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### Four new species of the genus Lacustriella (Bacillariophyta) from Lake Baikal

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Abstract: We add four new species to the recently established genus Lacustriella Kulikovskiy, Lange—Bertalot et Metzeltin and characterize morphology of the taxa based on LM and SEM observations. Lacustriella cuspidata, L. rombea, L. radiata and L. solita share all of the typical morphological features of the genus including longitudinal canal along each valve margin, radiate uniseriate striae terminated with large elongated areolae, and external distant raphe ends bent oppositely. L. cuspidata is peculiar among the other Lacustriella with rows of small pores look as if they continue striae on the surface of longitudinal canals as well as with the axial area sharply widened into the asymmetrical central area. L. rombea is different in combination of valve outline with unprotracted rostrate apices, irregular areolation at centre of the external valve, and slit—like areola occluded with volae. In L. radiata distinctive characters include protracted rostrate valve apices, rhombic central area and volated slit—like areolae. L. solita differs in the highest density of areolae in striae and an ornamented surface of longitudinal canals. New species were compared to the closest morphologically similar taxa. Some general ecological properties and distribution in the littoral zone of the ancient lake are given.

Key words: Lacustriella, Lake Baikal, new diatom species, SEM

### Introduction

The genus Lacustriella (Neidiaceae) was described in 2012 based on a set of morphological peculiarities including naviculoid shape, symmetry and raphe morphology. Typical characters of the genus include external distal raphe endings deflected to different sides; central ends straight or slightly deflected and lay into large dropshaped grooves; strong nonperforated marginal crest along the valve margins representing a hollow tube from the inner side – longitudinal canal as the authors have shown later (Kulikovskiy et al. 2016); radiate uniseriate striae with open round areolae; close to canal, striae end by transapically elongated slit-like areolae. These areolae forming a row along each canal are of typical character of the genus. Authors of the genus describe them as follows "... two or several areolae are confluent in transapically elongated, slit-like foramina" (pl. 152 figs 1, 2 in Kulikovskiy et al. 2012). The internal side of the valve areolae are covered with a hymen, and raphe endings terminate with helictoglossae, which centrally are merged into a compound structure (Kulikovskiy et al. 2012). The genus contains in total 9 taxa (Guiry & Guiry 2021) four of them are from Lake Baikal, the other species, transferred from other genera, are spread across the Northern Hemisphere water bodies (Table 1).

Here we present light and scanning electron

microscopical observations of four species of the genus *Lacustriella* from Lake Baikal, each with a combination of unusual morphological characters with those typical of the genus.

### MATERIALS AND METHODS

Material for the investigation was taken from the collection of Lake Baikal benthic diatoms established during the 'Darwin initiative' project (1997–1998) at the Limnological Institute, Irkutsk, Russia. Benthic diatoms studied in this assay were collected by scuba divers from depths of 2 to 20 m at three sampling sites. Station 6 was located near Anga bay in the middle basin of the lake (52.7921 °N, 106.6111 °E), station 41was situated next to the settlement Mangutie in Southern Baikal (51.6069 °N, 103.91028 °E) and station 25 was near cape Orlovy in the northern basin (51.5093 °N, 104.9161 °E). For sandy or silted samples the upper 0–1cm layer was taken directly, occasionally an Ekman grab sampler was used (FLOWER & CHAMBERS 2004; FLOWER et al. 2004). The obtained material was preserved with 96% ethanol. Samples were processed in the laboratory more than 24h by hot digestion (under 80 °C) with 30% hydrogen peroxide, then with concentrated HCl. Each treatment followed by 3–5 cycles of centrifugation (1500 rpm), discarding the supernatant and rinsing the precipitate with distilled water (Krammer & Lange-Bertalot 1986). Valve morphology was studied using an Axiostar Plus light

microscope (Zeiss, Gottingen, Germany) with a  $100\times$  oil immersion objective. LM photographs were taken with an attached TOUPCAMTM DC UA1000CA camera. For scanning electron microscopy 50  $\mu$ l aliquot of the suspension of cleaned valves was placed on aluminium stub, dried on air, coated with a gold in Balzers SCD 004 sputter coater and examined with an FEI Quanta 200 scanning electron microscope (Eindhoven, Netherland) at a working distance of 10 mm and acceleration voltages of 25–30 kV. Measurements of valves, density of striae and areolae in striae were taken for each photographed cell; at least 20 individuals of each taxon were studied. Valve structure terminology follows Krammer & Lange-Bertalot (1986), Round et al. (1990) and Kulikovskiy et al. (2012, 2016).

### RESULTS

# *Lacustriella cuspidata* sp. nov. (Figs. 1–5, 16–22) Description

**Light microscopy** (Figs 1–5): Valves elliptic–lanceolate with apiculate apices. Valve length  $36.5–55.5~\mu m$ , width  $18.0–22.5~\mu m$ . Axial area narrow at apices expanded to the wide asymmetrical central area. Raphe weakly lateral with central endings slightly bent to the same side, terminal ends bent oppositely. Striae uniseriate radial,  $11–13~in~10~\mu m$ . One longitudinal canal along each margin.

**Scanning electron microscopy** (Figs 16–22): Externally, areolae in striae round and open lay within surface depressions. Areolae around axial and central area large, become smaller towards mantle. Areolae in striae in central part of valve irregular, 13–16 in 10 μm (Figs 16, 18). Areolae in striae along border of longitudinal canal

transapically elongated with slit—like open foramina (Figs 16, 19, 20). The surface of the longitudinal canal is covered with a row of 1–5 transapically oriented small pores (Figs 19, 20). Proximal raphe endings slightly deflecting to the same side lay in drop—shaped depressions (Fig. 18). Distal endings bent oppositely almost at right angle, extending to valve mantle ornamented with areolae (Fig. 19). Internally, areolae in striae covered with a hymen, raphe endings terminate centrally on raised coalescing helictoglossae; distal ends terminate on thickened helictoglossae (Figs 21, 22).

**Holotype**: NS1000200 (Central Siberian Botanical Garden, Novosibirsk, Russia, Herbarium of Vascular Plants). Holotype specimen is illustrated in Fig. 1.

**Isotype**: slide 0086–BK, Limnological Institute of the Siberian Branch of the Russian Academy of Sciences Irkutsk, Russia.

**Type locality**: northern basin of Lake Baikal, Cape Orlovy (52.7921 °N, 106.6111 °E), sample collected on July, 5, 1997.

**Etymology**: the specific epithet *cuspidata* refers to acuminate valve ends.

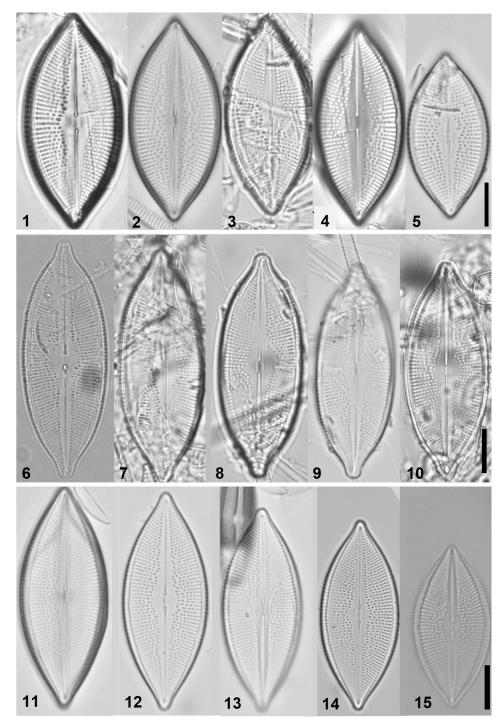
**Habitat and distribution**: yet found in Lake Baikal, in the type locality, benthic, sand at 12 m.

# Lacustriella radiata sp. nov. (Figs 6–10, 23–31) Description

**Light microscopy** (Figs 6–10): Valves widely lanceolate with rostrate apices. Valve length 45.5–56.0 μm, width 16.0–19.5 μm. Axial area narrow at apices gradually expanded to slightly widened rhomboid central area.

Table 1. Species composition of the genus Lacustriella established in 2012.

Species names	basionym
*L. acutocuneata Metzeltin, Lange–Bertalot et Kulikovskiy	
L. gibbosa (J. Brun) Lange–Bertalot et Kulikovskiy	Diploneis lacus-lemani var. gibbosa J. Brun
L. herrmannii (Reichelt) Lange–Bertalotet Kulikovskiy	Navicula herrmannii Reichelt
L. lacus-lemani (J. Brun) Lange-Bertalot et Kulikovskiy	Diploneis lacus-lemani J. Brun
L. lacustris (Gregory) Lange–Bertalotet Kulikovskiy	Navicula lacustris W. Gregory
L. parallela (Wislouch et Kolbe) Kulikovskiy et Lange–Bertalot	Navicula lacusris var. parallela Wislouch et Kolbe
*L. perapiculata Metzeltin, Lange–Bertalot et Kulikovskiy	
*L. superlacustris Metzeltin, Lange–Bertalot et Kulikovskiy	
*L. elongata Metzeltin, Lange–Bertalot et Kulikovskiy	



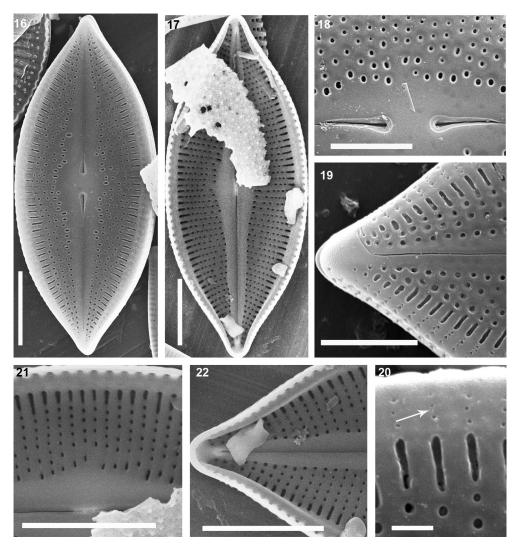
Figs 1–15. LM, variation of the valve outline: (1–5) *Lacustriella cuspidata* sp. nov., (1) holotype specimen; (6–10) *Lacustriella radiata* sp. nov., (6) holotype specimen; (11–15) *Lacustriella rombea* sp. nov., (12) holotype specimen. Scale bar 10 μm.

Raphe filiform, central endings straight, terminal ends bent oppositely. Striae uniseriate radiate, 13-14 in 10  $\mu m$ . One longitudinal canal along each margin.

Scanning electron microscopy (Figs 23–31): Externally, areolae in striae rounded, open, within surface depressions, 17–18 in 10 µm. Areolae around axial and central areas larger in size (Figs 23, 25). Areolae in striae along border of longitudinal canal transapically elongated with slit–like openings covered with volae (Fig. 25). Surface of longitudinal canals smooth (Fig. 25). Proximal raphe

endings straight, slightly widened, within drop—shaped depressions (Figs 23, 24). Distal endings deflecting oppositely extend to valve mantle (Fig. 26). Internally, areolae in striae covered with a hymen, raphe endings terminate centrally on raised coalescing helictoglossae; distal ends with thickened helictoglossae (Figs 28–30).

**Holotype**: NS1000201 (Central Siberian Botanical Garden, Novosibirsk, Russia, Herbarium of Vascular Plants). Holotype specimen is illustrated in Fig. 6.



Figs 16–22. Lacustriella cuspidata sp. nov. from type material: (16, 18–20) SEM external views, (16) whole valve, (18) valve centre with proximal raphe endings and areolae, (19) terminal fissure, (20) areolae of the longitudinal canal (arrow), areolae decreasing in size towards the valve mergins, slit–like areolae; (17, 21–22) SEM internal views, (17) whole valves, (21) central nodule, proximal raphe endings terminate in merged helictoglossae, (22) distal fissure, raised helictoglossae. Scale bars 10 µm (16, 17, 21–22), 5 µm (18, 19), 2 µm (20).

**Isotype**: slide 0016–BK, Limnological Institute of the Siberian Branch of the Russian Academy of Sciences Irkutsk, Russia.

**Type locality**: Lake Baikal, middle basin, Anga Bay (52.7921 °N, 106.6111 °E), sample collected on June, 27, 1997.

**Etymology**: the specific epithet *radiata* refers to the type of valve striation.

**Habitat and distribution**: yet found in Lake Baikal, in the type locality, benthic, silted sand at 17 m.

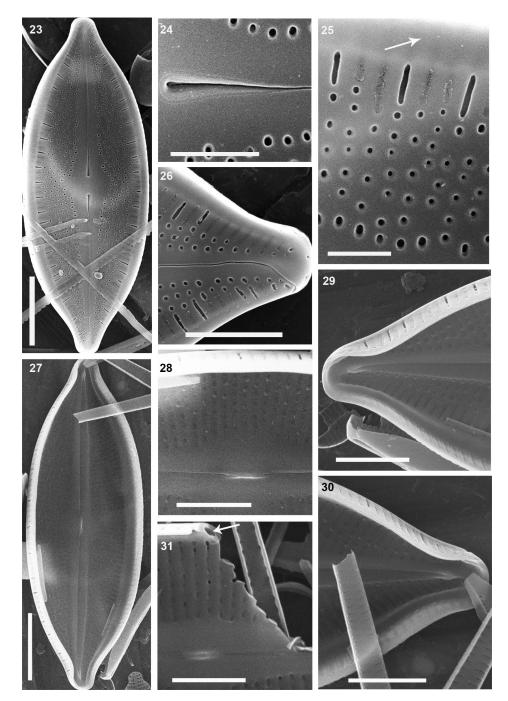
# Lacustriella rombea sp. nov. (Figs 11–15, 32–40) Description

**Light microscopy** (Figs 11–15): Valves from elliptic–lanceolate to rhombic in smaller cells with convex margins and unprotracted rostrate apices. Valve length  $37.0–51.0~\mu m$ , width  $17.0–19.5~\mu m$ . Axial area narrow at apices gradually expanded to the centre. Central area slightly widened. Raphe filiform. Central endings slightly curved to the same side, terminal ends bent oppositely.

Striae uniseriate slightly radiate at centre, radiate towards apices, 13–14 in 10  $\mu m.$  One longitudinal canal along each margin.

Scanning electron microscopy (Figs 32–40): Externally, areolae in striae round, open, within surface depressions, at central part irregular arranged in stria, 16–20 in 10 µm. Terminal areolae in striae at border of longitudinal canal transapically elongated slit–like occluded with volae (Figs 32, 33, 35). Surface of longitudinal canals smooth (Fig. 20). Proximal raphe endings straight, slightly widened, in drop–shaped depressions. Distal endings deflect oppositely, expand to the valve mantle (Figs 34, 36, 37). Internally, areolae covered with a hymen, raphe endings terminate centrally on the raised coalescing helictoglossae; distal ends with thickened helictoglossae (Figs 38–40).

**Holotype**: NS1000202 (Central Siberian Botanical Garden, Novosibirsk, Russia, Herbarium of Vascular Plants). Holotype specimen is illustrated in Fig. 12.



Figs 23–31. *Lacustriella radiata* sp. nov. from type material: (23–26) SEM external views, (23) whole valve, (24) proximal raphe ending, (25) the surface of canals (arrow), areolae, slit–like areolae with volae, (26) terminal fissure; (27–31) SEM internal views, (27) whole valve, (28) central nodule, proximal raphe endings terminate in merged helictoglossae, (29, 30) distal fissure, raised helictoglossae, (31) broken valve showing section of the longitudinal canal (arrow). Scale bars 10 μm (23, 27), 5 μm (26, 28–31), 3 μm (24), 2 μm (25).

**Isotype**: slide 0138–BK, Limnological Institute of the Siberian Branch of the Russian Academy of Sciences, Irkutsk, Russia.

**Type locality**: Lake Baikal, south basin, near the settlement Mangutei (51.6069 °N, 103.91028 °E), sample collected on June 22, 1998.

**Etymology**: the specific epithet *rombea* refers to the valve shape.

**Habitat and distribution**: yet found in Lake Baikal in the type locality, benthic, silt at 2 m.

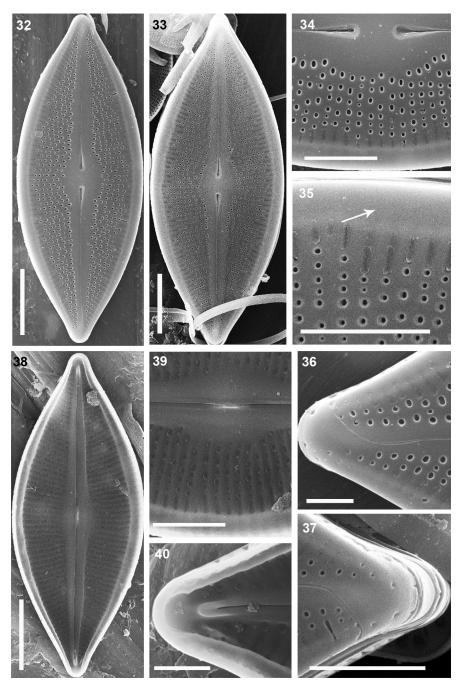
### Lacustriella solita sp. nov. (Figs 41–61) Description

**Light microscopy** (Figs 41–52): Valves linear–lanceolate to elliptic–lanceolate in smaller cells with linear or weakly convex margins and slightly protracted cuneate apices. Valve length 27.0–45.5  $\mu$ m, width 11.5–14.5  $\mu$ m. Axial area narrow at apices gradually expanded to slightly widened central area. Raphe filiform. Central endings straight, terminal ends bent oppositely. Striae uniseriate slightly radiate at centre, strongly radiate

Table 2. Comparison of morphological characters of new Lacustriella species to related similar taxa.

Taxon	Length / Width (µm)	Valve outline	Apices	Striae/ Areolae in 10 µm	Surface of longitudinal canals	structure of areolae in striae	Central area	structure of elongated areolae
L. acutocuneata <sup>a</sup>	45–49/ 17–18	Linear–elliptical– lanceolate	Protracted rostrate or subrostrate	12–13/ 9–14	n.d.	n.d.	III-defined	n.d.
L. cuspidata°	36.5–55.5/ 18.0–22.5	Elliptic-lanceolate	apiculate	11–13/ 13–16	Uniseriate small pores	Round, open irregular	large asymmetric	open
$L.lacustris^{ m a,b,e}$	30–62/ 12–20	Widely-lanceolate	Cuneately –apiculate to acutely–rostrate	14–16/ 12–14	Biseriate small pores	Round, open	Round	open
$L$ . $parallela^{\mathrm{a,d}}$	34–53/ 13–17	Lanceolate with straight or concave margins	rostrate or subrostrate	11–12/ n.d.	n.d.	n.d.	Small rounded	n.d.
L. perapiculataª	36-46/14-16	Elliptic-lanceolate to linear-lanceolate	Shortly rostrate	13–14/ 12–15	n.d.	Round, irregular	rhombic	n.d.
L. radiata°	45.5–56.0/ 1.0–19.5	Widely lanceolate	Rostrate	13–14/ 17–18	smooth	Round, open	rhomboid	volated
L. rombea °	37.0–51.0/ 17.0–19.5	Elliptic—lanceolate to rhombic in smaller cells	unprotracted rostrate	13–14/ 16–20	smooth	Round, open	Weakly widened	volated
L. solita°	27.0–45.5/ 12.5–14.5	Linear-lanceolate	Weakly protracted cuneate	13–14/ 17–22	Smooth at apices, uneven with rounded dents at centre	Round, open	III-defined	open
$L$ . $superlacustris^{a}$	58–80/ 25–29	Elliptic—lanceolate, broadly lanceolate	Short-subrostrate	10–12/ 7.5–12	n.d.	n.d.	Hardly separated or irregularly expanded	n.d.

Notes: n.d.: no data; \*Kulikovskiy et al. (2012); \*Kulikovskiy et al. (2016); \*present study; \*Zabelina et al. (1951); \*Krammer & Lange-Bertalot (1986).



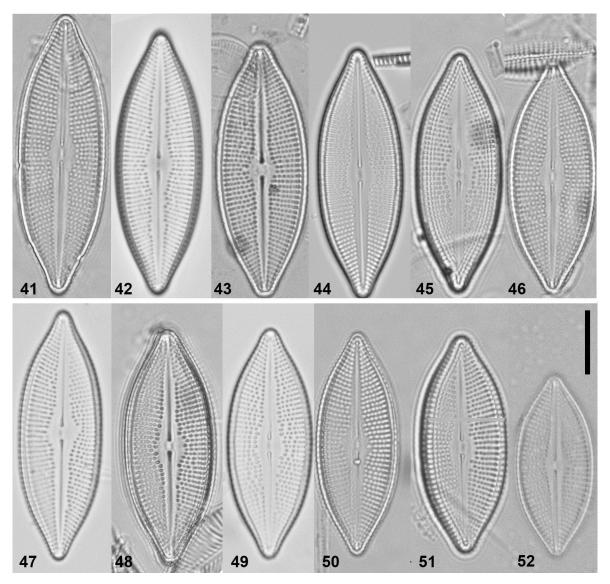
Figs 32–40. *Lacustriella rombea* sp. nov. from type material: (32–37) SEM external views, (32, 33) whole valves, (34) valve centre with proximal raphe endings and areolae, (35) longitudinal canal (arrow), areolae decreasing in size towards the valve margins, slit–like foramina with volae, (36) terminal fissure, (37) cingulum of few bands; (38–40) SEM internal views, (38) whole valve, (39) central nodule, proximal raphe endings terminate in merged helictoglossae, (40) distal fissure, raised helictoglossa. Scale bars 10 μm (32, 33, 38), 5 μm (34, 35, 37, 39), 2 μm (36, 40).

towards apices, 13-14 in  $10 \mu m$ . One longitudinal canal along each margin.

Scanning electron microscopy (Figs 53–61): Externally, areolae in striae rounded, open, within surface depressions, irregular in size at centre, 17–22 in 10 μm (Figs 37–39). Terminal areolae in striae at border of the longitudinal canal transapically elongated, slit–like, open (Figs 53, 56). Surface of longitudinal canals with rounded dents (Fig. 56). Proximal raphe endings straight, slightly widened, in drop–shaped depressions. Distal endings deflecting oppositely expand to the valve mantle (Figs

55, 57, 59). Internally, areolae in striae covered with a hymen, raphe endings terminate centrally on raised coalescing helictoglossae; distal ends with thickened helictoglossae (Figs 60, 61).

**Holotype:** NS1000203 (Central Siberian Botanical Garden, Novosibirsk, Russia, Herbarium of Vascular Plants). Holotype specimen is illustrated in Fig. 41. **Isotype:** slide 0138–BK, Limnological Institute of the Siberian Branch of the Russian Academy of Sciences, Irkutsk, Russia.



Figs 41-52. Lacustriella solita sp. nov., LM: morphological variability of specimens in valve view, (41) holotype specimen. Scale bar 10 µm.

**Type locality**: Lake Baikal near the settlement Mangutei (51.6069 °N, 103.91028 °E), sample collected on June, 22, 1998.

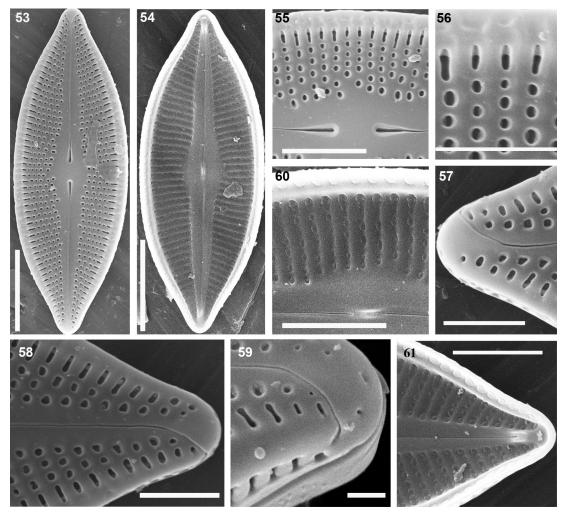
**Etymology**: the specific epithet *solita* means ordinary. **Habitat and distribution**: yet found in Lake Baikal in the type locality, benthic, silt at 2 m.

#### **DISCUSSION**

Lacustriella cuspidata, L. rombea, L. radiata and L. solita share the morphology of the raphe system and internal valve, as well as external striae having terminal conspicuous areolae with large and long foramina near longitudinal canals with other Lacustriella species. (Kulikovskiy et al. 2012, 2016). The comparison with the most morphologically similar taxa revealed considerable differences suggesting them to be independent species.

L. cuspidata is similar in the elliptic–lanceolate outline with L. lacustris and L. superlacustris (Kulikovskiy et al. 2012, figs150: 1–3, figs 152: 2–7, correspondingly). Comparing to L. superlacustris the new species is different in considerably smaller valve size, form of valve apices and densely spaced areolae in striae (13–16 against 7–12 in 10 µm). L. cuspidata is distinct from L. lacustris by more spaced striae, an asymmetric (against round) central area, uniceriate (not biseriate) rows of pores on the surface of longitudinal canals and uneven arrangement (in contrast to regular) of areolae in striae (Kulikovskiy et al. 2012, fig. 152: 2–7; Kulikovskiy et al. 2016, figs 2.30: 3, 5)

L. rombea and L. solita found in the same sample share the habitat and are similar in general view, especially in lanceolate valve form. However, in closer inspection one can see the outline of L. rombea is elliptic—lanceolate with convex margins, and smaller cells look almost rhombic (Figs 15, 32, 33,38), while valves of L. solita are more linear—lanceolate with weakly convex to almost



Figs 53–61. *Lacustriella solita* sp. nov. from type material: (53, 55–59) SEM external views, (53) whole valve, (55) valve centre with proximal raphe endings and areolae, (56) the surface of canals with dents, areolae, slit–like areolae, (57, 58) terminal fissure, (59) the valve margins with slit–like areolae; (54, 60, 61) SEM internal views, (54) whole valve, (60) central nodule, proximal raphe endings terminate in merged helictoglossae, (61) distal fissure, raised helictoglossa. Scale bars 10 μm (53, 54), 5 μm (55, 60, 61), 3 μm (56–58), 1 μm (59).

linear margins. In addition, rostrate valve ends of L. rombea differ from cuneate apices of L. solita. Valves of near length of the species significantly differ in width: maximal width in L. solita 14.5  $\mu$ m is less the smallest 17.5  $\mu$ m in L. rombea). One can notice a difference in the pattern of areolation in the central valve part: in L. rombea it looks unordered because of unaligned areolae in striae in contrary to regular arrangement in L. solita. SEM observations in addition to dissimilarity in density of areolae in striae (16–20 against 17–22) show additional morphological differences including volated slit—like areolae and smooth surface of canals in L. rombea in contrary to open areolae and uneven canals in L. solita. The sum of characters differing the close relative baikalian diatoms suggest them to be distinctive entities.

 $L.\ rombea$  also shares valve shape with unprotracted rostrate apices and density of striae with  $L.\ perapiculata$ . The new species can be distinguished by more rhombic, wider valves (17–19.5  $\mu$ m against 14–16  $\mu$ m), higher density of areolae in striae and, especially, volated slit–like areolae bordering longitudinal canals

(Figs 13–17, 18, 20; Kulikovskiy et al. 2012, fig. 151: 8–10). *L. solita* by linear–lanceolate valves and density of striae is similar to *L. acutocuneata* (13–14 in 10 μm). Cuneate apices (in contrast to rostrate or subrostrate ends), more narrower valves (12.5–14.5 in contrary to 17–18) and more denser areolae in striae are features separating *L. solita* as an independent species (Figs 25–37; Kulikovskiy et al. 2012, fig. 150: 4–7).

Due to valve size range and protracted rostrate ends *L. radiata* is similar to *L. parallela*. However, in *L. radiata* volated slit–like areolae at border of longitudinal canals, rather rhombic central area and more denser striation (13–14 against 11–12) were observed (Figs 46–51, 53, 55; Kulikovskiy et al. 2012, fig. 151: 11–12). The three features combined with the lanceolate valve outline (versus straight or concave valve margins) distinguish *L. radiata* from *L. parallela* and other species in the genus *Lacustriella*.

Lacustriella species are known exclusively from oligotrophic waters of northern fresh water habitats of Holarctic (ZABELINA et al. 1951; KRAMMER &

Lange-Bertalot 1986; Kulikovskiy et al. 2012). The new Lacustriella live in Lake Baikal in slightly alkaline oligotrophic low-mineralized (below 100 mg.l<sup>-1</sup>) waters on silted sand in deep areas (down to 20 m) of Northern and Central basins (L. cuspidata and L. radiata, correspondingly) as well as at 2 m in the South basin (L. solita and L. rombea). They developed during spring-summer season lasting in the lake from late June to early October (Kozhov 1955), when pH in the littoral zone ranges between pH 7.6-8.5 and water temperature raises from 4-7 °C to 14-16 °C (Khodzher et al. 2018; PODLESNAYA et al. 2021). The species are considerably rare, L. solita, the most common among them, comprised less 3% of total cells in a sample. Deep-water L. cuspidata belongs to the diatom assemblage dominated by widespread Gomphonema intricatum Kützing, Encyonema minutum (Hilse) D.G.Mann, Cocconeis placentula Ehrenberg and the endemic *Diploneis munge* Kulikovskiy et Lange-Bertalot. L. radiata occurred together with Sellaphora bacillum (Ehrenberg) D.G. Mann including also extremely abundant bentoplanktonic Ulnaria ulna (Nitzsch) Compère. Populations of Lacustriella rombea and L. solita were associated with Cocconeis placentula, the endemic species Aneumastus perodon Kulikovskiy, Metzeltin et Lange-Bertalot, A. odon Kulikovskiy, Metzeltin et Lange-Bertalot.

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