

Comparative morphology of glochidial shells of *Anodonta cygnea* and *Anodonta anatina* (Bivalvia: Unionidae)

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Abstract. Like other Unionidae species, members of *Anodonta* have parasitic glochidia larvae. Their complex life cycle, with a parasitic stage for metamorphosis, is characterized by complete morphological differences between the adult mollusk and the glochidium, so that the larval shell offers additional features for identification. The morphology of glochidia may vary among taxa, and such differences can be used to classify unionidans. Using extensive material, including samples from 26 localities in Germany, Kazakhstan, Poland, and Russia, we show that mature glochidia of *Anodonta anatina* and *A. cygnea* possess a species-specific microsculpture on their external shell surface: raised loops arranged in clear, parallel rows dorsoventrally in *A. anatina*, and loops that are not arranged in rows in any area of the glochidial valves in *A. cygnea*. While glochidia of the two species also differ in average height and length, in practice the size ranges are overlapping and it is impossible to identify the species without scanning electron microscopy. Based on sampling here, the previously predicted presence of *A. anatina* in water bodies of Krasnoyarsk Region, Tyva and Khakassia Republics (Enisey River basin), and Novosibirsk District (Ob' River basin) in Russia is validated for the first time. In addition to four known localities of *A. cygnea* in Russia from Kaliningrad Region (Baltic Sea drainage), Voronezh Region (Don River basin, Azov Sea drainage), and Moscow Region (Volga River basin, Caspian Sea drainage), three new localities of *A. cygnea* have been discovered in the Volga and Oka river basins (Caspian Sea drainage), all in the Moscow Region.

Keywords. *Anodonta* spp., larval morphology, external shell sculpture, species recognition, distribution range

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Introduction

Traditionally, the adult mollusk shell was the part of the animal whose characteristics were used to identify and distinguish between species, for example, in the latest printed monograph (Haas, 1969). On the other hand, the shape of the shells is subject to morphological plasticity influenced by varying environmental condi-

tions, making consistent identification a challenge (Zieritz & Aldridge, 2009, Yusseppone et al., 2018, Krylov & Bolshiyanov, 2020, Lyubas et al., 2023). In this way, the similarity of some morphotypes can make the distinction between species extremely difficult. Before the application of an integrative approach involving reproductive traits, anatomy, shell characters and DNA sequence data, the taxonomy of the family Unionidae in general and

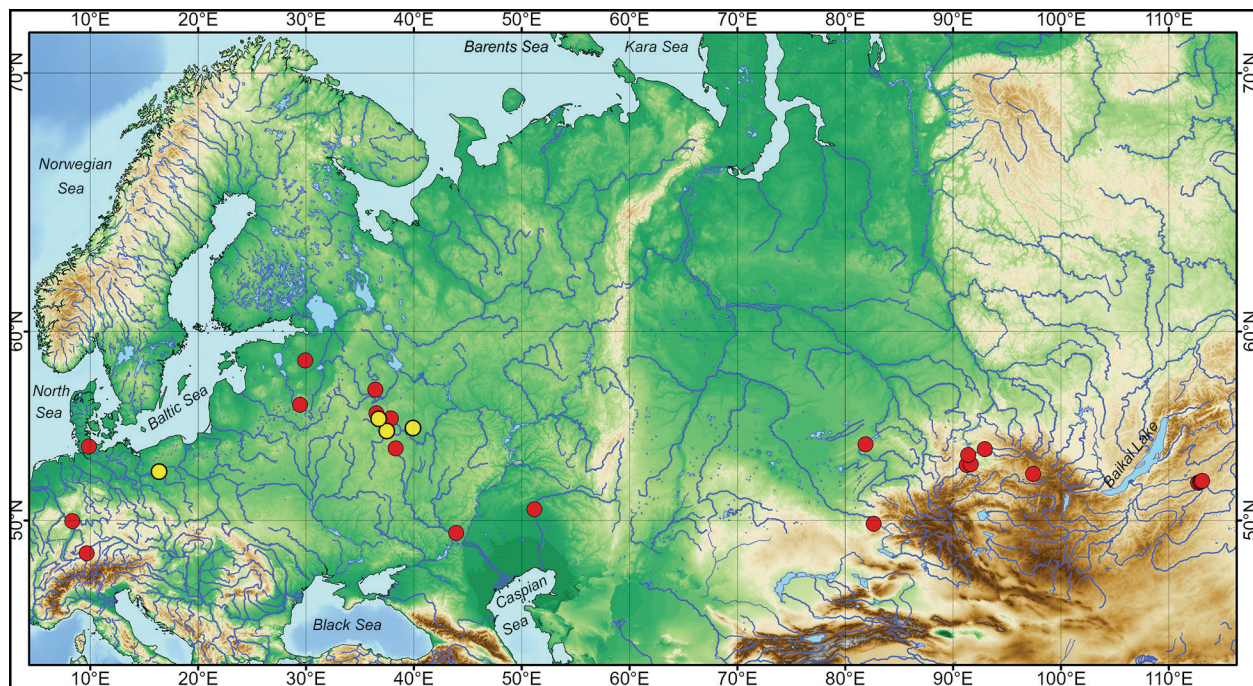


Figure 1. Map of the localities of *Anodonta cygnea* (yellow dots) and *Anodonta anatina* (red dots) used in the study.

the genus *Anodonta* Lamarck, 1799 in particular was very confusing. The high plasticity of freshwater mussel shells has led to different approaches in identification and taxonomy. The most important were the “Nouvelle École” (Bourguignat, 1881, Locard, 1884) and the “comparatory method” (Logvinenko & Starobogatov, 1971, Scarlato et al., 1990). Both significantly overestimated the number of Unionidae taxa (Starobogatov et al., 2004, Graf, 2007, Kantor et al., 2010).

Anodontine bivalves of Europe and Siberia were first thought to belong to the genus *Anodonta*, although the number of *Anodonta* species was unclear until the middle of the 20th century when in the most recent monograph of the order Unionida all nominal species were lumped together in a single taxon, *Anodonta cygnea* (Linnaeus, 1758) (Haas, 1969). Later genetic studies proved the composite nature of this taxon (Baagøe et al., 1985, Nagel et al., 1996). According to these studies, only the two species *Anodonta anatina* (Linnaeus, 1758) and *A. cygnea* live in Europe, excluding Southern Europe (Kodolova & Logvinenko, 1974, Nagel et al., 1998, Froufe et al., 2014). This conclusion had already been reached much earlier on the basis of shell characteristics, but was not accepted until later (Falkner, 1994). Based on the differences in adult shell size, shell surface, and umbo sculpture, some European-Siberian *Anodonta* species had been separated as the genus *Colletopterum* Bourguignat, 1880 (Zatravkin, 1983). Furthermore, following the comparatory method, the number of *Colletopterum* species has been gradually increased (Starobogatov et al., 2004, Bogatov et al., 2005, Prozorova & Bogatov, 2006) and the genus was divided into two subgenera (Bogatov et al.,

2005). It should be noted that some malacologists did not accept the validity and reliability of the genus *Colletopterum* (Graf, 2007, Lopes-Lima et al., 2017b).

New approaches in the description of freshwater bivalves became possible with the application of genetic methods, such as DNA-barcoding, allowing more reliable results (Chac & Thinh, 2023). Consequently, most of the comparatory species were recently synonymised (Klishko et al., 2018, Lopes-Lima et al., 2017a, 2020, Sayenko et al., 2017, 2021). The use of the comparatory method for species identification among Unionidae was recognized as unreliable even by one of the adherents of the method, as a result, three comparatory species was considered as junior synonyms of *Colletopterum anatinum*, the validity of the genus *Colletopterum* remained unchanged (Bogatov et al., 2018). Whereas, allozyme genetics and sequence data of the cytochrome c oxidase subunit I (COI), 16S rRNA and 28S rRNA genes refuted the validity of *Colletopterum* instead showing the presence of *Anodonta anatina* and *A. cygnea* (Mezhzherin et al., 2014, Froufe et al., 2014, 2017, Lopes-Lima et al., 2017b). In Russia, genetic information of *A. anatina* is only available from a few regions: the Azov Sea river basins, the Lake Baikal and Transbaikalia regions (Klishko et al., 2018, Bolotov et al., 2020, Tomilova et al., 2020), while vast regions of Khakassia, East Siberia and the adjacent territories are still missing.

Like other species of Unionidae, members of *Anodonta* have specific larvae (glochidia) in their life cycle which are obligate parasites of mostly fishes, before their metamorphosis to the juvenile forms (Wächtler et al., 2001). Such a life cycle is characterized by a complete mor-

Table 1. List of investigated *Anodonta* species and collection sites.

Species name*	COI GenBank accession number, reference	Collection number	Locality (listed E–W and S–N for river basins)	Latitude (N)	Longitude (E)	River basin, Sea drainage	N
<i>A. anatina</i>		Chita-1	Russia, Far Eastern part, Zabaykalsky Region, Chitinsky District, Ivan-Arakhley Lakes System, Ivan Lake	52°15'42.1"	112°59'46.6"	Lena River, Laptev Sea	1
<i>A. anatina</i>	<i>A. anatina</i> (MH062765) Klishko et al., 2018	Chita-6	Russia, Far Eastern part, Zabaykalsky Region, Chitinsky District, Ivan-Arakhley Lakes System, Tasey Lake	52°18'53.5"	113°06'15.5"		1
<i>A. anatina</i>		Chita-2 [gl-C1]	Russia, Far Eastern part, Zabaykalsky Region, Chitinsky District, Ivan-Arakhley Lakes System, Shakshinskoe Lake (Baikal Lake basin)	52°11'46.3"	112°43'05.3"		1
<i>A. anatina</i>		Chita-4 [gl-C2/C4]	Russia, Far Eastern part, Zabaykalsky Region, Chitinsky District, Ivan-Arakhley Lakes System, Arakhlei Lake (Baikal Lake basin)	52°14'38.4"	112°52'45.9"		3
<i>A. anatina</i>		6043-b	Russia, Eastern Siberia, Republic of Tyva, Todzhinsky District, Borzu-Khol Lake	52°42'33.5"	97°24'34.6"	Enisey River, Kara Sea	1
<i>A. anatina</i>		gl-C3	Russia, Eastern Siberia, Republic of Khakassia, Beisky District, Krasnoe Lake near Novonikolaevka village	53°12'34.1"	91°14'30.3"		2
<i>A. anatina</i>		gl-Col12	Russia, Eastern Siberia, Republic of Khakassia, Altaysky District, Novomikhailovskoe Lake at Novomikhailovskoe village	53°15'02.4"	91°37'25.9"		1
<i>A. anatina</i>		13-m	Russia, Eastern Siberia, Republic of Khakassia, Tasheba River at Abakan City	53°45'01.3"	91°24'30.5"		1
<i>A. anatina</i>		24-m	Russia, Eastern Siberia, Krasnoyarsk Region, Kuraginsky District, Bolshaya Irba River in Bolshaya Irba village	54°04'58.2"	92°56'03.4"	Ob' River, Kara Sea	1
<i>A. anatina</i>	<i>A. anatina</i> (MT027871–74) Tomilova et al., 2020	gl-KZ(1–3)	Kazakhstan, East Kazakhstan Region, Ulansky District, Ablaketka River	49°48'04.4"	82°37'32.2"		3
<i>A. anatina</i>		gl-Nov(1–2)	Russia, Siberia, Novosibirsk Region, Novosibirsk District, Novosibirskoye Reservoir at Ordynskoe village	54°20'50.3"	81°52'16.7"		2
<i>A. anatina</i>	<i>A. anatina</i> (MK603925–30) BOLOTOV et al. 2020	Ural-1	Kazakhstan, West Kazakhstan Region, Terekinsky District, Ural River at Ulken-Elbek village	50°40'17.1"	51°10'02.7"		1
<i>A. anatina</i>	<i>A. anatina</i> (KY328495–96) Bolotov et al., 2020	13Don	Russia, European part, Volgograd Region, Ilovinsky District, Piatidesiatnoe Lake (Don River basin)	49°14'31.8"	43°55'27.2"	Volga River, Caspian Sea	2
<i>A. anatina</i>		gl-T-Sh(1–3)	Russia, European part, Tula Region, Novomoskovsk District, water reservoir of Shat River near Znamenka village	54°06'46.3"	38°18'37.8"		3
<i>A. cygnea</i>		gl-M-Shp(1)	Russia, European part, Moscow Region, Serpukhovskiy District, pond near Sharapova Okhota village	55°02'55.7"	37°28'30.7"		1
<i>A. cygnea</i>		412Msc	Russia, European part, Moscow Region, Shatursky District, Yalma River (Oka River basin)	55°13'03.7"	39°55'24.6"		8
<i>A. cygnea</i>		gl-Mosc(4)	Russia, European part, Moscow Region, Zvenigorod District, Zvenigorog Biological Station of the Moscow State University, Moskva River	55°42'04.9"	36°43'16.7"		1

Species name*	COI GenBank accession number, reference	Collection number	Locality (listed E–W and S–N for river basins)	Latitude (N)	Longitude (E)	River basin, Sea drainage	N
<i>A. anatina</i>	<i>A. anatina</i> (MT027868–69) Tomilova et al., 2020	gl-Mosc(1–2)	Russia, European part, Moscow Region, Zvenigorod District, Zvenigorod Biological Station of the Moscow State University, Moskva River	55°42'04.9"	36°43'16.7"	Volga River, Caspian Sea	2
<i>A. anatina</i>		gl-Col18-19	Russia, European part, Moscow City, Kosino-Ukhtomsky District, Beloe Lake	55°43'15.5"	37°50'58.3"		2
<i>A. anatina</i>		gl-Col16	Russia, European part, Moscow City, Troitsky District, pond at Molodilnja River	55°58'37.1"	36°30'50.1"		1
<i>A. anatina</i>	<i>A. anatina</i> Bogatov et al., 2018	gl-Iv(1–4)	Russia, European part, Tver Region, Rameshkovsky District, Ivitsa River	57°11'43.4"	36°24'41.1"	Narva River, Baltic Sea	4
<i>A. anatina</i>		86Balt [gl-Col30]	Russia, European part, Pskov Region, Pustoshinsky District, Velikaya River	56°25'13.1"	29°26'13.7"		1
<i>A. anatina</i>		gl-ZIN	Russia, European part, Leningrad Region, Luzhsky District, Cheremenetskoe Lake	58°38'04.8"	29°55'49.8"		1
<i>A. cygnea</i>	<i>A. cygnea</i> (GU230749) Soroka, 2010	Ac281(H,G)	Poland, Wielkopolska Region, Czarnkowsko-Trzciancki District, Wieleń town, Hamrzysko Lake	52°49.27.5"	16°22.29.6"	Odra River, Baltic Sea	2
<i>A. anatina</i>		SMF 355123	Germany, Baden-Württemberg Region, Biberach District, Olzreuter See [lake] near Bad Schussenried town	48°00'38.2"	9°40'43.6"	Rhine River, North Sea	2
<i>A. anatina</i>		SMF 354414	Germany, Hessen Region, Groß-Gerau District, Ginsheim-Gustavsburg town, Ginsheimer Altrhein [side branch of the Rhine River]	49°57'45.4"	8°20'33.2"		2
<i>A. cygnea</i>		SMF 355410	Germany, Schleswig-Holstein Region, Rendsburg-Eckernförde District, Warder town, Mühlenau stream	54°12'47.9"	9°51'29.9"	Eider River, North Sea	1

* – species identification is made based on glochidia exterior microsculpture; N – number of investigated adult mussels.

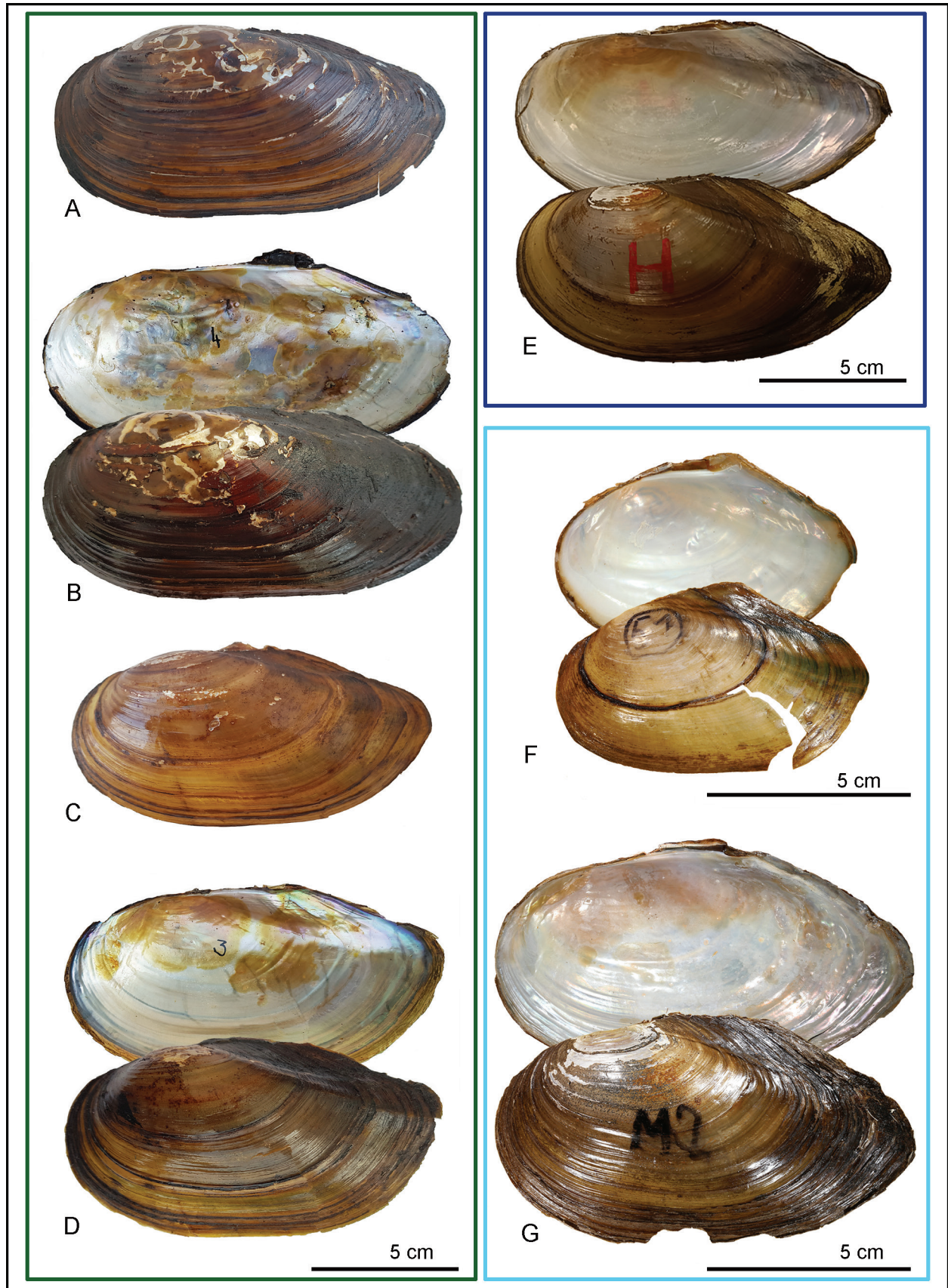
phological separation between the adult mollusk and the larvae, so that the larval shell offers additional features for identification. The shape and morphology of glochidia may vary among taxa and such differences were used to classify unionidans (Inaba, 1964, Hoggarth, 1999, Wächtler et al., 2001, Sayenko, 2006, Vikhrev et al., 2019, Klunzinger et al., 2023). However, the differences in dimensional characteristics of glochidia may not necessarily distinguish between species (Bonetto, 1961, Sayenko & Shedko, 2005, Sayenko, 2006) but the fine structure of the glochidial shells has the potential to be used as a taxonomic tool (Hoggarth, 1999, Mansur & Silva, 1999, Pimpão et al., 2012, Sayenko, 2014a, 2016, O'Brien et al., 2019, Sayenko et al., 2021). The present study is a description of the larvae of two species, *Anodonta anatina* and *Anodonta cygnea*, using specimens from genetically validated populations as a reference, and to assess the potential of various traits for species differentiation and taxonomy. Specifically,

this study aims (i) to compare the shapes of the glochidial shells, (ii) to investigate whether the fine structure of glochidial shells offers reliable distinguishing interspecific characteristics, and (iii) to add data to the knowledge of the species' distribution ranges.

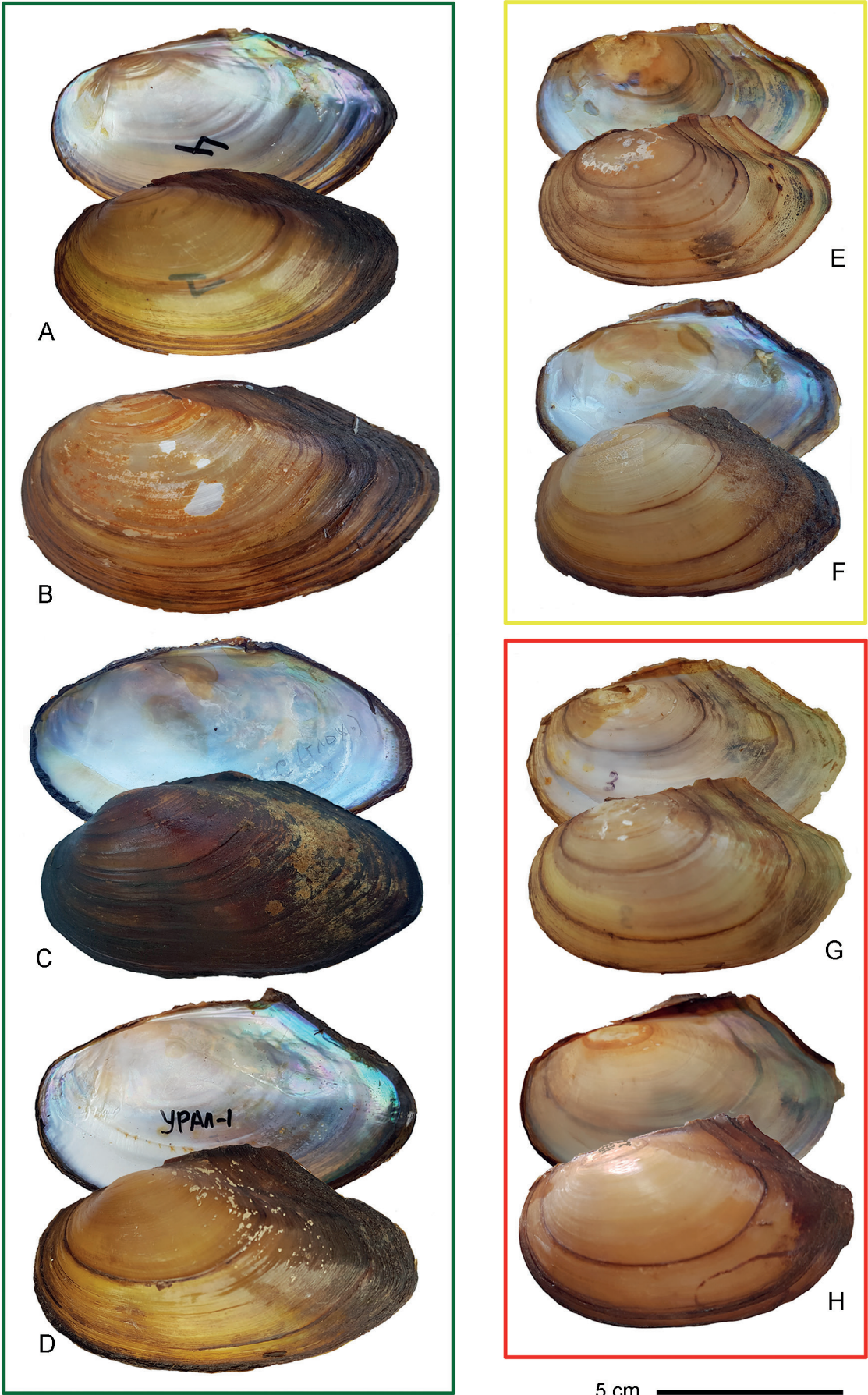
Material and methods

Specimen sampling

Specimens of *Anodonta* were collected from 26 localities in Germany, Kazakhstan, Poland and Russia (Figs 1–4). The degree of maturity of brooded glochidia was determined by the shape and colour of the outer demibranchs in adult mussels, when swollen dark-brown outer demibranchs indicates that the majority of larvae are already mature (Sayenko & Kazarin, 2022,



Figures 2. Shells of *Anodonta cygnea* (A–E, G) and *Anodonta anatina* (F) from some localities: Yalma River, Oka R. basin (A–D); Hamrzysko Lake, Odra R. basin (E); Rhine River (F, SMF 354414); Elbe River (G, SMF 355410). Colours indicate the following sea drainages: green – Caspian, dark blue – Baltic, blue – North.



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Table 2. Morphometric characteristics (in μm) of glochidia of two *Anodonta* species from three sea drainages. Limits of variation (min–max) of every characteristic with mean arithmetical value (bold) $\pm$ standard error of the mean are given; n – number of measured glochidia.

Feature	<i>A. anatina</i>			<i>A. cygnea</i>		
	Caspian Sea drainage (n = 45)	Baltic Sea drainage (n = 15)	North Sea drainage (n = 291)	Caspian Sea drainage (n = 14)	Baltic Sea drainage (n = 14)	North Sea drainage (n = 110)
height, H	317.8–390.0 353.9 $\pm$ 2.4	315.0–362.5 346.8 $\pm$ 3.1	299.6–390.0 338.7 $\pm$ 1.1	320.0–340.0 328.6 $\pm$ 1.4	300.0–330.0 315.7 $\pm$ 3.1	300.0–352.5 329.3 $\pm$ 1.2
length, L	322.1–390.0 363.7 $\pm$ 2.0	338.3–392.5 373.7 $\pm$ 4.1	300.0–358.4 345.7 $\pm$ 1.1	330.0–350.0 345.0 $\pm$ 2.0	310.0–340.0 323.9 $\pm$ 2.6	305.5–364.3 333.0 $\pm$ 1.3
H/L	0.93–1.05 0.97 $\pm$ 0.003	0.88–0.97 0.93 $\pm$ 0.007	0.91–1.10 0.98 $\pm$ 0.002	0.91–1.00 0.95 $\pm$ 0.01	0.90–1.03 0.98 $\pm$ 0.01	0.95–1.05 0.99 $\pm$ 0.002

Nagel & Sayenko, 2025). Mature infective glochidia were identified by snapping movements of live larvae or by the presence of a fully developed hook in fixed glochidia (Hoggarth, 1999, Sayenko, 2006, Sayenko & Kazarin, 2022). To confirm species identification, 15 specimens with mature glochidia were collected in 7 localities where genetic studies had already been carried out and local *Anodonta* species had previously been determined. In addition, to investigate the fine sculpture of glochidial shells, 36 adult mussels with mature glochidia from 20 localities were also collected and studied (Table 1). After identification, selected dry adult shells were photographed using a Canon EOS 6D camera (a Canon Macro 100 mm lens) and a Samsung Galaxy S8 smartphone.

Data availability

Glochidial samples from Kazakhstan, Poland and Russia and the adult shells from Kazakhstan and Russia are deposited in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok (FSCEATB FEB RAS, hereafter). Glochidia and adult shell samples from Germany are kept in the Senckenberg Research Institute and Natural History Museum Frankfurt (SMF), Malacology Section. The collection numbers of the samples are given in Table 1.

Procedures for light, scanning electron microscopy and glochidia morphometry

Glochidia preserved in 75% alcohol were used for morphological investigations, subsequently, only chemical cleaning of the larvae shells was applied following the standard procedures (Sayenko & Kazarin, 2022). The photographs of glochidia were obtained on 1) a Zeiss

MERLIN SEM (the Biology and Genetic Engineering Center for Collective Use of the FSCEATB FEB RAS) at 10–20.1 kV, chromium and gold coating 2–4 nm; 2) EVO SEM (the Biology and Genetic Engineering Center for Collective Use of the FSCEATB FEB RAS) at 17.3–20.23 kV, chromium and carbon coating 2–4 nm; 3) Cambridge Stereoscan 600 (Senckenberg Research Institute and Museum Frankfurt) at 25 kV, gold coating 50 nm; 4) Philipps SEM 505 (Institute of Mineralogy, Technical University Darmstadt) at 30 kV; 5) Leitz AMR 1200 (University Kassel) at 25 kV.

The description of the main conchological features of glochidial shells (glochidial valve height and glochidial valve length, anterior and posterior margins of the larval shells, the shape of glochidial hooks) (Fig. 5) follows Hoggarth (1999) and Sayenko (2006). Following Hoggarth (1999) we use the term stylet describing the form of the complex hook of the anodontine glochidia. The spiny formation covering the upper surface of glochidial hooks may contain microspines ($<1.0 \mu\text{m}$ long) or macrospines ($>1.0 \mu\text{m}$ long) (Hoggarth, 1999).

The external microsculpture of each larval shell was examined at a few points: closer to the ventral end (i.e., to the hook), at the center of the valve (the adductor region), near the central part of the ligament, and at the valve rim closer to the ligament (Fig. 5). The description of the exterior valve microsculpture follows the published data, when the main types are identified as smooth, rough (coarse), beaded (granule), rosette, net (divided into loose-looped, tight-looped, ribbed loose-looped), and vermiculate (Hoggarth, 1999, Panha & Eongprakornkeaw, 1995, Sayenko, 2016).

Larval shells were measured using light microscopes (Nikon Alphaphot-2 YS2, Leitz Ortholux II) with an ocular micrometer and scanning electron microscopes (Zeiss MERLIN, EVO) directly from SEM photographs, where *glochidial valve length* (L) was measured parallel

Figure 3. Shells of *Anodonta anatina* from some localities: Moskva River, Volga R. basin (A); Shat River, Volga R. basin (B); Ivitsa River, Volga R. basin (C); Ural River (D); Arakhley Lake, Enisey R. basin (E); Baikal Lake, Enisey R. basin (F); Ivan Lake, Lena R. basin (G); Tasey Lake, Lena R. basin (H). Colours indicate the following sea drainages: green – Caspian, yellow – Kara (Enisey River basin), red – Laptev.

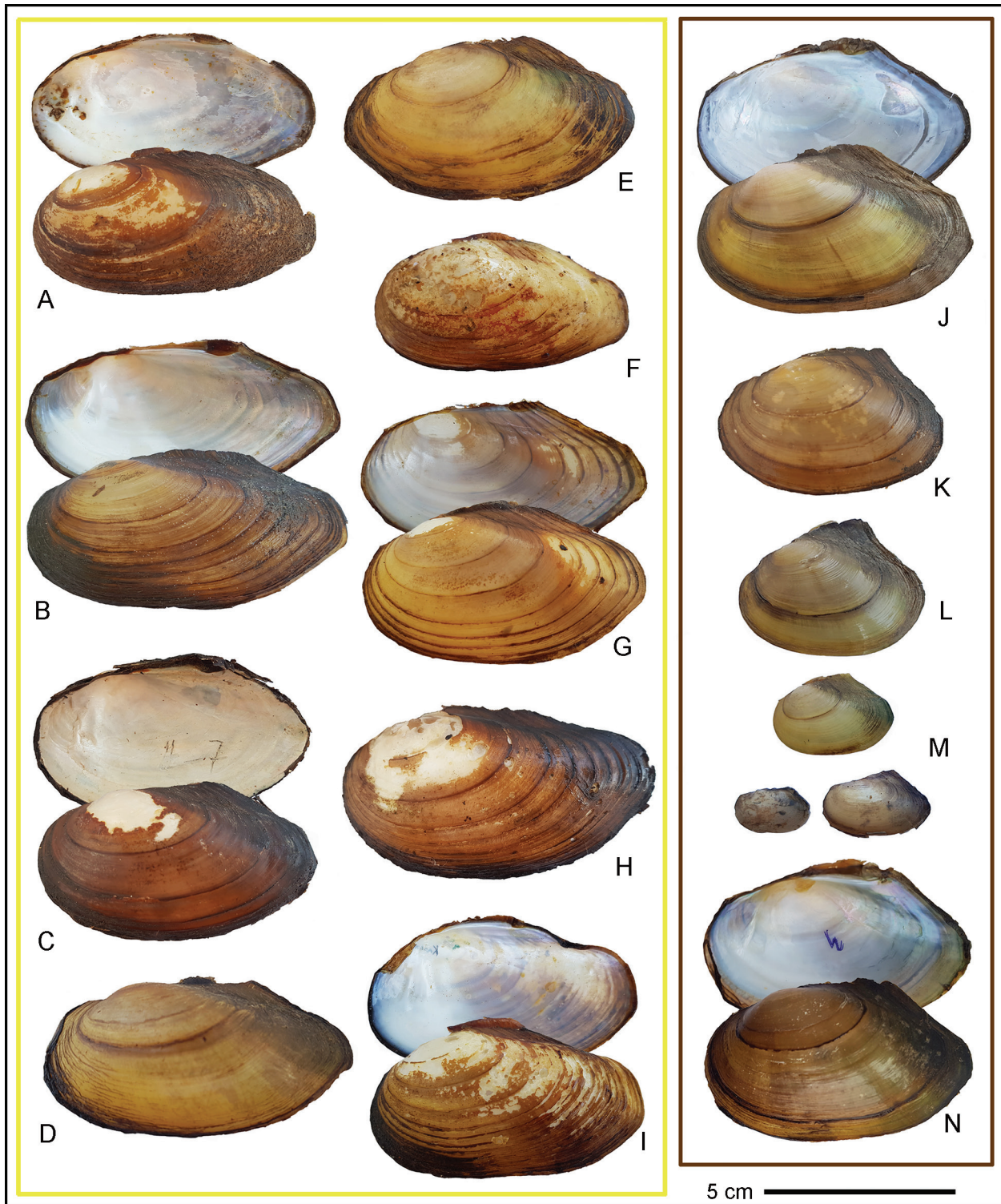


Figure 4. Shells of *Anodonta anatina* from some localities (Kara Sea drainage): Krasnoe Lake (A); Novomikhailovskoe Lake (B); Drena Lake (C); Krasnyi Dar Lake (D); Irba Lake (E); Borzu-Khol Lake (F–G); Azas Lake (H); Kadysh Lake (I); Ob' River, adult shells (J–K); Ob' River, juvenile shells (L–M); Ablakotka River (N). Colours indicate the following river basins: yellow – Enisey, brown – Ob'.

to the hinge as the greatest distance from anterior to posterior margins; *glochidial valve height* (H) was measured perpendicular to length as the greatest distance from dorsal to ventral margins (Fig. 5). At least 14 glochidia

shells taken from one adult mollusk were measured. To standardize the measurements and to estimate the shape of the glochidium shells, the ratio H/L was calculated. To analyse size variability of glochidia within each species,

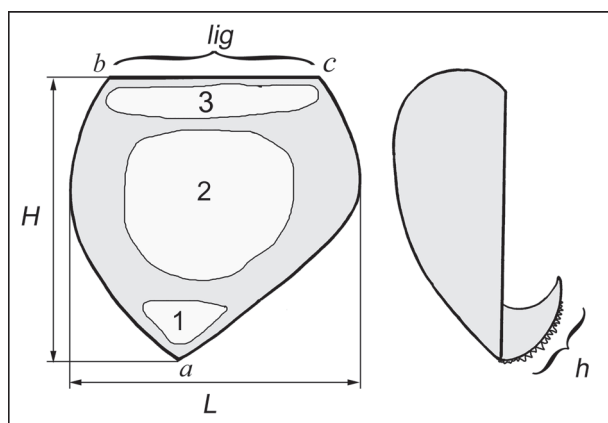


Figure 5. Scheme of glochidial valve in two views demonstrating main characteristics, measurements used in the study and valve regions examined by SEM for outer microsculpture. Abbreviations: *ab* – posterior (short) side of the valve; *ac* – anterior (long) side of the valve; *bc* – dorsal side of the valve; *a* – ventral angle (base of the hook); *lig* – ligament; *h* – hook; *L* – valve length; *H* – valve height; 1 – ventral tip of glochidial valve; 2 – center of the valve (the adductor region); 3 – valve rim close to the ligament.

we used measurements from the catchment areas where both species were collected, namely the Caspian, Baltic and North Seas drainages. Differences in morphometric characteristics between the discussed species were assessed using a two-way ANOVA and intra- and interspecific differences for all catchment areas were investigated applying a Nested ANOVA implemented in the STATISTICA software (version 10). Statistical results are given with the *p*-value, test statistic (*F*-value), degrees of freedom (*df*), and the sample size (*n* – the number of measured glochidia for a particular parameter).

Results and Discussion

Morphology of glochidia

The glochidial shells were of the anodontine type; i.e., a larval shell with two dorsally articulated hooked valves subtriangular in outline, a slightly protruding base edge forming a straight dorsal line and styliiform hooks (Giusti et al., 1975, Nagel, 1985, Antonova & Starobogatov, 1988, Hoggarth, 1999, Sayenko, 2014b, 2019). The valves of the glochidial shells of *A. anatina* and *A. cygnea* are moderately asymmetrical in contour (Fig. 6). Styliiform hooks at the ventral tips of the glochidial valves are large, up to 150 μm and not less than 1/3 of the valve height and are covered by micro- (up to 1 μm in height) and macrospines (Fig. 7). Macrospines are arranged in one row in the terminal half of the hook; there are up to 20 of them on a single hook. Our results

confirm previously published data on the genus-level diagnostic value of these different hooks (Nagel, 1988, Sayenko, 2006). The closely related genus *Pseudanodonta* Bourguignat, 1876 differs in the arrangement of the macrospines on the shell hooks with two rows in the terminal region.

Morphometric analysis showed that glochidia of *Anodonta anatina* were generally larger than those of *A. cygnea*, although the ranges of the checked features broadly overlapped in the taxa. Considering the entire data set, the mean glochidial height (*H*) of *A. anatina* was $341.3 \pm 0.7 \mu\text{m}$ (range 285.0–400.0 μm), compared to $327.3 \pm 2.2 \mu\text{m}$ (300.0–360.0 μm) in *A. cygnea*. The mean glochidial length (*L*) in *A. anatina* ($347.1 \pm 0.8 \mu\text{m}$; range 285.0–407.0 μm) is larger than in *A. cygnea* ($336.6 \pm 2.6 \mu\text{m}$; range 310.0–360.0 μm). The ranges of *H/L* ratio almost completely overlap, although on average the glochidia of *A. anatina* (0.98 ± 0.001 ; range 0.86–1.1 μm) are more elongated dorsoventrally but these include samples that are less elongated than any of the glochidia of *A. cygnea* (0.96 ± 0.01 ; range 0.90–1.1 μm). In three water catchment areas (the Caspian, Baltic and North Seas drainages), samples of both species were available, allowing us to directly compare the taxa. Glochidia of *A. anatina* were generally larger than those of *A. cygnea* (Table 2, Fig. 8). Statistically significant interspecific differences were found in all three parameters: the glochidial height ($p = 0.000001$, $F = 65.4$, $df = 1$, $n = 489$), the glochidial length ($p = 0.000001$, $F = 99.7$, $df = 1$, $n = 489$), and the index *H/L* ($p = 0.03$, $F = 4.7$, $df = 1$, $n = 489$). The size of glochidia of both species increases from north to the Caspian Sea drainage, with the smallest larvae in the North Sea drainage for *A. anatina* and in the Baltic Sea drainage for *A. cygnea*, but both species retains the same overall shape (the *H/L* ratio is completely retained in all areas) (Table 2, Fig. 8). Morphometric parameters, such as glochidial height (*H*), glochidial length (*L*), and *H/L* ratio, demonstrate that glochidial size metrics are geographically variable within species, with inter-species dimensional overlap, remain relatively unreliable characteristics for distinguishing *Anodonta anatina* and *A. cygnea*. Based on size characteristics, it is practically impossible to reliably assign a single mature glochidium to a specific species without identifying the adult mollusk from which this larva was taken.

The glochidial shell consists of two layers, a thick inner calcareous layer and a thin outer organic layer (Giusti, 1973). The inner layer has pores that are covered by the outer layer. The latter has a special microsculpture that is visible under the scanning electron microscopy at high magnification. This microsculpture shows clear differences between the two species (Figs 9, 10).

All specimens from localities where *A. cygnea* had been genetically identified exhibit glochidia whose outer valve microstructure consists of loose loops and numerous granules (Fig. 9). In contrast, the mature larvae of

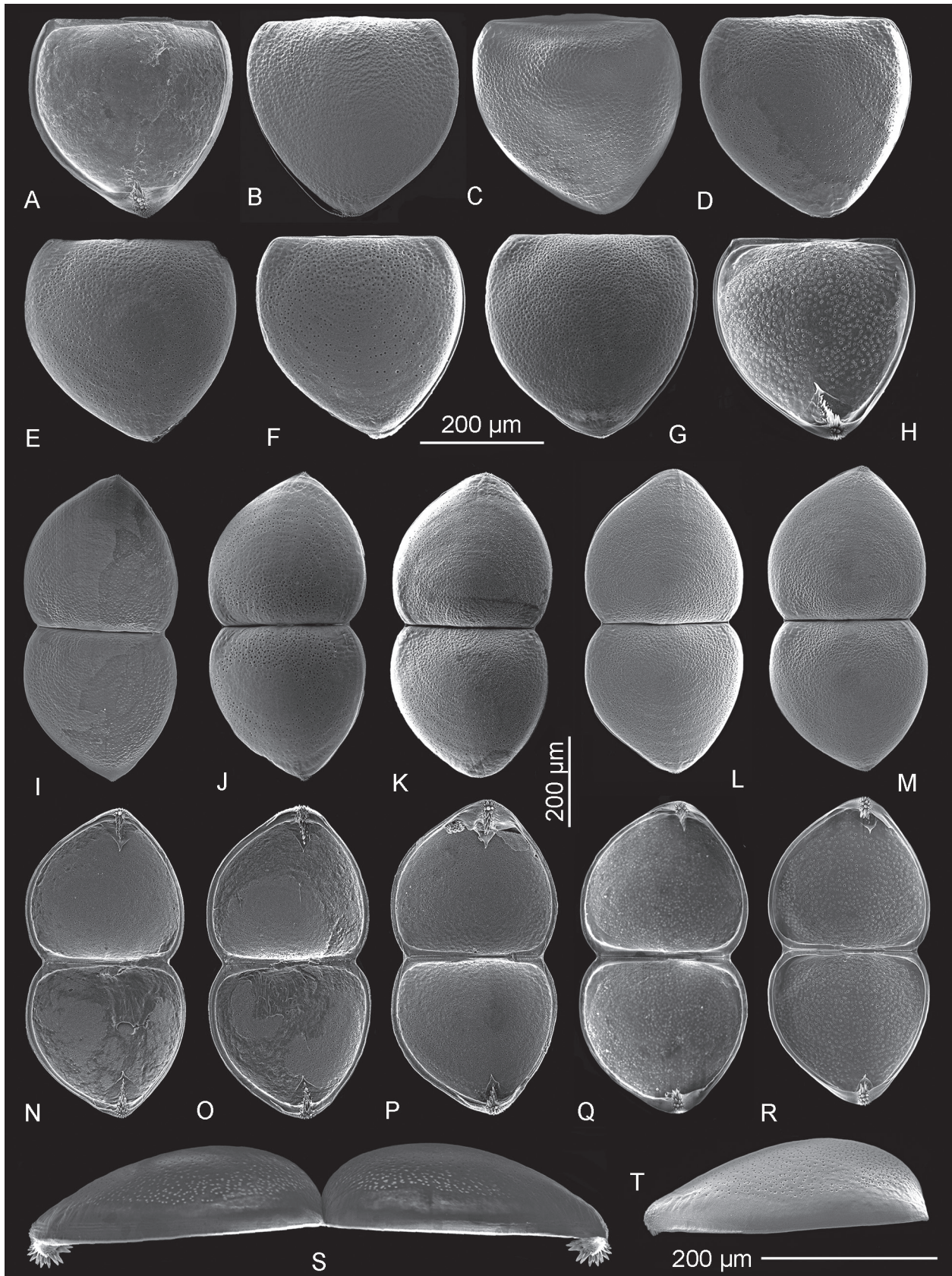


Figure 6. Glochidial shells of *Anodonta cygnea* (A, B, E, F) and *Anodonta anatina* (C, D, G–T): interior (A, H) and exterior valves (B–G); exterior (I–M) and interior (N–R) views of open shells; lateral views (S, T). Hamrzysko Lake (A, I); Yalma River (B, J); Velikaya River (C); Beloe Lake (D, K, O); Moskva River (E, P); Tasheba River (F); Bolshaya Irba River (G); Novomikhailovskoe Lake (H); Shat River (L, T); Molodilnja R. basin (M, N); Arakhley Lake (Q); Krasnoe Lake (R, S).

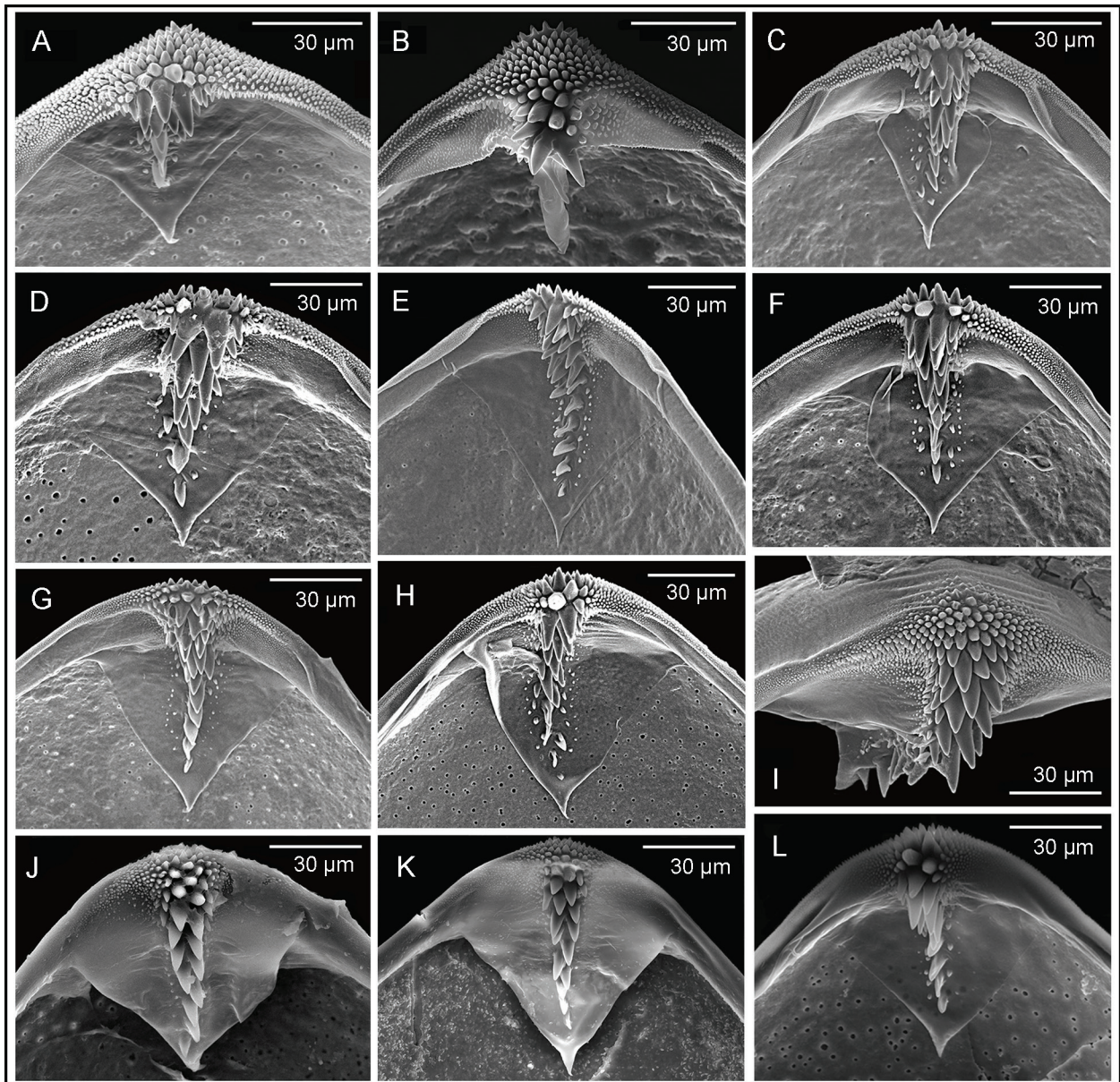


Figure 7. Glochidial hooks of *Anodonta cygnea* (A, B) and *Anodonta anatina* (C–L). Hamrzyso Lake (A); Yalma River (B); Velikaya River (C); Beloe Lake (D); Shat River (E–F); Molodilnja R. basin (G); Moskva River (H); Ablaketka River (I); Ob` River (J–K); Krasnoe Lake (L).

A. anatina have an external microsculpture consisting of raised loops arranged in clear parallel rows. The rows run dorsoventrally in the central part of the valves, from the ligament to the ventral tips. It should be emphasized that this pattern is unique in the genus *Anodonta* and does not occur in the North American species (O'Brien et al., 2019). In most specimens, such rows of raised loops are visible on all parts of the outer surface of the glochidial shells, including the areas along the edges of the valves. The rows of raised loops were most visible in the central part and along the edges of the valves. Glochidia collected from the Moskva River also had clearly visible rows except at the ligament, where the rows were

barely visible. Between the rows, narrow strips of the valve surface are often covered with granules (Fig. 10). The morphology of the glochidia of both *Anodonta* species had been studied previously using both light microscopy and scanning electron microscopy (Giusti, 1973, Wood, 1974, Giusti et al., 1975, Antonova, 1986, Antonova & Starobogatov, 1988, Wächtler et al., 2001, Sayenko, 2001, 2014a, 2014b, Lima et al., 2006, Başçınar & Düzgüneş, 2009). The first descriptions of the outer microsculpture of *Anodonta* glochidia from European waters indicated the presence of two different types. As more data became available, it became clear that freshwater mussels with the same type of glochidial valve

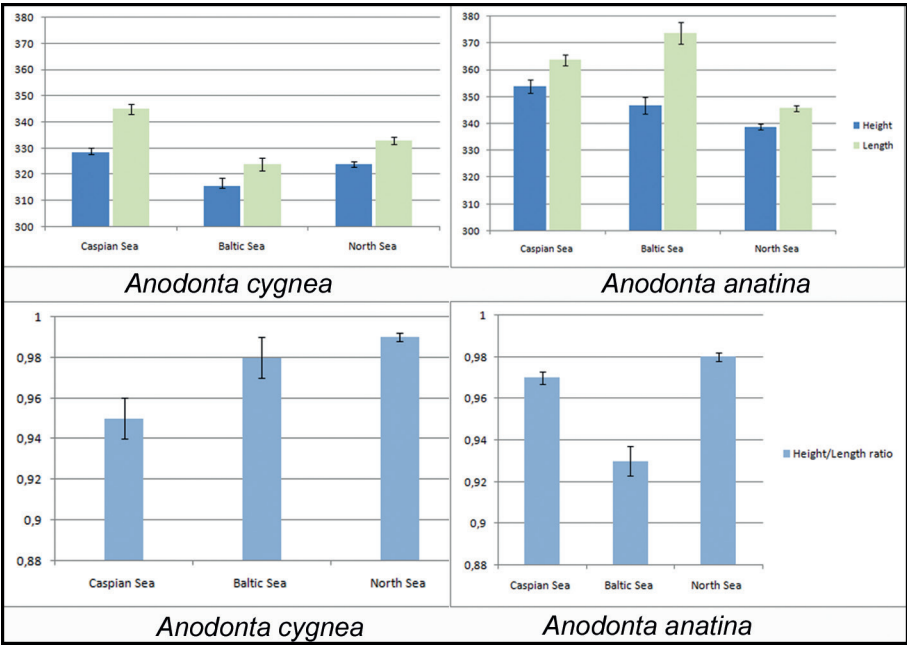


Figure 8. Comparison of the measurement parameters (height, length and height/length ratio) of *Anodontwa cygnea* and *Anodonta anatina* glochidia for the Caspian, Baltic and North Seas drainages.

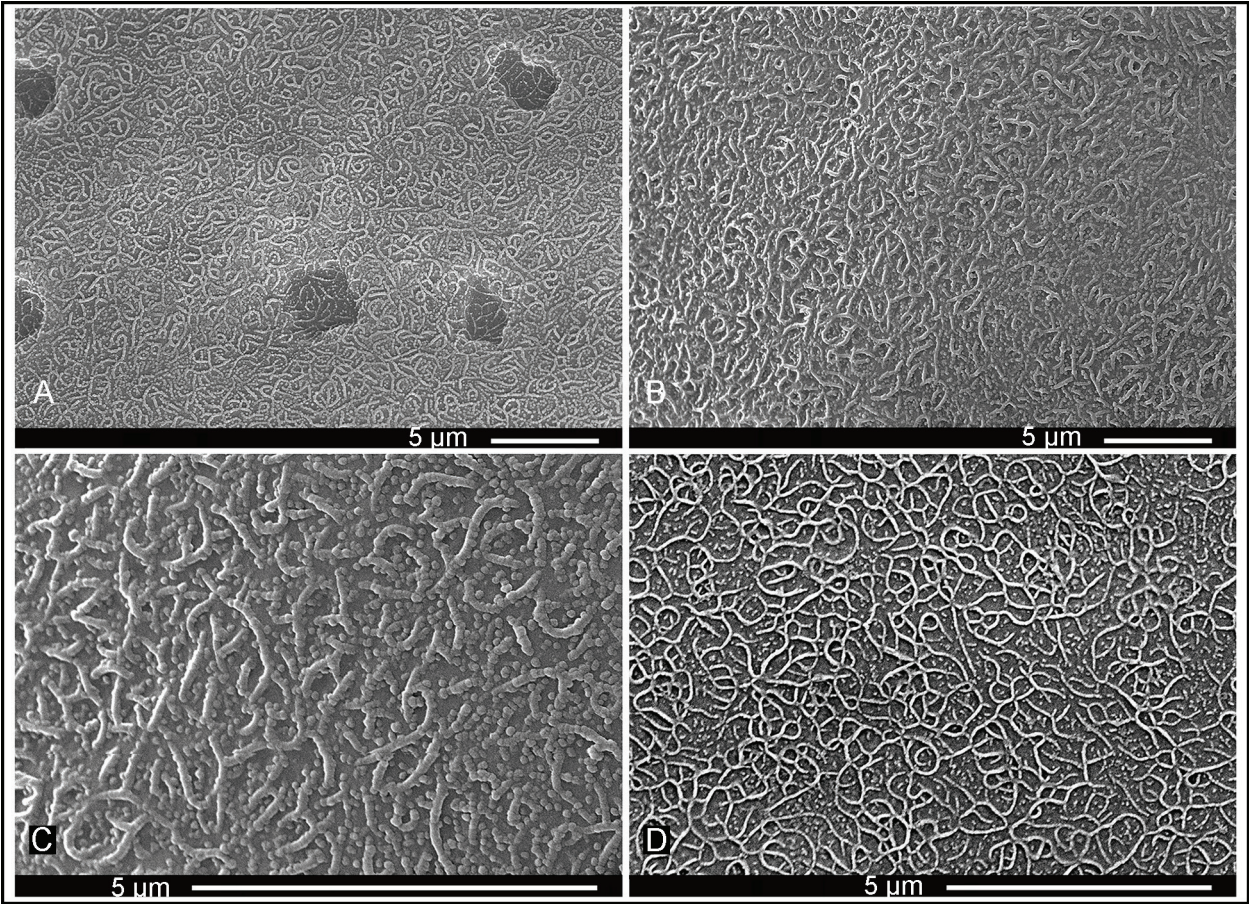


Figure 9. Exterior microsculpture of *Anodonta cygnea* glochidia observed in central part of the valve with adductor area (A) and near ligament (B–D): Hamrzysko Lake, Odra River basin (A–C) and Yalma River, Volga River basin (D).

microsculpture were assigned to different species. For example, the external looped structure forming parallel

rows was noted for glochidia of *A. piscinalis* (= *A. anatina*) from the Rhine River basin in Germany (Nagel,

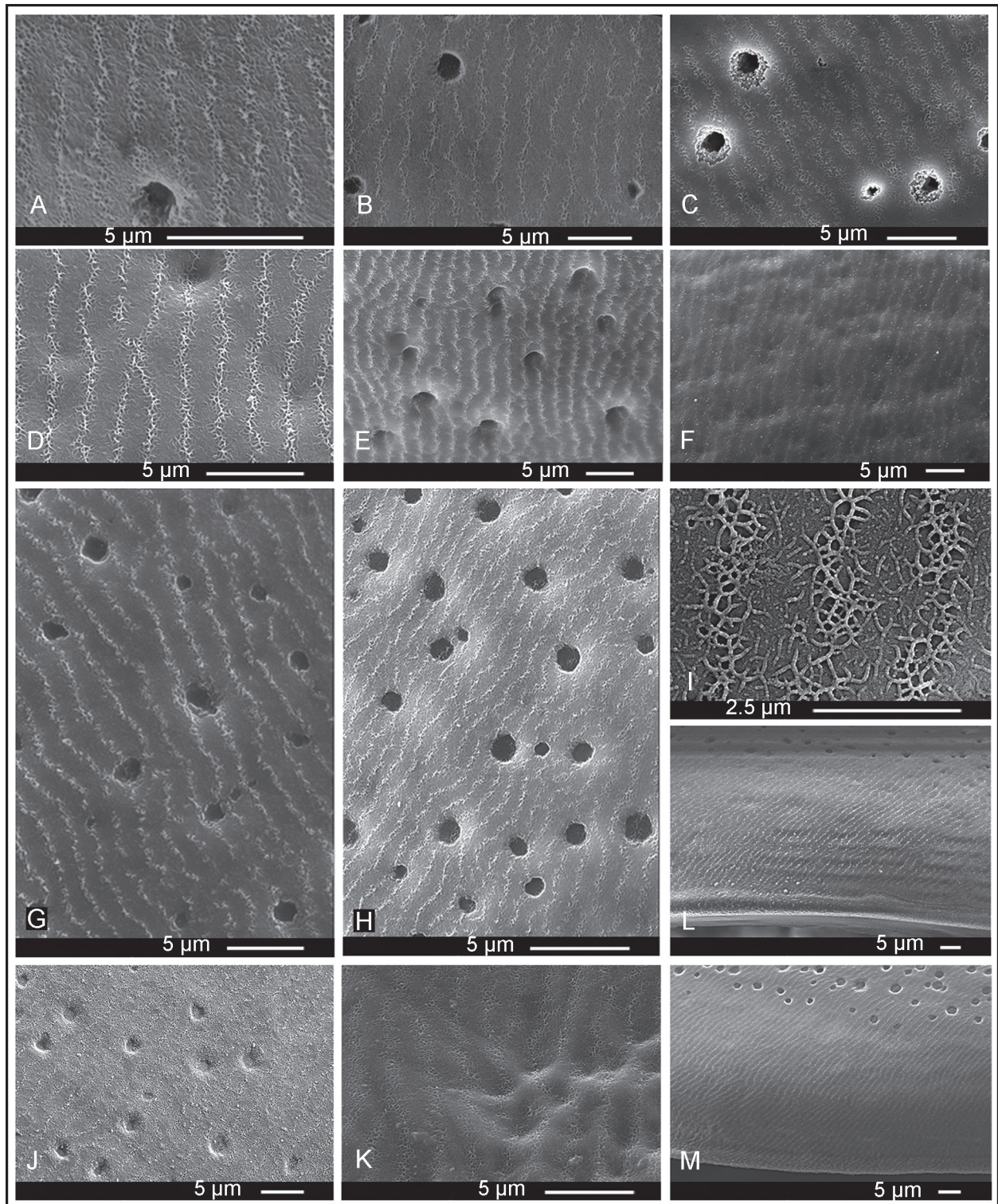


Figure 10. Exterior microsculpture of *Anodonta anatina* glochidia observed in central part of glochidial valve (A–I), at the ventral tip (J, K) and at the valve edge (L, M): Rhine River (A, B); Leningrad Region (C); Ablakotka River (D); Novomikhailovskoe Lake (E); Borzu-Khol Lake (F); Arakhley Lake (G); Shakshinskoe Lake (H); Krasnoe Lake (I); Moskva River (J–K); Velikaya River (L); Shat River (M).

1985), *A. cygnea* (but see the discussion below) from the Lake Maggiore (Po River basin, Lombardy region) and Lake Trasimeno (Tiber River basin, Umbria region) in Italy (Giusti, 1973, Giusti et al., 1975), and *A. anatina*

from the Ixelles Ponds in Belgium (Hoggarth, 1999). A completely different sculpture with looped structures that do not form rows was described for *A. cygnea* glochidia from the Genval Lake basin in Belgium (Hoggarth,

1999) and the Mira Lagoon in Portugal (Lima et al., 2006). Based on conflicting data of exterior sculpture, Hoggarth (1999) first pointed out the unclear situation regarding taxon identification and suggested distinguishing the two *Anodonta* species based on the external microsculpture of the glochidia, highlighting dorsoventral parallel rows as a unique feature of *A. anatina*. However, at the time, confirming this hypothesis without genetic verification was impossible.

In most cases, the exterior microsculpture with parallel rows was observed across the entire surface of the glochidial valves including the areas along the margins of the valves (Sayenko, 2014a, 2014b). In some cases, the presence of such an exterior microsculpture with parallel rows was noted, but it was not clearly indicated whether it was actually distributed across the entire surface of the glochidial valves or in which part of the larval valve the SEM photographs were taken (Nagel, 1985, Hoggarth, 1999). A recent study of *A. anatina* glochidia from Dagestan showed the presence of rows of raised loops on almost the entire outer surface of glochidial shells, with the exception of the area near the ligament (Sayenko & Palatov, 2022). The same variation in the exterior microsculpture without parallel rows near the ligament occurred in the glochidia of *A. anatina* from the Moskva River (sampling locality as before). We can assume that such variations are not an exception. Therefore, in order to consider parallel rows as a diagnostic feature for distinguishing *A. anatina*, special attention must be paid to the central part of the glochidial valves.

Molecular comparisons showed that both species, *A. anatina* and *A. cygnea*, inhabit Lake Maggiore alongside *A. exulcerata* Porro, 1838 (Riccardi et al., 2020). This suggests that the species described by Giusti (1973) and Giusti et al. (1975) as *A. "cygnea"*, whose glochidia had a shell surface with a microsculpture of dorsoventral rows of raised loops, actually represent *A. anatina* in the current sense.

Recent studies have confirmed the differences between genera *Anodonta* and *Pseudanodonta* in terms of the shape and the external shell morphology of the glochidia (Sayenko et al., 2025). Both *P. complanata* (Rossmässler, 1835) and *A. cygnea* have an external microsculpture of the glochidial shells with loops. However, there are some differences. While glochidia of *P. complanata* have loose loops that form a net-like pattern, the glochidia of *A. cygnea* have complex loops with intersecting lines. The microsculpture of *A. cygnea* glochidia also has a much larger number of granules compared to the glochidia of *P. complanata*.

Distribution of the species in the study area

The two *Anodonta* species occur in most European countries and are distributed widely from Norway to Finland in the north, Portugal, Spain and the UK in the west to Russia in the east (Lopes-Lima et al., 2017b,

Bolotov et al., 2020, Tomilova et al., 2020).

While *A. anatina* inhabits both lentic and lotic waters, *A. cygnea* is essentially a species of stagnant (lakes, backwaters, ponds, gravel pits) or slow-flowing (bays, reservoirs) water bodies. Among these two native European species, *A. anatina* has a much wider distribution, can occupy a wider variety of habitats (Lopes-Lima et al., 2017b), has higher infestation rates and rates of juvenile mussels' excystment than *A. cygnea* (Huber & Geist, 2019). Although both *Anodonta* species were confirmed host generalists with a broad host range (Huber & Geist, 2019), *A. anatina* produces the bigger glochidia (Schar-sack, 1994, Ćmiel et al., 2021) and attaches to more body parts of a host fish (Huber, 2020). This might lead to a higher initial infestation rate and higher distribution of *A. anatina* in contrast to *A. cygnea*.

The presence of *A. anatina* is confirmed for many river basins from Europe to the east across Russia, including Elbe, Rhine, Odra, Volga, Ob', Enisey (with the Baikal Lake basin), with the Lena River as the easternmost boundary of the range. The wide range of this species covers the water bodies of the North, Baltic, Black, Caspian, Kara and Laptev Sea drainages. In Russia, the presence of *A. anatina* in water bodies of Krasnoyarsk Region, Tyva and Khakassia Republics (Enisey River basin) and Novosibirsk District (Ob' River basin) was previously predicted by Bolotov et al. (2020) and validated here for the first time.

The distribution of *A. cygnea* in Russia is still poorly studied. Currently, there are only four records verified by genetic data: Lebedinoe Lake in Kaliningrad Region (Baltic Sea drainage), Tikhaya Sosna River in Voronezh Region (Don River basin, Azov Sea drainage), Glubokoe Lake and Medvezhii lakes in Moscow Region (Volga River basin, Caspian Sea drainage) (Tomilova et al., 2019, 2020, Bolotov et al., 2020). In many cases, older records are not always reliable because adult shells have often been misinterpreted (Graf, 2007, Klishko et al., 2018). In addition to four localities of *A. cygnea* in Russia, the first records of this species are noted for three localities: Shatursky, Odintsovsky and Serpukhovskiy districts of the Moscow Region (Volga River basin, Caspian Sea drainage).

The International Union for Conservation and Nature Red List of Threatened Species considers both species as Least Concern. However, in Poland *A. cygnea* is under protection (Piechocki & Wawrzyniak-Wydrowska, 2016), and the German national Red List considers the species as threatened (Jungbluth & Knorre, 2011).

Conclusion

The comparison of glochidia morphometric features showed that the glochidia of *A. anatina* are more elongated dorsoventrally than the glochidia of *A. cygnea*.

However, since both the range of values of the two species overlap, size data alone cannot be used for accurate species identification. For both species, geographic variability in glochidia size was demonstrated.

Based on glochidia samples from localities where a molecular barcoding technique was used for valid identification, it was shown that two different types of the external microsculpture of mature glochidial shells correspond to two different *Anodonta* species and can be used as a reliable characteristic for species identification. The external valve sculpture of *A. anatina* glochidia consists of raised loops that are arranged in clear parallel rows running dorsoventrally mainly over the entire surface of the valves or, in some cases, only at the ventral tip of the valves. In contrast, the exterior microsculpture of *A. cygnea* glochidia consists of loose loops and numerous granules, and the loops are nowhere arranged in rows.

Based on the features of glochidia morphology, the presence of *A. anatina* in Krasnoyarsk Region, Novosibirsk District, Tyva and Khakassia Republics is confirmed. The first record of *A. cygnea* is reported for three localities in the European part of Russia, namely in Shatursky, Odintsovsky and Serpukhovskiy districts of the Moscow Region (Volga River basin, Caspian Sea drainage).

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Author contributions

All authors had full access to all the data in the study and took responsibility for its integrity and the accuracy

of the analysis. Study concept and design: E.M.S. with the help of K.-O.N. Collection of biological material: E.M.S. (Republic of Khakassia and Krasnoyarsk Region in Russia), D.M.P. (European part of Russia, Kazakhstan), K.-O.N. (Germany). Data analysis and interpretation: glochidia morphology – E.M.S., K.-O.N.; glochidia measurements – E.M.S., K.-O.N.; statistical analysis – M.V.I. Drafting of the manuscript: E.M.S., with input from K.-O.N. Glochidia images: E.M.S., K.-O.N. Adult shells images: E.M.S., K.-O.N. All authors read and approved the final manuscript. The authors declare that there are no conflicts of interest.

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