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Five new Achnanthidiaceae species (Bacillariophyta) from Jiuzhai Valley, Sichuan Province, Southwestern China

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Abstract

Five new species are described in this study: *Achnanthidium jiuzhaienis sp. nov.*, *Achnanthidium epilithica sp. nov.*, *Achnanthidium limosua sp. nov.*, *Achnanthidium subtilissimum sp. nov.*, and *Kolbesia sichuanenis sp. nov.* Classification was based on light microscopy (LM) and scanning electron microscopy (SEM). *A. jiuzhaienis sp. nov.* belongs to the "*A. pyrenaicum* complex" of the genus *Achnanthidium*, this classification was based on certain characteristics, such as the possession of transpically-elongated areolae and deflected external distal raphe fissures. *A. epilithica sp. nov.*, *A. limosua sp. nov.*, and *A. subtilissimum sp. nov.* belong to the "*A. minutissimum* complex" of the genus *Achnanthidium*, this classification was based on certain characteristics, such as the possession of straight external distal raphe fissures and round or elliptical areolae. *Kolbesia sichuanenis sp. nov.* was found to belongs to the *Kolbesia* genus, based on its elongated areolae and striae comprised of one macroareola. All five species were sufficiently different from other similar species to be considered unique separate species based on their valve outline, shape of the axial and center areas, and density of striae of each taxon. These new species derived from the epilithic and epiphytic collections of four alkaline lakes in Jiuzhai Valley, Sichuan Province, Southwest China.

Keywords: Diatoms, *Achnanthidium*, *Kolbesia*, Monoraphid, Morphology

Introduction

Kützing (1844) initially described the genus *Achnanthidium* at the subgenus level, originally within genus *Achnanthes* Bory (Bory 1822: 79), when *A. microcephalum* Kützing (Kützing 1844: 75) was the type of species belonging to that genus (Kingston 2003, Pérès *et al.* 2014). Later, Round *et al.* (1990) and Round & Bukhtiyarova (1996) re-established *Achnanthidium* as a new separate genus. Species belonging to *Achnanthidium* commonly, abundantly, and frequently occur in various freshwater habitats, such as streams, rivers, and lakes (Potapova & Ponader 2004, Kobayasi *et al.* 2006, Novais *et al.* 2011, Wojtal *et al.* 2011, Pinseel *et al.* 2015, Karthick *et al.* 2017, Krahn *et al.* 2018). Moreover, *Achnanthidium* species can occur across a broad range of freshwater habitats, from oligotrophic to eutrophic waters (Potapova & Hamilton 2007, Karthick *et al.* 2017, Krahn *et al.* 2018, Yu *et al.* 2019). However, species recognition within the genus *Achnanthidium* has presented some challenges in terms of research. Such species are usually small in size, possess fine striations, and have a limited number of morphological characteristics that are clearly visible in light microscopy (LM), and these variable diagnostic features often overlap when measured (Ponader & Potapova 2007, Hlúbiková *et al.* 2011, Morales *et al.* 2011, Karthick *et al.* 2017, Marquardt *et al.* 2017, Krahn *et al.* 2018). For now, to obtain adequate identification characteristic, use of scanning electron microscopy (SEM) is often necessary. (Krahn *et al.* 2018).

To date, three complexes have been characterized in the *Achnanthidium* genus: (1) members of the *A. minutissimum* (Czarnecki 1994: 157) complex, have straight external distal raphe ends, and striae density that increase towards the apex; (2) members of the *A. pyrenaicum* (Kobayashi 1997: 148) complex, have external distal raphe ends that deflect or hook to one side of the valve; and (3) members of the *A. exiguum* (Czarnecki 1994: 157) complex, have external distal raphe ends curved in opposite directions (Round & Bukhtiyarova 1996, Krahn *et al.* 2018, Pinseel *et al.* 2015, Compère & Van de Vijver 2011, Jüttner *et al.* 2011, Yu *et al.* 2018, 2019). Currently, more than 200 *Achnanthidium* species have been reported in the literature worldwide (Marquardt *et al.* 2017, Kociolek *et al.* 2018, Yu *et al.* 2019). In

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China, roughly 60 *Achnanthidium* species have been reported (Liu *et al.* 2013, 2015, 2016, Wang & Deng 2017, Yu *et al.* 2017), but only a few have been reported as new species (Yu *et al.* 2018, 2019, Liu *et al.* 2016).

The genus *Kolbesia* Round & Bukhtiyarova ex Round (Round 1998: 181) was distinguished by Round & Bukhtiyarova (1998) with *K. kolbei* (Hustedt) Round & Bukhtiyarova ex Fourtanier & Kociolek (Fourtanier & Kociolek 1999: 81) as the type species. The primary characteristics of this genus include valves that are elliptic to elliptic-lanceolate with drawn-out and bluntly round ends, and striae that are slightly radial on both valves. Furthermore, the areolae are elongated and separated, with a few in each stria, and the raphe filiform and straight in an elliptical sternum (Round & Bukhtiyarova 1996). Currently, there are only 7 species have been reported in this genus: *K. amoena* (Hustedt) Kingston (Kingston 2000: 410), *K. gessneri* (Hustedt) Aboal (Aboal *et al.* 2003: 160), *K. kolbei* (Hustedt) Round & Bukhtiyarova (Fourtanier & Kociolek 1999: 81), *K. nitidiformis* (Lange-Bertalot) Lange-Bertalot (Bukhtiyarova 2006: 90), *K. ploenensis* (Hustedt) Round & Bukhtiyarova (Round 1998: 181), *K. sinica* Krzywda, Witkowski & Chunlian Li (Witkowski *et al.* 2016: 183), and *K. suchlandtii* (Hustedt) Kingston (Kingston 2000: 410). Only one species *K. sinica* is marine species, while the others are all freshwater species. In China, only one marine species *K. sinica* has been reported in the Bohai and Yellow Seas of Yantai (Witkowski *et al.* 2016).

The Jiuzhai Valley Nature Reserve (32°51’–33°19’ N, 103°46’–104°4’ E) is located in Sichuan Province, in southwestern China, where the elevation ranges from 1990–4764 m. The Reserve lies in a transition zone on the eastern edge of the Qinghai-Tibet Plateau, between the Qinghai-Tibet Plateau and Sichuan Basin. The Jiuzhai Valley has a subtropical to temperate monsoon climate (Zhou *et al.* 2009). Valleys are warm and dry, while the middle mountain slopes are cold and damp. The annual average temperature is above 7.3 °C. The total annual rainfall is 580–780 mm (Zhou *et al.* 2006, Gan *et al.* 2010, Yu *et al.* 2018,). During an investigation of freshwater diatoms from the Jiuzhai Valley, four unknown *Achnanthidium* species and one *Kolbesia* species were discovered. This paper presents the morphology of these five new species by light microscopy (LM) and scanning electron microscopy (SEM), as well as compares those new species with morphologically similar species from the genera *Achnanthidium* and *Kolbesia*.

Material and Methods

In this study, diatom samples were collected from 51 sampling sites from the Jiuzhai Valley in July 2013. These new species were found in 4 lakes (Fig. 1). Five-Flower Lake is a landslide-caused barrier lake, that is 450 m long, 227–313 m wide, and 5 m deep, with an area of 90,000 m^2 and is 2472 m in elevation. Tiger Lake is a glacier-caused barrier lake, that is 310 m long, 171–194 m wide, and 25 m deep, with an area of 200,000 m^2 and is 2298 m in elevation. Mirror Lake is a glacier-caused barrier lake, that is 1115 m long, 123–241 m wide, and 31 m deep, has an area of 190,000 m^2 and is 2390 m in elevation. Swan Lake is a swampy lake, that is 720 m long, 50–100 m wide, and 6 m deep, has an area of 55,000 m^2 and is 2905 m in elevation.

Several water chemistry characteristics were recorded in the field, including water pH, temperature, total dissolved solids (TDS), and conductivity, which were measured using a YSIPro Plus multiparameter meter (YSI, Inc., Yellow Springs, O.H., U.S.A.). Diatom samples were removed from their natural substrata, including stones, plant roots, and other substrates (e.g., wood, *Chara*) using a knife and/or clean toothbrushes, and form sediments using a turkey baster. Samples were placed in bottles, preserved with formalin (4% final concentration), and kept in sealed bottles.

Diatom valves were cleaned with concentrated nitric acid using a Microwave Accelerated Reaction System (CEM Corp., Matthews, N.C., U.S.A.) (Parr *et al.* 2004), with a predetermined programmed digestion scheme (temperature, 180 °C) (Yu *et al.* 2017). Then, samples were alternately centrifuged for 5 min at 3500 rpm (TDZ5-WS, Luyi Corp., Shanghai, China) and washed about six times using distilled water to remove nitric acid until the pH of each sample was roughly 7.0. Finally, cleaned diatom samples were preserved in 95 % ethanol. Cleaned diatom frustules were mounted in Naphrax for LM or air-dried onto coverslips and mounted onto copper stubs for SEM observations. LM observations were conducted using an Olympus BX-53 and ZEISS AXIO Imager A2 microscope fitted with DIC optics and a 1.4 numerical aperture, 100× oil immersion objective. SEM was conducted using an SU8010 SEM at 2 kV, and WD less than 6 mm (Hitachi High-Technologies Corp., Tokyo, Japan). Images were compiled using Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, C.A., U.S.A.). Morphological terminology followed Round *et al.* (1990). All diatom samples and permanent slides are housed in the Biology Department Diatom Herbarium, Shanghai Normal University (SHTU), Shanghai, China.

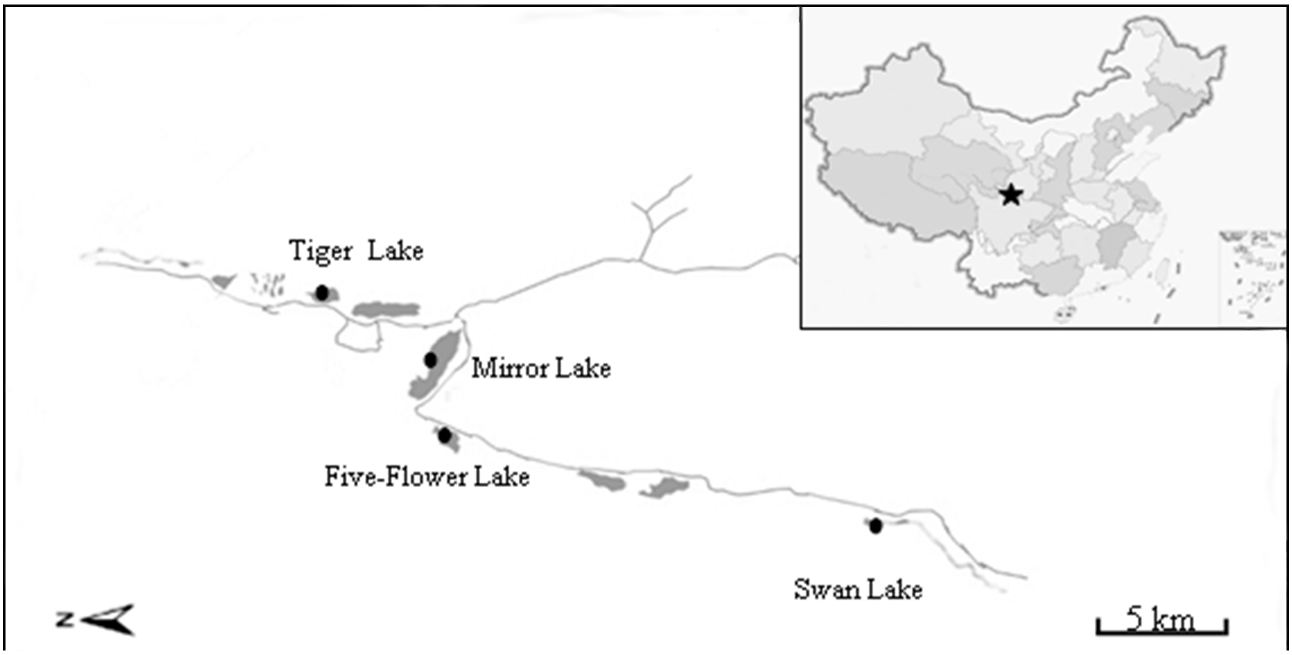


FIGURE 1. Location of the Jiuzhai Valley. Sample collection sites are indicated by black dots.

Results

Achnanthidium jiuzhaienis **P. Yu, Q-M. You & Q-X Wang *sp. nov.* (Figs 2–129)**

Holotype: SHTU! slide JZG–1307027, holotype illustrated in Figs 12, 41. Diatom samples are housed in the Biology Department Diatom Herbarium, Shanghai Normal University, China.

Isotypes: COLO!, slide 628094, Samples are housed in the Kociolek Collection, University of Colorado, Museum of Natural History Diatom Herbarium, Boulder, U.S.A.

Type locality: CHINA. Samples collected from Jiuzhai Valley Nature Reserve, Sichuan Province, 33°09'34"N, 103°52'39"E, altitude: 2432 m, collected by *Q.X. Wang*, on July 5, 2013.

Etymology: Species was named for the Jiuzhai Valley Nature Reserve where the samples were collected.

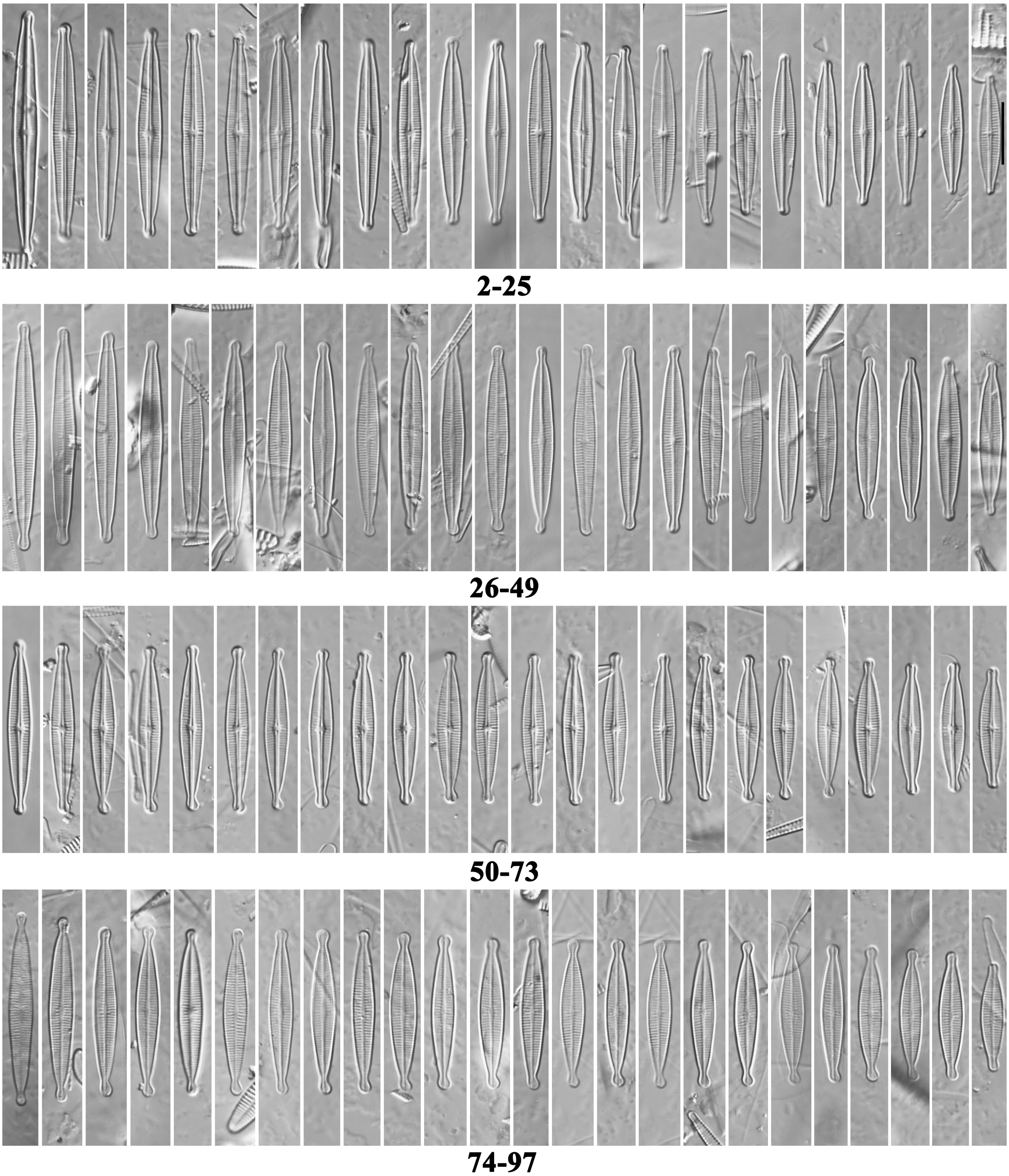
Description: According to LM observations (Figs 2–97), valves were wide lanceolate with subcapitate to capitate ends. The raphe valve was concave and the rapheless valve was convex. Valve length was 19–40 µm, width was 3.2–4.5 µm (n=200). Both valves possessed a narrowly linear-lanceolate axial area that widens slightly towards the middle portion; a differentiated central area was absent. On both valves, striae radiate to nearly parallel. Striae number was 22–28/10 µm at the center, and up to 30–38/10 µm near the apices on the raphe valve. On the rapheless valve, striae were 22–27/10 µm in the middle and 28–36/10 µm near the apices. Individual areolae were not visible with an LM.

SEM observations of both valves revealed that the valve face had a mantle junction bordered by a narrow hyaline area (Figs 98, 106, 115, 123). The mantle had a single row of linear areolae (Figs 100, 108, 125–126). On the raphe valve, externally, the raphe were filiform and straight (Figs 98,115). The external terminal raphe fissures hooked toward the same side of the valve (Figs 98, 115). The proximal raphe endings slightly expanded (Figs 101, 118). Striae were comprised of 2–5 transapically-oriented areolae in the middle portion (Figs 101, 118) and 1–3 transapically-oriented areolae at the apices (Figs 100, 117). Internally, the raphe terminated distally as an elevated helictoglossa (Figs 99, 102, 116, 119), and the proximal raphe endings gently deflecting in opposite directions (Figs 103, 120). Areolae appeared to be oblong, and the openings occluded with fine hymenate structures that included small openings on the periphery and a central occlusion (Figs 104–105, 121–122).

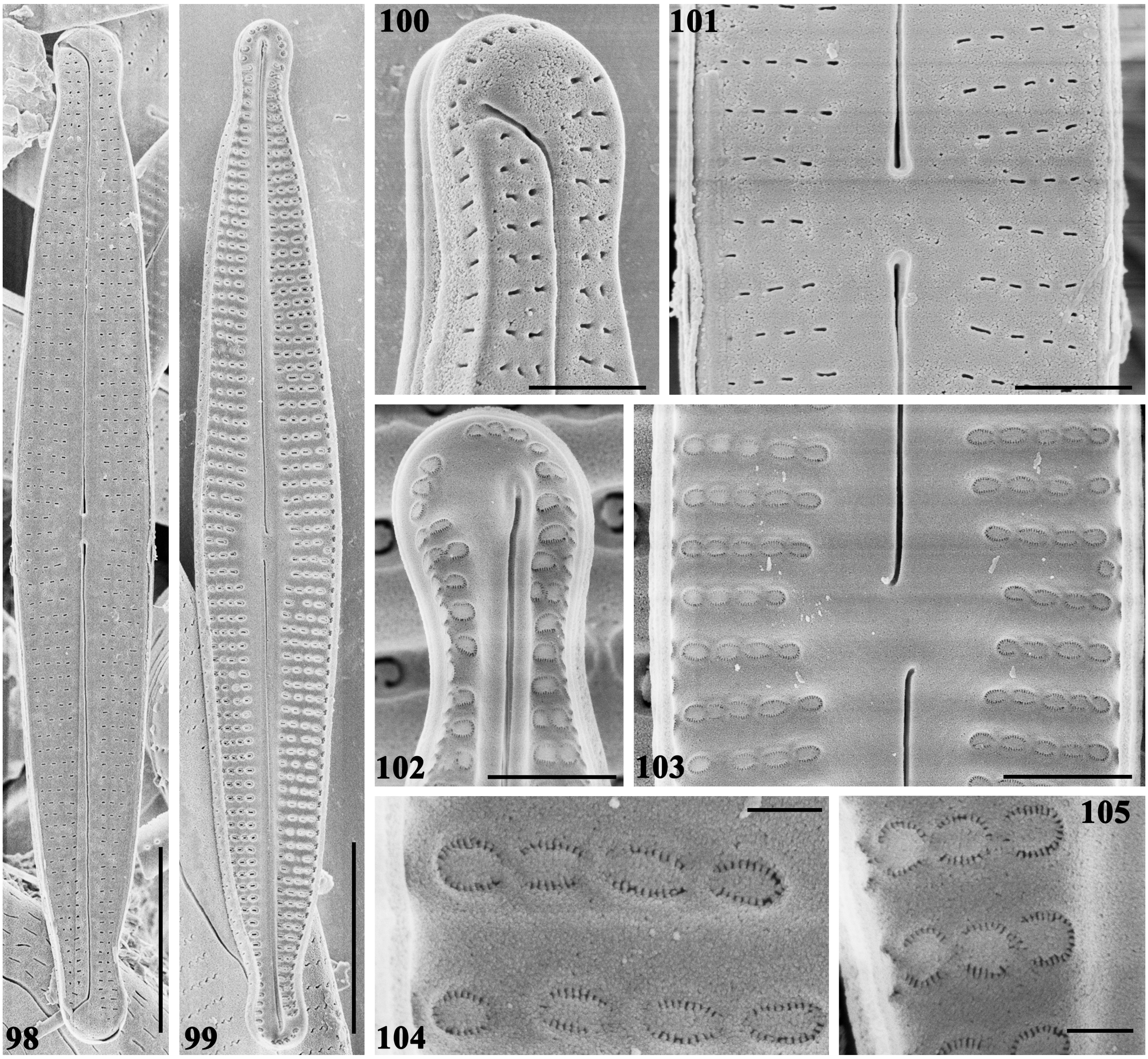
On the rapheless valve, the axial area had raphe vestiges at the apices (Figs 106, 108–109, 123, 125–126). Striae consisted of 2–5 transapical slit-like areolae at the middle portion (Figs 110–111, 127, 129), becoming 1–3 irregular transapical slit-like areolae at the apices (Figs 108–109, 125–126). Internally, the raphe vestiges at the apices consisted of 1–2 openings or absent (Figs 107, 112–113, 124, 128). The shape of areolae were internally oblong, and areolar openings had fine hymenate occlusions (Figs 114).

Ecology: Collected in one sample, JZG–201307027, on filamentous algae (pH 8.4, water temperature 9.8 °C, TDS 0.246 g/L, conductivity 367 µs/cm). This new species occurred at 4.3 % relative abundance (total counted, 400 valves) in sample JZG–201307027. There were 6 species that accounted for more than 5 % of this sample: *Cymbella vulgata* Krammer (Krammer 2002: 55) (19 %), *Delicata delicatula* (Kützing) Krammer (Krammer 2003: 113) (17.3 %), *Achnanthidium minutissimum* (Kützing) Czarnecki (10.8 %), *Denticula elegans* Kützing (Kützing 1844: 44) (9.3 %), *Achnanthidium pyrenaicum* (Hustedt) Kobayasi (6%), and *Achnanthidium macrocephalum* (Hustedt) Round & Bukhtiyarova (Round & Bukhtiyarova 1996: 75) (5.8 %).

Distribution: Thus far, the new species was collected only at the type locality in Jiuzhai Valley.



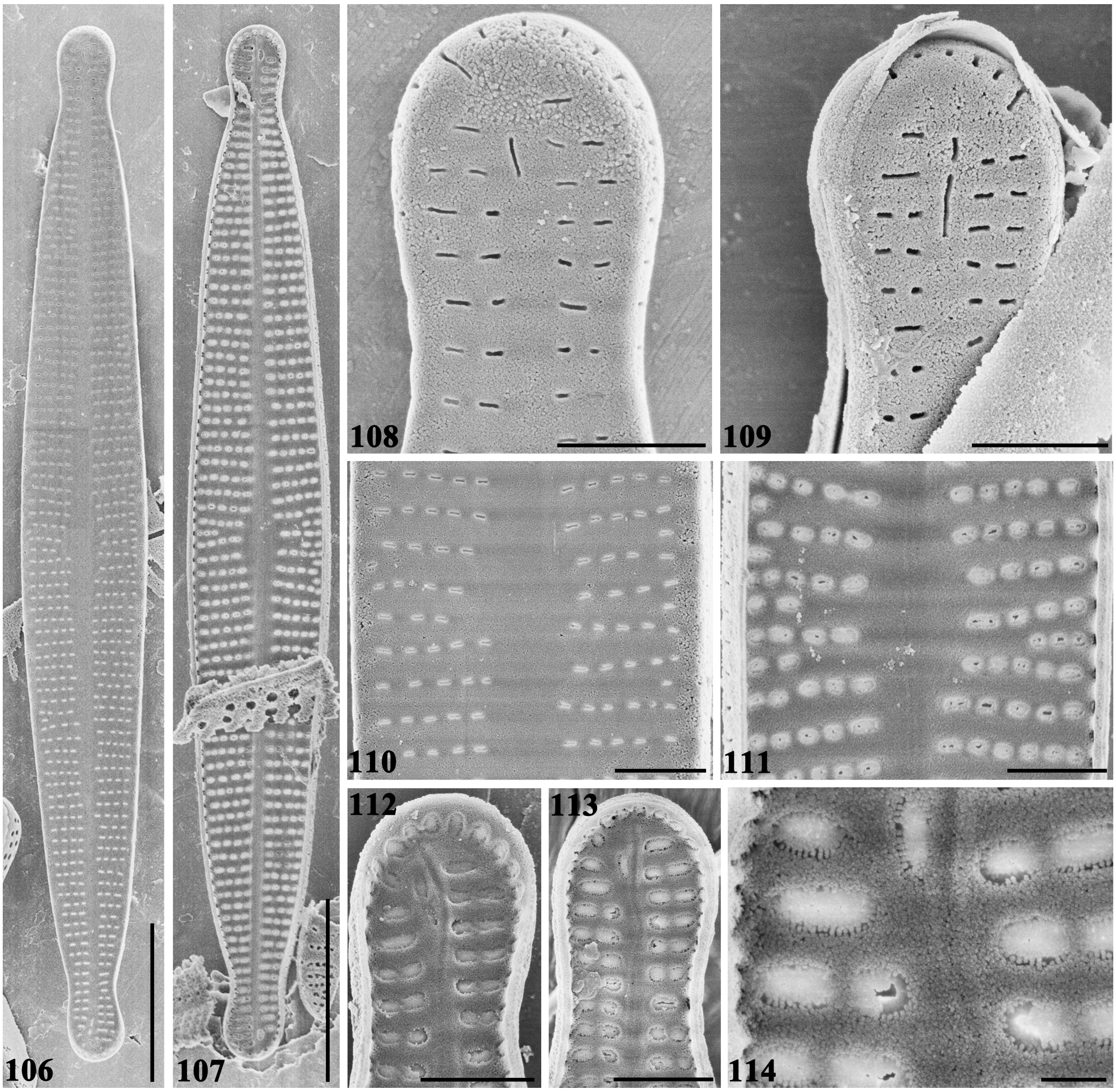
FIGURES 2–97. *Achnanthidium jiuzhaienis sp. nov.*, LM. 12, 41. Holotype; 2–49. Longer morphotype, (2–25) Raphe valves; (26–49) Rapheless valves; 50–97. Shorter morphotype, (50–73) Raphe valves; (74–97) Rapheless valves. Scale bar 10 µm.



FIGURES 98–105. *Achnanthidium jiuzhaienis sp. nov.* Longer morphotype, SEM views of raphe valve. 98. External view of an entire raphe valve. 99. Internal view of an entire raphe valve. 100–101. Details of the apices and the central area on the external of the raphe valve. 102–103. Detail of the apices and the central area on the internal of the raphe valve. 104–105. Internal areola openings with fine hymenate structures. Scale bars 5 µm (98–99), 1 µm (100–103), 0.2 µm (104–105).

Remarks: *Achnanthidium jiuzhaienis* can be compared to several species in the same genus, based on similarities in the outline and structure of the valve, including *A. gracillimum* (Meister) Lange-Bertalot (Meister 1912: 234, Krammer & Lange-Bertalot 2004: 430), *A. acerosum* Vijver, Lange-Bertalot & Jarlman (Vijver *et al.* 2011: 198), and *A. ertzii* Vijver & Lange-Bertalot (Vijver *et al.* 2011: 200). The morphological characteristics of *A. jiuzhaienis*. and these similar species are summarized in Table 1 to facilitate a comparison. In terms of features viewed with an LM, the outline of the valves of A. *jiuzhaienis*. were wide lanceolate with subcapitate to capitate ends, while that of *A. gracillimum* were linear-lanceolate with broadly rounded ends, *A. acerosum* were narrowly lanceolate with subcapitate ends, and *A. ertzii* is linear-lanceolate with capitate ends. Additionally, the valves of *A. jiuzhaienis* were longer (19–40 µm) than the valves of *A. acerosum* (15–19 µm) and *A. ertzii* (18–22 µm), as well as wider (3.2–4.5 µm) compared to (*A. gracillimum* (3–4 µm), A. *acerosum* (1.9–2.4 µm), and A. *ertzii* (2.2–2.8 µm). No central area was observed in the new species, but the other species did have a central area. With respect to features observed with an SEM, *A. jiuzhaienis*. was distinguished from these similar species by the presence of hooked external distal raphe fissures; the external distal raphe ends were straight in *A. acerosum* and *A. ertzii*.

Two morphotypes appeared to be present in this population of *A. jiuzhaienis*. One morphotype was longer with subcapitate ends (Figs 2–49), while the other morphotype was shorter with capitate ends (Figs 50–97). Additionally, on the raphe valve, striae radiated in the middle portion in the longer morphotype (Figs 115, 118), and were nearly parallel in the shorter morphotype (Figs 98, 101).



FIGURES 106–114. *Achnanthidium jiuzhaienis sp. nov.* Longer morphotype, SEM views of rapheless valve. 106. External view of an entire rapheless valve. 107. Internal view of an entire rapheless valve. 108–109. Raphe vestiges at the two apices of the same valve. 110. Detail of the central area on the external of the rapheless valve. 111. Detail of the central area on the internal of the rapheless valve. 112–113. Raphe vestiges at the apices with usually 1–2. 114. Internal areola openings with fine hymenate structures. Scale bars 5 µm (106–107), 1 µm (108–113), 0.2 µm (114).

TABLE 1. Comparison of morphological characteristics of *Achnanthidium jiuzhaienis sp. nov.* and closely related taxa.

Species/Feature	*A. jiuzhaienis sp. nov.*	*A. gracillimum* (Meister) Lange-Bertalot	*A. acerosum* Vijver, Lange-Bertalot & Jarlman	*A. ertzii* Vijver & Lange-Bertalot
Valve length (µm)	19–40	25–40	15–19	18–22
Valve width (µm)	3.2–4.5	3.0–4.0	1.9–2.4	2.2–2.6
Valve outline	Wide lanceolate	Linear-lanceolate	Narrowly lanceolate	Linear-lanceolate
Valve apices	Subcapitate to capitate	Broadly rounded	Subcapitate	Capitate
Raphe valve				
Axial area	Linear-lanceolate	Linear	Linear	Linear
Central area	Absent	Small	Elliptical to round	Round

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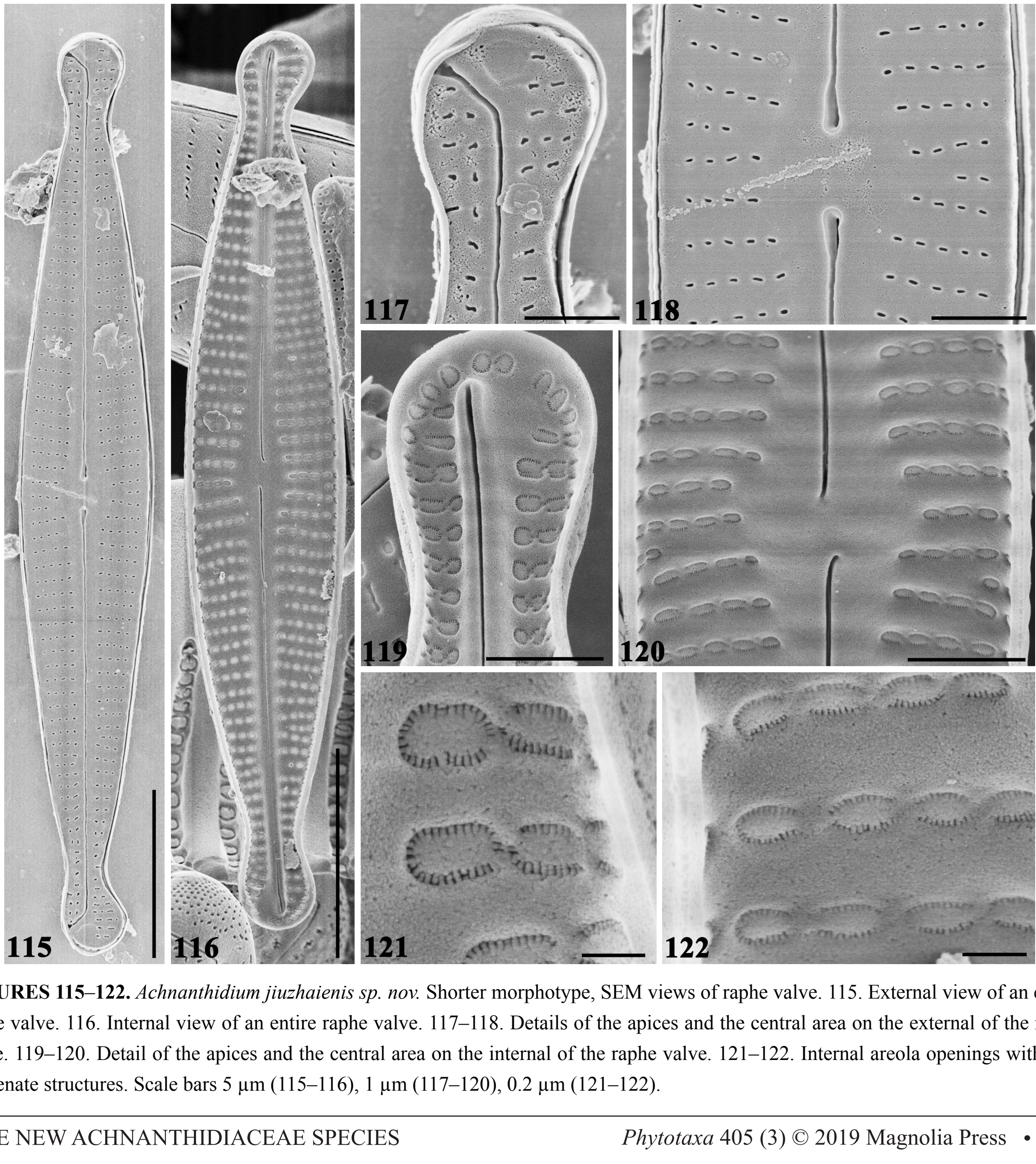
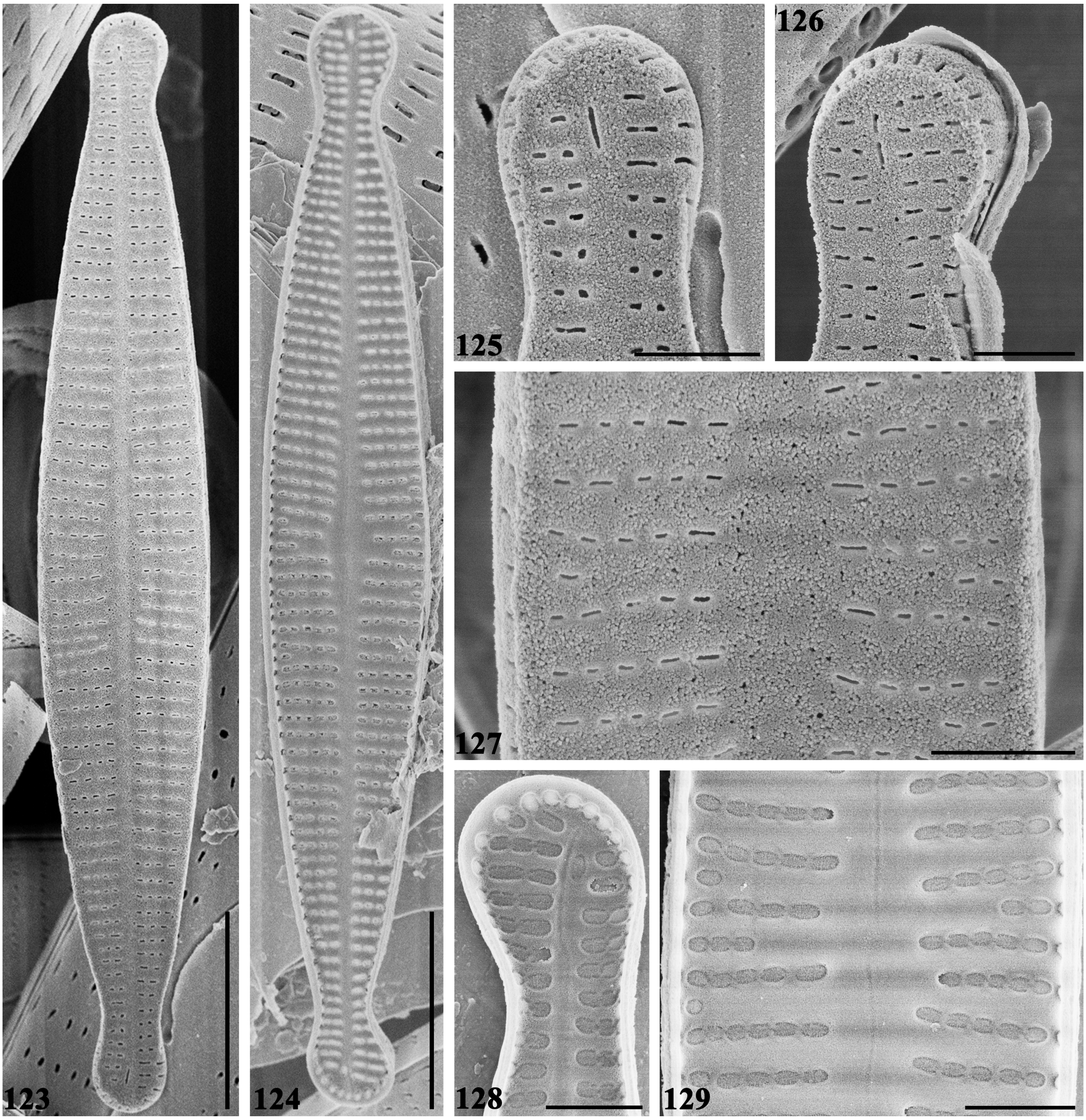
TABLE 1. (Continued)

Species/Feature	*A. jiuzhaienis sp. nov.*	*A. gracillimum* (Meister) Lange-Bertalot	*A. acerosum* Vijver, Lange-Bertalot & Jarlman	*A. ertzii* Vijver & Lange-Bertalot
Raphe	Filiform, terminal raphe fissures hooked towards the same side	Filiform	Filiform, straight and enlarged proximal raphe endings	Filiform, distal ends are short and straight
Density of striae (10 µm)	22–28 (M), 30–38 (A)	30	36–38	28–30
Number of areolae per striae	2–5 (M), 1–3 (A)	–	2–4 (M), 1–2 (A)	1–2 (M), 2–3 (A)
Rapheless valve				
Axial area	Linear-lanceolate	-	Narrowly linear	Linear-lanceolate
Central area	Absent	-	Absent	Absent
Density of striae (10 µm)	22–27 (M), 28–36 (A)	30	36–38	28–34
Number of areolae per stria	2–5 (M), 1–3 (A)	–	2–4 (M), 1–2 (A)	2–3
References	Current study	Meister (1912), Krammer & Lange-Bertalot (2004)	Vijver *et al.* (2011)	Vijver *et al.* (2011)

Note: "M" means middle; "A" means apices.



FIGURES 115–122. *Achnanthidium jiuzhaienis sp. nov.* Shorter morphotype, SEM views of raphe valve. 115. External view of an entire raphe valve. 116. Internal view of an entire raphe valve. 117–118. Details of the apices and the central area on the external of the raphe valve. 119–120. Detail of the apices and the central area on the internal of the raphe valve. 121–122. Internal areola openings with fine hymenate structures. Scale bars 5 µm (115–116), 1 µm (117–120), 0.2 µm (121–122).



FIGURES 123–129. *Achnanthidium jiuzhaienis sp. nov.* Shorter morphotype, SEM views of rapheless valve. 123. External view of an entire rapheless valve. 124. Internal view of an entire rapheless valve. 125–126. Raphe vestiges at the two apices of the same valve. 127. Detail of the central area on the external of the rapheless valve. 128. Raphe vestiges at the apices. 129. Detail of the central area on the internal of the rapheless valve. Scale bars 5 µm (123–124), 1 µm (125–129).

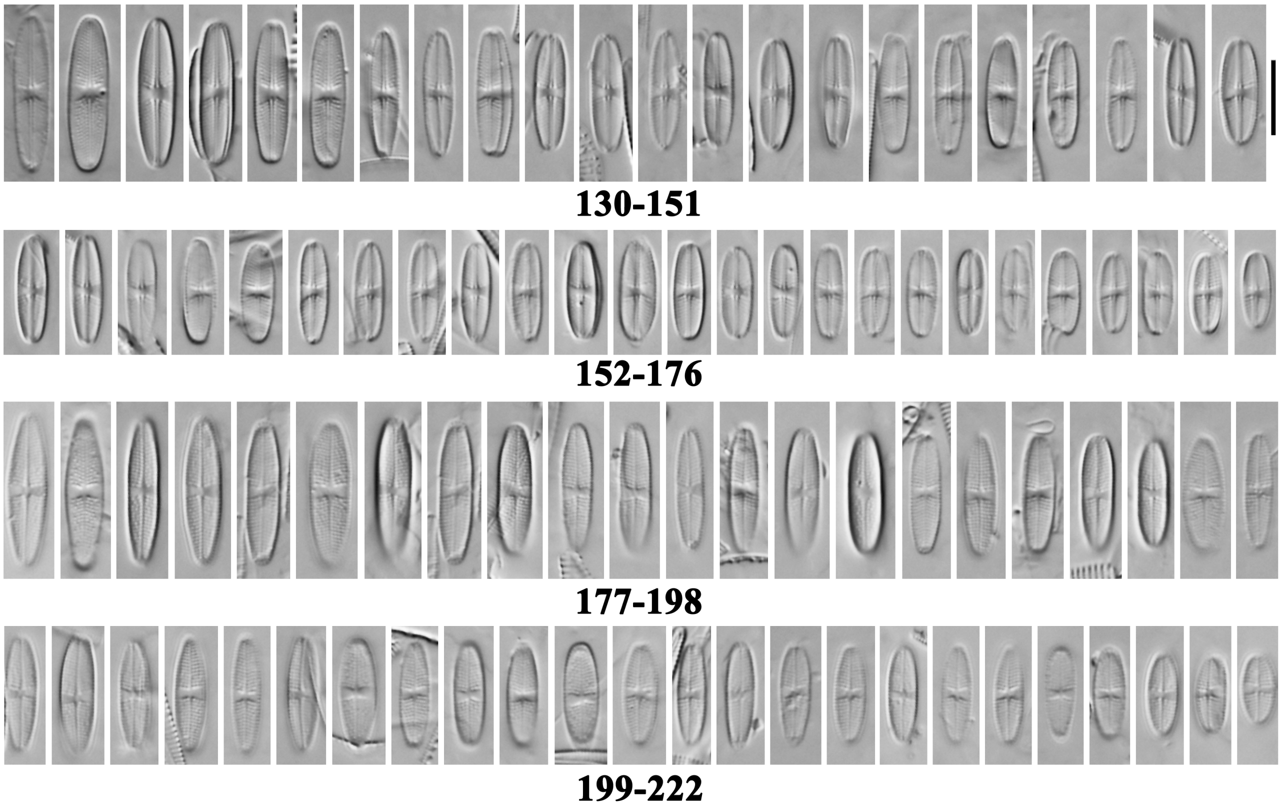
Achnanthidium epilithica **P. Yu, Q-M. You & Q-X Wang** ***sp. nov.*** **(Figs 130–231)**

Holotype: SHTU!, slide JZG–201307043, holotype illustrated in Figs 158, 217. Diatom samples are housed in the Biology Department Diatom Herbarium, Shanghai Normal University, China.

Isotypes: COLO!, slide 614008, Samples are housed in the Kociolek Collection, University of Colorado, Museum of Natural History Diatom Herbarium, Boulder, U.S.A.

Type locality: CHINA. Samples collected from Jiuzhai Valley Nature Reserve, Sichuan Province, 33°11'40"N, 103°53'34"E, altitude: 2250 m, collected by *Q.X. Wang*, on July 7, 2013.

Etymology: Species was named after the diatom species collected on the face of stones and dead wood.

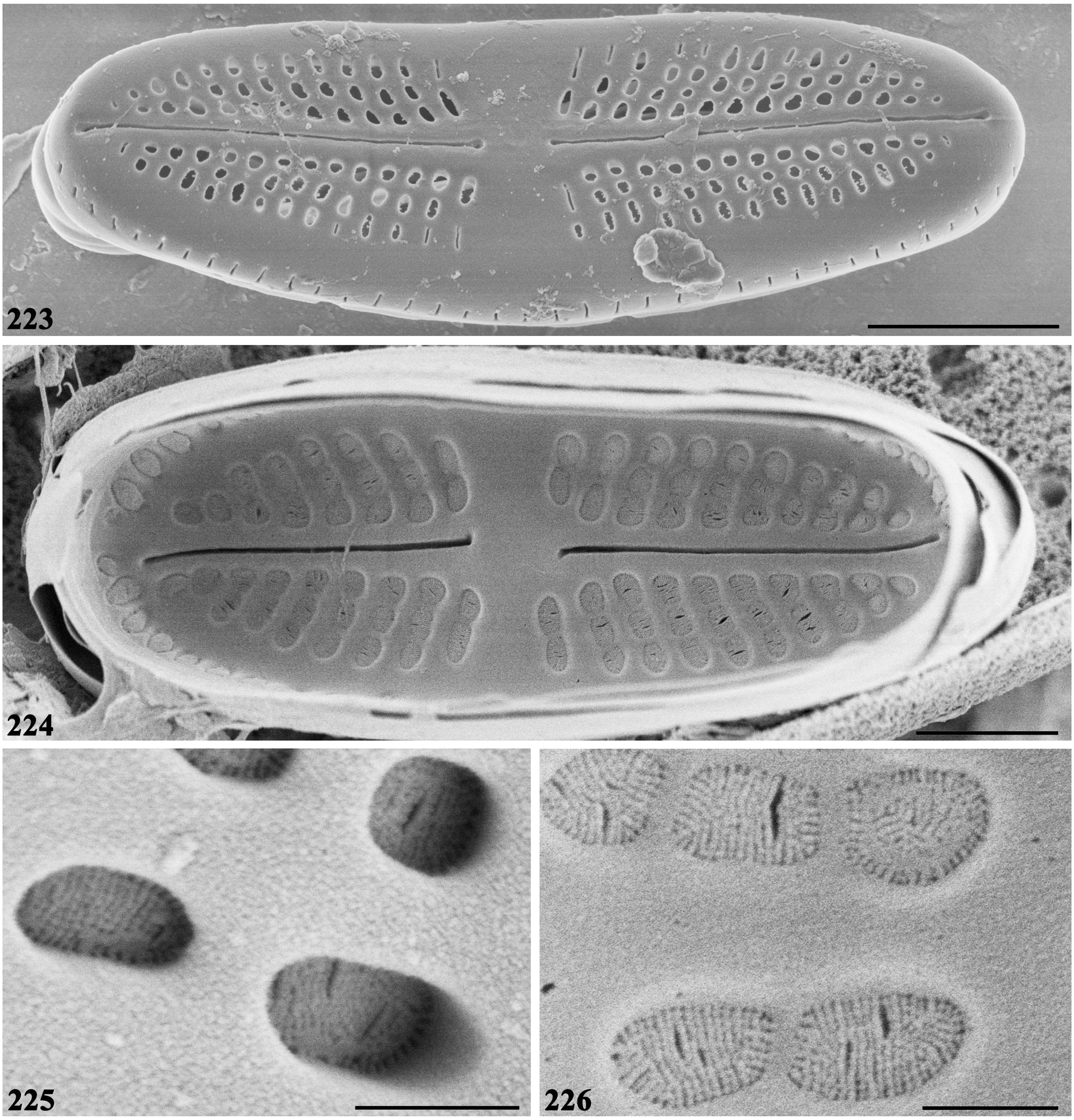


FIGURES 130–222. *Achnanthidium epilithica sp. nov.*, LM. 158, 217. Holotype; 130–176. Raphe valves. 177–222. Rapheless valves. Scale bar 10 µm.

Description: According to LM observations (Figs 130–222), valves were linear-elliptical to elliptical in shape, with broadly rounded ends. Valve length was 9.3–21.3 µm, and width 3.3–5.3 µm (*n*=200). Both valves possessed a linear axial area, and the central area was in a bow tie shape. Striae radiated along the entire valve. Striae number was 22–28/10 µm at the center, and up to 25–34/10 µm near the apices on the raphe valve. On the rapheless valve, striae radiated along the middle portion and were nearly parallel near the apex, becoming denser toward the valve apices, as well as 26–30/10 µm in the middle and 30–35/10 µm near the apices. Individual areolae were not visible with an LM.

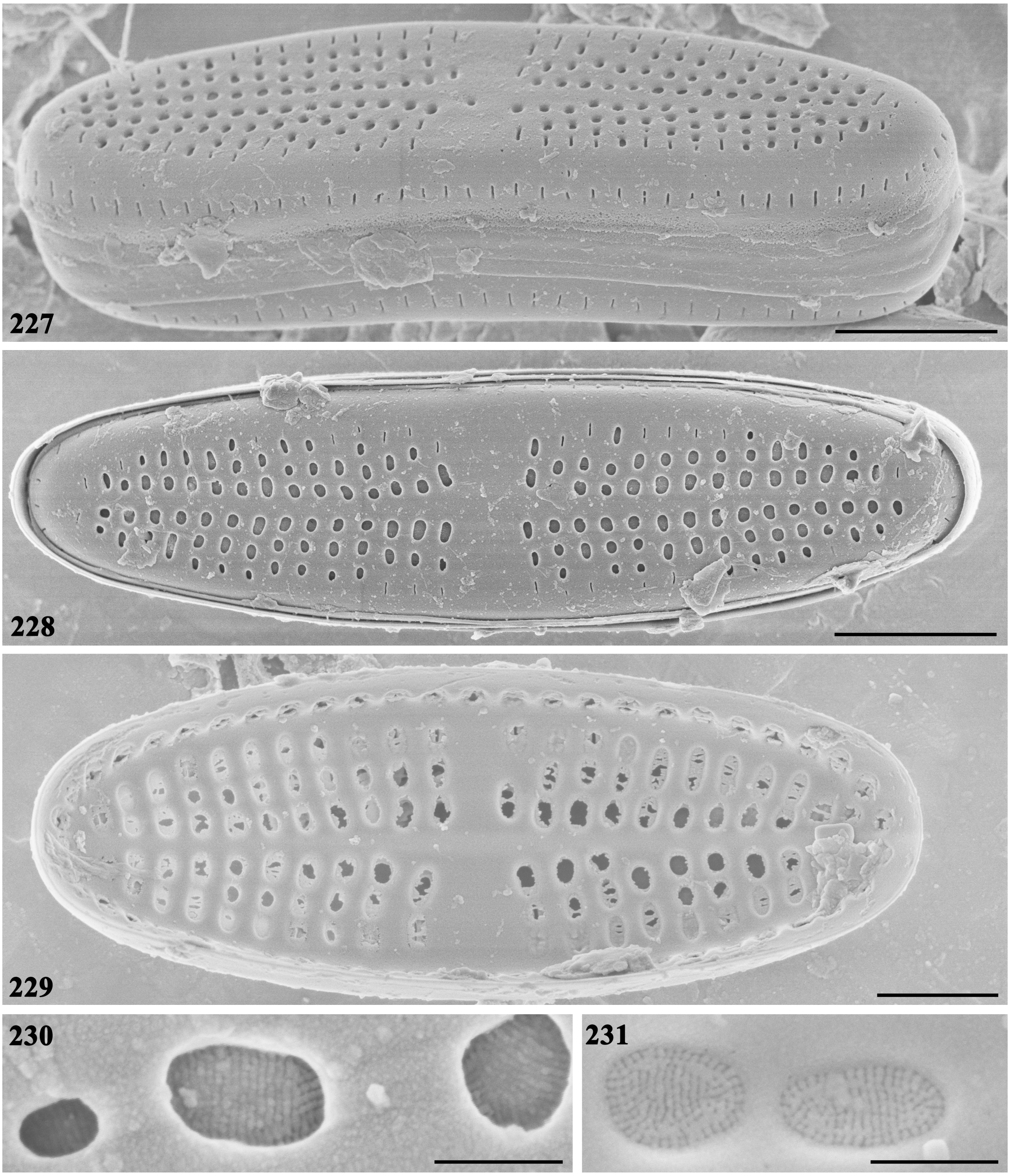
SEM observations of both valves revealed that the valve face had a mantle junction bordered by a wider hyaline area (Figs 223, 227–228), and the mantle had a single row of linear areolae (Figs 223, 227). On the exterior of the raphe valve, the raphe was filiform and straight; proximal raphe ends were straight, and simple; and the distal raphe ends were straight, small, and drop shaped and did not extend on the valve mantle (Fig. 223). Striae were uniseriate, comprised of 1–3 oblong areolae or transapically-oriented areolae in the center of the valve, with 1–2 large, irregularly-round, or transapically-oriented areolae elsewhere (Fig. 223). One slit-like areola occasionally appeared at the end of the striae close to the valve margin (Fig. 223). Areolae occlusions are positioned within the opening and could be seen from the exterior (Fig. 225). Internally, the raphe terminated distally as an elevated helictoglossa, and the proximal raphe endings were short, deflecting in opposite directions (Fig. 224). Areolae were large and irregularly-round to oblong in shape and the openings are occluded with fine hymenate structures that included small openings around the periphery (Fig. 226).

On the external side of the rapheless valve, the axial area was linear, and possessed a bow tie-shaped central area (Figs 227–228). Striae were uniseriate, comprised of 1–3 round to transapically elongate areolae in the middle portion of the valve, and 1–2 irregularly round or transapically elongate areolae at the ends (Fig. 228). One slit-like areola occasionally appeared at the end of the striae close to the valve margin (Figs 227–228). The areolae were occluded with a fine hymen structure that could be seen externally (Fig. 230). Internally, areolae were round to transapically-elongated, occluding on the valve face and mantle, and the occlusions comprised a fine hymenate structure (Figs 229, 231).



FIGURES 223–226. *Achnanthidium epilithica sp. nov.* SEM views of raphe valve. 223. External view of an entire raphe valve. 224. Internal view of an entire raphe valve. 225. Detail of the areolae on the external of the raphe valve. 226. Internal areola openings with fine hymenate structures. Scale bars 2 µm (223), 1 µm (224), 0.2 µm (225–226).

Ecology: Collected in two samples: JZG–201307008 on dead wood (pH 8.4, water temperature 8.2 °C, TDS 0.198 g/L, conductivity 296 µs/cm); and JZG–201307043 on stones (pH 8, water temperature 12.8 °C, TDS 0.227 g/L, Conductivity 338 µs/cm). The samples of this new species occurred at 5.5 % and 4.5 % relative abundance (total counted 400 valves) in samples JZG–201307008 and JZG–201307043, respectively. There were 7 species that accounted for more than 5 % of sample JZG–201307008: *Denticula elegans* Kützing (Kützing 1844: 44) (29.3 %), *Delicata delicatula* (Kützing) Krammer (18 %), *Adlafia bryophila* (Petersen) Lange-Bertalot (Moser *et al.* 1998: 89) (9 %), *Staurosirella ovata* Morales (Morales & Manoylov 2006: 357) (6.5 %), *Achnanthidium minutissimum* (Kützing) Czarnecki (5.8 %), *Encyonopsis descripta* (Hustedt) Krammer (Krammer 1997: 123) (5.5 %), and *Achnanthidium epilithica sp. nov.* (5.5 %). There were 4 species that accounted for more than 5 % at sample JZG–201307043: *Denticula elegans* Kützing (30.8 %), *Delicata delicatula* (Kützing) Krammer (26.8 %), *Encyonopsis descripta* (Hustedt) Krammer (18.8 %), and *Adlafia bryophila* (Petersen) Lange-Bertalot (13.3 %).



FIGURES 227–231. *Achnanthidium epilithica sp. nov.* SEM views of rapheless valve. 227. Girdle view, a frustule with convex rapheless valve. 228. External view of an entire rapheless valve. 229. Internal view of an entire rapheless valve. 230. Detail of the areolae on the external of the rapheless valve. 231. Internal areola openings with fine hymenate structures. Scale bars 2 µm (227–228), 1 µm (229), 0.2 µm (230–231).

Distribution: thus far, the new species was only collected only from the two sampling localities in Jiuzhai Valley.

Remarks: *Achnanthidium epilithica* can be compared to several species in the same genus, based on similarities in outline and structure of the valve, including *A. dolomiticum* Cantonati & Lange-Bertalot (Cantonati & Lange-Bertalot 2006: 1185), *A. subexigua* Hustedt (Krammer & Lange-Bertalot 2004: 40), and *A. straubianum* Lange-Bertalot (Hlúbiková *et al.* 2011: 37). The morphological characteristics of *A. limosua* and these similar species are summarized in Table 2 to facilitate comparison. The overall lengths of individuals of the three species do not typically exceed 16 µm, and their widths are less than 4.5 µm, while *A. epilithica* was longer (21.3 µm) and wider (up to 5.3 µm) than the three similar taxa. On the exterior of the valve, the mantle junction of *A. epilithica* was bordered by a wider hyaline area, while the similar species have a narrow hyaline area. Furthermore, both valves of *A. epilithica* possess a bow tie-shaped central area, while the central area of *A. dolomiticum* has a narrow fascia on the raphe valve and rectangular to lanceolate on the rapheless valve, *A. subexigua* has a bow tie-shaped central area in the raphe valve, and *A. straubianum* has a central area that is entirely absent. Additionally, on the rapheless valve, *A. epilithica* possess a linear axial area, while *A. subexigua* has a lanceolate to rhombus-lanceolate axial area. *A. epilithica* appears to be clearly different from the three similar *Achnanthidium* species based on its bow tie-shaped central area of both valves and dense striation.

TABLE 2. Comparison of morphological characteristics of *Achnanthidium epilithica sp. nov.* and closely related taxa.

Species/Feature	*A. epiphytica sp. nov.*	*A. dolomiticum* Cantonati & Lange-Bertalot	*A. straubianum* Lange-Bertalot	*A. subexigua* Hustedt
Valve length (µm)	9.3–21.3	5.0–10.1	6.5–8.5	10–16
Valve width (µm)	3.3–5.3	2.5–3.5	2.6–3.5	3–4.5
Valve outline	Linear-elliptical to elliptical	Rectangular to linear-elliptical	Elliptical	Linear
Valve apices	Broadly rounded	Broadly rounded	Broadly rounded	Broadly rounded
Raphe valve				
Axial area	Linear	Narrowly linear	Narrowly lanceolate	Linear
Central area	Bow tie	Narrow fascia	Almost absent	Bow tie
Raphe	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight
Density of striae (10 µm)	20–28 (M), 25–34 (A)	33–36	27–30	32
Number of areolae per stria	1–3 (M), 1–2 (A)	4–5 (M), 2–4 (A)	4–5 (M), 2–4 (A)	–
Rapheless valve				
Axial area	Linear	Narrowly linear	Linear	Lanceolate to rhombus-lanceolate
Central area	Bow tie	Narrowly fascia	Almost absent	Absent
Density of striae (10 µm)	26–30 (M), 30–35 (A)	33–36	27–30	32
Number of areolae per stria	1–3 (M), 1–2 (A)	4–5 (M), 2–4 (apices)	4–5 (M), 2–4 (A)	-
References	Current study	Cantonati & Lange-Bertalot (2011)	Hlúbiková *et al.* (2011)	Krammer & Lange-Bertalot (2004)

Note: "M" means middle; "A" means apices.

***Achnanthidium limosua* P. Yu, Q-M. You & Q-X Wang *sp. nov.* (Figs 232–292)**

Holotype: SHTU!, slide JZG–201307018, holotype illustrated in Figs 242, 273. Diatom samples are housed in the Biology Department Diatom Herbarium, Shanghai Normal University, China.

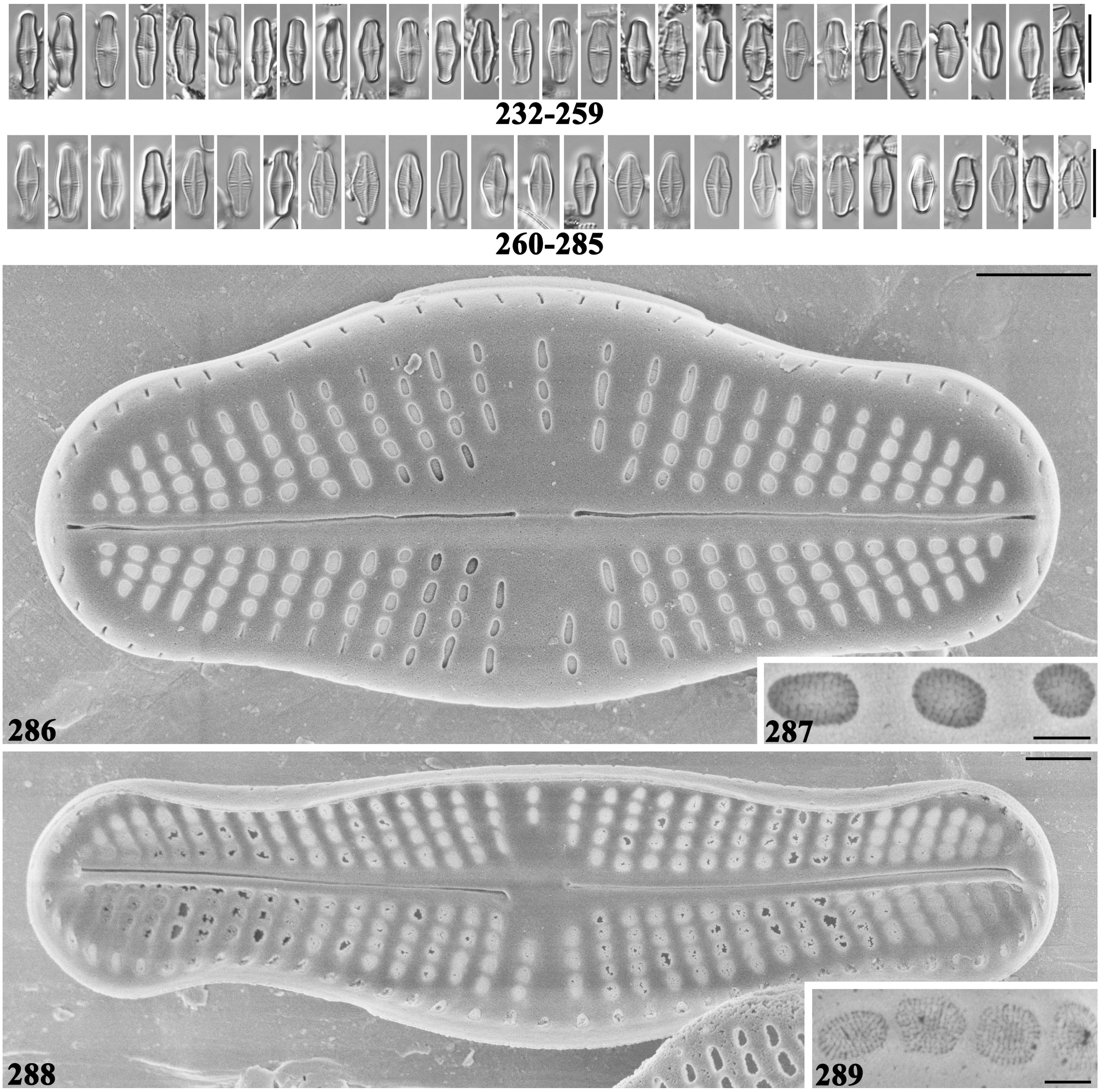
Isotypes: COLO!, slide 614007, Samples are housed in the Kociolek Collection, University of Colorado, Museum of Natural History Diatom Herbarium, Boulder, U.S.A.

Type locality: CHINA. Samples collected from Jiuzhai Valley Nature Reserve, Sichuan Province, 33°09'43"N, 103°53'34"E, altitude: 2332 m, collected by *Q.X. Wang*, on July 5, 2013.

Etymology: Species was named after the diatom species collected on sediment.

Description: According to LM observations (Figs 232–285), valves were linear-elliptical in shape, with subcapitate apices. Valve length was 7.4–11.4 µm, and width 3.2–4 µm (*n*=100). Raphe valves possessed a linear axial area with a small oval central area. Rapheless valves possessed a narrowly linear-lanceolate axial area that widened slightly toward the middle portion. On both valves, striae slightly radiated along the middle portion and become radiated more near the apices. Striae number was 26–30/10 µm at the center, and up to 35–40/10 µm near the apices on the raphe valve. On the rapheless valve, striae were denser toward the valve apices, 27–32/10 µm in the middle and 32–40/10 µm near the apices. Individual areolae were not visible with an LM.

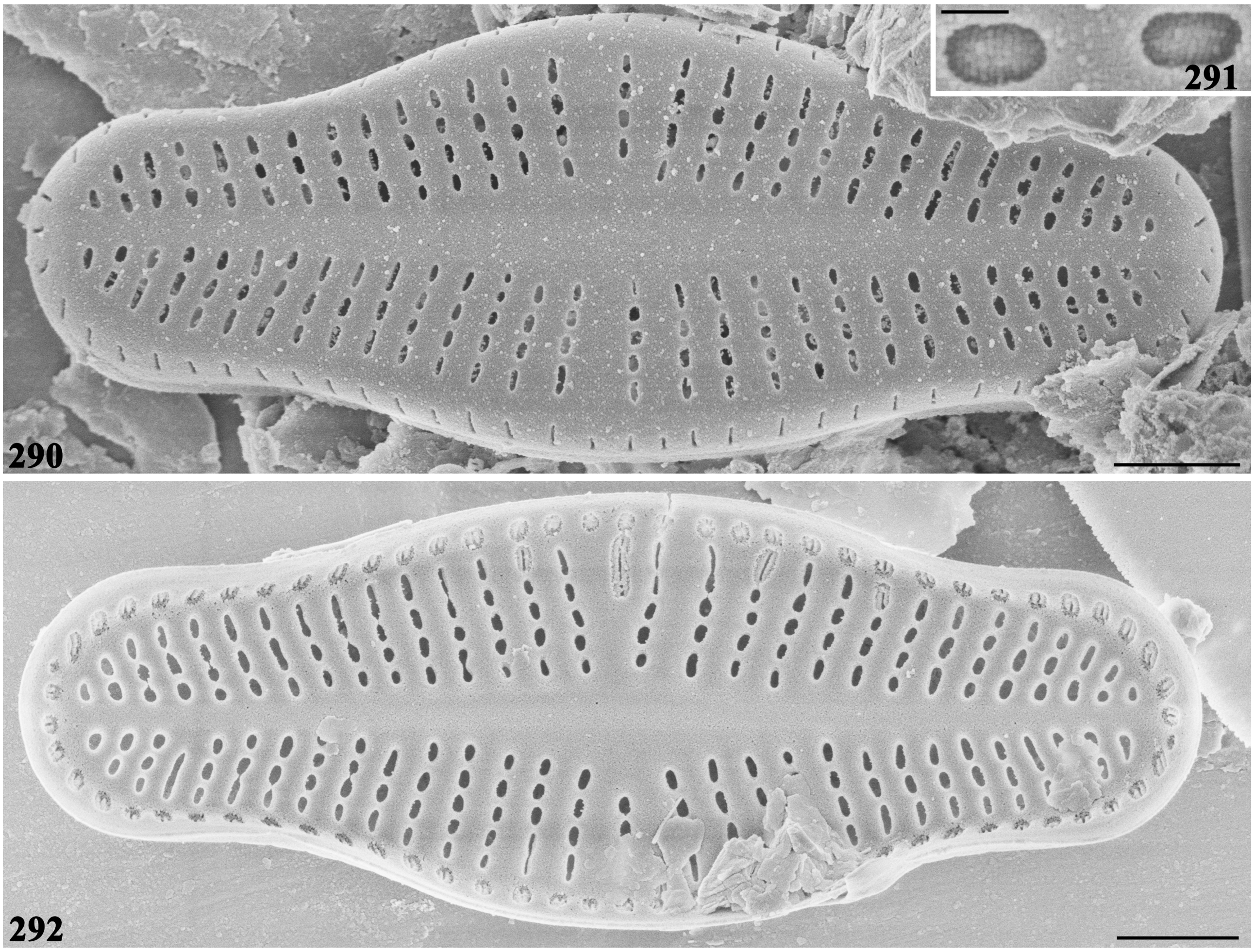
SEM observations of both valves revealed that the valve face: had a mantle junction bordered by a narrow hyaline area and the mantle had a single row of linear areolae (Figs 286, 290). On the exterior of the raphe valve, the raphe was filiform and straight, proximal raphe ends were straight, and simple, while the distal raphe ends were straight and extended on the valve mantle (Fig. 286). Striae were uniseriate, comprised of 2–4 oblong areolae in the middle portion, and 1–3 irregularly-round or oblong areolae elsewhere (Fig. 286). One slit-like areola occasionally appeared at the end of the striae close to the valve margin (Fig. 286). Areolae occlusions were positioned within the opening and could be seen from the exterior of the internal valve (Fig. 287). Internally, the raphe terminated distally as an elevated helictoglossa, and the proximal raphe endings were short, deflecting in opposite directions (Fig. 288). Areolae were large and irregularly-round to oblong in shape, while the openings occluded with fine hymenate structures that included small openings around the periphery (Fig. 289).



FIGURES 232–289. *Achnanthidium limosua sp. nov.* 232–259. LM of raphe valve. 260–285. LM of rapheless valve. 242, 273. Holotype. 286–289. SEM views of raphe valve. 286. External view of an entire raphe valve. 287. Detail of the areolae on the external of the raphe valve. 288. Internal view of an entire raphe valve. 289. Internal areola openings with fine hymenate structures. Scale bars 10 µm (232–285), 1 µm (286, 288), 0.1 µm (287, 289).

On the rapheless valve, externally, the axial area was linear-lanceolate that widens slightly toward the middle portion (Fig. 290). Striae were uniseriate, comprised of 4–5 oblong to transapically elongated areolae in the middle partition of the valve, and 1–3 irregularly round or transapically elongated areolae at the ends (Fig. 290). The areolae occluded with fine hymen structures that could be seen externally (Fig. 291).

Ecology: Collected in one sample JZG–201307018 on sediment (pH 8.42, water temperature 9.9 °C, TDS 0.246 g/L, conductivity 367 µs/cm). This new species occurred at 2.5 % relative abundance (total counted 400 valves) in sample JZG–201307018. There were 5 species that accounted for more than 5 % of this sample: *Staurosirella ovata* Morales (30.3 %), *Staurosira venter* (Ehrenberg) Cleve & Moller (Cleve & Möller 1879: 242) (20 %), *Pseudostaurosira brevistriata* var. *inflata* (Pantocsek) Edlund (Edlund 1994: 12) (18.8 %), *Achnanthidium straubianum* Lange-Bertalot (6.5 %), and *Encyonema silesiacum* (Bleisch) Mann (Round *et al.* 1990: 667) (6 %).



FIGURES 290–292. *Achnanthidium limosua sp. nov.* SEM views of rapheless valve. 290. External view of an entire rapheless valve. 291. Detail of the areolae on the external of the rapheless valve. 292. Internal view of an entire rapheless valve. Scale bars 1 µm (290, 292), 0.1 µm (291).

Distribution: thus far, the new species was collected only at the type locality in Jiuzhai Valley.

Remarks: *Achnanthidium limosua* can be compared to several species in the same genus, based on similarities in the outline and structure of the valve, including *A. lusitanicum* Novais & Morales (Novais *et al.* 2015: 129), *A. eutrophilum* Lange-Bertalot (Hlúbiková *et al.* 2011: 30), *A. caravelense* Novais & Ector (Novais *et al.* 2011: 142), *A. acsiae* Wojtal, Morales et al. (Wojtal *et al.* 2011: 226), and *A. minutissimum* (Kützing) Czarnecki (Morales *et al.* 2011: 97). The morphological characteristics of *A. limosua* and these similar species are summarized in Table 3 to facilitate comparison. Valves of *A. limosua* are wider (3.2–4.0 µm) than *A. subtilissimum* P. Yu, Q-M. You & Q-X Wang (2.2–2.8 µm), *A. lusitanicum* (2.3–3.0 µm), *A. acsiae* (7.0–15.8 µm), and A. *minutissimum* (3.0–3.5 µm), but narrower than *A. eutrophilum* (to 5.8 µm). Additionally, on the raphe valves of *A. limosua* possess a linear axial area, while the axial area is linear-lanceolate in *A. subtilissimum*, *A. eutrophilum*, and *A. minutissimum*. Moreover, *A. limosua* has a small oval central area, while *A. caravelense* has a round to elliptical central area, and *A. lusitanicum*, *A. eutrophilum*, and *A. acsiae* have a rectangular fascia central area. Furthermore, on the rapheless valve, the new species had no central area, but *A. caravelense* and *A. lusitanicum* possess an elliptical central area, while *A. minutissimum* has an elliptical to lanceolate central area. Additionally, the density of striae at the apices of *A. limosua* is higher on both the raphe (35–40/10 µm) and rapheless (32–40/10 µm) valves than in *A. lusitanicum* (30–35/10 µm on the rapheless valve), *A. eutrophilum* (Hlúbiková *et al.* 2011) (25–30/10 µm on the rapheless valve), and *A. minutissimum* (Morales *et al.* 2011) (22–24/10 µm on raphe valve, 24–30/10 µm on the rapheless valve).

TABLE 3. Comparison of morphological characteristics of *Achnanthidium limosua sp. nov.* and closely related taxa.

Species/Feature	*A. limosua sp. nov.*	*A. lusitanicum* Novais & Morales	*A. eutrophilum* Lange-Bertalot	*A. caravelense* Novais & Ector	*A. acsiae* Wojtal, Morales *et al.*	*A. minutissimum* (Kützing) Czarnecki
Valve length (µm)	7.4–11.4	5.3–13.0	7.5–16	9.6–17	7.0–15.8	5–20
Valve width (µm)	3.2–4.0	2.3–3.0	3.2–5.8	2.5–4.2	2.3–3.1	3.0–3.5
Valve outline	Linear-elliptical	Elliptical to linear-lanceolate	Narrowly rhombie	Narrowly elliptical	Linear - lanceolate	Linear-elliptical to linear-lanceolate
Valve apices	Subcapitate	Rostrate, protracted	Bluntly rounded to broadly cuneate	Bluntly rounded	Subcapitate to protracted	Protracted, capitate
Raphe valve						
Axial area	Linear	Narrowly linear	Linear-lanceolate	Linear	Lanceolate	Linear-lanceolate
Central area	Small oval	Absent	Absent	Round to elliptic	Rectangular fascia	Irregular
Raphe	Distal raphe fissures straight	Distal raphe fissures straight	Distal raphe fissures straight	Distal raphe fissures straight	Distal raphe fissures straight deflected	Distal raphe fissures straight
Density of striae (10 µm)	26–30 (M), 35–40 (A)	35 (M), 40 (A)	18 (M), 36–40 (A)	30 (M), 35–40 (A)	28–36(M), 38 (A)	30
Number of areolae per stria	2–4 (M), 1–3 (A)	3–4 (M), 1–3 (A)	3–5 (M), 2–3 (A)	2–4	3–4	3–4 (M), 1–3 (A)
Rapheless valve						
Axial area	Linear-lanceolate	Linear	Linear-lanceolate	Linear	Lanceolate	Linear-lanceolate
Central area	Absent	Small elliptic	Absent	Elliptic	Small	Elliptic to lanceolate
Density of striae (10 µm)	27–32 (M), 32–40 (A)	30–35	25–30	30 (M), 35 (A)	30–31(M), 38 (A)	24–34
Number of areolae per stria	4–5 (M), 1–3 (A)	3–4 (M), 1–3 (A)	4–6 (M), 1–3 (A)	3–4	3–4	4–5 (M), 1–3 (A)
References	Current study	Novais *et al.* (2015)	Hlúbiková *et al.* (2011)	Novais *et al.* (2011)	Wojtal *et al.* (2011)	Morales *et al.* (2011)

Note: "M" means middle; "A" means apices.

Achnanthidium subtilissimum **P. Yu, Q-M. You & Q-X Wang** ***sp. nov.*** **(Figs 293–355)**

Holotype: SHTU!, slide JZG–201307018, holotype illustrated in Figs 300, 328. Diatom samples are housed in the Biology Department Diatom Herbarium, Shanghai Normal University, China.

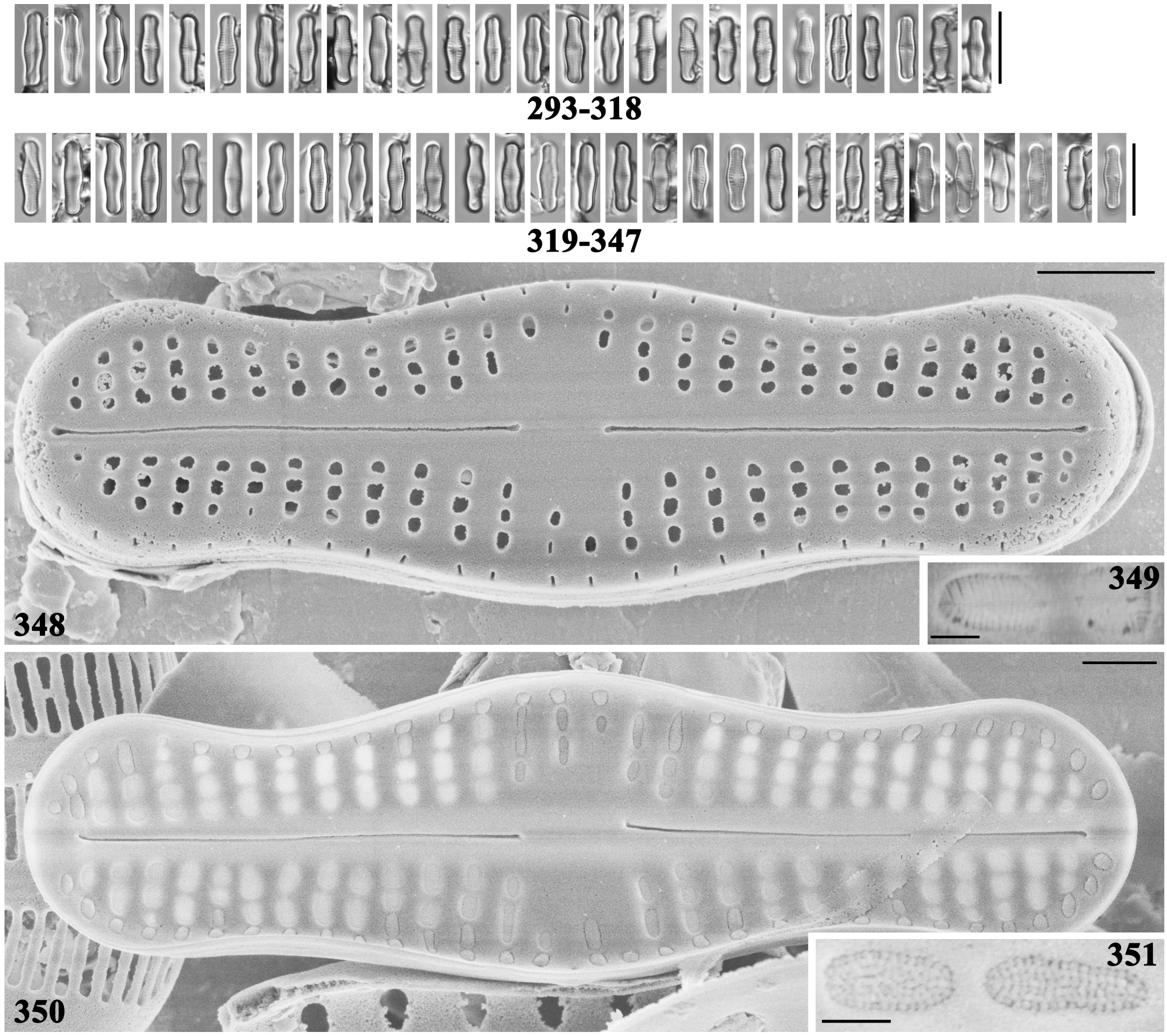
Isotypes: COLO!, slide 614007, Samples are housed in the Kociolek Collection, University of Colorado, Museum of Natural History Diatom Herbarium, Boulder, U.S.A.

Type locality: CHINA. Samples collected from Jiuzhai Valley Nature Reserve, Sichuan Province, 33°09'43"N, 103°53'34"E, altitude: 2332 m, collected by *Q.X. Wang*, on July 5, 2013.

Etymology: Species was named based on the narrow valves observed in this new species.

Description: According to LM observations (Figs 293–347), valves were linear-elliptical in shape, with wide capitate ends, and valves had a central inflation that was slightly larger in width than the apical expansions. Valve length was 8.5–10.2 µm, and width 2.2–2.8 µm (n=80). Raphe valves possessed a linear-lanceolate axial area with a small oval central area. Rapheless valves possessed a linear-lanceolate axial area that widened toward the middle portion. On both valves, striae are nearly parallel along the entire valve. Striae number was 26–32/10 µm at the center, and up to 32–36/10 µm near the apices on the raphe valve. On the rapheless valve, striae number was 27–30/10 µm in the middle, and up to 30–36/10 µm near the apices. Individual areolae were not visible with an LM.

SEM observations of both valves revealed that the valve face: had a mantle junction bordered by a narrow hyaline area and the mantle has single row of linear areolae (Figs 348, 352). On the exterior of the raphe valve, the raphe was filiform and straight; proximal raphe ends were straight, and simple; and the distal raphe ends were straight, small and drop shaped and they do not extend on the valve mantle (Fig. 348). Striae were uniseriate, comprising 1–3 round or oblong areolae in the entire valve (Fig. 348). Some striae had one slit-like areola on the valve mantle (Fig. 348). Areolare occlusions were positioned within the opening and could be seen from the exterior (Fig. 349). Internally, the raphe terminated distally as an elevated helictoglossa, and the proximal raphe endings were short, deflecting in opposite directions (Fig. 350). Areolae were large and round to oblong in shape, and the openings occluded with fine hymenate structures that included small openings around the periphery on the internal valve (Fig. 351).



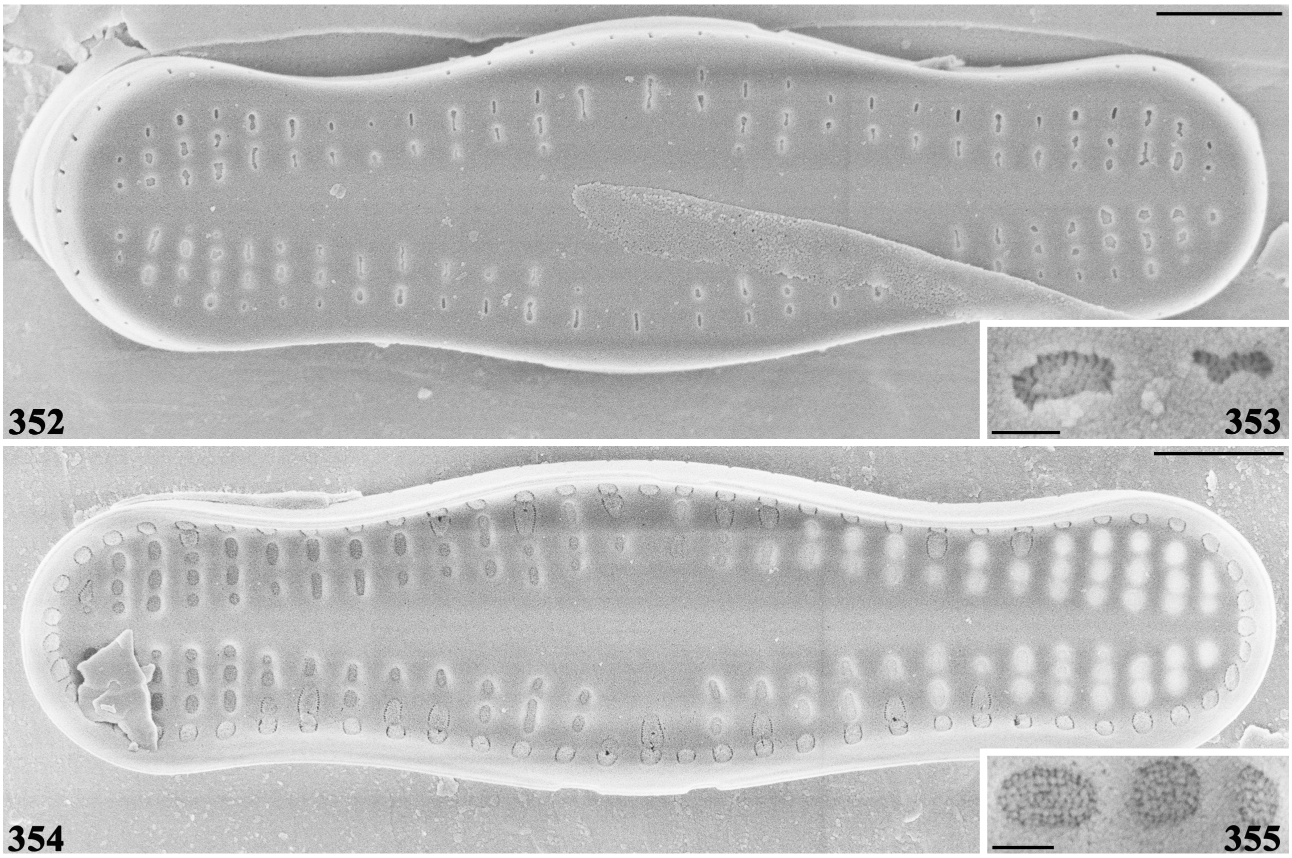
FIGURES 293–351. *Achnanthidium subtilissimum sp. nov.* 293–318. LM of raphe valve. 319–347. LM of rapheless valve. 300, 328. Holotype. 348–351. SEM views of raphe valve. 348. External view of an entire raphe valve. 349. Detail of the areolae on the external of the raphe valve. 350. Internal view of an entire raphe valve. 351. Internal areola openings with fine hymenate structures. Scale bars 10 μm (293–347), 1 μm (348, 350), 0.1 μm (349, 351).

On the external portion of the rapheless valve, the axial area was linear-lanceolate which widened toward the middle portion (Figs 352, 354). Striae were uniseriate, comprised of 1–3 transapically-oriented areolae in the middle partition of the valve, and 2–3 irregularly round or small round areolae at the ends (Fig. 352). The areolae were occluded with a fine hymen structure velum that could be seen externally (Fig. 353). Internally, areolae were round to oblong, and occluded on the valve face, which comprised a fine hymenate structure (Figs 354–355).

Ecology: Collected in one sample JZG–201307018 on sediment (pH 8.42, water temperature 9.9 °C, TDS 0.246 g/L, conductivity 367 μs/cm). This new species occurred at 2.8 % relative abundance (total counted, 400 valves) in sample JZG–201307018.

Distribution: Thus far, the new species was collected only at the type locality in Jiuzhai Valley.

Remarks: *Achnanthidium subtilissimum* can be compared with several species in the same genus, based on similarities in the outline and structure of the valve, including *A. acsiae* Wojtal, Morales, Vijver & Ector (Wojtal *et al.* 2011: 226), *A. catenatum* (Bily & Marvan) Lange-Bertalot (Hlúbiková *et al.* 2011: 23), *A. microcephalum* Kützing (Novais *et al.* 2015: 111), *A. sibiricum* Kulikovskiv, Lange-Bertalot, Witkowski & Khursevich (Kulikovskiv 2011: 79), *A. saprophilum* (Kobayashi & Mayama) Round & Bukhtiyarova (Hlúbiková *et al.* 2011: 33), *A. lineare* Smith (Vijver *et al.* 2011: 170) and *A. lusitanicum* Novais & Morais (Novais *et al.* 2015: 129). The morphological characteristics of *A. subtilissimum* and these similar species are summarized in Table 4 to facilitate a comparison. The length of *A. subtilissimum* does not exceed 10 µm, while other species are longer including *A. acsiae* (up to 15.8 µm), *A. catenatum* (up to 17.8 µm), *A. microcephalum* (up to 14.2 µm), *A. saprophilum* (up to 14.5 µm), *A. lineare* (up to 13.5 µm), and *A. lusitanicum* (up to 13 µm) compared with the new species. Additionally, *A. subtilissimum* is wider (2.2–2.8 µm) than *A. sibiricum* (1.7–2.1 µm), but narrower than *A. saprophilum* (3.0–3.6 µm). Moreover, *A. subtilissimum* has wide capitate ends and possesses a small oval central area on the raphe valve that is different from other similar taxa. Furthermore, the density of the striae at the apices of *A. subtilissimum* is higher on both the raphe (32–36/10 µm) and rapheless (30–36/10 µm) valves than in *A. catenatum* (30–32/10 µm at the apices on the raphe, 30–34/10 µm on the rapheless valve), *A. saprophilum* (28–31/10 µm on both valves), and *A. lineare* (28–32/10 µm on both valves). The density of striae in the new species is lower than in *A. acsiae* (36–38/10 µm at the apices on the raphe valve and 38/10 µm on the rapheless valve), and *A. lusitanicum* (40/10 µm at the apices on the raphe valve). Additionally, fewer areolae per striae are present on *A. subtilissimum* on both valves (1–3) than in *A. acsiae* (3–4 on both valves), *A. catenatum* (5–6 on the raphe valves, 4–5 on the rapheless valves), *A. microcephalum* (2–5 on the raphe valves, 3–4 on the rapheless valves), and *A. microcephalum* (2–5 on the raphe valves, 3–4 on the rapheless valves).



FIGURES 352–355. *Achnanthidium subtilissimum sp. nov.* SEM views of rapheless valve. 352. External view of an entire rapheless valve. 353. Detail of the areolae on the external of the rapheless valve. 354. Internal view of an entire rapheless valve. 355. Internal areola openings with fine hymenate structures. Scale bars 1 µm (352, 354), 0.1 µm (353, 355).

TABLE 4. Comparison of morphological characteristics of *Achnanthidium subtilissimum sp. nov.* and closely related taxa.

Species/Feature	*A. subtilissimum sp. nov.*	*A. acsiae* Wojtal, Morales, Vijver & Ector	*A. catenatum* (Bily & Marvan) Lange-Bertalot	*A. microcephalum* Kützing
Valve length (µm)	8.5–10.2	7.0–15.8	10.0–17.5	6.2–14.2
Valve width (µm)	2.2–2.8	2.3–3.1	2.8–3.6	2.4–3.6
Valve outline	Linear-elliptical	Linear-elliptical to linear-lanceolate	Linear-lanceolate	Linear-elliptical to linear-lanceolate
Valve apices	Widely capitate	Protracted, subcapitate	Capitate to broadly subcapitate	Protracted rostrate to subcapitate
Raphe valve				
Axial area	Linear-lanceolate	Narrowly linear-lanceolate	Narrowly linear-lanceolate	Narrowly linear

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TABLE 4. (Continued)

Species/Feature	*A. subtilissimum sp. nov.*	*A. acsiae* Wojtal, Morales, Vijver & Ector	*A. catenatum* (Bily & Marvan) Lange-Bertalot	*A. microcephalum* Kützing
Central area	Small oval	Rectangular fascia	rounded	Absent
Raphe	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight
Density of striae (10 μm)	26–32 (M), 32–36(A)	28–36 (M), 36–38 (A)	30–32	30–32 (M), 35 (A)
Number of areolae per stria	1–3	3–4	5–6	2–5
Rapheless valve				
Axial area	linear-lanceolate	Linear-lanceolate	Linear	Linear
Central area	Absent	Small	Absent	Absent
Density of striae (10 μm)	27–30 (M), 30–36 (A)	30–31(M), 38 (A)	30–34	30 (M), 35 (A)
Number of areolae per stria	1–3 (M), 2–3 (A)	3–4	4–5	3–4
References	Current study	Wojtal *et al.* (2011)	Hlúbiková *et al.* (2011)	Novais *et al.* (2015)

Note: "M" means middle; "A" means apices.

TABLE 4-1. Comparison of morphological characteristics of *Achnanthidium subtilissimum sp. nov.* and closely related taxa.

Species/Feature	*A. sibiricum* Kulikovskiv, Lange-Bertalot, Witkowski & Khursevich	*A. saprophilum* (Kobayashi & Mayama) Round & Bukhtiyarova	*A. lineare* Smith	*A. lusitanicum* Novais & Morais
Valve length (μm)	8.0–10.3	9.5–14.5	9.0–13.5	5.3–13.0
Valve width (μm)	1.7–2.1	3.0–3.6	2.2–2.8	2.3–3.0
Valve outline	Linear	Broadly linear	Linear	Elliptical, linear-elliptic to linear-lanceolate
Valve apices	Broadly rounded	Broadly rounded	Broadly rounded	Rostrate, protracted
Raphe valve				
Axial area	Linear	Linear lanceolate	Linear-lanceolate	Narrowly linear
Central area	Bow-like	Variable	Rectangular fascia	Absent to small rounded
Raphe	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight
Density of striae (10 μm)	33–36	28–31	28–32	35 (M), 40 (A)
Number of areolae per stria	2–3 (M), 1–2 (A)	4–7	2–3	3–4 (M), 1–3 (A)
Rapheless valve				
Axial area	Linear	Linear-lanceolate	Linear-lanceolate	Linear
Central area	Absent	Narrowly rhombic	Absent	Small elliptical
Density of striae (10 μm)	33–36	28–31	28–32	30–35
Number of areolae per stria	2–3 (M), 1–2 (A)	4–6	1–3	3–4 (M), 1–3 (A)
References	Kulikovskiv (2011)	Hlúbiková *et al.* (2011)	Vijver *et al.* (2011)	Novais *et al.* (2015)

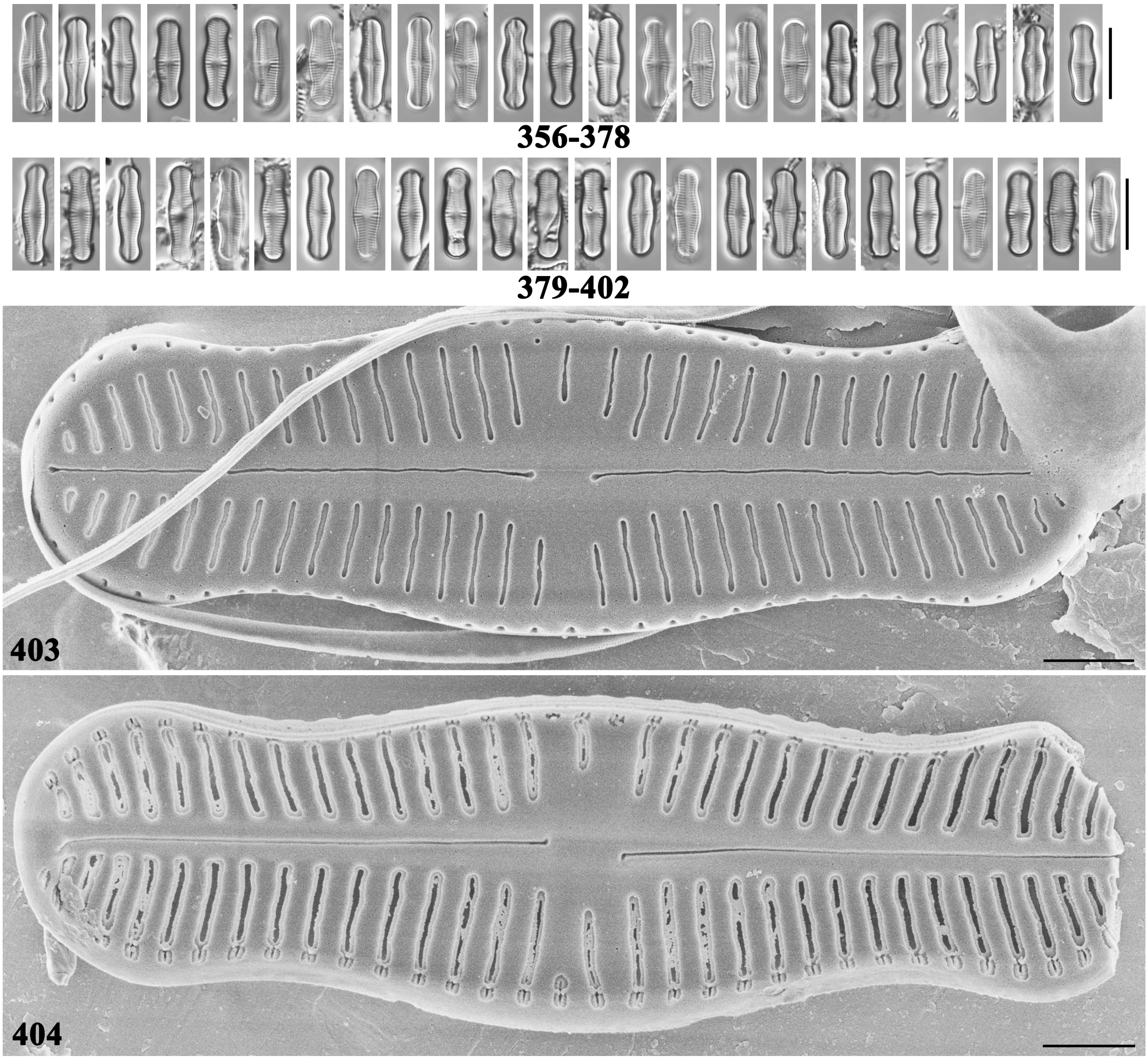
Note: "M" means middle; "A" means apices.

***Kolbesia sichuanenis* P. Yu, Q-M. You & Q-X Wang *sp. nov.* (Figs 356–408)**

Holotype: SHTU!, slide JZG–201307018, holotype illustrated in Figs 356, 379. Diatom samples are housed in the Biology Department Diatom Herbarium, Shanghai Normal University, China.

Isotypes: COLO!, slide 614007, Samples are housed in the Kociolek Collection, University of Colorado, Museum of Natural History Diatom Herbarium, Boulder, U.S.A.

Type locality: CHINA. Samples collected from Jiuzhai Valley Nature Reserve, Sichuan Province, 33°09'43"N, 103°53'34"E, altitude: 2332 m, collected by *Q.X. Wang*, on July 5, 2013.

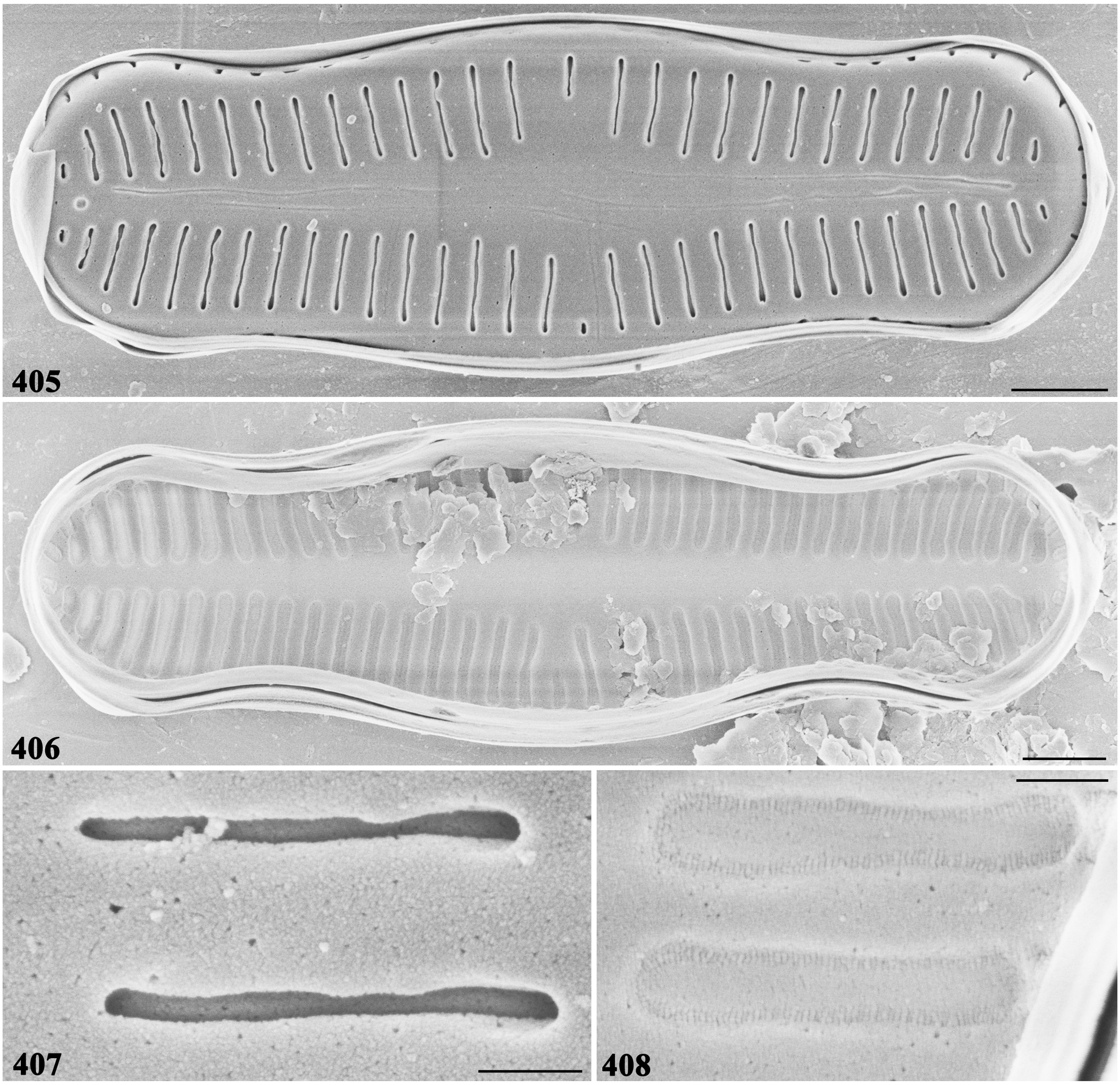


FIGURES 356–404. *Kolbesia sichuanenis sp. nov.* 356–378. LM of raphe valve. 379–402. LM of rapheless valve. 356, 379. Holotype. 403–404. SEM views of raphe valve. 403. External view of an entire raphe valve. 404. Internal view of an entire raphe valve. Scale bars 10 µm (356–402), 1 µm (403–404).

Etymology: Species was named based on its finding in Sichuan Province.

Description: According to LM observations (Figs 356–402), frustules were heterovalvar, and monoraphid. Valves were linear-lanceolate in shape, with broadly capitated ends, and valves had a central inflation slightly larger in width at the apical expansions. Valve length was 10.8–14.1 µm, and width 3.2–3.7 µm (*n*=50). Raphe valves possessed a narrowly linear-lanceolate axial area that had a small oval central area, and striae slightly radiated along the entire valve. Striae number was 22–26/10 µm at the center, and up to 28–30/10 µm near the apices on the raphe valve. Rapheless valves possessed a narrowly linear-lanceolate axial area that widened slightly towards the middle portion, and striae slightly radiated along the entire valve. Striae number was 24–28/10 µm in the middle and 28–32/10 µm near the apices. Individual areolae were not visible with an LM.

SEM observations of both valves revealed that the valve face: had a mantle junction bordered by a narrow hyaline area and the mantle had a single row of linear areolae (Figs 403, 405). On the exterior of the raphe valve, the raphe was filiform and straight; proximal and distal raphe ends were straight, small, and drop shaped, and the distal raphe did not extend along the valve mantle (Fig. 403). Striae were uniseriate, and narrow striae comprised 1 macroareola (rarely 2 or 3) in the entire valve (Fig. 403). Internally, the raphe terminated distally as an elevated helictoglossa, and the proximal raphe endings were short, deflecting in opposite directions (Fig. 404).



FIGURES 405–408. *Kolbesia sichuanenis sp. nov.* SEM views of rapheless valve. 405. External view of an entire rapheless valve. 406. Internal view of an entire rapheless valve. 407. Detail of the areolae on the external of the rapheless valve. 408. Internal areola openings with fine hymenate structures. Scale bars 1 µm (405–406), 0.2 µm (407–408).

On the external surface of the rapheless valve, the axial area was narrowly linear-lanceolate and had some cutting lines that widened slightly towards the middle portion (Fig. 405). Striae were uniseriate with one macroareola on the external valve (Fig. 405). Areolae occluded with a fine hymen structure that could be seen externally (Fig. 407). Internally, macroareolae were on the valve face, and the occlusions comprised a fine hymenate structure (Figs 406, 408).

Ecology: Collected in one sample JZG–201307018 on sediment (pH 8.42, water temperature 9.9 °C, TDS 0.246 g/L, conductivity 367 µs/cm). This new species occurred at 2 % relative abundances (total counted 400 valves) in sample JZG–201307018.

Distribution: Thus far, the new species was collected only at the type locality in Jiuzhai Valley.

Remarks: *Kolbesia sichuanenis* can be compared to several species in the genus *Achnanthidium*, based on similarities in the outline and structure of the valve, including *A. rosenstockii* (Lange-Bertalot) Lange-Bertalot (Krammer & Lange-Bertalot 2004: 433), *A. rosenstockii* var. *inareolatum* Lange-Bertalot (Krammer & Lange-Bertalot 2004: 433), *A. capitatum* Riaux Gobin, Romero, Compère & Al Handal (Riaux-Gobin *et al.* 2011: 13) and *A. trinode* Ralfs (Krammer & Lange-Bertalot 2004: 286). The morphological characteristics *A. uniporatus* and these similar species are summarized in Table 5 to facilitate a comparison. The width of *K. sichuanenis* is narrower (3.2–3.7 µm) than *A. rosenstockii* var. *inareolatum* (4.2–5.1 µm), *A. capitatum* (4–5 µm), and *A. trinode* (5–6 µm). On the raphe valve, *K. sichuanenis* has a small oval central area, while *A. rosenstockii* var. *inareolatum* and *A. capitatum* have no central area, and *A. trinode* possesses a round central area. Additionally, the density of striae at the apices of *K. sichuanenis* is higher on both the raphe (28–30/10 µm) and rapheless (28–32/10 µm) valves than in *A. rosenstockii* var. *inareolatum* (20/10 µm at the apices on both valves) and *A. capitatum* (22–25/10 µm on the raphe valve), but less than *A. trinode* (30–35/10 µm on both valves). Furthermore, the distal ends of raphe are straight, small, and drop shaped in *K. sichuanenis*, but in *A. capitatum* they are gently and doubly hooked on the same side. Moreover, the proximal raphe ends are straight, small, and drop shaped in *K. sichuanenis*, while *A. capitatum* are relatively close. Additionally, striae are comprised of one macroareola on both valves in this new species, but the number of areolae per striae of *A. rosenstockii* is 2–4 on the raphe valves and 3–4 on the rapheless valves, and *A. trinode* is 5–9 on the raphe valves and 4–7 on the rapheless valves.

Kolbesia sichuanenis is clearly different from the other species when compared to the genus *Kolbesia* based on the new species linear-lanceolate shape, while the other species are elliptical, linear-elliptical, elliptical-lanceolate (e.g., *K. amoena*, *K. gessneri*, *K. kolbei*, *K. nitidiformis*, *K. ploenensis*, and *K. suchlandtii*), or linear (*K. sinica*). Additionally, the width of *K. sichuanenis* usually does not exceed 4 µm, while other species are wider, including *K. amoena*, *K. nitidiformis*, *K. suchlandtii* (up to 5 µm), *K. kolbei* (up to 7.5 µm), *K. ploenensis* (up to 6 µm), *K. gessneri* (up to 7 µm), and *K. suchlandtii* (up to 5 µm).

TABLE 5. Comparison of morphological characteristics of *Kolbesia sichuanenis sp. nov.* and closely related taxa.

Species/Feature	*K. sichuanenis* sp. nov.	*A. rosenstockii* (Lange-Bertalot) Lange-Bertalot	*A. rosenstockii* var. *inareolatum* Lange-Bertalot	*A. capitatum* Riaux Gobin, Romero, Compère & Al Handal	*A. trinode* Ralfs
Valve length (µm)	10.8–14.1	6–14	9.6–15.1	9–15	15–28
Valve width (µm)	3.2–3.7	3–4	4.2–5.1	4–5	5–6
Valve outline	Linear-lanceolate	Linear-elliptical	Linear-elliptical	Linear-elliptical	Linear
Valve apices	Broadly capitate	Subcapitate	Subcapitate	Widely capitate	Broadly capitate
Raphe valve					
Axial area	Linear-lanceolate	Linear	Linear-lanceolate	Linear	Linear
Central area	Oval	Elliptical	Absent	Absent	Round
Raphe	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures straight	Filiform, distal raphe fissures gently doubly hooked on same side, proximal raphe ends close	Filiform, distal raphe fissures straight
Density of striae (10 µm)	22–26 (M), 28–30 (A)	27–32	20	22–25	30–35
Number of areolae per stria	1	2–4	1	1	5–9
Rapheless valve					
Axial area	Linear-lanceolate	Lanceolate	Linear	Linear to lanceolate	Linear-lanceolate
Central area	Absent	Absent	Absent	Absent	Lanceolate
Density of striae (10 µm)	24–28 (M), 28–32 (A)	27–32	20	27–38	30–35
Number of areolae per stria	1	3–4	1	1	4–7
References	Current study	Krammer & Lange-Bertalot (2004)	Krammer & Lange-Bertalot (2004)	Riaux-Gobin *et al.* (2011)	Krammer & Lange-Bertalot (2004)

Note: "M" means middle; "A" means apices.

Discussion

A. jiuzhaienis, *A. epilithica*, *A. limosua*, and *A. subtilissimum* are new species that possess certain characteristics that justify their classification in the genus *Achnanthidium*. Such characteristics include having small linear-lanceolate to lanceolate elliptical valves, uniseriate striae, a fine raphe and straight or deflected external distal raphe fissures

(Ponader & Potapova 2007). The new species, *A. jiuzhaienis* belongs to the "*Achnanthidium pyrenaicum* complex" based on deflected external distal raphe fissures. Meanwhile *A. epiphytica*, *A. limosua*, and *A. subtilissimum* belong to the "*A. minutissimum* complex" based on their possession of straight external distal raphe fissures. Additionally, *Kolbesia sichuanenis* is a new species that possesses certain characteristics that support its assignment to the genus *Kolbesia*. Such characteristics include striae that are uniseriate, and narrow striae comprised of one macroareola (rarely 2 or 3) on the both valves. And *K. sichuanenis* is a new freshwater species of this genus reported for the first time in China. Following the survey of samples retrieved from the Jiuzhai Valley Nature Reserve, in the terms of diatom diversity, the number of species in the *Achnanthidium* genus were found to be relatively large in most samples, such as *A. minutissimum*, *A. affine*, *A. exile*, *A. rivulare*, *A. convergens*, *A. pyrenaicum*, and *A. duthiei*. While only one species belonged to the genus *Kolbesia*. This new species WAS collected only at the type locality in Jiuzhai Valley.

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