Observations on the lectotype material of *Fragilariforma virescens* (*Fragilariaceae, Bacillariophyceae*)

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Fragilariforma virescens (Ralfs) D.M.Williams & Round (1988b: 265) is a frequently observed freshwater diatom thriving predominantly in oligotrophic, electrolyte-poor, weakly acid streams, springs, and lakes (Lange-Bertalot & al. 2017: 280) and considered an indicator of excellent water quality, not tolerating moderate (anthropogenous) acidification. The species was originally described as *Fragilaria virescens* Ralfs (1843: 110, pl. II: fig. 6) from several English freshwater locations near Penzance (Madron and Chyenhal Moor, Penzance, Cornwall, UK) and Cold Bath, Tunbridge Wells (Kent), the latter sample sent to him by Mr Jenner (Ralfs 1843: 110, 'In freshwater pools. Cold Bath, Tunbridge Wells, Mr. Jenner. Madron and Chyanhâl Moor [sic] near Penzance'). Ralfs (1843: 110) described his new species as follows: "*Frustules broad, with two evident puncta at each end; lateral surfaces turgid-lanceolate, constricted near the ends; striae none, or indistinct.*" He illustrated it with several line drawings depicting the species in girdle and valve face view. As he described the species within the genus *Fragilaria*, Ralfs clearly indicated that way that the species formed long filaments ("Filaments free, fragile", Ralfs 1843: 106). Smith (1856: 22) added more morphological information to the original description: "*V(alve) linear or elliptical, suddenly attenuated towards the produced extremities, which are obtuse; striae 44 in ·001". Length of frustule·0005" to·0027*"" Grunow (1862: 373) refined the ecological preferences and its distribution (adding multiple localities in Austria and Hungary) reporting that *F. virescens* seems to be absent from calcareous areas ("*im Kalkgebiete gänzlich zu fehlen*") and that it was mostly present in samples taken from *Sphagnum*. He also separated two varieties, transferring a species William Smith had described in 1856 as *F. undata* W.Smith (1856: 24, including the var. α, β and γ), splitting them between the var. β *diatomacea* Grunow and the var. γ *undata* Grunow based on the drawings in Smith (1856, fig. 377). *Fragilaria undata* var. β and var. γ are now known as *Fragilariforma undata* (W.Smith) Heudre, C.E.Wetzel & Ector (Heudre & al. 2017: 264) whereas the var. β *diatomacea* should be considered to represent the nominate variety, *F. virescens* var. *virescens*.

Grunow (in Van Heurck 1881, pl. XLIV: figs 1–6) illustrated a population he observed in Eulenstein sample 44 (Stuttgart, Baden-Württemberg, Germany) of *F. virescens* (fig. 1), together with several other populations that he separated as *F. virescens* var. *exigua* Grunow (now *Stauroforma exiguiformis* (Lange-Bertalot) R.J.Flower, V.J.Jones & Round; figs 2–3), *F. virescens* var. *subsalina* Grunow (fig. 5), *F. virescens* var. *oblongella* Grunow (fig. 6) and *F. virescens* [var. *oblongella*] f. *clavata* Grunow (now considered a synonym of *Fragilaria schultzii* C.Brockmann, fig. 4). An initial study of the drawings indicated that the latter varieties should no longer be considered as being related to *F. virescens* and will be subject to a thorough revision based on the original Grunow material (Van de Vijver & al., unpubl. res.).

Over the following 100 years, many infraspecific taxa were separated from the nominate *F. virescens*, often only based on (slight) differences in valve outline, shape of the apices and valve

dimensions (Reid et al. 1995). AlgaeBase (Guiry & Guiry 2023) lists some 56 varieties and forms under the name *Fragilaria virescens*, such as *F. virescens* var. *birostrata* A.Cleve (1953: 49), *F. virescens* var. *obtusa* Skvortzov (1976: 121), often with very restricted distributions. Many of these names, however, are now known to represent quite different taxa or are simply synonyms. Examples include *Fragilaria virescens* f. *parva* Kützing that is now considered to belong to the genus *Pseudostaurosira* as *P. cubonii* D.M.Williams & C.E.Wetzel (Williams & Wetzel 2020). Mayer (1937, pl. 1) illustrated, together with seven drawings of the nominate *F. virescens*) several of these varieties and forms. Literature data erroneously show that the species is highly variable in valve outline, but this is more a result of the incorrection appreciation of this morphological variability. Moreover, the numerous records from all continents worldwide (as listed in Guiry & Guiry 2023 under 'detailed distribution with sources') may point, misleadingly, to a cosmopolitan distribution. This was contradicted by a critical examination of all specimens conserved in **BM** (Webb & Williams 1996, Yesilyurt & Williams 2016). Reid & al. (1995) pointed out that it is important to know how many of these varieties are different from the nominate *F. virescens* and have unique distributions. As not all published records are accompanied with illustrated observations, it is very difficult to verify the exact distribution. Analysis of published illustrations, however, casts serious doubts upon the conspecificity of all these records. An illustrated valve from Utah (USA) shows a specimen with large virgae, quite clearly separating the striae (Johansen & Rushforth 1981), which is morphologically sufficient to exclude conspecificity. The same can be said from a valve illustrated from Sierra Leone (Carter & Denny 1982), Sudan (Abdel Karim 1975), Ontario (Canada) (Sreenivasa & Duthie 1973) and Nepal (Saxena & Venkateswarlu 1968), all localities outside Europe. But even within Europe, several illustrated records show morphological differences. Carter & Baily-Watts (1981: pl. 9: fig. 30) shows a valve observed on the Shetland Islands with slightly concave margins and elongated, rostrate apices. Also Foged (1977: 63) reported the presence of a very long, elongated valve with a valve length almost 10 times the valve width.

It is likely that most of this confusion arose due to a combination of 1) a lack of a good knowledge of the morphology of the type material of *F. virescens*, and 2) force-fitting local populations into names found in easy-to-get floras to identify these populations specimens. A good example can be found in Krammer & Lange-Bertalot (1991, pl. 126: figs 1–10) that illustrate broad morphological variation in *F. virescens*, but none of the valves shown were observed from the type material. Patrick & Reimer (1966, pl. 3: figs 7–9) illustrated three long valves with a broad sternum and almost strictly parallel margins.

When Williams & Round (1988a: 280) described the new genus *Neofragilaria* D.M.Williams & Round, *nom. illeg.* to accommodate *Fragilaria virescens*, they typified the species using slide **BM** 81303. This slide was from Tunbridge Wells, Ralfs material from Jenner, and specimens from herbarium sheet BM 001222540, but they did not document the species with LM pictures from the original material and the SEM pictures used to highlight the morphological features lacked a specific geographical designation. As the genus name *Neofragilaria* was introduced validly by Desikachary & al. (1988) a few weeks before the Williams & Round (1988a) publication, a replacement name was proposed: *Fragilariforma* D.M.Williams & Round (1988b: 265). Williams & Round (1988a) had, however, listed both syntypes Ralfs (1843) had mentioned.

In 2001, Williams reported on the post-auxospore valves of *F. virescens* listing all slides containing the species kept in **BM**, including slide **BM** 81303, collected from Cold Bath, Tunbridge Wells, UK (Williams 2001: 107). The illustrated LM observation shows a valve with a length of 75 µm, although a scale bar is lacking.

In the William Smith collection, kept in the Van Heurck collection in **BR** (Meise Botanic Garden, Belgium, Hoover 1976), several original samples collected by Mr. Jenner from Tunbridge Wells were found, although given the dates on the samples (December 1842 and March 1843), they

cannot be considered to belong to one gathering. Volume LXXV of The Annals and Magazine of Natural History in which Ralfs (1843) published his description of *F. virescens* was published in August of that year and as Ralfs only mentioned that the sample was collected by Mr Jenner, without specifying an exact date, we cannot be certain which of the samples he used. In this contribution, we document the morphological variation of *F. virescens* in the sample collected in December 1842, using both light and scanning electron microscopy observations, providing a good written description of the species. This information can be useful to avoid force-fitting vaguely similar populations worldwide into *F. virescens*, allowing that way for a better understanding of the ecology and worldwide distribution of *F. virescens*. The Tunbridge Wells material kept in **BR** is added as isolectotype.

Fragilariforma virescens (Ralfs) D.M.Williams & Round (1988b: 265) Figs 1–43
Basionym: *Fragilaria virescens* Ralfs (1843: 110: pl. II: fig. 6)
Syntype localities: "In freshwater pools. Cold Bath, Tunbridge Wells [Kent], Mr. Jenner. Madron and Chyanhâl [sic] Moor near Penzance [Cornwall]." (Ralfs 1843: 110),
Lectotype: slide **BM** 81303 designated by Williams & Round (1988a: 280). Although not stated at the time, this slide was made from Jenner material from Cold Bath, Tunbridge Wells (herbarium sheet BM 001222540, coll. date December 1842) as mentioned by Williams (2001, p. 105)
Isolectotype: **BR**-4832 (Meise Botanic Garden, Belgium), slide made from original material also from Cold Bath, Tunbridge Wells, Dec. 1842, leg. Mr. Jenner, **BR**! We therefore conclude that this is the same material as was used by Williams & Round (1988a) to designate the lectotype.
Registration (of isolectotype): http://phycobank.org/104405

Description: Frustules rectangular in girdle view, linked to each other forming long, ribbon-like chains using spatulate linking spines with a narrow base and broad apex. Chains with up to more than 100 cells observed. Cingulum composed of 5 open, ligulate girdle bands perforated by a single row of small, rounded poroids (Figs 34–37). Valvocopula with a dentated pars interior and a distinct septum at the closed pole (Figs 35, 36). Valves linear-lanceolate in larger specimens to elliptic-lanceolate in smallest specimens. Valve margins straight to clearly convex. Apices shortly protracted in smaller valves to elongated rostrate in longest valves. Capitate apices so far not observed. Valve dimensions (n=50): valve length 16–38 µm, valve width 6–8 µm, frustule width ca 10 µm. Mantle broad with broad advalvar part bearing long striae and narrow abvalvar hyaline edge. Mantle edge with thick mantle plaques, irregularly scattered along the entire valve length (Fig. 34). Valve face flat (Figs 38, 39). Continuous series of marginal linking spines, regularly placed on each virga at the valve face edge (Fig. 39). At one apex, row of spines interrupted (Fig. 41) whereas on the other apex, spines present on the apical pore field (Fig. 40). Occasionally valves with acute separation spines (Fig. 38). Sternum variable, but usually very narrow. Central area absent. Striae uniseriate, composed of small, rounded areolae, 16–17 in 10 µm. Areolae not discernible in LM. Striae either continuous over the sternum or alternating, often irregularly spaced. Large apical pore fields present on both apices, composed of an irregular pattern of short series of small, rounded poroids, continuing on one apex from the valve face onto the mantle, whereas on the other apex, pore field only present on the valve face. Pore fields not equal in size and shape, giving the valves a heteropolar outlook, only discernible in SEM. One rimoportula per valve, slit-like, present near the smaller apical pore field with circumpolar spines, replacing some of the areolae in the last stria (Figs 38–41). Internally, sternum well visible. Rimoportula labiate, perpendicular to the apical axis (Figs 41, 43).

The valve dimensions differ from the reported length and width ranges in Williams & Round (1988a). Despite a scan of the isolectotype slide for the longest and shortest specimens, no valves longer than 38 µm and shorter than 15 µm have been observed among the hundreds of valves in the sample. Williams & Round (1988a) reported a valve length range of 12–120 µm with a valve width of 5–10 µm. A similar width range was also not observed. The reported ranges in Williams &

Round (1988) included auxospore sizes to capture the measurements from the entire cell cycle (Williams 2001). This underlines not only the limitations of type material; frequently, observed specimens in such material are captured at one point in the life cycle, but also may lead to the description of a lot of varieties as part of the life cycle, such as for instance *Fragilariforma virescens* var. *elliptica* (Hustedt) Aboal. A better analysis of *F. virescens* populations in Europe will be necessary to verify whether these longer valves also belong to *F. virescens sensu stricto*.

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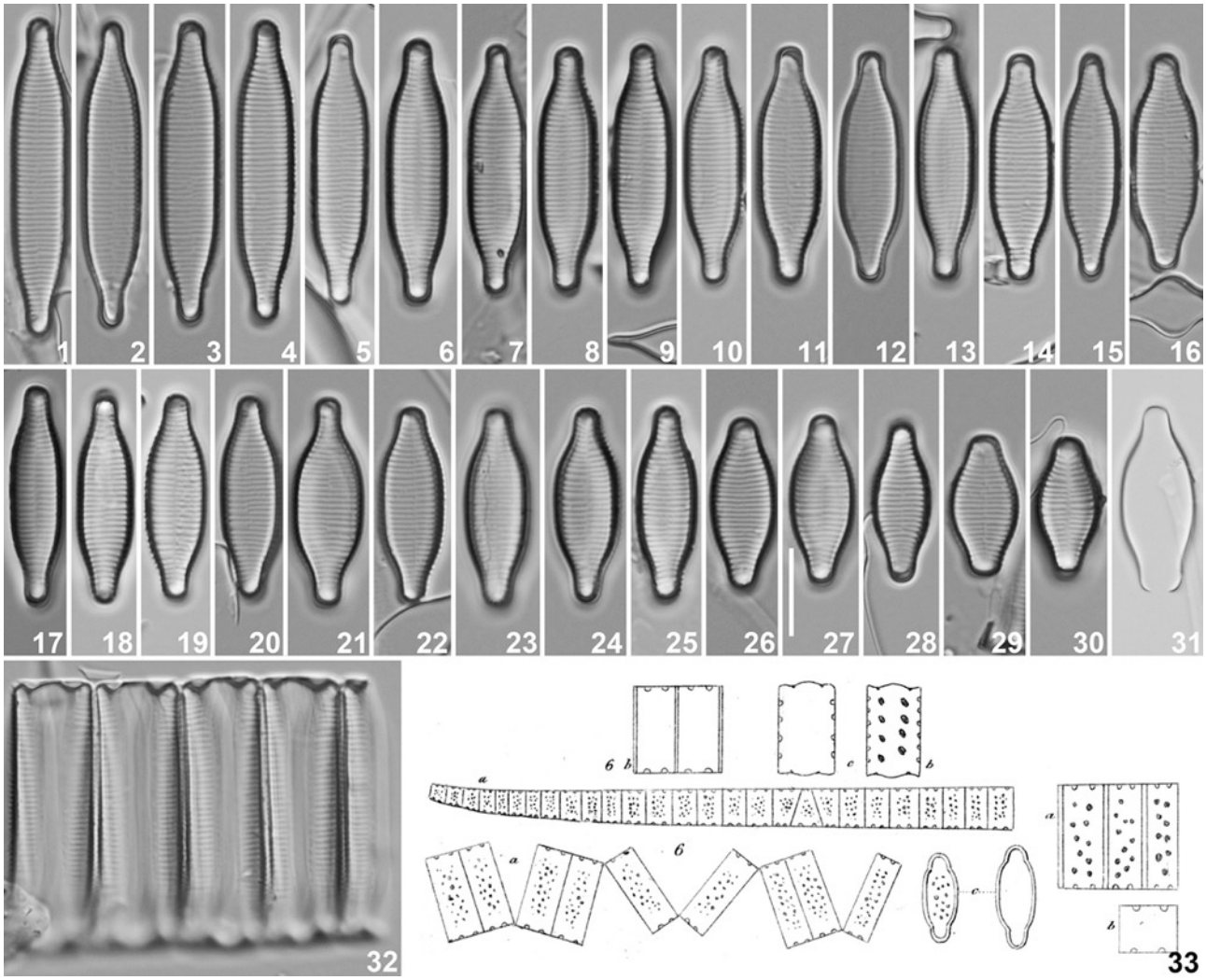
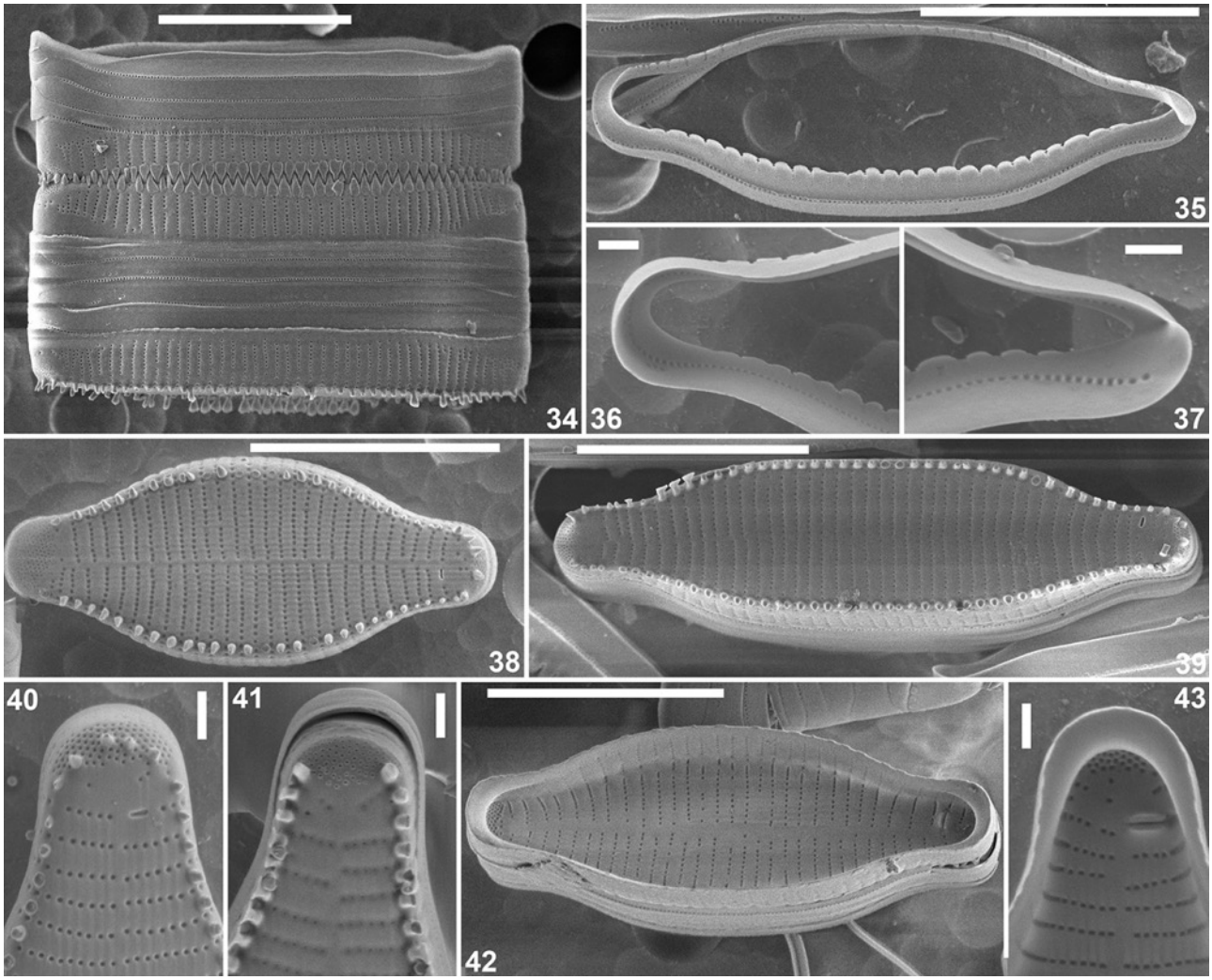


Figs 1–33. *Fragilariforma virescens* (Ralfs) D.M.Williams & Round. LM pictures taken from the isolectotype material (**BR**-4832, Tunbridge Wells, coll. date xii.1842, leg. Mr. Jenner). **Figs 1-30.** LM views of a size diminution series. **Fig. 31.** LM view of an open girdle band. **Fig. 32.** LM view of 4 frustules in girdle view connected in a ribbon-like chain. **Fig. 33.** Original drawing of *Fragilaria virescens* from Ralfs (1843, pl. 1: fig. 6). Scale bar = 10 µm.



Figs 34–43. *Fragilariforma virescens* (Ralfs) D.M.Williams & Round. SEM pictures taken from the isolectotype material (**BR**-4832, Tunbridge Wells, coll. date xii.1842, leg. Mr. Jenner). **Fig. 34.** Frustules in girdle view, connected to each other with linking spines. **Fig. 35.** SEM view of a girdle band (valvocopula?) with dentated pars interior and perforated pars exterior. **Fig. 36.** Closed part of a copula with distinct septum and short second row of small poroids. **Fig. 37.** Open part of a copula. **Fig. 38.** SEM external view of an entire valve with possible acute separation spines. **Fig. 39.** SEM external view of an entire valve with (broken and eroded) linking spines. **Fig. 40.** SEM external detail of a valve apex with slit-like rimoportula, most likely of a valve with acute, separating spines. Note the circumpolar, acute spines. **Fig. 41.** SEM external detail of a valve apex with larger apical pore field, lacking rimoportula. Note the interruption in the linking spines at the tip. **Fig. 42.** SEM internal view of entire valve with the position of the rimoportula. Note the broad sternum. **Fig. 43.** SEM internal detail of the labiate rimoportula and the apical pore field. Scale bar = 10 µm (Figs 34, 35, 38, 39 & 42), = 1 µm (Figs 35, 36, 40, 41 & 43).