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Disentangling the Taxonomic History of the Widespread and Overlooked Centric Diatom *Stephanodiscus makarovae* and Its Transfer to *Cyclostephanos*

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Abstract: *Stephanodiscus makarovae*, a taxon originally described from Russia, is morphologically similar to several other taxa within *Cyclostephanos*, namely *C. invisitatus*, *C. delicatus* and *C. tholiformis*. However, it has not yet been transferred into *Cyclostephanos*, perhaps due to the difficulty in identifying it, as its original description is available only in the Russian language. To investigate its morphology, a detailed morphological comparison of *S. makarovae* and *C. invisitatus* was done from 286 SEM micrographs of 12 monoclonal strains. We performed a three-gene phylogenetic analysis with strains from eight additional taxa to independently confirm the position of *S. makarovae*. The morphology of *S. makarovae* shows key features of the genus *Cyclostephanos* and this attribution is supported by the phylogeny. Here we propose the transfer of the taxon *S. makarovae* to *Cyclostephanos*, considering the morphological and molecular data. According to both the molecular and morphological data, *C. delicatus* has a unique position within the genus; *S. makarovae* and *C. invisitatus* are morphologically very similar but genetically distinct. Furthermore, based upon the results, it was possible to reassign the authority of the transfer of *S. delicatus* into *Cyclostephanos*.

Keywords: *Cyclostephanos*; *makarovae*; morphological and molecular analyses

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1. Introduction

Scientific and methodical advancements in diatom taxonomy have given rise to conflicting species and generic concepts, resulting in frequent transfers. This of course also applies to centric diatoms. These difficulties are especially clear when examining the taxonomic relationships within the many genera erected in the mid-19th century, many of which have since been revised, forgotten or resurrected. In this paper, we will examine the links between four taxa derived from or still in the genus *Stephanodiscus* Ehrenberg 1845: *Stephanodiscus makarovae* Genkal 1978, *Cyclostephanos invisitatus* (M.H.Hohn and Hellermann) E.C.Theriot, Stoermer and Håkasson 1988, *C. tholiformis* Stoermer, Håkansson and Theriot 1988 and *C. delicatus* (Genkal) S.J.Casper and W.Scheffler 1990. All these taxa are small and morphologically quite similar.

Ehrenberg first published the genus *Stephanodiscus* in 1845 [1]. The type species, named by Boyer 1927 [2], is *Stephanodiscus niagarae* Ehrenberg. Round 1982 the authors of [3] invalidly erected the genus *Cyclostephanos* mainly due to the alveolar structures in some species, but this lacked a valid type reference, which was subsequently established as

C. novae-zeelandiae (Cleve) in 1987 [4]. Theriot et al. 1987 [5] then highlighted the significance of the position and form of the external opening of the rimoportula to distinguish between both genera, which is inconspicuous and below the spines in *Cyclostephanos* and spine-like in *Stephanodiscus*. This was also the basis for their subsequent transfer of *S. invisitatus* to the genus *Cyclostephanos.* Shortly after in 1990, both Casper and Scheffler [6] and Håkansson and Kling [7] published, in close succession, revisions of *S. delicatus* to *C. delicatus*. Håkansson and Kling also amended the description of *C. tholiformis* to separate it from *C. delicatus*. Dreßler and Hübener 2006 [8] later attributed the transfer of *C. delicatus* to Casper and Scheffler and also assumed that *C. tholiformis* is conspecific to *C. delicatus*.

S. makarovae is morphologically quite similar to the aforementioned taxa. It has, however, not been transferred to the genus *Cyclostephanos* and has had little to no recognition in taxonomic publications outside of Russia, possibly due to the fact that the original publication [9] was written in Russian. Based on the results of this study, we will also clarify the priority for the transfer of *C. delicatus* to the genus *Cyclostephanos*. Furthermore, we will use integrative morphological and molecular data from new isolates from Canada and Europe to transfer the taxon currently known as *S. makarovae* to the genus *Cyclostephanos*.

2. Materials and Methods

2.1. Sampling, Cultivation and Electron Microscopy

Table 1 lists the strains used and their relevant metadata. Water samples of 0.5–1 L were taken from each waterbody near the surface. Single cells were isolated using an inverse microscope–micromanipulator–micropipette system and clonal cultures from these cells cultivated for at least two weeks in 0.2 µm filtered mineral water enriched with 4 mL $\times$ L^{-1} f/2 medium (Guillard and Ryther 1962) and 60 mg $\times$ L^{-1} metasilicate ($Na_2SiO_3 \times 9\ H_2O$). Liquid cultures were maintained in 50–150 mL Erlenmeyer flasks at 14–18 °C in a light/dark photocycle of 14.5:9.5 h and moderate shaking. Finally, subsamples of each clonal culture were used for light microscopy (LM) and scanning electron microscopy (SEM) analyses. The rest of each culture was concentrated by centrifugation and the resulting pellets were resuspended with dH_2O in 1.5 mL tubes and stored at −20 °C for DNA analysis.

For LM and SEM, ~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. These cleaned cell suspensions were pipetted onto coverslips and glued on aluminum stubs for SEM analysis. Prepared stubs were coated with ~25 nm Au and viewed under a ZEISS Merlin VP compact SEM.

In valve view the following parameters were recorded: undulation of the valve, maximum number of areolae per fascicle at the valve-mantle junction, total diameter, number of striae, number of marginal fultoportulae, number and position of central fultoportulae and number of rimoportulae. The striae density is given as the number of striae in 10 µm circumference [10]. Internal views additionally revealed the orientation of the labium, the number of accompanying cowlings [8,11] of the marginal and central fultoportulae (satellite pores) and the structure of the cribra.

Table 1. The strains used in this study and their strain ID, localities, coordinates, collector and isolator information, and GenBank accession numbers for the respective gene loci.

Taxon	Strain	Locality	Coordinates	Collector	Isolation	D2D3 LSU	*rbc*L	*cox*1
Cyclostephanos makarovae	MIC7	Lake Mickowsee, Germany	53.70459°, 11.62498°	K. Schultz	K. Schultz	OL436661	OL493032	OL628836
	QC2	Lake Lac Saint-Augustin, Canada	46.75047°–71.39313°	O. Jacques	K. Schultz	OL436662	OL493031	OL628834
	SN29	Lake Schweriner See, Germany	53.63405°, 11.46698°	S. Schultz	K. Schultz	OL436665	OL493033	OL628837
	US5	Lake Untersee, Germany	52.94595°, 12.44498°	M. Dreßler	K. Schultz	OL436664	OL493034	OL628835
	STB1	Lake Sternberger See, Germany	53.71738°, 11.84062°	K. Schultz	K. Schultz	OL436668	OL493037	OL628832
	M13	Lake Mälaren, Sweden	59.4325°, 17.70446°	S. Gottschalk	M. Dreßler	OL436666	OL493036	OL628833
	RIE1	Lake Scharmützelsee, Germany	52.21957°, 14.02552°	M. Knie	T. Hübener	OL436663	OL493038	OL628831
	W36	River Warnow, Germany	54.04522°, 12.16332°	T. Hübener	T. Hübener	OL436667	OL493035	OL628838
Cyclostephanos invisitatus	W13	River Warnow, Germany	54.04522°, 12.16332°	T. Hübener	T. Hübener	OL436670	OL493042	OL628841
	M10	Lake Mälaren, Sweden	59.4325°, 17.70446°	S. Gottschalk	M. Dreßler	OL436671	OL493041	OL628840
	GC4	River Guadalquivir, Spain	37.86848°, 4.78506°	S. Haupt	K. Schultz	OL436672	OL493040	OL628842
	SN34	Lake Schweriner See, Germany	53.63405°, 11.46698°	S. Schultz	K. Schultz	OL436669	OL493039	OL628839
Cyclostephanos dubius	HZ1	Lake Herzsee, Austria	47.24854°, 11.45539°	E. Rott	M. Dreßler	OL436674	OL493045	OL628843
	DOL38	Lake Dolgener See, Germany	53.95012°, 12.24597°	T. Hübener	T. Hübener	OL436673	OL493044	OL628844
Cyclostephanos delicatus	QN14	St. Lawrence River, Canada	46.75062°–71.26741°	O. Jacques	K. Schultz	OL436675	OL493043	OL628845
Stephanodiscus niagarae	LD1	Lake Lac Daviault, Canada	52.81044°–67.07239°	O. Jacques	K. Schultz	OL436676	OL493051	OL628851
Stephanodiscus neoastraea	DOL10	Lake Dolgener See, Germany	53.95012°, 12.24597°	T. Hübener	T. Hübener	OL436679	OL493046	OL628848
Stephanodiscus hantzschii	CAS3	Lake Cambser See, Germany	53.68989°, 11.53306°	M. Dreßler	M. Dreßler	OL436677	OL493049	OL628846
	TO6	Lake Lago di Toblino, Italy	46.05274°, 10.96444°	K. Fink	M. Dreßler	OL436678	OL493050	OL628847
Stephanodiscus binatus	QC3	Lake Lac Saint-Augustin, Canada	46.75047°–71.39313°	O. Jacques	K. Schultz	OL436681	OL493048	OL628849
	S4	Lake Stechlinsee, Germany	53.15284°, 13.02772°	L. Krienitz	T. Hübener	OL436680	OL493047	OL628850
Pantocsekiella ocellata	RL1	Ross Lake, Ireland	53.37257°–9.21186°	U. Nitzschke	T. Hübener	OL436682	OL493052	OL628852
Lindavia sp.	DR1	Lago di Landro, Italy	46.63116°, 12.23037°	K. Fink	K. Schultz	OL436683	OL493053	OL628853

2.2. DNA Extraction, Purification, Amplification

Genomic DNA of frozen cultures was extracted using the salt-extraction technique modified after Aljanabi and Martinez [12]. For DNA-amplification the following primer combinations were used: T16N and T24U [13] for D2 and D3 regions of the large rDNA subunit (LSU), Wawrik_for and Wawrik_rev [14] for partial *rbc*L, and CoxF and CoxR [15] as well as CO1_for and CO1_rev [16] for partial *cox*1. The respective PCR programs for the primers were implemented as described in their references.

PCR products were visualized in 1.5% agarose gel and relevant bands were cut out. Gel extraction and purification of PCR products were conducted by applying an innuPREP Gel Extraction kit (Analytik Jena, Jena, Germany). Final products were sequenced using Sanger sequencing with PCR primers as sequence primers. Sequences were edited and aligned with the software BioEdit v7.2.5 [17].

The molecular phylogeny was calculated using the software Geneious 8.1.9 (Biomatters Ltd., v. 8.1.9, Auckland, New Zealand) with the add-ons for RAxML (ML) and MrBayes (BI), respectively. Both ML and BI analyses used the GTR + G + I model with four rate categories, with the –f a option with 10,000 bootstrap replicates to calculate branch support for the best-scoring tree in ML. All analyses were conducted under random seed 12354. The following settings were used for BI: runs with four incrementally heated Metropolis-coupled Monte-Carlo Markov Chains with five million generations, burn-in 1,250,000 generations, with a subsampling frequency of 1000; heated chains = 4, temp. = 0.2; random seed = 10,464. The effective sample size (ESS) value was > 200 and the trace plot indicated convergence. Introns were ignored for the phylogenetic analyses, since they contributed to artificially high differences in the resulting trees. Final tree files were edited in Powerpoint (Microsoft Office, Standard 2013, Redmond, WA, USA). The outgroups were *Lindavia* sp. and *Pantocsekiella ocellata*.

3. Results

3.1. Molecular Phylogeny

Figure 1 shows the phylogeny generated from the concatenated dataset (D2D3, *rbc*L and *cox*1). All branches had exceptionally high support, above 0.9 BI and 90 ML. Two genera, *Cyclostephanos* and *Stephanodiscus*, were recovered from the phylogeny with high support (1.00/100), with *Stephanodiscus* clearly separate from *Cyclostephanos*. *S. niagarae*, the type species for *Stephanodiscus*, fell within the *Stephanodiscus* clade. *Cyclostephanos* consisted of four clades (1.00/95)—representing *C. delicatus*, *S. makarovae*, *C. invisitatus* and *C. dubius*, with high support for all the nodes.

3.2. LM and SEM

Figures 2 and 3 (**2**–**25**) detail the morphological structures of *C. makarovae* and Figure 4 (**26**–**37**) that of *C. invisitatus;* Figure 5 (**38**–**46**) is an overview of other relevant centrics. Table 2 shows a summary of the measured morphometrical parameters of all the studied monoclonal cultures as well as references from past morphological studies on *C. makarovae* and *C. invisitatus*. The results of this study are in good agreement with the literature references (Table 2). However, looking at most parameters it becomes clear that both taxa share many morphological traits. The number of central fultoportulae, the number of areolae per fascicle at the valve-mantle junction and the density of marginal fultoportulae are basically identical between both taxa. The diameter of *C. invisitatus* is on average higher as well as the number of marginal fultoportulae, which is correlated to the diameter. However, the ranges of both parameters widely overlap in both taxa, leaving only the undulation and descriptive features such as the areolation pattern as distinguishing traits.

The number of satellite pores accompanying the marginal fultoportulae was always two for all strains in the *Cyclostephanos* clade, except for one *C. delicatus* strain (QN14), which always had three satellite pores (N = 15).

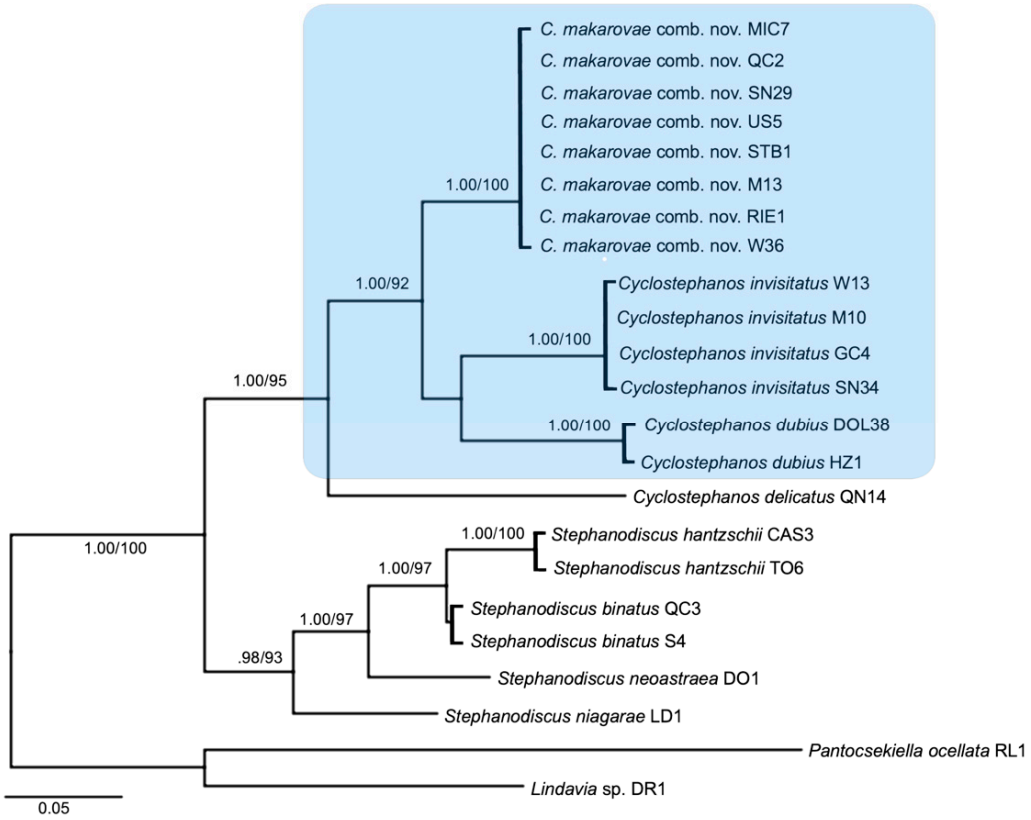

Figure 1. Concatenated gene tree of LSU D2/D3, *rbc*L and *cox*1 sequences of the dataset strains. Strains within the blue box possess 2 satellite pores marginal to the fultoportulae. Left branch support values = BI, right branch support values = ML; BI/ML branch support values over 90/50 are displayed.

Table 2. Morphometric measurements of the strains studied and summaries, compared with literature references by Genkal 2007 for *C. makarovae*, Houk et al. 2014 for *C. invisitatus* and *C. delicatus* and Håkansson and Kling 1990 for *C. delicatus*. Und = undulation (1 = flat, 2 = somewhat undulated, 3 = distinctly undulated); Arae/Fas = maximum number of rows of areolae per fascicle at the valve-mantle-junction; D = diameter in µm; S/D = striae in 10 µm circumference [10]; N MFP = number of marginal fultoportulae; N CFP = number central fultoportulae; MFP/D = marginal fultoportulae in 10 µm circumference.

Taxon	N	Strain	Und.	Arae/Fas	D	S/D	N MFP	N CFP	MFP/D
C. makarovae	22	MIC7	1–2	2	6.7–8.3	14.1–18.0	7–10	1 (–2)	3.1–4.3
	21	QC2	1–3	2 (–3)	6.0–8.6	14.3–18.0	5–8	1	2.5–3.8
	25	SN29	1–3	2 (–3)	4.9–7.0	12.9–16.6	5–7	1	2.7–3.7
	21	US5	1–3	2 (–3)	6.1–9.3	12.7–17.3	6–10	1	2.6–3.7
	22	STB1	1–3	2	7.8–10.4	13.5–16.2	7–9	1	2.5–3.3
	26	M13	1–3	2 (–3)	5.8–7.6	12.4–16.7	5–8	1	2.5–4.0
	21	RIE1	1–3	2 (–3)	6.4–9.2	12.2–16.2	6–11	1 (–2)	2.8–4.8
	24	W36	1–2	2 (–3)	5.8–7.4	13.0–17.1	6–9	1	3.0–4.4
	182	**all**	**1–3**	**2 (–3)**	**4.9–10.4**	**12.2–18.0**	**5–11**	**1 (–2)**	**2.5–4.8**
C. makarovae [18]	447			2–3	3–10	14–25			
C. invisitatus	28	W13	1	2 (–3)	9.5–10.0	14.3–16.9	8–11	1	2.9–3.7
	36	M10	1	2–3	8.9–9.7	12.5–16.1	8–11	1 (–2)	2.6–3.6
	20	GC4	1	2–3	11.3–12.2	10.6–15.0	12–16	1 (–2)	3.2–4.4
	20	SN34	1	2 (–3)	8.4–9.8	10.2–15.5	7–11	1	2.7–3.8
	104	**all**	**1**	**2–3**	**8.4–12.2**	**10.2–16.9**	**7–16**	**1 (–2)**	**2.6–4.4**
C. invisitatus [19]			1	2	6–18	9–19		1	
C. delicatus [7]			2–3	2–4	6–14	8–20		1	
C. delicatus [19]			2–3	(2–) 3–4	5–15			1 (–2)	

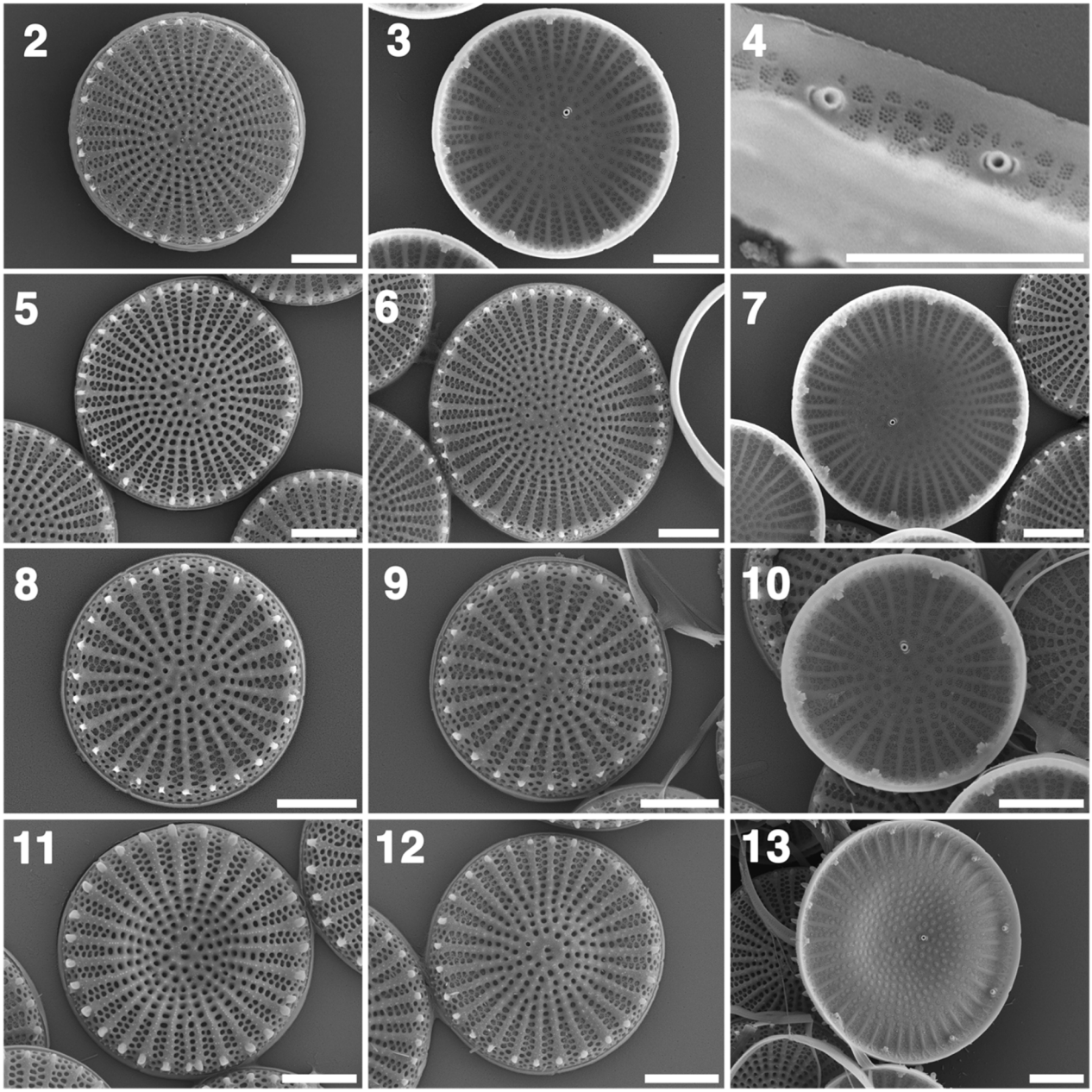

Figure 2. (**2–13**) SEM photographs of the internal and external ultrastructure of *Cyclostephanos makarovae* comb. nov. (**2–4**) strain MIC7; (**5–8**) strain QC3; (**8–10**) strain SN29; (**11–13**) strain US5. Scale bars = 2 μm.

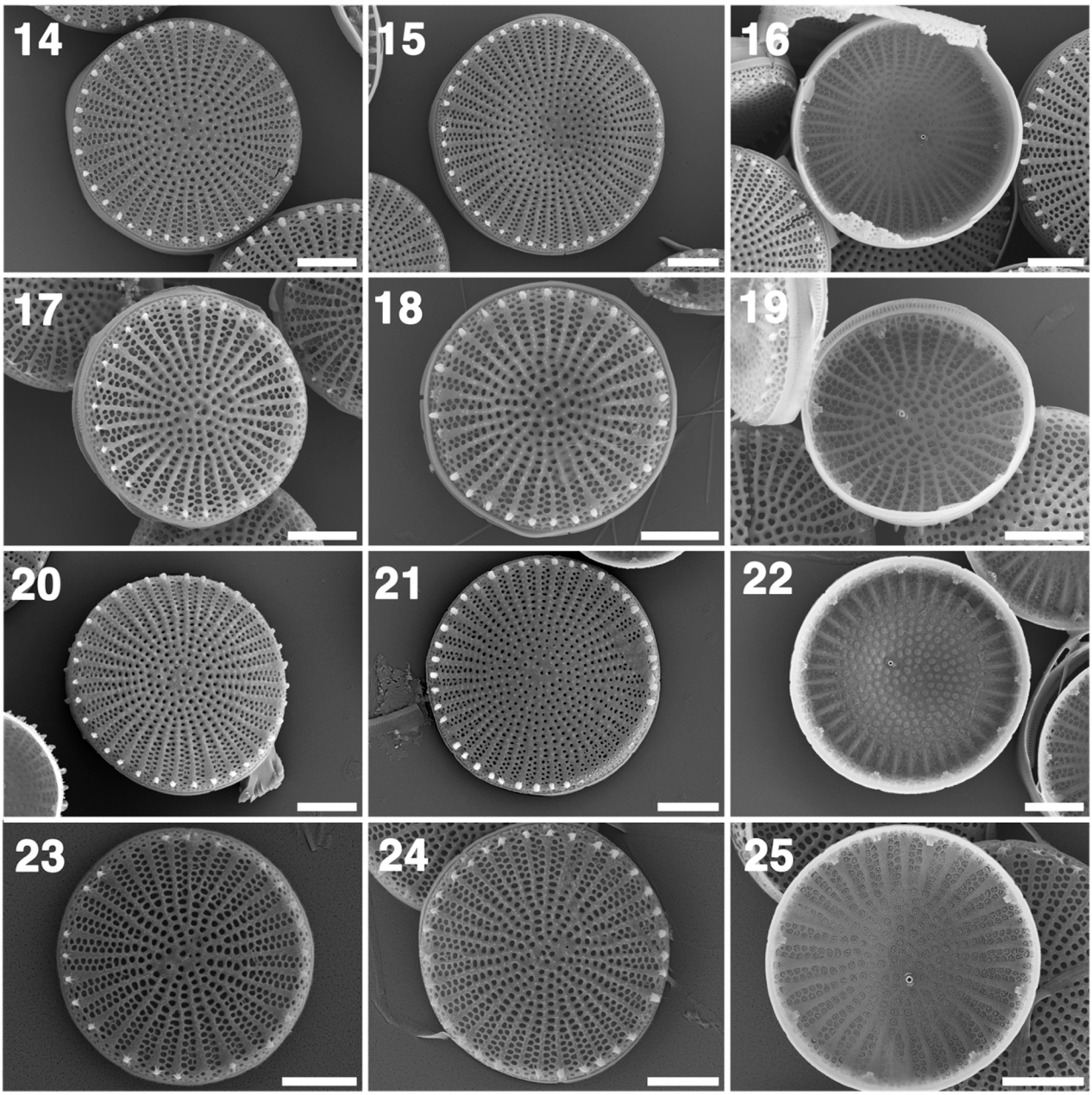

Figure 3. (**14**–**25**) SEM photographs of the internal and external ultrastructure of *Cyclostephanos makarovae* comb. nov. (**14**–**16**) strain STB1; (**17**–**19**) strain M13; (**20**–**22**) strain RIE1; (**23**–**25**) strain W36. Scale bars = 2 μm.

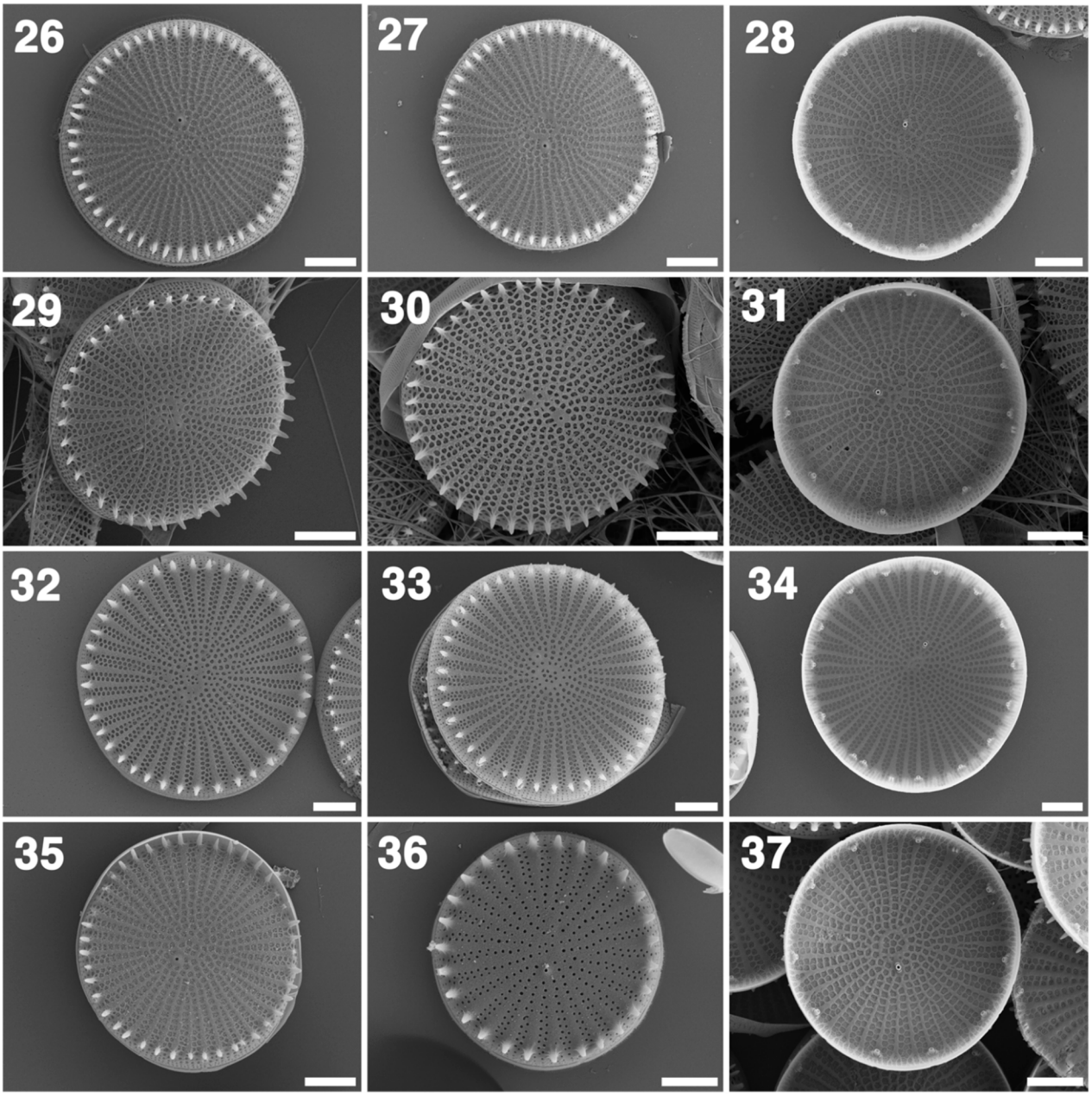

Figure 4. (**26**–**37**) SEM photographs of the internal and external ultrastructure of *Cyclostephanos invisitatus*. (**26**–**28**) strain W13; (**29**–**31**) strain M10; (**32**–**34**) strain GC4; (**35**–**37**) strain SN34. Scale bars = 2 μm.

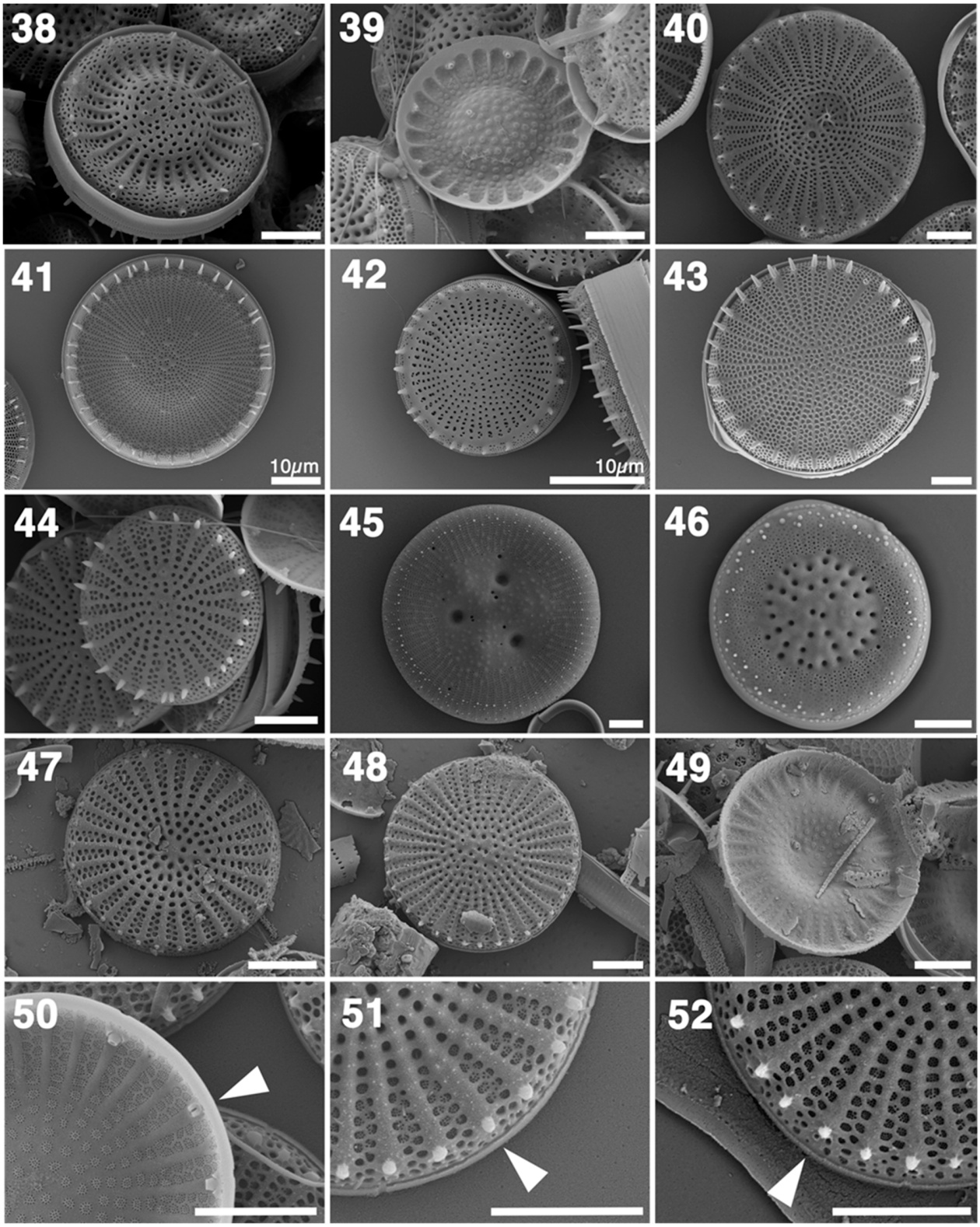

Figure 5. (**38**–**52**) SEM photographs of the internal and external ultrastructure of various strains. (**38**–**39**) strain HZ1 *Cyclostephanos dubius*; (**40**) strain QN14 *C. delicatus*; (**41**) strain LD1 *Stepha-nodiscus niagarae*; (**42**) strain DOL10 *S. neoastraea*; (**43**) strain TO6 *S. hantzschii*; (**44**) strain QC3 *S. binatus*; (**45**) strain RL1 *Pantocsekiella ocellata*; (**46**) strain DR1 *Lindavia* sp.; (**47**–**49**) *C. makarovae* from Lake Vielbecker See, Germany; (**50**–**52**) arrowheads mark the position of the internal and external openings of the rimoportula in *C. makarovae* comb. nov., (**50**–**51**) strain US5, (**52**) strain RIE1. Scale bars = 2 μm unless otherwise marked.

4. Discussion

4.1. The Transfer of S. makarovae into Cyclostephanos

Based on the results of this study we find that *S. makarovae* should be transferred to *Cyclostephanos*, as has been done with the similar taxa *S. invisitatus* and *S. delicatus* in the past. The issue first came to light when eight strains from eight distinct water bodies first identified in the light microscope as *C. invisitatus* were molecularly distinct from other *C. invisitatus* strains in culture as well as from all other sequenced *Cyclostephanos* strains (Figure 1). These strains had morphological characters of the genus *Cyclostephanos* but were yet unidentified. Upon combing the literature for a diagnosis for similar small-valved *Cyclostephanos*, a description of the taxon *S. makarovae* was discovered and fitted the strains in question well. The diagnosis was also confirmed by Genkal, who originally described the taxon (personal communication).

The concatenated three-gene phylogeny also indicates that isolates identified as *S. makarovae* are distinct from *Stephanodiscus*, including *S. niagarae*, the generitype of the genus (1.00/100), as well as other strains within *Cyclostephanos*. Furthermore, the gene locus sequences for all eight newly identified strains are identical, which strongly suggests that they belong to one taxon.

Delineating *S. makarovae* from *Stephanodiscus* requires a detailed look at their morphological features and the subsequent comparison to *Cyclostephanos*. *S. makarovae* has multiple morphological features in common with *Cyclostephanos* that are distinct from *Stephanodiscus*:

- Two satellite pores accompanying the marginal fultoportulae (Figure 2 (**2**)).
- External openings of the rimoportula are inconspicuous, not spine-like (Figure 5 (**51**–**52**)).
- Slight alveolar structures can be present (Figure 2 (**13**)).

This morphological separation is echoed by the phylogeny (Figure 1). We hence make the following taxonomic and nomenclatural transfer:

***Cyclostephanos makarovae* (Genkal) Schultz comb. nov.**

Basionym: *Stephanodiscus makarovae* Genkal 1978: Novyi vid iz roda *Stephanodiscus* Ehr. (Bacillariophyta) [New species from the genus *Stephanodiscus* Ehr. (Bacillariophyta)]. Novosti Sistematiki Nizshykh Rasteniy 15: 11–14, 2 pls. pl. I [1]: figs. 3–6; pl. II [2]: figs. 1–6. The Latin diagnosis of *S.makarovae* is on pages 13–14).

Type locality: Russian Federation, Tver region, Ivankovo Reservoir on Volga River below Tver town (old name–Kalinino).

(Genkal 1978: 11–14); Type: S.I. Genkel; ix. 1972; Inst. Biol. Aquarum Internarum Acad. Sc. URSS, Borok district, Yaroslavl Oblast (Index Nominum Algarum).

4.2. Reprioritizing the Name of C. delicatus

Because *S. makarovae* has been largely ignored outside of Russia, it can be assumed that it has been mistaken for similar taxa in the past. The example of the transfer of *S. delicatus* into *Cyclostephanos* illustrates this.

Within a week of each other in November 1990, both Casper and Scheffler and Håkansson and Kling transferred *Stephanodiscus delicatus* into the genus *Cyclostephanos*. In 2006, Dreßler and Hübener assigned priority to the transfer made by Casper and Scheffler. However, based on examination of the valve morphology of the specimen material from Casper and Sheffler, it appears to belong to *C. makarovae*, not *C. delicatus* as the depicted valves are mostly biseriate and the marginal fultoportulae have two satellite pores (also see *4.3 Comparative Morphology*). The authors themselves pointed these differences out but attributed it to intraspecific variation. Genkal, in 2007, had already come to the same conclusion that their material shows *C. makarovae* [18].

This resulted in a valid nomenclatural transfer but invalid in terms of the true identity of the specimen. Genkal [20] stated that *S. delicatus* was indeed similar to *S. makarovae* and both taxa have probably been confused on numerous occasions.

The feature that most clearly distinguishes the two taxa is the number of satellite pores associated with the marginal fultoportulae. *C. delicatus* has three while *S. makarovae*

has two. However, *C. delicatus* has a confusing history concerning the number of satellite pores: The original description [21] does not mention this feature. When *C. tholiformis* was first described by Stoermer et al. 1987 12/3/2021 8:56:00 AM [22], three satellite pores accompanying the marginal fultoportulae of this taxon were mentioned. In 1990 Håkansson and Kling amended the species description of *C. tholiformis* and pointed out that in the SEM material at least two *Cyclostephanos* species could be found: *C. delicatus* with three and *C. tholiformis* with two satellite pores.

In 2006 Dreßler and Hübener compared environmental samples containing *C. delicatus* (Lake Vielbecker See, Germany) and the type of material of *C. tholiformis* (Lazy Lagoon, USA). In both materials they found *Cyclostephanos* valves with two or three satellite pores and concluded that both taxa can have two or three. However, in the case of the *C. tholiformis* material, this had already been called into question by Håkansson and Kling 1990 as mentioned before.

In this study we re-examined the material from Lake Vielbecker a(s used by Dreßler and Hübener in 2006). Amongst *C. delicatus*, *C. makarovae* was abundant in the samples (Figure 5 (**47**–**49**)), providing a likely explanation as to why marginal fultoportulae with three, as well as two, satellite pores can be found in the material.

Our study confirms Håkansson and Kling 1990 in that all investigated valves of the *C. delicatus* strain (QN14) have three satellite pores associated with the marginal fultoportulae while all valves of all other *Cyclostephanos* taxa present in this study always had two. This is also reflected in the basal position and long branch length of *C. delicatus* in the phylogeny (Figure 1). In addition, we know of no other verified example of intraspecific variation of this feature within the Stephanodiscaceae. This example also highlights the difficulties working solely with environmental material, where single cells may be isolated and decontextualized from its true taxonomic placement.

Complicating things further, the specimen chosen by Håkansson and Kling appears to be the correct specimen matching the diagnosis of *C. delicatus*, with but not given priority in terms of publication time. To avoid further confusion, we propose to prioritize the specimen and nomenclatural transfer made by Håkansson and Kling and to designate the specimen from Casper and Scheffler to be of *C. makarovae*. Based on the reprioritization of the Håkansson and Kling 1990 specimen, the taxon *C. delicatus* will reprise its former epithet:

***Cyclostephanos delicatus* (S.I. Genkal) H. Kling and H. Håkansson in H. Håkansson and H. Kling 1990**

4.3. Comparative Morphology

In SEM the distinction between *C. makarovae* and *C. delicatus* is relatively simple. The marginal fultoportulae of *C. delicatus* are accompanied by three satellite pores, while *C. makarovae* and *C. invisitatus*, like most other *Cyclostephanos* species, have two satellite pores (Figure 2 (**4**)). Moreover, *C. delicatus* has broader striae with 3–4 areolae per fascicle (e.g., Figure 5 (**40**)) whereas *C. makarovae* and *C. invisitatus* have mostly biseriate (more rarely triseriate) fascicles (Figures 2 and 3 (**2**–**25**)). The concentric undulation is usually even more distinct in *C. delicatus*.

In contrast, *C. invisitatus* and *C. makarovae* are differentiated mainly by habitus. *C. makarovae* is mostly somewhat undulated but can also be flat (e.g., Figure 2 (**2**)) or distinctly undulated (e.g., Figure 2 (**11**)). The valve face of *C. invisitatus* is always flat (Figure 4 (**26**–**37**)). In most cases the central area of valve faces of *C. makarovae* is more strongly silicified with the areolae becoming increasingly round and small towards the center, giving *C. makarovae* a coarser appearance in SEM images. Due to this feature, a ring-like structure, sometimes referred to as annulus, in the center of the valve may infrequently be seen in *C. makarovae*, but is common in *C. invisitatus* (e.g., Figure 4 (**33**)). The fascicles in both *C. makarovae* and *C. invisitatus* are typically biseriate at the valve–mantle junction. While in *C. makarovae* triseriate fascicles occur relatively rarely, they seem to be more frequent in *C. invisitatus* (Figure 4 (**33**)) and may even be the dominating type in some valves. Furthermore, the spines of *C. makarovae* are often less developed and shorter than

those of *C. invisitatus* (e.g., Figure 3 (**23**)). A direct comparison of the strains in our study to the type of material is unfortunately not possible, because there is no type material suitable for SEM (Genkal, personal communication).

Another taxon that is potentially similar to these two is *C. tholiformis*. According to the amended description of Håkansson and Kling 1990, this taxon has, in contrast to *C. delicatus*, two satellite pores associated with the marginal fultoportulae. The taxon has often been confused with *C. delicatus* and there is generally not much data available. It could be conspecific with *C. makarovae* or *C. invisitatus* and in both cases the latter taxa would have priority. However, only judging by the micrographs available in Håkansson and Kling 1990, it could well be a distinct taxon. In contrast to *C. makarovae* and *C. invisitatus*, the striae seem to be triseriate and the interstriae are distinctly raised. More morphological and molecular data for this taxon is needed. In future investigations, morphological diagnoses should always be complemented by molecular gene sequence data for small Stephanodiscaceae.

4.4. Environmental Distribution

C. makarovae was described by Genkal 2007 [18] to be widespread throughout Russia in meso-eutrophic water bodies of 10–16 °C, with pH of 6–8.8, although valves also have been found in brackish waters of the Northern Caspian Sea. In this study the sampling sites where *C. makarovae* are found include Germany, Sweden, and Canada; it is thus possible that the taxon occurs throughout North America. It is especially common in the nutrient-rich water bodies of the German lowlands. Even considering that the ecological data on this taxon is still sparse, these conditions are so common in the northern hemisphere, that it is likely that *C. makarovae* is quite common.

C. makarovae is also found in the same water bodies as *C. invisitatus* (e.g., River Warnow, Lake Schweriner See, Lake Mälaren) hinting at similar ecological preferences and further complicating species delimitation.

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