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# *Cymbella pamirensis sp. nov*. (Bacillariophyceae) from an alpine lake in the Pamir Mountains, Northwestern China

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## Abstract

This paper describes a new *Cymbella* species from an alpine lake in the Pamir Mountains, NW China, with the aid of light and scanning electron microscopy and morphometric analyses. The morphology of the new species, named *Cymbella pamirensis*, is discussed and compared to similar species. The main morphometric features distinguishing *Cymbella pamirensis* from similar species of *Cymbella* are the outline and size of the valves. *Cymbella pamirensis* has been observed in surface sediment and core samples from Lake Sate Baile Dikuli, an alkaline, mesotrophic lake of the Pamir Mountains.

**Key words:** China, *Cymbella,* diatom, morphometric analysis, taxonomy

## Introduction

The genus *Cymbella* was established almost 200 years ago by Agardh (1830: 1), but no generitype species was designated. Håkansson & Ross (1984: 525) proposed that *Cymbella cymbiformis* C.Agardh (1830: 10) should be accepted as *typus conservandus* (da Silva *et al.* 2013). The taxonomy of the genera belonging to the Cymbellaceae has long been problematic until the extensive revisions proposed by Krammer (1997a, b, 2002, 2003). In recent years, our understanding of the taxonomy and species diversity in the genus *Cymbella* has considerably improved, as the descriptions of many species have been emended or clarified and new species of *Cymbella* are being described at a constant rate (Van de Vijver & Lange-Bertalot 2008, Cantonati *et al.* 2010, Gong & Li 2011, Stancheva & Ivanov 2011, da Silva *et al.* 2013, 2015, Gong *et al.* 2013, Hu *et al.* 2013, Recasens & Maidana 2013, Rodionova *et al.* 2013, Pomazkina & Rodionova 2014, Buczkó *et al.* 2015, Le Cohu *et al.* 2015, Vishnyakov *et al.* 2015, Garcia & Dutra 2016). In the online database AlgaeBase, Guiry & Guiry (2017) currently lists 1464 species in the genus, of which 313 have been accepted as valid taxonomically. Among those, 49 species and 5 varieties have been reported in China (Li *et al.* 2003, 2007, Shi 2013) with eight of them described as new (Gong & Li 2011; Gong *et al*. 2013; Hu *et al*. 2013). In this paper, we report on a new *Cymbella* species, named here as *Cymbella pamirensis sp. nov.*, using light (LM) and scanning electron microscopy (SEM). The new species was found in sediment samples from Lake Sate Baile Dikuli, an alpine lake in the Pamir Mountains, NW China.

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## Material & methods

The study presented here was carried out in the context of an ongoing project investigating the Holocene paleoclimate and palaeoenvironment from two alpine lakes located in the Pamir Mountains, in Xinjiang Province, northwestern China. Work on Lake Kalakuli, the larger of the two lakes, has focused on geochemical analyses (Liu *et al.* 2014; Aichner *et al*. 2015; Yin *et al.* 2016), while the smaller lake called "Sate Baile Dikuli" (38°27′52″ N, 75°02′6″ E, 3650 m a.s.l.) is being investigated using diatom analysis.

Lake Sate Baile Dikuli is a lake of glacial origin, with a 1.47 $km^2$ area and a shallow basin with a maximum depth of 22.8 m (Fig. 1, Table 1). In summer the lake has no surface inflow or outflow. Sometimes in spring however, glacier melt-water coming from the eastern flank of the Pamir Mountains enters the lake from the south-west via a small stream. The vegetation on the catchment of the lake is very sparse, dominated by annual plants, perennial dwarf shrubs and cushion plants (Yang *et al*. 2012, Yin *et al.* 2016). The bedrock in the basin is mainly composed of granodiorite and monzogranite (Yin *et al.* 2016). Data from Tashikuergan, the nearest meteorological station located about 75 km to the south from the study lake, indicate a very cold and dry climate. The mean annual temperature is 0.7°C and the mean annual precipitation is 127 mm, while the annual evaporation is > 1500 mm (Liu *et al.* 2014).

In June 2013, a 6.3 m long core was retrieved using a UWITEC corer near the deeper part of the lake (at ~22 m water depth). Preliminary work on the chronology, based on the $^{14}C$ AMS dating of 14 samples (including 10 plant macrofossil, 3 mollusc shells and 1 fish bone), suggests that the core records the last ~7000 years. In September 2014 epilithic, epiphytic and surface sediment samples were collected. In addition, two surface water samples, collected in October 2013 and April 2016, were analyzed to determine their chemical composition (Table 1). Water temperature and pH were first measured in the field using a Hanna HI8424® meter, then re-measured in the laboratory along with the variables listed in Table 1. Electrical conductivity (EC), total alkalinity, total phosphorus (TP) and total nitrogen (TN) were measured on unfiltered lake water. Anions ($Cl^-$, $SO_4^{2-}$, $NO_3^-$, $F^-$), cations ($Ca^{2+}$, $Mg^{2+}$, $K^+$, $Na^+$, $NH_4^+$), dissolved organic carbon (DOC) and dissolved Si were measured on water filtered through Whatman GF/F® glass fiber filters. A complete list of the laboratory methods used for water chemistry analyses is given in Rioual *et al.* (2013).

**TABLE 1.** Physical and chemical parameters for Lake Sate Baile Dikuli. Water chemistry data were obtained from the analyses of two samples collected in October 2013 and April 2016. Except for water temperature and pH that were also measured in the field, all other variables were measured in laboratory conditions. <d.l. = below detection limits; n.a. = not available.

| Parameters | Lake Sate Baile Dikuli | |
|---|---|---|
| Elevation (m a.s.l.) | 3650 | |
| Latitude (N) | 38°27′52″ | |
| Longitude (E) | 75°02′6″ | |
| Max depth (m) | 22.8 | |
| Area ($km^2$) | 1.47 | |
| | 24th October 2013 | 25th April 2016 |
| Remark on location | Eastern shore | SW shore, ice-cover |
| Water temperature (field, °C) | 6.9 | 3.3 |
| pH (field/lab at 25°C) | 8.9 / 7.3 | 8.1 / 6.5 |
| Elec. Conductivity (μS/cm) | 390 | 175 |
| Alkalinity (μeq/L) | 1585 | 550 |
| TP (μg/L) | 31 | 30 |
| TN (μg/L) | 1610 | 890 |
| DOC (mg/L) | 4.2 | 0.8 |
| $Cl^-$ (mg/L) | 53.2 | 11.1 |
| $NO_3^-$ (mg/L) | <d.l. | 2.5 |
| $SO_4^{2-}$(mg/L) | 23.9 | 24.8 |
| $F^-$ (mg/L) | 0.6 | 0.7 |
| $Ca^{2+}$ (mg/L) | 20.2 | 11.8 |
| $K^+$ (mg/L) | 6.2 | 5.0 |
| $Mg^{2+}$ (mg/L) | 13.1 | 9.3 |
| $Na^+$ (mg/L) | 16.9 | 10.3 |
| $NH_4^+$ (mg/L) | <d.l. | n.a. |
| Dissolved Si (mg/L) | 1.7 | 0.8 |

Diatom samples were prepared using the water-bath method (Renberg 1990). Freeze-dried sediment samples were treated with 30% hydrogen peroxide ($H_2O_2$) to remove organic matter. The sample were then rinsed in distilled water and let to settle for 24 hours. Subsamples of the homogenized suspensions were diluted by adding distilled water and air-dried onto glass coverslips. Then the coverslips were mounted onto slides using Naphrax®. Specimens were observed under an Olympus BX51 light microscope (LM) using bright field and phase contrast oil immersion optics at ×1000 magnification. Both samples and prepared slides are archived in the Diatom Laboratory, Institute of Geology and Geophysics, Chinese Academy of Sciences, Beijing City, People's Republic of China. Coordinates of the type specimen are specified according to the method described by Sterrenburg *et al.* (2012) and using an England finder.

For scanning electron microscopy (SEM), a subsample of the clean material was air-dried onto 12 mm diameter glass coverslips attached to aluminum stubs using Ted Pella® double-coated carbon conductive adhesive tape and were sputter coated with 15 nm of gold. At the Institute of Geology and Geophysics, Chinese Academy of Sciences, SEM photographs were taken with a Nova Nano SEM 450 (FEI, USA) using15 kV.

For morphometric analysis with LM, measurements of valve length, width, stria density, chord length and sagitta length were made on digitized images calibrated against a slide micrometer using the public domain ImageJ software (Rasband 2013). In total, 40 valves were investigated under the LM. Valve curvature was calculated following the method from Bixby & Zeek (2010). The formula for the estimator of valve curvature is:

$$\mathrm{k} = \frac{2h}{l^2 + h^2}$$

in which k is the valve curvature, *l* is the chord length and *h* is the sagitta length (Fig. 5).

The distributions of single characters representing the new *Cymbella* were illustrated using histograms while the relationships for each pair of variables were illustrated using biplots (bivariate analyses). All the plots were drawn using the free software environment R and the package "GGally" (R Core Team 2016).

In addition to these analyses using LM, SEM images were used to assess areola density. In practice, the linear length containing 10 areolae was measured on five striae situated in the mid-area between the center and apex of the valve and the mean values were converted to a number of areolae per 10 µm (Cortese & Gersonde 2007). Seven valves were analyzed in this manner.

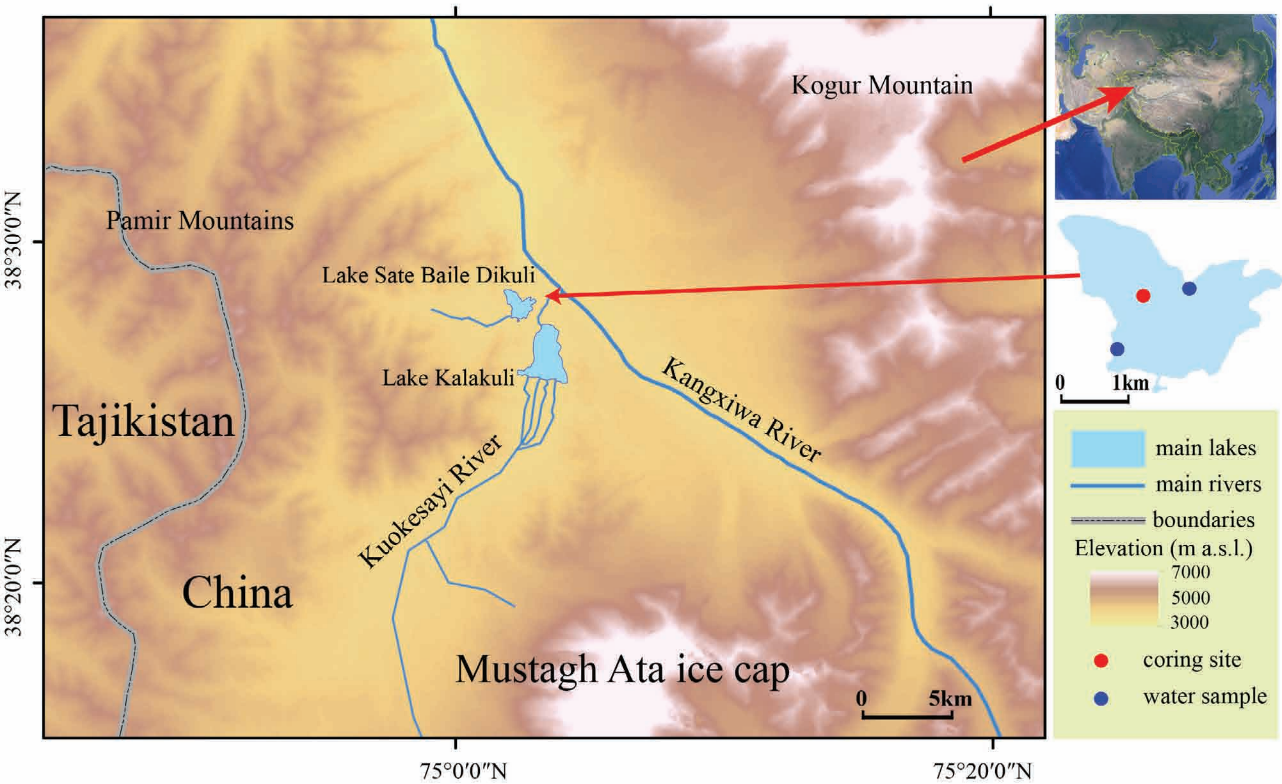
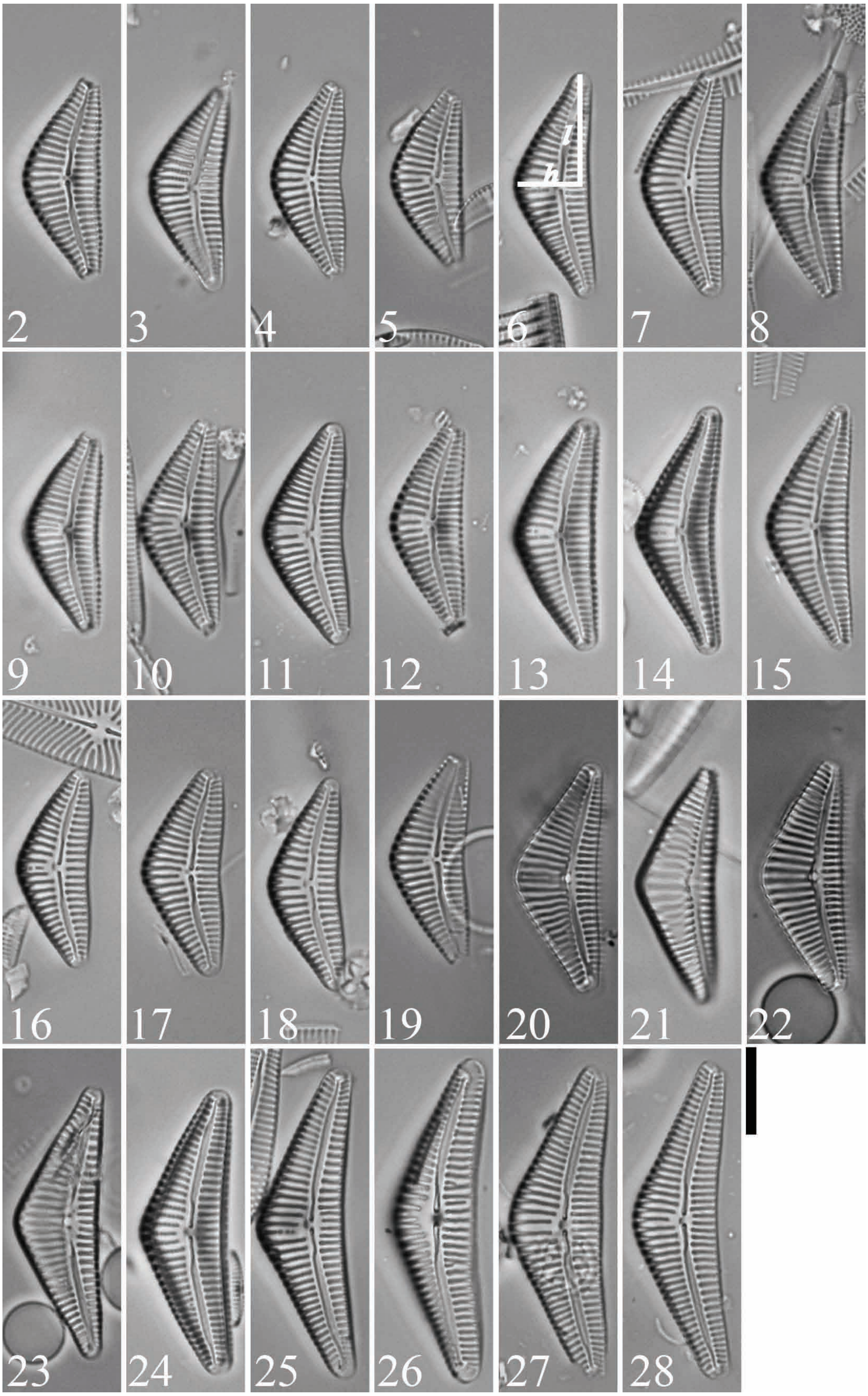



**FIGURE 1.** Locations of Lake Sate Baile Dikuli in the Pamir Mountains in western China and of the coring site.




**FIGURES 2–28.** *Cymbella pamirensis*. Light micrographs showing variation in size and valve outline. Scale bar: 10 µm. In Fig. 6, *l* represents chord length and *h* represents sagitta length.

## Results

### Taxonomic description

Division **Bacillariophyta**
Class **Bacillariophyceae**
Subclass **Bacillariophycideae**
Order **Cymbellales**
Family **Cymbellaceae**
Genus ***Cymbella***

***Cymbella pamirensis* Z.Zhang & Rioual, *sp. nov.* (Figs 2–34)**

**LM observations (Figs 2–28)**

Valves strongly dorsiventral and triangular. Dorsal margin strongly arched. Ventral side nearly straight or slightly concave with slightly tumid center part. Ends variable from broadly round to weakly protracted. Valve dimensions (n=48): length 21–39 μm, width 7–10.5 μm, length/width ratio 2.6–4.3 (median 3.3), curvature value 0.038–0.083. Axial area linear, narrow, widening slightly at mid-valve on the dorsal side, almost in the middle of the valve. Central area generally lacking on the ventral side or very small (Figs 27, 28), but present dorsally where striae gradually shortened. Raphe branches lateral, becoming narrower near distal ends; terminal fissures curved dorsally. Striae moderately radiate in middle of valve. No isolated stigmata discernible under the LM.

**SEM observations (Figs 29–34)**

Externally, apical pore fields situated on margin and mantle and striae composed of 6–15 elongated areolae extending to mantle, 8–14 in 10 μm (Figs 29–31). Central pores present at end of proximal raphe (Fig. 31). Terminal raphe fissures hooked toward dorsal margin of valve (Fig. 30). Striae continuing without interruption on mantle. Sometimes on dorsal side of central area, one shorter or discontinuous stria is interposed at mid-valve and composed by smaller, less elongated areolae. Three rounded stigmata open at end of central ventral striae (Fig. 31). Internally, distal raphe terminate as raised helictoglossae (Figs 32, 34). Striae form depressions in which rectangular areolae open. Stigmata (2 or 3) visible as elongated furrows directly connected to striae (Fig. 33).

**Type locality:**—CHINA, Xinjiang Province, Pamir Mountains, Lake Sate Baile Dikuli, 3650 m, 38°27′52″N, 75°02′6″E (Holotype slide IGGDC-XKL-355! specimen illustrated in Fig. 2, located using England Finder K13 or at coordinates 15.6 and 113.5 with an Olympus BX51 light microscope. Isotype: BM slide 101/855, Natural History Museum, London, UK).

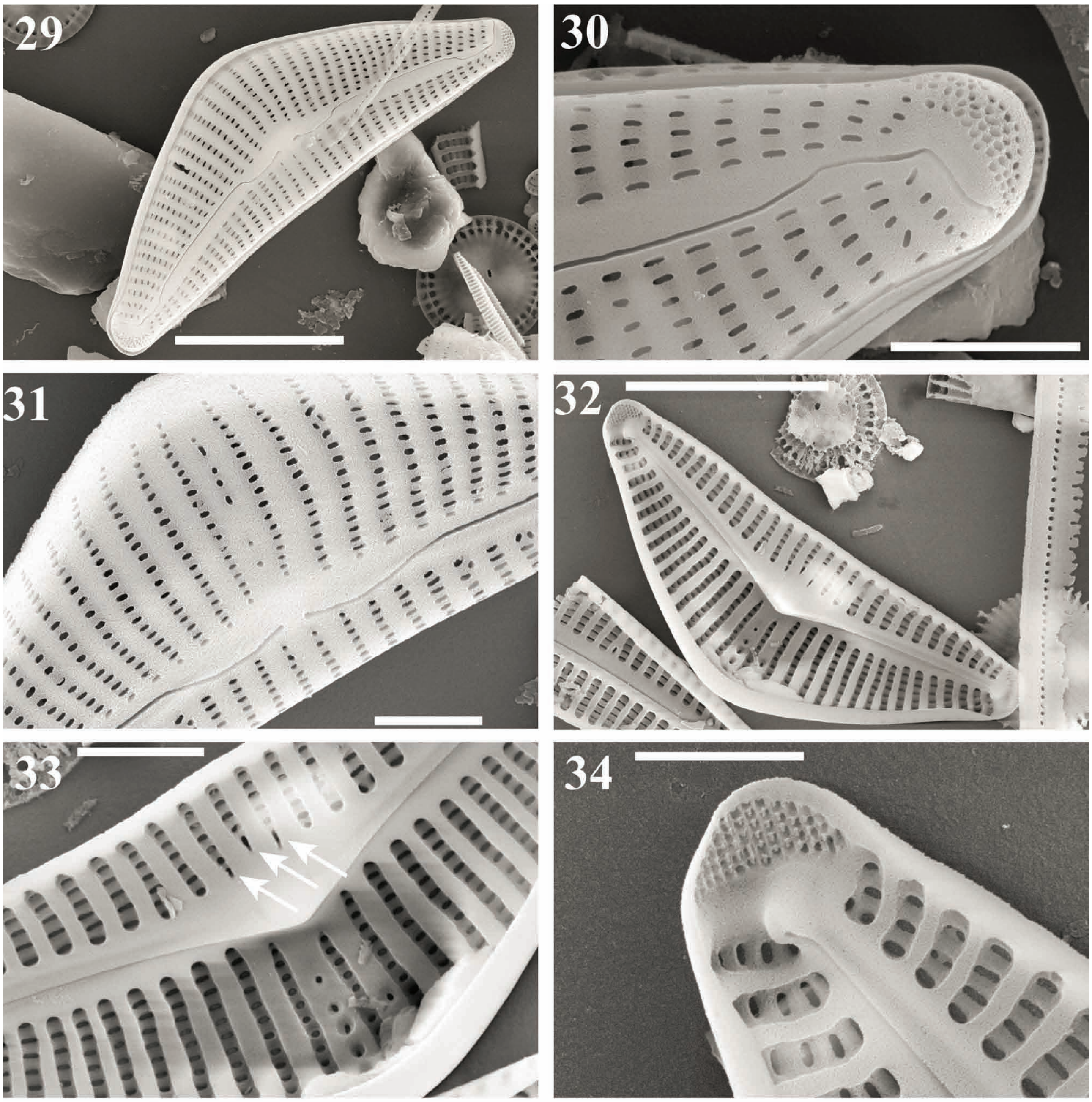
**Etymology:** The epithet of the new species refers to the Pamir Mountains.

***Morphological characters and comparison with similar taxa***

*Cymbella pamirensis* clearly belongs to the genus *Cymbella* as it shows the main diagnostic features of this genus including the dorsi-ventral shape, the asymmetry to the apical axis, symmetry to the transapical axis, and distal raphe ends deflected dorsally (Krammer 2002).

The histograms of the morphometric characters of *C. pamirensis* show right-skewed, unimodal distributions for valve length and width (Fig. 35). This suggests that our measurements are representative of the population investigated (Krammer 1997a). The matrix of biplots for comparison of morphometric characters of *Cymbella pamirensis* (Fig. 35) shows that the valve length is highly correlated with the other characters, especially curvature (with a negative correlation coefficient of 0.97), valve width ($r = 0.69$) and to a lesser extent with stria density ($r = 0.61$). Despite the strong correlation between length and curvature, curvature remains a useful character in separating species with similar lengths as shown by Bixby & Zeek (2010) for the genus *Hannaea*. In our core samples, curvature is useful to distinguish valves of *C. pamirensis* from those of *C. hantzschiana* Krammer (2002: 47, pl. 27, figs 8–14) and *C. excisiformis* Krammer (2002: 31, pl. 11, figs 1–23), which have a similar size range but much lower curvature.

In the literature, three other taxa appear morphologically similar to *Cymbella pamirensis*: *Cymbella terrafuegiana* Krammer (2002: 88, 169, pl. 76, figs 1–3), *Cymbella shudunensis* Y.L.Li & Metzeltin in Hu *et al.* (2013: 362, figs 7–13) and *Cymbella gravida* Recasens & Maidana (2013: 468, figs 2–15). These three species share a similar outline with *C. pamirensis*, with strongly dorsi-ventral triangular shape and a dorsal side semi-rhomboid. The first two species, however, have a much larger valve size compared to *C. pamirensis* (Table 2). The minimum valve lengths of *C. terrafuegiana* and *C. shudunensis* are 64 µm and 55 µm respectively, while the maximum valve length of *C. pamirensis* is 40 µm. In addition, *C. pamirensis* has a higher number of striae in 10 µm (23–34 µm) than *C. terrafuegiana* (19–21 µm) and *C. shudunensis* (17–29 µm). *Cymbella gravida* is the most similar species to *C. pamirensis* in terms of valve length and stria density but differs by being wider (12–17 instead of 7–11 µm) and by having a very tumid ventral side.




**FIGURES 29–34.** *Cymbella pamirensis* SEM. 29. Valve in external view. 30. Close-up on apex in external view. 31. Close-up on proximal raphe endings in external view. 32. Valve in internal view. 33. Close-up on central area in internal view, note the three stigmata visible as elongated furrows connected to the striae (arrows) and the discontinuous stria interposed at mid-valve. 34. Close-up on apex in internal view showing the distal raphe fissure ending in helictoglossae and the apical pore field. Scale bars: 10 µm in Figs 29 and 32; 3 µm in Figs 30, 31 and 33; 2 µm in Fig. 34.

In addition to these morphometric differences, *C. pamirensis* can be distinguished from similar species according to several features of its ultrastructure. The most obvious one, is the position of the stigmata. Under the LM, stigmata cannot be distinguished in *C. pamirensis* while they are obvious in *C. gravida, C. terrafuegiana* and *C. shudunensis*. Under the SEM, the stigmata are clearly detached from the striae in *C. gravida* and *C. terrafuegiana* (as shown by Recasens & Maidana 2013) as well as in *C. shudunensis* (Hu *et al*. 2013). By contrast, in *C. pamirensis* images of the valve interior show that stigmata are directly connected to the striae (Fig. 33). Stigmata with a similar position have been observed in *Cymbella marvanii* Le Cohu & Tudesque in Le Cohu *et al.* (2015: 258, figs 1–16). There is however no obvious difference in the shape of stigmata as seen under the SEM, as in all species considered here they appear rounded in external views of the valve and slit-like in internal views. The apical pore fields in *C. gravida* (fig. 18 in Recasens & Madaina 2013) appear similar to that of *C. pamirensis* while those of *C. terrafuegiana* were not clearly illustrated. The apical pore fields are missing in *C. shudunensis* (fig. 13 in Hu *et al.* 2013).

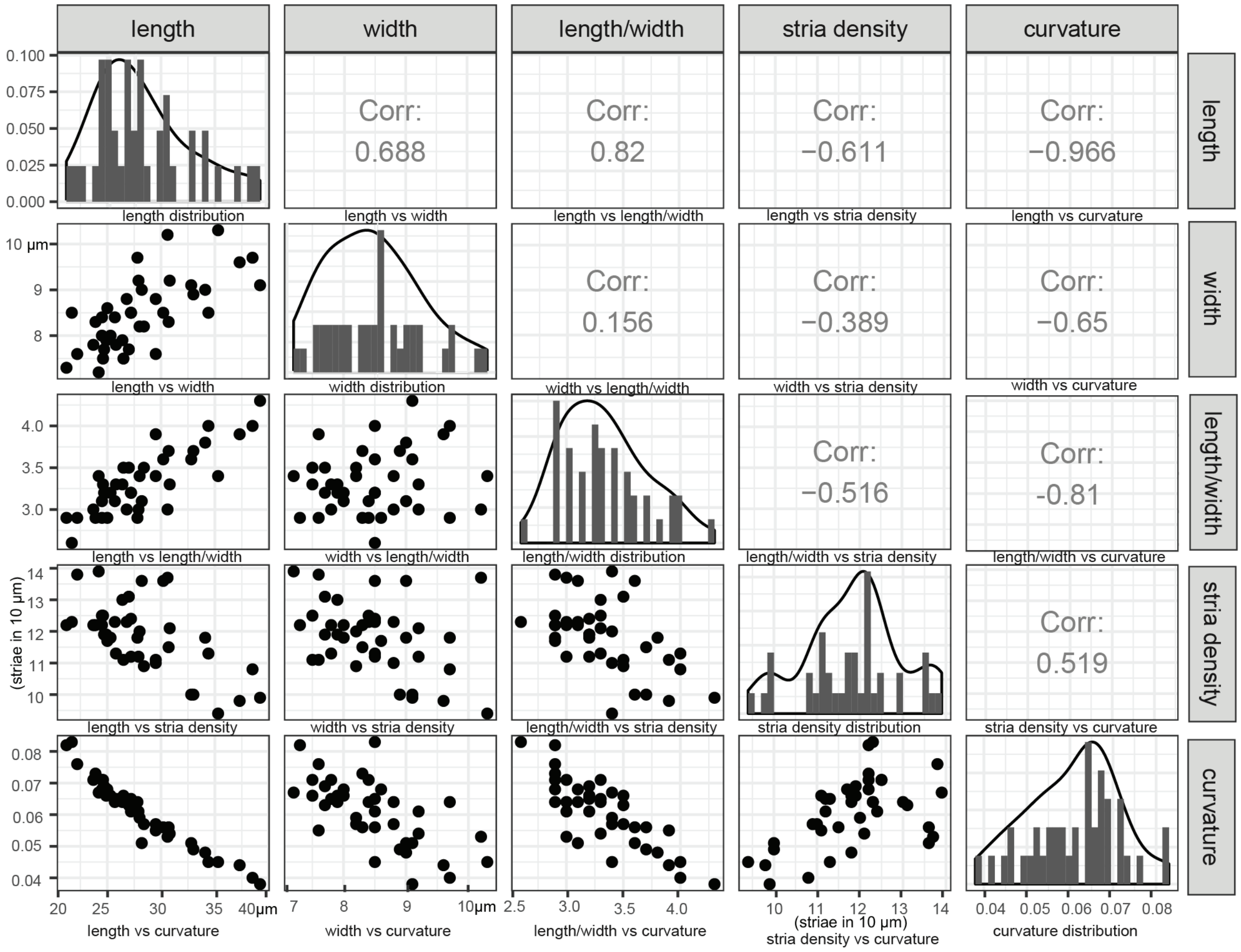



**FIGURE 35.** Histograms showing the distributions of morphometric characters of *Cymbella pamirensis* are shown in the main diagonal of the matrix (n=40). Biplots and the correlation coefficients for each pair of characters are shown below and above the main diagonal, respectively. Measurements are in µm for valve length and width. Stria density is in number of striae per 10 µm. Valve curvature is calculated using the formula $k=2h / (l^2+h^2)$, see text for details.

**TABLE 2.** Comparison between *Cymbella pamirensis* and similar *Cymbella* species with a semi-rhomboid valve shape.

| | *C. pamirensis* | *C. gravida* | *C. terrafuegiana* | *C. shudunensis* |
|---|---|---|---|---|
| Dorsal side | semi-rhomboid | semi-rhomboid | semi-rhomboid | semi-rhomboid |
| Ventral side | slightly concave with slightly tumid center | concave with tumid center | straight or very slightly convex | slightly concave with tumid center |
| Apex | broadly rounded to weakly protracted | rounded to subrostrate | acutely rounded | broadly rounded |
| Valve length (µm) | 21–40 | 25–57 | 64–74 | 55–87 |
| Valve width (µm) | 7–11 | 12–17 | 18–22 | 24–27 |
| Length/width ratio | max 4.3 | max 3.8 | max 3.6 | max 3.3 |
| Striae in 10 µm | 9–14 | 8–12 | 8–9 | 7–12 |
| Areolae in 10 µm | 23–27 | 18–26 | 19–21 | 17–19 |
| Stigmata | ventral side: 2–4 | ventral side: 1–4 | ventral side: 2–4 | ventral side: 2–4 |
| Central area | absent or indistinct | small, rounded | absent or roundish | rounded, larger on ventral side |
| References | this study | Recasens & Maidana (2013) | Krammer (2002) | Hu *et al.* (2013) |

The course of the raphe of *C. pamirensis* is similar to that of *C. gravida* (Recasens & Maidana 2013) but in *C. shudunensis* the raphe fissure is characterized by scythe-shaped distal endings and drop-like proximal endings (figs 12–13 in Hu *et al*. 2013).

In addition to these morphological differences, *C. gravida* and *C. terrafuegiana* appear to have never been reported outside South America. *Cymbella gravida* is also a fossil species, possibly brackish (Recasens & Maidana 2013).*C. shudunensis* was found in Lake Shudu, a lake in the Hengduan Mountains of southwestern China situated at an elevation similar to that of Lake Sate Baile Dikuli but under a much warmer and humid climate.

***Ecology and sedimentary record***

*Cymbella pamirensis* has been observed in the surface sediment sample and core samples from Lake Sate Baile Dikuli. The chemical analyses of the two water samples we collected show that this lake is not saline. The lake is mesotrophic and has relatively large seasonal variations in its alkalinity, ionic composition and concentration of dissolved organic carbon. The discrepancy in pH values measured in the field and in the laboratory may be due to several factors as discussed by Latysh & Gordon (2004). A primary factor is contamination during sample handling, transport, and processing when hydrogen-ion differences may be influenced by surface chemistry and the adsorption of ions to the container walls. Other factors influencing pH differences include delayed neutralization of entrained particulates in the water sample, measurement errors in the field or laboratory, and influences from organic acids.

Preliminary diatom analysis of the sedimentary record from Lake Sate Baile Dikuli indicates that *C. pamirensis* has been present in that lake since at least ~4000 years BP. It is most abundant (~2% relative abundance) in the sample taken at 355 cm core depth, which corresponds to an age of ~1800 years BP. It is however not a strictly fossil species as it is present in some of the modern samples investigated, albeit with very low abundance, accounting for much less than 1% of the assemblages. The assemblages are mainly dominated throughout the sediment sequence by *Pantocsekiella comensis* (Grunow in Van Heurck) K.T.Kiss & Ács in Ács *et al.* (2016: 65) (syn. *Cyclotella comensis* Grunow in Van Heurck 1882, pl. 93, figs 16, 17) and *Amphora indistincta* Levkov (2009: 69). Variations in the relative percentages of these two species are the most noticeable changes observed in the sequence and may be related to changes in lake level. Further work, including the geochemical analyses of the sediment, is necessary to clarify the environmental changes that took place in Lake Sate Baile Dikuli during the course of the Holocene.

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