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REVISION OF THE SYNECHOCOCCALES (CYANOBACTERIA) THROUGH RECOGNITION OF FOUR FAMILIES INCLUDING OCULATELLACEAE FAM. NOV. AND TRICHOCOLEACEAE FAM NOV. AND SEVEN NEW GENERA CONTAINING 14 SPECIES

Truc Mai
John Carroll University, tmai18@jcu.edu

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REVISION OF THE SYNECHOCOCcales (CYANOBACTERIA) THROUGH RECOGNITION OF FOUR FAMILIES INCLUDING OCULATELLACEAE FAM. NOV. AND TRICHOCOLEACEAE FAM NOV. AND SEVEN NEW GENERA CONTAINING 14 SPECIES

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By
Truc T. Mai
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The thesis of Truc T. Mai is hereby accepted:

Reader – Christopher A. Sheil  
05 DECEMBER 2016  
Date

Reader – Michael P. Martin  
12-5-16  
Date

Reader – Nicole Pietrasiaik  
12-7-16  
Date

Advisor – Jeffrey R. Johansen  
Dec. 5, 2016  
Date

I certify that this is the original document

Author – Truc T. Mai  
Dec 5th, 2016  
Date
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ABSTRACT

A total of 48 strains of thin, filamentous cyanobacteria in the Synechococcales were studied by sequencing 16S rRNA, *rpoC1*, and *rbcL* gene fragments. A subset of these were carefully characterized morphologically. Bayesian analysis of the 16S rRNA gene data in a large alignment of Synechococcales (345 OTU’s) was in agreement with the phylogeny based on the *rpoC1* gene for 59 OTU’s. Both indicated that the large family-level grouping formerly classified as the Leptolyngbyaceae could be further divided into four family-level clades. Two of these family-level clades have been recognized previously as Leptolyngbyaceae and Prochlorotrichaceae. The Oculatellaceae *fam. prov.* and Trichocoleaceae *fam. prov.* are proposed for the other two families. The Oculatellaceae was studied in greater detail, and seven new genera containing 14 species were characterized and named. These new taxa are: *Pegeithrix botrychoides sp. prov.*, *P. olivacea sp. prov.*, *P. convoluta sp. prov.*, *P. indistincta sp. prov.*, *Francisia lurida comb. prov.*, *F. hepatica sp. prov.*, *F. fasciculata sp. prov.*, *Cartusia fontana comb. prov.*, *Joesphinia torsiva sp. prov.*, *J. nuda sp. prov.*, *Gardneria angustata sp. prov.*, *Kaiparowitsia implicata sp. prov.*, *Thallothrix obliquedivisa sp. prov.*, *T. radians sp. prov.*
INTRODUCTION

The cyanobacteria currently contain eight orders supported by molecular sequence data: Gloeobacteriales, Synechococcales, Spirulinales, Chroococcales, Pleurocapsales, Oscillatoriales, Chroococcidiopsidales, and Nostocales (Komárek et al. 2014). There are over 300 genera, with over 50 described since 2000. Despite the rapidly growing number of genera and species in recent years, relatively little revisionary work has occurred at the family level. Only a third of the genera have 16S rRNA gene sequence data for the generitype (Komárek et al. 2014), so most families contain a preponderance of unsequenced and unverified genera (which are often poly-/paraphyletic) based primarily on morphology. Families of cyanobacteria are thus not confirmed as lineages, and researchers consequently have been reluctant to revise this middle tier of higher level taxonomy.

The Synechococcales are especially problematic. This group used to contain only coccoid and bacilloid unicellular and colonial genera, with the related Pseudanabaenales containing simple filamentous forms. Phylogenetic analyses demonstrated that the genera of the two families were intermixed, and consequently all of the genera in the order were consolidated into Synechococcales (Komárek et al. 2104), which currently contains 11 families. Of these, the families containing simple filamentous forms with peripherally arranged thylakoids are Pseudanabaenaceae, Leptolyngbyaceae, Romeriaceae, Heteroleibleiniaceae, and Schizotrichaceae. However, even a casual phylogenetic analysis of these families reveals problems. Representatives of Romeria (type genus of Romeriaceae), Schizothrix (type genus of the Schizotrichaceae), and Tapinothrix (member of Heteroleibleiniaceae) are all phylogenetically positioned within
the Leptolyngbyaceae (Osorio-Santos et al. 2014, see Fig. S1), although none of these sequences are of the type species of these genera (Table 1). Consequently, these families may disappear if it is found that the type species and other sequenced species are in the same generic clade (e.g. if Tapinothrix bornetii Sauvageau (1892: CXXIII) is found to be phylogenetically related with the molecularly characterized T. clintonii, in Leptolyngbyaceae). Alternatively, the families based on these genera will be retained if the type species are outside of other described family-level groupings (e.g. if T. bornetii is found to be very distantly related to T. clintonii and is well outside of Leptolyngbyaceae), and the current existing sequences will need to be assigned to other genera. In all recently published trees of the Leptolyngbyaceae, there appears to be stable phylogenetic structure that suggests the family as currently constructed could be further divided (Johansen et al. 2008, 2011, Mühlsteinová et al. 2014, Osorio-Santos et al. 2014, Song et al. 2015, Vaz et al. 2015, Li & Li 2016, Miscoe et al. 2016). Thus, the families in the Synechococcales require revision that combines some families and creates new families for monophyletic clusters of genera.

The largest family in the Synechococcales is the Leptolyngbyaceae, and this group has received significantly more study in recent years than any other group in the order. Leptolyngbya has repeatedly been shown to be polyphyletic, and numerous genera have been split out from the genus. The revisionary work has been facilitated by availability of sequence data for the type species, L. boryana (Gomont 1899: 36) Anagnostidis & Komárek (1988: 391), which provides a clear benchmark against which morphologically similar taxa can evaluated (Johansen et al. 2008, 2011). Newly described genera in the Leptolyngbyaceae include Planktolyngbya, Prochlorothrix,
Trichocoleus, Halomicronema, Phormidesmis, Plectolyngbya, Nodosilinea, Haloleptolyngbya, Oculatella, Neosynechococcus, Stenomitos, Kovacikia, Pantanalinema, Alkalinema, Scytolyngbya, Thermoleptolyngbya, and Limnolyngbya (Table 2). All of these new genera and a few previously described genera in the family (Geitleribactron, Tapinothrix, Schizothrix, Romeria) have sequence data, making the Leptolyngbyaceae one of the better taxonomically resolved families in the cyanobacteria.

Despite the progress made in the Leptolyngbyaceae, the family still requires considerable alpha-level taxonomy. Many strains have been sequenced that are not assigned to a particular species of *Leptolyngbya* that clearly are phylogenetically distant from the group of *Leptolyngbya* containing the generitype, which has been called *Leptolyngbya sensu stricto* (Bohunická et al. 2011, Johansen et al. 2011, Perkerson III et al. 2011, Mühlsteinová et al. 2014, Osorio-Santos et al. 2014). The problem lies in the fact that *Leptolyngbya* is broadly circumscribed by a very small number of morphological characters (Komárek & Anagnostidis 2005). Many of the species in the group are also phenotypically plastic, making identification of species (and genera) very difficult based on morphology alone. Despite the morphological limitations of the genus, it is evident that many more genera need to be described if *Leptolyngbya* is to become a monophyletic genus (see fig. S1 in Osorio-Santos et al. 2014). Adding to this problem is that many (if not most) of the more than 100 species in this genus are poorly characterized, often without illustrations, and overlapping morphological traits. Most of these species have not been sequenced, and are very infrequently reported in the literature. Many of them likely belong in other, as yet to be described genera. Thus alpha level taxonomy will include incorporating some historical species names into new taxa, as well as describing
species truly new to science that cannot be assigned to existing taxa. The availability of 16S rRNA gene sequence data greatly facilitates the taxonomy and revisionary process now under way.

However, many scientists have recently turned their attention to other genes that are taxonomically informative, as the 16S rRNA gene is considered insufficient to resolve all taxonomic questions (Komárek 2006). The 23S rRNA gene region has been suggested in some studies to be a good phylogenetic marker, because the sequence agrees well with 16S rRNA data (Cedergren et al. 1988; Rijket & Peer 1995). It potentially contains more phylogenetically informative nucleotides across its length than the 16S rRNA gene (Ludwig & Schleifer 1994). In the past, the length of 23S rRNA gene has been the major obstruction to its amplification efficiency, as many internal primers are required to obtain the whole sequence. This problem can be bypassed by using a partial sequence of the 23S rRNA gene. For example, the Universal Plastid Amplicon (UPA) is a large fragment of the 23S rRNA that can be easily sequenced, and has been recently demonstrated to improve phylogenetic support when concatenated with 16S rRNA sequence data (Sherwood et al. 2007, 2015). With the introduction of novel and high-quality whole-genome amplification methods, and better broad-range primers designed for conserved regions (Hunt et al. 2006), this information-rich gene will likely see increasing phylogenetic use in the near future.

Several protein coding genes have been shown promising for phylogenetic analyses in Cyanobacteria. The \textit{rpo} gene family encodes for different beta subunits of DNA-dependent RNA polymerase and is present as a single copy in the genome, making members of this gene family a subject of interest for phylogenetic studies. The \textit{rpo}B and
rpoC1 loci, which encode for the β and β' subunits of RNA Polymerase, have been reported to be more discriminatory markers than the 16S rRNA sequences (Case et al. 2007, Gaget et al. 2011), and several works have been done on the basis of these genes, mostly concerning toxic taxa (Fergusson & Saint 2000, Wilson et al. 2000). Data mined from rbcL, a gene encoding the large subunit of RuBisCO, a critical protein in CO₂ fixation, has also been shown to support ribosomal sequence data. (Tomitani et al. 2006; Andersen 2013). The previous use of cpcA-cpcB (phycocyanin subunit A and B) intergenic spacer (IGS) in the phylogeny of Geitlerinema (Bittencourt-Oliveira et al. 2009) has also sparked some interest in further application of this locus to study Synechococcalean taxa, particularly at the species level.

A number strains of Leptolyngbyaceae were isolated as part of a study of the aquatic and subaerial cyanobacterial flora of the Grand Staircase-Escalante National Monument (Krautová 2008). The study had also morphologically characterized these isolates. They are currently housed within the Cyanobacterial Culture Collection at John Carroll University, and were the focus of this study, which sequenced and described them. The collection also has numerous other strains in order Synechococcales, from diverse sites and habitats including desert soils in North and South America, the Great Smoky Mountains National Park, Hawaii, and Europe. Within this broader collection are many Leptolyngbyaceae sensu lato, for which 16S rRNA data already exist. Previously, a lot of thin filamentous strains were morphologically characterized in Krautová (2008). This manuscript begins the revisionary process for Leptolyngbyaceae by breaking the family into four monophyletic families, describing Trichocoleaceae and Oculatellaceae, and redefining two older families, Leptolyngbyaceae and Prochlorotrichaceae. The
Oculatellaceae are more completely characterized through description of seven new genera and fourteen species (either new to science or based on earlier taxa) based upon the strains available in the JCU collection. These new taxa will be discussed in this thesis as putative new taxa, and will be subsequently formally described in published manuscript.

MATERIALS AND METHODS

Molecular techniques. Genomic DNA was extracted from selected strains (Table 2) located in the Cyanobacterial Culture Collection of John Carroll University (JCU), using UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) or a CTAB (cetyl trimethylammonium bromide)-based extraction following Burke et al. (2006). PCR amplification of the 16S rRNA gene was performed using primers VRF1: 5’-CTC TGT GTG CCT AGG TAT CC-3’ (Wilmotte et al. 1993, Boyer et al. 2001) and VRF2: 5’-GGG GAA TTT TCC GCA ATG GG-3’ (Nübel et al. 1997, Boyer et al. 2001). All PCR reactions contained 1X GoTaq® Flexi Buffer, 0.025 units/μL GoTaq® Flexi DNA Polymerase, 3 mM MgCl₂ (Promega, Madison, WI, USA.), 0.2 mM dNTPs, 0.5 μg/μL of BSA (NEB, Ipswich, MA, USA.) and 0.5 μM each of primer VRF1 and VRF2, (NEB, Ipswich, MA, USA). Reactions were performed in a BioRad PCR Thermocycler (Bo-Rad Laboratories, Inc., France) with a 3 minute incubation at 94°C to minimize non-specific DNA amplifications. Subsequently, reactions underwent 35 cycles of 94°C (30 s), 53°C (30 s) and 72°C (60 s), followed by an incubation at 72°C (300 s) to complete synthesis. A representative of each genus was selected for PCR amplification of DNA-dependent RNA Polymerase subunit Gamma
(rpoC1) and Rubisco 1,5-biphosphatase carboxylase/oxygenase large and small subunit (rbcLX). Primer sequences for rpoC1 were rpc/MF: 5´-GGT GAR GTN ACN AAR CCA GAR AC-3´ and rpc/CR-1: 5´-CCA GAR TAG TCN ACC CGT TTA CC-3´ (Seo & Yokota 2003). Primer sequences for rbcLX were CW: 5´-CGT AGC TTC CGG TGG TAT CCA CGT-3´ and CX: 5´-GGG GCA GGT AAG AAA GGG TTT CGT A-3´ (Rudi et al. 1998). The cycling conditions followed those described in the above-cited publications. For the 16S rRNA gene which occurs as multiple copies across genomes, PCR products were cloned according to manufacturer instructions (La Jolla, CA, USA.). Plasmid purification proceeded with QIAPrep Miniprep Spin Kit (Qiagen, Carlsbad, CA, USA.) prior to EcoRI digestion to select successful clones. For PCR products from rbcLX and rpoC1 genes, mono-product reactions were directly purified, whereas multi-product reactions were excised from an agarose gel and purified using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). All plasmid DNA and purified PCR products were sent to Functional Biosciences, Inc. (Madison, WI) for sequencing, and processed with Sequencher v. 4.10.1 software (Gene Codes Corp, Ann Arbor, MI, USA.). Sequencing primers for sequencing includes primer M13 forward, M13 reverse, primer 5 (5´–TGT ACA CAC CGG CCC GTC–3´) (Wilmotte et al. 1993), primer 7 (5´–AAT GGG ATT AGA TAC CCC AGT AGT C–3´) and primer 8 (5´–AAGGAGGTGATCCAGCCACA–3´) (Nübel et al. 1997).

Phylogenetic analysis. 16S rRNA sequences were aligned with ClustalW (Larkin et al. 2007). Processed sequences were aligned with MUSCLE in MEGA6, and were curated manually using our 348-sequence database of Synechococcales to correctly align sequences by conservation of secondary structure of conserved helices in the 16S rRNA.
molecule (identified based on Řeháková et al. 2014). The alignment was submitted to MrBayes on XSEDE (3.2.6) available on CIPRES Science Gateway v.3.1 (Miller et al. 2011) with the following parameters: NST=6, Rates=equal, MCMC Ngen=5,000,000. All other parameters were left as defaults. The 16S–23S rRNA internal transcribed spacer regions (ITS) were not aligned, but secondary structures including D1-D1’, Box B, V2 and V3 helices were identified and predicted using the Mfold web server (Zuker 2003). Additional conserved domains (all helices plus D2, D3, Box A, D4, and D5) were identified for comparison of lengths. All structures were redrawn in Adobe Illustrator in the CS5 software package. For rbcLX and rpoC1, sequences were blasted in the NCBI protein database using BLASTX to identify the start codon. Two individual DNA alignments of 55 rpoC1 sequences and 55 rbcLX sequences composed of data from our selected strains and strains available on NCBI (from single PCR or from genomes) were submitted to JModelTest2 2.1.6 (Darriba et al. 2012) on XSEDE (2.01) to find the appropriate empirical evolutionary models (highest BIC). Tree topology with rpoC1 gene was constructed with MrBayes using model TrN+I+G with the following parameters: NST=6, Nucmodel=codon, Rates=invgamma, Revmatpr=fixed (1.0000, 4.0631, 1.0000, 1.0000, 6.4511, 1.0000), Pinvarpr=0.3400, Shapepr=0.6700; MCMC Ngen=10,000,000. Tree topology with rbcLX was constructed using model TPM1uf+I+G with the following parameters: NST=6, Nucmodel=codon, Rates=invgamma, Revmatpr=fixed (1.0000, 2.4878, 0.8459, 2.4878, 1.0000), Pinvarpr=0.0930, Shapepr=0.7740; MCMC Ngen=15,000,000.

Microscopy. Cyanobacteria were cultured in solid Z8 media (Carmichael 1986), or liquid Z8 medium when necessary, over the course of approximately 8 months.
Microscopic images were taken when growth started to occur in a new transfer to represent exponential phase after 2–3 months, and after 6–8 months to represent stationary phase. All images were taken with an Axioskop HBO 50 (Carl Zeiss AS, Norway) and processed when necessary with Adobe Photoshop in the CS5 software package.

*Type materials preparation.* All strains were cultured in liquid Z8 medium until biomass was sufficient to prepare three dried preparations. Liquid cultures were vacuum filtered on to sterile glass fiber filters, which were allowed to dry at room temperature in covered glass petri dishes for a week. These filters were then placed in wax-paper envelopes, mounted on card, and placed in protective covers. A portion of the type materials were also preserved in 4% Formaldehyde. All materials were deposited in the Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Brigham Young University, Provo, Utah, USA.

**RESULTS**

*Phylogenetic results based on 16S rRNA phylogeny.* Our analyses of 345 OTUs belonging within the Synechococcales with *Gloebacter violaceus* Rippka *et al.* (1974: 436) (Gloeobacteriales) as outgroup indicated the existence of six distinct family-level clades (Figs 1–3). These clades have been evident in numerous other phylogenetic analyses with different taxon sampling and are stable in all phylogenies we have run using 16S rRNA gene sequences (Bruno *et al.* 2009, Komárek *et al.* 2009, Moro *et al.* 2010, Bohunická *et al.* 2011, Mühlsteinova *et al.* 2014, Osorio-Santos *et al.* 2014, Sciuto & Moro 2016).
The first of these clades is the Leptolyngbyaceae. Many of the taxa in the past have been placed in *Leptolyngbya sensu lato* (untapered trichomes less than 3.5 μm wide, with sheaths, and without aerotopes). Recently, *Leptolyngbya* has been revised in part with the separation of several genera formerly in the genus (e.g. *Nodosilinea*, *Phormidesmis*, *Oculatella*, *Plectolyngbya*, *Thermoleptolyngbya*). The type species of *Leptolyngbya* is *L. boryana*, and a number of strains belonging to this species have been sequenced. Other *Leptolyngbya* species including *L. angustata* Casamatta & Johansen in Casamatta *et al.* (2005: 430–431), *L. corticola* Johansen & Kovačík in Johansen *et al.* (2011: 291–293), *L. foveolarum* (Gomont 1892: 164) Anagnostidis & Komárek (1988: 391), *L. tenerrima* (Hansgirg 1892b: 87) Komárek in Anagnostidis (2001: 374), and *L. crispata* (Playfair 1915: 350) Anagnostidis & Komárek (1988: 391) are molecularly and morphologically close to *L. boryana*, and have been come to be known in published phylogenies as *Leptolyngbya sensu stricto* (Bohunická *et al.* 2011, Johansen *et al.* 2011, Perkerson III *et al.* 2011, Mühlsteinová *et al.* 2014, Osorio-Santos *et al.* 2014). The family level clade containing *Leptolyngbya sensu stricto* by definition must belong in the Leptolyngbyaceae, and based on our Bayesian analysis of a 1162 nucleotide partial sequence of the 16S rRNA gene (Fig. 1), this family should contain *Leptolyngbya sensu stricto*, *Plectolyngbya*, *Tapinothrix*, *Romeria*, *Myxacorys*, *Planktolyngbya*, *Alkalinema*, *Phormidesmis*, *Stenomitos*, *Neosynechococcus*, *Arthronema*, *Pantanalinema*, and *Scytolyngbya* (Table 1). There are still a number of lineages of uncertain generic affinity that are putative new taxa to science and need to be described. Most of these are called “*Leptolyngbya sp.*” even though they are well outside of *Leptolyngbya sensu stricto*, although a few are misidentified “*Phormidesmis*”.
The definition of genus *Leptolyngbya* is still questionable. As we have defined it in this paper, *Leptolyngbya sensu stricto* and *Plectolyngbya* OTUs form a well-supported generic clade (Fig. 1, top clade sister to *Tapinothrix clintonii*). *Plectolyngbya* was originally separated due to its ability to produce double false branches as opposed to the single false branches common in *Leptolyngbya*. Given the high molecular and morphological similarity between *Leptolyngbya* and *Plectolyngbya*, it is possible that these species will be combined with *Leptolyngbya* in the future. The alternative is to retain *Plectolyngbya* and recognize a number of *Leptolyngbya* in the third clade (containing the SEV strains from the Sevielleta LTER) as a new genus (Fig. 1). Because this work is focused on definition of the Oculatellaceae, we consider this revisionary process beyond the scope of this paper. There still remains a great deal of taxonomic work to be done in Leptolyngbyaceae *sensu stricto*, as we have identified over 20 genus-level taxa, of which only 12 are currently described (Fig. 1).

Sister to the Leptolyngbyaceae is another well supported clade of diverse taxa containing the recently described genera *Oculatella* and *Thermoleptolyngbya* (Fig. 2). Because *Oculatella* is the older of these two genera, we propose naming this family-level grouping Oculatellaceae *fam. prov.* This family has a number of previously undescribed genera based on both morphological and molecular differences. These taxa were mostly isolated from subaerial environments including wet rocks and soils, and will be described in the paper based on this thesis as *Pegethrix, Francisia, Thallothrix, Cartusia, Josephinia, Gardeneria*, and *Kaiparowitsia*.

Three family-level clades occur in a position basal to the Leptolyngbyaceae-Oculatellaceae clade. The largest of these is the clade containing *Nodosilinea*, 
Halomicronema, and Prochlorothrix, as well as a number of unspeciated taxa of uncertain generic identity (Fig. 3). The invalidly described “Xeronema” (Garcia-Pichel et al. 2001) occurs in this clade, as well as two sequences submitted as L. appalachiana which are very distant from the reference sequence for this species (Fig. 1). Of the three described genera, Prochlorothrix is the oldest name, and at the time of its description it was placed in its own family, Prochlorotrichaceae (Burger-Wiersma et al. 1989).

*Prochlorothrix hollandica* Burger-Wiesma et al. (1989: 256) and Prochlorotrichaceae were described under the International Code of Nomenclature of Bacteria, but are valid under the International Code of Nomenclature of Plants, Algae and Fungi (McNeill et al. 2012), Art 45.1. In fact, *P. hollandica* is cited as an example (Art. 45.1, Ex 3) of this rule. Consequently, we adopt this validly described family name to contain members of this clade.

The fourth family-level clade contains two species in the genus *Trichocoleus* (Fig. 3). Although low in diversity, this clade cannot be included in any of the above three groups without creating paraphyletic genera. We consequently propose the name *Trichocoleaceae fam. prov.* for this group of taxa. The fifth family-level clade contains two uncharacterized strains isolated from soil at a single site in the Atacama Desert, which at present are only identified to order (Synechococcales), so no family name is proposed for this possible new family. At the base of the Synechococcales (defined by its proximity to the outgroup taxon *Gloeobacter*) is the Pseudanabaenaceae, a family containing *Pseudanabaena* and *Limnothrix* (Fig. 3).

Our findings are not in agreement with the recent revision of the higher level taxonomy of the Cyanobacteria (Komárek et al. 2014). Pseudanabanaceae included

Alyssophoron, Limnothrix, Prochlorothrix Burger-Wiersma et al. (1989), 
Pseudanabaena, and Yonedaella Umezaki (1962). Our work indicates that Arthonema 
and Prochlorothrix belong outside of this family (Leptolyngbyaceae and 
Prochlorotrichaceae, respectively). Tapinothrix was placed in the Heteroleibleiniaceae 
instead of the Leptolyngbyaceae. The rest of the Leptolyngbyaceae taxa in Komárek et 
al. (2014) were scattered throughout the three families recognized in this work: 
Leptolyngbyaceae, Oculatellaceae, and Prochlorotrichaceae.

Oculatellaceae is being separated from Leptolyngbyaceae in this paper because 
the separation between the two clades is well supported phylogenetically, both families 
are speciose with substantial diversity in genera, and finer division at the family level will 
be of assistance when sequences from environmental samples are being assigned to taxa. 
A correct family-level designation is considered preferable by us to an incorrect generic-
level epithet (e.g. Leptolyngbya). Our intent in creating this taxonomic scaffold is to 
encourage further taxonomic work in this diverse and important group of cyanobacteria.

Phylogenetic results based on rpoC1 phylogeny. Analysis of 57 sequences of the 
rpoC1 gene shows the Oculatellaceae to be the most divergent group of genera in the 
Synechococcales. The groups of Oculatellaceae and Prochlorotrichaceae are relatively 
stable and agree with 16S rRNA phylogeny, but the Leptolyngbyaceae appears 
polyphyletic by this gene (Fig. 4). Most Oculatellaceae form a near monophyletic clade 
according to rpoC1 sequence data, except for J. nuda, which is in the Leptolyngbyaceae 
clade in this phylogeny. Five taxa identified as members of the Leptolyngbyaceae by
16S rRNA gene phylogenies were positioned outside of the family-level clade containing the type for *Leptolyngbya, L. boryana* (Fig. 4). Perhaps most concerning was the position of *P. hollandica*, the namesake of the Prochlorotrichaceae. It fell well outside of any family grouping. *Trichocoleus* in the Trichocoleaceae was monophyletic, but separated *Neosynechococcus* and several Hawaiian “*Leptolyngbya* species” from the primary clade containing Oculatellaceae and Leptolyngbyaceae. We have general but not absolute agreement between the two phylogenies.

*Analysis of 16S rRNA p-distance for family separation.* Mean percent difference in 16S rRNA gene sequence among genera of different families is 7.71–10.63% (Table 3). This is higher than the mean percent difference between genera of the same families (7.31–8.21%). However, if one examines the ranges of difference among genera, both within and between families, no clear similarity cut-off exists that would allow recognition of families by similarity alone. The lowest between-family generic difference for all pairwise family comparisons ranges 4.74–7.04%, whereas the highest within-family generic difference within each family is in every case >7.5%. This means that if a value below 4.74% is chosen to designate families, every family would need to be split further, or alternatively, if a value above 7.5% were chosen, numerous genera in different families would appear to belong to the same family. Consequently, families cannot be separated based on an arbitrary cut-off limit in percent dissimilarity between their constituent genera. An outcome of this finding is that environmental sequences should not be assigned to family in the Synechococcales based on a BLAST search, unless such a search clearly places an environmental sequence within a genus within one
of the four recognized families. Assigning environmental sequences to family should be done based upon other molecular evidence, e.g. phylogeny or criteria given below.

*Molecular diagnosis of families in Synechococcales.* Based on the 16S rRNA gene sequence alignment of 1162 nucleotides for 345 OTUs, we looked at phylogeny-informative sites for family-specific signals. Of 440 sites examined, we identified 7 nucleotide positions in 5 different helices that were consistent indicators of family identity. In all cases, secondary structure of the helices was conserved among sequences (see examples in Fig. 5). These distinctive nucleotides were always found in at least 90% of the OTUs compared in this study (Table 4). We consider conformation to these sequence patterns to be useful evidence for definition of the families, but given the occasional variability within some OTUs, this should not be the only way in which family-level assignments are made in the future. We consider phylogeny most useful, and this measure as supporting evidence.

We also looked for family-level signals in the conserved domains of the 16S–23S ITS region (Figs 6–9). While there were clear genus-specific signals in many of these structures, the ITS region was too variable in sequence and structure to find any differences that were consistent across all genera in any one family.

*Taxonomic descriptions.*

Class: *Cyanophyceae*

Subclass: *Synechococcophycidae*

Order: *Synechococcales*
Trichocolaceae *fam. prov.*

*Description.* A monophyletic assemblage of genera based on 16S rRNA gene sequence phylogeny, with filaments containing one to many trichomes, obligately forming sheaths except in hormogonial stages. Sheaths thin and firm to soft and wide, lacking pigmentation. Trichomes slightly to distinctly constricted at the crosswalls, straight or flexuous, less than 5 µm wide. Cells shorter than wide, isodiametric, or longer than wide, with peripheral thylakoids, obligately without aerotopes, facultatively forming polyphosphate granules in the centroplasm. End cells cylindrical, rounded, rounded conical, conical, or attenuated to a point.

*Type genus:* *Trichocolus* (West & West) Anagnostidis

Oculatatellaceae *fam. prov.*

*Description.* A monophyletic assemblage of genera based on 16S rRNA gene sequence phylogeny, with filaments without sheaths in actively growing populations, but facultatively developing sheaths in established populations, without false branching in some genera, but typically falsely branched in most genera. Sheaths thin, firm, lacking pigmentation. Trichomes slightly to distinctly constricted at the crosswalls, straight, flexuous, spirally twisted, or knotted into loose nodules, less than 3 µm wide. Cells shorter than wide, isodiametric, or longer than wide, with peripheral thylakoids, obligately without aerotopes, facultatively forming granules in the centroplasm. End cells mostly cylindrical, rounded, but rounded conical in some genera.

*Type genus:* *Oculatella* Zammit, Billi & Albertano
**Pegethrix gen. prov**

*Description:* Filaments mostly solitary, at times with multiple hormogonia in a common sheath, or with loose nodule formation, with infrequent double and single false branching. Sheath clear thin and firm to soft and widened, but never diffuent. Trichomes straight, flexuous, or entangled within a sheath into a loose nodule, sometimes spirally coiled, slightly constricted at the crosswalls, with slow gliding motility observed in trichomes lacking sheath, not tapered. Cells mostly shorter than wide, becoming isodiametric to slightly longer than wide before division, without aerotopes, sometimes with granules in cytoplasm; with parietal thylakoids. Apical cells rounded, without calyptra. Involution cells with axillary bud-like structures rare. Reproduction by trichome fragmentation via disintegration at necridia, without necridia, or in meristematic regions.

*Etymology:* Pege (Gr): water, stream or spring; thrix (Gr.): hair

*Type species:* Pegethrix bostrychodes

**Pegethrix bostrychodes sp. prov.** (Fig. 10)

*Diagnosis:* Differing from other species in the genus based on the frequent formation of spirals; the basal 6 bp clamp on the D1-D1’ helix of the ITS region (5’-GACCUU:AAGGUC-3’) (Fig. 6a), the bilateral bulge near the base of the Box B helix of the ITS region (5’-AU:UC-3’) (Fig. 7a), and unique V2 and V3 helices of the ITS region (Figs. 8a, 9a).

*Description:* Colony bright blue green, with radial fasciculation, penetrating the agar. Filaments long or short, rarely singly or doubly false branched (Figs. 10d, f, g, h), frequently loosely to tightly spirally coiled (Figs. 10k, l, o–q), sometimes forming
nodules (Figs. 10a, c), 2.0–6.0 µm wide (to 14 µm wide at nodules). Sheath firm, colorless, usually attached to trichome, occasionally softer, widened (Figs. 10b, c, e, f), sometimes irregular and stratified (Fig. 10f). Trichomes untapered, more or less constricted at the distinctly visible cross-walls, occasionally with tight, regular, screw-like coils (Figs. 10k, l, o–q), necridia not observed, 1.5–2.5–(3.0) µm wide. Cells slightly shorter than wide to longer than wide, rarely with a single central granule, with parietal thylakoids, 1.0–3.0 µm long. End cells rounded.

D1-D1’ helix 85 nucleotides long, with basal 3’ unilateral bulge of 8 unpaired nucleotides, with mid-helix region with one dinucleotide side bulge at position 14–15, one bulge at position 22–25/56–60, one small bulge at position 32–33/48–49, with terminal loop of 4 nucleotides, having terminal sequence GAAA (Fig. 6a). Box B helix 36 nucleotides long, bearing 4 nucleotides at terminal loop, and one small bilateral bulge at position 5–6/31–32 (Fig. 7a); V2 helix 24 nucleotides long, with terminal loop of 6 nucleotides (Figs. 8a); V3 helix 96 nucleotides long, most similar in structure to V3 helix of P. olivacea, but with different sequence (Fig. 9a).

Etymology: bostrychos (Gr.): curl, anything twisted; Latinized to bostrychodes

Type locality: Drip Tank Seep Wall site, sandstone seep wall with small moist area and larger pond below the rock face, within Strait Cliffs Formation, in the Grand Staircase-Escalante National Monument (GSENM), Kane County and Garfield County, Utah, USA. Eukaryotic green algae to one side, 37°19’12.79”N, 111°31’50.59”W.

Holotype to be designated: BRY 37770, Monte L. Bean Museum, Provo, Utah.

Notes: The coiling pattern is very characteristic and likely key to species identification. *Spirulina rosea* Crouan & Crouan (1867: 111) ex Gomont (1892: 253) has trichome width and pattern of coiling very similar to this species; however trichome coloration and especially the intensive, obligate motility observed in *Spirulina* sp. is not observed in this species. Several species of *Planktolyngbya* have also been described to have such coils, including *Planktolyngbya holsatica* (Lemmermann 1904: 306) Anagnostidis & Komárek (1988: 394). *Planktolyngbya bipunctata* (Lemmermann 1899: 133) Anagnostidis & Komárek (1988: 394), *Planktolyngbya circumcreta* (West 1907: 174) Anagnostidis & Komárek (1988: 394) and *Planktolyngbya contorta* (Lemmermann 1898a: 202) Anagnostidis & Komárek (1988: 394). *P. holsatica* has dimensions that best fit with this species description (filaments up to 3.5 µm wide, trichomes 2.7–3.0 µm wide). All aforementioned *Planktolyngbya* sp. have homogeneous cell content, with no constrictions at cross-walls except for *P. contorta*, but this species has cells distinctively longer than wide. All *Planktolyngbya* sp. were originally described from planktonic communities, and the single species sequenced for this genus is in the Leptolyngbyaceae (Fig. 1). Several *Leptolyngbya* species described with coiling behavior include, *Leptolyngbya protospira* (Skuja 1939: 50) Anagnostidis (2001: 367) and *Leptolyngbya spiralis* (Jao 1948: 169) Anagnostidis (2001: 367). However the identity of this new species as either of the described *Leptolyngbya* sp. is questionable, as both were described with thinner trichome widths compared to this species (*L. protospira* 0.16–1.4 µm wide; *L. spiralis* 1–1.5 µm wide), with non-granulated cell content, and non-stratified sheaths. *L. protospira* and *L. spiralis* are found in either brackish water or marine.
environments, which additionally suggests that this is a new, undescribed species to science that differs from all previously described taxa in other genera.

*P. bostrychodes* is distinct from the four other species in this genus which we recognize in this study. It differs morphologically based on the frequent formation of spirals. Sequence identities based on p-distance of the 16S rRNA gene sequences in the genus do not provide evidence of species separation in this genus, with values between species ranging 98.6–99.9 (Table 6). However, the phylogeny separates the species fairly well (Fig. 2), with *P. bostrychodes* being sister to *P. olivacea*. The separation of species is further supported by the large p-distance of the 16S-23S ITS region (≥8.2%, see Table 7), which several papers have found useful as evidence of cryptic species separation in other cyanobacterial genera (Erwin & Thacker 2008, Osorio-Santos et al. 2014, Pietrasiak et al. 2014, Bohunická et al. 2015).

**Pegethrix olivacea sp. prov.** (Fig. 11)

*Diagnosis:* Morphologically most similar to *P. convoluta* in the formation of nodules, however, differing from this and other species in the dirty, olive-green coloration of trichomes; similar to *P. bostrychodes* in conserved domains of ITS region (Fig. 6b), but separated by longer V2 helix (Fig. 8b, Table 5) and differences in sequence in the apical half of the V3 helix. (Fig. 9b).

*Description:* Colony dark olive-green, hairy, spreading radially, flat and mucilaginous or mounded. Filaments long or short, frequently irregularly bent due to uneven cell division along filament (Figs. 11 g–j, l), false branched, sometimes loosely coiled to form irregular nodules (Figs. 11a, e), 2.0–3.3–(3.7) µm wide. Sheath firm,
colorless, usually attached to trichome, occasionally widened (Fig. 11f), up to 1.7 µm wide. Trichomes constricted at indistinctly visible cross-walls, cell division along trichomes often irregular, producing cells with variable shape and width (Figs. 11d, f–j, l), with necridia, 1.9–2.8 µm wide in young trichomes, 2.4–3.5 µm wide in actively dividing trichomes. Hormogonia few-celled (Figs. 11c, h). Cells occasionally isodiametric (1.7–2.6 µm long), shorter than wide in meristematic regions (1.1–1.7 µm long), often with a large central granule. End cells typically rounded, but sometimes elongated and/or irregularly shaped (Fig. 11b).

D1-D1’ helix similar to that of *P. bostrychodes* in structure and sequence, 87 nucleotides long, with basal 3’ unilateral bulge of 9 unpaired nucleotides, opposed on the 5’ strand by a single unpaired cytosine residue, with mid-helix region with one dinucleotide side bulge at position 14–15, one bulge at position 22–25/56–60, one small bulge at position 32–33/48–49, with terminal loop of 4 nucleotides, having terminal sequence GAAA (Fig. 6b); Box B helix 36 nucleotides long, bearing 4 nucleotides at terminal loop, and one small bilateral bulge at position 5/32 (Fig. 7b); V2 helix 24 nucleotides long, with terminal loop of 4 nucleotides; V3 helix 96 nucleotides long, having structure almost identical to that in *P. bostrychodes*, with terminal loop with sequence of GUAA instead of GAGA.

*Etymology:* *olivacea* (L.): olive-green coloration of trichomes.

*Type locality:* Drip Tank Seep Wall site, sandstone seep wall with small moist area and larger pond below the rock face, within Strait Cliffs Formation, in the Grand Staircase-Escalante National Monument (GSENM), Kane County and Garfield County, Utah, USA. Eukaryotic green algae to one side, 37°19’12.79”N- 111°31’50.59”W.
**Holotype to be designated:** BRY 37771, Monte L. Bean Museum, Provo, Utah.

**Reference strain:** GSE-PSE-MK46-15A

**Notes:** *P. olivacea* is phylogenetically separated from all previously described Synechococcales, for which sequence data exist. Comparisons with previously described Synechococcales but for which no sequence data exist reveal no exact matches to this species, so we conclude that this taxon is a species new to science, not a previously described species that should be a new combination into *Pegethrix*. *Leptolyngbya subtilissima* corresponds in part to this taxon, with olive-green coloration, coiled filaments, thin, and colorless and attached sheath; however, filament width is much narrower (1–1.8 µm wide). The zig-zag bent growth form of some trichomes was illustrated in both *Planktolyngbya undulata* Komárek & Kling (1991: 30) and *P. limnetica* (Lemmermann 1898b: 154) Komárková-Legnerová & Cronberg (1992: 23), but trichome dimensions, color, and benthic versus planktonic habitat are significantly different.

*P. olivacea* is most similar to *P. bostrychodes* in the formation of nodules; molecular characteristics such as the presence of a V2 helix and sequence length of conserved domains within the ITS region, particularly the D1-D1' helix, V2 spacer, pre and post Box-B spacer, and V3 helix (Table 5). The 16S rRNA gene phylogeny also supports the close relationship between these two species (Fig. 2). However, the species is distinguished from *P. bostrychodes* in trichome coloration, presence of irregularly-shaped cells, and hormogonia production, as well as nucleotide differences within the ITS region. Color of trichomes and cell characteristics also distinguish *P. olivacea* from other *Pegethrix* sp. Compared with other species of *Pegethrix*, p-distance based on aligned ITS
regions of the species to all other species is in the range of 8%–23%, and the sequence lengths of the ITS between *P. olivacea* and *P. convoluta, P. indistincta* and the Antarctic *Pegethrix* sp. are also very different (Table 5)

**Pegethrix convoluta sp. prov.** (Fig. 12)

* Diagnosis: Differing from other species in the genus based on the frequent formation of loose irregular nodules; with conserved ITS domains most similar to those of *P. indistincta*, but differing in the cytosine residue opposite the basal 3’ unilateral bulge of the D1-D1’ helix (Figs. 6c, d) and the V3 helix sequence and structure (Figs. 9c, d).

* Description: Colony bright blue green, radially spreading, growing into the agar. Filaments fasciculated, long, sometimes singly or doubly false branched (Figs. 12f, g), straight or slightly bent (Figs. 12d–k), frequently forming loose to compact nodules (Figs. 12 a–c), 1.4–3.9–(4.9) µm wide. Sheath firm, colorless, usually attached to trichome, occasionally widened (Fig. 12f), rarely irregular and stratified, up to 1.3 µm wide. Trichomes untapered, not or slightly constricted at distinctly visible cross-walls, with necridia (Figs. 12b, c, e), lacking meristematic zones, with cell division occurring throughout trichome, 1.3–2.5 (3.2) µm wide. Hormogonia few-celled (Figs. 12f, g). Cells slightly shorter than wide to longer than wide, sometimes with a single central granule, with parietal thylakoids, 1.0–2.5–(3.7) µm long. End cells rounded.

D1-D1’ helix of the 16S-23S ITS region 91 nucleotides long, with basal 3’ unilateral bulge of 9 unpaired nucleotides, two bilateral bulges at bp 14–15/69–70 and 26–27/56–57, and a subterminal bilateral bulge at bp 32–33/50–51, with terminal loop with 4
nucleotides, having terminal sequence GAGA (Fig. 6c). No V2 helix present between two tRNA\textsuperscript{Ala} and tRNA\textsuperscript{Ile}. Box B helix with 36 nucleotides, bearing 6 nucleotides at terminal loop (Fig. 7c). V3 helix 110 nucleotides long, with a unilateral bulge at bp 12–14, one small bilateral bulge at bp 32–33/76–77, and three larger bulges at bp 27–29/81–83, 39–41/68–71 and 45–48/61–64, with terminal loop of 6 nucleotides (Fig. 9c).

*Etymology:* *convoluta* (L.): rolled up; referring to the nodules in the trichomes.

*Type locality:* Lower Calf Creek Falls site, large seep wall and waterfall in Navajo Sandstone, in the Grand Staircase-Escalante National Monument (GSENM), Kane County and Garfield County, Utah, USA. Small pool with black soil and microbial layer at the base of seep wall, 37°49′44.77″N - 111°25′12.58″W.

*Holotype to be designated:* BRY 37772, Monte L. Bean Museum, Provo, Utah.

*Reference strains:* GSE-PSE-MK22-07D, GSE-PSE-MK38-07D.

*Notes:* Based on the ecological preference for subaerophytic environments, morphological traits such absence of constrictions at cross-walls, attached sheaths, and rounded apical cells, this species keys to several possible species in *Leptolyngbya*, including *Leptolyngbya* "Albertano/Kováčik -green" 1992, *L. compacta* (Kützing ex Hansgirg 1892b: 88) Komárek in Anagnostidis (2001: 374), *L. subtilissima* (Kützing ex Hansgirg 1892b: 87) Komárek in Anagnostidis (2001: 374), and *L. schmidlei* (Limanowska 1912: 364) Anagnostidis & Komárek (1988: 392). The closest morphospecies using both morphological and ecological criteria is *L. compacta*. Compared to *L. compacta* and *L. subtilissima*, *P. convoluta* has larger trichome width, and isodiametric to shorter than wide cells compared to the isodiametric to longer than wide cells in those two species. Trichomes of *L. schmidlei* have average width larger than
P. convoluta. L. compacta, L. subtilissima and L. schmidlei are poorly understood species based on the absence of illustrations in the original diagnoses and later accounts (Komárek & Anagnostidis 2005), and so these names should likely be avoided in modern taxonomic treatments. We conclude that this species has not been described before in any other genus, and represents a new species to science.

P. convoluta is morphologically similar to P. olivacea, but differs in trichome color and in the sequence of the 16S-23S ITS region (p-distance = 8.23). It is molecularly most similar to P. indistincta, with highly similar secondary structures (identical in the Box B helix) and fairly low p-distance between ITS sequences. P-distance between P. convoluta and P. indistincta is intermediate between levels normally separating species and populations of the same species (p = 4.11). The trichome widths overlap, although P. indistincta typically has wider trichomes than P. convoluta.

Pegethrix indistincta sp. prov. (Fig. 13)

Diagnosis: Morphologically intermediate between other species, differing in the absence of spiraling and nodule formation; with conserved ITS domains most similar to those of P. convoluta, but differing in the adenine residue opposite the basal 3’ unilateral bulge of the D1-D1’ helix (Figs. 6c, d) and the V3 helix sequence and structure (Figs. 9c, d).

Description: Colony bright blue green or olive-green. Filaments long, with variation in width between young and mature trichomes (Figs. 13a–c, i), rarely singly or doubly false branched (Figs. 13c, e), rarely with more than one trichome sharing a common sheath (Figs. 13a, b), 2.3–4.0 µm wide. Sheath firm, usually attached to
trichome, occasionally widened, rarely irregular and stratified (Fig. 13d), absent in immature filaments (Figs. 13f, j). Trichomes untapered, not or slightly constricted at distinctly visible crosswalls, with necridia, with meristematic zones, 1.9–3.3 \( \mu \)m wide. Hormogonia short (Figs. 13h, i). Cells typically isodiametric, often shorter than wide especially in meristematic zones (Fig. 13a), slightly longer than wide in young trichomes (Fig. 13f), (1.3)–1.7–2.7 \( \mu \)m long. End cells rounded.

D1-D1' helix of the 16S-23S ITS region 92 nucleotides long, having shape almost identical to D1-D1' helix of \( P. \) convoluta, but with differences in sequence in the basal portion of the helix (Figs. 6c, d), with basal 3' unilateral bulge of 8 unpaired nucleotides. Box B helix is identical to \( P. \) convolute with 36 nucleotides, bearing 6 nucleotides at terminal loop (Fig. 7c). No V2 helix present between two tRNA\(^{Ala}\) and tRNA\(^{Ile}\). V3 helix 110 nucleotides long, very similar in secondary structure and sequence to \( P. \) convoluta but 5 nucleotides longer (Fig. 9d).

**Etymology:** indistinctus (L.): indistinct, without morphological apomorphies that distinguish it from the other species.

**Type locality:** Lower Calf Creek Falls site, large seep wall and waterfall in Navajo Sandstone, in the Grand Staircase-Escalante National Monument (GSENM), Kane County and Garfield County, Utah, USA. Black compact mat, 37°49'44.77''N - 111°25'12.58''W.

**Holotype to be designated:** BRY 37773, Monte L. Bean Museum, Provo, Utah.

**Reference strains:** GSE-TBC-7GA, GSE-TBC-7GB, GSE-TBD1-7G.

**Notes:** This species is molecularly very similar to \( P. \) convoluta, but differs in the V3 helix sequence and structure and in the apparent lack of nodules in the filaments.
**Pegethrix sp. prov**

*Note:* The two strains representing this taxon were collected from Antarctic water bodies by others. Based on the description, isolated distribution, molecular data and site information provided by those authors (Taton *et al.* 2006, Sabbe *et al.* 2014), this is likely an undescribed species of *Pegethrix*. We do not name it here as we do not have the culture in our possession and consequently cannot prepare valid type materials. The description below is taken from the original work.

*Description:* Filaments rarely false branched. Sheath present. Trichomes brownish, constricted, without necridia, 1.73 ± 0.23 µm wide. Cells are generally isodiametric, 1.76 ± 0.94 µm long, but Brown *et al.* (2010) also reported two different morphotypes within one single species culture, with cells both longer and shorter than wide.

D1-D1' helix 76 nucleotides long, with basal 3' unilateral bulge of 9 unpaired nucleotides, opposed on the 5' strand by a single unpaired adenine residue with mid-helix region with one dinucleotide bilateral bulge at position 14–15/53–54 and 26–27/40–41, one large bulge at position 20–23/44–48, and a terminal loop of 4 nucleotides, having sequence of GAAA (Fig. 6e). Box B helix 37 nucleotides long, bearing 5 nucleotides in terminal loop (Fig. 7d). No V2 helix present between two tRNA^Ala^ and tRNA^Ile^ helix. V3 helix 94 nucleotides long, similar in basal sequence and structure to the V3 helices of *P. convoluta* and *P. indistincta.*
Type locality: Strains were collected in a water body of Larsemann Hills, located in the Prydz Bay region which consists of two major ice-free regions in continental east Antarctica.

Reference strains: ANT.LH70.1, ANT.LMA.1

Francisia gen. prov.

Description: Filaments mostly solitary, at times consolidated into fascicles, with infrequent single false branching. Sheath clear, thin, and firm, occasionally widened. Trichomes untapered, straight, flexuous, or spirally coiled, but not in nodules, slightly constricted at the crosswalls. Cells mostly longer than wide, becoming isodiametric to slightly shorter than wide in dividing trichomes, without aerotopes, rarely with a central granule in the cytoplasm; with parietal thylakoids. Apical cells cylindrical, untapered, rounded, without calyptra. Reproduction by trichome fragmentation via disintegration without necridia.

Etymology: Francisia: named in honor of Francis Drouet, a prominent North American phycologist of the late 20th century whose monographic works still serve as a primary bibliographic reference into the nomenclature of the cyanobacteria.

Type species: Francisia lurida (Gomont) comb. prov.

Francisia lurida (Gomont) comb. prov. (Fig. 14)

Later Synonyms: *Leptolyngbya lurida* (Gomont) Anagnostidis & Komárek 1988:

*Archiv für Hydrobiologie, Supplement* 80: 392

*Locality of reference strain:* Town Most, Czech Republic. Found in dumps, cainozoic clay.

*Epitype to be designated:* BRY 37774, Monte L. Bean Museum, Provo, Utah.

Dried material based on the strain *Francisia lurida* Lukesova 1986/6.

*Reference strain:* *Francisia lurida* Lukesova 1986/6

*Description of reference strain:* Colony reddish brown or brown in actively growing cultures, turning olive-green as culture senesces. Filaments long, without false branching, 2.0–2.6 (3.4) µm wide. Sheath firm, thin, colorless, up to 0.8 µm wide. Trichomes not or slightly constricted at distinct cross-walls, without necredia, lacking meristematic zones, with cell division occurring throughout the length of the trichomes, 1.7–2.1 µm wide. Hormogonia absent. Cells mostly longer than wide (Figs. 14 a–e), rarely isodiametric after division, with parietal thylakoids, with up to three granules usually in the middle of the cell, (2.1) 2.9–3.8 (5.4) µm long. End cells untapered, rounded (Figs. 14 c–e).

D1-D1’ helix 64 nucleotides long, with side loop of 7 nucleotides, at mid-helix with one large bilateral bulge at position 14–17/41–44, with terminal loop of 5 nucleotides. Box B 34 nucleotides long. V2 helix absent.

*Notes:* Our strain conforms very well to the original description of *Phormidium luridum* Gomont, being nearly identical in filament, trichome, and cell dimensions, degree of constriction at crosswalls, and the purplish brownish color in actively growing populations, and with olive green coloration in less actively growing parts of the mat. *P.*
luridum (subsequently Leptolyngbya lurida) was described as having dark blue-green to dull violet or blackish mats with the subsurface layers being greyish green, which is slightly different than having cultures that have thin mats which change color with age. However, our material is so close morphologically to P. luridum, and is additionally from a European location, that we felt it could not be reasonably separated from that species. Komárek & Anagnostidis (2005) indicate that L. lurida is very widespread, and in need of revision. It clearly does not fit Leptolyngbya sensu stricto, which consistently has isodiametric cells and false branching. We do designate an eptitype which we characterize fully to help cement the concept of the species.

F. lurida is morphologically similar to F. hepatica in exponential phase. The 16S rRNA gene phylogeny shows that F. lurida is more closely related to the species cluster of F. hepatica and Antarctic Francisia than to F. fasciculata (Figure 2, Table 8). However, in examining the p-distance of the 16S-23S ITS region, F. lurida is >17.0% different to all other species in the genus, strong evidence of separation of species (Bohunická et al. 2015). F. lurida is further set apart by a D1-D1' helix that has a bilateral bulge in the lower middle region of the helix (Fig. 6i), a shorter Box B helix (Fig. 7e), and the absence of a V2 helix.

Francisia hepatica sp. prov. (Fig. 15)

Diagnosis: F. hepatica is distinguished from the other species of the genus by its meristematic zones and false branching, and by its wide variability in trichome width. Distinguished from both F. fasciculata and F. lurida by the presence of a long (39 nucleotide) V2 helix.
**Description:** Colony brownish or purplish-brown, forming floating, mucilaginous mats in liquid culture. Filaments long or short. Young filaments narrower, 2.3–2.8 µm wide (Figs. 15a, b, f), mature filaments 2.8–3.7 µm wide (Figs. 15c–e, h, i), occasionally with false branching (Fig. 15f). Sheath firm, colorless, thin to occasionally enlarged (Fig. 15f, g, i) and lamellate (Fig. 15d), up to 4.4 µm wide. Trichomes not or slightly constricted at cross-walls, cylindrical throughout or slightly widening towards apices (Fig. 15d), with compacted coils at meristematic zones (Fig. 15d, e), 1.5–2.1 µm wide in young trichomes and 1.7–3.0 µm wide in mature trichomes. Hormogonia absent. Cells longer than wide and occasionally elongated, (2.2) 3.1–4.5 µm long, or in meristematic regions isodiametric, 2.0–2.6 µm long, with one central granule present in both young and mature cells. End cells cylindrical, rounded.

D1-D1' helix 64 nucleotides long, with 3' unilateral bulge of 7 nucleotides, with mid to upper helix with two bilateral bulges, with a terminal loop of 5 nucleotides (5'-AAUCA-3') (Fig. 6f). Box B helix 34 nucleotides long, with a 5'-A:CC mismatch near the base typical of many Synechococcals, with a terminal loop of 4 nucleotides (5'-GAGA-3') (Fig. 7e). V2 helix 39 nucleotides long (Fig. 8c). V3 helix 52 nucleotides long, with terminal loop of 4 nucleotides (5'-UUAG-3') (Fig. 9f).

**Etymology:** hepaticus (L.): of or pertaining to the liver, in reference to the dark reddish brown (liver-colored) color of the colonies.

**Type locality:** Waterfall Kaskady, gorge Sucha Bela, National Park Slovak Paradise, Slovakia. Found on subaerial limestone. No record on specific coordinates available.

**Holotype to be designated:** BRY 37775, Monte L. Bean Museum, Provo, Utah.
Reference strain: *Francisia hepatica* Uher 2000/2452

Notes: The characteristic coloration of the trichomes is similar to *Leptolyngbya cebennensis* (Gomont 1899: 38) Umezaki & Watanabe (1994: 203), *L. carnea* (Kützing *ex* Lemmermann 1910: 206) Anagnostidis & Komárek (1988: 391) and *Lyngbya roseola* Richter *ex* Hansgirg (1892: 491). Trichomes and cell dimensions fit well with *Leptolyngbya cebennensis* and *Lyngbya roseola*, although the characteristic of heterogeneity in width of young and mature trichomes as well as the absence of pseudobranches separate *F. hepatica* from these taxa. *L. carnea* has granulated cell contents, tortuous trichomes and irregular sheath outlines which match filaments of *F. hepatica*. However, *F. hepatica* has wider trichomes, and brownish rather than pinkish coloration. No lamellate sheath was reported in any of the above species.

*F. hepatica* is distinct from the other named *Francisia* species based on p-distance of the ITS region, which is >18% (Table 9). This very well satisfies the criterion for species distinction based on p-distance of >7.0% (Bohunická *et al.* 2015). The closest taxon to *F. hepatica* is a strain that was named *Leptolyngbya frigida* ANT.LH52.2 by the researchers that found it and subsequently reported on it (Taton *et al.* 2006, Sabbe *et al.* 2004). ANT.LH52.2 has high 16S rRNA gene similarity to *F. hepatica* (99.5% identity), an ambiguous dissimilarity in the p-distance of ITS regions (6.9%, which is near the >7.0% cut-off broadly adopted for differentiating species, but well above the <4% level used as evidence of conspecificity), and identical domain lengths in the ITS region (Table 5). The most striking similarity between the two taxa is the near complete similarity of secondary structure of the conserved ITS domains (Figs. 6–9). We do not know the morphology of ANT LH52.2, so a decision as to whether or not to consider it conspecific.
with *F. hepatica* must be postponed until more information is available, but the evidence that this strain and associated sequence belong to *Francisia* is unequivocable.

**Francisia fasciculata sp. prov.** (Fig. 16)

*Diagnosis:* *F. fasciculata* is phenotypically distinct from other *Francisia* species due to its bright blue-green color and fasciculation of trichomes.

*Description:* Colony bright blue green, composed of fasciculated (Fig. 16a) and solitary filaments, growing into the agar. Filaments long, without false branching, frequently slightly coiled and entangled (Figs. 16b, c, g), 2.7–3.2 µm wide. Sheath firm, usually attached to trichome, occasionally distinct, clear, up to 1.3 µm wide (Fig. 16c). Trichomes not constricted at the cross-walls, with necridia, lacking meristematic zones, with cell division occurring throughout trichome, 1.5–2.4 (3.0) µm wide. Hormogonia absent. Cells longer than wide, occasionally isodiametric after division, with peripheral thylakoids, with one large or two smaller central granules, 3.1–4.4 (5.4) µm long. End cells untapered, rounded.

D1-D1’ helix similar to other species in the genus but with a large subapical bilateral bulge caused by multiple mutations (GAUUA→AAAUA at bp 34–38), with terminal loop of 5 adenosine residues (Fig. 6h). Box B helix 39 nucleotides long (Fig. 7g). V2 helix 11 nucleotides long (Fig. 8e). V3 helix 51 nucleotides long, similar in shape to *F. hepatica* but with a larger bilateral bulge (5’-AGAA:GAAA-3’) and several other differences in sequence (Fig. 9h).

*Etymology:* *fasciculatus* (L.): fasciculate, referring to the ability of this species to form fascicle of trichomes.
**Type locality:** Lower Calf Creek Falls site, large seep wall and waterfall in Navajo Sandstone, in the Grand Staircase-Escalante National Monument (GSENM), Kane County and Garfield County, Utah, USA. Found in mats with filamentous algae, 37° 49’ 44.77” N, 111° 25’ 12.58” W.

*Holotype to be designated:* BRY 37776, Monte L. Bean Museum, Provo, Utah.

*Reference strain:* GSE-PSE-MK29-07A

**Notes:** The simple morphology of *F. fasciculata* keys to multiple species of *Leptolyngbya*. The subaerophytic species, *Leptolyngbya gracillima* (Hansgirg 1892b: 41) Anagnostidis & Komárek (1988: 391) is similar in dimensions, but differed in the possession of false branching. *Leptolyngbya lagerheimii* (Gomont ex Gomont 1892: 147) Anagnostidis & Komárek (1988: 391) is similar but was described from stagnant waters in a tropical climate (Brazil). *Leptolyngbya subtruncata* (Woronichin 1930: 69) Anagnostidis (2001: 368) was close to *F. fasciculata* in size of cells, but was described as having truncate apical cells. *L. subtruncata* is an incompletely described species, and is sufficiently ecologically different from *F. fasciculata* that we do not feel using this name is appropriate for our populations. Several other species was also described to have similar morphology, especially the irregular coils, including *Leptolyngbya fritschii* Anagnostidis (2001: 366), *Leptolyngbya mucosa* (Gardner 1927: 43) Anagnostidis & Komárek (1988: 392), *Leptolyngbya patinae* (Schwabe 1944: 180) Anagnostidis (2001: 367), *Leptolyngbya spiralis* and *Lyngbya jacutica* Kisselev (1935:73) ex Elenkin (1949: 1663). All of these species were described from habitats very different from the temperate climate, subaerophytic habitat in which *F. fasciculata* was found, and it appears to be a
new species. It is sufficiently morphologically similar to these species that it could not be assigned to any one of them with confidence.

The morphological separation of *F. fasciculata* from the other species in the genus is supported by molecular evidence, in which the D1-D1’ helix of *F. fasciculata* is structurally different from others, the Box B helix is the longest of any of the *Francisia* species (Fig. 7g, Table 5), the V2 helix is very short (Fig. 8e), and the V3 helix is shorter than *F. hepatica* (Fig. 9h, Table 5). The p-distance between the ITS region of this species and the other taxa is >25% (Table 9).

**Cartusia gen. prov.**

*Description:* Filaments straight or flexuous, sometimes with more than one trichome in a common sheath, sometimes forming fascicles of trichomes, without false branching. Sheaths firm, thin, colorless. Trichomes not tapering, not or only slightly constricted at the crosswalls, up to 3.5 µm wide. Cells mostly shorter than wide up to isodiametric.

*Etymology:* named for the ruins of the Cartusian Monastery in National Park Slovak Paradise, from which the reference strain was collected.

*Type species:* *Cartusia fontana* comb. prov.

**Cartusia fontana comb. prov.** (Fig. 17)


Locality of reference strain: Ruins of the Cartusian Monaster, National Park Slovak Paradise, Slovakia. Found in pale green biofilm on wall at interior of church. No record on specific coordinates was available.

Epitype to be designated: BRY 37777, Monte L. Bean Museum, Provo, Utah.

Dried material based on the strain Cartusia fontana Kovacik 1999/1.

Reference strain: Cartusia fontana Kovacik 1999/1.

Description: Colony bright blue-green, becoming olive-green with age, fasciculated. Filaments straight or flexuous, sometimes with more than one trichome in a common sheath (Fig. 17a), sometimes forming fascicles of trichomes, without false branching, with variations in width between young and mature filaments (Figs. 17a, c), up to 5.4 μm wide in filaments with sheath. Sheath colorless, firm, usually thin, but occasionally widened (Fig. 17h), up to 1.6 μm wide. Trichomes not or slightly constricted at crosswalls, with necredia, with meristematic zones often occurring in wider trichomes (Figs. 17a, b, h), young trichomes narrower, 1.8–2.7 μm wide (Figs. 17b–e), mature trichomes larger, 2.7–3.5 μm wide (Figs. 17a, i). End cells rounded (Figs. 17c–e). Cells in young trichomes isodiametric or slightly shorter than wide, 1.3–2.0 μm long, in mature trichomes shorter than wide, 1.0–1.3 μm long, with one large single central granule commonly visible in cells.

D1-D1’ helix 107 nucleotides long, with two side branches (Fig. 6m), a length and structure unique within the family Oculatellaceae, with basal 3’ unilateral bulge of 8 nucleotides (Fig. 6m). Box B helix 60 nucleotides long, with one large bilateral bulge at
position 15–19/42–46. No V2 helix present. V3 helix not identified due to sequence truncation.

**Notes:** The strictly subaerophytic condition where the species was found limited our comparison to only subaerophytic species. Three morphospecies of *Leptolyngbya* were similar to our strain: *L. fontana* (Hansgirg 1892: 85) Komárek in Anagnostidis (2001: 374), *Leptolyngbya cataractarum* (Rabenhorst 1853 ex Hansgirg 1885: 292) Komárek (2001: 374), and *L. fallax* (Hansgirg ex Forti 1907: 185) Komárek (2001: 374).

*L. fontana* was a perfect match to our strain, matching in every regard to the morphological and ecological characteristics. Furthermore, *L. fontana* was described from mountainous regions of the Czech Republic, a close geographical match to National Park Slovak Paradise. *L. fontana* was incompletely described (no illustration, few details on morphology), but given the absence of any variance in characters, we feel that it is good to use this species epithet for our strain. The other two species are also not illustrated, but differ morphologically in terms of sheath characteristics and cell morphology.

Compared to other species belonging to Oculatellaceae, the morphology of *C. fontana* is similar to *Francisia fasiculata* and several species of *Pegethrix* (*P. bostrychodes*, *P. convolute* and *P. indistincta*). *C. fontana* was observed to have meristematic zones of cell division, which were absent in *F. fasiculata*, as well as cells shorter than wide. With regards to *Pegethrix* species, *C. fontana* does not form pseudobranches or nodules. Furthermore, they are separated by their position on the 16S rRNA phylogeny (Fig. 2), and the distinctive sequences and shape of the secondary structures of ITS regions. The D1-D1’ helix in *C. fontana* is distinct from the typical

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structure found in the vast majority of Synechococcales, with several small helices branching off from large internal loop structures (Fig. 6m). This structure was probably formed after a large insertion and several mutations starting from position 14–41/54–86, as the basal structure and sequence at position 1–13/87–107, as well as of the terminal loop and helice (42–45/50–53) in C. fontana was observed to be typical for the Synechococcales D1-D1' helix. Box B helix structure of the species is also unique in length, much longer compared to other species described in this family (Table 5).

**Josephinia gen. prov.**

*Description:* Filaments with or without sheath, straight, flexuous or spirally coiled. Sheath thin, firm, colorless when present. Trichomes untapered, not constricted to slightly constricted at the crosswalls, with thin translucent crosswalls that are sometimes hardly visible, under 3 µm wid, without necrdia and hormogonia. Cells longer than wide. End cells rounded. Phylogenetically distinct from all other genera in the Oculatellaceae. *Etymology:* named for Josephine Tilden, a prominent American cyanobacteriologist of the mid-20th century.

*Type species:* **Josephinia torsiva** sp. prov

**Josephinia torsiva sp. prov** (Fig. 18)

*Description:* Colony fasciculated, spreading irregularly, forming irregular clumps on the agar, with filaments penetrating the agar, bright blue green, becoming olive green with age. Filaments with rare false branching in older cultures (Fig. 18e), often entangled (Figs. 18b, d), 1.7–2.5 µm wide. Sheath firm, thin, colorless, up to 0.7 µm wide, often
not evident. Trichomes untapered, straight, curved, or sometimes spirally coiled (Fig. 18a, b), slightly constricted at the cross-walls, with cell division occurring throughout the trichome, 1.4–1.9 µm wide. Hormogonia and necridia absent. Cells rarely isodiametric, mostly longer than wide, with contents usually homogeneous, without granulation, varying from 1.5–2.7 µm long. End cells rounded (Figs. 18c–e).

D1-D1’ helix 65 nucleotides long, with basal 3’ unilateral bulge of 6 nucleotides, with a large bilateral bulge in mid-helix at position 20–22/37–40, with terminal loop of 4 nucleotides (Fig. 6n). With ribosomal operons with both tRNA\(^{\text{Ala}}\) and tRNA\(^{\text{Ile}}\) genes and with no tRNA genes amplified and sequenced. Box B helix 44 nucleotides long in operons without tRNA genes, 49 nucleotides in operons with tRNA genes (Fig. 7m), with variable terminal loops of 3–4 nucleotides, respectively. V2 helix not present. V3 helix 92 nucleotides long, with three bilateral bulges near the base, with a terminal 4 nucleotides loop, with a unique basal clamp shorter than all other V3 helices due to a A:A mismatch present in both reference strains (5’-GUC:GAC-3’ compared to 5’-UGUC:GACA in others) (Fig. 9l)

*Etymology:* *torsivus* (L.): spirally coiled.

*Type locality:* *Josephinia torsiva* Hubel 1974/233 was collected in bay Barther Bodden near bridge Meiningen Germany.

*Paratype locality:* *Josephinia torsiva* Uher 1998/13d was collected in gorge Prielom Hornadu, National Park Slovak Paradise, Slovakia, found in limestone wall near the tourist walkway.

*Holotype to be designated:* BRY 37778, Monte L. Bean Museum, Provo, Utah.

*Paratype to be designated:* BRY 37779, Monte L. Bean Museum, Provo, Utah.

Notes: Spirally coiled and contorted filaments as well as the variation in cell length from isodiametric to distinctly longer than wide in this species are considered most characteristic. This species is a close morphological match to *Leptolyngbya thermobia* Anagnostidis (2001: 368) and *L. lagerheimii* (Gomont 1890 ex Gomont (1892: 147) Anagnostidis & Komárek (1988: 391). *L. thermobia* was described from thermal waters, and is consequently physiologically distinct from *J. torsiva*.

*L. lagerheimii* is very similar to *J. torsiva*, having similar cell dimensions and loose spiral coiling. *L. lagerheimii* was originally described from Brazil as *Spirocoleus lagerheimii* Möbius (1889: 312). Gomont 1890 transferred the species into *Lyngbya lagerheimii* Gomont (1890: 354) ex Gomont (1892:147), and this species was again transferred into *Leptolyngbya* in the same publication in which that genus was described, designating the type as *L. boryana*. When Leptolyngbya was described, it was not realized that *Spirocoleus lagerheimii* had been validated post-starting point by Crow: *Spirocoleus lagerheimii* (Gomont) Möbius ex Crow (1927:147). *Leptolyngbya* was subsequently conserved against *Spirocoleus* because it was in much wider use (McNeill *et al.* 2006). The name *Spirocoleus* is available for use if it can be documented to be phylogenetically outside of *Leptolyngbya sensu stricto*. However, the loose spirals in trichomes are a trait that is not confined to *Josephinia* or *Spirocoleus*, and so we do not have convincing evidence that these European strains isolated from subaerophytic habitats in Europe belong to the same lineage as *S. lagerheimii* isolated from stagnant waters in Brazil. If *S. lagerheimii* could be isolated from Brazil near the type locality, and
if its sequence places it in equivalency with *J. torsiva*, then it would be necessary to transfer *J. torsiva* to *Spirocoleus*. For now we feel it is more conservative to simply describe this lineage as a genus new to science.

**Josephinia nuda sp. prov.** (Fig. 19)

*Description:* Colony fasciculated, not penetrating the agar, forming a compact mat, with small evenly distributed, rounded clumps of trichomes, bright blue-green. Filaments without false branching. Sheath usually absent, thin, firm and colorless when present, 1.0–1.4 µm wide. Trichomes pale blue-green, clearly constricted at cross-walls, cell division occurring throughout the trichome, 1.0–1.2 µm wide. Hormogonia and necridia absent. Cells always longer than wide, sometimes elongated (Fig. 19j), with mostly homogeneous content, rarely with small granules at polar regions of cells, 2.0–4.3 (5.1) long. Apical cells cylindrically rounded, sometimes apically swollen (Figs. 19g, h). D1–D1’ helix 65 nucleotides long, having basal 3’ unilateral bulge of 7 nucleotides, with terminal loop of 5 nucleotides (Fig. 6o). Box B helix 38 nucleotides long, with terminal loop 8 nucleotides (Fig. 7n). V2 helix 77 nucleotides long, with one large bulge at position 24–26/51–55, with terminal loop of 6 nucleotides (Fig. 8i). V3 helix unavailable due to sequence truncation.

*Etymology:* *nudus* (L.): naked, referring to the frequent absence of sheath.

*Type locality:* Wet stone wall in Stansstaad, Switzerland. No record on specific coordinates was available.

*Holotype to be designated:* BRY 37780, Monte L. Bean Museum, Provo, Utah.

*Reference strain:* Zehnder 1965/U-I40
Notes: This species is character poor, lacking features useful for recognition such as necridia, hormogonia, coiling or nodule formation, or false branching. Given its lack of features, it could fit a number of species belonging within Leptolyngbyaceae *sensu* Komárek & Anagnostidis (2005). The cell morphology (length to width ratio, dimensions) is similar to most *Oculatella* species, but *J. nuda* lacks the characteristic apical cells of that genus. The closest fit is *L. hansgirgiana* Komárek in Anagnostidis (2001: 374), but this taxon has a complicated taxonomic history. It was first described as *Leptothrix tenuissima* Nägele ex Kützing (1849: 265), then transferred to *Hypheothrix tenuissima* (Nägele) Rabenhorst (1865: 77, and finally to *Lyngbya tenuissima* (Nägele) Hansgirg (1891: 346). However, these were all pre-starting point names (i.e. pre-Gomont 1892, see McNeill *et al.* 2015). The taxon was validated post-starting point as *Lyngbya tenuissima* (Nägele) Hansgirg ex Hansgirg 1892. When many of the thin simple Oscillatoriales were transferred into *Leptolyngbya* (Komárek & Anagnostidis 1988), *Plectonema tenuissimum* Gardner (1927) was used as the basionym for *Leptolyngbya tenuissimum* (Gardner) Komárek & Anagnostidis (1988), and consequently was occupied at the time of further revision, when Nägele’s taxon was given a new name, *L. hansgirgiana*. Our taxon is a close fit to the protologue in Kützing (0.7 um wide, blue-green color only, terrestrial), but the description in Komárek & Anagnostidis (2005) has been broadened considerably based on the many subsequent records by multiple authors and is consequently not as apparent a fit. We hesitate to use this taxon with complicated, long history, broadly circumscribed morphology, and absence of sequence data or clear holotype material as the basis for the species name for Zehnder’s strain shown here to belong to *Josephinia*, and thus have created a new name.
*J. nuda* is considered to be distinctive from other species within the Oculatellaceae with thin trichomes and high variation in cell length within trichomes. Molecularly, the species is closely related to *J. torsiva*, with very high similarity in the 16S rRNA gene (99.1% identity, Table 10). However, substantial difference exists in the sequence and secondary structure of the D1-D1’ helix, Box B helix, and especially in the presence of the long V2 helix (Fig. 8i) between *J. nuda* and *J. torsiva*.

**Gardneria gen.prov.**

*Description:* Filaments simple, unbranched, with variation in width between post-hormogonial and mature filaments. Sheath firm, thin, colorless. Trichomes constricted at cross-walls, rarely tapering. Hormogonia and necridia present. Cells with parietal thylakoids, shorter than wide to longer than wide, mostly isodiametric.

*Etymology:* named for Nathaniel L. Gardner, a prominent American cyanobacteriologist of the early 20th century.

*Type species:* *Gardneria angustata* sp. prov.

**Gardneria angustata sp. prov.* (Fig. 20)

*Description:* Colony fasciculated, penetrating the agar, bright blue-green. Filaments lacking false branching, with variation in width between post-hormogonial and mature filaments, up to 3.9 µm wide (Figs. 20b–e). Sheath firm, thin, colorless, up to 0.7 µm wide. Trichomes constricted at cross-walls, rarely tapering (Figs. 20b, h), post-hormogonial trichomes 1.8–2.9 µm wide, mature trichomes 3.3–3.9 µm wide (Figs. 20b,
Hormogonia (Figs. 20f, i) and necridia (Fig. 20e) present. Cells with parietal thylakoids, with up to 3 small granules per cell, sometimes with one small orange granule, commonly cylindrical, especially in young trichomes, becoming isodiametric in actively growing trichomes; in more mature barrel-shaped, and pancake-like in meristematic zones (Fig. 20c), 2.2–5.7 µm long.

D1-D1’ helix similar to *Francisia* spp., 64 nucleotides long, with basal 3’ unilateral bulge 7 nucleotides long, with one large bilateral bulge at position 21–23/33–37 isolated from the terminal loop by two GC pairs, with terminal loop of 5 nucleotides (ACAGU) (Fig. 6p). Box B helix 41 nucleotides long, with a 3’ bilateral bulge at position 5/32–37, with one small bilateral bulge at position 10–11/26–27, with terminal loop of 4 nucleotides (Fig. 7o). V2 helix 20 nucleotides long, with terminal loop 4 nucleotides (Fig. 8k). V3 helix 96 nucleotides long, with six small bilateral bulges due to nucleotide mismatches, with terminal loop of 4 nucleotides (Fig. 9m).

_Etymology:_ angustatus (L.): narrowed, referring to the trichomes which are occasionally narrowed towards the end, as well as the narrowed hormogonia.

_Type locality:_ Water fall in El Yunque National Forest, Puerto Rico, 18°27.811’ N- 66°7.005’W. Benthic algae found in restaurant.

_Holotype to be designated:_ BRY 37781, Monte L. Bean Museum, Provo, Utah.

_Reference strain:_ EY01-AM2

_Notes:_ The heterogeneity in trichome width and absence of false branches is characteristic for this monotypic genus, and could be compared to tapering species in *Leptolyngbya: L. tenuis* (Gomont 1892) Anagnostidis & Komárek 1988, *L. laminosa*
(Gomont) Anagnostidis & Komárek 1988 and *L. fragilis* (Gomont) Anagnostidis & Komárek 1988. However, in every comparison the trichomes of *G. angustata* are wider. The secondary structure of the ITS region in *G. angustata* is indistinct in the D1-D1’ helix (which is very similar to those in the *Francisia* spp.) and V3 helix (which is very similar to those in the *Pegethrix* species). However, the Box B helix structure is very distinctive, being the only Box B helix with a basal unilateral bulge. Phylogenetically, the genus is sister to *Josephinia* in the 16S rRNA analysis (Fig. 1), but it is separate from that taxon according to the *rpoC1* phylogeny. It also has very low 16S rRNA gene sequence identity with the other genera in the Oculatellaceae (>94.5% for all genera), strong evidence that it represents a separate genus-level lineage. However, morphologically it is character-poor and will likely be difficult to determine in studies employing only light microscopy.

**Kaiparowitsia gen. prov**

*Description:* Filaments flexuous, entangled, sometimes fasciculated, with one to several trichomes in a common sheath, unbranched. Sheath thin, colorless. Trichomes bent, flexuous, entangled, sometimes forming nodules, less than 2.0 µm wide. Hormogonia and necridia absent. Cells cylindrical, longer than wide, sometimes with outgrowths. End cells rounded.

*Etymology:* Named for the Kaiparowits Plateau, a major geological formation in the Grand Staircase-Escalante National Monument from which the taxon was isolated.

*Type species:* *Kaiparowitsia implicata* sp. prov
**Kaiparowitsia implicata sp. prov** (Fig. 21)

*Description:* Colony radially spreading, becoming mucilaginous with age, bright blue-green, green, or olive green. Filaments flexuous, entangled, sometimes fasciculated (Fig. 21a), sometimes with multiple trichomes in a common sheath (Figs. 21i, j), without false branching, 1.3–1.5–(2.3) µm wide. Sheath thin, occasionally expanded (Fig. 21c), sometimes absent, up to 0.7 µm wide. Trichomes untapered, bent, flexuous, entangled, sometimes forming nodules (Figs. 21b, e), with cell division occurring throughout trichome length, not constricted at the indistinct cross-walls, 1.3–1.5 µm wide.

Hormogonia and necridia absent. Cells cylindrical, longer than wide, with *Arthronema*-like involution cells with outgrowths (Figs. 21g, h, at arrows), with homogenous content, typically 3.1–3.7 µm long, but abnormally long cells (as much as 20 µm) commonly encountered. End cells rounded or conically rounded.

D1-D1’ helix much longer than other members of Oculatellaceae, 142 nucleotides long, with basal 3’ unilateral bulge of 7 nucleotides, with additional large 3’ unilateral bulge at nucleotides 100–113, with 5–6 smaller bulges due to adenosine and guanosine mismatches of 3–5 nucleotides which possibly could form non-canonical pairings, with terminal loop of 6 nucleotides (5’-UUAAUU-3’) (Figs. 6q, r). Box B helix 36 nucleotides long, with one small basal bulge similar to other Oculatellaceae, but with different sequence (5’-C:AA-3’), with terminal loop of 4 nucleotides (Fig. 7p). V2 helix absent.

V3 helix 113 nucleotides long (Fig. 9n).

*Etymology:* *implicatus* (L): tangled

*Type locality:* Camp Spring Site 1, small horizontal seep wall in sandstone of Kaiparowits Plateau formation, colored intensely orange by iron bacteria, in the Grand
Staircase-Escalante National Monument (GSENM), Kane County and Garfield County, Utah, USA. Found in wet soil under the overhang with pH 7.7–8.1 (with mottled coloration), 37°32'35" N latitude - 111°38'26" W longitude.

**Holotype to be designated:** BRY 37782, Monte L. Bean Museum, Provo, Utah.

**Reference strains:** GSE-PSE-MK54-09C, GSE-TBC-9CA, GSE-TBC-9CA2

**Notes on other morphospecies:** *Kaiparowitsia implicata* is distinguished from previously described species in the Synechococcales by the characteristic involution cells with outgrowths on the side of the cell. These strange cells are reported only for *L. yellowstonensis* and *Oculatella subterranea* Zammit, Billi and Albertano 2012 (see Albertano & Grilli-Caiola 1988 and Komárek & Anagnostidis (2005: 222)). However, these two species are molecularly distinct from *Kaiparowitsia*.

*K. implicata* is distinguished molecularly by the exceptionally long D1-D1’ helix (Fig. 6q, r, Table 5), and a distinguishing unilateral bulge at nucleotide position 100–113. The structure of the basal helix and 3’ unilateral bulge was retained, but a transition mutation occurred in the in the bulge (nucleotide 131) of C→U which was unique among Oculatellaceae that have been sequenced (Fig. 6q, r). Box B helix structure was also unique among the taxa sequenced. V3 helices in *K. implicata* are of great length, comparable only to the *Pegethrix* spp.

**Thallothrix gen. prov.**

*Description:* Filaments untapered or clearly widened in basal regions, with repeated single and double false branching. Sheath thin to widened, colorless. Trichomes
not constricted to constricted at distinctly visible cross-walls, <4.0 µm wide. Cells shorter than wide to longer than wide, with parietal thylakoids.

*Etymology:* *thallos* (Gr.): branch, *thrix* (Gr.): hair, referring to the extensively branched, thin trichomes.

*Type species:* *Thallothrix obliquedivisa* sp. prov.

**Thallothrix obliquedivisa sp. prov.** (Fig. 22)

*Description:* Colony radially spreading, compact, firm, leathery, sometimes mounded, dark green, with yellowed margins near senescence. Filaments, untapered to slightly tapered (Figs. 22c, f, g), with repeated single and double false branching (Figs. 22 a–f), 2.0–3.2 (3.9) µm wide. Sheath usually thin, soft, colorless, rarely extended past trichome apex, visible mostly at points of branching (Fig. 22e), up to 1.3 µm wide. Trichomes false branched, with some branches erect and almost perpendicular to the original axis of the trichome (Fig. 22h), not constricted at distinctly visible cross-walls, occasionally becoming almost biseriate due to oblique division and compression of cells (Fig. 22i), 2.0–2.9 µm wide. Necridia present, hormogonia rare. Cells cylindrical, shorter than wide to longer than wide, with parietal thylakoids, often with one large central granule, 1.8–2.7–(3.7) µm long.

D1-D1’ helix 63 nucleotides long, having basal sequences similar to *Thallothrix* sp. WMT-WP7-NPA but differing in sequence and length in terminal region. Side loop similar in sequence to other species of Oculatellaceae. Mid-helix with one small bulge at position 14–15/41–42 and one large bulge at position 20–23/32–36, separated to the terminal loop by GC base pairs at position 24–25/30–31. Box B helix 49 nucleotides long.
with one small bulge at position 5/44–45; terminal loop of 5 nucleotides. V2 between two tRNA<sub>Ala</sub> and tRNA<sub>Ile</sub> present, 29 nucleotides long, with one bulge at position 5–6/23–25; terminal loop of 4 nucleotides. V3 61 nucleotides long, with three bulges at position 5/55–57, 10–11/47–50 and 23/34–35; terminal loop 6 nucleotides long.

*Etymology:* *obliquus* (L.): oblique; divisus (L.) divided; referring to the obliquely dividing cells that give rise to biseriate trichomes.

*Type locality:* Big Horn seep site, small horizontal seep wall in sandstone of Kaiparowits formation, colored intensely orange by iron bacteria, in the Grand Staircase-Escalante National Monument (GSENEM), Kane County and Garfield County, Utah, USA. Found in wet soil under the overhang with pH 7.7–8.1 (with mottled coloration), 37°32'35"N - 111°38'26"W; hanging garden on a sandstone rock wall in Carmel-Page formation, partly covered with organic debris material, mosses and vascular plants, in the Grand Staircase-Escalante National Monument (GSENEM), Kane County and Garfield County, Utah, USA. Found in cyanobacterial mat (8A) or olive calcified mat (8B), 37°43.012' N - 111°28.273' W.

*Holotype to be designated:* BRY 37783, Monte L. Bean Museum, Provo, Utah.

*Reference strains:* GSE-PSE-MK28-08A, GSE-PSE-MK23-08B

*Notes:* Attenuated trichomes, erect false branches, and oblique division leading to biseriate trichomes are the defining morphological characteristics of *T. obliquedivisa*. No previously described species is a match for these characteristics. The secondary structures of the conserved ITS domains are unique in comparison with the same structures from other Oculatellaceae, but without exceptional features; they look similar to other Oculatellaceae in the basal clamps and absence of side branches.
T. obliquedivisa and Thallothrix species WMT-WP7-NPA collected from the White Mountains share 99.05% identity in the 16S rRNA gene sequence, but they greatly differ in the ITS region (p=18.3%), and in the different lengths of the D1-D1’, Box B, and V2 helices (Figs. 6–8). Their close phylogenetic relationship is supported by the 16S rRNA gene phylogeny (Fig. 1), but they are evidently different species.

Thallothrix sp. prov

Description: This strain was lost after sequencing, and has no morphological description. The lengths of the D1-D1’, Box B, and V2 helices are different from T. obliquedivisa and T. radians, and so we consider this a different species, but we are not able to describe it at this time.

Collection locality: White Mountain Wilderness, California, USA

Thallothrix radians sp. prov.

Description: Colony mounded, leathery, dark olive-brown or dirty olive-green. Filaments relatively short, often clearly widened in meristematic zones (Fig. 23d), sometimes forming radial colonies (Figs. 23a, b), with characteristic consecutive false branching (Fig. 23f). Sheath usually thin and scarcely visible to widened and evident, rarely extended past the apical end of trichome. Trichomes brownish when actively growing, olive-green when approaching senescence, constricted at distinctly visible cross-walls, occasionally forming compact rope-like coils at meristematic or active division zone (Figs. g–j), 1.8–3.7 µm wide. Necridia absent. Hormogonia without sheaths (Fig. 23k). Cells in hormogonia and young filaments isodiametric, becoming shorter than
wide with maturation 1.2–2.2 µm um long, often with one large central granule. Apical cell shape rounded, conically rounded, or pancake-like rounded.

D1-D1’ helix 81 nucleotides long, with one large bulge at position 20–23/50–54, with terminal loop of 4 nucleotides (Fig. 6I). Box B helix 33 nucleotides long (Fig. 7k). V2 helix 12 nucleotides long, with one large terminal loop of 6 nucleotides (Fig. 8i). V3 helix 59 nucleotides long, similar in length and structure to the V3 helix in the other Thallothrix spp. (Fig. 9i–k).

Etymology: radians (L.): radiating, for the radiating pattern of filaments in the colonies.

Type locality: Lower Calf Creek Falls site, large seep wall and waterfall in Navajo Sandstone, in the Grand Staircase-Escalante National Monument (GSENM), Kane County and Garfield County, Utah, USA. Mats in waterfall, 37°49′44.77”N - 111°25′12.58”W.

Holotype to be designated: BRY 37784, Monte L. Bean Museum, Provo, Utah.

Reference strain: GSE-UNK-7R, GSE-TBD6-7R

Notes: Our strain closely resembles Leptolyngbya gracillima (Zopf ex Hansgirg 1892: 41) Anagnostidis & Komárek (1988: 391). It matches the recent description of this widely-reported taxon in Komárek & Anagnostidis (2005), with the exception that cells in that species are isodiametric to longer than wide instead of isodiametric to shorter than wide, as seen in T. radians. The radial arrangements of filaments and the occasional coiling have also not been observed in L. gracillima material. This taxon is interesting as it was originally described as Glaucothrix gracillima Zopf (1882:44), named Plectonema gracillimum Hansgirg (1887: 108), and then validated post-starting point (Gomont 1892)
by Hansgirg (1892:41). A lectotype specimen for the taxon was designated by Drouet (1968:41), who chose a herbarium sample collected by P. Richter from wet walls in a warm spring near Anger, Bavaria, Germany deposited in the Drouet Collection at the Smithsonian Institution (Alg. Exs. No. 593a). This was perhaps an unfortunate choice for the lectotype, as the original material of Zopf was not collected from a warm spring, but it remains a validly designated lectotype. Even though the descriptions of Zopf (1882) and subsequent authors matches our taxon fairly closely, this lectotype does not, as thermal springs in Germany are a very different habitat than a waterfall in a desert in North America.

*T. radians* is morphologically very distinct from *T. obliquedivisa*, and we considered putting the two species into separate genera. However, they were similar in 16S rRNA gene sequence (96.45%, Table 11), had very similar lengths of conserved domains in the ITS region (Table 5 and 12), and had similar ITS structures.

**DISCUSSION**

*Taxonomic decision-making.* Historically, families of cyanobacteria (and indeed all taxa governed by the Botanical Code, McNeill *et al.* 2015) were defined as assemblages of genera that shared morphological characteristics, i.e. they were defined phenetically on the basis of similarity. This was true for genera as well, which were defined as assemblages of morphologically similar species. With the advent of phylogenetic analysis based on molecular data, we have moved to a different standard for genera, families, and all other higher-level taxa. This is problematic for taxonomists, as
these higher level taxa are being recognized phylogenetically, even though morphological separation is not always evident. In this work, we could see the family level clades defined by phylogenetic analysis, but the families did not possess morphological synapomorphies that allow morphological recognition of the families. We did observe molecular synapomorphies in the 16S rRNA gene (Table 4) that help delineate family membership, but they were not 100% reliable for classification. The best we can say for recognition of Cyanobacterial families in the Synechococcales is that they form discreet, supported phylogenetic clusters of genera, many of which can be recognized morphologically, or at least with a combination of morphology and phylogeny.

Taxonomists working in other groups have been faced with the same dilemma. Adeolu & Gupta (2013) recognized two families in the Neisseriales on the basis of phylogeny, but defined the families based on conserved signature indels in 20 proteins. Fučíková et al. (2014) described 10 new families in the Sphaeropleales, an order of coccoid green algae, bringing the total number of families in the order to 17, justified almost entirely on phylogenetic grounds (although some minor morphological separation between some families exists). Many of the families in the Sphaeropleales are presently monotypic (Fučíková et al. 2014). Some workers might ask why we should recognize so many families in this single order. The answer is that if families must be monophyletic assemblages of genera, then the choice facing these taxonomists was to combine a number of pre-existing, well-established families into fewer families in order to make monophyletic taxa, or recognize the new phylogenetically distinct lineages in separate families. We agree with the latter approach. We feel that a more finely divided hierarchical taxonomy is preferable to one in which a multitude of species are housed in
relatively fewer higher level taxa, a guideline applicable to both genera and families. This is in agreement with the spirit of generic delineation by Anagnostidis & Komárek (1985), in which they recommend the recognition of many narrowly defined genera over a few broadly defined large genera. Families should be monophyletic assemblages of genera, but could in order to preserve monophyly be monotypic.

Genera are best defined as monophyletic clades containing one or more species which have at least some morphological characters separating them from other genera. Morphologically similar (or even indistinguishable) taxa in distinctly phylogenetically separated clades should also be described as separate genera if including them in a single genus would result in the loss of morphologically and molecularly distinct genera. In an example already mentioned but not resolved in this paper, combining the three clades of *Leptolyngbya*-like species into *Leptolyngbya sensu stricto* would require either transfer of *Plectolyngbya* species into *Leptolyngbya*, or recognition of a group of *Leptolyngbya* outside of the *Leptolyngbya/Plectolyngbya* clade as a new genus (Fig. 1). This is not an easy decision, and so other characters must be considered to make a decision that will result in recognition of evolutionary lineages in a taxonomy that is stable in the face of ever-increasing polyphasic characterization of taxa. One such character is 16S rRNA gene identity. Bacteriologists currently accept the idea that genetic similarity less than 94.5% is strong evidence that the compared strains belong to different genera (Yarza *et al.* 2014). The problem with accepting this cutoff is that identities above 94.5% cannot be used as evidence that compared strains belong in the same genus, and so in instances when similarities are high it is not useful for recognition of genera.
We have found that the secondary structures of the 16S–23S ITS region often have genus-specific structures that provide additional evidence of deep genetic separation of lineages. This was the case for all of the genera recognized and described in this paper (Figs 6–9). In addition, the lengths of conserved domains in the ITS can also provide evidence of separateness (Table 5). For example, whereas *Pegethrix* and *Cartusia* have high 16S rRNA sequence similarity (96.20%, Table 13), an examination of the length of ITS domains shows that *Cartusia* differs greatly in the length of the D1-D1’ helix, the pre-Box B spacer, Box B helix, and post-Box-B spacer (Table 5). Indeed the long length of the post-Box B spacer of *Cartusia* sets it apart from all other genera in the Oculatellaceae.

In this study, in all instances where similarity values between pairs of species were below 96%, they were in separate genera. In addition, no two genera had species more than 97% similar. However, pairwise comparisons of species within genera could be found to have identities of 96–97%, as could a few comparisons of species in different genera. As has been stated in many previous papers, percent identity can support or inform taxonomic decisions, but it should not be the only basis for such decision-making.

Separation of species within the same genera of cyanobacteria is generally less ambiguous than the separation of genera. Considerably more attention has been devoted in the literature to species concepts (Mishler & Theriot 2000, Rosselló-Mora & Aman 2001, Johansen & Casamatta 2005), and to criteria for species recognition (Řeháková et al. 2007, Osorio-Santos et al. 2014, Johansen et al. 2014, Pietrasiak et al. 2014, Bohunická et al. 2015). The 16S rRNA gene is often insufficient to resolve species of cyanobacteria (Perkerson III et al. 2011, Bohunická et al. 2015). However the 16S–23S
ITS region is increasingly useful for species separation. This region provides two criteria useful for recognition of species 1) the secondary structures can differ markedly between species, and always differ at least in some minor way, and 2) the p-distance between members of the same species is generally below 4.0%, with an average below 2.0%, while between members of different species the difference is over 7.0%, with an average over 9.0%, providing a large discontinuity that is easy to recognize (Erwin & Thacker 2008, Osorio-Santos et al. 2014, Bohunická et al. 2015).

We have found that morphological, biogeographical, ecological, and molecular evidence are often congruent, and taken together allow for clearer recognition of morphologically similar species. This is the essence of the polyphasic approach, and we have followed it in making our species-level determinations. For example, *P. bostrychodes* is molecularly most similar to *P. olivacea*, but the p-distance between the two species is 8.23% (Table 7), a clear separation. P-distance between *P. bostrychodes* and other species in the genus is even greater. All three *Francisia* species recognized in this paper are at least 7.0% dissimilar in ITS sequence, and can have p-distances as high as 25.0% (Table 9). The only instance in which this criterion was ambiguous was in the case of the separation of *P. convoluta* from *P. indistincta*. These taxa had a p-distance of 4.11%, which was in the traditional gap separating species (4–7%). We chose to separate these taxa based on morphological differences, and until many more strains of *Pegethrix* are sequenced, it will be difficult to assess whether these species should be retained or combined.

Applying the criteria given above, it is possible to derive the rationale for recognition of the genera in the Oculatellaceae. The reasoning for taxonomic decisions in
this paper was as follows. First, genera must be monophyletic, and so phylogenetic analysis was conducted as a prerequisite to identifying genus-level clusters. Second, we wished to preserve existing morphologically distinct and named genera, which at the initiation of this study were *Oculatella, Thermoleptolyngbya*, and the as-yet unnamed provisional genus *"Trichotorquatus"* (Komárek *et al.* 2014). These three taxa broke the Oculatellaceae into three groups. Third, we applied the 94.5% identity cutoff so that all species groups \( \leq 94.5\% \) similar were considered to be separate genera. By this standard, the following genera were separated: *Pegethrix, Francisia, Thermoleptolyngbya, Oculatella, Thallothrix, Josephinia, Gardneria, "Trichotorquatus",* and *Kaiparowitsia*. Fourth, we considered other separation criteria, including morphology, ITS secondary structures, and length of ITS conserved domains.

Species of *Cartusia* were 95.4–96.9% similar to species of *Pegethrix*, which is strong evidence of generic separation for one species pair, but not all species pairs. Logically, if even one pair of species are in different generic clusters, all members of both clusters should be considered to be separate genera. However, additional evidence was sought for separating these sister taxa. *Pegethrix* species have false branching and nodule formation, whereas *Cartusia* has neither of these characteristics. *Cartusia* had a longer and very unique D1-D1' helix in the 16S-23S ITS region (Fig. 6m), and the Box B helix and post-Box B spacer were both considerably longer than in any of the *Pegethrix* species (Table 5). Given this combination of evidence for separateness of the lineages, we recognized both *Cartusia* and *Pegethrix* as distinct genera.

Separation of species in this paper was done based on morphological differences and p-distance based on aligned ITS sequences. All of the species were separable with
this combination of criteria. The only ambiguous species pair were *P. convoluta* and *P. indistincta*, which had a p-distance of 4.1%, which fell into the typical gap separating cyanobacterial species (4–7%). These species were separated based on differences in morphology, the structure of the V3 helix, and their position in the 16S rRNA phylogeny (Fig. 2).

The model for separation of genera and species given above has been followed in numerous other papers on cyanobacterial taxonomy (Perkerson III *et al.* 2011, Johansen *et al.* 2011, 2014, Kaštovský *et al.* 2014, Osorio-Santos *et al.* 2014, Pietrasiak *et al.* 2014, Bohunická *et al.* 2015, Berrendero *et al.* 2016, Sciuto & Moro 2016, Hentschke *et al.* 2016). This model was also used to reject the genus name *Cronbergia* Komárek, Zapomelova & Hindak (2010:329) (Johansen *et al.* 2014), which had a phylogenetic position within a large cluster of *Cylindrospermum* species, as well as high genetic identity and ITS structures similar to *Cylindrospermum*.

**Remaining taxonomic problems.** In this work a number of genera new to science were recognized in the Oculatellaceae, but it is clear that more genera remain to be characterized and named. *Pegethrix, Cartusia, Francisia, Oculatella*, and *Thallothrix* all have unspeciated strains in the phylogenies that are based on unnamed cultures, misnamed cultures, and environmental samples (Fig. 2). There are also sequences attributed to *Leptolyngbya* sp. that are unequivocably not in that genus, but also not in any of the Oculatellaceae described in this work (e.g. CENA112, CENA103, CENA131, CENA129 discussed in Furtado *et al.* 2009, Genuario *et al.* 2016). Oscillatoriales
cyanobacterium JSC-1, a well-characterized strain (Brown et al. 2004), is undoubtedly a new genus and species, but has likewise not been named.

As phylogenetically defined lineages continue to be recognized taxonomically, it will likely become more difficult to morphologically separate genera. Whereas we were able to separate the genera morphologically in this study, the differences in some cases are fairly minor and ambiguous. Both cryptic species and cryptic genera likely exist in the Oculatellaceae. Such morphologically obscure taxa have been described in cyanobacteria by other workers (Nübel et al. 2000, Osorio-Santos et al. 2014, Dvořák et al. 2015).

The Oculatellaceae are diverse in terms of both morphology and ecological preference. The family contains representatives which vary in the expression of false branching, fasciculation, spiralling, length to width ratio in cells, sheath production, tapering of trichomes, hormogonia production, necridia formation, granulation in cells, and shape of end cells. Ecologically they have been found in tropical, temperate, and desert climates, in freshwater and subaerophytic habitats, as well as thermal waters. None of the genera or species are marine. Some of the genera share features with taxa in other families. For example, members of *Leptolyngbya sensu stricto* typically possess false branching and necridia, members of *Nodosilinea* produce nodules, *Josephinia nuda* resembles *Stenomitos* species. If these genera were not described, it would lead to further confusion and misnaming of strains.

The Leptolyngbyaceae and Prochlorotrichaceae have similar needs for revisionary work. Like the Oculatellaceae, both families have strains assigned to *Leptolyngbya* that fall outside of the clade containing *L. boryana*, as well as strains
misidentified as *Phormidesmis, Phormidium, Plectonema, Pseudophormidium*, and LPP-group. Some strains are incompletely classified (e.g. Leptolyngbyaceae, Pseudanabaenales, Pseudanabaenaceae). These strains require study and taxonomic diagnosis. The Trichocoleaceae currently has only one genus, and the sequenced members of the Pseudanabaenaceae are currently limited to *Pseudanabaena* and *Limnothrix*. However, as more representatives of these families are characterized and sequenced, there will likely be new taxa discovered in them as well.

Some might still argue that there is little point in naming obscure cryptic species or even cryptic genera. There was a recommendation for delay in taxonomic revision and taxon description until more genomes become available in a roundtable of cyanobacteriologists, although this recommendation was opposed by others in the same roundtable (Hoffmann 2005). The authors of this paper recommend both the description of cryptic taxa (when sufficient evidence is gathered) and immediate efforts to catalogue species and generic level diversity in the cyanobacteria. If the filamentous Synechococcales were not revised and studied, we would still be using general morphogenera such as *Leptolyngbya* sp. or LPP group B for most of the strains in our phylogenies, with few to no species named (Figs. 1–3). This would not only entail a loss of information and detail now, it would greatly slow progress in the field. Without a finely divided taxonomy, researchers could assign little meaning to finding thin, filamentous cyanobacteria in the environment. Generalizations about physiology and habitat preference would be difficult to make. Together, the Leptolyngbyaceae, Oculatellaceae, Prochlorotrichaceae and Trichocoleaceae are very diverse genetically, physiologically, morphologically, and biogeographically. We would not understand the
ecology and evolution of the group without named taxa. Naming taxa is the first act of progression in biological science. Once organisms are named, linkage between studies of the same taxon can be made. No ecologist would be satisfied with a taxonomy that had just mammals, reptiles, and birds. Without species, ecology and evolutionary studies could not progress. We consider this true for cyanobacteria as well, and promote the characterization and description of species, genera, and higher level taxa.

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Koniglichen Böhmischen Gesellschaft der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe 1–34.


Zammit, G., Billi, D. & Albertano, P. (2012). The subaerophytic cyanobacterium
Oculatella subterranea (Oscillatoriales, Cyanophyceae) gen. et sp. nov.: a
cytomorphological and molecular description. European Journal of Phycology
Verlag von Veit & Comp, Leipzig, pp. 1–74.
**TABLE 1.** List of genera and their families affinity within Synechococcales based on the 16S rRNA phylogeny in this study. **Annotations:**—Seq. Type: Sequence of the type species/type specimen is available; Seq. Nontype: Sequence of species other than the type is available; No Seq.: No molecular sequence is available for any species of the genus.

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<td><em>Arthronema</em> Komárek &amp; Lukavský (1988:32)</td>
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<td><em>Kovacikia</em> Miscoe &amp; Johansen (2016: 83)</td>
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<td><em>Thermoleptolyngbya</em> Sciuto &amp; Moro (2016: 33)</td>
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<td>Anagnostidis &amp; Komárek (1988: 395)</td>
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<td><em>Schizothrix</em></td>
<td>Kützing (1843: 230) ex Gomont (1892: 292)</td>
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</table>

*Sequence of the type species of *Jaaginema* is *J. subtilissimum* (Kützing 1847 ex De Toni 1907) Anagnostidis & Komárek (1988: 396) can be found on NCBI, however identity of this sequence as belonging to cyanobacteria is uncertain. Consequently, a lot of sequences submitted under *Jaaginema* has not been ascertain.*
### TABLE 2. Strains used in the phylogenetic analyses in this paper, with accession numbers. Sequences available on NCBI marked as such.

<table>
<thead>
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<th>Strain names</th>
<th>Family</th>
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<th>DNA Sequences available</th>
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<td>Leptolyngbyaceae</td>
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<td>NCBI</td>
</tr>
<tr>
<td>2 Myxacorys ATA2-1-KO14</td>
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<td>Atacama Desert, Chile.</td>
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</tr>
<tr>
<td>3 Plectolyngbya WJT66-NPBG17</td>
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<td>Wonderland of Rock site, Joshua Tree National Park, Mojave Desert, CA, USA.</td>
<td>NCBI</td>
</tr>
<tr>
<td>4 Plectolyngbya HA4277-MV3</td>
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<td>Palolo Valley Trail, Honolulu, Oahu, HI, USA.</td>
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</tr>
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<td>5 WOS-LAB13</td>
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<td>Whiteoak Sink, Great Smoky Mountain National Park, TN, USA.</td>
<td>NCBI</td>
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<td>6 Leptolyngbya (cf.) HA4303-MV7</td>
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<td>8 Phormidesmis WJT36-NPBG15</td>
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<td>Wonderland of Rocks site, Joshua Tree National Park, Mojave Desert, CA, USA.</td>
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<td>9 Phormidesmis WJT36-NPBG12</td>
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<td>Northern Cottonwood–Western Eagle Mountains, Joshua Tree National Park, CA, USA.</td>
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</tr>
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<td>10 Phormidesmis WJT24-NPBG8</td>
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</tr>
<tr>
<td>11 Phormidesmis TAA2-2HA3</td>
<td>Leptolyngbyaceae</td>
<td>Fort Irwin National Training Center site, Mojave Desert, CA, USA.</td>
<td>NCBI</td>
</tr>
<tr>
<td>12 Tapinothrix GSE-PSE06-7G</td>
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<td>Lower Calf Creek Falls, GSENEM, UT, USA.</td>
<td>NCBI</td>
</tr>
<tr>
<td>13 Leptolyngbyaceae EY07-AM2</td>
<td>Leptolyngbyaceae</td>
<td>Waterfall rocks, El Yunque National Forest, Puerto Rico.</td>
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<tr>
<td>No.</td>
<td>Accession</td>
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<td>-----------</td>
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<td>Leptolyngbyaceae</td>
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<td>Stenomitos rutilans HA7619-LM2</td>
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<td>Kauai. HI, USA.</td>
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<tr>
<td>17</td>
<td>Oculatella atacamiensis ATA3-4Q-CV5</td>
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<td>Atacama Desert. Chile.</td>
</tr>
<tr>
<td>18</td>
<td>Oculatella kauaiensis HA4348-LM1</td>
<td>Oculatellaceae</td>
<td>Kauai. HI, USA.</td>
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<tr>
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<td>Oculatellaceae</td>
<td>Locality N/A</td>
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<td>21</td>
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<td>27</td>
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<td>Drip Tank Seep Wall, GSENM. UT, USA.</td>
</tr>
<tr>
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<td>Lower Calf Creek Falls, GSENM. UT, USA.</td>
</tr>
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<td>GSE-UNK-7R</td>
<td>Oculatellaceae</td>
<td>Lower Calf Creek Falls, GSENM. UT, USA.</td>
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<td>GSE-TBD6-7R</td>
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<td>Species</td>
<td>Location</td>
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<tr>
<td>-----</td>
<td>------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>33</td>
<td>Luboš31 Hubel 1974/223</td>
<td>Oculatellaceae</td>
<td>Bay Barther Bodden near bridge Meiningen, Germany</td>
</tr>
<tr>
<td>34</td>
<td>Luboš34 Uher1998/13d</td>
<td>Oculatellaceae</td>
<td>National Park Slovak Paradise, Slovakia.</td>
</tr>
<tr>
<td>35</td>
<td>Luboš25 ZEHNDER 1965/U140</td>
<td>Oculatellaceae</td>
<td>Wet stone wall, Stansstaad, Switzerland.</td>
</tr>
<tr>
<td>36</td>
<td>EY01-AM2</td>
<td>Oculatellaceae</td>
<td>Waterfall rocks, El Yunque National Forest. Puerto Rico.</td>
</tr>
<tr>
<td>37</td>
<td>GSE-PSE-MK54-09C</td>
<td>Oculatellaceae</td>
<td>Camp Pring Site, GSENM. UT, USA.</td>
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<td>38</td>
<td>GSE-TBC-9CA2</td>
<td>Oculatellaceae</td>
<td>Camp Pring Site, GSENM. UT, USA.</td>
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<tr>
<td>39</td>
<td>GSE-TBC-9CA</td>
<td>Oculatellaceae</td>
<td>Camp Pring Site, GSENM. UT, USA.</td>
</tr>
<tr>
<td>40</td>
<td>Schizothrix arenaria (cf.) HA4233-MV05</td>
<td>Oculatellaceae</td>
<td>Kawai Nui Marsh Nature Preserve, Kailua. HI, USA.</td>
</tr>
<tr>
<td>41</td>
<td>Nodosilinea GSE-PSE-MK27-15A</td>
<td>Prochlorotrichaceae</td>
<td>Drip Tank Seep Wall, GSENM. UT, USA.</td>
</tr>
<tr>
<td>42</td>
<td>Nodosilinea GSE-PSE-MK55-09B</td>
<td>Prochlorotrichaceae</td>
<td>Camp Pring Site, GSENM. UT, USA.</td>
</tr>
<tr>
<td>43</td>
<td>Nodosilinea Luboš26 Hubel 1974/235</td>
<td>Prochlorotrichaceae</td>
<td>Bay Barther Bodden near bridge Meiningen, Germany</td>
</tr>
<tr>
<td>44</td>
<td>Nodosilinea nodulosa UTEX 2910</td>
<td>Prochlorotrichaceae</td>
<td>South China Sea</td>
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<tr>
<td>45</td>
<td>Xeronema WJT66-NPBG5</td>
<td>Prochlorotrichaceae</td>
<td>Wonderland of Rocks site, Joshua Tree National Park. Mojave Desert. CA, USA.</td>
</tr>
<tr>
<td>46</td>
<td>Trichocoleus desertorum ATA4-8-CV3</td>
<td>Trichocoleaceae</td>
<td>Atacama Desert. Chile.</td>
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<tr>
<td>47</td>
<td>Trichocoleus desertorum ATA4-8-CV12</td>
<td>Trichocoleaceae</td>
<td>Atacama Desert. Chile.</td>
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<td>48</td>
<td>Pseudanabaena GSE-PSE-MK21-19D</td>
<td>Pseudanabanaceae</td>
<td>Left Hand Collet Canyon, GSENM. UT, USA.</td>
</tr>
</tbody>
</table>
**TABLE 3.** Percent difference in 16S rRNA within and between families. Bold font: between genera within the same family, regular font: between genera in two different families; values are mean (range). Trichocoleaceae currently has only one genus, so no between-genus comparison is possible for this family.

<table>
<thead>
<tr>
<th>Family</th>
<th>Leptolyngbyaceae</th>
<th>Oculatellaceae</th>
<th>Prochlorotrichaceae</th>
<th>Trichocoleaceae</th>
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</thead>
<tbody>
<tr>
<td>Leptolyngbyaceae</td>
<td><strong>7.89</strong> <strong>(3.38–12.47)</strong></td>
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<td></td>
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<tr>
<td>Oculatellaceae</td>
<td>8.98 (5.35–15.11)</td>
<td><strong>7.12</strong> <strong>(2.75–10.82)</strong></td>
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<tr>
<td>Trichocoleaceae</td>
<td>8.40 (4.74–11.70)</td>
<td>7.71 (5.17–9.62)</td>
<td>8.34 (6.94–12.03)</td>
<td><strong>NA</strong></td>
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</table>
TABLE 4. Nucleotides variable between families but consistent within families of the Synechococcales (relevant nucleotides in bold font). Nucleotides that varies within the consensus sequences are underlined.

<table>
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<th>Family</th>
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<th>Sequence</th>
<th>Percent presence in family</th>
</tr>
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<td>Leptolyngbyaceae</td>
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<td>TGCCAGCAGCCGCAGTCGAATA</td>
<td>100%</td>
</tr>
<tr>
<td>Oculatellaceae</td>
<td>18</td>
<td>TGCCAGCAGCCGCAGTCGAATA</td>
<td>99%</td>
</tr>
<tr>
<td>Prochlorotrichaceae</td>
<td>18</td>
<td>TGCCAGCAGCCGCAGTCGAATA</td>
<td>90%</td>
</tr>
<tr>
<td>Trichocoleaceae</td>
<td>18</td>
<td>TGCCAGCAGCCGCAGTCGAATA</td>
<td>100%</td>
</tr>
<tr>
<td>Leptolyngbyaceae</td>
<td>20</td>
<td>ACTGACACTGAGGGACGAAA</td>
<td>99%</td>
</tr>
<tr>
<td>Oculatellaceae</td>
<td>20</td>
<td>ACTGACACTGAGGGACGAAA</td>
<td>100%</td>
</tr>
<tr>
<td>Prochlorotrichaceae</td>
<td>20</td>
<td>ACTGACACTGAGGGACGAAA</td>
<td>90%</td>
</tr>
<tr>
<td>Trichocoleaceae</td>
<td>20</td>
<td>ACTGACACTGAGGGACGAAA</td>
<td>100%</td>
</tr>
<tr>
<td>Leptolyngbyaceae</td>
<td>23</td>
<td>ATGGGAAGAACACCCACGCC</td>
<td>92% and 95%</td>
</tr>
<tr>
<td>Oculatellaceae</td>
<td>23</td>
<td>ATGGGAAGAACACCCACGCC</td>
<td>98% and 99%</td>
</tr>
<tr>
<td>Prochlorotrichaceae</td>
<td>23</td>
<td>ATGGGAAGAACACCCACGCC</td>
<td>98% and 98%</td>
</tr>
<tr>
<td>Trichocoleaceae</td>
<td>23</td>
<td>ATGGGAAGAACACCCACGCC</td>
<td>100% and 100%</td>
</tr>
<tr>
<td>Leptolyngbyaceae</td>
<td>27</td>
<td>GGGAGTACGCACGCAGTGTGAAACTC</td>
<td>99% and 99%</td>
</tr>
<tr>
<td>Oculatellaceae</td>
<td>27</td>
<td>GGGAGTACGCACGCAGTGTGAAACTC</td>
<td>96% and 97%</td>
</tr>
<tr>
<td>Prochlorotrichaceae</td>
<td>27</td>
<td>GGGAGTACGCACGCAGTGTGAAACTC</td>
<td>95% and 99%</td>
</tr>
<tr>
<td>Trichocoleaceae</td>
<td>27</td>
<td>GGGAGTACGCACGCAGTGTGAAACTC</td>
<td>100% and 100%</td>
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<tr>
<td>Leptolyngbyaceae</td>
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<td>94%</td>
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<tr>
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<td>CGTCAAGTCAGCATGCACCC</td>
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<tr>
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<td>CGTCAAGTCAGCATGCACCC</td>
<td>95%</td>
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<td>Spacer + D2 + spacer</td>
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<td><em>Oculatella hafneriensis</em> Hindak 1982/12</td>
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<td><em>Oculatella cataractarum</em> GSE-PSE-49-07D</td>
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<td><em>Oculatella kauaiensis</em> HA4348-LM1</td>
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<tr>
<td><em>Pegethrix indistincta</em> GSE-TBC-7GB</td>
<td>7</td>
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<td>33</td>
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<td>GSE-UNK-7R</td>
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<td>&quot;Trichotorquatus sp.&quot;</td>
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</table>

**TABLE 6.** 16S rRNA distance of Pegethrix species.
### TABLE 7. ITS p-distance between *Pegethrix* species.

<table>
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<th>Strain</th>
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<th>3</th>
<th>4</th>
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<th>10</th>
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</thead>
<tbody>
<tr>
<td>7 <em>P. bostrychodes</em> GSE-TBD4-15B</td>
<td>98.62</td>
<td>98.62</td>
<td>98.71</td>
<td>98.80</td>
<td>98.80</td>
<td>100.0</td>
<td>-</td>
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</tr>
<tr>
<td>8 <em>Pegethrix</em> sp. ANT.LH70.1</td>
<td>99.74</td>
<td>99.74</td>
<td>99.83</td>
<td>99.91</td>
<td>99.91</td>
<td>98.88</td>
<td>98.88</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 <em>Pegethrix</em> sp. ANT.LMA.1</td>
<td>99.48</td>
<td>99.48</td>
<td>99.57</td>
<td>99.66</td>
<td>99.66</td>
<td>98.62</td>
<td>98.62</td>
<td>99.74</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10 <em>P. olivacea</em> GSE-PSE-MK46-15A</td>
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<td>98.88</td>
<td>98.97</td>
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<td>99.05</td>
<td>99.14</td>
<td>98.88</td>
<td>-</td>
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<tr>
<td>11 <em>Leptolyngbya</em> sp. 1T12c</td>
<td>98.28</td>
<td>98.28</td>
<td>98.36</td>
<td>98.45</td>
<td>98.62</td>
<td>98.62</td>
<td>98.54</td>
<td>98.80</td>
<td>98.10</td>
<td>-</td>
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<tr>
<td>12 Uncultured bacterium sp. GBe-058</td>
<td>97.80</td>
<td>97.80</td>
<td>98.08</td>
<td>97.99</td>
<td>97.99</td>
<td>97.62</td>
<td>97.62</td>
<td>97.89</td>
<td>98.17</td>
<td>97.43</td>
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<tr>
<td>13 <em>Leptolyngbya</em> sp. VP3-07</td>
<td>97.16</td>
<td>97.16</td>
<td>97.42</td>
<td>97.33</td>
<td>96.90</td>
<td>96.90</td>
<td>97.24</td>
<td>97.50</td>
<td>97.24</td>
<td>97.26</td>
</tr>
</tbody>
</table>

### TABLE 8. 16S rRNA p-distance of *Francisia* species

<table>
<thead>
<tr>
<th>Strain</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 <em>Leptolyngbya frigida</em> ANT-LH52.2</td>
<td>-</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2 <em>Francisia hepatica</em> UHER2000/2452</td>
<td>99.50</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 <em>Francisia lurida</em> LUKESOVA1986/6</td>
<td>97.52</td>
<td>97.40</td>
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</tr>
<tr>
<td>4 <em>Francisia fasiculata</em> GSE-PSE-MK29-07A</td>
<td>96.90</td>
<td>96.63</td>
<td>96.42</td>
<td>-</td>
</tr>
</tbody>
</table>
### TABLE 9. ITS p-distance between *Francisia*

<table>
<thead>
<tr>
<th>Strain</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 <em>Leptolyngbya frigida</em></td>
<td>ANT</td>
<td>LH52.2</td>
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<td>2 <em>Francisia hepatica</em></td>
<td>UHER2000/2452</td>
<td>93.07</td>
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<tr>
<td>3 <em>Francisia lurida</em></td>
<td>LUKESOVA1986/6</td>
<td>82.35</td>
<td>81.77</td>
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</tr>
<tr>
<td>4 <em>Francisia fasiculata</em></td>
<td>GSE-PSE-MK29-07A</td>
<td>74.99</td>
<td>75.12</td>
<td>76.35</td>
</tr>
</tbody>
</table>

### TABLE 10. 16S rRNA p-distance of *Josephinia* species

<table>
<thead>
<tr>
<th>Strain</th>
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<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 <em>Josephinia torsiva</em></td>
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<tr>
<td>Hubel1974/223</td>
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<tr>
<td>2 <em>Josephinia torsiva</em></td>
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</tr>
<tr>
<td>UHer1998/13d</td>
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<td></td>
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</tr>
<tr>
<td>3 <em>Josephinia nuda</em></td>
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<tr>
<td>ZEHNDER1965/U140</td>
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### TABLE 11. 16S rRNA p-distance of *Thallothrix* species

<table>
<thead>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>1 Uncultured cyanobacterium A10_3.6</td>
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<tr>
<td>2 Uncultured cyanobacterium RJ094</td>
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</tr>
<tr>
<td>3 <em>Thallothrix</em> sp. WMT-WP7-NPA</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 <em>Thallothrix obliquedivisa</em> GSE-PSE28-08A</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 <em>Thallothrix obliquedivisa</em> GSE-PSE-MK23-08B</td>
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<tr>
<td>6 <em>Thallothrix radians</em> GSE-UNK-7R</td>
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</tr>
<tr>
<td>7 <em>Thallothrix radians</em> GSE-TBD6-7R</td>
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</tbody>
</table>

### TABLE 12. ITS p-distance between *Thallothrix* species

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<th>Strain</th>
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<th>2</th>
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<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 <em>Thallothrix</em> sp. WMT-WP7-NPA</td>
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<tr>
<td>No.</td>
<td>Genera</td>
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<td>----------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>2</td>
<td>Thallothrix obliquedivisa GSE-PSE28-08A</td>
<td>81.72</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>Thallothrix obliquedivisa GSE-PSE-MK23-08B</td>
<td>81.72</td>
<td>100.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Thallothrix radians GSE-UNK-7R</td>
<td>74.62</td>
<td>76.97</td>
<td>76.97</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Thallothrix radians GSE-TBD6-7R</td>
<td>74.62</td>
<td>76.97</td>
<td>76.97</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**TABLE 13.** 16S rRNA percent dissimilarity between described Oculatellacean genera (Mean ± SD)
FIGURE LEGENDS

FIGURE 1. 16S rRNA phylogeny of the filamentous group of Synechococcales cyanobacteria, showing Leptolyngbyaceae. Black polygons represents genera that have been described or description in provision, with length correspond to the distance from the most basal OTU to the most diverged OTU of the genus. Posterior probabilities of MrBayes are given above branch.

FIGURE 2. 16S rRNA phylogeny of the filamentous group of Synechococcales cyanobacteria, showing Oculatellaceae. Black polygons represents genera that have been described or description in provision, with length correspond to the distance from the most basal OTU to the most diverged OTU of the genus. Posterior probabilities of MrBayes are given above branch.

FIGURE 3. 16S rRNA phylogeny of the filamentous group of Synechococcales cyanobacteria, showing Leptolyngbyaceae. Black polygons represents genera that have been described or description in provision, with length correspond to the distance from the most basal OTU to the most diverged OTU of the genus. Posterior probabilities of MrBayes are given above branch.

FIGURE 4. rpoC1 phylogeny of the filamentous Synechococcales cyanobacteria with representatives of family Leptolyngbyaceae, Oculatellaceae. Prochlorotrichaceae and Pseudanabaenaceae. OTUs demonstrated to be within specific families defined in 16S rRNA phylogenies are annotated accordingly. A large portion of Oculatellaceae (except Josephinia nuda) remains stable. Several previously listed under Leptolyngbyacea, Oculatellaceae and Prochlorotrichaceae have changed their positions.
FIGURE 5. Molecular diagnosis to the families proposed or described in this study. Only helices 23 and helices 27 are shown as they are considered to be the most straightforward and useful for family distinction.

FIGURE 6. D1-D1’ stems of species described to be in Oculatellaceae. The stem structures of genera described previously in other publications (Oculatella, Thermoleptolyngbya) or in publications under provision (Trichotorquatus) are not shown here.

FIGURE 7. Box B helices of species described to be in Oculatellaceae. The structures of genera described previously in other publications (Oculatella, Thermoleptolyngbya) or in publications under provision (Trichotorquatus) are not shown here.

FIGURE 8. V2 helices of species described to be in Oculatellaceae. Several species do not have this structure, including Pegethrix convoluta, P. indistincta, Antarctic Pegethrix species, Cartusia fontana, Kaiparowitsia implicata.

FIGURE 9. V3 helices of species described to be in Oculatellaceae. All species described have this structure, excluding Cartusia aeruginosa, because we do not have the full length of the ITS region.


sheath, indistinct with no diacritical characters. Scale bar of 10μm in 400X (A,B) and 1000X magnification (C–E).

**FIGURE 15.** *Francisia fasiculata.* A. Fasiculated filaments with individual sheath. B. Trichomes with one to two large central granules. C. Extended sheath in filaments rarely observed. D. Necridia. E. Hormogonia. F. Cells barrel-shaped, not constricted and separated by somewhat translucent cross-walls. G. Wavy and flexuous to spirally coiled trichomes commonly observed. H–I. Bright blue-green color in healthy filaments, compared to the discolored, yellowish green of older trichomes in B. Scale bar of 10μm in 400X (A) and 1000X magnification (B–J).

**FIGURE 16.** *Francisia hepatica.* A. Young filaments isodiametric to slightly cylindrical. B. Elongated apical cells rarely observed. C. Heterogeneity in trichome width between young and mature filaments. D. Rapid cell division in meristematic zones, with frequent necridia along trichomes. C–E. Rapid cell division sometimes caused twisted trichomes. F. False-branching very rare, sometimes also occur in young trichomes. G–I. Variation in sheath characteristics of filaments. Scale bar of 10μm in 1000X magnification.

**FIGURE 17.** *Cartusia fontana.* A. Fasicle of trichomes within one common sheath. B. variation in width between young and mature trichome, with cell division in meristematic zones. C-E. Cells isodiametric, with one large central granules in healthy filament. F–I. Meristematic zone of cell division in mature trichomes, with necridia; necridia in both young and senescing trichomes, Filaments in mature trichomes occasionally with thick, slightly layered sheath. Scale bar of 10μm in 1000X magnification.


FIGURE 20. *Gardneria angustata*. A. Trichomes constricted at slightly translucent cross-walls, sometimes with one to several orange granules on cell walls (at arrows). F. Variation in trichome width between mature and young filament or hormogonia. B–H. Rapid regional cell division along trichomes results in basal and apical parts of filaments. D–E. Necridia abundant in both young and mature trichomes. D, J. Variation in cell shapes between young and mature trichomes: isodiametric, slightly longer than width or barrel-shaped. Scale bar of 10μm in 1000X magnification.


Figure 1
Figure 3
Figure 4

Key to Node Recognition

- Marks Family level node
- Marks 16S rRNA Oculatelliteae OTUs
- Marks 16S rRNA Leptolyngbyaceae OTUs
- Marks 16S rRNA Nodosillinea OTUs
- Marks 16S rRNA Pseudoanabaenaceae OTUs
Figure 5

Key to base pairings
- Canonical bp (A:U or C:G)
- Wobble bp G:U
- Non-canonical bp A:G
- Other non-canonical bp
D1-D1' Stem

Figure 6
Figure 7
Figure 9
Figure 13
Figure 15
Figure 21
Figure 22
Figure 23