

# Homothallism, morphology and phylogenetic position of a new species of *Sellaphora* (Bacillariophyta), *S. pausariae*

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**Background and aims** – The eutrophic Blackford Pond in Edinburgh has already provided the holotypes of six other *Sellaphora* species. A further undescribed species is present and requires description and characterization.

**Methods** – Clones of the new species are characterized by light (LM) and scanning electron microscopy (SEM) and molecular phylogenetics (from a concatenated five-gene alignment of 18S rDNA, 28S rDNA, 23S rDNA, *cox1* and *rbcL*, and a two-gene alignment of *cox1* and *rbcL*).

**Key results** – *Sellaphora pausariae* sp. nov. is named in honour of Dr Eileen Cox ('pausaria' = a lady coxswain). In molecular phylogenies, small-celled *Sellaphora* species ('*minima*' and '*seminulum*' morphologies) branch off at the base of *Sellaphora*, though nodes are not well supported. Species and demes previously classified in either "*Navicula pupula*" or "*Navicula bacillum*" group into three very well supported clades (numbered 1–3). Although appearing in LM and SEM like a smaller, more delicate version of *S. obesa*, *S. pausariae* (clade 1) is not closely related to *S. obesa* (clade 2). Features of *Sellaphora pausariae* not confirmed previously in any *Sellaphora* but possibly widespread are: (a) hymenes with pores arranged in a regular scatter; (b) a stepped mantle near the poles; and (c) a 'primodominant' girdle comprising a wide band 1, a segmental band 2, and two extremely thin bands at the abvalvar end of the girdle. *Sellaphora pausariae* is homothallic; a deficiency of interclonal pairings in two-clone mixtures is interpreted as reflecting the tendency of cells to mate with their immediate neighbours.

**Conclusions** – Morphologically, the new species can be differentiated from existing described species, though only problematically from some informally named demes. Molecularly, it is clearly characterized by the five genes sequenced. Girdle terminology needs expansion (e.g. to distinguish primodominant girdles from 'graded' ones, in which the bands gradually decrease in width and structural complexity from the valve outwards).

**Key words** – Bacillariophyta, *cox1*, diatoms, rDNA, mating data, molecular systematics, new species, *rbcL*, *Sellaphora*, taxonomy.

## INTRODUCTION

The genus *Sellaphora* (Mereschkowsky 1902) was revived in 1989, following recognition of the many morphological

and cytological characteristics, for example raphe structure, areola shape, and chloroplast number and position, that separate it from *Navicula* (Mann 1989a, Round et al. 1990). At that time, only eight species were transferred to *Sellaphora*,

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all from *Navicula*, but these have been followed by transfers or elevations (from varietal status) of 109 more (Guiry & Guiry 2018) from *Navicula* (72 species), the former genus *Naviculadicta* (21 species), *Eolimna* (11 species), *Stauroneis* (4 species) and *Sellaphora* (1 species). A few examples of studies resulting in transfers are Potapova & Ponader (2008), Falasco et al. (2009), Wetzel et al. (2015) and Ács et al. (2017). Five old names in *Sellaphora* (given by Merschowsky 1902, and Müller 1910) remain to be clarified. In addition, however, 116 new species have been described, so that the total for the genus now approaches 240 spp.

The huge number of newly described species, in what was previously a “modestly sized diatom genus”, was partly to be expected given the general tendency for species diversity to have been underestimated in diatoms (e.g. Droop et al. 2000, Mann & Vanormelingen 2013), but it has probably been encouraged further by work on *Sellaphora* itself. This has indicated that subtle morphological differences between populations are often correlated with the presence of reproductive isolation and clear differences in gene markers such as *rbcl* and *cox1* (e.g. Mann 1984, 1989b, Evans et al. 2007, 2008). Mann et al. (2008) reviewed the state of *Sellaphora* taxonomy and made a preliminary catalogue of the morphological diversity of the larger *Sellaphora* species occurring in the British Isles, illustrating and discussing 54 demes that were argued to probably represent separate species. Subsequently a further deme was described from the UK by Mann (2008), making 55 in all. Of these demes, only about a quarter could be identified with moderate to total confidence as known, named species; the remainder were given informal names, e.g. “*Sellaphora* [*pupula* K-LB]  $\Phi$  ‘mini-obese’”; in such names, the phenodeme ( $\Phi$ ) is identified as belonging to a species complex (*S. pupula*, as defined by Krammer & Lange-Bertalot 1986 [=“K-LB”]) but is distinguished from other members of the complex by an informal English epithet, in this case ‘mini-obese’. Formal naming was not attempted by Mann et al. (2008) because (1) it was not certain that all of the distinctions were valid, (2) molecular barcodes could not be provided for all of the demes. Furthermore, although ultrastructural data were suggested to be not very useful for species distinctions in *Sellaphora* (Mann et al. 2008: 67), it has to be admitted that in 2008 few of the 55 demes had actually been studied in SEM, apart from the species introduced by Mann et al. (2004).

In the present paper, we describe a new *Sellaphora* species, *S. pausariae*, from natural populations of epipelton from Blackford Pond, Edinburgh, Scotland, and c. 35 clones, all isolated from the same locality. The species corresponds to *Sellaphora* [*pupula* K-LB]  $\Phi$  ‘spindle’ as defined by Mann (2008, not as in Mann et al. 2008) and is typified by preserved material of clone BLA16 (= KE68), for which *cox1*, *rbcl* and 18S rDNA sequences are already available (Evans et al. 2007, 2008). SEM observations of *S. pausariae* extend our knowledge of *Sellaphora* ultrastructure, especially of the girdle and pore occlusions, while observations of single clones and mating experiments were undertaken to determine the mating system. In order to determine the systematic position of the new species, we made a molecular phylogenetic analysis of *Sellaphora*, based on new compilations of data and concatenated alignments of two- and five-gene data. The name we

have chosen for the new species honours Eileen Cox, both by a play on words and in recognizing the pivotal role she has played in diatom research during more than 45 years.

## MATERIAL AND METHODS

Mixed ‘seminatural’ populations of epipelton (i.e. natural populations transferred to the laboratory, harvested from the sediments and manipulated) were obtained from Blackford Pond, Edinburgh. Methods for harvesting, processing, and cleaning epipelton for microscopy were as described by Mann et al. (2008). Briefly, 1- to 2-litre samples of a slurry derived from the top few millimetres of mud and overlying water were placed in plastic boxes (c. 25 × 25 cm), allowed to stand for at least 3 h, and the supernatant water removed. The mud surface was then covered with lens tissue (as a filter that allows motile diatoms to pass through) and epipelton harvested by placing further layers of lens tissue or cover-slips on top. Living cells were observed by mounting the cover-slips in drops of culture medium. Clones of *S. pausariae* (table 1) were isolated either (1) by streaking harvested epipelton on agar plates (WC medium + silicate, as specified by Guillard & Lorenzen 1972, but solidified with 2% agar), incubating the plates in low light (generally < 5  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) at c. 15°C for 2–3 weeks, and then transferring cells from the margins of discrete homogeneous colonies to liquid WC medium (with silicate, as above); or (2) by isolating single cells directly from the agar plates (under a dissecting microscope with 100× magnification) within a day of streaking the harvested epipelton (for isolations made in 2008). The cultures were subsequently maintained in 50-mm Petri dishes at 15–18°C, with 10–14 h light per day (c. 5–20  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Cultures were inspected using a Zeiss Axiovert inverted microscope.

Cleaned valves were prepared by total oxidation of the lens tissue, or of clonal material, in 1:1 70%  $\text{HNO}_3$  : 98%  $\text{H}_2\text{SO}_4$ , followed by mounting in Naphrax (Brunel Microscopes, Chippenham, UK): voucher accession numbers [for the diatom herbarium of the Royal Botanic Garden Edinburgh (E)] are given in electronic appendix 1. Bright field (BF) light microscopy (LM) was carried out with a plan-apochromat ×100 objective (nominal numerical aperture 1.32) on a Reichert Polyvar 2 photomicroscope fitted with a Polaroid DMC2 digital camera capable of 1600 × 1200 pixel resolution. Differential interference contrast (DIC) observations were made with a Zeiss AxioImager M2 microscope (Zeiss, Oberkochen, Germany), using a ×100 objective (nominal numerical aperture 1.4) and an AxioCam HRc camera with 2776 × 2080 pixels set as the resolution; 10 images were averaged to reduce noise.

For scanning electron microscopy (SEM), suspensions of cleaned diatoms were dried onto 13-mm diameter round cover-slips, which were then attached to aluminium stubs by carbon discs, with silver dag painted around the edges to promote conduction. Stubs were coated with platinum for 1–2 min in an Emitech K575X sputter coater and examined using a LEO Supra 55VP Field Emission SEM operated at 5 kV (c. 4 mm working distance; aperture 20  $\mu\text{m}$ ). Specimens were imaged either flat or with 25° tilt; in no case were the images of tilted specimens rotated, so that in our plates measure-

ments made parallel to the  $x$  (horizontal) axis are true. Images were captured as  $2048 \times 1536$  or  $3702 \times 2304$  pixel TIFF files.

Plates were assembled using Adobe Photoshop CS6 Extended (Adobe Systems, San Jose, California). Individual photographs were adjusted by general application of the Levels, Curves, Brightness and Contrast tools in Photoshop, and rarely also using Unsharp Mask and local alteration of contrast (in fig. 3D, for the raphe slit). Measurements were made from the images using the Ruler tool in Photoshop.

For crossing experiments, aliquots were taken from exponentially growing cultures and transferred, singly or in two-clone combinations, into new WC medium in 25-well square Repli dishes (100 mm, Sterilin™; Fisher Scientific, Loughborough, UK). The cultures were examined at least daily following inoculation for pairing and estimates made at intervals of the frequencies of intra- and inter-clonal pairs in those combinations where the two clones differed sufficiently in cell length. Experiments on the frequencies of intra- and interclonal reproduction began on 3 December 2004 and observations were made thereafter at 12 h intervals. The first pairs were observed on 5 December, fusing gametes on 6 December at 18.00 h, zygotes by 07.30 on 7 December, and enlarged auxospores on 9 December. Since sexual clones of *S. bisexualis* D.G.Mann & K.M.Evans were also available at the time, these were also included in the mating experiments, even though these species are not especially closely related according to molecular data (see analyses below). The results are nevertheless instructive because they cast some light on the limits to interbreeding between species (cf. De Decker et al. 2018).

Selectivity of pairing (inter- vs. intraclonal) was investigated by examining, via  $\chi^2$  tests, the goodness-of-fit between observed and expected frequencies of intra- and interclonal pairs. Many *Sellaphora* species, including *S. pausariae*, are fully sexual organisms, in which two (sometimes more) cells pair, undergo meiosis, and produce a zygote. If two clones are mated, one with smaller cells ( $s$ ), the other with bigger cells ( $b$ ), there are three possible types of pairs:  $s \times s$ ,  $b \times b$  and  $s \times b$ . Let the frequencies of each type of pair be  $S$ ,  $B$  and  $SB$ , respectively, and the total number of pairs  $S + B + SB = N$ . Then the proportion of  $s$  cells in pairs of any kind will be  $(2S + SB)/N$  and that of  $b$  cells  $(2B + SB)/N$ . If these proportions are representative of the proportions of sexualized cells of  $s$  and  $b$  when pairing took place (i.e.  $s$  and  $b$  cells that have been triggered to enter meiosis and sexual reproduction and that subsequently pair), then if the mating system is homothallic and pairing is random the expected proportions of  $s \times s$ ,  $b \times b$ , and  $s \times b$  will be  $[(2S + SB)/N]^2$ ,  $[(2B + SB)/N]^2$ , and  $[(2S + SB)/N] \times [(2B + SB)/N]$ , respectively. The key comparison of interest in the present work is the balance between interclonal ( $s \times b$  pairs) and intraclonal mating ( $s \times s$  and  $b \times b$  pairs). Using the terms referred to above, we wish to compare  $(S + B)$  with  $N\{[(2S + SB)/N] + [(2B + SB)/N]^2\}$  and  $SB$  with  $N\{[(2S + SB)/N] \times [(2B + SB)/N]\}$ , calculating  $\chi^2$  with 1 degree of freedom.

For phylogenetic analyses, all available published *Sellaphora* sequences were downloaded from GenBank and assessed for use in concatenated multigene analyses. Some of

these are newly published since our earlier two-gene phylogenies of the Sellaphoraceae (Evans et al. 2008, Pouličková et al. 2015). No genes had 100% coverage among the strains represented in GenBank, but moderate to excellent datasets were available for nuclear 18S rDNA and 28S rDNA, plastidial 23S rDNA, plastidial *rbcl*, and mitochondrial *cox1*; except for 18S rDNA and *rbcl*, the GenBank sequences comprised only a minor part of each gene (sequences used are listed in electronic appendix 7). The GenBank sequences are mostly derived from our earlier studies (principally Evans et al. 2007, 2008, for *cox1*, *rbcl* and 18S rDNA; Hamsher et al. 2011, for 23S and 28S rDNA). Selection of clones for inclusion in the five-gene set was based on availability of sequences for a majority of the five genes, except in a few cases where only the two longest genes (18S rDNA and *rbcl*) were available, or only *cox1* and *rbcl* (which had the best coverage overall). In addition, a concatenated matrix was constructed of all clones with both *cox1* and *rbcl*, for comparison with the five-gene set and with the earlier two-gene analysis of Pouličková et al. (2015). Alignment of 18S rDNA was done using MAFFD (<https://mafft.cbrc.jp/alignment/server/>), with further adjustment by eye in MEGA7 (Kumar et al. 2016). The other genes were aligned using MUSCLE (Edgar 2004), as implemented in MEGA7, with adjustments by eye for 28S rDNA. Finally, doubtfully aligned regions and autapomorphic insertions (mainly in loop regions of 18S and 28S rDNA) and incomplete 5' and 3' tails were omitted: the original and trimmed alignments are available as electronic appendices 3–6. Maximum likelihood analyses were performed using RAXML (Stamatakis 2014), operated via raxmlGUI v. 1.5 (Silvestro & Michalak 2012). The data were partitioned according to gene and, in *cox1* and *rbcl*, by codon position. The raxmlGUI default model (GTRGAMMA) was applied to all partitions and the analysis run using the ML+rapid bootstrap option in RAXML, with 1000 bootstrap replicates. The outgroup was *Sellaphora*'s sister genus *Fallacia* (see e.g. Witkowski et al. 2014). Trees were prepared for publication using iTOL (Letunic & Bork 2016), Adobe Illustrator CS6 (Adobe Systems, San Jose, California) and Adobe Photoshop.

## RESULTS AND DISCUSSION

### New species

*Sellaphora pausariae* Pouličková & D.G.Mann, sp. nov.

Figs 1, 2A–G, I, 3 & 4

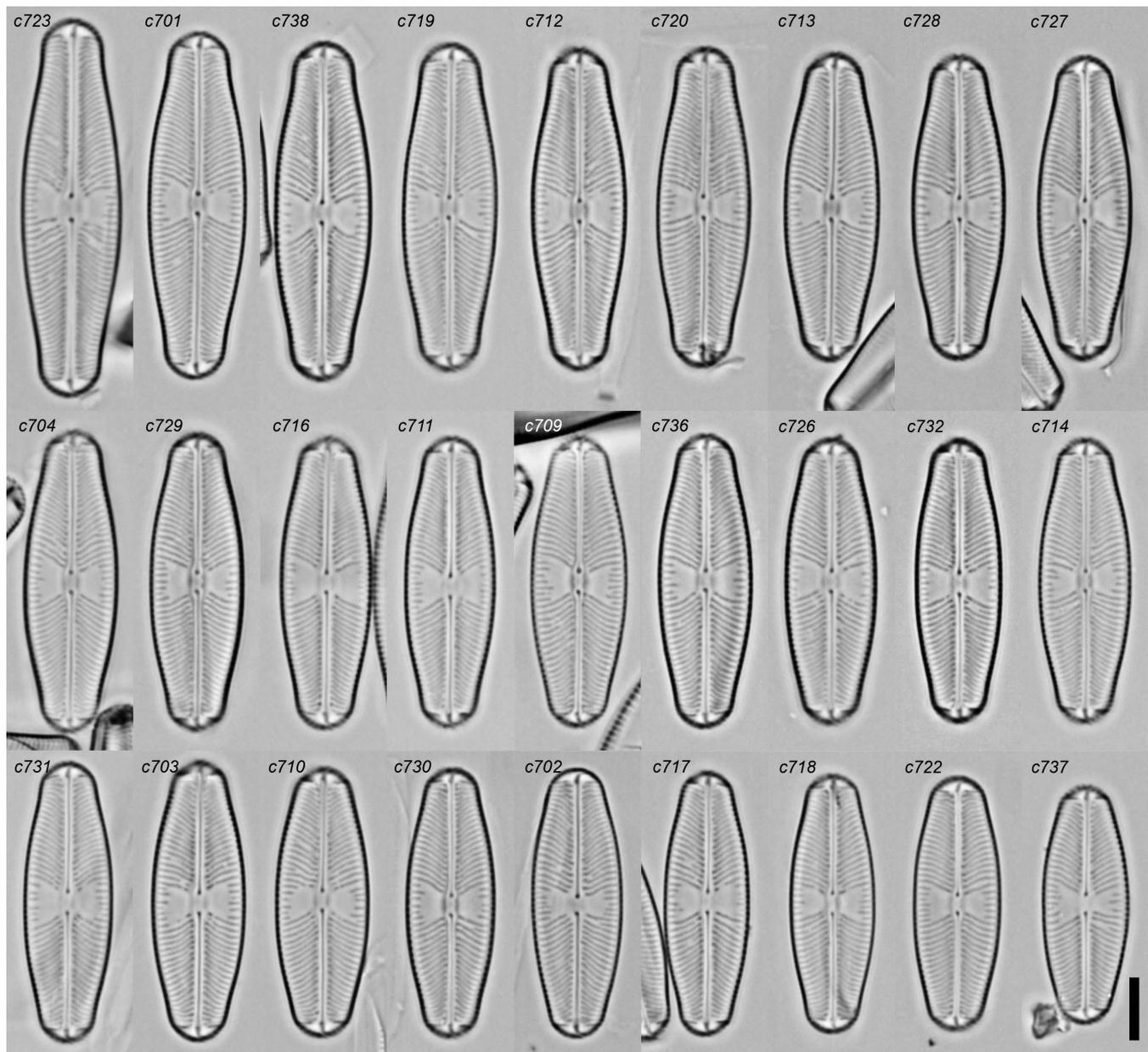
**Synonym** – *Sellaphora* [*pupula* K–LB]  $\Phi$  ‘spindle’ *sensu* Mann (2008: 118).

**Description** – Valves narrowly elliptical, shallow, with broad, slightly rostrate poles (therefore appearing ‘lanceolate’ overall: fig. 1), 16–32 (40 in post-auxospore valves)  $\mu\text{m}$  long  $\times$  7.0–8.3 (–9.0 in post-auxospore valves)  $\mu\text{m}$  wide. Striae slightly curved, radiate, becoming parallel or even slightly convergent in the longest specimens near the poles, where they are sometimes angled (fig. 2A), with some intercalated shorter striae at the centre, 19.5–22.5 in 10  $\mu\text{m}$  (21.1  $\pm$  0.80, [mean  $\pm$  standard deviation] for a sample of 29 valves representative of 29 clones); areolae invisible in LM. Axi-

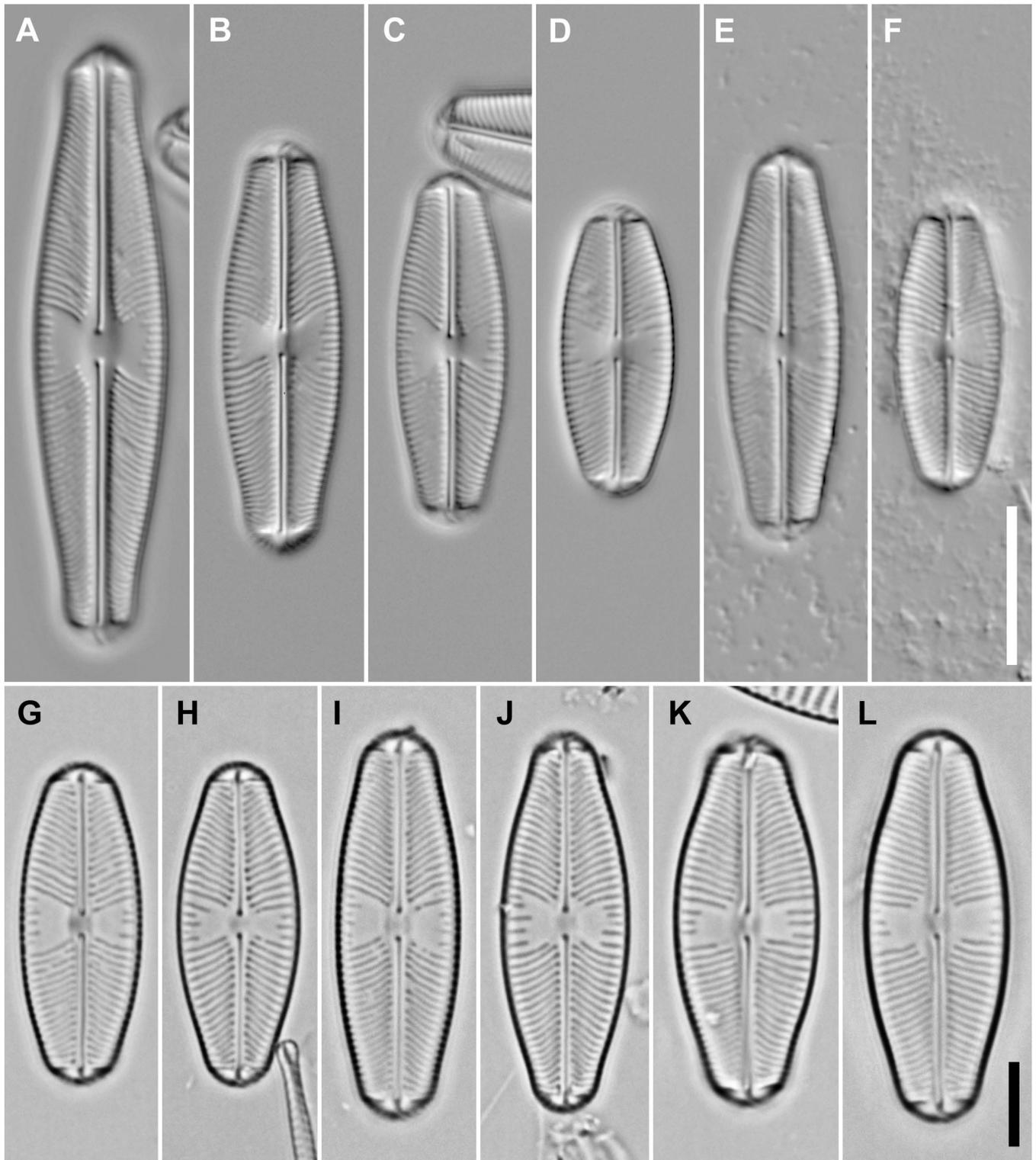
al area very narrow. Central area expanded (to 60–75% of the valve width), somewhat irregular, ± bowtie-shaped (fig. 2A–C & E) to transversely rectangular in the very smallest valves (fig. 2D & F). Grooves present alongside the raphe-sternum but difficult to detect in LM except at the centre (fig. 3A), not extending to the poles on the secondary side of the valve (figs 3B & 4A). Polar bars present, parallel or slightly convergent (in long specimens) (figs 1, 2A–G & I, 3C). Valve mantle stepped at the poles (figs 3B & 4B).

**Type material** – United Kingdom, Scotland, Edinburgh, Blackford Pond (UK National Grid Reference NT253708 = 55°55'29"N, 3°11'49"W), soft organic mud under 40–60 cm water in a eutrophic parkland pond, clone KE68, 3 Nov. 2005, *K.M. Evans* s.n. (holo-: E, slide E3658, preserved DNA EDNA 08-01116).

**Type material characteristics** – Material of clone KE68 (= BLA 16 of Evans et al. 2007, 2008), as preserved on slide E3658 (E), illustrated in fig. 2A & C, barcoded in GenBank by accessions EF164951 (*cox1*), EF143298 (*rbcL*) and EF151974 (18S rDNA), and with preserved DNA at (E) as EDNA 08-01116. Clone KE68 was isolated by K.M. Evans in 2005 from a sample collected from Blackford Pond, Edinburgh. The holotype slide and material contains both small valves (from vegetative and gametangial cells: fig. 2C) and large valves (from initial and post-auxospore cells: fig. 2A), since the clone exhibited vigorous intraclonal sexual reproduction. The presence of this variation tends to obviate the need for epitypes to be specified, in contrast to *S. caput* K.M.Evans & D.G.Mann, where the holotype comprises



**Figure 1** – *Sellaphora pausariae*: representative valves of 28 clones isolated from Blackford Pond in January 2008, LM, bright field optics. The clone number (see electronic appendix 1) is indicated on each photograph (e.g. c723 = Sel723B:). Scale bar = 5 µm.

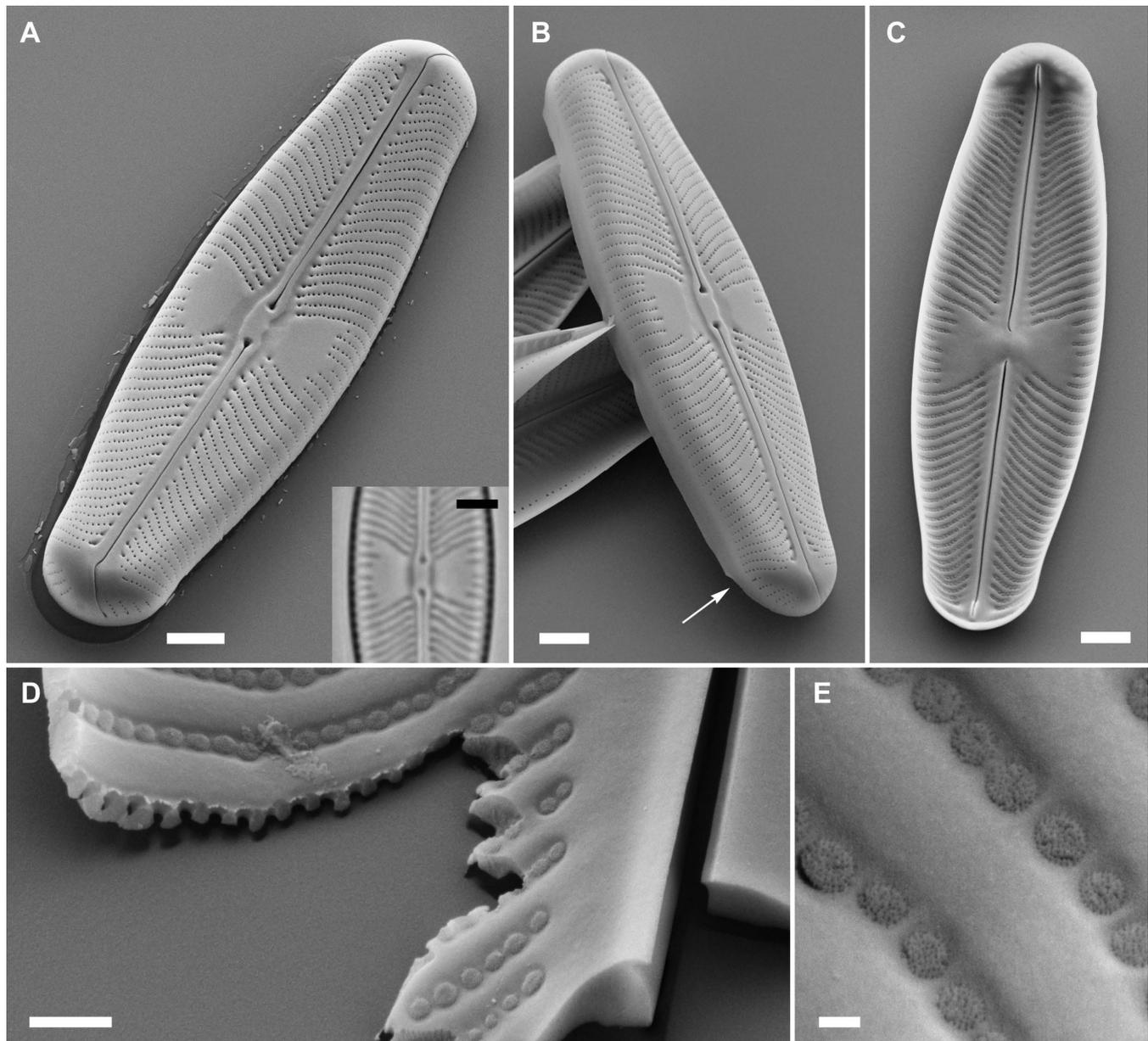


**Figure 2** – *Sellaphora* valves. A–F, LM, differential interference optics: A, holotype, clone KE68 (= BLA 16): enlarged, post-auxospore valve; B, clone KE65; C, holotype, clone KE68 (= BLA 16): valve of gametangial size; D, clone KE64: valve of gametangial size; E, clone SEL576B; F, clone 583B. G–L, valves of *Sellaphora pausariae* and similar species, LM, bright field optics: G & I, *Sellaphora pausariae*, clones KE64 and SEL726B; H & J, comparable valves of *Sellaphora* [*pupula* K–LB]  $\Phi$  ‘mini-obese’, from a natural population, Cole Mere, Cheshire, UK (see Mann et al. 2008); K & L, *Sellaphora obesa* from a natural population, Blackford Pond: valves were chosen that were a close match in length to those of *S. pausariae* and  $\Phi$  ‘mini-obese’ shown in I & J. Scale bars: A–F = 10  $\mu\text{m}$ ; G–L = 5  $\mu\text{m}$ .

only small valves close to the lower limit for the species (Evans & Mann 2009).

**Etymology** – We dedicate this new species to Eileen Cox. The epithet derives from the Latin ‘*pausarius*’, i.e. the officer of a Roman galley who ensured that the rowers kept in time (by beating a drum). He was thus the coxswain or ‘cox’ of the ship. We think that the name is also appropriate because Eileen has always been at the forefront in driving her field of activity forward, whether it is scientific research (e.g. phe-

notypic plasticity, relationships among naviculoid diatoms, ontogeny), communication (e.g. ensuring we know what we are talking about via good terminology), or the successful functioning of scientific societies like the International Society for Diatom Research or the British Phycological Society. In the present case, we have had to update the Latin by inventing a feminine equivalent of the *pausarius*, *pausaria*. The name of the species is therefore “the saddle-bearer [Sellaphora] of the Cox”.



**Figure 3** – *Sellaphora pausariae* valves, SEM; all are from clone KE65, except fig. 17 (KE64): A, valve exterior, primary side to left, showing uniseriate striae, grooves alongside the raphe, bent terminal raphe fissures, central raphe endings deflected slightly towards the primary side (on the left) and expanded. Inset: light micrograph for comparison: note that the grooves can be detected as shadows alongside the raphe; B, obliquely orientated valve exterior, showing the step in the mantle (white arrow) near the pole and the groove alongside the raphe and central raphe endings. Note the greater extension of this groove on the primary side (nearer) than on the secondary side here and on fig. 4A; C, valve interior, primary side to the right. Note the deflection of the central raphe endings; D, broken valve, seen from the interior, showing that the hymenes lie at the internal apertures of the poroids; E, hymenes with pores in a regular scatter. Scale bars: A–C = 2  $\mu$ m; D = 500 nm; E = 100 nm. All specimens tilted 25°, images not rotated.

**Table 1. – Metric data for the ‘obesa’ series of demes and species.**

Length and width are given as minimum–maximum, with outliers indicated in parentheses. For stria density and pole width, the figures are minimum–average–maximum. <sup>1</sup> The stria densities given are those published by Mann et al. (2008) or Metzeltin et al. (2009), except for *S. pausariae*, which was measured again for this paper (minimum and maximum from a survey of 83 valves; average derived from representative single valves from 29 different clonal cultures, with  $s \pm 0.80 \mu\text{m}$ ). <sup>2</sup> Measured at the point of inflection of the outline near the pole (cf. fig. 2A–L). Data sources were as follows. For *Sellaphora* [*pupula* K–LB]  $\Phi$  ‘corpulent’,  $\Phi$  ‘cf. corpulent’,  $\Phi$  ‘meso-obese’  $\Phi$  ‘mini-obese’,  $\Phi$  ‘little’ and *S. obesa*, measurements were made from the original images of six or seven representative valves illustrated by Mann et al. (2008). For *S. perobesa*, we made our own measurements from the micrographs published by Metzeltin et al. (2009: pl. 61, figs 1–7) and similarly we measured *S. meridionalis* from Potapova & Ponader (2008: fig. 2A–I). <sup>3</sup> In order to check that any differences between *S. perobesa* and *S. meridionalis* and the other species might have resulted from discrepancies in how stria density was measured by ourselves and Metzeltin et al. (2009) and Potapova & Ponader (2008), we measured stria densities in the micrographs published by these authors. <sup>4</sup> Initial cells and the largest post-auxospore cells of *S. pausariae* attain sizes of  $40 \times 9 \mu\text{m}$ . Such cells are rare in natural populations where the maximum size encountered is usually c.  $30 \times 8 \mu\text{m}$ .

Species or deme	Data sources	Length $\mu\text{m}$	Width $\mu\text{m}$	Stria density <sup>1</sup> # in $10 \mu\text{m}$	Pole width <sup>2</sup> # in $10 \mu\text{m}$
<i>Sellaphora</i> [ <i>pupula</i> K–LB] $\Phi$ ‘corpulent’	Mann et al. (2008) and this paper	28.5–37.5	9.75–10.5	19.2–19.9–21.0	7.1–7.2–7.3
<i>Sellaphora</i> [ <i>pupula</i> K–LB] $\Phi$ ‘cf. corpulent’	Mann et al. (2008) and this paper	25.5–46.5	9.5–10.25	19.1–20.1–20.9	6.4–6.6–6.7
<i>Sellaphora perobesa</i>	Metzeltin et al. (2009)	30–45	9–10	18–21	–
Metzeltin, Lange-Bert. & Nergui	This paper	–	–	18.6–19.2–19.6 <sup>3</sup>	6.4–6.8–7.2
<i>Sellaphora obesa</i> D.G.Mann & M.M.Bayer	Mann et al. (2008) and this paper	(20) 21.5–47.5	(8) 8.75–10.0	18.0–18.7–20.7 (–21.5)	5.6–6.0–6.3
<i>Sellaphora</i> [ <i>pupula</i> K–LB] $\Phi$ ‘meso-obese’	Mann et al. (2008) and this paper	26–32.5	8.25–8.75	18.3–19.6–21.0	5.3–5.4–5.6
<i>Sellaphora</i> [ <i>pupula</i> K–LB] $\Phi$ ‘little’	Mann et al. (2008) and this paper	21–28.5	7.0–7.25	18.7–19.8–20.9	4.5–4.7–4.8
<i>Sellaphora</i> [ <i>pupula</i> K–LB] $\Phi$ ‘mini-obese’	Mann et al. (2008) and this paper	13.5–37.5	7.0–8.75	18.7–19.2–20.5	4.3–4.5–4.9
<i>Sellaphora pausariae</i> sp. nov.	Mann et al. (2008) and this paper	16–32 (–40) <sup>4</sup>	7.0–8.3 (–9.0)	19.4–21.1–22.5	4.6–4.9–5.2
<i>Sellaphora meridionalis</i> Potapova & Ponader	Potapova & Ponader (2008)	18–32	6.8–7.8	22–23	–
	This paper	–	–	21.7–22.1–22.4 <sup>3</sup>	4.7–4.8–5.0

### Separation from similar species and aids to identification

*Sellaphora pausariae* would have been included within *Sellaphora* (‘*Navicula*’) *pupula* s. lat. by Hustedt (1930) and Krammer & Lange-Bertalot (1986). It has already been shown that the *S. pupula* (Kütz.) Mereschk. group of species is not monophyletic (e.g. Evans et al. 2008), but its species share a similar morphology: the central area is rectangular or bow-tie-shaped, not circular or elliptical (as in *S. bacillum* (Ehrenb.) D.G.Mann and similar species), and there are polar bars, i.e. small transverse thickenings of the valve at the poles, which appear as short dark lines in LM; these bars are also present in *S. bacillum* and similar species, but they are absent in the *S. laevisissima* (Kütz.) D.G.Mann and *S. americana* (Ehrenb.) D.G.Mann groups of species (Mann et al. 2008) and in many small-celled species (e.g. those studied by Falasco et al. 2009 and Wetzel et al. 2015; for example, *S. saugerresii* (Desm.) C.E.Wetzel & D.G.Mann, *S. seminulum* (Grunow) D.G.Mann and *S. stroemei* (Hust.) D.G.Mann). Within the *S. pupula* group (e.g. Mann et al. 2008: 19–21, 36–61), *S. pausariae* is part of a series of species – the *obesa* group (‘group’ refers to the morphological similarity and does not imply monophyly) – characterized by a linear-elliptical outline, broad and slightly rostrate poles, rather coarse striation ( $\leq 23$  in  $10 \mu\text{m}$ ), and a strongly widened, rectangular

(rarely bow-tie-shaped) central area. They lack the conopea present alongside each raphe slit in e.g. *S. lange-bertalotii* Metzeltin (Metzeltin & Lange-Bertalot 2002), *S. elliptico-lanceolata* Metzeltin, Lange-Bert. & Nergui (Metzeltin et al. 2009), *S. californica* Potapova (Potapova & Charles 2004) or *S. hohnii* Potapova & Ponader (2008) and the very wide rectangular central area separates them from species such as *S. mantasoana* Metzeltin & Lange-Bert. and *S. omuelleri* Metzeltin & Lange-Bert. (Metzeltin & Lange-Bertalot 2002). Other species within the *S. pupula* group (e.g. *S. capitata* D.G.Mann & S.M.McDonald, *S. blackfordensis* D.G.Mann & S.Droop) differ from all the above in having more strictly linear valves (Mann et al. 2004, 2008).

The *obesa* group includes *S. obesa* D.G.Mann & M.M.Bayer itself (fig. 2K & L), *S. perobesa* Metzeltin, Lange-Bert. & Nergui, the informally named demes *Sellaphora* [*pupula* K–LB]  $\Phi$  ‘corpulent’,  $\Phi$  ‘cf. corpulent’,  $\Phi$  ‘meso-obese’,  $\Phi$  ‘little’ and  $\Phi$  ‘mini-obese’ (for which, see fig. 2H & J), and *S. meridionalis* Potapova & Ponader. Differences among these demes and species are subtle and mostly comprise small differences in valve width and stria density (table 1). Together, the *obesa* series seem to form a continuum with respect to width at the centre, width of the valve poles (measured at the inflection in the outline where

the linear elliptical valves become strongly or weakly rostrate) and stria density, which all vary more or less in tandem, from the largest and coarsest of the series (*S. [pupula K–LB]* Φ ‘corpulent’) to the smallest and most delicate (*S. meridionalis*). Morphological data alone are unlikely to provide further clear evidence about species boundaries in this group and so it is currently pointless arguing whether or not all the demes and species in table 1 are truly distinct, since gene sequence data, which would probably give definitive conclusions, are absent for all except *S. obesa* and *S. pausariae*. Hence it is possible that the close morphological resemblance between *S. pausariae* and *S. [pupula K–LB]* Φ ‘mini-obese’ (fig. 2G–J, table 1) will be found in due course to reflect conspecificity. In that case, the circumscription of *S. pausariae* will need to be expanded to accommodate ‘mini-obese’ morphologies (with their slightly coarser structure and more tapering valves). However, it may be significant that even though *S. pausariae* is morphologically very similar to *S. [pupula K–LB]* Φ ‘little’ and is its sister species in phylogenetic analyses (see below), the two are well separated in gene sequences, by roughly the same distance as *S. auldreekie*, *S. [pupula K–LB]* Φ ‘southern-auldreekie’ and *S. [pupula K–LB]* Φ ‘coarse-auldreekie’ are from each other; these three are also morphologically similar to each other, but are reproductively isolated (Vanormelingen et al. 2013).

Of the named species in table 1, which seem to us to be the only named species with which *S. pausariae* is likely to be confused, *S. pausariae* is closest morphologically to *S. meridionalis*, and then to *S. obesa*. Metric data (especially central and polar widths, and to a lesser extent also stria densities) clearly separate *S. pausariae* from *S. obesa*, which is illustrated for comparison in fig. 2K & L. *Sellaphora meridionalis* matches *S. pausariae* in its dimensions and striation density, but two characters appear to be helpful in separating them: (1) the striae are much less strongly radial in *S. meridionalis*, even in longer valves (Potapova & Ponader 2008: their fig. 2A, I) (in *Sellaphora* species the striae tend to become more parallel as valve size decreases during the life cycle: see illustrations in Mann et al. 2008). (2) The raphe sternum is accompanied on either side by shallow grooves in *S. pausariae* (see below and figs 3A–C & 4A), but not in *S. meridionalis* (Potapova & Ponader 2008: their fig. 2J–L).

### Ecology and distribution

*Sellaphora pausariae* is known mainly from Blackford Pond, where it was noted from the early 2000s onwards; from 1983 to 1990, during which pond epilimon was sampled frequently, it was either not present or rare. Poulíčková et al. (2008) studied ecological differentiation of *Sellaphora* species in 22 British lakes, covering a gradient from oligotrophic mountain lakes to eutrophic shallow ponds, including Blackford Pond. Twenty-eight *Sellaphora* phenodemes were analysed, including *S. obesa*, *S. [pupula K–LB]* Φ ‘mini obese’, and *S. pausariae* (as *S. [pupula K–LB]* Φ ‘spindle’). Blackford Pond was found to belong to a group of eutrophic ponds with water quality class “poor”. Corresponding to this, the *Sellaphora* phenodemes occurring in Blackford Pond were found in the eutrophic region of ordination space. Of the phenodemes mentioned above, only *S. obesa* and ‘mini obese’

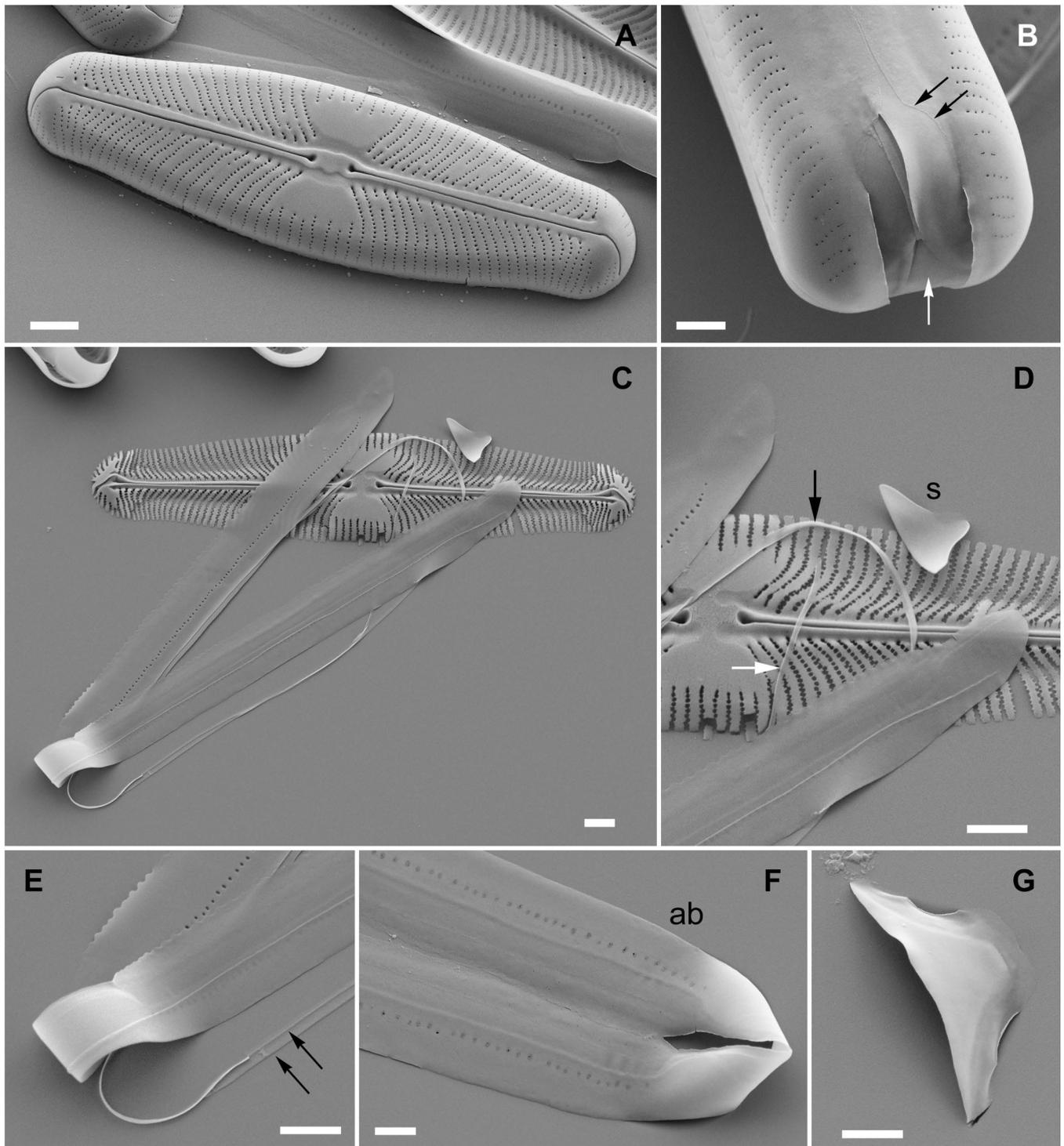
were found in more than five lakes and were used for further analyses. *Sellaphora obesa* was found to indicate mesotrophic conditions, together with *S. capitata* D.G.Mann & S.M.McDonald, *S. blackfordensis* D.G.Mann & S.Droop and *S. [pupula K–LB]* Φ ‘small lanceolate’. Unfortunately *S. pausariae* seems to be relatively rare in British epilimon and its requirements are therefore unclear; it occurred only in Blackford Pond and the Loch of Clunie, which seems to be mesotrophic. The introduction of metabarcoding approaches to freshwater biomonitoring (e.g. Vasselon et al. 2017, Kelly et al. 2018) will hopefully lead to better documentation of the distributions and ecology of *S. pausariae* (and other pseudocryptic and cryptic species), since the DNA sequences available for *S. pausariae* cover the most commonly used ‘barcode regions’ that are amplified for high-throughput sequencing.

### Morphology and cytology

The protoplast of *S. pausariae* is like that of many other *Sellaphora* species, with a single H-shaped chloroplast and an eccentric tetrahedral invaginated pyrenoid (not illustrated; cf. Mann 1989). In most respects, the ultrastructure of *S. pausariae* also agrees with what has been described previously for *Sellaphora* by Mann (1989), Mann et al. (2004), Metzeltin et al. (2002, 2009), and others. The striae are uniseriate, containing small round poroids (figs 3A–C & 4A); the poroids possess hymenate occlusions lying near their internal apertures (fig. 3C & D); and the raphe slits bend slightly towards the primary side at the centre, both internally (fig. 3C) and externally (figs 3A, B & 4A), and externally they are also expanded, forming rounded pores (fig. 3A). The terminal fissures curve towards the secondary side of the valve and are usually quite abruptly bent above the helictoglossa (fig. 3A). In this respect *S. pausariae* resembles *S. capitata* and *S. auldreekie* more closely than *S. obesa* (Mann et al. 2004), where the terminal fissures curve more gently, despite the similarity of *S. pausariae* to *S. obesa* in valve outline and stria pattern. On the other hand, *S. pausariae* resembles *S. obesa* in the presence externally of shallow grooves on either side of the raphe sternum (figs 3A, B & 4A). The grooves continue across the central area, where they can often be detected also with LM. On the primary side they extend to the poles, whereas on the secondary side, the grooves are shallower and disappear some distance from the poles (figs 3B & 4A).

The SEM used for the current study allowed better resolution of poroid structure than was possible for Mann (1989) and Mann et al. (2004) and revealed that the hymenate occlusions have pores arranged in a regular scatter *sensu* Mann (1981) (fig. 3E). It is not known if other *Sellaphora* species are similar.

It was also possible to document the girdle of *S. pausariae* more fully than has been possible in other *Sellaphora* species. In *S. bisexualis*, Mann et al. (2009) suggested from incomplete observations that there were probably “four bands in the mature cingulum: a wide band 1, a segmental band 2, and two further, very narrow bands” and exactly this arrangement can be confirmed for *S. pausariae*. In its wide pars exterior, band 1 (fig. 4C, E & F) bears a single row of



**Figure 4** – *Sellaphora pausariae*, valve and girdle, SEM: A, valve exterior; B, apex of frustule in girdle view, showing the steps in the mantles (e.g. black arrows), also the gap left by the open ends of band 1 (white arrow), which is filled by the ligulate band 2; C, dismembered cingulum, including the wide first band (b1), narrow bands (e.g. b4), and a forming valve; D, detail of C, showing details of the two narrow girdle bands (b3 and b4 : white and black arrows, respectively). The open end of band 3 is visible and the closed end of band 4. To one side is the ligulate second band(s), seen here from the inside; E, detail of C, showing the closed end and undulate pars interior of band 1, and the two narrow bands (b3 and b4 : arrows); F, detail of the closed end of band 1, showing the single line of poroids, which curves away from the abvalvar margin of the band (ab) towards the poles, and also the narrowing of the pars interior (facing inwards in both arms of the band) near the poles; G, the ligulate band 2, seen from its exterior: note the narrow pars interior. Scale bars: A & C–E = 2  $\mu\text{m}$ ; B, F & G = 1  $\mu\text{m}$ . All specimens tilted 25°, images not rotated.

small poroids, which curves towards the valve near each pole. It is open at one pole (fig. 4B & C). Band 1 narrows at the poles mainly through a decrease in the width of the pars interior, which has a plain margin at the poles but an undulate margin elsewhere (fig. 4E & F). The poroids are closed externally by hymenes (fig. 2F), as in the valves. Band 2 is segmental (fig. 4F & G), comprising a ligula that closes the gap left by the open end of band 1. It is non-porous. Bands 3 and 4 are also non-porous; they are both open and extremely narrow (fig. 4C–E).

The first band (valvocopula) is generally the widest band in diatoms, whatever the lineage, centric or pennate (though there are examples in *Nitzschia* where an abvalvar band is as wide as or wider than the first: Trobajo et al. 2006, 2013) – but it is often not overwhelmingly the widest. For example, in the *Eunotia* species illustrated by Round et al. (1990), the first band is wider than any of the others, but exceeds band 2 only by about as much as band 2 exceeds band 3, etc. However, there are several naviculoid genera in which, as in *Sellaphora*, the first band (the valvocopula) is by far the widest of the bands, and the bands further from the valve (the abvalvar bands *sensu* von Stosch 1975) are narrow strips or reduced to ligulae. This occurs, for example, in species of *Pinnularia*, *Trachyneis*, *Rhoikoneis* and *Diploneis* (Round et al. 1990, Idei 2013). Because this type of girdle is prevalent in a number of genera, some not closely related, and presumably has some functional significance, it seems worthwhile to have a term to describe it. We suggest ‘primodominance’, as in “the girdle of *Sellaphora* exhibits primodominance” (or “*Sellaphora* has a primodominant girdle structure”). In contrast, girdles in which the bands diminish in width and structural complexity more or less gradually from the valve outwards towards the girdle’s abvalvar margin, can be referred to as ‘graded’.

A feature present in *S. pausariae* and not remarked upon in previous papers, though with hindsight it can be found in photographs of several other *Sellaphora* species (our unpublished observations), is that the valve mantle is ‘stepped’, i.e. its depth decreases fairly abruptly near the poles (figs 3B & 4B). The place where the mantle narrows seems to correspond to the place where band 1 also narrows and where the single row of poroids on this band curves inwards towards the valve. The full distribution of this characteristic in diatoms is unknown, since rather few authors present images of tilted valves showing the mantle, but it seems that stepped mantles are not uncommon in pennate diatoms, occurring for instance in *Pinnularia* (Pouličková et al. 2018), *Adlafia* (Liu et al. 2017), *Cosmioneis*, *Reimeria*, *Placoneis* (Round et al. 1990), and *Cymbella* (e.g. Liu et al. 2018).

As with primodominance of the girdle, the significance of stepped mantles is unclear. However, we suggest that the possession of stepped mantles at the poles may perhaps be related to the development and maintenance of rostrate and capitate poles during the life cycle. This is because, if the valve mantles are shallower at the poles, and if the transapical circumference of the cell is therefore occupied proportionately more here by girdle bands than by the two valves, then the cell wall is probably more flexible in this region than towards the centre of the cell and will deform more in response to the outward hydrostatic pressure exerted by

the turgid cell within. As a result, an outline that is initially smooth and ±elliptical in the initial cells will, in time and with successive cell divisions, give way to one that bulges or projects at the apices. Two tests of this idea might be suggested: (1) an analysis of the variation pattern to see whether the presence of rostrate or capitate poles correlates with stepped mantles (or with equivalent differentiation of the girdle itself, such as partes exteriores of the bands that become narrower or thinner at the poles), and (2) finite element analyses of valve and girdle deformability, such as those undertaken for other purposes by Friedrichs et al. (2012) and Lu et al. (2015). Surveying for stepped mantles will need not only suitably orientated specimens, but also the assurance that specimens are intact, without significant erosion. In *S. marvanii*, for example, some of the specimens studied by Mann et al. (2011: their fig. 2f) seem to have lacked stepped mantles, but this appears to have been because of dissolution: the edge of the mantle is delicate and specimens of *S. marvanii* prepared without vigorous oxidative cleaning did possess stepped mantles (e.g. in the original of fig. 4a of Mann et al. 2011).

### Mating system

All of the *S. pausariae* clones used for experimental matings (table 2) were vigorously self-compatible and produced auxospores in monoclonal culture, i.e. each clone freely produced both male and female cells (in contrast, clones of the heterothallic *S. capitata* are almost completely self-incompatible and are either male or female: Mann et al. 1999). Tests of compatibility between *S. pausariae* clones, using cell size as a marker of clone identity, clearly demonstrated interclonal reproduction in four out of seven cases (and in these cases it was clear that either clone could act as the male during fertilization), and there was a high likelihood of interclonal reproduction in the three others.

Counts of pairs that appeared to represent interclonal matings (i.e. pairs with a clear difference in cell length, small × big), as opposed to those that seemed to be intraclonal (with similar size, small × small or big × big), were always less than expected on the basis of random pairing (table 3), though the deficiency was not always great enough to reject the null hypothesis ( $P \leq 0.05$ ). It is possible, therefore, that there is an intrinsic mechanism promoting selfing in *S. pausariae*. However, it is also possible that the deficiency of interclonal crosses is due to inhomogeneity in the distribution of the two clones in the experimental culture vessels. Even if the clones are evenly spread across the base of a culture vessel after inoculation, inhomogeneities are likely to arise through cell division, daughter cells of the same clone being closer together, on average, than they are to cells of the other clone. Hence, if all the cells are equally compatible, whether they belong to the same or different clones, it is likely that intraclonal pairing will occur more frequently than interclonal. Of course, the outcome is similar, whatever the mechanism that increases intraclonal reproduction: namely, a likely increase in homozygosity, the unmasking of recessive traits, an increase in the rate of fixation of deleterious or beneficial alleles, etc.

**Table 2 – Results of mating experiments within and between *Sellaphora pausariae* and *S. bisexualis*.**

All clones exhibited vigorous intracolonial reproduction. The most advanced stages observed in monoclonal cultures (after 6 days) are indicated along the diagonal (GF = gamete fusion, A = auxospores, PI = enlarged cells including initial cells and their descendants). For interclonal crosses, inter- and intracolonial pairs were distinguished on the basis of the *relative* sizes of the paired cells, not absolute measurements. Hence, if there was wide variation in size within either clone, the frequency of interclonal pairing could have been overestimated. Key: nd = no data (the cross was not made because the cell sizes of the two clones were so similar that interclonal crosses would not have been distinguishable from intracolonial); + = interclonal crosses certainly occurred; (+) = variability within each clone was enough that some intracolonial pairings may have been interpreted as interclonal; 0 = no interclonal pairing. Cell length data are also given (at the bottom). <sup>1</sup> s = standard deviation, n = number of measurements.

Species	<i>S. pausariae</i>	<i>S. bisexualis</i>	<i>S. bisexualis</i>	<i>S. bisexualis</i>	<i>S. bisexualis</i>								
Clone	575	576	583	512	514	515	574	581	579	582	516		
<b><i>S. pausariae</i></b>													
575	PI						0	0	0	0	0		
576	+	A					nd	0	0	0	0		
583	nd	+	PI				0	0	0	0	0		
512	+	nd	(+)	PI			nd	0	0	0	0		
514	nd	(+)	nd	nd	PI		0	0	0	0	0		
515	+	nd	(+)	nd	nd	PI	0	0	0	0	0		
<b><i>S. bisexualis</i></b>													
574							GF						
581							+	PI					
579							(+)	nd	A				
582							+	nd	nd	GF			
516							(+)	nd	nd	nd	GF		
mean length	16.6	23.8	17.7	21.9	19.6	21.9	25	30	30.5	35.3	27.3		
s <sup>1</sup>	0.84	0.75	0.71	0.55	0.87	0.38	0.89	0	0.72	0.58	1.50		
n <sup>1</sup>	10	6	9	9	3	7	6	5	2	3	7		

**Table 3 – Analysis of inter- and intraclonal pairing in *Sellaphora pausariae*.**

As in table 2, inter- and intraclonal pairs were distinguished mainly on the basis of the *relative* lengths of the paired cells, not absolute measurements, and the frequencies of interclonal pairing may therefore have been overestimated in the 583 × 512 and 583 × 515 crosses. Counts of small × small (*SS*) and big × big (*BB*) were merged. In every case, the number of small × big (*SB*) pairs was lower than expected on the basis of random pairing, though in many cases Chi<sup>2</sup> values did not exceed the 95% level. In a further cross (514 × 576), in which there was again the possibility of overestimation of interclonal pairing because of high intraclonal variation in cell length, too few pairs were counted to allow statistical analysis; however, there was again an imbalance between *SS+BB* and *SB* [counts of 8 : 1 of *SS+BB*: *SB* on each of 6.12.2004 and 7.12.2004]. <sup>1</sup> In these rows length variation within each of the two clones was high enough for some pairings to be misinterpreted as interclonal when they were in fact intraclonal (see text).

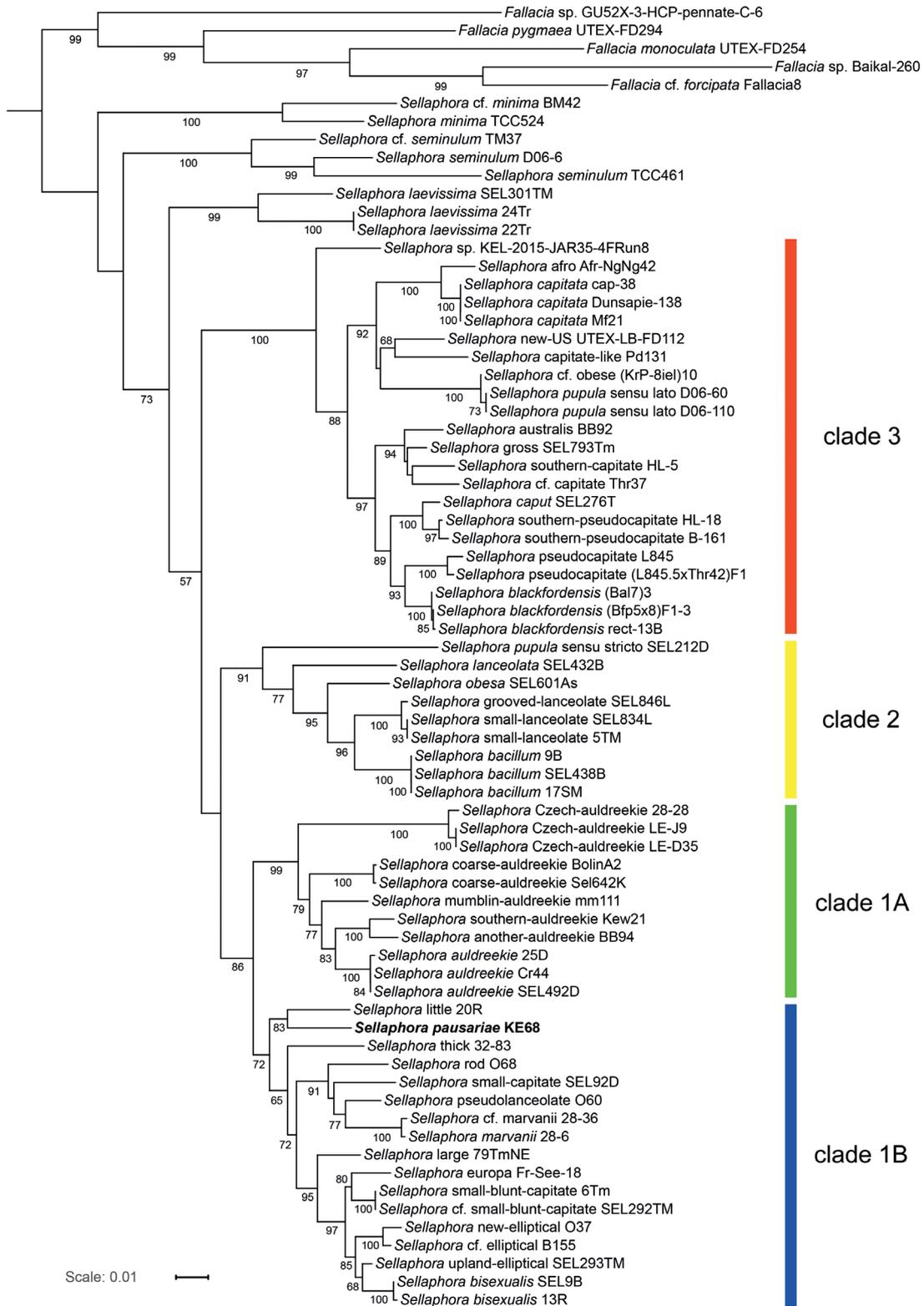
Cross (date of count)	Pairs	Counts	Expected	<i>SB</i> frequency	Chi <sup>2</sup>	90%	95%	99%
575 × 576 (6.12.2004)	<i>SS+BB</i>	34	32.8		0.24	no	no	no
	<i>SB</i>	6	7.2	deficient				
575 × 576 (7.12.2004)	<i>SS+BB</i>	36	27.5		5.25	yes	<b>yes</b>	no
	<i>SB</i>	19	27.5	deficient				
575 × 576 (9.12.2004)	<i>SS+BB</i>	22	19.2		0.81	no	no	no
	<i>SB</i>	16	18.8	deficient				
575 × 512 (6.12.2004)	<i>SS+BB</i>	29	22.9		3.36	yes	no	no
	<i>SB</i>	15	21.1	deficient				
575 × 512 (7.12.2004)	<i>SS+BB</i>	26	16.0		12.82	yes	<b>yes</b>	<b>yes</b>
	<i>SB</i>	5	14.9	deficient				
576 × 583 (6.12.2004)	<i>SS+BB</i>	18	14.5		2.01	no	no	no
	<i>SB</i>	7	10.5	deficient				
576 × 583 (7.12.2004)	<i>SS+BB</i>	19	16.5		0.76	no	no	no
	<i>SB</i>	14	16.5	deficient				
583 × 512 (6.12.2004) <sup>1</sup>	<i>SS+BB</i>	18	10.6		10.42	yes	<b>yes</b>	<b>yes</b>
	<i>SB</i>	3	10.4	deficient				
583 × 512 (7.12.2004) <sup>1</sup>	<i>SS+BB</i>	21	14.9		6.14	yes	<b>yes</b>	no
	<i>SB</i>	4	10.1	deficient				
583 × 515 (6.12.2004) <sup>1</sup>	<i>SS+BB</i>	21	13.1		8.67	yes	<b>yes</b>	<b>yes</b>
	<i>SB</i>	8	15.9	deficient				
583 × 515 (7.12.2004) <sup>1</sup>	<i>SS+BB</i>	20	11.6		12.20	yes	<b>yes</b>	<b>yes</b>
	<i>SB</i>	3	11.4	deficient				

Attempts to cross *S. pausariae* and *S. bisexualis* were always unsuccessful (table 2), even though cells of both species became sexualized and produced auxospores in the mixed cultures; pairing took place only between *pausariae* cells or between *bisexualis* cells. *Sellaphora bisexualis* has been kept for three inbred generations without obvious loss of vitality (Mann et al. 2009).

### Phylogeny

As a result of previous studies (especially Evans et al. 2007, 2008, Hamsher et al. 2011, Vanormelingen et al. 2013, Poulíčková et al. 2015), there are already many *Sellaphora* sequences in GenBank and no new ones were deposited for the present paper, apart from three extra sequences documenting the *cox1* haplotype of Blackford *S. pausariae* (see

below). However, the information available has not previously been exploited to the full. The five-gene dataset assembled for the current study goes beyond the concatenated two-gene sets analysed by Evans et al. (2008: *SSU+rbcL*) and Poulíčková et al. (2015: *cox1+rbcL*), both in the length of the alignment and the diversity of *Sellaphora* included. Nevertheless, the topology of the five-gene ML tree (fig. 5) is reassuringly similar to the topologies found earlier, with even better support for many features, including the existence of three major clades. Thus clades 1, 2 and 3 received 84, 88 and 99% support in the two-gene tree of Poulíčková et al. (2015), and 86, 91 and 100% in the current study. For comparison with the concatenated *cox1+rbcL* tree constructed by Poulíčková et al. (2015), we also constructed a new *cox1+rbcL* tree (electronic appendix 2), adding sequences that became available after the earlier study was undertaken.



**Figure 5** – Phylogenetic tree of *Sellaphora* species (italicized epithets) and demes (Roman epithets) based on a concatenated five-gene dataset (partial nuclear 18S rDNA and 28S rDNA, plastid 23S rDNA and *rbcL*, and mitochondrial *cox1*). The tree was rooted by *Fallacia* and shows bootstrap support values (1000 bootstrap replicates, values > 50%). The *Sellaphora* species and demes possessing ‘polar bars’ form a poorly supported monophyletic group comprising three well-supported clades, which are numbered 1–3 in accordance with Evans et al. (2008) and Pouličková et al. (2015), despite a few differences in clade composition. Clade 1 is itself split into two subclasses (1A and 1B). *Sellaphora pausariae* (formerly *Sellaphora [pupula K–LB] Φ ‘spindle’*) is shown in bold.

The following features of the two concatenated gene trees seem noteworthy:

- In contrast to previous studies using two-gene concatenations (Evans et al. 2008, Poulíčková et al. 2015), *Sellaphora* cf. *minima* (clone BM42) now branches off at the base of the tree, sister to a clone of “*S. minima*”; its previous position within the *S. pupula*–*S. bacillum* complex (clades 1–3) was anomalous because, unlike these species *S. minima* and similar species lack polar bars. Early branching of ‘*seminulum*-like’ *Sellaphora* lineages is also evident, in both the five- and two-gene trees.

- The topologies of both trees confirm previous analyses showing that the 20<sup>th</sup> century concept of *S. pupula* (*Navicula pupula sensu* Hustedt 1930, 1961, Krammer & Lange-Bertalot 1986) represents a paraphyletic group: *S. bacillum* is nested within clade 2, while the remainder of this clade and all of clades 1 and 3 correspond to *S. pupula* in the traditional sense.

- Within clade 2, there is a well-supported subclade comprising *S. bacillum*, *S. [pupula K–LB]* Φ ‘grooved lanceolate’ and *[pupula K–LB]* Φ ‘small lanceolate’. All three species possess conopea adjacent to the raphe-sternum, differing in whether the conopea are continuous across the central area (*S. bacillum*) or interrupted (‘grooved lanceolate’ and ‘small lanceolate’). Morphology predicts that *S. [pupula K–LB]* Φ ‘lordly’ may be sister to the *bacillum* group, since it has continuous conopea like *S. bacillum* but a stria pattern (transversely elongate central area) corresponding to that of ‘grooved lanceolate’ and other clade 2 members, and it will be interesting to test this hypothesis when molecular data become available.

- As noted by Evans et al. (2008), the phylogenetic position of *S. bacillum* implies that *Sellaphora* evolved well back in the Tertiary, since *S. bacillum* morphologies are already present in the Miocene (e.g. Ognjanova-Rumenova 2005).

- *Sellaphora obesa* is also included within clade 2, as is the type species of *Sellaphora*, *S. pupula*.

- *Sellaphora pausariae* belongs to clade 1, most likely subclade 1B, and its sister species (83% BS) is apparently *S. [pupula K–LB]* Φ ‘little’. This relationship was evident in the 18S+*rbcL* tree of Evans et al. (2007), but without high support, and it was absent in the *cox1+rbcL* tree of Poulíčková et al. (2015), the sister group instead comprising ‘little’ and *S. auldreekie*.

Thus, despite seeming to belong to a graded series of ‘*obesa*-like’ *Sellaphora* species, all with lanceolate outline and similar central areas, *S. pausariae* is not closely related to *S. obesa*, even though the two species share similar grooves externally alongside the raphe-sternum. As yet, no morphological synapomorphies can be identified for clade 1, nor for its two subclades. However, confirmation that there are none must await further detailed ultrastructural studies, which have been undertaken thus far only for *S. auldreekie*, *S. bisexualis* and *S. marvanii* (Mann et al. 2004, 2009, 2011) and *S. pausariae*.

Four extra *cox1* sequences documenting clones isolated from Blackford Pond have been deposited in GenBank as accessions MK210626 (clone KE22: electronic appendix 1), MK210625 (KE30), MK210624 (KE75) and MK210623

(KE80). All are identical to the *cox1* sequence of the type strain, KE68 (GenBank EF164951).

#### SUPPLEMENTARY DATA

Supplementary Data are available at *Plant Ecology and Evolution*, Supplementary Data Site (<https://www.ingentaconnect.com/content/botbel/plecevo/supp-data>) and consists of: (1) clones of *Sellaphora pausariae* and *S. bisexualis* isolated and studied for this paper, all from Blackford Pond, Edinburgh (pdf); (2) phylogenetic tree of *Sellaphora* species (italicized epithets) and demes (Roman epithets) based on a concatenated two-gene dataset (*cox1* and *rbcL*) (pdf); (3) concatenated alignment (fasta format) of five-gene dataset before exclusions and trimming; (4) final concatenated alignment (fasta format) of five-gene dataset after exclusions and trimming; (5) concatenated alignment (fasta format) of *cox1-rbcL* dataset before exclusions and trimming; (6) final concatenated alignment (fasta format) of *cox1-rbcL* dataset after exclusions and trimming; (7) list of taxa and GenBank accessions included in the molecular phylogenetic analyses (Excel spreadsheet).

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#### REFERENCES

- Ács É., Wetzel C.E., Buczkó K., Kiss K.T., Nagy K., Trábert Z., Földi A., Pozderka V., Werner P., Heudre D., Ector L. (2017) Biogeography and morphology of a poorly known *Sellaphora* species. *Fottea* 17: 57–64. <https://doi.org/10.5507/fot.2016.021>
- De Decker S., Vanormelingen P., Pinseel E., Seftom J., Audoor S., Sabbe K., Vyverman W. (2018) Incomplete reproductive isolation between genetically distinct sympatric clades of the pennate model diatom *Seminavis robusta*. *Protist* 169: 569–583. <https://doi.org/10.1016/j.protis.2018.05.003>
- Droop S.J.M., Mann D.G., Lokhorst G.M. (2000) Spatial and temporal stability of demes in *Diploneis smithii*/D. *fusca* (Bacil-

- lariophyta) supports a narrow species concept. *Phycologia* 39: 527–546. <https://doi.org/10.2216/i0031-8884-39-6-527.1>
- Edgar R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Evans K.M., Mann D.G. (2009) A proposed protocol for nomenclaturally effective DNA barcoding of microalgae. *Phycologia* 48: 70–74. <https://doi.org/10.2216/08-70.1>
- Evans K.M., Wortley A.H., Mann D.G. (2007) An assessment of potential diatom “barcode” genes (cox1, rbcL, 18S and ITS rDNA) and their effectiveness in determining relationships in *Sellaphora* (Bacillariophyta). *Protist* 158: 349–364. <https://doi.org/10.1016/j.protis.2007.04.001>
- Evans K.M., Wortley A.H., Simpson G.E., Chepurnov V.A., Mann D.G. (2008) A molecular systematic approach to explore diversity within the *Sellaphora* pupula species complex (Bacillariophyta). *Journal of Phycology* 44: 215–231. <https://doi.org/10.1111/j.1529-8817.2007.00454.x>
- Falasco E., Blanco S., Bona F., Gomà J., Hlúbiková D., Novais M.H., Hoffmann L., Ector L. (2009) Taxonomy, morphology and distribution of the *Sellaphora stroemii* complex (Bacillariophyceae). *Fottea* 9: 243–256. <https://doi.org/10.5507/fot.2009.025>
- Friedrichs L., Maier M., Hamm C. (2012) A new method for exact three-dimensional reconstructions of diatom frustules. *Journal of Microscopy* 248: 208–217. <https://doi.org/10.1111/j.1365-2818.2012.03664.x>
- Guillard R.R.L., Lorenzen C.L. (1972) Yellow-green algae with chlorophyllide c. *Journal of Phycology* 8: 10–14. <https://doi.org/10.1111/j.1529-8817.1972.tb03995.x>
- Guiry M.D., Guiry G.M. (2018) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from <http://www.algaebase.org> [accessed 1 Aug. 2018].
- Hamsher S.E., Evans K.M., Mann D.G., Pouličková A., Saunders G.W. (2011) Barcoding diatoms: exploring alternatives to COI-5P. *Protist* 162: 405–422. <https://doi.org/10.1016/j.protis.2010.09.005>
- Hustedt F. (1930) Bacillariophyta. In: Pascher A. (ed.) *Die Süßwasser-Flora Mitteleuropas*, vol. 10 (ed. 2). Jena, G. Fischer.
- Hustedt F. (1961) Die Kieselalgen Deutschlands, Österreichs und der Schweiz unter Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete. In: Dr L. Rabenhorst’s *Kryptogamenflora von Deutschland, Österreich und der Schweiz*, vol. 7(3:1): 1–160. Leipzig, Akademische Verlagsgesellschaft.
- Idei M. (2013) Three new species of freshwater *Diploneis* from Japan. *Diatom Research* 28: 1–11. <https://doi.org/10.1080/0269249X.2012.734527>
- Kelly M., Boonham N., Juggins S., Kille P., Mann D., Pass D., Sapp M., Sato S., Glover R. (2018) A DNA based diatom metabarcoding approach for Water Framework Directive classification of rivers. SC140024/R. Bristol, Environment Agency.
- Krammer K., Lange-Bertalot H. (1986) Bacillariophyceae 1. Teil: Naviculaceae. In: Ettl H., Gerloff J., Heynig H., Mollenhauer D. (eds) *Süßwasserflora von Mitteleuropa*, 2/1: 1–876. Stuttgart & New York, G. Fischer.
- Kumar S., Stecher G., Tamura K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular biology and evolution* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Letunic I., Bork P. (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research* 44(W1): W242–W245. <https://doi.org/10.1093/nar/gkw290>
- Liu B., Williams D.M., Ou Y. (2017) *Adlafia sinensis* sp. nov. (Bacillariophyceae) from the Wuling Mountain Area, China, with reference to the structure of its girdle bands. *Phytotaxa* 298: 43–54. <https://doi.org/10.11646/phytotaxa.298.1.4>
- Liu B., Williams D.M., Liu Q. (2018) A new species of *Cymbella* (Cymbellaceae, Bacillariophyceae) possessing valve with both uniseriate and biseriata striae. *Phytotaxa* 344: 39–46. <https://doi.org/10.11646/phytotaxa.344.1.5>
- Lu J., Sun C., Wang J. (2015) Mechanical simulation of a diatom frustule structure. *Journal of Bionic Engineering* 12: 98–108.
- Mann D.G. (1981) Sieves and flaps: siliceous minutiae in the pores of raphid diatoms. In: Ross R. (ed.) *Proceedings of the 6th Symposium on Recent and Fossil Diatoms*: 279–300. Koenigstein, O. Koeltz.
- Mann D.G. (1984) Observations on copulation in *Navicula pupula* and *Amphora ovalis* in relation to the nature of diatom species. *Annals of Botany* 54: 429–438. <https://doi.org/10.1093/oxford-journals.aob.a086813>
- Mann D.G. (1989a) The diatom genus *Sellaphora*: separation from *Navicula*. *British Phycological Journal* 24: 1–20. <https://doi.org/10.1080/00071618900650011>
- Mann D.G. (1989b) The species concept in diatoms: evidence for morphologically distinct, sympatric gamodemes in four epipelagic species. *Plant Systematics and Evolution* 164: 215–237.
- Mann D.G. (2008) The genus *Sellaphora*: an addition and corrections. *Fottea* 8: 117–119.
- Mann D.G., Vanormelingen P. (2013) An inordinate fondness? The number, distributions and origins of diatom species. *Journal of Eukaryotic Microbiology* 60: 414–420. <https://doi.org/10.1111/jeu.12047>
- Mann D.G., Chepurnov V.A., Droop S.J.M. (1999) Sexuality, incompatibility, size variation, and preferential polyandry in natural populations and clones of *Sellaphora pupula* (Bacillariophyceae). *Journal of Phycology* 35: 152–170. <https://doi.org/10.1046/j.1529-8817.1999.3510152.x>
- Mann D.G., McDonald S.M., Bayer M.M., Droop S.J.M., Chepurnov V.A., Loke R.E., Ciobanu A., Du Buf J.M.H. (2004) Morphometric analysis, ultrastructure and mating data provide evidence for five new species of *Sellaphora* (Bacillariophyceae). *Phycologia* 43: 459–482.
- Mann D.G., Thomas S.J., Evans K.M. (2008) Revision of the diatom genus *Sellaphora*: a first account of the larger species in the British Isles. *Fottea* 8: 15–78.
- Mann D.G., Evans K.M., Chepurnov V.A., Nagai S. (2009) Morphology and formal description of *Sellaphora bisexualis*, sp. nov. (Bacillariophyta). *Fottea* 9: 199–209. <https://doi.org/10.5507/fot.2009.021>
- Mann D.G., Pouličková A., Sato S., Evans K.M. (2011) Scaly incunabula, auxospore development, and girdle polymorphism in *Sellaphora marvianii* sp. nov. (Bacillariophyceae). *Journal of Phycology* 47: 1368–1378. <https://doi.org/10.1111/j.1529-8817.2011.01058.x>
- Mereschkowsky C. (1902) On *Sellaphora*, a new genus of diatoms. *Annals and Magazine of Natural History*, series 7, 9: 185–195.
- Metzeltin D., Lange-Bertalot H. (2002) Diatoms from the “Island Continent” Madagascar. *Iconographia diatomologica* 11: 1–286.
- Metzeltin D., Lange-Bertalot H., Soninkhishig N. (2009) Diatoms in Mongolia. *Iconographia diatomologica* 20: 3–686.

- Müller O. (1910) Bacillariaceen aus dem Nyassalande und einigen benachbarten Gebieten. IV. Engler's Botanische Jahrbücher 45: 69–122.
- Ognjanova-Rumenova N. (2005) Upper Neogene siliceous microfossils from Pelagonia Basin (Balkan Peninsula). *Geologica carpathica* 56: 347–58.
- Potapova M., Charles D.F. (2004) Potential use of rare diatoms as environmental indicators in U.S.A. rivers. In: Poulin M. (ed.) *Proceedings of the 17th International Diatom Symposium*: 281–295. Bristol, Biopress Limited.
- Potapova M., Ponader K. (2008) New species and combinations in the diatom genus *Sellaphora* (Sellaphoraceae) from the South-eastern United States. *Harvard Papers in Botany* 13: 171–181.
- Pouličková A., Špačková J., Kelly M.G., Duchoslav M., Mann D.G. (2008) Ecological variation within *Sellaphora* species complexes (Bacillariophyceae) – specialists or generalists? *Hydrobiologia* 614: 373–386. <https://doi.org/10.1007/s10750-008-9521-y>
- Pouličková A., Sato S., Evans K.M., Chepurnov V.A., Mann D.G. (2015) Repeated evolution of uniparental reproduction in *Sellaphora* (Bacillariophyceae). *European Journal of Phycology* 50: 62–79. <https://doi.org/10.1080/09670262.2014.985259>
- Pouličková A., Kollár J., Hašler P., Dvořák P., Mann D.G. (2019) A new species *Pinnularia lacustrigibba* sp. nov. within the *Pinnularia subgibba* group (Bacillariophyceae). *Diatom Research* 33: 273–282. <https://doi.org/10.1080/0269249X.2018.1513869>
- Round F.E., Crawford R.M., Mann D.G. (1990) *The diatoms. Biology and morphology of the genera*. Cambridge, Cambridge University Press.
- Silvestro D., Michalak I. (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* 12: 335–337. <https://doi.org/10.1007/s13127-011-0056-0>
- Stamatakis A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Stosch H.A. von (1975) An amended terminology of the diatom girdle. *Nova Hedwigia, Beiheft* 53: 1–28.
- Trobajo R., Mann D.G., Chepurnov V.A., Clavero E., Cox E.J. (2006) Taxonomy, life cycle, and auxosporulation of *Nitzschia fonticola* (Bacillariophyta). *Journal of Phycology* 42: 1353–1372. <https://doi.org/10.1111/j.1529-8817.2006.00291.x>
- Trobajo R., Rovira L., Ector L., Wetzel C.E., Kelly M., Mann D.G. (2013) Morphology and identity of some ecologically important small *Nitzschia* species. *Diatom Research* 28: 37–59.
- Vanormelingen P., Evans K.M., Chepurnov V.A., Vyverman W., Mann D.G. (2013) Molecular species discovery in the diatom *Sellaphora* and its congruence with mating trials. *Fottea* 13: 133–148. <https://doi.org/10.5507/fot.2013.012>
- Vasselon V., Rimet F., Tapolczai K., Bouchez A. (2017) Assessing ecological status with diatoms DNA metabarcoding: Scaling-up on a WFD monitoring network (Mayotte island, France). *Ecological Indicators* 82: 1–12. <https://doi.org/10.1016/j.ecolind.2017.06.024>
- Wetzel C.E., Ector L., Van de Vijver B., Compère P., Mann D.G. (2015) Morphology, typification and critical analysis of some ecologically important small naviculoid species (Bacillariophyta). *Fottea* 15: 203–234. <https://doi.org/10.5507/fot.2015.020>
- Witkowski A., Barka F., Mann D.G., Li C., Weisenborn J.L.F., Ashworth M.P., Kurzydłowski K.J., Zgłobicka I., Dobosz S. (2014) Description of *Biremis panamae* sp. nov., a new diatom species from the marine littoral, with an account of the phylogenetic position of *Biremis* D.G. Mann et E.J. Cox (Bacillariophyceae). *PLoS ONE* 9: e114508. <https://doi.org/10.1371/journal.pone.0114508>

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