

Luticola jinshaensis sp. nov. (Bacillariophyta), a new freshwater species from Jinsha River, China

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Abstract: A new species, *Luticola jinshaensis* sp. nov. is described from Xiangjiaba reservoir in Jinsha River, China. Observations on the new species were based on light microscopy (LM) and scanning electron microscopy (SEM). *Luticola jinshaensis* sp. nov. is distinguished from all other species of the genus in having an unusual external areolae structure, where each areola has a cribrum recessed and ornamented with tiny, irregular projections connected with inner wall of areolae raphe. This cribrum is fragile so that the structure may become destroyed by processing of the specimens. A second feature that distinguishes this new taxon is that the deflection of the distal raphe fissures which, in most case, are first deflected towards the same side as the proximal endings and then towards the opposite side and continuing onto the mantle. Occasionally, the external distal raphe fissures are first hooked towards the same side of the proximal endings, then towards the opposite side and, finally, then bent to the same side as the proximal endings. The new species is epilithic and lives in freshwater habitats, notes on its ecology and distribution are added.

Keywords: Diatom, morphology, new species, taxonomy, Xiangjiaba reservoir, valve ultrastructure.

INTRODUCTION

Luticola was established by MANN (1990), with *L. mutica* (Kützinger) Mann as the type species, for species of the *Navicula* subgroup “*Naviculae punctatae*” (CLEVE 1895; see also HUSTEDT 1964). When originally established, 30 taxa have been transferred from *Navicula* Bory to *Luticola* (MANN in ROUND et al. 1990). The genus *Luticola* was distinguished from other genera by: 1) distinctly uniseriate striae composed of round to elliptical areolae; 2) a conspicuous isolated pore in the central area (termed a “stigma” in the older literature, which is quite different morphologically from the feature that term is now applied to: isolated pores in the genus *Cymbella* Agardh (MANN in ROUND et al. 1990; COX et al. 2012; KRAMMER 2002; LEVKOV et al. 2013); 3) two internal longitudinal canals, one on either side of the axial area and placed marginally along the valve face/mantle junctions. The genus also has areolae that are internally occluded by hymens, and a strongly-thickened internal central nodule (LEVKOV et al. 2013). *Luticola* is considered a widely distributed genus with a very broad ecological, range. There are

freshwater, estuarine, marine and aerophilous species (e.g. HUSTEDT 1964; LEVKOV et al. 2013; KOHLER et al. 2015; RYBAK et al. accepted) with both cosmopolitan and endemic species (LEVKOV et al. 2017; PAVLOV et al. 2009; KOPALOVÁ et al. 2009, 2011; VAN DE VIJVER et al. 2002, 2006, 2008, 2011, 2012; ESPOSITO et al. 2008; WETZEL et al. 2010). To date, *Luticola* has approximately over 250 known taxa, according to the most recent revision of LEVKOV et al. (2013) and later studies (ZIDAROVA et al. 2014; GLUSHCHENKO et al. 2015, 2017; KOHLER et al. 2015, WOODARD et al. 2016; BAĞ et al. 2017; BUSTOS et al. 2017; CHATTOVÁ et al. 2017; KALE et al. 2017; KOCIOLEK et al. 2017; LIU et al. 2017; STRAUBE et al. 2017; Noga et al. 2017; BAĞ et al. 2019; KULICHOVÁ et al. 2019; DA SILVA–LEHMKUHL et al. 2019; SIMONATO et al. 2020; HINDÁKOVÁ et al. 2021; RYBAK et al., accepted). In China, early work on taxa that can be referred to *Luticola* included *Navicula nivalis* var. *chinensis*, *N. hongkongensis*, and *Luticola lagerheimii* (as *Navicula lagerheimii*), all reported by SKVORTZOW (1928, 1937, 1938, 1975). Subsequently, species of *Luticola* from China have been reported by Liu et al. (2013) and LI

& QI (2018). LEVKOV et al. (2013) described three new species from China, while LIU et al. (2017) reported two new species from the Wuling Mountains Area, China. It is well known that there are extremely diverse inland freshwater ecosystems in China, which would lead to the expectation that diatom species will be found.

The present paper describes LM and SEM observations on one *Luticola* species from a single sample collected from the hull of the boat in the Xiangjiaba reservoir area. The sample contained numerous other diatom taxa and we provide ecological data to help circumscribe the parameters helping to define this species' distribution.

MATERIALS AND METHODS

The Jinsha River is located in the upper reaches of the Yangtze River, the largest river in China, and spans Sichuan and Yunnan provinces. The river has a total length of 2,326 km, is situated at an altitude of about 3,280 m, its watershed covers an area of 473,000 km², and annual average flow of 4,750 m³.s⁻¹. The Jinsha River accounts for ~26% of the Yangtze River basin area (GAO et al., 2019).

Periphytic samples were collected on November 5, 2019, from the Jinsha River (28°60'11"N, 103°96'2"E) from the surfaces of the hull of the boat. Samples were analyzed for total phosphorus (TP) using the ammonium molybdate spectrophotometric method (GB 11893–1989) and for total nitrogen (TN) using alkaline potassium persulfate digestion and the UV spectrophotometric method (HJ636–2012). Water temperature, conductivity, salinity, DO and pH were measured on-site with a YSIPro Plus multiparameter meter (YSI, Yellow Springs, OH, USA).

Original samples were treated with concentrated nitric acid using a microwave accelerated reaction system (MARS) (CEM Corporation, Charlotte, NC, USA) and a pre-programmed digestion scheme (temperature, 180 °C; ramp, 15 min; hold, 15 min) (LUO et al. 2018). To remove the acid from the samples, they were washed six times with distilled water, and the cleaned diatoms were then mounted air dried onto coverslips. To obtain permanent slides for observation with light microscopy (LM), the coverslips with the dried diatom material were mounted onto microscope slides with Naphrax®. LM studies were made with a ZEISS AXIO Imager A2 microscope. A total of 400 valves were identified to the level of species or variety (or numbered in the case of new species) and counted (BODÉN 1991) to assess the relative abundance of all taxa (including those new to science) in the sample. For scanning electron microscope (SEM) observations, cleaned specimens were air-dried on glass cover slips and attached to copper stubs, coated with ~15 nm gold–palladium using a sputter coater (HITACHI E–1045), and examined using a SU 8010 (Hitachi High–Technologies Corp., Tokyo, Japan) SEM (2 kV) at Shanghai Normal University, Shanghai, China. Both the cleaned material and slides are stored at the Laboratory of Algae and Environment of Shanghai Normal University. Diatom images were compiled with Photoshop CS4 (Adobe Photoshop CS4 Extended). Morphological terminology follows ROUND et al. (1990) and LEVKOV et al. (2013).

RESULTS

Class Bacillariophyceae Haeckel emend. Medlin et Kaczmarska 2004

Subclass Bacillariophycideae Mann in Round et al. 1990
Order Naviculales Bessey 1907

Family Diadesmidaceae Mann in Round et al. 1990
***Luticola jinshaensis* Yang et Wang sp. nov. (Figs 1–44)**

Morphological observations in LM (Figs 1–24): Valves linear–lanceolate to lanceolate with rostrate to subrostrate apices. Valve length 24.0–50.0 µm; valve width 10.0–13.5 µm. Axial area narrow, linear to lanceolate, expanded near central area. Central area asymmetric, rectangular, delimited by 3–4 striae. Slit–like isolated pore in the central area, halfway between valve center and margin. Raphe branches slightly curved. Proximal raphe endings deflected to the opposite side of the isolated pore. Striae radiate, 12–14 in 10 µm, each composed of 3–6 rounded to elliptic areolae, 14–16 in 10 µm. Marginal channel narrow, usually evident in LM.

Morphological observations in SEM (Figs 25–36): Externally, valve mantle with 1–3 rows of round areolae, varying in number between the valve apices (1–2 rows) and mid–valve (3 rows) (White arrow in Fig. 28). Striae composed of 3–6 rounded or elongated areolae (Fig. 25). The areolae are usually covered with recessed or ornamented cribra (Fig. 31). Areolae near valve margin have cylindrical opening compared to areolae located next to the axial area (Fig. 30). Isolated pore with a slit–like opening (Figs 25, 29). Proximal raphe endings deflected towards the side opposite the isolated pore (Fig. 29). Distal raphe fissures are, in most case first hooked, deflected towards the same side as the proximal endings and then towards the opposite side, continuing onto the mantle (Fig. 27); occasionally they are first hooked towards the same side of the proximal endings, then towards the opposite side and finally bent the same side as the proximal endings, extending onto valve mantle and continuing onto the mantle (arrow in Fig. 25). Cingular bands have a single row of round pores (arrow in Fig. 26).

Internally, the central area is narrow, laterally expanded to the valve margin (Figs 31, 34). Isolated pore foramina small with round opening occluded by a circular lipped structure (Fig. 33). Poroids of valve face are occluded by hymens forming a continuous strip on each stria, a thin and small silica flap covers the end of the central areolae (arrow in Fig. 34). Proximal raphe ends curved towards the same side of isolated pore (Figs 31, 34); distal external raphe ends hooked towards the isolated pore, extending onto valve mantle, terminating in small helictoglossae (Figs 32, 36). Areolae next to valve margin transversely elongated (arrow in Fig. 35).

Holotype: SHTU! Naphrax slide Z–JSJ–2–036(2)–1,

Lab of Algae and Environment, College of Life Sciences, Shanghai Normal University, Shanghai, China. Holotype illustrated in Fig.4.

Isotype: Slide 650061 is deposited in the Kociolek Diatom Collection at the University of Colorado, Boulder (COLO).

Type locality: China, Jinsha River, Xiangjiaba reservoir, 28°60'11"N, 103°96'2"E. collected by PAN YU, 5th November 2019.

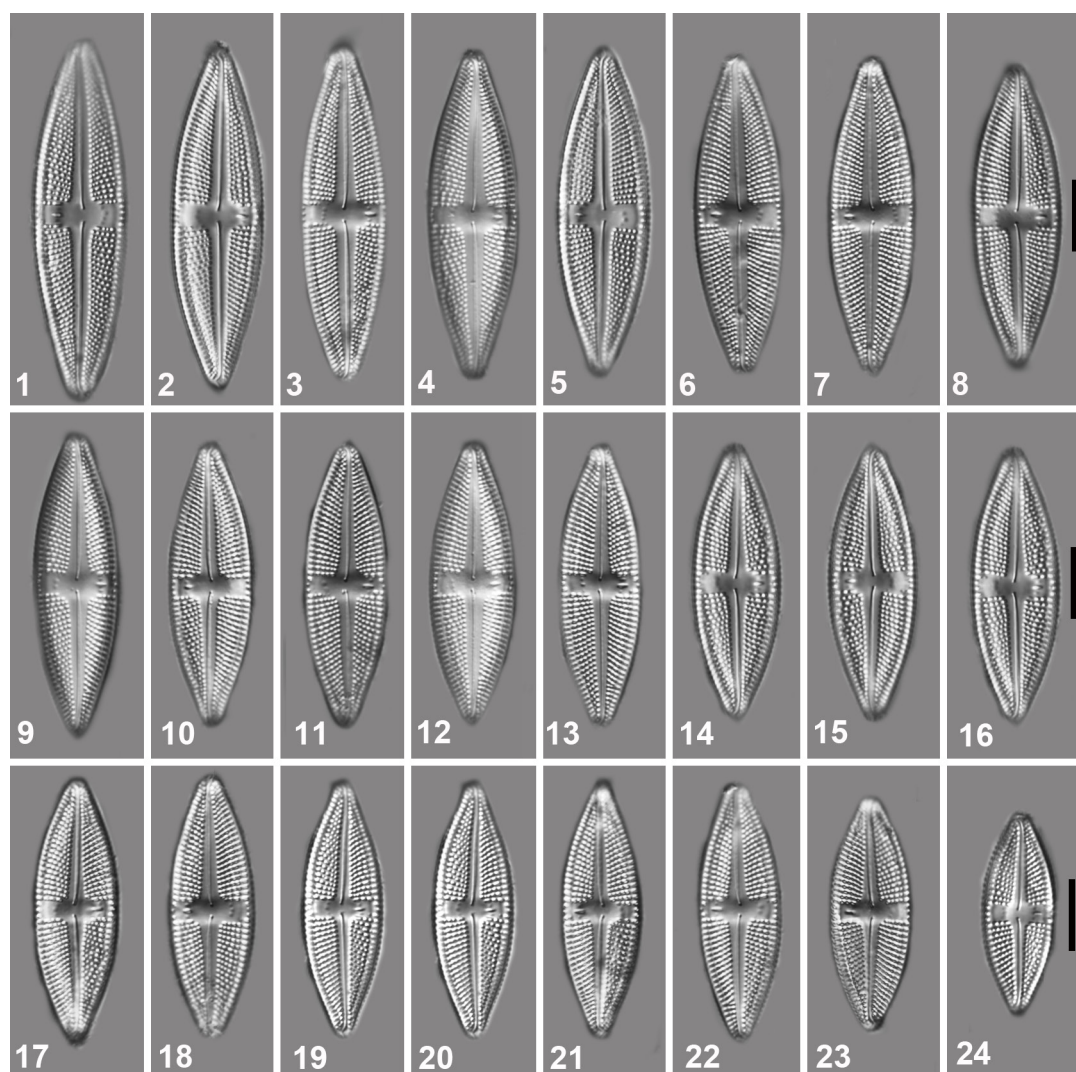
Etymology: The species is named refers Jinsha River where the species was found.

Ecology: During sampling, the water temperature was 19.8 °C and salinity was 0.2 ppt. Based on the salinity value, the environment is considered freshwater. pH 8.72, DO 7.73 mg.l⁻¹, TDS 267.8 mg.l⁻¹, Conductivity 370.8 µs.cm⁻¹, collected in one sample (Z-JSJ-2-036(2)) on the hull of the boat.

Distribution: The species has been found so far only in the type locality.

DISCUSSION

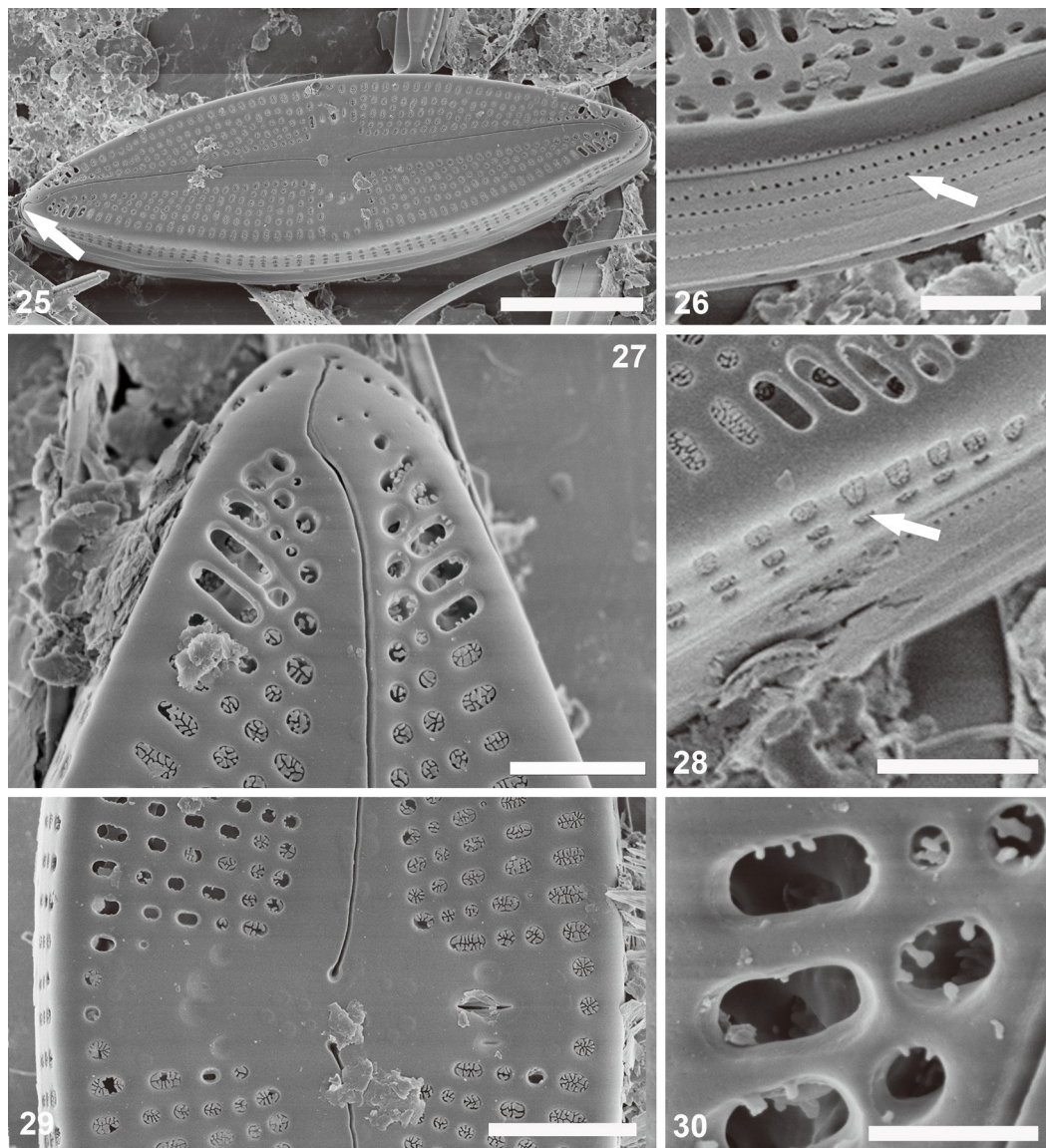
Members of the genera *Luticola* was previously considered to belong to the genus *Navicula* sensu lato, and accordingly possess certain similar morphological characters, including the shape and symmetry of the biraphid frustule, defined striae and more or less simple raphe system (HUSTEDT 1930; PATRICK & REIMER 1966; KRAMMER & LANGE-BERTALOT 1986). In the context of a more narrowly-defined concept for *Navicula* (sensu stricto), *Luticola* species are distinguishable with respect to the structures of areolae, a conspicuous isolated pore in the central area (commonly referred to as a 'stigma' in older literature), and internal, longitudinal, marginal canals on each side of the valve (LEVKOV et al. 2013; but see several species found only in Colombia that lack marginal canals—SIMONATO et al. 2020). The internal structure of the 'stigma' is unique (MANN in ROUND et al. 1990; COX 2012; LEVKOV et al. 2013) and differs from that in other genera of diatoms for which the term stigma (KRAMMER 2002).



Figs 1–24. *Luticola jinshaensis* sp. nov., LM. Scale bar 10 µm, valve views showing size diminution series.

HUSTEDT (1964) and LEVKOV et al. (2013) distinguished several groups of taxa with *Luticola* based on the size and shape of the valves. *Luticola jinshaensis* sp. nov. resembles *L. grupcei* Pavlov, Nakov et Levkov, *L. iporangensis* Silva–Lemhkuhl et Ludwig, *L. bartolomeii* Silva–Lemhkuhl, Luwig, Tremarin et Bicudo, *L. dapaloides* (Frenguelli) Metzeltin et Lange–Bertalot, *L. plausibiloides* (Hustedt) Mann, *L. uruguayensis* Metzeltin, Lange–Bertalot et García–Rodríguez, and *L. hilgenbergii* Metzeltin, Lange–Bertalot et García–Rodríguez in overall valve shape (see also Table 1). The most obvious characteristics of *Luticola jinshaensis* sp. nov. that differentiate it from those other, similarly-shaped taxa are the structure of areolae and the deflection of distal raphe endings. The most similar is *Luticola grupcei*, but this species can be differentiated from *L. jinshaensis*

sp. nov. by striae density and the structure of areolae. *Luticola grupcei* has 15–17 striae in 10 μm contrary to *L. jinshaensis* sp. nov. that has only 12–14 striae in 10 μm . *L. jinshaensis* sp. nov. has areolae covered with recessed cribra that are ornamented with short tiny projections, but this structure of areolae was not demonstrated in *L. grupcei* (Levkov et al. 2013). *Luticola dapaloides*, *L. uruguayensis* and *L. hilgenbergii*, all described from Uruguay and NE Argentina (Frenguelli 1953; Metzeltin et al. 2005) can be mainly differentiated from *L. jinshaensis* by the valve size (*L. dapaloides*: length 49–88 μm , width 19–25 μm ; *L. uruguayensis*: length 62.7–79 μm , width 16.7–23.7 μm ; *L. hilgenbergii*: length 42–83 μm , width 14–20 μm). Also, *L. dapaloides*, has slightly undulated margin, and the valve mantle has a single row of large, elongated areolae which are also characteristics

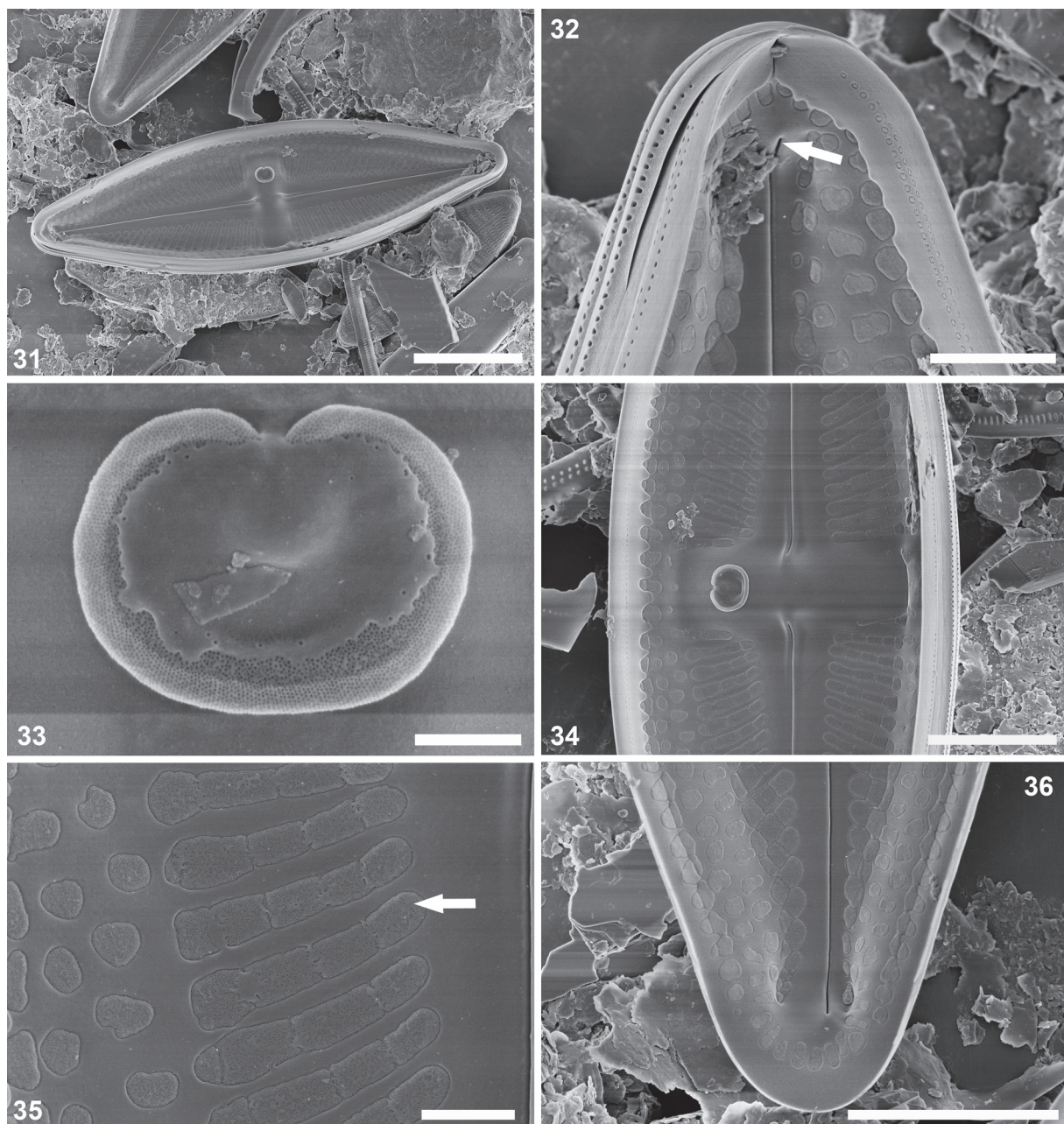


Figs 25–30. *Luticola jinshaensis* sp. nov., SEM external valve view: (25) note external view of an entire valve; (26) note single row of round poroids on the girdle band (arrow); (27) note external view of apex, detailing the distal raphe ends; (28) detail of valve apices and mantle striae with 1–3 round areolae (arrow); (29) detail of the central view in external view; (30) note detailing of the external view of areolae. Scale bar 10 μm (25), 2 μm (26, 27, 28, 29), 0.5 μm (30).

that distinguish this species from *L. jinshaensis* sp. nov. (LEVKOV et al. 2013; STRAUBE et al. 2017). Additionally, *Luticola plausibiloides* differs from *L. jinshaensis* sp. nov. by having rounded, not subrostrate to protracted, apices. *Luticola iporangensis* and *L. bartolomeii* have valve outlines that are similar to *L. jinshaensis* sp. nov. However, they can be distinguished from *L. jinshaensis* sp. nov. based on the density of areolae and striae (*L. iporangensis* has 18–19/10 μm striae composed of 5–8 areolae; *L. bartolomeii* has 18–20/10 μm striae composed of 5–9 areolae vs *L. jinshaensis* sp. nov. has 12–14/10 μm striae composed of 3–5 areolae) (ANGELA et al. 2019).

Additional details regarding the comparison between *L. jinshaensis* and these morphologically similar taxa are shown in Table 1.

Luticola jinshaensis sp. nov. appears unique among *Luticola* species to possess both external occlusions of the areolae (as small, variously-shaped extensions into the areolar openings) and internal, hymenate occlusions. This is not only a unique situation in the genus, but amongst nearly all raphid diatoms. It has been discussed that groups of raphid diatoms may be identified as having only internal coverings (in, for example, many genera of the Stauroneidaceae; see KULIKOVSKIY et al. 2019) or



Figs 31–36. *Luticola jinshaensis* sp. nov., SEM internal valve view of small valves: (31) note internal view of an entire valve; (32, 36) distal raphe branch terminating in small helictoglossa; (33) detail of the stigma surrounded by a large, circular, lipped structure; (34) detail of the central area with focus on the stigma, the cover of the longitudinal canal (arrow); (35) note close-up of the valve interior showing the hymenate occlusion of areolae (arrow). Scale bar 10 μm (31), 5 μm (34, 36), 2 μm (32), 1 μm (35), 0.5 μm (33).

Table 1. Morphological comparison between *L. jinshaensis* sp. nov. and morphologically similar taxa.

Character	<i>L. jinshaensis</i> sp. nov.	<i>L. iporangensis</i>	<i>L. bartolomei</i>	<i>L. dapuloides</i>	<i>L. plausibiloides</i>	<i>L. uruguayensis</i>	<i>L. grupeei</i>	<i>L. hilgenbergii</i>
Valve length (µm)	24–50	31.5–49.7	30–53.3	49–88	30–66	62.7–79.0	24.5–38	42–83
Valve width (µm)	10–13.5	10.7–12.4	12.2–13.1	19–25	12–20	16.7–23.7	8.5–10.5	14–20
Striae (in 10 µm)	12–14	18–19	18–20	12–13	13–15	12	15–17	15–16
Areolae (per stria)	3–6	5–8	5–9	8–12	5–6	5–8	5	14–16
The structure of areolae	covered with recessed cribrum ornamented with short tiny projections	round or elongate areolae	round or elongate areolae	covered with recessed cribrum ornamented with short tiny projections	round or elongate areolae	covered with recessed cribrum ornamented with short tiny projections	round or elongate areolae	round or elongate areolae
Valve outline	linear–lanceolate to lanceolate	linear–lanceolate to lanceolate	lanceolate	linear–lanceolate	elliptical–lanceolate	lanceolate	lanceolate	lanceolate to rhombic, undulate margins
Valve apices	rostrate to subrostrate	subrostrate to protracted	round to slightly protracted	round to slightly protracted	rounded to linear–elliptical	rostrate to subrostrate	protracted	protracted, subcapitate to rostrate–capitate
Central area	asymmetric, rectangular	elliptic	asymmetric, elliptic, wedge-shaped		asymmetric, rectangular	asymmetric, rectangular	bow-tie-shaped	asymmetrical, transversally elliptic to wedge-shaped
Valve mantle	1–3 rows of round areolae	valve apices (1–3 rows) and mid-valve (3 rows)	two rows of round areolae	single row of elongate areolae	one row of elongate areolae	single row of elongate areolae	two rows of round to elliptic areolae	1–3 rows of round areolae

Table 1 Cont.

Character	<i>L. jinshaensis</i> sp. nov.	<i>L. iporangensis</i>	<i>L. bartolomei</i>	<i>L. dapaloides</i>	<i>L. plausibiloides</i>	<i>L. uruguayensis</i>	<i>L. grupcei</i>	<i>L. hilgenbergii</i>
Distal raphe endings	in most case first hooked, deflected towards the same side as the proximal endings and then towards the opposite side; but occasionally first hooked towards the same side of the proximal endings, then towards the opposite side and finally bent the same side as the proximal endings	deflected towards the same side as proximal endings, continuing onto the valve mantle, extending and ending with a hook towards the same side as isolated pores	hooked, first deflected towards the same side as the proximal endings and then hooked towards the opposite side, continuing onto the mantle	slightly hooked over the mantle	hooked towards the isolated pores	hooked towards the isolated pores, extending onto valve mantle	hooked towards the isolated pores	hooked towards the isolated pores
Reference	This study	ANGALE et al. 2019	ANGALE et al. 2019	STRAUBE et al. 2017	LEVKOV et al. (2013)	LEVKOV et al. (2013), STRAUBE et al. 2017	PAVLOV et al. (2009),	ANGALE et al. 2019

external coverings (in, for example, the genus *Nupela* Vyverman et Compere; see KULIKOVSKIY et al. 2020). NIKOLAEV and HARWOOD (2000) used the position of areolar occlusions to differentiate amongst major grouping of ‘centric’ diatoms. The situation of occlusions both externally and internally is rare. A number of studies have shown species of *Neidium* Pfitzer to have external areolae openings with projections and internal hymenate occlusions (METZELTIN & LANGE–BERTALOT 2002; SIVER et al. 2003; SIVER et al. 2005; LIU et al. 2014, 2020). In analyses of phylogenetic relationships of raphid diatoms, *Luticola* species have been shown to be closely related to *Neidium* (KULIKOVSKIY et al. 2019, 2020).

Luticola jinshaensis sp. nov. has only been found so far on the “hull” of the boat in Xiangjiaba reservoir, which has slightly alkaline pH (8.72) and slightly high conductivity (370.8 $\mu\text{S}\cdot\text{cm}^{-1}$). Although a large number of samples were collected from aquatic plants, stones as well as phytoplankton, *L. jinshaensis* was never observed in any of these samples. *Luticola jinshaensis* sp. nov. was the dominant taxon in the sample in which it was found (representing nearly 30% of all counted valves), the diatom community on the the hull of the boat, it occurred with several other freshwater diatom species including, *Nitzschia amphibia* Grunow, *N. filiformis* (Smith) Van Heurck, *N. dissipata* (Kützing) Rabenhorst, *Amphora pediculus* (Kützing) Grunow, *Melosira varians* Agardh, *Tabularia fasciculata* (Agardh) Williams et Round, *M. capsularum* Yang et Wang, *Gomphonema pseudosphaerophorum* Kobayasi, *Bacillaria paxillifera* (Müller) Marsson, *Navicula novaesiberica* Lange–Bertalot and *N. lundii* Reichardt. All of these other species are commonly found in other habitats, and are not exclusively found on the the hull of the boat in Xiangjiaba reservoir, as it is the case of *Luticola jinshaensis*. Further research is needed to determine whether the geogrpahical distribution of this enigmatic diatom.

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