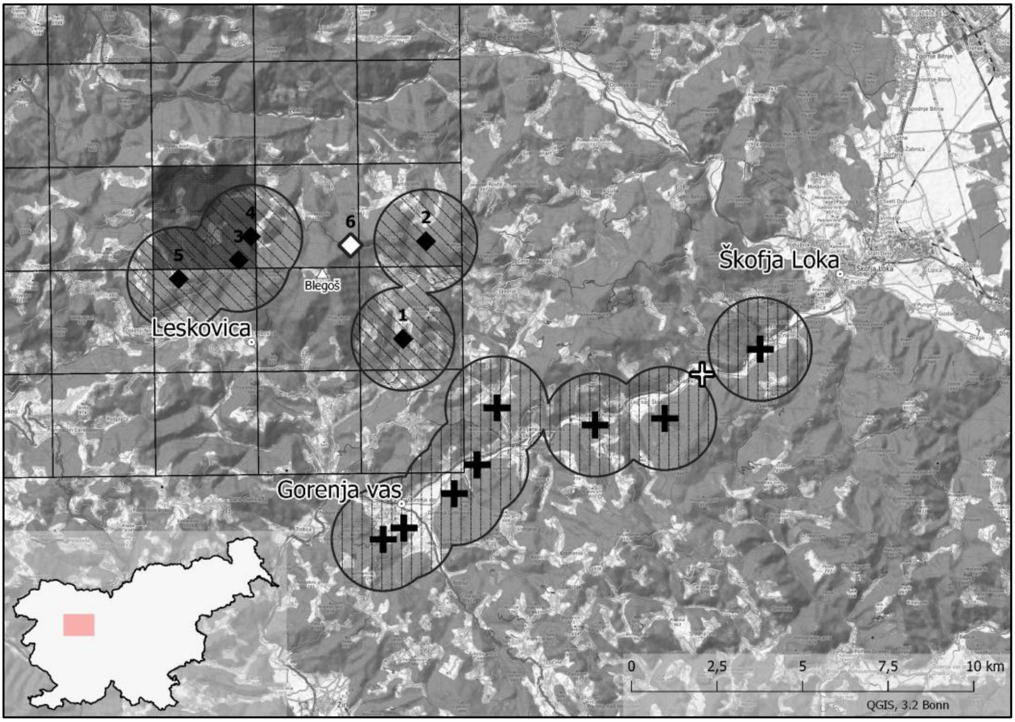

Figure 1: A map showing locations where we performed howling survey of the Eurasian golden jackal (*Canis aureus*) (plus signs) and grey wolf (*Canis lupus*) (diamond signs) territorial groups, with wolf national monitoring 3 × 3 km grid shown. Dark grey shows forested areas in the Poljanska Sora river valley and Škofjeloško hribovje hills. The empty signs of both plus and diamonds show locations where we could not perform the survey due to too excessive human activities or rough terrain. Shaded area shows the area surveyed with acoustic methods. Coloured square shows location of wolf pack recorded with howling survey in 2019 (Bartol et al. 2020).

Slika 1: Zemljevid s prikazom lokacij in okvirno popisanega območja v Poljanski dolini in Škofjeloškem hribovju, kjer smo predvajali posnetke tuljenja zlatega šakala in volka. Temno siva označuje gozdnata območja. Polno obarvani plus znaki prikazujejo mesta predvajanja tuljenja šakala (*Canis aureus*), polno obarvani karo znaki pa mesta predvajanja tuljenja volka (*Canis lupus*) z mrežo kvadrantov 3 × 3 km, uporabljeno pri nacionalnem monitoringu volka. Nebarvane oznake prikazujejo predvidene lokacije za popis, a ta tam ni bil opravljen zaradi preveč motečih dejavnosti človeka ali nedostopnega terena. Senčena območja prikazujejo območje, pokrito z zvočnimi popisi. Obarvani kvadrant prikazuje lokacijo volčjega tropa, zaznano leta 2019 (Bartol et al. 2020).

This study confirms the results of the national scale jackal survey from 2019 (Potočnik et al. 2019), which reported only occasional sightings of jackals in the Poljanska Sora valley. Those were most probably sightings of young individuals in dispersion, which can travel several hundred kilometres far (Spassov & Acosta-pankov 2019). The closest territorial groups were detected in a field near Škofja Loka NE of our study area and in the Idrija valley SW of it. The models of habitat suitability recognized the study area of the Poljanska Sora river valley as a less appropriate habitat for jackal occurrence (Potočnik et al. 2019). However, with current population expansion rate (Trouwborst et al. 2015), we can expect that it is only a matter of time when jackals will inhabit also less suitable areas like this valley. We could therefore assume that the number of jackal groups have not yet reached their maximum in the adjacent areas with higher habitat suitability.

On the contrary, the grey wolf population in Slovenia has only recently expanded towards the pre-Alpine and Alpine regions; the first territorial packs were detected in 2019 (Bartol et al. 2020). The newly established pack caused some damages on livestock, and thus quickly generated conflict with the livestock herders (ARSO 2021). The presence of a large carnivore species has also induced fear among local residents. Due to the reluctance of the locals to the wolves, also shown in public opinion questionnaires (Bartol et al. 2020), or seen on social media, there were several appeals on intensifying the population control measures, and showing dissatisfaction with national management plans. Until prevention measures could be properly introduced to the area, the government allowed limited cull of several individuals at sites where most damages to livestock occurred. This resulted in death of the reproductive male, which most probably caused pack disintegration (Brainerd et al. 2008, Bartol et al. 2020). Though there are no hard facts of it, the wolves in this area could also have been subjected to poaching. Whatever the underlying cause, our survey results suggest that a territorial pack of wolves, which has formed in the pre-Alpine region after decades of absence only a year ago, might have disappeared.

In conclusion, our findings highlight the importance of continuous monitoring which enables us to evaluate the effects of management measures, especially if they involve lethal control of a protected species. Immediate feedback on the outcome of culling is especially meaningful in situations where species is re-appearing in a geopolitically important area, such as the Alps, after decades of absence. Even if the status and consequently the management regime of a species changes, such as it did in the case of the jackal (Ur. l. RS 2019), it is even more important to monitor the development of the population and evaluate whether the main management measure (culling) has the desired effect or not. Only using baseline data from uninterrupted, standardized monitoring, management decisions can be informed rather than politically driven, which is too often the case for large carnivore species (Darimont et al. 2018).

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NATURA SLOVENIAE 23(1): 41-44
FIELD NOTE

Prejeto / Received: 18. 1. 2021
Sprejeto / Accepted: 17. 6. 2021

Two new capture records of the greater noctule bat *Nyctalus lasiopterus* (Schreber, 1780) in Slovenia

Nova podatka o ujetju velikega mračnika *Nyctalus lasiopterus* (Schreber, 1780) v Sloveniji

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The greater noctule bat (*Nyctalus lasiopterus*) is a very large bat species distributed in the Mediterranean region as well as in central and eastern Europe (Alcaldé et al. 2016) and has until recently only been assumed to occasionally appear in Slovenia (Petrinjak 2009). Until Presetnik & Knapič (2015) rediscovered *N. lasiopterus* in the country after more than 85 years, the only known confirmed location came from the Slovenian Littoral (Dal Piaz 1927). After discovering the species in 2013 and 2014 (Presetnik & Knapič 2015), a few new localities, detected by surveying bat echolocation calls, have been recorded in the south-western part of the country (Presetnik 2017, 2019, Presetnik & Šalamun 2019). In 2017, the first capture record of the species for the country followed when Zidar (2020) deployed mist-nets at a pond in the Istria region. This capture of *N. lasiopterus* was then followed in 2019 in two different regions by Gojznikar et al. (2020) in the Branica river valley, and Pavlovič et al. (2020) at Babna Polica. With this contribution, we wish to present two new capture records of the species, which add to the knowledge of its distribution and presence in Slovenia.

On 28 July 2020 we revisited the pond 350 m east from Poletiči (45.495674 °N, 13.867912 °N, 343 m a.s.l.), where Zidar (2020) made the first capture record in 2017. Mist-nets were erected along the pond's western, northern and eastern edges, covering around three quarters of the pond's perimeter (the southern bank was not enclosed). The total length of five nylon/polyester mist-nets covered 75 metres, with nets being erected approximately 4 metres in height. The field session was conducted between 20:36 (sunset) and 01:15 h. On another occasion, the mist-nets were erected on 9 August 2020 at a pond 700 m SE of Bukov vrh hill at the Poček military training ground near Postojna (hereinafter referred to as the Poček pond, 45.728980 °N, 14.245737 °E, 621 m a.s.l.). The shallow circular concrete-lined pond with a diameter of approximately 12 metres was completely surrounded by four nylon/polyester mist-nets in total length of 39 metres, erected approximately 3 metres in height. The area in the immediate vicinity of the pond consisted mainly of dry meadows, with closest mixed woodland edge c. 35 metres away. The field session began at 20:20 and ended at 23:20 h.

All caught bats were quickly removed from the mist-nets, then measured using a calliper (0.1 mm accuracy), weighed with a spring scale (0.5 g accuracy, max. range of 50 g) and determined using an identification key (Dietz et al. 2009). Sex, sexual activity, and age of each individual were determined (Haarsma 2008).

At the pond near Poletiči we caught two adult male *N. lasiopterus* (Tab. 1), which both showed signs of sexual activity (enlarged testes). The second caught male had a distinctly paler face (Fig. 1). According to Haarsma (2008), this could indicate an older individual. This conclusion, however, was not supported by absence of dental wear, which was similar to that of the first male. Additionally, we captured 17 individuals of *Pipistrellus kuhlii*, 14 of *Hypsugo savii,* 2 of *N. leislerii* and 1 of *Plecotus macrobullaris.*

Table 1. Measurements of captured *Nyctalus lasiopterus* individuals. Due to limitation with the maximum range of the spring scale (50 g), most individuals were too heavy to be weighed accurately. Abbreviations: M – male, AD – adult, AB – forearm length, T – testes, E – epididymis. Estimates of sexual activity: / - no signs; + - slightly swollen, ++ - swollen, +++ - very swollen, ++++ - extremely swollen.

Tabela 1. Meritve ujetih osebkov *Nyctalus lasiopterus*. Zaradi presežene zgornje vrednosti vzmetne tehtnice (50 g) je bila večina netopirjev pretežka za natančno tehtanje. Okrajšave: M – samec, AD – odrasel, AB – dolžina podlakti, T – moda, E – obmodki. Ocene spolne aktivnosti: / - ni sledov, + - rahlo napolnjeni, ++ - napolnjeni, +++ - zelo napolnjeni, ++++ - izredno napolnjeni.

Location	Date	Sex [Age]	AB (mm)	Mass (g)	Sexual activity	Remarks
Pond near Poletiči	28. 7. 2020	M [AD]	66.2	>48.0	T ++ E /	
Pond near Poletiči	28. 7. 2020	M [AD]	64.0	45.0	T + E /	pale facial skin
Poček pond	9. 8. 2020	M [AD]	65.8	>48.0	T ++++ E +(+)	
Poček pond	9. 8. 2020	M [AD]	64.0	>48.0	T +++ E +	
Poček pond	9. 8. 2020	M [AD]	67.8	>48.0	T +++ E /	

Figure 1. The pale-faced male *Nyctalus lasiopterus* (a) compared to its more usually coloured male conspecific (b) (both photographed at pond near Poletiči; photo: Jan Gojznikar).

Slika 1. Samec *Nyctalus lasiopterus* z bledo kožo obraza (a) primerjan z bolj običajno obarvanim sovrstnikom (b) (oba fotografirana na kalu pri Poletičih; foto: Jan Gojznikar).

At the Poček pond we caught three adult males of *N. lasiopterus* (Tab. 1) – all three with traits indicating sexual activity. We also caught two other *Nyctalus* species known to occur in Slovenia (Presetnik et al. 2009), with 2 individuals of both *N. leisleri* and *N. noctula*. Additionally, we recorded one individual of *Myotis myotis*, *M. nattereri* and *Pl. auritus* each.

So far, all capture records of the species in Slovenia have encountered exclusively male individuals (Zidar 2020, Gojznikar et al. 2020, Pavlovič et al. 2020, this study). Although Pavlovič et al. (2020) conclude that there is no proof of reproduction for Slovenia, we cannot exclude this possibility. Apparent signs of sexual activity in our caught males and those caught by Zidar (2020) and Gojznikar et al. (2020) could indicate that at least mating of *N. lasiopterus* may occur in Slovenia.

Our capture of *N. lasiopterus* at the pond near Poletiči shows that the capture by Zidar (2020) was not a chance finding of vagrant individuals. Instead, it suggests that the species is constantly present in the area, at least during the mid-summer months. After the long absence of record, our captures also suggest, together with other recent findings (Presetnik & Knapič 2015, Presetnik 2017, Presetnik 2019, Presetnik & Šalamun 2019, Zidar 2020, Gojznikar et al. 2020, Pavlovič et al. 2020), a possibility of the species becoming more common in the south-western part of the country. During the previous visit of the same pond at Poček twelve years ago, Zagmajster (2008) did not record *N. lasiopterus*, although a single mist-netting with no caught individuals does not prove the species absence. One of the possible explanations for the species becoming more common in Slovenia is perhaps climate change. Using modelling approaches, Rebelo et al. (2010) have suggested a future northward range expansion for many bat species of the Mediterranean biogeographic group, including *N. lasiopterus*, under the influence of climate change. Even though it has been demonstrated that this phenomenon has impacted distribution ranges of some other bat species, like *Pipistrellus kuhlii* (e.g., Ancillotto et al. 2016), further continental-wide research is required to corroborate this possibility for *N. lasiopterus*. Recent records of the species in Slovenia for now only imply that the species has become more common than previously observed, yet it would be nonetheless sensible to start considering this species in future conservation plans in Slovenia, at least within the south-western part of the country. Estók et al. (2007) have suggested that the species uses older trees with sufficient natural crevices for roosting, and since forested areas in Slovenia have been under increased pressure from logging in the last few years (e. g. Pisek & Poljanec 2020), *N. lasiopterus* should probably also be included in forest management plans. Additional research is also needed to unravel the status of the species in the country.

Acknowledgements

We are grateful to Matija Mlakar Medved, Rok Lobnik, Maruša Penca Kocjan, Anja Bolčina, Luka Poljanec, Jon Poljanec, Matevž Bervar and Teja Barašin for their participation in field work excursions. Special thanks also goes to Simon Zidar, who kindly recommended the locality at Poletiči and gave some valuable advice. Rudi Kraševec pointed to some valuable literature sources. The field work at the Poček pond was conducted as part of »Dijaški biološki tabor« (Youth Biology Camp), organized by the Slovenian Herpetological Society.

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NATURA SLOVENIAE 23(1): 45-46
FIELD NOTE

Prejeto / Received: 6. 4. 2021
Sprejeto / Accepted: 1. 7. 2021

The first find of Bechstein's bat *Myotis bechsteinii* (Kuhl, 1817) summer roost in Slovenia

Prva najdba poletnega zatočišča velikouhega netopirja *Myotis bechsteinii* (Kuhl, 1817) v Sloveniji

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The Bechstein's bat (*Myotis bechsteinii*) is a medium sized bat, easily distinguished from other species of the genus *Myotis* due to its size (forearm length = 39.0 – 47.1 mm) and remarkably large, over 20 mm long ears (Dietz & von Helversen 2004). The species is distributed in Western, Central and Eastern Europe, where it inhabits mature deciduous forests at different altitudes (Dietz & Kiefer 2016). The Bechstein’s bat is considered to be the most woodland dependent (Koselj 2009) out of 30 bat species currently living in Slovenia (Presetnik & Šalamun 2019). The distribution of the species in Slovenia is based mostly on mist-netting data from swarming sites at cave entrances. At the time when hibernating individuals were found on two occasions, no maternity or any other roosts were known in the country (CKFF 2021). This bat is known to roost in trunk crevices and tree holes, while roosts in buildings are scarce (Dietz & Kiefer 2016).

In July 2020 (17.7.–26.7.2020), the biological summer research camp (Raziskovalni tabor študentov biologije, RTŠB) was organised by the Biology Students’ Society (Društvo študentov biologije) at Gorenja vas in north-western Slovenia. Even though both authors worked as members of the Ornithological Group, we collected other records as well. On 25.7.2020, we examined the drainage pipes underneath a bridge over one of the intermittent torrents flowing into the Cerknica River. The 5 m long, 2 m wide and 4 m high concrete bridge is located on the asphalt road connecting the settlements Gorenji Novaki and Podpleče within the municipality of Cerkno (WGS 84 Lat./Long.: 46.143583, 14.049306). We examined two vertical drainage pipes with a diameter of 5 cm and depth of about 30 cm. In one of them we found a bat, which could be identified visually as Bechstein’s bat (Fig. 1). We took a photograph and did not disturb the individual.

Figure 1: Bechstein's bat (*Myotis bechsteinii*) at its roost, a drainage pipe underneath a bridge at Gorenji Novaki on 25.7.2020 (photo: D. Knez).
Slika 1: Velikouhi netopir (*Myotis bechsteinii*) v zatočišču v odtočni cevi mostu v Gorenjih Novakih 25.7.2020 (Foto: D. Knez)

The presence of the species in the area was not surprising, as it had been mist-netted at a cave entrance about 10 km north several times before (Presetnik et al. 2020). The interesting aspect of the find is that it is the first summer roost of the Bechstein’s bat recorded in Slovenia. Furthermore, the bat utilized an artificially made roost, and not a tree crevice, where the species usually roosts (Dietz & Kiefer 2016). Some individuals of this species have also been reported from under bridges in Bulgaria, where they roosted not in drainage pipes, but in crevices between bricks (Petrov 2006). This makes our finding interesting for understanding the roosting ecology of the species in general.

Acknowledgements

We wish to thank Primož Presetnik for accessing the CKFF database and confirming our find as the first of its kind, and Jan Gojznikar for making some useful suggestions on improving this manuscript. We also thank DŠB for organising the student's camp, which enabled us to conduct the fieldwork.

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LITERATURE
References should be cited in the text as follows: a single author is cited, as Schultz (1987) or (Schultz 1987); two authors would be (Parry & Brown 1959); if a work of three or more authors is cited, (Lubin et al. 1978); and if the reference appears in several works, (Ward 1991, Pace 1992, Amman 1998). If several works by the same author published in the same year are cited, the individual works are indicated with the added letters a, b, c, etc. (Lucas 1988a, b). The literature should be arranged in alphabetical order.

Examples (use the the following forms):

- articles from journals:

Schultz J.W. (1987): The origin of the spinning aparatures in spiders. Biol. Rev. 62: 123-134.
Parry D.A., Brown R.H.J. (1959): The hydraulic mechanism of the spider leg. J. Exp. Biol. 36: 654-657.
Lubin Y.D., Eberhard W.G., Montgomery G.G. (1978): Webs of Miagrammopes (Araneae: Araneaidae) in the neotropics. Psyche 85: 1-13.
Lucas S. (1988a): Spiders in Brasil. Toxicon 26: 759-766.
Lucas S. (1988b): Spiders and their silks. Discovery 25: 1-4.

- for books, chapters from books, reports, and congress anthologies:

Foelix R.F. (1996): Biology of spiders, 2. edition. Harvard University Press, London, pp. 155-162.
Nentwig W., Heimer S. (1987): Ecological aspects of spider webs. In: Nentwig W. (Ed.), Ecophysiology of Spiders. Springer Verlag, Berlin, 211 pp.
Edmonds D.T. (1997): The contribution of atmospheric water vapour to the formation of a spider's capture web. In: Heimer S. (Ed.), Proceedings of the 17th European Colloquium of Arachnology. Oxford Press, London, pp. 35-46.