*Orthoseira groenlandica* sp. nov., a new aerophilic diatom (Orthoseirales, Bacillariophyta) species from Greenland

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Abstract

During the survey of the moss-inhabiting diatoms in samples from Greenland, an unknown *Orthoseira* taxon was observed that was originally identified as *O. roeseana* but differed from the recently published lectotype population of the latter. Comparison with all known *Orthoseira* taxa worldwide showed that the unknown taxon represented a new species that is described as *O. groenlandica*, sp. nov. based on detailed light and scanning electron microscopy. The combination of the unique, rather chaotic valve face and mantle surface ornamentation, the absence of caverns and marginal pore fields, the presence of robust, short spines, and the absence of chain-like colonies has not been observed so far in any known *Orthoseira* species. The morphology of the new species is discussed comparing it with the morphology of all other *Orthoseira* species worldwide by carefully analysing the different morphological features observed so far in members of the genus *Orthoseira*. Brief notes on the ecology of the sample where the new species was found are added.

**Keywords:** *Orthoseira*, Greenland, new species, morphology,
Introduction

The diversity in the aerophilic diatom genus *Orthoseira* Thwaites (1848: 7) sensu Crawford in Round et al. (1990, p.174) has long been underestimated, not in the least by the continuous force-fitting of populations worldwide in only a handful of generally known names, such as *Orthoseira roeseana* (Rabenhorst 1853: 13) Pfitzer (1871: 134) or *O. dendroteres* (Ehrenberg 1848: 219) Genkal & Kulikovskiy (in Kulikovskiy et al. 2010: 14). This inevitably led to the creation of cosmopolitan catch-all taxa with a worldwide distribution. *Orthoseira roeseana* [in older literature often referred to under its basionym *Melosira roeseana* Rabenhorst (1853: 13)] is probably one of the most reported species with records on all continents including Asia (Radhakrishnan et al. 2022), South America (Rodrigues Maciel et al. 2022) and the sub-Antarctic region (Van de Vijver et al. 2002). A quick analysis of published images revealed a very broad variability in morphological features most likely suggesting the presence of a much higher species diversity identified under the name *O. roeseana* than currently accepted. The past fifteen years, however, more and more new species have been described in the genus *Orthoseira*, mostly from unique habitats such as lava caves (Lowe et al. 2013), remote islands such as Gough Island in the southern Atlantic Ocean (Van de Vijver & Kopalová 2008) or from geographic localities with an often endemic diatom flora such as Hawaii (Danz & Kociolek 2022), Rapa Nui Island (Easter Island, Rybak et al. 2022) or Yunnan (China, Kociolek et al. 2021). This considerably increased the currently known diversity in this genus with now almost 30 accepted names according to AlgaeBase (Guiry & Guiry 2023) and DiatomBase (Kociolek et al. 2023).

The recent analysis of the type material of both *Orthoseira (Melosira) roeseana* and *Orthoseira spinosa* W.Smith (1855: 8) proved that a lot of the presumable *O. roeseana* populations in fact represent independent, often undescribed species (Kochman-Kędziora et al. 2023).
The genus *Orthoseira* is characterised by the presence of carinoportulae in the central area, a term introduced by Crawford (1981, p. 186) for the tube-like passages through the valve, with a simple, often filled, internal opening and a more complex, slightly raised, well-defined collar on the outside (Houk *et al.* 2017). *Orthoseira* taxa are most commonly found in subaerial habitats such as wet walls, tree trunks, moist soils, waterfall spray zones and comparable wet but not submerged habitats (Houk *et al.* 2017). They are often, though not exclusively, filamentous, forming long chains. Several species, such as *O. biportulata* Van de Vijver & Beyens (in Van de Vijver *et al.* 2002: 75), described from the sub-Antarctic Crozet archipelago (Van de Vijver *et al.* 2002), has solitary cells or two cells attached to each other, whereas filaments so far have not been observed. The frustules can connect either via linking spines of variable length, linking plates, or, as suggested by Round *et al.* (1990) by mucilage excreted via the carinoportulae. The presence and function of the marginal spines has been briefly discussed in Danz & Kociolek (2022).

Although the taxonomic identity of the genus *Orthoseira* is unclear, (see Danz *et al.* 2022 for a complete overview of the taxonomic history of the genus) with Danz *et al.* (2022, p. 23) recommending to restrict the name *Orthoseira* only for the typus generis [*Melosira americana* Kützing (1844: 55)] given the absence of carinoportulae, it is important to document new taxa in this group to allow a more detailed phylogenetic analysis based on the morphological features of a higher number of species than currently known. During a survey of the moss-inhabiting diatom flora of Qeqertarsuaq (Disko Island, Greenland), two *Orthoseira* species could be distinguished. One was identified as *Orthoseira spinosa*, but the second one could not be identified using all currently available literature. Following a detailed morphological analysis using light (LM) and scanning electron microscopy (SEM), the unknown taxon is described as a new species:
*Orthoseira groenlandica* Goeyers, Kochman-Kędziora & Van de Vijver, *sp. nov.* The morphology of the new species is discussed and illustrated and compared with the morphology of similar *Orthoseira* species worldwide. Based on the accompanying diatom flora in the sample, the new species is also ecologically characterised.

**Material & Methods**

The Greenlandic island of Qeqertarsuaq (=Disko Island) is the second largest island of Greenland and is situated on the western coast of Greenland, about halfway on the frontier of the lower and middle Arctic. During a field campaign in July 2002, more than 100 moss samples were collected from various sites along the southern coastline of the island (in the vicinity of the Danish Polar Center). One of these samples, DM027, was taken from a rather wet inland moss vegetation (69°15′23.3″N/53°30′26.5″W at an elevation of 73m) on the shore of a small brook coming out of a shallow cave filled with dripping water. Water was squeezed from the moss vegetation and stored in a small 50 ml PVC-bottle fixed with 75% ethanol. PH of the dripping water was 9.0 with a conductivity of 76 µS/cm.

A subsample of the collected material was prepared for LM and SEM observations following the method described in van der Werff (1955). A small volume was cleaned by adding 37% H2O2 and heating to 80°C for about 1 h. The reaction was completed by addition of saturated KMnO4. Following digestion and centrifugation (three times for 10 minutes at 3700 × rpm), the resulting cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Cleaned diatom material was mounted in Naphrax (refraction index 1.73). The resulting slides were analysed using an Olympus BX53 microscope at 1000x magnification (N.A. 1.30), equipped with Differential Interference Contrast (Nomarski) optics and the
Olympus UC30 Imaging System, connected to the Cell Sense Standard program. For each taxon, the number of specimens, measured at random on the slide, is indicated (n=X).

For SEM, part of the suspension was filtered through 5-μm Isopore™ polycarbonate membrane filters (Merck Millipore), pieces of which were fixed on aluminum stubs after air-drying and coated with a platinum layer of 20 nm, and studied using a JEOL-JSM-7100F field emission scanning electron microscope at 2 kV. Material, slide, and stub are stored at the BR-collection (Meise Botanic Garden, Belgium). Plates were prepared using Photoshop CS5.

Terminology used in the description of the various structures of the siliceous cell wall is based on Ross et al. (1979, areola structure), Cox & Ross (1981, stria structure), Round et al. (1990, genus features for Orthoseira), and Houk et al. (2017, genus features for Orthoseira).


For typification of the species, we chose to use the entire slide as the type, following article 8.2 of the International Code for Botanical Nomenclature (Turland et al. 2018). Diatoms show a broad variability along their cell cycle, making the choice for the entire population on the slide more obvious, but because of admixtures, one valve was indicated to illustrate the taxon best. All novelties are registered proactively according to Art. 42.3 (Turland et al. 2018).

**Results**

**Division Heterokontophyta Cavalier-Smith**

**Diatoms (Diatomeae, Bacillariophyceae s.l., Bacillariophyta)**
Class Mediophyceae (Jousé & Proshkina-Lavrenko) Medlin & Kaczmarska

Subclass Chaetocerotophycidae Round & R.M.Crawford

Order Orthoseirales R.M.Crawford

Family Orthoseiraceae R.M.Crawford

Genus *Orthoseira* Thwaites sensu Crawford in Round et al. (1990)

*Orthoseira groenlandica* Goeyers, Kochman-Kędziora & Van de Vijver, *sp. nov.* (Figs 1–30)

**Type:**—GREENLAND, Qeqertarsuaq, sample DM27 (coll. date 27.VII.2002, 69°15′23.3″N/53°30′26.5″W, leg. P. Ledeganck), holo-BR-4801! (Meise Botanic Garden, Belgium), iso-slide 428! (University of Antwerp, Belgium). The holotype is represented by Fig. 8.

**PhycoBank registration:**—http://phycobank.org/XXXX

LM (Figs 1–22): Frustules cylindrical in girdle view, solitary or occasionally in pairs, with their valve faces connected (Figs 1–6, frustules in pairs not shown). Longer filaments of more than two connected frustules up to now not observed. Cells 15–25 μm, mantle height ca. 8 μm. Valves disc-shaped (Figs 7–22). Valve diameter (n=30): 10–35 μm. Valve surface flat, sloping abruptly at the valve face/mantle junction, visible in LM as a darker circular edge. Central area small, max. 1/5 of the total valve diameter, irregular in shape, hyaline, with often scattered areolae present between the carinoportulae. 2–3 (occasionally 4) large, rounded carinoportulae present. No relationship noted between number of carinoportulae and valve diameter. Striae on the mantle uniseriate, composed of densely packed irregular series of areolae, up to 25 in 10 μm. Valve face areolae large, arranged in radiate, uniseriate striae of variable length, 14–18 in 10 μm. Large, scattered marginal spines distinctly present at the valve face/mantle junction. No caverns, internal undulations or internal valves observed. Copulae number variable. (Figs 1–6).
SEM (Figs 23–35): Cingulum composed of several broad, open copulae entirely covered with small siliceous papillae (Figs 23, 24) and bearing several irregularly arranged, often incomplete rows of small poroids (Figs 23, 24). Mantle shallow, often with a clear step halfway the valve mantle (Figs 25, arrow, 26) giving the abvalvar mantle edge a thickened appearance compared to the advalvar edge. Marginal pore fields absent. Mantle covered by an irregular pattern of flattened but thickened ridges, giving the entire mantle a dirty outlook. Ridges extending from the valve face onto the advalvar edge of the mantle, continuing into a scattered pattern of small siliceous plates (Figs 26, 26). Areolae visible between the small siliceous plates (Fig. 26). Valve face/mantle junction abruptly sloping with rounded edge (Figs 25, 26). Short, radiating ridges surrounding the entire valve margin (Figs 25–29). Short, robust, marginal spines irregularly scattered on the valve margin (Figs 25–32). Valve face flat (Figs 27–29), covered in the center by a dense pattern of irregular small and large siliceous plates, obscuring the carinoportulae (Figs 27–30). Radiating striae extending from the central area to the valve margin, composed of distinct, rimmed, rounded areolae. In oblique view, the marginal zone of the valve face presenting a dense, irregular pattern of shorter and longer, small spines and papillae between and around the areolae (Figs 30, 32). Internally, valve face flat, smooth (Fig. 33). Areolae appearing as small, rounded poroids (Figs 34, 35). Striae often interrupted by short, slit-like openings (Fig. 34, arrows). Carinoportulae unoccluded (Fig. 30), pit-like, ornamented with a rough pattern (Figs 34, 35). Several slit-like openings present between the carinoportulae (Figs 34, 35). Internal caverns not observed (Fig. 33).

Ecology and associated diatom flora: —The sample was collected from wet (unidentified) terrestrial bryophytes, covered by cyanobacteria such as Phormidium sp., in a splash zone under
an overhanging cliff from where water was continuously dripping on the soil and mosses. Water squeezed out of the mosses had a pH of 9 and a conductivity of 76 µS/cm and contained almost no nutrients (Van de Vijver, unpubl. res.). The diatom flora was entirely dominated by only a handful of species with *Luticola arctica* Levkov & Metzeltin (in Levkov *et al.* 2013: 67) and *Humidophila perpusilla* (Grunow 1860: 552) R.L.Lowe (2014: 358) as the most dominant species, and *Stauroneis obtusa* Lagerstedt (1873: 36) and *Orthoseira groenlandica* being frequent but not dominant, pointing to the aerophilic character of the environment.

**Discussion**

At present, only two genera possess the typical central carinoportulae: *Orthoseira* and the recently described genus *Guarreraea* Kociolek, Guerrero & Van de Vijver in Guerrero *et al.* (2018: 129). The main feature separating both genera is the presence of rimoportulae in the latter, lacking entirely in *Orthoseira* (Guerrero *et al.* 2018), although several *Orthoseira* species possess slit-like structures internally visible between the carinoportulae. *Orthoseira groenlandica* only presents the slit-like structures (see Figs 34 & 35) but lacks rimoportulae excluding it from the genus *Guarreraea*.

*Orthoseira groenlandica* presents at first sight few discriminating features when only observed in LM, that can help to separate this new species from all other *Orthoseira* species known so far. The species possesses 2–4, usually 3, central carinoportulae, a dense striation pattern with clearly discernible areolae, robust, irregularly spaced marginal spines and broad girdle band. Recently described *Orthoseira* species such as *O. rapanuiensis* M.Rybak, Witkowski & Lange-Bertalot (in Rybak *et al.* 2022: 9), *O. mauiana* Danz & Kociolek (2022: 19) and even *O. roeseana*, present a similar combination of morphological features in LM (Rybak *et al.* 2022, Danz & Kociolek *et al.*
2022, Kochman-Kędziora et al. 2023). But analysis of the ultrastructural features, usually only visible in SEM, allows for a clear separation of this new species from all other known Orthoseira species. These features include the formation of long filaments, the shape of the marginal spines, the presence of internal marginal caverns, the presence of marginal porefields, the superficial structure of the valve face, the structure of the girdle bands, the presence of short slit-like structures amongst the areolae, and the structure of the mantle ornamentation.

A first discriminating feature that can also be used in LM, is the absence so far of colonies composed of at least three frustules. At present, only solitary cells or, very occasionally, pairs of frustules have been observed, a feature not often observed in Orthoseira. Orthoseira biportulata usually has solitary frustules although two frustules connected to each other were observed when analysing untreated material (Van de Vijver, unpubl. res.). Another, recently described, species that is not forming filamentous colonies is Orthoseira mauiana, but contrary to the previous two taxa, the latter lacks spines. Although in the genus Orthoseira frustules are usually connected to each other via linking spines or plates, Round et al. (1990) also had shown that some Orthoseira taxa seem to be able to connect their frustules via mucilage and that the spines might be for torsional control (Danz & Kociolek 2022).

A second feature is the shape of the marginal spines. Based on shape and structure of the spines four (five when counting the non-spinose O. mauiana) groups of Orthoseira species can be distinguished and therefore this feature can be used to discriminate the different Orthoseira species. A first group has large, plate-like linking structures connecting the frustules to each other. Typical examples include O. roeseana, O. tropica (Krasske 1948: 422) Metzeltin & Lange-Bertalot (2007: 186), O. ursula Metzeltin & Lange-Bertalot (2007: 187), O. tatrica Houk et al. (2017: 34), O. distinctiareolata M.Rybak, Glushchenko & Kulikovskiy (in Rybak et al.
2022: 6), *O. radiata* M.Rybak, Glushchenko & Kulikovskiy (in Rybak et al. 2022: 7) and *O. oregoniana* Danz & Kociolek (2022: 29). A second group shows plate-like ridges on the mantle edge (eventually interrupted by marginal pore fields) but with short, protruding, irregularly scattered teeth-like spines, as observed for instance in *O. spinosa* (see Kochman-Kędziora et al. 2023, fig. 30). *Orthoseira groenlandica* seems to belong to this group as the species clearly presents plate-like marginal ridges with short, conical spines. But contrary to *O. spinosa*, which forms long filaments, only solitary cells or very rarely pairs of frustules were observed, making it clear that these spines and ridges seem to play no role in filament formation for *O. groenlandica.*

The third group possesses irregularly scattered, robust, marginal spines with a stellate base, as observed in for instance *O. loweana* Danz & Kociolek (2022: 21). Other *Orthoseira* species with this kind of spines include *O. cylindrica* M.Rybak, Glushchenko & Kulikovskiy (in Rybak et al. 2022: 5), *O. rapanuiensis*, *O. biportulata*, and *O. hawaiiensis* Danz & Kociolek (2022: 24). A fourth group includes species with robust, bifurcating, often flattened species such as in *O. gremmenii* Van de Vijver & Kopalová (2008: 108), *O. verleyenii* Van de Vijver (in Lowe et al. 2013: 41) and *O. johansenii* R.L.Lowe & Kociolek (in Lowe et al. 2013: 46). And finally, *O. mauiana* is at present the only *Orthoseira* species not bearing spines.

Thirdly, the presence of marginal caverns, often well discernible in LM, can be used to separate *Orthoseira groenlandica* from this group of *Orthoseira* species as the latter does not possess these caverns. Cavern-bearing species include *O. loweana, O. hawaiensis, O. radiato-sinuata* (Chen 1980: 255) Kociolek, Q.Liu & Danz (in Kociolek et al. 2021: 349), and *O. madagascarensis* Spaulding & Kociolek ex Kociolek et al. (in Kociolek et al. 2021: 349), the latter two species with a distinct series of marginal caverns (Spaulding & Kociolek 1998, Kociolek et al. 2021, figs 1–7).
The presence or absence of distinct marginal pore fields can also be used as a fourth criteria to separate Orthoseira species. A typical example of such marginal pore field was observed in the lectotype population of Orthoseira spinosa (Kochman-Kędziora et al. 2023, figs 30 & 31).

Occasionally, the normal striation and spine pattern at the margin is interrupted by a dense grouping of rimmed areolae extending from the valve mantle onto the valve face. Similar pore fields were observed in O. hawaiiensis (Danz & Kociolek 2022, fig. 49), O. dendrophila (Ehrenberg 1848: 219) Round et al. (1990: 174) nom. inval. (Houk et al. 2017, plate 82, figs 6 & 8).

A fifth feature that can be used to distinguish O. groenlandica from other Orthoseira species is the ornamentation of the valve face and the structure of the mantle. The central area on the valve face is entirely covered by an irregular pattern of smaller and larger siliceous plate-like ‘scales’ entirely obscuring the carinoportulae. A similar obscuring of the carinoportulae was observed in the lectotype of O. roeseana (Kochman-Kędziora et al. 2023, fig. 18), and in O. distinctiareolata (Rybak et al. 2022, fig. 71) although in the latter two, there is no formation of extra irregular scales covering the carinoportulae but rather an entire siliceous plate covering the entire valve face that got irregularly incised. In O. circularis (Ehrenberg 1848: 219) M.Rybak, Glushchenko & Kulikovskiy (in Rybak et al. 2022: 4), the carinoportulae are obscured by thin siliceous plates as in O. groenlandica but they are surrounded by a complex network of ridges and not by thin spines and papillae as in O. groenlandica. Central covering plates are absent in almost all other Orthoseira species. Moreover, the area around the central area where the striae are visible has a very irregular unique surface structure with short, very thin spines and small blunt papilla-like structures, so far not observed in any other Orthoseira species. This irregular pattern can also be seen on the valve mantle, separated in two parts by a clear step, about halfway the mantle height.
The cingulum, composed of several broad, perforated girdle bands presents a dense pattern of small granules, contrary to the often very smooth girdle bands observed in other Orthoseira species such as *O. johansennii* (Lowe *et al.* 2013, fig. 47).

Apart from *O. groenlandica*, only *O. distincteareolata* possesses slit-like openings between the areolae, only visible in the valve interior. Rybak *et al.* (2022) did not comment on this structure, but it seems to be a rather unique feature that is only found so far in two species, although in *O. cylindrica*, it seems that more distinct pores can be seen on the valve interior, scattered between the areolae (see Rybak *et al.* 2022, fig. 51).

Finally, some *Orthoseira* species possess unique features such as internal valves (*O. gremmenii*). These internal valves are lacking in *O. groenlandica* excluding all conspecificity (Van de Vijver & Kopalová 2008).

**Conclusion**

The separation of *Orthoseira groenlandica* as a new species can be justified when considering the entire set of morphological features. The combination of the unique, rather chaotic valve face and mantle surface ornamentation, the absence of caverns and marginal pore fields, the presence of robust, short spines, and the absence of chain-like colonies has not been observed so far in any known *Orthoseira* species. Most likely, the new species has been reported in the past as *Orthoseira (Melosira) roeseana* but the recent analysis of the type of *O. roeseana* clearly contradicts these observations justifying the separation of *O. groenlandica* as a separate species.

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Figure captions

LM pictures taken from the holotype material (BR-2801, Qeqertarsuaq, Greenland, sample DM27). 1–6. Solitary frustules in girdle view. 7–22. LM pictures of valves in valve face view showing the rather constant number of carinoportulae and the irregularly spaced marginal spines. Figure 10 represents a teratological valve. Scale bar = 10 µm.

SEM pictures taken from the holotype material (BR-2801, Qeqertarsuaq, Greenland, sample DM27). 23. Frustule in girdle view showing the structure of the cingulum. 24. Entire girdle band. 25. SEM external view of a valve in oblique view showing the step in the mantle (arrows). Fig. 26. SEM external view of a valve in oblique view lacking the step in the mantle. 27–29. Three external views of an entire valve. Note the typical siliceous scales in the valve center, the marginal ring of ridges and the scattered, short teeth-like spines. 30. SEM external oblique detail showing the irregular valve face ornamentation with papillae, thin spines and siliceous plates in the valve center. 31. SEM external detail of the marginal spines. Note the granulate surface structure of the girdle band. 32. SEM external detail of the surface structure in the central area obscuring the view on the carinoportulae. Scale bars = 10 µm (Figs 23–31), 1 µm (Fig. 32).

SEM pictures taken from the holotype material (BR-2801, Qeqertarsuaq, Greenland, sample DM27). 33. SEM internal view of a complete valve. 34–35. SEM internal details of the carinoportulae showing the slits between the carinoportulae. The white arrows in Fig. 34 indicate
the slit-like openings scattered between the areolae. Scale bars = 10 µm (Figs 33–34), 1 µm (Fig. 35).