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Detailed description of the glochidium of *Pseudanodonta complanata* (Rossmässler, 1835) (Bivalvia: Unionidae), Depressed River Mussel, from distant regions of its geographic range

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Abstract. The morphology of mature larvae (glochidia) of *Pseudanodonta complanata* is described using scanning electron microscopy. In river basins of Germany and Russia, mature glochidia of *P. complanata* were recorded from July to late April. Mature glochidia are hooked and large, not less than 280 μm high and 390 μm long. A single styliform hook, up to 160 μm long, at the ventral tip of each glochidial valve is covered by lanceolate microstylets, each up to 20 μm. The glochidial shell is characterized by the broad and slightly protruded anterior and posterior margins covered with distinct comarginal lines, running parallel to the valve edge. The external sculpture of glochidial shells is a consistent character and comprised of loose-looped folds distributed over the entire surface of the valves.

Key words. Freshwater molluscs, scanning electron microscopy, morphometry, Russia, Germany, Unionida

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Introduction

Pseudanodonta complanata (Rossmässler, 1835) (Bivalvia: Unionidae), Depressed River Mussel, is a European freshwater bivalve whose range includes tributaries of the Atlantic Ocean and Baltic, Black, Azov, and Caspian seas, from the catchment of the river Garonne in the west to water bodies of European Russia in the east (Haas 1969, Gittenberger et al. 1998, Zettler 1998, Gural-Sverlova & Gural 2009, Yanovich & Pampura 2010, Lopes-Lima et al. 2017a). According to the International Union for the Conservation of Nature Red List of Threatened Species, *P. complanata* is categorized as either Near Threatened or Vulnerable in countries where it occurs (Van Damme 2011, Soroka 2020). In Russia, the species has been assessed as Least Concern with its status as a "rare species sporadically distributed over an extensive area" (Bolotov et al. 2020). In France, its conservation status is Endangered (UICN et al. 2021), and in Austria and Germany, it is "threatened by extinction" and has a high conservation priority (Reischütz & Reischütz 2007, Jungbluth et al. 2011).

As a result of using predominantly conchological characters of adult shells for identification, the many subspecies and forms of Anodontinae, including *Pseudanodonta*, have been raised to the level of species (Moskvicheva 1973, Zatravkin & Bogatov 1987, Starobogatov et al. 2004). In Europe, at least 3 geographically separated conchological *Pseudanodonta* species have been recognized: *P. complanata*, *P. elongata* (Holandre, 1836), and *P. middendorffii* (Siemaschko, 1848). The last taxon has been used to designate *Pseudanodonta* living in river systems of the Black Sea basin (Haas 1969, Cuttelod et al. 2011). Later revisions recognized 4 species—*P. complanata*, *P. elongata*, *P. klettii* (Rossmässler, 1835), and *P. nordenskioldi* Bourguignat, 1880—while *P. middendorffii* was

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treated as a synonym of *P. klettii* (Starobogatov et al. 2004, Vinarski & Kantor 2016). However, based on genetic markers all these taxa belong to a single species, *P. complanata* (e.g. Källersjö et al. 2005, Mezhzherin et al. 2014, Lopes-Lima et al. 2017b, Bolotov et al. 2020, Yunitsyna et al. 2023). However, *P. complanata* tends to have a unique shell morphology in different river systems, which allows for a geographical allocation of shells (for France see Falkner et al. 2002). In Central Europe, for example, *P. complanata* is very flattened and narrow in the middle Danube (Nesemann 1993).

Like the other freshwater mussels (order Unionida), the life cycle of *P. complanata* includes an obligate fish-parasitic larval stage. These larvae (called glochidia) develop in the outer gill demibranchs (marsupia) of adult females. As in *Unio crassus* Philipsson, 1788, another species of European freshwater mussel, the fully developed larvae remain in the marsupia over the winter until they are released into the water near a passing host fish, which is attracted using a "lure" formed by the mantle of female mussels (Rock 2024). If the glochidia succeed in attaching themselves to a suitable fish, they remain there for several weeks before freeing themselves from their enveloping cyst once they have undergone metamorphosis (e.g. Fukuhara et al. 1990, Rogers-Lowery & Dimock 2006, Barnhart et al. 2008, Chumnanouen et al. 2011, Fritts et al. 2013).

Schierholz (1889) first described the glochidia of *Pseudanodonta*, and for a long time, authors used this description with some small additions (e.g. Ortmann 1912, Israel 1913). In Russia, a first very brief description of *Pseudanodonta* glochidia was made using light microscopy (Shadin 1938). Later, the description was updated to include observations using both light (Antonova 1987, Antonova & Starobogatov 1988) and scanning electron microscopy (Antonova & Starobogatov 1989, Sayenko 2006) of *Pseudanodonta* larvae from the Volga River delta (Caspian Sea drainage) and ponds of the Curonian Spit (Baltic Sea drainage). The first detailed scanning electron microscope studies of *P. complanata* glochidia from Germany were conducted by Kinzelbach and Nagel (1986) using specimens from the north of that country.

The purpose of this work is to study *P. complanata* larvae from some previously unexplored localities in Germany and the European part of Russia; to clarify the characters based on new and previously known morphological data as a contribution to the taxonomy of these freshwater bivalves, and to confirm new localities of the species in European Russia.

Materials and Methods

The samples have been deposited at the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia (**FSCEATB FEB RAS**) and in the malacology collection of the Senckenberg Research Institute Frankfurt, Frankfurt am Main, Germany (**SMF**).

The following samples of glochidia were investigated:

1) Chaika Lake basin, Curonian Split, Kaliningrad Oblast, Russia, 17.x.1982, Ya.I. Starobogatov leg., FSCEATB FEB RAS.
2) Velikaya river at the outlet of Khvoyno lake (Narva river basin), Shchukinskaya volost, Pustoshkinsky District, Pskov Oblast, Russia (56° 33′ 20.5″ N, 029° 30′ 49.9″ E), 22.x.2016; D.M. Palatov leg.; FSCEATB FEB RAS BALT86.
3) Derkul river (a tributary of the Seversky Donets River), Rostov Oblast, Russia, 23.vii.1895, Kalashnikov leg., FSCEATB FEB RAS.
4) Kinzig river (a tributary of Main river) near Hanau, Hessen, Germany (50° 07.7′ N, 08° 57.0′ E), 27.iv.1923, A. Seidler leg.; SMF 3192/5.
5) Neckar river near Heidelberg, Baden-Württemberg, Germany (49° 24.7′ N, 008° 40.0′ E), 27.i.1910, F. Haas leg.; SMF 346006 (glochidia), SMF 3156/9 (soft bodies only).

The identification of adult bivalves, including gravid specimens, was based on the key shell characters described by Haas (1969) and Starobogatov et al. (2004). The shells of animals from which mature glochidia were taken were photographed using a digital camera (Fig. 1A, B, H, I, L). In addition, specimens of mussels in the SMF from which the morphology of glochidia had been previously studied were photographed (Fig. 1C–G, J, K, M):

- Eider river near Hohenhude, Schleswig-Holstein, Germany (54° 17′ 24″ N, 009° 59′ 41.6″ E), 23.iii.1986, K.-O. Nagel leg.; SMF 346335/3 (3 shells and separate soft bodies, 1 soft body without shell).
- Schierenseebach stream, Schleswig-Holstein, Germany (54° 16′ 54.0″ N, 009° 58′ 29.3″ E), 19.ii.1984, K.-O. Nagel & G. Roth leg.; SMF 346374/1 (1 shell and separate soft body, 1 soft body shell in Henri Caillot-Girardi collection, Montfavet), 353847/5 (shells only).
- Schwalm river, Otto mill near Bad Zwesten, Hessen, Germany (51° 03′ 22.5″ N, 009° 11′ 49.4″ E), 19.iii.1986, K.-O. Nagel leg.; SMF 354303/5 + ½ (shells only).
- Altmühl river shortly downstream of Solnhofen, Bavaria, Germany, 15.iv.1912, F. Haas & E. Schwarz leg.; SMF 369723/20 (shells only) and SMF 3045/3 (soft bodies only).
- Regen river at Zeitlarn, Bavaria, Germany (49° 04′ 22.0″ N, 012° 06′ 00.5″ E), 12.iv.1985, K.-O. Nagel leg.; SMF 346375/1 (1 shell and separate soft body).

Glochidia samples 1–3 and 5 were taken from the fresh marsupial gills initially preserved in 75% ethanol. The original type of fixation is not known for sample 4, presumably the complete animals (opened shell

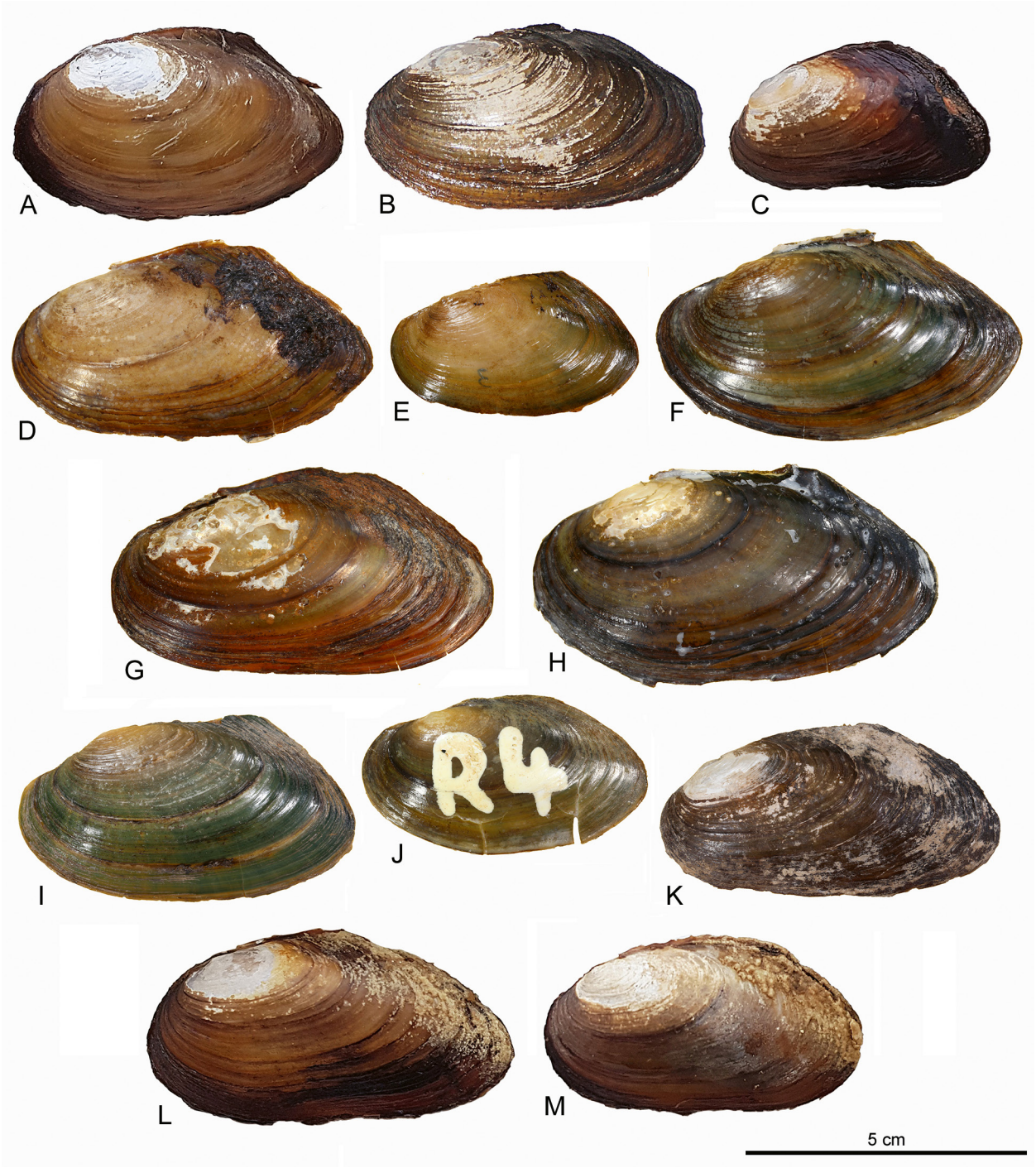

Figure 1. Adult shells of *Pseudanodonta complanata*. **A.** From basin of Chaika Lake, Curonian Split, Kaliningrad Oblast (specimen with glochidia). **B, C.** From Velikaya river, Pskov Oblast: (**B**) specimen with glochidia. **D, E.** From Schierenseebach river, Schleswig-Holstein (SMF 346374/1, SMF 353847/5, specimens with glochidia). **F.** From Eider river, Schleswig-Holstein (SMF 346335/3, specimen with glochidia). **G.** From Kinzig river, Hessen (SMF 6304/3). **H.** From Schwalm River, Hessen (SMF 354303/5+1/2). **I.** From Neckar river, Baden-Württemberg (SMF 342844/5). **J.** From Regen river, Bavaria (SMF 346375/1). **K.** From Altmühl river, Bavaria (369723/20). **L, M.** From Derkul river, Rostov Oblast: (**L**) specimen with glochidia.

with soft body or only the detached soft bodies) were transferred directly into alcohol. To prevent deformation or destruction of the hooks, only chemical cleaning was used. Before chemical cleaning, the glochidia were washed several times in distilled water and cleaned of tissue in a 5% KOH solution. During this procedure, the glochidia were examined under a light microscope (LM) every half an hour. The cleaned shells were washed several times in distilled water and an alcohol series (80, 90, and 96%), after which they were mounted as permanent slides for LM. To avoid the crushing of glochidia by the application of the coverslip during their mounting on LM permanent slides, each group of larvae was surrounded by a separate hair. For samples subjected to

scanning electron microscopy (SEM), glochidia were sputter coated with chromium or gold immediately after drying and mounted on a stub to prevent the deformation of the hooks and outer shell layer.

The description of the main conchological characters of glochidia shells follows Hoggarth (1999) and Sayenko (2016). The following characters were measured: valve length (**L**); l valve height (**H**); ligament length (**lig**); hook length (**h**) (Fig. 2). Measurements were made on at least 20 glochidia (if available and suitable) per adult female by LM (Nikon) with an ocular reticle and directly from SEM images (Zeiss EVO 40, Zeiss MERLIN, and Hitachi TM4000Plus II). For the LM, both temporary water–glycerin and permanent glycerin–gelatin preparations were used. Three ratios (H/L, lig/L, and h/H) were calculated.

The microsculpture of each larval shell was examined in at least 3 points: close to the ventral tip with the hook, at the centre of the valve in the region of the adductor muscle, and at the valve edge near the ligament (Fig. 2).

The photographs of glochidia were taken at the Biology and Genetic Engineering Center for Collective Use of the FSCEATB FEB RAS (Russia) and at the Senckenberg Research Institute Frankfurt (Germany).

Results and Discussion

Pseudanodonta complanata is a long-term winter brooder, holding larvae during autumn and winter and releasing glochidia in spring or summer (Hüby & Wächtler 1989). In river basins of Germany and Russia, mature glochidia of *P. complanata* were recorded from July to late April (Table 1). Depending on the conditions (primarily water temperature), maturation of larvae and spawning may vary even at the same place. However, in comparison with *Anodonta anatina* (Linnaeus, 1758), the timing of maturation of the glochidia of *P. complanata* is more variable (Pekkarinen & Englund 1995), and a short non-gravid interval was shown to be in March–May in Austria (Schierholz 1889), in June in Finland (Pekkarinen 1993), in May–July in the United Kingdom (Aldridge 1999, McIvor & Aldridge 2007), and in July–August in Russia (Shadin 1952).

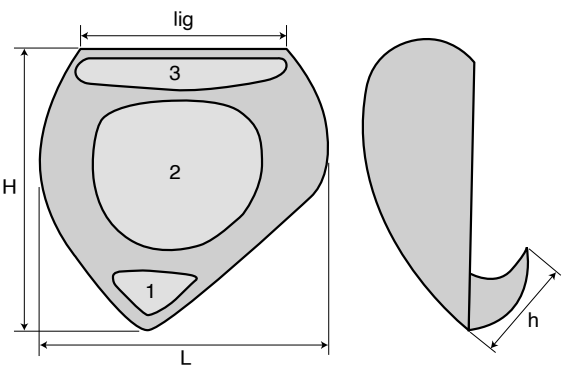

Figure 2. Schematic diagram of measurements of glochidia taken in this study and valve regions examined by SEM for outer microsculpture. L = valve length, H = valve height, lig = ligament length, h = hook length, 1 = ventral edge of glochidial valve, 2 = centre of valve near adductor muscle, 3 = valve margin near ligament.

Mature glochidia of *P. complanata* are hooked, triangular, with dorsally elongated shells (Fig. 3), and the shell length is always greater than height (Table 1). By the size of the larvae and by using the index GIn (glochidium shell length / shell height in mm), Davis & Fuller (1981) divided glochidia into 3 types: small (<0.036), medium (c. 0.050), and large (c. 0.100). Based on this approach, mature glochidia of *P. complanata* are large, not less than 280 µm high and up to 390 µm long (Table 1). A large, single, styliform hook (to 160 µm) at the ventral tip of each valve is covered by lanceolate microstylets (to 20 µm) (Fig. 4).

The conchological characteristics of the larvae are quite variable. Mature glochidia vary in size not only among different localities but also among different individuals collected at the same time at the same locality (Table 1). Indices, as well as absolute sizes, are also variable. H/L ranges from 0.83 to 0.88, and lig/L ranges from 0.73 to 0.78. The relative sizes of the hooks are approximately half the valve height (Table 1).

The larvae of *P. complanata* and of the closely related *Anodonta* species belong to the anodontine type and have similar shapes and sizes. However, glochidia of *P. complanata* are characterized by being broad and having slightly protruding anterior and posterior margins (Fig. 3F–H, M). This glochidial shell outline is not immediately formed, so immature glochidia are more rounded-triangular without a strongly pronounced margin (Fig. 3N, O). Margins of mature glochidia are covered with distinct raised lines, running parallel to the valve edge (Fig. 5A). While the thickness of the glochidial shell margin in *P. complanata* is not less than 40 µm (Kinzelbach & Nagel 1986, Sayenko 2006, our data), in *Anodonta* the thickness of the shell margin does not exceed 30 µm (Sayenko 2006). Raised lines on glochidial valve margins are either absent or they are fewer and less visible in *Anodonta* larvae.

In contrast to variable conchological features, external shell microsculpture in mature shells of *P. complanata* glochidia is a consistent character (Fig. 5B–H). The external sculpture consists of loosely looped folds, with the loops loose near the ventral tips of the valves, chaotic multilayered loops along the anterior and posterior valves edges and at the ligament, and with variously sized loops in the centre of the valves. The thickness of the folds is 0.034–0.067 µm.

The inner layer of the glochidial valves has continuous pores (Fig. 5I), with each pore 0.04–1.89 µm in diameter.

Our study, which is based on materials originally fixed in different ways, collected in different places, and

Table 1. Glochidia dimensions (mean values in µm) of *Pseudanodonta complanata*: published and our new data. Abbreviations: *N* = number of gravid mussels from which glochidia were examined; *n* = number of glochidia measured; L = length of glochidial shell; H = height of glochidial shell; lig = length of ligament; h = length of hook; dash means no data.

Catchment (N–S, W–E)	Locality	Sampling date	*N*	*n*	L	H	lig	h	H/L	lig/L	h/H	Source*
Lake Kirmustenjärvi (Baltic Sea)	parish of Sammatti, Uusimaa region, Southern Finland	1991–1992	—	—	390	331	—	—	0.85	—	—	Pekkarinen & Englund 1995
Vantaa River (Baltic Sea)	Vantaa River in Helsinki, Southern Finland	1988–1992	—	—	367	313	—	—	0.85	—	—	Pekkarinen & Englund 1995
Curonian Lagoon (Baltic Sea)	Chaika Lake basin, Curonian Split, Kaliningrad Oblast, Russia	17.X.1982	1	26	377	329	292	150	0.88	0.78	0.46	Sayenko 2006; new data
Narva River (Baltic Sea)	Velikaya River, Pskov Oblast, Russia	22.X.2016	1	50	354	304	273	137	0.86	0.77	0.45	New data
Eider River (North Sea)	Schierenseebach stream, Schleswig-Holstein, Germany	19.II.1984	1	30	341	288	—	132	0.84	—	0.46	Nagel 1984, 1988, Kinzelbach & Nagel 1986 (collection numbers SMF 346374/1, 353847/5)
			1	20	349	295	—	—	0.85	—	—	
	Eider River, Schleswig-Holstein, Germany	23.III.1986	1	50	343	295	—	—	0.86	—	—	Nagel 1988 (collection number SMF 346335/3)
			1	17	358	308	—	—	0.86	—	—	
	Schierenseebach stream and Eider River, Schleswig-Holstein, Germany	1986–1987	—	—	340	290	—	—	0.85	—	—	Hüby 1988
Weser River (North Sea)	Schwalm River, Hessen, Germany	19.III.1986	1	50	320	282	—	—	0.88	—	—	Nagel 1988 (collection number SMF 354303/5+1/2)
Rhine River (North Sea)	Kinzig River, Hessen, Germany	27.IV.1923	1	1	330	282	241	—	0.85	0.73	—	new data (collection number SMF 3192)
	Neckar River, Baden-Württemberg, Germany	27.I.1910	1	8	342	292	265	—	0.85	0.78	—	new data (collection number SMF 346006)
Danube River (Black Sea)	Regen River, Bavaria, Germany	12.IV.1985	1	50	367	314	—	—	0.86	—	—	Nagel 1988 (collection number SMF 346375/1, 353816/4)
			1	50	366	312	—	—	0.85	—	—	
			1	50	340	296	—	—	0.87	—	—	
	Altmühl River, Bavaria, Germany	15.IV.1912	1	50	383	319	—	—	0.83	—	—	Nagel 1988 (collection number SMF 3045/3)
Don River (Sea of Azov)	Derkul River (a tributary of Seversky Donets River), Rostov Oblast, Russia	23.VII.1895	1	15	345	300	261	135	0.87	0.76	0.45	Sayenko 2006; new data
Volga River (Caspian Sea)	Delta of Volga River, Astrakhan Oblast, Russia	1985	1	—	360	300	—	160	0.83	—	0.53	Antonova 1987

*For samples from the Malacology Collection of the Senckenberg Research Institute Frankfurt, Frankfurt am Main (Germany), the collection numbers are given.

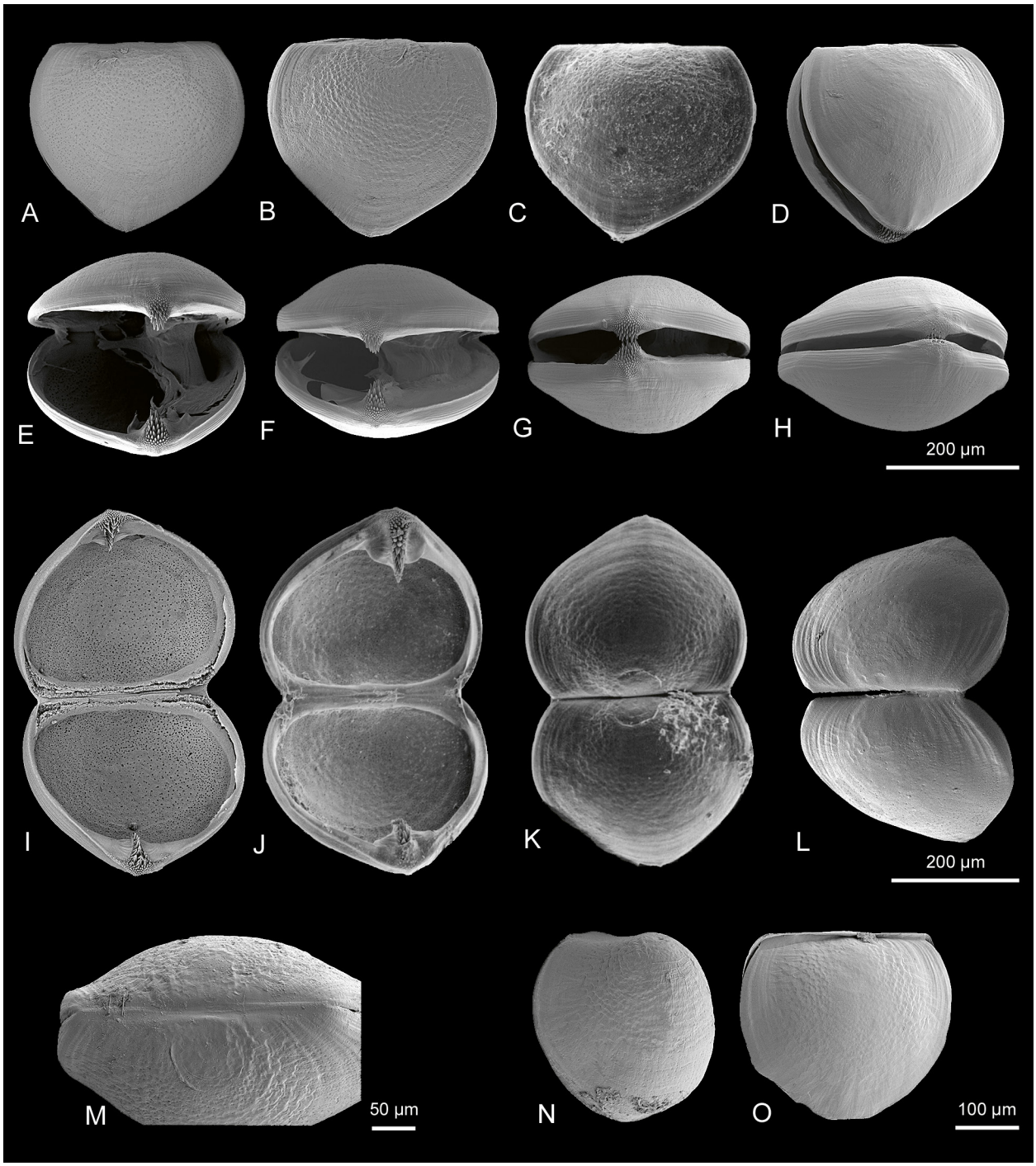

Figure 3. Glochidial shells of *Pseudanodonta complanata* at different views. **A–H.** Mature glochidia with closed and slightly open valves: (**A–D**) in lateral view showing outline of the valves; (**E–H**) showing hooks. **I–L.** Mature glochidia with valves open: (**I, J**) interior; (**K, L**) exterior. **M.** Hinge and umbo of mature glochidium. **N, O.** Immature glochidial shells. From Kinzig river (A); Neckar river (I); basin of Chaika Lake (B, C, J, K, M, N); Velikaya river (D–G, H, L); Derkul river (O).

stored in different conditions at 2 museums, shows comparable results among these samples (Table 1) and with previously published data (Nagel 1984; Kinzelbach & Nagel 1986; Sayenko 2006). Although sizes of the glochidial shells vary quite widely, their indices are within a fairly narrow range of values (Table 1). We also investigated the external microsculpture of glochidial shells using samples prepared differently, using both SEM and LM (under different microscopes). For the first time, the variability of the external microsculpture of larval shells was studied using SEM, which showed consistent microsculpture among samples (Fig. 5) and confirm the possibility of using glochidial shell microsculpture in future investigations.

In contrast to low genetic diversity of *P. complanata* over a wide area from the basin of the Volga to the Oise river in France, populations in the basin of the Don form a separate genetic lineage (Vikhrev et al. 2023).

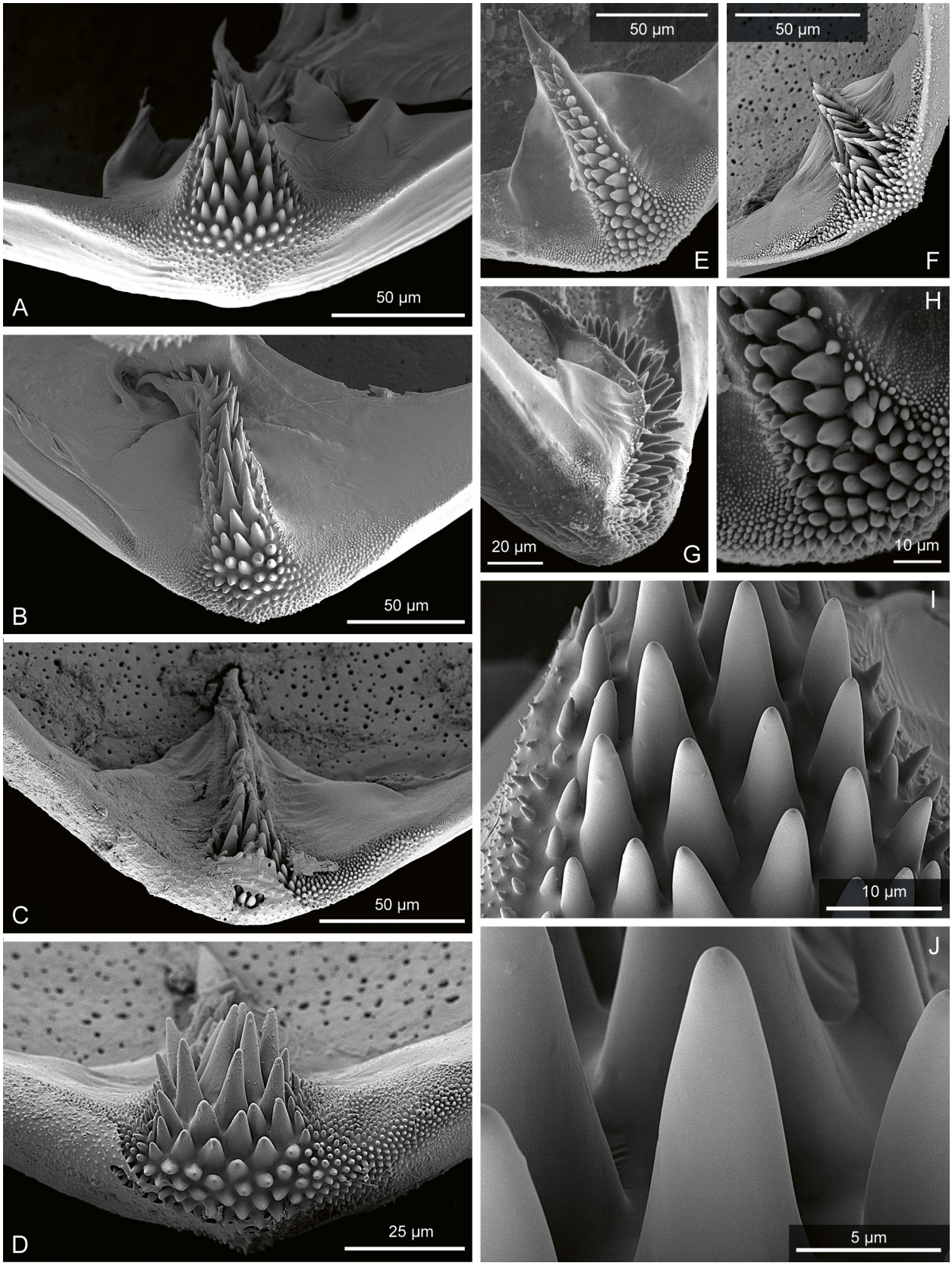

Figure 4. Hooks and spines of *Pseudanodonta complanata* glochidia. **A, B, I.** From Velikaya river. **C–E, G, H.** From basin of Chaika Lake. **F.** From Neckar river.

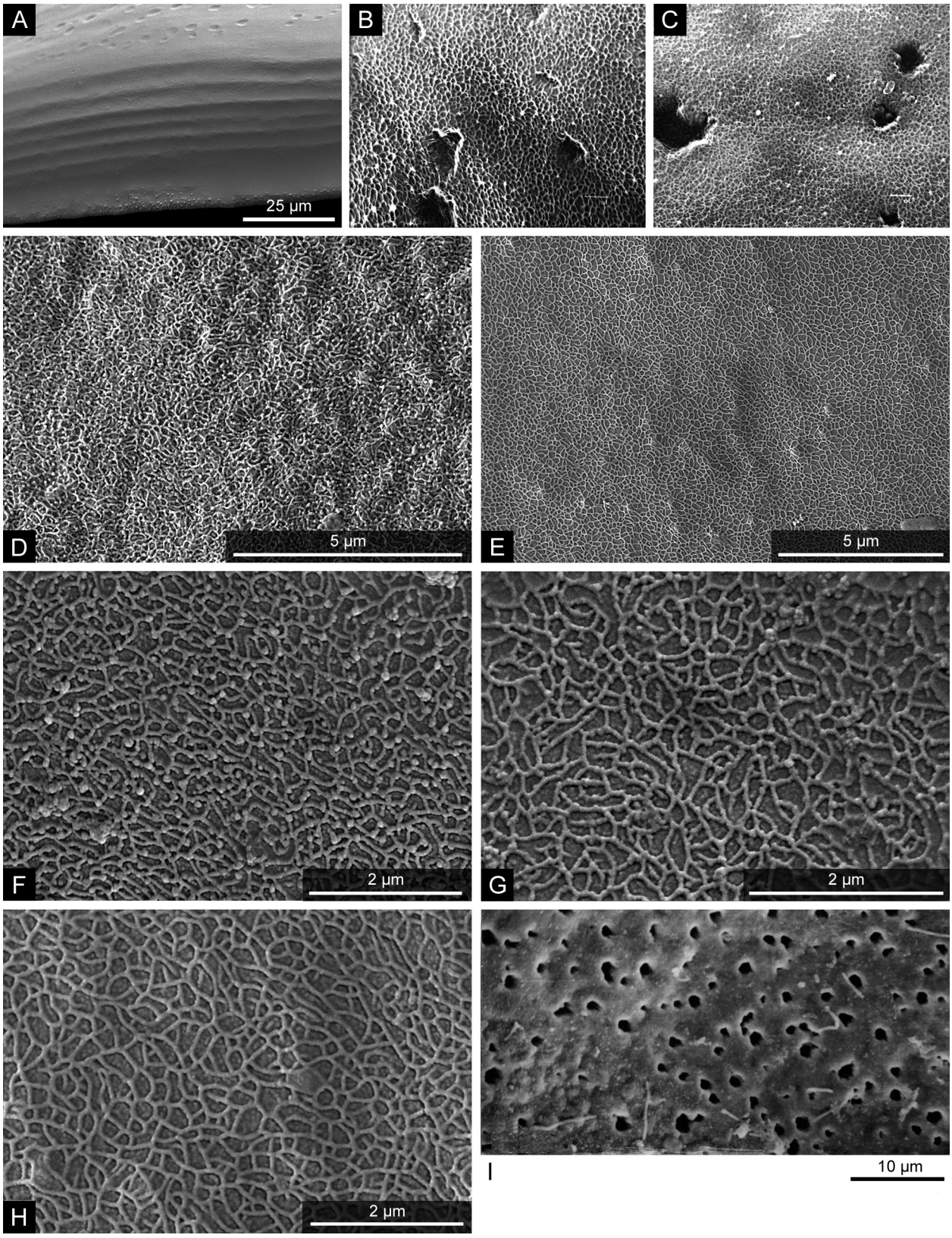

Figure 5. Shells surface of *Pseudanodonta complanata* mature glochidia. **A.** Shell edge with comarginal sculptured lines, exterior valve surface. **B–H.** Exterior microsculpture in different parts of the valves and at different magnifications: (**D, E**) close to the ventral edge; (**B, C, G**) at the centre; (**F, H**) at the valve edge near the ligament. **I.** Inner surface of the shell with pores. From Velikaya river (A, E, H); Derkul river (D); Regen river (B, C); basin of Chaika Lake (F, G, I).

Our data on glochidium morphology, both conchological features and external microsculpture, did not show differences between populations, including those of the Don river basin. Larvae are practically indistinguishable, although adult shells are quite variable. However, many watersheds in the south of the Russian Plain have yet to be studied, and data on *Pseudanodonta* localities are very limited. Further studies are needed to better understand the importance of the Russian Plain for the genetic and morphological variability of the species.

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The authors declare that there are no conflicts of interest.

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