
**Data on ultra-sculpture of glochidia of
Cristaria tuberculata (Unionidae: Anodontinae)
from the Khanka Lake (Russian Far East)¹**

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New data on morphology of glochidia of freshwater anodontine bivalvia *Cristaria tuberculata* Schumacher, 1817 from the Khanka Lake, Russian Far East are obtained with scanning electron microscopy. Ultra-sculpture of the outer valve is described and compared with the data on glochidia of *Cristaria plicata* (Leach, 1815) from the Dongting Lake, Hunan Province, China. Glochidia of the compared species have the same size differing by the bigger hook in *C. tuberculata*. In both species, external microsulpture is intermediate between the tight-looped and vermiculate.

Key words: microsculpture, glochidia, morphology, Unionidae, *Cristaria tuberculata*.

**Данные по микроскульптуре глохидиев
Cristaria tuberculata (Unionidae: Anodontinae)
из озера Ханка (российский Дальний Восток)**

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Данные по морфологии глохидиев пресноводных анодонтин *Cristaria tuberculata* Schumacher, 1817 из озера Ханка (российский Дальний Восток) дополнены с помощью сканирующей электронной микроскопии. Размерные характеристики и признаки микроскульптуры наружной поверхности глохидиев *C. tuberculata* сравниваются с данными для *Cristaria plicata* (Leach, 1815) из озера Дунтиху, провинция Хунань, КНР. Глохидии обоих видов имеют одинаковые размеры, отличаясь более крупным прикрепительным аппаратом (крючком) у *C. tuberculata*. У обоих видов микроскульптура наружной поверхности личиночных створок промежуточная между плотно-петлевидной и вермикулярной.

Ключевые слова: микроскульптура, глохидии, Unionidae, *Cristaria tuberculata*.

Based on different taxonomic approaches, two to eight Recent species are listed in the genus *Cristaria* Schumacher, 1817 [Liu et al., 1979; Liu, Zhang, 1982; Starobogatov et al., 2004; Graf, Cummings, 2007; He, Zhuang, 2013; Klishko et al., 2014]. Combining all data, the list of the species with their distribution is as follows: *Cristaria*

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plicata (Leach, 1814) (Indochina and China, Mongolia, perhaps, Japan), *C. tuberculata* Schumacher, 1817 (Khanka Lake including Ussury River basins), *C. tenuis* (Griffith et Pidgeon, 1833) (widespread in southeastern Asia, from Yangtze River south to Cambodia), *C. herculea* (Middendorff, 1848) (basins of the Amur, Ussury rivers and Khanka Lake; Buir Lake in Mongolia), *C. radiata* Simpson, 1900 (Anhui Province in China), *C. truncata* Dang et al., 1980 (northern Vietnam), *C. beirensis* Liu et Zhang, 1982 (Inner Mongolia and China), *C. discoidea* (Lea, 1834) (Yangtze-Huang in Indochina).

Features of larvae (glochidia) shells can provide additional data for taxonomic revision of anodontine bivalves. As to genus *Cristaria*, glochidia have been studied mainly with light microscopes. The first description and a schematic picture of *C. herculea* glochidia from Lefu River (now Ilistaya River, Primorsky Krai), formerly known as *C. plicata*, were given by Shadin [1938]. Brief descriptions and schematic illustrations of *C. discoidea* and *C. plicata* (= *C. spatiosa* Clessin, 1875) glochidia from Honshu and Hokkaido islands (Japan) have been published by Inaba [Inaba, 1941, 1964]. Description and a schematic illustration of *C. tuberculata* glochidia were made by Antonova and Starobogatov [1988]; measurements and the photos made by light microscope were published by Prozorova and Sayenko [2001]. For *C. plicata*, the first light microscope photos of glochidia were published in Japan [Higashi, Hayashi, 1964]; morphometric characteristics of *C. plicata* glochidia were also studied by Chinese malacologists [Wei et al., 1994]. Scanning electron microscopy was the next step in the study of *Cristaria* glochidia. SEM investigations were conducted for *C. herculea*, *C. tuberculata* and *C. plicata* [Wu et al., 2000; Sayenko et al., 2005; Shu, Wu, 2005; Sayenko, 2006, 2013]. Among *Cristaria* species, data on microsculpture of the outer surface of glochidial valves are obtained only for *C. plicata*.

Morphology of glochidia of the freshwater anodontine *C. tuberculata* from Khanka Lake, the largest freshwater lake in the Russian Far East, was examined by the light and scanning electron microscopy with a purpose to investigate the ultra-sculpture of glochidial valves and to compare new data with the characteristics of *C. plicata* from Dongting Lake, the second largest freshwater lake (after Poyang Lake) in China (Hunan Province).

Material and methods

Multiple mature glochidia were obtained from two gravid females of *C. tuberculata* collected in the Khanka Lake nearby Vostochnyi Cordon (28.10.1999; collector L.A. Prozorova).

Specimens and samples of mature glochidia were stored in 75% ethanol and deposited at the Laboratory of Freshwater Hydrobiology, Institute of Biology and Soil Science, Far Eastern Branch, Russian Academy of Sciences (IBSS FEB RAS), Vladivostok. Species identification of glochidia was made based on adult individuals, from the gills of which they were extracted. Classification of the genus *Cristaria* is given after Starobogatov et al. [2004].

To prepare for work on a light and scanning electron microscopes, mature glochidia were cleaned from the soft tissues in a 5% KOH solution. To prevent a risk to «over-clean» the shells during this procedure, when the thin outer layer, which forms the microsculpture, is removed, the glochidia were examined every half an hour under a light microscope. The glochidia were washed several times in distilled water and stored in 75% ethanol, after that they were ready for investigations by light microscopy. For scanning electron microscopy, the cleaned shells were washed in a series of alcohols (80, 90, and 96%), mounted on a stub and covered by gold.

Measurements to be used in the investigation were made using light microscopy on at least 25 glochidia per adult female, but not all measurements could be made on every glochidium. The microsculpture of each larval shell was examined at four points: closer to the ventral end (i.e., to the hook), at the center of the valve (the adductor region), between the adductor and central part of the valve rim, and at the ligament.

A Topcon ABT-60 Scanning Electron Microscope (Delaware Museum of Natural History, Wilmington, Delaware, USA) was used to view the glochidia while the Zeiss EVO 40 scanning microscope (Biology and Genetic Engineering Center for Collective Use at the IBSS FEB RAS, Vladivostok, Russia) was used to obtain data on glochidial ultra-sculpture.

Results and discussion

Bivalves of the genus *Cristaria* have dorso-ventrally elongated glochidia, when valve height is always greater than its length ($H > L$, where H – height of glochidial shell, L – length of glochidial shell). The ventral (hooked) edge of each valve is strongly displaced posteriorly and the anterior edge is longer and it has a more prominent curve than the posterior edge. Outer and inner surfaces of the valves have evenly distributed pores. Previously it was shown that glochidia of *Cristaria* have the smallest pores among Far Eastern anodontines [Sayenko, 2006, 2013]. Glochidial shells of *C. tuberculata* are also elongated vertically (Fig. 1), H/L ratio is 1.03–1.06 for *C. tuberculata* and 1.01–1.05 for *C. herculea* [Sayenko et al., 2005], 1.01–1.1 for *C. plicata* [Sayenko, 2013]. Differing by shell proportions, glochidia of the compared species do not differ by size. Glochidia of *C. tuberculata* with bigger hooks: the length of the hook (h) is 37–42% of the valve length while for *C. plicata* the length of the hook is 29–37% of the valve length (see Table). Large styliform hook has 15–20 macrospines (Fig. 2). In comparison, glochidial hooks of *C. plicata* with at least 25 macrospines [Sayenko, 2013]. Glochidia of *C. tuberculata* and *C. herculea* from the Khanka Lake basin have the same sizes and shell proportions differing only by length of ligament and hook that are bigger for *C. tuberculata* [Sayenko, 2006].

The shells of anodontine glochidia consist of two layers [Kinzelbach, Nagel, 1986]. The inner layer is thick, punctuated by pores, though their outer ends are covered by the thin outer layer that forms a special external microsculpture. This microsculpture

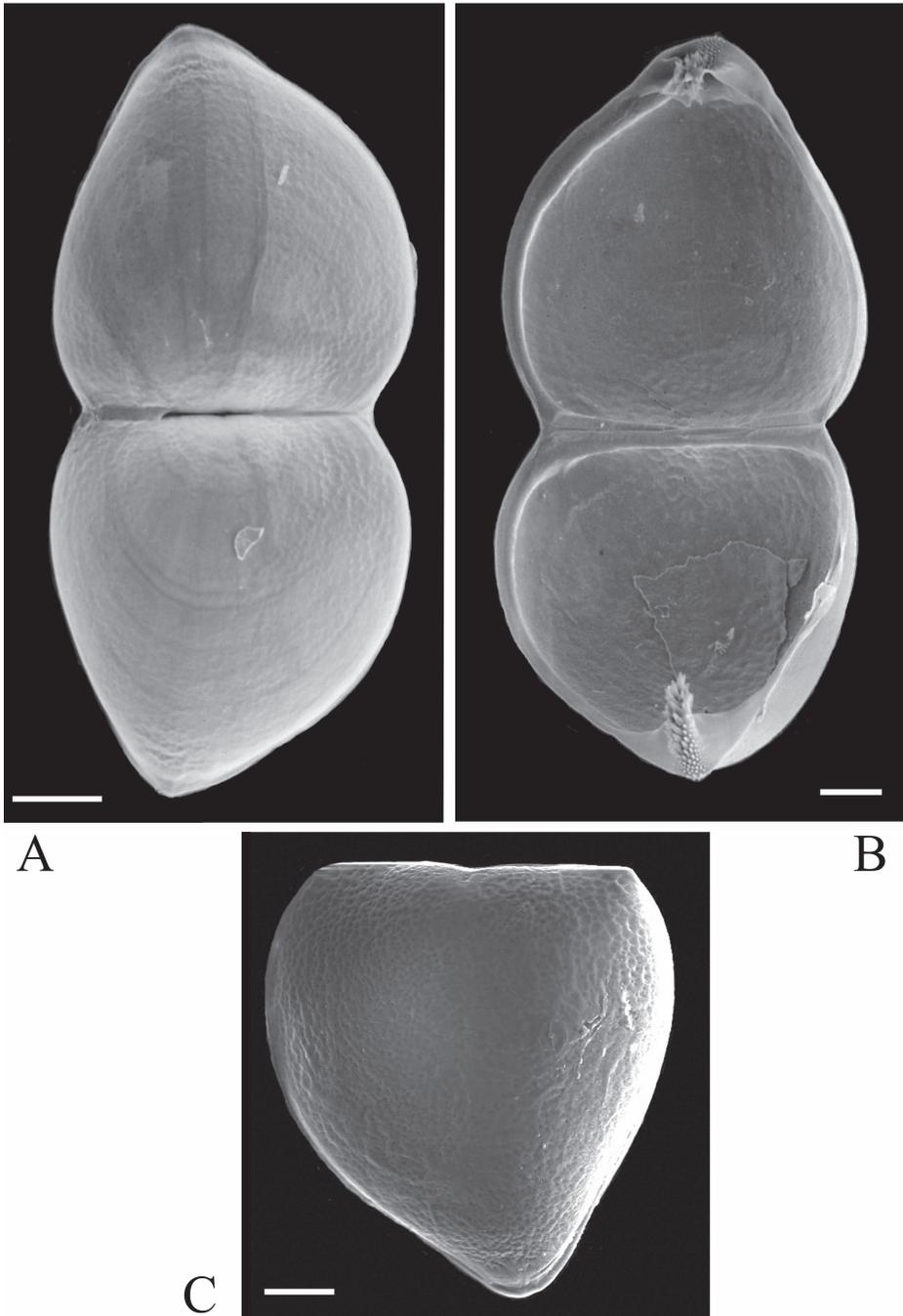


Fig. 1. Shells of mature glochidia of *Cristaria tuberculata*: **A, B** – with open valves, exterior (A) and interior (B) views; **C** – glochidium with closed valves. Scale bars: 62.5 μm (A); 50 μm (B); 40 μm (C).

Conchological features of glochidia of the *Cristaria* species (in μm)

Locality	Height of glochidial valve (H)	Length of glochidial valve (L)	Length of ligament (lig)	Length of the hook (h)	H/L
<i>Cristaria tuberculata</i> Khanka Lake	<u>278.5–299.9</u> 286.6 ±7.06	<u>271.3–285.6</u> 279.0 ±5.92	<u>207.1–221.3</u> 215.3 ±5.46	<u>107.1–114.2</u> 111.2 ±3.51	<u>1.03–1.05</u> 1.04 ±0.01
<i>Cristaria herculea</i> Khanka Lake (after Sayenko et al. [2005])	<u>271–293</u> 281.2 ±4.9	<u>271–285</u> 274.2 ±4.1	<u>193–207</u> 199.9 ±4.5	<u>100–114</u> 106.2 ±4.9	<u>1.01–1.05</u> 1.03 ±0.01
<i>Cristaria plicata</i> Southern Dongting Lake (after Sayenko [2013])	<u>246–310</u> 279.7 ±13.48	<u>232–270</u> 257.2 ±6.2	<u>178–202</u> 194.8 ±4.37	<u>64–100</u> 86.8 ±7.88	<u>1.04–1.12</u> 1.08 ±0.04

Note. Above the line – min-max of measurements. Under the line – mean arithmetic value (bold type) with standard deviation.

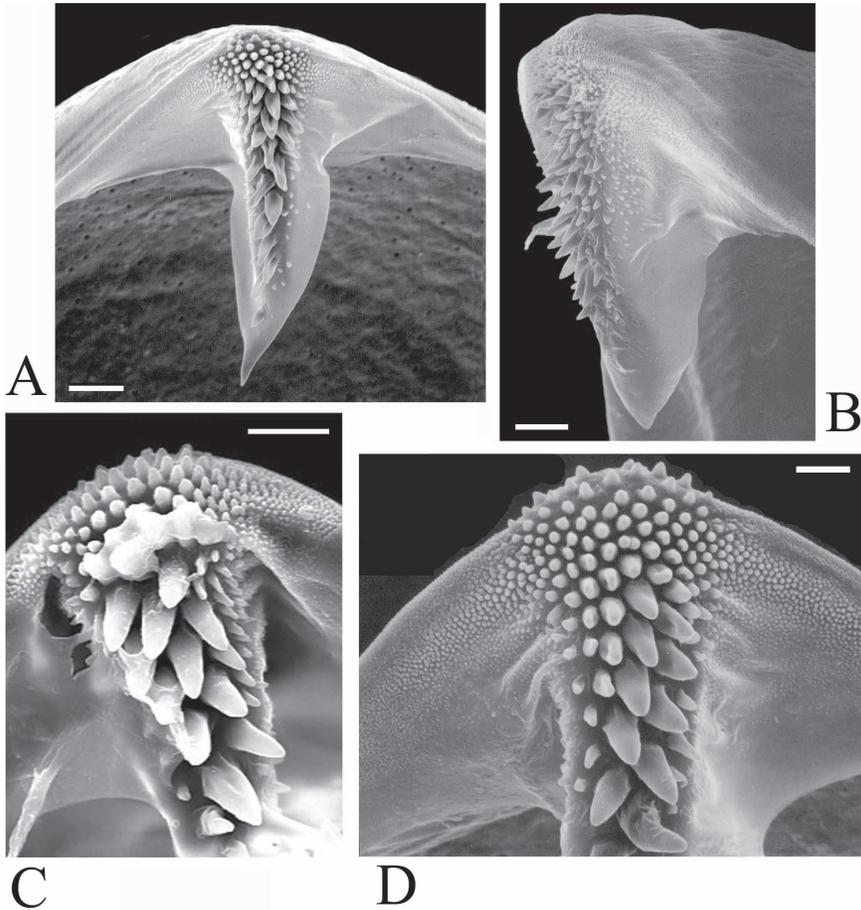


Fig. 2. Glochidial hooks and spines of *Cristaria tuberculata*: **A** – frontal view; **B** – lateral view; **C**, **D** – macrospines. Scale bar: 12.5 μm (A); 10 μm (B, C); 6.66 μm (D).

of the discussed species *C. tuberculata* could be described as intermediate between the tight-looped and vermiculate because chaotic and unstructured loops are packed so tight that only their tops are visible (Fig. 3). Replacement tight-looped sculpturing on

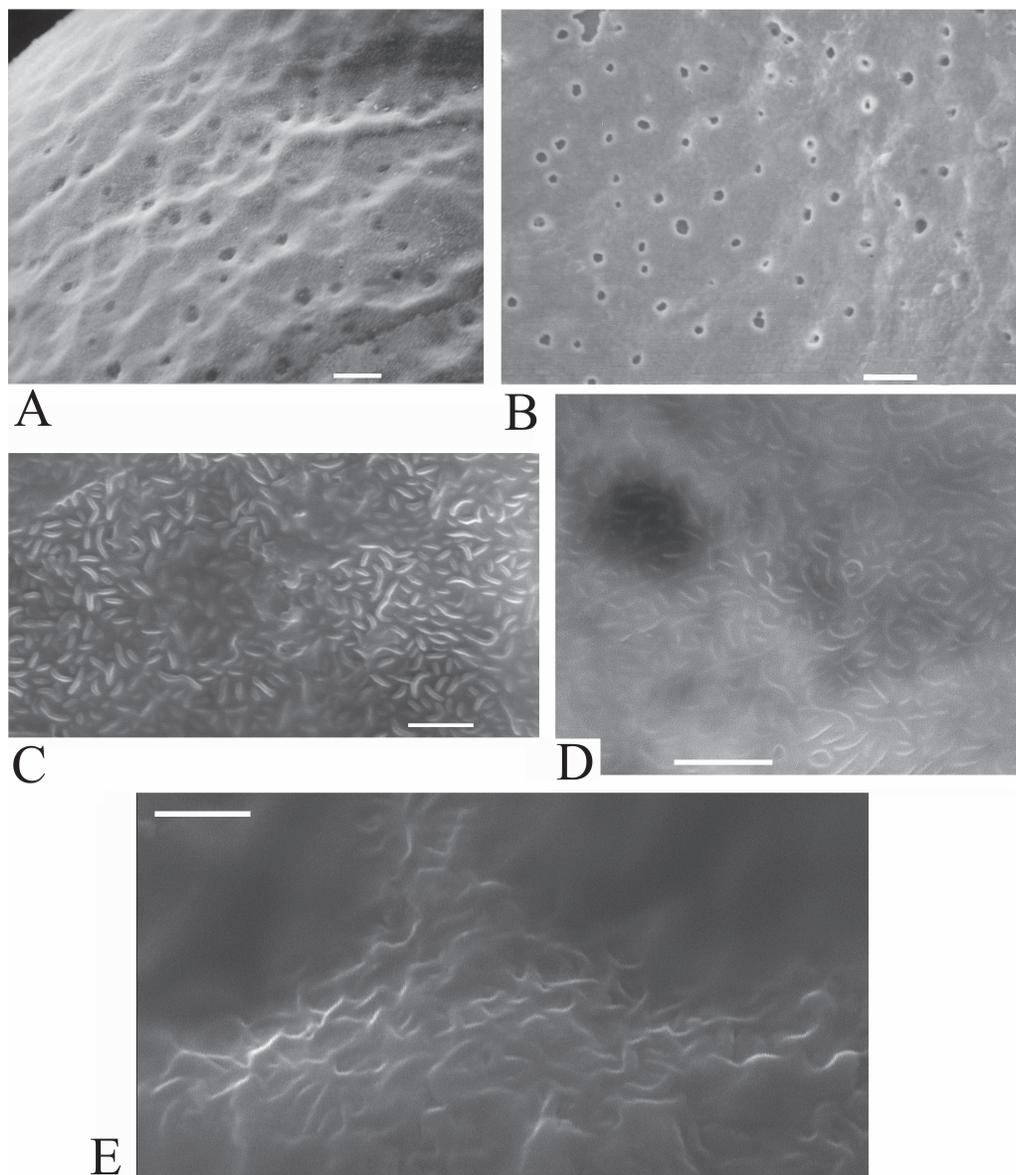


Fig. 3. Glochidial pores and microsculpture of *Cristaria tuberculata*: **A** – exterior valve sculpture at ligament near valve rim; **B** – interior valve surface with pores; **C** – exterior valve sculpture at the hook; **D** – exterior valve sculpture at the central part of the valve rim; **E** – exterior valve sculpture at the adductor. Scale bars: 5 μ m (A, B); 1 μ m (C, D, E).

vermiculate comes from the central part (adductor) to the valve edge (hook, ligament or valve rim) (Fig. 3C–E). Ultra-sculpture of *C. plicata* glochidia have the same features; in addition, very few microgranules are observed among tight loops [Sayenko, 2013].

It is interesting to compare external glochidial micrisculpture for *Cristaria* and *Sinanodonta* Modell, 1944 as previously a similarity of these two genera by glochidial shells shape was shown [Prozorova, Sayenko, 2001]. Glochidial micrisculpture of *Sinanodonta* is described as net type and consists of convex overlapping loop-like lines, which form a sufficiently dense fine net structure, withal granules are completely absent. Unlike *Cristaria*, glochidial micrisculpture of *Sinanodonta* is identical across the valve surface [Sayenko, 2014].

In summary, glochidia of *C. tuberculata* differ from both *C. herculea* and *C. plicata* by bigger hooks in *C. tuberculata*. External microsculpture features of glochidial shells of *C. tuberculata* and *C. plicata* are identical.

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