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A study of the morphology and distribution of four *Achnanthyidium* Kütz. species (Bacillariophyta), implications for ecological status assessment, and description of two new European species

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COUVERTURE / *COVER*:

Vue d'une valve du raphé de *Achnanthyidium tirolense* sp. nov. de Plansee, Autriche / View of a raphe valve of *Achnanthyidium tirolense* sp. nov. from Plansee, Austria

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A study of the morphology and distribution of four *Achnantheidium* Kütz. species (Bacillariophyta), implications for ecological status assessment, and description of two new European species

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

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ABSTRACT

Six species of the genus *Achnanthyidium* Kütz. with straight terminal raphe fissures including *Achnanthyidium caledonicum* (Lange-Bert.) Lange-Bert., *A. sieminskae* Witkowski, Kulikowski & Riaux-Gob., *Achnanthyidium neomicrocephalum* Lange-Bert. & F. Staab and *Achnanthyidium minutissimum* (Kütz.) Czarn. were studied using light and scanning electron microscopy, and shape analysis. The type of *Achnanthes microcephala* f. *scotica* J.R. Carter (synonym: *A. caledonica*) and a population of *A. neomicrocephalum* from its type locality were investigated. Two new species, *Achnanthyidium tirolense* sp. nov. and *Achnanthyidium lacuslustense* sp. nov., found in two oligotrophic lakes in Germany and Austria, were described. *Achnanthyidium tirolense* sp. nov. is distinguished by its rhombic-lanceolate valves with a slightly inflated valve centre and an acute-angled fascia on the raphe valve, and *Achnanthyidium lacuslustense* sp. nov. by its large capitate poles, broad fascia on the raphe valve and strongly curved frustules. The distribution of *A. caledonicum*, *A. sieminskae*, *A. neomicrocephalum* and *A. minutissimum* in relation to land use and their species associations were investigated in 52 rivers, streams, and lakes of Scotland. *Achnanthyidium sieminskae*, *A. caledonicum*, and *A. neomicrocephalum* were found in areas with low human impact where seminatural vegetation was dominant. The latter two species are typical in mountainous areas. In contrast, *A. minutissimum sensu stricto* was found in locations where human impact was greater. Although further data are required to determine which environmental variables underlie these distributions, our results suggest that the treatment of these species should be revised in ecological status assessments, and taxa aggregated under *A. minutissimum sensu lato* should be distinguished in ecological and biogeographical studies.

KEY WORDS

Europe,
Achnanthyidiaceae,
species distribution,
new species.

RÉSUMÉ

Étude de la morphologie et de la distribution de quatre espèces d'*Achnanthyidium* Kütz. (Bacillariophyta), implications pour l'évaluation de l'état écologique, et description de deux espèces nouvelles européennes. Six espèces du genre *Achnanthyidium* Kütz. à fissure terminale droite du raphé incluant *Achnanthyidium caledonicum* (Lange-Bert.) Lange-Bert., *A. sieminskae* Witkowski, Kulikovskiy & Riaux-Gob., *A. neomicrocephalum* Lange-Bert. & F. Staab et *A. minutissimum* (Kütz.) Czarn. ont été étudiées en utilisant microscopie optique, microscopie électronique à balayage et analyse de forme. Le type d'*Achnanthes microcephala* f. *scotica* J.R. Carter (synonyme: *A. caledonica*) et une population d'*A. neomicrocephalum* provenant de sa localité type ont été étudiés. Deux espèces nouvelles, *Achnanthyidium tirolense* sp. nov. et *A. lacuslustense* sp. nov., observées dans deux lacs oligotrophes en Allemagne et Autriche, ont été décrites. *Achnanthyidium tirolense* sp. nov. se distingue des autres espèces du genre par ses valves rhombiques-lancéolées avec un centre valvaire légèrement gonflé et un fascia à angle aigu sur la valve du raphé, et *Achnanthyidium lacuslustense* sp. nov. par ses grands pôles capités, un large fascia sur la valve du raphé et des frustules fortement incurvés. La distribution de *A. caledonicum*, *A. sieminskae*, *A. neomicrocephalum* et *A. minutissimum* en relation avec l'utilisation des terres et leurs associations d'espèces ont été étudiées dans 52 rivières, ruisseaux et lacs d'Écosse. *Achnanthyidium sieminskae*, *A. caledonicum* et *A. neomicrocephalum* ont été récoltées dans des zones à faible impact humain où une végétation semi-naturelle dominait. Ces deux dernières espèces sont typiques des régions montagneuses. En revanche, *A. minutissimum sensu stricto* a été récoltée dans des endroits avec un impact humain plus important. Bien que des données supplémentaires soient nécessaires pour identifier les variables environnementales qui déterminent ces distributions, nos résultats suggèrent que l'identité taxonomique de ces espèces devrait être révisée dans les systèmes de bio-indication, et que les taxons groupés sous le nom d'*A. minutissimum sensu lato* devraient être séparés dans les études écologiques et biogéographiques.

MOTS CLÉS

Europe,
Achnanthyidiaceae,
distribution des espèces,
espèces nouvelles.

INTRODUCTION

The high number of studies on the genus *Achnanthyidium* Kütz. (Achnanthyidiaceae, Bacillariophyta) reflects its importance in freshwaters both with respect to their distribution across a wide range of environmental conditions and the abundance and diversity of the species. Over 35 new taxa have been described in the last ten years alone from aquatic and subaerial habitats including several species with straight terminal raphe fissures (e.g. Taylor *et al.* 2011; Van de Vijver

et al. 2011a; Van de Vijver & Kopalová 2014; Pinseel *et al.* 2017; Krahn *et al.* 2018; You *et al.* 2019; Miao *et al.* 2020). Despite this progress in understanding species diversity in *Achnanthyidium*, there are still considerable problems with the identification of taxa, in particular of the small capitate species, including in well studied regions such as Europe (Gottschalk & Kahlert 2012 and references therein; Wetzel *et al.* 2019; Kennedy & Buckley 2021). In the present study we therefore investigated the morphology of six *Achnanthyidium* species with short, straight terminal raphe fissures from

several locations in Europe, four of which are more widely distributed, using light (LM) and scanning electron microscopy (SEM) and elliptic Fourier shape analysis. Based on the results, we describe two new species from two oligotrophic lakes in Germany and Austria.

Achnanthidium caledonicum (Lange-Bert.) Lange-Bert. was first described as a new form, *Achnanthes microcephala* f. *scotica* J.R.Carter (Carter & Bailey-Watts 1981: 534) and its nomenclatural history was recently summarized by Blanco (2016). Carter (Carter & Bailey-Watts 1981: pl. 1, fig. 31), who did not designate a holotype, provided two drawings, one showing the raphe valve, the other showing the rapheless valve. In the description, Carter noted that the new form differs from the nominate taxon by the parallel margins, capitate poles and a 'raphe-valve [sic] with transverse central area' ('*a specie differt quod margine parallela, apicibus capitatis. Raphovalva area centrali transversa*'). Lange-Bertalot & Krammer (1989: 106) proposed a new combination and change of rank to *Achnanthes minutissima* var. *scotica* (J.R.Carter) Lange-Bert., because they regarded *Achnanthes microcephala* (Kütz.) Grunow (synonym: *Achnanthidium microcephalum* Kütz.) to be a synonym of *Achnanthidium minutissimum* (Kütz.) Czarn. (Czarnecki 1994). Five LM pictures were shown from Carter's slide (BM[BM 94890], ex J.R. Carter 3665) (Lange-Bertalot & Krammer 1989: 276, pl. 55, figs 11, 12, 300, pl. 67, figs 35-36); this slide was later lectotypified by Williams & Reid (2002: 139). Lange-Bertalot (1993: 8) elevated the taxon to species rank as *Achnanthes scotica* (J.R. Carter) Lange-Bert., creating a later homonym of a different species, *Achnanthes scotica* Flower & V.J.Jones, and therefore the new name *Achnanthes caledonica* Lange-Bert. was introduced (Lange-Bertalot & Moser 1994: 95). The latter was transferred to *Achnanthidium caledonicum* (Lange-Bert.) Lange-Bert. (Lange-Bertalot 1999: 277). To illustrate the size diminution series, Lange-Bertalot & Krammer (1989: pl. 55, figs 4-12) and Krammer & Lange-Bertalot (1991: pl. 34, figs 1-6) presented micrographs of valves from the type locality in Scotland and, in addition, from Brunnsee, Bavaria, Germany. Some of the specimens shown from Brunnsee (Lange-Bertalot & Krammer 1989: pl. 55, fig. 4, valve on left, fig. 10; Krammer & Lange-Bertalot 1991: pl. 34, figs 4, 6), however, are narrower and have smaller poles, which might have led to misidentifications of other specimens as *Achnanthidium caledonicum*. The sampling location at Brunnsee was revisited for this study to investigate the variability in valve shape of *A. caledonicum* across its size range. The Brunnsee sample also contained other *Achnanthidium* taxa including specimens corresponding to the narrower valves shown as *A. caledonicum* in Lange-Bertalot & Krammer (1989) and Krammer & Lange-Bertalot (1991), which we believe to be a distinct taxon. The latter taxon, together with other populations from Germany, Austria and the United Kingdom, was studied in detail and compared with some recently described species such as *Achnanthidium sieminskae* Witkowski, Kulikovskiy & Riaux-Gob. (Witkowski *et al.* 2012: 65), *A. ertzii* Van de Vijver & Lange-Bert. (Van de Vijver *et al.* 2011a: 200) as well as *A. minutissimum* (Czarnecki 1994: 157).

Achnanthidium neomicrocephalum Lange-Bert. & F.Staab was described from the oligotrophic Lustsee in southern Bavaria (Krammer & Lange-Bertalot 2004: 431, pl. 89, figs 1-13). According to these authors, the species was rarely observed, although sometimes found in abundance, in oligotrophic and calcium-rich freshwaters of the foothills of the European Alps. Cantonati & Lange-Bertalot (2010) reported the species from an alpine spring in the Berchtesgaden National Park, Germany. Illustrated records from other locations in Europe are rare. *Achnanthidium neomicrocephalum* has been observed in Britain, and is shown in Hartley *et al.* (1996: pl. 6, fig. 3A) as *Achnanthes microcephala* var. *gracillima* (F.Meister) Lange-Bert., and is also shown from one location in the Rhone-Alps region (France) (Bey & Ector 2013: 109, figs 1-23). Silva-Lehmkuhl *et al.* (2019: fig. 3X-AD) illustrated the taxon from an oligotrophic reservoir in Brazil, and Bahls (2009) included records from Idaho and Montana in a checklist of diatoms from the north-western United States. For this study we collected specimens from its type locality, Lustsee (Bavaria, Germany), to study in detail its valve ultrastructure. We illustrated several populations from other locations in Germany, Wales, and Scotland. Finally, we studied the distribution of four species, *A. caledonicum*, *A. sieminskae*, *A. neomicrocephalum* and *A. minutissimum*, in 52 streams, rivers and lakes in Scotland in relation to catchment land use, and their species associations, to gain some insights into their environmental preferences.

MATERIAL AND METHODS

MATERIAL

Samples used for the taxonomic investigation and morphological observations of the species included the lectotype of *Achnanthes microcephala* f. *scotica* (BM[BM 94890], ex J.R. Carter 3665) and populations of *A. caledonicum* from Scotland, Austria and Germany, *A. sieminskae* from Scotland, England, Wales, Austria and Germany, *A. neomicrocephalum* collected from the type locality in Germany, and from Scotland and Wales, *A. minutissimum* from Scotland, *Achnanthidium tirolense* sp. nov. from Austria, and *Achnanthidium lacuslustense* sp. nov. from Germany (Fig. 1; Table 1). The populations from Germany and Austria are subsequently referred to as 'populations from Europe'.

SAMPLE PREPARATION

Diatom samples were collected from streams, rivers, and lakes by removing the biofilm from stone surfaces with toothbrushes, or by taking small amounts of plant material (macrophytes or bryophytes). All samples, except those from Saxony (Germany), were processed using hot peroxide oxidation and HCl to remove organic and carbonate content. The samples from Saxony were processed using HCl, H₂SO₄, KCl and HNO₃. The suspensions were cleaned with multiple rinses of distilled water and mounted on glass slides using Naphrax®. All species were investigated in light and scanning electron microscopy, except *A. minutissimum* which was studied in



FIG. 1. — Study sites and regions where *Achnanthydium* Kütz. species were collected in the United Kingdom, Germany, and Austria. Scotland: 1, Petta Water, Mainland, Shetland Islands; 2, Lochan na Ba Ruaidhe; 3, Ullapool River; 4, unnamed stream west of Arnisdale; 5, Loch Tarff; 6, River Feshi; 7, Loch Morlich; 8, River Spey; 9, Burn of Savoich; 10, Belti Burn. England: 11, River Ehen. Wales: 12, Llyn y Fan Fawr. Germany: 13, Ammelsheiner See; 14, Harthsee; 15, Speicher Dreiwiebern; 16, Lustsee; 17, Fischkaltersee; 18, Brunnsee. Austria: 19, Vilsalpe; 20, Plansee.

detail elsewhere (Novais *et al.* 2015). For light microscopy (LM), diatoms were investigated and photographed using a NIKON E600 microscope (DIC, $\times 100$), with an IMAGINGSOURCE camera (DFK NME72AUC02) and the

NIS-Elements D Software (Amgueddfa Cymru – Museum Wales), and a Zeiss Axio Scope A1 microscope (DIC) with a MikroLive camera (USB39 UX178 F) and the MikroLive v.3.0.0.0 Software (Freiburg, Germany). For scanning elec-

Table 1. — Material used in this study from the United Kingdom, Germany and Austria. Abbreviations: **SEPA**, Scottish Environment Protection Agency; **BrUL**, Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft Sachsen.

Species	Collection number	Location	Geographical coordinates	Date	Collector
<i>Achnanthes microcephala</i> f. <i>scotica</i> Carter	Lecto-, BM 94890 (ex J.R. Carter 3665)	Scotland, Ardislaigh Lochan		VI.1976	J.R.Carter
<i>Achnanthidium caledonicum</i> (Lange-Bert) Lange-Bert.	NMWC.2012.011.2012.Scotland.44sto NMWC.2011.006.2010.Austria.2 NMWC.2021.06.2019.Austria.Plansee.1sto.filialg NMWC.2013.010.2013.Germany.7.mac	Scotland, West Sutherland, Lochan na Ba Ruaidhe Austria, Tyrol, Vilsalpsee at outflow Austria, Tyrol, Plansee, Kleiner Plansee Germany, Upper Bavaria, Seesoner Lake Area, Brunnsee	58°23'35.658"N, 5°6'45.14"W 47°28'10.092"N, 10°30'20.592"E 47°28'34.756"N, 10°46'50.861"E 47°59'3.599"N, 12°26'7.861"E	12.III.2012 23.IX.2010 18.IV.2019 28.IV.2013	I. Jüttner I. Jüttner I. Jüttner I. Jüttner
<i>Achnanthidium sieminskæ</i> Witkowski, Kulikovskiy & Riaux-Gob.	NMWC.2012.018.SEPA.2047968 NMWC.2011.050.SEPA.1803904 NMWC.2011.050.SEPA.1803898 NMWC.2012.011.2012.Scotland.10.bryo Collection Martyn Kelly, UK ring test, slide 28 NMWC.2021.05.01/19.Brecon.Beacons.Llyn y Fan Fawr.sto	Scotland, Highland, Loch Morlich Scotland, Highland, River Spey Scotland, Highland, River Feshie Scotland, Highland, unnamed stream west of Arnsdale England, Cumbria, River Ehen Wales, Powys, Llyn y Fan Fawr	57°9'47.462"N, 3°42'47.559"W 57°1'12.31"N, 4°26'12.04"W 57°6'55.354"N, 3°53'53.386"W 57°8'47.238"N, 5°36'28.547"W 54°31'27.332"N, 3°25'16.071"W 51°52'48.464"N, 3°41'49.794"W	V.2012 27.IV.2011 27.IV.2011 8.III.2012 19.III.2013 20.I.2019	Staff of SEPA Staff of SEPA Staff of SEPA I. Jüttner M.G. Kelly I. Jüttner
<i>Achnanthidium neomicrocephalum</i> Lange-Bert. & F.Staab	NMWC.2021.06.2019.Austria.Plansee.1sto.filialg NMWC.2013.010.2013.Germany.6.mac Collection Lydia King, slide 001-013 BrUL Nossen, Saxony, Germany, S00210-13, B13115, S00210-15, B15030 BrUL S02400-15, B15048, S02400-17, B17093, S02400-18, B18008, BrUL S04600-13, B13049, S04600-15, B15092	Austria, Tyrol, Plansee, Kleiner Plansee Germany, Upper Bavaria, Seesoner Lake Area, Brunnsee Germany, Upper Bavaria, Osterseen area, Fischkaltensee Germany, Sachsen, Amneisheimer See Germany, Sachsen, Hartensee Germany, Sachsen, Speicher Dreiwelbern	47°28'34.756"N, 10°46'50.861"E 47°59'4.2"N, 12°26'14.46"E 47°46'54.39"N, 11°19'23.696"E 51°17'52.318"N, 12°36'14.194"E 51°5'13.34"N, 12°33'25.07"E 51°5'21.962"N, 12°32'59.269"E 51°23'38.166"N, 14°24'20.236"E	18.IV.2019 28.IV.2013 15.VI.1993 2013, 2015 2015, 2017, 2018 2018 2013, 2015	I. Jüttner I. Jüttner L. King Staff of BrUL Staff of BrUL Staff of BrUL Staff of BrUL
<i>Achnanthidium minutissimum</i> (Kütz.) Czarn.	NMWC.2013.010.2013.Germany.1.mac.sed Collection Lydia King, slide 001-013 NMWC.2012.018.SEPA.2124599 NMWC.2013.014.SEPA.2264470 NMWC.2021.05.01/19.Brecon.Beacons.Llyn y Fan Fawr.sto Collection Martyn Kelly, UK ring test, slide 59	Germany, Upper Bavaria, Osterseen area, Lustsee Germany, Upper Bavaria, Osterseen area, Fischkaltensee Scotland, Highlands, Loch Tarff Scotland, Highlands, Ullapool River Wales, Powys, Llyn y Fan Fawr Scotland, Shetland Islands, Mainland, Petta Water	47°48'33.509"N, 11°17'36.959"E 47°46'54.39"N, 11°19'23.696"E 57°9'3.852"N, 4°36'0.169"W 57°54'0.446"N, 5°9'51.804"W 51°52'48.464"N, 3°41'49.794"W 60°18'58.702"N, 1°14'57.016"W	14.IV.2013 15.VI.1993 IX.2012 24.IV.2013 20.I.2019 V.2013	I. Jüttner L. King Staff of SEPA Staff of SEPA I. Jüttner M.G. Kelly
<i>Achnanthidium tirolense</i> sp. nov.	NMWC.2013.014.SEPA.2337268 NMWC.2012.018.SEPA.2138134 NMWC.2021.06.2019.Austria.Plansee.1sto.filialg	Scotland, Aberdeenshire, Burn of Savoch Scotland, Aberdeenshire, Belti Burn Austria, Tyrol, Plansee, Kleiner Plansee	57°37'2.269"N, 1°55'5.653"W 57°3'29.556"N, 2°32'59.838"W 47°28'34.756"N, 10°46'50.861"E	1.X.2013 21.IX.2012 18.IV.2019	Staff of SEPA Staff of SEPA I. Jüttner
<i>Achnanthidium lacustense</i> sp. nov.	NMWC.2013.010.2013.Germany.1.mac.sed	Germany, Upper Bavaria, Osterseen area, Lustsee	47°48'33.509"N, 11°17'36.959"E	14.IV.2013	I. Jüttner

tron microscopy (SEM) a small amount of suspension was air dried on stubs (Amgueddfa Cymru – Museum Wales) or filtered through polycarbonate membrane filters with a pore diameter of 3 µm, mounted on stubs using double-sided carbon tape (Luxembourg Institute of Science and Technology). They were then sputter-coated with a layer of platinum and studied either with a Zeiss Gemini Ultra plus SEM microscope (working distance 3–12 mm, 3–5 kV, at the Natural History Museum, London, United Kingdom, Imaging and Analysis Centre), or with a Hitachi SU-70 (working distance 10 mm, 5 kV, at Luxembourg Institute of Science and Technology). The photo plates and the map were made using CorelDraw v.12. Morphological terminology follows Anonymous (1975), Ross *et al.* (1979) and Round *et al.* (1990). The diatom slides and suspensions are stored in the diatom collection of Amgueddfa Cymru – Museum Wales (NMW), and in the collections of Martyn Kelly (United Kingdom), Lydia King and BfUL Saxony (Germany), and types at NMW, at the Natural History Museum (BM), London, Meise Botanic Garden (BR), Belgium, and Canadian Museum of Nature (CANA), Canada.

SHAPE ANALYSIS

LM size diminution series for populations and taxa were used in this study to evaluate shape differences between species. Shape outlines from LM images for individual specimens were manually outlined using the drawing tools of PowerPoint (Office 2019) and saved as tiff files. In total, 328 valves were analysed, more specifically *Achnantheidium caledonicum* (49), *A. sieminskae* (116), *A. neomicrocephalum* (76), *A. minutissimum* (45), *Achnantheidium tirolense* sp. nov. (30) and *Achnantheidium lacuslustense* sp. nov. (12). Individual shapes were then extracted, and elliptic Fourier shape harmonics generated using the DiaOutline application (Wishkerman & Hamilton 2018). Multivariate analyses on the shape harmonics using Principal Component Analysis (self-learning) to show the variation in shape and Linear Discriminant Analysis (determined discrimination) to show the separation between taxa were performed in R 3.5.0 (R Core Team 2018) with the following packages installed: MASS (Venables & Ripley 2002), ggplot2 (Wickham 2016), GGally (Schloerke *et al.* 2018), doBy (Højsgaard & Halekoh 2018), data.table (Dowle & Srinivasan 2018), plyr (Wickham 2009, 2011), gridExtra (Auguie 2017) and Momocs (Bonhomme *et al.* 2014). To examine possible sample size bias, *Achnantheidium lacuslustense* sp. nov. with less than $n = 30$, was removed and 30 bootstrap simulations for even sample size of $n = 30$ were run for the remaining five taxa and compared. The resulting biplots for the complete dataset were modified for presentation using PowerPoint (Office 2019).

NUMERICAL ANALYSIS

To study the distribution of *A. caledonicum*, *A. sieminskae*, *A. neomicrocephalum* and *A. minutissimum* in relation to land use, 300–400 diatom valves were counted in 52 samples from rivers, streams and lakes in Scotland, United Kingdom, and relative abundances were calculated. Using Google Maps,

land use was assessed in the catchments upstream of each river or stream site, and in the lake catchments, within an area of 40 km², or less when the stream or lake catchments were smaller. The following categories were used to assess land use: ‘Urban/houses’, ‘Arable’, ‘Improved grassland’, ‘Unimproved – grass/bracken’, ‘Unimproved – heath, moorland, marsh’, ‘Woodland/forestry – broadleaf’, ‘Woodland/forestry – conifers’ and ‘Mountains – rocks’. The presence/absence of these categories were assessed using six categories: 0 = absent, 1 = rare (< 5 % of cover), 2 = occasional (5–20 %), 3 = regular (20–50 %), 4 = abundant (50–80 %), 5 = dominant (> 80 %).

Non-metric multidimensional scaling (NMDS) of diatom assemblages was carried out using procedures in PRIMER7 (Clarke & Gorley 2015). Ordination of diatom assemblages are based on Bray-Curtis similarities following square-root-transformation of the relative abundances of species. Vector plots, showing the directions of increase in eight land use types were superimposed on the NMDS ordination plot of the diatom assemblages. Bubble plots were used for species data with circle sizes illustrating the relative abundances of four *Achnantheidium* species in the diatom assemblages of each site. The plots were created in PRIMER7 and edited using CorelDraw v.12.

OBSERVATIONS

Class BACILLARIOPHYCEAE Haeckel
Order ACHNANTHALES P.C.Silva
Family ACHNANTHIDIACEAE D.G.Mann
Genus *Achnantheidium* Kütz.

Achnantheidium caledonicum (Lange-Bert.) Lange-Bert.
(Figs 2–4)

Iconographia Diatomologica 6: 270–283 (Lange-Bertalot 1999). — *Achnanthes microcephala* f. *scotica* J.R.Carter in Carter & Bailey-Watts, *Nova Hedwigia* 33: 534, pl. 1, fig. 31 (Carter & Bailey-Watts 1981). — *Achnanthes minutissima* var. *scotica* (J.R.Carter) Lange-Bert. in Lange-Bertalot & Krammer, *Bibliotheca Diatomologica* 18: 106, pl. 55, figs 4–12, pl. 57, fig. 11, pl. 67, figs 35–56 (Lange-Bertalot & Krammer 1989). — *Achnanthes scotica* (J.R.Carter) Lange-Bert., *Bibliotheca Diatomologica* 27: 8 (Lange-Bertalot 1993), *nom. illeg.*, non *Achnanthes scotica* Flower & V.J.Jones, *Diatom Research* 4: 228, figs 1–7, 42–53 (Flower & Jones 1989). — *Achnantheidium minutissimum* var. *scoticum* (J.R.Carter) H.Cremer in Cremer & Wagner, *Polar Biology* 26: 108 (Cremer & Wagner 2003). — Basionym: *Achnanthes caledonica* Lange-Bert. in Lange-Bertalot & Moser, *Bibliotheca Diatomologica* 29: 95 (Lange-Bertalot & Moser 1994).

DESCRIPTION

LM (Fig. 2A–AN)

Frustules moderately arched, with convex rapheless valves, concave raphe valves, and recurved poles. Valves linear with large, subcapitate to capitate poles, the latter sometimes wider than the maximum valve width at valve centre. Valve margins tapering slightly towards a small constriction before poles. At

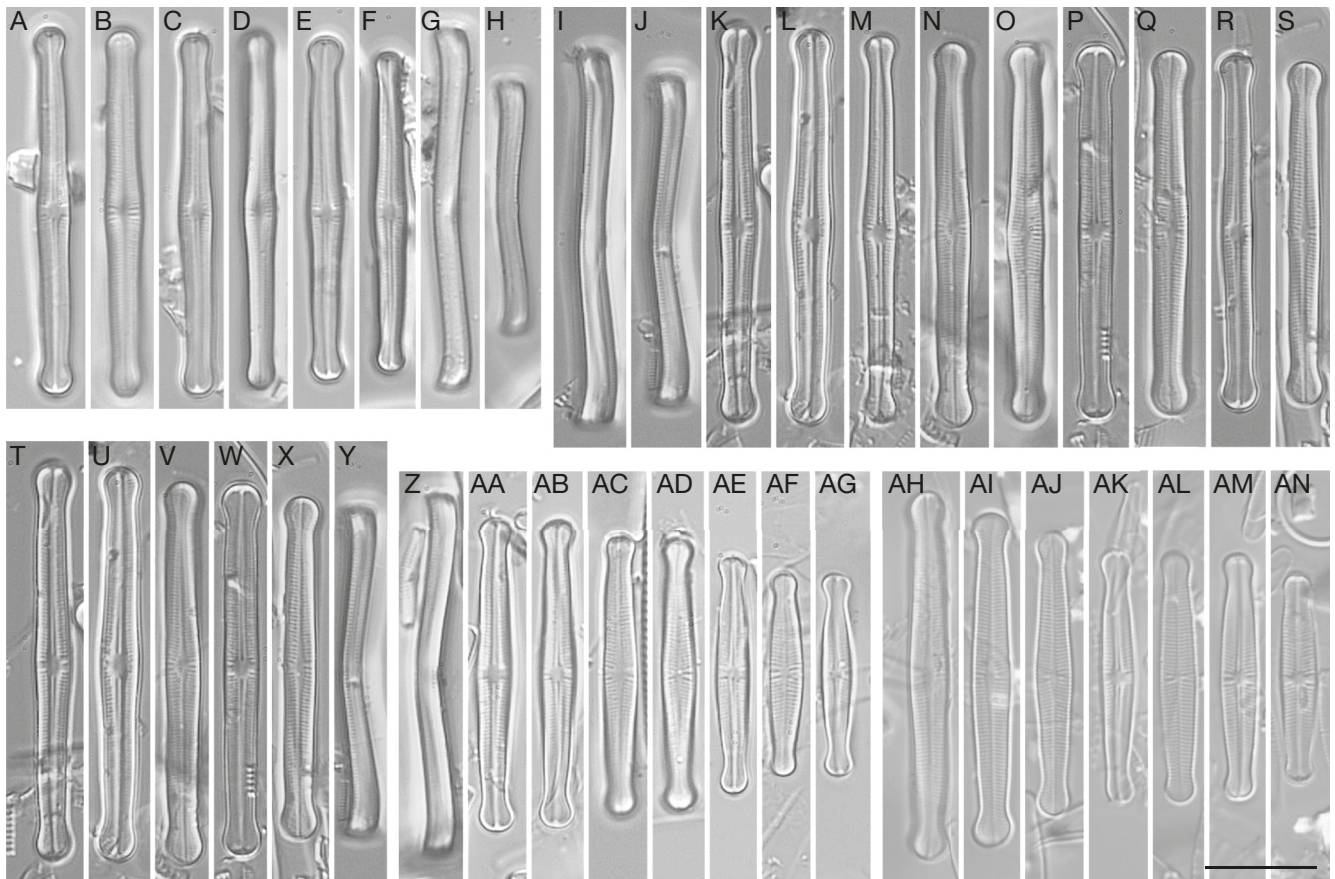


FIG. 2. — **A–H**, *Achnanthes microcephala* f. *scotica* J.R.Carter, type from Ardislaigh Lochan, Scotland; **I–AN**, *Achnanthyidium caledonicum* (Lange-Bert.) Lange-Bert.; **I–S**, specimens from Lochan na Ba Ruaidhe, West Sutherland, Scotland; **T–Y**, specimens from Vilsalpsee, Austria; **Z–AG**, specimens from Brunnsee, Germany; **AH–AN**, specimens from Plansee, Austria; **A–F**, **K–X**, **AA–AN**, LM views of valves; **G–J**, **Y**, **Z**, LM views of frustules in girdle view. Scale bar: 10 µm.

valve centre, margins slightly inflated, sometimes unilaterally, or straight. Valve dimensions ($n = 20$): length 17.5–37.5 µm, width at valve centre: 3.0–3.5 µm. On raphe valve (RV), shape of central area variable, either a small transverse fascia, or oval to round, bordered by one or several slightly shortened striae. Raphe filiform, straight, central pores hardly widened, distantly placed at margin of central area. Central area on rapheless valve (RL) narrow lanceolate. Axial area narrow linear on both valves. Striae radiate throughout on both valves, more distantly placed in valve centre, 30–32 in 10 µm.

SEM (Figs 3A–F; 4A–D)

Raphe located on slightly raised sternum, terminal fissures straight terminating at valve/mantle junction. Internally, central raphe endings shortly deflected to opposite sides, terminal raphe endings form small helictoglossae. Striae composed of 2–4 (RV) and 2–5 (RL) areolae. Areolae round or oval, areolae adjacent to valve margin slit-like. Row of transversally elongated areolae on mantle separated from areolae on valve face by hyaline area.

On the type slide (lecto-, BM[BM 94890], ex *J.R. Carter 3665*), the species is common, but many specimens are positioned in girdle view. The central area is often a fascia, as mentioned by Carter (Carter & Bailey-Watts 1981), but

sometimes less conspicuous, irregular, and formed by more widely spaced central striae.

Achnanthyidium sieminskae

Witkowski, Kulikowski & Riaux-Gob.
(Figs 5–8)

Witkowski *et al.* in Wołowski *et al.*, *Current Advances in Algal Taxonomy and its Applications: Phylogenetic, Ecological and Applied Perspective*: 65, fig. 6 (Witkowski *et al.* 2012).

DESCRIPTION

LM (Figs 5A–BN; 7A–BQ)

Frustules moderately arched, convex RL, concave RV, poles slightly to moderately recurved. Valves linear to linear-lanceolate. Valve margins slightly or moderately curved, tapering towards subcapitate or capitate poles. Valves occasionally slightly asymmetrical with one valve margin almost straight or less curved than the other (more commonly observed in United Kingdom populations). Valve dimensions: length 11.0–26.0 µm (populations United Kingdom, $n = 20$), 10.0–19.5 µm (populations Germany, Austria, $n=15$), width at valve centre 2.0–3.0 µm. On RV, central area small rounded or indistinct, bordered by

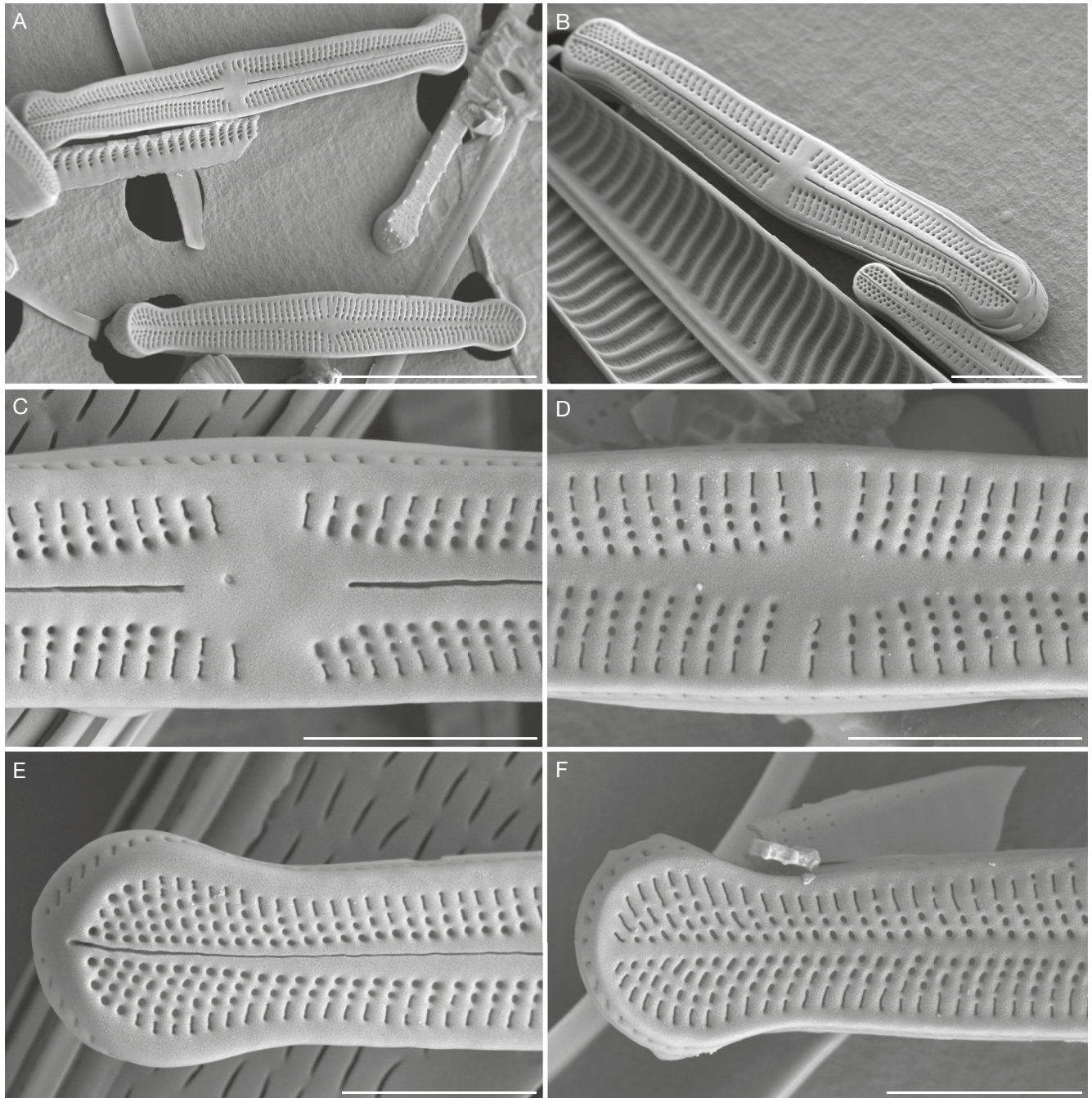


FIG. 3. — *Achnanthydium caledonicum* (Lange-Bert.) Lange-Bert.: **A–B**, specimens from Brunnssee, Germany; **C–F**, specimens from Scotland, 2012, 44sto; **A**, SEM external view of whole raphe valve and rapheless valve; **B**, SEM external view of whole raphe valve, mantle and girdle bands at pole; **C**, SEM external view of valve centre of raphe valve; **D**, SEM external view of valve centre of rapheless valve; **E**, SEM external view of pole of raphe valve; **F**, SEM external view of pole of rapheless valve. Scale bars: A, 10 µm; B, 5 µm; C–F, 3 µm.

1–2 slightly shortened, more widely spaced striae on each side, central striae can be absent. Raphe filiform, straight, central pores slightly widened, terminating close to margin of central area. Central area on RL indistinct or narrow rhombic. Axial area narrow linear on both valves, slightly widening towards valve centre. Striae weakly radiate in valve centre, becoming more strongly radiate towards poles on both valves, RV 32–34 in 10 µm, RL 30–34 in 10 µm (populations United Kingdom), 30–32 in 10 µm (RV and RL, populations Germany, Austria).

SEM (Figs 6A–H; 8A–F)

Raphe located on slightly raised sternum, terminal fissures straight terminating at valve/mantle junction. Internally, central raphe endings shortly deflected to opposite sides, terminal raphe endings form small helictoglossae. Striae composed of 2–4 (RV) and 3–5 (RL) areolae. Areolae round, oval or slit-like, those adjacent to valve margin often slit-like. A row of transapically elongated areolae on the mantle separated from the areolae on the valve face by a hyaline area.

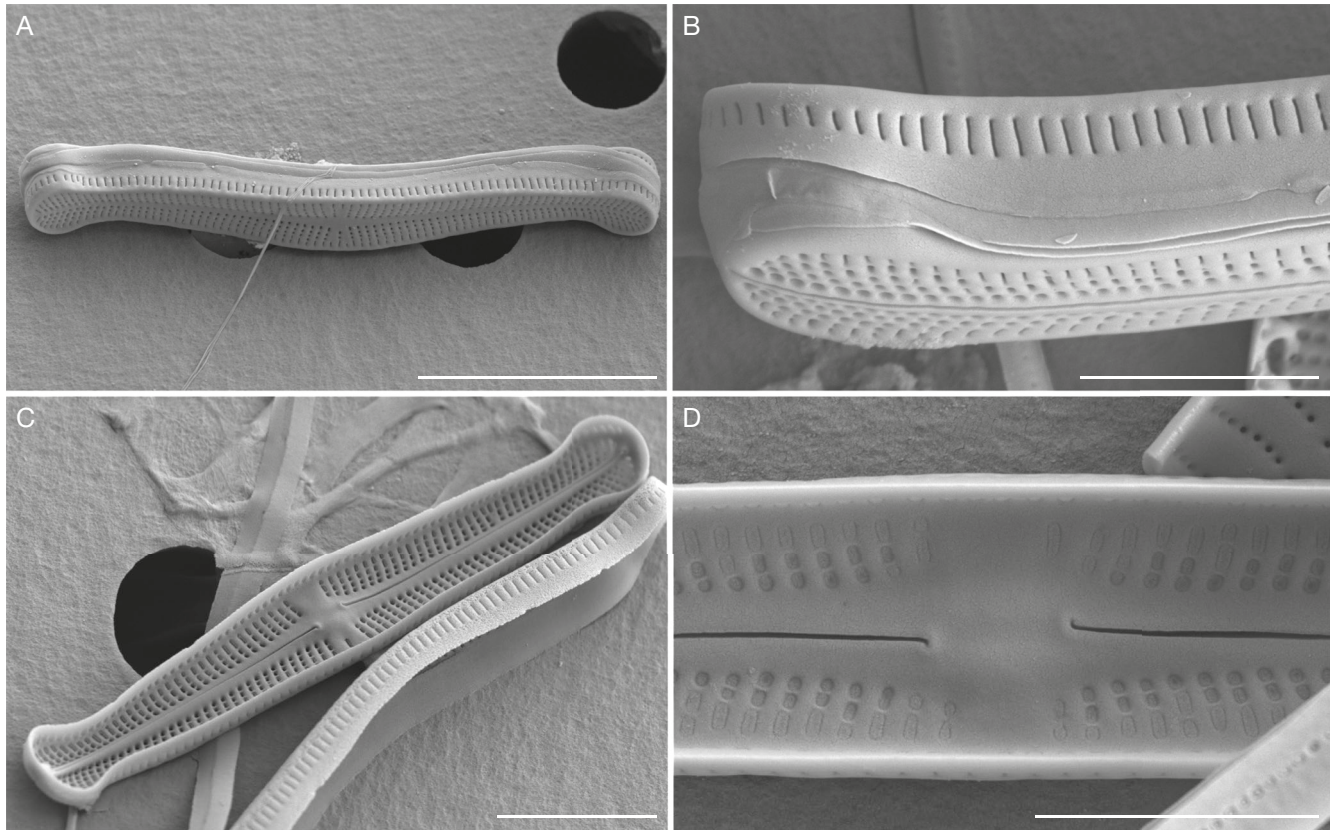


FIG. 4. — *Achnanthydium caledonicum* (Lange-Bert.) Lange-Bert.: **A, C**, specimens from Brunnsee, Germany; **B, D**, specimens from Scotland, 2012, 44sto; **A**, SEM external view of frustule in girdle view and valve face of rapheless valve; **B**, SEM external view of pole in girdle view and valve mantle; **C**, SEM internal view of whole raphe valve; **D**, SEM internal view of valve centre of raphe valve. Scale bars: A, 10 µm; B, D, 3 µm; C, 5 µm.

Achnanthydium neomicrocephalum Lange-Bert. & F.Staab
(Figs 9-13)

Süßwasserflora von Mitteleuropa, Bacillariophyceae. 4. Teil Achnanthaceae: 431, pl. 89, figs 1-13 (Krammer & Lange-Bertalot 2004).

DESCRIPTION

LM (Fig. 9A-DZ)

Frustules slightly arched, with convex RL, concave RV. Valves linear-lanceolate. Margins slightly tapering towards small protracted, capitate poles. Valve margins slightly inflated in valve centre, sometimes more strongly on one side. Valve dimensions ($n = 31$): length 12-30.5 µm, width at valve centre 2-2.5 µm. On RV, central area small rounded, bordered by 1-2 slightly shortened, more widely spaced striae on each side, or small transverse fascia. Raphe filiform, straight, central pores terminating close to margin of central area. Central area on rapheless valve indistinct. Axial area narrow, linear on both valves, slightly widening towards valve centre. Striae radiate throughout on both valves, 30-32 in 10 µm (RV and RL).

SEM (Figs 10A-H; 11A-F; 12A-H; 13A-D)

Raphe located on slightly raised sternum, terminal raphe fissures straight terminating at valve/mantle junction. Internally, central raphe endings shortly deflected to opposite sides. Striae composed of 2-3 areolae (RV and RL). Areolae round or oval

adjacent to axial area, sometimes slit-like adjacent to valve margin. Row of transapically elongated areolae on mantle separated from areolae on valve face by hyaline area.

Achnanthydium minutissimum (Kütz.) Czarn.
(Fig. 14)

Memoirs of the California Academy of Sciences 17: 157 (Czarnecki 1994). — Basionym: *Achnanthes minutissima* Kütz., *Linnaea* 8 (5): 578, pl. 16, fig. 54 (Kützing 1833); Kützing, 50, pl. 4, fig. 54 (Kützing 1834); Kützing, Dec. VIII, no. 75 (Kützing 1834), in Kützing, *Algarum Aquae Dulcis Germanicum*, Decades I-XVI, numbers 1-160. Halis Saxonum, 1833-1836.

DESCRIPTION

LM (Fig. 14A-AW)

Frustules slightly arched, with convex RL, concave RV. Valves linear-lanceolate. Margins tapering gradually towards short protracted rounded, rostrate poles. Valve dimensions ($n = 46$): length 8.5-15 µm, width 2.5-3.5 µm. Central area on raphe valve varies from small rounded to a narrow rectangular fascia on one or both sides. Striae more widely spaced in valve centre. Raphe filiform, straight, central pores slightly widened, terminating close to margin of central area. Central area on rapheless valve indistinct or narrow elliptical. Axial area narrow linear on both valves, slightly widening towards valve

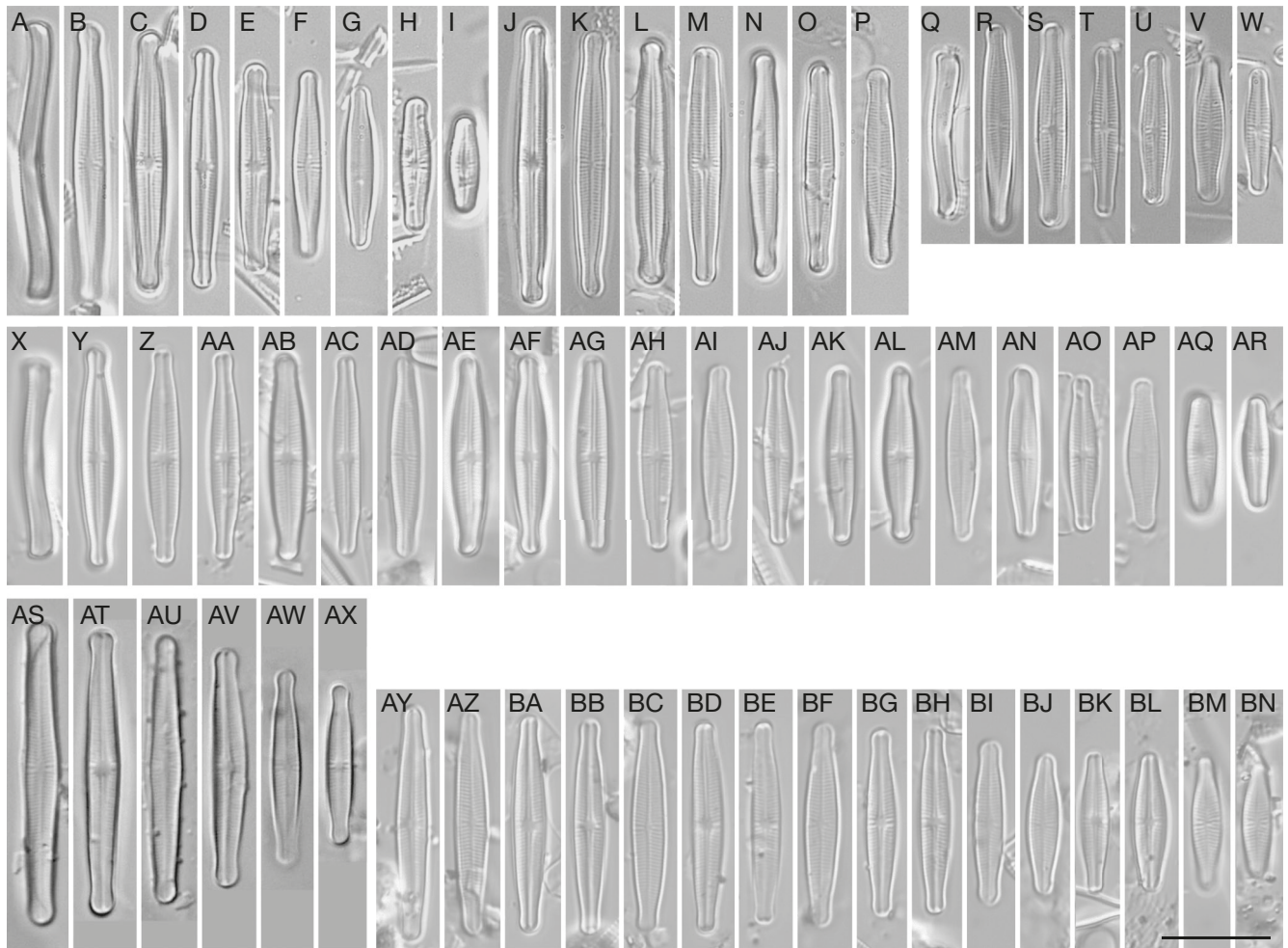


FIG. 5. — *Achnanthyidium sieminskae* Witkowski, Kulikowsky & Riaux-Gob.: **A-I**, specimens from Loch Morlich, Scotland; **J-P**, specimens from River Spey, Scotland; **Q-W**, specimens from River Feshie, Scotland; **X-AR**, specimens from unnamed stream west of Arnisdale, Highland, Scotland; **AS-AX**, specimens from River Ehen, England; **AY-BN**, specimens from Llyn y Fan Fawr, Wales; **B-P**, **R-W**, **Y-BN**, LM views of valves; **A**, **Q**, **X**, LM views of frustules in girdle view. Scale bar: 10 µm.

centre. Striae radiate throughout on both valves, 30-35 in 10 µm (RV and RL), becoming denser at poles.

Achnanthyidium tirolense sp. nov.
(Figs 15; 16)

HOLOTYPE. — **Austria**. Plansee, 18.IV.2019, I. Jüttner (holo-, NMW[NMW.C.2021.06.2019]; iso-, BM, BR, CANA).

TYPE LOCALITY. — **Austria**. Tyrol, Kleiner Plansee, Plansee, 47°28'34.756"N, 10°46'50.861"E.

HABITAT. — Epiphytic and epilithic in lake littoral.

ETYMOLOGY. — The species was named after the region where it was found, Tyrol, Austria.

REGISTRATION. — <http://phycobank.org/103379>

DESCRIPTION

LM (Fig. 15A-AG)

Valves rhombic-lanceolate with slightly inflated valve centre. Margins tapering gradually towards long protracted, broadly

rounded, rostrate poles. Valve dimensions (n = 25): length 14-23.5 µm, width at valve centre 3-4 µm. Central area on RV an acute-angled fascia. Raphe filiform, straight. Central pores slightly expanded, distantly placed at margin of central area. Central area on RL narrow lanceolate, sometimes a narrow acute-angled fascia on one or both sides formed by more widely spaced central striae. Axial area narrow linear on both valves. Striae radiate throughout on both valves, 28-30 in 10 µm (RV and RL).

SEM (Fig. 16A-F)

Raphe located on slightly raised sternum, central raphe endings small, round, terminal raphe fissures straight with tear drop-shaped ends, terminating at valve/mantle junction. Internally, central raphe endings slightly deflected to opposite sides, terminal raphe endings form small helictoglossae. Striae straight or slightly curved, on RV composed of 3-5 areolae, on RL composed of 2-5 areolae. Areolae round, those adjacent to valve margin round or slit-like. One row of transapically elongated areolae on mantle separated from areolae on valve face by a hyaline area.

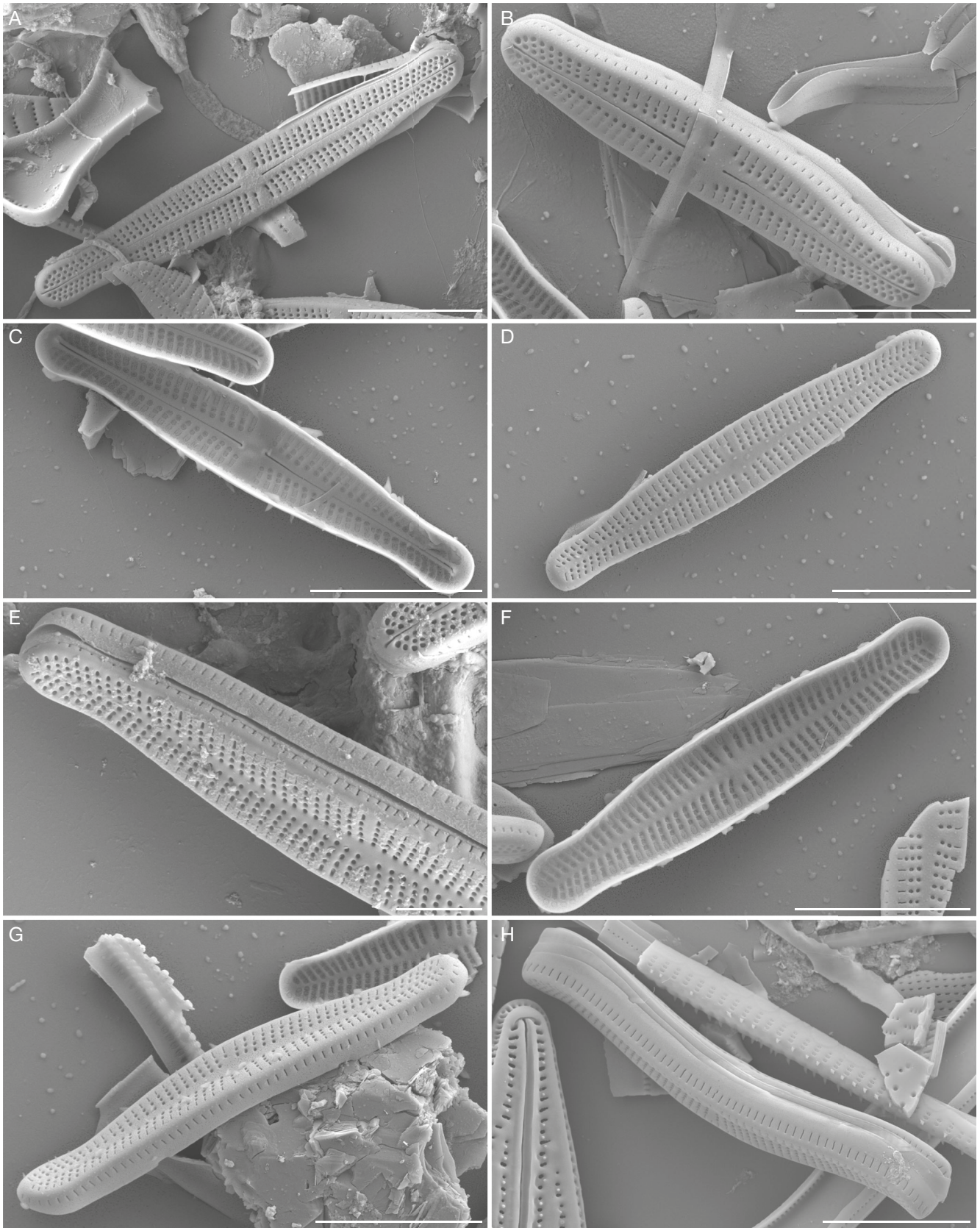


FIG. 6. — *Achnanthyidium sieminskae* Witkowski, Kulikowskij & Riaux-Gob.: **A, E**, specimens from River Spey, Scotland; **B-D, F-H**, specimens from River Feshi, Scotland; **A, B**, SEM external views of raphe valve; **C**, SEM internal view of raphe valve; **D, E**, SEM external views of rapheless valves; **F**, SEM internal view of raphe valve; **G**, SEM external view of rapheless valve and mantle; **H**, external view of frustule in girdle view. Scale bars: A-D, F-H, 5 μ m; E, 2 μ m.

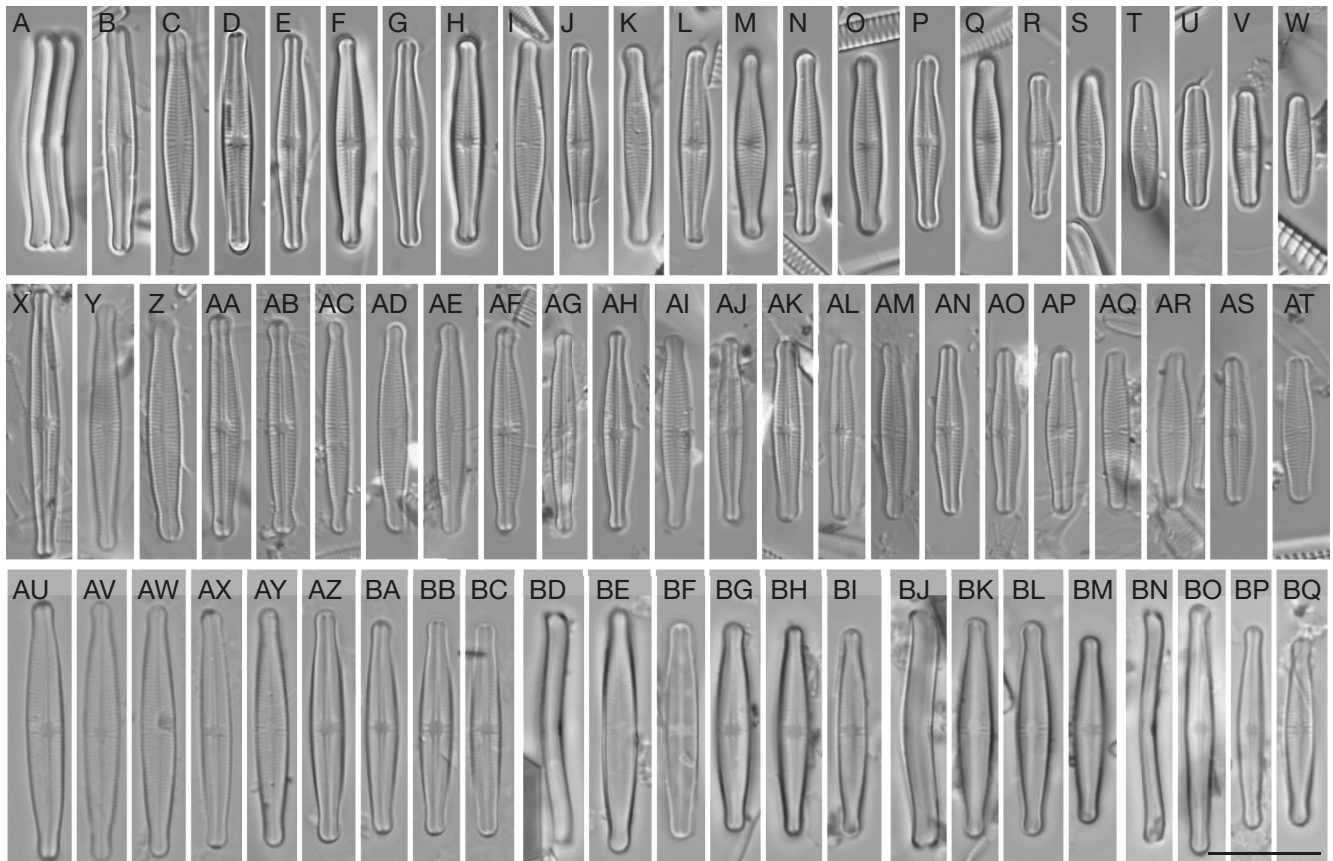


FIG. 7. — *Achnanthyidium sieminskai* Wittkowski, Kulikowski & Riaux-Gob.: **A–W**, specimens from Brunnssee, Germany; **X–AT**, specimens from Plansee, Austria; **AU–BC**, specimens from Fischkaltersee; **BD–BI**, specimens from Ammelsheiner See; **BJ–BM**, specimens from Harthsee; **BN–BQ**, specimens from Speicher Dreiwelbern; **B–BC**, **BE–BI**, **BK–BM**, **BO–BQ**, LM views of valves; **A**, **BD**, **BJ**, **BN**, LM views of frustules in girdle view. Scale bar: 10 µm.

Achnanthyidium lacuslustense sp. nov.
(Figs 17; 18)

HOLOTYPE. — **Germany**. Lustsee, 14.IV.2013, I. Jüttner (holo-, NMW[NMW.C.2013.010.2013]; iso-, BM, BR, CANA).

TYPE LOCALITY. — **Germany**. Upper Bavaria, Osterseen area, Lustsee, 47°48'33.509"N, 11°17'36.959"E.

HABITAT. — Epiphytic and epipsammic in lake littoral.

ETYMOLOGY. — The species was named after the location where it was found, the Lustsee in Upper Bavaria, Germany.

REGISTRATION. — <http://phycobank.org/103380>

DESCRIPTION

LM (Fig. 17A–M)

Frustules with strongly concave RV and convex RL with recurved poles. Valves lanceolate, margins tapering gradually towards large capitate poles. Width of poles slightly less or as wide as the maximum width at valve centre. Valve dimensions (n = 14): length 15.5–29.0 µm, width at valve centre 3–4 µm. Central area on RV a moderately broad fascia. Raphe filiform, straight. Central pores slightly expanded, terminating at margin of central area. Central area on RL rectangular, bordered by 2 short striae on each side. Axial area narrow linear on both valves. Striae radiate throughout on both valves, 28–30 in 10 µm (RV), 30 in 10 µm (RL).

SEM (Fig. 18A–H)

Raphe located on raised sternum, central raphe endings slightly expanded, tear drop-shaped, terminal raphe fissures straight with tear drop-shaped ends, terminating at valve/mantle junction. Internally, central raphe endings very slightly deflected to opposite sides, terminating on side of central node, terminal raphe endings form small helictoglossae. Striae straight on RV, composed of 3–4, rarely 2, areolae. Areolae round adjacent to axial area and at poles, others often slit-like. On RL striae straight, slightly curved at poles, composed of 3–4, rarely 2 or 5, areolae. Areolae mostly round adjacent to axial area, elsewhere often transapically elongate, internally occluded by individual hymenes. A row of transapically elongated areolae on mantle separated from areolae on valve face by a hyaline area.

RESULTS AND DISCUSSION

SHAPE ANALYSIS

Discriminant analysis (LDA) was based on the examination of 328 valves assigned to 6 taxa with the separation and overlap of the groups being illustrated in Figure 19. The results indicated that most of the explained variance was found in the first two axes and that different samples sizes did not alter shape associ-

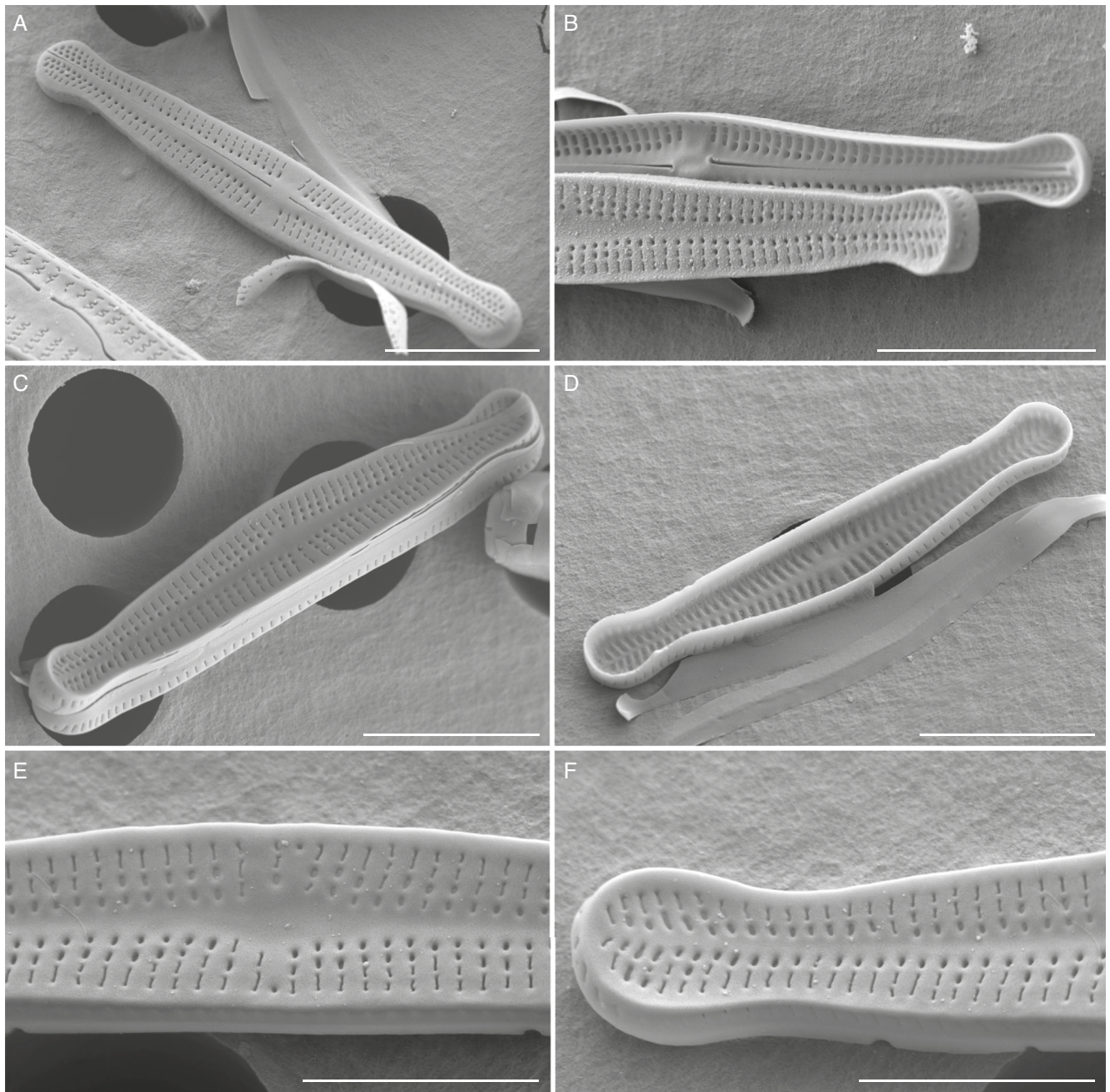


FIG. 8. — *Achnanthydium sieminskae* Witkowski, Kulikowski & Riaux-Gob., specimens from Brunsee, Germany: **A**, SEM external view of raphe valve; **B**, SEM external view of rapheless valve, internal view of raphe valve; **C**, SEM external view of rapheless valve; **D**, SEM internal view of rapheless valve; **E**, SEM external view of rapheless valve at centre; **F**, SEM external view of rapheless valve at pole. Scale bars: A-D, 5 μ m; E, F, 3 μ m.

ations. LDA showed that *Achnanthydium lacuslustense* sp. nov. had a distinct shape, while other taxa, except *A. sieminskae* and *A. neomicrocephalum*, showed minimal overlap. Three specimens of each, *A. minutissimum*, *A. caledonicum* (populations from Europe and populations from the United Kingdom), and *A. tirolense* sp. nov. showed some similarity to *A. sieminskae*. One specimen from each, *A. caledonicum* (population from Europe) and *A. tirolense* sp. nov., overlapped in shape with *A. neomicrocephalum*. There were no shape differences between the populations of *A. caledonicum* from Europe and those from the United Kingdom, or between *A. neomicrocephalum* and *A. sieminskae*.

The PCA results showed the shorter and relative to their length broader valves (smaller length/width ratio) of *A. minutissimum* present on the right side of axis one, and the more elongate valves (higher length/width ratio) of *A. caledonicum*, *A. neomicrocephalum*, and *A. sieminskae* on the left side of the biplot (Appendix 1). *Achnanthydium tirolense* sp. nov. and *A. lacuslustense* sp. nov. had valve widths and shapes transitioning between *A. minutissimum* and *A. caledonicum*, *A. sieminskae* and *A. neomicrocephalum*. Along axis two, *A. neomicrocephalum* and *A. sieminskae* were more elliptic in outline, whereas *A. caledonicum*, both populations from

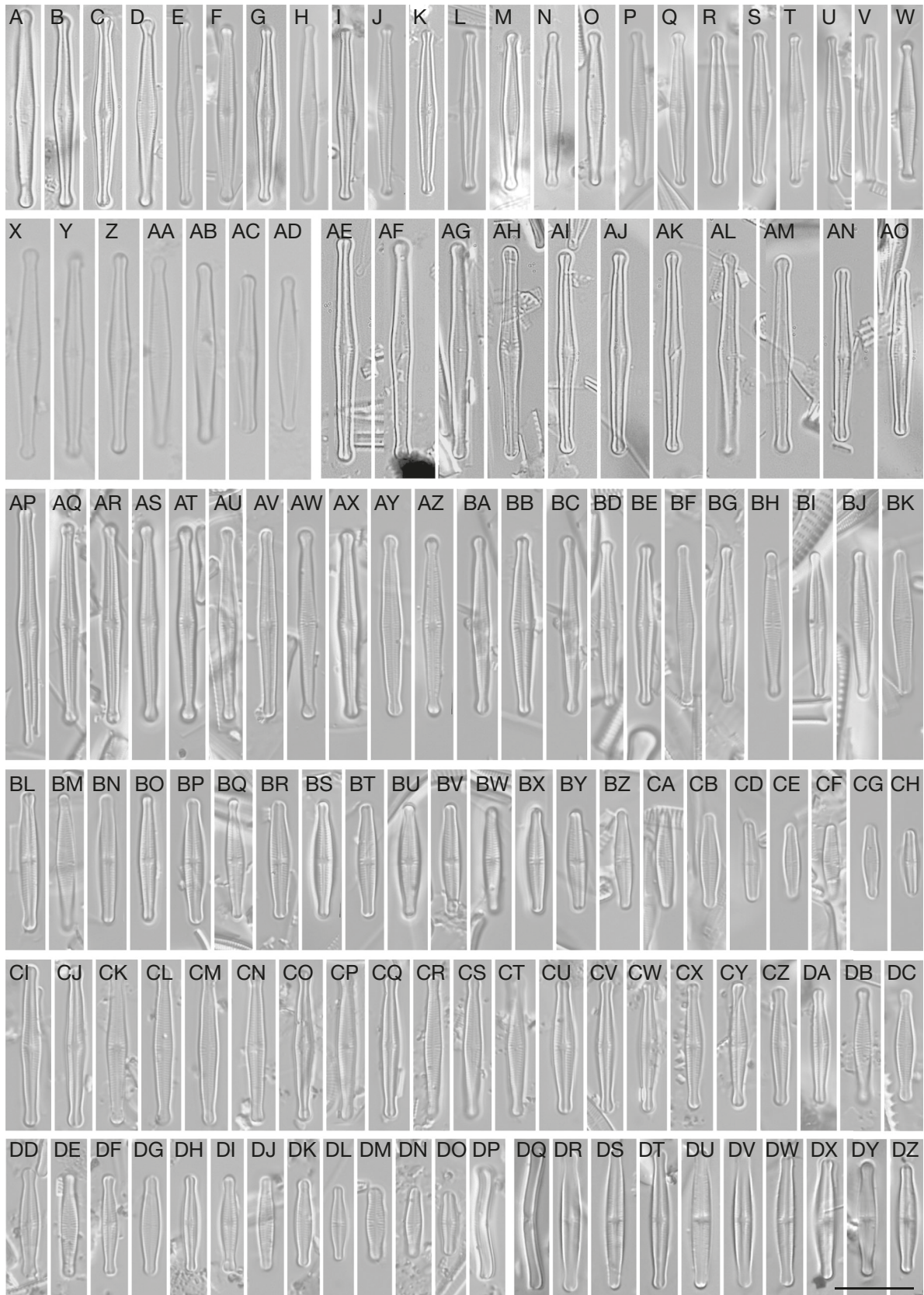


FIG. 9. — *Achnanthyidium neomicrocephalum* Lange-Bert. & F.Staab: **A–W**, specimens from Lustsee, Germany; **X–AD**, specimens from Fischkaltersee, Germany; **AE–AO**, specimens from Loch Tarff, Scotland; **AP–CH**, specimens from Ullapool River, Scotland; **CI–DP**, specimens from Llyn y Fan Fawr, Wales; **DQ–DZ**, specimens from Petta Water, Shetland Islands, Scotland; **A–DO**, **DR–DZ**, LM views of valves; **DP–DQ**, LM views of frustules in girdle view. Scale bar: 10 µm.

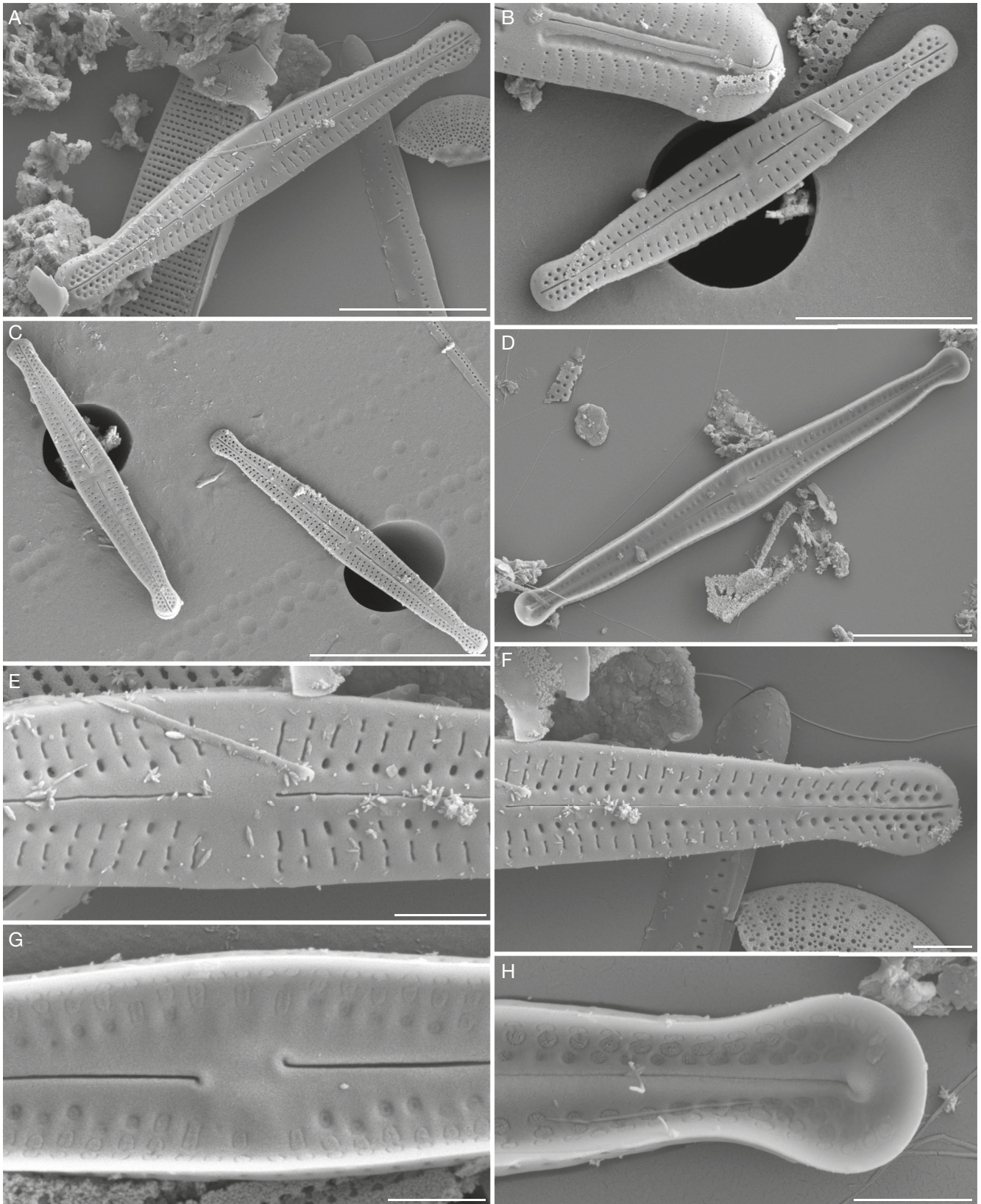


FIG. 10. — *Achnanthyidium neomicrocephalum* Lange-Bert. & F.Staab, specimens from Lustsee, Germany: **A-H**, SEM views of raphe valves; **A-C**, SEM external views of raphe valves; **D**, SEM internal view of raphe valve; **E**, SEM external view of valve centre; **F**, SEM external view of half of valve and pole; **G**, SEM internal view of valve centre; **H**, SEM internal view of valve pole. Scale bars: A, B, D, 5 μ m; C, 10 μ m; E-H, 1 μ m.

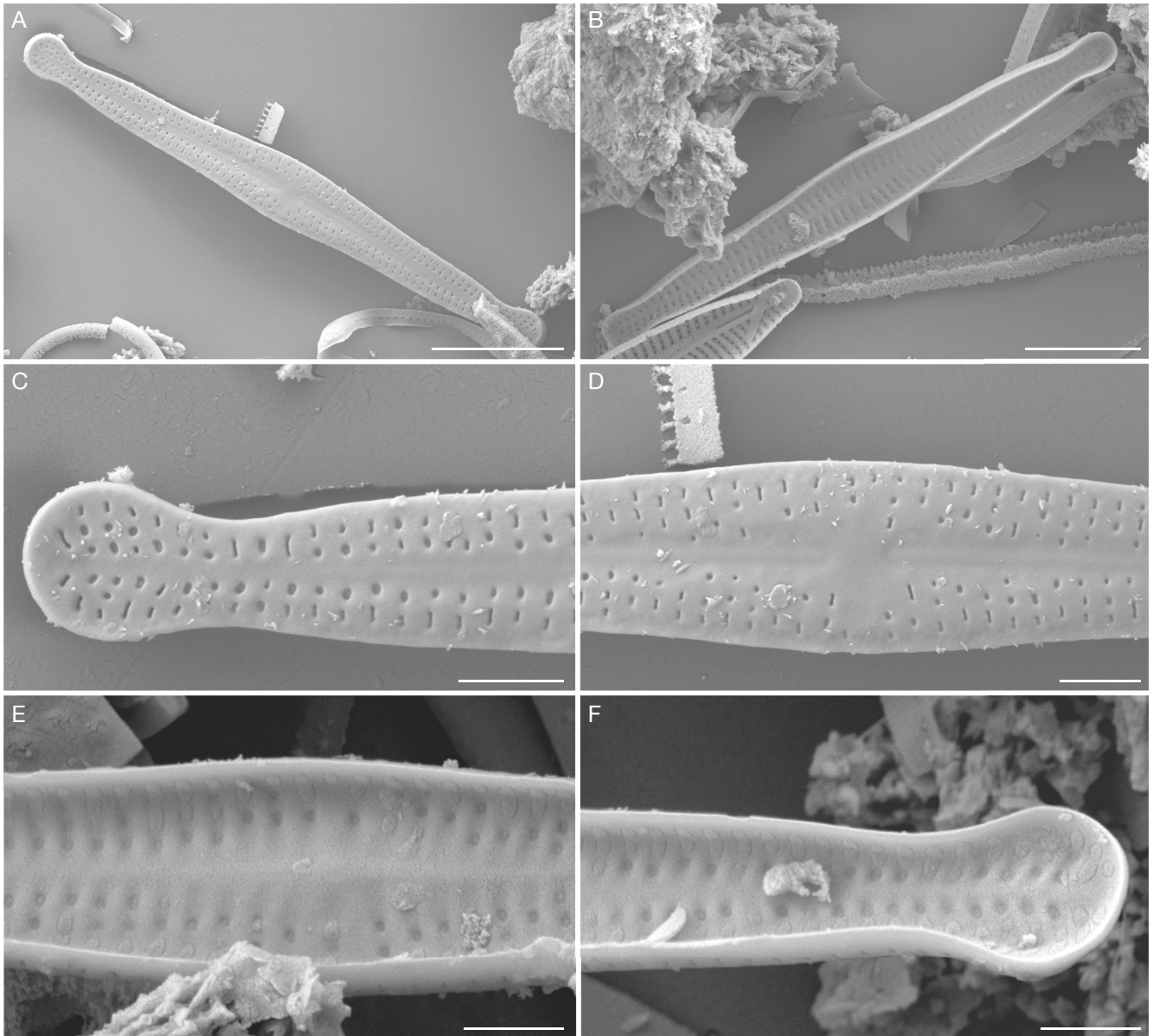


FIG. 11. — *Achnanthidium neomicrocephalum* Lange-Bert. & F.Staab, specimens from Lustsee, Germany: **A–F**, SEM views of rapheless valves; **A**, SEM external view of valve; **B**, SEM internal view of valve; **C**, SEM external view of pole; **D**, SEM external view of valve centre; **E**, SEM internal view of valve centre; **F**, SEM internal view of pole. Scale bars: A, B, 5 µm; C–F, 1 µm.

Europe and the United Kingdom, had linear valves. Likewise, *A. tirolense* sp. nov. was more elliptic compared to the more linear form of *A. lacuslustense* sp. nov.

Excluding the intermediate taxa (*A. tirolense* sp. nov. and *A. lacuslustense* sp. nov.), the PCA results showed that *A. caledonicum* was separated in shape from *A. sieminskae*, except with a small overlap from Europe. Within *A. sieminskae* there was a separation between populations from different locations (Fig. 20). The populations from England and from Europe on the right are more capitate, in contrast the populations from Scotland and Wales were more linear with less capitate poles. The LDA results comparing the different populations of *A. sieminskae* showed the same trends (Appendix 2).

TAXONOMIC TREATMENTS

The challenge of identifying species similar to *A. minutissimum* and the recognition that these taxa differ in their ecological preferences prompted a number of studies investigating the morphology and distribution of the species in more detail (Morales *et al.* 2011; Wojtal *et al.* 2011; Novais *et al.* 2015).

Based on their valve shape, the *Achnanthidium* species studied here are clearly separated in the elliptic Fourier shape analysis except for *A. sieminskae* and *A. neomicrocephalum* (Fig. 19). *Achnanthidium caledonicum* differs from the other taxa by its longer and, except *A. lacuslustense* sp. nov., wider valves, the linear shape of the valve and the large poles, which are as wide, or wider, than the maximum valve width in the centre. In all other species, the valve margins taper more clearly towards

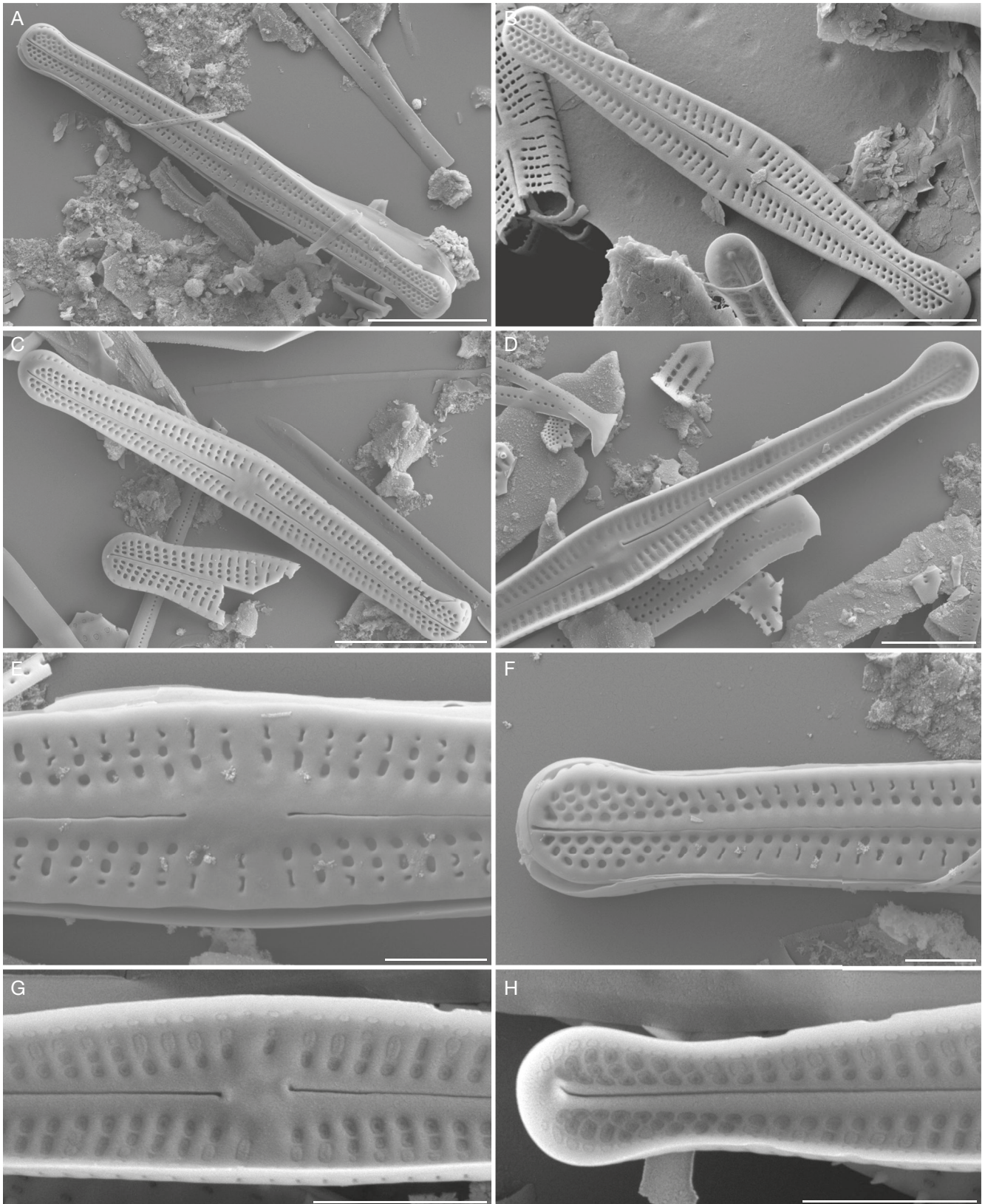


FIG. 12. — *Achnanthyidium neomicrocephalum* Lange-Bert. & F. Staab: **A, C–F**, specimens from Loch Tarff, Scotland; **B, G, H**, specimens from Llyn y Fan Fawr, Wales; **A–H**, SEM views of raphe valves; **A–C**, SEM external views of valves; **D**, internal view of valve; **E**, SEM external view of valve centre; **F**, SEM external view of valve pole; **G**, SEM internal view of valve centre; **H**, SEM internal view of valve pole. Scale bars: A–C, 5 µm; D, 3 µm; E–H, 1 µm.

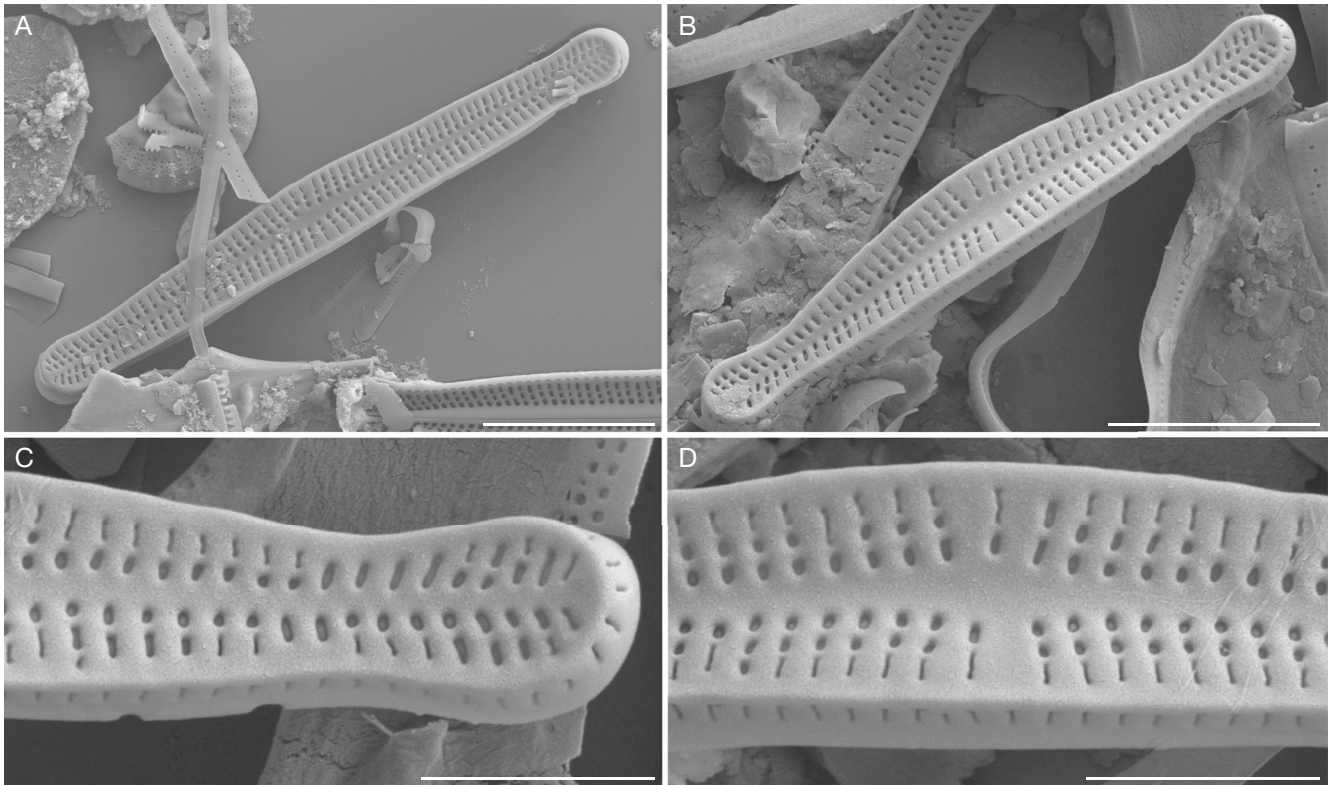


FIG. 13. — *Achnanthidium neomicrocephalum* Lange-Bert. & F.Staab: **A**, specimen from Loch Tarff, Scotland; **B-D**, specimens from Llyn y Fan Fawr, Wales; **A-D**, SEM views of rapheless valves; **A**, **B**, SEM external views of valves; **C**, SEM external view of valve pole; **D**, SEM external view of valve centre. Scale bars: A, B, 5 μ m; C, D, 2 μ m.

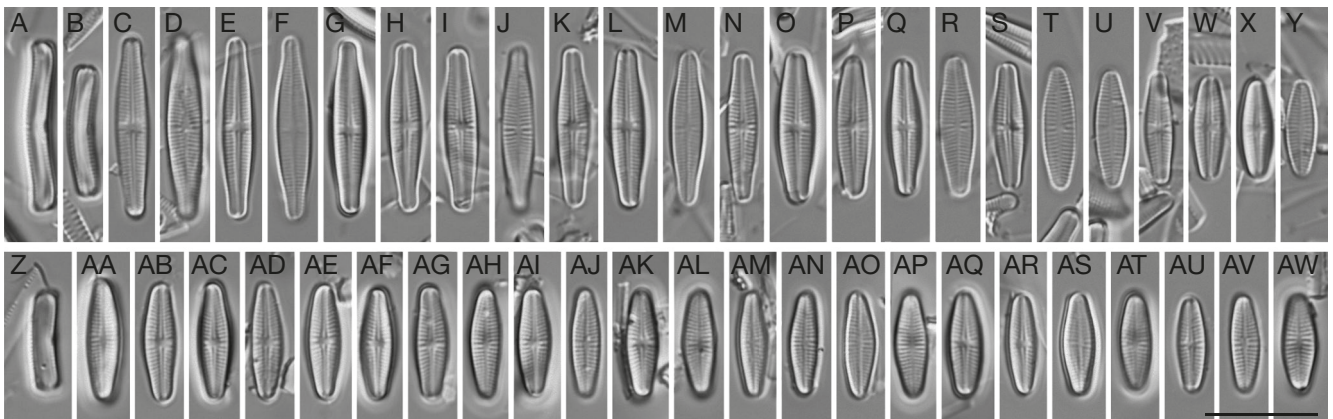


FIG. 14. — *Achnanthidium minutissimum* (Kütz.) Czarn.: **A-Q, S, T, W, Y**, specimens from Burn of Savoch, Scotland; **R, U, V, X, Z-AW**, specimens from Belti Burn, Scotland; **A, B, Z**, LM views of frustules in girdle view; **C-Y, AA-AW**, LM views of valves. Scale bar: 10 μ m.

smaller poles, except in *A. lacuslustense* sp. nov. which has large poles that can be as wide as the valve centre. *Achnanthidium caledonicum* and *A. lacuslustense* sp. nov. differ from each other in valve outline, and they differ from the other species in ultrastructure by a larger number of areolae at the poles.

Achnanthidium caledonicum is similar in valve outline to *Achnanthidium mangelsdorffii* Metzeltin & Lange-Bert. described from Cuba (Metzeltin & Lange-Bertalot 2007: 24, pl. 109, figs 11-23) although the valves of the latter are slightly narrower, 2.5-2.8 μ m vs 3.0-3.5 μ m, the poles are not as clearly

set apart as in *A. caledonicum* and the striae are less discernible. *Achnanthidium duthei* (Sreen.) Edlund, described from Ontario, Canada (Sreenivasa 1971: 79-80, figs 1A-D, 2A-C) and so far, only found in North America in nutrient poor but alkaline waters, has similar linear valves with broad poles (Edlund 1994). *Achnanthidium longissimum* P.Yu, Q.-M. You & J.P. Kociolek (Yu *et al.* 2018: 340, figs 1-40), described from Sichuan Province, China, is larger (36-48 μ m long, 4.0-4.5 μ m wide) with valve margins tapering gradually and more strongly from the valve centre towards capitate ends which are

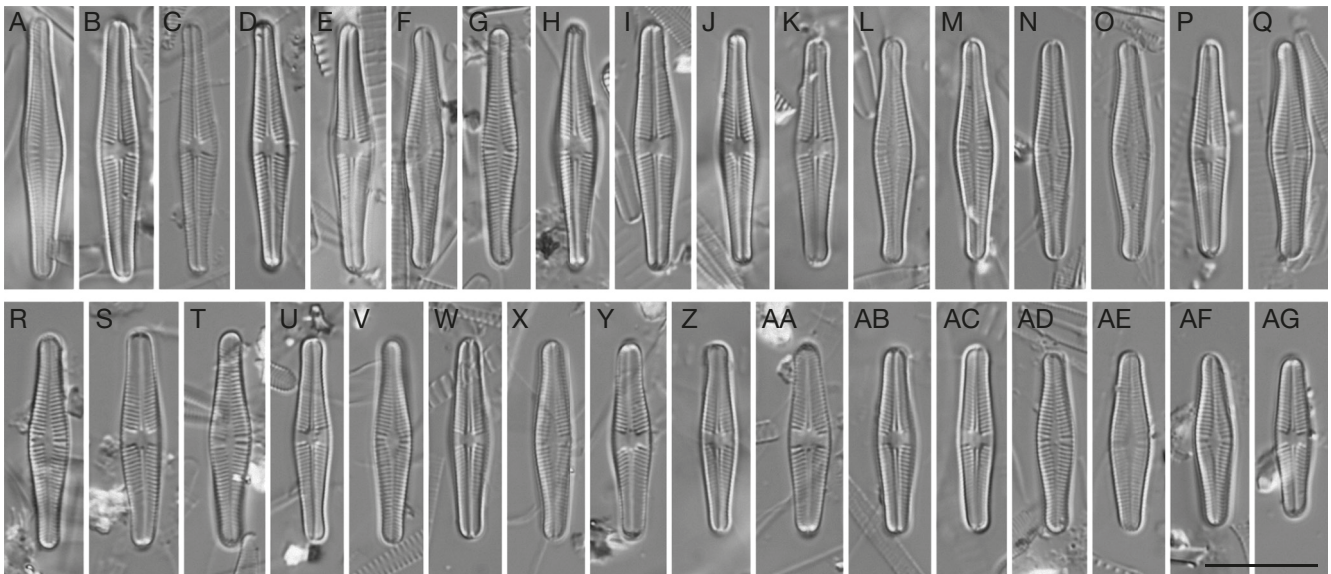


FIG. 15. — *Achnanthyidium tirolense* sp. nov., specimens from Plansee, Austria: **A–AG**, LM views of valves. Scale bar: 10 µm.

less wide than the valve centre. *Achnanthyidium chitrakootense* Wojtal, Lange-Bert. & P. Nautiyal (Wojtal *et al.* 2010: 58–59, figs 2–32), described from the Central Highlands, India, has linear valves which are slightly wider (3.4–4.2 µm). In larger valves the ends are slightly broadened but not subcapitate or capitate as in *A. caledonicum*. *Achnanthyidium chitrakootense* also differs by an indistinct or narrowly oval central area and unilaterally deflected terminal raphe fissures.

The specimens found in Brunnsee that were previously identified as smaller valves of *A. caledonicum* (Lange-Bertalot & Krammer 1989; Krammer & Lange-Bertalot 1991) and other populations from Germany, Austria and the United Kingdom resemble *A. sieminskae* which was described from and frequently found in the subantarctic region (Witkowski *et al.* 2012: figs 1–40; Van de Vijver & Kopalová 2014: figs 78–103). The populations of *A. sieminskae* from the southern hemisphere, the United Kingdom and from continental Europe cannot be separated morphologically, although there were small differences between the populations found in Scotland and Wales and those from England, Austria, and Germany. The valves of the latter are often more lanceolate, with the constriction before the poles more prominent, two areolae per striae in this part of the valve, and the poles can be more clearly protracted. Using shape analysis, both LDA and PCA showed this trend. We cannot rule out that *A. sieminskae* and *A. caledonicum* as understood in this study are in fact a complex of phenotypes or cryptic species and that genetically, the populations from the United Kingdom, continental Europe and the southern hemisphere are separate; but without further evidence from molecular studies this remains a hypothesis. A molecular phylogeny of representatives of the *A. minutissimum* complex including specimens from the Arctic, sub-Antarctic, Europe, Russia and North America showed several distinct lineages, with some being morphologically distinct while others were difficult to separate (Pinseel *et al.* 2017). Variability of the

valve outline, central area and areola numbers within strains assigned to the same morphodeme suggested clear intraspecific phenotypic variation although the extent of it requires further morphological and molecular studies across a large geographical area. Pérez-Burillo *et al.* (2021) showed that amplicon sequence variants of a short *rbcL* barcode within the *A. minutissimum* complex were linked to distribution and ecological groups with opposite responses to chemical conditions, i.e., calcium and conductivity. It is conceivable that, as in *A. minutissimum*, there are also several lineages in *A. sieminskae*. Unfortunately, the current study lacks data on water chemistry across all study sites, and no data are available from most locations in the United Kingdom, or from Bavaria (Germany) and Austria. We assume, however, that locations in the calcareous northern Alps (Plansee) and their foothills (Brunnsee) are likely more alkaline and more calcium-rich compared to the locations in the United Kingdom. The lakes in Saxony had much higher conductivity (> 400 µS/cm) than the lake Llyn y Fan Fawr in Wales (30 µS/cm), and both contained *A. sieminskae*. While morphologically, our populations are difficult to distinguish, further molecular studies could reveal whether genetic variability in this species also correlates with patterns in distribution and differences in water chemistry.

Small and medium sized valves of *A. sieminskae* can be difficult to distinguish from *A. neomicrocephalum*, and both taxa are not separated in shape analysis. However, medium and larger valves of *A. neomicrocephalum* have a small inflation at the valve centre and the valve margins taper from the valve centre to small capitate poles. In *A. sieminskae* the margins taper closer to the poles. Both taxa also differ in ultrastructure. The striae in *A. neomicrocephalum* are composed of a smaller number of areolae, often two and sometimes three, while in *A. sieminskae* the striae are composed mostly of 3–4 areolae. *Achnanthyidium sieminskae* is distinguished in shape analysis from *A. minutissimum*, the valves are longer and have protracted

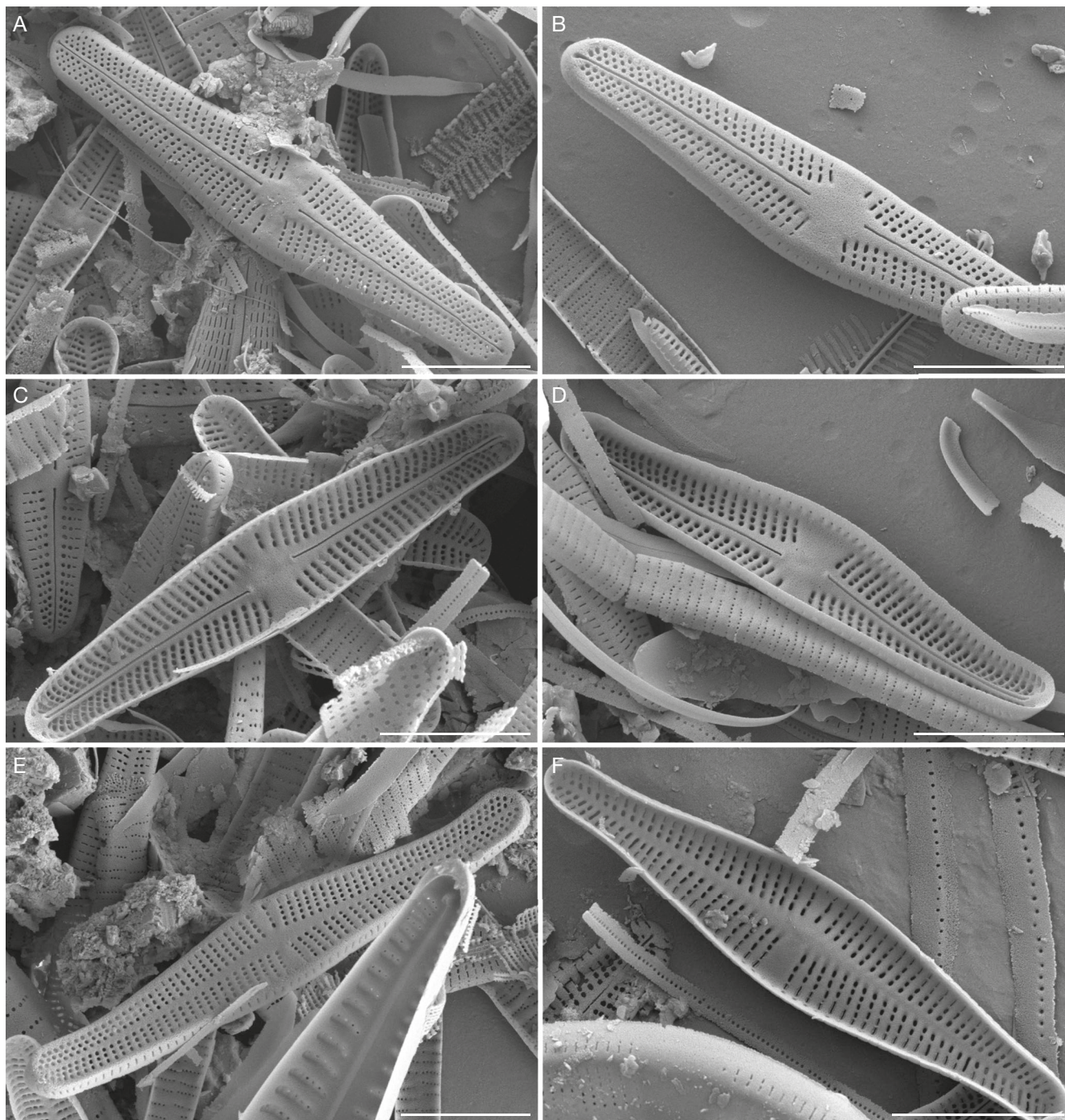


FIG. 16. — *Achnanthidium tirolense* sp. nov., specimens from Plansee, Austria: **A, B**, SEM external views of raphe valves; **C, D**, SEM internal views of raphe valves; **E**, SEM external view of rapheless valve; **F**, SEM internal view of rapheless valve. Scale bars: 5 μ m.

subcapitate to capitate poles in contrast to short protracted rostrate poles in *A. minutissimum*, as seen in the populations from Scotland. Our description of the latter conforms to the type of *A. minutissimum* examined in Novais *et al.* (2015) and Marquardt *et al.* (2017). *Achnanthidium sieminskae* is similar to a number of species. *Achnanthidium ertzii* (Van de Vijver *et al.* 2011a: 200, figs 26-47), described from Madeira, Portugal, has linear-lanceolate valves, with margins tapering to long protracted subcapitate to capitate poles, and the striae on

the raphe valve are composed of 2-3 areolae. *Achnanthidium barbei* Le Cohu & Peres (Peres *et al.* 2014: 388, figs 1-40) has slightly wider (2.6-3.7 μ m) lanceolate valves with margins tapering more strongly closer to the valve ends. The valve margins in *Achnanthidium polonicum* Van de Vijver, Wojtal, E. Morales & Ector (Wojtal *et al.* 2011: 223, figs 131-154), a species described from a spring-fed flush in Poland, also taper gradually from the valve centre but the central area is a broad transverse fascia. *Achnanthidium ennediense* (Compère) Com-

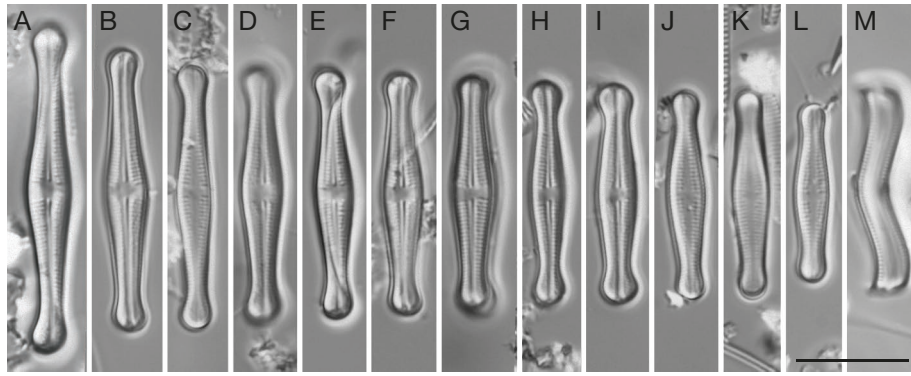


FIG. 17. — *Achnanthyidium lacuslustense* sp. nov., specimens from Lustsee, Germany: **A–L**, LM views of valves; **M**, LM view of frustule in girdle view. Scale bar: 10 μ m.

père & Van de Vijver (Compère & Van de Vijver 2011: 7, 11, figs 1–58) from the Ennedi mountains, Republic of Chad, has linear-lanceolate valves with margins that taper gradually from the valve centre and short protracted, rostrate ends. *Achnanthyidium acsiae* Wojtal, E. Morales, Van de Vijver & Ector (Wojtal *et al.* 2011: 226–227, figs 155–184), found in sulphur-rich springs in Poland, is a smaller species without recurved poles (7–16 μ m vs 10–26 μ m long). *Achnanthyidium neotropicum* K.J. Krahn & C.E. Wetzel (Krahn *et al.* 2018: 91–92, figs 2–88), described from a volcanic crater lake in El Salvador, is more linear in shape, and hence similar to some of the specimens observed in the United Kingdom populations. The central area of *A. neotropicum* is often a broad, rectangular fascia and longer valves can be constricted in the central part of the valve.

Achnanthyidium tirolense sp. nov., clearly separated from the other taxa in shape analysis, has a characteristic rhombic-lanceolate valve outline. It is similar to *Achnanthyidium affine* (Grunow) Czarn. (Czarnecki 1994: 156) whose type material from Belgium was recently investigated (Van de Vijver *et al.* 2021: figs 1–50). In contrast to the latter *Achnanthyidium tirolense* sp. nov. is rhombic-lanceolate in valve outline with a clearly inflated valve centre, the margins taper from the valve centre and the poles are long protracted. In *Achnanthyidium jackii* Rabenh. (Rabenhorst 1861, no. 1003) valve ends are short protracted and more narrowly rounded, the valve centre is not inflated, and the central area (RV) is a rectangular fascia (Van de Vijver *et al.* 2018: figs 1–22). *Achnanthyidium eutrophilum* (Lange-Bert.) Lange-Bert. (Lange-Bertalot 1999: 277; Hlúbíková *et al.* 2011: figs 104–117, 161–165; Novais *et al.* 2011: figs 37–77) and *Achnanthyidium exile* (Kütz.) Heib. (Heiberg 1863: 119) have rhombic valves without an inflated valve centre and more narrowly rounded ends, and the central areas are indistinct and small rounded or elliptic, respectively. *Achnanthyidium caravelense* Novais & Ector (Novais *et al.* 2011: 142, figs 1–36) from Portugal has linear-lanceolate valves without long protracted ends and lacks a central fascia. *Achnanthyidium lacustre* P.Yu, Q.-M. You & Kociolek (Yu *et al.* 2019a: 34, figs 1–20, 90–105), described from Taiping Lake, a large artificial reservoir in the Anhui Province, China, is similar in shape but the poles are more narrowly rounded. It also lacks the acute-angled fascia on the RV but has a small oval central area bordered by slightly shortened,

wider spaced striae. *Achnanthyidium limosua* P.Yu, Q.-M. You & Q.-X. Wang (Yu *et al.* 2019b: 158–159, figs 232–292) from the Jiuzhai Valley Nature Reserve, Sichuan Province, China, is smaller, 7.4–11.4 μ m vs 14–23.5 μ m long, has short protracted rostrate to subcapitate poles and a small central area formed by slightly shortened and wider spaced central striae. In *Achnanthyidium confusa* Bourr. & Manguin, a species described from the Iles Kerguelen (Bourrelly & Manguin 1954: 20, pl. 1, figs 12a, b), the valve margins do not taper gradually from the valve centre but taper more towards the slightly protracted valve ends, and the central area on the RV, and sometimes on the RL, is a rectangular fascia (Van de Vijver & Beyens 1996). *Achnanthyidium pseudolineare* Van de Vijver, Novais & Ector has linear-lanceolate to linear-elliptic valves with a narrow, rectangular fascia and without a central inflation (Van de Vijver *et al.* 2011b: 186, figs 208–256; Novais *et al.* 2015: figs 270–333). *Achnanthyidium palmeti* Gassiole, Le Cohu & M. Coste from La Réunion (Gassiole *et al.* 2013: 22–24, figs 1–27) and *Achnanthyidium costei* Peres & Le Cohu from France (Peres *et al.* 2014: 390, figs 41–80) have shorter protracted, rostrate or subcapitate poles and the former lacks a transverse fascia. *Achnanthyidium dolomiticum* Cantonati & Lange-Bert. from Italy (Cantonati & Lange-Bertalot 2006: 1185, figs 1–3) is smaller (5–10 μ m long, 2.5–3.5 μ m wide), linear-elliptic to linear-rhombic, lacks a central inflation and has short protracted, subrostrate ends (Hlúbíková *et al.* 2011: figs 35–72). *Achnanthyidium tropicocatenatum* Marquardt, C.E. Wetzel & Ector (Marquardt *et al.* 2017: 318, figs 3–5), described from a reservoir in Brazil, and *Achnanthyidium catenatum* (J. Bílý & Marvan) Lange-Bert. (Hlúbíková *et al.* 2011: figs 1–34; Marquardt *et al.* 2017: figs 6AG–AV, 8) have a similar valve outline with an inflated central part of the valve, but their poles are broader subcapitate to capitate, and in girdle view the poles are strongly recurved towards the rapheless valve. *Achnanthyidium sehuencoense* E. Morales from streams in Bolivia (Morales *et al.* 2009: 273, 275, figs 8–14, 21–26) and *Achnanthyidium peruvianum* E. Morales & Ector from a river in Peru (Morales *et al.* 2011: 121, figs 211–224) have a narrow rectangular fascia, elliptic-linear or more or less linear valves, respectively, and both species lack a central inflation.

Achnanthyidium lacuslustense sp. nov. is similar in valve outline to *A. catenatum* and *A. tropicocatenatum*. The latter species

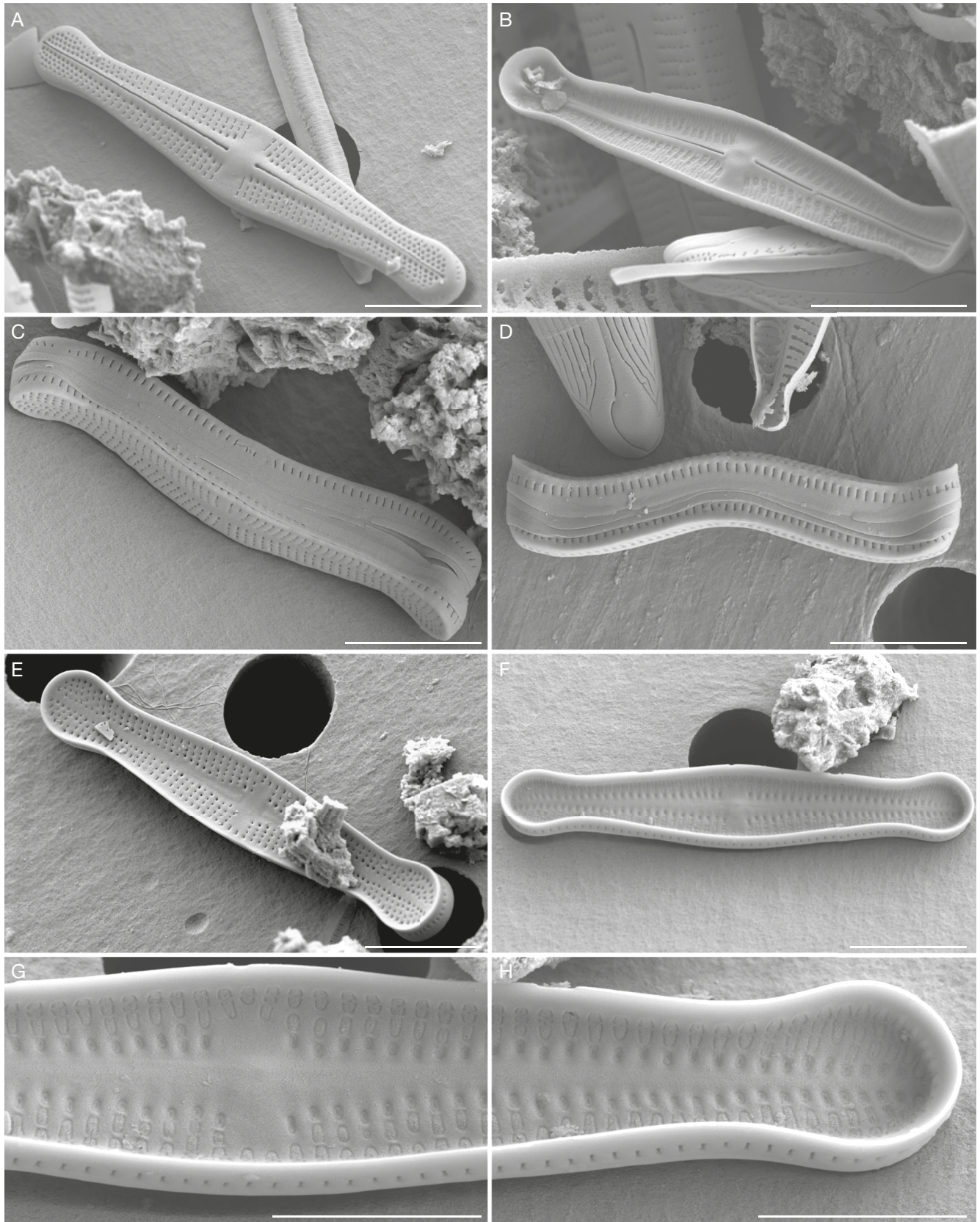


FIG. 18. — *Achnanthyidium lacuslustense* sp. nov., specimens from Lustsee, Germany: **A**, SEM external view of raphe valve; **B**, SEM internal view of raphe valve; **C**, **D**, SEM external views of frustules in girdle view; **E**, SEM external view of rapheless valve; **F**, SEM internal view of rapheless valve; **G**, SEM internal view of centre of rapheless valve; **H**, SEM internal view of pole of rapheless valve. Scale bars: A-F, 5 µm; G, H, 3 µm.

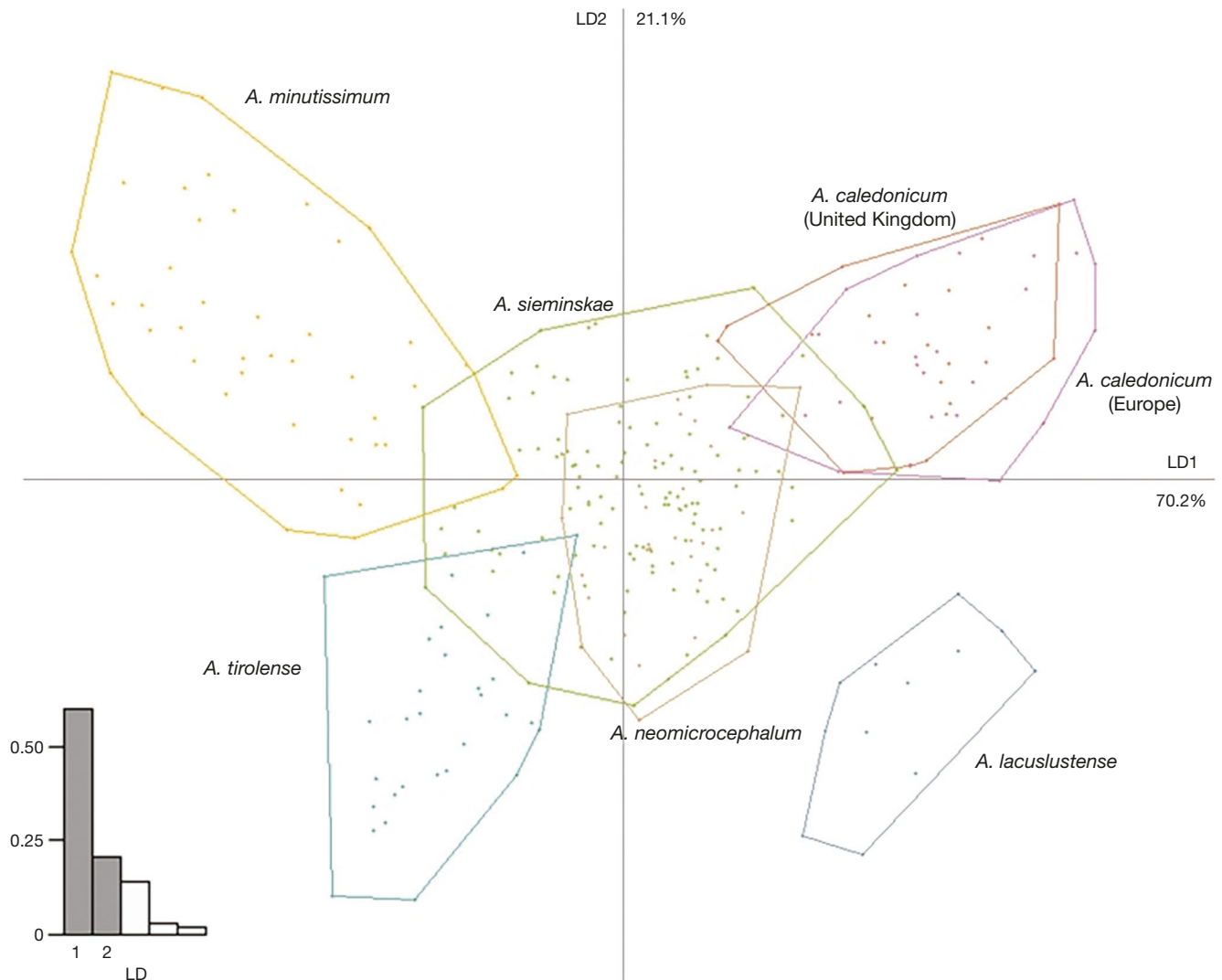


FIG. 19. — Linear discriminant analysis of elliptic Fourier shape harmonics for identified species. The biplot presents the first 2 axes with a total explained variance of 81.3%.

have broad ends which are almost as wide as the valve centre but lack a transverse fascia (RV) or rectangular central area (RL). In contrast to *Achnanthidium lacuslustrense* sp. nov. their frustules in girdle view are only slightly curved in the central part of the valve but they share the recurved poles. *Achnanthidium macrocephalum* (Hust.) Round & Bukht. (Round & Bukhtiyarova 1996: 349; Wetzel *et al.* 2019: figs 1A-M, 2A-K) and *Achnanthidium coxianum* Jüttner, Ector & C.E. Wetzel (Wetzel *et al.* 2019: 347, figs 1AL-BH, 5) have a similar valve outline but are much smaller taxa (7–12 µm long, 2.5–3.0 µm wide and 8–13 µm long, 2.5–3.0 µm wide, respectively) and have small rounded or oval central areas.

DISTRIBUTION

Achnanthidium species are abundant in freshwaters and found worldwide across a range of chemical and hydrological conditions, and habitat types (Cantonati & Lange-Bertalot 2006; Potapova & Hamilton 2007; Wojtal *et al.* 2011). Many are found in locations where human pressure is low and several

new species have been described from protected areas (e.g. Yu *et al.* 2019b; Cantonati *et al.* 2020). Some might be indicators of high habitat quality, for example *Achnanthidium acerosum* (Van de Vijver *et al.* 2011a) was described from a stream in Sweden with low concentrations of nutrients and a high diversity of diatom species. Other species, such as *A. eutrophilum* and *Achnanthidium saprophilum* (H. Kobayasi & Mayama) Round & Bukht., appear to be tolerant of higher nutrient levels and pollution (Mayama & Kobayasi 1984; Potapova & Hamilton 2007; Wojtal *et al.* 2011).

In *Achnanthidium* species identification and differentiation between populations using morphology can be challenging (Ector 2011; Kennedy & Buckley 2021). Due to this difficulty, the combination of morphologically similar species into groups is common for assessments of ecological status. This approach improves taxonomic consistency across analysts (Charles *et al.* 2021), and apparently similar classifications are found when using a less refined taxonomic resolution in acidity and pollution indices (Bigler *et al.* 2010). The first

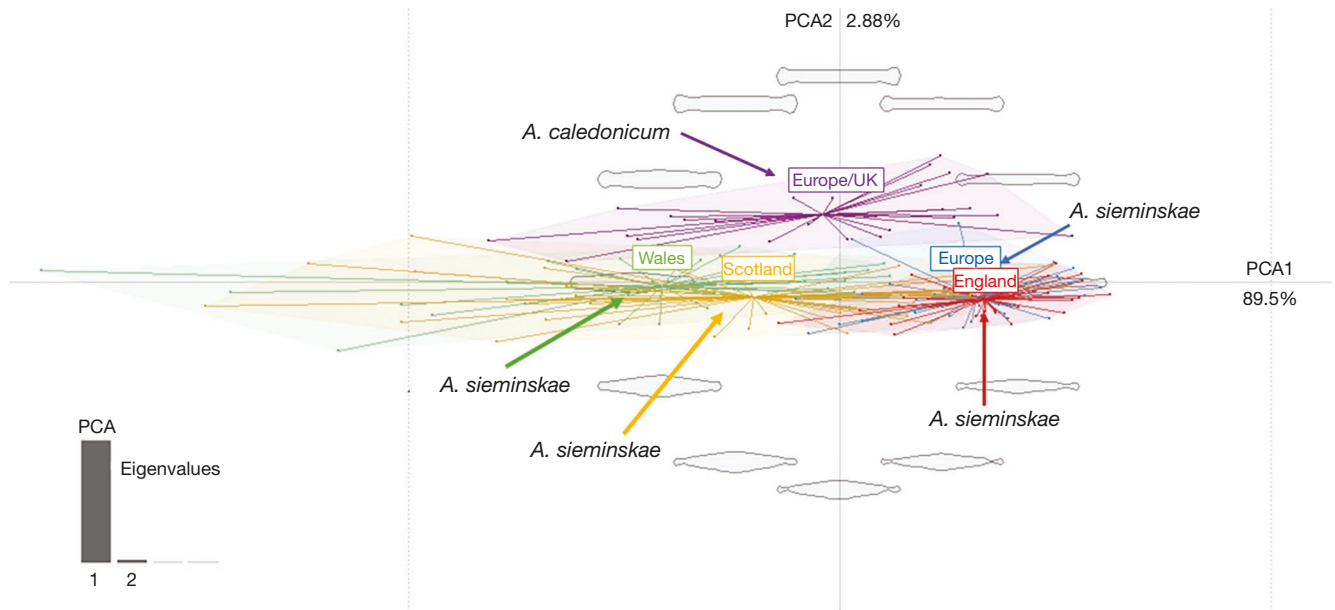


FIG. 20. — Principal component analysis of elliptic Fourier shape harmonics for specimens of *Achnanthyidum sieminskiae* Witkowski, Kulikowsky & Riaux-Gob. from different geographic regions (Europe, Wales, Scotland and England). Specimens of *Achnanthyidum caledonicum* (Lange-Bert.) Lange-Bert. are used as the outgroup. The biplot presents the first two axes with a total explained variance of 92.4 %.

version of the Trophic Diatom Index (TDI: Kelly & Whittton 1995), used in the United Kingdom and Ireland, for example, reflected the confusion in the taxonomic literature at the time, with many poorly described “Sippen-Komplexe” (Krammer & Lange-Bertalot 1991). In light of this, a decision was taken to differentiate only capitulate forms (using the old name “*Achnanthes microcephala*”), which were recognised as being more sensitive to nutrients than most other forms. Originally, other capitulate forms (such as the then yet-to-be described *A. neomicrocephalum*) would naturally have been included here. However, over the years, well-meaning attempts to keep nomenclature as current as possible has resulted in this category now being referred to as ‘*A. caledonicum*’, indicating high ecological status, with the assumption that *A. neomicrocephalum* and other capitulate forms are excluded. *Achnanthyidum minutissimum sensu lato* is regarded as an indicator of good ecological status (e.g. Almeida *et al.* 2014; Kelly *et al.* 2014). However, this often reflects a primary focus by regulators on the nutrient-organic pollution gradient and there are several reports that this group has been found frequently in waters polluted by toxic effluents and metals (e.g. Lainé *et al.* 2014; Falasco *et al.* 2021 and references therein). As an r-strategist, the ability of *A. minutissimum* to recolonise quickly and it being tightly attached to the substratum while other high-profile species are lost might help it to survive in some polluted environments (Biggs *et al.* 1998; Morin *et al.* 2008; Lainé *et al.* 2014). The abundance and wide distribution of *A. minutissimum* across a range of conditions in large scale surveys represented most likely the distribution of a complex of species (Heino *et al.* 2010; Virtanen *et al.* 2011). Hence studies that seek to explain pattern in diversity and geographical distribution as well as those primarily concerned with the assessment of water quality, would benefit from rec-

ognising species instead of recording groups which most likely comprise several species that are ecologically distinct (Jüttner *et al.* 2013). Potapova & Hamilton (2007) used geometric morphometric shape analysis to distinguish between different morphological groups within the *A. minutissimum* complex in North American rivers and showed that there were ecological shifts along gradients of pH, conductivity, phosphorus, and nitrogen. Using benthic diatom samples from the Catalan and French biomonitoring networks and the amplicon sequence variants (ASVs) of a *rbcl* barcode, Pérez-Burillo *et al.* (2021) showed that significant genetic variability within the *A. minutissimum* complex was linked with distribution of ASVs and environmental variation. To support a more refined approach in recording species in *Achnanthyidum*, there is a growing literature resource with clear documentation of the taxa in light microscopy and evidence for distinct habitat preferences for example for species found in Europe (Van de Vijver *et al.* 2011a, b; Wojtal *et al.* 2011; Novais *et al.* 2015) and in Asia (Wojtal *et al.* 2010; Karthick *et al.* 2017; Yu *et al.* 2018, 2019a, b; You *et al.* 2019; Miao *et al.* 2020).

In this study we examined the distribution of four *Achnanthyidum* species, identified based on their morphology, in rivers, streams, and lakes of Scotland in relation to land use (Fig. 21). They included *A. caledonicum* and *A. neomicrocephalum*, both regarded as important indicator species in oligosaprobic and oligotrophic freshwaters which can be distinguished from other *Achnanthyidum* species more tolerant of nutrient enrichment (Lange-Bertalot in Krammer & Lange-Bertalot 2004). Of the 52 locations investigated, 28 were in areas of low human impact and contained *A. sieminskiae* (23 sites), *A. neomicrocephalum* (18 sites) and/or *A. caledonicum* (8 sites). *Achnanthyidum caledonicum* was found in lakes and in one river of the north-west Scottish Highlands. All sites were in

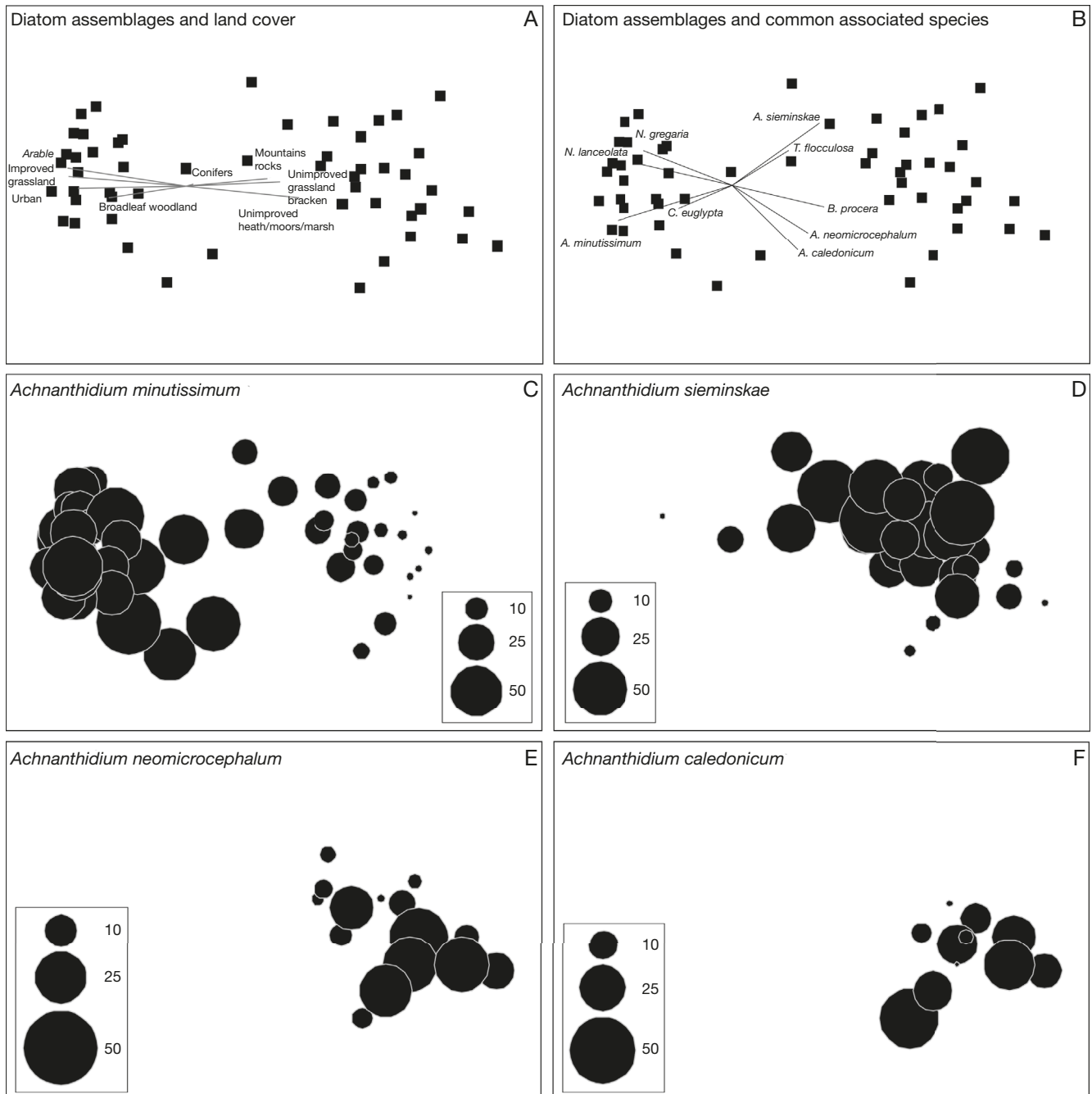


FIG. 21. — Relationships between diatom assemblages, four *Achnanthyidum* Kütz. species and land use in 52 streams, rivers and lakes of Scotland, United Kingdom. **A, B**, NMDS ordination (2D stress 0.1) of Bray-Curtis similarities of diatom assemblages: **A**, the vector plot shows the direction of linear increase in land use types and the multiple correlation of each of the land use categories with the diatom assemblages; **B**, the vector plot shows the direction of linear increase in the most abundant diatom species and the multiple correlation of each species with the diatom assemblages. **C–F**, bubble plots of species: the relative abundance of four diatom species in 52 diatom assemblages is represented by the circle size: **C**, *Achnanthyidum minutissimum* (Kütz.) Czarn.; **D**, *Achnanthyidum sieminskai* Witkowski, Kulikowski & Riaux-Gob.; **E**, *Achnanthyidum neomicrocephalum* Lange-Bert. & F.Staab; **F**, *Achnanthyidum caledonicum* (Lange-Bert.) Lange-Bert.

mountain areas and the dominant land cover comprised heath and upland unimproved grassland, and often rock outcrops. *Achnanthyidum caledonicum* occurred at these locations with *A. sieminskai* or *A. neomicrocephalum* or with both. Although *A. caledonicum* is regularly mentioned in the literature, there are few photographic records that resemble the type population. The presence of a second species in the sample from Brunsee that was earlier identified as *A. caledonicum* (Lange-

Bertalot & Krammer 1989: pl. 55, fig. 4, valve on left, fig. 10; Krammer & Lange-Bertalot 1991: pl. 34, figs 4, 6) might have led to erroneous records, and possibly, inaccurate reporting of the distribution of *A. caledonicum*. Lange-Bertalot (Lange-Bertalot & Krammer 1989) wrote that Carter found the species in several oligotrophic waters of Scotland. In central Europe *A. caledonicum* was reported from calcium-rich, oligotrophic to weakly mesotrophic lakes in the Alps and the

alpine foothills, but rarely from running waters, and scattered records exist from nutrient-poor lakes in the German central highlands and the northern lowlands (Lange-Bertalot *et al.* 2017). In southern Poland, *A. caledonicum* was common in a variety of habitats including springs, seepages, and lakes in the High Tatra Mountains, in circumneutral and slightly alkaline waters with low conductivity (Wojtal *et al.* 2011). For our records in Scotland, we have water chemistry data from only two lakes where conductivity was 66 and 171 µS/cm, and pH was 6.8 and 7.1.

The distribution of *A. minutissimum*, *A. sieminskae*, *A. neomicrocephalum* and *A. caledonicum* in Scotland was compared in relation to land use. For ecological status assessments using the United Kingdom trophic diatom index, *A. minutissimum*, *A. sieminskae*, and *A. neomicrocephalum* would be recorded as one group, ‘*Achnanthidium minutissimum* type’, and indicate good status. *Achnanthidium sieminskae* and *A. neomicrocephalum* were only found in streams or lakes in areas with low human impact (i.e., settlements and improved grassland were absent or very rare and seminatural vegetation was dominant). *Achnanthidium sieminskae* was more common and abundant than *A. caledonicum* and *A. neomicrocephalum*, probably because most of the sites sampled were lowland in character. The most dominant land use in the catchments of these sites was heath, and occasionally unimproved grassland and conifers. Outside Scotland, *Achnanthidium sieminskae* was found at several unpolluted sites, such as Llyn y Fan Fawr, an upland lake in the Brecon Beacons National Park, Wales, in the upper section of the River Ehen, Cumbria, England (a Special Area of Conservation), in the oligotrophic Brunnsee in Bavaria, Germany, and in some nutrient-poor lakes in Saxony, Germany. As with *A. caledonicum*, *A. neomicrocephalum* was more common in mountainous areas, but here more often in catchments with heath in contrast to unimproved upland grassland that seems more typical in catchments where *A. caledonicum* was found. Since the number of sites in mountain areas was relatively low in our study this would have to be confirmed by a wider survey at higher altitudes. Although a relatively small number of illustrated records of *A. sieminskae* and *A. neomicrocephalum* exist, they are all from unpolluted waters in the northern and southern hemisphere (Krammer & Lange-Bertalot 2004; Bahls 2009; Cantonati & Lange-Bertalot 2010; Van de Vijver & Kopalová 2014 and references therein; Peeters & Ector 2018; Silva-Lehmkuhl *et al.* 2019) confirming the ecological preferences for clean waters suggested by their distribution in Scotland. In contrast, *A. minutissimum* was the dominant species in 84 % of sites located in areas where either arable fields or improved grassland, or both, were the dominant land use and urban settlements were common. Species associated with *A. minutissimum*, most frequently *Navicula gregaria* Donkin and *Navicula lanceolata* Ehrenb., are known to be tolerant to pollution. In contrast, species associated with *A. caledonicum*, *A. neomicrocephalum*, and *A. sieminskae* in particular species in *Brachysira* Kütz., *Tabel-laria* Ehrenb. ex Kütz. and *Fragilaria* Lyngb., are typically

found in unpolluted waters (Kelly *et al.* 2008; Jüttner *et al.* 2012). Given the distribution and species associations of *A. minutissimum*, there is a possibility that this species is more pollution tolerant than *A. caledonicum*, *A. neomicrocephalum*, and *A. sieminskae*. On the other hand, land use is also linked to bedrock geology, so the possibility that alkalinity is also higher at sites with *A. minutissimum* and is influencing the diatom assemblages cannot be ruled out (Kelly *et al.* 2020).

Limitations of the present data allow us to conclude that these species have different ecological preferences but, without being able to test their response to more environmental variables, it is not possible to make authoritative statements of their tolerances to particular variables. Nonetheless, our results do suggest that it would be prudent to revise the treatment of *Achnanthidium* species in ecological status assessments, and to distinguish between taxa often aggregated under *A. minutissimum sensu lato* in ecological and biogeographical studies.

CONCLUSION

Species in the genus *Achnanthidium* are widely distributed in freshwaters and the correct evaluation in ecological status assessments is important for environmental assessments and subsequent decision making. The challenge of identifying these taxa, in combination with inadequate knowledge about their morphology, ecology and distribution, has led to simplifications in indices, ecological assessments, or wrong classifications. Thus, the use of diatom-based indices has been discouraged for certain types of pollution (Lainé *et al.* 2014). There is clearly a need for further investigation: a combination of molecular and morphological methods could help to delineate species and combined with simultaneous studies of their distribution lead to a better understanding of the ecology and biogeography of these important taxa. This would be useful in waters affected by different types of pollution but also in clean water environments where several *Achnanthidium* species can co-occur (Cantonati *et al.* 2020), and the combined use of DNA barcodes and identifications in microscopy have proven useful (Rimet *et al.* 2018).

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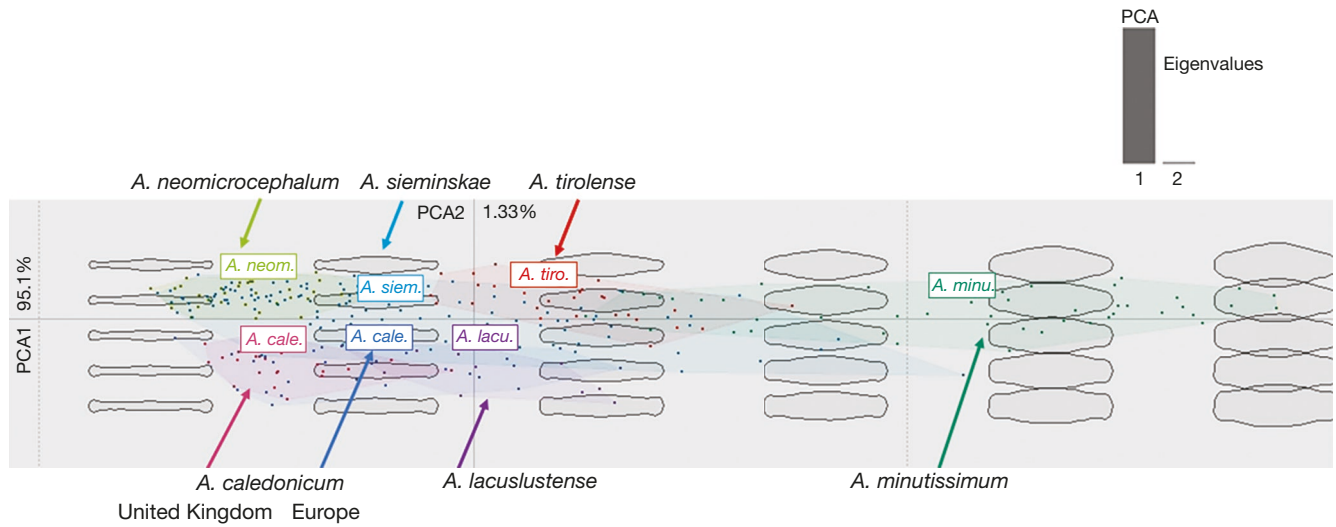
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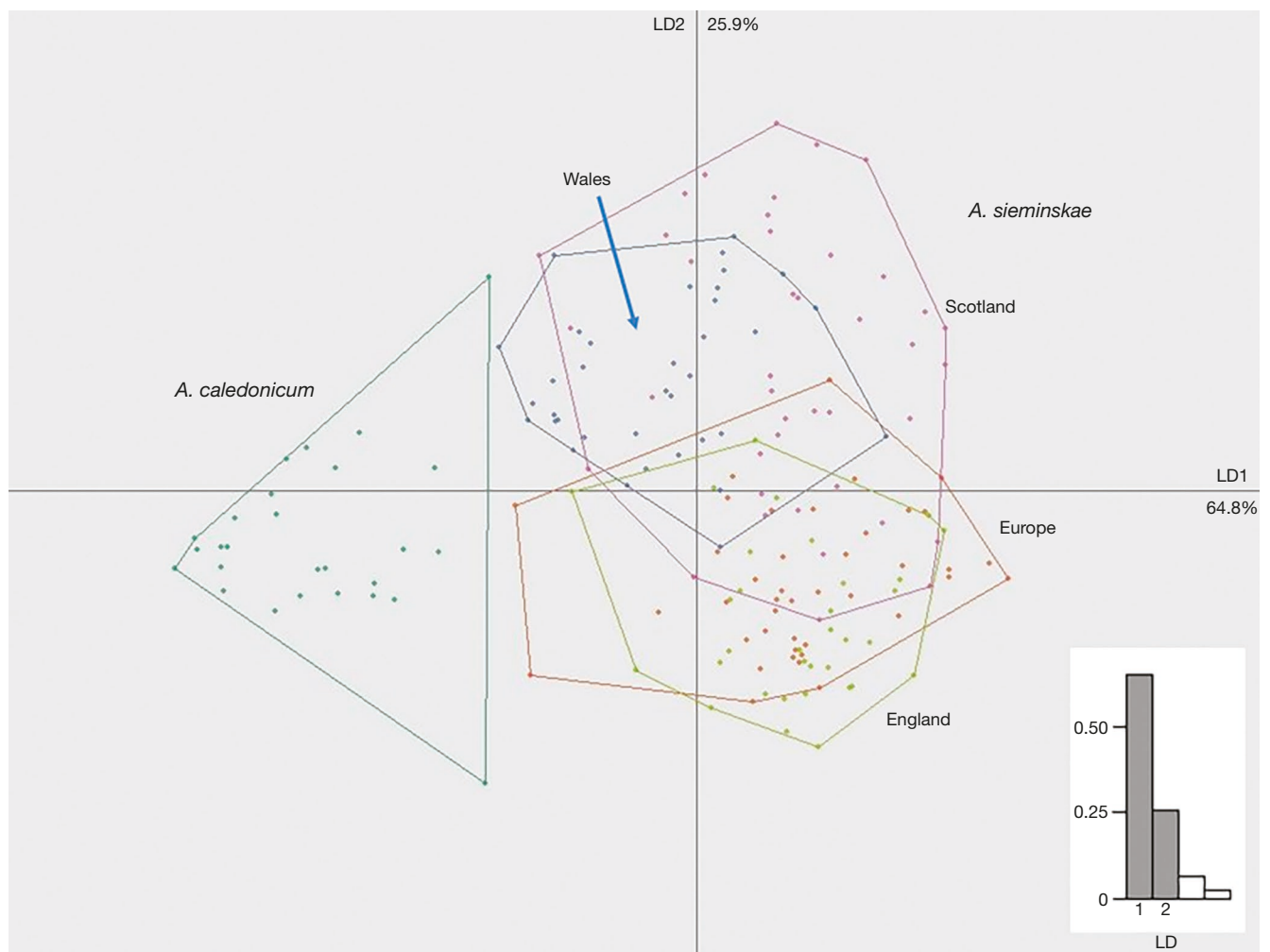
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APPENDICES



APPENDIX 1. — Principal component analysis of Fourier shape harmonics for identified species. The biplot presents the first two axes with a total explained variance of 96.4%.



APPENDIX 2. — Linear discriminant analysis of Fourier shape harmonics for *A. sieminskai* Witkowski, Kulikowski & Riaux-Gob. from different geographic regions (Europe, Wales, Scotland and England). Specimens of *A. caledonicum* (Lange-Bert.) Lange-Bert. are used as the outgroup. The biplot presents the first two axes with a total explained variance of 90.3%.