

Ultrastructure of *Tryblionella qinghainensis* sp. nov., *T. apiculata* and *T. levidensis* (Bacillariophyta) from Lake Qinghai (China), with reference to the marginal ridge

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Abstract: Three *Tryblionella* species found at the same locality in Lake Qinghai, the largest inland saline water lake of China, are studied by using light and scanning electron microscope. One species is proposed as new to science and named *Tryblionella qinghainensis* sp. nov. The other two species are identified as *T. apiculata* W. Gregory and *T. levidensis* W. Smith. *Tryblionella qinghainensis* sp. nov. is characterized by its linear–lanceolate valve outline, broad axial sternum, a stria density to fibula density ratio of ca. 1.9:1, alveolate striae, and a simple standing lamella–like marginal ridge unperforated. Several ultrastructural details for *T. apiculata* and *T. levidensis* are revealed for the first time, especially the ultrastructure of their marginal ridges. The structure and shape of the marginal ridge in these three *Tryblionella* species are different, and the ultrastructure at the junction between the marginal ridge and the distal mantle can also vary for each individual species. The ultrastructure of the marginal ridge and distal mantle may be used as important diagnostic characters for the species of the genus *Tryblionella*.

Key words: alveolate striae, distal mantle, marginal ridge, sternum, *Tryblionella qinghainensis*

INTRODUCTION

SMITH (1853) established the diatom genus *Tryblionella* and provided a brief description: Cells solitary. Valve elliptical or linear, valve surface plane. Alae submarginal or obsolete. Fibulae inconspicuous, parallel. He grouped six new species within this new genus but did not designate a type species for it. GRUNOW (1879) remarked that “long observation of this genus (*Nitzschia*) had convinced me that the genus *Tryblionella* was not sufficiently distinct to warrant their separation”. Therefore, he transferred the genus *Tryblionella* to *Nitzschia* and this status remained for more than 100 years until MANN (in ROUND et al. 1990) selected *Tryblionella acuminata* W. Smith as the lectotype and restored the generic status of *Tryblionella*, which includes most species traditionally placed in the following four sections of the genus *Nitzschia* Hassall: *Tryblionella*, *Circumsutae*, *Apiculatae* and *Pseudotryblionella*. This proposal was not accepted by KRAMMER & LANGE–BERTALOT (1997) who considered that the criteria to distinguish the different sections of *Nitzschia* were not clear. WITKOWSKI et al. (2004)

published four new species of *Nitzschia* sect. *Tryblionella* and did not accept the genus *Tryblionella* as well. Twelve years later, however, WITKOWSKI reconsidered his position on the status of *Tryblionella* and described a new species *Tryblionella gaoana* Witkowski et Li Ch. (WITKOWSKI et al. 2016). Later, BERTOLLI et al. (2019) described two new species of *Tryblionella* from Brazil. At present, AlgaeBase lists 69 accepted species names, 10 accepted varieties, and six accepted formae within the genus *Tryblionella* (GUIRY & GUIRY 2021).

The circumscription of a genus is usually based primarily on its type species, modified and expanded by the features of others species subsequently included. The lectotype species *Tryblionella acuminata* was originally described by SMITH (1853) as a species which valves have a linear outline with acuminate apices, a length range of 63–107 µm, and striae interrupted by an axial sternum with a density of 16 in 10 µm. Later, KRAMMER & LANGE–BERTALOT (1988), HARTLEY (1996), and WITKOWSKI et al. (2000) provided further light microscope (LM) observations for *T. acuminata*, such as the 1:1 ratio of its transapical ribs to striae. More recently, BERTOLLI et

al. (2020) provided scanning electron microscope (SEM) images of a single valve of *T. acuminata* (fig. 5 A–C). To our knowledge, the ultrastructure of *T. acuminata* has not been thoroughly investigated to date so that many important characters, such as the marginal ridge and the fibulae, have not been clearly illustrated or described yet.

ROUND et al. (1990) listed the shapes and structures of both fibulae and marginal ridge, stria types, the presence/absence of a sternum, and the undulation of the valve face along the apical axis, as diagnostic combinations of characters for the genus *Tryblionella*. Although the lectotype species *T. acuminata* has not been thoroughly investigated, it is likely that the 85 *Tryblionella* taxa currently listed in AlgaeBase all fit this loose circumscription. The fibula density, presence/absence of an axial sternum, and undulation of the valve face have been usually documented for the accepted *Tryblionella* taxa because these characters or character states can be readily observed under LM. On the other hand, both the fibulae whether apically elongate, striate, stubby, or bar-like, and the marginal ridge whether high, low, perforated, or unperforated, can only be revealed using SEM. Even for those taxa investigated under SEM, many fine details of the fibulae and the marginal ridges have not been revealed yet (Table 1). This is especially the case for the marginal ridge, which was defined in Ross et al. (1979) as “a ridge between the valve face and the valve mantle, continuous or interrupted, perforated or solid”, which may be an important character for the genus *Tryblionella*.

MANN et al. (2021) reassessed the diatom family Bacillariaceae and found that the *Tryblionella* taxa they included in their molecular analyses were not grouped within one clade. Instead, only three *Tryblionella* species (*T. apiculata* W. Gregory, *T. hungarica* (Grunow) Frenguelli, *T. gaoana*) were grouped within one clade (clade 5B) in their maximum-likelihood tree that was based on four genes (MANN et al. 2021, p. 7, fig. 3). These authors concluded that to achieve monophyly for the genus *Tryblionella* major changes in its circumscription was required.

Lake Qinghai is China’s largest inland brackish-water lake. Its diatom flora has been investigated since 1979 (e.g. Lanzhou Institute of Geology and Chinese Academy of Sciences 1979, YAO et al. 2011, PENG et al. 2013). These publications provided lists of diatom taxa but lacked any useful illustrations (drawings or micrographs) for these taxa. Recently however, new species have been described and well-illustrated with LM and SEM images (PENG et al. 2014, 2016; LIU et al. 2020; DENG et al. 2021; LONG et al. 2022; YUAN et al. 2022), suggesting that Lake Qinghai like other large ancient lakes, hosts endemic diatoms. In this paper another new species from Lake Qinghai, *Tryblionella qinghainensis* sp. nov. is described. In addition, emended descriptions of two known species of *Tryblionella* species also found in Lake Qinghai, are provided and the characters that are important for the taxonomy of this genus are discussed.

MATERIAL AND METHODS

Geographically, Lake Qinghai is located between longitudes 99°36’ and 100°47’, and latitudes 36°32’ and 37°15’ in Qinghai province, China. It is the largest inland brackish-water lake in China. The lake has a surface water area of ca. 4294 km² and the altitude of its surface level is ca. 3200 m a.s.l. Its climate belongs to the plateau continental climate. The average annual temperature is ca. –0.7 °C, the annual precipitation and the annual evaporation in the lake’s region are within 319–395 mm and 800–1000 mm respectively (LUO et al. 2017). More than 50 rivers/streams run into Lake Qinghai and there is no outlet to discharge the lake water as Lake Qinghai is hydrologically closed. The lake average and maximum water depths are 18.3 and 26.6 m, respectively. The average values for alkalinity and pH are 25.6 mmol.l^{–1} and 9.2 respectively (PENG et al. 2014). The salinity is relatively stable, varying between 9–12‰ (SUN et al. 2023). There is a three-month period of ice-cover (middle November to middle February) for Lake Qinghai so that the growth period for diatoms is mainly from May to October. The three sampling sites selected in Lake Qinghai for this study are the same as in YUAN et al. (2022, fig. 1). At these sites, there are many submerged stones with a yellow-brown surface which indicates that diatoms are growing abundantly on them. Each picked stone was placed on a plastic plate and its surfaces brushed using a toothbrush. The brushed-off diatoms were then washed onto the plate and transferred into 100 ml sampling bottles and fixed with 70% ethanol. Two bottles of diatom sample were collected for each sampling site. Together with details on the sites of collection, temperature, pH, and conductivity were measured in situ with a portable multimeter (HQ40D, HACH Company).

The samples were processed (cleaned of organic material) for microscope examination using 10% HCl and 30% H₂O₂. Permanent slides were prepared using the mountant Naphrax®. These slides were examined, and specimens photographed using a Leica DM3000 light microscope and a Leica MC190 HD digital camera. The holotype slide is deposited in the Herbarium of Jishou University, Hunan, People’s Republic of China (JIU) (Herbarium acronyms follow Index Herbarium <http://sweetgum.nybg.org/science/ih/>).

Samples were further examined under the SEM. Several drops of the selected cleaned diatom material were air-dried onto glass coverslips. Coverslips were attached to aluminium stubs using double-sided conductive carbon strip and sputter-coated with platinum (Cressington Sputter Coater 108auto, Ted Pella, Inc.). Samples were examined and imaged using a field emission scanning electron microscopy (FE-SEM) Sigma HD (Carl Zeiss Microscopy) available at Huaihua University, China. Diatom terminology largely follows ROUND et al. (1990), SIVER & HAMILTON (2005).

RESULTS

Tryblionella qinghainensis Bing Liu et Rioual sp. nov. (Figs 1–24)

Description

LM (Figs 1–6): Valve linear-lanceolate, slightly constricted in the middle on both sides of valve (Figs 1, 3, two wavy arrows respectively), with acute to apiculate apices. Valve dimensions (measured specimens, n = 19): length range 67.6–92.6 µm, width range 10.1–12.6 µm at the level of the mid-valve constriction. A broad axial sternum in the middle of valve surface, occupies

Table 1. Characters of *Tryblionella* taxa and three species of *Nitzschia* sect. *Tryblionella* investigated under SEM to date.

Taxon	Transapical ribs	Striae	Axial sternum	Fibulae	Marginal ridge	References
<i>T. acuminata</i>	Interrupted by axial sternum	Uniseriate	Present, occupying ca. 1/3 of valve width	Striae to fibulae ratio 1:1	Present, details unknown	SMITH 1853, KRAMMER & LANGE-BERLOT 1988, BERTOLLI et al. 2020
<i>T. adducta</i>	Across valve surface	Uniseriate	Absent	Striae to fibulae ratio 1:1	Unknown	Im et al. 2020
<i>T. calida</i>	Across valve surface	Uniseriate or biseriate	Absent	Regularly distributed	Present, details unknown	Bertolli et al. 2020
<i>T. circumscuta</i>	Absent	Uniseriate	Absent	Details unknown	Present, details unknown	Bertolli et al. 2020
<i>T. confusa</i>	Conspicuous on one side of valve face	Uniseriate	Absent	Details unknown	Present, with slit-like openings	Bertolli et al. 2019
<i>T. debilis</i>	One side with transverse ribs	Multiseriate	Absent	Details unknown	Present, with slit-like openings	MACHADO et al. 2023
<i>T. gaoana</i>	Interrupted by axial sternum	Uniseriate	Present	Rib-like	Unknown	Witkowski et al. 2016
<i>T. hungarica</i>	Interrupted by axial sternum	Biseriate	Present	Irregularly distributed	Present, details unknown	BERTOLLI et al. 2020
<i>T. ornata</i>	Across valve surface	Uniseriate	Absent	Details unknown	Present, with slit-like openings	Bertolli et al. 2019
<i>T. persuadens</i>	Across valve surface	Uniseriate, sometimes biseriate	Absent	Robust	Present, details unknown	Cavalcante et al. 2013
<i>T. perversa</i>	Across valve surface	Uniseriate	Absent	Details unknown	Present, details unknown	Bertolli et al. 2020
<i>T. plana</i>	Interrupted by axial sternum	Uniseriate	Present	Irregularly distributed	Present, details unknown	VAN DE VUIVER et al. 2021
<i>T. plana</i> var. <i>fennica</i>	Absent	Uniseriate	Present	Details unknown	Present, details unknown	Siver & Hamiltom 2005
<i>T. scalaris</i>	Across valve surface	Uniseriate	Absent	Details unknown	Present, details unknown	Siver & Hamiltom 2005
<i>T. victoriae</i>	Across valve surface	Uniseriate	Absent	Striated fibulae	Simple, very low	Bertolli et al. 2020
<i>Nitzschia busckbeckii</i>	Interrupted by axial sternum	Uniseriate	Present	Not striated fibulae	Simple, low	Witkowski et al. 2004
<i>N. ligowskii</i>	Interrupted by axial sternum	Uniseriate	Present	Not striated fibulae	Absent	Witkowski et al. 2004
<i>N. schweikertii</i>	Interrupted by axial sternum	Uniseriate	Present	Not striated fibulae	Present, very low	Witkowski et al. 2004

ca. 1/3 of the valve width, extends from pole to pole. Striae parallel becoming radiate approaching the apices, interrupted by sternum, 16–18 in 10 μm . Fibulae visible, short, 8–10 in 10 μm . The ratio of stria density to fibula density ca. 1.9:1 (not 2:1, Figs 3, 5, indicated by three arrows).

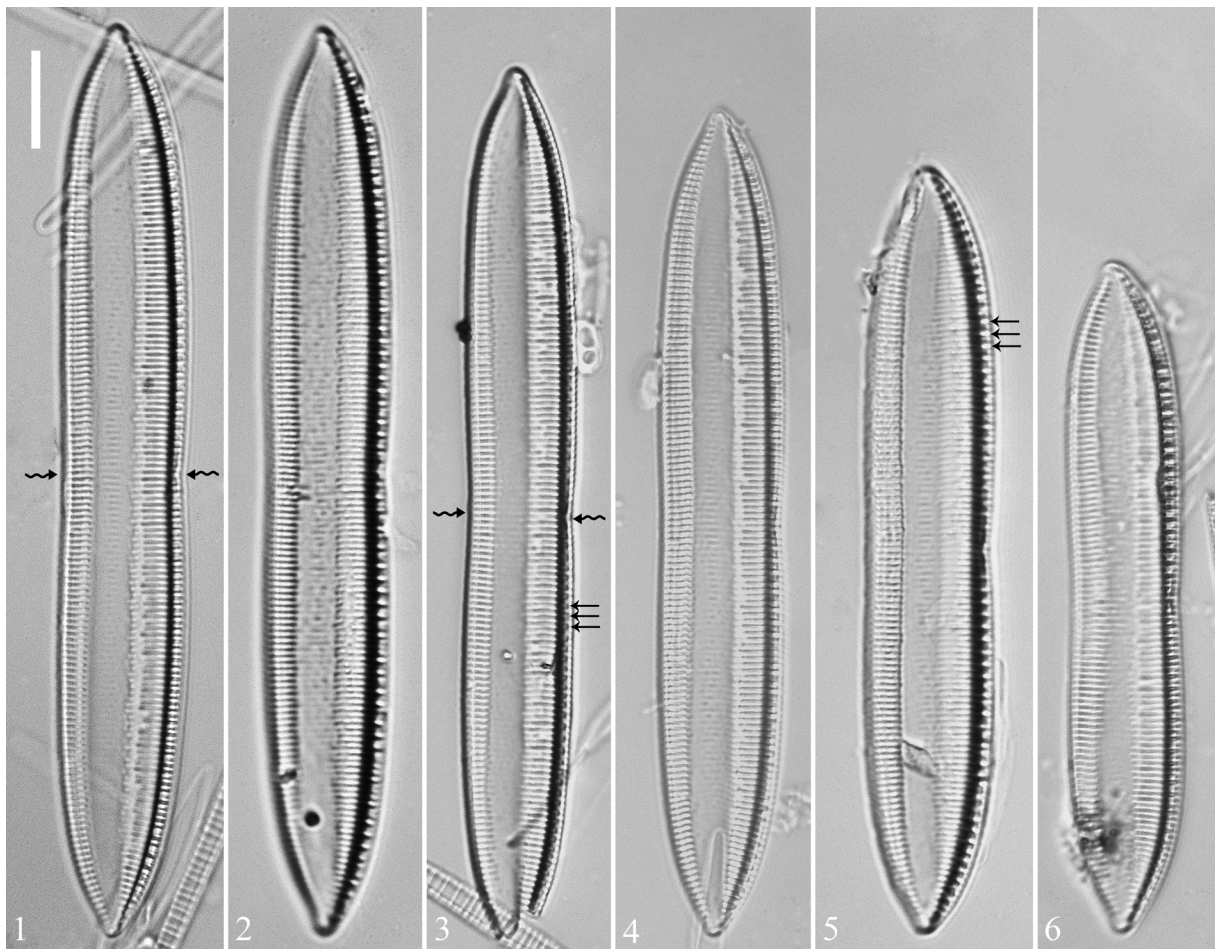
SEM (Figs 7–24): Axial sternum distinct, depressed, extending from pole to pole (Fig. 7). Transapical ribs elevated, parallel except approaching each apex, where becoming radiate; striae located between two adjacent transapical ribs. Striae interrupted by axial sternum but some transapical ribs continuing onto axial sternum (Fig. 8, two wavy arrows). Marginal ridge a simple standing lamella, erected along the valve margin opposite to the raphe canal, without perforations (Figs 8–12, arrows). Axial sternum broad with dense granules on its surface, not centrally situated, i.e., closer to the marginal ridge side. Fibula short, squat, not striate (Figs 13–20). Mostly, one fibula corresponding to two ribs (Figs 15–17, two wavy arrows respectively; Figs 19, 20, wavy arrow respectively), sometimes one fibula corresponding to one rib (Figs 15–18, arrows; Figs 19, 20, arrow respectively). Thus, the overall ratio of transapical ribs to fibulae is

not 2:1. Central nodule present, inconspicuous (Figs 13, 14, 21, arrow respectively). Striae interrupted by axial sternum internally, mostly mixed, i.e., within the same striae, part uniseriate, part biseriate which locations are variable (Figs 17–20, 22–24), whereas near each apex several uniseriate striae occurred. On the proximal mantle, thickened ribs arranged in the same pattern as those on the valve surface (Fig. 22, three arrows) and dense granules produced on the abvalvar margin of proximal mantle (Figs 22–24, wavy arrow respectively). Helictoglossa small (Fig. 24, arrow).

Diagnosis: Valve outline linear–lanceolate, 67.6–92.6 μm long, 10.1–12.6 μm wide; sternum occupies ca. 1/3 of the valve width; a ca. 1.9:1 ratio of the stria density to the fibula density; alveolate striae and a simple standing lamella–like marginal ridge which lacks perforations.

Holotype: Slide DIA2024002, specimen circled on slide, illustrated here as Fig. 1, deposited in the Herbarium of Jishou University (JIU), China. Registration: <http://phycobank.org/> (pending).

Type locality: A sampling point near the lakeshore



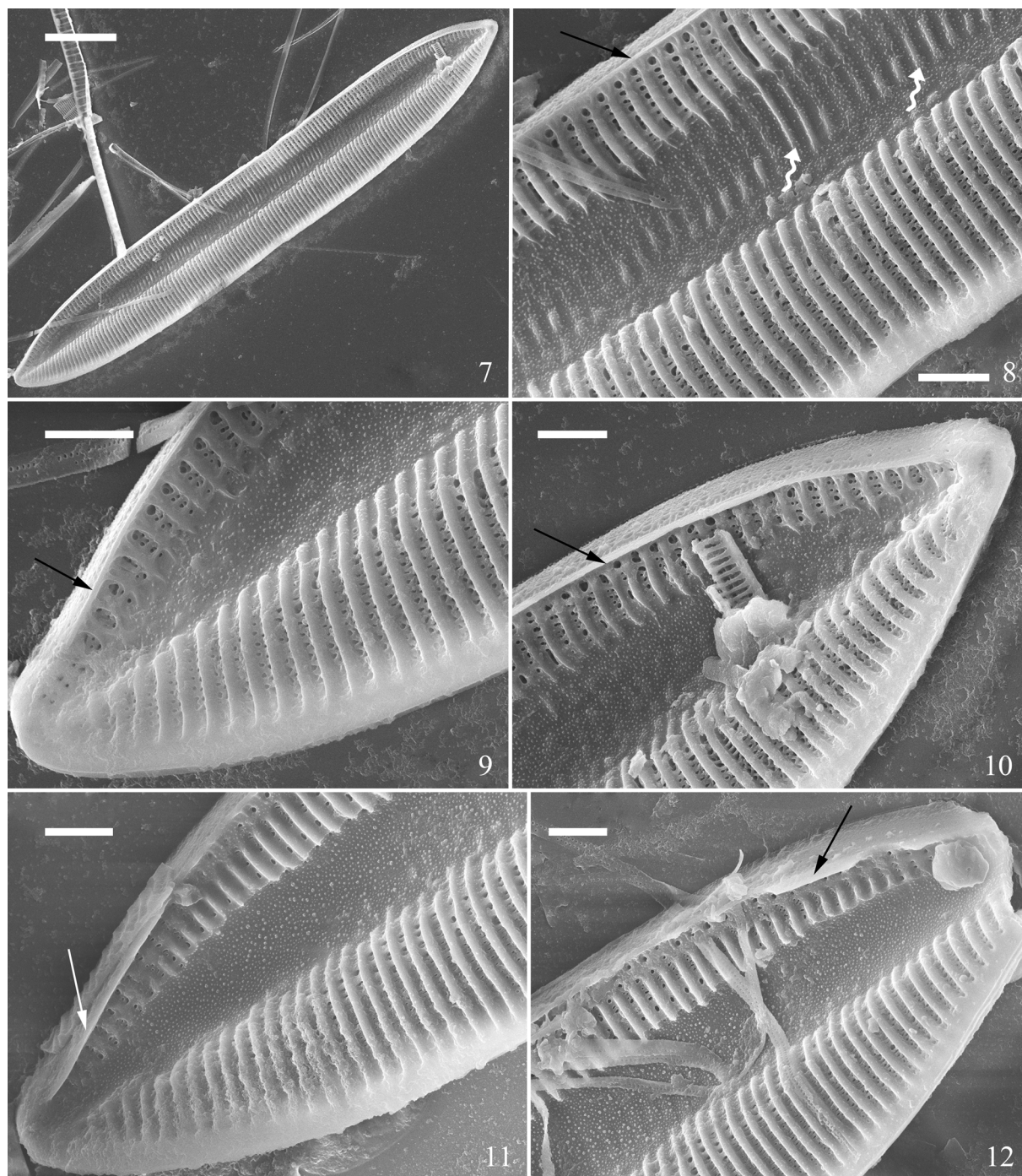
Figs 1–6. *Tryblionella qinghainensis* sp. nov., LM: (1–6) six valves showing a size diminution series, note that some valves are slightly constricted in their middle (Figs 1, 3, two wavy arrows respectively), with a broad axial sternum from pole to pole, and an overall ratio of transapical ribs to fibulae of 1.9:1 (Figs 3, 5, three arrows, respectively); (1) micrograph of holotype specimen. Scale bar 10 μm .

of Lake Qinghai (36°50'34" N, 99°42'39" E, 3210 m a.s.l.), Qinghai Province, China (collected by Bing Liu, July 19, 2019).

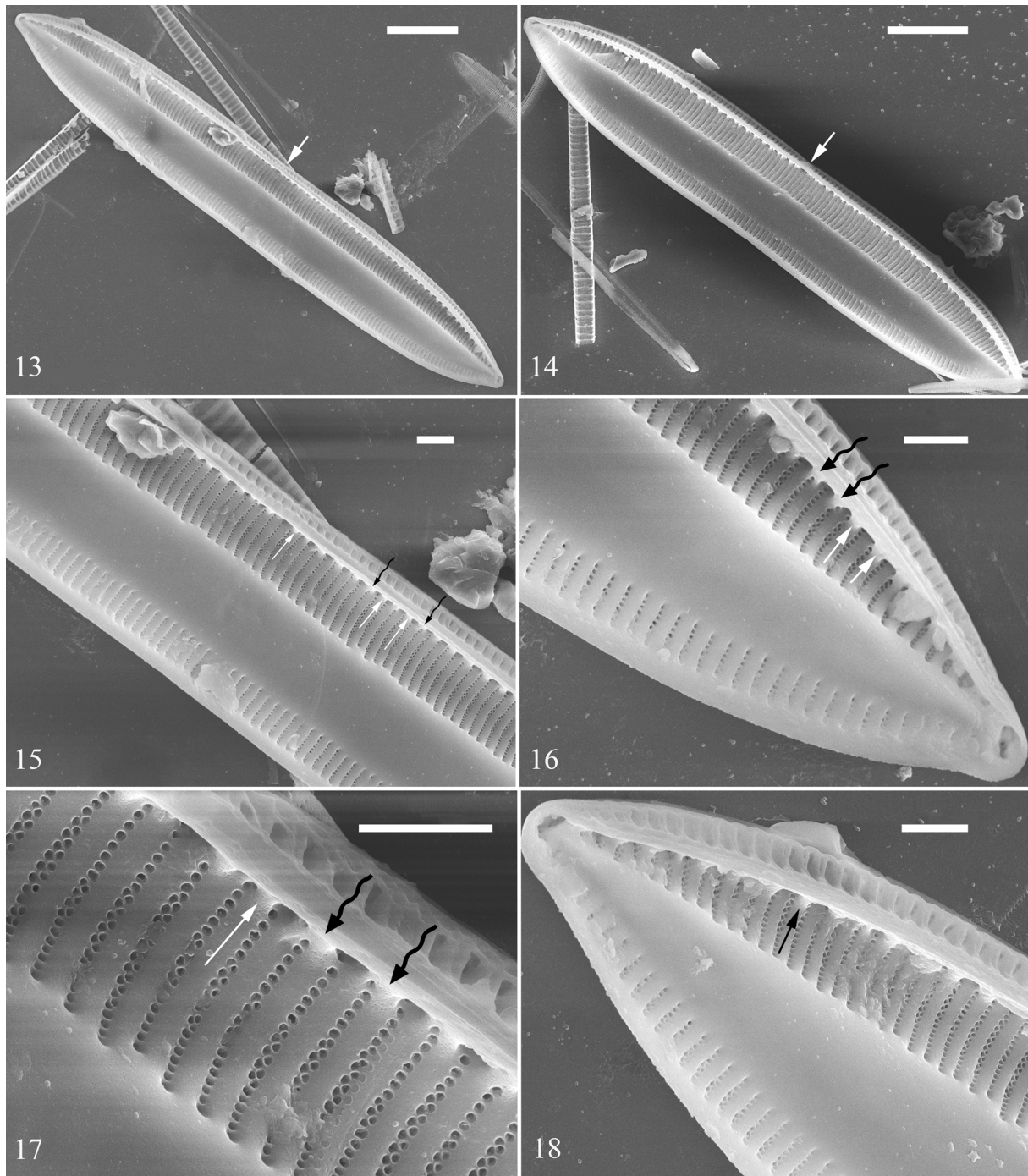
Habitat: The following environmental parameters were measured in the field. Conductivity was 16.3 ± 0.8 mS.cm⁻¹, pH was 9.14 ± 0.01 , and water temperature was 15.5 ± 0.3 °C. According to above data and because Lake Qinghai is a brackish–water lake, *T. qinghaiensis* sp. nov. is most likely a brackish–water diatom species.

So far, *T. qinghaiensis* has only been found in the type locality. The associated taxa include *Ctenophora sinensis* Bing Liu et D.M. Williams, *Diatoma sinensis* Bing Liu et Rioual, *Entomoneis paludosa* (W. Smith) Reimer, *E. qinghaiensis* Bing Liu et D.M. Williams, *E. sinensis* Bing Liu et D.M. Williams, *Pinnularia qinghaiensis* Bing Liu et S. Blanco, among others.

Etymology: Named after Lake Qinghai where the species was found.



Figs 7–12. *Tryblionella qinghaiensis* sp. nov., external view, SEM: (7) a complete valve, note the sunken axial sternum; (8–10) details from Fig. 7, note simple marginal ridge without perforations (arrows), some elevated ribs across axial sternum (Fig. 8, two wavy arrows), and granules present on axial sternum; (11, 12) apices from two other valves, note the marginal ridge (arrows) and granules on axial sternum. Scale bars 10 μ m (7), 2 μ m (8–12).



Figs 13–18. *Tryblionella qinghainensis* sp. nov., valve internal view, SEM: (13, 14) two complete valves, note the valves are slightly constricted centrally (two arrows) and the striae are interrupted by the broad axial sternum; (15–18) details from Fig. 13, note that mostly one squat fibula corresponds to two ribs (Figs 15, 16, 17, two wavy arrows respectively), sometimes one squat fibula corresponds to only one rib (arrows). Scale bars 10 μ m (13, 14), 2 μ m (15–18).

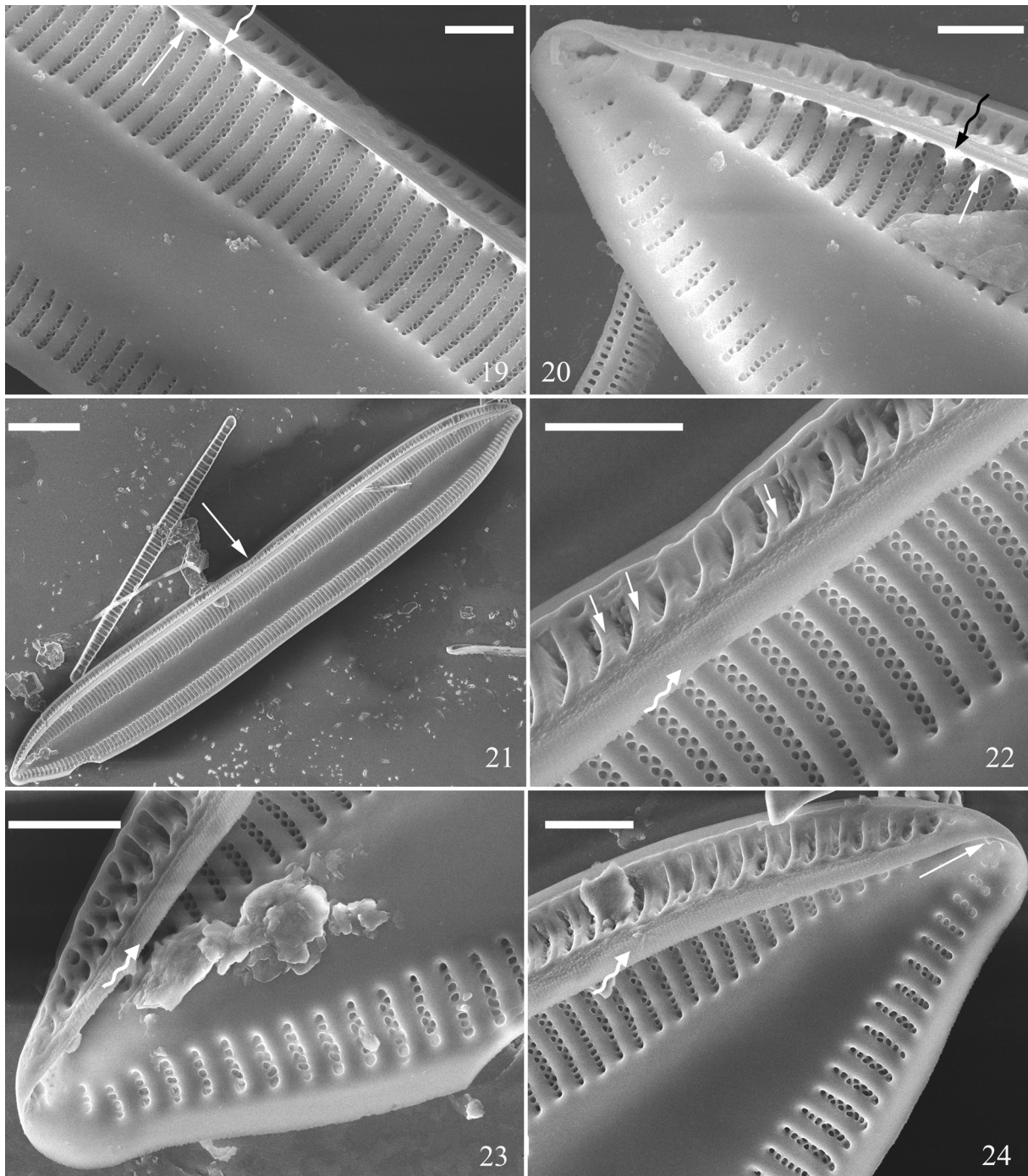
***Tryblionella apiculata* W. Gregory emend. Liu et Rioual (Figs 25–46)**

Description

LM (Figs 25–34): Valve panduriform with apiculate apices. Valve dimensions (measured specimens, $n = 34$): length range 23–45 μ m, width range 4.5–6.5 μ m at constricted middle parts. Axial sternum produced in the middle of valve surface, occupying ca. 1/4 of the valve width, extending from pole to pole. Striae parallel except

approaching apices, where becoming radiate. Striae interrupted by axial sternum, 15–17 in 10 μ m. Fibulae visible, short, 15–17 in 10 μ m. Transapical ribs and fibulae equal in number and aligned with one another, thus the ratio of transapical ribs to fibulae is 1:1.

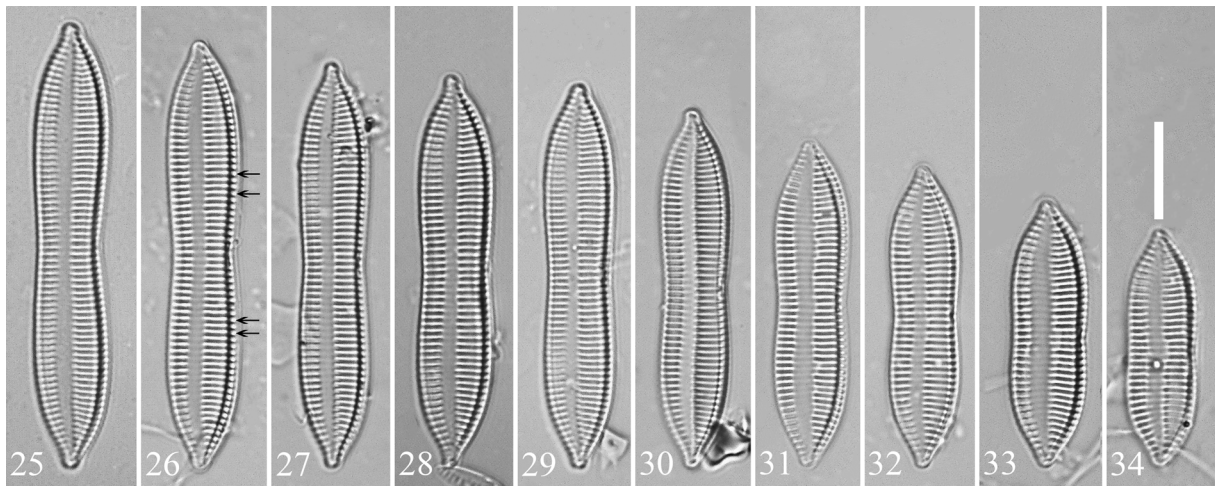
SEM (Figs 35–46): Axial sternum with granules on its surface, depressed, extending from pole to pole. Transapical ribs elevated, parallel except approaching each apex, where becoming radiate; striae located in two adjacent transapical ribs, multiserial alveolate. Marginal



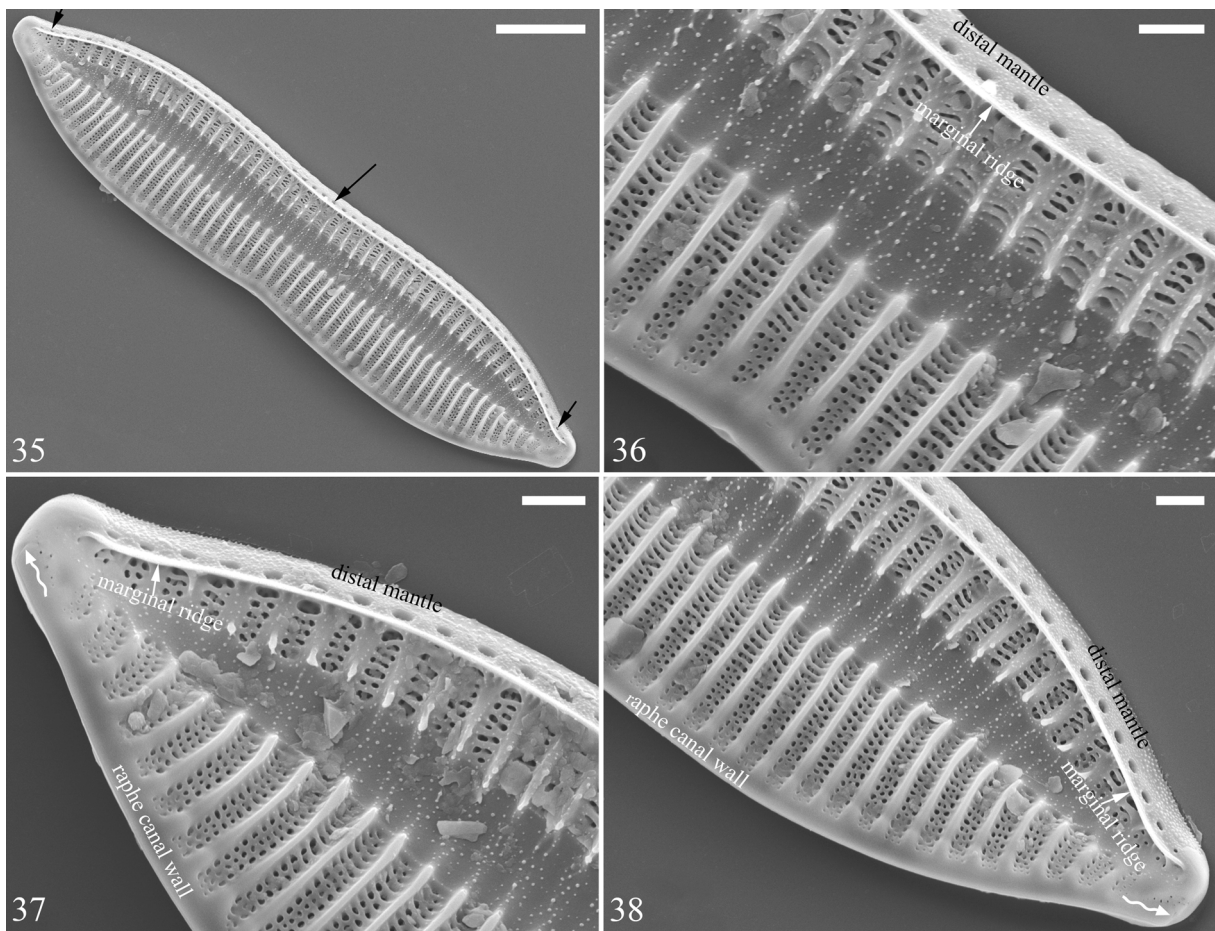
Figs 19–24. *Tryblionella qinghaiensis* sp. nov., internal view, SEM: (19, 20) details of another valve, note mostly one squat fibula corresponds to two ribs (wavy arrows), sometimes one squat fibula corresponds to only one rib (arrows); (21) a complete valve; (22–24) details from Fig. 21, note the costae on the proximal mantle (Fig. 22, three arrows) and granules on the abvalvar mantle margin (wavy arrows), mixed striae, and small helicoglossa (Fig. 24, arrow). Scale bars 2 μ m (19, 20, 22–24), 10 μ m (21).

ridge a simple standing lamella erected along the valve margin opposite to the raphe canal, no perforations in it, but a distinct row of large, rounded pores produced along the junction between the marginal ridge and the distal mantle (Fig. 35, three arrows; Figs 36–39). Distal mantle simple, shallow. Distal raphe fissures curved onto valve surface (Figs 37, 38, wavy arrow respectively; Figs 41, 42, arrow respectively). Valvocopula open (Fig. 42, wavy arrow), a row of poroids dividing pars interior from pars exterior (Fig. 41, double-headed arrow), and

a row of serrated projections corresponding to each rib internally (Fig. 40, three arrows). Internally, transapical ribs and striae interrupted by axial sternum (Figs 43–46). Inner openings of areolae covered by hymens (Figs 45, 46, two arrows respectively). The striae and costae in the proximal mantle having the same pattern as that on the valve face (Figs 44–46). Rows of granules produced on the abvalvar margin of the proximal mantle (Figs 44–46, abvalvar margin respectively). Helictoglossae small (Figs 45, 46, wavy arrow respectively).



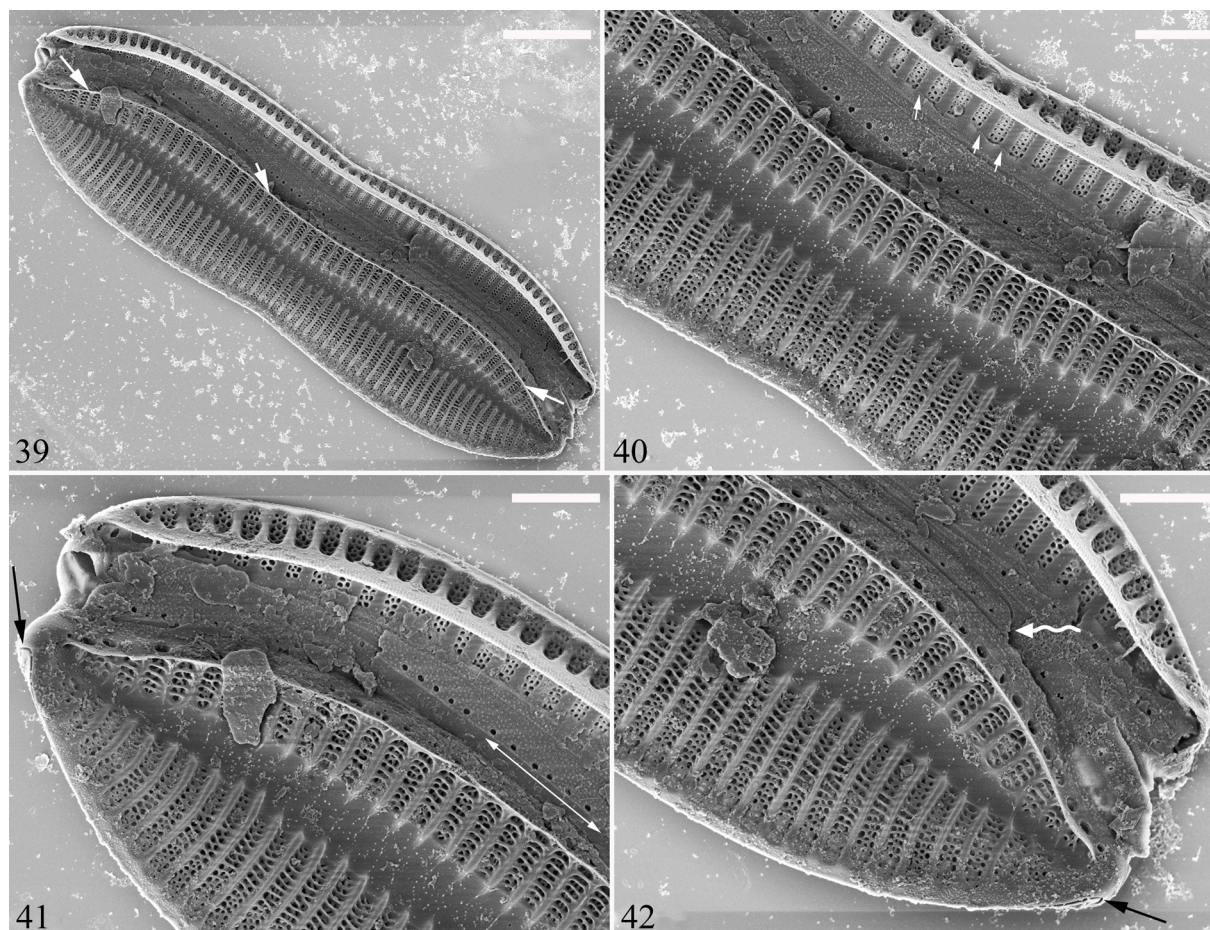
Figs 25–34. *Tryblionella apiculata*, LM: (25–34) ten valves showing a size diminution series, note panduriform valve outline, apiculate apices, axial sternum, and 1:1 ratio of transapical ribs to fibulae (indicated in Fig. 26, four arrows). Scale bar 10 μ m.



Figs 35–38. *Tryblionella apiculata*, valve external view, SEM: (35) a complete valve, note a row of large rounded pores produced along the junction between the marginal ridge and the distal mantle (three black arrows); (36–38) details from Fig. 35, note the low marginal ridge without perforations, granulated distal mantle, raphe canal wall without perforations, curved distal raphe fissures (Figs 37, 38, wavy arrow respectively), mostly triseriate alveolate striae, and elevated transapical ribs. Scale bars 5 μ m (35), 1 μ m (36–38).

Comments: *Tryblionella apiculata* is characterized by its panduriform valve outline, apiculate apices, distinct axial sternum, and simple, low marginal ridge with a distinct row of large, rounded pores produced along

the junction between the marginal ridge and the distal mantle. It was commonly found in the surface sediment collected in the brackish water of Lake Qinghai. Except for the original description of W. GREGORY (1857), there



Figs 39–42. *Tryblionella apiculata*, SEM: (39) a dismantled frustule, note the low marginal ridge (three arrows) and a row of pores located along the junction between the marginal ridge and the distal mantle; (40–42) details from Fig. 39, note the open nature of valvocopula (Fig. 42, wavy arrow), a row of poroids dividing pars interior from pars exterior (Fig. 41, white double-headed arrow), a row of serrated projections at the advalvar edge of valvocopula (Fig. 40, three arrows), and the curved distal raphe fissures (Figs 41, 42, black arrow respectively). Scale bars 5 μ m (39), 2 μ m (40–42).

are few reports of *T. apiculata* in the literature. Specimens of *Tryblionella apiculata* found in Lake Qinghai fit well with the original description and the report of PENTON (2013) who provided some LM images and one SEM image. The ultrastructure of its marginal ridge and distal mantle is revealed and described here for the first time.

Tryblionella levidensis W. Smith emend. Liu et Rioual (Figs 47–71)

Description

LM (Figs 47–59): Valve linear–lanceolate to elliptic–lanceolate with acute apices. Valve dimensions (measured specimens, $n = 29$): length range 32–59 μ m, width range 11–15 μ m at constricted middle parts. Valve midline almost bisecting the valve face into two different halves and no axial sternum produced. Fibulae visible, short, rectangular (e.g. Fig. 48, four arrows), 8–10 in 10 μ m. Transapical ribs elevated and more evident on the half near the marginal ridge than those on the other half near the raphe canal (e.g. Fig. 50). Marginal ridge discernible (e.g. Fig. 51).

SEM (Figs 60–71): Valve face divided by a longitudinal fold along the valve midline into two different halves:

one half close to the raphe canal lowered (Fig. 61, black double-headed arrow), the other half near the marginal ridge elevated (Fig. 61, white double-headed arrow). Transapical ribs parallel, some across valve surface continuously, some interrupted at the middle longitudinal fold (Figs 60–65). Three or four rows of striae located in between two adjacent transapical ribs (Figs 64, 65). Transapical ribs mostly not extending to the valve margin bearing the marginal ridge (Figs 60–64). Along the raphe canal wall, a row of lamellar ribs produced from pole to pole (Fig. 65, six arrows). Marginal ridge a high standing lamella, composed of a bottom part bearing a row of large, fenestra-like pores (Figs 60–64) which become smaller near each apex, and a top part with slightly undulate edge. A row of tiny poroids located along the junction between the marginal ridge and the distal mantle (Figs 63, 64, black double-headed arrow respectively). Distal mantle simple, smooth, without ornamentations (Figs 62–64). A row of tiny poroids produced in the canal raphe wall (Figs 63, 65, arrowheads). Central raphe endings close together, slightly expanded (Fig. 65, two wavy arrows); distal raphe fissure curved (Fig. 63, arrow). Internally,

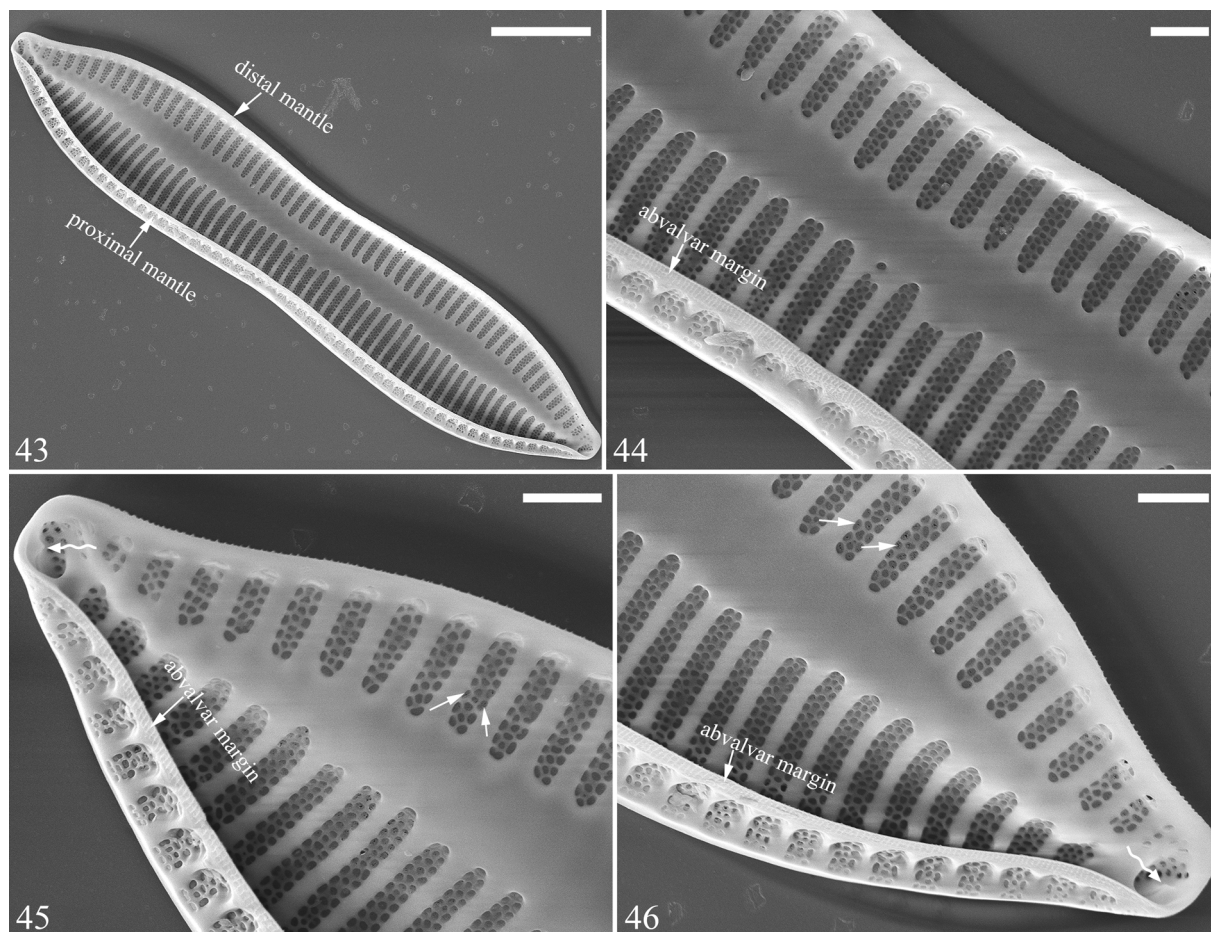
Table 2. Morphological features used for comparison of *Tryblionella qinghaiensis* sp. nov. with three other *Tryblionella* species.

Feature	<i>T. qinghaiensis</i> sp. nov.	<i>T. acuminata</i>	<i>T. acuminata</i>	<i>T. apiculata</i>	<i>T. apiculata</i>	<i>T. levidensis</i>	<i>T. levidensis</i>
Reference	This paper	WITKOWSKI et al. 2000	BERTOLLI et al. 2020	This paper	PENTON 2013	This paper	WITKOWSKI et al. 2000
Valve outline	Linear–lanceolate with acute to apiculate apices	Linear with acutely rounded apices	Linear–lanceolate with cuneate to subrostrate apices	Panduriform with apiculate apices	Panduriform with apiculate apices	Broad–lanceolate to elliptic–lanceolate with acute apices	Broadly linear–elliptic with cuneate apices
Valve dimensions (µm)	Length: 67–93; Width: 10.1–12.6	Length: 50–100; Width: 13–18	Length: 42.8–49.5; Width: 7.8–10.8	Length: 23–45; Width: 4.5–6.5	Length: 39–47; Width: 5–7	Length: 32–59; Width: 11–15	Length: 18–65; Width: 8–23
Stria type and density	Mixed striae, i.e., uniseriate, part biseriate, 16–18 in 10 µm	12–16 in 10 µm	Uniseriate, 13–16 in 10 µm	Multiseriate, 15–17 in 10 µm	Multiseriate, 15–17 in 10 µm	Uniseriate, 39–41 in 10 µm; coarse transapical ribs 8–14 in 10 µm	35–36 in 10 µm; coarse transapical ribs 5–12 in 10 µm
Fibula shape and density	Squat, short, not striate, 8–10 in 10 µm	12–16 in 10 µm	13–16 in 10 µm	15–17 in 10 µm	15–17 in 10 µm	Apically elongate, striate, short, 8–10 in 10 µm	6–12 in 10 µm
Ratio between striae and fibulae	Ca. 1.9:1	1:1	1:1	1:1	1:1	More than 3:1	More than 3:1
Marginal ridge	A simple standing lamella lacking ornaments	No data	Likely a simple standing lamella	A simple standing lamella with a row of large poroids located along the junction between the marginal ridge and the distal mantle	A simple standing lamella with a row of large poroids located along the junction between the marginal ridge and the distal mantle	A standing lamella perforated by a row of fenestra-like poroids and with a row of tiny poroids located along the junction between the marginal ridge and the distal mantle	No data
Proximal mantle	Costae alternate with striae	No data	No data	Costae alternate with striae	Costae alternate with striae	Costae alternate with striae	No data
Distal mantle	Solid, no costae and striae	No data	No data	Solid, no costae and striae	Solid, no costae and striae	Solid, no costae and striae	No data

a fold present along the valve midline (Fig. 66, white double-headed arrow). Proximal mantle composed of advalvar part and abvalvar part; advalvar part composed of lamellar ribs and elongate slits openings, abvalvar part lacking any ornamentations (Figs 67, 69, 71, black arrow respectively). Striae parallel except approaching apex, where becoming radiate, ca. 39–41 in 10 µm

(n=4). Internal openings of areolae rounded, rimmed (Figs 68–71). Fibulae short, apically elongated, striate (Figs 70, 71, two wavy arrows).

Comments: *Tryblionella levidensis* is characterized by its linear–lanceolate to elliptic–lanceolate valve outline, distinct transapical ribs elevated on two different halves of the valve, the lack of axial sternum, and a high marginal



Figs 43–46. *Tryblionella apiculata*, valve internal view, SEM: (43) a complete valve, note the striae are interrupted by the axial sternum, proximal mantle deeper than distal mantle; (44–46) details from Fig. 43, note the same patterns between the proximal mantle and the valve face, the multiserial alveolate striae covered by hymens (Figs 45, 46, two arrows respectively), the rows of granules on the abvalvar mantle margin, and small helicoglossae (Figs 45, 46, wavy arrow respectively). Scale bars 5 μm (43), 1 μm (44–46).

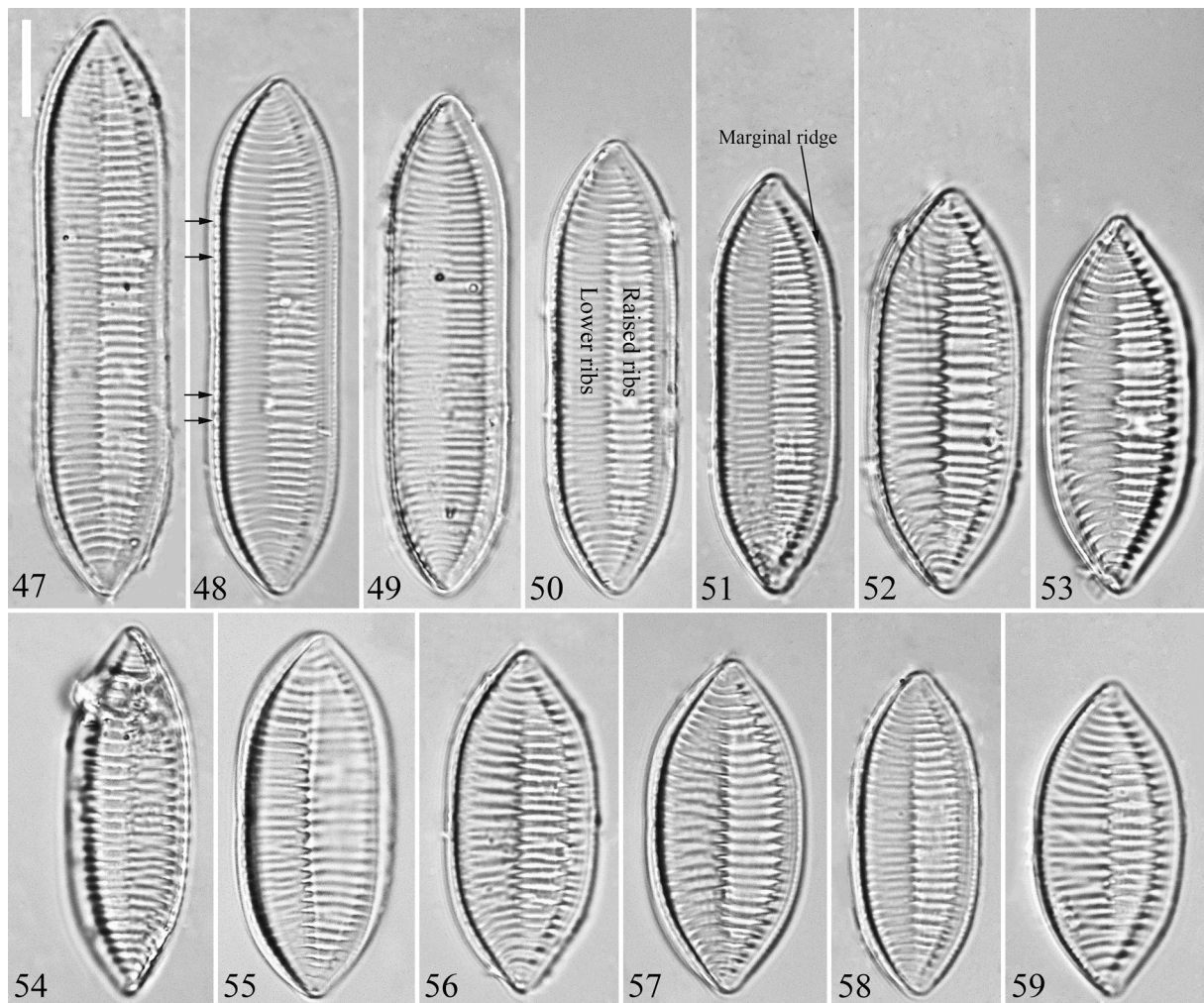
ridge composed of a bottom part bearing a row of large, fenestra-like pores and a top part with slightly undulate edge. *Tryblionella levidensis* is a brackish-water species according to the original description of SMITH (1856). KRAMMER & LANGE-BERTALOT (1988, fig. 28: 1–4) and WITKOWSKI et al. (2000, plate 180, figs 1–4) only provided four LM images for *T. levidensis* (as *Nitzschia levidensis* (W. Smith) Grunow) respectively, and the SEM morphological details, especially that of its marginal ridge, were unknown before this study. *Tryblionella levidensis* is very similar to *T. victoriae* under LM, especially for their smaller specimens. However, the former has much larger cells than the latter. Furthermore, the latter's marginal ridge is very low which is completely different from the former's (see Bertolli et al. 2020, p. 13, fig. 11).

DISCUSSION

Tryblionella qinghainensis sp. nov. is characterized by its linear-lanceolate valve outline, broad sternum, a ca. 1.9:1 ratio of the stria density to the fibula density,

alveolate striae, and a simple standing lamella-like marginal ridge which lacks perforations. Among the taxa of *Tryblionella*, the most similar species to *T. qinghainensis* is *T. acuminata* W. Smith which is a marine or brackish-water species according to the original description of SMITH (1853). Despite being the lectotype species of the genus *Tryblionella*, there are few reports of *T. acuminata*. KRAMMER & LANGE-BERTALOT (1988, fig. 34: 4–6) and WITKOWSKI et al. (2000, plate 188: 1–3) provided three LM images of *T. acuminata* respectively. The only SEM observations on *T. acuminata* are in PENTON (2013) and BERTOLLI et al. (2020). All these studies of *T. acuminata* confirmed that the ratio of its striae to its fibulae is 1:1, i.e., its stria density is equal to its fibula density (see Table 2), which excludes conspecificity with *T. qinghainensis*.

Both *Tryblionella apiculata* and *T. qinghainensis* have alveolate striae and a sternum interrupting the striae and transapical ribs. By contrast, *T. levidensis* does not possess alveolate striae (it has uniseriate striae) and does not bear a sternum to interrupt the striae and transapical ribs (there is only a fold along the midline of the valve) (Table 2). The structures of the marginal ridges and the distal mantle are different for the three



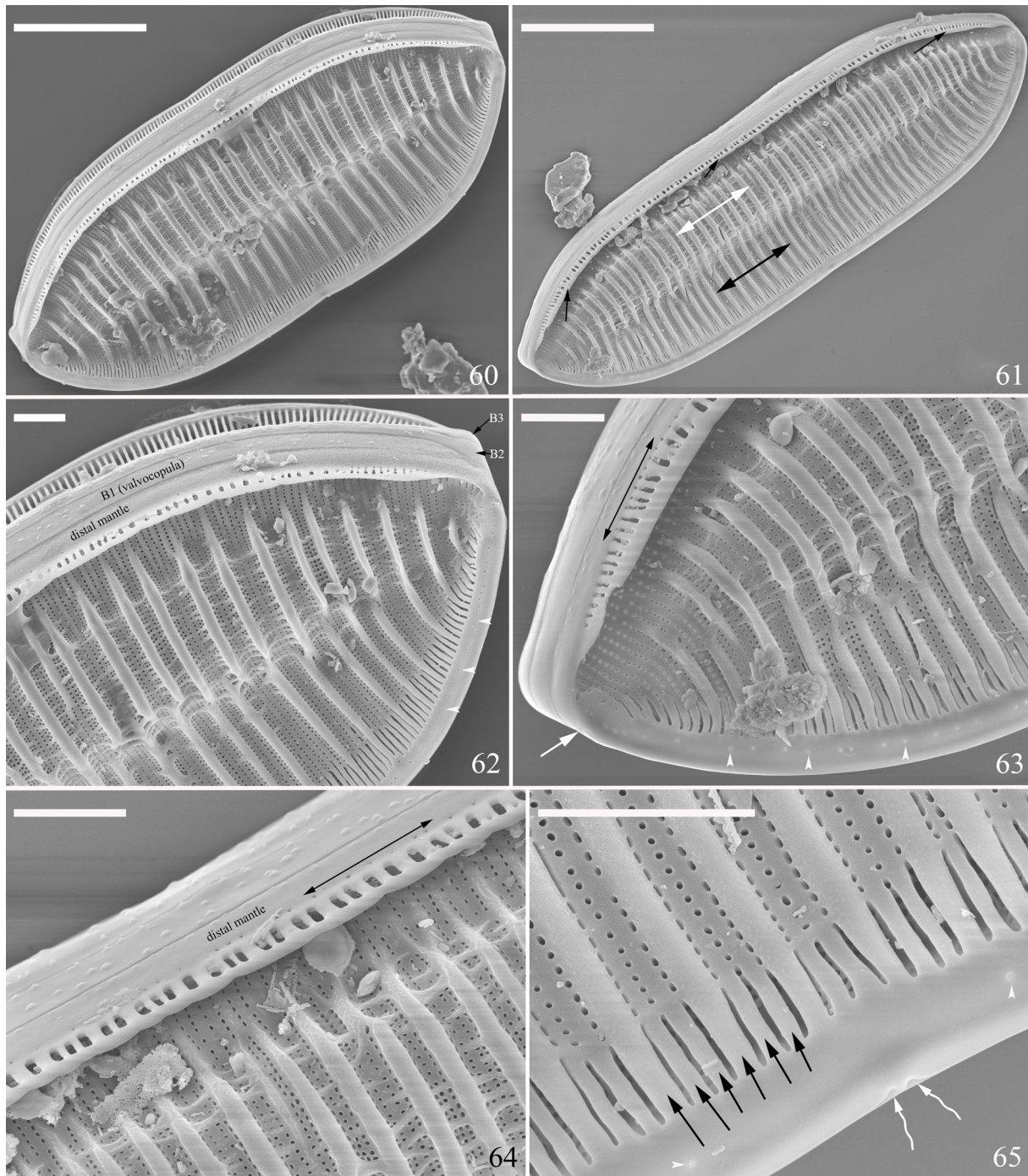
Figs 47–59. *Tryblionella levidensis*, LM: (47–59) thirteen valves showing a size diminution series, note lack of axial sternum, rectangular fibulae (Fig. 48, four arrows), valve midline bisects the valve face into two different halves (Fig. 50), and discernible marginal ridge (Fig. 51). Scale bar 10 μ m.

Tryblionella species discussed above. The marginal ridge in *T. levidensis* is more complex, being characterised by a high, standing lamella perforated by a row of fenestra-like pores and with a row of tiny poroids located along the junction between the marginal ridge and the distal mantle (Figs 60–64). On the other hand, the marginal ridge in *T. apiculata* is simple: a low, solid, standing lamella without perforations but with a row of large pores located along the junction between the marginal ridge and the distal mantle (Figs 35–42). The marginal ridge in *T. qinghainensis* is simple too: a low, solid, standing lamella without perforations and without poroids along the junction between the marginal ridge and the distal mantle (Figs 7–12). The structures of the marginal ridges and their junctions with the distal mantles are therefore highly variable and specific for these three *Tryblionella* species.

ROSS et al. (1979) defined the term of marginal ridge and ROUND et al. (1990) used it when they described the genus *Tryblionella*. Since then, however, the marginal ridge has often been neglected in the descriptions of new

Tryblionella species (see Table 1). For example, SIVER & HAMILTON (2005) noticed and mentioned the marginal ridges in *T. plana* var. *fennica* (p. 374, figs 62, 63) and *T. scalaris* (p. 376, figs 75, 76), but did not provide any descriptions in the text. BERTOLLI et al. (2019) illustrated the marginal ridges for *T. confusa* (p. 177, figs 16, 17) and *T. ornata* (p. 179, figs 32, 33), but did not mention the marginal ridges nor provided any descriptions in the text. Similarly, WITKOWSKI et al. (2016) did not mention the marginal ridge for *T. gaoana* when describing this species. One exception, is the study by CAVALCANTE et al. (2013) on *T. persuadens*, in which the authors mentioned the marginal ridge. However, the specimens illustrated in their SEM images (figs 14, 15) are not tilted enough to provide a clear view of the marginal ridge ultrastructure.

MANN et al. (2021) reassessed the genus *Tryblionella* and thought the modern and older definitions of *Tryblionella* need to be revised. As stated above, the current knowledge of the morphological structures of the established *Tryblionella* taxa is generally very incomplete. Our study indicates that it will be necessary,

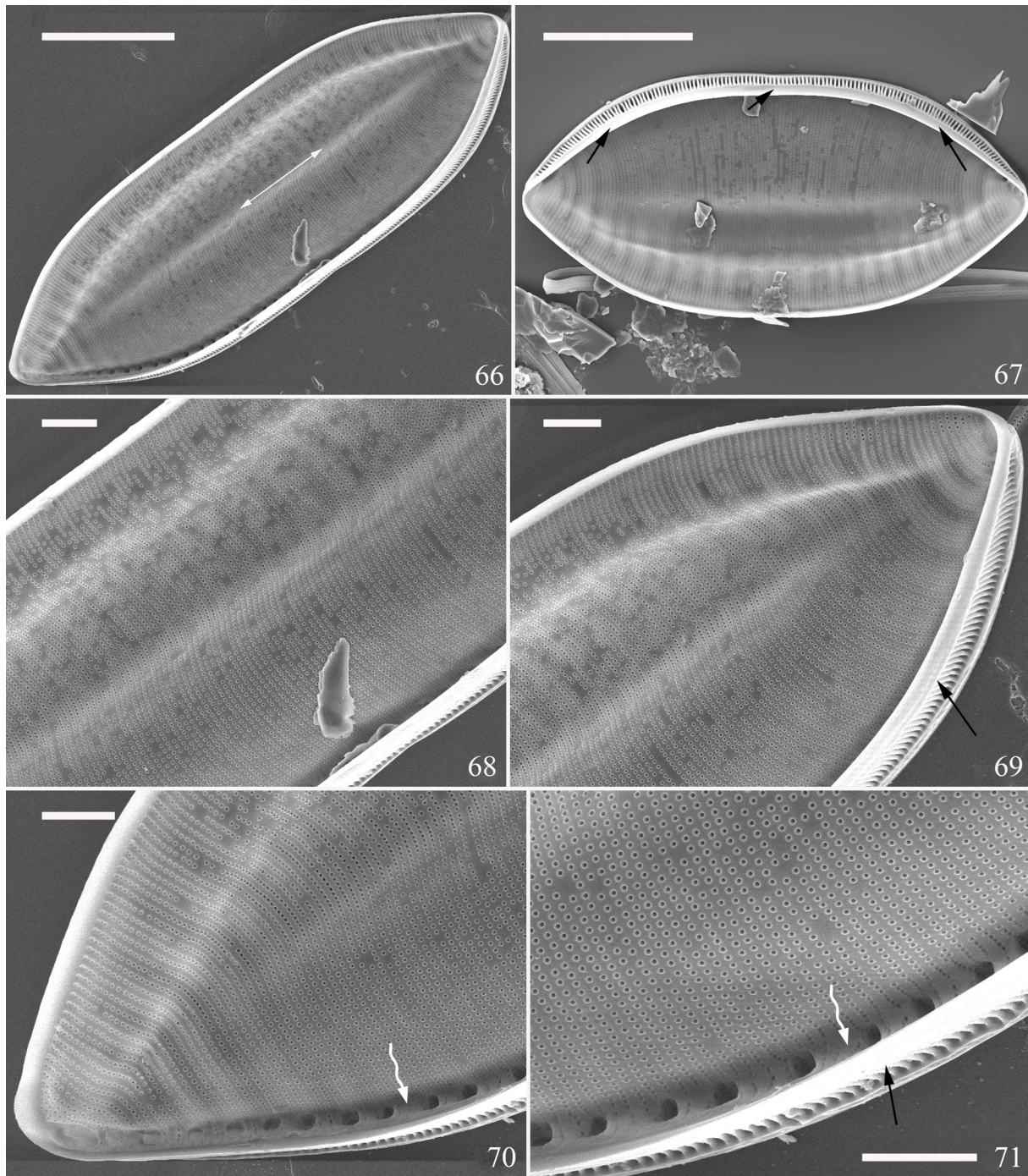


Figs 60–65. *Tryblionella levidensis*, valve external view, SEM: (60) a frustule; (61) a complete valve, note the high marginal ridge bearing a row of fenestrated pores (three black arrows), one elevated half valve surface (white double-headed arrow), and the lowered other half valve (black double-headed arrow); (62) detail from Fig. 60, note the high marginal ridge, simple distal mantle, three girdle bands (B1–B2), and a row of poroids produced in the raphe canal wall (three white arrowheads); (63, 64) details from Fig. 61, note a row of tiny poroids located between the distal mantle and the marginal ridge (one black double-headed arrow, respectively); (65) detail from Fig. 61, note a row of lamellar ribs along the raphe sternum (black arrows) and central raphe endings very close to each other, slightly expanded (two wavy arrows). Scale bars 10 μ m (60, 61), 1 μ m (62), 2 μ m (63–65).

to clearly reveal important characters such as the structures of the marginal ridge, proximal and distal mantle, striae, and fibulae for all known species of *Tryblionella* to make progress on the revision of *Tryblionella*. The ultrastructure of the marginal ridge in particular could represent a very useful morphological character for the revision of *Tryblionella*.

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Figs 66–71. *Tryblionella levidensis*, internal view, SEM: (66) a complete valve, note a fold along the valve midline (white double-headed arrow); (67) another complete valve, note the deep proximal mantle (three black arrows); (68) middle part detail from Fig. 66, note the rimmed internal openings of areolae; (69) apical detail from Fig. 66, note the proximal mantle (black arrow); (70, 71) details from Fig. 66, note the apically elongated, striate fibulae (Figs 70, 71, wavy arrow respectively) and the proximal mantle (black arrow). Scale bars 10 µm (66, 67), 2 µm (68–71).

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