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Three new cymbelloid species in the genera *Cymbella*, *Cymbopleura* and *Delicatophycus* from the mineral spring Crvena Voda near Skopje, Republic of North Macedonia

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Three new cymbelloid species in the genera *Cymbella*, *Cymbopleura* and *Delicatophycus* from the mineral spring Crvena Voda near Skopje, Republic of North Macedonia

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Mineral springs are unique habitats frequently inhabited by specific diatom species with restricted distributions. In the mineral spring Crvena Voda near Skopje, three cymbelloid taxa were observed, whose morphological characteristics differ from already known taxa. They are described here as: *Cymbella parahelvetica* sp. nov., *Cymbopleura angustilanceolata* sp. nov. and *Delicatophycus fontinalis* sp. nov. *Cymbella parahelvetica* sp. nov. is characterized by the absence of an apical pore field at the valve ends and by very densely arranged striae. *Cymbopleura angustilanceolata* sp. nov. can be distinguished from other *Cymbopleura* species by the valve outline, the shape of the central area and the stria density. *Delicatophycus fontinalis* sp. nov. can be differentiated from similar species by its strongly arched dorsal margin, its valve width and stria density.

Keywords: *biodiversity, diatoms, morphology, new species, taxonomy*

Introduction

The genus *Cymbella* C.A. Agardh is one of the largest diatom genera comprising more than 500 names (Guiry & Guiry 2017). *Cymbella* sensu lato is considered heterogeneous and since the 1990s around 20 genera have been separated off, based on the presence or absence of apical pore fields (APF), the orientation of the distal raphe ends and the structure of the striae (Videska et al. 2022). The revision of cymbelloid taxa began with Krammer (1997a, b, 2002, 2003) and continued with the description of genera such as *Oricymba* Jüttner et al. (2010), *Kurtkrammeria* Bahls (2015), *Celebesia* Kapustin, Kulikovskiy & Kociolek (Kapustin et al. 2018) and *Karthickia* Kociolek, Glushchenko & Kulikovskiy (in Glushchenko et al. 2019). The heterogeneity of cymbelloid taxa was also confirmed by molecular analyses (Nakov et al. 2014).

Cymbelloid genera are predominantly freshwater (Krammer 2002, 2003) although some species have been observed from brackish waters or waters with high conductivity (Bustos et al. 2018), including from thermo-mineral springs (Thomas & Gonzalves 1965). The number of diatom species in thermo-mineral and mineral springs is usually low (Leira et al. 2017, Beauger et al. 2022), but the species are often unique and have very restricted distributions (Beauger et al. 2016). In this type of habitat, the number of cymbelloid taxa is almost always low with one or very few species (Lai et al. 2022). However, cymbelloid species are abundant and diverse in other springs (Delgado et al. 2013), especially in alpine habitats (Levkov et al. 2005, Cantonati et al. 2022), often characterized by low conductivity and nutrient content.

Although most *Cymbella* taxa occur in oligotrophic habitats (Krammer 2002) several species, such as *C. excisa* Kützing, *C. tumida* (Brébisson) Van Heurck, *C. tumidula* Grunow, are frequently found in eutrophic lakes and ponds. The species group around *C. aspera* (Ehrenberg) Cleve are quite common in oligo-dystrophic alpine ponds at high altitude. Similarly, species of *Cymbopleura* (Krammer) Krammer are diverse in oligotrophic habitats (Levkov et al. 2005, Bahls 2013a, 2017, 2019). Nearly all species of the relatively small genus *Delicatophycus* M. J. Wynne (= *Delicata* Krammer) live in freshwater but some have restricted distributions and are considered as endemics (Le Cohu et al. 2020, Liu et al. 2022).

Recently, a project on the diatom flora of the Republic of North Macedonia was undertaken. This study included the examination of so called ‘extreme’ habitats, including halomorphic soils, subaerial habitats (entrances of caves, wet rocks, temporary ponds) and thermo-mineral and mineral springs. Several cymbelloid taxa were observed in a mineral spring in the vicinity of Skopje, including three taxa that possess unique characters and are here described as new species.

Material and methods

Study site: The region of the spring is hilly with an average elevation of 581 m a.s.l., and a peak elevation of 841 m

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a.s.l. A dry climate with a strong Mediterranean influence prevails. The region represents a very important biodiversity hotspot, since it is the largest serpentine massif in Macedonia and supports many rare and endemic plant species and communities (Melovski et al. 2013). The Radusha Massif is the most significant ophiolite massif, covering an area of 60 km^2. The mineral spring near the village of Radusha is located near the road to the village, ca. 20 km from the city of Skopje, on the shore of the River Vardar (Fig. 1). The spring has been captured and modified as a fountain, surrounded by a stone structure. The water from the fountain forms a small slow-flowing creek (ca. 10 m long) before entering the River Vardar. Macrophytes are not present in the stream or fountain.

Chemical analyses: Water chemistry was analysed at the Chemical Laboratory of the State Hydrometeorological Institute in Skopje, Republic of North Macedonia. Measurements of water temperature, pH and conductivity were carried out in situ with a portable multimeter (Hach HQ40d, Germany). Samples for water chemistry were collected and kept on ice during transport to the laboratory. The following parameters were measured in compliance with monitoring standards (CEN – EN 15708, 2009): nitrites (NO_2^-), nitrates (NO_3^-), ammonium (NH_4^+), phosphates (PO_4^{3-}), chloride (Cl^-) and sulphates (SO_4^-). Main cations and heavy metals were measured using an Atomic Absorption Spectrometer (Agilent 55A, USA).

Diatom samples: Diatom samples from the epilithon and epipelon were collected from the fountain outflow. Epilithic diatoms were collected using a toothbrush, while epipelon was collected with a spoon, transferred to plastic vials and fixed with 4% formalin. In the laboratory, diatom samples were cleaned with $KMnO_4$ and 37% HCl and boiled for 30 min at 80 °C. The samples were subsequently rinsed five times with distilled water and centrifuged for 10 min. Permanent diatom slides were prepared using Naphrax® and observed in light microscopy (LM) under oil immersion at 1500 × magnification with a Nikon Eclipse 80i microscope. Photographs were taken 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 and in the Natural History Museum in London, UK (BM). For scanning electron microscopy (SEM), the material was prepared by drying a clean diatom suspension onto cover slips attached to SEM stubs, coated with gold-palladium, and studied with a Zeiss Gemini Ultra plus SEM microscope (working distance 3–12 mm, 3–5 kV), in the Natural History Museum, London, UK (Imaging and Analysis Centre).

Results

Taxonomic diagnoses

Cymbella parahelvetica Levkov, Zaova & Jüttner sp. nov. (Figs 2–20).

Description: LM (Figs 2–8): Valves asymmetrical, distinctly dorsiventral, dorsal margin moderately arched, ventral margin slightly convex to almost straight. Valve ends broadly rounded, not protracted. Valve dimensions length 59–95 µm, width 12.0–13.5 µm (n = 20). Axial area narrow, linear, following raphe, central area indistinct, not differentiated from axial area. 6–9 stigmata present in central area on ventral side, difficult to distinguish from stria areolae. Raphe located in central part of valve or slightly ventrally displaced, distinctly lateral becoming filiform near central area and terminal ends. Striae finely punctate, parallel to slightly radiate in mid-valve, 12–14 in 10 µm dorsally and 13–16 in 10 µm ventrally. Apical pore field absent. Areolae visible in LM, 25–30 in 10 µm.

SEM (Figs 9–20): Externally, raphe slightly arcuate located in central part of valve (Figs 9, 11). Proximal raphe branches slightly deflected with ends clearly expanded, tear-drop-shaped (Figs 10, 12), distal raphe ends strongly dorsally deflected, extending onto valve mantle (Figs 13, 14). Central area absent with several stigmata (isolated pores) with round external openings. Striae uniseriate comprised of areolae with variable shapes, slit-like, Y-shaped and most frequently X-shaped. Apical pore field (APF) absent at valve end. Internally, proximal raphe ends covered by overgrowth of silica (Figs 15–18), distally raphe terminating in large knob-like helictoglossae (Figs 19, 20). Virgae (interstriae) strongly thickened, wider than striae (Figs 16, 18). Striae uniseriate, internal areolar openings round to oval (Fig. 19). Stigmata (isolated pores) with small transapically elongated to irregularly-shaped internal openings, not separated from striae (Figs 16, 18).

Holotype: Surface red sediment, collection date: 27.06.2020; Leg. Zlatko Levkov; Coordinates: 42.0553666° N, 21.2543255° E (Accession No. MKNCD 12910), Slide MKNDC 12910A (holotype),

Isotype: Slide BM 092323

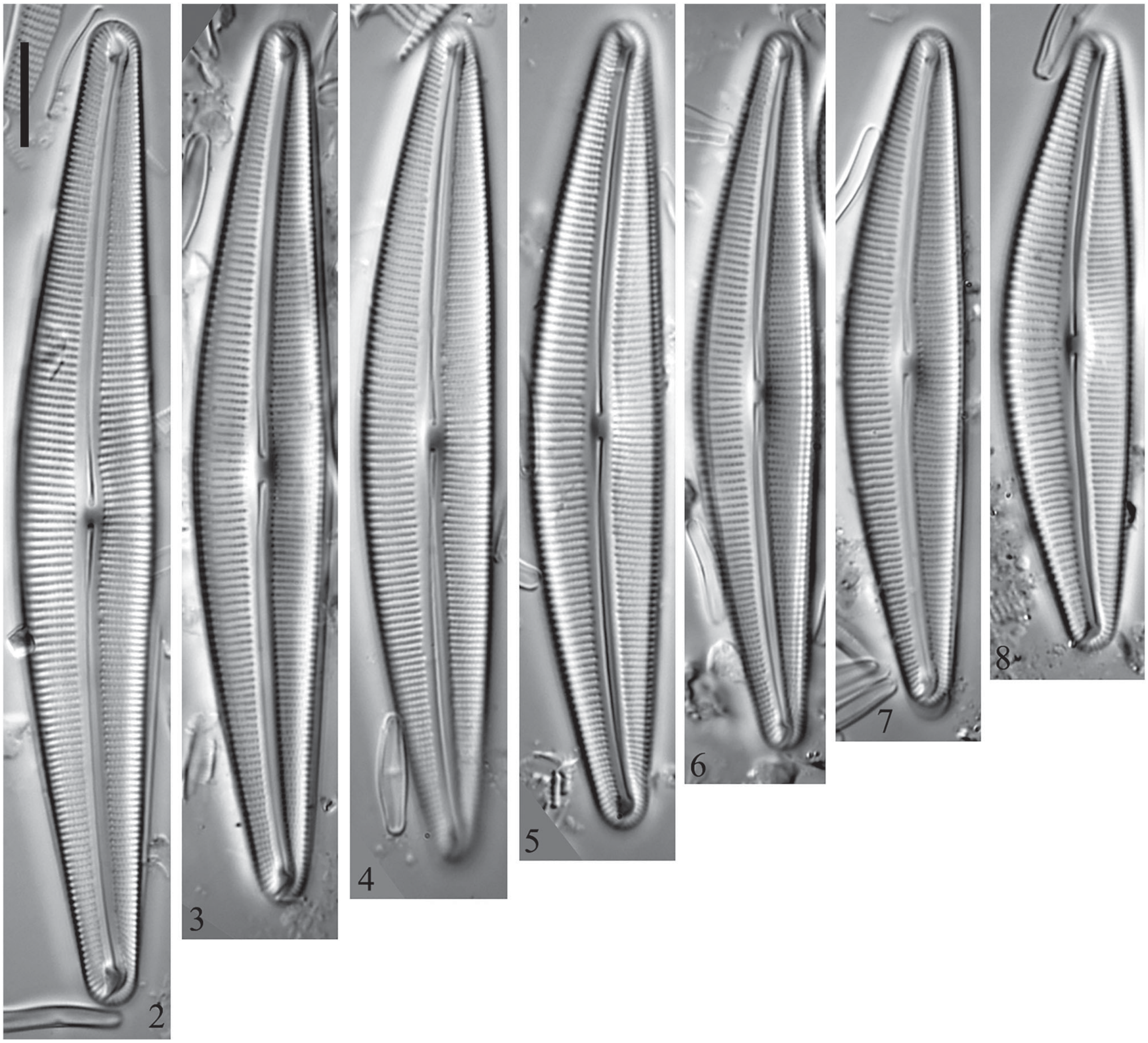
Type locality: Mineral spring near village Radusha, Skopje, R. North Macedonia

Etymology: The specific epithet '*parahelvetica*' refers to the similarity with *Cymbella helvetica* Kützing.

Distribution: The species was observed only at the type locality.

Ecology: *Cymbella parahelvetica* has only been found at the type locality, the mineral spring near Radusha.

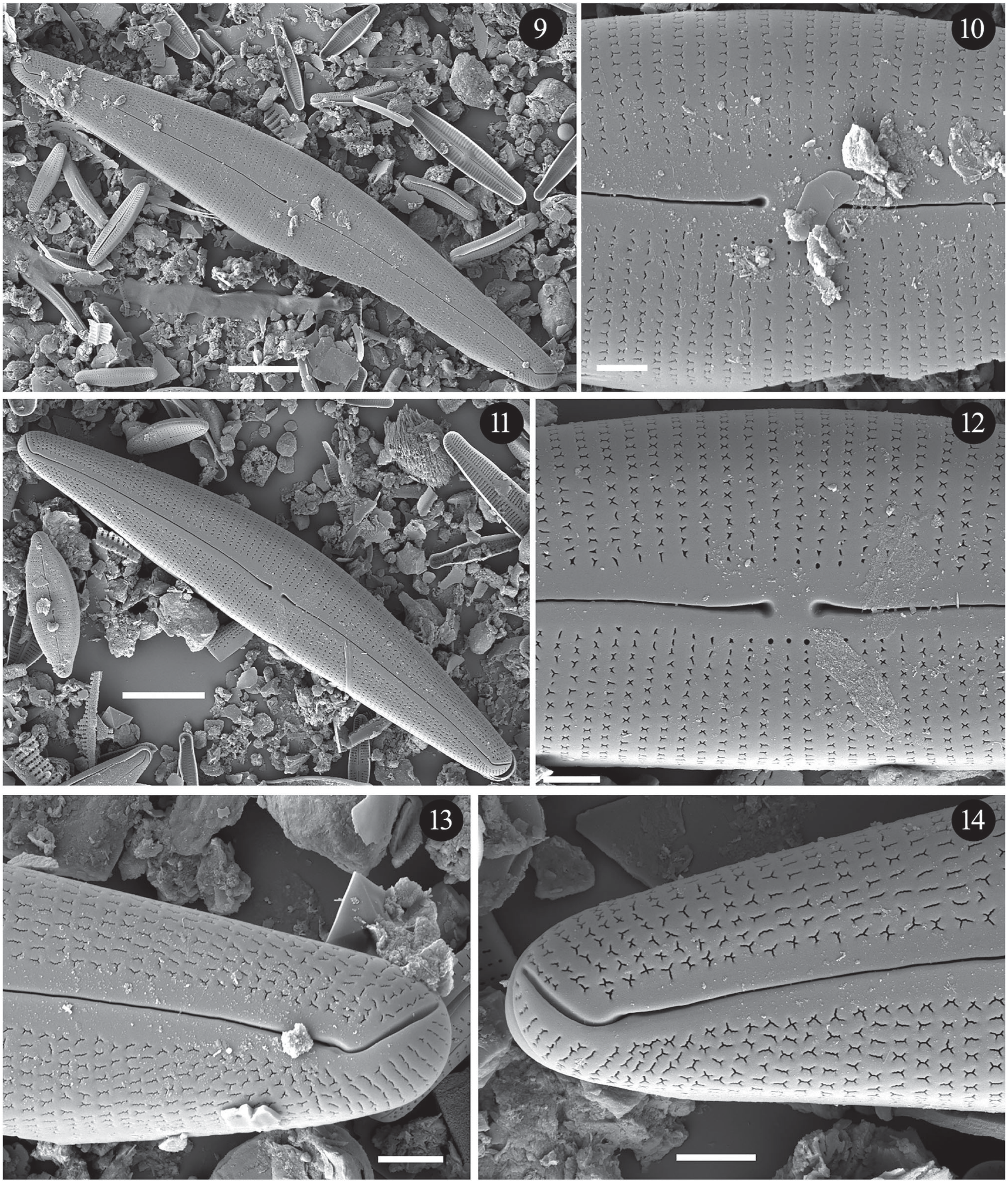
Fig. 1. Study area and location of the type locality spring Solenica.



Figs 2–8. *Cymbella parahelvetica* sp. nov. LM, valve views showing the size diminution series. Scale bar = 10 µm.

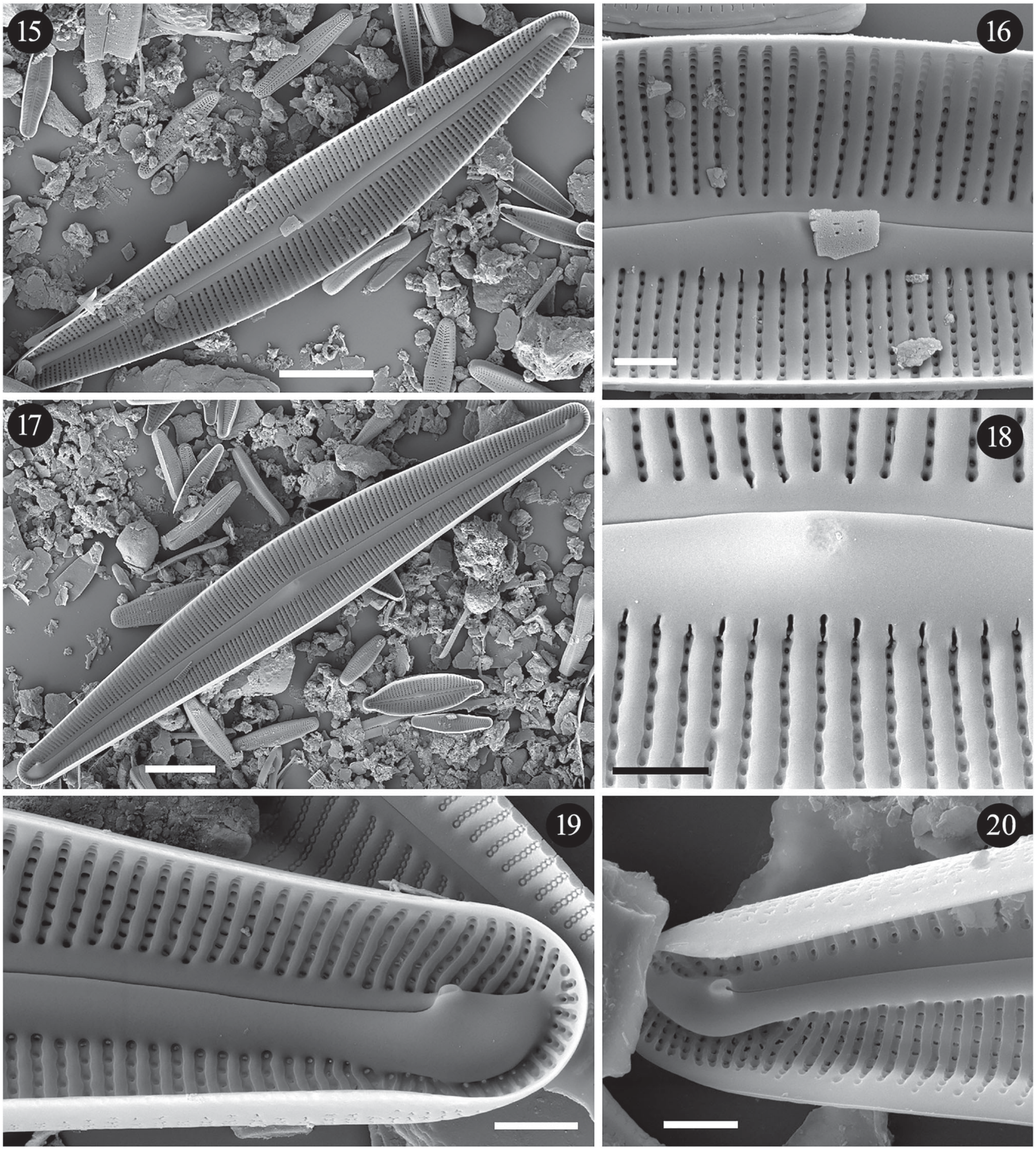
This spring is characterized by relatively high conductivity (569 µS/cm) and slightly alkaline pH (8.45) due to the high content of calcium (102.2 mg/L), magnesium ions (45.6 mg/L) chlorides (10.1 mg/L) and sulphates (14.9 mg/L) (Table 1). The associated flora is comprised of *Achnanthidium neocryptocephalum* (Grunow) Novais & Van de Vijver (59%), *Encyonopsis orientalis* Krammer (17%), *Achnanthidium* sp. (6%) *Cymbopleura acutilanceolata* sp. nov. (4%) and *Cymbella excisa* Kützing (4%).

Comparisons with similar taxa: The most similar species is *Cymbella cantonatii* Lange-Bertalot (in Krammer 2002, Fig. 172: 1–7) from Ca-rich springs in the Brenta mountains, Trentino, Italy, but this is smaller (valve length 30–55 µm) and has a higher areola density (29–33 in 10 µm), but like *C. parahelvetica*, also has X-shaped external areola openings and lacks apical pore fields. *Cymbella parahelvetica* is also similar to *C. helvetica* Kützing, depicted in Krammer (2002, Fig. 183: 1–3, Fig. 184: 1–4, Fig. 185: 1–6, Fig. 186: 1–3, Fig. 187: 1–5), but the latter is wider, 18–26 µm, and has a lower stria density (6–8 in 10 µm). The external areola openings of *C. helvetica* are apically elongate and slit-like, and the proximal raphe ends are deflected dorsally. *Cymbella lange-bertalotii* Krammer (Krammer 2002, Fig. 179: 1–6, Fig. 180: 1–8, Fig. 181: 1–6, 8, Fig. 182: 1–9) is comparable to *C. parahelvetica* in size (length = 38–100 µm, width 10–16 µm), but has lower stria (8–12 in 10 µm) and areola densities (18–24 in 10 µm), and the external areola openings of *C. lange-bertalotii* are apically elongate and slit-like. *Cymbella*



Figs 9–14. *Cymbella parahelvetica* sp. nov. SEM external views of whole valve (Figs 9, 11), central area (Figs 10, 12) and valve ends (Figs 13, 14). Scale bars = 10 µm (Figs 9, 11), 2 µm (Figs 10, 12–14).

jachalensis Bustos, Mattano & Maidana, was recently described from the Jáchal River, Argentina, and belongs to the group around *C. helvetica*, characterized by the absence of APFs (Bustos et al. 2018, Figs 2–27). *Cymbella jachalensis* has lower stria (8–11 in 10 µm) and areola densities (13–20 in 10 µm), and the external areola openings

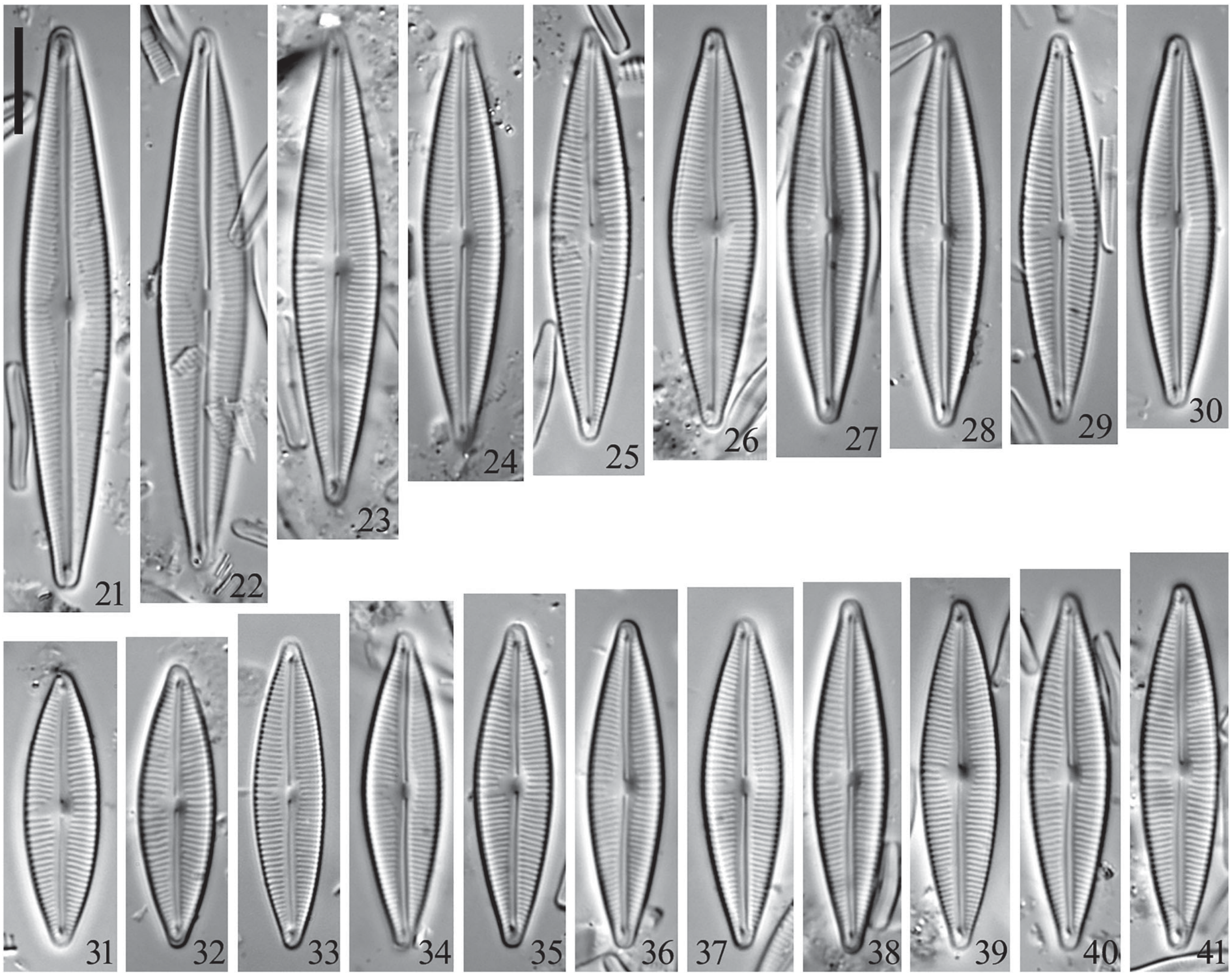


Figs 15–20. *Cymbella parahelvetica* sp. nov. SEM internal views of whole valve (Figs 15, 17), central area (Figs 16, 18) and valve ends (Figs 19, 20). Scale bars = 10 µm (Figs 15, 17), 2 µm (Figs 16, 18–20).

are slit-like. *Cymbella biseriata* Videska, Zaova & Levkov was recently described from an alpine pond on mountain Kozuf, North Macedonia (Videska et al. 2022, Figs 2–34). This species is smaller (length 29–38 µm, width 7–9 µm), has short protracted broadly rounded valve ends and entirely biseriate striae.

Cymbopleura acutilanceolata Levkov, Zaova & Jüttner sp. nov. (Figs 21–51).

Description: LM (Figs 21–41): Valves weakly dorsiventral to almost symmetrical, lanceolate, with both margins moderately convex. Valve ends not, or weakly, protracted, and



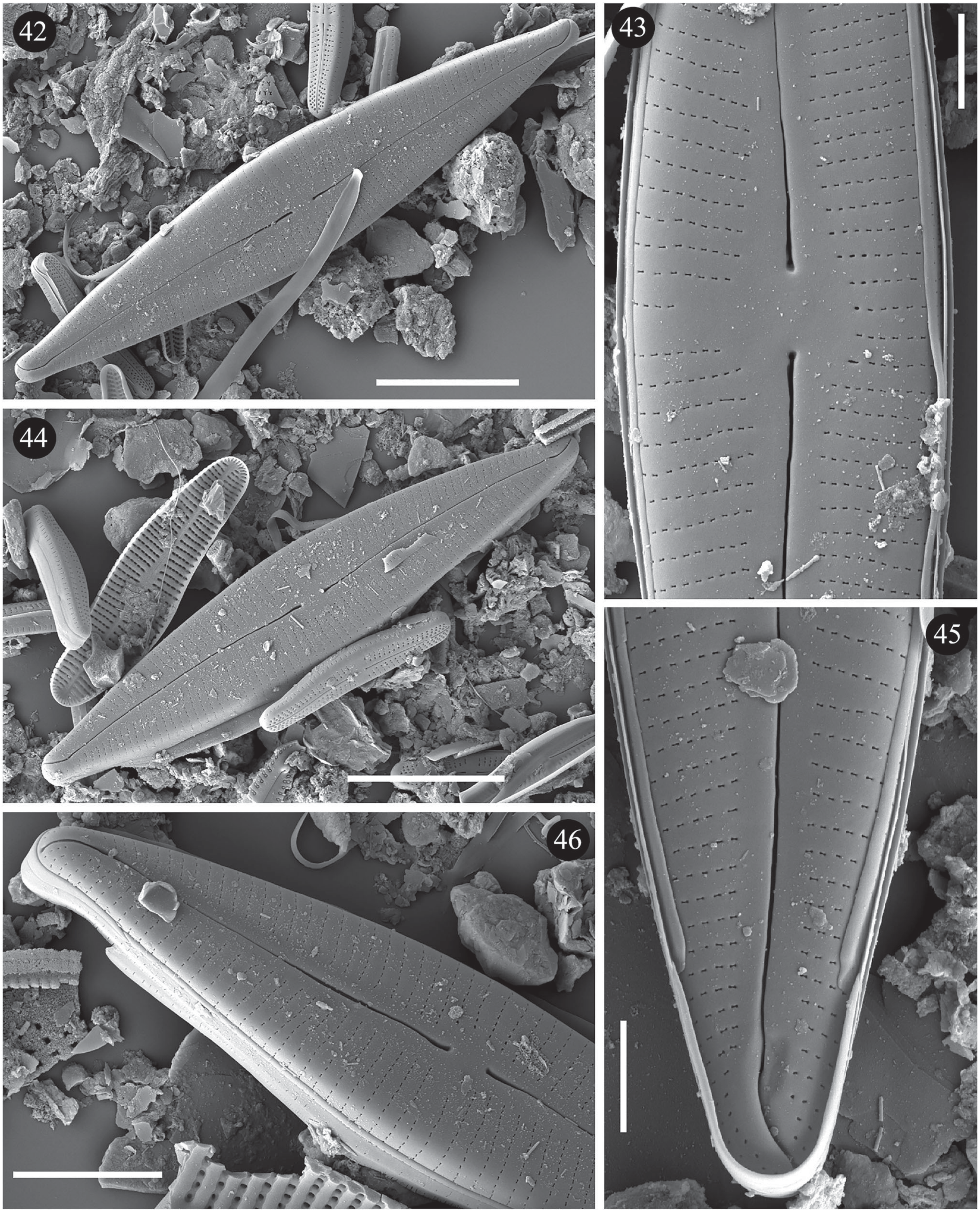
Figs 21–41. *Cymbopleura acutilanceolata* sp. nov. LM, valve views showing the size diminution series. Scale bar = 10 µm.

Table 1. Physico-chemical analyses of water from spring Solenica.

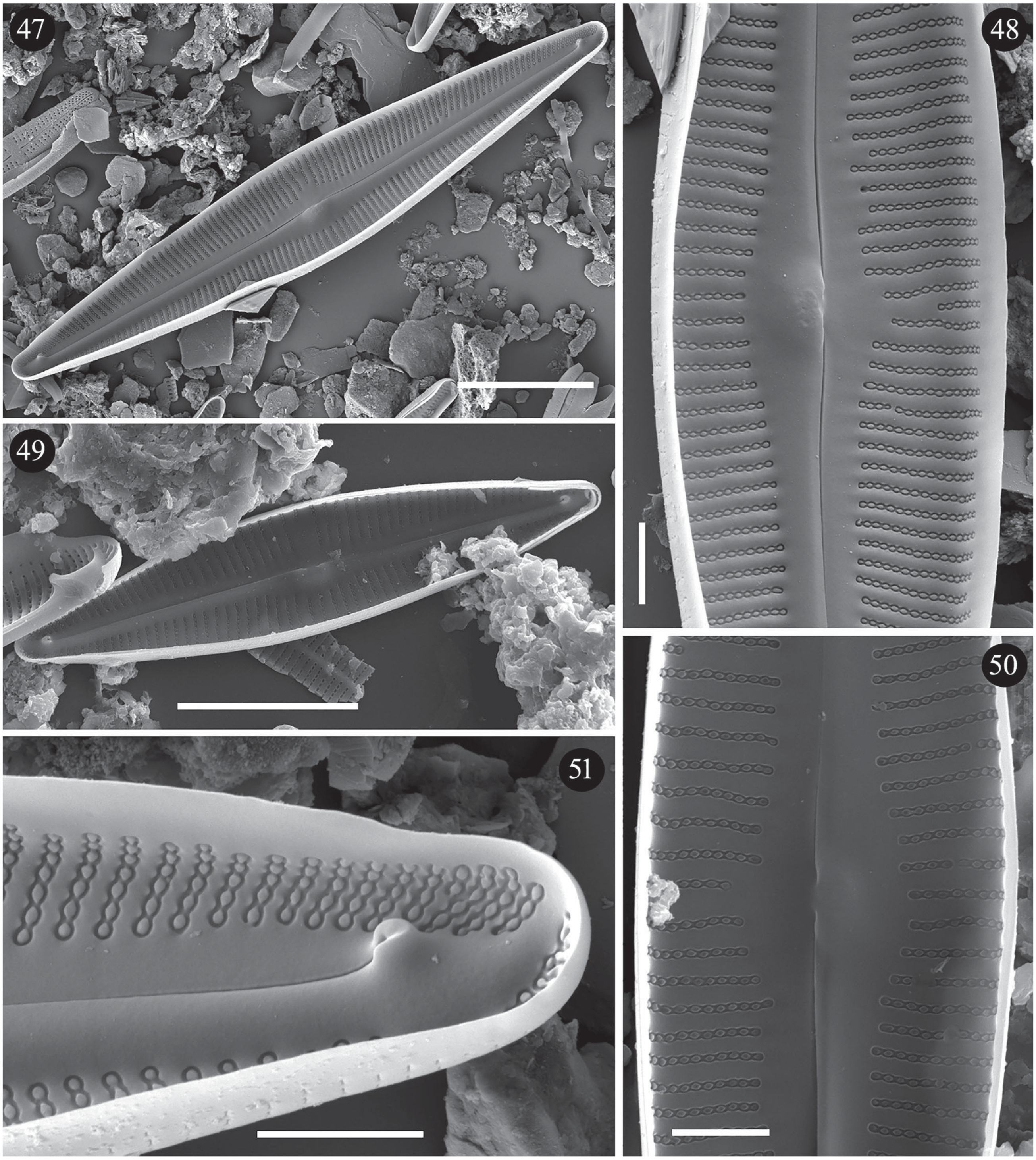
Parameter	
pH	8.4
conductivity	569 µS/cm
Nitrates	0.549 mg/L N
Nitrites	0.002 mg/L N
Ammonia	0.015 mg/L N
Phosphates	0.014 mg/L
Chlorides	10.1 mg/L
Sulphates	14.9 mg/L
Na	1.79 mg/L
K	0.57 mg/L
Ca	102.20 mg/L
Mg	45.60 µg/L
Fe	91 µg/L
Mn	17 µg/L
Zn	3.7 µg/L
Cu	0.056 µg/L
Pb	0.25 µg/L

acute, narrowly rounded. Valve length 26–52 µm, valve width 6.5–8.5 µm (n = 42). Axial area narrow, linear. Central area moderately large, occupying ca. 1/3 of the valve width, asymmetric elliptical, wider on dorsal side. Raphe weakly lateral. Proximal raphe ends simple to weakly inflated and almost straight, distal fissures comma-shaped and deflected dorsally. Striae radiate throughout, 18–22 in 10 µm, more densely spaced towards valve ends. Central striae in some specimens more distantly spaced. Areolae not discernible in LM.

SEM (Figs 42–51): External valve face flat, transition from valve face to mantle gradual (Figs 44, 46). Central area broad, bordered by three shortened striae on ventral side and 1–3 shortened striae on dorsal side (Fig. 43). Raphe slightly lateral, raphe branches slightly arcuate, proximal raphe ends expanded forming drop-shaped, slightly ventrally deflected central pores (Figs 43, 46). Distal raphe fissures deflected to dorsal side, extending onto valve mantle (Figs 45, 46). Striae uniseriate,



Figs 42–46. *Cymbopleura acutilanceolata* sp. nov. SEM external views of whole valve (Figs 42, 44), central area (Fig. 43) and valve ends (Figs 45, 46). Scale bars = 10 µm (Figs 42, 44), 2 µm (Figs 43, 45), 5 µm (Fig. 46).



Figs 47–51. *Cymbopleura acutilanceolata* sp. nov. SEM internal views of whole valve (Figs 47, 49), central area (Figs 48, 50) and valve apex (Fig. 51). Scale bars = 10 µm (Figs 47, 49), 2 µm (Figs 48, 50, 51).

composed of narrow, transapically elongated to dumb-bell-shaped areolae, ca. 40 in 10 µm. Internally, raphe with 'intermissio' i.e. proximal raphe ends not covered by overgrowth of silica. Proximal raphe ends not expanded, slightly curved to ventral side (Figs 48, 50). Distal raphe ends terminate in small, knob-like helictoglossae (Fig. 51). Virgae broad, separating narrow striae (Figs 48, 50, 51). Areola openings occluded by elliptical hymenes (Figs 48, 50). No apical pore field observed (Fig. 51). Striae at valve apex interrupted by narrow hyaline area (Fig. 51).

Holotype: Surface red sediment, collection date: 27.06.2020; Leg. Zlatko Levkov; Coordinates: 42.0553666˚ N, 21.2543255˚ E (Accession No. MKNDC 12910), Slide MKNDC 12910B (holotype)

Isotype: Slide BM 088059

Type locality: Mineral spring near village Radusha, Skopje, R. North Macedonia

Etymology. The specific epithet '*acutilanceolata*' refers to the valve shape, lanceolate with acute valve ends.

Distribution: The species was observed only at the type locality.

Ecology: *Cymbopleura acutilanceolata* was observed in the mineral spring near Radusha, in the same sample as *C. parahelvetica* (see above).

Comparisons with similar taxa: *Cymbopleura acutilanceolata* has a similar valve outline to *C. stauroneiformis* (Lagersedt) Krammer and *Cymbopleura lapponica* (Grunow) Krammer (Krammer 2003). *Cymbopleura stauroneiformis* (Krammer 2003, Figs 106: 8–10, 108: 1–16) is also characterized by almost symmetrical valves but the valve ends are more protracted and narrowly rostrate, the central area is large, transapically oval or an acute-angled subfascia, and the raphe is slightly reverse lateral near the proximal ends. Proximal raphe ends are simple and straight in C. *acutilanceolata.* The striae of *C. stauroneiformis* are finely punctate and less dense (15–18 in 10 µm) than in *C. acutilanceolata* (18–22 in 10 µm). *Cymbopleura acutilanceolata* can be differentiated from *C. lapponica* (Krammer 2003, Fig. 107:1–12) by the shape of the central area which is larger, round or rhomboid, occupying $^1/_3$ to ¾ of valve width in *C. lapponica* and by its lower stria density (15–17 in 10 µm). The valve size and central area of *Cymbopleura amicula* Gligora Udovič & Levkov (in Gligora Udovič et al. 2022, Figs 1–31) are similar to C. *acutilanceolata,* but the species can be easily differentiated by the valve shape of *C. amicula*, lanceolate with broadly rounded and ventrally bent ends, and its lower stria density (13–16 in 10 µm). *Cymbella dobsonensis* Krammer (2003, Fig. 116: 8–17) is similar in valve shape (rhomboid-lanceolate) and comparable in size (length 28–60 µm, width 7.4–8.5 µm), but can be differentiated from *C. acutilanceolata* by the shape of the central area (almost absent and not separated from axial area in *C. dobsonensis*), strongly lateral raphe branches becoming reverse-lateral proximally (in *C. dobsonensis*) and by the presence of APFs. Some *Encyonopsis* species such as *E. montana* Bahls (Bahls 2013b, 2019, Figs 33: 1–4) and *E. cesatiformis* Krammer (2003, Figs 106: 1–7b) have a similar valve and central area shape as C. *acutilanceolata,* but can be differentiated by their ventrally deflected distal raphe fissures.

Delicatophycus fontinalis Levkov, Zaova & Jüttner sp. nov. (Figs 52–77).

Description: LM (Figs 52–72): Valves dorsiventral, semi-lanceolate with moderately arched dorsal margin and slightly convex to almost straight ventral margin. Valve ends not protracted, narrowly rounded. Valve length 30.5–53.5 µm, valve width 6.0–7.5 µm (n = 32). Axial area narrow to moderately wide, gradually widening from valve ends towards the valve centre. Central area small, often not clearly differentiated from axial area, but when differentiated extended to dorsal side. Striae parallel to weakly radiate, becoming more strongly radiate towards valve ends, 17–20 in 10 µm. Central striae more distantly positioned. Areolae not discernible in LM.

SEM (Figs 73–77): Valve face flat, gradual transition from valve face to valve mantle (Figs 73, 74). Raphe branches arcuate with distinctly reverse-lateral proximal ends (Figs 73, 74) and distal fissures strongly deflected dorsally, extending onto valve mantle (Fig. 75). Isolated pores absent from central area, although a few areolae bordering the central area on ventral side have round external openings (black arrow in Fig. 74). Striae uniseriate composed of tilde-shaped areolae, mostly diagonally aligned, continuing without interruption onto mantle (Figs 73–75). No apical pore field observed (Fig. 75). Internally, proximal raphe ends covered by flap of silica covering the proximal raphe ends, while distal raphe ends terminate in slightly dorsally bent helictoglossae (Fig. 77). Striae separated by broad virgae (Fig. 76). Internal areola openings small, round to transversally elongated, located in shallow depressions (Fig. 76). Areolae bordering central area on ventral side ('pseudostigmoids' sensu Krammer 2003) with slightly larger openings (black arrow in Fig. 76), but true stigmata absent (Fig. 76). Internal areola occlusions observed on valve mantle and near valve ends, where areolae are densely packed, giving the impression that APF is present (white arrow in Fig. 77).

Holotype: Surface red sediment, collection date: 27.06.2020; Leg. Zlatko Levkov; Coordinates: 42.0553666˚ N, 21.2543255˚ E (Accession No. MKNDC 12910), Slide MKNDC 12910C (holotype)

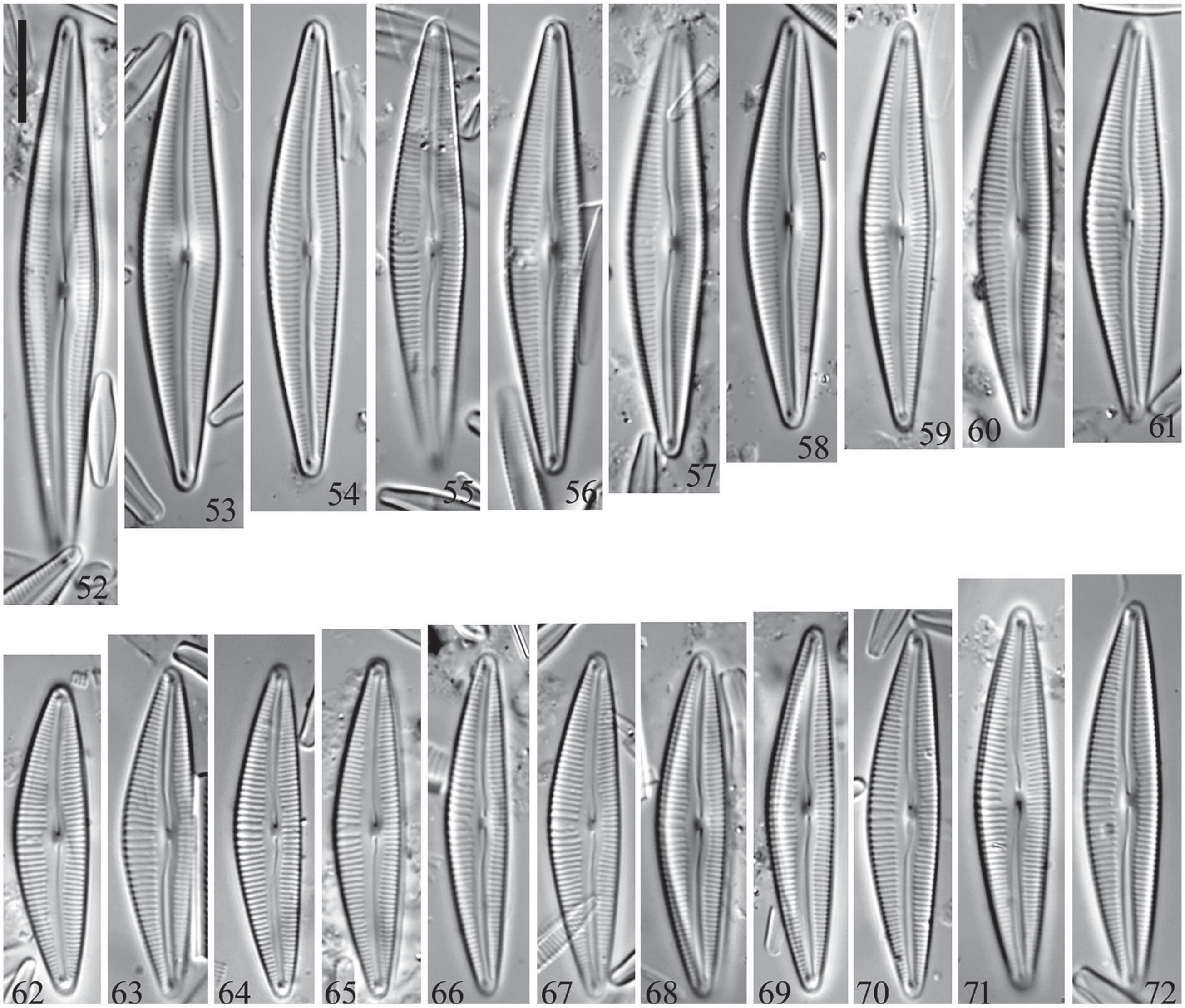
Isotype: Slide BM 092392

Type locality: Mineral spring near village Radusha, Skopje, R. North Macedonia

Etymology: The specific epithet '*fontinalis*' refers to the habitat (Lat. *fontinalis* = growing in or by springs).

Distribution: The species was observed only at the type locality.

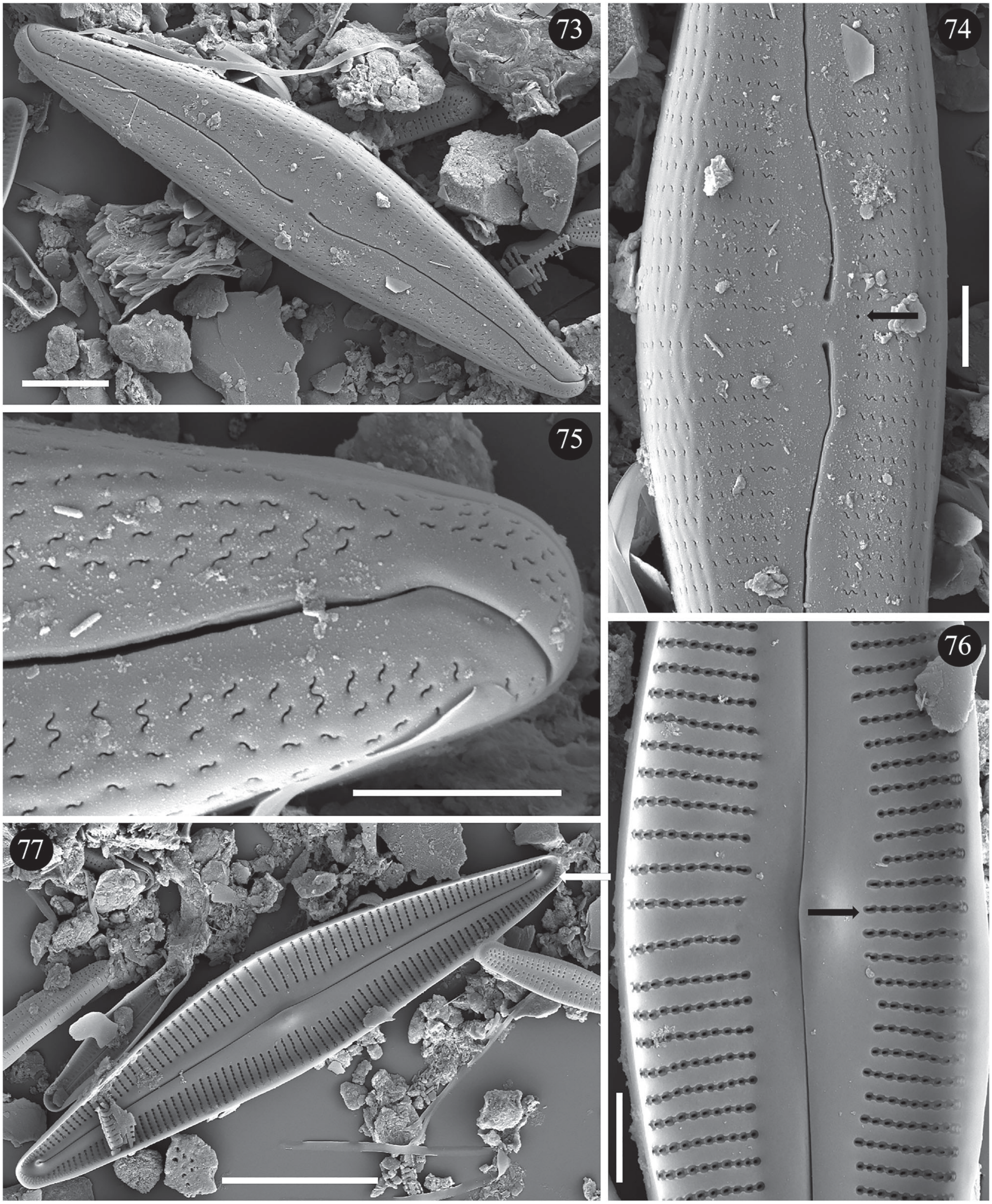
Ecology: *Delicatophycus fontinalis* was observed in the mineral spring near Radusha, in the same sample as *C. parahelvetica* and *C. acutilanceolata* (see above).



Figs 52–72. *Delicatophycus fontinalis* sp. nov. LM, valve views showing the size diminution series. Scale bar = 10 µm.

Comparisons with similar taxa: *Delicatophycus fontinalis* is most similar to *Delicatophycus verena* M. J. Wynne (= *Delicata verena* Lange-Bertalot & Krammer in Krammer 2003, Figs 137: 1–11) described from a spring on the Sinai, Egypt. The valves of *D. fontinalis* are more strongly dorsiventral with an almost straight or very slightly convex ventral margin. The ventral margin of the type population of *D. verena* is consistently convex. More specimens of these species require examination to determine whether the shape of the ventral margin is variable. *Delicatophycus porosus* Mironov, Chudaev & Jüttner, described from the River Adegoy, Russia (Mironov et al. 2022) is also very similar, but the valves are smaller (16.0–29.5 µm, 5.0–6.0 µm) than those of *D. fontinalis* and *D. verena*. The dorsal striae at the valve centre of *D. fontinalis* and *D. porosus* may be shortened and slightly more widely spaced. Dorsal striae at the valve centre in the type population of *D. verena* are of similar length and are not more widely spaced. Another similar species is *Delicatophycus judaicus* (Lange-Bertalot & Krammer) M. J. Wynne, depicted in Krammer (2003, Fig. 136: 1–5), which differs from *D. verena* in size but might also represent the lower size range of that species. *Delicatophycus nepouiana* Krammer (2003, Fig. 135: 10–12) is also similar in shape and size but has a slightly ventrally displaced axial area at the valve centre and a strongly reverse raphe towards the proximal raphe ends.

Delicatophycus fontinalis is similar in shape to *D. wulingensis* Bing Liu & S. Blanco (in Liu et al. 2022, Figs 7–50). Both species have comparable sizes (25–58 µm long, 5.1–7.9 µm wide for *D. wulingensis*) and stria densities (15–19 in 10 µm in *D. wulingensis*) but can be differentiated by the shape of the central area. *Delicatophycus wulingensis* has a broad fascia or subfascia bordered by one or two shortened and more distantly spaced central striae and possesses apical pore fields (APF). *Delicatophycus*



Figs 73–77. *Delicatophycus fontinalis* sp. nov. SEM external views of whole valve (Fig. 73), central area (Fig. 74) valve ends (Fig. 75), internal view of central area (Fig. 76), internal view of whole valve (Fig. 77). Fig. 74. Black arrow show the small, round external openings of areolae (pseudostigmoids) bordering the central area. Fig. 76. Black arrow show round internal openings of areolae (pseudostigmoids) bordering the central area. Scale bars = 5 µm (Figs 73, 77), 2 µm (Figs 74–76).

montana Wynne (= *Delicata montana* Bahls 2017) has rhombic-lanceolate valves with a tumid ventral valve margin and more broadly rounded valve ends, in contrast to the semi-lanceolate valves with narrowly rounded ends in *D. fontinalis*. Valves of *D. fontinalis* also have a more strongly arched dorsal margin and are consistently wider for any given length.

Discussion

In this study, three new cymbelloid taxa have been described, from the same sample from a mineral spring. The sample contains two other cymbelloid species, *C. excisa* and *Encyonopsis orientalis* Krammer. The latter species was described from a mineral spring in Israel (Krammer 1997) and, so far, there are no published records of this species in Europe. Interestingly, all five species belong to different genera according to the current taxonomic treatment of cymbelloid taxa. However, recent analyses by SEM and molecular techniques reveal that the delimitation of some genera is rather unclear.

As currently understood, *Cymbella* is morphologically very diverse with respect to the presence of stigmata, apical pore fields (APF), areola shape and raphe structure (Krammer 2002). One of the main differential characters for *Cymbella* is the presence of APFs. However, the species group around *C. helvetica* Kützing, (including the species *C. parahelvetica* described here) lack APFs, like species in *Cymbopleura*. Krammer (1982) mentioned that *C. helvetica* might belong to (subgenus) *Cymbopleura*, however, molecular phylogenetic analyses show that *C. helvetica* is more closely related to other 'typical' *Cymbella* species (Nakov et al. 2014; Glushchenko et al. 2022). One of the differential characters of *C. parahelvetica* is the presence of X-shaped areolae, a feature also found in *C. cantonatii* (Krammer 2002). This feature is not only found in the *C. helvetica* group but is one of the most important features for the separation of *Crucicostulifera* Taylor & Lange-Bertalot (2010) from *Navicula* Bory. More recently, *Crucicostulifera* was considered a cymbelloid taxon (Le Cohu et al. 2014).

Most species of *Cymbella* have uniseriate striae, but recently a new extant species with entirely biseriate striae was described (Videska et al. 2022). Biseriate striae in *Cymbella* were known from the extinct species, *C. diplopuncta* Krammer, and from *C. latarea* Maillard and *C. pernodensis* Maillard but only internally (Le Cohu et al. 2020). Partially biseriate striae are present in *Cymbella liyangensis* Zhang, Jüttner & E. J. Cox (Zhang et al. 2018), *Cymbella hubeiensis* Y. Li (Gong et al. 2013) and *Cymbella tridentina* Lange-Bert., M. Cantonati & A. Scalfi (Cantonati et al. 2010). One of the main distinguishing features for differentiating the genus *Celebesia* from *Cymbella* and *Cymbopleura* was the presence of biseriate striae in the former. However, the separation of these genera was based on a combination of ultrastructural characters.

Cymbopleura is also considered as a heterogenous group and recent molecular phylogenetic analyses suggested that it is not monophyletic (Glushchenko et al. 2019, Kezlya et al. 2022). Some of the species previously included in *Cymbopleura* have been transferred to other genera, while some *Encyonopsis* species have been transferred to *Cymbopleura* (Bahls 2019). The main differential characters of *Cymbopleura* are the absence of stigmata and APFs. *Delicatophycus* may or may not have APFs. In the original description of *Delicatophycus* (Krammer 2003), transapically undulate (tilde-shaped) areolae, and the lack of APFs were important features. However, more recently Liu et al. (2022) described three new *Delicatophycus* species from China with APFs. According to Liu (2022), tilde-shaped areolae and dorsally curved distal raphe fissures are the main differential characters. But dorsally deflected distal raphe fissures are typical for several other cymbelloid genera.

Despite the problems of generic delimitation in the cymbelloid group, based on a combination of characters the newly described species belong to *Cymbella*, *Cymbopleura* and *Delicatophycus*. *Cymbella parahelvetica*, although lacking APFs, shares all other characters (valve shape, presence of isolated pores, raphe morphology, areola structure) with *Cymbella*. *Cymbopleura acutilanceolata* belongs to *Cymbopleura* sensu lato based on valve symmetry, areola structure, absence of APFs and stigmoids, features typical for this genus. *Cymbopleura acutilanceolata* based on the symmetry of the valves, the structure of the areolae and the absence of APFs and stigmoids, belongs to *Cymbopleura* as currently understood. Having tilde-shaped areolae, pseudostigmoids and dorsally deflected distal raphe ends, *D. fontinalis* is a typical representative of *Delicatophycus*.

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