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ORIGINAL ARTICLE

Italobdella bieleckii sp. n. (Hirudinea: Piscicolidae), a new leech species from the Danube in Slovakia

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Abstract

Notwithstanding the genus *Italobdella* has been hypothesized to have doubtful taxonomic value, we decided to update its diagnosis by describing the new species – *Italobdella bieleckii* sp. n. The leeches were collected from the stony bottom along the shore of the Danube River in Slovakia. In the study, we implemented the model of leech body form and the set of external and internal morphological characters to present the similarity of the new species to the other fish leeches. As a result, we were able to construct the key for determining leeches belonging to the genus *Italobdella* hoping that the species could be found and determined by other researchers. This would enrich our knowledge about the actual distribution of these leeches and strengthen their systematic position in the genus.

Keywords *Italobdella* · Leeches · Piscicolidae · Morphology · Systematics · Danube

Introduction

Until now, leeches of the genus *Italobdell*a Bielecki, 1993 were found only in Europe. *Italobdella ciosi* Bielecki, 1993 was recorded in 17 locations: northern Italy (the Adda River), Germany (the Altmühl, Hawela, Elbe, Rhine, Danube rivers, the Wern River – a tributary of the Main River, and the Zusam River – a tributary of the Danube), Hungary (the Danube and Tisza rivers) and Austria (the Thaya River). *Italobdella epshteini* Bielecki, 1997 was found in seven locations: Germany (the Danube), Latvia and Poland (the Biebrza, Łyna, Narew and Zalew Zegrzyński rivers) (Bielecki 1997; Juhasz and Bekesi 2002; Juhasz and Tibor 2002; Nesemann 1994, 1997; Neubert and Nesemann 1999; Kaiser and Wittling, 2002; Jugg et al. 2004). The third species, described in the present paper, *Italobdella bieleckii* sp. n., was found in the Danube River in Slovakia.

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Leeches of the genus *Italobdella* have a reproductive system organized similarly as in marine leeches of the monotype genus *Galatheabdella* Richardson et Meyer, 1973 (Sawyer 1986; Bielecki 1993, 1997; Bielecki et al. 2012, 2013). They possess a vector tissue located before the opening of the oviduct, but conducting strands of vector tissue connect the tissue with ovisacs from one side and the posterior part of the copulatory bursa of atrium from the other side. The connection between conducting strands of vector tissue with the posterior part of atrium is lacking in the reproductive system of *Italobdella* spp. (Bielecki 1993; Bielecki et al. 2008).

The study includes a diligent description of the body form, external and internal morphology and the key for the identification of leeches belonging to the genus *Italobdella*.

Individuals from the genus *Italobdella* have been recorded in Europe on their hosts *Salmo trutta trutta* m. *fario*, *Barbus barbus* (Linnaeus, 1758), *Perca fluviatilis* Linnaeus, 1758, *Carassius carassius* (Linnaeus, 1758), *Cyprinus carpio* Linnaeus, 1758, *Esox lucius* Linnaeus, 1758, *Aspius aspius* Linnaeus, 1758, *Abramis brama* (Linnaeus, 1758), *Chondrostoma nasus* (Linnaeus, 1758), and on submerged plants and pebbles (Bielecki 1997; Bielecki and Cios 1997).

The aim of the study was a diligent morphological analysis of the body form and other external and internal morphological characters of the piscicolid leech found in Slovakia. Based on the results, the new leech species was described and classified within the genus *Italobdell*a Bielecki, 1993 and the diagnosis of the genus was updated.

Materials and methods

Material examined during this study was collected in Slovakia: the Danube, Kravany (Čenkov) (river kilometer 1733), 1.10.1992, one specimen collected by V. Košel; the Danube, Štúrovo, (river kilometer 1720), 17.10.1992, three specimens including one with a spermatophore collected by V. Košel; the Danube, mixed sample, six leeches: one specimen collected by E. Elexová.

The holotype and the paratype, as well as the other three collected specimens were preserved in 4% formalin (Košel leg., 1.10. 1992, 17.10. 1992). The holotype (no. MIZ 1/2021/1) and paratype (no. MIZ 1/2021/2) of the new *Italobdella* species have been deposited at the Museum of Zoology of the Polish Academy of Sciences in Warsaw. The other material is in the first author's collection.

All specimens were measured according to the model of the leech body by Bielecki (1997) (Table 1). Detailed dissections were performed on all specimens according to the procedure by Bielecki (1997). Leeches were dissected on their dorsal side under the stereomicroscope OLYMPUS SZ-ST. Using a

Table 1 Parameters describing the body form of *Italobdella bieleckii* sp. n

No.	L	d_1	d_2	d_3	d_4	d_5	d_6	d_7	S_1	S_2	S_3	S_4	S_5	S_6	C_1	C^1_1	R_1	M_1	C_2	C^1_2	R_2	M_2	N_1	N_2
1	9.92	0.50	1.00	0.80	0.90	1.50	1.40	0.60	1.40	1.00	1.60	1.80	1.70	1.30	1.00	1.00	0.60	0.10	1.30	1.30	0.60	0.45	0.50	1.70
2	10.80	0.50	1.00	0.90	0.90	1.50	1.50	0.70	1.40	1.10	1.70	1.90	1.70	1.50	1.00	1.00	0.70	0.10	1.40	1.40	0.80	0.45	0.52	1.80
3	10.00	0.70	1.00	1.35	1.60	1.60	1.60	0.90	1.50	1.20	2.00	2.00	1.80	1.70	1.00	1.00	0.70	0.10	1.50	1.50	1.00	0.55	0.55	1.80
4	11.45	0.90	1.70	1.52	2.30	2.60	2.60	0.90	1.40	1.70	2.00	2.10	2.00	1.50	1.10	1.10	0.75	0.25	1.60	1.60	1.00	0.55	1.20	2.50
5	15.70	1.10	1.60	1.85	2.40	2.90	3.95	2.10	2.00	1.80	2.30	2.50	2.50	2.60	1.70	1.70	1.10	0.25	2.30	2.30	0.90	0.70	1.40	2.50
Me-an	11.57	0.74	1.27	1.30	1.62	2.02	2.21	1.04	1.54	1.36	1.92	2.06	1.94	1.72	1.16	1.16	0.77	0.16	1.62	1.62	0.86	0.54	0.83	2.06

scalpel, several incisions of the integument were performed along the body midline, starting from the anterior sucker to the caudal sucker. Portions of the integument were spread aside using dissection needles to immobilize the specimen and reveal the alimentary tract. It is usually covered with various tissues (like muscles, coelomic structures and fat cell groups), which should be removed to interpret the tract structure better. In the next step, the structures of the alimentary tract should be resected to uncover the reproductive system. Bearing in mind that the area is very small, and the reproductive organs are very fragile, one should be extremely careful while taking out portions of the alimentary tract. After this is accomplished, it is possible to observe the positions of particular reproductive system structures and orient them in reference to ganglia.

Construction, description and application of the model

The model (Bielecki and Epshtein 1994; Cichocka and Bielecki 2015) presents the leech body on a plane, as two ellipses (suckers) and trapeziums situated between them (anterior body part – trachelosome – two trapeziums; posterior body part – urosome – four trapeziums) (Fig. 1a). Besides, transverse sections through the trachelosome and urosome are considered as two ellipses (Fig. 1b, c). An abundant material provides evidence that the model permits a sufficiently exact description of the body form of various leech species (Bielecki et al. 2012, 2013; Cichocka and Bielecki 2015).

The model is constructed according to the following parameters:

(1–4) Parameters describing the form of the anterior sucker: C_1 = vertical diameter; $C^1{}_1$ = horizontal diameter; R_1 = length of anterior part of sucker; M_1 = length of posterior part of sucker.
(5–12) Parameters describing the form of the trachelosome: d_1 = width at sucker junction; d_2 = width at outline narrowing; d_3 = width at border with urosome; D_1 = largest width of trachelosome; N_1 = largest height of trachelosome; S_1 = height of first trapezium; S_2 = height of second trapezium; $L_1 = (S_1 + S_2)$ = length of trachelosome.
(13–25) Parameters describing the form of the urosome: width at places of outline distortion (bases of consecutive trapeziums); d_4 = base of first trapezium; d_5 = base of second trapezium; d_6 = base of third trapezium; d_7 = base of fourth trapezium (width at sucker junction); D_2 = largest width of urosome; N_2 = largest height of urosome; $L_2 = (S_3 + S_4 + S_5 + S_6)$ = urosome length (height of consecutive trapeziums); S_3 = height of first trapezium; S_4 = height of second trapezium; S_5 = height of third trapezium; S_6 = height of fourth trapezium; K_1 = distance from d_3 to D_2; K_2 = distance from D_2 to d_7.
(26–29) Parameters describing the form of posterior sucker: $C^1{}_2$ = horizontal diameter; C_2 = vertical diameter; M_2 = length of anterior part of sucker; R_2 = length of posterior part of sucker.

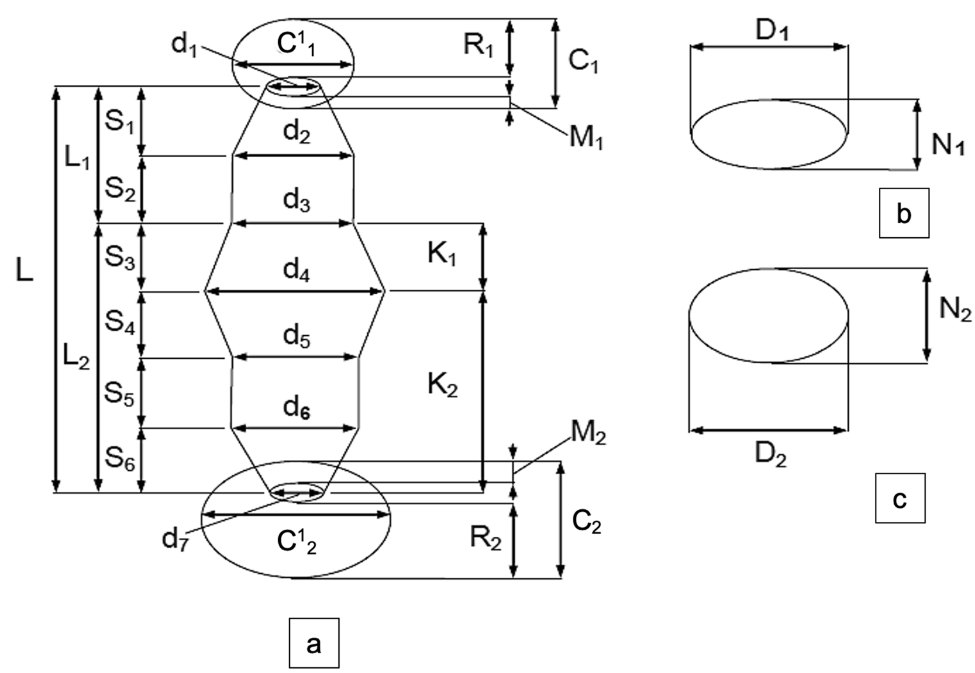


Fig. 1 Model of leech body form: **a** model of the body, **b** transverse sections through the trachelosome, **c** transverse sections through the urosome

The body shape and size are illustrated in Fig. 2 and Tables 1 and 2. The 19 body proportion indices (invariants) are:

1. Index describing L/D_2 = relative body length.
Indices describing anterior sucker: 2. $C^1{}_1/d_1$ = ratio of horizontal diameter of sucker to trachelosome width at sucker junction; 3. $C^1{}_1/D_1$ = ratio of horizontal diameter of sucker to greatest width of trachelosome; 4. R_1/M_1 = length ratio of the anterior part of sucker to its posterior part; 5. $C^1{}_1/C_1$ = ratio of horizontal diameter of sucker to its vertical diameter.
Indices describing trachelosome: 6. L_1/D_1 = ratio of trachelosome length to its greatest width; 7. D_1/N_1 = ratio of greatest trachelosome width to its greatest height; 8. S_1/S_2 = ratio of the height of first trapezium to the height of second trapezium.
Indices describing urosome: 9. L_2/D_2 = ratio of urosome length to its greatest breadth; 10. D_2/N_2 = ratio of greatest urosome width to its greatest height; 11. K_1/K_2 = ratio of the distance from d_3 to D_2 to the distance from D_2 to d_7.
Indices describing posterior sucker: 12. $C^1{}_2/d_7$ = ratio of horizontal diameter of sucker to urosome width at sucker junction; 13. $C^1{}_2/D_2$ = ratio of horizontal diameter of sucker to greatest body height; 14. R_2/M_2 = length ratio of the posterior part of sucker to its anterior part; 15. $C^1{}_2/C_2$ = ratio of horizontal diameter of sucker to its vertical diameter.
Indices describing relations between urosome and trachelosome: 16. L_2/L_1 = ratio of urosome length to trachelosome length; 17. D_2/D_1 = ratio of greatest width of urosome to greatest width of trachelosome; 18. N_2/N_1 = ratio of greatest height of urosome to greatest height of trachelosome.
Index describing proportions of suckers: 19. $C^1{}_2/C^1{}_1$ = ratio of horizontal diameter of posterior sucker to horizontal diameter of anterior sucker.

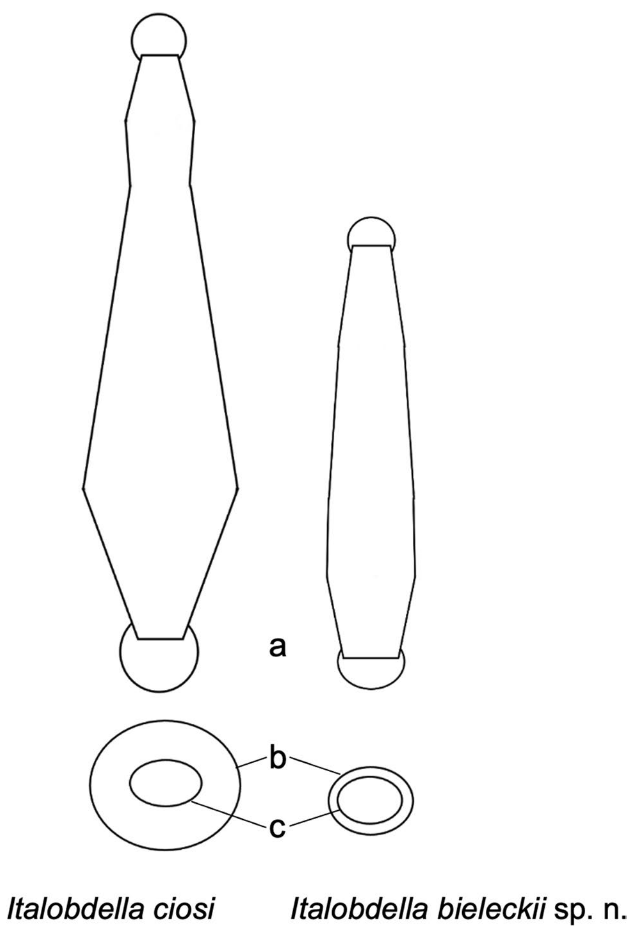


Fig. 2 *Italobdella ciosi* and *Italobdella bieleckii* sp. n.: **a** model of the body, **c, b** transverse sections through the trachelosome and urosome

The number of specimens examined and the parameters describing their body form are presented in Table 1, and mean values of the body proportion indices are presented in Table 2.

List and description of non-metric 113 character, states are presented in works by Bielecki (1997, 2001) and Thorp et al. (2019).

The joining or tree clustering algorithm was chosen to interpret the similarity of body form of the 24 piscicolid species. When selecting distance measures, city-block (Manhattan) distance was chosen, which minimizes the effect of more distant objects (morphotypes). Of amalgamation or linkage rules, Ward's method was used since it tends to yield small clusters and with such a low number of species gives a high resolution. The procedure allowed dividing the species morphotypes into two polytypic clusters (Figs. 3 and 4). Statistical analyses and figures were made using STATISTICA 12 (StatSoft, Inc.).

Taxonomy

Genus *Italobdell*a Bielecki, 1993

*Italobdell*a Bielecki, 1993: 67–78, Figs. 1–20.

Type species: *Italobdella ciosi* Bielecki, 1993.

Diagnosis. Considering the leech diversity in Palearctic, species of the genus *Italobdella* are most similar to the genus *Pawlowskiell*a Bielecki, 1997, *Caspiobdella* Epshtein, 1966 and *Acipenserodbella* Epshtein, 1969. In all these taxa spermatheca opening is located anterior to the first pair of respiratory vesicles. The female and male gonopores are well visible on clitellum. *Italobdella* differs from *Pawlowskiella* in mid-body somite 3-annulate and 4-annulate (in

Table 2 Mean values of 19 body proportion indices in 24 species of Piscicolidae

Species	Body proportion indices																		
	L/D_2	C^1_1/d_1	C^1_1/D_1	R_1/M_1	C^1_1/C_1	L_1/D_1	D_1/N_1	S_1/S_2	L_2/D_2	D_2/N_2	K_1/K_2	C^1_2/d_7	C^1_2/D_2	R_2/M_2	C^1_2/C_2	L_2/L_1	D_2/D_1	N_2/N_1	C^1_2/C^1_1
Cystobranchus mammillatus (Malm, 1863)	3.0	1.6	0.9	2.0	1.0	1.0	2.3	2.0	2.4	4.0	0.3	5.2	1.6	1.0	1.0	4.3	1.8	1.0	3.3
Acipenserobdella volgensis (Zykoff, 1903)	4.2	1.6	0.9	4.0	1.0	2.1	1.3	0.9	3.2	1.0	1.0	1.2	0.6	2.0	1.0	3.3	2.1	1.9	1.5
Caspiobdella fadejewi (Epshtein, 1961)	7.4	1.5	1.2	4.0	1.0	3.0	1.0	0.9	5.5	1.1	1.2	1.3	1.0	1.7	0.9	2.9	1.6	1.4	1.3
Italobdella epshteini Bielecki, 1997	3.4	1.3	0.8	5.0	1.1	1.3	1.7	1.3	2.6	1.0	1.1	0.9	0.7	1.7	0.9	2.9	1.5	2.6	1.2
Italobdella ciosi Bielecki, 1993	4.0	1.6	0.9	5.0	1.0	2.3	1.5	1.0	3.1	1.0	1.9	1.5	0.5	1.6	1.0	3.1	2.3	2.5	1.4
Iitalobdella bieleckii sp. n.	5.2	1.6	0.9	4.8	1.0	2.3	1.5	1.1	2.7	1.1	1.8	1.5	0.7	1.6	1.0	2.0	1.7	2.4	1.3
Piscicola burresoni Bielecki et al., 2013	6.7	1.2	1.0	2.4	1.0	2.2	1.8	1.1	5.6	1.7	0.2	1.2	1.0	3.0	1.0	4.4	1.9	1.1	1.9
Piscicola geometra (Linnaeus, 1761)	13.0	2.4	1.5	2.7	1.0	3.7	1.1	0.7	10.0	1.2	0.5	2.2	1.8	2.2	1.0	2.7	1.4	1.3	1.7
Piscicola respirans Troschel, 1850	4.4	1.6	0.9	3.0	0.8	1.5	2.4	1.9	3.4	2.8	0.9	1.9	1.0	1.6	1.0	3.7	1.7	1.4	1.7
Piscicola siddalli Bielecki et al., 2012	5.8	1.6	0.8	4.0	1.0	1.7	2.3	1.2	4.3	2.6	0.6	1.7	0.9	2.5	0.9	2.7	1.3	1.2	1.4
Piscicola fasciata Kollar, 1842	5.0	2.1	1.3	5.0	1.0	3.2	1.7	1.2	3.7	3.0	2.7	2.9	1.5	2.5	1.0	2.7	1.6	1.0	2.0
Piscicola pojmanskae Bielecki, 1994	6.7	1.1	0.7	2.7	0.8	2.1	1.1	2.2	5.3	1.0	1.8	1.4	1.0	2.2	0.9	4.0	1.6	1.6	2.5
Piscicola annae Bielecki, 1997	9.6	2.4	1.4	5.7	1.0	5.3	1.0	0.9	7.3	1.2	1.2	1.7	1.0	1.3	1.0	3.2	2.3	1.9	1.6
Piscicola borowieci Bielecki, 1997	23.0	2.0	1.3	3.0	1.0	6.0	1.2	1.1	17.6	1.0	0.3	2.1	2.1	2.0	1.0	3.4	1.2	1.4	1.9
Piscicola pomorskii Bielecki, 1997	5.6	2.3	1.6	3.3	1.0	3.1	1.7	0.9	4.3	3.1	0.8	1.6	0.8	2.6	0.9	3.5	2.5	1.3	1.2
Piscicola witkowskii Bielecki, 1997	6.5	1.8	1.0	3.0	1.0	2.2	1.1	1.5	5.2	1.2	2.9	1.3	1.0	1.0	1.0	3.9	1.7	1.6	1.7
Piscicola kusznierzi Bielecki, 1997	6.6	2.0	1.5	3.5	1.2	3.2	1.6	1.2	5.0	2.7	2.1	1.6	1.1	2.7	1.1	3.1	2.0	1.2	1.5
Piscicola wiktori Bielecki, 1997	15.0	1.0	0.8	5.0	0.6	6.2	1.7	0.9	11.7	2.5	0.9	1.1	0.9	3.5	0.8	3.8	2.0	1.3	2.3
Piscicola niewiadomskae Bielecki, 1997	6.1	2.2	1.3	4.0	1.1	3.1	1.3	0.9	4.8	0.9	0.9	1.3	0.8	2.0	1.1	3.5	2.3	2.9	1.4
Piscicola elishebae Bielecki, 1997	5.2	2.0	1.2	3.3	1.0	2.5	1.3	1.0	3.9	1.3	0.3	1.4	1.1	2.0	1.0	2.8	1.8	1.9	1.8
Piscicola margaritae Bielecki, 1997	6.5	1.3	0.8	4.0	0.8	2.4	1.4	0.9	5.5	2.5	0.3	1.4	0.7	1.3	0.8	5.3	2.3	1.3	1.9
Piscicola jarai Bielecki, 1997	11.0	1.3	0.8	6.0	1.0	3.1	2.5	1.8	8.5	2.6	0.3	1.7	1.5	1.3	1.0	3.6	1.3	1.3	2.4
Pawlowskiella stenosa Bielecki, 1997	19.6	1.7	1.2	4.0	1.2	6.3	2.0	1.2	14.1	1.4	3.3	1.3	1.4	1.7	1.0	2.6	1.2	1.7	1.4
Piscicola brylinskae Bielecki, 2001	7.0	1.5	0.7	4.0	1.5	2.7	2.7	1.0	5.4	2.8	0.2	1.6	0.9	2.7	1.3	3.5	1.7	1.7	2.2

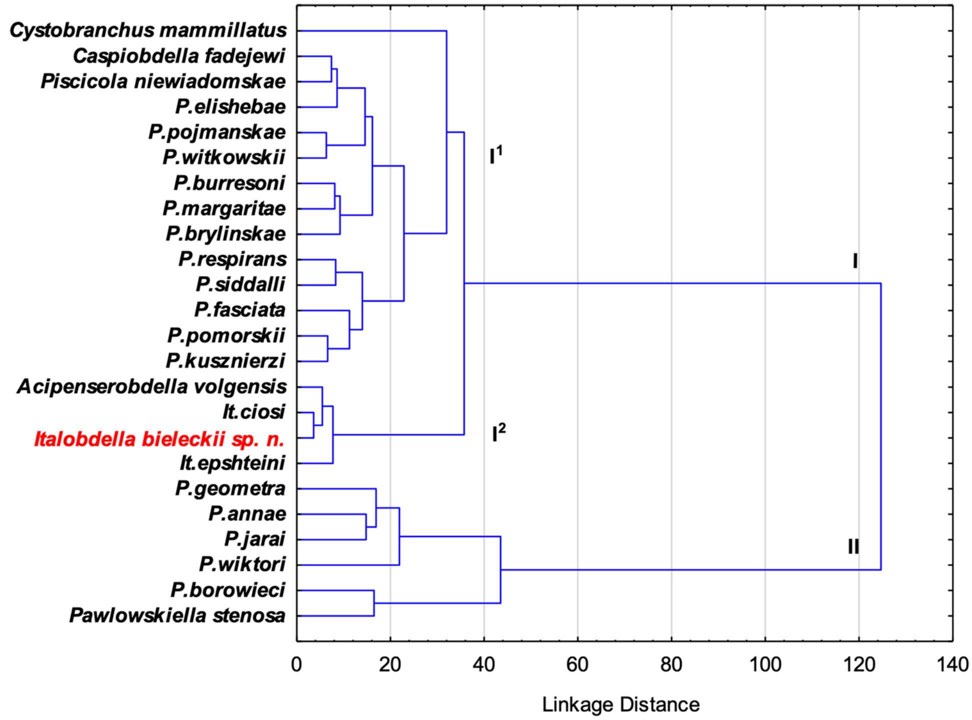


Fig. 3 Tree diagram for 24 species of Piscicolidae based on mean value of 19 body proportionindices (Ward's method, Manhattan distance)

Pawlowskiella and *Caspiobdella* 14-annulate, *Acipenesrobdella* 6-annulate), spermatheca opening is located between gonopores (Plate I: 4–6) (in *Pawlowskiella* posterior to the female gonopore), ovaries enter female gonopore posterior to the vector tissue (in *Pawlowskiella, Caspiobdella* and *Acipenserobdella* they enter through vector tissue); vector tissue is as a narrow plate transverse to the long body axis, and is located anterior to the oviduct outlet (in *Pawlowskiella, Caspiobdella* and *Acipenserobdella* as a circular plate situated on female gonopore). Oviducts of *Italobdella* do not enter the vector tissue like they do in *Pawlowskiella, Caspiobdella* and *Acipenserobdella*.

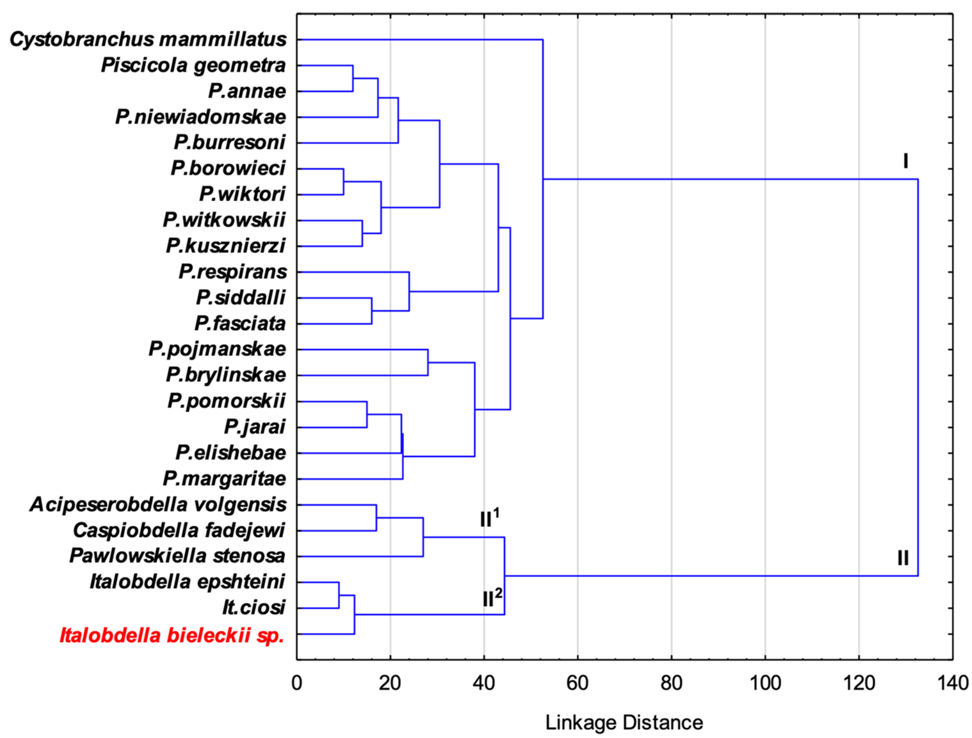
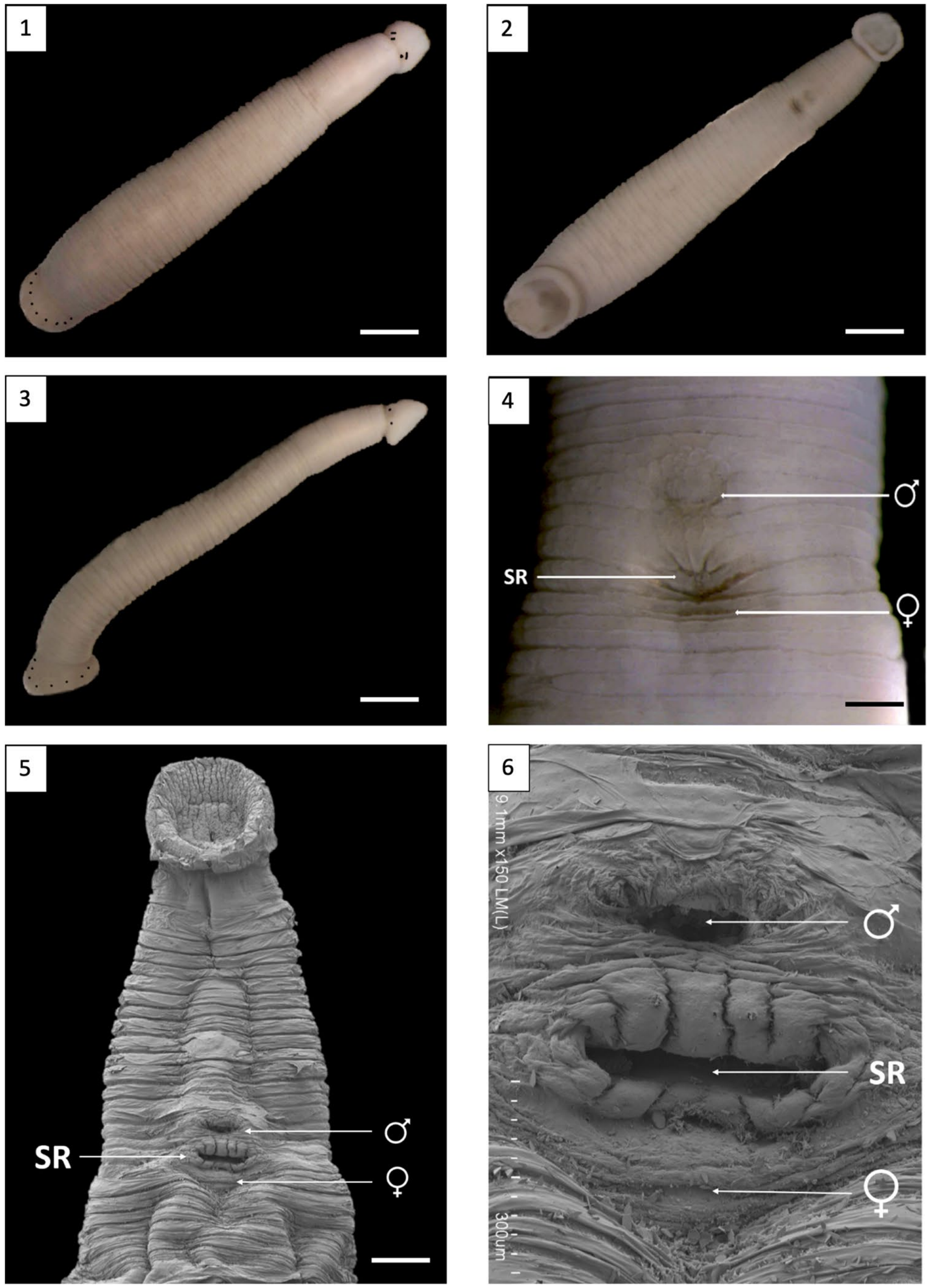


Fig. 4 Tree diagram for 24 species of Piscicolidae based on 113 character, states (Ward's method, Manhattan distance)

Plate I *Italobdella bieleckii* sp. n. (holotype 1–4): **1** dorsal side (scale bar = 1.73 mm); **2** ventral side (scale bar = 1.73 mm); **3** lateral side (scale bar = 1.73 mm); **4** part with gonopores and spermatheca, – male gonopore, SR – opening of spermatheca, – female gonopore, (scale bar = 0.26 mm); **5–6** part with gonopores and spermatheca in *Italobdella ciosi* (scale bar = 0.43), SEM



Description. Size very small (5.9 mm) or medium (15.7 mm) and large (21.1 mm). Body short. Division into trachelosome and urosome very well visible, trachelosome flattened and urosome cylindrical. Body smooth, with no papillae. Anterior sucker small or medium-sized, posterior also small and connected somewhat eccentrically. Respiratory vesicles (11 pairs) white, rather small but well visible, often white. Eyes on anterior sucker and eye-like spots on posterior sucker present. Mid-body somite three-annulate with secondary shallower grooves, up to six, or four-annulate with secondary shallower grooves, up to 12. Oesophagus with glands, crop and posterior crop caecum of two equal diverticula, both sides are equal in size or the first is much more developed than the other and it resemble ram horns. Posterior crop caecum incompletely fused; five fenestrae remain at the level of ganglia. Intestine well developed.

Spermatheca opening visible as a round white field with an opening in the middle (two annuli posterior to male gonopore). Copulatory area on clitellum small, surrounding spermatheca opening. Prostatic glands on atrium well developed. Ejaculatory ducts small, between fourth and fifth ganglia, or rather slightly protrude beyond fifth ganglion, bent once, with characteristic bulbs. Seminal vesicles reach the second pair of testisacs or are situated between the first and second pairs of testisacs. Vector tissue as a narrow, rectangular plate transverse to body long axis, anterior to oviduct outlet. Oviducts open to female gonopore posterior to vector tissue.

Distribution. Palaearctic genus - three species. Europe (N Italy, Germany, Austria, Hungary, NW Poland, Latvia and Slovakia).

Italobdella bieleckii **Cichocka, Košel et Świątek, sp. n.**
Plates I(1–4), II-III, Figs. 2a, b, c, 3, 4, Tables 1, 2

Diagnosis. Piscicolinae; freshwater; basically three-annulate; opening of spermatheca (receptaculum seminis) visible as transverse structure to the long body axis located between male and female gonopores (Plate I: 4–6). Fissure of spermatheca is bigger than male gonopore and located two annuli behind it. Female gonopore poorly visible, situated one annulus behind the opening of spermatheca. Copulatory area (CA) on clitellum absent (Plate I: 4). Vector tissue (VT) in the form of narrow plate arranged transverse to the long body axis, anteriorly to the outlet of oviducts. Conducting strands of vector tissue (CS) in the form of quite broad bands connecting ovisacs with VT (Plate III: 2, 6). Oviducts do not enter the VT but they go outside the clitellum below VT (Plate I: 4–6; III: 2, 6).

Considering morphotypes of all the analyzed species and also of other genera, in its body form *I. bieleckii* sp. n. is most similar to *I. ciosi* (Fig. 3). Very similar, almost identical, characteristics are: $C^1{}_1/d_1$, $C^1{}_1/D_1$, $C^1{}_1/C_1$, L_1/D_1, D_1/N_1, $C^1{}_2/d_7$, R_2/M_2, $C^1{}_2/C_2$.

It differs from *I. ciosi* in following characteristics: L/D_2, R_1/M_1, S_1/S_2, K_1/K_2, R_2/M_2, L_1/D_1, D_1/N_1, L_2/L_1, D_2/D_1, $C^1{}_2/C^1{}_1$, $C^1{}_2/D_2$, $C^1{}_2/d_7$, N_2/N_1.

In non-metric characteristics *I. bieleckii* sp. n. is most similar to *I. ciosi* (Plate I: 1–3, 5–6; Fig. 4). Both have the following characters in common: papillae (sensillae, tangoreceptors) absent, copulatory area absent, spermatheca opening is longitudinal to the body long axis at the level of the first pair of respiratory vesicles, crop and posterior crop caecum of two diverticules, with strongly folded walls, prostatic glands very large, well developed, ejaculatory ducts bent sharply once parallel to the body long axis, vasa deferentia straight, six pairs of testes, ovaries short reaching the first pair of testes (T_1), ovaries as flattened cylinders with posterior ends free, oviducts distant from CS, running parallelly, CS short, VT as a marked circular.

Italobdella bieleckii sp. n. differs from *I. ciosi* and *I. epshteini* in the following characteristics: mid-body somite of three (six) unequal annuli (in *I. ciosi* and *I. epshteini* four (12) unequal annuli), nephridiopores clearly visible in middle annuli of somite (in *I. ciosi* and *I. epshteini* nephridiopores are not visible), gonopores separated by 4.3 annuli (in *I. ciosi* and *I. epshteini* four annuli), two-piece parts of crop and posterior crop caecum unequal, first is significantly bigger than other in form of ram horns (in *I. ciosi* and *I. epshteini* two-piece parts of crop and posterior crop caecum are equal), seminal vesicles (VS) twisted many times, before the second pair of testes (T_2), (in *I. ciosi* and *I. epshteini* classically U shaped, before the first pair of testes (T_1) beginning of the ovary).

Description. Size and body form (Figs. 1, 2 and 3; Plate I: 1–3; III: 3–4; Tables 1 and 2). Medium-sized leeches. Of the five specimens, the largest was 15.70 mm long with the greatest width of 3.95 mm, and the smallest was 9.92 mm long with the greatest width of 1.50 mm. Average values were adopted for further analysis of the parameters. The length of the body exceeds the greatest width by 5.2 times. Ratio of the greatest width to the greatest height (thickness) of the urosome is 1.0. Ratio of the greatest width of the urosome to the greatest width of the trachelosome is 1.7. The diameter of the anterior sucker to the greatest width of the trachelosome is 0.9. The ratio of the diameter of the posterior sucker to the largest width of the urosome is 0.7. The diameter of the posterior sucker is slightly more than the diameter of the anterior sucker (1.3 times).

Separation into trachelosome and urosome is visible even in preserved and poorly fed individuals. Body is short, slightly flattened or only trachelosome is flattened and urosome is cylindrical. The anterior sucker is small or medium size, the posterior sucker is also small and connected to the urosome slightly eccentrically (Table 1).

The algorithm of combining or grouping the body forms of 24 Piscicolidae species allowed to divide the morphotypes of the species into two polytypic clusters. *Italobdella bieleckii* sp. n. was included in cluster I and subcluster I^2 together with *I. ciosi* as species with the most similar body forms. *Italobdella epshteini* and *Acipenserobdella volgensis* (Zykoff, 1903) were in the same subcluster (I^2), being external, less similar to these two species (Fig. 3).

Vesicles. Pulsating vesicles are white (11 pairs) small, but clearly visible. Body without papillae, smooth, although I. bieleckii sp. n. has distinct nephridiopores (Plate II: 1–2; III: 3, 5).

Somites. Full somites consist of 3 annulli. Further, shallower dividing furrows are visible on these 3 annuli. The first annulus, the shortest, is divided into two unequal parts, the second (with vesicle), slightly longer than the first, also divided into two unequal parts, and the third, longest, divided into two unequal parts. In total, the somite consists of three annuli separated by deep grooves, and shallower

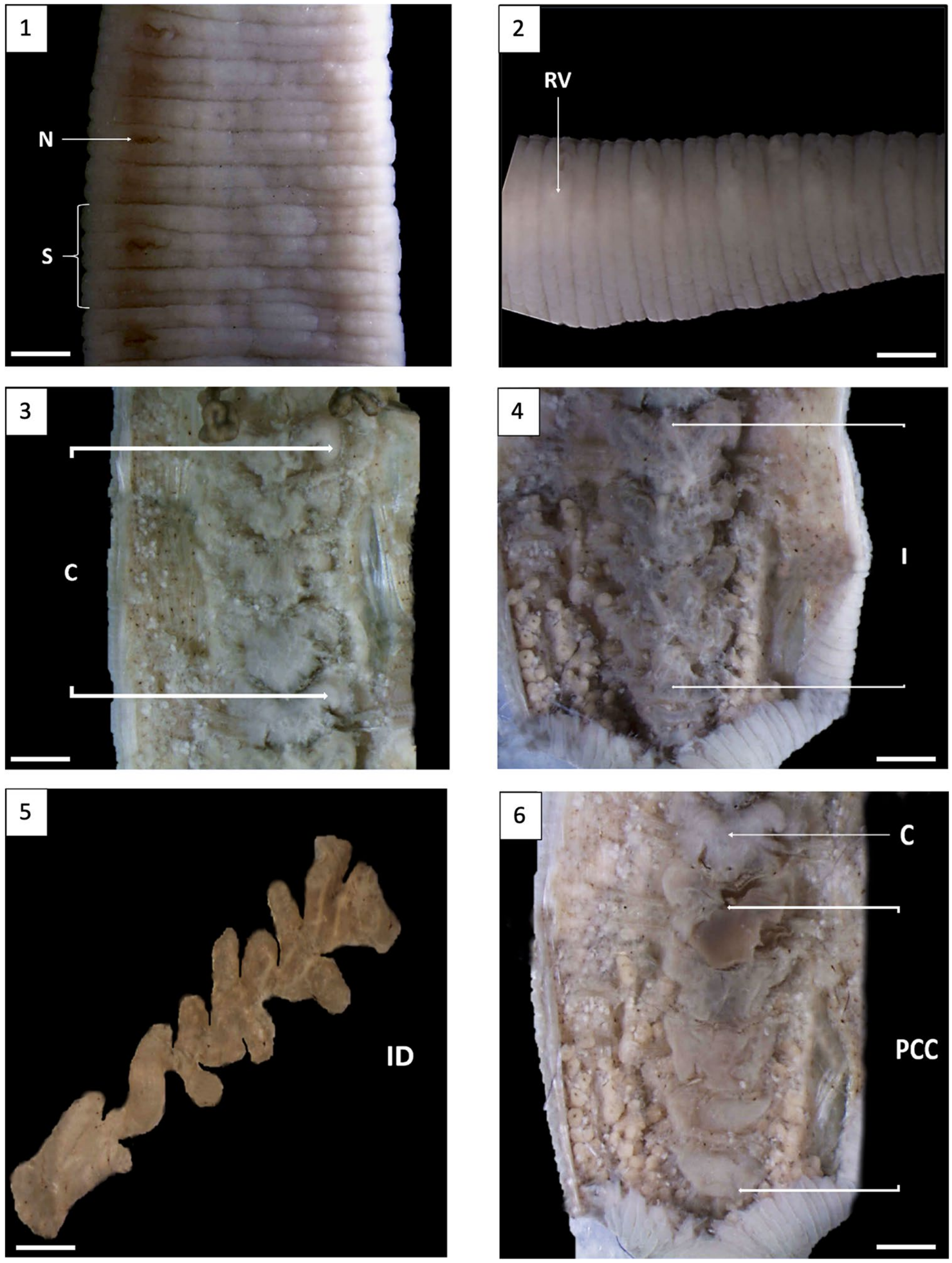


Plate II *Italobdella bieleckii* sp. n. (holotype): **1** ventral side, two full 3-annulte somites, N – nephridiopores, S – somite (scale bar = 0.23 mm); **2** lateral side, RV – respiratory vesicles (scale bar = 0.21 mm); **3–6** holotype, alimentary tract: **3** C – crop, pouches 3–7 (scale bar = 0.30 mm); **4** I – intestine (scale bar = 0.30 mm); **5** intestine separated from posterior crop, five intestine diverticula (ID) (scale bar = 0.39 mm); **6** PCC – posterior crop, caecum consisting of two diverticula, first is significantly bigger than the other (scale bar = 0.30 mm)

grooves divide annuli further giving finally six annuli in somite (Plate II. 1–2; III. 5).

Body colouration (Plate I: 1–3; III: 3–5). It results from the different arrangement of the two types of melanophores: brown and white. Their composition makes the color of the leeches quite monotonous. A characteristic feature is that both brown and white melanophores do not have protrusions and marked bodies. Even preserved specimens can hardly be confused with the other two species of the genus *I. ciosi* and *I. epshteini*. White melanophores on the trachelosome form 4 or 5 spots in the medial plane. To the left and right of the white medial spots, there are also white spots at the same height - paramedical (4 or 5) and on the sides of the body also 4 or 5 white spots. On the urosome, white spots maintain a similar order. There are 14 spots in the medial plane. On the left and right side of the medial spots are two rows of smaller white paramedical spots, with 13 on each side. The coloration, and especially the distribution of white spots on the trachelosome and urosome, resembles that of *I. ciosi*, but also of *Piscicola siddalli* Bielecki, Cios, Cichocka and Pakulnicka, 2012. The anterior sucker is uniformly brown. The posterior sucker has very narrow white and wide brown radial streaks. There are 14 of both.

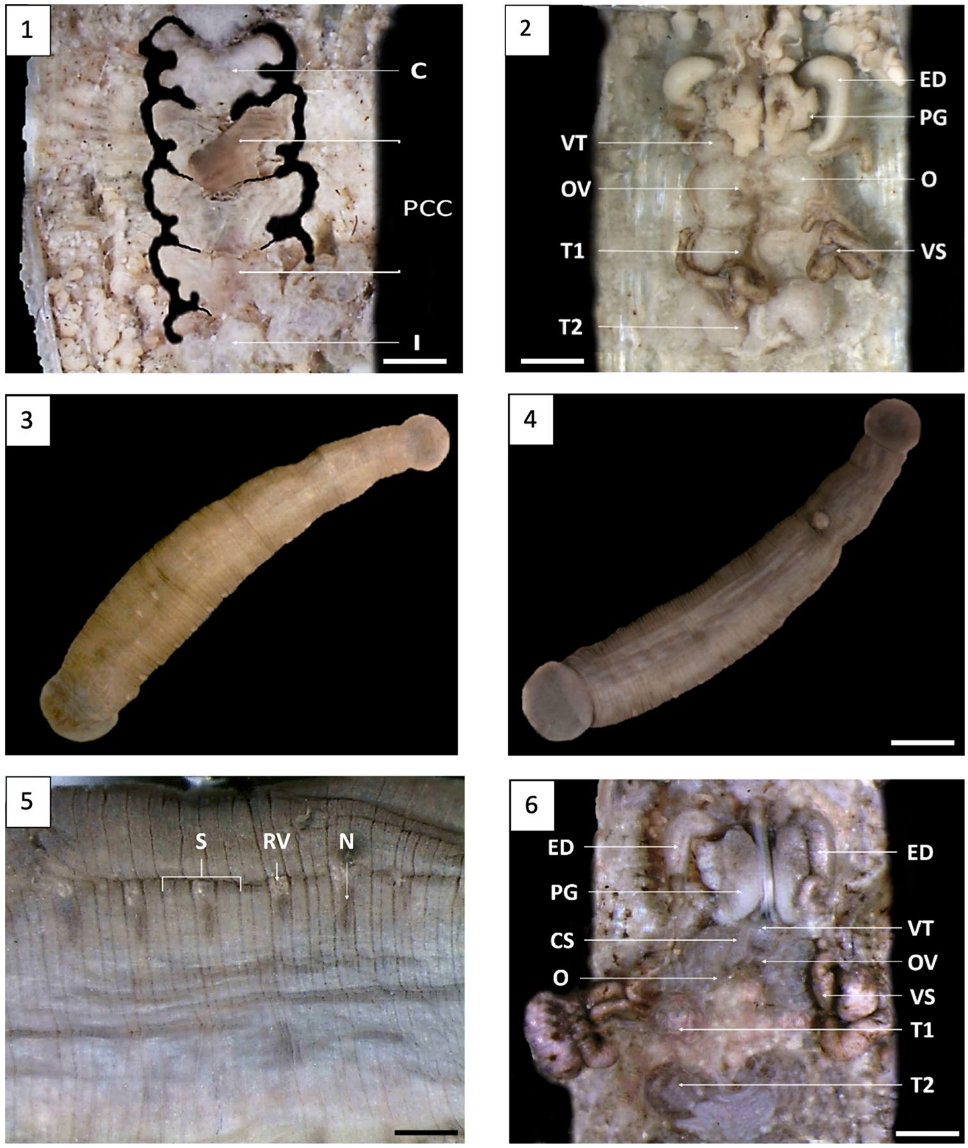


Plate III *Italobdella bieleckii* sp. n.: **1** alimentary tract of holotype: C – crop, seventh pouches; PCC – posteriori crop (3 pouches, each divided into two diverticula, similarly to the crop (retouched) (scale bar = 0.30 mm); **2** reproductive system of holotype (scale bar = 0.20 mm); **3** dorsal side of paratype (scale bar = 1.77 mm); **4** ventral side of paratype (scale bar = 1.77 mm); **5** part of ventral part with nephridiopores visible (scale bar = 0.22 mm); **6** reproductive system of paratype (scale bar = 0.23 mm); ED – ejaculatory duct, PG – prostate glands, VT – vector tissue (above the ovisacs), CS – conducting strands of vector tissue, O – ovisac, OV – oviduct, VS – seminal vesicle, T1-T2 – first and second pair of testisacs; 5. S – 3-annulate somite, RV – respiratory vesicle, N – nephridiopore

Eyes. On the anterior sucker, there are two pairs of eyes - the first one is slightly larger, obliquely to the sagittal plane, the second one is perpendicular and smaller. On the posterior sucker, there are 10 eye-shaped spots located on the edge of faintly defined dark radial streaks (Plate III: 3).

Digestive system (Plate II: 3–6; III: 1). The oral opening is eccentrically located at the posterior part of the anterior sucker and leads back to the proboscis. The position of the proboscis is between the first and third ganglia, starting with the cephalic ganglion mass (ganglia 1–6), what means, the proboscis reaches the ganglion of segment IX. There are salivary glands on the right and left sides of the proboscis. The thin-walled esophagus extends from IX to XII/XIII segment, and it enter the crop (C). There is a diverticulum (pair of pouches) that connects to the esophagus. A characteristic feature of the crop is presence of seven pouches located between the segments, they are paired along the right and left edges, each of them is divided into two parts (crop diverticula - CD). The first pair of diverticula is well developed and shaped like ram's horns (Plate II: 3–6; III: 1). In the XIX/XX somite below the seventh crop diverticulum, the intestine divides into a thin-walled caecum (posterior post caecum PCC) located ventrally, and a thick-walled intestine (I) located dorsally in relation to the previous one (i.e., on the posterior crop). The thin-walled posterior crop (PCC) consists of five diverticula which are not completely merged. There are five openings in its dorsal and ventral parts (at the level of the ganglia). Each diverticulum consists of two parts similar to the crop diverticula (CD) (Plate II: 6). The thick-walled intestine is very elongated with folded edges, so tight that it completely covers the PCC. It has five paired diverticula (ID), of which the first three pairs are well developed, directed to the sides and anteriorly. The fourth pair is not much smaller, and its branches are shifted to the sagittal plane. The fifth is very small and hardly visible. Behind it, the intestine forms a twisted tube, which continues into the rectum, ending with the anus (Plate II: 5).

Reproductive system (Plate I: 4; III: 2, 6). There are three openings on the clitellum. The first is a large, clearly visible male gonopore lying exactly in the center of the annulus. Three and a half annuli behind it, there is a second opening – spermatheca, very large, relatively narrow, in the form of a slit transversely arranged to the main axis of the body. The third opening is the female gonopore, small, hardly visible, lying ¾ of the annulus behind the spermatheca. The exact distance between the male and female gonopores is 4.25 annuli. The copulatory area (CA) on the clitellum is usually absent, however, if present, it is very small within the opening of the spermatheca. In the male reproductive system, there are six pairs of testes (T_1-T_6) located between the segments along the section from XIII/XIV segment to the XVIII/XIX segment. Seminal tubules (vasa eferentia) depart from the testicles and connect to the longitudinal vasa deferentia. At the front, the vasa deferentia pass into the seminal vesicles (VS) at the level of the testicles of the second pair (T_2). They form the ejaculatory ducts (ED) in the form of thick half loops that do not extend to the fifth ganglion and connect to the general part of the atrium and the sac of the copulatory capsule. In their natural position, the ejaculatory ducts bend in the sagittal plane towards the dorsal side. During dissection the ejaculatory ducts were bent to the sides (by 90°) to better visualise their structure (Plate III: 6). Prostate glands (PG) in the atrium are present. In the female reproductive system, the ovisacs are flattened, half-moon shaped, their posterior ends reach the testisacs of first pair (T_1). The anterior ends of the ovisacs (before passing into the oviducts) cover the vector tissue (VT), making the conductive strands of vector tissue (CS) invisible. The oviducts enter the female gonopore posterior to the vector tissue (VT) mass. *Italobdella bieleckii* sp. n. is characterized by a special structure and location of the vector tissue. It is in the form of a narrow plate arranged transversely to the longitudinal axis of the body and located above the outlet of the oviducts. Due to its location, paired conducting strands of vector tissue (CS) appear in the form of very short, relatively wide strands of fibers connecting each ovisac with the vector tissue. Thus, the space between the converging oviducts and the testisacs of the first pair (6th and 7th ganglia) is free. The vector tissue (VT) and paired conducting strands of VT (CS) are best seen when the ovisacs are exposed posteriorly or to the side by 180° (Plate III: 6).

The qualitative traits phenogram separated 24 Piscicolidae species into two polytypic clusters. *Italobdella bieleckii* sp. n. is included in cluster II and subcluster II^2 together with *Italobdella ciosi* and *Italobdella epshteini*, with *I. epshteini* being external, less similar to the other two species. Moreover, in the same cluster II but in the subcluster II^1 there were species from the genera *Acipenserobdella*, *Caspiobdella* and *Pawlowskiella*, similar mainly in terms of the reproductive and digestive systems (Fig. 4).

Etymology. Dedicated to Professor Aleksander Bielecki, Polish scientist, with broad scientific interests, with very good methodological skills, which is a unique example - deeply entering the area of the methodology of natural sciences. The main area of his scientific competence concerns comprehensive research (description, classification, and phylogenetic reconstruction) on leeches. He initiated topics, formulated and tested hypotheses in the field of developmental biology of Trematoda, but most of all he was involved in morphological, faunistic, ethological, hydrobiological, ecological, parasitological, taxonomic, molecular, and systemic research on leeches (Hirudinea), and in leeches parasitizing on fish (Piscicolidae). In his monograph, he described leeches parasitizing on Palearctic fish and solved the hypothesis about the positive phototactic mechanism of finding a host by some of these leeches, formulating the theory of positive phototaxis.

Discussion

According to the systematics based on internal and external morphology, there are 15 genera of fish leeches within the subfamily Piscicolinae Johnston, 1865 distinguished on the basis of the structure of the reproductive system (Caballero 1956; Sawyer 1986; Epshtein 1987, 1989; Bielecki 1993, 1997). However, the status of piscicolid subfamilies has been questioned due to the results of molecular studies in which they appeared to be non-monophyletic (Utevsky and Trontelj 2004).

Epshtein (2004) described the leeches from lakes of ancient origin in Asia, Europe and Africa with a particular emphasis on the leeches of the family Piscicolidae. Based on the analyzed distribution of leeches in these lakes, he made a hypothesis about the development and formation of two types of Palearctic leeches from their sea roots: the Eastern type, including the Far East, Baikal, Central Asia, the Eastern Trans-Caucasus and the Western (Caspian) type reaching from the Caspian Sea to the Apennine Peninsula. According to this division, *Italobdella bieleckii* sp. n. belongs to the Western (Caspian) type. The Caspian Sea (probably around 3 million years old), similarly to Lake Baikal, has an endemic fauna of leeches and is a refuge for the unique leeches of the family Piscicolidae. Some of them, although originally freshwater, are also tolerant of brackish water. From the Caspian Sea and the Danube basin to the Apennines, there is a region where the Caspian fauna occurs, which includes species of genera that differ from the Holarctic and Baikal Piscicolidae in terms of the structure of the female reproductive organs. These genera are as follows: *Caspiobdella*

Epshtein, 1966, *Italobdell*a Bielecki, 1993 and *Pawlowskiell*a Bielecki, 1997, with a total of six species: *Caspiobdella caspica* (Selensky, 1915), *C. fadejewi* (Epshtein, 1961), *C. tuberculata* Epshtein, 1966, *Italobdella ciosi* Bielecki, 1993, I. *epshteini* Bielecki, 1997 and *Pawlowskiella stenos*a Bielecki, 1997. Such a result was also obtained in the phenogram concerning the qualitative traits, mainly in terms of the similarity of the characteristics of the reproductive and digestive systems of Piscicolidae. *Italobdella bieleckii* sp. n. was grouped in cluster II and subcluster II^2 together with *I. ciosi* and *I. epshteini*. However, in the same cluster II and subcluster II^1 species from the genera *Acipenserobdella*, *Caspiobdella* and *Pawlowskiella* were assembled (Fig. 4). In the coastal waters of the Caspian Sea, there is *Piscicola geometra* (Linnaeus, 1761), while in much of the northern part of the basin only *Caspiobdella caspica* (Selensky, 1915) is present, and the presence of *P. geometra* was not reported. In the central part of the Caspian Sea, only one species of Piscicolidae has been described, *Caspiobdella tuberculata* Epshtein, 1966 (Epshtein 2013).

The occurrence of *I. ciosi* in Northern Italy is essential for understanding the origin of the Caspian Piscicolidae. This species is believed to be a relic of the waters flowing from the Tethys Sea and inhabits rivers flowing into the Mediterranean, Black and Azov Seas (Epshtein 2004, 2013). *Italobdella ciosi* is most likely found in the River Pad and its basin, which proves the western border of the Caspian fauna. Species of the genus *Piscicola* and species of the Caspian type genera, which never had a connection with the sea, after Martinson (1958), we name paleolimnic.

A disputable opinion was presented by Minelli (2006), who believed that *I. ciosi* was a species of low taxonomic value and was brought to Italy with fish. Unfortunately, he did not present any alternative hypothesis about the origin of this species, not to mention that he did not interpret the reproductive system of the leech. For these reasons, we believe that the approach by Minelli (2006) is within methodological anarchism (Feyerabend 1975; Bielecki et al. 2014). It must be clearly stated that the species *Italobdella ciosi*, *I. epshteini* and *I. bieleckii* sp. n. occur in their natural zone and have a very characteristic reproductive system (Bielecki 1993, 1997; Bielecki et al. 2008; Epshtein 2004, 2013). Interestingly, Epshtein (2004, 2013) presented the types of the reproductive system in leeches from the Piscicolidae family that correlate with their distribution. Within these types there is "*Caspiobdella*" type (subtribe Caspiobdellina), in which *Italobdella* is included.

Assuming that the distribution of *Italobdella* species is natural, one can hypothesize that, most likely, they are relics of the waters originated from the Tethys Sea, which inhabited (and still inhabit) the basins of rivers flowing into the Mediterranean Sea, the Black Sea and the Azov Sea. The study by Soes (2010) is significant, as he clearly presented that the collected specimens of *C. respirans* from Italy (at his disposal) were mistakenly identified, and most likely, they were specimens of *I. ciosi*, *I. epshteini* or *I. bieleckii* sp. n. or another new species of the genus *Italobdella*. To complete the picture of the Caspian Piscicolidae, the presence of two species on the other side (eastern) of the Adriatic Sea is also important. The first is *Caspiobdella hadzi* (Sket 1968) which was described as *Piscicola hadzi* Sket, 1968 but in 1997, Bielecki, after interpreting the reproductive system of this species, moved it to the genus *Caspiobdella*. The same happened with *Cystobranchus pawlowskii* Sket, 1968, which was transferred to the genus *Piscicola* (Sket 1968).

Concluding, in Eurasia we distinguish between two neolimic groups of the Piscicolidae family: the eastern one, which is represented by *Limnotrachelobdella* and genera related to Baikal leeches, and the western one, which contains the leeches classified in the Caspian type.

Key to the species of *Italobella*

1. Size very small (5.9 mm) and large (21.1 mm) ……………………………………………………………2

   – Medium size, body length up to 15.7 mm, on anterior sucker lack of "white spectacles", coloration of dorsal side of trachelosome and urosome distinct, uniform, formed by brown and white melanophores (without tabs and corps, white transverse segmental spots up to five in a row), somite consisting of three annuli (with further divisions into six annuli) ……………… ………………………………… ***I. bieleckii* sp. n.**

2. Relatively big, body length up to 21.1 mm, on anterior sucker large white "spectacles", coloration of dorsal side of trachelosome and urosome distinct - a characteristic pattern formed by brown, black, yellow and white melanophores whose arrangement results in white transverse segmental spots; somite consisting of 4 annuli (with further divisions into 12 annuli) ……………… …………………………………………………***I. ciosi***

   – Very small, body length up to 5,9 mm, on anterior sucker pigmented wavy strip, coloration of dorsal side of trachelosome and urosome not distinct, lack of specific pattern, only black melanophores present as very small dots, slightly concentrated in latero-medial lines; somite consisting of four annuli (with further divisions into 12 annuli) ………………………………………… ***I. epshteini***

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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