

Microcostatus elisabethianus, a new limnoterrestrial diatom species (Bacillariophyta) from the sub-Antarctic region

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Background and aims – During a survey of the soil diatom flora of the sub-Antarctic islands in the southern Indian Ocean, an unknown small-celled naviculoid taxon was discovered living in small cracks in cliffs bordering the ocean. The taxon showed a set of morphological features only found in the genus *Microcostatus*. However, the unknown taxon could not be identified using the currently available literature. **Methods** – Using both light microscopical and scanning electron microscopical techniques, the morphology of the unknown *Microcostatus* taxon was documented. The new taxon is described, illustrated and compared with all other similar *Microcostatus* taxa worldwide.

Key results – *Microcostatus elisabethianus* possesses a unique combination of morphological features that excludes conspecificity with all other members of the genus. It is characterized by a lanceolate valve outline with protracted, elongate apices, a very large, porous conopeum reaching the valve margins and uniseriate striae composed of only one macroareola and mantle areolae restricted to the valve apices. The morphological features of the genus *Microcostatus* are evaluated and discussed. Notes on the occurrence and ecology of the new taxon are added.

Keywords – Diatoms; sub-Antarctic region; Crozet archipelago; *Microcostatus*; new species; morphology.

INTRODUCTION

The freshwater diatom flora of the sub-Antarctic Region is characterized by a high number of endemic taxa (Van de Vijver et al. 2002a, 2005). Over the past twenty years, considerable effort has been made to revise the taxonomy of non-marine diatom species that were frequently reported from the different parts of the Antarctic Region. Earlier taxonomic work on the sub-Antarctic diatoms was done by Germain (1937), Bourrelly & Manguin (1954) and Le Cohu & Mailard (1983, 1986) all of which was, however, based on a rather broad species concept. Although a fairly high number of new species were described, it was clear that the majority of the diatom flora still was considered to have been cosmopolitan in nature (Van de Vijver & Beyens 1999).

In 2002, Van de Vijver et al. published the first illustrated overview of the diatom flora of the sub-Antarctic Iles Crozet, a small archipelago in the southern Indian Ocean and listed almost 220 different taxa; more than 35 of them described as new species. These included *Adlafia frenotii* Van de Vijver & Beyens and *Frustulia subantarctica* Van de Vijver & Beyens (Van de Vijver et al. 2002a). The Crozet Islands share a considerable number of species with the other sub-Antarctic islands in the Indian Ocean (Iles Kerguelen, Prince Edward Islands, Heard Island). Most of these species, however, have never been observed in the other sub-regions such as the Antarctic Continent and the Maritime Antarctic Region (Van de Vijver et al. 2004a, 2008), the latter both having their own endemic flora (Zidarova et al. 2016 and references therein).

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Further research on the sub-Antarctic diatom flora, using a narrower, more refined species concept (Mann 1999) resulted in the subsequent publication of several new genera, for instance *Labellicula* Van de Vijver & Lange-Bert. (Van de Vijver et al. 2005), *Ferocia* Van de Vijver & Houk (Van de Vijver et al. 2017a) and *Michelcostea* Van de Vijver et al. (Van de Vijver et al. 2017b). More than twenty new species were also described, many based on material from taxa previously assumed to be cosmopolitan in distribution and often formerly included in the 'catch-all' genus *Navicula* Bory s. lat.

For a long time, *Navicula* s. lat. was considered to be a very broad and heterogenous genus, grouping a large amount of biraphid diatoms with a bilateral symmetry and a naviculoid raphe. Hustedt (1961–1966) subdivided the genus into many sections in an attempt to classify the species in groups sharing similar sets of morphological features. Round et al. (1990) were the first to split off several of these sections, placing them in separate genera, as it was clear that only a very small number of species, i.e. section *Lineolatae*, belongs to the genus *Navicula* s. str. as described by Bory in 1822. In the last three decades, an increasing number of taxa have been reassigned to previously described genera (e.g. *Sellaphora* Mereschk.) or placed in new genera such as *Geissleria* Lange-Bert. & Metzeltin or *Microcostatus* J.R.Johans. & Sray.

Microcostatus was described in 1998 in the family Naviculaceae to accommodate species characterized by the presence of microcostae in the axial area, an enlarged central sternum flanked by two longitudinal depressions, a simple raphe structure with almost inconspicuous, only weakly deflected, central raphe endings and bent terminal raphe fissures (Johansen & Sray 1998; Van de Vijver et al. 2004b, 2010). Originally, the absence of a conopeum was reported to be one of the main morphological differences with another naviculoid genus, *Fallacia* Stickle & D.G.Mann (Round et al. 1990; Taylor et al. 2010; Stanek-Tarkowska et al. 2016). This was, however, contradicted in 2010 when *Microcostatus cholnokyi* J.C.Taylor et al. was described; it clearly showed the presence of a conopeum extending from the sternum onto the striae (Taylor et al. 2010). Other *Microcostatus* species showing a more or less conspicuous conopeum were described in 2013 (*M. australoshetlandicus* Van de Vijver et al.: Van de Vijver et al. 2013) and in 2016 (*M. salinus* Yu H.Li & Hidek.Suzuki: Li et al. 2016; *M. aerophilus* Stanek-Tarkowska et al.: Stanek-Tarkowska et al. 2016) confirming the presence of a conopeum as one of the key features of the genus *Microcostatus*.

During the survey of several wet soil samples, collected on the coastal cliffs of Ile de la Possession, the main island of the Crozet archipelago, a small, unknown naviculoid diatom was observed during scanning electron microscopy investigations, which showed the presence of a large conopeum entirely covering the striae and reaching the valve margins. A detailed morphological analysis confirmed that the unknown species belongs to the genus *Microcostatus* but that it could not be identified using the currently available literature. The present paper describes the new species as *Microcostatus elisabethianus* sp. nov. discussing and illustrating its main morphological characteristics and comparing it not only with

all known *Microcostatus* species but also with several other naviculoid taxa belonging to the genera *Chamaepinnularia* Lange-Bert. & Krammer, *Microfissurata* Lange-Bert. et al. and *Sellaphora*. Brief notes on the ecology of the new species are added.

MATERIAL & METHODS

Study site

The material used in this study was collected on Ile de la Possession, the main island of the Crozet Archipelago (45°48'–46°26'S, 50°14'–52°15'E), situated in the southern Indian Ocean northwards of the Antarctic Convergence. This rather small archipelago consists of five islands with an oceanic, cold climate (mean annual air temperature 5.3°C, annual precipitation of 2400 mm per year: Lebouvier & Frenot 2007). The main island, Ile de la Possession (total surface 156 km²) is roughly rectangular in shape with a topography dominated by a series of inland mountains culminating at 934 m (Pic du Mascarin) and deep glacial valleys such as Vallée des Branloires. The vegetation is dominated by grasses and mosses. More details on the topography and vegetation is given in Van de Vijver et al. (2002a).

During the austral summer of 1999, several soil samples were collected from cliffs bordering the ocean. One of the samples, BA105 was taken at the mouth of the small Vallée du Sphinx from relatively wet, bare soil under overhanging coastal cliffs. Water is continuously dripping from the cliffs onto the soil. Given the limited amount of available soil, it was impossible to perform physico-chemical analyses but samples taken nearby all had an acidic pH (5.7–5.9) and relatively high conductivity (> 500 µS/cm) (Van de Vijver et al. 2002b).

Sample preparation and analysis

The sample was prepared for LM and SEM observation following the method described in van der Werff (1955). A small part of the raw soil material was cleaned by adding 37% H₂O₂ and heating to 80°C for about 1h. The reaction was completed by addition of KMnO₄. Following digestion and centrifugation (three times 10 minutes at 3700 × g), cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides, and mounted in Naphrax. The slides were analysed using an Olympus BX53 microscope equipped with differential interference contrast (Nomarski) optics and the Olympus UC30 Imaging System. Samples and slides are stored at the BR collection (Meise Botanic Garden, Belgium). For SEM analyses, part of the oxidized suspension was filtered through a 5 µm Isopore™ polycarbonate membrane filter (Merck Millipore), air dried and glued to an aluminum stub. The stub was subsequently sputter-coated with a Gold-Palladium layer of 20 nm and studied on a ZEISS Ultra SEM microscope at 3 kV (Natural History Museum London, UK).

Diatom terminology follows Ross et al. (1979) (stria/areola structure), Johansen & Sray (1998) (genus morphology) and Round et al. (1990) (raphe structure). The morphology of the new species was compared with the ultrastructure of

similar species in the genera *Microcostatus* (Johansen & Sray 1998; Van de Vijver et al. 2004b, 2010, 2013; Taylor et al. 2010; Li et al. 2016; Stanek-Tarkowska et al. 2016), *Microfissurata* (Cantonati et al. 2009) and *Chamaepinnularia* (Van de Vijver et al. 2002a; Wetzels et al. 2013).

RESULTS

Microcostatus elisabethianus Van de Vijver & Ector, sp. nov.

Figs 1–3

Type material – Iles Crozet, Ile de la Possession, Vallée du Sphinx, sample BA105 (46°24'47"S/ 51°51'58"E), 1 Dec. 1999, *B. Van de Vijver s.n.* (holotype: BR, slide BR-4566; isotype: University of Antwerp, Belgium, slide PLP-361).

Description: LM – See fig. 1: valves lanceolate to almost rhombic-lanceolate with convex margins and acute, protracted, often elongated apices. Valve dimensions ($n = 25$): length 12–17 μm , width 3.5–4.0 μm . Axial area narrow near the apices, gradually widening towards the central area, clearly raised and thickened, with only weakly to not constricted central part. Central area very large, elliptical, forming a broad fascia in larger specimens whereas in smaller specimens, short marginal striae border the entire central area. Raphe barely visible. Central raphe endings straight, simple. Terminal raphe fissures not visible in LM. Striae distinctly radiate almost throughout the entire valve, becoming parallel to convergent at the apices. Areolae not discernible in LM.

Description: SEM – External and internal view, see figs 2, 3: Raphe filiform, straight, located in a distinctly raised, central, never constricted raphesternum (fig. 2A, D). External central raphe endings straight, never bent or deflected with slightly expanded central pores (fig. 2A, E). Terminal raphe fissures elongated, unilaterally bent, continuing onto the valve mantle, almost reaching the mantle edge (fig. 2A–C). Two large depressions running on both sides of the raphesternum (fig. 2D). Microcostae barely visible. Externally, a finely porous, well-developed conopeum extends outward from the raphesternum almost to the valve margins, covering the entire longitudinal depression of the valve with the microcostae (fig. 2A–C). Conopeum pores extremely fine (fig. 2E). Due to sample preparation, pores often fused forming large, transapical perforations on each side of the sternum (fig. 2C). Outer non-porous margin of the conopeum undulating, neatly following, but never fused with, the striae/virgae system of the valve (fig. 2B, C). Striae composed of one large mac-

roareola, clearly sunken between the distinctly raised virgae (fig. 2C). Areolae externally occluded by porous hymenes (fig. 2C). Valve face/mantle edge almost making a 90° angle (fig. 2B). Mantle short with mantle areolae only present at the apices, absent on the rest of the valve mantle (fig. 2B). Mantle edge straight. Internally, short macroareolae clearly visible, running from the mantle edge almost halfway toward the axial area (fig. 3A). Near the apices, a few mantle areolae separated from the valve face areolae visible (fig. 3B). Microcostae indistinct in the axial area, extending from the virgae into the axial area (fig. 3B, see arrows). Rest of axial and central area smooth, unornamented (fig. 3C). Internal central raphe endings very short, unilaterally deflected (fig. 3C). Distal endings terminating in very weakly developed helictoglossae (fig. 3B). The girdle is composed of several open, non-perforated copulae (fig. 2D).

Etymology – *Microcostatus elisabethianus* is named in honour of Her Royal Highness Princess Elisabeth of Belgium who already gave Her name to the Belgian research station on Antarctica.

Ecology and associated diatom flora – At present, the new *Microcostatus* species was only observed with certainty in soil sample BA105. Due to possible confusion with similar small-celled naviculoid species such as several small *Chamaepinnularia* species that are also present in the sub-Antarctic soil samples, it is unclear whether the species is more widely distributed on Ile de la Possession and on other islands of the southern Indian Ocean. The sample is dominated by a large number of small-celled taxa such as *Chamaepinnularia australomedicris* (Lange-Bert. & Rol. Schmidt) Van de Vijver, *Frankophila maillardii* (Le Cohu) Lange-Bert., several unidentified *Chamaepinnularia* taxa, *Planothidium cyclophorum* (Heiden) Van de Vijver, *P. subantarcticum* Van de Vijver et al. and *P. renei* (Lange-Bert. & Rol. Schmidt) Van de Vijver. A similar combination of diatom taxa was observed in other soil samples but *M. elisabethianus* has up to now not been observed in these (Van de Vijver, Belgium, pers. obs.).

DISCUSSION

The new species presents a rather unique combination of features that is not entirely in accordance with the original genus description of *Microcostatus* (Johansen & Sray 1998). *Microcostatus elisabethianus* is characterised by the presence of a large conopeum, microcostae that are hard to discern, striae composed of one large macroareola and elon-

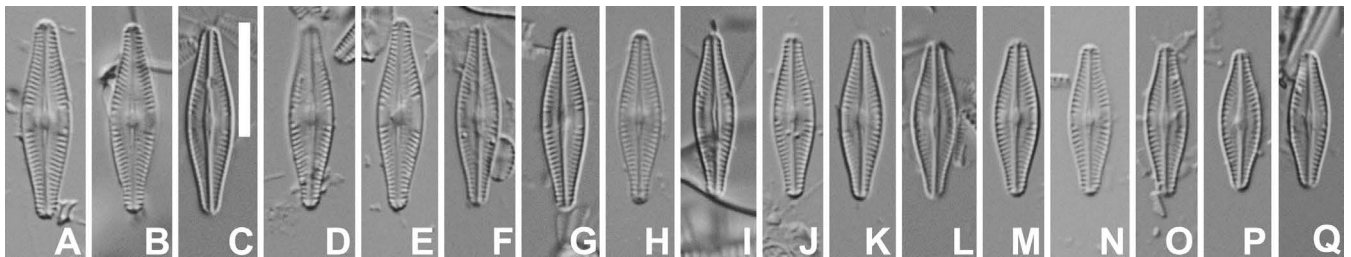


Figure 1 – *Microcostatus elisabethianus*, LM of the type population from Crique du Sphinx, Ile de la Possession, Iles Crozet (sample BA105). A–Q. LM views of several valves in valve face view showing the observed size range. Note the difference in central area between larger (presence of a large fascia) and smaller specimens (presence of marginal striae). Scale bar = 10 μm .

gated terminal raphe fissures, continuing almost up to the mantle edge.

Li et al. (2016) discussed the microcostae and concluded that their presence is not clearly visible in all members of the genus. In *Microcostatus zackenbergensis* Van de Vijver et al. and *M. naumannii* (Hust.) Lange-Bert. for instance, microcostae are probably absent (Van de Vijver et al. 2004b). Other taxa such as *M. krasskei* (Hust.) J.R.Johans. & Sray and *M. deslooveri* Iserentant & Van de Vijver have very well

developed microcostae (Johansen & Sray 1998; Van de Vijver et al. 2004b). Secondly, the presence of a conopeum was originally excluded in the genus *Microcostatus*. Johansen & Sray (1998) explicitly stated in their genus description that a conopeum was absent as the generitype, *M. krasskei*, did not have a conopeum but instead had a very well-developed, clearly centrally constricted raphesternum. Later species that were transferred to, or described in *Microcostatus*, such as *M. deslooveri* or *M. naumannii*, in concurrence with the ge-

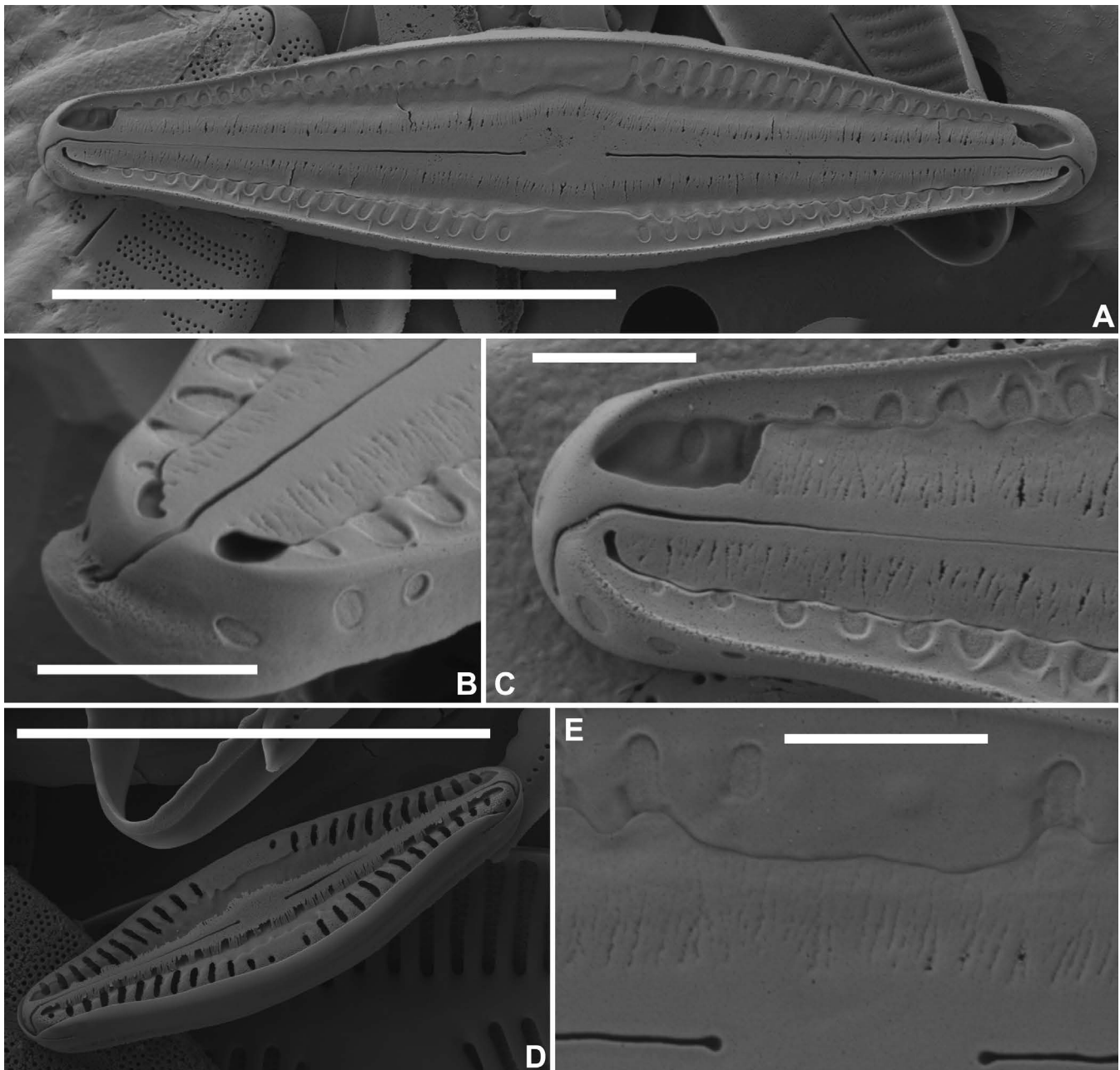


Figure 2 – *Microcostatus elisabethianus*, SEM of the type population from Crique du Sphinx, Ile de la Possession, Iles Crozet (sample BA105). **A.** External view of an entire valve. **B.** External detail of the valve apex showing the terminal raphe fissures, the mantle areolae and the conopeum together with the virgae and occluded macroareolae. **C.** External detail of the valve apex in valve face view with the conopeum and the striae in the longitudinal canal. **D.** External view of an entire valve with eroded conopeum showing the longitudinal depressions bordering the axial area. **E.** External detail of the central area with the straight, simple central raphe endings and the surface structure of the conopeum. Scale bars: A, D = 10 µm; B, C, E = 1 µm.

nus description and did not have a conopeum (Van de Vijver et al. 2004b, 2010). However, in 2010, Taylor et al. added the presence of a conopeum to the list of morphological features characterising (several members of) the genus *Microcostatus*. A second variable morphological feature in the genus *Microcostatus* is the structure of the striae. According to the genus description, the striae in *Microcostatus* are uniseriate, lacking hymenes, and, based on the illustrations shown in Johansen & Sray (1998) it is clear that they are composed of a series of very small, rounded to transapically elongated, slit-like areolae. Although most species in the genus such as *M. egregius* (Hust.) Lange-Bert. or *M. schoemaniai* J.C. Taylor et al. possess the same stria structure, the striae in *Microcostatus salinus* are composed of two elongated areolae whereas others have striae only consisting of one long macroareola (Li et al. 2016). Typical examples of the latter situation include *M. deslooveri*, *M. chalnoky* and *M. australoshetlandicus* (Van de Vijver et al. 2004b, 2013). The only feature that seems to be rather constant in all *Microcostatus* species known so far, is the presence of elongated terminal raphe fissures, usually continuing up to the mantle edge. Given the above-mentioned variability in the morphological features of

the genus *Microcostatus*, the placement of the new species within this genus is justified.

Several recently published papers present excellent comparison tables discussing the differences and similarities between the different *Microcostatus* (Van de Vijver et al. 2004b; Taylor et al. 2010; Li et al. 2016). These tables have been used for the following morphological comparison analysis. Based on its ultrastructure, *Microcostatus elisabethianus* cannot be confused with any other *Microcostatus* species. The main feature of this new species is its very large conopeum covering almost the entire valve face. Up to now, it is the only species where the conopeum is so developed. There are only a handful of *Microcostatus* species known worldwide possessing a conopeum or a pseudoconopeum (Lange-Bertalot et al. 1990). The largest conopeum is found in *M. chalnoky*, a species described from South Africa. The latter however can be distinguished from *M. elisabethianus* by its longer and more closely positioned marginal striae, the hyaline, irregular valve face margin, the more elliptic valve outline lacking elongated apices and the much higher stria density (45–50 in 10 μm vs. 23–24 in 10 μm) (Taylor et al. 2010). Another South African species, *M. schoemaniai*, has a smaller conopeum not reaching the valve margins, an ellip-

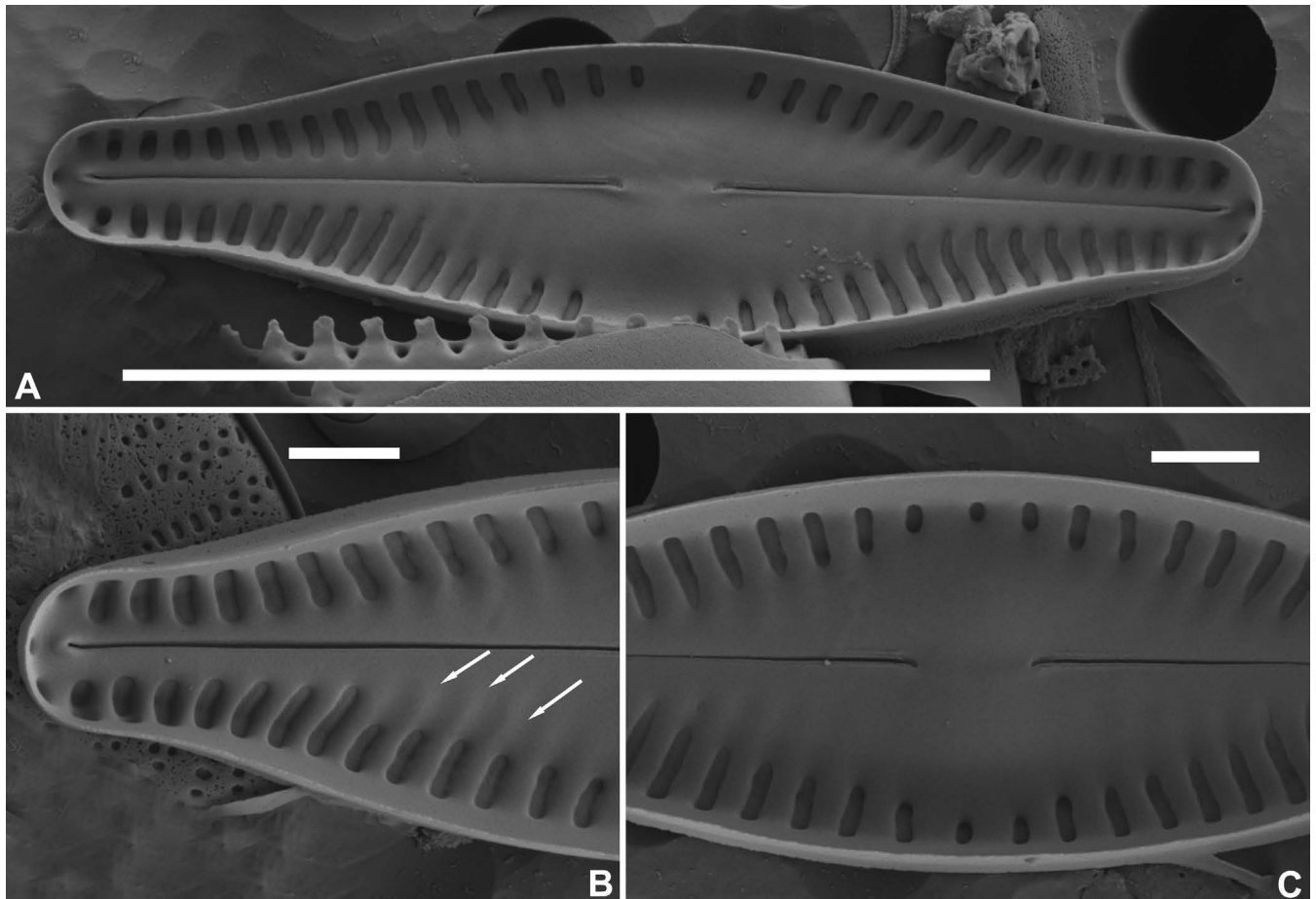


Figure 3 – *Microcostatus elisabethianus*, SEM of the type population from Crique du Sphinx, Ile de la Possession, Iles Crozet (sample BA105). A. SEM internal view of an entire valve. B. SEM internal detail of the valve apex showing the faintly present microcostae (see arrows) and the small helictoglossae. C. SEM internal detail of the valve central area with the shortly bent central raphe endings and the macroareolae. Scale bars: A = 10 μm ; B, C = 1 μm .

tic-lanceolate valve outline, a very high stria density (50–55 in 10 µm) and striae that are composed of a series of very small areolae, contrary to *M. elisabethianus* that possesses striae consisting of only one long macroareola (Taylor et al. 2010). *Microcostatus aerophilus* Stanek-Tarkowska et al. and *M. egregius* have a very narrow conopeum leaving the longitudinal canals visible in LM (Stanek-Tarkowska et al. 2016). The marginal striae in *M. aerophilus* are composed of a series of very small areolae and the microcostae are very well developed, clearly visible in SEM as radiate, slightly raised ribs (Stanek-Tarkowska et al. 2016). *Microcostatus salinus* has linear-elliptic valves with broadly rounded, never protracted apices (Li et al. 2016). The species possesses a well-developed conopeum, although it never reaches the valve margins, as is the case in *M. elisabethianus*. It was described from sandy sediments in Japan and is up to now the only brackish/marine species of the genus. *Microcostatus australoshetlandicus* Van de Vijver et al., described from the Maritime Antarctic Region, has a similar stria structure with only one macroareola covered externally by a porous hymen but differs in valve dimensions (valve length 6.5–9.0 µm), a higher number of striae (24–28 in 10 µm compared to 23–24 in 10 µm for *M. elisabethianus*) and a smaller conopeum (Van de Vijver et al. 2013). Two other *Microcostatus* species, from South America (Wydrzycka & Lange-Bertalot 2001; Metzeltin & Lange-Bertalot 2007), differ in the absence of a conopeum. *Microcostatus monsviridis* Metzeltin & Lange-Bert. and *M. ornicollegarum* Lange-Bert. & Wydrzycka have a similar stria structure with typical macroareolae but can be differentiated from *M. elisabethianus* in valve outline (both elliptic-lanceolate with cuneate apices instead of being lanceolate with long, protracted apices) and valve dimensions (max. length 12 µm).

Apart from a possible confusion with other *Microcostatus* species, using only light microscopy it is often difficult to distinguish the new species from members of the genus *Chamaepinnularia*, of which several are present in the sub-Antarctic region. Van de Vijver et al. (2002a) recorded several *Chamaepinnularia* taxa on Ile de la Possession that were either given provisional names (*Chamaepinnularia* sp.: see Van de Vijver et al. 2002a: plate 86) or wrongly identified as *C. evanida* (Hust.) Lange-Bert., *C. alienae* (Krasske) Van de Vijver or *Pinnularia* aff. *perirrorata* Krammer (Van de Vijver et al. 2002a: plates 85 & 96), but show some resemblance to *M. elisabethianus*. Most of these species require revision clarifying their correct taxonomic position as most of them probably represent new species (Van de Vijver, pers. comm.). The main similarity between *Chamaepinnularia* and the new *Microcostatus* species is based on the rather coarse striation pattern in the latter, giving the new *Microcostatus* species the impression, in LM, that it might actually belong to *Chamaepinnularia*. Most *Chamaepinnularia* have a stria density that is easily discernible in LM (usually between 20 and 25 µm) (Wetzel et al. 2013) and *M. elisabethianus* possesses between 23 and 24 striae in 10 µm. Moreover, the general valve outline of most of these *Chamaepinnularia* species is lanceolate with cuneately rounded, rostrate or elongated, protracted apices, quite similar to the valve outline of *M. elisabethianus*.

However, analysis of the ultrastructure of the new species using SEM excludes all conspecificity. *Chamaepinnularia* species are characterised by striae that are composed of two areolae, one on the valve face and a second one on the mantle. Moreover, microcostae and a conopeum are always absent.

Finally, the genus *Microfissurata*, described from the Italian Dolomites, also shows some similarities with the new species. This small genus has one representative (*M. australis* Van de Vijver & Lange-Bert.) on the island Ile Amsterdam, situated north of the Iles Crozet. The latter species can, however, be relatively easily separated based on its large macroareolae, the absence of the longitudinal depression, the more linear-lanceolate valve outline and broadly rounded apices (Cantonati et al. 2009). Moreover, the genus *Microfissurata* lacks a conopeum, that typifies *M. elisabethianus*, excluding all conspecificity.

Based on the results of this morphological comparison, the description of *Microcostatus elisabethianus* as a new species is justified.

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