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Determining geographical range and alien status in diatoms: three instructive case histories of species newly recorded in the British Isles, including a non-native marine species from the Pacific, *Diademoides luxuriosa*

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Abstract

Three benthic diatom species – *Diademoides luxuriosa*, *Navicula entoleia* and *Nitzschia ocellata* – have been discovered for the first time in Scottish coastal waters. All are highly distinctive but rarely recorded, with less than 100 mentions found worldwide in an exhaustive search of the literature, even after taking into account possible synonyms. Even so, one of the three, *D. luxuriosa*, can be argued to be non-native in the British Isles, being a recent introduction from the Pacific rim (with a distribution from New Zealand, Australia, Indonesia, China and Japan to W North America). Plausible vectors are via ship hulls or co-transport with Pacific oysters. The distributions of the other two species show no clear pattern, *Navicula entoleia* having been found in Europe and NW USA, while *Nitzschia ocellata*, or at least a lineage of similar species containing *N. ocellata*, occurs in Europe, S America and E Asia. The three case histories are used to define a series of criteria for establishing geographical range and alien status in diatoms and other microalgae. *Diademoides luxuriosa* may be the first example anywhere of an alien marine diatom, since previous claims of such status, e.g. for *Coscinodiscus wailesii* and *Trieres* (*Odontella*) *sinensis*, fail to satisfy the criteria outlined. The morphological characteristics of *D. luxuriosa* and *N. entoleia*, including cellular detail, are used to comment briefly on their systematic position.

Keywords: aliens, biogeography, endemism, introductions; marine diatoms; new records; taxonomy

Running head: *Diademoides luxuriosa*, a Pacific alien in Europe

Introduction

Changes in the distributions of organisms and their pathogens are accelerating as a result of the pressures imposed by an ever-growing human population, faster transport, and climate change. Some of these are to be welcomed. For example, given that anthropogenic climate change is occurring, movement of species may be essential to avoid extinction. Other changes are detrimental, disrupting ecosystems, sometimes severely. Among many examples of invasive marine algae is *Caulerpa taxifolia* (Vahl) C. Agardh, which was released into the Mediterranean in the 1980s and has expanded dramatically, displacing native seaweeds and seagrasses (Boudouresque *et al.* 1995, Duarte 2002). Contractions in the ranges of organisms can also occur, e.g. if climate change or habitat loss decrease the extent to which the niche space of a species is represented in the biotopes (cf. Colwell & Rangel 2009) of a particular region. For example, cold-water marine zooplankton species have apparently retreated in the N Atlantic as sea temperatures have increased (Beaugrand & Reid 2003) and major shifts are predicted for the geographical ranges of intertidal seaweeds in the boreal region (Müller et al. 2009) as a result of increases in sea temperature. On land, boreal and montane lichen species can simply run out of suitable habitat as they retreat northwards and upwards (Ellis et al. 2007).

Detecting range shifts requires that we know the natural distributions of organisms. In many higher plants and metazoa, these can often be determined accurately through visual surveys because: (1) the organisms (or, in the case of some pathogens, their effects on other organisms) are macroscopic, so that they are easily detected; (2) they are usually morphologically distinctive, so that we can identify them accurately; (3) their ecology and phenology are reasonably well known, so that we know where and when to look for them; and (4) there are enough people with the expertise and interest to survey all the areas where the species might occur. Thus we can be highly confident that the moss *Paludella squarrosa* (Hedwig) Bridel is now absent in Britain and reduced to a single locality in Ireland (Lockhart 1999) because it is a conspicuous and easily identified perennial moss species, its preferred habitat is known to be rich and intermediate fen (e.g. Rybníček 1966, Cooper 1991), and there are (and have been for well over a century and a half) many bryologists exploring and mapping the distributions of British and Irish bryophytes. For similar reasons we can be sure that some bryophytes have been introduced outside their native range and that a few of these, e.g. *Campylopus introflexus* (Hedwig) Bridel, are highly invasive, with significant effects on autochthonous vegetation (Essl et al. 2013, 2014).

It is doubtful whether any microscopic algae are as well understood geographically as *Paludella* and *Campylopus*, even though the health risks posed by some microalgae (e.g. toxin-producing dinoflagellates and diatoms) have made it a priority to determine their distribution and abundance. For example, *Pseudo-nitzschia*, *Alexandrium* and *Gambierdiscus* species are now monitored in many parts of the world because of their involvement in amnesic and paralytic shellfish poisoning and ciguatera, respectively (Hallegraeff 1993). However, even in these, establishing the geographical range is difficult, because the organisms cannot be identified morphologically without examination of the fine detail of the cell covering, while their pathogenic effects are often inconsistent and do not act as markers for particular species. For example, the same *Pseudo-nitzschia* species may or may not produce domoic acid, and even when domoic acid is produced, it may not cause noticeable damage to higher animals (Trainer *et al.* 2012). Hence, species distributions must be determined by critical microscopy of water samples – which requires considerable expertise – or the use of molecular methods, which are essential in some cases because the species are morphologically indistinguishable, e.g. in the *Alexandrium tamarense* (Lebour) Balech species complex, or *Pseudo-nitzschia delicatissima* (Cleve) Heiden and *Pseudo-nitzschia* *arenysensis* Quijano-Scheggia, Garcés & Lundholm (John *et al.* 2014, Quijano-Scheggia *et al.* 2009). Not surprisingly, therefore, even for groups of organisms that can directly affect human well-being, knowledge of the autecology and phenology of individual microalgal species is still in its infancy and does not allow detailed prediction of which areas are potentially able to support viable populations. Hence the four factors that give us confidence about the distribution of the moss *Paludella* only raise doubts in the case of toxic marine phytoplankton: we know where particular species have been recorded to occur, but we cannot say with much confidence where they do *not* occur. It is correspondingly difficult to have much confidence in suggestions that species distributions have changed.

In freshwaters, there have been claims that particular diatom species are endemic (earlier examples were reviewed by Vanormelingen et al. 2008; many more have since been put forward) and also that species have been introduced outside their natural range (e.g. Spaulding et al. 2010). Some of the most celebrated examples are *Asterionella formosa* Hassall (Harper 1994) and *Didymosphenia geminata* (Lyngbye) Martin Schmidt (Kilroy 2004), which are suggested to have been absent in New Zealand before European colonization. However, although a strong case can be made that *D. geminata* has been spread by humans to New Zealand (Kilroy & Novis 2018), genetic evidence indicates that the expansion of this species in South America is unlikely to be the result of recent introduction (Jones et al. 2019), while in most diatom species – freshwater or marine – there is no convincing evidence demonstrating that distributions have been extended through human intervention.

The aim of this paper is to examine the distributions and status – introduced or native – of three coastal marine diatoms recently detected for the first time in Britain and to use these case histories to illustrate criteria for determining whether a particular microalga has a restricted geographical range or can be claimed to be non-native. My analysis is predicated on the fact that the coastline of the British Isles is one of the best studied in the world, with long-established institutions and research labs for marine biological research (e.g. the Natural History Museum of London, the Scottish Association for Marine Science, and the Marine Biological Association of the United Kingdom) and checklists available for many groups of animals and plants (e.g. Howson & Picton 1997). British marine diatoms were first investigated in detail in the middle of the 19th century during a rather short period (from c. 1845 until 1870) in which it would probably be fair to claim that British researchers dominated research on marine diatoms and extensive collections were made from coasts in the British Isles and overseas (e.g. Smith 1853–1856, Brightwell 1853, 1858, Gregory 1857, Donkin 1858, 1870–3, Roper 1858, 1859, Ralfs 1861, Greville 1863, 1866). Since 1870, knowledge of the British marine diatom flora has continued to grow (albeit more slowly) through the contributions of various professional and amateur diatomists summarized in the checklists of Hendey (1954, 1974) and Hartley (1986). These culminated in a flora of British marine diatoms (Hendey 1964) and an iconograph that includes both named and unidentified species (Hartley et al. 1996). Nearby coastlines of the European continent have also been studied in detail (e.g. Peragallo & Peragallo 1897–1908, Hustedt 1927–66, Hustedt 1939, Brockmann 1950, Cleve-Euler 1951–55). Despite this promising history, new records continue to be made in the British Isles (e.g. Droop 1998, Mann 1998, Massé et al. 2001, Mann & Trobajo 2014), indicating that we are far from being able to say that the British marine diatom flora is ‘well known’ (in contrast, for example, to the British angiosperms, which have been endlessly catalogued and revised and illustrated during the 20th century, with minimal additions of previously undocumented native species, and have been mapped in detail for more than 60 years: Braithwaite & Walker 2012).

In each of the three cases considered here, I begin by describing the material found and then review the taxonomy and characteristics of the species, in order to provide a basis for discussing its biogeography and the significance that can be attached to its discovery in the UK. I then evaluate previous records of the species to determine whether it is possible to say whether any of them are ‘aliens’. Finally, the implications of the case studies for establishing the biogeography and native status of microalgae from morphology-based and molecular records are explored in a ‘General discussion’.

Materials and methods

Diademoides luxuriosa. As in almost every year for the last 25 years, samples were collected from the Firth of Forth near Edinburgh in 2014–2019 and 2022 to provide material for MSc practical classes on marine epipelic microalgae and for research. Specifically, at about 08.30 h on 8 October 2014, a sample of sand was collected during low tide from the flat lower foreshore of the Firth of Forth at Silverknowes, the City of Edinburgh, Scotland (55° 59′ 00″N, 3° 16′ 41″W). At this location, the Firth of Forth is essentially fully marine – an enclosed extension of the North Sea (Dyke 1987) – though the collection site is often subject to rainfall and hence lowered salinity during low tide. Enough sand was scooped from the top 1 cm in depressions and on the sides of sand ripples to fill a plastic box (c. 22 × 22 cm, with transparent lid) to a depth of 3 cm. In the laboratory, the sand surface was smoothed by hand, the box was tapped gently on the bench to settle and flatten the sand further, and then the box was allowed to stand for c. 6 h. Supernatant water was removed by suction and a single layer of lens tissue (Whatman, type 105, Sigma–Aldrich) placed on the sand in the late afternoon. At c. 09.00 h on 9 October, cover-slips (22 × 22 and 24 × 50 mm) were placed on the tissue to become colonized by diatoms migrating up from the sand. They were removed for microscopical examination after three or more hours, mounted on drops of seawater and sealed onto slides with petroleum jelly, as described by Jones *et al.* (2005). Further cover slips were added at intervals during the next day according to the same protocol. In addition, diatoms were cleaned *in situ* on cover-slips using the method described by Trobajo & Mann (2019), followed by mounting in high refractive index Naphrax medium (Brunel Microscopes, Chippenham, Wiltshire, UK).

Subsequently, further samples from the Firth of Forth were examined alive from the same Silverknowes site on 14 November 2015, 8 November 2017 and 17 October 2019; from Portobello, City of Edinburgh (55° 57′ 15.29″N, 3° 06′ 21.98″W), Musselburgh, East Lothian (55° 56′ 52.49″N, 3° 03′ 51.53″W), Port Seton, East Lothian (55° 58′ 28.49″N, 2° 56′ 45.48″W), Longniddry, East Lothian (55° 59′ 07.30″N, 2° 54′ 06.12″W), and Gosford, East Lothian (55° 59′ 54.44″N, 2° 53′ 22.50″W), all collected on 22 October 2016; from Portobello (location as above), collected on 13 February 2017; from Longniddry, Portobello and Gullane, East Lothian (56° 02′ 21″N, 2° 50′ 39″W) on 14 October 2018; from Silverknowes on 17 October 2019; and from Portobello on 16 March 2022. Diatoms from these collections were harvested and cleaned by the rapid cleaning method as above, or by using lens tissue (a double layer, placed on the single layer used as a filter as described above), which was subsequently oxidized using first nitric and then sulphuric acid, with intervening washes with deionized water to remove calcium salts; residual acids and oxidation products were removed by repeated sedimentation and dilution by deionized water. Slides were made as described above, with Naphrax mountant. Accession numbers for samples and slides are given in Supplementary Table 1.

Navicula entoleia. Two specimens were observed in the same sample from Portobello collected on 13 February 2017 in which *D. luxuriosa* was detected; they were photographed as through-focus stacks of images using a Zeiss AxioImager microscope (see below). In addition, examination of archived film (Kodak Technical Pan) and records in the Royal Botanic Garden Edinburgh revealed that *N. entoleia* was present in sublittoral sediments collected by R. Park in Loch Goil (the site is described by Mann & Trobajo 2014) on 17 February 1988, in December 1989 and on 10 January 1990.

Nitzschia ocellata. Silty sand was collected on 14 March 1994 from the sublittoral off Toscaig Pier, near Applecross, W. Scotland. Extensive reddish-brown diatom blooms were visible on sand deposits on a steep rock face and boulder slope; the fauna of the area included red cushion stars, crabs, squat lobsters and tunicates. A sample of sand from 12 m depth was cleaned in the 1990s using first nitric and then a mixture of 70% nitric and 98% sulphuric acids, with removal of dissolved oxidation products in each case by repeated washing with deionized water. Voucher material for the 12 m Toscaig sample was given accession number E2486. In 2014 it was discovered by accident that no slides had been made of this material. Aliquots of cleaned material were therefore dried onto circular coverslips (after removal of the coarsest sediment fraction by sedimentation for a couple of minutes to give modified accession E2486.1) and mounted in Naphrax as a series of slides numbered E2486.1/1, E2486.1/2, etc. *Nitzschia ocellata* was found in these slides. Since the species was not observed live when the Toscaig material was collected, it is important to record that at the time the voucher material of E2486 was cleaned in the 1990s, no material from outside the UK was being processed; nor was any non-UK material being handled when the E2486 slides were made. Contamination with non-UK material is therefore very unlikely.

Other benthic material examined. In addition to slides or material in which *N. entoleia, N. ocellata* and *D. luxuriosa* were found, numerous (> 100) other inter- and subtidal sediment samples were examined from various parts of the UK coastline, including at least annual samples from the Edinburgh shoreline (between Cramond on the west and Portobello on the east) between 1986 and 2014, and earlier more frequent, though irregular, sampling in 1983–1985. Apart from the samples listed here in which the three species were found, no others were observed to contain them.

Microscopy. Light microscope (LM) observations of living and cleaned cells were made using a Zeiss AxioImager photomicroscope with motorized *z*- (focus-) control and run using Axiovision software, as described by Mann & Trobajo (2014). Digital images were processed using Adobe Photoshop CS5 (Extended, version 12.0.4) or CS 6, by general application of the Levels, Curves and Unsharp Mask tools (in *N. entoleia* also the High Pass filter) without localized enhancement, and selective use of differential digital filters to optimize transformation of the original colour images to greyscale. Stria densities of *D. luxuriosa* and *N. entoleia* valves were measured adjacent to the axial area over a distance of 10 µm.

Results and Discussion

***Diademoides luxuriosa* (Greville) K.-D. Kemp & Paddock (1990, p. 199)**

SYNONYMS: *Navicula luxuriosa* Greville (1863, p. 18, pl. 1, figs 10, 11)
Diadema luxuriosa (Greville) K.-D. Kemp & Paddock (1989, p. 40), *nom. illeg.*

Mastogloia dubitabilis Meister (1937, p. 268)
Neidium opacelineatum Riznyk (1973, p. 128)

TYPE: AUSTRALIA, “Hab. Port Stephen, New South Wales, in a dredging communicated by Dr. Roberts” (Greville 1863, p. 18), lectotype designated here: Greville (1863), pl. 1, fig. 10 = Fig. 3 herein.

Discovery and characteristics of a diatom new to the UK. In 2014, while inspecting colonized cover-slips from the Edinburgh foreshore at Silverknowes to check beforehand which microalgae might be encountered by students during a practical class, a diatom was found that did not correspond to any seen previously during 40 years of observing marine littoral diatoms near Edinburgh and elsewhere in the UK, nor to any in the checklist and atlas of British diatoms (Hartley et al. 1986; Hartley *et al.* 1996). The species was rare, comprising less than 0.01% of the total epipelic diatom community (voucher slide E5895.1): on each of several 24 × 50 mm coverslips bearing many thousands of diatom and dinoflagellate cells, there were zero, one, or up to three of the unusual cells.

The species was among the larger-celled diatoms present (length × breadth ranging from ~50 ×24 µm to ~95 × 30 µm) and had lanceolate valves (Figs 1, 2, 6–8), which were very slightly rostrate in the shortest specimens. The transverse striae were coarse, 8–9 in 10 µm, very slightly radial, and composed of widely spaced areolae, which were aligned to form longitudinal striae; these were straight near the raphe, but clearly biarcuate nearer the margin (Figs 1, 2). The raphe was straight, except at the poles, where both terminal fissures curved off towards the secondary side of the valve (the primary and secondary sides were differentiated by the occurrence of Voigt discontinuities on the secondary side: cf. Mann 1981). Between the central raphe endings there was a short longitudinal ridge internally (Fig. 5). Parallel to each margin, there was a prominent dark line (Figs 1–5), which careful focusing revealed to be the inner wall of a longitudinal canal running the whole length of each side (see also Supplementary Fig. 2). The outer canal wall appeared to be porous (from the variation in optical density within the canals visible in Figs 4, 5).

Cells always lay in valve view and were motile. There was one chloroplast per cell, which was divided into two butterfly-like plates, one against each valve (Figs 6, 7), linked by a massive pyrenoid traversing the lumen of the cell (Fig. 8). The pyrenoid was slightly eccentric and had a rounded cross-section; the nucleus lay alongside it in a bridge of cytoplasm separating the large vacuole into two apical halves (Fig. 8). Sometimes, concentrations of worm-like organelles were visible along the side of the cell in the angle between valve face and mantle (Fig. 9); it was not possible to be sure whether these were within the longitudinal canal or just beneath it, within the cell. The nucleus contained a single nucleolus.

The species has been found in each year since 2014 (except 2020 and 2021, when there was no sampling because of the pandemic): viz. at Silverknowes in November 2015, November 2018 and October 2019, at Port Seton and Longniddry in October 2016, and at Portobello in February 2017 and March 2022 (Table 1). At each site it was rare, as at Silverknowes in 2014, and usually comprised much less than 0.1% of the epipelic diatom community; however, in the latest sample from Silverknowes (taken in October 2019), it reached 1%. It was not recorded at Portobello, Musselburgh or Gosford in October 2016.

Identification. In their shape, size, tendency to lie in valve view, and positioning of the chloroplasts principally against the valves (rather than against the girdle as in *Navicula* species: Round *et al.* 1990, Cox 1996) the cells superficially resembled *Petroneis* and *Lyrella* species (cf. Jones et al. 2005, Mann & Stickle 1993, 1997). These two genera are common on sandy UK shores and at least one *Lyrella* species has a near-central bridging pyrenoid like that shown in Fig. 8 (our unpublished observations of *Lyrella sandriana* (Grunow) D.G. Mann). However, the cells of the Silverknowes diatom differed structurally from *Petroneis* and *Lyrella* in two respects visible in LM: *Petroneis* and *Lyrella* species never have longitudinal canals within their valves, nor do they possess a ridge internally linking the central raphe endings (Round *et al.* 1990, Mann & Stickle 1997, Mann 1998, Witkowski *et al.* 2000, Jones *et al.* 2005).

Searches in the wider literature revealed that the Silverknowes diatom is *Diademoides luxuriosa*, first collected by a Dr. Roberts in 1862 from Port Stephens, New South Wales, Australia, and described as *Navicula luxuriosa* by Greville (1863). The original sample was a dredging from the sublittoral. Greville illustrated two valves from Port Stephen (op. cit., pl. 1, figs 10, 11; see my Fig. 3 and Supplementary Fig. 1) but unfortunately it appears that the slide or slides used to prepare these drawings have been lost (Williams 1988, p. 40): several slides of Port Stephens material are listed as containing the species in Greville's own handwritten catalogue, but these are currently untraced (D.M. Williams personal communication, February 2022). In the absence of these, I designate Greville's fig. 10 (Fig. 3) as the lectotype of *N. luxuriosa*. Clearly visible in the original drawings and noted in Greville's description are the straight or biarcuate longitudinal striae obvious in the Silverknowes material and, more importantly, the longitudinal canal near the mantle ("the intra-marginal line … forming a prominent ridge, leaving a sort of channel between it and the margin": Greville 1863, p. 18). A photograph of a specimen of *D. luxuriosa*, possibly from original material, was given by Kemp & Paddock (1989, fig. 1, from slide BM20539) and this too shows the marginal canal and longitudinally aligned areolae seen in the Scottish specimens.

Unfortunately, Greville gave no stria density measurements or valve widths in his description. However, assuming the stated magnification of Greville's illustrations (×400) is accurate, the dimensions (length × breadth) of the two valves are 95 × 34.5 and 72.5 × 34.5 µm (length × width). The lengths agree reasonably well with the range given in Greville's description ("Length .0030″ to 0.0038″", equivalent to 76.2 to 96.5 µm), suggesting that it is reasonable to make measurements of stria density from Greville's drawings. The longer specimen shown in fig. 10 (my Fig. 3) gives 9–10 striae in 10 µm, the shorter one in fig. 11, 8–9 in 10 µm, giving good agreement with the Silverknowes material. Kemp & Paddock (1989) also give no stria densities for *D. luxuriosa*. The specimen they illustrate from type material (Kemp & Paddock 1989, fig. 1) is said to be 45 µm long and on that basis measurements from the figure would indicate a valve breadth of 22 µm and a stria density of 11.9 in 10 µm. However, there are discrepancies in scale between the LM and SEM illustrations that Kemp & Paddock give of other material of *N. luxuriosa* (from Port Douglas, N Queensland, Australia) [using the dimensions and scale bar supplied, the valve shown by LM in Kemp & Paddock's fig. 2 would have a width of 12 µm and a stria density of 15.5 in 10 µm, whereas that shown by SEM in their fig. 5 would have a width of c. 21 µm and a stria density of 10.3 in 10 µm – only the latter seems plausible] and so it is unsafe to rely on their scale bars.

Later, and based apparently on material from Australia, China and Japan, Cleve (1894) gave dimensions of *D. luxuriosa* as 60–90 × 25–35 µm, with 7–9 striae in 10 µm, while Boyer (1927) stated the stria density to be 'about 8' in 10 µm. In the single photographs of *D. luxuriosa* provided by Foged (1978), Tynni (1986), and Joh (2013) the stria densities, measured from the figures, are 9.5, 7.5–8.5 and 8.5–9 in 10 µm, respectively, and the dimensions of the valves are 52 × 28, 75 × 32 and 46 × 22 µm. Huang (1984) gave dimensions of 56 × 27 µm, with 8–9 striae in 10 µm, and Meister (1937, as "*Mastogloia? dubitabilis*") gave 51 × 24 µm, with 10 striae in 10 µm; in both these reports, the measurements apparently refer to the single specimen illustrated. Finally, Riznyk (1973) gave dimensions of 45–60 × 24–30 µm, and measurements off his figure give 7.5–8 striae in 10 µm, and John (1983, 2012) give 47–80 × 21–34 µm with 9–11 striae and 7–10 areolae in 10 µm. Overall, then, the valve size and striation densities of the Silverknowes specimens (50 ×24 to 95 × 30 µm, with 8–9 in 10 µm) agree well with those of *D. luxuriosa* in the original description and subsequent reports (46–95 µm long, 21–35 µm wide, with 8–10 striae in 10 µm.

Diademoides luxuriosa cannot be easily confused with other species. Its unusual morphology – with curving longitudinal lines of widely spaced areolae and submarginal canals – is reflected in its current classification in a monospecific genus and its previous classification by Cleve (1894) in *Navicula* sect. *Luxuriosae* Cleve, which included no other extant species but only two fossil species from the Upper Eocene deposit at Oamaru, New Zealand (Cleve 1894, p. 74). One of these two, *Navicula decora* Grove & Sturt, has recently been illustrated from type material by Witkowski et al. (2017, fig. 121). Their photograph shows the accuracy of Grove & Sturt's drawing (Grove & Sturt 1887, pl. 10, fig. 13), which depicts submarginal interruptions of the striae like those drawn by Greville in *D. luxuriosa* (Fig. 3). However, there is no sign of canals in *N. decora*, which should be detectable by their walls, appearing as dark lines running parallel to the margin, as in *D. luxuriosa* (Figs 1, 2, 4 and 5). Hence a relationship to *D. luxuriosa* is currently non-proven and further progress awaits discovery of new material permitting SEM observations. Material of the third species of Cleve's sect. *Luxuriosae, Navicula trilineata* Grove & Sturt (1887, pl. 10, fig. 8), seems to have been lost (Witkowski et al. 2017).

The frustule ultrastructure reported by Kemp & Paddock (1989) allows a preliminary assessment of the systematic position of *Diademoides*, which I suggest belongs in the Neidiaceae–Scoliotropidaceae group sensu Round et al. (1990). These two families, which may not be separable (in the molecular phylogeny of Witkowski et al. 2014 the Scoliotropidaceae are paraphyletic) can be characterized by the possession of longitudinal canals that open externally by one or a few open foramina and internally by a finely porous plate, and by the presence of a double helictoglossa between the internal central raphe endings (Supplementary information).

The previously known distribution of D. luxuriosa. Until now, the known distribution of *D. luxuriosa* has been entirely Pacific (Table 1, Fig. 10) and the pattern of occurrence around the Pacific rim, from New Zealand to northwestern USA, was already evident by 1910. As far as I can determine, there has been no record of *D. luxuriosa* from Atlantic coasts, and there are none from Mediterranean or Indian Ocean localities, despite extensive (though patchy) sampling of the temperate and subtropical benthic marine diatom flora in Europe, eastern N America, S Africa, the Persian Gulf and, to a lesser extent, India (e.g. Gregory 1857; Donkin 1870–3, Van Heurck 1880–5; Schmidt 1874; Peragallo & Peragallo 1897–1908; Boyer 1916; Brockmann 1950; Hustedt 1955; Wood 1963; Hendey 1964, 1970; Cholnoky 1968; Giffen 1971, 1975; Foged 1975; Tynni 1983; Desikachary 1986–1989; Hartley et al. 1996; Navarro & Hernández-Becceril 1997). Tynni (1986, p. 20) refers to a record of *D. luxuriosa* from south Texas bays by Wood (1963), but there is no reference to this species in the paper Tynni refers to; he may have confused Wood's 1963 paper with others (Wood 1961a, b) in which *D. luxuriosa* was indeed recorded, but only from Australia and New Zealand (Table 1). A record of *D. luxuriosa* from Holocene sediments of the St Lucia coastal lagoon in SW Africa by Gomes (2016) was based on a preliminary identification and was subsequently determined not to be this species (M. Gomes and Dr M. Humphries, personal communication, January 2018).

Cleve's inclusion of *D. luxuriosa* in his (at the time) comprehensive survey of naviculoid diatoms (Cleve 1894, 1895) will have helped advertise its existence and characteristics, and many of the records listed in Table 1 are accompanied by photographs that confirm the validity of the identification. Nevertheless, the fact that the species has been 'rediscovered' and described again twice (as *Mastogloia? dubitabilis* and *Neidium opacelineatum*) shows (a) the danger of relying on names alone to deduce distributions of microalgae, even of a morphologically distinctive diatom, and (b) that *D. luxuriosa* was not well known by every diatomist, despite Cleve's work. Hence, in cases where *D. luxuriosa* is *not* reported, under its proper name or any of its synonyms, it is helpful (for interpretation) to be able to show that diatomists concerned would have been able to identify it, if it had been present. Table 1 shows that Peragallo, Tempère, Boyer, Cleve, Grunow, Foged, Tynni and Wood all had the competence to identify *D. luxuriosa* (since they made positive records of the species) and all these authors made studies of marine littoral diatoms outside the Pacific. For example, Peragallo & Peragallo (1897–1908) produced a fully illustrated account for the Mediterranean and Atlantic coasts of France and Tempère & Peragallo (1895, 1915) included samples from the coasts of all continents, including some (e.g. their 1915 exsiccatae 96–100, 148, 324 and 325 from Madagascar and Sri Lanka) that might be thought (from co-occurring species) to have been derived from habitats suitable for *D. luxuriosa*. Likewise, Boyer (1916) catalogued the diatom flora of the eastern seaboard of the USA near Philadelphia but did not record *D. luxuriosa* there, unlike in Washington State (Boyer 1927, see Table 1), and Foged produced illustrated floristic lists for various areas, e.g. Tanzania (Foged 1975) and Cuba (Foged 1984) but did not record *D. luxuriosa* there, only in Australia (Foged 1978, Table 1). Furthermore, Wood (1961a) recorded that *D. luxuriosa* from off Port Hacking and Jibbon Beach were "identified by N.I. Hendey", which validates Hendey as a further diatomist able to recognize the species and gives extra meaning to the absence of any record of *D. luxuriosa* in Hendey's monograph of British marine diatoms (Hendey 1964) or his studies of the littoral floras of W Africa and Kuwait (Hendey 1957, 1970). Furthermore, Hendey's monograph of the British marine diatom flora built on a long tradition of study of the littoral by amateur and professional diatomists (references listed in Hendey's 1964 bibliography), which has continued (e.g. Hartley 1986, Hartley et al. 1996, Jones et al. 2005, Massé et al., 2001). None of these diatomists recorded *D. luxuriosa* in the British Isles.

Evaluating sampling bias. Even though *D. luxuriosa* is distinctive and several diatomists working on the Atlantic coasts of N America and Europe, or S African shores during the last 150 years certainly had the competence to identify it, it might be argued that poor sampling could explain the disjunction between previous records around the Pacific rim and the present UK record. A major impediment in evaluating sampling bias is that there is very little information about the autecology and phenology of *D. luxuriosa*, as with most other benthic diatoms: it is therefore unclear whether suitable habitats have been overlooked. What can be said from existing records (Table 1) is that *D. luxuriosa* seems to be a marine or brackish-marine species that sometimes occurs on sediments and is sometimes associated with molluscs or macroalgae. Most importantly, it can occur intertidally as well as in the sublittoral, which greatly increases its chance of being recorded, since this zone is much more accessible than the sublittoral and hence is much better sampled. Altogether, then, I consider that the restriction of all previous records of *D. luxuriosa* to the Pacific rim, and the appearance of the species at Silverknowes in 2014, require a biological rather than a sociological explanation.

Biogeographical context. In assessing the significance of the observed distribution of *D. luxuriosa*, it is also helpful to consider whether it agrees with the distributions of any other, better studied marine organisms, such as seaweeds, for which more extensive data are available. These suggest that a *natural* disjunction between the Pacific rim and Europe is not to be expected: there are, as far as I know, no geological or ecological factors that would produce such distributions. Instead, a Pacific–W Europe distribution of seaweeds reflects long-distance transport by human agency. Examples are the red algae *Polyopes lancifolius* (Harvey) Kawaguchi & Wang (Mineur et al. 2010) and *Gracilariopsis chorda* (Holmes) Ohmi (Mineur et al. 2012), or the brown alga *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim (e.g. Verlaque et al. 2009).

However, seaweed distributions suggest that, even without the UK record, the distribution of *D. luxuriosa* would still be curious, since it seems that few if any taxonomically well-studied macroalgae are found in all of New Zealand, Australia (temperate and tropical), Indonesia, the NW Pacific and the NE Pacific (from Oregon northwards), but nowhere else. For example, among brown algae, none of the *Padina* species studied by Silberfeld et al. (2013) showed a similar distribution to *D. luxuriosa*: one, *Padina crassa* Yamada, occurs in China, Korea, Japan and Baja California (Mexico), but it is apparently absent from temperate Australasia and the NW Pacific coasts of North America. The natural distribution of *Rugulopteryx okamurae* is more similar to that of *D. luxuriosa*, extending around the western half of the Pacific rim from Australia and the Philippines (but not New Zealand) through China and Taiwan to Korea and Japan, and there are reports from Mexico, but it too is not reported from the temperate coasts of NW North America (Verlaque et al. 2009, Guiry 2021). Hence the Pacific distribution of *Diademoides* is wider than might be expected from seaweeds. Of course, it is not impossible that the range of *Diademoides* is wider now in the Pacific than it would have been without human intervention. However, the report of tsunami-driven rafting of macroscopic organisms across the Pacific by Carlton et al. (2017) suggests that no such intervention is needed to achieve transoceanic dispersion of marine littoral diatom species (at least in the Pacific, where major earthquakes are quite frequent), even though floating *natural* substrata (wood, mats of vegetation) are less durable than the Japanese flotsam Carlton et al. tracked from the 2011 earthquake. It may be significant that the Japanese tsunami material was washed ashore predominantly in Washington and Oregon, just where *D. luxuriosa* is found on the western US seaboard. Transport on pumice (e.g. Bryan et al. 2012, Naya & Hatanaka 2021) and by loggerhead turtles (e.g. Van de Vijver et al. 2020, Briscoe et al. 2021) may also help spread littoral diatoms (Van de Vijver et al.'s list of species on turtles is impressive, though these are not records of *living* diatoms).

Availability of vectors. The credibility of any claim that *D. luxuriosa* has been introduced to UK waters from the Pacific depends in part on whether an effective vector can be identified. In order to do this, it is helpful to know when *D. luxuriosa* arrived. No definite answer can be given. However, my colleagues (especially A.J. Stickle in 1983–86, who was searching specifically for naviculoid diatoms with unusual chloroplast arrangements, and S.J.M. Droop, studying *Diploneis* around the UK coastline) and I have sampled the southern coastline of the Firth of Forth extensively for over 30 years (particularly at Portobello but at times also at other sites, including Cramond, Silverknowes, Musselburgh, Prestonpans and Port Seton), using the same size of cover-slips in the same way as at Silverknowes to harvest and examine epipelon, and often at the same time of year (in autumn). However, we never found *D. luxuriosa* until 2014, either when the diatom flora was examined while still alive or afterwards in cleaned material. I therefore consider that the species has arrived recently and was not a 19th or early 20th century introduction.

Many different vectors have been shown to be effective in transporting marine algae and invertebrates. For example, Brawley et al. (2009) have shown by genetic analyses, coupled with detailed examination of shipping records, that the brown seaweed *Fucus serratus* almost certainly arrived in Nova Scotia in the first half of the 19th century, attached to ballast stones. This method of introduction is unlikely for *D. luxuriosa,* since ballast stones are no longer used by large, ocean-going ships travelling long distances. Instead, stabilization of ships travelling long distances is now achieved through use of ballast water and it has been shown (e.g. Hallegraeff 1993, Burkholder et al. 2007, Steichen et al. 2014) that this water can transport viable cells of dinoflagellates and diatoms. The *International Convention for the Control and Management of Ships' Ballast Water and Sediments*, formulated more than 15 years ago (IMO 2004), aims to reduce the risk of transporting and introducing invasive marine organisms, but so far the UK has not ratified it (IMO 2022) and the precautions that are taken to avoid introductions sometimes fail (e.g. SEPA 2011, cited by Smith et al. 2015). Conceivably, therefore, this could be a route by which *D. luxuriosa* could have recently entered UK waters. However, most viable diatoms recovered from ballast water are planktonic (Burkholder et al. 2007, Klein et al. 2010), which is unsurprising, not only because the ballast water is likely to come from the water column and might therefore be expected to contain few benthic organisms (and therefore very few if any *Diademoides*), but also because coastal planktonic diatoms often possess resting spores or other dormant stages (by which they survive unfavourable seasons in nature after sedimentation at the end of a bloom) and are consequently preadapted to long-distance transport in ballast water; such stages are almost unknown in epipelic diatoms like *Diademoides* (an exception is the freshwater genus *Craticula*: Schmid 1979).

Among other possibilities for transport is on ships' hulls or via aquaculture (e.g. Hewitt et al. 2007, Mineur et al. 2008, Minchin et al. 2013, Nunes et al. 2014). The Silverknowes site is close to Granton harbour (c. 3.5 km distant), which was formerly a harbour for fishing vessels, import of esparto grass for paper manufacture, local ferry services, and the servicing and scrapping of naval vessels (Wikipedia entry, accessed 2 May 2022: http://en.wikipedia.org/wiki/Granton,_Edinburgh) but is nowadays used mainly by small recreational craft; these are unlikely to be newly arrived from the Pacific. So, if the introduction of *D. luxuriosa* is recent, Granton is unlikely as the gateway. A little further away are two major ports – Leith (c. 6 km) and Rosyth c. 15 km) – which receive large commercial or naval vessels. Mostly, these ships ply routes in Europe or the Atlantic, but from time to time vessels arrive from further afield, such as the transporter of the 'Goliath' crane at Rosyth, which arrived in March 2011 after a three-month journey from Shanghai (http://www.bbc.co.uk/news/uk-scotland-edinburgh-east-fife-12634036). This could have transported many benthic diatom species.

Introductions of non-native macroalgae via marine aquaculture are well documented and, in several cases, have probably involved co-transport with Pacific oysters (*Crassostrea gigas*) (e.g. Verlaque et al. 2005, Mineur et al. 2010, 2012). It is therefore interesting, especially given the reports of *D. luxuriosa* on Japanese oysters, both in Japan and imported into China (Table 1), that a population of Pacific oysters has recently become established in Whitehouse Bay, less than 7 km upstream from Silverknowes on the south shore of the Firth of Forth (Smith et al. 2015). The estimated ages of the oysters ranged up to six years, giving 2007 as the latest date for establishment of the colony. Unfortunately, the source of the oysters at Whitehouse Bay is unknown: Smith et al. (2015) identified several possibilities, including via ballast water or ships' hulls or discards of oysters bought to eat, or as relics of attempts to establish oyster cultivation in the 1970s or 1980s (though these were apparently much further east than Silverknowes). Since Silverknowes and Whitehouse Bay are connected by a continuous expanse of silty sands, it is possible that *C. gigas* and *D. luxuriosa* were introduced together, in which case it may be instructive to look for *D. luxuriosa* in other UK and European sites where *C. gigas* is cultivated or has established feral populations.

To summarize: the evidence suggests that the natural range of *D. luxuriosa* is in the Pacific and that it has been introduced recently to the UK.

Navicula entoleia **Cleve (1896, p. 31, fig. 13)** ***sensu*** **Hustedt (1927–66, p. 661)**

SYNONYMS: "*Navicula cingulata*" *nom. illeg.* (Hustedt in A. Schmidt 1874–1959, pl. 403, figs 3, 4); *Pinnularia yaquinensis* Riznyk (1973, p. 130, pl. 12, fig. 13); ?*Navicula tripartita* Heiden & Kolbe (1928, p. 624, fig. 75)

TYPE: SWEDEN, Kattegat, north of Kullen (Cleve 1896, p. 13, , fig. 13 = Supplementary Fig. 3A herein). Cleve's collection, in the Swedish Museum of Natural History (S), Stockholm, is currently inaccessible and will remain so until 2025 (https://herbarium.nrm.se/, accessed 2 May 2022).

Characteristics of UK specimens. In February 2017, during a search for new localities of *D. luxuriosa*, living cells of a similar linear-lanceolate naviculoid diatom were found on Portobello beach, Edinburgh. Valves possessed a wide axial area (more than half the valve width) and short, slightly radial striae. Each short stria contained three very clearly defined areolae on the valve face, which were aligned to form longitudinal striae parallel to the valve margin. The raphe slits were not expanded at their central endings and there was no indication of accompanying ribs such as those present in *Navicula sensu stricto* (e.g. Round et al. 1990). Two living specimens were found and photographed: one is shown in Figs 11–15). Archived film recorded four specimens of a similar diatom in samples from the sublittoral of Loch Goil observed in February 1988 and December 1989 (Supplementary Fig. 4). The lengths and widths of the six specimens were (Portobello first, then Loch Goil): 32 × 14 µm, 33 × 14 µm, 26 × 11.5 µm, 28 × 11.5 µm, 33 × 13.5 µm and 36 × 13.5; the corresponding stria densities were 10, 10.3, 10.6, 10.6, 10.6 and 10.6 in 10 µm.

Cells lay in valve view. There was one chloroplast per cell, which, in its position and overall structure resembled that of *D. luxuriosa*. Differences were that the valve-appressed plates were not indented at the centre (making them H-shaped rather than butterfly-like), and that the single massive pyrenoid had a triangular cross-section and lay close to the girdle, rather than being almost central (Figs 13, 14). Another difference from *D. luxuriosa* was the presence of a prominent spherical volutin granule in each vacuole (Fig. 14, Supplementary Fig. 4C).

Identification and taxonomy. The valve morphology of the Portobello and Loch Goil specimens corresponds to '*Navicula cingulata*' as illustrated by Hustedt (1936, in Schmidt et al. 1874–1959, pl. 403, figs 3, 4) and photographed by Simonsen (1987, pl. 304, figs 1–5). *Navicula cingulata* was later synonymized with *N. entoleia* by Hustedt (1952), though *N. cingulata* was in any case an invalid name because Hustedt did not provide a formal description of the species in 1936, only illustrations. *Navicula entoleia* was first described by Cleve (1896) from a single specimen found in plankton from the Kattegat, north of Kullen, Sweden. This was very similar to the Portobello and Loch Goil specimens, with the same wide axial area, slightly radial striae, and the "puncta in rows parallel to the valve margin" (op. cit., p. 31), apart from the apices, which are more acute in Cleve's drawing. The dimensions given were 38 × 15 µm, with 11 striae in 10 µm, closely matching the Portobello and Loch Goil specimens. The illustration provided by Cleve (1896, fig. 13, which I reproduce here in Supplementary Fig. 3A) seems to show four rows of areolae on the valve face, rather than the three present in the Scottish material, but a fourth row can be seen along the edge of the valve (i.e. along the valve face–mantle junction or on the mantle) in Hustedt's (1936, in Schmidt et al. 1874–1959) illustrations of "*N. cingulata*" and this is confirmed in the photograph of one of Hustedt's specimens by Simonsen (1987, pl. 304, fig. 4). Cleve's specimen, like the Scottish specimens, had a simple raphe (i.e. the raphe appears as a narrow straight line in LM because the internal and external raphe fissures have exactly the same course), with unexpanded central raphe endings.

Cleve's daughter, Astrid Cleve-Euler (1951–55, part 3, published 1953), combined data from the original account of the species by Cleve (1896) and the illustrations of *N. cingulata* by Hustedt (in Schmidt et al. 1874–1959) and hers was the first flora to provide comparative information for the species and a key to identification, since Cleve described *N. entoleia* after his *Synopsis of naviculoid diatoms* was published. Cleve-Euler gave dimensions of 38–55 × 14–15 µm, with 11 striae in 10 µm, which are not far from the Portobello and Loch Goil specimens. Several specimens identified as *N. cingulata* or *N. entoleia* by Hustedt or R. Simonsen are present in the Hustedt collection, with photographs at http://hustedt.awi.de. These include the two specimens from Norway illustrated by Hustedt (1936) and catalogued by Simonsen (1987, p. 194) but also three from German sites (Table 2), Schlei, Stollergrund and Isefjord, which measure 31 × 13.5 µm (10.2 striae in 10 µm), 35 × 14 µm (10.2 striae in 10 µm) and 48 × ? µm (10.4 striae in 10 µm; this specimen was partly obscured and lay obliquely, so that its width could not be determined), which all agree in valve shape and pattern with the descriptions by Cleve (1896) and Cleve-Euler (1951–55).

In terms of valve outline, stria arrangement and the possession of a wide, linear-lanceolate axial area, *N. entoleia*, as illustrated by Cleve, resembles *Navicula palpebralis* Brébisson *ex* W.Smith (e.g. Hendey 1964, pl. 34, figs 13–19; Witkowski et al. 2000, pls 139, 140) and the two have similar dimensions and stria densities. However, in *N. palpebralis* the raphe is 'complex', i.e. the inner and outer raphe fissures do not follow exactly the same course, and the areolae are so fine as to be virtually undetectable in LM. Furthermore, *N. palpebralis* was well known to Cleve (e.g. 1895, p. 70), so that conspecificity with *N. entoleia* can be ruled out. Overall, then, it seems reasonable to conclude (1) that "*N. cingulata*" is indeed the same as *N. entoleia*, as concluded by Hustedt (1952, 1927–66) and Cleve-Euler (1951–55), (2) *N. entoleia* is not identical with *N. palpebralis*, and that consequently (3) the Scottish specimens, Hustedt's material and Cleve's specimen from the Kattegat can all be identified as *N. entoleia.*

Navicula entoleia is similar in shape, stria pattern and stria structure to two further described species: *Navicula tripartita* Heiden & Kolbe (1928: 46 × 12 µm with 9 striae in 10 µm) and *Pinnularia yaquinensis* Riznyk (1973: 35–40 × 13–14 µm with 10–11 striae in 10 μm). Riznyk photographed his new species (ibid., pl. 12, fig. 13) and it corresponds well to the specimens identified here as *N. entoleia*, even though the valve shown was lying obliquely. *Navicula tripartita* is more problematic. It seems that no original material exists (Simonsen 1992, p. 61) and no photographic evidence was provided by Heiden & Kolbe (1928). It is possible that Heiden & Kolbe's species is another younger synonym of *N. entoleia* and this was the conclusion of Hustedt (1927–66, part 3(4), p. 661, published 1966), who may have had access to Heiden's material (since it is in Hustedt's collection that Heiden's remaining types are located). However, although the wide stria spacing and coarse areolae of *N. tripartita* are reminiscent of *N. entoleia*, *N. tripartita* seems to be a more slender diatom and conspecificity is doubtful (Supplementary Fig. 3B, compare 3A). Neither Heiden, nor Kolbe, nor Riznyk are known to have ever to have identified *N. entoleia*, so they cannot be assumed to have been familiar with Cleve's description and illustration.

Although the arrangement of the areolae in longitudinal rows is reminiscent of *Navicula sensu stricto* (Round et al. 1990, Cox 1999), the chloroplast morphology of *N. entoleia* indicates that this species does not belong in *Navicula*: where known, all species of *Navicula sensu stricto* possess two rather simple, plate-like chloroplasts (these sometimes have lobed margins but they are not subdivided into two complex plates like those of *N. entoleia*), which lie one against each side of the girdle rather than against the valves (e.g. Cox 1996). Instead, the chloroplasts of *N. entoleia* resemble those of *Rhoicosphenia* (Mann 1982). It therefore seems unlikely that *N. entoleia* belongs in *Navicula sensu stricto*. Unfortunately, *N. entoleia* was so rare in our material that it has not been possible to find frustules to study in SEM to help establish its taxonomic position. The clearly punctate striae, lanceolate axial area and simple raphe are shared with *Austariella* (Witkowski et al. 2000, pl. 66; Joh 2013, pl. 4, figs 21–23).

A final taxonomic note is that *Navicula entoleia* might be thought to be the inspiration, and type species, for Cleve's *Navicula* sect. *Entoleiae*, which was characterized principally by the presence of a lanceolate axial area, the absence of a separate central area, and striae that are "fine, finely punctate" (Cleve 1894, p. 131). These characteristics certainly fit *N. entoleia*. However, the *Entoleiae* were defined before *N. entoleia* was described.

The previously known distribution of N. entoleia. *Navicula entoleia* is morphologically distinctive so that, even though the species has been described independently three (or possibly four) times, there is a low risk of it being confused with any other. In other words, all records of "*Navicula entoleia*", including any that exist but that I did not find, are very likely to be of the same species. I therefore accept the records by Razzhigaeva et al. (2009), even though they were not accompanied by photographic evidence. Simonsen (1962) also published no photographs but his records are backed by the photographs in the Hustedt collection, of which Simonsen was later the curator.

As with *D. luxuriosa*, *N. entoleia* was described more than 100 years ago, so that there has been a considerable time for geographical records to accumulate. After that, however, the similarities between the two species disappear. The few records of *N. entoleia* (Table 2) are from widely scattered localities in the Atlantic and Pacific Oceans and (if *N. tripartita* is a synonym) in Antarctica. No geographical pattern is evident now, nor at any time previously (the records from Sweden and Antarctica were made less than a decade apart). This remains true even if records of the species are reduced to those for which photographic evidence is available (from Germany, Scotland and Oregon). Nor does any clear habitat preference emerge from the data. The specimen that Cleve found in the plankton may have been dislodged from benthic communities elsewhere, but this is conjecture. Most records are from sublittoral sediments and in some cases from very considerable depths (–87 m at Nordåsot, –70 m at Gaussberg), but the Yaquina estuary and Portobello sites were intertidal. The tsunami deposits in which *N. entoleia* was recorded (Razzhigaeva *et al.* 2009) are by definition displaced material.

Nitzschia ocellata **Cleve in Cleve & Grunow (1880, p. 80)**

POSSIBLE SYNONYMS: ?*Amphiprora rugosa* Petit (1877, p. 187, pl. 5, fig. 17); ?*Amphiprora aculeata* Krasske (1941, p. 275, fig. 4: 12); ?*Nitzschia pustulata* Meister (Meister 1937, p. 270, pl. 11, fig. 10); ?*Nitzschia tenera* Meister (Meister 1937, p. 270, pl. 11, fig. 7)

TYPE: SPAIN, Balearic Islands (Cabrera?), collected Dr. Soderlund (Cleve 1881, p. 80, fig. 47a, b = Supplementary Fig. 5 herein), Cleve & Möller (1877–1882), exsiccatae 154, 155.

While searching slides in 2014 for cleaned valves to confirm identification of a *Psammodictyon* species photographed alive in 1984 from sublittoral sediments from Toscaig, W Scotland, I noticed a few (< 10) frustules of a bilobate *Nitzschia* species, including the one illustrated here (Figs 16–19). The frustules were highly compressed laterally and therefore lay in girdle view. The raphe was slightly eccentric and raised on a narrow keel, so that isolated valves lay slightly obliquely, with one side appearing foreshortened and the other displaying its full size. The few frustules found were nitzschioid (i.e. the narrower side of one theca lay adjacent to the wider side of the other theca: Figs 16, 18). The valves were c. 80 µm long, 7.5–8.5 µm deep at the centre and c. 10 µm (narrow side) or c. 13 µm (wide side) at the deepest point. The striae were delicate and slightly more widely spaced at the centre than elsewhere (20–23 rather than 24–25 in 10 µm). The areolae were distinct in LM (Fig. 19) and rather widely spaced. The most distinctive feature of the frustules was the presence of fibulae of different sizes at different levels beneath the raphe: close to the raphe there was an orderly row of small fibulae, with a density of 7–9 in 10 µm, while larger fibulae were present further from the raphe, forming an irregular longitudinal line containing 1–3 in 10 µm (Figs 16–18). Occasional extra fibulae were sometimes present between the two principal rows. The cingulum of each theca contained several narrow porous bands, some at least bearing two rows of pores (Fig. 19).

Identification and taxonomy. The specimens found have the same morphological and metric characteristics as *Nitzschia ocellata,* described by Cleve & Grunow (1880, p. 80) and Cleve (1881, p. 16) from Mediterranean material; the latter also illustrated the species. The metric data given by Cleve and Grunow were valve lengths (90–100 and 80–100 µm, respectively), stria densities (c. 20 and 22 in 10 µm, respectively) and fibula densities (8–9 and 8–10 in 10 µm for the row of fibulae adjacent to the raphe). Measurements from the illustrations of the two specimens (a frustule and a valve) given by Cleve (1881, pl. 4, fig. 47a, b, reproduced here as Supplementary Fig. 5) give lengths of 78 and 102 µm, maximum depths of c. 12 and 19 µm, and fibula densities of 7.5–9.5 and 8–9 in 10 µm, respectively; the central depth is c. 10.5 µm (Supplementary Fig. 5A). Cleve commented that “the striae are sharper and more distant in the centre of the valve”, agreeing with the Toscaig material, and Cleve’s claim that the striae contain “small elongate puncta” may reflect the rather distant spacing of the areolae noted above. As in Toscaig specimens, Cleve illustrated fibulae at different levels beneath the raphe. In the frustule he drew (Supplementary Fig. 5B), the fibulae were just as in the Toscaig frustule (Figs 16–19), with an orderly row of small fibulae bounding the raphe canal and a more irregular longitudinal row of large fibulae closer to the valve margin. However, in the separated valve shown by Cleve (Supplementary Fig. 5A), the larger fibulae occurred at several levels beneath the raphe, sometimes forming transverse rows of three or four.

Later authors have added little to Cleve's and Grunow's descriptions. De Toni (1892, p. 516) gave lengths of 90–108 µm, with 8–10 fibulae and 20–22 striae in 10 µm, while in the account that has probably been used most for the identification of marine benthic *Nitzschia* species, both in Europe and worldwide, Peragallo & Peragallo (1897–1908) gave similar figures of 90–110 µm long and 9–10 fibulae in 10 µm. The specimen they illustrate from Banyuls, France, has a length of 111 µm, a maximum depth of c 19 µm, a minimum depth (at the centre) of c. 12 µm, and a fibula density of 8–9 in 10 µm. It resembles the single valve of *N. ocellata* illustrated by Cleve (1881), with larger fibulae present at several levels beneath the raphe, sometimes forming short 'striae'. Lange-Bertalot & Krammer (1987, pl. 20, fig. 9) illustrate a valve c. 110 µm long, with 8–10 fibulae and 23 striae in 10 µm, from material distributed by Cleve & Möller (1877–82, preparation 155). In the Hustedt collection database (http://hustedt.awi.de/ accession H77408) there is an image of a specimen identified as *N. ocellata* from Hustedt slide 413-50 (from Pozzuoli, Naples, Italy) that is 124 µm long, has a maximum depth of 21 µm and a central depth of c. 15 µm, with 8–9 fibulae (in the row adjacent to the raphe) and 22–24 striae in 10 µm. Altogether, then, *N. ocellata* has been recorded as having valves 80–124 µm long, with a maximum depth of 10–21 µm (apparently depending on the stage of the life cycle, longer valves being deeper), a minimum depth (at the centre) of 7.5–15 µm, 7–10 fibulae in 10 µm in the row adjacent to the raphe canal, and 20–25 striae in 10 µm, the lower density occurring only at the centre of the valve.

Taxonomy. *Nitzschia ocellata* was first mentioned but not validly published by Cleve & Möller (1878). The species was first described two years later by Cleve & Grunow (1880). The morphology of the frustule – with its bilobate shape, the compressed and keeled valve, the neat row of closely spaced small fibulae parallel to the raphe, the more irregular line of scattered larger fibulae closer to the valve margins – is uncommon in the Bacillariaceae and led Grunow to place *N. ocellata* (with only one other species, namely *Nitzschia amphiprora* Grunow) in a separate section of *Nitzschia*, *Pseudo-amphiprora* (Cleve & Grunow 1880, p. 80). The morphology of *N. ocellata* is certainly reminiscent of *Amphiprora* (currently regarded as a synonym of *Entomoneis*), because of its constricted shape and multiple levels of fibulae. However, *N. ocellata* lacks the sigmoid twist to the frustule generally regarded as characteristic of *Amphiprora*. Since 1880 the species has remained classified in *Nitzschia* and the section *Pseudo-amphiprora* has been maintained (Mann et al. 2021, their supplementary fig. 1).

As with *D. luxuriosa* and *N. entoleia*, however, closer scrutiny shows that the taxonomy of *N. ocellata* is not simple, with several potential synonyms. In 1877, Petit described a new species "*Amphiprora rugosa*" from sublittoral sands off the subantarctic Campbell Island, near New Zealand. The valve shape and fibulae are like those of *N. ocellata*, and Grunow (in Cleve & Grunow 1880, p. 81) wrote "Hierher gehört vielleicht *Amphiprora rugosa* Petit"). However, the valve length (132 µm) is higher than recorded for *N. ocellata*, while the depth is proportionately less (with a maximum of c. 13 µm and a central minimum of 8.5 µm: compare with the Pozzuoli specimen mentioned above). There is insufficient detail of the striae to help confirm whether the two taxa are the same. Some of Petit's Campbell Island material was distributed by Tempère & Peragallo in their exsiccata collections but *A. rugosa* is not listed as being present (Tempère & Peragallo 1895, exsiccata 7; Tempère & Peragallo 1915, exsiccata 357). In 1889, Petit reported *N. ocellata* (as "*N.*

oscellata") but not *A. rugosa* in marine samples collected in Tierra del Fuego during the 1882 French expedition to Cape Horn to observe the transit of Venus. This implies that he did not regard *A. rugosa* and *N. ocellata* as synonymous, since otherwise his own epithet *rugosa* would have had priority and he presumably would have made this the basis of a new combination in *Nitzschia*. Unfortunately, no illustrations or specimen details were given for the Tierra del Fuego *N. ocellata*.

Meister (1937) described two new species that resemble *N. ocellata*, *N. pustulata* and *N. tenera*. Since he did not mention *N. ocellata* in his accounts, it is unclear whether he knew of *N. ocellata* and decided that neither of his species were conspecific with it, or was unaware of it. In fact, neither species agrees exactly with *N. ocellata*. *Nitzschia tenera* (Meister 1937, pl. 11, fig. 7) has exactly the same differentiation of a fairly dense row of small fibulae close to the raphe and an irregular more marginal row of large fibulae that is present in the Toscaig *N. ocellata*, and the fibula density is similar (8–9 in 10 µm), but it seems to be a more delicate diatom than *N. ocellata*, with a maximum depth of 8–10.5 µm and a minimum of 3 µm, with 28 striae in 10 µm. Lange-Bertalot & Krammer (1987) indicate possible synonymy between *N. ocellata* and *N. tenera*, suggesting that they differ only a little and could be local variations of the same species ("differieren nur wenig und könnten eher Ausdruck von Lokalvariationen derselben Art sein…"). *Nitzschia pustulata* (Meister 1937, pl. 11, fig. 10), though more closely resembling *N. ocellata* in its dimensions than *N. tenera* (76–86 µm long, maximum width 11–22 µm, 4–12 µm central width), has denser fibulae adjacent to the raphe (11–14 in 10 µm) than in *N. ocellata* and many more of the large fibulae per valve, which in LM appear to form a dense array of large 'pustules' on the keel. These are not obviously aligned in transverse rows, unlike in Cleve's fig. 47a (1881) of *N. ocellata* or the Hustedt collection photograph H77408.

In 1941, Krasske described a new species, *Amphiprora aculeata*, from the surface of the Pacific Ocean off Dichato, Chile. Photographs of the type specimen – apparently the only specimen Krasske ever found – have been provided by Lange-Bertalot et al. (1996, pl. 47, fig. 1). Measurements from the photographs, which show a complete frustule in girdle view, give a length of 77 µm (80 µm according to Krasske's protologue: see Lange-Bertalot et al. 1996, p. 37), which is the same as in the *N. ocellata* frustule illustrated by Cleve (1881, fig. 47b) and the Toscaig frustule: a maximum depth of 11 µm; a minimum depth of 5–6 µm; and a fibula density adjacent to the raphe of 10–11 in 10 µm. Unfortunately, the stria density cannot be determined from the photographs of *A. aculeata* and no information about it was provided either by Krasske (1941) or Lange-Bertalot et al. (1996), but the valves have a speckled appearance that does not correspond to what is seen in *N. ocellata* (Lange-Bertalot & Krammer 1987, contrast Figs 16–19). Although the appearance, structure and dimensions of Krasske's specimen agree well with *N. ocellata*, and although there is no evidence of a sigmoid twist to the frustule, Lange-Bertalot et al. apparently considered the species to belong in *Entomoneis* and mention a new combination "*Entomoneis aculeata* (Krasske) Lange-Bertalot" (Lange-Bertalot et al. 1993, p. 188). However, the combination was not formally proposed (contrast, for example, the transfers of *Navicula mutata* Krasske and *N. molesta* Krasske to *Sellaphora* and *Craticula*, respectively, in the same publication, on pp. 130, 131).

In summary, it is unclear whether any other named species are synonymous with *N. ocellata*. It seems quite possible that there is a group of morphologically similar and probably closely related species, all with similar valve shape and keel, and with multiple levels of fibulae beneath the raphe, but differing to some extent in their dimensions and the distributions of the larger fibulae. This interpretation is further supported by a valve present on slide GC90577, Academy of Natural Science of Philadelphia, Manila, that I examined in summer 1981. The slide was distributed by J. Tempère, who died in 1926 (www.ipni.org, searched 24 January 2018) and is labelled as containing "*Nitzschia ocellata* Cleve"; the provenance was Manila ("Manilla"), Philippines. The valve has the same overall appearance as *N. ocellata* (scanned drawing in Supplementary Fig. 6) and has similarly distantly spaced areolae but is very considerably larger and coarser, being 214 µm long, with a maximum depth of 23 µm (17 µm on the narrower side), a central depth of 14 µm (8 µm on the narrower side), 4–5 fibulae in 10 µm near the raphe, and 14 striae in 10 µm. Moreover, unlike *N. ocellata*, the fibulae closest to the raphe do not form a separate line. On the other hand, a single line of areolae can be detected within the raphe canal, as in the Toscaig frustule shown in Figs 16–19. The discrepancy between the dimensions of the Manila valve and those of Cleve's specimens and other European specimens of *N. ocellata* suggests that the valve represents a different, though probably closely related species.

Distribution. Most of the few reports of *N. ocellata* are from the Mediterranean. The first (Cleve & Möller 1878) was from the Balearic Islands, Spain, and this is the only record mentioned by Cleve & Grunow (1880) and Cleve (1881). Later reports extended the known distribution to other sites in the Mediterranean, in France, Italy, Croatia and Greece (Table 3). Not all of these records are certain, since they are not all illustrated.

The records from outside the Mediterranean are all doubtful, except ours from Toscaig, and depend on the interpretation of the possibly synonymous *Amphiprora aculeata*, *Nitzschia pustulata* and *N. tenera*, and the Manila *"N. ocellata*". In most cases where details of the sample location have been given, *N. ocellata* has been found in sublittoral sediments (Table 3). Since the sublittoral can only be sampled by divers, or from boats (or piers) using grab samplers or corers, it is far less well known than the intertidal, even in a well-studied area like the British Isles. This is illustrated by the ease with which new diatom species, even quite large-celled conspicuous forms such as *Nitzschia brachygramma* D.G. Mann & Trobajo and *Nitzschia dicrogramma* D.G. Mann & Trobajo, can be found in the sublittoral of the UK (e.g. Mann & Trobajo 2014): *Nitzschia ocellata* is probably underrecorded simply because its habitat is seldom sampled. Hence little biological significance can be attached to the absence of previous UK records of *N. ocellata* and it is quite possibly native to the UK, especially given its proven presence in the Mediterranean. What can be said with high confidence is that *N. ocellata* must be rare in the *intertidal* zone of the UK, since it would otherwise have been found by the many amateur and professional diatomists who have studied UK coasts (e.g. Hartley et al. 1996), including ourselves.

It is important to add that, even though I am not convinced that *N. ocellata* has yet been recorded anywhere outside Europe, the group of species to which it seems to belong – characterized by a 'bilobate' shape, laterally compressed valves, multiple levels of fibulae beneath the raphe, and widely separated areolae, and containing '*Amphiprora*' *aculeata*, *N. pustulata* and *N. tenera* and the Manila *"N. ocellata*" – are widely distributed, being recorded from the Atlantic and Pacific Oceans and the Mediterranean (Table 3). Thus, the perception of geographical distribution quickly changes as one ascends through the taxonomic hierarchy. This implies either that crossing major oceanic barriers is relatively easy, or that the *ocellata* lineage is old enough to predate the present configuration of the continents. As far as I know, however, there is as yet no record of the group from N America.

General discussion: criteria for establishing geographical range

In all three case histories considered here it is challenging to establish the natural geographical range, even though all the species have been known for over 100 years, with different complicating factors in each case. They lead me to list some criteria for assessing information on the geographical distributions and status (exotic vs native) of microscopic algae, especially diatoms. These modify and extend the analysis given for marine macrophytes by Boudouresque & Verlaque (2002) and reflect some of the same points made by Finlay et al. (2002). They apply to both marine and freshwater diatoms.

1. *Taxonomy*. The nomenclature and circumscription of the species must be clearly established, with no unknown synonymies that could hide records. Ideally, there should have been a peer-reviewed revision of related species and a full review of nomenclatural history (cf. Kociolek & Spaulding 2000, pp. 225, 228 ff), but there should at least have been a long history of demonstrably consistent use of the species name and its synonyms. Even after a detailed review, the only one of the three species considered here that satisfies these requirements is *D. luxuriosa*. In the case of new species and their distributions (including supposed endemism), great care needs to be taken to check that the species is not already known under a different, and possibly inappropriate name (e.g. the re-description of *D. luxuriosa* as *Mastogloia dubitabilis*'): this may be very difficult given that relevant literature is often scattered and of uneven quality.
2. *Recognition and identification*. The species must be sufficiently distinctive that it can be reliably separated from other similar species using the methods and instrumentation that have been routinely available to recorders over the period since the species was described. All three species satisfy this criterion: they are in effect 'flagship species' (sensu Tyler 1996, Foissner 2006, Hines et al. 2020). All three have moderate-sized cells (> 40 µm long) and possess distinctive features (e.g. the marginal canals of *D. luxuriosa*, the stria arrangement and structure of '*Navicula*' *entoleia*, the presence of fibulae at several levels beneath the raphe in *Nitzschia ocellata* sensu lato) that make them conspicuous in LM among co-occurring diatoms of similar shape and size. In many other diatoms, however, this criterion would not be met. Species described principally on the basis of SEM features cannot be expected to be identified reliably in LM; a diatom described as new has by definition not been available for identification previously; some diatoms are plain difficult (the *Lanceolatae* group of *Nitzschia*, small *Sellaphora*, name your favourite!).
3. *Availability of information and expertise*. It should be demonstrable that potential recorders have had access to literature or other resources (collections, photographs) that would have enabled them to identify the species. Inclusion in revisions and major floras helps enormously in this respect. The original description of *D. luxuriosa* (Greville 1863) and the first illustrated account of *N. ocellata* (Cleve 1881) both included excellent drawings that greatly facilitate identification. Moreover, while these two publications are arguably 'obscure' (both are now digitized and available online but will have been difficult to access for many people over most of the last 140 years), the two species were also included in key syntheses, such as Cleve (1894, 1895) and Peragallo & Peragallo (1897–1908). '*Navicula*' *entoleia* is more problematic, having been published after Cleve's synthesis and not included in any major work until it was invalidly redescribed as *N. cingulata* by Hustedt (1936); it did then appear in Cleve-Euler's flora of Fennoscandia (1951–55) and subsequently in Hustedt's third volume of

Rabenhorst's Kryptogamen-Flora (Hustedt 1966, in Hustedt 1927–1966) but it is quite possible that it has sometimes been mistaken for a variety of *N. palpebralis*. As a further illustration of the influence of identification texts (see also Kociolek & Spaulding 2000, p. 228), a Google search made in 2006 (and therefore before the massive recent proliferation of online databases and taxonomic resources and digitized literature) showed that the four *Cocconeis placentula* Ehrenberg varieties recognized in Hustedt's handbook of freshwater diatoms (1930: *euglypta*, *lineata*, *klinoraphis* and *pseudolineata* were included) have been mentioned much more than those excluded (*rouxii*¸ *intermedia*) or described slightly later (*tenuistriata*) (Supplementary Table 2). The last-described variety, var. *euglyptoides* (Geitler 1958), was omitted from the handbook of freshwater diatoms by Krammer & Lange-Bertalot (1991) and remains almost unreported (3 out of 845 mentions in 2006, 174 out of 48756 mentions on 1 July 2021), despite provision of a key for identifying the varieties (Geitler 1982).

4. *Vouchers and collections*. Unless there is no possibility of confusion, it must be possible to check records via adequate vouchers, which may be photographs but specimens are better (better still, specimens that still contain DNA). Listing all the herbarium specimens examined is common practice in revisions of angiosperm species, but rarer for diatoms (but see, for example, Williams 1996).The smaller the differences between species and their most similar relatives, the higher the quality required of voucher materials and the checks made using them. My searches revealed published or databased photographic documentation (available online) of all three species considered here and these were invaluable for establishing the reliability of records. Unfortunately, many researchers' collections are never transferred to permanent repositories in museums and herbaria, and many museums are not adequately funded to curate and document the collections in their charge. Photographic evidence is still very limited – often a few examples in published papers and expensive books – rather than being made available via the Web, though this is changing (e.g. https://diatoms.org/ https://naturalhistory.museumwales.ac.uk/diatoms/). Perhaps it is time to insist that biogeographical claims are always backed by photographs that are freely available online, together with publicly available material or slides in well-found institutions.
5. *Sampling adequacy*. A single record will prove presence, but absence can only be established as more or less probable, the probability depending on how intensely searches have been made in the likely places where the species might occur, at the times when it might be sufficiently abundant to be detected (cf. Foissner 2006: "13 sampling campaigns, distributed over a period of 17 months, were required to find 80% of the 160 ciliate species identified until [then] ... in just 100 m^2 of an Austrian beech forest soil"). Consequently, knowledge of the ecology and phenology of a species is important, because it can help reduce the scope of the searches we need to make. The *Paludella* example (Introduction) illustrates the point. Given its known preference for particular kinds of wetland that are rather rare in Great Britain, more or less exhaustive searches can be made for it over the whole country. The autecologies of diatoms are rarely so well understood as that of *Paludella* and none of the three species considered here are well known in this respect; there are hints that at least one of them (*N. ocellata*) would be reported more often if the deeper sublittoral (>2 m) was sampled more frequently. Where the ecology of a species is not well known, searches for it must be correspondingly wider and must have been done over a long period (at different

seasons and at different sites) if we are to have much confidence that the perceived range is real.

Williams & Reid (2006) point out (correctly of course) that it is always possible to "invoke undersampling ... on an *ad hoc* basis for never having found specimens of rare species." They then pose a question: "we haven't yet found kangaroos outside of Australia because they're (probably) very rare elsewhere – we should keep looking. But at what point do we admit that kangaroos do not, indeed, exist in Europe?". This appeal to extremes is, I think, not helpful, because it implies that we can have as much certainty about a diatom distribution as about that of kangaroos, which is simply not true. Again, the example of *Paludella* is relevant: if we know where to look, we can search efficiently; if we don't we will sometimes be lucky (cf. Hines et al.'s 2020 discovery of the 'flagship' ciliate *Condylostomides etoschensis* in N America, 12000 km from its only previous locality in Africa), more often not. Absolute certainty about a distribution is not the issue, instead avoiding claims that suggest that we can ever know a protist distribution as well as the distributions of large mammals and trees.

6. *Contamination*. The rarer a species is, the more important it is to take stringent precautions to ensure it is autochthonous. This is a problem that scarcely arises with larger, macroscopic organisms and my experience, especially while preparing vouchers of clones that have been grown for gene sequencing (and should therefore be pure), has shown how easy it is for a few valves or frustules to contaminate another sample. For instance, they can be transferred by 'inheritance' from a previous use of the glassware (we have had particular problems with re-use of filter-holders containing sintered glass supports), spitting of the hot oxidizing solutions used to clean diatoms, inadvertent use of the same pipette tip to transfer aliquots of different samples onto coverslips, and use of the same forceps to pick up and carry coverslips. Publications rarely include any information about what measures were taken to avoid cross-contamination, e.g. was glassware treated with strong alkalis to dissolve residual diatoms from a past use? were samples from different localities processed together? were forceps cleaned between samples and how? In the case of *D. luxuriosa* and '*Navicula*' *entoleia*, the new records self-validate, since the cells found were alive and there was no possible source of contamination. *Nitzschia ocellata*, on the other hand, was only found when dead, in prepared slides. I have noted above that contamination by non-UK material is very unlikely in this case, since no samples from outside the UK were collected and processed in the same period, and at the time (the early 1990s) it was common for us to clean glassware with hot concentrated NaOH solutions. However, I cannot now say that it is impossible that frustules could not have been transferred during oxidative cleaning, by aerial transfer from another UK sample being processed at the same time that we have not yet studied in detail. If so, however, that sample would have had to be particularly rich in *N. ocellata*.
7. *The limits of detection*. In order to judge the significance of 'absence', it is helpful if information is given about the limit of detection that applies to each set of observations, just as it is important to specify the limits of detection in toxicological studies (cf. de la Iglesia et al. 2013). In many biomonitoring studies, a relatively low number of valves are counted – 300–500 is often considered sufficient (e.g. CEN 2014) – and most rare species will be overlooked. Furthermore, the limit of detection will vary according to the organism (large or small, tending to occur as scattered individuals or in groups, etc) and the method of detection (microscopy of living cells or cleaned preparations, use of a

molecular probe, metabarcoding, etc). None of the three species considered here are likely to be overlooked if a slide containing them is determinedly scanned at moderate magnifications (c. ×20 objective) and hence they should be readily detectable even if comprising only 0.001–0.01% of the total diatom population examined, providing that frustules are not obscured by sediment and other extraneous material. In the case of *Diademoides*, the highly distinctive chloroplast arrangement, relative to co-occurring species, helped greatly during searches for the species at the different Firth of Forth sites. However, any previously unrecorded species that might have been present at the same relative abundance but with smaller cells or less distinctive chloroplasts (e.g. many species of *Nitzschia* or *Navicula*) would almost certainly have been missed. And of course, total enumeration of the diversity of microbial communities is impossible (cf. Finlay et al. 2002, p. 270). So, unless there is a limit to rarity set by the intrinsic characteristics of the organism (e.g. that sexual reproduction is obligate and will fail below a certain minimal population density) and without a matching sampling strategy, it is impossible to prove that a particular species is absent in a particular place or even to calculate a probability that the observations indicate it is absent. It is important to remember also that the limit of detection is not the same as the average frequency of the rarest species detected and needs to be tied to a particular predetermined level of significance. For example, if a species X comprises 0.01% of the valves in a sample and c. 10000 valves of this sample are placed on each of a number of slides, on average more than a third (36.8%) of the slides will not contain one specimen, even though the average number per slide is 1. The Poisson distribution specifies the probabilities that a rare species will be found 0, 1, 2, etc, times in a series of similar samples and indicates that, if we want to be 95% certain that species X has an average frequency below 1 valve per slide (i.e. a hypothesis of absence at 0.01%), we will have to scan the whole of three slides [the chance of recording zero valves in all three where the average number is 1 valve per slide is $(0.368)^3 = 0.05$: so, in 95% of trials, at least one valve should be recorded in at least one slide of the three], comprising in my example, c. 30000 valves. To be 99% sure (and this is still a very long way below the minimum standards of proof accepted in some physical sciences), we would have to scan the whole of 4.5 slides. Hence, a high degree of humility is always going to be appropriate when making claims that a particular diatom species is an ‘endemic’ or an ‘alien’, or has a particular specified geographical distribution, which all presuppose the absence of the species elsewhere, among all slides examined by anyone.

8. *Corroboration*. Because of the difficulties of being sure that particular records of diatoms and other microorganisms are ‘real’, as opposed to being the result of contamination or misinterpretation, and because of the difficulties in determining and interpreting absence, it is helpful to corroborate distributional data by reference to what else is known (even though it may not be much!) about species’ ecology, dispersal mechanisms, dormant stages, etc, and the external agencies that could have constrained its biogeography (such as tectonic movements, ocean currents or animal vectors – an example recently attracting attention is turtles: Van de Vijver et al. 2020). This may seem circular reasoning, given that it is the distributions that we want to detect and explain, but it helps to reduce the number of biogeographical hypotheses to be tested. It is also important to take into account the patchiness of sampling. The marine microphytobenthos has been sampled much less and much less evenly than the marine phytoplankton (and also

much less than freshwater microphytobenthos, especially of rivers). Moreover, particular geographical regions (e.g. a non-exhaustive list might include Europe in general, S Africa, parts of N America, the Caribbean, southern Brazil, and miscellaneous scattered localities in Asia and Oceania) have been sampled for marine benthos to the virtual exclusion of other areas. Such biases are well known but still seem to be underestimated while developing claims of ‘endemism’ for particular species. For the three species considered here, there seem to be fewer than 100 reports worldwide during the last 100 years (during more than 150 years for *D. luxuriosa*; Tables 1–3). All three are clearly under-recorded. Nevertheless, an apparently coherent pattern of distribution was evident for *D. luxuriosa* until 2014, indicating that it was restricted to the Pacific; no pattern can be established for the other two, lowering confidence that anything can yet be said about their full natural ranges.

An important further corroboration is whether unrelated species show similar patterns of distribution (independent of observational bias of course: works that focus entirely on the flora of a particular region are likely to generate data suggesting shared patterns of distribution), and whether the distributions of suites of phylogenetically related species are consistent with geological history (tectonic movements, appearance and disappearance of geographical barriers) and the ability of individuals to disperse (dispersion *sensu* Ebach & Williams 2016). Unfortunately, the relationships of the three species to other species are largely unknown and I am not aware of any marine littoral species that share the same circum-Pacific distribution as *D. luxuriosa*. Interestingly, a predominantly circum-Pacific distribution occurs among some freshwater diatoms, such as fossil and recent species of *Tetracyclus* (e.g. Williams 1996).

9. *The special problem of aliens*. Claims for disjunct distributions require an especially high standard of proof, because by definition the distribution will be difficult or impossible to explain from knowledge of the inherent dispersibility of the organism, even if this is available. Hence claims that a species has been recently introduced to a region should be accepted only when other explanations have been ruled out as implausible. It is also helpful if an effective vector can be demonstrated to exist. Several planktonic marine diatom species have sometimes been suggested to be non-native in Europe, notably *Coscinodiscus wailesii* Gran & Angst and *Trieres* (formerly *Biddulphia* or *Odontella*) *chinensis* (Greville) . Ashworth & E.C. Theriot. However, the same criteria that support *D. luxuriosa* as a recent introduction to the British Isles undermine the case made for the two planktonic species (Gómez 2008, Gómez & Souissi 2010). The taxonomic history of these species is poorly established, early records have not been re-examined in the light of later revisions, and no clear geographical pattern of distribution was established for either species before the supposed ‘introductions’. Hence *D. luxuriosa* may not only be a genuine alien in British waters but also represent the first plausible report of *any* introduced marine diatom *anywhere*. Of course, if *D. luxuriosa* has been transported long distances by human agency, then it is very likely that other marine benthic and planktonic diatoms have also been transferred. However, claims that particular species have been introduced need to be examined very carefully, because of the possibility that the ‘appearance’ of a species in an area where it was previously rare or absent will be attributed to introduction when the real reason is environmental change. In this connection, and given the inevitability of major shifts in the distributions of organisms as a result of climate change, environmental degradation and deliberate or accidental introductions, a

> major new international effort to systematically collect specimens and environmental DNA of benthic and planktonic diatoms would be timely.

Finally, a note on molecular screening methods, such as metabarcoding. These give new possibilities for studying marine and freshwater diatom distributions (e.g. Piredda et al. 2018, An et al. 2018, Pérez-Burillo et al. 2021) and are increasingly popular. There is no fundamental difference from microscopical approaches in the criteria that must be applied in validating molecularly-derived biogeographical data, although some of the requirements outlined above can be satisfied more easily. For example, metabarcoding approaches are essentially self-vouchering and the voucher – the sequence – is infinitely distributable without degradation or depletion, in contrast to physical specimens. Furthermore no sequence is any more ‘distinctive’ than any other, smaller diatoms are no more difficult to detect and identify than large ones (except that they may contain fewer copies of the marker gene: e.g. Vasselon et al. 2018), there is no problem of inaccessible literature, and it is common for >10,000 DNA reads to be available per sample and this number can be increased at will, with a cost implication but no decrease in the effectiveness of observation. In contrast, scanning slides microscopically for rare or small species is exhausting and increasingly unreliable as more specimens are examined and the observer tires. With the introduction of long-read molecular methods, moreover, there is a potential solution to the special problem of aliens, since it should become possible to use longer, more discriminatory markers to look for agreement between patterns of genetic variation within populations and hypothesized introductions (cf. Brawley et al. 2009). However, even with the explosive increase in metabarcoding studies that is currently taking place, it will be some time before coverage begins to match existing morphological surveys and collections (i.e. sampling adequacy is a severe problem) and problems of contamination are arguably much worse. There is also the problem of relating sequences to described taxa, despite the development of ‘curated’ DNA reference libraries (e.g. Rimet et al. 2019). The potential of existing collections of material (both preserved and mounted) thus remains huge relative to their actual and historical use, but new ways need to be found to make them widely available, such as automated slide-scanning and detection (e.g. Kloster et al 2017). Otherwise, the risks and costs of transferring and studying physical specimens, the lack of funding to help researchers to visit museums, and the shortage of museum curators who are also diatom researchers (and therefore know from personal experience the conditions needed for effective use of collections) will unfortunately make specimen-based approaches increasingly obsolete in biogeographical studies of diatoms and other microalgae, except in relation to fossils.

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Supplementary data

Supplemental data for this article can be accessed at
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Supplementary Information. Further discussion on the systematic position of *Diademoides.*

Supplementary Table 1. Voucher material for Firth of Forth sites sampled 2013–2019. In the case of the lens tissue samples, the vouchers comprise unmounted cleaned material in 70% ethanol together with two or more microscope slide preparations.

Supplementary Table 2. Records of *Cocconeis placentula* varieties found by Google searches in October 2006 and on 1 July 2021.

Supplementary Fig. 1. Greville's (1863) original illustrations of *Navicula luxuriosa* from Port Stephens, New South Wales, Australia. Scale bar = 10 µm (see main text for details).

Supplementary Fig. 2. Three picked specimens of *Diademoides luxuriosa* in a preparation by F. Sterrenburg from Sulawesi, Indonesia. A–C show different focuses of a medium-long valve, while D and E show later stages in size reduction- Scale bar = 10 µm.

Supplementary Fig. 3. Cleve's (1896) original illustration of *Navicula entoleia*, from the Kattegat (A), and Heiden & Kolbe's original illustration of *Navicula tripartita* from Antarctica (B). Scale bar = 10 µm.

Supplementary Fig. 4. Living cells of '*Navicula entoleia*' from the sublittoral of Loch Goil, collected in 1988 and 1989. A–C. Three focuses of a single cell, showing the valve and chloroplast lobes (A), peripheral focus of chloroplast bridge with droplets of reserve material (B) and mid focus (C) with nucleus (n) and volutin granules (e.g. v). D. Short specimen in mid focus: note the triangular profile of the pyrenoid (arrow: compare Figs 3 and 7). Figs E–H. A single cell, focused on a valve, upper plastid lobes, midsection, and lower plastid lobes, respectively, with nucleus (n) and triangular pyrenoid profile (arrow). Scale bar = 10 µm.

Supplementary Fig. 5. The drawings of *Nitzschia ocellata* provided by Cleve (1881, pl. 4, fig. 47a, b), showing a valve (A) and a frustule (B). Scale bar = 10 µm.

Supplementary Fig. 6. Valve identified as *Nitzschia ocellata* on Academy of Natural Sciences of Philadelphia slide GC90577, drawn by D.G. Mann in 1981. Note the absence of an orderly row of smaller fibulae close to the raphe. Scale bars = 10 µm.

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Table 1. Records of *Diademoides luxuriosa* (most were made under the name *Navicula luxuriosa*; other names used are given in the footnotes) from published literature, online databases, and slides in museum collections. Records are listed in geographical order, clockwise around the Pacific rim.

Site sampled	**Country**	**Date of sample**	**Identification or source of information**
Pacific Ocean			
Company Bay, near Dunedin, South Island	New Zealand	before 1959	Wood (1961b)
Beach sand, Auckland, North Island	New Zealand	1858[1]	Grunow (1867)
Macquarie Harbour, Tasmania	Australia	late Holocene	McMinn *et al.* (2003)
Vegetation on sandy-clay from 10–20 cm depth, lagoon, Victoria	Australia	1966	Foged (1978)
Sublittoral dredging, Port Stephens[2], New South Wales	Australia	1862[3]	Greville (1863)
Shells dredged from 6–8 fathoms, Port Jackson, New South Wales	Australia	?1874	BM 32060, Coll. Comber
Sublittoral dredging, Jibbon Beach, New South Wales	Australia	before 1961	Wood (1961a)
Sublittoral dredging, Port Hacking, New South Wales	Australia	before 1961	Wood (1961a)
Scrapings from sandy clay on banks with *Mesembryanthemum* and mangrove; brackish to salt water, Big River, 17 miles N of Emigrant Creek, New South Wales	Australia	1966	Foged (1978)
Caloundra, S Queensland	Australia	before 1902[4]	BM 32060, Coll. Comber
Scrapings and brown algae on beach boulders, Noosa Head, S Queensland	Australia	1966	Foged (1978)
Port Douglas, N Queensland	Australia	before 1989	Kemp & Paddock (1989)
North-western Australia	Australia	before 1927[5]	BM 31782, Coll. F.W. Payne
Swan River, Perth, W Australia	Australia	1977, 1981	John (1983, 2012)
Sulawesi	Indonesia	?1991[6]	Prepared slide of F. Sterrenburg

[No details given]	P.R. China	before 1894	Cleve (1894)[7]
Guangxi coast	P.R. China	1992	Fan et al. (1993)
On algae, Xiamen, Fujian	P.R. China	before 1985	Jin *et al.* (1985)
On algae, Pingtan Island, Fujian	P.R. China	before 1985	Jin *et al.* (1985)
Holothurians	P.R. China	1938	N5-97, Hustedt Coll. [8]
Along the China coast, "as far north as Qingdao and Weihaiwei [Weihai]"	P.R. China	before 1942	Voigt (1942)[9]
Porphyra mariculture sites, Chinmen Island, off P.R. China	Taiwan	1977	Huang (1984)[10]
Intertidal sandflats and beaches, Nakdong River estuary and adjoining coasts	S Korea	1986–2013	Joh (2013)
Nagasaki shellfish, offered for sale in Shanghai, China	Japan	1933/4[11]	Meister (1937)[9]
Hiragata Inlet, Kanazawa-Ku, Yokohama	Japan	20th century	MPC-01189, Tsumura Coll. (National Museum of Nature and Science, Japan)[12]
Obuchi, Rokkasho	Japan	20th century	MPC-01273 and -01294, Tsumura Coll. (National Museum of Nature and Science, Japan)[12]
Hakodate, Hokkaido	Japan	before 1927[5]	BM 39720, Coll. F.W. Payne
On oysters	Japan	1889/1900	Tempère & Peragallo (1915)
Holothurians	Japan	before 1891	Brun (1891)[13]
[No details given, probably the same as the record by Brun]	Japan	before 1894	Cleve (1894)
Sea Island, Fraser River, Richmond, British Columbia	Canada	before 1938[14]	Adams Coll. (BM), slide T.S. 630

On seaweeds, Port Townsend, near Seattle, N Washington State	USA	before 1908	Tempère & Peragallo (1915)[15]
Port Townsend, near Seattle, N Washington State	USA	before 1927	Boyer (1927)[16]; N5-07, Hustedt Coll.[8]
Long Beach, S Washington State	USA	1982/3	Tynni (1986)
Intertidal sandflats, Yaquina Estuary, Oregon	USA	before 1969	Riznyk (1973)[17]
Atlantic Ocean			
Intertidal sandflats at Silverknowes, City of Edinburgh, E Scotland	UK	2014, 2015, 2018, 2019	This paper[18]
Intertidal sandflats at Port Seton and Longniddry, E Lothian, E Scotland	UK	2016	This paper[18]
Intertidal sandflats at Portobello, City of Edinburgh, E Scotland	UK	2017	This paper[18]

[1] Date obtained from Frauenfeld (1860, p. 733).

[2] As Port Stephen.

[3] For evidence for the 1862 date of collection, see Kemp & Paddock (1989, caption to fig. 1).

[4] Thomas Comber died in 1902: see http://www.ipni.org/

[5] Frederick William Payne died in 1927: see http://www.ipni.org/

[6] Sterrenburg's slide is undated; he studied material from S Sulawesi collected in 1991 (Sterrenburg et al. 1995), but it is unknown to me whether this was the source of the *Diademoides* specimens. Three valves from the slide are illustrated here in Supplementary Fig. 2.

[7] According to Jin *et al.* (1985), Cleve's record refers to the South China Sea.

[8] Record in Hustedt collection database, Alfred Wegener Institut, Bremerhaven (http://hustedt.awi.de): search 11 January 2018

[9] As "*Mastogloia? dubitabilis* Meister".

[10] As "*Mastogloia dubitabilis* Meister?".

[11] Date and sample details are given by Meister (1935, p. 96).

[12] Slides and specimens in the Tsumura collection are illustrated online in the Japan Paleobiology Database at http://jpaleodb.org/hierarchy; the habitats recorded are marine (Hiragata) or brackish (Obuchi): see also Nomura et al. (2006).

[13] As "*Navicula luxuriosa* var. *cuneata*".

[14] Frederick Adams died in 1938: see http://www.ipni.org/

[15] As "*Navicula luxuriosa* var. *abrupta*".

[16] Boyer (1927) does not cite Tempère & Peragallo (1915) and includes information that indicates that he identified *N. luxuriosa* independently from them and with reference instead to Brun's (1891) description. It is therefore not certain that the material he examined was a Tempère & Peragallo slide, though it seems likely.

[17] As "*Neidium opacelineatum*"; see also Tynni (1986, p. 20).

[18] Voucher material (in the diatom herbarium, Royal Botanic Garden Edinburgh): see Supplementary Table 1.

Table 2. Records of '*Navicula*' *entoleia*, including those of possible synonyms.

Site sampled	**Country**	**Date of sample**	**Source of information**
Antarctic Ocean			
?Sublittoral sediment, Gaussberg	Antarctica	8 October 1902	Heiden & Kolbe (1928)[1]
Pacific Ocean			
Marine tsunami deposit, Ainu Bay, Matua (Kuril Islands: Pacific Ocean)	Russia	before 2009	Razzhigaeva et al. (2009)
Marine tsunami deposit, Simushir (Kuril Islands, Pacific Ocean)	Russia	before 2009	Razzhigaeva et al. (2009)
Intertidal sandflats, Yaquina Estuary, Oregon	USA	before 1969	Riznyk (1973)[2]
Atlantic Ocean			
Sublittoral sand, –6 m, Schlei estuary, Schleswig-Holstein	Germany	1954	image from slide SIM5-03, Hustedt collection database[3]
Sublittoral silty sand and sand, –16.5 and –9 m, Stollergrund, Kiel fjord, Schleswig-Holstein	Germany	1955	images from slides SIM5-15, SIM5-16, Hustedt collection database[3]
Sublittoral fine sand, –10 m, Isefjord, Schleswig-Holstein	Germany	1956	image from slide SIM7-10, Hustedt collection database[3]
Belt Sea	Germany	before 1962	Simonsen (1962)[4]
Sublittoral sediment, "Nordåsot"[5]	Norway	before 1936	Hustedt in Schmidt (1874–1959, pl. 403, figs 3, 4)[6]
Plankton, Kattegat off Kullen	Sweden	before 1896	Cleve (1896)[7]

Sublittoral sediments, Loch Goil, Argyll, W Scotland	UK	1988–1990	this paper
Intertidal epipelon, Portobello beach, Edinburgh, SE Scotland	UK	2017	this paper

[1] This record is doubtful, depending on the arguable synonymy of *Navicula tripartita* with *N. entoleia*

[2] As *Pinnularia yaquinensis*.

[3] At http:/hustedt.awi.de

[4] Simonsen remarked (p. 111) that *N. entoleia* is one of a group of species that is found in the Belt Sea but was not recorded along nearby North Sea coasts by Hustedt (1939) and Brockmann (1950).

[5] Exact location unknown.

[6] As *Navicula cingulata*.

[7] In the eventuality that Cleve's *N. entoleia* proves to differ from the concept of the species developed by Hustedt and Simonsen (if, for example, the type specimen still exists and its characteristics can be determined conclusively and shown to differ from the diatom considered here), it is arguable that Hustedt's (1952) concept should be conserved (following Article 14.9 on the International Code: https://www.iapt-taxon.org/nomen/pages/main/art_14.html), since all records of the species have relied on this concept, elaborated by Cleve-Euler (1951–55) and Hustedt (1927–66).

Table 3. Records of *Nitzschia ocellata* and similar diatoms classified in *Nitzschia* or *Amphiprora*. Not all of these records are necessarily independent: all the Balearic Islands records may derive from the same original sample used by Cleve & Möller in 1878, and the illustration of *N. ocellata* by Peragallo & Peragallo (1897–1908) may have been made from one of the samples distributed as exsiccata slides by Tempère & Peragallo (1895, 1915).

Site sampled	**Country**	**Date of sample**	**Identification**	**Source of information**
Mediterranean Sea				
Balearic Islands, Mediterranean Sea[1]	Spain	before 1878	*N. ocellata*	Cleve & Möller (1878), exsiccatae 154, 155; Cleve & Grunow (1880); Cleve (1881)
Balearic Islands, Mediterranean Sea	Spain	?	*N. ocellata*	R.W. Kolbe, slide S122854-01-023, Swedish Museum of Natural History, Stockholm [http://www.nrm.se/english/researchandcollections/palaeobiology/collections/databases/diatomaceae/ktdiatomtaxa/diatomtaxaneo.10860.html]; F. Hustedt, unpublished[2]
Dredging "No. 3", Banyuls, Mediterranean Sea	France	before 1895	?*N. ocellata*[3]	Tempère & Peragallo (1895), exsiccata 278
Dredging "No. 4", Banyuls, Mediterranean Sea	France	before 1895	*N. ocellata*	Tempère & Peragallo (1895), exsiccatae 310, 341
Dredgings, Banyuls-sur-Mer, Mediterranean Sea	France	before 1915	?*N. ocellata*[3]	Tempère & Peragallo (1915), exsiccatae 285–7
Banyuls, Mediterranean Sea	France	before 1908	*N. ocellata*	Peragallo & Peragallo (1897–1908), pl. 70, fig. 32
Sediments at 30–100 m depth, Gulf of Marseilles, Mediterranean Sea	France	1964	*N. ocellata*	Plante-Cuny (1969)
Naples, Mediterranean Sea	Italy	before 1908	*N. ocellata*	Peragallo & Peragallo (1897–1908), p. 273
Bocca Piccola strait, Gulf of Naples, Mediterranean Sea	Italy	before 1959	*N. ocellata*	F. Hustedt, unpublished[2]
Holothurians, 45 m depth, Pozzuoli, Gulf of Naples, Mediterranean Sea	Italy	before 1959	*N. ocellata*	F. Hustedt, unpublished[2]

Holothurians, Rovinj, Mediterranean (Adriatic) Sea	Croatia	before 1879	*N. ocellata*	Cleve & Möller (1877–1882), exsiccatae 208–210
Pula, Mediterranean (Adriatic) Sea	Croatia	1908	*N. ocellata*	F. Hustedt, unpublished[2]
Sediments at 3–5 m depth, N of Agios Nicolaos, Crete (35° 14′ 21.32″ N, 25° 44′ 06.80″ E), Mediterranean Sea	Greece	October 2011	*N. ocellata*	M. Loir [http://www.diatomloir.eu/Site%20Diatom/List%20diatoms%20from%20Crete.html]
Sediments near hydrothermal vents at 7 m depth, Milos, Mediterranean (Aegean)Sea	Greece	1996/7	*N. ocellata*	Drs I. Louvrou and D. Danielidis, personal communication
Atlantic Ocean				
Sublittoral sand at –12 m, off Toscaig pier, near Applecross, W Scotland, 12 m depth	UK	1994	*N. ocellata*	This paper
Red Sea				
Benthos, upper sublittoral at Sharm-el-Sheikh	Egypt	2014/15	*N. ocellata*	Gerasimyuk (2019)[4]
Pacific Ocean				
Unspecified marine material[5], Orange Bay (Bahia Nassau), Hoste Island, Tierra del Fuego	Chile	1882	?*N. ocellata*[6]	Petit (1889)
Surface waters, Dichato Bay	Chile	before 1941	*N.* cf. *ocellata*[7]	Krasske (1941)[8]
Sublittoral sediments, Perseverance Harbour, Campbell Island	New Zealand	1874	*N.* cf. *ocellata*[7]	Petit (1877)[9]
Manila	Philippines	before 1926[10]	*N.* cf. *ocellata*[7]	slide preparation GC90577, Academy of Natural Science of Philadelphia, examined by D.G. Mann, summer 1981
Nagasaki	Japan	1933/34[11]	*N. pustulata*	Meister (1937)
Nagasaki	Japan	1933/34[11]	*N. tenera*	Meister (1937)

Other				
Swamp, Lower Save River	Mozambique	2015	?*N. ocellata*	Christiansson (2016)[12]

[1] From the island of Cabrera according to Colmeiro (1889, p. 1055).

[2] Record in Hustedt collection, Alfred Wegener Institut, Bremerhaven: database search 24 February 2015. The collection database includes an image of a specimen from Pozzuoli (specimen i.d. H77408: http://hustedt.awi.de)

[3] As *Nitzschia* "*oculata*". No *Nitzschia* species has ever been described with the name '*oculata*'. Given that Peragallo & Peragallo (1897–1908) specifically record *N. ocellata* from Banyuls, and Tempère & Peragallo (1895, exsiccatae 310, 341) list *N. ocellata* from Banyuls dredgings, it seems likely that the two records of *N.* '*oculata*' from Banyuls dredgings are of *N. ocellata* and that the name is a misspelling.

[4] Unfortunately this record, one of the few outside the Mediterranean, was not backed by a photograph; in addition it is well known that 'leakage' of species does occur through the Suez canal, raising the possibility that *N. ocellata* could be nonnative there.

[5] The marine material studied by Petit included dredgings of sublittoral sediment, drift seaweeds, and mollusc shells.

[6] As *Nitzschia* "*oscellata*". Record not supported by any illustration or description.

[7] See text for an assessment of the identification

[8] As "*Amphiprora aculeata*".

[9] As "*Amphiprora rugosa*".

[10] The slide preparation was made and distributed by J. Tempère, who died in 1926 (www.ipni.org, searched 24 January 2018)

[11] The date is implied by the information given by Meister (1935, 1937) about the provenance – the sample was provided by M. Voigt.

[12] No voucher photographs were provided and the habitat would be a first record in less than fully marine conditions.

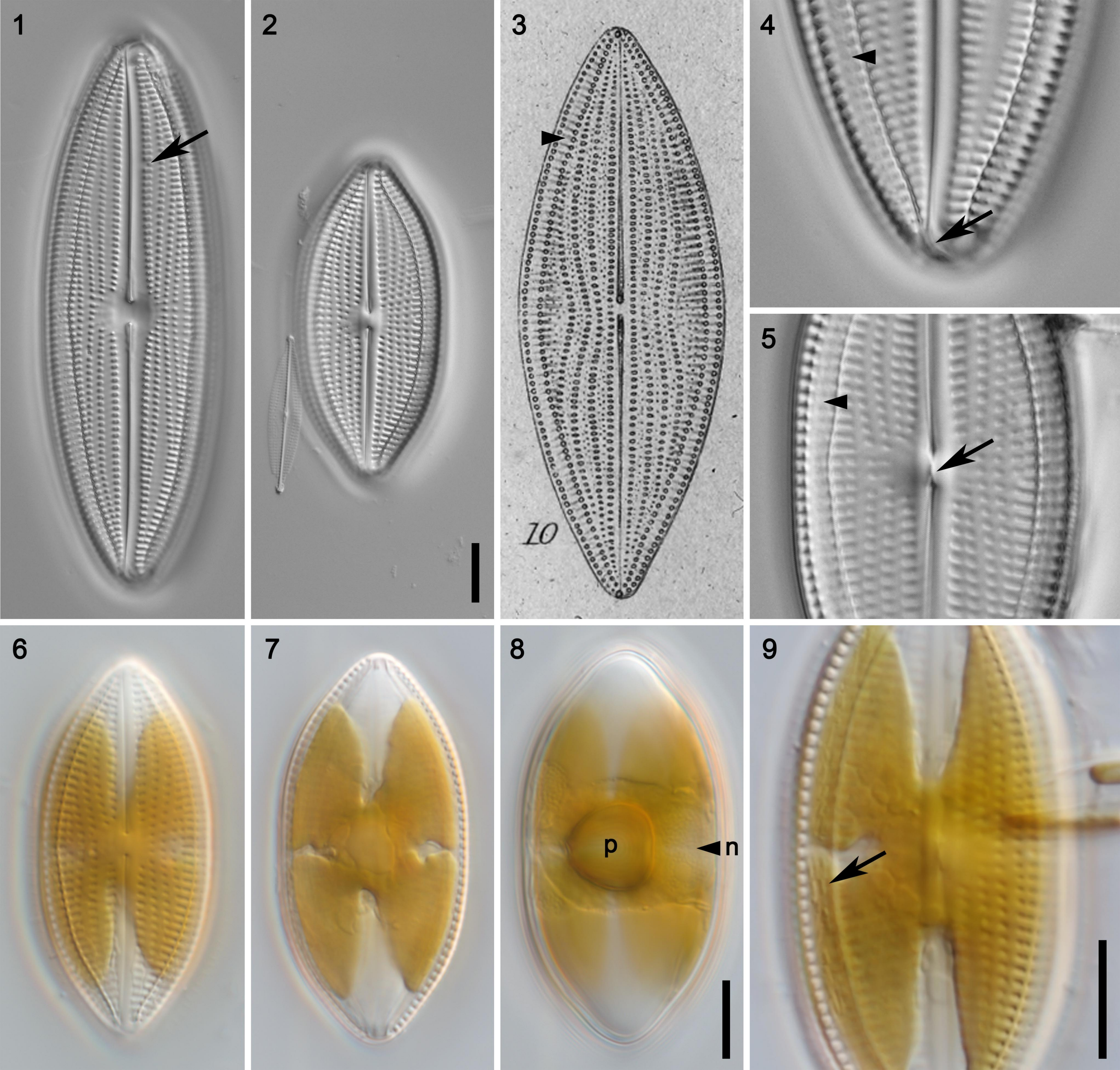
Figure legends

Figs 1–9. *Diademoides luxuriosa*, from the foreshore at Silverknowes, City of Edinburgh, Scotland: cleaned valves (Figs 1–5) and live cells (Figs 6–9), LM, differential interference contrast optics. Fig. 1. Large (post-auxospore?) valve. Note the organization of the areolae into slightly radial transverse striae and biarcuate longitudinal striae. A line running parallel to the valve margin on each side marks the boundary of a longitudinal canal. The secondary side of the valve is on the right, as demonstrated by the positions of the Voigt discontinuities (arrow). Fig. 2. Small valve. Fig. 3. Drawing by Greville (1863, fig. 10). Fig. 4. Detail of pole with deflected terminal raphe fissure (arrow); note that the striae appear to continue into the walls of the marginal longitudinal canal (arrowhead). Fig. 5. Detail of valve centre showing a short ridge (arrow) internally, between the raphe endings. The focal plane passes through the empty lumen of the longitudinal canal (arrowhead). Figs 6, 7. Peripheral focuses on the upper side of a cell showing butterfly-like lobing of the chloroplast; the lower (far) side of the cell exhibited a similar appearance. Fig. 8. Mid focus of cell, showing the stout cylindrical pyrenoid (p) that links the two butterfly-like halves of the chloroplast. The pyrenoid is positioned slightly to one side of the centre in a bridge of cytoplasm that also contains the nucleus (n). Fig. 9. Focus on the lumen of one of the longitudinal canals: irregular striations (e.g. at arrow) suggest the presence of bacteria within the canal. Scale bars = 10 µm (for Figs 1, 2, see Fig. 2; for Figs 4, 5 and 9, see Fig. 9; for Figs 6–8, see Fig. 8).

Fig. 10. The known distribution of *Diademoides luxuriosa* (see also Table 1). Green = the type locality; ochre = pre-1910 records; blue = records 1910 to present; red = new UK records; brown = imprecisely located records (‘NW Australia’, ‘China’, ‘Japan’). In addition, the map indicates (with yellow shading) a subjective assessment of the parts of the world’s coastlines that have been relatively well studied but in which *D. luxuriosa* has not been recorded.

Figs 11–15. *Navicula entoleia*, Portobello Beach, February 2017: a single living specimen photographed at different focal levels (selected photographs from a series). Similar photographs but of Loch Goil specimens are given in Supplementary Fig. 4. Fig. 11. Valve: the original image was processed using the High Pass filter in Adobe Photoshop, to suppress large-scale features such as the nucleus and chloroplast. Fig. 12. Upper chloroplast lobes. Fig. 13. Median focus showing nucleus suspended in a bridge of cytoplasm between two apical vacuoles (n = nucleus, p = pyrenoid, arrowheads = putative lipid droplets). Fig. 14. Lower focus, close to the outer boundaries of the vacuoles, showing spherical volutin granules (e.g. arrow). Fig. 15. Lower chloroplast lobes. Scale bar = 10 µm.

Figs 16–19. *Nitzschia ocellata*, from off Toscaig Pier, Applecross, W Scotland: a single cleaned frustule lying in girdle view. LM, differential interference contrast optics. The cell to which the frustule belonged had recently divided when it died and hence the frustule contains two fully formed hypothecae. The frustule is highly compressed, so that the raphe system (lying along the margins in this view) lies at the apex of a narrow keel. Figs 16–18. Whole frustule photographed in different planes, showing the nitzschioid symmetry of the parent cell and both daughter cells. The fibulae (appearing as refractile spots) form two series: in one the fibulae are small and closely spaced in a row parallel to the raphe, while the second comprises larger, more widely spaced fibulae in a somewhat irregular row (particularly irregular in the valve shown in-focus in Fig. 17) near the valve margins. Fig. 18. Detail of one theca, showing fine striae on the valve, each containing small widely spaced areolae. The first (most advalvar) girdle band bears two rows of areolae (arrow). Scale bars = 10 µm (for Figs 16–18, see Fig. 18).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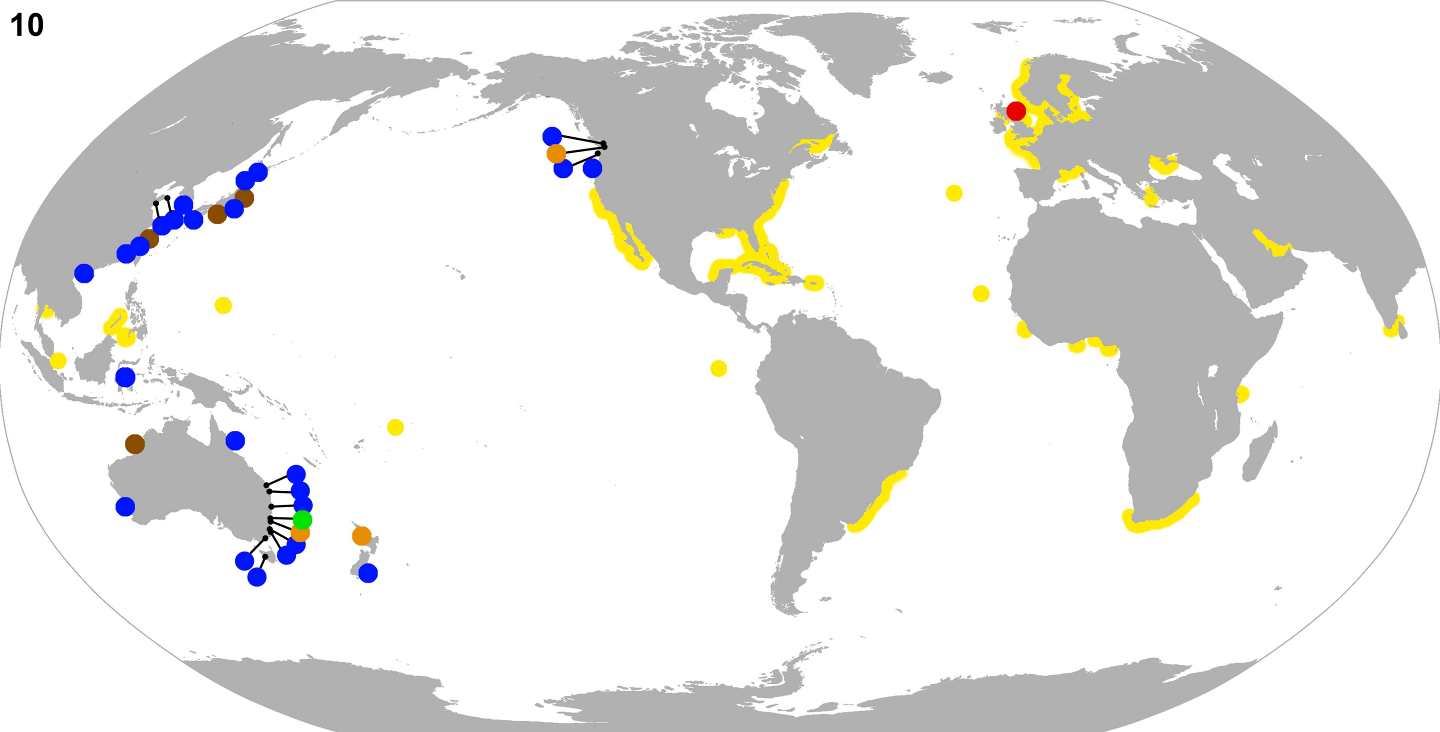
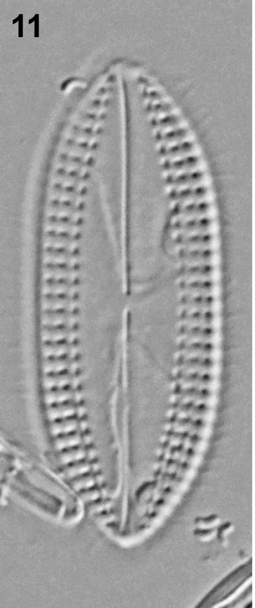
10

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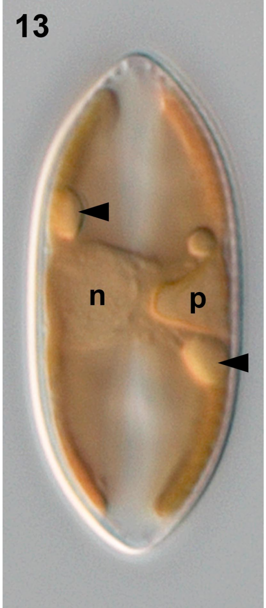
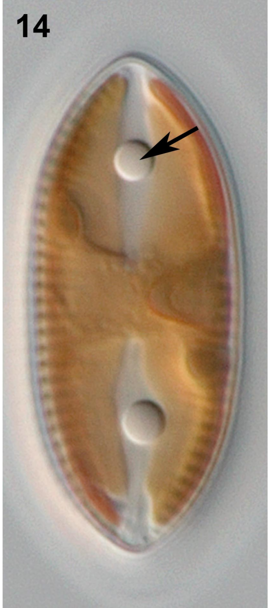

13
n
p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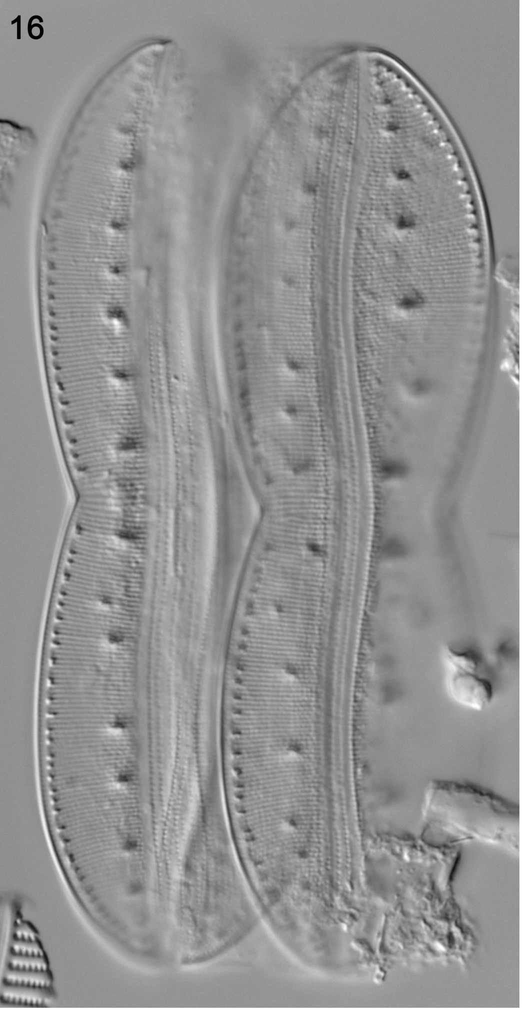
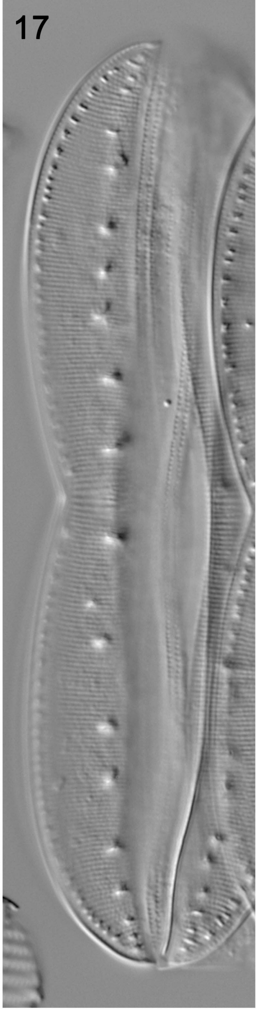
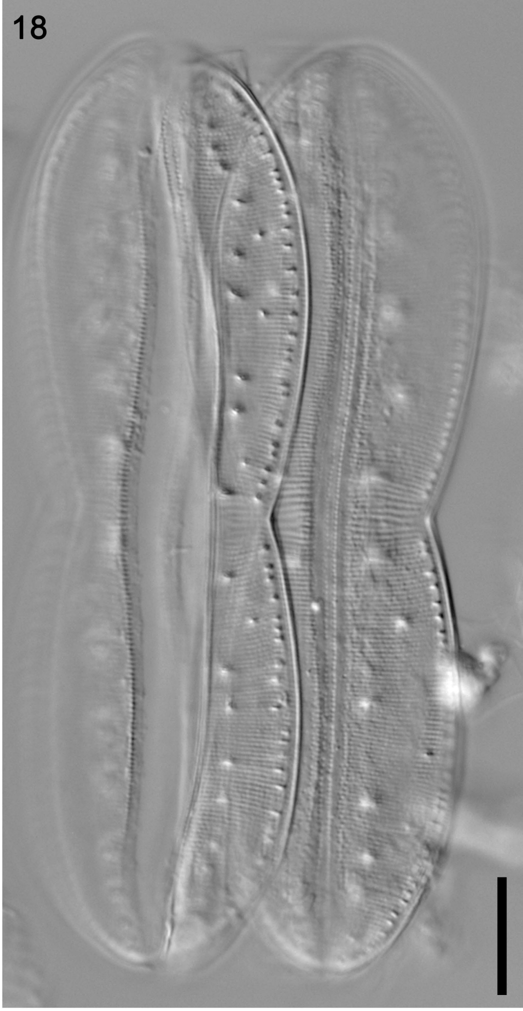


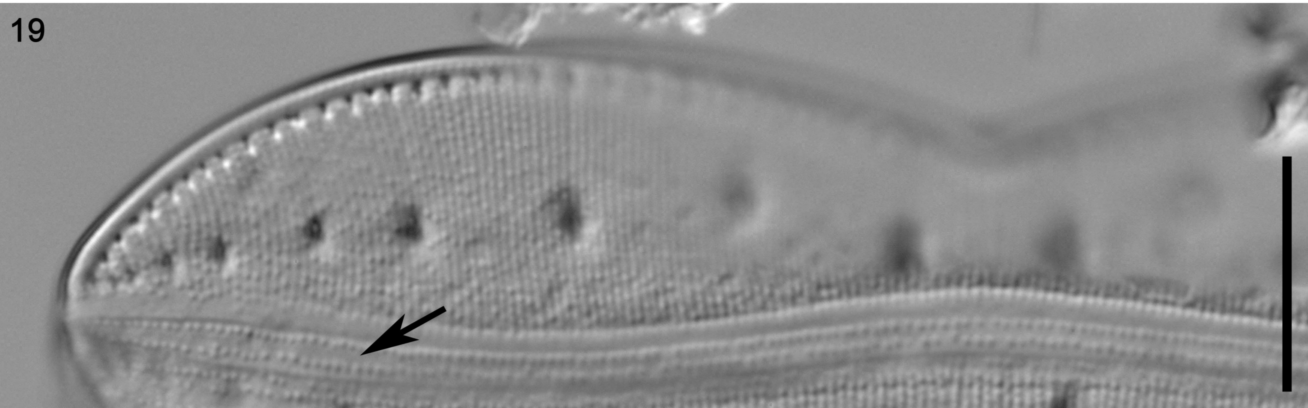
17



18



19



The systematic position of **Diademoides luxuriosa**

Diademoides luxuriosa has never been reported living before and so the current paper is the first to include cytological detail for the genus. Here I evaluate what frustule and chloroplast morphology may imply concerning the systematic position of *Diademoides.*

Some frustule characteristics, particularly the presence of longitudinal hollows in the valves and a double helictoglossa at the centre, connecting the internal raphe endings, suggest a link between *Diademoides* and *Neidium, Biremis* and related genera: it is perhaps not surprising that Riznyk (1973) classified *D. luxuriosa* in *Neidium*. Kemp & Paddock (1989) showed that there is a longitudinal canal at the margin of the valve face in *Diademoides* and that this is continuous from pole to pole. It opens to the outside by rows of unoccluded holes (foramina), while the inner wall of the canal is like a sieve, being perforated by multitudes of tiny pores. This kind of structure – longitudinal canals opening externally by unoccluded pores and internally by sieve-like occlusions or hymenes – is also present in *Neidium, Biremis* and *Scoliopleura* (Round *et al.* 1990, Witkowski *et al.* 2014), which group together in molecular phylogenies (Witkowski *et al.* 2014), and in *Muelleria*, *Progonoia* and *Scoliotropis*, for which no DNA sequences are yet available. The positions of the canals vary: in *Neidium* and *Diademoides*, they lie at the junction of valve face and mantle (Kemp & Paddock 1989, Round et al. 1990), whereas in *Scoliopleura, Scoliotropis* and *Muelleria*, the canals are adjacent to the raphe-sternum (Round et al. 1990, Spaulding et al. 1999). In *Biremis* and *Progonoia* there are marginal chambers with external foramina and internal sieves (Round et al. 1990) as in *Scoliotropis* and *Diademoides*, but it is unclear whether these are fully interconnected to form a continuous longitudinal canal. All of the genera mentioned in this paragraph are also linked by possession of a small ridge or beak between the central raphe endings internally, which resembles two helictoglossae fused back-to-back (Round et al. 1990, Spaulding et al. 1999, Van de Vijver et al. 2014); Kemp & Paddock (1989) do not refer to this feature in their description of *D. luxuriosa* but it is visible in their fig. 7.

Neidium also possesses longitudinal canals, but is differentiated from the other genera mentioned above in that the canals are not clearly differentiated from the remainder of the valve structure and their internal walls do not bulge into the cell lumen. However, *Neidium* groups with *Biremis* and *Scoliopleura* in molecular phylogenies (e.g.

Witkowski *et al.* 2014, Nakov et al. 2015) and, as already mentioned, also possesses double helictoglossae at the centre internally.

The single chloroplast of *Diademoides*, comprising two butterfly-like plates linked by a large, almost central pyrenoid, is unusual but by no means unknown among naviculoid diatoms: it is characteristic, for example, of *Placoneis* species, *Cavinula scutiformis*, and *Lyrella* cf. *sandriana* (unpublished observations of H.M. Jones, A.J. Stickle and D.G. Mann; for *Placoneis*, see also Mann & Stickle 1995 and Cox 1996). Furthermore, a similar chloroplast, but usually with a more strongly eccentric pyrenoid bridge (lying near or against the girdle) is present in (1) the naviculoid genera *Dickieia* (Cox 1985, Mann 1994), *Luticola* (Cox 1985, Poulíčková 2008) and *Anomoeoneis* (Pfitzer 1871, Cox 1985), and in '*Navicula*' *cluthensis* (one of the many naviculoid diatoms that do not belong in *Navicula sensu stricto* but have still not been reclassified) (my unpublished observations); (2) the heteropolar and heterovalvar *Rhoicosphenia* (Mann 1982); and (3) the heteropolar or dorsiventral genera *Cymbella*, *Encyonema*, *Gomphonema*, *Didymosphenia* and *Reimeria* (Cox 1996, Kulikovskiy et al. 2014), which are closely related to each other and also to *Placoneis* (including *Geissleria*) and *Anomoeoneis*, according to morphological and molecular data (Round et al. 1990, Bruder & Medlin 2007, Kermarrec et al. 2011, Nakov et al. 2014, 2018) – all these five genera will be referred to below as the Cymbellales, although the exact limits of this order have yet to be established (Nakov et al. 2014).

Mereschkowsky (e.g. 1903) classified diatoms with the kind of chloroplast present in *Diademoides*, Cymbellales, *Luticola* and the other genera listed above, in a group that he considered natural (i.e. monophyletic), which he referred to as the *Diatomea Pyrenophora* (or 'Pyrenophoreen'). However, molecular phylogenetic analyses indicate that, whereas *Rhoicosphenia* and *Dickieia* (especially the latter) are possible members of the Cymbellales (Nakov 2014), *Luticola* is probably related instead to diatoms with different types of chloroplast (Bruder & Medlin 2008, Nakov et al. 2014), while *Lyrella sandriana* is the only species of *Lyrella* known so far to have a pyrenophorean chloroplast (other species have two or four chloroplasts per cell and the pyrenoids are not linking structures: e.g. Mann & Stickle 1993, 1997). Thus, the pyrenophorean chloroplast is not, on its own, a foolproof guide to relationships and Mereschkowsky's *Diatomea Pyrenophora* is not a monophyletic group. Consistent with this, while some *Lyrella* species (e.g. *L. cassiteridum*: Mann 1998), *Rhoicosphenia*, *Anomoeoneis* and Cymbellales have hooked internal central raphe endings (though these may be obscured in fully formed valves of Cymbellales by an overgrowth of silica: e.g. Round et al. 1990), *Diademoides*, *Dickieia* and *Luticola* do not (Kemp & Paddock 1989, Round et al. 1990, Cox 1985).

Summarizing, *Diademoides* is linked to the *Neidium–Biremis* group by frustule characters, whereas the chloroplast morphology would seem to indicate a closer relationship to other naviculoid diatoms, perhaps *Dickieia* or *Luticola*. However, although no members of the *Neidium–Biremis* group are yet known that have a single pyrenophorean plastid like that in *Diademoides*, two plastids of not dissimilar structure are present in the cells of some *Biremis* species, such as that illustrated by Witkowski et al. (2014, fig. 7), though they are orientated with the butterfly-like plates against the girdle rather than the valves. This difference may not be as great as it appears, given that rearrangements of the chloroplasts and even a change in chloroplast number can occur during the life cycle of some *Biremis* in culture (Cox 1990).

Overall, then, and without a formal analysis (information about chloroplast characters is not available for all the taxa of interest, including *Scoliopleura* and *Progonoia*) the chloroplast data are currently equivocal, but I suggest on the basis of the valve structure that *Diademoides* belongs in the *Neidium*–Scoliotropidaceae group (*sensu* Round et al (1990), characterized by (1) the possession of an apparently unique type of longitudinal canal in the valves, which opens to the exterior by one or a few foramina opposite each transapical stria, and internally by a finely porous plate, and (2) a double helictoglossa at the centre internally. *Neidium* differs from the other genera of this group in that the longitudinal canals do not bulge into the interior of the cell but are instead integrated into the thickness of the valve. It is unclear whether the Neidiaceae can be separated from the Scoliotropidaceae as was done by Round et al. (1990). If the molecular tree of Witkowski et al. (2014) accurately reflects the course of evolution, the Scoliotropidaceae (represented there by *Biremis* and *Scoliopleura*) would be paraphyletic.

There is no evidence of a relationship to *Mastogloia*, contrary to the classification of '*Mastogloia dubitabilis*' by Meister (1937): the marginal chambers in *Mastogloia* are part of the girdle (internal developments of the first band = valvocopula), not the valve.

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Supplementary Table 1. Voucher material for Firth of Forth sites sampled 2013–2019. In the case of the lens tissue samples, the vouchers comprise unmounted cleaned material in 70% ethanol together with two or more microscope slide preparations.

Locality	**Sample information**	**Date of sample**	**Accessions**
Silverknowes, City of Edinburgh	intertidal silty sand, epipelon harvested by cover-slip	22 April 2013	Slides E6102.1, E6102.2, E6102.3
Silverknowes, City of Edinburgh	intertidal silty sand, epipelon harvested by cover-slip	8 October 2014, harvested 10 October 2014	Slides 5797.1, E5797.2, E5895.1, E5895.2
Silverknowes, City of Edinburgh	intertidal silty sand, epipelonm harvested by lens tissue	12 November 2015	Sample E5678
Silverknowes, City of Edinburgh	intertidal silty sand, epipelon harvested by cover-slip	17 October 2019, harvested 18 October 2019	Slides E5896.1–E5896.7
Portobello Beach, City of Edinburgh	intertidal sand near low tide mark, epipelon harvested by lens tissue	2 October 2016	Sample E6012
Portobello Beach, City of Edinburgh	intertidal sand near low tide mark, epipelon harvested by lens tissue	22 October 2016, harvested 24 October 2016	Sample E6015
Portobello Beach, City of Edinburgh	intertidal sand near low tide mark, epipelon harvested by lens tissue	13 February 2017, harvested 17February 2017	Sample E6021
Port Seton, East Lothian	intertidal sand near low water mark, epipelon harvested by lens tissue	2 October 2016	Sample E6013
Port Seton, East Lothian	intertidal sand near low water mark, epipelon harvested by lens tissue	22 October 2016, harvested 24 October 2016	Sample E6017
Musselburgh, East Lothian	intertidal sand near low water mark, epipelon harvested by lens tissue	22 October 2016, harvested 24 October 2016	Sample E6016
Longniddry, East Lothian	intertidal sand near low water mark, epipelon harvested by lens tissue	22 October 2016, harvested 24 October 2016	Sample E6018
Longniddry, East Lothian	intertidal sand, high shore, epipelon harvested by lens tissue	22 October 2016, harvested 24 October 2016	Sample E6019

Gosford Bay, East Lothian	intertidal sand near low water mark, epipelon harvested by lens tissue	22 October 2016, harvested 24 October 2016	Sample E6020
Gosford Bay, East Lothian	intertidal sand, mid- to low shore, epipelon harvested by lens tissue	11 November 2017	Sample E6025

Supplementary Table 2. Records of *Cocconeis placentula* varieties found by Google searches in October 2006 and on 1 July 2021.

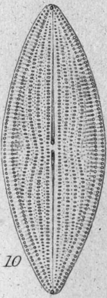
Google search for [Cocconeis placentula "var. XXX"], where XXX is as below

XXX	Date of description	Included in Hustedt 1930	Included in Hustedt 1933	Google records 2006	Google records 2021
lineata	1843	**Yes**	**Yes**	306	17400
euglypta	1854	**Yes**	**Yes**	404	19200
rouxii	1893	No	**Yes**	21	322
intermedia	1893	No	**Yes**	17	7800
klinoraphis	1927	**Yes**	**Yes**	30	773
pseudolineata	1927	**Yes**	**Yes**	56	2640
tenuistriata	1932	No	**Yes**	8	447
euglyptoides	1958	No	No	3	174

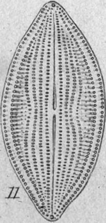
Google search for [Cocconeis placentula "var. XXX" YYY] This extra search was undertaken on 1 July 2021 to explore whether anyone had reported the informally named *Cocconeis* populations analysed by Geitler (1958)

XXX	YYY	
euglypta	Schladming	8
euglypta	Prater	106[1]

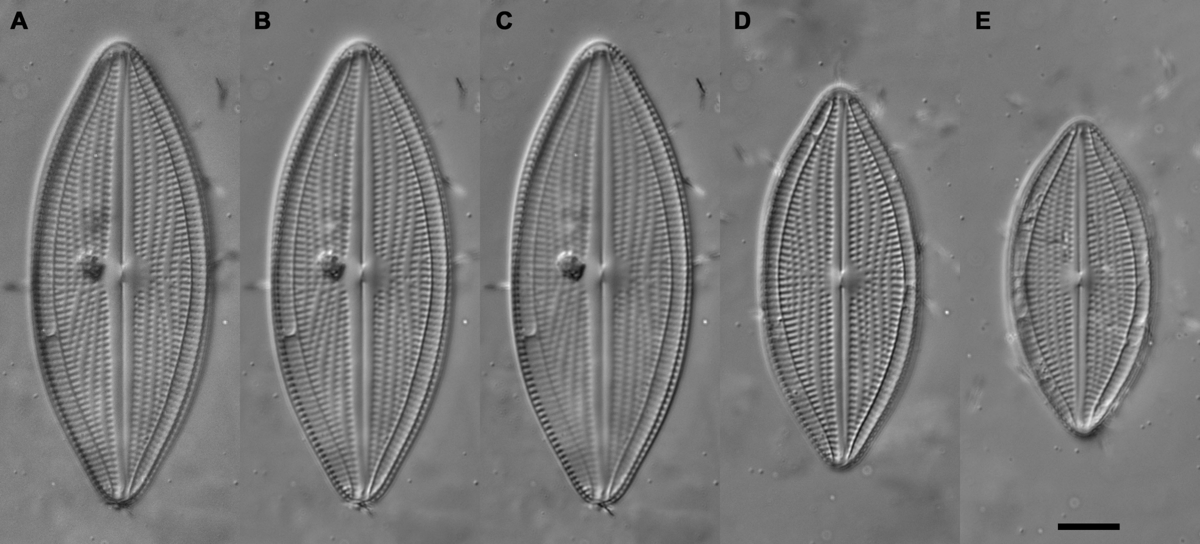
[1] However, the "Prater" mentioned was generally not the site in Austria from which Geitler reported the race of *C. placentula* var. *euglypta*!



10



11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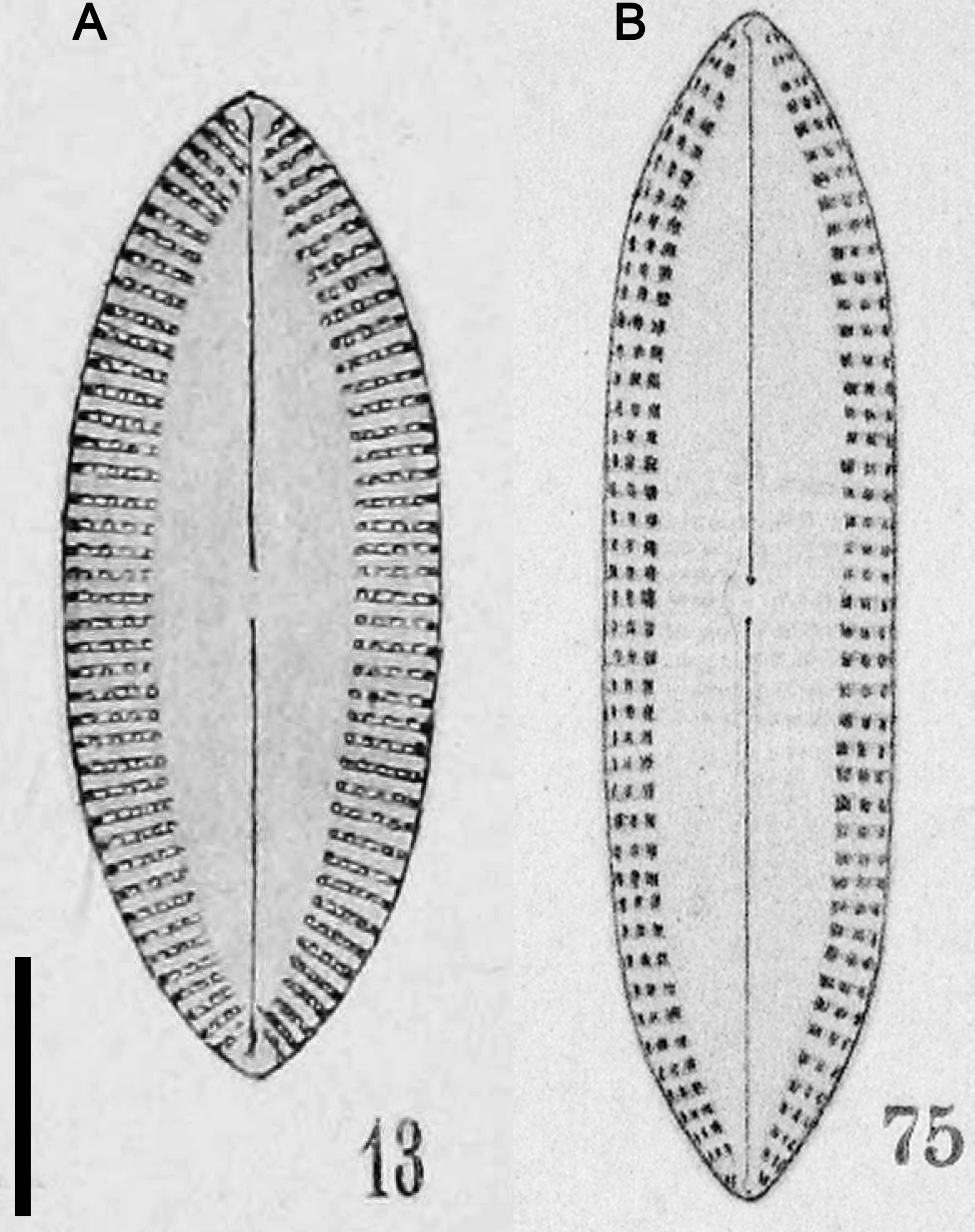
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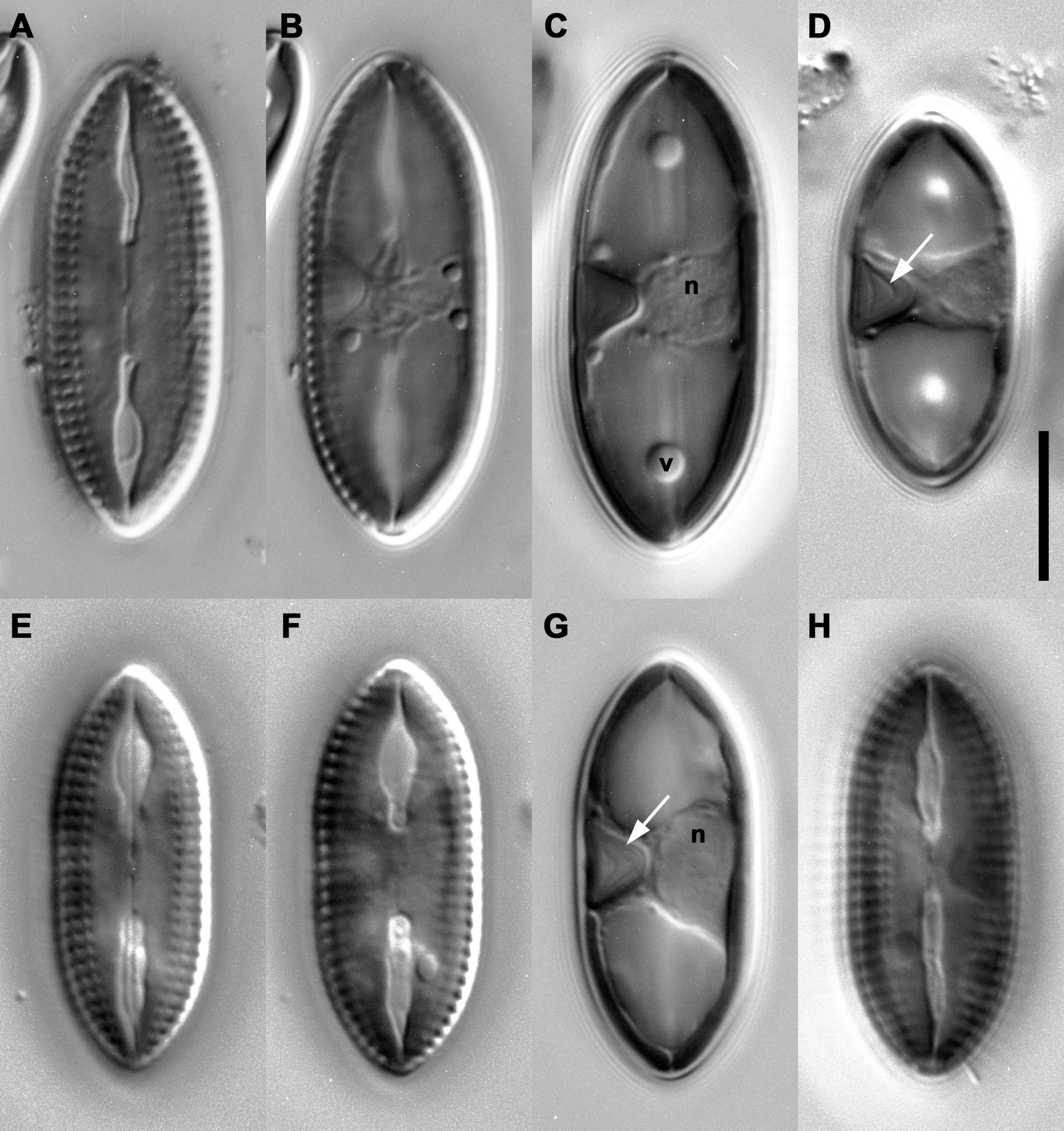
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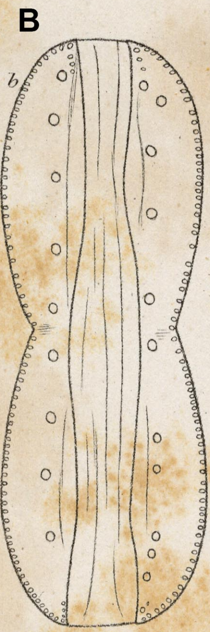
47.



51.

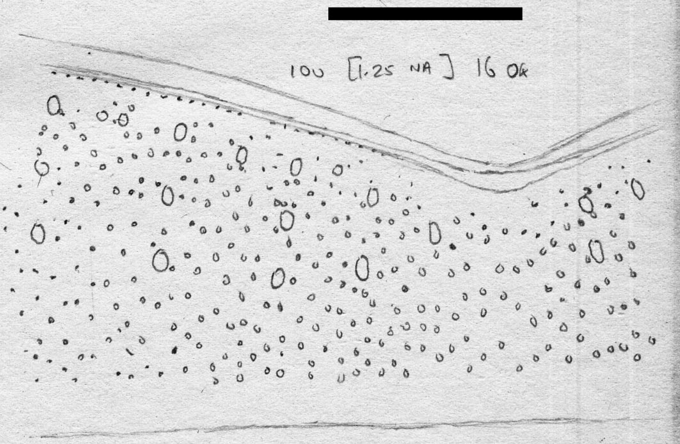
GC 90577

"Nitzschia ocellata Cleve
Manilla"

Ex Penn St. – Pease Coll.

Grez s/Loing

J. Tempère préparateur S.-M.



fibulae appear to tie an otherwise "turgid valve" into slight folds ie