

Resurrection of the diatom genus *Stephanocyclus* (Coscinodiscophyceae: Stephanodiscaceae) on the basis of an integrated molecular and morphological approach

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Abstract: The history of the description and subsequent consideration of the diatom genus *Stephanocyclus* Skabitschevsky (Thalassiosirales, Bacillariophyta) is discussed. The new genus was described on the basis of a new species, *S. planum* Skabitschevsky, originally collected from the River Ob. However, the genus has been more or less forgotten since its initial description in 1975. Type material from Skabitschevsky's collection has subsequently been lost. An epitype for this species is recognized from the same region as the original collection, and typified with a population from Lake Baikal and using molecular data. The population from Lake Baikal fully corresponds in terms of morphological features with the population originally investigated by Skabitschevsky. Morphology of the genus *Stephanocyclus* is discussed and, on the basis of an integrated molecular and morphological investigation, we propose to recognize the genus, including four species within it. Three taxa are transferred to the genus, including *Cyclotella cryptica*, *C. gamma* and *C. meneghiniana*. A comparison of *Stephanocyclus* with other cyclotelloid genera is given.

Key words: diatoms, *Cyclotella*, *Stephanocyclus*, molecular investigation, morphology, taxonomy

INTRODUCTION

The genus *Cyclotella* was described first as subgenus within the genus *Frustulia* by Kützing in 1833 and later elevated to generic level by Brébisson in 1838 (HOUK et al. 2010). This genus is a good example of a so-called “catch-all” taxon, to which many taxa were included on the basis of possessing circular, infrequently elliptical, valve outlines. This genus is easily recognized from other centric diatoms by having a valve face that has two different patterns of ornamentation, with a radially striated marginal part surrounded by distinctly different flat or tangentially, radially undulate central area. The central area can be smooth or with wrinkles and colliculate. Central fultoportulae may be present or absent on the valve face and rimoportulae are present on the mantle and, in some species, on the valve face (KULIKOVSKIY et al. 2016). Combining these features with the presence of spines, spinulae, granules, a striated marginal area, and alveolate striae differentiates *Cyclotella* sensu lato

from others in the family Stephanodiscaceae Glezer et Makarova, especially from *Stephanodiscus* Ehrenberg. The latter genus is characterized by having a single pattern of ornamentation, with uniseriate or biseriate striae near mantle with spines between valve face and mantle and presence central fultoportulae and rimoportulae on the mantle only (KULIKOVSKIY et al. 2016).

Using a broad taxonomical concept for the genera of centric diatoms allowed past researchers to put many taxa into the genus *Cyclotella* and this genus has included several hundred species. This level of species richness was high in comparison with the genus *Stephanodiscus* or others such as *Melosira* C.A. Agardh sensu lato (including taxa that were later included in the resurrected genus *Aulacoseira* Thwaites). So, the taxonomy of centric diatoms fully reproduced the situation seen in taxonomy of pennate diatoms. Amongst the pennate diatoms, the genus *Navicula* Bory sensu lato has had more than 50 genera separated from it, and the situation is similar with respect to the genus *Achnathes* sensu lato, which as had

more than 10 genera described and separated from it during last decades (CHUDAEV et al. 2020; KULIKOVSKIY et al. 2013, 2015, 2020a–e; RADHAKRISHNAN et al. 2020). Recognition of morphological subgroups within *Cyclotella* started with the work of LOWE (1975). Formal revision of *Cyclotella* started in 2002 (see HÅKANSSON 2002), as some new genera were described and older genera were resurrected. However, as we will show below, revision of the genus *Cyclotella* was not comprehensive. Especially, this is evident from treatment of HOUK et al. (2010), where many species were discussed under the genus *Cyclotella* sensu lato. KHURSEVICH & KOCIOLEK (2012) further divided the genus *Cyclotella* into 12 groups on the basis of morphological features, but without formal taxonomical interpretations. While molecular investigations and formal analysis are not comprehensive for centric diatoms, they have been important tools for understanding taxonomy at the generic level (see DULEBA et al. 2014; NAKOV et al. 2015; ÁCS et al. 2016).

In terms of some reconsidering of the morphological subgroups *Cyclotella* and the establishment of new genera, we can present several case studies. For example, HÅKANSSON (2002) discussed the morphology of *Cyclotella* species around *C. comta* (Ehrenberg) Kützing and its allies and suggested a new genus *Puncticulata* Håkansson. This genus is characterized by presence of circular to oblong–elliptical valves with different central area, presence of both areolae and valve face fuloportulae, or areolae only within the central area, arrangement of loculate areolae with the internal domed cribra and external foramina in short or long radial rows, presence of a complex alveolar structure composed primary thick costae internally bearing marginal fuloportulae, position of rimoportulae on the valve face surface and some others that distinguish this group from type species of the genus *Cyclotella*, *Cyclotella distinguenda* Hustedt (see HÅKANSSON 2002; HOUK et al. 2010; KHURSEVICH & KOCIOLEK 2012). HÅKANSSON (2002) also transferred to this new genus a previously described species, *Cyclotella austriaca* (M. Peragallo) Hustedt. However, HÅKANSSON (2002) did not recognize that this species was previously assigned to the genus *Handmannia* M. Peragallo in Handmann (1913). The name *Handmannia* thus had priority over *Puncticulata*, a situation recognized by HOUK et al. (2010), and KHURSEVICH & KOCIOLEK (2012) applied the name *Handmannia* to this group of cyclotelloid diatoms.

Another group of *Cyclotella* species was investigated by HOUK & KLEE (2004). They revised species around *Cyclotella stelligera* Cleve et Grunow, the so-called “stelligeroid” taxa, and suggested a new genus *Discostella* Houk et Klee on the basis of presence in the central area of the valve face of a star-shaped structure composed of alveolae or external ridges, presence in the marginal area of the alveolate striae separated by radial costae, presence of simple narrow alveolae, presence of marginal fuloportulae with 2 satellite pores internally and position of one rimoportula between two costae on

the valve margin or within an alveolar chamber (see HOUK & KLEE 2004; HOUK et al. 2010; KHURSEVICH & KOCIOLEK 2012). This genus was described without molecular data but the morphological distinction of the group is evident.

Important step in the revision of cyclotelloid taxa was prepared by NAKOV et al. (2015). These authors restored the genus *Lindavia* (Schütt) De Toni et Forti that was forgotten for a long time. Using features such as the position of rimoportula on the valve face as a synapomorphy for a group of taxa that included *Cyclotella comta* and *C. ocellata* and previously–described genera *Pliocaenicus* (Round et Håkansson 1992) emend. Khursevich et Stachura–Suchoples 2008 and *Puncticulata* / *Handmannia* allowed them to transfer all species from these taxa to the genus *Lindavia*. They pointed out that the position of the rimoportula is an important morphological feature for delimitation of genera such as *Cyclotella* sensu stricto (with rimoportula within the ring of marginal fuloportulae and located on a costa), *Discostella* (with rimoportula located within the ring of marginal fuloportulae and both types of processes are located between costae), and *Tertiarius* (now known as *Paleotertiarius* Blanco 2020) (with internal opening of the rimoportula positioned laterally on a fuloportula–bearing costa). This “synapomorphy–based” taxonomy justified by a single morphological feature was not supported by ÁCS et al. (2016). These authors, using an integrated morphological and molecular approach, suggested that the *Cyclotella ocellata* group within either *Cyclotella* or *Lindavia* sensu Nakov et al. could be raised as a separate genus. *Pantocsekiella* was described based on *Cyclotella ocellata* and its allies, which differ from other cyclotelloid diatoms by the presence of striae of unequal length and the central area without areolae. Description of *Pantocsekiella* K.T. Kiss et Ács was an example of using more narrow taxonomy for cyclotelloid centrics and separating taxa from (another) catch–all genus like *Lindavia*.

Another cyclotelloid genus, *Stephanocyclus*, was described by Skabitschevsky in 1975. *Stephanocyclus* was established on the basis of a newly–described species *Stephanocyclus planum* Skabitschevsky from the River Ob in Russia. This genus was forgotten for a long time, due in part to Skabitschevsky’s work being published in the Cyrillic alphabet (publication was prepared in Ukrainian) but also in Roman alphabet. This genus was cited and recognized by STOERMER & JULIUS (2003) who assigned *Cyclotella meneghiniana* Kützing to this genus, even though it was never formally transferred to this genus. These authors pointed that the genus *Stephanocyclus* includes species that have traditionally been classified under *Cyclotella meneghiniana* and its allies on the basis of structurally different marginal and central areas that are characteristic of *Cyclotella* and lack the two–layered wall characteristic of *Cyclotella* sensu stricto, and marginal chambers typical for *Cyclostephanos* (STOERMER & JULIUS 2003). FOURTANIER & KOCIOLEK (1999, 2011)

recognized *Stephanocyclus* as a valid, monotypic genus.

The aim of this publication is to clarify the taxonomic history of the description of the genus *Stephanocyclus* by Skabitshevsky and to understand its phylogenetic position within the Stephanodiscaeae, based on an integrated morphological and molecular approach.

MATERIALS AND METHODS

Samples. Samples from Vietnam were collected by E.S. Gusev. Samples from Ethiopia were collected by B.A. Levin (IBIWRAS). Physical and chemical water parameters were measured with a Hanna Combo (HI 98129) multiparameter probe (Hanna Instruments, Inc., USA). A list of all strains examined in this study with their GenBank accession numbers and geographic location of sampling sites with measured ecological parameters is presented in Table 1.

Preparation of microscope slides and microscope observations. Strains for LM and SEM investigations have been processed by means of a standard procedure involving treatment with 10% HCl and concentrated hydrogen peroxide ($\approx 37\%$) to dissolve organic matter. After treatment, the sample was washed with deionized water four times at 12 h intervals. Permanent diatom preparations have been mounted in Naphrax[®]. Light microscopic (LM) observations have been performed by means of a Zeiss Axiovert and Zeiss Axioscope A1 microscope equipped with an oil immersion objective (100/n.a. 1.4, DIC). Valve ultrastructure was examined with a JSM-6510LV field emission scanning electron microscope (Borok, Russia). The stubs were sputter coated with 50 nm of Au by means of a Eiko IB 3. Samples, cultures and slides are deposited in the collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia.

Culturing. A subsample of each collection was added to WC liquid medium (GUILLARD & LORENZEN 1972). Monoclonal strains were established by micropipetting single cells under an inverted microscope. Non-axenic unialgal cultures were maintained in WC liquid medium at 10 °C in a growth chamber with a 12:12 h light:dark photoperiod.

Molecular methods. Total DNA of monoclonal cultures was extracted using ChelexTM 100 Molecular Biology Grade Resin according to the manufacturer's protocol 2.2. Fragments of 18S rDNA (356–404 bp, including V4 domain), and partial rbcL plastid genes (600–772 bp) were amplified using primers D512for and D978rev from ZIMMERMAN et al. (2011) for 18S rDNA fragments and rbcL40+ from RUCK & THERIOT (2011) and rbcL1255– from ALVERSON et al. (2007) for rbcL fragments. Amplifications of the 18S rDNA fragments and partial rbcL gene fragment were carried out using the premade mix ScreenMix (Evrogen, Russia) for the polymerase chain reaction (PCR). The conditions of amplification for 18S rDNA fragments were: an initial denaturation of 5 min at 95 °C, followed by 35 cycles at 94 °C for denaturation (30 s), 52 °C for annealing (30 s) and 72 °C for extension (50 s), and a final extension of 10 min at 72 °C. The conditions of amplification for partial rbcL were: an initial denaturation of 5 min at 95 °C, followed by 45 cycles at 94 °C for denaturation (30 s), 59 °C for annealing (30 s) and 72 °C for extension (80 s), and a final extension of

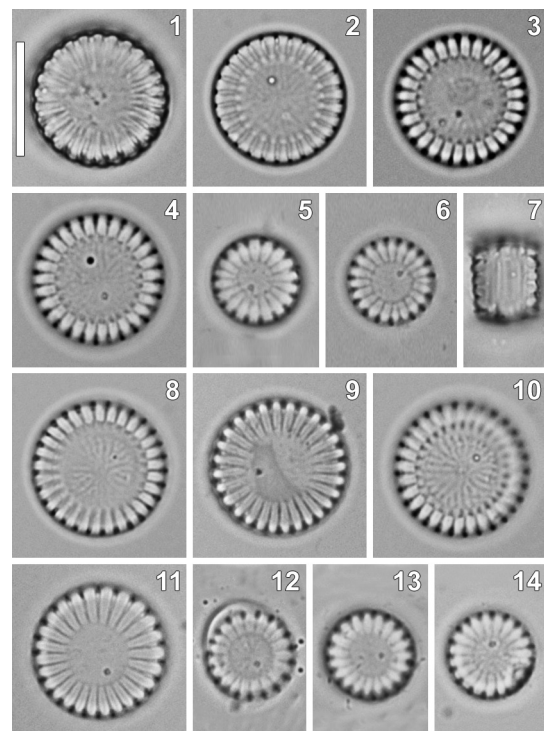
10 min at 72 °C.

The resulting amplicons were visualized by horizontal agarose gel electrophoresis (1.5 %), colored with SYBR Safe (Life Technologies, United States). Purification of DNA fragments was performed with the ExoSAP-IT kit (Affimetrix, USA) according to the manufacturer's protocol. 18S rDNA fragments and partial rbcL gene were decoded from two sides using forward and reverse PCR primers and the Big Dye system (Applied Biosystems, USA), followed by electrophoresis using a Genetic Analyzer 3500 sequencer (Applied Biosystems).

Editing and assembling of the consensus sequences were carried out by comparing the direct and reverse chromatograms using the Ridom TraceEdit program (ver. 1.1.0) and Mega7 (KUMAR et al. 2016). Newly determined sequences and DNA fragments from 98 other diatoms, which were downloaded from GenBank (taxa and Accession Numbers are given in the tree, Fig. 29), were included in the alignments. Diatom species from genera *Bellerochea*, *Ditylum*, *Helicotheca* and *Lithodesmium* were chosen as the outgroup.

The nucleotide sequences of the 18S rDNA and rbcL genes were aligned separately using the Mafft v7 software and the E-INS-i model (KATOH & TOH 2010). For the protein-coding sequences of the rbcL gene, we checked that the beginning of the aligned matrix corresponded to the first position of the codon (triplet). The resulting alignments had lengths of 410 (18S rDNA) and 1131 (rbcL) characters.

The data set was analyzed using Bayesian inference (BI) method implemented in Beast ver. 1.10.1. (DRUMMOND & RAMBAUT 2007) to construct phylogeny. For each of the alignment partitions, the most appropriate substitution model was estimated using the Bayesian information criterion (BIC) as implemented in jModelTest 2.1.10 (DARRIBA et al. 2012). This BIC-based model selection procedure selected the following models, shape parameter α and a proportion of invariable



Figs 1–14. *Stephanocyclus planum*, LM, DIC, size diminution series: (1–7) strain B012, sample 07501, (2) epitype; (8–14) strain B016, sample 07502. Scale bars 10 μ m (1–14).

Table 1. List of strains examined in this study with their GenBank accession numbers. Geographic locality of samples and measured ecological parameters indicated.

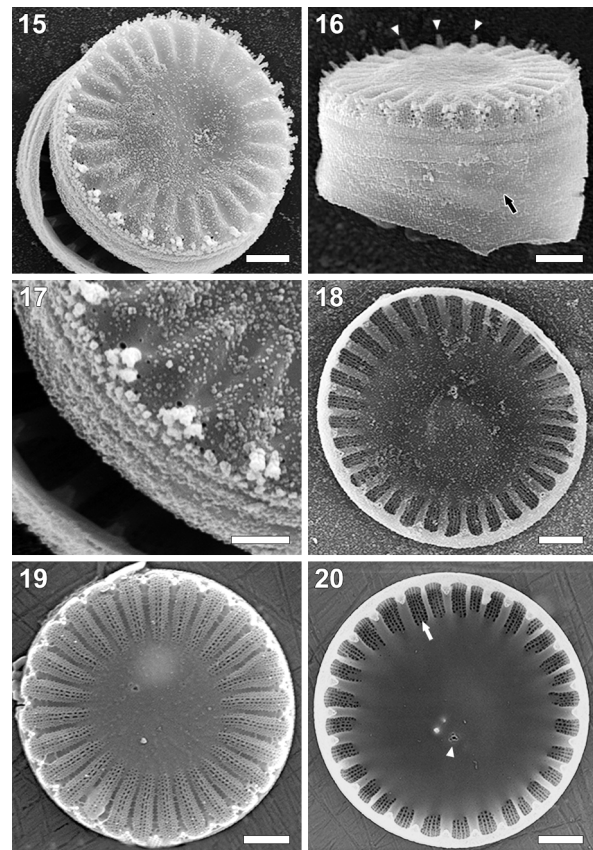
Strains	Sample No	Sample locality	Collection of date	Coordinates	t (°C)	pH	Cond. (µS.cm ⁻¹)	Substratum	GenBank accession number, SSU rDNA, partial	GenBank accession number, rbcl, partial
<i>Stephanocyclus plum B012</i>	07501	Jenhaluk, Baikal Lake, Russia	July 17, 2011	52°33'85.5"N 107°08'12.5"E	13.9	8.2	107	periphyton	MW728341	MW729352
<i>Stephanocyclus plum B016</i>	07502	Jenhaluk, Baikal Lake, Russia	July 15, 2011	52°28'99.7"N 106°57'60.1"E	15.2	8.5	120	periphyton	MW728342	MW729353
<i>Stephanocyclus meneghiniana M100</i>	07503	Moscow Botanical Garden of Academy of Sciences, Moscow, Russia	July 1, 2019	55°50'36"N 37°35'12"E	18.4	8.5	213	plankton	MW728344	MW729355
<i>Stephanocyclus meneghiniana M187</i>	06410	Moscow Botanical Garden of Academy of Sciences, Moscow, Russia	July 1, 2019	55°50'36"N 37°35'12"E	18.4	8.5	213	plankton	MW728345	MW729356
<i>Stephanocyclus meneghiniana M237</i>	07496	Beach "Levoberezhnyj", Moscow, Russia	July 1, 2019	55°52'36.9"N 37°27'51.8"E	20.7	7.9	275	plankton	MW728346	MW729357
<i>Stephanocyclus meneghiniana M238</i>	07497	Beach "Levoberezhnyj", Moscow, Russia	July 1, 2019	55°52'36.9"N 37°27'51.8"E	20.7	7.9	275	plankton	MW728347	MW729358
<i>Stephanocyclus meneghiniana M239</i>	07498	Bol'shoj Sadovyj prуд (Big Garden Pond), Moscow, Russia	July 1, 2019	55°49'56.5"N 37°32'20.6"E	20.8	7.8	281	plankton	MW728348	MW729359
<i>Stephanocyclus meneghiniana M240</i>	07499	Bol'shoj Sadovyj prуд (Big Garden Pond), Moscow, Russia	July 1, 2019	55°49'56.5"N 37°32'20.6"E	20.8	7.8	281	plankton	MW728349	MW729360
<i>Stephanocyclus meneghiniana M241</i>	07500	Beach "Levoberezhnyj", Moscow, Russia	July 1, 2019	55°52'36.9"N 37°27'51.8"E	20.7	7.9	275	plankton	MW728350	MW729361

Table 1 Cont.

<i>Stephanocyclus meneghiniana</i> Ryb8	01991	Rybinsk Reservoir, Yaroslavl oblast, Russia	August 23, 2014	58°12'22"N 38°30'58"E	23.7	7.8	253	plankton	MW728354	MW729365
<i>Stephanocyclus meneghiniana</i> Ryb10	02004	Rybinsk Reservoir, Yaroslavl oblast, Russia	August 23, 2014	58°17'03"N 38°43'33"E	23.1	7.6	251	plankton	MW728352	MW729363
<i>Stephanocyclus meneghiniana</i> Ryb14	01999	Rybinsk Reservoir, Yaroslavl oblast, Russia	August 23, 2014	58°23'00"N 38°16'49"E	23.5	7.6	252	plankton	MW728353	MW729364
<i>Stephanocyclus meneghiniana</i> Efl	05429	On road awash-mile hot spring, Ethiopia	March 30, 2016	58°42'59"N 38°16'12"E	23.5	7.8	251	plankton	MW728343	MW729354
<i>Stephanocyclus meneghiniana</i> Mmp198	03200	Bay in the eastern part of the Bayan Lake	July 13, 2015	48°26'24"N 95°13'73"E	10	8.5	260	periphyton	MW728351	MW729362

sites (pinvar): TIM3+I+G, $\alpha=0.6620$ and pinvar=0.4590 for 18S rDNA gene; TrN+I+G, $\alpha=0.2520$ and pinvar=0.6330 for the first codon position of the rbcL gene; F81+G, $\alpha=0.1920$ for the second codon position of the rbcL gene; TVM+I+G, $\alpha=0.8130$ and pinvar=0.1360 for the third codon position of the rbcL gene.

We used the HKY model of nucleotide substitution instead of TIM3 and TrN, the GTR model instead of TVM, given that they were the best matching model available for Bayesian inference. A Yule process tree prior was used as a speciation model. The analysis ran for 15 million generations with chain sampling every 1000 generations. The parameters—estimated convergence, effective sample size (ESS) and burn-in period were checked using the software Tracer ver. 1.7.1. (DRUMMOND & RAMBAUT 2007). The initial 25% of the trees were removed, the rest retained to reconstruct a final phylogeny. The phylogenetic tree and posterior probabilities of its branching were obtained on the basis of the remaining trees, having stable estimates of the parameter models of nucleotide substitutions and likelihood. Maximum Likelihood (ML) analysis was performed using the program RAxML (STAMATAKIS et al. 2008). The nonparametric bootstrap analysis with 1000 replicates was used. The statistical support values were visualized in FigTree ver. 1.4.4 and Adobe Photoshop CC (19.0).



Figs 15–20. *Stephanocyclus planus*, SEM: (15–18) strain B012, sample 07501, (15–17) external valve view, black arrow shows the girle bands, white arrowheads show the spines, (18) internal valve view; (19, 20) strain B016, sample 07502, (19) external valve view, (20) internal valve view, white arrow shows completely open alveoli, white arrowhead shows the central fuloportula. Scale bars 2 μm (15, 16, 18–20), 1 μm (17).

RESULTS

Typification of the genus

***Stephanocyclus* Skabitschevsky emend. Kulikovskiy**

Type species: *Stephanocyclus planum* Skabitschevsky
Lectotype (designated here): Fig. 1 in Ukr. Bot. Zhurn. 32. 1975.

Locus typicus: Novosibirsk reservoir on Ob River, Russia. In plankton.

Epitype (designated here): Slide no MW-D 07501 deposited in Diatom collection of the Department of Mycology and Algology, Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia. Fig. 2 illustrates the epitype.

Locus epitypicus: Lake Baikal, Russia.

Reference strain: B012 (see Figs 1–7, 15–18) from the culture collection of laboratory of molecular systematics of the aquatic plants (IPP RAS); molecular data for

18S V4 and *rbcL* of this strain is available under the NCBI Accession numbers MW728341 and MW729352 respectively.

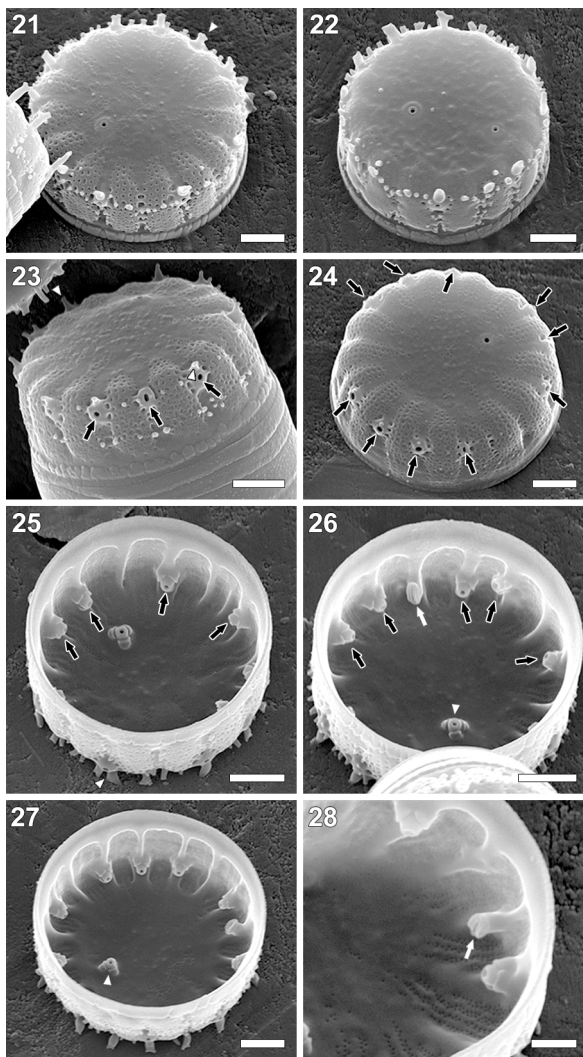
Representative specimens: list of all samples and strains examined in this study presented in Table 1.

Morphology of *Stephanocyclus planum* Skabitschevsky emend. Kulikovskiy, Genkal et Kociolek (Figs 1–28)

Cells cylindrical, circular, mostly solitary. Striation very coarse (Figs 1–28). Internally, alveoli are completely open (Fig. 20, white arrow). Externally, marginal fultoportulae are located on almost every costa with a circle of external openings with evident stellate reinforced bases (see description below) (Figs 23, 24, black arrows). Internally each marginal fultoportula has a short central tube surrounded by three satellite pores (Fig. 25, 26, black arrows). Spines can be inserted slightly below the valve face in mantle (Figs 16, 23, 25, white arrowheads) on each striae or rarely absent (Fig. 24). A single rimoportula is present within the ring of marginal fultoportulae, with a slit-like external opening. Internally, the rimoportula is present as a stalked labium, prominent and easily visible, slit is slightly oblique (Figs 26, 28, white arrows). Central area is slightly transversally undulate, appearing smooth. Central fultoportulae number one to two. Internally central fultoportulae present as short central tube with three satellite pores (Figs 20, 26, 27, white arrowheads). The marginal and central parts are indistinctly separated due to missing centripetal occlusions of internal alveoli. The girdle bands are smooth with several copulae (Figs 16, black arrow).

The population from Lake Baikal closely resembles the description of the species by Skabitschevsky. He described his new species as being 13–29 µm in diameter and having 5.0–7.3 striae per 10 µm. He noted that the new species is characterized by possessing visible spines. Our population from Lake Baikal is characterized by being 3.7–26.0 µm in diameter and having 5–15 striae per 10 µm. The smaller valves having a larger number of striae than described for a natural population in the River Ob by Skabitschevsky can be explained by the presence of small valves during long cultivation in culture (see Figs 21–28). In cultured specimens, we also note that valves may be without spines.

Molecular analysis (Fig. 29): The Stephanodiscaceae is shown to be subdivided into two distinct groups or clades. The first clade includes the freshwater genera *Stephanodiscus*, *Cyclostephanos*, several cyclotelloid genera (such as *Lindavia*, *Pantocsekiella*, *Discostella*) as well as some mostly estuarine–marine taxa. These include several distinct genera such as *Shionodiscus*, *Detonula*, *Bacterosira*, *Roundia* and *Skeletonema*, as well as a clades of taxa currently assigned to the genus *Thalassiosira*. The second distinct clade of taxa in the Stephanodiscaceae is comprised of several species of *Thalassiosira*, species of *Cyclotella* (*Cyclotella* sp., *C. choctawhatcheana*, *C. atomus* and *C. striata*). An independent branch includes *Cyclotella distinguenda*,



Figs 21–28. *Stephanocyclus planum*, SEM, strain B012, sample 07501: (21–24) external valve view, white arrowhead shows the spine, black arrows show the marginal fultoportulae; (25–28) internal valve view; note the marginal fultoportulae (black arrows), rimoportula (white arrow), and the central fultoportula (white arrowhead). Scale bars 1 µm (21–27), 0.5 µm (28).

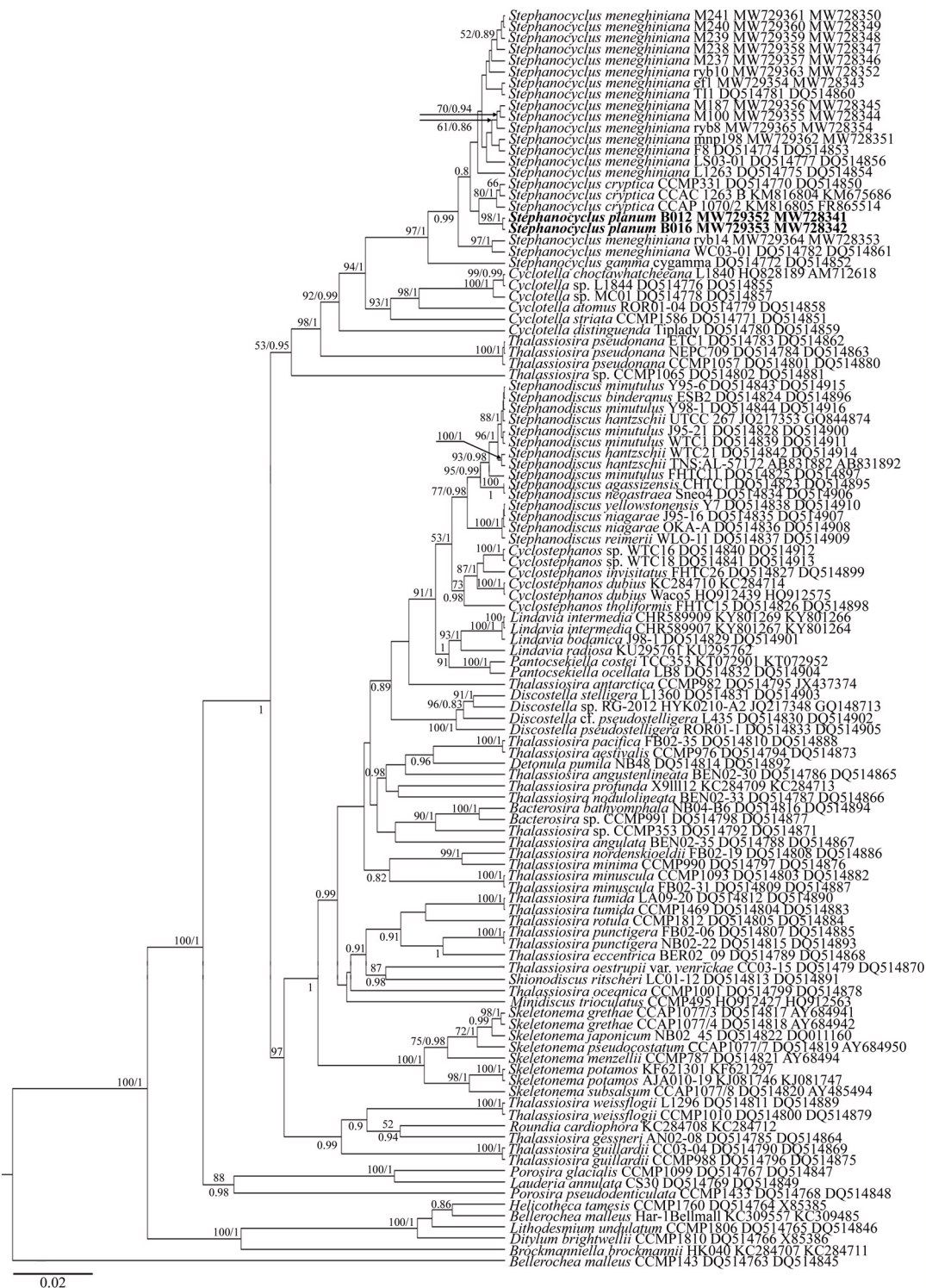


Fig. 29. Phylogenetic position of *Stephanocyclus planum* Skabitshevsky emend. Kulikovskiy, Genkal & Kocielek (indicated in bold) based on Bayesian analysis of 112 partial rbcL and partial 18S rDNA sequences of 1541 characters. Values above the horizontal lines are bootstrap support from ML analyses (<50 are not shown); values below the horizontal lines are Bayesian posterior probabilities (<0.8 are not shown). All sequences have strain numbers (if available) and GenBank numbers. Species from genera *Bellerrochea*, *Ditylum*, *Helicotheca* and *Lithodesmium* were used as an outgroup.

the type species of the genus *Cyclotella*, and taxa whose valve morphologies suggest their inclusion in the genus *Stephanocyclus*.

Of the taxa included in *Stephanocyclus*, there is first a branch that includes *Cyclotella gamma* (recognized

in the tree as *S. gamma*), two strains of *C. meneghiniana* (recognized as *S. meneghiniana*) and then the two strains of *Stephanocyclus planum* from Lake Baikal, which form a clade together (ML 98; BI 100). This branch is sister to branch with three strains of *Stephanocyclus cryptica*.

Table 2. Comparison of *Stephanocyclus* with other cyclotelloid genera.

Features	<i>Stephanocyclus</i>	<i>Cyclotella</i> s. str.	<i>Discostella</i>	<i>Lindavia</i>	<i>Paleotertiarius</i>	<i>Pantocskiella</i>	<i>Pliocenicus</i>
Central fulloportulae	+	-	-	+	+	+	+
Number of satellite pores of the central fulloportulae	3	-	-	3	2	2	2
Position of marginal fulloportulae	on the costae between the valve face and mantle	on the costae in the middle part of mantle	between the costae	on each costa	on the costae	on the costae in marginal part of the valve face	on the costae
Position of rimoportula	on a costa within the ring of marginal fulloportulae	on a costa within the ring of marginal fulloportulae	between costae and within the ring of marginal fulloportulae	on the valve face	laterally on a fulloportula-bearing costa	in the submarginal zone on the costa or just below it	in the middle or in the submarginal zone of the valve face

The other fifteen strains considered in this analysis form a monophyletic group. They were previously identified as *Cyclotella meneghiniana* (in the tree they are recognized as “*Stephanocyclus meneghinianum*”).

DISCUSSION

SKABITSCHESKY (1975) described a new genus with a new species *Stephanocyclus planum* from River Ob in Siberia, Russia. According to his opinion the genus *Stephanocyclus* differs from the *Cyclotella meneghiniana* by the presence of spines at the valve margin in his new genus. SKABITSCHESKY (1975) pointed that *Cyclotella meneghiniana* does not have spines. According to SKABITSCHESKY (1975) the new genus *Stephanocyclus* is phylogenetically closer to the genus *Stephanodiscus* based on the shared feature of presence spines at the valve margin. So, SKABITSCHESKY (1975) choose for the main morphological feature present of spines in the valve face near the mantle to distinguish his monotypic genus from other cyclotelloid diatoms and to align it with *Stephanodiscus*. However, presence of spines is a common feature for *Cyclotella meneghiniana* and many other species from the genus *Cyclotella* (see HOUK et al. 2010). Proposal of a new genus without evident morphological features was one reason why this genus not recognized for a long time in our opinion. Based on the description provide by SKABITSCHESKY (1975) and his discussion of the new genus and species it was evident that his new species belongs to the *Cyclotella meneghiniana* species complex.

This genus was forgotten for a long time in Russian literature (Soviet before 1991) and not used in foreign publications. HOUK et al. (2010) did not mention Skabitschessky’s genus in his comprehensive investigation of centric diatoms. However, *Stephanocyclus* was recognized by STOERMER & JULIUS (2003). However, they erroneously mentioned the name *Stephanocyclus meneghiniana* Skabitschessky, a formal combination never proposed and, in Skabitschessky’s opinion not closely related to his new genus and species. STOERMER & JULIUS (2003) recognized Skabitschessky’s new genus and differentiated it from other cyclotelloid genera. They noted it having two distinct patterns of ornamentation, like other cyclotelloid groups, but lacking the two-layered wall characteristic of *Cyclotella sensu stricto*, and the marginal chambers typical for *Cyclostephanos* (STOERMER & JULIUS 2003).

The type species of the genus *Cyclotella* is *Cyclotella distinguenda*. This species was thoroughly investigated by HOUK et al. (2010) using type material and material collected from a natural lake in Russia by GENKAL et al. (2019). Based on these two studies on *C. distinguenda*, we can make detailed comparisons between *Cyclotella sensu stricto* and *Stephanocyclus*. This species, as the type for the genus *Cyclotella*, is characterized by

Table 3. Morphological and morphometric comparisons among four species of *Stephanocyclus*.

Taxon	Valve diameter	Number of striae in 10 µm	Number of central fultoportulae	Reference
<i>Stephanocyclus planum</i>	13–29	5–7.3	2(3–4)	SKABITSHEVSKY (1975) This study
	3.7–26	5–15	1–2	
<i>Stephanocyclus cryptica</i>	5–25	6–10	1–5	REIMANN et al. (1963)
<i>Stephanocyclus gamma</i>	18–33	5–7	–	SOVEREIGN (1963) HOUK et al. (2010)
	6–50	5–9	1–5	
<i>Stephanocyclus meneghiniana</i>	5–45	6–10	1–3	HÅKANSSON (2002) HOUK et al. (2010)
	5–60	6–9(10)	1–9	

absence of central fultoportulae (a feature that is present in species of the genus *Stephanocyclus*). Rimoportulae in *Stephanocyclus* are characterized as mainly robust (bigger than in *Cyclotella* s. str.) processes. Striae in *Stephanocyclus* are multiseriate with more than 2 or 3 areolae. The valve mantle is fully perforated by smaller areolae without evident striae and interstriae as is seen in *Cyclotella*. External openings of the rimoportula and mantle fultoportulae are situated between the valve face and mantle but not in middle part of mantle as seen in *Cyclotella*. An interesting feature for many species from the genus *Stephanocyclus* is the more complicated morphology of external openings of marginal fultoportulae which have evident stellate reinforced bases. These bases are organized with an elevated ring of marginal fultoportulae or small ribs between pores in the mantle (see HOUK et al. 2010, tab. 144: 4). This structure was mentioned by Stoermer & Julius (2003) as marginal chambers typical for *Cyclostephanos*. Mantle fultoportulae are present almost on all ribs in *Stephanocyclus*, but in *Cyclotella* they occur on every 3 or more ribs.

All of the morphological features described above differentiate the genus *Stephanocyclus* not only from the genus *Cyclotella* but also from other cyclotelloid genera such as *Discostella*, *Paleotertiarius*, *Pantocskiella*, *Lindavia*, *Pliocaenicus*. The genus *Discostella* is characterized by a stelligeroid central area and presence of marginal fultoportulae and rimoportulae between marginal ribs. Marginal fultoportulae are present not in every rib and central fultoportulae are absent. *Paleotertiarius* is characterized by marginal region with stalked rimoportulae situated internally on the side thickening of a rib inside an alveolar opening and having a narrow slit oriented nearly radially. Central fultoportulae are several, each with two satellite pores (HOUK et al. 2010). *Lindavia* as a genus is characterized by the presence of several radiating rows of alveolar cribra and many central fultoportulae with three satellite pores. Rimoportulae are several and present as short-stalked processes situated in the marginal part of valve face. Marginal fultoportulae are present on each rib and many ribs have two marginal fultoportulae present on one rib. *Pantocskiella* as a genus is characterized by the presence of a few or many central

fultoportulae with two satellite pores and rimoportulae in marginal part of valve face. Marginal fultoportulae with two satellite pores are present but not on each rib. *Pliocaenicus* as a genus is characterized in having many small central fultoportulae and marginal fultoportulae positioned on the mantle under ribs; one rimoportula can be found near the mantle (Table 2).

Based on our interpretation of the results in Figure 29, we conclude that the genus *Stephanocyclus* is a monophyletic group and can be recognized and differentiated from all other cyclotelloid groups. Based on these results, we propose the following new transfers and combinations in the genus:

***Stephanocyclus cryptica* (Reimann, Levin et Guillard) Kulikovskiy, Genkal et Kociolek comb. nov.**

Basionym: *Cyclotella cryptica* Reimann, Lewin et Guillard 1963 in Reimann et al. *Cyclotella cryptica*, a new brackish–water diatom species. Phycologia, 3(2): p. 82; figs 4–11.

***Stephanocyclus gamma* (Sovereign) Kulikovskiy, Genkal et Kociolek comb. nov.**

Basionym: *Cyclotella gamma* Sovereign 1963. New and rare diatoms from Oregon and Washington. Proceedings of the California Academy of Sciences, Series 4. 31(14): p. 350, figs 1, 2.

***Stephanocyclus meneghiniana* (Kützing) Kulikovskiy, Genkal et Kociolek comb. nov.**

Basionym: *Cyclotella meneghiniana* Kützing 1844. Die Kieselschaligen Bacillarien oder Diatomeen. 50, pl. 30: fig. 68.

Morphological and morphometric comparison of four species of *Stephanocyclus* is presented in Table 3.

Recognition of *Stephanocyclus* as a distinct genus as proposed here has the implication that, based on the results presented herein, *Cyclotella*, comprised of the type species of the genus, as well as *C. gamma*, *C. atomus*, *C. striata* and *C. choctawhatcheeana* and *Cyclotella* sp., is non-monophyletic. More species need to be studied with molecular tools to assess the diversity in the genus and if further dissection of this taxon is warranted. A

further result of this work is that while authors may continue to use the term ‘cyclotelloid’ to refer to those centric diatoms of the Stephanodiscaceae that have two patterns of ornamentation on the valve face, in fact there are genera to whom that term has been applied that are distantly related to one another. “Cyclotelloid” refers to a grade of morphological organization, not a monophyletic clade of closely-related species.

Another result of our analysis is that recognition of *S. planum* as a distinct species renders strains given the species epithet “meneghiniana” as non-monophyletic. *Cyclotella meneghiniana* was a single, cosmopolitan taxon for a long time (KULIKOVSKIY & KUZNETSOVA 2014). However, BESZTERI et al. (2005, 2007) carried out morphometric and molecular-genetic analyses of widely distributed strains referred to as *C. meneghiniana*. It was shown that this species is a group of cryptic taxa. Our comprehensive material also shows that *C. meneghiniana* is not a single, natural group and can be divided into several species (KULIKOVSKIY et al. in prep.).

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