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UNRAVELLING THE RELATION OF THE MORPHOLOGICAL SPECIES CONCEPT TO THE MOLECULAR ONE IN THE GENUS *FRAGILARIA* (BACILLARIOPHYTA)[1]

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UNRAVELLING THE RELATION OF THE MORPHOLOGICAL SPECIES CONCEPT TO THE MOLECULAR ONE IN THE GENUS *FRAGILARIA* (BACILLARIOPHYTA)[1]

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Running title: UNRAVELLING THE GENUS *FRAGILARIA*

Abstract

This paper evaluates taxonomic relationships in *Fragilaria* sensu stricto, an abundant and ecologically important diatom genus, taking advantage of cultured and DNA-barcoded material. The ultimate goal is to facilitate identification of European taxa within this complex, providing a unified view on morphological, molecular and ecological relationships. This will simplify both research and environmental assessment, based on either microscopical or molecular analyses. There is general agreement that the separation of species within the group of *Fragilaria* is difficult and consequent confusion can blur potentially important ecological distinctions between species. Our study demonstrated that some species defined on morphological criteria could be confirmed using *rbc*L chloroplast gene as a genetic marker, e.g. *F. gracilis* Østrup, *F. tenera* (W. Sm.) Lange-Bert., *F. perminuta* (Grunow) Lange-Bert. by Tuji & Williams and *F. subconstricta* Østrup. However, even for those species, preliminary identifications based on morphology were often different to those finally established by phylogenetic clustering. A deeper study of morphological characters using both light and scanning electron microscopy confirmed that identification of *Fragilaria* by light microscopy is indeed difficult, and that phylogeny based on DNA barcodes may be a more precise means of differentiating species. Based on molecular and morphological data, we describe three new species: *Fragilaria agnesiae*, *Fragilaria heatherae*, and *Fragilaria joachimii*. Finally, we found well-defined subgroups within one morphological species (*F. gracilis*), whose biogeography and ecology require further study.

Key index words: cryptic taxa, DNA barcoding, *Fragilaria*, integrative taxonomy, *rbc*L, species delimitation, taxonomy

List of abbreviations: RBGE, Royal Botanic Garden Edinburgh, UK. TCC, Thonon Culture Collection, France. IPS, Indice de Polluosensibilité Specifique. TDI, Trophic Diatom Index.

LM, light microscopy. SEM, scanning electron microscopy. HTS, high-throughput sequencing. NCBI**,** National Center for Biotechnology Information. MOTU, molecular operational taxonomic unit.

Working name abbreviations for *Fragilaria* groups, assigned to preliminary identifications: FCAP, *F. capucina.* FGRA, *F. gracilis.* FPEM, *F. perminuta.* FTEN, *F. tenera.* FTNS, *F. tenuistriata.* FVAU, *F. vaucheriae.* FCRNAPA, *F. crotonensis*-*F. nanoides*-*F. pararumpens.*

Introduction

This paper evaluates taxonomic relationships in *Fragilaria* sensu stricto, an abundant and ecologically important diatom genus, taking advantage of cultured and barcoded material from two different collections (Royal Botanic Garden Edinburgh, UK (RBGE); Thonon Culture Collection, France (TCC)). The goal of this paper is to facilitate identification of European taxa within this complex, providing a unified view on morphological, molecular and ecological relationships and an improved basis for biomonitoring using DNA metabarcoding (cf. Vasselon et al. 2017, Kelly et al. 2018).

Species of *Fragilaria* sensu stricto are often an important part of the diatom assemblage in freshwater ecosystems, spanning a range of ecological conditions. For example, diatoms identified as *F. capucina* var. *vaucheriae* (Kütz.) J. B. Petersen were present in 642 of 2170 samples collected in the eastern part of France (Rimet and Bouchez 2012a), whilst *Fragilaria gracilis* Østrup was the third most commonly recorded diatom taxon in 1013 samples collected in Sweden (Kahlert 2011).

It is important to separate different taxa from each other because they can give different ecological information. For example, of the 37 freshwater *Fragilaria* taxa in the Swedish standard checklist of diatoms for biomonitoring (Kahlert et al. 2017), their Indice de Polluosensibilité Specifique (IPS) sensitivity values (Cemagref 1982) range from 3.4, indicating moderate pollution or eutrophication, to 5, indicating no pollution and very high water quality. The Trophic Diatom Index (TDI) sensitivity values (Kelly and Whitton 1995) for *Fragilaria* taxa range from 1, indicating that a taxon is mainly present at very low phosphorus concentrations to 3, indicating that a taxon can tolerate moderate phosphorus enrichment. The pH preferences of *Fragilaria* taxa range from acidophilous to alkaliphilous (Van Dam et al. 1994). Some *Fragilaria* species form colonies whilst others are found as single cells only (Rimet and Bouchez 2012b, Lange-Bertalot and Ulrich 2014), and whereas many live amongst the benthos of rivers or lakes, others are planktonic (e.g. Rimet and Bouchez 2012b) or maybe even alternate between the two (e.g. Lange-Bertalot and Ulrich 2014).

However, there is general agreement that separation of species within *Fragilaria* is challenging. Krammer and Lange-Bertalot (1991) stated that the taxa used up to then were often difficult to separate, sometimes due to issues with original type description and often because, even when type descriptions were adequate, morphological characteristics overlap. Since then, *Fragilaria* has received more attention: several groups of species have been split off into other genera, type material of many taxa has been studied, new taxa have been described, and comparisons between taxa have been made (Tuji and Williams 2013, Lange-Bertalot and Ulrich 2014, Delgado et al. 2015, Wetzel and Ector 2015, Almeida et al. 2016). Problems remain, however, despite these studies: there are no or only limited descriptions of the type for several species, scanning electron microscopy (SEM) observations are sometimes missing, and there may be no descriptions of traits such as colony formation, or habitat. It is also not helpful that comparisons of new with existing species often do not take all similar taxa into account, leaving readers wondering if certain taxa are no longer considered to be valid: for instance, *F. nanoides* Lange-Bert., is not mentioned in Lange-Bertalot and Ulrich (2014).

Different laboratories have developed their own strategies to handle these issues, leading to species concepts and descriptions that do not necessarily resemble each other. As an example, the common species *F. vaucheriae* (Kütz.) J. B. Petersen – known to be tolerant to high nutrient concentrations, organic pollution and even pesticides in contrast to other species within the *Fragilaria capucina* complex sensu Krammer and Lange-Bertalot (1991), which are seen as more sensitive (Cemagref 1982, Lecointe et al. 1993, Kelly and Whitton 1995, Larras et al. 2013, Lecointe 2018) – is differentiated using different criteria by different authors: The original type material, studied by Wetzel and Ector (2015), shows a taxon with a length of 14–50 µm, a width of 3.8–5.1 µm, and 11–14 striae per 10 µm. However, Hofmann et al. (2011) report 9–14 striae per 10 µm whilst the online Diatoms of the United States (Morales 2010) states 14–16 striae per 10 µm. In a compilation aimed to harmonize identification of diatoms from the Baltic Sea, *F. vaucheriae* is described as having a width of 2.5–4 µm (Snoeijs and Potapova, 1995) and, in the European Diatom Database (Battarbee et al. 2001), *F. vaucheriae* has been merged with *F. rumpens* (Kütz.) G. W. F. Carlson. The situation gets more complicated because Krammer and Lange-Bertalot (1991) omitted *F. pectinalis* (O. F. Müll.) Lyngb. which is narrower and has finer striae than *F. vaucheriae* whilst Wetzel and Ector (2015) have described *F. microvaucheriae* C. E. Wetzel & Ector sp. nov.

Fragilaria gracilis presents another example of confusion within this genus. Even though type material was studied and compared with similar taxa by Tuji (2007) and Lange-Bertalot and Ulrich (2014), its separation from other species has not necessarily become clearer. Lange-Bertalot and Ulrich (2014) give a key where the first character separating *F. gracilis* from other similar taxa is “no formation of colonies”. However, most diatom analyses are based on prepared slides with limited opportunities to check the unprepared sample for colony-formation. Moreover, even if there is access to fresh material, it can be difficult to directly link the unprepared specimens of *Fragilaria* to identifiable cleaned (i.e. oxidized) specimens on prepared slides or SEM stubs if there are several similar taxa present in a single sample (which is often the case). So colony formation, even if helpful for a species description, and important in ecology, is not a very helpful characteristic for routine diatom identification. The next criterion used by Lange-Bertalot and Ulrich (2014) is valve shape (“needle-shaped”), which then leads to a choice between “valve ends narrowly rounded, never subcapitate or capitate”, which would identify the diatom as *F. saxoplanktonica* Lange-Bert.

et Ulrich, or “valve ends capitate or at least weakly subcapitate, sometimes protracted”, which would be other species, among them *F. gracilis*. Tuji (2007), on the other hand, studied the type material of *F. gracilis* and reported that “the form of apex [of *F. gracilis*] can vary from acute to subcapitate”. So again this character is not really helpful in routine analysis. Finally, separation of *F. gracilis* from the recently described species *F. aquaplus* Lange-Bert. et S. Ulrich is based mainly on the number of striae (*F. gracilis* 19.5–21.5 per 10 µm, *F. aquaplus* 22–24 per 10 µm), which is a good character to use for routine analysis because it can be easily measured. However, the additional criterion of the striae “opposite or alternating” (*F. gracilis*) versus “mostly opposite” (*F. aquaplus*) is again weak, as the qualifier “mostly” requires the analyst to make value judgements about whether an exception to a general rule indicates a different species or simply phenotypic variation within a population. In summary, different experts analyzing diatom samples probably have a quite different picture of *F. vaucheriae* or *F. gracilis* and relatives, leading to problems when taxa lists from different laboratories, projects and countries are compared (e.g. Kahlert et al. 2009).

While traditional descriptions of diatom species and identification aids have been based solely on morphological characters, we now have an additional tool to gain deeper insights into taxonomy: DNA sequences. Using DNA sequences has provided a completely new set of characters to compare, helping to unravel species boundaries and names, and giving a level of clarity about species boundaries that was extremely difficult to obtain previously (Amato et al. 2007, Sarno et al. 2007, Evans et al. 2008, Poulíčková et al. 2010, Vanormelingen et al. 2013). Appropriate short regions within selected genetic markers can be used as ‘DNA barcodes’ for identification (e.g. Mann et al. 2010), especially during applications of high-throughput sequencing (HTS) technologies for DNA metabarcoding. Analyzing environmental samples with DNA metabarcoding is already being applied in ecological assessment and research (Vasselon et al. 2017, Kelly et al. 2018, Rimet et al. 2018b) and will also give new insights into diatom taxonomy as researchers investigate the biological meaning and phylogenetic significance of clusters of DNA sequences (molecular operational taxonomic units, MOTUs).

In this study we aim to unravel *Fragilaria* species taxonomy and names by comparing a phylogenetic tree created from molecular data with observed morphological characters, and with a list of morphological features compiled from published species descriptions and type material. Our goal is to ensure consistency in the use of species names, to supply reference sequences (and hence DNA barcodes for HTS analyses), and where possible to identify diagnostic morphological features. We aim to define monophyletic taxa based on molecular data because paraphyletic taxa are almost impossible to detect with DNA metabarcoding. This, in turn, will give more accuracy and robustness to ecological values of *Fragilaria* species, facilitating the development of DNA-based approaches for ecological assessment using diatoms.

Material and methods

Studied strains

The strains studied come from two different culture collections: the Royal Botanic Garden Edinburgh (RBGE) and the Thonon Culture Collection (TCC), France. They were initially identified to species using the light microscope (LM) and common diatom identification literature, before being sequenced and investigated by SEM. For this study, we selected all available strains of both collections identified as belonging to the genus *Fragilaria* and the neighbor genus *Ulnaria*. For detailed information on the studied strains see Table S1, and Rimet et al. (2016), Kelly et al. (2018), Mougin et al. (2018), and Rimet et al. (2018a).

128 strains were available from RBGE, collected and isolated from Scotland and England in 2012 (hereafter United Kingdom, UK); all were sequenced for *rbc*L as described by Kelly et al. (2018). The isolates were cultured only as long as required to generate enough material for DNA extraction. For voucher material, subsamples were dried onto small round cover slips (13 mm diameter for SEM, 18 mm diameter for LM). For LM, the diatoms adhering to the cover-slips were cleaned in situ using hot 70% HNO_3 and then washed with deionized water before mounting in Naphrax (Brunel Microscopes, Chippenham, UK). Light micrographs were taken (by S. Sato and D. Mann, for preliminary identification for the biomonitoring project of Kelly et al. 2018) prior to the present project but no SEM observations of the *Fragilaria* clones had been made. DNA extraction and *rbc*L sequencing of the RBGE clones is described by Kelly et al. (2018).

The TCC collection hosts isolates from different countries and geographical regions. Those used in our study were from Europe (France, Italy, Portugal, Luxembourg, Sweden), and a few from the over-sea territories of France (Ile de La Réunion). The TCC database also includes information about curated strains from NCBI (National Center for Biotechnology Information) (Rimet et al. 2016), and such strain information was also added to the present study when relevant. Altogether, *rbc*L barcodes were available for 66 strains in the TCC collection. The RBGE barcode and strain information has now also been added to the TCC collection. DNA extraction and *rbc*L sequencing of the TCC clones, plus information about the NCBI strains used, are described in Rimet et al. (2016).

Study of morphological characters

RBGE strains: three different analyses of the morphological characters were made for the present study: (1) examination of LM photographs of cleaned material on permanent slides,

(2) a study of the fragile unprepared voucher material dried onto cover slips, especially to see the form of any colonies present, and (3) a scanning electron microscopy (SEM) study of material dried on the 13 mm cover slips, after cleaning the material in situ with 70% HNO_3 and washing with water, as for the LM preparations. Due to time restrictions, detailed studies of voucher material (for colony formation and SEM) were done on a subset of the sequenced isolates (42 strains). For SEM, cover slips were attached to aluminum stubs by carbon discs, coated with platinum, and studied in a LEO Supra 55VP at 5 kV.

TCC strains: LM photographs were not available for all strains and no SEM observations had been made before our study. To obtain material for SEM, all still-living strains were cultured at the Swedish University of Agricultural Sciences, and 23 of these were examined using SEM at RBGE, prepared in the same way as for the RBGE strains. We then studied all available LM photographs of living cultures and cleaned material, and additionally SEM pictures of the 23 strains.

Combined dataset: The selection of strains for SEM was based on a preliminary phylogenetic analysis to analyze diversity, and to cover morphological variability within the clusters, within the constraints of strain availability. Using the morphological characteristics determined with LM and SEM, we then re-examined the strain identifications in relation to published information and, where possible, the morphological characteristics of type material. We also checked commonly used identifications and related literature to study how other authors evaluated this complex. The compilation of morphological features from published species descriptions and type material is available as Table S2.

General remark on morphological data derived from diatom cultures: We are aware that diatom cells in cultures, especially after some time, often decrease in cell size and develop valve deformations, and thus may not necessarily resemble the morphology of natural populations (Mann and Chepurnov 2004). We dealt with this problem by (a) where possible, using morphological characters derived from LM micrographs taken as soon after isolation as possible where cell length and morphology had not yet changed from the population that existed at the site at the time of sampling, and (b) taking care when interpreting populations with obviously reduced cell sizes coupled to clear deformities during our analyses.

Phylogenetic analyses

The *rbc*L chloroplast gene marker was used to produce a phylogeny of the *Fragilaria* strains. This gene was selected for having 1) available sequences for all our *Fragilaria* strains and b) good efficiency to identify diatoms at species level with DNA metabarcoding when compared to other genes (Kermarrec et al. 2013, Kermarrec et al. 2014). All *rbc*L sequences of the 194 selected strains from the RBGE and TCC collections were aligned using CLUSTAL W Multiple alignment (Thompson et al. 1994) in BioEdit (Hall 1999). The lengths of the Sanger sequences were 1440 base-pairs (bp) for the RBGE strains and at least 574 bp for the TCC strains. To establish a phylogenetic tree, we used a subset of the aligned part trimmed to 1087 bp. The best substitution model was identified with PhyML (Guindon et al. 2010). A first phylogenetic tree was then calculated with RAxML v.8.2.10 (Stamatakis 2014) with the substitution model GTR+I+G. The tree was rooted with *Ulnaria* (30 strains), a neighbor genus of *Fragilaria* (154 strains). To assess branch support, 1000 bootstraps were run. We then removed duplicate sequences, leaving 45 *Fragilaria* and 12 *Ulnaria* sequences. We re-calculated the phylogenetic tree, using the same analysis as before. We used a bootstrap threshold of 69% (Soltis and Soltis 2003) to define well-supported groups and thus help define MOTUs. A distance matrix of all long *rbc*L sequences (length > 1086; 178 strains) was calculated by MEGA7 (Kumar et al. 2016) to analyze the number of substitutions per base pair within well-supported phylogenetic clusters.

Material and data accessibility

All material associated with the RBGE strains is stored at the Royal Botanic Garden Edinburgh (for voucher slide accession numbers, see Table S1). All material associated with the TCC strains is accessible through the Thonon Culture Collection (Rimet et al. 2018a). All metadata of both the RBGE and the TCC strains are stored in the open-access R-Syst::diatom reference database; a detailed description of this database and its management is given in (Rimet et al. 2016).

Results

Overall, the phylogenetic tree based on the *rbc*L barcode and rooted with *Ulnaria* (Fig. 1) shows two groups of *Fragilaria* taxa: (1) a modestly supported clade with relatively few sub-branches (the FGRA clade), of which one subgroup was however well-supported; and (2) a very well supported but more heterogeneous clade containing some well supported subgroups together with other strains whose interrelationships are unclear. Below we define and characterize the *Fragilaria* clades that receive good support in the *rbc*L tree. An overview of the morphological characters visible in light and scanning electron microscopy respectively, and a summary of the molecular separations, can be found in Tables 1 and 2. To simplify reading, we used working name abbreviations for the *Fragilaria* groups. Thus, FCAP, FGRA, FPEM, FTEN, FTNS, FVAU, FCRNAPA refer to clones that were assigned, in preliminary identifications, to *F. capucina*, *F. gracilis*, *F. perminuta* (Grunow) Lange-Bert. by Tuji & Williams, *F. tenera* (W. Sm.) Lange-Bert., *F. tenuistriata* Østrup, *F. vaucheriae*, and the group of *F. crotonensis* Kitton-*F. nanoides* Lange-Bert.-*F. pararumpens* Lange-Bert., G. Hofmann & Werum, respectively.

The FGRA clade

The modestly supported FGRA clade included 77 strains in total, among which there was a well-supported (97%) subgroup, a clade, with 52 strains. Both groups contain many strains from UK, but also from other parts of Europe. 19 of the 77 strains were studied in SEM. Three of the TCC cultures had turned into dwarf forms at the time of study; therefore their morphological characters were considered with care. We first summarize the well-supported clade **FGRA2** separately from the rest of the strains, which form a paraphyletic grade and are referred to as **FGRA1.** All of the strains with an original identification as "*F. gracilis*" fell into the **FGRA** clade.

FGRA2 (52 strains, Fig 2a). 50 of the strains were from UK, 2 from Italy. Two haplotypes were recorded. *Morphology.* No aggregations of cells were observed. Valves mostly linear or linear-lanceolate, gradually narrowing towards bluntly rounded to weakly protracted or subcapitate ends. Length 12–45 μm, breadth 2–3.1 μm at the widest point. The central area is, in most cases, approximately square and the axial area is narrow and linear; sometimes the central area extends into a widened axial area, producing a rhombic shape. Striae always opposite. Stria density 20–24 per 10 μm (mean 22), no areolae visible in light microscope. SEM revealed a rimoportula on the valve face at one of the poles. Two oval to rectangular apical pore fields with 4-8 columns of pores, containing a maximum of four pores each. Spines absent. *Sequence distances.* The sequence distances within the group of FGRA2 of the studied part of *rbc*L of 1087 bp were 0–0.001 substitutions per site.

FGRA1 (25 strains, Fig. 2b). 18 of the strains were from UK, the rest from Italy, Portugal, Luxembourg and Sweden. Five haplotypes were included in this group. *Morphology.* No aggregation of cells was observed. Valves mostly linear, a few strains had linear-lanceolate outlines, gradually narrowing towards bluntly rounded to weakly protracted or subcapitate ends. Length 16–42 μm, breadth 1.8–2.7 μm at the widest point. The central area is, in most cases, approximately square and the axial area is narrow and linear; sometimes central area extends into a widened axial area, producing a rhombic shape. Striae always opposite, 18–23 per 10 µm (mean 20), no areolae visible in light microscope. SEM revealed a rimoportula on the valve face at one of the poles. Two apical pore fields with oval to rectangular form; 4–8 pore columns with up to four pores each. Spines absent. *Sequence distances.* The sequence distances within the group of FRGA2 of the studied part of *rbc*L of 1087 bp were 0–0.004 substitutions per site.

The heterogeneous **Fragilaria** *clade, containing the FVAU, FCAP1, FCAP2, FTNS, FTEN1, FTEN2 and FPEM subgroups and the residual FCRNAPA strains*

All other strains identified as *Fragilaria* species fell into the well-supported (98%) heterogeneous clade. Whereas all strains in the **FGRA** clade had opposite striae, all those in this clade had alternate striae.

Although the deeper relationships in this clade were unclear, it did contain well-supported subgroups. One clade with an identical sequence of the studied part of the *rbc*L barcode (hereafter called **FVAU**) contained several strains originally identified as *F. vaucheriae.* Two other clades contained many strains original identified as *F. capucina* (hereafter called **FCAP1**, 79% support and **FCAP2**, 84% support). Other groups were a clade containing strains all originally identified as *F. perminuta* (**FPEM**, 87% support), and a clade resembling *F. tenuistriata* (hereafter called **FTNS**, 79% support). We also found two clades containing strains with longer valves, resembling *F. tenera* (hereafter called **FTEN1**, 100%, and **FTEN2**, 79% support). There were also many additional strains in the heterogeneous **FCRNAPA** clade, which were irregularly distributed in the phylogenetic tree but still relatively closely related, according to their sequences. Many of these strains were originally identified as *F. crotonensis*, *F. nanoides* or *F. pararumpens***.**

FVAU (6 strains, Fig. 3). The clade was made up of strains from UK (1 strain), the Ile de La Réunion (3 strains), Luxembourg (1 strain) and Italy (1 strain), all strains with identical sequences regarding the studied part of *rbc*L. *Morphology.* LM pictures were available for four strains; these showed long ribbon-band colonies in three, but not in 041SynPO4; instead, cells were found in irregular dense clumps some of them loosely attached to each other. For further details on morphology, see Box 1.

FCAP1 (4 strains, Fig. 4). The clade was made up of one strain from Italy (haplotype 1) and three strains from UK (haplotype 2). *Morphology*. LM and SEM pictures were available for the UK strains. None of the strains formed ribbon-bands, but instead formed loosely irregular aggregates of about ten cells. However, in two strains with elongated cells (~37 µm) most cells came in pairs connected either in the middle or via the entire side. For further details on morphology see Box 2. *Sequence distances.* The sequence distances of the studied part of *rbc*L of 1087 bp were 0–0.003 substitutions per site.

FCAP2 (7 strains, Fig. 5). The clade was made up of two strains from Sweden with identical sequences and five strains from UK, with sequences that were identical to each other but different from the Swedish strains. *Morphology*. LM and SEM pictures were available for all strains. No clear pattern could be observed for colony formation within the clade. One of the Swedish strains formed long ribbon-like bands (>10 cells) relatively loosely connected in the central part of the cell, leaving the ends free. The other Swedish strain was found at most in pairs, some of which were connected by their ends to form irregular stellate colonies. Of the Scottish strains, three were found to form long bands (> 10 cells), one formed shorter bands (2–5 cells), and one occurred mostly as single cells with some pairs. All strains were also found to build irregular aggregates connected by the cell ends. The presence of spines was not correlated with the presence of colonies. For further details on morphology see Box 3.

Sequence distances. The sequence distances within this group for the studied part of *rbc*L of 1087 bp was 0–0.002 substitutions per site.

Box 1. *Fragilaria agnesiae* Kahlert & Rimet (Fig.3) (operational name for this article: FVAU)

Description: Valves mostly linear-lanceolate, becoming lanceolate in shorter cells, ends rostrate. Length of the studied strains 9-21 µm (to 65 µ in recently expanded post-auxospore cells). Cell width 4.0–5.4 µm at the center. Central area always unilateral, often strongly so, with a rimmed swelling on the outer valve side. Striae always alternate, 14–16 per 10 µm, with no areolae visible with LM. Most studied strains form long ribbon-like colonies, but shorter bands and irregular loose aggregates also occur. SEM characters: Rimoportula positioned on the valve face at one of the poles. Two apical pore fields with rectangular form, with 8–10 columns of pore columns containing a maximum of five pores each. Spines relatively small, with a round base ending in a round tip. No internal rimmed depression observed in the central area.

Holotype: Material of clone 041SynP04, as preserved on slide BC0041 (Royal Botanic Garden Edinburgh (herbarium abbreviation = E)) illustrated in Fig. 3c, e–i, barcoded by *rbc*L in GenBank accession XXXX, and with preserved DNA at E as EDNA13-0031229.

Authenticated material: Seven isolated strains form the basis of the species description: 041SynP04 (type strain), TCC541, TCC553, TCC558, TCC662, TCC681, TCC547. The TCC strains are all registered and conserved in the Thonon Culture Collection of the INRA at Thonon-les-Bains, France. All strains belong to one haplotype with respect to an identical part of the *rbc*L barcode sequence (1087 bp long).

Type locality: Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian (UK), sampled 19 May 2012 by David Mann, coordinates: 55°84′42.2″N, 3°31′10.9″W.

Name registration: http://phycobank.org/100270

Etymology: The specific epithet refers to the wife of one of the authors.

Similar taxa: This taxon resembles especially *F. rinoi* Almeida et C. Delgado, but this species is described as having no spines and a solitary habit. Other similar species matching in length and form are *F. vaucheriae, F. neointermedia* Tuji & Williams*, F. capucina, F. pectinalis* and *F. uliginosa* Kulikovskiy, Lange-Bert., Witkowski & Dorofeyuk. However, *F. vaucheriae,* which is the closest in width, should not have more than 14 striae per 10 µm and not form ribbon-like colonies, *F. pectinalis* and *F. uliginosa* also do not form colonies, the latter also has large spathulate spines. Other taxa matching *F. agnesiae* in length and striae density differ from it in the possession of prominent capitate heads (e.g. *F. recapitellata* Lange-Bert. et Metzeltin), or because they are thinner (e.g. *F. perminuta* and *F. microvaucheriae)*.

Ecology: The cluster of strains used to describe this new species was isolated from streams in UK (Pentland Hills, Green Cleuch, above Balerno, Midlothian), the Ile de La Réunion (rivière de Bras Caverne, rivière de Langevin – grand Galet, and rivière de Langevin, site amont prise EDF), Luxembourg (river Our at Vianden), and from Italy (Trentino, rivière de Regnana à Amont de Bedollo). There are no direct measurements of water chemistry for any of the collection places, but there is some information for the rivière de Langevin on Ile de La Réunion: pH was 8–8.4, conductivity 8.4-9.3 mS m^{-1}, and oxygen saturation was 113% at a temperature of 21–22 °C (measured 2006 and 2007). The dominant diatom taxa indicated moderately high nutrient concentrations at all three sampling sites, and a possible impact of organic pollution at the rivière de Langevin site amont prise EDF. *F. agnesiae* was recorded from this site with 0.7% in November 2006. The River Our at Vianden, Luxembourg is underlain by schist geology and has good water quality.

Box 2. *Fragilaria heatherae* Kahlert & M. G. Kelly (Fig. 4) (operational name for this article: FCAP1)

Description: Long valves are spindle-shaped with subcapitate ends; short valves are lanceolate with acute to rounded ends. Length of the studied strains 9–38 µm. Cell width 3.3–3.7 at the center. Central area of long cells weakly asymmetric and blurred, whilst, in short cells it is strongly unilateral and clearly separated from the axial area, with a rimmed swelling on the external valve side. Striae always alternate, no areolae visible with LM. Stria density 16 per 10 µm in long cells, 18 per 10 µm in short cells. Cells found in loosely irregular aggregates of about ten cells. SEM characters: Rimoportula positioned on the valve face at one of the poles. Two apical pore fields with rectangular form, with 9 (short cells) or 12–14 (long cells) columns of pores containing a maximum of six pores each. Spines absent.

Holotype: Material of strain 513FraK01 as preserved on slide BC0513 (E), illustrated in Fig. 4a, d, h, barcoded by *rbc*L in GenBank accession XXXX, and with preserved DNA at (E) as EDNA13-0031453.

Authenticated material: Four strains are the basis for the species description: 513FraK01 (type strain), 514FraK01 (in E as slide BC0514), 621FraP11 (in E as slide BC0621), and TCC682 (registered and conserved in the Thonon Culture Collection of INRA at Thonon-les-Bains, France). The RBGE strains belong to one haplotype with respect to an identical part of the *rbc*L barcode sequence (1087 bp long), the TCC strain to a second haplotype.

Type locality: Euden Beck (England, UK), sampled 20 June 2012 by Martyn Kelly, coordinates: 54°66′50.4″N, 1°89′76.5″W.

Name registration: http://phycobank.org/100271

Etymology: The specific epithet refers to the wife of one of the authors.

Similar taxa: Size and form resemble *F. capucina* sensu stricto Desm., but this species has spathulate spines in contrast to *F. heatherae* where spines are absent. The size and shape resemble also *Fragilaria pectinalis* (O. F. Müll.) Lyngb. and *Fragilaria microvaucheriae* C. E. Wetzel et Ector; however, the upper limit for the length:width ratio, which provides clear diagnostic characters for these two species, is lower (<8) than for *F. heatherae* (up to 11, wholly including the ranges of the other two). Moreover, the subcapitate form of the ends separates *F. heatherae* from *F. microvaucheriae* (rostrate ends). *F. perminuta* has a higher striae density (17–19), *F. vaucheriae* a lower striae density (9–14).

Ecology: The cluster of strains used to describe this new species was isolated from streams in UK (Euden Beck and River Tay, Pitlochry, Perth & Kinross) and Italy (Trentino rivière de Regnana à Amont de Bedollo). Euden Beck drains a largely forested catchment with some moorland and rough grazing in the upper catchment. The water is relatively soft and low in nutrients (mean chemistry for June 2011 to June 2012: pH: 7.4; alkalinity: 18 mg L^{-1} $CaCO_3$; conductivity: 10.4 mS m^{-1}; ammonia-N: 0.02 mg L^{-1}; nitrate-N: 0.22 mg L^{-1}; molybdate-reactive P: 0.015 mg L^{-1}). Long-term averages for the River Tay at Aberfeldy also show circumneutral, soft and low nutrient conditions (pH: 7.3, conductivity: 5.3 mS m^{-1}, alkalinity: 11.7 mg L^{-1} CaCO3, NO3-N: 0.103 mg L^{-1}, ammonia-N: 0.024 mg L^{-1}, reactive P: 0.013 mg L^{-1}, total P: 0.031 mg L^{-1}).

Box 3. ***Fragilaria joachimii*** Kahlert (Fig. 5) (operational name for this article: FCAP2)

Description: Long cells: linear; medium-sized cells: lanceolate to spindle-shaped; short cells: lanceolate. Length of the studied strains 5–34 µm. Cell width 3.3–4.6 measured at the center. Central area variable, mostly rectangular, or unilateral with a rimmed swelling. Striae always alternate, 14–16 per 10 µm (up to 19 in some short cells); no areolae visible in LM. No regular pattern of colony formation: cells found in long ribbon bands, or pairs and singular cells, all forming loosely connected aggregates. SEM characters: rimoportula positioned on the valve face at one of the poles. In one cell two rimoportulae were found, one at each pole of one valve. Two apical pore fields with rectangular form, with 5–14 columns of pores containing a maximum of five pores each. A central rimmed depression mostly present in various grades. Very tiny spines irregularly arranged on the valve edges and ends, no spines found on short valves. The presence of spines was not correlated with the presence of long ribbon-like colonies.

Holotype: Material of strain TCC887 (Thonon Culture Collection of the INRA at Thonon–les–Bains, France), illustrated in Fig. 5b, b2, c, j, m, o and p, barcoded by *rbc*L in GenBank accession XXXX.

Name registration: http://phycobank.org/100272

Authenticated strains: Seven isolated strains provide the basis of the species description: 042SynP04, 046SynP04, 054SynP04, 171FraB05, 435FraT01 (all in E, as slides BC0042, BC0046, BC0054, BC0171 and BC0435, respectively), and TCC877 and TCC887 (type strain) (registered and conserved in the Thonon Culture Collection of the INRA at Thonon-les-Bains, France). The five RBGE strains belong to one haplotype with respect to an identical part of the *rbc*L barcode sequence (1087 bp long), the two TCC strains to a second haplotype.

Type locality: Broströmmen near Norrtälje city, Sweden, sampled 24 September 2013 by Maria Kahlert, coordinates: 59°75′72.8″N, 18°72′06.0″E.

Etymology: The specific epithet refers to the husband of the author.

Similar taxa: In size and form, *F. joachimii* resembles *F. capucina* Desm. sensu stricto, but *F. capucina* has spathulate spines, in contrast to *F. joachimii*, where the spines are tiny and irregular. The size and form also resemble *F. pectinalis* and *F. microvaucheriae*; however, the length:width ratio (defined as clear diagnostic character for these two species) of *F. joachimii,* is wholly including the ranges of both those species. Moreover, the presence of spines, the formation of colonies and the subcapitate form of the ends separate *F. joachimii* from *F. pectinalis* (no spines or colonies) and *F. microvaucheriae* (rostrate ends). *F. perminuta* has a higher striae density (17–19 per 10 µm), *F. vaucheriae* a lower striae density (9–14 per 10 µm).

Ecology: The cluster of strains used to describe this new species was isolated from streams in Sweden (Norrtäljeån and Broströmmen, both close to Norrtälje city) and Scotland, UK (Pentland Hills, Green Cleuch, above Balerno, Midlothian; Allt a'Bhalachain, Argyll & Bute; and the River Tay, near Aberfeldy, Perth & Kinross). There is a detailed monitoring program for water quality available for the Swedish streams. Both catchments are covered by approx. 30–50% forest, and 20–40% agricultural land. Diatoms have generated IPS values of 13.1–15.8 over the course of several years. Mean pH in Broströmmen was 7.7, mean total phosphorus (TP) 0.046 mg l^{-1} and mean total nitrogen (TN) 1.162 mg l^{-1}. Mean Total Organic Carbon (TOC) was 12.7 mg l^{-1} and mean conductivity 36 mS m^{-1} (measured 2006–2008). Annual mean oxygen saturation was 78% at a mean temperature of 8.0°C (measured 2016). In Norrtäljeån, annual mean temperature was 8.0°C, pH 7.8, conductivity 37 mS m^{-1}, TOC 13 mg m^{-1}, oxygen saturation 83%, TP 0.049 mg $l^{-1}$and TN 1.666 mg l^{-1} (measured in 2016).

Long-term averages for the River Tay at Aberfeldy: pH: 7.3, conductivity: 5.3 mS m^{-1}, alkalinity: 11.7 mg L^{-1} CaCO3, NO3-N: 0.103 mg L^{-1}, ammonia-N: 0.024 mg L^{-1}, reactive P: 0.013 mg L^{-1}, total P: 0.031 mg L^{-1}).

FPEM (10 strains, Fig. 6). This well-supported clade of 10 strains (87%) comprised three haplotypes, of which the first was represented by two strains from UK and five from Sweden, the second by two, and the third by one strain from France, respectively. All were originally identified as *F. perminuta*. *Morphology.* LM data were available for all strains and SEM for five of them. All strains were rhombic, with a distinct strongly unilateral central area with a rimmed external swelling and internal depression (horse-shoe like structure). Length: 8–23 µm; width: 3.2–4.6 µm; stria density: 18 to 21 per 10 µm. No spines were observed in SEM, and the strains did not form colonies. *Sequence distances.* The sequence distances within the group of FPEM of the studied part of *rbc*L of 1087 bp was 0–0.001 substitutions per site.

FTNS (3 strains, Fig. 7). The next well-supported clade (79%) comprised a mixture of one UK and two TCC strains from Sweden, originally identified as *F. mesolepta* Rabenhorst and *F. tenuistriata*. The UK and one of the TCC strains belonged to one haplotype, the other TCC one to a separate one. *Morphology*. For the RBGE strain and one TCC strain both LM and SEM pictures were available; for the other TCC strain LM pictures only. All three strains formed long ribbon-like colonies, with no obvious separation cells. The strains were 34–40 µm long and 4.1–4.5 µm wide, with 16–17 alternating striae per 10 µm. Valves linear, center very slightly almost not constricted, ends bluntly rounded. In SEM, large spathulate spines were observed. The rimoportula was placed at the mantle face junction. *Sequence distances.* The sequence distances within the FTNS clade of the studied part of *rbc*L of 1087 bp were 0–0.001 substitutions per site. For comparison, we also studied the strain 653FraK08, which was also originally identified as *F. mesolepta*, but did not cluster together with the three FTNS strains. This strain had similar morphological features as FTNS, but a different valve outline (Fig. 7b).

FTEN1 (7 strains, Fig. 8). This clade of seven strains (100%) comprised two haplotypes, all from UK, one represented by six strains, the other by one. Of the seven strains, three were originally identified as *F. tenera* or *F.* cf. *tenera*, two as *F. pararumpens* and two as *F. gracilis***.** *Morphology*. LM data were available for all strains and SEM for six of them. The strains were quite similar morphologically, ranging from 40–59 µm long, 2.1–3 µm wide, with 18–20 alternating striae per 10 µm. Most cells were solitary or, at most, loosely aggregated. The valves were linear-lanceolate with slightly convex margins and subcapitate apices. The central area was quite broad and slightly inflated in six out of the seven strains and extended into the axial area due to shortened striae. In SEM, the central area was more difficult to observe and marked by ghost striae in all cases. SEM revealed that all strains had very regularly arranged pyramidal spines, often with a sharply bent tip. Spines were present at the poles as well. *Sequence distances*. The sequence distances within the group of FTEN1 of the studied part of *rbc*L of 1087 bp were 0–0.001 substitutions per site.

FTEN2 (3 strains, Fig. 8). This clade was made of three strains of two haplotypes (79%), one from UK and two from Sweden. The Swedish strains were originally identified as *F.* cf. *nanoides*, the Scottish strain as *F.* cf. *pararumpens*. *Morphology*. LM data were available for all strains and SEM for two of them. Like the FTEN1 clade, the strains were quite similar morphologically, being 50–85 µm long, 2.4–3 µm wide, with 21 alternating striae per 10 µm. Most cells were solitary or at most in pairs; rarely three to four cells were connected at the ends to form a stellate colony. The valves were spindle-shaped with slightly convex margins and subcapitate to capitate apices. The central area was even broader than in FTEN1, and also slightly inflated in all strains, extended far into the axial area due to shortened striae, creating a rhombic central area. Some cells had a quite long section of the central part of the valve having parallel sides, which then abruptly tapered to the ends. In SEM, the central area was also rhombic, and often marked by ghost striae. Spines were present, and often of pyramidal form. One rimoportula was present on each valve, close to the end. However, in contrast to FTEN1, spines were more irregular with various forms, and were often missing in the "neck" of the cell. Spines could have both round and rectangular quadratic bases, and the tip, often sharply bent, could also form a leaf-like structure in some cells, or was reduced to a wart. *Sequence distances*. The sequence distances within the group of FTEN2 of the studied part of *rbc*L of 1087 bp were 0–0.001 substitutions per site.

FCRNAPA: the residual strains of the heterogeneous clade. The original identifications of these strains as *F. crotonensis*, *F. nanoides* or *F. pararumpens* reflects the fact that all of them had very elongate valves.

Discussion

General. Overall, our phylogenetic tree based on *rbc*L and rooted with *Ulnaria* showed two *Fragilaria* clades well-separated both from each other and from the *Ulnaria* strains. One clade contained all strains identified as *F. gracilis*, whereas the other was a heterogeneous group including several well-supported subgroups. Below we try to unravel species taxonomy and names of the *Fragilaria* groups that received good support in the *rbc*L tree.

The FGRA clade. The original identifications of these strains were in most cases *F. gracilis*, except for some of the TCC strains, which had originally been identified as *F. rumpens*. The original description of *F. gracilis* was by Østrup (1910), who described it as having almost linear valves with subcapitate to slightly attenuated ends, giving a length of 63 µm and a width of 3.6 µm, with 20 striae per 10 µm. However, these length and width measurements cannot be confirmed when measuring the original picture of Østrup (1910, Tab V, Fig. 117). Instead, the original drawing has a length of 43 and a width of 2.1 µm, with 25 striae per 10 µm. The microphotographs from the lectotype slide, coll. Østrup 1342, given in Krammer and Lange-Bertalot (1991) and Tuji (2007), show lengths of 28-54 µm, widths of 2–2.7 µm, with

18–24 striae per 10 µm. Tuji (2007) describes the striae as “being parallel throughout”, with SEM pictures showing opposite striae with some irregular parts where striae are alternate. Lange-Bertalot and Ulrich (2014) define the striae as “opposite or alternating”. Note that the term “parallel” refers to the orientation of the striae to each other, while the terms “alternate/opposite” refers to whether the striae on either side of the sternum are opposite each other or alternate. Both Lange-Bertalot and Ulrich (2014) and Tuji (2007) show that *F. gracilis* has no spines, a single transapically orientated rimoportula per valve, and two apical pore fields.

All studied strains falling into the FGRA clade in this study, both those belonging to the well-supported FGRA2 as well as those that did not (FGRA1) fit the descriptions of the type of *F. gracilis* given by Lange-Bertalot and Ulrich (2014) and Tuji (2007). The main difference between the subgroup FGRA2 and the rest of the strains is a higher density of striae in FGRA2 (a mean of 22 vs. 20 per 10 µm), and a tendency to rather more linear valve outline in FGRA1. However, this presents a rather simplistic view of the situation, as the literature on *F. gracilis* and similar taxa is more complicated than this.

The first problem is that the species diagnosis is not clear. Lange-Bertalot and Ulrich (2014) refer to Hofmann et al. (2011) for a species diagnosis, and also refer to Hofmann et al. (2011) when giving size characters for *F. gracilis* in their Table 2. However, Lange-Bertalot and Ulrich (2014) give a length of 20–45 µm, width of 1.9–2.5 µm and 20–23 striae per 10 µm, whereas Hofmann et al. (2011) have 10–60 µm, 2–3 µm and ~20 striae per 10 µm. Furthermore, Table 2 in Lange-Bertalot and Ulrich (2014) gives quite detailed size descriptions, whereas Hofmann et al. (2011) just gives a few (range of length, width and approximate number of striae), thus it is not completely clear what is meant when Lange-Bertalot and Ulrich (2014) note that their size characters of Table 2 are based on “Hofmann et al. (2011) supplemented by own observations”. Other complications are that both Lange-

Bertalot and Ulrich (2014) and Hofmann et al. (2011) state that the ends are slightly protracted and rounded, whereas both have pictures that include weakly subcapitate forms. Both Østrup (1910) and Tuji (2007) note that some valves can have subcapitate ends. Next, whereas neither Tuji (2007) nor Lange-Bertalot and Ulrich (2014) comment on colony formation, Hofmann et al. (2011) state that this taxon is often found in ribbon-like aggregates or attached in stellate colonies.

The second problem is that *F. gracilis* is addressed as part of a ‘difficult to separate complex’, with Hofmann et al. (2011) listing *F. famelica* (Kütz.) Lange-Bert.*, F. rumpens, F. tenera* and *F. capucina* as “very similar taxa”, of which *F. tenera* would be so similar that it would be “impossible to separate from” *F. gracilis*. Lange-Bertalot and Ulrich (2014) then describe even more similar-looking new species, namely *F. tenuissima*, *F. saxoplanktonica* and *F. aquaplus*.

However, we reason that our FGRA clade fits the characters of *F. gracilis* and cannot be mistaken for any of the other species mentioned above. First, stria density is higher than described for *F. famelica, F. rumpens* and *F. capucina* (Table S2). Second, *F. tenera* has been described as possessing spines (Table S2), whereas none were seen on specimens from our clade. Third, in published LM illustrations of the above-mentioned taxa (see references in Table S2), alternate striae are clearly visible, whereas our clade had opposite striae throughout. The separation of FGRA from *F. saxoplanktonica* and *F. aquaplus* is more complicated than from the other taxa, since neither *F. saxoplanktonica* nor *F. aquaplus* possess spines, and both have clearly opposite striae, according to Lange-Bertalot and Ulrich (2014). However, *F. saxoplanktonica* is defined as having denser striae (23–28 according to Lange-Bertalot and Ulrich (2014) than *F. gracilis*, and also the valve form is different (fusiform to needle-shaped) to that of *F.gracilis*.

More difficult is the separation of *F. aquaplus*. Whereas its size and striae density are similar to that of *F. gracilis* (Table S2), Lange-Bertalot and Ulrich (2014) state that *F. aquaplus* can be distinguished by having a conspicuously narrow axial area and an arrangement of striae which is "opposing throughout". Actually, using Hofmann et al. (2011) for identification, *F. aquaplus* would be identified as *F. nanana* Lange-Bert. sensu Krammer and Lange-Bertalot (1991). Using instead Cantonati et al. (2017), *F. nanana* is synonymized with *F. saxoplanktonica*, even if those authors gave a differential diagnosis of these two taxa in Lange-Bertalot and Ulrich (2014). This confusion is caused by the fact that the name "*F. nanana*" was given to a mixture of two species both present in the type slide, as described in Lange-Bertalot and Ulrich (2014). We recommend therefore that the name "*F. nanana*" is not used anymore. The FGRA strains in our study fit the length description of *F. gracilis* and *F. aquaplus*, even if the strains with a width of more than 2.5 µm would not fit into Lange-Bertalot and Ulrich (2014)'s description of those two species anymore. There is no clear picture regarding axial area, striae density and form of the ends. Some of the strains in both FGRA2 and FGRA1 would fit the description of *F. gracilis*, others of *F. aquaplus*, others none at all. We conclude from this that separation of *F. gracilis* and *F. aquaplus* is not supported by the *rbc*L barcode. We recommend that the wider definition of *F. gracilis* is used for the whole cluster, as many strains are not identifiable to either species sensu Lange-Bertalot and Ulrich (2014), whereas all of them fit the characters of the type material of *F.gracilis*. Our results support a synonymization of *F. aquaplus* back into the wider concept of *F.gracilis* Østrup sensu Østrup. We consider that *F. gracilis*, if considered in this way, is no longer a part of a 'difficult to separate complex', but can be separated in LM from the members of the heterogeneous *Fragilaria* clade using the clear opposite arrangement of the striae, which gives the impression of regularly arranged parallel lines across the valve in LM. The heterogeneous *Fragilaria* clade has alternately arranged striae instead. We do not rule out the option that the FGRA2 might represent a cryptic species, but if so, it will need a new, different separation than that proposed by Lange-Bertalot and Ulrich for distinguishing between *F. gracilis* and *F. aquaplus*.

The heterogeneous Fragilaria clade, containing the FVAU, FCAP1, FCAP2, FTNS, FTEN1, FTEN2 and FPEM subgroups and the residual FCRNAPA strains

FVAU. The original identification of these strains was *F. vaucheriae* in the UK barcode strain and four TCC strains, and *F. rumpens* in two TCC strains. Wetzel and Ector (2015) used the original type material “Kützing 185” of *F. vaucheriae* to analyze this taxon in detail, including girdle views and SEM pictures. Wetzel and Ector (2015) also noted that the name was first published in Kützing’s exsiccata set Algarum Aquae Dulcis Germanicarum (Decas III, No. 24), albeit without a description or figure, which were added later by Kützing (1833b, p. 560, fig. 38). Less detailed analyses of the original material are also given by Tuji and Williams (2006a) Tuji and Williams (2013), Lange-Bertalot (1980), pl. 4, figs 82–94, 97–102), and Krammer and Lange-Bertalot (1991) (2004, pl. 108, figs 10–15). Wetzel and Ector (2015) also state that the concept for FVAU has been shifting from a broader to a narrower concept. Valves of *F. vaucheriae* type material are linear and narrow with rostrate to subcapitate ends with a unilaterally expanded central area. The length is 14–50 µm, the width 3.8–5.1 µm, and the stria density 11–14 per 10 µm. The striae are described by Wetzel and Ector (2015) in the text as ‘subparallel’, pictures showing an alternate arrangement. For SEM, Tuji and Williams (2013) noted one rimoportula per valve near the poles, large rectangular apical pore fields, and no or very small spines that do not link to sibling cells. Wetzel and Ector (2015) confirm these observations (their figs. 39–53), adding that the sibling cells can remain loosely attached to each other (their figs. 44, 47) but never more than two cells could be found together as observed by Petersen (1938) (e.g. their figs. 6, 18, 44 and 47).

Descriptions of *F. vaucheriae* as having linking spines and forming long ribbon-like colonies (e.g. in Tuji and Williams 2006a) are most probably an error originating from the confusing and shifting concepts of this species. Wetzel and Ector (2015) and Tuji and Williams (2013) both refer to Petersen (1938)'s comments on problems with the *F. vaucheriae* type slides. The taxon identified as *F. intermedia* (Grun.) Grun. in Van Heurck's (1881) Synopsis des diatomées de Belgique, pl. 45. fig. 11 (but not figs. 9 or 10) and present on slide 552 in Grunow's collection in Vienna agrees with the original illustration given in Grunow (1860), and should be considered as synonymous with *F. vaucheriae*, having no or very small spines and forming no colonies. On the other hand, the individuals from Grunow's slide 31, identified as *F. intermedia* sensu Grunow in Van Heurck (1881) and illustrated in Van Heurck's plate 45, figs. 9 and 10 (not fig. 11) have spines and form ribbon-band colonies and have been described as a new species, *F. neointermedia* Tuji et D.M. Williams (Tuji and Williams 2013).

The characters we found for the five strains with LM pictures available, and the one with SEM pictures (041SynPO4), do not, unfortunately, yield a single consistent diagnostic character for the FVAU clade, even within the same haplotype (for a summary of morphological characters of similar species, see Table S2). The form (linear-lanceolate with ± rostrate ends and ± unilateral central area), length 9-21 µm (to 65 µ in recently expanded post-auxospore cells), width (4.0–5.4 µm) and striae density (14–16 per 10 µm) of the observed specimens match only the recently described *F. rinoi* (Delgado et al. 2016). However, *F. rinoi* was described as having no spines at all, whereas we could frequently observe small spines in SEM. Furthermore, *F. rinoi* was described as solitary, whereas four of the strains in our study formed long ribbon-formed colonies. Other species matching FVAU in length and form (*F. vaucheriae, F. neointermedia, F. capucina, F. pectinalis* and *F. uliginosa*) are less than 5.1 µm wide, whereas our specimens were up to 5.4 µm wide. *F. vaucheriae* is closest in width and form to the specimens we have observed; however, this species should not have more than 14 striae per 10 µm (the lower limit for our specimens). Moreover, whereas the tiny conical spines observed in SEM in 041SynPO4 plus the lack of long ribbon-like colonies in this strain would match both *F. vaucheriae* and *F. pectinalis*, the long ribbon-like colonies of the other strains would exclude those two species. Instead, the colony-formation of these strains, combined with their form, length and stria density, matches *F. neointermedia* and *F. capucina*. As we do not have SEM pictures for the ribbon-forming strains, we are not able to tell something about the rimoportulae (two per valve would fit *F. capucina* only), or the presence of spines (both *F. neointermedia* and *F. capucina* should have linking spines). Other taxa matching in length and striae density fit neither, not *F. recapitellata* (because it has very prominent capitate heads), nor *F. perminuta* and *F. microvaucheriae* (because they should be < 4 µm wide, and most of our valves were at least 4 µm). In conclusion, no existing species fits the well-supported clade based on the *rbc*L barcode. Moreover, both morphological and molecular analysis of this taxon group will lead to inconsistencies in species identification and links to ecology if no attempt is made to name the well-supported groups. Therefore we describe here the new species *Fragilaria agnesiae* (Box 1) and also suggest that further studies of morphological plasticity of the "real" *F. vaucheriae*, and its phylogenetic relationship to *F. agnesiae* are needed, because one possibility is that *F. vaucheriae* might not form ribbon-like colonies under all circumstances.

FCAP. The original identification of strains in FCAP1 and FCAP2 was either *F. capucina* or *F. rumpens* (TCC) or "*F. capucina* cf. var. *capucina*", *F. vaucheriae* or "*F.* cf. *vaucheriae*" (RBGE). One strain was named "*F.* cf. *gracilis*". *F. capucina* is a taxon that still is not very well understood, even though its type material has been described at least three times (Krammer and Lange-Bertalot 1991, Tuji and Williams 2006b, Delgado et al. 2015). One difficulty is that the type material includes three different diatoms, which were defined as morphological variations of *F. capucina* by Krammer and Lange-Bertalot (1991). However, these three forms are actually quite distinct and have been suggested to represent three different species (Tuji and Williams 2006b, Delgado et al. 2015, not naming them though), differing not only in outline, but also in the presence of spines and the formation of colonies. (Table S2). Tuji and Williams (2006b) and Delgado et al. (2015) considered that *F. capucina* sensu stricto is the taxon with linear valves, rectangular central area, rostrate ends, two rimoportulae per valve, linking spines, and ribbon-like colonies, and Tuji and Williams (2006b) designated one of these valves as the lectotype of *F. capucina*.

Following this definition of *F. capucina*, we consider that the strains of our FCAP1 and FCAP2 clades must be something else, as none of them had linking spines. FCAP1 and FCAP2 were mainly differentiated by the absence of spines and colonies in FCAP1 (cells were connected, at most, in pairs). FCAP2 has irregular, very small spines in some strains, and forms colonies, either as bands or in some other form, even if these were sometimes rather loose. Taxa without linking spines which would fit the size measurements of our FCAP clusters (length 5–38 µm, width 3.3–4.6 µm, stria density- 14.5–16(–19) per 10 µm) are *F. pectinalis* or *F. microvaucheriae* for the six strains up to 3.8 µm in width, and *F. rinoi* for the strain that was wider than 4.2 µm. Both *F. pectinalis* and *F. microvaucheriae* would fit FCAP1. According to Wetzel and Ector (2015), the best character to separate those two species is the length to width ratio, being 2-6 in *F. microvaucheriae* and 6-8 in *F. pectinalis*. Our valves, however, had L:W ratios between 2.5 and 10 or 11 so, again, none of the taxa described in literature fit the strains in FCAP1. L:W ratios for cluster FCAP2 range from 5.3 to 9.4 (short ones 1.5–2), again spanning the quoted ranges for both *F. pectinalis* and *F. microvaucheriae*, so we are once more not able to give one consistent name for these three strains. In any case, the subcapitate ends of FCAP2 do not fit the descriptions of *F. microvaucheriae* or *F. rinoi*, both of which have rostrate ends. *F. pectinalis* also has rostrate ends as well as a similar outline; however, Wetzel and Ector (2015) did not see spines or colony formation, whereas our specimens had tiny irregular spines, and sometimes formed colonies. The two other taxa in the *F. capucina* type material would also fit our FCAP strains' outline, size and striae density, but no information is available on the presence of spines or colony formation. Even more confusing, we found two rimoportulae per valve on one specimen in one of the strains, the rest having only one rimoportula, indicating that the number of rimoportulae might not be a sufficiently stable character on which to separate species. If this is the case then it is not clear how *F. capucina* can be separated from similar taxa, as all other characters overlap with several others (but not *F. recapitellata* which has prominent capitate ends, *F. perminuta* which has a higher striae density (17–19) or *F. vaucheriae* which has a lower striae density (9–14)). In conclusion, we are unable to give an established species name to our FCAP clusters, not even to the well-defined subgroups, or to most of the strains at all, because one or another character of the described species do not fit, and it is not clear which of the morphological characters should have priority when identifying. Therefore, to move forward based on our morphological data and well-supported clades, we propose the name *Fragilaria heatherae* for the FCAP1 clade and *Fragilaria joachimii* for FCAP2 (Boxes 2, 3). It is, nonetheless, still necessary to do further studies to understand the morphological plasticity of *F. pectinalis* and *F. capucina*, if 'typical' forms can be found, isolated and sequenced, and to determine their phylogenetic relationship to *Fragilaria heatherae* and *Fragilaria joachimii*. It is clear from the current phylogenetic tree based on *rbc*L that taxa defined using morphology as *F. capucina* sensu lato fall into several clusters which could reflect both different species, but also morphological and molecular variability.

The **FPEM** clade was the only group of taxa beside the FGRA clade where the morphological identity seemed to be clear, reflected in the fact that all strains were originally identified as *F.*

perminuta. All strains were rhombic, with a clear, strongly unilateral central area with a rimmed external swelling and deep internal depression (horse-shoe like structure). Length, stria density, and in most cases also the width fit well to the definition of *F. perminuta* in the original description by Grunow in Van Heurck 1881). Even though three strains are too broad (> 4 µm), we still consider our strains to match descriptions of *F. perminuta* well, in that they lacked spines, did not form colonies and had valves with a rhombic outline along with a very clear rimmed swelling/depression, which was more distinct than in strains from other clades where this was observed (in 621FraP11 in FCAP1, in 435FraT01, 042SynP04, and in many valves of TCC887 in FCAP2, and in 041SynP04 in FVAU).

FTNS. The strains of this clade were originally named *F. mesolepta* or *F. tenuistriata*. Tuji and Williams (2008) analyzed the type material of *F. mesolepta, F. tenuistriata* and *F. subconstricta*, and noted that all three taxa had certain diagnostic characters (Table S2): whereas *F. mesolepta* has linear valves with a concave fascia and subcapitate ends, both *F. tenuistriata* and *F. subconstricta* have linear valves with a linear fascia and rostrate ends. *F. tenuistriata* and *F. subconstricta* in turn can be separated by the fact that *F. tenuistriata* has a rimoportula situated on the sternum on the valve face, whereas *F. subconstricta* (and *F. mesolepta*) have their rimoportulae on the mantle–valve face junction. Size, spines (spathulate) and colony-formation (long ribbon-like) are similar among all three taxa. The three strains in the FTNS clade all fit the description of *F. subconstricta*, whereas the single similar strain 653FraK08 that did not cluster with the other three is *F. mesolepta*, due to the valve outline (constricted median part, subcapitate poles) and the rimoportula on the mantle–valve face junction. Tuji and Williams (2008) noted that *F. tenuistriata* and *F. subconstricta* cannot be separated in LM other than by the position of the rimoportula. In conclusion, this well-supported FTNS clade is clearly *F. subconstricta* and the single strain not fitting the cluster is *F. mesolepta*, because all morphological characters fit these species. More research is needed to understand if the position of the rimoportula is sufficient to separate two species.

Our **FTEN1** clade fits the description of *F. tenera* var. *tenera*, as described by Krammer and Lange-Bertalot (1991), Lange-Bertalot and Ulrich (2014) and Almeida et al. (2016) (Table S2). This nicely clustered clade includes taxa originally assigned different names (*F. tenera, F. cf. tenera, F. gracilis*, *F. pararumpens*), showing, again that identification of *Fragilaria* using LM can be a challenge, and that we need clearer statements of which characters should be used to separate species. One character obviously is the arrangement of the striae, which is opposite in *F. gracilis*, whereas they are alternate throughout in *F. tenera*. Another character might be the ability to form colonies, as *F. pararumpens* has been described to be typically forming long ribbon-like-colonies, whereas *F. tenera* tends to be present mainly as single cells (occasionally pairs), which can be united at the base to form radiating clusters. Another character of the valve outline of our strains was a slightly swollen central area, whereas the illustrations of Hofmann et al. (2011) show a clearly inflated center. Unfortunately, no SEM pictures of *F. pararumpens* are given in the first description (Hofmann et al. 2011), nor did we find any in the literature, thus we do not have information about the presence or formation of spines. We conclude that our FTEN1 clade has all the morphological characters of *F. tenera*. However, more care is needed when describing species in order to be clear about characters that differentiate species from similar taxa. The most useful morphological characters in the case of *F. tenera* are the alternation of the striae (which separate it from *F.gracilis*) and the valve outline with no inflated center (which distinguishes it from *F. pararumpens*).

FTEN2. This clade was quite similar morphologically to FTEN1, identified as *F.* cf. *nanoides* and *F.* cf. *pararumpens* respectively, but it clusters separately. The main differences were a higher striae density (21 per 10 µm), more irregularly arranged and formed spines, and that some cells were elongated around the central part of the valve. The stria density is too high to match *F. tenera*, instead matching *F. tenuissima* (Table S2). That at least a few cells were connected at the ends would also match this to *F. tenuissima*, defined as "may form loose few-celled aggregates" (Lange-Bertalot and Ulrich 2014). However, FTEN2 cannot be identified as *F. tenuissima*, because we observed only one rimoportula per valve, and not two as defined for *F. tenuissima*. No other long *Fragilaria* species match the characters we observed for FTEN2 (Table S2), so again we here have a clade not matching any currently species described from morphological characters. We suggest to refer to this cluster as "*F. tenuissima* with one rimoportula" until more strains and information are available for further studies.

Finally, the phylogenetic relationships of **the residual strains of the heterogeneous clade FCRNAPA** were not clear, as no groups were well supported. Most strains were originally identified as *F. crotonensis*, *F. nanoides* or *F. pararumpens*, with a few (e.g. strains TCC562, TCC589 and TCC705) originally named *F. capucina* despite not resembling the description of *F. capucina* at all (Tuji and Williams 2008). Our attempt to give correct species names to the **FCRNAPA** strains was hampered on one hand because not all morphological characters matched already described species, but mostly because we were not able to make comparisons with some long-celled *Fragilaria* species, because not all characters are described in the original accounts. In particular, the SEM characteristics of *F. pararumpens* and *F. nanoides*, and information on colony formation in *F. nanoides*, are missing. Nonetheless, most strains originally identified as *F. pararumpens* did not match this taxon because they had a higher stria density than *F. pararumpens*. Despite so many long *Fragilaria* taxa having been described, there is no species description to which many of our strains can be fitted. Consequently there is a need to improve our knowledge of the identity and phylogenetic relationships of the **FCRNAPA** clade by collecting and analyzing more strains and sequences, but also by including the study of colony formation and SEM analysis in the morphological descriptions. More work is certainly needed to understand species diversity and taxonomic relationships in *Fragilaria*, and this entails consideration of all possible characters, morphological, ecological and molecular.

Conclusion

Species names and barcodes.

Our study indicates that a) some species, defined using morphological criteria were consistent with groupings established using *rbc*L sequences, and we recommend that these continue to be recognized. These are: *F. gracilis* Østrup sensu Østrup (synonym pro parte: *F. aquaplus* Lange-Bertalot and Ulrich), *F. perminuta* (Grunow) Lange-Bertalot by Tuji & Williams, *F. tenera* (W. Smith) Lange-Bertalot, *F. subconstricta* Østrup, b) even for those species, however, the original identification using LM sometimes differed from the final one confirmed by molecular and morphological characters (including those visible only with SEM), confirming that the identification of *Fragilaria* by LM is difficult. However using a single-gene 'barcode' approach provides an effective tool to overcome problems. We also conclude that c) more strains are needed to analyze relationships, especially between strains of long *Fragilaria* taxa, including some apparently clearly-defined species such as *F. crotonensis*. Moreover, we highlighted that d) some of our well-supported clades (FCAP1 and FCAP1, FVAU, FTEN2), as well as some of the less well-supported groups include strains that could not be identified using the current morphological literature, either because the morphological characters of the strains in the group did not fit any of the described taxa, or because several taxa would fit and there was not sufficient clarity on differentiating criteria. In order to ensure the recognition and reporting of the well-supported clusters in our reference database, especially during subsequent future DNA metabarcoding analysis, we decided to describe three new species: *Fragilaria agnesiae* (FVAU), *Fragilaria heatherae* (FCAP1), and *Fragilaria joachimii* (FCAP2). As a help to identify *Fragilaria* in LM, we also provide a summary of published information on *Fragilaria* morphology (Table S2) and a summary of the LM characters of the taxa we have studied, including suggestions how to solve identification problems (Table S3*).* Finally, we note that e) there are well-defined subgroups within one morphological species (here FGRA), whose geographical distribution and ecology need further study. If we can find meaningful ecological differences between FGRA1 and FGRA2, it would be worthwhile to define these as distinct (albeit cryptic) species that can be separated by DNA sequences more easily than using LM, where only the stria density differs between these groups, and even this character is overlapping.

Ecological assessment. A complete overhaul of the taxonomic mess in *Fragilaria sensu* stricto will take a long time. However, we need to find out which taxa can be pooled or need to be separated in curated databases in order to achieve harmonized taxa lists. Indeed, DNA approaches reinforce the need for curated and operational taxa lists, linked to their DNA barcodes, for both research and environmental monitoring of freshwater ecosystems. Our results show that our well-supported clusters originate in different parts of Europe, supporting the idea that those clades are not regionally restricted in distribution. Especially for biomonitoring purposes, we need to know how differences in a barcode relate to differences in ecology. Thus, the next step is to relate the different clades to differences in environmental variables in order to determine whether the better taxonomic discrimination obtained using DNA metabarcoding translates into better ecological understanding.

In this paper we have used molecular biology, in effect, to test and validate a taxonomy based on sometimes inadequate descriptions of morphological characters. Moving forward, we will continue trying to provide a classification that is operable simultaneously by traditional and molecular approaches and that allows maximum continuity with what has done before, and improved insights into both taxonomy and ecology of diatom species.

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Tables

Table 1. Summary of morphological data for the well-supported clusters of the studied *Fragilaria* strains in this study. Characters visible in light microscopy.

Table 2. Summary of morphological data for the well-supported clusters of the studied *Fragilaria* strains in this study. Characters visible in scanning electron microscopy. Information on molecular phylogeny based on a *rbc*L barcode included.

Figures

Figure legends

Fig. 1 Phylogeny of the studied strains of *Fragilaria*, rooted with strains of the genus *Ulnaria*, on a 1087 bp long part of the *rbc*L barcode. Bootstrap values above 69% are given for each node. Scale bar: number of substitutions per site.

Fig. 2a. *Fragilaria gracilis* Østrup*,* studied strains of the well-supported cluster FGRA2. (a–e) Light microscopy (LM); example of valve views (a) 166FraB05, (b) 139FraB04, (c) 177FraB05, (d) 148FraB04, (e) 170FraB05; (f–j) Scanning electron microscopy (SEM); (f) general external view of valve of 166FraB05, no spines present, (g) external and (h) internal view of central area of 166FraB05, (i) external and (j) internal view of head poles with pore fields and rimoportula (i) 726FraB12, (j) 166FraB05. Scale bar 10 µm (a–f), or 1 µm (g–j).

Fig. 2b. *Fragilaria gracilis* Østrup*,* studied strains of the cluster FGRA1. (a–e) Light microscopy (LM); (a) living culture of TCC869, not forming colonies, (b–e) examples of valve views (b) 907FraR05, (c) 185FraB06, (d) TCC869, (e) 005FraP02; (f-k) Scanning electron microscopy (SEM); (f) general external view of valve of 488FraR03, no spines present, (g) general internal view of valve of TCC869, (h) external and (i) internal view of central area of 185FraB06 (h) and 018FraP02, (j) external and (k) internal view of head poles with pore fields and rimoportula of 185FraB06. Scale bar 10 µm (a–g), or 1 µm (h, k).

Fig. 3. *Fragilaria agnesiae* (operational name for this article: FVAU): (a–d) Light microscopy (LM); (a, b) living cultures (a) of TCC541, forming ribbon-band colonies, (b) of TCC541,

valve and girdle view, (c) valve view of 041SynP04, (d) valve of TCC553. (e–i) Scanning electron microscopy (SEM) of 041SynP04; (e, f) general external view of valve and girdle view, small spines visible, (g) internal view of central area, no rimmed depression visible, external (h) and (i) internal view of head pole with transapical rimoportula on valve face and pore field visible. Scale bar 10 µm (a–d), 5 µm (e, f), or 1µm (g–i).

Fig. 4. *Fragilaria heatherae,* studied strains (operational name for this article: FCAP1): (a–c) Light microscopy (LM); (a) valve view of 513FraK01, (b) 514FraK01 and (c) 621FraP11. (d–h) Scanning electron microscopy (SEM); general external (d, 513FraK01) and internal (e, 514FraK01) view (no rimmed depression visible in central area), (f) external view of central area of 514FraK01, (g) internal view of central area of 621FraP11 with rimmed depression, external (h, 513FraK01) and internal (i, 514FraK01) view of head pole with transapical rimoportula on valve face and pore field visible. Scale bar 10 µm (a–c), 5 µm (d, e), 1 µm (f–i).

Fig. 5. *Fragilaria joachimii,* studied strains (operational name for this article: FCAP2): (a–j) Light microscopy (LM); (a), living culture of TCC877, forming ribbon-band colonies, (b) living culture of TCC887, forming stellate irregular colonies, (c) valve view of TCC887, (d–j) valve view of all strains forming the new described species: (d) 042SynP04, (e) 046SynP04, (f) 054SynP04, (g) 171FraB05, (h) 435FraT01 (i) TCC877, (j) TCC887; Scanning electron microscopy (SEM); (k) ribbon-band colonies of 042SynP04, (l) general external view of valve of 054SynP04, (m) external view of central area of 171FraB05, showing tiny spines, (m2–m5) internal views of central area showing various grades of a rimmed depression, (m2) 435FraT01, (m3) 171FraB05, (m4) TCC877, (m5) 046SynP04, (n-p) detailed view of head

poles with pore fields; (n) external, tiny spines of 046SynP04, (o) external, tiny spines, rimoportula of TCC887, (p) internal, rimoportula of 054SynP04. Scale bar 10 µm (a–i), 5 µm (l, m2–m5), or 1 µm (m–p).

Fig. 6. *Fragilaria perminuta* (Grunow) Lange-Bertalot by Tuji & Williams (operational name for this article: FPEM). (a, b) Light microscopy (LM); (a) living culture of TCC829, not forming colonies, (b) valve views of 038FraP04; (c–f) Scanning electron microscopy (SEM); (c) general external valve view of 043SynP04, (d) detailed internal view on central area with rimmed depression of 038FraP04, (e) external and (f) view of head pole with pore fields and rimoportula, (e) of TCC 866, (f) of 038FraP04. Scale bar 10 µm (a, b), 5 µm (c), or 1 µm (d–f).

Fig. 7. *Fragilaria subconstricta* Østrup (operational name for this article: FTNS). (a–e) Light microscopy (LM): (a) living culture of TCC867, forming long ribbon-band colonies, (b–d) valve views of (b) 015FraP02, (c) TCC867, (d) TCC868; (e–h) Scanning electron microscopy (SEM); (f) general external girdle view 015FraP02, (f) detailed external girdle view of head poles with pore fields and rimoportula on mantle/valve junction (arrow) of 015FraP02, (g) detailed external valve view of head poles with pore field of 015FraP02, (h) detailed external valve view of head poles with rimoportula on mantle/valve junction of 015FraP02. Scale bar 20 µm (a), 10 µm (b–d), 5 µm (e), or 1 µm (f–h).

Fig. 7b. *Fragilaria mesolepta* Rabenhorst, studied strain 653FraK08, to compare with *Fragilaria subconstricta* Østrup (Fig. 7). (a) Light microscopy (LM), general external view of valve; (b–d) Scanning electron microscopy (SEM); (b) general external girdle and internal valve view, (c) external and (d) internal view of head poles with close-up of the spathular spines, pore fields and rimoportula on mantle/valve junction. Scale bar 10 µm (a, b), or 1 µm (c, d).

Fig. 8. *Fragilaria tenera* (W. Smith) Lange-Bertalot (operational name for this article: FTEN1). (a–g) Light microscopy (LM); examples of valve views of (a) 343FraT01, (b) 575FraK01, (c) 613FraP11, (d) 625FraP11, (e) 662FraK09, (f) 726FraB12, (g) 732FraB12; (h-j) Scanning electron microscopy (SEM); (h) general external valve view of 343FraT01, (i) external view of central area of 613FraP11, showing pyramidal spines, (j) external view of head poles with pore fields and rimoportula of 613FraP11. Scale bar 10 µm (a–h), 5 µm (i, j).

Fig. 9. *Fragilaria* sp. 1 (Operational name for this article: FTEN2). (a–f) Light microscopy (LM); (a) living culture of TCC870, not forming colonies, (b–f) examples of valve views of (b-c) TCC870, (d-e) TCC871, (f) 032FraP02; (g–i) Scanning electron microscopy (SEM); (g) general external valve view of TCC871, (h) external view of central area of 032FraP02, showing pyramidal spines, (i) external view of head poles with pore fields and rimoportula of 032FraP02. Scale bar 20 µm (b–e), 10 µm (f, g), or 1 µm (h, i).

Figures (separate files)

Supplementary Tables *(separate files)*

Table S1. Strain information, including clone identifier, voucher slide identifier, initial and final taxon identifications and operational name for this article, collection information, *rbc*L sequences, and GenBank accession numbers (where appropriate).

Table S2. Compiled information on the genus *Fragilaria*, retrieved as correct as possible from published material. Length, width (at widest point of valve), number of striae per 10µm, and information on spines (visible in SEM only) and colony formation (visible on uncleaned material only) is given. More details and photographs can be found directly in the publications. This table does not claim to be exhaustive. Information taken from ^{t} text or ^{f} figures in the specified references. SEM: scanning electronic microscopy. LM: light microscopy. no info = no information is given in the reference. RP: rimoportula. Striae p = opposite and parallel, a = alternating, pa: both alternatively.

Table S3. Towards more harmonized taxa names: Suggestions on how to separate *Fragilaria* species by morphological characters in the LM (based on type descriptions).

Supplementary Figure *(separate file)*

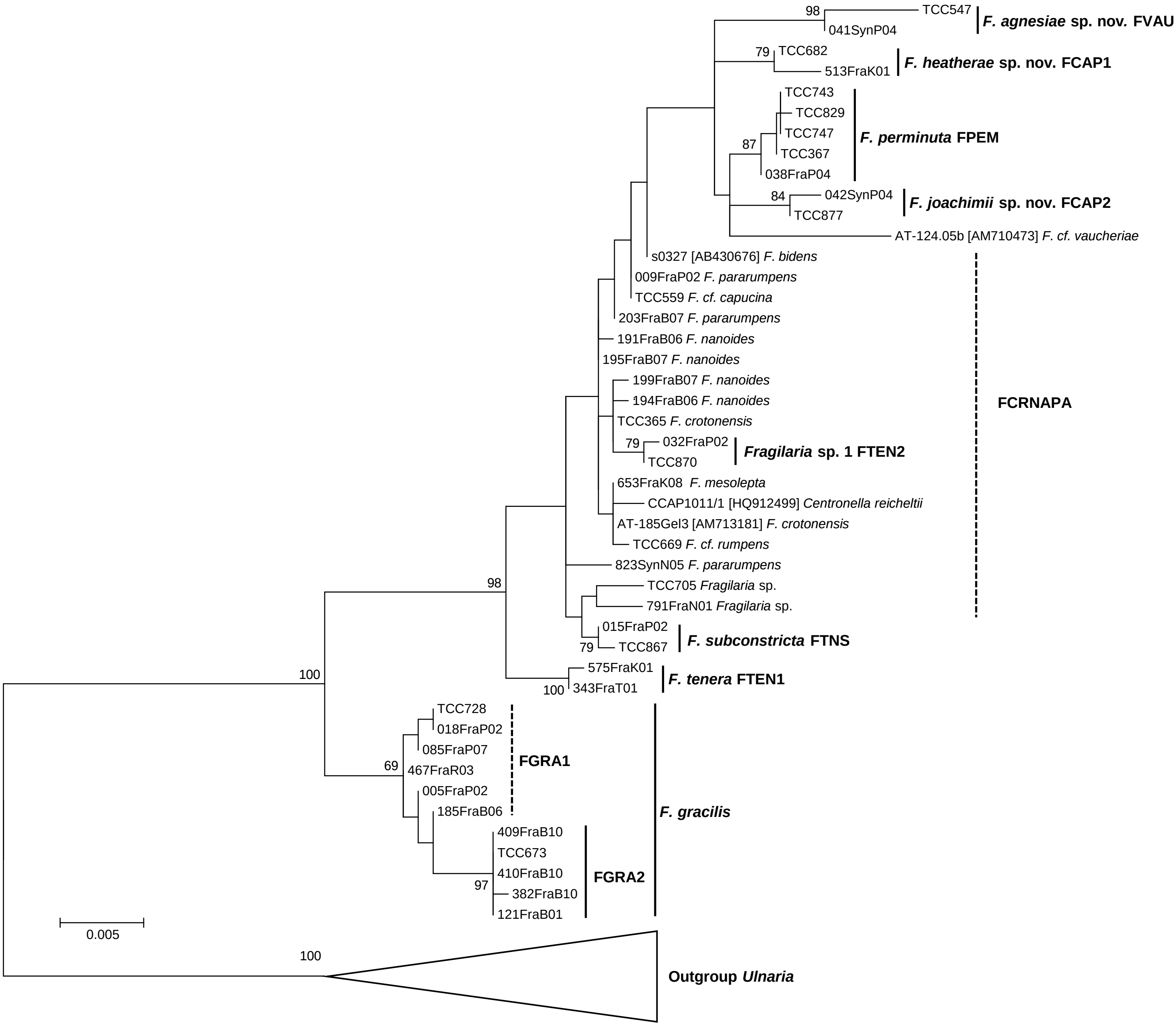
Fig. S1. Phylogeny of the studied strains of *Fragilaria*, rooted with strains of the genus *Ulnaria*, on a 1087 bp long part of the *rbc*L barcode. Bootstrap values above 69% are given for each node. Scale bar: number of substitutions per site. Full tree with geographical information included.

Table 1

		Fragilaria gracilis Østrup	*Fragilaria gracilis* Østrup	*Fragilaria agnesiae* nov. spec.	*Fragilaria heatherae* nov. spec.	*Fragilaria joachimii* nov. spec.	*Fragilaria perminuta* (Grunow) Lange-Bertalot by Tuji & Williams	*Fragilaria subconstricta* Østrup	*Fragilaria tenera* (W. Smith) Lange-Bertalot	*Fragilaria* ”sp. 1”
	working name in ms	FRGA2	FRGA1	FVAU	FCAP1	FCAP2	FPEM	FTNS	FTEN1	FTEN2
	nr of studied strains	52	25	7	4	7	10	3	7	3
	Geographical origin	UK, Italy	UK, Italy, Portugal, Luxembourg, Sweden	UK, Ile de La Réunion (France), Luxembourg, Italy	UK, Italy	UK, Sweden	UK, Sweden	UK	UK, France, Sweden	UK, Sweden
	nr of strains studied for LM	52	25	5	3	7	10	3	7	2
	Colony formation	none	none	long ribbon-like, or in short bands & loose aggregates	loose aggregates	long and short ribbon-like, also single cells and pairs, all forming loose aggregates	none	long ribbon-like	mostly single cells, occasional loose aggregates	none, occasionally, cells connected at ends, at most in pairs
Valve shape	general valve shape	linear or linear-lanceolate, gradually narrowing	mostly linear, a few strains linear-lanceolate, gradually narrowing	linear-lanceolate, short cell becoming lanceolate	long strains spindle-formed; short strain lanceolate	long cells linear, medium-sized cells lanceolate to spindle-formed, short cells lanceolate	rhombic	linear	linear-lanceolate with slightly convex margins	spindle-formed with slightly convex margins
	ends	bluntly rounded to weakly protracted or subcapitate	bluntly rounded to weakly protracted or subcapitate	rostrate	long cells subcapitate, short cells acute to rounded	long cells subcapitate, short cells acute to rounded	mainly subcapitate, sometimes capitate, rounded in smaller cells	bluntly rounded	(sub)capitate	(sub)capitate
	central area	rectangular, sometimes rhombic	rectangular, sometimes rhombic	unilateral with rimmed swelling	weakly asymmetric and blurred in long cells, clearly unilateral with rimmed swelling in short cells	varying, mostly rectangular, or unilateral with a rimmed swelling	clear strongly unilateral with a rimmed swelling	weak, bilateral, rounded	quite broad and somewhat inflated	broad, extending into axial area, and somewhat inflated
	axial area	linear narrow	linear narrow	linear narrow	wide proximal, narrow distal	narrow	linear narrow	narrow	wider proximal, narrow distal	wide proximal, narrow distal
	length [µm]	12–45	16–42	9-65	9-38	5-34	8-23	34-40	40-59	50-85
	width [µm]	2–3.1	1.8–2.7	4.0-5.4	3.3-3.7	3.3-4.6	3.2-4.6	4.1-4.5	2.1-3	2.4-3
Striae	striae per 10 µm	20-24 (mean: 22)	18-23 (mean 20)	14-16	16 in long cells 18 in short cells	14.5-16 (up to 19 in short cells)	18-21	16-17	18-20	21
	striae arrangement	opposite	opposite	alternate	alternate	alternate	alternate	alternate	alternate	alternate

Table 2

		Fragilaria gracilis Østrup	*Fragilaria gracilis* Østrup	*Fragilaria agnesiae* nov. spec.	*Fragilaria heatherae* nov. spec.	*Fragilaria joachimii* nov. spec.	*Fragilaria perminuta* (Grunow) Lange-Bertalot by Tuji & Williams	*Fragilaria subconstricta* Østrup	*Fragilaria tenera* (W. Smith) Lange-Bertalot	*Fragilaria* "sp. 1"
	working name in ms	FRGA2	FRGA1	FVAU	FCAP1	FCAP2	FPEM	FTNS	FTEN1	FTEN2
	nr of strains studied for SEM	9	10	1	3	7	5	2	6	2
Rimoportul	number of rimoportulae	1	1	1	1	1 (2 in one specimen)	1	1	1	1
	position of rimoportulae	on valve face at one of the poles	on valve face at one of the poles	on valve face at one of the poles	on valve face at one of the poles	on valve face at one of the poles	on valve face at one of the poles close to final pore	on mantle face junction	on valve face at one of the poles close to final pore	on valve face at one of the poles close to final pore
Apical pore fields	number of apical pore fields	2	2	2	2	2	2	2	2	2
	form of apical pore fields	oval to rectangular	oval to rectangular	rectangular	rectangular	rectangular	oval to rectangular	rectangular	triangular to rectangular	rectangular
	number of columns per field	4-8	4-8	8-10	long strains: 12-14; short strain: 9	5-14	6-10	15	5-7	6-8
	maximum number of pores per column	4	4	5	6	5	6	5	5	4
	Spines	absent	absent	irregular arranged, tiny to pyramidal with a round basis ending in a round tip	absent	irregular arranged, very tiny spines in long cells, absent in short cells	absent	gross spathulate	very regular arranged, pyramidal, often with a sharp bent tip	irregular to regular arranged; varying forms, tiny to pyramidal, then with a sharp bent tip; often lacking at the "neck" part
	Central area	rhombic	rhombic	no internal rimmed depression observed	internal rimmed depression in short cells, not observed in long cells	various grades of internal rimmed depression, clearest in short cells	clear internal rimmed depression	no observations	rhombic with external ghost striae	large rhombic, some cells with external ghost striae
molecular phylogeny	cluster support [%]	97% (as subgroup within FGRA)	69% (FGRA1 & FGRA2 together)	98%	79%	84%	87%	79%	100%	79%
	Evolutionary Divergence between Sequences (direct MEGA output)	0-0.001	0-0.004	0	0-0.003	0-0.002	0-0.002	0-0.001	0-0.001	0-0.001
	nr of haplotypes	2	5	1	2	2	3	2	2	2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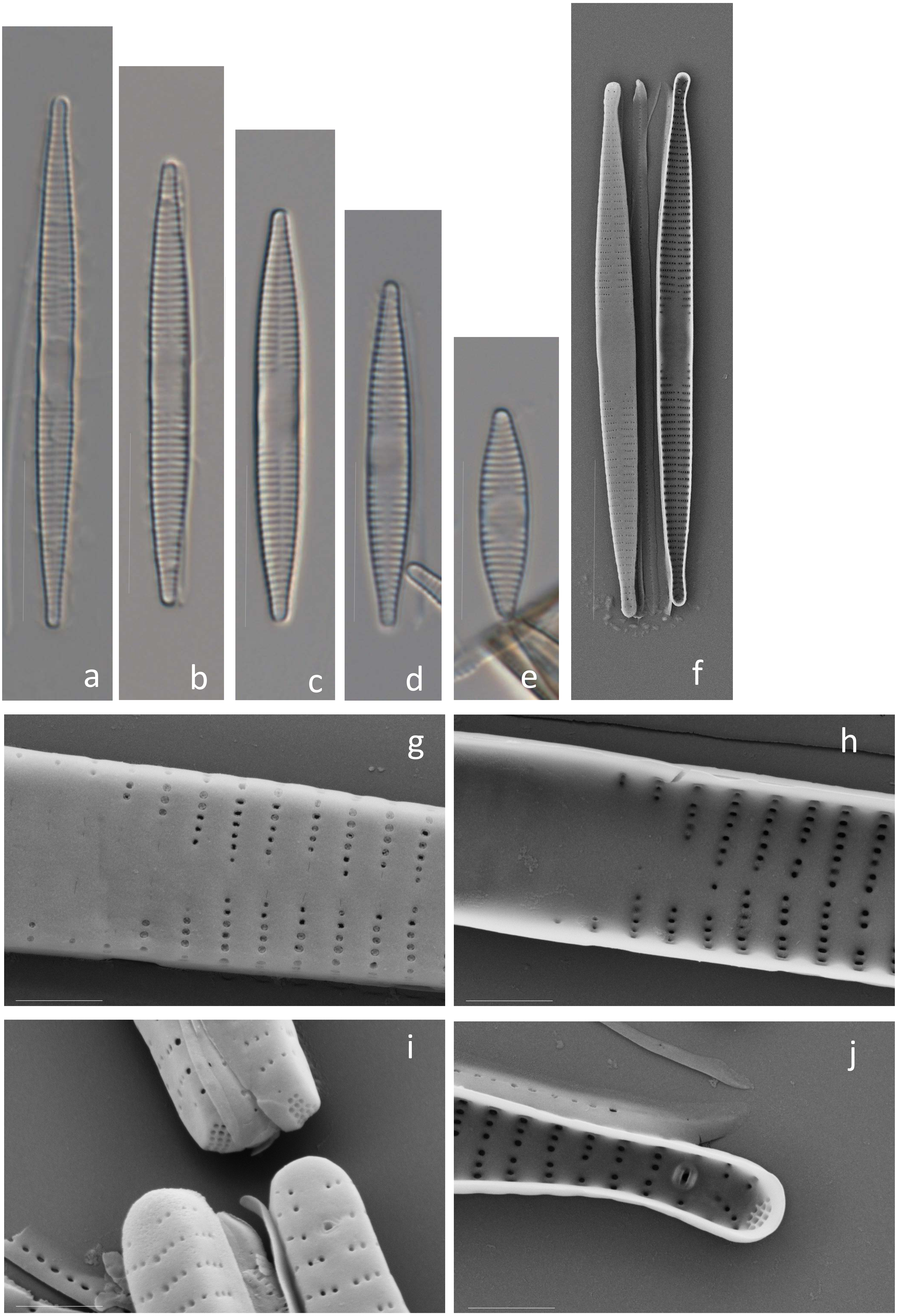
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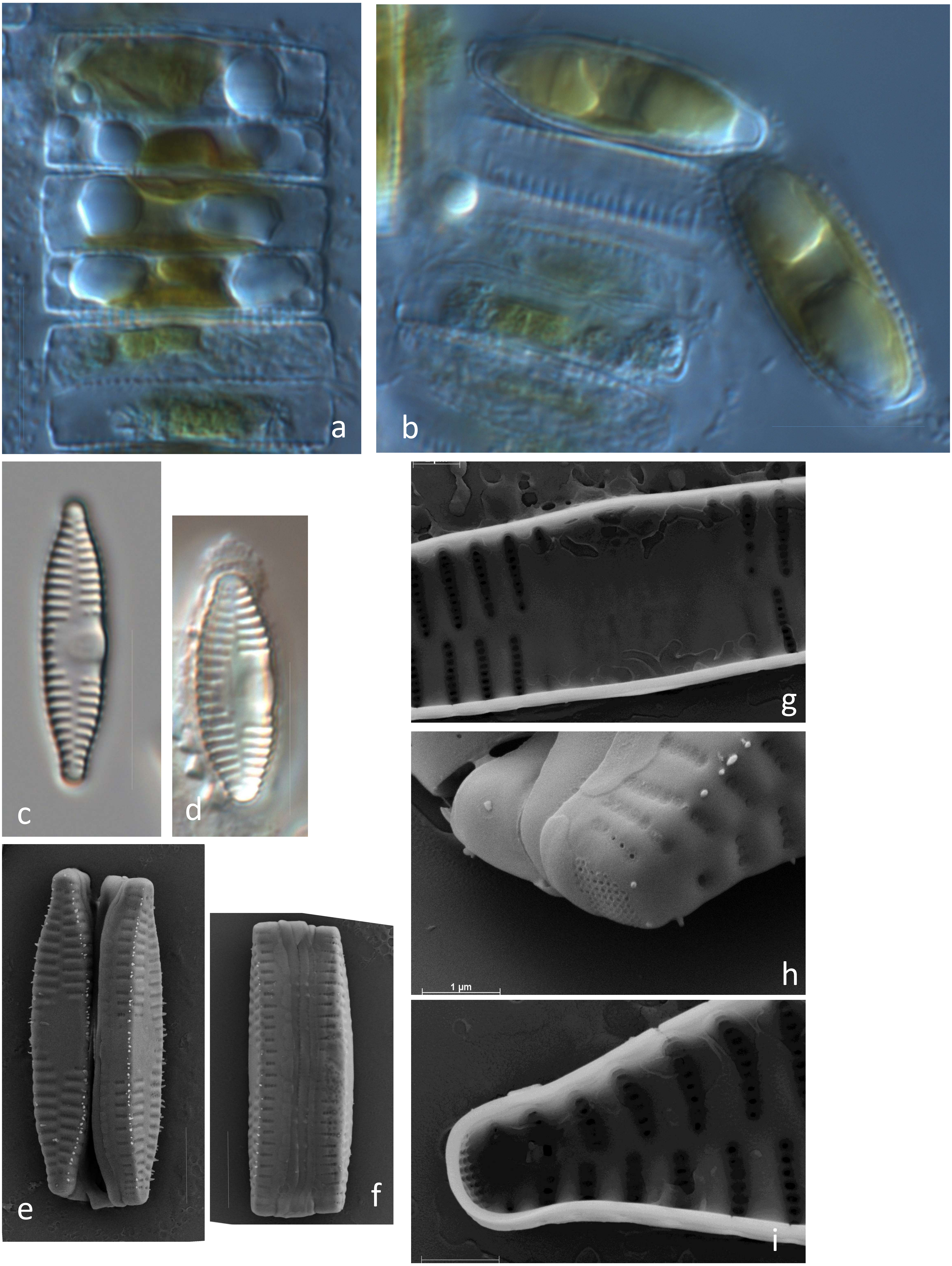
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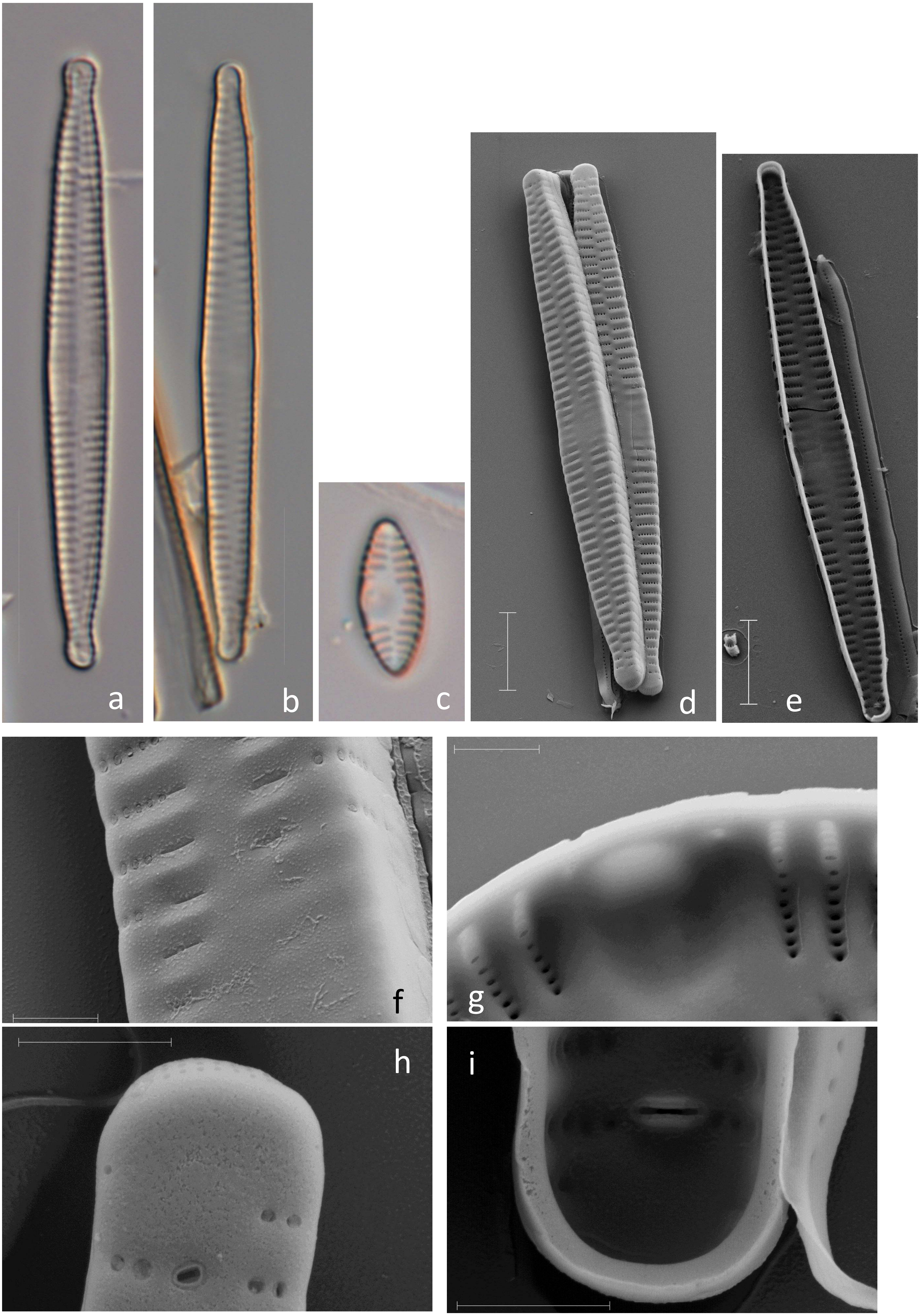
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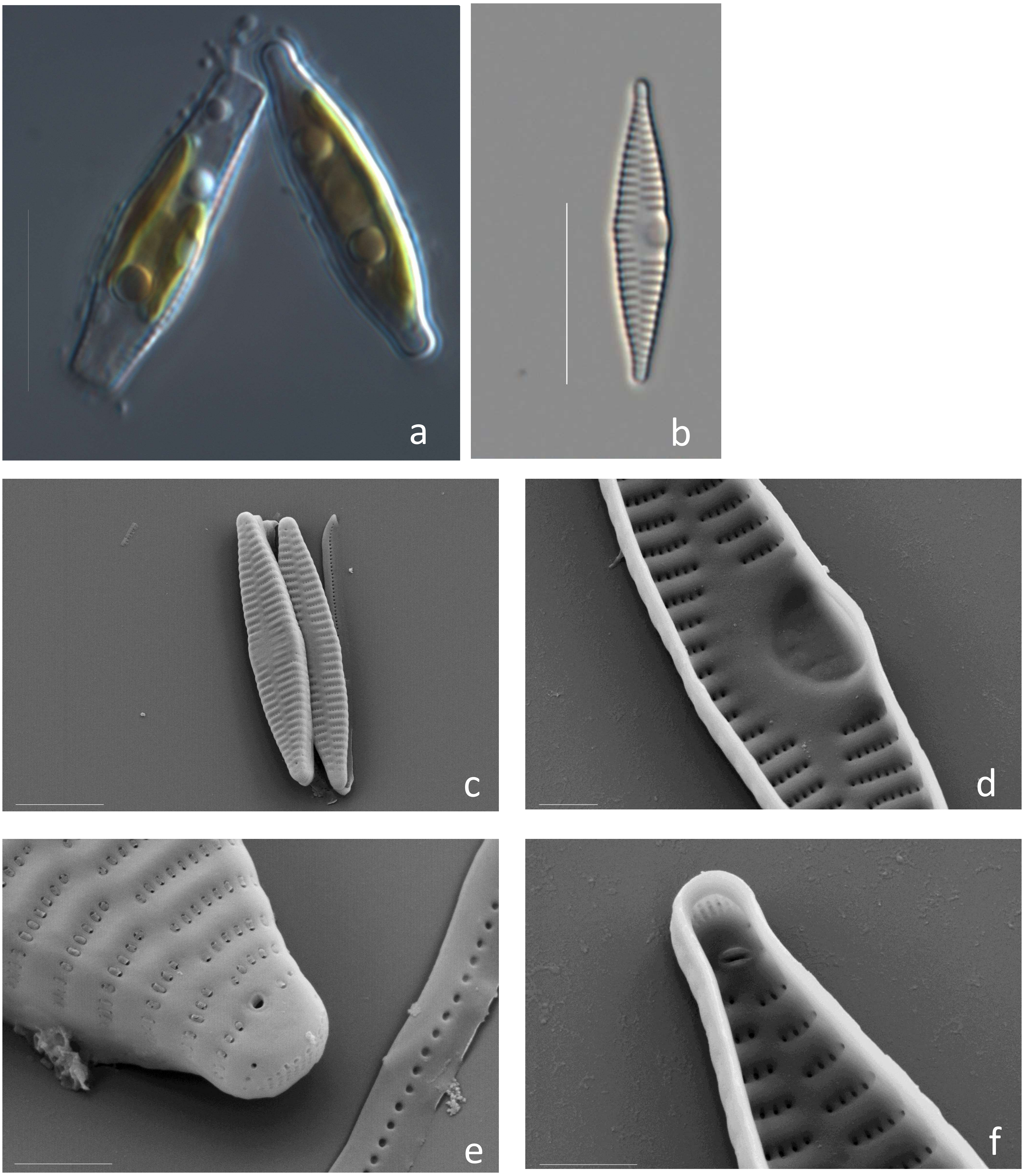
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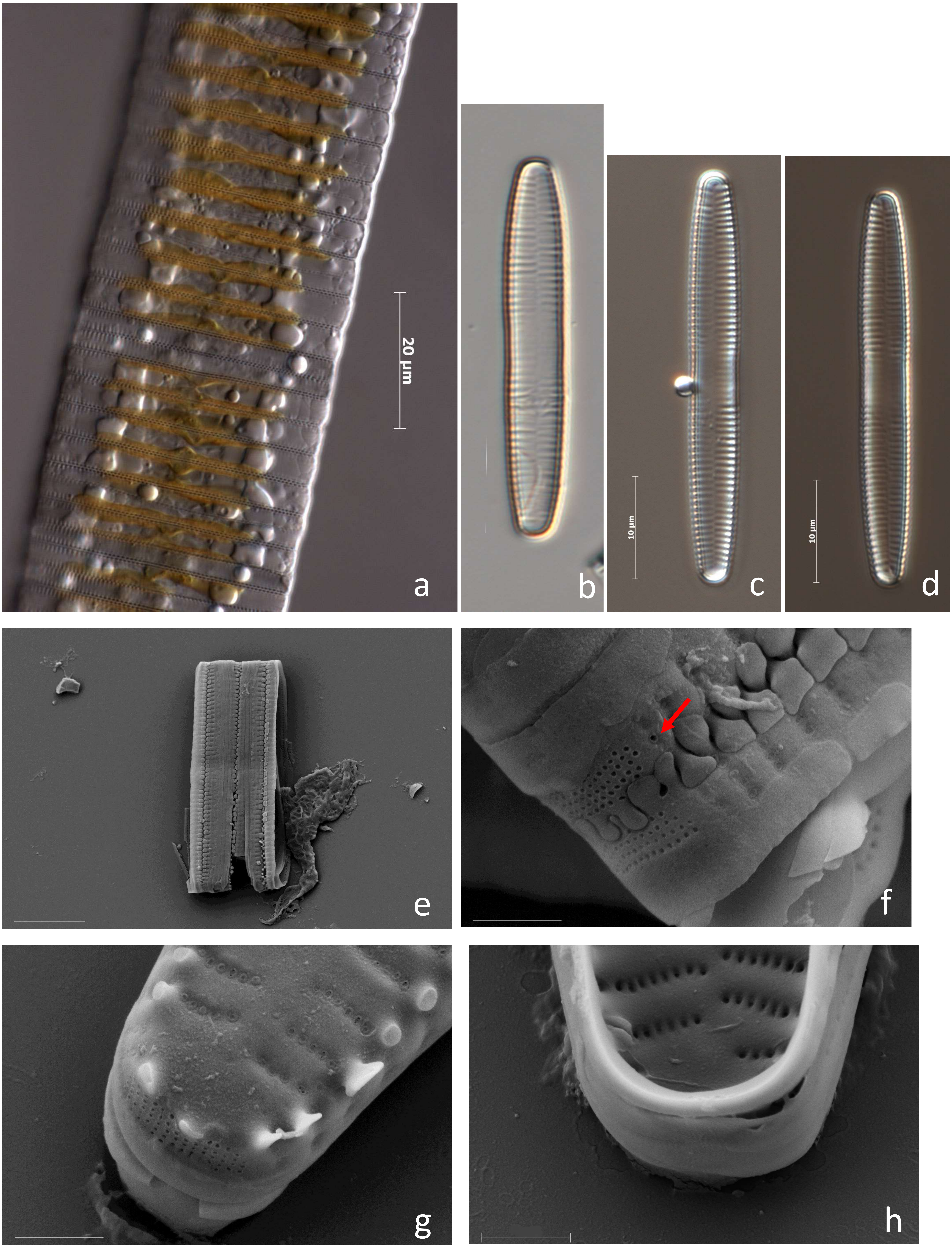
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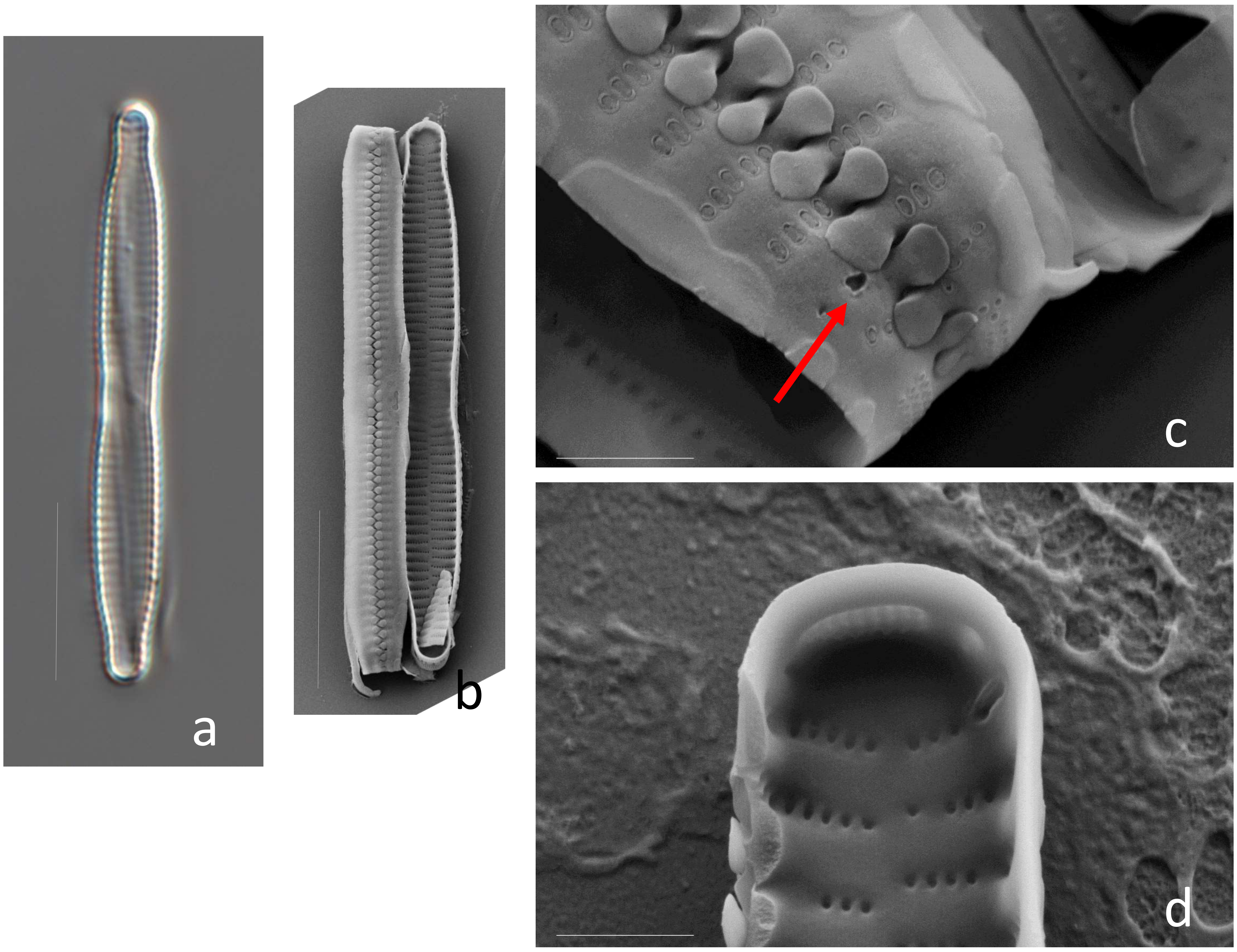
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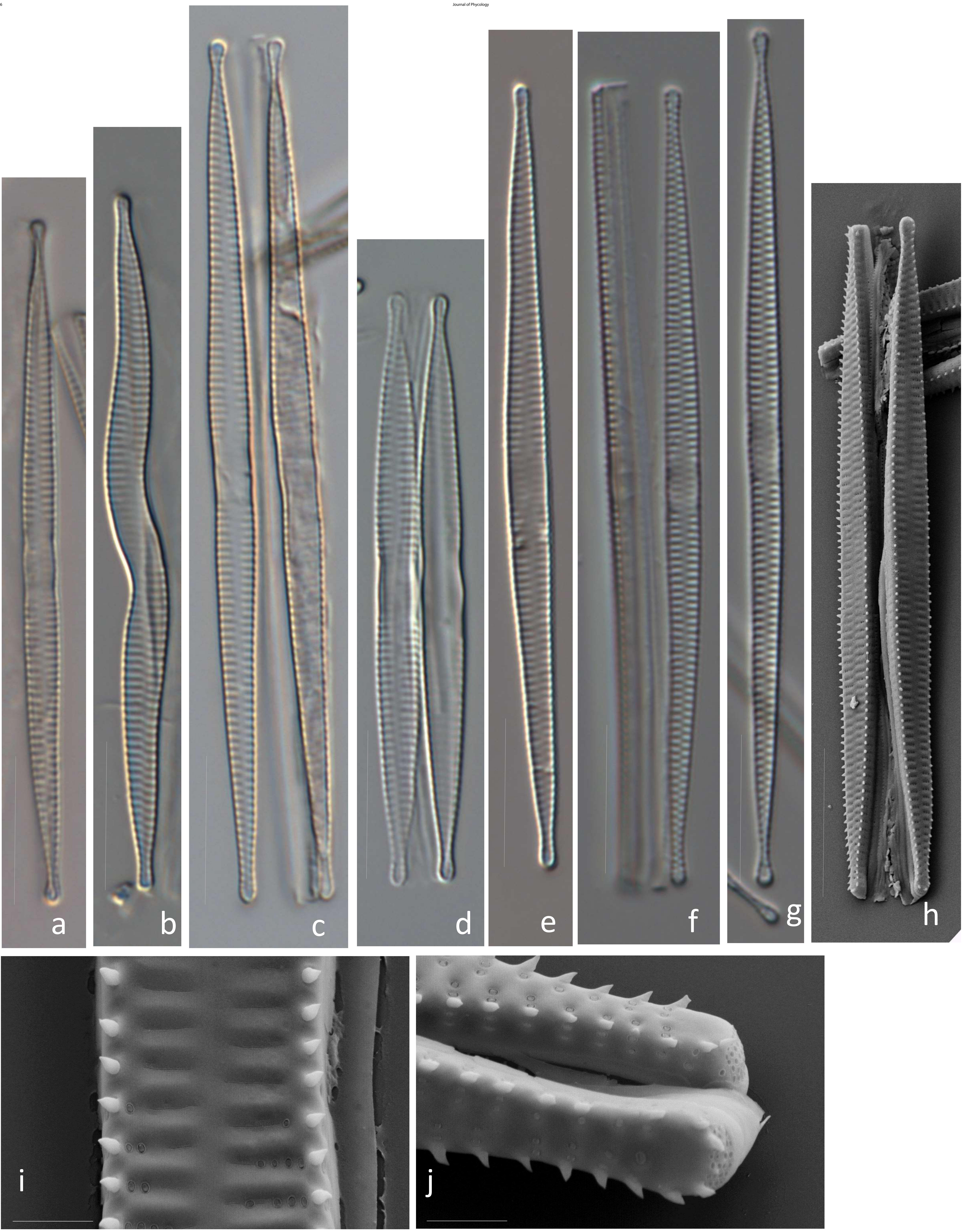
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Clone ID	Voucher slide	original name	operational na
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012FraP02	BC0012	Fragilaria gracilis	FGRA1
015FraP02	BC0015	Fragilaria sp	FTNS
018FraP02	BC0018	Fragilaria gracilis	FGRA1
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028FraP02	BC0028	Fragilaria gracilis	FGRA1
032FraP02	BC0032	Fragilaria sp	FTEN2
033SynP02	BC0033	Ulnaria acus	ULNA
034FraP03	BC0034	Fragilaria gracilis	FGRA1
038FraP04	BC0038	Fragilaria perminuta	FPEM
041SynP04	BC0041	Fragilaria vaucheriae	FVAU
042SynP04	BC0042	Fragilaria vaucheriae	FCAP2
043SynP04	BC0043	Fragilaria perminuta	FPEM
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085FraP07	BC0085	Fragilaria gracilis	FGRA1
091SynP07	BC0091	Ulnaria ulna	ULNA
121FraB01	BC0121	Fragilaria gracilis	FGRA2
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139FraB04	BC0139	Fragilaria gracilis	FGRA2
142FraB04	BC0142	Fragilaria gracilis	FGRA2
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170FraB05	BC0170	Fragilaria gracilis	FGRA2
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178FraB05	BC0178	Fragilaria gracilis	FGRA2
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220UlnB08	BC0220	Ulnaria ulna	ULNA
241FraB08	BC0241	Fragilaria gracilis	FGRA2
243FraB09	BC0243	Fragilaria pararumpens	FCRNAPA
245FraB09	BC0245	Fragilaria pararumpens	FCRNAPA
246FraB09	BC0246	Fragilaria pararumpens	FCRNAPA
247FraB09	BC0247	Fragilaria pararumpens	FCRNAPA
248FraB09	BC0248	Fragilaria pararumpens	FCRNAPA
249FraB09	BC0249	Fragilaria pararumpens	FCRNAPA
266FraB10	BC0266	Fragilaria gracilis	FGRA2
319FraW02	BC0319	Fragilaria gracilis	FGRA1
343FraT01	BC0343	Fragilaria sp	FTEN
344UlnT01	BC0344	Ulnaria ulna	ULNA
358UlnW01	BC0358	Ulnaria ulna	ULNA
378FraB10	BC0378	Fragilaria gracilis	FGRA2
379FraB10	BC0379	Fragilaria gracilis	FGRA2
380FraB10	BC0380	Fragilaria gracilis	FGRA2
381FraB10	BC0381	Fragilaria gracilis	FGRA2
382FraB10	BC0382	Fragilaria gracilis	FGRA2
383FraB10	BC0383	Fragilaria gracilis	FGRA2
385FraB10	BC0385	Fragilaria gracilis	FGRA2
387FraB10	BC0387	Fragilaria gracilis	FGRA2
388FraB10	BC0388	Fragilaria gracilis	FGRA2
404FraB10	BC0404	Fragilaria gracilis	FGRA2
409FraB10	BC0409	Fragilaria gracilis	FGRA2
410FraB10	BC0410	Fragilaria gracilis	FGRA2
412FraB10	BC0412	Fragilaria gracilis	FGRA2
414FraB10	BC0414	Fragilaria gracilis	FGRA2
435FraT01	BC0435	Fragilaria vaucheriae	FCAP2
467FraR03	BC0467	Fragilaria gracilis	FGRA1
488FraR03	BC0488	Fragilaria gracilis	FGRA1
511FraK01	BC0511	Fragilaria gracilis	FGRA2
513FraK01	BC0513	Fragilaria capucina	FCAP1
514FraK01	BC0514	Fragilaria capucina	FCAP1
515FraK01	BC0515	Fragilaria gracilis	FGRA2
516FraK01	BC0516	Fragilaria gracilis	FGRA2
517FraK01	BC0517	Fragilaria gracilis	FGRA2
551FraK01	BC0551	Fragilaria gracilis	FGRA1
555FraK01	BC0555	Fragilaria gracilis	FGRA2
575FraK01	BC0575	Fragilaria rumpens	FTEN
591FraK01	BC0591	Fragilaria gracilis	FGRA2
613FraP11	BC0613	Fragilaria sp	FTEN
621FraP11	BC0621	Fragilaria capucina	FCAP1
622FraP11	BC0622	Fragilaria gracilis	FGRA1
625FraP11	BC0625	Fragilaria rumpens	FTEN
643FraK06	BC0643	Fragilaria gracilis	FGRA1
653FraK08	BC0653	Fragilaria mesolepta	FCRNAPA

657UlnK08	BC0657	Ulnaria ulna	ULNA
662FraK09	BC0662	Fragilaria sp	FTEN
665SynK09	BC0665	Ulnaria ulna	ULNA
668SynK09	BC0668	Ulnaria acus	ULNA
698UlnK10	BC0698	Ulnaria ulna	ULNA
702UlnK11	BC0702	Ulnaria ulna	ULNA
706SynK11	BC0706	Ulnaria acus	ULNA
720UlnK13	BC0720	Ulnaria ulna	ULNA
726FraB12	BC0726	Fragilaria sp	FTEN
732FraB12	BC0732	Fragilaria tenera	FTEN
734FraB13	BC0734	Fragilaria gracilis	FGRA2
741FraB13	BC0741	Fragilaria gracilis	FGRA2
743FraB14	BC0743	Fragilaria gracilis	FGRA2
746FraB14	BC0746	Fragilaria gracilis	FGRA2
791FraN01	BC0791	Fragilaria sp	FRAS
819FraN02	BC0819	Fragilaria pararumpens	FCRNAPA
820SynN05	BC0820	Ulnaria acus	ULNA
823SynN05	BC0823	Fragilaria pararumpens	FCRNAPA
836UlnN05	BC0836	Ulnaria ulna	ULNA
842FraN04	BC0842	Fragilaria gracilis	FGRA2
844FraN04	BC0844	Fragilaria gracilis	FGRA2
845FraN04	BC0845	Fragilaria gracilis	FGRA2
852FraN04	BC0852	Fragilaria gracilis	FGRA2
853FraN04	BC0853	Fragilaria pararumpens	FCRNAPA
854FraN04	BC0854	Fragilaria gracilis	FGRA2
855FraN04	BC0855	Fragilaria gracilis	FGRA1
856FraN04	BC0856	Fragilaria gracilis	FGRA2
881UlnR05	BC0881	Ulnaria ulna	ULNA
907FraR05	BC0907	Fragilaria gracilis	FGRA1
s0327	s0327	Fragilaria bidens	FRAS
AT-185Gel3	n/a	Fragilaria crotonensis	FCRNAPA
AT_185Gel3	n/a	Fragilaria crotonensis	FCRNAPA
AT-124.05b	n/a	Fragilaria vaucheriae	FRAS
CCAP1011/1	n/a	Centronella reicheltii	FCRNAPA
UTEXFD404	n/a	Ulnaria ulna	ULNA
TCC134	TCC134	Ulnaria acus	ULNA
TCC301	TCC301	Fragilaria crotonensis	FCRNAPA
TCC302	TCC302	Fragilaria crotonensis	FCRNAPA
TCC304	TCC304	Fragilaria crotonensis	FCRNAPA
TCC306	TCC306	Ulnaria ulna	ULNA
TCC365	TCC365	Fragilaria crotonensis	FCRNAPA
TCC367	TCC367	Fragilaria perminuta	FPEM
TCC520	TCC520	Ulnaria ulna	ULNA
TCC522	TCC522	Ulnaria ulna	ULNA
TCC541	TCC541	Fragilaria vaucheriae	FVAU
TCC547	TCC547	Fragilaria vaucheriae	FVAU
TCC553	TCC553	Fragilaria vaucheriae	FVAU
TCC558	TCC558	Fragilaria vaucheriae	FVAU
TCC559	TCC559	Fragilaria capucina	FRAS
TCC562	TCC562	Fragilaria capucina	FCRNAPA

TCC584	TCC584	Ulnaria acus	ULNA
TCC589	TCC589	Fragilaria capucina	FCRNAPA
TCC626	TCC626	Ulnaria ulna	ULNA
TCC633	TCC633	Ulnaria ulna	ULNA
TCC634	TCC634	Ulnaria ulna	ULNA
TCC635	TCC635	Ulnaria ulna	ULNA
TCC654	TCC654	Ulnaria ulna	ULNA
TCC656	TCC656	Ulnaria ulna	ULNA
TCC662	TCC662	Fragilaria rumpens	FVAU
TCC666	TCC666	Fragilaria rumpens	FGRA1
TCC669	TCC669	Fragilaria rumpens	FGRA2
TCC670	TCC670	Ulnaria ulna	ULNA
TCC671	TCC671	Fragilaria rumpens	FGRA2
TCC673	TCC673	Fragilaria rumpens	FGRA2
TCC677	TCC677	Fragilaria rumpens	FGRA1
TCC681	TCC681	Fragilaria rumpens	FVAU
TCC682	TCC682	Fragilaria rumpens	FCAP1
TCC686	TCC686	Fragilaria rumpens	FGRA1
TCC695	TCC695	Ulnaria ulna	ULNA
TCC699	TCC699	Ulnaria ulna	ULNA
TCC705	TCC705	Fragilaria capucina	FRAS
TCC716	TCC716	Ulnaria ulna	ULNA
TCC722	TCC722	Fragilaria rumpens	FGRA1
TCC728	TCC728	Fragilaria rumpens	FGRA1
TCC729	TCC729	Fragilaria rumpens	FGRA1
TCC743	TCC743	Fragilaria perminuta	FPEM
TCC747	TCC747	Fragilaria perminuta	FPEM
TCC752	TCC752	Fragilaria perminuta	FPEM
TCC7a	TCC7a	Fragilaria vaucheriae	FCRNAPA
TCC829	TCC829	Fragilaria perminuta	FPEM
TCC862	TCC862	Fragilaria cf. nanoides	FCRNAPA
TCC863	TCC863	Fragilaria cf. nanoides	FCRNAPA
TCC865	TCC865	Fragilaria perminuta	FPEM
TCC866	TCC866	Fragilaria perminuta	FPEM
TCC867	TCC867	Fragilaria tenuistriata	FTNS
TCC868	TCC868	Fragilaria tenuistriata	FTNS
TCC869	TCC869	Fragilaria gracilis	FGRA1
TCC870	TCC870	Fragilaria cf. nanoides	FTEN2
TCC871	TCC871	Fragilaria cf. nanoides	FTEN2
TCC873	TCC873	Fragilaria perminuta	FPEM
TCC874	TCC874	Fragilaria perminuta	FPEM
TCC877	TCC877	Fragilaria capucina var. capucina	FCAP2
TCC878	TCC878	Fragilaria cf. nanoides	FCRNAPA
TCC882	TCC882	Fragilaria perminuta	FPEM
TCC887	TCC887	Fragilaria capucina var. capucina	FCAP2

final name
Fragilaria gracilis
Fragilaria pararumpens
Fragilaria gracilis
Fragilaria subconstricta
Fragilaria gracilis
Ulnaria acus
Fragilaria gracilis
Fragilaria sp. 1
Ulnaria acus
Fragilaria gracilis
Fragilaria perminuta
Fragilaria agnesiae
Fragilaria joachimii
Fragilaria perminuta
Fragilaria joachimii
Fragilaria gracilis
Fragilaria cf. pararumpens
Fragilaria joachimii
Fragilaria cf. pararumpens
Ulnaria ulna
Fragilaria gracilis
Ulnaria ulna
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
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Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria joachimii
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria nanoides
Fragilaria nanoides
Fragilaria nanoides
Fragilaria nanoides

Fragilaria nanoides
Fragilaria pararumpens
Fragilaria nanoides
Ulnaria ulna
Ulnaria ulna
Fragilaria gracilis
Fragilaria nanoides
Fragilaria nanoides
Fragilaria nanoides
Fragilaria cf. nanoides
Fragilaria cf. nanoides
Fragilaria cf. nanoides
Fragilaria gracilis
Fragilaria gracilis
Fragilaria tenera
Ulnaria ulna
Ulnaria ulna
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria joachimii
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria heatherae
Fragilaria heatherae
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria tenera
Fragilaria gracilis
Fragilaria tenera
Fragilaria heatherae
Fragilaria gracilis
Fragilaria tenera
Fragilaria gracilis
Fragilaria mesolepta

Ulnaria ulna
Fragilaria tenera
Ulnaria ulna
Ulnaria acus
Ulnaria ulna
Ulnaria ulna
Ulnaria acus
Ulnaria ulna
Fragilaria tenera
Fragilaria tenera
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria sp.
Fragilaria cf. pararumpens
Ulnaria acus
Fragilaria pararumpens
Ulnaria ulna
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria pararumpens
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Ulnaria ulna
Fragilaria gracilis
Fragilaria bidens
Fragilaria crotonensis
Fragilaria crotonensis
Fragilaria cf. vaucheriae
Centronella reicheltii
Ulnaria ulna
Ulnaria acus
Fragilaria crotonensis
Fragilaria crotonensis
Fragilaria crotonensis
Ulnaria ulna
Fragilaria crotonensis
Fragilaria perminuta
Ulnaria ulna
Ulnaria ulna
Fragilaria agnesiae
Fragilaria agnesiae
Fragilaria agnesiae
Fragilaria agnesiae
Fragilaria cf. capucina
Fragilaria cf. capucina

Ulnaria acus
Fragilaria cf. capucina
Ulnaria ulna
Ulnaria ulna
Ulnaria ulna
Ulnaria ulna
Ulnaria ulna
Ulnaria ulna
Fragilaria agnesiae
Fragilaria cf. gracilis
Fragilaria cf. rumpens
Ulnaria ulna
Fragilaria gracilis
Fragilaria gracilis
Fragilaria cf. gracilis
Fragilaria agnesiae
Fragilaria heatherae
Fragilaria cf. gracilis
Ulnaria ulna
Ulnaria ulna
Fragilaria sp.
Ulnaria ulna
Fragilaria gracilis
Fragilaria gracilis
Fragilaria gracilis
Fragilaria perminuta
Fragilaria perminuta
Fragilaria perminuta
Fragilaria sp.
Fragilaria perminuta
Fragilaria cf. nanoides
Fragilaria cf. nanoides
Fragilaria perminuta
Fragilaria perminuta
Fragilaria subconstricta
Fragilaria subconstricta
Fragilaria gracilis
Fragilaria sp. 1
Fragilaria sp. 1
Fragilaria perminuta
Fragilaria perminuta
Fragilaria joachimii
Fragilaria cf. nanoides
Fragilaria perminuta
Fragilaria joachimii

Sampling site	Country
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Streams, Pentland Hills, Green Cleuch, above Balerno, Midlothian	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom

Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Water of Leith, Currie, Edinburgh	United Kingdom
River Tay, near Aberfeldy, Perth & Kinross	United Kingdom
River Tay, near Aberfeldy, Perth & Kinross	United Kingdom
Water of Leith, Currie, Edinburgh	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
Allt a ‘Bhalachain, Argyll & Bute	United Kingdom
River Tay, near Aberfeldy, Perth & Kinross	United Kingdom
Royal Botanic Garden Edinburgh, pond	United Kingdom
Royal Botanic Garden Edinburgh, pond	United Kingdom
Euden Beck	United Kingdom
Euden Beck	United Kingdom
Euden Beck	United Kingdom
Euden Beck	United Kingdom
Euden Beck	United Kingdom
Euden Beck	United Kingdom
Euden Beck	United Kingdom
Euden Beck	United Kingdom
Euden Beck	United Kingdom
Euden Beck	United Kingdom
River Tay, Pitlochry, Perth & Kinross	United Kingdom
River Tay, Pitlochry, Perth & Kinross	United Kingdom
River Tay, Pitlochry, Perth & Kinross	United Kingdom
River Tay, Pitlochry, Perth & Kinross	United Kingdom
Cheriton Stream, Cheriton	United Kingdom
Pillhill Brook, Upper Clatford (112278)	United Kingdom

Pillhill Brook, Upper Clatford (112278)	United Kingdom
River Anton, Andover, “KFC”	United Kingdom
River Anton, Andover, “KFC”	United Kingdom
River Anton, Andover, “KFC”	United Kingdom
Lambourn, Bagnor (112280)	United Kingdom
River Kennet, Stitchcombe Mill	United Kingdom
River Kennet, Stitchcombe Mill	United Kingdom
River Wylye, Henford Marsh	United Kingdom
Inveruglas Water, by Ben Vane, Argyll & Bute	United Kingdom
Inveruglas Water, by Ben Vane, Argyll & Bute	United Kingdom
Inveruglas Water, by Ben Vane, Argyll & Bute	United Kingdom
Inveruglas Water, by Ben Vane, Argyll & Bute	United Kingdom
Allt Coiregrogain, by Ben Vane, Argyll & Bute	United Kingdom
Allt Coiregrogain, by Ben Vane, Argyll & Bute	United Kingdom
Wooler Water near Wooler, Northumbria	United Kingdom
Wooler Water near Wooler, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Harthope Burn, Northumbria	United Kingdom
Royal Botanic Garden Edinburgh, pond	United Kingdom
Royal Botanic Garden Edinburgh, pond	United Kingdom
Okinoshima Island, Shimane Prefecture	Japan
Wümme River, Schleswig-Holstein	Germany
Wümme River, Schleswig-Holstein	Germany
Zarrentiner Becken Lake, Mecklenburg-Vorpommern	Germany
Pluβsee, Rathjensdorf, Plön	Germany
Minnesota	USA
Lac d'Annecy	France
Lac du Bourget	France
Lac du Bourget	France
Lac du Bourget	France
Lac du Bourget	France
Lac Léman - SHL2	France
Lac Léman, Port de l'INRA	France
Ile de La Réunion rivière de des Galets site Marla	France
Ile de La Réunion rivière de des Galets site Marla	France
Ile de La Réunion rivière de Bras Caverne	France
Ile de La Réunion rivière de Langevin, grand Galet	France
Ile de La Réunion rivière de Langevin, grand Galet	France
Ile de La Réunion rivière de Langevin site amont prise EDF	France
Ile de La Réunion rivière de Sainte Suzanne	France
Ile de La Réunion rivière de Sainte Suzanne	France

rivière la Moselle à Bainville aux Miroirs	France
Canal de Nantes à Brest à Nort-sur-Erdre	France
rivière Le Gier à Givors	France
rivière Eischbaach à Boevange/attert	Luxembourg
rivière Eischbaach à Boevange/attert	Luxembourg
rivière Eischbaach à Boevange/attert	Luxembourg
rivière Alzette à Walfer-Steinsel	Luxembourg
rivière Attert à Colmar-Berg	Luxembourg
rivière Our à Vianden	Luxembourg
rivière Sûre à Camping Heiderscheidergrund	Luxembourg
Trentino Canal à Vérone	Italy
Trentino rivière de Avisio à Lavis	Italy
Trentino rivière de Brusago	Italy
Trentino rivière de Brusago	Italy
Trentino rivière de Brusago	Italy
Trentino rivière de Regnana à Amont de Bedollo	Italy
Trentino rivière de Regnana à Amont de Bedollo	Italy
Piemonte rivière de Rocciamelone à Foresto (Bussoleno)	Italy
Trento Torrente à Fersina	Italy
rivière de Carrión à Embalse de Compuerto	Spain
rivière de Valdavia à Osorno	Spain
rivière de Yuso à Boca de Huérgano	Spain
Rib. De Seixe à Zambujeira De Baixo	Portugal
Rib. De Seixe à Foz Do Arroio	Portugal
Rib. De Seixe à Foz Do Arroio	Portugal
Lac Léman, estuaire du Foron	France
Lac Léman, estuaire du Foron	France
Lac Léman, parc de Rovorée	France
rivière La Loue (Jura)	France
Lac Léman, Port de l'INRA, France	France
Siggeforasjön lake near Norrtälje city	Sweden
Siggeforasjön lake near Norrtälje city	Sweden
Erken lake near Norrtälje city	Sweden
Erken lake near Norrtälje city	Sweden
Järsöströmmen river near Norrtälje city	Sweden
Järsöströmmen river near Norrtälje city	Sweden
Siggeforasjön lake near Norrtälje city	Sweden
Siggeforasjön lake near Norrtälje city	Sweden
Siggeforasjön lake near Norrtälje city	Sweden
Erken lake near Norrtälje city	Sweden
Erken lake near Norrtälje city	Sweden
Norrtäljeån river near Norrtälje city	Sweden
Siggeforasjön lake near Norrtälje city	Sweden
Erken lake near Norrtälje city	Sweden
Broströmmen river near Norrtälje city	Sweden

Sampling site	Sampling site	Culture autho	Sampling date	Sequence ID	rbcl sequenc
55.844216	-3.311093	D. Mann, M. K	2012/05/19	005FraP02	ATGTCTCAAT
55.844216	-3.311093	D. Mann, M. K	2012/05/19	009FraP02	ATGTCTCAAT
55.844216	-3.311093	D. Mann, M. K	2012/05/19	012FraP02	ATGTCTCAAT
55.844216	-3.311093	D. Mann, M. K	2012/05/19	015FraP02	ATGTCTCAAT
55.844216	-3.311093	D. Mann, M. K	2012/05/19	018FraP02	ATGTCTCAAT
55.844216	-3.311093	D. Mann, M. K	2012/05/19	024SynP02	ATGTCTCAAT
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56.206051	-4.790649	D. Mann, M. K	2012/05/19	249FraB09	ATGTCTCAAT
56.206051	-4.790649	D. Mann, M. K	2012/05/19	266FraB10	ATGTCTCAAT
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53.55	10.91933	Medlin,L.K. - D	2002-07-29	AM710473	AAATGCATC
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-20.961426	55.578578	Gilles Gassiole	2010/11/15	TCC562-Rbcl-1	TTCATACGCT

48.433333	6.283333	Frédéric Rime	2010/11/15	TCC584-Rbcl-1	AAGTGACCG
47.43495865	-1.494850158	Lenaig Kerma	2010/11/15	TCC589-Rbcl-1	TGACCGTTC
45.59126173	4.778683296	Lenaig Kerma	2010/11/15	TCC626-Rbcl-1	GTGTCCGTT
50.04594805	5.932536216	Lenaig Kerma	2010/11/15	TCC633-Rbcl-1	CATACGCTG
50.04594805	5.932536216	Lenaig Kerma	2010/11/15	TCC634-Rbcl-1	CAAGTGTCC
50.04594805	5.932536216	Lenaig Kerma	2010/11/15	TCC635-Rbcl-1	TTCATACGC
49.658333	6.137222	Lenaig Kerma	2010/11/15	TCC654-Rbcl-1	CGTTCGAAT
49.81622394	6.094506835	Lenaig Kerma	2010/11/15	TCC656-Rbcl-1	TGTCCGTTA
49.93234814	6.209918968	Lenaig Kerma	2010/11/15	TCC662-Rbcl-1	TCAAGTGAC
49.90648554	5.959132095	Lenaig Kerma	2010/11/15	TCC666-Rbcl-1	AAAGTGACC
45.41931787	11.01891344	Luc Ector	2010/11/15	TCC669-Rbcl-1	TCAAGTGAC
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37.38510618	-8.649999999	Luc Ector	2010/11/15	TCC729-Rbcl-1	AAGTGACCG
46.34257721	6.379213126	F. Rimet	2010/12/10	TCC743-Rbcl-1	AAAGTGACC
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46.37282622	6.340542777	F. Rimet	2010/12/10	TCC752-Rbcl-1	GTGACCGTT
47.09462446	5.873719962	INRA	2004/05/06	TCC7a-Rbcl-1	TACTGGGAT
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59.757285	18.7206	Maria Kahlert,	2013/06/25	TCC868-Rbcl-1	ATTATTCCG
59.757282	18.720598	Maria Kahlert,	2013/06/25	TCC869-Rbcl-1	GGACAGATT
59.757282	18.720598	Maria Kahlert,	2013/06/25	TCC870-Rbcl-1	ACTGTTGTA
59.757282	18.720598	Maria Kahlert,	2013/06/25	TCC871-Rbcl-1	AACAGCTTG
59.707064	18.603658	Maria Kahlert,	2013/06/25	TCC873-Rbcl-1	TATGGACAG
59.707064	18.603658	Maria Kahlert,	2013/06/25	TCC874-Rbcl-1	GAAGCAGCA
59.75728	18.72059	Maria Kahlert,	2013/06/25	TCC877-Rbcl-1	TACTTTGCT
59.757282	18.720598	Maria Kahlert,	2013/06/25	TCC878-Rbcl-1	ACCGAGTAG
59.707064	18.603658	Maria Kahlert,	2013/07/02	TCC882-Rbcl-1	TGCAACTGA
59.757285	18.7206	Maria Kahlert,	2013/07/02	TCC887-Rbcl-1	GCTTTCATC

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KF959647		
	0	short rbcL sequence
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KT072942		
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	Length [µm]	Width [µm]	Striae $10\mu m^{-1}$	spines	colonies	reference
vaucheriae (Kütz.) J.B.Petersen	14.1-50.4	3.8-5.1	11-14	several conic small spines located along the valve face/mantle junction. Irregularly distributed	never more than two loosely attached cells could be found as also observed by Petersen (1938)	Wetzel and Ector (2015)[t]
vaucheriae	no info	no info	9-14	no info	ribbon like, attached whole way	Tuji and Williams (2006a)[t]
vaucheriae	no info	no info	no info	spines either do not exist or are very small	do not link with sibling cells	Tuji and Williams (2013)[t]
vaucheriae	<10-50	4-5	9-14	no info	no info	Hofmann et al. (2011)
intermedia (synonym to *vaucheriae*)	no info	no info	no info	SEM shows none, or very small spines	SEM shows single cells only	Tuji and Williams (2013)[t] Note: synonymized *F. intermedia* with *F. vaucheriae*
intermedia (Grunow) Grunow in van Heurck (1881)	16-30	3-4	11-14	no info	no info	Delgado et al. (2015), analyzing figures of Tuji and Williams (2013)[t] Note: refer to Tuji and Williams (2013), but still list *F. intermedia* as valid name
neointermedia Tuji & Williams	25-35[t]	3.5–4.5[t]	8-10[t]	spathulate linking	long colonies	Tuji and Williams (2013)[t]
neointermedia	$25–35^{\mathrm{LM}}$ $23-29^{\mathrm{SEM}}$	$3.5–4.5^{\mathrm{LM}}$ $3-4^{\mathrm{SEM}}$	$8–10^{\mathrm{LM}}$ $10-12^{\mathrm{SEM}}$	no info	no info	Delgado et al. (2015), re-analyzing figures in Tuji and Williams (2013)[t]
neointermedia	22.5-45	3.4-4.2	11-14	spathulate linking	no info	Delgado et al. (2015)[t] , analyzed type material
capucina Desmazières s.str.	24-65[f]	3-5[f]	13-17[f]	linking spines	ribbon like; (Tuji and Williams (2006b write it is attached)	Delgado et al. (2015)[t] re-analyzing figures in Tuji and Williams (2006b) (2 RP per valve)
capucina Desmazières s.str.	28-47	3.3-4.2	14-17	linking spines	no info	Delgado et al. (2015)[t] (2 RP per valve)
"capucina" 1 (aff.)	33.2-35.2	3.2-3.4	15	"lacking" marginal spines	no info	Tuji and Williams (2006b)[t, f]
"capucina" 2 (aff.)	18-20	3.4-3.6	15	no info in text; no spines visible on SEM (Tuji and Williams 2006b). (The info by Delgado et al. (2015)[t] of "linking spines" is probably a misinterpretation of Tuji & Williams (2006b)'s text).	no info	Tuji and Williams (2006b)[t, f]

capucina	20-75	3.5-4.5	12-17	no info	ribbon-bands OR stellate	Hofmann et al. (2011), discussing Krammer and Lange-Bertalot (1991), probably including all 3 types in typeslide
microvaucheriae C.E.Wetzel & Ector	5.7-23.4	2.5-3.8	15-16	small, conic. Usually absent	no colonies	Wetzel and Ector (2015)[t]
pectinalis (O.F.Müll.) Lyngb.	12.0-36.2	2.8-4.7	15-18	totally absent	no colonies	Wetzel and Ector (2015)
pectinalis	28-37	3,5-4	14-15	a few, very small, spines, visible in SEM	No information, but SEM shows a pair of cells	Tuji and Williams (2006b), Tuji and Williams (2008b)[t]
rinoi Almeida & C.Delgado	8.8-24.1	4.2-5.6	14-16	totally absent	no colonies	Delgado et al. (2016)[t]
uliginosa Kulikovskiy, Lange-Bert., Witkowski & Dorofeyuk	14.1-28.8	3.5-4.5	15-16[t] 16-17 (table)	wide, spathulated and usually located on the striae at the valve face/mantle junction	colonies were not observed and the species seems to be mainly found as solitary cells	Wetzel and Ector (2015)[t]
recapitellata Lange-Bert. & Metzeltin	20-39[t] 21-37[f]	3-4[t] 3-4[f]	17-19[t] 18-20[f]	no spines[f]	no info	Tuji and Williams (2008b), analysis of figures by Delgado et al. (2015)
recapitellata	21.4-30.4	2.8-4.2	17-19	no info	no info	Delgado et al. (2015), analyzed type material
recapitellata	11-38	3-5	14-18	no info		Hofmann et al. (2011)
recapitellata	15-27	4.1-5.6	16-20	no spines	no info	Bishop and Spaulding (2014)* (*"Note that the taxon illustrated here conforms more to the taxon illustrated in Metzeltin et al. (2009) than in the original description of *Synedra capitellata* Grunow in Van Heurck 1881")
perminuta (Grunow) Lange-Bert.	9-24	3-3.5	18-19	no material for SEM left, thus no info	no info	Tuji and Williams (2008b)[t]
perminuta	8-25[f]	3-4[f]	17-19[f]	no info	no info	Delgado et al. (2015), re-analysed Tuji and Williams (2008b)[t]
perminuta	7-40	3-4	17-21	no info	no info	Hofmann et al. (2011)
mesolepta Rabenhorst	23.8-55.9[f]	3.9-5[f]	13-15[f]	spathular linking	ribbon closed to ends	Tuji and Williams (2008a)[f] (note: RP on mantle/valve junction)
mesolepta	20-60	3.5-4.5	15-18	no info	no info	Hofmann et al. (2011)[t]
tenuistriata Østrup	50.4-74.8[f]	3.9-4[f]	15-16[f]	spathular linking	ribbon closed to ends	Tuji and Williams (2008a)[f] (note: RP on sternum)

subconstricta Østrup	39.4-47.4[f]	3.7[f]	15-16[f]	spathular linking	ribbon closed to ends	Tuji and Williams (2008a)[f] (notes: RP on mantle/valve junction, but different valve shape than *F. mesolepta*)
rhabdosoma Ehrenberg	~27	~3-4	15	spathular, nicely intertwined between cells	long ribbon bands	Tuji (2004) (considers F.bidens being a synonym to F. rhabdosoma)
"*bidens*" Heiberg	10-50	(2)3-4	(11?)15-18	no info	long ribbon bands	Krammer and Lange-Bertalot (1991), (complicated taxonomy and synonymy, including also F.socia, F. familiaris, F. parva, F. rhabdosoma, see also Lange-Bertalot (1980))
radians (Kütz.) Lange-Bert.	35-55	3.5-4.5	9-11	no SEM pictures	radiar colonies ("stellate")	Krammer and Lange-Bertalot (1991), Krammer and Lange-Bertalot (2004), Hofmann et al. (2011)
austriaca (Grunow) Lange-Bert.	20-60	3-4	12-15	no SEM pictures	ribbon-bands OR stellate	Krammer and Lange-Bertalot (1991), Krammer and Lange-Bertalot (2004), Hofmann et al. (2011)
amphicephaloides Lange-Bert.	40-75	2-3	10-14	no SEM pictures	ribbon-bands OR stellate	Krammer and Lange-Bertalot (1991), Krammer and Lange-Bertalot (2004), Hofmann et al. (2011)
henryi Lange-Bert.	35-60	3-4	11-13p	no SEM pictures?	ribbon-bands OR stellate OR singular cells	Krammer and Lange-Bertalot (1991), Lange-Bertalot and Genkal (1999), Krammer and Lange-Bertalot (2004), Hofmann et al. (2011)
acidoclinata Lange-Bert. & G. Hofmann	35-60	3-4	11-13p	no SEM pictures?	ribbon-bands OR stellate OR singular cells	Krammer and Lange-Bertalot (1991), Lange-Bertalot (1993), Krammer and Lange-Bertalot (2004), Hofmann et al. (2011)
famelica (Kütz.) Lange-Bert.	10-70	2.5-4	11-16p	no SEM pictures?	ribbon-bands OR stellate OR singular cells	Krammer and Lange-Bertalot (1991), Krammer and Lange-Bertalot (2004), Hofmann et al. (2011)
pararumpens Lange-Bert., G. Hofmann & Werum	25-50	2.5-3	16-18	linking spines, thorn-formed with cylindric base and flatted anchors, which are tightly connected at neighboring frustules; at the valve ends spines acute thorn-formed, not connecting	chain formed connected in the middle (double-comb), similar to *F. crotonensis*	Krammer and Lange-Bertalot (1991), Krammer and Lange-Bertalot (2004), Hofmann et al. (2011)
parva Tuji & D. M. Williams	30-40[f]	~3[f]	~20[f]	no info	no info	Tuji and Williams (2008c)

socia (Wallace) Lange-Bert.	16-72	3.5-4	17	no material for SEM left, thus no info	no info	Lange-Bertalot (1980), Tuji and Williams (2008b)
socia	14.1-42.9	3.4-4.1	16.4-18.5	no info	frustules are joined in colonies, attached to a benthic substrate by a mucilage pad (pictures: stellate)	LaLiberte and Vaccarino (2015)
rumpens (Kütz.) G. W. F. Carlson	25-63	3-4	18-20	irregular, located on the costae, at mantle-face junction, often deformed and rectangular at central area, very small and triangular at valve poles	ribbon like, adhering by valve faces, sometimes separated from each other at poles (tychoplanktonic to attached)	Tuji and Williams (2006a)[t]
rumpens	20-65	3.5-4.5	18-20	no info	ribbon band or stellate adnate	Hofmann et al. (2011)
gracilis Østrup	36	2-3/3.6	20p	absent	no info	Tuji (2007)
gracilis	no info	no info	18-20p	absent	no info	Schmidt et al. (2004)
gracilis	30-50	2-3	20-22*	absent	no info	Lange-Bertalot and Ulrich (2014) (* "alternating and opposing in the same valve")
gracilis	10-60	2-3	~20	no info	ribbon-bands OR stellate	Hofmann et al. (2011)
aquaplus Lange-Bert. & S. Ulrich*	30-50 22-45 (table)	1.5-2.5	22-24p	absent	solitary	Lange-Bertalot and Ulrich (2014) (*this taxon is one of the 2 taxa on the *Synedra nana* Meister 1912 typeslide. Was pooled by Krammer and Lange-Bertalot (1991) into *F.nanana* Lange-Bert.)
nanana sensu Lange-Bertalot (1991)	40-90	1.5-2	22-30	no info	no info	Krammer and Lange-Bertalot (1991)
nanana sensu Lange-Bertalot (1991)	40-90	1.5-2	22-30*	no info	no info	Hofmann et al. (2011) (* „striae coaxial, not alternating“)
nanana sensu Lange-Bertalot (1991)	no info	1.5-2	no info	absent	no info	Schmidt et al. (2004)
tenera var. nanana (Lange-Bertalot)	29-85 (table)	2-2.3 (table)	18.5–20	present marginal and apical	no colonies	Lange-Bertalot and Ulrich (2014) (*this taxon is one of the 2 taxa on the *Synedra nana* Meister 1912 typeslide. Was pooled by Krammer and Lange-Bertalot (1991) into *F.nanana* Lange-Bert.)
saxoplanctonica Lange-Bert. & Ulrich	40–170	1.5–2.5	23–28 pa	absent	single individuals, not in ribbon-like or stellate colonies	Lange-Bertalot and Ulrich (2014)

sepes Ehrenberg				small rectangular spines	yes, ribbon-like; Ehrenberg picture shows a kind of long ribbon band, could also be a double-comb	Tuji (2004)
sepes	47.0– 69.0	1.5–2.0	24–25 pa	small, pyramidal	nd.	Almeida et al. (2016), re-analyzed Tuji (2004)
tenuissima Lange-Bert. & Ulrich	40–150	1–3	16-20.5	marginal spines may be reduced	may form loose fewcelled aggregates	Lange-Bertalot and Ulrich (2014)
tenuissima	70–145	1.6–2.8	16.0–20.5	small, reduced or absent	single, or short ribbon-like	Almeida et al. (2016) re-analyzed Lange-Bertalot and Ulrich (2014)
tenera (W. Smith) Lange-Bert.	68.1–114.4	1.9–2.1	18–20	pyramidal spines	at most loose aggregates	Almeida et al. (2016)
tenera var. *tenera*	60–120	1.8-2.5	18-20	marginal and apical	may form loose fewcelled aggregates	Lange-Bertalot and Ulrich (2014)
tenera	30->100	2-3	17-20	no info	no info	Hofmann et al. (2011)
tenera	30-100	2-3	17-20	no info	never forms colonies, always solitary	Druart et al. (2007)
tenera var. *lemanensis* Druart, Lavigne & Robert	70-80	2-3.5	18-20	no info	stellate	Druart et al. (2007)
tenera var. *lemanensis*	70-90 (table)	2-3.5^{t}	18-20^{t}	marginal and apical	stellate	Lange-Bertalot and Ulrich (2014)
nanoides Lange-Bert.	40-90	1.8-2.4	22.5-24a	no info	no info	Lange-Bertalot (1996)
nanoides			>20	absent	occurrence of cell pairs	Schmidt et al. (2004)
spectra P.D.Almeida, E.Morales & C.E.Wetzel	40.5–73	1.5–2.5	24–25	absent	solitary	Almeida et al. (2016) (2 RP per valve)
neotropica P.D.Almeida, E.Morales & C.E.Wetzel	52-72	1.7-2	28-32	pyramidal, at junction valve/mantle	not observed	Almeida et al. (2016)
longifusiformis ssp. *longifusiformis* (Hains & Sebring) Siver et al.	50-175	2-4	26-34 pa	reduced marginal spines, few apical spines of different size	no info	Lange-Bertalot and Ulrich (2014)
longifusiformis ssp. *eurofusiformis* Lange-Bert. & S.Ulrich	70-120	3-4	29–31 pa	reduced marginal spines, few apical spines of different size	very rarely, bunch-like	Lange-Bertalot and Ulrich (2014)
grunowii Lange-Bert. & S.Ulrich	90-380	3–4	12–15 pa	no, at most small blunt apical dents	solitary	Lange-Bertalot and Ulrich (2014)

schroeteri (Meister) Lange-Bert. & S.Ulrich	300-450	2.5–4.5	12–15	none marginal, but 2 short apical	solitary	Lange-Bertalot and Ulrich (2014)
paludosa (Meister) Lange-Bert. & S.Ulrich	73–110	3.2-3.9	14-15	no info	no info	Lange-Bertalot and Ulrich (2014)
delicatissima (W.Smith) Lange-Bert.	30-100	2.5-3	14-16	no info	no info	Krammer and Lange-Bertalot (1991), Hofmann et al. (2011)
perdelicatissima Lange-Bert. & Van de Vijver	36-95	2-2.6	14-16	absent	Solitary (benthic)	Lange-Bertalot and Ulrich (2014), *F. delicatissima* (W. Smith) Lange-Bertalot sensu Krammer & Lange-Bertalot 1991, p. 129, fig. 115: 13
crotonensis ssp. *crotonensis* Kitton	40-170	2-4(5), mostly 2.5-3.5	15–18	spatula shaped linking spines, marginal spines short & acute (but no single valves with short, acute spines)	ribbon-like colonies, rarely single cells, cells connected only in the proximal inflated part	Lange-Bertalot and Ulrich (2014)
crotonensis ssp. *lacus-vulcani* Lange-Bert. & S.Ulrich	55-120	2-4 (long forms: 2-3, short forms 3-4)	14-17	spatula shaped linking spines, marginal spines short & acute (plus many single valves with short acute marginal spines)	cells mainly single or in few-celled aggregates	Lange-Bertalot and Ulrich (2014)
crotonensis “rod-form”: *F. crotonensis* var. *crotonensis* Kitton	34-100, rod-formed ends in girdle view	No info	14-15 center, 17-18 in apices	Interlocking spines are narrow at the base and broaden towards their spathulate tips, which interdigitate with those of the sibling valve. Outside the linking zone, the spines change in form, taper distally, and tend to slant toward the cell apex. Valves at the ends of intact filaments lack a linking zone and may be regarded as separation valves. On these valves all of the spines are tapered, simple, and small.	long raft-like chains, with wide mucilage envelope (up to 20 µm)	Crawford et al. (1985) (did not formally describe the 2 forms), difference to flared-form in parasite suceptibility
crotonensis ”flared form”: *F. crotonensis var. prolongata* Grunow ex van Heurck.	58-92 flared-formed ends in girdle view	No info	14-15 center, 17-18 in apices	same as before	long raft-like chains, with very narrow mucilage envelope (1-6 µm)	Crawford et al. (1985) (did not formally describe the 2 forms), difference to flared-form in parasite suceptibility

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Table S3. Towards more harmonized taxa names: Suggestions on how to separate Fragilaria species by morphological characters in LM (based on published analyses of type material). Fet style: typical character to use for separation in LM.

FRAGILARIA	length [µm]	width [µm]	striae 10µm^{-1}	colonies*	characters	reference	note
"medium-sized"							
F. neointermedia Tuji & Williams	23-35	3-4.5	**8-12**	yes	**few striae**	1	
F. vaucheriae (Kütz.) J.B.Petersen	14.1-50.4	3.8-5.1	**11-14**	no	**few striae**	2	
F. rinoi Almeida & C.Delgado	12-36	4.2-5.6	14-16	**no**	SEM: no spines	1	"*F.pectinalis* s.lat. without colonies" - complex **not separable in LM**
F. uliginosa Kulikovskiy, Lange-Bert., Witkowski & Dorofeyuk	14.1-28.8	3.5-4.4	15-17	**no**	SEM: large linking spines	2	"*F.pectinalis* s.lat. without colonies" - complex **not separable in LM**
F. microvaucheriae C.E.Wetzel & Ector	5.7-23.4	2.5-3.8	15-16	**no**	< 4µm wide; **L:W 2-6,** SEM: tiny or no spines	2	"*F.pectinalis* s.lat. without colonies" - complex **not separable in LM**
F. pectinalis (O.F.Müll.) Lyngb.	12-36	2.8-4.7	15-18	**no**	**L:W 6-8**, SEM: tiny spines	2, 4	"*F.pectinalis* s.lat. without colonies" - complex **not separable in LM**
F. heatherae sp. nov. (FCAP1)	9-38	3.3-3.7	16(18)	**± no**	< 4µm wide; L:W up to 11, SEM: no spines	13	"*F.pectinalis* s.lat. without colonies" - complex **not separable in LM**
F. capucina s.str. Desm.	28-47	3.3-4.2	14-17	**yes**	SEM: large linking spines	3, 1	"*F.capucina* s.lat. with colonies" - complex **not separable in LM**
F. joachimii sp. nov. (FCAP2)	5-35	3.3-4.6	14-16	**yes**	L:W ratio 5.3 to 9.4, SEM: tiny spines	13	"*F.capucina* s.lat. with colonies" - complex **not separable in LM**
F. agnesiae sp. nov (FVAU)	9-65	4.0-5.4	14-16	**yes**	SEM: tiny spines	13	"*F.capucina* s.lat. with colonies" - complex **not separable in LM**
F. perminuta (Grunow) Lange-Bertalot	8-25	3-4	17-21	no info	**< 4µm wide, rhombic valve form, rimmed central unilateral depression/swelling**	1, 6, 7	
F. recapitellata Lange-Bertalot&Metzeltin	20-39	2.8-4.2	17-20	no info	**capitate ends**	1, 4, 5	
"medium-long, thin, with ~20 striae 10 µm^{-1}"							
F. gracilis Østrup	30-50	2-3	20-22	no	**opposite, parallel striae; valve form ± linear**	8, 9	
F. tenera	60-120	1.8-2.5	18-20	no	**valves linear-lanceolate with only slightly convex margins**, subcapitate	9, 10	
F. rumpens (Kütz.) G. W. F. Carlson	25-63	3-4	18-20	**yes**	**width > 3µm**	11	
F. pararumpens	25-50	2.5-3	16-18	**yes**	**central swelling**, subcapitate ends	7	
F. saxoplanctonica	40-170	1.5-2.5	**23-28**	no		7, 9	
F. nanoides	40-90	1.8-2.4	**22.5-23**	no	subcapitate, getting thinner from middle to ends; central area 'empty'	12	
F.aquaplus Lange-Bert. & S. Ulrich	22-45^{9}	1.5-2	22-24	no	opposite, parallel very delicate striae; valve form ± needle-formed, rounded ends	6, 7, 9, 12, 13	suggestion to synomize *F.aquaplus* with *F.gracilis,* because not separated by rbcL & presence of intermedia forms former called "*F. nanana*" sensu Lange-Bert. 1991, part of the mixture of "Synedra nana Meister 1912"
F. nanana sensu Lange-Bertalot (1991)	40-90	1.5-2	22-30	no info	striae not alternating	6, 7, 12	part of the mixture of "*Synedra nana* Meister 1912", name should not be used further

* the presence of colony-formation needs to be analysed in adequately prepared slides

Literature used:

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13 this study

Fig. S1. Phylogeny of the studied strains of *Fragilaria*, rooted with strains of the genus *Ulnaria*, on a 1087 bp long part of the *rbcL* barcode. Bootstrap values above 69% are given for each node. Scale bar: number of substitutions per site. Full tree with geographical information included.