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Article

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Analysis of the type material of *Navicula brachysira* Brébisson with the description of *Brachysira sandrae*, a new raphid diatom (Bacillariophyceae) from Iles Kerguelen (TAAF, sub-Antarctica, southern Indian Ocean)

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Abstract

During a study of the freshwater diatom flora of some hot springs on the sub-Antarctic Kerguelen Islands, an unknown *Brachysira* species was observed. Detailed morphological analysis using both light and scanning electron microscopy observations revealed sufficient morphological differences to separate this species as *Brachysira sandrae* sp. nov. The new species belongs to the complex of taxa around *B. brebissonii*. The type material of *Navicula brachysira*, most likely the type of *B. brebissonii*, was studied to reveal its morphological ultrastructure. The new species is compared with *B. brebissonii* and with similar *Brachysira* taxa worldwide.

Keywords: *Brachysira*, *Navicula brachysira*, sub-Antarctica, Iles Kerguelen, morphology, new species

Introduction

Although described in 1836 by Kützing, it was not until 1981 that the genus *Brachysira* Kütz (1836: 153) was morphologically better characterized and separated from the, at that moment, more generally used genus *Anomoeoneis* Pfitzer (1871: 77). Despite this characterization, the genus remained quite species poor with only fifteen known taxa until 1994 when a new monograph was published by Lange-Bertalot & Moser (1994) containing 67 taxa, of which 34 were described as new to science. Fourtanier & Kociolek (2011) currently list 138 records of *Brachysira*, mostly from Lange-Bertalot & Moser (1994), Moser *et al.* (1998) and Metzeltin & Lange-Bertalot (2007). Morphologically, the genus is characterized by rather linear, lanceolate or rhombic valves with an ornamented valve face (spines, ridges, warts); uniseriate striae composed of transapically elongated areolae, internally closed by hymenes, two sets of ribs, one surrounding the valve margin and one bordering the raphe; and a narrow raphe sternum with straight raphe branches and simple proximal and distal raphe endings. The genus is globally widespread and often associated with acid, oligotrophic and oligosaprobic conditions (Round & Mann 1981, Lange-Bertalot & Moser 1994).

One of the globally most widespread taxa is *Brachysira brebissonii* R.Ross (in Hartley 1986: 607). The species was originally described in 1849 by Kützing as *Navicula aponina* var. *brachysira* Kütz. (1849: 69). Wolfe & Kling mention as one of the synonyms *Navicula brachysira* Bréb. (in Rabenhorst 1853: 66). In the collection of the Botanic Garden Meise, material from Falaise of *Navicula brachysira* Brébisson, sent by de Brébisson, was found. This material has never been investigated with scanning electron microscopy techniques.

Although the environmental conditions in the sub-Antarctic region (Van de Vijver & Beyens 1996, 1999, Van de Vijver *et al.* 2001, 2004, 2008) are comparable to the (sub-)Arctic region, the genus *Brachysira* is almost absent in this region. The only taxon that is present with 100% certainty is *Brachysira minor* (Krasske 1939: 377) Lange-Bert. (in Lange-Bert. & Moser 1994: 47), described from southern Chile (Krasske 1939) but reported from various islands and archipelagos in the southern Atlantic Ocean [sub-Antarctic and Maritime Antarctic localities such as South Georgia (Van de Vijver & Beyens 1996), South Shetland Islands (Zidarova 2008, Kopalová & Van de Vijver 2013) and James Ross Island (Kopalová *et al.* 2012, 2013, 2014)]. Kellogg & Kellogg (2002) list only three *Brachysira* taxa that are found in the (sub-)Antarctic region: *B. aponina* Kütz. (1836: n° 154) (2 records), *B. microcephala* (Grunow 1867: 19) Compère (1986: 26) (1 record) and *B. serians* (Bréb. In Kütz. 1844: 92) Round & D.G.Mann (1981: 227)

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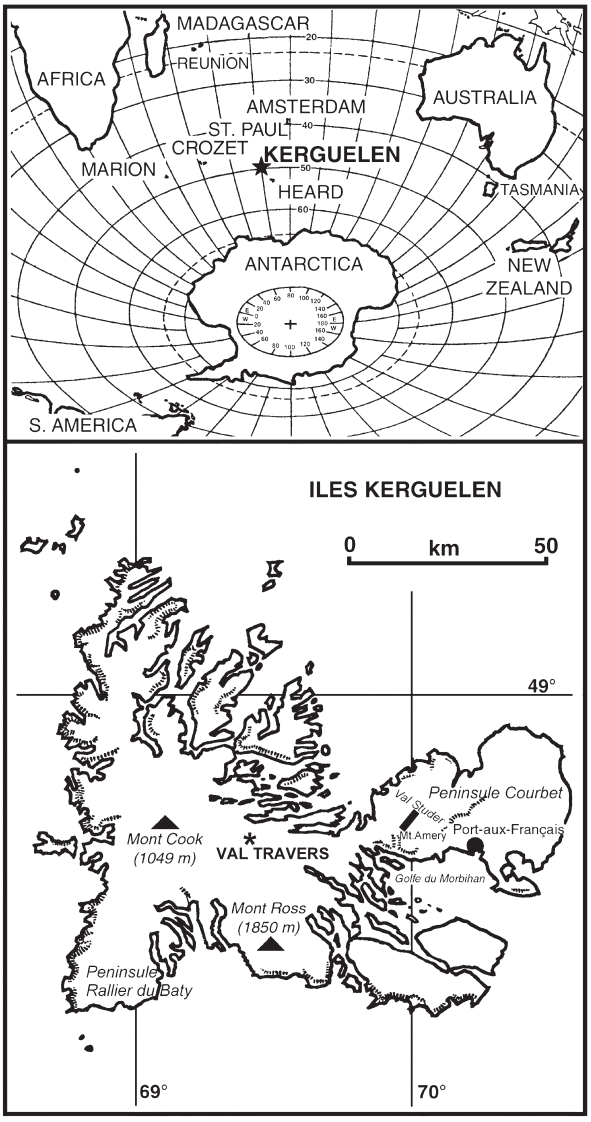


FIGURE 1. Geographical position of Iles Kerguelen in the southern hemisphere and the position of the study area on Grand Terre, the main island of Iles Kerguelen (indicated by *)

(1 record). None of the records could however be verified but considering the large amount of investigated samples during the ongoing taxonomic and ecological survey of the (sub-Antarctic) diatoms, their presence seems unlikely. In Lange-Bertalot & Moser (1994) a *Brachysira* population from Iles Kerguelen was mentioned as 'Morphotyp "aus der Antarktis"' within the *brebissonii*-complex. This *Brachysira* taxon was observed during a small ecological study of a hot spring area on Iles Kerguelen.

The present paper investigates the material of *Navicula brachysira*, present in the Botanic Garden Meise, and separates and describes a *Brachysira* population from Kerguelen as a new species, based on detailed light and scanning electron microscopy. Its morphology is compared with related *Brachysira* taxa.

Material & Methods

The French Iles Kerguelen (49ºS, 69ºE) is a large archipelago composed of one major island (Grand Terre), surrounded by more than 300 small outlying islands and islets covering a total land area of 7215 km². The island is mountainous, with the highest peak culminating at 1850 m a.s.l. The archipelago is very isolated, ca. 4000 km from the coasts of South Africa and Australia (Fig 1). The climate is oceanic and cold with a mean annual temperature at the Port-aux-

Français weather station of 4.6 ºC, with 7.7 °C (February) and 2.1 ºC (August) for the warmest and coldest month respectively. Wind speed is generally high. Annual rainfall varies from less than 800 mm in the eastern part to more than 3200 mm on the western coast (Frenot *et al.*, 1997).

The study area Val Travers is located in the center of the main island Grand Terre (69°23' S, 49°19' E). This valley contains one of the last remaining expressions of the ancient volcanic history of Iles Kerguelen. A total of 24 moss samples has been collected on a transect starting close to a hot spring along the river that runs through the Val Travers valley. The water temperature of the hot spring was 62 °C whereas the temperature in the mosses varied between 18 and 20 °C (measurements done on 31/01/2005). Almost all samples contained valves that belonged to the new *Brachysira* taxon. The largest population was observed in sample B13, thus selected for light and scanning electron microscopy.

Original material of *Navicula brachysira* from Falaise (leg. Alph. de Brébisson) was found in the collection of the Botanic Garden Meise and selected to study the type of this species.

The sample for LM observation was prepared 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 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 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), Round & Mann (1981), Round *et al.* (1990) and Lange-Bertalot & Moser (1994). The morphology of the new taxon has been compared with the ultrastructure of known species described worldwide (a.o. Lange-Bertalot & Moser 1994, Moser *et al.* 1998, Wolfe & Kling 2001, Werum & Lange-Bertalot 2004, Metzeltin & Lange-Bertalot 2007).

Observations

Division Bacillariophyta

Class Bacillariophyceae Haeckel emend. Medlin & Kaczmarska 2004

Subclass Bacillariophycidae D.G.Mann in Round *et al.* 1990

Order Naviculales Bessy 1907

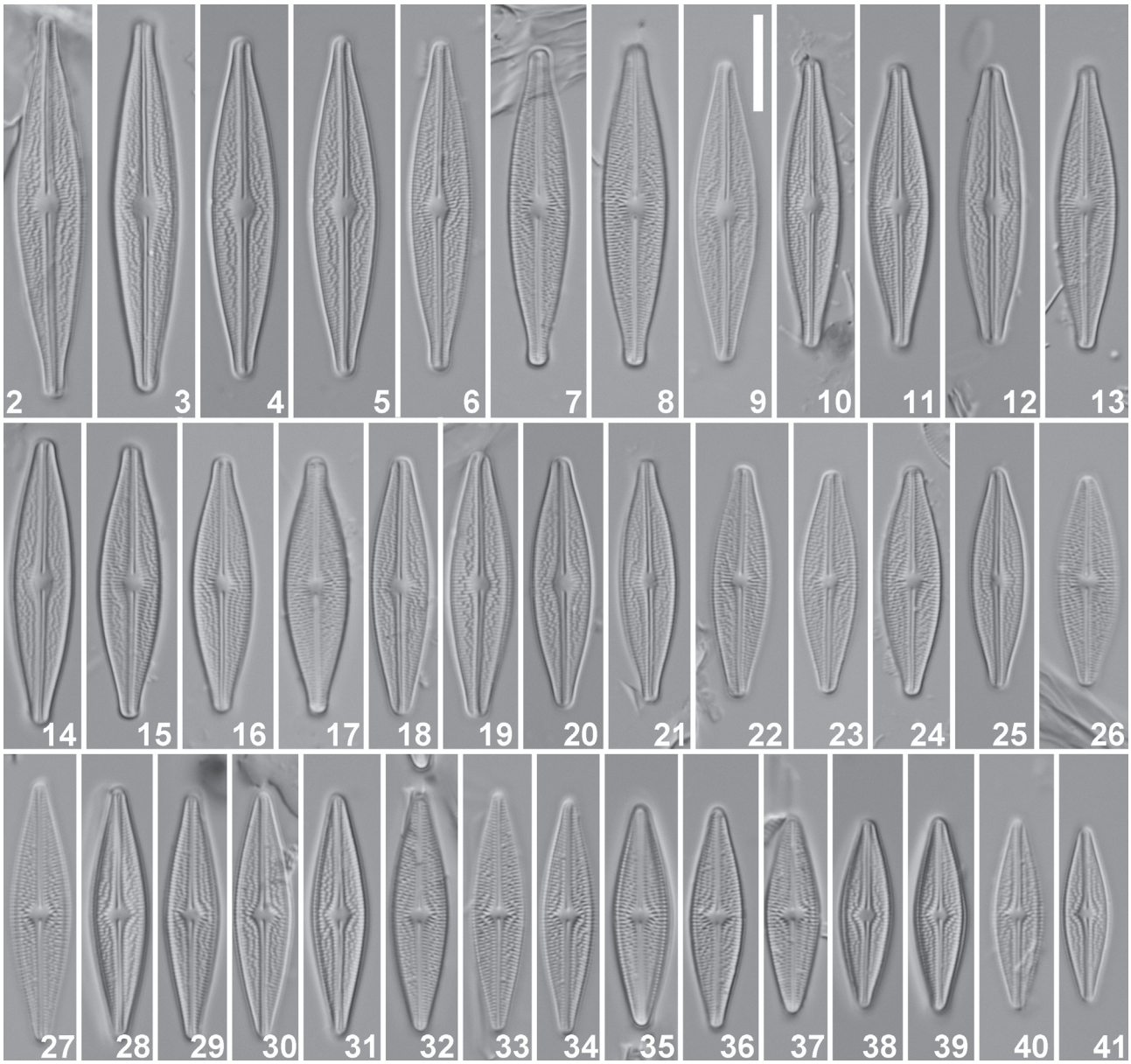
Family Brachysiraceae D.G.Mann in Round *et al.* 1990

Genus *Brachysira* Kütz. 1836

***Brachysira sandrae* sp. nov.** (Figs 2–26, 41–47)

Type:—KERGUELEN. Val Travers, sample B13, leg. M. Lebouvier, coll. date 31/01/2005 (holotype, slide no. BR–4390; isotype, slide PLP–275, University of Antwerp, Belgium).

LM (Figs 2–26):—Cells solitary. Valves narrowly lanceolate to rhombic lanceolate with convex margins and clearly protracted, rostrate, rounded apices. Smaller specimens with less protracted though still clearly rostrate apices. Valve dimensions (n=50): length 21–40 µm, width 5.4–7.1 µm. Axial area very narrow, linear. Central area small, symmetrical, rounded to elliptical due to shortened central striae. Raphe straight, filiform with simple, straight proximal endings. Distal raphe endings not visible in LM. Striae lineate, uniseriate, radiate throughout the entire valve, 29–31 in 10 µm, composed of 2–4 in LM clearly discernible areolae.

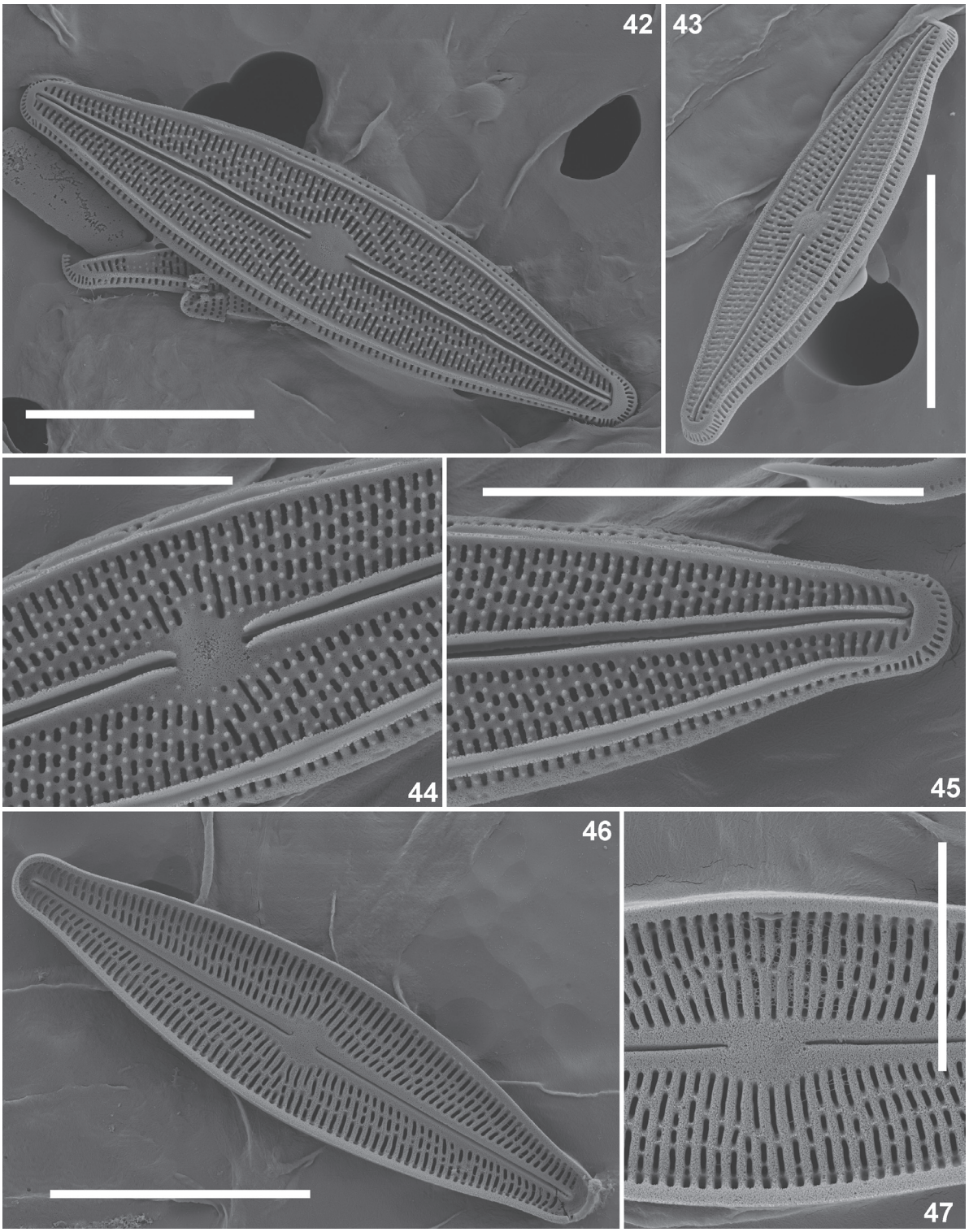


FIGURES 2–41. LM. *Brachysira sandrae* (2–26) and *Navicula brachysira* (27–41). Figs 2–26. *Brachysira sandrae*, pictures taken from the holotype population (sample B13) from the Val Travers area. Figs 27–41. *Navicula brachysira*, pictures taken from the type population found in material collected by A. de Brébisson in Falaise and deposited in the Botanic Garden Meise. Scale bar represents 10 µm.

SEM (Figs 42–47):–Valves entirely surrounded by an elevated ridge, clearly thickened near the valve apices (Figs 42, 43). Striae uniseriate composed of a series of 2–4 (near the apices usually 1) transapically elongated areolae. Areolae per stria showing an irregular length, the outer row usually being the longest, producing apically running hyaline undulations on the valve surface. Areolae foramina with small indentations (Figs 44, 45). Small papillae present on the interstriae, usually 5–7 per interstriae, but number rapidly declining towards the apices (Fig. 42). Mantle striae composed of one elongated bacilliform areola (Fig. 43). Near the apices, mantle striae closer together. Raphe branches bordered by an elevated ridge running from the central area to the apices, never fusing with the marginal ridge (Figs 44, 45). Raphe straight with inconspicuous, simple proximal endings (Fig. 44) and short, straight to weakly deflected distal endings (Fig. 45). T-shaped raphe endings only weakly and irregularly developed. Internally, central area symmetrical (Fig. 46) to asymmetrical (Fig. 47). Proximal raphe endings unilaterally bent (Fig. 46) to straight (Fig. 47). Distal raphe endings terminating on weakly raised helictoglossae. Due to erosion, internal areolar occlusions not observed.

Ecology and Distribution:–*Brachysira sandrae* was found in several samples collected in some brooks and rivers in the Val Travers area (Kerguelen). The samples were quite species-rich and dominated by *Achnanthidium sieminskae* Witkowski, Kulikovskiy & Riaux-Gobin (2012: 65), *A. modestiforme* (Lange-Bert. in Lange-Bertalot &

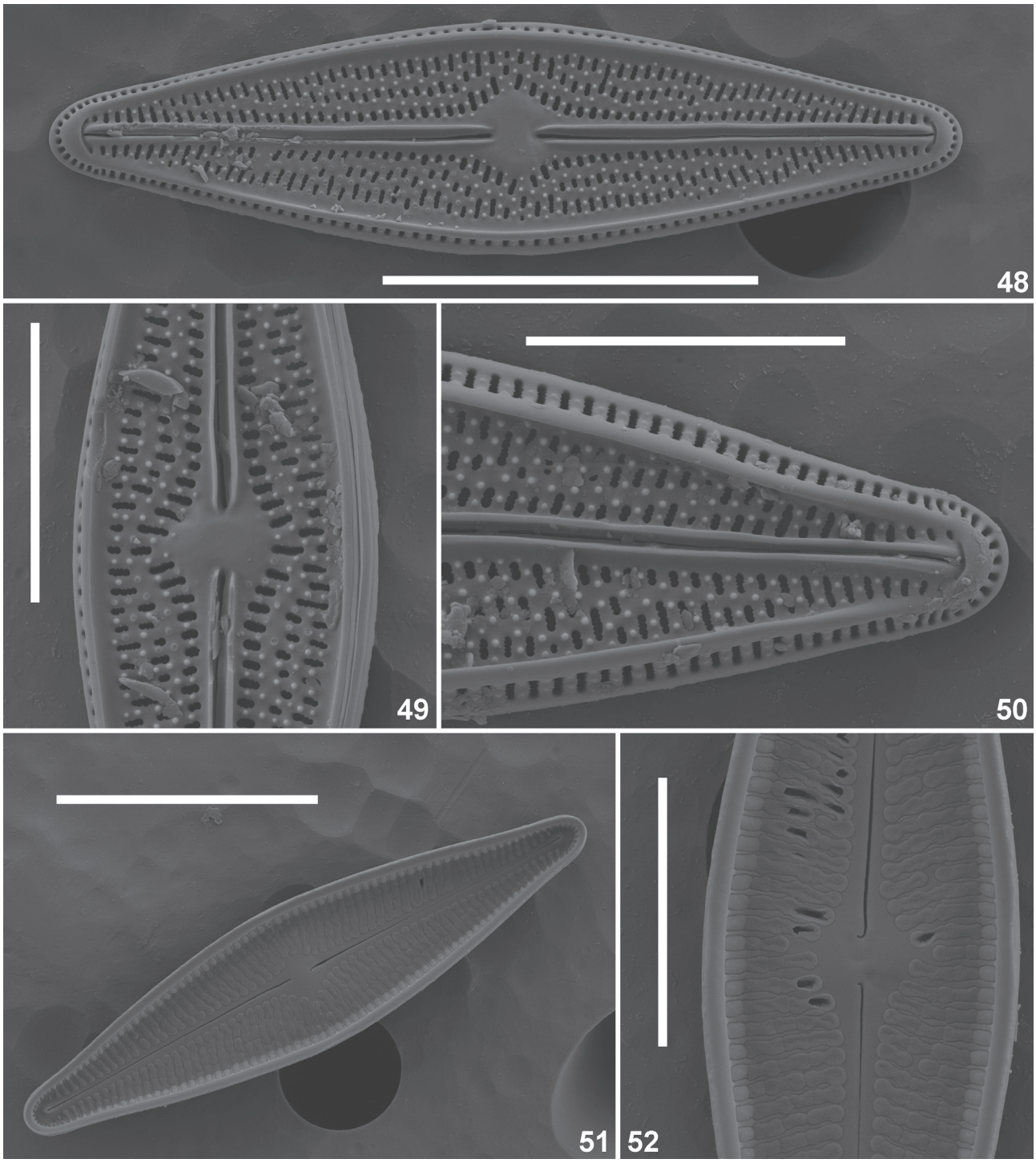
Krammer 1989: 107) Van de Vijver (in Van de Vijver *et al.* 2002: 17) and several *Psammothidium* taxa. The largest population was observed in sample B13, taken from the main hotspring at 70 m altitude with a water temperature of 62.4°C, a pH of 8 and a temperature of 18–20°C in the mosses floating on the surface.



FIGURES 42–47. *Brachysira sandrae*. All pictures taken from the holotype population (sample B13). Fig. 42. External view of an entire valve showing the most typical features such as the marginal ridge, the striae composed of 2–4 areolae, the simple raphe endings and the mantle striae. Fig. 43. External view of an entire valve in oblique position showing clearly the marginal ridge and the ridge bordering the raphe. Fig. 44. Detail view of the central area. Not the presence of the papillae, the irregular length of the areolae and the simple proximal raphe endings. Fig. 45. External detail of a valve apex with the typical axial ridge terminating before the marginal ridge, the simple, almost straight distal raphe endings and the diminishing number of areolae per stria. Fig. 46. Internal view of an entire valve. Fig. 47. Internal detail of the central area with the typical weakly bent proximal raphe endings. Scale bar represents 10 µm, except for Figs 44 & 47 where scale bar = 5 µm.

Etymology:—The species is named my dear friend, Mrs Sandra Scheerer (Jena, Germany) in recognition of her efforts in nature conservation and more specifically the protection of native European orchids.

Navicula brachysira **Brébisson ex Rabenhorst 1853** (Figs 27–41, 48–52)
(name replaced by R. Ross in Hartley (1986: 607) by *Brachysira brebissonii* R.Ross)



FIGURES 48–52. *Navicula brachysira*. All pictures taken from the type population (Falaise). Fig. 48. External view of an entire valve showing the most typical features such as the marginal ridge, the striae always composed of (2)3 areolae, the simple raphe endings and the mantle striae. Fig. 49. Detail view of the central area. Not the presence of the papillae, the regular length of the areolae and the simple proximal raphe endings. Fig. 50. External detail of a valve apex with the typical axial ridge terminating before the marginal ridge, the simple, almost straight distal raphe endings, the T-shaped raphe groove near the apex and the diminishing number of areolae per stria. Fig. 51. Internal view of an entire valve. Fig. 52. Internal detail of the central area with the typical weakly bent proximal raphe endings and the porous hymenate internal coverings of the striae. Scale bar represents 10 µm, except for Figs 49, 50 & 52 where scale bar = 5 µm.

LM (Figs 27–41):—Cells solitary. Valves rhombic-lanceolate with weakly rhombic to convex margins and almost non-protracted, acutely rounded apices. Valve dimensions (n=25): length 18.2–27.7 µm, width 4.9–5.8 µm. Axial area narrow, linear. Central area very small, rounded to rhombic. Raphe filiform, straight with simple proximal raphe endings. Distal raphe endings not discernible in LM. Striae lineate, uniseriate, radiate throughout the entire valve, 26–38 in 10 µm, composed of 3, near the apices 2, in LM clearly discernible areolae. Areolae separated by clear apically running, hyaline lines.

SEM (Figs 48–52):—Valves entirely surrounded by a narrow, elevated ridge, clearly thickened near the valve apices (Fig. 48). Striae uniseriate composed of a series of 3 (near the apices usually 1–2) transapically elongated areolae. Near the central area, striae usually composed of 2 longer areolae. Areolae per stria showing a rather similar length, separated by apically running hyaline undulations on the valve surface (Fig. 48). Areolae foramina with small indentations (Figs 49, 50). Small papillae present on the interstriae, usually 4–6 per interstria, but number rapidly declining towards the apices (Fig. 49). Mantle striae composed of one elongated bacilliform areola (Fig. 50). Near the apices, mantle striae closer together (Fig. 50). Raphe branches bordered by an elevated ridge running from the central area to the apices, never fusing with the marginal ridge (Figs 48–50). Raphe straight with inconspicuous, simple proximal endings (Fig. 49) and short, straight distal endings (Fig. 50). T-shaped raphe endings clearly developed. Internally, central area asymmetrical (Fig. 51) to symmetrical (Fig. 52). Proximal raphe endings unilaterally bent (Fig. 52). Distal raphe endings terminating on weakly raised helictoglossae (Fig. 51). Striae internally covered by continuous, though subdivided hymenes.

Discussion

Brachysira sandrae shares morphological features with *Brachysira brebissonii* and several species, comparable to *B. brebissonii* (hereafter referred to as the *brebissonii*-complex). Lange-Bertalot & Moser (1994) already reported a population from Iles Kerguelen as 'Morphotyp "aus der Antarktis"' and placed the valves in the *brebissonii*-complex. When comparing all the 'Morphotypes' illustrated in Lange-Bertalot & Moser (1994), it is clear that the entire *brebissonii*-complex is a typical example of the broader morphological concept suggested by Hustedt (1930). The application of a more narrow species concept (Mann 1999) nowadays and a more critical analysis of the morphological observations made clear that the broad morphological variability of *B. brebissonii* can no longer be maintained. Wolfe & Kling (2001) discussed the taxonomic history of *B. brebissonii* and at least one of the morphotypes in Lange-Bertalot & Moser (1994) has already been split off based on subtle but nevertheless distinct morphological differences and described as a separate taxon: *Brachysira arctoborealis* Wolfe & H.J.Kling (2001: 252).

Unfortunately, the type material of *Brachysira brebissonii* was never thoroughly investigated, most likely because of the confusion that is linked with defining what should be considered to be the type material. Kützing described the *Navicula aponina* β *brachysira* from Falaise material he received from de Brébisson and mentions in his description: "*Navicula brachysira et ordinata* Brébisson. However, the first (accepted) published record of *Navicula brachysira* dates only from four years later when Rabenhorst (1853) discussed the species and added "De Brébisson hat sie bei Falaise beobachtet". Unfortunately, no original description nor mentioning from de Brébisson regarding this species was retrieved. Nevertheless, it is highly likely that the material of *Navicula brachysira* from Falaise that was found in the collection of the Botanic Garden Meise, actually represents the type of *Brachysira brebissonii* and is probably even similar to the material that was used to Kützing in 1849 for his description.

Therefore, the structure of the Kerguelen population was compared with the type of *Navicula brachysira* to verify its relation to the latter (and hence to *Brachysira brebissonii*). This resulted in the separation of *B. sandrae* from *B. brebissonii*. Both taxa can been differentiated based on valve dimensions. Although both length and width overlap. *B. brebissonii* usually has slightly smaller, narrower valves (valve width 4.9–5.8 µm vs. 5.4–7.1 µm in *B. sandrae*). The main differences however are found in the valve outline and the structure of the striae. *Brachysira brebissonii* never shows protracted, rostrate nor capitate but always acutely rounded valve apices whereas all valves in the observed populations of *B. sandrae* (especially in the larger specimens) have clearly protracted, even elongated, rostrate apices (see for instance Figs 2–6) and even in smaller specimens, apices are always slightly protracted. The stria density in *B. sandrae* is always markedly higher than in *B. brebissonii* (29–31 vs. 24–27 in 10 µm). The striae themselves show a different structure with narrower apically running hyaline lines in *B. sandrae* than is the case in *B. brebissonii* (Lange-Bertalot & Moser (1994): "durch breitere wellige Apikallinien"). This is mainly due to the irregular number and size of the areolae in *B. sandrae* that usually has up to four areolae per stria whereas in *B. brebissonii* this is always strictly

limited to three, equally shaped areolae. The combination of these differences justify the separation of the Kerguelen populations from *B. brebissonii* and their description as a new species.

Brachysira neoexilis Lange-Bert. (in Lange-Bertalot & Moser 1994: 51) is usually smaller with narrower valves (valve width 3–5 µm) and clearly rostrate to even capitate apices giving the valves always a narrower outline. The species also shows a higher stria density (30–36 in 10 µm vs. 29–31 in *B. sandrae*). Conspecificity and confusion is hence to be excluded. *Brachysira lehmanniae* Lange-Bert. & Gert Moser (1994: 40) has a more rhombic outline with a larger valve width (8–9.5 µm) and never protracted, broadly rounded apices. *Brachysira manfredii* Lange-Bert. (in Lange-Bertalot & Moser 1994: 44) has more lanceolate valves with acutely rounded, never protracted apices and a lower stria density (24.5–26.5 in 10 µm) (Lange-Bertalot & Moser 1994). Finally, *B. guttiformis* Gert Moser, Lange-Bert. & Metzeltin (1998: 95) has a comparable, though slightly more rhombic-lanceolate valve outline but a lower stria density (22–24 in 10 µm) and has up to 5 areolae per stria (versus 2–4 in *B. sandrae*) (Moser *et al.* 1998).

Acknowledgements

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