Description of *Gomphothidium* gen. nov., with light and scanning electron microscopy: A New Freshwater Monoraphid Diatom Genus from Asia

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**Abstract:** We describe a new genus of monoraphid diatom based on *Achnanthidium ovatum* T.Watanabe et Tuji in Watanabe et al., a species described originally from Japan. We encountered this species from Yunnan Province in the southwestern part to eastern China, as well as in Luding, Sichuan Province and Xianju, Zhejiang Province, in pristine lotic environments. The species differs from the genus *Achnanthidium* by being asymmetrical to the transapical axis and has a pseudoseptum at the poles of the interior of the raphe valve which is fused with each helictoglossa. Moreover, a distinct group of condensed striae can be found at the narrower pole ('footpole') of the raphe valve. This appears to be the only known genus of freshwater monoraphid diatoms that is restricted to a single continent.

**Key words:** New Genus, Monoraphid diatoms, SEM, biogeography, Achnanthidiaceae, China, Asia

**INTRODUCTION**

Diatoms that have a single raphe system on their frustules (termed ‘monoraphid diatoms’) represent a diverse assemblage of species that totals over 2,000 taxa assigned to over 15 genera (Kociolek et al. 2019). These genera occur within at least 5 distinct lineages of biraphid diatoms, representing independent losses of the raphe system on one of the valves (Thomas et al. 2016; Kociolek et al. 2019). The monoraphid diatoms occur in marine ecosystems and freshwaters, and they all are symmetrical about the apical and transapical axes. An exception to this could be the genus *Sinoperonia* Kociolek, Liu, Glushchenko et Kulikovskiy in Liu et al. (2018), which belongs to the Eunotiales and forms predominantly monoraphid frustules but can also form biraphid and araphid frustules.

Over the last 30 years, there has been a large body of work revising the freshwater monoraphid diatoms, going from recognizing basically two genera in freshwaters (*Achnanthes* and *Cocconeis*; see Hustedt 1930; Patrick & Reimer 1966; Krammer & Lange–Bertalot 1991), to recognizing ca. 13 freshwater genera (Round & Bukhtiyarova1996; Round & Basson 1997; Krammer & Lange–Bertalot 2004; Wojtal 2013; Kulikovsky et al. 2013; Kulikovskiy et al. 2015; see Kociolek et al. 2015a) worldwide. Even with this relatively recent revisionary work and the proposal of new genera, there are no known freshwater genera of monoraphids that are endemic to a single continent (Kociolek, 2019).

In the last ten years there has been renewed interest in the biodiversity of freshwater diatoms from China, with several new genera (e.g. *Tibetiella* Li et al. 2010; *Gomphosinica* Kociolek et al. 2015; *Edtheriotia* Kociolek et al. 2016) and many new species described from across the country (Kociolek et al., in review). In the present report we use light and scanning electron microscopy to describe a new freshwater monoraphid diatom genus that occurs across the China, from Yunnan to Sichuan to Zhejiang Provinces as well as in Japan. Unique symmetry and other features of the valve distinguish this new species from all other freshwater monoraphid genera.
Material and Methods

Diatom samples were collected from the western Sichuan plateau and National Forest Park of Xianju, Zhejiang. The western Sichuan plateau is located in the SE edge of the Qinghai-Tibet plateau, and has high elevation mountains. Forest Park of Xianju is located in the southeast of Zhejiang. Diatom samples were removed from stones, plant roots and other substrates in lentic and lotic systems using tweezers or a knife, and from sediments using a turkey baster. Sample information is listed in Table 1, including location of samples, habitat, and elevations. Water temperature, pH and conductivity were measured using a YSIPro Plus multiparameter meter (YSI Inc., Yellow Springs, OH., U.S.A.).

In the laboratory, a Microwave Accelerated Reaction System (Model MARS, CEM Corporation, Mathews, NC, USA) was used to digest samples, following the procedures of Park 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. For Scanning Electron Microscopy observations, samples were observed with a SU8010 field emission SEM (Hitachi Corporation, Tokyo, Japan). Slides and cleaned material are preserved at SHNU and at COLO. Diatom images were compiled with Photoshop 6.0 software (Adobe Systems Incorporated, USA).

For the Yunnan samples, six samples were collected from high mountain lakes on the Yunnan Plateau (26°37'44.8″–26°38'29.89″N, 99°42'33.1″–99°43'31.9″E), in September and October, 2006 and 2007 (Table 1). The samples were collected by scraping rocks and then fixing the samples in 5% formalin. Organic matter was removed according to the method of Battarbee (1987). These samples are archived at Yunnan University. Permanent diatom slides were made with Naphrax. Diatoms were identified using oil immersion at 1000× magnification with an Olympus microscope (BX51–DIC). Cleaned material for SEM analysis was air-dried onto cover glasses, mounted onto stubs, and coated with 20 nm of Au. Samples were examined and photographed using a Leo 1530 SEM. Cleaned material and slides are preserved at the Institute for Ecological Research and Pollution Control of Plateau Lakes, School of Ecology and Environmental Science, Yunnan University, Kunming, P. R. China. Environmental parameters were measured, including conductivity and pH (measured by using YSI 6600 Multiparameter Instrument).

Results

Class Bacillariophyceae Hauckel emend. Medlin & Kaczmarska (2004)
Subclass Bacillariophycidae D.G.Mann in Round et al. (1990)
Order Cymbellales D.G.Mann in Round et al. (1990)
Family Achnanthidiaceae D.G.Mann in Round et al. (1990)

Gomphothidium Kociolek et al., gen. nov.
Description: Frustules flexed about the transapical axis, with one raphe system. Valves narrowly linear–lanceolate, clavate, asymmetrical about the transapical axis.

Figs 1–40. LM, *Gomphothidium ovatum*: (1–21) valve views of raphe valves; (22–37) valve views of rapheless valves; (38–40) girdle view of frustules. Scale var 10 µm.
Raphe valve with axial area narrow, raphe with dilated external proximal raphe ends. External distal raphe ends straight, not extending onto the valve mantle. Striae fine, radiate, coarse around the central area, compressed at the footpole. Internally, raphe with proximal raphe ends deflected in opposite directions. Helictoglossae thickened, connected to laterally-expanded pseudosepta at the poles. Areolae with hymenate occlusions on the valve face and mantle. Internally, areolae with hymenate occlusions, extending around the face and mantle. 

**Type species of the genus:** *Gomphothidium ovatum* (T.Watanabe et Tuji in Watanabe et al. 2008: 34) Kociolek et al. comb. nov.

**Basionym:** *Achnanthidium ovatum* T.Watanabe et Tuji in Watanabe et al. (2008): Epilithic diatom assemblages and two new species Achnanthidium ovatum and Gomphonema yakuensis from Yaku–shima Island, Kagoshima Prefecture, Japan. – Diatom 24: 34, figs 2–25.

In LM, the valves of *G. ovatum* are asymmetrical to the transapical axis, with the upper half of the valve being broader than the lower half. Valves are clavate or lanceolate with round ends. Valves are 9.0–37.5 μm long and 2.5–3.5 μm wide. Proximal raphe ends distinct, teardrop–shaped (Figs 1–21). Raphe valves with a linear axial area expanding slightly towards the center. Striae are most indistinct, easier to resolve in the middle part of valve where a few stria rows become resolvable due to a wider spacing (Figs 1–21). Rapheless valve with a linear axial area (Figs 22–37). Striae on the rapheless valves are indistinct (Figs 22–37). Frustules in girdle view are distinctly bent, nearly V–shaped (Figs 38–40).

In the SEM, the raphe valve with a narrow lanceolate axial area and an elliptic–lanceolate central area (Figs 41, 45, 50). The rapheless valve with a linear axial area and no distinct central area (Figs 51, 52). Externally, on the raphe valve the distal raphe fissures straight to very slightly deflected, while the central raphe endings dilated and teardrop–shaped (Figs 41, 50). At the headpole, areolar openings distinctly larger compared to the opposite apex. Internally, distal raphe ends slightly curved towards opposite sides. Thickening on either side of the raphe terminus, but helictoglossae appear merged into the pseudosepta, which appear shelf–like at both apices (Figs 45–47). Pseudosepta not present on the rapheless valves (Fig. 52). Internal proximal raphe endings curved in opposite directions. Striae radiate throughout on both valves (Figs 41, 48). Stria density 31–34 in 10 μm at the valve center and up to 36 in 10 μm near apices of raphe valve, 28–32 in 10 μm at the valve center and up to 36 in 10 μm near apices on the rapheless valve. Areolae external openings round, or transapically elongated, whereas internal openings transversely elliptical (Figs 41–44). Each stria in the middle part of the raphe valve composed of 1–2 (usually 2) areolae (Fig. 44). Each stria towards the base composed of 3–4 larger areolae (Figs 41, 43). Each stria in the middle part of the rapheless valve composed of 3–4 areolae (Figs 51–52). Around the central areas of raphe valves, striae more widely spaced and shortened; sometimes very short marginal striae (composed of 1–2 areolae) present. A row of slit–like areolae present on the valve mantle (e.g. Figs 50, 51).

**Discussion**

This new genus of monoraphid diatoms has fine hymenate occlusions and internal proximal raphe ends being deflected in opposite directions, and both of these features suggest it is closely allied with *Achnanthidium* (Yu et al. 2018, *Fottea*, Olomouc, 21(1): 1–7, 2021 DOI: 10.5507/fot.2020.011).
There are several other features that distinguish *Gomphothidium* from *Achnanthidium*, however, which argue for recognition of this taxon as a separate genus. In addition to the valve asymmetry, *Gomphothidium* has a distinct concentration of areolae at the narrower end of the valve (‘footpole’) bordered by a thickened rim. The areolae are suggestive of an apical pore field, but because the samples studied here were cleaned immediately, we cannot be sure if these pores function in mucilage secretion, as is the case for other apical pore field–bearing diatoms such as *Cymbella* C.A.Agarth, *Gomphonema* C.G.Ehrenberg and *Gomphonella* Rabenhorst (Krammer & Lange–Bertalot 1986; Kociolek et al. 2015b). Internally, raphe valves of *Gomphothidium* have internally–projected pseudosepta at both poles. At the broader pole (‘headpole’) the thickening is distinct and broadened beyond where the helictoglossa connects to it. At the narrower end of the valve the thickening is smaller. The helictoglossae are attached, and reduced in size as compared to species in *Achnanthidium*. These features, taken together, and compared to distinctions made between other monoraphid genera such as valve shape (to separate *Psammothidium* Bukhtiyarova et Round from *Achnanthidium*; but see Monnier et al. 2007), and cavum number (to separate *Gliwiczia* Kulikovskiy et al. from *Planothidium*), support the proposal of a new genus.
Within *Achnanthidium*, however, three morphological groups have been identified, recognized by their distinctive features of flexure of the external distal raphe ends and areolar structure, the *A. minutissimum* complex, the *A. pyrenaicum* complex and the *A. exiguum* complex. Members of the *A. minutissimum* complex have straight external distal raphe endings and round to elliptical areolae, members of the *A. pyrenaicum* complex have external distal raphe fissures deflect in the same direction and with lineate areolae, and members of the *A. exiguum* complex have external distal raphe fissures deflected in the opposite directions (Wojtal et al. 2011; Péres et al. 2012; Péres et al. 2014; Karthick et al. 2017). Based on this breakdown of the genus *Achnanthidium*, *Gomphophidium* most resemble members of the *A. minutissimum* group (Novais et al. 2015). There here are no formal analyses, however, to support the idea that these distinctions reflect phylogenetic affinities.

*Gomphophidium* may be endemic to Asia. The specimens on which we base our observations are from China, and these occurred from Yunnan eastward to Sichuan and to Zhejiang Provinces. The specimens of *G. obtusum* were described originally from Yakushima Island, Kagoshima Prefecture, Japan (Watanabe et al. 2008). The original authors of this species suggested that although it was abundant from the type locality (Anbou River) in 1976, by 2007 it was locally extinct. Another genus of freshwater diatoms that can be found in China and Japan is *Edtheriotia* (Kociolek et al. 2016), and there are many genera currently known as endemics in Asia. In fact, there are more endemic genera in Asia than any other continent (Kociolek 2019).

We place our new genus within the *Achnanthidiaceae*, and that family within the *Cymbellales*, per the analysis by Thomas et al. (2016). The phylogenetic relationships of some monoraphid genera such as *Achnanthidium* being part of the *Cymbellales* supports the classification system of Mireschkowski (1902) and his group “Monolepatae”. From a biogeographic point of view, Asia is home to the only endemic genus of *Cymbellales* (*Oricymba* Jüttner et al. 2010; Celebesia Kapustin et al. 2017; *Alveocymba* Kapustin et al. 2020). While this is the original presentation of *Gomphophidium*, (and the discovery of taxa beyond their initial distribution is common in freshwater diatoms), the currently available data suggest that this genus is also endemic to Asia.

Of the 13 genera of freshwater monoraphid diatoms recognized, none of them are endemic to a single continent. This may be due to having lumped species into currently–recognized genera, even though there were revisions of several genera in the last several decades. Kociolek & Williams (2017) and Kociolek (2019) suggested we may need a more fine–grained taxonomy to further elucidate, describe and interpret biogeographic patterns among genera of freshwater diatom genera.

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