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Investigation of the type material of *Microneis gracillima*, *Navicula pyrenaica, Achnanthes amphicephala*, *Achnanthes thienemannii* and *Achnanthidium rostropyrenaicum* (Achnanthidiaceae, Bacillariophyta) and additional populations of the species

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Abstract: The historic type material of *Microneis gracillima*, *Navicula pyrenaica*, *Achnanthes amphicephala*, *Achnanthes thienemannii* and *Achnanthidium rostropyrenaicum* was investigated together with additional European populations of these species using light and scanning electron microscopy. The morphology of the species was compared to similar taxa. *Achnanthes amphicephala* is transferred to the genus *Achnanthidium*. The species are distinguished from each other in the shape of their poles and in valve outline. There are currently very few photographic records of the species, but our results suggest that these species are typical in base–rich, oligotrophic waters, and might be important indicator species for these habitats.

Key words: *Achnanthidium*, distribution, indicators, morphology, oligotrophy, type material

†In memory of our friend and colleague Luc Ector (1962–2022)

Introduction

The type material together with additional populations of four *Achnanthidium* species, most likely important indicator species in unpolluted, carbonate–rich habitats were studied. *Navicula pyrenaica* W.Smith was described by Smith (1857, p. 8, pl. 2, fig. 5, here reproduced in Fig. 1) from the Pyrenees, where it was found in freshwater at Gave de Lizez near Cauterets, 803 m a.s.l., and at Gave de la Reine, 822 m a.s.l., France (Smith 1857). Later, Meister (1912, p. 97, pl. 12, figs 21–22, here reproduced in Fig. 16) described *Microneis gracillima* F.Meister from the basin of a fountain ('Brunnentrog') in the Alptal, Switzerland, without providing details about the locality (the slide from the Meister Collection marked as type is labelled '21.9.1907, Alptal, *Microneis gracillima* F.Meister'), and without reference to the taxon Smith (1857) described. Meister collected during many excursions from several hundred localities in all parts of Switzerland between 1901–1911 and we assume he collected the material of *M. gracillima* himself. Nothing to the contrary is said in Meister (1912, p. 5) where he listed friends and colleagues who donated their collections for his work. Lange–Bertalot & Krammer (1989) proposed the new combination *Achnanthes minutissima* var. *gracillima* (F.Meister) Lange–Bertalot, showing specimens from a spring–fed stream in the upper reach of the River Loisach in the European Alps (North Tyrol, Austria) (Lange–Bertalot & Krammer 1989, pl. 54, figs 21–32), from the Kützing Collection 192, BM 18205, (pl. 55, fig. 1), and from Soor in Belgium, Van Heurck Collection (pl. 55, figs 2, 3). *Achnanthes minutissima* var. *gracillima* was raised to species level in Lange–Bertalot (1993, p. 2) and given the new name *Achnanthes alteragracillima* Lange–Bertalot because of *Achnanthes gracillima* Hustedt (Hustedt 1927, p. 161, figs 10, 11; Foged 1952, p. 162, figs 9a, b), now transferred to *Nupela* Vyverman et Compère as *N. neogracillima* Kulikovskiy et Lange–Bertalot (Kulikovskiy et al. 2009). *Achnanthes alteragracillima* was included in *Achnanthidium* as *Achnanthidium altergracillima* (Lange–Bertalot) Round et Bukhtiyarova by Round & Bukhtiyarova (1996, p. 349), but the name is superfluous. The former epithet '*gracillima*' in *Microneis gracillima* is available in combination with *Achnanthidium* and has priority over '*altergracillima*' (Turland et al. 2018, Art. 11.4), and Krammer & Lange–Bertalot (2004, p. 430) introduced the new combination *Achnanthidium gracillimum* (F.Meister) Lange–Bertalot. *Microneis gracillima* F.Meister 1912 has also been transferred into *Achnanthidium gracillimum* (F.Meister) Mayama in (Kobayasi et al. 2006, p. 123, pl. 154, figs 1–14), two years after Krammer & Lange–Bertalot (2004), thus making it a superfluous combination.

Achnanthes amphicephala Hustedt was described from the river Gave d'Ossau (near Laruns, France), in the Pyrenees, and two line–drawings were shown of a raphe valve (RV) and a rapheless valve (RLV) (Hustedt 1939, p. 555, pl. 25, figs 3, 4). It was rare and found on stones. Images of the holotype were published in Simonsen (1987, pl. 371, figs 5–8).

Achnanthes thienemannii ('thienemanni') Hustedt was described from a spring ('sporadically', 'in water between stones short mosses [*Mniobryum albicans* var. *glaciale*]'), collected 28.8.1939 above the treeline at Mount Njulla, Abisko, Sweden (Hustedt 1942, p. 98, fig. 1: 2–5). The description of the new species was accompanied by four line–drawings showing two RVs and two RLVs. Light microscope images of the lectotype, slide MA2–92, and the isolectotype, slide MA2–93, were shown in Simonsen (1987, p. 302, plate 456, figs 12–17). Two LM images of a RV and a RLV, and two internal views in SEM, all from the type material, were shown in Potapova (2006, p. 406, figs 42–45). The new combination *Achnanthes biasolettiana* var. *thienemannii* (Hustedt) Lange–Bertalot (Lange–Bertalot & Krammer 1989, p. 29) was introduced because *A. thienemanni* differed from *Achnanthes biasolettiana* Grunow, a younger synonym of *Achnanthes pyrenaica* Hustedt [*Achnanthidium pyrenaicum* (Hustedt) H.Kobayasi] (Kobayasi 1997), mainly in valve outline (figs 60: 2, 12–18, ['10, 11, 30', these valves belong to a different taxon]). Krammer & Lange–Bertalot (2004, p. 431) raised the taxon to species level as *Achnanthidium thienemannii* (Hustedt) Lange–Bertalot.

Both *A. amphicephala* and *A. thienemannii* have rarely been encountered in Europe (Hustedt 1939, 1942; Krammer & Lange–Bertalot 2004; Rimet et al. 2003) and their ecology is insufficiently known (Potapova 2006).

Achnanthidium rostropyrenaicum Jüttner et E.J.Cox was described from a stream in the Manaslu region of the Nepalese Himalaya (Jüttner et al. 2011, p. 49, figs 2–11) and has since been found in Europe (Pérez–Burillo et al. 2020; Cantonati et al. 2022a).

In this study, using light (LM) and scanning electron microscopy (SEM), we reinvestigated the type material of *N. pyrenaica*, *M. gracillima*, *A. amphicephala*, *A. thienemannii* and *A. rostropyrenaicum,* and studied additional populations of *A. amphicephala*, *A. thienemannii* and *A. rostropyrenaicum* from the Republic of North Macedonia, Russia and Wales. We compared these taxa with similar species.

Material and Methods

Diatom samples were collected from streams, processed in the laboratories of Amgueddfa Cymru – Museum Wales (NMW), Institute of Biology (MKNDC), Lomonosov Moscow State University (MW), and Luxembourg Institute of Science and Technology (LIST). Diatom slides and suspensions are stored in the collections of NMW, MKNDC, MW–D and Botanic Garden Meise (BR). Diatom biofilms were removed from stone surfaces with toothbrushes and surface sand or sediment with a spoon, placed in plastic vials and fixed with alcohol or 4% formaldehyde. The collected samples were oxidised using H_2O_2 or with $KMnO_4$, and HCl, to remove organic material, cleaned by rinsing with distilled water and repeated centrifugation, and mounted on glass slides using Naphrax®. For LM, diatoms were investigated at 1000× magnification and photographed using a NIKON E600 microscope (DIC, 100× objective), with an IMAGINGSOURCE camera (DFK NME72AUC02) and the NIS–Elements D Software (NMW), at 1500× magnification using a Nikon Eclipse 80i microscope with a Nikon Coolpix P6000 camera (MKNDC), at 1000× magnification using a Leica DM750 microscope (bright field light, 100× objective) with a digital camera Leica ICC50 HD (MW), and at 1000× magnification using an Olympus BX53 microscope and the Olympus UC30 Imaging System (BR). For scanning electron microscopy (SEM) a small amount of suspension was air dried on cover glasses mounted on stubs (NMW, MW) or filtered through polycarbonate membrane filters with a pore diameter of 3 µm and mounted on stubs using double–sided carbon tape (LIST). They were then sputter–coated with a layer of platinum or with gold–palladium and studied with a Zeiss Gemini Ultra plus SEM microscope (working distance 3–12 mm, 3–5 kV, in BM), with a Hitachi SU–70 (working distance 10 mm, 5 kV, in LIST) or with a JEOL JSM–6380LA 20kV microscope (working distance 10 mm, in MW). The photo plates were made using CorelDraw v.12 (NMW), Adobe Photoshop® (MKNDC), and with GIMP 2.10.10 (MW). Morphological terminology follows Anonymous (1975), Ross et al. (1979) and Round et al. (1990).

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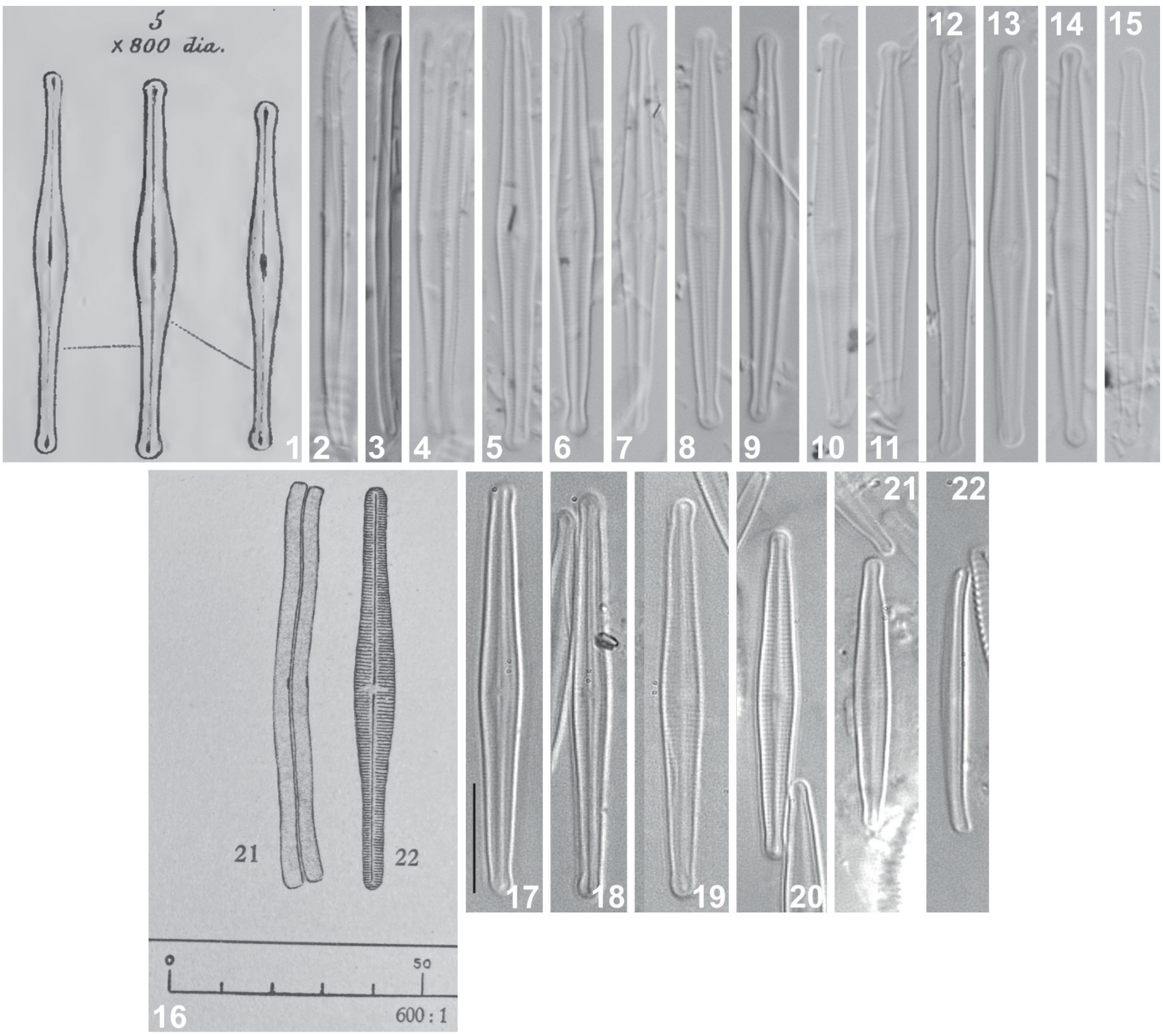
Results

Achnanthidium gracillimum **(F.Meister) Lange–Bertalot 2004 (Figs 1–38)**

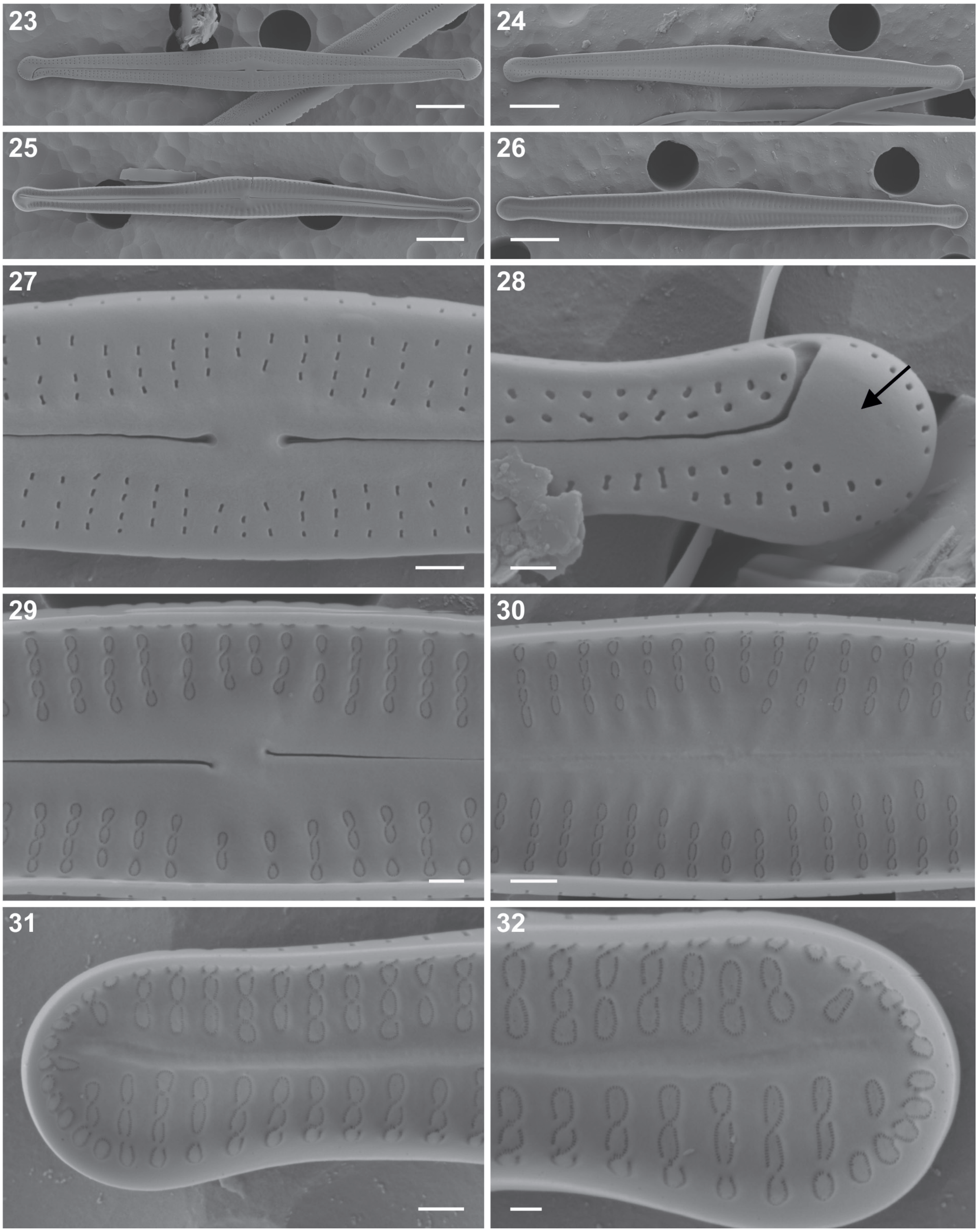
Basionym: *Microneis gracillima* Meister 1912 in Meister (1912, Die Kieselalgen der Schweiz: p. 97, pl. 12, figs 21–22).
≡ *Achnanthes minutissima* var. *gracillima* (F.Meister) Lange–Bertalot 1989
≡ *Achnanthes alteragracillima* Lange–Bertalot 1993
≡ *Achnanthidium altergracillimum* (Lange–Bertalot) Round et Bukhtiyarova 1996, nom. illeg. (Index Nominum Algarum 2022, an illegitimate name because *gracillimum* was available under *Achnanthidium*)
≡ *Achnanthidium gracillimum* (F.Meister) Mayama in Kobayasi et al. (2006)
Heterotypic synonym: *Navicula pyrenaica* Smith 1857 in Smith (1857, The Annals and Magazine of Natural History 19, p. 8, pl. 2, fig. 5).

The valve morphology of the type specimens for *Navicula pyrenaica* (Smith 1857) (Figs 1–15) is identical to those of the type for *Microneis gracillima* F. Meister (Meister 1912), (Figs 16–22). *Navicula pyrenaica* (Smith 1857) is the earliest name for the taxon currently known as *Achnanthidium gracillimum* (F. Meister) Lange–Bertalot. The combination *Achnanthidium pyrenaicum* (Hustedt) H.Kobayasi (Kobayasi 1997, p. 148) is in use for an unrelated species, so the earliest available alternative name at species rank is *Microneis gracillima* Meister 1912 and '*gracillimum'* is the correct epithet in *Achnanthidium gracillimum* (F.Meister) Lange–Bertalot (Turland et al. 2018, Art. 11.4) (Kusber pers. comm.).

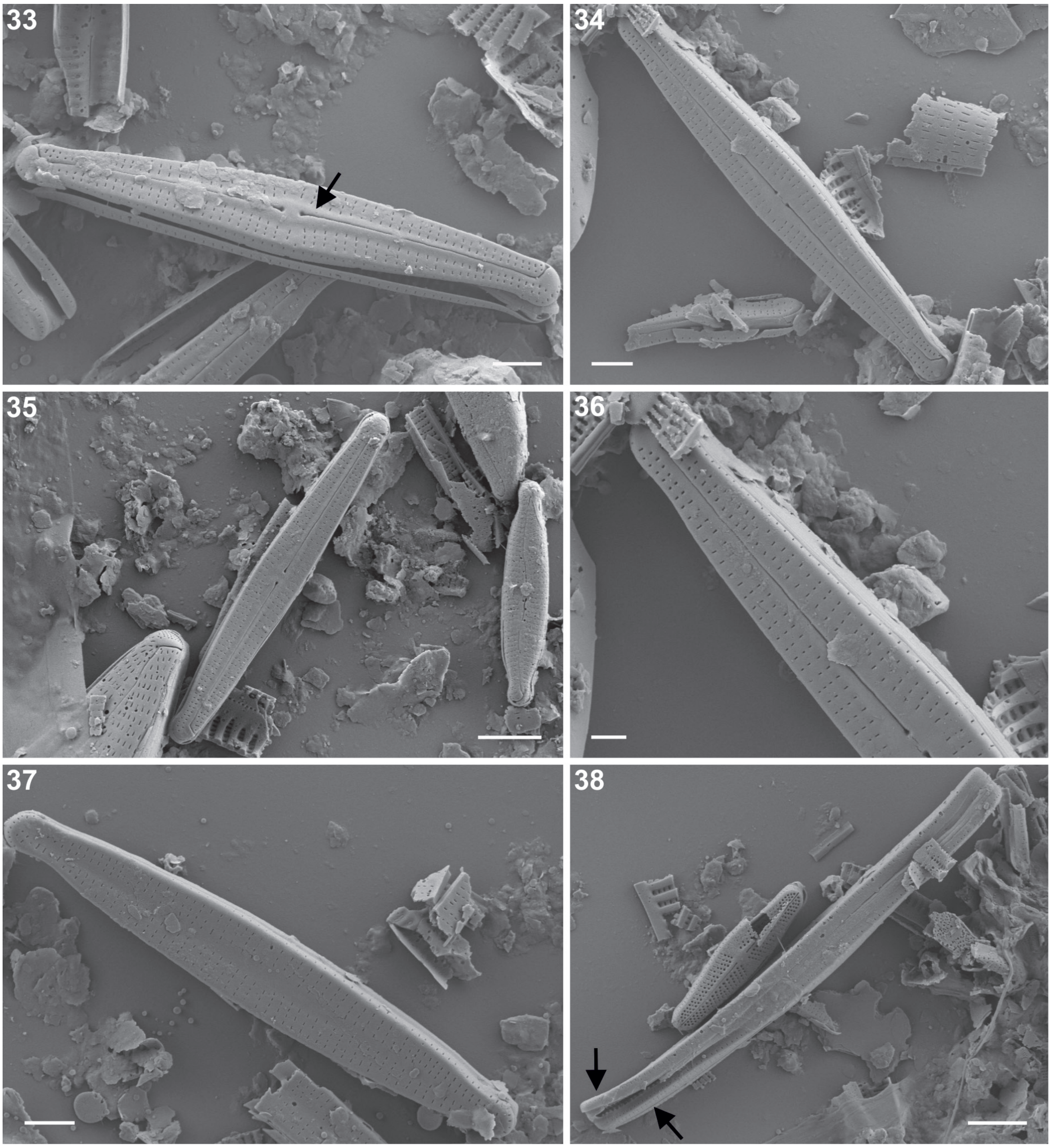
LM (Figs 1–22): Frustules in girdle view slightly arched, with weakly convex raphe valve and concave rapheless valve (Figs 2–4, 22). Frustules slightly more curved towards the poles than in the central part. Valves linear–lanceolate with margins gradually tapering from valve centre to subcapitate or capitate poles, the latter less wide than the maximum valve width at the valve centre (Figs 5–15, 17–21). Valves occasionally slightly inflated at the valve centre. Valve dimensions: *Navicula pyrenaica*, type slide (n=20): length 33–43 µm, width 3.5–4.0 µm; *Microneis gracillima*, type slide (n=15):



Figs 1–22. *Achnanthidium gracillimum*: (1) *Navicula pyrenaica* W. Smith. Drawings in Smith (1857), pl. II, fig. 5. (2–15). LM images taken of specimens on the type slide Smith from Gave de Lizez, VI–1–A12 (BR); (2–4) frustules in girdle view; (5–11) raphe valves; (12–15) rapheless valves; (16) Drawings of *Microneis gracillima* from Meister (1912, figs 21, 22); (17–22) LM images taken of specimens on the type slide 1010013 (Z+ZT), Horgen, Alptal, Switzerland; (17–21) raphe and rapheless valves; (22) frustule in girdle view. Scale bar 10 µm.



Figs 23–32. *Achnanthidium gracillimum*, SEM images taken of the type material of *Navicula pyrenaica* W. Smith: (23) external view of a raphe valve with a rhombic central area and terminal fissures deflected to the same side; (24) external view of a rapheless valve showing the axial area widening towards a rhombic central area; (25, 26) internal view of a raphe valve and a rapheless valve; (27) external view of the raphe valve centre showing the slightly shortened central striae; (28) external view of the pole of a raphe valve showing the large hyaline area adjacent to the deflected terminal fissure; (29) internal view of the valve centre of a raphe valve; (30) internal view of the valve centre of a rapheless valve; (31, 32) internal view of the poles of a rapheless valve. Scale bars 4 µm (23–26), 600 nm (27, 30), 400 nm (28, 29, 31), 200 nm (32).



Figs 33–38. *Achnanthidium gracillimum*, SEM images taken of the type material Nr. 203 (Z+ZT) of *Microneis gracillima* F.Meister: (33–35) external view of raphe valves showing the slightly raised sternum, very small, rhombic central areas and terminal fissures deflected to the same side; (36) external view of a raphe valve from the valve centre to the pole; (37) external view of a rapheless valve with a narrow, almost indistinct central area; (38) girdle view of a frustule showing the convex raphe valve and the concave rapheless valve. Scale bars 4 µm (35, 38), 2 µm (33, 34, 37), 1 µm (36).

length 25–38 µm, width 3.0–4.0 µm. Central areas indistinct on both valves, axial areas narrow, linear. Raphe straight, filiform, with hardly widened central pores. Striae parallel throughout both valves; *Navicula pyrenaica*, type slide (n=20): 23–26 in 10 µm (n=10) on the raphe valve (RV), 22–24 (n=10) on the rapheless valve (RLV); *Microneis gracillima* Meister, type slide (n=4): 22–26 in 10 µm (n=2) RV, 22–26 (n=2) RLV. Areolae not discernible in LM.

SEM (Figs 23–38): Axial area on both valves slightly widening towards a very small rhombic central area (Figs 23–27, 29, 30), bordered by slightly shortened central striae composed of 2–3 areolae (Figs 27, 29, 30). Other striae composed of three to four areolae, in the central part of the valve, and mostly two areolae per stria towards and at the poles. Areolae mostly transapically elongate, occasionally more rounded. Striae separated by a hyaline area at the valve face/mantle junction from one row of round to transapically elongated areolae on the mantle (Figs 27, 33, 36, 37, 38); a larger hyaline area present on one side of the pole adjacent to the terminal fissures (Fig. 28). Raphe located on a slightly raised sternum, terminating in small, tear–drop–shaped central pores. Terminal raphe fissures unilaterally deflected, terminating at the valve face/mantle junction (Figs 23, 27, 28, 33). Internally, central raphe endings shortly deflected to opposite sides, terminal raphe endings end in small helictoglossae (Figs 25, 29). Areolae internally occluded by porous hymenes (Figs 29–32).

Analysed material: *Navicula pyrenaica* W.Smith, Gave de Lizez, Pyrenées, hauteur 2634 feet, 1856, slide VI–1–A12 conserved in the Van Heurck collection (BR, Belgium); *Achnanthidium gracillimum*, F. Meister, Horgen, Alptal, Switzerland, 21 IX 1907, 1010013 (type slide) and 1010014 (Z+ZT).

Associated diatom flora: The Gave de Lizez slide is dominated by a large number of taxa including several *Cymbella* and *Delicatophycus* species, *Fragilaria perdelicatissima* Lange–Bertalot et Van de Vijver, *Brachysira neoexilis* Lange–Bertalot in Lange–Bertalot et Moser, *B. vitrea* (Grunow) R.Ross and *Odontidium informe* W.Smith, all indicating calciumcarbonate rich, oligotrophic conditions (Lange–Bertalot et al. 2017). Taxa associated with *Achnanthidium gracillimum* on slide 1010013 were *Cymbella* sp., *Encyonema* sp. and *Encyonopsis* sp.

Taxonomic remarks: Meister's drawing (Fig. 21) of *Microneis gracillima* implies that the frustules are curved towards the RV but our study shows that the RV is convex (Figs 2–4, 22, 33, 38). It is possible that the specimens shown by Smith (1857) and Meister (1912) do not represent the entire size range of the species. The specimens shown in Lange–Bertalot & Krammer (1989) encompass a wider size range, but analysis of the populations used for these illustrations will be necessary to confirm whether all illustrated specimens belong to *A. gracillimum*.

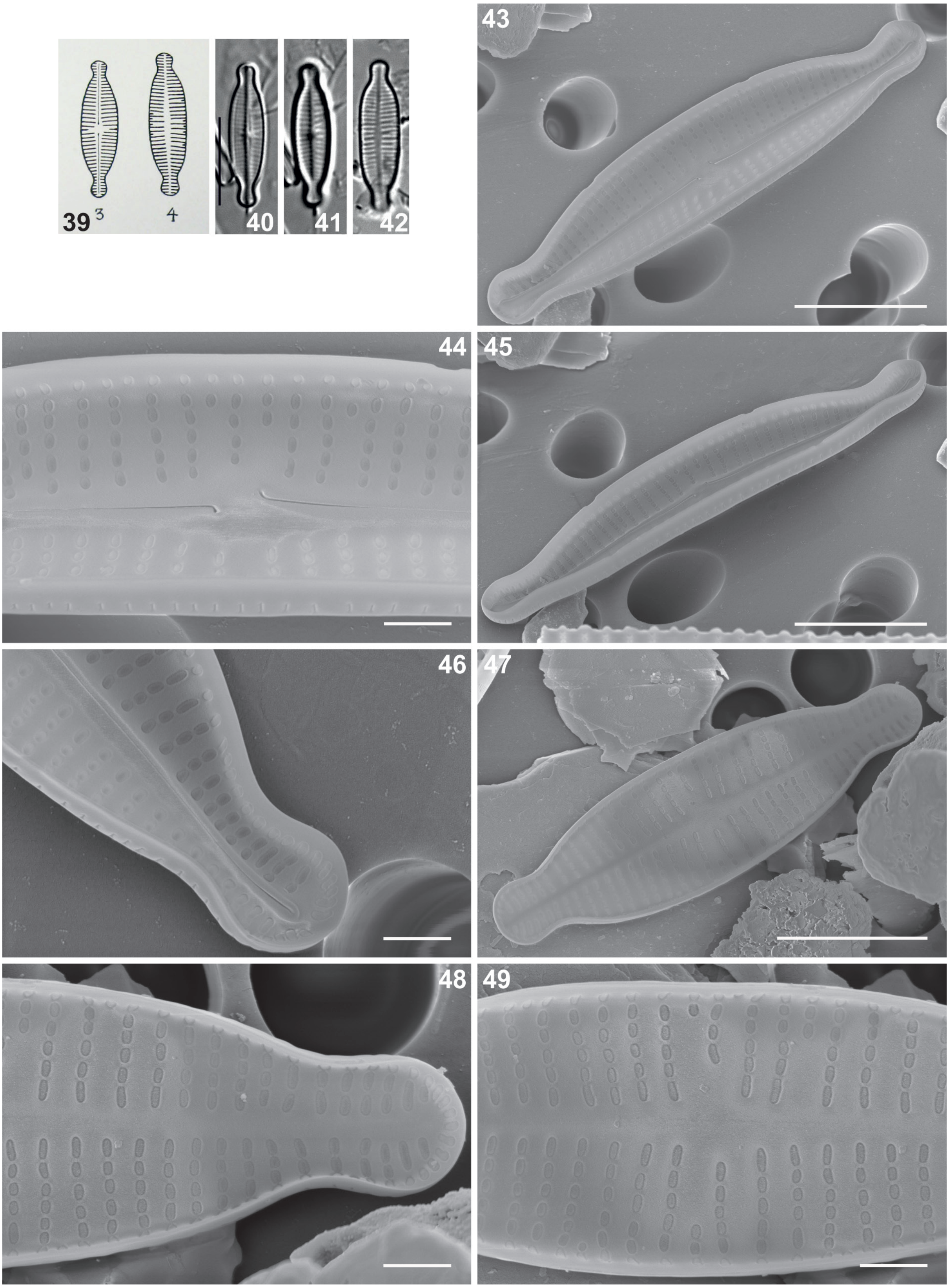
The morphology of the recently described *Achnanthidium longissimum* P.Yu, Q.–M.You et Kociolek (Yu et al. 2019a, p. 2, 4, figs 1–40), found in three high altitude lakes of the Jiuzhai Valley Nature Reserve, Sichuan Province, China, with a slightly alkaline pH (8.0–8.4) and intermediate conductivity (338–367 $\mu S.cm^{-1}$), does not show any significant differences with the type material of *A. gracillimum*, making conspecificity highly likely and *A. longissimum* a younger synonym of *A. gracillimum*. However, this should be confirmed by a reanalysis of the original *A. longissimum* material. *Achnanthidium jiuzhaienis* P.You, Q.–M.You et Q.–X. Wang (Yu et al. 2019b, p. 148, figs 2–129), also from the Jiuzhai Valley Nature Reserve, possesses similar deflected terminal raphe fissures as *A. gracillimum*, but has a linear–elliptical valve outline with small, distinctly capitate poles. *Achnanthidium gracillimum* has a similar valve outline and similar central and axial areas as *A. neomicrocephalum* Lange–Bertalot et Staab but the latter has straight terminal raphe fissures and a concave raphe valve with recurved poles. *Achnanthidium acerosum* Van de Vijver et Jarlman (Van de Vijver et al. 2011, p. 198, figs 18–25) has a more linear–elliptical valve outline with small rostrate to subcapitate poles, lower valve dimensions, and straight terminal raphe fissures. *Achnanthidium ertzii* Van de Vijver et Lange–Bertalot (Van de Vijver et al. 2011, p. 200, figs 26–47) has a linear–elliptical valve outline, short, straight terminal raphe fissures, and a concave raphe valve. Finally, *A. ennediense* (Compère) Compère et Van de Vijver has short protracted rostrate apices, straight terminal raphe fissures and a concave raphe valve (Compère & Van de Vijver 2011, figs 1–58).

Distribution: There are few published images of *A. gracillimum*, but the available records from the River Loisach (North Tyrol, Austria; Lange–Bertalot & Krammer 1989, pl. 54, figs 21–32) and from the Rhône–Alpes region suggest that it is a rare, pollution–sensitive species in nutrient–poor waters with approximately circumneutral pH and elevated Ca concentrations. It can be abundant in suitable environments as in the River Chassezac at Berrias–et–Casteljau (France; Bey & Ector 2013, p. 101, figs 1–44). The wider distribution of *A. gracillimum* remains unknown. Its correct identity was obscured because the type material of Smith and Meister was not properly studied and taxa identified as *A. gracillimum*, most likely belonged to *Achnanthidium pyrenaicum* or *A. thienemannii* and *Achnanthes amphicephala* (Lee et al. 1994, as *A. alteragracillima*; Ponader & Potapova (2007, as *A.* (cf.) *gracillimum*), pl. 3, figs 6–11, pl. 4, figs 1–10; Potapova 2010).

Achnanthidium amphicephalum (Hustedt) Jüttner, C.E.Wetzel et Levkov comb. nov. (Figs 39–77)

Basionym: *Achnanthes amphicephala* Hustedt 1939 in Hustedt (1939, Berichte der Deutschen Botanischen Gesellschaft: p. 555, pl. 25: 3, 4). Registration: http://phycobank.org/103184.

Based on the combination of morphological features observed in the material of *Achnanthes amphicephala* Hustedt, it is clear that the latter belongs to the genus *Achnanthidium*, and therefore the new combination *Achnanthidium amphicephalum* comb. nov. (Hustedt) Jüttner, C.E.Wetzel et Levkov is proposed. No valves were found in the holotype E809 material when examined in LM, but a few valves were examined in SEM. The holotype slide is damaged and now cannot be loaned. The LM images in Figs 40–42 are those shown on the website of the Alfred–Wegener–Institut, Germany, of the holotype (BRM 397–18, Gave d'Ossau, Pyrenees, leg. F. Hustedt). Specimens from the Republic of North Macedonia conform to the holotype specimens in size, stria density and valve features as described by Hustedt (1939): 'Schalen elliptisch mit schmalen geschnäbelten und mehr oder weniger kopfig abgeschnürten Enden… raphenlose Schale mit sehr schmal lanzettlicher Pseudoraphe, ohne Zentralarea, Transapikalstreifen … leicht radial … im mittleren Teil des Randes mit eingeschobenen sehr kurzen Streifen. Raphenschale, .. Axialarea sehr schmal, Zentralarea klein, infolge Verkürzung der Mittelstreifen



Figs 39–49. *Achnanthidium amphicephalum*: (39) *Achnanthes amphicephala* Hustedt. Drawings from Hustedt (1939, pl. XXV, figs 3–4); (40–42) LM images of one raphe valve and two rapheless valves, holotype, slide 397–18 (BRM), Copyright of images: Alfred–Wegener–Institut, Germany; (43–49) SEM images taken of the type material of Hustedt E809 (BRM); (43–46) internal view of raphe valves; (43) whole valve; (44) valve centre and mantle; (45) view of a tilted valve showing the mantle; (46) valve pole; (47–49) internal view of rapheless valves; (47) whole valve; (48) valve pole; (49) valve centre. Scale bars 10 µm (40), 5 µm (43, 45, 47), 1 µm (44, 46, 48, 49).

etwas quer verbreitet' [Valves elliptic with narrow beaked and more or less capitate ends… RLV with very narrow, lanceolate pseudoraphe, without central area, transapical striae… slightly radiate… in the central part of the margin with inserted very short striae. RV,…axial area very narrow, central area small, because of the shortening of the central striae somewhat transapically widened]. Hustedt (1939) mentions that the areolae on the RLV are slightly spotted ('leicht gepunktet'), something that could not be observed, but is apparent, also on the RV, in a population from France (Peeters & Ector 2018, p. 13, figs 1–36).

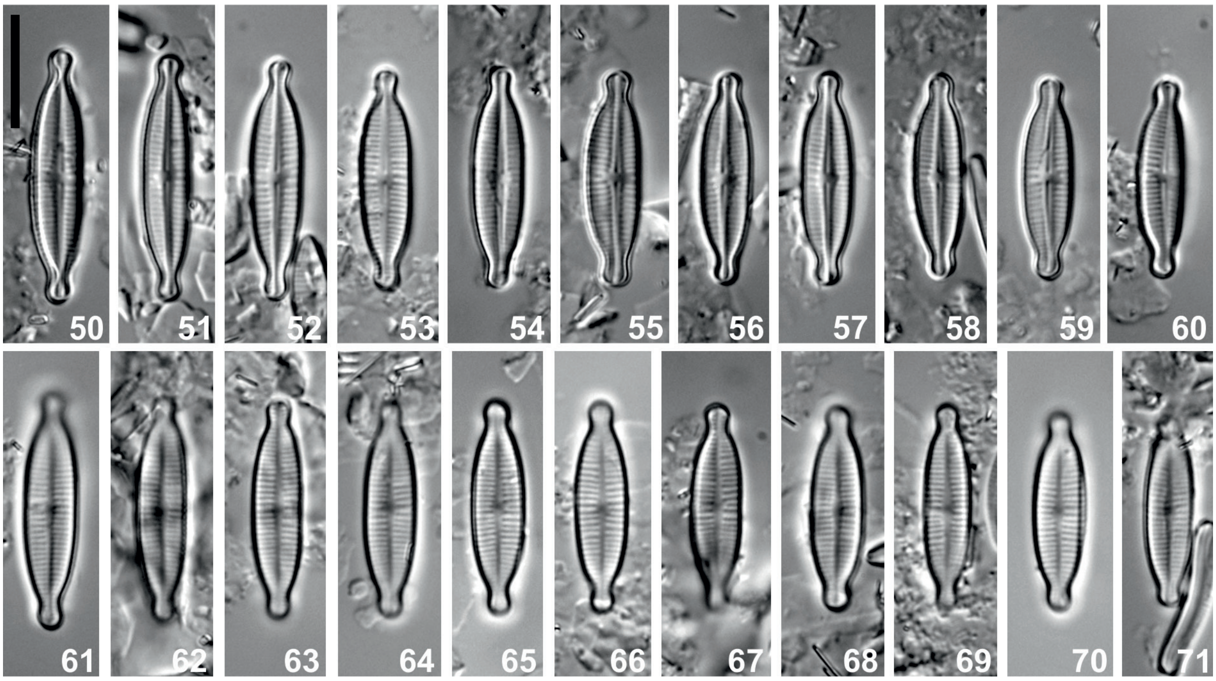
LM (Figs 39–42, 50–71): Valves linear–elliptic with almost straight or slightly convex margins at the valve centre, tapering more strongly and gradually close to the small, capitate poles. Valve dimensions: BRM 397–18 (Holotype) (n=3): length 16.5–17.0 µm, width 4.0–4.5 µm, Hustedt (1939): length 14.0–19.0 µm, width 4.5 µm; Acc. No. 12174 (MKNDC), Republic of North Macedonia, (n=56): length 16.5–22.5 µm, width 4.0–4.5 µm. On the RV, central area small, rounded or irregular, bordered by slightly shortened, occasionally more distantly spaced striae, or indistinct. Axial area narrow, linear. On the RLV, central area indistinct or slightly wider than the narrow linear axial area, central stria occasionally more widely spaced or slightly shortened. Raphe filiform, straight with slightly widened central pores. Striae parallel to slightly radiate on both valves; Hustedt type material E809 material (n=3): 21 in 10 µm on 1 RV and 2 RLV; Acc. No. 12174, Republic of North Macedonia, (n=56): 21–24 in 10 µm RV, 22–25 in 10 µm RLV. Areolae not discernible in the specimens from North Macedonia or in Figs (40–42) of the holotype.

SEM (Figs 43–49, 72–77): Raphe with slightly widened central raphe endings and short terminal raphe fissures, unilaterally deflected terminating on valve face. Small hyaline area present adjacent to the terminal raphe fissures at the pole (Figs 72–75). Internally, central raphe endings deflected to opposite sides, terminal raphe endings end in small helictoglossae (Figs 43–46). On both valves, striae uniseriate, usually composed of 4–6 transapically elongated, slit–like areolae. Near and at the poles, striae composed of 2–3 areolae; areolae 35–40 in 10 µm, on the RV ca. 40 in 10 µm, on the RLV ca. 35 in 10 µm. One row of transapically elongated areolae on the mantle separated from the areolae on the valve face by a hyaline area at the valve face/mantle junction (Figs 43–46, 74–77).

Analysed material: *Achnanthidium amphicephalum*, type material E809 (BRM), Pyrenees, Gave d'Ossau 2, near Laruns, 'on stone in still water', Simonsen (1987), p. 248, plate 371: 9–17; Acc. No. 12174 (MKNDC), North Macedonia, River Pena, Leshnica, Shara Mountain, Shar Planina, 42.0246111 ° N, 20.7865055 ° E, elevation 1462 m asl., leg. Dusica Zaova, 15.07.2018.

Associated diatom flora: Species associated with *A. amphicephalum* in the River Pena were *A. pyrenaicum*, *A. rostropyrenaicum*, and *Achnanthidium* spp., *Nitzschia* spp., and *Gomphonema* spp.

Taxonomic remarks: *Achnanthidium amphicephalum* is similar in shape to *A. latecephalum* H.Kobayasi (Kobayasi 1997) described from Japan, but also found elsewhere in Asia (Jüttner et al. 2011) and in Europe (Rimet et al. 2003). Both have linear–elliptic or elliptic valve outlines, but *A. latecephalum* has broader subcapitate to capitate poles.



Figs 50–71. *Achnanthidium amphicephalum*, LM images of specimens from the River Pena, Shara Mountain, North Macedonia, Acc. No. 12174 (MKNDC): (50–60) raphe valves, (61–71) rapheless valves. Scale bar 10 µm.

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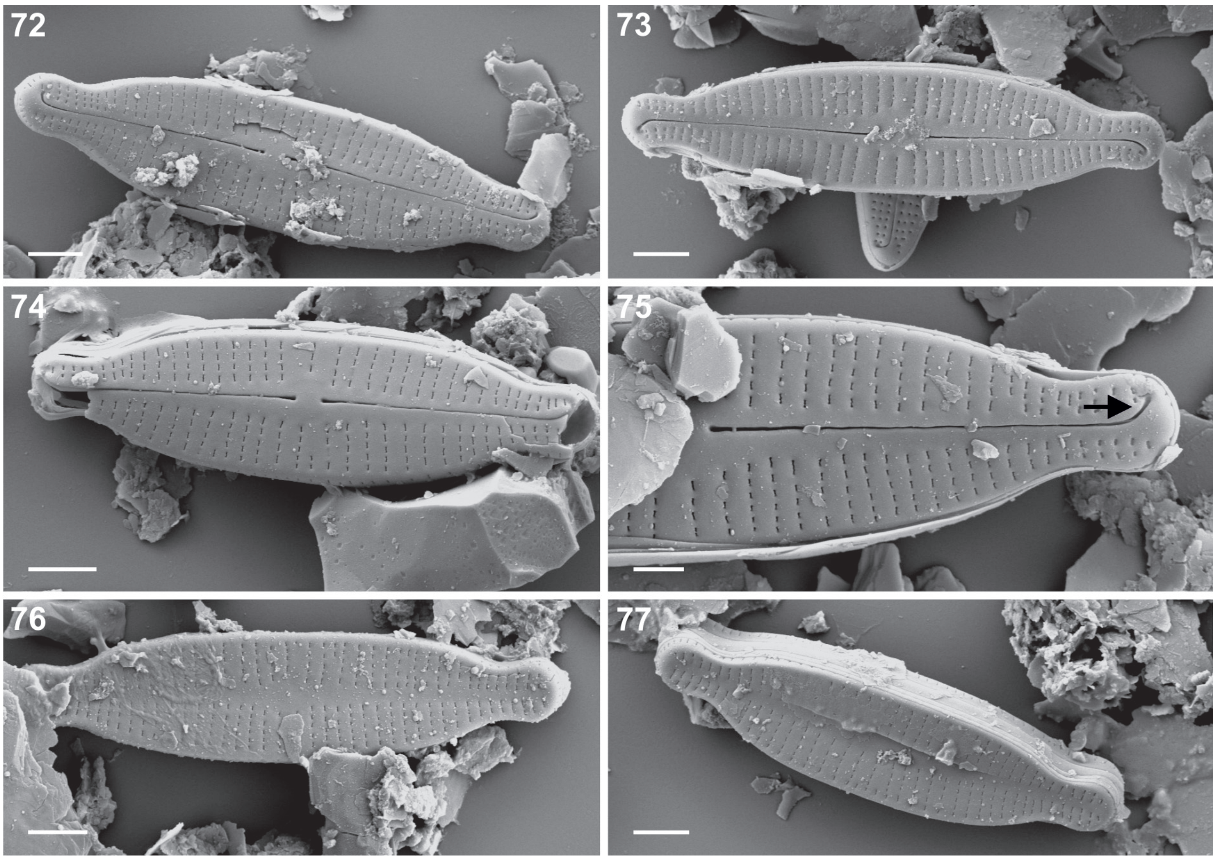
Distribution: The distribution of *A. amphicephalum* is poorly known. The specimens from North Macedonia occur in a fast flowing, cold, oligotrophic alpine river located in a karstic area. Two specimens in Ponader & Potapova (2007, pl. 4, figs 2, 4) resemble *A. amphicephalum*, although other specimens of this population from the Susquehanna River, Pennsylvania and the Holston River, Virginia, USA, have wider, subcapitate poles. Other reports, without photographic documentation, include records from Tibet (Ge et al. 2022), the Shanxi Province (Hu et al. 2012) and the Wei River basin (Liu et al. 2020) in China, and Serchina, Kurdistan, Iraq (Maulood & Hinton 1979). In the latter location it was most abundant in the autumn in the tychoplankton of spring pools on limestone at low oxygen concentration, pH 7.3, $CaCO_3$ up to 790 mg.l^{-1} (Maulood & Hinton 1979). Sládeček (1986) considered *A. amphicephalum* as a species typical for oligosaprobic waters.

***Achnanthidium thienemannii* (Hustedt) Lange–Bertalot in Krammer et Lange–Bertalot 2004 (Figs 78–126)**

Basionym: *Achnanthes thienemannii* (*'thienemanni'*) Hustedt 1942 in Hustedt (1942, Archiv für Hydrobiologie 39: p. 98, fig. 1: 2–5).
≡ *Achnanthes biasolettiana* var. *thienemannii* (Hustedt) Lange–Bertalot 1989.

No specimens of *A. thienemannii* were found on a new slide made using lectotype material E3846, but three valves from the type material were examined in SEM. The LM images in Figs 79–82 are those shown on the website of the Alfred–Wegener–Institut, Germany, of the lectotype (BRM MA2–92, Abisko 178, Quelle am Njulja 'spring at Njulja', d, 28.8.1939, leg. Thienemann, Moos). These valves were also illustrated in Simonsen (1987, pl. 456, figs 12, 13 [our Figs 79, 80], 14, 15 [our Figs 81, 82]), together with one additional valve of the isolectotype E3845, slide number MA2–93 (Simonsen 1987, pl. 456, figs 16, 17). Our specimens from Russia resemble Hustedt's in size and shape but there is more variability in the shape of the pole, and a slight constriction at the valve centre is often present. This reflects what is described in the protologue 'Schalen linear mit parallelen bis leicht konkaven Seiten und vorgezogenen, breit geschäbelten, zuweilen leicht kopfigen Enden' [Valves linear with parallel to slightly concave margins and protracted, broadly rostrate, sometimes slightly capitate poles]. The protologue also mentions the fascia, sometimes only unilateral, on the raphe valve 'Zentralarea eine bis an den Schalenrand reichende Querbinde, zuweilen nur einseitig ausgebildet', the narrow–lanceolate axial area with lanceolate central area on the RLV '..mit sehr enger, in der Mitte oft etwas lanzettlich erweiteter Pseudoraphe' and parallel to slightly radiate striae, 23 in 10 μm on the RV, and 25 in 10 μm on the RLV.



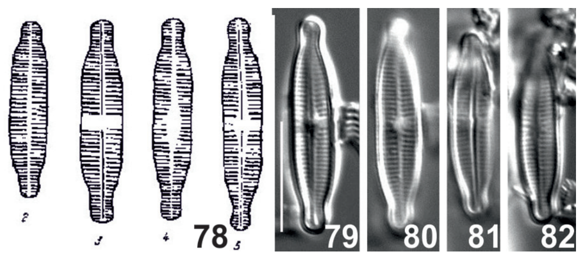
Figs 72–77. *Achnanthidium amphicephalum*, SEM images taken of specimens from the River Pena, Shara Mountain, North Macedonia, Acc. No. 12174 (MKNDC): (72–75) external view of three whole raphe valves and a raphe valve from the centre to the pole, showing the small central area and the short, deflected terminal raphe fissures (arrow); (76, 77) external view of whole rapheless valves. Scale bars 2 μm (72–74, 76, 77), 1 μm (75).

LM (Figs 78–82, 91–122): Valves linear with rostrate or subcapitate poles, less wide than the maximum valve width at the valve centre. Valve margins occasionally slightly constricted at the valve centre, tapering gradually close to the poles. Valve dimensions: population from the River Peschanyi, Russia (n = 32): length 20.0–22.5 µm, width at valve centre: 3.0–4.0 µm, Hustedt (1939, p. 555): length 14.0–19.0 µm, width 4.5 µm. On the RV, shape of the central area variable, either forming a transverse fascia, small rhombic bordered by slightly shortened, more widely spaced central striae, or asymmetrical with a unilateral fascia and a shortened stria on the opposite side. Axial area narrow, linear, slightly widening near the central area. Raphe straight, filiform, with hardly enlarged central pores. Axial area on the RLV narrow, widening towards a narrowly lanceolate central area. Striae parallel or slightly radiate throughout on both valves; population from the River Peschanyi, Russia: 21–25 in 10 µm on the RV, 21–26 in 10 µm on the RLV. Areolae not discernible in LM.

SEM (Figs 83–90, 123–126): Terminal raphe fissures short, unilaterally deflected, terminating on the valve face (Figs 83, 86, 123). Internally, central raphe endings shortly deflected to opposite sides, terminal raphe endings end in small helictoglossae (Figs 85, 87, 88, 125). Striae uniseriate, composed of 3–4, near the pole of 2–3 transapically elongated, slit–like areolae on both valves (Figs 83, 84, 89, 90, 123, 124). One row of transapically elongated areolae on mantle separated from the valve face areolae by a hyaline marginal area at the valve face/mantle junction (Figs 89, 90).

Analysed material: *Achnanthidium thienemannii*, type material E3846, slide MA2–92 (BRM), prepared by Hustedt, F. Sweden, Abisko 178, Quelle am Njulja, d, 28.8.1939, leg. Thienemann. Habitat: Moos (*Mniobryum albicans* var. *glaciale*). Simonsen finder: 466.1/467.2–3, Simonsen (1987, p. 302, plate 456: 12–17); Sample MW–D 332, Russia, Murmansk Region, Kandalaksha District, small River Peschanyi, 4.5 km west of Luven'ga village, 67.12364 ° N, 32.58968 ° E, scrapings from stones, leg. D.A. Chudaev, 20.06.2014.

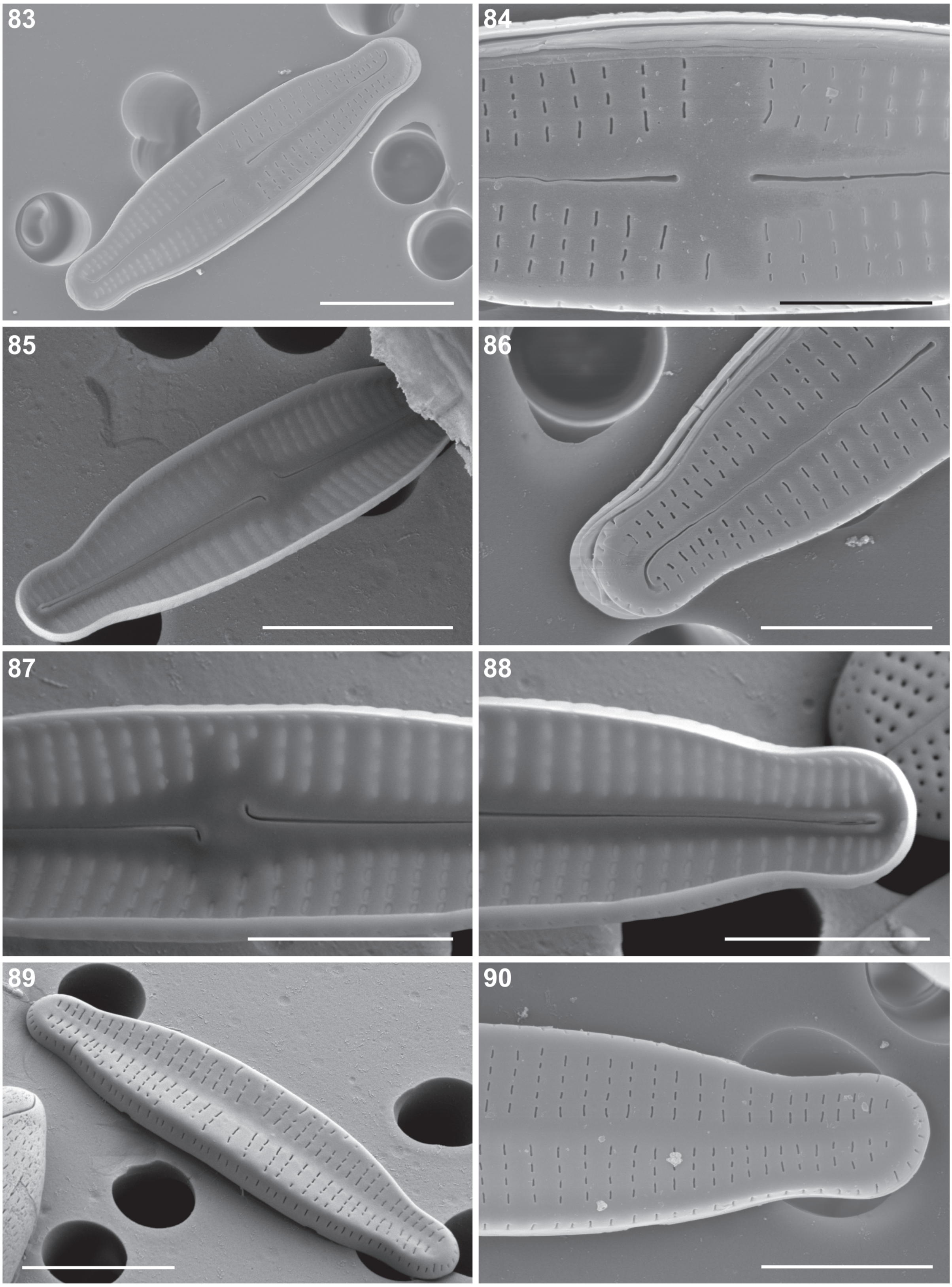
Associated diatom flora: The assemblage at the type location, a spring at Mount Njulla Abisko, Sweden, was dominated by *Staurosirella neopinnata* E.Morales and *Meridion circulare* (Greville) C.Agardh. Species present in the River Peschanyi, Russia, included *Achnanthidium kriegeri* (Krasske) P.B.Hamilton, D.Anton et Siver, *Achnanthidium minutissimum* (Kützing) Czarnecki, *Achnanthidium nodosum* (A.Cleve) Tseplik et Chudaev, *Achnanthidium* cf. *parallelum* J.R.Carter ex Jüttner et al., *Achnanthidium pusillum* (Grunow) Czarnecki, *Eucocconeis laevis* (Østrup) Lange–Bertalot, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange–Bertalot, *Psammothidium bristolicum* Bukhtiyarova, *Psammothidium helveticum* (Hustedt) Bukhtiyarova et Round and *Psammothidium subatomoides* (Hustedt) Bukhtiyarova et Round (Tseplik & Chudaev 2020).



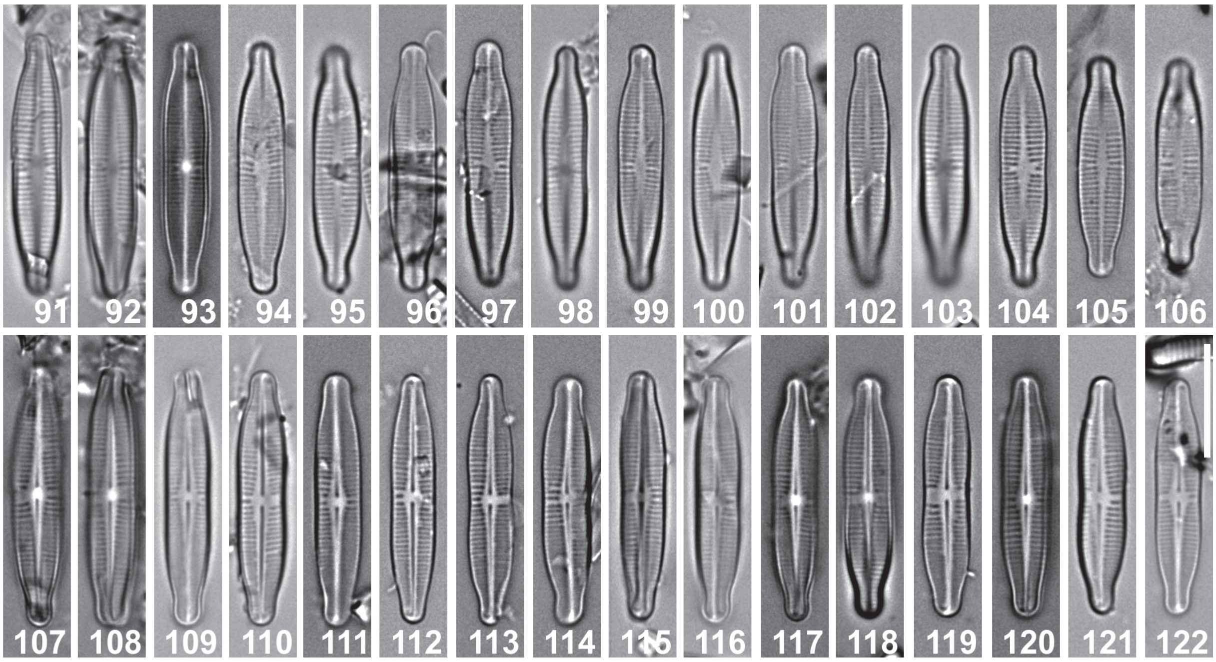
Figs 78–82. *Achnanthidium thienemannii*: (78) *Achnanthes thienemannii* Hustedt. Drawings in Hustedt (1942, pp. 98–9, fig. 1: 2–5); (79–82) LM images of the lectotype, slide number MA2–92 (BRM), valve views of (79) raphe valve, (80–82) rapheless valves, Copyright of images: Alfred–Wegener–Institut, Germany. Scale bar 10 µm.

Taxonomic remarks: *Achnanthidium thienemannii* is similar to *A. latecephalum*, *A. rostropyrenaicum*, and three species more recently described species from European freshwaters including *Achnanthidium druartii* Rimet et Couté (Rimet et al. 2010), *Achnanthidium polonicum* Van de Vijver et al. (Wojtal et al. 2011) and *Achnanthidium barbei* Le Cohu et Pérès (Peres et al. 2014). *Achnanthidium latecephalum* has an elliptical valve shape and broad subcapitate to capitate poles (see also Potapova 2006). *Achnanthidium druartii*, *A. polonicum* and *A. barbei* are linear–lanceolate in valve outline. The axial areas on the RLV in *A. druartii* and *A. barbei* are very narrow, in the latter sometimes slightly expanded to a small, round central area. The central area of the RV in *A. polonicum* is a rectangular fascia. *Achnanthidium rostropyrenaicum* is linear–lanceolate and often slightly asymmetrical; sometimes valve margins are straight and slightly constricted in the central part of the valve as in *A. thienemannii*, but margins taper gradually towards the poles and do not form 'shoulders'.

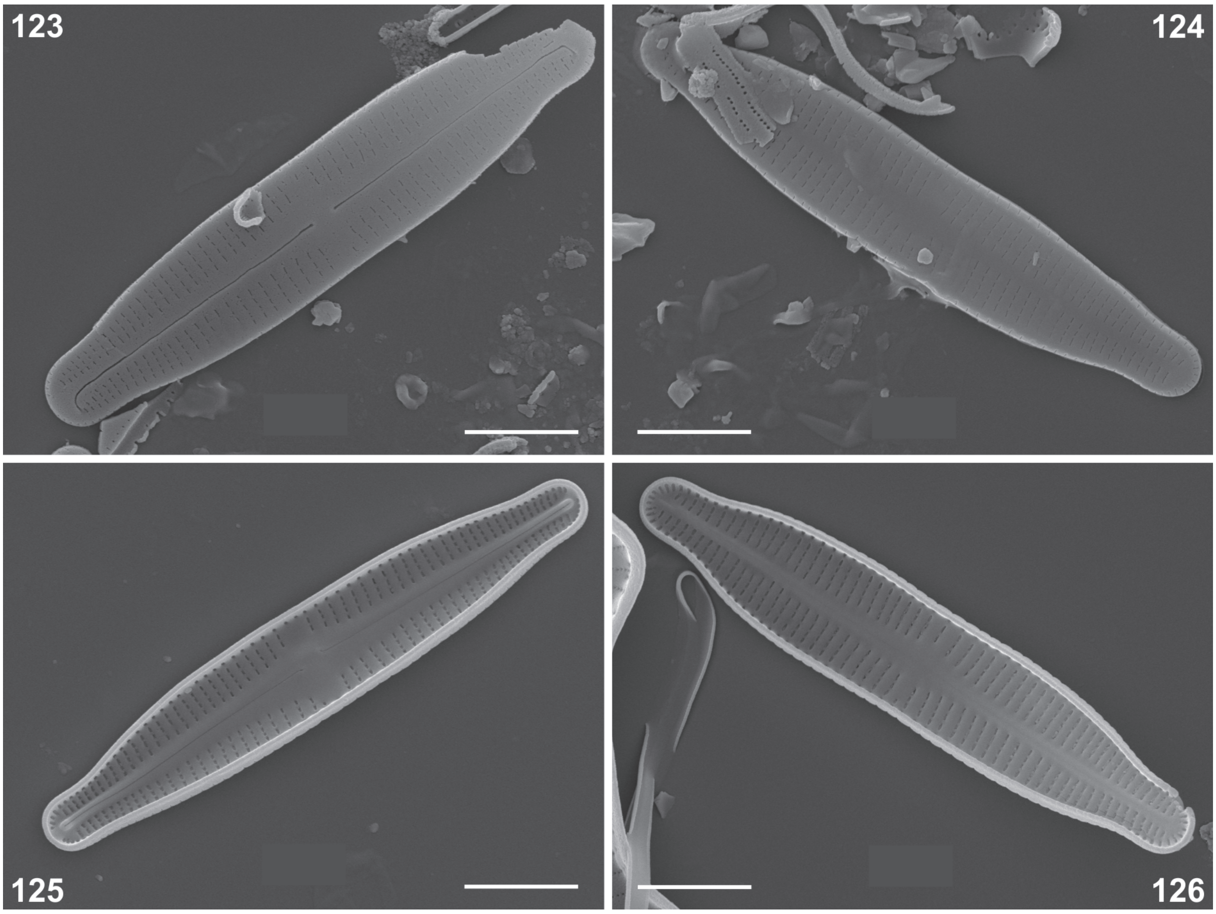
Distribution: *Achnanthidium thienemannii* was rare in the populations from Sweden, a spring (temperature 3.6 °C, pH 6.8, conductivity '[TH] 178') above the treeline at Mount Njulla (Nuolja), Abisko (Hustedt 1942), and in the River Peschanyi, Russia. The latter was located on siliceous bedrock in a forest with *Sphagnum* bogs in the taiga without anthropogenic activity in the vicinity and was the only sample of 41 taken in the region which contained *A. thienemannii* (Tseplik & Chudaev 2020). Currently available data suggest that the species is rare but has a wider distribution with records existing from locations elsewhere in the northern hemisphere. However, the species is most likely under recorded because it might have been previously identified as *A. pyrenaicum* (*Achnanthes biasolettiana*, *Achnanthidium biasolettianum*, see Potapova 2006) or identified with a preliminary name. Potapova (2006) reported and illustrated the first Russian records of *A. thienemannii* from six rivers and streams of the Izhora Plateau, fed by springs draining limestone aquifers, southwest of St. Petersburg. The species was most abundant at sites with 137–412 µS.cm^{-1} conductivity, pH 7.4–7.7, and low nitrate and phosphate concentrations. A study of diatoms in nine north–western states of the United States reported



Figs 83–90. *Achnanthidium thienemannii*, SEM images taken of specimens in the type material, Hustedt E3846 (BRM): (83, 84, 86) external view of raphe valves; (83) whole raphe valve; (84) valve centre; (85) internal view of the raphe valve; (86) half of the valve with pole; (87) internal view of the valve centre; (88) internal view of the pole; (89, 90) external view of rapheless valves; (89) whole rapheless valve; (90) valve pole. Scale bars 5 µm (83, 85, 89), 3 µm (86–88, 90), 2 µm (84).



Figs 91–122. *Achnanthidium thienemannii*, LM images of specimens from the River Peschanyi, Russia (MW–D 332): (91–106) valve views of rapheless valves; (107–122) valve views of raphe valves. Scale bar 10 µm.



Figs 123–126. *Achnanthidium thienemannii*, SEM images taken of specimens from the River Peschanyi, Russia (MW–D 332): (123, 124) external view of whole valves; (123) raphe valve; (124) rapheless valve; (125, 126) internal view of whole valves; (125) raphe valve; (126) rapheless valve. Scale bars 4 µm.

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A. thienemannii only from small and cold mountain streams with low conductivity in Montana, including a stream in the Gallatin Canyon, streams of the Flathead River catchment and a stream in the Yellowstone River catchment (Bahls 2006).

Achnanthidium rostropyrenaicum **Jüttner et E.J.Cox 2011 (Figs 127–221)**

in Jüttner et al. (2011, Algological Studies 136/137: p. 49, figs 2–11).

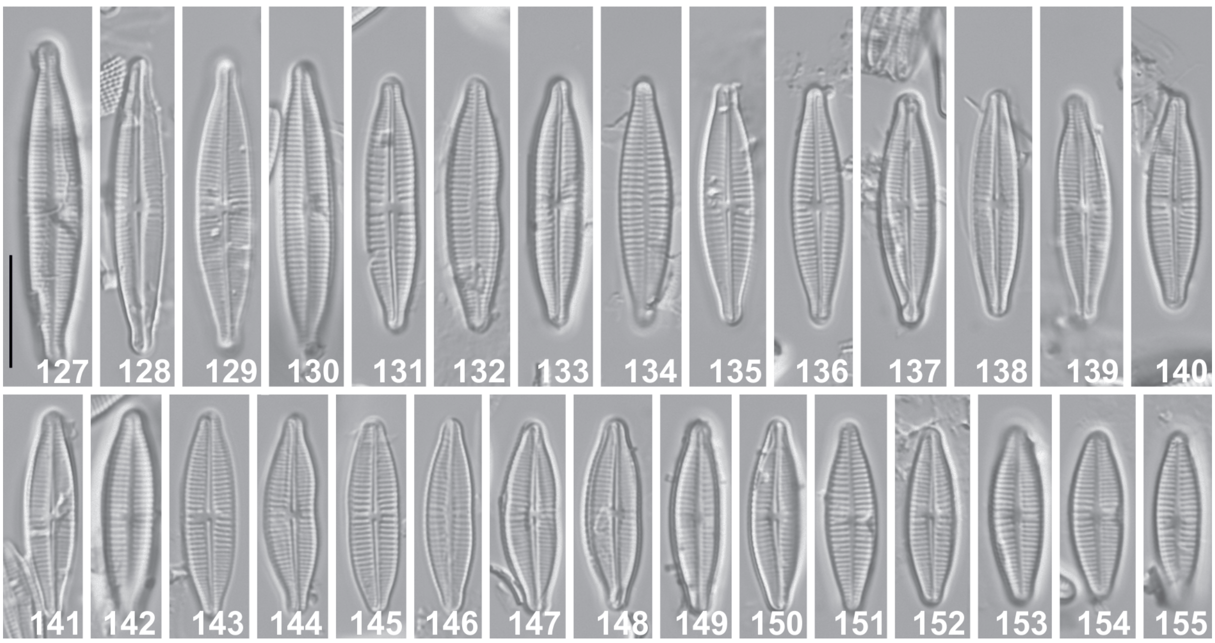
Specimens from the type population from the Nepalese Himalaya were reinvestigated, and from two additional populations from Wales and North Macedonia.

LM (Figs 127–155, 162–189, 198–219): Valves linear–lanceolate with rostrate poles. Valves often slightly asymmetrical with one or even both poles occasionally slightly deflected. Valve margins usually slightly convex in the central part of the valve but sometimes almost straight to slightly constricted in the valve centre. Margins gradually tapering towards the poles. Valve dimensions: stream MS43, Gorkha district, Nepal (n = 29): length 14.5–28.5 µm, width at valve centre: 4.0–5.0 µm; tributary to the Honddu, Mynydd Epynt, Wales (n = 32): length 11.5–17.0 µm, width at valve centre: 3.0–4.0 µm; River Pena, Shara Mountain, Republic of North Macedonia (n = 66): length 15.0–23.5 µm, initial cell 27.5 µm, width at valve centre: 3.5–5.0, initial cell 5.5 µm. On the RV, shape of the central area variable, either indistinct or bordered by more widely spaced, often shortened central striae (with spacing between striae sometimes differing on both sides) or forming a narrow fascia on one or both sides. Axial area narrow, linear, slightly widening near the central area. Raphe straight, filiform with hardly enlarged central pores. Axial area on the RLV narrow, widening towards a narrow lanceolate central area. Striae parallel or slightly radiate throughout on both valves; MS43, Gorkha district, Nepal: 20–26 in 10 µm on the RV and RLV; tributary to the Honddu, Mynydd Epynt, Wales: 24–28 in 10 µm on the RV, 22–28 in 10 µm on the RLV; River Pena, Shara Mountain, Macedonia 21–25 in 10 µm on the RV, 22–24 in 10 µm on the RLV. Areolae not discernible in LM.

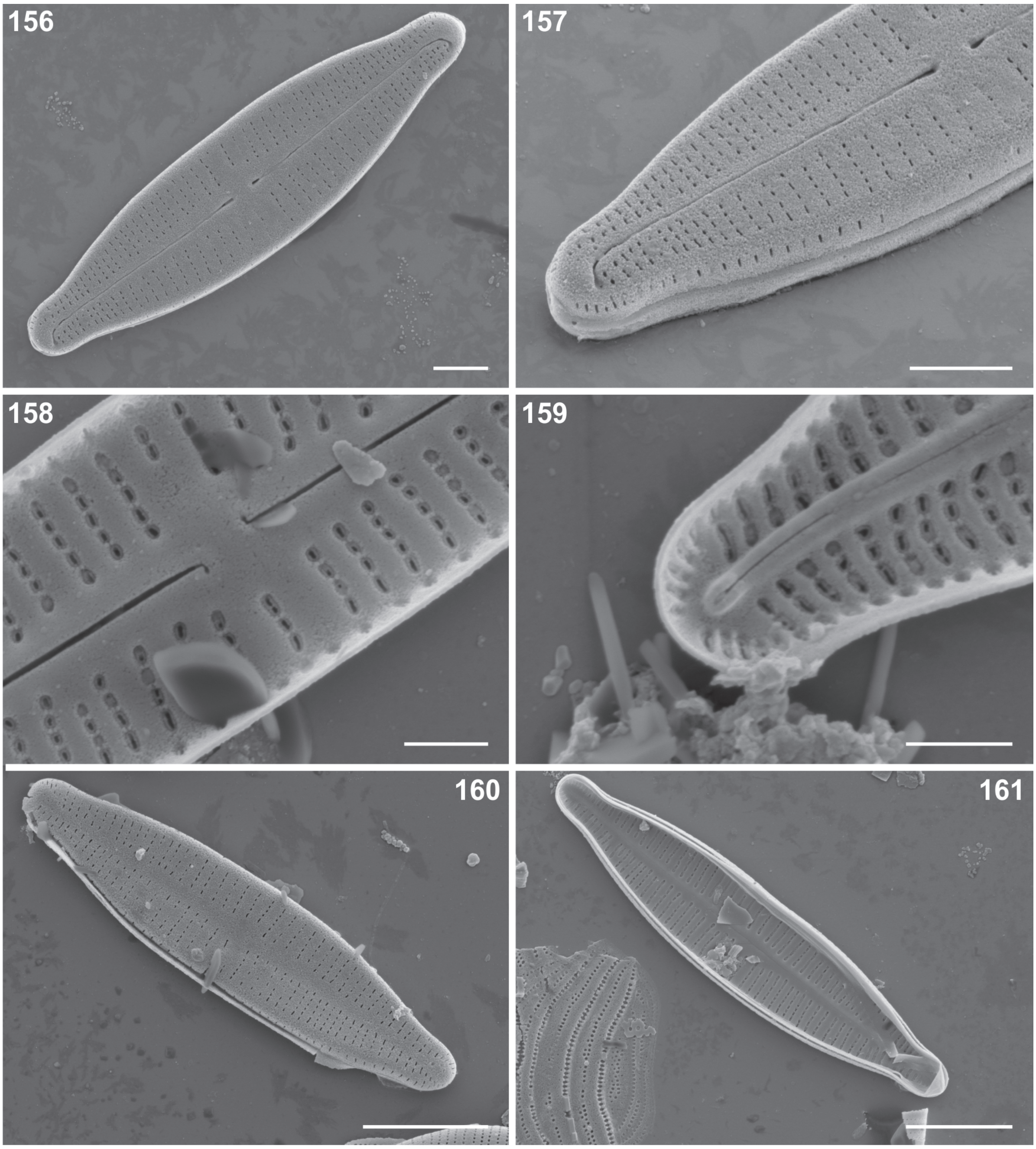
SEM (Figs 156–161, 190–197, 220–221): Terminal raphe fissures short, unilaterally deflected, terminating on the valve face (Figs 156, 157, 190, 191, 193). Internally, central raphe endings shortly deflected to opposite sides, terminal raphe endings end in small helictoglossae (Figs 158, 159, 196). Striae uniseriate, composed of 4–5 areolae in the central part of the valve, nearer and at the pole of 2–3. Areolae transapically elongated, slit–like to round on both valves (Figs 156, 157, 160, 190–195, 220, 221). In the Macedonian population, striae composed of 5–6 round to slit–like areolae, except near and at the poles. Areola density 40 in 10 µm on the RV, 45–50 in 10 µm on the RLV. One row of transapically elongate areolae on the mantle separated from the valve face areolae by a hyaline marginal area at the valve face/mantle junction (Figs 157).

Analysed material: *Achnanthidium rostropyrenaicum*, holotype NMW.C.2007.006.MS43, stream MS43, Gorkha district, western Nepal, 84.675735 °N, 28.570454 °E, leg. I. Jüttner, 16.10.1996; NMW.C.2022.05.Wales.2020.6sto, stream, tributary to Honddu south of Epynt Visitor Centre, Mynydd Epynt, Powys, Wales, 52.082916 °N, 3.470685 °W, leg. I. Jüttner, 19.08.2020; Macedonia 12174, North Macedonia, River Pena, Leshnica, Shara Mountain, Shar Planina, 42.0246111 °N, 20.7865055 °E, elevation 1462 m asl., leg. D. Zaova, 15.07.2018.

Associated diatom flora: Species associated with *A. rostropyrenaicum* in the stream in Nepal were *A.*



Figs 127–155. *Achnanthidium rostropyrenaicum*, LM images taken of specimens on the holotype slide (NMW.C.2007.006.MS43); valve views. Scale bar 10 µm.

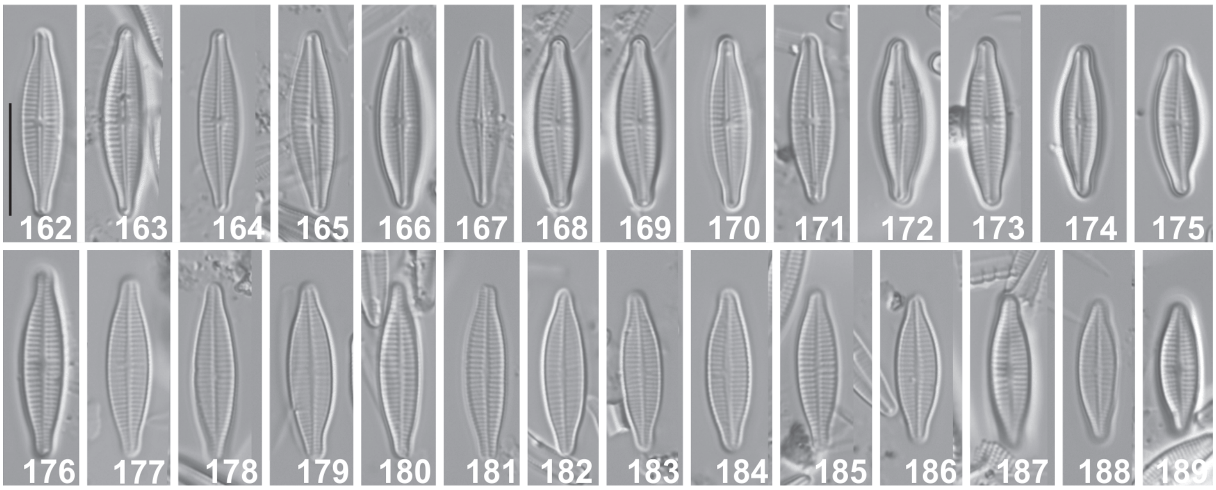


Figs 156–161. *Achnanthidium rostropyrenaicum*, SEM images taken of specimens of the type material (NMW.C.2007.006.MS43): (156, 157, 160) external view of valves; (156) whole raphe valve; (157) valve from the valve centre to the pole; (160) whole rapheless valve; (158, 159, 161) internal view of valves; (158) valve centre; (159) valve pole; (161) whole rapheless valve. Scale bars 5 µm (160, 161), 2 µm (156, 157), 1 µm (158, 159).

pyrenaicum, *A. minutissimum* and *Delicatophycus delicatulus* (Kütz.) M.J.Wynne, and associated species in the tributary to the Honddu were *A. pyrenaicum*, *A. minutissimum*, *Cymbella lange–bertalotii* Krammer and *Cymbella excisa* var. *procera* Krammer. In the River Pena *A. rostropyrenaicum* was associated with *A. pyrenaicum*, *A. amphicephalum*, and *Achnanthidium* spp., *Nitzschia* spp., and *Gomphonema* spp.

Taxonomic remarks: *Achnanthidium rostropyrenaicum* has more protracted rostrate poles compared to *A. pyrenaicum* whose type was studied by POTAPOVA & PONADER (2004). Both taxa can be consistently separated in localities where they co–occur using this difference in shape. *Achnanthidium barbei* is similar in shape but is smaller, the striae appear more radiate and the distal raphe ends are straight. *Achnanthidium druartii* is similar in shape but has broader poles and lower stria density.

Distribution: Current records suggest that *Achnanthidium rostropyrenaicum* is widely distributed in Europe and perhaps typical for calcareous waters. It was common and abundant in rheocrene and rheo–helocrenic springs in Berchtesgaden National Park, Germany, and was assigned



Figs 162–189. *Achnanthidium rostropyrenaicum*, LM images taken of specimens from the tributary to the Honddu, Mynydd Epynt, Wales (NMW.C.2022.05.Wales.2020.6sto): (162–175) raphe valves; (176–189) rapheless valves. Scale bar 10 μm.

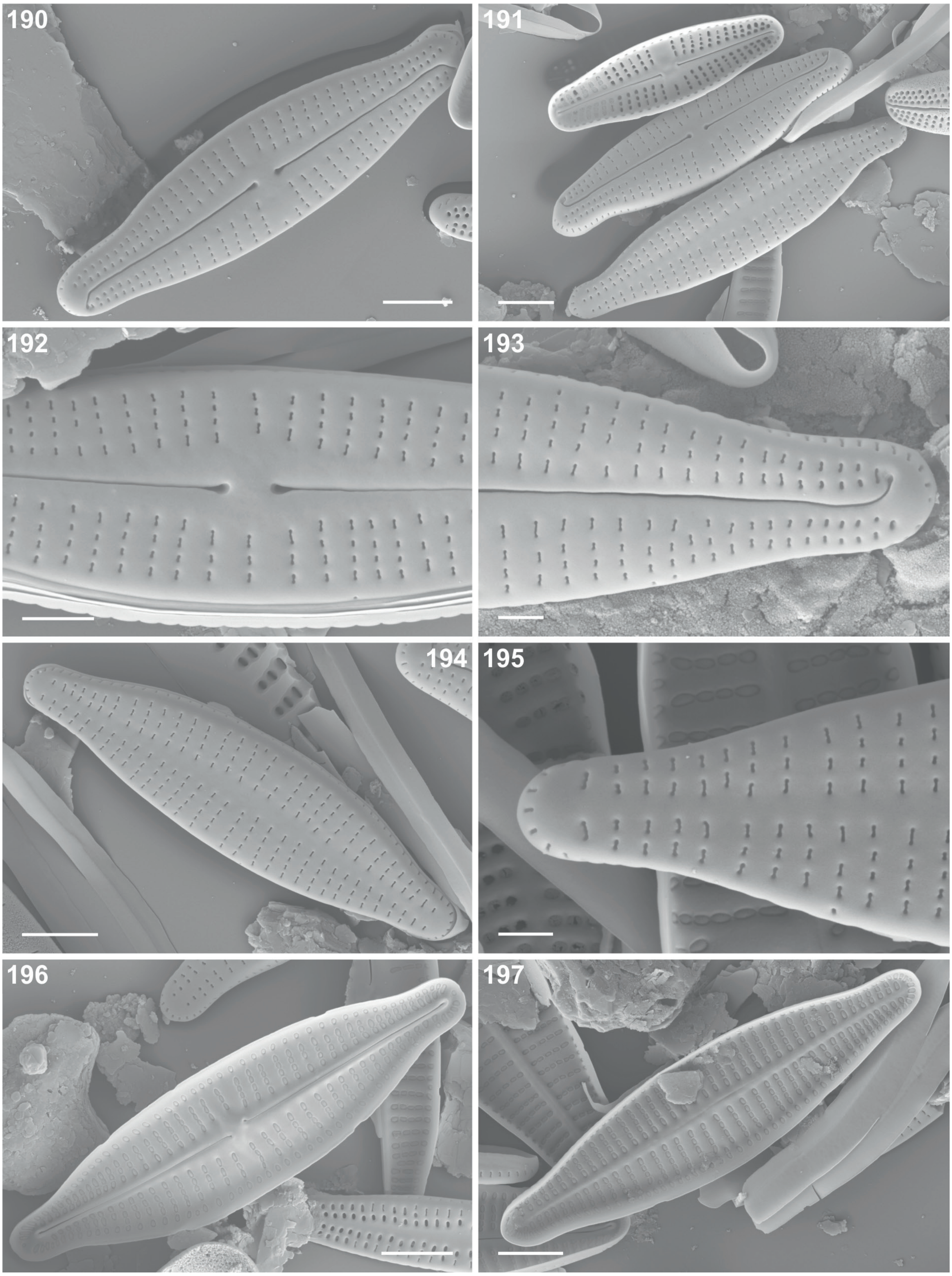
to the Red List category G (threat of unknown extent) based on experience of the researchers (Cantonati et al. 2022a). It was also found in springs of the Adamello–Brenta Nature Park (Cantonati 1998, as *Achnanthes biasolettiana* var. *biasolettiana*, Hofmann et al. 2018). It was an important species in unimpacted areas (‘reference sites’) of the River Llobregat and Ter catchment, Catalonia, Spain (Pérez–Burillo et al. 2020). In the Himalaya it occurred in a fast flowing, shaded stream at 3295 m a.s.l. altitude with high calcium concentration (54 mg/L). In Wales, no water chemistry data are available, but association with *A. pyrenaicum* suggests that it was a more base–rich site. In Macedonia, *A. rostropyrenaicum* was also associated with *A. pyrenaicum*, and with *A. amphicephalum*, and was found in several fast–flowing mountain streams and rivers in karstic areas on Shara Mountain.

Discussion and Conclusion

Achnanthidium amphicephalum (Hustedt) Jüttner, C.E.Wetzel et Levkov comb. nov., *A. thienemannii* and *A. rostropyrenaicum* are similar but can be distinguished in LM by differences in the shape of their poles and valve outline. There are no differences in ultrastructure between these species. *Achnanthidium gracillimum* has narrower valves with margins tapering from the valve centre and is more likely confused with *A. neomicrocephalum* and similar taxa.

Several new species in *Achnanthidium* with deflected terminal raphe fissures were described in recent decades (e.g. Yu et al. 2022; Pérès et al. 2012) and one species, *A. druartii*, is regarded as invasive in European freshwaters (Rimet et al. 2010). Their recognition in ecological studies and a general application of a more fine–grained taxonomy in this group would improve our understanding of their distribution as it has elsewhere (Kociolek et al. 2017). *Achnanthidium* species are abundant in unpolluted freshwaters, but many species are not included in taxa lists or commonly used floras that are used for monitoring indices (Ponader & Potapova 2007). Sequences of some taxa are unavailable and hence species, for example *A. rostropyrenaicum* although important in some locations, remain unidentified when DNA–based methods are used and do not contribute to indices (Pérez–Burillo et al. 2020). Even relatively recent morphology–based methods for the assessment of ecological status define reference conditions using a limited set of species (Pardo et al. 2018) based on broad species concepts which taxonomic research has shown to be inaccurate (Novais et al. 2015). It is essential to identify species in *Achnanthidium* using readily available taxonomic literature to understand their distribution particularly in less impacted headwater areas (Ponader & Potapova 2007). This will challenge common assumptions about the distribution of frequently used taxa (groups) and their value as indicators as currently applied (Jüttner et al. 2022).

While the abundance of *A. pyrenaicum* [= *A. biasolettianum* (Grunow) Bukhtiyarova] in base–rich, oligotrophic and oligosaprobic waters is well documented (Rimet et al. 2004; 2009), this study implies that all the species studied here are typical in base–rich, oligotrophic waters, an assumption supported by records from areas on carbonate–rich rocks elsewhere (Lange–Bertalot & Krammer 1989; Bey & Ector 2013; Potapova 2006; Pérez–Burillo et al. 2020). The rare reporting of these species is unlikely due to misidentifications, except perhaps for A. *rostropyrenaicum*, but perhaps more likely due to a study bias towards habitats impacted by pollution (Kociolek & Stoermer, 2009) and the uncertainty about their identity. Hence it is important to carry out further studies on difficult but abundant genera in oligotrophic freshwaters such as *Achnanthidium* to support the use of these species and unique communities in long–term monitoring and to recognise and reverse the loss of species in particular in base–rich oligotrophic habitats (Cantonati et al. 2022a, b).



Figs 190–197. *Achnanthidium rostropyrenaicum*, SEM images taken of specimens from the tributary to the Honddu, Mynydd Epynt, Wales (NMW.C.2022.05.Wales.2020.6sto): (190–195) external view of valves; (190, 191) whole raphe valve; (191, 194) whole rapheless valve; (192) valve centre; (193, 195) valve pole; (196, 197) internal view of valves; (196) whole raphe valve; (197) whole rapheless valve. Scale bars 2 µm (190, 191, 194, 196, 197), 1 µm (192), 600 nm (193, 195).

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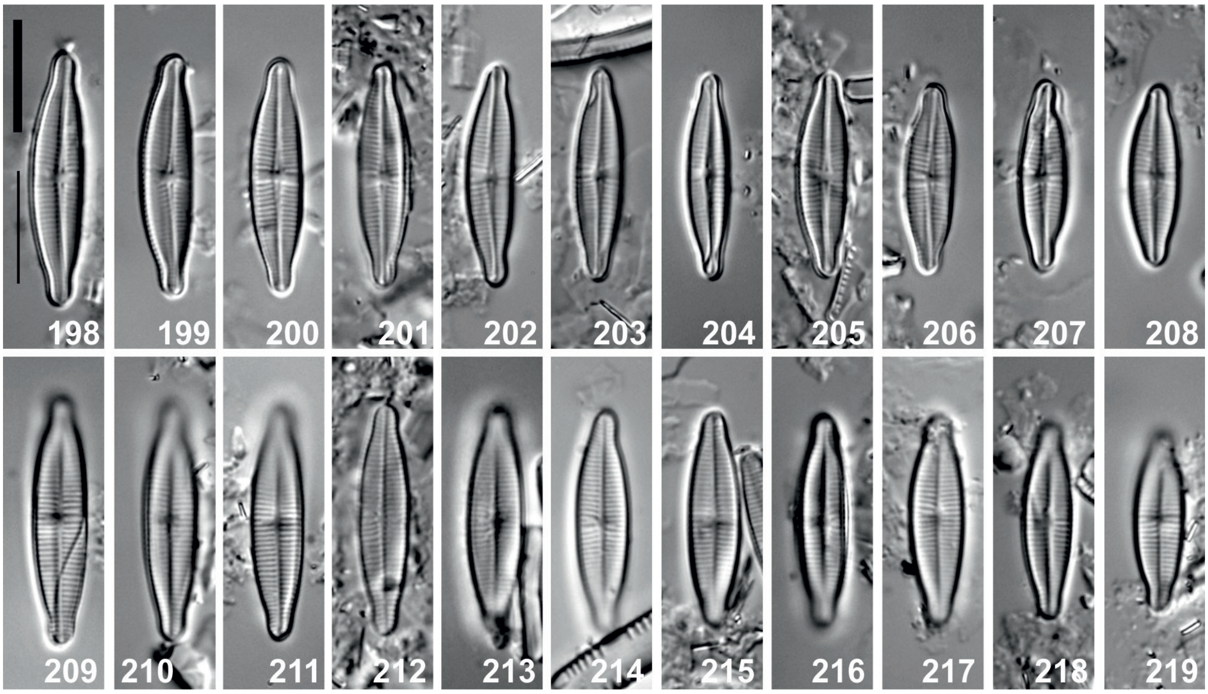
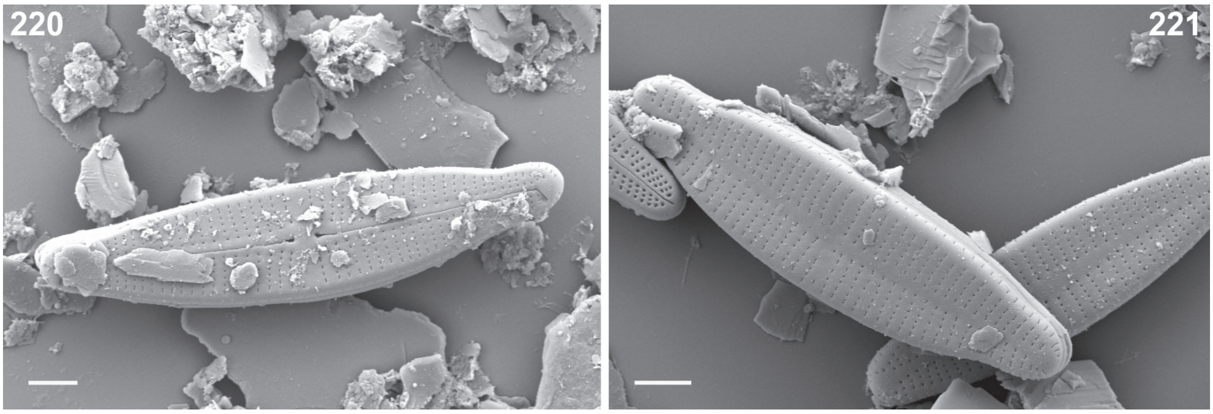


Figs 198–219. *Achnanthidium rostropyrenaicum*, LM images taken of specimens from the River Pena, Shara Mountain, North Macedonia, Acc. No. 12174 (MKNDC): (198–208) raphe valves; (209–219) rapheless valves. Scale bar 10 µm.



Figs 220, 221. *Achnanthidium rostropyrenaicum*, SEM images taken of specimens from the River Pena, Shara Mountain, North Macedonia, Acc.No. 12174 (MKNDC): (220, 221) external view of valves; (220) whole raphe valve; (221) whole rapheless valve. Scale bars 2 µm.

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