

**A morphology-based phylogeny of the diatoms formerly assigned to the Orthoseiraceae (Bacillariophyta): phylogenetic relationships, character analysis and classification.**

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## Abstract

A formal cladistic analysis of the morphological features of taxa assigned to the Orthoseiraceae, including *Phycavernosa*, *Guarreraea* and species of the genus *Orthoseira* is developed for 35 taxa based on 16 characters along with two *Melosira* species and four biddulphioid species as ingroup taxa as well as *Leptocylindrus danicus* as the outgroup. A consensus tree of 266843 most parsimonious topologies suggest that the Orthoseiraceae is monophyletic, and that *Guarreraea* plus all former species of *Orthoseira* represent a monophyletic group. This analysis of morphological data did not provide support for the members of the Orthoseiraceae being more closely related to melosiroid or biddulphioid diatoms. Relationships within the monophyletic orthoseiracean group are detailed. Due to the exclusion of the generitype of *Orthoseira* not possessing carinoportulae which are central tube processes, one of the diagnostic features of the former Orthoseiraceae, other taxon names are described and recognised herein. We recognise and describe a new order, the Liparogyrales, based on the genus *Liparogyra* for taxa that were previously assigned to *Orthoseira*. Two families, the Phycavernosaceae, comprising the genus *Phycavernosa*, and the Liparogyraceae, comprising *Guarreraea* and taxa formerly assigned to *Orthoseira*, are described. The genus *Liparogyra* is composed of three subgenera: the subgenus *Liparogyra*, the subgenus *Porocyelia*, and the subgenus *Insularis* which are diagnosed by synapomorphic features. Based on our analysis, the genus *Stephanosira* appears to lack synapomorphies. *Liparogyra*, *Porocyelia* and *Stephanosira* had previously been described as genera by Ehrenberg. *Guarreraea* and one newly-proposed genus are also included in the Liparogyraceae. Several other lineages are aphyletic, due to them either being monotypic or without diagnostic features. Thus, a total of 29 species in 5 genera are recognised within the newly-described order and two newly-described families. 18 new combinations are proposed for the transfer of taxa from *Orthoseira* to other genera. Homoplasy amongst some of the character states is discussed.

**Key words:** Bacillariophyta, cladistics, classification, homoplasy, phylogenetics

## Highlights

The diatom genus *Orthoseira* as typified by *O. americana* does not possess carinoportulae, requiring a new approach to the nomenclature and taxonomy of the group.

A phylogenetic analysis of the group using morphological data was performed, demonstrating patterns of relationships of the species for this lineage.

A new classification for this diatom lineage is presented.

## Introduction

*Orthoseira* Thwaites (1848), a genus of diatoms with a radial pattern centre in their valves found in sub-aerial or aerophilous habitats (Round *et al.*, 1990), is best known for its characteristic central tube processes, termed carinoportulae (Crawford 1981; Round *et al.* 1990). The genus was first described by Thwaites (1848), with *Melosira americana* Kützing (1844) as the generitype, even though Thwaites did not formally transfer the species to his new genus. The original figures of *Melosira americana* do not show any feature that might be interpreted as a carinoportula (Kützing 1844, l. 30, Fig. 69a-d). However, in the modern circumscription of the genus *Orthoseira*, carinoportulae are considered the defining character of the genus (Crawford 1981; Round *et al.* 1990). As a result of the generitype lacking the diagnostic feature of the genus, the status of *Orthoseira* has been regularly questioned (Houk 1993; Spaulding & Kociolek 1998; Van de Vijver & Kopalová 2008; Guerrero *et al.* 2018; Danz *et al.* 2022). Unfortunately, the type material of *Melosira americana* from tropical South America has been lost (Kützing 1844; Spaulding and Kociolek 1998; Danz *et al.* 2022).

In addition to the type material of the generitype being lost, *Orthoseira* has had a long nomenclatural history related to *Melosira* C.Agardh (1824), regularly being separated from and lumped with *Melosira* (see Danz *et al.* 2022 for a complete historic overview of the genus *Orthoseira*). Revision of *Melosira* taxa resulted in the carinoportulae-bearing taxa becoming associated with the group, most notably, when the taxa of three genera described by Ehrenberg in 1848 conspicuously bearing carinoportulae (*Liparogyra*, *Stephanosira* and *Porocyclia*) were moved to *Orthoseira* (Crawford 1981; Houk 1993; Danz *et al.* 2022). Crawford (1981), while discussing *Orthoseira*, commented on the presence of carinoportula-like features in these three genera, and how the taxa of these genera are more representative of the group (as diagnosed by Crawford 1981) than the type species *M. americana*. Crawford pointed to the date of Ehrenberg's (1848) publication, just three months after that of Thwaites, as a reason not to use all or any of these genus names to describe what is currently assigned to *Orthoseira*.

While all three of Ehrenberg's (1848) genera bear carinoportulae, the distinctions Ehrenberg made between his genera can still be seen in taxa currently assigned to *Orthoseira*. *Liparogyra* was described as having obvious or deflected girdle bands organised in a spiral arrangement (Ehrenberg 1848; Ehrenberg 1871), and this feature can be seen in, for instance, *Orthoseira dendroteres* (Ehrenberg) Genkal & Kulikovskiy (in Kulikovskiy *et al.* 2010), *O. circularis* (Ehrenberg) R.M.Crawford (as cited in Haworth & Kelly 2002; this is an invalid name), and in the recently described species *O. hawaiiensis* Danz & Kociolek (2022). *Stephanosira* was the least characterised of the three genera Ehrenberg described, as it was described as a group of species lacking any distinctive unique features, merely grouping the leftovers that did not fit into the other two genera. Thus, it was a “not-A” group, that is, a group characterised by a lack of synapomorphic features, and numerous species of *Orthoseira* could fit this characterization (Ehrenberg 1848, 1871; Danz *et al.* 2022). The third genus, *Porocyclus*, was described as having window-like openings at the valve margin (Ehrenberg 1848, 1871), a feature noted in Van de Vijver & Kopalová (2008) to be the result of an “internal valve”, and additionally, as the result of either “true caverns” or valvocopular extensions (Danz *et al.* 2022). This “windowed” appearance can also be seen in the taxa *Orthoseira dendrophyla* (Ehrenberg) Round, R.M.Crawford & D.G.Mann (Round *et al.* 1990), *O. gremmenii* Van de Vijver & Kopalová (2008), *O. loweana* Danz & Kociolek (2022), *O. circularis*, *O. hawaiiensis*, *O. dendroteres*, and *O. yunnanica* (J.Chen) Kociolek, Liu & Danz (Ehrenberg 1848; Chen 1980; Round *et al.* 1990, Van de Vijver & Kopalová 2008; Kociolek *et al.* 2022; Danz & Kociolek 2022).

Houk (1993) also identified distinctions between taxa in *Orthoseira*, forming six morphological groups, with varying numbers of subgroups, all within the *Orthoseira roeseana* (Rabenhorst) Pfitzer (1871) complex. These groups were: “*dendroteres*”, “*circularis*”, “*epidendron*”, “*dendrophyla*”, “*roeseana*”, and “*tatrica*” (Houk 1993). Groups “*dendroteres*” and “*circularis*” aligned with *Liparogyra* as described by Ehrenberg, having notable girdle bands, and these two groups are named for the two species Ehrenberg described for *Liparogyra* (Ehrenberg 1848; Houk 1993). Groups “*epidendron*” and “*dendrophyla*” corresponded to Ehrenberg's *Stephanosira* and *Porocyclus*, respectively, and are also named for the species Ehrenberg placed in those genera (Ehrenberg 1848; Houk 1993). The last two groups, “*roeseana*” and “*tatrica*” did not correspond to any taxa Ehrenberg described; however, both of

these groups bear ridge features (termed ‘rib’ by Houk 1993) along the valve margin, a feature consistent with the taxa *Orthoseira ursulae* Metzeltin & Lange-Bertalot (2007), *O. tropica* (Krasske) Metzeltin & Lange-Bertalot (2007), *O. oregoniana* (Danz & Kociolek 2022), *O. roeseana* (Pfitzer 1871; Lange-Bertalot *et al.* 1996; Metzeltin & Lange-Bertalot 2007; Danz & Kociolek 2022; Kochman-Kędziora *et al.* 2023), *O. distinctiareolata* Rybak, Glushchenko & Kulikovskiy (Rybak *et al.* 2022), and *O. radiata* Rybak, Glushchenko & Kulikovskiy (Rybak *et al.* 2022).

Since the type material of the type species of the genus *Orthoseira* has been lost, and as a result cannot be observed to see if there is a carinoportula-like feature present, and as the original description and drawings of that type material make no mention of a carinoportula-like feature, it is difficult to understand and circumscribe the genus, and thus have a rational basis on which to make decisions to assign species to the genus. At an initial glance, Ehrenberg’s (1848) work appears most appropriate in description and chronology to replace the name *Orthoseira*, but which of his three generic names to use, and what features might diagnose those groups in a phylogenetic perspective is unclear. The work done by Houk (1993), while further supporting Ehrenberg’s (1848) distinctions, also adds a few of its own. Additionally, the inclusion of recently-described genera *Guarreraea* Kociolek, J.M.Guerrero & Van de Vijver (in Guerrero *et al.* 2018), based on the presence of both carinoportulae and rimoportulae and *Phycavernosa* S.Blanco (2020), based on the presence of caverns and rimoportulae but lacking carinoportulae (Stidolph 1990; Guerrero *et al.* 2018) in the Orthoseiraceae Round, Crawford & D.G.Mann (1990) further clouds the relationships and distinctions within the family.

Gargas *et al.* (2018) recently suggested, based on an analysis of molecular data, that *Orthoseira* was more closely related to biddulphioid genera such as *Terpsinoë* Ehrenberg (1843) and *Hydrosera* G.C.Wallich (1858), contradicting the historic understanding of *Orthoseira* as closely related to the melosiroid diatoms, though there are some questions regarding the five-gene analyses that revealed this relationship (Medlin & Desdevises 2020). Additionally, while taxa in *Orthoseira* have been variously placed together or arranged in formal (Ehrenberg 1848; Guerrero *et al.* 2018) or informal taxonomic groupings (Houk 1993), there has been no formal phylogenetic analysis of the members of the Orthoseiraceae.

The purpose of the present study is to: 1) test the relationship of orthoseiracean diatoms with those from the Melosiraceae Kützing (1844) and Biddulphiaceae Kützing (1844), and 2) use

new and published morphological data to formally determine the phylogenetic relationships of members of the Orthoseiraceae, assessing previous formal and informal taxonomic assignments, and develop a natural classification of this group based on morphological features. Our intention is to describe the diversity and relationships expressed in the phylogenetic analyses in the context of historic interpretations (e.g. Ehrenberg 1848; Houk 1993), while also establishing a framework for future analyses.

## Methods and Material

### *Taxa selection, acquisition of morphological data, and building of matrix*

We selected taxa based on historic and traditional ideas of the diatom tree of life, influenced by the work of Theriot *et al.* (2015) and Gargas *et al.* (2018). The terminal taxa included in this analysis are presented in Table 1.

Characters and character states used in a parsimony analysis were acquired via observations made from the SEM and LM as described in Danz and Kociolek (2022), as well as from other published literature. Some historical and traditional references were also utilised. Reference locations for each terminal taxa can be found in Table 1. The characters, as well as their character states are summarised as follows:

1. *Carinoportula(e)*: A central process with the internal opening being simple, while the external opening usually bears a collar (Round *et al.* 1990, p. 174, Figs D, F, H, I, K, L; Diatoms of North America, term: “Carinoportula”). This feature is found in all the species of *Orthoseira* and in both species of *Guarreraea*, but not in *Phycavernosa* or taxa outside the Orthoseiraceae. Absence is coded as (0) and presence is coded as (1).
2. *Rimoportula(e)*: An opening that is slit-like and (usually) bordered by thick siliceous ridges, giving the impression of a pair of lips (hence the name labiate process) internally. It is present in nearly all diatoms (Round *et al.* 1990), though may vary in form. As a contradiction to the previous statement, the feature is lacking in all species of *Orthoseira*, coded as (0). When present, the rimoportula(e), internally, may be a slit, coded as (1), as seen in *Guarreraea* and *Ferocia* Van de Vijver & Houk (Guerrero *et al.* 2018, Figs 10, 20, 22, 23; Round *et al.* 1990, p. 231, Fig. i), sessile, coded as (2), as seen in taxa of

*Melosira*, stalked, coded as (3), as seen in *Phycavernosa* (Stidolph 1990, Figs 15, 19-21, 23).

3. *Internally etched slits*: A faint etching of a slit that may not fully pass through the valve, can be seen internally in some species of *Orthoseira* (Kociolek *et al.* 2022, Fig. 14; Danz & Kociolek 2022, Fig. 30). Unlike true rimoportulae, these slits lack thickenings on both sides of the opening. The extent of this character is variable within those taxa bearing it. Absence of this character is coded as (0) and presence is coded as (1).
  
4. *True caverns*: “Hollows” or “cavities” that are formed internally at the valve face/mantle junction (Stidolph 1990, Figs 19, 20, 22, 24; Kociolek *et al.* 2022, Figs 13, 15; Danz & Kociolek 2022, Figs 30, 31). Externally, when the valve face of a taxon bearing this feature is observed under LM, small, circular areas with a greater transparency near the margin can be observed (Stidolph 1990, Figs 1--3; Kociolek *et al.* 2022, Figs 1--7; Danz & Kociolek 2022, Figs 14-23). “True Caverns” can be seen in *Phycavernosa kapitiana* S.Blanco (2020), *Orthoseira dendrophylla*, *O. madagascarensis* S.A.Spaulding & Kociolek ex Kociolek *et al.* (2022), *O. loweana*, *O. yunnanica*, and *O. radiato-sinuata* (J.Chen) Kociolek *et al.* (2022). The external appearance of “True Caverns” appears remarkably similar to the external appearance of the next two characters: “Valvocopular Extensions” and “Internal Valves”, and the most reliable way to distinguish between them is via internal views of the valve. Absence of “True Caverns” is coded as (0) and presence is coded as (1).
  
5. *Valvocopula extensions*: Internally, the valvocopula forms siliceous projections that extend up the mantle and can lie flat along the internal valve face and possess fimbriae (Danz & Kociolek 2022, Figs 53-56). These projections vary in structure and can resemble panels or grow together more elaborately. This feature can be seen in *Orthoseira roeseana*, *O. hawaiiensis*, and *O. circularis*. Externally, this character has an appearance remarkably similar to the previous character, “True Caverns”, and the next character “Internal Valve”, and the most reliable way to distinguish between them is via internal views of the valve. Absence is coded as (0) and presence is coded as (1).

6. *Internal valve*: Among the members of this group, this character is seen only in the species *Orthoseira gremmenii*. It is described as “A corroded frustule with one valve (up) smooth with irregular pattern of areolae and with the ring of holes (circular patches in LM) at the valve margin (arrows) and in the valve centre” (Van de Vijver & Kopalová 2008). Internally, the “Internal Valve” appears somewhat similar to the “Valvocopular Extensions”, possessing a somewhat fimbriate margin and looking akin to small “windows” as described by Van de Vijver & Kopalová (2008); however, the “Internal Valve” is a feature of the valve, not the cingulum, and it forms a more complete covering than the “Valvocopular Extensions” (Van de Vijver & Kopalová 2008, Figs 28, 29). The description and appearance of the internal valve of *O. gremmenii* differs from the descriptions of the internal valves of other taxa (Van de Vijver & Kopalová 2008). As with the previous two characters, this is difficult to discern without an internal view of a valve with SEM. Absence is coded as (0) and presence is coded as (1). The species *Ferocia setosa* (Greville) Van de Vijver & Houk often has internal valves (Van de Vijver *et al.* 2017a).
7. *Differentiated pores near the margin*: Groupings of pores can be identified as being differentiated from the rest of the areolae in size and demarcation. The feature may be absent, coded as (0), as in *Melosira nummuloides* C.Agardh (1827) and *Melosira varians* C.Agardh (1827). It may be present, with the differentiation of the openings being smaller and with a distinct rim (ocellus; see Round *et al.* 1990, p. 40, and p. 230, Figs a, e, f, g), as seen in *Pleurosira laevis* (Ehrenberg) Compère (1982), coded as (1). Openings that are not differentiated by a rim but are still smaller than those of the regular areolae are termed pseudocelli (Round *et al.* 1990, p. 40, and p. 246, Figs a-d, f, g). This character state is found in the species of *Biddulphia* S.F.Gray (1821), *Terpsinoë*, and *Hydrosera*, and is coded as (2). The third character state, marginal pore fields, are groups of differently arranged areolae at the edge of the valve face which may correspond with internal caverns. These groupings are distinct from the regular pattern of striation, but they lack a distinct rim, and they are not smaller in size than the regular areolae (Ross & Sims 1978, Figs 1-4; Round *et al.* 1990, p. 175, Figs e, f, g; Kociolek, *et al.* 2022). The



marginal pore fields character state is coded as (3), and can be seen in *Orthoseira circularis*, *O. dendrophylla*, *O. hawaiiensis*, *O. madagascarensis*, *O. radiato-sinuata*, *O. spinosa* W.Smith (Kochman-Kędziora *et al.* 2023), and *O. yunnanica*. While the marginal pore field is similar to the pseudocellus, these character states were separated to best reflect the formal definition of a pseudocellus: “field of areolae decreasing in size from those on the main part of the valve” (Ross *et al.* 1979, p. 521).

8. *Thickenings along the girdle*: One undulation of thickly deposited silica is deposited on each copula of the girdle band for species bearing this feature (Danz & Kociolek 2022, Figs 46, 47, 50, 51, 52, 57, 58). Crawford in Round *et al.* (1990) mentioned a similar feature as helping define the genus *Orthoseira*: “a characteristic thickening of the girdle bands can be seen in mid focus” (176, Fig. C); however, we have only seen this feature in some, not all, taxa, whereas Crawford mentioned it in the generic description. There may be some pores through this thickened section, with grooved ornamentation, internally, at the base of these thickenings (Danz & Kociolek 2022, Fig. 57). Areolae and ornamentation can only be discerned by looking at internal views of a cell with a scanning electron microscope. When observing this feature with a light microscope, the thickenings can be seen as bands of alternating colour (Round *et al.* 1990, p. 174, Fig. c; Danz & Kociolek 2022 Figs 46, 47), and in some species these are deflected (Round *et al.* 1990, p. 175 Fig. n; Ehrenberg 1871, plate 2, Figs 5-8). Absence of this feature is coded as (0), and presence is coded as (1). This character is seen in *O. circularis*, *O. dendroteres*, *O. hawaiiensis*, and, to a lesser degree, in *Phycavernosa kapitiana* (Stidolph 1990, Fig. 4).
  
9. *Collum*: Presence of the collum can be distinguished by a lack of ornamentation towards the abvalvar edge of the mantle (Danz & Kociolek 2022, Fig. 11). In some taxa, in addition to being present, the collum is also thickened, and can project out and form a mantle step (Danz & Kociolek 2022, Fig. 11), while in others the thickening is represented internally (Danz & Kociolek 2022, Fig. 56). This feature can be seen in taxa of the genera: *Aulacoseira* Thwaites (1848), *Orthoseira*, *Guarreraea* and *Phycavernosa*. The collum at the base of the mantle is absent, coded as (0), or present, coded as (1).

10. *Mantle height*: This feature refers to the height/length of the valve. The mantle is thought to be short in most centric diatoms (Round *et al.* 1990) and is coded as (0). An elongated mantle, compared to most ‘centric’ diatoms, was suggested to be a feature that diagnosed the Melosirales R.M.Crawford (Round *et al.* 1990). Neither an absolute size, nor a ratio of mantle to frustule length has been quantitatively defined, so this is a highly subjective, and relative, term. An elongate mantle is coded as (1) and is present in taxa from the genera *Melosira*, *Orthoseira*, *Guarreraea* and *Phycavernosa* (Round *et al.* 1990; Cremer *et al.* 2011; Guerrero *et al.* 2018) based on the historic understanding of the Melosirales.
11. *Mantle shape*: This feature refers to whether the mantle is uniform in height between the valve edge and the valvocopula, coded as (0), or saddle, coded as (1). A uniform mantle is one that has a consistent depth all the way around the frustule and can be seen in most species of *Orthoseira*, and in taxa of *Melosira*, *Guarreraea*, *Phycavernosa*, *Biddulphia*, *Terpsinoë*, and *Hydrosera* (Guerrero *et al.* 2018, Fig. 25). A saddle mantle (Kociolek *et al.* 2022, Fig. 9) can be seen in the species *O. radiato-sinuata*, *O. yunnanica*, and *O. dendroteres*. In *O. dendroteres* the girdle bands are deflected as well (Ehrenberg 1871, pl. 2, Figs 5-8).
12. *Spines*: Spines are silica extensions that protrude away from the valve face and are either solid or hollow (Round *et al.* 1990, p. 175, Figs e, f, j; Danz & Kociolek 2022, Fig. 38). They may be located near the edge of the valve face, by the face-mantle junction, or along the valve. Absence of spines is coded as (0). Types of spines include: simple (Van de Vijver & Crawford 2014, Figs 6-7), coded as (1), and complex (Van de Vijver & Kopalová 2008, Figs 22-23), coded as (2).
13. *Ridges*: This feature refers to a series of indentations and extrusions that form ridge-, or canyon-like features along the valve margin (Metzeltin & Lange-Bertalot 2007, pl. 4, Figs 1-12; Round *et al.* 1990, p. 175, Fig. h; Danz & Kociolek 2022, Figs 59-65, 67-69). This feature has been called a “rib” by Houk (1993), though that term is easily conflated with other, unrelated features. Absence of this feature is coded as (0) and presence is

coded as (1). This feature can be seen in the species *Orthoseira groenlandica* Goeyers, Kochman-Kędziora & Van de Vijver, *O. distinctiareolata*, *O. oregoniana*, *O. radiata*, *O. roeseana*, *O. spinosa*, *O. tropica*, and *O. ursulae*.

14. *Valve-mantle interface/junction*: This feature refers to the shaping of the valve face, and to the junction between the valve face and the valve mantle. This shaping can either be rounded or convex, and have a rounded transition at the valve face-mantle junction, as in *Melosira nummuloides* (Crawford 1979), coded as (0), or it can be relatively flat/concave, and have a near right angle at the valve face-mantle junction, as typically seen in species of *Orthoseira* (Round *et al.* 1990, p. 174-175, Figs e-i; Danz & Kocielek 2022, Figs 22-23, 27, 46-47, 50-51), coded as (1).
15. *Copulae Form*: The girdle bands may either be closed (0), as in *Terpsinoë*, *Biddulphia*, and *Hydrosera* (Round *et al.* 1990, p. 247, Figs d, h; p. 251, Fig. j; p. 257, Figs b, f;), or open and ligulate (1), as in *Orthoseira*, *Guarreraea*, *Ferocia*, *Phycavernosa*, *Pleurosira*, and *Melosira* (Guerrero *et al.* 2018, Figs 31-33).
16. *Valve outline*: The outline of the valve is either bi- or multipolar (Round *et al.* 1990, p. 247, Fig. H, and p. 250, Fig. C), coded as (0), or radial (Round *et al.* 1990, p. 174, Fig. D), coded as (1). In *Biddulphia*, *Hydrosera*, and *Terpsinoë* the outline is bipolar or multipolar. In *Orthoseira*, *Guarreraea*, *Ferocia*, *Leptocylindrus*, *Phycavernosa* and *Melosira* the frustule outline is round/radial.

The matrix was built using Mesquite version 3.61 (Maddison & Maddison 2019). Tree searching was done in PAUP\* version 4.0a (Swofford 2003), and trees were observed using FigTree (Version 1.4.4). The full matrix is presented in Table 2.

#### *Parsimony analysis*

The major phylogenetic analyses for our dataset were performed using both the command line version and the GUI version of PAUP\*. For every analysis, an initial heuristic search was performed which was then followed by bootstrap iterations. The heuristic search utilised the

parsimony criterion, the outgroup method, with the taxon *Leptocylindrus danicus* Cleve (1889) as the outgroup informed by the work of Theriot *et al.* (2015), random sequence additions, 500 repetitions, and tree bisection-reconnection (TBR) as the swap criterion. Due to the large number of trees visited, auto increase of maximum trees was allowed. During the initial heuristic search, all of the most parsimonious trees were held and their branch lengths were stored. Upon completion of the heuristic search a majority rule consensus tree was generated. Bootstrap iterations utilised all of the same settings as the initial heuristic search. 500 bootstrap iterations were run for each analysis. For each iteration the most parsimonious trees were held and a majority rule consensus tree was generated upon completion of all runs. Resulting trees were saved in .tre format. The consistency index as well as other related information was also calculated in PAUP\*. A permutation tail probability (PTP) test with 100 re-samplings was also implemented in PAUP\* to determine if our data held any evolutionary significance (Archie 1989).

We did not compute bootstrap values for the consensus tree. Autapomorphic characters have been suggested as non-conducive to bootstrap analyses, as well as an increasing number of taxa, as both decrease the expected bootstrap value (Carpenter 1992; Soltis & Soltis 2003). There are some solutions aimed at solving these limitations, such as adding invariant characters to a matrix to increase the probability an informative character is selected, and corrective actions aimed at the bootstrap sampling specifically, but these solutions are highly computationally intensive, and do not always succeed (Soltis & Soltis 2003).

The majority rule consensus trees resulting from the initial heuristic search was opened in Mesquite along with the original matrix data. Using Mesquite's built-in parsimony function, we mapped the history of each character in order to determine at what point along the tree individual features evolved. This mapping was done solely to determine the hierarchical layout of each topology. No hypothesis was tested based on this mapping or these characters. For final presentation, the .tre file saved from the parsimony analyses were opened and oriented using the program FigTree. Once oriented, the file was then opened in Adobe Illustrator CS6 and the origin and character state changes for every character identified from Mesquite were added.

Registration of all novelties have been made in PhycoBank (Kusber *et al.* 2019) according to Turland *et al.* (2018, Art. 42).

## Results

The results of the permutation tail probability test indicate that this morphological data does have a significant evolutionary signal (p-value = 0.01).

The phylogenetic tree as presented in Fig. 1 was a majority rule consensus tree generated from the heuristic analysis. This analysis found 266843 equally parsimonious topologies, all 41 steps long. Scores were presented along the branches of Fig. 1 are not bootstrap values, as no bootstrap resampling occurred during this search. The scores were only indicative of how often a group was seen out of the 266843 equally parsimonious topologies. The tree shown in Fig. 1 had a consistency index value of 0.51, a homoplasy index of 0.49, a retention index value of 0.75, and a rescaled consistency index of 0.3841. A description of the tree, as well as analysis of characters follows. Character state changes were placed along branches solely for presentation purposes; the only informative information was that a change occurred at some point along a branch, and no information was included indicating if one change on a branch preceded a separate change on the same branch.

### *Heuristic tree description*

All the following distinctions were drawn from Fig. 1. Placement of characters along a branch did not include any time calibrated information and the orientation of one character before or after another is solely the result of presentation. At the root, the ancestor of all included taxa, including the outgroup *Leptocylindeus danicus*, had “open and ligulate girdle bands”, a “radial valve outline”, an uncertain placement of a “rounded valve-mantle junction”, and it had none or simple spines. Moving from the root there were two distinct clades — one composed of the terminal, and outgroup, taxon *L. danicus*, the other consisted of all the ingroup taxa. The branch leading to *L. danicus* was marked with “rounded valve-mantle junctions”, but it is possible that this evolution occurred earlier, hence its uncertain designation and the inclusion of this feature both at the root and along this line. *Leptocylindeus danicus* and its ancestor also lacked spines. The branch leading to the clade of all of the ingroup taxa was diagnosed by sessile rimoportulae and a flat valve-mantle junction.

At the next node two branches were present, one that led to a clade of the biddulphioid taxa while the other led to rest of the ingroup taxa. The branch leading to the biddulphioid clade was diagnosed by “bi-multi polar valves” and an uncertainty in the status of “differentiated

pores”. The biddulphioid clade was further split into a lone branch and a polytomy. The lone branch lead to *Pleurosira laevis*, diagnosed by the evolution of “ocelli” and the presence of both “slit and sessile rimoportulae”. An unresolved trichotomy comprised of *Terpsinoë musica* Ehrenberg (1843), *Hydrosera triquetra* G.C.Wallich, and *Biddulphia biddulphiana* (J.E.Smith) Boyer was present. This trichotomy was diagnosed by the evolution of “stalked rimoportulae”, “pseudocelli”, and “closed girdle bands.” The ancestor of *Terpsinoë musica* also lost spines.

The remaining ingroup taxa formed a clade opposite the biddulphioid group. The branch leading to the remaining ingroup taxa was united by the character “elongate mantle.” Two of the branches in this polytomic clade led to the terminal taxa *Melosira varians* and *M. nummuloides*, respectively. The branch leading to *M. varians* had no diagnostic features, while the branch leading to *M. nummuloides* had a “rounded valve-mantle junction” marked. The final branch of the polytomic clade led to another, larger polytomy with four subsequent branches. This polytomy was united by the character “collum”.

Three of the four branches in this next, larger polytomy led to the terminal taxa *Aulacoseira crenulata* (Ehrenberg) Thwaites, *Ferocia setosa*, and *Phycavernosa kapitiana*, respectively. The branch with *A. crenulata* was diagnosed by “complex spines” while the branch with *F. setosa* was diagnosed by a “rounded valve-mantle junction”, “slit rimoportulae”, and “complex spines”, *F. setosa* also often has an internal valve present, but not always, and the branch with *P. kapitiana* was diagnosed by “true caverns”, “thickened girdles”, and “stalked rimoportulae.” “Rounded valve-mantle junction” also evolved in the ancestor of *Melosira nummuloides*, and it had an uncertain position near the root of the tree and with the outgroup; “stalked rimoportulae” also independently evolved in the ancestor of the *Biddulphia-Hydrosera-Terpsinoë* trichotomy. Along the final branch of this polytomy the feature of “carinoporulae” arose.

The clade following “carinoporulae” was broken into two branches. One branch led to the terminal taxon *Guarreraea limnopolarensis* (Van de Vijver & R.M.Crawford) Kociolek & Van de Vijver, and is marked with “slit rimoportulae.” The taxon *G. stipata* (Frenguelli) Kociolek & J.M.Guerrero (in Guerrero *et al.* 2018) codes exactly the same as *G. limnopolarensis*. “Slit rimoportulae” also independently evolved in the ancestor of *Ferocia setosa*. Opposite the *Guarreraea limnopolarensis* branch was a clade with all taxa of *Orthoseira*. The ancestor of all *Orthoseira* taxa lost “rimoportulae.” This clade was also polytomic with two

of the three branches leading to the terminal taxa *O. biportulata* Van de Vijver & Beyens and *O. johansenii* R.L.Lowe & Kocielek, respectively. There are no synapomorphies for either branch. The third branch leads to an additional polytomic clade where “internal etchings” may have arisen.

Of the three branches in the polytomy, one branch led to a clade of seven terminal taxa. The feature “ridges” arose in the common ancestor of these seven taxa. This was the only lineage among all of the taxa presented in this analysis to have evolved the “ridge” feature. The clade of seven terminal taxa was polytomic, with one branch leading to the terminal taxon *O. tropica*. *Orthoseira tropica* has an uncertain status regarding the feature “internal etchings”. Another of the branches led to a clade of *O. ursulae* and *O. oregoniana*. The ancestor of these two taxa did not have “internal etchings”, and the ancestor of *O. oregoniana* additionally secondarily lost the “collum” feature. The final branch of this polytomy led to another polytomic clade of four terminal taxa: *O. roeseana*, *O. radiata*, *O. distinctiareolata*, and *O. groenlandica*; these four taxa had internal etchings present. The feature of “valvocopular extensions” was autapomorphic (derived and unique) for *O. roeseana*.

Opposite the branch with “ridges” were two additional branches. One branch led to the terminal taxon *O. epidendron* (Ehrenberg) Round, R.M.Crawford & D.G.Mann, which had an unknown “internal etching” status. The taxa *Stephanosira hamadryas* Ehrenberg (1848) and *Orthoseira europaea* (Ehrenberg) R.Jahn & Kusber (in Geissler *et al.* 2006) coded exactly the same as *O. epidendron*, and together formed a group. The final branch opposite “ridges” led to an additional polytomy; the ancestor of the taxa in this polytomy definitively had the “internal etching” feature. This polytomic clade had five sub-branches. The first of the five sub-branches had four terminal taxa, *O. mauiana*, *O. verleyenii*, *O. spinosa* and *O. gremmenii*. The ancestor of these four taxa had an uncertain “spine” status, with “simple” and “loss of” being equally parsimonious. *Orthoseira mauiana* diverged from *O. verleyenii*, *O. spinosa* and *O. gremmenii*, and at some point in its evolution had an ancestor that definitively lost spines. The ancestor of *O. verleyenii*, *O. spinosa* and *O. gremmenii* evolved “complex spines.” The ancestor of *O. spinosa* independently evolved “marginal pore fields”, and the ancestor of *O. gremmenii* independently evolved an “internal valve”, a feature also often seen in *Ferocia setosa*.

The second of the five sub-branches contained only the terminal taxon *O. ferrarioana* Guerrero, Kocielek & Sala, and it lacked any synapomorphic features. The taxon *Orthoseira*

*frenquellii* J.M.Guerrero, Kociolek & Vouilloud (in Guerrero *et al.* 2018) coded exactly the same as *O. ferrarioana*.

The third of the five sub-branches was comprised of four terminal taxa: *O. loweana*, *O. yunnanica*, *O. madagascarensis*, and *O. dendrophylla*. The ancestor of this lineage evolved the synapomorphy “true caverns.” True caverns also evolved in the ancestor of *Phycavernosa kapitiana*. *Orthoseira yunnanica*, *O. madagascarensis*, and *O. dendrophylla* form another grouping diagnosed by the presence of “marginal pore fields”. The ancestor for *Orthoseira yunnanica* and *O. madagascarensis* had no or simple spines. Diagnostic features of *O. yunnanica* include a “saddle” shaped mantle and a total “loss of spines.” *Orthoseira radiato-sinuata* codes the exact same as *O. yunnanica* and would also fall out here. “Loss of spines” can also be seen in the ancestor of *O. mauiana*.

The fourth sub-branch contained only the terminal taxon *O. cylindrica* Rybak, Glushchenko & Kulikovskiy in Rybak *et al.* (2022), and it lacked any synapomorphic features.

The fifth sub-branch is composed of four terminal taxa: *O. rapanuiensis* Rybak, Witkowski & Lange-Bertalot in Rybak *et al.*, *O. dendroteres*, *O. hawaiiensis*, and *O. circularis*. This ancestor of this lineage may have evolved “thickenings on the girdle bands”, a feature that is also seen in *Phycavernosa kapitiana*, here. *Orthoseira rapanuiensis* diverged from the remaining three taxa, though this is likely as a result of the uncertain girdle status. The original report of *O. rapanuiensis* did not include any description or images of the girdle elements, and the most parsimonious assumption is that this taxon does have thickened girdles, but this remains to be verified, hence its placement as adjacent. The remaining three taxa, *O. dendroteres*, *O. hawaiiensis*, and *O. circularis* shared an ancestor that definitively evolved “thickened girdle bands.” Of these three terminal taxa, *O. dendroteres* was more distantly related and is diagnosed by a “saddle shaped mantle”; a feature also seen in *O. yunnanica*. The ancestor of *O. hawaiiensis* and *O. circularis* evolved “valvocopular extensions” and “marginal pore fields”, the latter also found in the clade of *O. yunnanica*, *O. madagascarensis*, and *O. dendrophylla*; whereas “valvocopular extensions” were also observed in *O. roeseana*. An ancestor of *O. hawaiiensis* independently lost the “collum” feature.

## Discussion



The consensus heuristic search yielded trees that had moderate support and allowing further interpretation of the interrelationships of the orthoseiracean taxa (see sections below). Additionally, while not the same as bootstrap resampling, the frequent identification of certain groups (up to 100% of the trees out of the large consensus pool) indicates that there are uniting features in this group. As it stands, the characters used to generate this tree provide a framework for a hypothetical phylogeny. Ideally, this phylogeny will be tested by additional data and methods. Many of the characters included in this analysis appear to be highly specialised (apomorphic) and may be the result of species diversification of the Liparograceae fam. nov. after moving to aerophilous habitats and as new niches were encountered in this environment. Many of the groups introduced from this analysis are diagnosed by only one character, and it is possible they would be unrepresented in some of the bootstrap iterations.

#### *Relationship of the Orthoseiraceae to Melosiraceae and Biddulphiaceae*

Gargas *et al.*'s (2018), five-gene (nSSU, *rbcL*, *psbC*, *psbA*, and *psaB*) phylogenetic analysis revealed that the Liparograceae fam. nov. is more closely related to the genera *Hydrosera* and *Terpsinoë*, within the biddulphioid lineage, than it is to any genus of the melosiroid diatoms. This is a stark divergence from the historic understanding of relationships with the genus (see Danz *et al.* 2022 for a detailed history of the group), though there are questions regarding the five-gene analysis that revealed the relationships reported by Gargas *et al.* (2018) (see Medlin & Desdevises 2020). The majority rule consensus tree of the heuristic search did speak to this question, that is, the melosiroid taxa are more closely related to the members of *Orthoseira* and its relatives than the biddulphioid taxa. However, due to the existing questions regarding the 5-gene analysis that discovered the Biddulphioid-Orthoseiroid relationship and the lack of consensus in our data, we do not make a distinction regarding the status of the Liparograceae as either a melosiroid or biddulphioid diatom. As a result we are unable to definitively answer the first aim of this report, i.e., the relationship of Liparograceae to Melosiraceae and Biddulphiaceae, and further study is required to discern where exactly the Liparograceae fall out in the diatom tree of life.

#### *A proposed natural classification*

Based on the phylogeny presented herein, we can develop a classification system for the taxa previously assigned to the genus *Orthoseira*. Since *Orthoseira sensu* Thwaites (1848), typified by *O. americana* (Kützing) Kociolek & Spaulding (basonym: *Melosira americana* Kützing 1844), lacks carinoportulae (based on Kützing's original figure), we consider *Orthoseira*, and the family (Orthoseiraceae) and order (Orthoseirales) based upon the genus, as *Incertae sedis*, probable synonyms of *Melosira*, Melosiraceae and Melosirales, respectively. Thus, we propose a new order and two families for this group (Table 3).

Order Liparogyrales Danz & Kociolek, ord. nov.

Description: Cells cylindrical in girdle view, forming short chains. Method of chain formation unknown, hypothesised to be the result of linking spines, mucilage excretion, or a combination of both. Spines present or absent, when present providing torsional control in addition to linkage. Valves radial, with rows of striae radiating from the centre. Carinoportulae and/or rimoportulae present in the centre. Girdle bands open, ligulate, poroids present on the copulae. Taxa bearing a mantle collum, the latter occasionally thickened. Taxa found in aerophilous, or sub-aerial habitats, especially among freshwater bryophytes.

Type: *Liparogyra* Ehrenberg (automatically typified according to Turland *et al.* 2018, Art. 16.1, 10.10).

Registration: <http://phycobank.org/104342>

Family Phycavernosaceae Danz & Kociolek, fam. nov.

Description: Cells round. Rimoportulae distinct, elevated, placed in or around the centre of the valve. Valve exterior with rounded areolae. Granules and ridges occasionally present. Spines, with simple or stellate bases, located around the valve margin. Internally, caverns formed by depressions in the valve face and thickened ribs. Girdle bands ligulate, open. Valvocopula with a fimbriate margin on the pars interior.

Type: *Phycavernosa* S.Blanco (automatically typified according to Turland *et al.* 2018, Art. 10.9).

Registration: <http://phycobank.org/104343>

Genus *Phycavernosa* S.Blanco in *Notulae Algarum* 39: 5. 2020.

See Stidolph (1990) and Cremer *et al.* (2011) for a description of this genus under the name *Cavernosa* Stidolph (see Turland *et al.* 2018, Art. 20.2 according to Blanco 2000).

Generitype: *Phycavernosa kapitiana* S.Blanco.

Family Liparogyraceae Danz & Kociolek, fam. nov.

Description: Cells round, carinoportulae distinct, positioned in or around the centre of the valve. Rimoportulae absent. Otherwise, conforming to the description of the order.

Type: *Liparogyra* Ehrenberg (automatically typified according to Turland *et al.* 2018, Art. 10.9).

Genus *Liparogyra* Ehrenberg, Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlichen Preussischen Akademie der Wissenschaften zu Berlin 1848: 217. 1848. emend Danz & Kociolek.

The generitype of this genus is designated here:

*Liparogyra (Liparogyra) dendroteres* Ehrenberg, Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlichen Preussischen Akademie der Wissenschaften zu Berlin 1848: 219. 1848.

Registration: <http://phycobank.org/104363>

This genus was originally described by Ehrenberg in 1848 but was relegated in favour of the genus *Orthoseira* Thwaites (Crawford 1979). An emended description is presented below.

Ehrenberg did not designate a type species for this genus.

Emended description by Danz & Kociolek: Cells cylindrical in girdle view. Valve face flat to slightly concave, disc-shaped, forming a right-angle with the valve mantle. Caverned appearance visible in both LM and SEM. Marginal pore fields visible in SEM at the valve margin, corresponding with the caverned appearance. Rows of striae radiating from the centre, occasionally leaving a small hyaline central area. Carinoportulae present at the centre with faint etch-like slits present between them. Striae from the valve face continuing onto the valve mantle. Lower part of the mantle marked by a collum. Cingulum composed of open, ligulate copulae, poroids present. Individual copulae with a distinct thickening, visible in both LM and SEM.

Subgenus *Liparogyra* Ehrenberg

Nominate subgenus automatically established because of *Liparogyra* subgen. *Porocyclia* (Ehrenberg) Danz & Kociolek and *Liparogyra* subgen. *Insularis* Danz & Kociolek (see below; Turland *et al.* 2018, 22.3).

Description of the subgenus: Containing the features of the genus.

As presented, the subgenus *Liparogyra* should have three taxa assigned to it: *L. circularis*, *L. hawaiiensis*, and *L. dendroteres*. This subgenus has a wide distribution, with taxa having been described from Venezuela and Maui, Hawaii (Ehrenberg 1848; Chen 1980; Danz & Kociolek 2022). Some of these taxa have been seen outside of their type localities as well, with *L. circularis* and *L. dendroteres* reported from Central Europe, and *L. dendroteres* reported from the United States (Krammer & Lange-Bertalot 1991). These taxa are typically found growing on mosses or trees (Ehrenberg 1848; Danz & Kociolek 2022). The distinction of this group as unique matches the work done by Ehrenberg (1848), but slightly conflicts with the findings of Houk (1993). Where Ehrenberg (1848) saw one genus, Houk (1993) saw two groups. Houk's groups are emphasised by the species *L. circularis* and *L. dendroteres*. *Liparogyra circularis* lacks deflected girdle bands while *L. dendroteres* does. Houk (1993) questioned if this was sufficient to distinguish two groups, and ultimately depicted them as such. The topology presented in Fig. 3 shows two distinct groups within this suggested subgenus, with one marked by a caverned appearance as the result of extensions of the valvocopulae, while the other has true caverns, a non-uniform mantle, and deflected girdles. It is possible that there is a greater variability than we have presented here, but the presence of girdle thickenings, and the much earlier publication of Ehrenberg's (1848) generic name has led us to conclude there is 1 subgenus present. It differs from all other subgenera in the genus *Liparogyra* by the thickenings found on each copulae.

Ehrenberg (1848) described one other species: *Liparogyra (Liparogyra) circularis* Ehrenberg.

One new combination is proposed for this subgenus here:

*Liparogyra (Liparogyra) hawaiiensis* (Danz & Kociolek) Danz & Kociolek, comb. nov.

Basionym: *Orthoseira hawaiiensis* Danz & Kociolek (2022), *Diatom Research*, 37(1): 24, Figs 39-58.

Registration: <http://phycobank.org/104362>

Subgenus *Porocyclia* (Ehrenberg) Danz & Kociolek, subgen. nov., stat. nov.

Basionym: *Porocyclia* Ehrenberg, Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlich-Preussischen Akademie der Wissenschaften zu Berlin 1848: 217. 1848.

Type: Automatically typified by *Porocyclia dendrophyla*, type of the genus *Porocyclia* by monotypy (see below).

Registration: <http://phycobank.org/104345>

This subgenus was originally described as a genus by Ehrenberg in 1848 but was included within the genus *Orthoseira* (Crawford 1981).

Description: Cells cylindrical in girdle view. Valve face flat to slightly concave, disc-shaped, forming a right-angle with the valve mantle. Rows of striae radiating from the centre, leaving occasionally a small hyaline central area. Carinoportulae present at the centre. Faint etch-like rimoportulae present between the carinoportulae. Striae from the valve face continuing onto the valve mantle. Lower part of the mantle marked by a mantle collum. Cingulum composed of open, ligulate copulae, poroids present.

*Liparogyra dendrophyla* was originally described from Karsten's material from South America (Ehrenberg 1848), but it has also been interpreted to be present from Scotland (Ross & Sims 1978) and Central Europe (Krammer & Lange-Bertalot 1991). Given the regionalism of many species in this group, the reports of this taxon from Europe should be further analysed. *Liparogyra dendrophyla* has simple spines, true caverns, similar to those of *Phycavernosa*, and internal and external marginal pore fields associated with the caverns. This taxon was found growing on trees (Ehrenberg 1848). The distinction of this subgenus as a unique group matches the work done by Ehrenberg (1848) and Houk (1993). This subgenus differs from all others by the presence of etch-like rimoportulae between its carinoportulae, combined with simple spines and true caverns that have pores associated with them.

New combinations in *Liparogyra* in the subgenus *Porocyclia* include:

*Liparogyra (Porocyclia) dendrophyla* (Ehrenberg) Danz & Kociolek, comb. nov.

Basionym: *Porocyclia dendrophyla* Ehrenberg (1848), Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlich-Preussischen Akademie der Wissenschaften zu Berlin 1848: 219.

Registration: <http://phycobank.org/104364>

*Liparogyra (Porocyclia) yunnanica* (Chen) Danz & Kociolek, comb. nov., stat. nov.

Basionym: *Melosira radiato-sinuata* var. *yunnanica* Chen (1980), Acta Hydrobiologica Sinica 7(2): 256, pl. 2, Figs 5-7.

Homotypic synonym: *Orthoseira yunnanica* (Chen) Kociolek *et al.* 2022.

Registration: <http://phycobank.org/104365>

*Liparogyra (Porocyclia) radiato-sinuata* (Chen) Danz & Kociolek, comb. nov.

Basionym: *Melosira radiato-sinuata* Chen (1980). Acta Hydrobiologica Sinica 7(2): 255, Plate 2, Figs 2-4.

Homotypic synonym: *Orthoseira radiato-sinuata* (Chen) Kociolek *et al.* 2022.

Registration: <http://phycobank.org/104366>

*Liparogyra (Porocyclia) madagascarensis* (Spaulding & Kociolek ex Kociolek *et al.*) Danz & Kociolek, comb. nov.

Basionym: *Orthoseira madagascarensis* Spaulding & Kociolek ex Kociolek *et al.* (2022), Diatom Research 36(4): 349.

Registration: <http://phycobank.org/104367>

*Liparogyra (Porocyclia) loweana* (Danz & Kociolek) Danz & Kociolek, comb. nov.

Basionym: *Orthoseira loweana* Danz & Kociolek (2022), Diatom Research 37(1): 21, Figs 14-29.

*Subgenus Insularis* Danz & Kociolek, subgen. nov.

Registration: <http://phycobank.org/104346>

This subgenus was not treated previously.

Description: Cells cylindrical in girdle view. Valve face flat to slightly concave, disc-shaped, forming a right-angle with the valve mantle. Rows of striae radiating from the centre. Small hyaline central area occasionally present. Carinoportulae present at the centre. Faint etch-like rimoportulae present between the carinoportulae. Spines entirely lacking or present but then having a complex structure, linking cells similar to the spines in *Aulacoseira* (Round *et al.* 1990). Striae continuing from valve face onto valve mantle. Lower part of the mantle marked by a collum. Cingulum composed of open, ligulate copulae, poroids present.

As presented, four taxa should be assigned to this subgenus, including *L. gremmenii*, *L. verleyenii*, *L. mauiana*, and *L. spinosa*. *Liparogyra gremmenii* is unique in that it has a caverned appearance, but this is the result of an “internal valve” (Van de Vijver & Kopalová 2008). This genus has a wide distribution, with taxa having been described from Gough Island in the South Atlantic, Île Amsterdam (southern Indian Ocean) and Maui, Hawaii respectively (Van de Vijver & Kopalová 2008; Lowe *et al.* 2013; Danz & Kociolek 2022). Members of this genus have been reported growing on mosses in lava tubes and in freshwater to terrestrial habitats (Van de Vijver & Kopalová 2008; Lowe *et al.* 2013; Danz & Kociolek 2022). This subgenus has not been suggested as a group in the past. It differs from all other subgenera in *Liparogyra* by the presence of etch-like rimoportulae combined with either no spines or complex spines, but at present not simple spines.

Type: *Liparogyra (Insularis) mauiana* (Danz & Kociolek) Danz & Kociolek, comb. nov.

New combinations are presented below:

*Liparogyra (Insularis) mauiana* (Danz & Kociolek) Danz & Kociolek, comb. nov.

Basionym: *Orthoseira mauiana* Danz & Kociolek (2022), *Diatom Research* 37(1): 19, Figs 1-18.

Registration: <http://phycobank.org/104369>

*Liparogyra (Insularis) gremmenii* (Van de Vijver & Kopalová) Danz & Kociolek, comb. nov.

Basionym: *Orthoseira gremmenii* Van de Vijver & Kopalová (2008) *Cryptogamie Algologie*, 29: 108, Figs 1-29.

Registration: <http://phycobank.org/104370>

*Liparogyra (Insularis) verleyenii* (Van de Vijver in Lowe *et al.*) Danz & Kociolek, comb. nov.  
 Basionym: *Orthoseira verleyenii* Van de Vijver in Lowe *et al.* (2013), *Phytotaxa*, 111: 41, Figs 1-27.

Registration: <http://phycobank.org/104371>

*Liparogyra (Insularis) spinosa* (W.Smith) Danz & Kociolek, comb. nov.

Basionym: *Orthos[e]ira spinosa* W. Smith (1855), *Annals and Magazine of Natural History*, series 2 15:8, pl. 1, Fig. 12.

Registration: <http://phycobank.org/104372>

Genus *Guarreraea* Kociolek, J.M.Guerrero & Van de Vijver

See Guerrero *et al.* (2018, p. 129) for the description of the genus.

Generitype: *G. limnopolarensis* (Van de Vijver & R.M.Crawford) Kociolek & Van de Vijver

One additional taxon was assigned to the genus, *G. stipata* (Frenguelli) Kociolek & J.M.Guerrero.

Genus *Stephanosira* Ehrenberg

This genus was originally described by Ehrenberg in 1848 but was relegated in favour of the genus *Orthoseira* Thwaites (Crawford 1981). Ehrenberg (1848, 1853, 1854, 1856) included 6 species in this genus, and later Karsten (1905) added another.

Description: Cells cylindrical in girdle view. Valve face that flat to slightly concave, disc-shaped, forming a right-angle with the valve mantle. Rows of striae radiating from the centre. Small hyaline central area occasionally present. Carinoportulae present at the centre. Internal etchings occasionally present. Striae from the valve face continuing onto the valve mantle. Lower part of the mantle marked by a collum. Cingulum composed of open, ligulate copulae, poroids present. This genus is distinct from the others presented here, as in addition to carinoportulae, it can have slit-like rimoportulae at the centre.

*Stephanosira hamadryas* was an exact match for *S. epidendron*, and as a result is redundant and not included in the analysis, though it does fall out here. *Orthoseira europaea* also codes the exact same as these two taxa, but its taxonomic treatment is not being addressed here



as the type material needs to be confirmed before any nomenclatural action is taken. Additional taxa possibly belonging in this genus include: *Orthoseira antiqua*, *O. asiatica* (Skvortzov) H.Kobayasi in Mayama *et al.* 2002, *O. rotula*, and *O. tenuis* (Kützing) Rabenhorst. Based on what data could be collected, these taxa coded out the exact same as *S. epidendron*, though there were numerous gaps in information due to the age and technology of their most recent treatments. The status of these taxa will remain pending until the original material of these taxa can be analysed and a greater resolution achieved. Some final taxa that may be included in the genus but requiring further study are *S. symbolophora* Ehrenberg (1853), *S. caroli* Ehrenberg (1854), *S. mississippiica* Ehrenberg (1856) and *S. decussata* Karsten (1905), which appear to not have been treated since their original descriptions.

Taxa included in the genus *Stephanosira* are listed below:

*Stephanosira epidendron* Ehrenberg

Original description: *Stephanosira epidendron* Ehrenberg (1848), Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlich-Preussischen Akademie der Wissenschaften zu Berlin, 1848: 219.

*Stephanosira hamadryas* Ehrenberg

Original description: *Stephanosira hamadryas* Ehrenberg (1848), Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlich-Preussischen Akademie der Wissenschaften zu Berlin, 1848: 219.

Homotypic synonym: *Orthoseira hamadryas* (Ehrenberg) Houck, Klee & H.Tanaka 2017.

Genus *Aerophilia* Danz, Van de Vijver & Kociolek, gen. nov.

Generitype: *Aerophilia oregoniana* (Danz & Kociolek) Danz, Van de Vijver & Kociolek, comb. nov.

Registration: <http://phycobank.org/104347>

Description: Cells cylindrical in girdle view. Valve face flat to slightly concave, disc-shaped, forming a right-angle with the valve mantle. Rows of striae radiating from the centre. Small hyaline central area occasionally present. Carinoportulae present at the centre. Rimoportulae

lacking. Ridges present at the margins. Spines occasionally protrude from these ridges, linking between the ridges of adjacent cells, forming chains via linking spines. Rows of striae observed between the ridges, either as a single row or multiple rows. Ridges, and striae between the ridges continuing onto the valve mantle. Lower part of the mantle marked by a collum. Cingulum composed of open, ligulate copulae, poroids present.

As presented, seven taxa should be assigned to this genus: *Aerophilina distinctiareolata* (Rybak, Glushchenko & Kulikovskiy) Danz, Van de Vijver & Kociolek comb. nov., *A. groenlandica* (Goeyers, Kochman-Kędziora & Van de Vijver) Danz, Van de Vijver & Kociolek comb. nov., *A. oregoniana* (Danz & Kociolek) Danz, Van de Vijver & Kociolek comb. nov., *A. radiata* (Rybak, Glushchenko & Kulikovskiy) Danz, Van de Vijver & Kociolek comb. nov., *A. roeseana* (Rabenhorst) Danz, Van de Vijver & Kociolek comb. nov., *A. tropica* (Krasske) Danz, Van de Vijver & Kociolek comb. nov., and *A. ursulae* (Metzeltin & Lange-Bertalot) Danz, Van de Vijver & Kociolek comb. nov. This genus is presumed to have a worldwide distribution. Taxa have been found growing on mosses in lava tubes, on trees, and in other freshwater/terrestrial habitats on Maui, Hawaii, in the continental United States, in Greenland, in Costa Rica, in Brazil, in Peru, and in other Northern Hemisphere and South American locales (Lange-Bertalot *et al.* 1996; Metzeltin & Lange-Bertalot 2007; Danz & Kociolek 2022; Kochman-Kędziora *et al.* 2023; Goeyers *et al.* 2023). Houk (1993) did address two groups similar to *Aerophilina*, these were the “*tatica*” group and the “*roeseana*” group. Both of these groups have features homologous to the ridge described here, though Houk referred to them as “ribs” (Houk 1993). This genus differs from all other genera in the Liparogyraceae based on its synapomorphic feature “ridges”.

New combinations for this new genus are presented below:

*Aerophilina distinctiareolata* (Rybak, Glushchenko & Kulikovskiy) Danz, Van de Vijver & Kociolek, comb. nov.

Basionym: *Orthoseira distinctiareolata* Rybak, Glushchenko & Kulikovskiy in Rybak *et al.* (2022), Diatom Research 37(1): 6, 7, Figs 55-76.

Registration: <http://phycobank.org/104348>

*Aerophilina groenlandica* (Goeyers, Kochman-Kędziora & Van de Vijver) Danz, Van de Vijver & Kociolek, comb. nov.

Basionym: *Orthoseira groenlandica* Goeyers, Kochman-Kędziora & Van de Vijver (2023), *Phytotaxa* 60(1): 83, Figs 1-30.

Registration: <http://phycobank.org/104349>

*Aerophilina oregoniana* (Danz & Kociolek) Danz, Van de Vijver & Kociolek, comb. nov.

Basionym: *Orthoseira oregoniana* Danz & Kociolek (2022): *Diatom Research*, 37(1): 30, Figs 59-75.

Registration: <http://phycobank.org/104350>

*Aerophilina radiata* (Rybak, Glushchenko & Kulikovskiy) Danz, Van de Vijver & Kociolek, comb. nov.

Basionym: *Orthoseira radiata* Rybak, Glushchenko & Kulikovskiy in Rybak *et al.* (2022), *Diatom Research* 37(1): 7, Figs 77-106.

Registration: <http://phycobank.org/104373>

*Aerophilina roeseana* (Rabenhorst) Danz, Van de Vijver & Kociolek, comb. nov.

Basionym: *Melosira roeseana* Rabenhorst (1853), *Die Süßwasser-Diatomaceen (Bacillarien): für Freunde der Mikroskopie*. Leipzig, p. 13.

Homotypic synonyms: *Orthoseira roeseana* (Rabenhorst) O'Meara (1875), *Gallionella roeseana* (Rabenhorst) Petit 1880, p. 195; *Lysigonium roeseanum* (Rabenhorst) Kuntze (1891), *Melosira dendroteres* var. *roeseana* (Rabenhorst) R.Ross (1947).

Registration: <http://phycobank.org/104351>

*Aerophilina tropica* (Krasske) Danz, Van de Vijver & Kociolek, comb. nov.

Basionym: *Melosira roeseana* var. *tropica* Krasske, G. (1948). *Diatomeen Tropischer Moorsrasen*. *Svensk Botanisk Tidskrift*, 42: 422.

Homotypic synonyms: *Orthoseira tropica* (Krasske) Metzeltin & Lange-Bertalot 2007, p. 186. *Orthoseira roeseana* var. *tropica* (Krasske) Lange-Bertalot & Willmann in Lange Bertalot *et al.* 1996, p. 94.

Registration: <http://phycobank.org/104353>

*Aerophilina ursulae* (Metzeltin & Lange-Bertalot) Danz, Van de Vijver & Kociolek, comb. nov.

Basionym: *Orthoseira ursulae* ‘*ursula*’ Metzeltin & Lange-Bertalot (2007), *Iconographia Diatomologica*, 18: 186, pl. 4: Fig. 1; pl. 7: Fig. 2; pl. 8i: Figs 1-5; pl. 9; pl. 10: Figs 1-4.

Nomenclatural note: Because this species was named for Ursula [Wydrzycka], the basionym should be corrected to *Orthoseira ursulae*, in accordance with Turland *et al.* (2018: Art. 60.8a).

Registration: <http://phycobank.org/104354>

There are other groups identified in the phylogenetic analysis that lack synapomorphies. Until those diagnostic features can be identified we will leave them as unnamed groups and consider them as *Incertae sedis*. They include:

*Incertae Sedis*: “*Orthoseira*” *cylindrica*

This taxon has slit-like openings alternating with the c. 3 carinoportulae internally. Externally, there are small spines and thickenings around the carinoportulae and areolae. We found no features that diagnose this species from others in the context of this phylogenetic analysis. This species is known from Cambodia, Vietnam and Laos (Rybak *et al.* 2022).

*Incertae Sedis*: “*Orthoseira*” *ferrarioana* + *O. frenguelli* group

Taxa of this group have cells that are cylindrical in girdle view, with a valve face that is flat to slightly concave, disc-shaped, and forming a right-angle with the valve mantle. Rows of striae radiate from the centre, and a small hyaline central area may be present. Carinoportulae are present at the centre, with faint etch-like slits present between them. This group has simple, spike-like, spines. Striae from the valve face continue onto the valve mantle, and the lower part of the mantle is marked by a mantle collum. The cingulum is composed of open and ligulate copulae, with poroids present. As presented, this group is comprised of *O. ferrarioana* and *O. frenguelli*. This genus has a very narrow distribution, as both of these taxa are from a small creek near Copahue Volcano, Neuquén, Argentina (Guerrero *et al.* 2018). This group has been found growing in freshwater habitats (Guerrero *et al.* 2018), and it has not been suggested as a separate group in the past. This group does not have any specific feature that separates it from

the other genera treated here, instead the other genera have further distinctions that this genus lacks. The lack of a defining feature has led us to leave this group as Incertae sedis.

Incertae Sedis: “*Orthoseira*” *johansenii* only

This group includes a single taxon, *O. johansenii*, which has cells that are cylindrical in girdle view, with a valve face that is flat to slightly concave, disc-shaped, and forming a right-angle with the valve mantle. Rows of striae radiate from the centre, and a small hyaline central area may be present. Carinoportulae are present at the centre, there are no rimoportulae present in this genus. The taxon of this group has both simple and complex spines. Striae from the valve face continue onto the valve mantle, and the lower part of the mantle is marked by a collum. The cingulum is composed of open and ligulate copulae, with poroids present. *Orthoseira johansenii* was described from Pua Po’o lava tube, Volcanoes National Park, Hawai’i where it was growing on mosses in lava tubes (Lowe *et al.* 2013). The lack of a defining feature has led us to leave this genus as Incertae sedis.

Incertae Sedis: “*Orthoseira*” *biportulata* only

This taxon has cells that are cylindrical in girdle view, with a valve face that can be flat to slightly concave or slightly rounded and convex, is disc-shaped, and forms a right-angle or rounded angle with the valve mantle. Rows of striae radiate from the centre, and a small hyaline central area may be present. Carinoportulae are present at the centre, there are no rimoportulae present in this genus. The taxon of this genus has simple spines. Striae from the valve face continue onto the valve mantle, and the lower part of the mantle is marked by a collum. The cingulum is composed of open and ligulate copulae, with poroids present. *Orthoseira biportulata* was found growing on the soil of a cavern on Ile de la Possession, the main island of the Crozet Archipelago in the southern Indian Ocean (Van de Vijver *et al.* 2002). Another endemic genus from the (sub-)Antarctic region is *Michelcostea* Van de Vijver, Lange-Bertalot, C.E. Wetzel & Ector (2017) (Van de Vijver *et al.* 2017b; Kociolek 2019). Taxa of the genus *Ferocia* are also found to prefer aerophilous conditions (Van de Vijver *et al.* 2017a). This taxon does not have any specific feature that separates it from the other genera described here, instead the other genera have further distinctions lacking in this taxon. The lack of a defining feature has led us to leave this taxon as Incertae sedis.

### *Monotypic genera*

Of the 10 genera, subgenera, and uncertain groups, named and unnamed, included here in the Liparogyrales, 3 are monotypic. These monotypic groups are “*Orthoseira*” *cylindrica*, “*Orthoseira*” *johansenii* and “*Orthoseira*” *biportulata*. There is some debate on the appropriate way to deal with monotypic genera. Platnick (1976) argued that monotypic genera are counter-productive, as they are usually based on characters that are autapomorphic, and, as a result, not informative to phylogenies. Platnick (1976) also discussed the possibility that monotypic genera represent unclassified diversity, in relation to either unknown or extinct species, and with greater sampling and discovery, eventually these unknowns will come to fill the genus.

There are many instances of monotypic groupings in science. *Diprora* Main (2003) is one instance of a monotypic genus in the diatoms, reported as an endemic from the Hawaiian Islands (Main 2003). Another example of a monotypic diatom genus is *Cyclotubicoalitus* Stoermer *et al.*, described from the east coast of the USA (Stoermer *et al.* 1990). The diatom genus *Spicaticribra* J.R.Johansen, Kociolek & R.L.Lowe (2008) was originally described with a single species, *S. kingstonii* J.R.Johansen, Kociolek & R.L.Lowe (2008). Since 2008, nine additional species have been described for this genus (Guiry 2021). The centric genus *Arcanodiscus* Maidana & E.Morales (in Maidana *et al.* 2018) was described as being monotypic from Argentinian Patagonia, but, in the past 5 years, four additional new species were added from the sub-Antarctic region (Van de Vijver & Houk 2019; Goeyers & Van de Vijver 2020). More recently, two new species were described in the previously monotypic genus *Druehlado* Lobban & Ashworth (in Ashworth *et al.* 2017) (Majewska *et al.* 2021). Several examples of monotypic genera having new taxa assigned are also known from marine fossils. Outside the diatoms, Rana and Ranade (2009) indicate there are 236 genera of flowering plants in India alone that are monotypic.

Monotypic groupings have been proposed for many genera; however, over time and subsequent study, they do not usually remain with a single species. Contrary to the argument of Platnick (1976), recognition of monotypic groupings seems to have some benefit, as the designation of a new genus, or genera, seems to result in the description of more taxa. Possibly as the result of greater emphasis being placed on fine morphological detail after the challenging of long-established assumptions. Prior to this analysis, the argument for a single genus,

*Orthoseira*, to nomenclaturally encompass all of the diversity described herein was based on the assumption that species in the genus were highly plastic (Crawford 1981).

Ebach & Williams (2010), in relation to monotypic groupings, discussed Gregg's Paradox, where a single taxon cannot be included at two classification levels, and introduced the idea of aphyly, a classification status that indicates future revision is necessary, to replace paraphyletic and monotypic designations. The aphyletic distinction refers to a group not having a classification for some reason (paraphyletic, missing data, etc.), and in the specific case of monotypic genera, it recommends this classification scheme until more taxa can be added to determine monophyly (Ebach & Williams 2010). This is the situation we follow here with monotypic genera without synapomorphies (see Table 3). Application of aphyly to other situations in diatom classification (such as the genera *Epithemia* and *Rhopalodia*; Ruck *et al.* 2016; Vigneshwaran *et al.* 2021) may be a welcomed approach.

#### *Character analysis and diversification*

The topology presented in Figure 1 shows instances of homoplasy in several of the character states evaluated, including rimoportula(e), caverned appearance, girdle thickenings, and spines. In a data set that includes 35 taxa, and 16 characters, this seems like a fairly high number, especially in characters that seem highly specialised (i.e. the different methods of forming caverns and rimoportulae) (Kociolek *et al.* 2019). These homoplasious features have made the taxonomy of the group challenging and led to the suggestion that species of *Orthoseira* are highly variable (Crawford 1981). In fact, rather than being highly variable, each taxon has a unique set of features that, based on our observations and review of the literature, are well-defined. Instead of single taxon having multiple features, there have been several independent gains of features, such as spines and girdle thickenings. The appearance of caverns in this group can be seen as internally scalloped-shaped areas around the margin on the interior of the valve face (e.g. in *Phycavernosa* and *Liparogyra* subgenus *Porocyclia*), while in other species “caverns” are formed by elaborate folding and outline of the valvocopula, not the valve (as in certain species of *Liparogyra*). In some cases these characters can be synapomorphic for taxa (e.g. *L. circularis*, *L. hawaiiensis*), reminding us that even though they are shared with other taxa they can be a diagnostic feature (e.g. Kociolek 1998). In other cases, there has been loss of features, such as stalked rimoportulae or spines, creating evolutionary “noise” as we strive for

informative “signal”. However, both homoplasious character states and loss of features can represent evolutionary novelties and be diagnostic for lineages.

In the diatoms cases have been described where homoplasy is common (“rampant”) (Kociolek *et al.* 2019). The homoplasy seen in the Liparogyrales may suggest that when the in-group studied here transitioned to aerophilous environments numerous niches were exploited and there was a corresponding diversification within the group. At the same time, incidences of convergent evolution were realised between different lineages. Similar observations in which radiations in different lineages were accompanied by homoplastic features has been documented in spiders and small mammals (e.g. Blackledge & Gillespie 2004; Wölfer & Nyakatura 2019).

Also of note in these results relates to characters where the history of the state goes from absent to present, and then back to absent. Dollo’s Law of Irreversibility posits that it is extremely improbable for a character to revert back to a previous state, as some portion of the evolved trait should remain identifiable (Dollo 1893). In our work here, evidence suggests that both the ‘mantle collum’ and rimoportulae went through a series of gains and losses. The fact that this apparent reversal happened not once, but twice, in a single group, may further be related to diversification after invasion into aerophilous habitats.

Equally parsimonious interpretations of character state data can lead to uncertainty in the interpretation of character evolution. For example, the character “internal etchings” may have arisen in the ancestor of the genera *Liparogyra*, *Stephanosira* and *Aerophilia*, and then was subsequently lost in the terminal taxa *Aerophilia oregoniana* and *A. ursulae*. It is an equally parsimonious interpretation that “internal etchings” arose independently in the genus *Liparogyra*, and in the *groenlandica-distinctiareolata-radiata-roeseana* clade within the genus *Aerophilia*. Currently we cannot clarify this uncertainty as the internal area around the carinoportulae has not been observed for *Stephanosira epidendron* or *Aerophilia tropica*, preventing assignment of characters for this feature in these taxa. Future observations of these taxa will help us understand origin of this feature, and similar features where data is currently missing.

#### *Future work*

We recommend future analyses, with wider taxon sampling, be performed. Additionally, we recommend further review of the type material of historic taxa, namely those specimens attributed to the *Melosira roeseana* group, to resolve and thus more easily include/exclude those



with uncertain positions. Ideally, any future analyses looking into the phylogenetic placement of the discussed groups will also include molecular data. This will help both taxonomic and biogeographical understanding, such as the level of endemism and/or cosmopolitanism in the group. Unfortunately, this analysis was unable to provide any clarification regarding the status of Liparogyraceae with regards to Melosiraceae and Biddulphiaceae, and this remains an open-ended question in diatom systematics. Additionally, the uncertain relationship of the two *Melosira* taxa included here, *M. nummuloides* and *M. varians* suggests that there may be greater phylogenetic diversity within the group than has been previously reported.

## Acknowledgements

We would like to thank the two anonymous reviewers of this manuscript for their valuable feedback and constructive criticism. Their comments clarified the intention, language, and scope of this work, We would also like to thank Dr. Wiebe Kooistra and Dr. Juliet Brody for their final comments, and for their help in preparing this manuscript for publication. Their comments helped make this manuscript more cohesive and concise.

## Disclosure Statement

No potential conflict of interest was reported by the authors.

## Author contribution

AD participated in the development of the project, carried out the analysis, did the majority of the writing of the manuscript and participated in the revisions. JPK participated in the development of the project, writing and review of the manuscript and in its revisions. BV, W-HK, JG, AV and SS participated in writing and review of the manuscript, and in the preparation of the revisions.

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**Figure Legends:**

## Figure 1.

A consensus tree generated from a phylogenetic heuristic analysis of all of the included taxa. Newly proposed groupings are identified. Character state changes are also indicated. Placement of character state changes along specific branches does not carry any ordering or time sensitive data. All changes could have been made at any point along an indicated branch, and they are located as such solely for presentation purposes. The only phylogenetically informed information is that the indicated change did occur at some point along the indicated branch.

## Table 1.

Terminal taxa included in the phylogenetic analysis with references used to determine frustule features.

## Table 2.

The full matrix used in the phylogenetic analysis, with species, characters, and character state scores.

## Table 3.

A proposed classification for taxa from Orthoseiraceae.