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New species of *Iconella* Jurilj (Bacillariophyta) from tropical areas of China

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Abstract: Three distinctive species of the diatom genus *Iconella* Jurilj were described from tropical areas of China. These new species all have the canal raphe extending around valve margins, keel raised off the valve surface forming wings, fenestrae obvious and alar canals that are uniformly porous. Together these features suggest these species should be assigned to the newly re–established genus *Iconella*. Here we describe *Iconella shiwana* Yan Liu, Ziyi Jiang et Kociolek sp. nov., *Iconella pseudoconstricta* Yan Liu, Ziyi Jiang et Kociolek sp. nov. and *Iconella uniformis* Yan Liu, Ziyi Jiang et Kociolek sp. nov., all described on the basis of light and scanning electron microscope observations from material collected from Hainan and Southern Guangxi Provinces. *Iconella shiwana* differs from other taxa by its isopolar valves and broadly round apices, tri– to quadriseriate short striae and large elliptical hyaline area in the valve center. *I. pseudoconstricta* is also isopolar, but it has acute ends and a constricted valve center. *I. uniformis* has heteropolar valves with a broadly rounded head pole and acute foot pole, a large spine is present near the head pole, and hook–like helictoglossae present at the foot pole. Our previous studies in tropical areas in China showed the diatom flora includes endemic taxa, although additional investigations are needed to explore and describe the abundant biodiversity there.

Key words: *Iconella*, new species, tropical areas, China

Introduction

The genus *Iconella* Jurilj (Jurilj 1949) was re–established by Ruck et al. (2016) based on morphological and molecular research on the Surirellales and Rhopalodiales (Ruck et al. 2016). According to Ruck et al. (2016)'s new classification, the well–known *Surirella* Turpin (Turpin 1828) was split into two genera, *Surirella* and *Iconella*. The newly–circumscribed "*Surirella*" contains the "Pinnatae" group of *Surirella* species, exemplified by taxa such as *S. striatula* Turpin (Turpin 1828) and *S. sella* Hustedt (Hustedt 1925) as well as the genus *Cymatopleura* Smith (Smith 1851). *Iconella* was composed of the "Robustae" group of *Surirella*, "Robusti" group of *Campylodiscus* Ehrenberg ex Kützing (Kützing 1844) and the genus *Stenopterobia* Brébisson ex Van Heurck (Van Heurck 1896). The diagnostic morphological characters of *Iconella* were described by Jahn et al. (2017) as having a "raphe canal [that] rises above the valve face and mantle and has alar canals with fenestral openings occluded by fenestral bars with internally rimmed pores." Changes in nomenclature and new combinations needed to adhere to the new taxonomic scheme proposed by Ruck et al. (2016), have been proposed by Jahn et al. (2017) and Kapustin & Kulikovskiy (2018).

Tropical areas are renown for harboring high levels of biodiversity of freshwater diatoms, with numerous new and endemic species reported (e.g. Metzeltin & Lange–Bertalot 1998). *Surirella* was also found have high diversity in tropical areas with many new species were discovered from different continents, such as Asia (Hustedt 1937–1939; Bramburger et al. 2006; Karthick et al. 2012), Africa (Cocquyt & Jahn 2005a, b, 2007a,b,c; Cocquyt & Kusber 2010; Cocquyt & Taylor 2015), South America (Sala et al. 2013), and Oceania (Vyverman 1991).

The genus *Surirella* was first reported from China by Mereschkowsky (1906), and he described one new *Surirella* species (*Surirella tibetica* Mereschkowsky), which was later made into a new combination (as *Surirella helvetica* var. *tibetica* (Mereschkowsky) Cleve–Euler (Cleve–Euler 1952). Although several reports have focused on *Surirella* from China (Yang 1999; Zhu & Chen 2000; You et al. 2011; Wang 2018; Liu et al. 2019), only 102 taxa have been reported from China (unpublished data). Just over 10% of the *Surirella* taxa reported from China have been newly described, as 12 new species have been described from China (Table

1). Most of these new *Surirella* taxa were described by Skvortzow (1927; 1928; 1929a, b; 1930; 1976) based on his extensive studies on the freshwater diatoms of China. The *Surirella* records from tropical regions in China are few, with only 4 species recorded previously from Hainan Province (Wang 2018). Compared with the names reported worldwide (more than 2000 names in Kociolek et al. 2020), the diatoms of *Surirella* sensu lato are still poorly known in China.

During our investigation of diatoms from tropical areas of China, samples from Hainan and Southern Guangxi were collected, and three new species of *Iconella* were discovered. In this paper, the morphological characters were described based on LM and SEM observations for these 3 new species.

Materials and Methods

In 2014 and 2018 collections of diatoms were taken from tropical areas of China from Hainan Province and Southern Guangxi Province, which are located in the tropical monsoon climatic zone, exhibiting high temperature and humidity.

Samples were fixed with 4% formaldehyde in the field. Water temperature, pH (KL–009III pen–based high–precision pH meter) and conductivity (DDB–11A Portable digital conductivity meter) were measured during the collection time. Samples contained the new species are listed at Table 2.

Materials were boiled with HNO_3, then washed and settled using distilled water to remove all the organic matter and nitric acid, until the pH was neutral. For light microscope (LM) observations, cleaned diatoms were mounted with Naphrax. Permanent slides were examined with a Zeiss Imager A2 microscope equipped with a digital camera (AxioCam MRc 5) and observed with DIC (differential interference contrast) optics (Zeiss, Jena, Germany at Harbin Normal University), and Olympus BX 51 microscope with DIC optics, equipped with a Olympus DP 71 camera (Olympus, Tokyo, Japan at the University of Colorado, Boulder). For scanning electron microscope (SEM) observations, cleaned material was air–dried and coated with gold–palladium, and observations made with a Hitachi S–4800 field emission SEM (Hitachi, Tokyo, Japan at Harbin Normal University) at an operating voltage of 15kv. Diatom images were compiled with Photoshop 7.0. Terminology follows that of Ruck & Kociolek et al. (2004), Bramburger et al. (2006) and Jahn et al. (2017). Samples, cleaned material and permanent slides are all archived at the Key Laboratory of Biodiversity of Aquatic Organisms, Harbin Normal University.

Results

***Iconella shiwana* Yan Liu, Ziyi Jiang et Kociolek sp. nov. Figs (1–10, LM; 11–20, SEM)**

LM observations: Valves isopolar or slightly heteropolar, linear, with broadly rounded poles. Middle portion of the valve slightly constricted in larger valves, nearly parallel in smaller valves. Length 32.5–54.5 μm, breadth 11.5–12.1 μm, length to breadth ratio 2.8–4.5: 1 (n=30). Fibulae distinct, 6–7/10 μm. Porcae nearly parallel in the valve center, becoming radiate toward the apices. Axial area narrow and linear, interrupting the porcae.

SEM observations: Externally, raphe canal extends around the periphery of valve, discontinous at both apices, curved towards the mantle (Fig. 12). Keel elevated from the valve surface, fenestrae well–developed, containing 2–3 fenestral bars (Fig. 13). On the alar canals, striae are short, nearly 1/3 length of the half valve, triseriate (Fig. 14). Porcae smooth, without areolae (Figs 13–14). In the depressions between porcae, striae are

Table 1. *Surirella* taxa which were originally reported from China.

Species name	Literature
Surirella angusta var. *amoyensis* Skvortsov	Skvortzow 1929
Surirella didyma var. *hinganica* Skvortsov	Skvortzow 1976
Surirella fukiensis Skvortsov	Skvortzow 1930
Surirella helvetica var. *tibetica* (Mereschkowsky) Cleve–Euler	Cleve–Euler 1951
Surirella hinganica Skvortsov	Skvortzow 1976
Surirella ovalis [var. *minuta*] f. *tientsinensis* Skvortzow	Skvortzow 1930
Surirella ovata f. *curta* Skvortsov	Skvortzow 1930
Surirella ovata f. *mongolica* Skvortsov	Skvortzow 1930
Surirella robusta var. *manschurica* Skvortsov	Skvortzow 1928
Surirella saxonica var. *sinica* Skvortsov	Skvortzow 1930
Surirella tenera var. *hinganica* Skvortsov	Skvortzow 1976
Surirella tientsinensis Skvortsov	Skvortzow 1927

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long, extending to the axial area, uniseriate or biseriate (Figs 13–15). Areolae on the valve surface are covered by tiny siliceous granules (Fig. 15). Axial area smooth, without areolae or spines (Figs 11, 14). Internally, raphe discontinuous at both apices (Figs 17–18), helictoglossae are not expanded, a raised nodule–like structure formed between two distal raphe ends (Figs 17–18). Striae short internally, forming a large elliptical hyaline area in the valve center (Fig. 16). Striae mostly present on both valve and mantle sides of portulae (Fig. 19), 3–4 striae per portula, each stria composed by 3–4 rows of areolae (Figs 17, 18, 20). Several irregular striae occur on the fibulae, bi– to triseriate (Fig. 20).

Holotype: HANU! Individual in slide THHN2018004, here illustrated as Fig. 1.
Isotype: COLO! Kociolek Collection (University of Colorado, Boulder, U.S.A.), slide no. 650046.
Type locality: Small pond near the river, Shiwan Mts. National Forest Park., Guangxi Zhuang Autonomous Region. Coll. Y. Liu & X.X. Lu May 2018.
Etymology: This species is named for its type locality.
Remarks: Only known from the type locality.

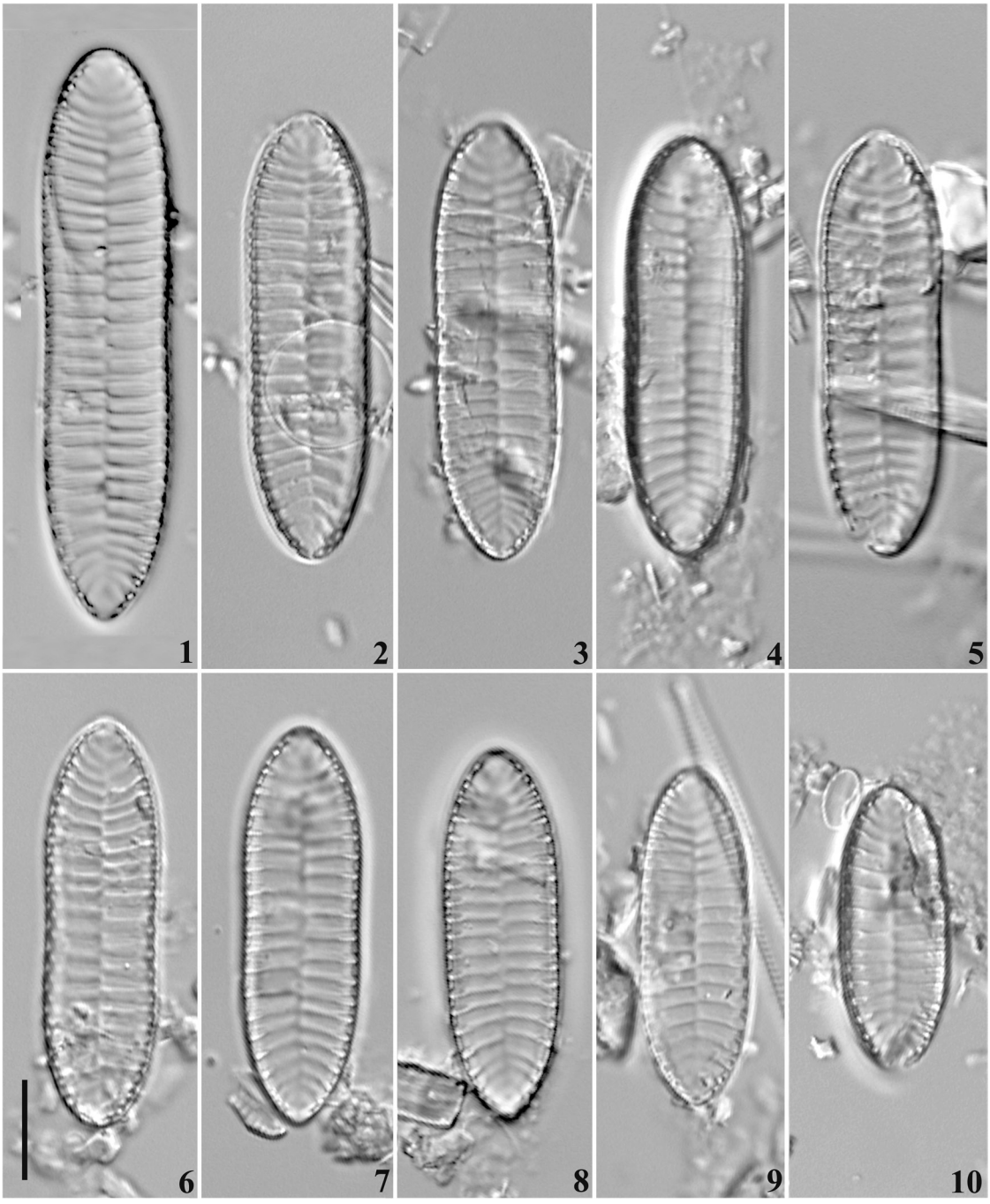
***Iconella pseudoconstricta* Yan Liu, Ziyi Jiang et Kociolek sp. nov. Figs (21–32, LM; 33–42, SEM)**
LM observations: Valves isopolar, linear, tapered to rounded apices, valve middle slightly constricted. Length 25–41μm, breadth at mid–valve 5–8 μm, breadth at widest portion 7–10 μm, length to breadth ratio is 5: 1 (n=30). Fibulae 7–8/10 μm. Axial area very narrow, linear. Porcae nearly parallel in the middle and slightly radiate towards the apices. Striae indistinct.
SEM observations: Externally, raphe canal runs along the periphery of the valve margin, discontinuous at both apices (Figs 34–35), distal raphe ends straight and flat (Figs 34–35). Keel elevated above the valve face, fenestrae well–developed. Each fenestra contains 1–2 fenestral bars (Fig. 36). Striae extending into the axial area, biseriate (Fig. 37). Areolar openings round and occluded (Fig. 37). Internally, raphe discontinuous at both apices, helictoglossae small and flat (Figs 39–40). Striae occur on both valve face and mantle, interrupted by the axial area in the middle (Fig. 42), areolar openings small and round, unoccluded and without rims (Fig. 41).

Holotype: HANU! Individual in slide THHN2014017, here illustrated as Fig. 21.
Isotype: COLO! Kociolek Collection (University of Colorado, Boulder, U.S.A.), slide no. 650047.
Type locality: Stream. Wuzhi Mountains. Hainan Province, Coll. Y. Liu & X.X. Lu July 2014.
Etymology: This species is named for its central constriction.
Remarks: This species also found from samples THHN2014162 and THGX2014267.

***Iconella uniformis* Yan Liu, Ziyi Jiang et Kociolek sp. nov. (Figs 43–61)**
LM observation: Valves heteropolar, lanceolate, head pole broadly round, foot pole narrowly rounded. Length 71.6–94.4 μm, breadth 30.4–38.0 μm, length to breadth ratio is 2.4–2.5: 1(n=30). Fibulae 18–22/100 μm. Axial area lanceolate, wider in the center, narrower toward the apices. A distinct spine is visible at the end of axial area near the head pole. Porcae slightly radiate along the whole valve. Striae indistinct.
SEM observations: Externally, raphe canal runs along the periphery of the margin, discontinuous at both apices. At head pole, distal raphe ends straight, slightly raised, tooth–like in appearance, positioned on the mantle of headpole (Fig. 53). Keel elevated off of the valve face. Fenestrae well–developed, each containing 4–5 fenestral bars. Striae continuous across the axial area, uni– to biseriate (Fig. 55). Numerous granules scattered in the depression between porcae (Figs 53–55). One robust spine present at the headpole of axial area (Fig. 54). Several needle–shaped small spines occur on some fenestral bars. Internally, raphe continuous at the headpole (Fig. 58), discontinuous at the footpole, forming two large hook–shaped helictoglossae (Fig. 59). Axial area has a lanceolate depression (Fig. 57). Striae uni– to biseriate, continuous across the axial area (Fig. 60). Areolar openings small, round and rimmed (Fig. 61).

Table 2. Sampling information for new species in this research.

Sample No.	Locality	Habitat	pH	Conductivity ($\mu S.cm^{-1}$)	WT (°C)
THHN2014017	Wuzhi Mts, Hainan Province	Stream	4.78	20	23
THHN2014078	Diaoluo Mts. Hainan Province	Stream	7.52	120	26.4
THHN2014153	Jianfengling Mts. Hainan Province	Stream	6.98	70	26.3
THHN2014162	Jianfengling Mts. Hainan Province	Lake	6.98	50	24.8
THGX2014267	Fangchengjiang	Wet–wall	4.78	–	26.9
THGX2018004	Shiwan Mts. Guangxi	Epiphyton on Ponds	4.49	15.31	–



Figs 1–10. *Iconella shiwana* Yan Liu, Ziyi Jiang et Kociolek, LM pictures, valve view showing size variation. Fig. 1 is of the holotype. Scale bar 10μm.

Holotype: HANU! Individual in slide THHN2014078, here illustrated as Fig. 44.
Isotype: COLO! Kociolek Collection (University of Colorado, Boulder, U.S.A.), slide no. 650048.
Type locality: Stream. Diaoluo Mountains. Hainan Province, Coll. Y. Liu & X.X. Lu July 2014.
Etymology: This species is named for its large, hook–shaped helictoglossae.
Remarks: This species was also found from sample THHN2014153.

Discussion

All 3 new species considered here possess features that would support their assignment to the genus *Iconella*, including canal raphe extending around the whole valve margin, keel raised off the valve surface, presence of wings, fenestrae obvious, and uniformly porous alar canals. However, the two isopolar species, *I. shiwana* and *I. pseudoconstricta* have discontinuous raphe ends at both apices, which were illustrated as the character of "Pinnatae" group of *Surirella* (Jahn et al. 2017).

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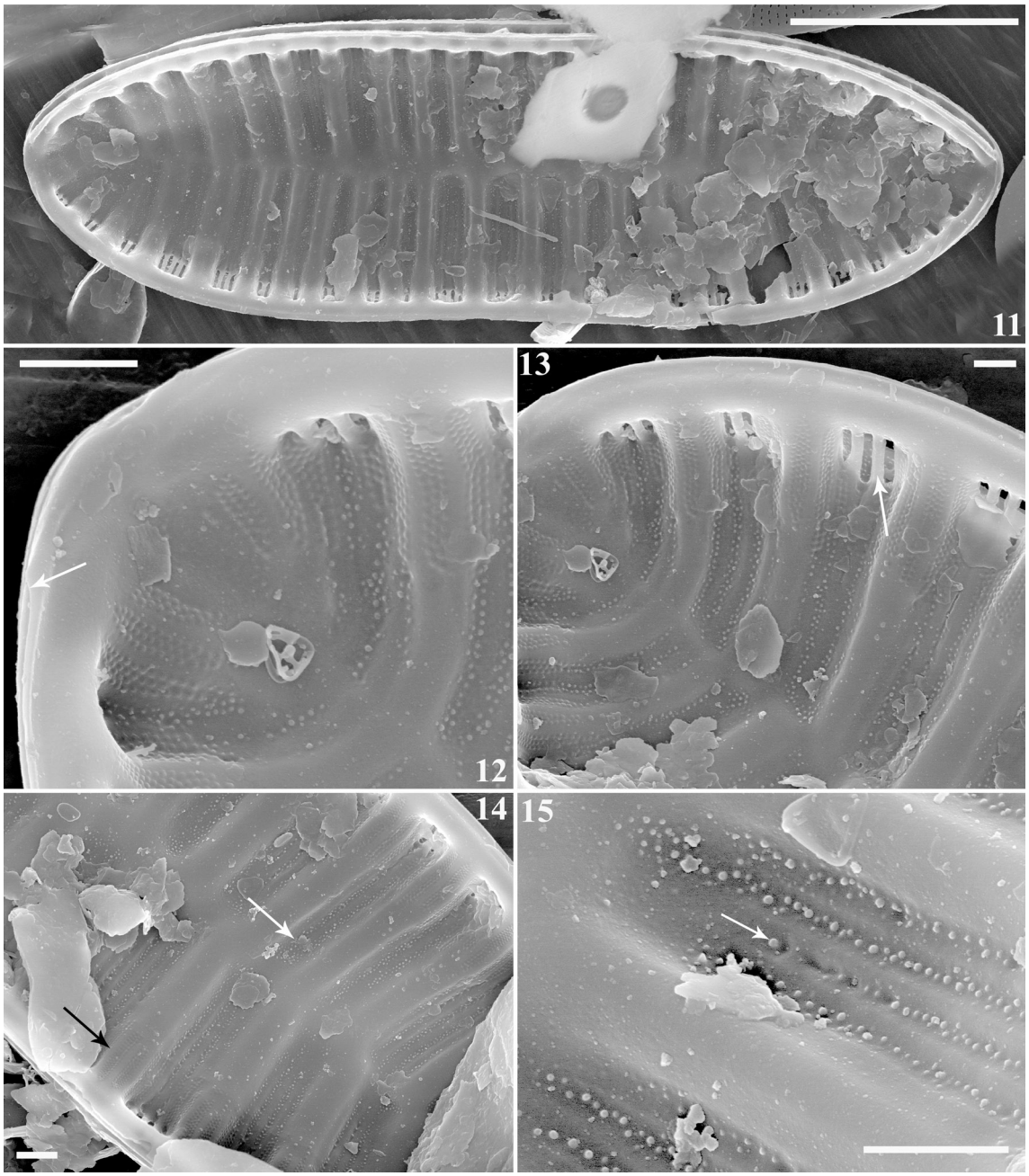
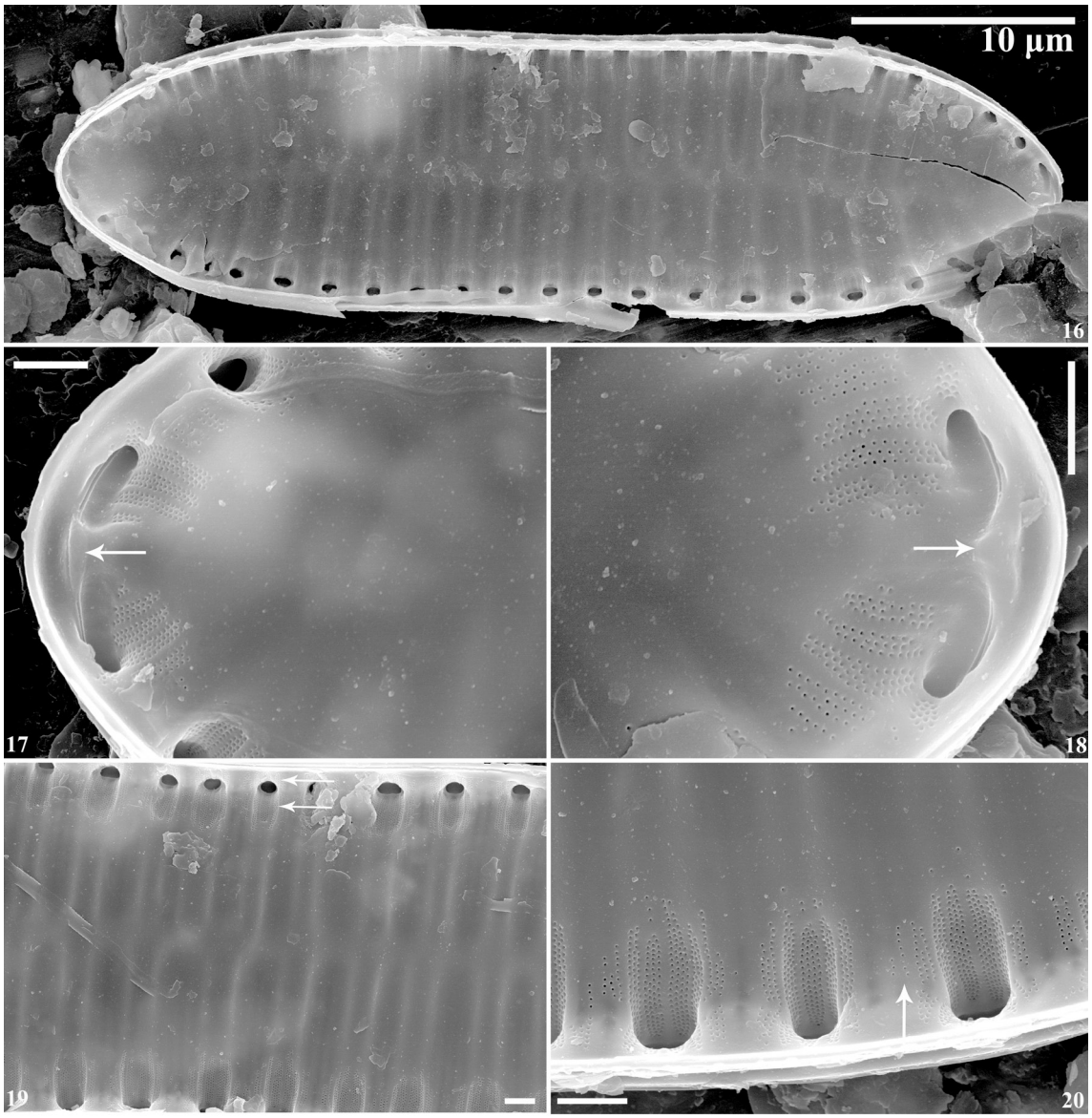


Figs 11–15. *Iconella shiwana* Yan Liu, Ziyi Jiang et Kociolek, SEM pictures, external views: (11) whole valve; (12) apex of the valve, showing the hooked distal raphe end (white arrow); (13) magnification of valve margin, showing the fenestral bars (white arrow); (14) valve face, showing the normal length striae (white arrow) and short striae at the porcae (black arrow); (15) striae showing areolae opening covered by the tiny silica granules. Scale bar 10μm (11), 1μm (12–15).

Additionally, these two taxa also do not have rimmed pores internally, a feature also missing in *Surirella congolensis* Cocquyt et Taylor (Cocquyt & Taylor 2015) and *Surirella leyana* Bramburger et Hamilton in Bramburger et al. (2006) (Karthick et al. 2012), which do not fit the illustrations for *Iconella* in Jahn et al. (2017). The number of taxa previously assigned to the genus *Surirella* sensu lato number over 1800 (Kociolek et al. 2020), yet fewer than 130 (7.2%) have been investigated with SEM (Gaul et al. 1993; Henderson & Reimer 2003). As suggested by the distribution of characters in the four taxa cited above, it is possible that features suggested to diagnose genera in the phylogeny of Ruck et al. (2016), and thus the recognition of taxa, may be in further need of review.

In terms of the distinctions of the three taxa considered herein, and their separation from similar forms, *Iconella shiwana*, is only known from Shiwan Mountains in Guangxi Zhuang Autonomous Region, an area with some very unique diatom taxa, including the genus *Sinoperonia* Kociolek et al. in Liu et al. (2018) and new species of *Neidium* Pfitzer (Pfitzer 1871) in



Figs 16–20. *Iconella shiwana* Yan Liu, Ziyi Jiang et Kociolek, SEM pictures, internal views: (16) whole valve, showing the elliptical hyaline area in the center; (17–18) apices of the same valve, showing the discontinuous raphe ends, and a nodule structure between the two distal raphe ends (white arrow); (19) valve face showing striae on both valve and mantle sides (white arrows) of the portulae; (20) scattered striae on fibulae (white arrow). Scale bar 10µm (16), 1µm (17–20).

Liu et al. (2020). *I. shiwana* differs from other taxa by the isopolar valve and linear outline with broadly round apices. SEM observations also showed very distinctive character of the striae internally. Most *Iconella* or *Surirella* species have the same organization of striae both externally and internally, but *I. shiwana* has tri– to quadriseriate, short striae internally, forming a large, elliptical hyaline area in the valve center but externally this species has striae that are long, extending to the axial area, and being uniseriate or biseriate. Helictoglossae are distinct, a fused nodule structure is present between the two raphe ends.

Iconella pseudoconstricta differs from *I. shiwana* by its acutely–rounded apices and more slender valves. It also resembles *Surirella rafaelii* Ramirez & Sala in Sala et al. (2013), which is also reported from a tropical area, but *S. rafaelii* can easily be separated from *I. pseudoconstricta* by its large valve, central ridge and tendrils at the base of fenestrae.

Iconella uniformis resembles the cosmopolitan species *Surirella splendida* (Ehrenberg) Kützing (Kützing 1844), the difference between these two species is that *I. uniformis* is smaller and has a spine at the end of axial area near headpole. Our specimens also resemble *S. splendidoides* Hustedt (Hustedt 1965) as illustrated in Metzeltin & Lange–Bertalot (1998, Taf. 209: 1–2), but the type specimens of Hustedt's taxon does not have a spine at the central area, as illustrated in Simonsen (1987, pl. 757: 4–5). A very distinctive feature of *I. uniformis* is the hook–like helictoglossae at the foot pole. The helictoglossae are usually overlooked by researchers (e.g.: Krammer & Lange–Bertalot 1997; Ruck &

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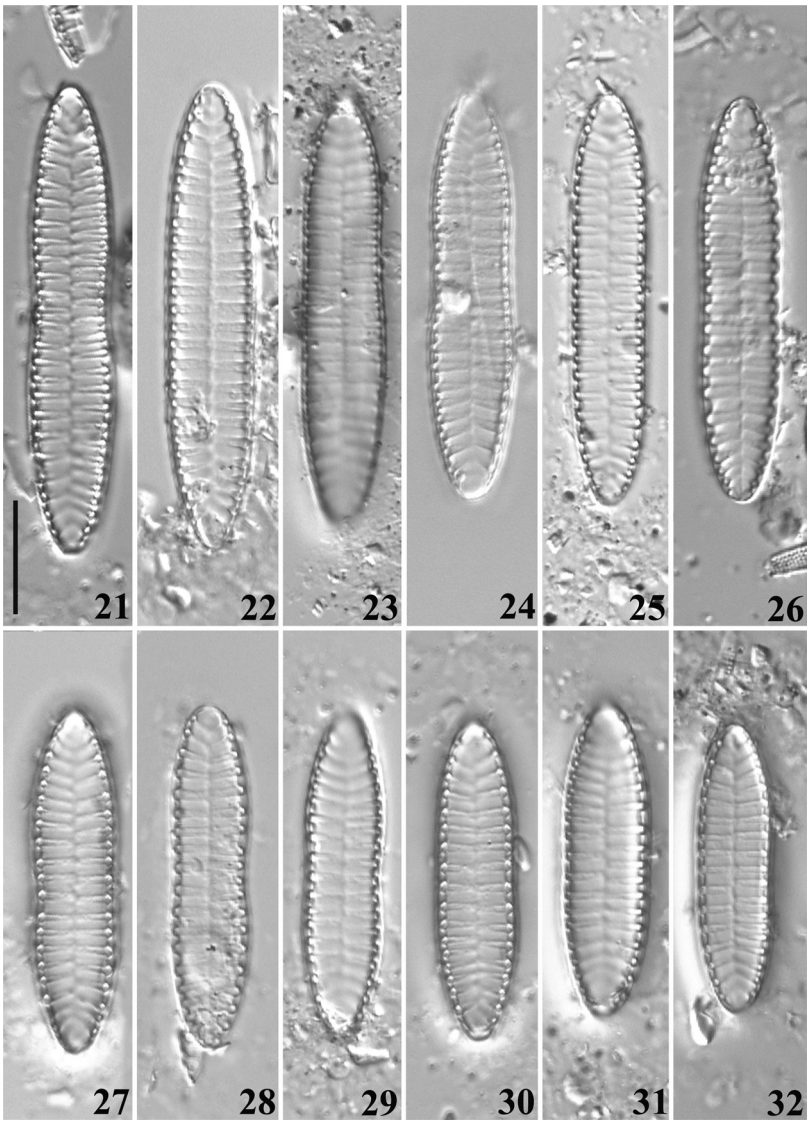

Figs 21–32. *Iconella pseudoconstricta* Yan Liu, Ziyi Jiang et Kociolek, LM pictures, valve views showing size variation: (21) is of the holotype. Scale bar 10μm.

Kociolek 2004), and the various morphologies of the structure of helictoglossae in *Iconella* or *Surirella* are poorly documented for new species, even in more recent treatments (e.g.: English & Potapova 2011; Blanco et al. 2012; Van de Vijver et al. 2013; Brindle et al. 2018). Ruck & Kociolek (2004) described helictoglossae in *S. robusta* Ehrenberg (Ehrenberg 1840) and *S. splendida* to have typical helictoglossae–like process at the footpole (Ruck & Kociolek 2004, pl. 23: 10; pl. 29: 14). Cocquyt & Taylor (2015) showed *S. ebalensis* to have a continuous raphe with a reduced helictoglossa, but a discontinuous raphe with ends at the footpole that formed two reduced helictoglossae separated farther away from the raphe endings (Cocquyt & Taylor 2015, Fig. 5 C–D). Another uncommon feature observed in our new species is the small teeth at the mantle of headpole. Cocquyt et al. (2017) also found similar structures in *Surirella chasei* Cholnoky (Cholnoky 1954) which is diagnosed by the various granules scattered on the entire valve face. More research is needed to understand the morphological diversity of helictoglossae and teeth–shaped structure of *Iconella*, and its position in the taxonomy of the Surirellales.

Our exploration of tropical areas in China has resulted in the report of several new and endemic species (e.g. Liu et al. 2014a,b, 2016, 2018), indicating the diatom flora is very unique in this region. However, diatom biodiversity is still poorly understood for this area. We expect as further collections are made in the region, and subsequent taxonomic works are carried out, additional new taxa will be described from tropical areas in China.

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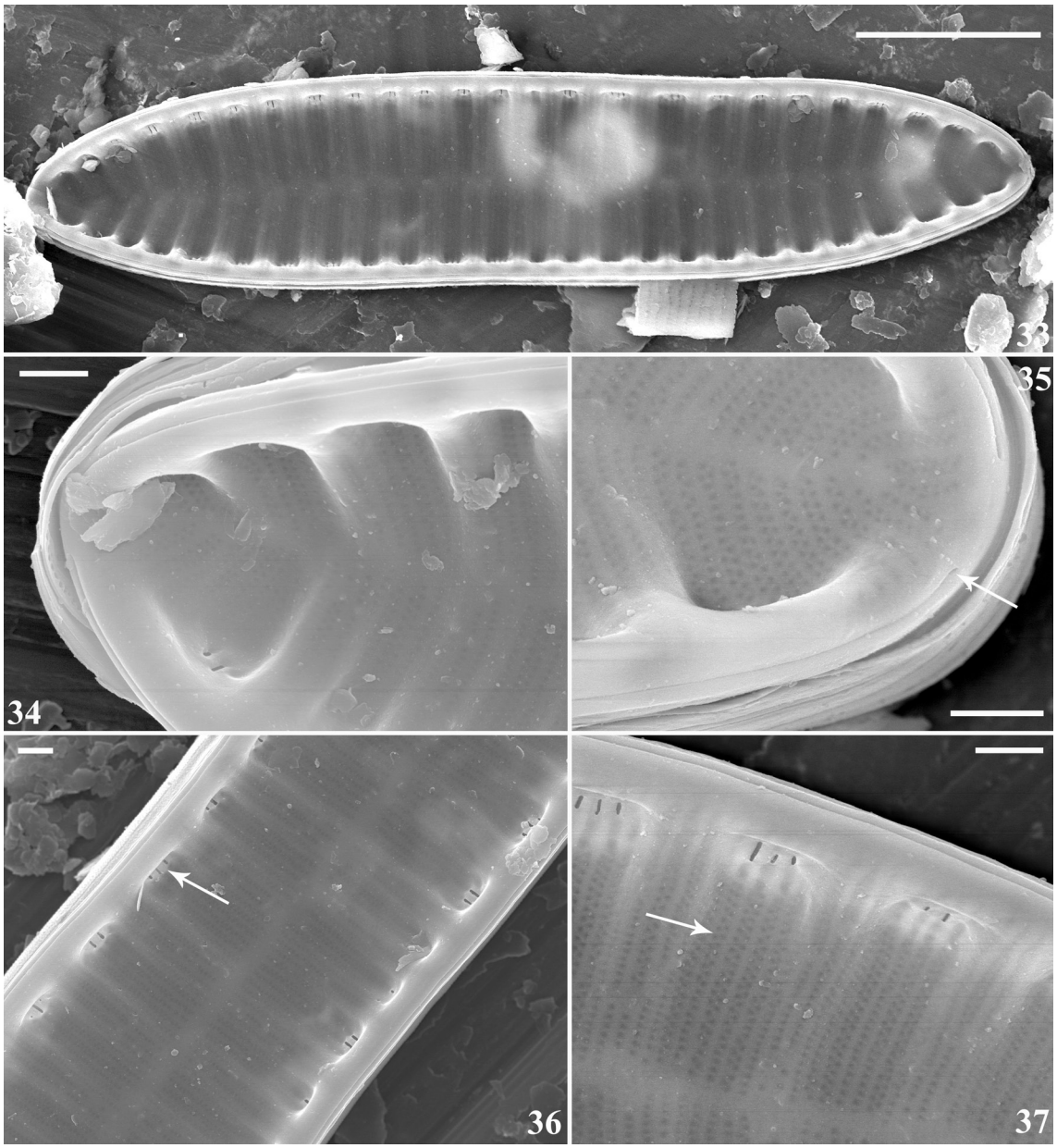

Figs. 33–37. *Iconella pseudoconstricta* Liu, Jiang, Kociolek et Fan, SEM pictures, external views: (33) whole valve; (34–35) apex of valve, showing the distal raphe ends (white arrow in fig. 35); (36) fenestral bars (white arrow); (37) biseriate striae (white arrow). Scale bar 10µm (33), 1µm (34–37).

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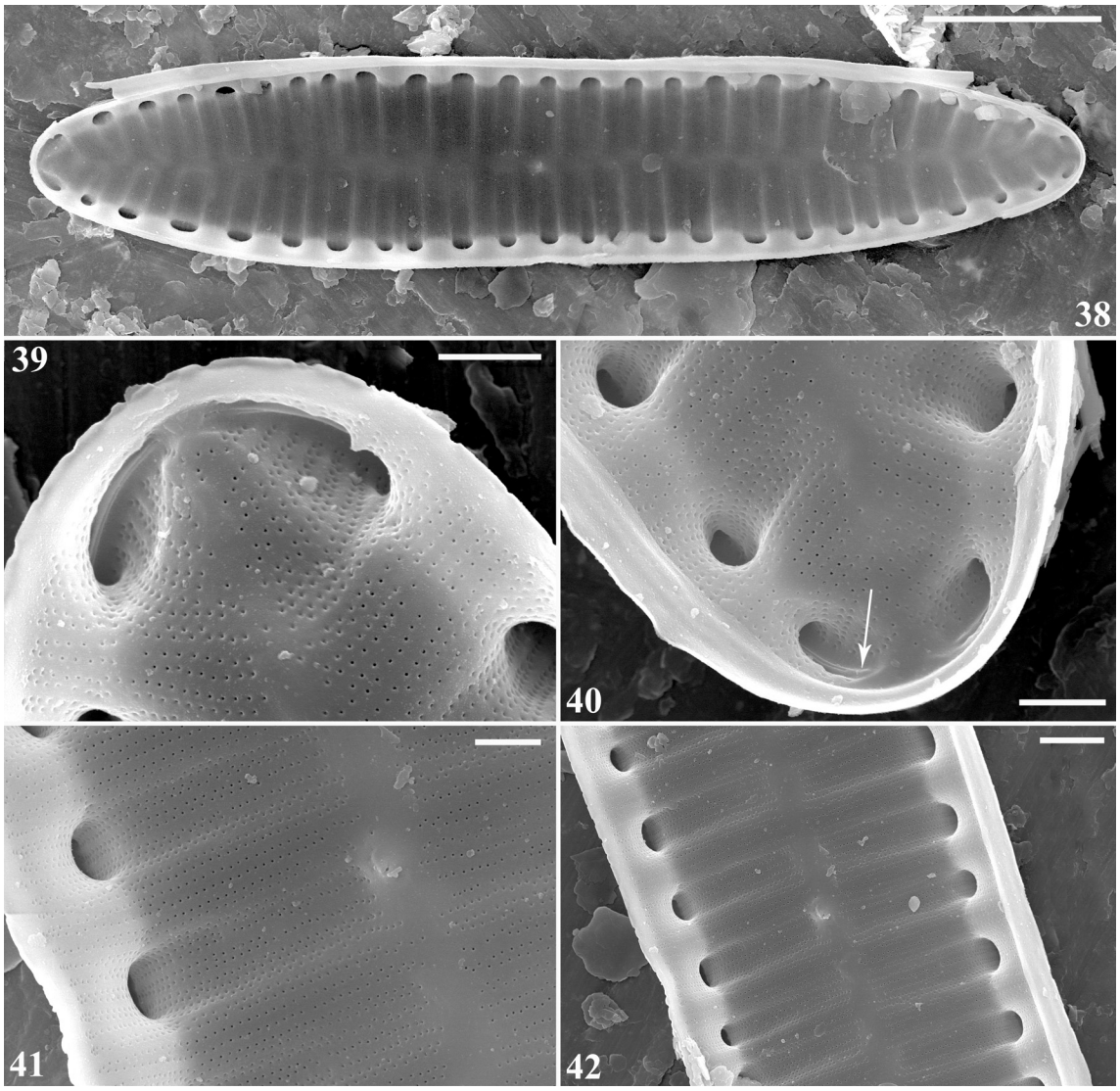

Figs 38–42. *Iconella pseudoconstricta* Yan Liu, Ziyi Jiang et Kociolek, SEM pictures, internal views: (38) whole valve; (39–40) apices of the same valve, showing the discontinuous distal raphe ends (white arrow in fig. 40); (41) small and round areolar opening; (42) striae discontinuous in the axial area. Scale bar 10μm (38), 1μm (39–41), 2μm (42).

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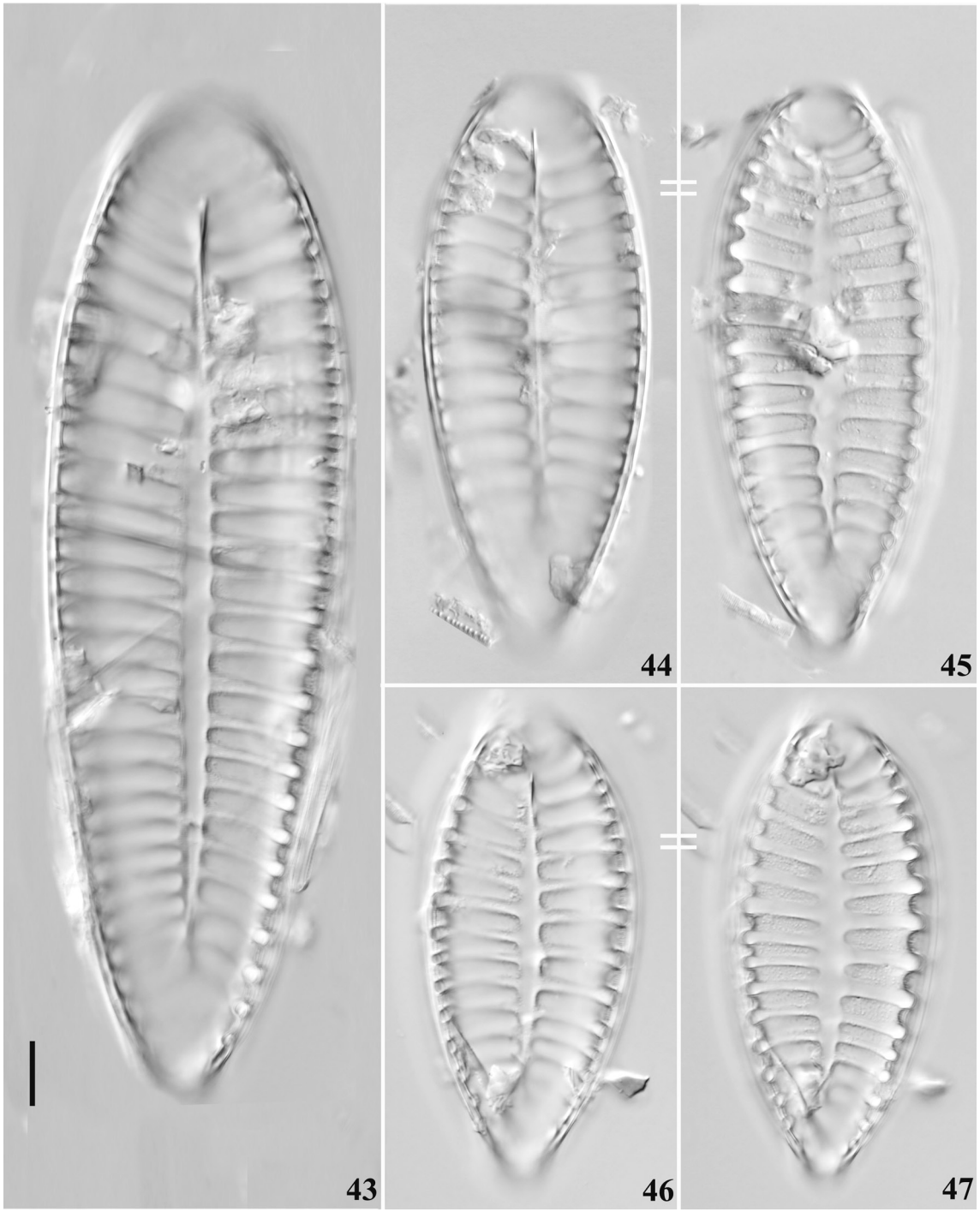

Figs 43–47. *Iconella uniformis* Yan Liu, Ziyi Jiang et Kociolek, LM pictures, valve view showing the size variation: (44) is of the holotype; (44–45) and (46–47) are each of the same valves at different focal planes. Scale bar 10μm.

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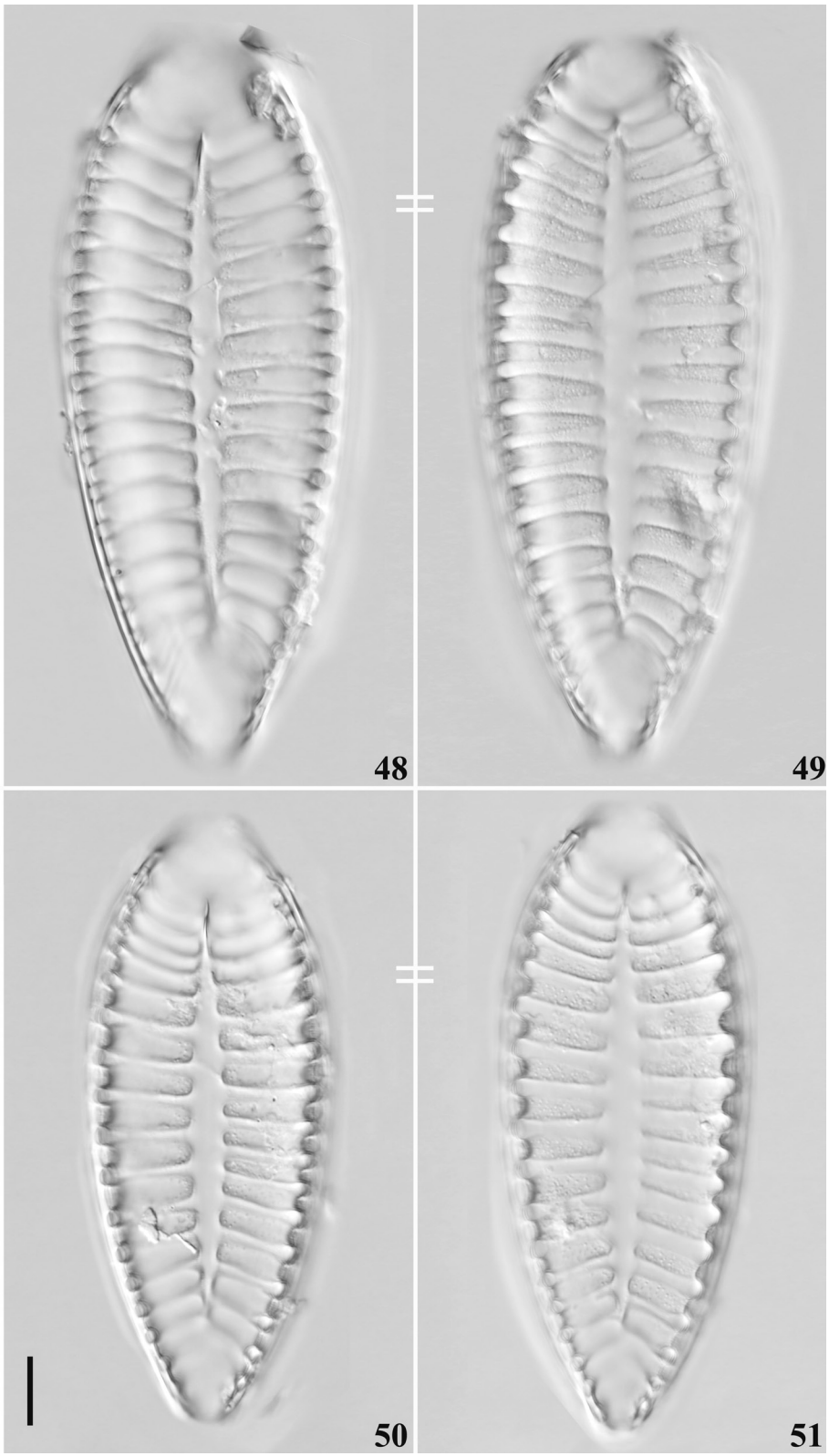

Figs 48–51. *Iconella uniformis* Yan Liu, Ziyi Jiang et Kociolek, LM pictures, valve view showing the size variation: (48–49) and (50–51) are each of the same valves at different focal planes. Scale bar 10µm.

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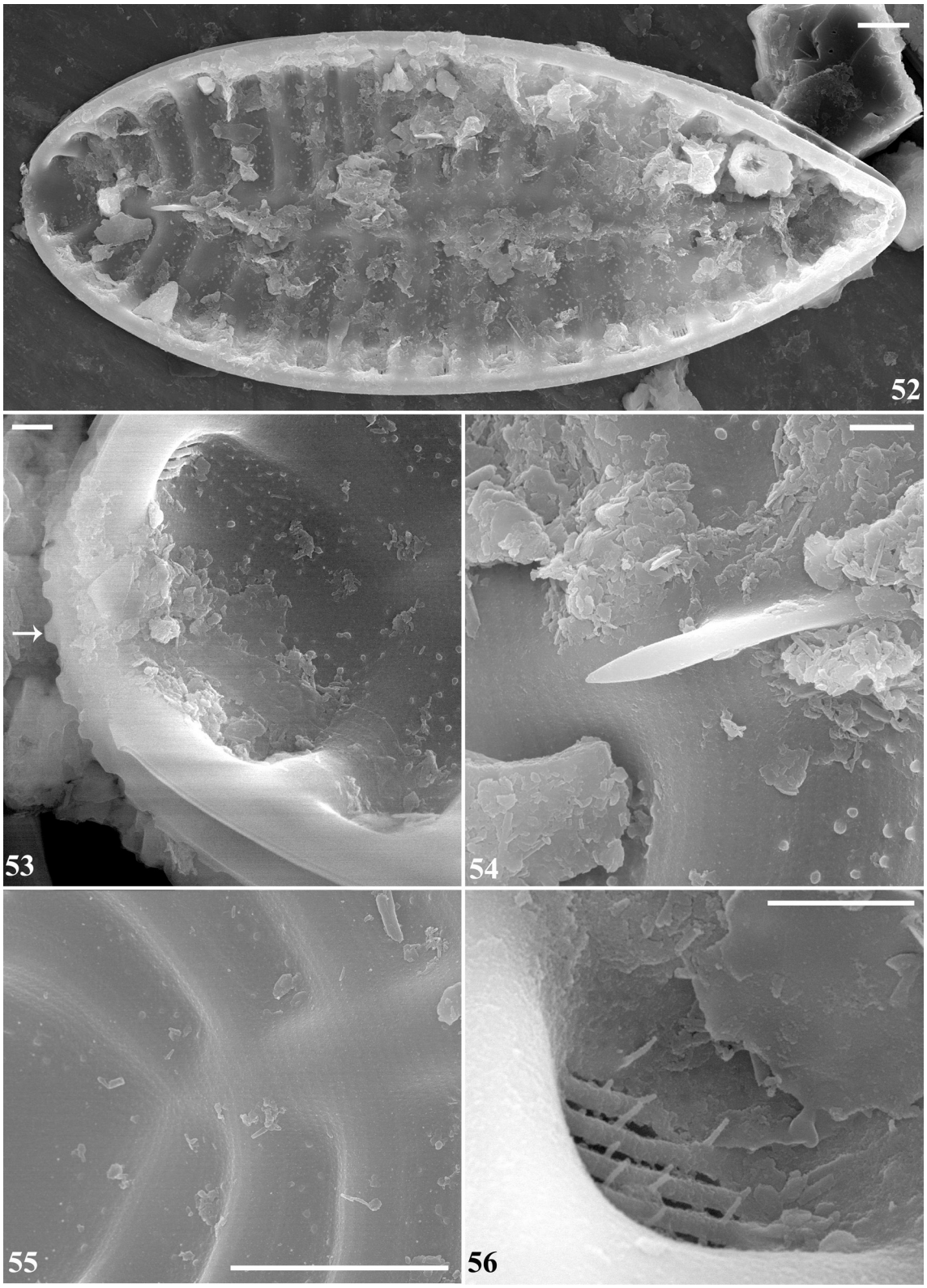

Figs 52–56. *Iconella uniformis* Yan Liu, Ziyi Jiang et Kociolek, SEM pictures, external views: (52) whole valve; (53) headpole of the valve, showing the distal raphe ends slightly raised, and teeth structure at the mantle of headpole (white arrow); (54) spine in the headpole of axial area; (55) axial area showing the striae continuous in the axial area; (56) a single fenestra, showing the small needle like spine on the fenestral bars. Scale bar 5µm (52), 1µm (53–56).

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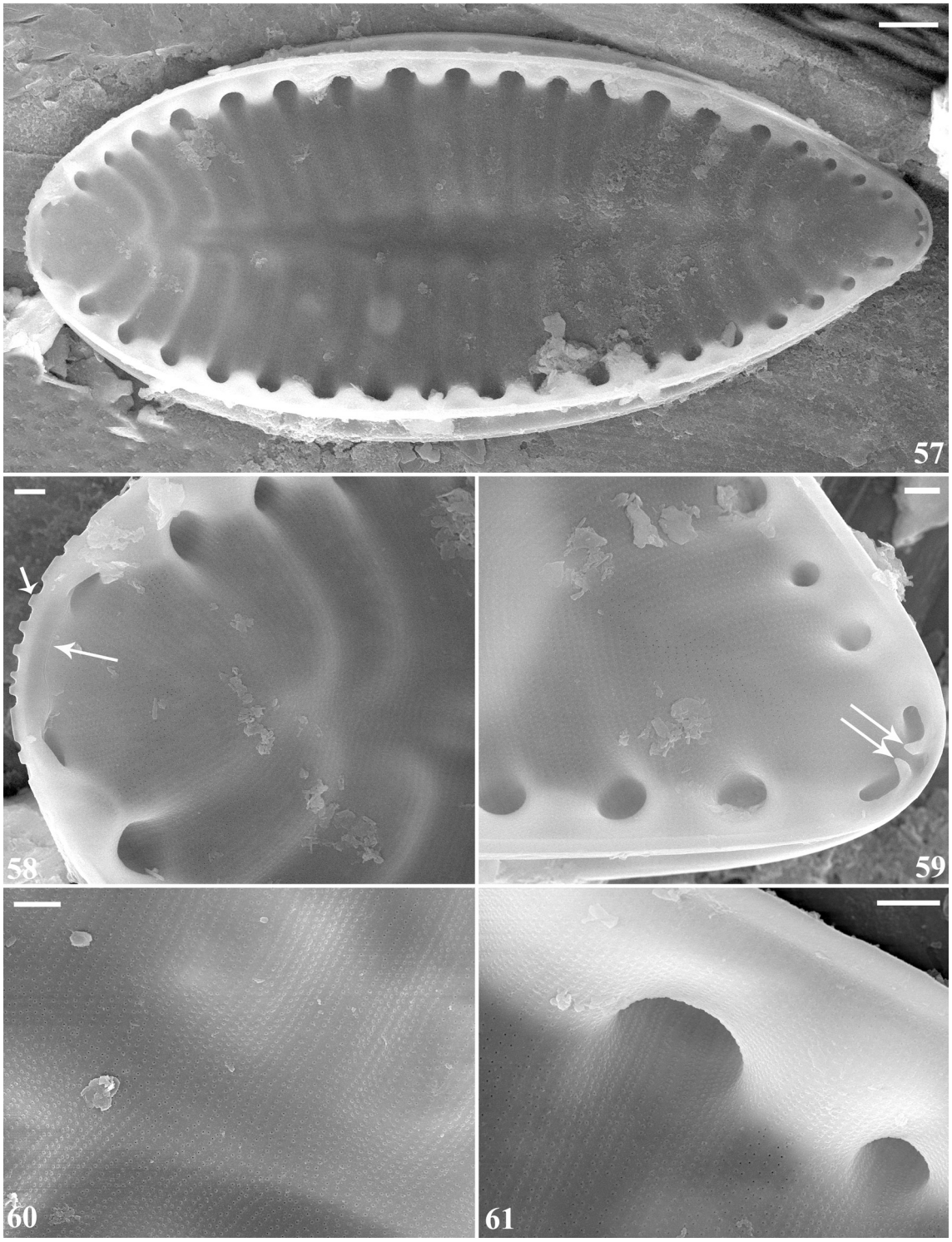

Figs 57–61. *Iconella uniformis* Yan Liu, Ziyi Jiang et Kociolek, SEM pictures, internal views: (57) whole valve; (58) headpole of the valve, showing the continuous distal raphe ends (white arrow); (59) footpole of the valve, showing the hook–shaped helictoglossae (white arrow); (60) axial area showing the striae continuous in the axial area; (61) valve margin showing the rimmed areolae openings. Scale bar 5μm (57), 1μm (58–61).

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