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Sellaphora jiuzhaienis sp. nov. (Bacillariophyceae)—A new diatom species from Jiuzhaigou National Nature Reserve, China

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Abstract

A new species *Sellaphora jiuzhaienis sp. nov*. was found in epilithic samples from Jiuzhaigou National Nature Reserve, China. The species is described herein as new to science based on light microscopy (LM) and scanning electron microscopy (SEM), and ecological information is provided. The species has linear to linear-elliptic valves, 17.5–33.5 µm long and 7.0–8.5 µm wide and very broad conopeum that occupies almost half of the valve width. 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 in the national protected areas of China.

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

Introduction

Diatom research in China has increased significantly in recent decades, leading to the description of many new species and genera (Kociolek 2019). One of the genera commonly observed in China is *Sellaphora* Mereschkowsky (1902: 186), and recently several new species have been described from different parts of the country (e.g., Li *et al.* 2010a, b, Liu *et al.* 2020). The genus *Sellaphora* in its original concept, included two taxa and later the concept was updated by Mann (1989) and Round *et al.* (1990). More recently, the concept of the genus was expanded by Wetzel *et al.* (2015) to include many small-sized species, mainly from the genera *Navicula* Bory (1822: 128) and *Eolimna* Lange-Bertalot & Schiller (in Schiller & Lange-Bertalot 1997: 166). Several molecular studies show that *Sellaphora* is a monophyletic genus (Evans *et al.* 2008, Kociolek *et al.* 2013), although it is characterized by high morphological and genetic diversity (Mann *et al.* 2004, 2008, Evans *et al.* 2008, Mann & Poulíčková 2019). Liu *et al.* (2020) proposed a subdivision of the genus into four groups (A-D) based on morphological characters. The differentiation of the groups is mainly based on the presence of the conopeum, the axial pit and the morphology of the stria. The group A comprises species with a conopeum: group B includes species with an axial trough beside the axial area but without a conopeum, while in the group C are placed species without a conopeum and axial groove but have apical pits. The group D is the smallest and includes species with biseriate striae and without a conopeum, axial groove or apical pits (Liu *et al.* 2020).

Most species of the genus *Sellaphora* inhabit freshwater habitats. They occur in a wide range of habitats, from oligotrophic lakes and streams (Genkal & Yarushina 2018) to subaerial/terrestrial environments (Wetzel *et al.* 2017),

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eutrophic ponds (Mann *et al.* 2004, Kochoska *et al.* 2021) and polluted rivers (Lange-Bertalot *et al.* 2017). The genus *Sellaphora* was observed in all continents and high diversity was observed in Asia (e.g. Metzeltin *et al.* 2009, Kulikovskiy *et al.* 2012). According to Li & Qi (2018), the genus *Sellaphora* in China is represented with 22 species and six varieties. Additionally, several new *Sellaphora* species has been described from various parts of the country (*e.g.,* Li *et al*. 2010a, 2010b, You *et al*. 2017, Liu *et al*. 2020, Ni *et al*. 2022). Recent observations of algae in Jiuzhaigou National Nature Reserve, reveal existence of 419 algal taxa (including varieties) belonging to seven phyla, 38 families, and 100 genera (Liu *et al.* 2007). Diatoms, among all algal groups have the largest diversity with 192 taxa. As a part of this work in Jiuzhaigou National Natural Reserve, a new *Sellaphora* species was observed, which we formally describe here using light (LM) and scanning electron microscopy (SEM).

Material and methods

Jiuzhaigou National Nature Reserve was declared a UNESCO World Heritage Site in 1992 and subsequently joined the Man and Biosphere Conservation Network in 1997. The park stretches over 72,000 hectares and located at elevations ranging from 1.990 m to 4.764 m above sea level (Liu *et al.* 2007). According to Liu *et al.* (2007) all the scenic spots of Jiuzhai Valley were formed by the accumulation of snow during the Quaternary period, which led to the development of Jiuzhai Valley glaciers. The lakes in 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.The present investigation was conducted in Jiuzhaigou National Nature Reserve, covering its fabled blue and green lakes, spectacular waterfalls, narrow conic karst landforms.

The newly discovered species was observed in the coastal region of Mirror Lake - Jìng Hǎi, spanning 190,000 m² and situated at an elevation of 2,382 m above sea level. The lake's mean depth is approximately 11 m, with its deepest point reaching 24.3 m. Encircled by dense forests, Mirror Lake exhibits a slender and elongated shape, extending up to 1,000 m in length. Epilithic diatom samples were gathered from six different locations, as shown in Fig. 1. These specimens were carefully removed from five randomly selected natural stones (averaging 15 to 30 cm in size) using a toothbrush. Before the collection process, each rock was lightly agitated in water to dislodge any loosely adhered sediments and non-epilithic diatoms. All the diatom suspensions were combined to make a single representative sample, which was then stored in a clearly marked 120 mL plastic container.

Water temperature, pH, and conductivity were analysed on site using a portable conductivity metre (Hanna Instrument Company, USA). Total nitrogen was analysed using a TOC/TNb multi N/C 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). The annual environmental parameters (April to May, from 2013 to 2016), were obtained from the Jiuzhaigou National Nature Reserve Management Bureau as part of the water quality monitoring process.

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 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 then centrifuged. The diatom slides were mounted with Naphrax. LM observations were made with Nikon Eclipse 80i microscope (Nikon Corporation, Japan) under oil immersion at 1000× 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). All diatom samples and permanent slides are housed at Croatian National Diatom Collection (University of Zagreb Faculty of Science - HRNDC) and North Macedonia (Macedonian National Diatom Collection MKNDC, at the Institute of Biology, Faculty of Natural Sciences, Ss Cyril and Methodius University).

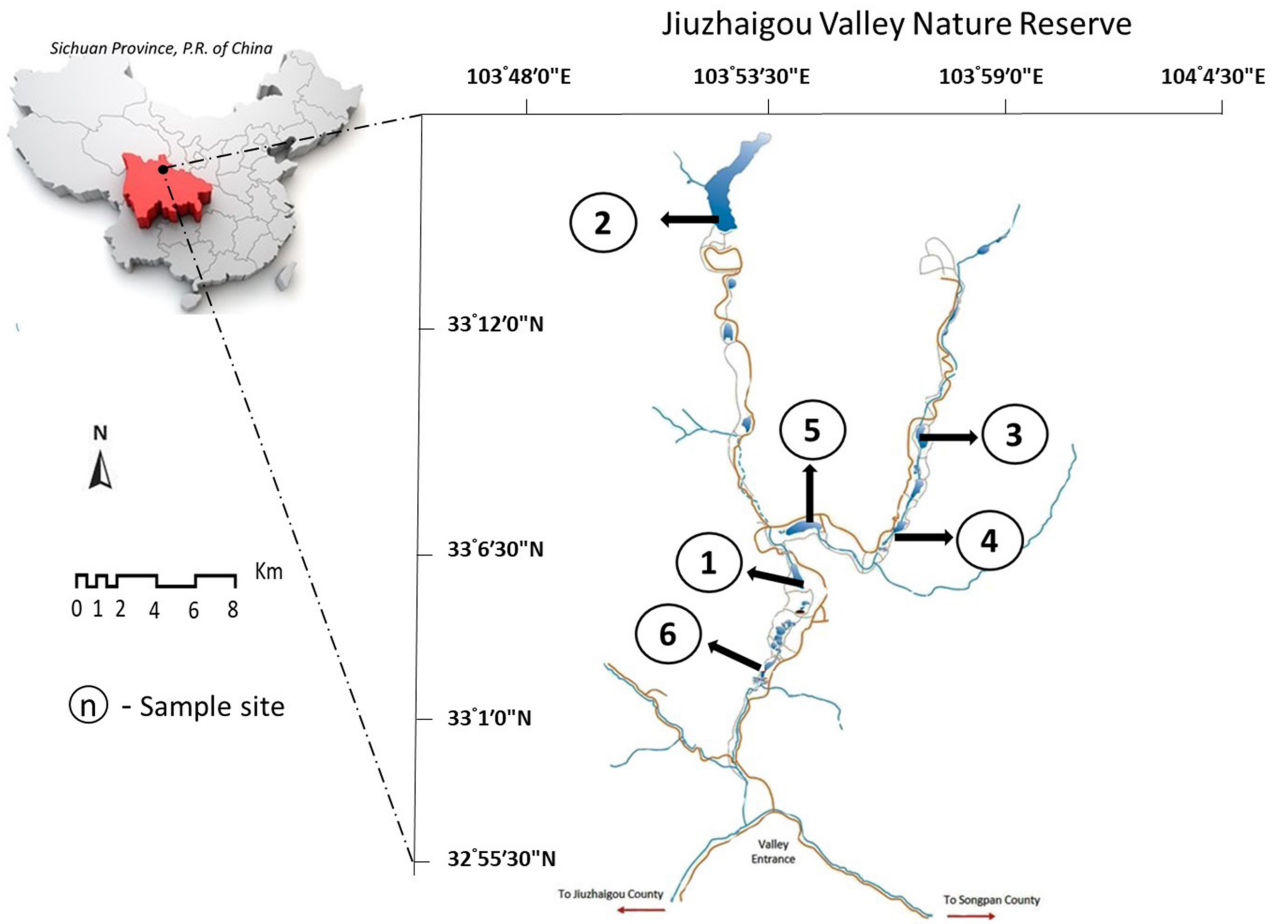


FIGURE 1. Map of Jiuzhaigou National Nature Reserve and location of the study sites (1= Rhinoceros Lake, 2=Long Lake, 3= Arrow Bamboo Lake, 4= Mirror Lake, 5= The Peacock Riverbed, and 6= Reed Lake)

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: 125
Order **Naviculales** Bessey 1907: 284
Suborder **Sellaphorineae** D.G. Mann in Round *et al.* 1990: 657
Family **Sellaphoraceae** Mereschowsky 1902: 193
Genus ***Sellaphora*** Mereschkowsky 1902: 186
Sellaphora jiuzhaienis Levkov, Plenković-Moraj & Sun *sp. nov.* (2–39)

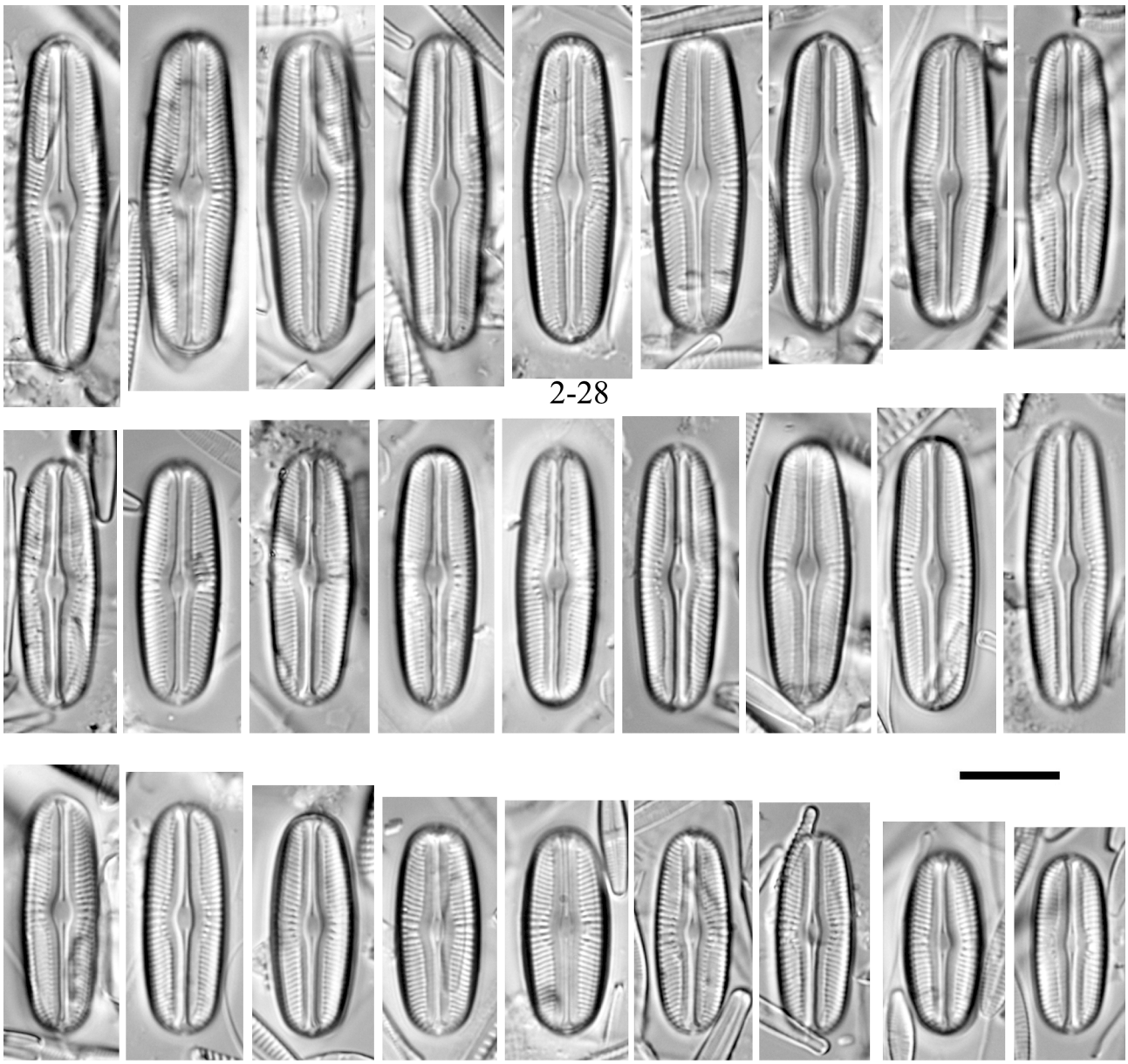
Description: LM (Figs 2–28): Valves symmetrical, linear to linear-elliptic with slightly convex valve margins and unprotracted and broadly rounded ends. Valve length 17.5–33.5 µm and 7.0–8.5 µm wide (n=38). Axial area moderately broad, widened towards central area. Central area broad, longitudinally elliptical to round bordered by 4–5 regularly shortened and more distantly spaced central striae. Broad conopeum visible in central area. Raphe linearly arranged within broad conopeum. Ends of central raphe slightly expanded into small central pores, distal raphe fissures long, slightly curved in the same directions and passing onto valve mantle. Striae strongly radiate throughout and slightly curved, 18–20 in 10 µm. Areolae not discernible with LM.

SEM (Figs 29–39): Valve surface flat and transition to valve mantle gradual (Figs 29, 31). Raphe situated on very wide conopeum surrounded on both sides by deep and narrow grooves (Figs 29–34, white arrows in Figs 33, 34). Conopeum very broad in central area and occupies half of valve width (Figs 32, 34). Raphe linear with slightly

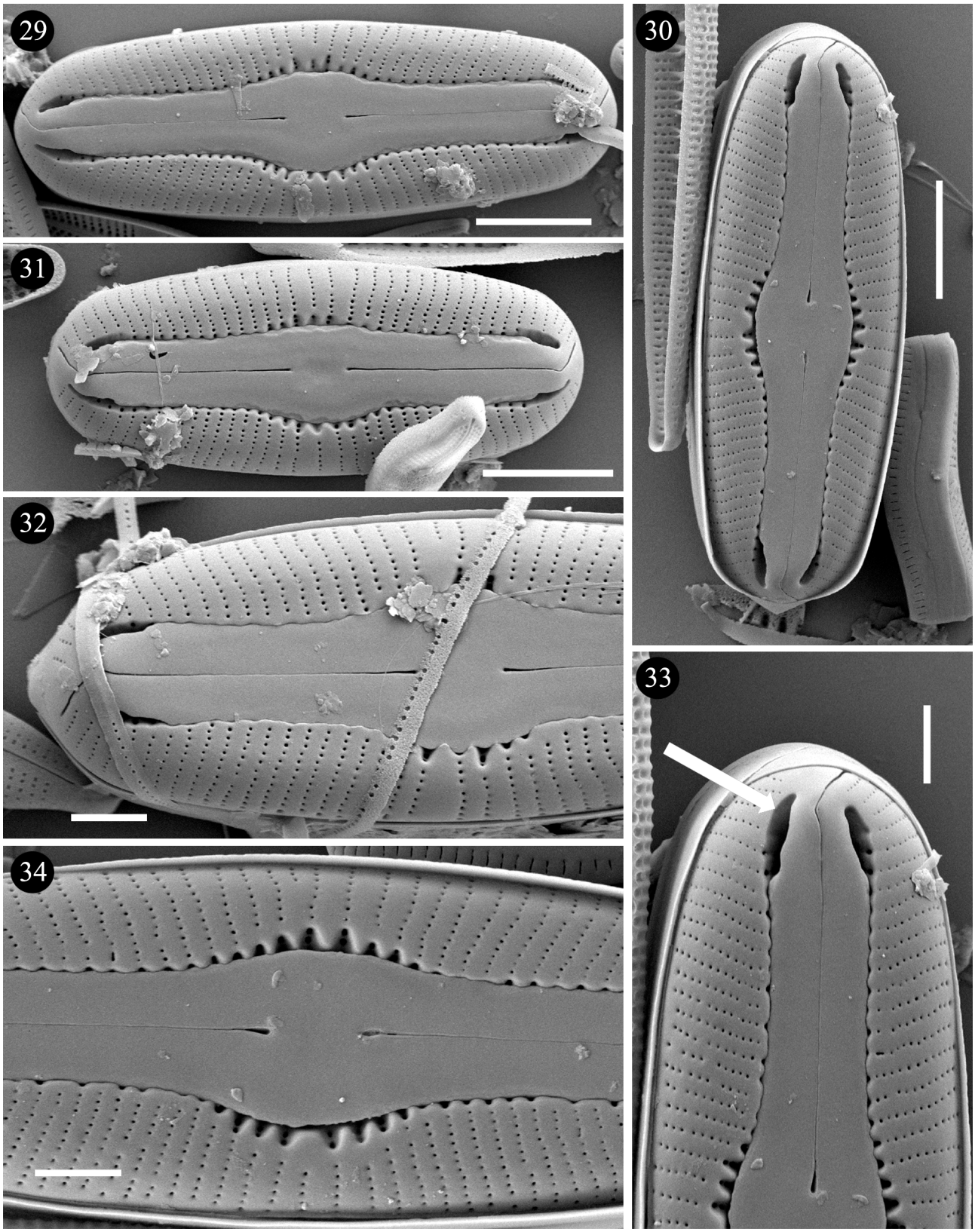
expanded central pores and slightly unilaterally deflected (Figs 32, 34). Distal raphe ends long, curved unilaterally towards secondary valve side and passing onto valve mantle (Fig. 33). Polar bars absent (Fig. 33). Internally, the raphe is linear with indistinct central ends located in a slightly elevated central area (Figs 35, 37). Distally, the raphe terminates in small helictoglossae (Figs 36, 38, 39). Large foramen-like (pit), which does not penetrate externally, present distally of the helictoglossae (Figs 36, 38, 39). Striae uniseriate, composed of small, round areolae (Figs 35–39). Areolae about 60 in 10 μm, towards apices and valve margin becoming smaller. Striae separated by thickened interstriae (Figs 37, 39). Internally, areolae occluded by dome-shaped hymens (white arrow in Fig. 38). In corroded specimens' areolae appear as open holes (Figs 36, 37, 39).

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 dates: 29.04.2015, Slide HRNDC 48J2016 (holotype), slide MKNDC! 14382/A (isotype)

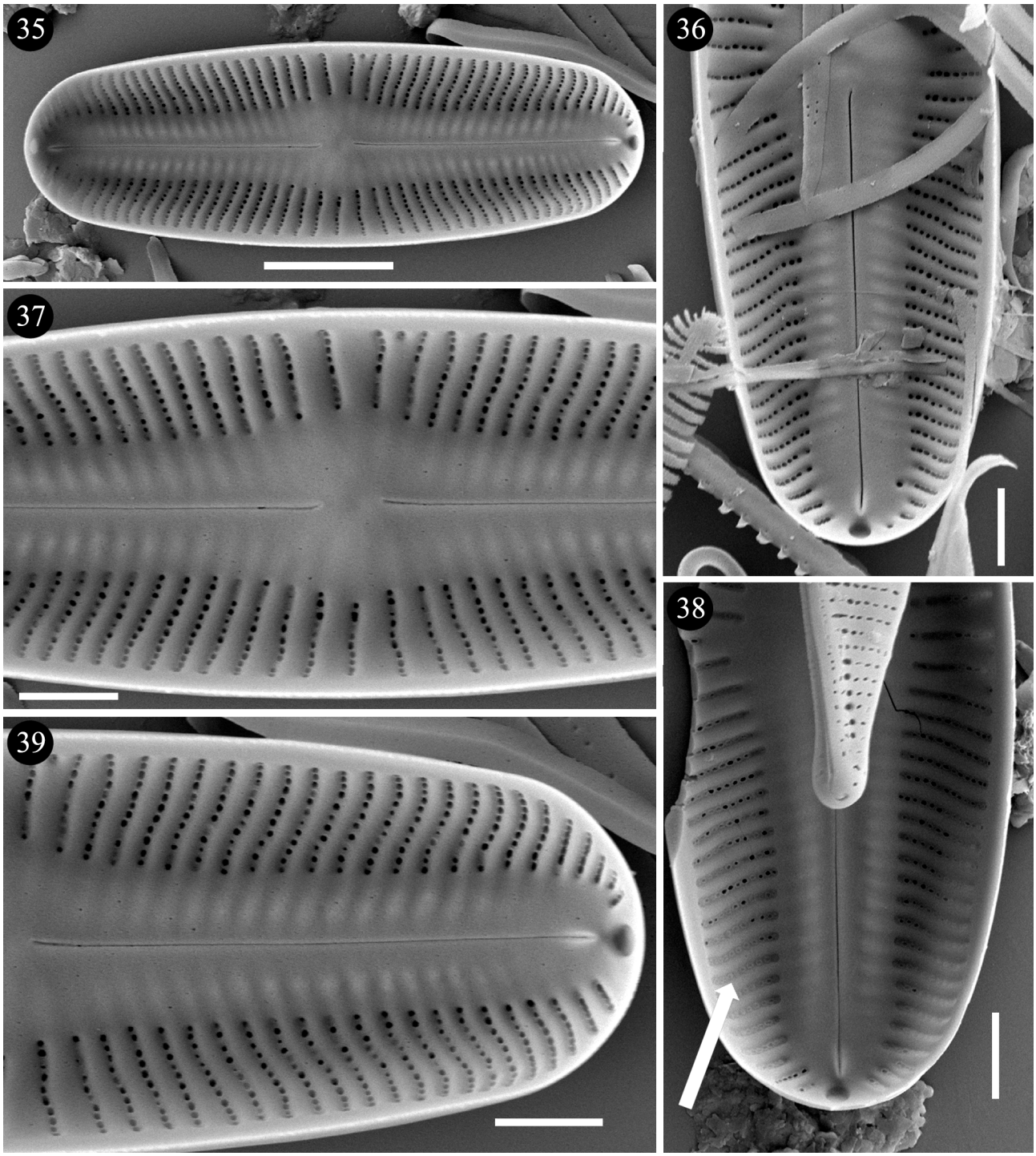
Etymology: the specific epithet ("*jiuzhaienis*") refers to the name of the Jiuzhaigou Nature Reserve from where this species is described.



FIGURES 2–28. *Sellaphora jiuzhaienis sp. nov.* Light microscopy (LM). Valve size diminution series. Scale bar = 10 μm.



FIGURES 29–34. *Sellaphora jiuzhaienis sp. nov.* Scanning electron microscopy (SEM). Figs 29–31. External view of entire valve. Figs 32, 33. External view of half of the valve. White arrow indicates narrow grooves surrounding the conopeum. Fig. 34. Detailed external view of the valve centre (white arrow indicates narrow grooves surrounding the conopeum). Scale bar = 5 μm (29–31), 2 μm (32–34).



FIGURES 35–39. *Sellaphora jiuzhaienis sp. nov.* Scanning electron microscopy (SEM). Fig. 35. Internal view of entire valve. Figs 36, 39. Internal view of half of the valve. Fig. 37. Detailed internal view of the valve centre. Fig. 38. Internal view of valve apex (white arrow indicates dome-shaped hymens), Scale bar = 5 μm (35), 2 μm (36–39).

Ecology: This new species occurred at 5.5 % 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) (18.6%), *Cymbella hantzschiana* Krammer (2002: 62) (16.2%), *Tryblionella angustata* W. Smith (1853: 36) (15.3%), *Nitzschia fossilis* (Grunow in Cleve & Grunow 1880: 98) Grunow (in Van Heurck 1881, fig. 68: 24) (10.2%), and *Gomphonema acuminatum* Ehrenberg (1832: 88) (6.2%). The 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.

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

Discussion

Several new *Sellaphora* species have been recently described from China. *Sellaphora tanghongqui* Y-L. Li (in Ni *et al.* 2022: 106, figs 2–26) is characterized by valves with weakly undulate and slightly constricted in the mid-valve margins, with lengths of 24.5–45.5 μm and widths of 9.0–11.0 μm. One of the most prominent features of the latter species is the undulated conopeum. It can be easily differentiated from *S. jiuzhaienis* by the valve shape (linear to linear-elliptic in *S. jiuzhaienis*) and size (7.0–8.5 μm *vs.* 9.0–11.0 μm), as well as size and morphology of the conopeum (broad and widened in the mid-valve *vs.* undulated conopeum in *S. tanghongqui*). A similar valve outline to *S. tanghongqui* is present in *S. constricta* Kociolek & Q-M. You (in You *et al.* 2017: 262, figs 1–17), described from the Maolan Nature Reserve in Guizhou Province, China. It also shares most of the characters, such as valve size (length 29.0–52.0 μm, width in the middle 9.0–10.5 μm, greatest width 9.5–11.0 μm) and stria density (14–16 in 10 μm), with *S. tanghongqui*. *Sellaphora jiuzhaienis* can be easily differentiated from *S. constricta* by the valve shape (not constricted in the mid-valve), valve width and shape of the conopeum. *Sellaphora wangii* Y. Liu & Kociolek (in Liu *et al*. 2020) is characterized by valves with almost parallel margins that are slightly constricted near the ends and slightly subcapitate to truncate apices and coarser striae (12–13 in 10 μm). The latter species can easily distinguish from *S. jiuzhaienis* by its valve shape, narrower conopeum and coarser striae. *Sellaphora yunnanensis* Li & Metzeltin (in Li *et al.* 2010a: 1160, figs 1–10) has broadly elliptical valves, 48–77 μm long and 22–24 μm wide, and thus can be easily differentiated from *S. jiuzhaienis* by its much larger valves. *Sellaphora sinensis* Li & Metzeltin (in Li *et al.* 2010a: 1162, figs 11–20) has even broader valves (width 36–42 μm in *S. sinensis*), which are much wider than those of *S. jiuzhaienis* (7.0–8.5 μm in *S. jiuzhaienis*). *Sellaphora fuxianensis* Li (in Li *et al.* 2010b: 65, figs 1–19) has a comparable valve size (length 10.5–25.5 μm, and width 4.5–7.5 μm), but it has a much narrower and linear conopeum and higher stria density (22–25 in 10 μm in *S. fuxianensis*).

Several *Sellaphora* species have been illustrated in Li & Qi (2018) from China. From those illustrated *S. boltziana* Metzeltin, Lange-Bertalot & Nergui (2009: 85, figs 252: 5–9; 254: 3–4) has a similar valve outline (linear) and a relatively broad central area but can be easily differentiated by the larger valves (length 45–65 μm, width 12–13 μm), narrower conopeum and densely spaced striae (19–21 in 10 μm). *Sellaphora krsticii* Levkov, Nakov & Metzeltin (in Levkov *et al.* 2006: 301, figs 14–29) sensu Li & Qi (2018, fig. 40: 1) has much larger valves with almost parallel valve margins. *Sellaphora gregoryana* (Cleve & Grunow) Metzeltin & Lange-Bertalot (1998: 204) is recorded from China (Jiaying & Yuzao, 2018, fig. 40: 6), but it can be distinguished from *S. jiuzhaienis* by its larger valves with constricted valve margin in the mid-valve and narrower conopeum.

Metzeltin *et al.* (2009) have described and recorded several new *Sellaphora* species from Mongolia. *Sellaphora interrupta* Metzeltin, Lange-Bertalot & Nergui (2009: 90, figs 60: 13, 14; 250: 3) has linear valves with broadly rounded ends. The valve size is comparable with *S. jiuzhaienis*, but both species can be easily differentiated by the width of the axial and central area. *Sellaphora mongolcollegarum* Metzeltin & Lange-Bertalot (in Metzeltin *et al.* 2009: 95, figs 59: 1–7) and *S. pseudobacillum* (Grunow) Lange-Bertalot & Metzeltin (in Metzeltin *et al.* 2009: 100, figs: 8–14), have much larger valves and thus differentiation from *S. jiuzhaienis* is straightforward. *Sellaphora* sp. sensu Metzeltin *et al.* (2009, fig. 37) is probably most similar taxon considering valve outline and size to *S. jiuzhaienis*, but it has much narrower and linear axial (conopeum), and central area.

Lake Baikal is considered as a hot spot for diatom diversity and many new species and even genera have been described from the lake (Kulikovskiy *et al.* 2012, 2015), including new species from the genus *Sellaphora*. Most of the endemic species from Lake Baikal belong to the group of *S. bacillum* (Ehrenberg) D.G. Mann (1989: 2) and *S. jiuzhaienis* can be differentiated from them by the valve size and shape. The most similar taxon from Lake Baikal is *Sellaphora* sp. 11. (Kulikovskiy *et al.* 2012, Fig. 106: 20), particularly considering the valve size and especially with respect to the large central area. The conspecificity between *S. jiuzhaienis* and the latter taxon cannot be evaluated since it is illustrated only by a single valve. Besides Lake Baikal, Lake Ohrid is also characterized by a large diversity of *Sellaphora* species (Levkov *et al.* 2007), both from *S. bacillum* and *S. pupula* (Kützing) Mereschkowsky (1902: 187) species complexes. *Sellaphora ohridana* Levkov & Krstic (in Levkov *et al.* 2007: 120) and *S. perbacilloides* Levkov, Nakov & Metzeltin (2006: 305) have similar valve outlines as *S. jiuzhaienis*, but the latter species has a much smaller valve size than the former species.

The type material and many other European populations of *Sellaphora stroemii* (Hustedt) H. Kobayasi (in Mayama *et al.* 2002: 90) has been observed by Falasco *et al.* (2009). This species is characterized by linear valves with subcapitate apices, 10–18 μm long and 4–5 μm wide. The conopeum is very narrow and flanked by parallel furrows. Differences between *S. jiuzhaienis* and *S. stroemii* can be noticed in the valve shape and size, as well as in the width of

the conopeum. *Sellaphora subbacillum* (Hustedt) Falasco & Ector (in Falasco *et al.* 2009: 251) has comparable valve outline as *S. jiuzhaienis*, but it is narrower (width 3.5–5.0 μm). *Sellaphora bacillum* complex has been observed in detail by Mann (1989) and Mann *et al.* (2008). The broader conopeum was observed in *Sellaphora* [*bacillum* K–LB] Φ 'butter' (Mann *et al.* 2008, figs 9a–f) and *Sellaphora* [*bacillum* K–LB] Φ 'buttermax' (Mann *et al.* 2008, figs 8a–f), but both taxa have broader valves than *S. jiuzhaienis*. Similar valve size and shape have *Sellaphora* [*bacillum* K–LB] Φ 'gibbous' but can be differentiated from *S. jiuzhaienis* by the narrower axial and smaller central area. *Sellaphora alastos* (Hohn & Hellerman 1963: 290) Lange-Bertalot & Metzeltin (1996: 101) is characterized by moderately broad axial and central area, but it has typical linear valves with parallel margins and is much broader than *S. jiuzhaienis*. *Sellaphora pelagonica* Kochoska, Zaova, Videska & Levkov (in Kochoska *et al.* 2021: 123, figs 2–39) has a narrow and linear conopeum surrounded by relatively deep and wide grooves, and densely spaced striae (22–24 in 10 μm).

Diatom research in China has been intensified in the last two decades with description of many new taxa, including six new genera (e.g., Yanling *et al.* 2009, Williams *et al.* 2010, Kociolek *et al*. 2015). According to Kociolek *et al.* (2020) since year 2000, 421 diatom species have been described from China, but only five species belong to the genus *Sellaphora*. The genus *Sellaphora* is considered one of the most diverse diatom genera with more than 250 taxa (Guiry & Guiry 2023). This number is most likely underestimated and will be increased in the future. Also, some of the taxa illustrated in Li & Qi (2018) from China, might belong to other, probably new species. Very likely many new species with restricted distribution in freshwater habitats in China will be discovered, especially from calcareous oligotrophic habitats (e.g., Plenković-Moraj *et al.* 2023).

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