

Morphological reconsideration of the araphid genus *Pseudostaurosira* (Bacillariophyceae), a revision of *Gedaniella*, *Popovskayella* and *Serratifera*, and a description of a new *Nanofrustulum* species

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Background and aims – As a result of the description of many new species, reanalyses of type material, and information becoming available on valve morphogenesis in small araphid diatoms lacking a rimoportulae, the existing classification scheme at the genus level needs revision. Because morphological information has increased manyfold since the system provided by Williams & Round (1987), it may now be possible to find distinguishing characters in order to produce a more stable and useful framework, encompassing a morphogenetic perspective, which could then guide the placement of newly discovered taxa. This new framework could also be used to help assess the molecular information generated for the group, based on which many new genera are being erected, but perhaps without proper pondering of morphological data.

Methods – A thorough review was made of available published information on the ultrastructure of small-celled araphid diatoms lacking rimoportulae. In addition, image databases were searched, and new light and scanning electron microscopical observations made of some hitherto undescribed species.

Key results and conclusions – We provide a table of putative distinguishing features for nine genera (*Nanofrustulum*, *Opephora*, *Pseudostaurosira*, *Pseudostaurosiropsis*, *Punctastriata*, *Sarcophagodes*, *Stauroforma*, *Staurosira* and *Staurosirella*), together with a discussion on their value for discriminating these small diatoms using a morphogenetic perspective. Based on our findings, we amend the genus *Pseudostaurosira*, establishing wide and short vimines as its most characteristic feature. We use our system in describing a new species from Bolivia, which we place in *Nanofrustulum* based on its quasifract copulae, the distinguishing trait of the genus. The new species is distinguished from its congeners by its heteropolar valves, apical pore field features, and the multiseriate areolae. We also examine the three genera *Popovskayella*, *Gedaniella*, and *Serratifera*, the latter two recently erected based on molecular information. Since none of these latter genera pass the morphogenetic evaluation we think is essential, we place them in synonymy with other genera and provide the consequent nomenclatural changes. Finally, we make several new combinations in *Nanofrustulum*, *Pseudostaurosira*, *Sarcophagodes* and *Staurosirella*.

Key words – Araphids, Bolivian Altiplano, Fragilariaceae, molecular phylogenies, Pantocsek, systematics, type material.

INTRODUCTION

While the many features currently used for small araphids lacking rimoportulae might be appropriate for species level delimitation, synapomorphies at the genus level are more complicated to single out (see Williams 2011, 2013 and Kociolek & Williams 2015). This difficulty has generated an alternate view to the taxonomy proposed by Williams & Round (1987), which is expressed, for example, in Lange-Bertalot (1989, 1993), Krammer & Lange-Bertalot (1991) and Ognjanova-Rumenova et al. (1994). This alternative has gained partial support from molecular analyses (Medlin et al. 2012). Nevertheless, the Williams & Round (1987) proposal, based completely on morphology and later complemented with additional genera (*Stauroforma* Flower, V.J. Jones & Round, *Pseudostaurosiropsis* E. Morales and *Sarcophagodes* E. Morales), has become widely used. Perhaps, one of the reasons for acceptance comes from the fact that the morphological diversity found in samples can be relatively easily ascribed to these genera. This, of course, is helpful when diatoms are used in applied fields such as bioindication. While molecular analyses strive to seek the best way to express evolutionary relationships among existing genera (e.g. Medlin et al. 1993, 2008, 2012, Medlin & Desdevises 2016), morphological data continue to be the most trusted basis for identifying taxa and indeed for classifying them, as shown through the plethora of new taxa described in recent decades using a combination of light (LM) and scanning electron microscopy (SEM) (e.g. Witkowski et al. 2010, Morales et al. 2012, Grana et al. 2018).

In the last 30 years, the number of araphid species has increased significantly (Guiry & Guiry 2018). Newly described features have broadened the range of morphological characters used for genera and species delimitation. Therefore, there is now a need to start modifying the definitions of taxa in order to reflect what is known about their morphology (Morales & Manoylov 2006b, Morales et al. 2012, García et al. 2017). One of the genera in need of such reconsideration is *Pseudostaurosira* D.M. Williams & Round in which at least 40 species, some new, others transferred from *Fragilaria* Lyngb., *Opephora* P. Petit and *Staurosira* D.M. Williams & Round have been included (e.g. Morales & Edlund 2003, Cejudo-Figueiras et al. 2011, Kociolek et al. 2014, Kulikovskiy et al. 2015, García et al. 2017). Taking as a basis the SEM analysis of type material of the generitype, *Pseudostaurosira brevistriata* (Grunow) D.M. Williams & Round (Morales et al. 2015), and published SEM information for the majority of species currently ascribed to this genus, it is now possible to reassess the original protologue and delimit the genus in a more concise manner. Among the features that have been better revealed by studies published after Williams & Round (1987) are, for example, the spines, which are now known to be absent in some species (e.g. *Pseudostaurosira parasitica* (W.Sm.) E. Morales; Morales et al. 2015: figs 95–98), growing on the virgae in others (e.g. *Pseudostaurosira pseudoconstruens* (Marciniak) D.M. Williams & Round; Marciniak 1986: pl. 6, figs 229–232), or even occurring along the striae in some others (e.g. *Pseudostaurosira microstriata* (Marciniak) Flower; Marciniak 1986: pl. 6, figs 233, 234). Among the totally new features are the flaps covering

the areolae externally (Morales et al. 2012: figs 45, 52, 53) and the stipulae at the base of spines (Morales et al. 2012: figs 46, 50).

But even with all this new information on *Pseudostaurosira*, it remains difficult to find derived characters that would unify all the species within the genus and at the same time separate them from other araphids without rimoportulae. Certainly, the characters mentioned before (spines and their associated structures) or even the areolae and their substructures (volae, rotae and flaps), the apical pore fields, and the girdle bands, which are traditionally used to separate small araphid genera, are not helpful in this task. Herein, we attempt to establish distinguishing traits through an analysis of features that are less frequently mentioned in araphid taxonomy and systematics, and also try to incorporate a morphogenetic perspective following the recommendations of Cox (2004, 2012) and Mann (1984, 2006).

During our search for SEM information to build a comparative table of distinguishing features, we reviewed several new genera that were erected in recent years, such as *Popovskayella* Kulikovskiy & Lange-Bertalot, *Serratifera* Chunlian Li, Witkowski & Ashworth and *Gedaniella* Chunlian Li, Witkowski & Ashworth, and revise them together with some other araphid species, based on the discriminating characters we propose, making all the required nomenclatural rearrangements.

We also describe a new species in *Nanofrustulum* Round, Hallsteinsen & Paasche from the Andes of Bolivia. This is a small-valved diatom that has unusual features and that we use to test our morphological feature analysis.

MATERIALS AND METHODS

The search for characters for all currently described genera of small fragilarioids lacking rimoportulae was done on the basis of published information (cited throughout the manuscript) and through examination of image databases at the Luxembourg Institute of Science and Technology (LIST) and the Water Laboratory, University of Évora, Portugal.

In addition, two samples were analysed specifically for the present work. The material used for the description of the new *Nanofrustulum* was an epipsammic sample collected from the Desaguadero River, Bolivia, described in Morales et al. (2012). The second sample was diatomite material from Lutilla (Hungarian Natural History Museum, HNHM-ALG-DC-0092), Slovakia, described in Morales et al. (2014b).

For LM analyses, an aliquot of the Bolivian material was boiled for 45 min in 70% nitric acid (1:1 by volume with the sample). The mixture was then rinsed with distilled water until neutrality. Permanent slides were mounted using Naphrax (Brunel Microscopes, Chippenham, UK) and analysed using a Zeiss Universal microscope (Zeiss, Jena) fitted with DIC (Differential Interference Contrast) and a Spot Insight 2 model 18.2 colour digital camera (Diagnostic Instruments Inc., Michigan). Microphotographs were taken at 2000× using a Zeiss PLAN 100×, 1.25 N.A., immersion objective and SpotSoftware version 4.6 (Diagnostic Instruments, Michigan). For the Lutilla material, a rehydrated raw sample was dried, mounted in Zrax (product no longer available) and an-

analysed with a LEICA DM LB2 microscope (Leica Microsystems, Wetzlar), equipped with a FinePix S2 Pro digital camera (Fuji Photo Film, Tokyo), at 1000× using a Leica DMLB 100×, 1.40 N.A., oil immersion objective.

For SEM analysis, a 2 ml subsample of the Bolivian clean material was heated with 100 ml of hydrogen peroxide (35%) for 36 h in a sand bath at 210°C. The supernatant was then removed and 1 ml of hydrochloric acid (37%) added, the material allowed to rest for 2 h, and subsequently rinsed with distilled water until neutrality. The *Lutula* diatomite was used untreated. All materials were then rinsed with deionized water over 3-µm glass fibre filters. Specimens were coated with platinum using a BAL-TEC MED 020 Modular High Vacuum Coating System (Leica Microsystems, Wetzlar) for 30 s at 100 mA and observed with a Hitachi SU-70 electron microscope (Hitachi, Chiyoda), operated at 5 kV and 10 mm working distance. All micrographs were digitally manipulated and plates containing LM and SEM pictures were mounted using CorelDraw X6®.

Morphological terminology follows Barber & Haworth (1981) for valve shape and stria pattern; Cox & Ross (1981) and Cox (2012) for lateral extensions from the sternum (or annulus) (*virgae*) and cross bars developing later (*vimines* and *viminules*) that delimit the areolae; and Williams & Round (1987) and Round et al. (1990) for areolar substructures, spine features, apical pore fields and girdle bands.

RESULTS AND DISCUSSION

Finding distinguishing characters at the genus level

The analysis of several hundred SEM images of various described and potentially new taxa from all continents yielded the list of potential traits displayed in table 1. This table was constructed with difficulty due to the large volume of information available, but also because current genus descriptions in the literature are ambiguous and do not present clear-cut boundaries. The existence of a range of opinions regarding the features to be used for identification and classification of known species is also confusing (for example, see discussion in García et al. 2017, for *Staurosira* Ehrenb.). Also, many of the species names that are currently used refer to several entities that are morphologically similar (i.e. they belong to complexes) but that are clearly differentiated when SEM information is used (Morales et al. 2013, 2014a). In addition, some widely used reference works (e.g. Metzeltin & Lange-Bertalot 1998) do not include a thorough argumentation, a necessary step in building a strong taxonomic basis (Silva in Hegewald & Silva 1988).

The analysed information shows that the characters traditionally used to distinguish genera of small araphids lacking rimoportula (Williams & Round 1987) are in fact problematic, since they are too variable within each genus and often overlapping among different genera. In the case of *Pseudostaurosira*; for example, Lange-Bertalot et al. (2017) stated that the “most characteristic feature” is the short striae. While this may be true for the small and rather heterogeneous (regarding spines, apical pore fields, etc.) group of species included by these authors, consideration of characters in other species reveals that stria length is in fact one of

the most variable characters in this genus and that there are overlaps with species in *Nanofrustulum*, *Pseudostaurosira* and *Sarcophagodes*. It is therefore not surprising that the same authors consider *Pseudostaurosira* as “weakly circumscribed” and that its generitype, *P. brevistriata*, is “a heterogeneous group of species”.

More robust and natural classifications can be achieved by using features that are shared only among the members of a genus and that do not appear in any other groups (Kociolek & Williams 2015). Such characters, synapomorphic for the species of the genus and autapomorphic for the genus, are difficult to recognize when limited morphological information is available. Fortunately, as many new species have been erected in recent years (e.g. Witkowski et al. 2010, Morales et al. 2012, Kulikovskiy et al. 2015) and type material has been reanalysed with both high resolution LM and SEM (e.g. Edlund et al. 2006, Cejudo-Figueiras et al. 2011, Morales et al. 2015), the ranges of variation of many features have become clearer and the status of particular characters – whether or not they are synapomorphies – can be assessed. Herein, we present only a first step in the recognition of these unique characters that separate genera. Later on we will present tests to evaluate their status as synapomorphies.

It must be added that having a well-delimited generitype gives an idea of the Bauplan (body plan) of that species and its evolutionary associates within the genus. However, as stated by Cox (2010), analysing characters that are fully formed can lead to a misappreciation of evolutionary relationship, since the morphogenetic basis for character variation remains hidden when only the end products of valve morphogenesis are considered. Therefore, pondering developmental pathways underlying character formation may be useful to establish the nature of these characters, why they vary, and whether they are homologous.

Unfortunately, there are not very many studies of valve morphogenesis in araphid diatoms, certainly not sufficient to represent the great diversity within the group. But some information available for diatoms in general lays a foundation for understanding the group of araphid diatoms treated here. For example, Cox & Kennaway (2004) and Cox (2010) analysed the origin of the sternum, *virgae*, *vimines*, *viminules*, areolae and associated structures, and the work of Kaluzhnaya & Likhoshway (2007) and Sato et al. (2008, 2009, 2011) showed how these features arise in the specific case of araphid diatoms.

Justification of the newly defined distinguishing characters

The characters contained in table 1 help to explain differences among the nine genera described to date, excluding *Gedaniella*, *Popovskayella* and *Serratifera*. These characters are arranged roughly in the order in which they develop during valve morphogenesis. For example, areolar substructures (*volae*, *rotae* and *flaps*) are the last to form when the valve is being constructed and girdle bands form synchronously with, or towards completion of, valve formation, depending on the species; growth of areolar substructures begins when the areolae are already well defined (e.g. Sato et al. 2011). At least part of the variation in the process of valve construction

Table 1 – Comparison of genera of small-celled fragilarioid diatoms lacking rimoportulae.
 Bold indicates important distinguishing features.

Feature	<i>Nanofrustulum</i>	<i>Opephora</i>	<i>Pseudostaurosira</i>	<i>Pseudostaurosiropsis</i>	<i>Punctastriata</i>	<i>Sarcophagodes</i>	<i>Stauroforma</i>	<i>Staurosira</i>	<i>Staurosirella</i>
Virgae shape	doubly flared	doubly flared	doubly flared	doubly flared	doubly flared	doubly flared	rectangular	singly flared towards mantle	doubly flared
Vimen shape	slender and short	without vimines	wide and short	slender and short	slender and short	wide and long	slender and short	slender and short	slender and long
Viminule production	present in parts of the striae	without viminules	without viminules	without viminules	present in the entire striae	without viminules	without viminules	present in parts of the striae	present in parts of the striae
Areolae shape	round to transapically elliptical	transapically elliptical	round to transapically elliptical	round	round to apically elliptical	apically reniform	round to apically elliptical	round to apically elliptical	apically oriented lineolae
Areolae number	always more than two	one	two to many	two to many	always more than two	two to many	always more than two	always more than two	always more than two
Striae type	uniseriate to multiseriata	uniseriate	uniseriate	uniseriate	multiseriate	uniseriate	uniseriate	uniseriate to multiseriata	uniseriate to multiseriata
Volae origin	inner perimeter of the areola	virgae	inner perimeter of the areola	without volae	inner perimeter of the areola	inner perimeter of the areola	inner perimeter of the areola	inner perimeter of the areola	inner perimeter of the areola
Rotae production	without rotae	without rotae	without rotae	present, originating from a single point within the areola	without rotae	without rotae	without rotae	without rotae	without rotae
Girdle element structure	valvocopula: open, without fimbriae, copula: open, ligulae in valvocopulae and copulae morphologically different	valvocopula: open, without fimbriae, copula: entire, open	valvocopula: open, without fimbriae, copula: entire, open	valvocopula: open, without fimbriae, copula: entire, open	valvocopula: open, with fimbriae, copula: entire, open	valvocopula: open, without fimbriae, copula: entire, open	valvocopula: open, without fimbriae, copula: entire, open	valvocopulae: closed, with fimbriae, copula: entire, open	valvocopula: open or closed, with fimbriae, copula: entire, open or closed
SEM references	Lee et al. 1980, Round et al. 1990, Witkowski et al. 2010, Li et al. 2018	Round et al. 1990, Sabbe & Vyverman 1995, Li et al. 2018	Williams & Round 1987, Cejudo-Figueiras et al. 2011, Morales et al. 2015	Morales 2001, Morales 2002	Williams & Round 1987, Hamilton & Siver 2008	Morales 2002	Flower et al. 1996, Talgatti et al. 2014	Williams & Round 1987, Morales 2006	Williams & Round 1987, Morales, 2006, Morales & Manoylov 2006a, 2006b

could arise from interactions with the environment through selection and adaptation, generating reproducible, inheritable patterns of valve formation (see discussions by Mann 1999, 2010); at least some of these variations could represent features expressing evolutionary relationship among the different genera.

We hypothesize that the formation of striae begins soon after the formation of the virgae (in *Opephora*), vimines (in *Pseudostaurosira*, *Pseudostaurosiropsis*, *Sarcophagodes* and *Stauroforma*) and viminules (in *Punctastriata* D.M. Williams & Round). We are herein using a restricted concept of *Opephora*, following Round et al. (1990) and Morales (2002). In exceptional cases, species of *Nanofrustulum*, *Staurosirella*, and *Staurosira* produce striae that are partially or completely multiserial (Morales 2005), but in these genera the striae appear with the formation of the vimines, the production of viminules being rather sporadic and insufficient to characterize these genera as multiserial as a whole. Only in the case of *Punctastriata* are all the striae always and entirely multiserial invariably in all species.

During stria development, the areolae near the axial area form first and the ones near the valve margin form last (Cox 1999, 2010, Sato et al. 2011). In all the genera considered here, the areolar size increases toward the valve face-mantle junction and from there to the margin the size decreases in size again. In the case of *Opephora* with a single areola running uninterrupted from the valve face to the mantle, the ends on both valve face and mantle taper so that a transapical elliptical shape is formed (Round et al. 1990: 382, 383, figs d–j). In genera having a multiserial areolar pattern, there are fewer areolae towards the extremes of the striae, so more or less elliptical striae shapes are produced (see illustrations for *Punctastriata* in Round et al. 1990; the case of the new species placed in *Nanofrustulum* is discussed below).

Because of this difference in the diameter or number of the areolae within the striae, the virgae in the genera considered should tend to be flared (i.e. they should become wider) at both ends; however, this is not always the case and genera tend to differ in specific patterns. We define the “shape of virgae” to be the geometrical pattern that the virgae assume in relation to neighbouring striae. Some taxa, such as *Stauroforma*, always have rectangular virgae and this is because these structures are thickened and raised in internal and external views, and remain somewhat independent from stria shape (Van de Vijver et al. 2002: pl. 15, figs 24–26, Genkal et al. 2011: pl. 19, figs 11, 12). *Staurosira* also has a unique virga shape. Here there is an enlargement of the valve face areolae as the striae proceed towards the valve margin, but the influence of smaller areolae on the shape of the virgae is compensated by the elevation of the latter. On the valve mantle, the virgae are less raised and therefore the smaller areolae found at the end of the striae make these virgae appear subtly flared (Lange-Bertalot 1989: pl. 5, figs 1, 2).

As stated before, there is an underlying mechanism for virga and areola formation, where the timing of areola production is the result of how the virgae and their associated structures (vimines and viminules) develop. The thickening of the virgae, as the striae and associated features develop, also has an influence on stria formation, especially on their

internal structure (Sato et al. 2011). The majority of genera possess a conspicuous depression along each stria, the end result of virgae and sternum reinforcing in a trans and perivalvar direction. The thickened structures grow up above the striae often giving the impression of a semi-chambered internal structure like an alveolus (see for example Morales et al. 2015: fig. 141, for *Pseudostaurosira*; Round et al. 1990: 359, fig. E, for *Punctastriata*; Grana et al. 2018: figs 47, 48, 51 for *Staurosira*; and Morales et al. 2010c: fig. 47, for *Staurosirella*).

The features of the vimines and viminules determine the characteristics of the areolae they delimit, except in *Opephora* where the single areolar aperture runs from valve face to mantle. Here, therefore, the extension and shape of this single opening is determined solely by the degree of development of the virgae and sternum (Round et al. 1990: 382, 383, figs d–j). In the case of *Staurosirella*, the virgae are well separated from each other and the vimines are long (length expressed as the apical distance between virgae) and narrow, delimiting closely spaced, elongated openings or lineolae. The length and reduced width of the vimines in *Staurosirella* are unique, while in the case of *Pseudostaurosira* and *Sarcophagodes* the vimines are also long and wide, but they are thicker (thickness expressed as the transapical distance between areolae) in the former. The remaining genera either lack vimines or have them slender and short.

As stated previously, the production of viminules is constant in all species of *Punctastriata*, therefore this is a defining characteristic for this genus. Although *Nanofrustulum*, *Staurosira* and *Staurosirella* also produce viminules, in this case the biserial nature of the striae is expressed only on portions along the same striae or on an entire stria within the same valve (Lange-Bertalot 1989, Morales 2005). Again, since there is an underlying ontogenetic mechanism and the end products are different, areolar shape and the varying features of vimines and viminules can be used as distinguishing features.

The stated differences in the formation of the virgae also determine the sites of production of structures within the areolae (Sato et al. 2011, Cox 2012). The volae arise from the virgae in *Opephora* and define and delimit the areolar openings (Round et al. 1990: 382, 383, figs e, f), while in the rest of the genera volae are either absent (e.g. *Pseudostaurosiropsis*: Morales 2001: figs i, j) or they are born from the inner perimeter of the areola, either on vimines or viminules (e.g. *Pseudostaurosira*: Morales et al. 2012: fig. 48). Rotae are produced only by *Pseudostaurosiropsis* (Morales 2002: pl. 2, figs 5, 6) and originate from a single point, usually near the base of a spine. In areolae that are not in close proximity to a spine, the rotae arise from a point on the side closest to where the spine is located (Morales 2001: figs 7a–d, g). The points of origin of the rotae are especially clear when the rotae are beginning to be formed or when they are somewhat eroded (Morales 2001: figs 7e, i, j). Rotae can be confused with ‘flaps’, but flaps originate from the base of spines or from other regions around the areolar perimeter, and a single areola can have several flaps (Morales et al. 2012: fig. 45). Flaps also differ from rotae in that they are produced on top of volae (Morales et al. 2012: figs 51–53), whereas rotae de-

velop in isolation within the areolar opening (Morales 2001: figs 7i, j).

Volae are very similar in the eight genera included in table 1 (excluding *Pseudostaurosira*). The only difference is in the degree of thickening and development. In general, these are highly (dichotomously) branched structures that originate from the inner areolar opening, at different points in *Pseudostaurosira* (Morales 2003: figs 60–65). Smaller, thinner and less branched volae are produced in *Punctastriata* (Lilitskaya 2016: pl. 4, fig. 2), while larger, thicker, and profusely branched are produced in the large-valved species of *Pseudostaurosira* (Morales & Edlund 2003: figs 45–50).

Although there is not sufficient information on girdle band formation to permit any generalization, as shown by Tiffany (2015), these structures begin their formation early in valve development, but the end of the process terminates after the margin of the valve has formed. The latter begins its solidification as the last mantle areolae complete their definition, once the distal ends of the virgae fuse (Sato et al. 2011). Though there are no specific studies for these rimoportula-lacking araphids showing the formation of individual girdle elements, it is known that for diatoms, in general, each of these elements forms within its own silica deposition vesicle and that there are differential interactions with previously formed parts of the valve, i.e. the mantle as a whole, composed of virgae and striae (Mann 1984, Round et al. 1990). Therefore, there is a morphogenetic basis for the structural difference between those girdle elements. The valvocopula, for example, has a pars interior that is recessed to accommodate the valve mantle and to attach internally to it, sometimes directly (as in, for example, *Nanofrustulum*, *Opephora*, *Pseudostaurosira*; e.g. Morales et al. 2012: fig. 49), otherwise by means of delicate, narrow extensions called fimbriae, that attach to the internal faces of the virgae (as in, for example, *Staurosira* and *Staurosirella*; Morales 2005: fig. 103). The rest of the girdle elements in small araphids lacking a rimoportula tend to be plainer, but they can be open or closed. Sometimes, the same girdle is composed of open and closed bands as in the case of *Staurosira* (Morales 2006), or the girdle may contain all closed or all open elements, as in *Staurosirella* (Morales 2006). In other cases, the morphological difference in the copulae is even more extreme, as in *Nanofrustulum*, in which the copulae are much smaller than the valvocopulae, arranged around the girdle and attached to other copulae by well developed ligulae (Round et al. 1999, Morales 2001, Witkowski et al. 2010, Wetzel et al. 2013). Such differences among elements of the same girdle are not exclusive to small araphids. For example, the large araphid *Glyphodesmis* Grev. (Round et al. 1990: 240, 241, figs c, i, j) and the raphid *Tursiocola* R.W.Holmes, S.Nagas. & Takano (Frankovich et al. 2015) also have these differences between valvocopulae and copulae.

This view (that the nature of the girdle bands is variable among closely related taxa) conflicts with the current consideration of the open and closed nature of girdle bands as a distinguishing feature in *Fragilaria* and *Ulnaria* (Nitzsch) Compère (Compère 2001) (closed in the latter) (Williams 2011). Molecular phylogenies using four genes show a weak difference between the two genera (2.5%), insufficient to justify a morphological separation based on known characteristics, as

stated by Medlin et al. (2012). The latter authors also stated that looking to other sources of information such as reproduction might reveal further differences. Until such studies are done, the open or closed feature of girdle bands remains a practical way of separating species in the two genera even though the synapomorphic nature of this feature is challenged from a morphogenetic standpoint. Besides, a wider analysis of other morphological features, in the light of the numerous new species that are being published for both genera, it could be possible to find synapomorphies; we reserve this task for later.

Are there any other distinguishing characters?

There are perhaps other sources of distinguishing features among the nine genera delimited in table 1. For example, morphogenesis of apical pore fields and spines might hold useful information. We have detected several variations in the structure of apical pore fields such as presence/absence, external and internal depressions, internal and external troughs, rims surrounding the pores, etc. It is known that apical pore fields appear earlier in the formation of the valve of araphids possessing a rimoportula, and they complete their formation before the completion of silicification of the valve (Tiffany 2002, Kaluzhnaya & Likhoshway 2007, Sato et al. 2011). However, the process of formation of associated structures such as the troughs seen in *Pseudostaurosira parasitica* (Morales 2003), the “volcano” shaped domes seen in individual poroids of *Stauroforma inermis* Flower, V.J.Jones & Round (Morales 2001), or the whitish rims frequently seen in species of *Staurosira* and *Staurosirella*, remain unknown.

Likewise, there are several character states for spines in araphids, which seem to be interesting from the ontogenetic standpoint. For example, certain taxa are reported as having hollow or solid spines (see discussion in Morales & Manolov 2006b), some even have spines with a soft core (Morales et al. 2015: fig. 45). Yet it is difficult to determine whether spine features can be regarded as distinguishing at some level, simply because we do not know the mechanism of their formation and the actual processes determining whether spines are solid or lack a “filling” material altogether. It is possible that at least some of the reports of hollow or solid spines may be more related to stages of development than to actual character states (e.g. Morales et al. 2010b: figs 45, 47). Whether spines are formed in a fashion similar to centric diatoms, in which there is an active deposition of siliceous material guided from within the cell (Pickett-Heaps 1998), merits study. What is known thus far about cell wall formation in araphids, however, suggests that a centric-like mechanism might be more likely when spines are produced along striae, since holes would be readily available for cytoplasmic expansion from within the cell. In the case of spines growing on the virgae, it is surprising that one never finds openings within virgae in internal views of the valve face–mantle junction under SEM. Since sites of production of features such as areolae and apical pore fields are fixed within the organic apparatus that originates the new valves, it is logical to assume that there are also sites for spine production. The suppression of such sites would be a relatively simple evolutionary step, perhaps triggered by genetic, environmen-

tally-induced, or even mechanical influences. Explaining the change of location of sites for spine production from striae to virgae or *vice versa* appears more complex. Yet, there are genera that contain spiny and spineless species (e.g. *Staurosira* and *Staurosirella*; Morales & Manoylov 2006a, 2006b, Morales et al. 2010a) and species that have spines along the stria or growing from the virgae as in *Pseudostaurosira*. Studying the mechanism of spine production-suppression, therefore, could elucidate evolutionary relatedness at different taxonomic levels.

We disregarded the features of the sternum as a source of distinguishing traits because the formation of the new valves begins with the formation of the sternum and the process seems to be common not only to all araphids, but to all pennates (Cox 2012).

Redefinition of the genus *Pseudostaurosira*

Pseudostaurosira D.M. Williams & Round emend.
E. Morales

Generitype – *Pseudostaurosira brevistriata* (Grunow) D.M. Williams & Round.

Microscopical study of generitype material – Morales et al. (2015: figs 107–127 (LM), 128–143 (SEM)).

Description – Frustules symmetrical in side view, forming chains in taxa that possess spines. Taxa with incipient or no spines, presumably attached by mucilage stalks. Valves cruciform, bigibbous, lanceolate, rhombic or elliptic. Sternum of variable width and shape. Transition from valve face to mantle varies from abrupt to more gradual, with formation of a transition zone. Striae composed of one to (rarely) a few rows of wide, round, transapically elliptical, or irregularly polygonal areolae running from valve face to mantle. Internally, areolae open into a single depression running from valve face to mantle, which may become chamber-like. Volae highly branched, usually dichotomously, arising from the inner perimeter of the areolae and at different depths. Virgae rectangular or flared at their proximal and distal ends. Vimines short and wide, the one at the valve face-mantle transition usually being wider, giving the impression of a single apical rib running along the valve face margin. Viminules seldom produced and, when formed, occupying only a part of a stria. Flaps or flat siliceous growths originate from several points around the external perimeter of the areolae, close to their external surface. Concentric discs sometimes present, partially occluding the depression into which the areolae open internally. Solid spines can grow from the enlarged vimen that connects the virgae along the valve-face edge or from the virgae themselves, or they can be absent. Spines of varying shape, but usually spatulate, with or without terminal branching, with a cylindrical base, and often bearing stipulae. Stipules produced near the base of spines and projecting downward. Apical pore fields absent, reduced or more fully developed, of the ocellulimbus type; in many cases they are sunken into the apical portion of the valve. They are composed of round poroids, which are sometimes arranged along external troughs parallel to the apical axis of the valve. Mantle plaques present in many species and situated along the abvalvar edge of the valve mantle. Cingulum

composed of a larger valvocopula and few to many ligulate copulae, always open, lacking fimbriae and perforations.

Distinguishing character – Wide and short vimines. Though these vimines are also found in other genera having round areolae, such as *Nanofrustulum* (Li et al. 2018: figs 286–289), *Pseudostaurosireopsis* (Morales 2001: figs 7a–d, g, i, j, l) and *Sarcophagodes* (Morales 2002: pl. 5, figs 1–3), in *Pseudostaurosira* they are wider due to transapical expansion during valve ontogeny, and shorter due to the areolae being transapically expanded, thus having their shortest axis parallel to the longitudinal axis of the vimines (Morales & Edlund 2003: figs 39, 45, Cejudo-Figueiras et al. 2011: figs 108–111, Morales et al. 2012: figs 42, 48).

Other salient, but not unique features – Rectangular or doubly flared virgae. The degree of flaring varies depending on the size reduction of terminal vimines (those located towards the axial area of the valve and towards the abvalvar edge of the mantle) and the shape of terminal areolae along a stria (Morales et al. 2015: figs 128, 129, 137, 141, 142).

The striae lie within an internal depression. This depression varies among species from shallow (Witkowski et al. 2010: figs 50–55) to deep, the striae appearing semi-chambered (Morales 2002: pl. 4, fig. 2).

There is a high variability in spine presence, position and associated structures. Spines can be absent (Morales et al. 2015: figs 95, 96, 99, 100, 103, 104), or produced on virgae (Round et al. 1990: 356, 357, figs d, e) or on vimines (Cejudo-Figueiras et al. 2011: figs 100, 101, Morales et al. 2015: figs 130, 132, 135). Spines are always solid, although the presence of a soft core in some species gives the impression of spines being hollow after breakage and erosion (Edlund et al. 2006: figs 18, 20).

Stipules and flaps are produced. The stipules, originating at the base of the spines and projecting at an angle, away from the valve mantle, are a rather common feature among spiny species of *Pseudostaurosira* (Morales et al. 2012: fig. 50). The shape of these structures varies among species (Morales 2002: pl. 4, fig. 6). Stipules are also present in other genera such as *Nanofrustulum* (Li et al. 2018: fig. 288, as “lateral projections”) and *Punctastriata* (Williams & Round 1987: fig. 43; Flower 2005, fig. 6). Stipules are frequently helpful in the SEM identification of chains in valve view, which can easily be confused with chains of *Staurosira*, but where stipulae are always lacking.

Flaps originate from different points along the inner perimeter of the areolae and sometimes resemble disks lying atop the volae. Morales et al. (2012: fig. 45) show a valve in which some mantle flaps are broken, while others (that originated very close to the basis of the spines on the valve face) are still attached. Figure 50 of the same publication (Morales et al. 2012) is helpful in the distinction of stipules and flaps; stipules can be seen projecting downward along the mantle and originate from the body of the spine.

At least two species (*Pseudostaurosira decipiens* E. Morales, G. Chávez & Ector and *Pseudostaurosira laucensis* var. *vulpina* Lange-Bert. & Rumrich) have been found to produce internal accumulations of siliceous material on the volae, depositions that appear as two concentric disks (Morales et al. 2012: figs 40, 44; E. Morales, unpublished obs.).

The functions of these are unknown, but they can be used to help identify the two species. However, in *P. laucensis* var. *vulpina*, eroded valves lack depositions (e.g. Rumrich et al. 2000: pl. 10, fig. 9).

Apical pore fields are variable among different *Pseudostaurosira*. In this genus they invariably occupy the mantle portion of the apex and never subtend striae, unlike in e.g. *Staurosira* (Morales et al. 2012: figs 57, 58, 62). Their development ranges from absent or present even in the same species (e.g. *Pseudostaurosira sajamaensis* E.Morales & Ector: Morales et al. 2012 and may be slightly developed (e.g. *P. brevistriata*: Morales et al. 2015) or fully developed (*P. parasitica*: Morales 2003). Internally, poroids open into a single, circular depression, as seen in the type of *P. brevistriata* (Morales et al. 2015: figs 140–142), but this feature might also be present in species of *Staurosirella* (e.g. Kulikovskiy et al. 2015: pl. 10, fig. 22). In some cases there are external transapical troughs along which there are lines of pores (Morales 2003: figs 54–58, 64).

Lange-Bertalot et al. (2017) considered that the width of the sternum is a diagnostic feature of *Pseudostaurosira* (see also the dichotomous key presented in Li et al. 2018). They even placed species such as *Pseudostaurosira subsalina* (Hust.) E.Morales in *Staurosira* due to their narrower axial area. There is no support for this, however, and such an area should be regarded as a variable character that can be diagnostic, but at the species level, e.g. to distinguish *Pseudostaurosira elliptica* (Schum.) Edlund, E.Morales & S.A.Spauld. (Edlund et al. 2006) from *Pseudostaurosira americana* E.Morales (Cejudo-Figueiras et al. 2011) (see discussion in Grana et al. 2018). *Pseudostaurosira* and *Staurosira* each have their distinguishing features and are well delimited genera (table 1, and compare our emended description herein with the discussion in García et al. 2017). Moreover, the sternum width is also variable in genera such as *Staurosirella* (Morales et al. 2010c) and *Staurosira* (Grana et al. 2018).

The placement of a newly discovered diatom in the nine genera scheme

Nanofrustulum rarissimum E.Morales, Novais, C.E.Wetzel & Ector, **sp. nov.**

Figs 1 & 2

Type material – Bolivia, Desaguadero River, Department of Oruro (17°23'51"S, 68°14'33"W, 3701 m a.s.l.), 5 Jul. 2009, G. Chávez s.n. (holo-: ANSP, slide ANSP GC 26815 partially illustrated here in figs 1A–F (LM), 1G–K, 2A–D (SEM); iso-: Diatomothea Bolivienensis, Cochabamba, Bolivia, slide DBOL-0246)

Description – Frustules rectangular in girdle view. Valves ovoid with broadly rounded head poles and narrower, almost cuneate foot poles (figs 1A–H, 2B & 2D). Length 7.3–9.5 µm, width 2.5–3.3 µm, stria density 13–14 in 10 µm. Sternum narrowly lanceolate (figs 1A, 2B & D). Virgae doubly flared raised externally and internally (figs 1G, 2A, 2D). Transition between valve face and valve mantle abrupt (figs 1G–K & 2A–C). Valve mantle edge parallel to valve face–mantle junction (fig. 2A). Siliceous plaques along valve mantle edge present (fig. 2A). One to several rows of are-

olae present on the same stria, progressing uninterruptedly towards valve mantle (figs 1G–K & 2A–C). Areolae square, trapezoid or elliptical, apically or transapically elongated, all opening internally into a single depression (figs 1G–K & 2A–D). Volae robust relative to areolar opening and arising from the inner periphery of each areola (figs 1G, I–K, 2B & 2D). Spines located on vimines, arising from at least two points along the width of striae (figs 1G–K, 2A & B). A wide, laminar, skirt-like stipula is present near the base of each spine (fig. 1G–K). Wart-like, whitish depositions sometimes present on the valve face, more numerous at the valve head pole (figs 1H, J & 2B). Apical pore fields composed of variable rows of poroids, present at both apices, but more developed at the foot pole (figs 1G–I, 1K & 2B–D). Pore field at head pole located closer to the mantle abvalvar edge (fig. 1G, I & J), while foot pole pore field lies at the transition between valve face and mantle (figs 1G, H, K, 2B & D). Poroids of latter pore field sit within cavernous troughs carved deep into the valve (figs 1G, K & 2C). Girdle composed of an open, ligulated valvocopula lacking perforations, and quasifract, ligulated copulae, also lacking perforations (fig. 2A).

Etymology – “*Rarissimum*” refers to the fact that the features of the new taxon are rather infrequent within the genus.

Taxonomic remarks – Using traditional morphological characters, the new taxon shows several features that link it to *Pseudostaurosira*. The individual areolae bearing well developed volae, the well developed apical pore fields composed of round poroids, and even the position of these poroids within troughs have all been described in other species of the genus. Regarding the areola structure, the new taxon resembles *P. brevistriata*. Observing figs 128–130 and 132 in Morales et al. (2015) and comparing with figures presented herein, the resemblance in areolar openings and the manner in which the volae are born and developing to the valve interior can be seen. Likewise, figs 140–142 in the same publication show how volae project into the valve interior and even collect extra siliceous material, similarly as what happens in the new *Nanofrustulum* species. Other features of the new taxon, similar to those in taxa currently ascribed to *Pseudostaurosira*, are the uneven development of the apical pore fields and the development of troughs at the foot pole, which have also been seen in *Pseudostaurosira clavatum* E.Morales (compare Morales 2002: pl. 4, figs 1, 4, with our figs 1G, K & 2C) and *Pseudostaurosira perminuta* (Grunow) Sabbe & Vyverman (Sabbe & Vyverman 1995: figs 54, 57, 58), though not as developed as in the new taxon.

However, as shown in table 1, *N. rarissimum* has a feature not present in *Pseudostaurosira* as defined here, namely quasifract valvocopulae (“segmented copulae” in Li et al. 2018), only present in representatives of *Nanofrustulum*. Quasifract copulae are present in the type of the genus *N. shiloi* (J.J.Lee, Reimer & McEnery) Round, Hallsteinsen & Paasche (Round et al. 1999: fig. 8). Quasifract copulae are also reported for *Pseudostaurosira cataractarum* (Hust.) C.E.Wetzel, E.Morales & Ector (Wetzel et al. 2013: figs 2F, G), but this species needs to be transferred to *Nanofrustulum* (see below). Viminules are lacking in *Pseudostaurosira* and *Nanofrustulum*, as currently circumscribed, but the new taxon produces them regularly. Due to the complexity of girdle band morphogenesis, the nature of this structure can be con-

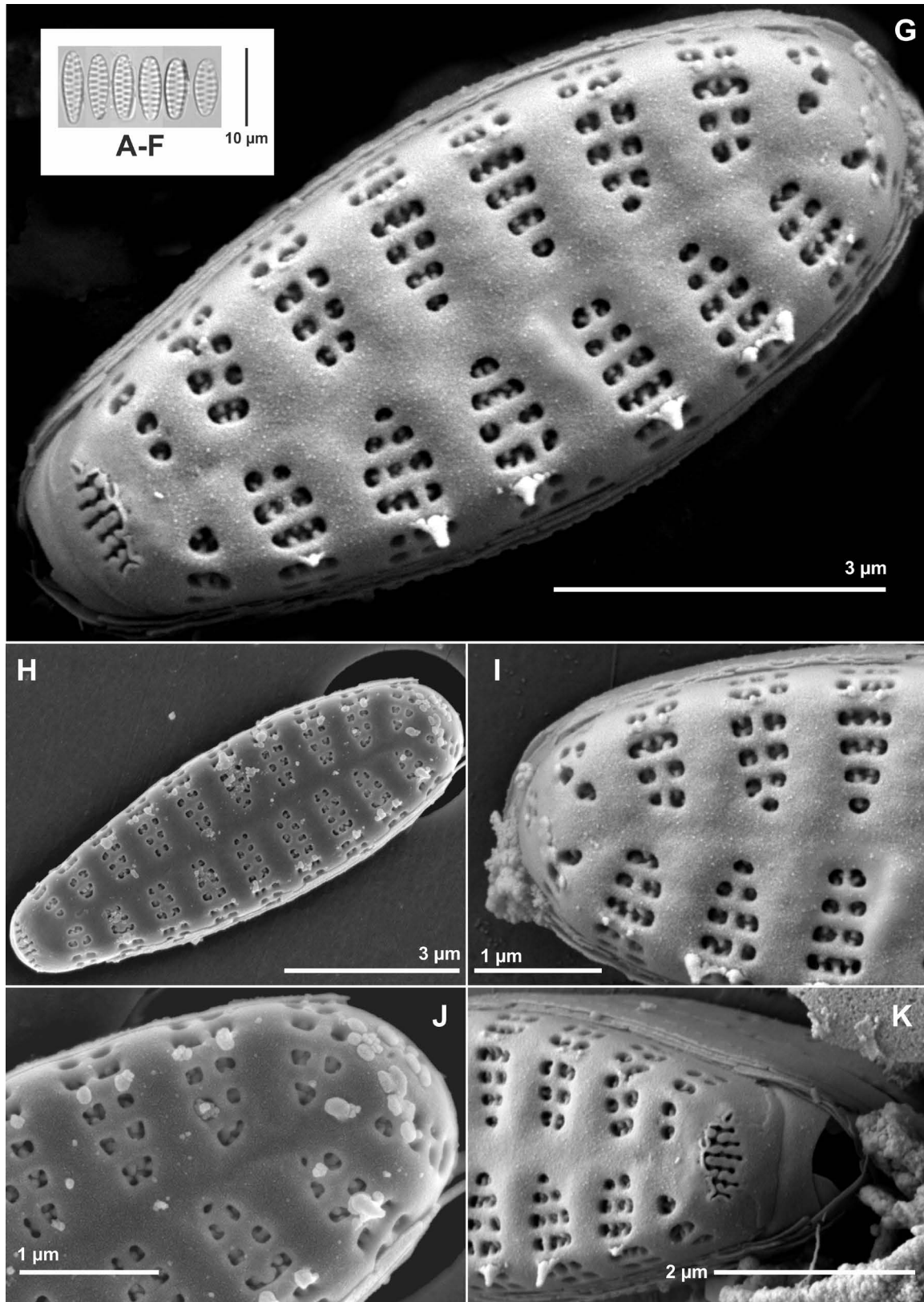


Figure 1 – *Nanofrustulum rarissimum*, LM and SEM images taken from type material (ANSP GC 26815): A–F, size diminution series in LM; G–K, SEM images of external details; G & H, view of entire outer surface showing details of sternum, virgae, striae, spines and apical pore fields. Notice heterovalvarity, also expressed in the apical pore fields, and the skirt-like appearance of the stipulae; I & J, detail of head pole. Notice small apical pore fields near the abvalvar edge of the mantle, and the volae; K, detail of foot pole showing the apical pore field with deep throughs, the closed extreme of the valvocopula and ends of some copulae.

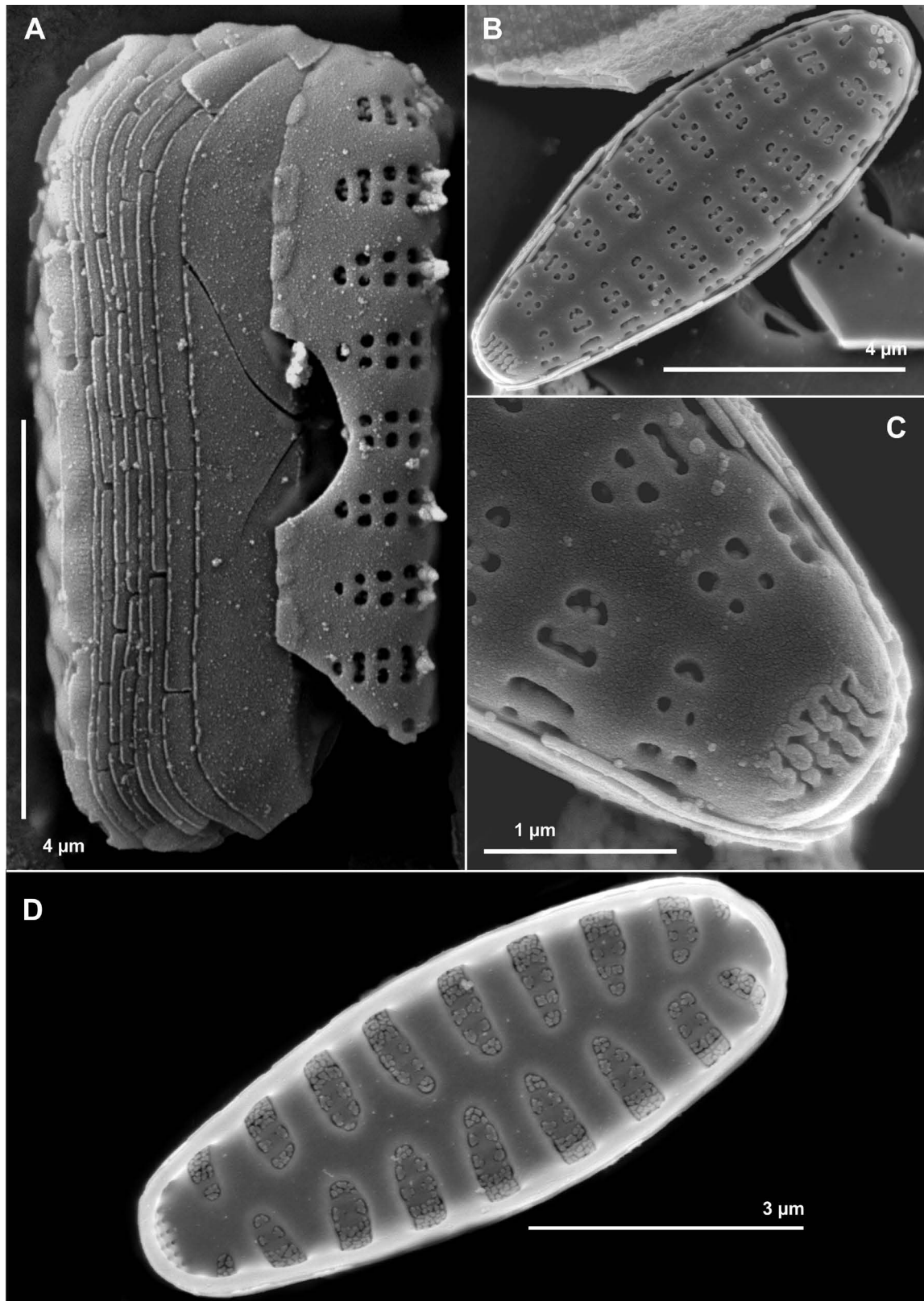


Figure 2 – *Nanofrustulum rarissimum*, SEM images taken from type material (ANSP GC 26815): A, detail of a broken frustule showing girdle structure. Notice entire valvocopula and quasifract copulae, as well as siliceous depositions or plaques on the abvalvar edge of the mantle; B & D, contrast of surfaces. Notice deposition of material on the volae in internal view, and the depressions in which the internal openings of the areolae are contained; C, detail of foot pole showing variability of areolar shape and infrequency of viminule formation.

sidered to have more weight as that of viminules, especially considering that viminule formation may simply require a mechanical reason, i.e. a wider spacing between virgae (Cox 2012). Therefore, *N. rarissimum* can be ascribed to *Nanofrustulum* as the only taxon that produces viminules, thus far.

Another genus that produces viminules is *Punctastriata*. However, the species of this genus have entirely multiseriata striae, with no partial production of viminules as in *N. rarissimum*. Additionally, *Punctastriata* lacks quasifract bands and its valvocopula have fimbriae.

The following combined features differentiating *N. rarissimum* from other taxa currently assigned to *Nanofrustulum* are: heteropolarity of the valves, production of developed apical pore fields and multiseriata areolae, the latter being the most prominent feature characterizing the new taxon. Another salient feature is the heteropolarity of the apical pore fields and the cavernous structure of the one at the foot pole.

The epipsammic sample from Bolivia in which *N. rarissimum* was found contains several new araphid taxa (Morales et al. 2012). It is characterized by a high taxon richness (228 species and varieties), with a high proportion of unknowns. *Nanofrustulum rarissimum* was rare in the material, and does not appear in a 500 valve count made by Morales et al. (2012: table 1). Analysis of nearby localities, other than the Desaguadero River (Morales, unpublished data) did not yield additional valves of the new taxon, suggesting that it is restricted to the river. One reason could be that the river is dominated by high sediment in the water and the entire river bed at the site of collection is dominated by sand and finer sediments, with no development of aquatic vegetation. The characteristics of sites nearby are quite different, varying from small streams with high flow of clear water (with beds covered by rock, moss and other High Andean vegetation), to shallow lentic systems that are ephemeral or permanent and fed by ice melting or ground water (with emergent vegetation and with much lower deposition of fine sediments and sand). These latter waterbodies are strongly affected by metamorphic rock due to their proximity to the Sajama volcano, while although the Desaguadero has the same rock bottom, it is isolated under several metres of fine sediments on top.

The richness of diatom taxa in the Desaguadero River sample is not uncommon for samples collected in the pristine area of the Titicaca-Sajama Volcano region, far from human-related activities (Morales et al. 2012, 2014c) and contrasting with the European view of the Andean diatom flora (Metzeltin & Lange-Bertalot 1998, Rumrich et al. 2000). The contrast could be explained by the areas that have been commonly collected by foreign scientists, which tend to be located more along roads and close to populated areas, where human activities affect ecosystems and tend to make habitats more uniform and similar in the assemblages they contain (Morales et al. 2012, Goldenberg Vilar et al. 2014).

Assessing the validity of *Popovskayella*

SEM images of *Popovskayella* from Lake Baikal are reminiscent of *Pseudostaurosira*. In fact, Kulikovskiy et al. (2015) state that the latter is the most closely related genus. The distinguishing features of *Popovskayella* are the presence of “one apical row of very small areolae” (which we interpret

as one row on the valve face and one on the valve mantle on each stria, based on the fact that none of its species has a single areola composing the striae), the lack of branched volae, the presence of three areolae on the transapical striae, and the presence of a single internal groove running from valve face to mantle, through which, the external openings of individual areolae can be observed. The protologue also mentions the presence of sponge-like silica membranes. Let us consider each of these features.

Because striae are formed independently from each other during valve ontogeny, it is difficult to conceive the existence of “apical striae”. That is, in araphid diatoms striae are morphogenetically transapical structures. Therefore, it does not seem useful, at least for small araphids, to consider that areolae are arranged in apical rows and use this as a taxonomic criterion. What is more, the single apical alignments of areolae (one on valve face and one on valve mantle) are also present in several representatives of *Pseudostaurosira*, such as *Pseudostaurosira tenuis* E.Morales & Edlund (Morales & Edlund 2003), *Pseudostaurosira elliptica* (Schumann) Edlund, E.Morales & S.A.Spauld., *Pseudostaurosira microstriata* (Marciniak) Flower, and the generitype *Pseudostaurosira brevistriata* (Grunow) D.M.Williams & Round (Morales et al. 2015). The areolar opening in the latter taxon is much wider than in any of the *Popovskayella* taxa, but areolar diameter is not a distinguishing character since all the genera in table 1 vary widely in the size of the areolar opening. Regarding volae, it is difficult to make out why Kulikovskiy et al. (2015) stated that there are no closing plates since these structures occur in the generitype *Popovskayella nanobaculum* Kulikovskiy & Lange-Bert. (Kulikovskiy et al. 2015: pl. 11, fig. 15), *Popovskayella simplex* Kulikovskiy & Lange-Bert. (Kulikovskiy et al. 2015: pl. 12, fig. 8), *Popovskayella minutula* Kulikovskiy & Lange-Bert. (Kulikovskiy et al. 2015: pl. 13, fig. 8) and *Popovskayella tenerrima* Kulikovskiy & Lange-Bert. (Kulikovskiy et al. 2015: pl. 14, fig. 8). These volae can obviously not be highly branched since the areolar aperture is narrow in all these taxa, impeding the profuse development of volae, but it is evident that they are in fact dichotomously branched (e.g. see Kulikovskiy et al. 2015: pl. 13, fig. 8).

What Kulikovskiy et al. (2015) regarded as a single areola in internal view is in fact the extreme expression of the depressed striae that are present in all nine genera in table 1. What they term an areola in internal view does not correspond to such a structure from a morphogenetic perspective. Areolae are formed by production of viminules, which in conjunction with the virgae delimit the areolar openings. A single internal opening, therefore, corresponds to a depression where individual areolae open to the valve interior, in this case much deeper due to heavy silicification of sternum and virgae. Because of the small size of the valves in all the *Popovskayella* species, it is expected that the stria depression becomes deeply marked as the sternum and the virgae thicken in a transapical and peralvar direction. In small-valved taxa of *Pseudostaurosira* such deep depressions can be seen, as in, for example, *Pseudostaurosira microstriata* (Marciniak 1982: pl. 2, fig. 6) and *Pseudostaurosira tenuis* (Morales & Edlund 2003: fig. 41).

The sponge-like silica membranes seem to be a misinterpretation of the structure shown in Kulikovskiy et al. (2015: pl. 12, fig. 5). This figure shows small volae projecting inward from the inner periphery of the areolae. The apical portions of the valve in this figure are covered by material which could correspond to excess amounts of the coating applied for SEM analysis or to an organic layer impregnated with mineral depositions, frequent in material that is only partially clean.

Using the information for *Pseudostaurosira* in table 1, we conclude that *Popovskayella* species are in fact members of the former genus and should be transferred to this genus (see section New combinations). They have as distinguishing features wide and short vimines, they lack viminules, their areolae are round to transapically ellipsoid and arranged in a single series along the striae. The volae originate from the inner perimeter of the areolae, and there is no evidence of rotae. Unfortunately, there is no information on girdle band structure for any of the species.

Evaluation of genera based on molecular information

The molecular phylogenies presented to date for small araphids lacking rimoportulae are based on very limited taxon sampling. Genera such as *Pseudostaurosira*, *Staurosira* and *Staurosirella* now contain dozens of taxa (Guiry & Guiry 2018) and including even ten species per genus in any phylogeny (many are currently represented by only one in most trees) might still be unrepresentative of the larger groupings. Thus far, the separation of small araphids from the larger fragilarioids having a rimoportula seems to be convincingly resolved; this seems to hold regardless of the genes used (Medlin et al. 2008). A more recent tree, used for many important taxonomic decisions (Li et al. 2018), does not offer better resolution than that of older phylogenies.

Ninety-nine strains were used by Li et al. (2018: table 3), representing 33 taxa (six of which are unknown) in eight known and one unknown genera. Eight of the nine genera included in our table 1 appear in that phylogeny (Li et al. 2018, S1), where they are represented by ten known species (with several strains) and nine undetermined strains. Of the strains used, 119 belong to eighteen genera, but twelve of them are represented by three strains or less. Although the phylogeny presented by Li et al. (2018) is informative, taxon sampling can be a serious shortcoming in any phylogenetic analysis (Theriot 2008, Theriot et al. 2010) and perceptions of relationships may change drastically as more taxa and genes are added (Sato et al. 2008, Theriot 2008). Additionally, the approach adopted by Medlin et al. (2012), Medlin & Desdevises (2016), and Li et al. (2016), which all have tried to fit morphological data into inferred molecular phylogenies *a posteriori* is risky (Theriot 2008, Williams 2013), because phylogenies and morphology are not treated using a common methodology, or because morphological data are not analysed at all before they are incorporated into phylogenetic reconstructions. This may explain why the morphogenetic approach we follow here, yielding a clear-cut pattern among the nine genera presented in table 1, appears to be in conflict with the conclusions of previous authors (Medlin et al. 2012, Medlin & Desdevises 2016, Li et al. 2018).

Superimposing ecological information *a posteriori* onto phylogenies also requires further and careful consideration. For example, Medlin & Desdevises (2016) stated that the members of their new family Staurosiraceae (comprising *Nanofrustulum*, *Opephora*, *Plagiostriata* and *Staurosira*) are bottom-dwelling or planktonic, never epiphytic. However, Frenguelli (1945) reported that, for example, *Opephora schwartzii* (Grunow) P.Petit ex Pelletan grows on marine littoral algae, and Sullivan (1978) listed the same species and two additional ones in the same genus from salt marsh spermatophytes. We add that ecological information should also be analysed using a common methodology, before being added to phylogenetic interpretations.

Williams (2013) took the phylogeny-morphology discussion a step further and expressed that the phylogenies for araphid diatoms presented in the literature do not provide evidence for the nodes formed during tree construction. For us, this deficiency is hard to justify given the amount of available morphological information on fossil and extant araphid diatoms. Indeed, the simultaneous treatment of morphological and molecular data is possible as demonstrated by Frankovich et al. (2018), and this approach might produce a more parsimonious consideration of species and their relationships.

Perhaps, one of the most conspicuous shortcomings in the *a posteriori* attempt to merge morphological and molecular data is that the morphological characterization of taxa used in phylogenies is poorly done. In Li et al. (2018), for example, the size diminution series for taxa presented in figs 39–129 contains a mixture of morphological variants that do not seem to fit with each other. Since the authors stated that all microscope analyses were performed using old and new cultures, as well as field collected material, their arrangement of LM photographs and SEM plates seems rather haphazard. This of course, is counterproductive for users of plates and figures at the bench, with the risk of producing misidentifications and ecological misinterpretations. This is precisely one of the implications of Theriot's (2008) and Williams' (2013) reasoning: the degree of attention given to the molecular part of these studies is not the same as that given to the morphological data, to the point that it seems rather impractical (and risky) to join them together.

As stated before, the large amount of work done on analyses of type material of many araphid species has greatly clarified the boundaries of taxa widely cited in the literature and used for applied purposes (e.g. Morales et al. 2015). Therefore, the rather hasty use of names for identification of strains used for phylogenetic reconstruction (e.g. Medlin et al. 2008) is completely unjustified (see also argumentation by Williams 2013). Thus, it seems that investing more effort in the so called "total evidence approach", combining molecular, physiological and ecological information, together with data from nomenclatural types, would add value to current phylogenies.

Clearly then, the elucidation of molecular phylogenies and the search of robust trees that would indicate stable, trustable and statistically plausible relationships among taxa is work in progress. Meanwhile, it is possible to turn our eyes to alternative, equally trustable and testable hypotheses that

find support from expressed features, which in the end are the targets of natural selection (Mann 1999). Besides, there is now evidence pointing to the evolutionary functionality of valve features for nutrient acquisition, control of diatom sinking rate and turbulence, as antipredator mechanisms, etc. (Aitken et al. 2016), features that undoubtedly have an underlying genetic mechanism for their generation.

Also using table 1, we can analyze the validity of *Serratifera* (Li et al. 2016) and *Gedaniella* (Li et al. 2018), two genera erected based on molecular information. Using the distinguishing feature approach, there is no one feature that would separate *Serratifera* from the rest of the nine genera included in table 1. The general Bauplan of this genus is reminiscent of *Pseudostaurosira*. Li et al. (2016) stated that their new genus can be characterized by lack of rimoportulae (all genera considered in table 1 do), possession of a “short, single round-to-elliptical areolae per stria” and lack of mantle areolae. Since in their ontogenetic explanation the authors refer to the striae as a single structure running continuously from the axial area to the mantle, considering striae as composed of single areola and then mentioning a lack mantle areola is an unnecessary redundancy. Their figs 2a–f show valve formation in *Serratifera varisterna* Chunlian Li, Ashworth & Witkowski, the generitype, which mirrors the process we described above: the areolae that appear in a mantle in related diatoms are simply covered with a further growth of the virgae – a slit between virgae is produced initially and then gradually reduces in length until only one areola is left on the valve face. During this process, a short and wide vimen (the distinguishing character of *Pseudostaurosira*) is formed at the future valve face-mantle transition (fig. 2c) and then the open virgae ends fuse and fill all the space on the mantle (fig. 2d). In fact, filling of the areolae is a common process in some species of *Pseudostaurosira*. In *P. subsalina*, for example, some of the areolae are filled in on the valve face (Cejudo-Figueiras et al. 2011: figs 94, 97), and this also happens in *Pseudostaurosira alvareziae* Cejudo-Figueiras, E.Morales & Ector (Cejudo-Figueiras et al. 2011: figs 103, 104). This latter species also presents filled-in areolae on the mantle (Cejudo-Figueiras et al. 2011: figs 101, 103), a process also occurring in *P. sajamaensis* (Morales et al. 2012: fig. 55).

Therefore, the valves produced in *S. varisterna* are congruent with those in *Pseudostaurosira*, except for the production of mantle areolae; we propose to consider the latter as a feature at the species level, not as a discriminating character for the genus. That is, the shape of the areolae, features of the volae, the entire, open, ligulate, non-areolate girdle bands, and even the way colonies are formed (Li et al. 2016: fig. 11) in species of *Serratifera*, are all shared by species currently placed in *Pseudostaurosira*.

In 2018, Li et al. amended their protologue of *Serratifera* to include species having two areolae per striae (sometimes even three), one located on the valve mantle. They added (Li et al. 2018: 63) “...we have been unable to find a synapomorphic character to define this genus”. Then, they stated that several combinations of characters can be used to distinguish the species they describe in the genus, which (we add) could be used to perfectly distinguish them once they are transferred into *Pseudostaurosira* instead. The fact that

three *S. varisterna* clones (*S. varisterna* is the generitype of *Serratifera*) cluster together with clones of *Nanofrustulum* and *Pseudostaurosira* in a subgrouping within their “Clade ‘A’ Fragilariaceae” is partial molecular support for our conclusion (Li et al. 2016: fig. 10).

The genus *Gedaniella* (Li et al. 2018) contains at least two forms that can be easily separated from each other: those with entire, open, unperforated girdle bands and those with quasifract copulae. Unfortunately, the type species *G. boltonii* Chunlian Li, Krawczyk, Dąbek & Witkowski is in itself a mixture of these two forms, which we separate in the new combinations section below. The larger valves shown by Li et al. (2018: figs 191–198) belong in *Sarcophagodes*, since they have wide and long vimines delimiting apically elongated areolae. They also have the entire, unperforated, open girdle bands characteristic of this latter genus (Li et al. 2018: fig. 198). At least some of the small forms presented as *G. boltonii*, such as the one depicted by Li et al. (2018: fig. 204), have quasifract copulae and they belong in *Nanofrustulum*, but we do not transfer these small forms until more precise information regarding their structure is available.

Gedaniella flavovirens (Takano) Chunlian Li, Witkowski & Ashworth, is also an undefined mixture of morphologically different small and large valves that do not seem to belong to the same diminution series. Takano (1986), when describing *Fragilaria flavovirens* Takano, the basionym of *G. flavovirens*, stated that the change from the small to the large forms he included in his taxon was never observed in culture, but the reasons were never stated. Therefore, the type material should be reanalysed and a diligent study using SEM and morphometrics (if needed) should clarify the relationship between small and large valves and the identity of this taxon as a whole. As for the material presented in Li et al. (2018), larger forms surely belong in *Pseudostaurosira* since they have short and wide vimines and entire and open bands (Li et al. 2018: figs 235–240), while smaller forms belong in *Nanofrustulum* since they have slender and short vimines and quasifract bands (Li et al. 2018: figs 249, 250).

Once redefined, *Fragilaria flavovirens* should be compared with *Pseudostaurosira americana*, *P. trainori* E.Morales, *Pseudostaurosira sopotensis* (Witkowski & Lange-Bert.) E.Morales, C.E.Wetzel & Ector (the latter two being transferred to *Nanofrustulum* below) and *P. ovalis* Lilitzkaya to confirm conspecificity. Thus far, there are no solid grounds to merge some of these species as suggested by Li et al. (2018, see comment for *Nanofrustulum sopotensis* comb. nov. and *N. trainori* comb. nov. below). Regarding *Pseudostaurosira americana*, there also seem to be differences with *Fragilaria flavovirens* in valve outline among larger forms, those of *P. americana* being cuneate to subrostrate and having the valve width rather stable (4.5–5 µm).

Other species currently under *Gedaniella*, such as *G. alfred-wegeneri* Chunlian Li, Shin.Sato & Witkowski, and *G. arenaria* Chunlian Li, Witkowski & Shin.Sato belong in *Sarcophagodes* (see below) since they have wide, long vimines and apically elongate areolae. Other species such as *G. paucistriata* Chunlian Li & Witkowski should be transferred to *Pseudostaurosira* based principally on its wide and short vimines. In the case of *G. panicellus* Chunlian Li, S.X.Yu

& Witkowski it is hard to decide in which genus to allocate it, given the mixed information presented in Li et al. (2018). Some of the forms seem to belong in *Sarcophagodes* due to their apically elongated areolae separated by wide and long vimines (e.g. Li et al. 2018: figs 269–274), while others seem to belong to *Staurosira* (e.g. Li et al. 2018: fig. 263). Their transfer is deferred until more information is available.

NOMENCLATURAL CHANGES

Some of the combinations demonstrated to be needed are given here, while others are pending until more SEM information is gathered (e.g. *Fragilaria gedanensis* Witkowski, for which there is no information on girdle band structure and confirmation of hymens in the areolae is required: see Witkowski 1993). Comments are provided for taxa requiring some clarification.

Li et al. (2018) stated that *Serratifera opephoroides* (Takano) Chunlian Li & Witkowski, based on *Fragilaria opephoroides* Takano (Takano 1988) is conspecific with *Pseudostaurosira naveana* (Le Cohu) E.Morales & Edlund. Until this is proven through analysis of type material of both species, we defer the transfer of *S. opephoroides* to the genus *Pseudostaurosira*.

Nanofrustulum cataractarum (Hust.) C.E.Wetzel, E.Morales & Ector, **comb. nov.**

Basionym – *Melosira cataractarum* Hust., Archiv für Hydrobiologie, Supplement 15: 142, pl. 9, figs 6, 7. 1938 (Hustedt 1938). – Type: Java, Tjibeureum Wasserfall, TJ2.III.c., BRM material AS1524 (holo-: BRM, slide no. A2/27).

Synonym – *Pseudostaurosira cataractarum* (Hust.) C.E.Wetzel, E.Morales & Ector in Wetzel et al. (2013).

Comment – This species, as shown by Wetzel et al. (2013: fig. 2F & G), has quasifract copulae, the distinguishing feature of the genus *Nanofrustulum*. All the morphological features of this taxon and its distinction from closely related taxa are presented by these authors.

Nanofrustulum krumbeinii (Witkowski, Witak & Stachura) E.Morales, **comb. nov.**

Basionym – *Opephora krumbeinii* Witkowski, Witak & Stachura in Lange-Bert. & Genkal, Iconographia Diatomologica 6: 80, pl. 3, figs 1–17, pl. 4, figs 1–3. 1999 (Lange-Bertalot & Genkal 1999). – Type: Poland, Baltic Sea, Puck Bay, coastal shallows, Oct. 1993, *A. Witkowski* s.n. (holo-: SZCZ, slide Chalupy X.1993A). Not seen.

Comment – This taxon also possesses quasifract copulae (Lange-Bertalot & Genkal 1999: pl. 3, fig. 12). Other features such as areolae, volae, spines and apical pore fields are similar to those in *N. shiloi* (Round et al. 1999).

Nanofrustulum sopotensis (Witkowski & Lange-Bert.) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Fragilaria sopotensis* Witkowski & Lange-Bert., Limnologica 23: 67, figs 6a–p. 1993 (Witkowski & Lange-Bertalot 1993). – Type: Poland, Puck Bay, salt meadow in Wladyslawowo, 1991, *A. Witkowski* s.n. (holo-:

: Institute of Oceanography, University of Gdansk, Gdynia, Poland, slide no. WL (A, IV. 91)).

Comment – The species has quasifract copulae (Witkowski & Lange-Bertalot 1993: figs 6m, o). All the rest of the features of this small species are reminiscent of other species ascribed to the genus. It is very similar to *P. trainori*, also transferred to *Nanofrustulum* below. Analysis of type material of both species is needed to establish further differences than those highlighted in Morales (2001) and Morales et al. (2010a). At the LM level, both species can be distinguished based on stria density (13–17 in *N. sopotensis* comb. nov. and 20–25 in *N. trainori* comb. nov.).

Nanofrustulum sourniae (Chunlian Li, Riaux-Gob. & Witkowski) E.Morales, Novais & M.Morais, **comb. nov.**

Basionym – *Serratifera sourniae* Chunlian Li, Riaux-Gob. & Witkowski in Li et al. 2018, Phytotaxa 355: 83, figs 36, 172–175, 437–448. 2018 (Li et al. 2018). – Type: Madagascar, Nosy Be coastal zone, 13°29'4.8"S, 48°14'13.2"E, rock surface, Jul. 2014, *A. Witkowski* & *C. Riaux-Gobin* s.n. (holo-: BM, slide BM 101907; iso-: SZCZ, slide SZCZE517). Not seen.

Comment – This species clearly belongs to *Nanofrustulum* since it has quasifract copulae. Unfortunately, Li et al. (2018) in their amended description of the genus *Serratifera* did not discuss the girdle bands, so it is difficult to find what their complete interpretation of this structure is, beyond what is written in their protologue on page 63. It is advisable to re-analyse type material of this taxon since the range of valve size and features seem too broad, with larger, clavate forms (Li et al. 2018, figs 439, 443–446, 448) being different from smaller, round to elliptical individuals, especially regarding the shape of the areolae and apical pore fields.

Nanofrustulum trainori (E.Morales) E.Morales, **comb. nov.**

Basionym – *Pseudostaurosira trainori* E.Morales, Proceedings of the Academy of Natural Sciences of Philadelphia 151: 113, 114, figs 6a–l. 2001 (Morales 2001). – Type: Connecticut, Avery Pond, surface sediment, *P.A. Siver* s.n. (holo-: ANSP, material no. 4198).

Comment – This taxon also has quasifract copulae, a feature that had not been mentioned in the original publication, but it was illustrated (Morales 2001: fig. k) and confirmed upon our re-analysis of material from the type locality. The remaining features of the taxon are similar to those of other species in the genus. It was originally thought that one of the main features of this species at the SEM level was the serrate spines, however, *N. shiloi* (Li et al. 2018: fig. 282) and *N. sopotensis* also have this feature (C.E. Wetzel, unpublished).

Pseudostaurosira andersonii (Chunlian Li, Dąbek & Wachn.) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Serratifera andersonii* Chunlian Li, Dąbek & Wachn. in Li et al., Phytotaxa 355: 63, figs 29, 120–129, 360–373. 2018 (Li et al. 2018). – Type: South Africa, Kraalbaai, 33°08'16.4"S, 18°01'34.6"E, muddy rock pools, May 2015, *P. Dąbek* s.n. (holo-: BM, slide BM 101898; iso-: SZCZ, slide SZCZCH1126). Not seen.

Pseudostaurosira brevis (Chunlian Li & Ashworth) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Serratifera brevis* Chunlian Li & Ashworth in Li et al., Phytotaxa 355: 66, figs 31, 138–142, 385–392. 2018 (Li et al. 2018). – Type: Hawaii, unspecified benthic environment, Jan. 2012, *N. Lecler* s.n. (holo-: BM, slide BM 101900; iso-: Theriot Lab Collection, University of Texas, Austin, slide HK446). Not seen.

Pseudostaurosira bronkei (Witkowski, Lange-Bert. & Metzeltin) C.E.Wetzel & E.Morales, **comb. nov.**

Basionym – *Fragilaria bronkei* Witkowski, Lange-Bert. & Metzeltin, Iconographia Diatomologica 7: 48 (English), 427 (Latin), pl. 12, figs 1–12. 2000 (Witkowski et al. 2000). – Type: Poland, Gulf of Gdansk, Mar. 1992, *A. Witkowski* s.n. (holo-: SZCZ Collection Cholnoky, slide SO5/III.93/BB). Not seen.

Pseudostaurosira coralina (Chunlian Li, Górecka & C.J.Kwon) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Serratifera corallina* Chunlian Li, Górecka & C.J.Kwon in Li et al. 2018, Phytotaxa 355: 71, figs 148–151, 399–404. 2018 (Li et al. 2018). – Type: Philippines, Cebu Island, Moal Boal, 09°56'57.7"N, 123°21'55.4"E, submarine rock cliff, water depth of 50 m, *Dr. Kwon* s.n. (holo-: BM, slide BM 101897; iso-: SZCZ, slide SZCZE1544). Not seen.

Pseudostaurosira frugalis (Kulikovskiy & Lange-Bert.) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Popovskayella frugalis* Kulikovskiy & Lange-Bert. in Kulikovskiy et al., Iconographia Diatomologica 26: 21, 22, pl. 13, figs 9–11. 2015 (Kulikovskiy et al. 2015). – Type: Lake Baikal, Bolshoi Ushkaniy Island, Jul. 1965, *A.P. Skabitschewsky* (holo-: Collection Maxim Kulikovskiy, I.D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences (IBIW), slide no. 15650m; iso-: SZCZ, slide SZCZ15650a). Not seen.

Pseudostaurosira liae E.Morales, **nom. nov.**

Replaced synonym – *Serratifera clavata* Chunlian Li, Tomczak & Witkowski in Li et al., Phytotaxa 355: 68, figs 32, 143–147, 393–398. 2018 (Li et al. 2018). – Type: Abu Dhabi, Persian Gulf, 24°28'53.4"N, 54°20'43.2"E, sand in shallow water, Oct. 2014, *M. Tomczak* s.n. (holo-: BM, slide BM 101901; iso-: SZCZ, slide SZCZCH752). Not seen.

Comment – A new name is required for this taxon in order to transfer it to *Pseudostaurosira* since the epithet *clavatum* is already taken by *P. clavatum* E.Morales (Morales 2002).

Etymology – This diatom is named after Dr. Chunlian Li for her contributions to diatom systematics.

Pseudostaurosira linearis (Pant.) E.Morales, Buczkó & Ector, **comb. & stat. nov.**

Lectotype (here designated): permanent slide HNHM-ALG-D002264, Algological Collection of Hungarian Natural History Museum Budapest (HNHM). English Finder Q32; Figs 3A–AC & 4A–L.

Basionym – *Fragilaria pinnata* var. *linearis* Pant., A pozsonyi Orvos-Természettudományi Egyesület közleményei 23: 30, pl. 2, figs 65, 68 (as *F. mutabilis* var. *linearis* in figure legend). 1913 (Pantocsek 1913).

Synonym – *Fragilaria pinnata* var. *ovalis* Pant., A pozsonyi Orvos-Természettudományi Egyesület közleményei 23: 30, pl. 2, figs 66, 67 (as *F. mutabilis* var. *ovalis* in figure legend). 1913 (Pantocsek 1913).

Comment – We synonymize these two taxa described by Pantocsek (1913: figs 16–43) since no differences were observed between them under SEM (fig. 4). Outer and inner valve features of sterna, areolae, spines (T-shaped, bearing M- or inverted V-shaped stipulae), and apical pore fields are the same for all valves of all sizes found in the sample from Lutilla (compare smaller and larger forms in fig. 3B–X). Both varieties have equal priority, but we choose “*linearis*” since it describes well the valve outline of the larger valves of this taxon, which can vary in the width of its apices, from more broadly rounded to almost cuneate. Based on new measurements, we expand the valve dimension ranges for this taxon to length: 2–34 µm, width: 2.5–4.8 µm. The stria density is 12–14 in 10 µm.

Pseudostaurosira linearis resembles *Pseudostaurosira polonica* (Witak & Lange-Bert.) E.Morales & Edlund (Witkowski et al. 1995); their valve dimensions intergrade to a large extent (length: 10–30 µm, width: 3.5–5 µm in *P. polonica*). We have found that the median for stria density in *P. linearis* is 13 and that for *P. polonica* is 16. Also, the areolae in *P. polonica* are more transapically elongated, while they are round in *P. linearis*. On the other hand, smaller forms of *P. linearis* could be confused with *P. elliptica*, but they can be distinguished mainly by the stria density, which is 14–16 per 10 µm in the latter.

One salient feature of *P. linearis* is the transitional zone between valve face and mantle. This is a feature also found in the generitype *P. brevistriata* (Morales et al. 2015) and in *P. polonica* (Witkowski et al. 1995). Thus far, *P. linearis* has only been reported from fossil material.

Pseudostaurosira marciniakae Ector, E.Morales, C.E.Wetzel, **nom. & stat. nov.**

Replaced synonym – *Fragilaria pseudoconstruens* var. *rhombica* Marciniak, Acta Geologica Academiae Scientiarum Hungaricae 25: 164–165, pl. 2, fig. 5. 1982 (Marciniak 1982). – Type: Poland, Tatra Mountains, Przedni Staw Lake, *B. Marciniak* core no. 2. (holo-: Collection of Institute of Geological Sciences, Polish Academy of Sciences, Warsaw, slide no. 3024).

Etymology – This species is named in honour of Dr. Barbara Marciniak for her contributions in paleoecology and the study of fossil diatoms.

Comment – An alternative name is necessary because the epithet *rhombica* is in use at species level in the genus (see below).

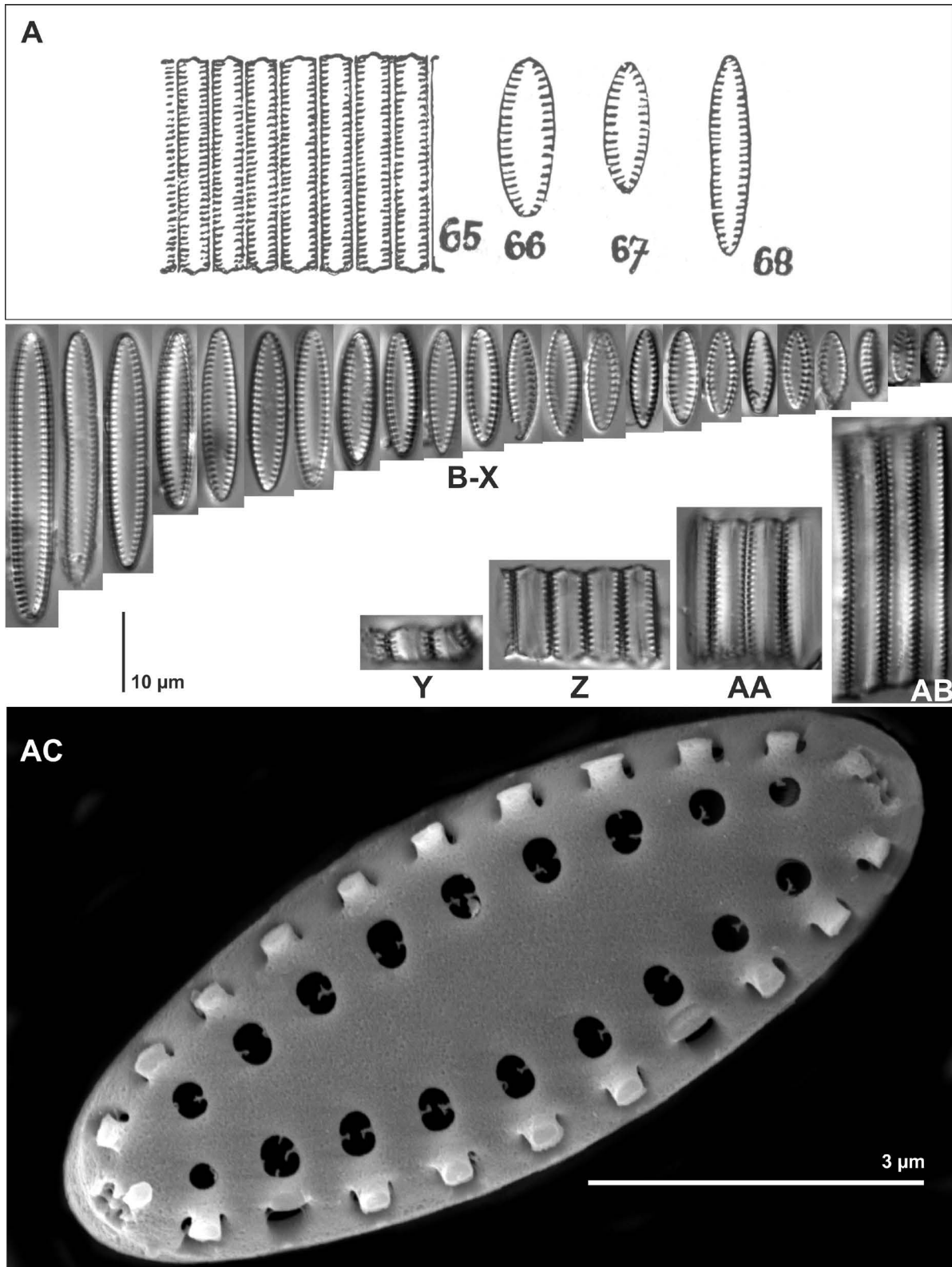


Figure 3 – *Pseudostaurosira linearis*, drawings, LM and SEM depictions from the fossil Lutilla material, Slovakia: A, reproduction of Pantocsek’s original drawings (1913: p. 14, pl. 2, figs 65–68). His figs 65 and 68 were originally labelled as *Fragilaria mutabilis* var. *ovalis*, while 66 and 67 were labelled as *Fragilaria mutabilis* var. *linearis*; B–AB, size diminution series and girdle views of colonies in LM. Notice that wider and slender valves have all other features (sternum, striae, frustule shape and valve outline) similar to each other; AC, external features of a valve (SEM) showing details of areolae, spine position, apical pore fields and the characteristic transition zone between valve face and abvalvar portion of mantle. Also note point of insertion within the areolae of eroded volae.

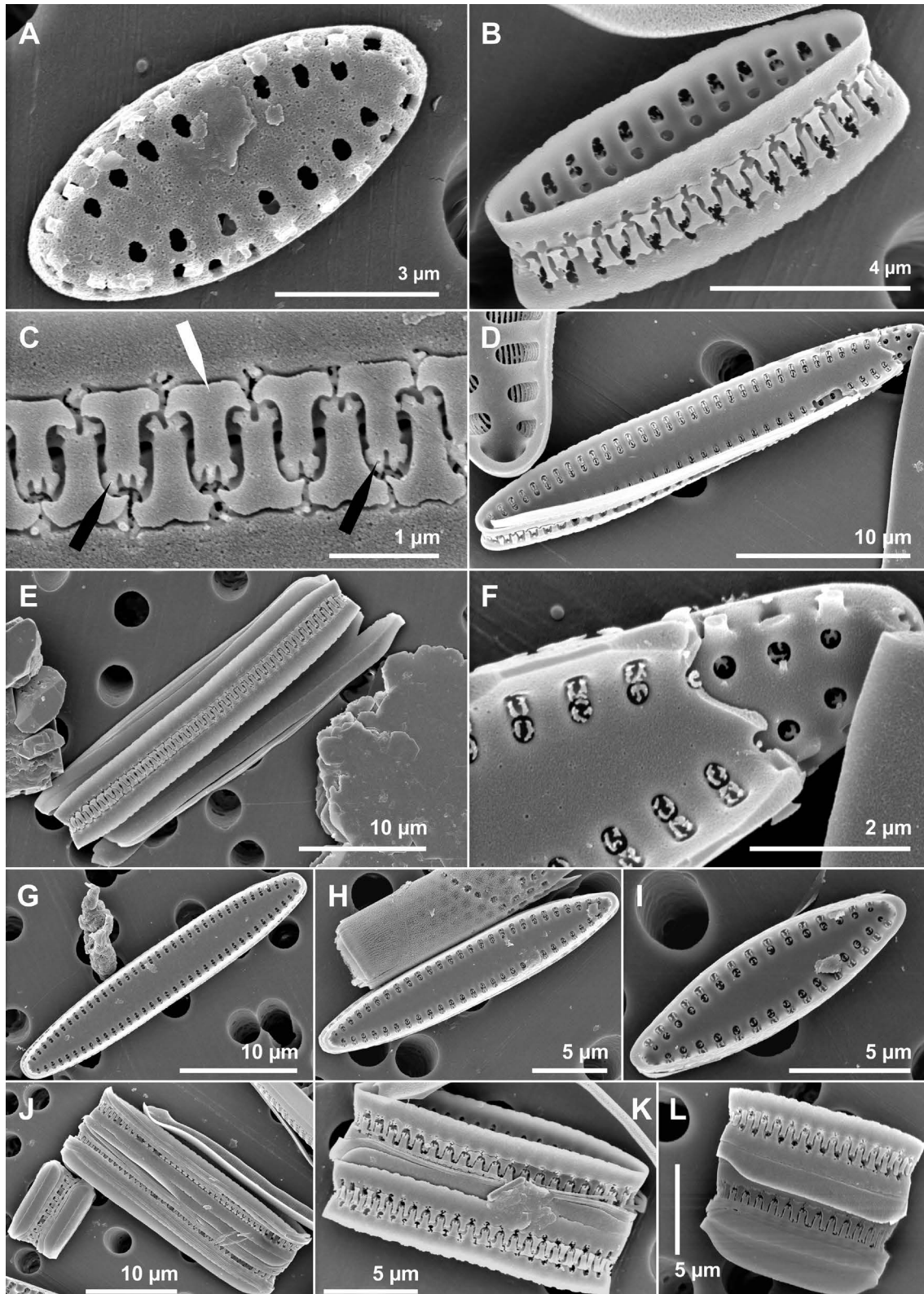


Figure 4 – SEM images of *Pseudostaurosira linearis*, from the fossil Lutilla material, Slovakia: A, external view; B & C, side view of attached neighbouring valves and detail of spine connection. Notice “T” shape of spines (white arrow) and “M” or inverted “V” shaped stipulae (black arrows); D & F, broken valve in inner view on top of a complete valve in outer view. Notice that in the zoomed image of this large specimen the characteristic transitional zone of the valve mantle is also present; E & J–L, side views of connected valves showing details of open, unperforated girdle bands and the resemblance among large and small connected valves; G–I, diminution series of internal views also showing similarities among larger and smaller representatives.

Pseudostaurosira minutula (Kulikovskiy & Lange-Bert.) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Popovskayella minutula* Kulikovskiy & Lange-Bert. in Kulikovskiy et al., *Iconographia Diatomologica* 26: 22, pl. 13, figs 1–8. 2015 (Kulikovskiy et al. 2015). – Type: Lake Baikal, Bolshoi Ushkaniy Island, Jul. 1965, *A.P. Skabitschewsky* s.n. (holo-: Collection Maxim Kulikovskiy, I.D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences (IBIW), slide no. 15646m; iso-: SZCZ, slide SZCZ15646a). Not seen.

Pseudostaurosira namibica (Chunlian Li & Witkowski) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Serratifera namibica* Chunlian Li & Witkowski in Li et al., *Phytotaxa* 355: 71, 72, figs 152–156, 405–412. 2018 (Li et al. 2018). – Type: Namibia, Sandwich Harbor lagoon, sediment core, Apr. 2013, *A. Witkowski & P. Dqbek* s.n. (holo-: BM, slide BM 101902; iso-: SZCZ, slide SZCZP88). Not seen.

Pseudostaurosira nanobaculum (Kulikovskiy & Lange-Bert.) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Popovskayella nanobaculum* Kulikovskiy & Lange-Bert. in Kulikovskiy et al., *Iconographia Diatomologica* 26: 22, 23, pl. 11, figs 1–18. 2015 (Kulikovskiy et al. 2015). – Type: Lake Baikal, Bolshoi Ushkaniy Island, Jul. 1965, *A.P. Skabitschewsky* s.n. (holo-: Collection Maxim Kulikovskiy, I.D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences (IBIW), slide no. 15646m; iso-: SZCZ, slide SZCZ15646a). Not seen.

Pseudostaurosira nosybeana (Chunlian Li, Witkowski & Riaux-Gob.) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Serratifera nosybeana* Chunlian Li, Witkowski & Riaux-Gob. in Li et al., *Phytotaxa* 355: 74, figs 157–160, 413–419. 2018 (Li et al. 2018). – Type: Madagascar, Nosy Be Island, 13°29'4.8"S, 48°14'13.2"E, rock surface, Jul. 2014, *A. Witkowski & C. Riaux-Gobin* s.n. (holo-: BM, slide BM 101903; iso-: SZCZ, slide SZCZCH92). Not seen.

Pseudostaurosira parkii (Chunlian Li & Ashworth) E.Morales, Novais & M.Morais, **comb. nov.**

Basionym – *Serratifera parkii* Chunlian Li & Ashworth in Li et al., *Phytotaxa* 355: 79, figs 33, 161–165, 420–424. 2018 (Li et al. 2018). – Type: South Korea, Tongyeong, vicinity of the LNG Terminal, 34°56'46.2"N, 128°25'46.2"E, mud from sand, Oct. 2014, *J. Park* s.n. (holo-: BM, slide BM 101904; iso-: Theriot Lab Collection, University of Texas, Austin, slide HK507). Not seen.

Pseudostaurosira paucistriata (Chunlian Li & Witkowski) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Gedaniella paucistriata* Chunlian Li & Witkowski in Li et al., *Phytotaxa* 355: 42, 46, 47, figs 15, 90–94, 275–281. 2018 (Li et al. 2018). – Type: Qingdao, China, 36°05'32" N, 120°28'10"E, sand at low tide, Jun. 2015, *A.*

Witkowski & S.X. Yu s.n. (holo-: BM, slide BM 101893; iso-: SZCZ, slide SZCZCH1281). Not seen.

Pseudostaurosira punctata (Shin.Sato & Chunlian Li) E.Morales, Novais & M.Morais, **comb. nov.**

Basionym – *Serratifera punctata* Shin.Sato & Chunlian Li in Li et al., *Phytotaxa* 355: 79, figs 34, 166–168, 425–430. 2018 (Li et al. 2018). – Type: Senegal, Dakar, Goree Island, sand in port, Sep. 2006, *S. Matsumoto* s.n. (holo-: BM, slide BM 101905; iso-: Sato Collection, Faculty of Marine Bioscience, Fukui Prefectural University, Fukui, Japan, slide s0386). Not seen.

Pseudostaurosira pusilla (Kulikovskiy & Lange-Bert.) E.Morales, Novais & M.Morais, **comb. nov.**

Basionym – *Popovskayella pusilla* Kulikovskiy & Lange-Bert. in Kulikovskiy et al., *Iconographia Diatomologica* 26: 23, pl. 13, figs 12–35. 2015 (Kulikovskiy et al. 2015). – Type: Lake Baikal, Bolshoi Ushkaniy Island, Jul. 1965, *A.P. Skabitschewsky* s.n. (holo-: Collection Maxim Kulikovskiy, I.D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences (IBIW), slide no. 15646m; iso-: SZCZ, slide SZCZ15646a). Not seen.

Pseudostaurosira rhombica (Shin.Sato, Chunlian Li & Witkowski) E.Morales, Novais & C.E.Wetzel, **comb. nov.**

Basionym – *Serratifera rhombica* Shin.Sato, Chunlian Li & Witkowski in Li et al., *Phytotaxa* 355: 81–83, figs 35, 169–171, 431–436. 2018 (Li et al. 2018). – Type: Okinawa, Iriomote Island, Japan, 24°12'36"N, 123°25'48"E, Oct. 2005, *T. Watanabe* s.n. (holo-: BM, slide BM 101906; iso-: Sato Collection, Faculty of Marine Bioscience, Fukui Prefectural University, Fukui, Japan, slide s0357). Not seen.

Pseudostaurosira simplex (Kulikovskiy & Lange-Bert.) E.Morales, Novais & M.Morais, **comb. nov.**

Basionym – *Popovskayella simplex* Kulikovskiy & Lange-Bert. in Kulikovskiy et al., *Iconographia Diatomologica* 26: 24, pl. 12, figs 1–8. 2015 (Kulikovskiy et al. 2015). – Type: Lake Baikal, Bolshoi Ushkaniy Island, Jul. 1965, *A.P. Skabitschewsky* s.n. (holo-: Collection Maxim Kulikovskiy, I.D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences (IBIW), slide no. 15645m; iso-: SZCZ, slide SZCZ15645a). Not seen.

Pseudostaurosira takanoi (Shin.Sato & Chunlian Li) E.Morales, Novais & M.Morais, **comb. nov.**

Basionym – *Serratifera takanoi* Shin.Sato & Chunlian Li in Li et al., *Phytotaxa* 355: 86, 87, figs 37, 176, 449–454. 2018 (Li et al. 2018). – Type: Okinawa, Iriomote Island, Japan, 24°12'36"N, 123°25'48"E, Oct. 2005, *T. Watanabe*, s.n. (holo-: BM, slide BM 101908; iso-: Sato Collection in Faculty of Marine Bioscience, Fukui Prefectural University, Fukui, Japan, slide s0308). Not seen.

Pseudostaurosira tenerrima (Kulikovskiy & Lange-Bert.) E.Morales, Novais & M.Morais, **comb. nov.**

Basionym – *Popovskayella tenerrima* Kulikovskiy & Lange-Bert. in Kulikovskiy et al., *Iconographia Diatomologica* 26: 24, pl. 14, figs 1–8. 2015 (Kulikovskiy et al. 2015). – Type: Lake Baikal, Bolshoi Ushkaniy Island, Jul. 1965, *A.P. Skabitschewsky* s.n. (holo-: Collection Maxim Kulikovskiy, I.D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences (IBIW), slide no. 15646m; iso-: SZCZ, slide SZCZ15646a). Not seen.

Pseudostaurosira varisterna (Chunlian Li, Ashworth & Witkowski) E.Morales, **comb. nov.**

Basionym – *Serratifera varisterna* Chunlian Li, Ashworth & Witkowski in Li et al., *Journal of Phycology* 52: 1021–1022, figs 1a–l, 2a–f. 2016 (Li et al. 2016). – Type: Texas, Mustang Island, Packary Channel, 27°37.070'N, 97°12.70'W, plankton net, Dec. 2013, *M. Ashworth, C.H. Li, A. Witkowski & E. Theriot* s.n. (holo-: BM, slide BM 101829; iso-: SZCZ, slide SZCZCH168). Not seen.

Comments – Wide and short vimines, the distinguishing feature of *Pseudostaurosira*, characterize the striae of this taxon, which are also composed of transapically elongated areolae. The spines located on the vimines, the reduced apical pore fields present at both valve apices and the entire, open, unperforated girdle bands, are features that also appear in other species included in the genus.

Pseudostaurosira wachnickiana (Chunlian Li, Witkowski & Ashworth) E.Morales, Novais & M.Morais, **comb. nov.**

Basionym – *Nanofrustulum wachnickianum* Chunlian Li, Witkowski & Ashworth in Li et al., *Phytotaxa* 355: 50, figs 18, 19, 97, 98, 300–311. 2018 (Li et al. 2018). – Type: Florida, Marquesas, sediment in shallow water, Aug. 2013, *A. Wachnicka* s.n. (holo-: BM, slide BM 101896; iso-: SZCZ, slide SZCZCH285). Not seen.

Comments – This taxon has entire, open, unperforated girdle bands. The distinguishing trait of *Pseudostaurosira*, wide and short vimines, is also present in this taxon. All the remaining features such as transapically elongated areolae, spines bearing stipulae and presence of flaps and plaques on the abvalvar edge of the mantle are characteristic of other species in *Pseudostaurosira*.

Sarcophagodes alfred-wegeneri (Chunlian Li, Shin.Sato & Witkowski) E.Morales, Novais & M.Morais, **comb. nov.**

Basionym – *Gedaniella alfred-wegeneri* Chunlian Li, Shin.Sato & Witkowski in Li et al., *Phytotaxa* 355: 30, 32–34, figs 8, 60–62, 217–221. 2018 (Li et al. 2018). – Type: Bremerhaven, River Weser, mud, Jul. 2005, *S. Sato* s.n. (holo-: BM, slide BM 101894; iso-: SZCZ, slide SZCZs0263). Not seen.

Comment – This taxon has wide and long vimines and the areolae are apically elongated, some of them having a reniform shape, the unique traits of the genus (table 1). Though the striae are longer since they are composed of a larger number of areolae, the general features of this taxon are similar to *S. delicatula* E.Morales, the generitype of *Sarcophagodes* (Morales 2002). As it happens in the latter species,

the vimines situated at the valve face–mantle transition are thicker and bear rudimentary spines or a ridge (Li et al. 2018: fig. 220). All the other features (heteropolarity of valves, volae, and apical pore fields) are similar in both taxa.

Sarcophagodes arenaria (Chunlian Li, Witkowski & Shin.Sato) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Gedaniella arenaria* Chunlian Li, Witkowski & Shin.Sato in Li et al., *Phytotaxa* 355: 34, figs 9, 63–67, 222–234. 2018 (Li et al. 2018). – Type: Międzyzdroje, Amber Baltic Hotel sand beach, Baltic Sea, Nov. 2006, *S. Sato & A. Witkowski* s.n. (holo-: BM, slide BM 101895; iso-: SZCZ, slide SZCZCH1765 (originally labeled as “s0393”). Not seen.

Comment – Although this taxon lacks the rudimentary spines or a ridge at the valve face–mantle junction, it does possess wide and long vimines and the apically elongated areolae, the features defining *Sarcophagodes* (table 1). In this taxon, the large valvocopula and the slender, open, unperforated copulae are also similar to those in *S. delicatula*, the generitype.

Sarcophagodes boltonii (Chunlian Li, Krawczyk, Dąbek & Witkowski) C.E.Wetzel, E.Morales & Ector, **comb. nov.**

Basionym – *Gedaniella boltonii* Chunlian Li, Krawczyk, Dąbek & Witkowski in Li et al., *Phytotaxa* 355: 24, 27, *pro parte*, figs 191–198. 2018 (Li et al. 2018). – Type: South Africa, Kraalbaai, 33°08'16.4"S 18° 01'34.6"E, muddy rock pool, May 2015, *P. Dąbek* s.n. (holo-: BM, slide BM 101891; iso-: SZCZ, slide SZCZCH1528). Not seen.

Comments – Since *G. boltonii* is a mixture of at least two morphological variants, we transfer here the larger form presented by Li et al. (2018) to the genus *Sarcophagodes*. This taxon has wide and long vimines and the areolae are apically reniform, the two distinguishing characters of the genus. We choose the SEM images of Li et al. (2018) as examples showing the features of this taxon, since the LM information presented by these authors is difficult to discern. *Sarcophagodes boltonii* has fully developed spines, located on a vimen situated at the junction of the valve face and mantle.

Sarcophagodes mutabilis (Grunow) E.Morales, **comb. nov.**

Basionym – *Sceptroneis mutabilis* Grunow (*marina* var.?) in Cleve & Möller, *Diatoms* (1877–1882), Part 5: no. 255. 1879 (Cleve & Möller 1877–1882). – Type: Pointe du Hourdel, Cayeux-sur-Mer, Hauts-de-France, mouth of the Somme river, *P. Petit* s.n. Not seen.

Comments – This taxon has the wide and long vimines and the areolae are apically reniform, the two discriminating features of the genus. The combination *Opephora mutabilis* (Grunow) Sabbe & Vyverman (Sabbe & Vyverman 1995) is untenable following the concept proposed by Round et al. (1990).

Sarcophagodes poulinii (Witkowski, Riaux-Gob. & Daniszewska-Kowalczyk) E.Morales, **comb. nov.**

Basionym – *Staurosirella poulinii* Witkowski, Riaux-Gob. & Daniszewska-Kowalczyk, *Vie et milieu* 60: 277, figs 68–78. 2010 (Witkowski et al. 2010). – Type: Kerguelen, Ile Haute, intertidal sediments, Dec. 1991, *C. Riaux-Gobin* s.n. (holo-: SZCZ, slide SZCZ7350_8; iso-: Collection C. Riaux-Gobin, U SR 3278 CNRS-EPHE, CRIOBE-Université de Perpignan, slide No. KER16; BRM, slide BRM ZU7/84). Not seen.

Comments – This species has wide and long vimines delimiting transapically elongated areolae, the distinguishing character of the genus *Sarcophagodes*. Additionally, the species has spines located on vimines, that is, they are situated along the striae at the valve face–mantle junction. *Staurosirella* has slender, long vimines, lineolae (unique features for the genus), and the spines are located on the virgae.

Staurosira viereckiana (Gerd Moser) C.E.Wetzel & E.Morales, **comb. nov.**

Basionym – *Fragilaria viereckiana* Gerd Moser, *Bibliotheca Diatomologica* 43: 127–129, pl. 2, figs 1–17, pl. 3, fig. 2. 1999 (Moser 1999). – Type: Nouvelle-Calédonie, Houaïlou, upstream, 10 km past village, Mar. 1994, *Moser et al.* PN 83 (holo-: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main, slide Neukaledonien OTL 70). Not seen.

Staurosirella guenter-grassii (Witkowski & Lange-Bert.) E.Morales, C.E.Wetzel & Ector, **comb. nov.**

Basionym – *Fragilaria guenter-grassii* Witkowski & Lange-Bert., *Limnologica* 23: 65, 66, figs 5a–l. 1993 (Witkowski & Lange-Bertalot 1993). – Type: Poland, Puck Bay, 1992, *A. Witkowski* s.n. (holo-: Institute of Oceanography, University of Gdansk, Gdynia, Poland, slide no. P3 (A, V. 92)). Not seen.

Comments – This species has the distinguishing trait for the genus, i.e., long and slender vimines. Thus the areolae are apically elongated. The volae are branched (Witkowski 1994: pl. 8, fig. 3). Apical pore fields are developed at the foot pole and are composed of round poroids in the manner of, for example, *S. minuta* E.Morales & Edlund (Morales & Edlund 2003: figs 33–38). For all this, the combination *Gedaniella guenter-grassii* (Witkowski & Lange-Bert.) Chunlian Li, Shin.Sato & Witkowski presented in Li et al. (2018) is untenable. Likewise, since we are following the concept of *Opephora* suggested by Round et al. (1990), the combination *O. guenter-grassii* (Witkowski & Lange-Bert.) Sabbe & Vyverman (Sabbe & Vyverman 1995) is unsupported.

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