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Revision of the *Synechococcales* (Cyanobacteria) through recognition of four families including Oculatellaceae *fam. nov*. and Trichocoleaceae *fam. nov*. and six new genera containing 14 species

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Abstract

A total of 48 strains of thin, filamentous cyanobacteria in Synechococcales were studied by sequencing 16S rRNA and *rpo*C1 sequence fragments. We also carefully characterized a subset of these by morphology. Phylogenetic analysis of the 16S rRNA gene data using Bayesian inference of a large Synechococcales alignment (345 OTU's) was in agreement with the phylogeny based on the *rpo*C1 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. Oculatellaceae *fam. nov.* and Trichocoleaceae *fam. nov.* are proposed for the other two families. The Oculatellaceae was studied in greater detail, and six new genera containing 14 species were characterized and named. These new taxa are: *Pegethrix botrychoides*, *P. olivacea, P. convoluta*, *P. indistincta*, *Drouetiella lurida*, *D. hepatica*, *D. fasciculata*, *Cartusia fontana*, *Tildeniella torsiva*, *T. nuda*, *Komarkovaea angustata*, *Kaiparowitsia implicata*, *Timaviella obliquedivisa*, and *T. radians*.

Keywords: *Pegethrix*, *Drouetiella*, *Cartusia*, *Tildeniella*, *Komarkovaea*, *Kaiparowitsia, Timaviella*, Oculatellaceae, Leptolyngbyaceae, Prochlorotrichaceae, Trichocoleaceae, 16S rRNA phylogeny, *rpoC*1 phylogeny, 16S rRNA synapomorphy, cyanobacteria taxonomy

Introduction

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. Less than 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 un-sequenced and unverified genera 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 is 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 are phylogenetically intermixed. 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 (see fig. S1 in Osorio-Santos *et al.* 2014), 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: 123) is found to be phylogenetically related with the molecularly characterized *T. clintonii* Bohunická *et* Johansen in Bohunická *et al.* (2011: 130 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.

Leptolyngbyaceae, as the largest family in the Synechococcales 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 be evaluated (Johansen *et al.* 2008, 2011). Recently described genera in the Leptolyngbyaceae include *Planktolyngbya*, *Prochlorothrix*, *Trichocoleus*, *Halomicronema*, *Phormidesmis*, *Plectolyngbya*, *Nodosilinea*, *Haloleptolyngbya*, *Oculatella*, *Pantanalinema*, *Alkalinema*, *Scytolyngbya*, *Kovacikia*, *Stenomitos*, *Thermoleptolyngbya*, *Pinocchia*, *Onodrimia*, *Chamaethrix*, *Elainella*, *Timaviella,* and *Limnolyngbya* (Table 1). All of these new genera and a few previously described genera in the family (*Geitleribactron* Komárek 1975: 265, *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 α-level taxonomy. Many strains have been sequenced having only the simple epithet "*Leptolyngbya* species". However, these strains are clearly 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 taxa closely related to *Leptolyngbya sensu stricto* need to be described as new genera so that *Leptolyngbya* can eventually 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 possess 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, yet-to-be-described genera. Thus, α-level taxonomy will include incorporating some historical species names into new genera, as well as describing species truly new to science that cannot be assigned to any existing taxa.

With the availability of the 16S rRNA and the ITS regions, α-level taxonomy and revision of existing taxa are rapidly progressing at the species and generic levels. However, 16S rRNA alone is considered insufficient to resolve all taxonomic questions (Komárek 2006). At the family level, 16S rRNA is ambiguous and ITS sequences and secondary structures are highly variable. Higher level taxonomy thus requires more information from other regions of the genome. Currently, with the introduction of novel and high-quality whole-genome amplification methods that facilitates whole-genome comparisons (Naushad *et al*. 2014), better broad-range primers designed for conserved regions (Hunt *et al.* 2006), and the continually falling cost of whole genome sequencing, many genomes are becoming available in public databases. A time may soon come when 16S rRNA-based taxonomy will be replaced by multi-locus or whole genome characterization. However, considering that high throughput sequencing technologies are still relatively costly and restricted in accessibility, and high quality downstream assembly requires time and effort, the use of 16S rRNA and marker genes still has some future for large scale taxonomic studies. Additional marker genes that may prove helpful include, but are not limited to, the 23S rRNA gene containing a large fragment called the Universal Plastid Amplicon (UPA), that can easily be sequenced (Sherwood *et al.* 2007, 2015); several protein coding genes, for example the *rpo*B and *rpo*C1 loci of the *rpo* gene family encoding for different beta subunits of DNA-dependent RNA polymerase (Case *et al.* 2007, Gaget *et al.* 2011, Fergusson & Saint 2000, Wilson *et al.* 2000); *rbc*L, a gene encoding the large subunit of RuBisCO, a critical protein in CO_2 fixation (Tomitani *et al.* 2006; Andersen 2013); *cpc*A-*cpc*B (phycocyanin subunit A and B) intergenic spacer (IGS) (Bittencourt-Oliveira *et al.* 2009) that performs taxonomic placement at the species level; and many more.

A number of strains of Leptolyngbyaceae were isolated and morphologically described as part of a study of the aquatic and subaerial cyanobacterial flora of the Grand Staircase-Escalante National Monument (Krautová 2008). They are currently housed within the Cyanobacterial Culture Collection at John Carroll University, and were the focus of this study. The collection also has numerous other strains in the 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. Our objective was to taxonomically study these thin filamentous strains in Synechococcales using a polyphasic approach including data on morphology, ecology, 16s rRNA and *rpo*C1 phylogeny, and secondary structures of the 16S–23S ITS region. 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 six new genera and fourteen species (either previously described taxa or taxa new to science) based upon the strains available in the JCU collection.

TABLE 1. List of genera with author citations and their family affinity within Synechococcales based on the 16S rRNA phylogeny in this study. **Annotations:—**Type: Sequence of the type species/type specimen is available; Nontype: Sequence of species other than the type is available; No Seq.: No molecular sequence is available for any species of the genus.

Taxon	**Status**
Leptolyngbyaceae (Anagnostidis & Komárek 1988: 439) Komárek *et al.* (2014: 316)	
Alkalinema Vaz *et al.* (2015: 302)	Type
Arthronema Komárek & Lukavský (1988:32)	Type
Kovacikia Miscoe & Johansen *in* Miscoe *et al*. (2016: 83)	Type
Leptolyngbya Anagnostidis & Komárek (1988: 390)	Type
Myxacorys Pietrasiak *et al.* 2015 provis. In Komárek *et al.* (2014: 332)	Type
Neosynechococcus Dvořák *et al*. (2013: 26)	Type
Pantanalinema Vaz *et al.* (2015: 301)	Type
Phormidesmis Turicchia *et al.* (2009: 179)	Type
Plectolyngbya Taton *et al.* (2011: 184)	Type
Planktolyngbya Anagnostidis & Komárek (1988: 394)	Type
Romeria (Raciborski) Koczwara *in* Geitler (1932: 916)	Nontype
Scytolyngbya Song & Li (2015: 74)	Type
Stenomitos Miscoe & Johansen *in* Miscoe *et al.* (2015:84)	Type
Tapinothrix Savageau (1892: 123)	Nontype
Limnolyngbya Li & Li (2016: 479)	Type
Pinocchia Dvořák *et al.* (2015: 114)	Type
Onodrimia Jahodářová *et al.* (2017: 30)	Type
Chamaethrix Dvořák *et al.* (2017: 270)	Type
Oculatellaceae *fam. nov.*	
Cartusia gen. nov.	Type
Elainella Jahodářová *et al.* (2014: 4)	Type
Drouetiella gen. nov.	Type
Komarkovaea gen. nov.	Type
Tildeniella gen. nov.	Type
Kaiparowitsia gen. nov.	Type
Oculatella Zammit *et al.* (2012: 352)	Type
Pegethrix gen. nov.	Type
Thermoleptolyngbya Sciuto & Moro (2016: 33)	Type
Timaviella Sciuto *et al.* 2017	Type
Trichotorquatus Pietrasiak & Johansen 2015 provis. In Komárek *et al.* (2014: 332)	Type

TABLE 1. (Continued)

Taxon	Status
Prochlorotrichaceae Burger-Wiersma *et al.* (1989: 255)	
Haloleptolyngbya Dadheech *et al.* (2012: 272)	Type
Halomicronema Abed *et al.* (2002: 59)	Type
Nodosilinea Pekerson & Casamatta *in* Perkerson *et al.* (2011: 1404)	Type
Prochlorothrix Burger-Wiersma *et al.* (1989: 255)	Type
Trichocoleaceae Mai *et al.* 2016	
Trichocoleus Anagnostidis (2011: 369)	Nontype
Pseudanabanaceae Anagnostidis & Komárek (1988: 374)	
Komvophoron subg. *Alyssophoron* Anagnostidis & Komárek (1988: 372)	No Seq.
Limnothrix redekei Meffert (1988: 10)	Type
Pseudanabaena Lauterborn (1915: 10)	Type
Yonedaella Umezaki (1962: 323)	No Seq.
Incertae familiae	
Cyanocatenula Joosten (2006: 34)	No Seq.
Dasygloea Thwaites (1848: pl. 2941) *ex* Gomont (1892: 346)	No Seq.
Heteroleibleinia (Geitler 1932: 1035) Hoffmann (1985: 76)	No Seq.
Jaaginema Anagnostidis & Komárek (1988: 395)	Type*
Schizothrix Kützing (1843: 230) *ex* Gomont (1892: 292)	Nontype
Tubiella Hollerbach (1935: 34)	No Seq.
Wolskyella Claus (1963: 32)	No Seq.

*Sequence of the type species of *Jaaginema* is *J. subtilitissimum* (Kützing 1847 *ex* De Toni 1907) Anagnostidis & Komárek (1988: 396) and can be found on NCBI. However, identity of this sequence with cyanobacteria is uncertain. Consequently, the phylogenetic position of *Jaaginema* is presently uncertain.

Material and methods

Molecular techniques:—Genomic DNA was extracted from selected strains (Table 2 and Table S1) 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) when UltraClean Kit could not retrieve DNA successfully. 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 $MgCl_2$ (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 (Bio-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*). Primer sequences for *rpo*C1 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). 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 using the StrataClone PCR cloning kit according to manufacturer instructions (La Jolla, CA, USA). Plasmid purification proceeded with QIAPrep Miniprep Spin Kit (Qiagen, Carlsbad, CA, U.S.A.) prior to EcoRI digestion to select successful clones. For each strain, 4 plasmids were sent out for sequencing. For PCR products from *rpoC1* gene, mono-product reactions were directly purified, whereas multi-product reactions (the primers lack specificity) were excised from an agarose gel (choosing amplifications of correct size) 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 of the cloned products included 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).

TABLE 2. List of sequences from our database used in this analysis, with accession numbers. Sequences available on NCBI marked as such. **Annotations:—F.** (Family): L=Leptolyngbyaceae; O=Oculatellaceae; Pr=Prochlorotrichaceae; T=Trichocoleaceae; Ps=Pseudanabaenaceae. **NCBI Accession Number:** N/A=Sequences not available; Accession number (regular) = Single bold) = Multiple sequence with single accession number on NCBI; Unpublished=Sequence available, pending submission. **Collection site:** JTNP=Joshua Tree National Park; GSENM=Grand Staircase-Escalante National Monument; GSMNP=Great Smokey Mountain National Park; EYNF=El Yunque National Forest.

Strain names		Collection site	NCBI Accession Numbers	
			16S	***rpoC1***
Scytolyngbya HA4215-MV1	L	Laie Falls, Oahu. HI, USA.	KF307599	KY498296
"Myxacorys" ATA2-1-KO14	L	Atacama Desert, Chile.	NCBI	KY498278
Plectolyngbya WJT66-NPBG17	L	Mojave Desert. CA, USA.	NCBI	KY498279
Plectolyngbya HA4277-MV3	L	Honolulu, Oahu. HI, USA.	NCBI	KY498280
Leptolyngbyaceae WOS-LAB13	L	GSMNP. TN, USA.	KY078761	KY498298
Leptolyngbya (cf.) HA4303-MV7	L	Maunawili stream, Oahu. HI, USA.	NCBI	KY498299
Leptolyngbya (cf.) HA4237-MV2	L	Taro fields, Oahu. HI, USA.	NCBI	KY498300
Phormidesmis WJT36-NPBG15	L	JTNP, Mojave Desert. CA, USA.	NCBI	KY498266
Phormidesmis WJT36-NPBG12	L	JTNP, Mojave Desert. CA, USA.	NCBI	KY498267
Phormidesmis WJT24-NPBG8	L	JTNP, Colorado Desert. CA, USA.	NCBI	KY498301
Phormidesmis TAA2-2HA3	L	JTNP, Mojave Desert. CA, USA.	NCBI	KY498301
Leptolyngbyaceae EY07-AM2	L	EYNF, Puerto Rico.	KU161656	KY498308
Leptolyngbyaceae GSE-TBD9-6B	L	GSENM. UT, USA.	KY078757	KY498289
Leptolyngbyaceae GSE-TBD7-7G	L	GSENM. UT, USA	KY078758	N/A
Leptolyngbyaceae GSE-UNK-8H	L	GSENM. UT, USA	KY078759	N/A
Arthronema africanum CCALA20 (SAG 1.89)	L	Wau en-Namus, Fezzan. Libya.	NCBI	KY498294
Stenomitos rutilans HA7619-LM2	L	Kauai. HI, USA.	NCBI	KY498295
Oculatella atacamensis ATA3-4Q-CV5	O	Atacama Desert. Chile.	NCBI	KY498276
Oculatella kauaiensis HA4348-LM1	O	Kauai. HI, USA.	NCBI	KY498277
"Trichotorquatus" ATA2-1-CV25	O	Atacama Desert. Chile	NCBI	KY498284
"Trichotorquatus" SMER-A	O	N/A	Unpublished	KY498293
Pegethrix convoluta GSE-PSE-MK38-07D	O	GSENM. UT, USA.	KY078763	KY498281
Pegethrix convoluta GSE-PSE-MK22-07D	O	GSENM. UT, USA.	KY078764	N/A
Pegethrix indistincta GSE-TBC-7GA	O	GSENM. UT, USA.	KY078765	N/A
Pegethrix indistincta GSE-TBD1-7G	O	GSENM. UT, USA.	KY078766	N/A
Pegethrix indistincta GSE-TBC-7GB	O	GSENM. UT, USA.	KY078767	N/A
Pegethrix bostrychoides GSE-PSE-MK47-15B	O	GSENM. UT, USA.	KY078768	N/A
Pegethrix bostrychoides GSE-TBD4-15B	O	GSENM. UT, USA.	KY078769	N/A
Drouetiella fasciculata GSE-PSE-MK29-07A	O	GSENM. UT, USA.	KY078770	KY498282
Timaviella obliquedivisa GSE-PSE23-08B	O	GSENM. UT, USA.	KY078772	KY498309

TABLE 2. (Continued)

Strain names		Collection site	NCBI Accession Numbers	
			16S	***rpoC1***
Timaviella obliquedivisa GSE-PSE28-08A	O	GSENM. UT, USA.	NCBI	KY498310
Timaviella radians GSE-UNK-7R	O	GSENM. UT, USA.	KY078773	KY498288
Timaviella radians GSE-TBD6-7R	O	GSENM. UT, USA.	KY078774	N/A
Tildeniella torsiva Hubel 1974/223	O	Bay Barther Bodden, Germany	KY498227	N/A
Tildeniella torsiva Hubel 1974/235	Pr	Bay Barther Bodden, Germany	NCBI	KY498290
Tildeniella torsiva Uher1998/13d	O	SPNP, Slovakia.	KY498228	N/A
Tildeniella nuda ZEHNDER 1965/U140	O	Stansstaad, Switzerland.	NCBI	KY498291
Komarkovaea angustata EY01-AM2	O	Puerto Rico.	NCBI	KY498308
Kaiparowitsia implicata GSE-PSE-MK54-09C	O	GSENM. UT, USA.	KY078776	KY498286
Kaiparowitsia implicata GSE-TBC-9CA2	O	GSENM. UT, USA.	KY078777	KY498285
Kaiparowitsia implicata GSE-TBC-9CA	O	GSENM. UT, USA.	KY078778	KY498287
Nodosilinea GSE-PSE-MK27-15A	Pr	GSENM. UT, USA.	KY078779	KY498306
Nodosilinea GSE-PSE-MK55-09B	Pr	GSENM. UT, USA.	KY078780	KY498307
Nodosilinea nodulosa UTEX 2910	Pr	South China Sea	NCBI	KY498292
"Xeronema" WJT66-NPBG5	Pr	JTNP, Mojave Desert. CA, USA.	NCBI	KY498305
Trichocoleus desertorum ATA4-8-CV3	T	Atacama Desert. Chile.	NCBI	KY498274
Trichocoleus desertorum ATA4-8-CV12	T	Atacama Desert. Chile.	NCBI	KY498275
Pseudanabaena GSE-PSE-MK21-19D	Ps	GSENM. UT, USA.	Unpublished	KY498297

Phylogenetic analysis:—After sequencing, all four clones per strain were inspected for number of tRNAs in the ITS region. Only operons with 2 tRNA genes were chosen to reduce error introduced by sequence differences between multiple paralagous operons. Orthologous operons were aligned with ClustalW to create one consensus sequence per strain (Larkin *et al.* 2007). 16S rRNA sequences were then submitted to MUSCLE in MEGA6. In addition, we also looked into the conserved regions of the 16S that have secondary structure (identified by Řeháková *et al.* 2014) to make sure that they were aligned correctly, i.e. different nucleotide sequences fold into the same secondary structures. 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=50,000,000. All other parameters were left as defaults. The BA had a mean estimated sample size (ESS) exceeding 270 for all parameters (ranging 271-16,086), above the average of 200 typically accepted as sufficient by phylogeneticists (Drummond *et al.* 2006). The final average standard deviation of split frequencies was <0.03. The potential scale reduction factor (PSRF) value for all the estimated parameters in the Bayesian analysis was 1.00, indicating that convergence of the MCMC chains was statistically achieved (Gelman and Rubin 1992).

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 (Adobe Systems Incorporated, San Jose, CA, USA.). Descriptions of secondary structures were based on nomenclature set forth by Bevilacqua and Blose (2008). For *rpoC1*, sequences were blasted in the NCBI protein database using BLASTX to identify the start codon. DNA alignments of 55 *rpoC1* 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 and obtain the appropriate parameters for those models (values for Revmatpr, Pinvarpr and Shapepr). Tree topology with the *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 specified by JModelTest2 2.1.6. (Darriba *et al.* 2012): 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. The *rpo*C1 BA had a mean estimated sample size (ESS) exceeding 250 for all parameters (ranging 274-1418). The final average standard deviation of split frequencies was <0.011. The potential scale reduction factor (PSRF) value for all the estimated parameters in the Bayesian analysis was 1.00, indicating that convergence of the MCMC chains was statistically achieved (Gelman and Rubin 1992).

Since the tree topology for *rbcLX* is in critical disagreement with 16S rRNA and *rpoC1* phylogenies, we decided to gather more information on this gene in anticipation of a future study, but do not include the analysis in this manuscript. Calculation of uncorrected p-distance in 16S rRNA and the 16S–23S ITS regions was done with PAUP 4.0 (Swofford 2002) and used to calculate sequence identity (100*(1-p)) for 16S rRNA data and percent dissimilarity (100*p-distance) for ITS data.

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 Axio Scope HBO 50 (Carl Zeiss AS, Norway) and processed when necessary with Adobe Photoshop in the CS5 software package (Adobe Systems Incorporated, San Jose, CA, USA). For each phase of growth, at least three cells in ten different filaments were measured, but also a search was made for maximum and minimum dimensions.

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 a 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 16S rRNA analyses of 324 OTUs belonging within the Synechococcales with *Gloeobacter violaceus* Rippka *et al.* (1974: 436) (Gloeobacteriales) as outgroup indicated the existence of five distinct family-level clades (Figs 1–3). The Leptolyngbyaceae (Fig. 1) contains *Leptolyngbya sensu stricto*, and several newly described and revised taxa of *Plectolyngbya*, *Tapinothrix*, *Romeria*, *Planktolyngbya*, *Alkalinema*, *Phormidesmis*, *Stenomitos*, *Neosynechococcus*, *Arthronema*, *Pantanalinema*, *Limnolyngbya, Pinocchia*, *Onodrimia*, *Chamaethrix*, and *Scytolyngbya* (Table 1). The family contains many strains that are incorrectly placed taxonomically, mostly either in *Leptolyngbya* or *Phormidesmis* (see genera in quotation marks, Fig. 1). *"Myxacorys"* is the name of a large clade of soil species that have been discussed in the literature (Komárek *et al.* 2014), but have not yet been validly published.

In a sister phylogenetic relationship to Leptolyngbyaceae is another large, highly supported clade, containing diverse subaerophytic taxa from both wet rocks and soils, some of which were separated from *Leptolyngbya sensu lato* (Fig. 2). The clade is referred to in this work as Oculatellaceae *fam. nov.*, a new family based on *Oculatella*, the first genus to be described in this group, and contains nine additional genera: *Thermoleptolyngbya*, *Elainella, Timaviella, Pegethrix gen. nov., Tildeniella gen. nov., Drouetiella gen. nov., Cartusia gen. nov., Kaiparowitsia gen. nov.* and *Komarkovaea gen. nov.* The Oculatellaceae also has a number of strains that are incorrectly placed (e.g. *"Leptolyngbya"* and *"Phormidium"*). Two genera lacking valid descriptions appear in this family. *"Marsacia ferruginose"* is a manuscript name for a characterized strain discussed in the literature (Brown *et al.* 2010), and *"Trichotorquatus"* represents a set of soil strains which may be described in the future (Komárek *et al.* 2014).

At the basal position to Leptolyngbyaceae and Oculatellaceae is a group we refer to as Prochlorotrichaceae, containing *Nodosilinea*, *Halomicronema*, and *Prochlorothrix*, as well as a number of taxa of uncertain generic identity (Fig. 3). 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. 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 families. We consequently establish the name Trichocoleaceae *fam. nov.* for this group of taxa. At the base of the Synechococcales (defined by its proximity to the outgroup taxon *Gloeobacter*) is the Pseudanabaenaceae, a family containing *Pseudanabaena* Lauterborn (1915: 437) and *Limnothrix* Meffert (1988: 269) (Fig. 3). Three representatives of the order Oscillatoriales were included in our analysis, and they fall between the Pseudanabaenaceae and the other families of the Synechococcales. If this position is stable in more extensively samples phylogenies, it could indicate that the order Pseudanabaenales may be narrowly defined and distinct from Synechococcales, in which it was recently subsumed (Komárek *et al.* 2014). In the phylogenomic study by Mareš (2017) the Synechococcales consisted of four clades at the base of the phylogenetic tree (rooted by Gloeobacteriales) that were paraphyletic to all other cyanobacteria. His tree supported the recognition of Pseudanabaenales as a separate order from Synechococcales, and indicated much more revision in the higher level taxonomy of simple filamentous forms is needed.

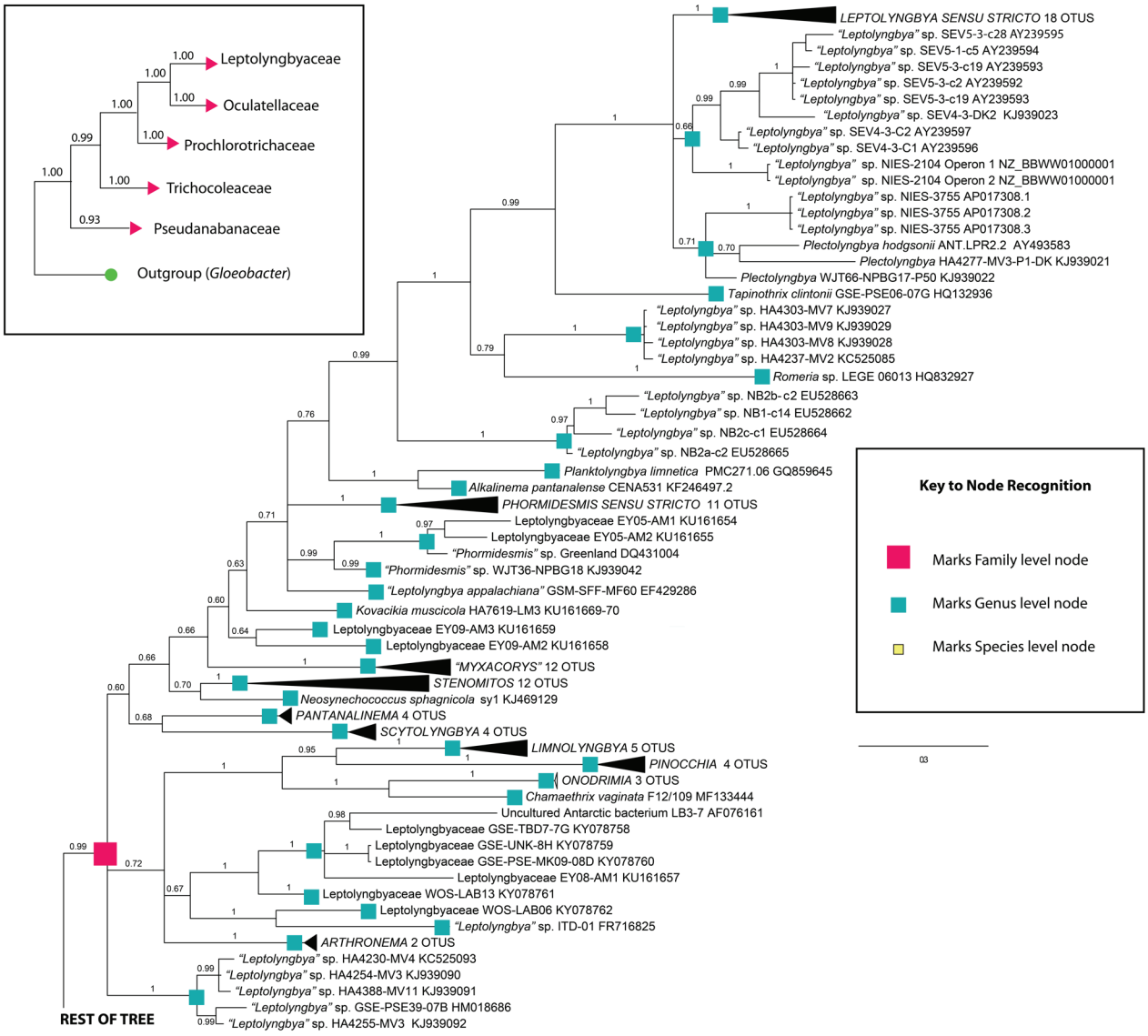

FIGURE 1. 16S rRNA Bayesian Inference analysis of the filamentous group of Synechococcales cyanobacteria, showing Leptolyngbyaceae. Black polygons represent genera that have been described or are named in provision (e.g., *"Myxacorys"*), with length corresponding to the distance from the most basal OTU to the most diverged OTU of the genus. Posterior probabilities for the BI analysis are given above the nodes. Taxa which we consider to be incorrectly named in NCBI or requiring revisionary work are in quotation marks.

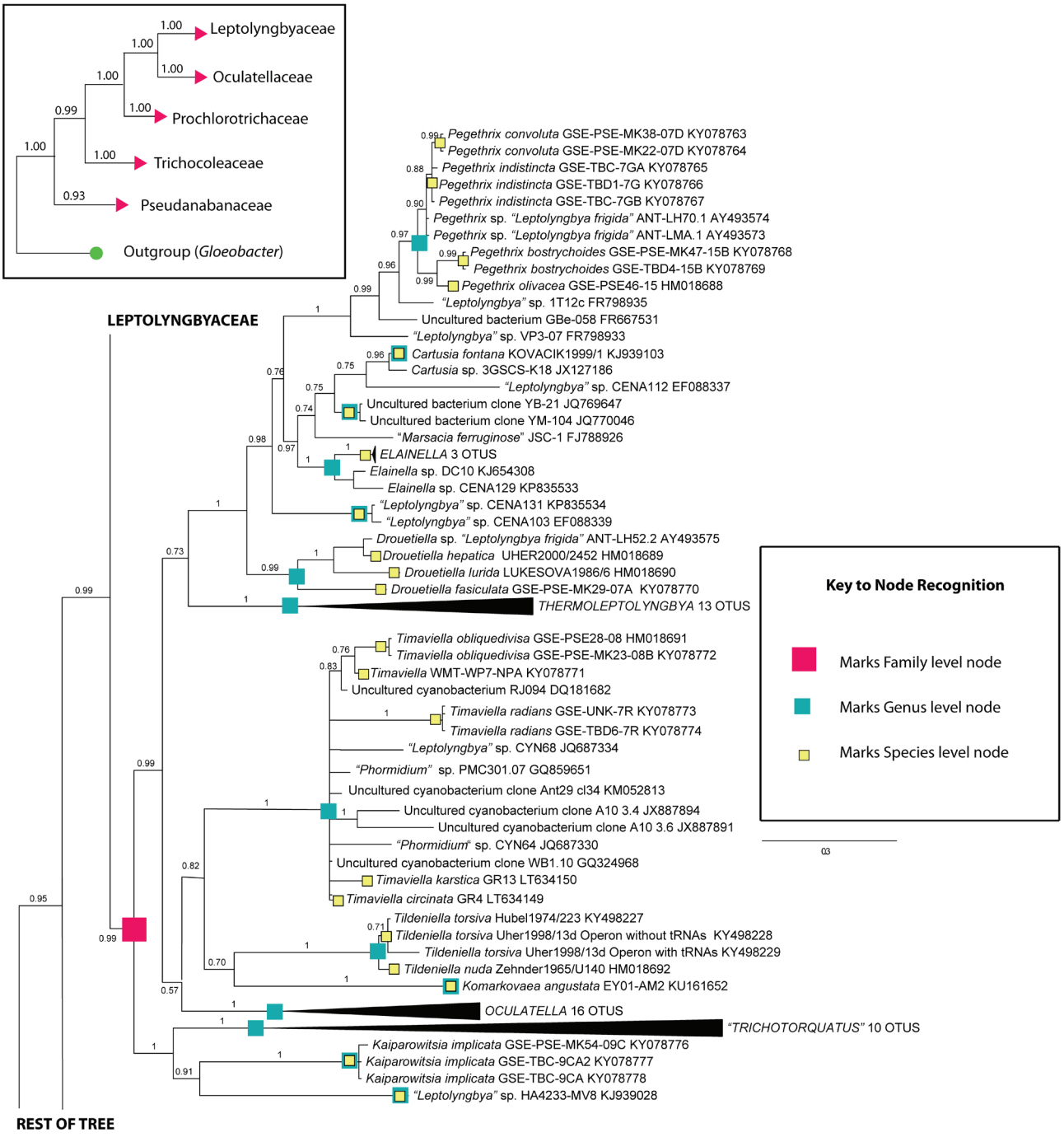

FIGURE 2. 16S rRNA Bayesian Inference analysis of the filamentous group of Synechococcales cyanobacteria, showing Oculatellaceae. Black polygons represent genera that have been validly described or are named in provision (e.g., *"Trichotorquatus"*), with length corresponding to the distance from the most basal OTU to the most diverged OTU of the genus. Posterior probabilities for the BI analysis are given above the nodes. Taxa which we consider to be incorrectly named in NCBI or requiring revisionary work are in quotation marks.

Phylogenetic results based on *rpoC1* phylogeny:—Analysis of 55 sequences of the *rpo*C1 gene shows the Oculatellaceae to be the most divergent and cohesive group of genera in the Synechococcales (Fig. 4, OTUs marked by yellow squares). The group is stable in the phylogenetic analysis, and would be monophyletic if *Tildeniella nuda* was excluded from the analysis. Leptolyngbyaceae is shown as the sister taxon to Oculatellaceae, which agrees with the analysis based on 16S rRNA gene sequence. Most taxa belonging to Leptolyngbyaceae in the 16S rRNA analysis

were also shown to be related in the *rpoC1* analysis (Fig. 4, clades marked with black hollow circles). This group contains *Leptolyngbya sensu stricto* and related taxa: *Plectolyngbya*, *"Myxacorys"*, *Arthronema*, *Phormidesmis* and *Stenomitos*. However, the inclusion of *T. nuda* within this group and the exclusion of several "Leptolyngbyaceae" which fall outside of the clade make the family polyphyletic when only the rpoC1 data are considered (Fig. 4). *Trichocoleus* taxa are shown to be at a position distinct from other taxa, but in this analysis appear to be more related to Oculatellaceae and Leptolyngbyaceae, while in the 16S rRNA analysis Prochlorotrichaceae was more related to the Oculatellaceae + Leptolyngbyaceae clade than the Trichocoleaceae (Fig. 3). The group of Prochlorotrichaceae splits into three clades, with *Prochlorothrix hollandica* in an unresolved position at the base of the tree and *Nodosilinea* with a number of Prochlorotrichacean OTUs aggregating to form a large group sister to the clade containing Oculatellaceae + Leptolyngbyaceae + Trichocoleaceae. The group of Pseudanabanaceae is poorly sampled, with only two strains of *Pseudanabaena* (Fig. 4). Compared to the 16S rRNA phylogeny, there are major position changes in the *rpo*C1 tree, although the families maintain their structure at least to some degree.

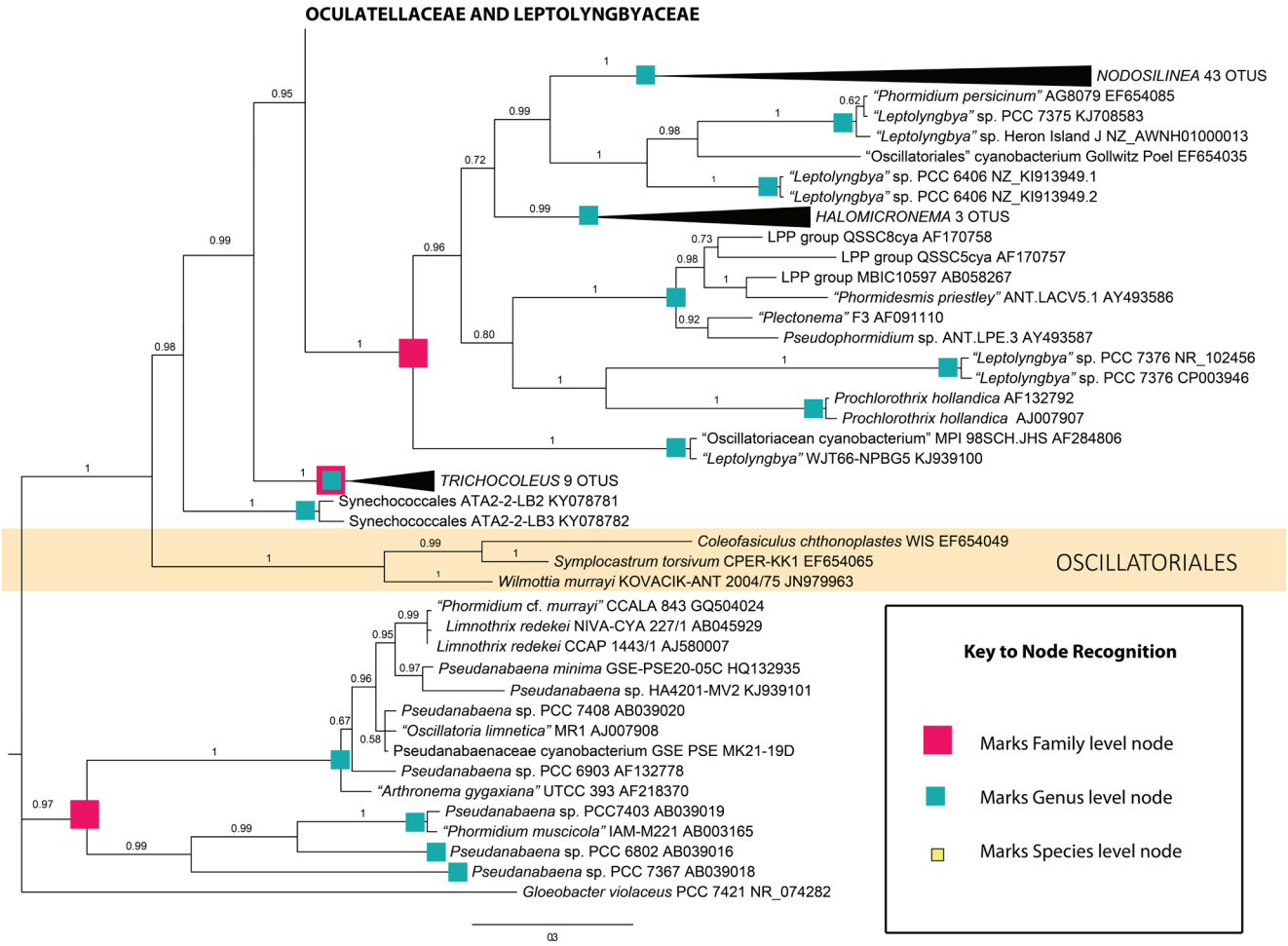

FIGURE 3. 16S rRNA phylogeny of the filamentous group of Synechococcales cyanobacteria, showing Prochlorotrichaceae, Trichocoleaceae and Pseudanabaenaceae. Black polygons represent genera that have been described, with length correspond to the distance from the most basal OTU to the most diverged OTU of the genus. Posterior probabilities for the BI analysis are given above the nodes. Taxa which we consider to be incorrectly named in NCBI or requiring revisionary work are in quotation marks.

Analysis of 16S rRNA dissimilarity for family separation:—Mean percent dissimilarity in 16S rRNA gene sequence among genera of different families is 8.2–11.8% (Table 3). This is broader than the mean percent difference between genera of the same families (6.7–8.5%). However, there is considerable overlap in the range between percent difference between genera of the same family and different families, making it not realistic to utilize a specific range or value for family recognition.

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.

Family	Leptolyngbyaceae	Oculatellaceae	Prochlorotrichaceae	Trichocoleaceae	Pseudanabaenaceae
Leptolyngbyaceae	**8.5 (4.0–13.1)**				
Oculatellaceae	9.4 (5.2–25.7)	**6.7 (2.8–8.5)**			
Prochlorotrichaceae	10.8 (7.1–15.7)	10.3 (6.9–17.3)	**8.4 (4.8–15.5)**		
Trichocoleaceae	8.2 (4.9–11.2)	7.7 (6.9–8.8)	9.15 (6.8–13.1)	**NA**	
Pseudanabaenaceae	11.3 (8.3–14.2)	10.4 (8.4–12.7)	11.8 (9.4–15.9)	9.1 (8.6–9.7)	**8.5 (7.2–10.4)**

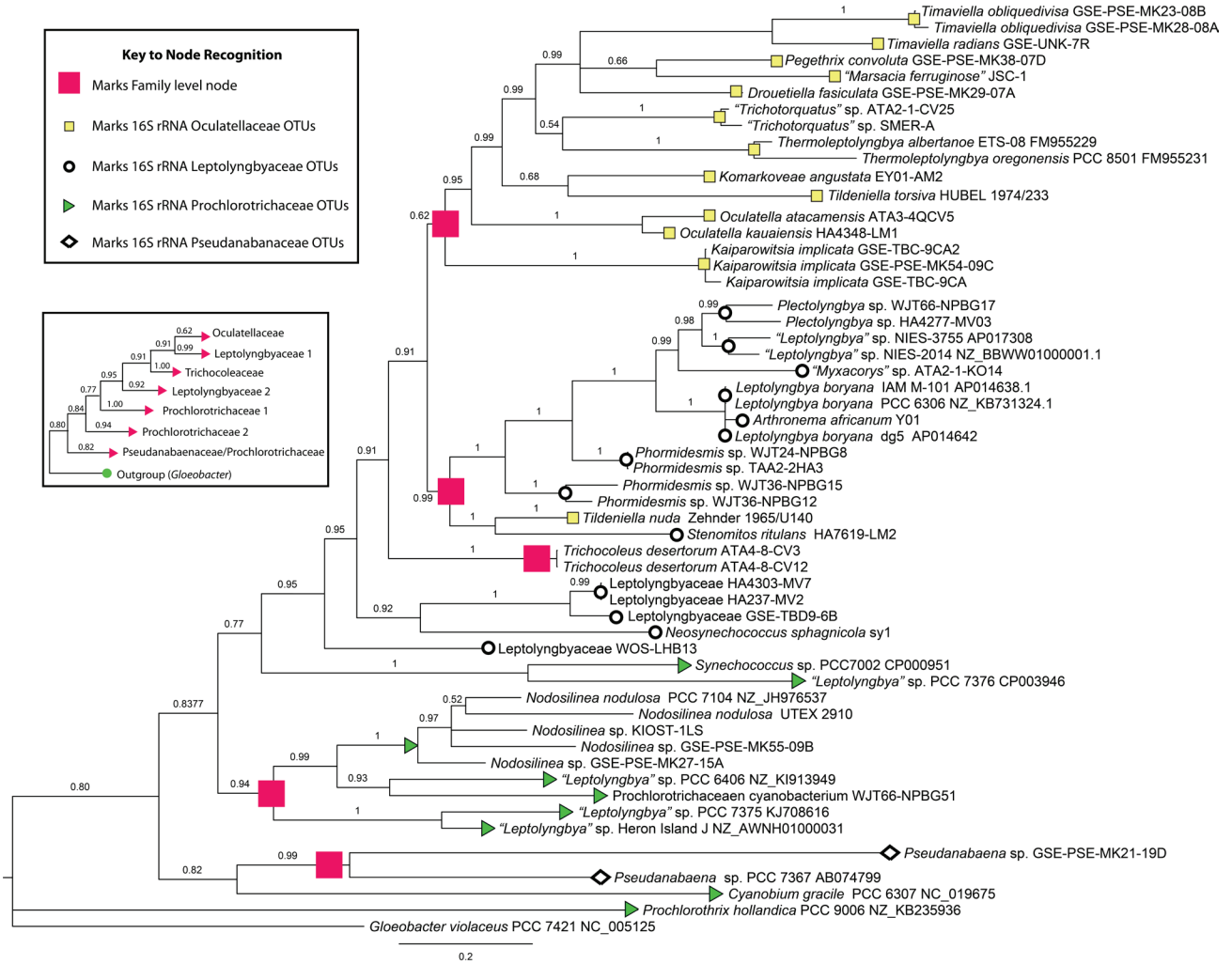

FIGURE 4. *rpoC1* Bayesian Inference analysis of the filamentous Synechococcales cyanobacteria with representatives of family Leptolyngbyaceae, Oculatellaceae. Prochlorotrichaceae and Pseudanabaenaceae. OTUs demonstrated to be within specific families defined in 16S rRNA phylogenies are anotated accordingly. The Oculatellaceae (top clade) has the same composition as the family in the 16S rRNA gene phylogeny, except for *Tildeniella nuda,* which is in the Leptolyngbyaceae clade in this analysis. Several OTUs previously listed under Leptolyngbyaceae and Prochlorotrichaceae have changed their positions with respect to the 16S rRNA gene phylogeny. Taxa which we consider to be incorrectly named in NCBI or requiring revisionary work are in quotation marks.

Molecular diagnosis of families in Synechococcales:—Of 440 sites in the 16S rRNA sequence that were considered parsimony-informative, we identified 8 nucleotide positions in 5 different helices that were considered consistent indicators of the family-level clades (Table 4). In all cases, secondary structure of the helices was conserved among sequences (see examples in Fig. 5). These distinctive nucleotides were found in more than 87% of the OTUs compared. In some cases, these distinctive nucleotides are shared between two families (e.g: Helix 18 with T in Leptolyngbyaceae, Oculatellaceae and Gloeobacteraceae and G in Prochlorotrichaceae, Trichocoleaceae and Pseudanabaenaceae, see Table 4).

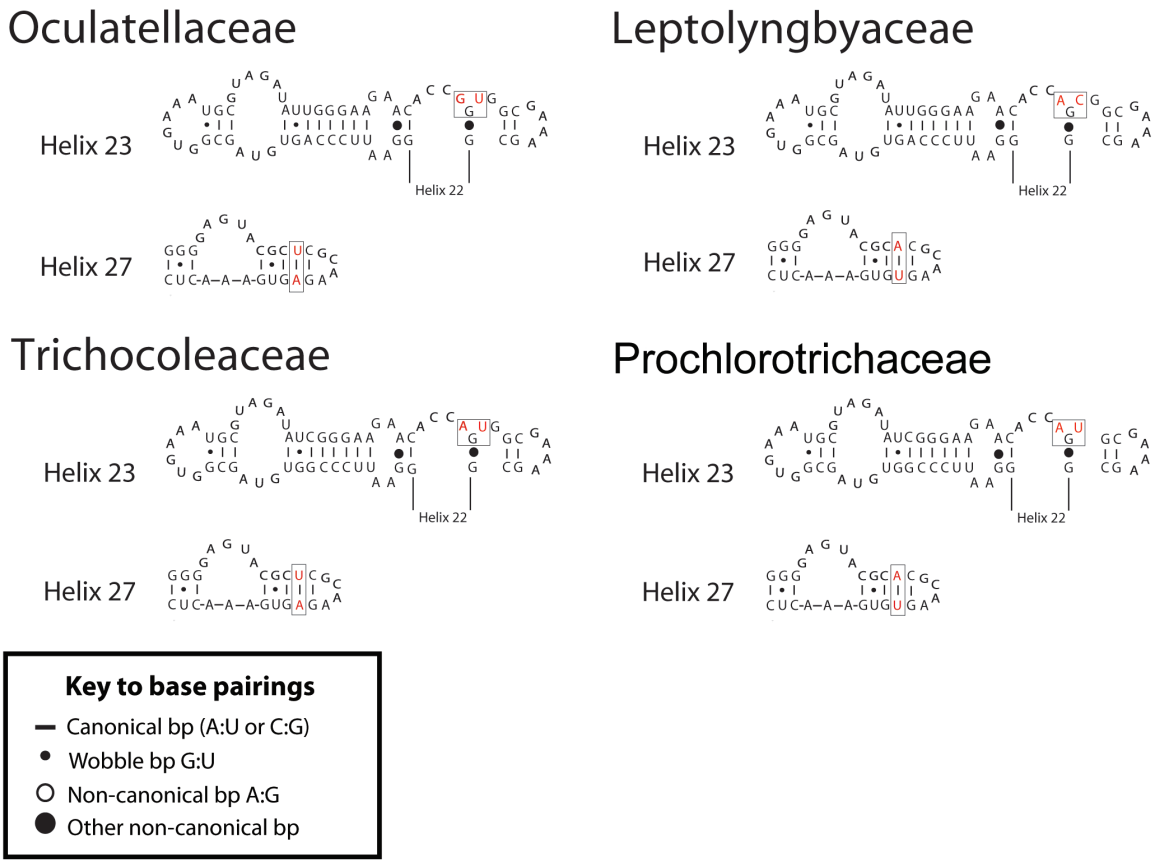

FIGURE 5. Molecular diagnosis of the families proposed or described in this study. Only helices 23 and 27 are shown as they are considered to be the most straightforward and useful for family distinction.

Taxonomic descriptions:—Families of Synechococcales are characterized on the basis of their 16S rRNA phylogenetic position and morphological features. We describe two new families in which monophyletic status is strongly supported. Genera of Oculatellaceae *fam. nov.* are separated based on a combination of the 16S rRNA threshold of 94.5 % (below 94.5% sequence similarity is strong evidence for different genera, see Table 5 for genera separated by this criterion), shapes and sequences of the ITS secondary structures (Figs. 6–9, and S1) as well as morphological features (Figs. 10–23). Species of Oculatellaceae *fam. nov.* are separated based on the 16S rRNA threshold of 98.7 % (below 98.7 % sequence similarity is strong evidence for different species), the 16S–23S ITS percent similarity threshold of less than 96%, the configuration, sequence (Figs. 6–9), and length (Table 6) of the ITS conserved domains, as well as morphological features (Figs. 10–23). Using these criteria, we recognize in this work six new genera of Oculatellaceae: *Pegethrix gen. nov.*, *Drouetiella gen. nov.*, *Cartusia gen. nov.*, *Tildeniella gen. nov.*, *Komarkovaea gen. nov.*, and *Kaiparowitsia gen. nov*, and a total of 14 named species belonging to these genera and the recently described *Timaviella*.

Class: Cyanophyceae

Subclass: Synechococcophycidae

Order: Synechococcales

TABLE 4. Nucleotides variable between families but consistent within families of the Synechococcales (relevant nucleotides in bold font). IUPAC code letters are given for those nucleotides that vary within the consensus sequences.

Family	Helix	Sequence	Percent presence in family
Leptolyngbyaceae	18	TGCCAGCAGCCGCGGTAA**T**A	93%
Oculatellaceae	18	TGCCAGCAGCCGCGGTAA**T**A	99%
Prochlorotrichaceae	18	TGCCAGCAGCCGCGGTAA**G**A	87.5%
Trichocoleaceae	18	TGCCAGCAGCCGCGGTAA**G**A	100%
Pseudanabaenaceae	18	TGCCAGCAGCCGCGGTAA**G**A	100%
Gloeobacteraceae	18	TGCCAGCAGCCGCGGTAA**T**A	100%
Leptolyngbyaceae	20	CTGAC**A**CTSAKGGACGAAA	94%
Oculatellaceae	20	CTGAC**A**CTGAKGGACGAAA	100%
Prochlorotrichaceae	20	CTGAC**G**CTGAKGGACGAAA	92%
Trichocoleaceae	20	CTGAC**A**CTGAGGGACGAAA	100%
Pseudanabaenaceae	20	CTGAC**R**CTGARGTACGAAA	100%
Gloeobacteraceae	20	CTGAC**G**CTGAGGTACGAAA	100%
Leptolyngbyaceae	23	ATTGGGAAGAACACC**A**G**C**G	87% and 90%
Oculatellaceae	23	ATTRGRAAGAACAYC**G**G**T**G	99% and 99%
Prochlorotrichaceae	23	ATYRGGAAGAACACC**A**G**T**G	92% and 98%
Trichocoleaceae	23	ATCGGGAAGAACACC**A**G**T**G	100% and 100%
Pseudanabaenaceae	23	ATCKGGAAGAACACC**A**G**T**G	100% and 80%
Gloeobacteraceae	23	ATCGGGAAGAACACC**A**G**C**G	100% and 100%
Leptolyngbyaceae	27	GGGAGTAYGC**A**CGCAAG**T**GTGAAACTC	99% and 98%
Oculatellaceae	27	GGGAGTACGC**T**CGCAAG**A**GTGAAACTC	98% and 98%
Prochlorotrichaceae	27	GGGAGTACGC**A**CGCAAG**T**GTGAAACTC	97% and 100%
Trichocoleaceae	27	GGGAGTACGC**T**CGCAAG**A**GTGAAACTC	100% and 100%
Pseudanabaenaceae	27	GGGAGTACGG**T**CGCAAG**A**TTGAAACTC	93% and 93%
Gloeobacteraceae	27	GGGAGTACGC**A**CGCAAG**T**GTGAAACTC	100% and 100%
Leptolyngbyaceae	34	YGTCAAGTCA**G**CATGCC**C**C	94% and 100%
Oculatellaceae	34	CGTCAAGTCA**G**CATGCC**C**C	99% and 100%
Prochlorotrichaceae	34	CGTCAAGTCA**T**CATGCC**C**C	100% and 100%
Trichocoleaceae	34	CGTCAAGTCA**G**CATGCC**C**C	100% and 100%
Pseudanabaenaceae	34	CGTCAAGTCA**T**CATGCC**C**C	80% and 100%
Gloeobacteraceae	34	CGTCAAGTCA**G**CATGGC**T**C	100% and 100%

Trichocoleaceae Mai *et* Johansen *fam. nov.*

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:—*Trichocoleus* (West & West) Anagnostidis

TABLE 5. 16S rRNA percent similarity among described Oculatellacean genera (Mean ± SD)

No	Genus	1	2	3	4	5	6	7	**8**	**9**	**10**	**11**
1	*Pegethrix*	-	-	-	-	-	-	-	-	-	-	-
2	*Cartusia*	98.0±0.57	-	-	-	-	-	-	-	-	-	-
3	*Elainella*	97.2±0.57	97.0±0.0	-	-	-	-	-	-	-	-	-
4	*Drouetiella*	95.4±0.65	94.6±0.40	94.6±0.36	-	-	-	-	-	-	-	-
5	*Thermoleptolyngbya*	92.3±0.90	92.2±0.93	92.2±0.90	92.9±0.95	-	-	-	-	-	-	-
6	*Oculatella*	92.1±1.08	91.8±0.58	91.9±0.63	92.0±0.83	91.8±1.09	-	-	-	-	-	-
7	*Timaviella*	89.5±1.48	90.9±1.29	90.1±1.23	90.0±1.35	88.5±1.50	88.5±1.12	-	-	-	-	-
8	*Tildeniella*	88.4±0.48	90.1±0.33	89.6±0.33	88.7±0.35	91.8±1.24	90.9±0.61	90.0±1.09	-	-	-	-
9	*Komarkovaea*	89.1±0.50	90.1	90.1±0.00	88.2±0.73	91.1±1.23	88.5±0.34	86.9±0.93	91.6±0.23	-	-	-
10	*"Trichotorquatus"*	89.5±1.39	91.0±1.48	90.6±1.22	89.7±1.58	90.6±1.84	88.4±1.37	86.5±1.64	88.3±1.13	88.4±0.53	-	-
11	*Kaiparowitsia*	91.4±0.79	91.9±0.00	91.9±0.00	90.6±0.58	92.3±1.21	91.3±0.62	90.2±1.10	92.9±0.21	92.7±0.00	90.8±0.72	-

TABLE 6. Length of regions within the ITS structure of species within family Oculatellaceae.

Strain ID	Leader	D1-D1' helix	Spacer + D2 + spacer	D3 + spacer	$tRNA^{Ile}$ gene	V2 spacer	$tRNA^{Ala}$ gene	Pre-Box B spacer	Box B helix	Post-Box B spacer	Box A	D4 + spacer	V3	D5
Oculatella neakameniensis Kovacik 1990/37	7	64	37	11	74	16	73	30	34	15	11	23	-	-
Oculatella atacamensis ATA2-1-KO17	7	63	37	11	74	15	73	33	34	15	11	23	52	14
Oculatella coburnii WJT55-NPBG-6A	7	64	38	11	74	15	73	30	34	15	11	23	52	14
Oculatella mojaviensis CMT-3BRIN-NPC87	7	64	36	11	74	15	73	13	34	14	11	23	52	14
Oculatella hafneriensis Hindak 1982/12	7	64	38	11	74	22	73	33	35	15	11	23	52	-
Oculatella cataractarum GSE-PSE-49-07D	7	64	39	12	74	22	73	34	34	15	11	24	54	13
Oculatella subterannea VRUC 135	7	64	41	11	74	82	73	37	34	15	11	23	55	14
Oculatella kauaiensis HA4348-LM1	7	124	38	11	74	76	73	22	32	15	11	23	55	14
Pegethrix convoluta GSE-PSE-MK38-07D	7	91	33	12	74	14	73	64	36	19	11	16	110	23
Pegethrix convoluta GSE-PSE-MK22-07D	7	91	33	12	74	14	73	64	36	19	11	16	109	23
Pegethrix indistincta GSE-TBC-7GA	7	91	33	12	74	14	73	64	36	19	11	16	110	23
Pegethrix indistincta GSE-TBD1-7G	7	91	33	12	74	14	73	64	36	19	11	16	110	23
Pegethrix indistincta GSE-TBC-7GB	7	91	33	12	74	14	73	64	36	19	11	16	110	23
Pegethrix bostrychoides GSE-PSE-MK47-15B	7	87	33	12	74	36	73	48	36	18	11	16	96	23
Pegethrix bostrychoides GSE-TBD4-15B	7	87	33	12	74	36	73	48	36	18	11	16	96	23
Pegethrix olivacea GSE-PSE46-15B	7	87	33	12	74	36	73	48	36	18	11	16	96	23
Pegethrix ANT.L70.1	7	75	33	12	74	14	73	43	37	18	11	16	94	23
Pegethrix ANT.LMA.1	7	75	33	12	74	14	73	43	37	18	11	16	94	23
Cartusia fontana Kovacik 1999/1-LC	8	107	33	13	74	11	73	62	60	53	11	-	-	-
"Marsacia ferruginose" JSC-1	-	63	35	13	74	58	73	99	47	16	11	-	-	-
Elainella saxicola E1	7	62	34	12	74	68	73	32	33	17	11	16	21	27
Drouetiella sp. ANT.LH52.2	7	64	35	12	74	42	73	39	34	19	11	14	52	29
Drouetiella hepatica Uher 2000/2452	7	64	35	12	74	42	73	39	34	19	11	14	52	51
Drouetiella lurida Lukesova 1986/6	7	64	35	12	74	43	73	45	33	19	11	14	-	-
Drouetiella fasciculata GSE-PSE-MK29-07A	7	65	40	12	74	13	73	46	39	17	11	14	52	26
Thermoleptolyngbya oregonensis PCC8501	7	64	33	12	74	83	73	41	59	15	11	19	74	-
Thermoleptolyngbya albertanoe ETS-08	7	64	33	12	74	161	73	72	47	16	11	19	74	-
Timaviella karstica GR13	8	85	39	13	74	13	73	43	44	17	11	14	59	27
Timaviella circinata GR4	8	85	39	13	74	13	73	44	50	17	11	14	59	26
Timaviella WMT-WP7-NPA	8	75	36	15	74	15	73	21	42	17	11	14	61	27
Timaviella obliquedivisa GSE-PSE28-08A	8	63	42	21	74	50	73	41	49	17	11	14	61	27
Timaviella obliquedivisa GSE-PSE-MK23-08B	8	63	42	21	74	50	73	41	49	17	11	14	61	27
Timaviella radians GSE-UNK-7R	8	81	42	22	74	14	73	41	33	18	11	14	59	34
Timaviella radians GSE-TBD6-7R	8	81	42	22	74	14	73	41	33	18	11	14	59	34
Tildeniella torsiva Uher 1998/13d	7	66	33	14	74	11	73	35	49	18	11	15	92	16
Tildeniella torsiva Hubel 1974/223	7	66	33	14	74	11	73	35	49	18	11	15	92	16
Tildeniella nuda Zehnder 1965/U140	7	65	34	12	74	84	73	46	38	17	11	13	-	-
Komarkovaea angustata EY01-AM2	7	64	33	12	74	29	73	94	41	16	11	14	96	16
"Trichotorquatus" sp. TAA2-2HA1	8	80	42	14	74	37	73	34	36	16	11	14	65	-
"Trichotorquatus" sp. ATA1-4-KO25A	7	101	38	12	74	28	73	64	36	18	11	87	49	13
"Trichotorquatus" sp. WJT66-NPBG9	7	81	38	12	74	28	73	39	36	18	11	40	96	12
Kaiparowitsia implicata GSE-PSE-MK54-09C	7	142	37	11	74	11	73	105	36	17	11	18	113	23
Kaiparowitsia implicata GSE-TBC-9CA	7	142	37	11	74	11	73	105	36	17	11	18	113	23
Kaiparowitsia implicata GSE-TBC-9CA2	7	142	37	11	74	10	73	105	36	17	11	18	113	23

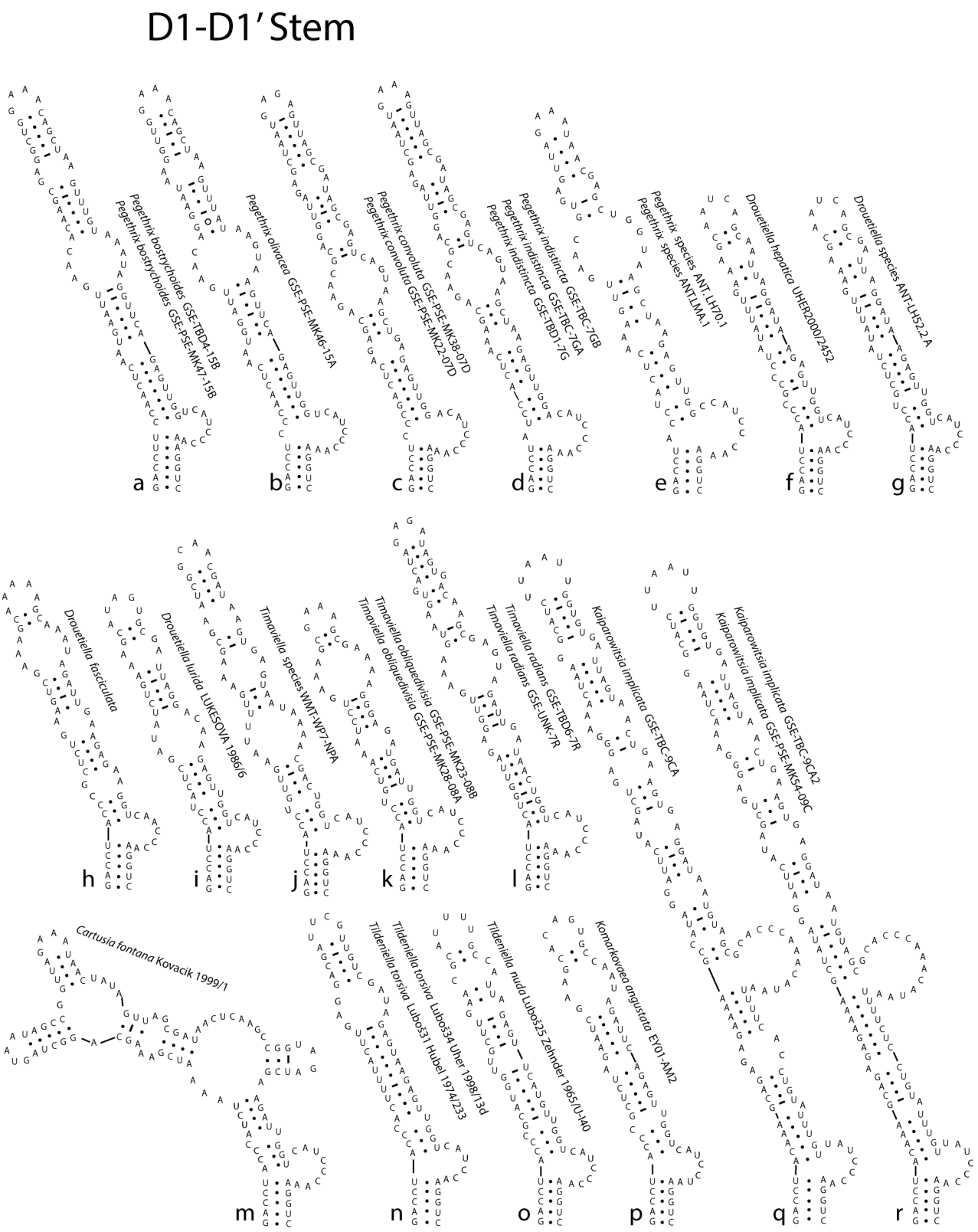

FIGURE 6. D1-D1' stems of species described in Oculatellaceae. The stem structures of genera described previously in other publications (*Oculatella*, *Thermoleptolyngbya, Timaviella*) or in publications under provision (*Trichotorquatus*) are not shown here.

Oculatellaceae Mai *et* Johansen *fam. nov.*

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* Mai, Johansen *et* Bohunická, *gen. nov.

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 diffluent. 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 or without the presence of necridia.

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

Type species:—*Pegethrix bostrychoides* Mai, Johansen *et* Bohunická, *sp. nov*.

***Pegethrix bostrychoides* Mai, Johansen *et* Bohunická, *sp. nov*.**

Diagnosis:—Differing from other species in the genus based on the frequent formation of spirals; the internal loop near the base of the Box B helix of the ITS region at position 5–6/31–32 (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, sometimes forming nodules (Fig. 10a), or loosely to tightly spirally coiled (Figs. 10b–g), rarely singly (Fig. 10i) or doubly false branched (Fig. 10h), 2.0–6.0 μm wide (to 14 μm wide at nodules). Sheath firm, colorless, usually attached to trichome, occasionally softer, widened (Figs. 10b, i–k), sometimes irregular and stratified (Fig. 10k). Trichomes untapered, more or less constricted at the distinctly visible cross-walls, occasionally with tight, regular, screw-like coils (Fig. 10l), 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' side loop of 8 unpaired nucleotides (5'-UCAUCCCA-3'), mid-helix region with two unpaired adenine residues at position 14–15, internal loops at position 22–25/56–60 and at 32–33/48–49. Terminal loop 5'-GAAA-3' (Fig. 6a). Box B helix 36 nucleotides long, bearing 4 nucleotides at terminal loop, and one small internal loop at position 5–6/31–32 (Fig. 7a). V2 helix 24 nucleotides long, with terminal loop of 6 nucleotides (Fig. 8a). V3 helix 96 nucleotides long, with several internal loops at position 5–6/92, 10–14/86–88, 18/81–82, 36–37/62–63, with two mismatches of 5'-A/G-3' and 5'-G/G-3' at 29/70 and 44/55. Terminal loop sequence 5'-GAGA-3' (Fig. 9a).

Etymology:—*bostrychos* (Gr.): curl, anything twisted; Latinized to *bostrychoides*

Type locality:—Drip Tank Seep Wall site, Grand Staircase-Escalante National Monument (GSENM), 37°19'12.79''N, 111°31'50.59"W, collected on 15 August 2006 by Markéta Bohunická. Sandstone seep wall with small moist area and larger pond below the rock face, within Strait Cliffs Formation, in the GSENM, Kane County, Utah, USA.

Holotype here designated:—BRY37770!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Isotype here designated:—BRY37771!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Reference strain:—GSE-PSE-MK47-15B, Algal Culture Collection at John Carroll University, Cleveland, USA.

Taxonomic 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.

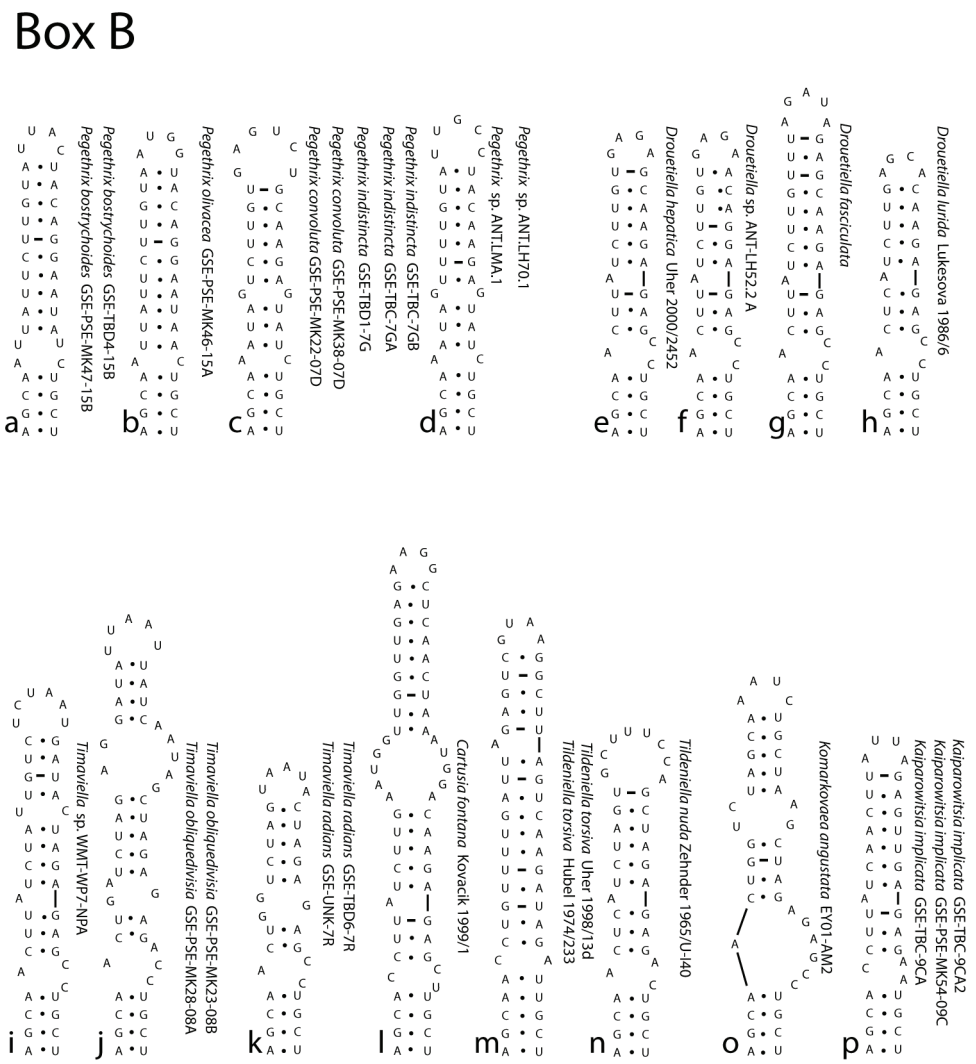

FIGURE 7. Box B helices of species described in Oculatellaceae. The structures of genera described previously in other publications (*Oculatella*, *Thermoleptolyngbya, Timaviella*) or in publications under provision (*Trichotorquatus*) are not shown here.

P. bostrychoides 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 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 7). However, the phylogeny separates the species fairly well (Fig. 2), with *P. bostrychoides* being sister to *P. olivacea*. The separation of species is further supported by the large percent dissimilarity of the 16S–23S ITS region (≥8.2%, see Table 8), 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).

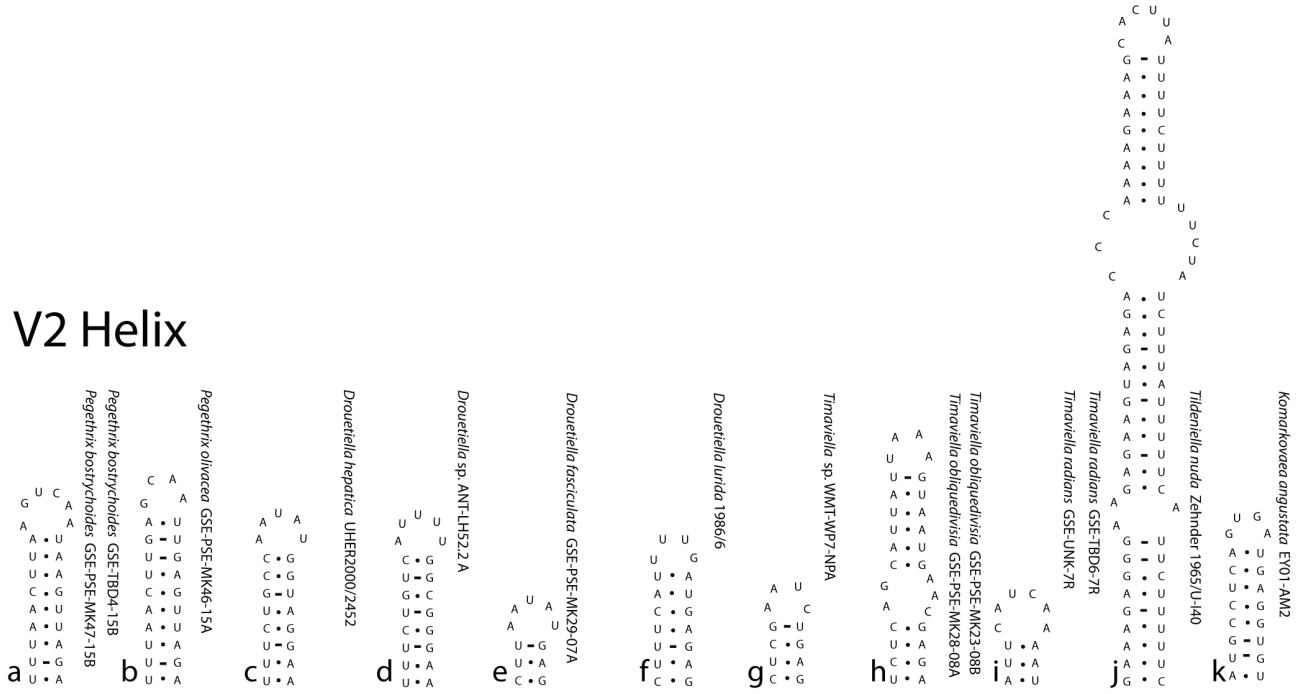

FIGURE 8. V2 helices of species described in Oculatellaceae. Several species do not have this structure, including *Pegethrix convoluta*, *P. indistincta*, Antartic *Pegethrix* species, *Cartusia fontana*¸ *Kaiparowitsia implicata.*

TABLE 7. 16S rRNA genetic similarity among *Pegethrix* species.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*P. convoluta* GSE-PSE-MK22-07D	-												
2	*P. convoluta* GSE-PSE-MK38-07D	100.0	-											
3	*P. indistincta* GSE-TBC-7GA	99.74	99.74	-										
4	*P. indistincta* GSE-TBD1-7G	99.83	99.83	99.91	-									
5	*P. indistincta* GSE-TBC-7GB	99.83	99.83	99.91	100.0	-								
6	*P. bostrychoides* GSE-PSE-MK47-15B	98.62	98.62	98.71	98.79	98.79	-							
7	*P. bostrychoides* GSE-TBD4-15B	98.62	98.62	98.71	98.80	98.80	100.0	-						
8	*Pegethrix* sp. ANT.LH70.1	99.74	99.74	99.83	99.91	99.91	98.88	98.88	-					
9	*Pegethrix* sp. ANT.LMA.1	99.48	99.48	99.57	99.66	99.66	98.62	98.62	99.74	-				
10	*P. olivacea* GSE-PSE-MK46-15A	98.88	98.88	98.97	99.05	99.05	99.05	99.05	99.14	98.88	-			
11	*Leptolyngbya* sp. 1T12c	98.28	98.28	98.36	98.45	98.45	98.62	98.62	98.54	98.80	98.10	-		
12	Uncultured bacterium sp. GBe-058	97.80	97.80	98.08	97.99	97.99	97.62	97.62	97.89	98.17	97.43	98.44	-	
13	*Leptolyngbya* sp. VP3-07	97.16	97.16	97.42	97.33	97.33	96.90	96.90	97.24	97.50	97.24	97.25	97.26	-

TABLE 8. ITS genetic similarity among *Pegethrix* species.

	Strain	1	2	3	4	5	6	7	8	9	10
1	*P. convoluta* GSE-PSE-MK22-07D	-									
2	*P. convoluta* GSE-PSE-MK38-07D	100.0	-								
3	*P. indistincta* GSE-TBC-7G$_A$	95.89	95.89	-							
4	*P. indistincta* GSE-TBD1-7G	95.89	95.89	100.0	-						
5	*P. indistincta* GSE-TBC-7G$_B$	95.89	95.89	100.0	100.0	-					
6	*P. bostrychoides* GSE-PSE-MK47-15B	76.95	76.95	76.75	76.75	76.75	-				
7	*P. bostrychoides* GSE-TBD4-15B	77.17	76.95	76.75	76.75	76.75	100.0	-			
8	*Pegethrix* sp. ANT.LH70.1	90.73	90.73	91.48	91.48	91.48	81.48	81.48	-		
9	*Pegethrix* sp. ANT.LMA.1	90.71	90.73	91.47	91.47	91.47	81.44	81.44	100.0	-	
10	*P. olivacea* GSE-PSE-MK46-15A	77.17	77.17	76.62	76.62	76.62	91.77	91.77	80.69	80.66	-

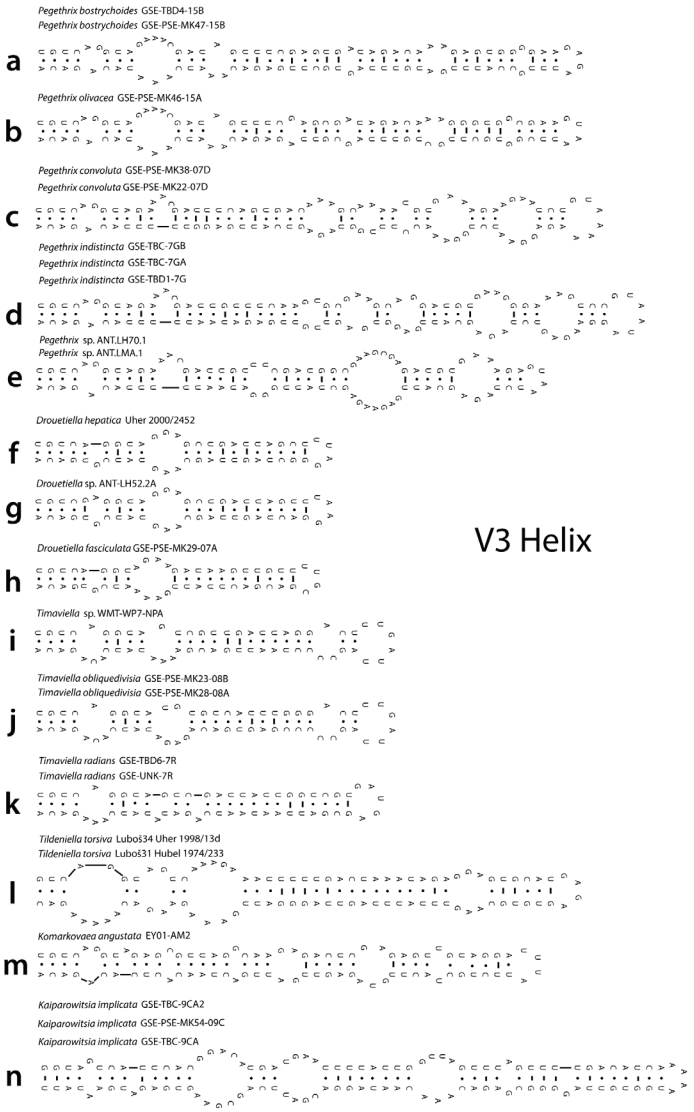

FIGURE 9. V3 helices of species described in Oculatellaceae. All species described have this structure. The V3 helix of *Cartusia aeruginosa* is missing because we do not have the full length of its ITS region.

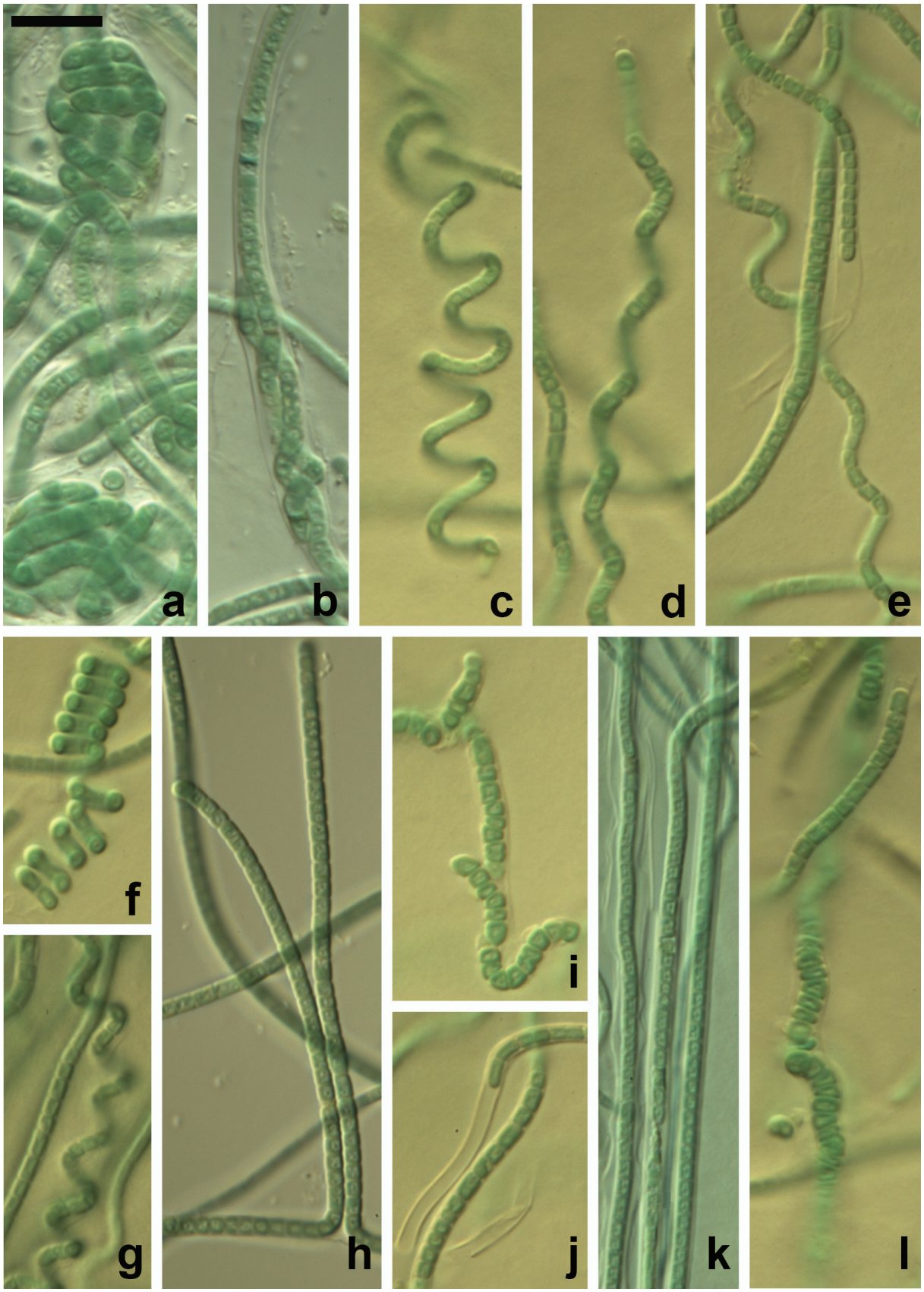

FIGURE 10. *Pegethrix bostrychoides*. A. Nodule formation. B. Existence of multiple trichomes within common sheath. C–G. Variation in the degree of filament coiling, from flexuous to spiral coils. H–I. Double false-branches and single false-branches. J–K. Sheath tightly embraces trichome or expands from trichome. L. Heterogeneity in cell shape and trichome width between young and mature trichomes. Meristematic zone of cell division shown on mature trichome. Scale bar 10μm in 1000X magnification.

Pegethrix olivacea* Mai, Johansen *et* Bohunická *sp. nov.

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.

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 (Fig. 11a–d), false branched (Fig. 11e), sometimes loosely coiled to form irregular nodules (Fig. 11d), 2.0–3.3–(3.7) µm wide. Sheath firm, colorless, usually attached to trichome, occasionally widened (Fig. 11h), 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. 11g–k), 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. 11h–k). 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. 11k).

D1-D1' helix similar to that of *P. bostrychoides* in structure and sequence, 87 nucleotides long, with basal 3' side loop of 9 unpaired nucleotides (5'-UCAUCCCAA-3'), opposed on the 5' strand by a single unpaired cytosine residue, with mid-helix region with two unpaired adenine residues at position 14–15, several internal loops at position 32–33/48–49 and 22–25/56–60. Terminal loop having sequence 5'-GAAA-3' (Fig. 6b). Box B helix 36 nucleotides long, bearing 4 nucleotides at terminal loop, and one 5'-A/C-3' mismatch at position 5/32 (Fig. 7b). V2 helix 24 nucleotides long, with terminal loop of 4 nucleotides (Fig. 8b). V3 helix 96 nucleotides long, with several internal loops at positions 5–6/92, 10–14/86–88, 18/81–82 and 36–37/62–63, and two mismatches of 5'-G/A-3' and 5'-A/G-3' at positions 24/75 and 29/70 respectively. Terminal loop having sequence 5'-GUAA-3' (Fig. 9b).

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

Type locality:—Drip Tank Seep Wall site, Grand Staircase-Escalante National Monument, 37°19'12.79"N, 111°31'50.59"W, collected on 15 August 2006 by Markéta Bohunická. Sandstone seep wall with small moist area and larger pond below the rock face, within Strait Cliffs Formation, in the GSENM, Kane County, Utah, USA.

Holoype here designated:—BRY37772!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Reference strain:—GSE-PSE-MK46-15A, Algal Culture Collection at John Carroll University, Cleveland, USA.

Taxonomic 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. bostrychoides* 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 6). The 16S rRNA gene phylogeny also supports the close relationship between these two species (Fig. 2). However, the species is distinguished from *P. bostrychoides* 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*, percent dissimilarity based on aligned ITS regions of the species to all other species is in the range of 8%–23% (Table 8), 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 6).

Pegethrix convoluta* Mai, Johansen *et* Bohunická *sp. nov.

Diagnosis:—Differing from other species in the genus based on the frequent formation of loose irregular nodules. Distinguishable from *P. indistincta* 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). Some similarity with *P. bostrychoides* and *P. olivacea* in nucleotides 1–15 on the 5' strand and their complement was observed, but the absence of the V2 helix and other dissimilarities clearly set them apart.

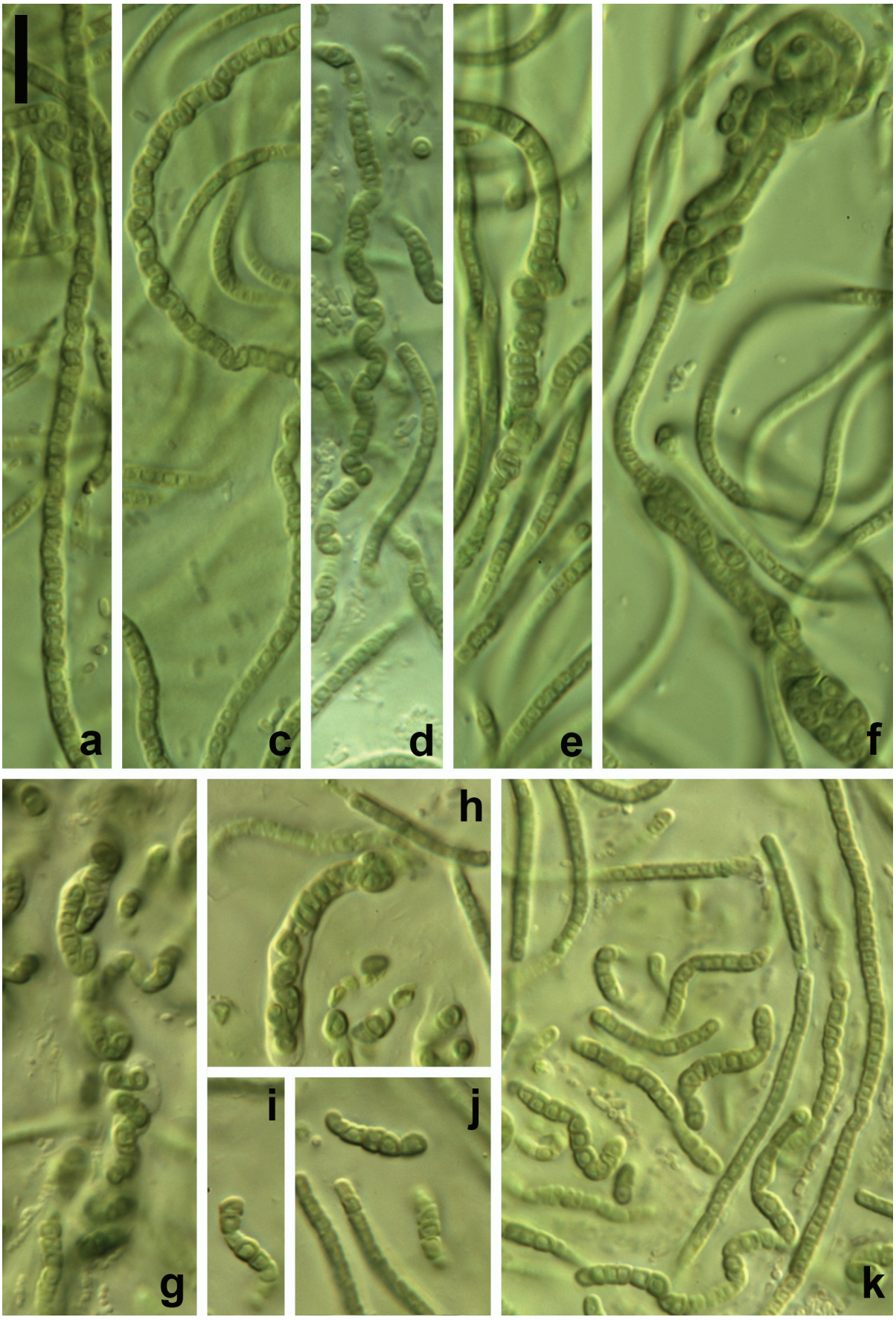

FIGURE 11. *Pegethrix olivacea*. A–D. Irregular filament shapes due to uneven cell division events along trichome. E. Single false-branching filament. F. Nodule formation. G–K. Irregular cell shape and trichome length, hormogonia few-celled, abundant. Scale bar 10μm in 1000X magnification.

Description:—Colony bright blue green, radially spreading, growing into the agar. Filaments fasciculated, long, sometimes singly or doubly false branched (Fig. 12a), straight or slightly bent (Figs. 12a–b, e), frequently forming loose to compact nodules (Figs. 12b–c), 1.4–3.9–(4.9) µm wide. Sheath firm, colorless, usually attached to trichome (Fig. 12e), occasionally widened (Figs. 12d–e), 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, e–f), lacking meristematic zones, with cell division occurring throughout trichome, 1.3–2.5 (3.2) µm wide. Hormogonia few-celled (Fig. 12a). 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' side loop of 9 unpaired nucleotides (5'-ACAUCCCAA-3') opposed by a single cytosine residue, with multiple small internal loops at position 14–15/69–70 (with sequence 5'-AG/GA-3'), at 26–27/56–57 and 32–33/50–51, one large asymmetrical internal loop at position 19–23/60–65. Terminal loop with 4 nucleotides, with sequence 5'-GAGA-3' (Fig. 6c). No V2 helix present between $tRNA^{Ala}$ and $tRNA^{Ile}$. Box B helix with 36 nucleotides, bearing 6 nucleotides at terminal loop (Fig. 7c). V3 helix 110 nucleotides long, with one basal internal loop (5–6/101), one unilateral bulge at positions 12–14, several small internal loops at positions 27–29/81–83, 32–33/76–77, 39–41/68–71 and 45–48/61–64 and a pair of mismatched 5'-U/C-3' at position 36/74. Terminal loop of 6 nucleotides, having sequences 5'-GUAAAA-3' (Fig. 9c).

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

Type locality:—Lower Calf Creek Falls site, Grand Staircase-Escalante National Monument, 37°49'44.77''N, 111°25'12.58"W, collected on 15 August 2006 by Markéta Bohunická. Large seep wall and waterfall in Navajo Sandstone, in the GSENM, Kane County, Utah, USA. Small pool with blackened soil and microbial layer at the base of seep wall.

Holotype here designated:—BRY37773!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Reference strain:—GSE-PSE-MK38-07D, Algal Culture Collection at John Carroll University, Cleveland, USA. Other reference strain of the species: GSE-PSE-MK22-07D, Algal Culture Collection at John Carroll University, Cleveland, USA.

Taxonomic notes:—Based on the ecological preference for subaerophytic environments, morphological traits such as the 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 (percent dissimilarity = 8.23%, see Table 8). It is molecularly most similar to *P. indistincta*, with highly similar secondary structures (identical in the Box B helix) and fairly low percent dissimilarity between ITS sequences. Percent dissimilarity between *P. convoluta* and *P. indistincta* is intermediate between levels normally separating species and populations of the same species (4.11%, see Table 8). The trichome widths overlap, although *P. indistincta* typically has wider trichomes than *P. convoluta*.

Pegethrix indistincta* Mai, Johansen *et* Bohunická, *sp. nov.

Diagnosis:—Morphologically intermediate between other species, differing in the absence of spiraling and nodule formation; with conserved ITS domains almost identical in shape to D1-D1' helix of *P. convoluta* (Fig. 6d). Distinguishable from this species in the adenine residue opposite the basal 3' unilateral bulge of the D1-D1' helix and sequence from position 1–15/69–90 (Figs. 6d), and the V3 helix sequence and structure (Figs. 9d).

Description:—Colony bright blue green or olive-green. Filaments long, with variation in width between young and mature trichomes (Figs. 13a), rarely singly or doubly false branched (Figs. 13a–b), rarely with more than one trichome sharing a common sheath (Fig. 13c), 2.3–4.0 µm wide. Sheath firm, usually attached to trichome, occasionally widened, rarely irregular and stratified (Fig. 13d), absent in immature filaments or hormogonia. Trichomes untapered,

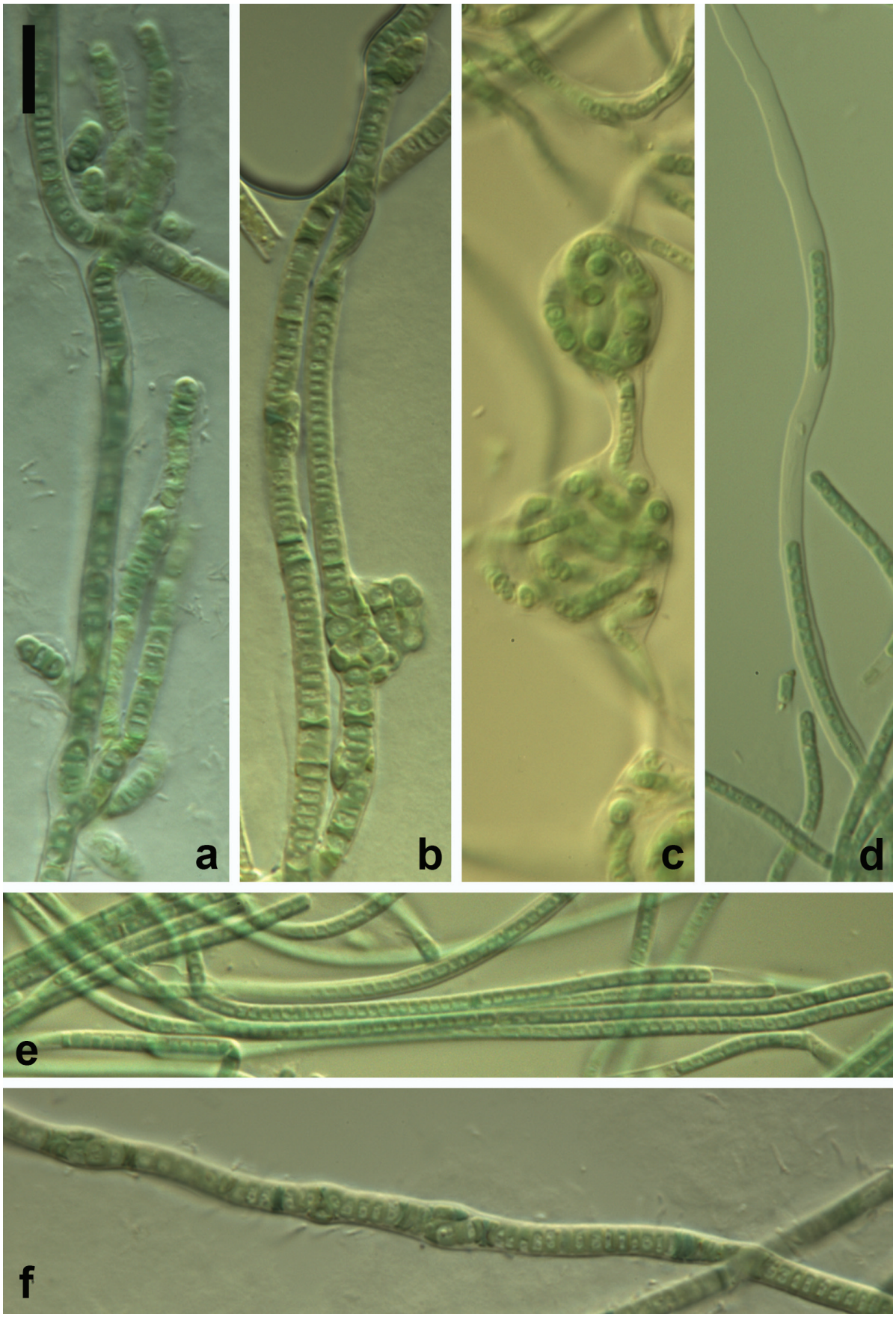

FIGURE 12. *Pegethrix convoluta*. A. Consecutively false-branching filaments. B–C. Compact nodules formed by rapid cell division in local region of trichomes. D–E. Sheath rarely irregular and stratified, more commonly tightly attached to trichomes. F. Necridia abundant in mature trichomes. Scale bar 10µm in 1000X magnification.

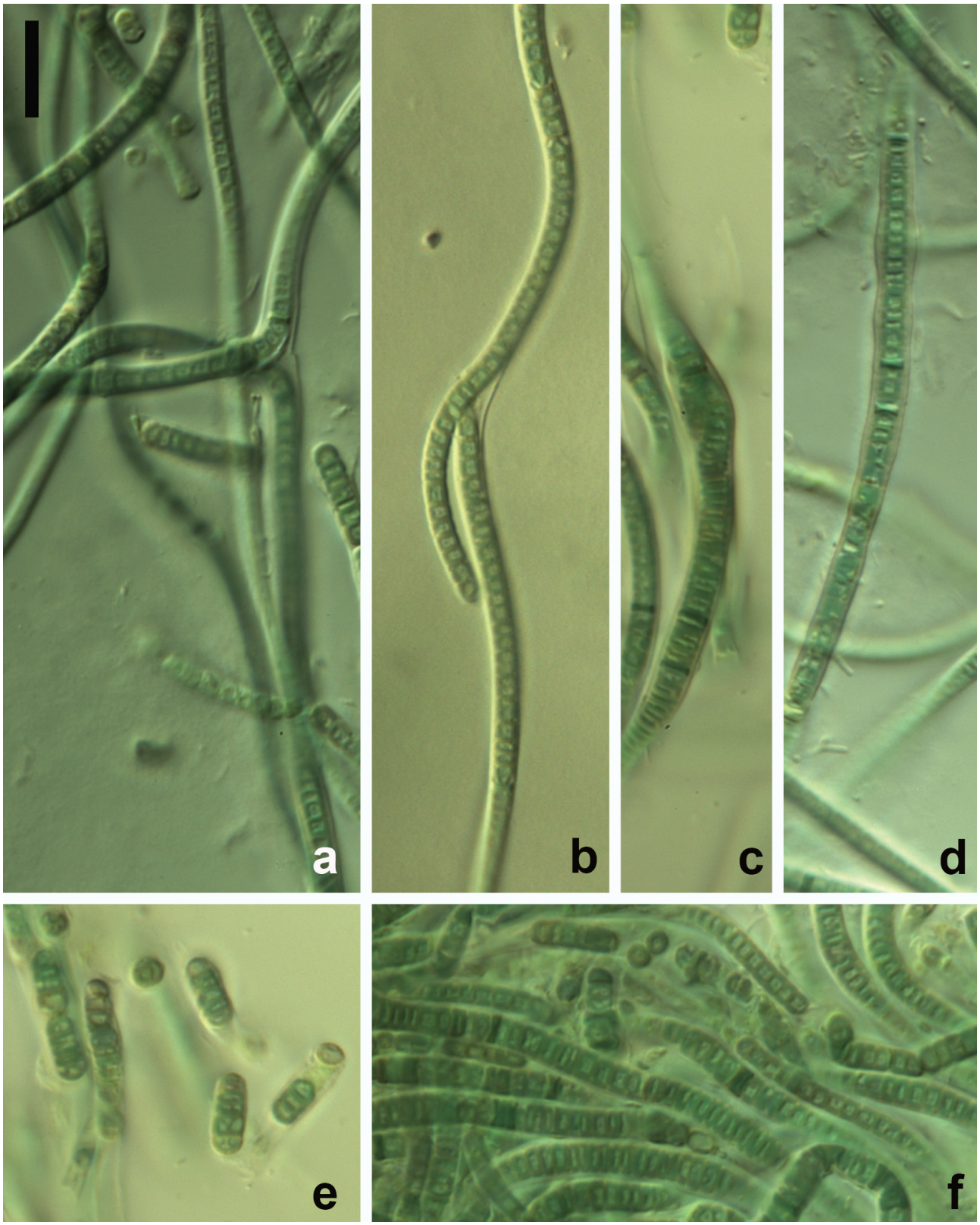

FIGURE 13. *Pegethrix indistincta*. A–B. Double and single-false branching filaments. C. Multiple trichomes coiling in one common sheath. D. Thick and firm sheath observed in mature trichomes. E–F. Hormogonia released from bundles of trichome undergoing rapid disintegration (note necridia). Scale bar 10μm in 1000X magnification.

not or slightly constricted at distinctly visible crosswalls, with necridia (Figure 13c–d, f), with meristematic zones, 1.9–3.3 µm wide. Hormogonia short (Figs. 13e–f). Cells typically isodiametric, often shorter than wide especially in meristematic zones (Fig. 13f), slightly longer than wide in young trichomes (Fig. 13a–b), (1.3)–1.7–2.7 µm long. End cells rounded.

D1-D1' helix of the 16S–23S ITS region 91 nucleotides long having the basal 3' side loop of 8 unpaired nucleotides (5'-CAUCCCAA-3') opposed by a single adenine residue, with multiple internal loops at positions 14–15/69–70, 19–23/60–65, 26–27/56–57 and 32–33/50–51. Terminal loop with 4 nucleotides, with sequence 5'-GAAA-3' (Fig. 6d). Box B helix is identical to *P. convoluta* with 36 nucleotides, bearing 6 nucleotides at terminal loop (Fig. 7c). No V2 helix present between $tRNA^{Ala}$ and $tRNA^{Ile}$. V3 helix 110 nucleotides long, with one basal internal loop (5–6/101), with one unilateral bulge at position 12–14, several small internal loops at positions 23–27/88–89, 30–32/84–85, 35–36/79–81, 42–45/71–73 and 49–51/64–67. Terminal loop of 6 nucleotides, bearing sequence of 5'-GUAAUA-3' (Fig. 9d).

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

Type locality:—Lower Calf Creek Falls site, Grand Staircase-Escalante National Monument, 37°49'44.77''N-111°25'12.58"W, collected on 15 August 2006 by Markéta Bohunická. Large seep wall and waterfall in Navajo Sandstone, in GSENM, Kane County, Utah, USA. Found in black compact microbial mat.

Holotype here designated:—BRY 37774!, Herbarium for Nonvascular Cryptogams, Monte L., Bean Museum, Provo, Utah.

Isotypes here designated:—BRY37775! and BRY37776!, Herbarium for Nonvascular Cryptogams, Monte L., Bean Museum, Provo, Utah.

Reference strain: —GSE-TBC-7GA, Algal Culture Collection at John Carroll University, Cleveland, USA. Additional reference strains: GSE-TBC-7GB and GSE-TBD1-7G, Algal Culture Collection at John Carroll University, Cleveland, USA.

***Pegethrix* sp. ANT.LH70.1 & ANT.LMA.1** (Fig. 6e, 7d, 9e)

Diagnosis:—Secondary structure of the conserved D1-D1' domain most similar to *P. bostrychoides* and *P. olivacea* in the presence of the largest internal loop at position 20–23/44–48 (Fig. 6e). Similar to *P. convoluta* and *P. indistincta* in the presence of the internal loop at position 14–15/53–54 of D1-D1' helix (Fig. 6e), the basal mismatch at position 5 on 5' strand of Box B (Fig. 7d) and the basal helix of V3 at position 1–18 on 5' strand and its 3' complement especially at the basal internal loop and the 5' unilateral bulge. Dissimilarities between the structure and sequence of these conserved domains of Antarctic *Pegethrix* sp. and other species of the genus clearly set them apart.

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' side loop of 9 unpaired nucleotides (5'-CCAUCCCAA-3'), opposed on the 5' strand by a single unpaired adenine residue. Mid-helix region with several internal loops at positions 14–15/53–54, 20–23/44–48 and 26–27/40–41, and one large bilateral bulge at position 20-23/44-48, and a terminal loop of 4 nucleotides, having sequence of 5'-GAAA-3' (Fig. 6e). Box B helix 37 nucleotides long, bearing 5 nucleotides in terminal loop (Fig. 7d). No V2 helix present between $tRNA^{Ala}$ and $tRNA^{Ile}$. V3 helix 94 nucleotides long, with several internal loops at positions 5–6/90, 21-22/77–78, 30–36/63–69 and 42–44/55–57, with a unilateral bulge at position 12–14 on the 5' strand. Terminal loop 4 nucleotides, 5'-GUAA-3' (Fig. 9e).

Collection 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.

Taxonomic notes:—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 above is taken from the original work.

Drouetiella* Mai, Johansen *et* Pietrasiak *gen. nov.

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:—*Drouetiella*: 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:—*Drouetiella lurida* (Gomont) Mai, Johansen *et* Pietrasiak *comb. nov*.

Taxonomic notes:—The genus *Drouetiella* currently contains three named species: *D. lurida*, *D. fasciculata*, and *D. hepatica*. This genus is most closely related to the cluster containing both *Cartusia* and *Pegethrix* (Fig. 2). Genetic identity analysis of the 16S rRNA of these species separated three species at the 98.8 % threshold (Table 9). However, their percent dissimilarity in the ITS regions strongly supported their separation into four different lineages (Table 10). There are also diagnosable differences among the species in their morphology (Figs. 14–16) and secondary structures of the ITS.

TABLE 9. 16S rRNA genetic similarity among *Drouetiella* species.

	Strain	1	2	3	4
1	*Drouetiella* species ANT-LH52.2	-			
2	*Drouetiella hepatica* UHER2000/2452	99.50	-		
3	*Drouetiella lurida* LUKESOVA1986/6	97.52	97.40	-	
4	*Drouetiella fasciculata* GSE-PSE-MK29-07A	96.90	96.63	96.42	-

TABLE 10. ITS genetic similarity among *Drouetiella* species.

	Strain	1	2	3	4
1	*Drouetiella* species ANT-LH52.2	-			
2	*Drouetiella hepatica* UHER2000/2452	93.07	-		
3	*Drouetiella lurida* LUKESOVA1986/6	82.35	81.77	-	
4	*Drouetiella fasciculata* GSE-PSE-MK29-07A	74.99	75.12	76.35	-

Drouetiella lurida* (Gomont) Mai, Johansen *et* Pietrasiak *comb. nov.

Basionym:—*Phormidium luridum* Gomont 1892, *Annales des Sciences Naturelles*, *ser*. 7 16:165, plate 4, figs 17, 18.

Later Synonyms:—*Leptolyngbya lurida* (Gomont) Anagnostidis & Komárek 1988: *Archiv für Hydrobiologie*, *Supplement* 80: 392

Diagnostic features:—Dissimilar to all other *Drouetiella* species in the shape and sequence of D1-D1' helix and V2 helix (Figs. 6, 8).

Description of epitype:—Colony reddish brown or brown in actively growing cultures, turning olive-green as culture senesces (Fig. 14a). Filaments long, without false branching, 2.0–2.6 (3.4) µm wide. Sheath firm, thin, colorless, up to 0.8 µm wide (Fig. 14c). Trichomes not or slightly constricted at distinct cross-walls, without necridia, 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. 14b, d), 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.

D1-D1' helix 64 nucleotides long, with basal side loop of 7 nucleotides (5'-CAUCCCA-3'), at mid-helix with one large internal loop at position 14–17/41–44, one pair of mismatched nucleotides at position 9 on the 5' strand (U/U) and one sub-terminal internal loop at 23–24/34–35 immediately separated from the terminal loop by a 5'-GC:GC-3' clamp. Terminal loop of 5 nucleotides, terminal sequence 5'-AUAGU-3' (Fig. 6i). Box B 34 nucleotides long, with one basal internal loop at position 5/27–28 and one unpaired adenine residue at position 9 of the 5' strand (Fig. 7h). V2 helix 18 nucleotides long, unique in sequence and shape, terminal loop of 4 nucleotides, sequence 5'-UUUG-3' (Fig. 8f). V3 helix was not reported as the sequence from NCBI terminated before the end of the ITS region.

Epitype:—Czech Republic: City of Most, collected in 1986 by Alena Lukesova (Epitype BRY37777!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah).

Reference strain:—Lukesova 1986/6, Algal Culture Collection at John Carroll University, Cleveland, USA. Found in tailings of Cainozoic clay.

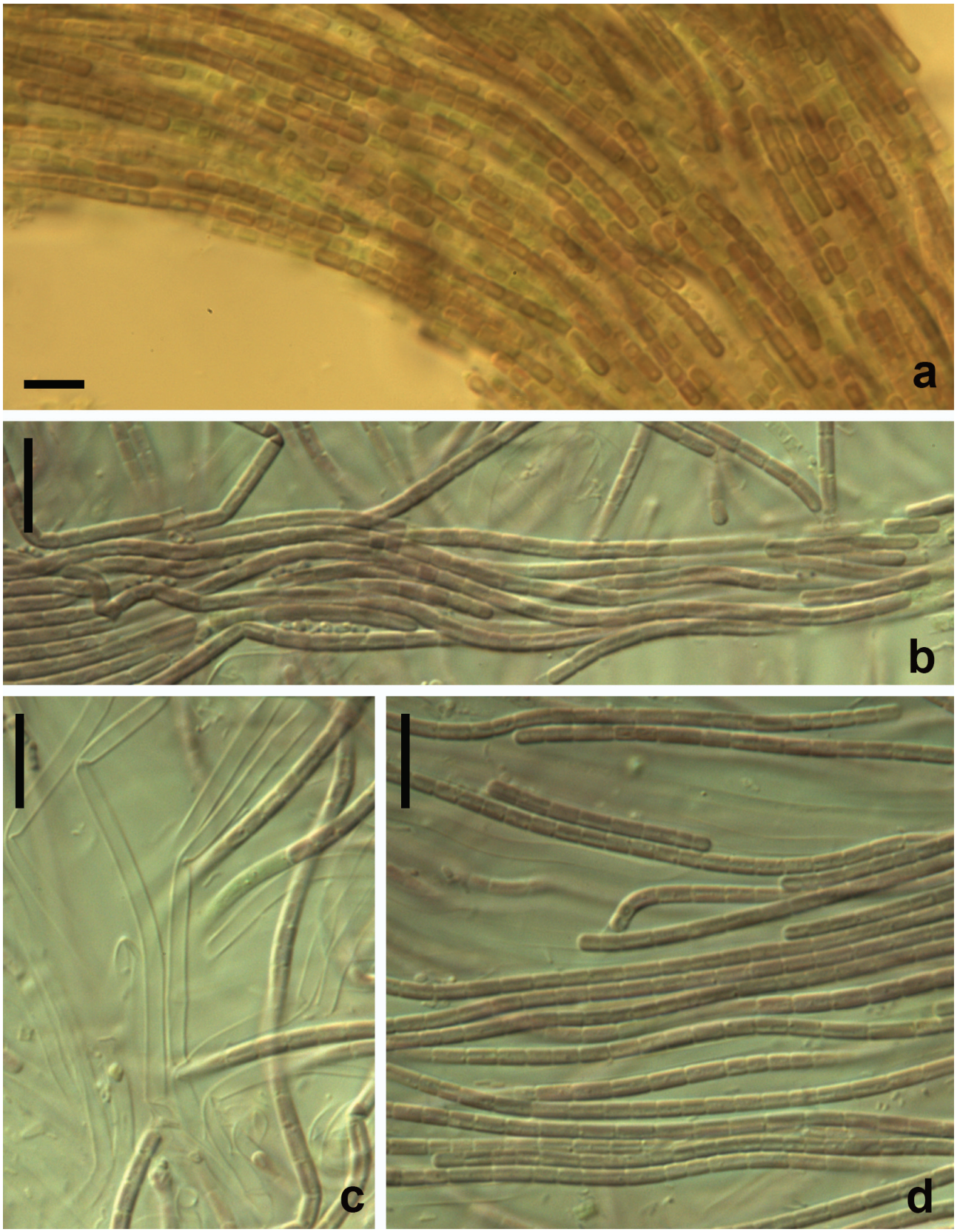

FIGURE 14. *Drouetiella lurida*. A. Olive-greenish trichome coloration of mature culture. B. Liver-brown filament coloration in healthy culture. C–D. Filaments with thin, attached sheath, indistinct with no diacritical characters. Scale bar 10μm in 400X (A) and 1000X magnification (B–D).

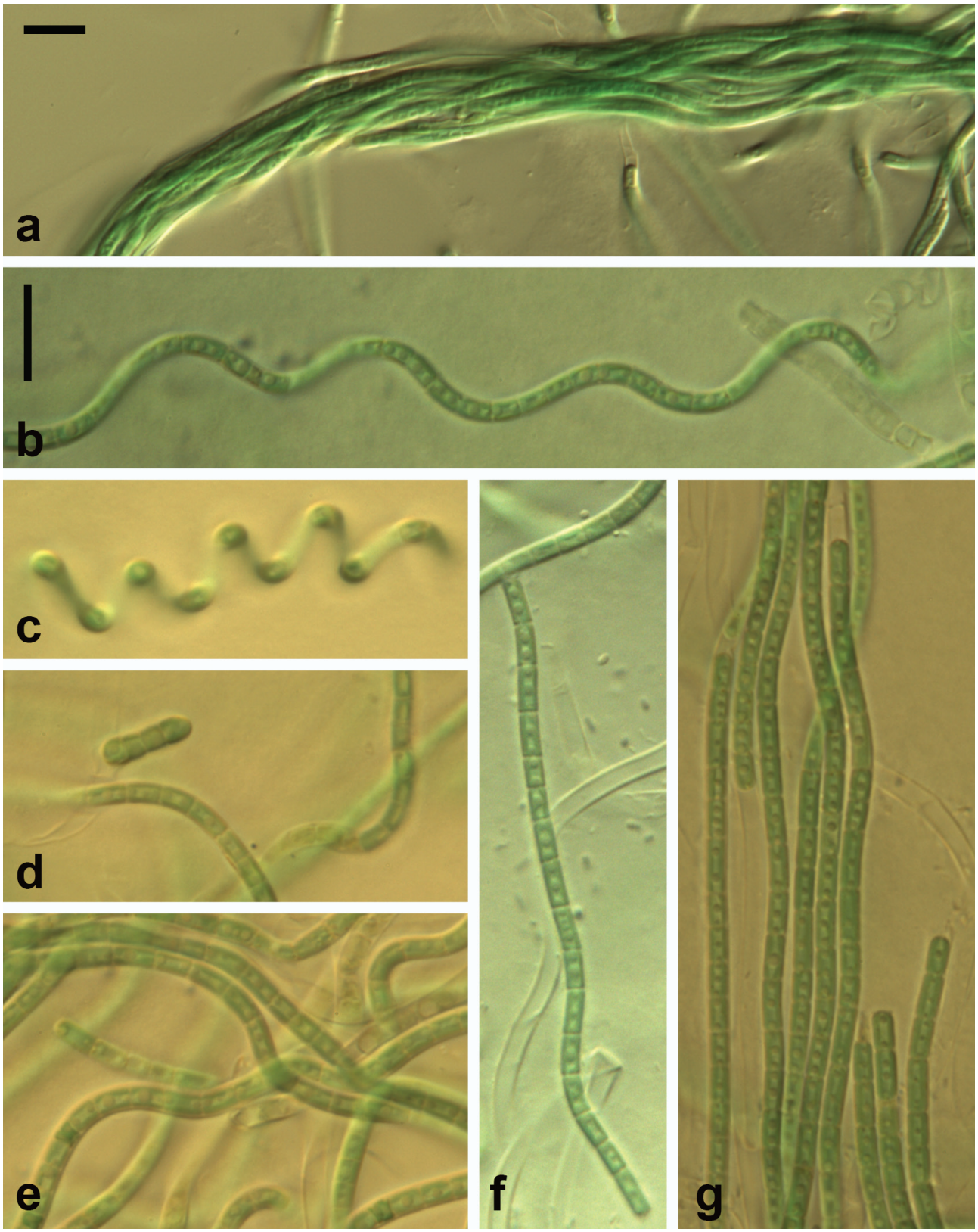

FIGURE 15. *Drouetiella fasciculata*. A. Fasiculated filaments with individual sheath. B. Mature trichomes with one to two large central granules. B–C. Different coiling patterns of filaments. D. Hormogonia. E–G. Cells barrel-shaped, not constricted and separated by somewhat translucent cross-walls. Scale bar 10μm in 400X (A) and 1000X magnification (B–J).

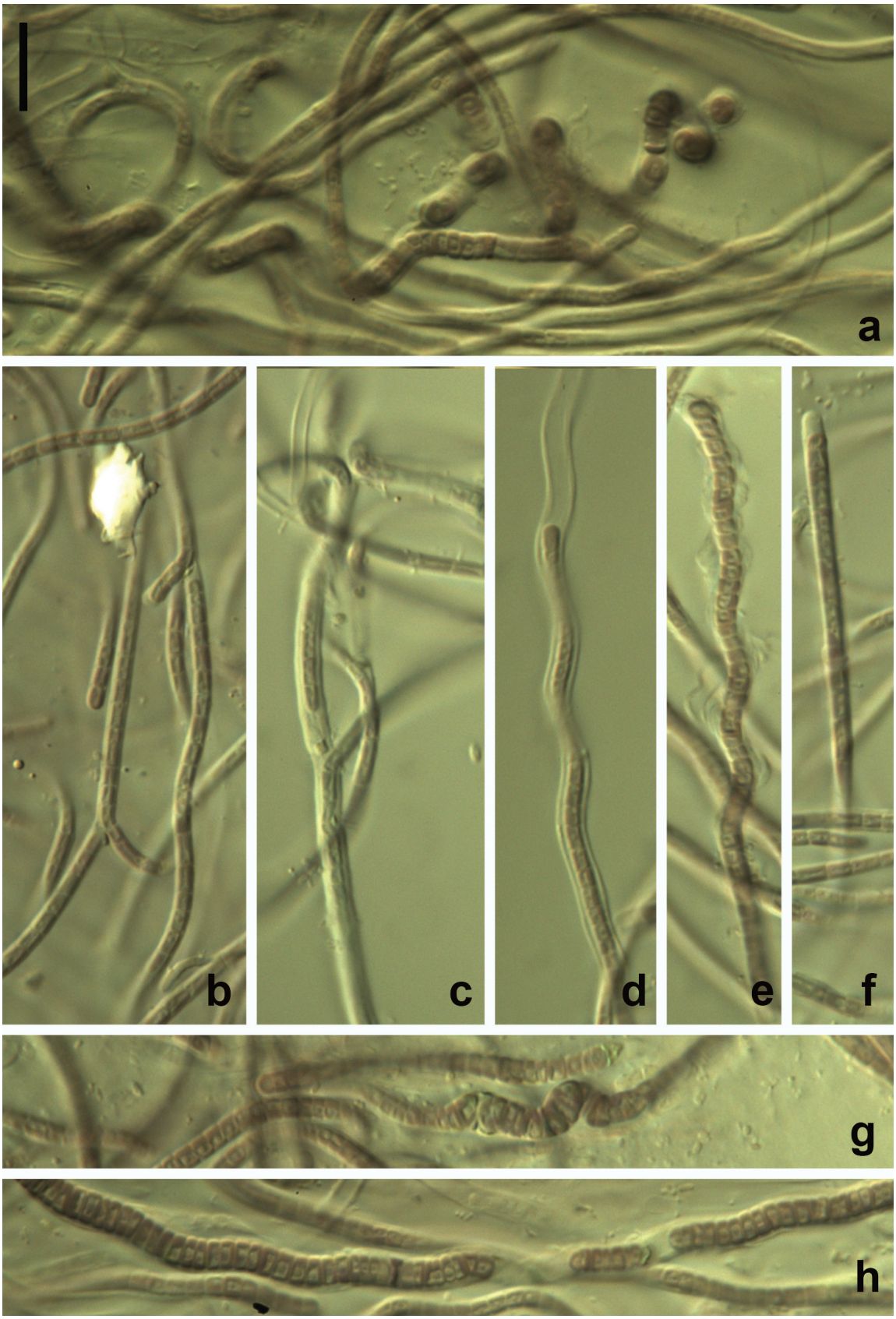

FIGURE 16. *Drouetiella hepatica*. A. Heterogeneity in trichome width between young and mature filaments, young filaments isodiametric to slightly cylindrical. B–C. False-branching very rare in young trichomes, only single false-branches observered. D–F. Sheath sometimes elongated and lamellate as in (E). G. Rapid cell division in meristematic zones sometimes caused twisted trichomes. H. Frequent necridia occur along actively dividing trichomes. Scale bar 10μm in 1000X magnification.

Taxonomic 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 cross-walls, 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 have designated an epitype which we have characterized fully to help cement the concept of the species.

The 16S rRNA gene phylogeny shows that *D. lurida* is more closely related to the species cluster of *D. hepatica* and Antarctic *Drouetiella* than to *D. fasciculata* (Fig.2, Table 9). However, in examining the percent dissimilarity of the 16S–23S ITS region, *D. lurida* is >17.0% (Table 10) different from all other species in the genus—strong evidence of separation of species.

***Drouetiella* sp. ANT.LH52.2** (Figs. 6g, 7f, 8d, 9g)

Molecular Diagnosis:—Secondary structures of conserved domain of the ITS are very similar to *D. hepatica*, almost identical in shape but with considerable difference in sequences (Figs. 6g, 7f, 8d, 9f), separated from *D. hepatica* by the extremely different habitats. Similar to other species in the presence of a 5'-GC:GC-3' closing pairs immediate to the terminal loop in the D1-D1' stem (Fig. 6g), and position 1–14 of the 5' strand and its 3' complementary of the Box B structure (Fig. 7f), but is variously different in other characteristics.

Molecular Description:—D1-D1'stem 64 nucleotides long, with basal 3' unilateral bulge of 7 unpaired nucleotides (5'-CAUCCCA-3'). Mid-helix region with one small internal loop at position 24/34–35 immediately separated from the terminal loop by a 5'-GC:GC-3' clamp, and two mismatches at position 9 (U/U) and 21 (G/A) on 5' strand. Terminal loop having sequence 5'-AAUCA-3' (Fig. 6g). Box B helix 34 nucleotides long, with one basal internal loop at position 5/29–30 and one unpaired adenine residue at position 9 of the 5' strand (Fig. 7f). V2 helix 21 nucleotides long, almost identical to *D. hepatica*, terminal loop of 5 nucleotides with sequence 5'-AUUUU-3' (Fig. 8d). V3 structure 52 nucleotides long, with one large internal loop at position 11–13/40–42 and one mismatch at position 6 (A/G) of the 5' strand. Terminal loop sequence 5'-UAAG-3' (Fig. 9g).

Reference strain:—*Leptolyngbya frigida* ANT.LH52.2.

Taxonomic notes:—This species of *Drouetiella* was isolated from an Antarctic environment without morphological description, and was identified on the basis of molecular characteristics only. Its original strain designation is *Leptolyngbya frigida* ANT.LH52.2, but it certainly does not belong to *Leptolyngbya*. Other Antarctic strains designated as *L. frigida* (ANT.LH70.1, ANT.LMA.1) are phylogenetically placed in *Pegethrix* (Fig. 2), so the correct placement of the species epithet *frigida* is ambiguous at this time. If the holotype specimen of *L. frigida* could be sequenced, it is possbile that it could be confirmed to belong in either *Pegethrix* or *Drouetiella*.

Drouetiella fasciculata* Mai, Johansen *et* Bohunická *sp. nov.

Diagnosis:—*D.fasciculata* is phenotypically distinct from other *Drouetiella* species due to its bright blue-green color and fasciculation of trichomes. The V3 helix has identical sequence and structure to *D. hepatica* in nucleotides 1–8 on the 5' strand and their complement on the 3' strand (Fig. 8e); however, in general it is distinctive in length and sequence from all other species (Table 6). The percent dissimilarity between the ITS region of this species and the other taxa is >25% (Table 10).

Description:—Colony bright blue green, composed of fasciculated (Fig. 15a) and solitary filaments, growing into the agar. Filaments long, without false branching, frequently slightly coiled and entangled (Figs. 15b–c), 2.7–3.2 μm wide. Sheath firm, usually attached to trichome, occasionally distinct, clear, up to 1.3 μm wide. 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 rare (Fig. 15d). 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 (Figs. 15e–f). End cells untapered, rounded (Fig. 15g).

D1-D1' 65 nucleotides long, with a 3' unilateral bulge of 7 nucleotides (5'-CAACCCA-3'), and several internal loops in the mid-helix region at positions 8–9/49–50 and 15–16/43–44, with the largest bilateral bulge at position 21–24/34–38 immediately subtending the terminal loop by a 5'-GC:GC-3' clamp (Fig. 6h). Box B helix 39 nucleotides long, with a basal bulge at position 5/34–35 and one unpaired adenine residue at position 9 on the 5' strand (Fig. 7g).

V2 helix 11 nucleotides long, with a 5-nucleotide terminal loop, having sequence 5'-AAUAU-3' (Fig. 8e). V3 helix 51 nucleotides long, with one basal unpaired guanine residue at position 46 of the 3' strand, and one large bilateral bulge at position 9–12/39–42. Terminal loop sequence 5'-UUGC-3' (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, in the Grand Staircase-Escalante National Monument, 37°49'44.77"N, 111°25'12.58"W, collected on 15 August 2006, by Markéta Bohunická. Large seep wall and waterfall in Navajo Sandstone, found in mats with filamentous algae, in the GSENM, Kane County, Utah, USA.

Holotype here designated:—BRY37779!, Herbarium for Nonvascular Cryptogams, Monte L., Bean Museum, Provo, Utah.

Reference strain:—GSE-PSE-MK29-07A, Algal Culture Collection at John Carroll University, Cleveland, USA.

Taxonomic notes:—The simple morphology of *D. fasciculata* keys to multiple species of *Leptolyngbya*. The subaerophytic species, *Leptolyngbya gracillima* (Hansgirg 1892b: 41) Anagnostidis & Komárek (1988: 391) is similar in dimensions, but differs 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 *D. 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 *D. fasciculata* that we do not feel using this name is appropriate for our populations. Several other species were 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* (C.-C.Jao 1948: 169) Anagnostidis (2001: 367), and *Lyngbya jacutica* Kisselev (1935:73). These five species were described from tropical, marine, or polar aquatic habitats very different from the temperate climate, subaerophytic habitat in which *D. fasciculata* was found. Furthermore, they differ in dimensions, and it appears to be a new species. It is equally morphologically dissimilar to these species, such that it could not be assigned to any one of them with confidence.

Drouetiella hepatica* Mai, Johansen *et* Pietrasiak *sp. nov.

Diagnosis:—Distinguished from the other species of the genus by its meristematic zones and false branching, and by its wide variability in trichome width.

Description:—Colony brownish or purplish-brown, forming floating, mucilaginous mats in liquid culture. Filaments long or short. Mature filaments 2.8–3.7 µm wide (Figs. 16a, g–h), young filaments narrower, 2.3–2.8 µm wide (Figs. 16b–f), occasionally with false branching (Fig. 16a–b). Sheath firm, colorless, thin to occasionally enlarged (Figs. 16c–e) and lamellate (Fig. 16e), up to 4.4 µm wide. Trichomes not or slightly constricted at cross-walls, cylindrical, with compacted coils at meristematic zones (Figs. 16a,g), 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. Necridia present (Fig. 16h). End cells cylindrical, rounded.

D1-D1' helix 64 nucleotides long, with 3' unilateral bulge of 7 nucleotides (5'-CAUCCCA-3'). Mid-helix region with a mismatch at position 24/34–35 immediately separated from the terminal loop by a 5'-GC:GC-3' clamp, and two mismatches at position 9 (C/U) and 21 (G/A) on the 5' strand. Terminal loop having sequence 5'-AAUCA-3' (Fig. 6f). Box B helix 34 nucleotides long, with one basal internal loop at position 5/29–30 and one unpaired adenine residue at position 9 of the 5' strand (Fig. 7e). V2 helix 21 nucleotides long, with terminal loop having sequence 5'-AAUAU-3' (Fig. 8c). V3 helix 51 nucleotides long, with one large internal loop at position 10–12/39–41 and one unpaired guanine residue at position 46 on 3' strand. Terminal loop sequence 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:—Slovakia. National Park Slovak Paradise: Waterfall Kaskady, gorge Sucha Bela, collected in 2000 by Bohuslav Uher. Found on subaerial limestone.

Holotype here designated:—BRY37778!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Reference strain:— UHER 2000/2452, Algal Culture Collection at John Carroll University, Cleveland, USA.

Taxonomic 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 *D. hepatica* from these taxa. *L. carnea* has granulated cell contents, tortuous trichomes and irregular sheath outlines that match filaments of *D. hepatica*. However, *D. hepatica* has wider trichomes, and brownish rather than pinkish coloration. No lamellate sheath was reported in any of the above species.

D. hepatica is distinct from the other named *Drouetiella* species based on percent dissimilarity of the ITS region, which is >18% (Table 10). This very well satisfies the criterion for species distinction based on percent dissimilarity of >4.0% (Erwin & Thacker 2008, Osorio-Santos *et al.* 2014, Pietrasiak *et al.* 2014, Bohunická *et al.* 2015). The closest taxon to *D. 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 *D. hepatica* (99.5% identity), but dissimilarity in the ITS regions between two taxa is well above the 4% level used as evidence of conspecificity. Both taxa have identical domain lengths in the ITS region (Table 6). 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 *D. hepatica* must be postponed until more information is available, but the evidence that this strain and associated sequence belong to *Drouetiella* is unequivocable. It is discussed further under Antarctic *Drouetiella* sp. above.

***Timaviella* sp. WMT-WP7-NPA**

Description:—D1-D1' helix 75 nucleotides long, with a 3' basal unilateral bulge of 8 nucleotides (5'-CAUCCCAA-3'), having several internal loops at positions 14–17/50–54, 21–23/44–46 and 26–27/40–41. Terminal loop 4 nucleotides, 5'-GCAA-3' (Fig. 6j). Box B helix 42 nucleotides long, with a basal internal loop at position 5/37–38, one unpaired adenine residue at position 9 and a U/C mismatch at position 13/29 (Fig. 7i). V2 helix 12 nucleotides long (Fig. 8g). V3 helix 61 nucleotides long, with three internal loops at positions 5/55–57, 10/48–50 and 23/34–35, separated from the terminal loop by a 5'-GU:AG-3' clamp (Fig. 9i).

Compared to other species, D1'-D1' helix structure has identical basal sequence (region 1–9/59–75) especially the 3' side loop and a U/U mismatch at position 9/75 (Fig. 6j), and the V3 helix is almost identical in shape to *T. obliquedivisa* with minor differences in sequence. The strain is similar in the base of Box B helix to *T. radians* at position 1–5 and its complement on the 3' strand (Fig. 7i); however, it differs in other molecular characteristics.

Collection locality:—White Mountain Wilderness, California, USA.

Taxonomic notes:—The reference material for this species did not survive in culture, and thus has no morphological description. The species has high similarity in the 16S rRNA sequence to other species of this genus (Table 11), but we do not have enough morphological information to formally describe this as a species within *Timaviella*.

TABLE 11. 16S rRNA genetic similarity among *Timaviella* species.

	Strain	1	2	3	4	5	6	7
1	*Timaviella karstica* GR13	-						
2	*Timaviella circinata* GR4	98.71	-					
3	*Timaviella* sp. WMT-WP7-NPA	98.45	98.97	-				
4	*Timaviella obliquedivisa* GSE-PSE28-08A	98.36	96.36	99.05	-			
5	*Timaviella obliquedivisa* GSE-PSE-MK23-08B	98.36	96.36	99.05	100.0	-		
6	*Timaviella radians* GSE-UNK-7R	96.82	97.08	97.25	96.81	96.81	-	
7	*Timaviella radians GS*E-TBD6-7R	96.82	97.08	97.25	96.81	96.81	100.0	-

Timaviella obliquedivisa* Mai, Johansen *et* Bohunická *sp. nov.

Diagnosis:—Similar to *T. radians,* but differing in the profuse single and double false-branching and the coloration of trichomes, as well as the shorter D1-D1' helix. V3 helix almost identical in shape to V3 helix of *Timaviella* species WMT-WP7-NPA in shape and sequence (Fig. 9j). Box B helix also similar to both other species, but with notable differences in all conserved domains of the ITS region (Figs. 6k, 7j, 8h, 9j).

Description:—Colony radially spreading, compact, firm, leathery, sometimes mounded, dark green, with yellowed margins near senescence. Filaments, untapered to slightly tapered (Figs. 17a–b), with repeated single and double false branching (Figs. 17c–e), 2.0–3.2 (3.9) µm wide. Sheath usually thin, soft, colorless, rarely extended past

trichome apex), up to 1.3 µm wide. Trichomes false branched, with some branches erect and almost perpendicular to the original axis of the trichome (Fig. 17f–h), not constricted at distinctly visible cross-walls, occasionally becoming almost biseriate due to oblique division and compression of cells (Fig. 17f), 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.

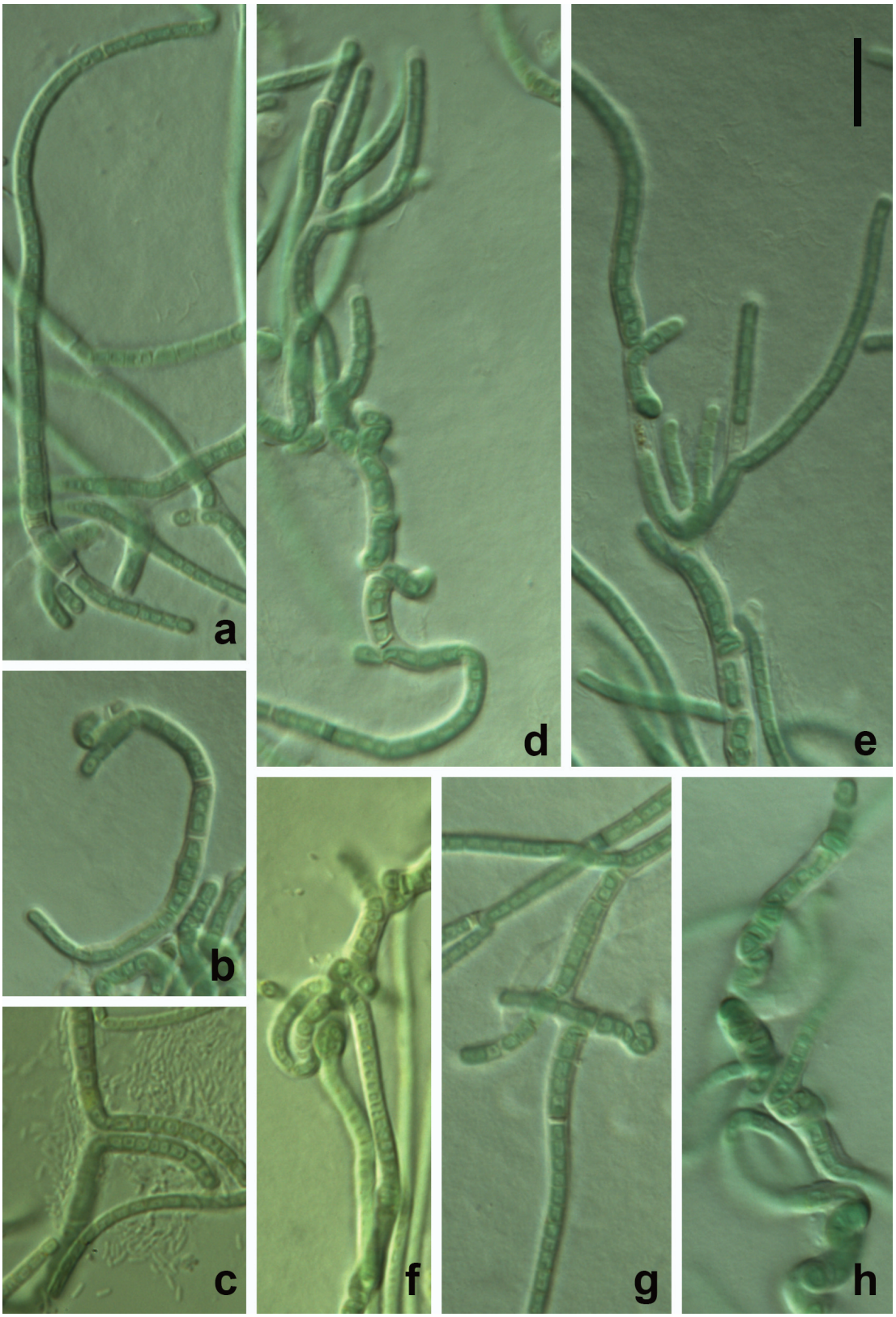

FIGURE 17. *Timaviella obliquedivisa*. A–B. Slightly tapering filaments. C–E. Consecutive double and single false-branches in filaments resulting in branch-like structures. F–G. Cell division in oblique angles, causing geminate, knot-like branching or resembling to true-branching. H. Compact coiling of trichomes within sheath. Scale bar 10µm in 1000X magnification.

D1-D1' helix 63 nucleotides long, with a 3' unilateral bulge of 8 nucleotides (5'-CAUCCCAA-3'). Mid-helix with a pair of nucleotide mismatch of U/U and two internal loops at position 14–15/41–42 and 20–23/32–36 which is separated from the terminal loop by a 5'-GC:GC-3' clamp. Terminal loop having sequence 5'-GAAA-3' (Fig. 6k). Box B helix 50 nucleotides long, with several internal loops at position 5/44–46, 8–9/41 and 25–25/31–35, an internal mismatch 5/44–46 probably due to an insertion of an adenine residue. Terminal loop 5 nucleotides long, 5'-UUAAU-3' (Fig. 7j). V2 helix 29 nucleotides long, with one internal loop at position 5–6/23–25 (Fig. 8h). V3 helix 62 nucleotides long, with several internal loops at positions 4/55–57, 11–12/47–50 and 23/34–35, separated from the terminal loop by a 5'-GU:AG-3' clamp (Fig. 9j).

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

Type locality:—Big Horn Seep Wall, Grand Staircase-Escalante National Monument, 37°43.012' N-111°28.273' W, collected on 15 August 2006 by Markéta Bohunická. Hanging garden (concave wet rock face in a grotto with vascular plants hanging from the rock) on a sandstone rock wall in the Carmel-Page formation, partly covered with organic debris, mosses and vascular plants, in the GSENM, Kane County, Utah, USA.

Holotype here designated:—BRY37787!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Isotype here designated:—BRY37788!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Reference strain:—GSE-PSE-MK28-08A, Algal Culture Collection at John Carroll University, Cleveland, USA. Additional reference strain: GSE-PSE-MK23-08B, Algal Culture Collection at John Carroll University, Cleveland, USA.

Taxonomic 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 (Sciuto *et al*. 2017). 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. The conserved domains of the 16S–23S had secondary structures unique to this species (see Sciuto *et al*. 2017).

Timaviella radians* Mai, Johansen *et* Bohunická *sp. nov.

Diagnosis:—Differing from *T. obliquedivisa* in the formation of radial microscopic colonies, color and occasional pigmentation of the trichomes. D1-D1' helix similar in structure to those of *T. obliquedivisa* and *T. radians*, especially in the unilateral bulge sequence, the internal loop at position 14–15/59–60 and the large internal loop at position 20–23/50–54, but differing in length of helix (Fig. 6j–l). Box B helix shorter than the Box B helix of other *Timaviella* species (Fig. 7i–k). V2 helix very short, 12 nucleotides (Fig. 8i).

Description:—Colony mounded, leathery, dark olive-brown or dirty olive-green. Filaments relatively short, often clearly widened in meristematic zones (Fig. 18a–b), sometimes forming radial colonies (Figs. 18c), with characteristic consecutive false branching (Fig. 18d). 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 (Fig. 18a, e), 1.8–3.7 µm wide. Necridia absent. Hormogonia without sheaths (Fig. 18d, f). Cells in hormogonia and young filaments isodiametric, becoming shorter than wide with maturation 1.2–2.2 µm long, often with one large central granule, often with purplish pigmentation (Fig. 18f). Apical cell rounded, conically rounded, or pancake-like rounded.

D1-D1' helix 81 nucleotides long, with a 3' unilateral bulge of 8 nucleotides (5'-CAUCCCAA-3'); mid-helix with several internal loops at position 14–15/59–60, 20–23/50–54, 26–27/46–47 and a nucleotide mismatch of G/G at position 30/43, with. terminal loop having sequence 5'-GAGA-3' (Fig. 6l). Box B helix 33 nucleotides long, with two internal loops at positions 5/28–29 and 8–9/25; terminal loop having sequence 5'-UAAUA-3' (Fig. 7k). V2 helix very short (Fig. 8i). V3 helix 59 nucleotides long, with one internal loop at position 5/53–55 and two unpaired adenine residues at positions 48 and 44 of the 3' strand (Fig. 9k).

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

Type locality:—Lower Calf Creek Falls site, Grand Staircase-Escalante National Monument, 37°49'44.77"N-111°25'12.58"W, collected on 15 August 2006 by Markéta Bohunická. Large seep wall and waterfall in Navajo Sandstone, in the GSENM, Kane County, Utah, USA. Mats in waterfall.

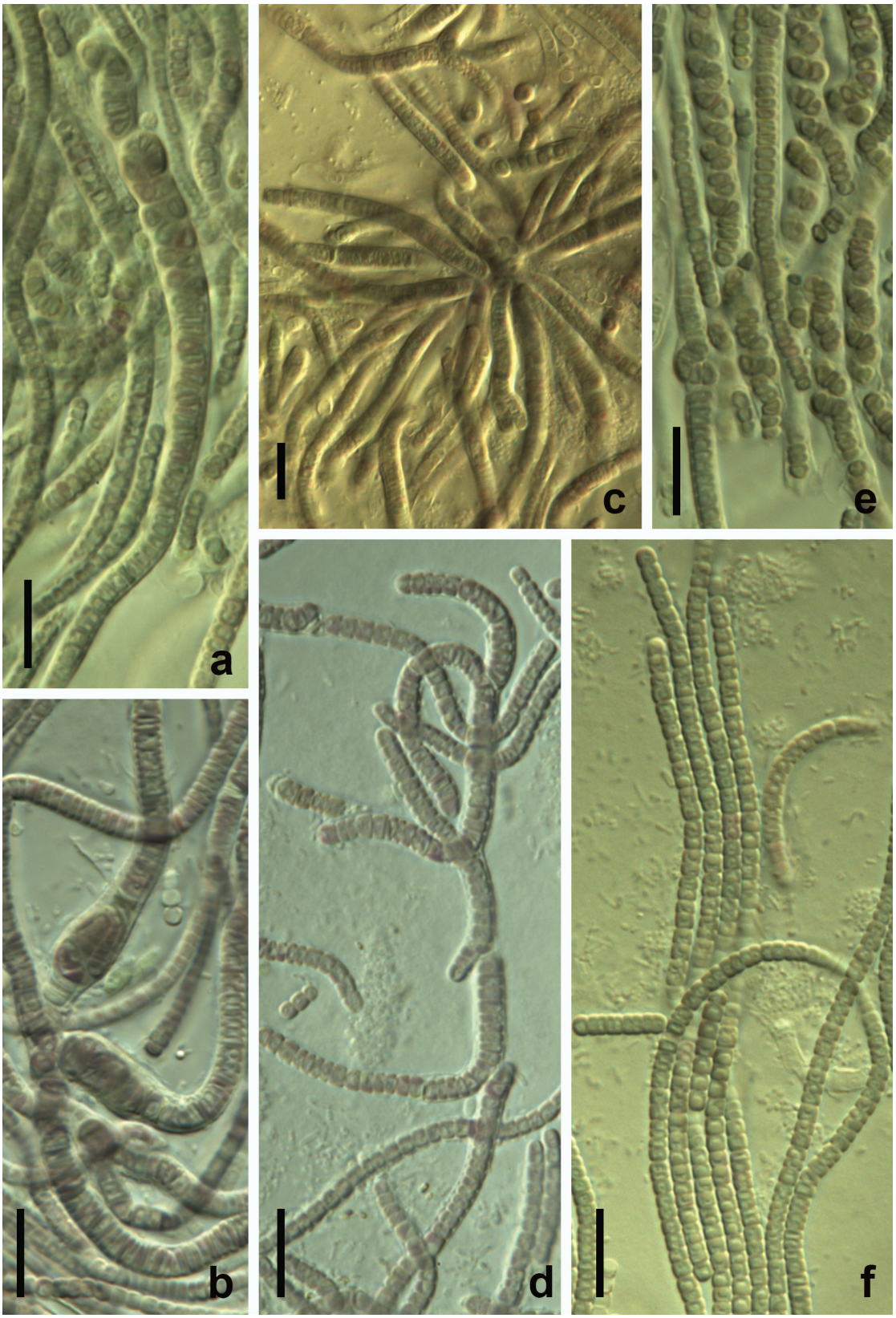

FIGURE 18. *Timaviella radians*. A–B. Rapid cell division at apical region commonly observed, forming a "basal" part to filament. C. Radial formation of colonies based on trichomes radiating from an attachment point. D. False-branches in singles or in pairs, consecutive, sometimes geminate branching, E. Compact rope-like coils. F. Rapid cell division causing twists and turn of trichome within sheath. Mature filaments isodiametric, trichomes constricted at distinct cross-walls, with one large central granule dominates cytoplasm. Scale bar 10μm in 400X (C) and 1000X magnification (A–B, D–F).

Holotype here designated:—BRY37789!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Reference strain:—GSE-UNK-7R, Algal Culture Collection at John Carroll University, Cleveland, USA.

Taxonomic 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* and *T. obliquedivisa* were similar in 16S rRNA gene sequence (96.45%, Table 11), had very similar lengths of conserved domains in the ITS region (Table 6), and had similar ITS structures, but distinctive molecular and morphological features well separate the two species. *T. radians* has a markedly different structure in the D1-D1', Box-B, and V3 helices than the earlier described species, *T. circinata* Sciuto *et al.* (2017: 319, figs. 3–5) and *T. karstica* Sciuto *et al.* (2017: 319, figs. 3–5). *T. obliquedivisa* and *Timaviella* species WMT-WP7-NPA collected from the White Mountains share 99.05% identity in the 16S rRNA gene (Table 11) sequence, but are greatly different in the ITS region (p=18.3%, Table 12) and their ITS structures (Figs. 6–9). All *Timaviella* species described to date are reported from wet rock faces in grottos or caves, although *T. circinata* and *T. karstica* are from karstic rocks in an alpine region in the Italian Alps, while the species described in this paper are from sandstone seeps in the arid canyon country of Utah, USA.

TABLE 12. ITS genetic similarity among *Timaviella* species.

	Strain	1	2	3	4	5	6	7
1	*Timaviella karstica* GR13	-						
2	*Timaviella circinata* GR4	86.28	-					
3	*Timaviella* sp. WMT-WP7-NPA	83.62	83.76	-				
4	*Timaviella obliquedivisa* GSE-PSE28-08A	84.23	83.82	81.72	-			
5	*Timaviella obliquedivisa* GSE-PSE-MK23-08B	84.23	83.82	81.72	100.0	-		
6	*Timaviella radians* GSE-UNK-7R	80.13	79.59	74.62	76.97	76.97	-	
7	*Timaviella radians GS*E-TBD6-7R	80.13	79.59	74.62	76.97	76.97	100.0	-

Cartusia* Mai, Johansen *et* Pietrasiak *gen. nov.

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 cross-walls, 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* Mai, Johansen *et* Pietrasiak, *comb. nov.*

Cartusia fontana* (Hansgirg) Mai, Johansen *et* Pietrasiak *comb. nov.

Basionym:—*Lyngbya fontana* Hansgirg 1892, *Archiv für die naturwissenschaftliche Landesdurchforschung von Böhmen* 8: 85.

Later Synonym:—*Leptolyngbya fontana* (Hansgirg) Komárek in Anagnostidis 2001, *Preslia*, *Praha* 73: 374.

Description of epitype:—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. 19a), sometimes forming fascicles of trichomes, without false branching, with variations in width between young and mature filaments (Figs. 19a, c), up to 5.4 µm wide in filaments with sheath. Sheath colorless, firm, usually thin, but occasionally widened (Fig. 19b–d), up to 1.6 µm wide. Trichomes not or slightly constricted at crosswalls, with necredia (Fig. 19b, d), with

meristematic zones often occurring in wider trichomes (Figs. 19b–c), young trichomes narrower, 1.8–2.7 µm wide, mature trichomes larger, 2.7–3.5 µm wide. End cells rounded. 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.

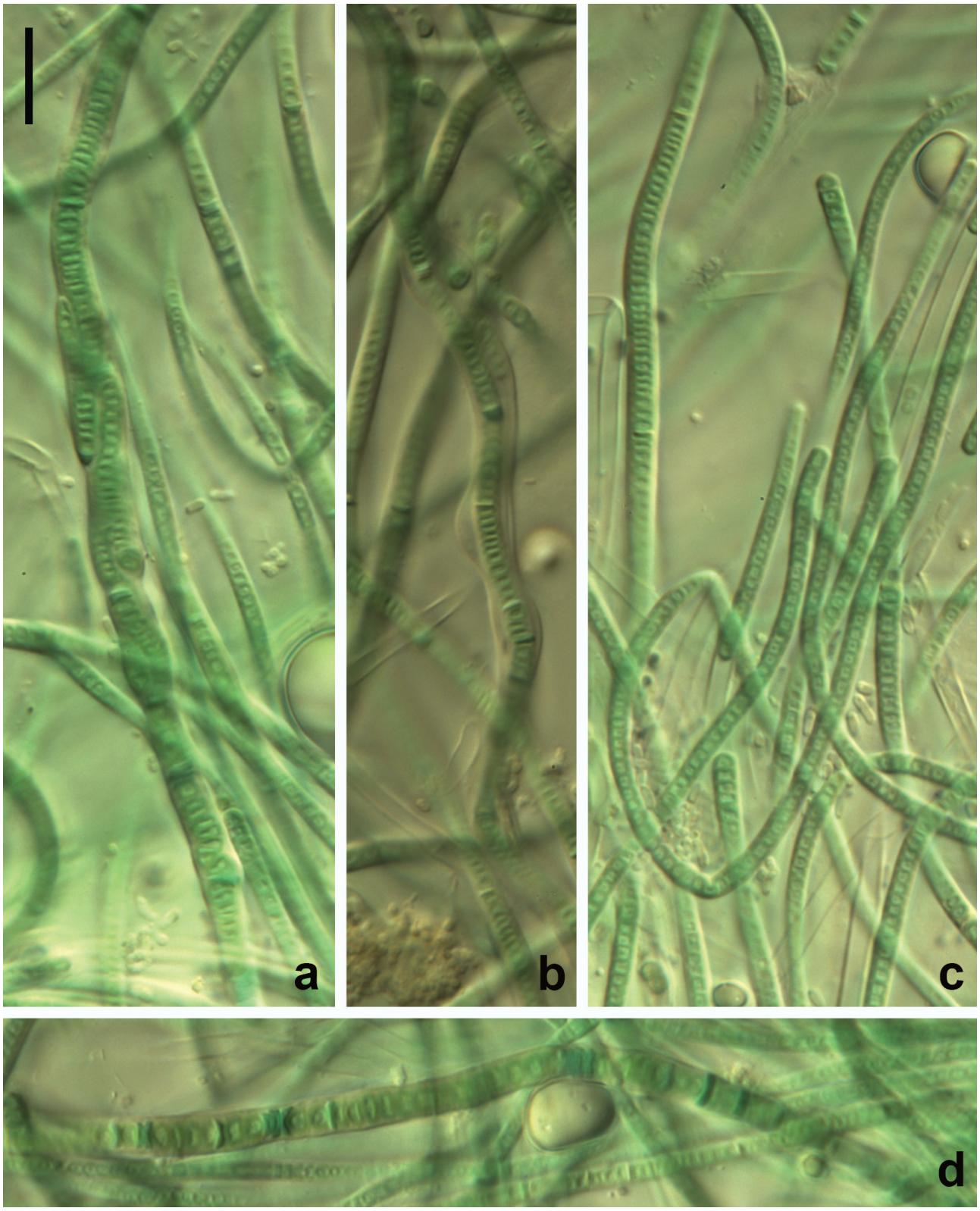

FIGURE 19. *Cartusia fontana*. A. Fasicle of trichomes within one common sheath. B. Expanded and lamellate sheath. C. Variation in width between young and mature trichome, with cell division in meristematic zones. D. Meristematic zone of cell division in mature trichomes, with necridia; necridia in both young and senescing trichomes, filaments in mature trichomes occasionally with thick, slightly layered sheath. Scale bar 10µm in 1000X magnification.

D1-D1' helix 107 nucleotides long, with a basal 3' unilateral bulge of 8 nucleotides (5'-CAUCCCAA-3'), and multiple side branches from two large internal loops (Fig. 6m), a length and structure unique within the Oculatellaceae (Fig. 6m). Box B helix 60 nucleotides long, with two internal loops at positions 5/55–56 and 15–19/42–46, and one unpaired adenine residue at position 9 on the 5' strand (Fig. 7l). V2 helix nonexistent. V3 helix not identified due to short end sequence.

Collection locality:—Slovakia: National Park Slovak Paradise, ruins of the Cartusian Monaster, collected in 1999 by Łubomir Kovácík. Found in pale green biofilm on wall at interior of church.

Epitype:—BRY37780!, Monte L. Bean Museum, Provo, Utah.

Reference strain:—Kovacik 1999/1, Algal Culture Collection at John Carroll University, Cleveland, USA.

Taxonomic 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, morphology of *C. fontana* is similar to *Drouetiella fasciculata* and several species of *Pegethrix* (*P. bostrychoides*, *P. convolute* and *P. indistincta*). *C. fontana* was observed to have meristematic zones of cell division, which were absent in *D. fasciculata*, as well as cells shorter than wide. In comparison to *Pegethrix* species, *C. fontana* does not form pseudobranches or nodules. Furthermore, the two genera are separated by their position in 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 structure found in the vast majority of Synechococcales, with several small helices branching off from large internal loop structures (Fig. 6 m). 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 helix (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 6).

Cartusia is in a clade with two other taxa (Fig. 2). "*Marsacia ferruginose*" JSC-1 is an undescribed genus and species (Brown *et al.* 2010) based on a strain from thermal springs in Yellowstone National Park and is 97.2% similar in 16S rRNA sequence. It is possible that this undescribed taxon could be described as another species of *Cartusia*. It currently is a *nomen nuda*, without description or a type specimen designated. *Elainella saxicola* Jahodářová *et al.* (2017: 4) was very recently published and is 97.0% similar in 16S rRNA sequence. *Elainella* differs morphologically in the repeated false branching and the restriction of only a single trichome per sheath. An uncultured cyanobacterium, NCBI accession JX127186 is 99.4% similar in 16S rRNA sequence. This is almost surely a *Cartusia* species, possibly *C. fontana*, sequenced in an environmental sample from building stone in Germany. Unfortunately, we do not have a strain of this material. Of these sequences, only *"Marsacia ferruginose"* JSC-1 has ITS sequence available, and the region is different in structure and lengths of domains.

Tildeniella* Mai, Johansen *et* Pietrasiak *gen. nov.

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 wide, 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:—*Tildeniella torsiva* Mai, Johansen *et* Pietrasiak *sp. nov.*

Tildeniella torsiva* Mai, Johansen *et* Pietrasiak *sp. nov.

Diagnosis:—Morphologically distinguished from *T. nuda* by the presence of spirally-coiled filaments and false-branches. Molecularly distinguished by from *T. nuda* by secondary structures of the D1-D1' and Box B helices (Figs. 6n, o, 7m, n), as well as by the absence of a V2 helix.

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. 20a), often entangled, 1.7–2.5 µm wide. Sheath firm, thin, colorless, up to 0.7 µm wide, often not evident. Trichomes untapered, straight, curved (Fig. 20b), or sometimes spirally coiled (Fig. 20c–d), 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.

D1-D1' helix 65 nucleotides long, with basal 3' unilateral bulge of 6 nucleotides (5'-CAUCCA-3'), with one C/U mismatch at position 9/51 and one internal loop at position 20–22/37–40. Terminal loop 4 nucleotides long, 5'-UUCG-3' (Fig. 6n). Box B helix 49 nucleotides long, with one C/A mismatch at position 6/44 and one unpaired adenine residue at position 18, with terminal loop sequence 5'-AAGG-3' (Fig. 7m). V2 helix absent.V3 helix 92 nucleotides long, with multiple internal loops along the helix at positions 4–5/85–89, 8–9/81–82, 12–16/75–78 and 35–37/54–56. Basal clamp of V3 helix shorter than all other species of Oculatellaceae due to a A/A mismatch (5'-AGUC:GACA-3' compared to 5'-UGUC:GACA-3' in other species of Oculatellaceae) (Fig. 9l).

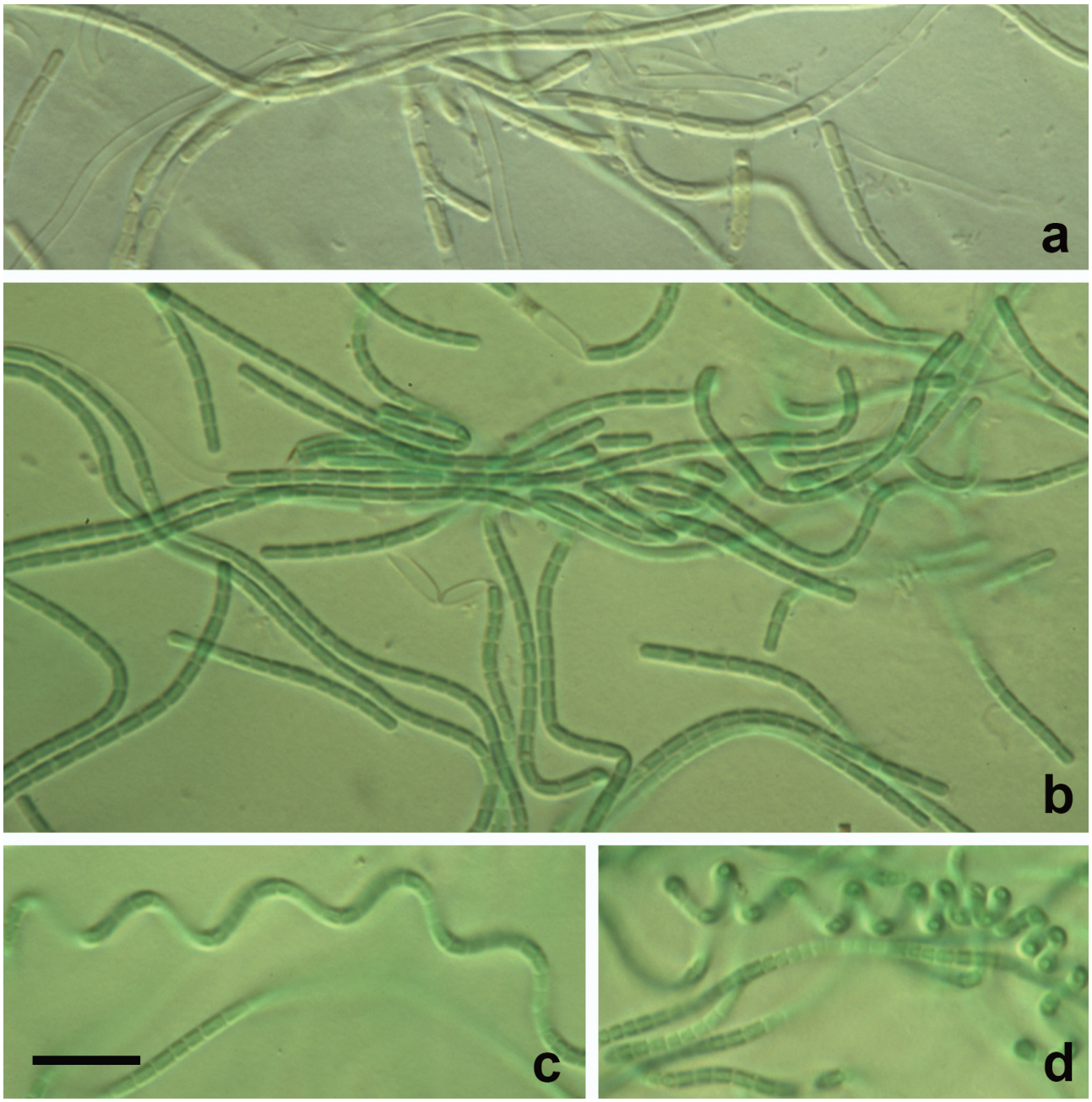

FIGURE 20. *Tildeniella torsiva*. A. Single false-branching very rarely observed, only in senescing culture. B. Cells isodiametric to slightly longer than width. C–D. Filaments sometimes wavy to strongly spirally coiled. Scale bar 10µm in 1000X magnification.

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

Type locality:—Slovakia. National Park Slovak Paradise: Gorge Prielom Hornadu, collected in 1998 by Bohuslav Uher. Found in limestone wall near tourist walkway (strain Uher 1998/13d). Other locality: Bay barther Bodden near bridge Meiningen, Germany (strain Hubel 1974/233).

Holotype here designated:—BRY37781!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Reference strain:—Uher 1998/13d, Algal Culture Collection at John Carroll University, Cleveland, USA. Other reference strain Hubel 1974/233.

Taxonomic notes:—The spirally coiled and contorted filaments as well as the variation in cell length from isodiametric to distinctly longer than wide are considered the most characteristic features in this species. This taxon 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 *T. torsiva*.

L. lagerheimii is very similar to *T. torsiva*, having similar cell dimensions and loose spiral coiling. *L. lagerheimii* was originally described from Brazil as *Spirocoleus lagerheimii* Möbius (1889: 312). Gomont transferred the species into *Lyngbya lagerheimii* Gomont (1890: 354) *ex* Gomont (1892:147), and this species was later transferred into *Leptolyngbya* in the same publication in which that genus was described and typified by *L. boryana*. It was not realized at that time 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 *Tildeniella* 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 *T. torsiva*, then it would be necessary to transfer *T. torsiva* and *T. nuda* to *Spirocoleus*. For now, we feel it is more conservative to simply describe this lineage as a genus new to science.

Tildeniella nuda* Mai, Johansen *et* Bohunická *sp. nov.

Diagnosis:—Different from *T. torsiva* in the occasional presence of swollen cells and much narrower trichome width. Molecularly distinguished by from *T. torsiva* by secondary structures of the D1-D1' and Box B helices (Figs. 6n–o, 7m–n), as well as by presence of a V2 helix.

Description:—Colony fasciculated, not penetrating the agar, forming a compact mat, with small evenly distributed, rounded clumps of trichomes, bright blue-green. Filaments relatively short (Fig. 21a), 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 the cross-walls (Figs. 21c–e), cell division occurring throughout the trichome, 1.0–1.2 μm wide. Hormogonia and necridia absent. Cells always longer than wide, sometimes elongated (Fig. 21b–c), with mostly homogeneous content, rarely swollen (Fig. 21d–e, g–h, at arrows) or with small granules at polar regions of cells, 2.0–4.3 (5.1) long. Apical cells cylindrically rounded, sometimes apically swollen (Fig. 21a, f at arrow).

D1-D1' helix 65 nucleotides long, basal 3' unilateral bulge of 7 nucleotides long, with sequence 5'-CAUCCCA-3', with mid-helix with two mismatches of C/U and G/A at positions 9/50 and 22/39, one internal loop at position 25/35–36 immediately separated from the terminal loop by a 5'-GC:GC-3' clamp, and two unpaired nucleotides at position 16–17 of the 5' strand, with terminal loop sequence 5'-AUUUU-3' (Fig. 6o). Box B helix 38 nucleotides long, with one internal loop at position 5/33–34 and one unpaired adenine residue at position 9 of the 5' strand (Fig. 7n). V2 helix 77 nucleotides long, with one small internal loop at position 10–11/68 and one large internal loop at position 24–26/51–55 (Fig. 8j). V3 helix not reported here due to short sequence missing the end of the ITS.

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

Type locality:—Switzerland, Stansstaad, collected by Zehnder in 1965; found on a wet stone wall.

Holotype here designated:—BRY37782!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Reference strain:—Zehnder 1965/U140, Algal Culture Collection at John Carroll University, Cleveland, USA.

Taxonomic 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 lato* Komárek & Anagnostidis (2005). The cell morphology (length to width ratio, dimensions) is similar to most *Oculatella* species, but *T. nuda* lacks the characteristic apical cells of

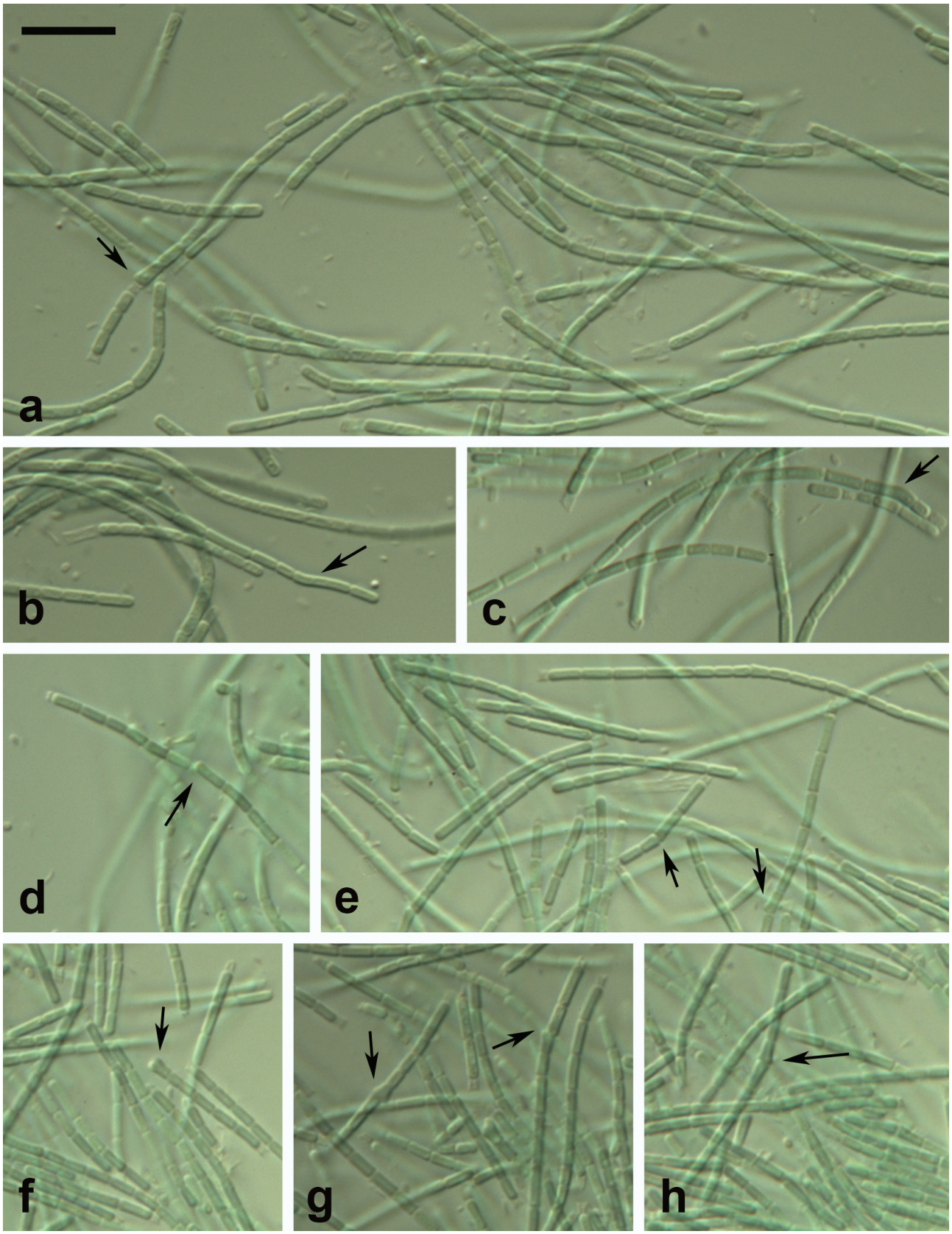

FIGURE 21. *Tildeniella nuda*. A. Filaments short, *Pseudanabaena*-like, trichomes not or only slight constricted, connected by somewhat translucent cell wall; B–H. Cells sometimes distinctively elongated, bent or involuted (arrows). Scale bar 10μm in 1000X magnification.

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* Nägeli *ex* Kützing (1849: 265), then transferred to *Hypheothrix tenuissima* (Nägeli) Rabenhorst (1865: 77), and finally to *Lyngbya tenuissima* (Nägeli) 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* (Nägeli) 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 μm 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 *Tildeniella*, and thus have created a new name.

T. nuda is distinct from other species within the Oculatellaceae by its thin trichomes and high variation in cell length within trichomes. Molecularly, the species is closely related to *T. torsiva*, with very high similarity in the 16S rRNA gene (99.1% identity, Table 13), and we were not able to compare the percent similiarty in the ITS structure due to sequence truncation of the *T. nuda* reference strain. However, substantial taxonomic features separate the two species well.

TABLE 13. 16S rRNA genetic similarity among *Tildeniella* species.

	Strain	1	2	3
1	*Tildeniella torsiva* Hubel 1974/223	-		
2	*Tildeniella torsiva* Uher1 998/13d	100.0	-	
3	*Tildeniella nuda* Zehnder 1965/U140	99.14	99.14	-

Komarkovaea* Mai, Johansen *et* Pietrasiak *gen. nov.

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 Jaroslava Komárková, a prominent Czech cyanobacteriologist, phytoplankton ecologist, and personal friend who recently passed away. She will be sorely missed, and we wish to honor her memory and contributions to science with the establishment of this genus.

Type species:—*Komarkovaea angustata* Mai, Johansen *et* Pietrasiak *sp. nov.*

Komarkovaea angustata* Mai, Johansen *et* Pietrasiak *sp. nov.

Diagnosis:—Phylogenetically closest to *Tildeniella* species, however, differing from that genus and all other genera in the Oculatellaceae by the basal unilateral bulge present in the Box-B helix (Fig. 7o).

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 (Fig. 22a–d). Sheath firm, thin, colorless, up to 0.7 μm wide. Trichomes constricted at cross-walls, rarely tapering (Figs. 22d), post-hormogonial trichomes 1.8–2.9 μm wide, mature trichomes 3.3–3.9 μm wide. Hormogonia (Figs. 22a–b) and necridia (Figs. 22b–c) present. Cells with parietal thylakoids, with up to 3 small granules per cell, sometimes with one small orange granule (Fig. 22e, at arrows), commonly cylindrical, especially in young trichomes, becoming isodiametric in actively growing trichomes; in more mature trichomes cells barrel-shaped, and pancake-like in meristematic zones (Fig. 22c–d), 2.2–5.7 μm long.

D1-D1' helix 63 ncleotides long, with basal 3' unilateral bulge 7 nucleotides long (5'-CAUCCUA-3'), with one C/U mismatch at position 9/48, one unpaired Adenine residue at position 15 of the 5' strand, and one large internal loop at position 21–23/33–37 immediately separated from the terminal loop by a 5'-GG:CC-3' clamp. Terminal loop sequence 5'-ACAGU-3' (Fig. 6p). Box B helix 41 nucleotides long, with one distinctive basal 3' unilateral bulge at position 5/32–37, one internal loop at position 10–11/26–27; terminal loop with sequence 5'-AAUC-3' (Fig. 7o). V2 helix 20 nucleotides long, with terminal loop of 4 nucleotides (Fig. 8k). V3 helix 87 nucleotides long, with multiple mismatches at position 13/77, 19/71, an unpaired adenine residue at position 9 on the 5' strand, and three internal loops at position 5–6/83, 23–24/66–67 and 30–31/58–60 (Fig. 9m).

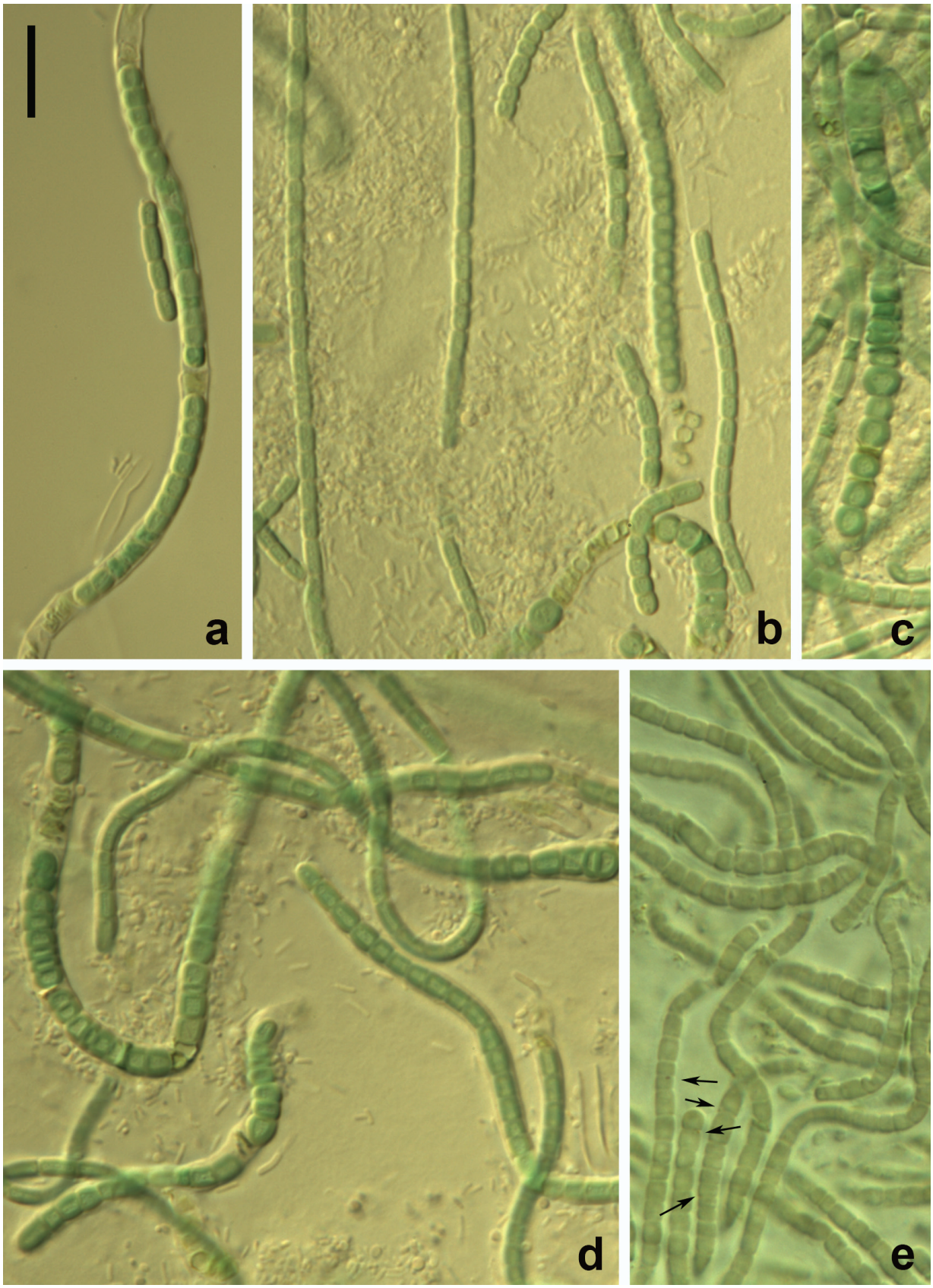

FIGURE 22. *Komarkovaea angustata*. A–B. Variation in trichome width between mature and young filament or hormogonia. C. Variation in cell shapes between young and mature trichomes: isodiametric, slightly longer than width or barrel-shaped, and abundance of necridia in mature trichomes. D. Rapid regional cell division along trichomes resulting in basal and apical parts of filaments. E. Reddish small granules occassionaly observed on cells. Scale bar 10μm in 1000X magnification.

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

Type locality:—Puerto Rico, El Yunque National Forest: Waterfall in the forest, 18°27.811' N–66°7.005'W collected in 2012 by Jay Hillery.

Holotype here designated:—Holotype BRY37783!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Reference strain:—EY1-AM2, Algal Culture Collection at John Carroll University, Cleveland, USA.

Taxonomic notes:—This genus is character-poor. The heterogeneity in trichome width and absence of false branches is characteristic, and could be compared to tapering species in *Leptolyngbya*: *L. tenuis* (Gomont 1892: 169) Anagnostidis & Komárek (1988: 393), *L. laminosa* (Gomont 1892: 167) Anagnostidis & Komárek (1988: 392) and *L. fragilis* (Gomont 1892: 163) Anagnostidis & Komárek (1988: 391). However, in every comparison the trichomes of *K. angustata* are wider. The species has low 16S rRNA gene sequence identity with the other genera in the Oculatellaceae (91.9-93.5%, see Table 5), strong evidence that it represents a separate genus-level lineage.

Kaiparowitsia* Mai, Johansen *et* Bohunická *gen. nov.

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* Mai, Johansen *et* Bohunická *sp. nov.*

Kaiparowitsia implicata* Mai, Johansen *et* Bohunická *sp. nov.

Diagnosis:—Considerably similar to the morphology of *T. nuda*, however differing in the occasional formation of *Arthronema*-like outgrowths (Figs. 23g–i at arrows). Other distinctive characteristics observed in the secondary structure of conserved ITS domains, especially in the long and unique sequence of D1-D1' helix and a likely C→ U mutation (position 101) in the 3' basal unilateral bulge unique among Oculatellaceae (Fig. 6q–r).

Description:—Colony radially spreading, becoming mucilaginous with age, bright blue-green, green, or olive green. Filaments flexuous, entangled, sometimes fasciculated (Fig. 23a–b), sometimes with multiple trichomes in a common sheath (Figs. 23d–f), without false branching, 1.3–1.5–(2.3) µm wide. Sheath thin, occasionally expanded (Fig. 23d), sometimes absent, up to 0.7 µm wide. Trichomes untapered, bent, flexuous, entangled, sometimes forming nodules (Figs. 23c–f), 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. 23g–i 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 142 nucleotides long, with basal 3' unilateral bulge of 7 nucleotides (5'-UAUCCCA-3'), with additional large 3' unilateral bulge at position 100–113; mid-helix with 5–6 internal loops due to adenine and guanine mismatches of 3–5 nucleotides, at positions 15–17/111–112, 29–30/92–93, 35–37/86–87, 41–42/81–82, 45–47/77–78 and 53/70–71; terminal loop having sequence 5'-UUAAUU-3' (Fig. 6q–r). Box B helix 36 nucleotides long, with one basal internal loop at position 5/31–32, one unpaired adenine at position 9, terminal loop of 4 nucleotides (Fig. 7p). V2 between $tRNA^{Ala}$ and $tRNA^{Ile}$ absent. V3 helix 113 nucleotides long, with a5'-G/A-3'and 5'-A/G-3' mismatches at positions 5/109 and 42/72, several internal loops at positions 14–18/95–99, 22–25/88–91, 34–37/77–79, two unpaired nucleotides of guanine and adenine at positions 67 and 105 respectively of 3' strand (Fig. 9n).

Etymology:—*implicata* (L): tangled

Type locality:—USA. Utah: Camp Spring Site 1, Grand Staircase-Escalante National Monument (GSENM), 37°32'35" N-111°38'26" W, collected on 15 August 2006 by Markéta Bohunická. 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, Utah, USA. Found in wet soil under the overhang with pH 7.7–8.1, with mottled coloration.

Holotype here designated:—BRY37784!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Isotypes here designated:—BRY37785! and BRY37786!, Herbarium for Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah.

Reference strain:—GSE-PSE-MK54-09C, Algal Culture Collection at John Carroll University, Cleveland, USA. Additional reference strains: GSE-TBC-9CA and GSE-TBC-9CA2, respectively.

Taxonomic notes:—*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 subterrannea* Zammit, Billi and Albertano 2012 (see Albertano & Grilli-Caiola 1988 and Komárek & Anagnostidis (2005: 222)). However, these two species are molecularly distinct from *Kaiparowitsia*.

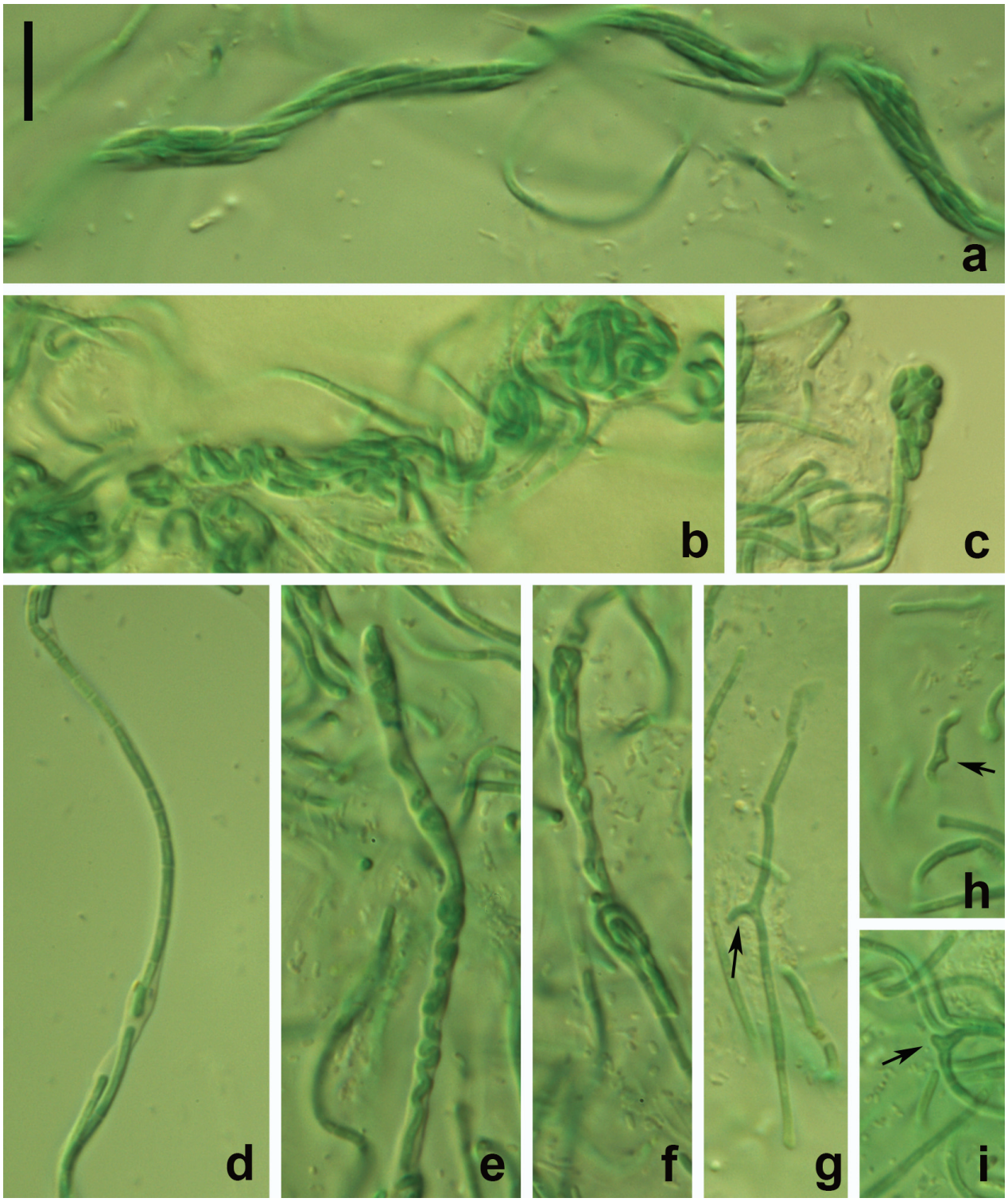

FIGURE 23. *Kaiparowitsia implicata.* A. Fasicles of trichomes without common sheath. B–C. Tightly entangled filaments forming compact coils. D. Disintegration of trichomes within common sheath occasionally observed. E–F. Nodule formation common. F–H. Distinctively longer than width common cells and crooked, *Arthronema*-like involution from cells along trichomes. G–I. Multiple trichomes entangled within one common sheath also observed. Scale bar 10μm in 1000X magnification.

Discussion

A family-level taxonomic scaffold for Synechococcales:—Previously, a family has been characterized by a collection of discrete morphological characters, for example polarity of trichomes, types of branching, akinete production and organisation, nodule formation, etc., which have been found to be context-dependent and do not necessary reflect evolutionary history (Komárek *et al.* 2014, Komárek 2006). Family establishment in prokaryotes is especially difficult if based only on morphology. In the order Synechococcales, difficulty in family circumscription is encountered due to both the lack of morphologically distinct characters within families (they are all simple filaments), as well as the very divergent characteristics in some families (Oculatellaceae and Prochlorotrichace), which have distinct autapomorphies in several genera that are absent at the family level. Taxonomists working in other groups of organisms have been faced with a similar dilemma. In such occasions, synapomorphies were found in the genetic codes of the organisms (Adeolu & Gupta 2013, Fučíková *et al.* 2014, Sawana *et al.* 2014, Gupta *et al.* 2015).

In this work, we attempted well-developed methods in cyanobacterial taxonomy to delimit families. We looked for an agreement between the 16S rRNA and *rpoC1* phylogenies to identify family groupings, as well as searching for diagnosable features of families, including the morphological characteristics,16S rRNA genetic identity cut-off values and synapomorphic nucleotides of the 16S rRNA sequence. Family level clades are well-defined by phylogenetic analysis, but the families often do not possess morphological synapomorphies that allow morphological recognition of the families. Instead, Řehákova *et al.* (2014) found that the 16S rRNA secondary structures might have characteristics useful for higher level taxonomy. However, when applying this approach to our sequences to identify subtypes of the variable 16S rRNA helices, we found secondary structure across the families we assigned in the Synechococcales was highly conserved and thus not taxonomically informative at the family level. We have identified the family-consistent single nucleotide variations within helices 18, 20, 23, 27 and 34 (Fig. 5 and Table 4) that help delineate family membership, but since they are not 100% consistent within family-level groups, they are not universally applicable for family separation. While these conserved differences provide a useful hint as to family placement, 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.

In another attempt to delimit family, Yarza *et al*. (2014) suggested that the threshold for sequence identity of 16S rRNA to separate taxa into two different families, is below 86.5% (i.e. greater than 13.5 sequence dissimilarity). One important thing to note when interpreting this threshold value is that it is uninformative in comparison between taxa with sequence similarity of above 86.5% (i.e. less than 13.5 % sequence dissimilarity). When applied to our case, the value suggests that there are more than one family existing in Prochlorotrichaceae, but it is not sensitive enough to detect polyphyly in other families. The best we can say for recognition of cyanobacterial families in the Synechococcales given current knowledge is that they form highly supported phylogenetic clusters of genera, many of which can be recognized morphologically, or at least with a combination of morphology and phylogeny. In this case, it is suggested that Oculatellaceae is the most stable and well-established group that deserves recognition (Figs. 2, 4). Genera are best defined as monophyletic clades containing one or more species which have at least some morphological characters separating them from other genera, so phylogenetic analysis was conducted as a prerequisite to identifying genus-level clusters. 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. 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). By this standard, the following genera were separated: *Pegethrix*, *Drouetiella*, *Thermoleptolyngbya*, *Oculatella*, *Timaviella*, *Tildeniella*, *Komarkovaea*, *"Trichotorquatus"*, and *Kaiparowitsia.* As discussed above, the use of cut-off values does have some limitations in the resolution it provides. We also considered other separation criteria, including morphology, ITS secondary structures, and length of ITS conserved domains. 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 6). For example, whereas *Pegethrix* and *Cartusia* have high 16S rRNA sequence similarity (96.20%, Table 5), 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 6). Indeed, the long length of the post-Box B spacer of *Cartusia* sets it apart from all other genera in the Oculatellaceae. In a similar manner, the high similarity of 16S rRNA sequences between *Cartusia* and *Elainella* was also observed (97.0%, Table 5), but they too had distinctively different lengths in the conserved domains of the ITS (Table 6).

Separation of species within the same genus 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 percent dissimilarity 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. bostrychoides* is molecularly most similar to *P. olivacea* (Table 7), but the percent dissimilarity in ITS sequence between the two species is 8.2% (i.e. 91.8% sequence similarity, Table 8), a clear separation. ITS percent dissimilarity between *P. bostrychoides* and other species in the genus is even greater (Table 8). 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 percent dissimilarity of 4.1%, 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. 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*.

Present taxonomic hierarchy in the Synechococcales:—Our current findings are not in agreement with the more recent revision of the higher level taxonomy of the Cyanobacteria in Komárek *et al.* 2014. Our work indicates that *Arthonema* and *Prochlorothrix* belong outside of the Pseudanabaenaceae (in Leptolyngbyaceae and Prochlorotrichaceae, respectively). In addition, *Tapinothrix* was placed in the Heteroleibleiniaceae instead of the Leptolyngbyaceae (Fig. 1). 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). In this report, we only revise taxa with molecular data in addition to morphological description.

However, the clades identified in our analysis of the 16S rRNA phylogeny agree with multiple analyses conducted in the past, even with different taxon sampling, and were also consistent with other studies of the group. The grouping we have in the Oculatellaceae has been consistent across multiple analyses with different taxon sampling and phylogenetic methods (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, Sciuto *et al.* 2017). The families of Trichocoleaceae and Prochlorotrichaceae are also phylogenetically stable. *Trichocoleus* forms a distinct clade with currently no closely related taxa. Prochlorotrichaceae was originally a monotypic family, created to contain *Prochlorothrix*, which lacks phycobilins and possesses chlorophyll a and b (Burger-Wiersma *et al.* 1989). We have expanded the Prochlorotrichaceae to include a number of other genera, particularly *Nodosilinea* and *Halomicronema* (Fig. 3). However, the Prochlorotrichaceae might possibly be split in the future, as suggested by our *rpo*C1 phylogeny (Fig. 4). Furthermore, the taxa apparently closely related to *Prochlorothrix* (Fig. 3) are known to only have chlorophyll *a* and are thus deeply phenotypically divergent from *Prochlorothrix* (Li & Brand 2007, Paul *et al.* 2014).

Historically, the type genus of Leptolyngbyaceae, *Leptolyngbya,* is a heterogeneous genus, containing all taxa that satisfy the criteria of untapered trichomes less than 3.5 μm wide, with sheaths, and without aerotopes. Recent efforts have been made to re-define the genus by recognizing *Leptolyngbya sensu stricto,* a clade that includes the type species*, L. boryana*, and excludes strains and sequences that fall in distant clades that bear the name *Leptolyngbya,* but cannot be put in that genus if monophyly is to be achieved in the genus. 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 included in *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).

Remaining taxonomic problems:—Despite the intensive revisions being made in the Synechococcales, there still exist a number of lineages of uncertain generic affinity that belong to this group, falling well outside of *Leptolyngbya sensu stricto*, or being misidentified as "*Phormidesmis*". Interestingly enough, there are a handful of strains that were fully investigated in genomic, metabolomics and biochemical aspects, yet still remain improperly classified. The aforementioned Oscillatoriales cyanobacterium JSC-1 or "*Marsacia ferruginose*" JSC-1, is a well-characterized strain, especially in photosynthetic pigmentation and chromatic adaptation (Brown *et al.* 2004, Gan 2014, Gan *et al.* 2015, Li & Chen 2015, Shen *et al.* 2016). The strain represents, undoubtedly, a new species (either in a new genus or in *Cartusia*) that possesses unique biochemical metabolites, but has not yet been named. A study by Shimura *et al.* (2015) is of importance in understanding what comprises *Leptolyngbya sensu stricto* (represented by *Leptolyngbya boryana* PCC 6306, *Leptolyngbya* IAM M-1-1, *Leptolyngbya* dg5, and *Leptolyngbya* PCC 73110). They demonstrated that the strains in this clade can fix nitrogen under anaerobic conditions, while other *"Leptolyngbya"* such as *"Leptolyngbya"* NIES 2104 and the *"Leptolyngbya"* SEV strains do not have this ability. These latter strains are not in *Leptolyngbya sensu stricto* based on phylogenetic evidence and appear to be in other, at present unspecified, genera awaiting description. A number of other strains scattered throughout our phylogenetic tree also share the same problem, either remaining undescribed or associated with the incorrect epithet (Sciuto *et al.* 2011, Jancusova *et al.* 2016).

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*, *Drouetiella*, *Oculatella*, and *Timaviella* all contain some uninformative or misleading OTUs 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 genera described in this work (e.g. CENA112, CENA103, CENA131, CENA129 discussed in Furtado *et al.* 2009, Genuario *et al.* 2016). As for the family Prochlorotrichaceae, the situation is less complicated, with only a few orphan OTUs. However, there also exist strains that were not named regardless of the available data (Coates *et al.* 2014, Paul *et al.* 2014, Baran *et al.* 2013). The invalidly described "*Xeronema*" (Garcia-Pichel *et al.* 2001) occurs in this clade, as well as two sequences submitted as *L. appalachiana* Johansen *et* Olsen in Johansen *et al*. (2008: 24) which are very distant from the reference sequence for this species (Fig. 1).

In conclusion, we are proposing a molecularly-supported initial family-level structure, in the hope that this will later serve as a scaffold for future discoveries in the Synechococcales. A correct family-level designation is considered preferable by us to an incorrect generic-level epithet (e.g. Oculatellaceae instead of *Leptolyngbya* sp.). 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 taxonomists is 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.

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TABLE S1. List of DNA sequences for *rbcLX* (Rubisco 1,5-biphosphate), *cpcBA* (Phycocyanin subunit βα and IGS), and 23S-5S ITS (or partial 23S) sequences. **Annotation:—F.** (Family): L=Leptolyngbyaceae; O=Oculatellaceae; Pr=Prochlorotrichaceae; T=Trichocoleaceae; Ps=Pseudanabaenaceae. **NCBI Accession Number:** N/A=Sequences not available; Accession number (bold) = Multiple clones with multiple accession numbers on NCBI; Accession number (regular) = Single sequence with single accession number on NCBI; **Collection site:** KNMNP=Kawai Nui Marsh Nature Preserve; JTNP=Joshua Tree National Park; GSENM=Grand Staircase-Escalante National Monument; GSMNP=Great Smokey Mountain National Park; EYNF=El Yunque National Forest.

Strain names	**F.**	**Collection site**	**NCBI Accession Number**		
			rbcLX	***cpcBA***	**23S-5S ITS**
Scytolyngbya HA4215-MV1	L	Laie Falls, Oahu. HI, USA.	KY498262	N/A	N/A
"Myxacorys" ATA2-1-KO14	L	Atacama Desert, Chile.	KY498252	**KY498234**	N/A
Plectolyngbya WJT66-NPBG17	L	Mojave Desert. CA, USA.	KY498253	**KY498237**	N/A
Plectolyngbya HA4277-MV3	L	Honolulu, Oahu. HI, USA.	N/A	**KY498241**	N/A
Leptolyngbyaceae WOS-LAB13	L	GSMNP. TN, USA.	KY498263	N/A	N/A
Leptolyngbya HA4303-MV7	L	Oahu. HI, USA.	KY498264	N/A	N/A
Leptolyngbya HA4237-MV2	L	Taro fields, Oahu. HI, USA.	KY498265	N/A	N/A
Phormidesmis WJT36-NPBG15	L	JTNP, Mojave Desert. CA, USA.	KY498266	N/A	N/A
Phormidesmis WJT36-NPBG12	L	JTNP, Mojave Desert. CA, USA.	KY498267	N/A	N/A
Tapinothrix clintonii GSE-PSE06-7G	L	GSENM. UT, USA.	KY498270	N/A	N/A
Leptolyngbyaceae EY07-AM2	L	EYNF, Puerto Rico.	KY498256	KY498245	N/A
Nodosilinea GSE-TBD7-7G	L	GSENM. UT, USA	N/A	N/A	**KY511268**
Leptolyngbyaceae GSE-UNK-8H	L	GSENM. UT, USA	N/A	N/A	**KY511264**
Oculatella atacamensis ATA3-4Q-CV5	O	Atacama Desert. Chile.	KY498250	N/A	N/A
Oculatella kauaiensis HA4348-LM1	O	Kauai. HI, USA.	KY498251	N/A	N/A
"Trichotorquatus" ATA2-1-CV25	O	Atacama Desert. Chile	KY498257	KY498233	N/A
"Trichotorquatus" SMER-A	O	N/A	KY498261	KY498247	**KY511272**
Drouetiella fasciculata GSE-PSE-MK38-07D	O	GSENM. UT, USA.	KY498254	N/A	N/A
Drouetiella fasciculata GSE-PSE-MK29-07A	O	GSENM. UT, USA.	KY498255	N/A	N/A
Tildeniella nuda Zehnder 1965/U140	O	Stansstaad, Switzerland.	N/A	KY498246	N/A
Komarkovaea angustata EY01-AM2	O	Puerto Rico.	KY498256	N/A	N/A
Kaiparowitsia implicata GSE-PSE-MK54-09C	O	GSENM. UT, USA.	KY498258	N/A	**KY511260**
Kaiparowitsia implicata GSE-TBC-9CA2	O	GSENM. UT, USA.	KY498272	N/A	N/A
Kaiparowitsia implicata GSE-TBC-9CA	O	GSENM. UT, USA.	KY498259	N/A	N/A
Schizothrix arenaria HA4233-MV05	O	KNMNP, Kailua. HI, USA.	KY498271	N/A	N/A
Nodosilinea GSE-PSE-MK55-09B	Pr	GSENM. UT, USA.	KY498268	N/A	N/A
Trichocoleus desertorum ATA4-8-CV3	T	Atacama Desert. Chile.	KY498248	N/A	**KY511274**
Trichocoleus desertorum ATA4-8-CV12	T	Atacama Desert. Chile.	KY498249	**KY498230**	N/A