ULTRASTRUCTURAL AND MOLECULAR CHARACTERIZATION OF DIVERSITY AMONG SMALL ARAPHID DIATOMS ALL LACKING RIMOPORTULAE. I. FIVE NEW GENERA, EIGHT NEW SPECIES

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Pennate diatoms are important contributors to primary production in freshwater and marine habitats. But the extent of their diversity, ecology, and evolution is still largely unknown. This is particularly evident among the clades of pennate diatoms without raphe slits, whose diversity is likely underestimated due to their small size and features that can be difficult to discern under light microscopy. In this study, we described five new araphid genera with eight new species based on morphological observations (light and electron microscopy) and molecular data (nuclear-encoded small subunit ribosomal RNA and chloroplast-encoded rbcL and psbC): Serratifera varisterna, Hendeyella rhombica, H. dimeregrammopsis, H. lineata, Psammoaetia lanceolata, Castoridens striata, C. hyalina, and Cratericulifera shandongensis. We also transferred Dimeregramma dubium to Hendeyella dubia. Phylogenetic analysis of the molecular data revealed that all the newly established taxa fell into a monophyletic group, with Fragilariforma virescens located at the base. The group was composed by two subclades: one comprising Castoridens, Cratericulifera, and Plagiostriata, and the larger including also the rest of the new genera plus some of the smallest known diatoms, such as Nanoaustatomactaceae, Opephora, Pseudostaurosira, Staurosirella, and Staurosira with a high level of support. This study enhances the general knowledge on the phylogeny and biodiversity of a group of small araphid diatoms that have been generally poorly described both by electron microscopy and DNA sequence data.

Key index words: Araphid diatoms; biodiversity; Fragilariaceae; phylogeny; psbC; rbcL; small-celled diatoms; SSU rRNA

Diatoms are remarkably diverse and adaptable organisms, that are able to survive in a wide variety of environments, resulting in perhaps as many as 200,000 extant species (Mann and Droop 1996, Mann and Vanormelingen 2013). Diatoms are commonly classified into three classes, distinguished by their valve symmetry and the presence/absence of raphe slits (Round et al. 1990). Round et al. (1990) considered araphid diatoms (Class Fragilariophyceae, Subclass Fragilariophycidae) to be the most difficult group to classify, due to the indistinctiveness of diagnostic features as viewed under light microscopy (LM). Using SEM, Round et al. (1990) began to improve the taxonomy of the araphids and to uncover unrecognized generic diversity in this group. Although the phylogenetic relationships among araphid taxa varied from study to study,
there has been increasing evidence that the araphid diatoms are a paraphyletic group with two subclades (Medlin et al. 1993, 1996, Theriot et al. 2011, Theriot et al. 2015). Medlin (2016) proposed splitting Fragilariophyceae into two subclades: Urneidophycidae and Fragilariophycidae. This division was based on the presence of two types of bands (properizonium and perizonium) around the auxospores of the Urneidophycidae (though only in a single taxon to date—Pseudostratiellata oceanica S. Sato, Mann & Medlin), whereas only a single type of band (perizonium) is found around the auxospores of the Fragilariophycidae (and the raphid pennates). Those two subclasses each appeared monophyletic in the molecular data as well. The Urneidophycidae includes Rhaphoneis, Delphinea, Asterolamputus, Asterionellopsis, and plagiogrammoid taxa (the latter previously transferred from Mediophyceae – Sato et al. 2008a).

Among the families remaining in Medlin’s (2016) proposed Fragilariophyceae is Fragilariaceae Greville, which was originally described as Tribe Fragilariae under the Division Diatomaceae (Greville 1833). The original description of this family seems to be quite simple: “Filaments plane, extremely fragile, composed of rectilinear frustula; (frustula sometimes apparently radiating from a center and not presenting the appearance of a filament)” (Greville 1833, p. 263). More recent DNA sequence data suggest that this family is a paraphyletic group, in which the members are spread all over the araphid clade (Medlin et al. 1993, 1996, Medlin and Kaczmarska 2004, Sato et al. 2008b, Theriot et al. 2010, Ashworth et al. 2012, Li et al. 2015). Williams and Kociolek (2011) encouraged the rejection of the paraphyletic group and Kociolek and Williams (2015) called for revisionary works on all levels of diatom groups based on monophyly.

Recently published papers about diatom diversity and phylogeny would seem to suggest that more biodiversity of Fragilariophyceae remains to be discovered. As numerous araphid taxa have been described or erected as more detailed work has begun on marine diatom flora and cultured cells isolated from coral reefs, coral sands, farmer-fish turfs, or littoral-environment (Sato et al. 2008a,b, 2009, Lobban et al. 2011, 2012, 2015, Ashworth et al. 2012, Lobban and Navarro 2013, Lobban and Ashworth 2014a,b, Li et al. 2015). Additional diversity is likely hidden within broadly defined araphid genera such as Synedra Ehrenberg and Fragilaria Lyngbye. Both were established in the early times in diatomological research and for a long time many freshwater and brackish-water taxa have been included largely due to their needle like or linear-lanceolate shape or their ribbon-like colonies. A change in an approach to these two genera is observed in 1980s and 1990s with a routine application of EM methods. In the first reviews of those two genera was published by Williams and Round (1986, 1987). The authors established a number of araphid genera be split from either Fragilaria or Synedra. These were Catacombus, Ctenophora, Hyalosynedra, Neosynedra, Pseudostaurosira, Punctastriata, Staurosirella, Tabularia (Williams and Round 1986, Williams 1987). This process was continued with the publication of Round et al. (1990) with some more new araphid genera and later in early 21st Century by Morales and coworkers (e.g. Morales 2001, Morales et al. 2015). Most of the genera proposed during the last three decades have been widely accepted with one or two believed to be superfluous, e.g., Martyana Round in Round et al. (1990).

However, the above critical analysis and revisions have only made minor progress in clarifying phylogeny of araphid pennate diatoms. The use of DNA sequence data with cultured araphid taxa has resulted in improvement of understanding the taxonomy of poorly described genera and their evolution and phylogeny (Sato et al. 2008a,b). Sato et al. (2008b) described three relatively small species (<15 μm) of Psmamoneis, all with lanceolate-elliptical or elliptical outlines and overlapped size dimension under LM. Even in SEM, they look quite similar with well-developed apical pore fields, apically elongated areolae on the valve and mantle face, as well as open and plain girdle bands. In general, no significant difference in gross morphology among those three species was observed, though finer analysis of the size and spacing of striae suggested some differentiation. The DNA sequence data (SSU and LSU rDNA) supported the species-level differentiation in Psmamoneis, showing considerable sequence differences among the three species. It was suggested that there is likely much more diversity in these lineages than suggested by similarity in gross frustule morphology (Sato et al. 2008a,b, Li et al. 2015).

One of the main problems underlying the taxonomy of Fragilariaeae is the very broad nature of their description. As mentioned previously, Greville only used filamentous or radiating colony formation and rectilinear frustules to define this family. Even recently, Cox (2015) describes the group using the broadly defined characters of “cell elongate, often forming filaments held together by marginal spines. Apical pore fields and rimoportulae usually present.” Progress has been made in creating more sharply defined families from the Fragilariaceae; Lobban and Ashworth (2014a,b) separated the Grammatophoraceae based on molecular data and morphology, and Cox (2015) removed nine genera from Fragilariaceae to Ulnariaceae, one genus to Rhaphoneidaceae, and three genera to Tabellariaceae, leaving 14 genera included in Fragilariaceae. Nevertheless, Fragilariaceae is still paraphyletic. One clade which appears in several molecular phylogenies is a small-celled “fragilarioid” group (<20 μm): Nanofrustulum, Opephora,
**Pseudostaurosira, Staurosirella, and Staurosira** (Lobban et al. 2011, Ashworth et al. 2012, Lobban and Ashworth 2014a,b, Li et al. 2015). One shared feature for this group is lack of a rimoportula (labiate process), much like the Plagiogrammaceae. In addition, most of the genera in that group above have uniseriate striae, linking spines in the interstriae, simple and open copula, simple apical pore field of some kind, and ribbon-like or radiating colony (Williams 1987, Round et al. 1990, Morales et al. 2015). These characters (or lack of characters) are also found on other araphid diatoms and non-araphid diatoms (Williams 1987, Round et al. 1990) making it difficult even for the most experienced diatomists to distinguish those genera based on the morphology. For instance, *Pseudostaurosira brevitriata* and *Staurosira construens*, were previously classified in *Fragilaria* Lyngb., on the basis of linear girdle bands, and a simple type of ocellulimbus at each pole, and became the types of new genera (Williams 1987).

To analyze the biodiversity of araphid diatoms and enhance the comprehension of their phylogenetic relationships, in particular for relatively small-sized taxa, we have collected and isolated numerous araphid strains from a wide range of marine or brackish habitats. Here, we describe eight araphid taxa, some of them represented by multiple strains, isolated from North America, northern China, Hawaii Island, and Guam. We analyzed the general characteristics and enhance the comprehension of their phylogenetic position among established araphid taxa, we have assigned all of the eight taxa as new for science.

**MATERIAL AND METHODS**

**Collections and cultures.** The detailed sampling information of the newly described taxa in this manuscript is summarized in Table 1. Clones SZCZCH1247, HK315, and HK391 were isolated from marine sand. Clone HK325 was isolated from macrophytes, whereas HK445 and HK446 from an unspecified benthic environment. The remaining clones were collected by plankton net from the shallow marine environment. Single cells were isolated from wild samples to obtain monoclinal cultures. *Serratirea varisterna* was grown in enriched seawater at a salinity of 35 f/2 medium (Guillard 1975) at 18°C under a 16:8 light:dark cycle, illuminated with 50 μmol photons \( \text{m}^{-2} \text{s}^{-1} \) of white light. *SZCZCH1247* was grown in the same condition as *Serratirea varisterna*, but at a salinity of 30. Clone HK385 was grown in a salinity of 40 f/2 medium, at 27°C under a 12:12 light:dark cycle, illuminated with 21 μmol photons \( \text{m}^{-2} \text{s}^{-1} \) of white light. With the same light conditions, clone HK315 was grown at a salinity of 35 f/2 medium, at 27°C. The remaining clones were grown at a salinity of 35 PSU f/2 medium at 20°C-24°C. The mounted slide of *Dimeregramma dubium* (Grunow slide no. 501) was obtained from the Grunow diatom collection deposited in the Naturhistorisches Museum, Vienna.

**Microscopic examination.** Microscopic work within this study has been shared between University of Szczecin and University of Texas at Austin. LM and SEM protocols at Austin and Szczecin follow Ashworth (2013) and Li et al. (2015), respectively.

**DNA extraction and PCR.** For clones SZCZCH1168 and SZCZCH1247, several milliliters of cell suspension from an exponentially growing culture were harvested to extract the genomic DNA using the Genomic DNA NucleoSpin Plant II kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. The primers for PCR amplification and sequencing of the small subunit (SSU) of ribosomal RNA, and two chloroplast genes (*rbcL* and *psbC*) were described in Alverson and Kolnick (2005) and Alverson et al. (2007). The volume of each PCR was 25 μL: 2 μL (20 ng) purified DNA template; 2.5 μL 10× Dream Taq buffer (includes 20 mM MgCl₂); 1 μL Ultrapure dNTPs Set (3 mM each dATP, dCTP, dGTP, dTTP); 0.5 μL each primer (10 μM); 0.15 μL Dream Taq DNA polymerase (5 U μL⁻¹); and ddH₂O to a final volume of 25 μL. PCR conditions for SSU were as follows: 94°C for 2 min, 35 cycles of (94°C for 1 min, 55°C for 1 min, 72°C for 1 min and 35 s), and final extension at 72°C for 7 min. PCR conditions for *psbC* and *rbcL* were the same as with SSU but with 53°C for annealing temperature and 1 min 15 s for extension time. PCR products were visualized in 1% agarose (Maximus, Lodz, Poland) gel and then purified using Exonuclease I & Pol-A (EUREs, Gdanisk, Poland) protocol. PCR products were sent to oligo.pl DNA Sequencing Laboratory (IBB PAS, Warsaw, Poland for Sanger sequencing with use of BigDye Terminator v. 3.1 chemistry and ABI3730xl sequencer. Regarding the remaining clones in Table 1, the DNA extraction and amplification follows Ashworth (2013).

**Phylogenetic analyses.** ML analysis was performed with a three-gene (SSU, *rbcL*, and *psbC*) data set (Appendix S1 in the Supporting Information) using two strains of *Bolidomonas pacifica* L. Guillou & M.J. Chrétiennot-Dinet as the outgroups. The secondary structural alignment of SSU primary sequences was aligned by SSU-align (Nawrocki 2009) using covariance models. The ambiguous sites with a posterior probability less than the default of 0.9 were removed. GenBank accession numbers of the taxa used in this study were listed in supplementary file Table S1 in the Supporting Information. In the ML analysis, the data set was partitioned by different genes, different codon positions (in case of chloroplast markers), and paired and unpaired sites (in case of SSU markers) with a GTR+G+I model. Phylogenetic tree was conducted with 1,000 bootstrap replicates using rapid Bootstrap analysis in RAxML v8.1 (Stamatakis 2014). The best-scoring ML tree was chosen as the final tree and bootstrap values were added to the corresponding nodes.

**RESULTS**

**Morphology.** *Serratirea* Ashworth, Chunlian Li & Witkowski **gen. nov.**

**Diagnosis.** Frustules rectangular in girdle view with rounded corners. Cells joined by the valve face to form ribbon-like colonies. Two plastids per cell, lying against the valve face. Valves linear to linear-lanceolate, with a very broad sternum. Transapical striae composed of single, circular areola positioned on the valve face, valve mantle free of marginal areolae. Areolae occluded by complex vela.
Rimoportulae are absent. Apical pore fields composed of several small, round apical pores. Copulae are open and plain. Valvocopula is several times wider that other copulae.

**Comment:** This genus could be distinguished from the other small araphid genera, which lack rimoportulae by the short, single round-to-elliptical areolae per stria on the valve face and lack of valve mantle areolae.

**Etymology:** The generic name refers to the shape of the contact line between the sternum and the striae, which resembles a “saw” (“serratifera” = “bearing saws”).

**Type species:** *Serratifera varisterna* Chunlian Li, Ashworth & Witkowski **sp. nov.**

**Serratifera varisterna** Chunlian Li, Ashworth & Witkowski **sp. nov.** (Figs. 1 and 2)

**Diagnosis:** Cells in girdle view rectangular with rounded corners. Valves linear to linear-lanceolate, 35–41 μm in length, and 4.1–4.6 μm in width. Sternum in well-developed specimens broadly lanceolate. Transapical striae composed of a single areola located along the valve margin, 13.5 in 10 μm. Areolae covered by branched vela, which grow from the edge of each areolae.

**Holotype:** Slide BM 101 829, in the Natural History Museum, London, UK, leg. M. Ashworth, C.H. Li, A. Witkowski, E. Theriot, December 2013

**Isotype:** Slide SZCZCH168, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin

**Type locality:** Collected by plankton net, Packary Channel, Mustang Island, Texas, USA, N 27°37.07′; W 97°12.7′, December 2013

**Etymology:** Species name refers to the sternum width changing from narrow, linear to broad lanceolate during the valve morphogenesis (for details see the description of the species morphology).

**Morphology:** Each cell appears to possess two chloroplasts (Fig. 1a). Valves are linear to linear-lanceolate (Figs. 1, b and c; 2, e and f). Frustules are rectangular in girdle view (Fig. 1d), and forming ribbon-like colonies by interlocking spines (Fig. 1, a, h, and l). Sternum linear to broadly lanceolate (Figs. 1, e and g; 2, a, c, and d) depending on the valve morphogenesis. In the early stage development of the valve sternum is distinct, but narrow and strictly linear (Fig. 2, a and c). As the valve development progresses, part of each areola is filled and the sternum significantly expands and becomes very broad (Figs. 1e; 2, d and e). Finally the striae are reduced in their size to a solitary row of oblong areolae positioned close to the valve margin (Fig. 2d). Striae uniseriate, composed of one round-to-elliptical (transapically elongate) areola on the valve face. The transapical striae never extend onto the valve mantle (Fig. 1h, l). Areolae occluded by delicate granulose decoration (Fig. 1, h and l).

| Species                        | Clones     | Locality               | Source               | Latitude/Longitude   | Collection date |
|-------------------------------|------------|------------------------|----------------------|----------------------|-----------------|
| *Serratifera varisterna*      | SZCZCH168  | Packary Channel, Mustang Island, USA | Plankton net | N 27°37.07′; W 97°12.7′ | December 2013 |
| *Serratifera varisterna*      | HK424      | Packary Channel, Mustang Island, USA | Plankton net over mud | Sand | N 27°37.08′; W 97°12.78′ | March 2014 |
| *Serratifera varisterna*      | HK315      | FSU Marine Laboratory, FL, USA | Sand | N 29°54.96′; W 84°30.78′ | October 2010 |
| *Hendeyella rhombica*         | HK445      | Hawaii, USA            | Unspecified benthic habitat | Sand | N 19°34.8′; E 155°58.2′ | January 2012 |
| *Hendeyella dimeregrammopis*  | HK391      | Cozumel, Mexico       | Epiphytic            | Sand | N 20°24.16′; W 86°51.51′ | January 2012 |
| *Hendeyella lineata*          | HK325      | Gab Gab Beach, Guam, USA | Plankton net over sand | Sand | N 39°40.38′; W 144°38.58′ | July 2011 |
| *Psammotaenia lanceolata*     | HK316      | St. George Island, FL, USA | Plankton net over sand | Sand | N 12°54.88′; W 97°27.41′ | June 2011 |
| *Castoridens striata*         | HK385      | Baffin Bay, TX, USA    | Plankton net over sand | Sand | N 30°20′; W 86°40′ | December 2013 |
| *Castoridens hyalina*         | HK444      | Destin-Choctawhatchee Bay, Florida | Plankton net over sand | Sand | N 36°5.58′; E 120°28.33′ | June 2015 |
| *Cratericulifera shandongensis* | SZCZCH1247 | Qingdao, NE China      | Sand from water depth of 10 cm | Sand | N 36°5.58′; E 120°28.33′ | June 2015 |

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kept in the Theriot Lab collection at the University of Texas, Austin. The third strain of *Serratifera varisterna* was isolated from the sand at the FSU Marine Lab, FL and USA and is also maintained in culture at the University of Texas. Austin. It is likely that *Serratifera varisterna* is benthic, as the strains were isolated either directly from the substrate (FSU Marine Lab) or from sediment-rich, well-mixed plankton net casts from shore (Packary Channel). *Serratifera varisterna* clones were only observed in marine temperate climate zone in this study.

*Comparison with established taxa:* When observed in LM, this species can be confused with small *Tabularia* and large *Pseudostaurosira* species, such as *Tabularia investiens* (W. Smith) D.M. Williams & Round, *Pseudostaurosira decipiens* E. Morales, G. Chavez & Ector and *Pseudostaurosira subsalina* (Hustedt) E.A. Morales, because of the broad sternum and linear-to-lanceolate valve shape. However, the striae of *S. varisterna* are very easy to observe under LM, which distinguishes this species from the *Pseudostaurosira* group, which have striae barely visible under LM. Furthermore, *Serratifera* spp. possess a single areola per stria and lacks areolae on the valve mantle, which distinguishes it from *Tabularia* and *Pseudostaurosira*, both having the striae with multiple areolae present on the valve face and at least one row of areolae along the valve mantle. Moreover, *Tabularia* spp. possess one or two rimoportula, whereas *S. varisterna* has none.

**Hendeyella** Ashworth, Wikowski & Chunlian Li *gen. nov.*
**Diagnostic** Frustules are rectangular in girdle view, forming ribbon-like colonies through interlocking spines. One or two chloroplasts per cell. Valve rhombic-lanceolate, linear or elliptical with obtusely rounded apices. Cell heteropolar or isopolar. Sternum linear and inconspicuous. Girdle composed of a few (three, where observed) plain copulae, the valvocopula is much broader than other copulae. Spines are solid and sometimes multi-branched, situated on the interstriae at the valve face/mantle junction. Below the marginal spines is one row of areolae on the valve mantle. Transapical striae...
uniseriate. Apical pore field present at both apices, and well-decorated externally. Areolae are occluded by branching volae. Rimoportulae are absent.

**Comment:** *Hendeyella* as a genus can be distinguished from other araphid genera and *Dimeregramma* in particular by the inconspicuous sternum and the single row of areolae beneath the marginal spines on the valve mantle. Although *Hendeyella* has well-branched spines and very broad valvocopulae, like *Dimeregramma*, the former has plain copulae and small apical pore fields restricted to the valve mantle or valve margin. *Dimeregramma* has perforated copulae and well-developed apical pore fields on the valve face and mantle.

**Etymology:** the generic name is dedicated late Dr. N.I. Hendey a distinguished diatomologist, to acknowledge his research on small marine benthic diatoms.

**Type species:** *Hendeyella rhombica* Ashworth sp. nov.

**Hendeyella rhombica** Ashworth sp. nov. (Fig. 3)

**Diagnosis:** Valve rhombic to rhombic-lanceolate with broadly rounded apices, valve 14–20.5 μm long, 5.7–7.2 μm wide, 12–13 striae in 10 μm.

**Holotype:** “araphid ribbon-10” NateSite1, HI; Slide BM 101 832, in the Natural History Museum, London, UK, leg. N. Leclear, January 2012

**Isotype:** Slide SZCZ23421, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin

**Type locality:** Benthic collection, Kona area, Hawaii, USA, N 19°34.8′; E 155°58.2′.

**Etymology:** the species epithet refers to the rhomboidal valve outline (“rhombica”).

**Morphology:** Frustules rectangular in girdle view (Fig. 3, d, f, and h). Two chloroplasts per cell, each lying against the valve face (Fig. 3a). Valve rhombic to rhombic-lanceolate with broadly rounded apices (Fig. 3, c, g, and i). Cells are heteropolar (Fig. 3, b, c, e, g, i, and l). Sternum is linear, and not obvious (Fig. 3, b, c, e, and g). Transapical striae uniseriate, parallel. Areolae are circular to oblong (Fig. 3e). Two to five areolae per stria on the valve face (Fig. 3, e, g, and i) and a single row of areolae on the valve mantle below the spines (Fig. 3, e, f, and h). Spines are solid and branched, positioned on the interstriae (virgae) between the areolae at the

![Fig. 3. Hendeyella rhombica (a–d, LM, e–l, SEM).](image-url)
junction of the valve face and valve mantle (Fig. 3, f and h). Areolae are occluded by branching volae (Fig. 3j). Rimoportulae absent (Fig. 3g). Apical pore field, present at both apices at the junction of valve face and valve mantle, well-decorated externally and composed of several porelli (Fig. 3, i and k). Copulae few, plain and open (Fig. 3h). In the material studied three copulae were observed (Fig. 3h). Valvocopula is very broad, almost five to six times bigger than other copulae (Fig. 3, f and h).

Comparison with established taxa: Under the LM, this new species has a gross morphology similar to Trachysphenia australis, in that both possess circular areolae, an indistinct sternum and heteropolar valve. However, the sternum is more distinct in T. australis, and the valve face of T. australis is arched, whereas H. rhombica has a flat valve. In addition, H. rhombica possesses very well-developed marginal linking spines, whereas in T. australis marginal spines have never been observed.

**Hendeyella dimeregrammopsis** Ashworth sp. nov. (Fig. 4)

**Diagnosis** Valves elliptical, 7.5–12 μm in length, 4.4–5.8 μm in width, 12–14 striae in 10 μm.

**Holotype.** “cf. Dimeregramma” Coz-1 Cozumel, Mexico; Slide BM 101 833, in the Natural History Museum, London, UK, leg. M. Yu, January 2012.

**Isotype.** Slide SZCZ23422, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin

**Type locality:** Collected from subtidal sand at Cozumel, Mexico, N 20°24.16’; W 86°51.51’

**Etymology:** the specific epithet refers to the superficial resemblance of this taxon to Dimeregramma Ralfs.

**Morphology:** One chloroplast per cell (Fig. 4a). Frustules rectangular in girdle view, forming ribbon-like colonies by interlocking spines (Fig. 4, a, e, and g). Valves elliptical and slightly heteropolar (Fig. 4, b–d, f, and h). Sternum is linear and narrow (Fig. 4, b–d, f, and h). Transapical striae uniseriate, parallel in the middle becoming radiate toward the apices, composed of one to three circular areolae on the valve face (Fig. 4f). Linking spines are solid, spatulate, sometimes multi-branched, located on the interstriae (virgae) at the valve face/mantle junction (Fig. 4, g and i). A single row of areolae occurs below the marginal spines on the valve mantle (Fig. 4, f, g, and i). Siliceous plaques present along the edge of the mantle (Fig. 4i). Areolae are occluded by complex, branching volae (Fig. 4, f and h). No rimoportulae present (Fig. 4h). Apical pore field, composed of several vertical rows of round areolae (Fig. 4j), located on the valve mantle, with extensions between the porelli above the valve surface into more or less regularly branched dentate structures with blunt ends (Fig. 4, i and k). Girdle broad, composed of plain, open copulae (Fig. 4g). The valvocopula is very broad and appears to occupy the whole cingulum (Fig. 4g).

**Hendeyella lineata** Ashworth & Lobban sp. nov. (Fig. 5)

**Diagnosis** Frustules rectangular in girdle view. Valves strictly linear with parallel margins and obtusely rounded apices. Transapical striae parallel throughout becoming slightly radiate only at the apices. For the culture, the valve is 15.7–50.8 μm in length, 3.7–4.2 μm in width, 12–14 striae in 10 μm. Valves from wild material showed great variation in length, 17–90 μm, but consistently ca. 4 μm wide, 11 striae in 10 μm, and four longitudinal rows of areolae.

**Holotype.** “wide ribbon” GU44AI-5; Slide BM 101 834, in the Natural History Museum, London, UK, leg. C. Lobban, September 2011.

**Isotype.** Slide SZCZ23423, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin

**Type locality:** Collected as epiphytes on farmer fish turf, Gab Gab Beach, Guam, USA, N 13°26.64’; E 144°38.58’

**Etymology:** the specific epithet is derived from strictly linear valves (“lineata”) of this taxon.

**Morphology:** One plate-like plastid appressed to both valves (Fig. 5a). Valves linear, elongate with parallel margins and obtusely rounded apices (Fig. 5b). Frustules rectangular in girdle view, forming ribbon-like colonies (Fig. 5, c and h). Valve surface flat, sternum inconspicuous and linear (Fig. 5, e–g). Transapical striae uniseriate, parallel throughout, becoming radiate only at apices and composed of four (one to three in culture) circular areola per stria on the valve face (Fig. 5, e–g). The valve mantle possesses one row of areolae, which is nearly completely covered by spatulate marginal spines (Fig. 5, f and i). At each pole on the valve mantle a small pore field is observed (Fig. 5e), composed of several vertical rows of round areolae arranged in apically oriented linear rows. Externally, apical pore fields are delicate, with branched dentate structure and blunt ends (Fig. 5i). Rimoportulae absent (Fig. 5, f and g). Areolae are occluded by complex branching volae (Fig. 5j). Siliceous plaques occur along the margin of the mantle (Fig. 5i). Girdle rather narrow, composed of three (where observed), plain copulae, with very broad valvocopula (Fig. 5i). Copulae open (Fig. 5i) with obvious ligulae (Fig. 5k).
Comparison with established taxa: This new species could be distinguished from *H. rhombica* and *H. dimeregrammopsis* by its much longer and narrower valves with parallel margins. In addition, it differs from them in the pattern of marginal spines. In the two aforementioned species, the linking spines are bifurcating, whereas for this new species, the linking spines are spatulate and multi-lobed on the tip of the spines. *H. lineata* resembles *D. dubium* under the LM due to the outline of the valve face and punctate striae, but the size dimensions of the two species are different: in *D. dubium*, the length of the valve is 20–60 μm, width is 6–8 μm and stria density is 8–10 in 10 μm (Hustedt 1932, Ricard 1987), whereas in *H. lineata*, the length is 15.7–50.8 μm, the width is 3.7–4.2 μm and stria density is 12–14 in 10 μm.

**Hendeyella dubia** (Grun. in van Heurck) Chunlian Li, Witkowski & Ashworth *comb. nov.* (Fig. 5d, Grunow no. 501).

**Basionym:** *Fragilaria dubia* Grunow 1862, Verhandlungen der kaiserlich-königlichen Zoologisch-Botanischen Gesellschaft, Wien 12:315–472.

**Synonym:** *D. dubium* (Grunow) Grunow in van Heurck Synopsis des Diatomées, Atlas; Pl.XXXVI, fig. 18 (1881).

**Nematoplata** (*Nematoplate*) *dubia* (Grunow) (Kuntze. 1898, p.416).

The locality of examined slide (Grunow no. 501): This sample was collected in Porto piccolo on 1 January 1858 by Dr. Lorenz.

**Type locality:** There is no indication of the type of *Fragilaria dubia* in Grunow diatom collections. A handwritten annotation in Grunow's own exemplar of van Heurck's Synopsis des Diatomées, Atlas; Pl.XXXVI, fig. 18 indicates that the material has been collected by Simon F. Söderlund on the Balears (Spain) and the Grunow number is 1751, but it is not mentioned whether the material is the type or not.
**Description:** Frustules rectangular in the girdle view with rounded ends, attached in chains by valve face to face contact, striae arranged parallel (Fig. 5d). The length of short chain from the mounted slide in Grunow diatom collection is 32.9–35.4 µm and the striae density is 9 in 10 µm. No valve view was observed. In Hustedt (1932) description and Ricard (1987), the illustration of *D. dubium* shows cells in girdle view rectangular, not constricted below the apices, the cells are connected in chains with clearly visible marginal spines (see Ricard 1987, fig. 617 and our Fig. 5d). Valves linear with parallel margins and weakly wedge shaped apices, 20–60 µm in length, 6–8 µm in width. Valve face coarsely areolar-punctate, transapical striae 8–10 in 10 µm, longitudinal rows straight. Sternum (pseudoraphe) very narrow (Hustedt 1932 and Ricard 1987, fig. 616). We propose to transfer *D. dubium* to the newly established genus *Hendeyella*.

**Psammotaenia** Ashworth, Chunlian Li & Witkowski gen. nov.

**Diagnosis:** Frustules rectangular in girdle view with rounded corners, one chloroplast per cell. Valves linear-lanceolate to linear-elliptical or oval, with valve face almost flat and shallow mantle. Sternum broad, lanceolate. Transapical striae uniseriate, extending from valve face margin onto the mantle continuously. Areolae small and slightly apically elongate. Marginal spines occur on the interstriae at the valve face and mantle face junction. Rimoportulae absent. Copulae are numerous, plain and open. Apical pore fields are relatively large and of equal size at both apices.

**Comment:** This monotypic genus can be mistaken for some *Pseudostaurosira* taxa, though *Pseudostaurosira* spp. possess a distinct apical bar that crosses the striae at the junction between valve face and the mantle. This character is missing in *Psammotaenia*, where the striae are composed of equally sized, sub-rectangular areolae. The apical pore fields also differ between these two genera; those of *Pseudostaurosira* are small and composed of only a few
scattered porelli, whereas those of *Psammotaenia* are larger and composed of apically oriented rows of porelli.

**Etymology**: the generic name refers to the habitat of the strain, which was isolated from the sand ("psammo-") and forms long, ribbon-like colonies ("taenia" = "ribbon").

**Type species**: *Psammotaenia lanceolata* Ashworth, Chunlian Li & Witkowski sp. nov.

*Psammotaenia lanceolata* Ashworth, Chunlian Li & Witkowski sp. nov. (Fig. 6)

**Diagnosis**: Frustules rectangular in girdle view with rounded corners. Plastid one per cell. Valves lanceolate to linear-lanceolate or elliptical, 12.5–17.3 μm in length, 1.0–3.2 μm in width. Transapical striae restricted to valve margin and mantle, comprised of 2–4 simple pores, 17.5–18 in 10 μm. Internally, striae lie in an elongate depression which does not have a corresponding feature on the exterior.

**Holotype**: “epipsammic ribbon” 10 × 10⁻² FL; Slide BM 101 835, in the Natural History Museum, London, UK, leg. M. Ashworth, October 2010.

**Isotype**: Slide SZCZ23428, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin

**Type locality**: Sand in the littoral zone of St. George Island, FL, USA, N 29°40.38”; W 84°52.14'.

**Etymology**: Specific epithet is derived from the overall shape of the valve outline.

**Morphology**: Frustule rectangular in girdle view (Fig. 6, j, q, and s). Sternum quite broad (Fig. 6, b–p). One chloroplast per cell (Fig. 6a). For large cells, valves are linear-lanceolate (Fig. 6, b, c, j, and n). As the cells decrease in length, the valve become linear-elliptical or oval (Fig. 6, d–i, k–m, o, and p). Transapical striae uniseriate, parallel throughout on the valve face along the sternum, restricted to the valve margin and mantle (Fig. 6, j–p). Areolae are small, sub-rectangular, slightly apically elongate with simple occlusions (Fig. 6, j–p and r). Close to the striae, there are numerous fine globose particles (Fig. 6, j–m). Rimoportulae absent (Fig. 6, n–p). Apical pore fields well-developed, expanding from valve face onto the valve mantle (Fig. 6, j–p), consisting of several apically oriented rows of circular poroids (Fig. 6j). Siliceous plaques were observed along the valve mantle (Fig. 6, j and s). Cingulum consists of numerous plain, open girdle bands (Fig. 6, j, q, and s). Valvocopula is wider than the other copulae (Fig. 6, j and q).

![Fig. 6. *Psammotaenia lanceolata* (a–i, LM; j–s, SEM). (a) Chain-like colony, attached by the valve face to face contact. (b–i) External valves, showing the size decrease in length. (j–m) External valves, showing broad sternum and variation in the outline of the cell, from linear-lanceolate to elliptical-lanceolate, and to oval. (n–p) Internal valves, showing the variation in the cell outline and note the absence of rimoportulae. (q) The whole frustule, showing the girdle structure and columnar spines. (r) Simple areolae occlusions (arrow). (s) The girdle view, showing open copulae (arrow); scale bars: a–c, i = 10 μm, j, k, l, o, p, s = 1 μm, n, q = 2 μm, r = 200nm, and as for d–h, they share the same scale bar as in i.](image-url)
Comparison with established taxa: Psammotaenia lanceolata in LM resembles some Pseudostaurosira species, such as *P. versiformae* Witkowski, Riaux-Gobin & Daniszewska-Kowalczyk. However, the latter species is strongly heterovalvate and also differs from *P. lanceolata* in terms of size dimension data. In addition, the linking spines of *P. versiformae* are positioned on the apical bars crossing the areolae.

*Psammotaenia* shows some degree of similarity in LM to *Hyaloneis* Amskopper, *Pravifusus* hyalinus Witkowski, Lange-Bertalot & Metzeltin and *Rimoneis* M. Garcia (Witkowski et al. 2000, Amskopper 2008, Garcia 2010). They all are characterized by the hyaline valves when observed in LM. In each of the above genera the structural characters are first observed in EM. When observed under EM, *Hyaloneis* and *Pravifusus* have well-developed linking spines and pore fields composed of small poroids arranged in a few rows, which are similar to *Psammotaenia*, however, they both lack transapical striae. In *Psammotaenia* transapical striae are readily resolvable in LM (see our Fig. 6). *Rimoneis* when observed in EM is characterized by a flat valve face with apical pore fields composed of two slits and transapical striae in a form of a single row of areolae which extend onto the valve mantle in several rows (Garcia 2010). The transition from the valve face to the mantle is abrupt, whereas in *Psammotaenia* it is gradual. The major difference between *Psammotaenia* and *Rimoneis* is the presence of apical pore fields in the form of two slits, abrupt contact between valve face and the mantle and distinctly areolated valve mantle in the latter genus (Garcia 2010).

**Castoridens** Ashworth, Chunlian Li & Witkowski

**gen. nov.**

**Diagnosis** Frustule is rectangular in the girdle view. One valve-appressed plastid per cell. Chain colony formed by secreting mucilage. Valves are linear-lanceolate or linear. Valve mantle is shallow. Striae uniseriate or biseriate, arranged alternately along the sternum and extending from valve face to mantle face without interruption. Areolae are round or quadrangular and appear unoccluded. No rimoportulae present. Marginal spines absent. Two apically elongate slits occur at both apices positioned in the valve face. Cingulum is relatively wide, composed of several copulae.

**Comment:** *Castoridens* has a distinctive apical pore field, composed of two apically elongate slits. *Plagiostriata gorreensis* S. Sato & Medlin also possess the pore fields composed of two pores, however, which are short and only located at the valve apices. While in *Castoridens*, it has two slits extending from the valve face to the valve margin. What is more, *Plagiostriata* differs from *Castoridens* by the oblique striae, the presence of rimoportulae and the relatively deep mantle. Apical pore field composed of slits can also be observed in some species of *Neogragilaria, Licmophora, Synedropsis, Falcula, Hustedtiella*, and *Rimoneis* (Round et al. 1990, Crawford et al. 1993, Hasle et al. 1994, Honeywill 1998, Garcia 2010, Lobban et al. 2012, Li et al. 2015), but the number of slits, their size, and their position are variable among different genera and differ from that in *Castoridens*.

**Etymology:** the generic name refers to a resemblance of the apical structure when observed in SEM to beaver’s incisors teeth (genus Castor), hence *Castoridens*.

**Type species:** *Castoridens striata* Ashworth, Chunlian Li & Witkowski sp. nov.

*Castoridens striata* Ashworth, Chunlian Li & Witkowski sp. nov. (Fig. 7)

**Diagnosis** Frustules rectangular in girdle view. Valves lanceolate with obtusely rounded apices, 23.9–24.2 μm in length, 3.9–4.4 μm width. Sternum indistinct, linear. Transapical striae arranged alternately throughout, 12 in 10 μm. Pores round and appear unoccluded. Valvocopula identical to other copulae, with single row of pores.

**Holotype.** “Baffin Bay araphid ribbon” 15VI11-2A TX; BM 101 836, in the Natural History Museum, London, UK, leg. M. Ashworth, E. Theriot, June 2011.

**Isotype** Slide SZCZ23427, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin

**Type locality:** collected by plankton net over sandy substrate, Baffin Bay, TX, USA, N 27°15'38.9'; W 97°27.41'

**Etymology:** species name is derived from the coarse striation along the valve, hence *C. striata*

**Morphology.** Frustules rectangular in girdle view with rounded corners (Fig. 7, d and h). One cup-shaped chloroplast per cell (Fig. 7a). Chain-like colonies observed in cultured material, but no linking spines have been observed on the valve surfaces (Fig. 7, e and f). Valves are lanceolate with acutely rounded apices (Fig. 7, b, c, and f). Sternum is narrow, linear (Fig. 7, b, c, e–g). Striae uniseriate, arranged alternately along the sternum (Fig. 7, e and g) and extend continuously onto the mantle (Fig. 7e). Areolae appear to be unoccluded (Fig. 7, e–g). Siliceous plaques are present along the margin of the valve mantle (Fig. 7, f and h). Two distinct slits occur on the apices, observed both externally and internally (Fig. 7, e–g). No rimoportulae present (Fig. 7g). Cingulum composed of several identical copulae (four, where observed), each bearing a single row of fine puncta (Fig. 7h).

**Comparison with established taxa:** Like *Plagiostriata gorreensis* S. Sato & Medlin, *Castoridens striata* has two slits on the apices instead of apical pores. However, the differences between these two taxa are quite straightforward. The most striking feature of the former is its striation, which is angled at ~60° across the robust sternum, whereas in the latter, the striae are perpendicular to the sternum. In addition, *Plagiostriata gorreensis* has plain girdle bands, deep valve mantle and has rimoportulae, whereas in *Castoridens*...
striata, copulae are perforated, valve mantle is shallow and the valves lack rimoportulae. Furthermore, Castoridens striata could be separated from the other species Castoridens hyalina (described below) by its larger size, perforated girdle bands and presence of striae in the middle portion of the valve.

Castoridens hyalina Ashworth, Witkowski & Chunlian Li sp. nov. (Fig. 8)

Diagnosis: Frustules rectangular in girdle view with rounded corners. Valves linear with acutely rounded apices, 5.4–6.3 μm in length, 1.3–1.5 μm in width. Under the LM, the striae structure not possible to be observed. Under the SEM, the sternum is narrow and linear, and observed only in the apical part of the valve. Likewise, transapical striae, observed in the apical valve part, parallel throughout. There is a big hyaline area in the middle part of the valve face. Copulae hyaline and valvocopula not distinct.

Holotype: “LS-B1” C1 6-1-14 FL; BM 101 837, in the Natural History Museum, London, UK, leg. J. Nie-now, December 2013.

Isotype: Slide SZCZ23424, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin

Type locality: Collected by plankton net over sand, Destin-Choctawhatchee Bay, Florida, U.S.A., N 30°20’; W 86°40’

Etymology: The species name is derived from its central hyaline area in the middle part of the valve.

Morphology: A single plate-like plastid is present per cell (Fig. 8, a and b). Cell is linear in valve view (Fig. 8, d–g), and frustule is rectangular in girdle view (Fig. 8, c and h). Biseriate striae, occur only near the apices (Fig. 8, j–l), and the rest of the valve face is hyaline (Fig. 8, j and l). Externally, the striae appear coated by a siliceous layer, which obscures the areolae (Fig. 8, h, i, and l). Rimoportulae and marginal spines are absent (Fig. 8, j–l). Apical pore fields are detected at both apices, consisting of two slit-like apical pores (Fig. 8, i and j). Cingulum consists of several plain copulae, identical in shape and size (Fig. 8h).

Comparison with established taxa: As in Plagiostriata gorreensis and Castoridens striata, the apical pores of Castoridens hyalina are two elongate slits. Nevertheless, the latter could be easily separated from the former two species in its hyaline areas occupying nearly two-thirds of the valve internally and
Cratericulifera Chunlian Li, Witkowski & Ashworth gen. nov.

Diagnosis: Cells rhombic with rounded corners, formed into stellate colony by the secreting mucilage. Girdle view has not been observed. Sternum is linear near the apices and broadly lanceolate in the middle. Uniseriate striae, composed by round, crater-like openings of areolae. Striae expand from the valve face to the mantle, with only one row of areolae positioned on the mantle. No rimoportulae present. No areolae occlusion has been observed.

Comment: This genus could be differentiated from other established araphid genera by the nature of its areolae. The external openings of the areolae are crater-like. Furthermore, the position and the number of the marginal spines in the intersstriae are also very unique.

Etymology: The name is derived from crater-like openings of areolae on the valve face.

Type species: Cratericulifera shandongensis Chunlian Li, Witkowski & Ashworth sp. nov.

Cratericulifera shandongensis Chunlian Li, Witkowski & Ashworth sp. nov. (Fig. 9)

Diagnosis: Cells are rhombic in the valve face view. The length of the valve is 12.6–14.8 μm, the width is 4.0–5.1 μm and striae density is 13–14 per 10 μm. The opening of each areola is crater-like.

Holotype: Slide BM 101 830, in the Natural History Museum, London, UK, leg. A. Witkowski, S. Yu, June 2015

Isotype: Slide SZCZCH1247, deposited in Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin

Type locality: Sand from the water depth of 10 cm, large sand beach, Qingdao, China, N 36°5.58′; E 120°28.35′

Etymology: The specific name is derived from the name of the Shandong province, NE China where this clone was collected.

Morphology: Chloroplast seem to be one per cell (Fig. 9, a and b). Most of the cells are slightly heteropolar (Fig. 9, c, d, f–h), and few cells are isopolar (Fig. 9e), perhaps it is the effect of the culture. Cells are rhombic (Fig. 9, c–d). Sternum is linear near both apices and expands toward the middle of the valve face (Fig. 9, f–h). Striae linear, uniseriate, extending continuously from the valve face to the mantle. There is a single row of areolae positioned on the mantle (Fig. 9g). Areolae rounded with crater-like openings (Fig. 9, f, g, i, k, and l), which is obviously elevated above the valve face (Fig. 9k, see arrow) and the elevation of the areolae also could be seen in focused ion beam preparations (Fig. 9l). Spines are short, situated at the junction of the valve face/mantle (Fig. 9, f, g, and k). Mostly, close to both sides of marginal areolae, there exists one spine, and rarely two spines in one side of the areolae, sometimes even absent (Fig. 9, j).
Apical pore fields composed of several areolae, and slightly lifted up internally (Fig. 9, h and j). Rimoportulae absent (Fig. 9, h and j). No complete frustule has been observed as yet. Copulae are broad, open and each possess a single row of small, round poroids in the middle (Fig. 9m). There is one row of plaques occurring along one side of the margin of copulae (Fig. 9m).

Comparison with other taxa. Because of rhombic valve outline and linear striae under the LM, *C. shandongensis* resembles *Odontidium tabellaria* W. Smith. However, the sternum in the *Cratericulifera* is linear to lanceolate, whereas in the *O. tabellaria* it is very narrow. Moreover, the width (6.0–7.0 μm) in the latter is larger and the striae (14–15 in 10 μm) are denser than that in *C. shandongensis*. When observed under the SEM, *C. shandongensis* could be easily separated from *O. tabellaria* by its crater-like openings of the round areolae and the position of the short spines situated near the side of the areolae on the mantle face, in contrast to *O. tabellaria*, in which the areole are apically linear and the spathulate marginal spines are located in the middle of the interstriae.

Phylogenetic results. A ML analysis was performed using sequence data from 179 diatoms strains, including eight taxa described in this manuscript (Fig. S1 in the Supporting Information). All the new taxa were positioned in araphid clade “A” (bootstrap value [bv] = 93%; Fig. 10), which also included strains from the genera *Fragilariforma*, *Plagiostriata*, *Staurosira*, *Opephora*, *Pseudostaurosira*, *Staurosirella*, and
Nanofrustulum, in which Fragilariforma virescens located at the base of the clade. The result of ML analysis confirmed the monophyly of the genus Serratifera (bv = 100%) which was represented by three clones, all from temperate zone (Table 1). This clade was sister to a clade consisting of three clones of Opephora (SZCZCH152, SZCZCH992, HK446) (bv = 91%). Likewise the tropical genus Hendeyella was also monophyletic (bv = 100%), with all three species—H. rhombica, H. lineata, and H. dimeregrammopsis—forming a clade (bv = 100%). This cluster was sister to Psammotaenia lanceolata (bv = 95%), followed by Staurosira construens (bv = 68%) and then Opephora pacifica (bv = 51%). The temperate species Castoridens striata and Castoridens hyalina, also formed a monophyletic group (bv = 66%), which was sister
to a clade (bv = 56%) containing Craticulifera and Plagiostriata.

DISCUSSION

In this study, we used morphological and molecular data to describe five new genera with eight new taxa of mostly small-celled araphid taxa: Serratifera varisterna, Hendeyella rhombica, H. dimeregrammopsis, H. lineata, Psammotaenia lanceolata, Castoridens striata, C. hyalina, and Craticulifera shandongensis. The phylogeny supported the monophyly in genera with multiple strains and species: Serratifera, Hendeyella, and Castoridens. We also transfer D. dubium, which was included in Plagiogrammaceae in the early literature, to the newly established genus Hendeyella (as H. dubia) based on morphological data. The DNA sequences data also showed that Hendeyella is not related to Plagiogrammaceae; they were far apart in the phylogenetic tree (Figs. 10 and S1).

Although there are several differences in Castoridens striata and C. hyalina, such as a large centrally positioned hyaline area and thin siliceous layer covering in the external valve surface and plain girdle bands in C. hyalina, we still included them in the same genus. We feel that despite some differences in morphology, both of these taxa are small, chain-forming diatoms with valves synapomorphies, such as distinctive apical pore fields and DNA data placed them in a clade exclusive to all other diatoms described here. As for their differences (hyaline area and plain girdle bands) mentioned above, they could be explained as only one difference: total pore occlusion in C. hyalina.

Regarding Serratifera, despite several similarities in the morphology with Pseudostaurosira and Tabularia (discussed in the morphology section above), their striae composed of single areolae that do not extend to the valve mantle readily distinguish Serratifera from those genera. The DNA sequence data support this interpretation, as Serratifera, Pseudostaurosira, and Tabularia are all found in different clades. At this time, both Psammotaenia and Craticulifera were monotypic. Although Psammotaenia was sister to Hendeyella (bv = 93%), to include the former in Hendeyella would have resulted in a genus with a very broad morphological description. For instance, even viewed under LM, all three Hendeyella spp. described here, shared common characters such as a linear and narrow sternum, distinctive areolae and branched marginal spines, and differed only in gross valve outline and striae density. On the other hand, Psammotaenia in LM is easily distinguished from all Hendeyella spp. by its short marginally striae composed of indistinct pores and wide linear-lanceolate sternum (resembling the unrelated Serratifera far more than the closely related Hendeyella). Furthermore, under SEM, all three Hendeyella spp., share a row of areolae occluded with dense vola, and apical pore fields restricted to the valve mantle, whereas Psammotaenia has several rows of small areolae occluded by 1–2 vola on the mantle and relatively large apical pore fields that extend from the valve face to the mantle. Based on these characters shared by Hendeyella, which are not expressed in Psammotaenia, we feel that the latter should be a distinct, but closely related genus. As for Serratifera, DNA data suggested that it had a sister relationship with Plagiostriata. However, with no corresponding morphological observations that suggested their close relationships, this result seemed to be questionable. It should be noted that the support of the sister relationships between Craticulifera and Plagiostriata were low (bv = 56%), so it was likely that the sister relationship of Craticulifera and Plagiostriata was still uncertain.

Our molecular results demonstrated that the Fragilariaceae was a paraphyletic group, with the members of the Fragilariaceae positioned in five different clades (Fig. 10). Previous publications showed that the Fragilariaceae was non-monophyletic (Medlin et al. 1993, 1996, Medlin and Kaczmarska 2004, Sato et al. 2008a,b, Theriot et al. 2010, Ashworth 2013, Li et al. 2015); however, there was a group of small-celled taxa belonging to Fragilariaceae, such as Nanofrustulum, Opephora, Staurosirella, Staurosira, Pseudostaurosira, Plagiostriata, and Fragilariforma and did form a monophyletic group (Sato et al. 2008b, Theriot et al. 2010, 2011, Ashworth et al. 2012, Lobban and Ashworth 2014a,b, Li et al. 2015). In this study, those small-celled genera mentioned above also fall into the same clade “A” along with the newly established genera described here (Fig. 10). While it is tempting to formally describe a new family around clade “A,” we remain hesitant to do so on the basis of DNA sequence data alone. While the DNA evidence suggests such a family level association of these genera exists outside of the Fragilariaceae, at this point we cannot identify a morphological synapomorphy—a character which is possessed by all taxa within clade “A” but not shared by taxa without the clade. Without a synapomorphy limiting circumscription of genera to the family, we feel a formal description at this time would only add to confusion in the future. However, we do feel the DNA data does strongly suggest such a synapomorphy exists, perhaps in the intracellular ultrastructure or sexual reproductive cycle. Furthermore, the phylogenetic result revealed Opephora and Nanofrustulum as paraphyletic groups (Fig. 10). Previous single-gene or four-gene phylogenies (Medlin et al. 2008, 2012) has shown that none of Pseudostaurosira, Staurosirella, Staurosira were monophyletic. The taxonomy of the above mentioned genera in the clade “A” clearly required more work to further be emended.

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Diversity among Small Araphid Diatoms

Christina E. E. Stoecker, John P. Kociolek, andYoko Takeuchi

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Diversity among Small Araphid Diatoms

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1. Introduction

Araphid diatoms are a group of small, typically barrel-shaped diatoms that lack a central axis (araphid). They are found in a variety of aquatic environments, including freshwaters, estuaries, and marine habitats. The study of these diatoms is significant for understanding the diversity and distribution patterns within the diatom community, as well as their ecological roles in various ecosystems. In this study, we focus on the diversity of small araphid diatoms, specifically those belonging to the genus *Cyclophora*.

2. Methods

The investigation was conducted using a multidisciplinary approach that combined morphological analysis, molecular phylogenetics, and ecological studies. Specimens were collected from various locations in the world, including freshwater and marine environments. Morphological characteristics were recorded, and samples were subjected to DNA extraction and sequencing to determine the phylogenetic relationships among different species.

3. Results

The analysis revealed a high diversity of small araphid diatoms, particularly within the genus *Cyclophora*. A total of 16 new species were identified, representing novel lineages that were previously undescribed. These new species were named and described based on their distinct morphological features and molecular signatures.

4. Discussion

The discovery of new araphid diatom species highlights the ongoing diversity within the diatom community. The molecular phylogenetic analysis provided insights into the evolutionary relationships among these taxa, suggesting that araphid diatoms have a wide distribution and adaptive flexibility in various aquatic habitats.

5. Conclusion

The study underscores the importance of continued research in diatom ecology and systematics. The new species identified contribute to the knowledge base of diatom diversity and enable a more comprehensive understanding of their ecological roles and potential applications in biotechnology and biogeochemistry.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web site:

**Figure S1.** A Maximum likelihood phylogeny of 179 diatoms (with bootstrap values at nodes) was inferred from a concatenated alignment of SSU rRNA, rbcL, and psbC markers. Taxa in bold are newly described species. Support values less than 50% were omitted. Two *Bolidomonas pacifica* species were used as out groups.

**Table S1.** Strain collection information and GenBank accession numbers of the diatom taxa used in the phylogenetic analyses in this manuscript. Newly generated sequences are listed in bold.

**Appendix S1.** Alignment of concatenated DNA sequence data of 179 taxa (SSU rRNA, rbcL, and psbC) in NEXUS format used for three-gene phylogenetic analysis in this study. Sequences for the three genes of each taxon are sequential, starting with nuclear-encoded ribosomal small subunit (bases 1–1,757), chloroplast-encoded rbcL (bases 1,758–3,230) and chloroplast-encoded psbC (bases 3,231–4,362).