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Three new *Craticula* species (Bacillariophyta) from the Maritime Antarctic Region

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Abstract

Three new diatom taxa belonging to the genus *Craticula* were found during the ongoing taxonomic revision of the Antarctic freshwater and limno-terrestrial diatom flora: *Craticula australis*, *sp. nov*., *C. obaesa*, *sp. nov* and *C. petradeblockiana*, *sp. nov*. Detailed light and scanning electron microscope observations are used to better characterize the morphology and ultrastructure of these three new taxa. A fourth *Craticula* taxon, formerly identified as *Navicula megacuspidata*, described from South Georgia, is transferred to the genus *Craticula* and its taxonomy is briefly discussed. Comparisons with similar taxa and the ecological preferences of each species are added. The revision of these species confirms once again the endemic nature of the Antarctic diatom flora.

Introduction

The genus *Craticula* Grunow (1867: 20) was described to accommodate a series of taxa that are characterized in having parallel, uniseriate striae, composed of round to elliptical areolae occluded internally by perforated hymenes, a thick raphe sternum, expanded, straight to deflected external proximal raphe endings, hook-like distal endings and the lack of accessory ribs next to the internal raphe branches (Round *et al*. 1990). Moreover, the areolae and the separating frets are aligned longitudinally giving the impression of longitudinal ribs running from pole to pole. Morales *et al*. (2014) discuss in detail the morphology of both valves and cell contents. The taxa within the genus *Craticula* can be roughly subdivided into two groups: a first groups contains all larger-celled species such as *C. cuspidata* (Kütz. 1833: 549) D.G.Mann in Round *et al*. (1990: 666) and *C. ambigua* (Ehrenb. 1843: 471) D.G.Mann in Round *et al*. (1990: 666) and a second group with smaller taxa such as *C. molestiformis* (Hustedt 1949: 86) Mayama (1999: 2) and *C. halophila* (Grunow in Van Heurck 1885: 100) D.G.Mann (in Round *et al*. 1990: 666). The first group typically forms inner valves, known as 'heribaudii' stages (Lange-Bertalot 2001) also known as 'craticula', whereas in the second group some taxa produce highly silicified valves (Kusber & Cocquyt 2007, Van de Vijver *et al*. 2010). At present, this freshwater genus has about 50 taxa (Fourtanier & Kociolek 2011), several of them recently described (Van de Vijver *et al*. 2002, 2010, Lange-Bertalot *et al*. 2003, Morales *et al*. 2014).

The number of *Craticula* species in the Antarctic Region is rather low and the list of records merely comprises observations of northern hemisphere taxa such as *Craticula cuspidata* (Kütz. 1833: 549) D.G. Mann in Round *et al*. (1990: 666) or *Craticula halophila*. Kellogg and Kellogg (2002) list all records. Most taxa have been reported from the Maritime Antarctic Region, although Van de Vijver *et al*. (2002) illustrated three taxa from sub-Antarctic Ile de la Possession (Crozet Archipelago) in the southern Indian Ocean, including one new species: *C. salsuginosa* Van de Vijver & Beyens in Van de Vijver *et al*. (2002: 30). One species, *C. glaberrima* (W. & G.S. West 1911: 282) Van de Vijver *et al*. (2013: 199) was described from the Antarctic Continent (West & West 1911, Van de Vijver *et al*. 2012) whereas Carlson (1913) described *Navicula megacuspidata* Carlson (1913: 14) from South Georgia, a species that, based on its structure, most likely should be transferred to the genus *Craticula*. Additionally, two other *Craticula* taxa, *C. subpampeana* Van de Vijver & Sterken (in Van de Vijver *et al*. 2010: 435) and *C. antarctica* Van de Vijver & Sabbe (in Van de Vijver *et al*. 2010: 433) were recently described from James Ross Island (Van de Vijver *et al*. 2010).

The present paper describes three new taxa from James Ross Island situated in the southern part of the Maritime

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Antarctic Region: *Craticula australis*, *sp. nov*., *C. obaesa*, *sp. nov* and *C. petradeblockiana*, *sp. nov.* based on detailed light and scanning electron microscopy observations. The three taxa are compared with similar taxa worldwide and notes on their ecological preferences are added. The transfer of *Navicula megacuspidata* Carlson (1913: 14) to the genus *Craticula* is made and discussed in the light of a possible conspecificity (and hence priority over) with *Craticula pampeana* (Frenguelli 1926: 43) Lange-Bert. in Lange-Bertalot *et al*. (2003: 39).

Materials and Methods

During a survey of the non-marine diatoms from James Ross Island (64° 10' S, 57° 45' W), a series of samples collected on the Ulu Peninsula and Clearwater Mesa, has been investigated. James Ross Island is located in the southern Maritime Antarctic Region, in the north-western part of the Weddell Sea, close to the northern tip of the Antarctic Peninsula. This fairly large island (2450 km²) is situated in the transition zone between the Maritime Antarctica and Continental Antarctica regions (Øvstedal & Lewis-Smith 2001). Only the northern part, the Ulu Peninsula, where a part of the samples was taken, is ice-free. The mean annual air temperature rarely exceeds -5 °C at sea level with the mean summer temperature slightly below 0 °C (Schwerdtfeger 1984). Precipitation is limited [150 mm/y in the northern part (Aristarain *et al*. 1987)] with high evaporation rates reducing the formation of large open water bodies. The terrestrial vegetation is limited to non-vascular plants forming a bryophyte-and lichen-tundra in places. Clearwater Mesa is a volcanic tableland composed of single hyaloclastite breccia unit capped with subaerial basalt of the James Ross Island Volcanic Group (Nelson 1966, Košler *et al*. 2009). On the flat mesa surface (mostly 250–300 m a.s.l.) tens of lakes evolved after the glacier retreat during the early Holocene (Nedbalová *et al*. 2013). These lakes with littoral zones covered by microbial mats, are generally shallow and could be regarded as permanent and old, with a persistence of thousands of years (D. Nývlt, pers. comm.). Sampling methods follow those described in Kopalová *et al*. (2012, 2013, 2014). Details on the physico-chemical analyses (pH, conductivity, nutrients) of Ulu Peninsula can be found likewise in these references.

Diatom samples were prepared for light microscopy (LM) observation following the method described in Van der Werff (1955). Small parts of the sample were cleaned by adding 37% H_2O_2 and heating to 80 °C for about 1 h. The reaction was completed by addition of $KMnO_4$. 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. Cleaned diatom material was mounted in Naphrax®. The slides were analyzed using an Olympus BX53 microscope, equipped with Differential Interference Contrast (Nomarski) and the Olympus UC30 Imaging System. Samples and slides are stored at the BR-collection, property of the Belgian federal government and given in permanent loan to the Botanic Garden Meise (Belgium). For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered through a 1-µm Isopore™ polycarbonate membrane filter (Merck Millipore). 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 London, UK). Diatom terminology follows Ross *et al*. (1979) (stria/areola structure), Round *et al*. (1990) (raphe structure) and Lange-Bertalot (2001) (genus morphology). The morphology of the new taxa has been compared with the ultrastructure of known species described worldwide (Lange-Bertalot 1993, 2001, Lange-Bertalot *et al*. 2003, Kusber & Cocquyt 2007, Van de Vijver *et al*. 2002, 2010, Morales *et al*. 2014).

Observations

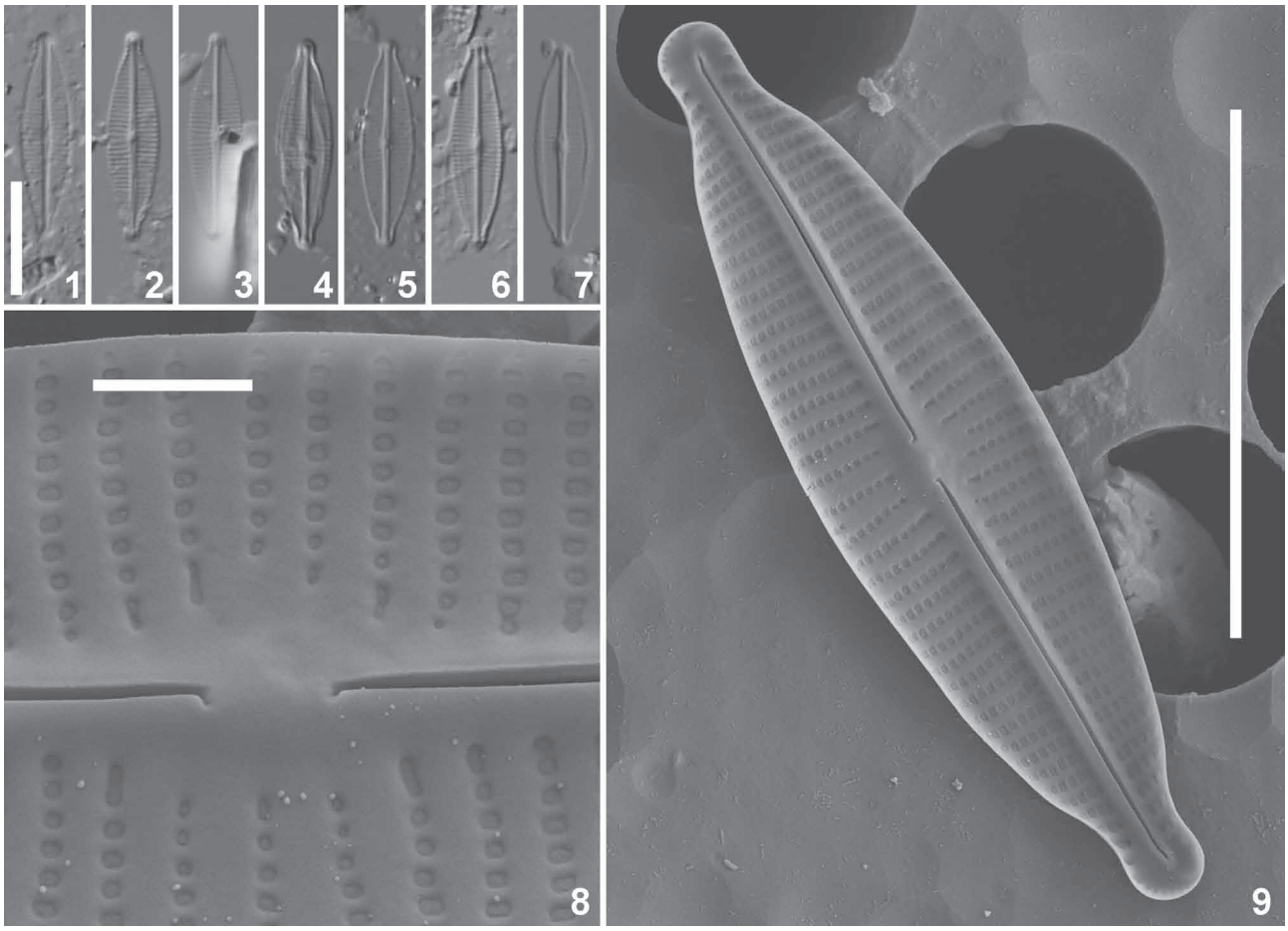
Division Bacillariophyta
Class Bacillariophyceae
Subclass Bacillariophycidae
Order Naviculales
Family Stauroneidaceae
Genus *Craticula*

Craticula australis Van de Vijver, Kopalová & Zidarova, *sp. nov*. (Figs 1–9)

LM (Figs 1–7): valves broadly lanceolate with clearly convex margins gradually tapering towards the protracted, rostrate to

weakly sub-capitate apices. Valve dimensions (n=10): length 18.0–19.5 µm, width 4.0–5.5 µm. Axial area very narrow, linear, forming a thickened, raised raphesternum. Central area absent. Raphe branches straight with weakly expanded proximal raphe endings. Distal raphe endings not discernible in LM. Striae almost parallel to very slightly radiate in the valve center, becoming slightly convergent (Fig. 3), 24–28 in 10 µm, showing no difference in stria density throughout the valve. Areolae not visible in LM. SEM (Figs 8–9): due to the rare nature of this taxon in the sample, SEM observations of the valve exterior were not possible. Only the valve interior could be examined. Striae uniseriate, composed (internally) of rounded to rectangular areolae. Areolae covered by perforated hymenes, located inside each areola (Fig. 8). Striae near the central area weakly more distant than in the rest of the valve, occasionally with one shortened stria inserted within the normal striation pattern. Central nodule weakly raised. Internal proximal raphe endings positioned closely together, unilaterally shortly hooked (Fig. 8). Distal raphe endings terminating on small helictoglossae (Fig. 9).

Type:—JAMES ROSS ISLAND. Lachman 1: sample D2, 63° 47” 34.9’ S, 57° 48” 13.8’ W, *L. Nedbalová*, *22 January 2008* (holotype BR! slide no. 4392, isotype PLP! Slide no. 276, University of Antwerp, Belgium).



FIGURES 1–9. *Craticula australis*. LM & SEM pictures taken from the holotype population (sample D02) from Lachman Lake I, James Ross Island. 1–7. Light microscopy observations. 8. Internal view of the central area with focus on the proximal raphe endings and the perforated areola occlusions. 9. Internal view of an entire valve showing the raphe and the variable distance between the striae. Scale bar represents 10 µm except for Fig. 8 where scale bar = 1 µm.

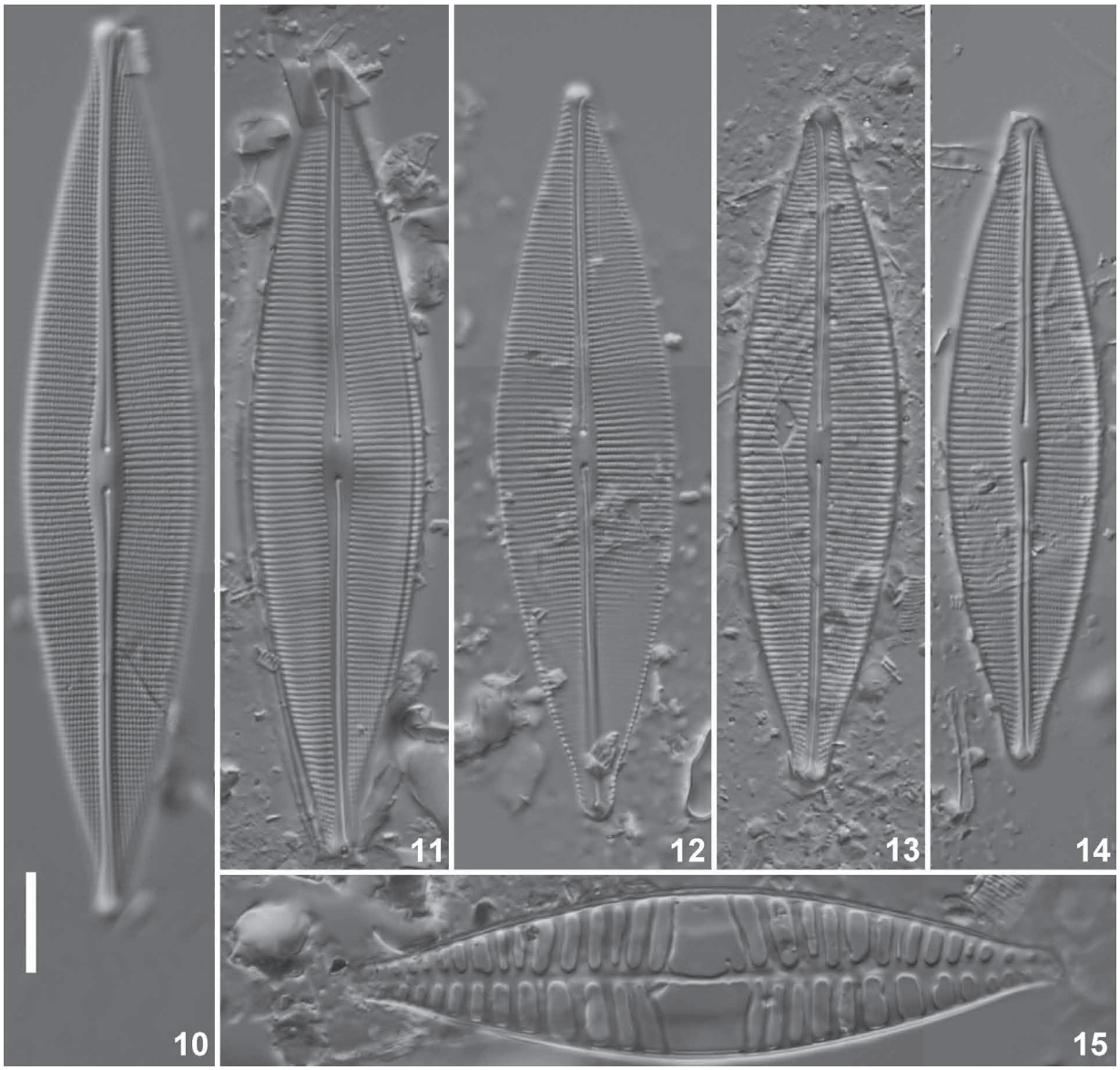
Ecology and Distribution:**—***Craticula australis* was so far only observed in one sample, taken from the epilithon of a shallow coastal lake (Lachman 1) on Ulu Peninsula (James Ross Island). The lake was slightly alkaline (pH 7.6) with a rather high specific conductance value (>1000 µS/cm) and high total phosphorus (TP) and sulphate levels (592 µg/l and 162 mg/l respectively). The sample was dominated by *Nitzschia homburgiensis* Lange-Bert. (1978: 650–651) and *Humidophila australis* (Van de Vijver & Sabbe in Van de Vijver *et al*. 2010: 438) Lowe *et al*. (2014: 357).

Etymology:—The specific epithet refers to the distribution the taxon in the southern hemisphere (Latin: *australis* = southern).

Craticula obaesa **Van de Vijver, Kopalová & Zidarova, *sp. nov*. (Figs 10–20)**

LM (Figs 10–15): valves lanceolate to elliptic-lanceolate with distinctly convex margins, tapering gradually towards the small, weakly protracted, subrostrate apices. Valve dimensions (n=20): length 59–90 µm, width 14–18 µm. Axial area linear, very narrow, formed by a raised sternum, slightly widening towards the small, narrowly elliptic central area. Raphe branches almost straight to weakly curved, slightly lateral for most of its length, almost filiform near the central area. Proximal raphe endings weakly deflected terminating into enlarged pores. Distal raphe endings elongated, distinctly hooked opposite to the proximal raphe endings (Figs 13, 14). Striae more distantly spaced near the central area, almost parallel to weakly radiate throughout the entire valve, 13–15 in 10 µm, becoming parallel and finally weakly convergent and more densely spaced near the apices, 16–18 in 10 µm. Areolae visible in LM, *ca*. 26–31 in 10 µm. Heribaudii-stages often observed (Fig. 15). SEM (Figs 16–20): external proximal raphe endings short, expanded, clearly hooked (Fig. 16). Distal fissures elongated continuing onto the mantle (Fig. 17). Striae composed of narrow, apically elongated slit-like areolae (Figs 16, 17). Rarely, areolae in the valve middle almost rounded or only weakly transapically elongated (Fig. 16). Frets separating the areolae weakly raised forming longitudinal grooves in which the areolae are positioned (Fig. 17). Near the central area and the apices, grooves absent. Internally, central nodule raised (Fig. 18). Proximal raphe endings short, unilaterally weakly deflected. Areolae occluded by perforated hymenes (Fig. 19). Heribaudii stage composed of a longitudinal, axial sternum with irregularly and distantly-spaced, thickened transverse bars (Fig. 20).

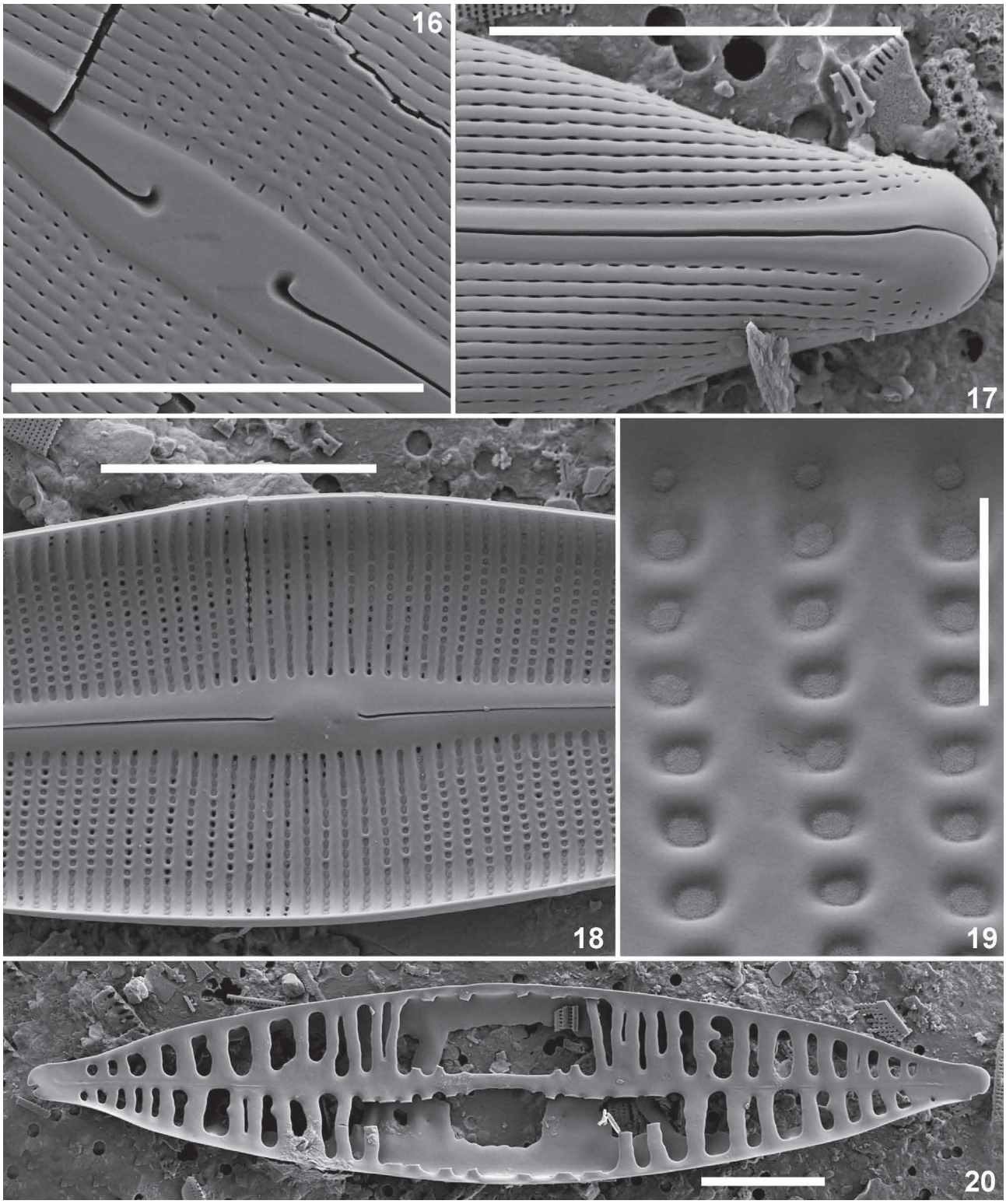
Type:—JAMES ROSS ISLAND. Clearwater Mesa: sample CLW60, *L. Nedbalová*, *09 February 2009* (holotype BR! slide no. 4393, isotype PLP! Slide no. 277, University of Antwerp, Belgium).



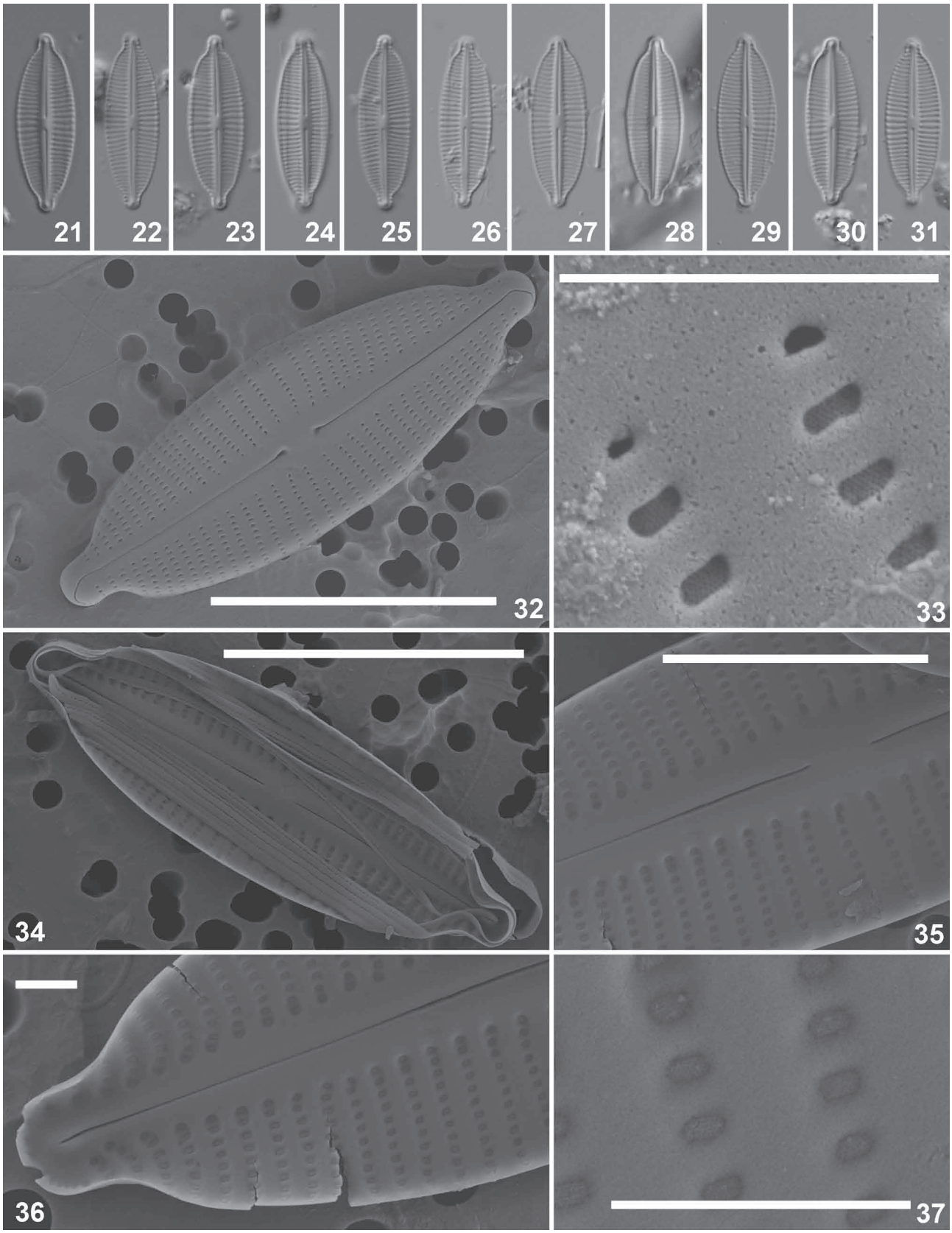
FIGURES 10–15. *Craticula obaesa*. LM pictures taken from the holotype population (sample CLW60) from Clearwater Mesa, James Ross Island. 15. LM view of a heribaudi stage. Scale bar represents 10 µm.

Ecology and Distribution:—*Craticula obaesa* has been found in several lakes on the Clearwater Mesa, a volcanic tableland in the northern part of James Ross Island. Their littoral zone is covered by microbial mats dominated by filamentous cyanobacteria (L. Nedbalová, pers. comm.). The largest populations of the new species were found in lakes with an alkaline pH (8.1–8.7) and a moderate to rather high conductivity (500–1000 µS/cm).

Etymology:—The specific epithet describes the general outlook of the species, showing a rather obese aspect.



FIGURES 16–20. *Craticula obaesa*. SEM pictures taken from the holotype population (sample CLW60) from Clearwater Mesa, James Ross Island. 16. Detail view of the central area. Note the difference in shape of the central areolae and the expanded, hooked proximal raphe endings. 17. External detail of a valve apex with the typical longitudinal lines formed by the frets separating the areolae. 18. Internal detail of the central area with focus on the central nodule and the differences in areola structure near the central area. 19. Internal detail of the areolae occlusions. 20. Heribaudii stage. Scale bar represents 10 µm, except for Fig. 19 where scale bar = 1 µm.



FIGURES 21–35. *Craticula petradeblockiana*. LM & SEM pictures taken from the holotype population (sample Vo4) from Water-Supply Creek, James Ross Island. 21–31. Light microscopy observations. 32. External view on an entire valve showing the raphe and the variation in stria distance in the central area compared to the rest of the valve. 33. External detail of the perforated areola occlusions in the areolar canal. 34. Internal view of an entire valve showing the raphe. 35. Internal detail of the central area. 36. Internal detail of a valve apex showing the small helictoglossa. 37. Internal detail of the areolae showing the position of the hymenes. Scale bar represents 10 µm except for Figs 33, 36 & 37 where scale bar = 1 µm and Fig. 35 where scale bar = 5 µm.

Craticula petradeblockiana **Van de Vijver, Kopalová & Zidarova, *sp. nov*. (Figs 21–35)**

LM (Figs 21–31): valves elliptical with clearly convex margins and shortly protracted, rostrate apices, set on well-developed shoulders. Valve dimension (n=40): length 20–22 µm, width 6.0–7.5 µm. Axial area narrow to moderately broad, linear to linear-lanceolate, weakly widened near the central area. Central area almost absent, only visible due to the widening of the axial area, and occasionally shortened striae inserted into the normal striation pattern (Figs 25, 30). Raphe branches straight, filiform with simple, weakly expanded proximal raphe endings and hooked distal endings (Figs 26, 27). Striae weakly radiate near the central area becoming parallel to slightly convergent near the apices, 20–22 in 10 µm. Near the central area, striae more distantly spaced contrary to the apices where striae more closely together positioned. Areolae not discernible in LM. SEM (Figs 32–35): striae externally composed of apically elongated, rectangular to rounded (bordering the axial area) areolae (Fig. 32). External proximal raphe endings droplike, straight (Fig. 32). Distal fissures hooked, slightly extending onto the valve mantle. Internally, central nodule very weakly raised (Figs 34, 35). Internal proximal raphe endings relatively distant, straight to very slightly unilaterally shortly bent (Figs 34, 35). Distal raphe endings terminating on small helictoglossae (Fig. 36). Areolae covered by perforated hymenes, located inside each areola (Figs 33, 37).

Type:—JAMES ROSS ISLAND. Ulu Peninsula: Water-supply Creek, sample Vo4, 63° 48” 58.2’ S, 57° 54” 55.1 W , *J. Elster*, *01 February 2006* (holotype BR! slide no. 4394, isotype PLP! Slide no. 278, University of Antwerp, Belgium).

Ecology and Distribution:**—***Craticula petradeblockiana* was found in a sample, collected from Water-Supply Creek, on Ulu Peninsula at 2.2 km from the ocean at an altitude of 39 m a.s.l. Unfortunately, more ecological information was not collected when sampling. On the nearby Livingston Island (South Shetland Islands), *C. petradeblockiana* has been rarely observed in samples taken from streams and ponds, though more valves were found only in a pond with turbid water, having a relatively low conductance (212 µS/cm) and NO_3^--N (0.4 mg/l) and PO_4^{3-}-P (0.21 mg/l) concentrations (Zidarova, pers. obvs.) In all cases the samples were dominated by various *Mayamaea* and *Fistulifera* taxa.

Etymology:**—**The species is named after our dear colleague, Dr. Petra De Block (Botanic Garden Meise, Belgium) in recognition of her taxonomic work, mainly on the angiosperm Rubiaceae family from Madagascar.

Craticula megacuspidata **(Carlson) Van de Vijver, Kopalová & Zidarova, *comb. nov*.**

Basionym: *Navicula megacuspidata* Carlson (1913) *Wissenschaftliche Ergebnisse der Schwedischen Südpolar-Expedition 1901–1903*, *unter leitung von Dr. Otto Nordenskjöld*, p. 14, Figs II: 1, 2.

Discussion

It is highly likely that the three new *Craticula* species have been overlooked in the past as independent species based on the (very) broad species concept that was commonly applied by former taxonomists such as Hustedt or Krasske, and hence force-fitted within European or North-American relatives (Tyler 1996). Although for *C. australis* and *C. petradeblockiana* only a few valves and/or populations were observed and the range in valve dimensions is apparently rather narrow, their separation from previously described species is justified. *Craticula australis* most likely was confused with taxa such as *C. molesta* (Krasske 1938: 528) Lange-Bert. & Willmann in Lange-Bertalot *et al*. (1996: 130), *C. molestiformis*, *C. submolesta* (Hust. 1949: 86) Lange-Bert. in Lange-Bertalot & Metzeltin (1996: 42) or even *C. accomoda* (Hust. 1950: 446) D.G.Mann in Round *et al*. (1990: 666). Kellogg & Kellogg (2002) list all these records. However, all taxa can be more or less easily separated from *C. australis*. *Craticula molesta* is a taxon only known from two valves observed in a sample in Spitsbergen (Arctic Region) and was frequently reported from the Antarctic Continent (Kellogg & Kellogg 2002), although the taxon from the Continent proved to be *C. antarctica* (Van de Vijver *et al*. 2010). The valves are however slightly narrower (width 4 µm) than *C. australis* with a much lower stria density (22 in 10 µm). Both *C. molestiformis* and *C. submolesta* have a different valve outline lacking the typical rostrate ends, showing more or less truncated apices. The striae in *C. molestiformis* are only resolvable in LM near the middle part of the valves as towards the apices the stria density increases to almost 40 in 10 µm (Lange-Bertalot 2001), whereas this is never the case in *C. australis*. *Craticula antarctica* is larger (width 6.5–8.0 µm *vs*. 4.3–5.3 µm in *C. australis*), with a lower stria density (max. 20 in 10 µm), showing a more elliptic-lanceolate outline with more elongated, protracted apices (Van de Vijver *et al*. 2010). Heavily silicified valves as often observed in *C. antarctica* (Van de Vijver *et al*. 2010, see Figs 24, 35, 36) have never been observed in *C. australis*. *Craticula accomoda* has more elliptical valves with an increasing stria density towards the apices (Lange-Bertalot 2001). The species can also be separated from *C.*

dissociata in having larger valves (width 3.6–4.0 µm in *C. dissociata*) with a lower number of striae (35–38 in 10 µm in *C. dissociata*). Finally, *C. halophiloides* has also distinctly narrower valves (max. 4.5 µm) and an increasing (and higher) stria density towards the apices (Lange-Bertalot 2001). Other similar taxa are *C. antarctica*, *C. dissociata* (E.Reichardt 1984: 44) E.Reichardt (1997: 305) and *C. halophilioides* (Hust. 1959: 106) Lange-Bert. (2001: 114).

Craticula obaesa was most likely confused with *C. cuspidata* or *C. ambigua* (Kellogg & Kellogg 2002) based on the overall shape and valve dimensions and the, at that moment, broad species concept. *Craticula cuspidata* is much larger with a minimum valve width of 17 µm up to 35 µm, long, protracted apices and a more rhombic-lanceolate outline. On the other hand, *C. ambigua* shows a similar elliptic-lanceolate valve outline but presents typical shoulders just below the elongated apices, contrary to *C. obaesa* that shows gradually tapering convex margins lacking the typical shoulders. Valves are usually also wider (width up to 24 µm). *Craticula fumantii* Lange-Bert. *et al*. (2003: 33) has a similar valve outline but presents narrower valves (max. valve width 15 µm) and usually shorter valves. Other taxa such as *Craticula nonambigua* Lange-Bert. *et al*. (2003: 38) or *C. acidoclinata* Lange-Bert. & Metzeltin (1996: 41) can be separated based on either valve dimensions or valve outline, with *C. nonambigua* much shorter (max. 66 µm) and *C. acidoclinata* much longer (up to 130 µm) with a more elongated, lanceolate valve outline. The most similar taxon, however, is *C. subpampeana*, recently described from James Ross Island (Van de Vijver *et al*. 2010). The latter shows much longer (up to 110 µm) though not wider valves with hence an elongated, strictly lanceolate, never elliptic-lanceolate valve outline. When both taxa are found in the same sample, a clear separation based on valve outline is always possible.

Craticula petradeblockiana was earlier reported from Livingston Island (South Shetland Islands) as *Craticula accomoda* (*i.e*. Zidarova 2008) and most likely in other past studies it has also been confused with the latter (see for instance Wasell & Håkansson 1992 on Horseshoe Island). *Craticula accomoda* can however easily be separated based on a higher stria density (20–28 in 10 µm *vs*. 18–20 in 10 µm for *C. petradeblockiana*) (Lange-Bertalot 2001). *Craticula accomoda* also shows a different, raised raphe sternum (see Lange-Bertalot 2001, Figs 86: 1, 2) contrary to *C. petradeblockiana* that lacks a raised raphe sternum. *Craticula buderi* (Hust. 1954: 279) Lange-Bert. in Rumrich *et al*. (2000: 101) has a different valve outline with less developed shoulders and a slightly higher stria density (up to 24 in 10 µm) (Rumrich *et al*. 2000, Lange-Bertalot 2001). Finally, *C. antarctica* has likewise a different valve outline with more lanceolate valves and longer, more elongated apices (Van de Vijver *et al*. 2010). Other *Craticula* taxa are either too small (see for instance *C. molesta* or *C. molestiformis*) or too large (e.g., *C. riparia* (Hust. 1942: 52) Lange-Bert. 1993: 14) to be confused with *C. petradeblockiana*.

The identity of *Craticula megacuspidata* is not entirely clear. The material from Carlson (1913) has not been retrieved in a collection, so at present it is impossible to study the morphology of the species in more detail. The species has so far only been found on South Georgia (*e.g*., Fukushima 1965, Birnie 1990), but only Fukushima (1965) provided a good photograph of the taxon. Based on this photograph and the illustrations and description in Carlson (1913), conspecificity with *C. pampeana* is not to be excluded which, if confirmed, would make *C. pampeana* a latter synonym of *C. megacuspidata*. Valve outline, dimensions and stria density match between both species, especially with *N. pampeana* var. *major* Frenguelli (1926: 44), considered a synonym by Van Landingham (1975) with *C. pampeana*. Nevertheless, until the morphology of both taxa can be analysed in more detail, we suggest that they are kept separate but *N. megacuspidata* should be transferred to *Craticula* as the illustrated valves show all typical features of the latter genus.

The description of the three new taxa doubles the total number of *Craticula* taxa in the Maritime Antarctic Region. At present, six different taxa are with certainty observed in this region, most of them described from and so far only found on James Ross Island. Only *C. glaberrima* was initially described from the Antarctic Continent by West & West (1911) but later observed from several localities on the South Shetland Islands and James Ross Island. During the diatom survey of the South Shetland Islands, only a few valves of the smaller *C. antarctica* and *C. glaberrima* have been found whereas larger taxa such as *C. obaesa* are absent (Zidarova *et al*., unpubl. res.). Literature data however mention the presence of *C. cuspidata* on the South Shetlands (*e.g*., Björck *et al*. 1993, Håkansson & Jones 1994), although none of these records could be verified. This highly specific *Craticula* composition, confirms previous findings on the endemic character of the Maritime Antarctic diatom flora, as described for the genera *Muelleria* (Van de Vijver *et al*. 2014), *Stauroneis* (Zidarova *et al*. 2014) and *Luticola* (Kopalová *et al*. 2011).

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