Revision of European Brachysira species (Brachysiraceae, Bacillariophyta) : IV. the Brachysira vitrea group

Revision of European *Brachysira* species (Brachysiraceae, Bacillariophyta): IV. the *Brachysira vitrea* group

Bart Van de Vijver[1,2], Tanja M. Schuster[3], Gabriele Hofmann[4], Bryan Kennedy[5], Joachim Hürlimann[6] & Wolf-Henning Kusber[7]

[1]Meise Botanic Garden, Research Department, Nieuwelaan 38, 1860 Meise, Belgium; (corresponding author: bart.vandevijver@plantentuinmeise.be)

[2]University of Antwerp, Department of Biology – ECOSPHERE, Universiteitsplein 1, 2610 Wilrijk, Belgium

[3]Natural History Museum, Department of Botany, Herbarium, Burgring 7, 1010 Vienna, Austria

[4]Hirtenstrasse 19, 61479 Glashütten, Germany

[5]Environmental Protection Agency, Castlebar, Co. Mayo, Ireland

[6]AquaPlus AG, Gotthardstrasse 30, CH-6300 Zug, Switzerland

[7]Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin, Königin-Luise-Strasse 6-8, 14195 Berlin, Germany

Abstract

This fourth paper revising the European *Brachysira* species deals with the complex of species and populations identified as *Brachysira vitrea* and *Brachysira hofmanniae*. The morphology of these species is characterised in detail based on a combination of analyses of original type material and additional historic and modern samples.

The analysis of the type material of *B. vitrea* clearly showed that this apparently highly variable species should be divided into several distinct species, described as new: *Brachysira ellipticovitrea*, *B. vitreoides* and *B. paraexilis*. All new species can be separated, both in LM and SEM, based on valve outline, shape of the apices, morphometric differences and structure of the areolae and spine ornamentation.

The related *B. hofmanniae* group also contains several species, as shown by the type material analysis. *Brachysira pseudovitrea* has a very dense pattern of small spines on the virgae and an elongated, clearly heteropolar valve outline. *Brachysira hamiltoniana* is characterised by a very large marginal ridge and enlarged longitudinal axial ridges in the axial area, covering almost entirely the valve face. Finally, *B. ectoriana* has a typical areola pattern in the central area and a large marginal ridge, partly covering the valve margins.

The ecological preferences of all the reported taxa is derived from the accompanying diatom flora in the investigated samples and from measured physicochemical parameters. All taxa in this complex are typically found in oligotrophic, more or less alkaline environments.

KEYWORDS

Brachysira; Europe; morphology; taxonomy; type material

Introduction

In this fourth paper in a series on the European species of the genus *Brachysira* (Brachysiraceae, Bacillariophyta), we aim to provide a detailed revision of species in the *Brachysira vitrea*-complex. Previous papers in this series dealt with the *B. microcephala-neoexilis* group (Van de Vijver et al. 2021a), the *B. styriaca-zellensis* group (Van de Vijver et al. 2021b), and the *B. serians-brebissonii* group (Van de Vijver et al. 2022). In this last paper of the series, the type material of two species of *Brachysira* showing a very fine striation pattern is discussed and illustrated. *Brachysira vitrea* (Grunow) R.Ross was originally described as *Gomphonema vitreum* Grunow from the Austrian Lake Erlauf (Erlafsee) (Grunow 1878), due to its slightly heteropolar valve outline, a feature usually observed in the genus *Gomphonema* (Round et al. 1990). *Brachysira vitrea* is a regularly observed species in Central-European alkaline, oligotrophic lakes (Lange-Bertalot et al. 2017), but is disappearing gradually due to increased pollution, as it does not tolerate mesotrophic conditions. However, its morphology seems rather variable, as was illustrated in Lange-Bertalot & Moser (1994, plate 3), including both clearly heteropolar, but also strictly isopolar valves. In 1994, Lange-Bertalot (in Lange-Bertalot & Moser 1994, p. 31) split off *Brachysira hofmanniae* Lange-Bert. based on valve outline, shape of the central area, and differences in the surface structure of the valve face. The illustrated type population in Lange-Bertalot & Moser (1994, plate 8, figs 1–18), however, also shows a morphologically highly variable taxon, including several valves that most likely should be recognised as an independent species. Moreover, some of the valves illustrating *B. hofmanniae* cannot be distinguished from some of the illustrated valves identified as *B. vitrea* (see Lange-Bertalot & Moser 1994, plate 3, figs 1–7), urging for a revision of this complex.

The main aim of the current series of papers revising the European species of *Brachysira* is to better document the variability of each species, refine our knowledge of their ecological preferences, and discuss their taxonomy by analysing and illustrating the original (= type) material using light (LM) and scanning electron microscopy (SEM). The revision of the other *Brachysira*-groups (Van de Vijver et al. 2021a,b, 2022) showed that the diversity within the genus *Brachysira* in Europe had been underestimated up until now. The results of this ongoing revision not only offered a more precise characterisation of some well-known species, such as *B. neoexilis* Lange-Bert. (Lange-Bertalot & Moser 1994) or *Brachysira brebissonii* R.Ross (Hartley 1986), but also led to the description of several new species, such as *B. elisabethiana* Van de Vijver, C.E.Wetzel & Ector (Van de Vijver et al. 2022).

In the present paper, eleven additional populations presumed to be *B. vitrea* and *B. hofmanniae* have been investigated from Central-Europe and Ireland (Table 1). Comparison of the morphology of the type material of both *B. vitrea* and *B. hofmanniae* with these other populations indicated that several similar, though morphologically distinct, species were lumped under the name *B. vitrea*.

Most of the investigated populations in this fourth paper were collected in Bavaria in Germany, Switzerland, and Austria, regions characterized by having several large, oligo- to mesotrophic, distinctly alkaline (calcium-carbonate rich) lakes (Hofmann 1994). Two populations from Ireland were reassessed as they were reported to contain *B. vitrea*-populations (Kennedy & Allott 2017). Based on the results, we propose to recognise an additional six species as new: *Brachysira ectoriana* Van de Vijver & G.Hofm. sp. nov., *B. ellipticovitrea* Van de Vijver, B.Kenn. & G.Hofm. sp. nov., *B. hamiltoniana* Van de Vijver & G.Hofm. sp. nov., *B. paraexilis*

Van de Vijver & B.Kenn. sp. nov., *B. pseudovitrea* Van de Vijver & G.Hofm. sp. nov., and *B. vitreoides* Van de Vijver & B.Kenn. sp. nov.

Material & Methods

The original (type) material for two previously described species of *Brachysira*, *B. vitrea* and *B. hofmanniae*, was investigated. *Gomphonema vitreum* was described from the Austrian Lake Erlauf (= previously, Erlafsee), a small mountain lake (0.58 km²) located at an altitude of 835 m a.s.l. on the border between the Austrian provinces of Styria (Steiermark) and Lower Austria (Niederösterreich), not far from the small town of Mariazell. Grunow listed several samples in his accession books from the Erlafsee but he only mentioned this species in his sample 36. Therefore, Grunow sample 36 (Erlafsee bei Mariazell, auf *Tolypothrix*, coll. date 9.X.1856, leg. A. Grunow, QR-code W: W0127017) has been chosen as original (type) material to be investigated. Unfortunately, unmounted material was nearly devoid of diatoms, but the original slide made by Grunow from sample 36, kept in the Grunow collection in Vienna (W), contained a large population of *B. vitrea*. The second species, *B. hofmanniae*, was described in 1994 from sample Hofmann_S292, collected by G. Hofmann from the Lustsee, Oberbayern, Germany (Lange-Bertalot & Moser 1994). A subsample from the original material was analysed in the present study.

In addition to this type material, eleven European samples (collected from Austria, Germany, Ireland, Switzerland) containing *Brachysira* populations, identified as *B. vitrea* or *B. hofmanniae*, were analysed to determine the variability of the species. Table 1 summarises all investigated samples together with any collection information that was available.

Subsamples of all selected materials were prepared for LM and SEM observations following the method described in van der Werff (1955). Small volumes of the samples were cleaned by adding 37% H_2O_2 and heating to 80°C for about 1 h. The reaction was completed by addition of saturated $KMnO_4$. Following digestion and centrifugation (three times for 10 minutes at 3700 × rpm), the resulting cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Cleaned diatom material was mounted in Naphrax (refraction index 1.73). The resulting slides were analysed using an Olympus BX53 microscope at 1000x magnification (N.A. 1.30), equipped with Differential Interference Contrast (Nomarski) optics and the Olympus UC30 Imaging System, connected to the Cell Sense Standard program. For each taxon, the number of specimens, measured at random on the slide, is indicated (n=X). For SEM, part of the suspension was filtered through 5-µm Isopore™polycarbonate membrane filters (Merck Millipore), pieces of which were fixed on aluminum stubs after air–drying and coated with a platinum layer of 20 nm, and studied using a JEOL-JSM-7100F field emission scanning electron microscope at 2 kV. Slides and stubs are stored at the BR-collection (Meise Botanic Garden, Belgium). Plates were prepared using Photoshop CS5.

Terminology used in the description of the various structures of the siliceous cell wall is based on Ross et al. (1979, areola structure), Cox & Ross (1981, stria structure), Round et al. (1990, ridge structure), Lange-Bertalot & Moser (1994, genus features for *Brachysira*), and Van de Vijver (2014, genus features for *Brachysira*).

For typification of the species, we chose to use the entire slide as the type, following article 8.2 of the International Code for Botanical Nomenclature (Turland et al. 2018). Diatoms show a broad variability along their cell cycle making the choice for the entire population on the slide

more obvious, but because of admixtures, one valve was indicated to illustrate the taxon best (see Figures). All novelties are registered proactively according to Art. 42.3 (Turland et al. 2018).

Results

1. The Brachysira vitrea group

Based on LM and SEM images published in the literature (for instance Lange-Bertalot & Moser 1994), *Brachysira vitrea* is a morphologically quite variable species, grouping isopolar and heteropolar populations, with a lanceolate but also elliptic to elliptic-lanceolate valve outline, and capitate, rostrate and even cuneate apices. Analysis of the lectotype population (Grunow sample 36, lectotype see below) and several other historic samples and recently sampled populations, showed that at least four different species should be distinguished: *Brachysira vitrea*, *Brachysira ellipticovitrea* Van de Vijver, B.Kenn. & G.Hofm. sp. nov., *Brachysira paraexilis* Van de Vijver & B.Kenn. sp. nov., and *Brachysira vitreoides* Van de Vijver & B.Kenn. sp. nov.

***Brachysira vitrea* (Grunow) R.Ross** (Figs 1–133)

Basionym: *Gomphonema vitreum* Grunow (1878), Naturwissenschaftliche Beiträge zur Kenntnis der Kaukasusländer, auf Grund seiner Sammelbeute. (Schneider, O. Ed.), pp. 98-132. Dresden: Dresden Burdach, p. 110.

Lectotype (here designated): historic slide made from Grunow sample 36 (W0164896) (Erlafsee bei Mariazell, Austria) in W! The lectotype is illustrated by Fig. 6.

Epitype (designated here for the above lectotype of *B. vitrea* (Grunow) R.Ross): BR-4790, slide made from Hürlimann sample 2464 (Walensee near Quarten (Unterterzen), Switzerland) (Meise Botanic Garden, BR). The epitype is illustrated by Fig. 25.

Comment: Grunow sample 36 was cited as holotype by Compère (1988), however the sample is a gathering of which several derivations exist but not a single specimen, thus the cited holotype cannot be accepted as a lectotype. Hence we here provide a specimen prepared from Grunow's sample 36 as the lectotype and basis for a modern epitype to fully uncover the identity of the species.

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Synonyms: *Navicula gomphonemacea* Grunow in Van Heurck (1880), nom. illeg., *Anomoeoneis vitrea* (Grunow) R.Ross (Patrick & Reimer 1966), *Navicula variabilis* R.Ross (Ross 1947), *Anomoeoneis variabilis* (R.Ross) Reimer (Reimer 1961), *Anomoeoneis vitrea* var. *gomphonemacea* (Grunow) Moghadam (Moghadam 1969)

Description of the lectotype population (Figs 1–12): Valves typically heteropolar throughout the entire cell diminution series. Valve outline lanceolate to elliptic-lanceolate with convex margins. Headpole protracted, acutely rostrate to cuneate. Footpole protracted, subcapitate to rostrate. Valve dimensions (n=50): length 16–22 µm, width 5.0–5.5 µm. Axial area very narrow, linear, hardly widening near the central area. Central area very small, mostly non-existent. Raphe weakly discernible. Central raphe endings simple, straight, quite close to each other. Striae weakly discernible, ca. 40 in 10 µm. Areolae not visible in LM.

Additional populations (Figs 13–133): Since unmounted material of Grunow sample 36 contained hardly any diatoms, which made SEM analysis impossible, a large population (identified as *B. vitrea* s.s.) collected from the oligotrophic Walensee near the Swiss village of Unterterzen (Quarten, Kanton Sankt Gallen) was studied instead. Additionally, several other populations from Switzerland [Blausee, Berner Oberland, sample Brun 210 (Figs 70–88)];

Austria [Erlafsee, Grunow sample 20 (Figs 57–69), Attersee, Grunow sample 2647 (Figs 89–99), Vienna, Grunow sample 157 (Figs 121–133)]; and France [Cascade de Poux, Parc de Vanoise, Courchevel, sample VAN2020-02 (Figs 100–120)] were investigated to establish the morphological variability of *B. vitrea*. As the populations were studied using both light and scanning electron microscopy, the valve ultrastructure of *B. vitrea* can also be reported. An emended description is therefore proposed here:

Emended description:

Brachysira vitrea (Grunow) R.Ross in Hartley *emend*. Van de Vijver, Hürlimann, T.M.Schust. & Kusber

Valves strictly heteropolar, lanceolate to elliptic-lanceolate with distinctly convex margins. Head pole protracted, rostrate to cuneate; foot pole more elongated, protracted, rostrate to weakly subcapitate. Distinct, narrow marginal ridge surrounding the entire valve at the valve face/mantle junction, almost absent at the valve apices. Valve dimensions (n=60): length 15–40 µm, width 5.5–6.5 µm, length/width ratio 3.2–4.5. Axial area very narrow, linear, hardly widening near the central area. Central area very small to almost absent, occasionally valves with small, elliptical central area observed. Raphe branches straight, bordered by two distinctly raised ridges. Central raphe endings spatulate, drop-like, positioned rather closely to each other (Figs 55 & 56). Terminal raphe fissures short, simple. Striation pattern very dense, striae weakly radiate throughout the entire valve, equidistant, 32–35 in 10 µm. Striae composed of 5–6 irregularly, transapically elongated areolae. Dense pattern of short, acute spines present on the virgae, 3–5 per virga (Fig. 56).

Ecology & associated diatom flora: *Brachysira vitrea* was found in several samples collected from oligotrophic, calcium-bicarbonate rich lakes. The lectotype population was collected from one such water body, Lake Erlauf. The sample was dominated by *Brachysira vitrea*. Other taxa in the sample include *Achnanthidium exile* (Kütz.) Heiberg, *Brachysira neoexilis*, *Denticula tenuis* Kütz., *Diatoma ehrenbergii* Kütz., *Encyonema leibleinii* (C.Agardh) W.J.Silva et al., *Gomphonema* cf. *angustatum* (Kütz.) Rabenh., and *G. italicum* Kütz. The other investigated samples have a comparable diatom flora. The Walensee diatom flora (=epitype population) was mainly composed of *Achnanthidium caledonicum* (Lange-Bert.) Lange-Bert., *A.* cf. *jackii* Rabenh., *Cymbella excisiformis* Krammer, *Denticula tenuis*, *Encyonopsis microcephala* (Grunow) Krammer, and *Fragilaria perdelicatissima* Lange-Bert. & Van de Vijver. The sample collected by Brun from the Blausee was dominated by several species of *Brachysira* such as *B. calcicola* var. *pfisteri*, *B. vitrea* and *B. zellensis*, together with *Achnanthidium trinode* Ralfs, *Cymbella subhelvetica* Krammer, *Delicatophycus delicatulus* (Kütz.) M.J.Wynne, *D. capitatus* M.J.Wynne, *Denticula tenuis*, and *Navicula radiosa* Kütz. Grunow sample 2647 was collected from the Attersee, the largest lake in the province of Upper Austria (Austria). The sample was dominated by *Fragilaria perdelicatissima* with several cymbelloid taxa, such as *Cymbella subhelvetica* and *Encyonopsis subminuta* Krammer being subdominant. Finally, Grunow sample 20 was mainly dominated by various cymbelloid taxa such as *Cymbella simonsenii* and *Delicatophycus delicatulus*. Other taxa in the sample included *Denticula tenuis*, *Epithemia sorex*, *Navicula gottlandica*, *Navicula radiosa*, and several other *Brachysira*, such as *B. liliana*, *B. styriaca*, and *B. zellensis*. The species composition of all these samples mostly points to an oligotrophic, oligosaprobic, calcium-bicarbonate rich, alkaline lake habitat indicating very high water quality (Lange-Bertalot et al. 2017). In this context, the presence of *G.* cf. *angustatum* and

G. italicum in the lectotype sample is unusual, as these taxa mostly thrive in more mesotrophic conditions.

Brachysira ellipticovitrea **Van de Vijver, B.Kenn. & G.Hofm. sp. nov.** (Figs 134–181)

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Light microscopy (Figs 134–150): Valves isopolar, very rarely slightly heteropolar. Valve outline distinctly elliptical with rounded, convex margins and clearly protracted, capitate to subcapitate apices. Valve dimensions (n=50): length 20–35 µm, width 7.0–8.0 µm, length/width ratio 3.8–4.4. Axial area very narrow, linear. Central area very small, usually absent. Raphe filiform, straight, with simple central endings, positioned close to each other (0.9–1.2 µm). Terminal raphe fissures not visible in LM. Striation pattern very dense, striae radiate, equidistant throughout the entire valve, 32–33 in 10 µm. Areolae weakly discernible in LM, at least 5–6 per stria. Valve face spines not visible in LM.

Scanning electron microscopy (Figs 151–154): Valve face flat, bordered by distinctly raised marginal ridge, diminishing in height towards the apices (Fig. 151). Apices lacking marginal ridge, weakly thickened (Fig. 151). Mantle striae, composed of one transapically elongated areola, surrounding the entire valve (Figs 151, 152). Valve face surface ornamented with dense pattern of long, acute spines, located on the virgae (Fig. 153). Striae composed of up to 8 irregularly transapically elongated areolae, bordered by very narrow virgae (Figs 151–153). Areola foramina with smooth edge (Fig. 153). Central area very small (Fig. 151) to almost absent (Fig. 152). Raphe branches bordered by distinct ridges running from apex to central area, leaving a very narrow gap at the central area (Fig. 153). Central raphe endings spatulate (Fig. 153). Terminal raphe fissures short, simple, terminated by a narrow transapical slit (Fig. 151).

Internally, valve face almost flat with virgae almost not raised (Fig. 154). Central raphe endings simple, terminal endings terminating onto indistinct helictoglossae (Fig. 154).

Holotype: BR-4791 (Meise Botanic Garden, Belgium). The holotype is illustrated by Fig. 137.

Isotype: Slide-424 (University of Antwerp, Belgium)

Type locality: Lustsee, southeastern shore, Bavaria, Germany, Sample Hofmann-S314, coll. date 6.XI.1990, leg. G. Hofmann.

Etymology: the specific epithet refers to the resemblance in morphology to *B. vitrea* and the typically elliptical valve outline.

Additional populations: This new species was observed in several samples from Austria [Attersee, Grunow sample 2647, W0127052 (Figs 155–163)], Ireland [Lough Bunny, Kennedy sample WE_27_114_PB_0020_BK_20150630 (Figs 164–172)] and Germany [Bavaria, Weitsee, Hofmann sample S862 (Figs 173–181)]. All populations have a rather similar morphology and comparable valve dimensions.

Ecology and associated diatom flora: The type sample was collected from the Bavarian Lustsee ('Lake Lustsee'), a small oligotrophic lake located south of Munich (Germany). The sample was scraped from *Phragmites* stems on the southeastern lake shore. Hofmann (1994) briefly discussed the history of the lake and the surrounding area. The lake is part of the so-called Osterseen ('Easter Lakes') and characterised by an alkaline pH and very low nutrient status, one of the lowest in all of Bavaria (Hofmann 1994, Seele 2000). The sample has very high species richness. Typical species in the sample include several species of *Brachysira* (*B. hofmanniae*, *B. liliana* Lange-Bert., *B. neglectissima* Lange-Bert.), a large number of cymbelloid taxa [such as *Delicatophycus delicatulus* (Kütz.) M.J.Wynne, *Encyonopsis cesatii* (Rabenh.) Krammer],

Eunotia alkalibiontica Lange-Bert., *Gomphonema helveticum* Brun, *G. vibrio* Ehrenberg, *Mastogloia lacustris* (Grunow) Grunow, *Navicula gottlandica* Grunow, and *N. radiosa* Kütz. This diatom composition clearly points to calcium-bicarbonate rich, strictly oligotrophic conditions (Hofmann 1994, Lange-Bertalot et al. 2017). The smaller population of *B. ellipticovitrea* found in the Attersee (Austria) was observed under similar conditions. Van de Vijver & Wetzel (2022) described a new species of *Fragilaria* from this lake (though from a different Grunow sample, i.e. Grunow 2646), that is also characterised as ultra-oligotrophic and calcareous. Kennedy & Allott (2017) discussed the species of *Brachysira* from Ireland and reported *B. ellipticovitrea* (as *B. vitrea*) to "reach maximum abundance (1.4%) in higher alkaline, calcareous lakes, which were often marl precipitating (68–155 mg/l $CaCO_3$; pH: 8.0–8.1)" (Kennedy & Allott 2017, p. 13). Recently, this species was also observed in a karstic lake in Dalmatia in the Republic of Northern Macedonia and seems to be fairly common in such waterbodies in the Plitvice and Krka region, both in Croatia (Levkov, pers. comm.).

Brachysira paraexilis **Van de Vijver & B.Kenn. sp. nov.** (Figs 182–205)

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Light microscopy (Figs 182–202): Valves mostly isopolar to occasionally weakly heteropolar, the latter valves having the head poles less expanded than the foot poles (e.g. Figs 184, 185, 195). Valve outline distinctly lanceolate with convex margins and clearly protracted, rostrate (head poles) to subcapitate (foot poles) apices. Valve dimensions (n=25): length 25–35 µm, width 5.5–6.0 (6.5) µm, length/width ratio (4.2) 4.5–5.8. Axial area very narrow, linear. Central area very small, elliptical to rounded, clearly raised. Raphe filiform, straight. Central endings simple, positioned ca 1.5 µm from each other. Terminal raphe fissures not visible in LM.

Striation pattern very dense, striae radiate, equidistant throughout the entire valve, 32–33 in 10 µm. Areolae weakly discernible in LM, at least 4 per stria. Valve face spines not visible in LM.

Scanning electron microscopy (Figs 203–205): Valve face flat, bordered by distinctly raised marginal ridge, diminishing in height towards the apices (Fig. 203). Apices lacking marginal ridge, weakly thickened (Fig. 203). Mantle striae, composed of one transapically elongated areola, surrounding the entire valve (Fig. 203). Valve face surface ornamented with short, acute spines, located on the virgae (Fig. 204). Striae composed of up to 5 irregularly transapically elongated areolae, bordered by very narrow virgae (Figs 203, 204). In the central area, striae often bifurcating (Fig. 204, arrow). Areola foramina edges with small protrusions (Fig. 204) giving the areolae an irregular shape. Raphe branches bordered by distinct ridges running from apex to central area, leaving a fairly broad gap at the central area (Figs 203, 204). Central raphe endings spatulate (Fig. 204). Terminal raphe fissures short, simple (Fig. 203). Internally, valve face almost flat with only weakly raised virgae (Fig. 205). Raphe endings simple, terminal endings terminating onto indistinct helictoglossae (Fig. 205).

Holotype: BR-4792 (Meise Botanic Garden, Belgium). The holotype is illustrated by Fig. 187.

Isotype: Slide-425 (University of Antwerp, Belgium)

Type locality: Lough Bunny, Co. Clare, Ireland, Kennedy Sample WE_27_114_PB_0020_BK_20150630, coll. date 30.VI.2015, leg. B. Kennedy.

Etymology: the specific epithet refers to the resemblance with *Brachysira neoexilis*, a species originally identified by Grunow as *Navicula exilis*.

Ecology and associated diatom flora: *Brachysira paraexilis* has so far only been identified with certainty at the type locality, Lough Bunny, a shallow hardwater freshwater lake in the karstic limestone region of the Burren (Co. Clare, Ireland). Kennedy (2021) reported a pH of 8.3 and a conductivity of 300 µS/cm. The lake is a typical marl lake with a total hardness of 128 mg/l CaCO3 and with microbialites developed extensively in the shallow littoral areas. A conspicuous layer of filamentous diatoms was present on the surface of calcified mats comprising of *Schizothrix fasciculata* Gomont and diverse other cyanobacteria and non-diatom algal assemblages. The diatom flora is dominated by *Brachysira neglectissima* (other *Brachysira* species included *B. ellipticovitrea* and rarely *B. styriaca* and *B. neoexilis*), small *Encyonopsis*-species (including *E. subminuta*, *E. krammeri* E.Reichardt, *E. carraensis* B.Kenn., Y.Buckley & Allot), *Achnanthidium* species (including *A. neomicrocephalum* Lange-Bert. & Staab), various cymbelloid taxa of which *Cymbella subhelvetica* and *Delicatophycus delicatulus* are the most common, and *Fragilaria perdelicatissima*.

***Brachysira vitreoides* Van de Vijver & B.Kenn. sp. nov.** (Figs 206–218)

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Light microscopy (Figs 206–214): Valves slightly to clearly heteropolar. Valve outline lanceolate to elliptic-lanceolate with protracted, rostrate, slightly truncated head and subcapitate foot poles. Valve dimensions (n=20): length 20–30 µm, width 6.0–6.5 µm, length/width ratio 3.6–4.5. Axial area very narrow, linear. Central area small, elliptical to rounded, clearly raised. Raphe filiform, straight. Central endings simple, positioned ca 1.0 µm from each other. Terminal raphe fissures not visible in LM. Striae radiate, equidistant throughout the entire valve, 30–32 in 10 µm. Around the central area, radiating series of longer areolae visible. Areolae weakly

discernible in LM, at least 4 per stria. Valve face ornamentation discernible as zigzag pattern running from apex to apex.

Scanning electron microscopy (Figs 215–218): Valve face flat, bordered by a weakly raised marginal ridge, running up until the apices (Fig. 215). Apices only very weakly thickened, lacking a marginal ridge (Fig. 215). Valve face surface ornamented with a dense pattern of long and short, acute spines, located on the virgae (Figs 216, 217). Striae composed of up to 5 irregularly, transapically elongated areolae, bordered by flattened virgae, almost as broad as the striae (Figs 215, 216). In the central area, areolae distinctly elongated, forming long, undulating grooves (Figs 216, 218). Areola foramina edges undulating with distinct protrusions (Fig. 216) giving the areolae an irregular outline. Raphe branches bordered by ridges running from apex to central area, leaving a fairly broad gap at the central area, slightly widening at the central area (Figs 215, 216). Central raphe endings spatulate (Fig. 216). Terminal raphe fissures short, simple, terminated by a transapical shallow slit (Fig. 217). Internally, valve face almost flat with only weakly raised virgae (Fig. 218). Elongated areolae around the central area clearly visible (Fig. 218). Raphe endings simple, terminal endings terminating onto indistinct helictoglossae (Fig. 218).

Holotype: BR-4793 (Meise Botanic Garden, Belgium). The holotype is illustrated by Fig. 209.

Isotype: Slide-426 (University of Antwerp, Belgium)

Type locality: Lough Nambrackmore, Co. Galway, Ireland, Kennedy Sample WE_32_500_M3_BK_20130815, coll. date 15.VIII.2013, leg. B. Kennedy.

Etymology: the specific epithet refers to the resemblance with *Brachysira vitrea*.

Ecology and associated diatom flora: *Brachysira vitreoides* has so far been found in two localities, Lough Nambrackmore (Ireland) and Koppaälven, a small river in south-central Sweden close to the town of Gullspång (no figures shown for the latter population). Lough Nambrackmore is a small lake, located in the Roundstone Bog Complex in the West of- Ireland (County Galway) and is characterised by an almost circumneutral pH (6.8), a low conductivity (95 µS/cm), low alkalinity (4.0 mg/L CaCO3) and very low total phosphorus concentrations (>0.005 mg/L-P) (Kennedy 2021). The sample was dominated by *Achnanthidium caledonicum* (Lange-Bert.) Lange-Bert., *Brachysira microcephala*, *B. praegeri* B.Kenn. & Allott, *Encyonema neogracile* Krammer, *Encyonopsis neerlandica* Van de Vijver et al., *Frustulia crassinervia* (Brébisson) Lange-Bert. & Krammer, *Kobayasiella parasubtilissima* (H.Kobayasi & T.Nagumo) Lange-Bert., *Navicula radiosa* and *Nitzschia* sp. The Swedish population is rather small and was found in a sample dominated by *B. neoexilis* together with *Achnanthidium* cf. *minutissimum* (Kütz.) Czarn., *A. neomicrocephalum*, *Encyonopsis cesatii*, *E. subminuta*, *Fragilaria perdelicatissima*, *Gomphonema* cf. *exilissimum*, *G. vibrio*, *Navicula radiosa*, and *Tabellaria flocculosa*. Both the Irish and Swedish populations seem to occur in similar ecological conditions: weakly acid to circumneutral, oligotrophic, dilute waters (Lange-Bertalot et al. 2017).

2. The Brachysira hofmanniae group

Brachysira hofmanniae was originally described in 1994 from the Bavarian Lustsee in southern Germany. The plate illustrating the species in Lange-Bertalot & Moser (1994, plate 8, figs 1–18) showed a broad variability in valve outline (ranging from undulate, strictly lanceolate to distinctly rhombic, isopolar, but also heteropolar valves), striation pattern and valve dimensions

(mainly a large valve width range). Analysis of the type material (Hofmann sample S292) and a second sample from the Lustsee (Hofmann sample S314) revealed that *B. hofmanniae* should be treated as a complex of several (some undescribed) species. Apart from *B. hofmanniae*, two new species can be distinguished and are described here: *Brachysira hamiltoniana* Van de Vijver & G.Hofm. sp. nov. and *B. pseudovitrea* Van de Vijver & G.Hofm. sp. nov.

Brachysira hofmanniae **Lange-Bert. in Lange-Bertalot & Moser 1994** (Figs 219–243)

Holotype Präparat Hofmann S292 (G. Hofmann) from Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main (in Lange-Bertalot & Moser 1994, p. 31, pl. 8, figs 1–18)

Type locality Lustsee, Bavaria, Germany, Sample Hofmann S292, coll. date 4.IX.1990, leg. G. Hofmann

Light microscopy (Figs 219–238) Valves usually isopolar to weakly heteropolar. Valve margins strictly lanceolate. Larger valves with distinctly undulate margins. Apices protracted, rostrate to usually capitate. Valve dimensions (n=40): length 25–40 µm, width 5.5–7.0 µm, length/width ratio 4.8–6.7. Axial area narrow, linear. Central area elliptical to rounded, thickened. Raphe branches straight. Central raphe endings simple, at ca. 1.5 µm distance from each other. Striae only very faintly visible in LM. Large, wartlike blunt spines present on the virgae, clearly discernible in LM, 3–4 per virga. Areolae only discernible in the central area.

Scanning electron microscopy (Figs 239–243): Valve face flat. Virgae flattened and very broad, almost 5 times as broad as the striae (Figs 239–242). Raised marginal ridge surrounding the entire valve, almost absent at the apices (Figs 239, 240). Thick, wartlike or blunt spines present on the virgae, maximally 4 per virga (Figs 241, 242). Striae externally very thin, most

likely because of broadening of the virgae, composed of maximally 4 transapically elongated, short areolae. Stria density 33–34 in 10 µm. Raphe branches bordered by two raised longitudinal axial ridges, running from apex to central area (Fig. 239–242). Central raphe endings clearly spatulate (Fig. 241). Internally, virgae flattened (Fig. 243). Central raphe endings simple. Terminal raphe endings terminating onto small helictoglossae (Fig. 243).

Ecology & associated diatom flora: Currently, the only verified population of *B. hofmanniae* has been reported from the Lustsee. The species was present in both samples analysed (Hofmann S292 & S314). The ecology and associated diatom flora of the Lustsee were already discussed under *B. ellipticovitrea* (see above).

Brachysira pseudovitrea **Van de Vijver & G.Hofm. sp. nov.** (Figs 244–269)

Registration: http://phycobank.org/103636

Light microscopy (Figs 244–264): Valves strictly heteropolar. Valve outline lanceolate with weakly convex margins. Head pole shortly protracted, rostrate to cuneate. Foot pole more elongated, protracted, subcapitate to capitate. Valve dimensions (n=40): length 25–45 µm, width 5.5–6.5 µm, length/width ratio 4.5–7.2. Axial area narrow, linear. Central area typically elliptical, clearly thickened. Raphe filiform, straight, with simple central endings, positioned rather distant from each other (1.5–2.0 µm). Terminal raphe fissures not visible in LM. Striation pattern dense, striae radiate, equidistant throughout the entire valve, 30–34 in 10 µm. Areolae weakly discernible in LM, at least 4 per stria. Valve face ornamentation pattern very dense, usually not well discernible in LM.

Scanning electron microscopy (Figs 265–269): Valve face flat, bordered by weakly raised marginal ridge, almost absent towards the apices (Figs 265, 266). Valve face surface ornamented

with dense, equidistant pattern of short, wartlike spines on the virgae, at least 5–6 per virga (Figs 267, 268). Striae composed of up to 5 irregularly transapically elongated areolae, bordered by virgae, almost equal in width (Figs 267, 268). Areolae near the central area largest (Fig. 268). Areola foramina with smooth edge (Fig. 268). Central area large, elliptical (Figs 265, 266). Raphe branches bordered by distinct longitudinal axial ridges running from apex to central area (Figs 265, 268). Central raphe endings small (Fig. 267). Terminal raphe fissures short, simple (Fig. 266). Internally, virgae clearly raised (Fig. 269). Raphe endings simple, terminal endings terminating onto indistinct helictoglossae (Fig. 269). Enlarged central areolae clearly visible.

Holotype: BR-4791 (Meise Botanic Garden, Belgium). The holotype is illustrated by Fig. 245.

Isotype: Slide-424 (University of Antwerp, Belgium)

Type locality: Lustsee, southeastern shore, Bavaria, Germany, Sample Hofmann-S314, coll. date 6.XI.1990, leg. G. Hofmann.

Etymology: the specific epithet refers to the resemblance to *Brachysira vitrea*, with whom it might have been confused in the past.

Ecology and associated diatom flora: So far, the species has only been reported with certainty from the Lustsee. The ecology and associated diatom flora of the Lustsee samples were already discussed under *B. ellipticovitrea* (see above).

***Brachysira hamiltoniana* Van de Vijver & G.Hofm. sp. nov.** (Figs 270–281)

Registration: http://phycobank.org/103637

Light microscopy (Figs 270–278): Valves strictly isopolar with a rhombic to rhombic-elliptical valve outline and protracted, capitate apices. Valve dimensions (n=20): length 25–45 µm, width

5.5–7.0 µm, length/width ratio 4.0–4.7. Axial area narrow, linear. Central area elliptical, clearly thickened. Raphe filiform, bordered by raised, thickened longitudinal axial ridges, straight, with enlarged central endings, positioned rather distant from each other (almost 2.0 µm). Terminal raphe fissures not visible in LM. Striation pattern dense, radiate, equidistant throughout the entire valve, ca. 35 in 10 µm. Marginal parts of the striae covered (Figs 275, 278, arrows). Areolae weakly discernible in LM, at least 4–5 per stria. Central areolae distinctly elongated, clearly delimited by raised structure (Fig. 275, arrow). Valve face ornamentation forming zigzag pattern, discernible in LM.

Scanning electron microscopy (Figs 279–281): Valve face flat. Marginal ridge very large, entirely covering the distal parts of the valve face, leaving only the central area open (Figs 279, 280). At the apices, marginal ridge small, weakly raised, not covering the apices (Fig. 281). Longitudinal axial ridges bordering the raphe branches very large, covering the axial area from the central area to the apices (Figs 279, 280). Central area large, elliptical (Fig. 280). Central striae composed of three, transapically elongated areolae, the longest present around the central area (Fig. 280). Areola foramina with smooth edge (Fig. 280). Short, bifurcating spines present on the virgae (Fig. 280). Central raphe endings simple (Fig. 280). Internal structure not observed so far, due to rareness of the species in the sample.

Holotype: BR-4791 (Meise Botanic Garden, Belgium). The holotype is illustrated by Fig. 273.

Isotype: Slide-424 (University of Antwerp, Belgium)

Type locality: Lustsee, southeastern shore, Bavaria, Germany, Sample Hofmann-S314, coll. date 6.XI.1990, leg. G. Hofmann.

Etymology: the species is named after our friend and colleague Paul B. Hamilton (Canadian Museum of Nature, Ottawa, Canada), in recognition of his important contributions to diatom science.

Ecology and associated diatom flora: So far, a small population of the species has only been reported with certainty from the Lustsee. The ecology and associated diatom flora of the Lustsee samples were already discussed under *B. ellipticovitrea* (see above).

3. Brachysira ectoriana

Finally, an additional new species was observed during the analysis of a presumable *B. vitrea* population in a sample from the Bavarian Königssee (Germany). Although the morphology of the species showed some resemblance to *B. ellipticovitrea*, the observed population is easily recognisable, justifying its description as a new species: *Brachysira ectoriana* Van de Vijver & G.Hofm. sp. nov.

***Brachysira ectoriana* Van de Vijver & G.Hofm. sp. nov.** (Figs 282–295)

Registration: http://phycobank.org/103638

Light microscopy (Figs 282–291): Valves isopolar. Valve outline elliptic-lanceolate with broadly convex margins and distinctly protracted, capitate apices. Valve dimensions (n=30): length 19–26 µm, width ca. 6.0 µm, length/width ratio 3.3–4.3. Axial area very narrow, linear. Central area small, only weakly expanded to elliptical. Raphe filiform, straight, with simple central endings, positioned rather close to each other (ca. 1.0 µm). Terminal raphe fissures not visible in LM. Striation pattern dense, striae only weakly radiate to almost parallel, becoming

more radiate towards the apices, equidistant throughout the entire valve, 30–32 in 10 µm. Areolae only very weakly discernible in LM. Valve face ornamentation pattern often discernible in LM (see Figs 288, 289).

Scanning electron microscopy (Figs 292–295): Valve face flat, bordered by a large, distinctly raised marginal ridge, covering the valve margins (Fig. 292), but reduced to a narrow crest at the apices (Fig. 294). Valve face surface ornamented with a loose, irregular pattern of bifurcating, thin spines on the virgae (Fig. 293). Striae composed of up to 7, usually short, rounded to irregularly transapically elongated areolae, bordered by virgae, almost equal in width (Figs 292, 293). Areolae near the central area largest, forming a distinct pattern (Fig. 293). Areola foramina with rather smooth edge (Fig. 294). Central area narrow, apically elongated, elliptical (Fig. 293). Raphe branches bordered by distinct flaplike longitudinal axial ridges running from apex to central area (Figs 292, 293). Flaps covering the axial area. Central raphe endings small (Fig. 293). Terminal raphe fissures short, simple (Fig. 294). Internally, valve face flat (Fig. 295). Raphe endings simple, terminal endings terminating onto small helictoglossae (Fig. 295). Enlarged central areolae clearly visible.

Holotype: BR-4794 (Meise Botanic Garden, Belgium). The holotype is illustrated by Fig. 284.

Isotype: Slide-427 (University of Antwerp, Belgium)

Type locality: Archenwand, Königssee, Bavaria, Germany, Sample Hofmann-S410, coll. date 08.IV.1994, leg. G. Hofmann.

Etymology: this new species was named after our dear friend and colleague Luc Ector (1962–2022), who passed away too early in April last year. His contributions to diatom science, his endless enthusiasm and motivation are sorely missed.

Ecology and associated diatom flora: So far, the species has only been reported with certainty from the Königssee and the Lustsee, two calcareous, oligotrophic lakes in Bavaria (southern Germany). The largest population was observed in the Königssee ('Kings Lake') in sample Hofmann S410, collected from a cliff face of the Archenwand, located centrally on the western side of the lake. The lake, situated south of the Bavarian town of Berchtesgaden (Germany), is characterised by rather low conductivity (150–200 µS/cm), a pH of 8.0–8.5, and a total P-level ranging between 5–10 µg/L (Nationalpark Berchtesgaden 1985). The sample is dominated by *Achnanthidium affine*, *Delicatophycus delicatus*, *Fragilaria perdelicatissima*, *Navicula* cf. *irmengardis*, and various species of *Brachysira*, such as *B. calcicola*, *B. styriaca*, and *B. vitrea*, a typical flora for oligotrophic, alkaline (calcareous), oligosaprobic lakes (Lange-Bertalot et al. 2017). The Königssee represents a similar environment as in the Lustsee, whose ecology and associated diatom flora were already discussed under *B. ellipticovitrea* (see above).

Discussion

The results of the current study showed that *Brachysira vitrea* should be treated as a complex of morphologically related species including *B. hofmannii*, described in 1994 from the Bavarian Lustsee (Germany). This complex has its main distribution in the oligotrophic, alkaline lakes in Central Europe (Austria, Germany, Switzerland). Part of the taxonomic confusion surrounding this complex most likely results from the of lack of analysis of the original material Grunow used to describe *Gomphonema vitreum*. Grunow (1878) indicated the Erlafsee bei Mariazell (presently, Lake Erlauf), as the locality where he found *Gomphonema vitreum*, although Grunow never specified a sample number in his original publication. The samples Grunow worked with, are listed in his accession books kept in the Grunow collection at the Naturhistorisches Museum

in Vienna (Austria) (Schuster et al. 2022). Aside from Grunow's samples and accession books, many of his original drawings are conserved there as well. Grunow usually added the sample number from which the drawing was made. In the original publication of *Gomphonema vitreum* (Grunow 1878), Grunow did not illustrate this new species, and the first drawing of it was only published two years later in Van Heurck (1880, plate XII, fig. 13) where it was named *Navicula gomphonemacea* Grunow. One of the sheets of Grunow's drawings in Vienna, filed under the name '*Navicula exilis* Grunow', contained a drawing marked as *Navicula gomphonemacea* with *Gomphonema vitreum* added in pencil. Sample number '36 Erlaf See' was noted next to the drawing. Compère (1988) mentioned Grunow sample 36 as original material for *G. vitreum*. In Van Heurck (1880), '*Gomphonema* ? *vitreum* Grun. Caspi See Alg.' is added in brackets, indicating that it was a new name for the species described in 1878. Although Grunow had raised doubts about the placement of the new species within the genus *Gomphonema* by adding a question mark, that name was validly published (Turland et al. 2018, ICN art. 36.1), and the name change proposed in Van Heurck (1880) is therefore superfluous, making *Navicula gomphonemacea* an illegitimate name.

When comparing the morphology of the populations in the different samples, presumably containing *Brachysira vitrea*, several distinct species could be recognised, each showing a relatively uniform, characteristic morphology. Tables 2 & 3 provide an overview of all morphological differences of the species in the *B. vitrea* (Table 2) and *B. hofmanniae* (Table 3) groups. All species share some features that have so far not been observed in other species of *Brachysira*: rather robust valves with typically convex, broadly rounded margins, a very dense, fine striation pattern with striae composed of at least four, but often up to eight areolae, a valve surface ornamentation composed of a dense pattern of acute spines, typical, distinctly raised

longitudinal axial ridges bordering the raphe branches, and a raised marginal ridge, situated on the valve face/mantle junction, with a straight mantle, entirely perpendicular to the valve face (contrary to, for instance, *B. neoexilis* where the valve mantle areolae are visible when observing the valve in valve face view, see Van de Vijver et al. 2021a, fig. 95). This set of morphological features separates the *B. vitrea* group from all other species of *Brachysira* worldwide (Lange-Bertalot & Moser 1994, Van de Vijver et al. 2021a, b, 2022). Only *B. heteropolaris* Van de Vijver and *B. chiarruccii* Cantonati et al. have a similar finely striated morphology, but both have a heteropolar, rhombic-lanceolate valve shape lacking protracted apices, a typical feature of the *B. vitrea* group (Cantonati et al. 2021, Van de Vijver 2023). The latter also lacks a perpendicular valve mantle, but has a more oblique mantle, as was observed in *B. neoexilis* (Cantonati et al. 2021, fig. 25).

The *Brachysira vitrea* type population from Lake Erlauf only contained typically heteropolar valves with isopolar valves not observed, even after scanning the entire original Grunow slide. In several other samples from different localities, similar, typically heteropolar populations were observed, sharing the same morphology. Only in the Swiss Blausee population documented by a slide made by Brun, several valves with a larger central area were mixed with valves lacking a distinct central area. Since all other morphological features appeared similar, the authors conclude that these specimens should be included in the morphological species concept of B. vitrea, despite the slightly different morphology. Lange-Bertalot & Moser (1994, plate 3), show several valves that also fit into the emended description of *B. vitrea* (see plate 3, figs 24–31). Compared to *B. vitrea*, where the valve margins are typically broadly rounded, *Brachysira pseudovitrea* is similar, but has a more elongated, strictly lanceolate valve outline. This is also expressed in the length/width ratio that is clearly larger in *B. pseudovitrea* (4.5–7.2) than in *B.*

vitrea (max. 4.5, usually less), giving the latter a more robust outline. Moreover, *B. pseudovitrea* has a very dense ornamentation pattern that was not observed in *B. vitrea*.

Other examples of heteropolar species of *Brachysira* are not uncommon. Anderson et al. (2013) described two heteropolar species from the Porcupine Mountains (Michigan, USA). Both species, *B. gatesii* Kociolek & R.Lowe and *B. ontonageniana* Kociolek & R.Lowe, lack protracted apices in comparison to *B. vitrea* and *B. pseudovitrea* and have a more rhombic-clavate valve outline. Only *B. gatesii* has a weakly protracted foot pole, but differs by its much lower valve width (4–5 µm). Another heteropolar species, *Brachysira archibaldii* M.Coste & M.Ricard, was described from Mauritius Island (Indian Ocean) but has much longer valves (up to 50 µm) and lacks protracted apices (Coste & Ricard 1982).

Contrary to these heteropolar species, several isopolar populations, previously included within *B. vitrea* s.l., are here split as distinct species, based on the following characters. *Brachysira ellipticovitrea* and *B. paraexilis* can be separated from each other based on their valve outline, with *B. ellipticovitrea* having typically elliptical to elliptical-lanceolate valves, and *B. paraexilis* having a strictly lanceolate outline. The latter shows a similar outline as *B. neoexilis*, but can be differentiated based on its larger valves (valve width 3.5–5.0 µm in *B. neoexilis* versus 5.5–6.0 µm in *B. paraexilis*). They also have a different valve surface ornamentation (absence of acute short spines in *B. neoexilis*) and a smaller central area in *B. paraexilis*. *Brachysira ellipticovitrea* was already reported in Lange-Bertalot & Moser (1994, plate 3, figs 1–19) as *B. vitrea*, but since the latter name should, according to our results, only be used for heteropolar populations, these elliptical, isopolar populations are here described as a new species. An additional difference between *B. paraexilis* and *B. ellipticovitrea* is the distance between the central raphe endings, which is about 50% larger in *B. paraexilis* than in *B. ellipticovitrea*. *Brachysira paraexilis* also

possesses bifurcating striae in the central area, a feature so far not observed in any population of *B. ellipticovitrea*. Finally, the structure of the areolae is different, where *B. ellipticovitrea* has smooth edges in the areolae and those in *B. paraexilis* are jagged. It is unlikely that this difference in structure is related to erosion of the valves due to treatment, as in Lough Bunny, both species occur sympatrically and the same difference was observed (Van de Vijver, pers. obs.).

Brachysira vitreoides is another weakly heteropolar species with broad rostrate to subcapitate apices, quite distinct from the cuneately rounded head pole and narrowly rostrate foot poles in *B. vitrea*. In addition, this new species can be distinguished from *B. vitrea* by the typical areola pattern in the central area with the elongated areolae organised in a radiating pattern around the central nodule. Apart from *B. vitreoides*, the other three species were all found in calcium-bicarbonate rich, strictly oligotrophic lakes. Only *B. vitreoides* was observed in weakly acidic to circumneutral, oligotrophic habitats. The presence of *B. vitrea* s.s. and *B. ellipticovitrea* was confirmed from Austria, Switzerland, southern Germany, and the Balkans (Croatia and Rep. Northern Macedonia), which may point to a distinct biogeography. This, however, can only be confirmed when more records of *B. vitrea* from other countries have been investigated. Unfortunately, records of *B. vitrea* are scarce and when available only very rarely illustrated, not allowing for direct verification. Shayler & Siver (2004, figs 79–81 & 140–141) showed three valves identified as *B. vitrea* that most likely represent *B. ellipticovitrea*, based on their isopolar, more lanceolate valve outline. *Brachysira paraexilis* was also reported from the Cheyenne River (Western South Dakota, USA) and Connelly Fen (Northwestern Great Plains, Montana, USA). The latter is a shallow, highly calcareous fen, with a pH of 7.60 and a specific conductance of 1500 µS/cm (Bahls 2014).

Brachysira ectoriana has a distinctive morphology that cannot be confused with any other species of *Brachysira* known so far. In LM, the valve outline looks somewhat similar to *B. ellipticovitrea*. Both species have a more elliptical outline, but *B. ectoriana* has smaller valve dimensions (valve width 5–6 µm versus 7–8 µm for *B. ellipticovitrea*). Another distinct feature easily seen in LM is the structure of the central area, where *Brachysira ectoriana* has distinct elongated areolae, which are lacking in *B. ellipticovitrea* where no difference in areola size is observed in the central area. Moreover, the absence of superficial (blunt) spines in this area in *B. ectoriana* gives this a more depressed appearance, a feature so far not observed in any of the *B. ellipticovitrea* populations. This structure becomes quite clear when the ultrastructure of *B. ectoriana* is observed in SEM. The elongated areolae in the central area are very distinct, both on the valve outside (Fig. 293) and in the valve interior (Fig. 295). The valve ornamentation in *B. ectoriana* has a less dense pattern with irregularly shaped, often weakly bifurcating short spine-like protrusions. The marginal ridge and the longitudinal axial ridges bordering the raphe branches are strongly developed, with the marginal ridge even covering the marginal parts of the striae. In the Lustsee sample S314, a small population of *B. ectoriana* was observed together with a large population of *B. ellipticovitrea*. As none of the typical features of *B. ectoriana* were observed on the *B. ellipticovitrea* valves, this justifies the recognition of these two species as separate.

Brachysira hofmanniae also has a very distinct morphology with the broadened virgae, reducing the striae and areolae to very narrow slits. Additionally, the blunt, raised spines, loosely scattered over the valve face surface, the elliptical central area and the often weakly undulating valve outline discriminate this species from all others. In Lange-Bertalot & Moser (1994, plate 8), several valves identified as *B. hofmanniae* have a more rhombic valve outline with a distinct

areola pattern centrally (Lange-Bertalot & Moser 1994, plate 8, figs 10, 11, 15, 17 & 18). Although found sympatrically in the Lustsee sample (S 314) where also a large population of *B. hofmanniae* was observed, the rhombic valves can easily be distinguished in LM, but for certain in SEM. It is at present the only species of *Brachysira* showing the enlarged longitudinal axial ridges and enlarged marginal ridge, covering the distal parts of the valve face leaving only a small opening to show the underlying striae near the central area. Therefore, these valves were recognised as a distinct species here named *B. hamiltoniana*. Its morphology shows some resemblance to *B. ectoriana* but differs in the presence of the enlarged longitudinal axial ridges, the enlarged marginal ridge covering most of the valve surface, and the more rhombic valve outline. Both species also differ from *B. hofmanniae* in the structure of the virgae, since the broad virgae seen in *B. hofmanniae* have so far neither been observed in *B. ectoriana*, nor *B. hamiltoniana*. Comparing these broad virgae with SEM pictures of other species of *Brachysira* did not reveal any others with a similar structure.

ORCID

Bart Van de Vijver https://orcid.org/0000-0002-6244-1886

Tanja M. Schuster https://orcid.org/0000-0003-0851-3372

Wolf-Henning Kusber https://orcid.org/0000-0003-4543-5764

Bryan Kennedy https://orcid.org/0000-0003-2640-8197

Gabriele Hofmann no ORCID

Joachim Hürlimann no ORCID

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Figure Captions

Figures 1–12. *Brachysira vitrea* (Grunow) R.Ross. LM images of the lectotype, Grunow sample 36 (Erlafsee bei Mariazell, Austria, slide W0164896). LM views of the population arranged in decreasing length. Scale bar represents 10 µm.

Figures 13–53. *Brachysira vitrea* (Grunow) R.Ross. LM images of the epitype material (BR-4790, Hürlimann sample 2464 (Walensee near Quarten (Unterterzen), Switzerland). LM views of the population arranged in decreasing length. Scale bar represents 10 µm.

Figures 54–56. *Brachysira vitrea* (Grunow) R.Ross. SEM images of the epitype material (BR-4790, Hürlimann sample 2464 (Walensee near Quarten (Unterterzen), Switzerland). Fig. 54. SEM external view of an obliquely lying valve. Fig. 55. SEM external view of an entire valve. Fig. 56. SEM external detail of the central area. Note the raised ridges and the valve face spines. Scale bar represents 10 µm except for Fig. 56. where scale bar = 1 µm.

Figures 57–69. *Brachysira vitrea* (Grunow) R.Ross. LM and SEM images taken from Grunow sample 20 (BR-4795, Erlaufsee, Austria). Figs 57–66. LM views of the population arranged in decreasing length. Fig. 67. SEM external view of an obliquely lying valve. Note the spines on the valve face. Fig. 68. SEM external detail of the central area. Note the valve face spines. Fig. 69. SEM internal view of an entire valve. Scale bar represents 10 µm except for Fig. 68. where scale bar = 1 µm.

Figures 70–99. *Brachysira vitrea* (Grunow) R.Ross. LM images of the Blausee (Figs 70–88, BR-4796, Brun sample 210, Switzerland, Van Heurck collection n° IX-35-B13) and the Attersee (Figs 89–99, BR-4797, Grunow sample 2647, W0127052, Austria). LM views of the populations arranged in decreasing length. Scale bar represents 10 µm.

Figures 100–133. *Brachysira vitrea* (Grunow) R.Ross. LM and SEM images taken from the Vanoise (Figs 100–120, BR-4798, Courchevel, France, sample VAN2020-02) and Vienna (Figs 120–133, Grunow sample 157, Austria, slide W0127050 & BR-4644). Figs 100–132. LM views of the populations arranged in decreasing length. Fig. 133. SEM external view of an entire valve. Scale bars represent 10 µm.

Figures 134–150. *Brachysira ellipticovitrea* Van de Vijver, B.Kenn. & G.Hofm. sp. nov. LM images of the holotype, Hofmann-S314 (BR-4791, Lustsee, southeastern shore, Bavaria, Germany). LM views of the population arranged in decreasing length. Scale bar represents 10 µm.

Figures 151–154. *Brachysira ellipticovitrea* Van de Vijver, B.Kenn. & G.Hofm. sp. nov. SEM images of the holotype material, Hofmann-S314 (BR-4791, Lustsee, southeastern shore, Bavaria, Germany). Fig. 151. SEM external view of an entire valve. Fig. 152. SEM external view of an entire valve in slightly oblique position, showing the raised marginal ridge. Fig. 153. SEM external detail of the central area. Fig. 154. SEM internal view of an entire valve. Scale bar represents 10 µm except for Fig. 153 where scale bar = 1 µm.

Figures 155–181. *Brachysira ellipticovitrea* Van de Vijver, B.Kenn. & G.Hofm. sp. nov. LM images of several localities: Attersee (Figs 155–163, BR-4799, Grunow sample 2647, W0127052, Austria), Lough Bunny (Figs 164–172, BR-4792, Kennedy sample WE_27_114_PB_0020_BK_20150630, Ireland), Weitsee (Figs 173–181, BR-4799, Hofmann-S862, Germany). LM views of the populations arranged in decreasing length. Scale bar represents 10 µm.

Figures 182–205. *Brachysira paraexilis* Van de Vijver & B.Kenn.sp. nov. LM and SEM images of the holotype, Kennedy sample WE_27_114_PB_0020_BK_20150630 (BR-4792, Lough Bunny, Co. Clare, Ireland). Figs 182–202. LM views of the population arranged in decreasing length. Fig. 203. SEM external view of an entire valve. Fig. 204. SEM external detail of the central area. The white arrows indicate the bifurcating striae. Fig. 205. SEM internal detail of an entire valve. Scale bar represents 10 µm except for Fig. 204 where scale bar = 1 µm.

Figures 206–218. *Brachysira vitreoides* Van de Vijver & B.Kenn. sp. nov. LM and SEM images taken from the holotype, Kennedy sample WE_32_500_M3_BK_20130815 (BR-4793, Lough Nambrackmore, Co. Galway, Ireland). Figs 206–214. LM views of the population arranged in decreasing length. Fig. 215. SEM external view of an entire valve. Fig. 216. SEM external detail of the central area showing the longer areolae in the central area. Fig. 217. SEM external detail of the central area in oblique view showing the short spines on the valve face. Fig. 218. SEM internal detail of an entire valve. Scale bar represents 10 µm except for Figs 216 & 217 where scale bar = 1 µm.

Figures 219–238. *Brachysira hofmanniae* Lange-Bert. LM images of the holotype material, Hofmann-S292 (BR-4800, Lustsee, southeastern shore, Bavaria, Germany). LM views of the population arranged in decreasing length. Note the undulating valve outline in the larger specimens. The valve face ornamentation is clearly visible in LM as a pattern of small dots on the valve face. Scale bar represents 10 µm.

Figures 239–243. *Brachysira hofmanniae* Lange-Bert. SEM images of the holotype material, Hofmann-S292 (BR-4800, Lustsee, southeastern shore, Bavaria, Germany). Fig. 239. SEM external view of an entire valve with non-undulating valve margins. Fig. 240. SEM external view of an entire valve with undulating valve margins. Fig. 241. SEM external detail of the central area showing the broad virgae. Fig. 242. SEM external detail of the central area in oblique view showing the short, blunt spines on the valve face. Fig. 243. SEM internal detail of an entire valve. Scale bars represent 10 µm except for Figs 241 & 242 where scale bar = 1 µm.

Figures 244–264. *Brachysira pseudovitrea* Van de Vijver & G.Hofm. sp. nov. LM images of the holotype material, Hofmann-S314 (BR-4791, Lustsee, southeastern shore, Bavaria, Germany). LM views of the population arranged in decreasing length. Scale bar represents 10 µm.

Figures 265–269. *Brachysira pseudovitrea* Van de Vijver & G.Hofm. sp. nov. SEM images of the holotype material, Hofmann-S314 (BR-4791, Lustsee, southeastern shore, Bavaria, Germany). Figs 265 & 266. SEM external view of two entire valves. Fig. 267. SEM external detail of the central area showing the very dense pattern of valve face spines. Fig. 268. SEM external detail of the central area in oblique view showing the short, blunt spines on the valve face. Fig. 269. SEM

internal detail of an entire valve. Scale bars represent 10 µm except for Figs 267 & 268 where scale bar = 1 µm.

Figures 270–281. *Brachysira hamiltoniana* Van de Vijver & G.Hofm. sp. nov. LM and SEM images of the holotype material, Hofmann-S314 (BR-4791, Lustsee, southeastern shore, Bavaria, Germany). Figs 270–278. LM views of the population arranged in decreasing length. Fig. 279. SEM external view of an entire valve. Note the extremely large marginal ridge and the longitudinal axial ridges in the central area covering either the valve margins or the axial area. Fig. 280. SEM external detail of the central area showing the longer areolae in the central area and the spines. Fig. 281. SEM external detail of the valve apex with reduced crest. Scale bars represent 10 µm except for Figs 280 & 281 where scale bar = 1 µm.

Figures 282–295. *Brachysira ectoriana* Van de Vijver & G.Hofm. sp. nov. LM and SEM images of the holotype material, Hofmann-S410 (BR-4794, Königssee, Archenwand, Bavaria, Germany). Figs 282–291. LM views of the population arranged in decreasing length. Fig. 292. SEM external view of an entire valve. Fig. 293. SEM external detail of the central area showing the longer areolae in the central area. Fig. 294. SEM external detail of the central area in oblique view showing the short spines on the valve face. Fig. 295. SEM internal detail of an entire valve. Scale bars represent 10 µm except for Figs 293 & 294 where scale bar = 1 µm.

Table 1. List of all samples used in this paper

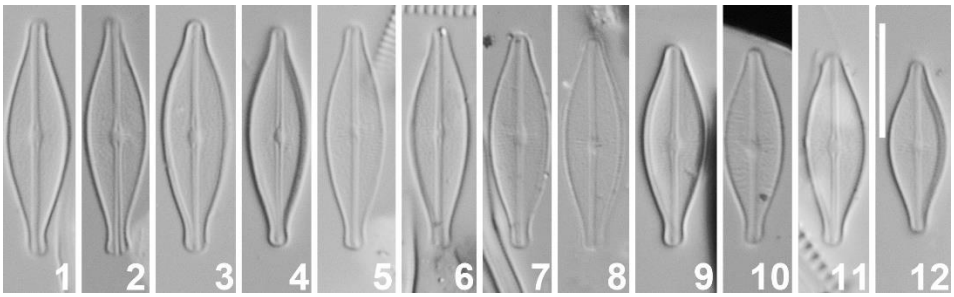
Sample	Locality	Investigated taxon	Collection date	Collector	Collection	Collection number
Grunow sample 36	Erlafsee [bei Mariazell (Austria)]	*Brachsyira vitrea*	09.X.1856	A. Grunow	Natural History Museum Vienna	W0164896
Hürlimann 2464	Walense (Quarten, Kanton Sankt Gallen, Switzerland)	*Brachsyira vitrea*	04.X.1996	J. Hürlimann	Meise Botanic Garden (Belgium)	BR-4790
Brun 210	Blausee (Berner Oberland, Switzerland)	*Brachsyira vitrea*	???	Brun	Meise Botanic Garden (Belgium)	BR-4796 (=IX-35-B13)
Grunow sample 20	Erlafsee [bei Mariazell (Austria)]	*Brachsyira vitrea*	09.X.1856	A. Grunow	Natural History Museum Vienna & Meise Botanic Garden (Belgium)	W0127016 & BR-4795
Grunow sample 157	Botanic Garden Vienna (Austria)	*Brachsyira vitrea*	1860 or earlier	A. Grunow	Natural History Museum Vienna & Meise Botanic Garden (Belgium)	W0127050 & BR-4644
Grunow sample 2647	Attersee (Austria)	*Brachsyira vitrea*	06.VIII.1862	von Mörl 55	Natural History Museum Vienna & Meise Botanic Garden (Belgium)	W0127052 & BR-4797
VAN2020-02	Courchevel (Parc de la Vanoise, France)	*Brachsyira vitrea*	12.VII.2020	B. Van de Vijver	Meise Botanic Garden (Belgium)	BR-4798
Hofmann-S314	Lustsee (Bavaria, Germany)	*Brachysira ellipticovitrea, B. pseudovitrea, B. hamiltoniana*	06.XI.1990	G. Hofmann	Meise Botanic Garden (Belgium)	BR-4791
WE_27_114_PB_0020_BK_20150630	Lough Bunny (Ireland)	*Brachysira ellipticovitrea, B. paraexilis*	30.VI.2015	B. Kennedy	Meise Botanic Garden (Belgium)	BR-4792
Hofmann-S862	Weitsee (Bavaria, Germany)	*Brachysira ellipticovitrea*	???	G. Hofmann	Meise Botanic Garden (Belgium)	BR-4799
WE_32_500_M3_BK_20130815	Lough Nambrackmore (Co. Galway, Ireland)	*Brachysira vitreoides*	15.VIII.2013	B. Kennedy	Meise Botanic Garden (Belgium)	BR-4793
Hofmann-S292	Lustsee (Bavaria, Germany)	*Brachysira hofmanniae*	04.IX.1990	G. Hofmann	Meise Botanic Garden (Belgium)	BR-4800
Hofmann-S410	Königssee (Bavaria, Germany)	*Brachysira ectoriana*	08.IV.1994	G. Hofmann	Meise Botanic Garden (Belgium)	BR-4794

Table 2. Comparison table of all *Brachsyira* taxa in the *B. vitrea*-group discussed in this paper

	Brachysira vitrea	***Brachysira ellipticovitrea***	***Brachysira paraexilis***	***Brachysira vitreoides***
Figures	1–133	134–181	182–205	206–218
length (µm)	15–40	20–35	25–35	20–30
width (µm)	5.5–6.5	7.0–8.0	5.5–6.0 (6.5)	6.0–6.5
length/width ratio	3.2–4.5	3.8–4.4	(4.2) 4.5–5.8	3.6–4.5
striae in 10 µm	32–35	30–34	32–33	30–32
valve outline	**strictly heteropolar**, lanceolate to elliptic-lanceolate with distinctly convex margins	**isopolar, very rarely slightly heteropolar, distinctly elliptical with rounded, convex margins**	mostly isopolar to occasionally weakly heteropolar, distinctly lanceolate with convex margins	slightly to clearly heteropolar, lanceolate to elliptic-lanceolate
apices	head pole protracted, rostrate to cuneate; foot pole more elongated, protracted, rostrate to weakly subcapitate	**clearly protracted, capitate to subcapitate**	clearly protracted, rostrate (head poles) to subcapitate (foot poles)	head pole protracted, rostrate, slightly truncated, foot pole subcapitate
central area	very small to almost absent, occasionally valves with small, elliptical central area observed	very small, usually absent	very small, elliptical to rounded, clearly raised	small, elliptical to rounded, clearly raised
marginal ridge	distinct, narrow marginal ridge surrounding the entire valve at the valve face/mantle junction, almost absent at the valve apices	distinctly raised marginal ridge, diminishing in height towards the apices	distinctly raised marginal ridge, diminishing in height towards the apices	**weakly raised marginal ridge, running up until the apices**
Areola shape	foramina irregular ranging from smooth edge to weakly undulating	foramina with smooth edge	foramina with small protrusions	**irregularly, transapically elongated areolae, foramina undulating with distinct protrusions**
spines	dense pattern of short, acute spines present on the virgae, 3–5 per virga	dense pattern of long, acute spines, located on the virgae	short, acute spines, located on the virgae	dense pattern of long and short, acute spines, located on the virgae

Table 3. Comparison table of all *Brachsyira* taxa in the *B. hofmanniae*-group discussed in this paper

	Brachysira hofmanniae	***Brachysira pseudovitrea***	***Brachysira hamiltoniana***	***Brachysira ectoriana***
Figures	219–243	244–269	270–281	282–295
length (µm)	25–40	25–45	25–45	19–26
width (µm)	5.5–7.0	5.5–6.5	5.5–7.0	ca. 6
length/width ratio	4.8–6.7	4.5–7.2	4.0–4.7	3.3–4.3
striae in 10 µm	33–34	30–34	ca. 35	30–32
valve outline	**usually isopolar to weakly heteropolar, strictly lanceolate, larger valves with distinctly undulating margins**	strictly heteropolar, lanceolate with weakly convex margins	strictly isopolar, rhombic to rhombic-elliptical	elliptic-lanceolate with broadly convex margins
apices	protracted, rostrate to usually capitate	head pole shortly protracted, rostrate to cuneate. Foot pole more elongated, protracted, subcapitate to capitate	protracted, capitate	**distinctly protracted, capitate**
central area	elliptical to rounded, thickened	**typically elliptical, clearly thickened**	elliptical, clearly thickened	small, only weakly expanded to elliptical
marginal ridge	raised, surrounding the entire valve, almost absent at the apices	weakly raised marginal ridge, almost absent towards the apices	**very large, entirely covering the distal parts of the valve face, leaving only the central area open, at the apices, small, weakly raised, not covering the apices**	**large, distinctly raised marginal ridge, covering the valve margins, but reduced to a narrow crest at the apices**
Areola shape	thin due to flattened and very broad virgae, almost 5 times as broad as the striae	foramina with smooth edge	foramina with smooth edge	**foramina with rather smooth edge, near the central area areolae largest, forming a distinct pattern**
spines	thick, wartlike or blunt spines present on the virgae, maximally 4 per virga	**dense, equidistant pattern of short, wartlike spines on the virgae, at least 5–6 per virga**	short, bifurcating spines present on the virgae	loose, irregular pattern of bifurcating, thin spines on the virgae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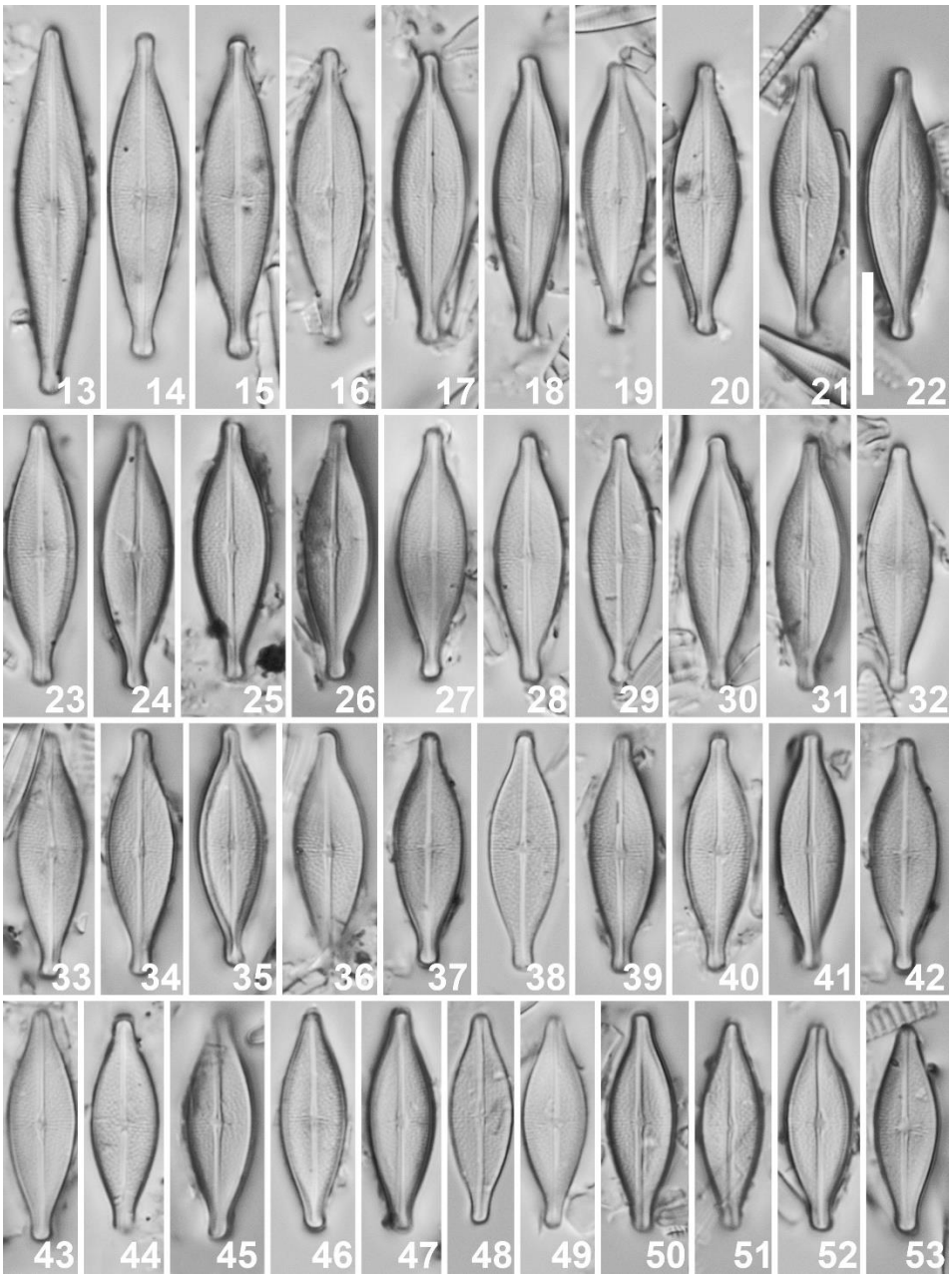
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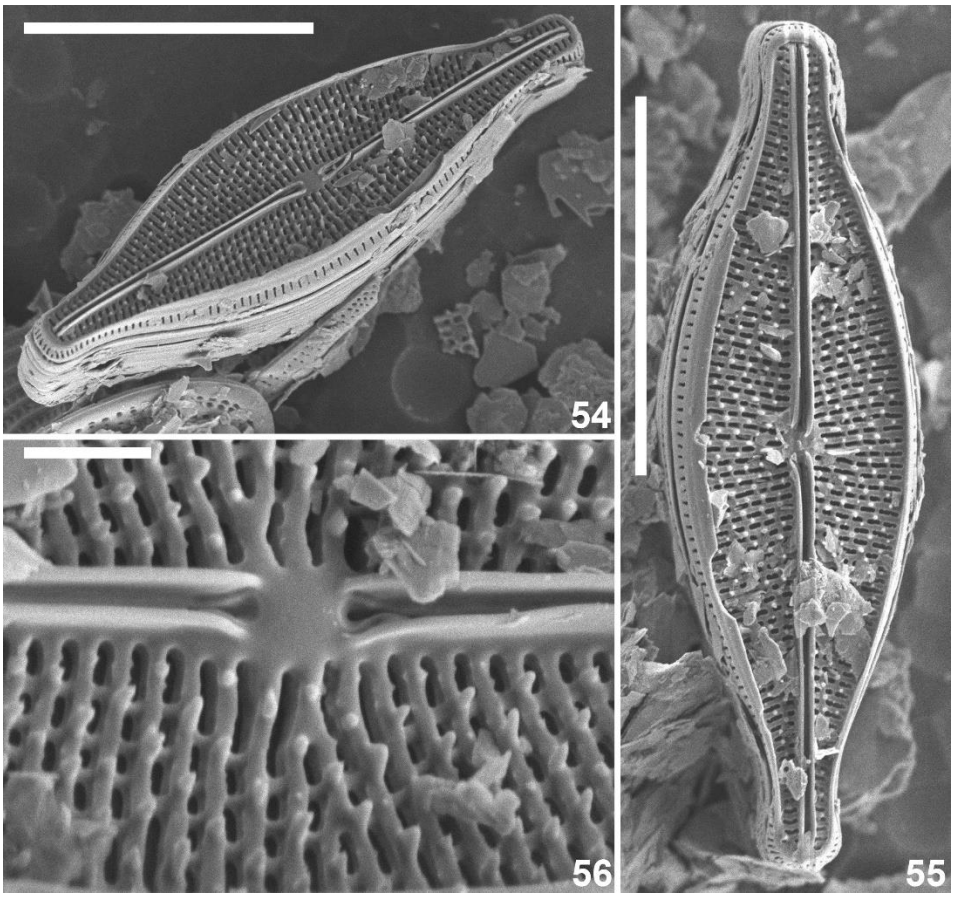
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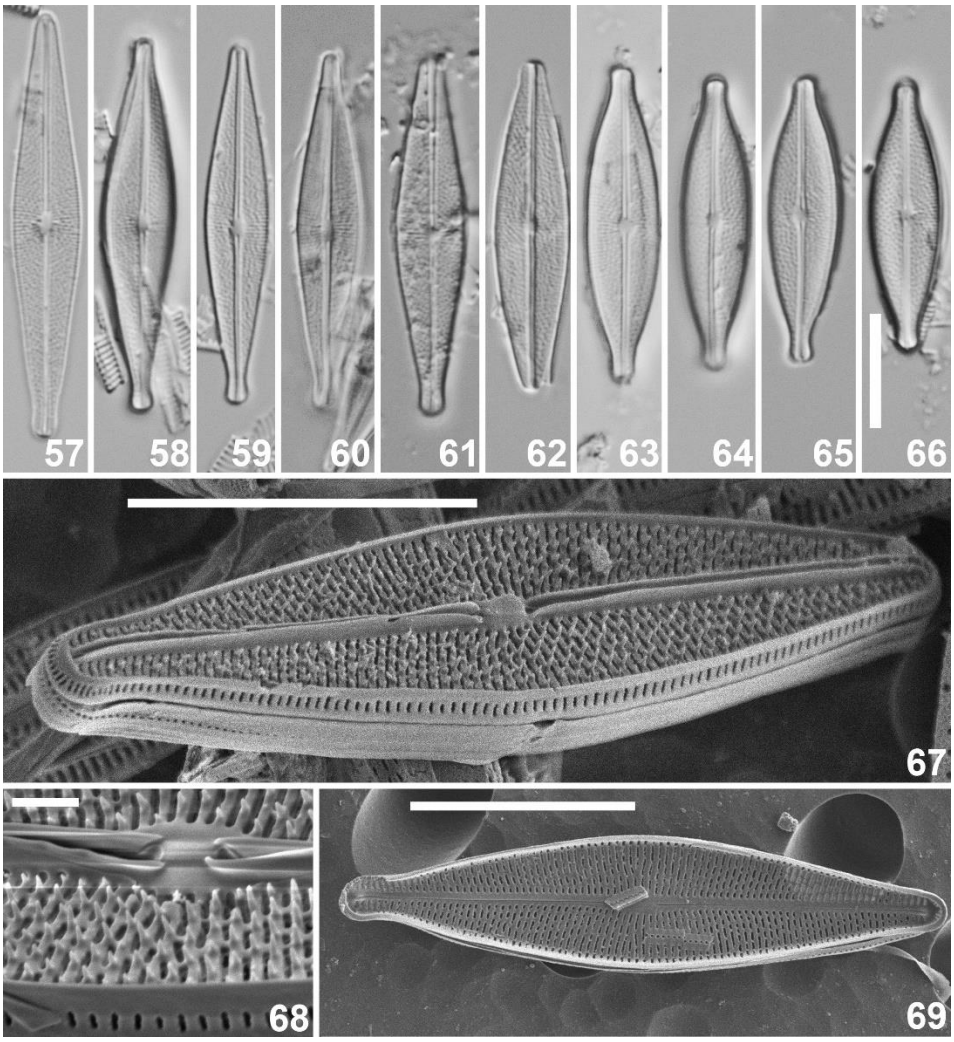
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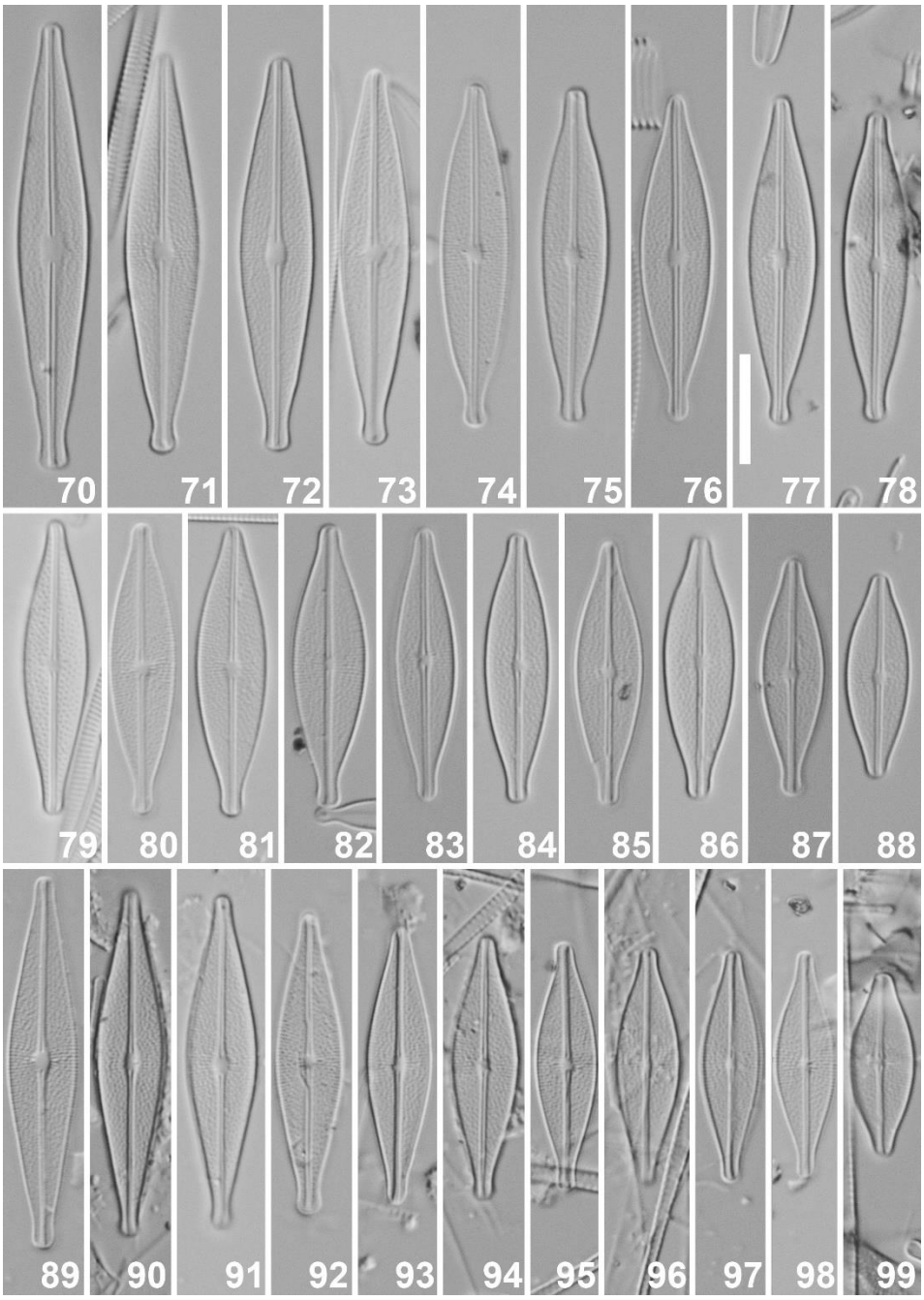
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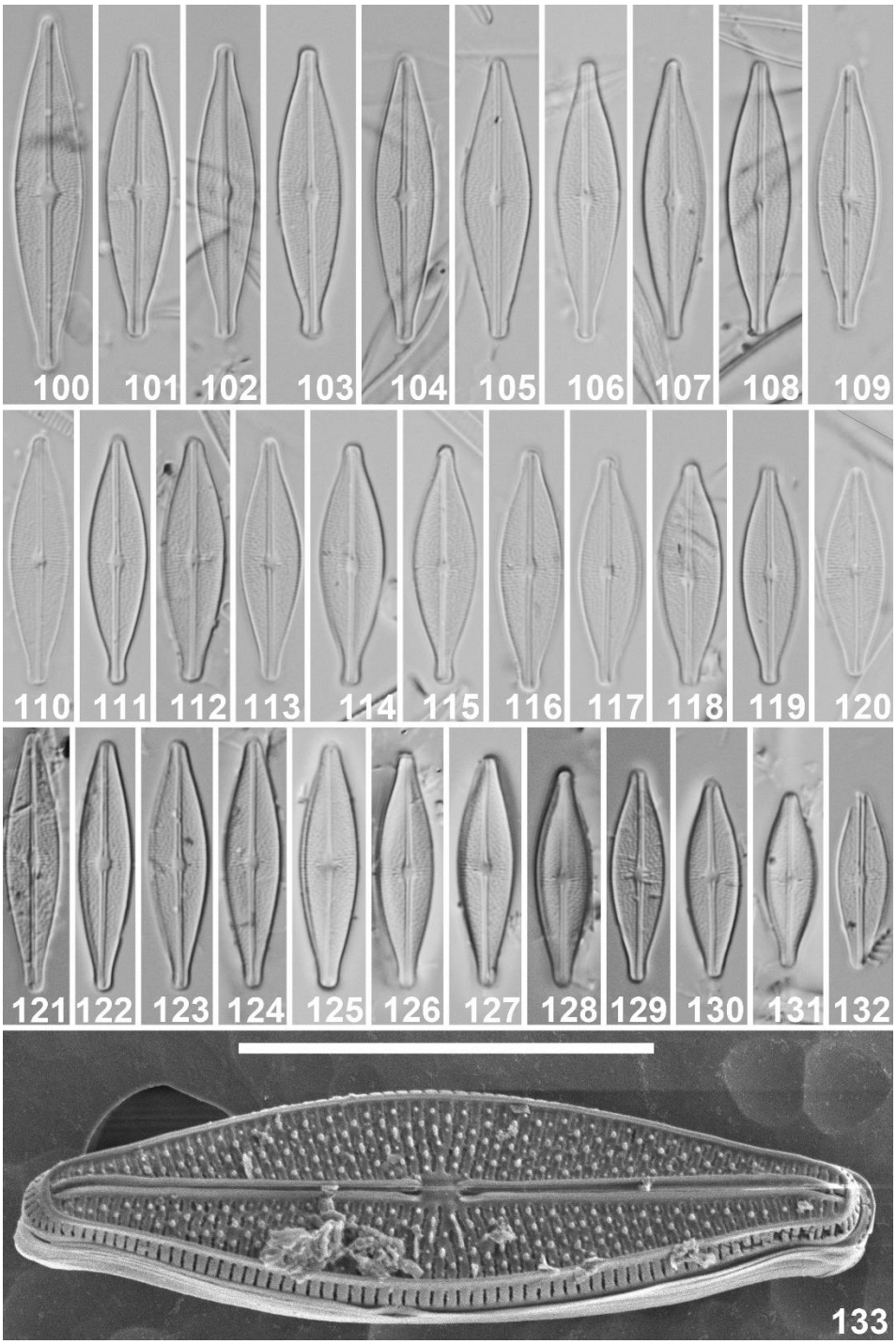
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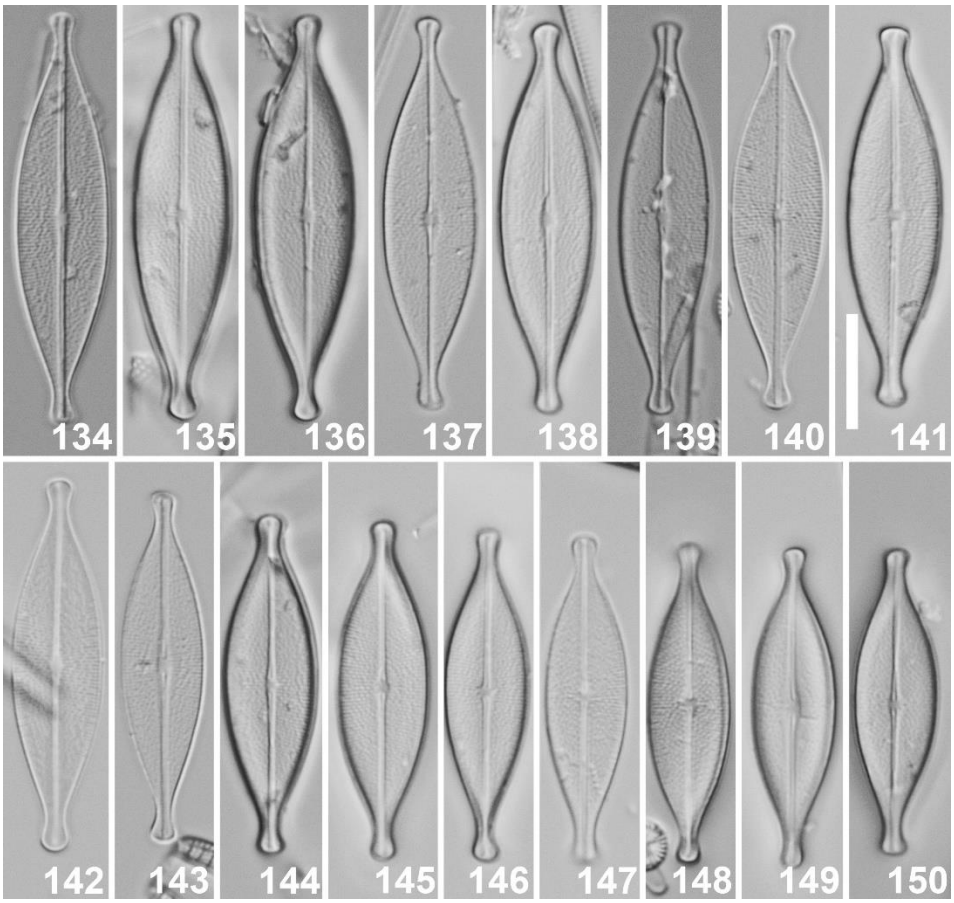
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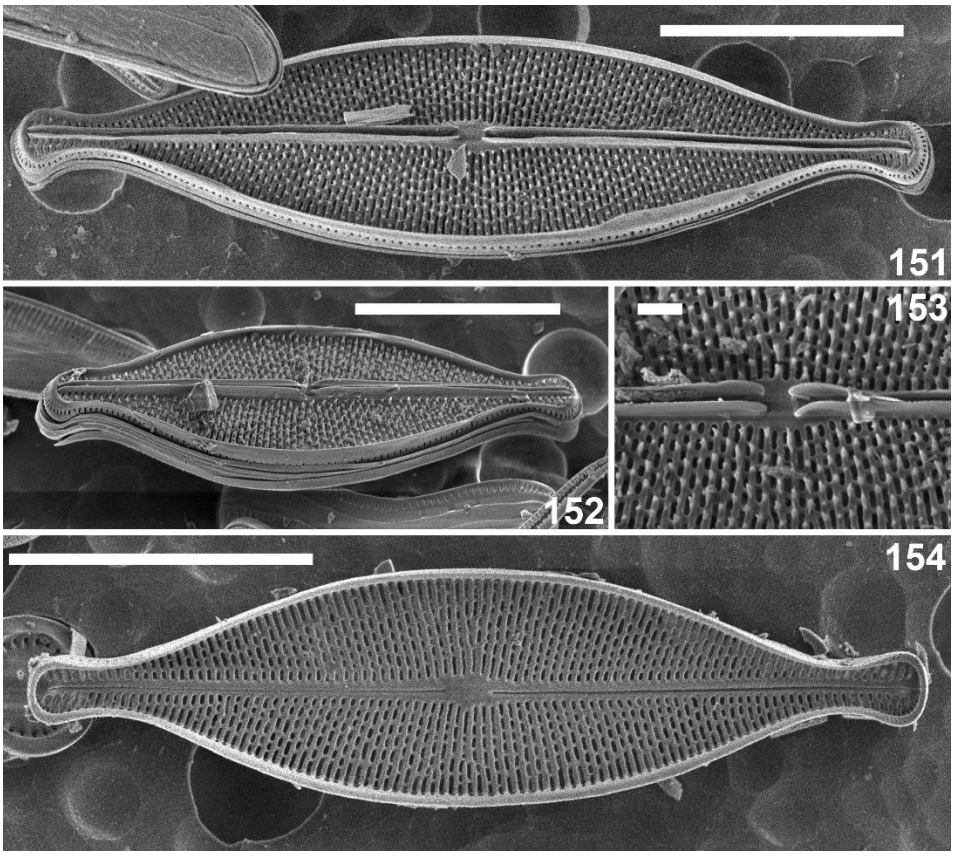
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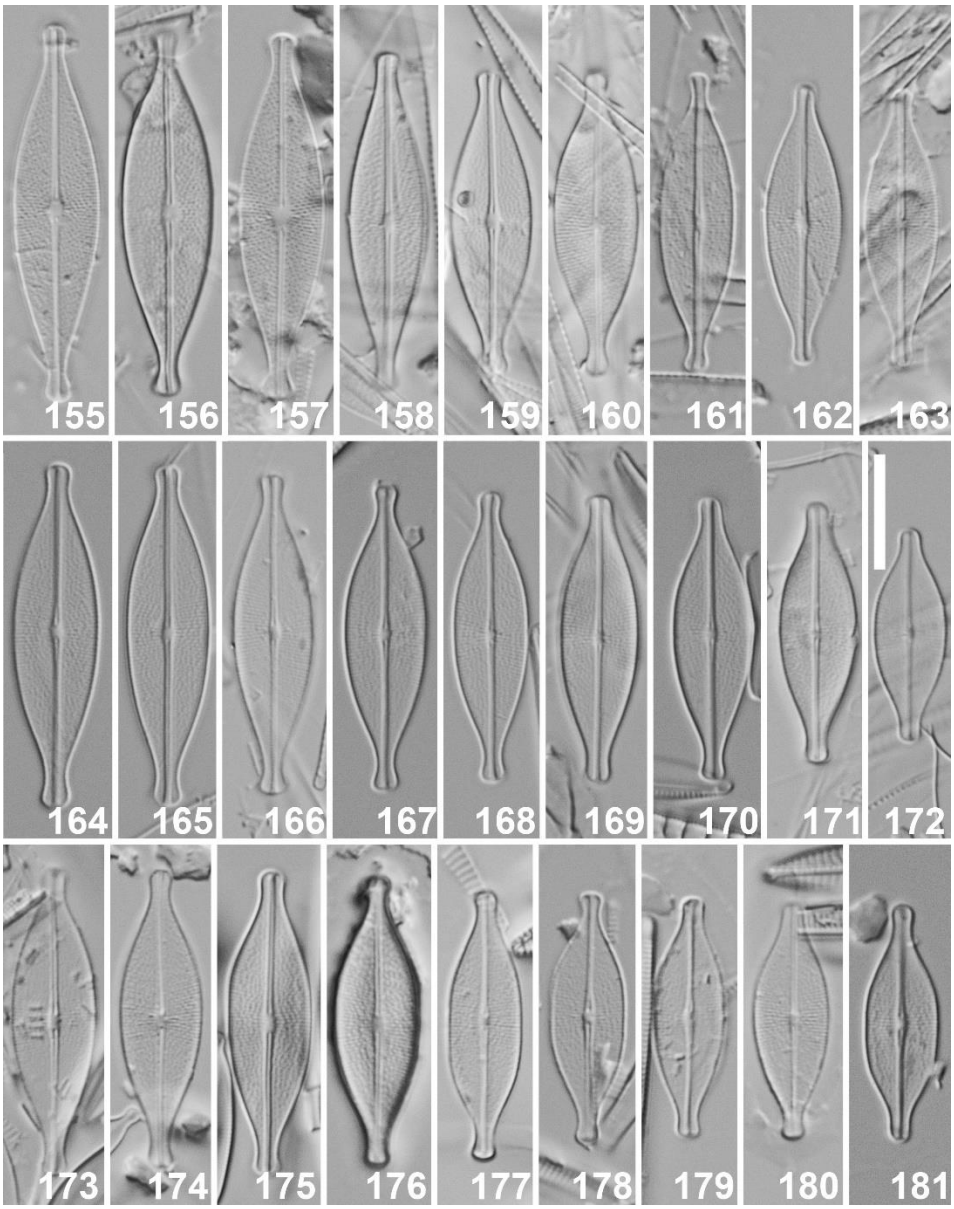
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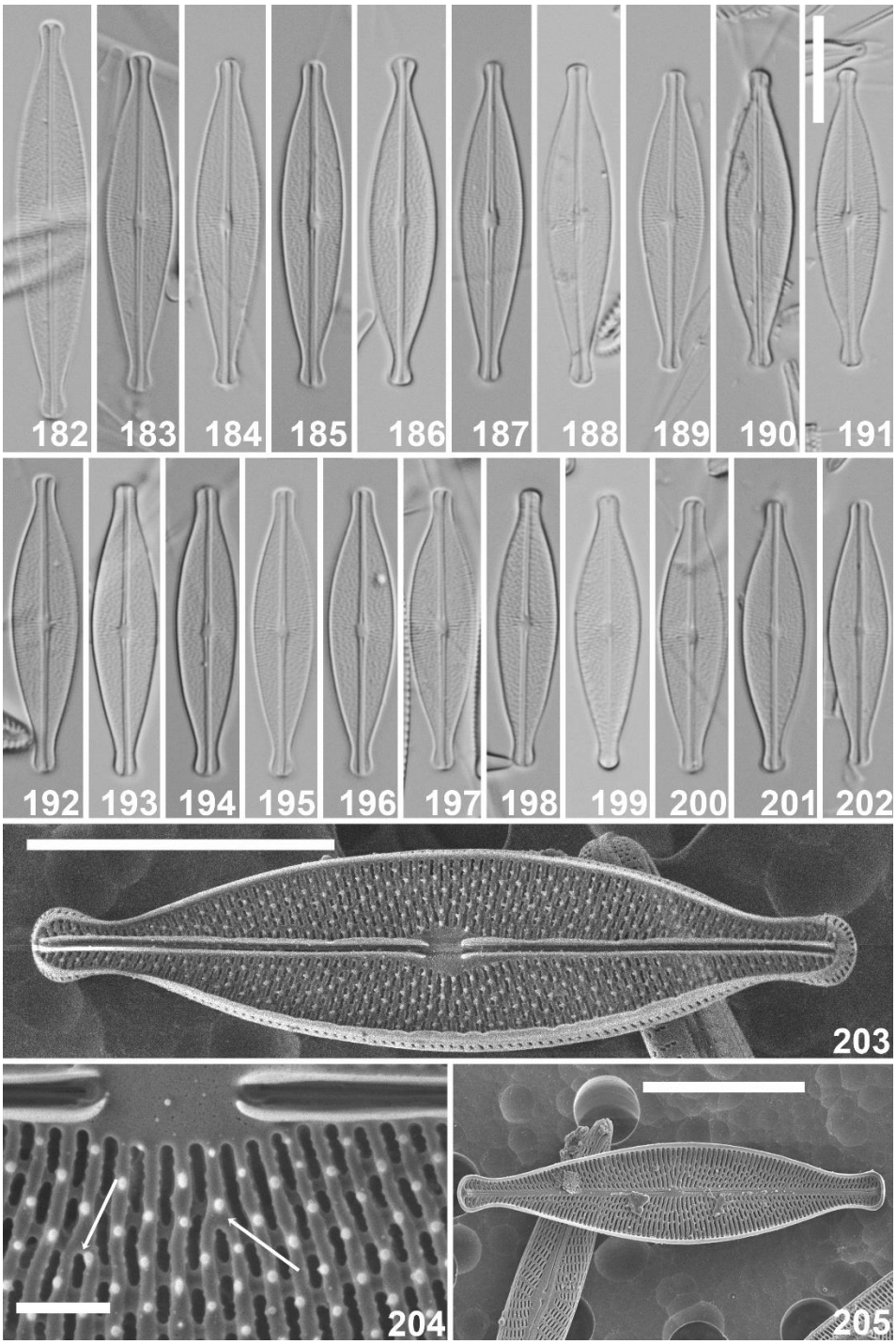
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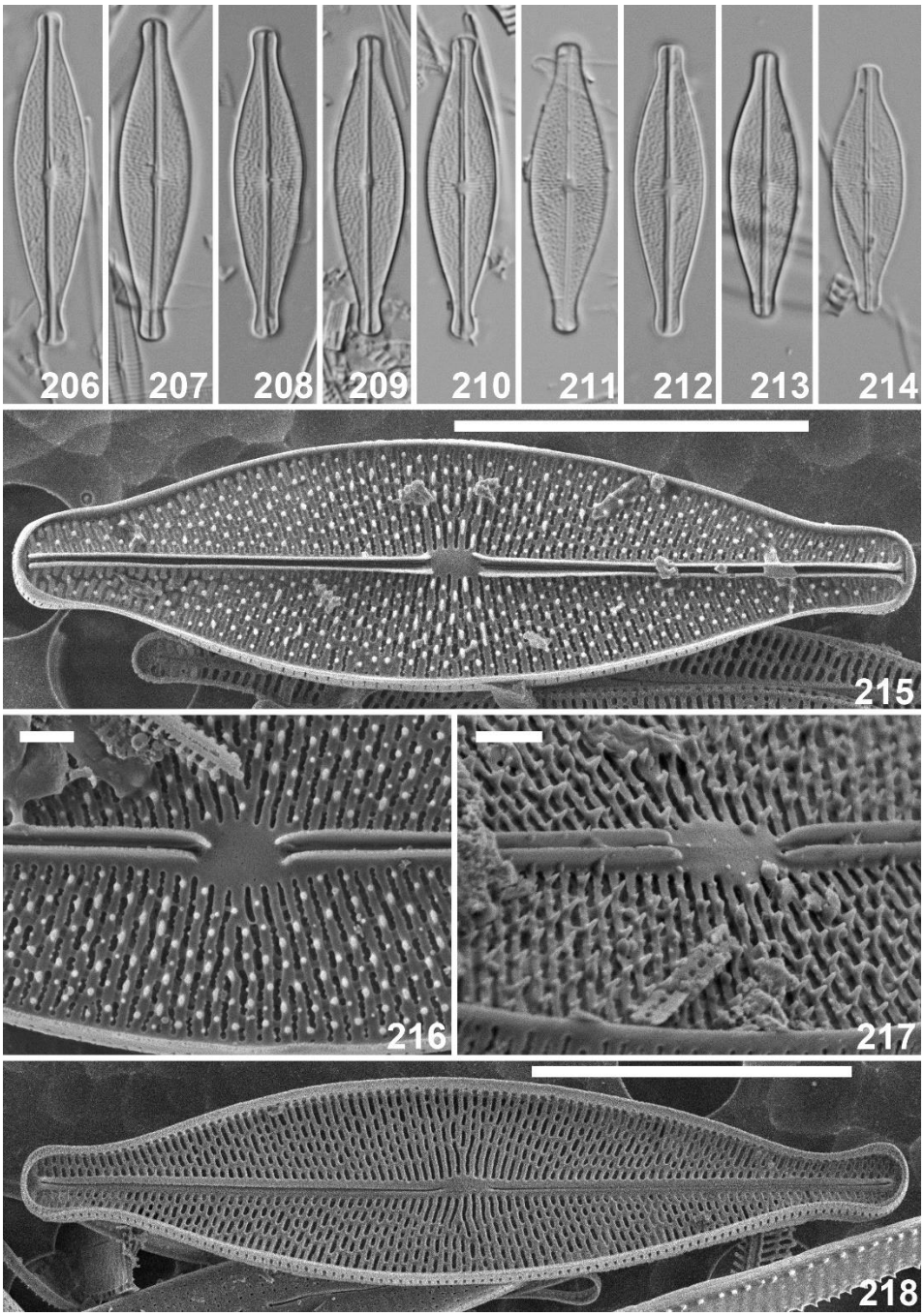
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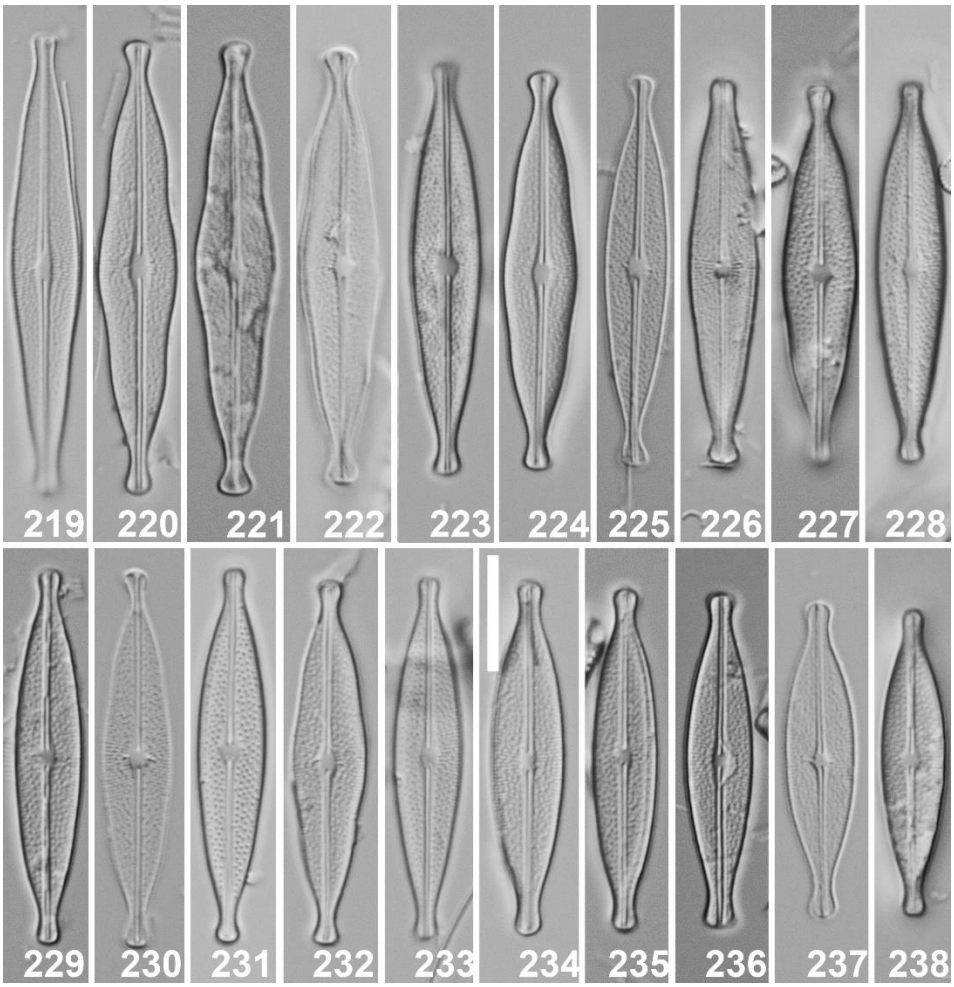
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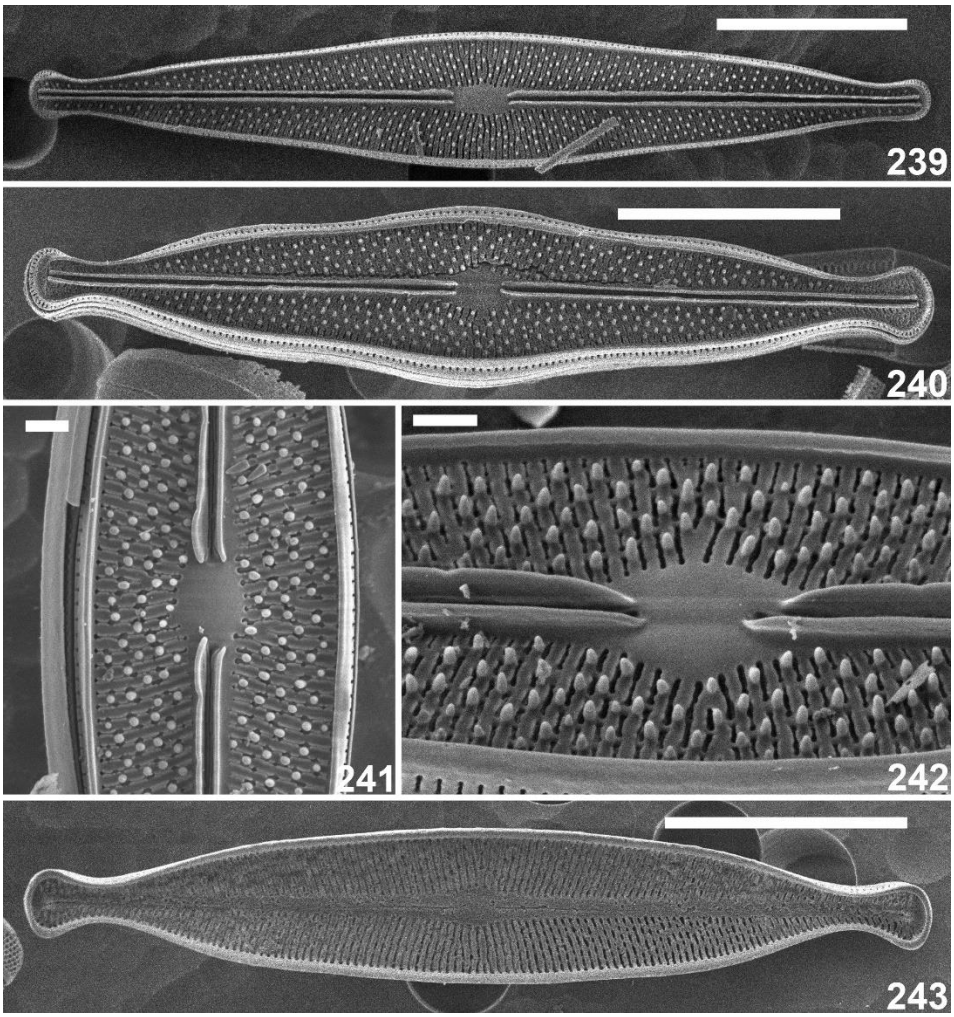
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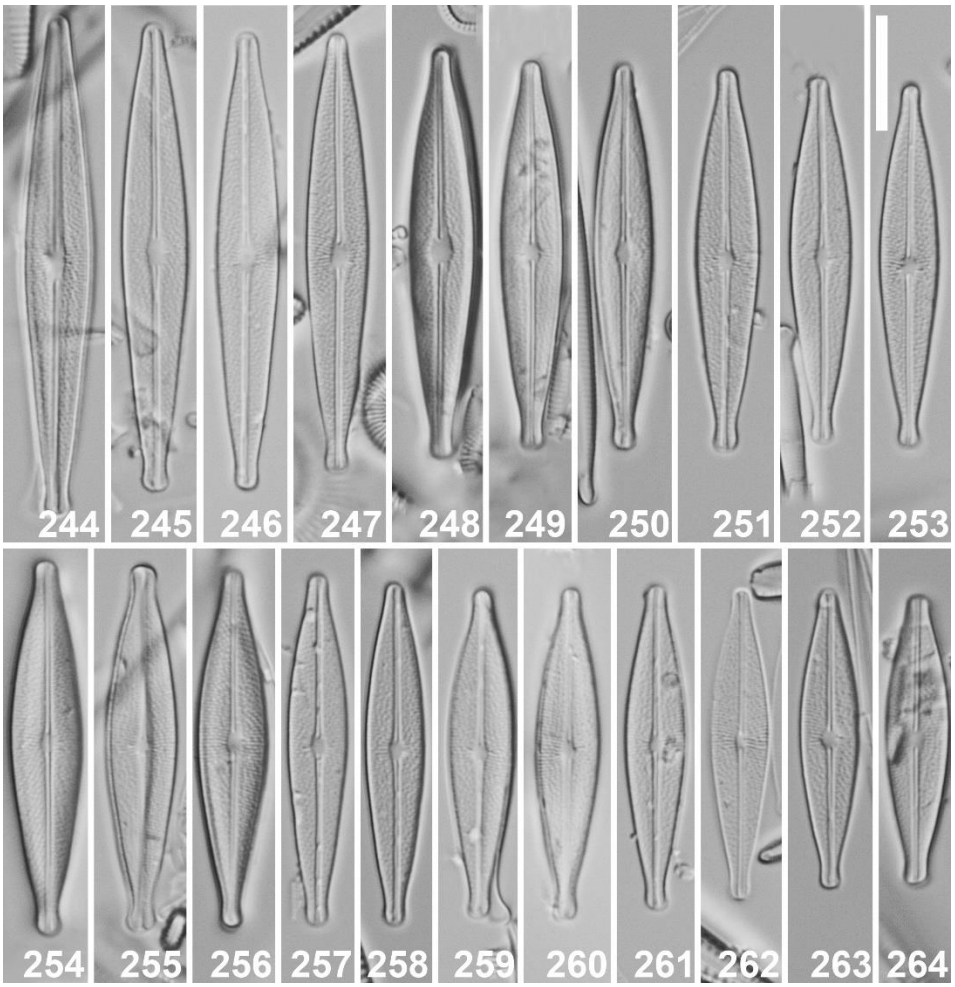
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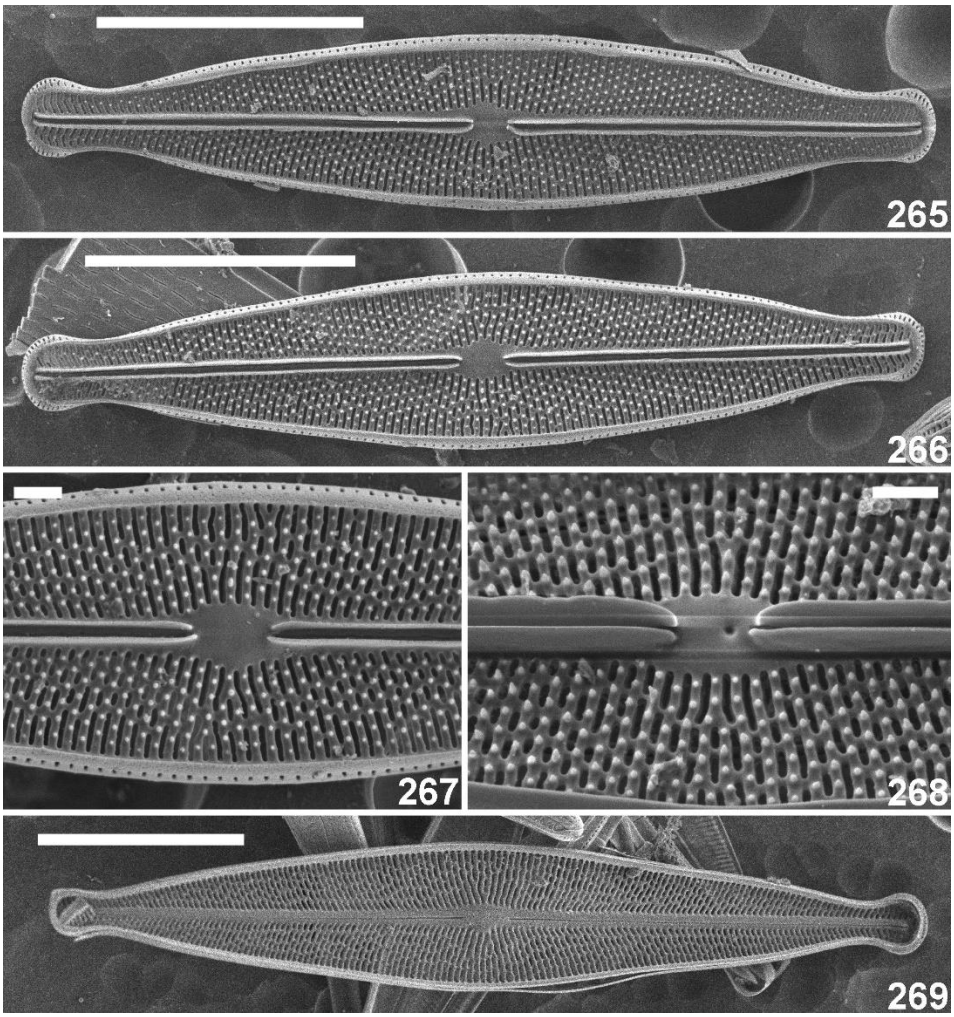
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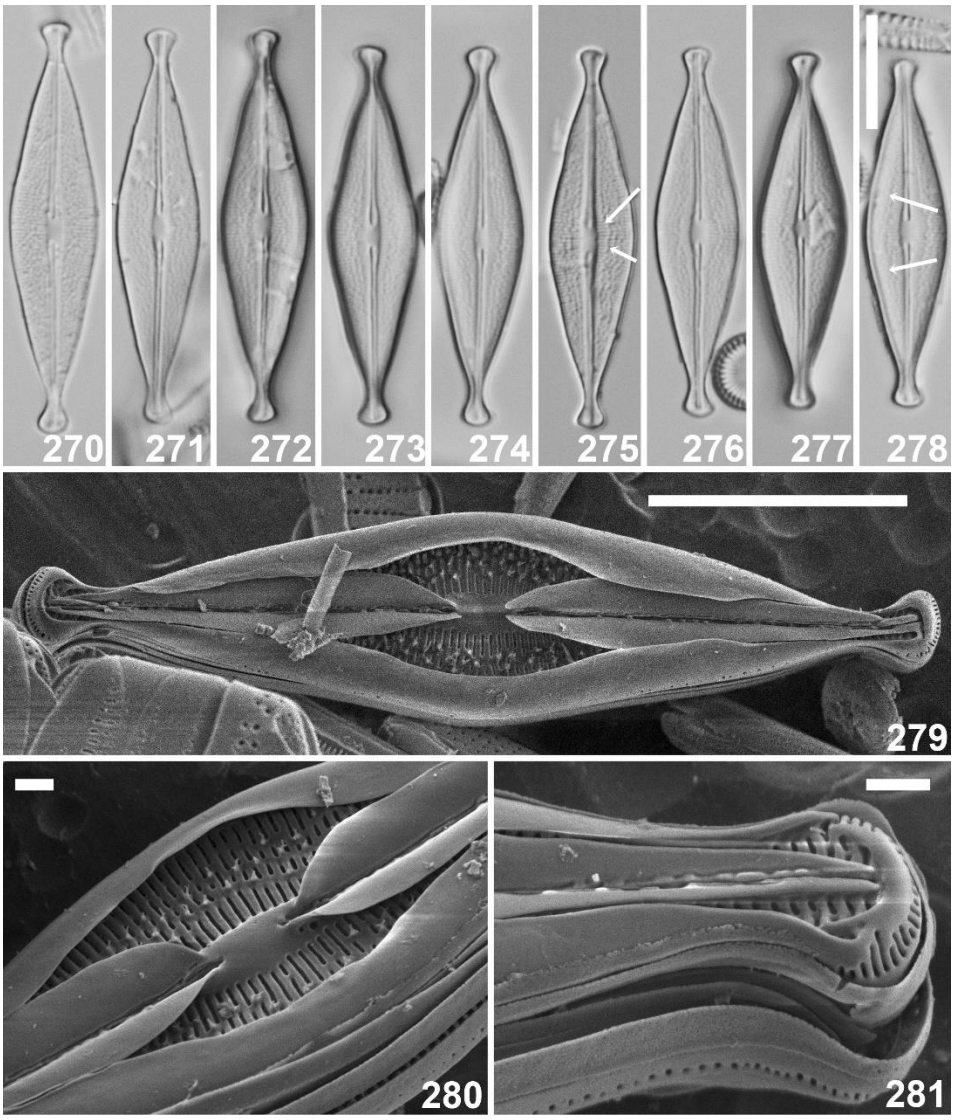
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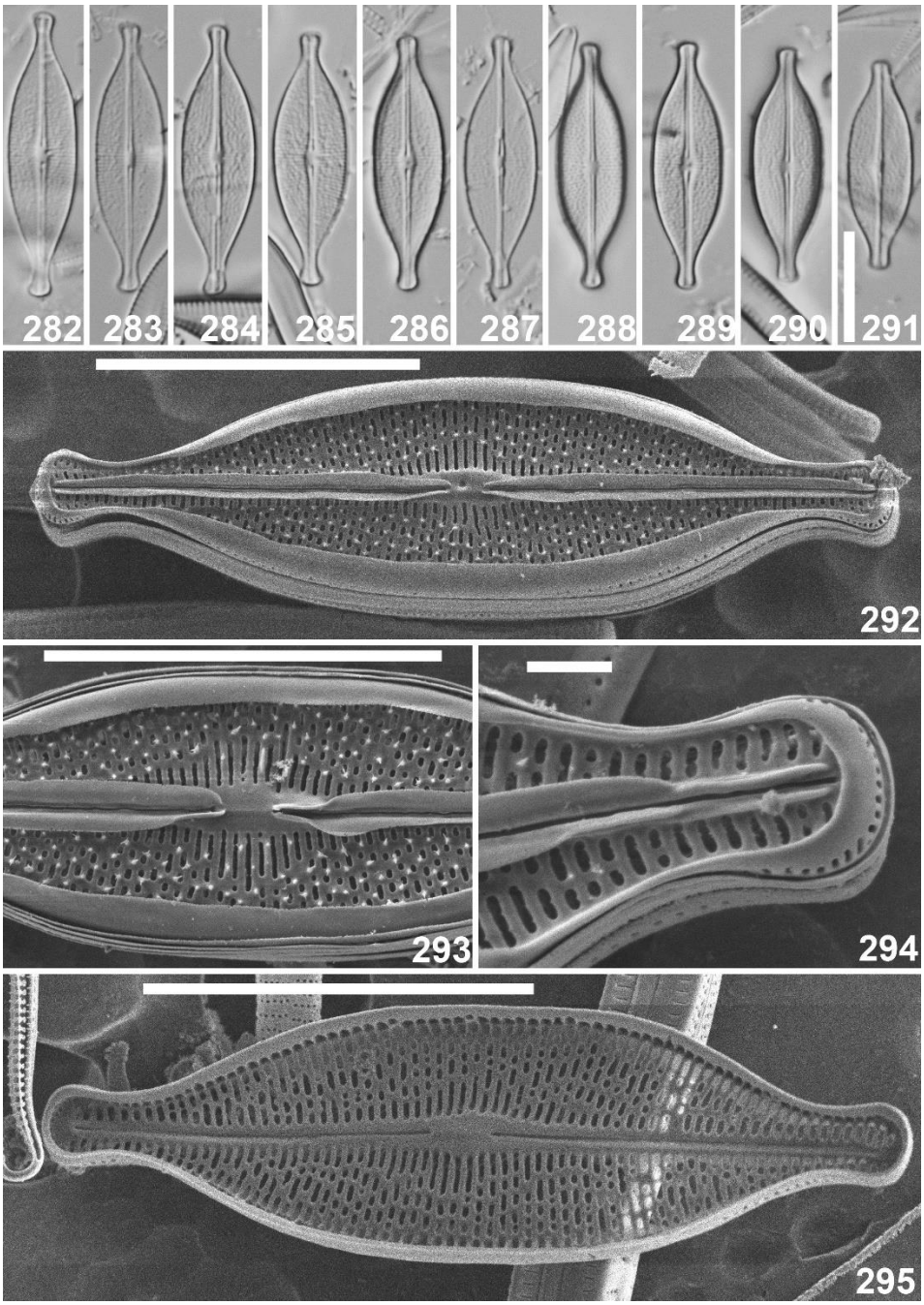
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