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Source: Cryptogamie, Algologie, 35(3):245-257. 2014.
Published By: Association des Amis des Cryptogames
DOI: http://dx.doi.org/10.7872/crya.v35.iss3.2014.245
URL: http://www.bioone.org/doi/full/10.7872/crya.v35.iss3.2014.245

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Cryptogamie, Algologie, 2014, 35 (3): 245-257
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Orthoseira limnopolarensis sp. nov. (Bacillariophyta), a new diatom species from Livingston Island (South Shetland Islands, Antarctica)

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Abstract – A new species of diatom, *Orthoseira limnopolarensis*, is described from a sediment core in Limnopolar Lake on Livingston Island, South Shetlands in the South Atlantic Ocean. The new species is distinctive in having an array of numerous (12-14) carinoportulae, a number of clearly defined rimoportulae which are described here for the first time in the genus, and a valve face surface that is covered with small, irregularly scattered spines. Linking spines are less well-developed than is generally observed in species of the genus *Orthoseira*. No caverns or internal undulations are present. Internal valves have not been encountered.

Maritime Antarctic Region / *Orthoseira* / taxonomy / morphology / rimoportula / carinoportula

Résumé – Une nouvelle espèce de diatomées, *Orthoseira limnopolarensis*, est décrite à partir d'une carotte de sédiments recoltée dans Limnopolar Lake sur l'île Livingston, îles Shetland du Sud dans l'Océan atlantique austral. La nouvelle espèce se distingue en ayant un réseau de nombreux (12-14) carinoportulae, un certain nombre de rimoportulae clairement définis qui sont décrits ici pour la première fois pour ce genre, et une surface de valve qui est couverte de petites épines irrégulièrement disséminées. Des épines de liaison sont moins bien développées que ce qui est généralement observé chez les espèces du genre *Orthoseira*. Des cavernes ou des ondulations internes sont absentes. Des valves internes n'ont jamais été observées.

Région maritime antarctique / *Orthoseira* / taxonomie / morphologie / rimoportula / carinoportula

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doi/10.7872/crya.v35.iss3.2014.245

INTRODUCTION

The number of non-marine centric diatoms in the different parts of the Antarctic Region (subdivided in sub-Antarctic, Maritime Antarctic and Continental Antarctic according to Chown & Convey, 2007) is very small and restricted to the genera *Aulacoseira* Thwaites, *Melosira* Agardh and *Orthoseira* Thwaites (Kellogg & Kellogg, 2002). Records from other centric genera such as *Stephanodiscus* Ehrenberg or *Cyclotella* (Kützing) Brébisson are, in most cases, doubtful and probably the result of contamination with non-Antarctic material or incorrect identification (e.g., Pankow *et al.*, 1991; Kawecka *et al.*, 1998). Recently, several new taxa were described from the Antarctic Region including *Aulacoseira principissa* Van de Vijver (Van de Vijver, 2012), *Orthoseira biportulata* Van de Vijver & Beyens (Van de Vijver *et al.*, 2002), *O. gremmenii* Van de Vijver & Kopalová (Van de Vijver & Kopalová, 2008) and *Orthoseira verleyenii* Van de Vijver (Lowe *et al.*, 2013). It is unclear why the number of centric taxa is so limited in the Antarctic Region but most likely it is related to the absence of diatom plankton in the Antarctic lakes as almost all recorded centric taxa show a preference for benthic or semi-terrestrial habitats. Le Cohu & Maillard (1986), Jones (1996) and Van de Vijver & Beyens (1999) discussed the absence of diatom plankton but the exact causes are still not entirely known. Spaulding *et al.* (2010) suggested that the perennial ice cover prevents the development of diatom plankton. This may be true for the lakes in the Maritime and Continental Antarctic localities but on the sub-Antarctic islands, lakes are seldomly to almost never ice-covered during a certain time of the year.

At present, the most diverse centric genus in the Antarctic Region is *Orthoseira* with four taxa. Apart from the above-mentioned three taxa, *Orthoseira roeseana* (Rabenhorst) O'Meara is quite widespread on the sub-Antarctic and Maritime Antarctic islands (Van de Vijver *et al.*, 2002) as it is elsewhere throughout the world (Krammer & Lange-Bertalot, 1991).

The genus *Orthoseira* (Coscinodiscophyceae, Orthoseirales) is typically found in subaerial habitats such as moist soils, wet walls, waterfall spray zones and comparable wet but not completely submerged habitats, making it hardly surprising, therefore, that the genus is the most species-rich of the three centric genera found in the Antarctic Region. *Orthoseira* often forms long chains in which cells are connected with linking spines of variable length. A unique and constant feature not shared with any other centric diatom genus, is the presence of carinoportulae on the valve surface, although the number varies considerably within the same species. These are tube-like passages through the valve, characterized by a slightly raised, well-defined collar on the outside and simple, internal openings (Crawford, 1981; Round *et al.*, 1990; Houk, 1993). Although the taxonomic and nomenclatural status of the genus is not entirely clear (Houk, 1993; Spaulding & Kociolek, 1998), a few new species have been described lately, such as *O. ursula* Metzeltin & Lange-Bertalot (2007) from Costa Rica and *O. gremmenii* from Gough Island (Van de Vijver & Kopalová, 2008).

The non-marine diatom flora of the Maritime Antarctic Region is currently under revision, which has resulted so far in the description of several new species of amongst others *Luticola* Mann (Van de Vijver & Mataloni, 2008; Kopalová *et al.*, 2011), *Pinnularia* Ehrenberg (Zidarova *et al.*, 2012) and *Navicula* Bory (Van de Vijver *et al.*, 2011). During a survey of the freshwater diatom flora of the Byers Peninsula, a large ice-free area located in the western part of Livingston Island (South Shetland Islands), an unusual *Orthoseira* taxon

showing a large number of small carinoportulae, was observed in Limnopolar Lake, one of the studied lakes. The present paper provides a description of this new species and illustrates its unique morphological features using detailed scanning electron microscopy to facilitate comparison with previously described *Orthoseira* taxa.

MATERIAL AND METHODS

Study area

The South Shetland Islands (ca. 63°00' S/60°00' W) constitute one of the principal archipelagos in the southern Atlantic Ocean, comprising a mountainous group of islands formed by rocky outcrops and 11 larger islands, situated within the so-called "Pack-Ice Zone", about 160 km from the northernmost tip of the Antarctic Peninsula. Livingston Island is the second largest island of the group and has a typical maritime Antarctic climate with a mean annual temperature of about –3 °C, high precipitation (mainly in the form of snow), and strong westerly winds. Permafrost might occur in some places. Several (semi-)permanent research stations have been established facilitating scientific research in the area. Recently, paleo-ecological research has been conducted in Limnopolar Lake, a relatively large (10.000 m^2) lake on Byers Peninsula, situated on the western side of Livingston Island (Toro *et al.*, unpublished results). A new species, *Pinnularia gemella* Van de Vijver from this lake was described in 2009 (Van de Vijver *et al.*, 2009).

Sample preparation and analysis

The sample containing *Orthoseira limnopolarensis* sp. nov. was taken from a sediment core collected from Limnopolar Lake, Byers Peninsula, Livingston Island (62°40'S, 61°00'W) using a modified Glew sediment corer. The 29.2 cm core was sectioned in the field at 0.2 cm intervals. In the laboratory, subsections of the core were filtered through a 44 µm sieve, and the collected material stored in ethanol for later analysis. The material described in this paper was recovered from a depth of 8.8-9.0 cm. More information on Livingston Island and Limnopolar Lake can be found in Toro *et al.* (2007).

Diatom samples for LM observation were prepared following the method described in Van der Werff (1955). Small parts of the samples were cleaned by adding 37% H_2O_2 and heating to 80°C for about 1h. The reaction was completed by addition of $KMnO_4$. Following digestion and centrifugation (three times 10 minutes at 3700 x g), cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Cleaned diatom material was mounted in Naphrax®. The slides were analyzed using an Olympus BX51 microscope, equipped with Differential Interference Contrast (Nomarski) and the Colorview I Soft Imaging System. Sample, slide and stub are stored at the BR-collection, property of the Belgian federal state and given in permanent loan to the Botanic Garden Meise (Belgium). For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered through polycarbonate membrane filters with a pore diameter of 1 µm, pieces of which were fixed on aluminum stubs after air-drying. The stubs were sputter-coated with a Gold-Palladium layer of 20 nm and studied in a ZEISS ULTRA SEM microscope at 3 kV (Natural History

Museum of London, UK). Some specimens were examined at the Friedrich Hustedt Study Centre, Alfred Wegener Institute in Bremerhaven using a Philips Quanta FEG SEM.

Due to the extreme rarity of this taxon, the number of photographed valves is very low. Most of the valves were lying in mantle view. Only a few acceptable LM micrographs were made, even after scanning several slides.

Comparisons are based mainly on information in Crawford (1981), Krammer & Lange-Bertalot (1991), Houk (1993), Spaulding & Kociolek (1998), Van de Vijver *et al.* (2002), Van de Vijver & Kopalová (2008) and Lowe *et al.* (2013). Terminology follows Round *et al*. (1990).

RESULTS

Orthoseira limnopolarensis sp. nov.

Cells cylindrical. Frustules in girdle view 75-85 µm long. Diameter 38-84 µm. Mantle height 40-48 µm. Central area forming a small hyaline zone (max. 1/5 of the total valve diameter) with a ring of 13-14 carinoportulae toward the valve centre. Marginal spines present, simple, irregularly positioned on the valve face/mantle margin. Areolae on the valve face arranged in radiating striae, 10-12 in 10 µm. On the mantle, striae parallel, distinctly areolated, 10-12 in 10 µm. A number of rimoportulae, 19 in one specimen, occur within the ring of carinoportulae and elsewhere on the valve. These open to the outside by small pores indistinguishable in the light microscope and bear striations on the inside of their tubes.

Holotypus: BR-4328 (Botanical Garden Meise).

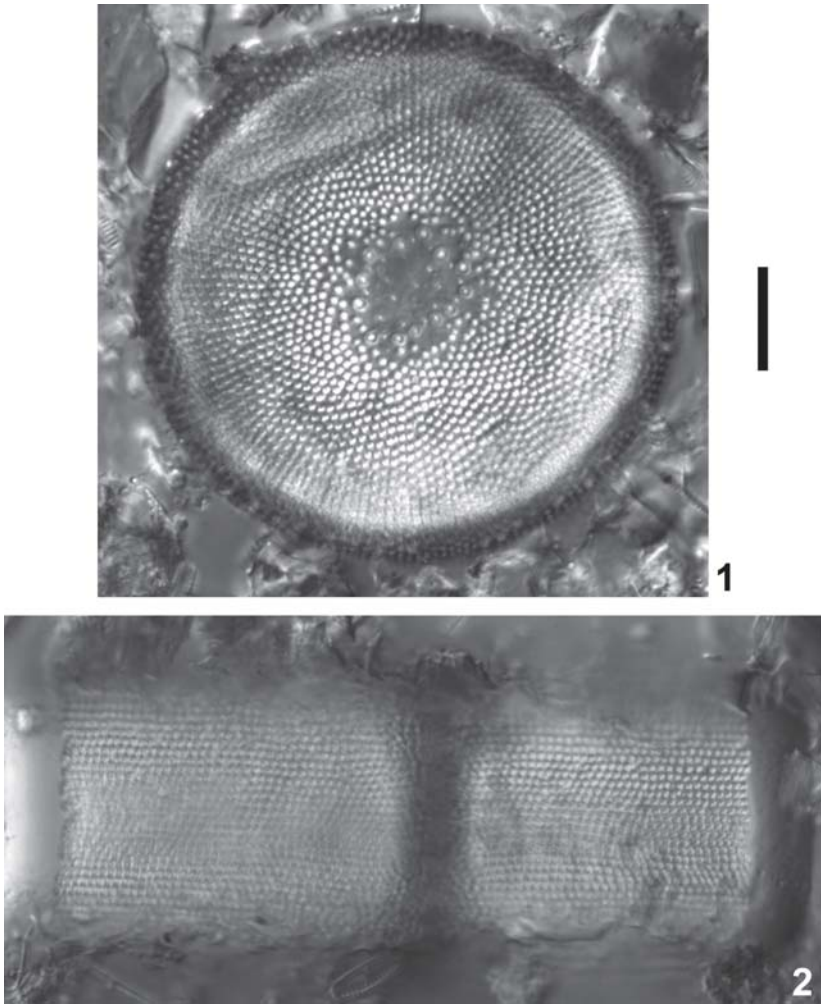
Isotypus: PLP-229 (University of Antwerp, Belgium).

Type Locality: Limnopolar lake, Byers Peninsula, Livingston Island (South Shetland Islands) (62°38'56.5"S, 61°06'16.9"W).

Etymology: The specific epithet *limnopolarensis* refers to Limnopolar Lake on Byers Peninsula where the sample was taken.

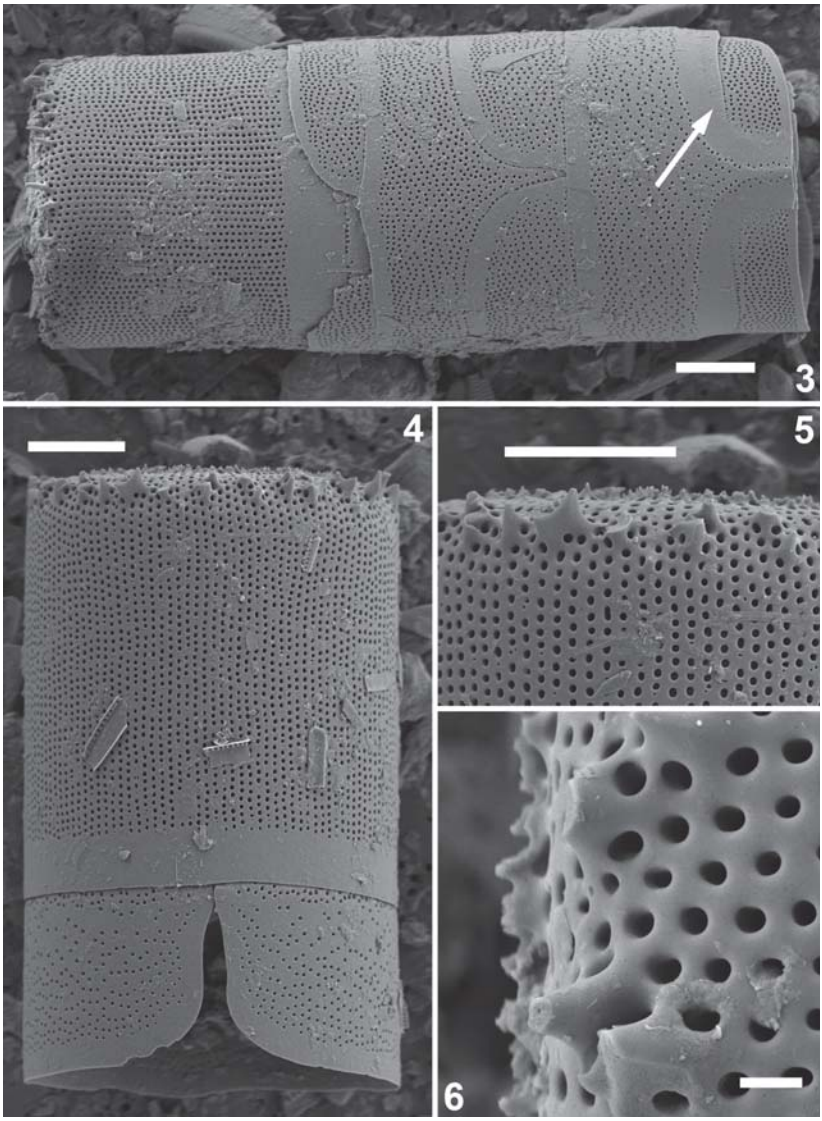
Morphology: Light Microscopy (Figs 1-2). Cells cylindrical in girdle view (Fig. 2), 75-85 µm, attached to each other with linking spines. Valves disc-shaped (Fig. 1). Valve dimensions (n = 10) diameter 38-84 µm, mantle height 40-48 µm. Valve surface flat, forming a right angle with valve mantle. Striae uniseriate, ca. 12 in 10 µm; valve face areolae small, radiate, leaving only a small central area without areolae, 10-12 in 10 µm. Towards the valve centre, areolae become more scattered, with the organized pattern becoming lost. Central area with 13-14 carinoportulae. Marginal linking spines present at the valve face/mantle junction but not well discernible. No special areas of pores/areolae here. No caverns or internal undulations such as observed in *O. gremmenii* (Van de Vijver & Kopalová 2008) present. No internal valves observed.

Scanning Electron Microscopy (Figs 3-14): Cingulum comprising several open copulae with a maximum observed number of five (Fig. 3). Each girdle band with a hyaline zone on both edges and a relatively long ligula fitting into the slit of the adjacent advalvar band. Pars interior fimbriate (Fig. 3, arrow). Pars exterior more solidly silicified with a dense pattern of irregularly scattered, large, circular poroids. Collum, *i.e.* the lowest part of the mantle, lacking areolae and making up



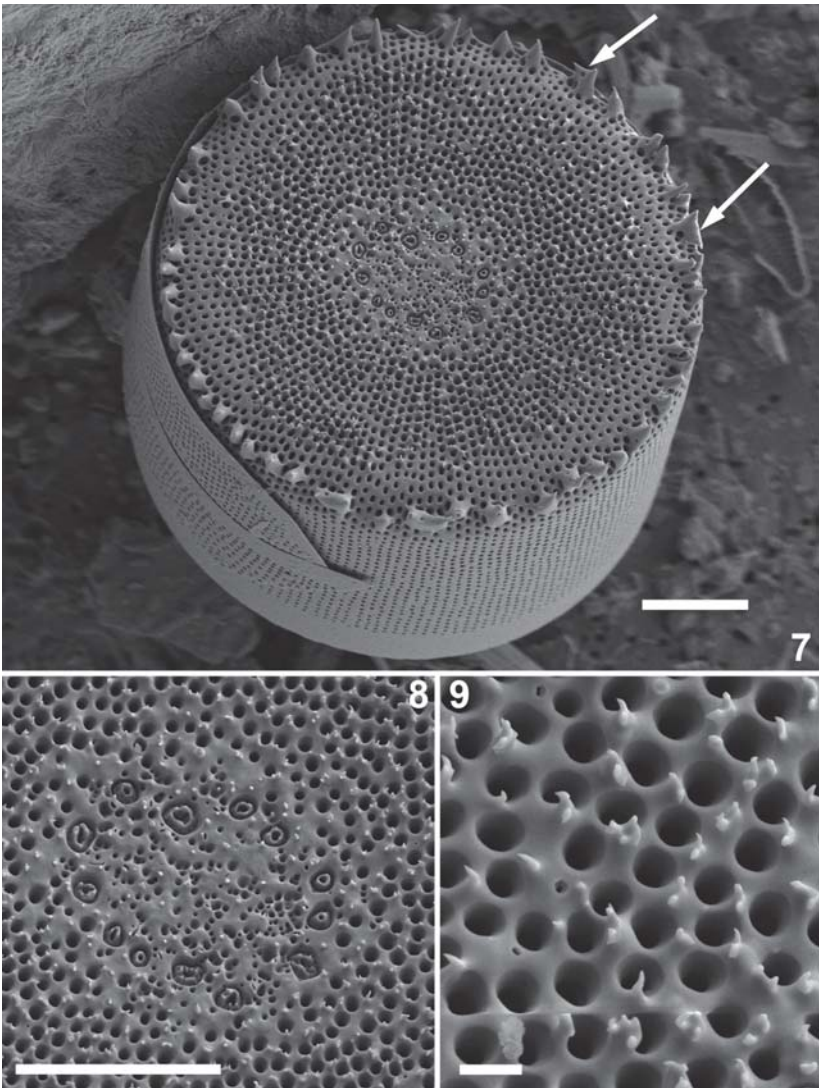
Figs 1-2. *Orthoseira limnopolarensis* sp. nov. Light Microscopical views. **1.** Valve view. **2.** Mantle view of two valves linked with linking spines. Scale bar represents 10 µm.

only 15-20% of the total mantle height, devoid of any ornamentation such as plaques or ribs (Figs 3, 4). Mantle striae composed of relatively small, round areolae, arranged in densely packed longitudinal rows, parallel to the pervalvar axis (Figs 3-5). Areola density per stria between 10 and 12 in 10 µm. Striae continuing without interruption over the mantle/valve face margin onto the spine face (Figs 4, 5). Valve face/mantle junction abrupt with rounded edge (Fig. 5). Ring of linking spines present (Figs 3-7), regularly scattered along the entire valve edge. Spines usually simple siliceous acute thickenings (Figs 5, 6), although sometimes spines merged to form plate-like structures (Fig. 7, arrow). No elaborate linking valves similar to other species of the genus have yet been found suggesting that this species was not forming long chains in the assemblage from which the sample was taken.



Figs 3-6. *Orthoseira limnopolarensis* sp. nov. Scanning Electron Microscopical views. **3.** Entire frustule in girdle view showing the structure and composition of the cingulum. Note the fimbriate pars interior of the copulae (see arrow). **4.** Valve in mantle view showing the hyaline collum and the mantle striae. **5.** Detail of the valve face/ mantle edge with simple spines. **6.** Detail of a marginal (broken) spine. Scale represents 10 µm except in Fig. 6 where scale bar = 1 µm.

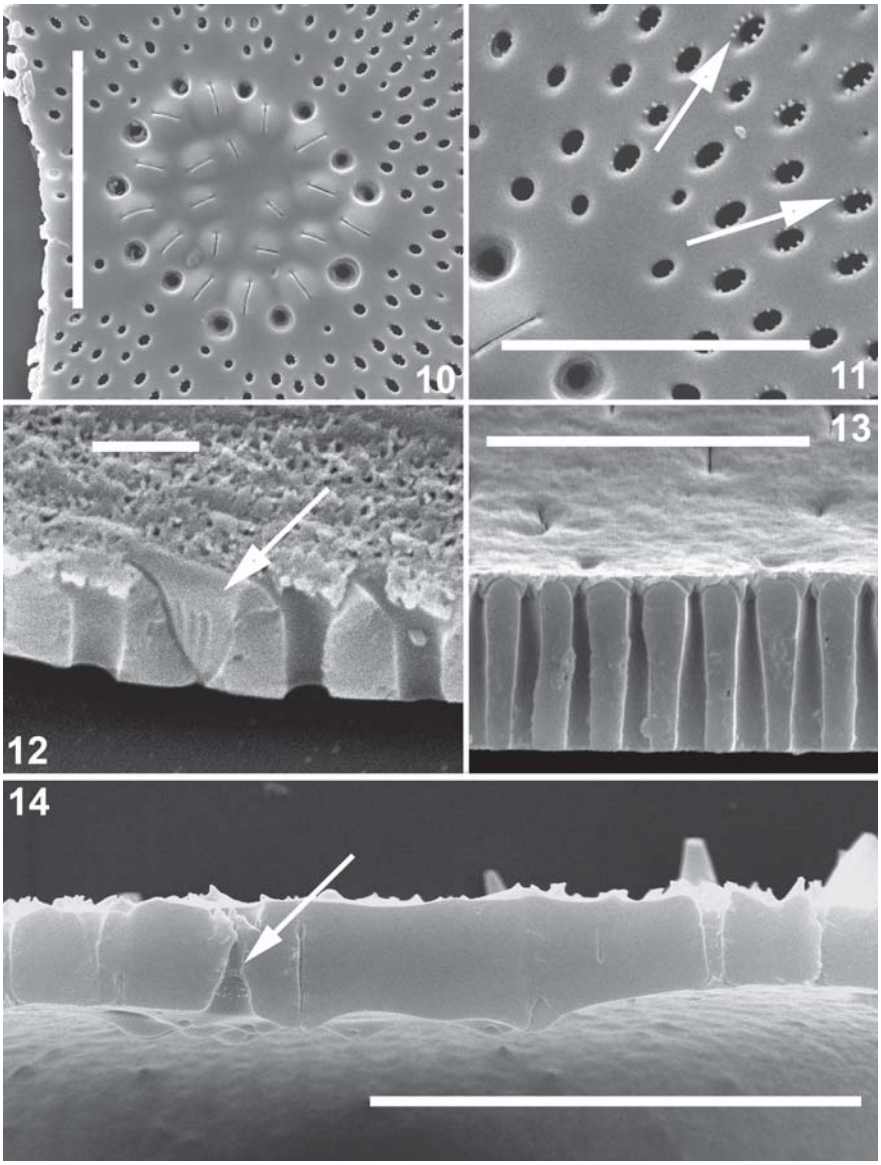
Valve face flat (Fig 7). Central area with an irregular pattern of more or less rounded areolae of different size (Fig. 8). Areolae very densely arranged in radiate striae (Fig. 7), small and pit-like, lacking raised rims (Figs 8, 9). Near the valve margin, areolae less pit-like. External marginal pore fields between spines absent (Figs 4, 5, 7). Carinoportulae small but still well distinguishable in the



Figs 7-9. *Orthoseira limnopolarensis* sp. nov. Scanning Electron Microscopical views, outer surface. **7.** Entire valve showing the carinoportulae, the marginal spines and the regular striae. Note the plate-like spines (arrow) and the difference in areola structure between the valve center and the areolae closer to the margin. **8.** Detail of the carinoportulae and the central area. **9.** Detail of the valve surface with the small, irregular, spine-like projections and the pit-like outer areola openings. Scale represents 10 μm except in Fig. 9 where scale bar = 1 μm.

central area, each surrounded by an irregular, pronounced, usually conical silica collar (Fig. 8). Between the areolae, small spine-like, irregularly shaped structures present, becoming less evident towards the valve margin (Figs 7, 9).

Internally, valve face flat, smooth (Fig. 10). Areolae appearing as small, rounded to elliptical poroids (Fig. 10). Vela never observed but are presumed to



Figs 10-14. *Orthoseira limnopolarensis* sp. nov. Scanning Electron Microscopical views of the inner surface facing up except figure 12. **10.** Broken valve showing the carinoportulae, the rimoportulae (slit-like) openings between the carinoportulae and the areola structure. **11.** Detail of the areolae. Note the silica granules in some areolae (see arrow). **12.** Cross-section of the valve face showing the internal structure of the rimoportula (see arrow). **13.** Cross-section of the valve mantle with the structure of the areolae. **14.** Cross-section of the valve face showing the entire slit of the rimoportula. Scale bar represents 10 µm except in Fig. 12 where scale bar = 1 µm and Figs 11 and 13 where scale bar = 5 µm.

have been lost due to the chemical preparation of the material. Areolae towards the mantle with small silica granules on the areolar edge (Fig. 11, arrow). Carinoportulae unoccluded, with well-defined, small, simple rounded pits (Fig. 10). Within the ring of carinoportulae, a large number of slits are present (Fig. 10). These are the openings of the first rimoportulae to be reported from the genus (Figs 10, 11). They have a well-defined, straight slit that is bordered by a ill-defined lip on both sides which can best be seen in profile (Fig. 14) and, as fracturing of the valve has shown, the inner surface of at least one side bears a series of ridges running from inside to outside of the tube (Fig. 12). The tube itself is sharply tapered towards a small pore on the outer surface of the valve that is difficult to distinguish from the areolae. Fig. 14 shows the narrow passage and small external opening of the rimoportula. Mantle areolae in the form of funnel-like tubes, occluded with a cribrum on the internal surface are shown in Figs 12, 13. The lower part of the valve mantle near the cingulum lacks areolae in a thickened hyaline zone (Fig. 11).

Ecology and associated diatom flora: It is not possible to identify the exact environmental preferences of *Orthoseira limnopolarensis* since the only sample in which the taxon was found so far, was taken from a subfossil sediment core. Ecological preferences can be partly deduced from the associated diatom flora in the sample. All taxa are typically freshwater such as *Hippodonta hungarica* (Grunow) Lange-Bertalot, Metzeltin & Witkowski, *Nitzschia perminuta* (Grunow) Peragallo and several species of *Stauroneis* Ehrenberg and *Muelleria* (Frenguelli) Frenguelli (such as *S. cf. subgracilior* Lange-Bertalot *et al.*, *S. latistauros* Van de Vijver & Lange-Bertalot, *M. variolata S*paulding & Kociolek, *M. cf. algida* Spaulding & Kociolek), *Psammothidium papilio* (Lange-Bertalot & Schmidt) Van de Vijver & Kopalová and *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot. This species composition clearly reflects lake water conditions. Typical aerophilic genera such as *Diadesmis* or *Luticola* (Fermani *et al.*, 2007) are only present in very low numbers (*Luticola*) or completely absent (*Diadesmis*).

The new species is extremely rare in the sample and was not found in recently collected surface samples from Limnopolar Lake or from nearby larger open waterbodies (Kopalová & Van de Vijver, 2013).

DISCUSSION

The new taxon clearly belongs to the genus *Orthoseira*, as described in Round *et al.* (1990), based on the presence of carinoportulae, linking spines, the relatively large mantle and the structure of the areolae. Of most significance is the first recognition of rimoportulae in the genus. The unique combination of features reduces all possible confusion with other, so far described, *Orthoseira* taxa. Markings similar to those of the rimoportulae in *O. limnopolarensis* have been found by scrutiny of *Orthoseira* (as *Aulacoseira*) *dendrophila* (Ehrenberg) Crawford (Crawford, 1981), in *O. verleyenii* (Lowe *et al.*, 2013, Fig. 25), in *O. gremmenii* (Van de Vijver & Kopalová, 2008, Fig. 18) and in an un-named *Orthoseira* illustrated by Spaulding & Kociolek (1998, figs 25 & 27). All above-mentioned taxa have the same slit-like structures in the center of the valve and it is most likely that these slits have the same internal structure as in

O. limnopolarensis. Figure 24 in Crawford (1981) even shows indications of the ridges shown in the present paper but a fortuitous fracture through the valve is needed in the other species to show whether this feature is unique to *O. limnopolarensis*. If these structures are investigated in more detail and found to be rimoportulae then the generic description will have to be altered. It might also provide a further character to evaluate species relationships within the genus and since there are now more than 15 species described so far, such a treatment might be welcome and even give some insight into the biology of the genus, being as it is, an organism of somewhat extreme environments. Unpublished results of a survey of the rimoportulae in diatoms by one of us (RMC) shows that if rimoportulae are present in one species of a genus then they tend to be present in all species but there are exceptions, The structure varies greatly in its form throughout the group and if we have to put a name to this structure in *O. limnopolarensis* then "rimoportula" is the obvious choice. One further question that must be answered is whether or not all species have the capability of producing internal valves. These internal valves have been observed in for instance *O. gremmenii* (Van de Vijver & Kopalová, 2008, figs 24-29) but in other taxa (for instance *O. verleyenii* and *O. biportulata*) such internal valves were never observed (Lowe *et al.*, 2013; Van de Vijver *et al.*, 2002).

The genus *Orthoseira* is still quite small with less than 20 taxa currently known worldwide (Fourtanier & Kociolek, 2011), yet even without the remarkable array of rimoportulae the separation of *O. limnopolarensis* is straightforward. Table 1 summarizes the morphological features of *O. limnopolarensis* and several related taxa.

Orthoseira limnopolarensis is the first taxon in this genus showing such a high number of carinoportulae. Usually, up to 5, rarely 7 carinoportulae are found (Crawford, 1981; Round *et al.*, 1990; Spaulding & Kociolek, 1998; Van de Vijver & Kopalová, 2008) whereas all observed valves of *O. limnopolarensis* present more than a doubling of this number. *Orthoseira gremmenii* has a large hyaline central area with a maximum of 4 carinoportulae. This large hyaline central area is absent in *O. limnopolarensis.* Moreover, *O. gremmenii* shows typical internal valves, a feature never observed in *O. limnopolarensis* (Van de Vijver & Kopalová, 2008). Both other sub-Antarctic *Orthoseira* species, *O. biportulata* and *O. verleyenii* are smaller with a maximum of 2-3 carinoportulae and show a different surface structure lacking the typical irregular spine-like structures. The latter surface structure is another unique feature that was never observed before in any known *Orthoseira* taxon. Spaulding & Kociolek (1998) report the presence of two unknown *Orthoseira* taxa from Madagascar. Both taxa however show a series of shallow undulating depressions, called caverns, on the valve face near the valve mantle, absent in *O. limnopolarensis*, whose valves always had a completely flat valve interior. Most of these taxa possess well developed linking spines making large chains possible (see for instance Van de Vijver & Kopalová, 2008, Fig. 22). On the contrary, valves with thick linking spines have so far never been observed in *O. limnopolarensis*. Whether this is an erosion effect due to the preservation of the specimens in a subfossil sediment core or a biological reality remains an open question and will only be solved when new material of the species will be investigated.

The observation of this unusual taxon in the Antarctic Region is not surprising. The taxonomic revision of the past years revealed the presence of a highly specific, even endemic diatom flora present on these islands. This unique flora has been neglected for a long time due to taxonomic uncertainties caused by species drift and force-fitting most Antarctic taxa in European or North-American

Table 1.

	Orthoseira biportulata	*Orthoseira gremmenii*	*Orthoseira johansenii*	*Orthoseira verleyenii*	*Orthoseira Madagascar1*	*Orthoseira Madagascar2*	*Orthoseira limnopolarensis*
	Van de Vijver & Beyens	Van de Vijver & Kopalová	Lowe & Kociolek	Van de Vijver	–	–	sp. nov.
Frustule length (µm)	12-18	40-62	?	45-95	± 60	?	75-85
Valve diameter (µm)	15-35	29-100	24-65	17-70	25-70	40-68	38-84
Mantle height (µm)	4-8	± 15	11-13	15-25	± 15	?	40-48
Central area	small, hyaline zone, max. 1/4 of total valve diameter	very large hyaline zone, 1/3-4/5 of total valve diameter	small hyaline zone, max. 1/3 of total valve diameter	small hyaline zone, max. 1/3 of total valve diameter	small hyaline zone, max. 2/5 of total valve diameter	very small hyaline zone	very small hyaline zone
Number of carinoportulae	2	2-4	1-3	1-6	2-4	4-5	13-14
Number of striae (in 10 µm)	16-17	14-17	17-19	15-18	10-15	6-13	10-12
Number of mantle striae (in 10 µm)	± 24	16-17	18-21	19-25	± 21	± 17	10-12
Presence of rimoportulae	no	yes	no	yes	yes	yes	yes
Marginal spines	short, blunt to acute spines, equally spaced	stellate, regulary spaced,linking spines plate-like	plate-like, bifurcated or complex	simple to plate-like, bifurcated	irregular in form and spacing	absent or small, stellate	simple acute thickenings
External marginal pore fields between spines	absent	absent	absent	absent	present	present	absent
Internal valves	absent	present	absent	absent	absent, but presence of caverns	absent, but presence of caverns	absent
Literature	Van de Vijver *et al.* (2002)	Van de Vijver & Kopalová (2008)	Lowe *et al.* (2013)	Lowe *et al.* (2013)	Spaulding & Kociolek (1998)	Spaulding & Kociolek (1998)	this study

names (Tyler, 1996). The presence of *O. roeseana* on most Antarctic localities [often incorrectly identified as *O. dendrophila*, see Kellogg & Kellogg, 2002 for references] adds to the underestimation of the diversity of the genus *Orthoseira* in the Antarctic region. At present, five different *Orthoseira* taxa are now reported for the (sub-)Antarctic Region, making this region one of the most species-diverse for this genus. The availability and broad variety of different, mostly terrestrial, microhabitats is clearly an important factor in developing this diversity since a similar high biodiversity is noted in other typical aerophilic genera such as *Luticola*, *Hantzschia* Grunow, *Muelleria* and *Diadesmis* Kützing (Van de Vijver & Mataloni, 2008; Van de Vijver *et al.*, 2002, 2010; Zidarova *et al.*, 2010).

Acknowledgments. The authors wish to thank Prof. Dr. A. Quesada (Universidad Autonoma de Madrid) for providing the sample containing *Orthoseira limnopolarensis*. Dr. Alex Ball, the staff of the EMMA laboratory and Dr. Eileen J. Cox at the Natural History Museum, London are thanked for their help with the scanning electron microscopy as is Friedel Hinz at the Alfred Wegener Institute in Bremerhaven, Germany. This study was supported by the BELSPO project CCAMBIO and an EU Synthesys grant to BVDV to visit the National History Museum in London, UK. The LIMNOPOLAR project has been funded by the grant CGL2005-06549-CO2-01 from the Ministerio de Educación y Ciencia of Spain.

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