

RESEARCH ARTICLE

What constitutes a stigma? A review of isolated pores in raphid diatoms (Bacillariophyceae) and the value of precise terminology

 Eileen J. Cox¹  | Bart Van de Vijver^{2,3} 
¹The Natural History Museum, London, UK

²Meise Botanic Garden, Research Department, Meise, Belgium

³Department of Biology – ECOSPHERE, University of Antwerp, Wilrijk, Belgium
Correspondence
 Eileen J. Cox, The Natural History Museum, London SW7 5BD, UK.
Email: e.j.cox@nhm.ac.uk
Funding information

EU Synthesys grants, Grant/Award Number: GB-TAF-1100 and GB-TAF-2012

Editor: B. Beszteri

Abstract

Scanning electron microscopy has revealed variation in the ultrastructure of distinctive isolated pores in or near the central area of raphid diatoms, with different types of pores being restricted to phylogenetic groups. Thus, the widespread use of the term stigma for all such pores not only hides the structural diversity but also obscures the phylogenetic distribution of the different types. This paper provides images of the different types of isolated pores, particularly refining the discrimination of variants within the Cymbellales, and reveals some interesting ecological patterns. Revised definitions of stigmata and stigmoids are proposed, together with the recognition and definition of another type of stigmoid. The restricted distribution of more precisely defined pore types shows the importance of consistent use of terminology and its relevance to phylogenetic studies.

KEYWORDS

buciniportula, cuniculus, fistula, isolated pores, SEM, stigma, stigmoid, terminology

INTRODUCTION

Distinctive isolated pores, of contrasting structure to the areolae composing the striae, occur near the central raphe endings in several raphid diatom genera. Van Heurck (1885, p. 64) noted the presence of a peculiar structure (“granules isolés”) in the central area of several *Cymbella* species such as *C. neocistula* (“2 à 5 granules isolés”) and *C. tumida* (“un ou deux granules isolés”). However, the first comment recognizing their significance seems to have been published by Cleve (1894), who introduced the term stigma for the isolated pores in five *Cymbella* species (*C. australica*, *C. tumida*, *C. janischii*, *C. mexicana*, and *C. punctifera*): “In one division of *Cymbella* there is in the middle of the central nodule or on its ventral side a peculiar punctum or pore, for which I use the name stigma” (Cleve, 1894, p. 157). But he

(Cleve, 1894) discriminated this from one or more isolated pores at the ends of striae in some other *Cymbella* species such as *C. cymbiformis*, *C. neocistula*, *C. affinis*, *C. turgidula*, and *C. tumidula*. (“At the ventral side of the central nodule are two small puncta, ending the median striae” Cleve, 1894, p. 171.) Elsewhere, in his introduction to *Gomphonema* Ehrenb., Cleve (1894, p. 178) not only refers to the presence of “an isolated punctum or stigma” or a “unilateral row of stigmas” in many species (including *Gomphonema geminatum* = *Didymosphenia geminata*) but also uses the presence or absence of “stigmata” in his artificial key to *Gomphonema* species. Cleve (1894, p. 129, 130) had also noted the presence of an isolated pore on one side of the central nodule in *Navicula mutica* (= *Luticola mutica*), although this was structurally different to the isolated pores in the Cymbellales. Clearly, Cleve (1894) was limited in the discrimination of the finer

Abbreviations: LM, light microscopy; SEM, scanning electron microscopy.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Author(s). *Journal of Phycology* published by Wiley Periodicals LLC on behalf of Phycological Society of America.

structure of such pores, being solely dependent on nineteenth-century light microscopy, and could only use number and position for comparison. But the term stigma was already being applied to what we now know are several contrasting structures within the cymbelloid and gomphonemoid diatoms.

With the introduction of scanning electron microscopy, it became clear that the ultrastructure of isolated pores and stigmata varied significantly between genera (Krammer, 1979, 1997a, 1997b; Appendix S1 in the Supporting Information). Krammer (1979) used the position of the pores in relation to the dorsiventrality of the valves (dorsal vs. ventral) as a discriminating feature, although (in both cases) ontogenetically, the pore position was on the primary side of the valve. Three years later, Krammer (1982) created the term *stigmoid* for isolated pores in the genera *Encyonema*, *Encyonopsis*, and *Gomphocymbella* that lacked the convoluted (cracked/fissured, according to Krammer) internal structure seen in *Cymbella* sensu stricto and opened at the end(s) of striae rather than separately. However, the internal openings of what he (Krammer, 1982) came to recognize as stigmata were also at the ends of striae, and as ontogenetically both ventral and dorsal sides could be the primary side of the valve, that particular criterion separating stigmata and stigmoids is called into question. In 2002, Krammer (2002, plates 4, 5) illustrated the variation in stigma (sensu Krammer) structure within cymbelloid taxa, both in position on the valve and internal structure. This showed that both stigmata and stigmoids (sensu Krammer) could open near the ends of central striae, although all his examples of stigmata (Krammer, 2002) were generally more complex internally (with rugged or serrated margins according to Krammer). It was also clear that these structures needed more careful discrimination.

Ross et al.'s (1979) earlier definition of a stigma had recognized that the internal structure and position of stigmata could differ, but they did not discriminate them any further. They also provided a definition of a cuniculus, another feature in which a distinct isolated pore is observed in the external central area of some *Parlibellus* species, opening internally at the ends of the raphe fissures (Cox, 1978, 1988). Later, Round et al. (1990) used stigma(ta) to refer to the isolated pore(s) in a broad array of genera: *Cymbella*, *Didymosphenia*, *Gomphoneis*, *Gomphonema*, *Gomphocymbella*, *Luticola*, and *Proschkinia*. They noted that *Encyonema* does not possess a true stigma and remained vague over the isolated pore in *Reimeria*, calling it “an isolated pore (stigma)” (Round et al., 1990, p. 486, 492, 494, 496, 498, 500, 532, 596), probably following Kociolek and Stoermer (1987) who had termed it a stigma.

Hustedt (1962) termed the isolated pore near the central nodule of several *Navicula* Bory species, now transferred to *Proschkinia*, either a stigma, an isolated pore, or a mucilage pore (“Gallertporus”), the latter later being

observed to be structurally similar to the isolated pore in *Fistulifera*, termed *fistula* by Lange-Bertalot (1997). More recently, Riaux-Gobin and Compère (2009) described a new genus, *Olifantiella*, with an unusual internal structure that they termed a *buciniportula*, showing some similarity to the isolated pore of *Luticola*. A similar structure (simply called a stigma) had been observed in the earlier described genus, *Labellicula* (Van de Vijver et al., 2005), with the similarity between *Labellicula* and *Olifantiella* being later discussed by Van de Vijver et al. (2016). Both genera are currently recognized as taxonomically valid (Guiry & Guiry, 2024). The small valve dimensions of both genera made it initially difficult to study the ultrastructure of these isolated pores in detail, and it is only by using high-resolution scanning electron microscopy (SEM) that it has become possible to begin to understand these ultrastructures in these genera in more detail and to consider them in a phylogenetic and ecological context.

Molecular phylogenetic analyses, based on one or more genes, are increasingly being applied to diatoms, including representatives of some of the above genera (Abarca et al., 2023; Glushchenko et al., 2022; Jahn et al., 2019; Kermarrec et al., 2011; Kezlya et al., 2021; Kim et al., 2020; Kulikovskiy & Kociolek, 2014; Majewska et al., 2019; Nakov et al., 2014; Tuji, 2020; Yana et al., 2022). These analyses allow us to see to what extent particular morphological features are distributed across clades and to infer their potential evolution. It is, however, clear that how we refer to these contrasting structures is relevant to their integration into phylogenetic analyses. If the same term is used to refer to significantly different structures, this potentially generates erroneous interpretations of relationships when working from published literature without going back to original illustrations every time (Cox, 2012). Does the terminology reflect homology, and are there any potential correlations with their physiology or ecology? This paper provides an overview of the morphological variation of isolated pores in raphid diatoms and recommends the use of terms that appropriately document that ultrastructural variation and reflect potential homologies. However, although it may be possible to infer the type of isolated pore in a particular specimen from its position on the valve and other morphological features allowing taxonomic identification, the presence of a particular pore type can only be confirmed by SEM study.

MATERIALS AND METHODS

Specimen preparation and microscopy

Subsamples from various materials containing species with isolated pores were prepared following the method described in van der Werff (1955). Small

volumes of the samples were cleaned by adding 37% hydrogen peroxide and heating to 80°C for about 1 h. The reaction was completed by the addition of saturated potassium permanganate. Following digestion and centrifugation (three times for 10 min 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 1.65) for light microscopy (LM). The resulting slides were analyzed using an Olympus BX53 microscope at 1000x magnification (UPlan FL N 100x objective, N.A. 1.30), equipped with differential interference contrast (Nomarski) optics and the Olympus UC30 Imaging System, connected to the cellSens Standard program. For scanning electron microscopy, part of the suspension was filtered through 5- μ m Isopore™ polycarbonate membrane filters (Merck Millipore), pieces of which were fixed on aluminum stubs after air-drying, coated with a 20-nm platinum layer, and studied using a ZEISS ULTRA field emission scanning electron microscope at 2 kV (Natural History Museum London, United Kingdom). Slides and stubs are archived in the BR-collection (Meise Botanic Garden, Belgium). Plates were prepared using Adobe Photoshop CS5.

Terminology with particular reference to the Cymbellales

The two sides of dorsiventral valves were traditionally discriminated by shape, with the wider, more convex side being designated dorsal and the usually narrower, less convex to concave side being designated ventral. Raphe paths, striae, positions of isolated pores, and orientations of cell contents (nucleus, pyrenoid) were also described in relation to this. Thus, the terminal raphe fissures of *Cymbella* are deflected toward the dorsal side, those of *Encyonema* to the ventral side. The nucleus of *Cymbella* is located against the ventral side, that of *Encyonema* against the dorsal side. However, ontogenetically, based on the position of the nucleus (against the primary side of the valve), the ventral side of *Cymbella* and dorsal side of *Encyonema* both represent the primary sides of the valves (Mann, 1981). In ostensibly bilaterally symmetrical valves, primary and secondary sides can be discriminated by the position of the Voigt discontinuity and the terminal fissure curvature of the raphe slits (Mann, 1981, 2006). We have therefore related isolated pore structures to the primary or secondary sides of the valve.

We also discriminated between Cleve's original use of stigma (stigma sensu stricto in *Didymosphenia*, *Oricymba*, and some *Cymbella* spp.) and its broader application by Krammer (1979, across other *Cymbella*

spp.), adopting Krammer's (1982) term stigmoid for the structures in *Encyonema*, *Gomphonema*, *Gomphoneis*, *Kurtkrammeria*, *Afrocymbella*, *Gomphocymbellopsis*, *Reimeria*, *Placoneis*, and *Geissleria*, although this is discussed in more detail below. There has been a lack of consistency in the application of these terms by recent authors, with stigma often being used to refer to all the different types of isolated pores across all these genera, most likely because of the application of the word stigma by Round et al. (1990) for almost all the genera with isolated pores that were known in 1990 (*Cymbella*, *Didymosphenia*, *Gomphoneis*, *Gomphonema*, *Gomphocymbella*, *Luticola*, and *Proschkinia*; e.g., Abarca et al., 2020; Kociolek et al., 2018; Levkov et al., 2013; Liu et al., 2021; Reichardt, 2015; Stone et al., 2020; Zhang et al., 2020). However, the isolated pores in some *Gomphoneis* have more recently been referred to as stigmoids (Kociolek et al., 2013; You et al., 2013). It should also be noted that the current definitions of stigmata in glossaries of online diatom sites (www.diatoms.org, <https://museumwales.ac.uk>) are also rather broad and somewhat out of date.

The introduction of the terms fistula and bucinipor-tula followed SEM studies leading to the recognition of *Fistulifera* and *Olifantiella* as new genera, which showed that the internal structure of their isolated pores contrasted with that of stigmata and stigmoids and warranted new terms (Lange-Bertalot, 1997; Riaux-Gobin & Compère, 2009).

RESULTS

Light microscopy

The limits to resolution of light microscopy restrict the information it can provide to the number and position of any pores that appear different from those forming the striae. Thus, the stigmata of *Didymosphenia* and *Cymbella mexicana* are clearly located within or close to the central nodule (Figure 1), whereas in *Cymbella* sensu stricto and *Oricymba*, they lie at or very close to the central ends of the striae on the ventral (primary) side of the valve (Figure 2). In both cases, the number of pores can vary between species, for example, *Oricymba* (1), *D. geminata* (2–5), *C. mexicana* (1), *C. affinis* (1), *C. cymbiformis* (1–2), *C. turgidula* (2–3), *C. necocistula* (3–5), and *C. aspera* (7–10; Jüttner et al., 2010; Krammer, 2002; Metzeltin & Lange-Bertalot, 2014).

When present, the isolated pores (stigmoids sensu Krammer) in *Encyonema*, *Kurtkrammeria*, *Gomphonema*, *Afrocymbella*, *Gomphocymbellopsis*, and *Reimeria* are usually distinctly different from the stria areolae and/or clearly separated from the central striae (Figure 3) on the primary side of the valve. However, in some (former) *Gomphonema*

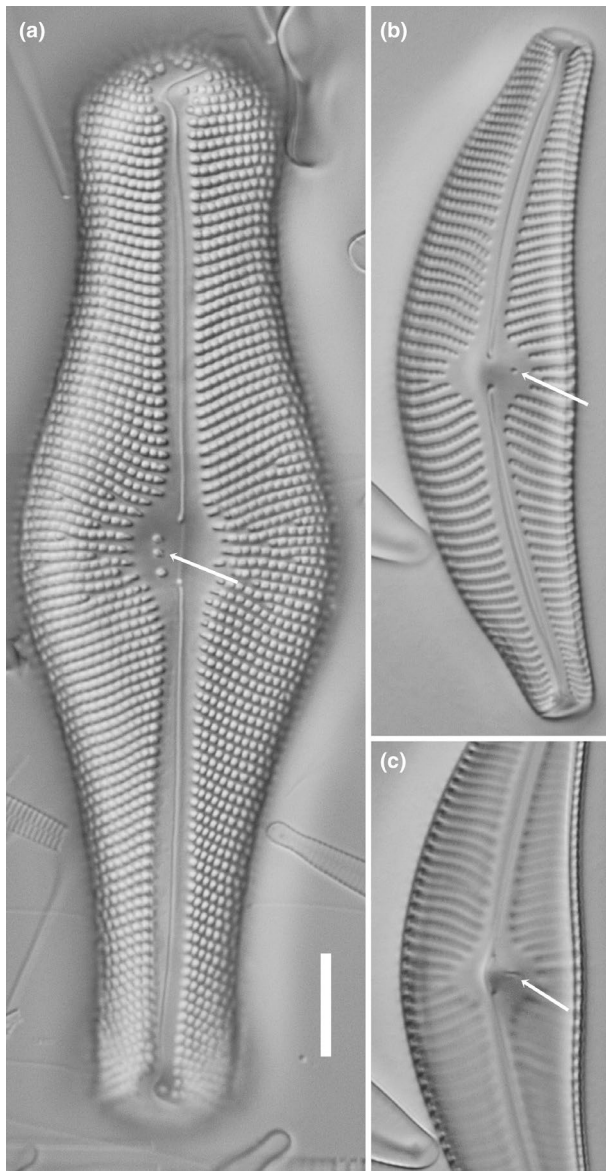


FIGURE 1 LM observations of taxa bearing a stigma. (a) *Didymosphenia geminata*. (b) *Cymbella tumida*, view of the valve exterior. (c) *Cymbella tumida*, view of the valve interior of the central area (different focal level). The arrows indicate the position of the stigma openings. Scale bar = 10 μ m.

or *Gomphoneis* species, now *Gomphonella* (Jahn et al., 2019), four stigmoids could be seen, one each at the ends of four striae abutting the central area (Figure 3d). However, it should be noted that in their definition of *Gomphonella*, Jahn et al. (2019) indicated that stigmata or stigmoids were absent (aside from the occasional few isolated puncta in the central area, set aside from the striae, Jahn et al., 2019; Abarca et al., 2023), although Tuji (2020) has suggested that *Gomphoneis tetrastigmata* (with stigmoids) belongs in *Gomphonella*. When present, the isolated pores (stigmoids) in species of *Geissleria*

and *Placoneis* were often paired and lay on the primary side of the central nodule, clearly separated from the central striae (Figure 3h,i). In *Brebbissonia*, a change from biseriate striae to single areolae was just visible beside the central area under LM and allowed their recognition as potential stigmata or stigmoids (Figure 3k,l).

Labellicula did not have an obvious isolated pore when seen with light microscopy, but there was asymmetry in the refractiveness of the valve beside the central nodule (Figure 4a). Isolated pores in *Fistulifera* (Figure 4b) and *Proschkinia* (Figure 4c) were usually only seen as more or less refractive dots near the central raphe endings, whereas in *Luticola* (Figure 4d,e) and *Olifantiella* (Figure 4f), they were more obviously positioned between the striae and central area. The more recently described genus *Luticolopsis* (Levkov et al., 2013), showing structural similarities with *Labellicula*, also had an isolated pore between the central area and a short central stria (see Levkov et al., 2013, plate 202). As in members of the Cymbellales, the isolated pores were on the primary side of the valve in *Fistulifera*, *Luticola*, and *Proschkinia*; however, in *Karthickia* and *Olifantiella*, they were on the secondary side of the valve. It was harder to determine the ontogenetic position of isolated pores in *Labellicula* and *Luticolopsis* due to the difficulty in identifying the position of the Voigt discontinuity or curvature of the terminal raphe endings. In *Parlibellus*, the isolated pore was within the central nodule.

Scanning electron microscopy

Stigmata sensu stricto

In external view under SEM, stigmata were seen as one or more unoccluded holes, within or to one side of the central area and the central raphe endings (Figure 5a,b,f). Internally, these opened as raised convoluted areas of silica on the primary side of the valve, often within the thickened central region of silica that overlay the internal central raphe endings, even if the external opening was more or less central within the central nodule (Figure 5c–e). With the use of a frustule cleavage method on *Cymbella janischii* (2021), Mayama and Mayama (2021) showed that that the convoluted internal surface was formed by radiating slits diverging from the simple external opening (figures 18, 21–23). The shape of the raised internal convoluted areas can be more or less circular in outline (*C. mexicana*, *Didymosphenia*: Figure 5c–e, and *C. janischii*: Mayama & Mayama, 2021, figure 18) or more elongated, as in, for example, *C. tumida*, extending onto the silica growth overlying the central raphe endings (Figure 5g).

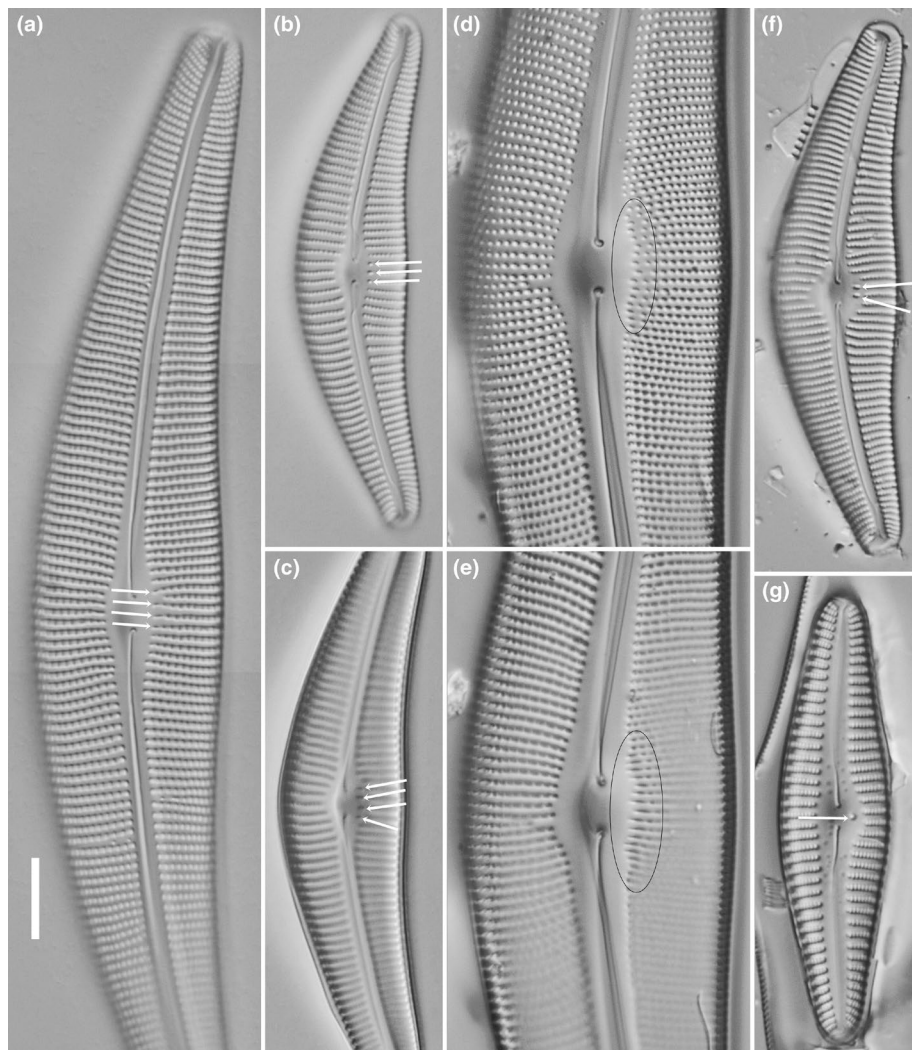


FIGURE 2 LM observations of taxa bearing a parastigma. (a) *Cymbella neolanceolata*. (b) *Cymbella neocistula*, view of the valve exterior. (c) *Cymbella neocistula*, view of the valve interior of the central area (different focal level). (d) *Cymbella aspera* view of the valve exterior. (e) *Cymbella aspera*, view of the valve interior of the central area (different focal level). (f) *Cymbella cymbiformis*. (g) *Oricymba* sp. The arrows indicate the position of the parastigma openings. The black circles on (e,f) show the position of the parastigmata. Scale bar = 10 μm .

Parastigmata (stigmata sensu Krammer 1979)

Externally, the pore openings in this group were usually simple and rounded, on the ventral (primary) side of the valve (Figure 6a,c,e). Internally, two types of parastigma could be distinguished: those in which the internal openings had irregular or dentate margins (Figure 6b,d) and those where the internal openings were simply elongate with smooth edges (Figure 6f). Krammer (1979) related their positions to the dorsiventrality of the valve, that is, as ventral, which in these taxa is the primary side of the valve. In both cases, the external openings were to one side of the central area, and the pores opened internally at the proximal ends of the central striae. In species such as *Cymbella neocistula*, *C. cymbiformis*, and *C. neolanceolata* the internal openings had somewhat dentate margins (Figure 6b); in others, such as *C. aspera* (Figure 6f), the internal

openings were more elongated than the usual areolae but had smooth margins. *Oricymba* species had internal openings with dentate margins (Figure 6d), as in the *C. neocistula* group (Jüttner et al., 2010; Krammer, 2002). Parastigmata with dentate margins were also present in some *Delicatophycus* species, for example, *D. williamsii* and *D. luiweii* (Liu et al., 2018, 2021).

Stigmoids—sensu Krammer

Stigmoids, as defined by Krammer (1982), are simpler isolated pores, lacking the rounded, convoluted internal closure of stigmata, and they have their internal openings at the end of a central stria. However, more recent use of the term stigmoid has deviated from Krammer's original use with respect to the position of the internal opening, although all

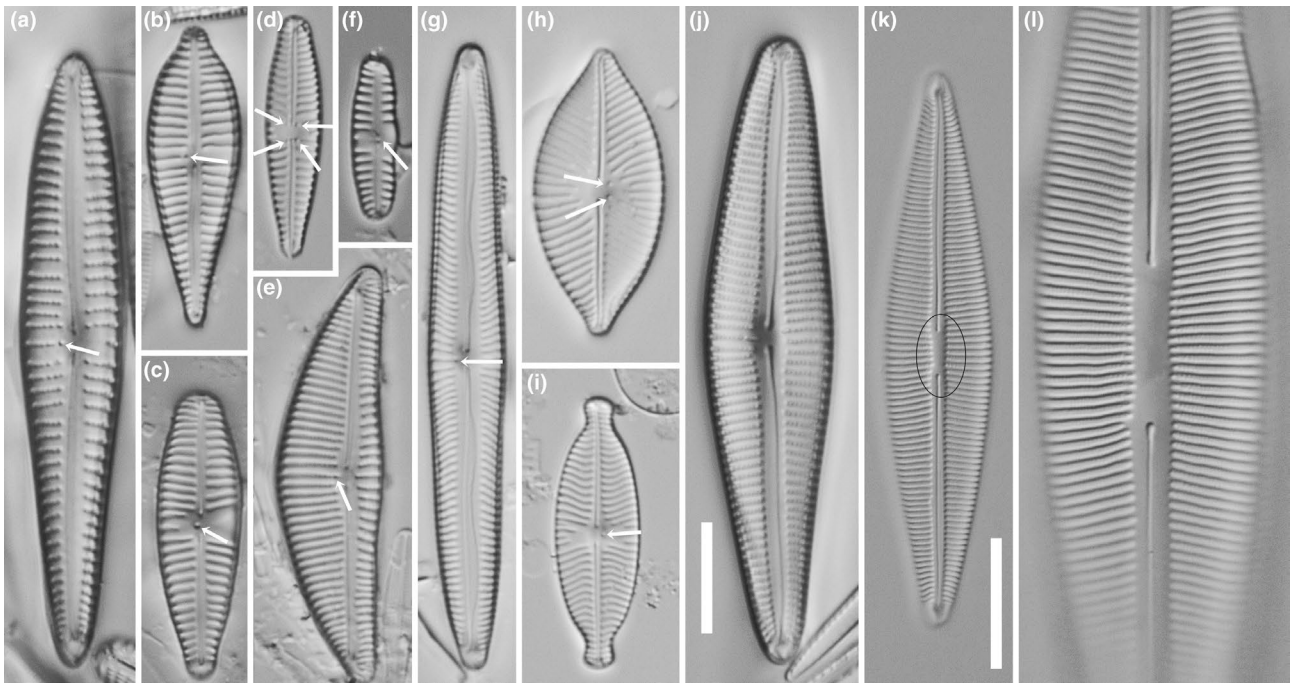


FIGURE 3 LM observations of taxa bearing a stigmoid. (a) *Gomphonema* sp. (b) *Gomphonema pseudoaugur* (c) *Gomphonema tergestinum*. (d) *Gomphonella* (e) *Encyonema*. (f) *Reimeria sinuata*. (g) *Kurtkammeria neoamphioxys*. (h) *Placoneis clementispronina*. (i) *Geissleria decussis*. (j) *Afrocybella barkeri*. (k) *Brebissonia lanceolata*. (l) *Brebissonia lanceolata*, detail of the central area. The arrows indicate the position of the stigmoid openings. The black circles on (k) show the position of the parastigmata. Scale bar = 10 μm except for (k) where scale bar = 20 μm .

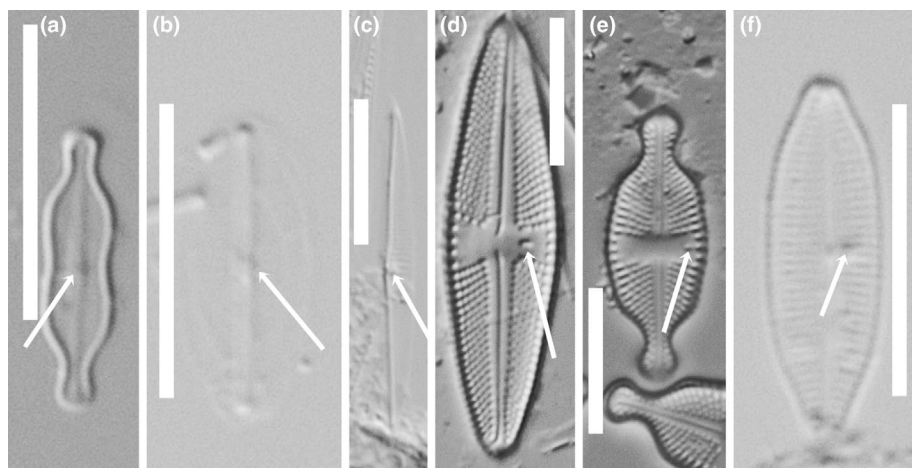


FIGURE 4 LM observations of taxa bearing a fistula (a–c) or a buciniportula (d–f). (a) *Labellicula subantarctica*. (b) *Fistulifera pelliculosa*. (c) *Proschkinia* sp. (d) *Luticola goeppertiana* (e) *Luticola ventricosa*. (f) *Olifantiella muscatinei*. The arrows indicate the position of the fistula (a–c) and buciniportula (d–f). Scale bars = 10 μm .

stigmoids lack any kind of convoluted or dentate siliceous ingrowths. Whereas stigmoid external openings were almost always round (Figures 7a,b,f and 8a,c,e,g), the internal openings varied in shape (Figures 7c–e, g, h and 8b,f,h): round in *Encyonema*, some *Gomphonema*, *Gomphonella* (according to Tuji, 2020), *Gomphocymbellopsis*, *Placoneis*, and *Geissleria* (Figure 8g,h); slit-like in *Kurtkammeria*, some *Gomphonema*, *Gomphadelphia* (although the

authors refer to these as stigmata), and *Afrocybella* (Figure 7d); and slightly elongate in *Reimeria* (Figure 8f). Single (occasionally two) stigmoids were observed on the primary side of the valve only, regardless of the dorsiventrality. External positions varied, not only sometimes clearly lateral to the central raphe endings, for example, *Reimeria* (Figure 8e). However, internal openings were always more

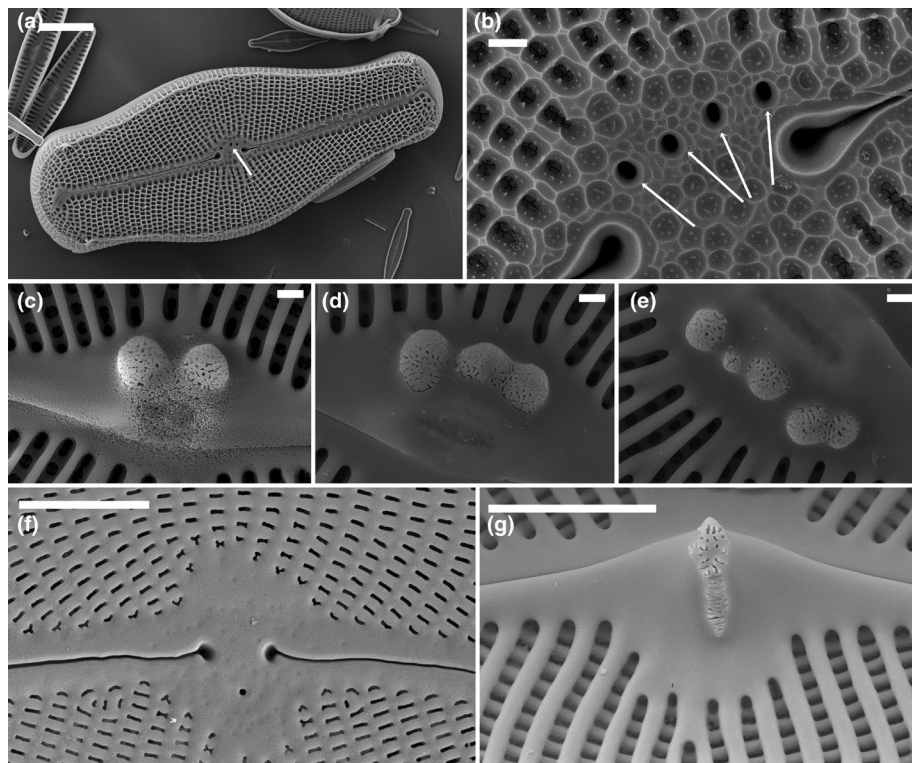


FIGURE 5 SEM observations of taxa bearing a stigma. (a) *Didymosphenia geminata*, external view of an entire valve. The arrow indicates the position of the stigmata. (b) *Didymosphenia geminata*, external view of the central area. The arrows indicate the external openings of the stigmata. (c–e) *Didymosphenia geminata*, three views of the stigmata in the valve interior indicating the variation in stigma numbers. (f) *Cymbella tumida*, external detail of the central area with rounded opening of the stigma. (g) *Cymbella tumida*, internal detail of the stigma. Scale bar = 10 μm (a), 5 μm (f and g), 1 μm (b–e).

displaced toward the primary margin, suggesting a tubular connection between the exterior and interior. In *Afrocymbella*, *Kurtkrammeria*, *Gomphodelpha*, and most *Gomphonema* (but see below), the internal opening was usually clearly slit-like and transapically orientated, varying in length between species (Figure 7c,d), although it could appear more rounded depending on the angle of view (Figure 7e). The slit-like internal opening often lay midway between the laterally deflected, central raphe endings, but in others species, it terminated at one of the central striae. This resembles the situation in *Encyonema*, where a stigmoid could appear as a slightly more distinct, more rounded pore at the end of a central stria (Figure 7f), internally opening as a larger, more elongate pore terminating that stria (Figure 7g). The internal openings of a few *Gomphonema* spp., for example, *G. angustum*, *G. angustius*, *G. paratergestinum*, *G. supertergestinum*, and *G. tergestinum*, were more or less circular but surrounded by small knobs of silica creating a flower-like appearance (Figure 7h).

Following the transfer of taxa in the *Gomphoneis tetrastigmata* species complex to *Gomphonella* (Tuji, 2020), some members of this genus are now considered to have four circular “stigmoids” at the central ends of biseriate striae close to both sides of the central raphe endings (Figure 8a,b). Internally, these

may have irregular depressions or teeth-like projections around their openings or are marked by one or a few transverse bars across the striae proximal to the central area (Figure 8b; Tuji, 2020, Figure 4a–d). However, the original description of *Gomphonella* (for species around *Gomphonema olivaceum*) stated that “there are no stigmoids or stigmata present on the valve face” (Jahn et al., 2019, p. 227), although there was a transition from biseriate to uniseriate striae in four striae approaching the central area, with the last areola in these striae often being slightly larger, more stigmoid-like (Jahn et al., 2019, figures 5A, 8B, 8H, 9B, 9I, and 12G).

Rexlowea was separated from *Placoneis* in part because it did not have stigmoids (although not all *Placoneis* spp. have stigmoids), but the pores at the central ends of the striae around the central area are often larger and more distinct externally and elongated internally (Kocielek & Thomas, 2010, figures 14, 21). This is also the case for the rarely illustrated *Brebissonia lanceolata*, which possesses stigmoids on both sides of the central nodule (Mahoney & Reimer, 1986). In this taxon, the stigmoids open externally by transapical slits, perpendicular to the central area in contrast to the areolae in the biseriate parts of the striae, which are aligned parallel to the long axis of the valve (Round et al., 1990: 488 figure c, figure 8c). Internally, the stigmoids open as

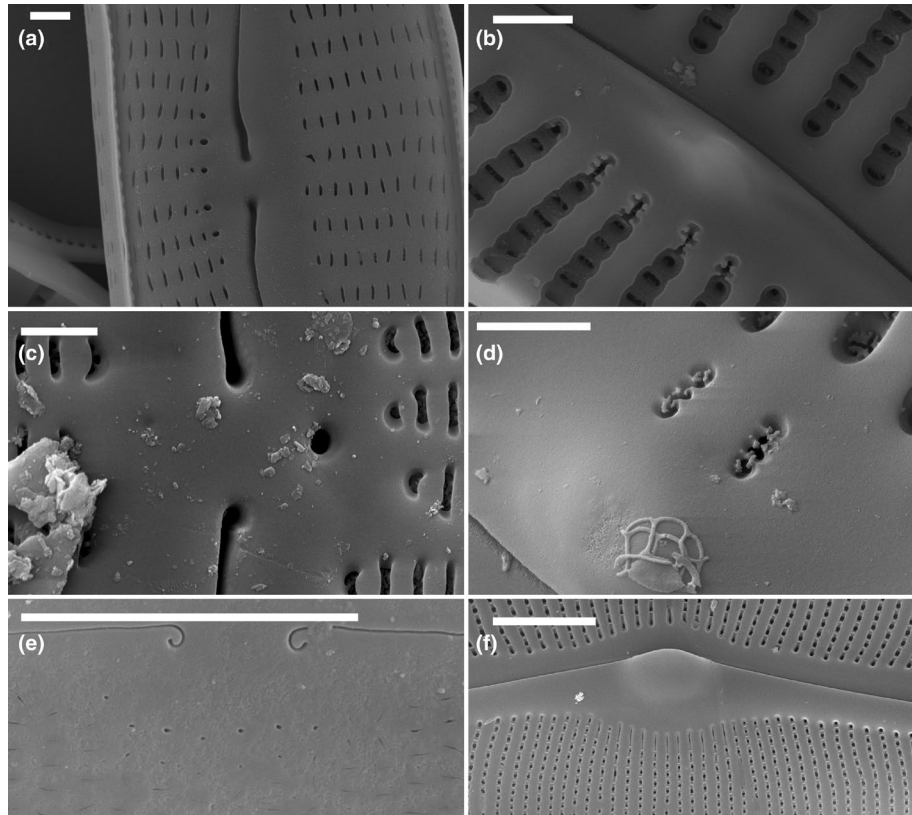


FIGURE 6 SEM observations of taxa bearing a parastigma. (a) *Cymbella neocistula*, external view of the central area showing the external openings of the parastigmata. (b) *Cymbella neocistula*, internal view of the central area showing the dentate openings of the parastigmata. (c) *Oricymba* sp., external detail of the central area with the large, rounded opening of the parastigma. (d) *Oricymba* sp., internal detail of the central area with the dentate openings of the parastigmata. (e) *Cymbella lanceolata*, external detail of the central area with the numerous very small, rounded openings of the parastigma. (f) *Cymbella aspera*, internal detail of the central area with the numerous slit-like openings of the parastigmata. Scale bar = 5 μm (e), 1 μm (a–d, f).

slightly elongate pores with smooth margins terminating the biseriate central striae alongside the central nodule (Round et al., 1990, p. 488 fig. g, figure 8d, arrows).

Stigmoids—*Karthickia*

Unlike the above taxa, the recently recognized cymbeloid genus *Karthickia* (Glushchenko et al., 2019) has a stigmoid on the secondary side of the valve with a more complex structure, showing a single external opening splitting to open internally at the ends of two striae (Yana et al., 2022, figures 20–22, 25, 27–31).

Fistulae

Lange-Bertalot (1997) defined a fistula based on the structure in *Fistulifera*, which has a simple, short slit externally (Figure 9a), with a raised, often almost hemiglobular, hymenate internal closure (Figure 9b). A similar structure was observed in *Proschkinia*, although the external opening in this genus was usually a longer slit, orientated along the apical axis and occasionally

somewhat hidden by a ridge of silica. Internally, there may be one or a series of knob-like, hymenate occlusions arranged perpendicular to the apical axis (Figure 9c,d). *Labellicula* has shown a similar structure, with internally two hemiglobular structures (Majewska et al., 2017, Van de Vijver et al., 2005; Figure 9e,f). Like the stigmata and stigmoids in the Cymbellales (except *Karthickia*), fistulae were observed on the primary side of the valve.

More recently, a similar structure with paired internal openings has been illustrated for *Luticolopsis vietnamica* (Levkov et al., 2013, plate 202: figures 22–25; plate 203: figures 5). This taxon has been considered to have a similar valve structure to *Luticola* (Levkov et al., 2013), but forms chains of cells, with the isolated pore displaced toward the margin of the valve, still opening to the exterior even when cells were linked as a chain. Its taxonomic position probably requires reassessment, possibly closer to *Labellicula* than *Luticola*.

Buciniportulae

Buciniportulae were first described in species of *Olifantiella* (Riaux-Gobin & Compère, 2009) as

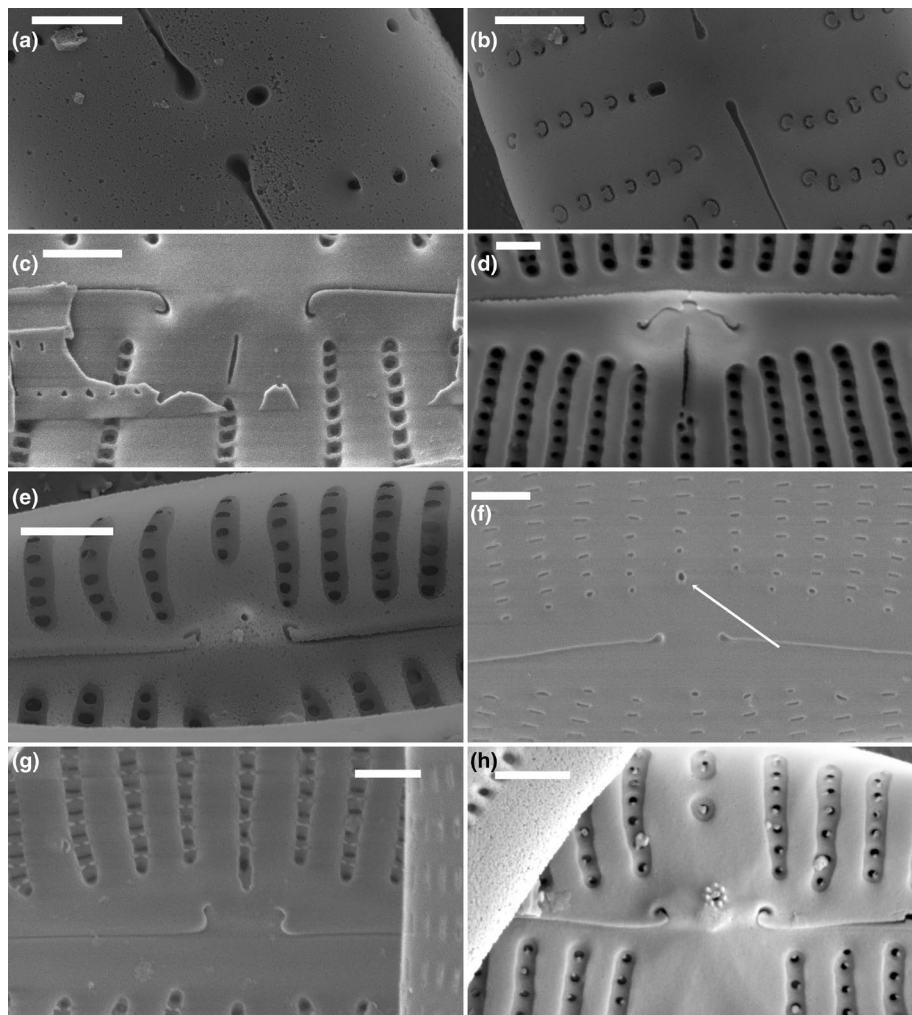


FIGURE 7 SEM observations of taxa bearing a stigmoid. (a) *Gomphonema* sp., external view of the central area showing the isolated external opening of the stigmoid, almost between the central raphe endings. (b) *Gomphonema* cf. *auritum*, external view of the central area showing the external opening of the stigmoid, at the end of a slightly shortened stria in the central area. (c) *Gomphonema* sp., internal detail of the central area with the slit-like opening of the stigmoid. (d) *Afrocybella barkeri*, internal detail of the central area with the slitlike opening of the stigmoid. (e) *Gomphonema* sp., internal detail of the central area showing the isolated internal opening of the stigmoid, almost between the central raphe endings. (f) *Encyonema silesiacum*, external detail of the central area with the rounded opening of the stigmoid (arrow). (g) *Gomphonema* cf. *pumilum*, internal detail of the central area showing the internal opening of the stigmoid, at the end of the middle stria. The stigmoid is located in a shallow depression. (h) *Gomphonema tergestinum*, internal detail of the central area with the more or less circular opening of the stigmoid, surrounded by small knobs of silica creating a flower-like appearance. Scale bar = 1 μ m.

structures with a simple external elongate opening connected to an internal tubular process with a flap-like occlusion (Figure 10a,b). The length of the tubular process differed between species ranging from very long (see, for instance, Riaux-Gobin & Al-Handal, 2012, figures 8–10; Riaux-Gobin & Compère, 2009, figure 18) to very short (Figure 10b). Other *Olifantiella* species have been described in which the internal opening is paired (e.g., *O. rodriguensis* Riaux-Gobin & Al-Handal, 2012, figures 20–21). Similarities have been drawn with *Luticola* (Riaux-Gobin & Compère, 2009, p. 184) because it has, internally, a raised, lipped, flap-like occlusion linked to a simple external opening, lacking, however, a protruding

tubular structure (Figure 10c,d). As in *Olifantiella*, the flap-like occlusion was very finely perforated, almost having a hymenate structure (Figure 10d). Mayama and Kobayasi (1986, figures 18–20, 44 and 45) described the internal occlusion as a “thin siliceous layer connected to the basal siliceous layer,” which represents exactly the same structure as in *Olifantiella* without the protruding tubular process (Mayama & Kobayasi, 1986, p. 175).

Although it can often be difficult to determine the primary and secondary sides of the valves in *Olifantiella* and *Luticola*, buciniportulae seem to be located on the secondary side of the valves, unlike stigmata and stigmoids, always present on the primary valve side.

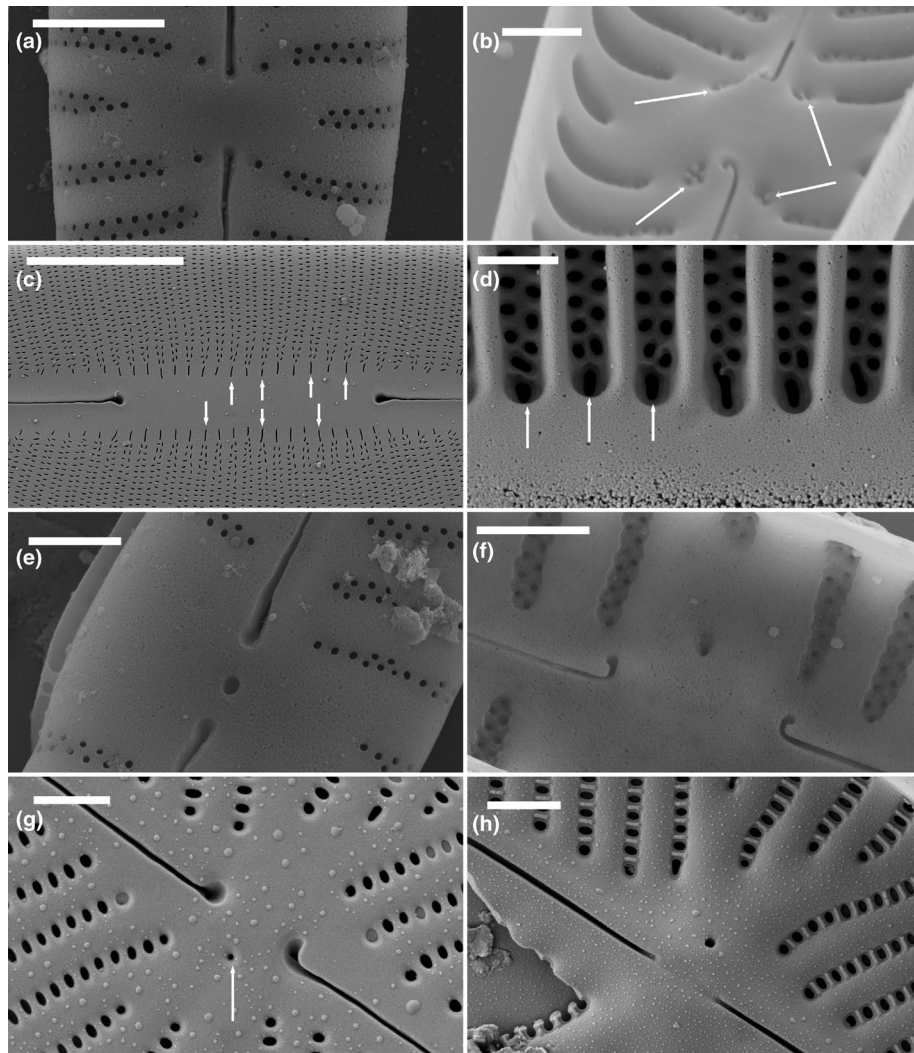


FIGURE 8 SEM observations of taxa bearing a stigma. (a) *Gomphonella* sp., external view of the central area showing the four small external openings of the stigmoids, at the end of the central striae (b) *Gomphonella* sp., internal view of the central area showing the four small internal openings of the stigmoids, indicated by the arrows (c) *Brebissonia lanceolata*, external detail of the central area with the slit-like openings of the stigmoids (arrows) bordering the axial area. (d) *Brebissonia lanceolata*, internal detail of the central area showing some of the internal stigmoid openings (arrows). (e) *Reimeria sinuata*, external detail of the central area showing the isolated rounded external stigmoid opening, located between the central raphe endings. (f) *Reimeria sinuata*, internal detail of the central area showing the isolated rounded external stigmoid opening. (g) *Geissleria decussis*, external detail of the central area showing the small rounded stigmoid opening of the stigmoid (arrow), near the central raphe endings. (h) *Geissleria decussis*, internal detail of the central area showing the rounded stigmoid opening of the stigmoid. Scale bar = 1 μ m.

Cuniculus

The term cuniculus was applied to the structure observed at the center of two *Parlibellus* species, *P. delognei* and *P. rhombicus*, which were illustrated by Cox (1978: figure 8–10, 16–22; 1988: figures 34, 36, and 38). This structure appeared as a simple external pore (occasionally two pores) at the center of the valve between the central raphe endings (Figure 10e) but had longitudinal perforated (hymenate) ridges terminating the raphe endings internally (Figure 10f; Cox, 1978: figures 8–10, 1988: figure 36).

DISCUSSION

It is clear that terminology around isolated pores, particularly for species within the Cymbellales has been confused (Cox, 2012), with some authors using stigma(ta) regardless of any variation in structure (Abarca et al., 2023; Van de Vijver et al., 2005), whereas other authors recognize differences in structure and use stigma, at least for some genera (Jahn et al., 2019). This, of course, assumes that ultrastructural details have been determined, and we suggest that if specimens have only been observed

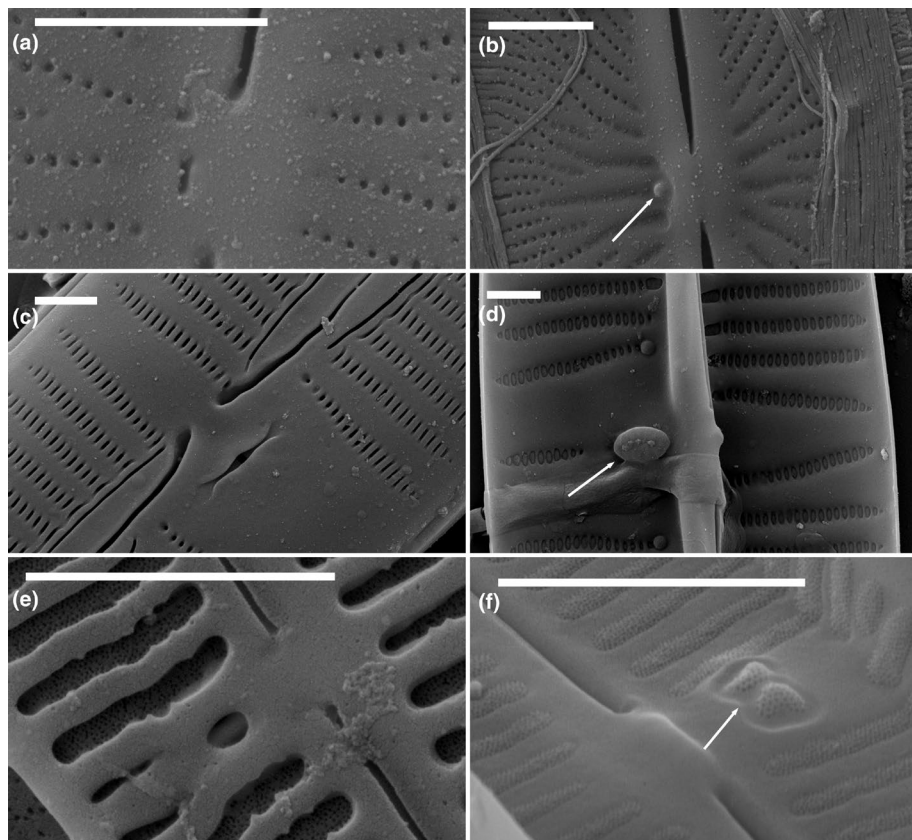


FIGURE 9 SEM observations of taxa bearing a fistula. (a) *Fistulifera pelliculosa*, external view of the central area showing the slit-like opening of the fistula between the central raphe endings. (b) *Fistulifera pelliculosa*, internal view of the central area showing the fistula, presenting the raised, almost hemiglobular, hymenate internal closure. (c) *Proschkinia* sp., external detail of the central area with the large slit-like opening of the fistula. (d) *Proschkinia* sp., internal detail of the central area with the knob-like occlusion of the fistula (arrow). (e) *Labellicula subantarctica*, external detail of the central area with the large opening of the fistula. (f) *Labellicula subantarctica*, internal detail of the central area with the double hemiglobular coverings of the fistula. Scale bar = 1 μ m.

with LM the neutral term “isolated pore” should be used. Although some homology between the different types of isolated pores has been assumed, molecular analyses that have included cymbelloid taxa (Abarca et al., 2023; Glushchenko et al., 2022; Jahn et al., 2019; Kermarrec et al., 2011; Kezlya et al., 2021; Kulikovskiy & Kocielek, 2014; Nakov et al., 2014; Tuji, 2020; Yana et al., 2022) have revealed that different stigmata and stigmoid types fall in different clades (Figure 11 is based on a tree in Nakov et al., 2014 because this most clearly shows the relationship between several cymbelloid taxa and isolated pore structure. Later trees—using the same sequences—do not conflict with this).

Thus, *Didymosphenia*, *Cymbella janischii*, and *C. mexicana* with stigmata sensu stricto consistently fall in the same clade, sometimes with *C. proxima* and *C. stuxbergii* (with dentate stigmata sensu Krammer) as a sister clade between *Didymosphenia* + *C. janischii* + *C. mexicana* and *C. tumida* (Nakov et al., 2014, figures 3–5, S1). Other taxa with dentate stigmata sensu Krammer, for example, *C. affinis*, *C. neocistula*, *C. cymbiformis*, *C. helvetica*, and *C. neolan- ceolata* group together but separate from a clade

with smooth stigmata sensu Krammer, for example, *C. aspera* and *C. baikalaspera*, which lie closer to *Cymboplectra* spp. (Nakov et al., 2014, figures 3–5, S1), which do not possess stigmata or stigmoids. We, therefore, suggest that these three types are different and propose that *stigma* be restricted for the isolated pores in *Didymosphenia*, *C. janischii*, *C. mexicana*, and *C. tumida*, in which the internal openings have raised, highly convoluted coverings. We propose a new term, *parastigma* (=stigma sensu Krammer), for the pores in most *Cymbella* species, with a relevant preceding descriptor. Thus, where the internal openings have teeth-like projections, we call these dentate parastigmata; where the internal openings do not have these projections, we call these smooth parastigmata. *Oricymba* spp., for instance, possess dentate parastigmata using this terminology. It should also be noted that *Oricymba* is unusual in that there were two internal parastigma openings but a single simple external one. We presume that there is bifurcation of the connecting external–internal tube, but this requires confirmation.

A potential variant “stigma” has been shown in the recently established genus *Gomphosinica*, in

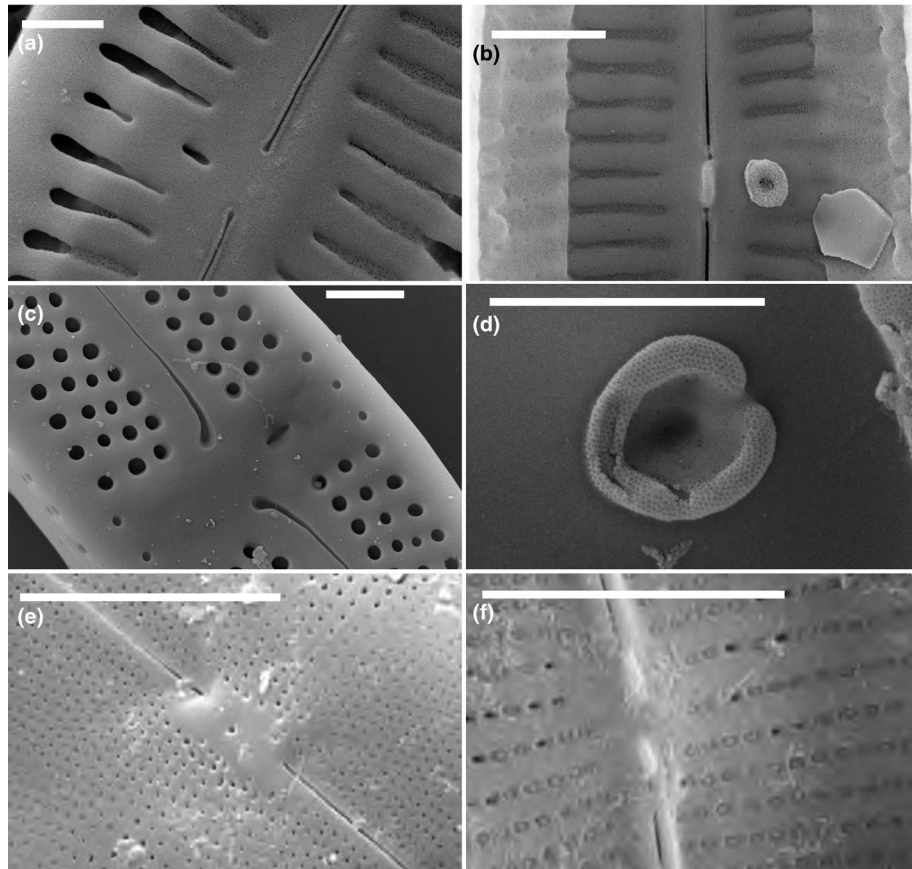


FIGURE 10 SEM observations of taxa bearing a buciniportula or a cuniculus. (a) *Olifantiella muscatinei*, external view of the central area showing the isolated slit-like opening of the buciniportula. (b) *Olifantiella muscatinei*, internal view of the central area showing the trumpet-like tubular buciniportula. (c) *Lutica denisei*, external detail of the central area with the large, isolated slit-like opening of the buciniportula. (d) *Lutica denisei*, internal detail of the finely perforated occluded process of the buciniportula. (e) *Parlibellus* sp., external detail of the central area with the small opening of the cuniculus. (f) *Parlibellus* sp., internal detail of the central area with the longitudinal ridges at the end of the central raphe endings. Scale bar = 10 µm (e), 5 µm (f), and 1 µm (a–d).

which the internal opening formed a perforated mound rather than having a more convoluted surface (Kociolek et al., 2015, figures 30, 45, 61, 82, 99, 110, 131). Although Kociolek et al. (2015, figure 133) presented an “evolutionary scenario of phylogenetic relationships of freshwater gomphonemoid diatoms” (p. 178), this genus has not been included in any molecular analyses, and its “stigma” may not be homologous with a stigma sensu stricto. Unlike all other taxa with stigmata or parastigmata, *Gomphosinica* does not have hidden internal central raphe endings and may be a member of the Gomphonemataceae rather than the Cymbellaceae in which true stigmata are seen. All *Cymbella* and *Didymosphenia* taxa are separated from *Encyonema*, *Gomphonema*, *Reimeria*, *Placoneis*, and *Geissleria* spp. with isolated pores (when present) that would be considered stigmoids (Glushchenko et al., 2022; Jahn et al., 2019; Kermarrec et al., 2011; Kezlya et al., 2021; Kulikovskiy & Kociolek, 2014; Nakov et al., 2014; Yana et al., 2022), while *Gomphonella* species form a clade of their own (Jahn et al., 2019; Tuji, 2020; Yana et al., 2022), separated from *Gomphonema*, *Encyonema*, *Reimeria*,

Placoneis, and *Geissleria*. When stigmoids are present in *Placoneis* and *Geissleria*, they open internally as simple round pores, immediately below their external openings, whereas in *Encyonema*, *Gomphonema* and *Reimeria*, the internal stigmoid openings can vary from almost round to elliptical to slit-like, with some taxa having characteristically long internal slits, for example. *Afrocymbella* and *Afrocymbella* have not been included in any molecular phylogenies to date, so their phylogenetic relationships to other stigmoid-bearing taxa remain unknown. *Reimeria* forms a sister clade to some *Gomphonema* species. Although external stigmoid openings in *Gomphonema* are usually lateral (primary side) to the central raphe endings, they open almost centrally in *Reimeria*, between the raphe endings, although the internal openings are more lateral on the primary side.

Although the distribution of different stigma, parastigma, and stigmoid types across taxa in the phylogenetic trees generally matches the clades including those taxa, some *Cymbella* spp. lack parastigmata, while stigmoids are not invariably present in all *Encyonema*, *Placoneis*, and *Geissleria* species. Additionally,

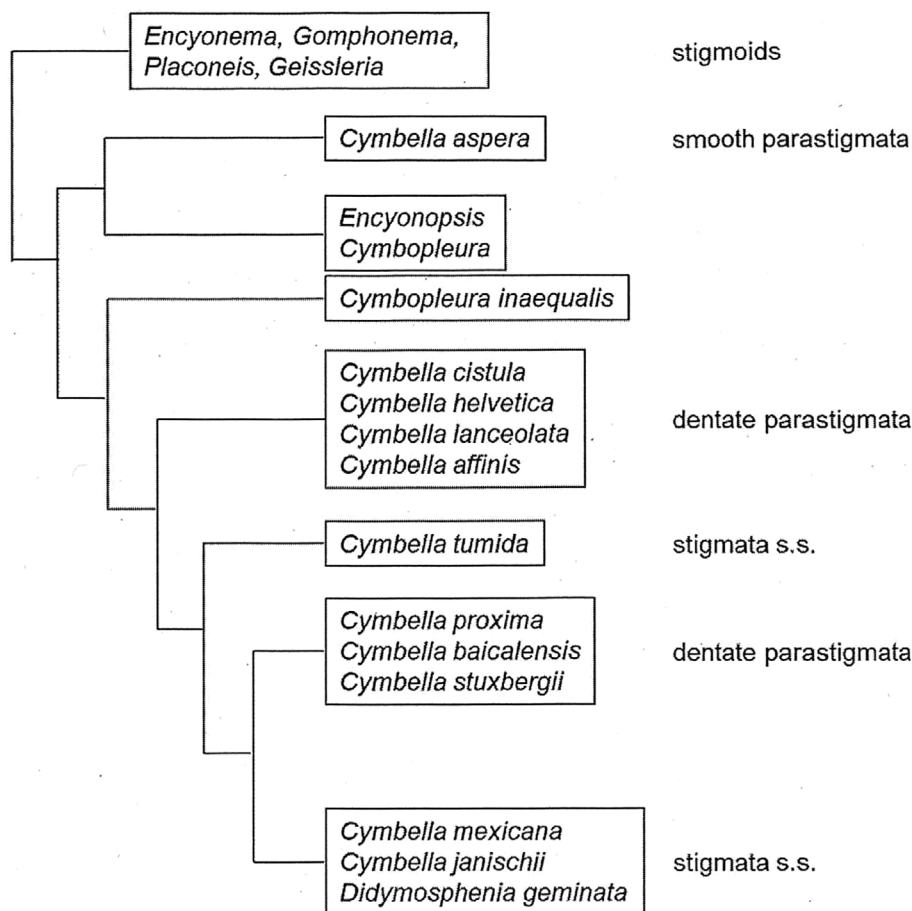


FIGURE 11 Diagram showing the distribution of different types of stigmata across *Cymbella* and *Didymosphenia* based on the maximum likelihood tree by Nakov et al. (2014, figure S1; adapted with permission).

whereas, as far as we know, all the above stigmata and stigmoids always have a one-to-one relationship between their external and internal openings, the parastigma in *Oricymba* and the dichostigmoid in *Karthickia* have single external openings connected to two internal ones, that is, the connecting “tube” bifurcates. More detail on the 3D structure of the parastigmata in *Oricymba* and the ontogeny of both genera would be invaluable for determining any homology between them.

There is a major structural difference between the isolated pore structures in the Cymbellales, which are never fully occluded, and the fistulae, buciniporulae, and cuniculi present in some members of the Naviculales, which have hymenate closures. Although the phylogenetic relationships of *Fistulifera* and *Proschkinia* have been explored in a few publications (Kim et al., 2020; Majewska et al., 2019; Tseplik et al., 2022; Zgrundo et al., 2013), there are no molecular studies focusing on *Olifantiella* or *Labellicula*, and *Luticola* has rarely been included (but see Han et al., 2018, Keziya et al., 2021). However, based on partial 18S rRNA gene sequences, a putative *Olifantiella* species grouped with *Luticola* (Han et al., 2018), distant from *Gomphonema*, which had been used as the outgroup. This could support the

identification of the isolated pores of both *Olifantiella* and *Luticola* as buciniporulae. Similarly, a phylogeny based on partial *rbcL* and SSU rDNA gene sequences placed *Parlibellus* in the Stauroneidaceae, also within the Naviculales and remote from the Cymbellales (Kulikovskiy et al., 2019).

Despite the morphological differences, on which their taxonomic affiliations are based, fistulae and buciniporulae occur in taxa from marine or high conductivity waters, unlike the freshwater members of the Cymbellales. The internal closure of a cuniculus in *Parlibellus*, a marine genus, is also finely perforate, like a hymen. The contrast in closure may be linked to their ecology: hymenate occlusions occurring in taxa located in marine and high conductivity environments and possibly being important in osmoregulation in more saline waters.

Revised definitions of terms

Stigma (=stigma sensu Cleve)

An isolated pore that opens externally near or within the central area, internally with a rounded or

elongate, domed crenulate surface. Observed in *Didymosphenia* and some *Cymbella* spp., including *C. mexicana*, *C. janischii*, and *C. tumida*. The pores lie on the primary side of the valve or, if they are central, open internally on the primary side of the valve, within the silica flange that overlies the internal raphe endings.

Parastigma (=stigma sensu Krammer)

A distinctive pore at or near the end of one or several central striae, on the primary side of the valve, larger and more rounded than the stria areolae, more elongate internally. The internal openings may be smooth (smooth parastigma) or with more convoluted, dentate margins (dentate parastigma). Observed in *Cymbella*, *Oricymba* and some *Delicatophycus* spp., for example, *D. williamsii* and *D. liuweii*. *Cymbella* spp., with those with smooth or dentate parastigmata usually falling in different parts of published phylogenetic trees.

Stigmoid

A distinctive pore, usually at least slightly removed from the central end of a stria on the primary side of the valve, with a simple round external opening, but varying in shape internally, although never occluded or partially occluded. Internal openings may be round to elongate or slit-like, sometimes curved. Some may be surrounded by small silica knobs, creating a flower-like appearance, for example, *Gomphonema tergestinum*. The presence of four stigmoids, one at each end of the central striae flanking the central area have been observed in *Gomphonella* and some *Gomphonema* spp., such as the *G. tetrastigmata* group. The distinctive pores flanking both sides of the central nodule in *Brebissonia* are stigmoids.

Dichostigmoid

A dichostigmoid has only been observed in *Karthickia*, differing from stigmoids in being positioned on the secondary side of the valve, with a simple round external opening internally into two pores at the ends of central striae.

Buciniportula

A distinctive isolated pore, opening externally as an elongate pore, to one side of the central area, internally as one or two, often raised, tubular structures, with thin siliceous, finely perforated, flap-like coverings. The

height of the internal tube varies between species, particularly in *Olifantiella*, in which examples in with double internal openings have occurred. In *Luticola*, the internal structure is shorter, and only single internal openings have been observed. Although it is often difficult to identify unequivocally primary and secondary sides of the valve in these genera, buciniportulae seem to lie on the secondary sides.

Fistula

An isolated pore with a slit-like external opening, aligned along the long axis of the valve, opening internally as one or more raised, spherical hymenate areas. The fistula lies on the primary side of the valve. It has been observed in *Fistulifera*, *Proschkinia*, and *Labellifera*.

Cuniculus

This structure appears as a simple external pore (occasionally two pores) at the center of the valve between the central raphe endings, but as raised, longitudinal perforated (hymenate) ridges terminating the raphe endings internally.

AUTHOR CONTRIBUTIONS

Eileen J. Cox: Conceptualization (equal); investigation (equal); methodology (equal); writing – original draft (lead); writing – review and editing (equal). **Bart Van de Vijver:** Conceptualization (equal); investigation (equal); methodology (equal); writing – original draft (supporting); writing – review and editing (equal).

ACKNOWLEDGMENTS

Carlos E. Wetzel, Luc Ector, and Ingrid Jüttner are thanked for the picture of *Gomphonema tergestinum* and *Cymbella tumida*. Christine Cocquyt is acknowledged for the picture of *Afrocymbella*. Alex Ball and the staff of IAC at the Natural History Museum are thanked for their assistance with the scanning electron microscopy observations. The European Union Synthesys programme provided the necessary funding allowing BVDV to visit the Natural History Museum in 2011, 2012 and 2013. We also thank the reviewers for their constructive comments, which have highlighted how much we still have to discover about the ways in which diatoms construct their frustules and what this can tell us about their relationships.

ORCID

Eileen J. Cox  <https://orcid.org/0000-0002-4522-3203>

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

REFERENCES

- Abarca, N., Stancheva, R., Skibbe, O., Schimani, K., Kusber, W.-H., Zimmermann, J., & Jahn, R. (2023). *Gomphadelphia* (Bacillariophyceae) – A new genus name for taxa formerly subsumed in the *Gomphoneis herculeana*-group. *Nova Hedwigia*, 117, 213–254.
- Abarca, N., Zimmermann, J., Kusber, W.-H., Mora, D., Van, D. T., Skibbe, O., & Jahn, R. (2020). Defining the core group of the genus *Gomphonema* Ehrenberg with molecular and morphological methods. *Botany Letters*, 167, 1–46.
- Cleve, P. T. (1894). *Synopsis of the naviculoid diatoms*. Part I. *Kongliga Svenska-Vetenskaps Akademiens Handlingar*, 26(2), 1–194, 5 pls.
- Cox, E. J. (1978). Taxonomic studies on the diatom genus *Navicula* Bory. *Navicula grevillii* (C.A.Ag.) Heiberg and *N. comoides* (Dillwyn) H. & M. Peragallo. *Botanical Journal of the Linnean Society*, 76, 127–143.
- Cox, E. J. (1988). Taxonomic studies on the diatom genus *Navicula* V. The establishment of *Parlibellus* gen. nov. for some members of *Navicula* sect. *Microstigmaticae*. *Diatom Research*, 3, 9–38.
- Cox, E. J. (2012). Ontogeny, homology and terminology – Wall morphogenesis as an aid to character recognition and character state definition for pennate diatom systematics. *Journal of Phycology*, 48, 1–31.
- Glushchenko, A. M., Kuznetsova, I. V., Kociolek, J. P., & Kulikovskiy, M. (2019). *Karthickia verestigmata* gen. et sp. nov. – An interesting diatom with frustular morphology similar to several different cymbelloid genera. *Phycologia*, 58, 605–613.
- Glushchenko, A. M., Maltsev, Y. I., Kociolek, J. P., Kuznetsova, I. V., & Kulikovskiy, M. S. (2022). Molecular and morphological investigations of two giant diatom *Cymbella* species from the Transbaikal area (Russia, Siberia) with comments on their distributions. *Plants*, 11(2445), 1–16.
- Guiry, M. D., & Guiry, G. M. (2024). *AlgaeBase*. World-wide electronic publication, University of Galway. <https://www.algaebase.org>
- Han, J., Zhang, L., Wang, P., Yang, G., Wang, S., Li, Y., & Pan, K. (2018). Heterogeneity of intron presence/absence in *Olifantiella* sp. (Bacillariophyta) contributes to the understanding of intron loss. *Journal of Phycology*, 54, 105–113.
- Hustedt, F. (1962). Die Kieselalgen Deutschlands, Österreichs und der Schweiz unter Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete. Bd. VII: Teil 3: Lieferung 2. In: *Rabenhorst's Kryptogamen Flora von Deutschland, Österreich und der Schweiz* (pp. 161–348).
- Jahn, R., Kusber, W.-H., Skibbe, O., Zimmermann, J., Van, A. T., Buczkó, K., & Abarca, N. (2019). *Gomphonella olivacea* (Bacillariophyceae) – A new phylogenetic position for a well-known taxon, its typification, new species and combinations. *Plant Ecology and Evolution*, 152, 219–247.
- Jüttner, I., Krammer, K., Van de Vijver, B., Tuji, A., Simkhada, B., Gurung, S., Sharma, S., Sharma, C. M., & Cox, E. J. (2010). *Oricymba* (Cymbellales, Bacillariophyceae), a new cymbelloid genus and three new species from the Nepalese Himalaya. *Phycologia*, 49(5), 407–423.
- Kermarrec, L., Ector, L., Bouchez, A., Rimet, F., & Hoffmann, L. (2011). A preliminary phylogenetic analysis of the Cymbellales based on 18S rDNA gene sequencing. *Diatom Research*, 26, 305–315.
- Kezlya, E., Glushchenko, A., Maltsev, Y., Gusev, E., Genkal, S., Kociolek, J. P., & Kulikovskiy, M. (2021). Three new species of *Placoneis* Mereschkowsky (Bacillariophyceae: Cymbellales) with comments on cryptic diversity in the *P. elginensis*-group. *Water*, 13, 3276.
- Kim, S.-Y., Witkowski, A., Park, J.-G., Gastineau, R., Ashworth, M. P., Kim, B.-S., Mann, D. G., Li, C., Igersheim, A., Płociński, T., Yoo, Y.-D., Chung, S.-O., & Theriot, E. C. (2020). The taxonomy and diversity of *Proschkinia* (Bacillariophyta), a common but enigmatic genus from marine coasts. *Journal of Phycology*, 56, 953–978.
- Kociolek, J. P., Kapustin, D., & Kulikovskiy, M. (2018). A new, large species of *Gomphonema* Ehrenberg from ancient Lake Matano, Indonesia. *Diatom Research*, 33(2), 241–250.
- Kociolek, J.P., Kulikovskiy, M. & Solak, C.N. (2013) The diatom genus *Gomphoneis* Cleve (Bacillariophyceae) from Lake Baikal, Russia. *Phytotaxa* 154(1), 1–37.
- Kociolek, J. P., & Stoermer, E. F. (1987). Ultrastructure of *Cymbella sinuata* and its allies (Bacillariophyceae), and their transfer to *Reimeria*, gen. nov. *Systematic Botany*, 12, 451–459.
- Kociolek, J. P., & Thomas, E. W. (2010). Taxonomy and ultrastructure of five naviculoid diatoms (class Bacillariophyceae) from the Rocky Mountains of Colorado (USA), with the description of a new genus and four new species. *Nova Hedwigia*, 90(1–2), 195–214.
- Kociolek, J. P., You, Q.-M., Wang, Q.-X., & Liu, Q. (2015). A consideration of some interesting freshwater gomphonemoid diatoms from North America and China, the description of *Gomphosinica* gen. nov. *Nova Hedwigia. Beiheft*, 144, 175–198.
- Krammer, K. (1979). Zur Deutung der Diatomeen-Feinstrukturen im Lichtmikroskop. *Mikrokosmos*, 68, 66–71.
- Krammer, K. (1982). Valve morphology in the genus *Cymbella* C.A. Agardh. *Morphology of diatom valves XI*, edited by Helmcke, J.-G. & Krammer, K. 1–199.
- Krammer, K. (1997a). Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil 1. Allgemeines und *Encyonema*. *Bibliotheca Diatomologica*, 36, 1–382.
- Krammer, K. (1997b). Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil 2. *Encyonema* part., *Encyonopsis* und *Cymbellopsis*. *Bibliotheca Diatomologica*, 37, 1–469.
- Krammer, K. (2002). *Cymbella*. In *Diatoms of Europe: Diatoms of the European Inland Waters and Coparable Habitats*, edited by H. Lange-Bertalot, Vol. 3, 1–584. Koeltz Botanical Books.
- Kulikovskiy, M., & Kociolek, J. P. (2014). The diatom genus *Gomphonema* Ehrenberg in Lake Baikal. I. Morphology and taxonomic history of two endemic species. *Nova Hedwigia. Beiheft*, 143, 507–518.
- Kulikovskiy, M., Maltsev, Y., Andreeva, S., Glushchenko, A., Gusev, E., Podunay, Y., Ludwig, T. V., Tusset, E., & Kociolek, J. P. (2019). Description of a new diatom genus *Dorofeyukea* gen. nov. with remarks on phylogeny of the family Stauroneidaceae. *Journal of Phycology*, 55, 173–185.
- Lange-Bertalot, H. (1997). *Frankophila*, *Mayamaea* und *Fistulifera*: drei neue Gattungen der Klasse Bacillariophyceae. *Archiv für Protistenkunde*, 148, 65–76.
- Levkov, Z., Metzeltin, D., & Pavlov, A. (2013). *Luticola* and *Luticolopsis*. *Diatoms of Europe*, 7, 1–698.
- Liu, B., Blanco, S., & Lan, Q.-Y. (2018). Ultrastructure of *Delicata sinensis* Krammer et Metzeltin and *D. williamsii* sp. nov. (Bacillariophyta) from China. *Fottea*, 18, 30–36.
- Liu, B., Zhou, Y.-Y., Blanco, S., & Williams, D. M. (2021). Three new species of *Delicatophycus* M.J. Wynne (Bacillariophyta) from China, all possessing apical pore fields. *Fottea*, 22, 137–151.
- Mahoney, R. K., & Reimer, C. W. (1986). Studies on the genus *Brebissonia* (Bacillariophyceae). I. Introduction and observation on *B. lanceolata* comb. nov. In M. Ricard (Ed.), *Proceedings of the 8th international diatom symposium, Paris 1984* (pp. 183–190). Koeltz Scientific Books.
- Majewska, R., Bosak, A., Frankovich, T. A., Ashworth, M. P., Sullivan, M. J., Robinson, N. J., Iazo-Wasem, E. A., Pinou, T., Nel, R.,

- Manning, S. R., & Van de Vijver, B. (2019). Six new epibiotic *Proschkinia* (Bacillariophyta) species and new insights into the genus phylogeny. *European Journal of Phycology*, 54(4), 609–631.
- Majewska, R., De Stefano, M., & Van de Vijver, B. (2017). *Labellicula lecohuiana*, a new epizoic diatom species living on green turtles in Costa Rica. *Nova Hedwigia. Beiheft*, 146, 23–31.
- Mann, D. G. (1981). A note on valve formation and homology in the diatom genus *Cymbella*. *Annals of Botany*, 47, 267–269.
- Mann, D. G. (2006). Specifying a morphogenetic model for diatoms: An analysis of pattern faults in the Voigt zone. *Nova Hedwigia. Beiheft*, 130, 97–118.
- Mayama, N., & Mayama, S. (2021). A simple method for making transverse cleavages of diatom frustules for scanning electron microscopy. *Diatom Research*, 36, 305–311.
- Mayama, S., & Kobayasi, H. (1986). Observations of *Navicula mobilensis* var. *minor* Patr. and *N. goeppertiana* (Bleisch) H. L. Sm. In M. Ricard (Ed.), *Proceedings of the 8th international diatom symposium, Paris, 1984* (pp. 173–182). Koeltz Scientific Books.
- Metzeltin, D., & Lange-Bertalot, H. (2014). The genus *Didymosphenia* M. Schmidt. A critical evaluation of established and description of 11 new taxa. *Iconographia Diatomologica*, 25, 1–293.
- Nakov, T., Ruck, E. C., Galachyants, Y., Spaulding, S. A., & Theriot, E. C. (2014). Molecular phylogeny of the Cymbellales (Bacillariophyceae, Heterokontophyta) with a comparison of models for accommodating rate variation across sites. *Phycologia*, 53, 359–373.
- Reichardt, E. (2015). The identity of *Gomphonema clavatum* Ehrenberg (Bacillariophyceae) and typification of five species of the genus *Gomphonema* described by C.G. Ehrenberg. *Diatom Research*, 30, 141–149.
- Riaux-Gobin, C., & Al-Handal, A. Y. (2012). New species in the marine diatom genus *Olifantiella* (Bacillariophyta, Biraphidineae) from Rodrigues Island (Western Indian Ocean). *Fottea*, 12(2), 199–217.
- Riaux-Gobin, C., & Compère, P. (2009). *Olifantiella mascarenica* gen. & sp. nov., a new genus of pennate diatom from Réunion Island, exhibiting a remarkable internal process. *Phycological Research*, 57(3), 178–185.
- Ross, R., Cox, E. J., Karayeva, N. I., Mann, D. G., Paddock, T. B. B., Simonsen, R., & Sims, P. A. (1979). An amended terminology for the siliceous components of the diatom cell. *Nova Hedwigia. Beiheft*, 64, 513–533.
- Round, F. E., Crawford, R. M., & Mann, D. G. (1990). *The diatoms*. Cambridge University Press.
- Stone, J. R., Wilson, M. C., & Jovanovska, E. (2020). The genus *Afrocybella* (Bacillariophyceae) from lakes Malawi and Tanganyika, with description of new fossil and extant species. *Phytotaxa*, 556, 21–48.
- Tseplik, N., Glushchenko, A., Maltsev, Y., Kuznetsova, I., Genkal, S., Kociolek, J. P., & Kulikovskiy, M. (2022). A new monoraphid diatom species from the genus *Karayevia* sensu lato (Bacillariophyceae: Stauroneidaceae) with remarks on taxonomy and phylogeny of the genus. *Diatom Research*, 37, 39–50.
- Tuji, A. (2020). Transfer of the *Gomphoneis tetrastigmata* species complex and related taxa to the genus *Gomphonella* (Bacillariophyceae). *Bulletin of the National Museum of Nature and Science. Series B, Botany*, 46(2), 65–73.
- Van de Vijver, B., Frenot, Y., Beyens, L., & Lange-Bertalot, H. (2005). *Labellicula*, a new diatom genus (Bacillariophyta) from Île de la Possession (Crozet Archipelago, Subantarctica). *Cryptogamie, Algologie*, 26, 125–133.
- Van de Vijver, B., Mertens, A., & Van Dam, H. (2016). *Olifantiella elisabethiana*, a new raphid diatom species (Bacillariophyta) observed in the port of Antwerp (Belgium). *Phytotaxa*, 261, 251–259.
- Van der Werff, A. (1955). A new method of concentrating and cleaning diatoms and other organisms. *Verhandlungen - Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 12(1), 276–277.
- Van Heurck, H. (1885). *Synopsis des diatomées de Belgique*. Anvers: Texte, 235.
- Yana, E., Nakkaew, S., Pekkoh, J., Peerapornpisal, Y., Tuji, A., Davis, M. P., Julius, M. L., & Mayama, S. (2022). Valve and 'stigma' structure and phylogeny of an enigmatic cymbelloid diatom *Karthickia verestigmata* Glushchenko, Kulikovskiy & Kociolek. *Diatom Research*, 37, 293–305.
- You, Q., Kociolek, J. P., & Wang, Q. (2013). New *Gomphoneis* Cleve (Bacillariophyceae: Gomphonemataceae) species from Xinjiang Province, China. *Phytotaxa*, 103(1), 1–24.
- Zgrundo, A., Lemke, P., Pniewski, F., Cox, E. J., & Latąta, A. (2013). Morphological and molecular phylogenetic studies on *Fistulifera saprophila*. *Diatom Research*, 28, 431–443.
- Zhang, W., Gui, X.-W., Bao, M.-Y., Kociolek, J. P., Blanco, S., & Li, Y. (2020). *Kurtkrammeria tiancaiensis* sp. nov. a new cymbelloid species (Bacillariophyceae) from Lijiang Laojunshan National Park in Yunnan Province, v. *Phytotaxa*, 45, 223–230.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. List of published images of different types of isolated pores.

How to cite this article: Cox, E. J., & Van de Vijver, B. (2024). What constitutes a stigma? A review of isolated pores in raphid diatoms (Bacillariophyceae) and the value of precise terminology. *Journal of Phycology*, 60, 1498–1513. <https://doi.org/10.1111/jpy.13522>