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New diatom species *Diploneis zhui sp. nov.* from Jiuzhaigou National Nature Reserve, China

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Abstract

Diploneis zhui sp. nov. was observed in epilitic diatom communities from Jiuzhaigou National Nature Reserve. The species is described herein based on light microscopy (LM) and scanning electron microscopy (SEM) and ecological information of the type locality is provided. The species is characterized by linear to linear-elliptic valves with broadly rounded ends, 18–39 µm long, and 9.5–11.0 µm wide and partly biseriate striae, 16–18 in 10 µm. The species is compared with most similar taxa from other regions. The discovery of a new diatom species confirms the importance of Jiuzhaigou not only in terms of species richness and endemicity, but also for the biodiversity of freshwater karst ecosystems.

Key words: biodiversity, diatom, *Diploneis*, freshwater, taxonomy, Jiuzhaigou National Nature Reserve

Introduction

The diatom research in China in the last two decades has significantly amplified and resulted in the description of a large number of species and even new genera (Kociolek *et al.* 2015). New species has been described from almost all groups of diatoms: monoraphid (e.g. Luo *et al.* 2021), biraphid (e.g. Lowe *et al.* 2017), gomphonemoid (e.g. Liu *et al.* 2013), nitzschioid (e.g. You *et al.* 2015). Also, several new species have been described from Jiuzhai Valley (Xu *et al*. 2017, Yu *et al*. 2019a, b). However, still many species are unidentified and need proper taxonomic analyses (Plenković-Moraj *et al.* 2023).

The genus *Diploneis* (Ehrenberg) Cleve (1894: 76) is one of the largest diatom genera and comprise species living both in marine and freshwater habitats (Round *et al.* 1990). It was considered mainly as marine and brackish water genus (Hustedt 1937, Pennesi *et al.* 2017), but recent studies of the ancient lakes revealed large species diversity in *Diploneis* (Jovanovska *et al.* 2013a, 2015, 2023, Kulikovskiy *et al.* 2015). In addition to ancient lakes, also *Diploneis* species can be found in various freshwater, even subaerial habitats (Lange-Bertalot & Fuhrman 2016, Lange-Bertalot *et al.* 2020, Jovanovska & Levkov 2020). Besides its large species diversity, *Diploneis* is characterized by large morphological and ultrastructural variability. In the past, the genus has been divided into several subgroups: group I freshwater and groups II to VII marine species (Hustedt 1931–1959). More recently, based on areola structure, the genus was subdivided into three subgenera *Diploneis*, *Cribradiploneis*, and *Volaediploneis* (Lange-Bertalot *et al.* 2020). Most of the freshwater species belong to the subgenus *Cribradiploneis* (Lange-Bertalot *et al.* 2020; Jovanovska & Levkov 2020).

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Most of the *Diploneis* species have predominantly elliptical valve outline (or linear-elliptic) or more or less constricted (Cleve 1894, 1895, Hustedt 1931–1959). The most prominent feature of all *Diploneis* species is the presence of a longitudinal canal system running alongside the raphe. The raphe complex is consists of raphe branches lying in a narrow and shallow internal sternum which is bordered by a distinct canal. In the subgenus *Cribradiploneis* areolae and canal pores are externally occluded by cribra while internally areolae are occluded by a thin silica layer. The valve wall is composed of two layers and within these layers are the chambers (Droop 1998) or loculate areolae (Round *et al.* 1990). In the subgenus *Volaediploneis*, alveoli are restricted to valve's margins and mantles and not subdivided into separate areolae and occluded usually by a single vola. In the subgenus *Diploneis* areolae externally are covered by a continuous narrow and slits covering all marginal and submarginal parts of the valve face and mantle (Lange-Bertalot *et al.* 2020). One of the most important distinguishing features of the subgenus *Cribradiploneis* is the morphology of the areolae. Based on recent observations with scanning electron microscope (SEM), stria might be uni- to biseriate (Lange-Bertalot *et al*. 2020; Jovanovska & Levkov 2020), or partly biseriate towards valve margin. This feature is considered as a stabile character and used for separation of morphologically similar taxa (Jovanovska *et al.* 2013b).

The genus *Diploneis* is quite diverse in freshwater habitats in China (Li & Qi 2010) with 28 recorded taxa (22 species and six infraspecific taxa). According to Kociolek *et al*. (2020) nine *Diploneis* taxa have been described from China (Mereschkowsky 1906, Skvortzov 1929a, b, 1938, Huang *et al.* 1998, Kulikovskiy *et al*. 2012). Recently two new species have been described one freshwater *D. ellipticasinensis* Lange-Bertalot & Fuhrmann (in Lange-Bertalot *et al.* 2020: 42, figs 181: 1–10) and one marine *D. guangdongensis* W.W.Wu, Y.H.Gao & C.P.Chen (in Wu *et al.* 2022: 234, figs 1–32) from China. During the study of diatoms from Jiuzhaigou National Park, one new *Diploneis* species was observed and here we are providing its formal description based on light (LM) and scanning electron microscopy (SEM).

Material and methods

Study area

Jiuzhai Valley was formed by the accumulation of snow during the Quaternary period, which led to the development of Jiuzhai Valley glaciers (Liu *et al*. 2007). The Jiuzhaigou River, the largest river in the reserve, rises on Ga'erna Peak in the Minshan Mountain Range. It is a tributary of the headwaters of the Baishui River, the right tributary of the Bailong River west of the source of the Jialing River in the Yangtze River system, and covers an area of 642.97 km^2.The lakes of Jiuzhaigou are close to each other and are called group lakes. The group lakes are mostly connected with waterfalls, like the Shuzheng and Nuorilang sights.

Environmental parameters

Water temperature, pH, and conductivity were analysed at each site using a portable conductivity metre (Hanna Instrument Company, USA). Total nitrogen was analysed using a TOC/TNb analyser (Analytic Jena Company, Germany) and total phosphorus was analysed using the ammonium molybdate spectrophotometry method (Ministry of Environmental Protection of the People's Republic of China 2002).

Sampling and preparation

The epilithic diatom samples were collected from six sampling sites (Fig. 1). The samples were scraped off and removed with a toothbrush from five randomly collected natural rocks (average-size rock of about 15 to 30 cm). Prior to sampling, the stones were gently shaken in water to remove any loosely attached sediments and non-epilithic diatoms. Diatom suspensions were pooled to form a single sample that was then put in a labelled plastic container (120 mL). The new species was found in the coastal area of Mirror Lake—Jìng Hǎi, which covers an area of 190,000 m^2 at an elevation of 2,390 m. The average depth the Lake is 11 m (deepest 24.3 m). Surrounded by forests, Mirror Lake is a narrow and long lake with a length of 1,000 m.

Diatom samples for LM and SEM were fixed in the field with a 2% final concentration formaldehyde solution. In the laboratory, they were treated with $KMnO_4$ and 37% HCl and left overnight to remove carbonates. To oxidize the organic matter, 37% HCl was added and the samples were boiled at 80 °C for 45 minutes. The samples were rinsed

five times with distilled water and centrifuged. The diatom slides were mounted with Naphrax. LM observations were made with Leicka DMLB (at Chengdu Institute of Biology, Chinese Academy of Science) and Nikon Eclipse 80i microscope, under oil immersion at 1500× magnification. Diatom images were captured using a Nikon Coolpix P6000 camera. For SEM, aliquots of diatom sample extracts were dried on a coverslip attached to stubs with carbon tape and coated with a thin gold-palladium layer approximately 20 nm for 120 seconds with a sputtering current of 20 to 25 mA (Polaron SC7640 Sputter, Quorum Technologies, Ashford, UK). Scanning electron microscopy was performed at 5 kV and 5 mm working distance using a Zeiss Gemini Ultra plus SEM (Cambridge Instruments Ltd, Cambridge, UK).

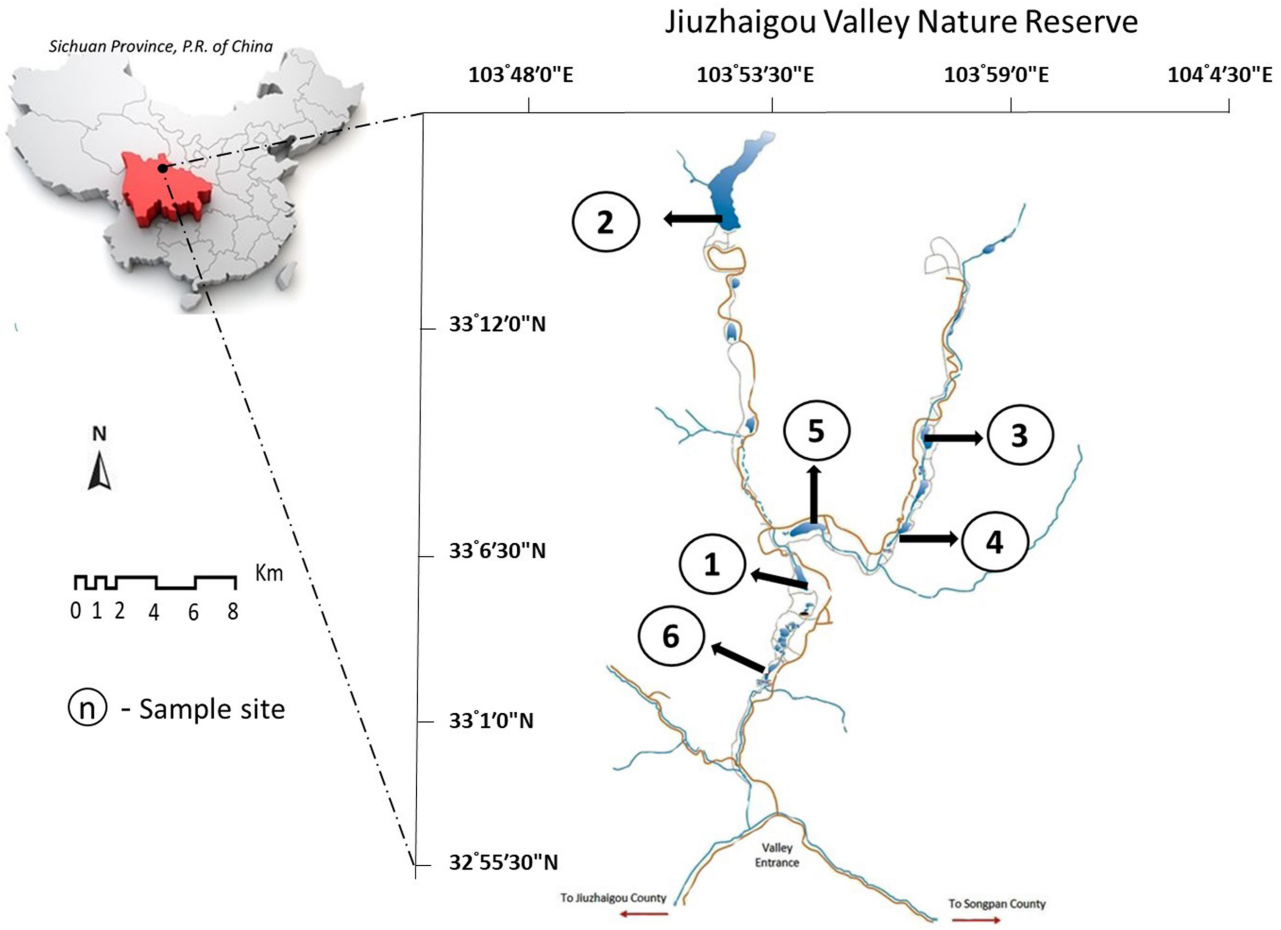


FIGURE 1. Map of Jiuzhaigou National Nature Reserve and location of the study sites (1= Rhinoceros Lake, 2=Long Lake, 3= Arrow Bamboo Lake, 4= Mirror Lake, 5= The Peacock Riverbed, and 6= Reed Lake).

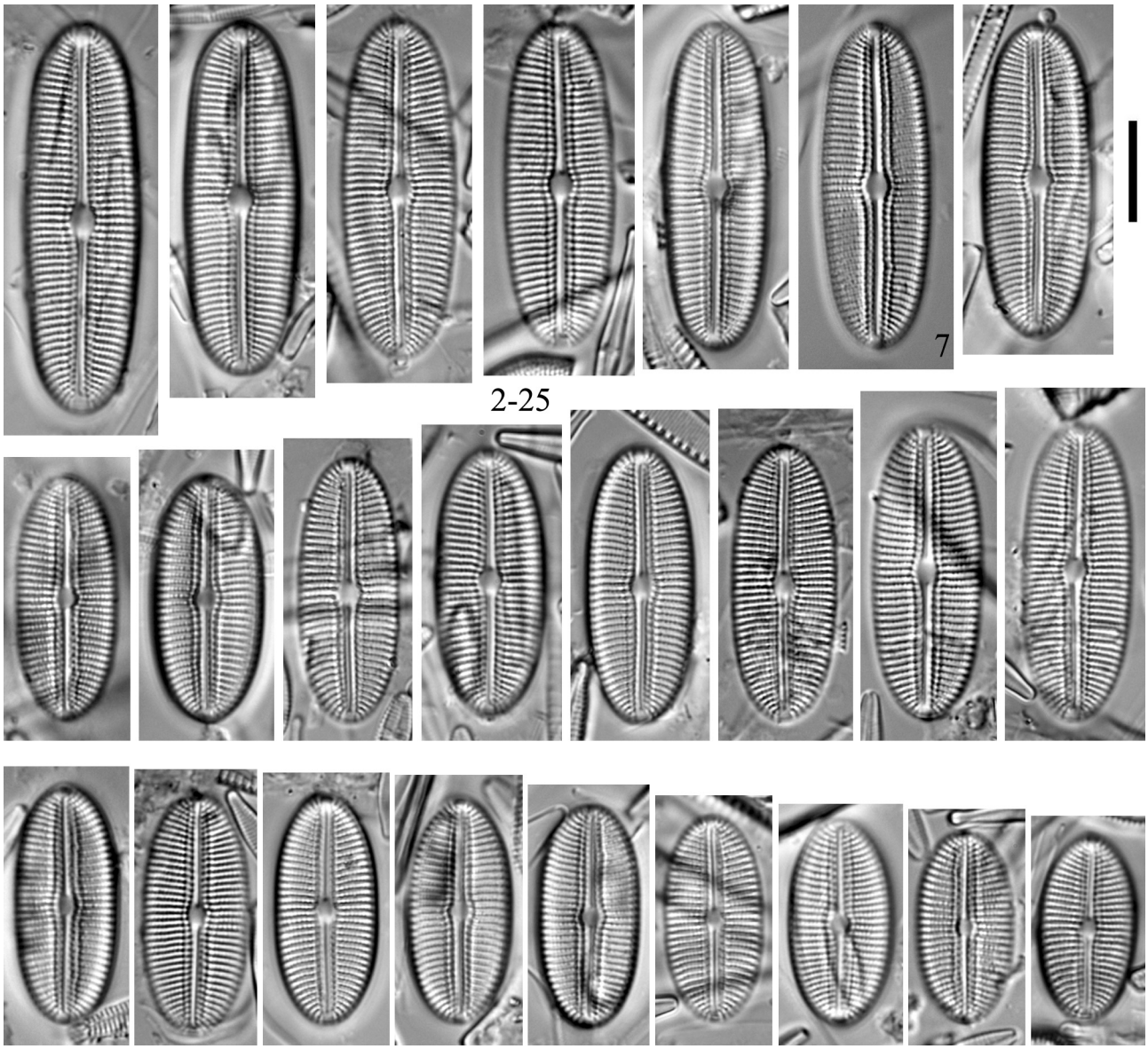
Results

Division Bacillariophyta Haeckel 1878: 95
Class Bacillariophyceae Haeckel 1878 emend D.G. Mann in Round *et al*. 1990: 651
Subclass Bacillariophycidae D.G. Mann in Round *et al*. 1990: 651
Order Naviculales Bessey 1907: 284
Suborder Diploneidineae D.G.Mann in Round *et al*. 1990: 656
Family Diploneidaceae D.G.Mann in Round *et al*. 1990: 660
Genus *Diploneis* Ehrenberg ex Cleve 1894: 76

Diploneis zhui Levkov, Plenković-Moraj & Sun *sp. nov.* (LM 2–25, SEM 26–35)

Description: LM (Figs 2–25): Valves linear to linear-elliptic with broadly rounded ends. Length 18–39 µm, breadth 9.5–11.0 µm. Axial area narrow, linear, central area small, longitudinally elliptical to round. Raphe filiform, with slightly expanded central pores. Longitudinal canals present on both sides of raphe, linear, opened with two small

areolae. Striae almost parallel at mid-valve to strongly radiate towards valve apices, 16–18 in 10 μm. Areolae fine, visible with LM, ca. 25 in 10 μm.

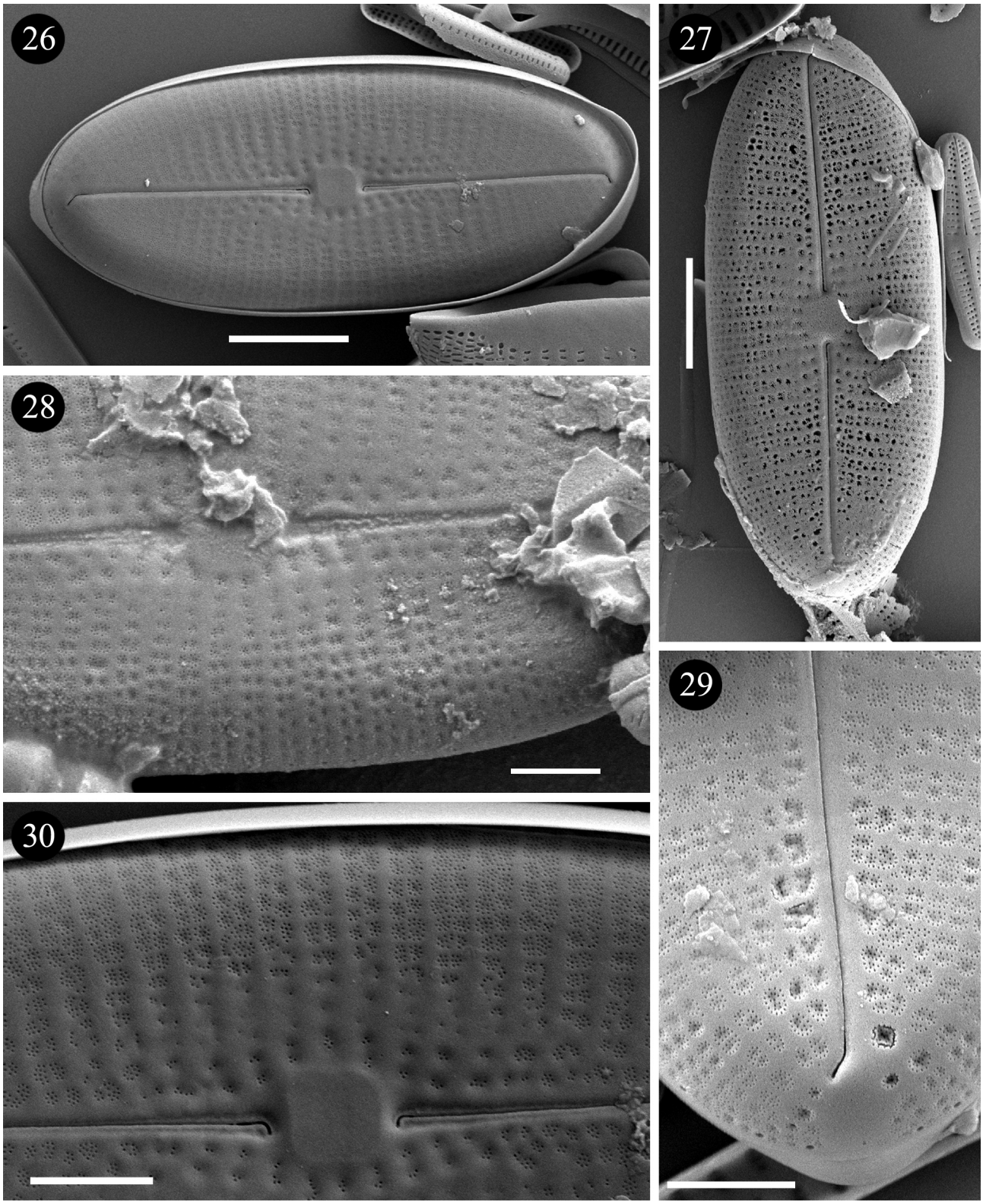


FIGURES 2–25. *Diploneis zhui sp. nov.* Light microscopy. Valve size diminution series. Scale bar = 10 μm.

SEM (Figs 26–35): Valve face is flat and transition to the valve mantle is gradual (Figs 26, 27). Central area is slightly raised (Figs 28, 30). Raphe is linear with unilaterally deflected proximal raphe ends (Figs 28, 30). Distal raphe ends short and unilaterally bent, not passing on valve mantle (Figs 26, 29). Raphe is not surrounded with raphe ribs (Fig. 28, 30). Externally, axial area very narrow, linear bordered by longitudinal canal (Figs 26, 27). Longitudinal canal is linear with two rows of areolae occluded by cribra (Figs 29, 30). Striae uniseriate near raphe becoming biseriate towards valve margin (Figs 26, 30). Biseriate pattern of striae at marginal part visible internally in corroded specimens (Figs 33, 34). Internally, raphe is linear with simple proximal and distal raphe ends (Figs 33–35), placed in depression formed by longitudinal canal (Figs 31–35). Internally, axial area broad covered by silica plate that encloses longitudinal canal (Figs 31–35). Striae covered with thin silica layer (Fig. 35).

Type:—CHINA: Jiuzhaigou National Nature Reserve, Mirror Lake, rocks from 0.5 to 1 m deep on the east coast, coordinates 103°53′57.80” E, 33°9′34.90” N (Accession No. 42); Leg. N. Moraj & W. Xiao; collection date: 29.04.2015, Slide HRNDC! 48J2016 (holotype), Fig. 7 represents the holotype specimen; slide MKNDC! 14382/B (isotype)

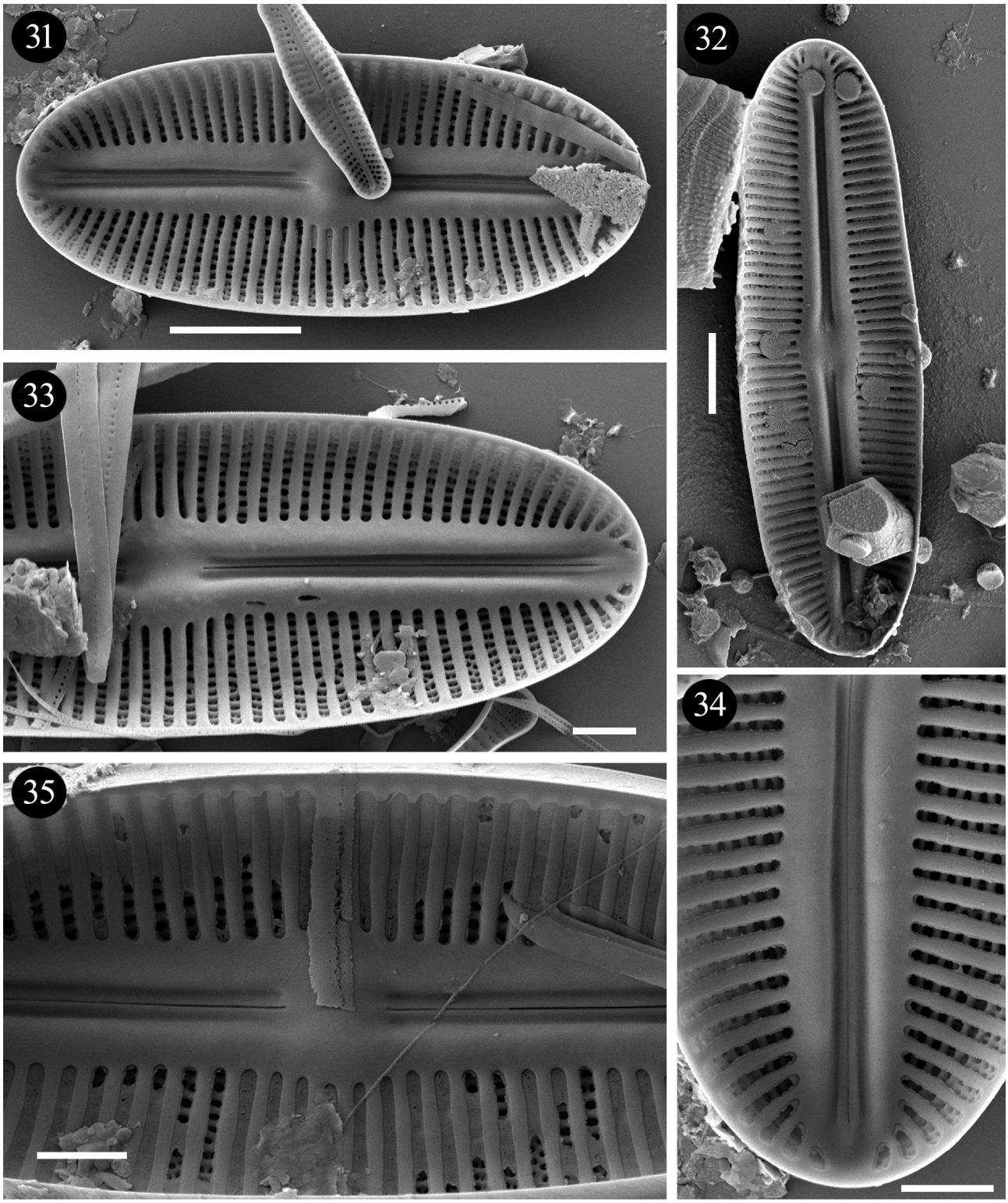
Etymology:—The species is named in honor of Zhongfu Zhu, a researcher, scientist and irreplaceable colleague of Jiuzhaigou Administrative Office, Sichuan, China, who sadly passed away.



FIGURES 26–30. *Diploneis zhui sp. nov.* Scanning electron microscopy. Figs 26, 27. External view of the entire valve. Figs 28, 30. External view of the middle of the valve. Fig. 29. Detailed external view of the valve end. Scale bar = 5 μm (26, 27), 2 μm (28–30).

Ecology:—*Diploneis zhui* occurred at 5,0 % relative abundance (total counted, 400 valves) in samples together with species that accounted for more than 5 %: *Navicula cryptotenella* Lange-Bertalot (in Krammer & Lange-Bertalot 1985: 62) (18.5%), *Cymbella hantzschiana* Krammer (2002: 47) (16.0%), *Tryblionella angustata* W. Smith (1853: 36) (15.0%), *Nitzschia fossilis* (Grunow) Grunow (in Van Heurck 1881, fig. 68: 24) (10.0%), *Gomphonema acuminatum* Ehrenberg (1832: 88) (6.0%) and *Sellaphora* Mereschkowsky (1902: 186) sp. (5.5%). Mean values of environmental

parameters were: pH = 8.22, water temperature = 9.72 °C, conductivity = 351 μs/cm, Total N = 417 μg/L and Total P = 8 μg/L.



FIGURES 31–35. *Diploneis zhui sp. nov.* Scanning electron microscopy. Figs 31, 32. Internal view of the entire valve. Figs 33. Internal view of half of the valve. Fig. 34. Detailed internal view of the valve end. Fig. 35. Detailed internal view of the valve centre. Scale bar = 5 μm (31, 32), 2 μm (33–35).

Distribution:—So far, the new species was observed only at the type locality Mirror Lake in Jiuzhai Valley.

TABLE 1. Comparison of numerical and morphological features of Diploneis species related to *D. zhui*.

Species	Valve outline	Length (µm)	Width (µm)	Stria density (in 10 µm)	Areola density (in 10 µm)	Central area	Row of areolae on longitudinal canal	Stria morphology	Reference
D. boldtiana	Elliptic to linear-elliptic	20–30	11.0–12.0	13–14	35–38	Small, longitudinally elliptical	two	biseriate to multuseriate towards margin	Idei & Kobayashi 1989
D. potapovae	Linear-elliptic to elliptic	14–30	8.0–12.0	15–16	20–21	Small, longitudinally elliptical	single	uniseriate on valve face	Lange-Bertalot *et al.* 2020
D. minerovicіae	Oblong-elliptic to linear-elliptic	20–25	8.0–9.0	14	30–35	Small, longitudinally elliptical	single	uniseriate on valve face	Lange-Bertalot *et al.* 2020
D. navahoarum	Elliptic to elongate-elliptic	7.5–21	5.5–9.0	13–15	<30	Almost absent	single	biseriate	Lange-Bertalot *et al.* 2020
Diploneis praetermissa	Elliptic to linear-elliptic	8–30	5.5–8.0	17–19	32–35	Almost absent	single	biseriate	Lange-Bertalot & Fuhrmann 2016
D. modica	Linear-elliptic	10.5–17.5	5.0–6.8	18–24	-	Almost absent	two	biseriate	Jovanovska & Levkov 2020
D. ellipticasinensis	Elliptic to lanceolate-elliptic	25–52	14–24	10–11	12–15	Longitudinally elliptical	single	uniseriate	Lange-Bertalot *et al.* 2020
D. zhui sp. nov.	linear to linear-elliptic	18–39	9.5–11.0	16–18	25	Almost absent	two	partly biseriate	This study

Discussion

The most similar species to *D. zhui* is *D. boldtiana* Cleve (1891: 43, fig. 2: 12). Detailed revision of the species has been done by Idei & Kobayashi (1989) and Lange-Bertalot *et al*. (2020). Idei & Kobayashi (1989, fig. 2) were able to find a single specimen in the type material and several specimens in the recent materials from Finland. The valves are linear-elliptic, 20–30 µm long, 11–12 µm wide, with 13–14 striae in 10 µm. SEM images observations reveal that striae are biseriate (internally and externally) that might become tri to multiseriate towards valve margin (Idei & Kobayashi 1989, fig. 12). Differences between *D. zhui* and *D. boldtiana* might be seen in the valve shape (*D. boldtiana* has more elliptical outline), shape and size of the central area (almost absent in *D. boldtiana*) and especially in the stria morphology: striae in *D. boldtiana* are biseriate becoming triseriate towards margin and areolae cannot be resolved with LM, opposite to uniseriate striae in the mid-valve (see Figs 33, 34) and areolae visible with LM in *D. zhui* (Figs 2–25). Additionally, differences can be noticed in the valve width (11.0–12.0 µm in *D. boldtiana* vs. 9.5–11.0 µm in *D. zhui*) and stria density (13–14 in 10 µm *D. boldtiana* vs. 16–18 in 10 µm in *D. zhui*). According to Lange-Bertalot (2020: 25), *D. boldtiana* is very rare species that occur only in Finland and Alaska and other records might belong to other species. *Diploneis boldtiana* was also recorded in China (Li & Qi 2010, fig. 36: 4) and the illustrated valve appear very similar to *D. zhui. Diploneis boldtiana* was recorded in Lake Hövsgöl, Mongolia (Jovanovska *et al.* 2015, figs 220–235) and it closely resembles populations from Skandinavia.

Three other species have comparable characteristics with *D. zhui*. *Diploneis potapovae* Lange-Bertalot, Fuhrmann & Cantonati (in Lange-Bertalot *et al.* 2020: 108, figs 88: 1–35, 89: 1–4) has a similar valve outline (linear-elliptical) and size (length 14–30 µm, width 8–12 µm), but differences can be noticed in the stria morphology (uniseriate on the valve face coarsely punctate in *D. potapovae* vs. partly biseriate in *D. zhui*), stria density (15–16 in 10 µm) and number of areolae on longitudinal canal (single row in *D. potapovae* vs. double row in *D. zhui*). *Diploneis minerovicia*e Lange-Bertalot, Fuhrmann & Werum (2020: 80, figs 90: 1–15) is characterized with oblong-elliptic to linear-elliptical valves with cuneately rounded ends and uniseriate striae on the valve face. Differences between *D. zhui* and *D. minerovicia*e can be noticed in valve shape and stria morphology and density (uniseriate and coarsely punctate 14 in 10 µm in *D. minerovicia*e). *Diploneis navahoarum* Lange-Bertalot, Fuhrmann & Werum (2020: 88, figs 86: 1–26) has biseriate striae that near the valve margin become triseriate. However, *D. zhui* can be easily distinguished from *D. navahoarum* by the valve shape (elliptical valves with rounded ends in *D. navahoarum*) and higher stria density (13–15 in 10 µm in *D. navahoarum*). *Diploneis praetermissa* Lange-Bertalot & Fuhrmann (2016: 169. figs 34–40, 134ab–138) has comparable valve shape as *D. zhui*, but it has smaller valves (5.5–7.8 µm), longitudinal canal with a single row or areolae and the distal raphe ends are long and passing on the valve mantle. Another species with biseriate striae is *D. modica* Hustedt (1945: 912, figs 42: 23, 24) but it can be easily differentiated from *D. zhui* but its narrower valves (5.0–6.8 µm) and higher stria density (18–24 in 10 µm) (Jovanovska & Levkov 2020). *Diploneis ellipticasinensis* Lange-Bertalot & Fuhrmann (in Lange-Bertalot *et al.* 2020: 42, figs 181: 1–10) was recently described from Lake Dali Er Hai, China. This species belongs to the group of *D. ellipica* Kützing (Cleve 1894: 92) and is characterized by elliptical to broadly lanceolate-elliptical, obtusely rounded at the ends, 25–52 µm long and 14–24 µm wide, and thus easily differentiated from *D. zhui*.

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