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Two new *Encyonema* species from Europe compared to the type material of *E. minutum* and *E. silesiacum* (Gomphonemataceae, Bacillariophyceae)

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Abstract

Cymbella minuta Hilse and *C. silesiaca* Bleisch, transferred in 1990 to the genus *Encyonema*, are two species frequently observed in our European rivers. The original material of these two species was included in Rabenhorst's historical exsiccata series (numbers 1261 & 1802) and illustrated by Kurt Krammer in 1997. Despite this publication, the morphological variability of these two species is little known and often too widely interpreted.

Recently, several populations of *Encyonema* have been observed in Europe with an intermediate morphology between *E. minutum* and *E. silesiacum*, making a reliable identification of these taxa impossible. Based on the currently available literature, two taxa could not be identified and are described as new: *E. morvanensis* and *E. vandammeana*. The present paper illustrates and discusses the morphology of the type material of *E. minutum* and *E. silesiacum* and compares it with the new species. *Encyonema morvanensis* is characterised in having usually protracted, rostrate apices, a low length/width ratio, the presence of an isolated pore, and areolae that are discernible in LM. On the other hand, *E. vandammeana* also possesses protracted apices, but a higher length/width ratio, only very weakly discernible areolae, and lacks an isolated pore. Comparisons with other *Encyonema* species, described in 1997 by Krammer (such as *E. brevicapitatum*, *E. minutiforme* and *E. simile*), are added in order to better understand the differences and similarities between these species.

Keywords: *Encyonema*, new species, Europe, morphology, type material analysis

Introduction

Encyonema minutum (Hilse) D.G.Mann and *E. silesiacum* (Bleisch) D.G.Mann are two commonly reported European diatom species, typically found on siliceous bedrock in oligo- to mesotrophic habitats with low to medium electrolyte contents (Lange-Bertalot et al. 2017). Both species were originally described in the genus *Cymbella* and later transferred *Encyonema* (Round et al. 1990, p. 667). Kützing (1834) had originally described the genus *Encyonema* with *Encyonema paradoxum* Kützing as typus generis, a species observed in July 1832 from a standing water body in Merseburg (near Leipzig, Germany). Kützing (1834) described his new genus as follows "*fila gelatinoso-membranacea, tenerrima, hyalina, simplicia, continua, libera, intus cymbellas longitudinaliter simplici serie dispositas continentia*" [gelatinous-membranous filaments, very tender, hyaline, simple, continuous, free, within the cymbellas longitudinally arranged in a simple series]. *Encyonema* species are known to live epiphytically or epilithically in mucous coatings (Krammer 1997a). At present more than 300 names are reported in the genus *Encyonema*, including 287 accepted species names (Guiry and Guiry 2023). The genus is characterized in having biraphid, dorsiventral valves with a clearly convex dorsal margin and a straight to slightly convex or concave ventral margin. The raphe is eccentrically displaced towards the ventral margin with simply central raphe endings and long terminal raphe fissures, bent towards the ventral margin. All *Encyonema* species have uniseriate striae composed of transapically elongated, slit-like areolae, internally located between distinctly raised virgae. Areolae are separated internally by thin narrow struts. Occasionally, an isolated pore can be observed in the central area, note that this was commonly but erroneously named a stigma (Round et al. 1990, Krammer 1997a). In 1997, Krammer published two volumes on the genera *Encyonema* revising all taxa known worldwide and describing 125 new taxa (including several varieties) (Krammer 1997a, b). Since then, a lot more *Encyonema* species have been described, mainly from understudied continents such as Asia (Kulikovskiy et al. 2012) and Central and South America (e.g. Rumrich et al. 2000; Novelo et al. 2007; Vouilloud et al. 2010; Marquardt et al. 2016). Only a very small number of new species was found in Europe after 1997. Levkov et al. (2006) for instance described *E. macedonicum* Levkov et al. from the iconic Lake Ohrid (Republic of North Macedonia).

During the routine biomonitoring of rivers in France and Belgium, several populations of unknown *Encyonema* taxa were observed that could not be identified using all currently available literature. Comparison with the taxa described by Krammer (1997a, b) did not result in a positive identification. As the unidentified taxa showed some resemblance to *E. minutum* and *E. silesiacum*, the type material of these common *Encyonema* species in Europe is reanalysed to establish their morphological variability and illustrate their ultrastructure using SEM as this is insufficiently documented in Krammer (1997a). After comparison with the type material and with all species described and discussed in Krammer (1997a, b), two new species are finally described as new: *Encyonema morvanensis* Van de Vijver & V.Peeters, sp. nov. and *E. vandammeana* Van de Vijver & Wilfert, sp. nov. The new species are morphologically characterised using detailed LM and SEM observations, their ecology is briefly discussed, and they are compared with similar taxa (including varieties) in Europe and worldwide.

Material and Methods

Four samples containing large *Encyonema* populations have been selected for the current analysis.

Two historic samples were retrieved from the diatom collection, kept in Meise Botanic Garden (BR, Belgium). The samples were designated as lectotypes in Patrick & Reimer (1975, p. 50) and Krammer (1997a, p. 53).

- *Cymbella minuta* Hilse, sample Rabenhorst 1261, Bei Gerbendorf in Schlesien, coll. date summer 1861, leg. W. Hilse (lectotype: slide ZU 3/8 HB) (Gerbersdorf most likely is, or is near, the actual Sokołowsko in Poland)
- *Cymbella silesiaca* Bleisch, sample Rabenhorst 1812, Brunnentrog des Gehöftes in Sedlitz bei Strehlen in Schlesien, coll. date November 1864, leg. E.J.Bleisch (lectotype Reimer, A-G.C 11486) (Strehlen is the actual Strzelin in Poland)

Two recently collected samples containing the unknown *Encyonema* species were also studied:

- Masblette river, Nassogne, Belgium, sample BERW 03803, coll. date 13/05/2019, leg. Service Public de Wallonie
- Yonne river, Amazy, France, sample 03024840, coll. date 28/06/2022, leg. V. Peeters

Subsamples of the four selected samples were prepared for LM and SEM observations following the method described in van der Werff (1955). Small amounts of each sample were cleaned by adding 37% H_2O_2 and heating to 80°C for about 1 h, after which 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) and 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). Stria density was determined by counting striae from the central area onwards to the apices. Middle striae are often more spaced, underestimating the actual stria density. For SEM analysis, part of the suspension was filtered through 5-µm Isopore™ polycarbonate membrane filters (Merck Millipore), pieces of which were affixed with conductive double-sided adhesive carbon-tabs to aluminum stubs after air–drying. Stubs were coated with a platinum layer of 15 nm, and studied using a JEOL-JSM-7100F field emission scanning electron microscope at 2 kV and a working distance of 4 mm. 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 and Ross (1981, stria structure), Round et al. (1990, raphe structure), and Krammer (1997a, genus features for *Encyonema*). The new species was compared with different *Encyonema* taxa described worldwide (Krammer 1997a, b).

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

Class Bacillariophyceae Haeckel

Subclass Bacillariophycidae D.G.Mann

Order Cymbellales D.G.Mann

Family Cymbellaceae Kützing

Genus *Encyonema* Kützing

***Encyonema minutum* (Hilse) D.G.Mann in Round et al. 1990 (Figure 1)**

Basionym

Cymbella minuta Hilse in Rabenhorst 1862: Algen Europa's, Fortsetzung der Algen Sachsens, Resp. Mittel-Europa's. Decasdes 27–28, n° 1261

≡ *Cymbella gracilis var. minuta* (Hilse) Rabenhorst 1864

≡ *Encyonema ventricosum f. minuta* (Hilse) Grunow in Van Heurck 1880

≡ *Encyonema ventricosum var. minuta* (Hilse) Schmidt 1881

≡ *Cymbella ventricosa f. minuta* (Hilse) Mereschkowsky 1906

≡ *Cymbella ventricosa f. minuta* (Hilse) Mayer 1913

≡ *Cymbella ventricosa f. minuta* (Hilse) A.Cleve 1932

≡ *Encyonema ventricosum var. minutum* (Hilse) Mayer 1947

≡ *Cymbella ventricosa var. minuta* (Hilse) A.Cleve 1955

Light microscopy (Figs 1a–1ab). Valves strongly dorsiventral with convex dorsal and straight to weakly concave ventral margin. Central part of ventral margin often weakly gibbous. Apices not to very weakly protracted, rostrate, acutely rounded, bent to the ventral side. Valve dimensions (n=40): length 10–21 µm, width 4–6 µm, length/width ratio 2.5–3.6. Axial area narrow, linear, displaced towards the ventral margin. Central area almost absent, very weakly widened at the dorsal side. Raphe filiform, with dorsally deflected central, simple raphe endings. Terminal raphe fissures ventrally bent, discernible in LM. Striae on the dorsal side straight to weakly radiate, 18–22 in 10 µm, the middle stria clearly more spaced than the others. Ventrally, striae very short, radiate, 17–19 in 10 µm, not or only very weakly more dense at the apices. Areolae not discernible in LM. Isolated pore absent.

Scanning electron microscopy (Figs 1ac–1ag). Valve face flat. Striae uniseriate, composed of transapically elongated, slit-like areolae, 35–40 in 10 µm. Several areolae in the valve centre smaller, more rounded (Figs 1ac–1ae). Raphe branches curved with dorsally deflected, simple, weakly expanded central raphe endings. Terminal raphe fissures ventrally hooked and weakly recurved, terminating on the valve face (Figs 1ac–1ae). Internally, areola foramina slit-like, located in long, shallow, transapical grooves (Figs 1af, 1ag). Areolae separated by short, siliceous, incomplete struts (Figs 1f, 1g). Internal central raphe endings with typical intermissio, sigmoidally hooked (Fig. 1ag). Terminal raphe endings terminating onto small helictoglossae. Isolated pore not visible. Two central areolae more rounded but not differing in structure from the other areolae (Fig. 1ag).

Encyonema silesiacum **(Bleisch) D.G.Mann in Round et al. 1990 (Figure 2)**

Basionym

Cymbella silesiaca Bleisch in Rabenhorst 1865: Algen Europa's, Fortsetzung der Algen Sachsens, Resp. Mittel-Europa's. Decades 81–82, n° 1802

≡ *Cymbella ventricosa var. silesiaca* (Bleisch) A.Cleve 1955

≡ *Cymbella minuta var. silesiaca* (Bleisch) Reimer in Patrick and Reimer 1975

Light microscopy (Figs 2a–2s). Valves strongly dorsiventral with convex dorsal and straight to weakly convex ventral margin. Central part of ventral margin often clearly gibbous. Apices not to very weakly protracted, rostrate, acutely rounded, bent to the ventral side. Valve dimensions (n=25): length 20–39 µm, width 7–10 µm, length/width ratio 2.8–4.1. Axial area moderately broad, lanceolate, displaced towards the ventral margin. Central area formed by a dorsal, occasionally also ventral, widening of the axial area. Raphe filiform, with dorsally weakly deflected central, simple raphe endings. Terminal raphe fissures ventrally bent, discernible in LM. Striae on the dorsal side weakly radiate, 14–15 in 10 µm, the middle stria clearly more spaced than the others. Ventrally, striae very short, radiate, 13–15 in 10 µm, more densely spaced near the apices. Areolae often discernible in LM. Isolated pore often visible in the central area, separated from the middle stria (Figs 2b, 2c, 2n).

Scanning electron microscopy (Figs 2t–2x). Valve face flat. Striae uniseriate, composed of transapically elongated, slit-like areolae, 25–32 in 10 µm. Virgae as wide as or slightly wider than the striae. Several areolae in the valve centre smaller, more rounded (Fig. 2u). Isolated pore visible as a rounded opening (Figs 2t, 2u, arrows). Raphe branches curved, weakly undulating, with dorsally deflected, weakly expanded central raphe endings. Terminal raphe fissures ventrally hooked, terminating on the valve face almost at the valve face/mantle junction (Figs 2t, 2v). Internally, areola foramina slit-like, located in long, shallow, transapical grooves (Figs 2w, 2x). Areolae separated by short, siliceous, usually incomplete struts (Fig. 2x). In the central area, struts often complete (Fig. 2w). Internal central raphe endings with typical intermissio, sigmoidally hooked (Fig. 2w). Terminal raphe endings terminating onto small helictoglossae (Fig. 2x). Isolated pore internally distinct as a long transapical slit at the end of the central stria (Fig. 2w).

***Encyonema morvanensis* Van de Vijver & V.Peeters, sp. nov. (Figure 3)**

Holotype

BR-4810 (Meise Botanic Garden, Belgium). Fig. 3d illustrates the holotype.

Isotype

Slide 429 (University of Antwerp, Belgium)

Type material

Yonne river, Amazy, France, sample 03024840, coll. date 28/06/2022, leg. V. Peeters

Etymology

The specific epithet '*morvanensis*', refers to the Morvan Massif in the Bourgogne-Franche-Comté Region (eastern France), where the new species has been regularly observed.

Registration

http://phycobank.org/XXXX

Description

Light microscopy (Figs 3a–3z). Valves elongated, dorsiventral with convex dorsal and straight to very weakly convex ventral margin. Central part of ventral margin often very weakly gibbous. Apices usually protracted, rostrate, acutely rounded, bent to the ventral side. Smaller specimens showing strictly non-protracted apices. Valve dimensions (n=40): length 12–22 µm, width 5.0–6.5 µm, length/width ratio 2.2–3.5. Axial area narrow, linear, displaced towards the ventral margin. Central area almost absent, formed by a very slight dorsal widening of the axial area.

Raphe filiform, with dorsally weakly deflected central, simple raphe endings. Terminal raphe fissures ventrally bent, discernible in LM. Striae on the dorsal side weakly radiate, 12–14 in 10 µm, the middle stria almost not more spaced than the others. Ventrally, striae very short, radiate, 12–13 in 10 µm, more densely spaced near the apices. Areolae often discernible in LM. Isolated pore visible as a distinct point in the central area, separated from the middle stria.

Scanning electron microscopy (Figs 3aa–3ad). Valve face flat. Striae uniseriate, composed of transapically elongated, broad, slit-like areolae, 30–35 in 10 µm. Virgae as wide as or slightly wider than the striae. Several areolae in the valve centre smaller, more rounded (Fig. 3ab). Isolated pore visible as a rounded opening (Fig. 3ab, arrow). Raphe branches curved, with dorsally deflected, weakly expanded central raphe endings (Figs 3aa, 3ab). Terminal raphe fissures ventrally hooked, terminating on the valve face (Fig. 3aa). Internally, areola foramina slit-like, located in long, shallow, transapical grooves (Figs 3ac, 3ad). Areolae separated by short, siliceous, usually incomplete struts (Fig. 3ad). Internal central raphe endings with typical intermissio, hooked (Fig. 3ad). Terminal raphe endings terminating onto small helictoglossae (Fig. 3ac). Isolated pore internally distinct bordered by an irregularly dentated margin at the end of the central stria (Figs 3ac, 3ad).

Encyonema vandammeana **Van de Vijver & Wilfert, sp. nov. (Figure 4)**

Holotype

BR-4811 (Meise Botanic Garden, Belgium). Fig. 4e illustrates the holotype.

Isotype

Slide 430 (University of Antwerp, Belgium)

Type material

Masblette river, Nassogne, Belgium, sample BERW 03803, coll. date 13/05/2019, leg. Service Public de Wallonie

Etymology

The species is named after Prof. dr Stefan Van Damme (University of Antwerp, Belgium) in recognition of his important contributions to aquatic ecological research.

Registration

http://phycobank.org/XXXX

Description

Light microscopy (Figs 4a–4x). Valves elongated, dorsiventral with convex dorsal and very weakly convex ventral margin. Central part of ventral margin weakly gibbous. Apices throughout the entire cell cycle clearly protracted, rostrate, bent to the ventral side. Valve dimensions (n=30): length 20.0–24.5 µm, width 6–7 µm, length/width ratio 2.9–3.6. Axial area very narrow, linear, displaced towards the ventral margin. Central area almost absent, a very slight dorsal widening of the axial area very rarely observed. Raphe filiform, with dorsally weakly deflected central, simple raphe endings. Terminal raphe fissures ventrally bent, discernible in LM. Striae on the dorsal side weakly radiate, 14–16 in 10 µm, the middle stria weakly more spaced than the others. Ventrally, striae very short, radiate, 14–16 in 10 µm, slightly more densely spaced near the apices. Areolae occasionally visible in LM (Figs 4e, 4j). Isolated pore not clearly discernible in LM, integrated into the middle stria in the central area.

Scanning electron microscopy (Figs 4y–4ab). Valve face flat. Striae uniseriate, composed of transapically elongated, slit-like areolae, ca 35 in 10 µm. Virgae slightly wider than the striae. Several areolae in the valve centre bordering the axial area mostly on the dorsal side smaller, clearly rounded (Figs 4y, 4z). Isolated pore visible as a small, rounded opening (Fig. 4y). Raphe branches curved, with dorsally deflected, weakly expanded central raphe endings (Figs 4y, 4z). Terminal raphe fissures ventrally hooked, not recurved, terminating on the valve face (Fig. 4y). Internally, areola foramina slit-like, located in long, shallow, transapical grooves (Figs 4aa, 4ab). Areolae separated by short, siliceous, usually incomplete struts (Fig. 4ab). Internal central raphe endings with typical intermissio, sigmoidally hooked (Fig. 4ab). Terminal raphe endings terminating onto small helictoglossae (Fig. 4aa). Isolated pore internally visible as a small, rounded point in a short groove (Fig. 4ab).

Ecology and associated diatom flora: *Encyonema vandammeana* was observed in several rivers in Wallonia, the southern region of Belgium. One of the largest populations was found in the Masblette river, a small 14 km long river running from the town of Saint-Hubert to the river Lomme. This river is characterised by an alkaline pH (7.4–8.1) and low levels of nitrate (<0.7 mg/l), phosphate (< 0.01 mg/l), and sulphate (<15 mg/l) and its diatom flora is dominated by several *Achnanthidium* species [*A.* cf. *crassum* (Hustedt) Potapova & Ponader, *A. lineare* W.Smith, *A. microcephalum* Kützing, *A. subatomus* (Hustedt) Lange-Bertalot], several *Fragilaria* species [*F. radians* (Kützing) D.M.Williams & Round, *F.* cf. *rinoi* Almeida &

C.Delgado, *F. sandellii* Van de Vijver & Jarlman], *Meridion circulare* (Greville) C.Agardh, and *Reimeria sinuata* (W.Gregory) Kociolek & Stoermer. Following Lange-Bertalot et al. (2017), these species point to more alkaline, oligo- to mesotrophic, low electrolyte running water conditions. The other species, *Encyonema morvanensis*, is typically found in the Morvan Massif in the eastern part of France. The species was described from the River Yonne, a 292 km long tributary of the Seine river. This river is characterized by a weakly alkaline pH (7.4), a rather low conductivity (138 µS/cm). The type sample was dominated by *Craticula subminuscula* (Manguin) C.E.Wetzel & Ector, *Gomphonema minutum* (C.Agardh) C.Agardh, *G. parvulum* (Kützing) Kützing, *G. pumilum* var. *rigidum* E.Reichardt & Lange-Bertalot, *Mayamaea permitis* (Hustedt) K.Bruder & Medlin, *N. cryptotenella* Lange-Bertalot, and *N. germainii* Wallace. This species composition is typical for alkaline running waters with higher trophic conditions, higher electrolyte contents and higher saprobic levels, often characterised as highly impacted (Lange-Bertalot et al. 2017).

Discussion

The analysis of the type material of *Encyonema silesiacum* showed that the latter in fact show a high morphological variability in the type material. In the type population, valve outline, areola density, and presence of the isolated pore vary considerably. This variability most likely has led Kurt Krammer to distinguish in 1997 a plethora of new varieties, often based on subtle differences with the valves he reported from the type material. These varieties were almost always described from different localities in Germany but in our analysis of the type material of *Cymbella silesiaca*, several of these varieties seem also to be present in the original material of the nominate form and hence represent merely expressions of the broad morphological variability of the species (Krammer 1997a). *Encyonema silesiacum* var. *distinctepunctatum* Krammer was separated based on a lower areola density but as can be seen in the series of illustrated valves of Figure 2, specimens with a lower areola density were also found in the *C. silesiaca* type material. Similarly, *Encyonema silesiacum* var. *ventriformis* Krammer should have a more gibbous central part on the ventral side but our Figs 2c & 2d show a similar ventral gibbosity. A more careful analysis of all these different varieties based on observations of the respective type materials is necessary to verify the usefulness of these separations. Moreover, the presence of these varieties in relation to ecological parameters of their environment should be evaluated.

Our observations of the stria density of both *E. minutum* and *E. silesiacum* show somewhat higher values than reported in Krammer (1997a), a discrepancy most likely due to differences in stria measurements. Although Krammer (1997a, p. 27–30) elaborately discussed striae and their density, he did not mention how he actually measured the density. As the middle striae are more distant, counting over the middle striae will lead to lower stria densities. In the present study, stria densities have been determined by avoiding counting over the middle. Therefore, the observed stria density in the present study for *E. minutum* is 18–22 in 10 µm (versus 15–18 in 10 µm in Krammer 1997a, p. 53) and for *E. silesiacum*, we measured 14–15 in 10 µm (versus 11–14 in 10 µm in Krammer 1997a, p. 73). This clear difference shows the importance of a clear description of the applied methods. Similarly, the stria density for *E. morvanensis* using the Krammer (1997a) method would be 12–13 in 10 µm (compared to 14–15 in 10 µm following our method) and for *E. vandammeana* would be 13–14 in 10 µm (compared to 14–16 in 10 µm in this study). It is important to take this into account when comparing the new species with all previously described species reported in Krammer (1997a, b).

The analysis of the types of *C. minuta* and *C. silesiaca* also showed that both species clearly differ from each other, based on valve dimensions, stria and areola density, the presence of an isolated pore and the terminal raphe fissures. Table 1 highlights a comparison of the morphology and morphometry of all four discussed species together with similar taxa based on Krammer (1997a).

Based on the morphological comparison, both new taxa differ from *E. minutum* and *E. silesiacum*. *Encyonema silesiacum* is much larger with a valve width of minimum 7 µm, a value not reached in both *E. morvanensis* and *E. vandammeana*. In LM, the species has a distinct isolated pore and clearly discernible areolae, separating the species from *E. vandammeana* that has in LM only weakly visible areolae and an isolated pore that, in LM, cannot be separated from the middle stria. *Encyonema morvanensis*, on the other hand, has more visible areolae in LM and in many valves, a separated isolated pore can be seen at the end of the middle stria, although this pore is never as distinct as in *E. silesiacum*. *Encyonema minutum* has a much higher stria density (18–22 in 10 µm) and a more strongly dorsiventral valve outline than both new species, making confusion based on morphology less likely. The differentiation from the taxa described by Krammer (1997a) is less clear. All discussed Krammer species were described from very oligotrophic habitats in northern Europe. They all present a very elongated, more slender valve outline compared to *E. morvanensis* and *E. vandammeana*, and have less to not protracted, acutely rounded apices. The comparison is based on the type populations of the different species. In Krammer (1997a), additional populations are illustrated for several of the species. Two different morphotypes are shown for *E. brevicapitatum* with only morphotype 1 representing the type. Morphotype 2 was also observed in Finnish-Lapland but has more curved valves with clearly protracted, bent, rostrate apices and slightly broader valves. It is highly unlikely that both morphotypes belong to the same species, given the above mentioned differences. The same can be said for *E. fogedii* where morphotype 2 has clearly protracted, distinctly rostrate, even weakly inflated apices and narrower, more slender valves. Both are recorded on Spitsbergen and according to Krammer (1997a, p. 105) can be easily separated (*lassen sich … gut unterscheiden*).

Another species with a similar morphology is *E. simile* Krammer, described from northern Sweden (Krammer 1997a). A distinction should be made between the different illustrated populations in Krammer (1997a). The *E. simile* population from the Andes (Krammer 1997a, plate 19, figs 9–16) clearly differs from the type population of *E. simile* in having protracted, more elongated apices and a more strongly dorsiventral valve outline. Whether these different morphotypes and/or populations represent different species or simply the expression of the morphological variability of these species, can only be established after checking the original material. Unfortunately, the entire Krammer collection, currently included within the Hustedt collection in Bremerhaven, will be transferred to the Botanic Garden of Berlin (Germany), a process that is taking a lot of time without an exact final date of execution (N.Abarca, Botanic Garden of Berlin, pers. comm.). Nevertheless, based on the available information in Krammer (1997a) *E. simile* can be separated from *E. morvanensis* in having a much higher length/width ratio (3.8–4.1 versus 2.2–3.5 in *E. morvanensis*) giving *E. simile* a more elongated outline, an isolated pore that is not visible in LM (although due to the poor quality of the images in Krammer 1997a, plate 8, figs 5–10, it cannot be verified with 100% certainty), less protracted, blunt apices and an almost straight, never gibbous ventral margin. The areola density apparently also differs with *E. simile* have a lower density, although this is not observed in the illustrated valves.

Given also the ecological preferences of the new species (being more typical for eutrophic waterbodies) and the differences in morphology with all currently known species, the description of both taxa as new species can be justified. The analysis of the type material of the two commonly reported species, *E. minutum* and *E. silesiacum*, showed a higher morphological variability that excluded conspecificity with the newly described species.

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Author contributions

Bart Van de Vijver

Contribution: generation and analyses of LM and SEM materials, discussion of results, development, writing, revision and editing of the manuscript.

Valérie Peeters

Contribution: analyses of LM materials, discussion of results, revision and editing of the manuscript.

Katharina Wilfert

Contribution: analyses of LM materials, discussion of results, revision and editing of the manuscript.

Myriam de Haan

Contribution: analyses of SEM materials, discussion of results, revision and editing of the manuscript.

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Table 1. Morphology and morphometric details of species showing some similarity with *E. morvanensis* and *E. vandammeana*.

	Encyonema silesiacum	*Encyonema minutum*	*Encyonema morvanensis*	*Encyonema vandammeana*	*Encyonema brevicapitatum*	*Encyonema simile*	*Encyonema minutiforme*	*Encyonema fogedii*
	this study	this study	this study	this study	Krammer (1997a)	Krammer (1997a)	Krammer (1997a)	Krammer (1997a)
Type locality	Silesia, Poland	Silesia, Poland	eastern France	southern Belgium	Finland	northern Sweden	northern Norway	Spitsbergen
length (µm)	20-39	10-21	12-22	20.0-24.5	12-25	16-25	19-26	12-19
width (µm)	7.0-10.0	4.0-6.0	5.0-6.5	6.0-7.0	4.5-5.0	5.1-6.0	5.0-6.0	4.0-5.0
length/width ratio	2.8-4.1	2.5-3.6	2.2-3.5	2.9-3.6	max. 5	3.8-4.1	max. 4.3	max 3.8
valve outline	strongly dorsiventral with convex dorsal and straight to weakly convex ventral margin	strongly dorsiventral with convex dorsal and straight to weakly concave ventral margin	rather strongly dorsiventral, elongated, with convex dorsal and straight to very weakly convex ventral margin	rather strongly dorsiventral, elongated, with convex dorsal and very weakly convex ventral margin	dorsiventral, with convex dorsal and straight to weakly convex ventral margin	dorsiventral, narrowly elongated, with weakly convex dorsal and straight ventral margin	dorsiventral, narrowly elongated, with weakly convex dorsal and straight ventral margin	dorsiventral, with convex dorsal and straight to weakly concave ventral margin
apices	not to very weakly protracted, rostrate	not to weakly protracted, rostrate	usually protracted, rostrate, acutely rounded	clearly protracted, rostrate	clearly protracted, rostrate	not protracted, acutely rounded	not or very weakly protracted, acutely rounded	weakly protracted, rostrate
isolated pore	visible in LM	not discernible in LM	visible in LM	not discernible in LM	not discernible in LM	not discernible in LM	not discernible in LM	not discernible in LM
striae (in 10 µm)	14-15	18-22	12-14	14-16	14-17*	10-13*	12-16*	15-18*
areolae (in 10 µm)	25-32	35-40	30-35	ca. 35	28-32	24-28	27-30	37-40
areolae (in 10 µm)	discernible in LM	not discernible in LM	discernible in LM	weakly discernible in LM	weakly discernible in LM	discernible in LM	discernible in LM	almost not discernble in LM

*stria density differently measured, i.e. over the middle striae. Actual stria density most likely slightly higher.

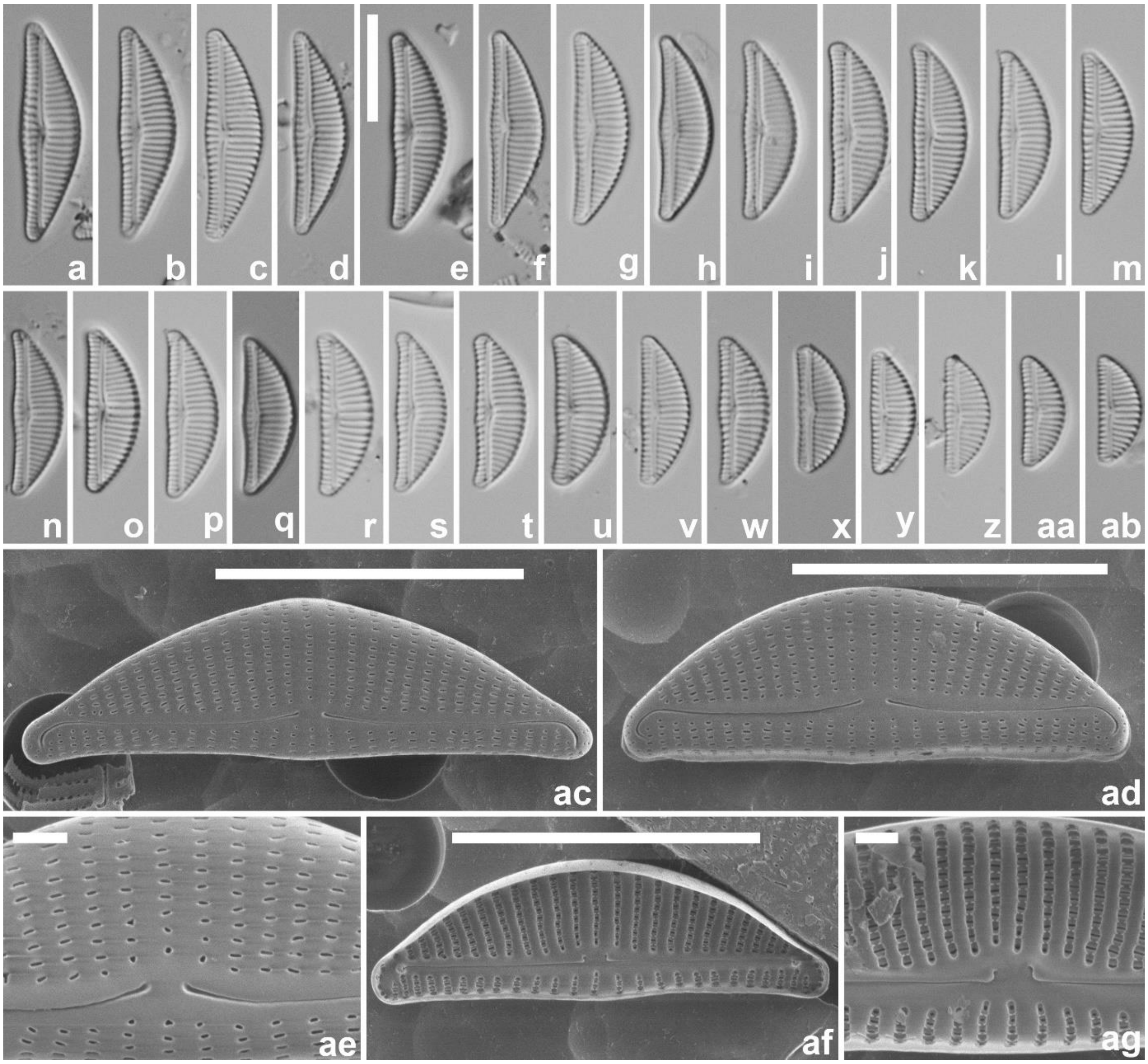
Figure Captions

Figure 1. *Encyonema minutum* (Hilse) Rabenhorst. LM and SEM images taken from the lectotype sample (Rabenhorst 1261, Bei Gerbendorf in Schlesien, coll. date summer 1861, leg. W. Hilse). Figs 1a–1ab. LM views of a size diminution series. Figs 1ac–1ad. SEM external view of two entire valves. Fig. 1ae. SEM external detail of the central area with the deflected central raphe endings. Note the absence of an isolated pore. Fig. 1af. SEM internal view of an entire valve. Fig. 1ag. SEM internal detail of the central area with the intermission, the central raphe endings, the absence of an isolated pore and the short siliceous struts. Scale bars represent 10 µm except for figs 1ae & 1ag where scale bar = 1 µm.

Figure 2. *Encyonema silesiacum* (Bleisch) Rabenhorst. LM and SEM images taken from the lectotype sample (Rabenhorst 1812, Brunnentrog des Gehöftes in Sedlitz bei Strehlen in Schlesien, coll. date November 1864, leg. E.J.Bleisch). Figs 2a–2s. LM views of a size diminution series. Fig. 2t. SEM external view of an entire valve. The arrow indicates the isolated pore. Fig. 2u. SEM external detail of the central area with the deflected central raphe endings. The external opening of the isolated pore is indicated with a white arrow. Fig. 2v. SEM external view of an entire valve in oblique view showing the dorsal margin. Fig. 2w. SEM internal detail of the central area with the intermission, the central raphe endings, the isolated pore, and the short siliceous struts. Fig. 2x. SEM internal detail of the valve apex with the helictoglossa. Scale bars represent 10 µm except for figs 2u, 2w & 2x where scale bar = 1 µm.

Figure 3. *Encyonema morvanensis* Van de Vijver & V.Peeters, sp. nov. LM and SEM images taken from the holotype sample (BR-4810, Yonne river, Amazy, France, sample 03024840, coll. date 28/06/2022, leg. V. Peeters). Figs 3a–3z. LM views of a size diminution series. Fig. 3aa. SEM external view of an entire valve. Fig. 3ab. SEM external detail of the central area with the deflected central raphe endings. The arrow indicates the external opening of the isolated pore. Fig. 3ac. SEM internal view of an entire valve. Fig. 3ad. SEM internal detail of the central area with the intermission, the central raphe endings, the isolated pore with the dentated margin and the short siliceous struts. Scale bars represent 10 µm except for figs 3ab & 3ad where scale bar = 1 µm.

Figure 4. *Encyonema vandammeana* Van de Vijver & Wilfert, sp. nov. LM and SEM images taken from the holotype sample (BR-4811, Masblette river, Nassogne, Belgium, sample BERW 03803, coll. date 13/05/2019, leg. Service Public de Wallonie). Figs 4a–4x. LM views of a size diminution series. Fig. 4y. SEM external view of an entire valve. Fig. 4z. SEM external detail of the central area with the deflected central raphe endings. Note the absence of the isolated pore. Fig. 4aa. SEM internal view of an entire valve. Fig. 4ab. SEM internal detail of the central area with the intermission, the central raphe endings, and the short siliceous struts. Scale bars represent 10 µm except for figs 4z & 4ab where scale bar = 1 µm.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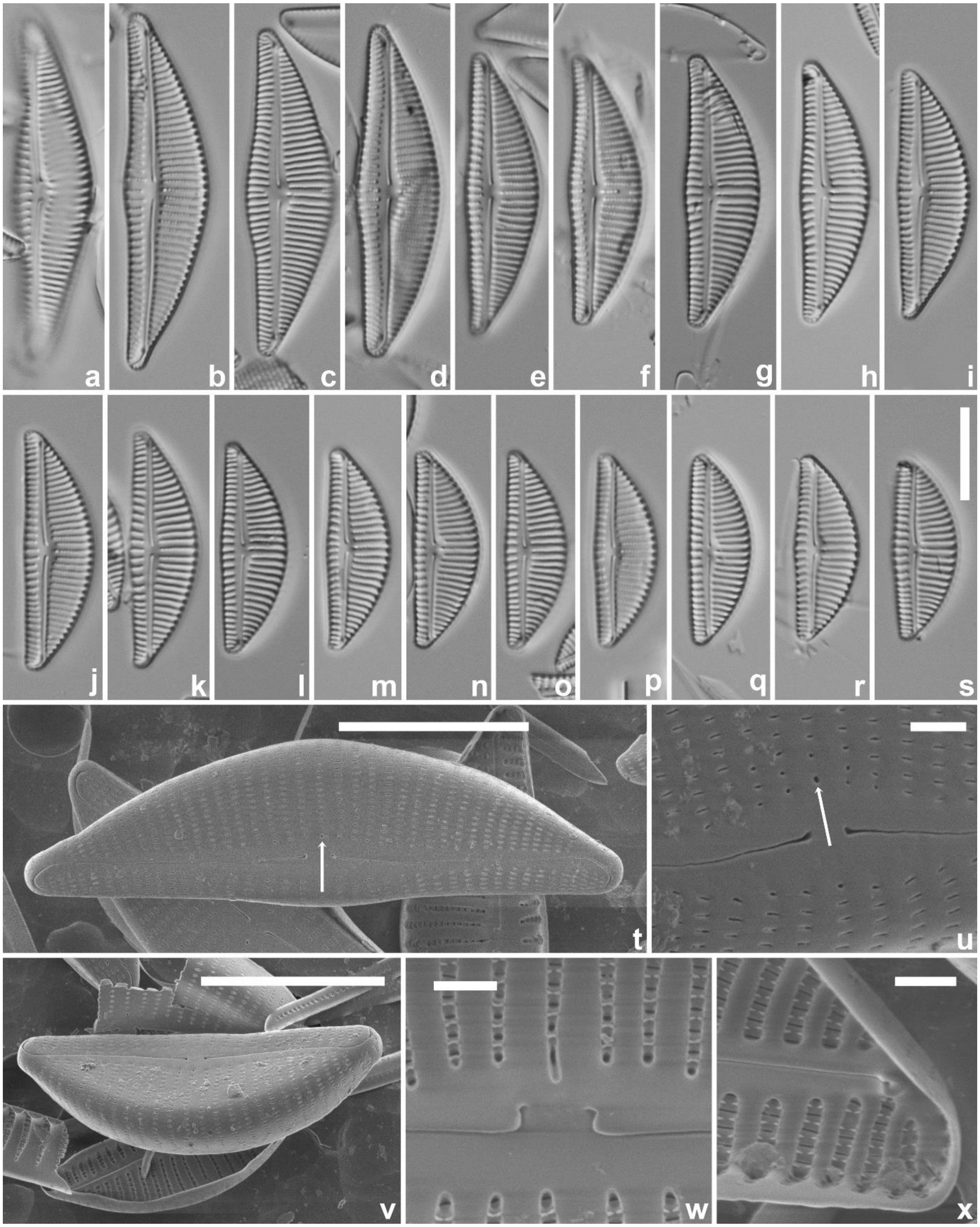
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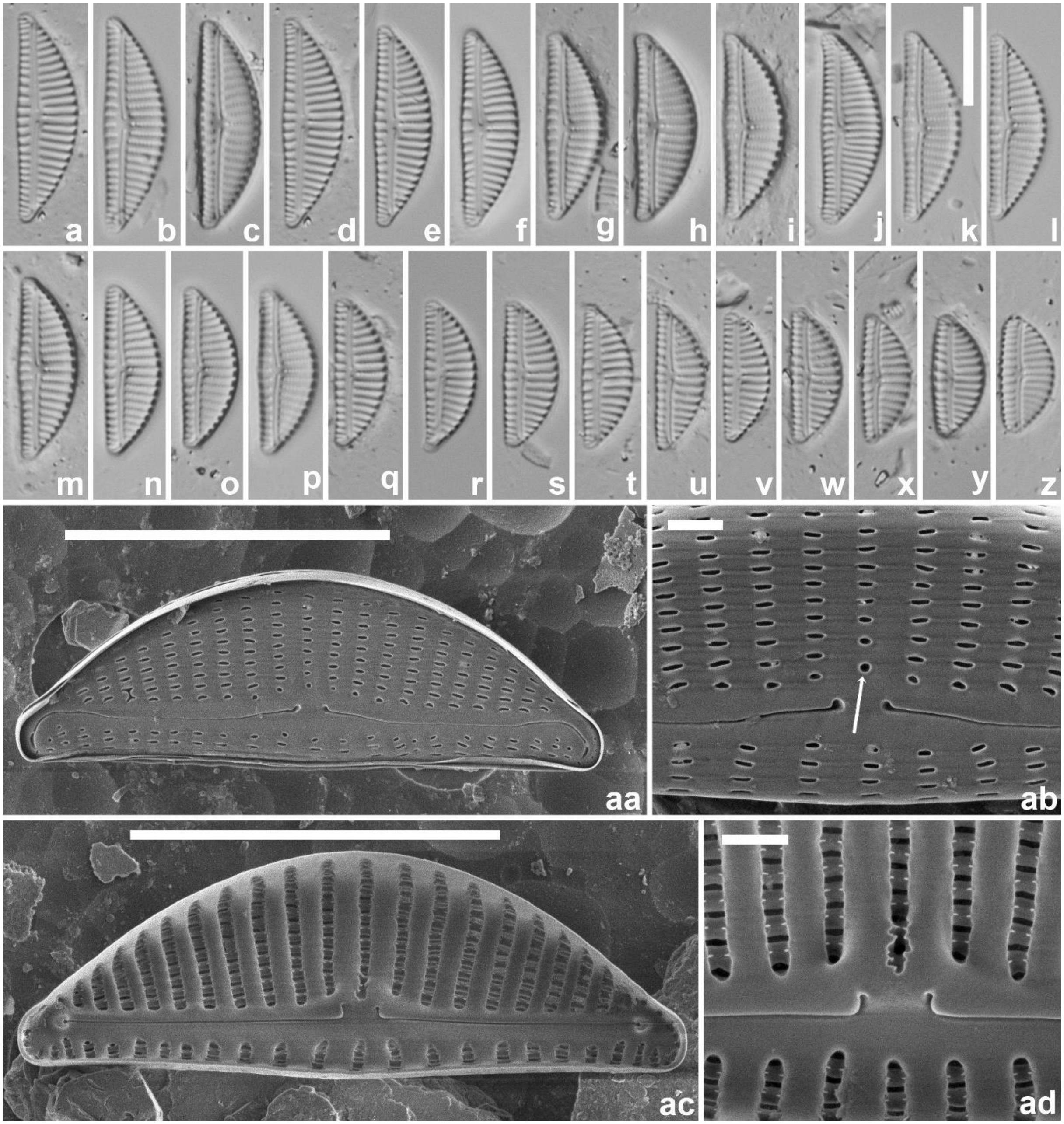
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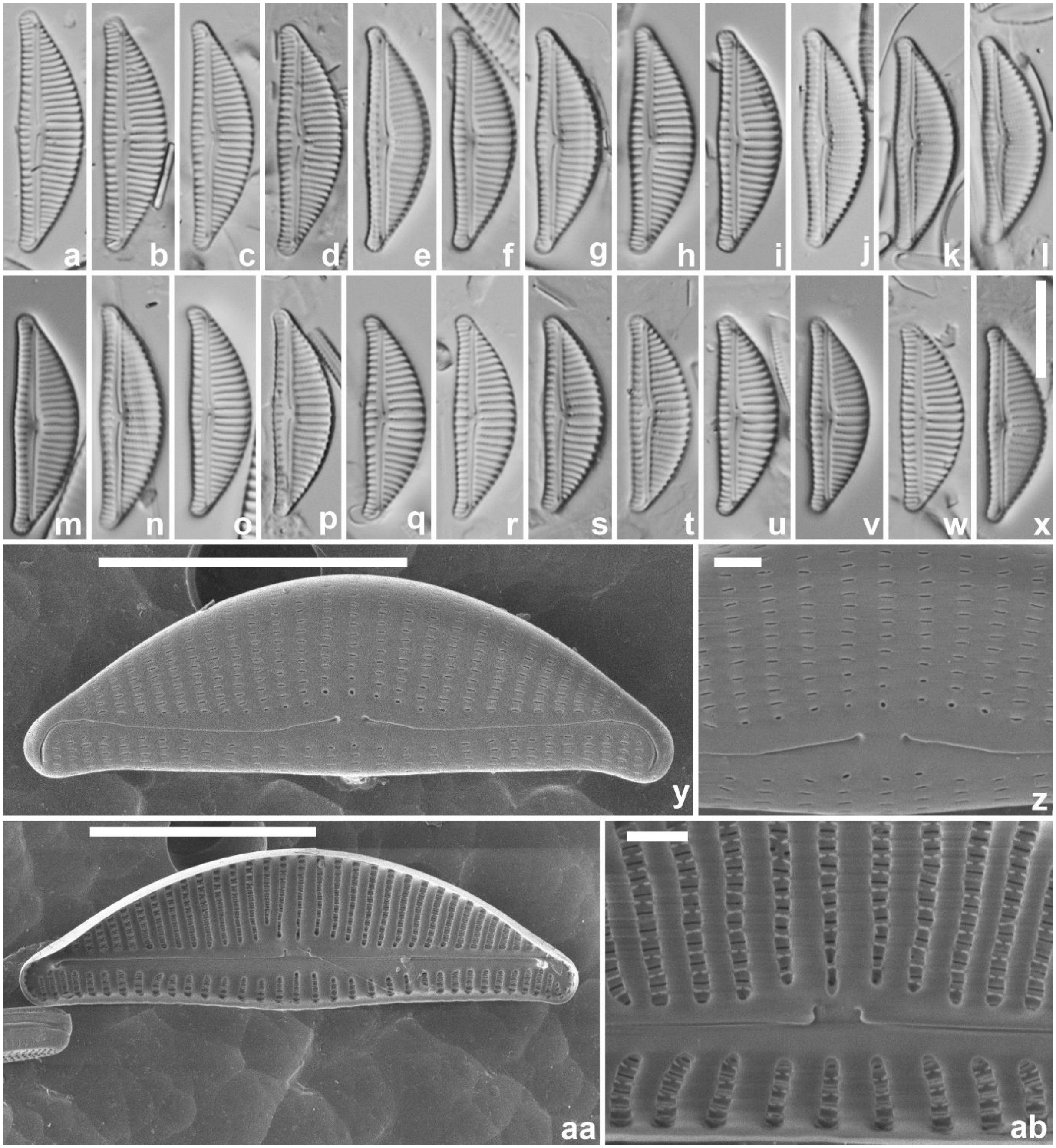
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