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Neotypification of Pleurocapsa fuliginosa and epitypification of P. minor (Pleurocapsales): resolving a polyphyletic cyanobacterial genus

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Abstract

Strains with complete morphological match to *Pleurocapsa fuliginosa* and *P. minor* were isolated from Oahu (Hawaii, USA), with another strain matching *P. minor* isolated from a wet rock face in Utah (USA). Phylogenetically these baeocyte and pseudofilament producing strains fell in a single well-supported clade among a number of pleurocapsalean strains. They were sister to a clade of baeocyte-producing strains that lack the ability to form pseudofilaments and likely belong in an as-yet-to-be-described genus. Strains putatively named *Pleurocapsa* are scattered throughout the Pleurocapsales and Chroococcales, indicating a need for clear definition of the genus so that revisionary work and alpha-level taxonomy can move forward. To satisfy this need, *P. fuliginosa* HA4302-MV1 and *P. minor* HA4230-MV1 were chosen as neotype and epitype, respectively, establishing the genus based on molecular sequence data. In addition to the distinctive morphology of the genus, all *Pleuro-capsa* species for which 16S-23S ITS regions are available have an unusually long, branched D5 helix at the termination of the ITS region. The sister clade of strains that lack the ability to form pseudofilaments also possess an unusually long and branched D5 helix as well, suggesting that this feature of the ITS region may be a family-level synapomorphy.

Key words: cyanobacteria, *Pleurocapsa*, Pleurocapsales, neotype, epitype, 16S rRNA phylogeny, ITS, Hawaii archipelago, Grand Staircase-Escalante National Monument

Introduction

The Pleurocapsales is an order of coccoid cyanobacteria with mostly polarized growth of the vegetative cells which subsequently divide by multiple fission forming beaocytes. Recently, members of *Chroococcidiopsis* Geitler (1933: 625) have been separated from the Pleurocapsales, into their own order, Chroococcidiopsidales, based on molecular evidence (Komárek *et al.* 2014). Presently, Pleurocapsales contains 4 families, 25 genera, and 247 species (Komárek *et al*. 2014, Oliveira Alvarenga *et al*. 2016, Guiry, M.D. & Guiry 2018). This group has been recognized as taxonomically confusing to many researchers (Cumbers & Rothschild 2014, Mareš 2017). Confusion arises due to inconsistencies in morphological character states between genera, their rare occurrence in nature, and the lack of sequence data of all representative genera. A particularly problematic genus is *Pleurocapsa* Thuret in Hauck (1885: 515), which has been attributed to many strains deposited in culture collections all over the world, of which many have been sequenced. However, in DNA based phylogenies these strains intermix with sequences assigned to *Chroococcidiopsis, Hyella* Bornet & Flahault (1888: 162), *Myxosarcina* Printz (1921: 35), *Stanieria* Komárek & Anagnostidis (1986: 208), *Xenococcus* Thuret in Bornet & Thuret (1880: 73, 74), and *Dermocarpella* Lemmermann (1907: 349) (Ishida *et al.* 2001, Mareš & Cantonati 2016, Oliveira Alvarenga *et al*. 2016).

The genus *Pleurocapsa* is an old, established taxon, originally described from limestone in the intertidal zone in the Adriatic Sea near Trieste, Italy (Hauck 1885). This genus was diagnosed by its ability to produce baeocytes, as well as to form irregular to radial or sarcinoid colonies with tightly appressed cells (Fig. 1A). Soon after, several

species were described in the genus that had the capability of producing pseudofilaments: *P. minor* Hansgirg (1891: 89), *P. concharum* Hansgirg (1891: 90), and *P. crepidinum* Collins (1901: 136). However, *P. fuliginosa* Hauck (1885: 515), the type species, was not reported to produce pseudofilaments in the protologue, a significant departure from the modern conception of the genus. Subsequently, Ercegović established *Scopulonema* Ercegović (1930: 368) to accommodate those *Pleurocapsa* species capable of producing pseudofilaments, most species in the genus at the time. Later, Komárek (1972) examined herbarium material of *P. fuliginosa* from Musée d'Histoire naturelle, Paris. He reported and illustrated pseudofilaments found in the original material, leading him to subsume *Scopulonema* into *Pleurocapsa* (Komárek & Anagnostidis 1995, Komárek & Anagnostidis 1998). Forms of *Pleurocapsa* not developing pseudofilaments have been moved to other genera: *P. fluviatilis* Lagerheim (1888: 430) into *Chroococcopsis fluviatilis* (Lagerheim) Komárek & Anagnostidis (1995: 17) and *P. muralis* Lagerheim in Wittrock & Nordstedt (1893: 195) into *Chroococcidiopsis muralis* (Lagerheim) Miscoe & Johansen in Miscoe *et al*. (2016: 87). In the botanical literature, a total of 18 valid species of *Pleurocapsa* are now listed in Komárek & Anagnostidis (1998), along with seven additional species clearly requiring further characterization or revision.

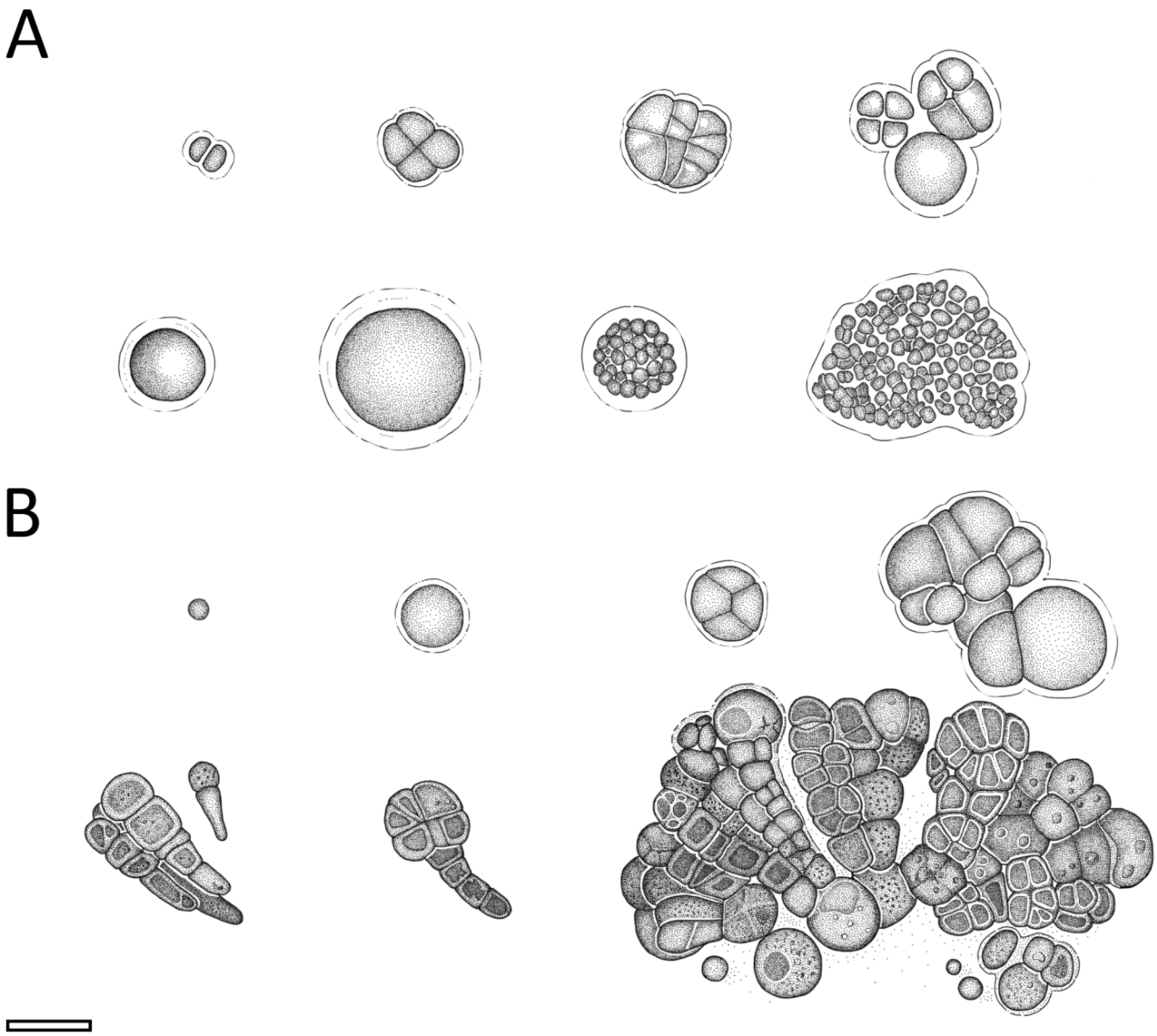

FIGURE 1. Line drawings of the type species of genus *Pleurocapsa*, *P. fuliginosa*: (A) Copy of *P. fuliginosa* from original publication (Shalygin after Hauck 1885); (B) Original images of *P. fuliginosa* from the neotype from Hawaii archipelago (cultural material) with additional morphological trait-pseudofilaments. Scale bar equals 10 µm.

Using microbiological methods and a strains-based approach, Waterbury & Stanier (1978) and Waterbury (1989) completed two important studies on strains assigned to the Pleurocapsales, and their findings became the foundation of the review of the order in Bergey's Manual (Rippka *et al*. 2001, Rippka *et al*. 2015). They recognize only a subset of the described genera, including *Cyanocystis* Borzì (1882: 314) which is synonymous with *Dermocarpa* Crouan & Crouan (1858: 70)*, Dermocarpella, Stanieria, Xenococcus, Chroococcidiopsis, Myxosarcina,* and the *Pleurocapsa*-group including *Pleurocapsa, Hyella*, and *Solentia* Ercegovic (1927: 80). Although few species were recognized in these works, detailed life cycles based on cell division and baeocyte formation were reported, and the morphological characteristics of many strains were shown. Consequently, sequences that were later determined for these strains are of exceptionally high value given the morphological and physiological characterizations that are linked to these sequences.

Despite the value of the previous microbiological work on the Pleurocapsales, the work is incomplete as it stands. More genera will likely need to be recognized in order to achieve monophyletic genera in the Pleurocapsales, and this will be accomplished either by assigning some of the sequenced *Pleurocapsa* taxa to other existing genera or to new genera (Mishler & Theriot 2000, Johansen & Casamatta 2005, Komárek *et al*. 2014, Dvořák *et al*. 2015, Shalygin *et al*. 2017). However, a challenge to revisionary work is that some of the most abundantly sequenced and clearly polyphyletic genera have not had their generitype species sequenced and definition of all of the genera in the Pleurocapsales is consequently presently ambiguous.

In the present paper we establish a neotype for the generitype, *P. fuliginosa*, and an epitype for a long-established species, *P. minor*. We base these typifications on sequenced strains that closely conform to descriptions for each species using the monophyletic species concept sensu Johansen & Casamatta (Mishler & Theriot 2000, Johansen & Casamatta 2005), following the rules for epitypification established in the International Code for Algae, Fungi and Plants (ICN, Turland *et al*. 2018). Furthermore, we establish the phylogenetic benchmark for *Pleuracapsa sensu stricto* including the two species mentioned. We exclude some strains, formerly assigned to the genus *Pleurocapsa*, from *Pleurocapsa sensu stricto* based on both phylogenetic and morphological evidence. Finally, we make recommendations for designating reference strains and sequences for six additional pleurocapsalean genera: *Chamaecalyx* Komárek & Anagnostidis (1986: 199)*, Hyella, Foliisarcina* Oliveira Alvarenga, Rigonato, Branco, Melo & Fiore (2016: 694)*, Xenococcus, Stanieria*, and *Chroococcopsis* Geitler (1925: 342).

Materials and Methods

Field Methods

Pleurocapsa fuliginosa HA4302-MV01 and *P. minor* HA4230-MV01 were isolated from environmental samples collected by Vaccarino and Johansen on Oahu, Hawaii, on the 23rd and 25th of July, 2009. Samples were placed in 2.0 ml eppendorf tubes. Environmental samples were preserved in 2.5% $CaCO_3$-buffered glutaraldehyde and deposited in the Bernice Pauahi Bishop Museum, along with dried herbarium mounts. Fresh material was kept for culturing at John Carroll University and isolates from this material are available upon request from the John Carroll University Algal Culture Collection. Additionally, information about habitat and morphology are available in the Table 1 or on the web through CRIS (Melechin *et al*. 2013).

An additional strain, *P. minor* GSE-CHR-MK-17-07R, was obtained from the John Carroll University Culture Collection. This strain was originally isolated from a collection made by Bohunická and Johansen from Lower Calf Creek Falls, Grand Staircase-Escalante National Monument, Utah, on the 18th of August, 2006 (Table 1).

TABLE 1. Main environmental features of the members of Pleurocapsaceae.

	Locality/Coordinates	Altitude (m)	Habitat	Substrate/Moisture
Pleurocapsa fuliginosa HA4302-MV1	Hawaii/21° 21' 05"N latitude, 157° 46' 08"W longitude	~107	Tropics: Maunawili stream (freshwater)	Rock face near the stream/wet
Pleurocapsa minor HA4230-MV1	Hawaii/21° 22' 01" N latitude, 157° 47' 31" W longitude	~412	Tropics: Nuuanu Pali Lookout (subaerophytic)	Cement/wet
Pleurocapsa minor GSE-CHR-MK17-07R	Utah/37° 49' 44.77" N latitude, 111° 25' 12.58" W longitude	~1694	Desert: Lower Calf Creek Falls (freshwater) within Grand Staircase-Escalante National Monument	Sandstone near waterfall/wet

Laboratory work

Fresh samples were diluted and inoculated onto Z8 medium (Carmichael 1986) agar plates. The dilution plates were then grown under low light (<200 μE) conditions. Colonies were picked after an extended growth period (1–2 months or more) and isolated into unialgal culture for microscopy and molecular analysis. All strains were characterized using Olympus BX60 and Zeiss Axioscope photomicroscopes with high resolution Nomarski DIC optics. Morphological measurements were made using Zeiss AxioVision 4.8 software (Oberkochen, Germany).

Healthy cultured cells were scraped from unialgal slants, and genomic DNA isolation was performed using the UltraClean Microbial DNA Isolation Kit from MO BIO (Carlsbad, CA). DNA was eluted into 50 μl of solution MD5 and stored at –20°C.

In order to amplify the promoter/leader region of the rRNA operon for the Hawaiian strains, we used the 16S forward promoter primer (5'-GGA TAT ATT GGA TAA GTG CC-3') developed by Lukešová *et al*. (2009) and the 16S reverse (5'-CCC ATT GCG GAA AAT TCC-3') primer to amplify the leader region and first 359 nucleotides of the 16S rRNA gene. Fifty microliter reactions were performed in a Bio-Rad DNA Engine PTC200 (Hercules, CA), and these reactions resulted in a ~540 bp PCR product. PCR conditions were 35 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 60 s; a 300 s extension at 72°C and 4°C hold followed. Final concentrations of reagents in the reactions were 1X Taq polymerase buffer (USB, Cleveland, Ohio), 1.5 mM $MgCl_2$, 2.5 pmol/μl of each primer, 1 μl of template DNA (100–200 ng total), 0.2 mM dNTPs (USB), and 1.25 units Taq polymerase (USB).

A PCR product of ~1600 nucleotides containing the 16S rRNA gene from bp 325 to the end of the gene, the 16S–23S ITS region, and the beginning of the 23S–5S ITS was generated using primers VRF1 and VRF2 (Boyer *et al*. 2001). The final reagent concentrations were the same as the leader PCR reactions. A 25 μL reaction for each strain was run in a C1000 Thermocycler (BIORAD).

Cycling conditions for the leader region and beginning of 16S rRNA gene were 35 cycles of 94°C for 45 s, 57°C for 45 s, and 72°C for 135 s. A 5 min extension at 72°C was performed, and the reactions were held at 4°C indefinitely. Cycling conditions for the ending 16S rRNA gene and 16S–23S ITS were a melting cycle of 95°C for 5 minutes; followed by 35 cycles of: 95°C for 60 s, 57°C for 45 s, and 72°C for 240 s; ending in an additional 300 s at 72°C and finally an indefinite hold at 4°C. The final concentrations of reagents used were as in Lukešová *et al*. (2009).

Amplification of the 23S–5S ITS region was performed by using the 23S end forward (5'-GCTGAAAGCATCTAAGTGGG-3') and 5S reverse (5'-CCTGGCRTCGAGCTATTT-3') primers (Lukešová *et al.* 2009). Reaction conditions were 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s followed by a 72°C extension for 300 s and a 4°C incubation. Reagent concentrations were the same as indicated above.

All PCR products were analyzed on 1% agarose gels before being TA-cloned into the pSC-amp/kan plasmid of the Stratagene Cloning Kit (La Jolla, CA). Putative clones were isolated using QIAprep Spin kits (Qiagen, Carlsbad, CA) with elution in 50 μl of sterile water. The presence of an insert was confirmed by *Eco*R I digestion.

At least two of each cloned PCR amplifications were sequenced for each of the three reactions by Functional Biosciences, Inc. (Madison, WI) using the M13 forward and M13 reverse primers present in the plasmid. Additionally, the 16S–23S ITS-containing plasmids were sequenced with internal primers VRF5 (5'-TGTACACACCGCCCGTC-3'), VRF7 (5'-AATGGGATTAGATACCCCAGTAGTC-3'), and VRF 8 (5'-AAGGAGGTGATCCAGCCACA-3'). Sequences were assembled and proofread using Sequencher software (version 4.8, Ann Arbor, MI).

Secondary structures of the 16S–23S internal transcribed spacer (ITS) region were determined for all Pleurocapsales strains for which ITS sequence was available (our strains and those on NCBI GenBank). The 16S–23S ITS regions recovered for *Pleurocapsa* all had both tRNA genes. Conserved domains within the 16S–23S ITS were identified through employment of comparative analysis with the ITS of other cyanobacteria, particularly with respect to the basal portions of each helix. The V3 and D5 domains were identified by folding the end of the ITS with the 23S–5S ITS region.

Phylogenetic Analysis

Phylogenetic analysis was based on the complete 16S rRNA gene (bp 1–1,558) sequences. We recovered two distinct ribosomal operons for *Pleurocapsa minor* HA4230-MV01, and both were used in the phylogenetic analyses. Approximately, 122 other sequences were chosen from GenBank for the phylogenetic analyses. Sequences were picked based on 1) similarity to our sequences of *Pleurocapsa* as determined by BLAST searches, 2) the strain name in a search for *Pleurocapsa, Xenoccoccus, Stanieria, Dermocarpella, Myxosarcina* and *Chroococcidiopsis* (i.e. the baeocyte-producing taxa), and 3) sufficient base length of sequence. Five outgroup taxa were included in the phylogeny, representing the Gloeobacterales, Synechococcales, Chroococcidiopsidales and Nostocales. The 16S rRNA gene sequences were aligned manually in Microsoft Word (Microsoft Corp., Redmond, Washington, USA) with the aim

of preserving secondary structure (Lukešová *et al*. 2009, Řeháková *et al*. 2014). Bayesian Inference (BI), Maximum Likelihood (ML), and Maximum Parsimony (MP) generated the phylogenies, however only the Bayesian tree topology is shown with additional ML probabilities and MP bootstrap values mapped onto the nodes represented on FIGURE S1. For ML and BI we used the GTR+I+G model, which was given by jModeltest2 (Darriba *et al*. 2012). During BI, which was running on MrBayes v.3.2.6, two runs of eight Markov chains were applied with 20 million generations, sampling every 100 generations, with 25% burn-in, using the sump command in Mr. Bayes (Ronquist *et al*. 2012). ML analysis was performed utilizing RaxML v. 7.2.8 with 1,000 bootstrap pseudoreplicates (Stamatakis *et al*. 2008). Both BI and ML analyses were run using CIPRES (Miller *et al*. 2015). MP analysis was completed on PAUP v. 4.02b with steepest descent, tree bisection and reconnection (TBR) branch swapping, and 1,000 bootstrap replicates, p-distance values were also calculated with PAUP (Swofford 2002).

Phylogenetic trees, ITS structures folded in Mfold (Zuker 2003) and line drawings of the species were post-produced in Adobe Photoshop CS5 (Adobe Systems Inc., San Jose, California, USA).

Results

16S rRNA phylogeny

The three phylogenetic analyses were in overall agreement with each other and with recent taxonomic revisions of cyanobacteria, which were based on conserved sequences of multiple protein coding genes (Komárek *et al*. 2014, Mareš 2017). The most derived lineages, furthest from *Gloeobacter violaceus* Rippka, J.B. Waterbury & Cohen-Bazire (1974: 436) were Nostocales and Chroococcidiopsidales (Figs. 2, S1). Both of these clades contained coccoid members. We assume that the taxonomically-undetermined coccoids in the Chroococcidiopsidales were members of that clade which have not been shown to produce baeocytes because of insufficient study. The unknown coccoids as well as genus *Chlorogloeopsis* Mitra & Padney (1967: 112) within the Nostocales are more problematic, but we have also seen *Nostoc* species from soils which produced such compact colonies that their filamentous nature is not evident, and which do not produce heterocytes unless grown in N-free medium. These "coccoids" require further study before taxonomic determinations can be made. The overall topology from the three different analyses (BI, ML, and MP) were highly congruent. The Bayesian Inference analysis had the greatest node support in comparison to ML and MP (Fig. S1).

While the Pleurocapsales clade had a posterior probability support value of 0.95 in the BI analysis, it was nested in the Chroococcales. The Bayesian Inference analysis had at least three lineages basal and paraphyletic to the Pleurocapsales with representatives of Gomphosphaeriaceae, Aphanothececaceae, Merismopediaceae, Coelosphaeraceae, Microcystaceae, and Chroococcaceae (following Komárek *et al*. 2014). *Chroococcus turgidus* (Kützing) Nägeli (1849: 46) was attributed to strains in very different phylogenetic positions, within the clades containing *Limnococcus* Komárková, Jezberová, O.Komárek & Zapomelová (2010: 79), *Gomphosphaeria* Kützing (1836: no I) and *Chroococcus* Nägeli (1849: 46). Additionally, the *Pleurocapsa concharum* group including *P. concharum* VP4-07 was located among the Microcystaceae (Fig. S1), and needs to be moved to another genus in the future if the strains indeed have the distinctive morphology of this species. Resolution of problems within Chroococcales and Nostocales is outside the scope of the present study.

The Pleurocapsales are morphologically defined by the presence of baeocytes and distant phylogenetic relationship to another baeocyte producers from Chroococccidiopsidales. Based on the literature (Waterbury & Stanier 1978) and our robust phylogeny we propose that some of the NCBI sequences need to be renamed to reflect more correct taxonomic determination. For example, *Dermocarpella incrassata* Lemmermann (1907: 349) was transferred into *Chamaecalyx incrassatus* (Lemmermann) Komárek & Anagnostidis (1986: 199) consequently all sequences designated as *D. incrassate,* such as SAG 29.84, need to be updated in NCBI (Fig. 2; Table 2). The clade which contains our two species, *P. fuliginosa* and *P. minor,* forms a well supported subcluster within the Pleurocapsales, and we are identifying this subcluster as *Pleurocapsa sensu stricto*. Even though *Pleurocapsa* sp. HRC17 is closely related to our *Pleurocapsa,* it certainly belongs to a different species. Its genetic identity with *Pleurocapsa sensu stricto* is only 95.5%, but given that only a 700 bp sequence exists for this species from a hyersaline habitat, little weight can be given to comparisons of genetic identity.

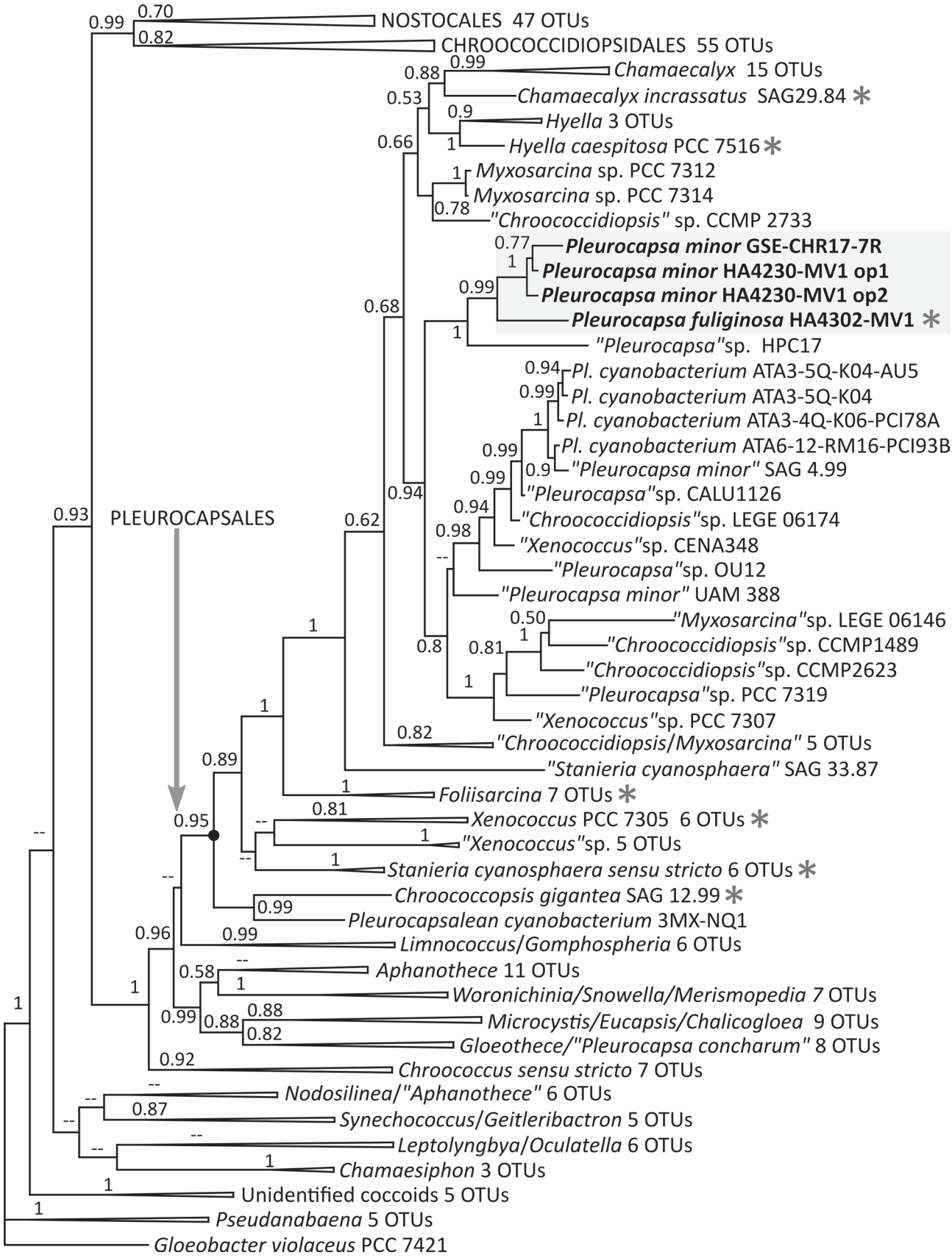

FIGURE 2. Collapsed 16S rRNA gene phylogeny of Pleurocapsales based on 253 OTUs with maximum length of 1,483 bp. Support values are shown as BI. Nodes lacking support are indicated by "–". The entire uncollapsed tree can be found in the Supplementary Materials as Fig. S1. Strains we consider to be correctly identified and representative of the genus are followed by an asterisk. Taxa which need revision are placed in the quotes.

TABLE 2. New suggested names for NCBI sequences, based on phylogenetic clustering and modern taxonomic treatments.

Old name	Our proposed name	Accesion
Dermocarpella incrassata SAG 29.84	*Chamaecalyx incrassatus* SAG 29.84	AJ344559
Stanieria sp. PCC 7301	*Hyella* sp. PCC 7301	AB039009
Stanieria sp. PCC 7302	*Hyella* sp. PCC 7302	KM019985
Uncultured *Stanieria* sp. clone CrN-P11	*Hyella* sp. CrN-P11	DQ072926
Pleurocapsa sp. PCC 7516	*Hyella caespitosa* PCC 7516	X78681
Uncultured *Xenococcus* sp. Cl. CrV-P5	*Hyella* sp. CrV-P5	DQ072929
Pleurocapsa sp. HA4230-MV1 (clones 2B and 2C)	*Pleurocapsa minor* HA4230-MV1 (clones 2B and 2C)	KC525080–KC525081
Pleurocapsa sp. HA4302-MV1	*Pleurocapsa fuliginosa* HA4302-MV1	JN385285
Pleurocapsalean cyanobacterium 3MX-NQ1	*Chroococcopsis* sp. 3MX-NQ1	MG710500

The members of *Pleurocapsa sensu stricto* have been observed to form pseudofilaments as well as pseudodichotomously branching aggregates in contrast to representatives of the sister clade directly below *Pleurocapsa* (Figs. 2, S1). This group of taxa is ambiguously separated from *Pleurocapsa sensu stricto*, having genetic identities with that group >95.3% (Table 3), which is above the cut-off indicative of separate genera (≤94.5%, see Yarza *et al*. 2014). However, this level of genetic demarcation is almost identical to that separating *Chaemaecaly*x and *Pleurocaps*a, which are morphologically very different and unquestionably belong to independent lineages. Furthermore, this sister group consists mostly of coccoid baeocyte producers unable to produce pseudofilaments that are morphologically similar to *Chroococcidiopsis*, and some even bear this generic epithet. *Pleurocapsa minor* SAG 4.99 is in this group, but it does not have pseudofilaments according to images available on the SAG website, and so it is morphologically consistent with other clade members. Finally, the sister group is ecologically distinct, containing many strains isolated from desert soils and quartz rocks. For now we leave this group of strains in *Pleurocapsa*, recognizing that they should be moved to other (likely new) genera in the future. Given the strong morphological similarity of our strains to *Pleurocapsa*, we are confident in our taxonomic determination of the Hawaiian strains, and will neotypify *P. fuliginosa* and epitypify *P. minor* so that these sequenced strains can provide a firm benchmark for the genus and for the order Pleurocapsales.

TABLE 3. Percent similarity of different representatives of Pleurocapsales, members of genus *Pleurocapsa* higlighed with bold font, "*Pleurocapsa concharum*" from Choococcales served as outgroup. The length of the sequences was ~ 1500 bp.

Strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 *Pleurocapsa fuliginosa* HA4302-MV1																
2 *Pleur. minor* GSE-CHR-MK17-07R	**97.28**															
3 *Pleurocapsa minor* HA4230-MV1 2C	**98.02**	**98.76**														
4 *Pleurocapsa minor* HA4230-MV1 2B	**98.27**	**99.01**	**99.75**													
5 "*Pleurocapsa* sp." HPC17	95.54	95.79	96.53	96.53												
6 *Xenococcus* sp. PCC7307	97.03	97.28	97.52	97.77	96.04											
7 "*Pleurocapsa* sp." PCC7319	95.79	95.79	96.04	96.29	96.29	96.53										
8 Pleurocapsales ATA3-5Q-K04 AU5	95.79	96.04	96.78	96.78	98.76	96.29	96.53									
9 Pleurocapsales ATA3-5Q-KO4	95.54	95.79	96.53	96.53	98.51	96.04	96.29	99.75								
10 Pleurocapsales ATA3-4Q-KO6	95.54	95.79	96.53	96.53	98.51	96.04	96.29	99.75	99.50							
11 Pleurocapsales ATA6-12-RM16	95.54	95.79	96.53	96.53	98.51	96.04	96.29	99.75	99.50	100.00						
12 "*Pleurocapsa minor*" SAG 4.99	95.30	95.54	96.29	96.29	98.27	95.79	96.04	99.50	99.26	99.75	99.75					
13 *Chamaecalyx* sp. HSC1	96.29	95.54	95.79	96.04	94.55	97.77	95.05	95.05	94.80	95.30	95.30	95.05				
14 *Chamaecalyx incrassatus* SAG 29.84	96.53	95.05	95.30	95.54	94.06	96.29	94.06	94.55	94.31	94.80	94.80	94.55	98.02			
15 *Hyella caispitosa* PCC 7516	96.53	96.04	96.29	96.53	96.29	97.28	96.04	96.53	96.29	96.78	96.78	96.53	96.04	96.53		
16 *Stanieria cyanosphaera* AB039008	93.32	92.33	93.07	93.32	91.83	93.56	93.81	91.58	91.34	91.58	91.58	91.34	92.57	92.33	91.83	
17 "*Pleurocapsa concharum*" VP4-07	90.59	89.60	90.35	90.59	90.10	89.85	90.35	90.10	89.85	89.85	89.85	90.10	89.60	89.85	89.85	92.33

Characterization of 16S–23S ITS and 23S–5S ITS regions

All Pleurocapsaceae studied strains had both tRNA genes ($tRNA^{Ile}$ and $tRNA^{Ala}$) and a V2 helix between the genes (Table 4). They also all had unusally long end regions following the V3 helix (Table 4).

Secondary structures of the conserved domains in *Pleurocapsa* were distinctive. The D1-D1' helices for both *P. fuliginosa* HA4302-MV1 and *P. minor* HA4230-MV1 both had a short helix in the 3' side of the unilateral basal bulge (Fig. 3 A, B), and formation of this short helix caused an unusual unpairing of the 5' side of the bulge which had four unpaired nucleotides. This structure also formed in two closely related pleurocapsalean strains (Fig. 3 D, F). In three of the strains, an alternate structure could form that resembles the typical structure of this helix in other cyanobacteria (Fig. 3 C, E, G). However, the alternate structure could not be formed in the case of *P. fuliginosa* due to a C→G transversion mutation in the 5' strand of the helix (Fig. 3 A).

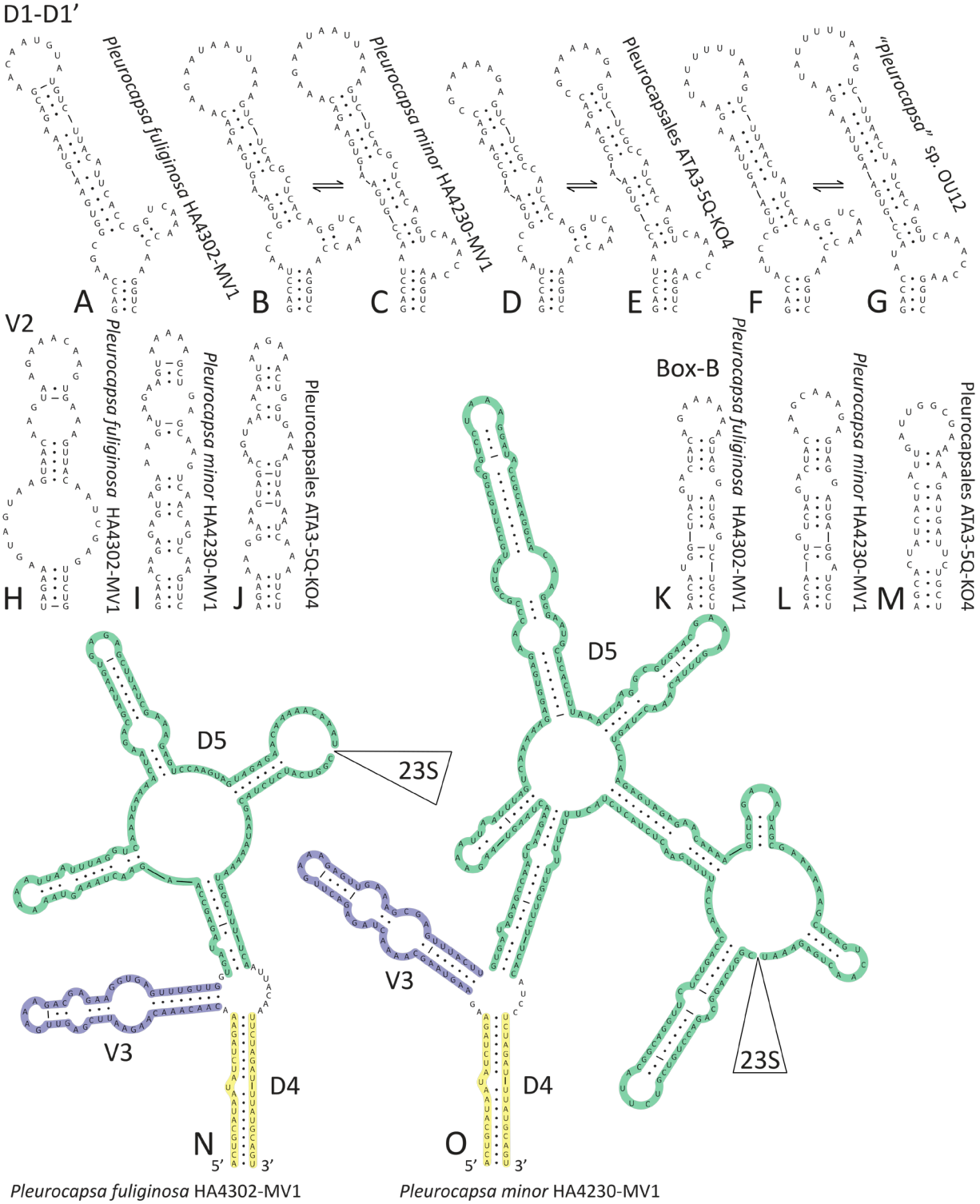

FIGURE 3. Secondary structures of conserved domains in the 16S–23S ITS region. A–G. D1-D1' helices for four strains; alternative structures for the same strain are indicated with equilibrium arrows. H–J. V2 helices situated between $tRNA^{Ile}$ and $tRNA^{Ala}$ genes. K–M. Box-B anti-terminator helix. N–O. End region of 16S–23S ITS showing D4 helices (yellow), V3-helices (purple), D5 region (green), and position of attachment of 23S rRNA gene within the D5, with 3' end of 16S–23S ITS paired to 5' end of 23S–5S ITS.

TABLE 4. Length of ITS conserved domains for species in *Pleurocapsa sensu stricto* and three close genera for which ITS regions are available. *Chroococcidiopsis thermalis* and *C. muralis* lacked the gene for $tRNA^{Ala}$ and the V3 helix; *Pleurocapsa concharum* (belonging to Chroococcales) lacked both tRNA genes.

Strain	Leader	D1-D1' helix	Spacer + D2 + spacer	D3 + spacer	tRNA-Ile	V2 helix	tRNA-Ala	Spacer	Box-B helix	Spacer	Box-A	D4 + spacer	V3	End region
***Pleurocapsa fuliginosa* HA4302-MV1**	**7**	**61**	**51**	**45**	**74**	**79**	**73**	**28**	**41**	**21**	**11**	**19**	**46**	**108**
***Pleurocapsa minor* HA4230-MV1 clone 2C**	**7**	**64**	**47**	**49**	**74**	**82**	**73**	**35**	**42**	**21**	**11**	**19**	**47**	**211**
***Pleurocapsa minor* HA4230-MV1 clone 2B**	**7**	**64**	**47**	**49**	**74**	**82**	**73**	**35**	**42**	**21**	**11**	**19**	**47**	**218**
Pleurocapsalean cyanobacterium ATA3-4Q-KO6	7	60	77	42	74	79	73	67	42	21	11	19	46	154
Pleurocapsalean cyanobacterium ATA12-6-RM16	7	60	77	42	74	79	73	66	42	21	11	19	46	154
Pleurocapsalean cyanobacterium ATA3-5Q-KO4	7	60	78	42	74	79	73	65	40	21	11	19	46	155
Pleurocapsa sp. PCC7319	7	63	44	28	74	53	73	45	40	22	11	19	44	134
Stanieria cyanosphaera PCC 7437	8	62	44	14	74	86	73	34	31	19	11	32	25	26
Stanieria cyanosphaera NIES 3757	8	62	42	14	74	86	73	41	31	19	11	32	26	26
Chroococcidiopsis thermalis PCC 7203	8	93	52	28	74		49		89	16	11		57	
Chroococcidiopsis thermalis CBG1-NQ12	8	93	52	28	74		49		89	16	11		57	
Chroococcidiopsis muralis HA8275-LM2	8	97	52	28	74		41		91	16	11		57	
Pleurocapsa concharum PCC 7327	8	95	31			19			45	15	11	27	66	63

The V2 helices were highly variable within the pleurocapsalean taxa represented, indicating they were separate species (Fig. 3 H–J). All three Box-B helices had identical basal clamp regions (5'-AGCA:UGCU-3'), but differed significantly in the three nucleotides adjacent to the clamp region on both the 5' and 3' strands (Fig. 3 K–M). The two *Pleurocapsa* species were identical in sequence in the central portion of the Box-B helix, but differed in sequence and length in the terminal loop (Fig. 3 K, L).

The most distinctive region of the ITS was the 3' end region (Fig. 3 N, O). The D4 helix, which pairs with the 23S–5S ITS, was typical in structure and length (17–18 nucleotides), as was the V3 helix (46–47 nucleotides). However, the D5 helix is typically a short unbranched helix formed from the 3' end of the 16S–23S ITS and the 5' end of the 23S–5S ITS. The D5 helices of *Pleurocapsa* were very long and branched, and were highly unusual in comparison to all other cyanobacterial D5 helices characterized thus far. These helices were long in both *Pleurocapsa sensu stricto* and the sister Pleurocasalean strains (Table 4, first seven sequences).

Characterization of strains

Class **Cyanophyceae**

Subclass **Oscillatoriophycidae**

Orber **Pleurocapsales**

Family **Pleurocapsaceae**

***Pleurocapsa fuliginosa* Hauck 1885**

Description of our material:—(Figs. 1A, B, 4A) Colony dark blackish brown, hard, dry, in discrete small clumps, arising from the substrate, consisting of large sarcinoid clusters of coccoid cells, with pseudofilament production uncommon, not obviously divaricately branching, but sarcinoid divaricating clusters may expand outward as they divide radially from a central point, forming compact disc-like expanses. Sheaths colorless, thin, tight, scarcely apparent. Pseudofilaments uni- or multiseriate, 4–8 μm wide when uniseriate, up to 20 μm wide in multiseriate filaments. Cells spherical or more commonly ovoid with sides appressed by neighboring cells, typically in large sarcinoid packets, at first the color of red wine, becoming bluish gray, with mature cells russet-brown to dull copper-brown, with thin cross walls, with few but distinct spherical granules, diameter (2) 4–17(20) μm, sometimes individual cells becoming

rounded, enlarged, up to 25 µm in diameter. Chromoplasm often appearing in a parietal position. Baeocytes uncommon, rounded, 3–3.5 µm in diameter.

Collection locality:—Maunawili stream, approximately 20 minutes hike downstream from Maunawili Falls, Oahu. 21° 21’ 05”N latitude, 157° 46’ 08”W longitude; July 25, 2009.

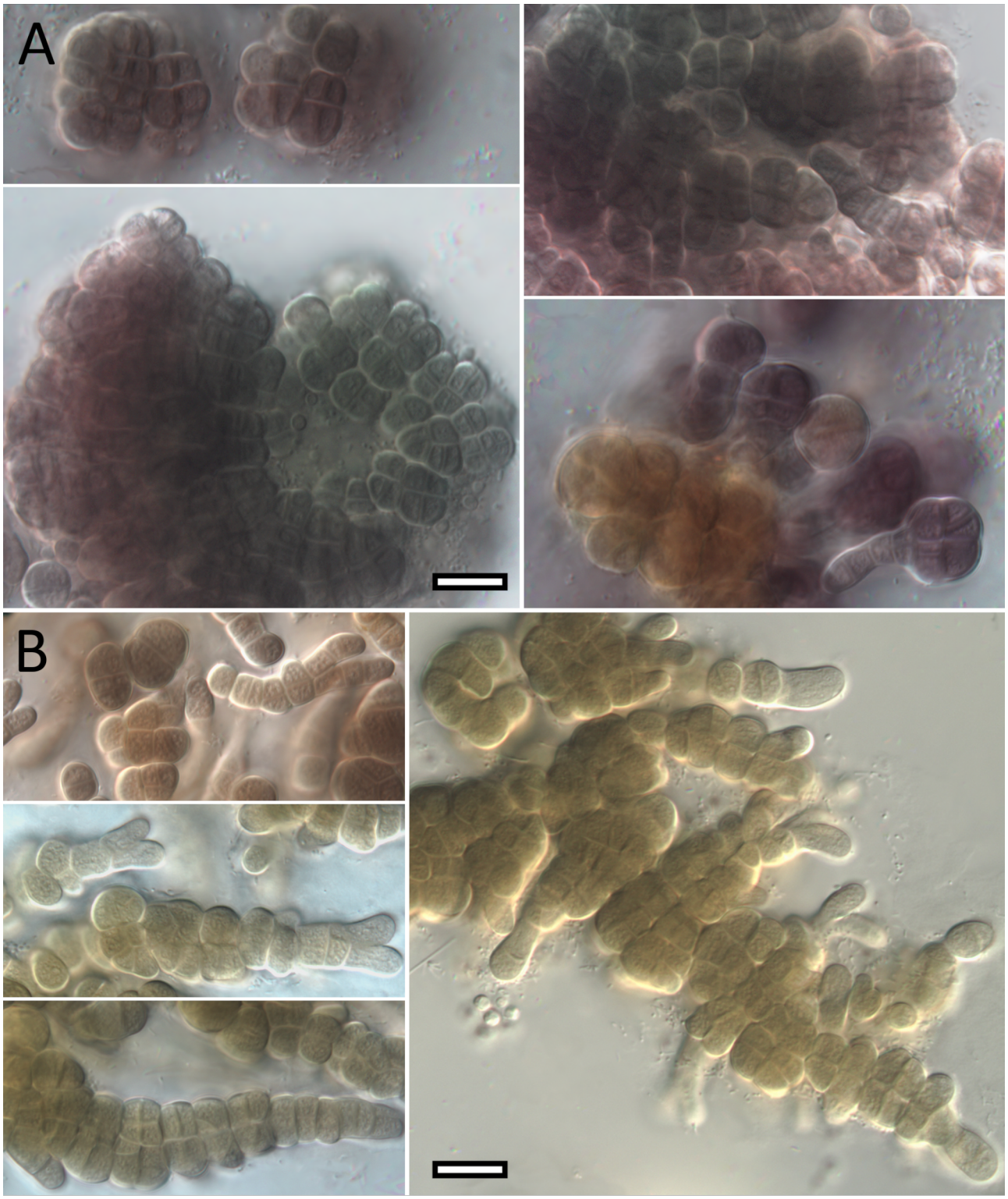

FIGURE 4. Light photomicrographs of *Pleurocapsa* spp.: (A) *P. fuliginosa* from neotype material, note pseudofilaments as well as peripheral and irregular thylakoid arrengment; (B) *P. minor* from Grand Staircase-Escalante National Monument (cultured isolates), with classical morphology for that taxon, which is formation of long pseudofilaments, and pseudodichotomous branchings. Scale bar equals 10 µm.

Holotype:—Possibly in the Musée d'Histoire naturelle, Paris, according to Komárek (1972). Not available for external loan. Material from North America Illustrated in Komárek & Anagnostidis 1998, Fig. 622 c, p. 472.

Neotype here designated:—BISH 755070, Bernice Pauahi Bishop Museum, Honolulu, Hawaii, USA., herbarium mount prepared from the strain, HA4302-MV1.

Reference Strain:—HA4302-MV1, John Carroll University Culture Collection, University Heights, Ohio, USA.

Taxonomic notes:—*P. fuliginosa* was originally described from limestone in the intertidal zone of the Adriatic Sea near Trieste, Italy by Hauck (1885) and a specimen was deposited in Thuret´s collection in the Musée d'Histoire naturelle, Paris. Here is original Hauck's description of *P. fuliginosa*:

"Macrocolonies as blackish aggregates. Cells 5–20 µm wide, single or 2–4 celled, in the large subcolonies up to 50–100 µm. Cell golden-brown, reddish to dirty violet, sometimes blackish, cell content homogeneous. Envelopes colorless".

However, none of the three deposited specimens which are available on the official web site Musée d'Histoire naturelle, Paris fit the type locality of the species. They all originate from different marine environments in North America. Some of them, such as *P. fuliginosa* MNHN-PC-PC0560503, were originally determined as *Coccochloris deusta* Meneghini (1841: 173) which is an earlier name for *Entophysalis deusta* (Menegh.) Drouet & Daily (1948: 79). Members of that genus are incapable of baeocyte production. Komárek (1972) most likely examined one of the three specimens which were mentioned to be epiphytic on the marine macroalgae, in contrast to the original material of Hauck who described *P. fuliginosa* from the stony intertidal. Here is an excerpt from correspondence with Prof. Jíři Komárek concerning *P. fuliginosa* sent in 2017:

"I have studied the type material of *Pleurocapsa fuliginosa* almost 50 years ago in the laboratory of Prof. Bourrelly in Paris. As I remember, we have studied there with Prof. Bourrelly the isotype material of *Pl. fuliginosa*, which was originally from Thuret´s collection and was deposited in the herbarium of Musée d'Histoire naturelle, Paris. I have studied these types just with the supervision and initiative of Prof. Bourrelly. Unfortunately, I do not remember the locality of the original Haucks material, because I was oriented mostly on my strains in that time, which are described in my short study. Prof. Bourrelly gave me this type material only for comparison and it was my omission that I did not describe better this original Haucks specimen. However, maybe that this type material is still deposited in cryptogamic herbarium in Paris, where the original locality should be found."

Unfortunately, Musée d'Histoire naturelle, Paris does not allow loan or use of biomass for molecular investigation of type materials for algae. Given the fact that the museum catalogue does not have material from the type locality or type substrate, we question whether or not the type is actually in the collection. Subsequently, following ICN (Article 9.7, ICN) we consider type materials of *Pleurocapsa fuliginosa* as being lost, and consequently we are establishing a neotype for that particular species, which is the type species of the genus.

The strain HA4302-MV01 which have been primarily characterised by Sherwood *et al*. (2014) exactly fits the morphological description and illustrations of the original description given by Hauck (1885). In particular, the sarcinoid radiating colony is identical, cell dimensions are the same, and the color of the cells are all a close match (Figs. 1, 3A). The dirty-violet (wine red) color is only found in this species of *Pleurocapsa*. The only difference we can detect is in ecological habitat. *P. fuliginosa* occurs in brackish (e.g. Baltic Sea) to marine (e.g. Adriatic Sea) waters, whereas our strain is only in freshwater in Hawaii. However, the sample from which our *P. fuliginosa* derived was collected in a Maunawili stream which is <5 kilometers from the coast. We are making the assumption that the neotype population could ultimately have had a marine origin.

Class **Cyanophyceae**

Subclass **Oscillatoriophycidae**

Orber **Pleurocapsales**

Family **Pleurocapsaceae**

***Pleurocapsa minor* Hansgirg 1891**

Synonym:—*Scopulonema minus* (Hansgirg) Geitler (1942: 93)

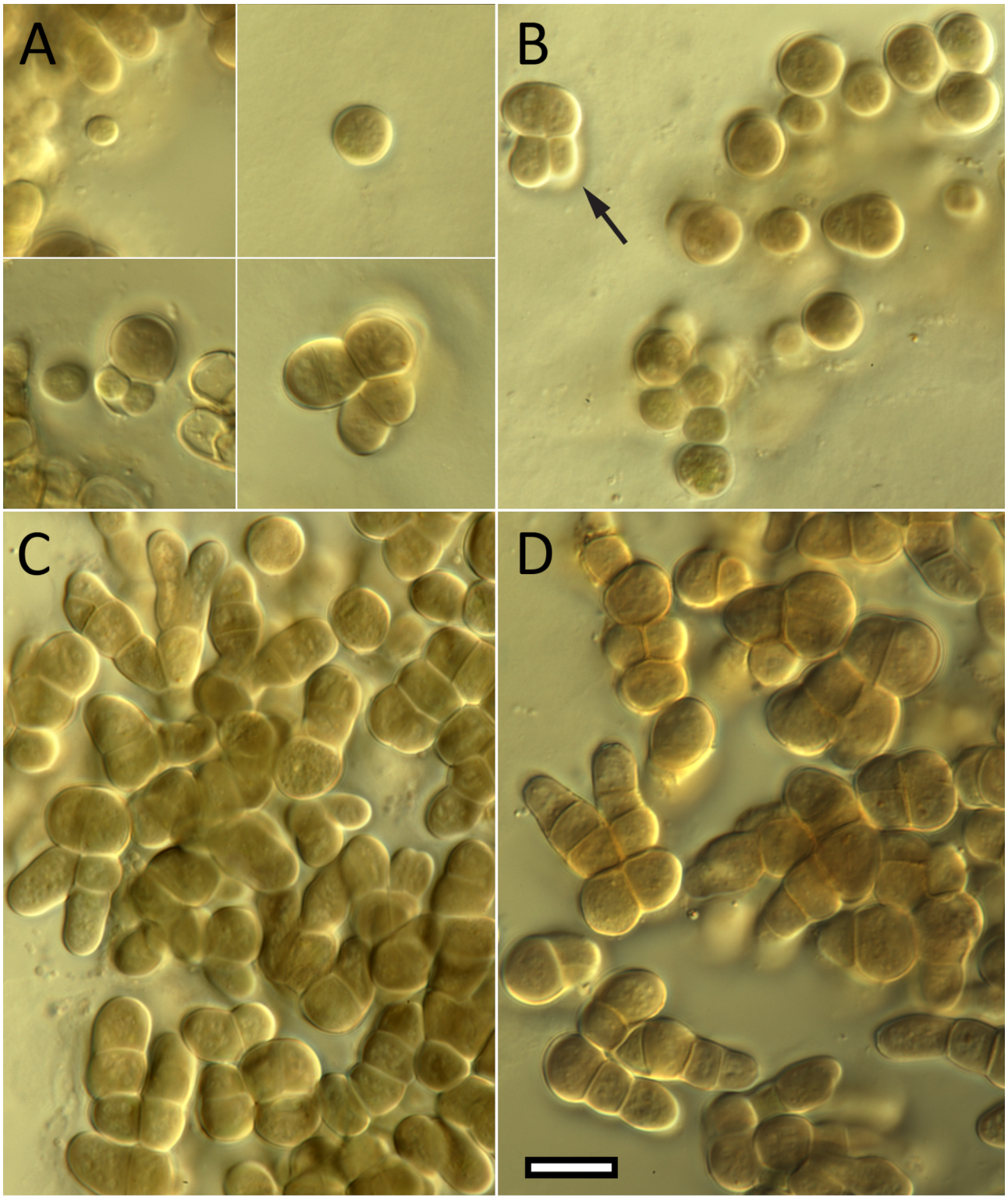

FIGURE 5. Light microphotographs of the epitype of the *Pleurocapsa minor* HA4230-MV1 from Hawaii: (A) Stages of germination; (B) Young subcolonies, derived from baeocytes, and initial stages of pseudodichotomously branched pseudofilaments (indicated with arrow); (C) Mature stages with defined rows of the cells, and clear pseudodichotomous branches; (D) Older stages with yellowish-orange, more or less robust sheaths. Scale bar equals 10 µm.

Description of our material:—(Figs. 4 B, 5) Colony compact, olive-brown, soft to hard, dry, in clumps or small irregular mounds, arising from the substrate with extensions that are higher than wide, consisting of aggregates of coccoid cells, in diads or tetrads, but not forming large cubical sarcinoid clusters, containing pseudofilamentous, pseudodichotomously branching aggregates, united laterally by the confluence of thin gelatinous sheaths. Sheaths colorless, thin, scarcely apparent, later, in the older stages, more robust with yellowish-orange color. Pseudofilaments

uni- or biseriate, 5–12.5 µm wide, with rounded end cells lighter in color than central cells, 4–10 µm wide, 3.5–22 µm long. Cells of the central portion of the thallus irregular, with binary to multiple fission, but mainly sarcinoid, dark olive-green, brown, to brownish orange in older parts of colony, nongranular, with reticulate nature of the cytoplasm often evident, 2.5–10 µm wide, up to 11.5 µm long. Baeocytes uncommon, 1.4–2.8 µm in diameter.

Collection localities:—GSE-CHR-MK-17-07R: Lower Calf Creek Falls, Grand Staircase-Escalante National Monument, Utah, USA. Growing in algal mats on sandstone continually wetted by a waterfall. 37° 49' 44.77" N latitude, 111° 25' 12.58" W longitude, August 16, 2006 (Fig. 2B). HA4230: Nuuanu Pali Lookout, HWY 61, Oahu, USA. Growing on cement trail by the cement wall. 21° 22' 01" N latitude, 157° 47' 31" W longitude; July 23, 2009.

Holotype:—Hansgirg (1891), as a pressed isotype specimen stored in the Department of Botany Collection, housed in the Smithsonian National Museum of Natural History, Washington, USA.

Epitype here designated:—dried material BISH 751766 is derived from HA4230-MV1, Bernice Pauahi Bishop Museum, Honolulu, Hawaii, USA.

Reference Strain:—HA4230-MV1, John Carroll University Culture Collection, University Heights, Ohio, USA.

Taxonomic notes:—Our strains are an excellent fit to the morphological description of *Pleurocapsa minor* Hansgirg (1891), and the subsequent expanded description of Geitler (1932). *P. minor* is typically found in waterfalls. *P. minor* HA4230-MV1 has a genetic identity of 98.8–99.0% to *P. minor* GSE-CHR-MK17-07R (Fig. 5), but is ≤98.7% similar to to all other species in our Pleurocapsales clade (98.0–98.3% similarity to *P. fuliginosa*, ≤96.8% similarity to all other taxa in the phylogeny).

Discussion

Cyanobacterial type specimens can be problematic as a source of taxonomic resolution for several reasons. Herbarium materials are dried and often chemically preserved, and have limited usage for molecular characterization, and frequently are not even of value for morphology. Types were not regularly designated prior to the type concept becoming prevalent in botany, and many times the original material is not described in sufficient detail to match old material from a collection with the material from which a taxon was described. Many types are inaccessible, with herbaria limiting access to the material, and often reluctant to allow any of the material to be destructively analyzed for sequencing. And finally, many potential types have been lost or destroyed. Consequently, many taxa have no type or an ambiguous type which cannot be identified with the original description with certainty. Epitypification and neotypification are both processes designed for addressing this problem and designating types that are useful in a modern context. By choosing new type specimens (neotypes) or more modern materials which can serve as unambiguous supplemental type specimens (epitypes) some of the ambiguity can be eliminated (Turland *et al*. 2018). Cyanobacteria are governed by both the International Code of Nomenclature for Fungi, Algae, and Plants (ICN) and the International Code of Nomenclature for Prokaryotes (ICNP) (Oren & Ventura 2017), but almost all taxa were described under the ICN. The ICN allows for both neotypes and epitypes, and recently several taxa have been epitypified, including *Geitlerinema splendidum* (Greville ex Gomont) Anagnostidis (1989: 43), *Anagnostidinema amphibium* (Agardh ex Gomont) Strunecký *et al*. (2017: 119), and *A. pseudoacutissimum* (Geitler) Strunecký *et al*. (2017: 119). *Phormidium penicillatum* Gomont (1893: 88–89) served as the basionym for the new genus, *Caldora penicillata* (Gomont) Engene *et al*. (2015: 679) which was epitypified with a recently isolated strain. Some other filamentous taxa such as *Oscillatoria princeps* Vaucher ex Gomont (1892: 206) in Mühlsteinova *et al*. (2018) and *Drouetiella lurida* (Gomont) Mai & Johansen in Mai *et al*. (2018: 27) have also been epitypified. The practice of epitypification is employed not only for cyanobacteria, but for other algal groups as well, including Euglenaceae (Karnkowska-Ishikawa *et al*. 2010, Karnkowska-Ishikawa *et al*. 2012), Eustigmataceae (Kryvenda *et al*. 2018), and Klebsormidiophyceae (Rindi *et al*. 2017). An epitype can be a specimen or an illustration selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, cannot be identified for the purpose of the precise application of the name to a taxon (ICN, Article 9.9). We feel this is the situation at hand for *P. minor*.

Pleurocapsales is the poorest known order within cyanobacteria, with few molecularly established taxa (Ishida *et al*. 2001, Oliveira Alvarenga *et al*. 2016). An essential task for this order is to establish the type species of the genus, so that it serves as benchmark for the genus, the family, and the order based upon it. Our *Pleurocapsa sensu stricto* group contains 3 geographically distant lineages which formed a well-supported, tight clade. *Pleurocapsa fuliginosa* is

established with a neotype (ICN, Atricle 9.8) in this work because type material has likely been lost (the "type" material from Muséum National d'Histoire Naturelle Paris comes from a locality different from the type locality the Adriatic sea of Treiste, Italy). An epitype for *Pleurocapsa minor* was designated since the type specimen was unofficially moved from the Naturhistorisches Museum Wien (Vienna, Austria) to the Smithsonian National Museum of Natural History (Washington, USA). The original illustrations were ambiguous, and so were not chosen as lectotypes.

There are still existing inconsistencies in the taxonomy of the Pleurocapsales. *Pleurocapsa* has been placed both in the Hyellaceae and the Pleurocapsaceae (Komárek *et al*. 2014). The correct placement must be the Pleurocapsaceae if Pleurocapsales is accepted as distinct from Chroococcales. The other families assigned to the order, Hydrococcaceae, Xenococcaceae, and Dermocarpellaceae, have representatives that have >93% genetic identity with *Pleurocapsa*, and we consider it likely that when sufficient taxon sampling is complete these families may be subsumed into Pleurocapsaceae. We consider the resolution of family-level taxonomy to be beyond the scope of this work, and will require further study before resolution is possible. However, we can make recommendations for reference strains for some of the pleurocapsalean genera present in our phylogeny for which morphological data are available. We recommend that the clades containing *Chamaecalyx incrassatus* (Lemmermann) Komárek & Anagnostidis SAG 29.84, *Hyella caespitosa* Bornet & Flahault (1888: 162) PCC 7516, *Foliisarcina bertiogensis* Oliveira Alvarenga, Rigonato, Branco, Melo & Fiore (2016: 694) CENA 333 (in collapsed *Foliisarcina* clade, see Fig. 2, Fig. S1), *Xenococcus* sp. PCC 7503, *Stanieria cyanosphaera* (Komárek & Hindák) Komárek & Anagnostidis (1986: 208) *sensu stricto,* and *Chroococcopsis gigantea* Geitler (1925: 342) SAG 12.99 be considered as the correct representatives of those genera. Strains assigned to one of these genera outside of the clade containing the reference strain for the genus need reassignment to existing or new genera. *Myxosarcina* is still polyphyletic, but we cannot make a recommendation at this time as to which clade contains *Myxosarcina sensu stricto*.

Several modern phylogenies have shown a paraphyletic relationship between Pleurocapsales and Chroococcales (Calteau *et al*. 2014, Komárek *et al*. 2014, Beccati *et al*. 2017, Mareš 2017). However, generitypes for these two orders were not sequenced: *Chroococcus rufescens* (Kützing) Nägeli (1849: 46) and *Pleurocapsa fuliginosa*, respectively. Assuming that Pleurocapsales with our typified sequences will be part of Chroococcales as Stigonematales became as a part of the Nostocales (Komárek *et al*. 2014), if only one order is recognized the name of the order must be Chroococcales since *Chroococcus rufescens* is the older taxon. For now, we leave Pleurocapsales intact, as a separate order with members bearing baeocytes with few exceptions. The final decision about inclusion of Pleurocapsales into the Chroococales should be made after a sequence of type species of *Chroococcus*, *C. rufescens* becomes available and taxon sampling within these two groups increases. With the establishment of a neotype for *Pleurocapsa fuliginosa*, it will now be possible to revise the numerous other pleurocapsalean taxa that bear the name *Pleurocapsa*, such as *P. concharum, Pleurocapsa* sp., or Pleurocapsales cyanobacterium.

The ITS region was highly unusual in the occurrence of a very long and branched D5 helix region. The D5 is typically under 40 nucleotides (Johansen *et al*. 2011, Hentschke *et al*. 2016, Mai *et al*. 2018, Becerra-Absalón *et al*. 2018), and is not branched. Since the exceptionally long D5 helix was characteristic of both *Pleurocapsa sensu stricto* and the sister group lacking pseudofilaments, this may be a feature of the family Pleurocapsaceae. Greater taxon sampling is needed to evaluate this character as a family-level marker. If consistent with greater taxon sampling, it will be the first instance of a family-level synapomorphy based on ITS sequence data.

Acknowledgements

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Literature Cited

Anagnostidis, K. (1989) *Geitlerinema*, a new genus of oscillatorialean cyanophytes. *Plant Systematics and Evolution* 164: 33–46. https://doi.org/10.1007/BF00940428

Beccati, A., Gerken, J., Quast, C., Yilmaz, P. & Glöckner, F.O. (2017) SILVA tree viewer: interactive web browsing of the SILVA phylogenetic guide trees. *BMC Bioinformatics* 18: 433. https://doi.org/10.1186/s12859-017-1841-3

Bornet, É. & Thuret, G. (1880) *Notes algologiques recueil d'observations sur les algues*. *Fascicule II*. G. Masson, Paris, 196 pp.

Bornet, É. & Flahault, C. (1888) Note sur deux nouveaux genres d'algues perforantes. *Journal de Botanique* 2: 161–165.

Borzì, A. (1882) Note alla morfologia e biologia delle Alghe Ficocromacee. Fam. IIIa. Rivulariacee. *Nuovo Giornale Botanico Italiano* 14: 272–315.

Boyer, S.L., Flechtner, V.R. & Johansen, J.R. (2001) Is the 16S–23S rRNA internal transcribed spacer region a good tool for use in molecular systematics and population genetics? A case study in cyanobacteria. *Molecular Biology and Evolution* 18: 1057–1069. https://doi.org/10.1093/oxfordjournals.molbev.a003877

Calteau, A., Fewer, D.P., Latifi, A., Coursin, T., Laurent, T., Jokela, J., Kerfeld, C.A., Sivonen K., Piel, J. & Gugger, M. (2014) Phylum-wide comparative genomics unravel the diversity of secondary metabolism in Cyanobacteria. *BMC Genomics* 15: 977. https://doi.org/10.1186/1471-2164-15-977

Carmichael, W.W. (1986) Isolation, culture, and toxicity testing of toxic freshwater cyanobacteria (blue-green algae). *Fundamental Research in Homogeneous Catalysis* 3: 1249–1262.

Collins, F.S. (1901) Notes on algae – III. *Rhodora* 3:132–137.

Crouan, P.L. & Crouan, H.M. (1858) Note sur quelques algues marines nouvelles de la rade de Brest. *Annales des Sciences Naturelles, Botanique, Quatrième série* 9: 69–75.

Cumbers, J. & Rothschild, L.J. (2014) Salt tolerance and polyphyly in the cyanobacterium *Chroococcidiopsis* (Pleurocapsales). *Journal of Phycology* 50: 472–482. https://doi.org/10.1111/jpy.12169

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Drouet, F. & Daily, W.A. (1948) Nomenclatural transfers among coccoid algae. *Lloydia*: 77–79.

Dvořák, P., Poulíčková, A., Hašler, P., Belli, M., Casamatta, D.A. & Papini, A. (2015) Species concepts and speciation factors in cyanobacteria, with connection to the problems of diversity and classification. *Biodiversity and Conservation* 24: 739–757. https://doi.org/10.1007/s10531-015-0888-6

Engene, N., Tronholm, A., Salvador-Reyes, L.A., Luesch, H. & Paul, V.J. (2015) *Caldora penicillata* gen. nov., comb. nov. (Cyanobacteria), a pantropical marine species with biomedical relevance. *Journal of Phycology* 51: 670–681. https://doi.org/10.1111/jpy.12309

Ercegovic, A. (1927) Tri roda litofitskih cijanoficeja sa jadranske obale. *Acta Botanica Croatica* 2: 78–84.

Ercegovic, A. (1930) Sur quelques types peu connus des Cyanophycées lithophytes. *Archiv für Protistenkunde* 71: 361–376.

Geitler, L. (1925) Über neue oder wenig bekannte interessante Cyanophyceen aus der Gruppe der Chamaesiphonaceae. *Archiv für Protistenkunde* 51: 321–360.

Geitler, L. (1932) Cyanophyceae. *In*: Rabenhorst, L.(Ed.) *Kryptogamen-Flora von Deutschland, Österreich und der Schweiz.* Vol. 14. Akademische Verlagsgesellschaft, Leipzig, 1196 pp.

Geitler, L. (1933) Diagnosen neuer Blaualgen von den Sunda-Inseln. *Archiv für Hydrobiologie* 12: 622–634.

Geitler, L. (1942) Schizophyta: Klasse Schizophyceae. *In*: Engler, A. & Prantl, K. (Eds.) *Die Natürlichen Pflanzenfamilien.* Duncker & Humblot, Leipzig, 232 pp.

Gomont, M. (1892) Monographie des Oscillariées (Nostocacées homocystées). *Annales des Sciences Naturelles, Botanique Série* 7, 16: 91–264.

Gomont, M. (1893) Sur quelques *Phormidium* à thalle rameux. Société Botanique de France 40: 86–90.

Guiry, M.D. & Guiry, G.M. (2018) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org (accessed 16 October 2018)

Hansgirg, A. (1891) Physiologische und algologische Mitteilungen. *Sitzungsberichte Der Königl. Böhmischen Gesellschaft Der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe Jahrgang* 2: 83–140.

Hauck, F. (1885) Die Meeresalgen Deutschlands und Österreichs. *In*: Rabenhorst, L. (Ed.) *Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Zweite Auflage*. Eduard Kummer, Leipzig, pp. 513–575.

Hentschke, G.S., Johansen, J.R., Pietrasiak, N., Fiore, M.F., Rigonato, J., Sanť Anna, C.L. & Komárek, J. (2016) Phylogenetic placement of *Dapisostemon* gen. nov. and *Streptostemon*, two tropical heterocytous genera (Cyanobacteria). *Phytotaxa* 245 (2): 129–143. https://doi.org/10.11646/phytotaxa.245.2.4

Ishida, T., Watanabe, M.M., Sugiyama, J. & Yokota, A. (2001) Evidence for polyphyletic origin of the members of the orders of Oscillatoriales and Pleurocapsales as determined by 16S rDNA analysis. *FEMS Microbiology Letters* 201: 79–82. https://doi.org/10.1111/j.1574-6968.2001.tb10736.x

Becerra-Absalón, I., Johansen, J.R., Muñoz-Martín, M.A. & Montejano, G. (2018) *Chroakolemma* gen. nov. (Leptolyngbyaceae, Cyanobacteria) from soil biocrusts in the semi-desert Central Region of Mexico. *Phytotaxa* 367 (3): 201–218. https://doi.org/10.11646/phytotaxa.367.3.1

Johansen, J.R. & Casamatta, D. (2005) Recognizing cyanobacterial diversity through adoption of a new species paradigm. *Algological Studies* 117: 71–93. https://doi.org/10.1127/1864-1318/2005/0117-0071

Johansen, J.R., Kovacik, L., Casamatta, D.A., Iková, K.F. & Kaštovský, J. (2011) Utility of 16S-23S ITS sequence and secondary structure for recognition of intrageneric and intergeneric limits within cyanobacterial taxa: *Leptolyngbya corticola* sp. nov. (Pseudanabaenaceae, Cyanobacteria). *Nova Hedwigia* 92: 283–302. https://doi.org/10.1127/0029-5035/2011/0092-0283

Karnkowska-Ishikawa, A., Milanowski, R., Kwiatowski, J. & Zakryś, B. (2010) Taxonomy of the *Phacus oscillans* (Euglenaceae) and its close relatives-balancing morphological and molecular features. *Journal of Phycology* 46: 172–182. https://doi.org/10.1111/j.1529-8817.2009.00765.x

Karnkowska-Ishikawa, A., Milanowski, R., Triemer, R.E. & Zakryś, B. (2012) Taxonomic revisions of morphologically similar species from two euglenoid genera: *Euglena* (*E. granulata* and *E. velata*) and *Euglenaria* (*Eu. anabaena*, *Eu. caudata*, and *Eu. clavata*). *Journal of Phycology* 48: 729–739. https://doi.org/10.1111/j.1529-8817.2012.01140.x

Komárek, J. (1972) Reproduction process and taxonomy of unicellular endosporine Blue-green algae. *Proceedings of the Symposium on Taxonomy and Biology of Blue-green Algae*: 41–47.

Komárek, J., Kaštovský, J., Mareš, J. & Johansen, J.R. (2014) Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. *Preslia* 86: 295–335.

Komárek, J. & Anagnostidis, K. (1986) Modern approach to the classification system of cyanophytes, 2–Chroococcales. *Algological Studies* 43: 157–226.

Komárek, J. & Anagnostidis, K. (1995) Nomenclatural novelties in chroococcalean cyanoprokaryotes. *Preslia, Praha* 67: 15–23.

Komárek, J. & Anagnostidis, K. (1998) Cyanoprokaryota 1. Teil: Chroococcales. *In*: Ettl, H., Gärtner, G., Heynig, H. & Mollenhauer, D. (Eds.) *Süsswasserflora von Mitteleuropa 19/1*. Gustav Fischer, Jena-Stuttgart-Lübeck-Ulm, 548 pp.

Komárková, J., Jezberová, J., Komárek, O. & Zapomělová, E. (2010) Variability of *Chroococcus* (Cyanobacteria) morphospecies with regard to phylogenetic relationships. *Hydrobiologia* 639: 69–83. http://dx.doi.org/10.1007/s10750-009-0015-3

Kryvenda, A., Rybalka, N., Wolf, M. & Friedl, T. (2018) Species distinctions among closely related strains of Eustigmatophyceae (Stramenopiles) emphasizing ITS2 sequence-structure data: *Eustigmatos* and *Vischeria*. *European Journal of Phycology* 53 (4): 471–491. https://doi.org/10.1080/09670262.2018.1475015

Kützing, F.T. (1836) *Algarum aquae dulcis germanicarum Decas* XVI. In commissis C.A., Schwetschkii, pp. 1–25.

Lagerheim, G. (1888) Sopra alcune alghe d'acqua dolce nuovo o rimarchevoli. *Notarisia* 3: 430.

Lemmermann, E. (1907) Algen I (Schizophyceen, Flagellaten, Peridineen). *In*: *Kryptogamenflora der Mark Brandenburg und angrenzender Gebiete herausgegeben von dem Botanishcen Verein der Provinz Brandenburg*. Verlag von Grebrüder Borntraeger, Leipzig, 304 pp.

Lukešová, A., Johansen, J.R., Martin, M.P. & Casamatta, D.A. (2009) *Aulosira bohemensis* sp. nov.: further phylogenetic uncertainty at the base of the Nostocales (Cyanobacteria). *Phycologia* 48: 118–29. http://dx.doi.org/10.2216/08-56.1

Mai, T., Johansen, J.R., Pietrasiak, N., Bohuniská, M. & Martin, M.P. (2018) 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. *Phytotaxa* 365 (1): 1–59. https://doi.org/10.11646/phytotaxa.365.1.1

Mareš, J. (2017) Multilocus and SSU rRNA gene phylogenetic analyses of available cyanobacterial genomes, and their relation to the current taxonomic system. *Hydrobiologia*: 1–16. https://doi.org/10.1007/s10750-017-3373-2

Mareš, J. & Cantonati, M. (2016) Phylogenetic position of *Geitleribactron purpureum* (Synechococcales, Cyanobacteria /Cyanophyceae) and its implications for the taxonomy of Chamaesiphonaceae and Leptolyngbyaceae. *Fottea* 16: 104–111. https://doi.org/10.5507/fot.2016.002

Melechin, A.V., Davydov, D.A., Shalygin, S.S. & Borovichev, E.A. (2013) Open information system on biodiversity cyanoprokaryotes and lichens CRIS (Cryptogamic Russian Information System). *Bulletin MOIP* 118: 51–56.

Meneghini, G. (1841) Sulle Alghe mediterranee italiane. *Atti della Seconda Riunione degli Scienziati Italiani:* 172–173.

Miller, M., Schwartz, T., Pickett, B., He, S., Klem, E., Scheuermann, R., Passarotti, M., Kaufman, S. & O'Leary, M.A (2015) A RESTful API for access to phylogenetic tools via the CIPRES Science Gateway. *Evolutionary Bioinformatics* 11: 43–48 https://doi.org/10.4137/EBO.S21501

Miscoe, L.H., Johansen, J.R., Kociolek, J.P., Lowe, R.L., Vaccarino, M.A., Pietrasiak, N. & Sherwood, A.R. (2016) Novel cyanobacteria from caves on Kauai, Hawaii. *Bibliotheca Phycologica* 120: 75–152.

Mishler, B.D. & Theriot, E.C. (2000) The phylogenetic species concept (sensu Mishler and Theriot): monophyly, apomorphy, and phylogenetic species concepts. *In*: Wheeler, Q. & Meier, R. (Eds.) *Species Concepts and Phylogenetic Theory: A Debate*. Columbia University Press, New York, pp. 44–54.

Mitra, A.K. & Pandey, D.C. (1967) On a new genus of the blue-green alga *Chlorogloeopsis* with remarks on the production of heterocysts in the alga. *Phykos* 5: 106–114.

Mühlsteinova, R., Hauer, T., De Ley, P. & Pietrasiak, N. (2018) Seeking the true *Oscillatoria*: a quest for a reliable phylogenetic and taxonomic reference point. *Preslia* 90: 151–169.

Nägeli, C. (1849) Gattungen einzelliger Algen, physiologisch und systematisch bearbeitet. *Neue Denkschriften der Allgemeines Schweizerischen Gesellschaft für die Gesammten Naturwissenschaften* 10 (7): i–viii, 1–139.

Oliveira Alvarenga, D., Rigonato, J., Zanini Branco, L.H., Melo, I.S. & Fiore, F.M. (2016) *Phyllonema aviceniicola* gen. nov., sp. nov. and *Foliisarcina bertiogensis* gen. nov., sp. nov., epiphyllic cyanobacteria associated with *Avicennia schaueriana* leaves. *International Journal of Systematic and Evolutionary Microbiology* 66: 689–700. https://doi.org/10.1099/ijsem.0.000774

Oren, A. & Ventura, S. (2017) The current status of cyanobacterial nomenclature under the "prokaryotic" and the "botanical" code. *Antonie van Leeuwenhoek* 110: 1257–1269. https://doi.org/10.1007/s10482-017-0848-0

Printz, H. (1921) Subaërial algae from South Africa. *Kongelige Norske Videnskabers Selskabs Skrifter* 1: 1–41.

Řeháková, K., Johansen, J.R., Bowen, M.B., Martin, M.P. & Sheil, C.A. (2014) Variation in secondary structure of the 16s rRNA molecule in cyanobacteria with implications for phylogenetic analysis. *Fottea* 14: 161–178. https://doi.org/10.5507/fot.2014.013

Rindi, F., Rysanek, D. & Skaloud, P. (2017) Problems of epitypification in morphologically simple green microalgae: a case study of two widespread species of *Klebsormidium* (Klebsormidiophyceae, Streptophyta). *Fottea* 17: 78–88. https://doi.org/10.5507/fot.2016.017

Rippka, R., Waterbury, J.B., Herdman, M. & Castenholz, R.W. (2001) Subsection II. (Formerly Pleurocapsales Geitler 1925, emend. Waterbury and Stanier 1978). *In:* Boone, D.R. & Castenholds, R.W. (Eds.) *Bergey's Manual of Systematic Bacteriology*. Second Edi. Springer-Verlag, New York, pp. 514–539.

Rippka, R., Waterbury, J.B., Herdman, M. & Castenholz, R.W. (2015) *Pleurocapsa*-group. *In Bergey's Manual of Systematics of Archaea and Bacteria*. pp. 1–9. https://doi.org/10.1002/9781118960608.gbm00431

Rippka, R., Waterbury, J.B. & Cohen-Bazire, G. (1974) A cyanobacterium which lacks thylakoids. *Archives of Microbiology* 100: 419–36. https://doi.org/10.1007/BF00446333

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B. Liu, L., Suchard, M.A. & Huelsenbeck, P.J (2012) Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–42. https://doi.org/10.1093/sysbio/sys029

Shalygin, S., Shalygina, R., Johansen, J.R., Pietrasiak, N., Berrendero Gómez, E., Bohunická, M., Mareš, J. & Sheil, C.A. (2017) *Cyanomargarita* gen. nov. (Nostocales, Cyanobacteria): convergent evolution resulting in a cryptic genus. *Journal of Phycology* 53: 762–77. https://doi.org/10.1111/jpy.12542

Sherwood, A.R., Carlile, A.L., Vaccarino, M.A. & Johansen, J.R. (2015) Characterization of Hawaiian freshwater and terrestrial cyanobacteria reveals high diversity and numerous putative endemics. *Phycological Research* 63: 85–92. https://doi.org/10.1111/pre.12080

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* 57: 758–771.
https://doi.org/10.1080/10635150802429642

Strunecký, O., Bohunická, M., Johansen, J.R., Čapková, K., Raabová, L., Dvořák, P. & Komárek, J. (2017) A revision of the genus *Geitlerinema* and a description of the genus *Anagnostidinema* gen. nov. (Oscillatoriophycidae, Cyanobacteria). *Fottea* 17: 114–126.
https://doi.org/10.5507/fot.2016.025

Swofford, D.L. (2002) *PAUP*. *Phylogenetic Analysis of Parsimony (and Other Methods) ver*. *4.02*. Sinauer Associates, Sunderland, Massachusetts.

Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A.M., Prado, J., Price, M.J. & Smith, G.F. (Eds.) (2018) *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017*. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books.
https://doi.org/10.12705/Code.2018

Waterbury, J.B. (1989) Subsection II Order Pleurocapsales. *In:* Buchanan, R.E. & Gibbons, N.E. (Eds.) *Bergey's Manual of Systematic Bacteriology*. Williams & Wilkins, Baltimore, pp. 1746–1770.

Waterbury, J.B. & Stanier, R.Y. (1978) Patterns of growth and development in pleurocapsalean cyanobacteria. *Microbiological Reveiws* 42: 2–44.

Wittrock, V. & Nordstedt, C.F.O. (1893) Algae aquae dulcis exsiccatae praecipue scandinavicae quas adjectis chlorophyllaceis et phycochromaceis distribuerunt - Fasc. 22, 23, 24 & 25. *Botaniska Notiser* 1893: 185–200.

Yarza, P., Yilmaz, P., Pruesse, E., Glöckner, F.O., Ludwig, W., Schleifer, K.-H., Whitman, W.B., Euzéby, J., Amann, R. & Rosselló-Móra, R. (2014) Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Reviews Microbiology* 12: 635–645.
https://doi.org/10.1038/nrmicro3330

Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* 31: 3406–3415.
https://doi.org/10.1093/nar/gkg595

FIGURE S1

FIGURE S1. Uncollapsed 16S rRNA gene phylogeny. Support values are given as BI/ML/MP; if boot straps=100 in ML or MP, or posterior probabilities=1.00 in BI, this full support is indicated by "*". Nodes lacking support are indicated by "–". *Pleurocapsa sensu stricto* marked in bold.