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Burliganiella gen. nov. (Bacillariophyta, Eunotiales): another case of raphe reduction based on the type material of *Fragilaria siolii* Hustedt

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Abstract – A new monotypic diatoms genus from Brazil is described based on gatherings and descriptions from the Amazon made by Hustedt in the early 50's. *Burliganiella* gen. nov. is described using light and scanning electron microscope and has as generitype *Burliganiella siolii* (Hustedt) comb. nov., originally described within *Fragilaria* Lyngb. The main diagnosed features include valves slightly asymmetrical to the apical axis with a sternum located near the ventral margin (*Eunotia*-like) while internally having slits that are not associated with a helictoglossa and no rimoportula (which is completely absent). While some characters link the species to the 'araphid' genus *Fragilariforma* D.M. Williams & Round, *Burliganiella* has more shared characters with the Eunotiales. For instance, the lack of a raphe in other species of eunotioid diatoms, suggests multiple lineages within the Eunotiales have reduced or lost raphe systems, such as in the case of *Burliganiella*.

Amazon / *Eunotia* / *Eunotiaceae* / *Fragilariforma* / raphe system / type material

INTRODUCTION

Among the *ca* 60 species described by Hustedt from the Amazon basin (e.g., Hustedt 1952a,b, 1955a,b, 1956 and 1965), several interesting species of the genus *Fragilaria* Lyngb. (*sensu lato*) were proposed. One of them, *Fragilaria braunii* Hustedt (1952b) was recently reconsidered (Costa *et al.* 2017), and based on SEM observations, including its possession of a central sternum, shown to have a small raphe system on the junction of the valve face and mantle. It was recently transferred to the genus *Peronia* Bréb. A second interesting *Fragilaria* species described in that same publication (1952b) was *F. siolii* Hustedt. This diatom, unlike most assigned to *Fragilaria* Lyngb., differs by being slightly asymmetrical to the apical axis. Hustedt's original description noted the presence of small spines along the margin, and also noted the sternum was not central, but rather placed towards the ventral margin. This position of the sternum suggested to Hustedt that the species might be phylogenetically close to the Eunotiales ('Rhaphidioideen'):

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Besonders charakteristisch für F. siolii *ist die transapikale Verschiebung der Längsrippe gegen den einen Schalenrand, und es ist möglich, dass darin phylogenetische Beziehungen zu den Rhaphidioideen angedeutet werden (Hustedt 1952b).*

A single figure (#43, here reproduced as Fig. 1) was offered in the original description, which also showed what would be interpreted as a rimoportula at one end of the valve. Simonsen (1987, plate 581, figures 1-5) illustrated two specimens from the type slide. One of the Hustedt specimens was pictured again by Metzeltin & Lange-Bertalot (2007), but otherwise this taxon has not since been recorded in modern treatments of the freshwater diatom flora of the Neotropics (e.g. Hohn, 1966; Uherkovich, 1976, 1981; Uherkovich & Rai, 1979; Uherkovich & Franken 1980; Fukushima & Xavier, 1988; De Oliveira & Steinitz-Kannan, 1992; Silva-Benavides, 1996; Souza-Mosimann *et al.,* 1997; Metzeltin & Lange-Bertalot, 1998, 2007; Michels, 1998a,b; Sala *et al.,* 1999, 2002, 2015; Rumrich *et al.,* 2000; Wydrzycka & Lange-Bertalot, 2001; Michels-Estrada, 2003; Díaz-Castro *et al.,* 2004; Raupp *et al.,* 2009; Montoya-Moreno *et al.,* 2013).

Through the courtesy of Dr. Friedel Hinz, one of us (CEW) obtained the type material of *F. siolii* from the Hustedt Collection in Bremerhaven (BRM). The current report attempts to use light (LM) and scanning electron microscopy (SEM) to detail the valve ultrastructure of *F. siolii* and to ascertain its systematic position.

MATERIALS AND METHODS

The materials AM1018 and AM1027 from BRM containing *Fragilaria siolii* have been examined using LM and SEM (Table 1).

Small portions of the 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 allowed to rest for 2 h followed by three repetitions of rinsing and decantation using deionized water. The material was then filtered and rinsed with additional deionized water through a 3-µm Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminum stubs and coated with platinum using a BAL-TEC MED 020 Modular High Vacuum Coating System for 30 s at 100 mA. Cleaned material was examined using a Hitachi SU-70 (Hitachi High-Technologies) ultra-high-resolution analytical field emission (FE) scanning electron microscope operated at 5 kV and 10 mm working distance. LM images were taken with a Leica DMRX equipped with a DFC500 digital camera.

Table 1. List of samples from Hustedt collection in Bremerhaven (BRM) containing *Fragilaria siolii*

Accession number	*Slide*	*Finder*	*Locality*
AM1018	318-02	527.5-6	Brazil, leg. Braun 58, Lago Jurucuí, 'Plankton', 13.11.1947
AM1027	318-23	312.1-4	Brazil, leg. Braun 252, Lago Jurucuí, 'an Pflanzen', 25.10.1947
AM1196	322-20b	316.5-6	Brazil, leg. Braun 211a, Igarapé Grande, 'Uferschlick', 27.11.1947

Table 2. List of samples from Instituto de Botânica de São Paulo (SP), Brazil, containing *Fragilaria siolii*

Accession number (SP)	*Sample*	*Sampling Site*	*Type*	*W*	*S*	*Date*
400007	RN53	Rio Negro	Phytoplankton	62.804406	1.047389	02/03/2005
400290	RN75	Adairá creek, Rio Negro	Periphyton	63.312616	0.417885	03/03/2005
400429	RN214	Rio Negro	Phytoplankton	67.023290	0.171812	08/03/2005
400474	RN259	Rio Negro	Phytoplankton	63.704072	0.372952	11/03/2005
400476	RN261	Rio Negro	Phytoplankton	63.448277	0.575747	11/03/2005
400490	RN275	Rio Negro	Phytoplankton	62.928512	0.967526	11/03/2005
400512	RN299	Rio Negro	Phytoplankton	61.510067	1.664768	12/03/2005

Permanent slides as well as cleaned samples deposited at the 'Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo, Secretaria do Meio Ambiente do Estado de São Paulo', Brazil (SP), collected at the Rio Negro basin (Wetzel *et al.,* 2012a), were re-assessed to verify the distribution of the taxon (Table 2). The following accession numbers deposited at the herbarium SP (samples from the Rio Negro basin, Amazonas, Brazil) were confirmed to contain *Fragilaria siolii*.

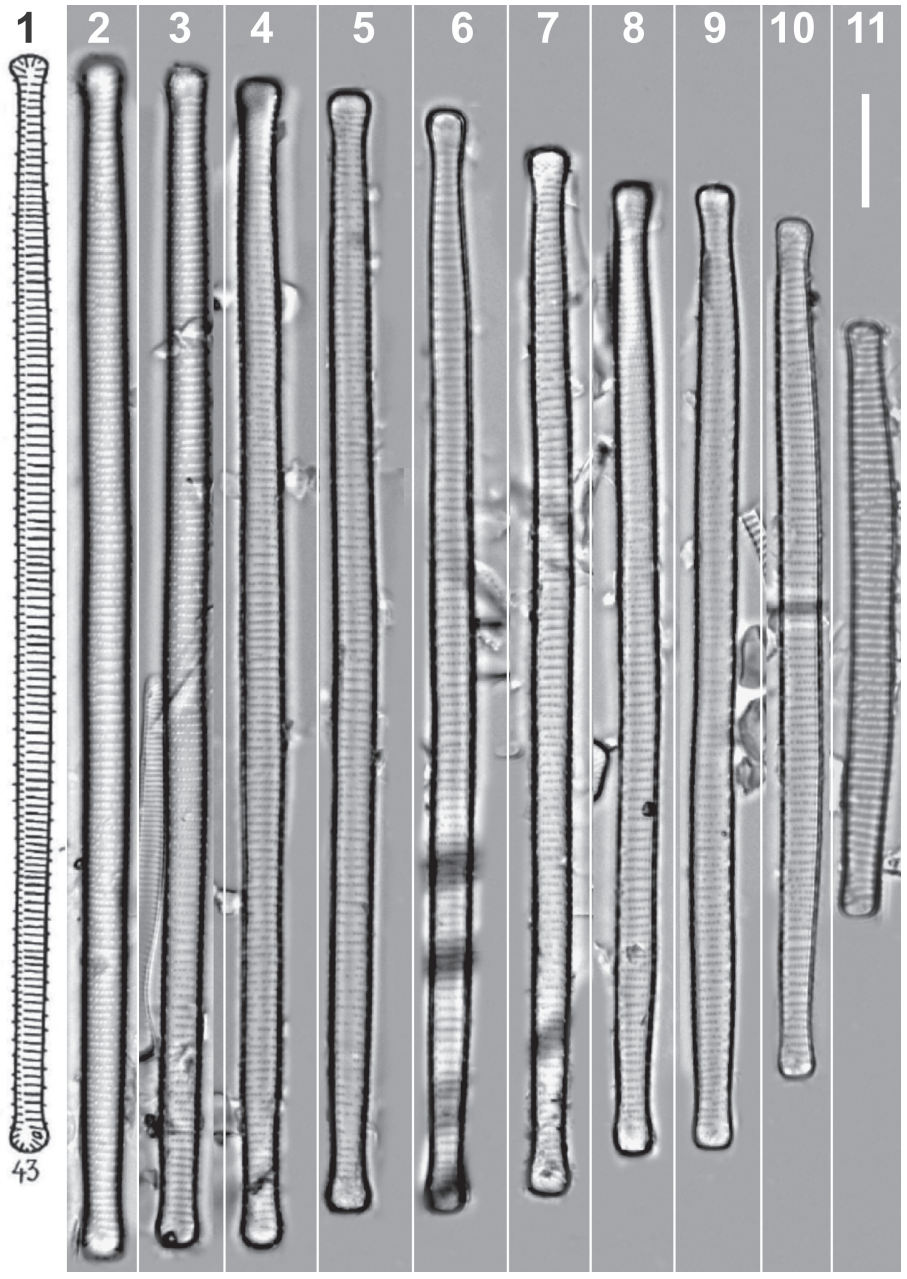
Additionally, SEM observations were made on samples containing specimens of the genera *Bicudoa* C.E. Wetzel, Lange-Bertalot & Ector (SP-400480), *Eunotia* Ehrenberg (SP-469534, Hedberg reservoir; São Paulo, Brazil) and *Fragilariforma* D.M. Williams & Round (Sample AM1027, Brazil, Lago Jurucuí) in order to illustrate morphological similarities and possible relationships among them.

RESULTS

Fragilaria siolii Hustedt **Figs 1-30**

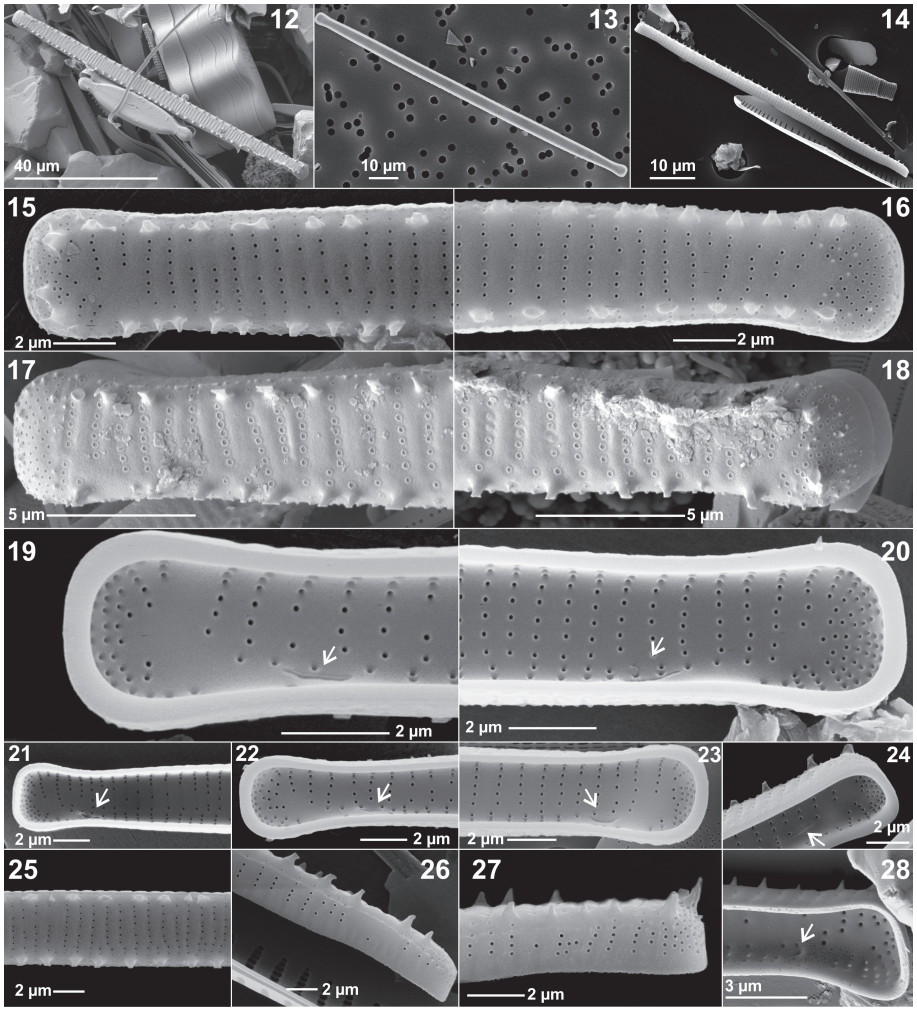
Valves linear-lanceolate, slightly asymmetrical to the apical axis, with poles protracted and apices subcapitate (Figs 1-11). Length 52-120 µm (Hustedt gives 70-145 µm), breadth 3.6-4.5 µm. Striae parallel, distinctly punctate, sometimes with shortened striae intercalated along the ventral margin, 10-14/10 µm. Striae interrupted by a sternum located near the ventral margin. Irregular marginal spines surround the valve (except at the apices; and less dense than the striae).

In SEM, the elongate, linear shape of the valves is evident (Figs 12-14). Small spines occur along the mantle (Figs 14-18, 25-28). Areolae are round in shape (Figs 15-16) with a thin rim around them (Figs 17-18, 30). Externally, the areolae appear to be occluded, this is especially apparent near the apices (Fig. 30). Areolae occur in shallow troughs running parallel (rather irregular) along most of the valve face (e.g. Figs 15-18, 25). At the apices, striae are radiate around the valve face and extend onto the mantle (Figs 15-18, 30); apical pore field is absent; smaller poroids are located at the apices (Figs 17-18). Internally, areolae are small, round and not occluded (Figs 17-22).

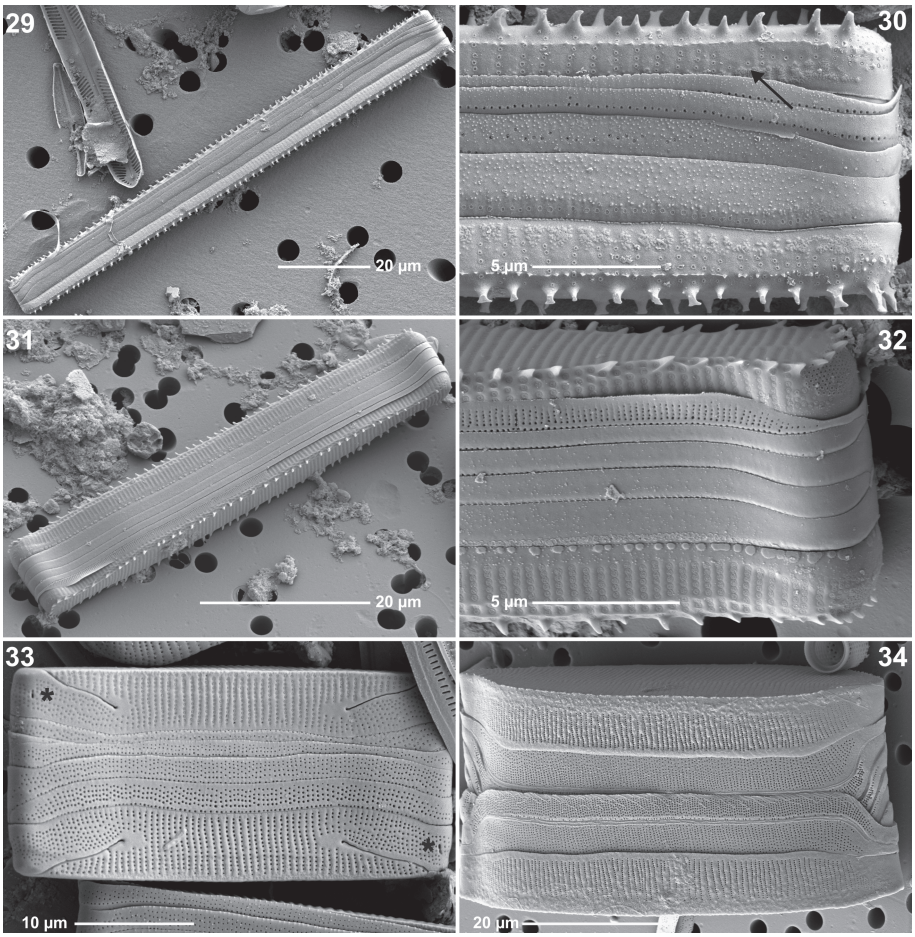


Figs 1-11. *Burliganiella siolii* (Hustedt) C.E. Wetzel & Kociolek *comb. nov.* **1.** Reproduction of *Fragilaria siolii* Hustedt (1952b, p. 380, fig. 43). **2-11.** LM images from sample AM1018 (Hustedt Collection, BRM) from Brazil (Lago Jurucuí). Note the eccentric sternum and slightly dorsiventrality of the valves.

Internal views of the valve apices show a small slit located on the valve face or mantle (Figs 19-24, 28). This slit is not associated with a helictoglossa, and no rimoportula have been found. There are no external expressions of this slit (Figs 15-16, 24-25); except for a hypothetical lack of areolae in area corresponding to the internal raphe slit (Fig. 30).



Figs 12-28. *Burliganiella siolii* (Hustedt) C.E. Wetzel & Kociolek *comb. nov.* SEM images. Note the extreme internal raphe reduction (white arrows) and the absence of external aperture. Internal raphe slits occur on the same 'ventral' part of the valve and are always present at both apices. Note as well the rimmed Eunotia-like areolae on figs 17-18. **13-16, 19-27.** Sample AM1018, Brazil (Lago Jurucuí). **12, 17-18, 28**. AM1027, Brazil (Lago Jurucuí).



Figs 29-34. Girdle bands and connective view of discussed genera. **29-30.** *Burliganiella siolii* (Sample AM1027, Brazil, Lago Jurucuí). Note the lack of areolae corresponding to the internal reduced raphe (black arrow); valve and cingulum of *Burliganiella siolii* showing marginal spines and open connective bands with one row of areolae. Striae uniseriate composed by rimmed areolae. **31-32.** *Fragilariforma amazonica* (Sample AM1027, Brazil, Lago Jurucuí); Connective view (Fig. 31) showing mantle margin with siliceous plaques (Fig. 32), spines present on valve face/mantle border; open girdle bands. **33.** *Eunotia yberai* Frenguelli (Sample SP-469534, Hedberg reservoir, São Paulo, Brazil) showing a common reduced eunotioid raphe. Black asterisk showing external raphe aperture. **34.** *Bicudoa amazonica* C.E. Wetzel, Lange-Bertalot & Ector (Sample SP-400480, Rio Negro basin, Amazonas, Brazil. Whole frustule in valvar view showing the complete absence of raphe aperture system; cingulum in girdle view showing perforated *Eunotia*-like open copulae with multiporoid striae and numerous open connective bands.

DISCUSSION

Comparison of the valve morphology of this species with others in the genus *Fragilaria* Lyngb. (e.g. Round *et al.,* 1990) suggest significant differences. These morphological differences include an interruption of the striae that is located near the ventral margin (instead of the center of the valve) and a slight asymmetry about the apical axis. These features all differ from *Fragilaria* (Williams & Round, 1997). The interruption of the striae positioned near the ventral margin in these valves with asymmetry about the apical axis is typical in most members of the Eunotiaceae. Burliga *et al.* (2013) suggested that with the exception of *Eunotioforma* Burliga *et al.*, other members of the family could be diagnosed by the morphological synapomorphy of a ventrally-placed interruption of the striae. If that is correct, it would suggest that *Fragilaria siolii* is a member of the Eunotiaceae. Based on symmetry features it might be assigned to the genus *Eunotia*, but the lack of rimoportulae and lack of a complete raphe would argue against that placement. Even though there are some *Eunotia* species without a rimoportula (e.g. *Eunotia emedii* C.E. Wetzel *et al.,* 2011), the raphe system has been reduced or lost only in few eunotioid diatoms. Kociolek *et al.* (1997) and Kociolek & Rhode (1998) examined species assigned to the genus *Asterionella* from Madagascar, and found they had small raphe slits, evident both internally and externally. Rimoportulae in those species were sometimes present and sometimes absent. The symmetry of the valves, asymmetrical to the apical and transapical axes, suggested a placement within the genus *Actinella*, within the Eunotiaceae. The raphe reduction was suggested to be a secondary loss as the diatoms adapted to a planktonic lifestyle. A total loss of the raphe has been suggested to have happened within *Eunotia* several times (Hustedt, 1952a). A new genus and species, *Bicudoa amazonica* Wetzel *et al.*, (Fig. 34) was described from planktonic and periphytic samples collected in northwestern Brazil (Wetzel *et al.,* 2012). Contrary to species recognized as *Eunotia* (Fig. 33), the species from this monotypic genus has no rimportulae, and only a short slit without any external expression and helictoglossae are wanting (Table 3). Even though these diatoms in *Actinella* and *Eunotia* have a small raphe, or lack a raphe altogether, the features of symmetry and interruption of the striae being displaced towards the ventral margin, suggest they cannot be assigned to the "araphid" diatoms, a group shown to be non-monophyletic. Since classification systems should reflect the evolutionary relationships (i.e. recognition of monophyletic groups in classification schemes), we continue to recognize these groups within the Eunotiaceae.

Based on these features, we propose the transfer of *Fragilaria siolii* to a new genus:

Burliganiella C.E.Wetzel & Kociolek**, *gen. nov*.**

Description: *Frustules rectangular in girdle view. Solitary cells; colonies not observed. Valves isopolar, moderately dorsiventral in valvar view; apices are drawn out and capitate. Axial area or sternum extremely narrow and displaced toward ventral side of valve. Transapical striae parallel often irregularly spaced, becoming strongly radiate toward the ends. Plastids unknown. Striae uniseriate situated both on valve face and mantle, interrupted by lateral area developing on junction between valve face and mantle. Areolae small and circular. Sternum narrow, sometimes displaced toward ventral side of valve. Condensed poroids at the apices, absent. Cingulum composed of 4-5 open copulae, perforated by pervalvar multiporoid striae*

Table 3. Comparison of morphological characteristics of the genera *Burliganiella* gen. nov., *Bicudoa* (incertae sedis), *Eunotia* (Eunotiophycidae) and *Fragilariforma* (Fragilariophycidae)

	Burliganiella *C.E.Wetzel & Kociolek, gen. nov.*	Bicudoa *C.E. Wetzel, Lange-Bert. & Ector*	Eunotia *Ehrenberg*	Fragilariforma *D.M. Williams & Round*
Typus generis	*Burliganiella siolii* (Hustedt) C.E Wetzel & Kociolek	*Bicudoa amazonica* C.E. Wetzel, Lange-Bertalot & Ector	*Eunotia arcus* Ehrenberg	*Fragilariforma virescens* (Ralfs) D.M. Williams & Round
Raphe system	Absent; vestigial raphe slits rarely present in internal view; when present, on both apices; never opened externally; helictoglossae absent	Absent; vestigial raphe slits rarely present in internal view; when present, on both apices; never opened externally; helictoglossae absent	Present; usually short and located at the poles on the ventral mantle, terminal fissures on the valve face	Absent
Rimoportulae	Absent	Absent	Present, mostly 1 per valve, at opposite poles; sometimes absent, sometimes 2 or 3	Present, usually near the apex; its internal slit aligned with the striae
Apical pore field	Absent	Absent	Absent	Usually well-developed (e.g. *Fragilariforma virescens*) or absent (e.g. several *Fragilariforma* spp. from Amazon basin)
Valve outline	Symmetric about median transapical plane; slightly asymmetrical about the apical axis	Symmetric about median transapical plane; slightly asymmetrical about the apical axis	Symmetric about median transapical plane; strongly dorsiventral	Symmetric, lanceolate-linear-elliptical, and sometimes centrally constricted
Striae/Areolae	Striae uniseriate, containing small round poroids, hymenes or other occlusions not observed	Striae uniseriate, containing small round poroids, hymenes or other occlusions not observed	Striae uniseriate, containing round poroids that can completely lack hymenes or other occlusions, but can also be covered externally by a flat velum	Striae uniseriate, alternate, occasionally extending across the valve face; areolae with simple volae
Marginal spines	Irregularly present	Absent	Usually absent, but can be present in some species	Present; spines occur marginally between the striae
Copulae/Cingulum	4-5 open bands; one to two rows of areola	6-10 open bands; several rows of areolae	6-10 open bands; several rows of areolae	Copulae 4-6, all open alternating and ligulate, with a single row of areolae on the advalvar side; in some species with few additional areolae
Colony formation	Cells solitary; life-form and habitat not observed	Band-like (no more than 4 joined cells observed)	Cells solitary or joined into band-like colonies, occasionally attached by mucilage pads	Cells forming linear or zig-zag colonies

arranged in discernible rows. Helictoglossae and rimoportulae lacking. Remnant raphe system visible internally.
Etymology: The genus is dedicated to our friend and colleague Dr. Ana Luiza Burliga.

Burliganiella siolii (Hustedt) C.E.Wetzel & Kociolek, ***gen. et comb. nov.***

Basionym: *Fragilaria siolii* Hustedt, 1952, Neue und wenig bekannte Diatomeen. IV. *Botanisk notiser* 1952: 380.
Type: Slide n° 318-23 at Hustedt Collection (Bremerhaven, BRM), Sample AM1027. leg. Braun 252 ('an Pflanzen'); 25.10.1947.
Type locality: Lago Jurucuí, Pará (PA), Brazil.
Remarks: Species that are also present in the Rio Negro material are assigned to the genus *Fragilariforma* by virtue of their narrow central sternum and robust spines (Table 3). Included among these are *Fragilariforma amazonica* C.E. Wetzel, P.D. Almeida et Ector and *Fragilariforma brasiliensis* (Grunow) P.D. Almeida, C.E. Wetzel, E. Morales & D.C Bicudo described from Brazil (Almeida *et al.* 2017), and *Fragilariforma javanica* (Hustedt) C.E. Wetzel *in* Wetzel *et al.* 2013, described from SE Asia by Hustedt (1937). There are some similarities between these species and *Burliganiella siolii*, including the coverings on the areolae, open connective bands and irregular spines (see Figs 29-30 and 31-32). These planktonic 'araphid' diatoms may have evolved from one or more eunotioid ancestors, a process similar to the "*Asterionella*" species that are now recognized as *Actinella* (Kociolek & Rhode, 1998). Their relationship to *Fragilariforma*, as typified with *Fragilariforma virescens* (Ralfs) D.M.Williams & Round may be circumspect, however, since *Fragilariaforma virescens* was shown by Theriot *et al.* (2010) to occupy a phylogenetic position outside the raphid diatoms. These species are worthy of future research to ascertain their phylogenetic position. Lack of a raphe in *Bicudoa* species (Fig. 34), and in other species of eunotioid diatoms, suggests multiple lineages within the Eunotiales have reduced or lost raphe systems (e.g. Kociolek & Rhode 1998; Wetzel *et al.* 2010, Wetzel *et al.* 2012b, Liu *et al.* 2018).

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