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One new species of *Cymbella* C.A. Agardh (Bacillariophyta) from high altitude lakes in the Hengduan Mountains of Southwest China

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Abstract – This paper describes a new species of the genus *Cymbella* C.A. Agardh from an alpine lake in the Hengduan Mountains of southwestern China. A detailed morphological description of the new species, named *Cymbella luoyulanae* sp. nov., is presented; the description is based on light and scanning electron microscopy. The main features of *C. luoyulanae* are strongly dorsiventral valves with strongly reverse-lateral raphe branches near the proximal ends, a large central area occupying approximately a half of the width with the valve and slit-like areolae comprising striae that may be unseriate or partially biseriate. The new species has morphological characteristics that resemble those of *C. heihainensis* Y.Li et Gong, *C. modicepunctata* Krammer and *C. asiatica* Metzeltin, Lange-Bertalot et Y.Li, but it differs from these three species in details of size, valve shape, striae density, central area, and number of stigmata.

Keywords: China, *Cymbella*, diatom, new species, taxonomy, SEM

Introduction

Diatoms from the Yunnan plateau have been only cursorily studied in the past (Skuja 1937, Zhu and Chen 1994, Li et al. 2007a,b). *Cymbella* C.A. Agardh (1830: 1) is one of the largest diatom genera, representing nearly two thousand described taxa (Kociolek et al. 2020a). However, in the past 30 years, the family Cymbellaceae has been split by some researchers more finely (Kociolek and Stoermer 1987, Krammer 1997a,b, Lange-Bertalot and Genkal 1999, Krammer 2002, 2003, Jüttner et al. 2010a, Bahls 2015, Kulikovskiy et al. 2014, 2015, Kulikovskiy and Kuznetsova 2016, Kapustin et al. 2017, 2020, Kociolek et al. 2017, Glushchenko et al. 2019, Kezlya et al. 2020). As a result of this recent work about 20 different genera are recognized (Tab. 1).

Since the revision of *Cymbella*, a number of other new species have been described from China (summarized in Kociolek et al. 2020b, see also Li et al. 2019, Liu et al. 2020, Zhang et al. 2020). In southwestern China, specifically, Li et al. (2003a,b) described new species of *Cymbella* respectively from northwest Tibet and Qinghai Province. Gong and Li (2011) described a new species of *Cymbella* from the Yunnan Plateau. Hu et al. (2013) described three new species of *Cymbella* from the high altitude lakes of the Hengduan Mountains region. In the present paper, we describe one new *Cymbella* species from the Hengduan Mountains area based on light and scanning electron microscopical investigations and compare its morphology with similar species.

Material and methods

Samples were taken using a Kajak-type gravity core (Renberg and Hansson 2008) from the surface sediments of Lake Shadecuo (29°44′35.8″ N, 101°21′39.8″ E, Tab. 2), located in the eastern Hengduan Mountains region of Southwest China. The pH and specific conductivity of the lake water were measured in the field using a YSI 650 multi-parameter display system (650 MDS, YSI Incorporated 1700/1725 Brannum Lane, Yellow Springs, OH 45387 USA) with a 600 XL probe. Water samples were taken from 50 cm under the water surface. Transparency was estimated using a Secchi disk. Total nitrogen (TN) and total phosphorus

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Tab. 1. A chronological listing of genera based on species formerly in the genus *Cymbella* or closely related genera (Zhang et al. 2021). * indicates the genus may be more closely related to freshwater gomphonemoid diatoms.

Genera	References
Encyonema Kützing (1833: 589)	Kützing 1833
**Reimeria* Kociolek et Stoermer (1987: 457)	Kociolek and Stoermer 1987
Pseudencyonema Krammer (1997: 156)	Krammer 1997a
Encyonopsis Krammer (1997: 156)	Krammer 1997b
Cymbellopsis Krammer (1997: 157)	Krammer 1997b
Cymbopleura Krammer (1999: 292)	Krammer 2003
Navicymbula Krammer (2003: 123)	Krammer 2003
Delicata Krammer (2003: 110; recently renamed as *Delicatophycus* M.J. Wynne 2019: 1)	Krammer 2003
Gomphocymbellopsis Krammer (2003: 127)	Krammer 2003
**Afrocymbella* Krammer (2003: 129)	Krammer 2003
Krsticiella Levkov in Levkov et al. (2007: 14–15)	Levkov et al. 2007
Oricymba Jüttner et al. (2010: 408)	Jüttner et al. 2010a
Ochigma Kulikovskiy, Lange-Bertalot et Metzeltin (2012: 214)	Kulikovskiy et al. 2012
Khursevichia Kulikovskiy, Lange-Bertalot et Metzeltin (2012: 157)	Kulikovskiy et al. 2012
Kurtkrammeria Bahls (2015: 6)	Bahls 2015
Celebesia Kapustin, Kulikovskiy et Kociolek (2017: 153)	Kapustin et al. 2017
Karthickia Kociolek, Glushchenko et Kulikovskiy (2019:606)	Glushchenko et al. 2019

(TP) were measured by a Shimadzu UV 2450 ultraviolet-visible spectrophotometer at Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, using the alkaline potassium persulfate digestion-UV spectrophotometric method (Nydahl 1978) and the ammonium molybdate spectrophotometry method (Ebina et al. 1983), respectively.

Diatom samples were kept under 4 °C in the refrigerator before laboratory treatments. In the laboratory, the diatoms were treated with HCl and H_2O_2 (Battarbee 1986). Permanent slides were made from cleaned materials and mounted in Naphrax® for observation with light microscopy (LM; Olympus, BX-51, DIC). Relative abundances of diatoms in the samples were determined with a count of 300 valves.

Cleaned materials were investigated with a Leo 1530 scanning electron microscope (SEM). Samples and slides are preserved in the Herbarium of Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, China.

Tab. 2. Physical and chemical parameters in Lake Shadecuo.

	Lake Shadecuo
Latitude (°N)	29°44′35.8″
Longitude (°E)	101°21′39.8″
Altitude (m a.s.l.)	4428
Maximum depth (m)	8
pH	7.66
Secchi disk depth (m)	4.8
Total nitrogen ($mg \cdot L^{-1}$)	0.288
Total phosphorus ($mg \cdot L^{-1}$)	0.004
Conductivity ($\mu s \cdot m^{-1}$)	33

Results

Taxonomy

Class Bacillariophyceae Haeckel 1878
Subclass Bacillariophycidae D.G. Mann in Round et al. 1990
Order Cymbellales D.G. Mann in Round et al. 1990
Family Cymbellaceae Greville 1833
Genus Cymbella C.A.Agardh 1830
Cymbella luoyulanae Y.Li sp. nov. Fig. 1 A-E; Fig. 1C is the holotype

LM (Fig. 1): Valves moderately dorsiventral. Dorsal margin strongly convex. Ventral margin concave, slightly tumid in middle. Valve ends slightly truncated and broadly rounded. Length 59-82 µm, width 16.0-18.5 µm, maximum length/width ratio ca. 4.5. Axial area moderately broad. Central area elliptical, about 1/4-1/3 of width of valve. Raphe distinctly lateral, abruptly reverse-lateral near proximal ends. Striae slightly radiate, becoming more radiate towards valve ends. 3-5 stigmata, some larger than others, occur ventrally from central nodule, distant from middle ventral striae. Striae 7-8/10 µm at centre becoming 10-12/10 µm towards apices, with 20-22 areolae in 10 µm.

In SEM external valve view (Fig. 2): Striae uniseriate, partly biseriate with elliptical and transapically oriented areolae. Areolae slit-like with apically-orientated openings in transapical striae (Fig. 2 A-F). Stigmata rounded, separated from the areolae at central area on ventral side. Small round depressions may occur on the dorsal side of the central area (Fig. 2 C, D). Raphe centrally located on the valve face in a

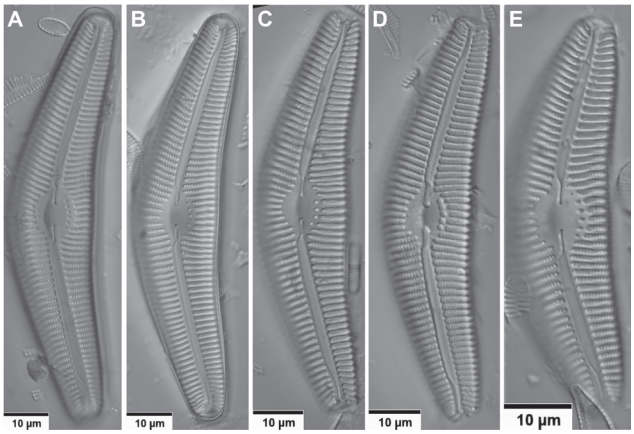

Fig. 1. Light microscopy micrographs of the type population of *Cymbella luoyulanae* sp. nov. in Lake Shadecuo, China. A-E – valve views, showing the valve variability of the holotype population.

narrow hyaline axial area with straight to slightly dorsally arched proximal ends (Fig. 2 A, B). External distal raphe ends deflected dorsally (Fig. 2 E, F); external proximal raphe ends dilated (Fig. 2 C, D). The apical pore fields present and comprised by a group of porelli, almost entirely on the valve mantle (Fig. 2 E, F).

In SEM internal valve view (Fig. 3): Striae with internal areolae openings lack any occlusions (Fig. 3 A, B). The central area distinct, projecting internally, and lacking an intermissio. Stigma opening round, with a slight expanded depression evident on the central nodule. Stigmata lack any ingrowths or occlusion (Fig. 3 C, D). Distal raphe endings bent slightly towards the dorsal margin, terminating in helictoglossae. (Fig. 3 E, F).

Type: – CHINA. Sichuan Province: Kangding City, Lake Shadecuo, 29°44′35.8″ N, 101°21′39.8″ E, elevation 4428 m a.s.l., samples collected by Dr. Yulan, Luo, 14th October 2017. Holotype Shadecuo 1-1 in Coll. Li Yanling, Yunnan University., Kunming, China. Fig. 1 is of the holotype.

Etymology: –The specific epithet '*luoyulanae*' refers to the collector of the sample on which these observations are based.

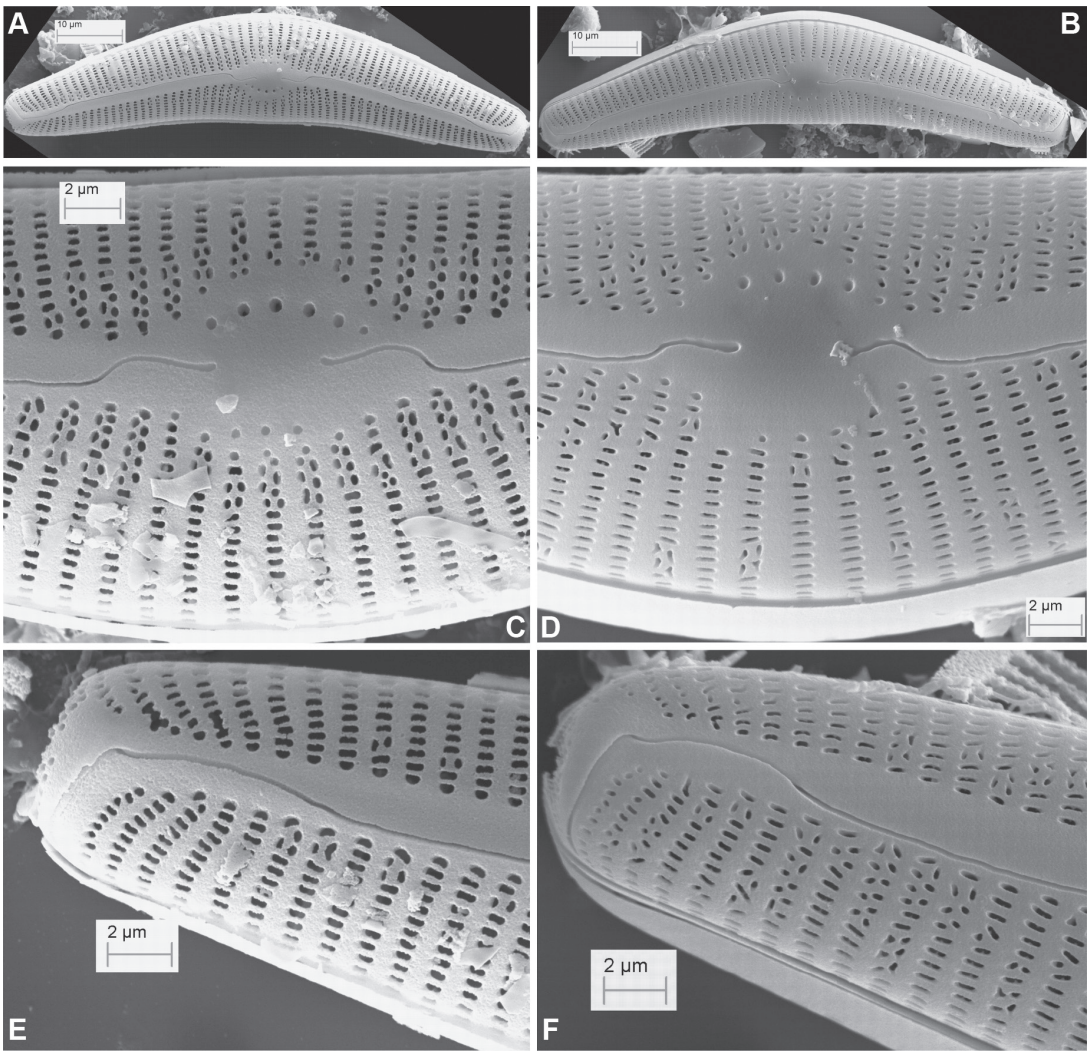

Fig. 2. External valve view of *Cymbella luoyulanae* sp. nov. by scanning electron microscope. A, B – external view of an entire valve. C, D – external view of valve center, the proximal raphe endings and the very large central area with 4-5 stigmata. E, F – valve apices, striae with slit-like areolae, some of them biseriate, and pore field separated by the distal raphe fissures.

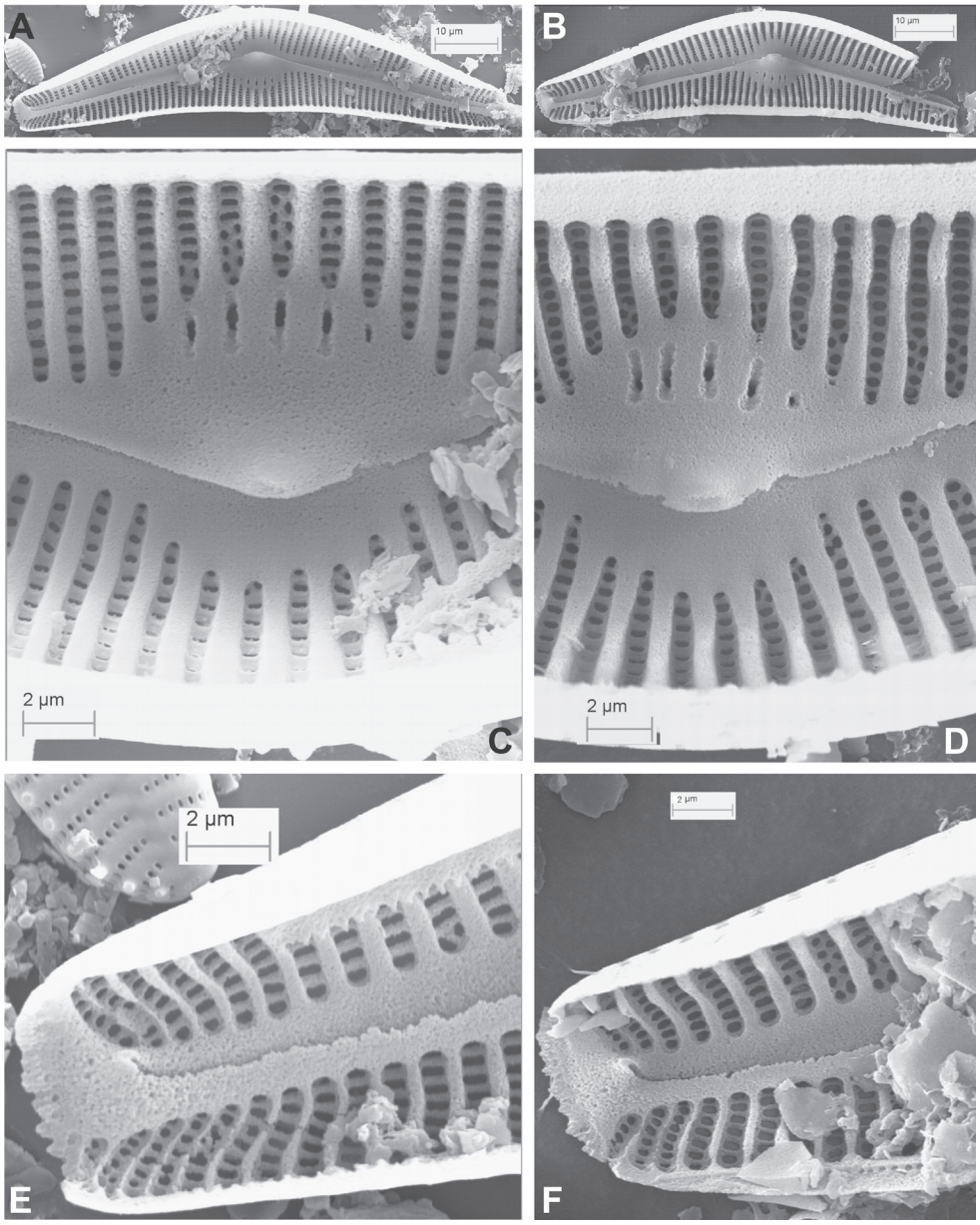

Fig. 3. Internal valve views of *Cymbella luoyulanae* sp. nov. by scanning electron microscope. A, B – internal view of entire valve. C, D – showing the valve centre and four big and one small stigmata as elongated furrows connected to the striae. E, F – internal view of valve apex with prominent helictoglossa deflecting to the dorsal side.

Ecology

Cymbella luoyulanae has been observed in the surface sediment sample from Lake Shadecuo. In Lake Shadecuo, this species was associated with *Cyclotella ocellata* Pantocsek (1902: 134) (20%), *Achnanthidium minutissimum* (Kützing) Czarnecki (Czarnecki 1994: 157) (15%), *C. comensis* Grunow in Van Heurck (1882, pl. 93, Fig. 3 E, F) (14%), *Pseudostaurosira pseudoconstruens* (Marciniak) Williames et Round (1987: 275) (15%), and *Staurosirella pinnata* (Ehrenberg) D.M. Williames et Round (1987: 274) (5%).

Discussion

The features found in *Cymbella luoyulanae* are compared to all other known species of the genus in Tab. 3. The taxon most similar to *C. luoyulanae* is *C. heihainensis*. The valves of this species are wider, have a larger central area, drop-like proximal raphe ends, and a higher number of areolae in 10 µm, distinguishing it from *C. luoyulanae*. *Cymbella modicepunctata* has larger valves with a more distinct central area, and fewer striae in 10 µm, distinguishing it from *C. luoyulanae*. *Cymbella luoyulanae* has stigmata that may vary in size along the ventral margin, while *C. modicepunctata* has uniformly-sized stigmata on the ventral side. The same stigmata feature of *C. luoyulanae* is also found in *C. schimanskii* Krammer and its variety var. *excelsa* (Meister) Krammer. In SEM view, *C. schimanskii* has Y-shaped areolae openings in the middle of the valve, which is different from the biseriate of *C. luoyulanae,* and they differ from *C. luoyulanae* by having larger valves, a smaller central area,

Tab. 3. Morphological characteristics of *Cymbella luoyulanae* sp. nov. and *Cymbella* species sharing similar morphological features.

Species /Feature	*C. luoyulanae*	*C. heihainensis* Li et Gong	*C. modicepunctata* Krammer	*C. asiatica* Metzeltin, Lange-Bertalot et Li, Y.	*C. schimanskii* Krammer	*C. arctissima* Metzeltin
Reference	this study	Hu et al. (2013)	Krammer (2002)	Metzeltin et al. (2009)	Krammer (2002)	Krammer (2002)
Valve length, µm	59-94	82-100	102-140	50-105	100-200	98-105
Valve width, µm	16.0-19.5	19.0-22.5	21-22	14-18	29-36	17-18
Length/ width ratio	4.8	max 4.7	max 6.4	max 5.8	max 5	max 5.8
Striae in 10 µm	7-10	7-10	5.5-6	6-7	7-12	7-10
Areolae in 10 mm	20-22	14-16	14-16	18-22	10-16	18-21
Areolae	Slit-like, biseriate	Slit-like, Y-, X- shaped	Lineolate	not known	Slit-like, Y-shaped	Slit-like
Stigmata	ventral side: 3-5 big and some smaller, dorsal side: 0-3	ventral side: 7-10 big and some smaller, dorsal side: 0–5	ventral side: 7-10	ventral side: 4-6	ventral side: 6-10 big and some smaller, dorsal side: sometimes some	ventral side: 6-8
Central area	1/2 width	1/2 width	2/3-3/4 width	1/2 width	1/3 width	2/3 width
Central pores of raphe	expanded drop-like, slightly ventrally deflected	expanded drop-like, slightly ventrally deflected	distinct, very slightly reverse-lateral	distinct, abruptly reverse-lateral	small, slightly reverse-lateral	small, dorsally deflected

and in the shape of the central pores (rounded vs expanded as in *C. luoyulanae*). *Cymbella luoyulanae* can be distinguished from *C. asiatica* by valve size and striae density. *Cymbella asiatica* varies between 50 and 105 µm in length and 14-18 µm in breadth, has no stigmata-like depressions on the dorsal side, and only 6-7 striae in 10 µm. *Cymbella luoyulanae* is also similar to *C. arctissima* Metzeltin, which can be distinguished from our new species which has dorsally deflected proximal raphe endings, is shorter (98-105 µm vs. 59-94 µm), and has a broader central area (2/3 width vs. 1/2 width).

Another member of the genus *Cymbella*, *C. distalebiseriata* Bing Liu et D.M. Williams in Liu et al. (2018: 41), was described from Hunan Province, China. *Cymbella distalbiseriata* shares the feature of having striae with uniseriate and partially biseriate striae. The only other species of *Cymbella* exhibiting (a few) biseriate striae include *C. yakii* Jüttner et Van de Vijver in Jüttner et al. (2010b), *C. duplopunctata* Krammer 2002 and *C. buechleri* Krammer 2002 (both described from a fossil deposit in western North America). Three of the five species exhibiting biseriate striae are found in Asia, and two have been described from China. The other two taxa with biseriate striae are known from a fossil deposit in western North America (apparently of Miocene age, see Krammer 2002, p. 109). These are distinct from one another in size, shape and other morphological features (Krammer 2002, Jüttner et al. 2010b, Liu et al. 2018). It is unclear if the possession of biseriate striae is a homologous feature for this group or not; formal phylogenetic analysis is required to understand character evolution in this regard. However, if the taxa with biseriate striae are indeed closely related among themselves and their distribution remains between Asia and western North America, it supports the notion, noted by Ehrenberg over 180 years ago (Ehrenberg 1849) and supported by additional diatom examples (Kociolek and Stoermer 1989, Kociolek et al. 2013, 2015, Genkal and Kulikovskiy 2016, Kociolek 2019) as well as some genera of higher plants (Xiang and Soltis 2001, Nie et al. 2006, Kadereit and Baldwin 2012), of there being a close relationship between the floras of these two areas.

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