



The Genus *Branchiobdella* Odier, 1823 (Annelida: Branchiobdellida) in Lithuania, with an Overview and an Identification Key to the Species in the Baltic Countries

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Abstract: Branchiobdellidans dwelling on *Astacus astacus* (Linnaeus, 1758) from 13 localities in Lithuania were collected in 2018 and 2020. Four *Branchiobdella* species were identified: *B. astaci* Odier, 1823, *B. pentadonta* Whitman, 1882, *B. kozarovi* Subchev, 1978 and *B. bulgariensis* Subchev & Rimcheska, 2021. Specimens of *B. astaci* were found only in two localities, lakes Ramis and Krakavas. The most widely distributed species is *B. pentadonta* being found on crayfish at all localities investigated, with the exception of Lake Kaukinė. The latter locality is the only one where crayfish were infested by *B. kozarovi*. *B. bulgariensis* was only found at one locality, and possibly at a second, and *A. astacus* is a new host for *B. bulgariensis*. *B. kozarovi* and *B. bulgariensis* are new to the Lithuanian fauna list. An overview of the genus *Branchiobdella* in the Baltic countries and a key to its species are also presented.

Key word: *B. astaci*, *B. bulgariensis*, *B. kozarovi*, *B. parasita*, *B. pentadonta*, distribution

Introduction

Branchiobdellidans (Annelida: Branchiobdellida) are small leech-like worms dwelling on crayfish. The only indigenous genus of this group occurring Europe is *Branchiobdella* Odier, 1823. Summarized information about European *Branchiobdella* species diversity and distribution on the continent is presented in SUBCHEV's (2014) review paper. According to the latter author there are eight species occurring in Europe, of which the most widely distributed

are those described already in the 19th century: *B. astaci* Odier, 1823, *B. parasita* (Braun, 1805), *B. pentadonta* Whitman, 1882 and *B. hexadonta* Gruber, 1883. From the species described later the most widely distributed species is *B. balcanica* Moszynski, 1937. The distribution of both *B. italica* Cane-gallo, 1928 and *B. kozarovi* Subchev, 1978 is restricted to their respective crayfish hosts species: *B. italica* on *Austropotamobius pallipes* (Lereboullet, 1858) in Italy, Croatia, and parts of Bosnia and Herzegovina (KLOBUČAR et al. 2006) and *B. kozarovi*

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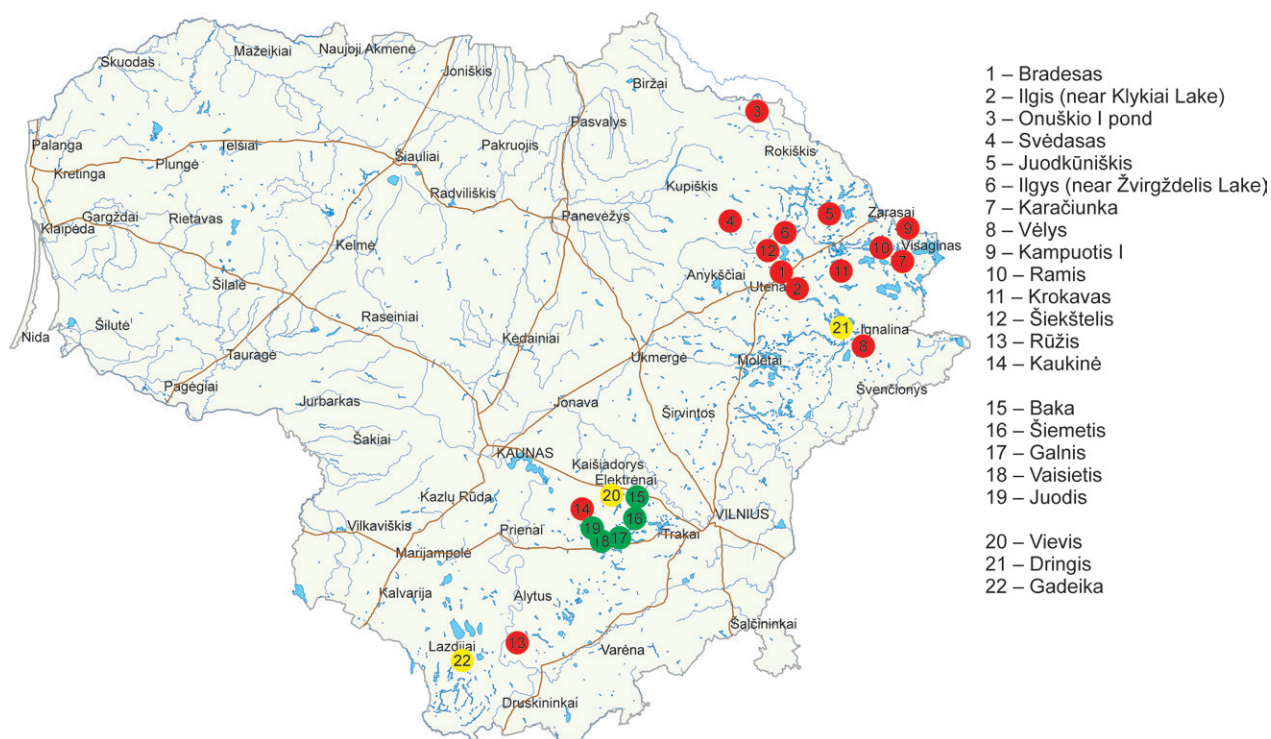


Fig. 1. Localities in Lithuania where *Branchiobdella* species have been recorded: 1–14 (this paper); 15–19 (MAŽYLIS & GRIGELIS 1979); 20–22 (CUKERZIS 1970).

on *Pontastacus leptodactylus* (Eschscholtz, 1823) in Eastern Europe, Turkey and some adjacent countries in Western Asia (FARD & GELDER, 2011; RIMCHESKA *et al.* 2016). Subsequently, *B. papillosa* Nesemann & Hutter, 2002, was described and is known only from its type locality at Voralberg, Austria. Recently, a new species similar to *B. parasita*, *B. bulgariensis* Subchev & Rimcheska, 2021, was described from Bulgaria (SUBCHEV & RIMCHESKA, 2021) with an additional location in Slovakia.

Originally, *Astacus astacus* (Linnaeus, 1758) was the only crayfish species in Lithuania (SOUTY-CROSSET *et al.*, 2006). Recently, three more crayfish species were introduced into the country: *P. leptodactylus* at the end of the 19th century, *Pacifastacus leniusculus* (DANA, 1852) in 1872, and *Faxonius limosus* (Rafinesque, 1817) was established for the first time in 1994 (BURBA, 1996).

The first information on the genus *Branchiobdella* in Lithuania is given in CUKERZIS's (1970) monography on *A. astacus*. The author mentioned two species, *B. astaci* and *B. pentadonta* found by him, but his data about their distributions refer only to *Branchiobdella* sp. Much more precise were MAŽYLIS (1973) and MAŽYLIS & GRIGELIS (1979), who presented valuable information about the biology and distribution of each of these two species in Lithuania. A third *Branchiobdella* species in this country, *B. parasita*, was reported by MAŽEIKIA *et al.*

(1999) but the authors gave no information about the locality where the species was found.

In the present paper, we report for the first time on the occurrence of *B. kozarovi* and *B. bulgariensis* in Lithuania and add new data on the distribution of *B. astaci* and *B. pentadonta* in this country. In addition, a key to *Branchiobdella* species in Lithuania and the other two Baltic countries, Estonia and Latvia, is compiled and an overview on the genus in the region is presented.

Materials and Methods

All branchiobdellidans were collected from *A. astacus* from 15 sites in Lithuania in 2018 and 2020 (Fig. 1; Table 1). In most cases the branchiobdellidans were removed from live crayfish. The crayfishes were placed into circulating 10% NaCl solution for 5–10 min. After this, the solution was filtered and the branchiobdellidans found were transferred into 80% alcohol. In addition, three crayfishes from Lake Rūžis were collected in 2020, fixed in 80% alcohol and then examined in laboratory under a stereomicroscope. The gills were removed from the crayfish and examined for the presence of branchiobdellidans. All branchiobdellidans found on the gills, exposed host surface and in the jar bottom debris were transferred into 80% alcohol for species identification.

Table 1. *Branchiobdella* species found in different localities in Lithuania in 2018 and 2020.

Locality*	District	Branchiobdella species			
		B. astaci	B. bulgariensis	B. pentadonta	B. kozarovi
2018					
Bradesas	Utena	?	-	+	-
Ilgys (near Lake Žvirgždelis)	Utena	?	-	+	-
Ilgis (near Lake Klykiai)	Utena	?	-	+	-
Juodkūniškis	Zarasai	?	-	+	-
Kampuotis I	Zarasai	?	-	+	-
Karačiunka	Ignalina	?	-	+	-
Krokavas	Zarasai	+	-	+	-
Ramis	Zarasai	+	+	+	-
Rūžis	Alytus	?	+	+	-
Šiekštelis	Utena	?	-	+	-
Svėdasas	Anykščiai	?	-	+	-
Vėlys	Ignalina	?	-	+	-
Onuškis pond	Biržai	?	-	+	-
2020					
Kaukinė	Kaišiadorys	?	-		+
Rūžis	Alytus	-	+	+	-

*All the localities are lakes with the exception of Onuškis pond.

The collected branchiobdellidans were identified by the specific body form and size and the specific form of the jaws as summarised by SUBCHEV (2014). For investigation of the jaws the head of the specimens was dissected with entomological needles under stereomicroscope, and the jaws were mounted on temporary microscopic slides with a drop of glycerin and examined under a light microscope. Two specimens from Lake Rūžis were dissected to determine the species as being *B. parasita* or *B. bulgariensis*. The dissection removed the spermatheca, which was mounted temporarily on a microscopic slide in a drop of 70% alcohol and observed under a light microscope.

Results

As a result of our investigations, four *Branchiobdella* species were found in Lithuania: *B. astaci*, *B. pentadonta*, *B. kozarovi* and *B. bulgariensis* (Table 1).

***Branchiobdella astaci*.** This species was found in only two localities: lakes Ramis and Krakavas in 2018 (Table 1, Fig. 2). Only two specimens were recorded from each of the two localities. However, the presence of *B. astaci* in any of the other lakes investigated in 2018 and 2020 is not excluded as far as this species inhabits the gill chamber of the host

and could be overlooked if the gills are not purposefully dissected. *B. astaci* was not present in the gill chambers of the three crayfishes collected in Lake Rūžis in 2020.

***Branchiobdella bulgariensis*.** Specimens provisionally identified based on the body form and the form of jaws as *B. parasita* were found only in the lakes Ramis and Rūžis in 2018. Altogether 18 more specimens, including juveniles, with the same body form were found on two *A. astacus* from Lake Rūžis in 2020 (Table 1, Fig. 2). Based on the spermatheca shape (a short duct followed by a spherical part and ending with a cylindrical part) in one of the two dissected specimens, we concluded it was *B. bulgariensis*. The spherical part of the spermatheca of the second specimen, however, was not clearly expressed and the organ looked more like a cylinder with a slightly narrower distal part. We had no opportunity to dissect any branchiobdellidans from Lake Ramis and thus accepted only provisionally that the two “parasita” branchiobdellidans collected in 2018 are also *B. bulgariensis*.

***Branchiobdella kozarovi*.** Numerous specimens of this species were collected on crayfish from Lake Kaukinė in 2020 (Table 1, Fig. 2).

***Branchiobdella pentadonta*.** This species was found in all of the investigated lakes in 2018 and in

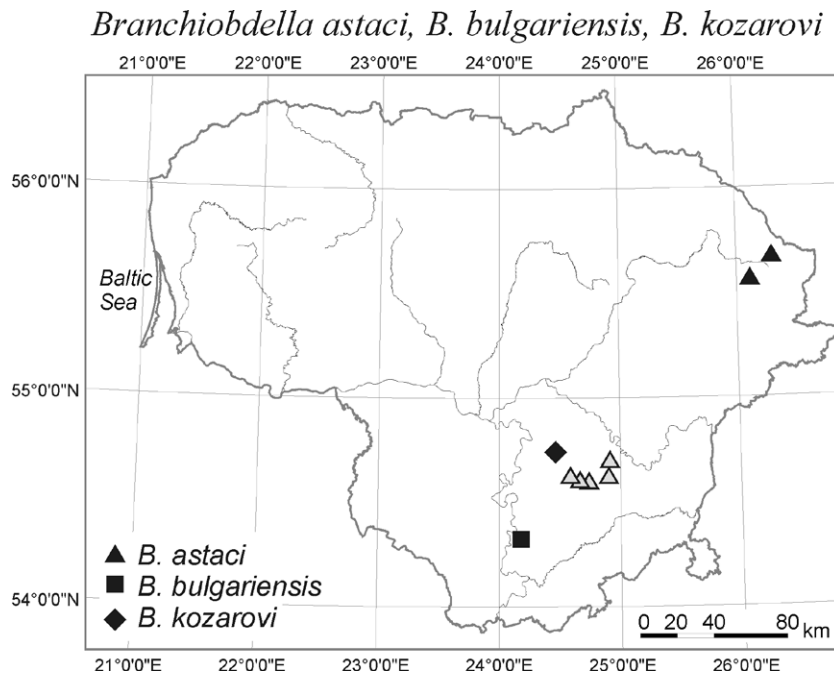


Fig. 2. Localities in Lithuania where *B. astaci* (triangle), *B. kozarovi* (diamond) and *B. bulgariensis* (square) have been recorded: black marks – our results, grey triangles – records from literature.

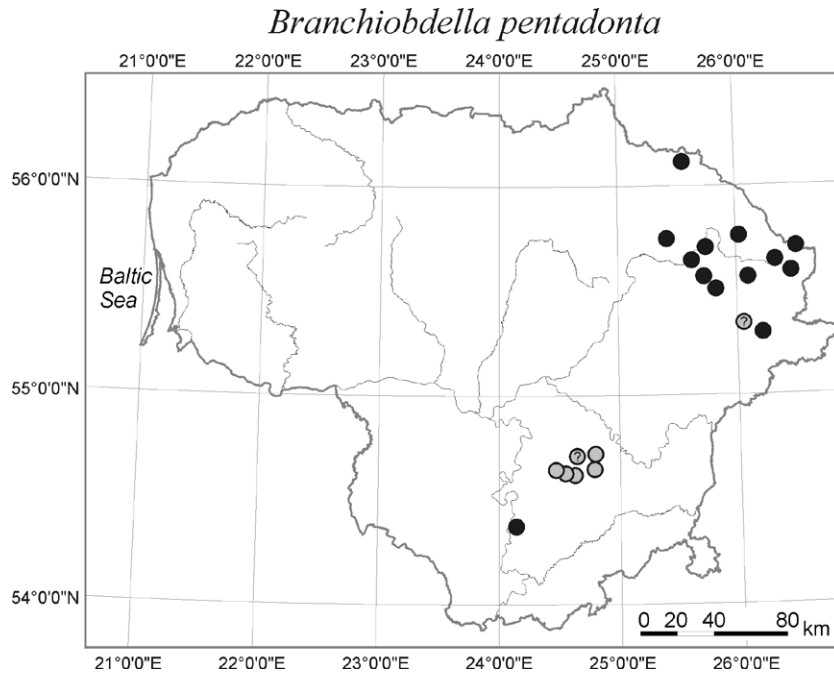


Fig. 3. Localities in Lithuania where *B. pentadonta* has been recorded: black dots – our results, grey dots – records from literature; uncertain records (see in the text) are marked with “?”.

Lake Rūžis in 2020 (Table 1, Fig. 3). *B. pentadonta* was absent only in Lake Kaukinė where the crayfish were infested by *B. kozarovi*. The highest numbers of branchiobdellidans of the species on a single crayfish were observed in the lakes Ilgys (near Lake Žvirgždelis) (125) and Bradesas (122), while in Lake Šiekštelis no more than three specimens were found on a single crayfish. In the rest of the lakes

the average number of *B. pentadonta* individuals per crayfish varies between 14 and 76.

An overview of the genus *Branchiobdella* in the Baltic countries

Lithuania, Latvia and Estonia are known as the Baltic states or the Baltic countries (https://en.wikipedia.org/wiki/Baltic_states, accessed 01 Dec. 2022).

Lithuania. Before our recent investigations, only three *Branchiobdella* species, *B. astaci*, *B. parasita* and *B. pentadonta* have been reported in Lithuania. The earliest information about Lithuanian branchiobdellidans was presented in CUKERZIS' (1970) monograph on *A. astacus* followed by CUKERZIS (1989) on all Lithuanian crayfish species. Unfortunately, the data presented there are not supported by detailed information. In both publications, after mentioning that the most widely distributed *Branchiobdella* species are *B. pentadonta* and *B. astaci*, the author just stated that "according to our data the intensity of infection by these parasites is quite high". In CUKERZIS (1970) three lakes are mentioned: Vievis and Dringis with intensity of *Branchiobdella* "infection" up to 74% and Gadeika with 100%. It is not clear which of the two species the author meant, but most probably the data refer to the most common surface-dwelling species in Lithuania, *B. pentadonta*. Later, a similar investigation was performed by BURBA et al. (1996). The author gave only summarized results about occurrence of *Branchiobdella* sp. in 31 unspecified lakes in Lithuania: mean of 66.7% and 89.0% of the infected waterbodies in 1994 and 1995 respectively and 60–90% and 60–90% infected crayfish in 1994 and 1995 respectively. Again, most probably the data refer to *B. pentadonta*.

The first reliable data on the genus *Branchiobdella* in Lithuania were those of MAŽYLIS (1973) and MAŽYLIS & GRIGELIS (1979) who investigated 3280 specimens of *A. astacus* from five lakes in the Trakai region, viz. Baka, Šiemetis, Juodis, Vaisietis and Galnis, and found *B. astaci* in the gill chambers and *B. pentadonta* on the exposed surface of the crayfishes. The same authors reported that the highest percentage (up to 83%) of crayfish infested by *B. astaci* was found in lakes Šiemetis in 1971 and Vaisietis in 1977. In the same lakes up to 20 specimens of *B. astaci* were found on a single crayfish. MAŽEIKI et al. (1999) studied *A. astacus* collected from unspecified localities that had been reared in a semi-intensive crayfish culture. As a result, the authors identified two *Branchiobdella* species, *B. astaci* and *B. parasita*. Thus, this was the first report of the latter species in Lithuania, but without any data on its natural occurrence in this country. However, because of the absence of details on the form of the spermatheca, the main feature separating *B. parasita* from *B. bulgariensis* (SUBCHEV, RIMCHESKA, 2021), it is not clear which of these two similar species was actually found by them. We could only suppose that this was *B. bulgariensis*, the species demonstrated by us to occur in Lithuania, but, at the same time,

we cannot exclude the possibility it was *B. parasita*.

Latvia. To the best of our knowledge there is only one short note about Latvian *Branchiobdella*; GRAPMANE et al. (1976) reported the occurrence of *B. astaci* and *B. pentadonta* in lakes Kaitras and Rešetnieku in Krāslava District.

Estonia. Intensive investigations on branchiobdellidans dwelling on *A. astacus* were provided in Estonia in 1952–1956 by JÄRVEKÜLG (1957). He examined more than 4000 crayfish from more than 50 sites on the mainland part of the country as well as on Saaremaa Island. As a result of his explorations *B. astaci* was found on both the mainland and the island, *B. parasita* proved to be rare on the mainland but widely distributed on the island, while *B. pentadonta* was established only in the mainland. It is interesting to note the author's finding that *B. parasita* is widely distributed on the Saaremaa Island but is rare on the mainland where it never co-occurs with *B. pentadonta*. The results of special observations presented in the same paper have shown that *B. astaci* is "... very pathogenic, injuring crayfish since sucking of blood from gills and causing abscesses on the places of attaching cocoons. In the case of mass occurrence of cocoons, the crayfish gills can become almost completely destroyed." (JÄRVEKÜLG, 1957). An assessment on the pathogenicity of *B. astaci* is presented in SUBCHEV et al. (2020). Again, as the case in Lithuania, further investigations need to establish which of the two similar species, *B. parasita* and *B. bulgariensis*, actually occurs in Estonia.

Morphological notes of the species of *Branchiobdella* in the Baltic countries

B. astaci (Fig. 4, 5). This species is very easily recognizable by the specific microhabitat on the host, unique body form and unique jaws. It dwells only in the crayfish gill chambers and leaves it only in the case of death of the host or abnormal external conditions. According to MAŽYLIS (1973), when treating crayfish with NaCl for 6 min, 40–50% of *B. astaci* were swept away from the gill chambers by the water stream, and the remaining ones left the gill chamber after treating and placing the host in clean water. The body undergoes a certain level of shrinking depending on the fixative used. Nevertheless, the senior author of this paper (MAS) has never observed fixed *B. astaci* exceeding 6 mm. The body of this species is thick, stout and usually slightly arch-shaped; the head is somewhat sharpened. The jaws are triangular, the dorsal one being about twice as large as the ventral one. The difference in the jaw dimensions clearly separates this species from the other Baltic species with similar body length and

triangular jaws, e.g., *B. parasita* and *B. bulgariensis*, in which the dorsal and ventral jaws are almost equal in size and with well-defined lateral teeth.

B. pentadonta (Fig. 4, 5). This species differs from the rest of the Baltic species by its specific leaf-shaped, dorsoventrally flattened body in fixed specimens reaching up to about 4 mm in length (MAS, unpublished personal observations). The jaws of this species are pectinated and almost equal in size, the dorsal one being slightly larger, with 5–7 denticles of which the lateral and median ones are larger. *B. pentadonta* can be easily distinguished from the other Baltic species with similar pectinated jaws, e.g., *B. kozarovi*, by body form and size. In contrast to the leaf-like body of *B. pentadonta*, the body of *B. kozarovi* is almost terete and considerably smaller (see below). In addition, the width to height ratio of the jaw plate in the two species is different – about 2 in *B. pentadonta* and less than 1.5 in *B. kozarovi*.

B. kozarovi (Fig. 4, 5). This is a species with almost terete form of the body, usually arched after fixation, which could be easily distinguished from the rest of Baltic *Branchiobdella* by its small dimensions measuring up to 2 mm in length in fixed adults (SUBCHEV, 1978). As already mentioned above, its small body size is enough to distinguish it from the other species with similar body forms, *B. parasita* and *B. bulgariensis*; in addition, the latter two species have completely different jaws. On the other hand, the only other species with similarly pectinated jaws, *B. pentadonta*, is larger and with leaf-shaped body (see above). For the difference in jaws of these two species see above.

B. bulgariensis (Fig. 4, 5, 6). The body of this species is almost terete and reaches up to 7 mm (SUBCHEV & RIMCHESKA, 2021). This body form resembles to some extent that of *B. kozarovi*, but even the youngest specimens of *B. bulgariensis* are equal and even larger than mature *B. kozarovi*, which measure less than 2 mm. The two species have completely different jaw forms – triangular in *B. bulgariensis* and pectinated in *B. kozarovi*. The difference between *B. bulgariensis* and the other Baltic species with similar body size and triangular jaws, *B. astaci*, is given in the descriptive notes of the latter species (see above). However, *B. bulgariensis* is very similar to another European species included in this key, *B. parasita*. Externally, the only feature differing the two species is a presence of four lobes on the dorsal lip of *B. bulgariensis* and the absence of such lobes in *B. parasita*, although these lobes are not always clearly visible in the former species. A feature clearly distinguishing these two species is the spermatheca shape. This organ has a short duct

and ampulla with an almost spherical proximal part and a narrower (cylindrical to bulb-like) distal part in *B. bulgariensis*, and a short duct and ovoid ampulla in *B. parasita*. It is worth noting that the juveniles of both *B. bulgariensis* and *B. parasita* have a specific dorsal ridge and thus differ by body form from the adults of these species as well as from both juveniles and adults of all other European *Branchiobdella* species.

B. parasita (Fig. 4, 5, 6). The body form and size, which separate this species from the other Baltic species (*B. astaci*, *B. pentadonta* and *B. kozarovi*), are the same as those mentioned above for *B. bulgariensis*, a species very similar to *B. parasita*. The differences between the latter two species, which include the form of the dorsal lip of the head and the form of the spermatheca, are shown in the descriptive notes on *B. bulgariensis* (see above).

Identification of the species of *Branchiobdella* in the Baltic countries

European *Branchiobdella* species inhabit either gill chambers or external surface of crayfishes (SUBCHEV 2014). In the Baltic countries the only species known to inhabit gill chambers is *B. astaci*, while *B. parasita* (this species is included because of the reasons shown above: see Overview part), *B. pentadonta*, *B. kozarovi* and *B. bulgariensis* dwell on the crayfish's exposed surface. Thus, if crayfishes are fixed immediately after their catching, we can expect to find in their gill chambers only one species, *B. astaci*, and not the latter species on crayfish's exposed surface or in jar debris. However, it is possible for *B. astaci* to leave the gill chamber of its host either after long keeping of the host in a small container or, according to MAŽYLIS (1973), after treating with salt solution.

The key is designed for fixed adult specimens. Mature *Branchiobdella* species occurring in the region are quite well distinguishable from each other even using the body form and size alone. Another stable feature, clearly different in all the species, except *B. pentadonta* and *B. kozarovi*, is the form of the jaws. For identification of Baltic *Branchiobdella*, with an exception of *B. parasita* and *B. bulgariensis*, there is no need for dissection and investigation of the reproductive system, unless the collected material differs significantly from the description of each of the five species presented below. We propose the identification to be done in two steps: by body form and size (step one) and by the form of jaws (step two). The second step is needed for a final identification, especially in case of any hesitations after completing the first one. In addition, a third step is added only for distinguishing *B. parasita* and *B. bulgariensis*.

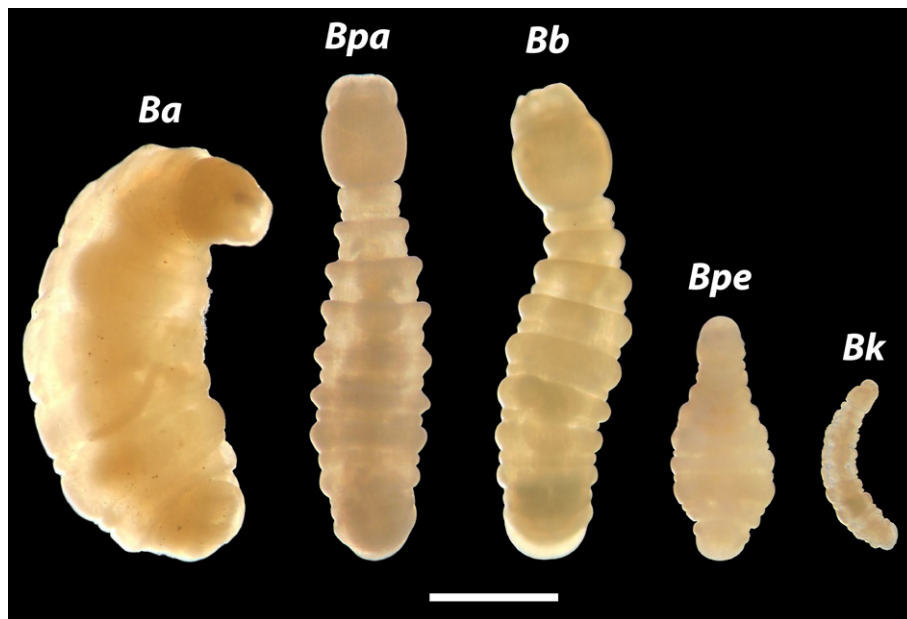


Fig. 4. External body view of *B. astaci* (Ba), Bulgaria; *B. parasita* (Bpa), Slovakia; *B. bulgariensis* (Bb), Lithuania; *B. pentadonta* (Bpe), Lithuania; and *B. kozarovi* (Bk), Bulgaria. Scale bar: 1 mm

Key to the species of *Branchiobdella* in the Baltic countries

Step 1, body (Fig. 4):

- 1a. Body dorsoventrally flattened, leaf-shaped*B. pentadonta*
- 1b. Body of other form2
- 2a. Body thick and stout*B. astaci*
- 2b. Body almost terete or slightly spindle-shaped.. 3
- 3a. Body less than 2 mm long*B. kozarovi* (conform to step 2)
- 3b. Body longer than 2-3 mm*B. parasita* or *B. bulgariensis* (conform to step 2) – step 3

Step 2, jaws (Fig. 5):

- 1a. Jaws pectinated with 5–6 (rarely 7) denticles, the lateral and median ones being larger2
- 1b. Jaws triangular3
- 2a. Ratio between width and height of jaw plate c. 2:1*B. pentadonta* (conform to step 1)
- 2b. Ratio between width and height of jaw plate less than 1.5:1*B. kozarovi* (conform to step 1)
- 3a. Dorsal jaw clearly larger than ventral one*B. astaci*
- 3b. Jaws almost equal in size – *B. parasita* or *B. bulgariensis*step 3

Step 3, spermatheca (Fig. 6):

- 1a. Spermatheca consisting of a short duct and an ovoid ampulla*B. parasita*
- 1b. Spermatheca consisting of a short duct and an ampulla with nearly spherical proximal part and a narrower (cylindrical to bulb-like) distal part*B. bulgariensis*

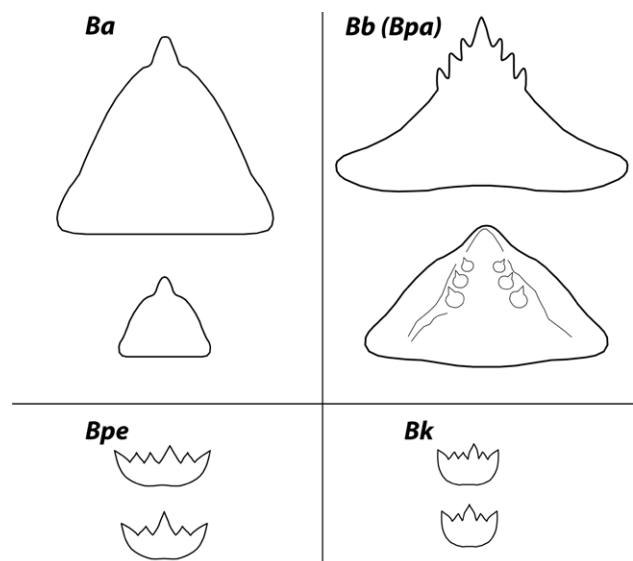


Fig. 5. Schematic view of the jaws of *B. astaci* (Ba), *B. bulgariensis* (Bb), *B. pentadonta* (Bpe) and *B. kozarovi* (Bk). Because of the great similarity between the jaws of *B. bulgariensis* and *B. parasita* (Bpa) separate drawings of jaws of the latter species are not presented.

Discussion

In the present paper, we report new data on the distribution of four *Branchiobdella* species dwelling on *A. astacus* in Lithuania: *B. astaci*, *B. pentadonta*, *B. kozarovi* and *B. bulgariensis*. The latter two species, *B. kozarovi* and *B. bulgariensis*, are new to the Lithuanian fauna list. The occurrence of *B. parasita* in this country reported by MAŽEIKA et al. (1999) is doubtful and needs further examination.

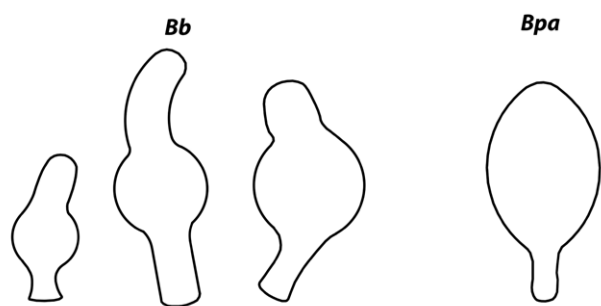


Fig. 6. Schematic view of spermathecae of *B. bulgariensis* (Bb) from SUBCHEV & RIMCHESKA (2021) and *B. parasita* (Bpa) based on: HENLE (1853), KARAMAN (1967), KOLESNYKOVA & UTEVSKY (2013) and investigations on material from Slovakia (M. Subchev, B. Rimcheska; unpublished data). For *B. bulgariensis* spermathecae of a young (left), a medium-aged (middle) and an old (right) specimen are presented.

The most widely distributed species was *B. pentadonta*, while the other three species were found only in single localities. So far, the only known host of *B. kozarovi* has been *A. leptodactylus* (SUBCHEV 2014). Thus, we report for the first time *A. astacus* as a host of this *Branchiobdella* species. Actually, *B. kozarovi* has been previously reported on *A. astacus* (SUBCHEV 1978), but this was later suspected to be a case of misidentification of the host (SUBCHEV 2014). Our finding of *B. kozarovi* on *A. astacus* in Lake Kaukinė could be explained by the fact that in the same lake *A. leptodactylus* also occurs. It is worth noting that possible future investigations on the specific host of *B. kozarovi*, *A. leptodactylus*, could reveal a much wider distribution of this species in Lithuania. So far, *B. bulgariensis* has been known only in Bulgaria and Slovakia (SUBCHEV & RIMCHESKA 2021) so our finding of this species in Lithuania significantly widens its distribution area. In addition, SUBCHEV & RIMCHESKA (2021) reported *Austropotamobius torrentium* (von Paula Schrank, 1803) as the only host of this species and thus we here report a new one, *Astacus astacus*.

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