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Taxonomic and ecological characterization of three symmetric biphid diatom species from streams in Cyprus

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Abstract: Environmental factors and diatoms were studied in 151 stations in Cypriot streams. Since *Navicula simplex* Krasske [new combination: *Craticula simplex* (Krasske) Levkov] is based on the small–scale drawing of a single valve and the type material is no longer available, we provide additional ecological, morphological, and ultrastructural data, and an epitype for *C. simplex*, which is distinguished by valve outline and stria density, and occurs in small, nutrient–enriched streams. Two other symmetric biraphid species are proposed as new to science based on light and scanning electron microscopy, careful comparison with similar established taxa, and on the analysis of our multi–annual databases. *Mastogloia cyprica* Lange–Bert. et Cantonati sp. nov. differs from similar species by raphe undulation, stria density, and rare occurrence in oligo– to slightly–eutrophic streamlets and lakes with medium–high conductivity. *Navicula loumatensis* Lange–Bert. et Cantonati sp. nov. is characterized by the combination of valve outline, central area, and by a high areola density, and occurs in medium–high conductivity, low–N but P–enriched streams. In–depth knowledge of Mediterranean stream diatoms is of pivotal importance in these critical decades of climate change.

Key words: Diatoms, streams, Cyprus, *Craticula*, *Mastogloia*, *Navicula*, new species, taxonomy, biogeography

INTRODUCTION

Diatom assemblages on the island of Cyprus show peculiar characteristics due to biogeographical and ecological reasons. The latter are mainly due to climate conditions rendering hydrology as the ecological factor having an overwhelming influence on stream biota (Cantonati et al. 2020a,b). Cyprus is the third largest island in the Mediterranean, with the Republic of Cyprus having effective control of the southern and western parts where this study was carried out. Its eastern Mediterranean location increases the gradient of aridity of the island and forms a distinctive climate with particularly severe summer droughts (Giannakopoulos et al. 2010). These conditions are of high concern for Cypriot water resources and cause the greatest water stress among member states in the EU. Such water scarcity places the country on the top of 20 water–deprived countries worldwide (Giannakopoulos et al. 2010; Sofroniou & Bishop 2014). The impact of droughts on Mediterranean streams has recently intensified and is likely to be exacerbated over the coming years (Giorgi & Lionello 2008). Specific geology of the region is present as the ophiolitic Troodos and carbonate rocks (WDD 2016). Various types of Mediterranean streams flow through basic/ultrabasic rocks that saturate waters with calcium, magnesium, and sodium, causing high conductivity, alkalinity and sulphate as well as high chloride concentrations (Neal & Shand 2002).

Thanks to previous detailed investigations on the

Cypriot Mediterranean streams, *Ulnaria acuscypriacus* Lange–Bert. et Cantonati was described as new to science, and *Ulnaria monodii* (Guermeur) Cantonati et Lange–Bert. was described as a new combination of a "forgotten" species (Cantonati et al. 2018). Relatively frequent findings in Cypriot streams enabled the ecological characterization of *Navicula veronensis* Lange–Bert. et Cantonati, which was described as a new species from a spring in the urban area of Verona, Italy (Cantonati et al. 2016).

Craticula Grunow is a medium–sized genus of symmetric biraphid (naviculoid) diatoms, numbering about 65 taxa in total (Kociolek et al. 2021). Since the publication of an overview of all European taxa (Lange–Bertalot et al. 2001), several new species were described and distinguishing features have been added to already existing taxa (e.g. Solak et al. 2020). The external raphe branches are located on a thickened conopeum although in some cases the raphe runs externally on the valve face (Morales & Le 2005). Striae are usually parallel to weakly radiate in the mid–valve, becoming slightly convergent towards the valve apices, uniseriate, composed of a single row of slightly elongated to lineolate (occasionally rounded) areolae, internally covered by porous hymenes (Round et al. 1990; Lange–Bertalot 2001). The genus *Craticula* can be found worldwide, observed from the tropics (Rumrich et al. 2000; Morales et al. 2014) to Antarctica (Sabbe et al. 2003; Van de Vijver et al. 2010), and is widely distributed in Europe (Lange–Bertalot 2001; Levkov et al. 2016). Although a large number of species are common in brackish (and even saline) conditions and (hyper)eutrophic and heavily polluted waters, some species have been observed in acidic, oligotrophic, and alkaline waters (Lange–Bertalot 1993, 2001; Lange–Bertalot et al. 2003; Levkov et al. 2016). In the Mediterranean region the most frequent and abundant *Craticula* species include *Craticula buderi* (Hust.) Lange–Bert., *C. halophila* (Grunow) D.G. Mann, and *C. subminuscula* (Manguin) C.E. Wetzel et Ector (Delgado et al. 2012; Cantonati et al. 2020a).

The biraphid naviculoid genus *Mastogloia* Thwaites is highly diverse, with an estimated 809 taxa (Kociolek et al. 2021). *Mastogloia* has valves that are usually isopolar (with rounded to capitate apices), however in some cases they are slightly heteropolar. The unique diagnostic character of the genus is the presence of chambers called partecta (or partectal ring, chamber band, modified valvocopulae, modified girdle band), which represents a modification of the valvocopula. The partecta may be regular or variable in shape and size and have concave or convex free inner margins. On the valve face and mantle there are small locular pores arranged in rows (Paddock & Kemp 1990; Round et al. 1990; Sylvia et al. 2014; Pavlov et al. 2016). The genus is distributed worldwide in predominantly warm–water, marine and brackish areas, with a small number of representatives inhabiting freshwaters including oligotrophic rivers, eutrophic ponds, mine lakes, and thermo–mineral springs. Few species are known from fossil sediments (Lobban & Pennesi 2014; Pavlov et al. 2016). Some species are known for reaching a high abundance in freshwater bodies with high conductivity and pH levels (Laird et al. 1996; Townsend & Gell 2005). Ecologically, polyphenism in *Mastogloia* species has already been emphasized by Edlund & Burge (2019), where they reported that the variability in stria structure and density between type materials of *M. grevillei* and *M. danseyi* is most likely triggered by changing total dissolved solids and conductivity, and thus recognized *M. danseyi* f. *grevillei* as an ecophenotype of the nominate *M. danseyi*. The most frequent and abundant *Mastogloia* species in the Mediterranean streams (Delgado et al. 2012; Cantonati et al. 2020a) are *Mastogloia lacustris* (Grunow) van Heurck and *Mastogloia elliptica* (C.A.Agardh) Cleve.

Navicula J.B.M. Bory de Saint–Vincent (*sensu stricto*) is one of the most species–rich diatom genera (Lange–Bertalot 2001): the name yields 9917 records in DiatomBase (Kociolek et al. 2021), though many of these have been transferred to other genera separated from *Navicula* sensu lato. In AlgaeBase there are 1607 species names flagged as accepted taxonomically under the name *Navicula*. However, monographs available for European waters include from 130 (Lange–Bertalot 2001) to ca. 200 taxa (Witkowski et al. 1998). In the literature there are plenty of examples of identification and definition of features for correct distinction between similar taxa, new combinations, and new species descriptions (e.g., Sabbe et al. 2019; Chudaev et al. 2021). This richness is also explained by the wealth of diverse habitats colonized, however with a predominance of freshwater diatoms (Kociolek & Spaulding 2003; Wehr & Sheath 2003; Dobosz et al. 2017; Vidakovic et al. 2020). Valves of *Navicula* s.s. are elliptic and boat–shaped in outline. The apices may be capitate, acute, rounded, or not expanded. Striae are composed of lineate areolae or lineolae, which is a defining character of *Navicula s.s.* as well as the thickened central sternum. The central area may be expanded, but the silica is not thickened into a stauros. The raphe is straight and filiform, or lateral in some species. The uniseriate striae are composed of slit–like areolae, and there are two plate–like, girdle–appressed plastids (Round et al. 1990; Lange–Bertalot 2001). The genus *Navicula s.s.* is broadly distributed almost on all continents, including Sub–Antarctic and Antarctic areas. At the same time, several species have also been shown to harbour significant semicryptic and/or cryptic diversity (Poulíčková et al. 2010; van de Vijver et al. 2011; Bahls 2012). *Navicula* species occur across a wide range of ecosystems (low–conductivity waterbodies, wetlands, brackish, and marine waters, etc.) (Lange–Bertalot 2001; Watanabe 2004; Vidakovic et al. 2020). However, the great majority of *Navicula* taxa have eco–specific characteristics enabling them to play a major role in biomonitoring (Werum & Lange–Bertalot 2004; Metzeltin & Lange–Bertalot 2007). The most frequent and abundant *Navicula* species in the Mediterranean streams (Delgado et al. 2012; Cantonati et al. 2020a) are: *N. veneta* Kütz., *N. tripunctata* (O.F. Müller) Bory, *N. cryptotenella* Lange–Bert. / *N. cryptotenelloides* Lange–Bert., *N. caterva* Hohn et Hellermann / *N. metareichardtiana* Lange–Bert. et Kusber (=*N. reichardtiana* Lange–Bert., nom. illeg.), *N. antonii* Lange–Bert., and *N. capitatoradiata* H.Germ. ex Gasse.

Using light microscopy (LM) and scanning electron microscopy (SEM) observations, as well as a thorough morphological, physical, chemical, and biological characterization of the habitats, the present study aims at characterizing three symmetric biraphid (naviculoid) species from Cypriot streams: two are proposed here as new to science within the genera *Mastogloia* and *Navicula*, and additional ecological, morphological, and ultrastructural data, and an epitype are provided for a *Craticula* species (*C. simplex*).

Material and Methods

The samples on which this study is based were collected during the national surveillance monitoring for the implementation of Article 8 of the Water Framework Directive (WFD, European Commission 2000), in the context of which diatoms were collected for bioassessment, from streams in the areas under the effective control of the Republic of Cyprus. Diatom samples were collected in eight different sampling expeditions in 2005, 2006, 2011, 2012, 2018, 2019, 2020, and 2021. The sampling was conducted twice per year, once in February–March and once April–June. Not all stations were sampled twice, which was greatly dependent on the local flow regime in each station. Overall, 151 different stations were sampled, over the years and 627 samples of diatoms, were collected for analysis. The studies of Cypriot stream network covered an area of about 5550 km^2. The following ecological parameters were considered: ecomorphology, detailed hydrology, physical and chemical variables.

The material was cleaned using hydrogen peroxide and mounted in Naphrax (refractive index of 1.74) (EN 13946 2003). Relative abundances were determined by identifying and counting a total of at least 400 valves using a light microscope (LM) Zeiss Axioskop 2 (Zeiss, Jena, Germany) and ×1000 magnification. SEM observations were made primarily at the University of Frankfurt using a Hitachi S–4500 (Hitachi Ltd., Tokyo, Japan [high vacuum, gold coated stubs]). Applying the most updated taxonomic concepts using both light (LM) and scanning electron microscopy (SEM) allowed us to uncover new species for the Cyprus diatom microflora.

The materials from other populations or of similar species with which the new *Mastogloia* species was compared were as follows: Lake Laach population; *Mastogloia danseyi sensu* Levkov et Metzeltin = Acc. No. MKNDC 003009, mosses, peat–bog, Ceripashina, Shara Mountain.

As concerns the typification of the new species and epitype, we chose to use the entire slide as the holotype following article 8.2 of the International Code for Botanical Nomenclature (Turland et al. 2018). The choice for the entire population on the slide is, in our opinion, more consistent with the fact that most diatom species show an extensive variability during their population cell cycle. However, we also clearly described / definined the new (or intended) species, and designated the most representative image taken from the type material as image illustrating the holotype (epitype) specimen.

Materials (slides, prepared material, and aliquots of the original samples), including the holotypes of the two new species and the epitype, were held at the Diatom Collection of MUSE – Museo delle Scienze (TR) of Trento (Northern Italy). Isotype slides and aliquots of prepared material from the same locality and substratum were deposited at the Diatom Collection of the Natural History Museum, London (BM) (UK), and the Diatom Collection of the Botanical Garden and Botanical Museum of the Freie University of Berlin (B) (Germany). If not otherwise stated, measurements on 30 different specimens representative of the size–diminution series were made to obtain ranges and averages of the morphological and ultrastructural features. Terminology to describe valve morphology is based on Round et al. (1990) and Lee et al. (2014) for *Mastogloia*. The ecomorphological characteristics of each station were recorded covering a 100 m stretch along the station. Information on the physical and chemical parameters of each station were provided by the Water Development Department (see Cantonati et al. 2020a for details).

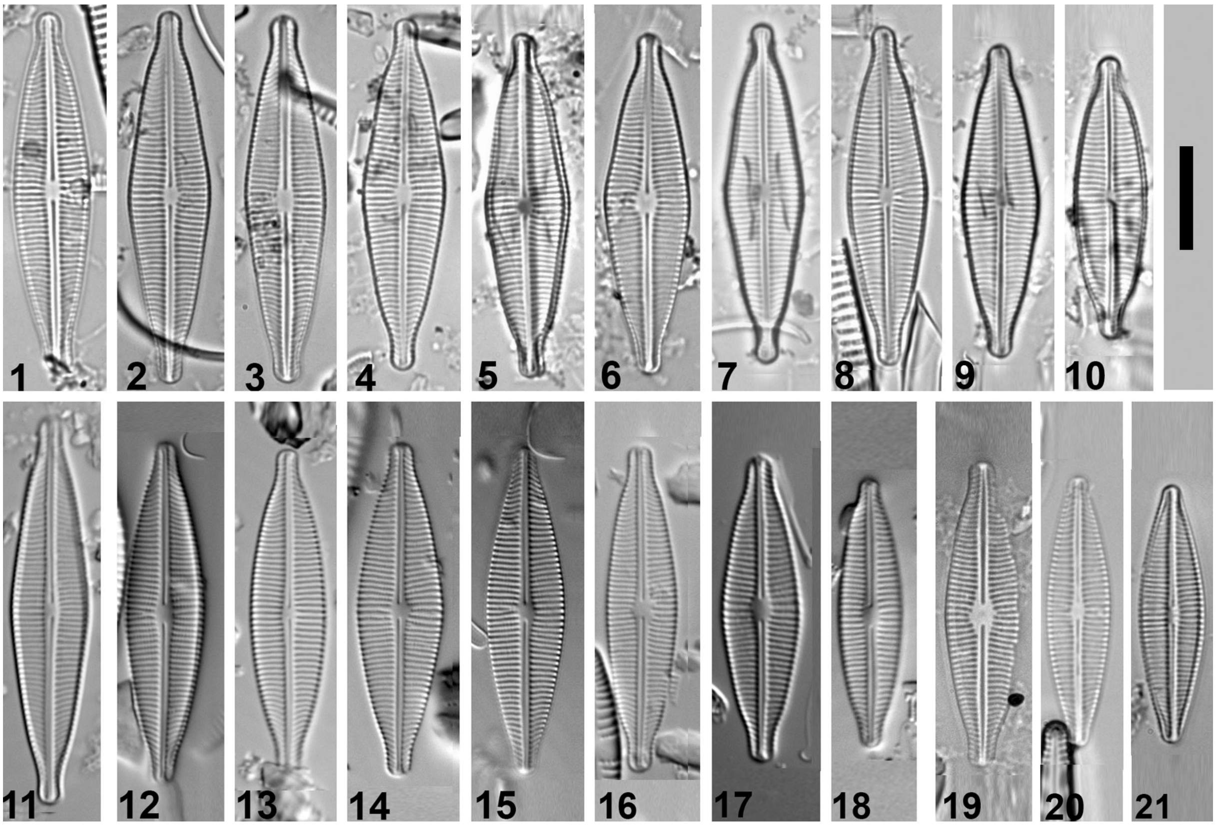
Results

***Craticula simplex* (Krasske) Levkov (Figs 1–21, 22–24)**
Since *Navicula simplex* Krasske [new combination: *Craticula simplex* (Krasske) Levkov] is based on the small–scale drawing of a single valve and the type material is no longer available, we provide additional ecological, morphological, and ultrastructural data, and an epitype for *C. simplex*.

Expanded description

LM (Figs 1–21): Valves rhombic–lanceolate, exceptionally lanceolate (Figs 16, 20–21); ends protracted rather broadly rostrate to rostrate–subcapitate; valve margins wavy (sometimes distinctly triundulate in larger specimens) (Figs 1–18). Length 30–44 µm, breadth 7–9 µm. Length to breadth ratio 4.3–4.9. Raphe filiform, appearing almost straight and weakly expanded toward the central ends which become moderately wider spaced in the longest specimens. Axial area narrows throughout, almost straight. Central area weakly expanded (Figs 6, 14, 19), indistinctly set off from the axial area. Striae radiate to subparallel (almost perpendicular) in the proximal part of the valve, 17–18 in 10 µm, becoming progressively convergent from the Voigt discontinuity to the ends and up to 20 in 10 µm. Areolae recognizable with oblique lighting, particularly in proximal striae.

SEM (Fig. 22 external view, Figs 23–24 internal views): Externally, central ends of the raphe, lying in an external sternum, shortly deflected to the primary side of the valve while the terminal raphe fissures are bent to the opposite (secondary) side where the Voigt fault is clearly visible. Extension of the sternum is ca. ¼ of the valve width. Areola foramina lineolate, orientated in apical lines throughout. Virgae appear approximately



Figs 1–21. Light micrographs of *Craticula simplex* (Krasske) Levkov, showing valve size–diminution series. Samples taken in 2011 (Figs 1–10), 2018 (Figs 11–18), and 2019 (Figs 19–21). Scale bar 10 μm (×1500).

equally broad centrally but narrower in subcentral and terminal parts of the valve (Fig. 22). Areola number is 33–35 in 10 μm. Internally, raphe sternum elevated, slightly asymmetric as externally (Fig. 23); central raphe ends almost straight to shortly and weakly bent, and rather widely spaced (Fig. 24). Terminal ends lying in poorly developed helictoglossae (Fig. 23). Approximately circular areolae aligned in striae and situated in troughs between virgae; striae become progressively strongly convergent at the ends (Figs 23–24).

Epitype: Diatom collection of the MUSE – Museo delle Scienze, Trento, Italy, TR, slide cLIM004 DIAT 4237. Fig. 8 illustrates the epitype specimen.

Basionym (designated here): [icon] Pl. 2: Fig. 33 in Krasske: Krasske, G. 1925: Die Bacillariaceen–Vegetation Niederhessens. Abhandlungen und Bericht des Vereins für Naturkunde zu Kassel 56: 51. 1925.

Isotypes (designated here)**:** Botanical Museum of the University of Berlin, Germany: B 40 0046488. The Natural History Museum London (UK), Cryptogamic Herbarium – Diatoms, BM 98362.

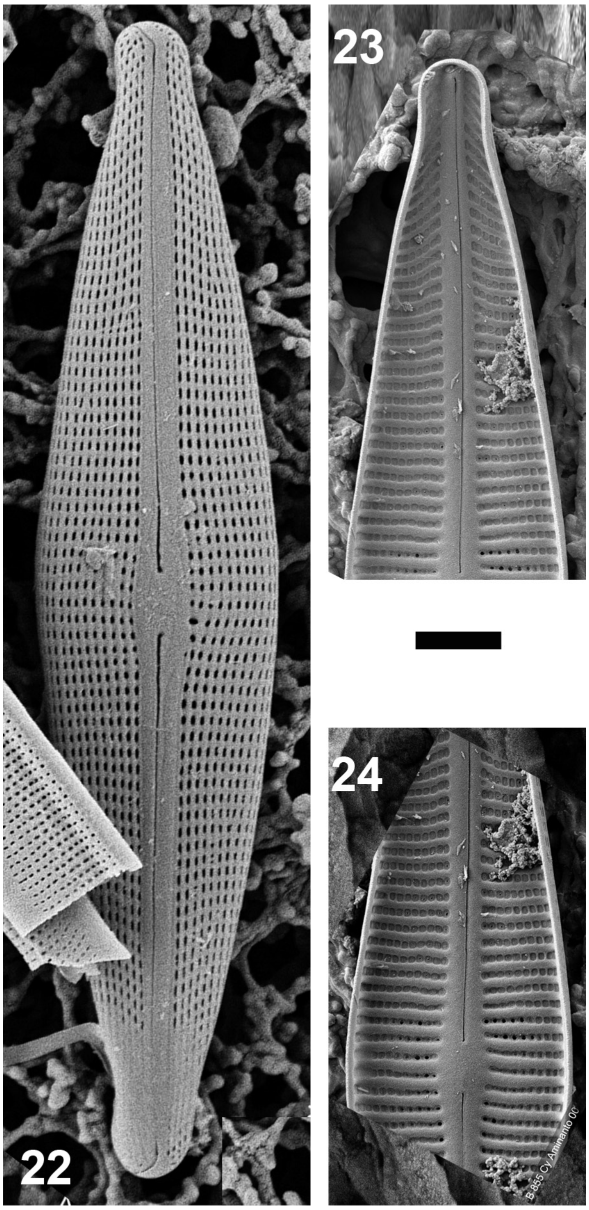
Registration: http://phycobank.org/103776

Epitype locality: Stream sampling site Treminthos near Agia Anna, Monitoring Code r8–4–3–40 (date of sampling 26/03/2021): 135 m a.s.l. Coordinates: East. 544130, North. 3866670 UTM Zone 36 North WGS 84. Geology: Upper pillow lavas. Catchment Area (km^2): 92.9. Source distance (km): 17.3. Bed stability: stable. Prevailing channel substrate type: gravel and cobble. Channel shading: Completely exposed. Prevailing flow type: Smooth. Main aquatic vegetation: *Cladophora* sp. (green algae), *Veronica anagallis–aquatica* (vascular plants). Environmental conditions (seasonal averages): Discharge (L.s^{-1}): 90, Temperature (°C): 14.13, conductivity (μS.cm^{-1}): 1460, pH: 8.2, dissolved–oxygen saturation (%): 93.5, nitrate nitrogen (mg.L^{-1}): 3.66, TP (μg.L^{-1}): 56, SRP (μg.L^{-1}): 40.

Microhabitat preference in the study area: Observed in the epilithon of Mediterranean streams. Here associated with *Nitzschia palea* (Kütz.) W.Smith and predominantly calciphilous diatom taxa. We did not observe "heribaudii" or "craticula" stages at this location where the conditions are only barely comparable with ephemeral water bodies or high fluctuations of specific conductivity.

Distribution in the study area: Frequent in Cypriot streams (24 sites) but never abundant. Observed qualitatively (i.e., relative abundance <0.25%) on 9 slides and counted on 15 (relative abundances: 0.25–2.5%). It's distribution in the island covers the southern and western parts. It has been recorded in 24 locations, in 23 different streams, within 16 catchments.

Most common associated species (> 15% on slides where *Craticula simplex* (Krasske) Levkov was recorded): *Nitzschia palea* (Kütz.) W.Smith, *Adlafia minuscula* (Grunow) Lange–Bert., and *Nitzschia inconspicua* Grunow.



Figs 22–24. SEM images of *Craticula simplex* (Krasske) Levkov: (22) external view of an entire valve showing the raphe structure, and details of the striae and of the central area; (23–24) internal views of the pole and central regions of the valve depicting the elevated and slightly asymmetric raphe sternum, the central nodule, occluded areolae, proximal and distal raphe fissures, and simple helictoglossa. Scale bar 3 µm.

Mastogloia cyprica Lange–Bert. et Cantonati sp. nov. (Figs 25–36, 37–40)

Synonyms: *Mastogloia elliptica* var. *danseyi* (Thwaites) Cleve sensu Krammer et Lange–Bert. (1986, p.435, Figs 202:1a, 1b, 2). *Mastogloia recta* Hust. sensu Germain 1981, p. 124, Figs. Pl. 45, Figs 8–11 and Pl. 167: 2, non typus from tropical SE–Asia.

Excluded from synonymy and conspecificity, respectively: *Mastogloia elliptica* (Agardh) Cleve 1893; *Mastogloia (Dickieia) danseyi* (Thwaites) W.Smith 1856; *Mastogloia (Dickieia) danseyi* Thwaites, 1848.

Description

LM (Figs 25–36): Frustules in girdle view rectangular with moderately depressed proximal parts of both valves. Mantles with areolated striae (Fig. 36). Partectal rings with ca. 15–25 uniform partecta along each side, 6–7 in 10 µm (Figs 34–36). Valves of the shortest cell cycle stages elliptical (Figs 32–33), medium–sized and larger stages linear–elliptic to linear with cuneately narrowed, finally obtusely–rounded to subrostrate ends (Figs 25–31). Length 40–66 µm, breadth 12–14 µm. Raphe very strongly undulate; proximal raphe endings hardly expanded, deflected in the same direction, terminal fissures appearing indistinct (Figs 25–33). Axial area rather broad in comparison to similar taxa, ca. 1.5–2 µm, narrowed in terminal parts. Central area distinct, rhomboidal to almost circular in shape, 1/3 or somewhat wider than the valve width, approximately symmetrical. Striae areolate, radiate throughout, 14–15 in 10 µm, becoming slightly denser near the ends. Areolae circular to somewhat transapically elliptic, 12–13.5 in 10 µm (Figs 25–33).

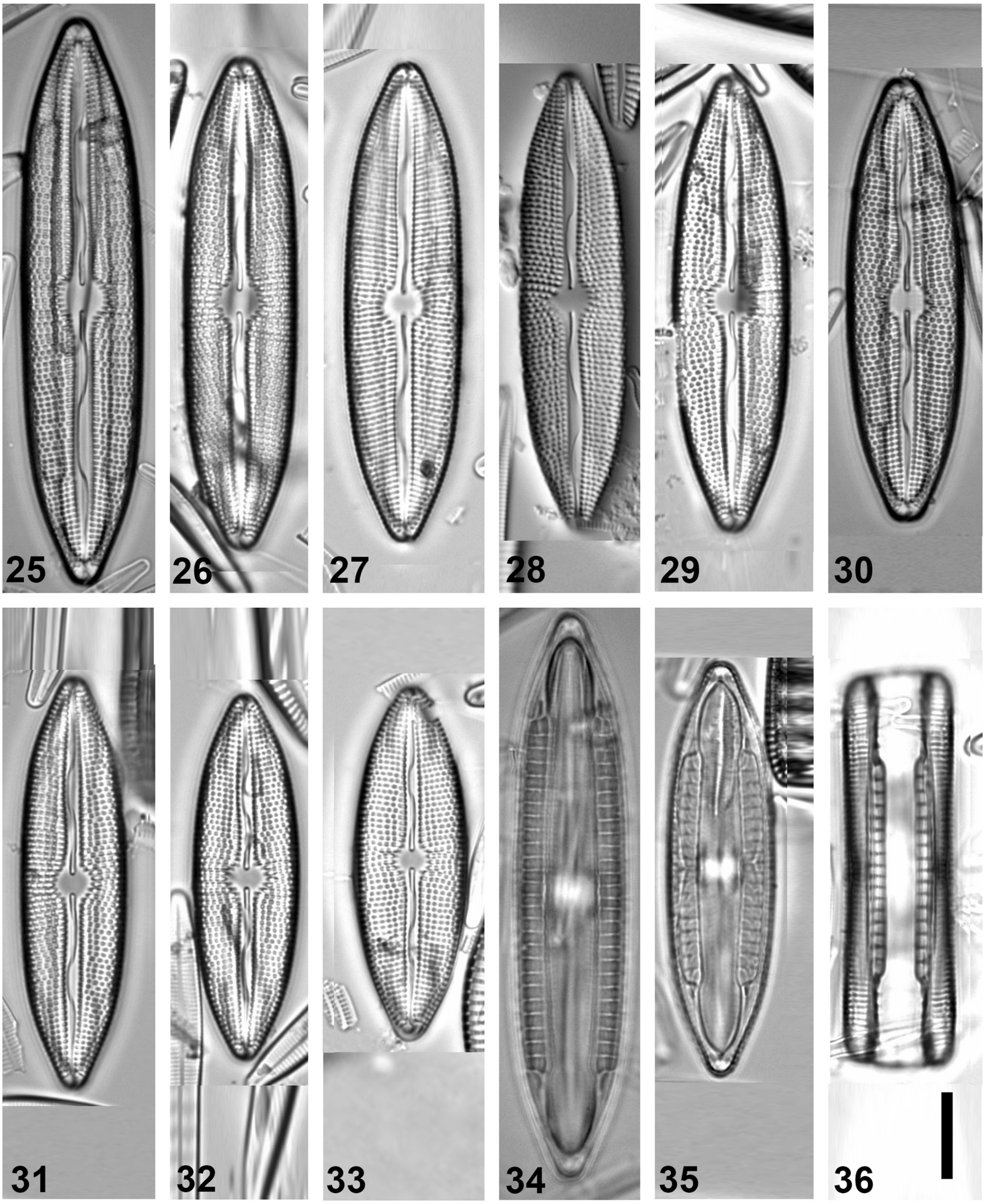
SEM (Figs 37, 40 external view, Figs 38–39 internal views): Externally the raphe undulates approximately over the full width of the axial area and hardly expanded at the proximal and terminal ends; terminal raphe ends deflect to the opposite side of the proximal raphe ends onto the mantle (Fig. 37). Areolae run first with unchanged arrangement over the junction of the valve face and mantle but finally become slit–like in pervalvar direction on the mantle (Fig. 40, arrow). Slit–like pervalvar areolae visible externally on the mantle together with the external partectal pores (Fig. 39, arrow), ca. 11 pores on each external end of the valve associated with the partectal ring (Fig. 38). Internally, polar raphe endings flanked by pseudopartecta (Fig. 38). The pseudoseptum is visible below the pyriform lacuna (pear–shaped gap) with a narrow cleft at the apical end of the partectal ring (Figs 39). Areolae with cribral pores are aligned in striae, which are separated by the virgae (Figs 39–40). Raphe sternum encasing the straight raphe slit (Figs 38–39). For comparison of valve morphology in SEM with similar but distinct taxa including *Mastogloia lacustris* (Grunow) Grunow and *M. calcarea* Lee, Gaiser, Van de Vijver, Edlund et Spaulding see Lee et al. (2014).

Holotype: Diatom collection of the MUSE – Museo delle Scienze, Trento, Italy, TR, slide cLIM004 DIAT 3521. Fig. 27 illustrates the holotype.

Isotypes: Botanical Museum of the University of Berlin, Germany: B 40 0046489. The Natural History Museum London (UK), Cryptogamic Herbarium – Diatoms, BM 98363.

Registration: http://phycobank.org/103773

Type locality: Stream sampling site Argaki tou Monastiriou near Amyrou Monastery, Monitoring Code r9–2–4–27, Date of sampling 03/03/2018, Sample collection A. Papatheodoulou, 350 m a.s.l, Coordinates: East. 504260, North. 3850960 UTM Zone 36 North WGS84. Geology: Serpentinite. Catchment Area (km^2): 3.99. Source distance (km): 1.8. Bed stability: Firmly bedded, Prevailing channel substrate type: bedrock and cobble, Channel shading: Completely exposed. Prevailing flow type: Rippled. Main aquatic vegetation: *Gloeocapsa* sp. (cyanoprokaryotes



Figs 25–36. Light micrographs of *Mastogloia cyprica* sp. nov., showing the valve variability and girdle view of the holotype population. Scale bar 10 μm (×1500).

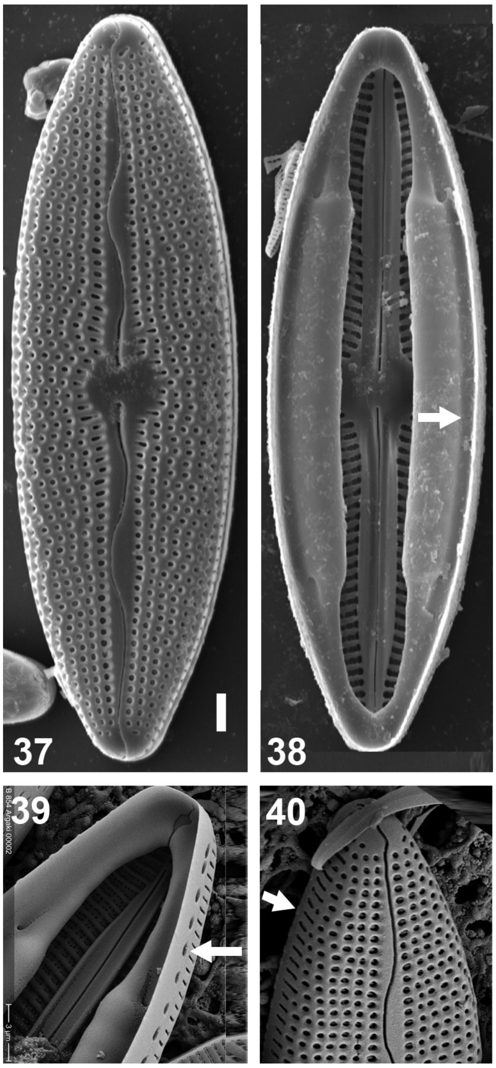
adapted to intermittent wetting), *Schoenus nigricans* and *Saccharum ravennae* (vascular plants).

Habitat: So far known only from the epilithon of a Mediterranean small–discharge spring–fed stream, and of a German lake of volcanic origin.

Etymology: The specific epithet refers to the finding of this rare diatom in a streamlet of the Republic of Cyprus.

Ecology: Environmental conditions at the type locality (seasonal averages): Discharge ($L.s^{-1}$): 3, Temperature (°C): 15.66, conductivity ($\mu S.cm^{-1}$): 958, pH: 9, dissolved–oxygen saturation (%): 98, nitrate nitrogen ($mg.L^{-1}$): 0.02, TP ($\mu g.L^{-1}$): 58, SRP ($\mu g.L^{-1}$): 30.

Distribution: Rare, in Cyprus, with very narrow distribution, recorded only in one location at very low abundance of one or few individuals. Overall, counted on 2 slides with relative abundances of 0.25%.



Figs 37–40. SEM images of *Mastogloia cyprica* sp. nov.: (37) external view of the valve showing the strongly undulating raphe, striae structure and central area; (38) internal view of the whole valve showing the partectal ring with partectal pores (arrow); (39) internal (oblique) close–up view on the pole region of the valve showing pseudopartecta, cleft and lacuna, the raphe sternum encasing the straight raphe slit, and the external openings of partectal pores (arrow); (40) external (oblique) close–up view on the pole region of the valve: Note the single slit–like areola on the mantle in prolongation of each stria (arrow). Scale bar 2 µm.

Obviously, *M. cyprica* was illustrated photographically in the past from comparable freshwater habitats in Central and Western Europe but misidentified under various names. A population observed recently in a medium–conductivity crater Lake Laach (Figs 45–47), Eifel Mountains, West Germany of young volcanic origin, varies moderately by narrower valves about 10–11 µm width (*vs.* 12–14), 15–16 striae (*vs.* 14–15) in 10 µm and areolae 14–15 (*vs.* 12–13.5) in 10 µm (Figs 41–44). However, the overall conforming complex of characters seems to represent the same species. *M. cyprica* was depicted under the name *M. danseyi* in Pavlov et al. (2016; Figs 1–32), who studied freshwater *Mastogloia* (Bacillariophyceae) taxa from Macedonia.

For comparison, here we show the similar species *Mastogloia danseyi* (Thwaites) W. Smith. The type specimens (Figs 41–54) from British River Ouse (photos by B. Van de Vijver) and specimens from the Poiano Spring in the northern Apennines , Italy, are illustrated in Figs (48–52). For this last population, we could also document the chromoplasts (Figs 51–52), which are two plastids, one towards each pole, lying under one valve face and extending on the sides of the girdle (compare Cox 1996).

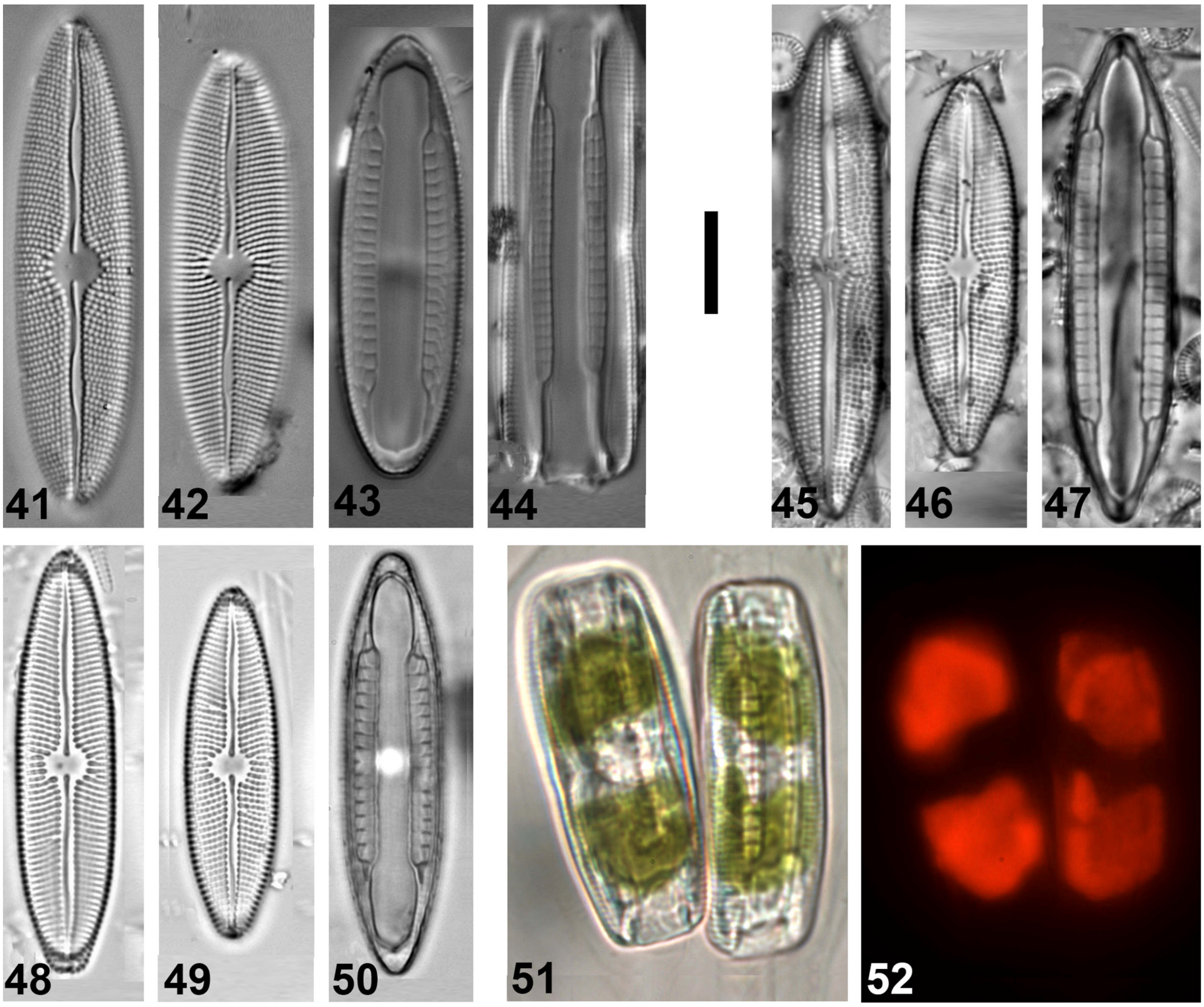
Most common associated species (> 15% on slides where *Mastogloia cyprica* was recorded): *Achnanthidium jackii* Rabenh.

Navicula loumatensis **Lange–Bert. et Cantonati sp. nov. (Figs 53–80, 81–83)**

Description

LM (Figs 53–80): Valves rather narrowly lanceolate tapering from the centre to the simply rounded non–protracted ends. Length 30–40 µm, breadth 6–7.5 µm. Ratio length–to–breadth 4.5–5.8. Raphe branches narrowly lateral, begin in a narrow, straight lanceolate axial area with central endings that are placed at an average (for *Navicula* s.s.) distance from one another but slightly deflected to the same (secondary) side as the distal ends of the terminal fissures. Central area somewhat variable in shape from approximately circular (e.g., Figs 55, 72–73) to rhombic (e.g., Figs 53, 57–59) extended a little less than half of the valve width. Striae rather strongly radiate from the centre to the Voigt fault where they become gradually less radiate to subparallel, finally slightly or moderately convergent, commonly 14, sometimes 13 or 14.5 in 10 µm, and finer near the ends, up to ca. 16 in 10 µm. In the centre one or two shorter striae irregularly intercalated. Areolae cannot be discerned with light microscopes even using strongly oblique lighting.

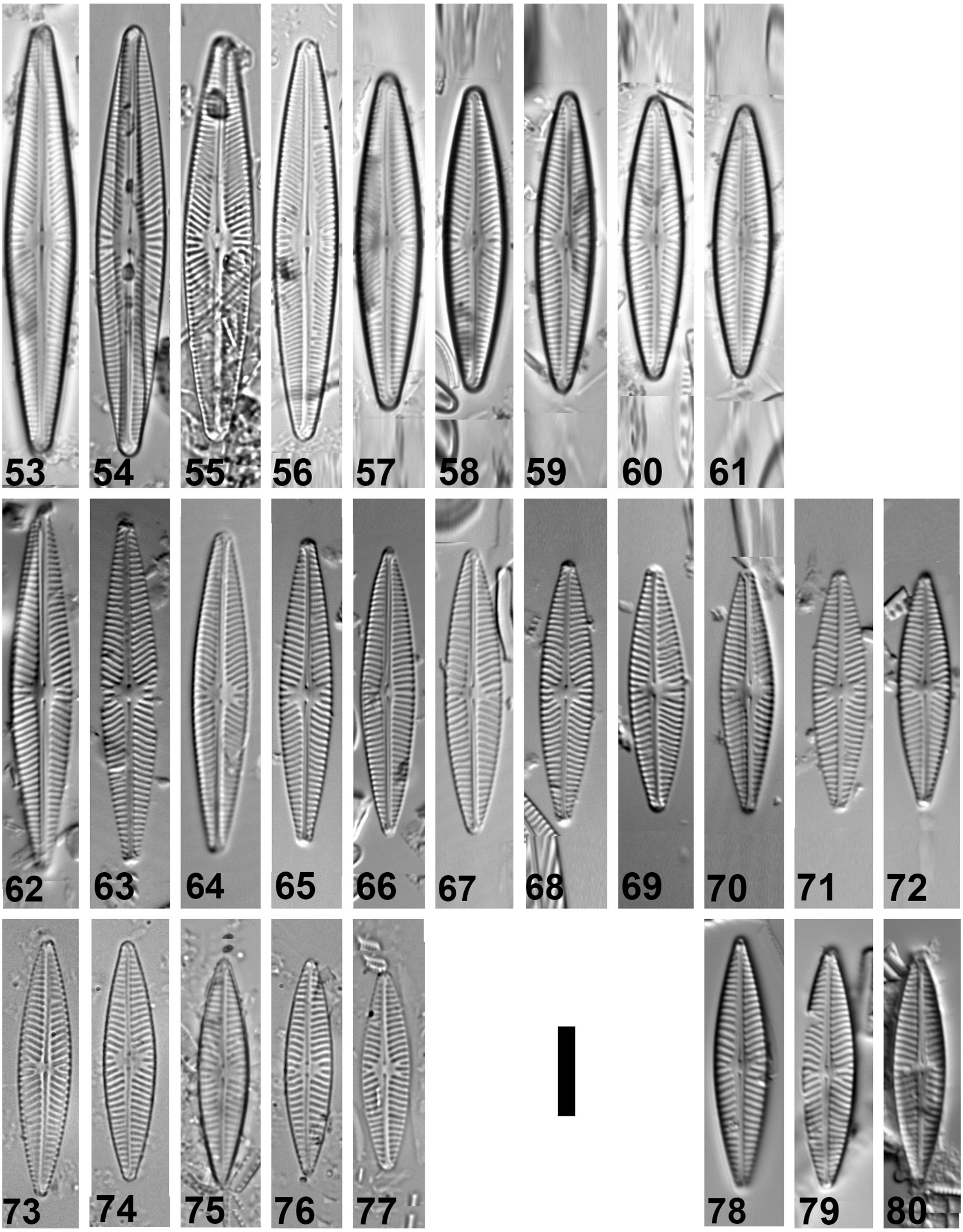
SEM (Figs 81–82 external views, Fig. 83 internal view): Externally, axial area and raphe sternum distinctly raised above the valve face, the proximal part extended transapically into a prominent central area (Figs 81–82). The central raphe ends drop–like and very slightly deflected to the secondary side of the valve; distal raphe ends are widely hooked terminal fissures and also deflect to the secondary side of the valve with the Voigt fault. Slit–like foramina of the areolae are expanded apically as long as the width of virgae, areolae density 46–50 in 10 µm; this is uncommonly high and hence an appropriate feature to distinguish the species from other large–celled *Navicula* spp. (Figs 81–82). Internally, the raphe slit is in a raphe sternum that is slightly narrower than the axial area; terminal raphe ends are small helictoglossae. The sternum expands at the central nodule similar to the majority of species in *Navicula* section *Navicula* (Fig. 83). Likewise, other main characters such as virgae separating troughs with "lineolate" areolae, the axial area expanding as a rib at the Voigt fault, and a hyaline and almost symmetrical central area do not



Figs 41–52. Light micrographs of the second population of *Mastogloia cyprica* sp. nov. (Lake Laach, 45–47), and *Mastogloia danseyi* shown for comparison: type material from British River Ouse (photo by B. Van de Vijver, 41–44), population from Poiano spring, Northern Apennines (48–52); (51–52) chromoplasts (bright field and autofluorescence) in fresh materials from the Poiano spring *M. danseyi* population. Scale bar 10 μm (×1500).

differ significantly from other large–celled taxa (see for comparison e.g. *N. parahasta* (LANGE–BERTALOT 2001, Figs 55:91, *N. hastatula* (Fig. 56:7) or *N. capitoradiata* (Fig. 78:6)). In oblique view (Figs 19, 20), a rather deep apical depression separates the raphe rib with central nodule from other parts of the sternum in a broad sense.

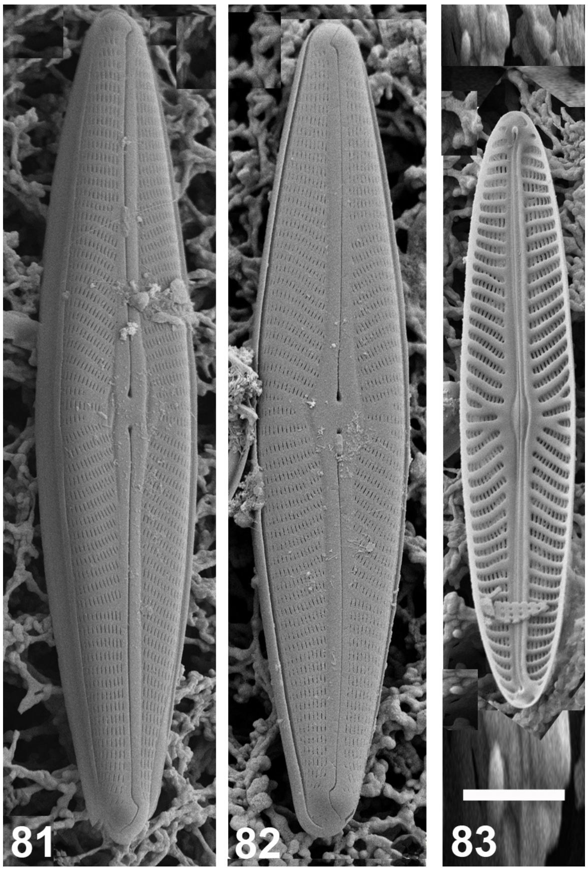
Holotype: Diatom collection of the MUSE – Museo delle Scienze, Trento, Italy, TR, slide cLIM004 DIAT 3545. Fig. 65 illustrates the holotype.
Isotypes: Botanical Museum of the University of Berlin, Germany: B 40 0046490. BM, slide BM 81900. The Natural History Museum London (UK), Cryptogamic Herbarium – Diatoms, BM 98364.
Registration: http://phycobank.org/103774
Type locality: Stream sampling site Stavros tis Psokas near Sarama quarry, Monitoring Code r2–2–6–35, Date of sampling 14/03/2018, Sample collection A. Papatheodoulou, 210 m a.s.l. Coordinates: East. 456240, North. 3867880 UTM Zone 36 North WGS 84. Geology: Upper pillow lavas. Catchment Area (km^2): 67.6. Source distance (km): 16.8. Bed stability: stable, Prevailing channel substrate type: gravel and cobble, Channel shading: Broken–medium. Prevailing flow type: Rippled. Main aquatic vegetation: *Cladophora* sp. (green alga), *Chara vulgaris* f. *longibracteata* (Kütz.) H.Groves et J. Groves (stonewort), *Alnus orientalis* Decne (vascular plant).
Distribution: Frequent (27 sites) in Cypriot streams but usually not abundant. Observed qualitatively (i.e., relative abundance <0.25%) on 6 slides and counted on 21 (relative abundances: 1–7%). Its distribution in the island covers the southern and western part. Recorded in 27 locations, in 24 different rivers, within 18 Cypriot catchments.
Habitat: Epilithon of Mediterranean streams.
Etymology: The species was first identified in 2018, at the station Loumata d/s Almyrolivadhon (boy scout camp) (Monitoring code r9–6–3–12). Both the species and the station were named after the toponym “Loumata tous Atous” which in Greek means “Eagles’ Baths”. The toponym refers to the homonymous spring, in which according to the tradition, eagles when bathed were reborn.



Figs 53–80. Light micrographs of *Navicula loumatensis* sp. nov. depicting valve size–diminution series. Samples taken in 2011 (Figs 53–61), 2018 (Figs 62–72), 2019 (Figs 73–77), and 2020 (Figs 78–80). Scale bar 10 μm (×1500).

Ecology: Environmental conditions at the type locality (seasonal averages): Discharge (L.s^{-1}): 100, Temperature (°C): 15.85, conductivity (μS.cm^{-1}): 943, pH: 8.1, dissolved–oxygen saturation (%): 84, nitrate nitrogen (mg.L^{-1}): 0.14, TP (μg.L^{-1}): 35, SRP (μg.L^{-1}): 14.

Most common associated species (> 15% on slides where *Navicula loumatensis* was recorded): *Achnanthidium minutissimum* (Kütz.) Czarn. and *Nitzschia inconspicua* Grunow.



Figs 81–83. SEM images of *Navicula loumatensis* sp. nov.: (81–82) external view of entire valves showing details of the sternum, polar and central raphe fissures, and slit–like areolae; (83) internal view of an entire valve with raphe slit, prominent central nodule, and helictoglossae. Scale bar 5 µm.

Discussion

Our *Craticula* species from Cyprus discussed here corresponds to *Craticula simplex* (Krasske) Levkov Morphotype I (Levkov et al. 2016, Fig. 17: 1–18 and Fig. 19: 1–6). Levkov et al. (2016) provided a new combination for *Navicula simplex* Krasske. Since *Navicula simplex* Krasske is based on the small–scale drawing of a single valve and the type material is no longer available (Lange–Bertalot et al. 1996), we provided additional ecological, morphological, and ultrastructural data, and an epitype for *C. simplex*. No established taxon is known from Europe or elsewhere that could be confused with *Craticula simplex* Morphotype I (see Supplementary Table S1). Several species might have similar taxonomic features; however, *C. simplex* still differs in other aspects of valve outline (i.e., rhombic–lanceolate to lanceolate with wavy margins and sometimes triundulate in larger specimens) and striation density (17–18 in 10 µm and up to 20 near the poles). The other morphologically close *Craticula* have distinctly different valve morphology and dimensions, and in general much higher stria density. *C. anatoliana* readily differs by its smaller and thinner valves (17.2–26.1 µm long and 4.1–5.1 µm wide *vs.* 30–44 µm long and 7–9 µm wide in *Craticula simplex* (Krasske) Levkov), almost indistinguishable central area (*vs.* weakly expanded and sometimes indistinctly set off from the axial area in *Craticula simplex* (Krasske) Levkov), parallel striation pattern, becoming convergent near the apices (*vs.* subparallel to radiate striae in *Craticula simplex* (Krasske) Levkov), higher areola density (40–45 in 10 µm *vs.* 33–35 in 10 µm in *Craticula simplex* (Krasske) Levkov), and ecological preferences (typically found in brackish waters *vs.* freshwaters for *Craticula simplex* (Krasske) Levkov). *Craticula buderi* (Hust.) Lange–Bert. is roughly similar based not on the mass culture of the type material, but an apparently conspecific population from Lake Tegel in Berlin (see Lange–Bertalot 2001, Figs 90: 6–13). Other populations which have been identified as questionable *C. buderi* (Lange–Bertalot 2001, Plates 90, 91) represent independent and yet undescribed species, and do not conform to *C. simplex*. *C. buderi* can be easily distinguished by L/W ratio (2–5 *vs.* 4.3–4.9 in *C. simplex*), shape of the central area (elliptical, oval or circular *vs.* weakly expanded and indistinctly set off from the axial area in *C. simplex*), and areola density [(28–)34–52 in 10 µm *vs.* 33–35 in 10 µm in *C. simplex*]. In other Cypriot samples, specimens appear (we have photographed a single one in the assemblage of Loumata river upstream of the Amiantos Reservoir but are looking for more in other stream habitats of Cyprus) that come close to the smallest stages of *C. simplex,* but all have rhomboidal valve outlines combined with wavy (sometimes triundulate) margins. Apart from the clear discrepancies in valve outlines and ends, *C. accomoda* also differs from *C. simplex* by its typically smaller valves (17–25 µm vs. 30–44 µm long), smaller L/W ratio (3.1–3.4 vs. 4.3–4.9), much higher stria density (up to 25 in 10 µm at the middle and 28 in 10 µm near the poles *vs.* 17–18 and up to 20 near the poles in *C. simplex*), and occurrence in organically polluted habitats. As compared to *C. riparia* var. *mollenhaueri*, there are some quite sharp differences that could be used to readily differentiate it from *C. simplex*. *C. riparia* var. *mollenhaueri* is mainly characterized by absence of valve margin undulation, much smaller valves (22–35 µm long), relatively higher stria density, and the presence in weakly acidic–to–circumneutral waters most often with humic acids, while *C. simplex* is usually found in alkaline freshwaters.

Mastogloia cyprica sp. nov. is distinguished by a combination of taxonomic features that could be applied to readily differentiate it from the other close taxa (*M. recta*, *M. danseyi*, *M. elliptica*, *M. calcarea*, and *M. lacustris*): 1) valves elliptical in small specimens and linear–elliptic to linear in larger specimens; 2) raphe is very strongly undulate; 3) striation density (14–15 in 10 µm and hardly denser near the apices); 4) ecological distribution in oligosaprobic, oligo– to slightly–eutrophic calcium carbonate rich flowing or stagnant waters with medium conductivity (never brackish) (Supplementary Table 2).

The most closely related species appears to be the lectotypified single valve and paralectotype of *Mastogloia recta* Hust. (documented by Simonsen 1987, Figs 403:1–3) from Lake Towoeti on paleotropical Sulawesi Island (known as Celebes Island, governed by Indonesia). Further illustrations by Simonsen (1987) (Pl. 403, Figs 7–11) from two other lakes of the same island are probably not conspecific. All of them are distinguished from the European *M. cyprica* populations by a significantly narrower, < 1 µm wide axial area in which the external raphe slit undulates much less strongly. The valve ends are bluntly rounded rather than cuneate. Partecta are ca. 8 (*vs.* 6–7) in 10 µm. Ecologically, *M. recta* prefers high–electrolyte fresh and brackish waters, while *M. cyprica* seems to prefer calcium carbonate–rich flowing or stagnant waters with medium conductivity (never brackish). Less similar is *M. danseyi* (Thwaites) W. Smith; for comparison, see type specimens from British River Ouse (Figs 41–44) and specimens from the Poiano Spring (Figs 48–52) in the northern Apennines, Italy (Cantonati et al. 2020c). All are living in brackish waters. The valve shape of longer specimens of *M. danseyi* is not strictly linear, ends are bluntly rounded, not cuneate. Axial area is narrower, the central raphe ends less distinctly curved. Areola density is higher, 15–20 (vs. 12–13.5) in 10 µm. *Mastogloia elliptica* (Agardh) Cleve is an often confused and has and inappropriately expanded species concept (see e.g. Krammer & Lange–Bertalot, 1986, illustrating heterogeneous specimens). The "nominate variety" of *M. elliptica* is characterized as a brackish water taxon, living frequently at marine coasts, for example of the North and Baltic Sea. Except for its autecology, *M. elliptica* is mainly distinguished by lacking linear cell cycle stages with parallel margins and a narrower axial area. *M. elliptica* specimens investigated by Pavlov et al. (2016, Figs 33–67) showed a combination of diagnostic features that make differentiation of the two species is much easier: 1) weakly protracted and rounded ends (*vs.* cuneately narrowed and obtusely rounded ends in *M. cyprica*); 2) much smaller valve dimensions (length 31.5–44.0 µm and width 9.0–10.5 µm *vs.* 40–66 µm long and 12–14 µm wide in *M. cyprica*); 3) higher stria density (15–17 in 10 µm); 4) higher partecta density (7–9 *vs.* 6–7 in 10 µm in *M. cyprica*); 5) the external proximal raphe fissure is expanded into round to tear–drop shaped central pores (vs. hardly expanded in *M. cyprica*); 6) the external distal raphe fissure forms a question mark shape (vs. not so but extended on the mantle). Besides the quite sharp discrepancies in the valve outlines and raphe course, both *M. calcarea* and *M. lacustris* differ from *M. cyprica* by their narrower valves, much higher stria and areola densities, the external distal raphe fissures with a small hyaline area, as well as ecological preferences (Supplementary Table S2).

Navicula loumatensis sp. nov. belongs to the *Navicula* section based on the central raphe endings which are weakly deflected towards the secondary side (Lange–Bertalot 2001). Although morphologically similar to several lanceolate European *Navicula* species, *N. loumatensis* could not be identified using the currently available literature. However, *N. loumatensis* can be distinguished by a combination of diagnostic features including rather narrowly lanceolate valve outline, the circular to rhombic central area, stria density (13–14.5 in 10 µm and up to 16 towards the apices), a much higher lineola density (46–50 in 10 µm), and internally the raphe slit is distinctly twisted. The first species that might be confused with the new taxon is *Navicula microdigitoradiata* Lange–Bertalot (1993, Pl. 58, Figs 9–15) with regard to the entire suite of morphologic characters. *Navicula microdigitoradiata* is 15–40 µm long, 5–7 µm broad, has 14–17 striae in 10 µm, and the areolae are likewise not discernible in LM. *Navicula microdigitoradiata* is distinguished, however, by more obtusely rounded ends of the elliptic–lanceolate to linear–lanceolate valves. The central raphe pores are also distinctly more closely spaced. The second comparable taxon is *N. cataracta–rheni* Lange–Bertalot (1993, Pl. 59, Figs 13–15), which is mainly distinguished by areola density, ca. 32 areolae in 10 µm (*vs.* 46–50 in 10 µm in *N. loumatensis*). Besides the clear discrepancies in the valve outlines and ends, the recently established species *N. similecataracta–rheni* Sagnet, Van de Vijver et Tudesque (2022, Figs 2–51) still differs by its much smaller valve length (20–32 µm long vs. 30–40 µm long in *N. loumatensis*), and a lower lineola density (27–30 in 10 µm) (Supplementary Table S3). The fourth and closest resembling "non–taxon" is *Navicula* spec. from the central and northern Alps, Austria (see Lange–Bertalot 2001, Fig. 7:9–11 and 12–13), which has length 35–48 µm, breadth 6.5–7.5 µm, stria density 13.5–14 in 10 µm, stria position, areola not discernible with light microscopic techniques, raphe area, and valve shape widely conforming to *Navicula loumatensis*; unfortunaely SEM observation was not possible on *Navicula* spec. Another morphologically similar species is *N. cincta* (Ehrenberg) Ralfs, which is broadly similar with regard to valve shape and a lower stria density, but in SEM, the oblique external and internal valve view shows areola density to be much lower, ca 40 in 10 µm. In contrast, *N. loumatensis* areola density (46–50 in 10 µm) is uncommonly high and hence an appropriate feature to distinguish the proposed new species from other large–celled *Navicula* spp. under the SEM. Lastly, *N. cryptotenella* can be separated from *N. loumatensis* in the shape and size of the central area (usually small with rather irregular border shape *vs.* most often circular to rhombic in *N. loumatensis*), having a higher stria density (14–16 vs. 13–14.5 in 10 µm in *N. loumatensis*), and a lower lineola density (38 in 10 µm vs. 46–50 in 10 µm in *N. loumatensis*).

Conclusion

Mediterranean streams are affected by multiple stressors, some of which, such as organic pollution, are global pressures, whilst others, such as olive mill wastewater, are more specific for geographic areas with this climate (e.g., Cantonati et al. 2020b). Moreover, the biota they host, including diatom microalgae, is highly influenced by the natural, often extreme, hydrological variability, which will be exacerbated by climate change, especially in the eastern Mediterranean. However, knowledge on the specific features of some components of the biota, that are also of pivotal importance for monitoring, namely diatoms, is still incomplete. This paper describes, illustrates, and characterizes three naviculoid diatom species, in the genera *Craticula*, *Mastogloia* and *Navicula*, from streams of the Island of Cyprus (Eastern Mediterranean) as a contribution to a more in–depth knowledge of the diatom communities of Mediterranean streams.

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