

Gomphonella olivacea (Bacillariophyceae) – a new phylogenetic position for a well-known taxon, its typification, new species and combinations

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Background and aims – Within the project “German Barcode of Life – Diatoms” common diatoms of German waters were routinely isolated and cultivated. In order to understand the taxonomy and phylogeny of the genus *Gomphonema*, one of the most common taxa of Central Europe, known currently either under the name *Gomphonema olivaceum* in Europe or *Gomphoneis olivacea* in America, was studied.

Methods – Twenty unialgal strains were established from five different water bodies in Germany and one from Lake Balaton, Hungary, which supplied molecular data (18S V4 and *rbcl*) besides morphometric and ultrastructural data. In addition, on eight populations from different water bodies including the type from Denmark, morphometric and micromorphological studies by light and scanning electron microscopy were performed.

Key results – Molecular and micromorphological data show that the target taxon neither belongs to *Gomphonema* Ehrenb. nor to *Gomphoneis* Cleve. By reinstating the genus name *Gomphonella* Rabenh., the nomenclatural and taxonomic enigma of this taxon is solved, and with the presentation of the type by Hornemann the authorship of the epithet is clarified. Molecular data for the unialgal strains and several environmental clones show that there is more diversity in the *Gomphonella olivacea* clade than can be identified morphologically. In addition, the establishment of the new species *Gomphonella coxiae* and *Gomphonella acsiae* is supported. The molecular data classified *Gomphonella* species as belonging to the Cymbellales but not to the Gomphonemataceae. In addition, molecular data put *Gomphoneis tegelensis* R.Jahn & N.Abarca also into *Gomphonella*. In order to make the genera *Gomphoneis* and *Gomphonema* monophyletic, their astigmatid members are transferred to *Gomphonella*.

Conclusions – The results clarify that the gomphonemoid outline is not restricted to the family Gomphonemataceae but seem to be distributed across the entire order Cymbellales. This is shown in this paper for the revived genus *Gomphonella*, which contains the astigmatid group of *Gomphoneis* and *Gomphonema* besides the longly disputed *G. olivacea*. Only a polyphasic approach, combining molecular and micromorphological data for taxonomy, nomenclatural evaluation, and observations from clonal cultures can reveal the full intricacies of evolutionary relations.

Key words – Bacillariophyceae, diatoms, genus emendation, *Gomphoneis*, *Gomphonema*, nomenclatural types, phylogeny, polyphasic approach.

INTRODUCTION

The taxon currently known under the name *Gomphonema olivaceum* (Hornem.) Ehrenb. or *Gomphoneis olivacea* (Hornem.) P.A.Dawson ex R.Ross & P.A.Sims is a very common diatom in fresh waters of Central Europe. Its habit of living on stalks or being free-living and producing fair amounts of mucus had originally placed it into different genera since, at the beginning of diatom research, life forms were thought to be decisive for phylogeny. In 1810 Hornemann pictured an olive coloured mass of mucus from a Danish river and named it *Ulva olivacea* Hornem. (fig. 1). Lyngbye (1819) used his material to describe and draw *G. olivacea*-like cells and recombined the name as *Echinella olivacea* (Hornem.) Lyngb. Kützing (1833) named it *Frustulia olivacea* (Hornem.) Kütz. and Brébisson & Godey (1835) *Cymbella olivacea* (Hornem.) Bréb. & Godey. When it was recognized that those two growth habits – attached on stalks or freely moving – were just two different life forms of the same species, the species was subsumed under the genus *Gomphonema* Ehrenb. In 1838 *Ulva olivacea* was transferred twice: in July/August by Ehrenberg and in October by Brébisson. In 1853 Rabenhorst published the new genus name and combination *Gomphonella olivacea* (Hornem.) Rabenh., a name which was reduced to a section of *Gomphonema* by Brun (1880) and which, since then, has apparently been neglected by the diatom community; nevertheless, in the Index Nominum Genericorum (2018) *Gomphonella* Rabenh. is listed as a genus with an unassigned type of the name of the genus even though a type was given in Round et al. (1990).

In parallel to the above sketch of the nomenclatural history of the epithet *olivacea*, there are further names which seem to refer to the same taxon. Agardh (1824) gave it the superfluous name *Meridion vernale* C.Agardh. In 1830 Leiblein, using diatom material from waters near Würzburg, Germany, published a picture of an unnamed gomphonemoid diatom and discussed the taxonomic identity of *Meridion vernale*. Leiblein sent this material among others to C. Agardh in Lund who described *Gomphonema leibleinii* C.Agardh validly from this material (Agardh 1830). Kützing

(1833) accepted Agardh's name whereas Ehrenberg (1838) put *Gomphonema leibleinii* into synonymy with his *Gomphonema clavatum* Ehrenb., which he had described validly in 1832 (in Ehrenberg 1830, it was a *nomen nudum*). Ehrenberg (1838) also used (and transferred) the taxon name *Gomphonema olivaceum*, which meant that for him *Gomphonema olivaceum* and *Gomphonema clavatum* (= *Gomphonema leibleinii*) were not conspecific. In 1844 Kützing put *Gomphonema leibleinii* into synonymy with *Gomphonema olivaceum* and also put *Gomphonema clavatum* into synonymy with *Gomphonema subramosum* C.Agardh (see also Reichardt 2015).

Despite its complex early history, this taxon has been known for 140 years as *Gomphonema olivaceum*. With the advent of the electron microscope it became obvious that its micromorphology was different from most *Gomphonema*. Dawson (1974) proposed that, because of its biseriate striation, it should be separated from *Gomphonema* and put into the genus *Gomphoneis* Cleve, which had been erected by Cleve (1894); this proposal was formally correctly executed by Ross & Sims in 1978. Since that time in the USA (Kocielek 2011) this taxon has been assigned to the genus *Gomphoneis* as *Gomphoneis olivacea* (Hornem.) P.Dawson ex R.Ross & P.A.Sims (1978) whereas in Central Europe it stayed within *Gomphonema* (Hofmann et al. 2013, Levkov et al. 2016) because biseriate striation was seen as common in this genus also and therefore not identified as a differentiating feature (Reichardt 2007). In addition, since it lacks an axial plate and mantle lamella it was seen as not fitting the genus *Gomphoneis* (Krammer & Lange-Bertalot 1985).

The logical approach for solving nomenclatural and taxonomic enigmas is to locate the type specimen or original material that was in the hands of the first describer. Locating this material was quite challenging, since 200 years ago Prof. Hornemann had worked in Copenhagen (Denmark), and Lyngbye, who had studied this material in more detail and published figures, had gotten his education in Copenhagen but was later based in Lund (Southern Sweden). Requests were made to both Herbaria, resulting in the finding of Agardh's *Gomphonema leibleinii* in the Lund Herbarium (plus comments made in 1982 by an earlier researcher). The type material of *Ulva olivaceum* had been loaned in 1965 to the Diatom Herbarium in Philadelphia, USA, by the Copenhagen Herbarium (C) but fortunately, an intensive search in Philadelphia resulted in the finding of this material after 52 years!

Within the framework of the project "German Barcode of Life – Diatoms" we were finally successful in isolating and cultivating several strains of this taxon. In our studies to understand the taxonomy and phylogeny of the genus *Gomphonema* (Abarca et al., in prep.), we also questioned its phylogenetic position and taxonomic affiliation with morphological as well as molecular data. Since these data are very different from the core group of *Gomphonema* yet similar to *Gomphoneis tegelensis* R.Jahn & N.Abarca (Skibbe et al. 2018), we are publishing the results here separately.

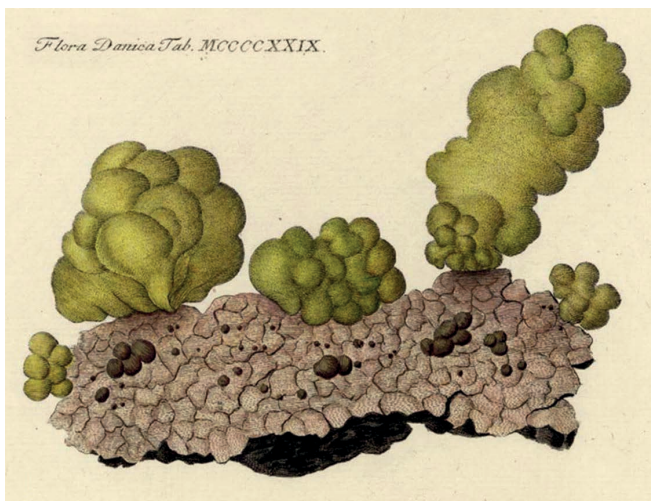


Figure 1 – *Ulva olivacea*. Reprint of *Flora Danica* Tab MCCCCXXIX 1429 (Hornemann 1810).

MATERIAL AND METHODS

The original material from Denmark of 1810 was studied (Lectotype C-A9208; see Typification below). In addition, data from eight populations and 38 strains are included in the present study (see electronic appendices 1 & 2). Thirty-two strains were established by the authors. The sequence data for the other six strains were downloaded from ENA/Genbank (as part of the International Nucleotide Sequence Database Collaboration, INSDC). All sequences downloaded from INSDC were BLASTed (basic local alignment search tool) against the INSDC database to test for taxonomic consistency.

Field collection and cultivation

Freshwater samples were collected from Germany and Hungary between 2004 and 2017. Twenty unialgal strains of the target taxon were isolated from nine samples of six different waters in Germany and from Lake Balaton in Hungary (for details see electronic appendices 1 & 2).

Clonal strains were established by micropipetting single cells using a stereo microscope (Olympus, Japan) and an inverted LM (Olympus, Japan). All strains were treated according to Romero & Jahn (2013). Non-axenic unialgal cultures were maintained at room temperature (19–25°C for cultures until 2016), 10°C (in 2016) and at 20°C (in 2017) in a growth chamber. A 12:12 h light/dark photoperiod from a daylight LED light source following Jahn et al. (2017) was applied. In addition, seven populations from the original samples from which clonal cultures were established were used for supporting documentation of the morphologies of the clones (for details see electronic appendix 2).

Documentation and vouchering

For all newly established strains the frustule preparation and morphological documentation were executed following Zimmermann et al. (2014). LM pictures of live cells (fig. 2) and of permanent specimens on slides were taken with a Zeiss AxioImager.M2 (Zeiss, Germany). SEM images were taken with a Hitachi FE SEM 8010 (Hitachi, Japan) of unsputtered material. The vouchers for all new strains are deposited at B (Herbarium Berolinense), where long-term stable and semantic web compatible identifiers for specimens are used according to Güntsch et al. (2017). Molecular data for all isolates are deposited in INSDC (see electronic appendix 1). DNA samples are stored in the Berlin DNA Bank and are available via the Genome Biodiversity Network (GGBN, Droege et al. 2014); nomenclatural acts are registered (Turland et al. 2018, Art. 42) in PhycoBank (continuously updated). Data are available through AlgaTerra (Jahn & Kusber continuously updated).

Morphological criteria

Besides valve outline and morphometric measurements of each clone (length, width, number of striae in 10 µm (electronic appendix 2) valves were investigated under SEM to compare internal and external valve and girdle views (fig. 5A–C). Special attention was given to the presence or lack of

stigmata or stigmoids, the form of the striae and covering of the areolae, and the porelli of the footpole. For comparison, SEM images of the same features of *Gomphonema minutum* are presented (fig. 5D–F).

DNA extraction, sequencing and alignment

Cultured material was transferred to 1.5mL tubes. The DNA was isolated using the NucleoSpin® Plant II Mini Kit (Macherey and Nagel, Düren, Germany) or Qiagen® Dneasy Plant Mini Kit (Qiagen, Valencia, CA) following the respective product instructions. The DNA fragment size and concentrations were measured via gel electrophoresis (1.5% agarose gel) and Nanodrop® (PeqLab Biotechnology, Erlangen, Germany), respectively. The DNA samples were stored at –20°C for future use and finally deposited in the Berlin collection of the DNA bank network (Droege et al. 2014). The polymerase chain reaction (PCR) for *rbcL* was conducted following Abarca et al. (2014). The V4 section of the 18S SSU rRNA gene locus (18S V4) was amplified and PCR performed following Zimmermann et al. (2011). PCR products were visualized in a 1.5% agarose gel and cleaned with MSB SpinPCRapace® (Invitex LLC, Berlin, Germany) following standard procedures. DNA concentrations were measured using Nanodrop® (PeqLab Biotechnology) and samples were normalized to a total DNA content >100 ng µL⁻¹ for sequencing.

Sanger sequencing was conducted by Starseq® (GENterprise, Mainz, Germany), *rbcL* gene according to Abarca et al. (2014) and 18S V4 according to Zimmermann et al. (2011). In both cases the same primers were used for amplification and sequencing. The editing, as well as the quality control of the pherograms for the new sequences, were done in Phyde® (Müller et al. 2010). The evaluated sequences were aligned using MUSCLE (Edgar 2010), as implemented in MEGA6 (Tamura et al. 2013) with subsequent manual adjustments in

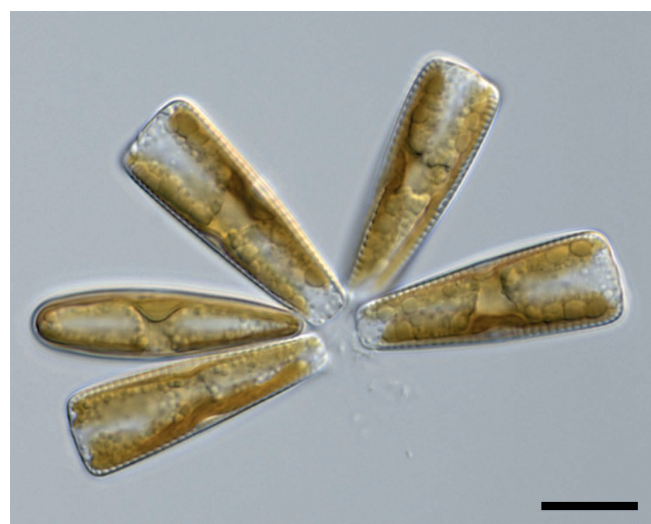


Figure 2 – Five living cells of *Gomphonella olivacea* (Strain D129_007): one in valve view and four in girdle view. Note the single chloroplast, consisting of two H-shaped lobes connected by a bridge which contains the pyrenoid. These cells contain abundant reserve material. Scale bar = 10 µm.

case of 18S V4. The lengths of the newly generated sequences were 432 bp for 18S V4 and 979 bp for *rbcL*. For comparison, the alignments for 18S V4 and *rbcL* also included other sequences of our own and some others from INSDC representing the Cymbellales D.G.Mann, as well as *Achnanthydium saprophyllum* (H.Kobayasi & Mayama) Round & Bukht., which was added as the outgroup for the phylogenetic tree generation following Kermarrec et al. (2011). All added sequences were trimmed to fit to the newly generated sequences for 18S V4 as well as *rbcL*. The accessions used are given in electronic appendix 1).

Phylogenetic analyses

Two different data sets (18S V4, *rbcL*) were used for the phylogenetic analyses. Each dataset was analysed using Maximum Likelihood (ML) as implemented in RAxML (Stamatakis 2006, 2014, Stamatakis et al. 2008) using the CIPRES platform (Miller et al. 2010) in both cases.

For the ML analysis of the molecular datasets, the optimal model of sequence evolution that best fits the sequence data was calculated under the hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC) using model test 3.7 (Posada & Crandall 1998). The best fitting model was GTR+G+I (Tavaré 1986). A ML analysis was conducted using RAxML 8.2.8 (Stamatakis 2006, 2014, Stamatakis et al. 2008), ML search option (GTR+G+I) and 1,000 bootstrap replicates (model GTRCAT as implemented in RAxML for the rapid bootstrap algorithm). Additionally, Bayesian phylogenetic inference was conducted for both data sets using MrBayes v. 3.1.2. (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) with the same model. The default settings were used, runs with four incrementally heated Metropolis-coupled Monte-Carlo Markov Chains and runs with 10 million generations were executed. The runs were sampled every 1000 generations, the first 25% generations being discarded as burn-in; the rest were used to calculate a 50% majority rule consensus tree. The best ML tree found by RAxML and the 50% majority rule tree of the BI analysis were compared for *rbcL* as well as 18S V4. In all cases the trees showed no different topologies and were therefore summarized in one tree for each marker, showing bootstrap statistics (> 75) for ML (LB) and (> 0.90) posterior probabilities (BI). Trees were drawn using FigTree v. 1.4.2 (Rambaut 2008) and Adobe Illustrator (Adobe Systems, San Jose, CA). Genetic distances for 18S V4 and *rbcL* were calculated using MEGA6 (Tamura et al. 2013) and the implemented p-distance option.

RESULTS

Molecular data (figs 3, 4)

The molecular markers 18SV4 and *rbcL* were used and both provided similar results, clustering all target strains into one main clade (figs 3 & 4) with 99/1.00 (18SV4) and 76/1.00 (*rbcL*) ML bootstrap support/posterior probabilities. We will refer to this clade from now on as the *Gomphonella* clade, in anticipation of the conclusions drawn later. Four clades, numbered 1, 2, 3 and 4 with even better bootstrap support are separated within the *Gomphonella* clade (details see be-

low), which is well separated from taxa of the genera *Encyonopsis* (18S V4, p-distance 10%) or *Cymbella* (*rbcL*, ca 5%). The *Gomphonema* clade, with *G. acuminatum* Ehrenb., *G. minutum* (C.Agardh) C.Agardh and *Gomphoneis minuta* (J.L.Stone) Kociolek & Stoermer, is well separated and supported by 99/1.00 (18SV4) and 100/1.00 (*rbcL*) bootstrap value/posterior probabilities and p-distances of around 12% (18SV4) and 6% (*rbcL*) from *Gomphonella* spp.

Clade 2 is the sister group to Clade 3 in the 18S V4 (1.00 posterior probabilities, fig. 3) as well as in the *rbcL* tree (83/1.00 bootstrap value/posterior probabilities, fig. 4). In the case of Clade 4 the two trees show slight differences in the topologies regarding the sister group relation. For 18SV4 (fig. 3) Clade 4, supported by 99/1.00 bootstrap value/posterior probabilities, is the sister group to the branch with Clades 1a, b, 2 and 3. Clades 2 and 3 for their part are building the sister group to Clade 1a, b supported by 75/1.00 bootstrap value/posterior probabilities. In contrast, for the *rbcL* tree (fig. 4) Clade 4 is the sister group to the branch with Clade 2 and 3 with a support of 87/0.98 bootstrap value/posterior probabilities and these three Clades (Clades 2, 3 and 4) are the sister group to Clade 1A, B with a support of 76/1.00 bootstrap value/posterior probabilities.

Clades 1A and 1B – From a molecular point of view the 13 clones (for details see electronic appendix 1) from Tegeler See (samples D128 and D130), Müggelsee (D129) and river Main (D135_024) are the same (Clade 1A). They show well supported but small differences – p-distance = 0.7% for 18SV4 and 0.4% for *rbcL* – to the two clones of river Spree (D03_184), and Saale (D132_024) (Clade 1B). The molecular data from the environmental sample named *Cymbellales* from brook Westerhöver Bach (Brinkmann et al. 2015) sits in Clade 1A.

Clade 2 – The two strains with data for 18S V4 and the four strains for which *rbcL* data are available, are all from Lake Balaton (D140; see electronic appendix 1). They show 0% differences between each other in 18SV4 and *rbcL*. There are well supported differences from the other three clades: For 18SV4 the p-distances are 1.6–2.3% to Clade 1, 1.6% to Clade 3 and 5.1% to Clade 4; for *rbcL* they are 3.5% to Clade 1, 2.9% to Clade 3 and 5.1% to Clade 4.

Clade 3 – This clade is defined by the data of strain D201_007 for which 18S V4 and *rbcL* data are available. For 18S V4 the p-distances are 2.3–2.8% to Clade 1, 1.6% to Clade 3 and 5.3% to Clade 4; for *rbcL* they are 2.9% to Clade 1, 2.2% to Clade 2 and 4.6% to Clade 4.

Clade 4 – This clade is defined by the data of the isolate D221_Gt, which has been separated into a new species, *Gomphoneis tegelensis* R.Jahn & N.Abarca (Skibbe et al. 2018). Concerning 18S V4 data, this strain has a p-distance of 4.9–5.3% to Clade 1, 5.1% to Clade 2 and 5.3% to Clade 3; concerning *rbcL* data, this strain has a p-distance of 5.0% to Clade 1, 5.1% to Clade 2 and 4.6% to Clade 3.

Morphology (figs 5–14)

The most conspicuous trait of all studied strains is their pronounced variability in outline: from the typical ovate-oblancoolate to symmetrically lanceolate, with apices that are

broadly rounded (i.e. fig. 6Q) to pointed (i.e. fig. 6R & S). They often grew fan-like (strains D129_043 and D132_024) or in lumps (strain D132_036) producing plenty of gelatinous material. Since the strains have such variable and often untypical outlines which might be due to long lasting cultivation, we also studied the populations of the samples from which the strains were isolated such as the populations from Tegeler See (figs 6AI–AO & 7A–I), Müggelsee (fig. 6I–P), river Main (fig. 7AO–AV), Saale (fig. 7S–AA), river Spree for Clade 1, Lake Balaton (fig. 11A–F) for Clade 2 and

Heleneesee (fig. 13A–F) for Clade 3. Specimens of Clade 4 are selectively isolated cells of clonal origin (Skibbe et al. 2018) which were not kept in culture.

Concerning morphological synapomorphies, the most important and conspicuous feature of clades 1–4 visible in LM is that they do not have any stigmata (fig. 5A & B); occasionally, there are a few isolated puncta visible in the central area (e.g. figs 7AR, AS & 8K) but they look like the areolae and seem to be continuations of the striae, just separated by a gap. In SEM, these puncta have no internal structure and

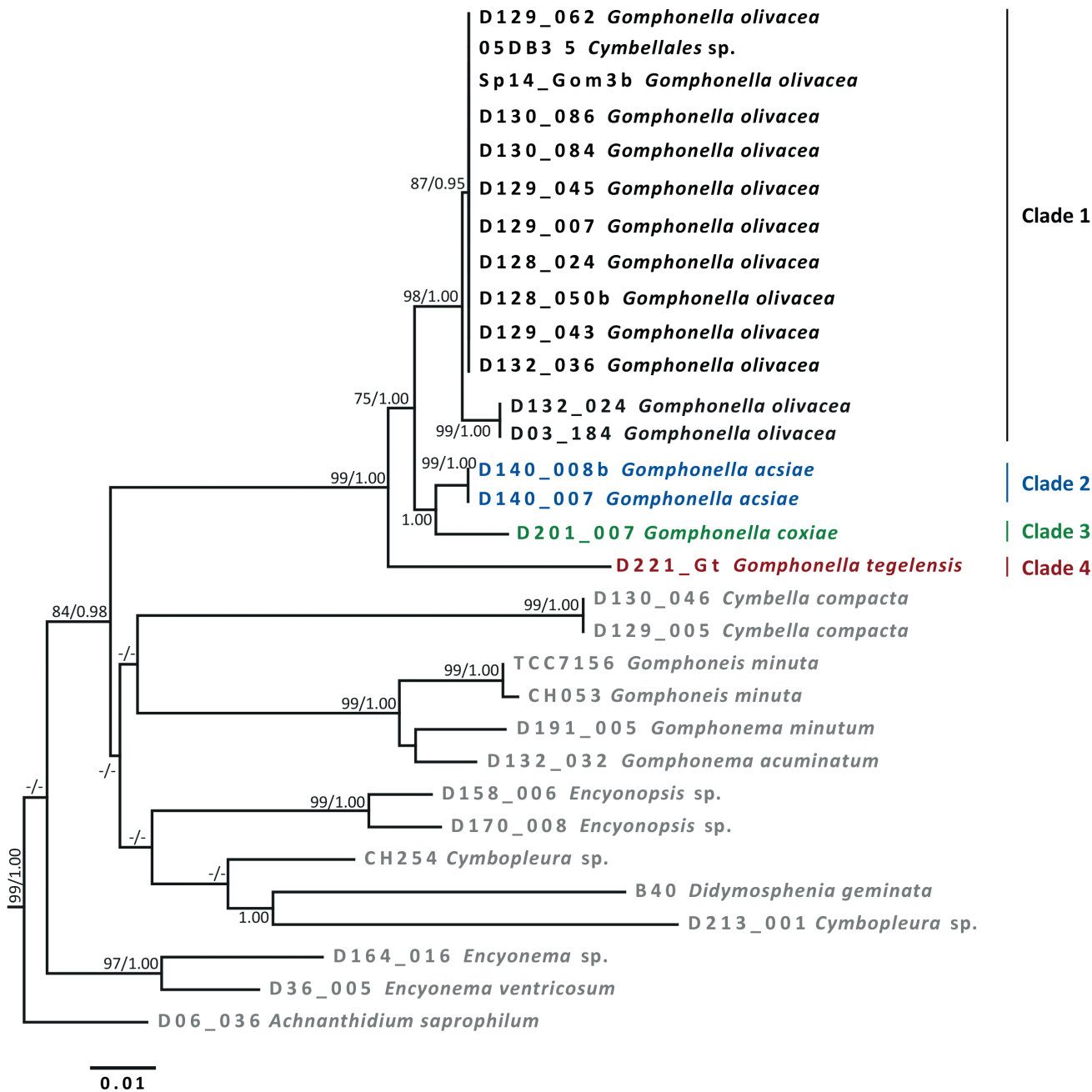


Figure 3 – Phylogenetic tree using maximum likelihood of the dataset of the 18SV4 molecular marker with bootstrap statistics (> 75) for ML (LB) and (> 0.90) posterior probabilities (BI). Black: Clade 1 *Gomphonella olivacea* (upper branch: genodeme 1 and lower branch genodeme 2); blue: Clade 2 *Gomphonella acsiae*; green: Clade 3 *Gomphonella coxiae*; purple: Clade 4 *Gomphonella tegelensis*.

are therefore not true stigmata (see also definition in www.diatoms.org). A further conspicuous trait visible only in SEM is their biseriate striation (in Clade 4 only, some striae are triseriate) with small round uniform areolae not occluded by siliceous flaps (see Skibbe et al. 2018: figs 17–21). The

striae are not interrupted near the valve face/mantle junction and continue onto the valve mantle (fig. 5C). These double rows of areolae can terminate either as single or as double rows along the axial area and mantle. But all striae taper into only a single row of areolae at the central area (fig. 5A). The

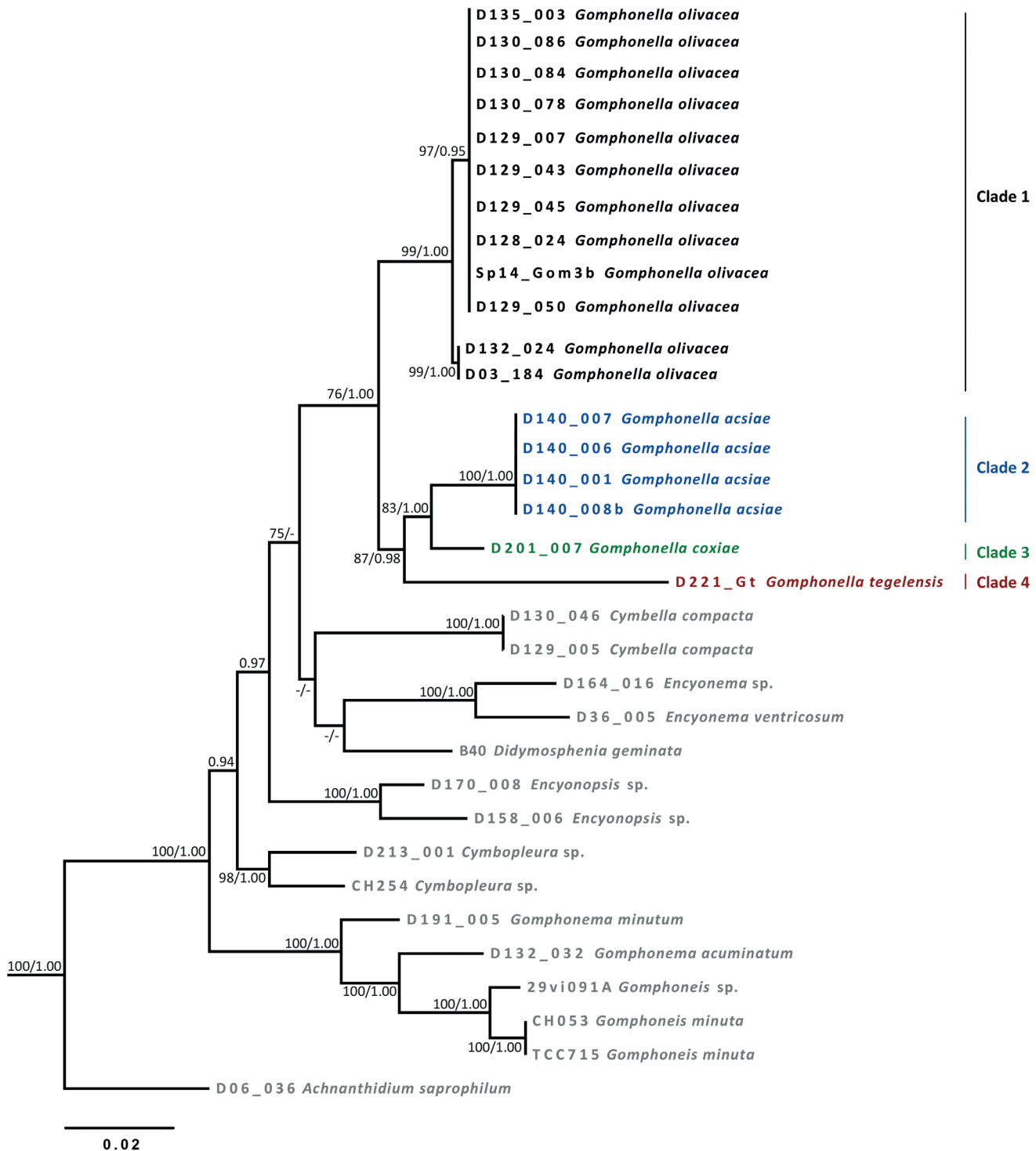


Figure 4 – Phylogenetic tree using maximum likelihood of the dataset of the *rbcL* molecular marker with bootstrap statistics (> 75) for ML (LB) and (> 0.90) posterior probabilities (BI). Black: Clade 1 *Gomphonella olivacea* (upper branch: genodeme 1 and lower branch genodeme 2); blue: Clade 2 *Gomphonella acsiae*; green: Clade 3 *Gomphonella coxiae*; purple: Clade 4 *Gomphonella tegelensis*.

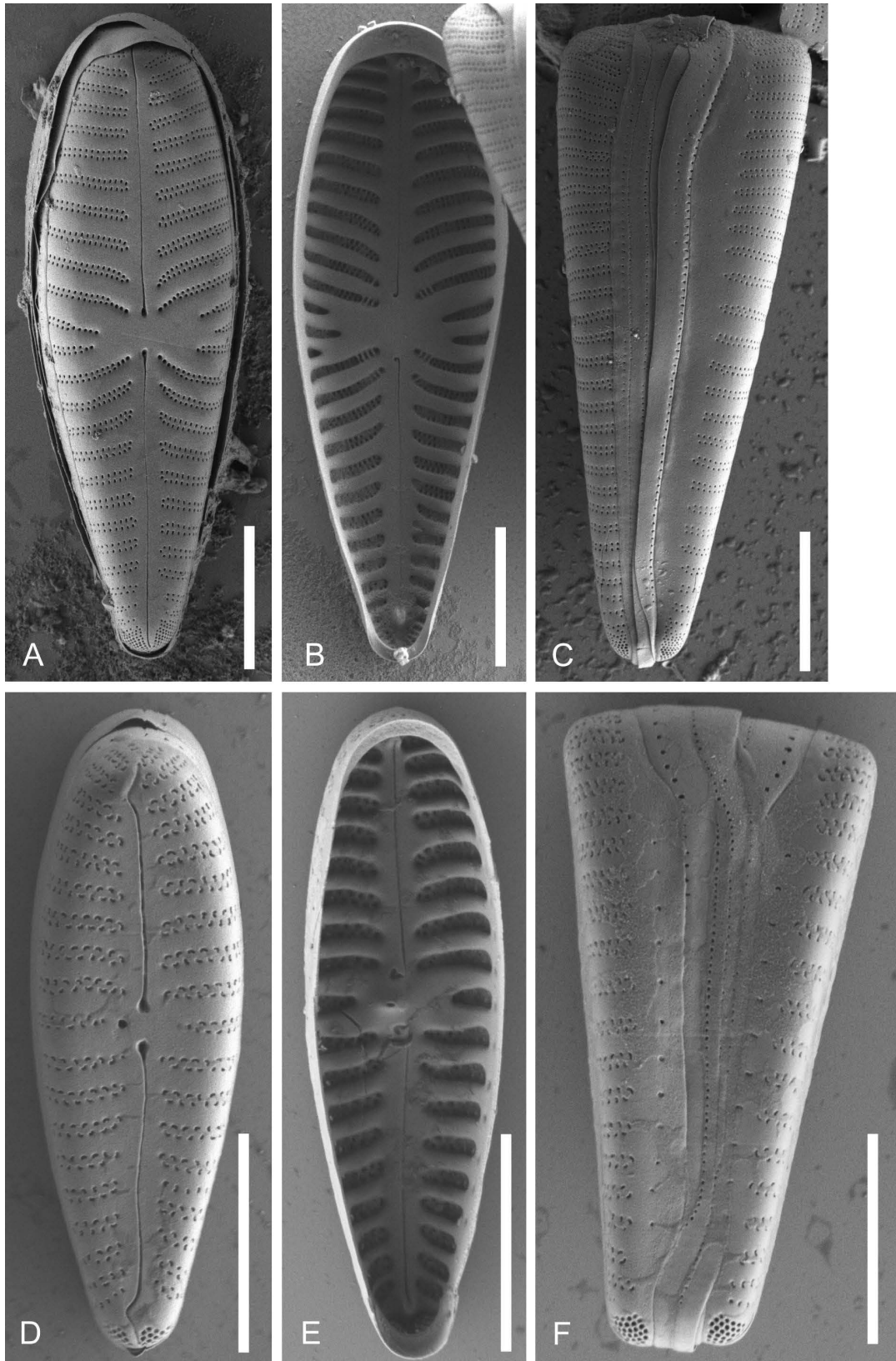


Figure 5 – SEM-comparison of autapomorphies: A–C, *Gomphonella olivacea*, C_A9208 lectotype population; D–F, *Gomphonema minutum*, strain D191_005. A & D, external valve view, note the differences in the areolae of the biseriate striae, the form of the raphe slit and the stigma in D; B & E, internal valve view, note the differences in the central raphe endings and helictoglossae as well as the stigma in E; C & F, girdle view, note the abrupt endings of the striae in C and the tapering into a single punctum or slit in F. Scale bars = 5 μm .

foot pole is composed of a bilobed field of porelli. The outside distal ends of the raphe extend through these porelli but not all the way to the end of the mantle. The raphe is either straight or very slightly undulated at both apices. Internally, the pseudosepta are wide, distinct and prominent at both apices. Also the helictoglossae are prominent at both poles and in some clones lie well away from the valve terminus.

The autapomorphies separating the clades (and species) are aspects of the striation, such as the number of striae in 10 μm , their parallel or radial direction throughout the valve face, and the form of the central area. Of special importance too are the porelli at the footpole, which are either of similar size and shape to the areolae of the striae or relatively larger and distinct; they are either arranged in double rows or with-

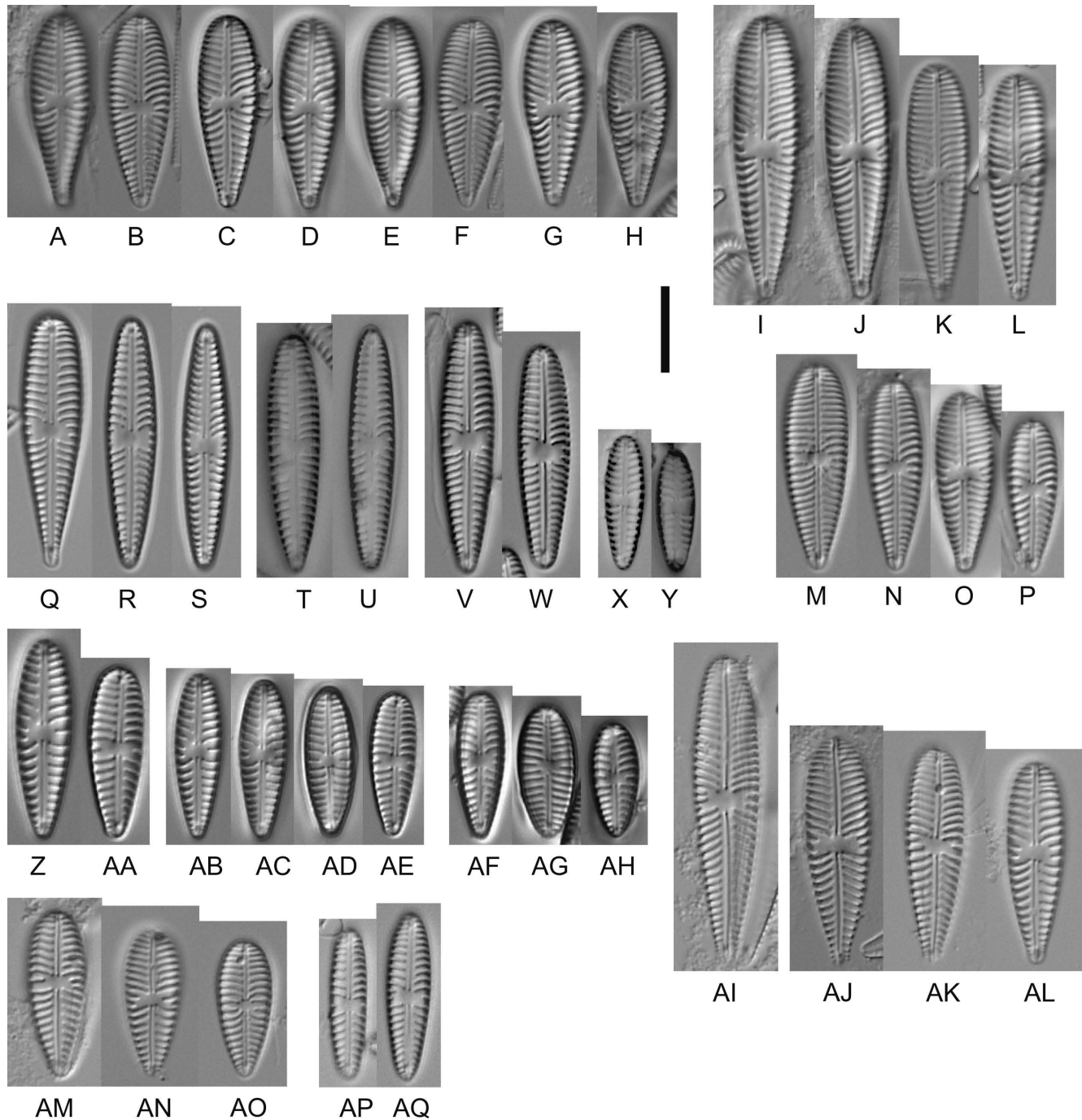


Figure 6 – *Gomphonella olivacea*, LM: A–H, C_A9208, lectotype population from Denmark. D represents the lectotype; I–AH, specimens from sample D129, Müggelsee, Berlin, Germany; I–P, natural population; Q–S, strain D129_43, Q represents the epitype; T & U, strain D129_050, V,W, strain D129_007; X & Y, strain D129_045; Z & AA, strain D129_065; AB–AE, strain D129_065; AF–AH, strain D129_062; AI–AQ, specimens from sample D128, Tegeler See, Berlin, Germany; AI–AO, natural population; AP & AQ, strain D128_024. Scale bar = 10 μm .

out any order and they are located close to the striae or well separated.

Clade 1 (figs 6–10 & 14A) – The valves are heteropolar, clavate with broadly rounded headpoles and acutely rounded footpoles (fig. 6A–P). There is wide variation within the clone cultures: the valves can be heteropolar, clavate with broadly rounded headpoles and acutely rounded footpoles (see fig. 6Q), but they can also be heteropolar, lanceolate to linear lanceolate (see fig. 6R), or even be only slightly heteropolar valves with almost parallel to slightly convex margins and rounded headpole and footpole (see fig. 6S). The axial area is narrow, straight, expanded at the centre to form a rectangular, bow-tie-shaped to transversally elliptical central area bordered at the margins by 1–3 approximately equally-shortened striae (fig. 8B & E). Transapical striae are strongly radiate in the central part of the valve and towards the footpole (fig. 8H & K), becoming slightly radiate towards the headpole (figs 8A, G & 9A). The footpole has a large apical pore field with porelli of the same size and structure as the areolae (figs 8C, I, 9C, I & 10C). The porelli appear to be arranged in double rows, located close to the striation of the valve face (figs 8C, I, 9C, I & 10C) and therefore undifferentiated structurally and spatially from them.

Clade 1A and 1B – Morphological differences between the Clades 1A and 1B are not discernible. They are very similar and uniform in shape.

Clade 2 (figs 11, 12 & 14B) – The valves are slightly heteropolar, lanceolate in larger specimens (fig. 11A–C & G–I) to clavate in smaller specimens, with narrowly rounded headpoles and acutely rounded footpoles. Wide variation of shape occurs within the clone cultures, since the valves can be slightly heteropolar, lanceolate and widest at the centre, or they can be heteropolar, clavate with broadly rounded headpoles and acutely rounded footpoles. The valves of the population of Clade 2 are on average longer and have a higher stria density than the club-shaped populations of Clade 1, but both have the same average width. Valves of Clade 2 are differentiated from valves of Clade 1 by a transapically widened central area, which is smaller and more rectangular (fig. 12B, E, H & K) than the central area of Clade 1 (which resembles a bow tie). Clade 2 possesses parallel striae which become radial at the centre (fig. 11A–Q), whereas in Clade 1 the striae are radial throughout the valve face. The footpole has a large apical pore field with relatively large porelli, which differ in size from the areolae of the striae and are well separated from the striation of the valve face (fig. 12C & I).

Clade 3 (figs 13A–O & 14C) – The valves of the population are slightly heteropolar, lanceolate in larger specimens to clavate in smaller specimens, with narrowly rounded headpoles and acutely rounded footpoles (fig. 13A–F). The valves of the clone cultures are linear-clavate or with a slight tumid swelling at the centre, headpoles narrowly rounded and footpoles rounded (fig. 13G–I). The axial area is narrow, straight, expanded at the centre to form a broad, bow-tie-shaped to rectangular central area bordered at the margin by two or three approximately equally-shortened striae (fig. 13L & M). The striae are composed of two alternating rows of areolae (fig. 13J). The footpole has a large apical pore field with relatively large porelli, which differ in size from the areolae of

the striae and are well separated from the striation. Valves of Clade 3 possess parallel transapical striae, which become radial at the centre as in Clade 2 (fig. 13A–I), whereas in Clade 1 the striae are radial throughout the valve face.

Clade 4 (illustrated in Skibbe et al. 2018) – The most prominent differences from Clade 1, 2 and 3 are the striation, which is bi- to triseriate, and the small axial plate and mantle lamella (for details see Skibbe et al. 2018). Otherwise, there are no stigmoids and the areolae are small, round and uniform, and are not occluded by siliceous flaps. The striae are not interrupted near the valve face/mantle junction and continue onto the valve mantle (Skibbe et al. 2018: figs 17, 18, 23).

Typification and nomenclature

Clades 2 and 3 cannot be identified with already known taxa and need to be described as new (see below). Specimens of Clade 4 were recently described as *Gomphoneis tegelensis* (Skibbe et al. 2018). Specimens of Clade 1 were described more than 200 years ago as *Ulva olivacea* (fig. 1), which went through a number of name changes until today. We were able to study this material in LM and SEM for the first time (see figs 5A–C & 6A–H).

Since clade 1 obviously does not belong to the genus *Gomphonema* (fig. 5D–F) and an earlier valid genus name exists, namely *Gomphonella* Rabenhorst (1853), we are here reinstating this name. Rabenhorst introduced this genus name and made the combination *Gomphonella olivacea* (1853: 61), describing it as “Eine gestielte *Gomphonema* in einer gestaltlosen Gallertmasse” [a stalked *Gomphonema* in an amorphous gelatinous mass].

Gomphonella Rabenh. (Rabenhorst 1853: 61, pl. IX)

Original description – “bis 2/100 Mm lang, verkehrt-eiförmig-lanzettlich; Nebenseiten breit keilförmig, am Rande mit zarten Querstreifen. Durch ganz Europa.” (Rabenhorst op. cit.) [up to 20 µm long, ovate oblanceolate; sides broadly wedge-shaped, with delicate bars at the margin. Throughout the whole of Europe].

Type species (lectotype) – *Gomphonella olivacea* (Hornem.) Rabenh. (*Ulva olivacea* Hornem.), designated in Round et al. (1990: 691).

Registration – <https://phycobank.org/100348>

Emended description – The most important and conspicuous feature of this genus visible in LM, separating it from other gomphonemoid taxa, is that there are no stigmoids or stigmata present on the valve face. A further conspicuous trait separating it from *Gomphonema* s. str. but visible only in SEM is the bi- to triseriate striation with small round uniform areolae not occluded by siliceous flaps. The diameter of the areolae is about 100 nm. The striae sit in moderately deep alveolae between thicker vimines. The striae continue onto the valve mantle. The apical foot pole is composed of a bilobed field of porelli which are round and similar to the normal areolae in the striae (= undifferentiated AFPs). The raphe is filiform and either straight or very slightly undulate at both apices. Internally, both polar raphe endings end in prominent helictoglossae well away from the valve terminus and there is an intermissio in the centre with the raphe end-

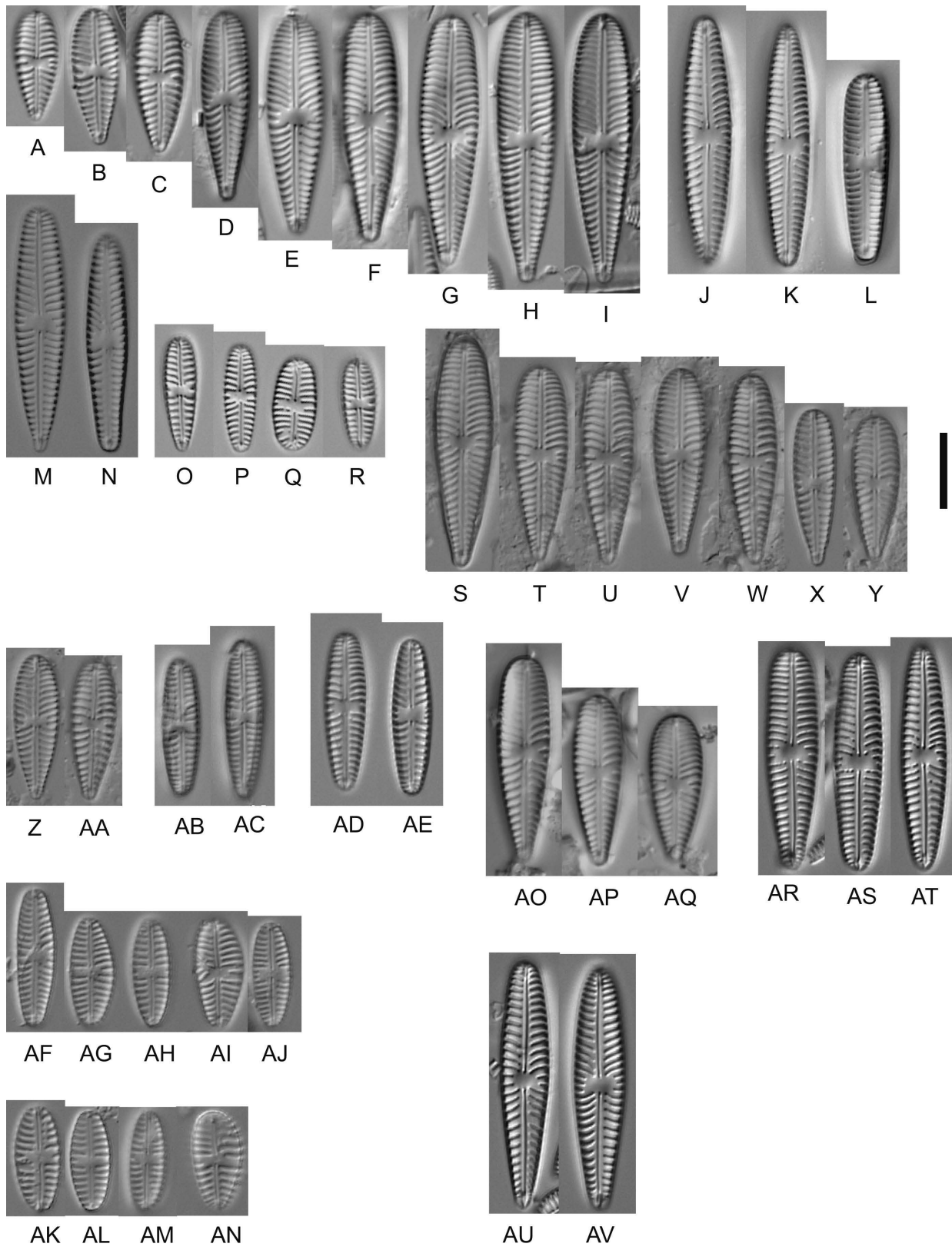


Figure 7 – *Gomphonella olivacea*, LM: A–R, specimens from sample D130, Tegeler See, Berlin, Germany; A–I, natural population; J–L, strain D130_086; M & N, strain D130_078; O–R, strain D130_084; S–AE, specimens from sample D132, Saale, Germany; S–AA, natural population; AB & AC, strain D132_036; AD & AE, strain D132_024, genodeme 2; AF–AN, strain D03_184, genodeme 2, river Spree, Berlin, Germany; AO–AV, specimens from sample D135, river Main, Germany; AO–AQ, natural population; AR–AV, strain D135_003. Scale bar = 10 µm.

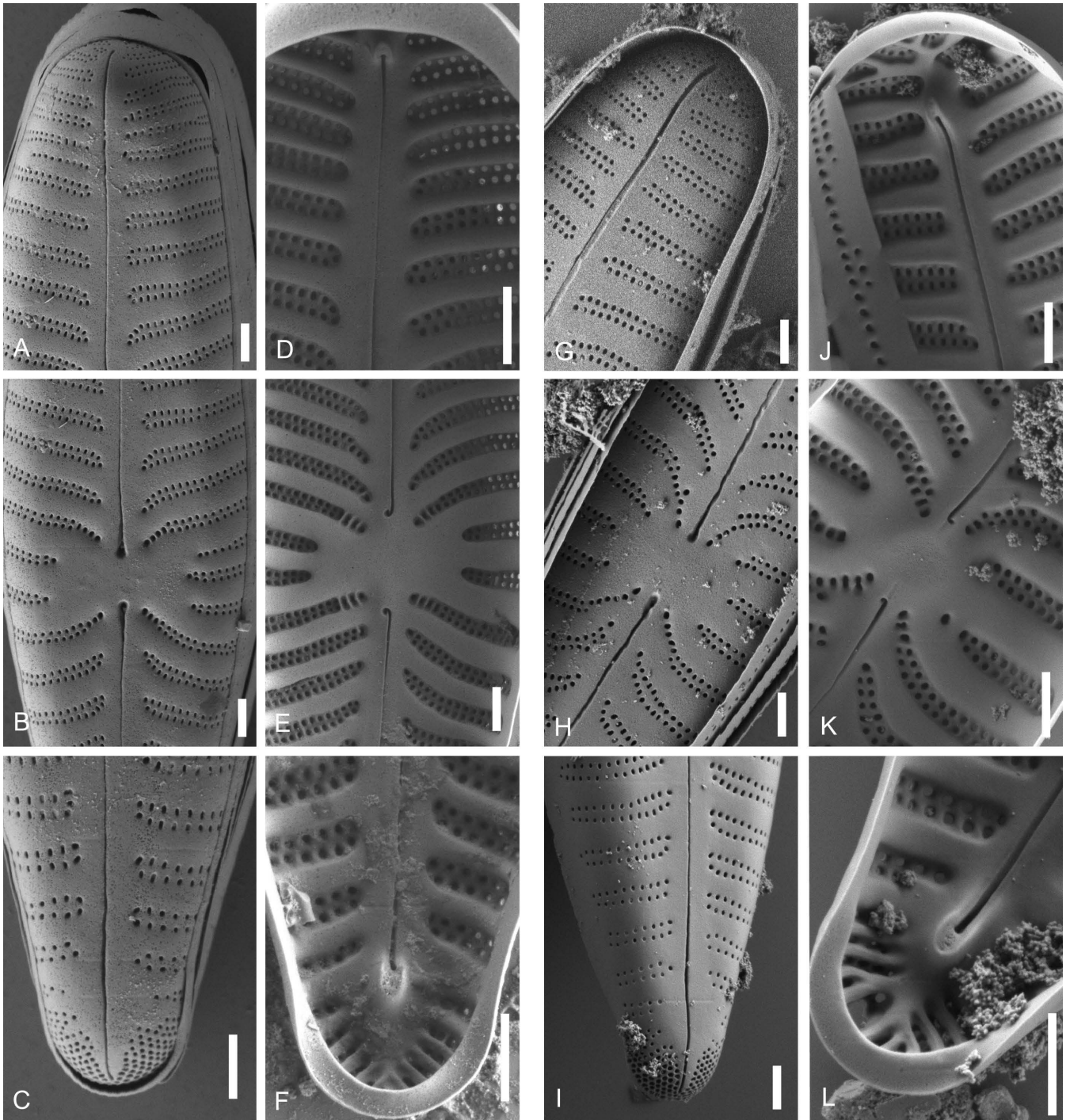


Figure 8 – *Gomphonella olivacea*, SEM: A–F, C-A9208 lectotype population, Denmark; G–L, epitype population, specimens from sample D129; Müggelsee, Berlin, Germany; A–C & G–I, external valve views; D–F & J–L, internal valve views; A, D, G & J, headpole; B, E, H & K, central area, note the arched striation in the centre tapering into a row of single areolae and the rectangular bow-tie to transversely elliptical form; C, F, I & L, footpole, note the porelli organized in double rows and no gap of striation between striae and porelli; Scale bars = 1 µm.

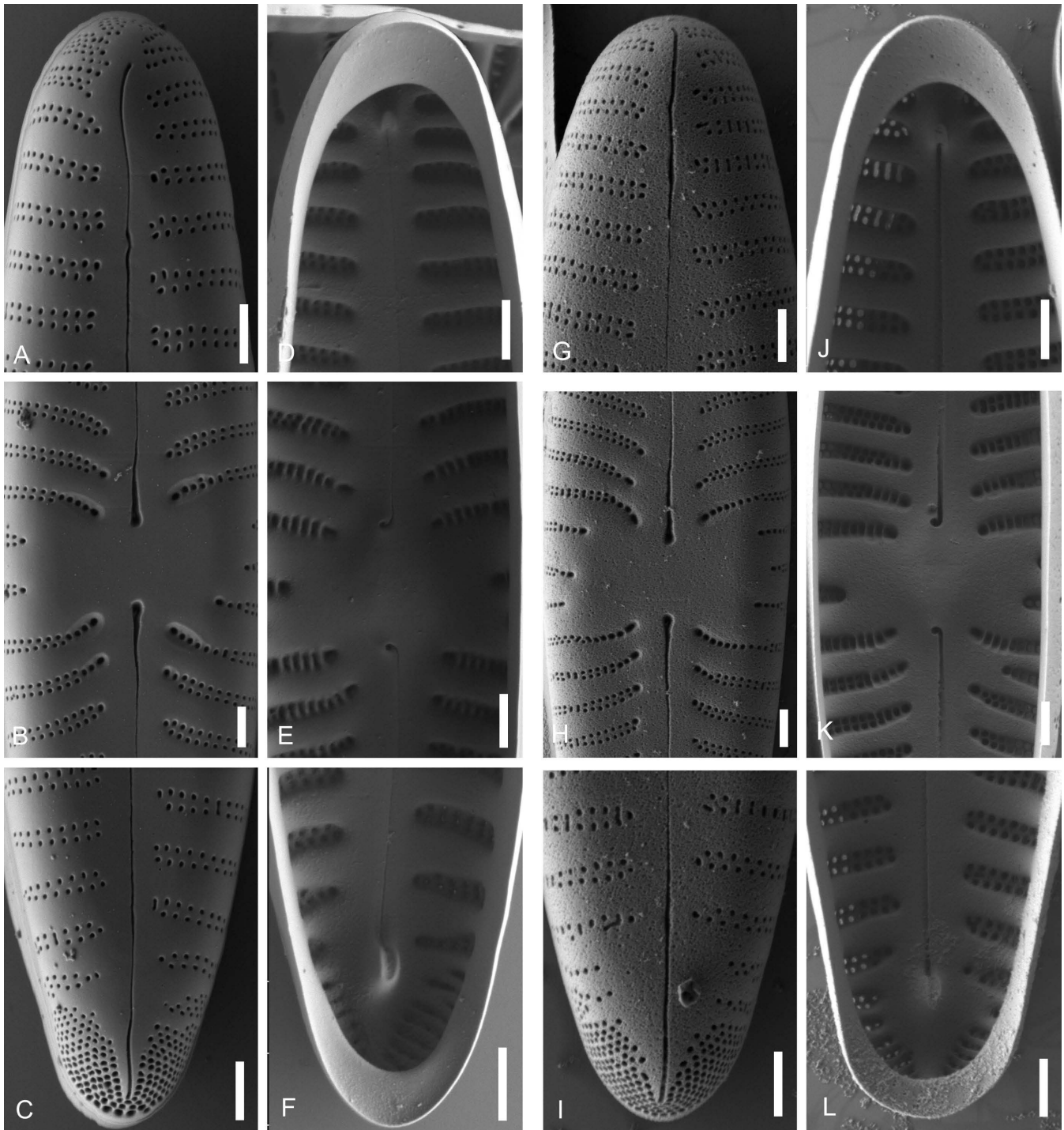


Figure 9 – *Gomphonella olivacea*, SEM, Müggelsee, Berlin, Germany: A–F, epitype strain D129_043; G–L, strain D129_007; A–C & G–I, external valve views; D–F & J–L, internal valve views; A, D, G & J, headpole; B, E, I & K, central area, note the arched striation in the centre tapering into a row of single areolae and the rectangular bow-tie to transversely elliptical form. C, F, I & L, footpole; note the porelli organized in double rows and no gap of striation between striae and porelli. Scale bars = 1 μ m.

ings slightly bent to the same side. The pseudosepta are distinct and prominent at both apices. At least two girdle bands belong to each valve and open alternately at either pole; each bears a line of pores along the junction between pars exterior and pars interior.

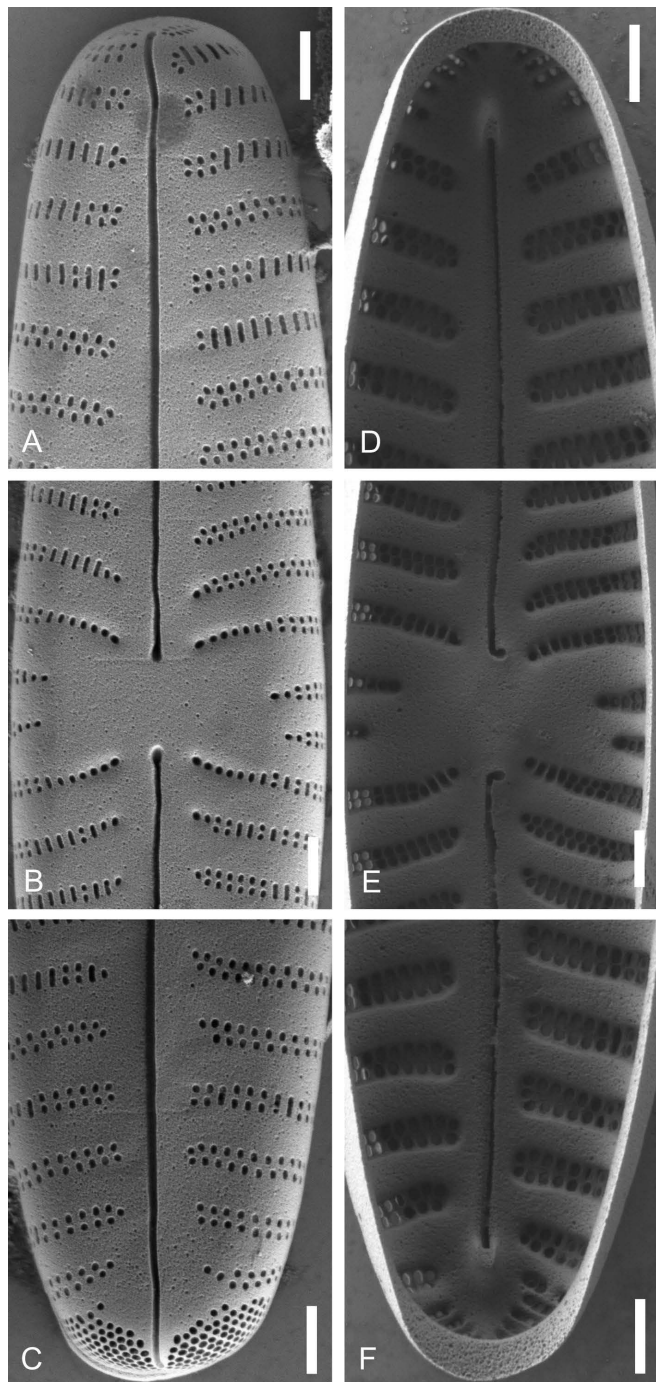


Figure 10 – *Gomphonella olivacea*, SEM, strain D132_024, genodeme 2, Saale, Germany: A–C, external valve views; D–F, internal valve views; A & D, headpole; B & E, central area, note the arched striation in the centre tapering into a row of single areolae and the rectangular bow-tie to transversely elliptical form; C & F, footpole; note the porelli organized in double rows and no gap of striation between striae and porelli. Scale bars = 1 μ m.

Gomphonella olivacea (Hornem.) Rabenh. (Rabenhorst 1853: 70).

Figs 1, 2, 5A–C, 6, 7A–AC, AO–AV, 8, 9 & 14A)

Ulva olivacea Hornem. (Hornemann 1810: 5, pl. MCCC-CXXIX (1429)).

Original description – “*Ulva olivacea* (mihi): frondibus cylindricis obtusis subrotundis v. oblongis sinuatis olivaceis, minutissime punctatis. Obs. Substantia gelatinosa lubrica suppelucida.” (Hornemann op. cit.)

Type – Denmark, “In rivulo prope Dams Mølle Sielladniae inveni, saxis innascentem”, 1810 (lecto-: C, material C_A9208, **designated here**, represented in fig. 6D; isolecto-: B, slide B 40 0042052, **designated here**, SEM and material available).

Epitype – Germany, Berlin, Müggelsee (52.443233°N, 13.676318°E), 13 Feb. 2016, *R. Jahn* D129 (epi-: B, slide B 40 0042144, **designated here**, prepared from strain D129_043 isolated by O. Skibbe, represented in fig. 6Q).

Registration – <https://phycobank.org/100349>

Echinella olivacea (Hornem.) Lyngb. (Lyngbye 1819: 209). 1819. – *Frustulia olivacea* (Hornem.) Kütz. (Kützing 1833: 556) – *Cymbella olivacea* (Hornem.) Bréb. & Godey (Brébisson & Godey 1835: 51) – *Gomphonema olivaceum* (Hornem.) Ehrenb. (Ehrenberg 1838: 218) – *Gomphonema olivaceum* (Hornem.) Bréb. (Brébisson 1838: 14) – *Gomphoneis olivacea* “olivaceum” (Hornem.) P.A.Dawson ex R.Ross & P.A.Sims (Ross & Sims 1978: 162) – *Gomphonema clavatum sensu* Reichardt (2015) non Ehrenberg (1832: 88) – *Gomphonema leibleinii sensu* Reichardt (2015) non Agardh (1830: 33).

Emended description – The morphometric data of the populations ($n = 113$) are: length 14.3–42.2 μ m, width 5.5–8.7 μ m and 8.0–15 striae in 10 μ m. Valves are heteropolar, clavate with broadly rounded headpole and acutely rounded footpole (figs 6A–P, AI–AO, 7A–I, S–Y & AO–AQ). The morphometric data of the clone cultures ($n = 207$) are: length 9.5–33.9 μ m, width 4.1–9.3 μ m and 8–15 striae in 10 μ m (figs 6Q–S, T–AH, AP, AQ, 7J–R, AB–AC & AR–AV). The valves have a wide variation within the clone cultures: they can be heteropolar, clavate with broadly rounded headpoles and acutely rounded footpoles (i.e. fig. 6Q), or heteropolar and lanceolate to linear lanceolate (i.e. fig. 6R), or be slightly heteropolar with almost parallel to slightly convex margins and rounded headpole and footpole (e.g. fig. 6S). In both natural populations and clone cultures, an axial plate and mantle lamella is lacking. The axial area is narrow, straight, expanded at the centre to form a rectangular, bow-tie-shaped to transversely elliptical central area bordered at the margins by 1–3 approximately equally-shortened striae arched around the central area (figs 5A, B, 8B, E, H, K, 9B, E, H, K, 10B, E). Except for occasional isolated puncta, which seem to be simple areolae as continuations of the central striae (figs 7AR–AS &

9K), structures similar to stigmoids or stigmata are lacking. Raphe lateral, with external proximal ends dilated or drop-shaped (figs 8B, H, 9B, H & 10B), extending into the central area; the external distal raphe endings extend straight (in some valves slightly deflected) onto the valve mantle at both poles (figs 8A, C, G, I, 9A, C, G, I & 10A, C). Internal proximal raphe endings curved in the same direction and located on a raised central nodule (figs 8E, 9E, K & 10E). Internal distal raphe endings - terminal nodules or helictoglossae - are distinct, positioned well before valve terminus (figs 8F, L, 9F, L & 10F). The striae are biseriate composed of small, round areolae not occluded by siliceous flaps that terminate as single arched rows around the central area (figs 8B, E, H & K). The internal structure of the single row of areolae is more pronounced in some valves, bordered by thickened vimines (fig. 8E). At the headpole, the striae are composed of two alternating rows of areolae (fig. 8A); some areolae are slit like (figs 9C, I & 10A). The striae are not interrupted near the valve face/mantle junction and continuing onto the valve mantle. (figs 5C & 14A-C). Transapical striae are strongly

radiate in the central valve and towards the footpole (fig. 8B, C, E & F), becoming slightly radiate towards the headpole (figs 8A & 10A). The footpole has a large apical pore field with porelli of the same size and structure as the areolae. The porelli appear to be arranged in double rows, located close to the striation and therefore undifferentiated structurally and spatially from them (figs 8C, I, & 9C, I). Both apices have distinct pseudosepta (figs 8D, F, J, 9D, F & 10D, F).

***Gomphonella olivacea* genodeme 2**

Fig. 7AD-AN

Fourteen strains show the same molecular data for 18SV4 and *rbcL* (see electronic appendix 1), but two strains (D03_184 Spree, fig. 7AF-AN; and D132_024 Saale; figs 7AD-AE & 10A-F) are slightly different and show p-distances from the others of 0.7% (18SV4) and 0.4% (*rbcL*). These two strains could represent a separate variety but since no morphological differences have been found, we are refraining here from naming and ranking this taxon, just using instead the neutral

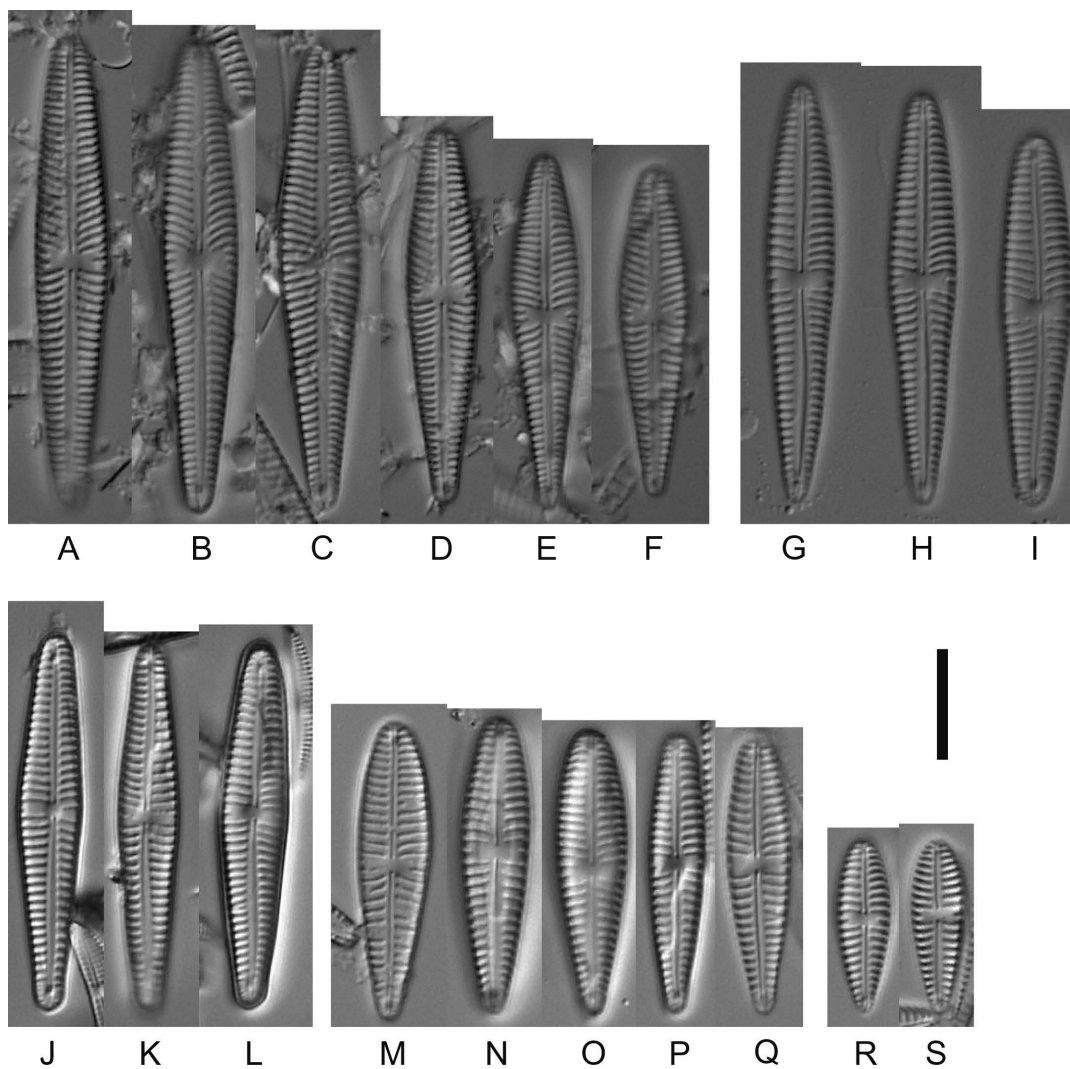


Figure 11 – *Gomphonella acsiae*, LM, specimens from Lake Balaton, Hungary: A-F, population from sample D140; G-I, type strain D140_006; H represents the holotype; J-L, strain D140_008b; M-Q, strain D140_001; R & S, strain 140_007. Scale bar = 10 µm.

term genodeme to mark molecular differences from genodeme 1 (i.e. the epitype of *G. olivacea*, see above).

***Gomphonella acsiae* R.Jahn & N.Abarca, sp. nov.**

Figs 11–12 & 14B

Type – Hungary, Tihany, Lake Balaton (46.914021°N, 17.892833°E), 23 Apr. 2016, *R. Jahn, K. Buczkó* D140 (holotype: B, slide B 40 0042417, prepared from strain D140_006 iso-

lated by O. Skibbe, represented in fig. 11H; iso-: BP, slide HNHM-ALG-D002300).

Description – The morphometric data of the type population ($n = 37$) are: length 19.2–44.5 μm , width 6.6–8.8 μm and 12–15 striae in 10 μm (fig. 11A–F). Valves are slightly heteropolar, lanceolate in larger specimens to clavate in smaller specimens with narrowly rounded headpoles and acutely rounded footpoles. The morphometric data of the clone cultures (n

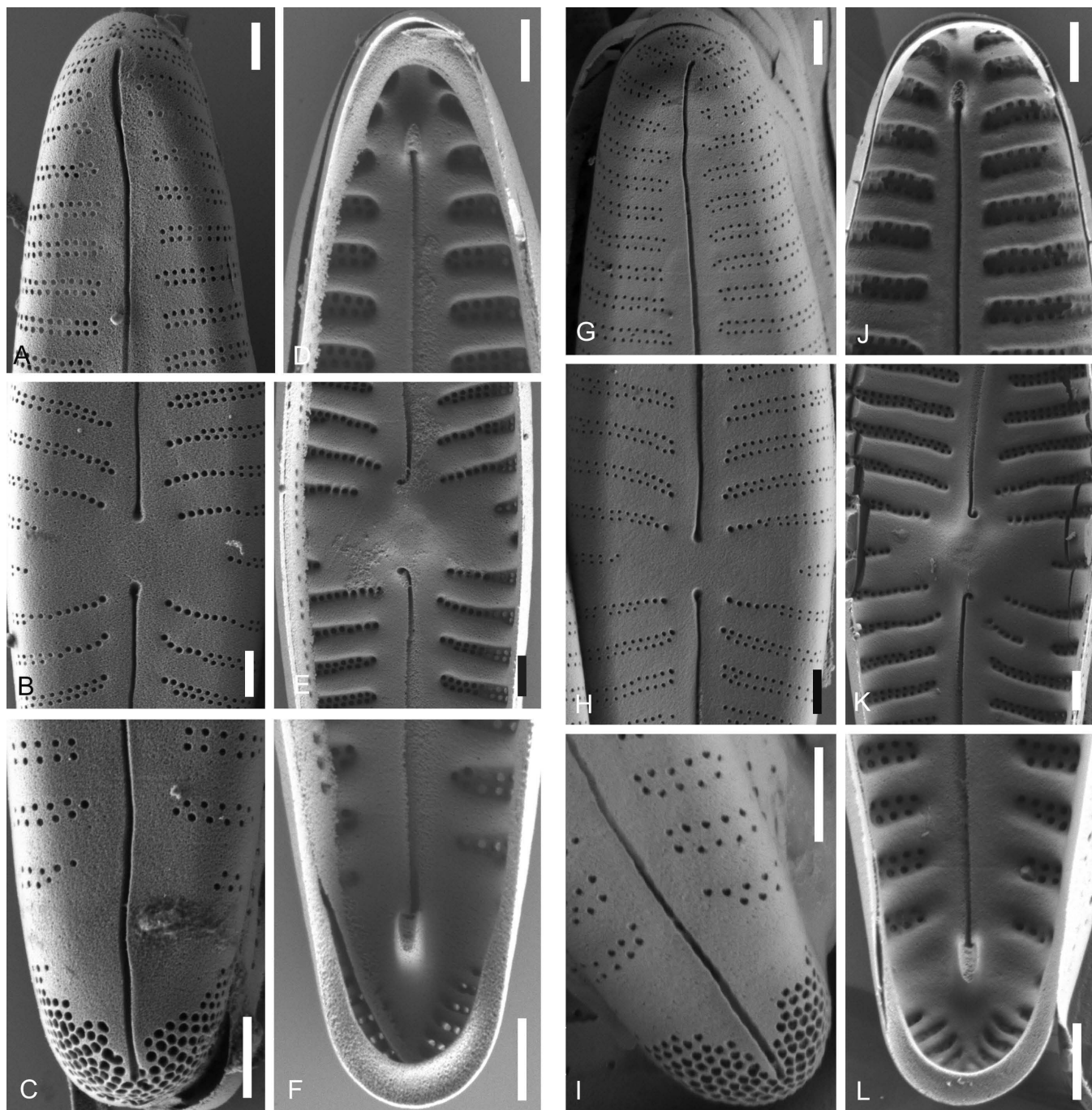


Figure 12 – *Gomphonella acsiae*, SEM, Lake Balaton, Hungary: A–F, epitype strain D140_006; G–L, strain D140_001; A–C & G–I, external valve views; D–F & J–L internal valve views; A, D, G & J, headpole; B, E, H & K, central area, note the slightly arched striae and the rectangular form; C, F, I & L, footpole, the porelli are larger than the areolae; note the disordered porelli and the large gap of striation between the striae and the porelli. Scale bars = 1 μm .

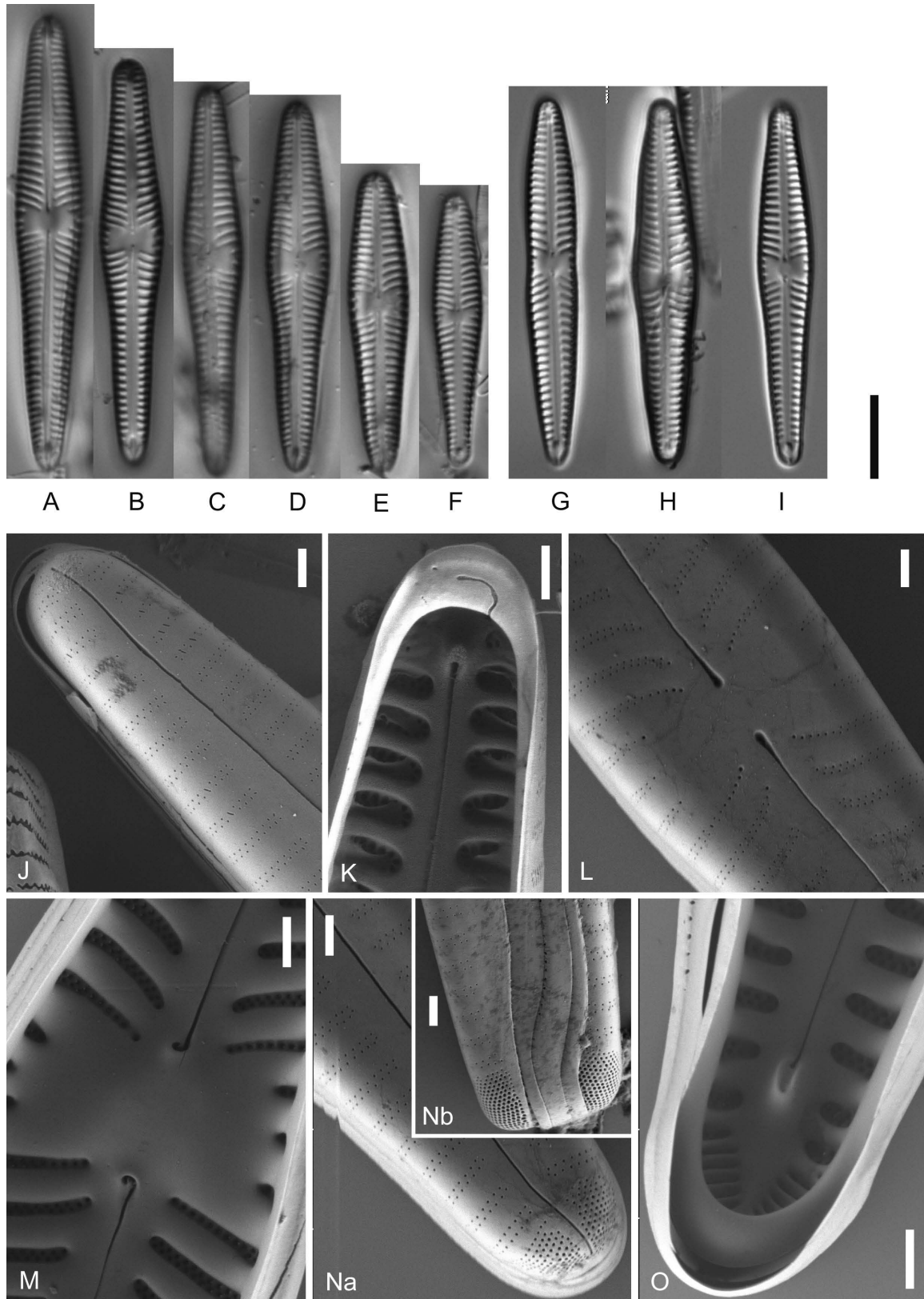


Figure 13 – *Gomphonella coxiae*, specimens from sample D201, Heleneesee, Brandenburg, Germany: A–I, LM; J–O, SEM; A–F, population; G–O, strain D201_007; I represents the holotype; J, L & Na, show external valve view and Nb an external girdle view of the footpole; K, M & O, show internal valve views; J & K, headpole; L & M, central area, note the bow tie and the straight striae; Na, Nb & O, footpole, the porelli are larger than the areolae. Note the densely arranged porelli and no gap of striation between the striae and the porelli (compared to Clade 1). Scale bars: A–I = 10 μ m; J–O = 1 μ m.

= 58) are: length 13–39.7 μm , width 4.5–6.8 μm and 11–15 striae in 10 μm . The valves have a wide variation of shape within the clone cultures: they can be slightly heteropolar, lanceolate and widest at the centre, or heteropolar, clavate with broadly rounded headpoles and acutely rounded footpoles (figs 11G–S). In both, the natural population and clone cultures, an axial plate and mantle lamella is lacking. The axial area is narrow, straight, expanded at the centre to form a small, rectangular central area bordered at the margins by 1–3 irregularly-shortened striae slightly arched around the central area (fig. 12B, E, H & K). Stigmoids in the central area are lacking. Raphe lateral, with external proximal ends slightly dilated (fig. 12B & H), extending into the central area; external distal raphe endings extend straight (fig. 12A) (in some valves slightly deflected, fig. 12C) onto the valve mantle at both poles. Internal proximal raphe endings curved in the same direction and located on a raised central nodule (fig. 12E & K). Internal distal raphe ends -terminal nodules or helictoglossae - are distinct, in the foot pole positioned

well before valve terminus (fig. 12F & L). The striae are biseriate composed of small, round areolae not occluded by siliceous flaps that terminate as single rows around the central area (fig. 12B, E, H & K). The internal structure of the single rows of areolae is more silicified in some valves, bordered by thickened vimines at the central area (fig. 12E). At the headpole, the striae are composed of two alternating rows of areolae (fig. 12A & G). Transapical striae are parallel, becoming radial at the centre 11–15 in 10 μm . The footpole has a large apical pore field with relatively large porelli, different in size from the areolae of the striae and well separated from the striation (fig. 12C & I). Both apices have distinct pseudosepta (figs 12D, F & L).

Registration – <https://phycobank.org/100350>

Etymology – We are dedicating this species to Dr. Éva Ács who has dedicated her scientific life to promote algae research in Hungary.

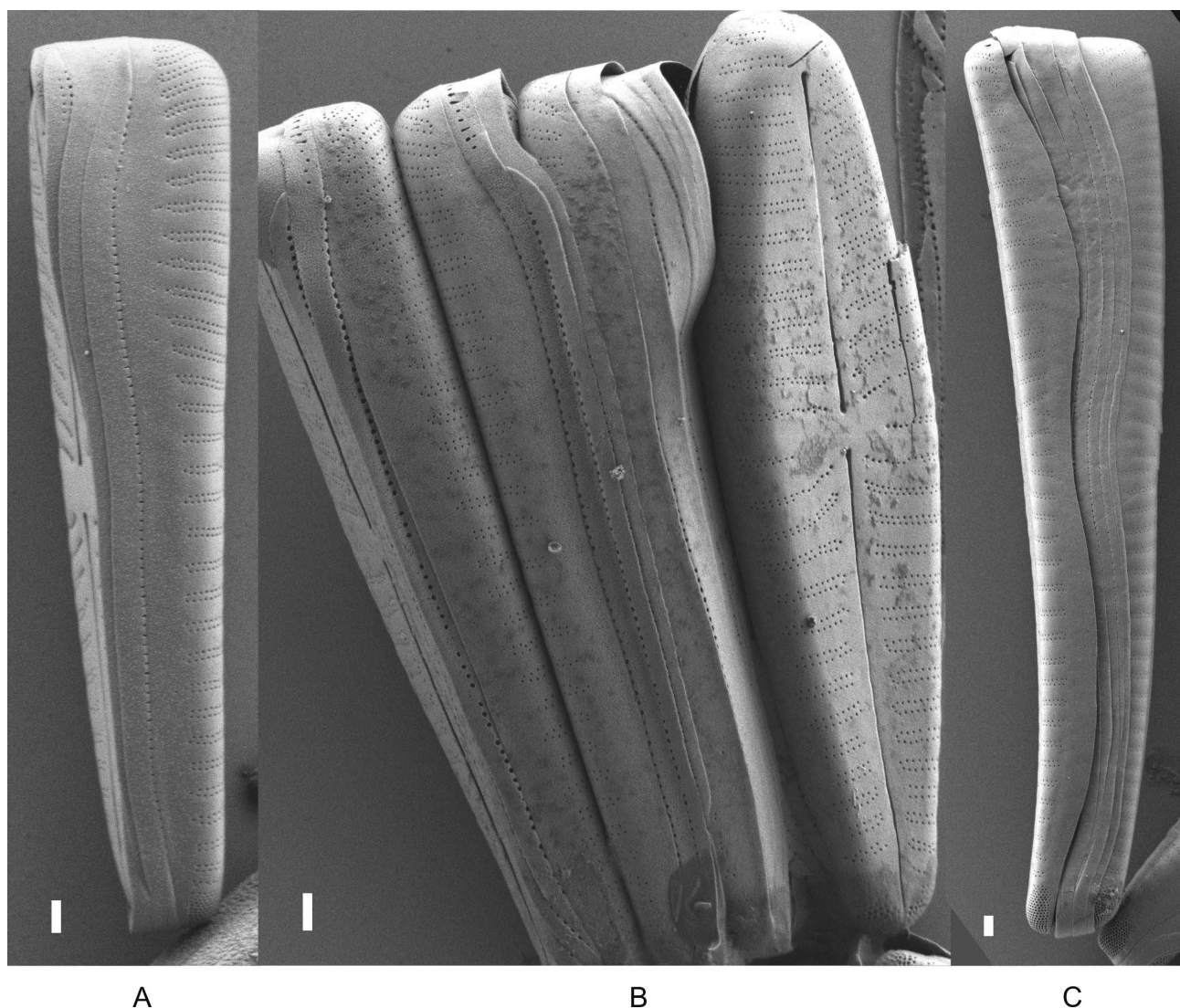


Figure 14 – Comparison of girdle views of *Gomphonella* species, SEM: A, *Gomphonella olivacea*, strain 129_007; B, *Gomphonella acsiae*, strain D140_001; C, *Gomphonella coxiae*, strain D201_007. Scale bars = 1 μm .

Gomphonella coxiae R.Jahn & N.Abarca, **sp. nov.**

Figs 13A–O & 14C

Type – Germany, Brandenburg, Helensee (52.267597°N, 14.503299°E), 13 Jul. 2017, R. Jahn & J. Zimmermann D201 (holo-: B, slide B 40 0042914, prepared from strain D201 007 isolated by O. Skibbe, represented in fig. 13I).

Description – The morphometric data of the type population ($n = 10$) are: length 33.5–55.7 μm , width 6.7–8.6 μm and 9–11 striae in 10 μm . Valves are slightly heteropolar, lanceolate in larger specimens to clavate in smaller specimens with narrowly rounded headpoles and acutely rounded footpoles (fig. 13A–F). The morphometric data of the clone culture ($n = 18$) are: length 42.8–46.1 μm , width 5.8–7.4 μm and 8–10 striae in 10 μm . The valves of the clone cultures are linear-clavate or with a slight tumid swelling at the centre; headpoles narrowly rounded and footpoles rounded (fig. 13G–I). In both the natural population and clone cultures the axial plate is lacking. The axial area is narrow, straight, expanded at the centre to form a broad, bow-tie-shaped to rectangular central area bordered at the margin by 2 or 3 approximately equally-shortened striae (fig. 13L–M). Stigmoids are lacking. Raphe lateral, with external proximal ends dilated, extending into the central area (fig. 13L); external distal raphe slightly bent before the apical point and extending onto the valve mantle at both poles (fig. 13J & N). Internal proximal raphe endings curved in the same direction and located on a raised central nodule (fig. 13M). Internal distal raphe endings - terminal nodules or helictoglossae - are distinct, positioned well before valve terminus (fig. 13O). The striae are biserial and composed of two small, round, and alternating but not occluded rows of areolae (fig. 13J); they terminate as single rows but are not arched around the central area. Transapical striae parallel, becoming radial at the centre. The footpole has a large apical pore field with relatively large porelli, which differ in size from the areolae of the striae and are well separated from the striation (fig. 13Na, b). Both apices have distinct pseudosepta (fig. 13K & O).

Registration – <https://phycobank.org/100352>

Etymology – We are dedicating this species to Dr. Eileen Cox for her outstanding contributions to diatom research as an author, editor and colleague. In addition, she organized and hosted the first German Speaking Diatom Meeting when she was doing research at the Limnological Station in Schlitz, Germany, in 1987.

New combinations

As explained further in the Discussion section, astigmatate taxa of the genera *Gomphoneis* and *Gomphonema* from several localities around the world need nomenclatural transfer to the genus *Gomphonella*. We effect those transfers here. But we are refraining from recombining taxa where SEM data is missing for an unambiguous demonstration that there is no stigma, typical striation, areolae and porelli (see table 1). This means that we had to rely on recent descriptions or interpretations. And we are refraining from recombining many of the infraspecific taxa of *G. olivacea* because we think that they need to be studied also molecularly in order to find out if we are dealing here with species or only outline variations. Differentiating features are listed in table 1.

Gomphonella baicaliana (Kociolek & Kulikovskiy) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis baicaliana* Kociolek & Kulikovskiy, Phytotaxa 154: 18, figs 283–286. 2013 (Kociolek et al. 2013). – Type: Russia, Lake Baikal, 1998 (holo-: COLO).

Registration – <https://phycobank.org/100353>

Gomphonella baltica (Cleve) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema balticum* Cleve, Öfversigt af Förhandlingar: Kongl. Svenska Vetenskaps-Akademien 25: 231, pl. 4, figs 10–16. 1868 (Cleve 1868). – Type: Sweden, Gotland.

Registration – <https://phycobank.org/100355>

Gomphonella basiorobusta (Q.You & Kociolek) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis basiorobusta* Q.You & Kociolek, Phytotaxa 103: 12, figs 55–66. 2013 (You et al. 2013). – Type: P.R.China, Xinjiang, Kalakule Lake, 16 Jul. 2007, Wang & You 071018 (holo-: SHTU).

Registration – <https://phycobank.org/100356>

Gomphonella calcarea (Cleve) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema calcareum* Cleve, Öfversigt af Förhandlingar: Kongl. Svenska Vetenskaps-Akademien 25: 231, pl. 4, figs 7–9. 1868 (Cleve 1868).

Synonym – *Gomphonema olivaceum* var. *calcareum* (Cleve) Van Heurck, Synopsis des diatomées de Belgique: explanation of pl. 25, fig. 23. 1880 (Van Heurck 1880). – Type: Sweden, Gotland.

Registration – <https://phycobank.org/100357>

Gomphonella densistriata (Levkov) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema densistriatum* Levkov, Phytotaxa 30: 30, figs 210–221, 253–258. 2011 (Levkov & Williams 2011). – Type: Macedonia, Lake Ohrid, 17 Mar. 2007 (holo-: BM).

Registration – <https://phycobank.org/100358>

Gomphonella distorta (Q.You & Kociolek) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis distorta* Q.You & Kociolek, Phytotaxa 103: 19, figs 97–108. 2013 (You et al. 2013). – Type: P.R.China, Xinjiang, Little Kalakule Lake, 16 Jul. 2007, Wang & You 071015 (holo-: SHTU).

Registration – <https://phycobank.org/100359>

Gomphonella fourtanierae (Kociolek & Kulikovskiy) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis fourtanierae* Kociolek & Kulikovskiy, Phytotaxa 154: 18, figs 287–306, 402–404.

Table 1 – *Gomphonella* taxa, their morphometrics and their ultra-structural differentiating features as documented in the corresponding reference.

Taxon name Corresponding reference	Length (in µm)	Width (in µm)	Striae (in 10 µm)	Areolae per striae	Axial plate	Mantle lamella	Shape of central area	Striae in central area	Porcelli at footpole	Pore field close to striae	Pore field arranged in double rows
<i>G. acstae</i> this study	13.0–44.5	4.5–8.8	11.0–15.0	biseriate	no	no	rectangular	slightly arched	relatively large, distinct	no	no/ discernible
<i>G. baicaliana</i> Kociolek et al. (2013: figs 283–286)	35–60	9–11	14–17	?	?	?	irregularly expanded	slightly arched	distinct	?	?
<i>G. baltica</i> Levkov (2016: figs 189: 1–19)	34–53	8–10	10–11	?	?	?	small, round to irregular	slightly arched	distinct	?	?
<i>G. basiorobusta</i> You et al. (2013: figs 61–66)	28.5–52.0	5.0–8.2	13–15	biseriate	no	no	rectangular to bow-tie	slightly arched	distinct	yes	yes
<i>G. calcarea</i> Cleve (1868: figs 4: 7–9), Levkov (2016: figs 188: 1–19).	22–55	7.5–10	9–11	?	?	?	bow-tie to round	arched, to slightly arched	?	?	?
<i>G. coxiae</i> this study	33.5–55.7	6.7–8.6	9–11	biseriate	no	no	bow-tie	straight	relatively large, distinct	yes	no
<i>G. densistriata</i> Levkov & Williams (2011: figs 253–258)	27–44	7.0–8.5	15–18	biseriate	no	no	small, elliptical to rhombic	straight	similar size and shape as areolae	yes	discernible
<i>G. distorta</i> You et al. (2013: figs 102–108)	30–45.2	5.5–7.5	10–14	biseriate	no	no	small, round to elliptical	slightly arched	distinct	yes	yes
<i>G. fonicola</i> Levkov et al. (2007: pl. 176, fig. 20)	21–42	5.5–7.5	10–14	biseriate	no	?	rectangular to bow-tie	slightly arched	similar size and shape as areolae	yes	?
<i>G. fourtanierae</i> Kociolek et al. (2013: figs 402–404)	19–55	5–11	15–18	biseriate	no	no	rectangular to bow-tie	straight/ slightly arched	?	?	?
<i>G. linearoides</i> Levkov & Williams (2011: figs 246–250)	24–35	5–6.5	7–10	biseriate	no	no	rectangular to bow-tie	straight/ slightly arched	similar size and shape as areolae	yes	discernible
<i>G. ohridana</i> Levkov et al. (2007: pl. 178, figs 1–9)	64–135	12–22	7.5–9.5 at centre	biseriate	yes	?	rectangular	slightly arched	?	?	?
<i>G. olivacea</i> this study	9.5–42.2	4.1–9.3	8.0–15	biseriate	no	no	rectangular bow-tie to transversely elliptical	arched	similar size and shape as areolae	yes	yes/ discernible
<i>G. olivacea 2</i> this study	13.2–23.3	4.6–7.0	11.0–15	biseriate	no	no	see above	arched	see above	yes	discernible

Table 1 (continued) – *Gomphonella* taxa, their morphometrics and their ultra-structural differentiating features as documented in the corresponding reference.

Taxon name	Length (in µm)	Width (in µm)	Striae (in 10 µm)	Arcolae per striae	Axial plate	Mantle lamella	Shape of central area	Striae in central area	Porelli at footpole	Pore field close to striae	Pore field arranged in double rows
<i>G. olivaceolacua</i> Lange-Bertalot (1993: fig. 81; 3, 4 & fig. 82: 1–4)	23–35	8.5–10.5	14–16	biseriate	no	no	rectangular to elliptical-rhombic	slightly arched	relatively large, distinct	yes	yes
<i>G. erolvaccacolacua</i> Levkov & Williams (2011: figs 241–245)	31–53	9.5–11	12–17	biseriate	no	?	small, elliptical to elongated	slightly arched	similar size and shape as areolae	yes	yes
<i>G. potapovae</i> Kocotolek et al. (2013: figs 417–419)	13–28	4–6	16–20	biseriate	no	no	narrow, rectangular to X-shaped	straight	relatively large, distinct	?	discernible
<i>G. prespanensis</i> Levkov et al. (2007: pl. 180, figs 1–6)	47–105	12–16.5	9–12.5 at centre	biseriate	yes	yes	rectangular to elliptical	slightly arched	?	yes	?
<i>G. pseudosubtiloides</i> You et al. 2013: figs 19–25)	45.4–51	8.1–8.6	9–12	biseriate	no	no	rectangular to bow-tie	slightly arched	relatively large	yes	yes
<i>G. qii</i> You et al. (2013: figs 31–38)	39–42	6.5	10–12	biseriate	no	no	rectangular	straight/ slightly arched	similar size and shape as areolae	yes	no
<i>G. reedinae</i> Levkov et al. (2016: pl. 187: fig. 31)	24–35	5.5–6.6	11–14	biseriate	no	no	bow-tie	straight/ slightly arched	?	?	?
<i>G. rostratoides</i> You et al. (2013: figs 91–96)	35.8–45	5.9–6.0	12–14.5	biseriate	no	no	small, round to elliptical	slightly arched	similar size and shape as areolae	yes	discernible
<i>G. russica</i> Kocotolek et al. (2013: figs 353–359)	47–68	11–14	11–15	biseriate	no	no	rectangular	straight	?	?	?
<i>G. stauroneiformis</i> Van Heurck (1880: pl. 25, fig. 22), this study	42	12	10–12	?	no	no	bow-tie	straight	?	?	?
<i>G. stoermeri</i> You et al. (2013: figs 77–83)	36.0–54.7	5.9–7.6	10–13	biseriate	no	no	round to elliptical	slightly arched	distinct	yes	yes
<i>G. strelnikovae</i> Kocotolek et al. (2013: figs 412–416)	12–18.5	3–5	19–22	biseriate	no	no	narrow, rectangular to X-shaped	straight	yes but absent from valve face	yes	no
<i>G. subolivacea</i> Levkov et al. (2007: pl. 176, figs. 10–15)	13–28	6.5–8.5	15–20	biseriate	no	no	small variable	straight/ slightly arched	similar size and shape as areolae	yes	yes

Table 1 (continued) – *Gomphonella* taxa, their morphometrics and their ultra-structural differentiating features as documented in the corresponding reference.

Taxon name Corresponding reference	Length (in µm)	Width (in µm)	Striae (in 10 µm)	Areolae per striae	Axial plate	Mantle lamella	Shape of central area	Striae in central area	Porelli at footpole	Pore field close to striae	Pore field arranged in double rows
<i>G. subrossica</i> Kociolek et al. (2013): figs 360–364)	29–41	7–9	11–13	biseriate	no	no	small irregular	slightly arched	?	?	?
<i>G. subtiloides</i> You et al. (2013): figs 7–14)	35–63	6.4–9.4	11–13	biseriate	no	no	rectangular to bow-tie	slightly arched	similar size and shape as areolae	yes	no
<i>G. tegeleensis</i> Skibbe et al. (2018): figs 6–10 & 13–26)	99.6–124.2	16.6–19.3	7–8	bi-triseriate	yes	yes	rectangular	straight/ slightly arched	relatively large, distinct	yes	no
<i>G. transylvanica</i> Skibbe et al. (2018): figs 11, 12)	72–100	14–16	10–12	biseriate	yes	yes	bow-tie	straight	distinct	yes	?
<i>G. xinjiangiana</i> You et al. (2013): figs 48–54)	22.4–32.7	5.3–6.3	14–16	biseriate	no	no	rectangular	slightly arched	slightly larger	yes	yes

2013 (Kociolek et al. 2013). – Type: Russia, Lake Baikal, 1998 (holo-: COLO).

Registration – <https://phycobank.org/100360>

Gomphonella fonticola (Hust.) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema olivaceum* var. *fonticola* Hust., Archiv für Hydrobiologie 40: 942, pl. 40, figs 19–22. 1945 (Hustedt 1945).

Synonyms – *Gomphonema fonticola* ‘fonticulum’ (Hust.) Levkov & Krstic (Levkov et al. 2007). – *Gomphoneis fonticola* (Hust.) Kociolek & Kulikovskiy (Kociolek et al. 2013). – Type: Macedonia, Lake Ohrid, St. Naum (lecto-: BRM).

Registration – <https://phycobank.org/100361>

Gomphonella linearoides (Levkov) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema linearoides* Levkov, Phytotaxa 30: 30, figs 199–209, 246–250. 2011 (Levkov & Williams 2011). – Type: Macedonia, Lake Ohrid, 25 Apr. 2003 (holo-: BM).

Registration – <https://phycobank.org/100362>

Gomphonella ohridana (Levkov) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis ohridana* Levkov, Iconographia Diatomologica 16: 60, pl. 178, figs 1–9. 2007 (Levkov et al. 2007). – Type: Macedonia, Lake Ohrid, 25 Apr. 2003 (holo-: MKNH).

Registration – <https://phycobank.org/100363>

Gomphonella olivaceolacua (Lange-Bert. & E.Reichardt) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema olivaceum* var. *olivaceolacuum* Lange-Bertalot & E.Reichardt, Bibliotheca Diatomologica 27: 67, pl. 80, figs 1–8; pl. 81, figs 3, 4; pl. 82, figs 1–4. 1993 (Lange-Bertalot 1993).

Synonyms – *Gomphonema olivaceolacuum* (Lange-Bert. & E.Reichardt) Lange-Bert. & E.Reichardt (Werum & Lange-Bertalot 2004). – *Gomphoneis olivaceolacua* (Lange-Bert. & E.Reichardt) Kociolek & Kulikovskiy (Kociolek et al. 2013). – Type: Switzerland, Lake Geneva, Mar. 1976 (holo-: FR).

Registration – <https://phycobank.org/100364>

Gomphonella perolivaceolacua (Levkov) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema perolivaceolacuum* Levkov, Phytotaxa 30: 28, figs 185–198, 241–245. 2011 (Levkov & Williams 2011). – Type: Macedonia, Lake Ohrid, 17 Mar. 2007 (holo-: BM).

Registration – <https://phycobank.org/100365>

Gomphonella potapovae (Kociolek & Kulikovskiy) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis potapovae* Kociolek & Kulikovskiy, Phytotaxa 154: 18, figs 305–315, 417–419. 2013 (Kociolek et al. 2013). – Type: Russia, Lake Baikal, 1998 (holo-: COLO).

Registration – <https://phycobank.org/100366>

Gomphonella prespanensis (Levkov, Metzeltin & Krstic) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis prespanensis* Levkov, Metzeltin & Krstic, Iconographia Diatomologica 16: 62, pl. 174: figs 1–6; pl. 178: fig. 10; pl. 179, figs 1–8; pl. 180, figs 1–6. 2007 (Levkov et al. 2007). – Type: Macedonia, Lake Prespa, 31 Jan. 2003 (holo-: MKNH).

Registration – <https://phycobank.org/100367>

Gomphonella pseudosubtiloides (Q.You & Kociolek) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis pseudosubtiloides* Q.You & Kociolek, Phytotaxa 103: 5, figs 15–25. 2013 (You et al. 2013). – Type: P.R.China, Xinjiang, Little Kalakule Lake, 16 Jul. 2007, Wang & You 071015 (holo-: SHTU).

Registration – <https://phycobank.org/100368>

Gomphonella qii (Q.You & Kociolek) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis qii* Q.You & Kociolek in You et al., Phytotaxa 103: 6, figs 26–38. 2013 (You et al. 2013). – Type: P.R.China, Xinjiang, Bulunkou township, 16 Jul. 2007, Wang & You 071013 (holo-: SHTU).

Registration – <https://phycobank.org/100369>

Gomphonella reediae (Levkov, Mitić-Kopanjanja & E.Reichardt) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema reediae* Levkov, Mitić-Kopanjanja & E.Reichardt, Diatoms of Europe 8: 112, pl. 187: figs 1–31. 2016 (Levkov et al. 2016). – Type: Macedonia, Doiran Lake, 29 Jul. 1997, Levkov s.n. (holo-: MKNH).

Registration – <https://phycobank.org/100370>

Gomphonella rostratoides (Q.You & Kociolek) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis rostratoides* Q.You & Kociolek, Phytotaxa 103: 17, fig. 84–96. 2013 (You et al. 2013). – Type: P.R.China, Xinjiang, Little Kalakule Lake, 16 Jul. 2007, Wang & You s.n. (holo-: SHTU).

Registration – <https://phycobank.org/100371>

Gomphonella russica (Kociolek & Kulikovskiy) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis russica* Kociolek & Kulikovskiy, Phytotaxa 154: 21, figs 353–359. 2013. (Kociolek et al. 2013). – Type: Russia, Lake Baikal, 1998 (holo-: COLO).

Registration – <https://phycobank.org/100372>

Gomphonella stauroneiformis (Grunow) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema stauroneiforme* Grunow in Schneider, Naturwissenschaftliche Beiträge zur Kenntniss der Kaukasusländer: auf Grund seiner Sammelbeute: 106. 1878 (Schneider 1878).

Synonym – *Gomphonema olivaceum* var. *stauroneiforme* ‘stauroneiformis’ Grunow in Van Heurck, Synopsis des diatomées de Belgique. Atlas: pl. 25, fig. 22. 1880 (Van Heurck 1880). *Gomphoneis stauroneiformis* ‘stauroneiforme’ (Grunow) Q.You & Kociolek in You et al. (2013). – Type: Type: Tirol und Schweiz, Gebirgsbäche.

Registration – <https://phycobank.org/100374>

Gomphonella stoermeri (Q.You & Kociolek) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis stoermeri* Q. You & Kociolek, Phytotaxa 103: 14, figs 67–83. 2013 (You et al. 2013) – Type: P.R.China, Xinjiang, Little Kalakule Lake, 16 Jul. 2007, Wang & You 071015 (holo-: SHTU).

Registration – <https://phycobank.org/100375>

Gomphonella strelnikovae (Kociolek & Kulikovskiy) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis strelnikovae* Kociolek & Kulikovskiy, Phytotaxa 154: 19, figs 316–327, 412–416. 2013 (Kociolek et al. 2013). – Type: Russia, Lake Baikal, 1998 (holo-: COLO).

Registration – <https://phycobank.org/100376>

Gomphonella subolivacea (Levkov & Nakov) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema subolivaceum* Levkov & Nakov, Iconographia Diatomologica 16: 69, pl. 176, figs 1–15. 2007 (Levkov et al. 2007).

Synonym – *Gomphoneis subolivacea* (Levkov & Nakov) Kociolek & Kulikovskiy (Kociolek et al. 2013). – Type: Macedonia, Lake Ohrid, 17 Oct. 2002 (holo-: MKNH).

Registration – <https://phycobank.org/100377>

Gomphonella subrussica (Kociolek & Kulikovskiy) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis subrussica* Kociolek & Kulikovskiy, Phytotaxa 154: 22, figs 360–364. 2013 (Kociolek et al. 2013). – Type: Russia, Lake Baikal, 1998 (holo-: COLO).

Registration – <https://phycobank.org/100378>

Gomphonella subtiloides (Q.You & Kociolek) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis subtiloides* Q. You & Kociolek, Phytotaxa 103: 3, figs 1–14. 2013 (You et al. 2013). – Type: P.R.China, Xinjiang, Little Kalakule Lake, 16 Jul. 2007, Wang & You 071014 (holo-: SHTU).

Registration – <https://phycobank.org/100379>

Gomphonella tegelensis (R.Jahn & N.Abarca) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis tegelensis* R.Jahn & N.Abarca in Skibbe et al. Diatom Research 33: 256, figs 4–10, 13–26. 2018 (Skibbe et al. 2018). – Type: Germany, Berlin, Tegeler See, 11 Sep. 2017, Jahn s.n. (holo-: B).

Registration – <https://phycobank.org/100380>

Gomphonella transylvanica (Pant.) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphonema transylvanicum* Pant., Beiträge zur Kenntnis der Fossilen Bacillarien Ungarns 3: pl. 14, figs 219, 220. 1893 (Pantocsek 1893).

Synonym – *Gomphoneis transylvanica* ‘transsilvanica’ (Pant.) Krammer (Krammer & Lange-Bertalot 1985). – Type: ‘Bibarczfalva, Bodos et Köpecz in Transilvania’ (lecto-: [icon] pl. 14, fig. 220 in Pantocsek (1893); epi-: HNHM).

Registration – <https://phycobank.org/100381>

Gomphonella xinjiangiana (Q.You & Kociolek) R.Jahn & N.Abarca, **comb. nov.**

Basionym – *Gomphoneis xinjiangiana* Q.You & Kociolek, Phytotaxa 103: 11, figs 39–54. 2013 (You et al. 2013). – Type: P.R.China, Xinjiang, Little Kalakule Lake, 16 Jul. 2007, Wang & You 071014 (holo-: SHTU).

Registration – <https://phycobank.org/100382>

DISCUSSION

Identification

The most conspicuous trait of all valves studied is that they are very variable in outline, making it difficult to identify individual valves and clones if the initial population is not taken into consideration. This study shows with clones and populations that identification via outline alone is not possible in this group: data on density and direction of striation, besides SEM data on the porelli of the footpole (see also You et al. 2013) as well as molecular data, provide important autapomorphies for the differentiation of species.

As can be seen in the above results, the taxon *Gomphonella olivacea* contains two molecular entities, which we named genodeme 1 (Clade 1A) and 2 (Clade 1B). Both were isolated from the same sample from Saale; they seem to occur side by side. The molecular data of the clone *Cymbellales* sp. from the environmental sample Westerhöver Bach (Brinkmann et al. 2015) have no associated morphological data but the molecular data show that it belongs to *Gomphonella olivacea* genodeme 1. In 18S V4 eDNA metabarcoding samples, more sequences of *Gomphonella olivacea* genodemes 1 and 2 were recorded, showing that they both occur in the Swiss rivers Thur at Niederneunforn and Töss at Kyburg in the valley Leisentäl at four sites each (J. Zimmermann & J. Hürlimann, unpubl. obs.) which might indicate that we are dealing here with a number of morphologically cryptic species.

Gomphonella acsiae, on the other hand, shows a diverse outline in cultured and environmental material but all have

the same genotype in our four samples. In his paper on the diatoms of Lake Balaton, Pantocsek (1901) described a number of new *Gomphonema* taxa in the group of Symmetrica Grunow (in Van Heurck, 1885: 123): *Gomphonema balatonis* Pant., *Gomphonema naviculaceum* Pant., *Gomphonema ovulum* Pant., and *Gomphonema olivaceum* var. *subacutum* Pant. He also reported the presence of *Gomphonema olivaceum* (Lyngb.) Ehrenb., *Gomphonema exiguum* Kütz. and *Gomphonema telographicum* Kütz. Unfortunately, none of Pantocsek’s drawings concerning the central area and striation (1901), nor the morphometric data of these taxa (length, width and striae/10 µm), fit our new taxon. A recent extensive search in Pantocsek’s material (K. Buczkó, pers. comm.) has revealed no valves which match our new taxon. This taxon is apparently new to Lake Balaton but it has evidently been seen since 2007 in Lake Balaton by Éva Ács (pers. comm.) and by Crossetti et al. (2013) but not correctly identified. The form of the central area and the striation pattern of *G. acsiae* are similar to *Gomphonella calcarea* (Cleve) R.Jahn & N.Abarca but the new species is differentiated by its stria density of 9–11 striae/10 µm versus 11–15 striae/10 µm. *Gomphonella periolivaceolacua* (Levkov) R.Jahn & N.Abarca possesses a clavate valve with a broadly rounded headpole, the central area is small, elliptical to transversely elongated and the apical pore field is composed of round porelli, with similar size and shape to the areolae. In *G. baltica* (Cleve) R.Jahn & N.Abarca the central area is smaller, round to irregular in shape, possesses a broadly-rounded headpole, and has a stria density of 10–11 striae/10 µm (see Levkov et al. 2016).

Gomphonella coxiae R.Jahn & N.Abarca has some resemblance to *G. densistriata* (Levkov) R.Jahn & N.Abarca but differs by having transapical striae that are strongly radiate in mid-valve, and by the stria density of 15–18 striae/10 µm, versus 9–11 striae/10 µm in *G. coxiae*. Levkov et al. (2016: pl. 186) also described a ‘*Gomphonema olivaceum* morphotype 3’ with a similar outline to *Gomphonella coxiae* but this taxon possesses 16–18 striae/10 µm. *Gomphonella coxiae* has some resemblance to *Gomphonema stauroneiforme sensu* Krammer & Lange-Bertalot (1991: pl. 88, fig. 16) and Hofmann et al. (2013) (this is not the same diatom as *Gomphonema stauroneiforme* Grunow). *Gomphonella coxiae* has a narrower valve (width-to-length ratio < 0.2) in contrast to *G. stauroneiformis* (Grunow) R.Jahn & N.Abarca as depicted by Grunow in Van Heurck (1880).

Gomphoneis tegelensis R.Jahn & N.Abarca has been described and treated in detail by Skibbe et al. (2018) concerning its ultrastructure and its occurrence as a neobiota in Germany. However, since it has more in common with *G. olivacea* than with *Gomphoneis* s. str. (it has only some triseriate striae and a small axial plate and mantle lamella) and it is molecularly well supported within the *Gomphonella*-Clade, we have decided to put this taxon also into the genus *Gomphonella*.

Habitus and habitat

An important feature of the living diatom *Gomphonella* is its mucus production. For Hornemann (1810), who was not aware of the individual cells producing his “*Ulva*” *olivacea*,

the jelly was the most conspicuous character (“Substantia gelatinosa lubrica supellucida”). Rabenhorst (1859) also used this feature to differentiate his new genus *Gomphonella* from *Gomphonema*: a stalked *Gomphonema* in an amorphous gelatinous mass (“Eine gestielte *Gomphonema* in einer gestaltlosen Gallertmasse”). When we took *Gomphonella olivacea* into culture we often noted that it grew in thick spherical lumps (O. Skibbe, unpubl. obs.). When we collected *Gomphonella coxiae* from reeds in Helenesee, it was surrounded by a 1 cm thick periphyton covering. When we sampled Lake Balaton, the stones at the shore of the lake were covered with a thick jelly which we expected to be *Didymosphenia* M.Schmidt (also known as ‘rock snot’ because of its jelly production). In the lab though, we did not find a single *Didymosphenia* but lots of *Gomphonella* specimens and some *Cymbella*. *Gomphonella olivacea* – identified as *Cymbellales* sp. because of missing taxonomic reference data – seems to be involved in the biogenic calcite precipitation and tufa formation of karstic streams (Brinkmann et al. 2015).

Distribution

Identified by its morphology alone (e.g. Kociolek 2011, Levkov & Williams 2011, Hofmann et al. 2013, Levkov et al. 2016), *Gomphonella olivacea* seems to be cosmopolitan, occurring with its many described varieties all over the world. For Central Europe, Hofmann et al. (2013) considered it to be the most frequent “*Gomphonema*”. Many of its varieties have been raised to species rank but it needs to be studied if these represent real species or just outline variations. When searching recent literature for SEM pictures of *Gomphoneis* and *Gomphonema* species which unambiguously show relevant synapomorphies [astigmatate valves, bi (to tri)-seriate striae with small round uniform areolae not occluded by siliceous flaps continuing unto the valve mantle, an apical foot pole composed of a bilobed field of porelli], we noticed that a diversity of apparently related species has been recently described from Asia such as China (You et al. 2013) and Lake Baikal (Kociolek et al. 2013) and to a lesser extent from Europe, such as from Lake Ohrid (Levkov & Williams 2011, Levkov et al. 2013) and Germany (this study). However – except for *Gomphonella olivacea* – none have been reported from the Americas, such as the USA (Spaulding & Edlund 2009) or Mexico (Vázquez et al. 2011, Mora et al. 2017). Whether this is a biogeographical pattern due to limited distribution or to missing investigations needs to be determined in future studies, when the autopomorphies of the genus *Gomphonella* in contrast to the genera *Gomphoneis* and *Gomphonema* have become better established. Nevertheless, these non-occurrences of *Gomphonella* in the Americas are paralleled by Cleve’s (1894) original remark on the distribution of *Gomphoneis* taxa: that this genus is found in the freshwaters of North and Central America.

Classification

From a molecular point of view *Gomphonella olivacea* is not closely related to *Gomphonema* as defined by its type *G. acuminatum*; the gene trees show that it does not even belong to the Gomphonemataceae (figs 3 & 4; and Skibbe et al. 2018: fig. 27). This is also supported by the different

morphologies, which are even discernible in LM since *Gomphonella* has no stigmoids, although occasionally there can be a few isolated puncta in the central area, set apart from the striae (figs 7AR, AS & 8K). The biseriate striation of the *Gomphonella* taxa previously included in *Gomphonema*, which has been explicitly assumed to be non-differentiating by Reichardt (2007) and Hofmann et al. (2013), is in fact constructed differently to the biseriate striation of *Gomphonema* s. str., which is clearly discernible if the valves are studied in the SEM (already seen by Dawson, 1974). To illustrate this fact, we here compare the micromorphology of *Gomphonella olivacea* (fig. 5A–C) with the taxon *Gomphonema minutum* (fig. 5D–F), since both have the same outline. In LM the only difference is the stigma in *Gomphonema minutum*. But in SEM, they look clearly different: *Gomphonema minutum* has biseriate areolae which are reniform and irregularly arranged and covered internally with flaps, in contrast to the round and regularly parallel arranged areolae with clear cut edges and no noticeable covering in *Gomphonella*. Also, the striae continue onto the valve mantle and end in a single well separated pore in *Gomphonema minutum*; in contrast to the abrupt stria endings in *Gomphonella*. Other differences include that the raphe is slightly bent at the foot pole and head pole in *Gomphonema minutum*; in contrast to the straight filiform raphe of *Gomphonella*; and the porelli at the foot pole are well set apart from the striae and the pores have a different pattern than the areolae of the striae (= differentiated APF) in *Gomphonema minutum*; in contrast to the undifferentiated apical porelli at the footpole of *Gomphonella*.

Molecularly, *Gomphonella olivacea* is also not closely related to *Gomphoneis minuta* (J.L.Stone) Kociolek & Stoermer (1988a) as published in Genbank. In contrast, it is closely related to *G. tegelesensis* as studied by Skibbe et al. (2018). Most interesting though is that these two taxa (*minuta* and *tegelesensis*), which had until now been put into the same genus *Gomphoneis*, are only distantly related (*rbcL* p-distance: 7.2–8.3%; 18SV4: 11.89–12.59%), with *Gomphoneis minuta* sitting closer to the *Gomphonema acuminatum* group (18SV4 and *rbcL* p-distances 3.3–3.5%) (Abarca et al. in prep.) within the Gomphonemataceae, whereas *G. tegelesensis* is sister to other *Gomphonella* species. This shows that our current concept of the genus *Gomphoneis* is polyphyletic. Unfortunately, the type of the name of the genus *Gomphoneis* – *Gomphonema elegans* Grunow in Van Heurck 1880 as *Gomphoneis elegans* (Grunow) Cleve 1894 according to Boyer (1928) – has not been studied molecularly in order to guide us to understand where the real *Gomphoneis* belongs phylogenetically; we presume that the true *Gomphoneis* will also not belong to the Gomphonemataceae (Cox 2015). This means that currently we have to rely on morphological differences only to delimit *Gomphonella* from *Gomphoneis*.

Cleve (1894: 73) described *Gomphoneis* and defined it as (*italics added*): “Valve elongated, clavate, or asymmetrical with the transverse axis. Median line straight, more or less oblique. Terminal fissures straight. Axial area narrow, linear. Central area small, rounded, with *one or more stigmata*. On both sides of the median line are longitudinal lines. Structure double: slightly radiate costae, and fine *puncta, forming obliquely decussating lines*. - Zone broader in the upper than in the lower end, not complex. Cell contents unknown. I have

Table 2 – Differentiating features of the genera *Gomphonema*, *Gomphoneis*, *Gomphosinica* and *Gomphonella* according to their corresponding reference.

<i>Taxon name</i> Corresponding Reference	Isolated puncta (stigma/stigmoid)	Striae	Areolae/puncta	Apical pore field (APF)	External raphe	Mantle	Axial plate/mantle lamella
<i>Gomphonema</i> Abarca et al. in prep.	yes, internally slit-like	uni- to biseriate	c- to reniform shaped and occluded by siliceous flaps	differentiated porelli, separated from the striae and mostly located on the mantle	undulate, bent at the poles and recurved	striae continue onto the mantle and end in separated pore(s)	none
<i>Gomphonella</i> this study	no, lacking	bi- to triseriate	round areolae with clear cut edges	undifferentiated porelli	straight, also straight at the poles	striae end abruptly on the mantle	some taxa
<i>Gomphoneis</i> Cleve 1894	yes, 1 or more	structure double	fine puncta, forming obliquely decussating lines	?	terminal fissures straight	?	on both sides of the median line are longitudinal lines
<i>Gompholeana</i> -group Kociolek & Stoermer 1988a	yes, internally slit-like	two rows of alternating puncta	irregular or angular shaped (similar to <i>Gomphonema</i>)	differentiated porelli (similar to <i>Gomphonema</i>)	slightly undulate, bent at the poles	striae continue onto the mantle and end in separated pore(s) (see fig. 31)	axial plate and marginal lamella
<i>Gomphoneis</i> <i>elegans</i> -group Kociolek et al. 2013	yes, 4 or more, internally simple openings have siliceous ingrowths - or lacking (see <i>Gomphonella</i>)	biseriate	round and unoccluded	undifferentiated porelli	straight, bent at the poles or nearly straight	striae end abruptly on the mantle (see fig. 378)	marginal lamella but no axial plate
<i>Gomphosinica</i> Kociolek et al. 2015	covering or hood comprised of many small, round openings	bi- to triseriate	round and unoccluded	undifferentiated porelli and mostly located on the mantle	slightly undulate, straight at the poles	striae end abruptly on the mantle (see fig. 126 & 128)	?

formed this new genus for some species formerly considered as belonging to *Gomphonema*, but differing from it both in the structure and the presence of the *longitudinal lines*. In these characteristics they agree nearly with *Scoliotropis*, but differ in the straight median line, and the asymmetrical form of the valve. To *Gomphoneis* may perhaps also belong *G. eriense* Grun. The few known species of *G.* are all of freshwater habitat and are found in North and Central America. *G. elegans* Grunow 1880, *G. herculeanum* Ehrenb. 1845. plus var. *robusta* Grun. 1878.”

In contrast to Cleve’s definition of *Gomphoneis*, *Gomphonella olivacea* has no stigma or stigmoid at all, no decussating lines of the double puncta and also no longitudinal lines. Nevertheless, Dawson (1974) transferred *G. olivaceum* and *G. quadripunctatum* to *Gomphoneis* (correctly done formally by Sims & Ross 1978) because of their double rows of simple round pores in contrast to the reniform poroidal structures of *Gomphonema* taxa.

In their paper on the phylogenetic relationship of *Gomphoneis*, Kociolek & Stoermer (1989) used morphological-cladistic methods to introduce two subgeneric groups, namely the *elegans*-group which contains the type of the genus, and the *herculeana*-group. This grouping helps to define the true *Gomphoneis*. *Gomphoneis minuta* (= *Gomphoneis herculeana* var. *minuta* J.L.Stone) belongs to the *herculeana*-group and therefore to the Gomphonemataceae and will not be discussed here any further (Abarca et al. in prep.). In a more recent paper on the *Gomphoneis* of Lake Baikal, Kociolek et al. (2013) defined the *elegans*-group further as having biseriate striae, undifferentiated porelli at the footpole, septa and pseudosepta, and a central nodule with internal proximal raphe ends recurved in the same direction. They proposed a further division into the typical *elegans*-group with four or more stigmoids (simple openings around the central area that may have siliceous ingrowths), and a group lacking stigmoids. We think that the taxa of the last group, those lacking stigmoids, should be recombined under the genus *Gomphonella* because the synapomorphies of *Gomphonella olivacea* are very similar to *G. tegeleensis*: the same size and details of the areolae, the undifferentiated porelli at the footpole, the straight raphe and the missing stigmoids or stigmata (compare Skibbe et al. 2018). Conclusions drawn from these micro-morphologies are supported by the molecular data, which cluster *Gomphonella olivacea*, *G. acsiae*, *G. coxiae* and *G. tegeleensis* into one group with very high bootstrap support (figs 3 & 4). The axial plate and mantle lamella, which are small but prominent features of *G. tegeleensis* (see Skibbe et al. 2018) and, according to Cleve of the genus *Gomphoneis* in general, do not seem to play such an important differentiating role on the genus level as proposed by Kociolek & Stoermer (1988b, 1988c, 1989, 1993).

Puzzling are the unclear differentiating features - autapomorphies - of the genus *Gomphosinica*, which was recently described by Kociolek et al. (2015), having been split off from *Gomphoneis* (for a morphological comparison see table 2). Although it looks like a *Gomphonella* concerning the areolae, undifferentiated porelli at the footpole and straight raphe endings, it has a stigmoid with internal stigmoid covering that seems to be typical for the *Gomphoneis elegans* group (containing the type species of *Gomphoneis*). This suggests to us

that, also in diatom research, new genera should be described with molecular data supporting ultrastructural features. Otherwise taxonomical artefacts are produced which hide the true evolutionary scenario. Recently, You et al. (2013) reiterated that the genus *Gomphoneis* is monophyletic and that the small species without stigmoids – *Gomphonella* species in this study – are highly derived members of *Gomphoneis*, as Kociolek & Stoermer (1989, 1993) had postulated on morphological cladistical evidence alone. Even Nakov et al. (2014), who used molecular data, stated that *Gomphoneis* is monophyletic and *Gomphonema* polyphyletic even though molecular data for only one *Gomphoneis* species – several strains of *Gomphoneis minuta* – was available. However, the genus *Gomphoneis* was clearly polyphyletic until now, because unrelated groups of taxa – the *herculeana*-group and the two *elegans*-groups – were subsumed under this genus name. The polyphyly of *Gomphoneis* supported by molecular data was first shown by Skibbe et al. (2018); in this paper we are resolving at least part of this polyphyly by moving the astigmat taxa from *Gomphoneis* to the reinstated genus *Gomphonella*.

CONCLUSION

This study shows that the gomphonemoid outline is not restricted to the family Gomphonemataceae but seems to be distributed across the entire order Cymbellales. This had already been shown for *Didymosphenia* but now it is clear also for the revived genus *Gomphonella*, which contains the astigmat group of *Gomphoneis* and *Gomphonema* besides the long disputed *G. olivacea*. Although the *Herculeana*-group of the polyphyletic genus *Gomphoneis* seems to belong to the Gomphonemataceae, we presume that the true members of the genus *Gomphoneis* – the *elegans* group – will also not cluster within the Gomphonemataceae, although this cannot be tested since no molecular data of *G. elegans* are currently available. The outcome of this study shows that only a polyphasic approach, combining molecular and micromorphological data for taxonomy, nomenclatural evaluation, observations from clonal cultures, and ecology, will reveal the full intricacies of evolutionary relations within specific organism groups, laying the foundation for future evolutionary, taxonomical and ecological studies as well as the sound application in monitoring approaches using diatoms.

SUPPLEMENTARY DATA

Supplementary Data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (<https://www.ingentaconnect.com/content/botbel/plecevo/supp-data>) and consists of: (1) list of material examined; and (2) morphometric data of the studied *Gomphonella* strains or populations.

FUNDING

This work was supported by the Federal Ministry of Education and Research [German Barcode of Life 2 Diatoms (GBOL2), grant number 01LI1501E] and the Deutsche Forschungsgemeinschaft (DFG) [PhycoBank, grant number JA 874/8-1].

ACKNOWLEDGEMENTS

Dr. David Mann, Dr. Bart Van de Vijver and one anonymous reviewer provided valuable comments on the manuscript. Many thanks are due to the curators who were involved in locating the original material: to Patrik Frödén (Herbarium Lund University, Sweden) who provided information on the deposition of the material of Hornemann, Lyngbye and Agardh; to Nina Lundholm (Herbarium Copenhagen, Denmark) who searched for the material and found out that its loan to Philadelphia from 1965 was still open; to Marina Potapova and her graduate student Nina Desianti (Diatom Herbarium, Philadelphia) who discovered it in a corner of one of Ruth Patrick's former offices. Thanks are also due to Juliane Bettig for technical assistance with SEM investigation and Jana Bansemer in the molecular lab.

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Managing Editor: David G. Mann

Submission date: 1 Oct. 2018

Acceptance date: 19 Dec. 2018