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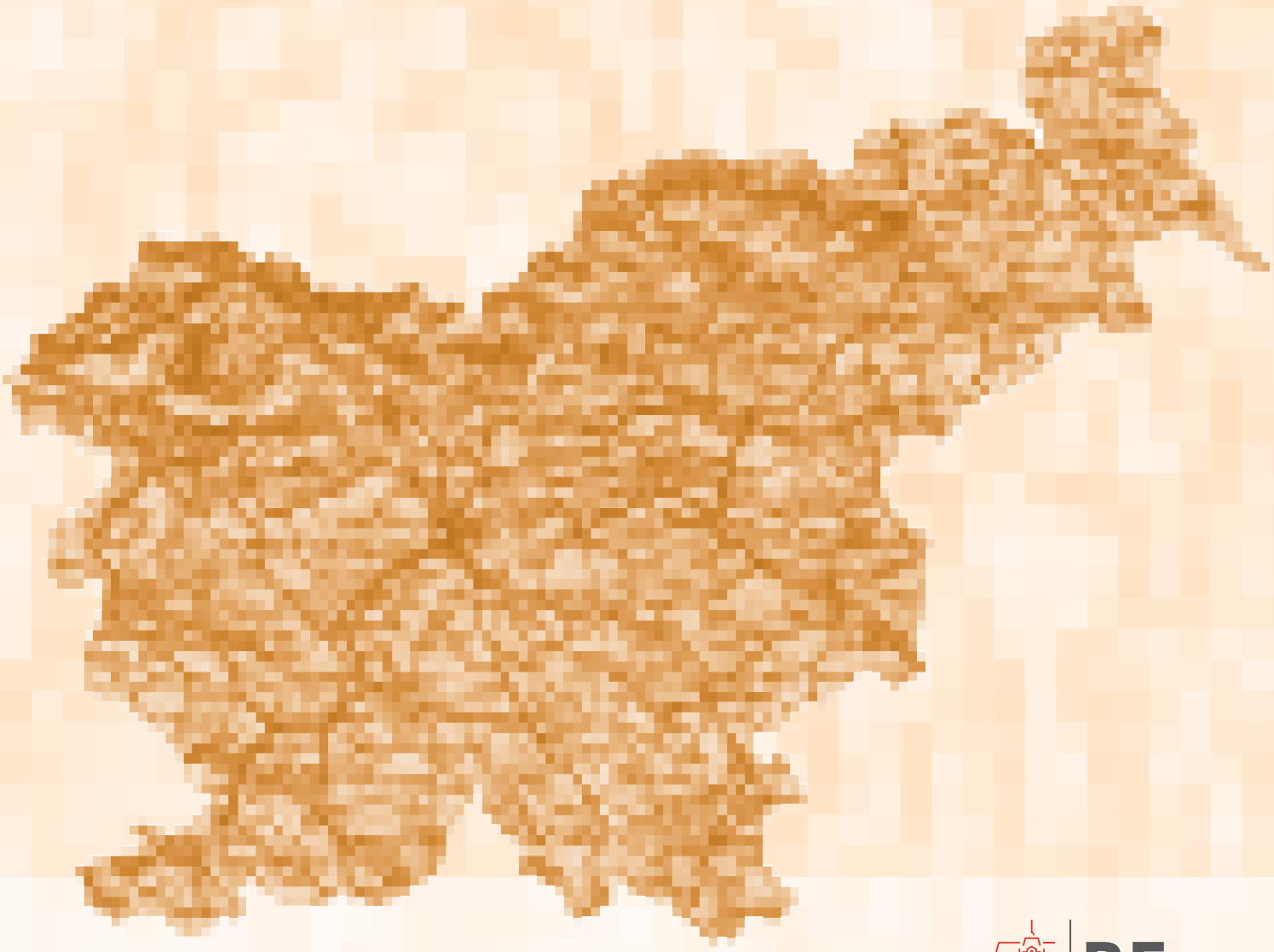

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Diversity, distribution and host interactions of branchiobdellidans (Clitellata: Branchiobdellida) in the Western Balkans

Mariia SHRESTKHA[1,2],
Serge UTEVSKY[1],
Peter TRONTELJ[2*]

ABSTRACT

Branchiobdellidan samples obtained from host crustaceans from 60 localities in the Balkan region (Slovenia, Croatia, Montenegro, Bosnia and Herzegovina, North Macedonia, Serbia) were studied based on collections of the University of Ljubljana, Biotechnical Faculty, Department of Biology (Slovenia) and new sampling. The crayfish worms were assigned to seven species: *Branchiobdella balcanica*, *B. pentadonta*, *B. italica*, *B. parasita*, *B. hexadonta*, *B. astaci* and *Xironogiton victoriensis*. Species-specific microlocations of the different branchiobdellidan species and their cocoons on the crayfish body are described. The wider type locality of the endemic subspecies *Branchiobdella balcanica sketi* was re-surveyed, and fresh samples were obtained. Based on morphological analysis of these samples, we formally reject the subspecies status of *B. balcanica sketi*.

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KEY WORDS:
Branchiobdellida, crayfish, distribution, symbiotic interactions, *B. balcanica sketi*, Western Balkan

IZVLEČEK

Diverziteta, razširjenost in gostitelji branhiobdelidov (Clitellata: Branchiobdellida) na zahodnem Balkanu

Članek obravnava vzorce branhiobdelidov ali račjih pijavčic z gostiteljskih rakov deseteronožcev, nabrane na 60 lokalitetah na območju zahodnega Balkana (Slovenija, Hrvaška, Bosna in Hercegovina, Črna gora, Makedonija, Srbija in Bolgarija). Vzorci so bili pridobljeni iz zoološke zbirke Oddelka za biologijo Biotehniške fakultete Univerze v Ljubljani ter z nedavnim terenskim delom. Ugotovljenih je bilo sedem vrst branhiobdelidov: *Branchiobdella balcanica*, *B. pentadonta*, *B. italica*, *B. parasita*, *B. hexadonta*, *B. astaci* in tujerodna *Xironogiton victoriensis*. Opisana so vrstno specifična mesta pričvrščanja odraslih branhiobdelidov in njihovih kokonov na telesu gostiteljev. Podrobno je bilo pregledano širše območje tipske lokalitete endemičnega taksona *Branchiobdella balcanica sketi*. Morfološka analiza novih vzorcev je razkrila, da razlikovalni znaki niso zanesljivi, zaradi česar je predlagana formalna ukinitev statusa samostojne podvrste za *B. balcanica sketi*.

KEY WORDS:
Branchiobdellida, potočni raki, razširjenost, simbiotske interakcije, *B. balcanica sketi*, zahodni Balkan

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INTRODUCTION

The Balkan Peninsula encompasses several biogeographical regions with a complex geological history and a variety of aquatic habitats. Its river network contributes to the drainage basins of the Black, Adriatic and Aegean Seas. This area is known for its high freshwater biodiversity and endemism (Bănărescu 2004; Wilke et al. 2010; Ivković & Plant 2015). The Western Balkan region, with its numerous small Adriatic river catchments and fragmented network of surface water bodies in the Dinaric Karst, hosts a rich freshwater crayfish fauna, both in terms of nominal taxa and highly divergent genetic lineages (Trontelj et al. 2005; Klobučar et al. 2013). Likewise, the Western Balkan fauna of crayfish-associated commensals, symbionts and parasites – the branchiobdellidan worms (Annelida: Branchiobdellida) – is diverse and relatively well explored (Subchev 2014).

Branchiobdellidans are a group of freshwater leech-like clitellates characterized by the possession of a peristomium (»head« with lips) and a posterior sucker disk (Fig. 1A). They are found mainly within the Holarctic, reflecting the geographical distribution of their hosts – crayfish in the family Astacidae. The diversity and distribution of European branchiobdellidans have been extensively studied, including Subchev's (2014) comprehensive review of all European species. Bláha et al. (2017) examined their associations with non-native crayfish, while Vedia et al. (2016) analyzed their distribution patterns relative to host crayfish ranges.

The species diversity of Balkan branchiobdellidans, their geographical distribution and host associations have been reported in several studies. The first report on the branchiobdellidans of former Yugoslavia is that of Moszyński (1938), who mentioned the presence of five European species and described three new species from Kosovska Mitrovica: *Branchiobdella balcanica*, *B. insolita* and *Pterodrilus karamani*. The latter two species were later synonymized with *B. balcanica* and *B. parasita*, respectively (Pop 1965). Georgévitch (1957) described 30 new species from Lake Dojran and other fresh waters of Yugoslavia, though Pop (1965) subsequently reduced that diversity to four species, including three subspecies (*B. astaci*, *B. parasita*, *B. hexadonta*, *B. pentodonta pentodonta*, *B. pentodonta orientalis*, and *B. pentodonta italica*). However, Karaman (1967, 1970) considered *B. pentodonta orientalis* a junior synonym of *B. balcanica balcanica* and reclassified the other two subspecies as *B. pentodonta* and *B. italica*. Karaman (1967) also described a new subspecies, *Branchiobdella balcanica sketi*, from Slovenia, which remains known only from its type locality and a subsequent report from Poland (Śmietana & Wierzbicka 1999). Further records from Slovenia were documented in the collections of the Vienna Natural History Museum (Subchev & Gelder 2010), while Füreder et al. (2009) reported branchiobdellidan species from Austria and Italy. Klobučar et al. (2006), Šarić et al. (2018) and Dražina et al. (2018) contributed to the knowledge of the branchiobdellidan fauna of Croatia. According to the literature, the Western Balkans harbour six of the nine known indigenous European branchiobdellidan species, with only *B. kozarovi* Subchev, 1978, *B. papillosa* Nesemann & Hutter, 2002 and *B. bulgariensis* Subchev & Rimcheska, 2021 not recorded from this region.

In this study we mostly focus on the part of the Western Balkan region that lies within the historical political boundaries of former Yugoslavia. Hydrologically, this region encompasses the Black Sea basin (drained by Danube tributaries such as

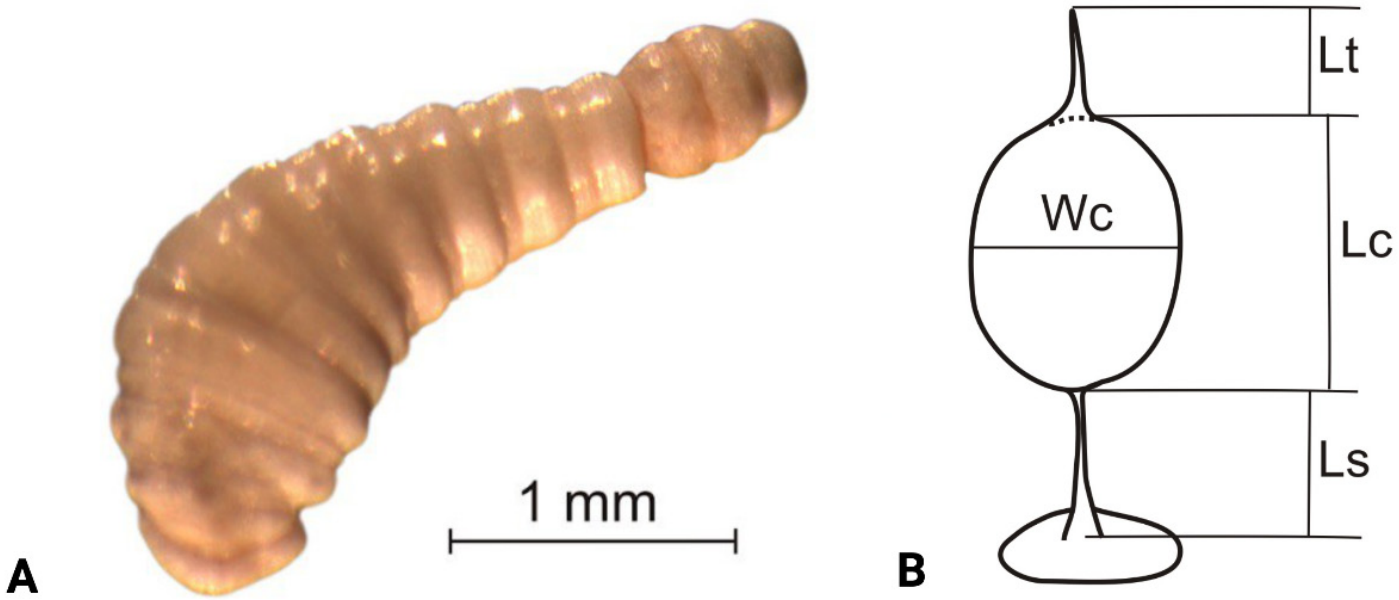

Figure 1. General view of an adult branchiobdellidan belonging to the species *Branchiobdella hexadonta* (A). Illustration of branchiobdellidan cocoon (B) with dimensions reported in this paper: Lc – capsule length, Lt – cap length, Ls – stem length, Wc – cocoon width. Figures A and B have different scales; for cocoon dimensions see Results (photo: M. Shrestkha).

Slika 1. Fotografija odraslega branhiobdelida vrste *Branchiobdella hexadonta* (A). Ilustracija kokona branhiobdelidov (B) z merami: Lc – dolžina kapsule, Lt – dolžina čepice, Ls – dolžina peclja, Wc – širina kokona. Sliki A in B imata različno merilo; mere kokonov so razvidne iz opisov v poglavju Results.

the Sava and Drava Rivers), the Adriatic Sea drainage basins (including the Reka, Zrmanja, Cetina, and Neretva Rivers) and the Aegean Sea basin (drained by the Vardar River). We present new records from existing crayfish collections at the University of Ljubljana and from recent fieldwork, using these data to revise and update species diversity, distribution, morphology, taxonomy and host associations of the region's branchiobdellidan fauna.

MATERIALS AND METHODS

We primarily studied the Zoological Collection of the Department of Biology at the Biotechnical Faculty of the University of Ljubljana (Zoološka zbirka Oddelka za biologijo Biotehniške fakultete Univerze v Ljubljani). Samples date from 1929 to 2018. Further field sampling was carried out by the authors in Slovenia, focused on sites critical for clarifying the taxonomic and distributional status of *Branchiobdella balcanica sketi*. Site selection was informed by historical records of branchiobdellidan presence and accessibility to complement gaps in museum data. In total, 207 samples of different crayfish species from 60 localities across Slovenia, Croatia, Montenegro, Bosnia and Herzegovina, Serbia, Bulgaria and North Macedonia were included in this study (Fig. 2, Tab. 1). Precise coordinates of new collections were recorded using a global positioning system (GPS) device. Sampling sites in museum collection were identified from their labels, and the Google Earth (version 4.3) online application (http://earth.google.com/) was used to determine coordinates. The record points were mapped using ArcGIS 10.1. and QGIS 3.34.

Crayfish samples were mostly preserved in 96% ethanol, with a few older samples stored in 4% formaldehyde. Branchiobdellidans were removed from the crayfish when still *in situ*, or picked from the containers containing the crayfish when dislodged. The taxonomic identification of branchiobdellidans is primarily based on a combined analysis of morphological features, including jaw structure, the number of denticles, external morphology and reproductive organs. These characteristics serve as distinguishing traits for species that may be subject to taxonomic dispute (Chekanovskaya 1962; Karaman 1970; Kozarov 1972; Boshko 1983; Gelder 1994; Nesemann & Neubert 1999). Specimens selected for identification were mounted on slides in glycerin under a cover glass. Since slide mounting permits observation from only one viewing angle, we examined multiple specimens to verify all diagnostic characters. For ambiguous cases, dissected reproductive organs were preserved in ethanol (96%) alongside the original specimen to enable future observation and DNA analysis. The reproductive system was examined in cases where the external morphology and the structure of the jaws were insufficient to identify the specimens. Microscopic examination of the jaws and details of the reproductive system was carried out using an AxioImager Z1 (Carl Zeiss). In order to examine morphological details of the cocoons, parts of the host carapace and gills were removed and placed in tubes with 96% ethanol and further examined under the microscope. We provide detailed descriptions of the cocoons of branchiobdellidans, including their dimensions (Fig. 1B). Cocoon location on the host was recorded for each branchiobdellidan species.

Table 1. Overview of sampling sites. Abbreviations for countries (ISO-3): BGR – Bulgaria, BIH – Bosnia and Herzegovina, HRV – Croatia, MKD – North Macedonia, MNE – Montenegro, SRB – Serbia, SVN – Slovenia.

Tabela 1. Pregled obravnavanih lokalitet. Okrajšave držav (ISO-3): BGR – Bolgarija, BIH – Bosna in Hercegovina, HRV – Hrvaška, MKD – Makedonija, MNE – Črna gora, SRB – Srbija, SVN – Slovenija.

Site No.	Country	Sampling site	Lat. (N)	Long. (E)	Date	Collector
1	BGR	River Timok, Bregovo	42.319722	19.148055	22. 8. 1965	unspecified
2	BIH	Stream near Uvir Cave, Kladanj	44.223611	18.674722	22. 7. 2004	P. Trontelj
3	BIH	Živašnica stream, Begića Česma, Konjic	43.675625	17.984746	1. 10. 1968	unspecified
4	BIH	Source of Sanica River, Sanica	44.616111	16.660277	10. 1. 1969	unspecified
5	BIH	Veliki ponor, Livno	43.825833	17.002500	unspecified	unspecified
6	BIH	Šuica River, Tomislavgrad (Duvno)	43.697478	17.258354	9. 1. 1987	unspecified
7	HRV	Lake Vrana, Cres Island	44.865555	14.388333	23. 9. 1997	unspecified
8	HRV	Stream near Butoniga, Pazin, Istra	45.310833	13.929722	25. 5. 1996	Mrakovčič
9	HRV	Lake Modro Oko, Rogotin, Ploče	43.045555	17.474166	1. 10. 2000	C. Fišer
10	HRV	Mirna River near Škuljari, Buzet, Istra	45.401780	13.900681	28. 4. 2001	B. Sket
11	HRV	Ljuta River, Konavoski Dvori, Dubrovnik	42.529975	18.368582	20. 9. 2000	C. Fišer
12	MKD	Stream near Izbište, Resen	41.127222	21.002500	16. 7. 2008	M. Bedjanič
13	MKD	Osojnica stream, 2.5 km NW of Laki village, Berovo	41.807777	22.646111	26. 7. 2008	M. Bedjanič

Site No.	Country	Sampling site	Lat. (N)	Long. (E)	Date	Collector
14	MNE	River Rijeka Crnojevića at Rijeka Crnojevića village, W part of Lake Skadar	42.355042	19.029075	30. 7. 2002	P. Trontelj
15	SRB	Riorska Reka, Valjevo	44.131666	20.103888	1. 10. 2009	unspecified
16	SVN	Lake Vučja Jama, near Lipovci, Beltinci	46.631666	16.223055	20. 9. 1993	unspecified
17	SVN	Stream Bača, Baška grapa, Podbrdo	46.201591	13.950899	1. 5. 2001	B. Sket
18	HRV	River Pazinčica, Pazin, Istra	45.248055	13.944444	1. 9. 1960	B. Sket
19	SVN	River Drava near Šturmovci, Ptuj	46.369386	15.945316	1. 7. 1975	unspecified
20	SVN	Stream Kožbanjšček, Neblo, Nova Gorica	46.008910	13.504900	30. 7. 1967	unspecified
21	SVN	Rižana River, Koper	45.551383	13.773090	6. 6. 1975	unspecified
22	SVN	Slovenia, unspecified	—	—	unspecified	unspecified
23	SVN	Belica stream, Osilnica, Kočevje	45.567500	14.714166	6. 9. 2001	C. Fišer
24	SVN	Creek near Bohinjska Bela, Bled	46.339440	14.058125	14. 10. 2000	C. Fišer
25	SVN	Lonjerce stream, Mlake, Vipava	45.821719	13.964592	15. 6. 2001	G. Bračko
26	SVN	Kozmanjka stream at Karlovica, Velike Lašče	45.803059	14.582214	11. 8. 1971	unspecified
27	SVN	Sušački potok at Novokrajska Jama, Ilirska Bistrica	45.491388	14.301388	unspecified	B. Sket, F. Velkovrh
28	SVN	Trnava stream, Silova, Velenje	46.324699	15.135485	29. 6. 1989	unspecified
29	SVN	Osapska reka, Osp, Koper	45.573055	13.849166	16. 6. 2003	C. Fišer
30	SVN	Lovšetov potok, Ribče, Litija	46.103011	14.764857	20. 4. 1980	unspecified
31	SVN	Martinjščica River, Lake Cerknica	45.774120	14.402164	1. 4. 1968	unspecified
32	SVN	Unica River on Planinsko polje, Planina	45.841944	14.283055	1. 6. 1966	B. Sket
33	SVN	Pendirjevka stream, Cerov Log, Šentjernej	45.797392	15.314138	14. 5. 2005	M. Bedjanič
34	SVN	Zala stream, Godovič, Idrija	45.970362	14.064909	17. 9. 2000	B. Sket
35	SVN	Stream at Rakitna Lake, Rakitna	45.884615	14.433062	16. 9. 2000	B. Sket
36	SVN	Grivački potok, Grivac, Kočevje	45.467500	14.843055	25. 10. 2004	P. Trontelj, F. Kljun
37	SVN	Tršovka stream, Poljane, Cerkno	46.140555	14.012500	24. 9. 2000	B. Sket
38	SVN	Iška River, Iški Vintgar, Ljubljana	45.930277	14.517222	23. 9. 2000	B. Sket
39	SVN	Nežica stream, Fara, Kočevje	45.478808	14.881217	25. 10. 2004	P. Trontelj, F. Kljun
40	SVN	Petkovšca stream, Zaplana, Logatec	45.965280	14.208846	24. 10. 2000	C. Fišer
41	SVN	Hotenjka stream, Hotedršica, Logatec	45.930476	14.147552	17. 9. 2000	B. Sket
42	SVN	Jamski zaliv, River Cerkniščica, Cerknica	45.784722	14.338611	20. 10. 1969	unspecified
43	SVN	Stream Bela, Vrhnika	45.962086	14.270597	1. 8. 1990	unspecified
44	SVN	Zala River, Cerknica	45.871878	14.479669	unspecified	unspecified
45	SVN	Zala stream, Godovič, Idrija	46.183055	14.104722	28. 9. 2009	M. Kolesnykova, L. Kebe
46	SVN	Kolpa River near Dolenja Žaga, Kočevje	45.516666	14.912777	22. 7. 2002	C. Fišer
47	SVN	Unspecified pond in Logatec	45.920601	14.202916	1993	unspecified
48	SVN	Nevljica River near Buč, Kamnik	46.215564	14.717009	1955	unspecified
49	SVN	Glinščica stream, Ljubljana	46.065911	14.453323	unspecified	B. Sket

Site No.	Country	Sampling site	Lat. (N)	Long. (E)	Date	Collector
50	SVN	Glinščica stream, concrete canal, Ljubljana	46.052510	14.462085	4. 10. 2000	C. Fišer
51	SVN	Bloščica River, Ravnik, Cerknica	45.801933	14.509333	2. 9. 2009	P. Trontelj, M. Kolesnykova
52	SVN	Bloščica River, Volčje, Cerknica	45.794811	14.506018	21. 5. 2018	M. Shrestkha, L. Kebe
53	SVN	Šmartinsko jezero, Celje	46.275000	15.261666	1. 5. 1979	unspecified
54	SVN	Mura River, Gornja Radgona	46.660883	16.026041	28. 9. 2003	U. Arnuš
55	SVN	Drava River, Maribor	46.538888	15.715833	8. 10. 2009	M. Kolesnykova
56	SVN	Rateški potok, Petelinjek, Novo mesto	45.809166	15.237777	17. 3. 2002	R. Verovnik, C. Fišer
57	SVN	Spring near Osp, Koper	45.571388	13.853055	12. 9. 2011	C. Fišer
58	SVN	Cave Velika Karlovica, Cerkniščica River, Cerknica	45.775000	14.325000	21. 5. 1969	unspecified
59	SVN	Postojna Cave, Tartar canal, Postojna	45.782667	14.203468	1. 2. 1973	unspecified
60	SVN	Dragonja River, Sečovlje	45.476944	13.616388	1. 5. 1979	unspecified

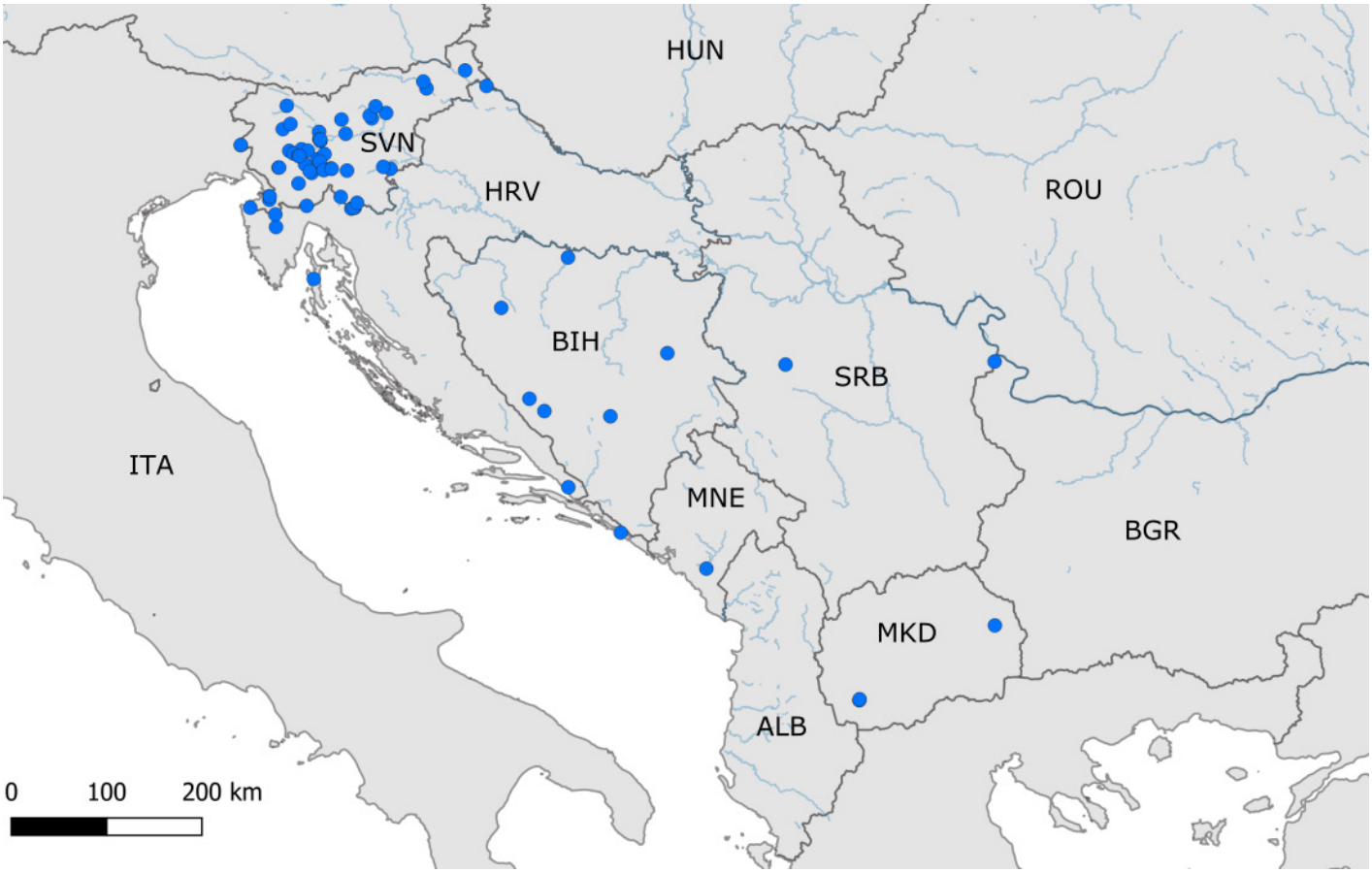

Figure 2. Map illustrating the geographical sites of branchiobdellidan samples analyzed in this study within the Western Balkan region. ISO country codes: ALB – Albania, BGR – Bulgaria, BIH – Bosnia and Herzegovina, HRV – Croatia, HUN – Hungary, ITA – Italy, MKD – North Macedonia, MNE – Montenegro, ROU – Romania, SRB – Serbia, SVN – Slovenia.

Slika 2. Prikaz obravnavanih najdišč branhiodbelidov. Okrajšave držav (ISO): ALB – Albanija, BGR – Bolgarija, BIH – Bosna in Hercegovina, HRV – Hrvaška, HUN – Madžarska, ITA – Italija, MKD – Makedonija, MNE – Črna gora, ROU – Romunija, SRB – Srbija, SVN – Slovenija.

RESULTS

SAMPLES

The examined crayfish belonged to four species: the native *Astacus astacus* (Linnaeus, 1758), *Austropotamobius torrentium* (Schrank, 1803) and *Austropotamobius pallipes* (Lereboullet, 1858), and the introduced North American *Pacifastacus leniusculus* (Dana, 1852). The presence of *P. leniusculus* was documented in this study at two localities in north-east Slovenia. Six European species of branchiobdellidans, *B. balcanica*, *B. pentadonta*, *B. italica*, *B. parasita*, *B. hexadonta*, *B. astaci*, and the non-indigenous North American species *X. victoriensis* were found on fresh or museum samples. We recorded the location of the cocoons of different branchiobdellidan species on the crayfish body (Figs. 3 and 4). Details of the geographical distribution of crayfish and associated branchiobdellidans are given in Tabs. 1 and 2.

Table 2. Occurrence of branchiobdellidan species on different crayfish hosts (* denotes that only cocoons were found).

Tabela 2. Pojavljanje branhiobdelidov na različnih gostiteljskih vrstah sladkovodnih deseteronožcev (* označuje vzorce, na katerih so bili najdeni le kokoni).

Site No.	Country	Crayfish species	Branchiobdellidan species
1	BGR	*Au. torrentium*	*B. parasita**
2	BIH	*Au. pallipes*	*B.pentadonta & B. hexadonta*
3	BIH	*Au. torrentium*	*B.pentadonta*, B. astaci, B. hexadonta*
4	BIH	*Au. torrentium*	*B.pentadonta & B. hexadonta*
5	BIH	*As. astacus*	*B. hexadonta**
6	BIH	*As. astacus*	*B.pentadonta*
7	HRV	*Au. pallipes*	—
8	HRV	*Au. pallipes*	—
9	HRV	*Au. pallipes*	*B. italica*
10	HRV	*Au. pallipes*	*B. italica*
11	HRV	*Au. pallipes*	*B. italica*
12	MKD	*Au. torrentium*	*B. parasita & B. astaci*
13	MKD	*Au. torrentium*	*B. parasita & B. pentadonta*
14	MNE	*Au. pallipes*	*B. italica*
15	SRB	*unspecified*	*B. parasita, B. pentadonta*
16	SVN	*As. astacus*	*B. parasita*
17	SVN	*Au. torrentium*	—
18	HRV	*Au. pallipes*	*B. astaci*
19	SVN	*As. astacus*	*B. parasita, B. hexadonta, B. italica, B. astaci*
20	SVN	*Au. pallipes*	*B. parasita & B. astaci*
21	SVN	*Au. pallipes*	*B. astaci*
22	SVN	*Au. pallipes*	*B. parasita & B. hexadonta*
23	SVN	*Au. torrentium*	*B. pentadonta*
24	SVN	*Au. torrentium*	*B. pentadonta & B. hexadonta*
25	SVN	*Au. pallipes*	*B. hexadonta & B. italica*
26	SVN	*Au. torrentium*	*B. astaci*
27	SVN	*Au. pallipes*	—
28	SVN	*Au. torrentium*	*B. parasita & B. pentadonta*
29	SVN	*Au. pallipes*	*B. italica*
30	SVN	*Au. torrentium*	*B. parasita**
31	SVN	*Au. torrentium*	*B. parasita*
32	SVN	*Au. torrentium*	*B. parasita & B. pentadonta*
33	SVN	*Au. torrentium*	—
34	SVN	*Au. torrentium*	—

Site No.	Country	Crayfish species	Branchiobdellidan species
35	SVN	*Au. torrentium*	—
36	SVN	*Au. torrentium*	*B. pentadonta*
37	SVN	*Au. torrentium*	*B. parasita*
38	SVN	*Au. torrentium*	—
39	SVN	*Au. torrentium*	*B. pentadonta & B. hexadonta*
40	SVN	*Au. torrentium*	*B. parasita*, *B. hexadonta*, *B. italica*, *B. balcanica*
41	SVN	*Au. torrentium*	*B. hexadonta & B. italica*
42	SVN	*Au. torrentium*	*B. astaci*
43	SVN	*Au. torrentium*	*B. parasita*
44	SVN	*Au. torrentium*	*B. parasita*
45	SVN	*Au. torrentium*	*B. parasita & B. pentadonta*
46	SVN	*Au. torrentium*	*B. hexadonta*
47	SVN	*As. astacus*	*B. parasita & B. pentadonta*
48	SVN	*As. astacus*	*B. pentadonta*
49	SVN	*Au. torrentium*	*B. pentadonta*
50	SVN	*Au. torrentium*	*B. parasita*
51	SVN	*As. astacus*	*B. parasita & B. balcanica*
52	SVN	*As. astacus*	*B. hexadonta & B. balcanica*
53	SVN	*As. astacus*	*B. parasita*
54	SVN	*P. leniusculus*	*X. victoriensis*
55	SVN	*P. leniusculus*	*X. victoriensis*
56	SVN	*unspecified*	*B. hexadonta*
57	SVN	*Au. pallipes*	*B. pentadonta*
58	SVN	*Au. torrentium*	*B. parasita*
59	SVN	*unspecified*	*B. parasita*
60	SVN	*Au. pallipes*	*B. pentadonta*

OVERVIEW OF SPECIES

Branchiobdella hexadonta Gruber, 1883

Cocoon description and location: Cocoon capsule oval-shaped with a brown cap (Fig. 3). Capsule length (Lc) 0.2 mm, capsule width (Wc) 0.18 mm, stem length (Ls) 0.1 mm (N = 6). Cocoons were not found dislodged in crayfish containers but only within the gill chambers, specifically attached to the gill filaments. Some filaments showed noticeable damage (Fig. 3), and gill fragments were present within the gut contents of this species.

Distribution: Western Palaearctic. In the Western Balkans, this species was reported for the first time in Croatia by Klobučar et al. (2006) and in Montenegro by Gelder (1999). Museum specimens from Croatia (»Plitvice« in 1939) and Montenegro (near Nikšić, Zeta River, 1998) are present in the collection of the Swedish Museum of Natural History (Subchev 2009). Recent studies have reported frequent occurrence of this species on *Au. torrentium* and *Au. pallipes* in Croatia (Šarič et al. 2018). The first report of this species in North Macedonia was from Lake Dojran (Karaman 1967). Later, Subchev (2007) noted its presence in museum samples collected in North Macedonia, but with no detailed sampling data. Samples from Bosnia and Herzegovina in the crayfish collection of the Natural History Museum in Vienna (Austria) included branchiobdellidans associated with *Au. pallipes*, but also with no precise information on their geographical sites (Subchev & Gelder 2010). The first record for Bosnia and Herzegovina (BIH) was from the western part of the country, in the Glamoč area (Karaman 1967). Our examination of the collection at the University of Ljubljana confirmed previous records of *B. hexadonta* from BIH (stream near Uvir, Kladanj, 2004). We also confirmed a number of sites in Slovenia: Drava River (1975), a stream near Bohinjska Bela, Bled (2000), a stream near Zaplana (2000), the Hotenjka stream (2000), the waterfall Nežica next to the Kolpa River (2004) and the Kolpa River itself (2002) (see Tab. 1). Previously, the only Slovenian record of this species was that of Karaman (1967), from the small river Vrhniški Obrh in southern Slovenia.

Host association and branchiobdellidan co-occurrence: *As. astacus*, *Au. torrentium*, *Au. pallipes* alone and coexisting with the following branchiobdellidans: *B. pentadonta*, *B. astaci*, *B. parasita*, *B. italica*, *B. balcanica* (Tab. 3).

Branchiobdella parasita (Braun, 1805)

Cocoon description and location: Cocoon ovoid in shape, widest at the apex. Capsule length (Lc) 0.4–0.6 mm, capsule width (Wc) 0.28–0.36 mm, stem length (Ls) 0.24–0.27 mm, about on half of capsule length (N = 30). Cocoon cap flat (Fig. 3). Cocoons located latero-posteriorly on crayfish cephalothorax (usually symmetrically on both sides). Where cocoons were already present on the lateral cephalothorax, other cocoons could be present on the second to fourth pleopods (Fig. 3). The pattern of location was the same on different host species.

Distribution: *Branchiobdella parasita* is widely distributed in Europe. It was recorded by Karaman (1967) from the Western Balkans in Serbia (Kosovska Mitrovica), BIH and Slovenia. We recorded this species in Serbia (Riorska reka), in the Osojnica stream in Berovo and in Lake Resen (Prespa; North Macedonia). It has previously been reported from North Macedonia in Lake Dojran (Karaman 1967). We present new data on the wide distribution of this species in Slovenia (Tab. 1).

Host association and branchiobdellidan co-occurrence: *As. astacus*, *Au. torrentium*, *Au. pallipes* alone and coexisting with the following branchiobdellidans: *B. pentadonta*, *B. astaci*, *B. hexadonta*, *B. italica*, *B. balcanica* (Tab. 3).

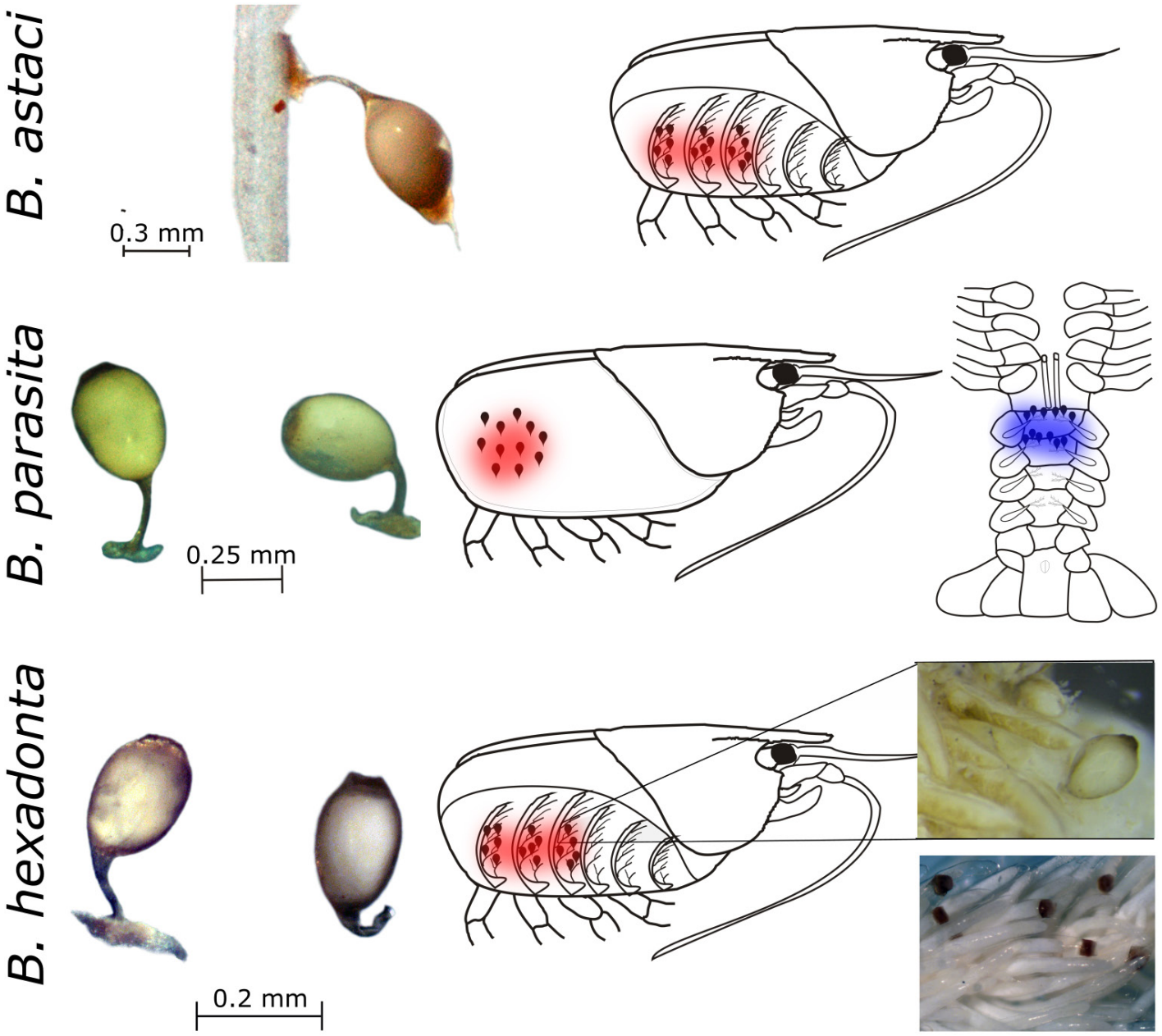

Figure 3. Structure and location of cocoons of *B. astaci*, *B. parasita* and *B. hexadonta* on their crayfish hosts. Primary cocoon locations are marked in red »🡇«, while secondary locations (observed during high infestation) are indicated in blue »🡇« (photos: M. Shrestkha).

Slika 3. Zgradba in lokacija kokonov vrst *B. astaci*, *B. parasita* in *B. hexadonta* na telesu gostiteljev. Oznaka »🡇« na rdeči podlagi označuje glavno mesto pričvrščanja kokonov, oznaka »🡇« na modri podlagi pa dodatno mesto pričvrščanja v primeru močne infestiranosti.

Branchiobdella astaci Odier, 1823

Cocoon description and location: Cocoon oval or fusiform. Capsule length (Lc) 0.43–0.50 mm, capsule width (Wc) 0.25–0.30 mm (N = 30). Cocoon stem thin, longer than half the capsule length; cocoon cap spiky (Fig. 3). Cocoons located symmetrically on both sides of the host gill, covering middle parts of central and posterior gill filaments (Fig. 3).

Distribution: In the Western Balkans this species is known from Serbia (Ibar River at Kosovska Mitrovica), Montenegro and Dalmatia (Cetina) (Moszyński 1938). In 1929, material of this species of unknown provenance was deposited in the French National Museum (Subchev 2008); similarly, a specimen from North Macedonia in the Humboldt Museum lacks a detailed label (Subchev 2007). In Croatia (Klobučar et al. 2006), it has been reported in Istria on *As. astacus* (Borutski stream, Dausi, Pazin) and *Au. pallipes* (Lake Vransko on Island Cres and Racice, Buzet) (Klobučar et al. 2006; Šarič et al. 2018). We report new sites for this species in Croatia (Lake Modro Oko and a stream in the Dalmatian Region) and North Macedonia (south-western part of Lake Resen). All samples examined from Slovenia were historical, with collection dates as follows: 1955 (River Nevljica, Buč), 1960 (River Fojba), 1967 (Kožbanjšček stream, Neblo), 1969 (Lake Cerknica, Rakovski mostek), 1971 (Karlovica), and 1975 (River Drava; River Rižana). One locality (Glinščica, Ljubljana) lacked date information on its label. (Tab. 1).

Host association and branchiobdellidan co-occurrence: *Au. torrentium*, *Au. pallipes* (alone and coexisting with *B. parasita*); *Au. torrentium* (coexisting with *B. pentadonta*); *As. astacus* (coexisting with *B. parasita*, *B. hexadonta*, *B. italica* (Tab. 3).

Branchiobdella pentadonta Whitman, 1882

Cocoon description and location: Cocoon rounded-oval (Fig. 4). Capsule length (Lc) 0.3–0.5 mm, capsule width (Wc) 0.27–0.34 mm, cocoon stem (Ls) short and thick, 0.07–0.11 mm long, cocoon cap (Lt) flattened with a small spine, 0.02–0.04 mm long (N = 30). Cocoons attached to central part of cervical furrow and ventro-posterior part of carapace, rostrum tip, maxillipeds, base of antennae, outer and inner sides of claw bends and at base of claws (Fig. 4).

Distribution: Widely distributed throughout the Danube Basin. Karaman (1970) recorded this species in the Balkans from southern North Macedonia (near Konsko), western Serbia, and the River Sana in western BIH. Šarić et al. (2018) recorded this species in Croatia. We provide new records of *B. pentadonta* in eastern North Macedonia (the Osojnica stream in Berovo), and in eastern and southern BIH (streams near Kladanj and Konjic). We also recorded this species from Croatia, BIH (stream near Uvir, Kladanj), Montenegro (River Reka Crnojevića and Lake Skadar), Serbia (Riorska Reka) and Slovenia (Tab. 1). There is one historical report from north-western Slovenia (Bled) (Karaman 1967).

Host association and branchiobdellidan co-occurrence: *As. astacus*, *Au. torrentium*, *Au. pallipes* alone and coexisting with the following branchiobdellidans: *B. parasita*, *B. astaci*, *B. hexadonta*, *B. balcanica* (Tab. 3).

Branchiobdella italica Canegallo, 1928

Cocoon description and location: Cocoon oval (Fig. 4). Capsule length (Lc) 0.32–0.40 mm, capsule width (Wc) 0.22–0.27 mm, cocoon stem (Ls) short and thick, 0.06–0.12 mm long; cocoon cap (Lt) with small spine, 0.01–0.06 mm long (N = 30). Cocoons located along the edge of the latero-posterior part of the carapace, sometimes covering the first abdominal segment. Also on the cervical groove, in claw bends, at the base of antennae and maxillipeds (Fig. 4).

Distribution: The first report of this species was that of Canegallo (1928) in northern Italy. Karaman (1967) recorded it in Yugoslavia and from the Adriatic coast region of Croatia. Recent studies have reported it associated with *Au. pallipes* and *Procambarus clarkii* in northern Italy (Gelder et al. 1999). Klobučar et al. (2006) and Šarić et al. (2018) presented more detailed information on the distribution of this species in Croatia. We provide new records of this species from Croatia, Slovenia and BIH (Tab. 1).

Host association and branchiobdellidan co-occurrence: *Au. pallipes*, *Au. torrentium*, *As. astacus* alone and coexisting with *B. hexadonta*, *B. parasita*, *B. astaci*, *B. balcanica* (Tab. 3).

Branchiobdella balcanica Moszynski, 1938

Cocoon description and location: Cocoon flask-shaped (Fig. 4). Capsule length (Lc) 0.25–0.38 mm, capsule width (Wc) 0.2–0.3 mm, cocoon stem (Ls) short, 0.07–0.10 mm long, broadening toward capsule base; cocoon cap (Lt) mitriform, length 0.1–0.15 mm, almost equal to or exceeding stem length (N = 30). Cocoons present on the inner side of claws and their bends, along the edge of the lateral side of the carapace, at the base of pereopods, on the lower part of the cervical groove of the carapace and on pleopods. Cocoons occasionally attached to the base of antennae and maxillipeds (Fig. 4).

Distribution: This species was described by Moszyński (1938) from crayfish from the Ibar River near Kosovska Mitrovica in Serbia. Later, it was recorded from rivers and streams in Romania, Hungary (Subchev 1984) and former Yugoslavia (North Macedonia, Serbia and Slovenia) (Moszyński 1938; Georgevitch 1957). Klobučar et al. (2006) reported this species in Croatia on *As. astacus* and Kovačs & Juhász (2007) recorded it from Hungary on *As. astacus* and *Au. torrentium*. We report this species from three new localities in Slovenia on *As. astacus* and from one locality on *Au. torrentium* (Zaplana, Logatec) (Tab. 1).

Host association: *As. astacus*, *Au. torrentium* coexisting with *B. hexadonta*, *B. parasita*, *B. italica*, *B. pentadonta* (Tab. 3).

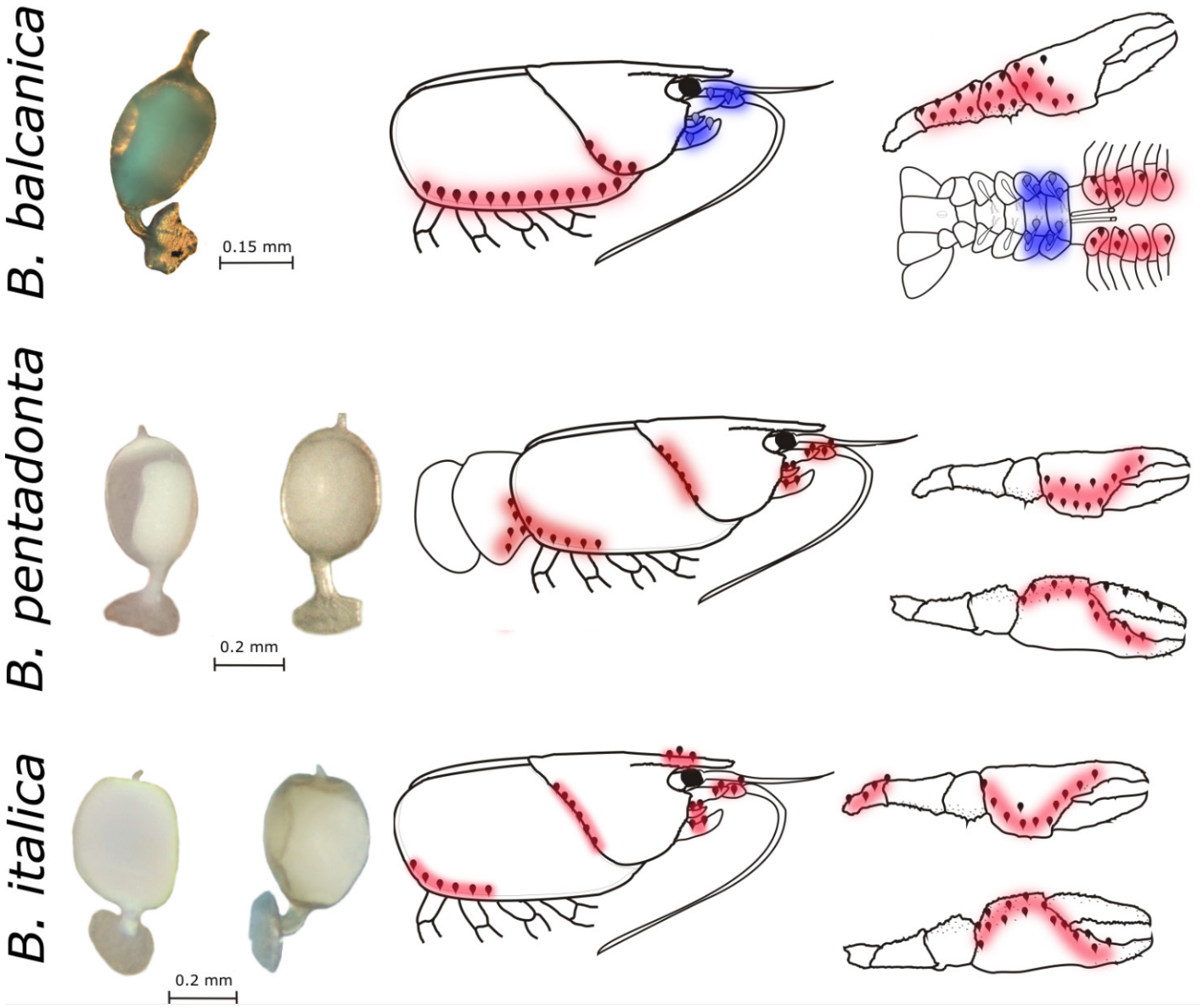

Figure 4. Structure and location of cocoons of *B. balcanica*, *B. pentadonta* and *B. italica* on their crayfish hosts. Primary cocoon locations are marked in red »♀«, while secondary locations (observed during high infestation) are indicated in blue »♀« (photos: M. Shrestkha).

Slika 4. Zgradba in lokacija kokonov vrst *B. balcanica*, *B. pentadonta* in *B. italica* na telesu gostiteljev. Oznaka »♀« na rdeči podlagi označuje glavno mesto pričvrščanja kokonov, oznaka »♀« na modri podlagi pa dodatno mesto pričvrščanja v primeru močne infestiranosti.

Xironogiton victoriensis Gelder & Hall, 1990

Cocoon description and localization: Cocoons were not observed.

Distribution: *Xironogiton victoriensis* is native to the western drainage catchments of Canada and the USA (Gelder 1990), where it is widely distributed. This species was introduced to Europe with the signal crayfish, *Pacifastacus leniusculus* (Dana, 1852). The first population of *X. victoriensis* in Europe was reported in Sweden, initially identified as *X. instabilis* Moore, 1894 (Franzén 1962). Surveys in northern Spain have since reported *X. victoriensis* on *P. leniusculus* (Gelder 1999; Oscoz et al. 2010) and *Procambarus clarkii* (Vedia et al. 2014). *Xironogiton victoriensis* has been reported from Auenbachl in Northern Italy (Quaglio et al. 2002) and from the River Lot, a tributary of the River Garonne, in south-western France (Laurent 2007). We report the first records of this species in Slovenian fresh waters in the River Mura (Gornja Radgona) and River Drava (Maribor) (Tab. 1).

Host association: *Pacifastacus leniusculus*

Taxonomic considerations relating to *Branchiobdella balcanica sketi* Karaman, 1967

The subspecies *B. balcanica sketi* is currently known from its single type locality in the Western Balkan area and a later record from Poland (Śmietana & Wierzbicka 1999). The latter record does not include detailed morphological information and cannot be confirmed. This subspecies was described by Karaman (1967) from Loško polje, specifically from a river he named »Verhniski Obrh« and that is nowadays known as Veliki Obrh. Further illustrations of the same specimens were published by Nesemann & Neubert (1999). We examined historical material collected from the Lake Cerknica area (Lake Cerknica,

Table 3. Number of documented cases of single occurrence or multiple co-occurring branchiobdellidan species on different crayfish hosts in the Balkans.

Tabela 3. Število dokumentiranih pojavljanj posameznih ali več so-pojavljajočih se vrst branhiobdelidov na gostiteljskih vrstah potočnih rakov na Balkanu.

Branchiobdellidan species	Crayfish				
	Au. torrentium	*Au. pallipes*	*As. astacus*	unspecif.	*P. leniusculus*
B. parasita	8		2	1	
B. pentadonta	3	2	2		
B. parasita + *B. pentadonta*	4		1	1	
B. italica		5			
B. astaci	2	2			
B. hexadonta	1		1	1	
B. pentadonta + *B. hexadonta*	3	1			
B. parasita + *B. astaci*	1	1			
B. italica + *B. hexadonta*	1	1			
B. parasita + *B. hexadonta*		1			
B. hexadonta + *B. balcanica*			1		
B. parasita + *B. balcanica*			1		
B. hexad. + *B. ital.* + *B. ast.* + *B. paras.*			1		
B. hexad. + *B. ital.* + *B. balc.* + *B.paras.*	1				
B. astaci + *B. pentadonta* + *B. hexadonta*	1				
Xironogiton victoriensis					2

Martinščica (1968); Lake Cerknica, Rakovski mostek (1969)) and newly collected material from an area close to the type locality. We could confirm the presence of only *B. parasita* and *B. astaci*. Additionally, we conducted a survey of the type locality from which *B. balcanica sketi* was originally described. The specimens described by Karaman were originally collected by Boris Sket, who directed us to this site. However, at that site the stream is now overgrown by vegetation and, based on informal conversation with local residents, no crayfish have been observed since 2007. Further exploration of the nearby stream of Bloščica (sites no. 51 and 52 in Tabs. 1 and 2) in 2009 and 2018 led to the discovery of *As. astacus* and associated branchiobdellidans including *B. balcanica.* According to Karaman (1967), the key difference between the two subspecies – *B. balcanica balcanica* and *B. balcanica sketi* – is the size of the copulatory bursa and the spermatheca. Our newly collected material revealed considerable variation in the size of these organs. Based on the level of variation observed, we suggest that material currently assigned to *B. balcanica sketi* lies within the range of variation that typifies *B. balcanica* as described by Moszyński (1938).

DISCUSSION

FAUNA AND HOSTS OF BRANCHIOBDELLIDANS IN THE WESTERN BALKANS

The most thorough revision available to date of branchiobdellidans of the Western Balkans is that of Karaman (1967, 1970). Subsequent studies have provided inventories of branchiobdellid collections held by some European museums (Subchev 2007, 2008, 2009, 2014; Subchev & Gelder 2010), along with recent syntheses of the branchiobdellidans of Croatia (Klobučar et al. 2006), Albania (Subchev 2011), Serbia (Živić et al. 2022) and North Macedonia (Rimcheska et al. 2014). The current study, based on thorough morphological analyses of both existing records and collections as well as new field material from multiple sites, documents the occurrence of seven branchiobdellidan species in the Western Balkans, six native species and one introduction. None of these species is endemic to the region. Our observations show that the morphological traits used to define the subspecies *B. balcanica sketi* Karaman, 1967 are inconsistent and unreliable. We therefore reject this subspecies.

Some *Branchiobdella* species recorded here appeared to be flexible in host selection and have wide geographical distributions. The parasitic gill-chamber-dwelling species *B. hexadonta* was found across all crayfish species and occurred in various combinations with all identified branchiobdellidan species (Tab. 3). The micro-predatory *B. parasita* was observed on *As. astacus* and *Au. torrentium*, but not on *Au. pallipes*, co-occurring with the ectocommensal European species *B. pentadonta*. The cohabitation of these species on the same host has been widely reported (Śmietana & Wierzbicka 1999).

The two largest branchiobdellidans, the gill-dwelling *B. astaci* and the micro-predatory *B. parasita*, were also found together on the same host, suggesting niche separation and lack of food competition between them. We identified *B. astaci* on *Au. torrentium* and *Au. pallipes* (Tab. 2, Tab. 3). A specimen of *B. astaci* was recorded on *As. astacus* collected from the River Drava in 1975. Our attempts to rediscover this species have been unsuccessful, suggesting it may now be rare. Recent findings of *B. astaci* are limited to only a few reports (Klobučar et

al. 2006; Rosewarne et al. 2012). This drop in abundance could be linked to the general decline of *As. astacus*. Similarly, the decline of the host species *As. astacus* has been reported to impact negatively its associated symbiont *B. parasita* (Szenejko et al. 2023).

In most cases, *B. balcanica* has been found associated with *As. astacus*, with only a single instance of occurrence on *Au. torrentium*. Importantly, *Au. pallipes* has not been reported as a host for *B. balcanica* in the existing literature, nor did we identify it during our examination of museum collections in this study (Tab. 3). Our findings are consistent with the recent report of Shrestkha & Utevsky (2024) that *B. balcanica* is primarily associated with *As. astacus*. In contrast, *B. italica* was predominantly attached to *Au. pallipes*, particularly on specimens from Montenegro, Croatia and Slovenia. It is noteworthy that we only detected *B. italica* on *Au. torrentium* in two specimens from Slovenia (Tab. 1, Tab. 2).

The introduction of the cultured American crayfish *Pacifastacus leniusculus* (Dana, 1852) and *Procambarus clarkii* (Girard, 1952) to Europe (Souty-Grosset et al. 2006), has resulted in the spread of the North American branchiobdellid species *Xironogiton victoriensis*. So far, this species has been recorded from Sweden, Spain, Italy, France and Czechia (Franzén 1962; Gelder 1999; Oscoz et al. 2010; Vedia et al. 2014; Quaglio et al. 2002; Laurent 2007; Blaha 2018). We present the first Slovenian records of *X. victoriensis* (Mura River at Gornja Radgona and Drava River in Maribor), on *P. leniusculus*. A potential introduction route may have been through translocation of *P. leniusculus* from Italian populations, where this symbiont is known to occur (Vedia et al. 2016).

ARRANGEMENT OF BRANCHIOBDELLIDAN COCOONS

The arrangement and characteristics of branchiobdellidan cocoons on the host body provide a useful cue for identification. Creed et al. (2015) documented that a substantial proportion of viable cocoons are typically located on the first and second abdominal somites of crayfish hosts, indicating a clear microlocation preference for cocoon deposition. This pattern may be influenced by physical characteristics of the host, including size and the availability of suitable microhabitats for developing embryos (Skelton et al. 2015). We outline several key structures that vary between species, including the shape of the cocoon capsule, the ratio of capsule length to capsule width, details of the cocoon cap, and stem length (Figs. 2 and 3). On many of the crayfish specimens examined in this study, only adult worms were present. However, on some specimens cocoons were present on different parts of the crayfish exoskeleton and gills, and were frequently loose in the containers in which the crayfish were preserved. Our observations indicate that branchiobdellidans preferentially lay cocoons on the most mobile areas of their host, specifically on the mouthparts, pleopods, pereiopods, antennae and gills, which are constantly exposed to water currents. Cocoons are often attached to the host surface via flexible stems that mitigate the effects of mechanical forces. Typically, branchiobdellidans occupy areas of the host body large enough to accommodate several dozen cocoons. However, our observations suggest that the density of cocoons can vary widely and does not necessarily correlate with the size of the area occupied. Cocoon density varies with the crayfish molt cycle, showing reductions following molting and increases prior to exoskeleton loss. The number of mature worms per host may be an additional contributing factor. However, controlled experiments will be necessary to verify these patterns.

Our observations showed that locations occupied by *B. pentadonta* and *B. italica* overlap (Fig. 4). Potentially, competition for these locations may explain why these two species were not found together on the same host individual, although, as noted above, most records of *B. italica*, including in our study, are from the single host species, *Au. pallipes*. *Branchiobdella balcanica* deposits cocoons on various sites on the host body, overlapping with those of *B. pentadonta* (Fig. 4). *Branchiobdella parasita* can be distinguished easily by the large cocoons located symmetrically latero-posteriorly on both sides of the crayfish cephalothorax (Fig. 3). No other species deposits cocoons in these locations (Kolesnykova & Utevsky 2013). The specific parasitic lifestyle of *B. parasita* may enable this species to co-occur on the same individual host with almost all Western European species of branchiobdellidans. *Branchiobdella astaci* is another parasitic species, present only in the gill chamber where it also deposits its cocoons (Fig. 3). We often found this species on crayfish that also hosted the ectocommensal *B. pentadonta*, although it seems likely that the individual worms would not interfere with each other given their specific locations. Cocoons of *B. hexadonta* were located on gill filaments (Fig. 3). Additionally, fragments of gill filaments were present within the gut contents of this species. These observations suggest that the primary habitat for adults of this species is the gill chamber, where they feed on the epithelial tissue of the gill filaments. This is consistent with previous studies that have indicated a similar feeding strategy among some branchiobdellidans (Chekanovskaya 1962). The location of cocoons in the gill chamber may indicate a reproductive strategy that maximizes offspring survival in a protected environment in proximity to a nutrient-rich food source. This ecological niche highlights the parasitic relationship between some branchiobdellidans and their crayfish hosts, emphasizing the importance of gill chambers as critical habitats for both feeding and reproduction.

POVZETEK

V članku so predstavljeni favna, razširjenost, gostiteljske vrste ter vrstno specifična mesta pričvrščanja odraslih branhiobdelidov ali račjih pijavčic in njihovih kokonov na telesu gostiteljev, kakor tudi morfološke karakteristike kokonov posameznih vrst. Poleg pregleda virov so podrobneje predstavljeni in analizirani novi podatki s 60 lokalitet na območju zahodnega Balkana (Slovenija, Hrvaška, Bosna in Hercegovina, Črna gora, Makedonija, Srbija in Bolgarija). Vzorci so bili pridobljeni iz zoološke zbirke Oddelka za biologijo Biotehniške fakultete Univerze v Ljubljani ter z nedavnim terenskim delom. Ugotovljenih je bilo sedem vrst branhiobdelidov: *Branchiobdella balcanica*, *B. pentadonta*, *B. italica*, *B. parasita*, *B. hexadonta*, *B. astaci* in tujerodna *Xironogiton victoriensis*. Slednja je nova vrsta za celotno območje, najdena pa je bila na tujerodnem signalnem raku *Pacifastacus leniusculus* v Muri pri Gorni Radgoni in v Dravi pri Mariboru. Na območju Slovenije je bilo najdenih vseh sedem vrst, s tem da *B. astaci* po letu 1975 ni bila potrjena. Primerjava diverzitete med državami znotraj obravnavanega območja zaradi zelo neenakomerne raziskanosti na tem mestu ni smiselna. S šestimi od devetih evropskih domorodnih vrst je branhiobdelidna favna zahodnega Balkana dokaj bogata, kar večinoma velja tudi za sladkovodno favno v splošnem. Nobena vrsta ni endemična. Domnevno endemične podvrste *Branchiobdella balcanica sketi*, opisane iz Obrha v Loški dolini, ni bilo mogoče najti na tipski lokaliteti. Pregled dokaj bogatega materiala z bližnje Bloške planote je pokazal, da gre pri opisanih diagnostičnih znakih najverjetneje le za variabilnost znotraj vrste *Branchiobdella balcanica*.

Pri izboru gostiteljev se je kot najbolj fleksibilna pokazala parazitska *B. hexadonta*, ki naseljuje škržno votlino vseh vrst gostiteljev in se lahko na istem gostitelju pojavlja z večino drugih vrst branhiobdelidov. Podobno fleksibilna je prav tako v škržni votlini živeča *B. astaci*, ki pa smo jo našli le na gostiteljih iz rodu *Austropotamobius* in ne na iz rodu *Astacus*, kot je znano iz literature. Mikroplenilska *B. parasita* se pojavlja na vrstah *Astacus astacus* in *Austrpotamobius torrentium* pogosto skupaj z ektokomenzalno *B. pentadonta*.

Mesta pričvrščanja kokonov na gostitelju deloma odsevajo mesto zadrževanja odraslih živali, pogosto so na telesnih delih, ki so najbolj izpostavljeni vodnemu toku, v primeru velike gostote odloženih kokonov pa so možna tudi alternativna mesta. Domnevamo lahko, da je sobivanje dveh ali več vrst branhiobdelidov na istem gostitelju vsaj delno omogočeno tudi prek razdelitve na mikro-niše, tako de se mesta pričvrščanja kokonov izključujejo med sabo.

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Overview of the slow worm *Anguis fragilis* Linnaeus, 1758 (Reptilia: Anguidae) findings in caves of Slovenia

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ABSTRACT

The slow worm (*Anguis fragilis*) is among the best-known reptiles in Slovenia, distributed throughout the country. Here, we report observations from caves based on information from local caving clubs' magazines and blogs, as well as our own fieldwork. Observations originate from different parts of the country. Most individuals were found under cave entrance pits (in five caves), while two observations were made in deeper cave sections away from entrance pits. In one cave, Velika jama nad Trebnjem, two dead individuals were found during two separate winter observations at the horizontal cave entrance. Although slow worms use underground shelters such as burrows of other animals or voids under stones during summer droughts and winter hibernation, their occurrences in karst caves can be considered accidental.

IZVLEČEK

Pregled najdb slepcev *Anguis fragilis* Linnaeus, 1758 (Reptilia: Anguidae) v jamah Slovenije

Slepec (*Anguis fragilis*) je med najbolj poznanimi plazilci v Sloveniji, razširjen je po vsej državi. V prispevku poročamo o opažanjih iz jam na podlagi podatkov, zbranih iz glasil in blogov lokalnih jamarskih društev, kot tudi lastnega terenskega dela. Opažanja izvirajo iz različnih delov države. Večina osebkov je bila najdena pod vhodnimi brezni jam (pet jam), medtem ko sta bila v dveh jamah osebka globlje v jami, oddaljena od vhodnih brezen. V Veliki jami nad Trebnjem sta bila v času dveh ločenih zimskih opažanj najdena dva poginula osebka na vodoravnem vhodu v jamo. Čeprav se slepci med poletnimi sušami ali med zimskim mirovanjem skrivajo v podzemnih zatočiščih, kot so rovi drugih živali ali vdolbine pod kamni, pa lahko njihovo pojavljanje v kraških jamah obravnavamo kot naključno.

INTRODUCTION

The slow worm (*Anguis fragilis* Linnaeus, 1758) is the only legless reptile living in Slovenia (Tome 1996; Krofel et al. 2009). Due to its recognisability, it is among the best-known reptiles, distributed all over the country, with findings from lowlands to up to 1,500 m altitudes (Tome 1996; Krofel et al. 2009). At a broader scale, the slow worm has one of the widest geographic ranges among Eurasian reptiles, extending far into northern Europe and approaching the Arctic Circle (Gasc et al. 2004; Sillero et al. 2014). The species is a habitat and dietary generalist, living in forests and semi-open environments and feeding on earthworms, slugs and other invertebrates. It has a semi-fossorial lifestyle and is viviparous, traits that together with relatively low body temperatures and a predominantly thigmothermic mode of thermoregulation (Brown & Roberts 2008) facilitate persistence in cooler climates.

Despite its tolerance for low temperatures, it is generally not expected, as a reptile species, to visit caves in temperate zones. Yet, it is reported among Pleistocene fossil remains of herpetofauna in some caves in Europe, like in Belgium (Blain et al. 2014), Italy (Delfino 2004), Serbia (Đurić et al. 2017) and

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UK (Holman 1993). On the other hand, surprisingly, there are virtually no recent reports of the species from caves in searchable scientific literature and portals. In the citizen science portal iNaturalist, there is a single observation of the species from a cave, reporting on the finding of a dead individual from »the water of a small cave pool« in Küsnacht in Switzerland (Spyr 2025). Additional records come from Italy, including a note in the Italian Alpine club's magazine (Ruocco 2007), and a photographic record published on the web page of a Ligurian speleological club (Bartolini 2016). Interestingly, even one Italian cave right on the border with Slovenia has been named after this species (CGEB 2025).

Own recent findings of slow worms in caves, and a lack of published data in the literature, encouraged the preparation of this contribution, where all records of the species from caves of Slovenia are gathered. Besides our own records, they include findings reported in grey literature (blogs, local cave clubs' magazines), that are not easily accessible to international readership.

MATERIAL AND METHODS

Our own field observations derive from visits to caves, performed during cave explorations to search for cave invertebrates or counts of bats. If a slow worm individual was found, photographs of the animal were taken and, in most cases, the individual was taken out of the cave for release at an appropriate site on the surface. In one case, the length of the animal was measured in the field and weighed, while in most cases, the length was estimated from the photographs, including sexing them based on characteristic body colour pattern (Arnold & Ovenden 2004).

Data on reported observations were taken from grey literature, including blogs and caver clubs' magazines. These sources were scrutinised during the routine work carried out by the Centre for Cartography of Fauna and Flora (CKFF), which collects distributional data of species in Slovenia in private database (CKFF 2025). All records reporting on slow worms were gathered, with all relevant details presented here.

RESULTS AND DISCUSSION

Slow worms were reported from eight different caves in Slovenia (Tab. 1, Fig. 1). Firstly, we present the findings taken from grey literature.

In Ferranova buža, an individual was found deeper in the cave some 35 m away from the cave entrance pit (Ferran 2003). From Medvedjak, there are two different observations of animals, in both cases found under the cave's 40 m deep entrance pit. The first observation is from Simić (1992), referring to the finding of a live animal. According to the notes in the registry of cave excursions by the Ljubljana Cave Exploration Society, this cave visit took place in March 1989 (Tab. 1). The second mention of an animal from Medvedjak is by Jazbec (2024), reporting on a dead individual, also under the cave entrance pit. As bats were also reported from this visit (CKFF 2025), the exact date of the excursion in November 2003 could be determined (Tab. 1). Apart from these records, only the observation from Bokalovo brezno v Kovkah included a photograph of the animal published on a blog, taken at the bottom of about 9 m deep entrance shaft (Ramšak 2015). This small cave is not registered in the Slovenian Cave Registry (eKataster 2025), so its position could be determined approximately based on the description. From the photograph on the blog, we estimated the animal being a male.

Further seven records, originating from five different caves, are reported here for the first time.

In July 2003, one slow worm was observed at the bottom of the few metres deep entrance pit in the cave Turške jame in the North of the country (Tab. 1, Fig. 1).

In October 2023, one slow worm was found in the cave Dolenca in Western Slovenia (Tab. 1, Fig. 1). The individual was observed in a cave chamber, approximately 15 m deep and 20 m horizontal distance from the cave's gated entrance pit. When discovered, the animal was lying on rocks on the cave floor and was moving slowly. It was measured and weighed (length 33 cm, weight 13 g), and released after returned to the surface at least 50 m away from the cave entrance at the edge of the forest. According to the body colour pattern, it was most likely a pale female (Fig. 2).

In January 2024, one individual was found at the bottom of the 15 m deep entrance pit of the cave Habjanova jama in Central Slovenia (Tab. 1, Fig. 1), resting on a mixture of decaying wood, leaves and soil. After visiting the cave, and despite inhospitable conditions (snow) on the surface, the individual was released under a large boulder, approximately 100 m from the cave entrance. According to the body colour pattern, it was most likely a female of at least 28 cm in length (Fig. 2).

In summer 2025, we found two different slow worms upon two visits to the cave Logarček in Central Slovenia (Tab. 1, Fig. 1). In both cases, individuals were found on the muddy bottom, among leaves and soil, right under the about 20 m deep entrance pit of the cave. We collected the animals on both occasions and released them in the forest at least 50 m away from the cave entrance. The animal collected in June 2025 was about 30 cm in length, and with colouration of a female; the animal in July was smaller, about 16 cm long and most likely a female (Fig. 2).

Here we also report on two different observations of slow worms at the horizontal entrance to the cave Velika Jama nad Trebnjem (Tab. 1, Figs. 1, 3). In both cases, dead animals were lying approximately 1 m inside the cave. In the first case (February 2009), the animal was found lying in snow, with the posterior part missing, suggesting that a predator might have found the animal hibernating at the edge of the cave entrance (Fig. 3). In the second case, the cave was visited after a snowfall in January 2018. The animal was found lying on a bed of moist leaves immediately after the cave entrance.

Table 1. Caves with records of slow worms. Numbers in brackets after cave name refer to the official registry numbers in the Slovenian Cave Registry (eKataster 2025). Loc. No. – Consecutive number of the cave; X, Y – latitude (North) and longitude (East) in WGS84 decimal degrees of the cave entrance (x – cave not registered, coordinates given approximately according to description of the position); Alt. – altitude of the cave entrance; No. – number of individuals; Alive – whether live individuals were found live or not; M – male, F – female, nd – sex not determined; Pos. – position within the cave: pit – under entrance pit, deep – deeper in the cave, h.entr. – at the horizontal entrance. The published records are listed first, followed by new observations. Dates marked with * are determined from secondary sources (see text for details).

Tabela 1. Jame s podatki o najdbah slepcev. Številke v oklepajih za imeni jam se nanašajo na katastrsko številko v Katastru jam Slovenije (eKataster 2025). Loc. No. – zaporedna številka navedene jame; X, Y – severna širina in vzhodna dolžina jamskega vhoda (WGS84 decimalne stopinje) (x – jama ni registrirana, koordinate so približne glede na opis lege); Alt. – nadmorska višina jamskega vhoda; No. – število osebkov; Alive – ali je bil najden osebek živ (yes) ali ne (no); M – samec, F – samica, nd – spol ni določen; Pos. – položaj znotraj jame: pit – pod vhodnim breznom, deep – globlje v jami, h.entr. – na vodoravnem vhodu. Najprej so navedeni objavljeni podatki, nato pa nova opažanja. Datuma označena z * sta povzeta iz sekundarnih virov (podrobnosti v besedilu).

Loc. No.	Cave	Town near	X, Y	Alt. [m]	Date	*Anguis fragilis* individuals				Reference/Legators
						No.	Alive	M/F	Pos.	
1	Medvedjak (881)	Markovščina	45.55728 14.00778	520	11. 3. 1989*	1	yes	nd	pit	Simič (1992)
					29. 11. 2003*	1	no	nd	pit	Jazbec (2004)
2	Ferranova buža (8085)	Stara Vrhnika	45.97632 14.25218	657	Not given	1	yes	nd	deep	Ferran (2003)
3	Bokalovo brezno v Kovkah[x]	Dol pri Hrastniku	46.1241[x] 15.1325[x]	unknown	15. 7. 2015	1	yes	M	pit	Ramšak (2015)
4	Turške jame (4461)	Koroška Bela	46.43098 14.10464	640	30. 7. 2003	1	yes	nd	pit	Primož Presetnik, Katerina Jazbec
5	Velika jama nad Trebnjem (104)	Trebnje	45.90050 15.00486	440	6. 2. 2009	1	no	F	h.entr.	Primož Presetnik, Monika Podgorelec, Tomaž Miklavčič
					8. 1. 2018	1	no	nd	h.entr.	Primož Presetnik, Tomaž Miklavčič
6	Dolenca jama (785)	Brestovica na Krasu	45.80922 13.64050	40	7. 10. 2023	1	yes	F	deep	Maja Zagmajster, Hans Recknage
7	Habjanova jama (4419)	Novi Svet	45.92575 14.12496	575	21. 1. 2024	1	yes	F	pit	Teo Delić, Aja Zamolo
8	Logarček (28)	Laze pri Planini	45.86493 14.26832	498	26. 6. 2025	1	yes	F	pit	Maja Zagmajster, Teo Delić
					21. 7. 2025	1	yes	F	pit	Maja Zagmajster, Teo Delić, Svit Zagmajster Lovrek

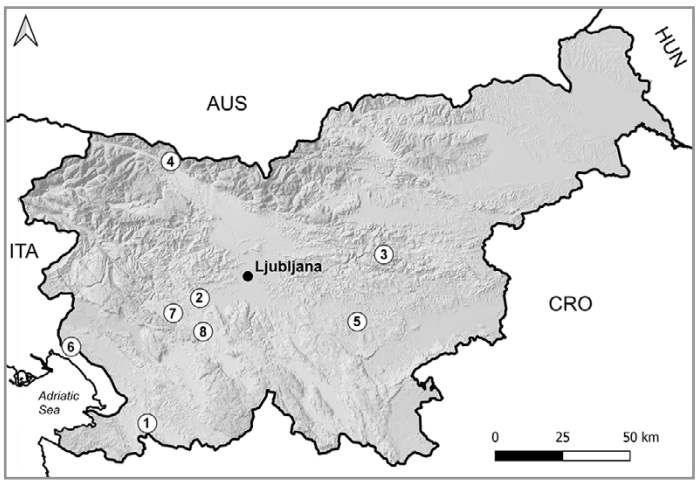

Figure 1. Map with cave localities in Slovenia (white circles), where slow worms were found. Numbers refer to names in Tab. 1.

Slika 1. Zemljevid lokacij jam v Sloveniji (beli krogi), kjer so bili najdeni slepci. Številke se nanašajo na imena v Tab. 1.

As almost all findings of slow worms occurred in caves with relatively deep pits at their entrance, we find it very unlikely that they entered these caves intentionally, even though the species can tolerate low temperatures. Slow worms exhibit a semi-fossorial lifestyle, seeking daily shelter under stones, within soil and among debris, while being primarily active during the night. During winters, in the period from October to February, they search for underground shelters where they semi-hibernate. While searching for appropriate subterranean refuges, individuals may have inadvertently fallen into caves with deep entrance shafts; their fossorial tendencies likely increased the risk of such accidental encounters. The findings of dead individuals or remains (especially in fossil studies), also indicate a possibility that their carcasses were brought to the caves by predators.

Figure 2. Slow worms observed in three caves in Slovenia: A – Dolenca, October 2023; B – Habjanova jama, January 2024; C – Logarček, June 2025; D – Logarček, July 2025 (photo taken upon being released on the surface). Hands on the photos for scale (Photo: M. Zagmajster – A, C, D; A. Zamolo – B).

Slika 2. Slepci, najdeni v treh jamah v Sloveniji: A – Dolenca, oktober 2023; B – Habjanova jama, januar 2024; C – Logarček, junij 2025; D – Logarček, julij 2025 (fotografija posneta ob izpustu na površini). Roke na fotografijah so za merilo (foto: M. Zagmajster – A, C, D; A. Zamolo – B).

Figure 3. Dead slow worm, found dead approximately 1 m inside the entrance to Velika jama nad Trebnjem cave, lying on the snow (photo: M. Podgorelec).

Slika 3. Mrtev slepec, najden približno 1 m za vhodom v Veliko jamo nad Trebnjem, kjer je ležal na snegu (foto: M. Podgorelec).

We found it surprising that we could not get any published resources in international literature on observations of slow worms in caves. It could be assumed that such encounters do happen more often, but are not reported, or remain in local magazines in national languages (ex. Jazbec 2004; Bartolini 2016). Even though such findings of slow worms are accidental, they should be systematically reported to improve the knowledge of the species' biology.

POVZETEK

Slepec (*Anguis fragilis*) je edini breznogi plazilec, ki živi v Sloveniji, kjer je splošno razširjen (Tome 1996; Krofel et al. 2009). Je prehranski generalist, živoroden in lahko preživi tudi v razmeroma hladnih okoljih. Čeprav je toleranten na nizke temperature, ga ne bi pričakovali v jamah zmernih klimatov. Obstaja kar nekaj najdb slepcev v kraških jamah iz pleistocenskih nanosov (Blain et al. 2014; Đurić et al. 2017), opažanj o sodobnih najdbah pa v dostopni znanstveni literaturi ni.

Zbrali smo podatke o opažanjih slepca v kraških jamah, objavljene v glasilih in na blogih jamarskih društev ter lastnega terenskega dela. Opažanja iz osmih jam izvirajo iz različnih delov države. Prve navedbe so iz jame Medvedjak, kjer sta bila v dveh različnih primerih najdena en živ in en mrtev osebek (Simić 1992; Jazbec 2004). V Bokalovem breznu v Kovkah je bil najden živ samec na dnu 9 m brezna (Ramšak 2015). V dveh jamah, v Ferranovi buži (Ferran 2003) in Dolenci, so bili osebki najdeni globlje v jami, odmaknjeni od vhodnih brezen. Živali so bile najdene še v treh jamah, v Turških jamah, Habjanovi jami in Logarčku (Tab. 1, Sl. 1). V Veliki jami nad Trebnjem sta bila v dveh različnih zimah najdena mrtva slepca, obakrat tik za vhodom v jamo.

Čeprav se slepci med poletnimi sušami ali med zimskim mirovanjem skrivajo v podzemnih zatočiščih, je njihovo pojavljanje v jamah naključno. Nadaljnja objava najdb v jamah bo pripomogla k boljšemu poznavanju biologije tega plazilca.

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A new record of wandering glider *Pantala flavescens* (Fabricius, 1798) (Odonata: Libellulidae) in Croatia

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ABSTRACT

In Europe, a cosmopolitan dragonfly species *Pantala flavescens* is scarce. Along the Adriatic coast, only two records, from Montenegro in 1972 and from Croatia in 2010, have been known so far. On 30. 7. 2025, a female of the species was observed in the canteen of Božava Hotel on the island Dugi otok in Dalmatia. This represents only the second record of the species for Croatia.

IZVLEČEK

Novo opazovanje ploščca vrste *Pantala flavescens* (Fabricius, 1798) (Odonata: Libellulidae) na Hrvaškem

V Evropi je kozmopolitska vrsta kačjega pastirja *Pantala flavescens* na splošno zelo redka. Z območja ob jadranski obali sta bila doslej znana le dva podatka, iz Črne gore leta 1972 in iz Hrvaške leta 2010. Dne 30. 7. 2025 je bila samica te vrste opažena v jedilnici hotela Božava na Dugem otoku v Dalmaciji, kar je šele drugi podatek za vrsto na Hrvaškem.

Commonly known as the wandering glider or globe skimmer, *Pantala flavescens* is a cosmopolitan dragonfly known to occur on every continent except Antarctica (Dijkstra et al. 2020). It is renowned for its long-distance, complex transoceanic migrations, with individuals travelling thousands of kilometres across continents, oceans and mountain passes (Anderson 2009; Hobson et al. 2012; Kalkman & Monnerat 2015). It is common and often abundant in the tropics and subtropics of both the Old and the New World. In large parts of its range, it is a migratory species, which appears in huge swarms along the monsoon fronts. Beyond the intertropical range, the species migrates as far north as Canada, northern Europe, Central Asia and the Kamchatka Peninsula (Kalkman & Monnerat 2015).

In Europe, *Pantala flavescens* is generally very scarce, frequent only in Sicily, Cyprus and southern Turkey (Kalman & Monnerat 2015; Piretta & Assandri 2019). Recent records are known from Bulgaria, some Italian islands, Gran Canaria, Azores, as well as from Baltic Sea in Kaliningrad, Poland, Germany and Switzerland (Ober 2008; Kalkman & Monnerat 2015; Piretta & Assandri 2019; Dijkstra et al. 2020). At the end of August in 2010, the first record of *Pantala flavescens* for Croatia was published. At least 3 individuals were spotted flying alongside a beach in a small bay near Stara Baška on the island of Krk (Finkenzeller 2010). Along the Adriatic coast, an old record from 1972 is also known from Hercegnovi in Montenegro (Ober 2008).

In the present note, I'm reporting on a second record of *Pantala flavescens* in Croatia. A female was found stuck in a canteen at Božava Hotel south of Božava (Lat. 44.137507, Lon. 14.907786) in the northern part of Dugi otok island on the 30. 7. 2025. The canteen has large windows, which in total measure around 5 m in height and 10 m in length. The female most likely entered the area from one of the glass doors that were open during the day. At first, it was observed perching on the ceiling, afterwards it began flying, repeatedly crashing into windows. When the individual ascended low enough, it was caught by hand and photographed (Fig. 1).

While handling the caught individual, presumably due to stress, it started to extract eggs. No freshwater bodies are present near the observation site. Upon closer inspection, slight damage on front two wing tips was noted (Fig. 1). After handling, the female was released into the wild, near the location where caught.

On the day of observation, the weather was sunny with no wind and temperatures reaching approximately 37°C. During the week before the observation, stormy weather with heavy rainfall and temperatures dropping down to 20–25 °C had been reported. The usage of storms for insect migration has been documented (Russell et. al. 1998; Srygley 2003), and the changes in temperature, cloud cover, wind speed and wind direction may also influence dispersal (Wikelski et al. 2006; Brattström et al. 2008; Chapman et al. 2015). These factors could explain the presented unexpected observation of *Pantala flavescens* on the Dugi otok island

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Figure 1. Female *Pantala flavescens* caught on 30. 7. 2025 in Božava town on Dugi otok in Dalmatia (Foto: Mitja Dobovišek).

Slika 1. Samica vrste *Pantala flavescens*, ujeta 30. 7. 2025 v mestu Božava na Dugem otoku v Dalmaciji (foto: Mitja Dobovišek).

Although the species has not yet been recorded in neighbouring countries, e.g. in Slovenia, Hungary, Bosnia and Herzegovina or Serbia, its occasional and scattered appearance in the region even north of the Alps suggests that more systematic fieldwork could increase the likelihood of additional observations and would provide additional insights into the biology and ecology of the species.

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