Analysis of the type of *Achnanthes exigua* Grunow (Bacillariophyta) with the description of a new Antarctic diatom species

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Abstract: A study was made of the type material of *Achnanthidium exiguum* (Grunow) Czarnecki. This often encountered and apparently ubiquitous species was originally described as *Stauroneis exilis* by Kützing from the island of Trinidad and later re–named as *Achnanthes exigua* by Grunow. The type material for this taxon (Kützing sample 397), housed in the Van Heurck collection at the National Botanic Garden of Belgium, Meise was investigated and the ultrastructure of this taxon was documented using both LM and SEM observations. A new Antarctic taxon, formerly identified as *A. exigua*, was described as a new species, *Achnanthidium australexiguum* sp. nov. The position of both taxa within the genus *Achnanthidium* is briefly discussed.

Key words: *Achnanthidium australexiguum, A. exiguum*, Maritime Antarctic Region, morphology, type material

Introduction

Widely distributed diatom taxa such as *Planothidium lanceolatum* (Brébisson) Lange–Bertalot, *Stauroneis anceps* Ehrenberg or *Achnanthidium minutissimum* (Kützing) Czarnecki were often described by the early diatom taxonomists who developed a species concept based on light microscopy analysis, illustrating this concept by line drawings (e.g. Ehrenberg 1843; Van Heurck 1880–1885; Krasske 1939; Hustedt 1937; Cleve–Euler 1953). The result is that we usually have a drawing that is essentially the author's interpretation of a species concept that later became coupled in Europe and North–America with a modern taxonomic concept based on light and electron photomicrographs. However, since careful examination of the type or the original material of diatom taxa is usually lacking even when this material originated from another continent, this has often led to a too broad interpretation of the original species concepts and subsequently to force–fitting similar populations from other continents into these old names (Tyler 1996) essentially creating species complexes or what is referred to in German as 'Sippen'. Moreover, it becomes quite problematic when species closely related to the original taxon need to be described as new but no clear concept of the type is available. As these common taxa are often important in biomonitoring and (paleo–)ecological studies, it is very difficult to assign exact ecological preferences to them making them less useful in environmental studies. Although, in the last couple of years, more and more of these common taxa such as *Nitzschia inconspicua* Grunow, the *Stauroneis anceps*–complex, *Nitzschia palea* (Kützing) W. Smith, *Planothidium lanceolatum* and the *Achnanthidium minutissimum*–complex have been restudied based on a thorough and detailed examination of their type material using both LM and SEM observations (Reichardt 1995; Trobajo et al. 2009, 2013; Van de Vijver et al. 2013; Wetzel et al. 2013), many of these taxa still await better characterization and delimitation. One of these taxa is *Achnanthidium exiguum* (Grunow) Czarnecki, reported from almost all continents including the Antarctic Region (Schoeman & Archibald 1976; Lange–Bertalot & Krammer 1989, Kellogg & Kellogg 2002; Metzeltin et al. 2005; Alakananda et al. 2013).

Achnanthidium exiguum was originally described from Trinidad and Tobago by Kützing in 1844 as *Stauroneis exilis* Kützing and illustrated by a rather minute line diagram (Fig. 1). Kützing's original sample register kept at the National Botanic Garden of Belgium, states with reference to this species: "397 *Stauroneis exilis*, stagnant water near a watermill Tacarigua XVI No 42". The original Kützing material of *S. exilis* (present in the British Museum and the Grunow collection in Vienna) was studied and illustrated by Schoeman &

Archibald (1976) by means of light microscopy (LM, Figs 15–22b) and transmission electron microscopy (TEM, Figs 41–45) observations. The latter authors however were rather suspicious about the origin of the material that was used for TEM since the samples were labeled *Achnanthes exigua* (and not *S. exilis*). However, the sample register of the Kützing collection was annotated by Grunow (signed 'Gr') with the following "*Achnanthes exigua* Gr *Nitz palea* var. *debilis* (Kz)" indicating that the Kützing sample 397 indeed contained the type material for this species. Based on observations of the Kützing material, Grunow (in Cleve & Grunow 1880) renamed this taxon as *Achnanthes exigua* Grunow. As Kützing had already described in 1833 another species under the name *Achnanthes exilis*, it was impossible for Grunow to use the species epithet '*exilis*' for the transferred taxon.

During a survey of the freshwater diatom flora in the Maritime Antarctic Region, several populations of a taxon identified as *A. exiguum* were found. A literature search based on Kellogg & Kellogg (2002) resulted in a list of almost 20 Antarctic references for this species. Almost all these records originate from the South Shetland Islands (Maritime Antarctic Region), making it likely that they all represent the same species. Comparing the ultrastructure of some of the Antarctic populations with the type material, revealed several significant morphological differences making conspecificity highly unlikely.

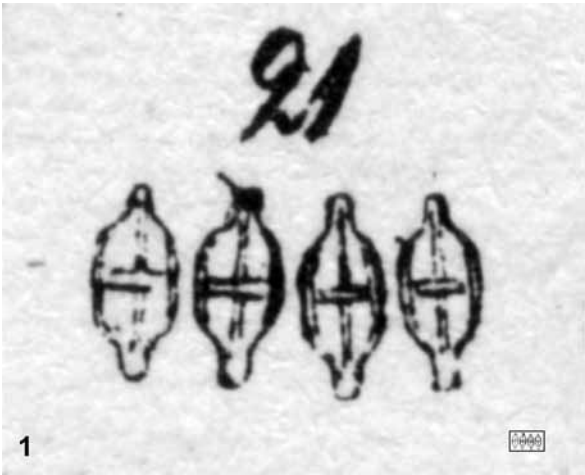


Fig. 1. *Stauroneis exilis* Kützing. Original line drawing from Kützing (1944, plate 30, fig. 21). Inset shows the approximate scale of the original illustration.

The present paper discusses the morphology of the type of *Achnanthidium exiguum* (*Stauroneis exilis*) using both LM and scanning electron microscopy (SEM) observations of "sample 397" from the Kützing collection housed at the National Botanic Garden of Belgium. Additionally, the *Achnanthidium* populations formerly identified as *A. exiguum* from the Maritime Antarctic Region are described as *A. australexiguum* Van de Vijver sp. nov.

Material and Methods

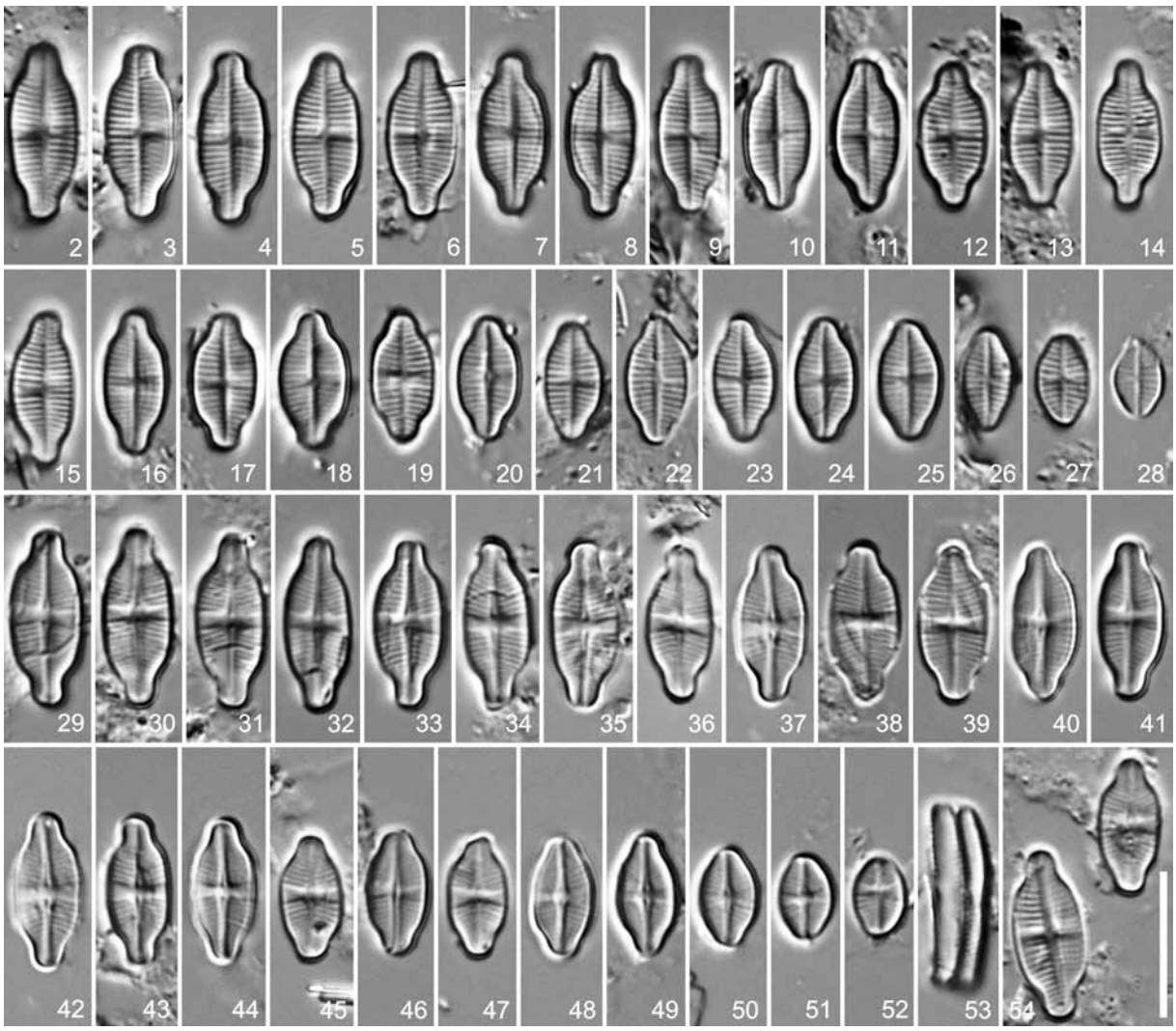
A duplicate of Kützing's sample 397 was found in the Kützing Collection deposited in the Van Heurck Collection, currently housed at the National Botanic Garden (Meise) of Belgium. A subsample of this material was prepared for LM and SEM analysis.

During the past 5 years, sediment samples from a large number of lakes were collected during several field campaigns on Livingston Island (South Shetland Islands) and James Ross Island. Table 1 lists all samples with populations formerly identified as *A. exiguum* that have been analysed during this study. Kopalová & Van de Vijver (2013) and Kopalová et al. (2013) discuss in detail the physic–chemical characteristics of the studied waterbodies.

Diatom samples for LM observation were prepared following the method described in Van der Werff (1955). Subsamples of the original material were oxidized using 37% H_2O_2 and heated to 80 °C for approximately 1 h. The reaction was further completed by the addition of $KMnO_4$. Following digestion and centrifugation (three times 10 minutes at 3700× g), the material free of organic matter was diluted with distilled water for sample mounting to avoid excessive concentrations of diatom valves and frustules on the slides. A subsample from the organic–free material was mounted in Naphrax® for diatom community studies. The slides were analyzed using an Olympus BX53 microscope, equipped with Differential Interference Contrast (Nomarski), and the Colorview I Soft Imaging System. For scanning electron

Table 1. List of samples used in this study.

Sample ID	Geographic locality	Source	Habitat	sample type
LIV–BY051	Livingston Island	Kopalová & Van de Vijver (2013)	Lake	algal mat
JRI2008–D3	James Ross Island	Kopalová et al. (2013)	Lake	epipelon
JRI2008–D10	James Ross Island	Kopalová et al. (2013)	Lake	epipelon
JRI2008–D11	James Ross Island	Kopalová et al. (2013)	Lake	epilithon
JRI2008–D29	James Ross Island	Kopalová et al. (2013)	Lake	epipelon
JRI2008–D37	James Ross Island	Kopalová et al. (2013)	Lake	epilithon



Figs 2–54. *Achnanthidium exiguum*. LM. Sample 397 *Stauroneis exilis*, stagnant water near a watermill Tacarigua XVI No 42. (2–28) rapheless valve views; (29–52) raphe valve views; (53) girdle view; (54) rapheless and raphe valve view. Scale bar 10 µm.

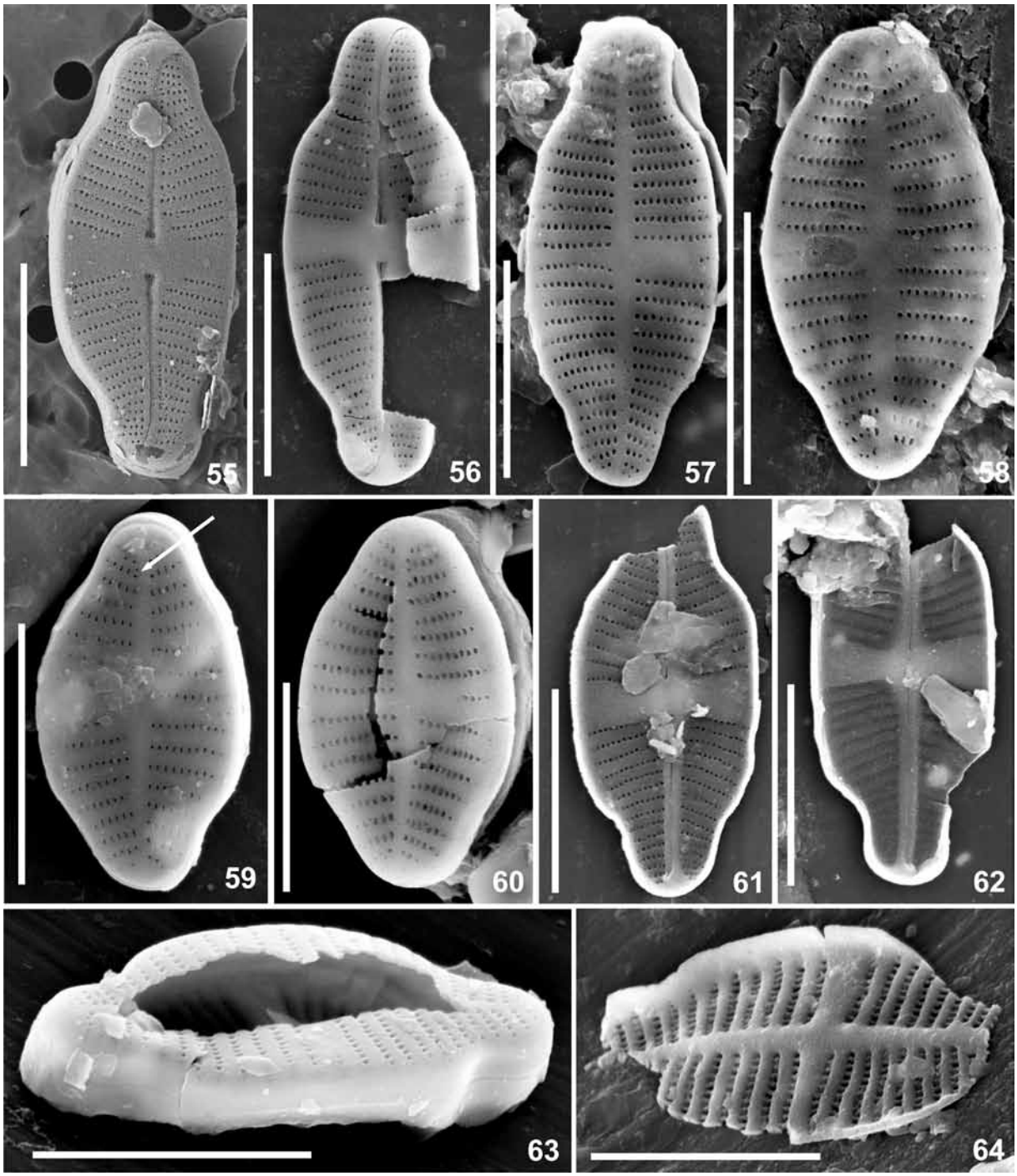
microscopy (SEM), aliquots of the oxidized suspensions were filtered through 1µm pore size polycarbonate filters that were cut into small pieces and fixed on aluminum stubs after air–drying. The stubs were sputter–coated with 50 nm of Au and examined at the Natural History Museum (London, UK) using a Zeiss Ultra plus SEM at 8 kV. Micrographs were digitally manipulated and plates containing light and scanning electron microscopy images were created using Adobe Photoshop 4.0®. Diatom terminology follows Ross et al. (1979), Round et al. (1990) and Bukhtiyarova & Round (1996).

Results

Achnanthidium exiguum (Grunow) Czarnecki 1994 (Figs 2–64)

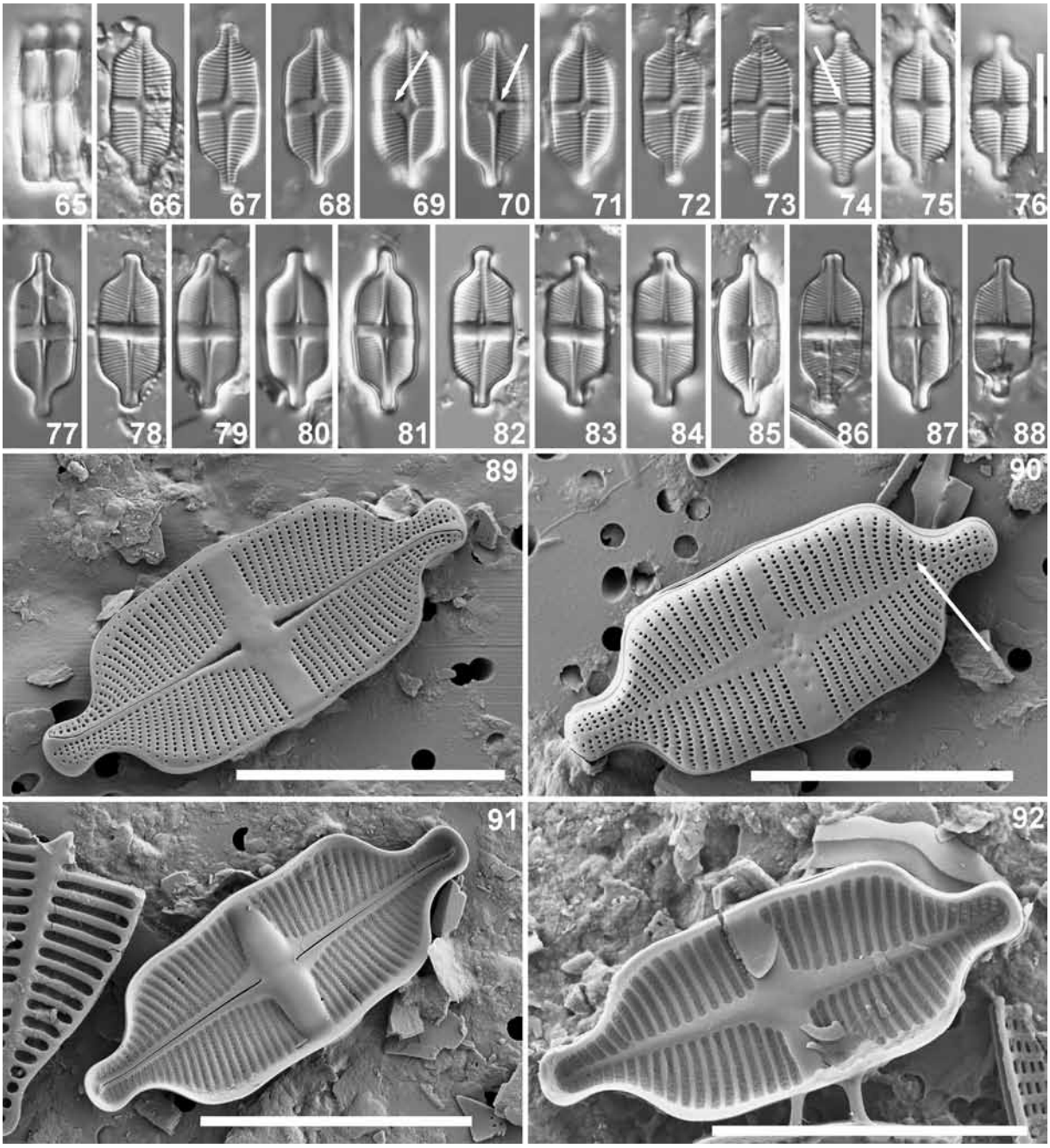
Basionym: *Achnanthes exigua* Grunow in Cleve & Grunow 1880, p. 21.; Replaced synonym: *Stauroneis exilis* Kützing 1844, p. 105, fig. 30: 21.

Light microscopy (Figs 2–54): Frustules in girdle view narrow, rectangular forming short chains, weakly curved (Fig. 53). Valves narrowly lanceolate with clearly convex margins and protracted, short rostrate, broadly rounded apices. Smaller specimens almost elliptical lacking protracted apices (Figs 28, 51, 52). Shoulders poorly developed. Valve dimensions (n=125): length 5.1–12.3 µm, width 3.3–5.1 µm. Axial area very narrow, linear, opening rather abruptly to the central area. Rapheless valve: central area asymmetrical, on one side forming a hyaline area up to the valve margin, on the other side formed by weakly more distant striae; striae almost parallel becoming more radiate towards the apices, 24–30 in 10 µm. Raphe valve: central area forming a more or less symmetrical narrow, rectangular to wedge–shaped fascia reaching the valve margins; no shortened striae present in the central area; striae radiate throughout, more strongly radiate near the apices, 27–31 in 10 µm. Raphe branches straight with straight, clearly expanded proximal raphe endings. Distal raphe fissures indistinct in LM. Areolae not discernible in LM.



Figs 55–64. *Achnanthidium exiguum*. SEM. Sample 397 *Stauroneis exilis*, stagnant water near a watermill Tacarigua XVI No 42. (55–56) raphe valve, entire view of the valve exterior; (57–60) Rapheless valve, entire view of the valve exterior. The arrow in Fig. 59 indicates the biseriate part of the striae. (61–62) raphe valve, entire view of the valve interior; (63) girdle view; broken rapheless valve situated on top; (64) rapheless valve, partial view of the valve interior. Note the raised virgae. Scale bar 5 µm (55 – 59, 61 – 63), 4 µm (60, 64).

Scanning electron microscopy (Figs 55–64): Striae of the raphe valve narrower than the virgae, uniseriate composed of very small, usually rounded areolae (Figs 55, 56). Proximal raphe endings straight, clearly expanded, forming a groove around the raphe (Fig. 56). Distal raphe fissures deflected to opposite sides (Fig. 55), terminating in droplike pores on the valve face (Fig. 56). Striae of the rapheless valve uniseriate, composed of apically elongated to rounded areolae (Figs 57–60, 63). Near the apices, striae very occasionally biseriate near the axial area (Fig. 59, see arrow). Internally, areolae covered by individual hymenes (Fig. 62). Central area of the raphe valve forming a thickened stauros (Figs 61, 62). Proximal raphe endings shortly deflected to opposite sides (Fig. 61). Distal raphe endings terminating on small helictoglossae (Fig. 62).



Figs 65–92. *Achnanthidium australexiguum*. LM and SEM. Sample D11, Monolith lake, Ulu Peninsula, James Ross Island. (65–88) LM, (65) girdle view, (66–76) rapheless valve views, (77–88) rapheless valve views; (89–92) SEM, (89) raphe valve, entire view of the valve exterior, (90) raphe valve, entire view of the valve interior, (91) rapheless valve, entire view of the valve exterior, (92) rapheless valve, entire view of the valve interior. Scale bar 10 µm.

Central area of the rapheless valve not or only slightly more enlarged compared to the virgae (Fig. 64).

Achnanthidium australexiguum **Van de Vijver sp. nov. (Figs 65–92)**

Description

Light microscopy (Figs 65–88): Frustules in girdle view narrow, rectangular forming short chains, weakly bent in the middle making the view somewhat slightly V–shaped (Fig. 65). Valves always linear with parallel, clearly undulating margins and protracted, distinctly rostrate apices. Shoulders always clearly developed. Valve dimensions (n=50): length 14.7–18.8 µm, width 6.3–7.5 µm. Populations on Livingston Island slightly larger than the type population (17.7 vs. 18.8 µm). Axial area very narrow, opening rather abruptly to the central area. Rapheless valve: central area forming a narrow, rectangular, asymmetrical fascia, lacking any shortened striae bordering the central area. Irregular markings sometimes present in the central area (Figs 69, 70, 74, see arrow); striae parallel becoming radiate

towards the apices, 23–24 in 10 µm. Raphe valve: central area forming a rather broad, rectangular, weakly asymmetrical fascia, lacking any shortened striae bordering the central area; striae radiate throughout, more strongly radiate near the apices, 28–30 in 10 µm. Raphe branches straight with straight, clearly expanded proximal raphe endings. Distal raphe fissures deflected, almost invisible in LM. Areolae not discernible in LM.

Scanning electron microscopy (Figs 89–92): Striae of the raphe valve narrower than the virgae, uniseriate composed of very small, rounded areolae (Fig. 89). Areolae becoming larger towards the valve margins. Proximal raphe endings clearly expanded, forming a groove around the raphe (Fig. 89). Distal raphe fissures deflected to opposite sides, terminating in droplike pores on the valve face (Fig. 89). Striae of the rapheless valve uniseriate, composed of apically elongated to rounded areolae (Fig. 90). Near the apices, striae occasionally biseriate (Fig. 90, see arrow). Shallow depressions sometimes present in the central area (Fig. 90). Internally, areolae covered by cribrate structures (Figs 91, 92). Central area on both valves forming a thickened stauros, more developed in the raphe valve than in the rapheless valve (Figs 91, 92). Proximal raphe endings shortly deflected to opposite sides (Fig. 91). Distal raphe endings straight and terminating on small helictoglossae (Fig. 91).

Holotype (designated here): BR–4347 (National Botanic Garden, Meise, Belgium)
Isotypes (designated here): PLP–241 (University of Antwerp, Belgium), BRM–ZU9/43 (Hustedt Collection, Bremerhaven, Germany)
Type locality: Monolith Lake, Ulu Peninsula, James Ross Island, sample JRI–D11 (Coll. L. NEDBALOVÁ and J. ELSTER) (01/02/2008).
Etymology: The specific epithet refers to the close resemblance to *Achnanthidium exiguum* and its geographic distribution in the southern hemisphere ('*australis*').

Ecology and Distribution: At present, confirmed records of this new taxon exist from James Ross Island (this study), Livingston Island (this study), King George Island (YANG & CHEN 1994, based on analysis of their illustrations) and Beak Island (STERKEN et al., unpubl. data). It is likely that the species is more widely distributed in the Maritime Antarctic Region as literature data reported *A. exiguum* from Horseshoe Island (WASELL & HÅKANSSON 1992) and the South Orkney Islands (HÅKANSSON & JONES 1994) but as illustrations are lacking in these publications, an unambiguous identification is not possible at the moment.
The largest population of *Achnanthidium australexiguum* was observed in the epipelon and epilithon of Monolith Lake on James Ross Island, a large lake with circumneutral pH (7.24), a low specific conductance (120 µS.cm^{-1}) and low values of total phosphorus (9.7 µg.l^{-1}) and sulphate (14 µg.l^{-1}). The diatom flora in this lake was dominated by *Nitzschia* cf. *perminuta* (GRUNOW) PERAGALLO, *Navicula cremeri* VAN DE VIJVER et ZIDAROVA, *Diadesmis australis* VAN DE VIJVER et SABBE and *Fragilaria capucina* s.1. DESMAZIÈRES. Other (but smaller) populations were found in weakly alkaline lakes (pH 7.6–7.7) with low specific conductance values (110–190 µS.cm^{-1}).

DISCUSSION

The type material of *Achnanthes exigua* shows some features that separate this taxon from the genus *Achnanthidium*. Based on the emended description given by ROUND & BUKHTIYAROVA (1996), the genus is characterized in having cells with a valves less than 30 µm long and 5 µm wide, concave raphe and convex rapheless valves and distal raphe fissures that are either straight or curved to one side. *Achnanthes exigua* has slightly wider valves (3.3–5.1 µm), although still within the range for the genus *Achnanthidium*. However, the distal raphe fissures are curved into opposite directions. CZARNECKI (1994) transferred *A. exigua* to *Achnanthidium* but did not justify this new combination. The presence of the distal raphe endings curved into opposite directions is unique within the genus *Achnanthidium*. POTAPOVA & PONADER (2004) discuss several *Achnanthidium* species with unilaterally curved distal raphe endings but do not refer to taxa with raphes curved in opposite directions. MOSER et al. (1995, 1998) reported on four *Achnanthes* taxa from New Caledonia with a short sigmoid raphe, later transferred by LANGE–BERTALOT (1999) to the genus *Achnanthidium*. Within the monoraphid diatom genera, two other genera present taxa with curved raphe endings. All species in the genus *Eucocconeis* Cleve have a typical sigmoid raphe with distal fissures curved to opposite directions (ROUND et al. 1990) and within the genus *Psammothidium*, several species are known with a similar sigmoid raphe such as *P. germainii* (MANGUIN) SABBE (SABBE et al. 2003). Both genera however present sufficient morphological differences with *A. exiguum* to justify a transfer to either of these two genera. Nevertheless, it is clear that in the present concept of *Achnanthidium*, the taxa with the sigmoid raphe represent a separate entity. At present however, a transfer of these *Achnanthidium* taxa to a new genus solely based on the structure of their raphe seems too premature and should ideally be supported by molecular or cladistic data. Therefore, the new Antarctic species, showing a similar sigmoid raphe is described in the genus *Achnanthidium*.

After a careful analysis of the type material, it is clear that the Maritime Antarctic populations of *Achnanthidium exiguum* are not conspecific with the type material, justifying the separation of the former populations

as a new taxon. *Achnanthidium exiguum* shows rather narrow, elliptic–lanceolate valves with short, broadly rounded rostrate apices, contrary to the larger, strictly linear valves with longer, rostrate apices. The shoulders seem less developed in *A. exiguum* contrary to *A. australexiguum*. Apart from additional clear differences in the morphometric data (with *A. australexiguum* being much larger than *A. exiguum*, 14.7–18.8 µm and 5.1–12.3 µm respectively), the biggest difference however is the presence of a clear, well–developed stauros in the rapheless valves of *A. australexiguum* whereas in *A. exiguum*, the valves only show an asymmetrical widening of the central area on one side of the valve whereas on the other side, the striae are only very faintly more spaced. In several cases, one complete stria is present in the central area on one side. In all cases, shallow pit–like depressions, as often seen in the central area of *A. australexiguum* were lacking in *A. exiguum*. The proximal raphe endings are more expanded and larger in *A. australexiguum* although both taxa seem to possess a certain groove around the proximal raphe ending. The axial area widens more clearly into the central area in *A. australexiguum* contrary to *A. exiguum* where no widening of the axial area was observed. Based on these differences, a separation of both taxa can be justified.

Our observations of the type of *A. exiguum* will hopefully also lead to a better understanding of the true identity of *A. exiguum*. Inevitably, using a more refined species concept, it will lead to a multiplicity of new taxa that need to be described, taxa that were formerly all included within *Achnanthes exigua* s.l. The taxon shown in Schoeman & Archibald (1976) for instance has a rapheless valve bearing a complete, though asymmetrical stauros and probably represents a separate taxon (see Schoeman & Archibald 1976, figs 2–10). In the past, *A. exiguum* was considered as being a highly variable taxon resulting in the description of a large number of infraspecific taxa (varieties and forms) (Fourtanier & Kociolek 2011). Several of these forms and varieties of *A. exiguum* show some similarities with the new taxon but present sufficient differences to separate them from *A. australexiguum*. The most similar one to *A. australexiguum* is *Achnanthes exigua* var. *angustirostrata* (Krasske) Lange–Bertalot, described from southern Chile in 1939. The latter shows the presence of a similar complete fascia in both rapheless and raphe valve although in the original line drawing of the rapheless valve that accompanied the description (Krasske 1939, fig. 11: 6) this was not visible. However, Lange–Bertalot et al. (1996) clearly show the presence of the fascia on the rapheless valve. Differences between both taxa include a higher stria density (ca 33 vs. 28–30 in 10 µm) on the raphe valve and more acutely shaped, rostrate apices compared to the broad rostrate apices in *A. australexiguum*.

Torka (1909) described *A. exigua* var. *constricta* (Torka) Hustedt [originally described from Poznan (Posen) as *Cocconeis*, in 1930 transferred by Hustedt to the genus *Achnanthes*] showing a similar complete fascia but with a clear constriction in the middle giving the taxon a well–developed undulating valve outline which is lacking in all investigated populations of *A. australexiguum*. Moreover, the apices are much broader in the former variety than in the new taxon.

In 1934, Cleve–Euler described *Achnanthes rostellata* Cleve–Euler but this taxon can be separated by its rapheless valve lacking a fascia in its central area and by a very narrow fascia on the raphe valve, contrary to *A. australexiguum* where a clear fascia is found on both valves.

So far, *A. australexiguum* has only been found in the Maritime Antarctic Region. Careful analysis of samples from both the sub–Antarctic Region and the Antarctic Continent did not reveal any presence of this species in these regions (Van de Vijver, pers. obs.). Literature data from the sub–Antarctic Region reporting on the monoraphid diatom flora never mentioned the presence of *Achnanthidium exiguum*, *Achnanthes exigua*, *Stauroneis exilis* or later synonyms (Le Cohu & Maillard 1983; Le Cohu 2005; Van de Vijver et al. 2002). This restricted distribution confirms once more the unique character of the diatom flora of the Maritime Antarctic Region that has been demonstrated in several other genera such as *Muelleria* (Van de Vijver et al. 2010), *Hantzschia* (Zidarova et al. 2010), *Luticola* (Kopalová et al. 2011) and *Navicula* (Van de Vijver et al. 2011).

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