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Cymbella biseriata sp. nov.—a unique living species of *Cymbella* Agardh (Bacillariophyceae) with biseriate striae

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Abstract

Alpine ponds are known biodiversity hot-spots and refugia for many rare and endangered species. They are also considered to be vulnerable habitats in terms of acidification, eutrophication, modification and climatic changes. Recently, a detailed study of diatoms in these habitats in North Macedonia has been started to evaluate their status and threats. During these studies, specimens of a species of *Cymbella* that lacked apical pore fields and had a small valve size were recorded. Scanning electron microscope observations revealed biseriate striae composed of small, slit-like to tilde-like areolae, a character so far not observed in any living representatives of the genus *Cymbella*. Herein, these specimens are assigned to the new species *Cymbella biseriata sp. nov.* based on detailed observations with light and scanning electron microscope. Comparison with similar taxa from the *C. helvetica* species complex and fossil species with biseriate striae is provided.

Keywords: alpine pond, diatom, freshwater, mountain Kozuf, taxonomy

Introduction

The genus *Cymbella* was first described by Carl Adolph Agardh to include all biraphid taxa with dorsiventral symmetry (Agardh 1830: 1). It has since been considered heterogenous since many included taxa exhibit a broad range of ultrastructural features. With a narrower generic concept, *Cymbella* sensu lato has since been separated into 15 genera plus *Cymbella*: *Encyonema* Kützing (1833: 589), *Pseudencyonema* Krammer (1997: 156), *Encyonopsis* Krammer (1997: 156), *Reimeria* Kociolek & Stoermer (1988: 457), *Cymbellopsis* Krammer (1997: 157), *Cymbopleura* Krammer (1999: 292), *Navicymbula* Krammer (2003: 123), *Delicatophycus* Wynne (2019: 1) (= *Delicata* Krammer 2003: 110), *Gomphocymbellopsis* Krammer (2003: 127), *Afrocymbella* Krammer (2003: 129), *Cymbellafalsa* Lange-Bertalot & Metzeltin (Metzeltin *et al.* 2009: 28, 29), *Oricymba* Jüttner *et al.* (2010: 408), *Kurtkrammeria* Bahls (2015: 6), *Celebesia* Kapustin, Kulikovskiy & Kociolek (Kapustin *et al.* 2017) and *Karthickia* Kociolek, Glushchenko & Kulikovskiy (in Glushchenko *et al.* 2019: 2). Krammer (2002) provides a generic definition using a combination of features to diagnose *Cymbella* sensu stricto, but it still remains a complex genus.

Several structural characteristics have been used to describe the genus *Cymbella* sensu stricto:

(1) "Cymbelloid" outline of the valve (as opposed to the more symmetrical "Naviculoid" outline);

(2) dorsally deflexed terminal raphe fissures;

(3) two apical pore fields on each valve (in *C. helvetica* Kützing (1844: 79) the apical pore fields are absent or initially present);

(4) the possession of one or more stigmata on the ventral side of the central nodule, for most taxa;

(5) an intermissio not visible in light microscopy (LM);

(6) dorsally arranged chloroplasts;

(7) found in fresh water, marine taxa are unknown.

One of the main characters that defines *Cymbella* sensu stricto is the presence of apical pore fields (APF) that are not bisected by the distal raphe ends. However, one species complex, *Cymbella helvetica* is characterized by absence of APF (Krammer 1982), although molecular evidence showed that this complex clearly belongs to the genus *Cymbella* (Nakov *et al.* 2014: 336, fig. 4, 367, fig. 5). Another important feature is the stria morphology. Most species are characterized by uniseriate striae composed of areola which are slit-like or *x*-shaped, but a few species have biseriate striae such as *Cymbella duplopunctata* (Krammer 2002: 109) and *Cymbella buechleri* Krammer (2002: 110). Both are considered extinct and, so far, recent species with entirely biserate striae have not been recorded (Liu *et al.* 2018). However, several species with partly biseriate striae, such as *Cymbella distalebiseriata* Liu & D.M. Williams (in Liu *et al.* 2018: 41, 43), *Cymbella liyangensis* Zhang, Jüttner & E.J. Cox (in Zhang *et al.* 2018: 16), *Cymbella yakii* Jüttner & Van de Vijver (in Jüttner *et al.* 2010), *Cymbella tridentina* Lange-Bertalot, Cantonati & Scalfi (in Cantonati *et al.* 2010: 778, 780); *Cymbella hubeiensis* Li (in Gong *et al.* 2013: 34), have been documented.

Observations of diatoms from alpine ponds on mountain Kozuf, North Macedonia, yielded a population having entirely biseriate striae and lacking APFs. Such a combination has so far not been observed in any other species of *Cymbella* and is described herein as *Cymbella biseriata* Videska, Zaova & Levkov *sp. nov*.

Materials and methods

Mountain Kozuf is located in the south-east part of the Republic of North Macedonia (Fig. 1). To the east it is limited by the Vardar Valley, to the west with Mountain Kozjak, and to the north with Tikvesh Valley. The mountain is mainly composed of Paleozoic shales and above them are Triassic limestones and volcanic rocks. Due to the high altitude of the mountain, several freshwater alpine ponds are present. One alpine pond is present on Adjibarica at an elevation of 1690 m asl, which represents a small oligo-dystrophic pond. The field sampling campaign was conducted in June 2020. Epiphyton (on moss) were collected manually and fixed with 4 % formalin. The samples 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 30 min at 80 °C. Using distilled water, the samples were rinsed five times and then subsequently centrifuged at least five times in period of 10 minutes. Permanent diatom slides were prepared using Naphrax.

Slides were observed under oil immersion at 1500× magnification with a Nikon Eclipse 80i microscope (objective magnification capacity of 100× and numerical aperture of 1.4) and photomicroscopy was done using a Nikon Coolpix P6000 camera. The slides were deposited in the Macedonian National Diatom Collection (MKNDC) at the Institute of Biology, Faculty of Natural Sciences, Skopje.

For scanning electron microscopy (SEM), the material was prepared by drying a clean diatom suspension onto cover slips attached to the SEM stubs using carbon tape, and coated with gold-palladium (Polaron SC7640 sputter coater, Quorum Technologies, Ashford, UK). SEM observations were performed using a Cambridge S4 Stereoscan at 10 kV and working distance of 10 mm (Cambridge Instruments Ltd, Cambridge, UK) at the Friedrich Hustedt Study Centre for Diatoms (BM), Bremerhaven, Germany.

Results

***Cymbella biseriata* Videska, Zaova & Levkov** *sp. nov.* **(LM Figs 2–22, SEM Figs 23–34)**

Description:—LM(Figs 2–22): Valves slightly dorsiventral, semilanceolate and asymmetrical. Valve ends slightly subcapitate. Dorsal margin slightly arched, ventral side almost straight. Valve dimensions: length 29–38 µm, width 7.0–9.0 µm (n=48). Axial area very narrow, following raphe, central area small, slightly expressed on dorsal side, absent on ventral side. 4–7 stigmata present in central area on ventral side. Raphe ventrally displaced, distinctly lateral becoming reverse-lateral near central area; filiform at terminal ends. Dorsal striae slightly radiate, 12–15 in 10 µm. APF absent. Areolae not visible in LM.

SEM(Figs 23–34): Externally, raphe branches curved (Figs 23–25), proximally reverse lateral with large drop-shaped central pores, slightly dorsally bent (Fig. 26). Distally, raphe ends abruptly dorsally bent (Figs 27, 28). APF absent at both valve apices (Figs 24, 26). Central area narrow, more expressed on dorsal side, semi-lanceolate. Stigmata externally with small round openings (Fig. 26). Striae entirely biseriate composed of narrow slit-like to tilde-like areolae

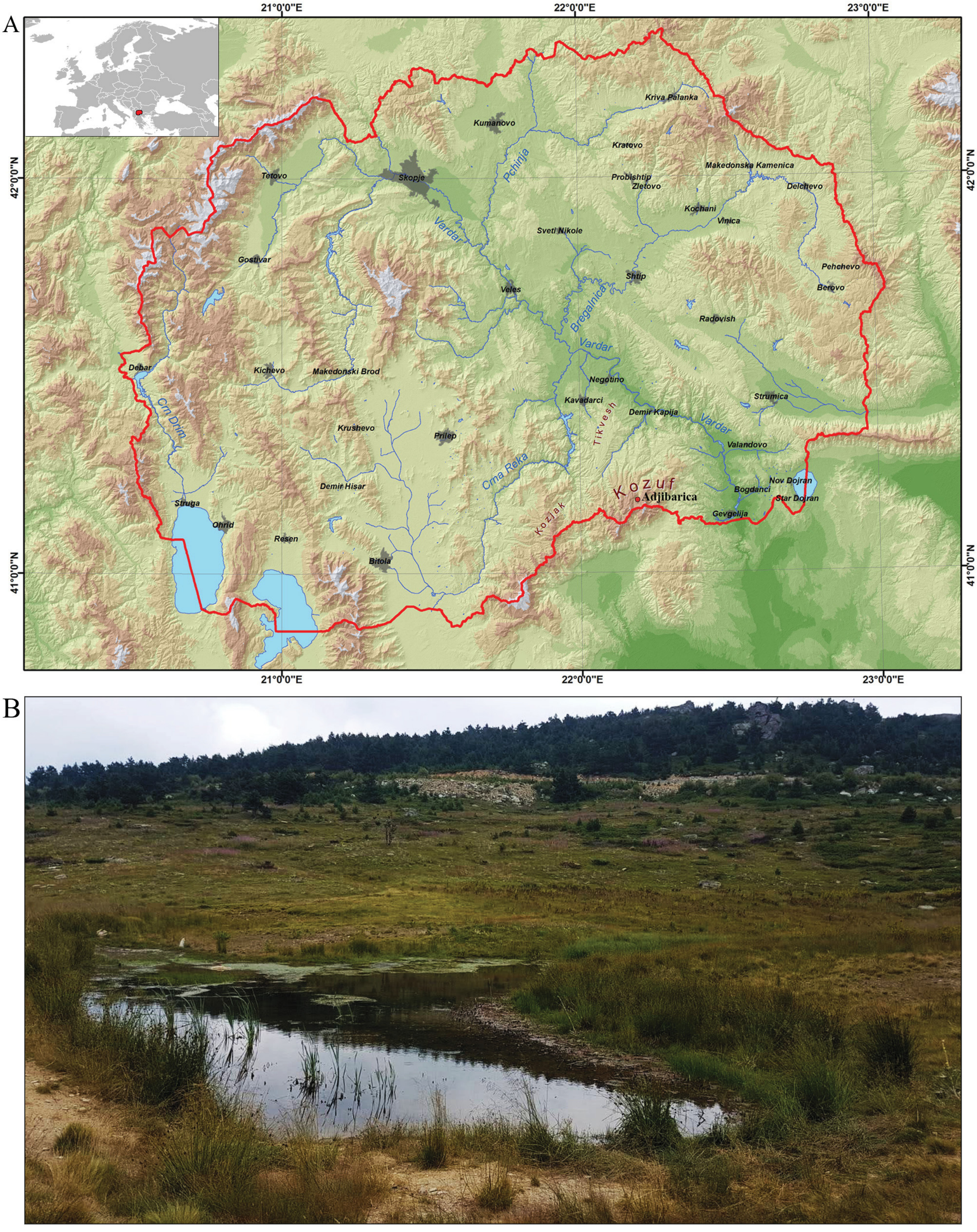
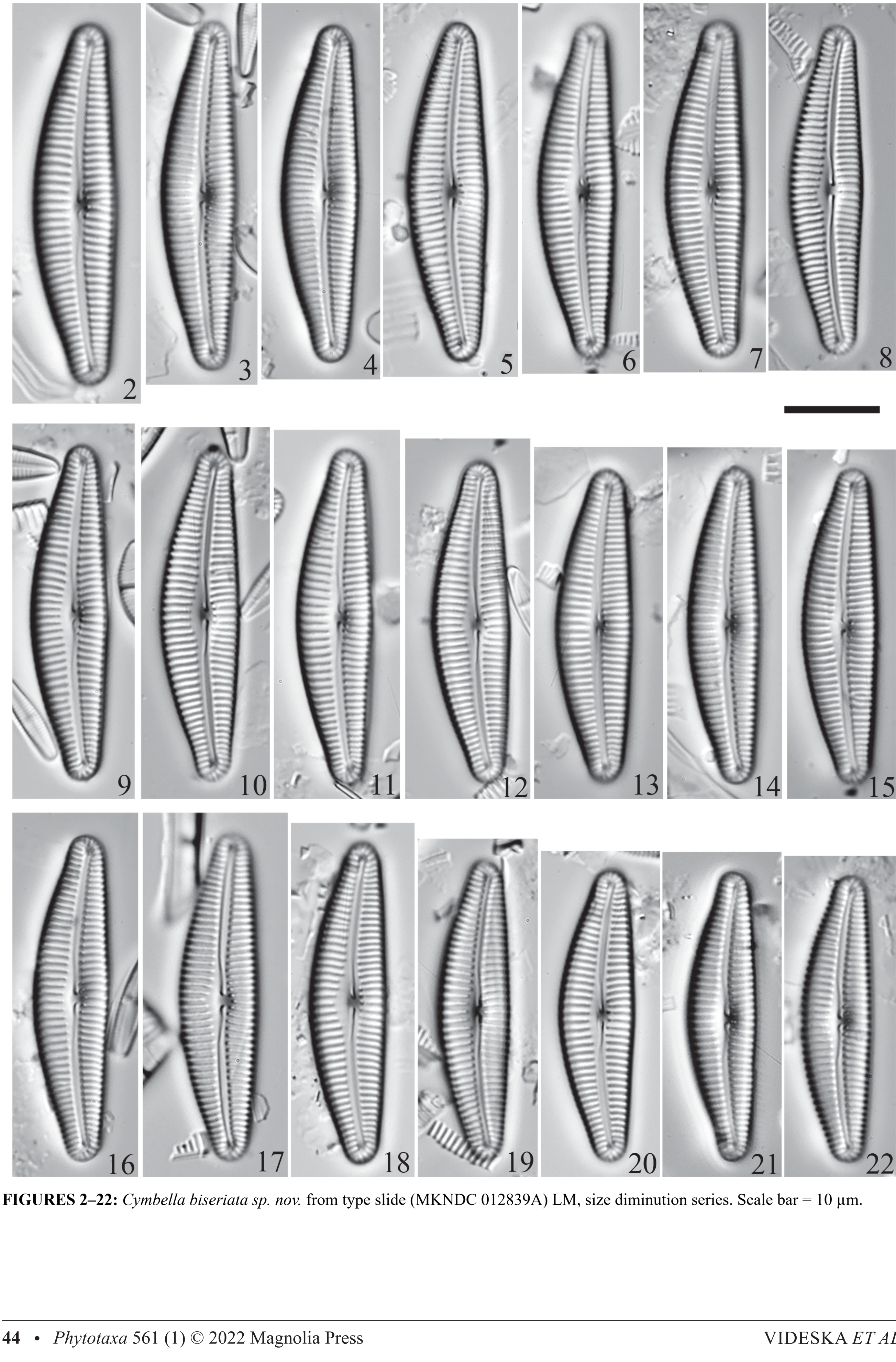
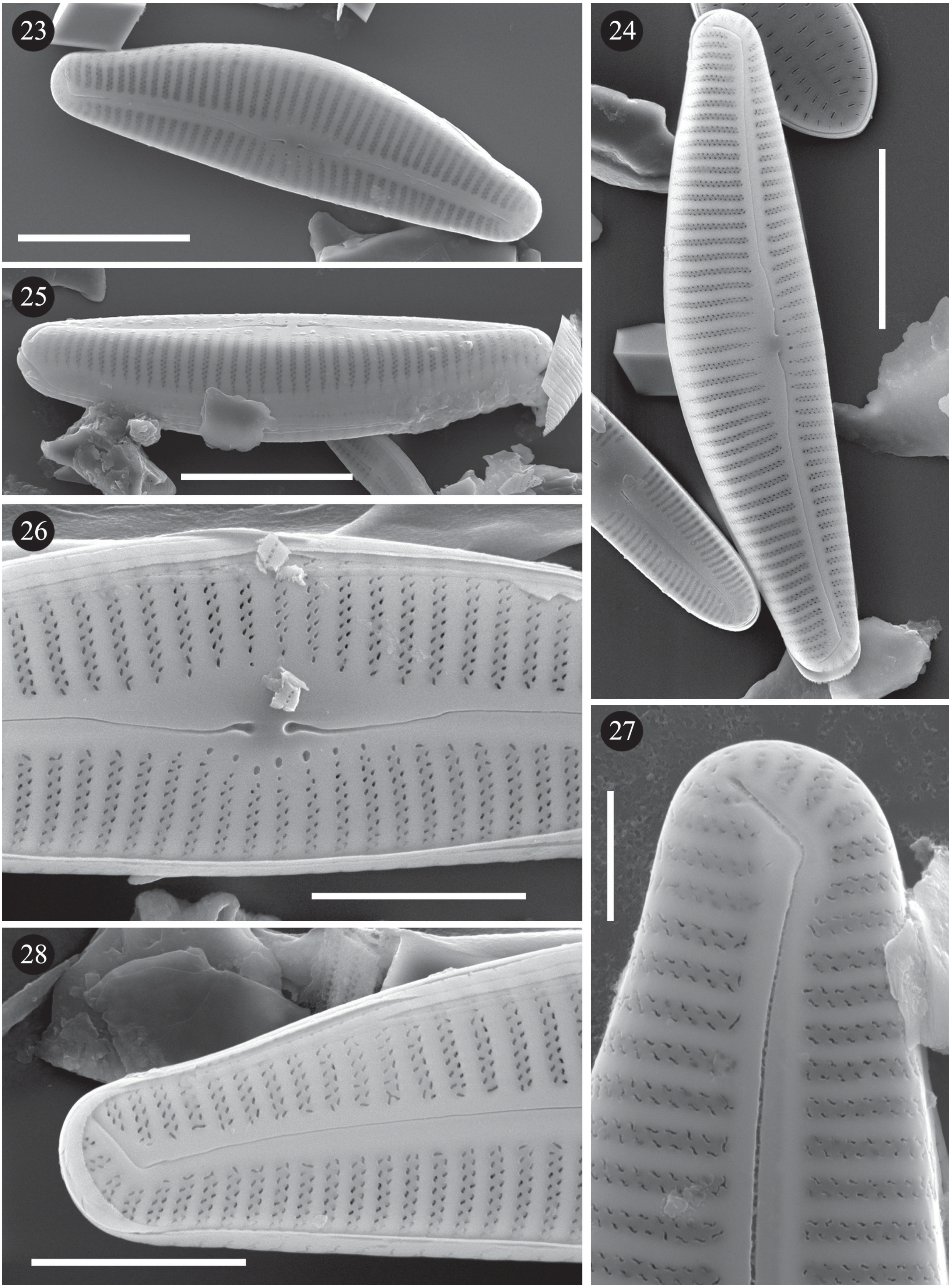


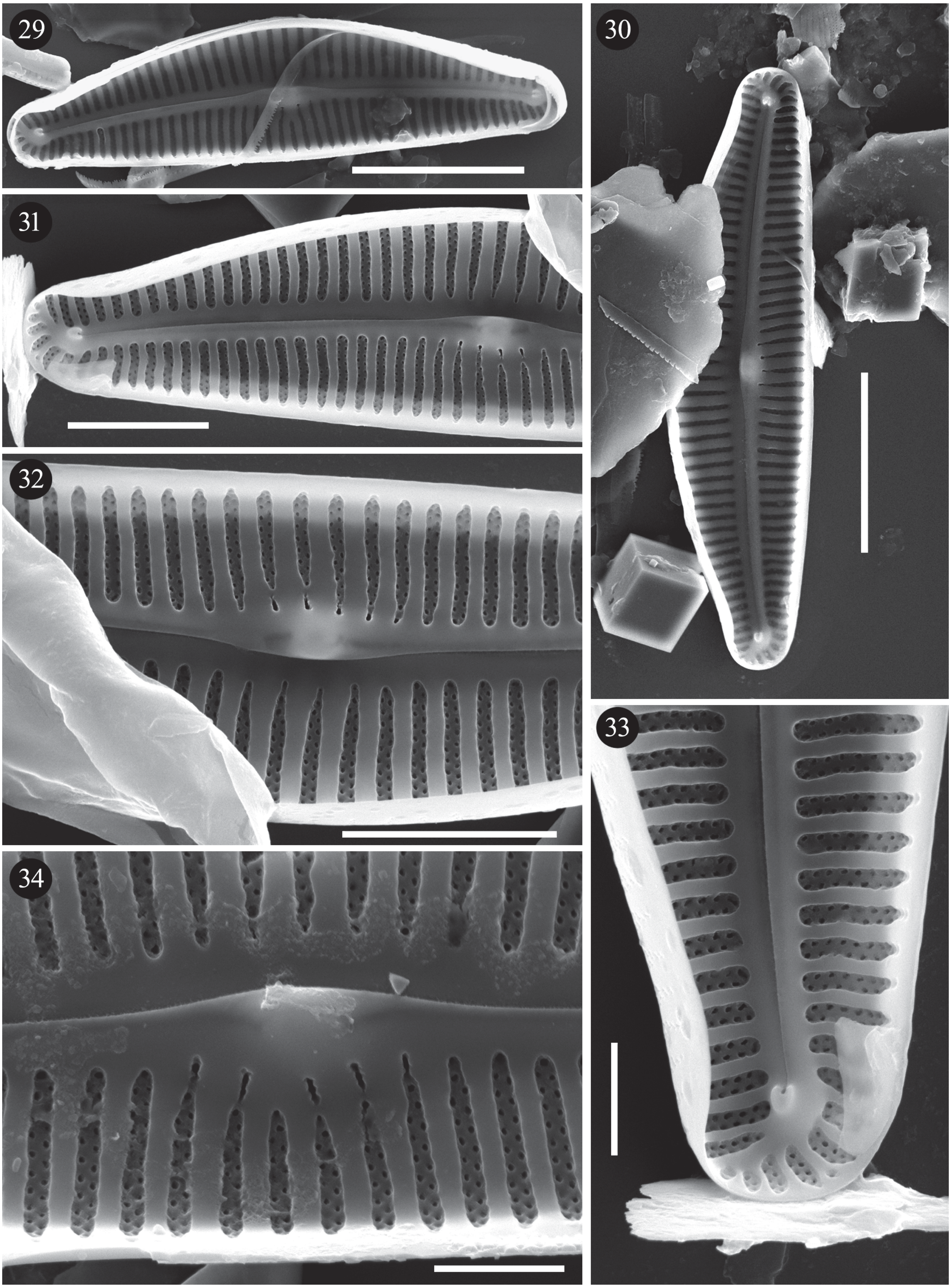
FIGURE 1: Map of North Macedonia with location of mountain Kozuf and the type locality, and image of the latter.



FIGURES 2–22: *Cymbella biseriata sp. nov.* from type slide (MKNDC 012839A) LM, size diminution series. Scale bar = 10 μm.



FIGURES 23–28: *Cymbella biseriata sp. nov*. SEM from type material (MKNDC 012839). 23, 24. External views of whole valve. 25. External view of dorsal side of frustule. Transition between valve face and mantle is gradual and striae are biseriate and not interrupted on valve mantle. 26. Detail views of the mid-valve showing biseriate striae and external round openings of stigmata. 27, 28. Detail view of valve apices showing distal raphe ends and absence of APF. Scale bar = 10 µm (Figs 23–25), 5 µm (Figs 26, 28) and 2 µm (Fig. 27).



FIGURES 29–34: *Cymbella biseriata sp. nov*. SEM from type material (MKNDC 012839). 29, 30. Internal views of the whole valves. 31, 32, 34. Detail view of the mid-valve. 33. Internal view of valve apices showing raphe distally terminates with large knob-like helictoglossae. Scale bar = 10 μm (Figs 29, 30), 5 μm (Figs 31, 32) and 2 μm (Figs 33, 34).

usually in oblique orientation, ca. 40 in 10 µm (Figs 23, 24). Internally, raphe curved (Figs 29, 30) distally terminates with large knob-like helictoglossae (Figs 31, 33), proximally covered by overgrowth of silica (Figs 32, 34) hiding proximal raphe ends (lacking "intermissio"). Striae entirely biseriate, areolae internally with small round openings (Figs 32–34). Striae separated with strongly thickened vimines with same width or wider than striae (Figs 31, 32). Stigmata with small elongated internal openings, weakly or not separated from striae (Figs 32, 34).

Type:—NORTH MACEDONIA. Mountain Kozuf: Adzibarica, moss in alpine pond, 41.1841904 N, 22.2035631 E. 1690 m asl, *A Videska, 4th June 2020* (holotype MKNDC 012839A! isotype BM 81943).

Etymology:—The epithet '*biseriata'* refers to entirely biseriate striae.

Ecology:—*Cymbella biseriata* has been observed only at the type locality, an oligo-dystrophic alpine pond on Adzibarica, mountain Kozuf. Data on the physical and chemical characteristics of the type locality have not been obtained, but floristical composition (ass. *Caricetum macedonicae* with *Carex macedonica* Bornmüller, *C. paniculata* Linnaeus, *C. stellulata* Goodenough, *Eriophorum latifolium* Hoppe, *Geum coccineum* Sibthorp John & Smith James Edward indicates oligo-dystrophic conditions of the habitat. Diatom species present in the sample are: *Encyonema venticosum* (Kützing) Grunow (in Schmidt *et al.* 1874–1959, pl. 10, fig. 59), *Odontidium mesodon* (Ehrenberg) Kützing (1844, p. 44), *Gomphonema elegantissimum* E.Reichardt & Lange-Bertalot, (in Hofmann *et al.* 2011: 302), *G. confusum* Levkov, Mitic-Kopanja & E.Reichardt, (2016: 41) *Achnanthidium microcephalum* Kützing (1844: 75), *Cymbella alpestris* Krammer (2002: 163), *C. excisiformis* Krammer (2002: 160), *Delicatophycus alpestris* Wynne (2019: 1), *Nitzshia perminuta* (Grunow) M. Peragallo (1903: 672).

Discussion

Based on its morphological features, *Cymbella biseriata* resembles the group of species morphologically close to the *C. helvetica* species complex (Table 1) that comprises several taxa characterized by the absence of APFs such as *C. balatonis* Grunow (in Schmidt 1874–1959 pl. 10, fig. 19), *Cymbella cantonatii* Lange-Bertalot (in Krammer 2002: 174); *C. compacta* Østrup (1910: 54), *C. helvetica* Kützing (1844: 79), *C. lange-bertalotii* Krammer (2002: 174), *C. ohridana* Levkov & Krstic (in Levkov *et al.* 2007: 43), *C. oligocenica* Schiller & Krammer (in Krammer 2002: 157), *C. suavis* Pantocsek (1892, pl. 15, fig. 229) and *C. subhelvetica* Krammer (2002: 174). All the above mentioned species have uniseriate striae composed by slit-like areolae, whereas *C. biseriata* has biseriate striae throught the whole valve. *Cymbella biseriata* can be easily distinguished from all these taxa by several other morphological features.

Differences between *C. cantonatii* and *C. biseriata* are valve width (10–13 µm in *C. cantontii* vs. 7.0–9.0 µm in *C. biseriata*), areolae density (29–33/10 µm in *C. cantonatii* vs. 40–50 in 10 µm in *C. biseriata*), areolae shape (x-shaped or modified x-shaped in *C. cantonatii* vs. small slit-like to tilde-like in *C. biseriata*), and, the most prominent morphological feature, the presence of biseriate striae in *C. biseriata*. Smaller specimens of *C. compacta* have a comparable valve shape (slightly dorsiventral, lanceolate-elliptical) and length as *C. biseriata* (length 28–76 µm in *C. compacta*), but it has broader valves (11–15 µm) with coarser striae (with 18–24 areolae in 10 µm). *Cymbella subhelvetica* has a comparable valve width (8.0–10.7 µm) to *C. biseriata*, but can be easily differentiated by valve shape (lanceolate in *C. subhelvetica*), valve length (33–67 µm), and stria (9–11 in 10 µm) and areola (20–25 in 10 µm) densities. *Cymbella biseriata* can be easily differentiated from *C. lange-bertalotii* by its valve size (length 38–100 µm, width 10–16 µm in *C. lange-bertalotii*), and stria (8–12 in 10 µm) and areola densities (17–25 in 10 µm). *Cymbella helvetica* is characterized by much larger valves (length 75–154 µm, width 18–26 µm) and thus can be easily differentiated from *C. biseriata*. Similarly, differentiation between *C. balatonis*, with broadly rhomboid valves and large cells (length 78–120 µm, width 24–30 µm), and *C. biseriata* (length 29–38 µm, width 7–9 µm), is straightforward. *Cymbella suavis* is a fossil species with similar valve size and shape as *C. balatonis* but it can be easily distinguished by its size, shape, stria and areola densities, as well as absence of stigmata in the central area. *Cymbella oligocenica*, another fossil species, is characterized by lanceolate-elliptical valves, 60–66 µm long and 23–24 µm wide with very coarse striae (12–14 areolae in 10 µm) composed of x-shaped areolae (Krammer 2002); these features make it easy to differentiate from *C. biseriata*. More recently, a new species from this complex was described from Lake Ohrid as *C. ohridana*, which is characterized by strongly dorsiventral valves with bluntly rounded apices, 59–103 µm long and 18.0–26.6 µm wide, with uniseriate striae composed of 16–18 areolae in 10 µm. *Cymbella biseriata* can be easily differentiated from the latter species by its smaller valve size, shape and areola density.

TABLE 1: Morphological features and measurments of *Cymbella biseriata sp. nov*. and comparison with similar *Cymbella* species.

Taxon	Valve length (in µm)	Valve width (in µm)	Striae/ 10 µm	Areolae/ 10 µm	Striae type	Stigma	Central area	Apices	APF	Reference
C. biseriata sp. nov.	29–38	7–9	12–15	40–50	Biseriate	4–7	Nearly absent or slight broaden of axial area	Slightly subcapitated	Absent	This study
C. cantonatii	30–55	10–13	10–13	29–33	Uniseriate	4–8	More pronounced on dorsal side	Rounded, not distinguished from valve body	Absent	Lange-Bertalot in Krammer (2002)
C. compacta	28–76	12–15	9–12	18–24	Uniseriate	4–8	Lacking or not well expressed	Rounded, only in large specimens distinguished form valve body	Absent	Østrup (1910)
C. subhelvetica	33–70	8.5–11	9–11	20–25	Uniseriate	4–8	Lacking on dorsal side, not well expressed on ventral side	Narrowly rounded, not or barely distinguished from valve body	Absent	Krammer (2002)
C. lange-bertalotii	38–100	10–16	8–12	18–24	Uniseriate	4–8	Lacking or not well expressed	Narrowly rounded, not or barely distinguished from valve body	Absent	Krammer (2002)
C. helvetica	75–154	18–26	6–8	14–18	Uniseriate	Up to 10	Roundish to elliptical longest at apical axis	Rounded, not or barely distinguished from valve body	Absent	Kützing (1844)
C. balatonis	78–120	24–30	7–8	15–17	Uniseriate	3–8	Elliptical or rhomboidal, longest at apical axis	Narrowly rounded, not or barely distinguished from valve body	Absent	Schmidt *et al.* (1874–1959)
C. suavis	81–120	28–32	6–7	8–13	Uniseriate	Not well observed in LM	Elliptical, longest at apical axis	Rounded to moderately convex	Absent	Pantocsek (1892)
C. oligocenica	60–66	23–24	11–12	12–14	Uniseriate	3–4	Very small, more pronounced on dorsal side	Rounded, not distinguished from valve body	Absent	Krammer (2002)
C. ohridana	59–103	18–26.6	10–12	16–18	Uniseriate	8–12	Asymmetrical to elliptical, more developed dorsally	Bluntly rounded, not protracted	Absent	Levkov *et al.* (2007)
C. duplopunctata	125–150	26–32	7–9	12–15	Biseriate	9–12	Large, orbicular, 1/3–1/2 the width of the valve	Not protracted, broadly rounded	Present	Krammer (2002)
C. buechleri	175	32	6–9	12–13	Biseriate	5	Large, orbicular, 1/3–1/2 the width of the valve	Rounded, not protracted	Present	Krammer (2002)

As mentioned above, there are two known, fossil species of *Cymbella* with biseriate striae: *C. duplopunctata* and *C. buechleri*. Besides their large size (longer than 125 µm and wider than 26 µm), both have distinct APFs at the valve apices and large central areas with clearly separated stigmata from central striae. These features make the separation of *C. biseriata* from both *C. duplopunctata* and *C. buechleri* very simple. Only a single image of *C. duplopunctata* has been published (Krammer 2002, fig. 116: 6) showing the internal view of the striae, so the ultrastructure remains largely unknown. Recently several *Cymbella* species with partly biseriate striae were described. All of them share features typical for *Cymbella* sensu stricto. As was mentioned by Liu *et al.* (2018: 45), up to now, living species with entirely biseriate striae were unknown, thus *C. biseriata* represents the only recent species with this feature.

Several diatom genera include species with uniseriate and biseriate striae such *Hippodonta* Lange-Bertalot, Witkowski & Metzeltin (1996: 249), *Sellaphora* Mereschkowsky (1902: 186) and *Rossithidium* F.E. Round & Bukhtiyarova (1996: 350). *Gomphonema* Ehrenberg (1832: 87) is one of the few genera with species that have uniseriate (most of the species), partly biseriate such as *G. acuminatum* (Ehrenberg 1832: 88) and *G. juriljii* Levkov, Mitic-Kopanja & E. Reichardt (Levkov *et al.* 2016: 68) and entirely biseriate striae such as *G. minutum* (Agardh) Agardh (1831: 34), *G. confusum* Levkov, Mitic-Kopanja & E. Reichardt (Levkov *et al.* 2016: 41, 42) and *G. irroratum* Hustedt (1945: 42). Both *Gomphonema* and *Cymbella* are considered to be paraphyletic and one possible solution would be to subdivide them into narrower genera representing monophyletic lineages (Nakov *et al.* 2014). Further molecular study might yield novel information about the relationships and phylogenetic position of *C. biseriata* (and entire *C. helvetica* complex) to other *Cymbella* sensu stricto species. At the moment, although *C. biseriata* has few ultrastructural features different to that of *Cymbella* sensu stricto, we consider it as part of this genus.

According to Liu *et al*. (2022) presence of tilde-shaped areolae and dorsally deflected distal raphe fissures are the characters defining genus *Delicatophycus*. However, the dorsally deflected raphe fissure is present in all *Cymbella* sensu stricto species, thus can not be used as differential character. Therefore, the only morphological character that can be used for separation of genera *Cymbella* and *Delicatophycus* is the shape of areolae. Part of the areolae in *C. biseriata* have tilde-shaped openings, but they are much smaller, and the shape is not pronounced as in other typical *Delicatophycus* taxa and thus cannot be treated as a member of the genus *Delicatophycus*. All morphological and ultrastructural features, such as absence of APF, raphe morphology (proximal and distal raphe ends), stigmata openings are identical to the *C. helvetica* species group and thus we consider this species as part of *Cymbella* sensu lato.

Alpine ponds and small lakes are considered as highly diverse habitats and play a key role in preserving freshwater biodiversity (Williams *et al*. 2004, Biggs *et al*. 2005). They are essential for freshwater biodiversity and especially recognized as stepping stone habitats (Ewald *et al.* 2010). In Europe establishing freshwater habitat protection, especially pond protection is led by Directive 92/43/EEC, known as the Habitat Directive. Biodiversity in alpine ponds is highly related to altitude, with increasing altitude a reduction of species occurs and communities become simpler compared to lowland environments (Hinden *et al*. 2005, Oertli *et al*. 2009). However, there is very scarce information about the biodiversity in alpine ponds (Hinden *et. al*. 2005), especially in North Macedonia (Levkov *et al.* 2005). Several studies indicate that freshwater alpine ecosystems are considered as a source of high diatom diversity (Cantonati *et al*. 2011, Falasco *et al.* 2012, Blanco *et al.* 2020). Additionally, all of these studies highlighted the contribution of alpine ponds to the conservation of diatom biodiversity (Beauger *et al.* 2020). This is due to the fact that mountain freshwater ecosystems are more conserved from anthropogenic impact than lowland environments (Müller *et al.* 1998).

However, alpine ponds may be potentially vulnerable ecosystems in terms of acidification, eutrophication and climate changes, and therefore are primarily influenced by species loss (Lotter *et al.* 1999, Körner 2004). These habitats are now considered as “refugia” for many rare and sensitive species and inhabit by endangered species (Hofmann *et al.* 2018). Their protection is essential for conservation of regional freshwater biodiversity and thus to reduce global biodiversity loss.

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References

Agardh, C.A. (1830) *Conspectus Criticus Diatomacearum*. Part 1. Lundae. Literis Berlingianus, pp. 1–16.

Agardh, C.A. (1831) *Conspectus criticus diatomacearum*. Part 3. Lundae. Litteris Berlingianis, pp. 33–48.

Bahls, L.L. (2015) *Kurtkrammeria*, a new genus of freshwater diatoms (Bacillariophyta, Cymbellaceae) separated from *Encyonopsis*. *Nova Hedwigia* 101 (1–2): 165–190.
https://doi.org/10.1127/nova_hedwigia/2015/0263

Beauger, A., Allain, E., Voldoire, O., Wetzel, C.E., Ector, L. & Van de Vijver, B. (2020) Temporal Evolution of Diatoms in a Temporary Pond Situated in the Massif du Sancy Mountains (Massif Central, France) and Description of a New *Pinnularia* Species. *Diversity* 12: 367.
https://doi.org/10.3390/d12100367

Biggs, J., Williams, P., Whitfield, M., Nicolet, P. & Weatherby, A. (2005) 15 years of pond assessment in Britain: results and lessons learned from the work of Pond Conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 15: 693–714.
https://doi.org/10.1002/aqc.745

Blanco, S.; Olenici, A.; Ortega, F.; Jiménez-Gómez, F.; Guerrero, F. Identifying environmental drivers of benthic diatom diversity: The case of Mediterranean mountain ponds. *PeerJ* 8: e8825.
https://doi.org/10.7717/peerj.8825

Cantonati, M., Lange-Bertalot, H., Scalfi, A. & Angeli, N. (2010) *Cymbella tridentina* sp. nov. (Bacillariophyta), a crenophilous diatom from carbonate springs of the Alps. *Journal of the North American Benthological Society* 29 (3): 775–788.
https://doi.org/10.1899/09-077.1

Cantonati, M., Lange-Bertalot, H., Decet, F. & Gabrieli, J. (2011) Diatoms in very-shallow pools of the site of community importance Danta di Cadore Mires (south-eastern Alps), and the potential contribution of these habitats to diatom biodiversity conservation. *Nova Hedwigia* 93: 475–507.
https://doi.org/10.1127/0029-5035/2011/0093-0475

Ehrenberg, C.G. (1832) Über die Entwicklung und Lebensdauer der Infusionsthiere; nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. *Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin* 1831: 1–154, 4 pls.

Ewald, N., Nicolet, P., Oertli, B., Della, Bella, V., Rhazi, L., Reymond, A.-S., Minssieux, E., Saber, E.-R., Rhazi, M., Biggs, J., Bressi, N., Cereghino, R., Grillas, P., Kalettka, T., Hull, A., Scher, O. & Serrano, L. (2010) A preliminary assessment of Important Areas for Ponds (IAPs) in the Mediterranean Basin and Alpine Arc. *European Pond Conservation Network (EPCN)*.

Falasco, E., Ector, L., Ciaccio, E., Hoffmann, L. & Bona, F. (2012) Alpine freshwater ecosystems in a protected area: a source of diatom diversity. *Hydrobiologia* 695 (1): 233–251.
https://doi.org/10.1007/s10750-012-1114-0

Glushchenko, A., Kuznetsova, I., Kociolek, J.P. & Kulikovskiy, M. (2019) *Karthickia verestigmata* gen. et sp. nov. – an interesting diatom with frustular morphology similar to several different cymbelloid diatom genera. *Phycologia* 58 (2): 605–613.
https://doi.org/10.1080/00318884.2019.1626605

Gong, Z., Li, Y., Metzeltin, D. & Lange-Bertalot, H. (2013) New species of *Cymbella* and *Placoneis* (Bacillariophyta) from late Pleistocene fossil, China. *Phytotaxa* 150 (1): 29–40.
https://doi.org/10.11646/phytotaxa.150.1.2

Hinden, H., Oertli, B., Menetrey, N., Sager, L. & Lachavanne, J.B. (2005) Alpine pond biodiversity: what are the related environmental variables? *Aquatic Conservation: Marine and Freshwater Ecosystems* 15: 613–624.
https://doi.org/10.1002/aqc.751

Hofmann, G., Werum, M. & Lange-Bertalot, H. (2011) Diatomeen im Süßwasser-Benthos von Mitteleuropa. *Koeltz Scientific Books, Königstein*, 908 pp.

Hofmann, G., Lange-Bertalot, H., Werum, M. & Klee, R. (2018) Rote Liste und Gesamtartenliste der limnischen Kieselalgen (Bacillariophyta) Deutschlands. *In:* Metzing, D., Hofbauer, N., Ludwig, G. & Matzke-Hajek, G. (eds.) *Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutschlands*, Band 7: Pflanzen. Landwirtschaftsverlag Münster, Naturschutz und Biologische Vielfalt, Münster, pp. 601–708.

Hustedt, F. (1945) Diatomeen aus Seen und Quellgebieten der Balkan-Halbinsel. *Archiv für Hydrobiologie* 40: 867–973, 12 pls.

Jüttner, I., Krammer, K., Van de Vijver, B., Tuji, A., Simkhada, B., Gurung, S., Sharma, S., Sharma, C. & Cox, E.J. (2010a) *Oricymba* (Cymbellales, Bacillariophyceae), a new cymbelloid genus and three new species from the Nepalese Himalaya. *Phycologia* 49: 407–423.
https://doi.org/10.2216/09-77.1

Jüttner, I., Gurung, S., Sharma, C., Sharma, S., De Haan, M. & Van de Vijver, B. (2010b) Morphology of new taxa in the *Cymbella aspera*

and *Cymbella neocistula* groups, *Cymbella yakii* sp. nov. and *Cymbella* cf. *hantzschiana* from Everest National Park, Nepal. *Polish Botanical Journal* 55: 73–92.
https://doi.org/10.11646/phytotaxa.344.1.5

Kapustin, D.A., Kulikovskiy, M. & Kociolek, J.P. (2017) *Celebesia* gen. nov., a new Cymbelloid diatom genus from the ancient lake Matano (Sulawesi Island, Indonesia). *Nova Hedwigia* 146: 147–155.
https://doi.org/10.1127/1438-9134/2017/147

Kociolek, J.P. & Stoermer, E.F. (1988) A preliminary investigation of the phylogenetic relationships among the freshwater, apical porefield-bearing cymbelloid and gomphonemoid diatoms (Bacillariophyceae). *Journal of Phycology* 24: 377–385.

Körner, C. (2004) Mountain biodiversity, its causes and function. *AMBIO: A Journal of the Human Environment* 33 (13): 11–17.
https://10.1007/0044-7447-33.sp13.11

Krammer, K. (1982) Valve morphology in the genus *Cymbella* C.A. Agardh. *In:* Krammer, K. (ed.) *Micromorphology of diatom valves*, Vol. 11. J. Cramer, Vaduz, 299 pp.

Krammer, K. (1997a) Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil 1. Allgemeines und Encyonema Part. *Bibliotheca Diatomologica* 36: 1–382.

Krammer, K. (1997b) Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil. 2. *Encyonema* part., *Encyonopsis* and *Cymbellopsis*. *Bibliotheca Diatomologica* 37: 1–469.

Krammer, K. (1999) *Annotated Diatom Micrographs.* Iconographia Diatomologica 6. pp. 1–292.

Krammer, K. (2002) The genus *Cymbella*. *In: Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats.* Vol. 3. pp. 1–584.

Krammer, K. (2003) *Cymbopleur*a, *Delicata*, *Navicymbula*, *Gomphocymbellopsis*, *Afrocymbella*. *In: Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats.* Vol. 4. pp. 1–530.

Kützing, F.T. (1833) Synopsis Diatomacearum oder Versuch einer systematischen Zusammenstellung der Diatomeen. *Linnaea* 8 (5): 529–620, pls. XIII–XIX.

Kützing, F.T. (1844) *Die kieselschaligen Bacillarien oder Diatomeen*. Nordhausen, 152 pp.
https://doi.org/10.5962/bhl.title.64360

Lange-Bertalot, H., Metzeltin, D. & Witkowski, A. (1996) *Hippodonta gen. nov.* Iconographia Diatomologica 4. pp. 247–276.

Levkov, Z., Krstic, S., Nakov, T. & Melovski, Lj. (2005) Diatom assemblages on Shara and Nidze Mountains, Macedonia. *Nova Hedwigia* 81 (3–4): 501–538.
https://doi.org/10.1127/0029-5035/2005/0081-0501

Levkov, Z., Krstic, S., Metzeltin, D. & Nakov, T. (2007) *Diatoms of Lakes Prespa and Ohrid (Macedonia).* Iconographia Diatomologica 16. pp. 1–603.

Levkov, Z., Mitić-Kopanja, D. & Reichardt, E. (2016) The diatom genus *Gomphonema* from the Republic of Macedonia. *In: Diatoms of Europe* 8. pp. 1–552.

Liu, B., Williams, D.M. & Liu, Q.Y. (2018) A new species of *Cymbella* (Cymbellaceae, Bacillariophyceae) from China, possessing valves with both uniseriate and biseriate striae. *Phytotaxa* 344: 39–46.
https://doi.org/10.11646/phytotaxa.344.1.5

Liu, B., Zhou, Y-Y. Blanco, S. & Williams, D.M. (2022) Three new species of *Delicatophycus* M.J. Wynne (Bacillariophyta) from China, all possessing apical pore fields. *Fottea*, *Olomouc* 22 (1) 137–151.

Lotter, A.F., Pienitz, R. & Schmidt, R. (1999) Diatoms as indicators of environmental change near Arctic and Alpine treeline. *In:* Stoermer, E.F. & Smol, J.P. (eds.) *The diatoms: application for the environmental and earth sciences*. Cambridge University Press, Cambridge, 205–226 pp.

Mereschkowsky, C. (1902) On *Sellaphora*, a new genus of diatoms. *Journal of Natural History*, Series 7, 9 (51): 185–195.

Metzeltin, D., Lange-Bertalot, H. & Nergui, S. (2009) *Diatoms in Mongolia.* Iconographia Diatomologica 20. pp. 1–684.

Müller, B., Lotter, A.F., Sturm, M. & Ammann, A. (1998) Influence of Catchment Quality and Altitude on the Water and Sediment Composition of 68 Small Lakes in Central Europe. *Aquatic Sciences* 60: 316–337.
https://doi.org/10.1007/s000270050044

Nakov, T., Ruck, E.C., Galachyants, Y., Spaulding, S.A. & Theriot, E.C. (2014) Molecular phylogeny of the Cymbellales (Bacillariophyceae, Heterokontophyta) with a comparison of models for accommodating rate variation across sites. *Phycologia* 53 (4): 359–373.
https://doi.org/10.2216/14-002.1

Oertli, B., Céréghino, R., Hull, A. & Miracle, R. (2009) Pond conservation: from science to practice. *Hydrobiologia* 634: 1–9.
https://doi.org/10.1007/s10750-009-9891-9

Østrup, E. (1910) *Danske Diatoméer*. C.A. Reitzels Boghandel, Kjøbenhavn, 323 pp., 5 pls.

Pantocsek, J. (1892) *Beiträge zur Kenntnis der Fossilen Bacillarien Ungarns*. 3 Teile. Julius Platzko, Nagyy-Tapolcsany. Teil III, Susswasser Bacillarien. Anhang-analysen 15 neuer Depots von bulgarien, Japan, Mahern, Russland und Ungarn. Nagy-Tapolcsány, Buchdrucherei von Julius Platzko, 42 pls.

Peragallo, M. (1903) *Le Catalogue Général des Diatomées* 2. Clermont-Ferrand. pp. 472–973.

Round, F.E. & Bukhtiyarova, L. (1996) Four new genera based on *Achnanthes* (*Achnanthidium*) together with a re-definition of *Achnanthidium*. *Diatom Research* 11 (2): 345–361. https://doi.org/10.1080/0269249X.1996.9705389

Schmidt, A., Schmidt, M., Fricke, F., Heiden, H., Müller, O. & Hustedt, F. (1874–1959) *Atlas der Diatomaceen-kunde*. Heft 1–120, Tafeln 1–460 (Tafeln 1–216 A. Schmidt; 213–216 M. Schmidt; 217–240 F. Fricke; 241–244 H. Heiden; 245, 246 O. Müller; 247–256 F. Fricke; 257–264 H. Heiden; 265–268 F. Fricke; 269–472 F. Hustedt). Aschersleben-Leipzig. https://doi.org/10.5962/bhl.title.64396

Williams, P., Whitfield, M. & Biggs, J. (2004) Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. *Biological Conservation* 115: 329–341. https://10.1016/S0006-3207(03)00153-8

Wynne, M.J. (2019) *Delicatophycus* gen. nov.: a validation of "*Delicata* Krammer" inval. (Gomphonemataceae, Bacillariophyta). *Notulae algarum* 97: 1–3.

Zhang, W., Jüttner, I, Cox, E.J., Chen, Q. & Tan, H. (2018) *Cymbella liyangensis* sp. nov., a new cymbelloid species (Bacillariophyceae) from streams in North Tianmu Mountain, Jiangsu province, China. *Phytotaxa* 348: 14–22. https://doi.org/10.11646/phytotaxa.348.1.2