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Taxonomy and ecology of *Fragilaria microvaucheriae* sp. nov. and comparison with the type materials of *F. uliginosa* and *F. vaucheriae*

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Abstract – A new diatom species from freshwater environments in France is described. *Fragilaria microvaucheriae* sp. nov. is illustrated and discussed based on populations from the Loire-Brittany basin and compared with the original (type) material of *Fragilaria vaucheriae* (Kütz.) J.B.Petersen (= *Exilaria vaucheriae* Kütz.) from Germany and *Fragilaria uliginosa* Kulikowskiy *et al*., recently described from a peat bog in Mongolia. Additional populations of *Fragilaria pectinalis* (O.F.Müll.) Lyngb. from the Rhône Alpes region (France) are also investigated in detail including its environmental preferences in order to compare with the new species. *Fragilaria microvaucheriae* is similar to *F. vaucheriae* but present smaller cell dimensions and higher density of striae. It occurs in slight acidic waters. The new species has been identified in the past as *F. vaucheriae, Fragilaria capucina* sensu auct., *Fragilaria austriaca* (Grunow) Lange-Bert. or *Fragilaria pectinalis*. The morphology of the new species and similar taxa is documented by light (LM) and scanning electron micrographs (SEM) and discussed in light of recent advances in the taxonomy of the genus *Fragilaria* Lyngb. from freshwater environments.

Taxonomy / Fragilariaceae / *Fragilaria* / France / morphology / type material

Résumé – Une nouvelle espèce de diatomée de milieux d'eau douce est décrite en France. *Fragilaria microvaucheriae* sp. nov. est illustrée et discutée à partir de populations du bassin Loire-Bretagne et comparée avec le matériel original (type) de *Fragilaria vaucheriae* (Kütz.) J.B.Petersen (= *Exilaria vaucheriae* Kütz.) de l'Allemagne et de *Fragilaria uliginosa* Kulikowskiy *et al*., récemment décrite d'une tourbière en Mongolie. D'autres populations de *Fragilaria pectinalis* (O.F.Müll.) Lyngb. de la région Rhône-Alpes (France) ont également été étudiées en détail, y compris leurs préférences environnementales, afin de pouvoir les comparer avec la nouvelle espèce. *Fragilaria microvaucheriae* est similaire à *F. vaucheriae* mais présente une taille plus petite des cellules et une densité plus élevée des stries. Elle affectionne les eaux légèrement acides. Cette nouvelle espèce a été identifiée par le passé comme étant *F. vaucheriae*, *Fragilaria capucina* sensu auct., *Fragilaria austriaca* (Grunow) Lange-Bert. ou *Fragilaria pectinalis*. La morphologie de la nouvelle espèce et des taxons semblables est documentée en microscopie optique (MO) et électronique à balayage (MEB) et est discutée par rapport aux progrès récents de la taxonomie des diatomées du genre *Fragilaria* Lyngb. de milieux d'eau douce.

Taxonomie / Fragilariaceae / *Fragilaria* / France / morphologie / matériel type

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INTRODUCTION

Diatoms are routinely used in Europe after the Water Framework Directive, WFD (European Commission, 2000) required member states to assess the ecological status of their rivers using these organisms. Since then many floristic and taxonomic works dealing with the most common species in aquatic environments have been developed and updated in order to tackle this mission in most European Union (EU) countries. Some books and guides are routinely and widely used as a common source for these assessments (e.g. Krammer & Lange-Bertalot, 1986, 1988, 1991a, b; Blanco *et al*., 2010; Coste & Rosebery, 2011; Hofmann *et al*., 2011; Bey & Ector, 2013). In France particularly, many new "Atlas" of common species conceived by distinct geographical/hydrological regions are being published in order to support water agencies on the biomonitoring task (Bey & Ector, 2013). Similarly, the United States Geological Survey has also published a similar on-line tool as a guide aiming to provide everyone with accurate information about freshwater diatoms of the United States (Spaulding *et al*., 2010).

In this context, several harmonization studies have been conducted in order to standardize some methodological aspects when dealing with taxonomic lists (e.g. Kahlert *et al*., 2009; Kahlert & Gottschalk, 2014; Dreßler *et al*., 2015). It should be noted that a few years ago (ca. 10-15 years) many species that are common today were simply not recognized by earlier "practical" diatomists. Nowadays, as soon a species is well illustrated and described, many people starts using the proposed names (see for example *Achnanthidium druartii* Rimet & Couté (Rimet *et al*., 2010), *Achnanthidium delmontii* Pérès, Le Cohu & Barthès (Pérès *et al*., 2012) and *Nitzschia soratensis* E.Morales & M.L.Vis (Trobajo *et al*., 2013). Several studies have demonstrated the indispensable role of collections for investigation on the diversity and distribution of diatom species, and as such many of them are the basis to complement the work started by Krammer & Lange-Bertalot (1986, 1988, 1991a, b). The lack of clear circumscriptions is still common even for supposedly well know diatom species (see for example the recent work of Reichardt, 2015a, b).

In many cases, species are treated as a complex of species that seems "impracticable" to identify (see the lumping position taken by Krammer & Lange-Bertalot 1986, 1988, 1991a, b) and because sometimes functional diversity among clones from contrasting environmental factors (usually in culture conditions though), can hardly be distinguished morphologically (see Rovira *et al*., 2015).

Concerning "araphid" taxa, Lange-Bertalot & Ulrich (2014) recently investigated with light microscopy (LM) and scanning electron microscopy (SEM) historic documents as well as original slides and type material from different authors concerning several planktonic species of the genera *Fragilaria* Lyngb., *Synedra* Kütz. and *Ulnaria* Compère in order to characterize morphologically and discuss their taxonomical context. In this paper, seven new taxa were described and many others that were historically documented, but not well known, were for the first time illustrated and clearly circumscribed. Since many species are still poorly known or often wrongly identified, one of the recommendations is to "merge" some of the complexes until a better defined morphology is clarified (see Kahlert *et al*., 2009). This often is the case for species around the "*Fragilaria capucina*-complex" and other complexes in the genus *Fragilaria* whose tackle in the clarification of names has been taken mainly by Tuji and Williams in a series of recent papers (*i.e.* Tuji, 2004, 2007; Tuji & Williams 2006a, b, 2013) based exclusively on the identity of the original materials and typification of important species. However many species

commonly found during ecological assessments of rivers and lakes in Europe do not fit in the original concepts and descriptions and thus many "morphological" species still lacks a formal description.

The *Fragilaria capucina/vaucheriae* species complex sensu Lange-Bertalot in Krammer & Lange-Bertalot (1991a, 2004) was recently revised by Tuji & Williams (2006b, 2013) and *Fragilaria capucina* Desm. or *F. vaucheriae* are understood to be cosmopolitan (and common) species, appearing in many freshwater diatom floras. A quick search using internet engines promptly shows us a profusion of images and citations of these names all over the world. But in spite of its frequency of occurrence, the identity of these species (as well as other within the species complex) remained for a long time problematic until recently. The objective of our paper is to describe a new species similar to *Fragilaria vaucheriae* and to better illustrate the type material of the later. Additionally, the type material of *Fragilaria uliginosa* Kulikowskiy *et al*. from Mongolia and several populations of *Fragilaria pectinalis* from France were also studied in detail in order to compare their morphologies with the new species.

MATERIAL AND METHODS

The following species and samples have been examined under LM and SEM microscopy:

1) *Fragilaria vaucheriae* (Kütz.) J.B.Petersen: Kützing's unmounted material "Kützing 185" from Botanic Garden Meise, Belgium (BR), Van Heurck's collection. "Exilaria vaucheriae *Kg. an* Vaucheria clavata *im Quellen bei Leisling". Leißling* (or Leissling) is a village and a former municipality in Saxony-Anhalt, Germany and it is part of the town *Weißenfels*. Kützing's notebook concerning the sample 185 is here reproduced in the Figure 1. The type locality indicated agrees the material observed by Tuji & Williams (2006a, 2013) published in Kützing's exsiccata set Algarum Aquae Dulcis Germanicarum in Decas III, No. 24 in 1833 (Kützing, 1833a). A new slide was prepared with Naphrax and morphometric data were gathered from 48 specimens.

2) *Fragilaria uliginosa* Kulikovskiy, Lange-Bert., Witkowski & Dorofeyuk: Unmounted material corresponding to the slide 12529 (Holotype) in Collection A. Witkowski, Institute of Marine Sciences, University of Szczecin (Poland) (SZCZ), from the *Sphagnum* bog Nur, northem part of Hentei highlands, Mongolia published

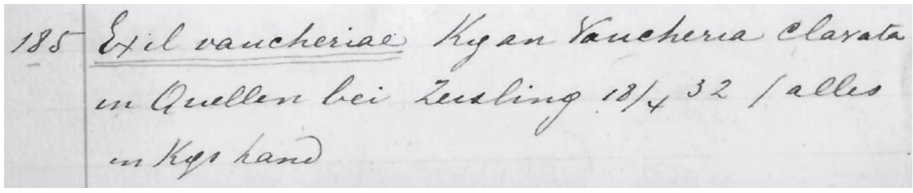


Fig. 1. Excerpt from Kützing notebook housed in the Botanic Garden Meise H. Van Heurck Collection, concerning sample n° 185, one of the original materials of *Exilaria vaucheriae* Kütz. "*an Vaucheria clavata im Quellen bei Leisling*".

by Kulikovskiy *et al*. (2010). A new slide was prepared for observation of the material and morphometric values were gathered from 16 specimens using LM.

3) *Fragilaria pectinalis* (O.F.Müll.) Lyngb.: Four populations from France (Rhône-Alpes region) illustrated were studied as follows:

• Epilithic sample from the Beaume River at Rosières, France; coll. date: 18/05/2011. Morphometric values were from 19 specimens using LM.

• Epilithic sample from the Drôme River at Livron-sur-Drôme, France; coll. date: 04/07/2007. Morphometric values were from 18 specimens using LM.

• Epilithic sample from the Eygues River at Saint-Maurice-sur-Eygues, France; coll. date: 28/06/2011. Morphometric values were gathered from 10 specimens using LM.

• Epilithic sample from the Romanche River at Le Bourg d'Oisans, France; coll. date: 04/04/2007. Morphometric values were gathered from 14 specimens using LM.

Light micrographs images of the four populations here observed were also published by Bey & Ector (2013). We observed the same materials now using SEM. Environmental parameters of the four sampling sites (rivers Beaume, Drôme, Eygues and Romanche) were provided by the DREAL Rhône-Alpes in order to characterize the ecology of the taxa. The data comprises monthly water samples from the sampling sites 3 months before, 3 months after and during the diatom sampling indicated above (n = 7). Boxplots of conductivity, biochemical oxygen demand (BOD), dissolved organic carbon (DOC), dissolved oxygen (DO), nitrates, nitrites, pH and phosphates are shown in the Fig. 106.

Additional measures of *F. pectinalis* (and its heterotypic synonyms) were taken from Tuji & Williams (2008, page 130, figs 14-27 concerning *F. pectinalis*; and from page 135, figs 54-64 concerning *Synedra capitellata* var. *cymbelloides* Grunow and *Synedra capitellata* f. *striis-distantioribus* Grunow).

4) *Fragilaria microvaucheriae* C.E.Wetzel & Ector sp. nov.: Two populations from the Loire-Brittany basin are illustrated and deposited at the Botanic Garden, Meise, Belgium (BR) with the following accession numbers:

• Sample BR-4424 (Botanic Garden Meise, Belgium). Epilithic sample from the Inam River at Lanvénégen (Breton: Lannejenn), a commune in the Morbihan department of the region of Brittany, north-western France; leg. Luc Ector, coll. date: 09/08/2006.

• Sample BR-4425 (Botanic Garden Meise, Belgium). Epilithic sample from the Tarun River at Baud, a commune in the Morbihan department of the region of Brittany, north-western France; leg. Luc Ector, coll. date: 18/09/2008.

Environmental parameters of the two sampling sites (rivers Inam and Tarun) were provided by the *Agence de l'Eau Loire-Bretagne*. The data comprises monthly water sampling at the site three (3) months before and during the diatom sampling campaign (n = 4) indicated above. Boxplots of conductivity, dissolved organic carbon (DOC), dissolved oxygen (DO), total nitrogen (TN), nitrites, nitrates, pH and total phosphorous are showed in the Fig. 143.

Subsamples of raw materials were digested using concentrated H_2O_2 and heating for 24 h using a sand bath. Preparations were then allowed to cool and settle (ca. 1 cm h^{-1}), and 80 to 90% of the supernatant was eliminated by vacuum aspiration. A volume of 1 ml of HCl (37%) was then added and the mixture was allowed to rest for 2 h followed by three repetitions of rinsing and decantation using deionized water. Permanent slides were mounted with Naphrax® and LM observations and morphometric measurements were performed using a Leica® DMRX brightfield

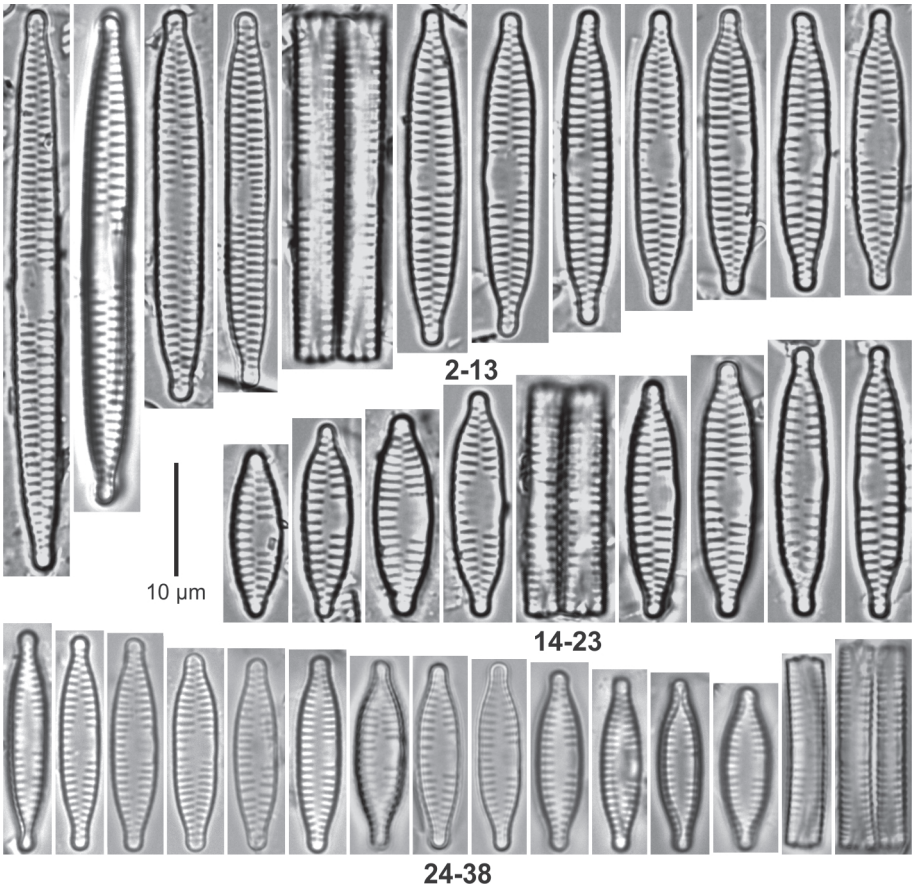
microscope with 100x oil immersion objective and light micrographs were taken with a Leica® DC500 camera. For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered with additional deionized water through a 3-µm Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminium stubs and coated with platinum using a Modular High Vacuum Coating System BAL–TEC MED 020 (BAL–TEC AG, Balzers, Liechtenstein). An ultra-high-resolution analytical field emission (FE) scanning electron microscope Hitachi SU–70 (Hitachi High-Technologies Corporation, Japan) operated at 5 kV and 10 mm distance was used for the analysis. SEM images were taken using the lower (SE-L) and upper (SE-U) detector signal and up to 28° tilted. Micrographs were digitally manipulated and plates containing light and scanning electron microscopy images were created using CorelDraw X6.

Additional measures of *Fragilaria recapitellata* Lange-Bertalot & Metzeltin in Metzeltin *et al*. (2009) published by Delgado *et al*. (2015) (n = 36) and *Fragilaria perminuta* (Grunow) Lange-Bertalot by Tuji & Williams (2008) (n = 6, p. 128, figs 1-9) were further digitally measured and included in comparative boxplots and scatterplots here discussed (Fig. 144). Whenever possible (i.e. when valves were more than 20 µm long) four measures of striae density were taken from each valve. Therefore striae measures are the median values for each valve. Boxplots and scatterplots were made using test using R version 3.1.2 (R Core Development Team, 2014) and the differences in morphometric data were examined using a Kruskal-Wallis non-parametric test using the package "Rcmdr" version 2.1-7 (Fox 2005, 2007).

RESULTS AND DISCUSSION

Fragilaria vaucheriae (Kütz.) J.B.Petersen LM: Figs 2-23, SEM: Figs 39-53

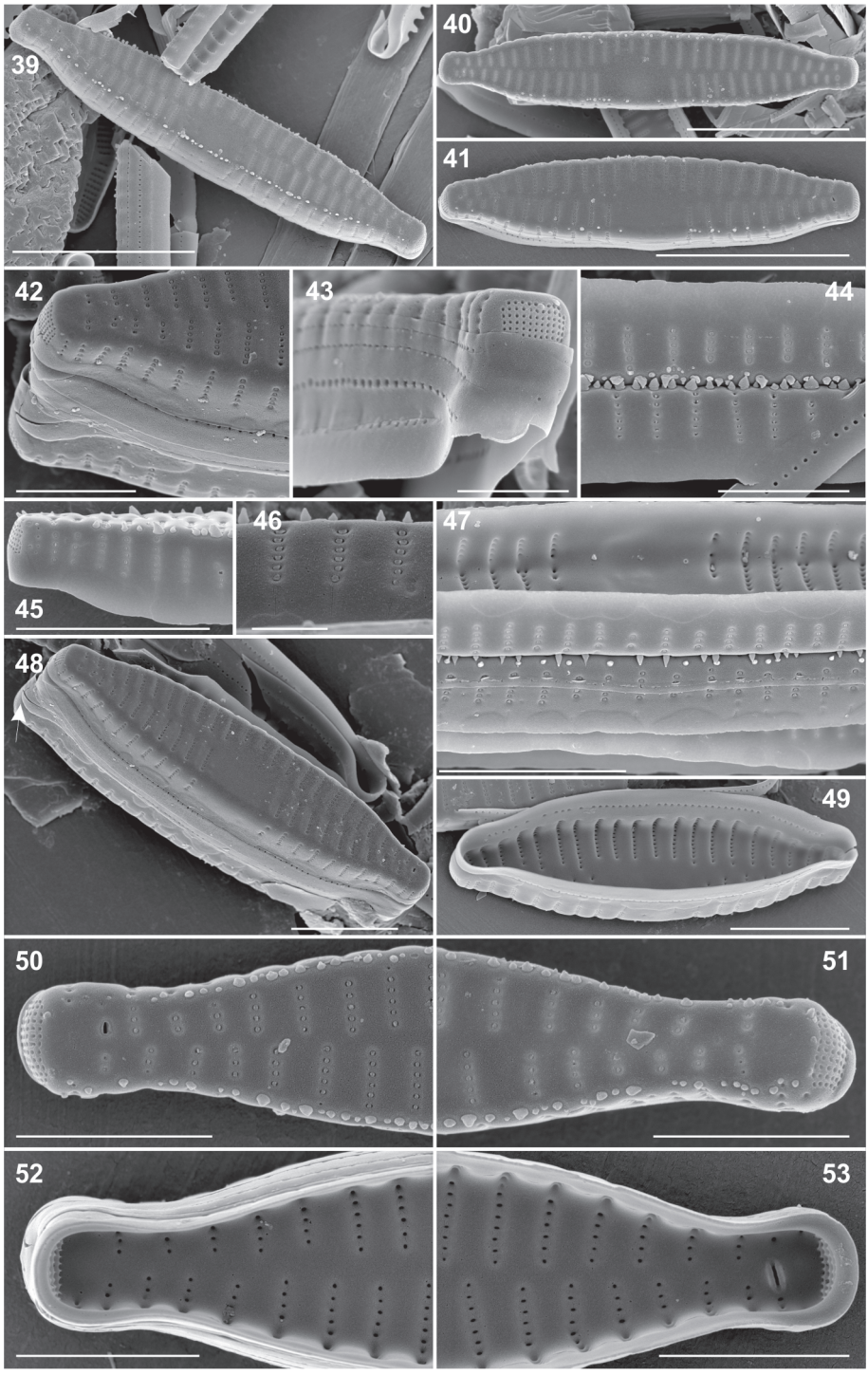
Exilaria vaucheriae Kütz. (Kützing 1833a) was published in Kützing's exsiccata set *Algarum Aquae Dulcis Germanicarum* (Decas III, No. 24) without description or figure, which were described and illustrated later in Kützing (1833b, p. 560, fig. 38). The original material from Germany ("*Quelle bei Weißenfels*") used by Kützing was recently observed by Tuji & Williams (2006b, 2013) and had been previously observed by Lange-Bertalot (1980, pl. 4, figs 82-94, 97-102) with a broader concept that was narrowed later in Krammer & Lange-Bertalot (2004, pl. 108, figs 10-15). However the lack of LM girdle views and SEM images in the latter two publications and the relatively poor quality of the first prompted us to better illustrate this material which is here presented in detail. Valves are linear and narrow with rostrate to subcapitate ends and the central margin and area are unilaterally expanded (Figs 2-23). In girdle view (Figs 6, 18), frustules are rectangular. The striae are mostly subparallel, to slightly radiate toward the valve apices. Striae are alternate and are often interrupted at the valve face/mantle junction (Figs 39, 42, 48). The spines are small, conic and delicate (Figs 44, 47). They are present along the valve face edge sometimes including the apices (Figs 45, 50, 51). The spines may be absent independent of the size of the specimens, but in general large cells have more spines. Well-developed apical pore fields with round poroids are present at both apices (Figs 43, 50, 51). A single rimoportula is present on each valve, aligned with the first stria at the valve face apex (Fig. 53). Girdle bands are open (Figs 42, 43, 48) with small unoccluded perforations. Valve dimensions taken from



Figs 2-38. **2-23.** *Fragilaria vaucheriae*. Type material from package n° 185 from Weißenfels (Germany) of *Exilaria vaucheriae* Kütz., housed the in Botanic Garden Meise. **24-38.** Type material of *Fragilaria uliginosa* Kulikovskiy, Lange-Bert., Witkowski & Dorofeyuk from the *Sphagnum* bog Nur, Hentei highlands, Mongolia.

48 specimens in LM: length 14.1-50.4 µm, width 3.8-5.1 µm, striae 11-14 in 10 µm. Ghost striae may be present in the central area (Fig. 4). Tuji & Williams (2013) noted that the rimoportulae occur near the poles, one per valve and the large apical pore fields are rectangular, spines either do not exist or are very small, and they do not link with sibling cells. We confirm these observations in the material 185 from Kützing (Figs 39-53), adding that the sibling cells can remain loosely attached to each other (Figs 44, 47) but never more than two cells could be found as observed by Petersen (1938) (e.g. Figs 6, 18, 44 and 47).

Figs 39-53. SEM of *Fragilaria vaucheriae*. Type material from package n° 185 from Weißenfels (Germany) housed the in Botanic Garden Meise. Scales = 10 µm (39-41); 5 µm (45, 47-49); 4 µm (44) 3 µm (42, 50-53); 2 µm (43); 1 µm (46). ▶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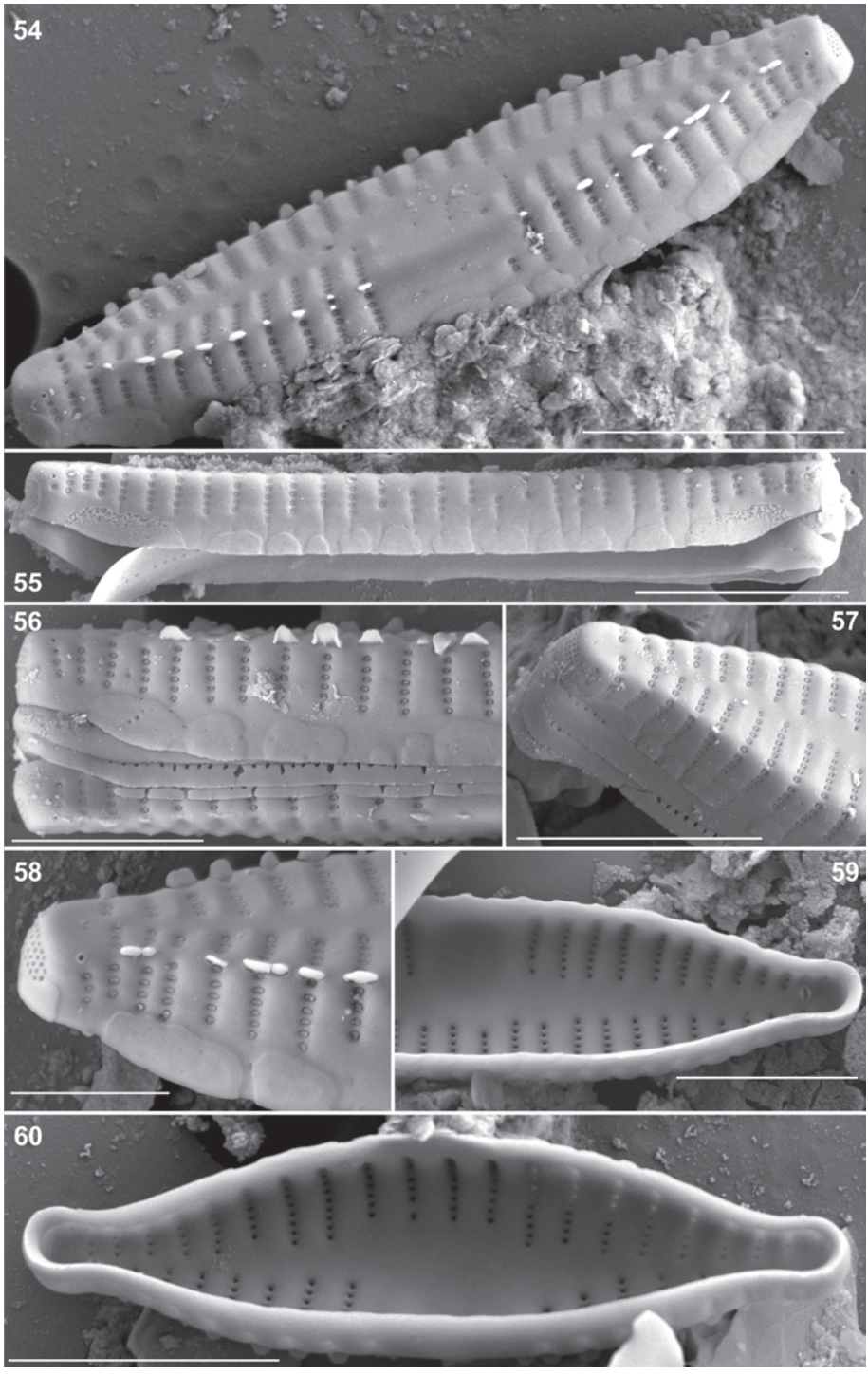
***Fragilaria uliginosa* Kulikovskiy, Lange-Bert., Witkowski & Dorofeyuk**
LM: Figs 24-38, SEM: Figs 54-60

Fragilaria uliginosa was recently described from a peatbog in Mongolia by Kulikovskiy *et al*. (2010, p. 37, pl. 9, figs 11-18, 20, 21) and is characterized by valves with linear-elliptic to linear-elliptic-lanceolate with apices abruptly rostrate to rostrate-subcapitate. Our observations from the type material expands the morphometric ranges of the species: valve length: 14.1-28.8 µm (instead 15.3-20.0 µm), valve width 3.5-4.5 µm (instead 3.7-4.5 µm) while the number of striae remains the same as observed by Kulikovskiy *et al*. (2010): 15 to 16 in 10 µm. Compared to the most similar species in LM (*i.e. Fragilaria vaucheriae* and *Fragilaria pectinalis*) the species shows differences in the wider axial area which is narrower in *F. vaucheriae* and *F. pectinalis* (Figs 59, 60).We could not find long cells and in general it has a short mean length with less than 20 microns (Fig. 144). The alternating striae on both sides are subparallel in the middle becoming slightly radiate to the ends. Most of the characters are barely different from most comparable species as observed by Kulikovskiy *et al*. (2010). The most striking difference are the spines, only visible in SEM, sometimes slightly irregularly distributed on the junction of the valve face/mantle and mostly located in the striae (Figs 54, 56, 58), but also on the middle of costae or very close to the series of areolae. The distal part of the spines is irregularly spathulate. *Fragilaria uliginosa* is wider than *F. microvaucheriae* and *F. perminuta* and possess less striae in 10 µm (median equal to 16) than both. For instance *F. uliginosa* has only been reported for the type locality in Mongolia and no other illustration could be found in the literature. As in *F. vaucheriae*, colonies were not observed and the species seems to be mainly found as solitary cells.

***Fragilaria pectinalis* (O.F.Müll.) Lyngb.** **LM: Figs 61-89, SEM: Figs 90-105**

Fragilaria pectinalis is the generitype of the genus *Fragilaria* Lyngb. as typified by Tuji & Williams (2006b). The species was viewed for a long time as a synonym of *Fragilaria capucina* and remained unknown or at least forgotten until the work by Tuji & Williams (2006b) further detailed by Tuji & Williams (2008) who explained the classification history of this taxon. In part due to its complicated and entangled taxonomic and nomenclatural history (regarded as a synonym of *Fragilaria capucina*) the species is not present in the *Süsswasserflora* series of Krammer & Lange-Bertalot (1991a, 2004). In those years the species was neither clearly circumscribed nor typified. But despite the posterior clarifications the species continues to be absent in most common guides for identification of benthic species (e.g. Blanco *et al*., 2010; Spaulding *et al*., 2010; Hofmann *et al*., 2011) and thus the name remains somehow poorly known while the species seems to be more common than previously registered. Tuji & Williams (2008) concluded that some of the common species illustrated by Grunow in Van Heurck (1881) identified as *Synedra capitellata* var. *cymbelloides* Grunow, *S. capitellata* f. *striis-distantioribus* Grunow and *S. gloiophila* Grunow were indeed synonyms of *Fragilaria pectinalis*.

Figs 54-60. SEM of *Fragilaria uliginosa*. *Sphagnum* bog Nur, Hentei highlands, Mongolia, corresponding to the sample 12529 (Holotype) in Collection A. Witkowski, Institute of Marine Sciences, University of Szczecin. Scales = 5 µm (54, 55, 57, 60); 4 µm (56, 59); 2 µm (58). ▶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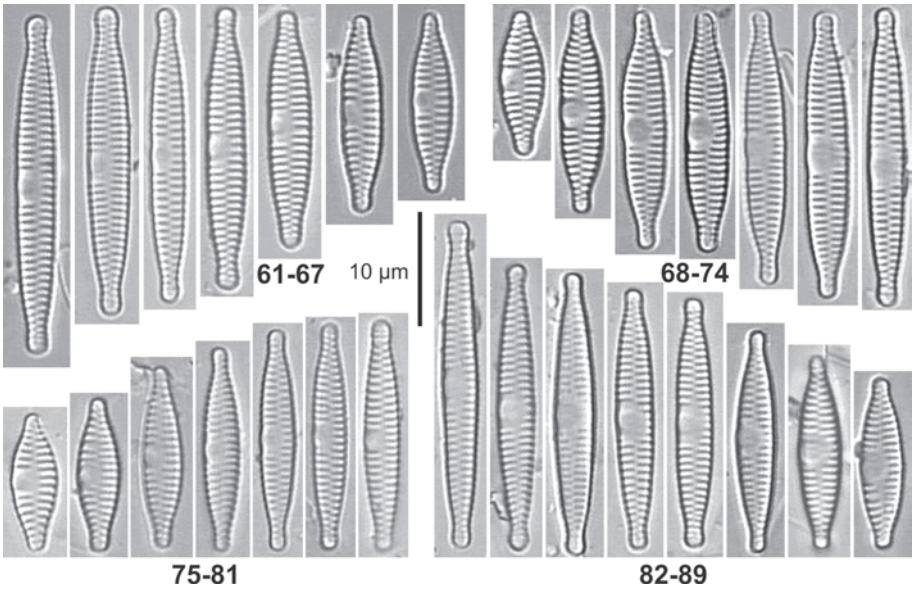
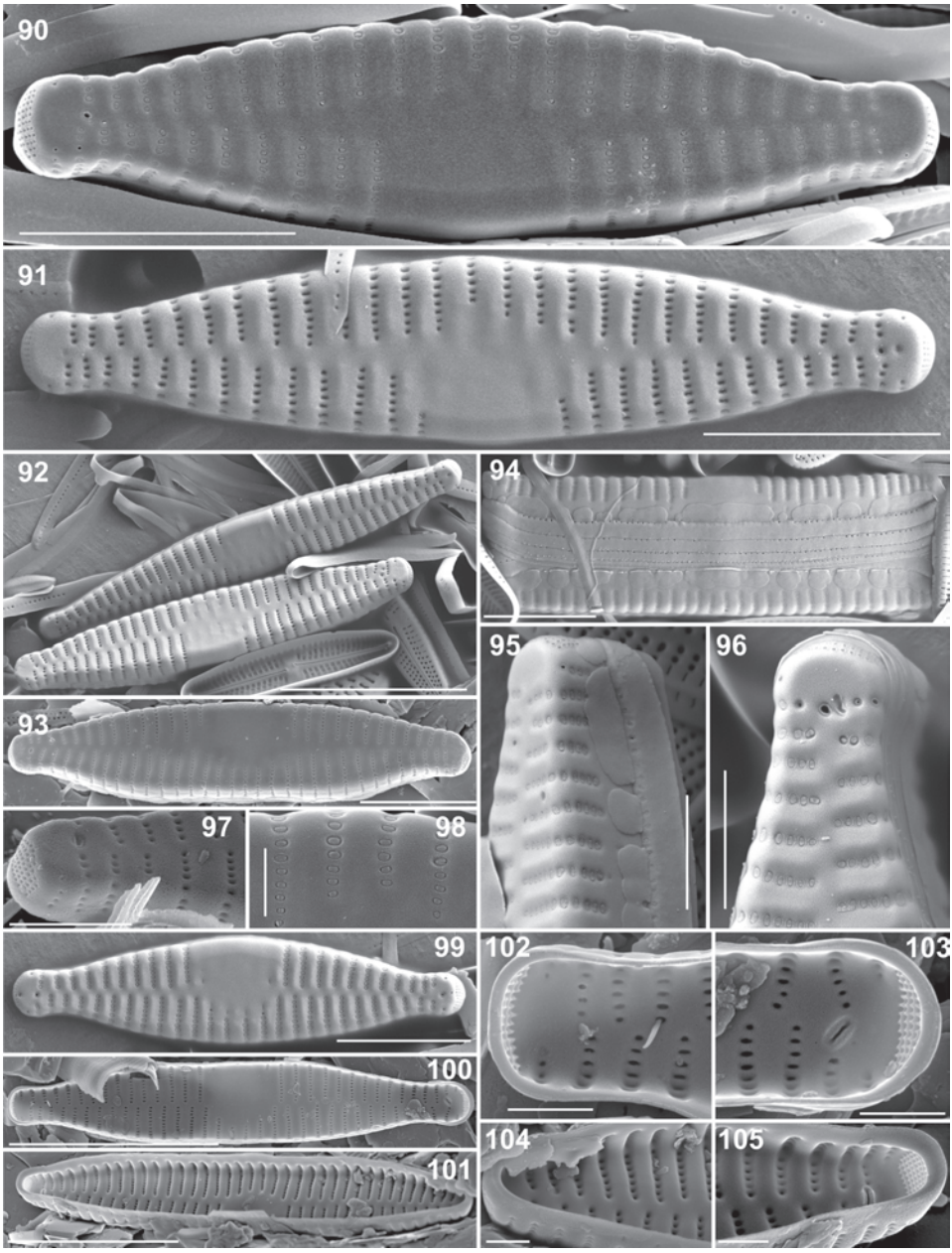


Figs 61-89. *Fragilaria pectinalis*. Populations from Rhône-Alpes region (France). **61-67.** Drôme River at Livron-sur-Drôme (04.07.2007), **68-74.** Romanche River at Le Bourg d'Oisans (04.04.2007), **75-81.** Eygues River at Saint-Maurice-sur-Eygues (28.06.2011), **82-89.** Beaume River at Rosières (18.05.2011).

Fragilaria pectinalis was for a long time called *F. vaucheriae* in many inventories from France monitoring databases (LE pers. obs.) and in many cases the species was informally recognised as a "small *vaucheriae*". All populations here presented were illustrated and identified as *Fragilaria pectinalis* by Bey & Ector (2013, p. 229, figs 1-64), but were previously confounded as *Fragilaria* sp. in the Drôme River (Figs 61-67), as *F. capucina* var. *capucina* in the Romanche River (Figs 68-74), as *F. austriaca* in the Eygues River (Figs 75-81) and finally as *F. capucina* var. *vaucheriae* in the Beaume River (Figs 82-89) showing the difficulties in the taxonomic fitting of this taxon. *Fragilaria pectinalis* appears among the cells illustrated by Krammer & Lange-Bertalot (2004, pl. 113, figs 6-12) as "*Fragilaria capucina* "*capitellata*-*Sippe*". The morphology of *F. pectinalis* has already been illustrated and discussed in the literature by Tuji & Williams (2008). We therefore consider it unnecessary to re-describe its morphology herein since the four populations from the Rhône-Alpes region agrees with the specimens illustrated by Tuji & Williams (2008, figures 50-64) more precisely with the specimens depicted as *Synedra capitellata* var. *cymbelloides* and *S. capitellata* f. *striis-distantioribus*, both heterotypic synonyms of *F. pectinalis*. There are small ultrastructural differences when compared to *F. vaucheriae*: *F. pectinalis* never showed any type of spines (Figs 90-99) and the striae are not interrupted in the valve face/mantle junction. The species does not make colonies and have one rimoportula per valve. Additionally, there is significant differences between both species concerning the number of striae (which is much more dense in *F. pectinalis*) and valve width (thinner than *F. vaucheriae*). Our populations appear to have preferences for hard waters with relatively good water quality [Specific Polluosensitivity Index (IPS) values between



Figs 90-105. *Fragilaria pectinalis*. Populations from Rhône-Alpes region (France). **90-91.** External view. Beaume River at Rosières. **92, 94-96.** Drôme River at Livron-sur-Drôme. **93, 97-98, 100-105.** Romanche River at Le Bourg d'Oisans; **99.** Eygues River at Maurice-sur-Eygues. Scales = 10 µm (92, 100, 101); 5 µm (90, 91, 93, 94, 99); 2 µm (95-97); 1 µm (98, 102-105).

15.1 and 18.8; $n = 4$). The four populations comes from poor mineralized rivers with low to medium conductivity (38.0-499.0 µS cm^{-1}), alkaline pH (7.6-8.6) but with relatively high nitrate concentration (up to 44.0 mg NO_3 L^{-1}). Dissolved oxygen concentrations varied between between 93.0% to 132.0%. The common species associated with *F. pectinalis* (with up to 5% of relative abundance) were *Achnanthidium lineare* W.Smith, *A. minutissimum* (Kütz.) Czarn., *A. pyrenaicum* (Hust.) H.Kobayasi, *A. rivulare* Potapova & Ponader, *Cocconeis euglypta* Ehr., *Cymbella excisa* Kütz., *Diatoma ehrenbergii* Kütz., *D. moniliformis* Kütz., *Encyonopsis minuta* Krammer & E.Reichardt, *Gomphonema minutum* (C.Agardh) C.Agardh, *G. olivaceum* (Hornemann) Bréb., *G. pumilum* (Grunow) E.Reichardt & Lange-Bert., *G. rosenstockianum* Lange-Bert. & E.Reichardt, *Hannaea arcus* (Ehr.) R.M.Patrick and *Nitzschia fonticola* Grunow. The Drôme River was the only one showing a slightly higher degree of pollution indicated by the presence of *Fistulifera saprophila* (Lange-Bert. & Bonik) Lange-Bert. with 7.5% of relative abundance also verified in a higher content of nitrates (Fig. 106).

Fragilaria microvaucheriae **C.E.Wetzel & Ector sp. nov.**
LM: Figs 107-136, SEM: Figs 137-142

Cellulae solitariae. Valvae lanceolatae quoad specimina maiora sed rhombico-lanceolatae quoad specimina minora, apicibus protractis, rostratis vel capitatis (quoad specimina maiora) ad cuneatis (quoad specimina minora). Longitudo valvae 5.7-23.5 µm, latitudo valvae 2.5-3.8 µm. Area axialis angusta linearis. Striae uniseriatae sub-parallelae vel leviter radiantes 15-16 in 10 µm. Spinae inconspicuae irregulariter in limbis praesentes vel vacantes. Rimoportula una pro valva. Areae pororum apicales in forma ocellulimbi adsunt. A Fragilaria vaucheriae *(Kütz.) J.B.Petersen differt proprie dimensionibus inferirioribus, etiam 15-16 striis in 10 µm (nec 11-14).*

Holotype: Slide BR-4424, Botanic Garden Meise, Belgium.

Type locality: FRANCE, Inam River at Lanvénégen (Lannejenn), a commune in the Morbihan department of the region of Brittany, north-western France; leg. Luc Ector, coll. date: 09/08/2006.

Fragilaria microvaucheriae is characterized by solitary cells with valves lanceolate in larger specimens to rhombic-lanceolate in smaller specimens with slightly rostrate (in larger specimens) to cuneate ends (in smaller specimens). Valve length 5.7-23.4 µm, valve width 2.5-3.8 µm. Axial area narrow, linear, central area large, unilateral. Striae coarse, uniseriate, radiate throughout, 15-16 in 10 µm. External valve face (Figs 137, 139) flat with small conic spines (spinules) present (Figs 137, 140) or absent (Fig. 139). Sternum narrow, gradually widening towards central area (Fig. 137). Central area wide and unilateral (bilateral in large specimens, e.g. Figs 107, 139). Striae uniseriate, composed of rimmed areolae (Fig. 140) with round foramina. Striae continuing onto valve mantle, not interrupted at the mantle-face junction (Fig. 137). A single rimoportula is present at one pole. External opening simple, rimmed, slightly larger than areolae. Apical pore fields present on both poles (Fig. 137); pore field composed of simple fine porelli arranged in regular rows parallel to the apical axis (Fig. 139). Internally (Fig. 141) costa well-developed and wider than the striae. Girdle bands are open (Figs 138, 140), with small unoccluded perforations (Fig. 142).

Fragilaria microvaucheriae was found in epilithon samples at two sampling sites in France (Loire-Brittany basin), with relatively low abundances.

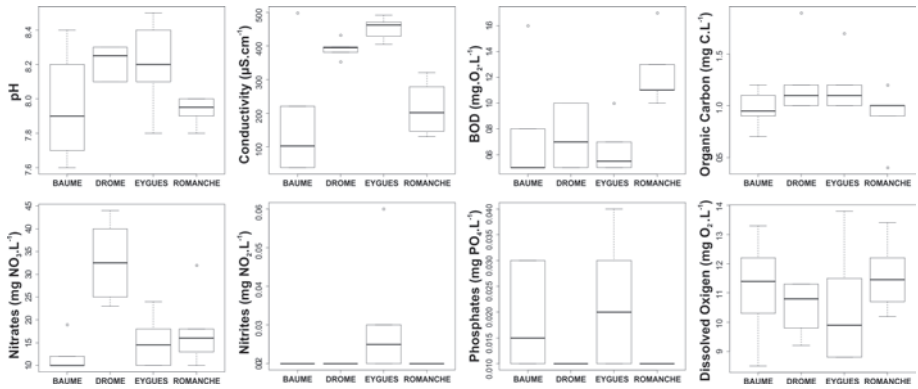
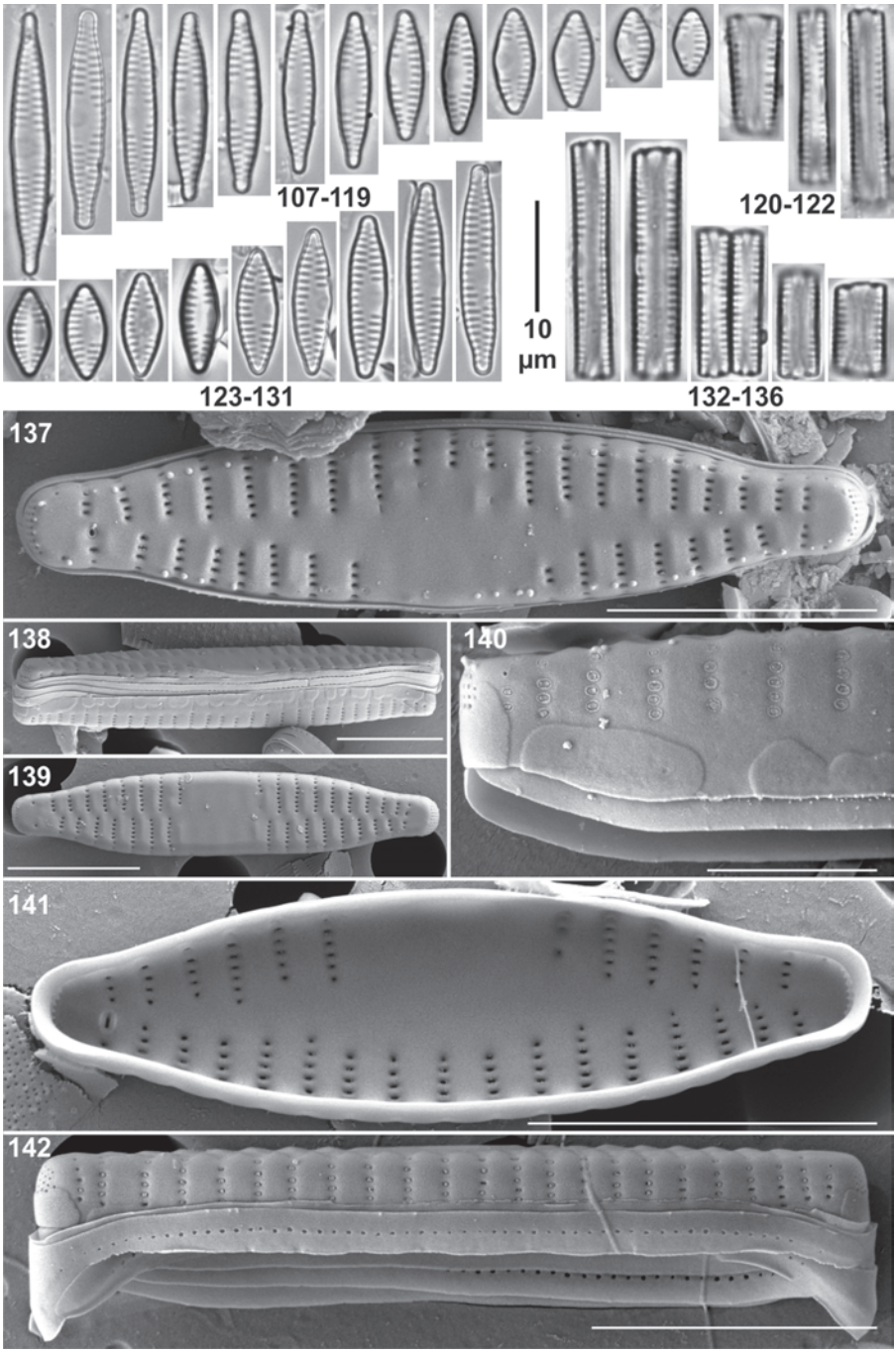


Fig. 106. Boxplot of mean values (n = 6) of chemical and physical parameters at the four studied rivers in the Rhône-Alpes region (France), where *F. pectinalis* was present. Data from Drôme and Romanche (2007) and Beaume and Eygues rivers (2011) were collected bimonthly.

At the Inam River it was identified as *Fragilaria capucina* Desm. var. *vaucheriae* (Kütz.) Lange-Bert. and reached 6% of relative abundance while at the Tarun River it was identified as *Fragilaria perminuta* (Grunow) Lange-Bert. and reached 6.4%. At the Inam River the most abundant species were *Achnanthidium minutissimum* (Kütz.) Czarn., *Cocconeis euglypta* Ehr., *Eolimna minima* (Grunow) Lange-Bert., *Fragilaria rumpens* (Kütz.) G.W.F.Carlson, *Mayamaea permitis* (Hust.) Bruder & Medlin, *Navicula gregaria* Donkin and *Psammothidium oblongellum* (Østrup) Van de Vijver, while at the Tarun River the community was basically the same with a dominance of *Navicula lanceolata* (C.Agardh) Ehr. (26%) and the presence of *Karayevia oblongella* (Østrup) Aboal with ca. 6%. *Fragilaria microvaucheriae* was present in poor mineralized rivers with low conductivity (170-230 µS cm^{-1}), acid to neutral pH (6.7-7.4) and low nutrient concentration (see Fig. 143).

Both populations of *Fragilaria microvaucheriae* from Inam River at Lanvénégen (Figs 107-122, 137-142) and Tarun River at Baud (Figs 123-136) may be confused at a first glance with *Fragilaria pectinalis* or *Fragilaria vaucheriae*. However these species have significant difference concerning basic morphometric measurements and valve outline. The smaller cells in *F. pectinalis* (Figs 66-69, 75-76, 88-89) will always present rostrate to subcapitated apices while *F. microvaucheriae* show almost cuneate ends in median to small specimens (Figs 114-119, 123-128). The same is valid for *F. vaucheriae* in which the whole population shows rostrate to subrostrate apices (Figs 2-23), additionally to the differences in width and number of striae. *Fragilaria microvaucheriae* also present slight difference in the central area which tends to form a semi-circle in *F. pectinalis* not clearly defined in *F. microvaucheriae*. The clear character however, is the length:width ratio (Fig. 144) that discriminate between *F. pectinalis* and the new species *F. microvaucheriae*. This length:width ratio in only comparable to the type population of *Fragilaria perminuta* (Grunow) Lange-Bert. illustrated by Tuji & Williams (2008) which have a higher density of striae (19-20 in 10 µm). The observed difference in the width and number of striae of cells between the studied population was statistically significant (Kruskal-Wallis test, $p < 0.05$), fig. 144).



Figs 107-142. LM and SEM images of *Fragilaria microvaucheriae* sp. nov. 107-122, 137-142: Type population from Inam River at Lanvénégen, region of Brittany, France. 123-136: specimens from the Tarun River at Baud, region of Brittany, France. SEM scales = 5 µm (137-139, 141-142); 2 µm (140).

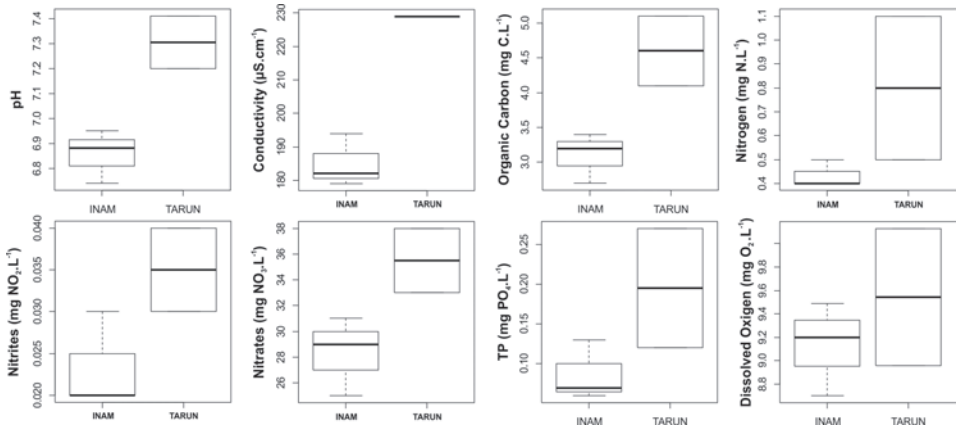


Fig. 143. Boxplot of mean values (n = 4) of chemical and physical parameters at the two studied rivers in the region of Brittany (France), where *F. microvaucheriae* was present. Data from Inam and Tarun rivers, Loire-Brittany basin, France.

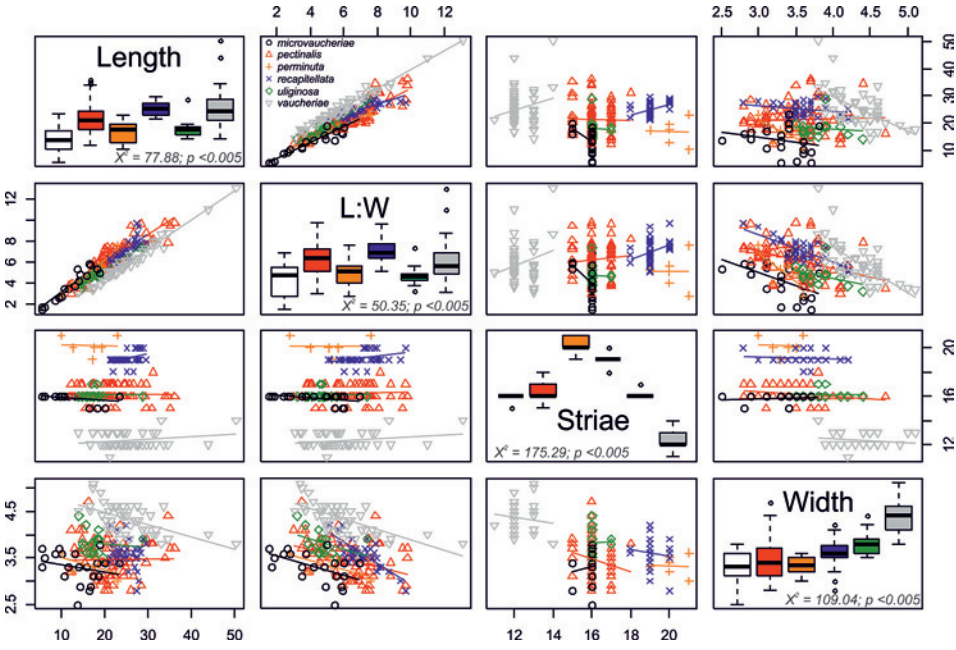


Fig. 144. Scatterplot matrix of measurements of different *Fragilaria* species. Variation in the valve length, width, striae in 10 µm and length width ratio (L:W). The boxes represent the 25-75% quartiles, the median is shown with a horizontal line inside the box, and minimum and maximum values are shown with short horizontal lines (whiskers). Outliers are plotted as individual points. *Fragilaria microvaucheriae* (n = 21), *Fragilaria pectinalis* (n = 77), *Fragilaria perminuta* (n = 6), *Fragilaria recapitellata* (n = 36), *Fragilaria uliginosa* (n = 16), *Fragilaria vaucheriae* (n = 48).

Table 1. Main morphological characters of the studied *Fragilaria* populations. (S.D. = standard deviation). (1) = Data from Tuji & Williams (2008); (2) = data from Delgado *et al.* (2015)

		F. microvaucheriae	*F. pectinalis*	*F. perminuta (1)*	*F. recapitellata (2)*	*F. uliginosa*	*F. vaucheriae*
	n =	21	77	6	36	16	48
Length (µm)	Min.-Max. Mean ± S.D Median.	5.7-23.4 13.7 ± 5.0 13.9	12.0-36.2 21.4 ± 5.5 20.9	10.1-23.0 16.7 ± 4.7 17.5	21.3-29.7 24.4 ± 5.9 23.5	14.1-28.8 17.9 ± 11.1 17.6	14.1-50.4 25.4 ± 4.6 24.4
Width (µm)	Min.-Max. Mean ± S.D Median and center median	2.5-3.8 3.3 ± 0.3 3.3	2.8-4.7 3.5 ± 0.4 3.4	3.0-3.6 3.3 ± 0.2 3.3	2.8-4.2 3.6 ± 0.3 3.6	3.5-4.4 3.8 ± 0.3 3.8	3.8-5.1 4.3 ± 0.3 4.4
Striae in 10 µm	Min.-Max. Mean ± S.D Median and center median	15-16 15.9 ± 0.4 16	15-18 16.2 ± 0.7 16	19-21 20.2 ± 0.8 20	18-20 19.1 ± 0.5 19	16-17 16.1 ± 0. 3 16	11-14 12.4 ± 0.6 12
Apical pore fields		Present at both apices, composed by round poroids. Apical pore fields with approximately 7-9 columns of pores, with ca. 3-5 pores per column	Present at both apices, composed by round poroids. Apical pore fields with approximately 9-12 columns of pores, with ca. 4-7 pores per column	–	–	Present at both apices, composed by round poroids. Apical pore fields with approximately 8-10 columns of pores, with ca. 2-5 pores per column	Present at both apices, composed by round poroids. Apical pore fields with approximately 10-13 columns of pores, with ca. 6-7 pores per column
Apices		Slightly rostrate to cuneate	Capitate to slightly rostrate	Slightly rostrate to cuneate	Strongly apiculate	Rostrate to subcapitate	Rostrate to subcapitate
Central area		Wide, unilateral, with a “quadratic” to rectangular aspect	Unilateral, with a “roundish” or semi-circle aspect	Strongly unilateral	Unilateral, with a “roundish” or semi-circle aspect	Central area somewhat variable in shape, occasionally lacking but mostly unilateral	Wide, unilateral, with a “quadratic” to rectangular aspect
Spines		Small, conic and irregularly located on the costa. Usually absent	Absent	–	–	Wide, spathulated and usually located on the striae at the valve face/mantle junction	Several conic small spines located along the valve face/mantle junction. Irregularly distributed
Sternum		Narrow linear to slightly lanceolate	Linear, narrow	Linear, narrow	Linear, narrow	Narrow linear to slightly lanceolate	Linear, narrow

FINAL REMARKS AND CONCLUSIONS

The diatoms studied here are often abundant and sometimes dominant in freshwater benthic communities. However, there have been many changes in their circumscription and concept since they were first described (see the series of paper by A. Tuji and D.M. Williams). The lack of analysis of original materials has led to the propagation of many errors in taxonomy and identification. For example, some older, largely unused names (like *Fragilaria pectinalis*) are much more commonly found against well-known names (like the rare *Fragilaria capucina*). The confused history of these "fragilarioid" taxa is of course partly because of their entangled nomenclatural and taxonomic classification. In this study, we described a new species comparing it with the most similar type materials (*F. vaucheriae* and *F. uliginosa*) to which the taxon was usually confounded with. Many new papers dealing with type materials (*i.e.* the series of paper by Tuji and Williams, Delgado *et al*., 2015) shed light into new concepts of some common European *Fragilaria* species. *Fragilaria microvaucheriae* was differentiated from other similar species using basic morphometric characters possible using a light microscope.

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