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Hidden complexity: An assessment of species diversity within the genus *Discostella* (Bacillariophyta)

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Abstract: The centric diatom genus *Discostella* is common in freshwater communities worldwide. Sixteen extant species are currently known, many of which are morphologically very similar. In applied taxonomy mostly broader concepts of *D. stelligera* and *D. pseudostelligera* are used. To assess the species diversity of the genus, 58 strains were cultivated and investigated using morphometric and molecular methods, obtaining the first multi–gene phylogeny of *Discostella*. Up to 22 operational taxonomic units were identified. For the taxonomic assignment of the OTUs a detailed morphological analysis including several type materials was performed. Up to 15 OTUs did not fit any existing taxon, revealing previously undetected diversity in the genus. A comparison of the different molecular markers revealed hints of a more complex intrageneric structure possibly due to reticulate evolutionary processes. The identity and generic placement of *D. glomerata* is resolved and *D. angainor* sp. nov. is described.

Key words: Bacillariophyta, cox1, *Discostella*, DNA barcoding, LSU, phylogeny, rbcL, species diversity, SSU

Introduction

Centric thalassiosiroid diatoms are abundant in phytoplankton communities of many water bodies worldwide. They are important primary producers and components of food webs of oceans, lakes and rivers. Within thalassiosiroid diatoms the genus *Discostella* Houk et Klee encompasses several species of planktonic freshwater diatoms formerly included in *Cyclotella* (Kützing) Brébisson. The genus is cosmopolitan and the different species can be found as solitary cells or chain–like colonies in the plankton of lakes, rivers and ponds of all trophic states. In 2004, Houk & Klee erected the genus based on the valve morphology. The most unique morphological feature of the genus is the position of the marginal fultoportulae (MFP) between the costae instead of on the costae. Since then several phylogenetic studies have shown that the genus *Discostella* is indeed not closely related to *Cyclotella* s. str. but is closer to the rest of Stephanodiscaceae (Alverson 2007; Jung et al. 2010). In Table 1 all extant taxa, including a short morphological description of the type, are listed and ranked after their publication year. In the description of the genus by Houk & Klee (2004) nine extant taxa are named (Table 1), some of which are seemingly restricted to more remote regions like Tasmania or Reunion Island. In 2010, Houk et al. documented all these taxa with the exception of *Discostella elentarii* (Alfinito et Tagliaventi) Houk et Klee. Since the description of the genus in 2004, seven additional extant species have been described or transferred (Table 1). With now 16 extant species the genus is relatively small compared to related genera like *Lindavia* (Schütt) De Toni et Forti, *Pantocsekiella* Kiss et Ács or *Stephanodiscus* Ehrenberg. Likely due to difficulties in species identification and taxonomical problems of the group, in applied taxonomy often only two taxa or groups are used: *D. stelligera* for larger, strongly silicified forms and *D. pseudostelligera* for smaller forms with prominent MFP (Adesalu & Julius 2017). Some authors even treat many of those taxa as

a species complex *D. stelligera* s.l. (Rott & Kofler 2021) due to the challenges concerning species identification. This is somewhat reminiscent of the related genus *Pantocsekiella*, where the two taxa groups around *Pantocsekiella comensis* (Grunow) Kiss et Ács and *P. ocellata* (Pantocsek) Kiss et Ács each comprise several different morphospecies. At the same time some studies report unknown morphotypes (Houk & Klee 2004; Guerrero & Echenique 2006; Öberg et al. 2009) and the sparse sequence data mainly used in broader phylogenetic studies indicates molecular differences in morphologically similar strains (Alverson 2007; Jung et al. 2010). Whether, in the case of *Discostella*, this is truly due to a lack of diversity or a consequence of the use of broad taxonomical concepts and species complexes will be a subject of this work. In this study we investigated 58 clonal strains from 50 water bodies from 13 countries across Eurasia and North America to assess the species diversity and phylogenetic structure of the genus *Discostella*. Like other recent studies (e.g. Kahlert et al. 2019; KOLLÁR et al. 2019; Jahn et al. 2021; Schultz et al. 2022), we use an integrative approach to species delimitation and taxonomy, combining morphological and molecular methodology. The obtained molecular and morphological data is used to update the taxonomy of the genus. In the effort to connect all strains to existing taxa, all strains were investigated under SEM and available type materials of critical taxa (*D. stelligera, D. tatrica*, *D. woltereckii*, *D. pseudostelligera* and *D. stelligeroides*) were also examined.

Material and Methods

For the morphometrical and molecular investigation 58 strains of *Discostella* spp., given in Table S1 (supplementary material), were used. Six strains of additional species (*Cyclostephanos makarovae* (Genkal) Schultz, *Stephanodiscus niagarae* Ehrenberg, *Pantocsekiella ocellata* (Pantocsek) Kiss et Ács, *Lindavia* sp., *Thalassiosira lacustris* (Grunow) Hasle and *Conticribra weissflogii* agg.) were used as references for the molecular analysis.

Sampling and cultivation. Water samples of 0.5–1 L were taken from each water body near the surface. From each water sample, single cells or chains were isolated using an inverse microscope and a micromanipulator–micropipette system and cultivated for at least one week in filtered (0,2 µm) mineral water (Volvic®) added with 4 mL.L^{-1} f/2 medium (Guillard & Ryther 1962) and 60 mg.L^{-1} metasilica ($Na_2SiO_3 \times 9\ H_2O$). Liquid cultures were maintained in 50–150 mL Erlenmeyer flasks at 14–20 °C in a light/dark photocycle of 14.5:9.5 h and moderate shaking. During the cultivation, the purity was checked. In case of doubt a single cell was isolated again. Finally, subsamples of each clonal culture were used for LM and SEM analysis and DNA extraction.

SEM and morphometric analysis. For SEM and LM analysis, ca. 5 mL of each culture were oxidized with 35% H_2O_2 for four to six weeks and finally the suspensions were washed by centrifugation four times with distilled water. The cleaned cell suspensions were pipetted onto cover slips and, after drying, fixed in Naphrax® for LM slides and dried on aluminium stubs for SEM analysis. Prepared stubs were coated with ca. 25 nm Au and viewed under a ZEISS Merlin VP compact SEM. The following morphometric parameters were recorded: undulation of the valve, total diameter, diameter of the central area, pattern of the central alveoli (none, point–like, irregular, stellate or ring), number of striae, number of shortened striae (= nodes), number of marginal fultoportulae (MFP), structure of MFP (simple pores; simple or complex [bilateral], short or prominent tubes), number of rimoportulae (RP), structure of RP (stalked or sessile). The density of the striae and MFP is given as the number in 10 µm circumference (Genkal 1977). Because the number of striae is lower towards the valve centre in many cases (due to shortened striae), the maximum number at the valve face–mantle junction was used to calculate the stria density. The following type materials were used for SEM investigation: *D. stelligera,* Kryptogamae exsiccatae No. 2046, W; *D. tatrica*, R1005, BRM; *D. woltereckii*, AS1329, BRM; *D. pseudostelligera*, E524, BRM and *D. stelligeroides*, E761, BRM.

Molecular analysis. Genomic DNA was extracted using a QIAGEN DNeasy® Plant Mini kit or the salt–extraction technique modified after Aljanabi & Martinez (1997). For DNA–amplification the following primer pairs were used: D512for and D978rev (Zimmermann et al. 2011) for the V4 region of the small rDNA subunit (18S), T16N and T24U (Hamsher et al. 2011) for D2 and D3 regions of the large rDNA subunit (LSU), Wawrik_for and Wawrik_rev (Wawrik et al. 2002) for partial rbcL, and CoxF and CoxR (Iwatani et al. 2005), GazF2 and KEdtmR (Evans et al. 2007) as well as CO1_for and CO1_rev (Kistenich et al. 2014) for partial cox1. The PCR mix followed Kistenich et al. (2014). PCR programmes were used according to Zimmermann et al. (2011) for 18S, Wawrik et al. (2002) for rbcL, Iwatani et al. (2005) and Evans et al. (2007) for cox1 and Hamsher et al. (2011) for LSU. PCR products were visualized in 1.5% agarose gel and relevant bands were cut out for Gel extraction and purification of PCR products. Final products were sequenced using Sanger sequencing. In case of deficient sequence quality or length additional sequencing and or PCR was performed. The same was done when ambiguous base signals were present and if these were persistent in different PCRs or sequencing directions, the IUPAC code for ambiguous bases was used. Sequences were edited and aligned with the software BioEdit v7.2.5 (Hall 1999). For the phylogenetic analysis maximum likelihood trees were constructed using the software MEGA (Kumar et al. 2018). The tree of the nuclear and chloroplast sequences is based on the Tamura–Nei model (Tamura & Nei 1993) and 10000 bootstrap replicates. A discrete Gamma distribution (G) was used to model evolutionary rate differences among sites. The rate variation model allowed for some sites to be evolutionarily invariable (I). The cox1 tree is based on the Tamura–3–parameter model (Tamura 1992) and 10000 bootstrap replicates. A discrete Gamma distribution was also used to model evolutionary rate differences among sites. Models were chosen according to the lowest Bayesian information criterion. In the same way a phylogeny for each individual marker was produced, using the following models: Kimura–2–parameter model (Tamura 1980) for 18S and for LSU (+G, +I) and Tamura–3–parameter model (Tamura 1992) for rbcL. Publicly available sequences were neglected for the creation of the overall phylogeny and definition of the clades, because a taxonomic interpretation would not have been possible as there is usually no morphological documentation available.

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Furthermore, no available strains had sequences for all four markers. However, available sequences were included into the phylogenies of the individual markers to compare taxonomic assignments and genetic types to the results of this study. In the case that the additional sequences changed the topology of the resulting tree, one additional neighbour joining tree was produced to show both, the Maximum likelihood phylogeny of the individual marker and the clustering of public sequence data. Sequences were taken from the NCBI database (Sayers et al. 2022) using the BLAST function (Altschul et al. 1990) and by manually checking the Diat.barcode database (Rimet et al. 2018).

Results

All nuclear (18S and LSU) and chloroplast (rbcL) markers were successfully amplified and sequenced for all strains. For all strains with the exception of XA4, KCH4 (*D. asterocostata*) and HSD15 a partial cox1 sequence could be obtained using different primer combinations. Based on the molecular and morphological differences or similarities we identified 15 different clades (A–P) each containing strains with similar sequences and morphology in the phylogeny of the nuclear and chloroplast markers (Fig. 1). The clades were defined in a way that the contained strains share at least one morphological feature that is distinct from the closest neighbouring clade. Morphologically similar molecular subclades are indicated as types (I, II, …) of a given clade. Eight of 15 clades were genetically and morphologically homogenous without distinct subclades (D, E, H, J, K, L, M and N) and three clades (B, F and O) only included a single strain. The other four clades (A, C, G and P) included two to five different molecular types, at least some of which also showed morphological differences (e.g. in clades A and G). Depending on whether these subclades are included, this results in a total of 15 to 22 OTUs out of 58 strains. Genetic distances (18S+28S+rbcL) between the clades ranged from 0.3% (between clades D and E) to 4.9% (between clades A and M). Within the clades the strains differed between 0% and 1.1% (between clade G type II and IV). The intergeneric distances ranged from 1.7% (*C. makarovae and S. niagarae*) to 9.6% (*D. stelligera* agg. and *T. lacustris*).

Only seven clades or subclades could be linked to known morphospecies ((A) *D. stelligera*, (D) *D. glomerata,* (E) *D. stelligeroides,* (G) (type I) *D. lacuskarluki,* (G) (type III) *D. guslyakovyi,* (M) *D. asterocostata* and (P) *D. pseudostelligera*), while a definitive identification was only possible for two (*D. asterocostata*, clade M and *D. glomerata*, clade D). The phylogenies of the nuclear (V4 18S and D2–D3 28S) and chloroplast (rbcL) loci produced the same clades (Figs S1–S4, supplementary material). The mitochondrial marker (cox1), however, reproduced most of those clades, but also revealed some contradictions (Fig. 1). Although generally the most variable marker, cox1 sequences of clades C, J and K were identical or significantly more similar than in the nuclear and chloroplast markers. This is a severe but interesting contradiction of both phylogenies, as cox1showed by far the highest divergence rates between the remaining clades. The relative arrangement of the clades varied between the markers and deeper nodes are more weakly supported (Figs S1–4).

The results of the morphometrical measurements for all subclades (types) and the investigated type materials are presented in Table 2. Some or all of the strains of the clades A, C and P underwent cell enlargement either due to homothallic sexual or asexual production of initial cells. In these cases, the complete size range could be studied. Concerning the type materials (Figs 2–13) investigated here, the materials of *D. pseudostelligera* and *D. woltereckii* were of poor quality and in the type material of *D. stelligeroides* we were unable to find valves belonging to the genus *Discostella*. The valve morphology across the clades was very diverse, but it was often difficult to differentiate a given clade from all other clades. Only four clades can be distinguished by a single morphological feature: the collar like external tubes of the MFP of clade H, the root–like ornamentation of the external pores of the MFP of clade C, the high density of MFP of *D. glomerata* (clade D), and the ring–like alveolar pattern in the central area of *D. asterocostata* (clade M). In all other cases, a combination of characters is necessary or a morphological distinction remains unclear as of yet. Ultrastructure features such as the morphology of the external tubes of the MFP, density of MFP and the structure of the RP (stalked or sessile) proved useful regarding the morphological differentiation of the clades, while more classical or LM–based parameters like stria density, undulation, degree of silicification or diameter are very variable and overlap widely. However, clade H includes strains from six different populations and is distinct within both phylogenies. The strains share a unique morphotype and there is no genetic variation within the clade. It is therefore described as a new species.

***Discostella angainor* Schultz sp. nov. (Figs 14–41)**

Description

LM (Figs 14–32): Cells solitary or forming chain–like colonies of up to 20 cells. Valves circular, flat to convex or concave, 3.8–8.3 µm in diameter. The central area occupies 22–64% of the valve diameter and shows point–like, stellate, irregular or no alveolar patterns. 14.1–29.1 striae in 10 µm circumference. Between 0 and 27 shortened striae.
SEM (Figs 33–41): Costae not or very slightly raised. 3.1–6.6 marginal fultoportulae in 10 µm circumference, internally placed between the costae, at the valve face–mantle junction and surrounded by two satellite pores. The external projections of the marginal fultoportulae are prominent complex tubes with a collar–like rim that is broadened parallel to the valve outline, somewhat reminiscent of the shape of an anchor. The arrangement of the marginal fultoportulae may be symmetrical or asymmetrical. One rimoportula is present, situated within the ring of marginal fultoportulae, internally distinctly stalked,

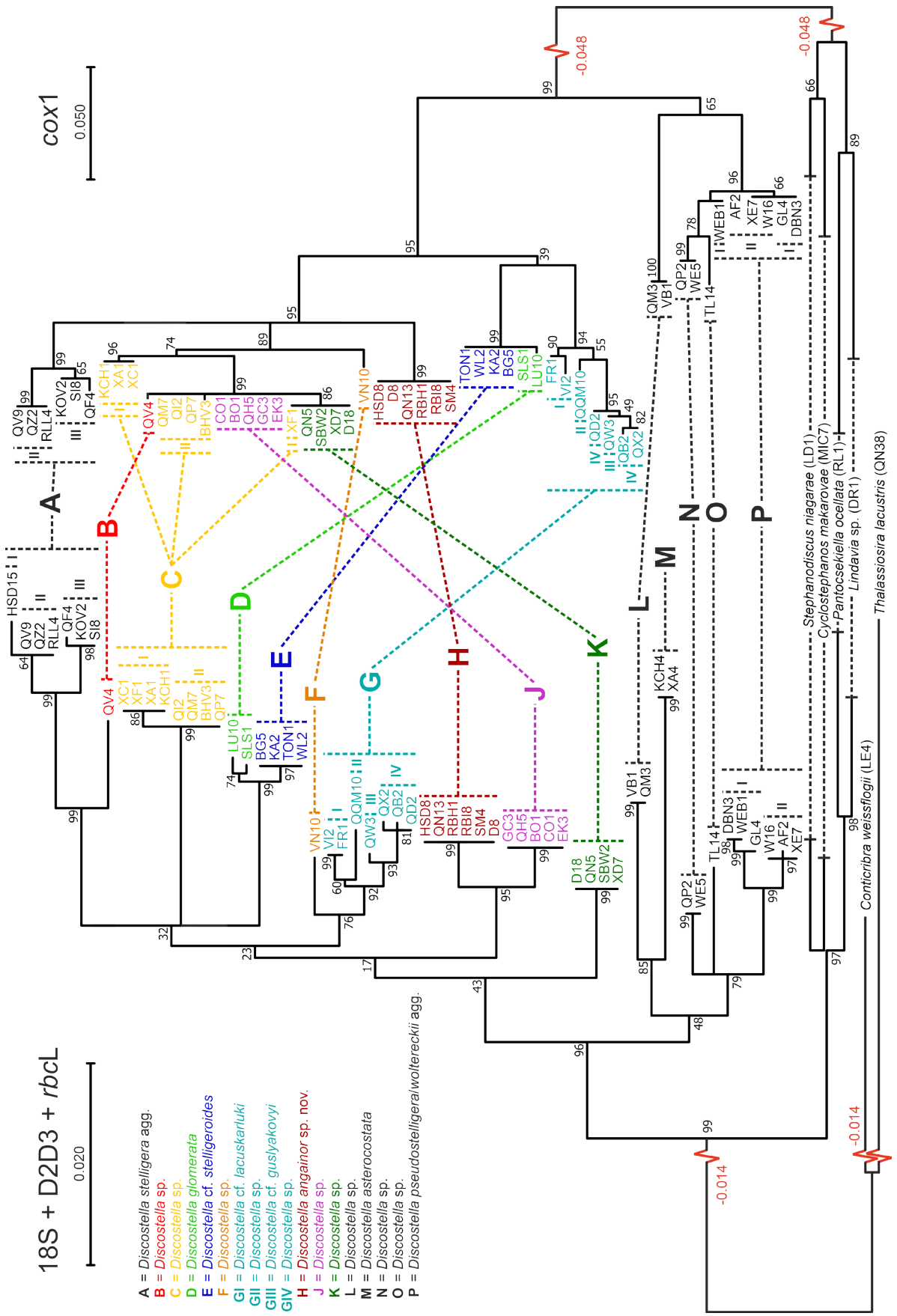

Fig. 1. Maximum Likelihood trees of the nuclear and chloroplast markers (partial 18S+D2D3 LSU+rbcL, left side) and the mitochondrial marker (partial cox1, right side) showing the clustering of all strains used in this study. Capital letters (A–P) indicate the clades defined in the nuclear and chloroplast tree. Roman numerals (I–IV) indicate molecular subtypes within a given clade. Dashed lines connect the clades between both trees and overlapping lines are color–coded for better distinguishability.

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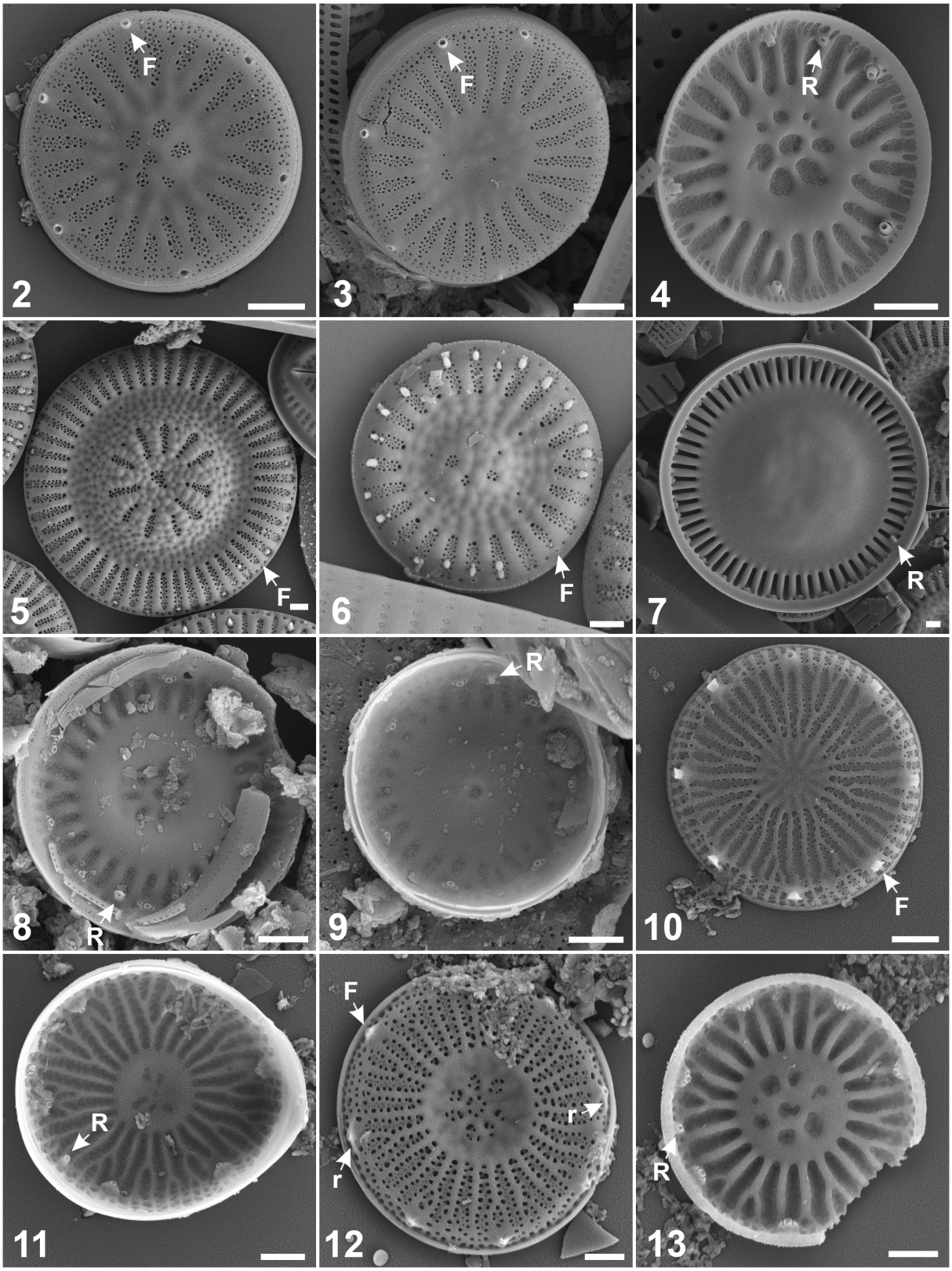
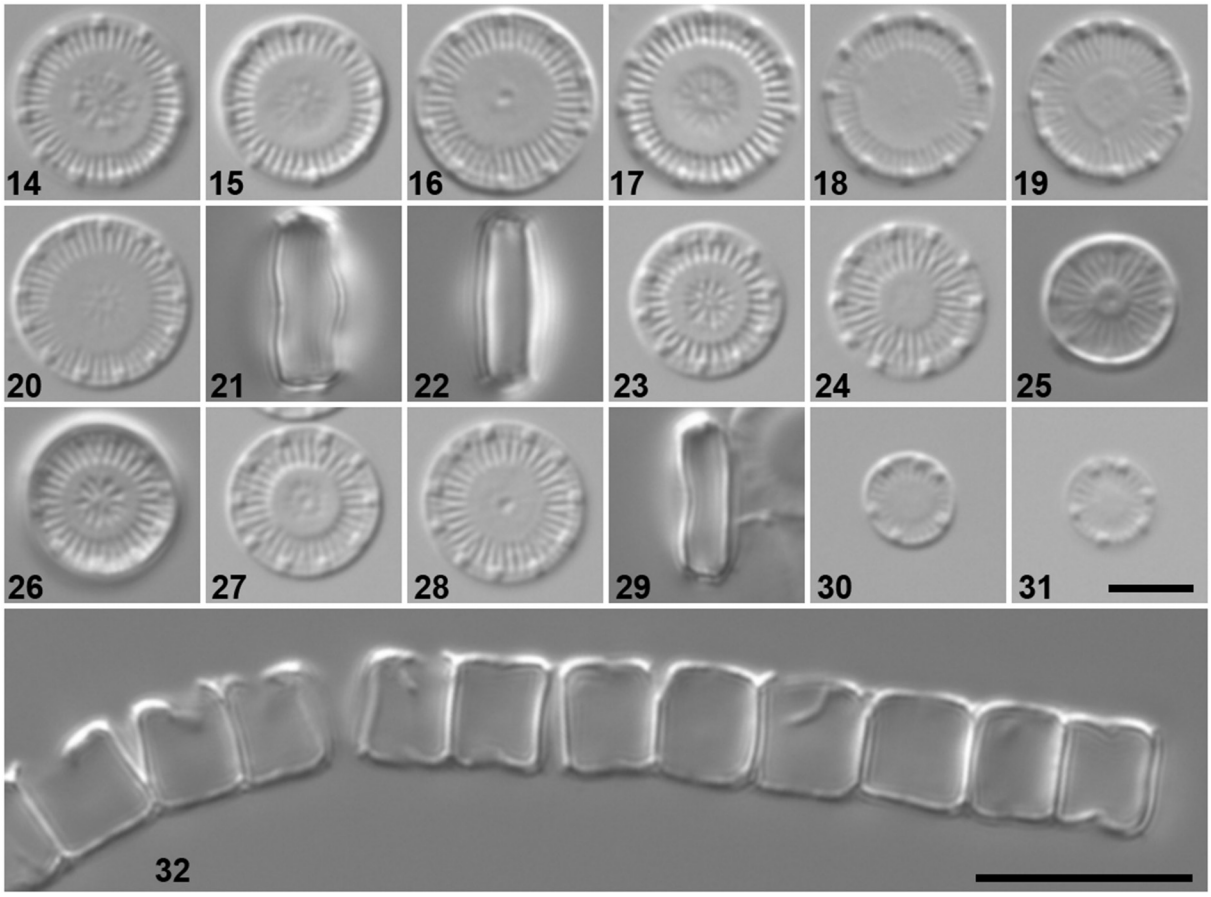


Figs 2–13. SEM images of investigated type materials: (2–4) *D. tatrica*, material R1005, BRM; (5–7) *D. stelligera,* Kryptogamae exsiccatae No. 2046, W; (8–9) *D. pseudostelligera*, material E524, BRM; (10–13) material AS1329, BRM (10–11) morphotype I = *D. woltereckii* (12–13) morphotype II = *Discostella* sp.; arrows give exemplary indications of important features, (F) external openings of the MFP, (R) rimoportula, (r) root–like structures. Scale bars 1 µm.



Figs 14–32. LM images of *D. angainor* sp. nov. (clade H): (14–22) type material, strain D8; (23–29) strain QN13; (30–31) strain SM4; (32) chain–like colony of strain RBH1. Scale bar 4 µm (14–31) and 10 µm (32).

externally a simple pore. In the central area, concentrical ridges or ghost–striae may be present.

Holotype: B! Material number B 40 0045362, strain D8 deposited at the botanical museum of Berlin.
Isotype: D8, Hübener–Dreßler–Schultz culture collection, University of Rostock.
Type locality: River Danube, Tulln, Austria; 48°20'10.6"N, 16°03'36.9"E.
Habitat: Plankton of large streams and lakes.
Etymology: After Angainor, the chain from the Silmarillion by J. R. R. Tolkien, due to the chain–like colonies.
Distribution: *D. angainor* was until now found in River Rhine, Germany; River Seine, France; River Danube, Austria; St. Lawrence River, Canada and Lake Hồ Suối Đá, Vietnam.

Different patterns for the relation of the molecular and morphological data can be seen. The strains of clade D (*D. glomerata*) and clade E (*D.* cf. *stelligeroides*) differ significantly on a morphological level, but both species seem to be very closely related according to the molecular data. On the other hand more abundant are the cases in which the morphology is very similar but the strains clearly belong to different clades (e.g. clades L and P). Some clades may be species complexes such as A, C and P defined by similar (not identical) morphology and somewhat diverse genetic types, while several other clades are, despite different geographic origins, internally molecularly identical or almost so, such like the clades E, H, J, K, L, M and N. While a morphological differentiation may be difficult in single cases, overall the molecular clades based on the nuclear and chloroplast markers are not in contradiction with the morphology. All clades consist of morphologically similar strains and some morphological features can are even be linked to deeper nodes (e.g. structure of the MFP).

Discussion

Phylogeny

According to Alverson et al. (2007) *Discostella* is the basal group within the Stephanodiscaceae (excluding *Cyclotella* s. str.) and thus important to understand the evolution of this family. According to the present study, there is no reason to believe that the genus is not monophyletic, as all strains clustered together with high support (96%) and are distinct from the outgroups. While the defined clades and subclades (types) generally have a high support (74–99%) within the phylogeny of the

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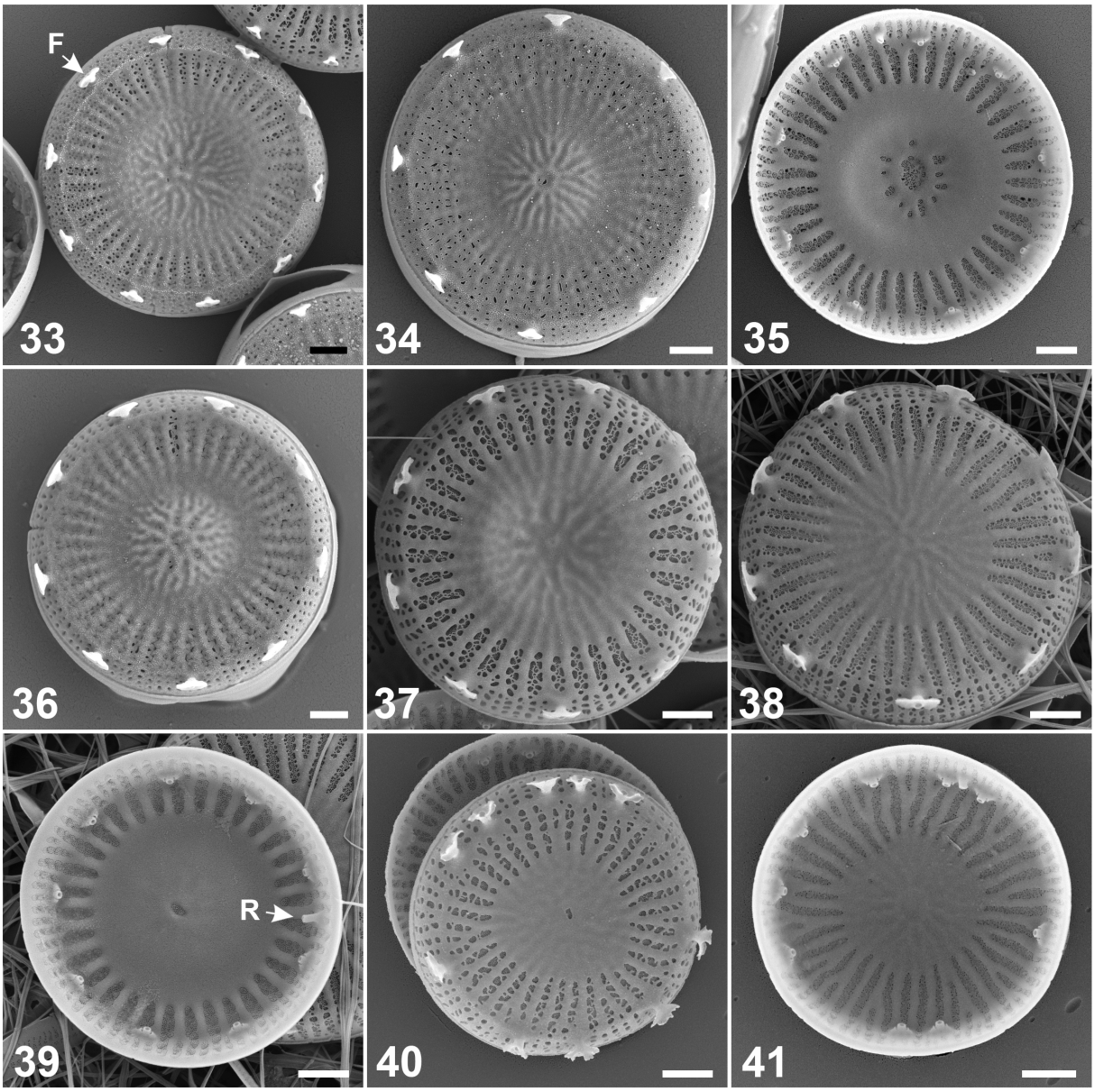
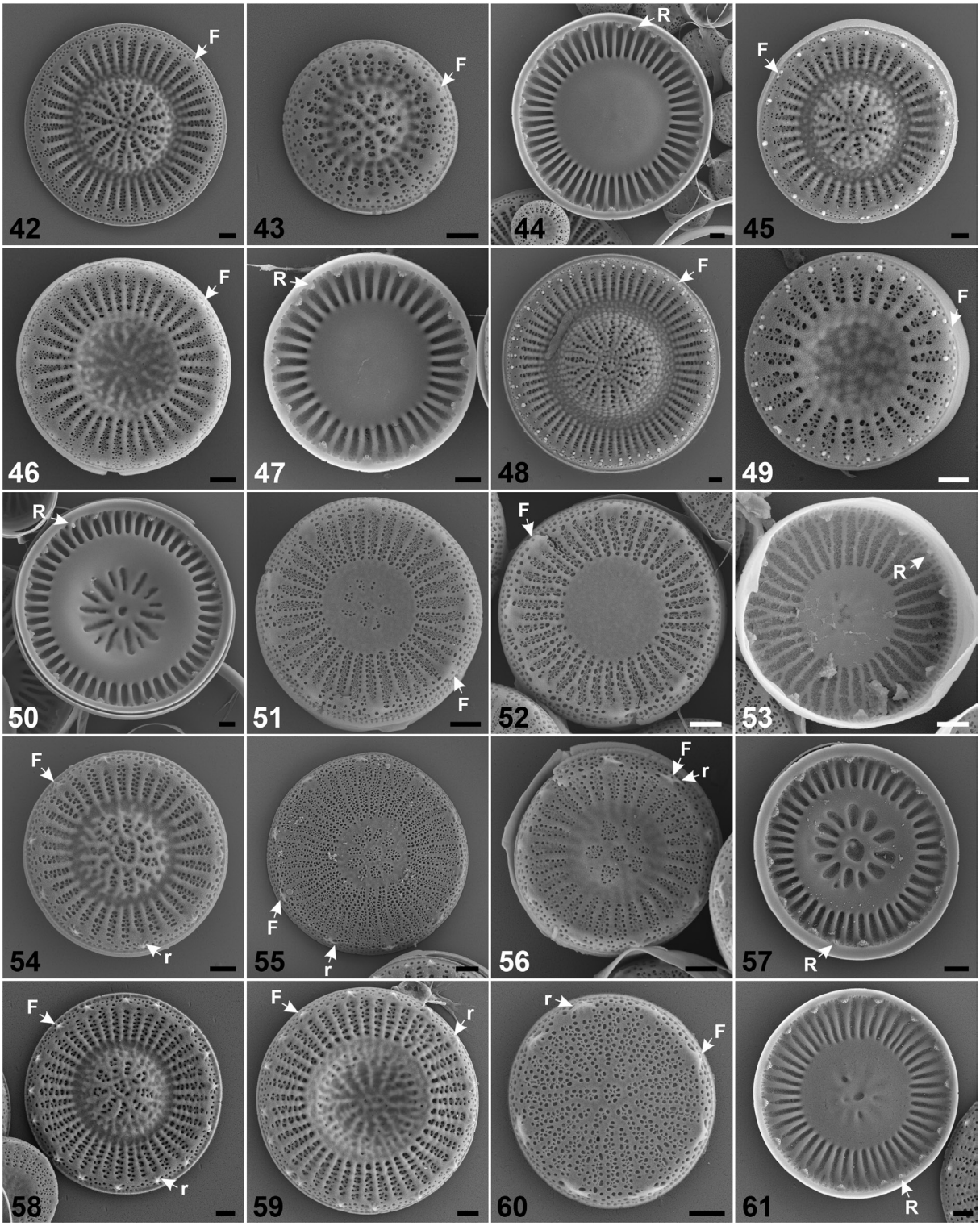


Figs 33–41. SEM images of *D. angainor* sp. nov. (clade H): (33–36) type material, strain D8; (37–39) strain QN13; (40–41) strain RBI8; arrows give exemplary indications of important features, (F) external openings of the MFP, (R) rimoportula. Scale bars 1 µm.

nuclear and chloroplast markers, deeper nodes within *Discostella* are generally more weakly supported, which is similarly true for the phylogenies of the individual markers (Figs S1–4). This issue may be related to the high number of OTUs in the tree. As the concatenated alignment does not lack variable sites, it is unlikely that the reason is a lack of information in the dataset. Homoplasy in variable regions of the alignment may also be a contributing factor. Another possible interpretation would be rapid diversification in the past. A lack of species sampling would also be conceivable in this case, especially considering the diversity newly found in this study.

Hints of reticulate evolution

Contradictory to the phylogeny of the nuclear and chloroplast genes, the clades B, C, J and K form a cluster of similar sequences in the cox1 phylogeny (Fig. 1). Clade C type II is even identical with clade J and the strain XF1 (clade C type I) is identical to clade K. However, all clades involved are distinctly different concerning morphology and the nuclear and chloroplast markers. Considering that the mitochondria might well be inherited uniparentally, this may indicate past or recent hybridization events. Other explanations like selection or incomplete lineage sorting seem to be less likely as the cox1 sequences show the highest degree of divergence (compared to the other individual markers) between the other clades and at least some of the affected clades are not closely related according to the less divergent nuclear and chloroplast markers (e.g. clades C and K). The morphology is furthermore quite different and in



Figs 42–61. SEM images of clades A–C: (42–50) clade A = *D. stelligera* agg., (42–44) type I (HSD15), (45–47) type II (45: QV9, 46–47: QZ2), (48–50) type III (48: KOV2, 49–50: QF4); (51–53) clade B = *Discostella* sp. (QV4); (54–61) clade C = *Discostella* sp.; (54–57) type I (54: XC1, 55: XF1, 56–57: XA1), (58–61) type II (58: QP7, 59: QI2, 60–61: QM7). Arrows give exemplary indications of important features, (F) external openings of the MFP, (R) Rimoportula, (r) root–like structures. Scale bars 1 μm.

accordance with the nuclear and chloroplast markers in those cases. Another hint of possible hybridization or genetic exchange is the fact that the LSU sequence of the strain GL4 (clade P type I) is a mixture of the sequence types of clade P type I (strains DBN3 and WEB1) and clade P type II (at the positions where both types differ, this sequence has ambiguous signals of both respective bases) and while both types of this clade are otherwise distinct concerning morphology and rbcL, the cox1 sequences are identical between both types. Moreover,

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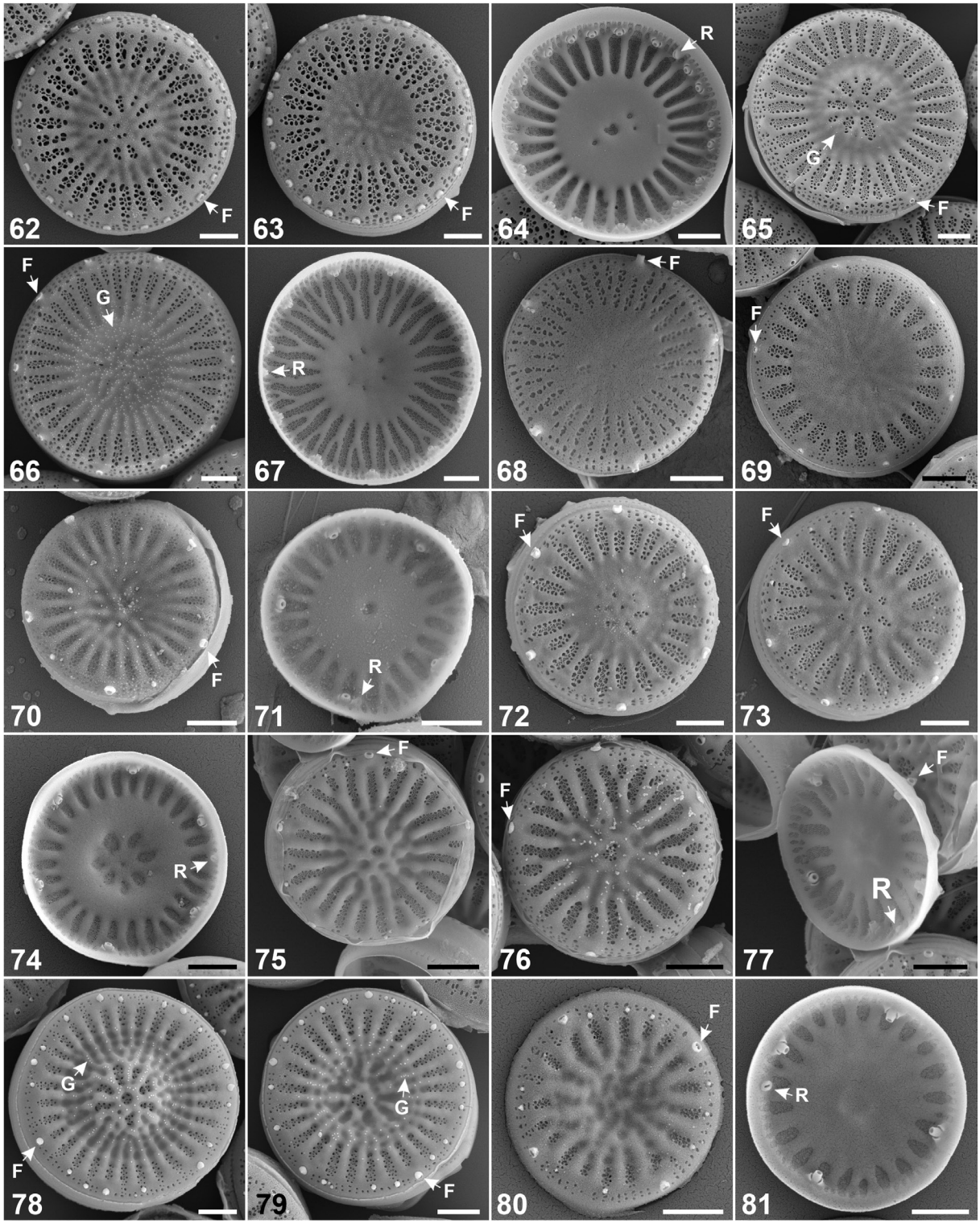
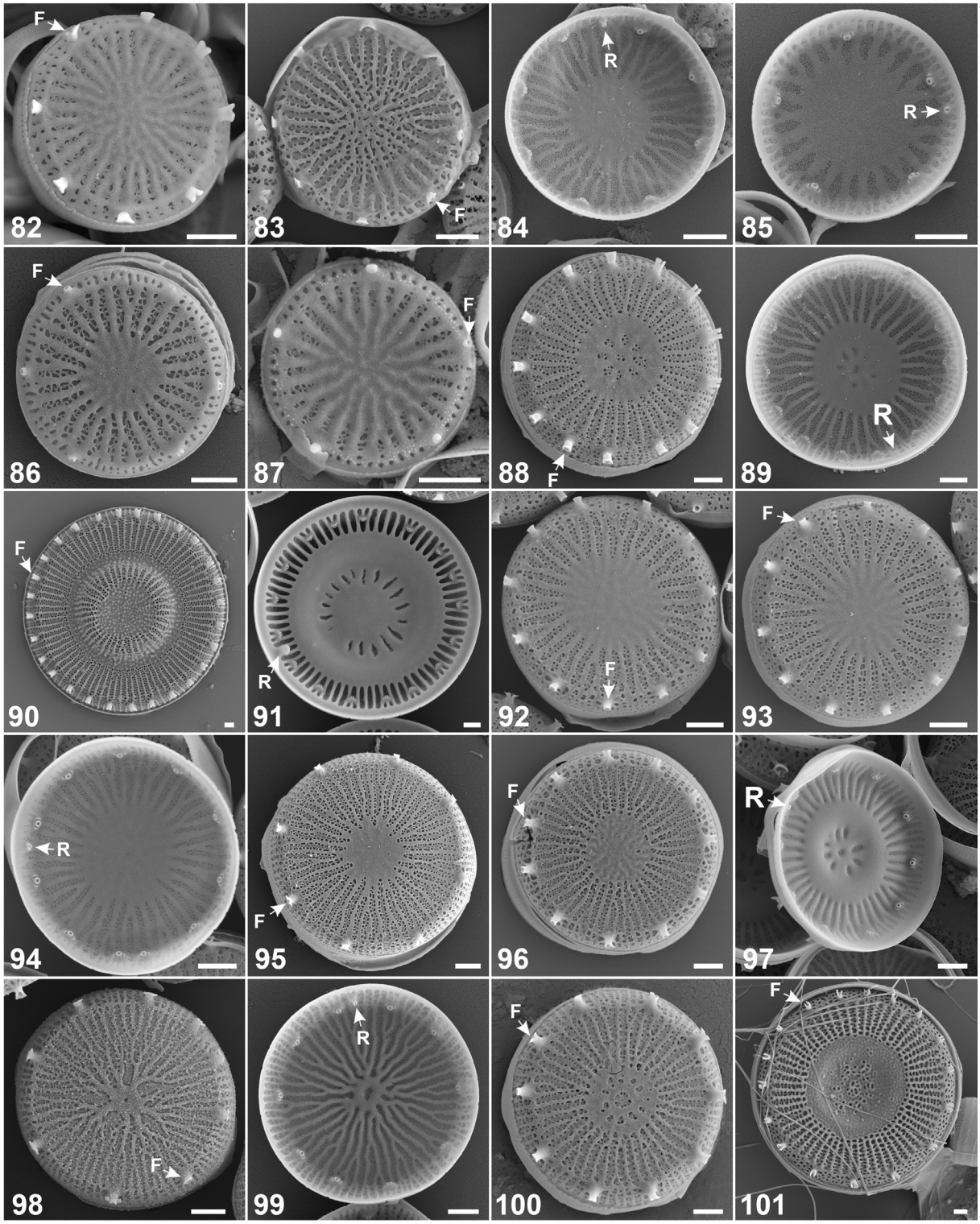


Figs 62–81. SEM images of clades D–G: (62–64) clade D = *D. glomerata* (62–63: LU10, 64: SLS1); (65–67) clade E = *D.* cf. *stelligeroides* (65: KA2, 66–67: TON1); (68) clade F = *Discostella* sp. (VN10); (69–81) clade G, (69–71) type I = *D.* cf. *lacuskarluki* (69: VI2, 70–71: FR1), (72–74) type II = *Discostella* sp. (QQM10), (75–77) type III = *D.* cf. *guslyakovyi* (QW3), (78–81) type IV = *Discostella* sp. (78–79: QX2, 80–81: QB2). Arrows give exemplary indications of important features, (F) external openings of the MFP, (R) Rimoportula, (G) granuli. Scale bars 1 µm.

LSU sequences of clade A type II contain ambiguous base signals, some of which are a mix with type I or II and others cannot be linked to any known type. However, it is also possible that there is some intragenomic variation of different divergent LSU copies within this clade. Furthermore, depending on chance or primer choice different copies may be amplified during the PCR. All these anomalies might be hints of processes of reticulate evolution, which may be a driver of diversification in this group.



Figs 82–101. SEM images of clades J–P: (82–84) clade J= *Discostella* sp. (82: BO1, 83–84: GC3); (85–87) clade K = *Discostella* sp. (85–86: SBW2, 87: QN5); (88–89) clade L = *Discostella* sp. (QM3); (90–91) clade M = *D. asterocostata* (90: XA4, 91: KCH4); (92–94) clade N = *Discostella* sp. (QP2); (95) clade O = *Discostella* sp. (TL14); (96–101) clade P = *D. pseudostelligera* agg., (96–98) type I (96–97: GL4, 98: WEB1), (99–101) type II (99: XE7, 100: W16, 101: AF2). Arrows give exemplary indications of important features, (F) external openings of the MFP, (R) Rimoportula. Scale bars 1 µm.

Table 1. Overview of the extant *Discostella* taxa. (Y) year of transfer or description; (CA/D) ratio of the diameter of the central area and the total diameter in %; (Pattern) pattern of the central area, (0) none, (1) point–like, (3) stellate, (i) irregular; (U) Undulation, (cx) convex, (cc) concave, (f) flat; (S/C) number of striae in 10 µm circumference; (MFP/S) density of the marginal fultoportulae (MFP) as MFP on every N. stria; (MFP) structure of the MFP, (SP) simple pores, (SP+R) simple pores with root = like ornamentation, (SST) short simple tubes, (PST) prominent simple tubes, (PCT) prominent complex tubes, (SCT) short complex tubes; (RP) structure of the rimoportula, (se) sessile, (st) stalked.

Taxon (reference)	Y	Type Locality	Diameter	CA/D	Pattern	U	S/C	MFP/S	MFP	RP
Discostella stelligera (Cleve et Grunow) Houk et Klee (Houk et al. 2010)	2004	Lake Rotoaira, NZ	5–40	60–75	0,3,i	cx, cc	8–12	2.–5.	SP	
Discostella tasmanica (Haworth etTyler) Houk et Klee (Haworth et Tyler 1993)	2004	Lake Laura, AU	7.5–35		0,3	cx, cc	9–14	2.–5.	SP, SCT	
Discostella glomerata (Bachmann) Houk et Klee (Klee et Houk 2007)	2004	Lake Zugersee, CH	4–8	33–50	0,3,i	cx, cc	12–14	1.–2.		
Discostella asterocostata (Lin, Xie et Cai) Houk et Klee (Houk et al. 2010)	2004	several lakes in China	10–40		ring	cx, cc	11–16	3.–4.	PCT	
Discostella stelligeroides (Hustedt) Houk et Klee (Houk et al. 2010)	2004	Plitvice Lakes, HR	6–12	ca. 50	0,3,i	cx, cc	ca. 20	2.–6.	SP	se
Discostella mascarenica (Klee, Houk et Bielsa) Houk et Klee (Houk et al. 2010)	2004	Lake Mare á Poule d'Eau, RE	4.2–15.7	40–60	0,3	cx, cc	5.5–8.5	1.	SST, PST	
Discostella woltereckii (Hustedt) Houk et Klee (Klee et Houk 1996)	2004	Pond in Bogor, ID	4.5–12.9	0–40	0,1,3,i	f, cx, cc	18–23	2.–6.	SP+R, PCT	se, st
Discostella pseudostelligera (Hustedt) Houk et Klee (Hustedt 1939)	2004	River Ems, DE	7–8	50	3	f, cx, cc	ca. 18		PCT	
Discostella elentarii (Alfinito et Tagliaventi) Houk et Klee (Alfinito et Tagliaventi 2002)	2004	Lake Monowai, NZ	6–25			f	9–10	2.		
Discostella nipponica (Skvortzov) Tuji et D. M. Williams (Tuji et Williams 2006 A)	2006	Lake Kizaki, JP	3–4				18			se
Discostella guslyakovyi Genkal, Bondarenko et Popovskaya (Genkal et al. 2007)	2007	Lake Nichatka, RU	2.8–5.7			f	20–30	4.–6.	SST	
Discostella hellae (Chang et Steinberg) Chang (Chang et Steinberg 1989)	2008	Rott Reservoir, DE	3–5			f		4.–7.	SST, SCT	
Discostella tatrica Procházková, Houk et Nedbalová (Procházková et al. 2012)	2012	Lake Nižné Temnosmrečinské pleso, SK	3.5–8.5		3	cx, cc	14,5–18	5.–8.	SST	
Discostella oyanensis Adesalu et Julius (Adesalu et Julius 2017)	2017	Oyan Reservoir, NG	4–13	55–66	3	cx, cc		3.	SP	
Discostella lakuskarluki (Manguin ex Kociolek et Reviers) Potapova, Aycock et Bogan (Potapova et al. 2020)	2020	Karluk Lake, US	2.6–5	30–50	0,3,i	cx, cc	13–19	4.–6.	SST	
Discostella gabinii Paillès et Sylvestre (Paillès et al. 2020)	2020	Lake Petén–Itzá, GT	8–18	ca. 33	punctae	f	10–14	3.–5.	SP	

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Table 2. Results of the morphometric measurements for all subclades (types) or clades: (N) number of measured valves; (Und) undulation, (0) flat, (0.5)slightly undulated, (1) distinctly undulated; (D) Diameter, (*) initial valves observed; (CA/D) ratio of the diameter of the central area to the valve diameter; (S/C) stria density/number of striae in 10 µm circumference; (Pattern) alveolar pattern of the central area, (n) none, (p) point–like, (s) stellate, (i) irregular, (r) ring; (Nodes) number of nodes/shortened striae; (MFP) structure of the MFP, (SP) simple pores, (SP+R) simple pores with root–like ornamentation, (SST) short simple tubes, (PST) prominent simple tubes, (PCT) prominent complex tubes, (SCT) short complex tubes; (MFP/D) number of marginal fultoportulae in 10 µm circumference; (RP) structure of the rimoportula, (se) sessile, (se+) sessile, but occasionally somewhat stalked, (st) distinctly stalked.

Clade/Type	Taxon	Strains	N	Und.	D (µm)	CA/D (%)	S/C	Pattern	Nodes	MFP	MFP/C	RP
Clade A Type I	*D. stelligera* agg.	HSD15	11	0–1	4.0–15.0*	46.0–67.4	10.7–20.6	n, p, s, i	0–1	SP	3.2–4.1	se+
Clade A Type II	*D. stelligera* agg.	QV9, QZ2, RLL4	34	0–1	5.8–12.4*	48.6–65.8	8.9–21.3	n, p, s, i	0–5	SP	3.6–5.8	se+
Clade A Type III	*D. stelligera* agg.	KOV2, SI8, QF4	32	0–1	5.6–17.5*	49.9–70.4	10.3–16.0	n, s, i	0–4	SP	2.7–4.5	se+
Clade B	*Discostella* sp.	QV4	13	0	7.2–8.0	38.5–47.8	16.2–22.5	n, p, s, i	6–21	SP (SST)	3.2–3.9	se+
Clade C type I	*Discostella* sp.	KCH1, XA1, XC1, XF1	50	0–1	5.4–11.4*	35.7–63.6	11.8–19.8	n, p, s, i	0–22	SP+R	3.0–5.4	se
Clade C type II	*Discostella* sp.	BHV3, QI2, QM7, QP7	40	0–1	5.6–12.5*	16.9–60.0	11.1–21.5	n, p, s, i	0–21	SP+R	3.2–5.1	se
Clade D	*D. glomerata*	LU10, SLS1	21	0–0.5	5.2–6.6	42.6–53.3	14.0–18.8	n, p, s, i	1–13	SP, SST	9.5–12.8	se
Clade E	*D.* cf. *stelligeroides*	BG5, KA2, TON1, WL2	44	0–0.5	5,6–7,0	26.1–58.4	14.7–20.8	n, p, s, i	0–18	SP, SST	4.8–6.6	se+
Clade F	*Discostella* sp.	VN10	10	0	4.3–4.6	38.1–61.1	21.3–29.8	n, p, s, i	1–14	PST, PCT	3.5–4.3	se
Clade G Type I	*D.* cf. *lacuskarluki*	FR1, VI2	23	0–1	3.6–5.2	43.0–63.4	14.6–21.8	n, p, s, i	0–9	SST, SCT	2.4–4.5	se
Clade G Type II	*Discostella* sp.	QQM10	13	0–1	3.2–4.5	49.1–61.5	17.3–24.8	n, s, i	0–4	SST, SCT	3.6–5.0	se
Clade G Type III	*D.* cf. *guslyakovyi*	QW3	10	0–0.5	3.7–4.1	40.5–56.8	18.1–20.8	n, p, i	2–7	SST, SCT	3.3–4.9	se
Clade G Type IV	*Discostella* sp.	QB2, QD2, QX2	43	0–1	3.6–6.3	48.1–69.1	14.0–22.7	n, p, s, i	0–4	SP, SST, SCT	2.4–4.2	se+
Clade H	*D. angainor* sp. nov.	HSD8, QN13, RBH1, RBI8, SM4, D8	64	0–1	3.8–8.3	21.8–63.9	14.1–29.1	n, p, s, i	0–27	PCT	3.1–6.6	st
Clade J	*Discostella* sp.	BO1, CO1, EK3, GC3, QH5	53	0	3.7–5.5	0–65.5	17.0–29.7	n	1–38	SCT, PCT	3.7–7.0	st
Clade K	*Discostella* sp.	SBW2, QN5, XD7, D18	50	0	3.3–4.9	9.1–70.8	19.3–31.1	n	0–28	SST, SCT	3.5–5.6	se
Clade L	*Discostella* sp.	QM3, VB1	24	0–1	7.6–9.3	38.7–54.0	13.4–22.8	n, p, s, i	3–24	PST, PCT	4.1–5.9	se+
Clade M	*D. asterocostata*	KCH4, XA4	23	0.5–1	12.9–24.5	57.3–69.7	11.7–16.2	n, r	0	PCT	3.6–4.4	se+
Clade N	*Discostella* sp.	QP2, WE5	22	0	4.9–6.2	19.0–52.7	19.4–29.1	n	7–35	PCT	4.8–6.9	se
Clade O	*Discostella* sp.	TL14	11	0	7.9–8.8	17.5–39.2	21.5–26.6	n	23–44	PCT	3.1–3.8	se
Clade P Type I	*D. pseudostelligera* agg.	DBN3, GL4, WEB1	31	0–0.5	6.0–7.4	11.4–53.8	14.5–31.8	n, p, s, i	0–54	PCT	3.8–5.6	se
Clade P Type II	*D. pseudostelligera* agg.	AF2, W16, XE7	40	0–1	4.5–15.4*	6.0–60.9	15.4–30.6	n, p, s, i	0–49	PCT (SST)	3.3–5.3	st
Type material	*D. tatrica*	N/A	12	0–0.5	4.4–5.9	39.8–51.3	15.4–20.5	s, u	5–15	SST	3.1–4.4	se
Type material	*D. stelligera*	N/A	20	0.5–1	7.6–24.0	37.5–76.8	9.6–12.2	n, s, i	0–2	SP	2.8–4.7	se
Type material	*Discostella* sp. (woltereckii material)	N/A	13	0–1	4.2–9.3	32.5–59.0	13.6–20.4	s	0–21	SP+R	3.5–4.7	se
Type material	*D. woltereckii*	N/A	4	0	5.2–8.3	6.8–34.9	23.9–24.6	n, i	19–29	PCT	3.2–4.5	st
Type material	*D. pseudostelligera*	N/A	19	0–1	2.8–7.6	28.2–62.4	14.9–22.1	n, p, s, i	0–6	PCT	4.5–5.6	st

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Species delimitation

As most proposed species concepts (SC) have a variety of problems and exceptions, the choice of a SC for a given group ideally requires knowledge on the population dynamics. For example, it would not be ideal to apply the biological SC to a group in which asexual processes dominate. Many of these factors are unknown in the case of *Discostella*, which is only reinforced by the here found diversity and the possible occurrence of reticulate evolutionary processes. Therefore, we argue that caution is advised when taxonomically assessing the found groups and that more research is needed. The basis of species conceptualisation in diatom taxonomy is morphology, either applying the morphological SC or inferring the limits of species boundaries in the context of the biological SC via morphology (Mann 1999). We likewise use morphological evidence as the basis for our analysis as all *Discostella* species so far were described morphologically and a comparison and assignment is only possible on a morphological level. However, species conceptualisation and species delimitation should be viewed as separate issues (de Queiroz 2007). Adopting a realist view, which implies that species are real biological entities, a morphological approach can be viewed as one possible line of evidence on the basis of a more fundamental conceptualisation like the unified SC, in which species are defined as separately evolving metapopulation lineages (de Queiroz 2007). Equally, DNA barcoding itself does not represent a SC or easily align with another SC, but similar to the morphological SC it should be viewed as another line of evidence for or against the hypothesis of lineage separation and can therefore be used to corroborate and complement the morphological approach (Rach et al. 2008). In fact, it has been argued that neither morphological nor genetic data alone are sufficient for species discovery (DeSalle 2006). We use morphological and molecular data in an integrative approach (Rubinoff 2006; Kollár et al. 2019) to assess the species diversity and flag potentially unknown species (Rach et al. 2008) in the following taxonomic evaluation. Clades A–P were defined as the smallest groups in which both lines of evidence support discriminability and are therefore in good support of lineage separation. However, in the case of subclades that differ only on the morphological or molecular level, one line of evidence still supports separation (de Queiroz 2007), albeit less strongly.

Taxonomy

Clade A – *Discostella stelligera* agg. (Figs 42–50)

This clade contains morphotypes similar to *D. stelligera*. Three different genetic types can be identified (Fig. 1). Types II and III were both found in Europe and North America, which rules out a phylogeographic explanation of the different types. The three types are morphologically very similar and overlap in all parameters. However, type II shows a higher MFP density than types I and III. The diameters of initial cells differ between the types, but even the highest diameter (17,5 µm in type III) is distinctly lower than the maximum of the type material of *D. stelligera* (24 µm). This and the fact that the type material stems from a completely different region of the world than the investigated strains leads to the possibility that *D. stelligera* is not among the three types in this study. Molecular data on the extant population of the type locality (Lake Rotoaira) will be needed to assign one of the types found in this study or a new genetic type to the taxon *D. stelligera*. In the light of these findings *D. stelligera* should be considered a species complex. *D. oyanensis* is most similar to the morphotypes of this clade. Adesalu & Julius (2017) state that it differs from *D. stelligera* in that the central area occupies 55–60% of the valve diameter, while this ratio is higher than 70% in *D. stelligera*. This, however, is not confirmed by our examination of the type material of *D. stelligera* (Figs 5–7), in which the central area occupies 38–77% of the diameter (46–70% for the strains of clade A). In our view, this taxon needs a confirmation on the molecular level and should be considered a part of the *D. stelligera* species complex.

Clade B – *Discostella* sp. (Figs 51–53)

This clade contains only the strain QV4. Morphologically, it is similar to clade E but differs in a higher diameter, slightly higher stria density, more shortened striae and a lower MFP density, which makes the morphotype of QV4 unique within the dataset. Genetically, it is distinct in nuclear and chloroplast markers, but falls into the cox1 – cluster which also contains clades C and J. It may either be a distinct species or the result of genetic exchange between clades C or J and an unknown species, depending on the reason for the ambiguous cox1 position.

Clade C – *Discostella* sp. (Figs 54–61)

All strains in this clade share a distinct morphological feature: root–like ridges around the external pores of the MFP (Figs 54–56, 58–60). Otherwise the morphology in this clade is quite variable spanning from strongly silicified valves similar to the *D. stelligera* agg. (clade A) to more weakly silicified, flat valves with higher stria density and more shortened striae. In the phylogeny of nuclear and chloroplast genes two molecular types can be found, with no apparent morphological differences between them. In the phylogeny of the cox1 gene (Fig. 1) three different types are apparent: One distinct sequence type (KCH1, XA1 and XC1), one identical with the cox1 sequences of clade J (= clade C type II) and the strain XF1 which shares the same sequence type as clade K. However, all three groups cluster in the same clade. Clade C corresponds to one of two morphotypes that can be found in the type material of *D. woltereckii* (see discussion of clade P). We did not find this clade in Middle Europe, but specimens from Austria depicted by Rott & Kofler (2021) named *D. stelligera* s. l. (Fig. 11 A, B) fit the morphology of this clade. Despite the anomaly of the mitochondrial phylogeny this clade likely represents

either a morphologically distinct species or possibly a species complex.

Clade D – *Discostella glomerata* (Figs 62–64)
Morphologically, *D. glomerata* is easily distinguished from all other *Discostella* morphotypes in this study by the highest MFP density, bearing MFP between almost every pair of costae. According to the molecular data, the clade is closely related to clade E, which was identified as *D*. cf. *stelligeroides*. Both strains of *D. glomerata* differ somewhat in the nuclear and chloroplast markers and the strain SLS1 shares the same cox1 haplotype as clade E, while LU10 slightly differs. This is interesting considering that both clades were found in similar regions and water bodies. Whether both clades are just very closely related species or a more complex situation including gene flow is the reason for these results, can only be decided by studying more populations in the future. Both clades are, however, morphologically distinct. There has been some confusion concerning the identity and generic placement of *D. glomerata* in the past. The taxon was transferred to *Discostella* (formerly *Cyclotella glomerata*) in the course of the description of the genus by Houk & Klee (2004). The type material contains both a *Pantocsekiella* (formerly also *Cyclotella*) and *Discostella* species. Because Bachmann's original drawings clearly show a colonial species, Tuji & Williams (2006 A) and Adesalu & Julius (2017) concluded that the *Discostella* species could not be the taxon in question. The latter argued that the genus lacks central fultoportulae and no other known *Discostella* species forms colonies, while *Pantocsekiella* species frequently form chain–like colonies. Based on the two stains acquired in this study (SLS1, LU10) we can now settle this issue and confirm that this taxon indeed belongs to the genus *Discostella*. It forms long and stable chain–like colonies (Figs S5–10, supplementary material). It is unclear how exactly these colonies are formed, but it seems likely that the very abundant chitin fibrils associated with the MFP might be responsible for the connection of the cells (Fig. S5–6). This makes *Lindavia glomerata* (H. Bachmann) Adesalu et Julius a synonym of *D. glomerata* and confirms the interpretation of Klee & Houk (2007) and Houk et al. (2010) who gave a detailed account of the morphology of *D. glomerata* in accordance with the strains used in this study.

Clade E – *Discostella* cf. *stelligeroides* (Figs 65–67)
The strains of this clade are morphologically very similar to the type material of *D. stelligeroides* (Houk et al. 2010). All strains are molecularly identical and share a distinct morphotype characterized by a high density of MFP, relatively strongly silicified valves and simple pores to very short simple tubes as external openings of the MFP. Many of the valves show very small granuli across the valve face (Figs 65–66), a feature shared by the type material shown by Houk et al. (2010). *D. tatrica* is somewhat similar especially concerning the external projections of the MFP. Procházková et al. (2012) state that *D. tatrica* differs from *D. stelligeroides* in the possibility of having an incomplete stellate pattern in its convex central parts and possessing alveoli in its concave central areas. Both of these characteristics could be found in most of the clades and strains with alveolate structures, including clade E. However, the measurement of the type material of *D. tatrica* (Figs 2–4) showed that it differs from *D. stelligeroides* and clade E in a lower density of MFP. In our investigation of the type material of *D. stelligeroides* we were unable to find *Discostella* valves. However, the unexpected diversity found in this study leaves the possibility that this clade may not represent *D. stelligeroides* but another closely related species.

Clade F – *Discostella* sp. (Fig. 68)
VN10 is the only strain within this clade. Morphologically, the strain is similar to clades G and K. It differs from clade G by more prominent external projections of the MFP and a higher stria density. The only difference to clade K is the presence of alveolar patterns in the central area. In the phylogeny of the nuclear and chloroplast markers clade F is closely related to clade G, but within the mitochondrial phylogeny it lies in the proximity of the cox1–cluster containing clades C, J and K with similar bootstrap support. Despite this different phylogenetic placement, it likely a distinct unknown species, but more data on the molecular and morphological variability is needed.

Clade G – *Discostella* cf. *lacuskarluki* and *Discostella* cf. *guslyakovyi* (Figs 69–81)
This clade contains rather small (3.2–6.3 µm) morphotypes with short external projections (complex or simple) of the MFP. At least four different genetic types are present in this clade. The two European strains (FR1, VI2 = type I) have identical sequences, while the five strains from Quebec (QB2, QD2, QQM10, QW3, QX2 = types II–IV) all differ on the molecular level. This genetic diversity is unique within the dataset, especially considering that all these Canadian populations were sampled within a radius just over 100 km, while other clades display identical sequences despite diverse origins of their strains (e.g. clades E, H, J, K). The morphology also somewhat differs between the genetic types. Especially the stria density (e.g. type II and IV) and the relief of the valve face (e.g. type I and III) can differ distinctly. The strain QX2 (type IV) shows small granuli across the central area of the valve face (Figs 78–79). Morphologically, type I is very similar to *D. lacuskarluki* and type III resembles *D. guslyakovyi* with the characteristic ridges on the valve face (Figs 75–76). However, as long as the reasons and scope of the genetic diversity within this clade remain unclear and the genetic information is not connected to the taxonomic types or type populations, the taxonomic assignment remains uncertain. We conclude that this clade likely comprises a species complex, probably including *D. lacuskarluki, D. guslyakovyi* and possibly *D. tatrica* and *D. nipponica*. Although it is possible that some of these

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taxa are conspecific as Potapova et al. (2020) proposed, in the light of these findings it would be necessary to use molecular data to confirm conspecificity.

Clade H – *Discostella angainor* sp. nov. (Figs 14–41)
Concerning all markers – this clade is distinct on the molecular level and there are no intraspecific differences despite the diverse origins of the strains. All strains share a common morphotype with a stalked RP and prominent complex MFP, leaving only clade J and P type II as similar morphotypes. The most distinctive feature of *D. angainor* is the broad collar–shaped external projection of the MFP (Figs 36–38, 40), which separates the taxon from the other morphotypes with distinctly stalked RP (clades J and P type II). The valves of clade J are also smaller and lack patterns in the central area and in clade P the striae are more branched. *D. angainor* seems to be a facultative colony former. The strains were mainly chain forming (RBI8, RBH1 and SM4) or both, solitary and colonial (HSD8, D8, QN13). The chains (Fig. 32) have a length of ca. 2–20 cells. Unlike the colonies of *D. glomerata*, they are shorter, less stable and the valves are tightly connected with the help of the external projections of the MFP. The notable characteristic of an asymmetric placement of the MFP (Figs 40–41) occurred solely in the strains that formed the longest colonies (RBI8, RBH1 and SM4). It seems that only larger valves have an alveolar pattern in the central area as the smaller strains (3.8–6.3 μm) show no such patterns, but the two strains with the largest valve diameter (6.4–8.3 μm) do. Under the name *D. pseudostelligera* Houk et al. (2010) show valves of *D. angainor* in material from Schäferweiher, a cut–off meander of the Rhine (p. 476, Figs 1–6).

Clade J – *Discostella* sp. (Figs 82–84)
This clade corresponds to a small, inconspicuous morphotype characterized by a stalked RP and the lack of alveolar patterns in the central area. In the phylogeny of the nuclear and chloroplast markers the clade is distinct and forms the sister clade of *D. angainor* (clade H). Within the cox1 phylogeny the strains fall into the cluster including clades B, C and K and even show identical sequences to clade C type II. However, the morphology and other markers are completely different from clade C and it can be ruled out that both clades belong to the same species. We therefore treat this clade as an unknown species. This species seems to be thriving under high nutrients conditions as cells often only emerged in the water samples after the addition of additional nutrients (e.g. in the cases of BO1 and QH5).

Clade K – *Discostella* sp. (Figs 85–87)
This clade shares a small inconspicuous morphotype without alveolar patterns in the central area, which differs from the similar clades H and J in the sessile RP. The stria density is higher than in the otherwise similar clade G. It differs from clade F in the lack of alveolar patterns in the central area, but is otherwise very similar. On the molecular level clade K is distinct concerning the nuclear and chloroplast markers and is even the basal clade within the genus. However, this relative position is only reproduced in the phylogeny of 18S (Fig. S1), which contributes to the low overall support within the concatenated dataset. In the cox1 phylogeny, it falls into the cluster of the clades B, C and J. Similarly to clade J and clade C type II which share the same cox1–sequences, clade K shares the same cox1 sequence as the strain XF1 (clade A type I) for which the explanation is equally unknown. However, as in the case of clade J the morphology of clade K completely differs from clades B and C and we suspect that it is a distinct species.

Clade L – *Discostella* sp. (Figs 88–89)
This clade contains the strains QM3 from Quebec and VB1 from North Germany. Molecularly, it is quite isolated, but the morphology is inconspicuous and reminiscent of the clades H, N, O or P. It remains especially unclear how to separate it from clade P by morphology only. In this data set, it is the closest relative to *D. asterocostata* (clade M). Due to the high separation in the molecular data, we argue that it is likely an unknown species. However since only two strains are known, it is unclear if it belongs to a species complex or what the morphological range of this species is.

Clade M – *Discostella asterocostata* (Figs 90–91)
The investigated strains of *D. asterocostata* are morphologically and molecularly distinct. Morphologically, the ring–shaped pattern in the central area seems to be the diagnostic feature of the taxon.

Clade N – *Discostella* sp. (Figs 92–94)
The two Canadian strains QP2 and WE5 would have likely been placed into the *D. pseudostelligera* agg. according to the current taxonomic literature. They are indeed morphologically similar to clade P, but differ from Clade P in the absence of alveolate structures in the central area and a higher MFP density. Furthermore, unlike Clade P type II, the RP is sessile. Clade N is distinct in all molecular markers. It is likely a distinct species, but more data on the morphological range and differences to other species is needed.

Clade O – *Discostella* sp. (Fig. 95)
The strain TL14 is molecularly unique, but is morphologically very similar to clades N and P and falls into the broad concept of *D. pseudostelligera*. The only known strain differs from clade P in the lack of alveolar patterns in the central area and from clade N in a lower density of MFP. It is likely a separate taxon, but more populations need to be studied.

Clade P and the identity of *Discostella pseudostelligera* and *Discostella woltereckii* (Figs 96–101)
A highly variable morphology was observed in this clade,

which is characterised by prominent complex tubes of the MFP, a medium to very high stria density and alveolar patterns in the central area. The morphotypes range from more strongly silicified forms with distinct costae, medium stria density and a low number of shortened striae (Figs 97, 101) to more weakly silicified, densely striated forms in which the striae sometimes almost reach the centre of the valve face (Figs 98–99). Within this spectrum, morphotypes fitting the concepts of *D. pseudostelligera* and *D. woltereckii* in the taxonomic literature can be found. Two different genetic types can be distinguished in the phylogeny of the nuclear and chloroplast markers, morphologically only differing by the structure of the RP (type I sessile and type II stalked). However, the genetic situation seems to be more complex as the LSU sequences of the strain GL4 of type I seems to represent a mixture of type I and type II possibly hinting to genetic exchange between both types at some point. Additionally, both types mix concerning the mitochondrial marker, leaving the rbcL sequences and the structure of the RP as clear differences. The type material of *D. woltereckii* has been analysed first by Klee & Houk (1996) and later by Tuji & Williams (2006 B). In the latter publication, the authors identified two different *Discostella* morphotypes based on position and structure of the RP. Our investigation of the type material confirmed these results. Two different *Discostella* morphotypes are present:

Morphotype I, *Discostella woltereckii*: stalked RP and prominent complex external openings of the MFP, similar to clade P type II (Figs 10–11)

Morphotype II, *Discostella* sp.: sessile RP and simple pores with root–like or wing–shaped (Klee & Houk 1996) ornamentation of the external openings of the MFP, similar to clade C (Figs 12–13)

Following Hustedt's original drawings, only morphotype I can be *D. woltereckii* as the MFP are very prominent in the drawings (Hustedt 1942). The fact that the striae are close to the centre and merge with the central pattern is often thought to be characteristic of *D. woltereckii*, however, this feature can be found for both morphotypes of the type material as well as for several of the clades in this study (clades C, J, N, P, O and K) and is therefore not of a high diagnostic value. The name *D. pseudostelligera* (sensu lato) is often used for all kinds of small *Discostella* morphotypes that would include several clades of this study. However, the investigation of the type material narrows down the possibilities. The type is characterized by a stalked RP, prominent complex openings of the MFP and alveolar patterns in the central area. This is in agreement with the investigation of Houk et al. (2010) and only leaves clade P type II as a candidate for *D. pseudostelligera* within the dataset of this study. Some of the valves found in the type material could also represent clade J, however clade J is not in agreement with Hustedt's original drawing that depicts a stellate pattern in the central area. If clade P type II indeed represents both taxa, that would make *D. woltereckii* a synonym of *D. pseudostelligera*. However, to confirm this hypothesis more data on more populations is needed as it is also possible that neither *D. pseudostelligera* nor *D. woltereckii* is represented by this clade. Until then, this group should be considered a species complex. In the light of these results, studies on the morphology of populations of *D. pseudostelligera* and *D. woltereckii* should be reappraised with care. The population studied by Hübener (1999) fits clade P and more specifically type I, as the RP seems to be sessile. Alongside morphotypes corresponding to clade P, Genkal (2015) shows several different morphotypes that could be linked to several clades found in this study, all under the name *D. pseudostelligera*. This concept of *D. pseudostelligera* is therefore shown to be too broad.

Hidden Diversity

The results of this study reveal previously unknown diversity within the genus *Discostella*. Taking the morphological data into account, the 15 clades (22 genetic types) defined in this study are a conservative estimate of species diversity and should each represent either species or species complexes. Four extant taxa (*D. tasmanica*, *D. mascarenica*, *D. elentarii*, and *D. gabinii*) were not among the studied strains. Other taxa like *D. stelligera*, *D. pseudostelligera*, *D. woltereckii* etc. may or may not be part of the found clades and further research is needed to connect molecular data to the taxonomic types. *D. hellae* (Chang & Steinberg) Chang could not be assigned to any morphotype due to a lack of characterization in the original description. Adding the 16 extant taxa from the current literature to those clades and types in this study that cannot clearly be identified as any of these species, we estimate that there is a minimum of 25–36 extant species. This is the most conservative estimate which considers only the dataset of the present study and the current taxonomic literature. However, there are reasons to presume that the diversity is even greater. Twenty–two different genetic types in 58 cultures give an average of one new genetic type discovered in every second to third culture. Considering the bias of regional sampling and the many regions that remain unsampled, this genus very likely still has even more undiscovered diversity. Furthermore, this hypothesis is also reinforced by studies like Guerrero & Echenique (2006), who found several different morphotypes in the Rio Limay basin (Argentina). Based on the findings of this study it should also be of interest to take a closer look at morphotypes at subspecies level (e.g. *Discostella stelligera* var. *tenuis* (Hustedt) Houk et Klee, *D. stelligera* var. *hyalina* (Hustedt) Houk et Klee, *D. stelligera* var. *elliptica* (Frenguelli) Guerrero et Echenique, *D. stelligera* var. *microrobusta* R.J.Flower, *D. stelligera* var. *robusta* (Hustedt) Houk et Klee, *D. woltereckii* var. *minor* Öberg, Risberg et Stabell and *D. asterocostata* var. *striata* (J.Y.Chen) V.Houk et R.Klee).

Distribution of the clades

So far, data on the distribution of the found clades is very

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preliminary as the number of strains and the sampling are very limited as of now. However, some patterns for the approximate sampling regions can already be seen (Table S2, supplementary material). For example, most clades containing at least two populations are not restricted to a certain region (clades A, C, G, H, J, K, L and P). Only clades D and E are restricted to Middle Europe and clade N is restricted to North America so far. Equally, *D. asterocostata* was only isolated from sites in South East Asia. This pattern also applies to the different types, which do not seem to be too restricted to certain regions, with the exception of clade C type I (only found in South East Asia) and clade G type II–IV (only found in Quebec). This indicates that most clades may not be restricted on smaller geographical scales at least in the present day, as it is unclear how human activities may have influenced these distributions. On the other hand, despite the fact that most sampling locations were in Middle/Western Europe, many clades/types cloud not be found there as of yet (clades A, B, C, F, G types II–IV, M and N), indicating that there may be some large scale patterns. In the case of *D. asterocostata*, this species is believed to have been originally restricted to parts of Asia, but has recently been reported as an introduced species in North America (Alverson et al. 2021). This is interesting considering that according to the molecular data this species is rather distant from all other known clades and therefore lineages around this species should have had plenty of time to reach an equally large distribution range like the many other more recently diverged clades. It is also noteworthy that some of the clades were found in the same water bodies (in most cases that means in the same sample). Table S3 (supplementary material) indicates, which species were found together in the present dataset. The co–occurrence of different *Discostella* species further complicates the issue of species identification. There are no indications that the cases of mixed LSU sequences or shared mitochondrial sequences are related to specific locations. When comparing the two main sampling regions in Quebec and North East Germany, it becomes clear that the species diversity in the latter region is much lower than in Quebec. In the course of our cultivation attempts in North East Germany, six different clades/types were found. Despite less than half the number of sampling sites, in Quebec twelve different clades/types have been discovered. The diversity and importance of the genus may therefore distinctly vary depending on the region.

Comparison with public sequence data

Concerning 18S, ten compatible *Discostella* sequences could be retrieved from the NCBI database. Five labelled as *Discostella* sp. clustering in clades A, C, H, J and E (Fig. S1), two sequences labelled as *D. pseudostelligera* clustered with clades E and P, where only the latter assignment would be consistent with the results of the present study. One sequence named *D. stelligera* clustered with clade A and one named *D. woltereckii* with clade P, which is consistent with our assessment. Another sequence labelled *D. nipponica* fell into clade K. However, Tuji & Williams (2006 A) demonstrate a different morphology for *D. nipponica* than the one which we observed for clade K in the present study and the assignment therefore seems to be incorrect. All sequences were identical to our clades except for the *Discostella* sp. sequence that clustered with clade E. Another sequence labelled *Discostella* sp. was found in the Diat. barcode dataset and clustered with clade J. Furthermore, fourteen sequences from Dinophyceae endosymbionts were found to fit within *Discostella*. Seven belonging to the genus *Unruhdinium* Gottschling, all of which are somewhat different and outside of the defined clades and six belonging to the genus *Peridiniopsis* Lemmermann, which clustered with clades H, J and K. The fact that the endosymbiont sequences of *Peridiniopsis* exactly fit extant populations while those associated with *Unruhdinium* all somewhat differ from the clades and among each other could hint at the fact that the endosymbiosis event in *Peridiniopsis* may have been more recent. Alternatively, the *Unruhdinium* endosymbionts could represent a set of unknown *Discostella* lineages. In the case of LSU, two compatible *Discostella* sequences could be found in the NCBI database. One *Discostella* sp. clustering with clade C and the mentioned strain named *D. nipponica* again with clade K (Fig. S2). For rbcL ten compatible *Discostella* sequences were found in the NCBI database (Fig. S3). Four labelled as *Discostella* sp. clustering with clades C, E and J. Three named *D. pseudostelligera* clustering all with different clades E, J and P (type II), only the last of which would be consistent with the results of our study. One sequence named *D. stelligera* correctly clustered with clade A (but not with a known type) and another sequence named *D. woltereckii* fell into clade P (type II), which is equally consistent with our results. The sequence of the strain identified as *D. nipponica* again falls into clade K raising doubt about the identification. Additionally, two sequences of *Peridiniopsis* endosymbionts were retrieved by the BLAST algorithm, clustering with clades J and K (again with identical sequences like for 18S). Another rbcL sequence was found in the Diat. barcode dataset, named *D. woltereckii*, but clustering with clade J, thus wrongly assigned. And lastly only two environmental sequences matching *Discostella* (wrongly assigned as unknown metazoa) could be found in the case of cox1. Both clustering with clade P (Fig. S4), one with type I and the other with neither type I nor II. Most of the publicly available sequences are identical to a clade or type defined in the present study. It is unclear if minor deviations represent additional molecular types or methodological artefacts as the sequences stem from different methodologies. Generally, this means that common sequence types in researched regions are represented within this study. In the light of our findings, it is not surprising that several taxa may be incorrectly assigned and that *D. pseudostelligera* is the most used name, confirming a broad taxonomical concept of this taxon in applied taxonomy.

Species identification

Smaller taxa with more weakly silicified valves (e.g. clades J and K) are practically impossible to determine via LM even on genus level. They can easily be mistaken for other small thalassiosiroid species such as *Thalassiosira pseudonana* Hasle et Heimdal as in Kiss (1984; Figs 10–19, 21–23) or Krammer & Lange–Bertalot (2004; pl. 60, Figs 6a, 6b). Generally, many important characters cannot be seen in LM such as the RP (sessile or stalked) or the number and structure of the MFP (especially when short or inconspicuous). SEM can help to characterize populations morphologically, but DNA barcoding can be a real alternative in this case, given a sound reference library. This also applies to describing and synonymising taxa as old and new taxonomic types should be linked to molecular data to avoid future confusion.

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References

Adesalu, T.A. & Julius, M.L. (2017): *Discostella oyanensis*, sp. nov., a new planktonic diatom species from Nigeria, West Africa. – Diatom Research 32: 163–173.

Alfinito, S. & Tagliaventi, N. (2002): Morphology and taxonomy of *Cyclotella elentarii* spec. nova a newly described centric diatom from a New Zealand Lake. – Algological Studies 105: 29–38.

Aljanabi, S.M. & Martinez, I. (1997): Universal and Rapid Salt–Extraction of High Quality Genomic DNA for PCR–Based Techniques. – Nucleic Acids Research 25: 4692–4693.

Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W. & Lipman, D.J. (1990): Basic local alignment search tool. – Journal of Molecular Biology 215: 403–10.

Alverson, A.J.; Jansen, R.K. & Theriot E.C. (2007): Bridging the Rubicon: phylogenetic analysis reveals repeated colonizations of marine and fresh waters by thalassiosiroid diatoms. – Molecular Phylogenetics and Evolution 45: 193–210.

Alverson, A.J.; Chafin, T.K.; Jones, K.A.; Manoylov, K.M; Johnson, H.; Julius, M.; Nakov, T.; Ruck, E.C.; Theriot, E.C.; Yeager, K.M. & Stone, J.R. (2021): Microbial biogeography through the lens of exotic species: the recent introduction and spread of the freshwater diatom *Discostella asterocostata* in the United States. – Biological Invasions 23: 2191–2204.

Chang, T.P. & Steinberg, C. (1989): Identifizierung von nanoplanktischen Kieselalgen (Centrales, Bacillariophyceae) in der Rott und im Rott–Stausee (Bayern, Bundesrepublik Deutschland). – Archiv für Protistenkunde 137: 111–129.

de Queiroz, K. (2007): Species Concepts and Species Delimitation. – Systematic Biology 56: 879–886.

DeSalle, R. (2006): Species Discovery versus Species Identification in DNA Barcoding Efforts: Response to Rubinoff. – Conservation Biology 20: 1545–1547.

Evans, K.M.; Wortley, A.H. & Mann, D.G. (2007): An assessment of potential diatom 'barcode' genes (cox1, rbcL, 18S and ITS rDNA) and their effectiveness in determining relationships in *Sellaphora* (Bacillariophyta). – Protist 158: 349–364.

Genkal, S.I. (1977): Method of Calculation of Some Taxonomically Important Structural Elements of the Diatom Valve from Family Thalassiosiraceae Lebour Emend Hasle (Bacillariophyta). – Botanicheskii Zhurnal 62: 848–851.

Genkal, S.I.; Bondarenko, N.A. & Popovskaya, G.I. (2007): New representative of the genus *Discostella* Houk & Klee from the Eastern Baikal area (Russia). – International Journal on Algae 9: 359–364.

Genkal, S. (2015): Morphological variability, taxonomy and ecology of *Discostella pseudostelligera* (Bacillariophyceae, Centrales) and similar species. – Nova Hedwigia 101: 427–449.

Guerrero, J.M. & Echenique, R.O. (2006): *Discostella* taxa (Bacillariophyta) from the Rio Limay basin (northwestern Patagonia, Argentina). – European Journal of Phycology 41: 83–96.

Guillard, R.R.L. & Ryther, J.H. (1962): Studies on marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. – Canadian Journal of Microbiology 8: 229–239.

Hall, T.A. (1999): BioEdit: A User–Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. – Nucleic Acids Symposium Series 41: 95–98.

Hamsher, S.E.; Evans, K.M.; Mann, D.G.; Poulíčková, A. & Saunders, G.W. (2011): Barcoding Diatoms: Exploring Alternatives to COI–5P. – Protist 162: 405–422.

Haworth E.Y. & Tyler P.A. (1993): Morphology and taxonomy of *Cyclotella tasmanica* spec. nov., a newly described diatom from Tasmanian lakes. – In: H. Van Dam (ed.): Proceedings of the Twelfth International Diatom Symposium, Renesse, The Netherlands, 30 August–5 September, 1992. – pp. 49–56, Hydrobiologia 269/270, Springer, Berlin.

Houk, V. & Klee, R. (2004): The stelligeroid taxa of the genus *Cyclotella* (Kutzing) Brebisson (Bacillariophyceae) and their transfer into the new genus *Discostella* gen. nov. – Diatom Research 19: 203–228.

Houk, V.; Klee, R. & Tanaka, H. (2010): Atlas of freshwater centric diatoms with a brief key and descriptions, Part III., Stephanodiscaceae A, Cyclotella, Tertiarius, *Discostella*. – Fottea 10 (Supplement): 1–498.

Hübener, T. (1999): Morphology and ultrastructure of a population of *Cyclotella woltereckii* Hustedt (Bacillariophyceae) in Northern Germany. – Nova Hedwigia 68: 469–476.

Hustedt, F. (1939): Die Diatomeenflora des Küstengebietes der Nordsee vom Dollart bis zur Elbemündung. I. Die Diatomeenflora in den Sedimenten der unteren Ems sowie auf den Watten in der Leybucht, des Memmert und bei der Insel Juist. – Abhandlungen des Naturwissenschaftlichen Verein zu Bremen 31: 571–677.

Hustedt, F. (1942): Süßwasser–Diatomeen des indomalayischen Archipels und der Hawaii–Inseln. Nach dem Material der Wallacea–Expedition. – Internationale Revue der gesamten Hydrobiologie und Hydrographie 42: 1–252.

Iwatani, N.; Murakami, S. & Suzuki, Y. (2005): A Sequencing Protocol of Some DNA Regions in Nuclear, Chloroplastic and Mitochondrial Genomes with an Individual Colony of *Thalassiosira nordenskioeldii* Cleve (Bacillariophyceae). – Polar Bioscience 18: 35–45.

Jahn, R.; Abarca, N.; Al–Handal, A.; Kusber, W.; Zimmermann, J. & Skibbe, O. (2021): Integrative taxonomic description of the marine species *Cocconeis crawfordii* (Bacillariophyceae) based on unialgal strains. – Nova Hedwigia, Beihefte 151: 85–105.

Jung, S.W.; Han, M.S. & Ki, J.S. (2010): Molecular genetic divergence of the centric diatom *Cyclotella* and *Discostella* (Bacillariophyceae) revealed by nuclear ribosomal DNA comparisons. – Journal of Applied Phycology 22: 319–329.

Kahlert, M.; Kelly, M.G.; Mann, D.G.; Rimet, F.; Sato, S.; Bouchez, A. & Keck, F. (2019): Connecting the morphological and molecular species concepts to facilitate species identification within the genus *Fragilaria* (Bacillariophyta). – Journal of Phycology 55: 948–970.

Kimura, M. (1980): A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. – Journal of Molecular Evolution 16: 111–120.

Fottea, Olomouc, 24(1): 42–60, 2024
DOI: 10.5507/fot.2023.010

Kistenich, S.; Dressler, M.; Zimmermann, J.; Hübener, T.; Bastrop, R. & Jahn, R. (2014): An Investigation into the morphology and genetics of *Cyclotella comensis* and closely related taxa. – Diatom Research 29: 423–440.

Kiss, K.T. (1984): Occurrence of *Thalassiosira pdeudonana* Hasle et Heimdal (Bacillariophyceae) in some rivers of Hungary. – Acta Botunica Hungarica 30*:* 277–287.

Kollár, J.; Pinseel, E.; Vanormelingen, P.; Poulíčková, A.; Souffreau, C.; Dvořák, P. & Vyverman, W. (2019): A polyphasic approach to the delimitation of diatom species: a case study for the genus *Pinnularia* (Bacillariophyta). – Journal of Phycology 55: 365–379.

Krammer, K. & Lange–Bertalot, H. (2004): Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. – In: Ettl, H.; Gerloff, J.; Heynig, H. & Mollenhauer, D. (eds): Süsswasserflora von Mitteleuropa. Band 2/3. – 599 pp., Spektrum Akademischer Verlag, Heidelberg.

Klee, R. & Houk, V. (1996): Morphology and Ultrastructure of *Cyclotella woltereckii* HUSTEDT (Bacillariophyceae). – Archiv für Protistenkunde 147: 19–27.

Klee, R. & Houk, V. (2007): Valve ultrastructure studies of *Discostella glomerata* (Bachmann) Houk & Klee. – Diatom Research 22: 89–103.

Mann, D.G. (1999): The species concept in diatoms. – Phycologia 38: 437–495.

Öberg, H.; Risberg, J. & Stabell, B. (2009): Morphology, valve ultrastructure and stratigraphical variability of *Discostella* taxa in a tropical crater lake, northern Tanzania. –Diatom Research 24: 341–356.

Paillès, C.; Sylvestre, F.; Tonetto, A.; Mazur, J. & Conrod, S. (2020): New fossil genus and new extant species of diatoms (Stephanodiscaceae, Bacillariophyceae) from Pleistocene sediments in the Neotropics (Guatemala, Central America): adaptation to a changing environment? – European Journal of Taxonomy 726: 1–23.

Potapova, M.G.; Aycock, L. & Bogan, D. (2020): *Discostella lacuskarluki* (Manguin ex Kociolek and Reviers) comb. nov.: a common nanoplanktonic diatom of Arctic and boreal lakes. – Diatom Research 35: 55–62.

Procházková, L.; Houk, V. & Nedbalová, L. (2012): *Discostella tatrica* sp. nov. (Bacillariophyceae) – a small centric diatom from the Tatra Mountain lakes (Slovakia/Poland). – Fottea 12: 1–12.

Rach, J.; Desalle, R.; Sarkar, I.N.; Schierwater, B. & Hadrys, H. (2008): Character–based DNA barcoding allows discrimination of genera, species and populations in Odonata. – Proceedings of the Royal Society B: Biological Sciences 275(1632): 237– 47.

Rimet, F.; Chonova, T.; Gassiole, G.; Gusev, E.; Kahlert, M.; Keck, F.; Kelly, M.; Kochoska, H.; Kulikovskiy, M.; Levkov, Z.; Maltsev, Y.; Mann, D.; Pfannkuchen, M.; Trobajo, R.; Vasselon, V.; Wetzel, C.; Zimmermann, J. & Bouchez, A. (2018): "2022–05–25–Diat.barcode–release–version 11.1.xlsx", Diat.barcode, an open–access barcode library for diatoms. – Recherche Data Gouv, V15. DOI: https://doi.org/10.57745/BE9TJK

Rubinoff, D. (2006): DNA Barcoding Evolves into the Familiar. – Conservation Biology 20: 1548–1549.

Sayers, E.W.; Bolton, E.E.; Brister, J.R.; Canese, K.; Chan, J.; Comeau, D.C.; Connor, R.; Funk, K.; Kelly, C.; Kim, S.; Madej, T.; Marchler–Bauer, A.; Lanczycki, C.; Lathrop, S.; Lu, Z.; Thibaud–Nissen, F.; Murphy, T.; Phan, L.; Skripchenko, Y.; Tse, T.; Wang, J.; Williams, R.; Trawick, B.W.; Pruitt, K.D. & Sherry, S.T. (2022): Database resources of the national center for biotechnology information. – Nucleic Acids Research 50: D20–D26. DOI: https://doi.org/10.1093/nar/gkab1112

Schultz, K.; Hübener, T.; Jacques, O.; Springer, A.; Frank, M. & Dressler, M. (2022): DNA barcoding reveals a new species of *Stephanodiscus* Ehrenberg. – Diatom Research 37: 89–106.

Tamura, K. (1992): Estimation of the number of nucleotide substitutions when there are strong transition–transversion and G + C–content biases. – Molecular Biology and Evolution 9: 678–687.

Tamura, K. & Nei, M. (1993): Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. – Molecular Biology and Evolution 10: 512–526.

Tuji, A. & Williams, D.M. (2006 A): The Identity of *Cyclotella glomerata* Bachmann and *Discostella nipponica* (Skvortzov) Tuji & Willims comb. et stat. nov. (Bacillariophyceae) from Lake Kizaki, Japan. – Bulletin of the National Museum of Nature and Science, Series B 32: 9–14.

Tuji, A. & Williams, D.M. (2006 B): Type Examination of *Cyclotella woltereckii* Hust. (Bacillariophyceae) with Special Attention to the Position of Its Rimoportula. – Bulletin of the National Museum of Nature and Science, Series B 32: 15–17.

Wawrik, B.; Paul, J.H. & Tabita, F.R. (2002): Real–Time PCR Quantification of rbcL (Ribulose–1, 5–Bisphosphate Carboxylase/Oxygenase) mRNA in Diatoms and Pelagophytes. – Applied and Environmental Microbiology 68: 3771–3779.

Zimmermann, J.; Jahn, R. & Gemeinholzer, B. (2011): Barcoding diatoms: evaluation of the V4 subregion on the 18S rRNA gene, including new primers and protocols. – Organisms Diversity & Evolution 11: 173–192.

Supplementary material

The following supplementary material is available for this article:

Fig. S1. A: Maximum likelihood tree of the 18S sequences. B: Neighbour joining tree of the 18S sequences including public sequence data (red). Genbank accession numbers are given, strain/voucher labels in brackets. DES = diatom endosymbiont. Bootstrap values above 25% are given for each node.
Fig. S2. Maximum likelihood tree of the LSU sequences including public sequence data (red). Genbank accession numbers are given, strain/voucher labels in brackets. Bootstrap values above 25 % are given for each node.
Fig. S3. A: Maximum likelihood tree of the rbcL sequences. B: Neighbour joining tree of the rbcL sequences including public sequence data. Genbank accession numbers are given, strain/voucher labels in brackets. Bootstrap values above 25% are given for each node.
Fig. S4. Maximum likelihood tree of the cox1 sequences including public sequence data (red). Genbank accession numbers are given, strain/voucher labels in brackets. Bootstrap values above 25% are given for each node.
Figs S4–S10. Colonies of *D. glomerata* (strain SLS1): (S1–S2) SEM images of dried colonies, note the abundant chitin–fibrils; (S3–S6) LM images of live colonies. Scale bars 1 μm (S1) and 20 μm (S2–S6).
Table S1. List of the strains used in this study including taxa, sampling sites and GenBank accession numbers for the respective gene loci.
Table S2. Distribution of the clades/types.
Table S3. Co-occurances of the Discostella clades. Numbers indicate how many times two clades were found in the same water body.

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)

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