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Lagosinema tenuis gen. et sp. nov. (Prochlorotrichaceae, Cyanobacteria): a new brackish water genus from Tropical Africa

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**Abstract:** A novel filamentous, nonheterocytous cyanobacterium was isolated from Lagos Lagoon, Nigeria. The isolate was <3.0 µm wide, untapered, with small rounded polar bodies (aerotopes or cyanophycin granules) visible at the crosswalls, and consequently fit the morphological description of *Limnothrix planctonica*. Although morphologically inseparable from that species, it was molecularly distant from that taxon, with genetic identities between the two taxa ranging 90.73–92.49%, a degree of separation typical of different genera. Both taxa, as well as *Limnothrix rosea*, are phylogenetically in the Prochlorotrichaceae, distant from the type species of *Limnothrix*, *L. redekei*, which is in the Pseudanabaenaceae. The isolate is herein described as *Lagosinema tenuis* gen. et sp. nov.

**Key words:** 16S rRNA phylogeny, 16S–23S ITS, cyanobacteria, Lagos Lagoon, *Limnothrix*, Nigeria, phytoplankton, polyphasic approach, Synechococcales, taxonomy

**INTRODUCTION**

There are over 300 genera of cyanobacteria in current use, of which over 50 have been described since 2000 (Komárek et al. 2014). Most of the genera described before 2000 were found in Europe and North America and are contained in the seminal review works of Gomont (1892), Bornet & Flahault (1886–1888), and Geitler (1932). These reviews are by nature of the cyanobacteria in temperate and subpolar climates, and they were broadly used by phycologists throughout the world to identify taxa outside of the geographic region from which they were described (e.g. Setchell 1924; Yoneda 1938a, 1938b, 1939, 1940, 1941; Ley 1947a, 1947b, 1948; Chu 1952; Desikachary 1959; Smith et al. 2015). Recently, many workers have posited that using European cyanobacterial literature for identification of tropical and subtropical collections on other continents fails to recognize cyanobacterial diversity, and a call has been made for modern study of cyanobacterial taxa outside of northern hemisphere temperate climate regions.

Studies of cyanobacteria using a polyphasic approach have led to discovery of novel species and genera in tropical regions. For example, a particularly active cyanobacterial research group in Brazil have described a number of new genera from the Mata Atlantica and other parts of Brazil, including *Brasilonema* Fiore et al. (2007), *Cyanoaggregatum* Werner et al. (2008), *Ophiothrix Sant’Anna* et al. (2010), *Streptostemon Sant’Anna* et al. (2010), *Cephalothrix da Silva Malone* et al. (2015), *Halotia Genuário* et al. (2015), *Alkalinema Vaz* et al. (2015), *Pantanalinema Vaz* et al. (2015), *Dapisiothemon Hentschke* et al. (2016), *Komarekiella Hentschke* et al. (2017), *Potamolinea Martins & Branco* (2016), and *Phyllonema Alvarenga* et al. (2016). All of these new genera were described just in the past decade, and there is little evidence to suggest that many new additional genera will not be described from this part of the world in the near future. In the tropical Hawaiian Archipelago, four new genera were described from just five caves on the island of Kauai: *Goleter Miscoe* et Johansen, *Kovacikia Miscoe*, *Pelatocladus Johansen & Vaccarino*, and *Stenomitos Miscoe* et Johansen (Miscoe et al. 2016). We can expect that other tropical areas, which may not yet have experienced researchers employing a combination of morphological and molecular methods, will yield similar new discoveries.
Of the tropical regions of the world, the continent of Africa is particularly in need of modern floristic and taxonomic studies. Many floristic lists have been compiled using some of the classical literature (e.g. Geitler 1932; Prescott 1962; Desikachary 1959; VanLandingham 1982; Whitford & Schmacher 1984), but since these lists do not have illustrations, it is difficult to confirm the cyanobacterial identifications given (Nwankwo & Akinson 1988; Nwankwo & Onyema 2003; Kadiri, 2006; Ekwu & Sikoki 2006; Onyema 2008; Adesalu & Nwankwo 2010; Onuoha et al. 2010; Akagha & Nwankwo 2015; Smith et al. 2015; Akagha 2017). To our knowledge, researchers have not yet undertaken molecular studies of cyanobacteria of tropical Africa.

The Lagos Lagoon is a large coastal ecosystem east of the city of Lagos, Nigeria, a city of 20 million people. It is part of the Lagos Lagoon Complex that spreads to the west (Lagos, Epe, Lekki, Kuramo, Mahin lagoons) as well as to the west (Yewa, Badagry, Ologe, Iyagbe lagoons). Several of these lagoons have received floristic study and published algal species lists (Lagos Lagoon—Nwankwo 1988, Akagha 2017; Epe Lagoon—Nwankwo 1998a, Akagha 2017; Lekki Lagoon—Adesalu & Nwankwo 2010; Kuramo Lagoon—Nwankwo et al. 2008; Ologe Lagoon—Onuoha et al. 2010; Iyagbe Lagoon—Onyema 2008). Lagos Lagoon has been especially studied due to the anthropogenic impacts from the city of Lagos, Nigeria, a city of 20 million people. Lagos Lagoon is a lotic system, its trophic state varies over the period of a year based on the degree of flushing during wet and dry seasons.

As part of the ongoing study of Lagos Lagoon, we sampled diverse sites in the lagoon during August 2014, and made a number of cyanobacterial isolations. One of our isolates bore a resemblance to Limnothrix Meffert in the possession of polar bodies resembling aerotopes, but did not fit any described species in that genus. Upon sequencing the 16S rRNA gene, it was discovered that this tropical brackish water species does not belong to Limnothrix, but rather to an as yet undescribed genus and species. The purpose of this paper is to describe this genus and species, which we will refer to hereafter as Lagosinema tenuis gen. et sp. nov., based on the polyphasic approach, and thus begin the modern characterization of the cyanobacterial flora of tropical West Africa.

### Materials and Methods

**Site Description.** Lagos Lagoon is shallow, brackish and the largest of the nine lagoons located in south–west Nigeria. Fresh water input from adjoining wetlands, creeks and small first–order streams as well as sea water incursion through the channel to the sea, associated with the semi–diurnal tidal regime, are important contributors to the hydrological conditions of Lagos Lagoon. Observable horizontal and vertical environmental gradients exist along the length of the lagoon because of microtidal dynamics. Domestic, municipal and industrial waste effluent empty into the lagoon through storm water channels. Human activities within the Lagos Lagoon vicinity include; indiscriminate dumping of waste, artisanal fishing, dredging, illegal sand mining, and inland water transportation.

**Sampling, Isolation and Characterization.** Water samples were collected from the Okobaba wood waste waste effluent empty into the lagoon through storm water channels. Human activities within the Lagos Lagoon vicinity include; indiscriminate dumping of waste, artisanal fishing, dredging, illegal sand mining, and inland water transportation. Water samples were collected from the Okobaba wood waste deposition part of the Lagos Lagoon (N 6°29.462’, E 3°23.810’) for cyanobacteria isolation. Isolations were carried out using the capillary isolation method under a compound microscope. Isolates were established in ESAW medium (Birgisi et al. 2001), maintained at 25°C, illuminated under a 12:12 h light–dark cycle with an average photon flux density of 20 mmol photons.m⁻².s⁻¹ (warm white fluorescent lamps). Strains were characterized using an Olympus BH–2 photomicroscope with Nomarski DIC optics and an Olympus DP25 camera system with CellSens software. At least 50 trichomes and 50 cells were examined to obtain cell dimension. Cultures were examined at 2 weeks and 3 weeks after transfer.

**Molecular characterization.** DNA was isolated from unialgal cultures using a phenol–chloroform protocol that was modified for this study. Unialgal cultures (20 ml) were harvested in mid to late exponential phase by centrifugation (5,000 rpm for 10 min at room temperature) in a sterile 50 ml centrifuge tube. This was followed by three freeze–thaw cycles, a process that involved alternating freezing in liquid nitrogen, thawing at room temperature, and simultaneously grinding using mortar and pestle to break the cell wall to release cell contents for further enzymatic lysis. The cells were suspended in 0.5 ml TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and transferred into fresh 2 ml tubes. Thereafter, 1% SDS and 50 μl of 50 mg.ml⁻¹ lysozyme were added for enzymatic cell wall lysis. The samples were immediately incubated at 70°C for 15 min to degrade the protein. The mixture was then centrifuged at 12,000 rpm for 10 min at room temperature and the supernatant transferred to a fresh 1.5 ml centrifuge tube. An equal volume of Tris phenol was added and centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was transferred into a 1.5 ml centrifuge tube. The nucleic acid was thereafter isolated by adding an equal volume of phenol: chloroform: isooamyl alcohol (25:24:1, Sigma–Aldrich, St. Louis, Missouri) and then centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was transferred into a fresh tube and twice the volume of iced absolute ethanol stored at −20°C was added. The mixture was frozen at −80°C for 30 min, centrifuged at 15,000 rpm for 30 min at 10°C and the supernatant discarded. The DNA pellet was washed by adding 1 ml of cold 70 % ethanol, centrifuged at 12,000 rpm for 10 min at 4°C and the supernatant was discarded. The DNA pellet was air dried before being suspended in 100 μl TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

Two separate polymerase chain reaction amplifications were conducted. The first amplified a long partial sequence of the 16S rRNA gene, using primers 27F and 1492R (Lane 1991). The amplified products were run in a Takara PCR thermal cycler (Takara Holdings, Kyoto, Japan) using the following PCR cycle: 94°C for 5 min followed by 34 cycles of: denaturation temperature of 94°C for 30 s, annealing temperature of 55°C for 30 s, and elongation temperature of 72°C for 90 s; ending in an additional 5 min elongation at 72°C, and finally an indefinite hold at 16°C. The amplified product was directly sequenced. The second set of PCR amplifications recovered a smaller fragment of the 16S rRNA gene and the full 16S–23S ITS region using primer VF1 and primer VF2 sensu Boyer et al. (2001, 2002), based on the original primers of Wilmotte et al. (1993) and Nübel et al. (1997). The amplified products were run
in a C1000 Thermocycler (BIORAD) using the following PCR cycle: 95 °C for 5 min followed by 35 cycles of: denaturation temperature of 95 °C for 1 min, annealing temperature of 57 °C for 45 s, and elongation temperature of 72 °C for 4 min; ending in an additional 5 min elongation at 72 °C, and finally an indefinite hold at 4 °C. All PCR products were visualized on 1% agarose gel and cloned into the pSC–A–amp/kan plasmid of the StratataClone PCR Cloning kit (La Jolla, California, USA). The clones were isolated using QIAprep Spin kit from QIAGEN (Venlo, The Netherlands) with elution in 50 μl of sterile water. The presence of an insert was confirmed by EcoRI digestion. Plasmids containing inserts were sent for sequencing to Functional Biosciences, Inc. (Madison, Wisconsin, USA). We used the sequencing primers M13 forward, M13 reverse and internal primers 3, 5 and 8 (BOYER et al., 2001, 2002).

**Bayesian Inference analysis** was conducted with MrBayes XSDE V3.2.6 (RONQUIST et al. 2012) through the CIPRES Science Gateway, applying the GTR+G+I model of nucleotide substitutions. A total of 38 million generations were run. The Bayesian analysis had an estimated sample size (ESS) exceeding 920 for all parameters (average ESS ranging 975–20,550), well above the average of 200 typically accepted as sufficient by phylogeneticists (DRUMMOND et al. 2006). The final average standard deviation of split frequencies was 0.010 The potential scale reduction factor (PSRF) value for all the estimated parameters in the Bayesian analysis was 1.00, indicating that convergence of the MCMC chains was statistically achieved (GELMAN & RUBIN 1992).

Maximum Likelihood (ML) analysis was run using RAxML–HPC v.8 on XSEDE available through the CIPRES Science Gateway v. 3.3. Maximum Likelihood was run using the GTR+I+G model, with 1000 bootstrap replicates. Maximum Parsimony (MP) analysis was run using PAUP (SWOFFORD 1998), version 4b.10, with a heuristic search with steepest=yes, multtrees=no, swap=TBR, nreps=1000, with a subsequent bootstrap analysis with nreps=1000.

Trees were viewed using FigTree (RAMBAUT 2007). The Bayesian Inference analysis was reported herein with bootstrap values for the ML and MP analysis mapped on to the BI tree. The final composite tree was re–drawn using Adobe Illustrator CS5.1. P–distance of 16S sequences was determined in PAUP to reveal similarity among our strains of interest (SWOFFORD 1998).

### Results

**Lagosinema Akagha et Johansen gen. nov.**

**Description:** Filaments solitary, lacking sheath material. Trichomes motile, without branching, straight to curved, flexuous, thin, less than 3 μm in diameter. Hormogonia formed by simple fragmentation without necridia. Cells nongranular or with colored elongated granules, with polar bodies resembling aerotopes typically at crosswalls, with parietal thylakoids.

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Fig. 1. *Lagosinema tenuis* LM photomicrographs. Arrows: bluish and brownish crystalline bodies. Scale 10 μm.
**Type species:** Lagosinema tenuis Akagha et Johansen sp. nov.

**Etymology:** Lagosinema = Lagos—site of origin + nema (GR)—filament.

*Lagosinema tenuis* Akagha et Johansen sp. nov. (Fig. 1, 2)

**Description:** Thallus in young cultures a thin mat coating the walls and bottom of culture vessel, later forming a floating mat, consistently pale blue–green. Sheaths always absent. Trichomes motile, straight to sinuous, flexuous, not coiled, without false branching, not constricted to slightly constricted at the crosswalls, 2–2.5 µm wide. Hormogonia form by simple fragmentation, without necridia, as short as a single cell in length. Cells nongranular or possessing elongate bluish and brownish granules, often with polar bodies resembling aerotopes at the crosswalls, with parietal thylakoids which are detectable in some cells as the presence of peripheral chromatoplasm, dividing by simple binary fission and achieving full length before next division, not in meristematic zones, 3.2–7.5 µm long. Apical cells cylindrical with rounded apices, not different in length or morphology from internal cells.

**Type Locality:** Lagos Lagoon, Okobaba (wood waste dump site), N 6° 29.462', E 3° 23.810', in brackish (15–20‰) water, sampled August 2014.

**Holotype here designated:** BRY37768, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Brigham Young University, Provo, Utah.

**Reference strain:** NGPC151

**Etymology:** tenuis (L) = thin, named for thin width of trichomes.

**Analysis of Molecular Data**

In all analyses conducted, Lagosinema was sister to a large clade of strains assigned to Limnothrix sp., *L. planctonica* (Woloszyńska) Meffert, and *L. redekei* (Goor) Meffert (Fig. 3). This clade of putative *Limnothrix* strains is well studied, and many have been illustrated and found to contain small polar aerotopes (Zhu et al. 2012). The reference strain for *L. redekei*, the type species for *Limnothrix*, is PCC 9416 (=SAG 3.89, originally isolated by Marie–Elisabeth Meffert from Eastholstein), and the short 16S rRNA partial sequence associated with this strain is most similar (100% identity) to the *L. redekei* strains in the Pseudanabaenaceae at the base of our phylogeny, CCAP 1443/1 and NIVA–CYA 227/1 (Fig. 3). This indicates that the *L. planctonica* clade of Zhu et al. (2012) contains strains that cannot belong to *Limnothrix* sensu stricto, and they will need to be transferred into another, new genus at some future time.

*Limosochara rosea* Meffert IAM–M220 falls outside of *Limnothrix* sensu stricto, *L. planctonica*, and *Lagosinema tenuis*, and is consequently in a genus separate from these three taxa. *Lagosinema*, *L. planctonica*, and *L. rosea* strains all fall into a family level clade that includes Prochlorothrix Burger–Wiersma, Stal et Mur, Halomicronema Abed, Garcia–Pichel et Hernández–Maríné, and Nodosilinea (Z. Li et J.Brand) Perkerson et Casamatta (1.0 BI posterior probability, 83% ML bootstrap support). *Prochlorothrix* was described under the International Bacteriological Code of Nomenclature and placed in the family Prochlorotrichaceae (Burger–Wiersma et al. 1989). We consequently place *Lagosinema* in the family Prochlorotrichaceae.

The p–distance based on 16S rRNA gene between *Lagosinema tenuis* and all members of the *L. planctonica* clade was 0.0751–0.0927 (Table 1), or 90.73–92.49% genetic identity. This level of sequence similarity is well below the 94.5% identity level most recently recommended as a cut-off value justifying recognition of separate bacterial genera (Stackebrandt & Goebel 1994; Stackebrandt & Ebers 2006; Kim et al. 2014; Yarza et al. 2014). It was not more similar to any other set of sequences in the Prochlorotrichaceae. The low genetic identity alone provides sufficient evidence to separate *Lagosinema* from all other Prochlorotrichaceae.

We recovered two ribosomal operons from *L. tenuis* NGPC151 based on the differences noted in the 16S–23S ITS. The ITS regions of the two operons had percent dissimilarity = 10.8%, a level high enough to rule out PCR error. The D1–D1’ and Box–B helices of the two operons were identical in structure, even with a few nucleotides different in both (Figs 4 A, 5 A, B). The V3 helices differed in structure and sequence within strain NGPC151 (Fig. 6 A, B). The ITS structures
Table 2. Nucleotide lengths for conserved domains in the 16S–23S ITS region of Lagosinema tenuis, closest relatives in the Prochlorotrichaceae (L. planctonica, P. hollandica, H. hongdechloris, L. rosea, Prochlorotricaceae Gollwitz, and Nodosilinea nodulosa), and more distantly related Pseudanabaenaceae (Limnothrix redekei). For operons lacking the tRNA\textsubscript{Ala} gene, the spacer following the tRNA\textsubscript{Ile} gene extends to the Box B helix. 

**Table 1. Comparison of percent genetic identity based on 16S rRNA gene sequence among taxa phylogenetically related to Lagosinema tenuis.** Cyanobium NGPC10GR and Synechococcus MBIC10598 are representatives of the Nodosilinea/Halomicronema group in Fig. 6. Oscillatoria rosea is an earlier synonym of Limnothrix rosea.

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<tr>
<th>Strain</th>
<th>Lagosinema tenuis NGPC151</th>
<th>Limnothrix B14</th>
<th>Limnothrix planctonica NGPC10/37</th>
<th>Limnothrix planctonica JQ004021</th>
<th>Limnothrix planctonica JQ004025</th>
<th>Limnothrix planctonica GQ859647</th>
<th>Synechococcus NGPC10GR</th>
<th>Synechococcus MBIC10598</th>
<th>Prochlorothrix hollandica</th>
<th>Limnothrix rosea IAM–M220</th>
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Table 2. Nucleotide lengths for conserved domains in the 16S–23S ITS region of Lagosinema tenuis, closest relatives in the Prochlorotrichaceae (L. planctonica, P. hollandica, H. hongdechloris, L. rosea, Prochlorotricaceae Gollwitz, and Nodosilinea nodulosa), and more distantly related Pseudanabaenaceae (Limnothrix redekei). For operons lacking the tRNA\textsubscript{Ala} gene, the spacer following the tRNA\textsubscript{Ile} gene extends to the Box B helix. H. hongdechloris lacked a D3 consensus sequence. We are unsure that the L. planctonica sequences were complete at the 3’ end of the sequence available.
in Lagosinema were very different from those in other Prochlororichaeaceae, another indication of evolutionary distance (Figs 4–6). Of special note was the presence of only a single tRNA gene (tRNA\(^{\text{Ala}}\)) in the ITS region. This pattern occurs in other cyanobacteria, but it is rarer than either two tRNA genes or none. The sister taxon, _L. planctonica_, likewise had only had the tRNA\(^{\text{Ala}}\) gene, but all other Prochlororichaeaceae had both tRNA\(^{\text{Leu}}\) and tRNA\(^{\text{Ala}}\) (Table 2).

The Prochlororichaeaceae had a number of unusual patterns in the ITS region. *Halomicronema hongdechloris* Chen, Li, Birch et Willows lacked a D3 region (consensus sequence 5′–GGTTCC–3′, after the D2 region and before the tRNA\(^{\text{Leu}}\) gene, see Johansen et al. 2017), the only instance we know of this absence in cyanobacteria. *Limnothrix planctonica, L. redekei*, *H. hongdechloris*, and *N. nodulosa* (Z. Li et J. Brand) Perkerson et Casamatta all lacked a D5 helix (Table 2). This lack of the D5 occurs only very seldom in the cyanobacteria. The D1–D1′ helix and spacer following tRNA\(^{\text{Ala}}\) in *Prochlorothrix* and the V3 helix in *Lagosinema* and *Prochlorothrix* were unusually long (more than 100 nt each, see Table 2).

**Discussion**

In the original description of *Limnothrix*, Meffert (1988) transferred three species into the new genus: Oscillatoria redekei Goor, Oscillatoria planctonica Woloszyńska, and Oscillatoria rosea Utermöhl. The taxonomic transfer was the conclusion of a long series of papers on these thin, aerotrope producing taxa which detailed aspects of the lifecycle and ultrastructure as visualized in LM and TEM (Meffert 1971; Meffert & Krambeck 1977; Meffert & Chang 1978; Meffert & Overbeck 1981; Meffert 1987; Meffert & Oberhäuser 1982). *Limnothrix redekei*, the type species for the genus, is difficult to maintain in culture without special treatment (Meffert 1972, 1973a, 1973b), and has this to its led from some culture collections. The reference strain, SAG 3.89—originally CCAP1443/3, still exists in the SAG collection, and a short ribosomal sequence exists (NCBI AJ544070, 489 nt). This sequence is 100% identical to another short sequence, PMC8502 (NCBI AJ544069, 489 nt) and a long sequence, CCAP1443/1 (NCBI AJ580007, 2431 nt), a strain originally isolated and studied by Meffert. There is little doubt that this set of strains, which together with NIVA–CYA227/1, all belong to the same species and genus, and could be considered reference strains of _L. redekei_. This is significant because other strains have been assigned to _L. redekei_ and sequenced, and they are undoubtedly incorrectly identified; they occur in the _L. planctonica_ clade (Fig. 3) and should be reassigned to that species. _L. planctonica_ differs from _L. redekei_ in that its polar aerotopes are much smaller and more spherical. _Lagosinema tenuis_ bears a strong resemblance to _L. planctonica_ in that it shares the small spherical polar bodies (Figs 1, 2). The nature of these polar bodies is unclear in the LM. Zhu et al. (2012) observed polar bodies that were iridescent and clearly aerotopes in _L. planctonica_ in the light microscope in fresh material, but in TEM of cultures they did not observe aerotopes, but rather saw spherical granules that are more likely cyanophycin granules. Due to this ambiguity in the sister taxon of _Lagosinema_, we are uncertain at this time whether they have aerotopes, cyanophycin granules, or the possibility of either at the poles of the cells. _L. tenuis_ was collected from the plankton of the water column, suggesting the presence of aerotopes, but at this time we refer to them simply as polar bodies in the formal description of the taxon.

Mai et al. (2018) split the Leptolyngbyaceae into four families. Leptolyngbyaceae and Prochlororichaeaceae were existing families, and Oculutellaceae and Trichocoleaceae were described as new families. The Pseudanabaenaceae was outside of Leptolyngbyaceae sensu lato in a basal position sister to Gloeobacter violaceus. The phylogeny in this work supported their analysis, with the same family level cladest being apparent.

*Limnothrix planctonica* and _L. rosea_ are both in the Prochlororichaeaceae, and are in separate generic cladest from each other and from *Lagosinema*. They both clearly are neither *Lagosinema* or *Limnothrix*, and must be eventually placed in their own genera. *Limnothrix redekei* is itself in jeopardy. Whitton (2011 p. 110) attempted to transfer _L. redekei_ to _Pseudanabaena redekei_ (Goor) Whitton, but the transfer was invalid due to incorrect pagination of the basionym citation (in violation of ICN Article 41.5 and Note 1 under 41.5). _L. redekei_ lies within the _Pseudanabaena_ Lauterborn clade as it is presently defined, and may eventually be transferred into _Pseudanabaena_. Alternatively, the subgenera in _Pseudanabaena_ may be raised to genus level, in which case _Limnothrix_ might retain its taxonomic status.

Our phylogenetic analysis demonstrates the need for considerable taxonomic work in the Prochlororichaeaceae. In addition to the _L. planctonica_ and _L. rosea_ clades, the following clades need corrected generic definition: 1) clade containing *Phormidium persicinum* Gomont (3 OTUs), 2) Oscillatoriales Gollwitz Pohl, 3) Leptolyngbya PCC 6406, 4) clade containing LPP group MBIC 10597 (6 OTUs), and 5) Leptolyngbya WJT66–NPBG5. In addition to these seven new genera (which will also require description of the species), the genus _Nodosilinea_ now has many sequenced strains that have not been identified to species. Many new species of _Nodosilinea_ will subsequently be described. This work on _Nodosilinea_ has already commenced (Heidari et al. 2018; Vázquez–Martínez et al. 2018).

The confusion surrounding _Limnothrix_ is likely due to the broad characterization of the genus: filaments less than 1–6 μm, untapered, with polar or central aerotopes (Komárek & Anagnostidis 2005). _L. bicudoi_ Azevedo et C.A. de Souza, _L. redekei_, and _L. vacuolifera_ (Skuja) Komárek ex McGregor possess enlarged, often irregular,
polar aerotopes, and may represent *Limnothrix* sensu stricto. The taxa with distinctive, large, central aerotopes may comprise another genus; these types include *L. borgertii* (Lemmermann) Anagnostidis, *L. brachynema* (Skuja) Hindák et Trifonova, *L. hypolimnetica* Hindák et Trifonova, *L. lauterbornii* (Schmidle) Anagnostidis, *L. pseudospirulina* (Pascher) Anagnostidis, *L. pseudovacuolata* (Utermöhl) Anagnostidis, and *L. rosea*. *Limnothrix planctonica* was illustrated by both MEFFERT (1988) and ZHU et al. (2012) as having small rounded polar aerotopes. Full support is indicated by an “*” (1.0 or 100%), less than 0.50/50% support indicated by “–”. Taxa which we consider to be incorrectly identified or needing taxonomic revision are set off in quotation marks.
Fig. 4. Secondary structure of the D1–D1’ helix in Lagosinema and representative Prochlorotrichaceae for which 16S–23S ITS sequence data are available. Circled bases in *L. tenuis* represent base pair differences between the different operons. *L. planctonica* also had two operons, but the D1–D1’ helices differed in structure between the two operons of that taxon.

Fig. 5. Secondary structure of the Box–B helix in Lagosinema and representative Prochlorotrichaceae for which 16S–23S ITS sequence data are available. Note the differences between the bilateral bulges just above the basal clamp sequence (5’–AGCA:UGCU–3’), consistent with the conclusion that these taxa are all in separate genera.

considered cryptogenera (Komárek et al. 2014).

The Synechococcales contains a great number of molecularly and morphologically divergent taxa. Seventy–four genera were recognized in the order in the newest taxonomic system (Komárek et al. 2014), with (counting Lagosinema) 18 additional genera being published since that treatment (Dvořák et al. 2015; Song et al. 2015; Vaz et al. 2015; Li & Li 2016; MisCole et al. 2016; Sciuto & Moro 2016; Dvořák et al. 2017; Jahodárová et al. 2017a, 2017b; Sciuto et al. 2017; Mai et al. 2018). Furthermore, there are numerous unnamed or incorrectly named sequenced species that appear in our phylogenetic analysis that certainly need to be placed in new genera if genera are to become monophyletic taxa (Fig. S1). There is a great deal of α–level taxonomy remaining to be completed in the order, and the next few years will likely see many efforts to expand the taxonomy of this group so that it conforms with modern species and generic concepts. We look forward to both seeing and being a part of this work.

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References


Whitton, B.A. (2011): Cyanobacteria (Cyanophyta). – In:


