

Three new species of *Delicatophycus* M.J. Wynne (Bacillariophyta) from China, all possessing apical pore fields

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Abstract: *Delicatophycus wulingensis* sp. nov., *D. qinghaiensis* sp. nov., and *D. menyuanensis* sp. nov., are described, illustrated, and compared to similar taxa. Two types of apical pore fields (APFs) in the genus *Cymbella* are defined. The three new *Delicatophycus* all possess the type II APF found in some species of *Cymbella*, i.e. the APF is divided by the distal raphe fissure into two areas, one larger than the other. They also share a further three distinct features: variable central dorsal portion of valve, stigmata, and a notch on the dorsal side of the distal raphe fissure. Since there are many species of *Delicatophycus* without APFs, such as *D. sinensis* M.J. Wynne, *D. williamsii* M.J. Wynne and *D. chongqingensis* M.J. Wynne; *D. williamsii* and *D. liuweii* Li both possess stigmata, thus proposed diagnostic characters for *Delicatophycus* are the tilde-shaped areolae and the dorsally curved distal fissures.

Key words: central area, dorsoventral, Qinghai, tilde-shaped, Wuling Mountains

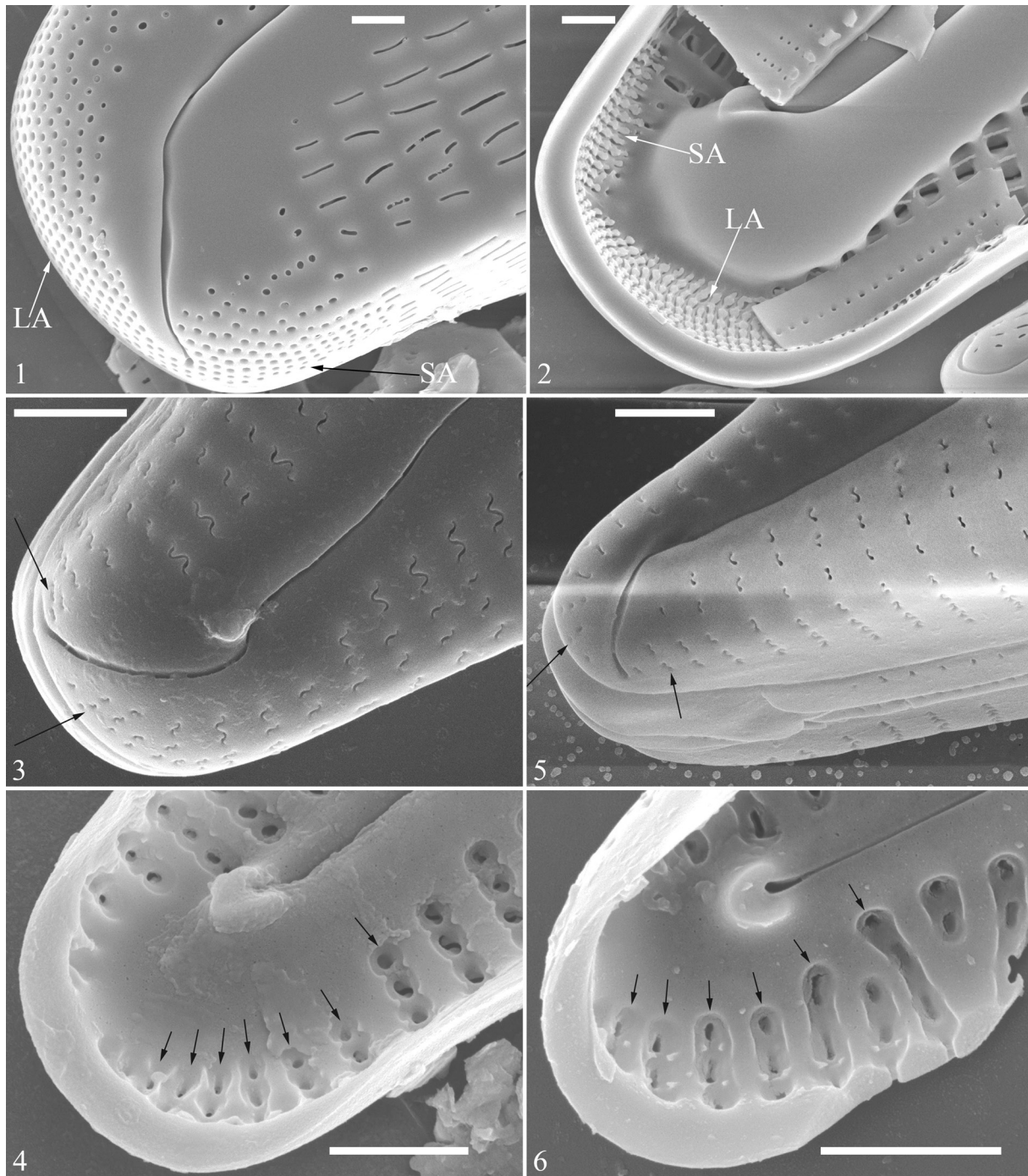
INTRODUCTION

KRAMMER (2003) erected the diatom genus *Delicata* Krammer for 19 taxa previously included in *Cymbella* sensu lato. *Delicata* was based on a combination of characters which included the transapically undulated foramina of the areolae (KRAMMER 2003, p. 111). In addition, species in *Delicata* were distinguished from *Cymbella* C. Agardh by their lack of apical pore fields (APFs) (KRAMMER 2003, p. 111). With many new species found in *Delicata* since KRAMMER, some presented additional diagnostic characters which KRAMMER had not discussed. For example, LIU et al. (2018) noted 3–4 stigmata located in the ventral sternum of *Delicata williamsii* Bing Liu et S. Blanco and LE COHU et al. (2018) noted that *Delicata pouemboutensis* Tudesque et al. has 5–6 pseudostigmoids and a notch on the dorsal side of the distal raphe fissure.

Some 16 years after its description, WYNNE (2019) corrected a nomenclatural issue with the name *Delicata* as the word is used as a technical term in morphology, which is not permitted by the current Code (Art. 20.2, in TURLAND et al. 2018). WYNNE (2019) proposed *Delicatophycus* M.J. Wynne as a replacement name for *Delicata* and introduced 26 replacement names for

invalid designations in *Delicata*. Since WYNNE proposed his new name, several further combinations have been made (BAHLS 2019; LE COHU et al. 2019) and the new species *D. liuweii* Li added (LIU et al. 2021). Thus, to date, there are 30 species within the genus *Delicatophycus*. From these published data, all authors consider the tilde-shaped areolae as a defining character of the genus *Delicatophycus*. Can the APF also be a defining character for *Delicatophycus*?

KRAMMER studied the APFs in *Cymbella* (KRAMMER 2002, pp. 193, 195, 197, pls 1–3). In *Cymbella aspera* (Ehrenberg) H. Peragallo, the APFs are externally divided by the distal raphe fissures into two distinct areas, one larger than the other, and both composed of small, round foramina (Fig. 1, LA = larger area, SA = smaller area; see also KRAMMER 2002, p. 193, fig. 5), whereas internally an undulate flap-like silica strip is above the apertures of each row of foramina (Fig. 2; see also KRAMMER 2002, p. 195, figs 1–3). COX (2012) reviewed diatom APFs and provided a definition: “Areas of unoccluded pores associated with polysaccharide secretion, to form pads or stalks for attachment, at valve apices are found in both raphid (e.g. *Cymbella*, *Gomphoneis*, *Gomphonema*) and araphid (e.g. *Asterionella*, *Meridion*, *Synedra*) diatoms, at one or both poles of the cell”. This definition presumes



Figs 1–6. Apical ultrastructure in *Cymbella aspera*, *Delicatophycus sinensis*, and *D. williamsii*: (1, 2) apical pore fields (APFs) of *C. aspera*, showing its APF composed of two parts: one is a larger area (LA) and the other is a smaller area (SA), an undulate flap-like silica strip above internal apertures of each row of foramina (Fig. 2, two arrows); (3, 4) apical details of *D. sinensis*, note no differentiation between the apical areolae and the other areolae, i.e. APF absent (arrows); (5, 6) apical details of *D. williamsii*, note no differentiation between the apical areolae and the other areolae, i.e. APF absent (arrows). Scale bars 1 μm .

a number of items. First, besides possessing the specific external and internal structure, as in *C. aspera*, an APF should have the function of secreting polysaccharide, knowledge of which is lacking in most species that possess APFs. Second, it presupposes that all APFs are covered by this one definition when, for example, *Cymbella*, *Gomphoneis*, and *Gomphonema* are not closely related to *Asterionella*, *Meridion*, or ‘*Synedra*’ [= *Ulnaria*]). In

the genus *Delicata* [= *Delicatophycus*] from Diatoms of North America (Spaulding & Edlund 2008), four taxa (*Delicatophycus canadensis* M.J. Wynne, *D. montana* M.J. Wynne, *D. delicatula* M.J. Wynne, and *D. alpestris* M.J. Wynne) were demonstrated to have APFs in valve external views whereas the authors neither provided the internal details of the APFs nor mentioned the APFs present at each apex of the above four taxa. Thus, to

Table 1. Sampling sites for the three new species of *Delicatophycus* from rivers in the Wuling Mountains and Qinghai province, China, with associated pH, conductivity, and temperature (three replicates).

Species	Sampling site	Coordinates and elevation (m a.s.l.)	pH	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	Temperature ($^{\circ}\text{C}$)	Sampling Date
<i>D. wulingensis</i> sp. nov.	Zhongjian River	29°43'40"N, 109°13'15"E; 690	8.50 ± 0.02	271 ± 1	9.2 ± 0	February 20, 2018
<i>D. qinghainensis</i> sp. nov.	Chengduo County	33°22'21"N, 97°0'15"E; 3710	8.40 ± 0.20	416 ± 1	12.2 ± 0.2	July 22, 2019
<i>D. menyuanensis</i> sp. nov.	Menyuan County	37°27'28"N, 101°23'15"E; 2940	8.36 ± 0.03	448 ± 1	11.9 ± 0.5	July 18, 2019

Table 2. Comparison among similar *Delicatophycus* species.

Feature	<i>D. wulingensis</i>	<i>D. qinghainensis</i>	<i>D. menyuanensis</i>	<i>D. sinensis</i>	<i>D. williamsii</i>	<i>D. chongqingensis</i>
Valve outline	Rhombic-lanceolate with not protracted apices	Lanceolate with slightly protracted, rostrate apices	Lanceolate in larger specimens, semi-lanceolate in smaller ones, with rostrate apices	Lanceolate with slightly protracted, rostrate apices	Semi-lanceolate with slightly protracted apices	Semi-lanceolate with slightly protracted, rounded apices
Valve length (L) and width (W) (μm)	L: 25–58 W: 5.1–7.9	L: 21–39 W: 4.4–5.8	L: 15–35 W: 4.3–5.9	L: 21–41 W: 5.9–8.0	L: 15–30 W: 4.7–6.2	L: 21.0–30.5 W: 6.0–8.3
Maximum length/width ratio	7.8	6.7	6.1	5.4	5.2	4.0
Stria number in 10 μm	15–19	16–18	14–19	14–16	12–16	13–15
Number of stigmata	3–4	1–5	2–4	0	3–4	0
Notch on the dorsal side of distal raphe fissure	Present	Present	Present	Absent	Absent	Absent
APF	Present	Present	Present	Absent	Absent	Absent
References	This paper	This paper	This paper	LIU et al. 2018	LIU et al. 2018	YANG et al. 2019

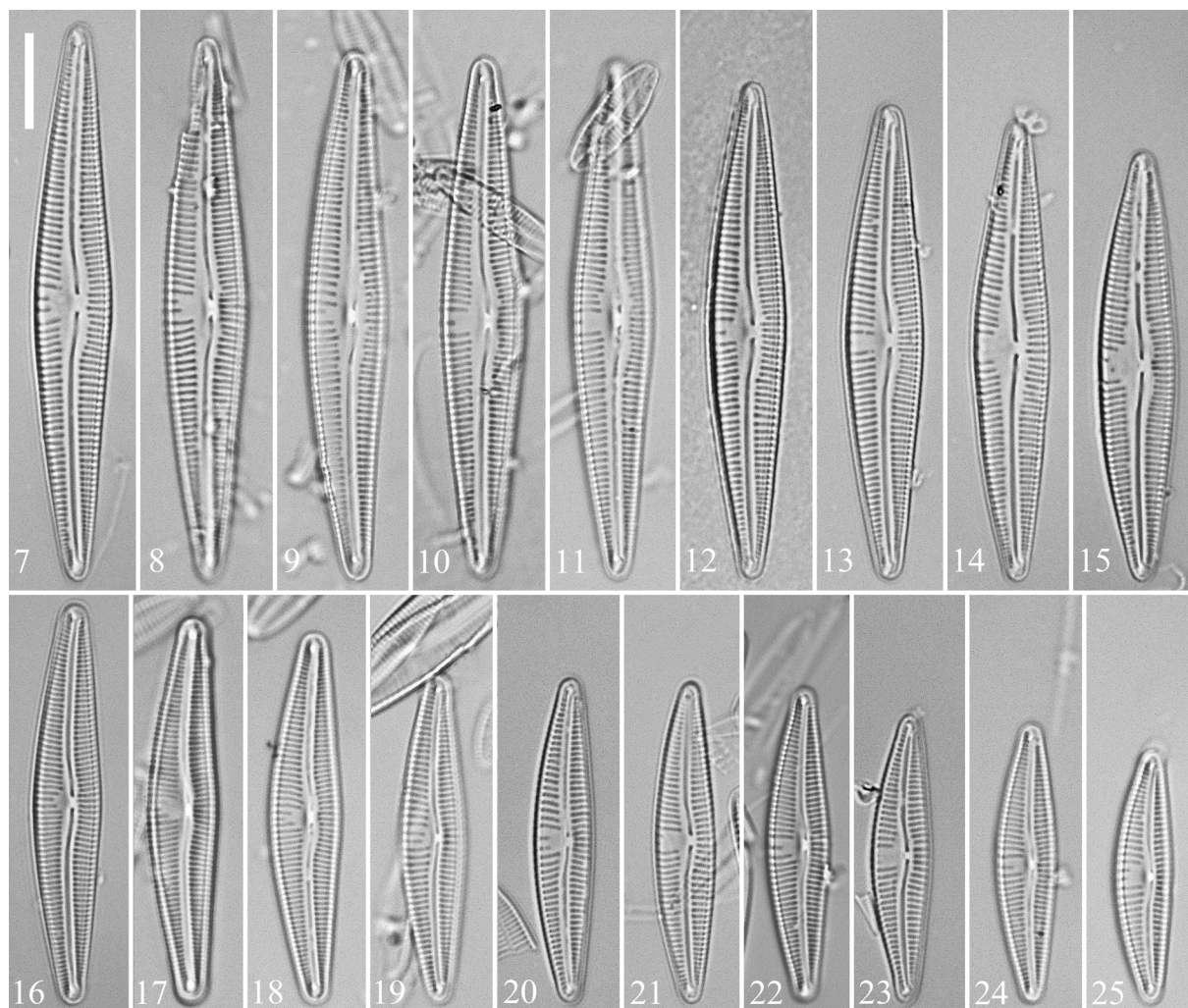
date, APFs have not yet been verified for any species of *Delicatophycus*. But some taxa lacking the APFs have been confirmed. Examples are *Delicatophycus sinensis* M.J. Wynne and *D. williamsii* M.J. Wynne, both of which do not have the APFs because the areolae at each apical field are still tilde-shaped externally (not differentiated into rounded foramina) and not covered by silica strips internally (Figs 3–6; see also LIU et al. 2018). One more example is *Delicatophycus chongqingensis* M.J. Wynne, which lacks APFs as well (see YANG et al. 2019, p. 61, figs 12, 13, 18, 19).

This study described three new species of *Delicatophycus* from the Wuling Mountains and Qinghai

province, China: *Delicatophycus wulingensis* sp. nov., *D. qinghainensis* sp. nov., and *D. menyuanensis* sp. nov. All three species possess APFs. This is discussed in the context of the definitions and descriptions of the genera *Cymbella* and *Delicatophycus*.

MATERIAL AND METHODS

One study site is at the course of Zhongjian River in Zhongjian River Giant Salamander National Nature Reserve, which is located in Wuling Mountains, China under a sub-tropical to



Figs 7–25. *Delicatophycus wulingensis* sp. nov., LM: (7–25) 19 valves showing size diminution series, note extremely variable dorsal central areas; (7) micrograph of holotype specimen; (12) micrograph of isotype specimen. Scale bar 10 μ m (in Fig. 7) for all figures.

warm temperate type climate. The other two study sites (a river in Chengduo County and a river in Menyuan County) belong to Qinghai province, which is on the north-east area of the Qinghai–Tibet plateau, with an average elevation of >3000 m a.s.l. Qinghai has a highland continental climate that is very different from Wuling Mountains. Information about sampling sites is summarized in Table 1. At these three sampling sites, epilithic algae were collected at numerous submerged stones showing yellow–brown surfaces that indicate the presence of diatoms. Each stone was placed on a plastic plate and its surface was brushed using a toothbrush, with the brushed-off diatom samples being washed into the plate. The diatom samples were transferred into a 100 ml sampling bottles and fixed with 70% ethanol. Two samples were collected from each site. Together with the sample collection, temperature, pH, and conductivity were measured in situ with a portable multimeter (HQ40D, HACH Company) — details are presented in Table 1.

The specimens for permanent slides were air-dried onto coverslips then mounted onto microscope slides using Naphrax. The slides were examined, and specimens photographed by using a Leica DM3000 light microscope (LM) at 1000 \times magnification (objective NA 1.25) and a Leica MC190 HD digital camera. The holotype slides are deposited in the Natural History Museum, London, United Kingdom (BM) and

isotypes slides are kept in the Herbarium of Jishou University, Hunan, People's Republic of China (JIU). Samples were further examined by using scanning electron microscopy (SEM). Several drops of the selected cleaned diatom material were air-dried onto glass coverslips which were then attached to aluminium stubs by using double-sided conductive carbon strips and sputter-coated with platinum for 20 seconds (Cressington Sputter Coater 108auto, Ted Pella, Inc.). Samples were examined and imaged by using a field emission scanning electron microscopy (FE–SEM) Sigma HD (Carl Zeiss Microscopy) available at Huaihua University, China.

Diatom valve terminology mainly follows KRAMMER (2003), LE COHU et al. (2018), and LIU et al. (2018).

RESULTS

Delicatophycus wulingensis Bing Liu et S. Blanco sp. nov. (Figs 7–50)

Description

LM (Figs 7–25): Valves dorsiventral, with markedly convex ventral margin, exhibiting rhombic–lanceolate outline (Figs 7–25). Apices not protracted, rounded.

Valve dimensions ($n = 100$): 25–58 μm long, 5.1–7.9 μm wide, length/width ratio range 4.7–7.8. Sternum narrow, widening when approaching central area. Two raphe branches mostly along valve mid-line but deflected towards ventral side when close to central area. Distal raphe fissures dorsally hooked and proximal raphe ends teardrop-like. Central area extremely variable. Ventral portion of central area nearly absent for all valves, dorsal portion of central area exhibiting four types: areas delimited by two marginal striae (e.g. Figs 7, 8, 20), areas delimited by one marginal shortened stria (e.g. Figs 9, 10, 12, 15, 22, 24), semi-fascia areas (e.g. Figs 11, 13, 14, 21, 23), and small areas delimited by slightly shortened striae (e.g. 16, 18, 19). Striae radiate throughout valve, 15–19 in 10 μm measured at ventral middle part, becoming denser towards apices.

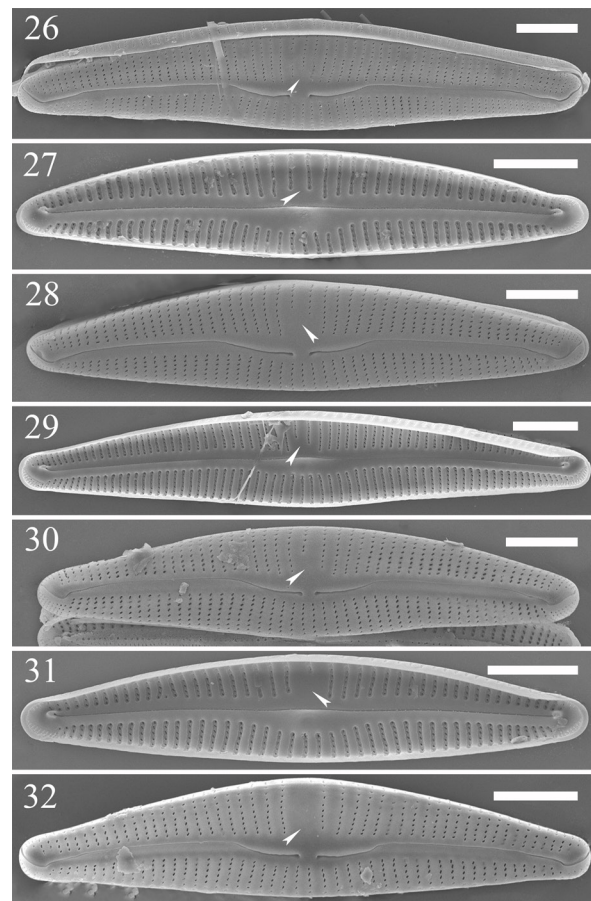
SEM (Figs 26–50): Dorsal central areas having four types: areas delimited by slightly shortened striae (Figs 26, 27, two arrowheads), areas delimited by two marginal shortened striae (Figs 28, 29, two arrowheads; Figs 33, 36, arrow; Fig. 39), areas delimited by one marginal shortened stria (Figs 30, 31, two arrowheads; Fig. 42), semi-fascia area (Fig. 32, arrowhead). Proximal raphe endings elongated and enlarged (Figs 33, 36), distal raphe fissures deflected towards dorsal side (Figs 34, 35, 37, 38). Notch on dorsal side of distal raphe fissure (Figs 34, 35, 37, 38, wavy arrow). Externally, striae composed of tilde-shaped areolae, continuing onto mantle. Internally, areolae diagonally aligned in shallow depressions bordered by very broad virgae. Internally, distal raphe ends terminating on helictoglossae, proximal raphe ends invisible because obscured by flap over central nodule, and few stigmata may be present on ventral side (Fig. 39, four arrowheads, Fig. 42, three arrowheads). APF divided by distal raphe fissure into two unequal sections: one larger than the other (Figs 45–48, LA, SA). The larger section composed of c. 7–9 rows of foramina whereas the smaller one composed of c. 3–5 rows of foramina. Externally, APF foramina rounded, which differ from tilde-shaped areolae on valve surface. Internally, each pervalvar row of inner openings covered by undulate flap-like silica strip above internal apertures but not completely occluded (Figs 46, 48, two wavy arrows, respectively). Each APF seems able to secrete polysaccharide stalks (Figs 49, one black and one white arrow, Fig. 50, two arrows).

Holotype: Slide BM 81897, the Natural History Museum, London. Fig. 7 is the micrograph of the holotype.

Isotype: Slide G202101, the Herbarium of Jishou University, Hunan, People's Republic of China. Fig. 12 is the micrograph of the isotype.

Type locality: The Zhongjian River course at a sampling point (29°43'40"N, 109°13'15"E, 690 m a.s.l.), Zhongjian River Giant Salamander National Nature Reserve, Xianfeng County, Hubei province, China (Collected by Bing Liu, February 20, 2018).

Habitat: Inhabiting surfaces of river immersed stones, thus, belonging to river epilithic diatom.



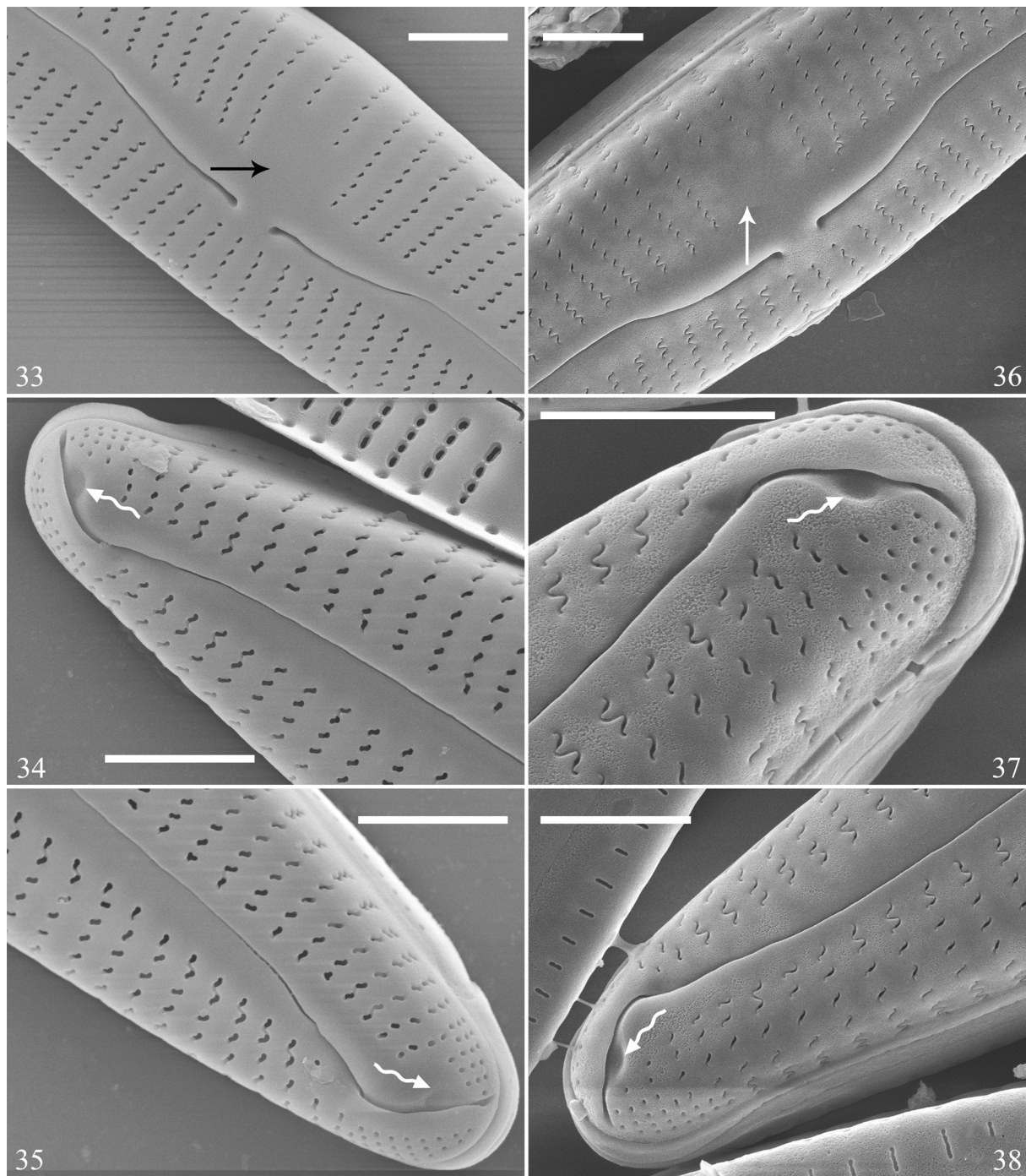
Figs 26–32. *Delicatophycus wulingensis* sp. nov., valve view, SEM: (26–32) seven valves showing four types of dorsal central areas; (26, 27) two valves showing two small dorsal central areas (two arrowheads); (28, 29) two valves showing two dorsal central areas delimited by two marginal shortened striae (two arrowheads); (30, 31) two valves showing two dorsal central areas delimited by one marginal shortened stria (two arrowheads); (32) one valve showing dorsal central area of semi-fascia (arrowhead). Scale bars 5 μm .

Etymology: Named after the Wuling Mountains, where the species was found.

Delicatophycus qinghainensis Bing Liu et S. Blanco sp. nov. (Figs 51–90)

Description

LM (Figs 51–72): Valves dorsiventral, with slightly convex ventral margin, exhibiting lanceolate outline (Figs 51–72). Apices slightly protracted, rostrate. Valve dimensions ($n = 45$): 21–39 μm long, 4.4–5.8 μm wide, length/width ratio range 4.5–6.7. Sternum narrow, widening while approaching central area. Two raphe branches mostly along valve mid-line but deflected towards ventral side when close to central area. Distal raphe fissures dorsally hooked and proximal raphe ends teardrop-like. Central area variable. Ventral portion of central area nearly absent for all valves, but dorsal portion of central area exhibiting three types: areas delimited by two marginal striae (e.g. Figs 51, 52, 54, 55, 58, 60–64, 69, 72), areas delimited by one marginal shortened stria (e.g. Figs 53, 56, 57, 59, 65), and semi-fascia areas (e.g.

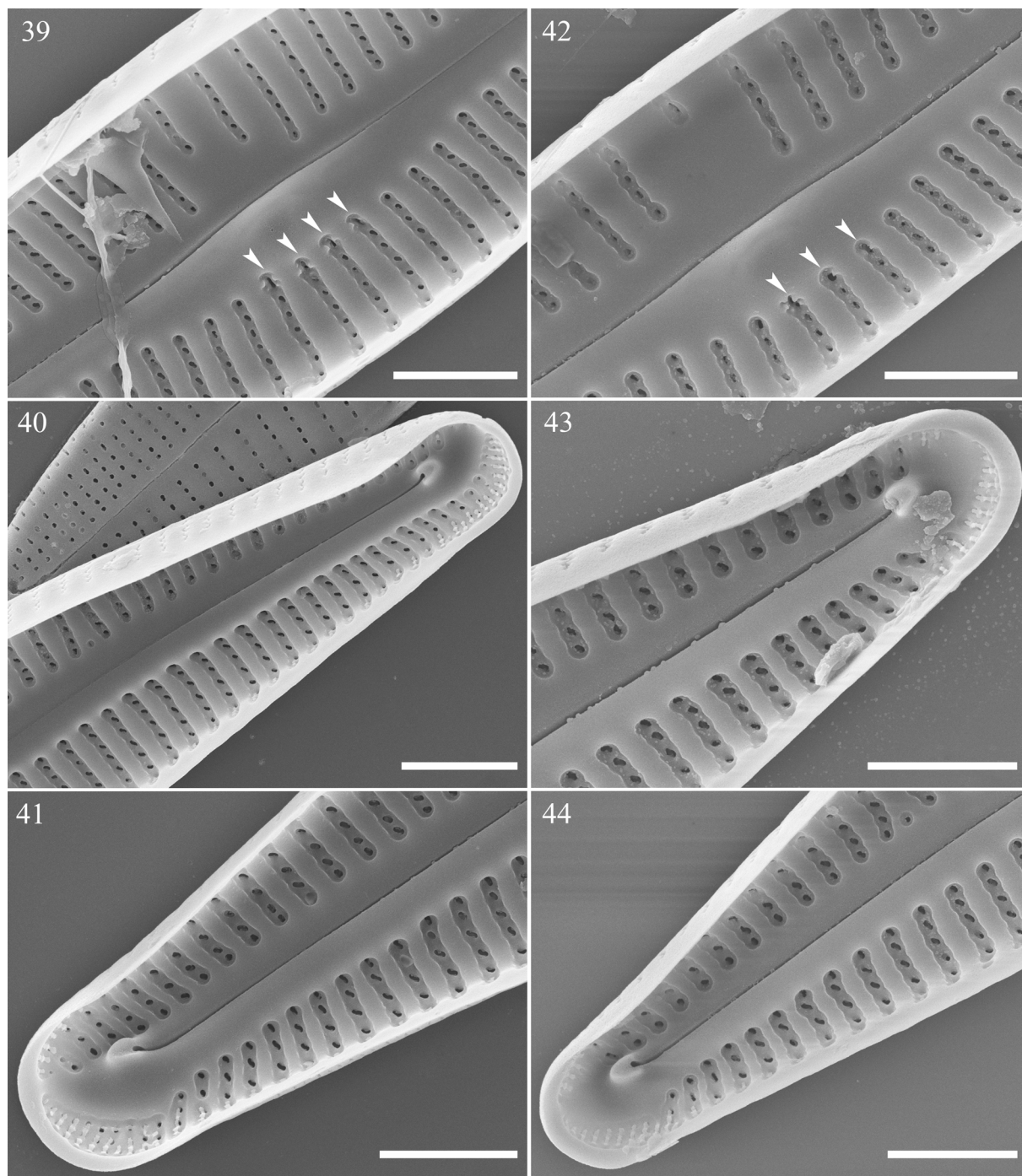


Figs 33–38. *Delicatophycus wulingensis* sp. nov., external valve view, SEM: (33–35) details of Fig. 28, note dorsal central area (Fig. 33, arrow), notch on the dorsal side of distal raphe fissure (Figs 34, 35, wavy arrow), and tilde-shaped areolae; (36–38) details of Fig. 49, note dorsal central area (Fig. 36, arrow), notch on the dorsal side of distal raphe fissure (Figs 37, 38, wavy arrow), and tilde-shaped areolae. Scale bars 2 μ m.

Figs 66–68). Striae radiate throughout valve, 16–18 in 10 μ m measured at ventral middle part, becoming denser towards two apices.

SEM (Figs 73–90): Dorsal central areas having three types: areas delimited by two marginal shortened striae (Figs 73, 74, arrowhead; Figs 79, 86), areas delimited by one marginal shortened stria (Figs 75, 76, arrowhead; Fig. 85), semi-fascia area (Figs 77, 78, arrowhead; Figs 80). One to five stigmata present on the ventral side (Fig. 79, four arrowheads; Fig. 80, three arrowheads; Fig. 85,

five arrowheads; Fig. 86, one arrowhead). Proximal raphe endings elongated and enlarged (Figs 79, 80), distal raphe fissures deflected towards dorsal side (Figs 81–84). Notch on dorsal side of distal raphe fissure (Figs 81, 82, wavy arrow). Externally, striae composed of tilde-shaped areolae, continuing onto mantle. Internally, areolae diagonally aligned in shallow depressions bordered by very broad virgae (Figs 85–88). Internally, distal raphe ends terminating on helictoglossae and proximal raphe ends invisible because obscured by flap over central nodule.



Figs 39–44. *Delicatophycus wulingensis* sp. nov., internal valve view, SEM: (39–41) details of Fig. 29, note four stigmata (Fig. 39, four arrowheads), diagonally aligned internal openings of areolae, radiate striae, and apical pore fields; (42–44) details of Fig. 31, note three stigmata (Fig. 42, three arrowheads), diagonally aligned internal openings of areolae, radiate striae, and apical pore fields. Scale bars 2 μ m.

APF divided by distal raphe fissure into two unequal sections (Figs 83, 84, 89, 90, APF): one larger than the other. Externally, APF foramina rounded, which differ from tilde-shaped areolae on valve surface. Internally, each perivalvar row of inner openings covered by undulate flap-like silica strip above internal apertures but not completely occluded (Figs 89, 90).

Holotype: Slide BM 81898, the Natural History Museum, London. Fig. 51 is the micrograph of the holotype.

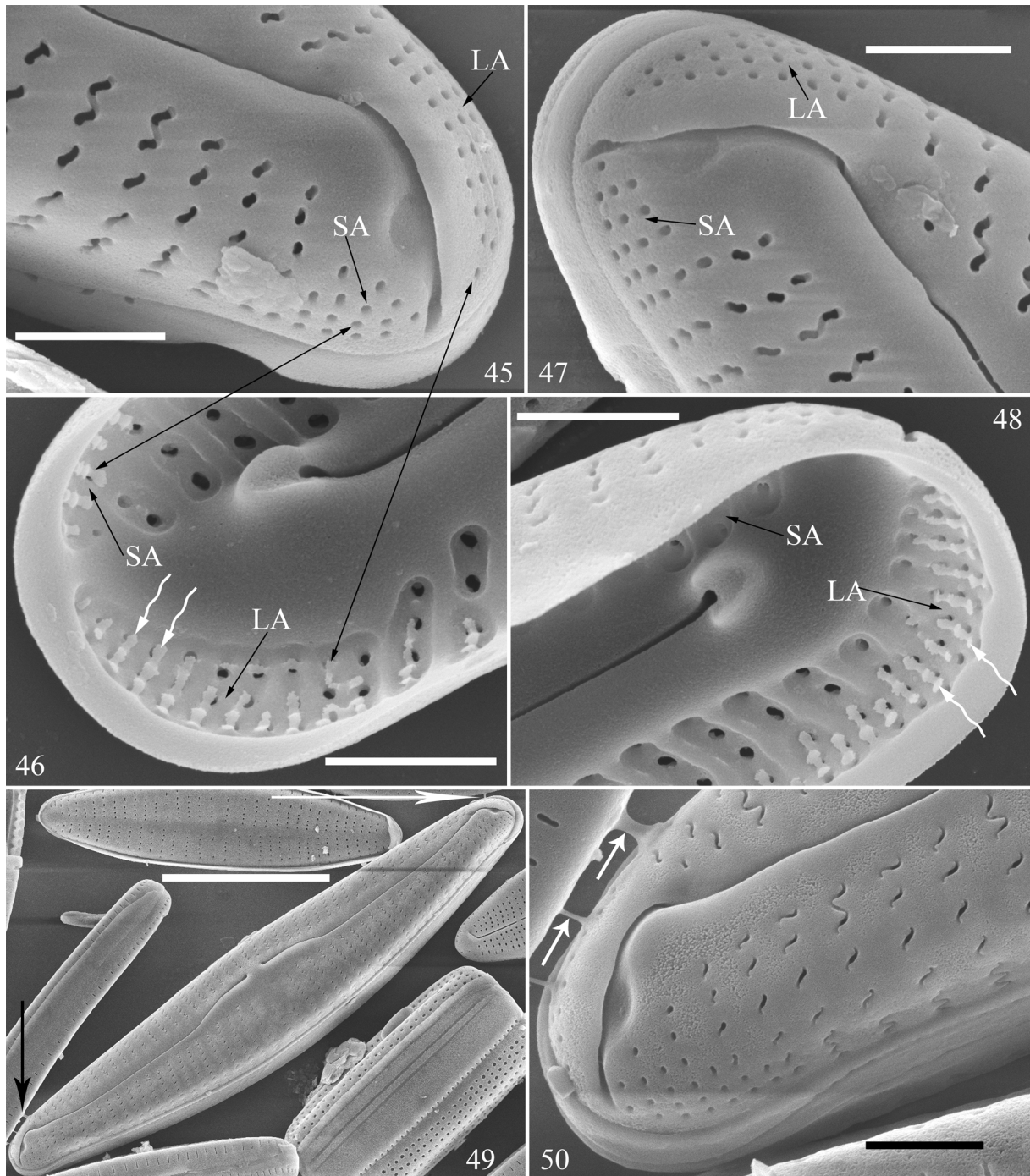
Isotype: Slide G202102, the Herbarium of Jishou

University, Hunan, People's Republic of China. Fig. 52 is the micrograph of the isotype.

Type locality: A river course at a sampling point (33° 22'21"N, 97°0'15"E, 3710 m a.s.l.), Chengduo County, Qinghai province, China (Collected by Bing Liu, July 22, 2019).

Habitat: Inhabiting surfaces of river immersed stones, thus, belonging to river epilithic diatom.

Etymology: Named after Qinghai province, where the species was found.



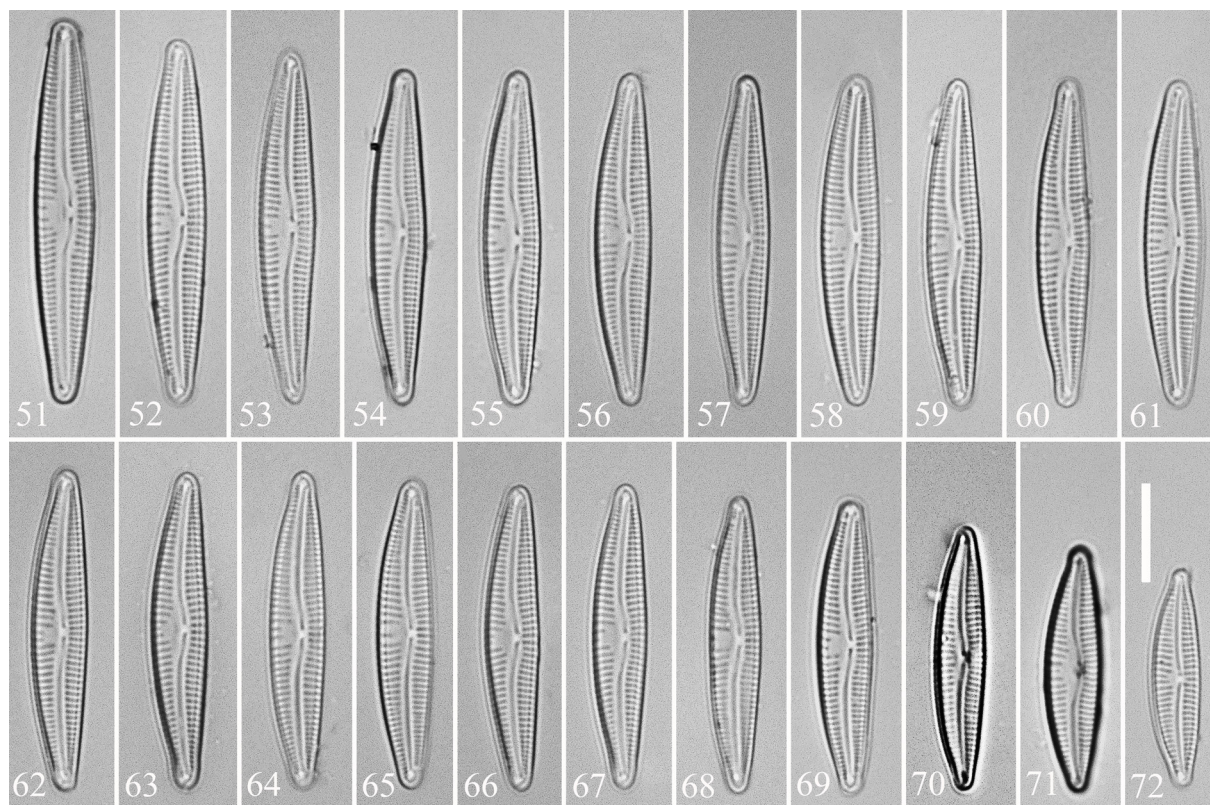
Figs 45–50. *Delicatophycus wulingensis* sp. nov., apical pore fields (APFs), SEM: (45, 46) details of two APFs, showing each APF composed of two areas: one is smaller (SA = smaller area), the other is larger (LA = larger area), the corresponding relationship from exterior to interior (two double-head arrows), and an undulate flap-like silica strip above internal apertures of each pervalvar row of foramina, not completely occluding internal apertures (Fig. 46, two curved arrows); (47, 48) another example of details of apical pore fields; (49, 50) a valve and its apical detail showing the polysaccharide stalks secreted from the apical pore fields (Fig. 49, white arrow and black arrow; Fig. 50, two arrows). Scale bars 1 μ m (45–48, 50), 8 μ m (49).

***Delicatophycus menyuanensis* Bing Liu et D.M. Williams sp. nov. (Figs 91–131)**

Description

LM (Figs 91–113): Valves dorsiventral, larger specimens with slightly convex ventral margins, exhibiting lanceolate outline (Figs 91–93); smaller specimens with nearly straight ventral margins, thus exhibiting semi-lanceolate

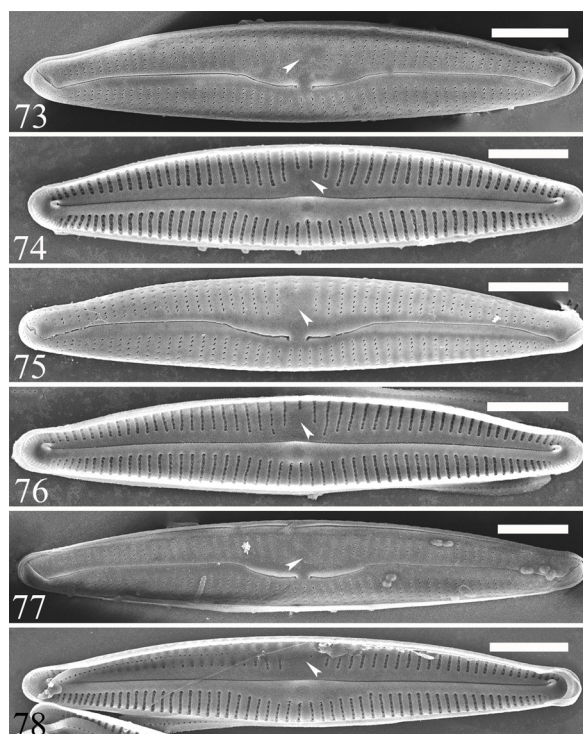
(Figs 94–113). Apices slightly protracted, rostrate. Valve dimensions ($n = 60$): 15–35 μ m long, 4.3–5.9 μ m wide, length/width ratio range 3.0–6.1. Sternum narrow, widening while approaching central area. Two raphe branches mostly along valve mid-line but deflected towards ventral side when close to central area. Distal raphe fissures dorsally hooked and proximal raphe ends



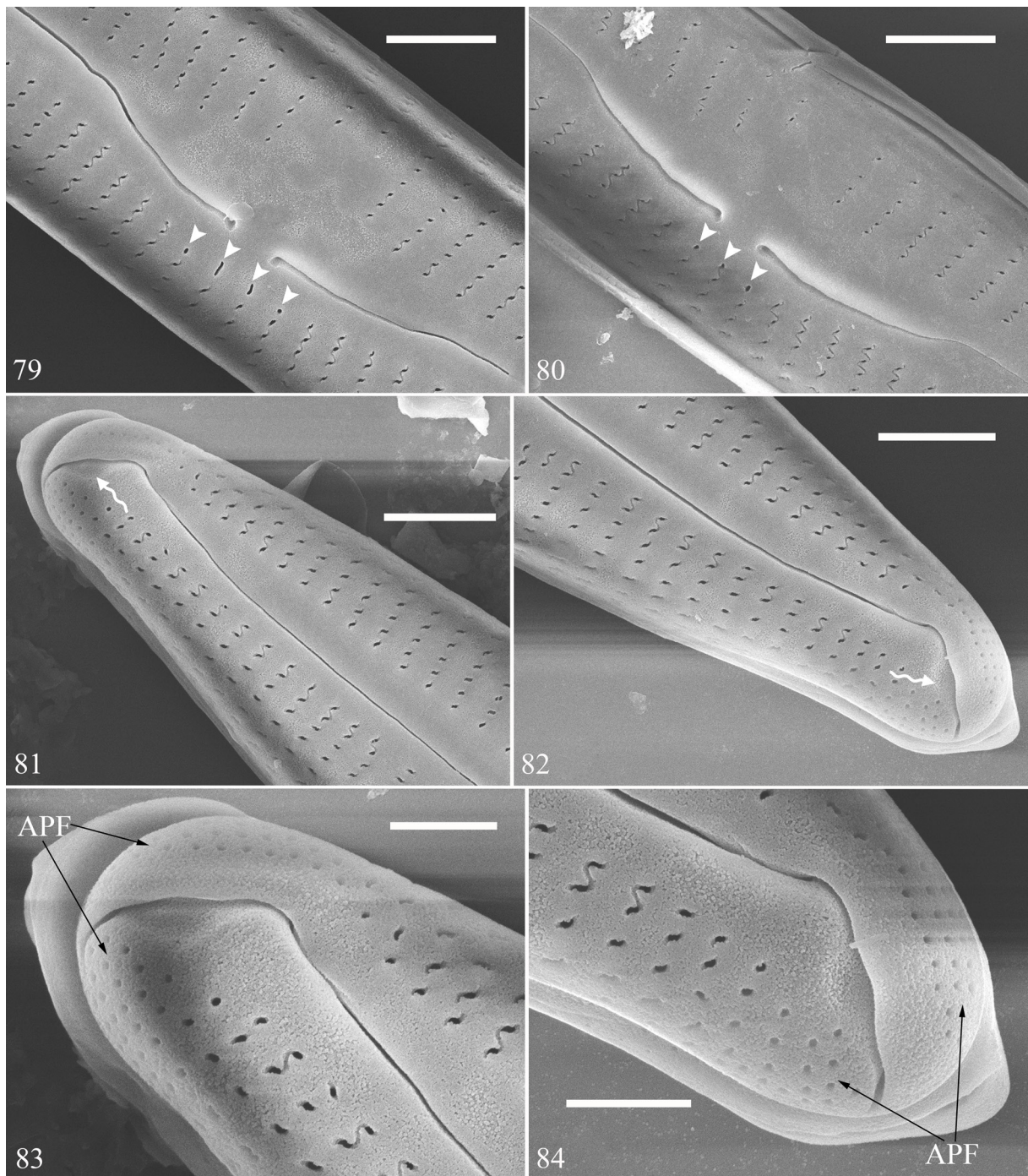
Figs 51–72. *Delicatophycus qinghaiensis* sp. nov., LM: (51–72) 22 valves showing size diminution series, note variable dorsal central areas; (51) micrograph of holotype specimen; (52) micrograph of isotype specimen. Scale bar 10 μ m (in 72) for all figures.

teardrop-like. Central area variable. Ventral portion of central area nearly absent for all valves, but dorsal portion of central area exhibiting two types: areas delimited by one marginal shortened stria (e.g. Figs 91–100, 106, 107, 109), and very small areas (e.g. Figs 101–105, 108, 110–113). Striae radiate throughout valve, 14–19 in 10 μ m measured at ventral middle part, becoming denser towards two apices.

SEM (Figs 114–131): Dorsal central areas having two types: areas delimited by one marginal shortened stria (Figs 114–117, arrowhead; Figs 121, 126), very small areas delimited by few distantly spaced striae (Figs 118, 119, arrowhead; Figs 120, 127). Two to four stigmata present on ventral side (Figs 120, 121, four arrowheads; Fig. 126, three arrowheads; Fig. 127, two arrowheads). Proximal raphe endings enlarged (Figs 120, 121), distal raphe fissures deflected towards dorsal side (Figs 122–125). Notch on dorsal side of distal raphe fissure (Figs 122, 123, wavy arrow). Externally, striae composed of tilde-shaped areolae, continuing onto mantle. Internally, areolae diagonally aligned in shallow depressions bordered by very broad virgae (Figs 126–131). Internally, distal raphe ends terminating on helictoglossae, and proximal raphe ends invisible because obscured by flap over central nodule. APF divided by distal raphe fissure into two unequal sections (Figs 124, 125, 130, 131, APF): one larger than the other. Externally, APF foramina rounded, different from tilde-shaped areolae on the other valve surface. Internally, each perivalvar row of inner openings



Figs 73–78. *Delicatophycus qinghaiensis* sp. nov., valve view, SEM: (73–78) six valves showing three types of dorsal central areas; (73, 74) two valves showing two dorsal central areas delimited by two marginal shortened striae (two arrowheads); (75, 76) two valves showing two dorsal central areas delimited by one marginal shortened stria (two arrowheads); (77, 78) two valves showing two dorsal central area of semi-fascia (two arrowheads). Scale bars 5 μ m.



Figs 79–84. *Delicatophycus qinghaiensis* sp. nov., external valve view, SEM: (79, 80) central details of Fig. 73 and Fig. 77, respectively, note four stigmata (Fig. 79, four arrowheads), three stigmata (Fig. 80, three arrowheads); (81–84) apical details of Fig. 77, showing tilde-shaped areolae, wavy striae, notch on the dorsal side of distal raphe fissure (Figs 81, 82, curved arrows), and apical pore field (Figs 83, 84, APF). Scale bars 2 μ m (79–82), 1 μ m (83, 84).

covered by undulate flap-like silica strip above internal apertures but not completely occluded (Figs 130, 131).

Holotype: Slide BM 81899, the Natural History Museum, London. Fig. 91 is the micrograph of the holotype.

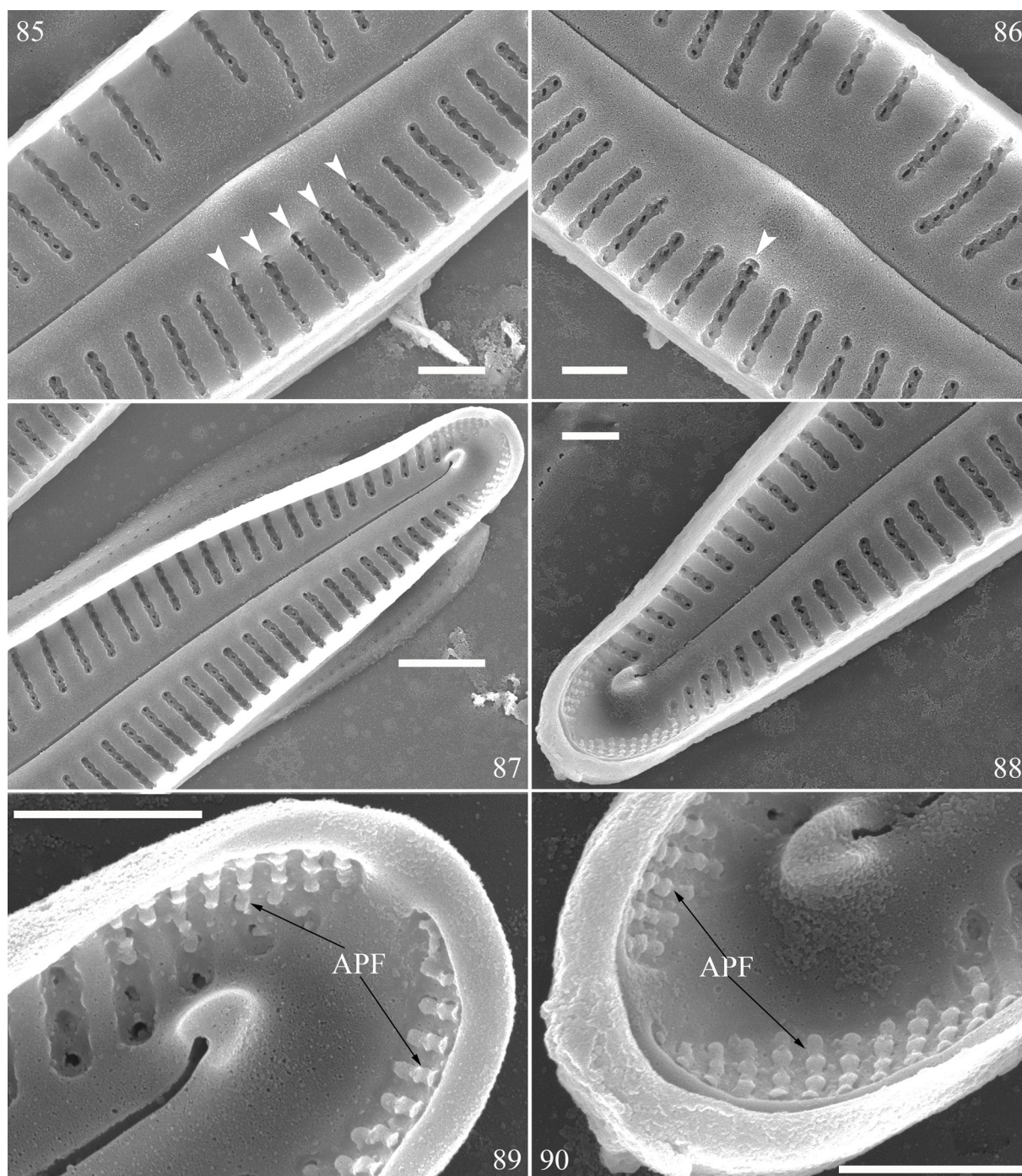
Isotype: Slide G202103, the Herbarium of Jishou University, Hunan, People's Republic of China. Fig. 92 is the micrograph of the isotype.

Type locality: A river course at a sampling point (37°27'28"N, 101°23'15"E, 2940 m a.s.l.), Menyuan

County, Qinghai, China (Collected by Bing Liu, July 18, 2019).

Habitat: Inhabiting surfaces of river immersed stones, thus, belonging to river epilithic diatom.

Etymology: Named after Menyuan County, where the species was found.

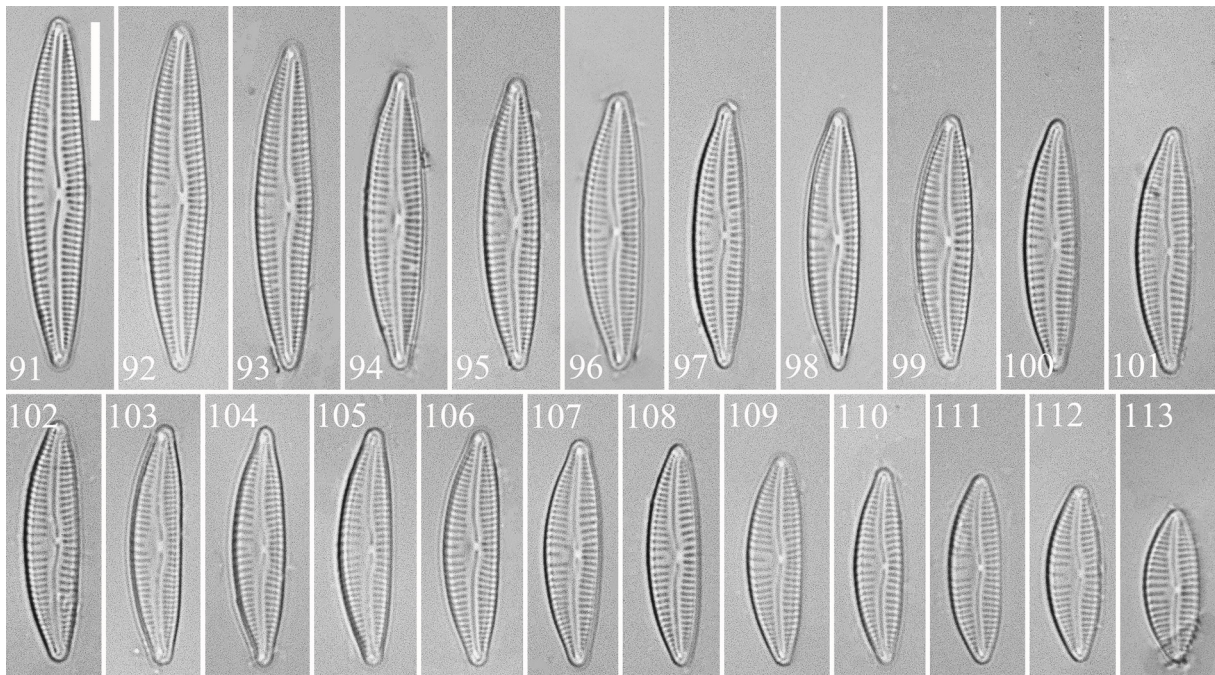


Figs 85–90. *Delicatophycus qinghaiensis* sp. nov., internal valve view, SEM: (85, 86) central details of Fig. 76 and Fig. 74, respectively, note five stigmata (Fig. 85, five arrowheads), one stigma (Fig. 86, one arrowhead); (87–90) apical details of Fig. 76, note diagonally aligned internal openings of areolae and apical pore fields (Figs 89, 90, APF). Scale bars 1 µm (85, 86, 88–90), 2 µm (87).

DISCUSSION

Delicatophycus wulingensis, *D. qinghaiensis* and *D. menyuanensis* all have tilde-shaped areolae and dorsally curved distal raphe fissures; thus, they belong in the genus *Delicatophycus*. They also share a further four distinct features: APFs, variable central dorsal portion of valve, stigmata, and a notch on the dorsal side of the distal raphe fissure (Table 2). However, *Delicatophycus wulingensis* has a rhombic–lanceolate valve outline and

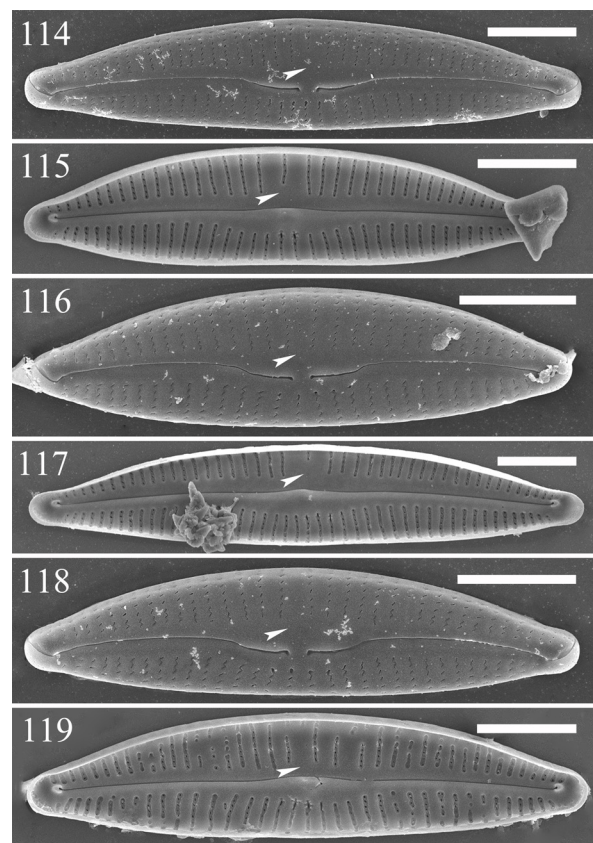
the largest valve length (58 µm long) recorded in the genus *Delicatophycus* (BAHLS 2017; Table 1 in LIU et al. 2018; LE COHU et al. 2018; and LIU et al. 2021); therefore, it can be clearly differentiated from *D. qinghaiensis* and *D. menyuanensis*. *Delicatophycus qinghaiensis*, for its part, has lanceolate valve outline whereas *D. menyuanensis* has semi-lanceolate valve outline except the larger specimens that have a lanceolate valve outline. Moreover, *D. qinghaiensis* may have a dorsal semi-fascia in the central area but *D. menyuanensis* lacks



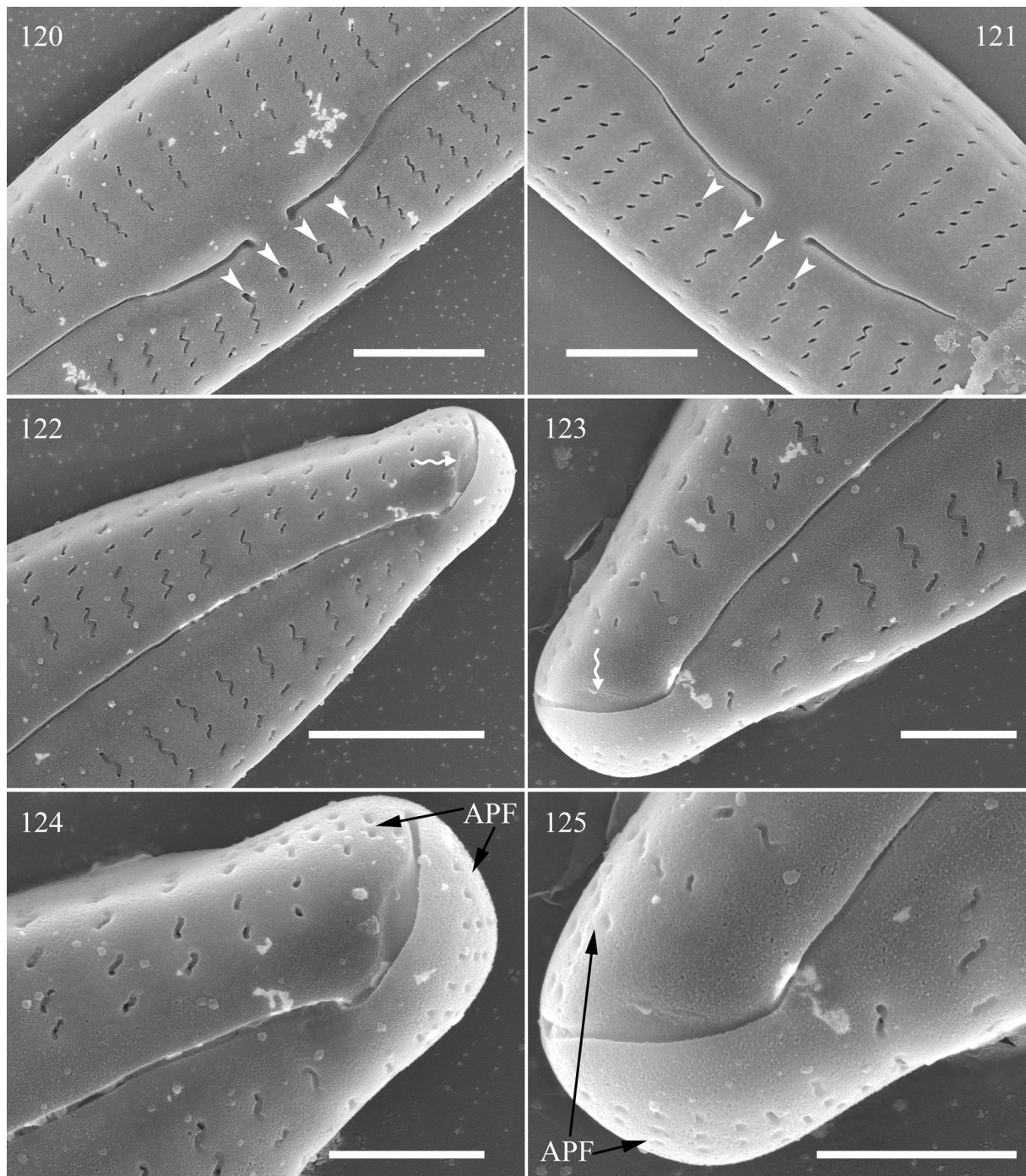
Figs 91–113. *Delicatophycus menyuanensis* sp. nov., LM: (91–113) 23 valves showing size diminution series, note variable dorsal central areas; (91) micrograph of holotype specimen; (92) micrograph of isotype specimen. Scale bar 10 µm (in 91) for all figures.

this feature. Comparatively, *D. sinensis*, *D. williamsii*, and *D. chongqingensis* all have variable dorsal central areas but neither have APFs nor a notch on the dorsal side of the distal raphe fissure (Table 2). Therefore, *D. wulingensis*, *D. qinghaiensis*, and *D. menyuanensis* are morphologically definable taxa.

In the APF-bearings Cymbelloid and Gomphonemoid diatoms, APFs can be divided into those that are differentiated and those that are undifferentiated, equal and unequal APFs, and bisected and non-bisected APFs (see KOCIOLEK & STOERMER 1988; Cox 2012). However, the above APF types need further investigations. The focus of this study is the genera *Cymbella* and *Delicatophycus*. APFs are a significant diagnostic character for most *Cymbella* species, but no systematic study of the various APFs has been undertaken. With examination of the published accounts concerning *Cymbella* (and some unpublished data), the APF can be divided into at least two types. Type I is a complete APF located in an uninterrupted area, not divided by the distal raphe fissure, e.g. *Cymbella lanceolata* (Agardh) Agardh (see KRAMMER 2002, p. 489, figs 1, 5), *C. latestriata* Pantocsek (see BUCZKÓ et al. 2015, p.177, figs 18, 23, 24), *C. stigmacentralis* B. Liu & D.M. Williams (see LIU et al. 2020, p. 102, fig. 20), and *C. compactiformis* B. Liu & D.M. Williams (see LIU et al. 2020, p. 106, figs 46, 47). Type II has an APF divided by the distal raphe fissure into two areas: one is larger than the other (e.g. Figs 1, 2), examples being *C. hustedtii* Krasske (see KRAMMER 2002, p. 577, figs 1, 4), *C. himalaspera* Jüttner et Van de Vijver (see JÜTTNER et al. 2010, p.77, fig. 3), *C. orientalis* var. *delicatula* Stancheva et Ivanov



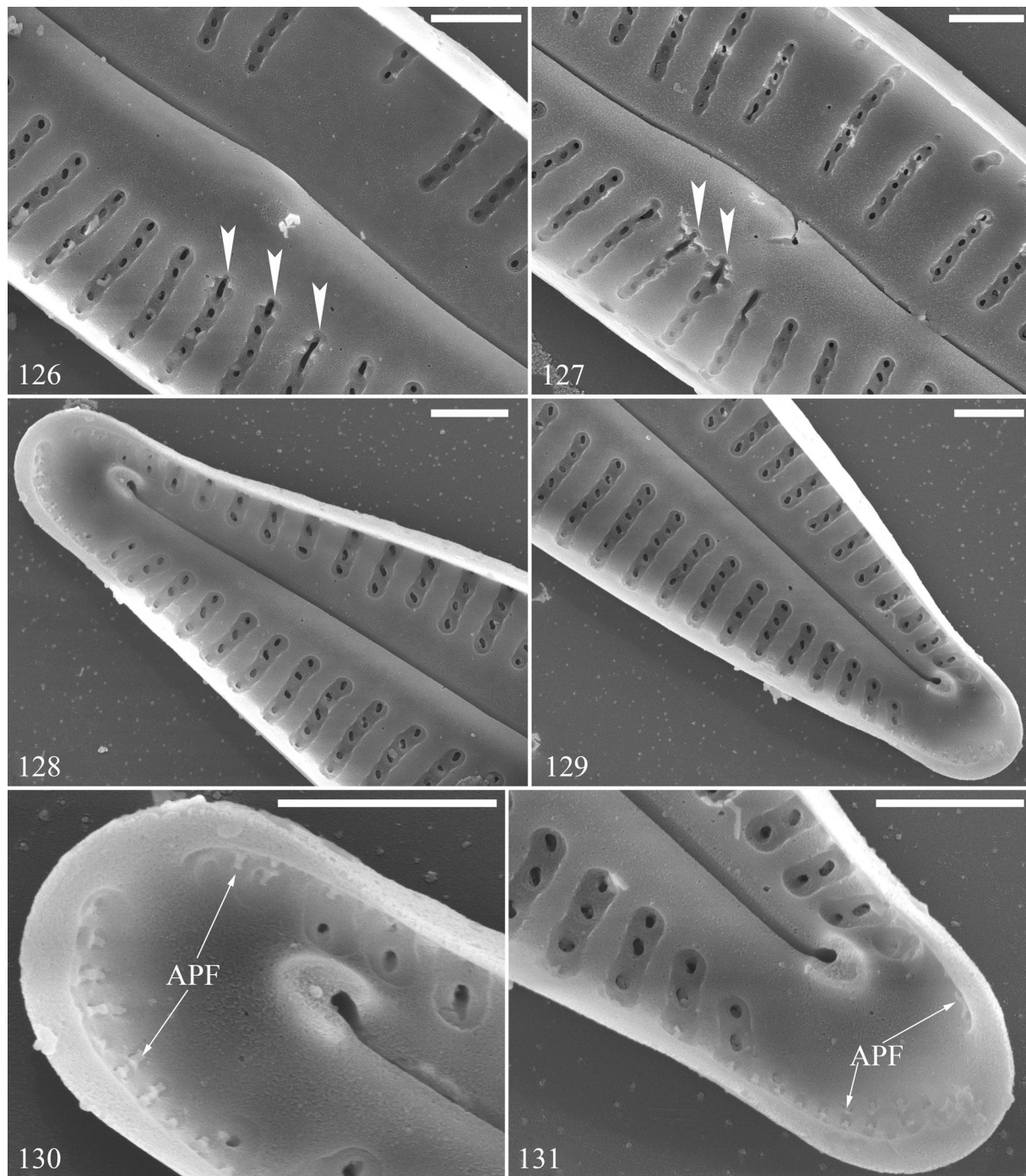
Figs 114–119. *Delicatophycus menyuanensis* sp. nov., valve view, SEM: (114–119) six valves showing two types of dorsal central areas; (114–117) four valves showing four dorsal central areas delimited by one marginal shortened stria (arrowheads); (118, 119) two valves showing two very small dorsal central areas, note the hyaline areas between two distantly spaced striae are not semi-fascia central areas (two arrowheads). Scale bars 5 µm.



Figs 120–125. *Delicatophycus menyuanensis* sp. nov., external valve view, SEM: (120, 121) central details of two valves, note four stigmata (Figs 120, 121, four arrowheads respectively); (122–125) apical details of Fig. 118, showing tilde-shaped areolae, wavy striae, notch on the dorsal side of distal raphe fissure (Figs 122, 123, curved arrows), and apical pore field (Figs 124, 125, APF). Scale bars 2 μ m (120–122), 1 μ m (123–125).

(see STANCHEVA & IVANOV 2011, p. 460, figs 16–19, 21), and *C. loescherae* M. Garcia et D.B. Dutra (see GARCIA & DUTRA 2016, p. 193, fig. 15). Within type I *Cymbella* taxa, *C. stigmacentralis* and *C. compactiformis*, LIU et al. (2020) found that the internal apertures of the APF's foramina are covered by an undulate flap-like silica strip (see LIU et al. 2020, p. 103, fig. 26; p. 107, fig. 53). For type II *Cymbella* taxon, *C. aspera*, the internal apertures of its APF's foramina are also covered by an undulate flap-like silica strip (Fig. 2). It is worth noting that the

three *Delicatophycus* species described herein all possess the type II APF. Thus, we can, for the first time, confirm that some species of *Delicatophycus* possess an APF like the one found in some *Cymbella*. Four *Delicatophycus* species from Diatoms of North America, *D. canadensis*, *D. montana*, *D. delicatula*, and *D. alpestris* appear to have the type II APFs from the valve external views (SPAULDING & EDLUND 2008). However, this can only be verified by further investigations of the internal sides of their APFs in the later researches.



Figs 126–131. *Delicatophycus menyuanensis* sp. nov., internal valve view, SEM: (126, 127) central details of Figs 117, 119, respectively, note three stigmata (Fig. 126, three arrowheads), two stigmata (Fig. 127, two arrowheads); (128–131) apical details of Fig. 117, note diagonally aligned internal openings of areolae and apical pore fields (Figs 130, 131, APF). Scale bars 1 μ m (126–131).

LIU et al. (2018) discussed whether stigma exists in the genus *Delicatophycus* and demonstrated that there are 3–4 stigmata in *D. williamsii*. LIU et al. (2021) noted that *D. liuweii* also possess 3–6 stigmata. Besides, LE COHU et al. (2018) found that both *D. pouemboutensis* and *D. gobiniana* Le Cohu et al. have a notch on the dorsal side of the distal raphe fissure, which the three species of *Delicatophycus* described herein possess. There are many species of *Delicatophycus* without APFs, such as *D. sinensis*, *D. williamsii*, and *D. chongqingensis*. All

these observations lead to pose the question as to what are the defining character(s) of *Delicatophycus*? From the current data, the tilde-shaped areolae and dorsally curved distal raphe fissures are proposed as two defining characters.

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