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A new species of *Achnanthes* (Bacillariophyceae) from a freshwater habitat in a karst landform from south-central China

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SUMMARY

Achnanthes maolanensis nov. sp. is a large diatom collected from subaerial, freshwater habitats in karst landforms in central-south China. Living cells have two chloroplasts. Valves of this new species are panduriform in outline, slightly constricted in the middle part of the margins, with uniseriate areolae; the raphe is filiform, and there is a linear, thickened stauros in the central area of the RV. On the ARV, there is no central area, and as well there are no terminal orbiculi, marginal ridge, or terminal spine externally. The axial area (rapheless sternum) is located down the centre, not offset from the center as in many other species of the genus and is narrow and almost straight. This suite of characters makes the new species easy to distinguish from other species in the genus. Its presence adds further documentation of the unique and diverse freshwater diatom flora from karst habitats in this region of China.

Key words: *Achnanthes maolanensis*; monoraphid diatom; Maolan Nature Reserve

INTRODUCTION

Although *Achnanthes* C. Agardh 1824 was previously a large genus more than 1000 species and subspecific epithets associated with it per DiatomBase (Kociolek *et al*. 2018), the genus is now considered more narrowly circumscribed, with most species being robust and being found in marine and brackish water environments (Mclntire & Overton 1971; Round *et al*. 1990; Toyoda *et al*. 2003, 2005a, b). In subaerial freshwater environments, *Achnanthes* species have been reported and described from the continental United States (Johansen *et al.* 2007), Antarctica (Kellogg & Kellogg 2002), Hawaii (Lowe *et al.* 2009) and Macedonia (Tofilovska *et al.* 2014).

Achnanthes sensu stricto differs from other monoraphid diatoms by the robust, large size of the species, growing on thick elongate stalks (Round *et al.* 1990; Novarino 1992; Lange–Bertalot & Compère 1997; Toyoda *et al.* 2003). The distinctly punctate striae have cribra in the areolae (Toyoda *et al.* 2003, 2005a; Toyoda & Williams 2004) and many species have a rapheless sternum on the rapheless valve that is offset to one side relative to the raphe sternum (e.g. Round *et al.* 1990; Tofilovska *et al.* 2014). Cox (2006) and Cox & Williams (2006) pointed out that *Achnanthes* could be transferred to Mastogloiales by evidence from protoplast and frustule data, based on cladistic analyses of valve morphology. Analyses of molecular data, however, have suggested a close relationship between *Achnanthes sensu stricto* and the Bacillariales (Sims *et al.* 2006), though that relationship has not been confirmed by shared, derived morphological features.

As a part of our continuing study of the freshwater diatoms of Guizhou Province, China (Kociolek *et al.* 2016a, b; You *et al.* 2016, 2017; Lowe *et al.* 2017; Yu *et al.* 2017), we encountered a species of *Achnanthes* for which we can find no previous description. The purpose of the present report is to present light and scanning electron microscope observations of this *Achnanthes* species from Guizhou, and to offer a formal description of it as a species new to science.

MATERIALS AND METHODS

Collection sites:—Algae samples were collected on the surface of stones beneath the small waterfall named Latan, in the Maolan Nature Reserve, Guizhou Province, China, on 4th October, 2015. Geographic coordinates of collection site are: N 25° 15′735″, E 108° 04′177″. Latan Waterfall is not open for visitors, so the habitat is almost untouched by human activities. Water temperature, pH, DO, Conductivity and TDS were taken at the time of collection were measured in situ with a YSI Pro Plus (YSI Instrument, Yellow Springs, OH, USA).

Processing and observations:—In the laboratory, Microwave Accelerated Reaction System (Model MARS, CEM Corporation, Mathews, NC, USA) were used to digest samples, following the procedures of Parr *et al.* (2004) and modified as described in You *et al.* (2015). Cleaned diatoms were mounted in Naphrax® (Robert Charles laboratories Ltd., UK) for light microscopy (LM), LM observations were made with an Olympus BX-53 microscope fitted with differential interference contrast (DIC) optics and an Olympus DP-80 digital camera. Samples conducted using a SU8010 field emission SEM (Hitachi Corporation, Tokyo, Japan) for ultra-structure observation. Diatom images were compiled with Photoshop 6.0 software (Adobe Systems Incorporated, USA). Terminology for the morphological description follows Cox (2006), Lowe *et al.* (2009), Sabbe *et al.* (2004), Round *et al.* (1990), Toyoda *et al.* (2005a, 2006, 2010).

RESULTS

Description

Achnanthes maolanensis P. Yu, J.P. Kociolek & Q-M. You sp. nov. (Figs 1-37)

Holotype: GZ-1510092, slide prepared from collected material deposited at the Diatom Museum of Biology Department, Shanghai Normal University (SHTU). Holotype specimen is represented by Figure 4 (raphe valve) and 11 (rapheless valve).

Isotype: JPK10464, cleaned collection material deposited at Kociolek Collection, University of Colorado Boulder (COLO), USA.

Type locality: N 25° 15′735″, E 108° 04′177″, Maolan Nature Reserve, Guizhou Province, CHINA. Materials were collected by Q-X. Wang & J.P. Kociolek, 4th October 2015.

Etymology: The species is named for the Maolan Nature Reserve, Guizhou, in which it was found.

Ecological information: Diatom samples were collected on stones beneath a waterfall and attached to a moss community. Altitude: 650 m, pH 8.2, water temperature 20℃, DO 9.48 mg/L, Conductivity 296 ms/cm, TDS 213 g/L.

Individual cells are panduriform in valve view, flexed in girdle view, with a convex ARV (araphid valves) and concave RV (raphid valves). Each cell contains two chloroplasts, separated by the median transapical plane. Cells usually form chain-like colonies (Fig. 1); no mucilage stalks were observed in the Guizhou samples.

Valves are 33.5 – 88.0 µm in length, slightly constricted at the centre, 12.5 – 24.0 µm in width. A single row of areolae is located between transapical costae, and there are 7-8 transapical costae in 10 µm on the RV and ARV. The central area of the RV is expanded into a linear, thickened stauros, reaching the valve margins. The raphe is filiform and appears arc-shaped because of the concavity of the valve. There is no central area in the ARV; the axial area (sternum) is located in the centre of the valve, appearing narrow and almost straight. Terminal orbiculi are absent at both ends of the ARV.

In the SEM, the external valve faces of RV are gently convave about the transapical axis (Fig. 32). Raphe has distinct central pores, which are slightly deflected to one side of the valve (Figs 14, 15), while the terminal fissures are obviously deflected in the opposite direction of the central fissures (Figs 14, 16, 17). Internally the RV has a distinct stauros and costae that extend from the raphe sternum. The stauros is robust (Figs 18, 20). Costae are more robust at the margin of valve (mantle) (Figs 19, 22). Occlusions are lacking in the areolae near the valve face (Figs 19, see arrows). The central raphe fissure appears as small and hooked-shaped (Fig. 20); the terminal fissures turn slightly in the opposite direction of the proximal ends and termiante in

small C-shaped helictoglossae (Fig. 19). The external valve surfaces of the ARV are slightly depressed compared to RV (Fig. 23), and there is slight depression around the valve margin (Figs 23, 24, 25). Internally, transapical costae are thickened, the sternum is central, elevated higher than costae for the most part, not evident near the end (Figs 29-31). On both valves, areolae are occluded by complex cribra, often supported by 3 – 8 pegs and at almost same level with the external valve (RV and ARV) surface (Figs 15, 17, 24, 26, 27, see arrows). There are no occlusions evident internally (RV and ARV) (Figs 19, 21, 30, 31). There are ca. 5 rows of areolae on the mantle. These areolae are similar to those on the valve face (Figs 33). Each girdle band is open at one end (Figs 34, 35), has areolae occluded by cribra (Fig. 36) and a serrated edge (Fig. 37).

DISCUSSION

Achnanthes maolanensis sp. nov resembles *A. coarctata* (Brébisson) Grunow, *A. yaquinensis* McIntire & Reimer, *A. longipes* C. Agardh , *A. pseudolongipes* Toyoda & Nagumo and *A. subconstricta* (Meister) Toyoda, all of these taxa have similar outlines, e.g. valves are panduriform in shape (McIntire & Reimer 1974; Toyoda *et al.* 2003, 2010; Krammer & Lange-Bertalot 2004; Lee *et al.* 2013). *A. maolanensis* differs from *A. coarctata* mainly in the end, it is more narrowly rounded in *A. maolanensis,* while in *A. coarctata* the ends are obviously widely rounded. The difference between *A. maolanensis* and *A. yaquinensis* is the latter species has orbiculi, a ridge and spines on the ARV, while *A. maolanensis* lacks these characters on both valves. *A. maolanensis* differs from *A. longipes* and *A. pseudolongipes* in the size and number of plastids. There are many small discoid plastids in the latter two species, while *A. maolanensis* has two large plastids. *A. maolanensis* and *A. subconstricta* are different in many respects, including that the latter species has orbiculi, a ridge and spines on the ARV, fewer costae and a greater number of striae between costae. More details about the differences among these species can be found in Table 1.

Species of the genus *Achnanthes* are variable with regard to cytological and valve features. While some species have many discoid plastids (Toyoda *et al.* 2006, 2010), most species illustrated have a few (2) large, plate-like chloroplasts (Mereschkowsky 1905). All of the species are monoraphid, robust in structure, having complex, external volate occlusions, and most are heterovalvate, with the position of the raphe being located in the center of the valve, while the central sternum of the rapheless valve is offset to one side. Nearly all of the species assigned to the genus are marine, though Tofilovska *et al.* (2014) list species recently described from freshwater. Many species possess a large opening at both ends of the rapheless valve called "terminal orbiculus" whose function is unknown. A robust central staurose is prominent on the raphe valve of many species. Some species also have marginal ridges, and there may be terminal spines on the rapheless valves. *Achnanthes maolanensis* sp. nov. has some of the features of the genus, including being robust, monoraphid, and having ornate occlusions in the openings of the areolae. But the species lacks other features seen in many marine species, including terminal orbicula, marginal ridges and spines. It is worth noting that we checked other freshwater *Achnanthes* species, e.g. *A. coarctata* (Brébisson) Grunow, *A. inflata* (Kütz.) Grunow, *A. brevipes* Agardh *et al*., and all of these species lack the characters mentioned here. *Achnanthes maolanensis* sp. nov. has the rapheless sternum being placed medially; this character is not common in the genus. A medially-placed rapheless sternum can be found in several freshwater species of the genus, including *A. naviformis* Van de Vijver & Beyens (Van de Vijver *et al*. 2002), *A. osteni* Frenguelli (Frenguelli 1933), *A. recava* Hohn & Hellerman (Hohn & Hellerman 1996), as well as several marine species, including *A. brevipes* var. *arctica* (Cleve) Kobayashi (Kobayashi 1965), *A. cocconeioides* Riznyk (Riznyk 1973), *A. longipes* Agardh (Toyoda *et al.* 2006), *A. radiata* Du & Cheng (Cheng and Du 1984), *A. subconstricta* (Meister) Toyoda (Toyoda *et al.* 2003), *A. pseudolongipes* Toyoda & Nagumo (Toyoda *et al.* 2010). A phylogenetic study of the group would be timely, to investigate the features used to diagnose the genus, describe the relationships of the taxa, and their relationships with the members of the

Bacillariales, and to study whether there was a single, or many invasions into freshwater from marine habitats or vice versa.

Achnanthes maolanensis sp. nov. was found on stones (subaerial environments) covered with mosses from a sampling site that is more or less undisturbed by human activities. In the sample we studied here, two large species may be found, *Nupela major* (Yu *et al.*, 2017) and *A. maolanensis* (both taxa having cells that are more than 30 μm long, with the largest cells reaching up to 90 μm in length). Most of the other species in this sample are very small, less than 20 μm long, e.g. *Achnanthidium* spp., *Sellaphora* spp., and *Navicula* spp., among others. These small species are difficult to distinguish in the LM, but they have interesting valve characters visible in the SEM. We will continue to document the biodiversity of the diatom flora from this unique region.

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Table 1: Comparison of *Achnanthes maolanensis* sp. nov. with other five species morphological characteristics.

Characters	*A. maolanensis sp. nov.*	*A. coarctata* (Brébisson) Grunow	*A. yaquinensis* McIntire & Reimer	*A. longipes* Agardh	*A. pseudolongipes* Toyoda & Nagumo	*A. subconstricta* (Meister) Toyoda
Valve Shape	Panduriform, gently constricted at the centre	Biundulate, constricted at the centre	Panduriform; gently constricted at the centre	Panduriform to linear-lanceolate	Panduriform to linear-lanceolate, gently constricted at the centre	Panduriform, constricted at the centre
End shape	Narrowly rounded	Widely Rounded	Narrowly rounded	Rostrated	Rounded	Rostrated
Chloroplast	Two large plastids	Two large plastids	Two large plastids	Many small discoid plastids	Many small discoid plastids	Two large plastids
Valve length	33.5–88 µm	17-48 µm	34–104 µm	77–128 µm	41–124 µm	22–94 µm
Valve width	12.5–24.0 µm	6-15 µm	10–21 µm	18–40 µm	15–26 µm	14–29 µm
Costae	7–8/10 µm on RV and ARV	10-14/10 µm on RV and ARV	8–9/10 µm on RV, 8.0–8.5/10 µm on ARV	6.5–7.5/10 µm on RV, 5–6.5/10 µm on ARV	8.0–9.5/10 µm on RV, 6.0–6.5/10 µm on ARV	5.5–6.0/10 µm on RV, 4.5–5.0/10 µm on ARV
Striae between costae	Single row	Single row	Single row	Two rows	Single row (RV), 2 or 3 rows (ARV)	2-5 rows
Areolae	Usually round	Usually round	Usually round or rectangular	Usually round	Usually round	Usually round
Rapheless sternum	Almost central	Eccentric	Almost central or eccentric	Almost central	Almost central	Almost central
Orbiculi on the ARV	No	No	Exist	Exist	Exist or not	Exist
Ridge on the external ARV	No	No	exist	No	No	Exist
Spine on the external ARV	No	No	2 terminal spines	No	No	2-6 marginal spines; 1 terminal spine
Habitat	Freshwater	Freshwater	Marine	Marine	Marine	Marine
Sources	This study	Krammer and Lange-Bertalot. 2004	McIntire and Reimer 1974; Toyoda *et al*. 2005b	Toyoda *et al.* 2006	Toyoda *et al.* 2010	Toyoda *et al.* 2003

Figure Legends

Fig. 1. LM micrograph of *Achnanthes maolanensis* sp. nov., showing chain-like colony and chloroplasts. Scale bar=10 μm.

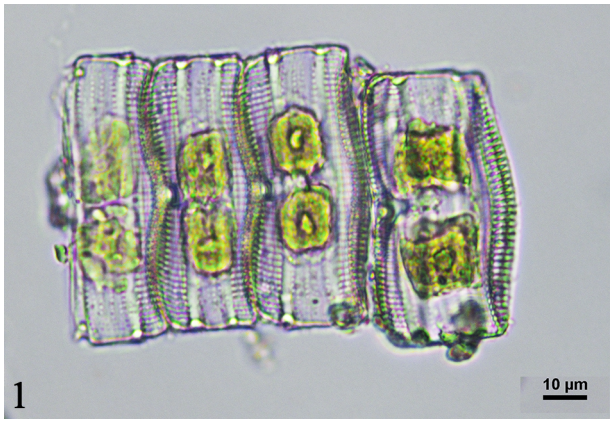
Figs 2–**13.** LM micrographs of *Achnanthes maolanensis* sp. nov. Scale bar=10 μm.

Figs 14–**17.** SEM micrographs of *Achnanthes maolanensis* sp. nov. on the external view of raphid valve (RV). 14. External view of the whole valve. 16. Central area of RV. 15, 17. Detail view of terminal raphe fissure and areolae.

Figs 18–**22.** SEM micrographs of *Achnanthes maolanensis* sp. nov. on the internal view of raphid valve (RV). 18. Internal view of the whole valve. 19. Detail view of areolae (arrows), terminal raphe fissure and helictoglossa. 20. Center stauros, central raphe endings are hook-shaped. 21. Detail of the areolae. 22. margin of valve, costae are protruded obviously.

Figs 23–**28.** SEM micrographs of *Achnanthes maolanensis* sp. nov. on the external view of araphid valve (ARV). 23. External view of the whole valve. 24. Detail view of the valve end, showing areolae in the valve and margin. 25. Central area of ARV. 26, 27. Detail of the areolae in the valve, show pegs (arrows). 28. Detail of the areolae in the margin.

Figs 29–**37.** SEM micrographs of *Achnanthes maolanensis* sp. nov. on the internal view of araphid valve (ARV). 29. Internal view of the whole valve. 30. End area of ARV. 31. Central area of ARV. 32-37. SEM micrographs of girdle view. 32. Girdle view of the frustule. 33. Areolae with different pegs in the mantle. 34. Open end of 2nd or 3rd girdle band (copula). 35. Open end of first girdle band, with close to valve mantle. 36. Areolae in the girdle band. 37. Expanded view of girdle band, with one serrated edge.



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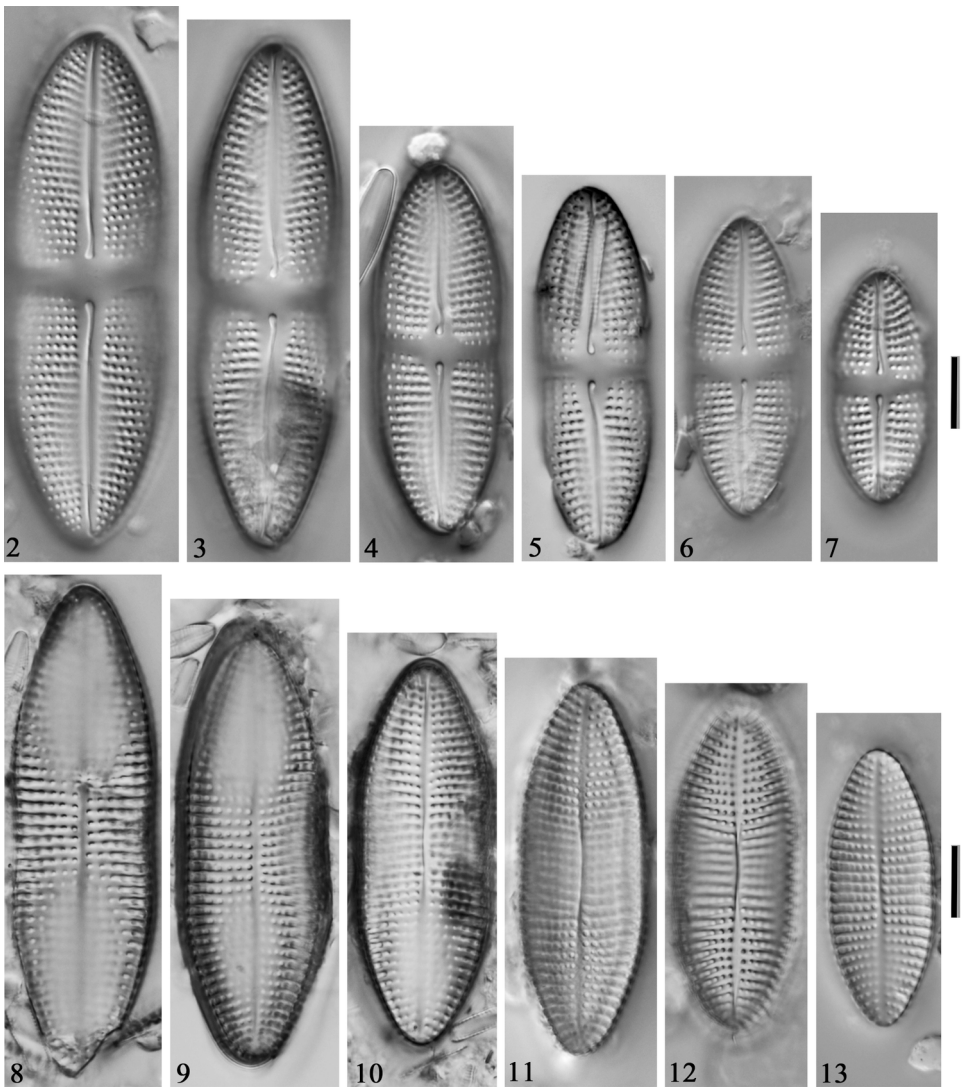

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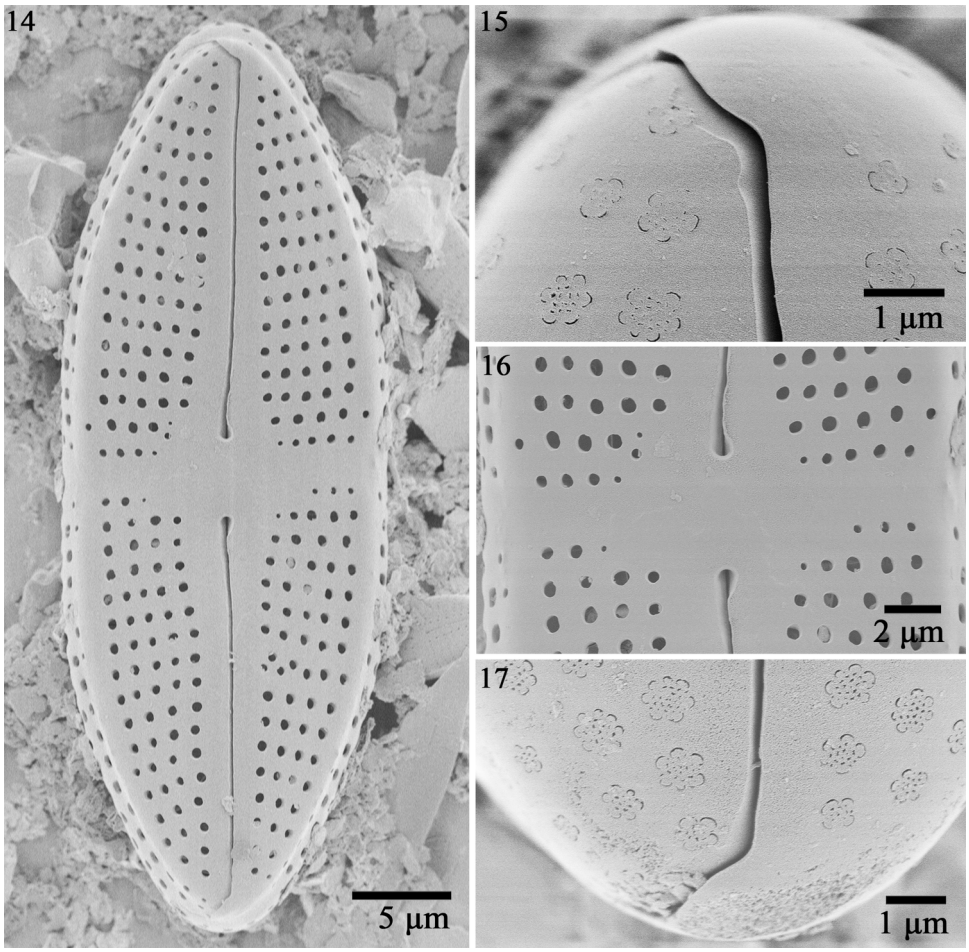
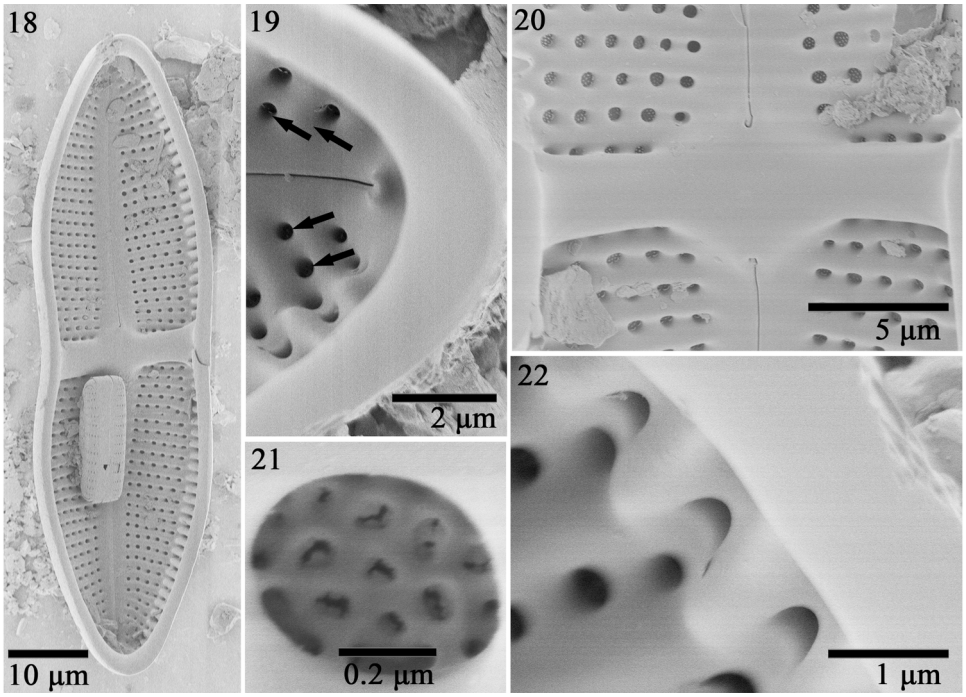
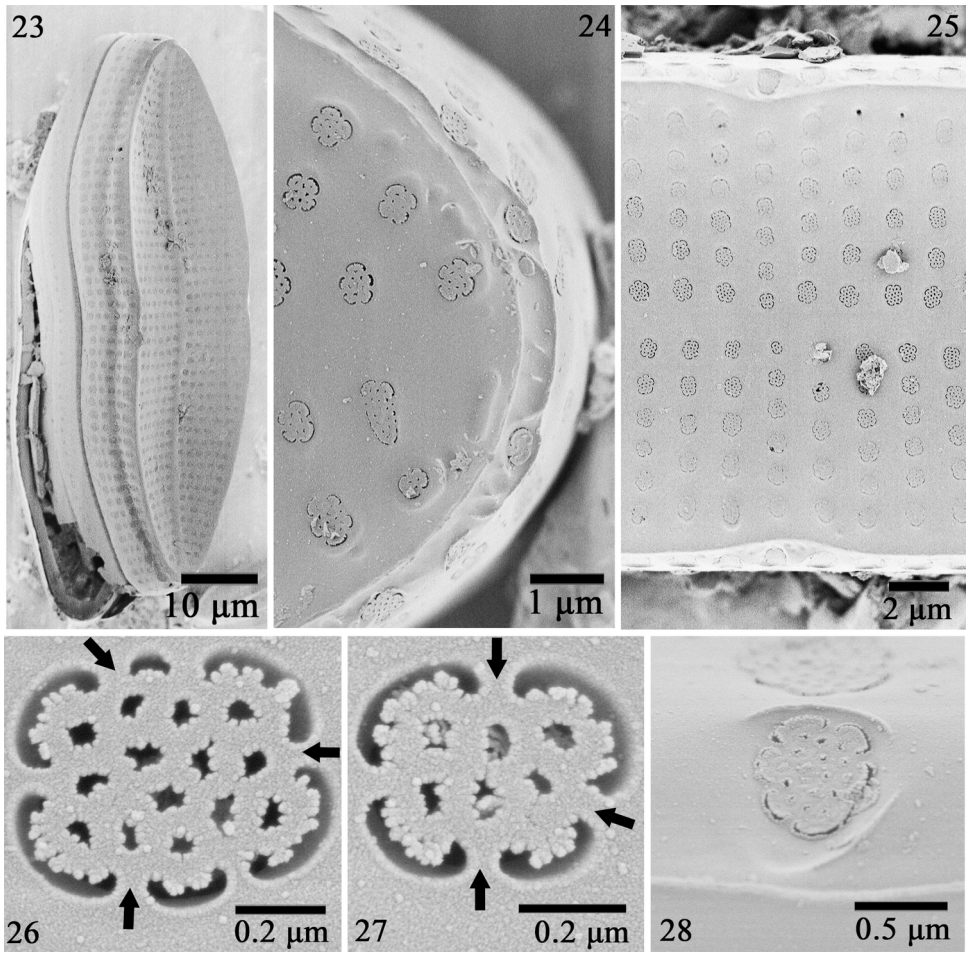


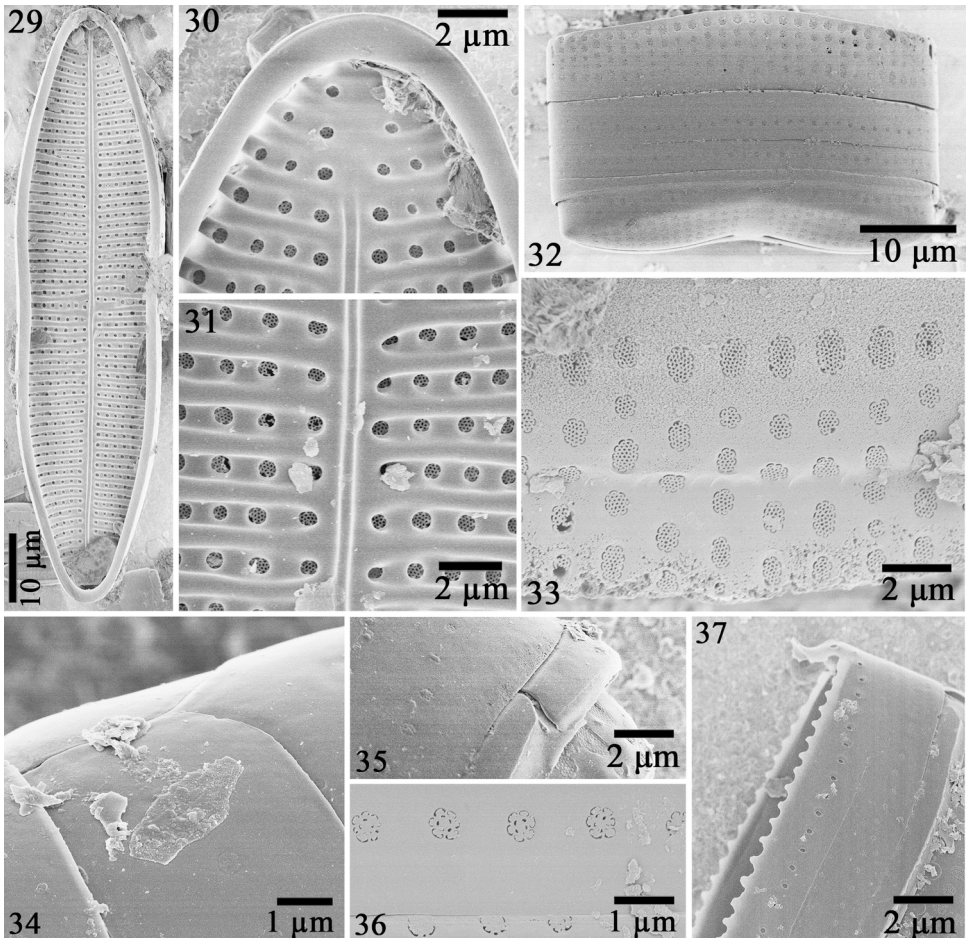
2. Raphe valve-1.tif



3. Raphe valve-2.tif



4. Rapheless valve-1.tif



5. Rapheless valve-2 6. Girdle view.tif