Phylogenetic analysis places Spicaticribra within Cyclotella

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A strong consensus has emerged that taxonomic classifications should be based on an underlying phylogenetic hypothesis. According to this view, named groups should be monophyletic, ensuring that a name uniquely matches the evolutionary history and biological attributes of a group of taxa. As originally conceived, the diatom genus Cyclotella is a large and morphologically diverse assemblage of taxa that we now know consists of several distantly related lineages. Considerable progress has been made in placing these lineages into different monophyletic genera. The genus Spicaticribra was originally described as monotypic and has features that suggest a close relationship to Cyclotella, but it has also retained some ancestral features that appear to differentiate it from Cyclotella. We sequenced two nuclear and two plastid genes to resolve the phylogenetic position of Spicaticribra and show that it is embedded within a clade that includes the type species of Cyclotella and, further, that maintaining Spicaticribra renders Cyclotella non-monophyletic. We transfer Spicaticribra species into Cyclotella, resolve related nomenclatural issues, and caution against using ancestral characters and character states for taxonomic classification.

Keywords: monophyly, monotypic, phylogeny, taxonomy, Thalassiosirales

Introduction

Among the 820 accepted species names in the Thalassiosirales, nearly half of them have been classified as either Thalassiosira Cleve (193) or Cyclotella Brébisson (170) (AlgaeBase, accessed 12 February 2021, Guiry & Guiry 2010). These numbers reflect similar and longstanding taxonomic challenges presented by these two genera. Both of them encomapss a broad range of morphological diversity, and their ‘defining’ morphological features include characters that, in some cases, appear to have been present since the origin of the Thalassiosirales. The use of ancestral characters for classification can lead to the bloating of taxonomic groups over time due to the inclusion of distantly related species. This situation applies to Thalassiosira, a large genus that phylogenetic analyses have shown to be polyphyletic (Alverson et al. 2007). Efforts to diagnose and name clades within the broadly and loosely defined Thalassiosira (Alverson et al. 2006, Stachura-Suchoples & Williams 2009) have left the remaining species polyphyletic, which we view as a transitional state of progress toward establishing a natural classification of this genus.

The challenge of Cyclotella is somewhat simpler because although it, too, has accumulated a large set of morphologically diverse species, decades of research have revealed an information-rich set of characters that have made it easier to first delineate phenetic morphological groups (e.g. Lowe 1975) and later to distinguish between ancestral and derived character states. As a result, many extant species can now be classified into one of several monophyletic genera that formerly fell under the Cyclotella umbrella. Examples include Discostella Houk & Klee (Houk & Klee 2004), Lindavia (Schütt) De Toni & Forti (Nakov et al. 2015), and Cyclotella sensu stricto (Alverson et al. 2011). Many challenges remain, however. For example, it can be hard to classify fossil taxa that have a mix of ancestral and derived character states (Stone et al. 2020), and the failure to make this distinction can lead to descriptions of new genera that make existing ones non-monophyletic.

Spicaticribra Johansen, Kociolek & Lowe is one such genus. It was first described as monotypic and was defined by the following diagnostic characters: continuous ‘spicate’ cribra on the interior valve face, absence of central strutted processes, and absence of external extensions of the marginal strutted processes (Johansen et al. 2008). Johansen et al. (2008) suggested that the lack of distinct regions on the valve face appeared to place it outside Cyclotella, whereas the presence of continuous cribra
indicated a closer relationship to *Thalassiosira*, another polyphyletic genus. Without a home for the newly discovered type species, *S. kingstonii* Johansen, Kociolek & Lowe, it was placed into its own genus. Other species were later placed into *Spicaticribra*, and the online nomenclatural database, AlgaeBase, lists a total of 10 *Spicaticribra* species (accessed 12 February 2021, Guiry & Guiry 2010).

We collected and cultured *S. kingstonii* from its type locality. Phylogenetic analyses of four DNA markers place *S. kingstonii* within *Cyclotella* sensu stricto. In an effort to preserve the phylogenetic integrity of *Cyclotella*, we transfer *S. kingstonii* and other *Spicaticribra* into *Cyclotella*.

**Methods**

We collected near-surface phytoplankton with a 10 μM mesh plankton net from the Tuckasegee River, North Carolina, USA, (35.439933, −83.55145) on 24 April 2017. We isolated individual cells with a micropipette and grew them at 21°C in WC medium (Guillard & Lorenzen 1972). We cleaned clonally cultured cells with nitric acid and rinsed them with deionized water until the solution reached a neutral pH. Cleaned cells were transferred onto coverslips and allowed to evaporate overnight before permanently affixing them onto microscope slides with Naphrax®. We identified and photographed cells at 600× magnification with a Zeiss compound microscope. For scanning electron microscopy, cleaned cells were dried onto 12 mm diameter coverslips and coated with 15 nm of iridium with a Cressington 208 Bench Top Sputter Coater (Cressington Scientific Instruments, Watford, UK). Scanning electron micrographs were taken with a Zeiss SUPRA 40 VP scanning electron microscope (Carl Zeiss Microscopy, Thornwood, NY, USA).

We collected live cells from a single clonal culture by centrifugation, vortexed them with 1.0 mm glass beads, and extracted DNA with a Qiagen DNeasy Plant Kit. We sequenced two nuclear (*SSU* and partial *LSU* rDNA) and two plastid (*rbcL* and *psbC*) genes. Primers, PCR conditions, and Sanger sequencing followed Alverson et al. (2007). We added *Spicaticribra* sequences to gene alignments from Alverson et al. (2011), using SSU-ALIGN version 0.1.1 (Navrocki et al. 2009) to align rDNA sequences with the covariance models included in the program for the *SSU* alignment, and a heterokont-based covariance model for the *LSU* alignment (Nakov et al. 2014). We removed poorly aligned sections of the alignment with SSU-MASK using the default settings, which retains columns with a Bayesian Posterior Probability of 0.95 of being correctly aligned. Multiple sequence alignment of the *psbC* and *rbcL* plastid genes was performed manually in AliView version 1.25 (Larsson 2014). We used trimAl version 1.4 (Capella-Gutiérrez et al. 2009) to remove alignment columns with gaps in more than 20% of the sequences. We concatenated sequences for all four genes into a single alignment with AMAS (Borowiec 2016) and used IQ-TREE version 1.6.4 (Nguyen et al. 2015) to reconstruct phylogenetic relationships. The concatenated sequences were partitioned by gene, and the best-fit substitution model for each partition was inferred using the ModelFinder algorithm implemented in IQ-TREE. We inferred the maximum-likelihood tree using the edge-linked partition model in IQ-TREE and applied the TIM2+F+R3 model to the *LSU* partition, TN + F + R3 to *SSU*, GTR + F + I + G4 to *psbC*; and the GTR + F + R4 model to the *rbcL* partition. Branch support was assessed with 100,000 bootstrap replicates using Ultrafast Bootstrap Approximation (Hoang et al. 2018) and the setting ‘-bnni’ to guard against overestimation of branch support.

Newly generated DNA sequences are available from the National Center for Biotechnology Information’s GenBank database under accession numbers MW327042, MW327043, MW326755, and MW326756. Multiple sequence alignments and tree files are available from a Zenodo online repository (10.5281/zenodo.4313346).

**Results**

We collected and cultured *S. kingstonii* from the Tuckasegee River, USA, which is part of the same riverine/reservoir system as Fontana Lake, the type locality for this species and for the genus (Johansen et al. 2008). Light microscope images confirmed the identity as *S. kingstonii* (Figs 1–2). Phylogenetic analyses of two nuclear and two plastid genes placed *S. kingstonii* as sister to a clade that includes *Cyclotella distinguenda* Hustedt, the type species of *Cyclotella*, and other *Cyclotella* species (Fig. 3). *Cyclotella nana* Hustedt was sister to the *Spicaticribra + Cyclotella* clade (Fig. 3). By separating *C. nana* from the remaining *Cyclotella*, *Spicaticribra* renders *Cyclotella* non-monophyletic.

**Discussion**

A strong consensus has emerged that taxonomic classifications should be natural, meaning that named groups are monophyletic, which ensures that a name corresponds to a shared, unique evolutionary history among the taxa bearing that name (Kociolek et al. 1989, Williams & Kociolek 2011, Kociolek & Williams 2015). A great deal of progress has been made in using phylogenetic trees and phylogenetic character interpretations to subdivide the Thalassiosirales into monophyletic genera. Most of this work, and much of the work that remains, concerns the two largest genera, *Cyclotella* and *Thalassiosira*. Molecular phylogenies have facilitated these efforts, but a phylogenetic tree is a hypothesis that can change, along with the classification that accompanies it, as new taxa are discovered (Kociolek & Williams 2015). A natural genus-level classification can also be disrupted in unintended ways by the discovery of new taxa without a clear generic affiliation. This is especially problematic when character interpretations of
Phylogenetic analysis places *Spicaticribra* within *Cyclotella*. New and challenging taxa are often placed into monotypic genera, either because of ambiguity about their relationship to other genera (Johansen et al. 2008), because they exceed some arbitrary threshold of difference to known genera (Williams 2009, 2013), or to intentionally communicate uncertainty about their relationship to other taxa (Williams 2013, Stone et al. 2020). Without ‘evaluation of all available evidence in terms of monophyly and synapomorphy’ (Williams & Kociolek 2011, p. 51), a newly described monotypic genus can make existing ones non-monophyletic, thereby undermining the *primum non nocere* principle that seems to underlie many of the guiding principles laid out by Kociolek & Williams (2015), an approach we see as having greatly improved our approach to diatom systematics and classification.

*Spicaticribra* was defined originally by the presence of a ‘spicate’ pattern of continuous cribra internally, absence of central strutted processes, and absence of external extensions of the marginal strutted processes (Johansen et al. 2008). Unless phylogenetic analyses demonstrate derived secondary loss of a character, we agree that character absences generally do not provide compelling evidence in support of a genus (Kociolek & Williams 2015), so we focus here on the internal cribra. Prasad et al. (2011) noted similarities between the cribral pattern of *Spicaticribra* and many *Conticribra* and *Thalassiosira* species (Prasad et al. 2011, Khursevich & Kociolek 2012). One possible subdivision of the continuous cribra character is whether a stria consists of multiple parallel rows of areolar pores (e.g. *T. gessneri* Hustedt and *T. livingstoniorum* Prasad, Hargraves & Nienow) or single pores evident in *S. kingstonii*, some *Conticribra guillardii* (Hasle) Stachura-Suchoples & Williams, and some *C. guillaumii*. Another possible distinguishing feature of *Spicaticribra* is that the internal cribra do not align with external openings of the areolae (Johansen et al. 2008).

The diagnosis of *Spicaticribra* was later broadened by Khursevich & Kociolek (2012) to include the following characters: loculate areolae with continuous or semicontinuous cribra and external foramina; plicated valve face, or not; one or more labiate processes that extend outwardly from the frustule, or not; strutted processes with 2–4 satellite pores that extend outwardly from the frustule, or not; absence of strutted processes on the valve face, or rarely not. By relaxing the required absence of central strutted processes and external extensions of the strutted processes, and allowing for presence or absence of other characters, the amended definition captures genera and species from across nearly all of the *Thalassiosirales*. Several species were identified as candidates for transfer to *Spicaticribra* under this new definition (Khursevich & Kociolek 2012), including *T. lacustris* (Grunow) G.R.Hasle, *T. gessneri*, *C. guillaumii*, *C. weissflogii* (Grunow) Stachura-Suchoples & Williams, and *C. nana* – a group of taxa whose common...
Phylogenetic position of *Spicaticribra kingstonii* based on maximum likelihood analysis of two nuclear and two plastid genes. Ultrafast bootstrap support values > 80% are shown. The grey box delimits the *Cyclotella* clade, including *S. kingstonii*. Black dots show a polyphyletic set of taxa that have been transferred into *Spicaticribra* since its inception. For simplicity, outgroups are not shown, and some monophyletic genera were collapsed into a single branch. Genus names are abbreviated as follows: *Porosira* (P), *Lauderia* (L), *Conticribra* (Co), *Thalassiosira* (T), *Cyclotella* (Cy), *Detonula* (D), *Minidiscus* (M).

A large body of research, dating back to early applications of the scanning electron microscope for describing frustule morphology, has highlighted the importance of strutted process ultrastructure in understanding the phylogeny (Theriot & Serieyssol 1994, Shiono 2000) and classification of the Thalassiosirales (Fryxell & Hasle 1979, 1980). *Spicaticribra* has a strutted process ultrastructure not found outside of the cyclotelloid and cyclostephanoid lineages of the Thalassiosirales (Alverson et al. 2007). The strutted processes of *Spicaticribra*, which feature robust cowlings and broad satellite pore covers (Fig. 4), resemble those of many *Cyclotella* species (e.g. *C. distinguenda*) (Fig. 5) and clearly indicate a closer relationship with...
et al. (2008) noted similarities between Thalassiosira these species than any Thalassiosira (Figs 6–7). Johansen et al. (2008) noted similarities between S. kingstonii and T. pseudonana. Phylogenetic analyses placed T. pseudonana/C. nana as sister to a clade of Cyclotella species, compelling resurrection of its original name, Cyclotella nana (Alverson et al. 2011). These results also helped circumcribe a monophyletic Cyclotella sensu stricto. Although ultimately discounted, comparisons of S. kingstonii to T. pseudonana/C. nana were prescient, as phylogenetic analyses of both morphological (Alverson et al. 2011) and molecular (Fig. 3; Tuji et al. 2012) datasets have shown that S. kingstonii falls in the middle of the grade between C. nana, C. distinguenda (the generitype), and the rest of Cyclotella – a result that we again recovered with our analyses of S. kingstonii from its type locality (Fig. 3). In short, Spicaticribra has rendered Cyclotella non-monophyletic, a result that closely matches one of the hypothetical consequences of describing monotypic genera without reference to a phylogenetic hypothesis (Kociolek & Williams 2015).

Two options are available for preserving monophyly of Cyclotella: retain Spicaticribra and place C. nana into a monotypic genus, or include Spicaticribra in Cyclotella (Fig. 3). The nomenclatural history of C. nana has been highly unstable, which has led to confusion about the biology and natural history of this important model species (Alverson et al. 2011). The placement of C. nana in Cyclotella dates back to its original description by Hustedt (1957), who correctly diagnosed its placement in Cyclotella despite the limited information available to him at the time. A solution to the conflict resulting from the creation and later expansion of Spicaticribra should prioritize historical precedence, taxonomic continuity, and maintain recent progress in stabilizing the taxonomy of Cyclotella. To this end, we recommend that Spicaticribra should not be recognized as a separate genus, and we therefore transfer the following species to Cyclotella:

**Cyclotella inlandica** M.L. Julius, K.M. Downey, E.C. Theriot, et A.J. Alverson comb. nov.

Basionym: Thalassiosira inlandica Hayashi in Hayashi & al. 2007, A fossil freshwater Thalassiosira, T. inlandica sp. nov. (Bacillariophyta), with semicircular cibra and elongated marginal fultoportulae. Phycologia, 46: 354, figs 2–40.

**Cyclotella kingstonii** M.L. Julius, K.M. Downey, E.C. Theriot, et A.J. Alverson comb. nov.

Basionym: Spicaticribra kingstonii J.R. Johansen, Kociolek & R.L. Lowe 2008, Spicaticribra kingstonii, gen. nov. et sp. nov. (Thalassiosirales, Bacillariophyta) from Great Smoky Mountains National Park, U.S.A. Diatom Research, 23: 369, figs 1–24.

**Cyclotella kodaikanaliana** M.L. Julius, K.M. Downey, E.C. Theriot, et A.J. Alverson comb. nov.

Basionym: Spicaticribra kodaikanaliana B. Karthick et al., 2011, Four new centric diatoms (Bacillariophyceae) from the Western Ghats, South India, Phytotaxa 22:29, figs 3 and 4.

**Cyclotella tanaka** M.L. Julius, K.M. Downey, E.C. Theriot, et A.J. Alverson nom. nov.

Replaced synonym: Stephanodiscus minutus Pantcsek 1889, Beiträge zur Kenntniss der fossilen Bacillarien Ungarns. II Thiel: Brackwasser Bacillarien. Nagy-Tapolcsány. Buchdruckerei von Julius Platzko, p.114, tafel XII, fig. 213.

Note 1: The replacement name Cyclotella tanaka is proposed for Stephanodiscus minutus/Spicaticribra minuta because its transfer to Cyclotella would create a later homonym to Cyclotella minuta (Skvortsov) Antipova 1956 (ICN Art. 53.1, also ICN Art. 11.8 Note 5 – ‘in accordance with Art. 53, later homonyms are illegitimate whether the type is fossil or non-fossil’).

Note 2: The species is renamed after Dr. Hiroyuki Tanaka in honour of his many research contributions involving the Thalassiosirales.

**Cyclotella rudis** M.L. Julius, K.M. Downey, E.C. Theriot, et A.J. Alverson comb. nov.

Basionym: Thalassiosira rudis P.I. Tremarin, T.A.V. Ludwig, V. Becker et L.C. Torgan in Ludwig et al., 2008, Thalassiosira rudis sp. nov. (Coscinodiscophyceae): A new freshwater species. Diatom Research 23:391, figs 1–57.
Additional taxa have been transferred to *Spicaticribra* that either do not possess the diagnostic characters of *Cyclotella* or lack sufficient illustration in the literature to determine if the transfer was warranted. These include:

**Spicaticribra guillardi** (Hasle) Khursevich *et* Kociolek

Note: Should be maintained as *Conticribra guillardi* (Hasle) K.Stachura-Suchoples *et* D.M.Williams because its marginal strutted processes do not conform with those expressed in *Cyclotella*.

**Spicaticribra weissflogii** (Grunow) Khursevich *et* Kociolek

Note: Should be maintained as *Conticribra weissflogii* (Grunow) Stachura-Suchoples *et* D.M.Williams because its marginal strutted processes do not conform with those expressed in *Cyclotella*.

**Spicaticribra kamszatica** (Lupikina) Khursevich *et* Kociolek

Note: Should be maintained as *Thalassiosira kamszatica* (Lupikina) Lupikina *et* Khursevich because sufficient illustrations do not exist in literature to accurately diagnose this taxon.

**Spicaticribra kilarskii** (Kaczmarska) Kociolek *et* Khursevich

Note: Should be maintained as *Thalassiosira kilarskii* I.Kaczmarska because sufficient illustrations do not exist in literature to accurately diagnose this taxon.

**Spicaticribra nevadica** (Khursevich & VanLandingham) Khursevich *et* Kociolek

Note: Should be maintained as *Thalassiosira nevadica* Khursevich *et* VanLandingham because its marginal strutted processes do not conform with those expressed in *Cyclotella*.

**Spicaticribra patagonica** (N.Maidana) Kociolek *et* Khursevich

Note: Should be maintained as *Thalassiosira patagonica* N.I.Maidana because its marginal strutted processes do not conform with those expressed in *Cyclotella*.

**Spicaticribra tricircularis** (Stachura-Suchoples *et* D.M.Williams) Kociolek *et* Khursevich[1]

Note: Should be maintained as *Conticribra tricircularis* Stachura-Suchoples *et* D.M.Williams because its marginal strutted processes do not conform with those expressed in *Cyclotella*.

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