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Original scientific paper

CONTRIBUTIONS TO THE BIOLOGY OF *DINA SKETI* GROSSER & PEŠIĆ, 2014 (HIRUDINEA: ERPOBDELLIDAE)

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Abstract

The known range of the species *Dina sketi* Grosser & Pešić, 2014 (Hirudinea: Erpobdellidae) includes several springs in the Cvrcka and Crna Rijeka basins (Republic of Srpska, Bosnia and Herzegovina). The biology of these leeches is insufficiently known. The aim of this paper was to present new knowledge about their ecology and reproductive biology, based on data collected in the springs of the Crna Rijeka basin. The analyzed parameters included characteristics of the biotope and macrozoobenthos assemblages of the studied springs, as well as characteristics of diet and the cocoons of *D. sketi*. The features of the biotope partially fit into the known range for this species. *D. sketi* is the only species of leech found in macrozoobenthos of the study area, where it is represented by less than 1%. The amphipod crustaceans and hydrobid gastropods are the most abundant members of the analyzed macrozoobenthos assemblages. The analysis of contents of the digestive tract revealed that these leeches prey on aquatic and terrestrial invertebrates. It seems that leeches prefer amphipods in their diet. All previous knowledge about the reproductive biology of *D. sketi* was based on description of their reproductive system. This paper presents the morphology and morphometric properties of the cocoons. They are "kiwi-shaped", 4.60–8.82 mm in length and 3.19–6.38 mm width.

Key words: *Dina sketi*, Crna Rijeka, ecology, reproductive biology, cocoons

INTRODUCTION

The erpobdellid leech *Dina sketi* Grosser & Pešić, 2014 (Hirudinea: Erpobdellidae) was described nine years ago from specimens collected in a spring in the basin of river Cvrcka (Grosser *et al.*, 2014). This leech species was also recorded in several other springs in the basins of river Cvrcka (see in Dmitrović, 2017) and Crna Rijeka (Grosser and Pešić, 2022). All these spring habitats of *D. sketi* are situated in the northwestern part of the Republic of Srpska in Bosnia and Herzegovina (Dmitrović and Pešić, 2020) (Figure 1), and therefore according to data available at the present time this leech species is considered an endemic crenobiont (Stanić-Koštroman *et al.*, 2022). At the suggestion of the first author of this paper (Dmitrović pers. com.), *D. sketi* was assigned the status of strictly protected wild species in the

Republic of Srpska in 2020 (Official Gazette of the Republic of Srpska, 65/20) due to its very small range.

Figure 1. Distribution map of *D. sketi* (A): springs of Crna Rijeka basin (green dot) and springs of Cvrcka river basin (brown dot). Spring habitat in June (B), adults (C) and deposited cocoons (C, D) with juvenile leeches before hatching (D) in Crna Rijeka basin

Grosser *et al.* (2014) provided the morphoanatomical description of *D. sketi*, including a detailed description of the reproductive system. Other studies on this species included molecular analyses (Grosser and Pešić, 2022) and discussions on phylogenetic relationships with other leech species (Grosser *et al.*, 2023). All other aspects of biology of this species may be considered poorly known, though it is included in several hydrobiological publications pertaining to springs in the basin of river Cvrcka (Pešić *et al.*, 2016; von Fumetti *et al.*, 2017; Pešić *et al.*, 2019).

The aim of this paper was to present new insights in ecology and reproductive biology of *D. sketi*, according to data collected in the springs of Crna Rijeka basin.

MATERIALS AND METHODS

Specimens of leech *D. sketi* were collected in springs of Crna Rijeka basin in early November 2018. Sampling included eucrenal and hypocrenal of three springs. The temperature boundaries between the selected sections of the springs were determined according to the guidelines provided by von Fumetti *et al.* (2007). Temperature and pH of spring water were measured with a field pH-meter (HI 98127), while composition and cover of the substrate were determined according to von Fumetti *et al*., (2006). Leeches were collected together with the other representatives of the macrozoobenthos, by using a hand net (mesh size: 350 μm) and according to the instructions for multihabitat sampling provided by Gerecke *et al.* (1998). These semi-quantitative samples were immediately fixed with 96% ethanol.

Qualitative and quantitative analysis of macrozoobenthos samples was performed in the laboratory of the Faculty of Natural Sciences and Mathematics at University of Banja Luka, under the magnification of a Leica EZ4D stereomicroscope and using the literature with identification keys for aquatic macroinvertebrates (Sládeček and Košel, 1984; Kerovec, 1986; Sundermann *et al*., 2007; Waringer and Graf, 2013; Grosser *et al*., 2016; Živić and Marković, 2017; Glöer, 2022). The body length of leeches was measured with a caliper, and after dissection the contents of their digestive tracts were collected for additional analysis.

This paper also presents important data on the reproductive biology of *D. sketi*. Thus, during the field studies of springs in the Crna Rijeka basin at the beginning of June 2019, it was noticed that numerous cocoons were attached to the lower surface of stones (Figure 1). Fourteen cocoons were collected and fixed in 96% ethanol. Morphological and morphometric analysis of the collected cocoons was performed in the same laboratory, using a Leica EZ4D stereomicroscope. Six morphometric features were measured for each cocoon (Figure 2), followed by descriptive statistical analysis.

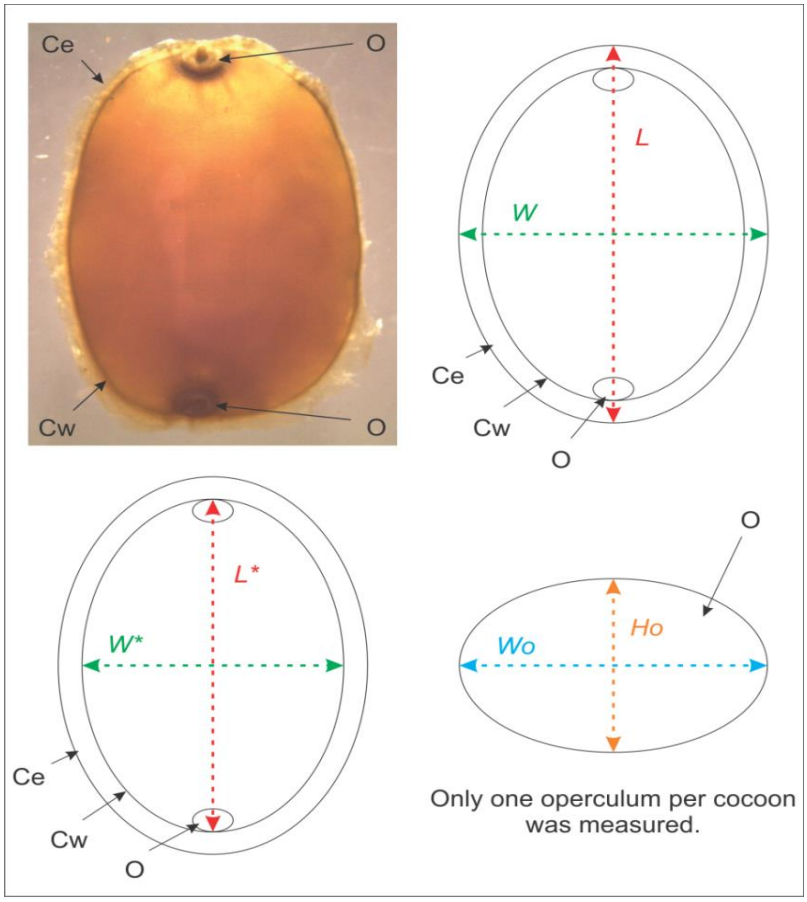

Figure 2. Cocoon morphology (dorsal side): cocoon edge (Ce), cocoon wall (Cw) and operculum (O). Analyzed morphometric traits of cocoons: length (L and L*), width (W, W* and Wo) and height (Ho)

All invertebrates collected in macrozoobenthos samples and the contents of the digestive tract of leeches, as well as collected cocoons, were preserved in 96% ethanol in order to form a reference collection.

RESULTS AND DISCUSSION

The measured temperatures of spring water in the Crna Rijeka basin vary from 8.0°C in the eucrenal of Spring II to 10°C in the hypocrenal of Spring I (Table 1). The water in all three springs is slightly alkaline (pH: 7.9–8.1). The determined properties of spring water fit within the previously recorded ranges for the habitats of *D. sketi*. The results of previous research showed that this leech species was recorded in the springs of the Cvrcka river basin, where water temperature values were 7.9–13.9°C while pH values were in the range of 7.7–8.1 (see in Dmitrović, 2017).

Table 1. Water and substrate characteristics of studied springs within the Crna Rijeka basin. Spring sections: eucrenal (E) and hypocrenal (H). Substrate type classes (based on percentage of areal coverage): 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (76–100%)

	Spring I		**Spring II**		**Spring III**	
	E	**H**	**E**	**H**	**E**	**H**
Temperature (°C)	9.0	10.0	8.0	9.0	8.7	9.7
pH	8.0	8.0	7.9	8.1	7.9	7.9
Leaf litter	3	3	4	4	3	3
Dead branches	1	1	1	1	1	1
Stones	2	2	2	3	1	2

The bottoms of all three springs from the Crna Rijeka basin are dominantly covered with leaf litter (Table 1). The cover of leaf litter was higher in springs of Crna Rijeka basin than in *D. sketi* habitats in Cvrcka river basin (see in Dmitrović, 2017). The studied springs within the Crna Rijeka basin are situated in a thinned deciduous forest. The field work was realized in the autumn, when nearby trees discarded their leaves.

The erpobdellid leech *D. sketi* is the only species of leech recorded in macrozoobenthos samples from eucrenal and hypocrenal of all three analyzed springs in the Crna Rijeka basin (Table 2). The lowest number of macrozoobenthos taxa was recorded in Spring III (13 taxa), and the highest in Spring I (21 taxa). The number of taxa recorded in the hypocrenal of the analyzed springs was lower than in the eucrenal (Table 2).

Table 2. Taxonomic composition of macrozoobenthos in the studied springs of the Crna Rijeka basin. Spring sections: eucrenal (E) and hypocrenal (H)

	Spring I			**Spring II**			**Spring III**		
Taxa	**E**	**H**	**Σ**	**E**	**H**	**Σ**	**E**	**H**	**Σ**
TRICLADA									
Dugesia sp.				+		+			
GASTROPODA									
Belgrandiella bozidarcurcici	+	+	+	+	+	+	+	+	+
OLIGOCHAETA	+		+	+		+	+	+	+
Eiseniella tetraedra	+		+	+		+			
HIRUDINEA									
Dina sketi	+	+	+	+	+	+	+	+	+
ACARI	+		+					+	+
AMPHIPODA									
Gammaridae	+	+	+	+	+	+	+	+	+
EPHEMEROPTERA									
Baetidae	+	+	+				+	+	+
Heptageniidae	+		+	+		+			
Rhitrogena sp.	+		+						
PLECOPTERA									
Nemouridae	+		+						

Protonemura sp.		+	+	+		+	+		+
DIPTERA									
Tipula sp.	+		+	+	+	+			
Limoniidae	+	+	+	+	+	+	+	+	+
Ceratopogonidae	+		+						
Chironomidae		+	+	+		+	+	+	+
Chelifera sp.							+		+
COLEOPTERA	+		+	+		+			
Scirtidae		+	+						
Elmis sp.	+	+	+	+		+	+	+	+
TRICHOPTERA									
Rhyacophilidae	+		+						
Goeridae	+	+	+	+	+	+		+	+
Limnephilidae	+	+	+	+	+	+	+		+
Molannidae				+		+			
Σ	18	11	21	16	7	16	11	10	13

The most abundant representatives of macrozoobenthos assemblages in the eucrenal and hypocrenal of all three analyzed springs in the Crna Rijeka basin were amphipod crustaceans from the family Gammaridae and hydrobid gastropods *Belgrandiella bozidarcurcici* (Table 2, Table 3). Leeches (*D. sketi*) represent less than 1% of the macrozoobenthos assemblages collected in both the eucrenal and hypocrenal of these springs. There is a slightly higher percentage of leeches (about 18%) in macrozoobenthos samples from springs of the Cvrcka river basin (Pešić *et al.*, 2016; Pešić *et al.*, 2019).

Table 3. Percentage share of major macrozoobenthos groups from the studied springs of the Crna Rijeka basin. Ind. is an abbreviation for the number of collected individuals

Spring sections	Major groups	Spring I		Spring II		Spring III	
		Ind.	%	Ind.	%	Ind.	%
Eucrenal	TRICLADA			1	0.27		
	GASTROPODA	37	17.70	93	25.20	96	45.50
	OLIGOCHAETA	4	1.91	8	2.17	5	2.37
	HIRUDINEA	2	0.96	3	0.81	1	0.47
	ACARI	1	0.48				
	AMPHIPODA	117	55.98	238	64.50	91	43.13
	EPHEMEROPTERA	8	3.83	2	0.54	1	0.47
	PLECOPTERA	13	6.22	1	0.27	4	1.90
	COLEOPTERA	13	6.22	12	3.25	9	4.27
	DIPTERA	3	1.44	5	1.36	3	1.42
	TRICHOPTERA	11	5.26	6	1.63	1	0.47
	Σ	209	100.00	369	100.00	211	100.00
Hypocrenal	GASTROPODA	129	33.86	55	39.57	185	51.39
	OLIGOCHAETA					3	0.83
	HIRUDINEA	1	0.26	1	0.72	1	0.28

ACARI					1	0.28
AMPHIPODA	207	54.33	76	54.68	129	35.83
EPHEMEROPTERA	4	1.05			11	3.06
PLECOPTERA	12	3.15				
COLEOPTERA	21	5.51			8	2.22
DIPTERA	3	0.79	2	1.44	20	5.56
TRICHOPTERA	4	1.05	5	3.60	2	0.56
Σ	381	100.00	139	100.00	360	100.00
Σ	590	100.00	508	100.00	571	100.00

Although leeches from the Erpobdellidae family are known to be predators (Kutschera and Wirtz, 2001), the exact diet of *D. sketi* used to be unknown. The samples from the digestive tract of individuals of this leech species from the springs of the Crna Rijeka basin included representatives of aquatic and terrestrial invertebrates (Table 4).

Table 4. Diet composition of *D. sketi* from the studied springs of the Crna Rijeka basin. Spring sections: eucrenal (E) and hypocrenal (H). The leech specimens (N=9) were categorized into three size classes based on differences in body length: small (S1–S3: 4.0–7.3 mm), medium (M1–M3 mm: 16.5–26.4 mm) and large (L1–L3: 46.6–48.6 mm)

	Spring I		**Spring II**		**Spring III**	
The content of the digestive tract	**E**	**H**	**E**	**H**	**E**	**H**
Gastropoda – shell-less terrestrial					L3	
Oligochaeta – terrestrial				L2		
Gammaridae	M1		M2		L3	
Unrecognizable remains		S1	S2		L3	
Empty digestive tract	L1		M3			S3

It seems that leeches of different body size prefer different prey items (Table 4). Thus, in addition to aquatic invertebrates, larger leeches also hunt larger terrestrial prey (shell-less gastropods and oligochaetes). For example, in the digestive tract of a leech L3 with a body length of 48.4 mm there were remains of an undigested 19.1 mm slug and some half-digested amphipods (Gammaridae). The amphipods were also recorded in the digestive tracts of medium-sized individuals, and they are the most common prey of *D. sketi*. Therefore, since amphipods and hydrobids are the most abundant members of the macrozoobenthos assemblages in the studied springs of Crna Rijeka basin (Table 3) and since hydrobids were absent in the contents of the digestive tract of leeches (Table 4), it seems that *D. sketi* prefers amphipods in diet. The relationship between the type and size of prey and the size of leeches was also observed in other species of family Erpobdellidae (Toman and Dall, 1997; Beracko and Košel, 2011; Beracko and Rogánska, 2014).

Numerous cocoons of *D. sketi* were observed attached to the lower surface of stones in eucrenal and hypocrenal of springs in the Crna Rijeka basin in early June 2019 (Figure 1). Fourteen cocoons were collected for analysis. The morphological analysis has shown that the collected cocoons had a more or less oval shape, while the ventral surface with which they were attached to the substrate was flat and the dorsal surface was convex, except in the zone marked as cocoon edge, which was also flat (Figure 2). The average length of the cocoon was

6.79 mm ± 1.23, and the average width was 5.15 mm ± 1.06 (Table 5). The data for L, L*, W and W* from Table 5 indicate that the cocoon edge represents a band with an average width of about 0.23 mm. Cocoons are brownish in color and more or less transparent. Each cocoon has two slightly oval opercula on the dorsal side, located at the opposite ends of the longer axis of the cocoon (Figure 2). The average height of the operculum was 0.73 mm ± 0.10, and the average width was 0.82 mm ± 0.11 (Table 5). According to Kutschera (2010), these paired structures, labeled as "plugs", are a feature characteristic of the cocoons of all species from the Erpobdellidae family. Cocoons of *Dina lineata* (O. F. Müller, 1774) are "lemon-shaped" (Sládeček and Košel, 1984; Elliott and Dobson, 2015), while those of *D. sketi* are more or less oval – "kiwi-shaped". Based on the only published photo in Pešić and Grosser (2022), the cocoon of *Dina serbica* Pešić & Grosser, 2022 from Serbia seem to have a similar shape.

Table 5. Descriptive statistics of analyzed morphometric traits of cocoons (N=14). The values are shown in mm

	L	W	L*	W*	Ho	Wo
Average	6.79	5.15	6.25	4.76	0.73	0.82
Minimum	4.60	3.19	4.00	3.07	0.59	0.68
Maximum	8.82	6.38	8.27	5.89	0.91	1.02
Standard deviation	1.23	1.06	1.21	0.89	0.10	0.11

The collected cocoons were in different stages of maturity, which will not be analyzed and discussed in this paper. Between 5 and 7 juvenile leeches were found in more mature cocoons (N=7), some of which were photographed (Figure 3).

Figure 3. *D. sketi* – juvenile leeches removed from the cocoon.

Although numerous cocoons of *D. sketi*, in different stages of maturity, were observed in the same locality in early June 2023, current knowledge is insufficient to assert with certainty whether cocoon deposition takes place only in that season. As recorded individuals of *D. sketi* were of different dimensions, clearly separated into three size classes (Table 4), it may indicate cocoon deposition at different times during the year, and also may indicate a two-year life cycle of leeches, as is known for some other representatives of the Erpobdellidae family. For example, according to data provided by Beracko and Košel (2011), *Dina punctata* from a small Carpathian stream have a maximal two-year life cycle and lays cocoons from April to the end of August, and most intensively in June.

CONCLUSION

Erpobdellid leech *D. sketi* inhabits the eucrenal and hypocrenal of the studied springs in the Crna Rijeka basin, where it constitutes less than 1% of macrozoobenthos assemblages. The measured values of the physicochemical parameters of water in the habitat (temperature and pH value) fit within the known ranges. The dominant component of the substrate is leaf litter. The most abundant members of the macrozoobenthos assemblages are amphipods and hydrobids. *D. sketi* preys on aquatic and terrestrial invertebrates, apparently preferring amphipods in its diet, while larger leeches also prey on some larger animals. Leech cocoons of this species at various stages of maturity were recorded in early June. Morphologically, they match the general structure of cocoons in other species of leeches from the Erpobdellidae family. They have two opercula at the distal ends of the dorsal convex side. The ventral side of the cocoon is flat, as is the cocoon edge on the dorsal side. The length of the cocoon ranges from 4.60 to 8.82 mm, and the width from 3.19 to 6.38 mm. The more mature cocoons may contain 5–7 juvenile leeches.

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PRILOZI POZNAVANJU BIOLOGIJE VRSTE *DINA SKETI* GROSSER & PEŠIĆ, 2014 (HIRUDINEA: ERPOBDELLIDAE)

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Sažetak

Poznati areal vrste *Dina sketi* Grosser & Pešić, 2014 (Hirudinea: Erpobdellidae) obuhvata nekoliko izvora u slivovima rijeke Cvrcke i Crne rijeke (Republika Srpska, Bosna i Hercegovina). Biologija ovih pijavica je nedovoljno poznata. Cilj rada bio je prezentovati

nova saznanja o njihovoj ekologiji i reproduktivnoj biologiji, a na bazi podataka skupljenih u izvorima sliva Crne rijeke. Analizirane su osobine biotopa i makrozoobentosa navedenih izvora, kao i osobine kokona i ishana ove vrste pijavica. Osobine biotopa djelimično se uklapaju u poznati opseg za ovu vrstu. *D. sketi* je jedina vrsta pijavica pronađena na predmetnom obuhvatu, gdje je zastupljena sa manje od 1% u odnosu na ostale članove makrozoobentosa. Najabundantniji članovi makrozoobentosa su amfipodni rakovi i hidrobidni puževi. Analizom sadržaja digestivnog trakta ovih pijavica utvrđeno je da njihov plijen čine akvatični i terestrični beskičmenjaci. Izgleda da pijavice u ishrani preferiraju amfipodne rakove. Sva dosadašnja znanja o reproduktivnoj biologiji vrste *D. sketi* bila su zasnovana na opisu njihovog reproduktivnog sistema. U ovom radu analizirani su morfologija i morfometrijske osobine kokona. Oni oblikom podsjećaju na plod kivija. Dugi su 4,60–8,82 mm, a široki 3,19–6,38 mm.

Kljčne riječi: *Dina sketi*, Crna Rijeka, ekologija, reproduktivna biologija, kokoni

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