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The distribution of branchiobdellidan worms (*Annelida: Clitellata*) on the noble crayfish, *Astacus astacus*, in the Transcarpathian region, Ukraine

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This study explores the distribution, abundance, and diversity of branchiobdellidans on freshwater crayfish in the Zakarpattia (Transcarpathian) region of Ukraine, focusing on populations in the Tereblyya River and Lake Synevyr. Surveys were conducted over the summer seasons of 2008, 2009, 2017, and 2022, examining a total of 68 noble crayfish (*Astacus astacus* Linnaeus, 1758). Four branchiobdellidan species were identified: *Branchiobdella parasita* (Braun, 1805), *B. balcanica* Moszynski, 1937, *B. pentadonta* Whitman, 1882, and *B. hexadonta* Gruber, 1883. This study presents the first records of *B. hexadonta* in Ukraine, expanding on the previously recorded presence of *B. parasita* and *B. balcanica*. Among the species observed, *B. balcanica* was the most prevalent, comprising 83% of the branchiobdellidans on individual crayfish in Lake Synevyr and 64% in the Tereblyya River. This species primarily occupied the claws, thoracic legs, and bases of the antennules, with cocoons mostly located in the lower cervical grooves. *B. pentadonta* was less frequent, accounting for 10% and 15% of branchiobdellidan specimens in Lake Synevyr and the Tereblyya River, respectively, and generally cohabited the same body regions as *B. balcanica*. *B. hexadonta*, detected exclusively in Lake Synevyr, was found within the gill chambers and represented only 3% of the total branchiobdellidans. Visible damage to some gill filaments suggests that this species may exhibit parasitic behavior. *B. parasita*, known for its considerable size and widespread presence across native European crayfish populations, accounted for approximately 21% of the branchiobdellidan specimens. It was commonly found on the eyes, maxillipeds, and lateral sides of the cephalothorax. This species displayed protective behavior around its cocoons, actively moving to shield them. In comparison, the recently described *B. bulgariensis*, identified in Bulgaria, differs from *B. parasita* primarily in spermathecal structure and is associated with *A. torrentium*. In this study, our samples exhibited distinct spermathecal structures from both *B. bulgariensis* and *B. parasita*, indicating potential taxonomic variation that needs further investigation through molecular genetic analysis. In this study, we enhance the existing descriptions of branchiobdellidan species by providing detailed morphological and anatomical parameters. Our findings are based on both original species descriptions and our own research contributing to the understanding of the diversity and morphology of European branchiobdellidans.

Key words: *Branchiobdella*, Ukraine, Transcarpathian, *Astacus astacus*, distribution

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Introduction

The Carpathian region is recognized as one of the most important biodiversity hotspots in Europe, with well-preserved ecosystems that support a wide range of animal and plant species (Bálint et al., 2011). This region provided a crucial glacial refugium during the Pleistocene, enabling temperate and cold-adapted species to survive climate fluctuations (Schmitt, 2009). Genetic studies indicate multiple glacial survival centers across the Carpathian area, which have contributed to the present distribution patterns of species in the region (Mráz and Ronikier, 2016; Mamos et al., 2021).

A key freshwater species in this region is the noble crayfish, *Astacus astacus* (Linnaeus, 1758), which likely colonized the Carpathian Danube basin during the late Pliocene or early Pleistocene (Trontelj et al., 2005; Klobučar et al., 2013). Populations of *A. astacus* have since been shaped over time by climatic shifts, with the Carpathians providing a relatively stable refuge (Schulz and Grandjean, 2005). In recent decades, however, these native crayfish have faced significant pressures, including habitat loss, competing invasive crayfish species and the crayfish plague, resulting in population declines across much of Europe (Holdich et al., 2009).

The Zakarpattia region of Ukraine, part of the Eastern Carpathians, hosts diverse and rich aquatic environments and associated flora and fauna (Kliment et al., 2016), with rivers, streams, and smaller water bodies interlacing to create ideal conditions for crayfish and their associated symbionts (Pârvulescu and Zaharia, 2014). This region also provides a core water resource in Ukraine (Gerenchuk, 1981). Thus, it is a key region for studying relationships between native *A. astacus* crayfish populations and their associated species such as branchiobdellidan ectosymbionts. Branchiobdellidans (*Annelida: Clitellata*) are small annelids that live on the surface of freshwater crayfish or in their gill chambers. Certain species of branchiobdellidans form mutualistic relationships with crayfish, benefiting both organisms. In these cases, Branchiobdellidans help keep the crayfish clean by removing debris and parasites, which supports the host's overall health. However, some branchiobdellidans exhibit parasitic behavior, feeding on crayfish tissues or bodily fluids. This can lead to stress, reduced growth, and increased vulnerability to disease for the host crayfish (Baker, 2003; Hollander, 2005).

In the Carpathian region, research on branchiobdellidans has been limited, despite the ecological significance of their freshwater crayfish hosts. This study set out to investigate branchiobdellidan species, associated with *A. astacus* in the Transcarpathian region of Ukraine and to increase knowledge of the interactions between crayfish and branchiobdellidans in the Carpathian refugium and to provide detailed descriptions of investigated species.

Materials and methods

Study sites

Surveys were conducted at two locations (Fig.1, A-D) in the Khust district of the Zakarpattia Region in Ukraine: Lake Synevir and the Tereblya River near the village of Kolochava. The field studies were carried out over several years, during the summer months in 2008, 2009, 2017, and 2022. A total of 68 crayfish specimens were examined for the presence of branchiobdellidans. These included 30 crayfish from the Tereblya River in 2008, 10 in 2009, 10 in 2017, and 10 in 2022. In Lake Synevir, 8 crayfish were examined in 2009.

1. **Lake Synevir** (48°37'01"N; 23°41'02"E). Collection date: July 16, 2009, 8 crayfish.
2. **Tereblya River** (48°26'13"N; 23°44'29"E). Collection dates: July 21, 2008 (30 crayfish); July 13, 2009 (10 crayfish); August 16, 2017 (10 crayfish); August 27, 2022 (10 crayfish).

Lake Synevir is a well-known tourist destination situated in the Synevir National Nature Park at an altitude of 989 meters above sea level. The lake is fed by three mountain streams, with marshy ground present at their respective discharge areas into the lake. Water from the lake flows through underground aquifers into the river Tereblya, one of the numerous headwaters of the River Danube. The Tereblya River is located in the same region and is a tributary of the Tisza River, which also subsequently joins the Danube.

Specimen collection and examination

Crayfish were collected by hand from the water bodies at both sites and visually examined for the presence of branchiobdellidans. These were gently washed off their crayfish hosts and preserved in tubes containing 96% ethanol for further analysis. Immediately after examination, most of the crayfish were returned to their natural habitats. Two crayfish from each locality were euthanized to allow for subsequent detailed examination of their gill chambers. Branchiobdellidans were identified using a stereomicroscope Leica M205 C. Species identification was based on examination of external morphology, jaw structure and reproductive system organization. Measurements of body proportions and microscopic examination of jaws were carried out performed using the microscopy imaging application Axiolmager Z1 (Carl Zeiss). The terminology used to describe morphological and anatomical features adheres to the standards outlined by Neubert and Nesemann (1999). We believe it is essential to provide detailed parameters and proportions related to the structure of the jaws and external morphology of the collected specimens, as traditional morphometry often focuses solely on length and breadth measurements. Additionally, we include microscopic photographs of the reproductive system, whereas most previous articles have primarily relied on illustrations. We present standardized descriptions of the species based on characteristics documented in the original species descriptions as well as our own research findings. These descriptions encompass size, external features, jaw structure, reproductive system, and cocoon structure.

Results

Branchiobdellidan species identification was based on both external morphological features and internal structures, including the spermatheca and atrium. Here, we provide brief descriptions of each species, highlighting the key features important for species differentiation. All crayfish from both study sites were identified

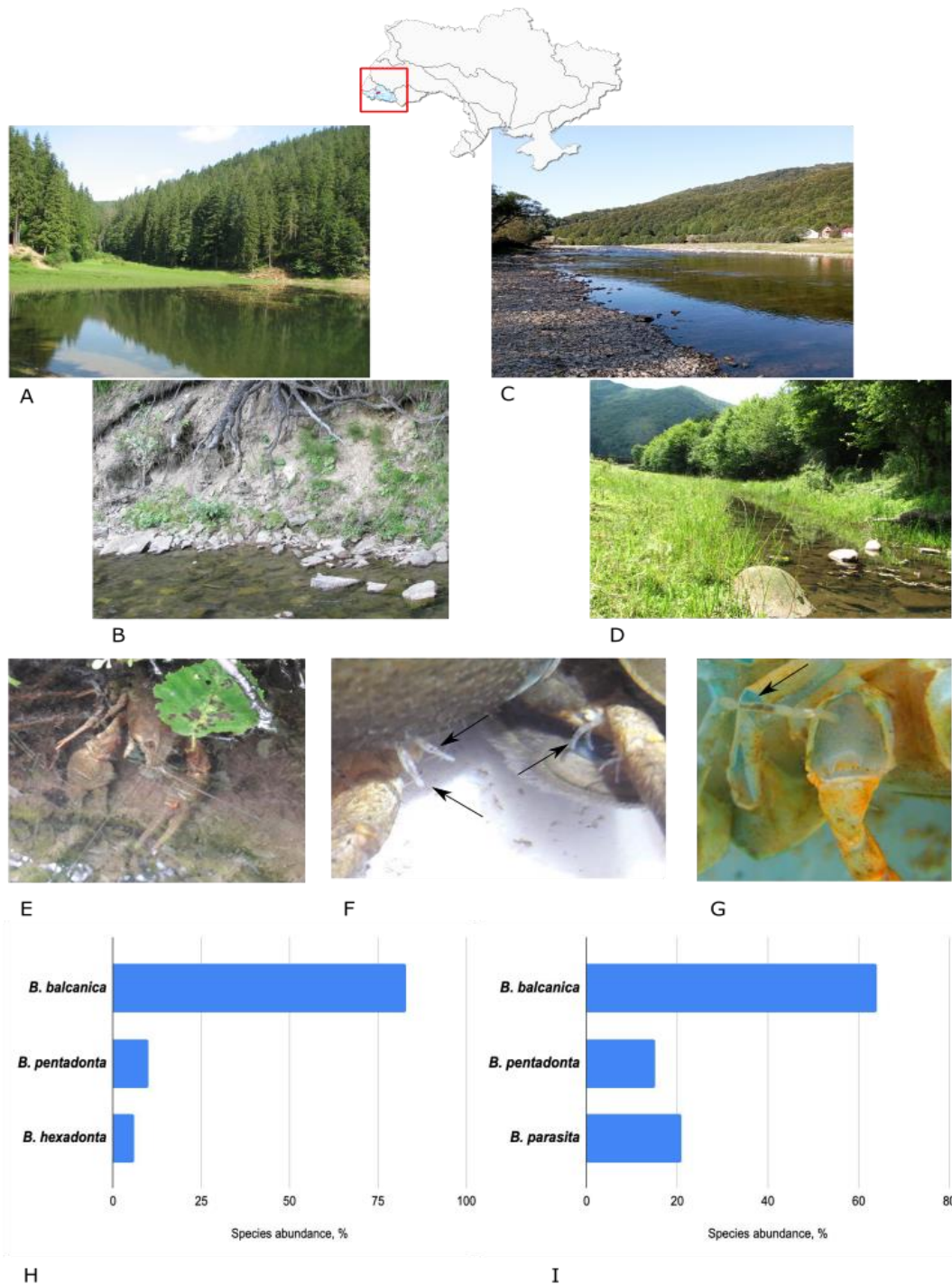


Figure 1. Surveyed locations in the Khust District, Zakarpattia Region, Ukraine (Photographs by M.Shrestkha). (A) General view of Lake Synevir; (B) Crayfish collection site in Synevir Lake; (C) General view of the Tereblya River; (D) Crayfish collection site in the Tereblya River; (E) *Astacus astacus* in its natural habitat; (F) *Branchiobdella balcanica* on its host; (G) *B. parasita* on its host; (H) The density and diversity of branchiobdellidans on the *A. astacus*, Lake Synevir; (I) The density and diversity of branchiobdellidans on the *A. astacus*, Tereblya River

as the noble crayfish, *A. astacus*. Three species of branchiobdellidan were recorded on crayfish collected from the Tereblya River, namely *B. parasita*, *B. pentadonta*, and *B. balcanica*. The most abundant species was *B. balcanica*, with 20 to 30 individuals per host. *Branchiobdella parasita* was less numerous, with 6 to 10 individuals per host, while *B. pentadonta* was present in smaller numbers, ranging from 5 to 7 individuals per host (Fig. 1, E-I). Three species of branchiobdellidan were also identified in Lake Synevir, *B. balcanica*, *B. hexadonta*, and *B. pentadonta*. The dominant species was *B. balcanica*, with 20 to 40 individuals found per host. *Branchiobdella pentadonta* was observed in smaller numbers, with 3 to 5 individuals per host, while *B. hexadonta* was the least common, with only 1 to 3 individuals per host (Fig. 1, H, I).

***Branchiobdella balcanica* Moszynski, 1937**

Size: The length of fixed animals varied from 1.8–4 mm

External features: The body is cylindrical and of uniform width from the 1st to the 3rd segments, and then dorso-ventrally flattened from the 4th to the 8th segments. It broadens significantly at the 4th segment, where the frontal annulus forms "shoulders" and doubles in width compared to the 1st segment, maintaining this width up to the 7th segment. The frontal annulus of the 8th segment has distinct lateral processes, while the 9th segment also exhibits lateral processes located on the dorsal annulus (Fig. 2, D–E). These processes are clearly visible in specimens that have been slowly fixed; in specimens fixed rapidly, however, the processes appear contracted.

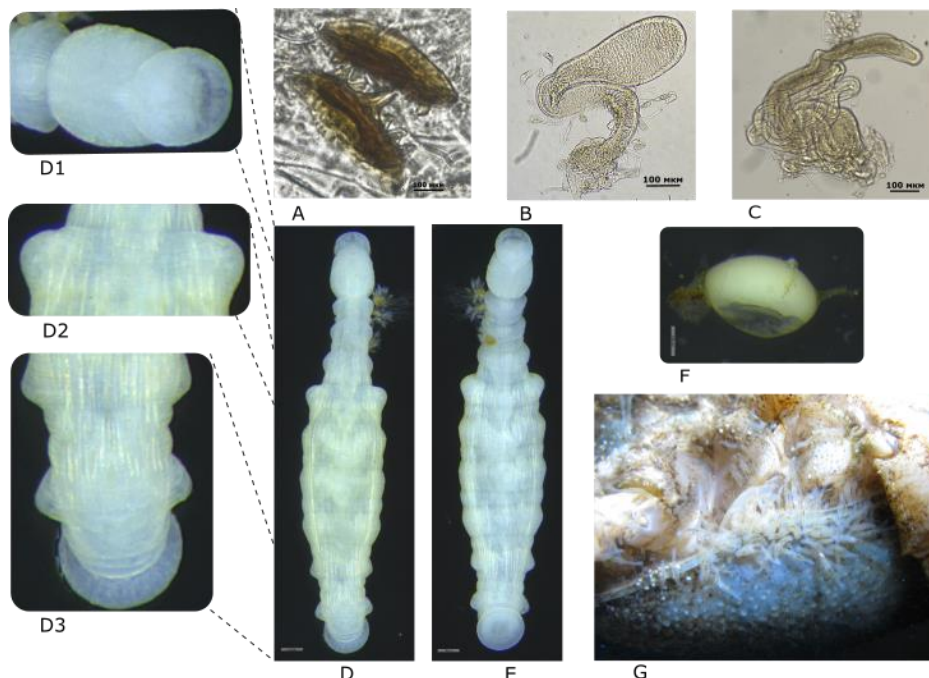


Figure 2. *Branchiobdella balcanica*: (A) dorsal and ventral jaws; (B) spermatheca; (C) atrium; (D) dorsal view; (D1) head region, (D2) 4th body segment, (D3) lateral processes on the frontal annulus of the 8th segment and the dorsal annulus of the 9th segment; (E) ventral view; (F) cocoon; (G) living worms on the crayfish host.

The head is oval, with a length approximately 10% greater than its width and nearly equal to the width of the 1st body segment. It comprises 15% of the total body length. The lips, which are distinctly separated from the head, are round (Fig. 2, D1) and account for 35% of the head length, lacking any lobes.

Jaw structure: The jaws are equal in size, dark brown, and elongated-oval in shape, with a width that is three times greater than their height. The teeth are arranged along the straight edge of each jaw (Fig. 2, A). The dorsal jaw bears five or six teeth, while the ventral jaw has five. On both jaws, the teeth are massive and generally uniform in size, with the middle tooth being distinctly larger than the others.

Reproductive system: In adult worms, the gonopores are clearly visible on the 5th and 6th body segments. The vesicle of the spermatheca has a bulbous shape, with a widened apical end and a narrower basal end. The ejaculatory canal is equal in length to the vesicle, curving beneath it to about half its length, then bending in the opposite direction toward the gonopore (Fig. 2, B). The glandular part of the atrium is

C-shaped, as described by Karaman (1967), and broad, while the tubular portion is thin and elongated, forming approximately four loops (Fig. 2, C). The copulative bursa is large.

Cocoon structure: The cocoon is flask-shaped, with a capsule measuring 0.4–0.5 mm in length and 0.3–0.35 mm in width. The cocoon stem is short, measuring 0.07–0.1 mm, and broadens toward the base of the capsule. The cocoon cap is mitriform, with a long spine measuring 0.1–0.15 mm, which is nearly equal to or exceeds the length of the stem (Fig. 2, F). Cocoons were observed on crayfish in various locations, including the inner side of the claws and their angular joints, along the lateral edge of the carapace, at the base of pereopods, on the lower part of the cervical groove, and on pleopods. Less frequently, cocoons were attached at the base of the antennae and maxillipeds (Fig. 2, G).

***Branchiobdella hexadonta* Grube, 1883**

Size: Length of fixed animals 3.2–3.7 mm

External features: The body is cylindrical along its entire length. The head is distinctly divided in the middle by a ring furrow, elongated, and makes up more than 30% of the total body length. Its width is comparable to, or slightly greater than, that of the first body segment and is about one-quarter of the head's length. The lips are separated from the head by a distinct furrow, with the upper lip divided into two lobes that slightly extend above the lower lip (Fig. 3, F). The lips constitute 30% of the head length. The body segments are generally uniform in width, with the widest point occurring at the 6th to 8th segments. The 4th segment is about 30% wider than the 1st segment (Fig. 3, G).

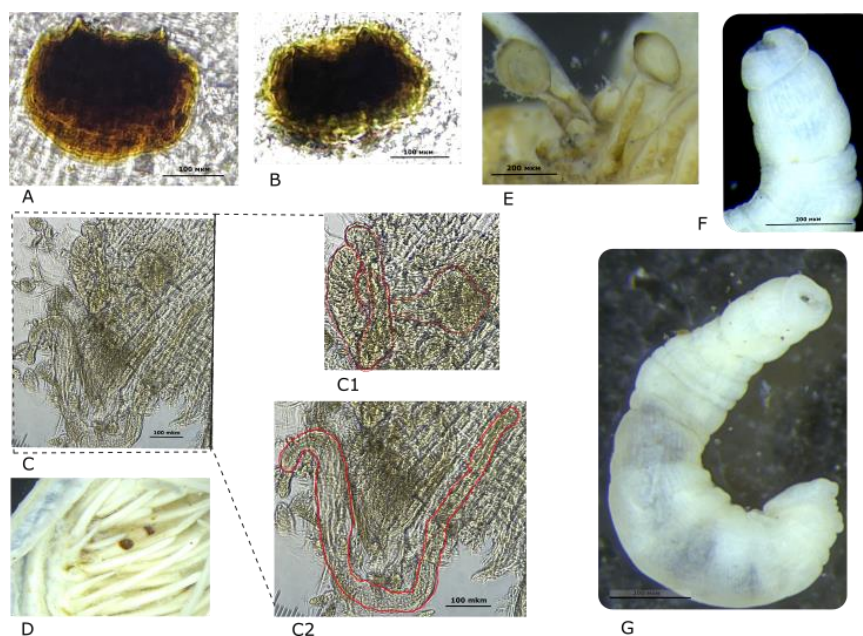


Figure 3. *Branchiobdella hexadonta*: (A) dorsal jaw; (B) ventral jaw; (C) reproductive organs: (C1) atrium, (C2) spermatheca; (D) damaged gills; (E) cocoon with a worm inside and an empty one nearby; (F) head region with deep furrow; (G) adult worm.

Jaw structure: The jaws are dense, dark brown, and have a rectangular-oval shape with a distinct concave upper margin. Nesemann (1999) describes this shape as reniform, or kidney-shaped. The dorsal jaw is slightly larger than the ventral jaw, with teeth arranged along the concave edge (Fig. 3, A, B). The dorsal jaw has six teeth, while the ventral jaw has five. The lateral teeth are significantly larger on both jaws.

Reproductive system: The gonopores are clearly visible in adult worms on the 5th and 6th body segments. The spermatheca resembles the shape of a fishing hook (Fig. 3, C2), with the vesicle and ejaculatory canal sharing the same width. The ejaculatory canal is twice the length of the vesicle, which is concave and scapular at its midpoint. The atrium is short, with a broad, short glandular section, while the tubular section is longer and forms a single loop (Fig. 3, C1).

Cocoon structure: The cocoon capsule is oval, featuring slightly elevated, with a flat brown cap (Fig. 3, E). The capsule measures 0.2 mm in length and 0.18 mm in width, with a stem length of 0.09 mm.

***Branchiobdella parasita* (Braun, 1805)**

Size: Length of fixed animals 2.4 – 5.5 mm

External features: Body cylindrical across entire length (Fig. 4, B). Frontal annulus of each body segment dorsally extended and in lateral view appears as a crest (Fig. 4, E). The body segments are broadly similar in width, reaching maximum width at the 5th to 7th segments. The 4th body segment is approximately 35% wider than the 1st segment.

The head is rectangular, 30% longer than wide, more than 20% of the total body length and 26% wider than the 1st body segment. The lips contribute 30% of the head length. The upper lip is divided into four lobes and slightly protrudes over the lower lip. The lobes are more distinct in young specimens and in well-fixed adult individuals (Fig. 4, F).

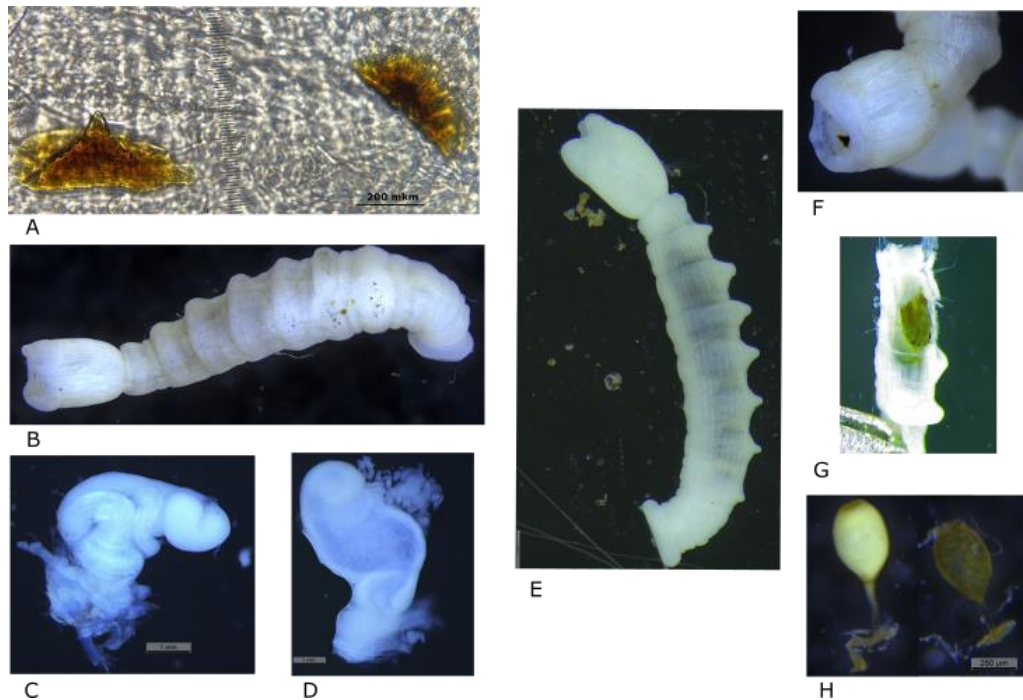


Figure 4. *Branchiobdella parasita*: (A) dorsal and ventral jaws; (B) dorsal view; (C) atrium; (D) spermatheca; (E) lateral view; (F) head region; (G) gut containing the head of a chironomid; (H) cocoon with a worm inside and an empty one nearby.

Jaw structure: Jaws large, light brown, triangular, height 30% of width. The upper jaw is 20% larger than the lower jaw. Teeth arranged along the triangle edges of the jaw. Each jaw bears seven or eight teeth. On both jaws, the lateral teeth are small and vary in number, while the middle tooth is noticeably larger (Fig. 4, A).

Reproductive system: Gonopores in 5th and 6th body segments are visible in adult worms. Vesicle of spermatheca has an oval-kidney shape with two folds on the concave edge. Fold on the apical end of the vesicle separates a round top. Another fold is located on the middle of the vesicle (Fig. 4, D). Ejaculatory canal is very short, forming a triangle widening at the base of the vesicle, which turns to the opposite direction from the fold.

Atrium has a massive glandular part and a double bend with a loop on its apical end (Fig. 4, C). Copulatory bursa large. Tubular part is wide in comparison with the other species considered here. Lengths of glandular and tubular parts are almost equal.

Cocoon description and location. Cocoon ovoid but with the widest part at the apex; capsule length 0.4–0.6 mm, width 0.28–0.36 mm. Cocoon stem 0.24–0.27 mm, nearly half of capsule length, cap flat (Fig. 4, H). Cocoons located latero-posteriorly on crayfish cephalothorax (usually symmetrically on both sides).

Remarks: The spermatheca of *B. parasita* exhibits high variability, despite the uniformity of the species' external morphology. The spermatheca differs in *B. bulgariensis* Subchev, 2021 and in *B. parasita* as described by Braun (1885), detailed by Henle (1835), and characterized by Karaman (1967) and

Kolesnykova (2013). Application of molecular genetic methods is now required to confirm the taxonomic validity of different morphotypes, including the material examined in this study and in the recently described *B. bulgariensis* (Subchev, 2021).

***Branchiobdella pentadonta* Whitman, 1882**

Size: Length of fixed animals 2.6 – 3.4 mm

External features: Body gradually broadening from 1st body segment to maximum width in 5th and 6th body segments and then gradually narrowing to the 9th segment. The first three segments are cylindrical, while segments 4– 8 are slightly flattened. The width of the 4th segment is 40% greater than the 1st while that of 6th segment is more than double that of the 1st. The frontal annulus of the 8th segment has small lateral processes which are more or less visible on all fixed specimens. Lateral processes are also present on the 9th segment but are located on the dorsal annulus (Fig. 5, C, E, F).

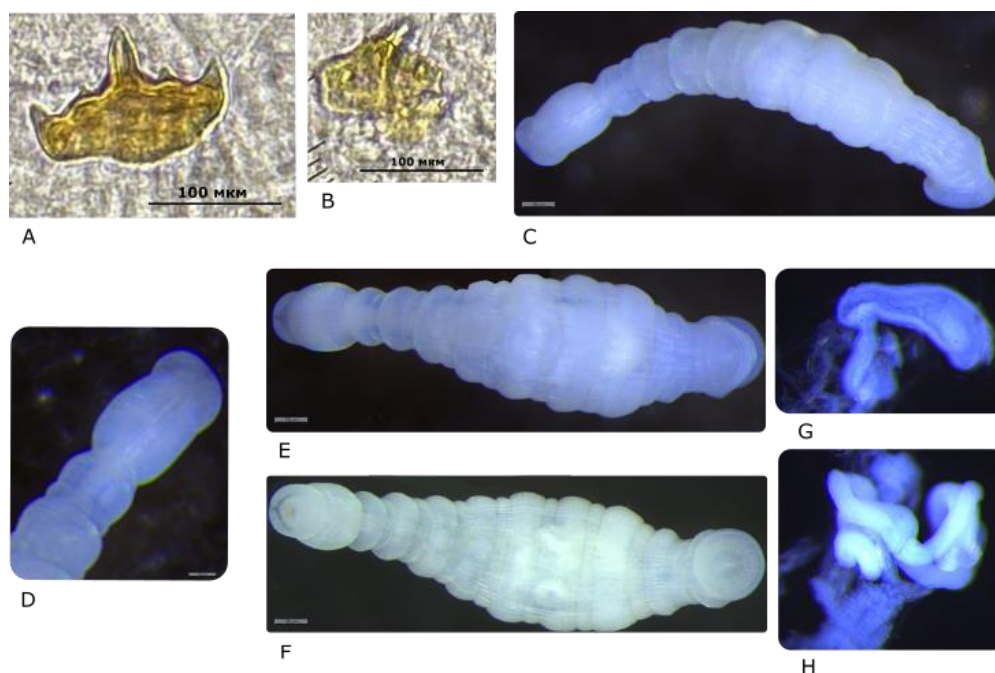


Figure 5. *Branchiobdella pentadonta*: (A) dorsal jaw; (B) lateral view of ventral jaw; (C) lateral view; (D) head region; (E) dorsal view; (F) ventral view; (G) spermatheca; (H) atrium.

Head rectangular-oval shaped, 30% longer than wide, about 20% of the total body length and 10% wider than the first body segment. The lips contribute about 25% of the head length. The upper lip has a small central furrow (Fig. 5, D).

Jaw structure: Jaws equal in size, light brown, rectangular, three times wider than high. Teeth arranged along the arc edge of the jaw (Fig. 5, A, B). Each jaw bears five or six teeth. On both jaws, the last lateral teeth are large and middle teeth are small, although the central of these is noticeably larger than the others.

Reproductive system: Gonopores in 5th and 6th body segments are visible in adult worms. Vesicle of spermatheca bulbous with a widened apical end with little narrowing (Fig. 5, G). Ejaculatory canal forms a small bend under the vesicle and then goes straight down, slightly bending at the end. The ejaculatory canal is half the length of the vesicle. Atrium has a short tubular section while its glandular part is long, forming several bends (Fig. 5, H). Copulatory bursa is small.

Discussion

Geographical distribution and hosts

The Carpathian Mountains continue to provide an important refugium for the noble crayfish, *A. astacus*, a species that has experienced considerable declines across its European range due to habitat degradation, pollution, and competition with anthropogenically introduced invasive species. They provide habitats with a number of environmental characteristics that are essential for the survival of *A. astacus* and

its ectosymbionts, including branchiobdellidans. The crayfish thrive in clean, well-oxygenated waters with stable substrata, such as gravel and cobbles, which are characteristic of the streams and rivers of the Carpathians (Pârvulescu et al., 2011). The region's relatively low levels of anthropogenic disturbance compared to other areas in Europe further contribute to the maintenance of suitable habitats for this crayfish species (Lovrenčić et al., 2022).

Until now, four species of branchiobdellidan, *B. kozarovi*, *B. pentadonta*, *B. parasita*, and *B. balcanica*, have been reported from Ukraine. All previous Ukrainian records derive from collections from the Dnieper River basin (Boshko, 2010; Kolesnykova, 2007, 2008) and the delta of the Danube River (Kolesnykova, 2013). Our data confirm the occurrence of ectosymbiotic branchiobdellidans on *A. astacus* in the Ukrainian Carpathians, specifically in Lake Synevyr and the Tereblyya River. *Branchiobdella hexadonta* is recorded for the first time in Ukraine. This species inhabits the gill chamber, where it feeds on crayfish gill filaments (see Fig. 3, D), and cannot be observed by examining the body surface alone. Known hosts of *B. hexadonta* include *A. astacus*, *A. torrentium* (Subchev and Stanimirova, 1998; Klobučar et al. 2006; Subchev, 2011) and *A. pallipes* (Obercofler et al. 2002; Füreder et al. 2009). It has also been found on the invasive crayfish *Orconectes limosus* (Duris et al., 2006). The distribution of *B. hexadonta* includes most of Europe but does not extend east of the Greece-Bulgaria-Romania-Poland line, with no reports from Nordic or Baltic countries, Russia or Ukraine (Subchev, 2014). Our findings therefore extend the documented range of *B. hexadonta* eastwards into Ukraine.

Our data also extend the known range of *B. pentadonta*, confirming its presence in the Tereblyya River. Previously, this species has been reported in Ukraine from waters near the city of Truskavets in association with *A. astacus* (Boshko, 1983) and from the Kharkiv region on *A. leptodactylus* (Kolesnykova, 2008). However, records of this species beyond the Carpathian region require confirmation due to the potential for confusion with the morphologically similar *B. kozarovi*. *Branchiobdella pentadonta* is widespread in European freshwater systems, where it is associated with *A. astacus* (Subchev 1986; Klobučar et al. 2006; Füreder et al. 2009), *A. leptodactylus* (Subchev and Stanimirova, 1998) and *A. torrentium* (Klobučar et al. 2006; Kovács & Juhász 2007; Füreder et al. 2009).

Branchiobdella balcanica was found in this study in both Lake Synevyr and the Tereblyya River. Previously, it has been reported in Ukraine near the city of Truskavets, in association with *A. astacus* (Boshko, 1983). Across its distribution range, this species is predominantly ectosymbiotic on *A. astacus* (Kozarov et al., 1972; Subchev and Stanimirova, 1998; Klobučar et al., 2006; Kovács and Juhász, 2007; Füreder et al., 2009) and on *A. torrentium* (Kovács and Juhász, 2007), occurring in European water bodies within the Danube River basin.

Branchiobdella parasita was found in this study only on crayfish from the Tereblyya River. Previous reports have briefly noted its presence in western Ukraine in association with *A. astacus* (Kupchinskaya, 1970). More recently, Kolesnykova (2013) confirmed its occurrence on *A. leptodactylus* in the Ukrainian Danube Delta. This species is notable for its large size and occurs on all native crayfish species across Europe. Recently, a similar species, *B. bulgariensis* Subchev, 2021, was described, differing primarily in spermatheca structure and associated with *A. torrentium* in Bulgaria (Subchev, 2021). Our samples demonstrate differences in spermatheca structure from *B. bulgariensis* and *B. parasita*. Szenejko (2023) recently investigated the genetic diversification of *B. parasita*. To confirm the taxonomic validity of the various morphotypes, including both our collected samples and the newly described *B. bulgariensis*, molecular genetic comparisons are now required.

Abundance, location and co-occurrence

The presence and abundance of branchiobdellidans on crayfish can serve as indicators of crayfish health and the overall quality of aquatic ecosystems. Branchiobdellidan presence is influenced by host tolerance and environmental conditions, with suggestions that a healthy crayfish population may support a diverse branchiobdellidan community (Skelton et al., 2014; Oberkofler et al., 2002; Klobučar et al., 2006). Although branchiobdellidans may cause some damage, they generally do not pose a significant threat to the host and, perhaps counter-intuitively, higher symbiont densities are thought to reflect less-stressed, more robust, crayfish populations. For instance, a study in Greece reported that branchiobdellidan diversity was positively linked to crayfish health, suggesting that monitoring these organisms could help in assessments of the ecological status of freshwater habitats (Subchev et al., 2007).

Our study confirmed the occurrence of four branchiobdellidan species on *A. astacus* in the Zakarpattia region of Ukraine. Previous research in this region, particularly in the Tereblyya River, recorded the co-occurrence of *B. parasita* and *B. balcanica* on *A. astacus* (Kolesnykova, 2013). Additional surveys

in 2017 and 2022 revealed a third species, *B. pentadonta*, cohabiting with *B. balcanica* and *B. parasita* on the same hosts. In the current study, among 60 crayfish surveyed from the Tereblya River and 8 from Lake Synevir, *B. balcanica* was the most prevalent species, comprising 83% of the branchiobdellidans present on individual crayfish in Lake Synevir and 64% in the Tereblya River (Fig. 1, H, I). This species primarily occupied the claws, thoracic legs (Fig. 1, F; Fig. 2, G), and base of the antennules, with cocoons attached mainly to the lower cervical grooves. *Branchiobdella pentadonta* was found less frequently, co-occurring with *B. balcanica* on the same body parts. It contributed 10% of branchiobdellidan specimens on individual crayfish in Lake Synevir and 15% in the Tereblya River (Fig. 1, H, I). *Branchiobdella hexadonta* was detected only in Lake Synevir and was located in the gill chamber, making its presence hard to assess without euthanizing the crayfish, as we did only with two individuals. This species accounted for only 3% of the branchiobdellidans observed (Fig. 1, H, I). Some gill filaments displayed visible damage, likely caused by *B. hexadonta*, perhaps, suggesting this species takes on a more parasitic rather than commensal function (Fig. 3, D). As with the previous surveys in the Tereblya River, we also observed *B. parasita*, recognizable by its large size and active movement. Representing ~21% of all observed branchiobdellidan specimens (Fig. 1, H, I), it was often found on the eyes, maxillipeds, and lateral sides of the cephalothorax (Fig. 1, G), where cocoons were also present. This species also exhibited protective behavior, actively moving around its cocoons.

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References

- Baker, A. M., W. J. H. (2003). Branchiobdellae: A review of the biology and ecology of the branchiobdellidan annelids. *Freshwater Biology*, 48(6), 1097-1109.
- Bálint, M., Ujvárosi, L., Theissinger, K., Lehrian, S., Mészáros, N., Pauls, S. U. (2011). The Carpathians as a major diversity hotspot in Europe. In: Zachos FE, Habel JC eds. *Biodiversity hotspots*. Berlin: Springer Verlag, 189–205.
- Boshko, E. (1983). Oligochaets inhabiting freshwater crayfishes in some waters in Ukraine. Report I. Branchiobdellidae. – *Vestnik Zoologii*, 2: 13-20.
- Boshko, E. G. (2010). Parasites and commensals of freshwater crayfish in the water bodies of Russia and Ukraine. *Proceedings of V.G. Belinsky Penza State Pedagogical University*, (21), 39-44.
- Braun, J. F. P. (1805). *Systematische Beschreibung einiger Egelarten: sowohl nach ihren äussern Kennzeichen als nach ihrem innern Bau*. GW Müller.
- Duris, Z., Horká, I., Kristian, J., Kozák, P. (2006). Some cases of macro-epibiosis on the invasive crayfish *Orconectes limosus* in the Czech Republic. *Bulletin Français de la Pêche et de la Pisciculture*, (380-381), 1325-1337. <https://doi.org/10.1051/kmae:2006038>
- Füreder, L., Summerer, M., Brandstätter, A. (2009). Phylogeny and species composition of five European species of *Branchiobdella* (Annelida: Clitellata: Branchiobdellida) reflect the biogeographic history of three endangered crayfish species. *Journal of Zoology*, 279(2), 164-172. <https://doi.org/10.1111/j.1469-7998.2009.00601.x>
- Gerenchuk, K.I. (Ed.) (1981). *Pryroda Zakarpats'koi oblasti. Vyscha shkola*, L'viv, 156 pp. (in Ukrainian).
- Holdich, D. M., Reynolds, J. D., Souty-Grosset, C., Sibley, P. J. (2009). A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems*, (394-395), 11. <https://doi.org/10.1051/kmae/2009025>
- Hollander, J., M. A. (2005). The role of Branchiobdellidans in the ecology of freshwater crayfish. *Aquatic Ecology*, 39(2), 215-222.
- Karaman, S. M. (1967). Branchiobdellidae Jugoslavije. *Buletin i Punimeve Shkencore të Fakulteti Filozofi k të Prishtinës*, IV: 39-64.
- Kliment J, Turis P, Janišová M (2016) Taxa of vascular plants endemic to the Carpathian Mts. *Preslia* 88:19–76.

- Klobučar, G. I., Podnar, M., Jelić, M., Franjević, D., Faller, M., Štambuk, A., ... Maguire, I. (2013). Role of the Dinaric Karst (western Balkans) in shaping the phylogeographic structure of the threatened crayfish *Austropotamobius torrentium*. *Freshwater Biology*, 58(6), 1089-1105. <https://doi.org/10.1111/fwb.12110>
- Klobučar G., I. Maguire, S. Gottstein-Matočec, S. Gelder (2006). Occurrence of Branchiobdellida (Annelida: Clitellata) on freshwater crayfish in Croatia. – *Annales de Limnologie*, 42 (4): 251-260. <https://doi.org/10.1051/limn/2006026>
- Kolesnikova, M. A., Utevsy, S., Utevsy, S. (2008). First record on *Branchiobdella kozarovi* (Clitellata: Branchiobdellida) from Eastern Ukraine. *Lauterbornia*, 65, 77-81.
- Kolesnikova, M. Y. (2007). New information on branchiobdellidans (Clitellata: Branchiobdellida) distribution in pools of Kharkiv region. *Visnik Harkivskogo nacional'nogo universitetu imeni VN Karazina. Serija: biologija*, 6(788), 97-103.
- Kolesnykova, M., Utevsy, S. (2013) New Ukrainian records of *Branchiobdella parasita* (Annelida: Clitellata: Branchiobdellida) from the Danube basin. *Scientific Annals of the Danube Delta Institute*, 19, 35-38.
- Kovács, T., Juhász, P. (2007). Data to the distribution of crayfish worms (Branchiobdellidae) in Hungary. *Folia Historico-Naturalia Musei Matraensis*, 31, 77-79.
- Kozarov, G., Michailova, P., Subchev, M. (1972). Studies on Branchiobdellidae (Oligochaeta, Annelida) in Bulgaria. – *Annuaire de l'Université de Sofia, Faculté de biologie*, 64 (1):77-89. (In Bulgarian).
- Kupchinskaya, O. S. (1970). Aquatic Oligochaetes and Their Parasites in the Fauna of the Western Regions of Ukraine. *Dissertation abstract for the degree of Candidate of Biological Sciences, Lviv*, Pp 21.
- Lovrenčić, L., Temunović, M., Gross, R., Grgurev, M., Maguire, I. (2022). Integrating population genetics and species distribution modeling to guide conservation of the noble crayfish, *Astacus astacus*, in Croatia. *Scientific Reports*, 12(1), 2040.
- Mamos, T., Jazdzewski, K., Čiamporová-Zaťovičová, Z., Čiampor Jr, F., Grabowski, M. (2021). Fuzzy species borders of glacial survivalists in the Carpathian biodiversity hotspot revealed using a multimarker approach. *Scientific Reports*, 11(1), 21629. <https://doi.org/10.1038/s41598-021-00320-8>
- Moszyński, A. (1937). Oligochetes parasites de l'ecrevisse (*Potamobius astacus*) de la Yougoslavie. *Glasnik (Bull.) Soc. Sci. Scopye*, 18: 6, 69-75.
- Mráz, P., Ronikier, M. (2016). Biogeography of the Carpathians: evolutionary and spatial facets of biodiversity. *Biological Journal of the Linnean Society*, 119(3), 528-559. <https://doi.org/10.1111/bij.12918>
- Nesemann, H., Neubert, E. (1999). Süßwasserfauna von Mitteleuropa. Bd. 6 Annelida. 2. Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea. *Spectrum Akademischer Verlag GmbH, Heidelberg, Berlin*. Pp 192.
- Oberkofler, B., Quaglio, F., Füreder, L., Fioravanti, M. L., Giannetto, S., Morolli, C., Minelli, G. (2002). Species of Branchiobdellidae (Annelida) on freshwater crayfish in south Tyrol (northern Italy). *Bulletin Français de la Pêche et de la Pisciculture*, (367), 777-784. <https://doi.org/10.1051/kmae:2002065>
- Pârvulescu, L., Pacioglu, O., Hamchevici, C. (2011). The assessment of the habitat and water quality requirements of the stone crayfish (*Austropotamobius torrentium*) and noble crayfish (*Astacus astacus*) species in the rivers from the Anina Mountains (SW Romania). *Knowledge and Management of Aquatic Ecosystems*, (401), 03. <https://doi.org/10.1051/kmae/2010036>
- Pârvulescu, L., Zaharia, C. (2014). Distribution and ecological preferences of noble crayfish in the Carpathian Danube basin: biogeographical insights into the species history. *Hydrobiologia* 726, 53–63.
- Schmitt, C. B., Burgess, N. D., Coad, L., Belokurov, A., Besançon, C., Boisrobert, L., Winkel, G. (2009). Global analysis of the protection status of the world's forests. *Biological Conservation*, 142(10), 2122-2130. <https://doi.org/10.1016/j.biocon.2009.04.012>
- Schulz, H. K., Grandjean, F. (2005). Roundtable session 3: phylogeny of European crayfish—improving the taxonomy of European crayfish for a better conservation. *Bulletin Français de la Pêche et de la Pisciculture*, (376-377), 829-836. <https://doi.org/10.1051/kmae:2005035>
- Skelton, J., Creed, R. P., Brown, B. L. (2014). Ontogenetic shift in host tolerance controls initiation of a cleaning symbiosis. *Oikos*, 123(6), 677-686. <https://doi.org/10.1111/j.1600-0706.2013.00963.x>
- Subchev, M., Koutrakis, E., Perdikaris, C. (2007). Crayfish epibionts *Branchiobdella* sp. and *Hystricosoma chappuisi* (Annelida: Clitellata) in Greece. *Bulletin Français de la Pêche et de la Pisciculture*, (387), 59-66. <https://doi.org/10.1051/kmae:2007017>
- Subchev, M. (2014). The genus *Branchiobdella* Odier, 1823 (Annelida, Clitellata, Branchiobdellida): a review of its european species. *Acta Zoologica Bulgarica*, 66(1), 5-20.
- Subchev, M. A., Rimcheska, B. J. (2021). Description of *Branchiobdella bulgariensis* sp. n. (Branchiobdellida) from Bulgaria. *Acta Zoologica Bulgarica*, 73(3), 331-338.

- Subchev, M. A., Stanimirova, L. S. (1986). The epibiont *Hystricosoma chappuisi* Michaelsen, 1926 (Oligochaeta, Aeolosomatidae) – new species for Bulgarian fauna. – *Acta Zoologica Bulgarica*, 32: 66-68. (In Bulgarian).
- Subchev, M. A. (2011). First record of *Branchiobdella* Odier, 1823 (Annelida: Clitellata) in Albania and an overview of the geographic distribution of *Branchiobdella hexadonta* Gruber, 1882 in Europe. – *Acta Zoologica Bulgarica*, 63,1: 109-112.
- Szenejko, M., Eljasik, P., Panicz, R., Śmietana, P. (2023). Declining populations of *Astacus astacus* drag European *Branchiobdella parasita* (Annelida: Clitellata) ectosymbionts to serious biodiversity loss. *Global Ecology and Conservation*, 48, e02719. <https://doi.org/10.1016/j.gecco.2023.e02719>
- Trontelj, P., Machino, Y., Sket, B. (2005). Phylogenetic and phylogeographic relationships in the crayfish genus *Austropotamobius* inferred from mitochondrial COI gene sequences. *Molecular Phylogenetics and Evolution*, 34(1), 212-226. <https://doi.org/10.1016/j.ympev.2004.09.010>

Поширення бранхіобделід (Annelida: Clitellata) на благородному раці *Astacus astacus* у Закарпатській області України

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Наше дослідження присвячене поширенню, чисельності та різноманіттю бранхіобделід на прісноводних раках у Закарпатському регіоні України, зокрема у річці Терєбля та озері Синевир. Під час літніх сезонів 2008, 2009, 2017 і 2022 років було обстежено 68 особин благородного рака (*Astacus astacus* Linnaeus, 1758), у яких було ідентифіковано чотири види бранхіобделід: *Branchiobdella parasita* (Braun, 1805), *B. balcanica* Moszynski, 1937, *B. pentadonta* Whitman, 1882 і *B. hexadonta* Gruber, 1883. У нашій роботі вперше для фауни України наводиться *B. hexadonta*, а також розширюються відомості про розповсюдження *B. parasita* та *B. balcanica*. Серед виявлених видів найбільш поширеним був *B. balcanica*, який складав 83% від загальної кількості бранхіобделід у зразках з озера Синевир та 64% у річці Терєбля. Цей вид переважно локалізувався на клешнях, грудних ніжках та основах антенул раків, а кокони розташовувалися у нижніх цервікальних борознах. Менш численним був *B. pentadonta*, частка якого становила 10% у зразках з озера Синевир та 15% у річці Терєбля; він зазвичай співіснував з *B. balcanica* на тих самих частинах тіла хазяїна. *B. hexadonta*, виявлений лише в озері Синевир, локалізувався в зябрових камерах та складав лише 3% загальної чисельності бранхіобделід; при цьому пошкодження зябрових філаментів може свідчити про паразитичну поведінку цього виду. *B. parasita*, відома своїми значними розмірами та широким ареалом у популяціях європейських раків, складала приблизно 21% від загальної кількості бранхіобделід і була виявлена переважно на очах, максиліпедах і боках головогрудей. Новоописаний вид *B. bulgariensis*, знайдений у Болгарії, відрізняється від *B. parasita* структурою сперматеки та асоціюється з *A. torrentium*. У нашому дослідженні зразки виявили відмінності у структурі сперматеки як від *B. bulgariensis*, так і *B. parasita*, що свідчить про можливі таксономічні відмінності, які потребують подальшого вивчення за допомогою молекулярно-генетичного аналізу. У цьому дослідженні ми розширюємо існуючі описи видів бранхіобделід Закарпатського регіону України надаючи детальні морфологічні та анатомічні параметри.

Ключові слова: *Branchiobdella*, Україна, Закарпаття, *Astacus astacus*, розповсюдження

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