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New information on the geographical distribution of *Piscicola brylinskae* Bielecki, 2001 (Hirudinea: Piscicolidae) in Poland and remarks on its systematic position

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Abstract: For the first time, *Piscicola brylinskae* was described from Lake Vechten in the village of Bunnik, near Utrecht – The Netherlands. Until now, *P. brylinskae* has been found in Poland in Lake Maróz and in the Łyna River near Olsztyn (the northern part of Warmian-Masurian voivodeship). Thanks to proper conservation the coloration of *P. brylinskae* was described for the first time. Applying 32 indexes resulted that *P. brylinskae* clustered to *Caspiobdella fadejewi.* Analysis based on 113 non-metric characteristics has shown that *P. brylinskae* is most similar to *Piscicola margaritae.* Though, *P. brylinskae* do not form with *P. margaritae* dichotomic branching nor with any other species from this cluster, it could confirm that it is an individual species of the *Piscicola* genus.

Key words: Hirudinea; new species; Poland

Introduction

The first specimen of *Piscicola brylinskae* was caught on August 2, 1979 from Vechten Lake in the village of Bunnik near Utrecht (The Netherlands) (52°03' N, 05°09' E). Vechten is a man-made lake isolated from other water bodies. It has an average depth of 6 m and maximum depth of 12 m, and the surface area comprises 4.7 ha. The lake is largely overgrown by willows (*Salix* sp.). In the lake littoral zone, there are plants such as *Iris*, *Phragmites*, *Glyceria* and *Mentha* (pers. comm.); the single specimen of *P. brylinskae* was found here in a combined sample from submerse vegetation, debris, stones and branches.

Based on the characteristics of their coelomic system, Piscicolidae are divided into three subfamilies: Pontobdellinae Llewellyn, 1966; Platybdellinae Epshtein, 1970; and Piscicolinae Caballero, 1956 (Caballero 1956; Sawyer 1986; Burreson & Dybdahl 1989). The subfamilies contain tribes, which are separated according to variants in the reproductive system (Epshtein et al. 1994; Epshtein 2004).

During the last ten years, there has been more emphasis on infraspecies-variability and molecular studies in order to explain phylogenetic relationships within the family Piscicolidae. The existing division to 18 tribes and 52 genera (Epshtein et al. 1994; Epshtein 2004) in the latest morphological and molecular studies have been examined (Siddall et al. 2001; Utevsky & Trontelj 2004). Moreover, some of the latest molecular studies questioned the previous division of Piscicolidae into 18 tribes (Utevsky et al. 2007; Williams & Burreson 2006). What is more, the new molecular studies have raised doubts on the monophyly of the Platybdellinae and Piscicolinae, and implied that Pontobdellinae could be paraphyletic (Williams & Burreson 2006). Recently, the Pontobdellinae have been described also as polyphyletic (Utevsky et al. 2007).

The subfamily Piscicolinae includes eight tribes, 20 genera and 52 species (Caballero 1956; Soós 1965; Sawyer 1986; Epshtein 1969, 1983, 1987, 1989; Epshtein et al. 1994; Davies 1991; Bielecki 1993, 1994, 1995, 1997, 2001; Bielecki & Epshtein 1994; Sket & Trontelj 2008). Sawyer (1986) based on the papers of Johansson (1896), Brumpt (1900), Selensky (1907), and Malécha (1979), determined the genus *Piscicola* as freshwater, with small respiratory vesicles and mid-body somite of 14 annuli, vector tissue and copulatory area with conductive tissue. Vector tissue may pass near the oviduct to the region of the male gonopore.

In Europe, there are 15 species of the *Piscicola* genus, and in the freshwaters of Poland, 14 of them have been recorded (Bielecki 1997). In 2005 specimens of *P. brylinskae* were found in Poland which makes the total number of 15 species (Bielecki 2001).

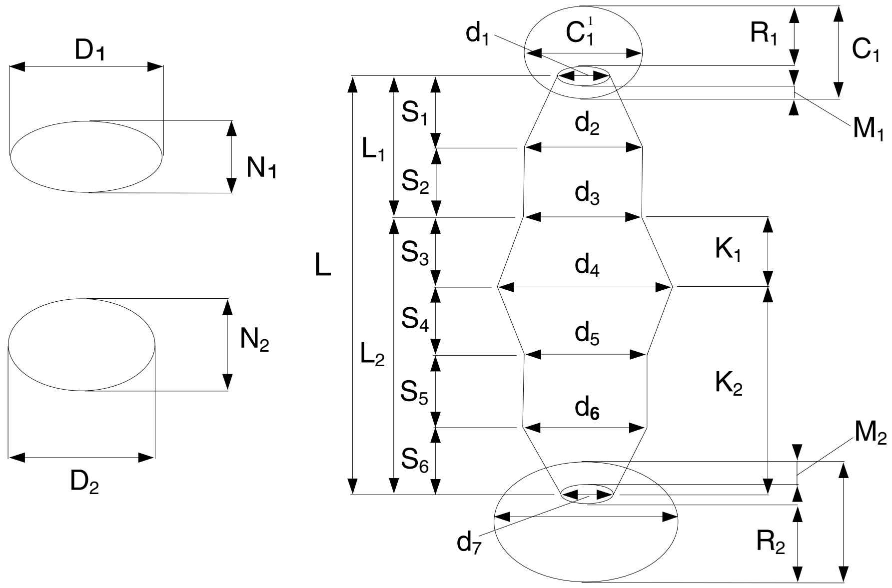

Fig. 1. Model of leech body form.

Material and methods

The first information about *P. brylinskae* from Poland is from August 2005. New specimens of *P. brylinskae* were caught in Lake Maróz (6 specimens) and Łyna River (5 specimens) near Olsztyn.

Leeches were anesthetized initially with 10% ethanol. Then, the mucus was washed in a solution of 50% ethanol and placed either in 70% alcohol solution or 3% formalin solution (Pawłowski 1936; Serafińska 1958). The material preserved that way was stored in the laboratory.

Leeches range in size and shape, depending on many factors such as the degree of fulfillment of the alimentary tract or the degree of sexual development. However, this was easily formalized and became an object of mathematical modeling (Bielecki 1993, 1997, 2001; Bielecki & Epshtein 1994; Bielecki et al. 2009). The model presents the leech body on a plane as two ellipses representing suckers and trapeziums situated between them reprezenting the anterior body part – trachelosome – 2 trapeziums; posterior body part – urosome – 4 trapeziums. In addition, transverse sections through the trachelosome and urosome are considered as two ellipses (Fig. 1).

The model was constructed according to the following parameters. Parameters for the form of the anterior sucker were: C_1 = horizontal diameter; C_1^1 = vertical diameter; R_1 = length of anterior part of sucker; M_1 = length of the posterior part of sucker. Parameters for the form of the trachelosome were: d_1 = width at the sucker junction; d_2 = width at outline narrowing; d_3 = width at border with the urosome; D_1 = greatest width of the trachelosome; N_1 = greatest height of trachelosome; S_1 = height of the first trapezium; S_2 = height of the second trapezium; $L_1 = (S_1 + S_2)$ = length of trachelosome.

Parameters for the form of the urosome were: d_4 = base of the first trapezium; d_5 = base of the second trapezium; d_6 = base of the third trapezium; d_7 = base of the fourth trapezium (width at sucker junction); D_2 = greatest width of urosome; N_2 = greatest height of urosome; $L_2 = (S_3 + S_4 + S_5 + S_6)$ = urosome length (height of consecutive trapeziums); S_3 = height of the first trapezium; S_4 = height of the second trapezium; S_5 = height of the third trapezium; S_6 = height of the fourth trapezium; K_1 = distance from d_3 to D_2; K_2 = distance from D_2 to d_7.

Parameters for the form of the posterior sucker were: C_2^1 = horizontal diameter; C_2 = vertical diameter; M_2 = length of the anterior part of sucker; R_2 = length of the posterior part of the sucker.

There were thirty-two body proportion indices (invariants):

1. Index describing L/D_2 = relative body length.

Indices describing the anterior sucker:

2. C_1^1/d_1 = ratio of the horizontal diameter of the sucker to trachelosome width at sucker junction;
3. C_1^1/D_1 = ratio of the horizontal diameter of the sucker to the greatest width of the trachelosome;
4. R_1/M_1 = ratio of the dorsal part of the sucker to its ventral part;
5. C_1^1/C_1 = ratio of the horizontal diameter of the sucker to its vertical diameter.

Indices describing the trachelosome:

6. L_1/D_1 = ratio of trachelosome length to its greatest width;
7. D_1/N_1 = ratio of the greatest trachelosome width to its greatest height;
8. S_1/S_2 = index describing the position of the greatest width of trachelosome;
9. L_1/L = ratio of trachelosome length to body length;
10. N_1/L_1 = ratio of the greatest height of the trachelosome to trachelosome length.

Indices describing the urosome:

11. L_2/D_2 = ratio of urosome length to its greatest breadth;
12. D_2/N_2 = ratio of greatest urosome width to its greatest height;
13. K_1/K_2 = ratio describing the position of greatest width of the urosome;
14. N_2/L_2 = ratio of the greatest height of the urosome to its length.

Indices describing the posterior sucker:

15. C_2^1/d_7 = ratio of the horizontal diameter of the sucker to urosome width at sucker junction;
16. C_2^1/D_2 = ratio of the horizontal diameter of the sucker to the greatest body height;

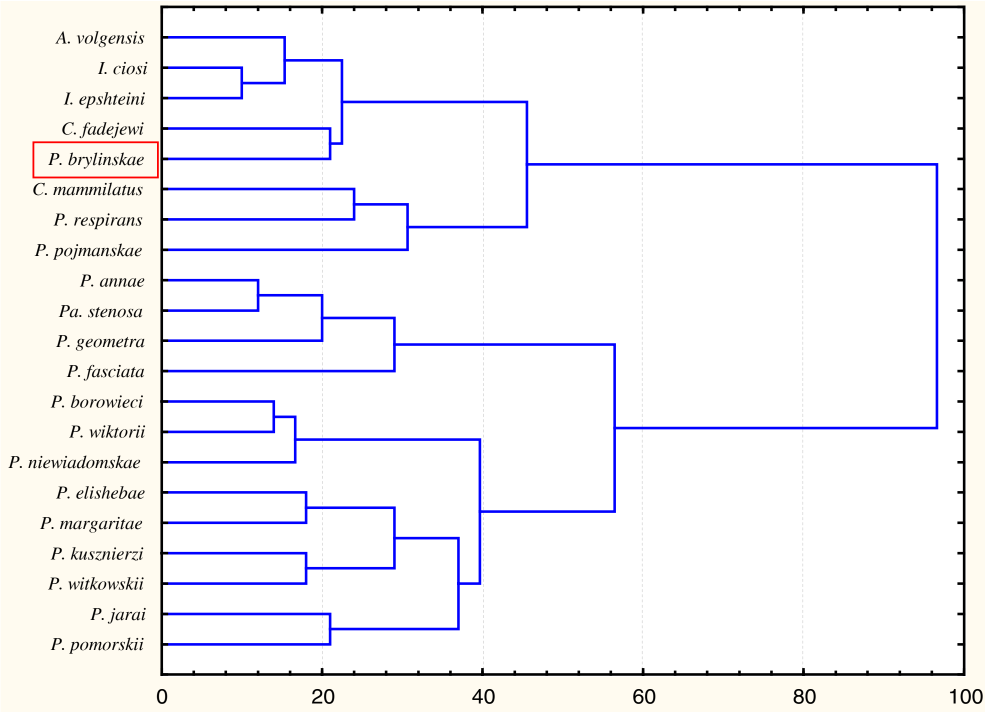

Fig. 2. Tree Diagram for 21 species (Piscicolidae) based on mean value of 32 body proportion indices (Ward's method, Manhattan distances).

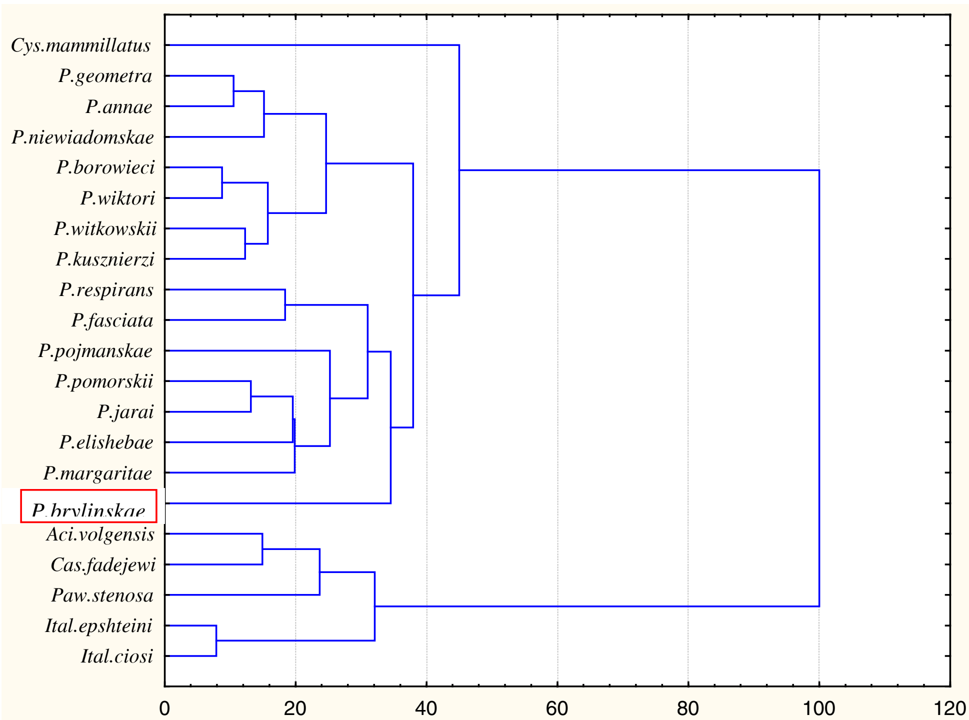

Fig. 3. Tree Diagram for 21 species (Piscicolidae) based on 113 non-metric characters (Ward's method, Manhattan distances).

17. R_2/M_2 = ratio of the dorsal part of the sucker to its ventral part;
18. C_2^1/C_2 = ratio of the horizontal diameter of the sucker to its vertical diameter.

Indices describing relations between the urosome and trachelosome:

19. L_2/L_1 = ratio of urosome length to trachelosome length;
20. L_2/L = ratio of urosome length to body length;
21. D_2/D_1 = ratio of the greatest width of the urosome to the greatest width of the trachelosome;
22. N_2/N_1 = ratio of the greatest height of the urosome to the greatest height of the trachelosome.

Indices describing proportions of the suckers:

Fig. 4. *Piscicola brylinskae*: posterior sucker with eye-like spots.

23. C_2^1/C_1^1 = ratio of the horizontal diameter of the posterior sucker to the horizontal diameter of the anterior sucker;
24. C_1^1/C_2 = ratio of the horizontal diameter of the sucker to the vertical diameter of the posterior sucker;
25. C_2^1/C_1 = ratio of the horizontal diameter of the posterior sucker to the vertical diameter of the anterior sucker;
26. C_2/C_1 = vertical diameter of the posterior sucker to the vertical diameter of the anterior sucker;
27. $(C_2\text{-}R_2\text{-}M_2)/(C_1\text{-}R_1\text{-}M_1)$ = attachment of the posterior sucker to the attachment of the anterior sucker.

Other indices:

28. C_2/N_2 = the vertical diameter of the posterior sucker to ratio of the greatest height of the urosome;
29. C_1/N_1 = the vertical diameter of the anterior sucker to ratio of the greatest height of the trachelosome;
30. $C_2/(C_2\text{-}R_2\text{-}M_2)$ = the vertical diameter of the posterior sucker to its attachment;
31. $C_1/(C_1\text{-}R_1\text{-}M_1)$ = the vertical diameter of the anterior sucker to its attachment; 32. d_7/d_1 = base of the fourth trapezium to the width at sucker junction.

The leeches were dissected under stereoscopic microscope (Olympus) on Petri dishes filled with parafine, according to Bielecki (1997).

Similarities of the body form and 113 non-metric characteristics (according to Bielecki 1997) were found using cluster analysis (Ward's method, Manhattan distance) (Figs 2, 3).

Material examined: 1 specimen (Holotype) – Lake Vechten in Bunnik village (near Utrecht) (The Netherlands); 6 specimens – Lake Maróz (NE Poland); 5 specimens – Łyna River (NE Poland).

Results

New specimens of *Piscicola brylinskae* were caught in Lake Maróz from the northern part of the Warmian-Masurian voivodeship (53°32′ N, 20°25′ E) and the Łyna River near Olsztyn. Maróz is an open lake and it belongs to a lake system with great natural and economic significance. The surface area of Lake Maróz covers 332 ha and has a maximum depth of 61 m. From three sides, the lake is overgrown by the forest, and in the littoral zone there are plants such as *Phragmites*, *Schoenoplectus* and *Acorus* (Darmochwał & Rumiński, 1996). Four specimens of *P. brylinskae* were found in substrates lying on the bottom of the lake and two of them were acquired from lavaret (*Coregonus* sp.). Another five, the newly collected, specimens were caught in the Łyna River flowing near Olsztyn (53°77′ N, 20°48′ E) (from pike *Esox lucius* L., 1758). The total length of this river is 263.7 km, of which 190 km falls on Polish territory, the rest part flows through the territory of Russia. The nature of the Łyna River near Olsztyn is quite varied. There are both, parts with mountainous character and parts that flow among the forests and wet meadows. In its upper reaches the river flows through the lakes: Brzeźno, Kiernoz Mały, Kiernoz Wielki,

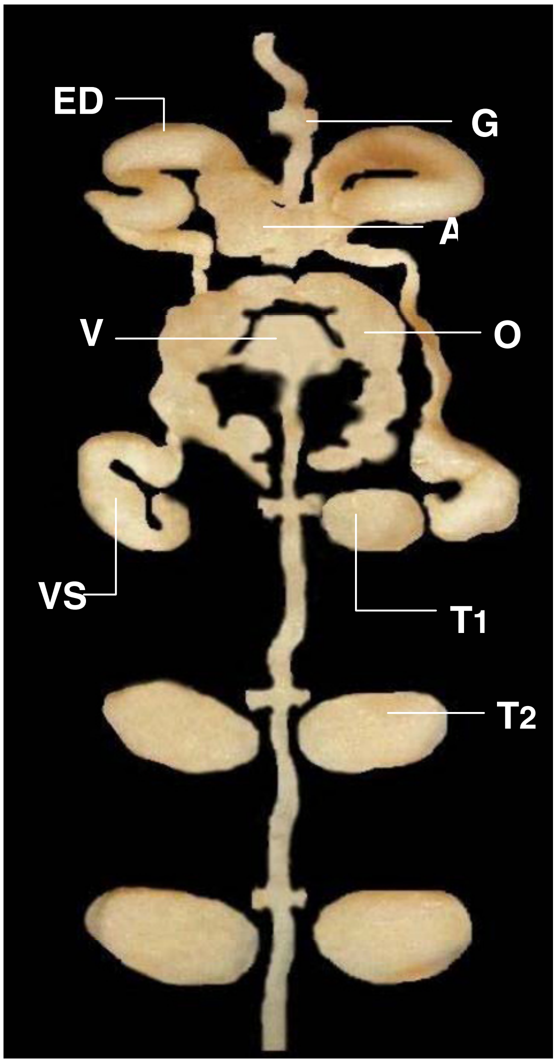

Fig. 5. Reproductive system of *Piscicola brylinskae*: G – ganglion, A – atrium, ED – ejaculatory duct, O – ovary, V – vector tissue, VS – seminal vesicle, T_1 – first pair of testes (lack of one testicle) and T_2 – the second pair of testes.

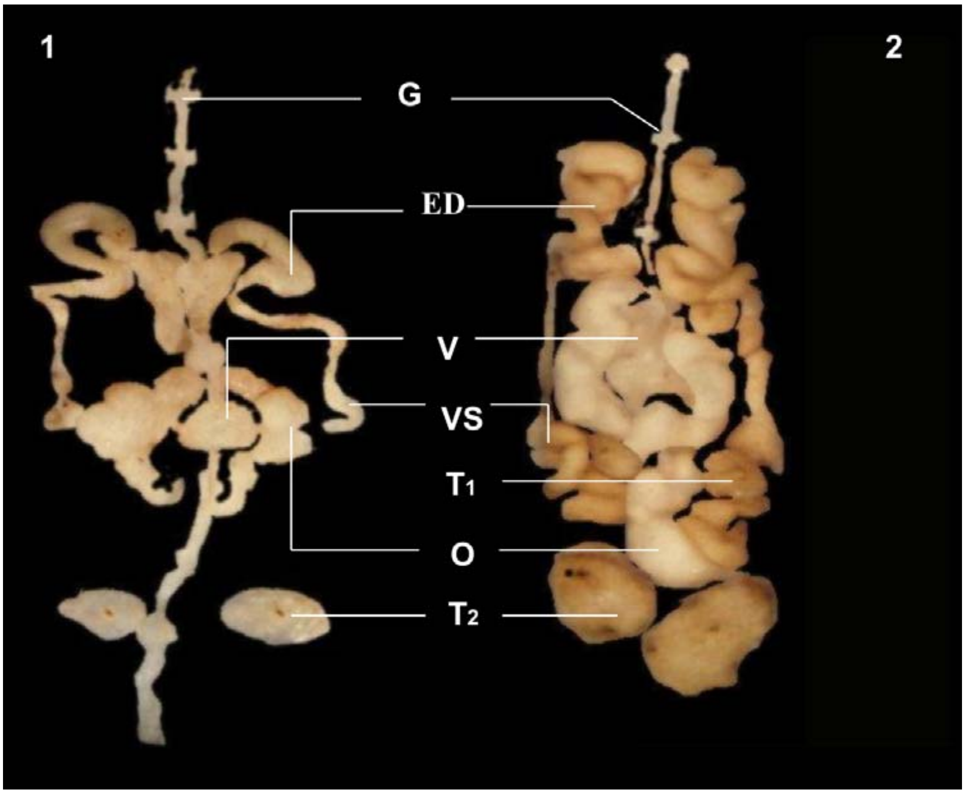

Fig. 6. Reproductive systems of *Piscicola brylinskae* (1) and *Piscicola margaritae* (2). G – ganglion, ED – ejaculatory duct, V – vector tissue, VS – seminal vesicles, O – ovary, T_1 – first pair of testes and T_2 – the second pair of testes.

Łańskie i Ustrych (Register of Tourism and Nature 2006).

Based on the indexes from the model of the body form, *P. brylinskae* is the most similar to *Caspiobdella fadejewi* Epshtein, 1961 (Fig. 2). Both species are similar in the following indexes: C_1^1/d_1 (1.78 and 1.81); S_1/S_2 (1.00 and 1.05); K_1/K_2 (0.42 and 0.41); R_2/M_2 (1.57 and 1.78); C_2^1/C_2 (0.99 and 0.96); C_2^1/C_1^1 (1.49 and 1.47); C_2/C_1 (1.52 and 1.31); C_2/N_2 (1.61 and 1.66); $C_2/(C_2\text{-}R_2\text{-}M_2)$ (4.85 and 4,.74); d_7/d_1 (1.68 and 1.62); D_2/D_1 (1.99 and 1.89) and N_2/L_2 (0.16 and 0.11).

Concering the 113 non-metric characters, *P. brylinskae* is similar to the following species from genus *Piscicola* (Fig. 3): *P. margaritae* Bielecki, 1997, *P. elishebae* Bielecki, 1997, *P. jarai* Bielecki, 1997 and *P. pomorski* Bielecki, 1997, but it is the most similar to *P. margaritae*. *P. brylinskae* and *P. margaritae* show the following common characteristics: (1) freshwater; (2) basically somit 14–annulate; (3) copulatory area extending from the clitellum to a part of the first urosomite; (4) present receptaculum seminis; (5) oviducts opening to the female gonopore just in front of the vector tissue.

Piscicola brylinskae differs from *P. margaritae* in the following characteristics: (1) short body, greatly flattened, while in *P. margaritae* elongate, relatively flattened; (2) anterior and posterior suckers elliptical or heart-shaped, while in *P. margaritae* rather circular; (3) gonopores separated by four annuli, while in *P. margaritae* by three annuli; (4) ejaculatory ducts semicircular, reach ganglion 5, while in *P. margaritae* they create a loop and reach ganglion 4; (5) seminal vesicles classically U-shaped, reach 1/2 of ovaries in *P. margaritae* round and reach the second pair of testes (Fig. 6); (6) ovaries spiraled, consisting of five sections, while in *P. margaritae* straight (Fig. 6); (7) vector tissue in the shape of a narrow plate, while in *P. margaritae* ellyptical (Fig. 6); (8) short conducting strands of the vector tissue, while in *P. margaritae* longer; (9) in typical individual five pairs of testes – testes of second pair (T_2) absent, but in *P. margaritae* six pairs of testes, (T_1–T_6).

The body shape of *P. brylinskae* is shown in Fig. 7, while the 32 indexes that describe this species are: $L/D_2 = 5.36$; $C_1^1/d_1 = 1.78$; $C_1^1/D_1 = 1.09$; $R_1/M_1 = 2.10$; $C_1^1/C_1 = 1.02$; $C_1/(C_1–R_1–M_1) = 8.58$; $L_1/D_1 = 3.31$; $D_1/N_1 = 1.87$; $S_1/S_2 = 1.00$; $L_1/L = 0.32$; $N_1/L_1 = 0.20$; $L_2/D_2 = 3.67$; $D_2/N_2 = 1.95$; $K_1/K_2 = 0.42$; $L_2/L = 0.68$; $N_2/L_2 = 0.16$; $C_2^1/d_7 = 1.57$; $C_2^1/D_2 = 0.83$; $R_2/M_2 = 1.57$; $C_2^1/C_2 = 0.99$; $C_2/(C_2–R_2–M_2) = 4.85$; $L_2/L_1 = 2.20$; $D_2/D_1 = 1.99$; $N_2/N_1 = 2.08$;$C_2^1/C_1^1 = 1.49$; $C_1^1/C_2 = 0.99$; $C_2^1/C_1 = 1.52$; $C_2/C_1 = 1.52$; $(C_2–R_2–M_2)/(C_1–R_1–M_1) = 3.37$; $C_2/N_2 = 1.61$; $C_1/N_1 = 1.90$; $d_7/d_1 = 1.68$.

From the ratios of indexes (invariants) it is understood that the body is short, $L = 6.62$ mm, and greatly flattened (individual with moderately filled alimentary tract).

Trachelosome and urosome. The division into trachelosome and urosome is poorly demarcated. However, closer examination shows that the first pair of respiratory vesicles is situated just behind a slight stenosis

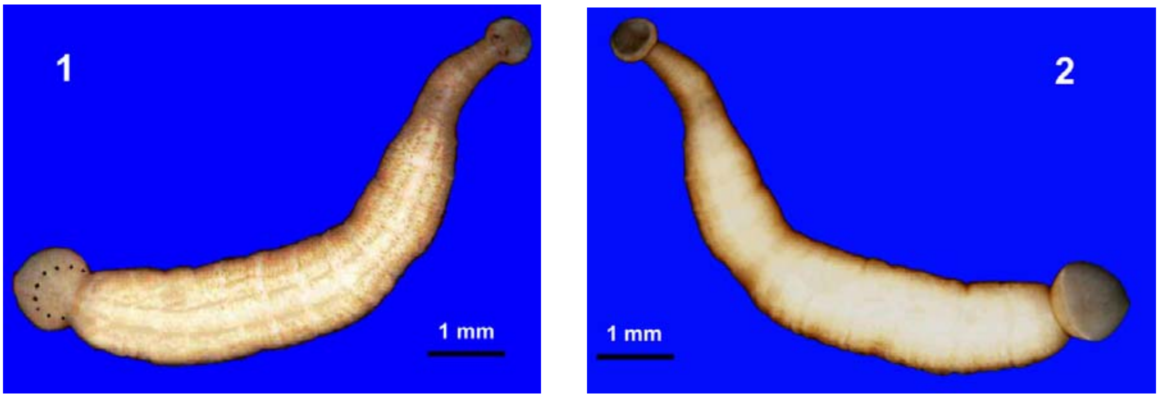

Fig. 7. *Piscicola brylinskae*: dorsal view (1) and ventral view (2).

between the trachelosome and urosome. The trachelosome slightly but distinctly widens just where, on the ventral side, male gonopore is situated.

Suckers (Fig. 4). The anterior sucker is relatively small, in a sagittal plane elliptical. This sucker bears two pairs of eyes. The first pair is large, semicircular, positioned obliquely to the sagittal axis, while the second pair is positioned perpendicularly. The posterior sucker is of medium size, not exceeding the greatest width of the urosome, with which it is connected eccentrically. It is strongly muscled, heart-shaped, elliptical in a vertical plane. Ten eye-like spots are situated closer to the margin of the posterior sucker.

Segmentation. Segmentation consists of typical somites of 14 annuli. The length of annuli is equal, except annulus 6, which is vesicular and somewhat broader. Papillae (sensillae, tangoreceptors) are absent. Eleven pairs of small, white respiratory vesicles are clearly visible.

Body coloration (Fig. 7). Thanks to proper conservation the coloration of *P. brylinskae* could be described for the first time. Body coloration of this leech is the result of the various arrangements of three kinds of melanophores: white, brown and yellow. Their disposition caused *P. brylinskae* to have interesting and characteristic markings. The dorsal side of the body is darker. Brown and yellow melanophores are settled in regular transverse stripes that are narrow on the trachelosome and at the end of the urosome. In the middle part of the urosome, the stripes are wider and well marked. In the middle part of the dorsal side, the brown-yellow stripes are divided by white strips. Along the middle of the dorsum and the entire leech body, there is a narrow white stripe between two dark streaks. The ventral side of the body is bright with very light, brown stripes. On the anterior sucker, close to the trachelosome junction, there is an area of randomly situated brown melanophores, which reach the two pairs of eyes. Such melanophores are also visible in the middle part of the sucker. The rest part of anterior sucker is white with single brown dots of melanophores. The posterior sucker has characteristic narrow stripes of brown and yellow melanophores. These stripes run parallel to each other from the junction of the urosome to the end of the sucker. Between these stripes, close to the margin of the sucker on the white background there are black eye-like spots. On the ventral side of the body suckers are white, smooth pigmented.

Alimentary tract. It is significant that in 9 specimens of *P. brylinskae* the structure of alimentary tract was recognizable and the same as in typical individual.

Reproductive system (Figs 5, 6). The reproductive system was not fundamentally different from the reproductive system of type specimens (in 8 typical specimens there were 5 pair of testes, the second pair of testes was absent). But, two specimens do not have the left testes of the first pair (the second pair of testes was present) (Fig. 5) while, one specimen do not have the both testes of the first pair (the second pair was present) (Fig. 6).

Discussion

In Maróz Lake *P. brylinskae* were caught on whitefish taken from the littoral. The fish leeches can not themselves occur in littoral. Because of that, it is very possible that *P. brylinskae* had to found their host in the deeper parts of the lake. The density filter variety of the whitefish could eat plankton and benthic organisms. Therefore, it is quite often seen in benthos as well as in water column (Szczerbowski 1981, 2000).

One of the biggest left-bank tributary of the Łyna River is Marózka that flows through Maróz Lake. It should be expected that specimens of *P. brylinskae* could be found not only in other tributaries of the Łyna River, but also in lakes by which the Łyna and Marózka flow.

It is interesting that five specimens of *P. brylinskae* differed from typical individual (from Lake Vechten) by lack of testes (Bielecki 2001) (Fig. 5). It is hard to tell whether these individuals, due to lack of testes, had reduced reproductive potential. In *P. geometra* and *C. fadejewi*, specimens are known in which the first pair of testes do not exist or there is lack of one of the testes from this pair (Epstein 1987; Bielecki, unpublished data.).

In a previous paper about the body form of fish leeches (Bielecki et al. 2009) and in present publication, the 32 indexes (invariants) were used for the first time. Moreover, the number of described specimens of

P. brylinskae is greater – one specimen in 2001, and currently 12 specimens (Bielecki 2001). Because of that, indexes obtained from the model are different than the first data and they are statistically more reliable.

Applying more indexes and the availability of more specimens resulted in a tree clustering algorithm where *P. brylinskae* cluster next to *C. fadejewi* (Fig. 2). Besides the metric characters of the body form, *P. brylinskae* and *C. fadejewi* are similar in non-metric characters: poorly marked distinction to trachelosome and urosome; 14–annualte somite; eleven pairs of small pulsative vesicles.

In earlier papers (Bielecki 1997, 2001), which were based on 19th indexes of body form, *P. brylinskae* correlated with *Piscicola margaritae* Bielecki, 1997, as well as now in the case of 113 non-metric characters. However, the body form in Hirudinea is not homogeneous and now, with more specimens available and information about body indexes, it should not be surprising that *P. brylinskae* cluster to *C. fadejewi*. Especially, when we consider that for example some Platybdellinae (*Truliobdella capitis*) have body form characteristic of species from the family Glossiphoniidae, and some Glossiphoniidae achieved body form typical of Piscicolidae (*Hemiclepsis marginata*) (Bielecki et al. 2009).

If we consider the 113 non-metric characteristics, we resume a large monotypic cluster which contains only species from the genus *Piscicola* (Fig. 3). This analysis has shown that *P. brylinskae* is most similar to *P. margaritae*. Though, *P. brylinskae* do not form dichotomic branching with *P. margaritae* nor with any other species from this cluster, which confirms that it is an individual species from the genus *Piscicola*.

Tree clustering algorithm based on the indexes of body form and non-metric characteristics, shows similarity of *P. brylinskae* to entirely different species. However, it should be taken into consideration that the body form is a feature, not having a clear diagnostic value on the generic level. Because of that analysis based on 113 non-metric characters is more important for biology and systematics.

Unfortunately, the diagnosis and, consequently, the systematic position of some of the purported members of the genus *Piscicola* are still debatable. The type species of the genus is the Palearctic *Piscicola geometra* (L., 1761). The congeneric species are: *P. milneri* (Verrill, 1871) and *P. punctata* (Verrill, 1871) from North America; *P. salmositica* Meyer, 1946 from western North America; *P. respirans* Troschel, 1850 from Western Europe; *P. fasciata* Kollar, 1842 from central-east Europe; *P. hadzii* Sket, 1985 from South Europe; *P. haranti* Jarry, 1960 from France; and *P. pawlowskii* from the former Yugoslavia (Sket 1968). Detailed information on the genus has been presented elsewhere (Troschel 1850; Verril 1872; Bielecki 1994, 1995, 1997, 2001; Bielecki et al. 2000a, b).

P. brylinskae has a few specific characters: unique ovary structure; five pairs of testes; a characteristic structure of the vector tissue that resembles a narrow plate; conducting strands of vector tissue as very short fibre strands connecting the ovaries; characteristic crop and posterior crop caecum. *P. brylinskae* differs from the species of the *Piscicola* genus in the following characteristics: (1) somite 14–annulate, annuli, except sixth, equal (in *P. punctata* 3–annulate; in *P. respirans*, *P. fasciata* 7–annulate; in *P. volgensis* 6–annulate; in *P. pojmanskae* 14– annulate, annuli 2, 6 and 11 being the widest); (2) gonopores separated by four annuli (in *P. punctata* by three or four annuli, *P. milneri* and *P. salmositica* by two annuli; in *P. pojmanskae* six annuli) (Bielecki, 2001).

P. brylinskae is comparable only with those species whose specific status is certain. These do not include *P. pawlowskii*, which is probably a species from genus *Acipenserobdella* Epshtein (1969) and *P. haranti*, which is well described but most likely do not belong to the *Piscicola* genus as well. Likewise, it is difficult to compare the new species with *P. hadzii* (Sket 1985) since it follows from the description (Sket 1968, 1985) that the species is probably a member of the genus *Caspiobdella* Epshtein, 1968.

P. brylinskae had been known to inhabit lakes and minor rivers, but now it is quite possible that it also appears in the same environment as *P. fasciata*, *P. respirans* and *P. geometra* (Malécha 1979; Bielecki et al. 2000a, b; Košel & Koščo 2006).

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