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Glochidial morphology of selected species of the genera *Cristaria* Schumacher, 1817 and *Sinanodonta* Modell, 1945 (Bivalvia: Unionidae) from Far Eastern Russia

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Abstract: Light and scanning electron microscopy (SEM) revealed similarities and differences in the glochidia of four species belonging to two unionid genera from Primorye Territory in Far Eastern Russia: *Cristaria herculea, Cristaria tuberculata, Sinanodonta amurensis,* and *Sinanodonta likharevi*. Glochidia of the two species of *Cristaria* Schumacher, 1817 differed from each other in morphological details of the hook (hook length, maximum height of hook microstylets) and hinge, but not in shape (height/length ratio) or size. Glochidia of the two species of *Sinanodonta* Modell, 1945 differed from each other in quantitative features such as height, length of hinge, and shape (height/length ratio). Glochidia of these two species of *Sinanodonta* lacked some characters possessed by other genera in the tribe Anodontini, in which *Sinanodonta* has been classified, and shared some characters with the two species of *Cristaria,* which are classified in the tribe Limnoscaphini, raising uncertainty about the correct classification of *Sinanodonta*.

Key words: Anodontini, *Cristaria herculea, Cristaria tuberculata,* Glochidia, Limnoscaphini, *Sinanodonta amurensis, Sinanodonta likharevi*

The adult shells of many unionid bivalves provide little information for reliable identification at the specific, generic, or even subfamilial level. The larvae (glochidia), however, are highly specialized because of their complex lifecycles and can provide characters that are useful for distinguishing among species and higher taxa. The shape, size, dentition, exterior sculpturing, and pit size of the glochidia all contribute useful taxonomic information (Hoggarth 1999).

There is disagreement about the number of species in the genus *Cristaria* Schumacher, 1817 (tribe Limnoscaphini Lindholm, 1932). Different authors include three (Haas 1969), four (Inaba 1964), or six (Moskvicheva 1973) species. Although Haas (1969) and Brandt (1974) considered both *Cristaria herculea* (Middendorff, 1847) and *Cristaria tuberculata* Schumacher, 1817 to be junior synonyms of *Cristaria plicata* (Leach, 1815), Russian malacologists have recognized *C. herculea* and *C. tuberculata* as full species for many years (Zatravkin and Bogatov 1987).

Forms identified as *Cristaria herculea* and *Cristaria tuberculata* inhabit the Amur River basin and Khanka Lake in eastern Russia. Shadin (1938) made the first short note on the glochidium of *C. herculea*. Antonova and Starobogatov (1988) reported on the glochidium of *C. tuberculata,* but their illustrations lack sufficient detail to be useful and their text measurements differ from those in the illustrations.

We studied the glochidia of *Cristaria herculea* and *Cristaria tuberculata* to see if glochidial characters could provide useful characters for addressing the species status of these two *Cristaria* species.

The species-rich Asian genus *Sinanodonta* Modell, 1944 has traditionally been classified in the tribe Anodontini Rafinesque, 1820, which also includes *Anemina*-group bivalves in the genera *Anemina* Haas, 1969, *Amuranodonta* Moskvicheva, 1973, and *Buldowskia* Moskvicheva, 1973. Shadin (1938) gave a preliminary description of the glochidium of *Sinanodonta woodiana* (Lea, 1834) (as *Anodonta woodiana*) without any illustrations. Inaba (1941, 1964) gave descriptions and schematic illustrations using light microscopy of the glochidia of *S. woodiana* (as *A. woodiana lauta* Martens, 1877 and *A. woodiana lauta tumens* Haas, 1910) and *Sinanodonta calipygos* (Kobelt, 1879) (as *A. woodiana calipygos*). Antonova and Starobogatov (1988) illustrated the glochidia of bivalves they tentatively identified as *Sinanodonta amurensis* Moskvicheva, 1973 using light microscopy, but again, their text measurements differed from those in the illustrations. Scanning electron microscopic studies on glochidia of *S. woodiana* (as *A. woodiana*) from Korea focused on the fine structure of the glochidia (Jeong 1989, Lee *et al.* 1989, Jeong *et al.* 1993, Kwon *et al.* 1993, Park and Kwon 1993). These studies compared the glochidia of species of *Sinanodonta* with those of species of *Anemina* and documented that glochidial features vary. Further examination of the glochidia of members of the genus *Sinanodonta,* especially of species that have not been examined

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previously, would improve our knowledge of this group of bivalves.

MATERIALS AND METHODS

Source and deposition of specimens

Glochidia were obtained from gravid females of *Cristaria herculea* (Primorye, Khanka Lake near Kamen'-Rybolov village; n = 1; 10 October 1996; collectors T.S. Vshivkova, T.V. Nikulina); *C. tuberculata* (Primorye, Khanka Lake near Vostochnyi kordon; n = 1; 28 October 1999; collector L.A. Prozorova); *Sinanodonta amurensis* (Primorye, Razdolnaya River; n = 1; 21 June 1999; collector L.A. Prozorova), and *S. likharevi* Moskvicheva, 1973 (Primorye, Ilistaya River basin near Sibirtsevo village; n = 1; 19 May 1996; collector V.A. Dvoryadkin). Sample sizes of adults were small because it was very difficult to find gravid females with mature glochidia. We assumed the developmental stages of all glochidia were the same across the species and could be compared directly because female unionids of the subfamily Anodontinae hold mature glochidia in their gills all winter, during which time the glochidia do not change appreciably in size (Shadin 1938, Antonova 1991, Chernyshev 1998).

Specimens of adult bivalves and glochidial samples were deposited at the Russian Academy of Sciences, Far Eastern Branch, Institute of Biology and Soil Sciences, Vladivostok, Russia. Samples of glochidia of three of the species were also deposited at the Delaware Museum of Natural History, Wilmington, Delaware, USA: *Cristaria herculea* (DMNH 221699), *Cristaria tuberculata* (DMNH 221700), and *Sinanodonta amurensis* (DMNH 221701).

Examining glochidia

Mature glochidia were recovered from the demibranchs of gravid females and fixed in 75% ethanol for investigation with both light and scanning electron microscopy (SEM). To extract the specimens from the gill tissue, we washed ethanol-fixed glochidia in three changes of distilled water, cleaned them in 5% KOH for 1.5-2 hours, and washed them at least five times with distilled water (following Kwon *et al.* 1993).

Measurements to be used in statistical comparisons were made using light microscopy on at least 20 glochidia per adult female, but not all measurements could be made on every glochidium. Both temporary water-glycerin and permanent glycerin-gelatin preparations were used for this purpose. We measured valve length parallel to the hinge (length) (n = 20-27 per species), valve height perpendicular to the hinge (height) (n = 15-22), length of hinge (hinge) (n = 20-22), and length of the hook (hook) (n = 5-19; no measurements of hook were made on *Sinanodonta likharevi*). We made these measurements using an Olympus light microscope fitted with an ocular reticle. For terminology of glochidia see Kondo and Yamashita (1980); regarding anterior-posterior orientation of glochidia see Hoggarth (1987). To standardize the measurements, we calculated three ratios: height/length, hinge/length, and hook/height.

Morphological details of the hooks and pits of the glochidia of *Cristaria herculea, Cristaria tuberculata,* and *Sinanodonta amurensis* were examined using SEM. The SEM details were used for qualitative descriptions. Glochidia of *Sinanodonta likharevi* were not available for SEM work. We made one SEM photomicrograph for each of seven views for each of the three species, sometimes photographing a different glochidium on the SEM stub for different views. We made images of glochidium shape, hinge, pits on the inner valve surface, pits on the outer valve surface, hook viewed perpendicular to the plane of the valve commissure, close-up of hook base, and hook in side view.

To prepare specimens for SEM, we pipetted glochidia to a 2-ml polyethylene centrifuge tube and shook them vigorously in distilled water for 30 seconds to separate soft parts from valves (Kinzelbach and Nagel 1986, Kwon *et al.* 1993). After the glochidial valves settled for 20-30 seconds, the liquid containing suspended soft parts was siphoned with a pipette. The shaking, settling, and siphoning procedure was repeated twice with distilled water; we used 95% ethanol in place of distilled water in the fourth and final iteration to hasten glochidia desiccation. Moist glochidia were removed from the centrifuge tube with a fine wooden pick and placed on double-sided carbon tape on a SEM stub. Using a Denton Vacuum Desk II sputter coater (Cherry Hill, New Jersey) we coated the glochidia with gold-palladium about 100 Å thick. A Topcon ABT-60 Scanning Electron Microscope (Topcon Technologies, Inc., 69406 Koll Center Parkway, Pleasanton, California 94566, USA) with a 10 kV electron beam was used to view the glochidia.

Intra- *vs.* interspecific glochidial variation: Glochidia from one female per species

We examined multiple glochidia from a single female from each species because additional gravid females were unavailable. However, if multiple glochidia from a single female do not adequately represent glochidial variation from the whole species, then interspecific comparisons using characters of those glochidia could give misleading conclusions. Furthermore, if variation of glochidia from different females of a single species were greater than the variation among females of different species, then glochidial features would not be reliable for discriminating among species. To address these concerns, we examined a published dataset to compare intraspecific variation in glochidia to interspecific variation to test whether glochidia from a single female could be used for discriminating among species.

We used data from Hoggarth (1999, table 2) to compare the morphological variation of glochidia within a single female to that between females of the same species and to the variation of glochidia among females of different species. We included species from the Anodontinae only because our species are members of that group. For 10 species in the Anodontinae, Hoggarth had measured glochidial characters from more than one female per species (with at least 2 glochidia per female). The species and the numbers of glochidia measured from each of two females are *Anodonta anatina* (Linnaeus, 1758) (5 glochidia, 2 glochidia), *Anodonta kennerlyi* Lea, 1860 (4, 2), *Pyganodon grandis grandis* (Say, 1829) (4, 3), *Pyganodon cataracta cataracta* (Say, 1817) (3, 2), *Utterbackia imbecillis* (Say, 1829) (8, 3), *Anodontoides ferussacianus* (Lea, 1834) (3, 4), *Lasmigona compressa* (Lea, 1829) (2, 2), *Lasmigona subviridis* (Conrad, 1835) (3, 2), *Lasmigona holstonia* (Lea, 1838) (3, 2), and *Lasmigona costata* (Rafinesque, 1820) (4, 3). We compared inter-female to inter-species variation for glochidial measurements of length, height, and hinge length using ANOVA for those 10 species.

While glochidia measurements differed significantly among species (length, height, hinge length all $p < 0.0001$), they did not differ significantly among females (length, $p = 0.454$; height, $p = 0.745$; hinge length, $p = 0.212$).

The results of this analysis of data from Hoggarth (1999) found greater interspecific than intraspecific variation in glochidia for these 10 species of Anodontinae. Furthermore, glochidia did not differ significantly between females of the same species. Using characters of the glochidia, we were not likely to misclassify conspecific females of these 10 species as different species.

If we could extrapolate this result to members of the Anodontinae from Far Eastern Russia, we should be able to determine whether single individual females are members of the same species by comparing their glochidia. In contrast, some Far Eastern Russian species of Anodontinae in the *Anemina* group (*Anemina* spp., *Amuranodonta* spp., *Buldowskia* spp.) do show large variation in their glochidial measurements among populations or even among individual females from the same population (Sayenko 1999b, 2000, 2003). However, species of other Far Eastern Russian anodonine genera that are more similar to species of *Cristaria*, including *Beringiana* Starobogatov in Zatravkin, 1983, do not show much intraspecific variation (Sayenko *et al*. 2001). Detailed investigations have not yet been made for glochidia of species in the genera *Cristaria* and *Sinanodonta*, but because these genera appear to be more closely related to *Beringiana* than to species in the *Anemina* group, we expect that their glochidia would not show much intraspecific variation. Therefore, finding statistically significant differences in glochidia from different females of *Cristaria* spp. and *Sinanodonta* spp. would be evidence supporting the idea that the females belong to different species.

Data analysis

For each species, we performed single-factor analysis of variance (ANOVA) on each glochidial character using SYSTAT 10 (SPSS Inc., 233 South Wacker Drive, 11th floor, Chicago, Illinois, 60606, USA). We used the least significance difference (LSD) post-hoc test to evaluate significance among the four species.

RESULTS

***Cristaria* species**

Cristaria herculea (Figs. 1A, 2A, 3A-B, 4A-B, 5). The valves of the glochidia of *C. herculea* were distinctly asymmetric because the ventral (hooked) edge of each valve was displaced posteriorly and because the anterior edge was longer and had a more prominent curve (Fig. 1A). Valve length (parallel to hinge) ranged from 271-285 μm (mean 274.2, st. dev. 4.1 μm) and valve height (perpendicular to hinge) ranged from 271-293 μm (mean 281.2, st. dev. 4.9 μm). Valve height was greater than valve length with the height/length ratio ranging from 1.01-1.05 (mean 1.032, st. dev. 0.013). Hinge length (Fig. 2A) was 193-207 μm (mean 199.9, st. dev. 4.5 μm), being 70-76% of valve length (mean 73.0, st. dev. 1.5%). The hook ranged from 100-114 μm (mean 106.2, st. dev. 4.9 μm), being from 36-41% of the valve height (mean 37.7, st. dev. 1.8%). The glochidium examined by SEM had approximately 20 large microstylets arranged on the hook in 2-3 poorly defined longitudinal rows (Fig. 3A-B). Maximum height of the microstylets on the hook was 12.7 μm. The pits on the inner valve surface (0.5-0.6 μm diameter) tended to be smaller than those on the outer surface (0.5-1.2 μm diameter) (Figs. 4A-B) and pits were situated on the entire valve including the adductor muscle attachment site.

Cristaria tuberculata (Figs. 2B, 3C-D, 4C-D, 5). The valves of *C. tuberculata* glochidia were distinctly asymmetric because the ventral (hooked) edge of each valve was displaced posteriorly and because the anterior edge was longer and had a more prominent curve (Fig. 2B). Valve length (parallel to hinge) ranged from 270-286 μm (mean 276.7, st. dev. 6.3 μm) and valve height (perpendicular to hinge) ranged from 271-300 μm (mean 284.4, st. dev. 7.5 μm). Valve height was greater than valve length with the height/length ratio ranging from 1.03-1.06 (mean 1.036, st. dev. 0.010). Hinge length (Fig. 2B) was 207-221 μm (mean 213.5, st. dev. 5.5 μm), being 75-80% of valve length (mean 77.0, st.

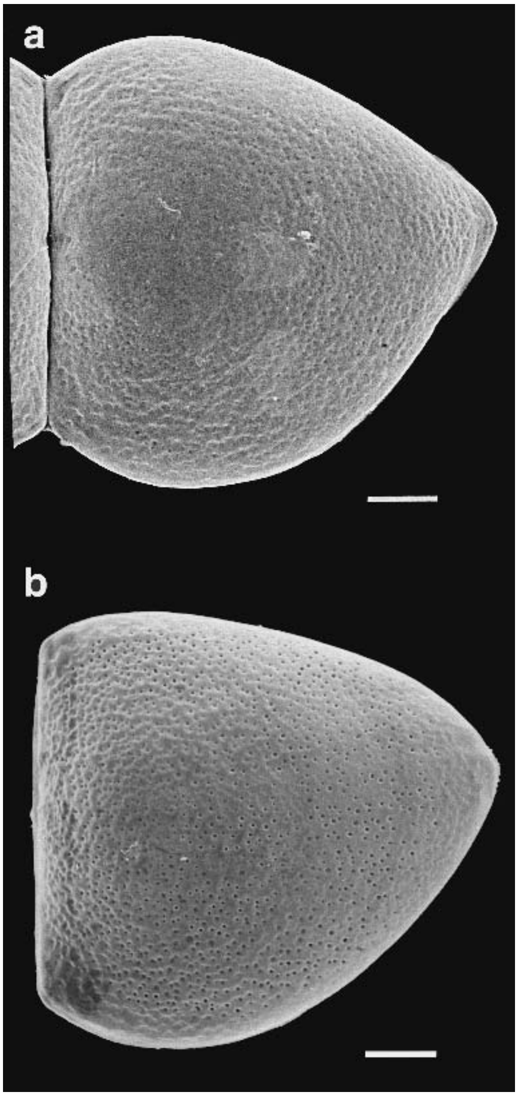

Figure 1. Scanning electron micrographs showing the shapes of the valves of the glochidia of A, *Cristaria herculea,* scale bar = 35 µm and B, *Sinanodonta amurensis,* scale bar = 40 µm.

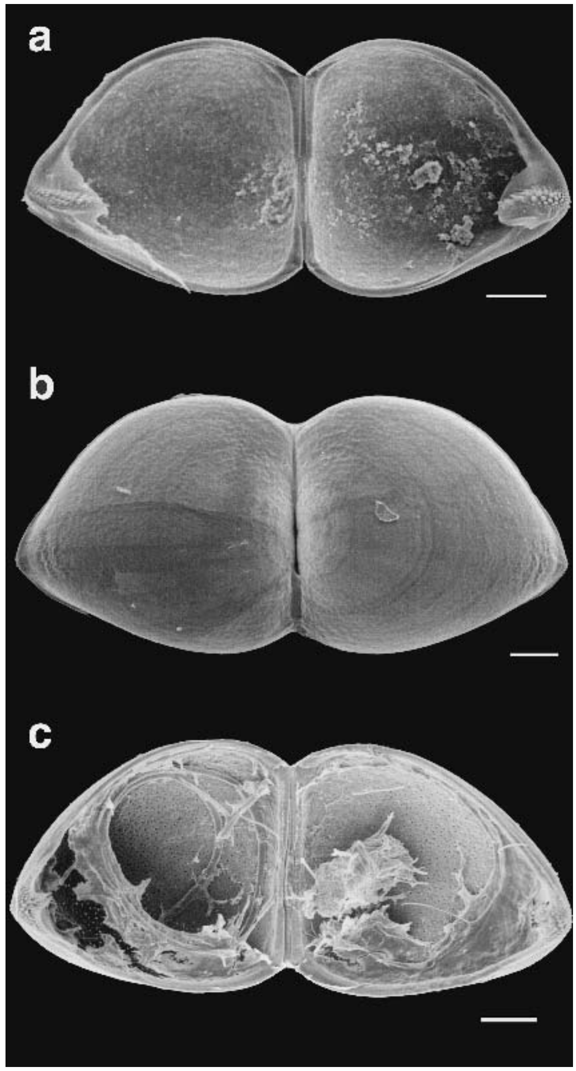

Figure 2. Scanning electron micrographs illustrating the hinge lengths of the glochidia of A, *Cristaria herculea* (internal view); B, *Cristaria tuberculata* (external view); and C, *Sinanodonta amurensis* (internal view). All scale bars = 50 µm.

dev. 1.7%). The hook ranged from 106-114 µm (mean 111.3, st. dev. 3.4 µm), being from 38-42% of the valve height (mean 39.9, st. dev. 1.8%). The glochidium examined by SEM had about 17 large microstylets arranged on the hook in 1-2 poorly defined longitudinal rows (Fig. 3C-D). Maximum height of the microstylets on the hook was 9.1 µm. The pits on the inner surface (0.5-1.2 µm diameter) tended to be slightly smaller than those on the outer surface (0.6-1.5 µm diameter) (Fig. 4C-D), although there was considerable overlap in pit diameters. Pits were situated on the entire valve including the site of adductor muscle attachment.

Similarities in the glochidia of *Cristaria herculea* and *Cristaria tuberculata* included valve shapes, both species having distinctly asymmetric valves with the anterior edge longer and with a more prominent curve (Figs. 1A, 2B). Glochidial valves of the two species did not differ significantly in size or shape (LSD post hoc tests after ANOVA for length, $p = 0.209$; height, $p = 0.131$; height/length, $p = 0.535$) (Fig. 5A-C). In one glochidium measured of each species there was overlap in the pit diameters on the internal surfaces of the glochidial valves of the two species (0.5-0.6 versus 0.5-1.2 µm, *C. herculea* and *C. tuberculata,* respectively)

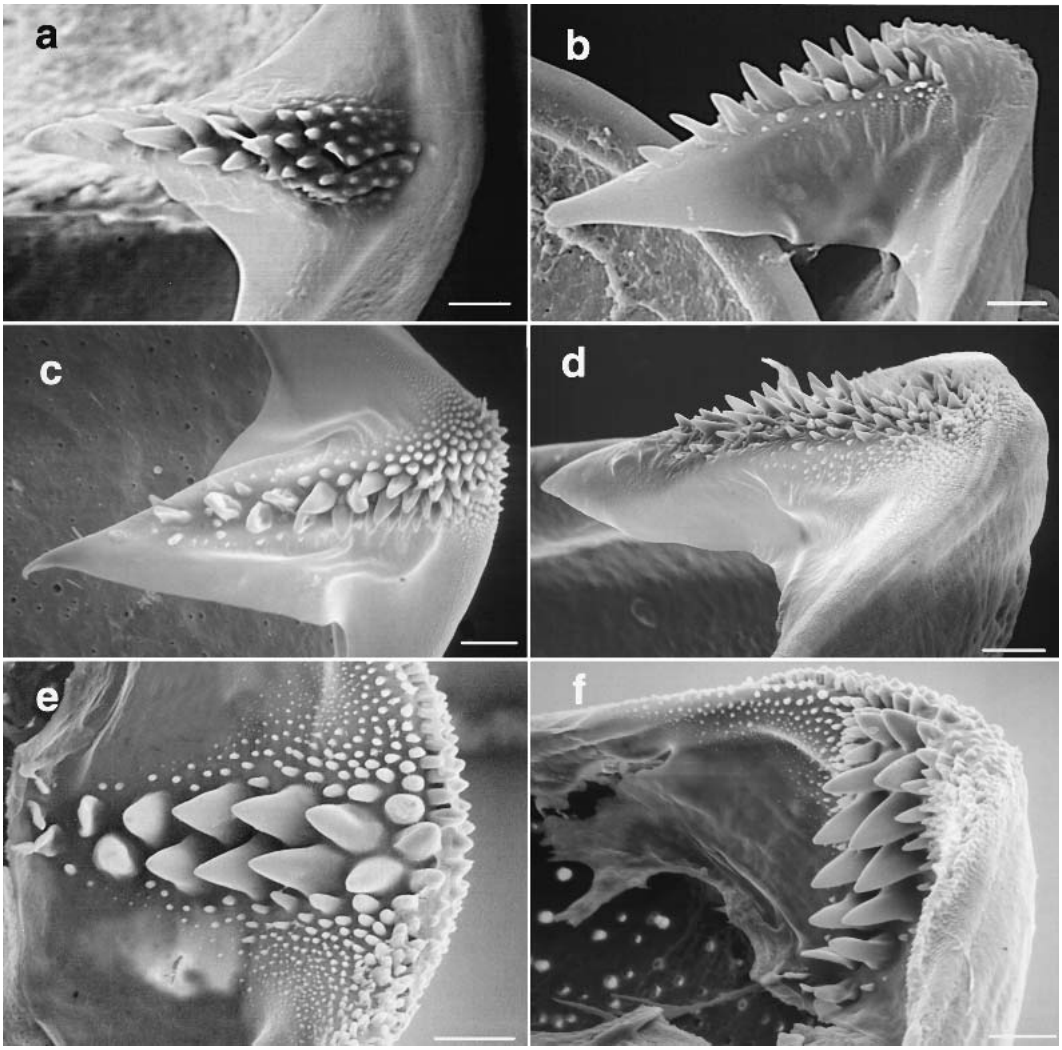

Figure 3. Scanning electron micrographs showing the hooks of the glochidia of A-B, *Cristaria herculea;* C-D, *Cristaria tuberculata;* E-F, *Sinanodonta amurensis*. Figures A, C, and E show the hook viewed perpendicular to the plane of the valve commissure. Figures B, D, and F show the hook in side view. All scale bars = 10 μm.

and on the external surfaces (0.5-1.2 versus 0.6-1.5 μm, respectively) (Fig. 4A-D).

Glochidia of the two species differed significantly in hinge and hook characters. Glochidia of *Cristaria tuberculata* had longer absolute and relative hinge lengths and longer absolute and relative hook lengths (LSD post hoc tests for hinge, $p = 0.000$; hinge/length, $p = 0.000$; hook, $p = 0.000$; hook/height, $p = 0.001$) (Fig. 5D-G). In one glochidium measured of each species, maximum height of the microstylets on the hook was greater in *Cristaria herculea* (12.7 μm) than in *C. tuberculata* (9.1 μm) (Fig. 3B,D). There were more small microstylets at the flex point of the hook in *C. tuberculata* than in *C. herculea* (Fig. 3A,C). Pits on the inner surfaces of the valves tended to be slightly smaller than those on the outer surfaces for both species of *Cristaria* (Fig. 4A-D).

Sinanodonta species

Sinanodonta amurensis (Figs. 1B, 2C, 3E-F, 4E-F, 5). The valves of the glochidia of *S. amurensis* were distinctly asymmetric because the ventral (hooked) edge of each valve was displaced posteriorly and because the anterior edge was longer and had a more prominent curve (Fig. 1B). Valve length (parallel to hinge) ranged from 254-264 μm (mean 258.6, st. dev. 3.7 μm) and valve height (perpendicular to hinge) ranged from 257-278 μm (mean 270.6, st. dev. 5.8

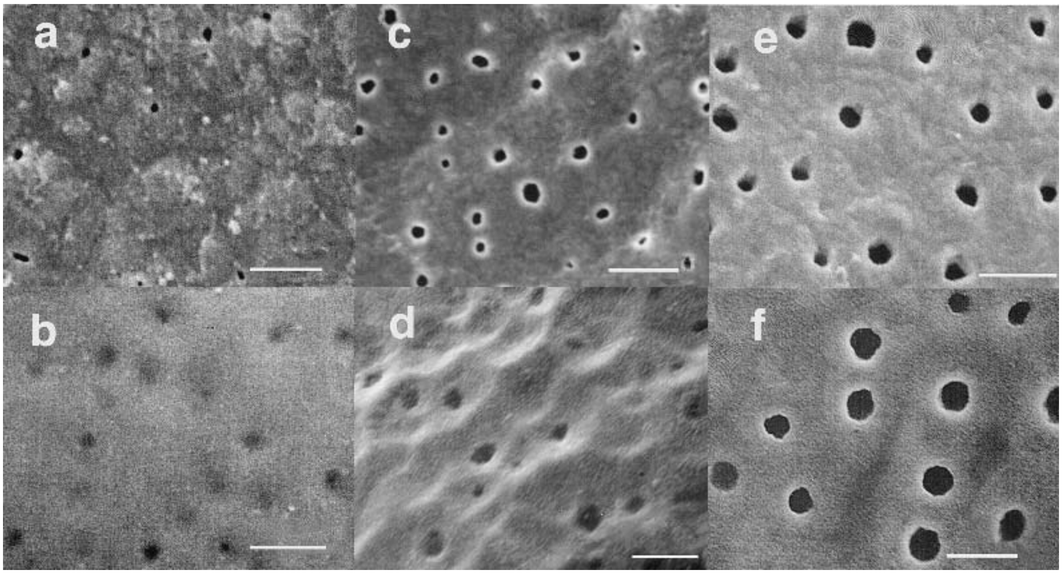

Figure 4. Scanning electron micrographs showing pits on the surfaces of the glochidial valves of A-B, *Cristaria herculea;* C-D, *Cristaria tuberculata;* E-F, *Sinanodonta amurensis*. Figures A, C, and E (top row) are of the internal valve surfaces. Figures B, D, and F (bottom row) are of the external valve surfaces. All scale bars = 5µm.

µm). Valve height was greater than or equal to valve length with the height/length ratio ranging from 1.00-1.07 (mean 1.041, st. dev. 0.020). Hinge length (Fig. 2C) was 186-214 µm (mean 202.7, st. dev. 6.8 µm), being 76-81% of valve length (mean 78.9, st. dev. 2.4%). The hook ranged from 96-100 µm (mean 99.2, st. dev. 1.6 µm), being from 36-38% of the valve height (mean 37.1, st. dev. 1.0%). The distorted appearance of the hook of *S. amurensis* in the SEM images (Fig. 3E-F) is an artifact of drying for SEM. Hooks were not distorted in wet specimens of this species that were measured for ANOVA. The glochidium examined by SEM had about 12 large microstylets arranged on the hook in two well-defined longitudinal rows (Fig. 3E-F). Maximum height of the microstylets on the hook was 13.6 µm. The pits on the inner surface (0.8-1.8 µm diameter) were smaller than those on the outer surface (1.6-2.0 µm diameter) (Fig. 4E-F). Pits were situated on the entire valve including the site of adductor muscle attachment.

Sinanodonta likharevi (Fig. 5). The valves of the glochidia of *S. likharevi* were distinctly asymmetric because the ventral (hooked) edge of each valve was displaced posteriorly and because the anterior edge was longer and had a more prominent curve. Valve length (parallel to hinge) ranged from 250-293 µm (mean 259.6, st. dev. 9.8 µm) and valve height (perpendicular to hinge) ranged from 264-314 µm (mean 283.6, st. dev. 10.9 µm). Valve height was greater than valve length with the height/length ratio ranging from 1.03-1.14 (mean 1.094, st. dev. 0.033). Hinge length was 171-218 µm (mean 194.4, st. dev. 11.0 µm), being 65-86% of valve length (mean 75.0, st. dev. 3.8%). We were unable to measure hook lengths of the glochidia of *S. likharevi* by light microscopy or to examine microstylets or pits by SEM because the hooks of the glochidia of *S. likharevi* were damaged.

Glochidial valves of *Sinanodonta amurensis* and *Sinanodonta likharevi* did not differ significantly in length (LSD post hoc test for length, $p = 0.654$) (Fig. 5A). The glochidia of *S. amurensis* and *S. likharevi* differed significantly in height and hinge length. The glochidia of *S. amurensis* were smaller in absolute and relative height (Fig. 5B-C) and larger in absolute and relative hinge length (LSD post hoc test for height, $p = 0.000$; height/length, $p = 0.000$; hinge, $p = 0.002$; hinge/length, $p = 0.000$).

Comparisons between *Cristaria* and *Sinanodonta*

ANOVA of the seven glochidial characters of the four species showed significant variation in all seven characters (Table 1). Letters in Fig. 5 indicate which species differ significantly in which characters from each other, as determined by LSD post-hoc tests.

There were a number of similarities in glochidia of the two species of *Cristaria* and the two species of *Sinanodonta*. In qualitative characters, glochidia of both genera were similarly asymmetric with the ventral (hooked) edge of each valve displaced posteriorly and with the anterior edge longer

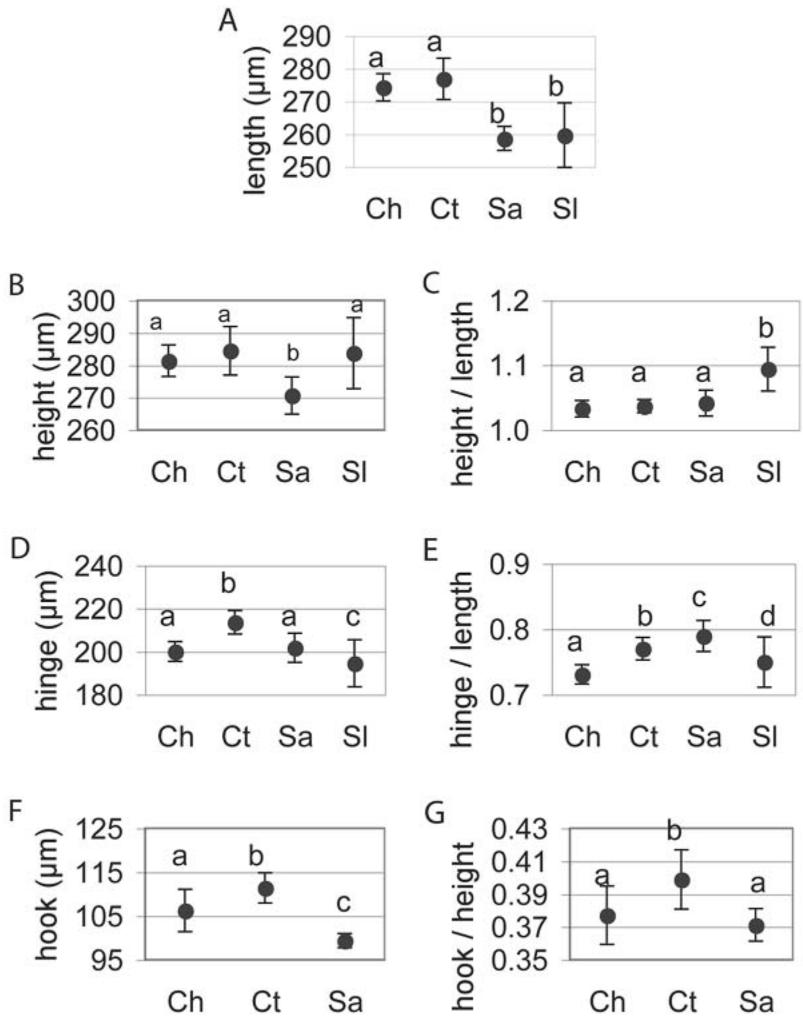

Figure 5. Mean values of seven characters of glochidia of *Cristaria herculea* (Ch), *Cristaria tuberculata* (Ct), *Sinanodonta amurensis* (Sa), and *Sinanodonta likharevi* (Sl) (five characters). Length is measured parallel to the hinge. Error bars are standard deviation. Different letters indicate values that differ significantly at $p < 0.05$.

and with a more prominent curve (Figs. 1-2). The pits on the inner surfaces of the glochidia of the two species of *Cristaria* and *Sinanodonta amurensis* tended to be smaller than those on the outer surfaces (Fig. 4) and pits were situated on the entire valve including the site of adductor muscle attachment.

Five quantitative characters (height, relative height, hinge length, relative hinge length, and relative hook length) varied from being significantly to not significantly different among the species, but they did not differ consistently between genera. Glochidial heights of the two species of *Sinanodonta,* ranging from 257-314 µm, completely overlapped the heights of the two species of *Cristaria* (271-300 µm) (Fig. 5B) and in both genera, the relative heights were always 1.00 or greater (1.00-1.14 height/length) (Fig. 5C). Glochidial hinge length overlapped considerably between the two genera (Fig. 5D), with the two species of *Cristaria* ranging from 193-221 µm, and the two species of *Sinanodonta* ranging from 171-218 µm. While the relative hinge lengths (standardized by valve length) of all four species differed significantly (LSD post hoc p-values ranged from $p = 0.000$ to $p = 0.031$), the direction of the difference was not consistent between genera (Fig. 5E). Relative hook lengths of the two species of *Cristaria* (36-42% of shell height) overlapped those of *Sinanodonta amurensis* (36-38% of shell height) (Fig. 5G).

On the other hand, there were some intergeneric differences in glochidia. The only quantitative character that differed significantly between genera but not within genera was length, with glochidia of *Cristaria* spp. being longer than those of *Sinanodonta* spp. (Fig. 5A) (species of *Cristaria* ranged 270-286 µm in length, species of *Sinanodonta* ranged 250-268 µm in length except one unusually large individual glochidium of *Sinanodonta likharevi* that was 293 µm long). Glochidial hooks in both species of *Cristaria* (hooks 100-114 µm long) were significantly longer than those of *Sinanodonta amurensis* (96-100 µm long) (LSD post hoc test after ANOVA for hook between *S. amurensis* and *Cristaria herculea,* $p = 0.002$; between *S. amurensis* and *Cristaria tuberculata,* $p = 0.000$) (Fig. 5F).

In characters examined on a single glochidium of each species, glochidial differences between the two genera included the position of the hook, valve curvature near the hinge, pit size, and arrangement and morphology of the microstylets on the hook. The hook area in *Sinanodonta amurensis* appeared less centered than that of either species of *Cristaria* (Fig. 2). The valve edge of the two species of *Cristaria* appeared to curve inward to the hinge more sharply than in *S. amurensis* (Fig. 2). Pits in the valves of the species of *Cristaria* tended to be smaller (0.5-1.5 µm diameter) than those in *S. amurensis* (0.8-2.0 µm) (Fig. 4).

The number and arrangement of microstylets on the hook differed in the one glochidium of each species examined. The two species of *Cristaria* had 15 to 20 large lanceolate microstylets that were more slender and shorter (maximum height 9.1-12.7 µm) and were arranged in 1-2 or 2-3 poorly-defined longitudinal rows (Fig. 3A,C). The glochidia of *Sinanodonta amurensis* had 10 or fewer large microstylets

Table 1. ANOVA of glochidial characters from *Cristaria herculea, Cristaria tuberculata, Sinanodonta amurensis,* and *Sinanodonta likharevi*. Analyses of Hook and Hook/Height do not include *S. likharevi.* Results of post-hoc tests are indicated in Figure 5 by letters.

Source	df	F-Ratio	p
Length	3	40.356	0.000
Error	74		
Height	3	17.210	0.000
Error	90		
Height/Length	3	36.452	0.000
Error	72		
Hinge	3	24.185	0.000
Error	79		
Hinge/Length	3	17.941	0.000
Error	71		
Hook	2	18.923	0.000
Error	38		
Hook/Height	2	6.863	0.004
Error	27		

that were more stout and longer (maximum height 13.6 µm) and were arranged in two discrete rows (Fig. 3E). The small microstylets in *S. amurensis* extended beyond the base of the hook to the outer margin farther than did the small microstylets in the two species of *Cristaria* (Fig. 3).

DISCUSSION

The question of whether *Cristaria herculea* and *Cristaria tuberculata* are different species has persisted among Russian malacologists (Kodolova and Logvinenko 1987, Prozorova and Sayenko 2001). Our examination of their glochidia suggests that these two forms differ significantly in glochidial hook and hinge characters, and we conclude that they are distinct species.

Our use of multiple glochidia from a single female of each species raised the question about variability of glochidia within a single female, among females of the same species, and among females of different species. If the variation of glochidia from different females of a single species were greater than the variation among females of different species, then glochidial features would not be reliable for discriminating among species. Our analysis of Hoggarth's (1999) data (reported above in the Methods section) suggests that for 10 species in the Anodontinae, characters of glochidia from a single female per species could be used for discriminating among species. We extrapolate that result on North American Anodontinae to Anodontinae from eastern Asia to conclude that significant differences in glochidia from different females indicate that the females are different species.

The glochidia of *Cristaria plicata* measured by Wu *et al.* (2000) had a smaller length (263.7 µm) and greater height (310.1 µm) resulting in more elongate valves (1.18 height/length ratio) than did those of *Cristaria herculea* and *Cristaria tuberculata* in this study. The hinge length in *C. plicata* (199.8 µm) was similar to what we found in *C. herculea,* and the number and arrangement of microstylets on the hook (15-18 microstylets arranged in 2 rows) was similar to what we found for *C. herculea* and *C. tuberculata*.

Our findings along with those of Wu *et al.* (2000) lead us to conclude that *Cristaria herculea, Cristaria tuberculata,* and *Cristaria plicata* are three separate species. This conclusion is in contrast to the conclusions of Haas (1969) and Brandt (1974), who synonymized *C. herculea* and *C. tuberculata* with *C. plicata*. Because Haas (1969) gave no justification for the synonymy, we cannot evaluate his evidence. On the other hand, it is not clear what species Brandt (1974) had. Brandt (1974: 279) wrote, referring to *C. plicata,* "As this species has semi-oval glochidia without hooks it cannot be placed among Anodontinae as Haas (1969), Vokes (1967: 213) a. o. suggested. … There is only one true Anodontinae known from SE-Asia, *Sinanodonta woodiana* (Lea), on whose various forms innumerable 'species' are based." The species of *Cristaria* we studied, and as studied by Wu *et al.* (2000), certainly do have anodontine glochidia. What Brandt (1974) was calling *C. plicata* was probably not *C. plicata,* and therefore the synonymy is suspect.

Antonova and Starobogatov (1988) compared glochidia of *Cristaria tuberculata* from Primorye with the data on *Cristaria herculea* from Primorye made by Shadin (1938) and with the description of the glochidia of *Cristaria* spp. from Iturup Island (southern Kuril Islands) made by Inaba (1941, 1964). However, recent examination of material collected during the 1994-1999 expeditions of the International Kuril Island Project, a biotic survey and inventory of the Kuril Archipelago (Pietsch *et al.* 2001, 2003), indicated that the specimens from Iturup Island are actually members of the genus *Kunashiria* Starobogatov in Zatravkin, 1983 (Sayenko and Bogatov 1998, Sayenko 1999a, Bogatov *et al*. 1999, Sayenko and Ohara 2001). Consequently, some of the data on the genus *Cristaria* reported by Antonova and Starobogatov (1988) should be attributed to *Kunashiria*. This mistaken identity likely explains why Antonova and Starobogatov (1988) incorrectly concluded that glochidia of *C. tuberculata* have large microstylets arranged in one row at the end of the hook, whereas those of *C. herculea* have large microstylets in two rows. Even if they had identified the species correctly, the number of microstylet rows would not be reliable for species identification because the arrangement of microstylets on hooks in species of *Cristaria* is variable (arrangement in 1-2 to 2-3 poorly defined rows, this paper).

Comparison of our findings on *Cristaria herculea* and *Cristaria tuberculata* with findings on glochidia of two other species of *Cristaria* from Japan examined by Inaba (1941, 1964), *Cristaria discoidea* (Lea, 1834) and *Cristaria spatiosa* (Clessin, 1875), reveals the variability in height, width, shape, and hinge length of glochidia in this genus. The hinge lengths of the glochidia of *C. discoidea* and *C. spatiosa* (203 μm and 213-214 μm, respectively) and the heights of glochidia of *C. discoidea* (277 μm) are within the ranges of those of *C. herculea* and *C. tuberculata*. However, the length of glochidia of *C. discoidea* (259 μm) is less than those of *C. herculea* and *C. tuberculata* and the heights and lengths of glochidia of *C. spatiosa* (312-330 μm and 284-307 μm, respectively) are considerably greater than those of the Russian *Cristaria* we studied (271-300 μm height, 270-286 μm length). Furthermore, glochidia of *C. discoidea* and *C. spatiosa* are slightly more elongate (height/length = 1.07 and 1.04-1.12, respectively) than those of *C. herculea* and *C. tuberculata* (1.01-1.06).

In addition to the species of *Sinanodonta* examined here, two other species of *Sinanodonta* have been studied extensively: *Sinanodonta calipygos* (as *Anodonta woodiana calipygos;* Inaba 1941, 1964), and *Sinanodonta woodiana* (as *A. woodiana lauta; A. woodiana lauta tumens;* and *A. woodiana;* Inaba 1941, 1964, Kwon *et al.* 1993, Park and Kwon 1993). In Japanese specimens, the heights of the glochidia of *S. calipygos* (298-313 μm) and *S. woodiana* (277-303 μm) and the lengths of the glochidia of *S. woodiana* (243-268 μm) are larger than those of the Russian *Sinanodonta amurensis* and *Sinanodonta likharevi* we studied. On the other hand, the lengths of the hinges of the glochidia of Japanese *S. calipygos* (180-191 μm) are in the same range as those of *S. likharevi* but considerably smaller than those of *S. amurensis*. Glochidia of *S. woodiana* from Korea do not differ from the Russian individuals of *Sinanodonta* in any measurements. Although the size of the glochidia of all species of *Sinanodonta* is mostly in the same range, the island specimens have larger glochidia than the continental specimens. Moreover, glochidia of the species of *Sinanodonta* studied in Japan by Inaba (1941, 1964) are more elongate (height/length = 1.18-1.23 for *S. calipygos,* and 1.13-1.14 for *S. woodiana*) than the species of *Sinanodonta* studied in Korea (1.04 for *S. woodiana* [Kwon *et al.* 1993, Park and Kwon 1993]) and in Russia (this paper). We conclude that the glochidia of *S. amurensis* are the smallest and that the glochidia of the Japanese *S. woodiana* are the largest of the species of *Sinanodonta* studied.

Regarding the hook features, the large microstylets of glochidia of *Sinanodonta woodiana* from Korea were centrally arranged in two uneven rows (Park and Kwon 1993), as we found in the glochidia of *Sinanodonta amurensis* from eastern Russia.

In contrast to other species of Anodontini (*Anemina*-group species), in which the height of the glochidia is less than the length and the glochidia are weakly asymmetric (Jeong 1989, Lee *et al.* 1989, Jeong *et al.* 1993, Kwon *et al.* 1993, Park and Kwon 1993, Chernyshev 1998, Sayenko 1999b), the glochidia of *Sinanodonta amurensis* and *Sinanodonta likharevi* were elongate (height > length), as were those in the species of *Cristaria* we examined. Furthermore, in the glochidia of *S. amurensis,* the pits were regularly distributed over the entire external and internal surfaces. This pit distribution is in contrast to that in glochidia of other species of Anodontini. For example, in the *Anemina*-group of species, the internal valve surface where the adductor muscle attaches either lacks pits, has fewer pits, or the pits are smaller than the pits on other parts of the glochidial valves (Chernyshev 1998).

In summary, we found significant differences in characters of multiple glochidia from a single parent of each species. Our analysis of data from Hoggarth (1999) showed significant variation in glochidial features among species, but no significant variation in glochidia among parents of the same species, suggesting that significant glochidial differences across parents would indicate different species. Consequently, our finding significant differences in multiple glochidia from single parents of *Cristaria herculea* and *Cristaria tuberculata* and differences in multiple glochidia from single parents of *Sinanodonta amurensis* and *Sinanodonta likharevi* support the recognition of these taxa as distinct species. Glochidial features of *Sinanodonta* spp. differ from those of other members of the tribe Anodontini (including the genus *Anemina*), casting doubt on whether *Sinanodonta* belongs in the tribe Anodontini. Glochidial similarities between *Sinanodonta* spp. and *Cristaria* spp. hint that *Sinanodonta* might belong in the tribe Limnoscaphini along with *Cristaria*. Future phylogenetic analyses that include glochidial characters of more members of the subfamily Anodontinae Ortmann, 1910 will provide additional insight into the relationships among the taxa.

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