Fottea, Olomouc, 24(2): 137–184, 2024
DOI: 10.5507/fot.2024.002

Revision of the pantropical genus *Brasilonema* (Nostocales, Cyanobacteria), with the description of 24 species new to science

Markéta Bohunická[1*], Jeffrey R. Johansen[2,3], Chelsea D. Villanueva[2,4], Jan Mareš[5], Lenka Štenclová[3,5], Itzel Becerra–Absalón[6], Tomáš Hauer[3] & Jan Kaštovský[3]

[1]*Department of Biology, Faculty of Science, University of Hradec Králové, Rokitanského 62, CZ–50003 Hradec Králové, Czech Republic; [*]Corresponding author e–mail: marketa.bohunicka@uhk.cz*
[2]*Department of Biology, John Carroll University, 1 John Carroll Blvd., University Heights, OH 44118, USA*
[3]*Faculty of Science, University of South Bohemia, Branišovská 1760, CZ–37005 České Budějovice, Czech Republic*
[4]*Department of Biological, Geological, & Ecological Sciences, Cleveland State University, Cleveland, OH, 44115 U.S.A.*
[5]*Biology Centre of the CAS, Institute of Hydrobiology, Na Sádkách 7, CZ–37005, České Budějovice, Czech Republic*
[6]*National Autonomous University of Mexico (UNAM), Facultad de Ciencias, Colonia Coyoacán, Código Postal 04510, P.O. Box 70–474, México, Distrito Federal, México*

Abstract: *Brasilonema* was separated from *Scytonema* only in 2007 (Fiore et al. 2007). It possesses diagnostic morphological characteristics such as vacuole–like structures in older cells, frequent purple pigmentation of cells, rare false branching and fasciculate growth of filaments. Prior to this study, *Brasilonema* was always found attached to the substrate in subaerophytic habitats, exclusively in tropical–subtropical biomes (Brazil, Guadeloupe, Hawaii, Mexico). We have gathered 76 new *Brasilonema* strains from North, Central and South America, central Africa, South and East Asia and Europe, including specialized subaerophytic habitats in temperate climates, and applied a polyphasic approach to their species delineation. All taxa were first examined morphologically and subsequently their relationships were tested using the traditionally applied 16S rRNA gene sequence together with three additional markers (nifD, rpoC1, rbcLX). The results revealed close relationships between specimens obtained from very distant localities (different continents) as well as phylogenetic distance between single *Brasilonema* strains collected from the same biotope. Our results provide evidence that *Brasilonema*, although previously overlooked (or misidentified), is a morphologically easy genus to distinguish that is common over the world in tropical and subtropical regions with humid climate. It can be also found in tropical greenhouses, power–plant cooling towers and other microhabitats that mimic subtropical to tropical conditions in other climatic regions. We conclude that *Brasilonema* is a pantropical genus, there are few geographical constraints in *Brasilonema* dispersal, and its absence in natural habitats in temperate and polar climatic zones may be due to intolerance to desiccation or winter freezing.

Keywords: 16S rRNA gene sequence, 16S–23S rRNA ITS, biodiversity, morphology, orthologous ribosomal operons, phylogenetic relationships, polyphasic approach, protein–coding genes, Scytonemataceae, cyanobacterial taxonomy

Introduction

The genus *Brasilonema* (Scytonemataceae) was first discovered in bromeliad cups in the Botanical Garden of São Paulo and in subaerophytic habitats surrounding the city. It is distinguished morphologically from *Scytonema* by its formation of fascicles and rare false branching (Fiore et al. 2007), and is phylogenetically distinct from all *Scytonema* sensu stricto. Other traits consistent within the genus are trichomes with vacuole–like structures in the cytoplasm and occurrence in subaerophytic habitats. Since the description of *Brasilonema*, populations of other species in the genus have been found in tropical and subtropical collections. Fifteen species have been described, including species new to science and existing species previously placed in other genera – *B. bromeliae*

Fiore et al. (Fiore et al. 2007), *B. octagenarum* Aguiar et al. (Aguiar et al. 2008), *B. epidendron* Sant'Anna et Komárek, *B. ornatum* Sant'Anna et Komárek, *B. roberti–lamyi* (Bourelly) Sant'Anna et Komárek, *B. sennae* (Komárek) Sant'Anna et Komárek, *B. terrestre* Sant'Anna et Komárek (all in Sant'Anna et al. 2011), *B. angustatum* Vaccarino et Johansen (Vaccarino & Johansen 2012), *B. tolantongense* Becerra–Absalón et Montejano (Becerra–Absalón et al. 2013), *B. burkei* Miscoe, Pietrasiak et Johansen in Miscoe et al. (2016), *B. lichenoides* Villanueva, P. Hašler et Casamatta (Villanueva et al. 2018), *B. geniculatum* Villanueva et Casamatta (Villanueva et al. 2019), *B. fioreae* D.E. Berthold et al., *B. santannae* D.E. Berthold et al., and *B. wernerae* D.E. Berthold et al. (Barbosa et al. 2021).

Other species are expected to be transferred to the genus, including for example several *Scytonema* species described by Gardner (1927) from Puerto Rico. The species sequenced thus far always form a tight clade (Sant'Anna et al. 2011; Vaccarino & Johansen 2012; Becerra–Absalón et al. 2013; Miscoe et al. 2016; Villanueva et al. 2018, 2019; Barbosa et al. 2021).

Brasilonema is an excellent example of the recurring phenomenon in cyanobacterial taxonomy that when a new cyanobacterial genus is described based on molecular evidence, other species of the genus are soon identified. *Oculatella subterranea* Zammit, Billi et Albertano (Zammit et al. 2012) was described only two years before seven additional species were reported and described (Osorio–Santos et al. 2014); later an additional five species were discovered (Vinogradova et al. 2017; Becerra–Absalón et al. 2020; Jung et al. 2020). *Mojavia pulchra* Řeháková et Johansen (Řeháková et al. 2007) was described at the same time as *Brasilonema* and two new species have been found since that time (Baldarelli et al. 2022). *Desmonostoc* (Bornet et Flahault) Hrouzek et Ventura with the type species *D. muscorum* was recognized as distinct from *Nostoc* in Hrouzek et al. (2013), and ten additional species in the genus have since been described (Miscoe et al. 2016; de Alvarenga et al. 2018; Cai et al. 2018; Saraf et al. 2018; Kabirnataj et al. 2020; Pecundo et al. 2021; Maltseva et al. 2022; Nowruzi et al. 2023). *Nodosilinea nodulosa* (Z. Li et J. Brand) Perkerson et Casamatta and three additional species within the new genus *Nodosilinea* were described by Perkerson et al. (2011), and subsequently an additional seven species were discovered (Heidari et al. 2018; Davydov et al. 2020; Radzi & Merican 2020; Strunecký et al. 2020; Vázquez–Martínez et al. 2020; Cai et al. 2022). Once these genera with molecular data are published, it is relatively easy to match them to newly sequenced strains through a nucleotide BLAST search, and this certainly contributes to the further discovery of species in these novel genera.

We recently made a concerted effort to find and isolate *Brasilonema* species in widely dispersed collections from tropical, subtropical, and temperate regions. It is thought by some that microbes are not geographically limited by dispersal, but rather are restricted by environmental conditions alone (Baas–Becking 1934; Garcia–Pichel et al. 1996; Zwart et al. 1998; Fenchel et al. 2003; Finlay & Fenchel 2004; Stanojković et al. 2022). If not dispersal–limited, we would expect to find some species widely distributed. If dispersal occurs readily, we might expect to find populations in permanently warm microhabitats in temperate areas, such as greenhouses and habitat created by geothermal activity or anthropogenic thermal activity, such as large scale industrial cooling systems, e.g. in power plants. We sampled tropical and subtropical areas as well as warm, damp microhabitats in the temperate zone and successfully isolated 76 strains. As part of the characterization of these strains, an extensive effort was made to sequence the 16S–23S ITS regions from multiple ribosomal operons. All strains were compared to existing species in the genus, and we here report the discovery of 24 new species as well as new populations of existing species. A thorough review of the genus together with descriptions of new species are given in the present work.

Materials and Methods

Isolation and maintenance of strains. Cyanobacterial strains examined in this study were isolated from samples collected in subaerial and terrestrial habitats on five continents and eight islands (Fig. 1, Table S1). Unialgal cyanobacterial strains were isolated from the samples by placing a small amount of the collected material onto agar–solidified (1.5%) Z8 medium (Carmichael 1986) and followed by series of transfers to fresh plates until clean/clonal strains were obtained. Unialgal strains were kept on Z8 slants under a natural daylight regime and dim light conditions (in a cultivation room with a window partly shaded by blinds) and temperatures of 18 to 21 °C. Each of the strains was thoroughly examined under an Olympus BX 51 light microscope equipped with Nomarski DIC optics and Olympus DP71 digital camera to confirm its morphological affiliation to *Brasilonema*. Cultures were repeatedly examined throughout all life–cycle stages, i.e. in successive time periods following transfer. Photographic plates were made from a subset of images taken and constructed using GIMP 2.10. Additional photographic plates showing wider variability within each illustrated species are given in supplemental material.

DNA extraction, PCR, and sequencing. Seventy–six strains determined as members of the genus *Brasilonema* (Table S1) were chosen for sequencing of the 16S rRNA gene and associated ITS region, together with partial sequences of nitrogenase molybdenum–iron protein alpha chain (nifD) and DNA–directed RNA polymerase gamma subunit (rpoC1) genes and part of the RuBisCO operon (rbcLX).

The first round of sequencing was conducted at the University of South Bohemia. Fresh biomass of each strain was collected in 2 ml Eppendorf microtubes and dried in

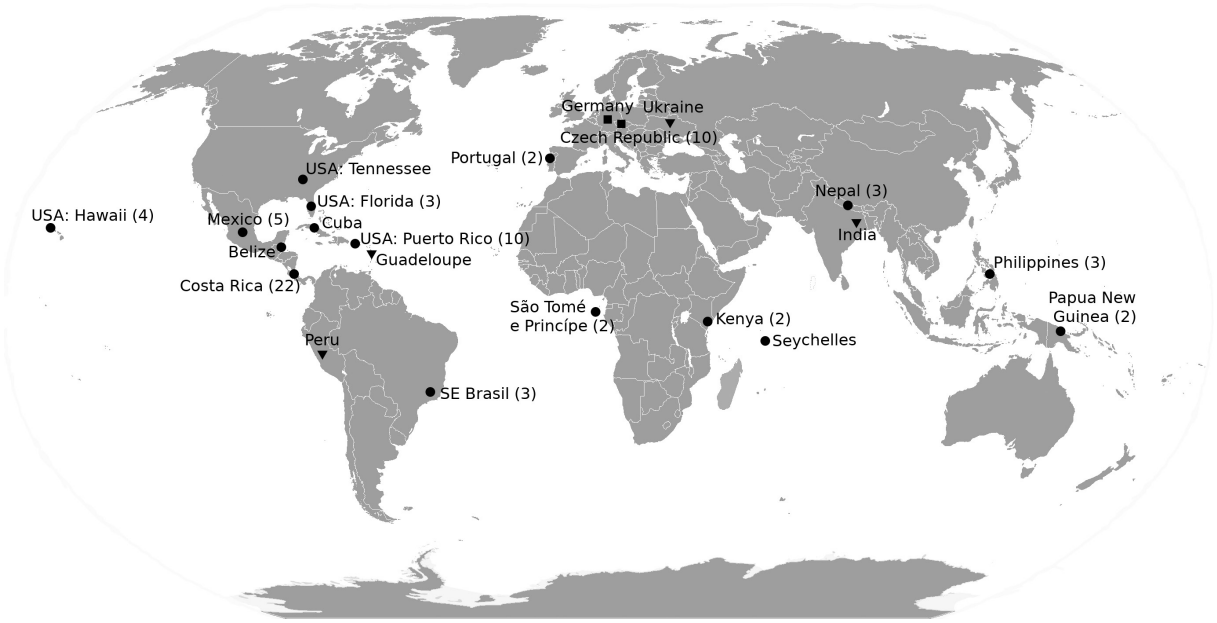

Fig. 1. Distribution of the 76 *Brasilonema* strains examined (morphologically and/or genetically) within this study. Number of strains from the area is specified in parentheses, if larger than one. Sites marked by circle are natural subtropical/tropical habitats, rectangle stands for artificial habitats of the temperate climate zone. Records of *Brasilonema* occurrence from additional localities reported by others are marked by triangle (Sant'Anna et al. 2011; Montoya et al. 2019; Romanenko et al. 2020; Mondal et al. 2022). The map base layer was downloaded from http://www.theworldmap.net/maps/big/world–map–to–download/.

a silica gel desiccator. The dry biomass for each strain was ground with 2 mm tungsten carbide beads using Mixer Mill MM200 (Retsch, Haan, Germany). Genomic DNA was isolated following the modified xanthogenate–SDS buffer protocol with the addition of 3% PVPP and PEG–MgCl2 precipitation (Yilmaz et al. 2009), respectively. A section of the ribosomal operon containing a substantial part of the 16S rRNA gene, the complete ITS region, and the very beginning of the 23S rRNA gene was amplified in a single PCR run using the primer combination VRF2F (5′–GGGGAATTTTCCGCAATGGG–3′) and VRF1R (5′–CTCTGTGTGCCTAGGTATCC–3′) (Wilmotte et al. 1993; Nübel et al. 1997; Boyer et al. 2001, 2002), or alternatively C106F (Nübel et al. 1997) and B23SR (Lepère et al. 2000). The PCR products were approximately 1550 or alternatively about 1800 nucleotides long depending upon primer combination used.

The 25 µl PCR reactions consisted of 12.5 µl of Plain PP Master Mix Combi (Top Bio, Prague, Czech Republic), 10 pmol of both primers, genomic DNA and MQ PCR water. The amplification was initiated with denaturation (94 °C, 5 min), continued with 40 cycles of denaturation (94 °C, 1 min), annealing (54 °C, 1 min) and elongation (72 °C, 2 min), and was finished by terminal elongation (72 °C, 10 min).

Two types of ribosomal operons of two slightly different lengths were often visible on the 1.5% agarose gel during the PCR control. However, it was difficult to separate the individual operons, so the whole PCR product was cut out of a 1.5% low melting agarose gel. Cut out bands were used directly as templates for ligation into plasmids. To get both types of operons, cloning using competent *E. coli* cells and a standard pGEM®–T Easy vector kit (Promega Corp., Madison, WI, USA) was conducted using white–blue selection according to the manufacturer's specifications. Transformed cells were cultivated on LB medium agar plates with the addition of ampicillin, treated with 64 µl of X–gal and 3.5 µl IPTG and incubated for 12 hours at 37 °C. White bacterial colonies with successfully integrated insert were used as templates for control PCR using the universal plasmid primers T7 promoter (5′–TAATACGACTCACTATAGGG–3′) and SP6R (5′–TATTTAGGTGACACTATAG–3′). Amplification was performed with the same settings as the initial PCR, except with annealing temperature set to 50 °C. Successful PCR products were commercially sequenced by SEQme Inc. (Dobříš, Czech Republic), using the above–mentioned PCR primers and internal primer VRF7 (5′–AATGGGATTAGATACCCCAGTAGTC–3′, Boyer et al. 2001). Sequences were assembled using SeqAssem© software (Hepperle 2004).

All three protein–coding housekeeping genes were amplified following previously published protocols, respectively: nifD (Roeselers et al. 2007), rpoC1 (Seo & Yokota 2003) and rbcLX (Rudi et al. 1998). All three sequences were successfully obtained for 54 strains, and for an additional five strains, two of the protein coding genes were obtained. These genes were also obtained for four close outgroup taxa (*Iphinoe,* Scytonemataceae spp.).

When it became clear from the initial round of sequencing that multiple ribosomal operons existed in *Brasilonema*, and that we were missing multiple operons for many strains, a more intensive effort was made to obtain multiple operons for every strain, particularly an operon with two tRNA genes in the ITS and an operon with no tRNA genes in the ITS. This effort proceeded at John Carroll University using the following methods.

Genomic DNA was extracted from *Brasilonema* isolates using 0.25 g of culture biomass and the DNeasy Powersoil™ DNA Extraction kit (Qiagen, Germantown MD). DNA quality was checked on ethidium bromide stained 1% agarose gels. Ribosomal operons were amplified in 25 µl volume reactions, using primer VRF2F and VRF1R with thermocycling parameters previously described in Boyer et al. (2001). PCR reactions included 1 µl of each primer (0.01 mM concentration), 12.5 µl LongAmp™ Taq 2× Master Mix (NEB, Ipswitch MA), 1 µl

template DNA (50 ng/µl), and nuclease free water to a final volume of 25 µl. PCR products were checked on ethidium bromide stained 1% agarose gels.

PCR products were ligated into pSC–A–amp/kan cloning vectors and ligands used to transform StrataClone competent cells, using the StrataClone PCR Cloning kit (Agilent, La Jolla, CA). Transformants were grown on LB plus ampicillin agar plates, prepared by coating with 40 µl of 2% X–gal, and incubated for 12–14 hours at 37 °C. For each strain cloned, 12 white colonies were transferred from agar plates to 3 ml of LB plus ampicillin broth and incubated at 37 °C for 12 hours with shaking. Plasmids were purified from overnight cultures, using QIAprep® Spin Miniprep Kits (Qiagen, Germantown MD). Purified plasmids were checked for inserts by restriction enzyme digestion with EcoR1 (NEB, Ipswitch MA) and subsequent gel electrophoresis. Plasmids containing inserts were Sanger–sequenced by Functional Biosciences (Madison, WI). For sequencing, two primers in the plasmid flanking the insert were used (M13F and M13R), as well as the internal primer VRF5F (5′–TGTACACACCGGCCCGTC–3′) (BOYER et al. 2001) to provide overlap for cloned sequences. The multiple contigs, when available (3–16 per strain), were used to create consensus sequences for each distinct operon. When single bases differed in the clones, the most commonly occurring base was chosen. When only two clones were available, disagreement in nucleotide was recorded by using the IUPAC code in the consensus. Without exception, only a single base among multiple clones differed from the rest in any particular location. Ribosomal operons from all strains were compiled, and additional sequences were harvested from NCBI. Using the Nucleotide database of NCBI, *Brasilonema* sequences with complete 16S rRNA sequences and mostly complete ITS sequences were obtained by downloading FASTA data.

The operons clearly amplify differentially, and we are uncertain if all species have all three operons or if the number of operons is variable among the different species. In all, 89 16S rRNA gene sequences and 117 ITS operon sequences were available with our efforts and with sequences already in public databases. These formed the primary source for subsequent phylogenetic analysis and polyphasic characterization of species.

16S rRNA phylogenetic analysis. An initial Maximum Likelihood (ML) analysis of 259 heterocytous strains together with five non–heterocytous outgroup taxa was conducted to confirm which strains belonged to *Brasilonema*. *Iphinoe* and *Symphyonemopsis* were found to be the closest genera to *Brasilonema* in this analysis. Subsequent analyses of the in–group *Brasilonema* strains with an outgroup consisting of *Iphinoe* and *Symphyonemopsis* were conducted. A total of 118 ribosomal sequences were aligned using ClustalW (LARKIN et al. 2007), and position of indels was manually corrected based upon secondary structure (ŘEHÁKOVÁ et al. 2014). Bayesian Inference (BI) analysis was implemented using MrBayes 3.2.6. (RONQUIST et al. 2012). The evolutionary model was chosen using J ModelTest2 on XSEDE and the BI analysis was subsequently conducted using the GTR+I+G evolutionary model, with both analyses being run in the CIPRES Science Gateway supercomputing facility (MILLER et al. 2015). The analysis was run for 90 million generations discarding the first 25% of samples as burn in. The final average standard deviation of split frequencies was 0.012. The average potential scale reduction factor (PSRF) for all parameter values was 1.000, indicating that convergence of the MCMC chains was statistically achieved (GELMAN & RUBIN 1992). The minimum estimated sample size (ESS) was > 6900 for all parameters, well above the average of 200 that is typically accepted as sufficient by phylogeneticists (DRUMMOND et al. 2006). For ML, we ran RaxML v. 8 (STAMATAKIS 2014) with rapid bootstrapping by choosing a fixed random number seed (12,345) and requesting 1,000 bootstrap iterations. All other options in CIPRES (MILLER et al. 2015) were left as default. Bootstrap values from the ML analysis were mapped to nodes in the Bayesian Inference tree, which was then post–edited in Adobe Illustrator CS5.1.

Four–loci phylogenetic analysis. Sequences of the three protein–coding loci (rpoC1, rbcLX, nifD) were aligned using MAFFT v. 7 (KATOH & STANDLEY 2013) and manually checked; from the RuBisCO operon only the coding regions were included in the phylogenetic analysis. A Maximum Likelihood (ML) phylogenetic analysis in RaxML v. 8 (STAMATAKIS 2014) employing the GTR+I+G substitution model was run with each of the protein–coding loci separately, with 1000 bootstrap pseudo–replications. Resulting phylogenies were manually checked to reveal and eliminate taxa exhibiting incongruent positions in individual gene trees. The three resulting concordant matrices were then concatenated with the 16S rRNA gene sequence prior to the final phylogenetic analysis for the four–loci tree. This alignment had 63 *Brasilonema* sequences, 19 additional heterocytous sequences, and four non–heterocytous taxa as outgroup. BI and ML analyses were run as described earlier in the text, employing a GTR+I+G substitution model in CIPRES. However, each of the loci was coded as a separate alignment partition under its own model of parameters in both types of analyses. An additional neighbor–joining (NJ) analysis was run in SeaView v. 4 (GOUY et al. 2010) using the BioNJ algorithm (GASCUEL 1997) and (default) Jukes–Cantor substitution model, with 1000 bootstrap iterations. Bootstrap support from the ML and NJ analyses were mapped onto nodes of the BI analysis, and this tree was post–edited in Adobe Illustrator CS5.1.

ITS analyses. The sequences of the 16S–23S ITS region were annotated for domain and tRNA gene inclusion. Secondary structures of helical domains were predicted in Mfold (ZUKER, 2003) RNA Folding Form with a structure draw mode of untangle with loop fix. Secondary structures and sequence alignments were used to identify putative orthologous ribosomal operons. We recognized the putative existence of seven orthologous operon lineages, but combined all operons with no tRNA genes into one alignment, all operons with a distinctively long D1–D1' helix alignment with two tRNA genes into a second alignment, and all other operons with two tRNA genes and a shorter D1–D1' helix into a third alignment. For all three alignments, BI and MP analyses were run as follows. For the BI analysis, indels were coded as standard data (1=nucleotide, 0=gap), the GTR+I+G evolutionary model was employed for DNA data, 90 million generations were requested with a stop value < 0.005, and the analysis was run in MrBayes v. 3.2.6 on XCEDE in the CIPRES Science Gateway. The MP analysis was run in PAUP on XSEDE v. 4.a168. For this analysis, the option GAPMODE=NEWSTATE was chosen, a heuristic search employing 10,000 nreps was run, with SWAP=TBR, MULTREES=YES, and STEEPEST=NO. A total of 10,000 bootstrap replicates were then run. The Bayesian trees are reported, with posterior probabilities and bootstrap values mapped to the nodes.

No single phylogenetic analysis based on the ITS region contained all species in our set, and there was not com-

plete topological agreement among these three analyses. This resulted in an unclear understanding of deeper evolutionary relationships among species. However, these three phylogenetic analyses were sufficient to clearly separate species based upon the criterion of lineage separation supporting existence of evolutionary species. The secondary structure and sequence of ITS sequences were also used in our polyphasic approach to recognizing species–level lineages. These structures as visualized in Mfold were post–edited in Adobe Illustrator CS5.

The role of genetic similarity and dissimilarity in species recognition. Species thresholds based on percent similarity (PS) of 16S rRNA sequence data (Table S2) and percent dissimilarity (PD) of the 16S–23S ITS regions (Tables S3–S5) were used to assist species recognition within *Brasilonema* (for all prokaryotic taxa, 16S rRNA PS values ≤ 98.7% are considered to be different species (Yarza et al. 2014). For cyanobacterial taxa, ITS PD ≥ 7.0% is considered strong evidence of lineage separation worthy of taxonomic recognition, while PD ≤ 3.0 % is considered evidence that two lineages belong to the same species (Erwin & Thacker 2008; Osorio–Santos et al. 2014). These thresholds were used in the present study, but both thresholds were problematic. First, 16S PS > 98.7% is not considered evidence that two lineages belong to the same species, but rather is considered uninformative (Stackebrandt & Ebers 2006). Likewise, when 3% < PD < 7% the results are inconclusive, although different species based on total evidence have been found to have PD in this range. These thresholds were used in the present study to establish lineage separation, but morphology, habitat preference, and phylogeny were combined with thresholds for a polyphasic approach to species recognition.

Similarity of 16S rRNA gene sequence was determined by calculating p–distance in PAUP with the SHOWDIST command, and then calculating percent similarity as PS=100*(1–p). Percent dissimilarity was determined by calculating p–distance in PAUP with the SHOWDIST command using the same alignments for ITS regions as described above, and then calculating percent dissimilarity as PD=100*p.

Establishment of species. With a strain set as large as ours, discrete species boundaries can be difficult to establish. We employed a polyphasic approach with the following taxonomic criteria. Strains with 16S rRNA sequence PS < 98.7% were considered to be different species. We considered strains falling in different locations within a phylogenetic analysis to be different species. Strains with ITS PD > 7% were considered to be different species. Strains failing to meet all three of these criteria were further examined in terms of morphology, habitat preference, ITS secondary structure, and ITS PD if 7% < PD < 3%. If evidence of lineage separation was supported by multiple lines of evidence among these character sets, then these lineages were recognized as separate species. If failure to establish lineage separation occurs, we consider it possible that in the future when further data are collected, that future researchers (including ourselves), may conclude that these lineages should be separated further, and more species may be recognized. Alternatively, if it is discovered that these strains plus additional strains that will likely be isolated have more than three operons each, then a re–evaluation of the ITS data may indicate that we have described too many species and synonymies among our taxa may be proposed. Taxonomy is a set of evolutionary hypotheses, each of which can be challenged or further supported with additional data, a fact the authors of this work well recognize.

Species were described following the requirements of the International Code of Nomenclature for Algae, Fungi, and Plants (hereafter the ICN, see Turland et al. 2018). Holotype materials consisted of air–dried cultures on glass fiber filters, included in archival paper envelopes deposited in the Herbarium of the University of South Bohemia (CBFS). In some instances, isolates failed after morphological characterization and molecular sequencing were complete but before a herbarium packet had been prepared. In these limited instances, it was not possible to prepare a physical specimen to be used as a holotype, and a figure was designated as holotype instead in accordance with ICN Article 8.1 combined with Article 40.5, which states that a type "may be an effectively published illustration if there are technical difficulties of specimen preservation or if it is impossible to preserve a specimen that would show the features attributed to the taxon by the author of the name" (Turland et al. 2018). The loss of all isolates upon which a species is based is considered a technical difficulty making it impossible to preserve a specimen that could be deposited in a curated herbarium.

Finally, we made continual use of AlgaeBase to assist in obtaining correct authorities and original publication information for taxa already described (Guiry & Guiry 2023). We also made use of the online articles in Notulae Algarum entitled Nomenclatural FAQs (https://www.notulaealgarum.com/nomenclature/index.html) and Validating a species name (https://www.notulaealgarum.com/nomenclature/is_my_name_valid.html), articles we recommend to all phycological taxonomists.

Results

Ribosomal operons in the genus *Brasilonema*

Between one and three operons with unique ITS regions were found in all species of *Brasilonema* that we and others have studied. In no instance were two operons found that lacked the tRNA genes. In instances where three operons were found, there were always two operons with both $tRNA^{Ile}$ and $tRNA^{Ala}$ genes. Based on these consistent findings, we assume that *Brasilonema* species have a maximum of three operons, and it is always two operons with tRNA genes and one operon with no tRNA genes. However, there was further structure in the ITS regions which could be noted. One set of ITS sequences had exceptionally long D1–D1' helices, and we denoted this set as Bt. However, the remaining ITS regions appeared to have further structure and sequence variation, and we recognized that three other D1–D1' helix types existed in the operons with two tRNA genes, which we labeled At, Ct, and Dt. Finally, we saw that the D1–D1' helix structure in At, Bt, and Ct also existed in the operons with no tRNA genes, and we labeled these operons Ao, Bo, and Co. Consequently, we had seven different orthologous operon types with four different D1–D1' helix types. This is the most complex ITS set we have found in cyanobacteria to date, and it affected our phylogenetic analysis.

All operons possessed D1–D1', Box–B, and V3 operons. Furthermore, all ITS regions with both tRNA genes possessed a relatively long V2 helix. A genus–specific feature of the ribosomal operon was the beginning of the

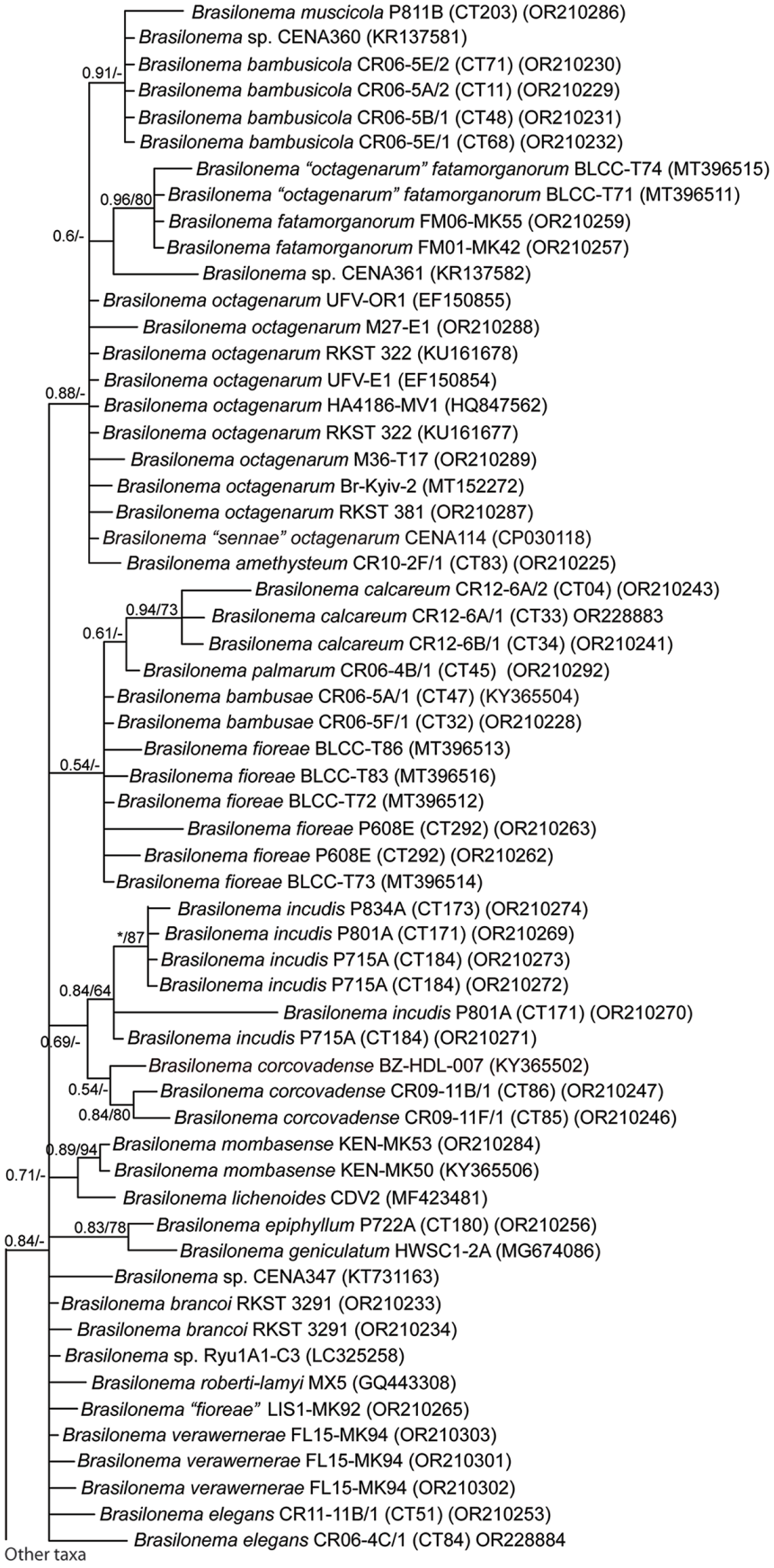

Fig. 2. Bayesian Inference analysis of aligned 16S rRNA gene sequences for the *Octagenarum* group of *Brasilonema* species covered in this study, with posterior probabilities at nodes together with bootstrap values from the Maximum Likelihood analysis. Alternate strain names are in parentheses after original strain designations. This is one part of an analysis that continues in Fig. 3.

23S rRNA gene. In almost all cyanobacterial taxa sequenced thus far, the 23S rRNA starts with 5'–GGUCAA–3'; in *Brasilonema* it starts with 5'–AGUCAA–3'. This feature can be used to separate *Brasilonema* from other Scytonemataceae genera. Specific differences based on the composition of ITS regions, phylogeny, and dissimilarity are given below in descriptions of taxa.

Phylogenetic analyses

The 16S rRNA based phylogeny in most cases showed strains that we considered to be species in supported clades (Figs 2, 3). However, some species groups did not show clear separation. For example, *B. amethysteum* was in a paraphyletic group containing *B. octagenarum* and *B. "sennae" octagenarum* in the *B. octagenarum* clade, and *B. bambusae* and *B. fioreae* were in a paraphyletic cluster at the base of the *B. calcareum/B. palmarum* clade. Other species pairs not separated by this analysis include *B. villosum/B. mata–atlanticum* and *B. xilitlae/B. hortense*. Furthermore, *B. brancoi, B. elegans, B. verawernerae*, and *B. roberti–lamyi* were not separated, and this cluster included strain LIS–MK92, identified as *B. fioreae* based on other analyses. These findings agree with other studies, which have found that 16S rRNA genes are not always sufficient for species recognition in cyanobacteria.

The four–loci tree also separated many species but failed to group all species correctly (Fig. 4). This was partly because we did not recover the protein–coding household genes in as many strains as we did for the ribosomal operons (Table S6). This tree had a different topology than the 16S tree, even though the 16S rRNA sequence was included in the analysis. Still, this tree appeared to resolve species with greater accuracy, and there were fewer conflicted species groups. *B. bromeliae* RKST 321 was distantly placed from other *B. bromeliae* strains, and *B. fioreae* LIS–MK92 was likewise widely separated from other strains found in that species. The two *B. roberti–lamyi* strains were not in the same clade,

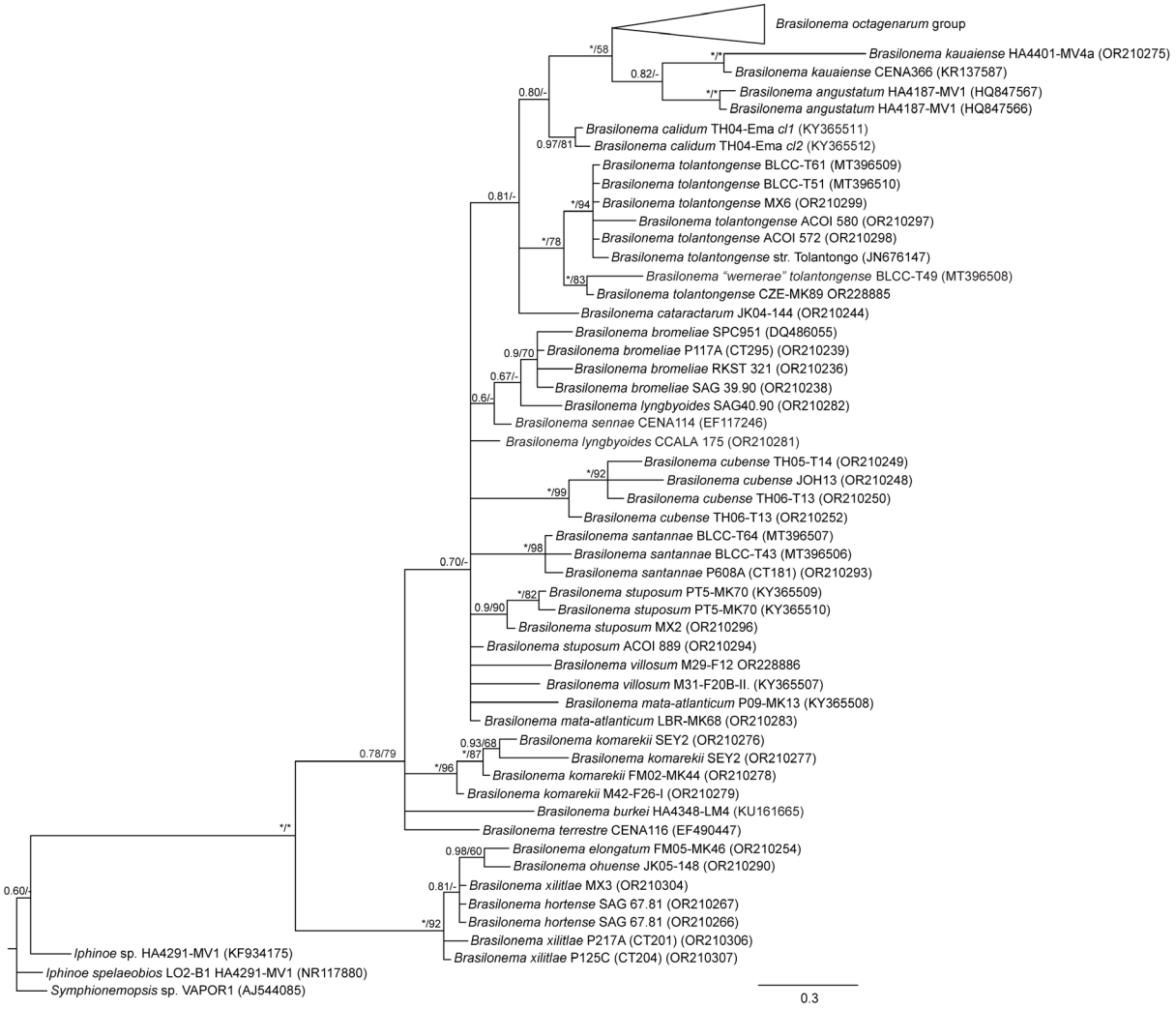

Fig. 3. Bayesian Inference analysis of aligned 16S rRNA gene sequences for the *Bromeliae* group of *Brasilonema* species covered in this study, with posterior probabilities at nodes together with bootstrap values from the Maximum Likelihood analysis. Alternate strain names are in parentheses after original strain designations. This is one part of an analysis that continues from Fig. 2, and shows the *Octagenarum* group only as a triangle at the top of the tree.

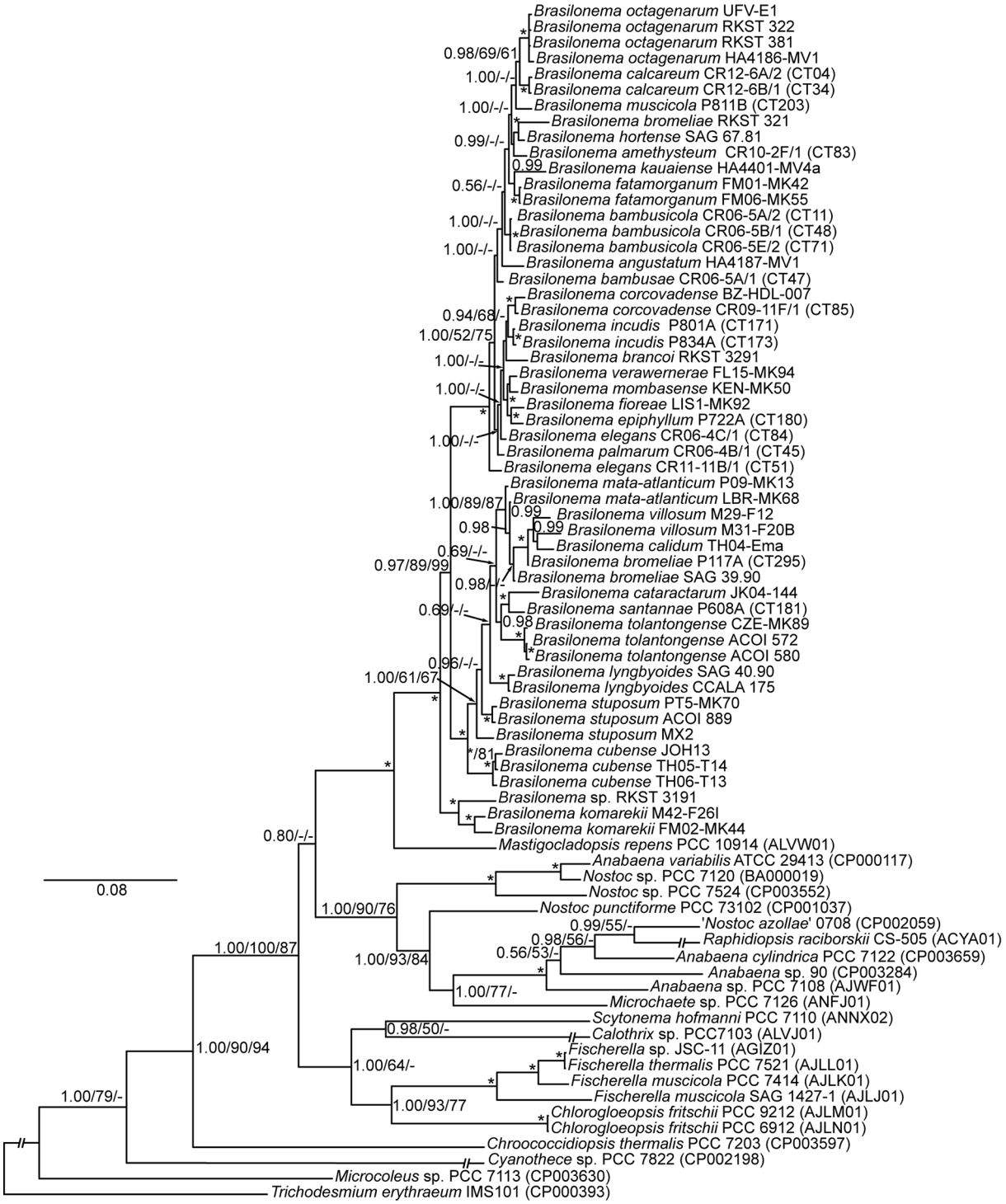

Fig. 4. Four–loci analysis based on concatenated sequences of the rpoC1, rbcLX, nifD, and 16S rRNA genes. The Bayesian Inference analysis is shown, with bootstrap values from the ML and NJ analyses mapped to the nodes.

but were proximal to each other in the tree.

The trees based on alignments of the ITS regions were consistently informative in separating our species and supporting their taxonomic recognition. The most thoroughly sampled set of operons were those with no tRNA genes in the ITS (all three orthologues). We had an operon with no tRNA genes for all but five species, *B. burkei, B. kauaiense, B. mata–atlanticum, B. muscicola*, and *B. terrestre*. None of the species we recognize were paraphyletic or polyphyletic in this analysis (Fig. 5).

The tree based on alignment of the Bt operons (long D1–D1' helix, both tRNA genes) had 39 sequences and was taxonomically informative and consistent (Fig. 6). However, this analysis revealed two possible taxonomic problems. *B. sennae*, which at the time of its description had only a 16S rRNA gene sequence (GenBank# EF117246, SANT'ANNA et al. 2011), has since had its genome sequenced (GenBank# CP030118), from which an Ao and

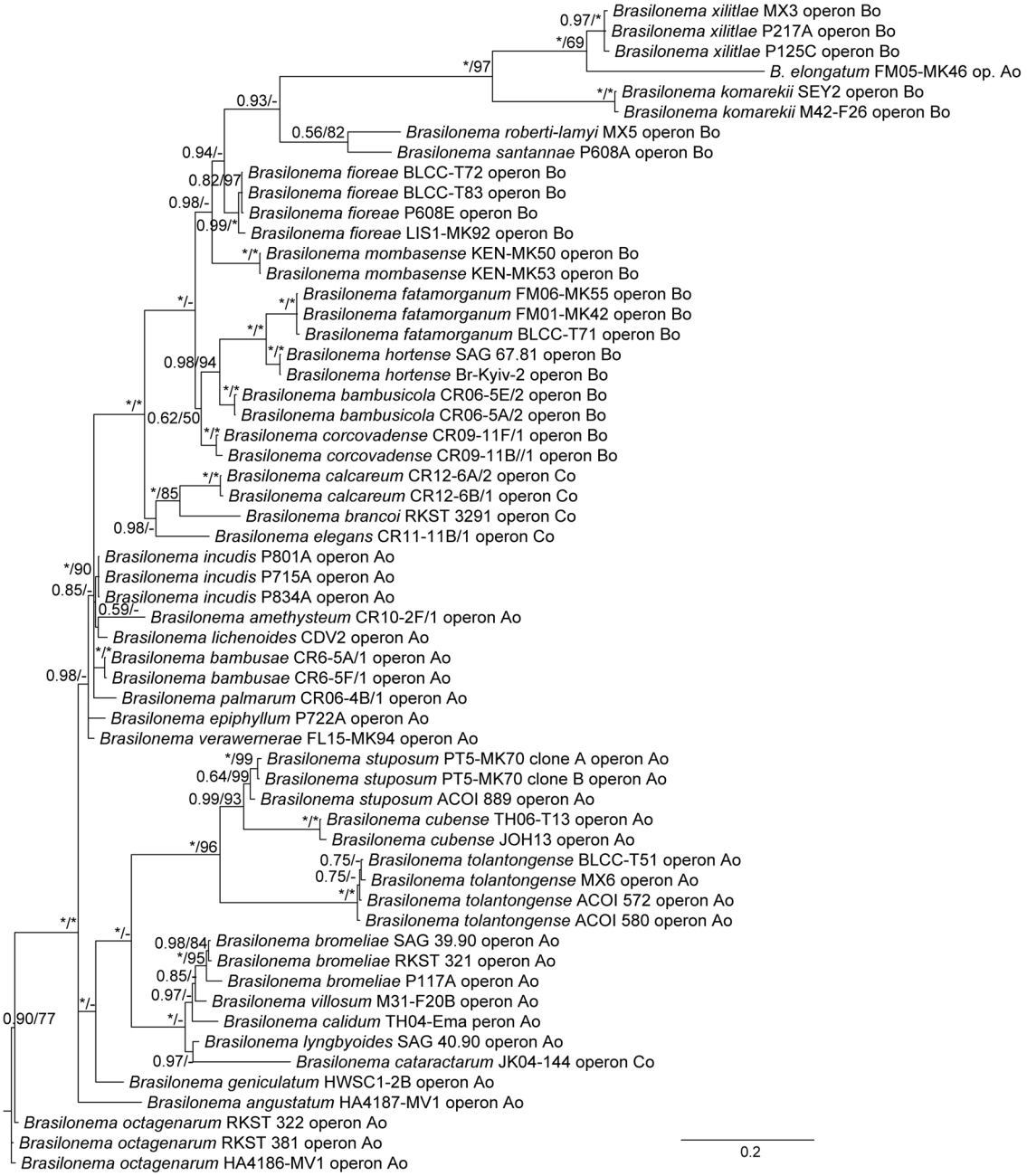

Fig. 5. Bayesian Inference analysis of all ITS sequences from operons lacking tRNA genes. Posterior probabilities from BI and bootstrap values from MP analysis are mapped to the nodes. Operon orthologue (Ao, Bo, Co) is shown for each strain at end of definition.

Bt operon were extracted. The operons from the genome suggest that this taxon belongs in *B. octagenarum*, and *B. sennae* (Sant'Anna et al. 2011) would therefore be a later synonym of *B. octagenarum* (Aguiar et al. 2008). However, the 16S rRNA sequence in the original publication of *B. sennae* differs significantly from the 16S rRNA sequence from the genome, resulting in its distant phylogenetic placement in our 16S tree (Fig. 3). The original sequence published for *B. sennae* is the reference sequence for this species, and therefore we consider it still valid, but the apparent problem with the genomic sequence will need to be addressed by the authors of that sequence.

The second taxonomic problem revealed by this analysis was the unequivocal placement of *B. wernerae* (Barbosa et al. 2021) in *B. tolantongense* (Becerra–Absalón et al. 2013). This was supported by the 16S phylogeny, which also placed *B. wernerae* in this taxon (Fig. 3). The conclusion to be drawn from these observations is that *B. wernerae* is a later synonym of *B. tolantongense* and should be subsumed into that species.

The last ITS phylogeny of the few taxa with an operon containing two tRNA genes with an At, Ct, or Dt operon clearly separated the species in that group (Fig. 7). Even though these are not orthologous, the stark differences in operons can be used as evidence of lineage separation in these taxa.

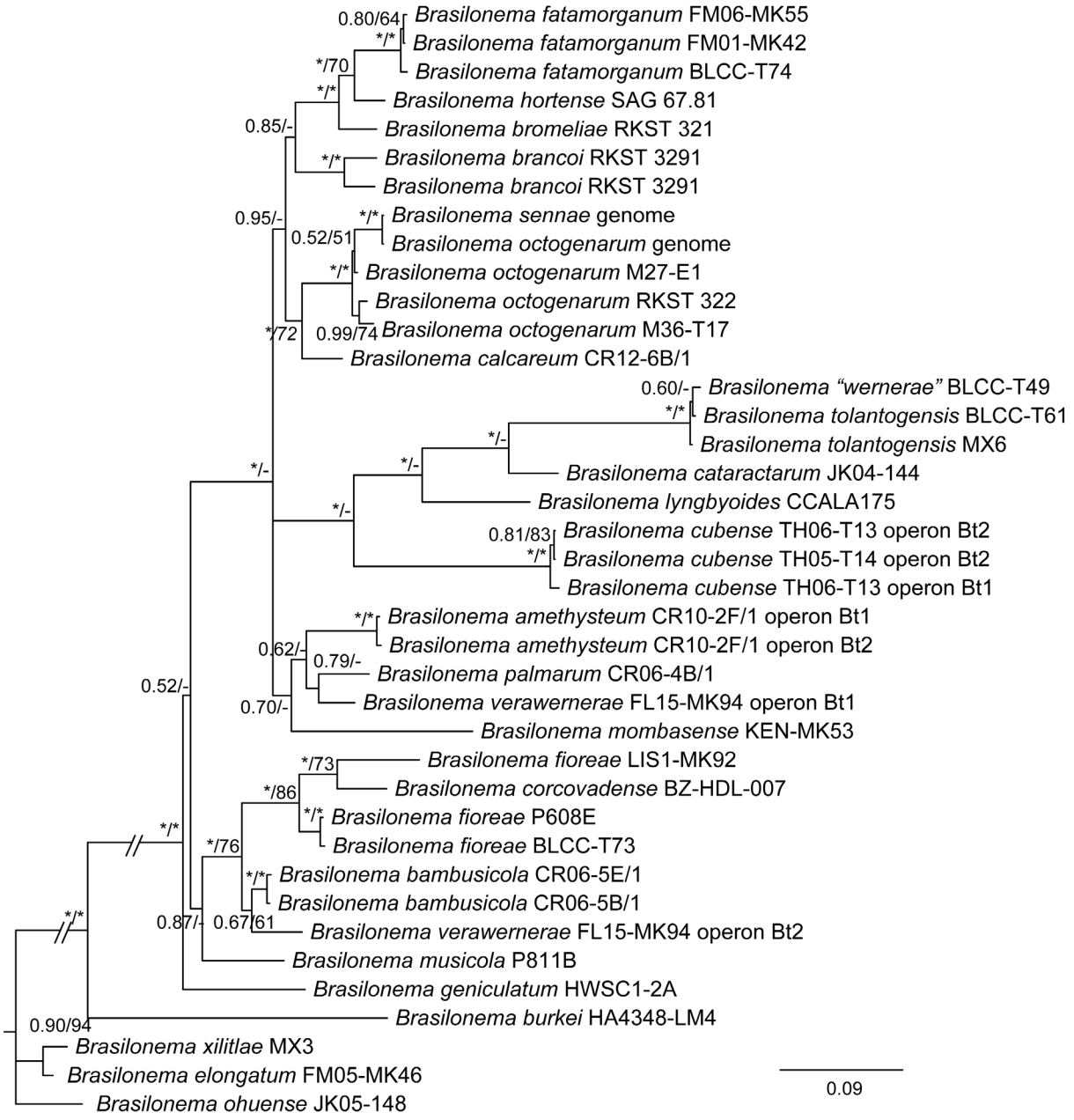

Fig. 6. Bayesian Inference analysis of all ITS sequences from operons possessing the Bt operon with two tRNA genes. Posterior probabilities from BI and bootstrap values from MP analysis are mapped to the nodes.

Percent similarity and dissimilarity

Percent similarity based on 16S rRNA gene sequence supported the recognition of many of our species, which were < 98.7% similar to other species (Table S2). However, this analysis revealed three sets of species with similarity > 98.7%. The group of species around *B. octagenarum* was a large group, containing the following species: *B. octagenarum*, *B. amethysteum*, *B. bambusae*, *B. bambusicola*, *B. brancoi*, *B. calcarium*, *B. corcovadense*, *B. elegans*, *B. epiphyllum*, *B. fatamorganum*, *B. fioreae*, *B. geniculatum*, *B. incudis*, *B. lichenoides*, *B. mombasense*, *B. muscicola*, *B. palmarum*, *B. roberti–lamyi*, and *B. verawernerae*. These taxa were together in a supported clade in the 16S rRNA phylogenetic analysis (Fig. 2). *B. angustatum*, *B. calidum*, and *B. tolantongense* straddled the 98.7% threshold in comparisons with this group of taxa, and fell outside of the supported clade in the 16S rRNA analysis (Fig. 3). A second group of taxa above the similarity threshold included *B. bromeliae, B. cataractarum, B. lyngbyoides*, *B. mata–atlanticum*, *B. santannae*, and *B. sennae* (Table S2), but this group did not form a supported clade in the 16S analysis (Fig. 3). A final third group of similar taxa included *B. elongatum*, *B. hortense*, *B. ohuense*, and *B. xilitlae* (Table S2), and these taxa were supported as the basal clade in *Brasilonema* (Fig. 3). Members of these three groups of taxa required evidence in the form of ITS

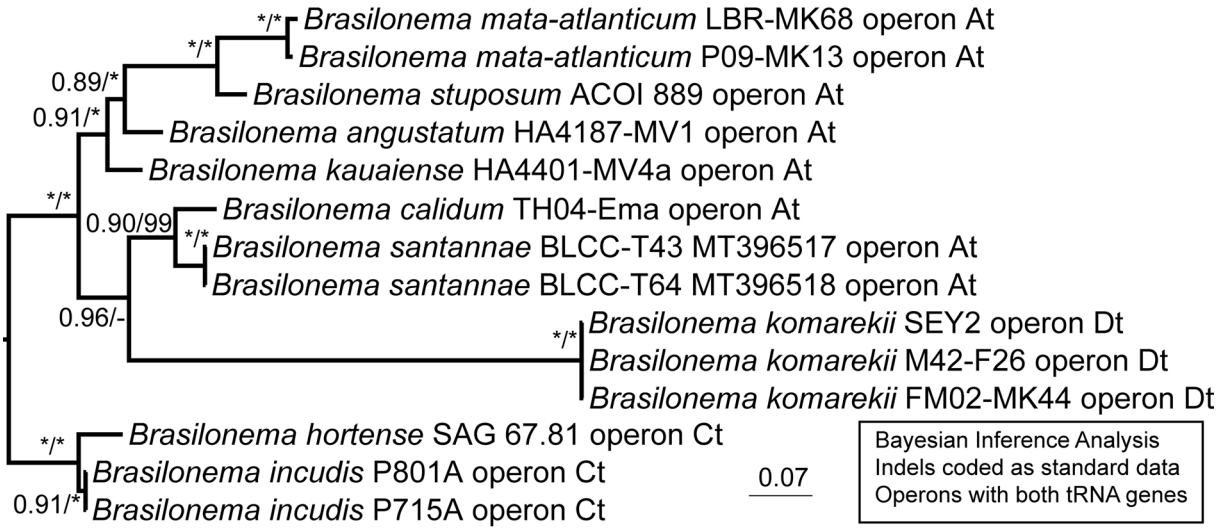

Fig. 7. Bayesian Inference analysis of all ITS sequences from operons possessing tRNA genes but belonging to the less frequently occurring orthologues At, Ct, and Dt. Posterior probabilities from BI and bootstrap values from MP analysis are mapped to the nodes.

phylogeny, ITS dissimilarity, ITS secondary structure, morphology, and ecology in order to tell them apart from one another.

Percent dissimilarities among the operons with no tRNA genes were effective at separating the taxa for which we had this operon, including those in the three similar groups indicated by 16S similarity (Table S3). The only exceptions were *B. lichenoides* and *B. incudis*, which had an average PD = 3.1, which we consider to be on the threshold of species recognition. Average percent similarity of the 16S rRNA gene (at 99.3%) was uninformative for this species pair. However, these two species were phylogenetically separate in both the 16S rRNA tree (Fig. 2) and the ITS tree based on the no–tRNA operon (Fig. 5). The within–species PD for *B. bromeliae* was high, and also at the threshold at 3.1%. However, in both phylogenies, *B. bromeliae* strains were all in the same node (Figs 2, 5). The only species for which we did not obtain a no–tRNA operon were *B. burkei*, *B. kauaiense*, *B. mata–atlanticum*, *B. muscicola*, and *B. terrestre*. Of these, *B. burkei*, *B. kauaiense*, and *B. terrestre* were < 98.7% similar in the 16S rRNA gene in all species comparisons, and so are well established. Consequently, *B. mata–atlanticum* and *B. muscicola* are the only species that must be established by other means than 16S rRNA percent similarity or ITS percent dissimilarity of the no–tRNA ITS sequence.

Percent dissimilarities in the Bt (both tRNA genes, long D1–D1' helix) operon phylogeny were high, with among–species comparisons mostly above 7.0%, and almost universally above 3.0%. The exception in this comparison was the *B. xilitlae* and *B. elongatum* comparison at 2.7% PD (Table S4). These two species also formed a node in the phylogenetic analysis based on this set of operons (Fig. 6). However, they are well separated in the no–tRNA gene ITS dissimilarity (Table S3), and in fact have paralogous no–tRNA operons, Ao for *B. elongatum* and Bo for *B. xilitlae*. Given that *Brasilonema* species consistently have only one no–tRNA gene, this difference provides clear lineage separation for these two taxa. The PD for this operon also clearly separated *B. muscicola* from all other taxa, with a minimum PD of 8.0%, and much higher PD (> 12%) for all other comparisons. The evolutionary relationships based on the no–tRNA operon set are not clear given the fact that they are not orthologous. Taxa are grouped more effectively by orthologue than by species.

Percent dissimilarities in the ITS phylogeny for At, Ct, and Dt operons (both tRNA genes, shorter D1–D1' helix) clearly separated all species (Table S5) shown in the phylogeny that went with this analysis (Fig. 7). Most importantly, analysis of this operon set clearly separated *B. mata–atlanticum* from all other taxa with the At, Ct, or Dt operons, thus establishing its separateness from other species in this study.

Secondary structure of ITS conserved helices

All of our species could be separated based on the combination of phylogeny, percent dissimilarity of the 16S rRNA gene, or percent dissimilarity among orthologous ITS regions, thus making use of the secondary structure of conserved domains of the ITS less necessary. We report all secondary structures for the D1–D1', Box–B, and V3 helices in supplementary materials to assist future taxonomic work on the genus (Figs S1–S9). The differences in the D1–D1' helix were most notable due to the existence of the four orthologous operons (Figs S1–S5). There was also significant variation within orthologous operons, such that only two groups of species had identical secondary structure for the D1–D1' helix: *B. bambusicola, B. calcareum* and *B. muscicola* (Bt operon, Fig. S3 C) and *B. corcovadense*

and *B. fioreae* (Bt operon, Fig. S3 G). There were also some very divergent D1–D1' helices that had branching helices or mid–helix unilateral bulges (Figs S1–S4).

The Box–B helices showed much less variability in structure and sequence than the D1–D1' helices. These structures in the *Octagenarum* group were particularly similar (Fig. S6), whereas the *Bromeliae* group showed variability in the size of the terminal loop (5–8 nucleotides, Fig. S7). The V3 helices were highly divergent, with only one species pair (*B. octagenarum* and *B. muscicola* Fig. S8 A, P) having identical sequence in this helix. The V3 was unusual among known structures of this helix in cyanobacteria in that the basal clamp was variable in length (1–5 base pairs) and sequence, whereas the upper part of the helix was highly conserved in sequence (Figs S8, S9). A set of species in the *Bromeliae* group had V3 helices that were longer, different in sequence, and very divergent in structure (Fig. S9 N–R).

Morphology and Ecology

In addition to the molecular separation of this set of *Brasilonema* species, morphology and ecology were at times informative. However, *Brasilonema* is consistently subaerial, occurring in repeatedly or continually dampened habitats, and not living in habitats exposed to freezing weather. Morphology differs primarily in color, some differences in size ranges, and some variation in ability to taper towards the ends. ITS secondary structures are informative in many instances, and these will be covered in the section that follows, in which species are described together with notes on distinctive morphology and ecology. We also review and comment on some existing taxa that have taxonomic problems revealed by our molecular analyses. We have organized taxa alphabetically into two major groups, those related to *B. octagenarum* (designated *Octagenarum* group) and those closer to *B. bromeliae* (designated *Bromeliae* group).

Some unusual characteristics found within single to multiple species of *Brasilonema* are first noted in this work. In many species, dark pigment was found entrapped in the sheath between trichomes that had separated. With careful and repeated examination, we conclude that necridia in *Brasilonema*, which are dark in coloration, lyophilize to release dark pigment in the space where necrida were present. In some instances, the apices of separating trichomes may release pigment into this space even without the formation of necridia. This interesting feature was noted late in our analysis, and is mentioned in the taxonomic treatments, but best illustrated in the supplemental materials.

Another very striking feature was the production of cellular apical caps in series, which could be separated from the trichome apex but then remained attached to the side of the trichome as the apical meristem produced additional vegetative cells and grew past the location of the caps. This characteristic was observed in *B. mata–atlanticum*. To our knowledge, this feature has only been observed in one other cyanobacterial species, *Aetokthonos hydrillicola* Wilde et Johansen.

Taxonomic descriptions
Class: Cyanophyceae
Order: Nostocales
Family: Scytonemataceae

Taxa lacking sequence data

***Brasilonema epidendron* Sant'Anna et Komárek (Sant'Anna et al. 2011, 52–54, figs 3–7, 9)**

This taxon was described based on morphology, no cultured material or sequence information is available. Its dimensions (reported filament and trichome width) are smaller than of any of the *Brasilonema* taxa morphologically examined within this study. Also, the color of the cell content was reported to be bright blue–green; the violet, purple or brown coloration typical for *Brasilonema* was not observed.

***Brasilonema gomontii* (Gutwinski) M. Bohunická et J.R. Johansen comb. nov.**

Basionym: *Scytonema gomontii* Gutwinski (Gutwinski 1901, 39: 17, fig. 7).

This species clearly belongs in *Brasilonema* based on its dark violet coloration, wooly appearance in nature, and tropical distribution (Indonesia). It is incompletely characterized in comparison to most modern species, with widths of filaments and trichomes overlapping with many species. Perhaps the most distinctive feature is the very long filaments, which were said to be 3–6 mm long in the protologue. It has also been recorded from Brazil (Komárek 2013).

***Brasilonema ornatum* Sant'Anna et Komárek (Sant'Anna et al. 2011: 54, fig. 10 A–D)**

This taxon was described based on morphology and ecology, no cultured material or sequence information is available. It can be distinguished by its lamellate, regularly ornamented colorless sheaths, a feature not observed in any of our *Brasilonema* strains. The reported color of cell content was grayish green.

***Octagenarum* group**

Defined as the group of *Brasilonema* species occupying the supported clade within the genus that contains *B. octagenarum.* Defined by 16S rRNA gene similarities above 98.7%. Further defined by the absence of any At type operons.

This group currently contains *B. octagenarum, B. amethysteum, B. bambusae, B. bambusicola, B. brancoi, B. calcareum, B. corcovadense, B. elegans, B. epiphyllum, B. fatamorganum, B. fioreae, B. geniculatum, B. incudis, B. lichenoides, B. mombasense, B. muscicola, B. palmarum, B. roberti–lamyi,* and *B. verawernerae* (Fig. 2).

Brasilonema octagenarum **R. Aguiar et al. (Aguiar et al. 2008, 1325–1326, figs 1, 4–6) (Fig. 8 a–e, S10 A–L)**
Emended description: Thallus macroscopic, velvet–like, with mostly short erect fascicles, creeping on the substrate, growing deeply into the substrate, tuft–like spreading with sites of denser biomass forming spherical fuzzy crumbs, black, black–brown, reddish brown, dark blue–green, dark green to green or yellow–brown when old. Filaments straight to slightly waved, irregularly coiled, of middle length, rarely to frequently single or double false branched, (9.8)12–20(24) µm wide, of the same width along the whole length or slightly gradually narrowed towards the ends. Sheath thin, firm, attached, mostly hyaline, sometimes widened or lamellated, up to 2 µm wide, colorless to greyish or yellow–brown. Trichomes not constricted up to distinctly constricted at crosswalls, (6)11–21(23) µm wide, sometimes narrowing towards the ends. Cells cylindrical to disc–like, mostly shorter than wide, sometimes up to isodiametric or longer than wide in old trichomes, (1.5)3–14(15) µm long, shorter towards the ends of the trichomes. Cell content finely granulated (structured), coarsely granulated when old, brown, grey–brown, violet or blue–green, olive green, yellow–green, bright or pale, slightly yellowish at crosswalls, often with typical vacuole–like structures in some parts of trichomes in rows of neighboring cells. Apical cells hemispherical or widely rounded, often yellowish, light colored, sometimes with hyaline calyptra–like structure. Heterocytes intercalary cylindrical, hemispherical or almost spherical, rarely in pairs, rarely terminal, yellow or yellow–green, light yellow often with sap vesicle, (8.4)13–20 µm wide, (5.5) 9–12(15.4) µm long. Hormogonia short, typically 1–8 cells, sometimes up to 16, formed by disintegration of filament, released from the ends, or sometimes from the middle part of the filament, short–celled, 4.8–12.6 µm wide, first isopolar, bluntly rounded or tapered slightly to both ends, becoming heteropolar with terminal heterocyte when germinating. Necridic cells of dark color present, lyophilizing to leave dark pigment between cells within the sheath. ITS operon types recovered include Ao and Bt.

Voucher preparations: CBFS A133–1! and CBFS A134–1!, dried material of strains HA4187–MV1 and RKST 381, respectively, immobilized on a glass fiber filter.
Habitat: The species has been found in bromeliad cups, as an epiphyte on leaves, on damp rocks, and on bark in humid tropical biomes (Brazil, Costa Rica, Hawaii) and subaerial in greenhouses in temperate climates (Teplice Botanical Garden, Czech Republic).
Reference strain: UFV–E1.
Materials analyzed: Sequences only UFV–E1, UFV–OR1, CENA114; strains and sequences M36–T17, M27–E1, RKST 322, RKST 381, HA4186–MV1.
Genbank accession numbers: 16S rRNA and ITS: NR_115956, EF150854–855, EF117246, OR210287–289, KU161677–678, HQ847562; WGS: NZ_QMEC01000404, CP030118.

Taxonomic notes: This species is represented by several strains from diverse locations. The reference strain has recently had its genome sequenced, and so the species is very well established phylogenetically. Belonging to a large clade that is similar in the sequence of the 16S rRNA gene (many strains with ≥ 98.7% genetic identity), *B. octagenarum* sensu stricto is a subset of that clade with the percent sequence dissimilarity in the Ao operon averaging 1.5%, but having ≥ 15% for all other strains with an Ao operon, and ≥ 23 % for all other strains with Bo or Co operons. All operons with tRNA genes occurring in *B. octagenarum* sensu stricto were Bt type, and were ~ 1.8% dissimilar within the species, but ≥ 6.5 % dissimilar from all other strains. While this species cannot be distinguished by 16S rRNA sequence alone, it is clearly delineated by the sequences of the ITS regions from both operons. In the phylogeny based on four loci, it is sister to *Brasilonema calcareum,* which differs through its possession of a Co type operon. The *Octagenarum* group is a difficult group, and likely specific determinations will not be possible without sequence data for multiple operons.

As detailed above, the genome of *B. sennae* (CP030118) has 16S rRNA gene sequence nearly identical to the sequence of *B. octagenarum*, but the original 16S sequence for *B. sennae* is quite different and places this species in a separate phylogenetic position, outside of the *Octagenarum* group 16S clade (Fig. 4). We consider *B. sennae* still a valid, unique taxon, but recognize that the genome of *B. sennae* needs to be examined further due to the confusion its sequence may cause. Unfortunately, when the strain was first characterized, no ITS sequence was reported.

Brasilonema amethysteum **M. Bohunická et J.R. Johansen sp. nov. (Fig. 8 f–k, S11 A–G)**
Description: Thallus black, with short dense, erect filaments, unevenly spreading. Filaments short, densely false branched, 11–20 µm wide. Sheath thin colorless to widened, hyaline or lamellate, colorless, orange, brown to black–brown at some spots. Trichomes not constricted to constricted at crosswalls, 8–18 µm wide. Cells shorter than wide, cylindrical, rarely with vacuole–like structures, purple, purple–brown, brown, olive–brown to grey, 2–8 µm long. Apical cells widely rounded, often of tan or lighter color, sometimes with light green spherical cells at the end. Heterocytes intercalary, cylindrical to disc–like, golden, smooth, 10–18 µm wide, (2)3–10 µm long. Hormogonia short, isopolar, dark dull green. Necridic cells of dark color present, lyophilizing to leave dark pigment between cells within the sheath. ITS operon types recovered include Ao, and two slightly different Bt operons.

Holotype here designated: CBFS A135–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in March 2010 near Corcovado Danta Lodge, Corcovado NP, Costa Rica (8°37'10.3" N, 83°28'31.48" W).

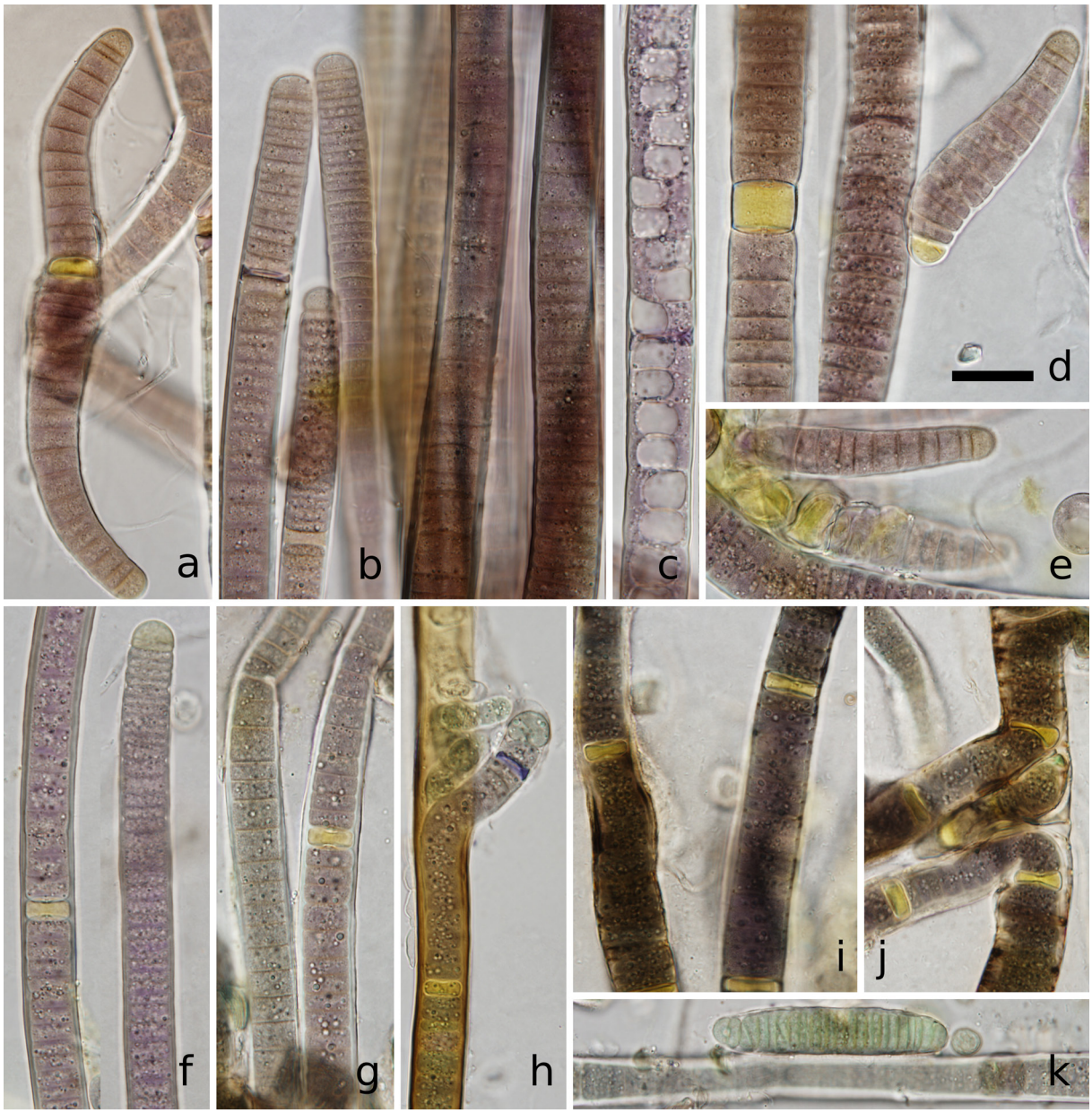

Fig. 8. (a–e) *Brasilonema octagenarum*, (a–b) strain RKST 381, (c–e) strain RKST 322; (f–k) *B. amethysteum* strain CR10–2F/1. Scale bar 20 µm.

Habitat: Subaerial on wood in tropical rainforest.
Etymology: L. *amethysteus* = the color of amethyst, named for the intense grayish purple of the trichomes.
Reference strain: CR10–2F/1.
Materials analyzed: Strain CR10–2F/1.
GenBank accession numbers: 16S rRNA and ITS: OR210225–227.

Taxonomic notes: This species, based on a single strain, was proximate to *B. bromeliae* and *B. hortense* in the four loci tree, and had 16S rRNA genetic identities above the 98.7% similarity threshold with numerous species scattered throughout the genus. However, it had a distinct position in the 16S rRNA phylogeny. The Ao operon ITS region was dissimilar to all other species possessing that operon (≥ 8%). The species had two different Bt operons, which were recognizable as different (0.3% PD) due to sequencing excess clones in both operon types and establishing that the minor sequence difference was in fact real, not just an artifact of sequence errors. The ITS region in the Bt operons was also quite dissimilar to all other species possessing that operon (≥ 8.7%). This species had the unusual morphological characteristic of blackish–brown sheaths in some filaments (Fig. S11 D).

***Brasilonema bambusae* M. Bohunická et J.R. Johansen sp. nov. (Fig. 9 a–f, S12 A–P)**
Description: Thallus with branched erect fascicles, black, brown to olive–green green when old. Filaments long, rarely false branched, sometimes gradually tapered towards the ends, 10–22 µm wide, 9 µm in narrowed

tips. Sheath thin, attached, to widened, lamellate, up to 3 µm wide, colorless to grey, rarely brown incrusted. Trichomes not constricted or slightly constricted at crosswalls, (7)10–17(21) µm wide. Cells shorter than wide to longer than wide, cylindrical to disc–like, of a range of various colors, typically grey–brown, but also (pale) blue–green, (pale) brown, dull purple, olive–brown or olive–green, green, yellow–green or grey, often with vacuole–like structures, 2–20 µm long, cells shorter than wide towards the end part of trichomes. Apical cells widely rounded, yellowish or of paler color than the rest of the trichome. Heterocytes intercalary, cylindrical, golden, yellow or yellow–green, with smooth content, sometimes with sap vesicle, 10–16 µm wide, 5–15 µm long. Hormogonia released from the ends of the filaments, short, with cylindrical cells shorter than wide, germinating usually to both ends or rarely to one end opposite to hemispherical terminal heterocyte, narrowing towards the growing tips, often brown or purple–brown when released, becoming blue–green during the germination. Necridic cells of various colors present. ITS operon types recovered only include Ao.

Holotype here designated: Fig. 9 d–f, images of CR06–5A/1.
Type locality: Collected in March 2010 from a bamboo trunk in a bamboo grove in the Costa Ballena region on the Pacific Coast, Uvita, Puntarenas, Costa Rica (9°09'05.6" N, 83°44'14" W).
Habitat: Tropical, subaerial, forming black mat on bamboo trunks in bamboo grove.
Etymology: L. *bambusa* = bamboo plant, named for its association with bamboo.
Reference strain: CR06–5A/1.
Materials analyzed: Strains: CR06–5A/1, CR06–5F/1.
GenBank accession numbers: 16S rRNA and ITS: KY365504–505, OR210228.

Taxonomic notes: This species is based upon two strains isolated from Costa Rica, CR06–5A/1 and CR06–5F/1. It possesses an ITS region similar to *B. lichenoides*, which also has an Ao type operon that is only 3.5% dissimilar. However, they are not sister taxa in either the 16S or the ITS phylogenies. We have separated these taxa so that we maintain monophyly in our recognized species. This species was isolated from the same sample in which *B. bambusicola* was isolated, but is phylogenetically very distinct. Unfortunately, the culture was lost during extended cultivation before manuscript preparation, making it technically impossible to prepare a physical type once the sequence data and analysis demonstrated the isolate represented a species new to science. We designate a figure as the holotype in accordance with ICN article 40.5, which states that the holotype "may be an effectively published illustration if there are technical difficulties of specimen preservation or if it is impossible to preserve a specimen that would show the features attributed to the taxon by the author of the name" (Turland et al. 2018).

***Brasilonema bambusicola* M. Bohunická et J.R. Johansen sp. nov. (Fig. 9 g–m, S13 A–J)**
Description: Thallus with long erect filaments, attached to substrate, (dark) brown, (dark) olive–green, becoming lighter when older, yellow to brown pigment sometimes released to the substrate. Filaments short to long, (rarely) single or double false branched, sometimes gradually narrowed towards the ends, 10–22 µm wide, narrowed up to 8 µm at the tips. Sheath thin, attached, firm or lamellate widened, hyaline to grey or yellowish, sometimes brown incrusted, up to 4 µm wide. Trichomes not constricted to constricted at crosswalls, 7–21 µm wide, 8 µm wide at the ends. Cells cylindrical, shorter than wide up to discoid, sometimes slightly longer than wide, sometimes with vacuole–like structures, of a range of various colors, typically purple, purple–brown, brown or olive–brown to olive–green, yellow–green, blue–green or grey, gradually lightening at the tips, 2–15 µm long. Apical cells hemispherical or widely rounded, tan or light green. Heterocytes intercalary, cylindrical, with sap vesicle(s), golden or yellow–green, 8–14 µm long, 10–16 µm wide. Hormogonia with short cells, released in branches or from the ends of filaments, typically C–bent and narrowed towards the growing tips when germinating, often with intercalary heterocyte, purple or purple–brown when released, becoming blue–green during elongation. Necridic cells of various colors present, lyophilizing to leave dark pigment between cells within the sheath. ITS operon types recovered include Bo, and Bt operons.

Holotype here designated: CBFS A136–1!, dried material of the reference strain immobilized on a glass fiber filter.
Paratypes here designated: CBFS A137–1! and CBFS A138–1!, dried material of strains CR06–5E/2 and CR06–5B/1, respectively, immobilized on a glass fiber filter.
Type locality: Collected in March 2010 in the Costa Ballena region on the Pacific Coast, Uvita, Puntarenas, Costa Rica (9°09'05.6" N, 83°44'14" W).
Habitat: Tropical, subaerial, forming black mat on a bamboo trunks in bamboo grove.
Etymology: L. *bambusa* = bamboo, *–cola* = dweller, the *Brasilonema* growing on bamboo.
Reference strain: CR06–5E/1.
Materials analyzed: CR06–5E/1, CR06–5E/2, CR06–5B/1, CR06–5A/2.
GenBank accession numbers: 16S rRNA and ITS: OR210229–232.

Taxonomic notes: This species is based upon four strains, all isolated from Uvita, Costa Rica. They consistently formed supported clades in the four loci, 16S rRNA gene, operon Bo ITS and operon Bt ITS phylogenies. In all cases internal dissimilarity of ITS regions was low (< 0.45% dissimilar), while other taxa had dissimilarities > 6.7% (Bt operon) or > 10% (Bo operon).

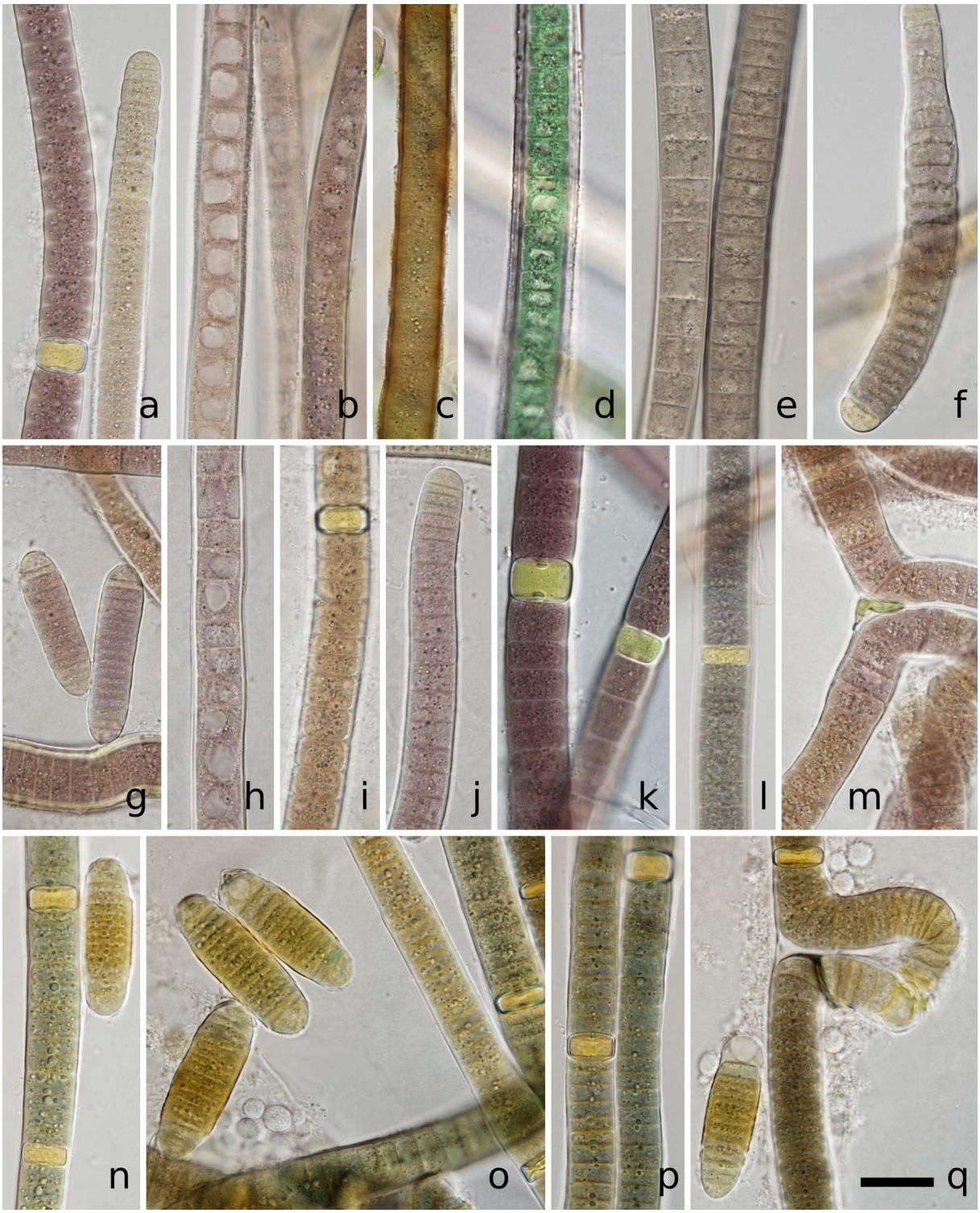

Fig. 9. (a–f) *Brasilonema bambusae*, (a–c) strain CR06–5F/1, (d–f) strain CR06–5A/1; (g–m) *Brasilonema bambusicola*, (g–i) strain CR06–5E/1, (j) strain CR06–5B/1, (k–m) strain CR06–5A/2; (n–q) *Brasilonema brancoi* strain RKST 3291. Scale bar 20 µm.

***Brasilonema brancoi* M. Bohunická et J.R. Johansen sp. nov. (Fig. 9 n–q, S14 A–F)**

Description: Thallus with short erect filaments, green–black or dark green, tuft–like spreading, slightly growing into the substrate. Filaments densely single or double false branched, 13–18 µm wide. Sheath colorless, yellow, orange or yellow–brown, attached to hyaline, irregularly widened. Trichomes slightly to distinctly constricted at crosswalls, crosswalls often yellow–brown, 10–15 µm wide. Cells cylindrical to disc–like, always shorter than wide, granulated, green, grey–green, olive–green or brown, rarely with vacuole–like structures, 3–5 µm long, shorter towards the ends of the trichomes. Apical cell hemispherical. Dark green concave necridia present.

Heterocytes cylindrical, yellow, 5–9 µm wide, 14–17 µm wide. Hormogonia formed at the ends of the filaments, typically 8–16 celled, with short cells and yellow to orange sheath, hyaline at both ends. ITS operon types recovered include Co, and two different Bt operons.

Holotype here designated: CBFS A139–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in March 2010 in San Gerardo de Rivas, San José, Cloudbridge Nature Reserve, Costa Rica (9°28'9" N, 83°36'16" W).
Habitat: Subaerial on a stone by the trail in tropical rain forest.
Etymology: Named in honor of prominent Brazilian cyanobacteriologist, Luis Henrique Zanini Branco.
Reference strain: RKST 3291.
Materials analyzed: Strain RKST 3291.
GenBank accession numbers: 16S rRNA and ITS: KU161679.

Taxonomic notes: This single strain does not cluster with any other strain in the 16S, four loci, no–tRNA operon or Bt operon ITS phylogenies. It has high 16S rRNA sequence similarity (> 99.0%) with most taxa in the *B. octagenarum* cluster, but with regards to the operon Co ITS sequence has ≥ 16 % dissimilarity with all strains possessing that operon. Among strains with operon Bt, its ITS sequence is consistently ≥ 6% dissimilar. The two Bt operons were distinctly dissimilar (4.2%), but these sequences were each determined as a consensus of several cloned sequences, so we are confident that the difference is real and not due to PCR error. This taxon was distinct in its morphology by having occasional orange coloration in crosswalls and abundant hormogonia production. The most distinctive feature was the morphology of hormogonia, which had hyaline ends but were brown in mid–hormognium (Fig. 9 n, o, q, S14 D–F). We did not see this particular morphological apomorphy in any other taxon.

***Brasilonema calcareum* M. Bohunická et J.R. Johansen sp. nov. (Fig. 10 a–d, S15 A–J)**
Description: Thallus grass–like, with short thick erect fascicles, sometimes creeping, evenly or unevenly spreading, growing into substrate, black, brown–black or black–green, light brown colored at the ends when older. Filaments short to long, rarely branched in young culture, short, rarely to densely false branched in older culture, 13–23 µm wide, sometimes slightly gradually narrowed towards the ends. Sheath thin, firm, attached, colorless or greyish, not structured or lamellate, becoming deep yellow, orange to brown when older. Trichomes mostly constricted at clearly visible crosswalls, often narrowed towards the ends, 8–20 µm wide. Cells cylindrical to disc–like, usually shorter than wide, rarely isodiametric or longer than wide (up to twice as long as wide), with vacuole–like structures, brown or purple, when young, also olive–green, grey–green or green when older, 2–17(20) µm long. Apical cells widely rounded or hemispherical, lightly tan colored. Heterocytes golden or light tan, mostly intercalary and cylindrical, but also terminal and widely rounded or hemispherical, sometimes in pairs, 7–20 µm wide, 3–14 µm long. Hormogonia short, cylindrical or tapered towards on end, often with terminal heterocytes, formed also by single false branching. Necridic cells present. ITS operon types recovered include Co and Bt.

Holotype here designated: CBFS A141–1!, dried material of the reference strain immobilized on a glass fiber filter.
Paratype here designated: CBFS A140–1!, dried material of strain CR12–6A/1 immobilized on a glass fiber filter.
Type locality: Collected in March 2010 in Barra Honda National Park, Guanacaste, Costa Rica (10°10'30.4" N, 85°22'19.5" W).
Habitat: This taxon includes three strains isolated from a limestone lookout building in the Barra Honda deciduous forest, Costa Rica.
Etymology: L. *calcareus* = limy, named for the species habitat, i.e. growing on limestone.
Reference strain: CR12–6B/1.
Materials analyzed: Strains CR12–6B/1, CR12–6A/1, and CR12–6A/2.
GenBank accession numbers: 16S rRNA and ITS: OR210240–243, OR228883.

Taxonomic notes: This species is based on three strains, CR12–6B/1, CR12–6A/1, and CR12–6A/2, all from a black mat growing on limestone in the Barra Honda deciduous forest in Costa Rica. All three strains occur together in supported nodes in the four loci, 16S rRNA gene, Bt and Co operon phylogenies. In the Bt operon comparison, *B. calcareum* had an ITS sequence that was ≥ 7.3% dissimilar to all other species having that operon. Internally, they have identical sequences in the Co operon ITS sequences. Possession of the Co is only shared with two other species, so in and of itself is fairly diagnostic and it differs notably from the other two species (≥ 16% dissimilar). Morphologically this species was somewhat distinctive because of the yellow to dark brown sheath and occurrence of bright dark green trichomes (Fig. S15 C–F, H–J).

***Brasilonema corcovadense* M. Bohunická et J.R. Johansen sp. nov. (Fig 10 e–g, S16 A–M)**
Description: Thallus with medium length to long, erect, grass–like filaments, soft, only weakly attached to the substrate or slightly growing into it, dark brown, black to olive–black. Filaments long, sometimes single or double false branched, at times repeatedly false branched, 14–23 µm wide. Sheath thin attached or thick, sometimes structured or lamellate, rarely also irregularly widened, hyaline to yellow–brown, orange, grey or brownish, up to 4 µm wide. Trichomes typically not constricted, sometimes constricted at crosswalls, crosswalls indistinct in young filaments, 10–27 µm wide. Cells shorter to longer than wide, cylindrical to disc–shaped, (coarsely)

Fig. 10. (a–d) *Brasilonema calcareum* strain CR12–6B/1; (e–g) *Brasilonema corcovadense* CR09–11F/1; (h–l) *Brasilonema elegans* strain CR11–11B/1. Scale bar 20 µm. Scale bar in (e) applies to all figures except (h).

granulated and vacuole–like structures especially when older, brown, purple–brown, violet, grey–brown, yellow–brown, olive–green, olive–brown or orange, 4–23 µm long, up to 2 µm wide at the ends. Apical cells widely rounded or hemispherical, light green, pale colored, yellow or tan. Heterocytes intercalary, cylindrical, yellow or yellow–green, sometimes granulated, 7–21 µm wide, 5–22 µm long. Hormogonia short, released from the ends of filaments, one to several cells, later when germinating with intercalary heterocyte or pair of heterocytes, slightly tapering, C–bent. Necridic cells concave, of dark color, lyophilizing to leave dark purple to black pigment between cells within the sheath. ITS operon types recovered include Bo and Bt.

Holotype here designated: CBFS A182–1!, dried material of the reference strain immobilized on a glass fiber filter.
Paratype here designated: CBFS A142–1!, dried material of strain BZ–HDL–007, immobilized on a glass fiber filter.
Type locality: Collected in March 2010 from Corcovado National Forest near La Leona Station, Costa Rica (8°26'52.08" N, 83°29'18.96" W).
Habitat: Tropical, terrestrial subaerial taxon, collected from Costa Rica and Belize.
Etymology: Named for the Corcovado National Park in Costa Rica, from which the species was first isolated.
Reference strain: CR09–11F/1.
Materials analyzed: Strains CR09–11F/1, CR09–11B/1, BZ–HDL–007.
GenBank accession numbers: 16S rRNA and ITS: OR210246–247, KY365502.

Taxonomic notes: This species forms distinct clusters of three strains, CR09–11F/1, CR09–11B/1 and BZ–HDL–007 in the four loci, 16S and operon Bo ITS phylogenies. In the Bt operon ITS tree it is embedded in the *B. fioreae* cluster (Fig. 6), and in both the 16S and four loci trees it is proximate to *B. incudis* (Figs 2, 3). BZ–HDL–007 is very distinct in its operon Bt ITS sequence, being ≥ 7.3% dissimilar in all comparisons among these sequences. Strains with the Bo operon have ITS regions that are ≥ 8.7% from all species having this operon. The Bt operon was not recovered in strains CR09–11F/1 and CR09–11B/1 and the Bo operon was not recovered from strain BZ–HDL–007, so an intraspecific comparison based on ITS sequences is not possible for this set of populations of this species. This species was highly variable in color, with violet to violet–brown coloration predominant. However, it was similar to many other species and morphologically cryptic.

***Brasilonema elegans* M. Bohunická et J.R. Johansen sp. nov. (Fig. 10 h–l, S17 A–H)**
Description: Thallus unevenly spreading in small subcolonies, with very short erect filaments, black, dark brown to green–black. Filaments short to long, (commonly) false branched, 13–24 µm wide. Sheath thin, attached, sometimes slightly widened, colorless, yellow to brownish. Trichomes cylindrical, (slightly) constricted or not constricted at crosswalls, crosswalls often orange or yellow, 11–22 µm wide. Cells cylindrical to disc–like, always shorter than wide, brown, grey–brown, olive–brown, olive–green, grey or bright blue–green, with interthylakoidal space in older trichomes, 2–11 µm long. Apical cells widely rounded or hemispherical, yellowish or of a lighter color that other cells of the trichome. Heterocytes abundant, intercalary, cylindrical or disc–shaped, golden, 12–24 µm wide, 3–12 µm long. Hormogonia with short cells released from the ends of filaments through trichome disintegration. Necridic cells present. ITS operon type recovered was only Co.

Holotype here designated: Fig. 10 h–l, images of CR11–11B/1, all taken on the same day from the same culture.
Type locality: Collected in March 2010 in Rincón de la Vieja, Guanacaste, Costa Rica (10°46'26.3" N, 85°20'60" W).
Habitat: Tropical, subaerial on rock in dry deciduous forest and epiphytic on a palm leaf in the Pacific shore of Costa Rica.
Etymology: L. *elegans* = elegant, named for its elegant appearance in the light microscope.
Reference strain: CR11–11B/1.
Materials analyzed: CR11–11B/1, CR06–4C/1.
GenBank accession numbers: 16S rRNA and ITS: OR210253 and OR228884.

Taxonomic notes: This species was unresolved in the *Octagenarum* group in the 16S rRNA tree (Fig. 3), but was in a supported clade with *B. brancoi* and *B. calcareum* in the no–tRNA ITS tree (Fig. 5). It was > 18% dissimilar to all other taxa in the ITS operon lacking tRNA genes, including *B. brancoi* and *B. calcareum.* It was found living in drier conditions than most *Brasilonema* species. It was one of only a few species which produced bright green cells (Fig. S17 C, G). The reference strain and additional strain were lost after morphological characterization and sequencing.

***Brasilonema epiphyllum* M. Bohunická et J.R. Johansen sp. nov. (Fig. 11 a–e, S18 A–I)**
Description: Thallus black to dark olive–green, evenly or unevenly spreading, with erect filaments. Filaments short to long, false branched, 12–18 µm wide. Sheath thin attached colorless, sometimes lamellate, to firm yellow, grey or brown. Trichomes cylindrical, not constricted to distinctly constricted at crosswalls, slightly gradually tapered towards the ends, 11–16 µm wide. Cells cylindrical to disc–like, shorter than wide, brown, grey–brown or olive–brown, light orange brown when old, with vacuole–like structures, 3–14 µm long. Apical cells of lighter color, rounded or hemispherical. Heterocytes common, intercalary, cylindrical, golden, 4–17 µm long, 14–16 µm wide. Hormogonia released by disintegration of the filament from its end, germinating typically into C–bent trichomes. ITS operon type recovered only includes Ao.

Holotype here designated: Fig. 11 a–e images of strain P772A, all taken on the same day from the same culture.
Type locality: Collected in August 2013 from a surface of a leaf in the tropical rainforest of El Yunque National Forest, Mina Falls trail, Puerto Rico (18°18'10.8" N, 65°46'29.5" W).
Habitat: Growing epiphytically on leaves in tropical conditions.
Etymology: L. *epiphyllus* = growing on leaves.
Reference strain: P772A.
Materials analyzed: Strain P772A.
GenBank accession numbers: 16S rRNA and ITS: OR210256.

Taxonomic notes: This single strain has an ITS region from operon Ao that is phylogenetically closest to *B. verawernerae* at ~ 5.6% dissimilarity. *B. incudis*, *B. bambusae,* and *B. palmarum* were also phylogenetically close but more dissimilar (5.8–7.2% dissimilar). All other species were considerably more distant. The 16S rRNA sequence percent similarity was high for all comparisons made in the *B. octagenarum* cluster (> 99.2%) indicating common ancestry with these species. In the 16S phylogeny it placed with *B. geniculatum*, but was separated from that species in the phylogeny of the ITS region of Ao operons (Fig. 5). It is morphologically cryptic with an appearance typical of many species.

***Brasilonema fatamorganum* M. Bohunická et J.R. Johansen sp. nov. (Fig. 11 f–i, S19 A–K)**

Description: Thallus velvet–like, with very short upright filaments, growing into substrate, black, brown, maroon, black–green, olive–green to orange when older, yellow–brown stain sometimes released into the substrate. Filaments often in fascicles, straight or bent, relatively short, densely single or double false branched, sometimes also long, easily disintegrating, sometimes gradually tapered towards the growing tips, abundance of very short germinating filaments of around 16 cells, tapered towards both ends, 14.5–24.1 µm wide. Sheath thin, attached, extending past trichome end, thicker near branching, hyaline or grey, up to 3.4 µm wide. Trichomes cylindrical, straight, always constricted at crosswalls, 10–22 µm wide. Cells shorter than wide, rarely up to isodiametric, or slightly longer than wide, cylindrical to disc–like, finely to coarsely granulated, often with larger granules confined to the center of the cytoplasm, light green, olive–green, blue–green, brown–green, dark brown, grey–green, purple–brown, or purple–black, 1.5–6–(17) µm long. Apical cells rounded or slightly conical, sometimes with calyptra, pale colored, sometimes hyaline. Heterocytes abundant, terminal or usually intercalary, single, in pairs or in rows up to 4 cells long, cylindrical, disc–like or hemispherical, often with one sap vesicle in the middle part, finely granulated, yellow–green or tan, 9–19.8 µm wide, 3–16 µm long. Hormogonia present in abundance. Necridic cells present. ITS operon types recovered include Bo and Bt.

Holotype here designated: CBFS A143–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in February 2007 from a tropical greenhouse Fata Morgana, Prague, Czech Republic (50°07'20.2" N, 014°24'49.8" E).
Habitat: Tropical greenhouses in temperate regions.
Etymology: Named for the type locality, the Fata Morgana Greenhouse. A Fata Morgana is a very complex superior mirage with more than three distorted erect and inverted images, and thus this species represents the taxonomic complexity and illusionary nature of *Brasilonema* specific taxonomy.
Reference strain: FM01–MK42.
Materials analyzed: Strains FM01–MK42, FM06–MK55, BLCC–T74, and BLCC–T71.
GenBank accession numbers: 16S rRNA and ITS: OR210257–260, MT396526, MT396522, and MT396511.

Taxonomic notes: This species is represented by four strains, FM06–MK55, FM01–MK42, BLCC–T74, and BLCC–T71 (both BLCC strains were originally identified as *B. octagenarum* by Barbosa et al. (2021)). They consistently cluster together in supported clades in the 16S, four loci, Bo ITS operon, and Bt operon ITS phylogenies. In both the Bo operon and Bt operon ITS sequences, all comparisons among these strains had < 1% dissimilarity, while they separated from all other species with the Bo ITS region by ≥ 10% dissimilarity. *B. hortense* was close in the Bt operon (3.7% dissimilarity), but phylogenetically distinct from that species in the 16S and four loci trees. This species is clearly defined by molecular characterization. Morphologically it is less clearly separated, but produces hormogonia that strongly taper, often produces heterocytes in pairs or even series of up to four, and has distinctive central granulation (Fig. 11G, S19 A–D, F–H, J, K).

***Brasilonema fioreae* D.E. Berthold et al. (Barbosa et al. 2021, 84, fig. 2) (Fig. 11 j–n, S20 A–I)**

Emended description: Thallus dense, formed by short erect fascicles, black, olive–black or forest green, sometimes to orange towards the ends of filaments. Filaments fasciculate, long to short, straight or bent, sometimes slightly tapering, false branched, 14–22.9 µm wide. Sheath thin, attached and hyaline, sometimes thickened at branching, to widened, structured and orange. Trichomes cylindrical, not constricted to constricted at crosswalls, crosswalls sometimes yellow or orange, 11–20.2 µm wide. Cells isodiametric to shorter than wide, mostly olive green, or light green to dark grey or orange, sometimes with irregular black pigmentation at some parts of the filaments (as immersed into ink), vacuole–like structures present, 3–10.1 µm long. Apical cells widely rounded, calyptra not present, of lighter color. Heterocytes intercalary, square and sometimes flattened, 12–18.3 µm wide, 5.3–16 µm long. Hormogonia motile, aerosolizing, released from the ends of the filaments, short, growing isopolar. ITS operon types recovered include Bo and Bt.

Voucher preparations: CBFS A144–1! and CBFS A145–1!, dried material of the strains LIS–MK92 and P608E, respectively, immobilized on a glass fiber filter.
Habitat: Subaerial, attached to walls, rock and other substrates of greenhouses, also on leaves of tropical plants in a tropical greenhouse in temperate climate locality (tropical greenhouse of the Botanical Garden in Liberec, Czech Republic). Subtropical to tropical distribution in natural conditions (Florida, USA, Puerto–Rico).
Reference strain: BLCC–T72.
Materials analyzed: Strains and sequences LIS1–MK92,

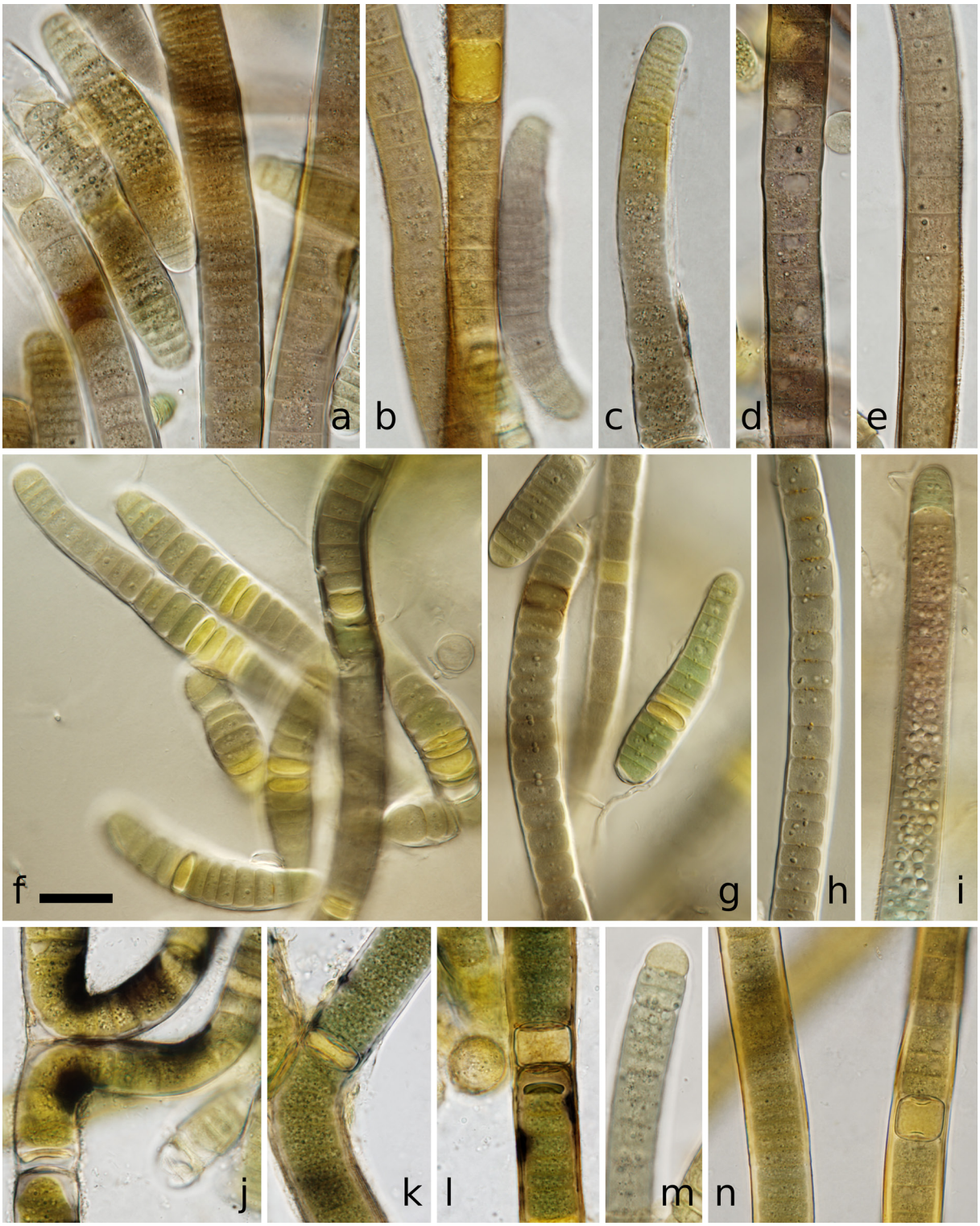

Fig. 11. (a–e) *Brasilonema epiphyllum* strain P722A; (f–i) *Brasilonema fatamorganum* strain FM06–MK55; (j–n) *Brasilonema fioreae* strain LIS1–MK92. Scale bar 20 µm.

P608E; sequences BLCC–T73 (MT396525, MT396514), BLCC–T83 (MT396524, MT396516), BLCC–T86 (MT396527, MT396513).

GenBank accession numbers: 16S rRNA and ITS: OR210261–265, MT396512–514, MT396516, MT396523–525, MT396527.

Taxonomic notes: One of our strains, P608E, was highly similar in 16S rRNA sequence (> 99%), and had low dissimilarity (< 1.5%) in both Bo operon and Bt operon ITS regions with strains previously identified in this species. It also grouped with the species in the 16S phylogeny, but in that tree *B. fioreae* was paraphyletic with

B. bambusae. One of our strains, LIS1–MK92, was in a well–supported node with *B. fioreae* in the no–tRNA ITS tree (Fig. 5) and had a PD in that ITS also < 1.5%, and we considered it to be *B. fioreae* during most of our study. However, LIS–MK92 is not in the *B. fioreae* clade in the 16S tree (Fig. 3), and is in a clade with both *B. fioreae* and *B. corcovadense* in the Bt ITS tree (Fig. 6). Finally, LIS–MK92 had a propensity to developing blackish pigmentation in the cytoplasm, a characteristic not seen in any other strains. We left this strain in *B. fioreae* with uncertainty, but felt it was not clearly separate enough to describe as a new species. *B. fioreae* is a well–established species represented by several strains isolated from Florida greenhouses (Barbosa et al. 2021).

***Brasilonema geniculatum* C.D. Villanueva et Casamatta (Villanueva et al. 2019, 9, fig. 9)**

Taxonomic notes: *B. geniculatum* was described from tombstones in a Jacksonville, Florida cemetery. We have not found additional populations of this taxon, which had morphology very similar to most other *Brasilonema* species, including purplish–brown coloration, slightly tapering filaments in fascicles, false branching, and highly vacuolated cells. It possesses both Ao and Bt operons. The authors considered it morphologically similar to *B. tolantongense* and phylogenetically close to *B. roberti–lamyi*. With our higher taxon sampling it remains phylogenetically distant from all other species, with the Bt operon ITS region ≥ 9% dissimilar and the Ao operon ITS region ≥ 14 % dissimilar to all other species with those operons.

***Brasilonema incudis* M. Bohunická et J.R. Johansen sp. nov. (Fig. 12 a–f, S21 A–I)**

Description: Thallus star–like spreading, with short erect filaments, with some filaments erect and some attached to the substrate, brown to black. Filaments very short to long, repeatedly false branched or long unbranched, 12–23 µm wide. Sheath thin, firm, attached, hyaline to grey or lamellate widened to yellow–brown. Trichomes slightly to distinctly constricted at crosswalls, slightly narrowed towards the ends, 10–20 µm wide. Cells cylindrical or disc–like compressed in older filaments, shorter than wide, with vacuole–like structures, sometimes with granulated content, violet, brown, olive–brown or olive–grey, light to deep colored, 2–14 µm long. Apical cells rounded, hemispherical to almost spherical, of a light color, often tan colored. Heterocytes intercalary, single or less often in pairs, cylindrical to concave, light yellow to golden, with smooth content, sometimes with sap vesicle, sometimes compressed in the trichome and wider than the trichome, 14–22 µm wide, 6–11 µm long. Hormogonia released by disintegration of the trichome, typically 1 to 4(8) celled. Necridic cells discoid, dark colored. ITS operon types recovered include Ao, and Ct.

Holotype here designated: CBFS A146–1!, dried material of the reference strain P801A immobilized on a glass fiber filter.

Paratypes here designated: CBFS A147–1! and CBFS A148–1!, dried material of the strains P834A and P715A, respectively, immobilized on glass fiber filters.

Type locality: Collected in August 2013 from the beginning of the Mt. Britton trail in El Yunque National Forest, Puerto Rico (18°17'55.2" N, 65°47'27.9" W).

Habitat: Tropical subaerial, covering firm surfaces in the shaded wet rainforest floor (rock, metal, asphalt road).

Etymology: L. *incudes* = anvil, named after El Yunque, which translates from the Spanish as the anvil.

Reference strain: P801A.

Materials analyzed: Strains P801A, P834A, and P715A.

GenBank accession numbers: 16S rRNA and ITS: OR210269–274.

Taxonomic notes: This species is comprised of three strains (P801A, P834A, P715A) isolated from El Yunque National Forest in Puerto Rico. It is phylogenetically associated with *B. corcovadense* in the four loci phylogeny, and has high 16S similarity to that taxon. However, *B. corcovadense* has a Bo ITS operon, while *B. incudis* has an Ao operon, which clearly separates those two species. Morphologically, this taxon can be distinguished by intense coiling and production of repeated false branches in series. This morphological character has only been seen in one other species, *B. burkei*, and the two are very separate phylogenetically.

***Brasilonema lichenoides* Villanueva, P. Hašler et Casamatta (Villanueva et al. 2018, 227, figs 1, 4)**

Taxonomic notes: This species is morphologically similar to other species in the genus. It was a lichen phycobiont, making it ecologically distinctive. The original authors placed it in a clade containing *B. octagenarum*, a result we confirm here with greater taxon sampling. Only the Ao operon was recovered during sequencing. The ITS of this operon lies on the dissimilarity threshold of *B. incudis* (≥ 3.1% dissimilar), but is phylogenetically separate from that taxon in both the 16S rRNA and no–tRNA ITS phylogenies (Figs 2, 5). This species is quite distinct in its habitat of origin, which was growing in association with lichens on gravestones. The only other taxon growing in association with lichens is *B. geniculatum*, growing on a gravestone in the same cemetery as *B. lichenoides*. However, the ITS of the Ao operon was very dissimilar in a comparison between these two species (> 15%).

***Brasilonema mombasense* M. Bohunická et J.R. Johansen sp. nov. (Fig. 12 g–j, S22 A–H)**

Description: Thallus grass–like with upright filaments, dark olive–green. Filaments densely false branched. Sheath hyaline to light orange, thin to widened and structured, lengthwise rugged or lamellate, sometimes widened at the ends of trichomes, up to 5 µm wide. Trichomes not constricted or constricted at crosswalls, 10–18 µm wide. Cells mostly cylindrical to disc like, isodiametric or shorter than wide, rarely longer than wide, granu-

Fig. 12. (a–f) *Brasilonema incudis*, (a) strain P801A, (b–e) strain P834A, (f) strain P715A; (g–j) *Brasilonema mombasense* strain KEN–MK50; (k–p) *Brasilonema muscicola* strain P811B. Scale bar 20 µm. Scale bar in (i) applies to all figures except (a) and (k).

lated, blue–green, olive green to greenish brown when old, 2.5–15(18) µm long. Apical cells widely rounded or hemispherical. Heterocytes intercalary, cylindrical, same size as vegetative cells, sometimes with one central sap vesicle, yellow, 10–18 µm wide, 5–8 µm long. Necridic cells bright blue–green to slate blue, abundant. Short hormogonia present. ITS operon types recovered include Bo, and Bt.

Holotype here designated: Fig. k–n images of strain KEN–MK50, all taken on the same day from the same culture.

Type locality: Collected in November 2011 from a bark of baobab tree in the vicinity of Mombasa, Kenya.
Habitat: Subaerial, growing on the bark of a baobab tree.
Etymology: Named for the city of Mombasa.
Reference strain: KEN–MK50.
Materials analyzed: Strains KEN–MK50 and KEN–MK53.
GenBank accession numbers: 16S rRNA and ITS: KY365506, OR210284–285.

Taxonomic notes: This species is comprised of two strains (KEN–MK50, KEN–MK53) isolated from the bark of a baobab tree in Kenya. It is a sister taxon of *B. lichenoides* based on the 16S rRNA gene tree, but differs from that taxon and all others with the Bo operon ITS sequence having dissimilarity ≥ 13%. The Bt operon ITS was also dissimilar (≥ 6.4%) to all other Bt operon ITS sequences. The D1–D1' helix of the Bt operon was unique among all such structures in *Brasilonema* due to its side helix on the 5' side mid–helix (Fig. S3 K). This effectively, clearly separates this species from all others in this study. Unfortunately, both cultures were lost during extended cultivation before manuscript preparation, making it technically impossible to prepare a physical type once the sequence data and analysis demonstrated the isolate represented a species new to science. This strain was unusual in its coloration. Most cells were blue–green to olive–green (Fig. S22 A–H), and violet coloration was not seen. We also did not observe vacuolization in the cytoplasm.

***Brasilonema muscicola* M. Bohunická et J.R. Johansen sp. nov. (Fig. 12 k–p, S23 A–H)**
Description: Thallus grass–like, with short erect filaments, evenly spreading, olive–black, growing into the substrate. Filaments short, single or double false branched, 11–20 µm wide. Sheath thin colorless or grey, attached. Trichomes not constricted to constricted at crosswalls, 9–19 µm wide. Cells cylindrical or disc–like, brown, violet–brown or dull blue–green, 2–10 µm long. Apical cells hemispherical, tan colored. Heterocytes terminal hemispherical or intercalary cylindrical, golden, 10–14 µm wide, 4–8 µm long. Hormogonia with terminal or intercalary heterocyte, isopolar or heteropolar, often C–bent. ITS operon types recovered include only the Bt operon.

Holotype here designated: CBFS A149–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in August 2013 from El Yunque National Forest, Puerto Rico (18°18'00.5" N, 65°47'04.76" W).
Habitat: Growing on mosses in tropical rain forest.
Etymology: L. *muscus* = moss, *–cola* = dweller; *muscicola* = growing on and among mosses.
Reference strain: P811B.
Materials analyzed: Strain P811B.
GenBank accession numbers: 16S rRNA and ITS: OR210286.

Taxonomic notes: This species was ≥ 8.6% dissimilar to the Bt operon ITS sequence in all possible comparisons of this taxon with other taxa containing the Bt operon. *B. muscicola* is morphologically similar to *B. bambusicola*. It was also phylogenetically close to *B. bambusicola* in both the 16S rRNA gene and Bt operon phylogenies, but is above the 7% dissimilarity threshold for ITS regions that are indicative of different species. This taxon was morphologically cryptic, with violet–brown coloration common but also brown and rarely blue–green. Vacuolization was not observed, and this may be an important feature in distinguishing the taxon. Growing among mosses in a tropical rainforest also seems undistinctive for the genus.

***Brasilonema palmarum* M. Bohunická et J.R. Johansen sp. nov. (Fig. 13 a–c, S24 A–I)**
Description: Thallus soft, with short, grass–like erect filaments, olive–black. Filaments long, rarely false branched. Trichomes not constricted to slightly constricted at crosswalls, 11–19 µm wide. Sheath thin attached, colorless to yellow–brown, widened up to 3 µm wide. Cells cylindrical, typically distinctly shorter than wide, up to isodiametric, brown, grey–purple, olive–green, grey–green, sometimes with vacuolization, 2–12 µm long, shorter at the end parts of the trichomes. Apical cells widely rounded, of lighter yellowish or greenish color. Heterocytes intercalary, regularly spaced, cylindrical rounded, with golden smooth content, 7–16 µm wide, 6–12 µm long. Hormogonia with short cells, usually grey–green. Necridic cells present. ITS operon types recovered include the Ao and the Bt operons.

Holotype here designated: CBFS A150–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in March 2010 in the Costa Ballena region on the Pacific Coast, Uvita, Puntarenas, Costa Rica (9°08'59.7" N, 83°44'07.3" W).
Habitat: Tropical, subaerial, on a palm leaf.
Etymology: L. *palma* = palm tree; gen. pl. *palmarum* = of palms.
Reference strain: CR06–4B/1.
Materials analyzed: Strain CR06–4B/1.
GenBank accession numbers: 16S rRNA and ITS: OR210292.

Taxonomic notes: This strain clustered with *B. verawernerae* FL15–MK94 in the operon Bt ITS phylogeny, but was 6% dissimilar in sequence in that operon, and 7% dissimilar in comparison with the ITS region from operon Ao. It clustered in the 16S phylogeny with *B. fioreae* strains, but was > 12% dissimilar in comparisons with both Bt operons and had the Ao operon as opposed to the Bo operon found in *B. fioreae*. The sheath was brown in midfilament with grey–green growing tips free of yellow color. The cells were uncharacteristically short, rarely reaching isodiametric, and this is a morphological feature that sets this species apart from others.

***Brasilonema roberti–lamyi* (Bourrelly) Sant'Anna et Komárek (Sant'Anna et al. 2011, 56, fig. 11).**
Basionym: *Tolypothrix roberti–lamii* Bourrelly (Bourrelly & Manguin 1952, 151, figs 78–84).
Reference strain: Los Manantiales (MX5) (Rodarte et al. 2014).
Materials analyzed: Strain MX5.
GenBank accession numbers: 16S rRNA and ITS: GQ443308.

Taxonomic notes: This species was described in Sant' Anna et al. (2013) but without the 16S rRNA or ITS sequence provided here (from operon Bo). The strain subsequently characterized molecularly (16S rRNA and cpcBA–IGS) was from Mexico (Rodarte et al. 2014), and we do not question its placement in this species. The Los Manantiales strain ITS was characterized by us subsequent to the work of Rodarte et al. (2014). Two strains of *B. elegans* consistently placed near *B. roberti–lamyi* in our 16S phylogeny, but had a different operon with no tRNA genes (Ao), and we consider this sufficient evidence to consider them to be separate species.

This species appears in the phycological record with two different spellings for the species epithet: *roberti–lamyi* and *roberti–lamii*. Bourelly gave the species name as *Tolypothrix Roberti–lamii*. It is named for Robert Lamy, and according to the ICN Art. 60.8, "y" is to be treated as a consonant, so the orthography should be corrected to *Brasilonema roberti–lamyi*, the spelling used in Sant'Anna et al. (2011).

***Brasilonema verawernerae* M. Bohunická et J.R. Johansen sp. nov. (Fig. 13 d–k, S25 A–I)**
Description: Thallus with short erect filaments, black or blackish–green. Filaments false branched, 12–20 µm wide. Sheath thin, attached or widened hyaline, colorless, greyish, yellowish to brown, up to 4 µm wide. Trichomes not constricted or constricted at crosswalls, crosswalls often yellowish, 11–19 µm wide. Cells cylindrical to disc–like, usually shorter than wide, sometimes isodiametric or longer than wide, finely granulated, brown, olive green, grey, 4–10(28) µm long. Apical cells hemispherical or widely rounded, yellowish. Heterocytes yellow, cylindrical, 4–12 µm long, 12–17 µm wide. Hormogonia short, formed by disintegration of filament. Necridic cells present, lyophilzing to leave black pigment between cells within the sheath, with black pigment also occurring between fragmented trichomes in sheaths not apparently associated with necridia. ITS operon types recovered include the Ao and two different Bt operons. Holotype here designated: CBFS A151–1!, dried material of the reference strain immobilized on a glass fiber filter.

Type locality: Collected in August 2013 in Kirby Storter Roadside Park, Big Cypress Natural Preserve, Florida, USA (25°52'03.5" N, 81°09'04.4" W).
Habitat: Subaerial on wet wood in subtropical climate.
Etymology: Named in honor of Brazilian phycologist Vera Regina Werner.
Reference strain: FL15–MK94.
Materials analyzed: strain FL15–MK94.
GenBank accession numbers: 16S rRNA and ITS: OR210301–303.

Taxonomic notes: This strain was unusual because it had two Bt operons and they were quite dissimilar (11.3%). The two operons were based on a number of clones each and represent robust consensus sequences. The strain clustered with different species in different analyses, and these are the taxa from which it must be excluded. It was in a weakly supported clade with *B. bambusicola* in the Bt operon ITS tree, but was dissimilar from this species (11.5%). It was proximal to *B. epiphyllum* in the no–tRNA ITS operon phylogeny, but also dissimilar (5.6%) to that taxon. While having relatively low dissimilarity in the Ao ITS to *B. incudis* (3.8%), none of the phylogenies show it as being phylogenetically close to that taxon. Consequently, despite the diverse phylogenetic analyses, it can be concluded based upon dissimilarity of three ITS regions to all other strains that FL15–MK94 is a separate, distinct lineage worthy of taxonomic recognition. The cells were characteristically gray to gray–green, lacking violet coloration.

***Bromeliae* group**
Defined as the group of *Brasilonema* species occupying the clade within the genus that contains *B. bromeliae.* Further defined by the common presence of At and Ao type operons, and the absence of Ct operons.
This group currently contains *B. bromeliae, B. angustatum, B. burkei, B. calidum, B. cataractarum, B. cubense, B. elongatum, B. hortense, B. kauaiense, B. komarekii, B. lyngbyoides, B. mata–atlanticum, B. ohuense, B. santannae, B. stuposum, B. terrestre, B. tolantongense, B. villosum, B. xilitlae* (Fig. 3).

***Brasilonema bromeliae* Fiore et al. (Fiore et al. 2007, 796–797, figs 1–5) (Fig. 13 l–o, S26 A–M)**
Updated description: Thallus with long, soft, erect fascicles, star–like or unevenly spreading, grass–like, growing into the substrate, dark olive–green, black–green, grey–green, blue–green when old. Filaments short to long, often to rarely single or double false branched, sometimes three branches germinating from one branching point, 14–38 µm wide, sometimes tapered towards the ends. Sheath thin, attached, colorless, grey hyaline or widened and lamellate or structured orange–brown. Trichomes not constricted or constricted at crosswalls, crosswalls sometimes orange, 11–30 µm wide, sometimes narrowing towards the ends. Cells shorter than wide and disc–like when young, length 1/3 of the width, often in "pairs" – each second crosswall being more deeply constricted, older cells cylindrical, isodiametric or slightly longer than wide, only rarely 1.5 times longer than wide,

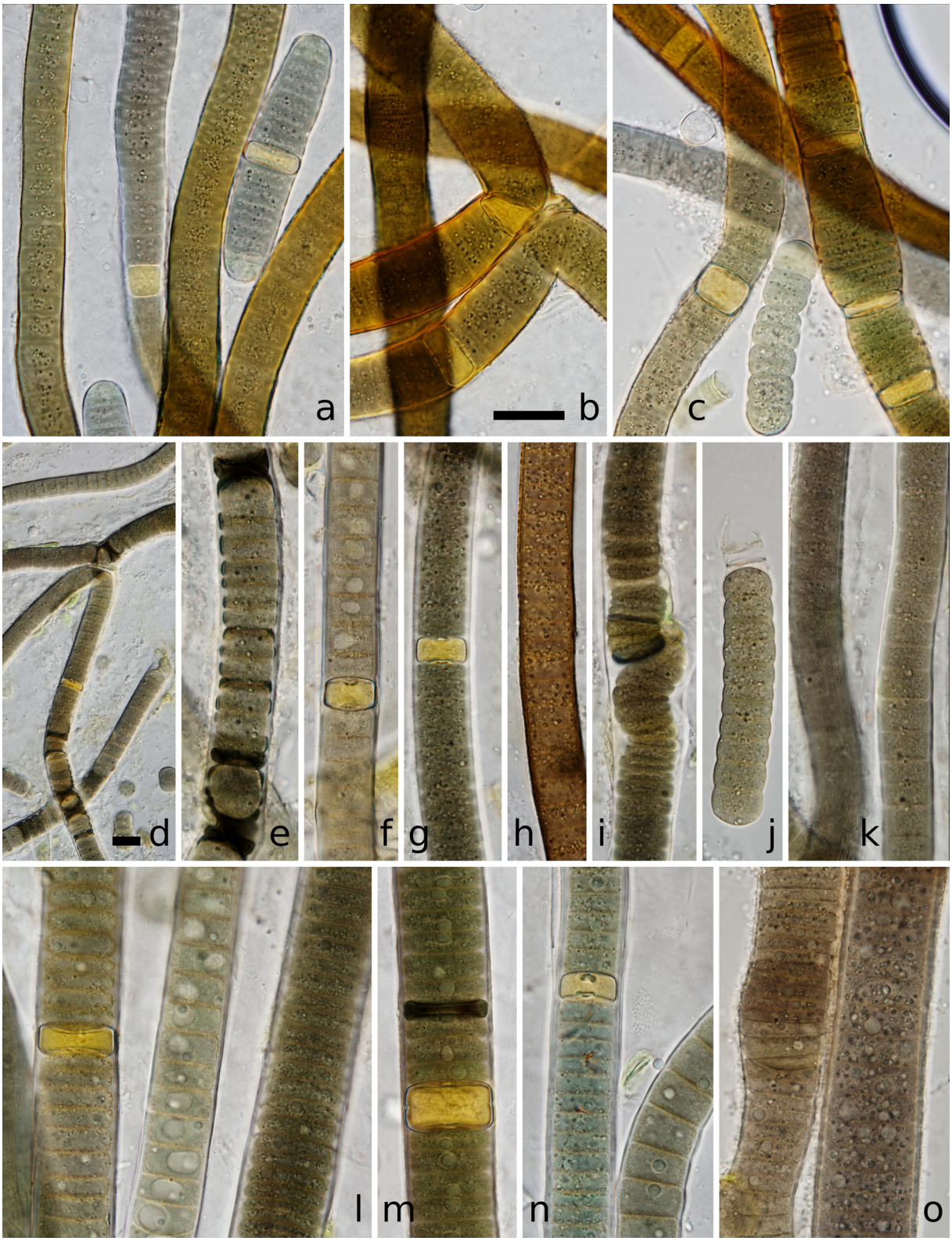

Fig. 13. (a–c) *Brasilonema palmarum* strain CR06–6B/1; (d–k) *Brasilonema verawernerae* strain FL15–MK94; (l–o) *Brasilonema bromeliae*, (l–n) strain RKST 321, (o) strain P117A. Scale bar 20 µm. Scale bar in (b) applies to all figures except (d).

3.5–14 µm long. Cell content typically violet or olive–green, or brown in healthy population, but also grey, grey–violet, olive–green to orange–brown, finely granulated, with spherical bodies, sometimes with vacuole–like structures. Apical cells widely rounded or hemispherical, tan colored, terminal meristematic zones Hassallia–like. Heterocytes intercalary cylindrical or almost spherical, single or

in pairs, tan, yellow, grey–yellow, yellow–green or golden, 13–26 µm wide, 5–25 µm long. Hormogonia released by filament disintegration, also in the middle of the filament, short, constricted at crosswalls, without sheath, with vacuole–like structures, often with intercalary heterocyte, germinating to one or both ends, meristematic zones with short cells, slightly narrowed towards growing tips, often bent, of pale olive–grey color. Necridic cells or rows of necridic cells of dark color present, with dark pigment also occurring between fragmented trichomes in sheaths not apparently associated with necridia. ITS operon types recovered include an Ao operon and a single Bt operon.

Voucher preparations: CBFS A151–1! and CBFS A152–1!, dried material of strains RKST 321 and SAG 39.90, respectively, immobilized on a glass fiber filter.
Habitat: In bromeliad cups in a tropical greenhouse in Brazil, but also found in nature in tropical climates growing on rock substrates in Puerto Rico, Costa Rica, and Nepal.
Reference strain: SPC951.
Materials analyzed: Strains and sequences RKST 321, SAG 39.90, P117A; sequences SPC951.
GenBank accession numbers: 16S rRNA and ITS: DQ486055, OR210236–239; WGS: NZ_QMEB00000000.

Taxonomic notes: Originally based on a strain isolated from bromeliad cups in a greenhouse in São Paulo, Brazil (strain SPC951), we are adding three strains to this species cluster. This is the type species of *Brasilonema*, but no ITS region was reported for the original reference strain, SPC951. We now have 16S rRNA gene sequence for four strains, SPC951, RKST 321, P117A, and SAG 39.90, with three strains having the ITS from the Ao, operon, and RKST 321 additionally having the ITS sequence from the Bt operon. *B. bromeliae* has a 16S rRNA sequence similarity above 98.7 % with *B. cataractarum, B. mata–atlanticum, B. santannae, B. lyngbyoides* and *B. sennae,* but forms a distinct clade only with the latter two species (Fig. 3). The phylogeny from the no–tRNA operon sequence, groups *B. bromeliae* into a monophyletic supported clade, with *B. villosum* M31–F20B II, *B. calidum* TH04–Ema, and *B. lyngbyoides* SAG 40.90 being proximate strains (Fig. 5). *B. calidum* and *B. lyngbyoides* strains are unambiguously different based on percent dissimilarity in the ITS sequence (≥ 8.4%). *B. villosum* M31–F20B II is more closely related to *B. bromeliae*, ranging from 4.1–7.2% dissimilarity. However, we consider this ITS dissimilarity sufficient difference to assume that it does not belong in the *B. bromeliae* clade, and this is further supported by our similarity and phylogenetic ananlyses of the 16S rRNA gene. We illustrate our strains of this species. They conform to the original description of the species.

***Brasilonema angustatum* M.A. Vaccarino et J.R. Johansen (Vaccarino & Johansen 2012, 1180–1181, figs 1b, 2, 3)**
Voucher preparation: CBFS A154–1!, dried material of the original reference strain HA4187–MV1 immobilized on a glass fiber filter.
GenBank accession numbers: 16S rRNA and ITS: HQ847566–567; WGS: JAHHGV000000000.

Taxonomic notes: This species was based on the morphological apomorphy at that time of tapering trichomes, a morphological character that has since been observed in a number of other species. Vaccarino & Johansen (2012) reported on ITS secondary structures and illustrated D1–D1′ helices for both the Ao and At ribosomal operons. It occupies a position just basal to the *Octagenarum* group in the 16S rRNA tree (Fig. 3) and is an isolated branch proximal to *B. octagenarum* in the no–tRNA ITS tree (Fig. 5). A whole genome shotgun (WGS) sequence was recently obtained for the reference strain, HA4187–MV1, but unfortunately only a single ribosomal sequence was reported (Ward et al. 2021). It is a common failing of WGS that ribosomal genes are not fully recovered. Its D1–D1' helices (Ao operon, Fig. S2 O, At operon Fig. S3 A) are unique in structure among all D1–D1' helices of *Brasilonema* species.

***Brasilonema burkei* Miscoe, Pietrasiak et J.R. Johansen nom. inval. (Miscoe et al. 2016, 88, figs 40–47)**
Miscoe et al. (2016) did not correctly designate the holotype for this species, listing the voucher specimen accession in the Bishop Museum that was meant to be the holotype, but not specifically saying "typus" or "holotypus", or its abbreviation, or its equivalent in a modern language as required by ICN Art. 40.6 (Turland et al. 2018). We here correct this error by validating this species below.

***Brasilonema burkei* Miscoe, Pietrasiak et J.R. Johansen sp. nov.**
Replaced binary designation: "*Brasilonema burkei* Miscoe, Pietrasiak et J.R. Johansen" in Miscoe et al. *Bibliotheca Phycologica* 120: 88, figs 40–47, 2016, nom. inval.

Description and illustrations: Miscoe et al. (2016: 88, figs 40–47).

Holotype: BISH 755080, deposited in the Bishop Museum, Honolulu, Oahu, Hawaii, USA. Representative illustrations in Miscoe et al. (2016: figs 40–47).
Type locality: Damp wet rock walls near the entrance of Maniniholo Cave, Kauai, Hawaii, USA, 22°12.183' N, 159°33.933' W, collected 28 July 2009 by J.R. Johansen, R.L. Lowe, A.R. Sherwood, and M.A. Vaccarino.
Etymology: Named in honor of David J. Burke, a well–

respected soil microbiologist and molecular ecologist.
Reference strain: HA4348–LM4, isolated by L.H. Miscoe.
GenBank accession numbers: 16S rRNA and ITS: KU161663–665.

Taxonomic notes: This species from a cave on Kauai, Hawaiian Islands, is distinct from all species based on its 16S rRNA genetic identity (≥ 98.3%), and also has a distinctive Bt ITS operon (≥ 24% dissimilarity) which causes it to occupy a long branch near the base of the phylogeny (Fig. 6). It is also morphologically distinct, producing extensively coiled filaments which give rise to series of loops leading to serial geminate false branching (see Miscoe et al. 2016, figs 40, 45–47). It also has pronounced tapering from widened central portions of the filaments. It is possibly the most morphologically distinct species of *Brasilonema* described to date.

***Brasilonema calidum* M. Bohunická et J.R. Johansen sp. nov. (Fig. 14 a–f, S27 A–J)**
Description: Thallus with soft, erect fascicles, growing into substrate, unevenly spreading, black–green. Filaments single or double false branched, 15–22 µm wide. Sheath thin, attached, hyaline, colorless or greyish. Trichomes not constricted or constricted at crosswalls, 14–20 µm wide. Cells cylindrical or disc–like, typically finely granulated/structured, brown in healthy population, also blue–green, green, olive–green, or olive–brown, vacuole–like structures present, 2–11 µm long. Heterocytes cylindrical intercalary or hemispherical terminal, yellow, yellow–green or green, 5–13 µm long, 15–17 µm wide. Apical cells hemispherical, yellowish. Necridic cells of dark color present. Hormogonia short, isopolar or heteropolar, often with intercalary heterocyte, germinating to both ends, meristematic zones with short cells, slightly narrowed towards growing tips, often bent. ITS operon types recovered include an Ao operon and an At operon.

Holotype here designated: CBFS A155–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in October 2010 from Ema Dump in Ostrava, Czech Republic (49°50'23.4" N, 18°18'52.8" E).
Habitat: Strain was isolated from moist soil in artificial habitat of burning coal tailings dump in temperate climate.
Etymology: L. *calidus* = warm, hot. Named for the habitat of the type locality, which has been continually warmed for many years by coal burning deep below the surface of the site.
Reference strain: TH04–Ema.
Materials analyzed: Strain TH04–Ema.
GenBank accession numbers: 16S rRNA and ITS: KY365511–512.

Taxonomic notes: This strain has high genetic identity (> 99%) with *B. epiphyllum*, *B. santannae* BLCC–T64, *B. roberti–lamyi, B. villosum*, and several strains of *B. tolantongense*. However, according to the 16S rRNA phylogeny, this strain stands separated from all of those strains. In the phylogeny based on four loci it is in a poorly supported clade containing two strains of *B. villosum* (M29–F12 and M31–F20B–II) and two strains identified as *B. bromeliae* (SAG 39.90 and P117A). Based on 16S similarity, three of these strains are < 98.7% identical, evidence that they are separate lineages representing different species. *B. villosum* M31–F20B–II is > 99% similar to *B. calidum* TH04–Ema, and so evidence for separation from the strain is needed. This is provided by the analysis of the ITS region in operon Ao, where they are phylogenetically separate in both the MP and BI analyses, and have dissimilarity > 7.0%, further evidence that they do not represent the same lineage. The species is unusual in its habitat. Few *Brasilonema* species grow directly on soil, and the Ema dump is a site exposed to temperate climate with snowy winters. The warming effect of the burning coal is similar to warming that might be experienced in the vicinity of geothermal springs, but so far *Brasilonema* has not been observed in such habitats. Morphologically cryptic.

***Brasilonema cataractarum* M. Bohunická et J.R. Johansen sp. nov. (Fig. 14 g–l, S28 A–K).**
Description: Thallus with short erect filaments, spreading in all directions, black, brown or olive green when old. Filaments isopolar, double or single false branched. Sheath colorless, thin, attached. Trichomes slightly to distinctly constricted at crosswalls, 18–30(40) µm wide. Cells always shorter than wide, cylindrical to disc–like, finely or coarsely granulated, blue–green, olive green, brown, purple, violet or grey, often with vacuole–like interthylakoidal space, 2.5–15 µm long. Apical cells widely rounded, pale colored, often with a thickened apical cap of refractive mucilage. Heterocytes usually intercalary, single or rarely in pairs, cylindrical, hemispherical or irregularly compressed, yellow or yellow–green colored, finely granulated, often with one (rarely more) sap vesicle in the middle part, 18–25 µm wide, 5–15 µm long, sometimes narrower than the trichome. Necridic and irregular cells commonly present. Hormogonia rounded, 1–8 celled, released off the end of the filaments or by the filament fragmentation. ITS operon types recovered include a Co operon and a Bt operon.

Holotype here designated: CBFS A156–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in 2008 from a wet stone in waterfall in Ohu, lowland tropical rain forest, Madang Province, Momase Region, Papua New Guinea (5°13'58.3" S, 145°40'44.5" E).
Etymology: L. *cataracta* = waterfall; gen. pl. *–arum*; *cataractarum* = of the waterfalls.
Habitat: Tropical subaerial, from a waterfall in lowland tropical rain forest.
Reference strain: JK04–144.
Materials analyzed: Strain JK04–144.

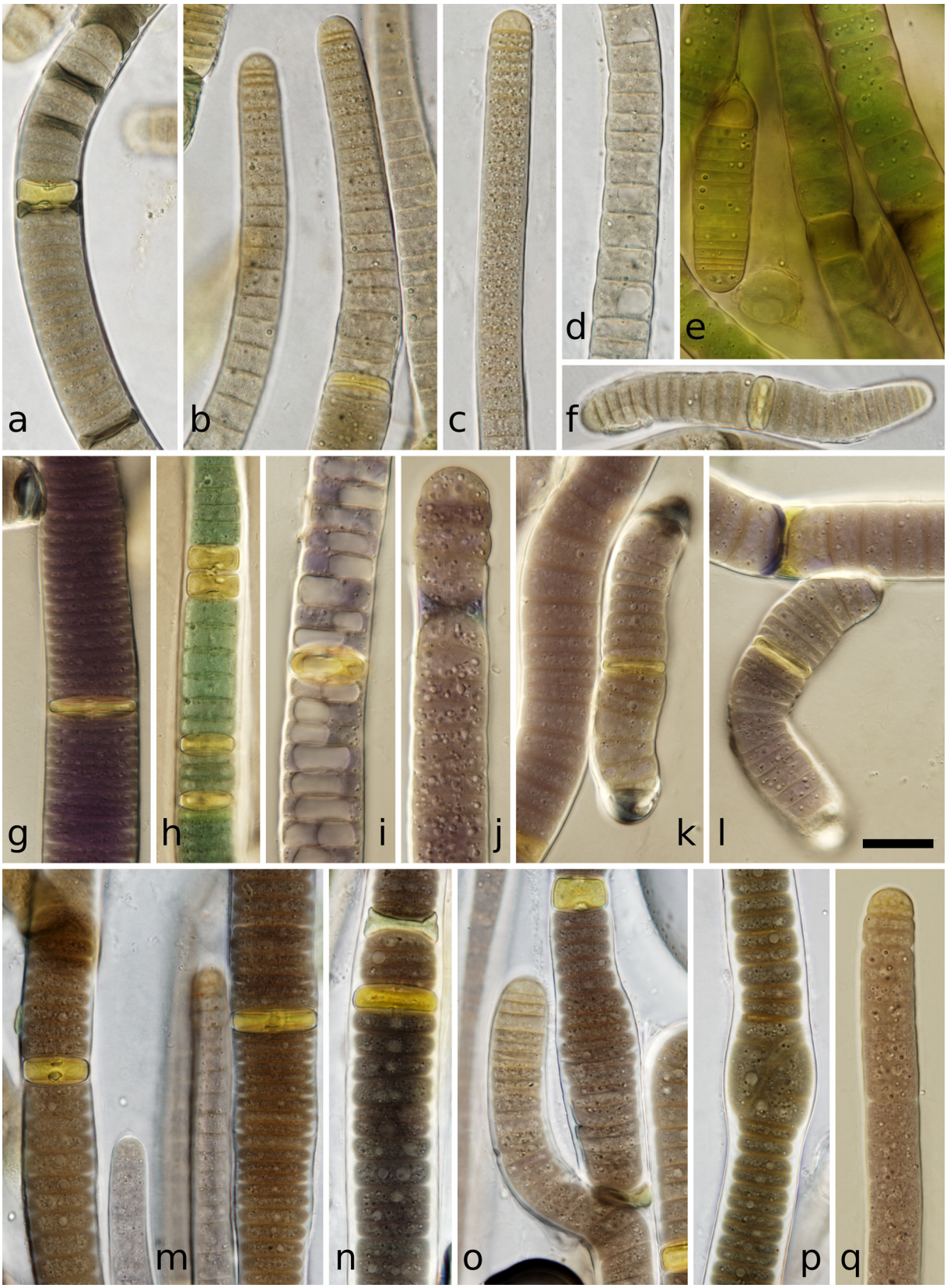

Fig. 14. (a–f) *Brasilonema calidum* strain TH04–Ema; (g–l) *Brasilonema cataractarum* strain JK04–144; (m–q) *Brasilonema cubense* strain TH06–T13. Scale bar 20 µm.

GenBank accession numbers: 16S rRNA and ITS: OR210244–245.
Taxonomic notes: This species is close to *B. fioreae* P608A in the four loci phylogeny, and the 16S rRNA genetic identity between the strain pair is high (98.9%). However, *B. fioreae* has a Bo operon whereas *B. cataractarum* has a Co operon. Furthermore, the Co operon is > 17% dissimilar in all comparisons with this ITS region, and *B. cataractarum* is > 12% dissimilar to all studied strains in the ITS region of operon Bt. Based on molecular data, it is clearly separated from all studied strains. Although the thinner filaments overlap size ranges of other species, this species produced some of the widest cells seen in this study (up to 40 µm wide). This taxon also produced numerous heterocytes, often appearing equidistantly placed in long trichomes. The refractive apical caps were also a very distinctive feature of this species (Fig. 14 k, l, S28 A–E, I, K).

***Brasilonema cubense* M. Bohunická et J.R. Johansen sp. nov. (Fig. 14 m–q, S29 A–L)**
Description: Thallus with medium length erect filaments, unevenly spreading, intensely branching, growing into the substrate, black or dark green. Filaments long, isopolar, single or double false branched, 15–26(30) µm wide. Sheath thin, attached, colorless or widened hyaline greyish or yellowish, sometimes up to 3 µm thick, lamellate. Trichomes not constricted or slightly constricted at crosswalls, crosswalls clearly visible, 13–24(29) µm wide. Cells shorter than wide, cylindrical, sometimes disc–like when older, granulated, typically brown, but also brown–green, olive–green, grey, purple–brown or green, 2–10 µm long, a row up to 8 cells in the trichome end pale colored, distinctly constricted off the rest of the trichome. Apical cells hemispherical or widely rounded, yellowish to achlorophyllous, sometimes with a mucilaginous apical cap. Heterocytes intercalary, cylindrical or rounded, golden or yellow–green, (13)15–24(27) µm wide, 5–19(27) µm long. Hormogonia short, formed by disintegration of filament, germinating to both ends, forming C–bent young filaments. Necridic cells present, lyophilizing to leave dark pigment between cells within the sheath. ITS operon types recovered include an Ao operon and two different Bt operons.

Holotype here designated: CBFS A159–1!, dried material of the reference strain immobilized on a glass fiber filter.
Paratypes here designated: CBFS A157–1! and CBFS A158–1!, dried material of strains TH06–13 and TH05–T14, respectively, immobilized on glass fiber filters.
Type locality: Collected in January 2004 from the bank of the stream La Pastora 2 km southwest of the village Cayajabos, Cuba (22°50'40.6" N 82°52'43.6" W).
Habitat: Terrestrial tropical, subaerial on concrete in stable conditions (high temperature and humidity) of a cooling tower of power plant in temperate zone. This species is represented by three strains, two from a concrete cooling tower in Ostrava, Czech Republic, and one from soil on the bank of a stream named La Pastora near Cayajabos, Cuba.
Reference strain: JOH13.
Materials analyzed: Strains JOH13, TH05–T14, and TH06–13.
Etymology: Named for Cuba, the country of origin for the reference strain.
GenBank accession numbers: 16S rRNA and ITS: OR210248–253.

Taxonomic notes: This species is based on three strains, JOH13, TH06–T13, and TH05–T14. All molecular evidence supports their inclusion in this species; 16S rRNA, four loci, and phylogenies all place them in supported clades. The three strains are highly similar in 16S sequence, but distant from all other strains reported in this manuscript. The no–tRNA operon ITS is < 1.5% dissimilar within the species, whereas the species is > 24% dissimilar to all other taxa for which this operon was obtained. The Bt operon ITS sequences were even more similar among species (~ 0.6% dissimilar), but had dissimilarity ≥ 14% in all other species comparisons. The formation of a series of several achlorophyllous apical cells was distinctive (Fig. S29 C, K), but otherwise the species is morphologically cryptic.

***Brasilonema elongatum* M. Bohunická et J.R. Johansen sp. nov. (Fig. 15 a–h, S30 A–N)**
Description: Thallus blackish blue–green to blue–green with orange tips when older, grass–like, spreading into all directions, with very long erect soft filaments. Filaments isopolar, long, false branched, tapered towards the growing ends. Sheath colorless, grey or yellow, sometimes transversely structured/ribbed or lamellate, up to 5 µm thick. Trichomes constricted or not constricted at crosswalls, 11–18 µm wide. Cells of various colors and shapes, cylindrical to disc–shaped, blue–green, green, olive–green, grey or grey–violet, with granulated content, isodiametric or shorter or longer than wide, rarely especially elongated, (3)–4–17 µm long. Apical cells widely rounded, hyaline structures at the ends observed. Heterocytes intercalary, yellow–green, usually with one sap vesicle in the middle part, cylindrical, 12–20 µm wide, (4)10–23 µm long. Hormogonia short, present. Necridic cells or rows of necridic cells together with deformed living cells present, lyophilizing to leave dark pigment between cells within the sheath. ITS operon types recovered include an Ao operon and a Bt operon.

Holotype here designated: CBFS A160–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in February 2007 from a tropical greenhouse Fata Morgana of Prague Botanical Garden, Czech Republic (50°07'20.2" N, 14°24'49.8" E).
Habitat: Subaerial, in artificially humid and warm conditions of tropical greenhouse.
Etymology: L. *elongatus* = elongated, named for its ability to produce especially elongated cells.

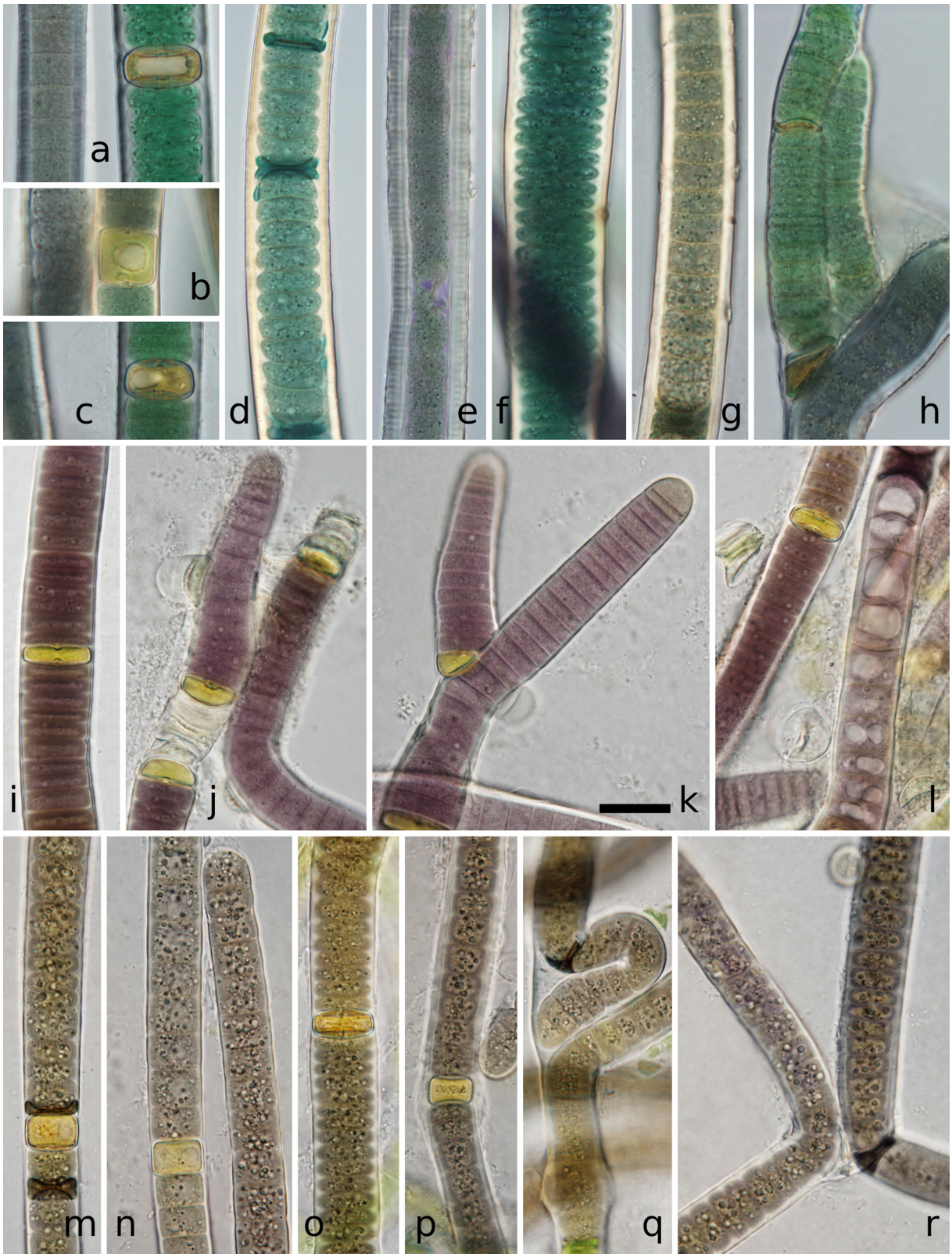

Fig. 15. (a–h) *Brasilonema elongatum* strain FM05–MK46; (i–l) *Brasilonema hortense* strain SAG 67.81; (m–r) *Brasilonema kauaiense* strain HA4401–MV4a. Scale bar 20 µm.

Reference strain: FM05–MK48.
Materials analyzed: Strain FM05–MK48.
GenBank accession numbers: 16S rRNA and ITS: OR210254–255.

Taxonomic notes: In the light microscope this species seems like a mixture of multiple taxa, but the macroscopic appearance is compact and gradual transitions between different filament types and colors of both

sheath and cells can be observed. The sheath can be structured (Fig. S30 F), and the unusually long cells are a distinct morphological feature. This species is similar in its 16S rRNA sequence and Bt operon ITS sequence to *B. ohuense*, and has very similar secondary structures for both the Box–B and V3 helices. However, it differs from *B. ohuense* based on the marked dissimilarity in the Ao operon sequence. In *B. ohuense* the D1–D1' helix is excessively long and has a branched secondary structure, such that it could not even be aligned with other operons and is excluded from the no–tRNA phylogeny (Fig. 5). *B. elongatum* is also similar in in its V3 helix to *B. xilitlae* (MX3) (Fig. S9 P, R). However, it differs from that taxon in that *B. elongatum* has an Ao operon, while *B. xilitlae* has a Bo operon. *B. elongatum* has a D1–D1' helix in the Ao operon that is distinct from all other D1–D1' helices in its possession of a short side helix on the 5' side of the helix (Fig. S1 P).

***Brasilonema hortense* M. Bohunická et J.R. Johansen sp. nov. (Fig. 15 i–l, S31 A–L)**

Description: Thallus with short erect or irregular creeping fascicles, brown–black, greenish–brown or blackish–green, somewhere in the old parts yellowish–brown. Filaments relatively short to long, straight or variously curved, often single or double false branched, single false branching with basal heterocyte, 11.4–25 µm wide. Sheath thin, attached, sometimes widened (in old filaments), (slightly) lamellate, hyaline, sometimes greyish or yellowish. Trichomes cylindrical, constricted or not constricted at crosswalls, sometimes slightly narrowed towards the growing tips, *Oscillatoria*–like cell division, (7.3)9–22(23.1) µm wide. Cells shorter than wide in apical parts and meristematic zones, to almost isodiametric in older parts, vacuole–like structures sometimes present, mostly grayish–violet or purple–brown, but can be olive–green, brown, olive–brown, blue–green, light emerald green, or even greenish yellow, (2)5–10 µm long. Heterocytes both terminal and intercalary, cylindrical or hemispherical, yellow, 9.3–20 µm wide, 5–12.5 µm long. Hormogonia common, often heteropolar, short–cylindrical or elongated–cylindrical, sometimes curved, sometimes narrowed at growing tips, with short granular cells, 9–13 µm wide, 23–66 µm long. Hormocytes ± spherical, 8.4–12.5 µm in diameter. Necridic cells present. ITS operon types recovered include Bo, Bt and Ct.

Holotype here designated: CBFS A161–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in 1980 from a bench with nutrient solution in greenhouse of Old Botanical Garden of Göttingen University (51°32'16.11" N, 9°56'09.41" E).
Habitat: Subaerial or epiphytic (on aerial roots of epiphytic and aerophytic orchids and bromeliads) in artificially warm and humid conditions of greenhouses in temperate climatic zone.
Etymology: L. *hortensis* = pertaining to gardens.
Reference strain: SAG 67.81.
Materials analyzed: strain SAG 67.81.
GenBank accession numbers: 16S rRNA and ITS: OR210266–268.

Taxonomic notes: *B. hortense* is grouped with *B. elongatum, B. ohuense* and *B. xilitlae* in a distinct clade sister to the node containing all other *Brasilonema* species in the 16S rRNA tree (Fig. 3). While *B. elongatum, B. ohuense,* and *B. xilitlae* occupy a supported node in the Bt ITS operon tree (Fig. 6), *B. hortense* is in a node with *B. fatamorganum* in the 16S tree (Fig. 3). It has a longer D1–D1' helix in the Bt operon than all other taxa except *B. mombasense* (Fig. S4 F). It has 16S rRNA PS < 98.7% for all species except *B. elongatum* and *B. ohuense.* However, its Bo ITS operon places it in a node distant from the above three species with *B. octagenarum* Br–Kyiv–2. We considered placing this latter strain in *B. hortense*, but Br–Kyiv–2 16S rRNA sequence places it definitively in the *B. octagenarum* clade (Fig. 2), even though its Bo ITS is identical to *B. hortense* SAG 67.81! We are unable to explain this strange discontinuity, and for now leave *B. octagenarum* Br–Kyiv–2 where it was placed by the researchers who originally sequenced it. This species is consistently violet in color, is vacuolated, has discoid cells, and has false branching, and thus shows features similar to a number of other taxa; morphologically cryptic.

***Brasilonema kauaiense* M. Bohunická et J.R. Johansen sp. nov. (Fig. 15 m–r, S32 A–I)**

Description: Thallus with long erect fascicles, black when young, green–black when old, tips of the filaments of lighter color in old culture. Filaments long, rarely to commonly false branched, 15–20 µm wide. Sheath thin, colorless, attached, or lamellate widened, sometimes greyish or brown. Trichomes not constricted, slightly or distinctly constricted at crosswalls, 7–17(20) µm wide. Cells intensely granular, cylindrical, barrel–shaped or disc–like, shorter than wide, brown, brown–olive, olive–green or grey, 2–10 µm long. Apical cells widely rounded, sometimes forming a bulbous end of several cells. Heterocytes intercalary, cylindrical, golden, 11–16 µm wide, 4–12 µm long. Hormogonia short, released from the ends of the filaments. Necridic cells blackish, lyophilizing to leave purplish pigment between cells within the sheath. ITS operon types recovered: Only the At.

Holotype here designated: Fig. 15 n–s, images of strain HA4401–MV4.
Type locality: Collected in July 2009 in McBryde National Tropical Botanical Garden from a tree near Lawai stream, Kauai, Hawaii, USA, (21°54'4.3" N, 159°30'21.6" W).
Habitat: Aerial from a tree bark near stream in tropical climate.
Etymology: Named for the type locality, the island of Kauai.
Reference strain: HA4401–MV4a.
Materials analyzed: Strain and sequence: HA4401–MV4a; sequence: CENA366.

GenBank accession numbers: 16S rRNA and ITS: OR210275, KR137587

Taxonomic notes: Strain HA4401–MV4a was isolated from Hawaii and is morphologically similar to *B. angustatum*, also from Hawaii. HA4401–MV4a is sister to CENA366, with 98.8% genetic identity based on 16S rRNA sequence. No ITS region has been sequenced in CENA 366, and we conclude from the 16S rRNA phylogeny that for now it also belongs to the same species as HA4401–MV4a. The strain pair is sister to *B. angustatum* HA4187–MV1, but HA4401–MV4a is only 97.4% similar in 16S rRNA sequence to *B. angustatum* HA4187–MV1, and so should be recognized as separate from that taxon. Furthermore, HA4401–MV4a is separated from *B. angustatum* based on ITS phylogeny (Fig. 7), and is 9.7% dissimilar in ITS sequence, further evidence of lineage separation. The intensely granular nature of the cytoplasm is the most distinctive feature of this species, but the occurrence of bulbous apices composed of several cells is also very distinctive (Fig. 32 D–G, I).

***Brasilonema komarekii* M. Bohunická et J.R. Johansen sp. nov. (Fig. 16 a–d, S33 A–M)**
Description: Thallus soft, spreading into all directions, with usually short erect branched filaments, spherical colonies, only slightly growing into the substrate, black, olive–black, olive–green or grey–green. Filaments of various lengths, often false branched, 15–26 µm wide. Sheath colorless, grey, yellow, orange to brown incrusted, lamellate or not lamellate, sometimes horizontally structured, attached or widened, up to 7 µm wide. Trichomes not constricted or constricted at crosswalls, 12–20 µm wide. Cells cylindrical to disc–like, always shorter than wide, coarsely to finely granulated, green, blue–green, olive–green, grey, violet–grey or brown, 2–12 µm long. Apical cells widely rounded, hemispherical, pale colored, light grey or tan. Heterocytes intercalary, cylindrical or rounded, smooth yellow–green, 8–16 µm long, 13–18 µm wide. Hormogonia of usually around 2–4 cells present, releasing from the end of the filament. Necridic cells commonly present, concave, dark. Necridic cells of various colors present, lyophilizing to leave dark pigment between cells within the sheath (Fig. S33 G). ITS operon types recovered: Bo and Dt.

Holotype here designated: CBFS A163–1!, dried material of the reference strain immobilized on a glass fiber filter.
Paratype here designated: CBFS A162–1!, dried material of strain FM02–MK44 immobilized on a glass fiber filter.
Type locality: Collected in April 2010 from a park on Antequera Plaza, Bohol, Philippines (9°46'51.5" N 123°53'52.8" E).
Habitat: Representatives of this species were collected from tree bark in the Seychelles Archipelago, Antequera Plaza Park, Bohol, Philippines, and the Fata Morgana Greenhouse in Prague, Czech Republic.
Etymology: Named in honor of Czech phycologist and leading cyanobacterial expert Jiří Komárek, friend and/or mentor to several of the authors of this manuscript.
Reference strain: M42–F26I.
Materials analyzed: Strains FM02–MK44, M42–F26I, and SEY2.
GenBank accession numbers: 16S rRNA and ITS: OR210276–280

Taxonomic notes: This species is based on three strains, M42–F26 I, SEY2, and FM02–MK44. They are well separated from all other species in the 16S rRNA, four loci, and no–tRNA operon phylogenies, and group together with high similarity in both the 16S rRNA gene sequence (99.2–99.8% similar) and ITS from operon Bo (0.9% dissimilar). However, the most distinctive feature is the existence of a distinct D1–D1' helix. This is the only species to have a Dt ribosomal operon, and this distinctive operon was found in all three strains of the species. The D1–D1' helix of this operon is similar in length to the helices in the Ao operons of other taxa, but has a distinctly different sequence. The V3 helix of *B. komarekii* is also very distinct in both the Bo and Dt operons, although this helix shared similar structure and sequence to the V3 found in *B. burkei* (Fig. S9 N, O). The sequence of the Dt operon from the reference strain is a consensus of 11 different clones (OR210279), and we have very high confidence in the accuracy of this sequence. This species was morphologically quite variable, with the different strains having different coloration in both sheath material and cytoplasm (Fig. S33).

***Brasilonema lyngbyoides* (Gardner) M. Bohunická et J.R. Johansen comb. nov. (Fig. 16 e–j, S34 A–O)**
Basionym: *Scytonema lyngbyoides* N.L. Gardner (Gardner 1927, 28, pl. 17: fig. 35)
Updated description: Thallus star–like spreading, with long, thick, erect fascicles, sometimes branched, black–brown to olive black–green when old. Filaments short to long, single or double false branched, 19–32(42) µm wide. Sheath unstructured or lamellate, thin attached to widened, colorless, grey to black or orange to brown. Trichomes not constricted to constricted at crosswalls, crosswalls yellow or orange, (14)16–29(38) µm wide. Cells shorter than wide, cylindrical to disc–like, dark violet, brown, olive–brown, grey–green, olive–green, slate blue, blue–green, grey or green, colors light to deep, cell content smooth to coarsely granulated, vacuole–like structures sometimes present, (3)4–15(20) µm long. Apical cells widely rounded, hemispherical, often of light color, tan or light green. Heterocytes cylindrical to disc–like, sometimes hemispherical, intercalary bipolar, golden, (15)17–30(35) µm wide, 4–21 µm long. Hormogonia short, released by trichome fragmentation. Necridic cells of dark color present. ITS operon types recovered: Ao and Bt.

Epitype here designated: CBFS A165–1!, dried material

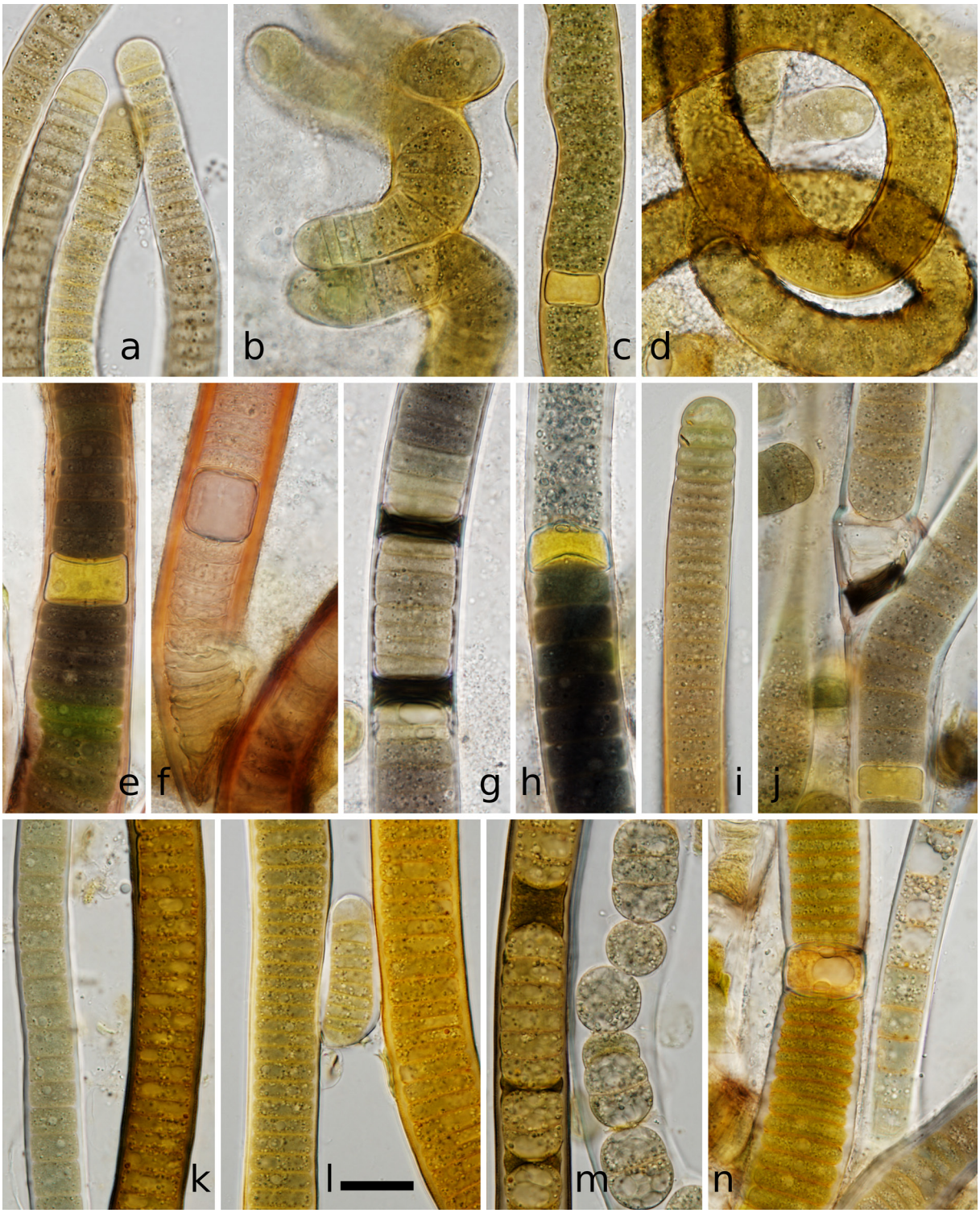

Fig. 16. (a–d) *Brasilonema komarekii* strain M42–F26I; (e–j) *Brasilonema lyngbyoides* strain SAG 40.90; (k–n) *Brasilonema mata–atlanticum* strain LBR–MK68. Scale bar 20 µm.

of the reference strain immobilized on a glass fiber filter.
Voucher preparation: CBFS A164–1!, dried material of strain CCALA 175 immobilized on a glass fiber filter.
Habitat: Collected in 1967 from a granite stone in Pokhara, Nepal.
Reference strain: SAG 40.90.
Materials analyzed: Strains SAG 40.90 and CCALA175.
GenBank accession numbers: 16S rRNA and ITS: OR210281–282.

Taxonomic notes: Strain CCALA 175 is a sub–isolate of strain SAG 40.90, but different operons were recovered from these two isolates. The Ao operon was recovered from SAG 40.90, while operon Bt was recovered from

CCALA 175. The 16S rRNA sequences differ between original strain and the sub–isolate (PS = 99.1%), and this could be due to actual differences in the 16S sequence of Ao and Bt operons, or some artifact, such as PCR error. They grouped together in the four loci tree (Fig. 4), but 16S sequences were separated (Fig. 3). The 16S rRNA similarity was > 98.7% in comparison to *B. bromeliae, B. sennae*, and *B. santannae*, but these were phylogenetically separated in the 16S rRNA tree. Furthermore, the percent dissimilarity of SAG 40.90 in operon Ao ITS regions was > 9% in all comparisons but one, which was still 6% dissimilar. The percent dissimilarity of CCALA 175 in operon Bt ITS regions was > 11% in all comparisons. This species was morphologically distinct from all other species in its ability to produce a reddish pigment in its sheath material that gave a pink cast to the sheath. It also produced the widest cells of any species. This is one of the species that could be considered morphologically recognizable.

***Brasilonema mata–atlanticum* M. Bohunická et J.R. Johansen sp. nov. (Fig. 16 k–n, S35 A–L)**

Description: Thallus spreading, grass–like with upright filaments, growing also into the substrate, black, dark brown to orange, especially at the ends of the filaments. Filaments of various length but often long, rarely to commonly single or double false branched, sometimes narrowed towards the ends, 15–26(30) µm wide. Sheath thin smooth attached, hyaline widened or lamellate, sometimes rugged at the surface, colorless, yellow, light grey to blackish. Trichomes not constricted to distinctly constricted at crosswalls, usually wider in the middle part, gradually tapered towards the ends, 12–20(25) µm wide. Cells usually shorter than wide, only rarely isodiametric up to slightly longer than wide, cylindrical to disc–like, often irregularly compressed, with finely to coarsely granulated content, greyish blue–green when young, olive–green to brown, bright violet, violet–brown or orange, sometimes with vacuole–like structures when older, often orange at the crosswalls, (2)4–10(18) µm long. End cells widely rounded to almost spherical, often of lighter color, constricted, sometimes with a series of flattened cellular apical caps, which can become separated from the apex but remain attached to the trichome, thus appearing to slide along the length of the trichome although the trichome is growing past the adherent set of caps. Heterocytes intercalary, cylindrical, sometimes hemispherical basal, with smooth yellow or yellow–green content, sometimes with sap vesicle, sometimes narrower than filament, forming constriction of the filament, not covered with sheath, found only rarely, (4)7–15 µm long, (12)17–22 µm wide. Hormogonia short, of 1–5 cells, constricted at crosswalls, light blue–green, with granulated content, formed by disintegration of the trichome. Necridic and irregular cells present, abundant. ITS operon types recovered: only the At.

Holotype here designated: CBFS A166–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in 2012 from Mata Atlântica, Brazil.
Habitat: Aerial in tropical regions.
Etymology: Named for the type locality – Mata Atlântica, from which the reference strain was isolated.
Reference strain: LBR–MK68.
Materials analyzed: Strains LBR–MK68 and P09–MK13.
GenBank accession numbers: 16S rRNA and ITS: KY365508 and OR210283.

Taxonomic notes: *B. mata–atlanticum* was in a small group of species having 16S PS > 98.7%, including *B. bromeliae, B. cataractarum, B. lyngbyoides, B. santannae*, and *B. sennae*. It was one of only six species with an At operon (which included *B. santannae* above), and was clearly distinct from all six of those species based on ITS phylogeny and ITS percent dissimilarities > 12%. *B. mata–atlanticum* LBR–MK68 had distinctive hormogonia, which were produced in long series in the trichome without the aid of necridia and were only 1–4 cells long. The sliding apical caps (Fig. S35 B–D, F) were very distinctive, and very similar to the detaching apical caps in *Aetokthonos hydrillicola* Wilde et Johansen (Wilde et al. 2014, see figs 2 F, G, 3 A–C). This highly unusual feature is found only in these two species at present.

***Brasilonema ohuense* M. Bohunická et J.R. Johansen sp. nov. (Fig. 17 a–f, S36A–N)**

Description: Thallus with long curved branched erect filaments, spreading into all directions, dark olive–green. Filaments isopolar, double or single false branched. Sheath colorless to blueish–black, thin up to 5 µm thick, not lamellate to lamellate. Trichomes constricted or not constricted at crosswalls, 14–23 µm wide. Cells isodiametric or shorter than wide, rarely longer than wide, cylindrical, finely or coarsely granulated, with olive green homogeneous content, 5–15(20) µm long. Apical cells widely rounded, pale olive–green, often with interthylakoidal space. Heterocytes intercalary, cylindrical, yellow–green, with smooth content, 15–20 µm wide, 9–23 µm long. Necridic and irregular cells present, Necridic cells of various colors present, lyophilizing to leave dark pigment between cells within the sheath. Hormogonia rounded, short, released apically. ITS operon types recovered: Ao and Bt.

Holotype here designated: CBFS A167–1!, dried material of the reference strain immobilized on a glass fiber filter.
Type locality: Collected in 2008 from a wet stone in waterfall in Ohu, lowland tropical rain forest, Madang Province, Momase Region, Papua New Guinea (5°13'58.3" S, 145°40'44.5" E).
Habitat: Tropical subaerial, on a wet stone in lowland tropical rain forest, waterfall in Ohu, Papua New Guinea.
Etymology: Named for the type locality, Ohu waterfall

in Papua New Guinea.
Reference strain: JK05–148.
Materials analyzed: Strain JK05–148.
GenBank accession numbers: 16S rRNA and ITS: OR210290–291.

Taxonomic notes: This species is sister to *B. elongatum*, and forms a supported clade with *B. xilitlae* and *B. hortense* in the phylogeny based on the 16S rRNA gene. However, dissimilarity in operon Bt (> 7 %) excludes it from being the same species as any of these three strains. *B. ohuense* had a distinctive autapomorphy in the Ao operon ITS. Its D1–D1' helix was exceptionally long and had two side helices branching from the main helix, a feature almost unique among cyanobacteria. The blueish–black sheath is very distinctive, and serves as a morphological apomorphy in this species.

***Brasilonema santannae* D.E. Berthold et al. (Barbosa et al. 2021, 84, fig. 3) (Fig. 17 g–m, S37 A–J)**
Updated description: Thallus dense, erect, green to dark grey in color. Filaments often long and fasciculate, straight or bent, slightly tapering, with false branching, usually double, 19.2–26.1 µm wide. Sheath thin, hyaline to yellow–brown or greyish, often encrusted. Trichomes cylindrical, straight to flexuous, not constricted to slightly constricted at crosswalls, sometimes constricted in the end parts (meristematic zones) and older filaments, 13.0–21.4 µm wide. Cells isodiametric cylindrical to disc–like, green, olive–green, olive–brown, to reddish–brown in color, (finely) granulated, with vacuole–like structures, (3)5.9–13.4(18) µm long. Apical cells of light color, often yellowish, widely rounded and blunt, hemispherical to almost spherical, often with sap vesicles, calyptra present. Heterocytes common, intercalary, regularly spaced in trichome, cylindrical, sometimes elongated, yellow or yellow–green, 16–26.1 µm wide, 2.4–18.2 µm long. Hormogonia rare, often with heterocyte in the middle and slightly narrowed towards both growing tips, C–bent. Necridic cells of dark color present, Necridic cells of various colors present, lyophilizing to leave dark pigment between cells within the sheath. ITS operon types recovered: Bo and At.

Habitat: Subaerial, attached to solid substrates in tropical regions (Florida, USA, Puerto–Rico).
Reference strain: BLCC–T43 (MT396517, MT396506).
Materials analyzed: Strains BLCC–T43, BLCC–T64, P608A.
GenBank accession numbers: 16S rRNA and ITS: MT396506–507, MT396517–518, and OR210293.

Taxonomic notes: Our strain (P608A) is in a supported clade with *B. santannae* based on 16S rRNA sequence. However, for the original *B. santannae* strain only operon At of the ITS was recovered, while only operon Bo of ITS was obtained for the strain P608A. Strain P608A was clearly separated from all strains with a no–tRNA operon (PD > 16%) and had a D1–D1' helix unique among all species due to its side helix on the 3' side of mid–helix (Fig. S4 O). The At operon ITS was also clearly separated from all other operons with two tRNA genes (PD > 9.8%). *B. santannae* P608A had hyaline hemispherical to subspherical end cells, copious geminate false branching, and regular heterocyte production, and was consequently quite morphologically distinct.

***Brasilonema sennae* (Komárek) Sant'Anna et Komárek (Sant'Anna et al. 2011, 57, figs 10 E–H)**
Taxonomic notes: This species was originally described as *Camptylonemopsis sennae* (Komárek 2003), and later transferred to *Brasilonema* (Sant'Anna et al. 2011). It certainly belongs to the *Brasilonema* clade, but raises disturbing concerns about whether or not *Camptylonemopsis* and *Brasilonema* may be synonymous. They share a number of morphological features, such as isopolar filaments with creeping central part and upturned ends, parallel arrangement of filaments and hormogonia, growth form in fascicles, and rare false branching. Both genera also have tropical to subtropical distribution. However, the type species of *Camptylonemopsis, C. lahorensis* (Ghose) Desikachary reportedly produces akinetes, a feature not found in any *Brasilonema* species.

B. sennae is morphologically cryptic in comparison to most *Brasilonema* species. A sequence of the 16S rRNA gene of *B. sennae* strain CENA114 (without the associated ITS) was published in GenBank in 2007, and included in Sant'Anna et al. (2011). In that early phylogeny, *B. sennae* was sister to *B. bromeliae*, in agreement with our placement of the taxon in the *Bromeliae* group. As mentioned elsewhere in this paper, a later ribosomal sequence was obtained as part of the genome of strain CENA114 (posted in GenBank in April 2020), but it is nearly identical to the 16S rRNA sequence for *B. octagenarum*. We consider *B. sennae* to be still nomenclaturally valid and accept the earlier sequence as most likely to be correct, but the discrepancy between 16S rRNA gene sequences in the reference strain needs to be examined.

***Brasilonema stuposum* (Bornet ex Bornet et Flahault) M. Bohunická et J.R. Johansen comb. nov. (Fig. 18 a–f, S38 A–J)**
Basionym: *Scytonema stuposum* [Kützing] Bornet ex Bornet et Flahault (Bornet & Flahault 1886, 92)
Updated description: Thallus firm, with short to long erect fascicles or irregular erect bundles, not growing into the substrate, black, brown–black or green–black. Filaments long, false branched, (12.5)14–35(39) µm wide. Sheath firm, hyaline or slightly lamellate, sometimes widened, colorless or greyish, up to 5 µm wide. Trichomes not constricted or constricted at crosswalls, crosswalls clearly visible, (11.5)14–34 µm wide. Cells cylindrical to disc–shaped, always

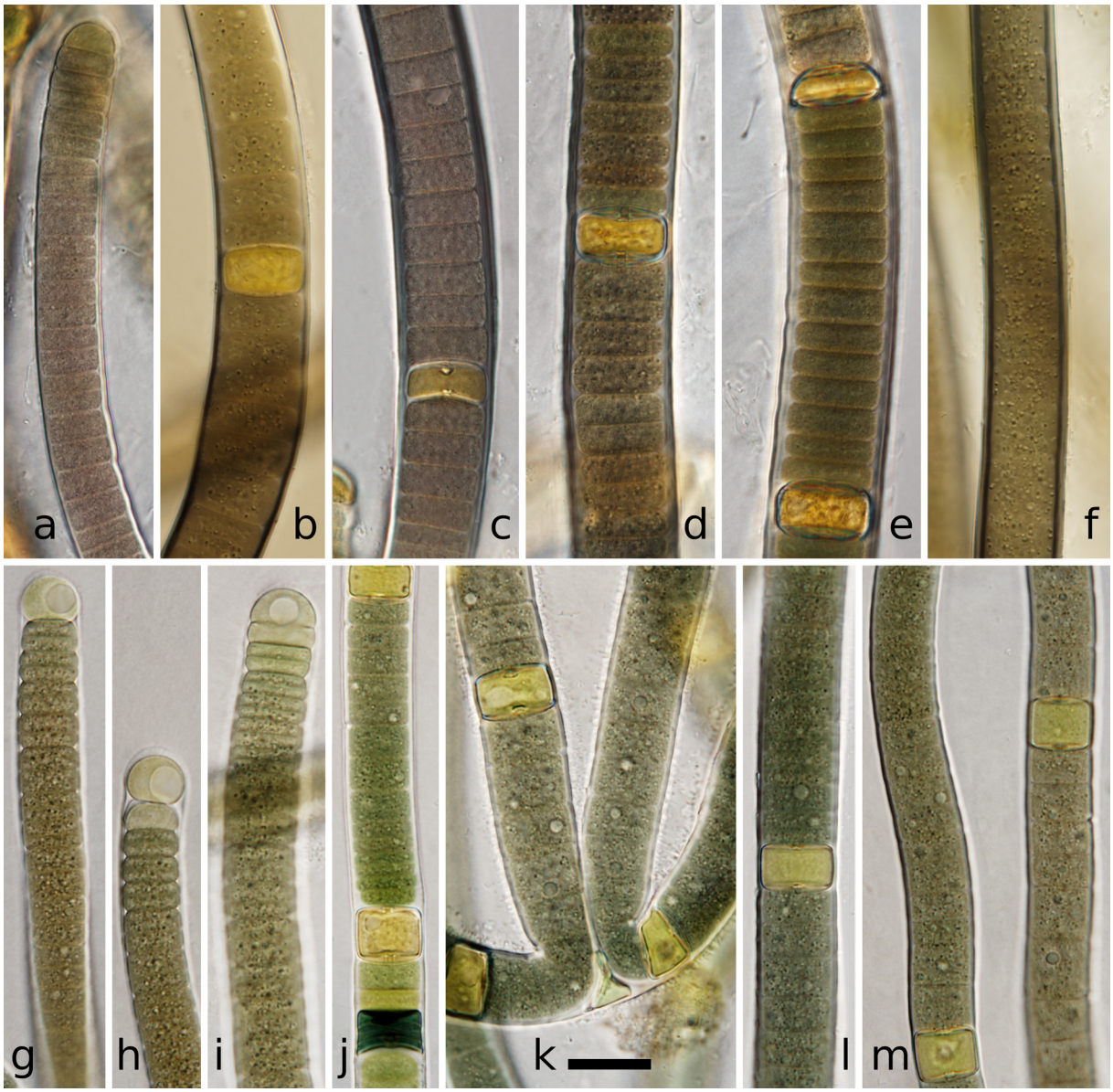

Fig. 17. (a–f) *Brasilonema ohuense* strain JK05–148; (g–m) *Brasilonema santannae* strain P608A. Scale bar 20 µm.

shorter than wide, granulated, sometimes with vacuole–like structures, intense dark brown, purple–brown, purple, olive–brown or blue–green, orange coarsely granular when old, 2–14 µm long. Apical cells widely rounded or hemispherical, yellowish, sometimes with a mucilaginous apical cap. Heterocytes mostly intercalary, single or in pairs, cylindrical, barrel–shaped or disc–shaped, sometimes terminal hemispherical or bluntly rounded, yellow or yellow–green, 11–25 µm wide, 4–21 µm long. Hormogonia isopolar, sometimes heteropolar with basal heterocyte, narrowed towards the growing tips, released with facilitation of necridic cells, sometimes attached to the sheaths of filaments, often remaining enclosed in sheath material. Necridic cells dark colored, lyophilizing to leave dark pigment between cells within the sheath. ITS operon types recovered: Ao and At.

Epitype here designated: CBFS A173–1!, dried material of the reference strain immobilized on a glass fiber filter.
Voucher preparations: CBFS A171–1! and CBFS A172–1!, dried material of strains MX2, and ACOI 889, respectively, immobilized on a glass fiber filter.
Habitat: Moist surfaces (limestone, granite, soil) in tropical and subtropical regions. Portugal (Porto, Arganil – Mata da Margaraça) and México (Cascada encantada waterfall, Los Manantiales, Morelos). Reported from warm temperate climates, and considered pantropical, but the classical taxon may represent many *Brasilonema* species.
Reference strain: PT5–MK70.
Materials analyzed: Strains PT5–MK70, ACOI 889, MX2.
GenBank accession numbers: 16S rRNA and ITS: KY365509–510 and OR210294–296.

Taxonomic notes: All strains within the species have 16S rRNA similarity > 99.7% and cluster together in a supported clade in all analyses in which they co–occur. Although two of the strains are phylogenetically separate in the four–loci tree, we consider it likely that they are the same species. This taxon has 16S rRNA similarity < 98.7% for all but six species, which are only slightly above the threshold. Both the Ao and At operons have PD > 12% in comparison to all orthologous operons. This species had wide filaments, although was not the widest of our set of species. It was a good morphological and ecological match to *Scytonema stuposum*.

***Brasilonema terrestre* Sant'Anna et Komárek (SANT' ANNA et al. 2011, 59, fig. 12)**

Taxonomic notes: Only 16S rRNA sequence data are available for this taxon. According to our data, only *B. terrestre* is molecularly similar to *B. stuposum* (98.8% PS), all others are below the 98.7% threshold. *B. terrestre* differs morphologically from *B. stuposum* primarily in width of trichomes, 9–15 µm wide as compared to 14–34 µm wide in *B. stuposum*. It is also gray–green to blue–green in color whereas *B. stuposum* is dark brown or purplish brown. This taxon awaits further characterization, but at present appears to be a species distinct from all other species.

***Brasilonema tolantongense* Becerra–Absalón et Montejano (BECERRA–ABSALÓN et al. 2013, 27, figs 1 a–f, 2 a–f as *'B. tolantongensis'*) (Fig. 18 g–l, S39 A–N)**

Updated description: Thallus with dense, erect fascicles, growing also into substrate, gold to dark brown, olive–green, black–green, or black–violet. Filaments long, often fasciculate straight or bent, sometimes slightly tapering, false branched, 15–32 µm wide. Sheath thin, attached, thicker near branching, sometimes widened, can extend past trichome, hyaline, lamellate or structured, colorless, yellowish to yellow at some parts. Trichomes cylindrical, mostly not constricted, or slightly constricted at crosswalls, crosswalls clearly visible, yellow or brown, 12–26.7 µm wide. Cells isodiametric or shorter than wide, cylindrical to disc–like, coarsely granulated when old, sometimes with vacuole–like structures, green, olive–green, brown–green, light brown, brown or brown–purple, 3–13(19) µm long. Apical cells rounded,

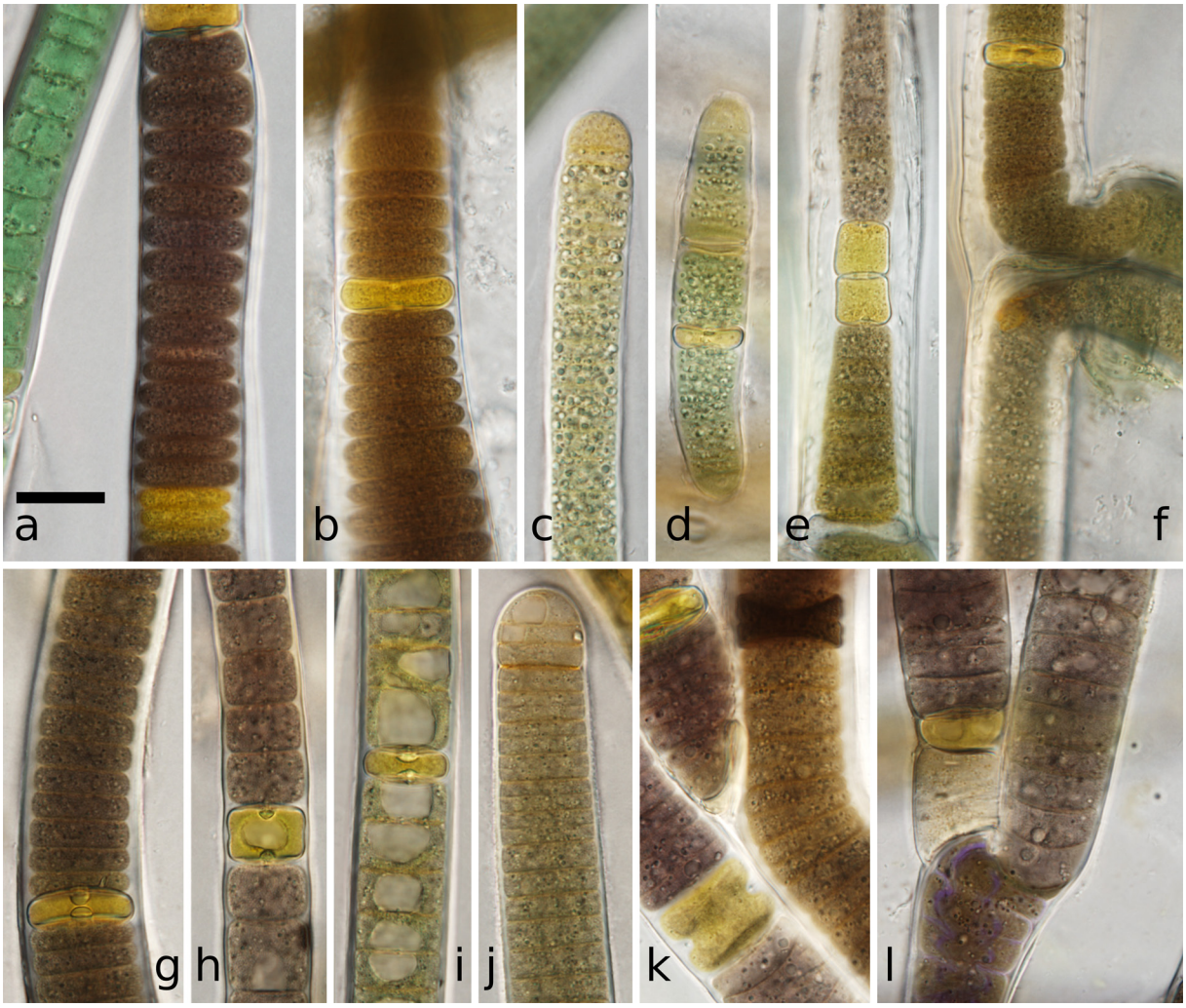

Fig. 18. (a–f) *Brasilonema stuposum* strain PT5–MK70; (g–l) *Brasilonema tolantongense* CZE–MK89. Scale bar 20 µm.

sometimes with calyptra, attenuated only in young trichomes. Heterocytes mostly intercalary, sometimes terminal, usually disc–like, sometimes isodiametric to elongated, yellow to orange, 13–26.5 µm wide, 5–18 µm long. Hormogonia motile, aerosolizing, isopolar, with very short cells, usually 3–5 µm long, when longer developing a firm, colorless sheath. Necridic cells dark colored. Morphologically greatly variable. ITS operon types recovered: Ao and Bt.

Epitype here designated: CBFS A174–1!, dried material of the reference strain MX6 immobilized on a glass fiber filter.
Voucher preparations: CBFS A175–1!, CBFS A176–1! and CBFS A177–1!, dried material of strains CZE–MK89, ACOI 580 and ACOI 572, respectively, immobilized on glass fiber filters.
Habitat: Pseudaerial, wet subaerial rock walls associated with waterfalls, also continually moistened substrates such as aeration tubes in outdoor fish tanks or surfaces in greenhouses. Subtropical to tropical distribution, also including artificially warmed habitats.
Reference strain: MX6/Tolantongo.
Materials analyzed: Strains CZE–MK89 ACOI 572, ACOI 580, BLCC–T51, BLCC–T61, BLCC–T49.
GenBank accession numbers: 16S rRNA and ITS: OR210297–300, MT396508–510, MT396519–521, and OR228885.

Taxonomic notes: *Brasilonema tolantongense* was originally described as *Brasilonema tolantongensis*, an orthographic error we correct here. *Brasilonema* is neutral, and requires the neutral ending *–ense*. This species has been collected several times since its inception. The reference strain was *B. tolantongense* Tolantongo (Becerra–Absalón et al. 2013), a strain that was lost before description necessitating an iconotype be designated as the holotype (Becerra–Absalón et al. 2013). Strain MX6 was isolated from the type locality. Two strains of *B. tolantongense*, BLCC–T51 and BLCC–T61, were isolated by Barbosa et al. (2021) from greenhouses in Apopka, Florida. Two other strains, ACOI 572 and ACOI 580, were isolated from São Tomé and Príncipe, an island country in the Gulf of Guinea off the coast of Central Africa. Strain CZE–MK89 was isolated from aquarium aeration tubes in a sturgeon fish tank in Liběchov, Czech Republic. A final strain, BLCC–T49, was isolated from a Florida greenhouse, but was described as a species new to science, *B. wernerae* (Barbosa et al. 2021). All members of the *B. tolantongense* cluster had 16S rRNA identities > 98.8%, with an average similarity of 99.6%. Operon Ao was sequenced for four strains, and they occurred in a well–supported clade in the ITS phylogeny based on this operon (Fig. 5). Percent dissimilarity among these strains averaged 1.3%. The ITS region for *B. wernerae* was from operon Bt, and the phylogeny based on the ITS alignment from this operon showed *B. wernerae* in a clade with the reference strain for *B. tolantongense* (Fig. 6) The percent dissimilarity among the three strains in this phylogeny were all < 1%, indicating that all should be considered to belong to a single species. The dissimilarity of the ITS regions to other strains is high, > 24% for operon Ao ITS regions, and > 12% for operon Bt ITS regions.

Although *B. wernerae* was reported to have heteropolar trichomes, a distinctive feature among *Brasilonema* species, the very high similarities in both 16S rRNA gene sequence and ITS sequences for both ITS operons recovered, along with phylogenetic placement, convince us that *B. wernerae* is a later synonym of *B. tolantongense*. We feel badly about discovering this taxon is a later synonym, and in another part of this paper have named *Brasilonema verawernerae* to honor Dr. Werner in agreement with our colleagues that she should be so recognized.

The hormogonia enclosed in sheath material are a distinctive feature of this species (Fig. S39 L–N). Such ensheathed hormogonia are defined as hormocytes in Komárek (2013).

***Brasilonema villosum* M. Bohunická et J.R. Johansen sp. nov. (Fig. 19 a–f, S40 A–J)**
Description: Thallus grey–green or dark olive green, with long erect fascicles, spreading in round star–like subcolonies. Filaments short to long, (sometimes) single or double false branched, 14–25(38) µm wide. Sheath thin, colorless or greyish, attached or lamellate orange–brown. Trichomes not constricted to constricted at crosswalls, crosswalls often orange, 11–22(30) µm wide. Cells shorter than wide, cylindrical to disc–shaped, green, olive–green, grey–violet, grey or brown, pale when older, finely granulated, with spherical bodies or vacuole–like structures, 3–10 µm long. Apical cells rounded. Heterocytes intercalary, cylindrical to almost spherical, single or in pairs, yellow, 13–22 µm wide, 5–22 µm long. Hormogonia released by disintegration of the filament in the end of the filament, also in the middle of the filament, short, constricted at crosswalls, without sheath, with vacuole–like structures, of pale olive–grey color. Necridic cells of various colors present, lyophilizing to leave dark pigment between cells within the sheath. ITS operon types recovered: only Ao.

Holotype here designated: CBFS A181–1!, dried material of the reference strain immobilized on a glass fiber filter.
Paratype here designated: CBFS A178–1!, dried material of strain M29–F12, immobilized on glass fiber filter.
Type locality: Collected in April 2009 from Loboc, Bohol, Philippines (9°39'36" N, 124°04'48" E).
Habitat: Subaerial on muddy wall.
Etymology: L. *villosus* = villous, i.e. shaggy, with fairly long soft straight not interwoven hairs. Describing the macroscopic appearance of the mat of filaments.
Reference strain: M31–F20B–II.
Materials analyzed: Strains M31–F20B–II, M29–F12.
GenBank accession numbers: 16S rRNA and ITS: KY365507 and OR228886.

Taxonomic notes: This strain clusters loosely with M31–F20B–II based on the 16S rRNA phylogeny, and the pair forms a paraphyletic sister group to *B. calidum* TH04–Ema in the four loci tree. We justify the separation of both strains of *B. villosum* from *B. calidum* above. M29–F12 and M31–F20B–II are on the threshold for recognizing different species based on 16S rRNA gene sequence similarity (98.7%). M31–F20B II is somewhat close to *B. bromeliae* based on both the four loci phylogeny and ITS no–tRNA operon sequence (average 5.2% dissimilar). However, this level of dissimilarity generally means that taxa are separate species, as we conclude here. We saw several heterocytes in this species that appeared to be dividing to produce two adjacent heterocytes. We are unaware of reports of this phenomenon, as heterocytes typically develop from vegetative cells. We do not know if this is a species–specific trait or a developmental anomaly that can occur in other *Brasilonema* species.

***Brasilonema xilitlae* I. Becerra–Absalón, M. Bohunická et J.R. Johansen sp. nov. (Fig. 19 g–l, S41 A–S)**
Description: Thallus soft, spreading spherically, with very long, erect filaments, growing into the substrate, black–brown to dark yellow–brown or olive–brown. Filaments long, single or double false branched, 16–22(32) µm wide. Sheath attached thin to firm, hyaline, grey, yellow orange to brown, grey–brown, purple or blue, structured, up to 7 µm wide. Trichomes isopolar, not constricted or only slightly constricted at crosswalls, young trichomes slightly curved to sigmoid, 11.7–18(29) µm wide. Cells shorter than wide to isodiametric, rarely longer than wide, cylindrical to disc–like, cell content brown, orange–brown, olive–brown, olive–green, or grey–brown when older, violet, with homogenous to profusely granulated cytoplasm, vacuole–like structures absent of rare, 5–19 µm long. Apical cells widely rounded, tan or light green colored, end of the trichome irregularly constricted, with cells shorter than wide. Heterocytes intercalary, cylindrical, barrel shaped or shorter than wide oval, yellow, yellow–green, yellow–orange or golden, 14–18(23) µm wide, 7–25 µm long. Hormogonia released from the end of filament by its disintegration, later growing at both ends, straight or C–bent. Necridic cells of dark brown color present, sometimes also in rows. ITS operon types

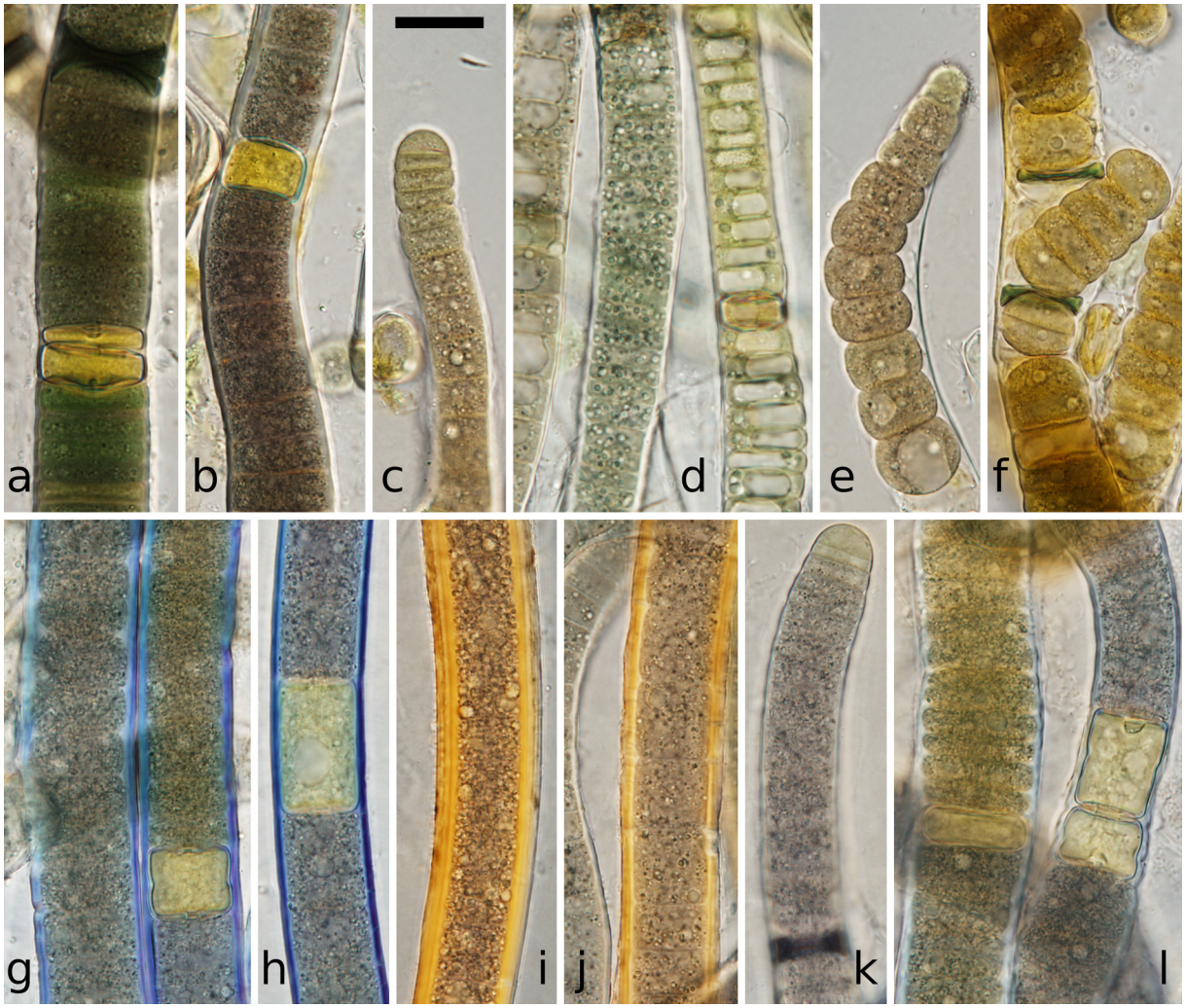

Fig. 19. (a–f) *Brasilonema villosum*, (a–e) strain M31–F20BII, (f) strain M29–F12; (g–l) *Brasilonema xilitlae* strain MX3. Scale bar 20 µm.

recovered: Bo and Bt.
Holotype here designated: CBFS A180–1!, dried material of the reference strain immobilized on a glass fiber filter.
Paratype here designated: CBFS A179–1!, dried material of strain P217A immobilized on a glass fiber filter.
Type locality: Collected in 2007 from a wet wall in Xilitla, San Luis Potosí, México.
Habitat: Populations in tropical, subaerial environments, on wet walls, hanging gardens (Mexico, Puerto–Rico).
Etymology: Named for the type locality, Xilitla, México.
Reference strain: MX3/Xilitla.
Materials analyzed: Strains MX3, P217A, P125C.
GenBank accession numbers: 16S rRNA and ITS: OR210304–307.

Taxonomic notes: This species consists of three strains with high similarity in the 16S rRNA (99.6–99.9% similar) and operon Bo ITS (0.9–1.6% dissimilarity). They cluster with *B. ohuense* and *B. elongatum* in the 16S phylogeny and structure of the V3 helices (Fig. S9 P–R), but can be separated from these strains based on dissimilarity levels in either operon Bo or operon Bt ITS sequences. Strain MX3 lacks the vacuole–like structures in the cells of other strains. In addition, hormogonia often develop a basal heterocyte in this strain. The most distinctive feature is the coloration of the sheaths, which can be bright orange or bright blue (Fig. 19 g–j, S41 P–R). We have not seen these intense sheath colors in any other cyanobacterial taxon.

Discussion

This study is unique among cyanobacterial taxonomic treatments for a number of reasons. A total of 24 new species, three new combinations, and review of 15 previously described species in a single genus were covered. Monographic treatments typically recognize many more species, but they primarily summarize previously described species in many different genera, rather than describe new taxa (Geitler 1932; Desikachary 1959; Starmach 1966; Komárek 2013; Komárek & Anagnostidis 1998, 2005). The same can be said for geographically limited algal floras (Joosten 2006; Kaštovský et al. 2018). This could be considered a monograph on the genus *Brasilonema*, covering at least to some extent all described species, and more than doubling the number of known species to a total of 40 species.

This treatment is also unusual for the nature of the treatment given species, particularly the description of new species. Polyphasic characterization was first limited primarily to the use of 16S rRNA phylogeny together with morphology and occasionally ecology (e.g. Fiore et al. 2007; Aguiar et al. 2008; Sant'Anna et al. 2011; Becerra–Absalón et al. 2013). More recently characterization of the secondary structure of conserved domains of the ITS region have been added to the list (Johansen et al. 2011; Osorio–Santos et al. 2014; Jung et al. 2020; Kumar et al. 2022; many others). In this paper we used 16S rRNA phylogeny, 16S rRNA percent similarity, ITS phylogeny for three operon categories of ITS region, secondary structure of the ITS conserved structures, a multilocus phylogenetic study, morphology, and ecology. So, a very thorough set of characters and criteria was used. The only current approach that was not employed was whole genome sequencing and given a set of 76 strains, this last approach must await a greater level of funding and evidence of need. The persistent effort to obtain as many operons as possible for each strain and each species was one characteristic that makes this study unique.

The search for multiple operons revealed the highest complexity in ITS regions so far observed in cyanobacteria. There were seven orthologous ribosomal gene lineages, four of which shared a common history evident in both operons with tRNA genes and those without. The origin of the four conserved D1–D1′ helix types is unknown, but a plausible hypothesis is that they represent at least some horizontal gene transfer (HGT) which occurred prior to speciation within the genus. We hypothesize that another source of the diversity in ITS regions is likely duplication of operons and divergence, as well as rearrangements in the ribosomal operons that lead to the removal or addition of tRNA genes to the ITS. The complexity in the evolutionary history of the ribosomal operons in *Brasilonema* is tantalizing, but presently we do not understand this complexity. Did an ancestor have all four operon types, and then subsequently lose one or more of these types? Has more recent HGT occurred? Is our assumption that every species has three operons correct? Do some taxa have more operons? Do some have less? Why is it so difficult to amplify some operons? This study places names on the species, but the study is just the first chapter in understanding phylogenetic complexity within this fascinating genus.

Another unusual discovery was the co–occurrence of different species in the same sample site. For example, *B. bambusae* and *B. bambusicola* were from bamboo stems on Uvita Pacific beach in Costa Rica, while *B. elegans* and *B. palmarum* were from palm leaves on the same beach. All four species are in the *Octagenarum* group and have high 16S rRNA similarity (99.1–99.8%, see Supplementary Table S2), but the ITS regions clearly separate them, with *B. bambusae* and *B. palmarum* having Ao operons (which are quite dissimilar), *B. bambusicola* having a Bo operon, and *B. elegans* having a Co operon. The Bt operons found in both *B. bambusicola* and *B. palmarum* are likewise quite different (PD = 12.7%). Consequently, four species of cyanobacteria with high 16S similarity can be found epiphytically in the same locale. In another instance, three species were found in the same locale, Cloudbridge Reserve in Costa Rica. *B. brancoi, B. bromeliae*, and *B. octagenarum* were all growing on stones next to the trail, with the latter two

scraped from the same rock. *B. bromeliae* is separated from the other two species by 16S rRNA PS (97.6%), whereas the other two species are separated by the PD of their Bt operons (9.1%) and the fact that they have different orthologues for the no–tRNA operons, *B. octagenarum* with Ao and *B. brancoi* with Co. Finally, three species were found in the Fata Morgana Greenhouse in Prague, *B. fatamorganum, B. elongatum*, and *B. komarekii*, but these three species are quite separate by all molecular criteria. This is somewhat disturbing, as these species are not only morphologically cryptic, requiring extra effort to sequence multiple operons, but they occur in essentially the same niche in the same geographical location. This gives us a message: cyanobacteria are much more diverse than previously thought, and with higher levels of isolation and sequencing effort, we can discover many more taxa from a small set of samples.

Brasilonema is easily recognized based on its wooly appearance in nature, its preference for subaerial and terrestrial habitats, its restricted distribution in warm moist climates (tropics and subtropics) or warm moist restricted habitats in temperate climates (greenhouses, cooling towers, soils warmed by burning subterranean coal). Furthermore, it has distinctive morphology, usually evidencing growth in fascicles, isopolar filaments, vacuolated cytoplasm, violet, blueish, brownish, or blackish cytoplasm, geminate false branching, and profuse hormogonia production. However, the species are morphologically cryptic or at best pseudocryptic. Only a few species have morphological apomorphies or combinations of traits that separate them from all other species. Dimensions, particularly filament width, trichome width, and cell length, show great overlap, and only the thinnest and widest species can be differentiated using these criteria.

The distribution of *Brasilonema* is also of interest. It occurs widely in tropical and subtropical climates (Fig. 1). Many of our species are restricted in their distribution, occurring as single or a few isolates from a limited region, such as the island of Puerto Rico or the country of Costa Rica. However, a number of species appear to be widely distributed. *B. octagenarum* has been found in Brazil, Hawaii, Costa Rica, and a greenhouse in Czech Republic. *B. bromeliae*, the type species, was described from bromeliads in Brazil, but we found it in Costa Rica, Nepal, and Puerto Rico. *B. fioreae* was described from Florida, but we have populations isolated from Puerto Rico and a greenhouse in Czech Republic. *B. komarekii* was isolated from the Philippines, the Seychelles Islands, and the Fata Morgana Greenhouse in Prague. Given that two species, *B. lichenoides* and *B. geniculatum*, were both described from granite headstones less than 50 years old, the wide distribution of *Brasilonema* species may additionally reflect high dispersal capabilities. To our knowledge, *Brasilonema* is not drought tolerant and produces no morphologically recognizable resting stages that could facilitate distribution over such long distances as were observed in this study. In the case of colonization of *Brasilonema* species in greenhouses there is an easy explanation for their presence, they came in with the soil and plants transported from tropical regions. On the other hand, the occurrence outside greenhouses is rather interesting. Are the nearby greenhouses sources of the inoculum? Or is the inoculum transported long distances from tropical regions in the air? *B. cubense* was collected from a natural locality in Cuba and from the concrete surface of the cooling tower of a power plant in the Czech Republic, a habitat presumably free of any cyanobacteria at the time it was built. Whatever the vector for transport may be, these species are widely distributed across the world, and we expect that with further study, many of the species we describe here will be found again in other places, perhaps very distant from their type localities.

We expect that more *Brasilonema* species will be discovered and described. We hope that future researchers will make significant efforts to sequence multiple ribosomal operons so that the new species will be accepted over time and not found to be later synonyms of previously described species. Reviewers should also be aware of the complexity of this genus and provide critical review. We are still concerned that *Brasilonema* could have more than three ribosomal operons, even though we have no evidence that this is so. Unfortunately, whole genome shotgun sequencing is notoriously poor at retrieving ribosomal operons. Furthermore, when multiple operons are sequenced, the ITS regions are identical, an artifact of the assembly of the contigs. Long–read sequencing, such as PacBio sequencing, could recover the diversity of ribosomal operons, but it is more costly and requires cultures of higher purity.

Whole genome sequencing is the next phase for understanding the evolution of ribosomal operons in *Brasilonema*, and for understanding diversity as well. This work provides a body of taxonomic hypotheses that can be tested against whole genome phylogenies and methods of recognizing species using genomes. We are hopeful that harmonization between the ribosomal operon approaches used here and whole genome approaches being developed by others can occur.

This in–depth and thorough look at a single cyanobacterial genus will hopefully be a template for similar studies in other genera. Targeted studies of individual cyanobacterial genera would be of great assistance in understanding speciation, distribution, and ecology of microbes. An effort has been made recently to describe ever more genera. It is time to describe the species.

Acknowledgements

This study was supported as a long–term research development project no. RVO 67985939, Czech Science Foundation project no. 15–11912S, and specific research project (no. 2102/2018) of the Faculty of Science, University of Hradec Králové, Czech Republic. We thank to Dirección de Asuntos del Personal Académico (DGAPA), from UNAM for supporting PAPIIT Project IA204718. John Carroll University provided funds to the Johansen lab for sequencing efforts conducted at that institution. Chelsea Villanueva received salary support for her dissertation research from Cleveland State University. Support for the final sequencing

effort was provided to Chelsea Villanueva from the Phycological Society of America. Jeffrey Johansen is grateful to the University of South Bohemia for continued research support. Many others helped us along the way, and we acknowledge those who provided us with some of their isolates or assisted in isolating strains in the Kaštovský lab: Radka Hauerová (RKST strains), Jana Widermannová (JK strains), Alena Lukešová (JOH strains), H.D. Laughinghouse (Belize Zoo strain BZ–HDL–007), Alžběta Vondrášková, Josef Juráň, Jana Veselá (CT strains). We also wish to acknowledge people who provided us with samples from which the strains were isolated: Pavel Hrouzek, Louis H.Z. Branco, Petr Čapek & Kateřina Čapková, and Otakar Stunecký.

References

Aguiar, R.; Fiore, M.F.; Franco, M.W.; Ventrella, M.C.; Lorenzi, A.S.; Vanetti, C.A. & Alfenas, A.C. (2008). A novel epiphytic cyanobacterial species from the genus *Brasilonema* causing damage to *Eucalyptus* leaves. – J. Phycol. 44: 1322–1334.

Baas–Becking, L.G.M. (1934): Geobiologie of inleiding tot de milieukunde. – 263 pp., WP Van Stockum & Zoon, Den Hague.

Baldarelli, L.M.; Pietrasiak, N.; Osorio-Santos, K. & Johansen, J.R. (2022): *Mojavia aguilerae* and *M. dolomitestris* – two new Nostocaceae (Cyanobacteria) species from the Americas. – J. Phycol. 58: 502–516.

Barbosa, M.; Berthold, D.E.; Lefler, F.W. & Laughinghouse IV, H.D. (2021): Diversity of the genus *Brasilonema* (Nostocales, Cyanobacteria) in plant nurseries of central Florida (USA) with the description of three new species: *B. fioreae* sp. nov., *B. santannae* sp. nov. and *B. wernerae* sp. nov. – Fottea 21: 82–99.

Becerra–Absalón, I.; Rodarte, B.; Osorio–Santos, K.; Alba–Lois, L.; Segal–Kischinevsky, C. & Montejano, G. (2013): A new species of *Brasilonema* (Scytonemataceae, Cyanoprokaryota) from Tolantongo, Hidalgo, Central Mexico. – Fottea 13: 25–38.

Becerra–Absalón, I.; Johansen, J.R.; Osorio–Santos, K. & Montejano, G. (2020): Two new *Oculatella* (Oculatellaceae, Cyanobacteria) species in soil crusts from tropical semi–arid uplands of Mexico. – Fottea 20: 160–170.

Bornet, É. & Flahault, C. (1886): Revision des Nostocacées hétérocystées contenues dans les principaux herbiers de France (Troisième fragment). – Annales des Sciences Naturelles, Botanique, Septième série 5: 51–129.

Bourrelly, P. & Manguin, E. (1952): Algues d'eau douce de la Guadeloupe et dépendances: recueillies par la Mission P. Allorge en 1936. – pp. 1–282, 31 pls. Paris, Société d'Edition d'Enseignement Supérieur.

Boyer, S.L.; Johansen, J.R.; Flechtner, V.R. & Howard, G.L. (2002): Phylogeny and genetic variance in terrestrial *Microcoleus* (Cyanophyceae) species based on sequence analysis of the 16S rRNA gene and associated 16S–23S ITS region. – J. Phycol. 38: 1222–1235.

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. – Mol. Biol. Evol. 18: 1057–1069.

Cai, F.; Yang, Y.; Wen, Q. & Li, R. (2018): *Desmonostoc danxiaense* sp. nov. (Nostocales, Cyanobacteria) from Danxia mountain in China based on polyphasic approach. – *Phytotaxa* 367: 233–244.

Cai, F.; Li, S.; Zang, H.; Yu, G. & Li, R. (2022): *Nodosilinea hunanesis* sp. nov. (Prochlorotrichaceae, Synechococcales) from a freshwater pond in China based on a polyphasic approach. – Diversity 14: 364.

Carmichael, W.W. (1986): Isolation, culture, and toxicity testing of toxic cyanobacteria (blue–green algae). – In: Shilov, V. (ed.): Fundamental Research in Homogenous Catalysis 3: – pp. 1249–1262. Gordon and Breach, New York.

Davydov, D.; Shalygin, S. & Vilnet, A. (2020): New cyanobacterium *Nodosilinea svalbardensis* sp. nov. (Prochlorotrichaceae, Synechococcales) isolated from alluvium in Mimer river valley of the Svalbard archipelago. – Phytotaxa 442: 61–79.

De Alvarenga, L.V.; Vieira Vaz, M.G.M.; Genuário, D.B.; Esteves–Ferreira, A.A.; Almeida, A.V.M.; de Castro, N.V.; Lizieri, C.; Souza, J.J.L.L.; Schaefer, C.E.G.R.; Nunes–Nesi, A. & Araújo, W.L. (2018): Extending the ecological distribution of *Desmonostoc* genus: proposal of *Desmonostoc salinum* sp. nov., a novel Cyanobacteria from a saline–alkaline lake. – Int. J. Syst. Evol. Micr. 68: 2770–2782.

Desikachary, T.V. (1959): Cyanophyta. – 686 pp., Indian Council of Agriculture Research, New Delhi.

Drummond, A.J.; Ho, S.Y.W.; Philips, M.J. & Rambaut, A. (2006): A relaxed phylogenetics and dating with confidence. – PLoS Biol. 2006, 4: e88.

Erwin, P.M. & Thacker, R.W. (2008): Cryptic diversity of the symbiotic cyanobacterium *Synechococcus spongiarum* among sponge hosts. – Mol. Ecol. 17: 2937–947.

Fenchel, T. (2003): Biogeography for bacteria. – Science 301: 925–926.

Finlay, B.L. & Fenchel, T. (2004): Cosmopolitan metapopulations of free–living microbial eukaryotes. – Protist 155: 237–244.

Fiore, M.F.; Sant'Anna, C.L.; Azvedo, M.T.P.; Komárek, J., Kaštovský, J., Sulek, J. & Lorenzi, A.S. (2007): The cyanobacterial genus *Brasilonema,* gen. nov., a molecular and phenotypic evaluation. – J. Phycol. 43: 789–798.

Garcia–Pichel, F.; Prufert–Bebout, L. & Muyzer, G. (1996): Phenotypic and phylogenetic analyses show *Microcoleus chthonoplastes* to be a cosmopolitan cyanobacterium. – Appl. Environ. Microbiol. 62: 3284–3291.

Gardner, N.L. (1927): New Myxophyceae from Porto Rico. – Mem. N.Y. Bot. Garden 7: 1–144.

Gascuel, O. (1997): BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. – Mol. Biol. Evol. 14: 685–95.

Geitler, L. (1932): Cyanophyceae: Rabenhorst's Kryptogamenflora von Deutschland, Österreich und der Schweiz, Band XIV. – 1196 pp., Akademische Verlagsgesellschaft m.b.h.

Gelman, A. & Rubin, D.B. (1992): Inference from iterative simulation using multiple sequences. – Stat. Sci. 7: 157–511.

Gouy, M.; Guindon, S. & Gascuel, O. (2009): SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. – Mol. Biol. Evol. 27: 221–224.

Guiry, M.D. & Guiry, G.M. (2023): AlgaeBase. World–wide electronic publication, National University of Ireland,

Galway. – https://www.algaebase.org; searched on 2 July 2023.

Gutwinski, R. (1901): Additamenta ad floram algarum India batavorum cognoscendam. – Rozpr. Akad. Umiej. Cracovie, Math.–Mrzyr., ser. 2, 39: 287–307.

Heidari, F.; Zima, J. Jr.; Riahi, H. & Hauer, T. (2018): New simple trichal cyanobacterial taxa isolated from radioactive thermal springs. – Fottea 18: 137–149.

Hrouzek, P.; Lukešová, A. & Ventura, S. (2013): Description of the cyanobacterial genus *Desmonostoc* gen. nov. including *D. muscorum* comb. nov. as a distinct and phylogenetically coherent taxon related to the genus *Nostoc*. – Fottea 13: 201–213.

Johansen, J.R.; Kováčik, L.; Casamatta, D.A.; Fučíková, K. & 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.

Joosten, A.M.T. (2006): Flora of the blue–green algae of the Netherlands 1. The non–filamentous species of inland waters. – 237 pp., KNNV Publishing, Utrecht.

Jung, P.; Mikhailyuk, T.; Emrich, D.; Baumann, K.; Dultz, S. & Büdel, B. (2020): Shifting boundaries: ecological and geographical range extension based on three new species in the cyanobacterial genera *Cyanocohniella, Oculatella*, and *Aliterella*. – J. Phycol. 56: 1216–1231.

Kabirnataj, S.; Nematzadeh, G.A.; Talebi, A.F.; Saraf, A.; Suradkar, A.; Tabatabaei, M. & Singh, P. (2020): Description of novel species of *Allinostoc, Desikacharya* and *Desmonostoc* using a polyphasic approach. – Int. J. Syst. Evol. Micr. 70: 3413–3426.

Kaštovský, J.; Hauer, T.; Geriš, R.; Chattová, B.; Juráň, J.;, Lepšová–Skácelová, O.; Pitelková, P.; Puztai, M.; Škaloud, P.; Šťastný, J.; Čapková, K.; Bohunická, M. & Mühlsteinová, R. (2018): Atlas sinic a řas ČR 1. – 384 pp., powerprint, Praha.

Katoh, K. & Standley, D.M. (2013): MAFFT multiple sequence alignment software version 7: improvements in performance and usability. – Mol. Biol. Evol. 30: 772–780.

Komárek, J. (2003): Two *Camptylonemopsis* species (Cyanoprokaryotes) from "Mata Atlantica" in coastal Brazil. – Preslia 75: 223–232.

Komárek, J. (2013): Cyanoprokaryota, part 3: heterocystous genera. – In: Büdel, B., Gärtner, G.; Krienitz, L. & Schagerl, M. (eds): Süsswasserflora von Mitteleuropa, Vol. 19/3. – 1130 pp., Springer Spektrum, Heidelberg.

Komárek, J. & Anagnostidis, K. (1998): Cyanoprokaryota, part 1. Chroococcales. – In: Ettl, H.; Gärtner, G.; Heynig, H. & Mollenhauer, D. (eds): Süsswasserflora von Mitteleuropa, Vol. 19/1. – 548 pp., Gustav Fischer, Jena–Stuttgart–Lubeck–Ulm.

Komárek, J. & Anagnostidis, K. (2005): Cyanoprokaryota, part 2. Oscillatoriales. – In: Büdel, B.; Krienitz, L.; Gärtner, G. & Schagerl, M. (eds): Süsswasserflora von Mitteleuropa, Vol. 19/2. – 759 pp., Elsevier/Spektrum, Heidelberg.

Kumar, N.; Saraf, A.; Pal, S.; Mishra, D.; Singh, P. & Johansen, J.R. (2022): Circumscription of *Fulbrightiella* gen. nov. and *Sherwoodiella* gen. nov., two novel genera in the Calotrichaceae (Nostocales, Cyanobacteria). – J. Phycol. 59: 204–220.

Larkin, M.A.; Blackshields, G.; Brown, N.P.; Chenna, R.; McGettigan, P.A.; McWilliam, H.; Valentin, F.; Wallace, I.M.; Wilm, A.; Lopez, R.; Thompson, J.D.; Gibson, T.J. & Higgins, D.G. (2007): Clustal W and Clustal X version 2.0. – Bioinformatics 23: 2947–2948.

Lepère, C.; Wilmotte, A. & Meyer, B. (2000): Molecular diversity of *Microcystis* strains (Cyanophyceae, Chroococcales) based on 16S rDNA sequences. – Syst. Geogr. Plants 70: 275–283.

Maltseva, S.; Bachura, Y.; Erst, T.; Kulikovskiy, M. & Maltsev, Y. (2022): Description of *Desmonostoc caucasicum* sp. nov. (Cyanobacteria) using an integrative taxonomic approach. – Phycologia 61: 514–527.

Miller, M.; Schwartz, T.; Pickett, B.; He, S.; Klem, E.; Scheuermann, R.H.; Passarotti, M., Kaufman, S. & O'Leary, M.A. (2015): A RESTful API for access to phylogenetic tools via the CIPRES Science Gateway. – Evol. Bioinform. 11: 43–8.

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

Mondal, A.; Mandal, S. & Rath, J. (2022): Seasonal diversity of cyanobacteria and new report of *Brasilonema* sp. colonizing the monuments of Santiniketan and Bishnupur (India). – Int. Biodeter. Biodegr. 167: 105350.

Montoya, H.T.; Gómez, J.C.; Mariano, M.A.; Jara, E.P. & Mayta, E.H. (2019): Phenotypic variability of the cyanobacterium *Brasilonema* (Nostocales, Scytonemataceae) of aerophytic community, new record for Peru. – Arnaldoa 26: 643–656.

Nübel, U.; Garcia–Pichel, F. & Muyzer, G. (1997): PCR primers to amplify 16S rRNA genes from cyanobacteria. – Appl. Env. Microbiol. 63: 3327–3332.

Nowruzi, B.; Becerra–Absalón, I. & Metcalf, J.S. (2023): A novel microcystin–producing cyanobacterial species from the genus *Desmonostoc, Desmonostoc alborizicum* sp. nov., isolated from a water supply system of Iran. – Curr. Microbiol. 80: 49.

Osorio–Santos, K.; Pietrasiak, N.; Bohunická, M.; Miscoe, L.; Kováčik, L.; Martin, M. P. & Johansen, J.R. (2014): Seven new species of *Oculatella* (Pseudanabaenales, Cyanobacteria): taxonomically recognizing cryptic diversification. – Eur. J. Phycol. 49: 450–470.

Pecundo, M.H.; Cai, F.; Chang, A.C.G.; Ren, H.; Li, N.; Li, R. & Chen, T. (2021): Polyphasic approach identifies two new species of *Desmonostoc* (Nostocales, Cyanobacteria) in the coralloid roots of *Cycas fairylakea* (Cycadales). – Phycologia 60: 653–668.

Perkerson, R.B.; Johansen, J.R; Kováčik, L.; Brand, J.; Kaštovský, J. & Casamatta, D.A. (2011): A unique pseudanabaenalean (cyanobacteria) genus *Nodosilinea* gen. nov. based on morphological and molecular data. – J. Phycol. 47: 1397–1412.

Radzi, R. & Merican, F. (2020): Validation of "*Nodosilinea signiensis* R.Radzi & F.Merican" nom. inval. (Prochlorotrichaceae, Cyanophyceae). – Notulae Algarum 125: 1.

Řeháková, K.; Johansen, J.R.; Casamatta, D.A.; Xuesong, L. & Vincent, J. (2007): Morphological and molecular characterization of selected desert soil cyanobacteria: Three species new to science including *Mojavia pulchra* gen. et sp. nov. – Phycologia 46: 481–502.

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.

Romanenko, P.A.; Vinogradova, O.M.; Romanenko, E.A., Mikhailyuk, T.I.; Babenko, L.M.; Ivannikov, R.V. & Scherbak, N.N. (2020): Morphological and molecular characterization of the representative of *Brasilonema* (Scytonemataceae, Cyanoprokaryota) from the tropical greenhouse in Kyiv (Ukraine). – Int. J. Algae 22: 103–122.

Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L; Darling, A.; Hohna, S.; Larget, B.; Liu, L.; Suchard, M.A. & Huelsenbeck, J.P. (2012): MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. – Syst. Biol. 61: 539–542.

Sant'Anna, C.L.; Azvedo, M.T.P.; Fiore, M.F.; Lorenzi, A.S.; Kaštovský, J. & Komárek, J. (2011): Subgeneric diversity of *Brasilonema* (Cyanobacteria, Scytonemataceae). – Revista Brasil. Bot. 34: 51–62.

Saraf, A.; Dawda, H.G.; Suradkar, A.; Behere, I.; Kotulkar, M.; Shaikh, Z.M.; Kumat, A.; Batule, P.; Mishra, D. & Singh, P. (2018): Description of two new species of *Aliinostoc* and one new species of *Desmonostoc* from India based on the polyphasic approach and reclassification of *Nostoc punensis* to *Desmonostoc punense* comb. nov. – FEMS Microbiol. Lett. 365: 1–9.

Stamatakis, A. (2014): RAxML version 8: a tool for phylogenetic analysis and post–analysis of large phylogenies. – Bioinformatics 30: 1312–1313.

Stanojković, A.; Skoupý, S.; Hašler, P.; Poulíčková, A. & Dvořák P. (2022): Geography and climate drive the distribution and diversification of the cosmopolitan cyanobacterium *Microcoleus* (Oscillatoriales, Cyanobacteria). Eur. J. Phycol. 57: 396–405.

Starmach, K. (1966): Cyanophyta–Sinice, Glaucophyta–Glaukofity. Flora Słodkowodna Polski, Tom 2. – 807 pp., Warsawa.

Strunecký, O.; Raabová, L.; Bernardová, A.; Ivanova, A. P.; Semanova, A.; Crossley, J. & Kaftan, D. (2020): Diversity of cyanobacteria at the Alaska North Slope with description of two new genera: *Gibliniella* and *Shackletoniella*. – FEMS Microbiol. Ecol. 96: fiz189.

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. – Koeltz Botanical Books, Glashütten.

Vaccarino, M.A. & Johansen, J.R. (2012): *Brasilonema angustatum* sp. nov. (Nostocales), a new filamentous cyanobacterium from the Hawaiian Islands. – J. Phycol. 48: 1178–1186.

Vázquez–Martínez, J.; Gutierrez–Villagomez, J.M.; Fonceca–García, C.; Ramírez–Chávez, E.; Mondragón–Sánchez, M.L.; Partida–Martínez, L.; Johansen, J.R. & Molina–Torres, J. (2020): *Nodosilinea chupicuarensis* sp. nov. (Leptolyngbyaceae, Synechococcales) a subaerial cyanobacterium isolated from a stone monument in central Mexico. – Phytotaxa 334: 167–182.

Villanueva, C.D.; Hašler, P.; Dvořák, P.; Poulíčková, A. & Casamatta, D.A. (2018): *Brasilonema lichenoides* sp. nov. and *Chroococcidiopsis lichenoides* sp. nov. (Cyanobacteria): two novel cyanobacterial constituents isolated from a tripartite lichen of headstones. – J. Phycol. 54: 224–233.

Villanueva, C.D.; Garvey, A.D.; Hašler, P.; Dvořák, P.; Poulíčková, A.; Norwich, A. R. & Casamatta, D.A. (2019): Descriptions of *Brasilonema geniculatum* and *Calothrix dumus* (Nostocales, Cyanobacteria): two new taxa isolated from cemetery tombstones. – Phytotaxa 387: 1–20.

Vinogradova, O.; Mikhailyuk, T.; Glaser, K.; Holzinger, A. & Karsten U. (2017): New species of *Oculatella* (Synechococcales, Cyanobacteria) from terrestrial habitats of Ukraine. – Ukrayins'kyi Botanichnyi Zhurnal 74: 509–520.

Ward, R.D.; Stajich, J.E.; Johansen, J.R.; Huntemann, M.; Clum, A.; Foster, B.; Roux, S.; Palaniappan, K.; Varghese, N.; Mukherjee, S.; Reddy, T.B.K.; Daum, C.; Copeland, A.; Chen, I.–M.A.; Ivanova, N.N.; Kyprides, N.C.; Shapiro, N.; Eloe–Fadrosh, E.A. & Pietrasiak, N. (2021): Metagenome sequencing to explore phylogenomics of terrestrial cyanobacteria. – Microbiol. Resour. Announc. 10: e00258–21.

Wilde, S.B.; Johansen, J.R.; Wilde, H.D.; Jiang, P.; Bartelme, B.A. & Haynie, R.S. (2014): *Aetokthonos hydrillicola* gen. et sp. nov.: Epiphytic cyanobacteria associated with invasive aquatic plants and implicated in bird deaths from Avian Vacuolar Myelinopathy. Phytotaxa 181: 243–260.

Wilmotte, A.; Van Der Auwera, G. & Dewachter, R. (1993): Structure of the 16S ribosomal RNA of the thermophilic cyanobacterium *Chlorogloeopsis* HTF (*Mastigocladus laminosus* HTF) strain PCC 7518, and phylogenetical analysis. – FEBS Letters 317: 96–100.

Yarza, P.; Yilmaz, P.; Pruesse, E.; Glöckner, F.O.; Ludwig, W.; Schleifer, K.; 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. Rev. Microbiol. 12: 635–645.

Yilmaz, M.; Phlips, E.J. & Tillett, D. (2009): Improved methods for the isolation of cyanobacterial DNA from environmental samples. – J. Phycol. 45: 517–521.

Zammit, G.; Billi, D. & Albertano, P. (2012). The subaerophytic cyanobacterium *Oculatella subterranea* (Oscillatoriales, Cyanophyceae) gen. et sp. nov.: a cytomorphological and molecular description. – Eur. J. Phycol. 47: 341–354.

Zuker, M. (2003): Mfold web server for nucleic acid folding and hybridization prediction. – Nucleic Acids Res. 31: 3406–15.

Zwart, G.; Hiorns, W.D.; Methé, B.A.; van Agterfeld; M.P., Huismans, R.; Nold, S.C.; Zehr, J.P. & Laanbroek H.J. (1998): Nearly identical 16S rRNA sequences recovered from lakes in North America and Europe indicate the existence of CLADES of globally distributed freshwater bacteria. – Syst. Appl. Microbiol. 21: 546–556.

Supplementary material

The following supplementary material is available for this article:

Fig. S1. D1–D1' helices for Ao operons lacking tRNA genes. (A–G) *Brasilonema Octagenarum* group. (A) *B. octagenarum* HA4186–MV1 and RKST381 (also structure for *B. octagenarum* RKST322, which differs by one nucleotide in the terminal loop); (B) *B. palmarum* CR06–4B/1; (C) *B. amethysteum* CR10–2F/1, *B. bambusae* CR06–5F/1, and *B. incudis* P834A; (D) *B. lichenoides* CDV2; (E) *B. verawernerae* FL15–MK94; (F) *B. epiphyllum* P722A; (G) *B. geniculatum* HWSC1. (H–P) *Brasilonema Bromeliae* group. (H) *B. bromeliae* SAG39.90, RKST321 and P117A; (I) *B. villosum* M31–F20BII; (J) *B. calidum* TH04–Ema; (K) *B. lyngbyoides* SAG40.90; (L) *B. cubense* JOH13, TH06–T13; (M) *B. stuposum* PT5–MK70, ACOI 889; (N) *B. tolantongense* MX6; (O) *B. angustatum* HA4187–MV1; (P) *B. elongatum* FM05–MK46; (Q) *B. ohuense* JK05–148.

Fig. S2. D1–D1' helices for type At operons with tRNA genes. (A) *B. angustatum* HA4187–MV1; (B) *B. kauaiense* HA4401–MV4; (C) *B. calidum* TH04–Ema; (D) *B. santannae* BLCC–T43; (E) *B. stuposum* ACOI 889; (F) *B. mata–atlanticum* P09–MK13 and LBR–MK68.

Fig. S3. D1–D1' helices for type B operons for *Brasilonema Octagenarum* group. D1–D1' (A–M) Type Bt operons with both tRNA genes. (A) *B. octagenarum* M36–T17; (B) *B. octagenarum* M27–E1; (C) *B. bambusicola* CR06–5B/1, CR06–5E/1, CR06–5E/2, *B. calcareum* CR12–6B1, and *B. muscicola* P811B (species differ by 3–4 nucleotides from each other but retain almost identical structure); (D) *B. fatamorganum* FM06–MK42; (E) *B. brancoi* RKST 3291; (F) *B. amethysteum* CR10–2F/1; (G) *B. corcovadense* BZ–HDL–007 and *B. fioreae* LIS1–MK92; (H) *B. fioreae* BLCC–T86 and P608E; (I) *B. fioreae* BLCC–T73; (J) *B. geniculatum* HWSC1–2A; (K) *B. mombasense* KEN–MK53; (L) *B. verawernerae* FL15–MK94; (M) *B. palmarum* CR06–4B/1; (N– R). Type Bo operons lacking tRNA genes. (N) *B. corvcovadense* CR09–11B/1 and CR09–11F/1; (O) *B. fatamorganum* FM06–MK42 and FM06–MK55; (P) *B. fioreae* BLCC–T72, BLCC–T83, P608E and LIS1–MK92; (Q) *B. hortense* SAG 67.81; (R) *B. mombasense* KEN–MK50; (S) *B. roberti–lamyi* MX5.

Fig. S4. D1–D1' helices for type B operons for *Brasilonema Bromeliae* group. (A–I) Type Bt operons with both tRNA genes. (A) *B. bromeliae* RKST321; (B) *B. cataractarum* JK04–144; (C) *B. ohuense* JK05–148; (D) *B. burkei* HA4348–MV1; (E) *B. cubense* TH05–T14; (F) *B. hortense* SAG 67.81; (G) *B. lyngbyoides* SAG40.90; (H) *B. tolantongense* MX6; (I) *B. elongatum* FM06–MK46; (J) *B. xilitlae* MX3. (K–O) Type Bo operons lacking tRNA genes. (K) *B. hortense* SAG 67.81; (L) *B. komarekii* M42–F26I; (M) *B. komarekii* SEY2; (N) *B. xilitlae* MX3, P125C; (O) *B. santannae* P608A.

Fig. S5. D1–D1' helices for type C and type D operons. (A) *B. hortense* SAG 67.81; (B) *B. incudis* P715A and P801A; (C) *B. cataractarum* JK04–144; (D) *B. brancoi* RKST3291; (E) *B. calcareum* CR12–6A/2 and CR12–6B/1; (F) *B. elegans* CR11–11B; (G) *B. komarekii* M42–F26I, SEY2, and FM02–MK44.

Fig. S6. Box–B helices for *Brasilonema Octagenarum* group, all operon types (operon types labeled above each helix). (A) *B. octagenarum* M36–T17, RKST381, HA4186–MV1, RKST322; (B) *B. octagenarum* HA4186–MV1; (C) *B. octagenarum* M27–E1, CENA114; (D–E) *B. amethysteum* CR10–2F/1; (F) *B. bambusae* CR6–5A/1, CR6–5F/1, *B. corcovadense* CR9––11B/1, CR9–11F/1, *B. elegans* CR11–11B/1, *B. mombasense* KEN–MK50, KEN–MK53; (G) *B. bambusicola* CR6–5A/2, CR6–5B/1, CR6–5E/1, CR6–5E/2; (H) *B. brancoi* RKST3291; (I) *B. calcareum* CR12–6A/2, CR12–6B/1; (J) *B. corcovadense* BZ–HDL–007; (K) *B. epiphyllum* P722A, *B. verawernerae* FL15–MK94; (L) *B. fatamorganum* BLCC–T71, FM01–MK42, FM06–MK55; (M) *B. fatamorganum* BLCC–T74, FM01–MK42; (N) *B. fiore*ae P608E, BLCC–T72, BLCC–T83, BLCC–T86, LIS–MK92; (O) *B. fioreae* P608E; (P) *B. fioreae* P608E, BLCC–T73; (Q–R) *B. geniculatum* HWSC1; (S) *B. incu*dis P801A, P715A; (T) *B. incudis* P715A, P801A, P834A, *B. lichenoides* CDV2; (U) *B. mombasense* KEN–MK53; (V). *B. muscicola* P811B; (W) *B. palmarum* CR6–4B/1; (X). *B. palmarum* CR6–4B/1; (Y) *B. roberti–lamyi* MX5.

Fig. S7. Box–B helices for *Brasilonema Bromeliae* group, all operon types (operon types labeled above each helix). (A) *B. bromeliae* RKST321, P117A, SAG 39.90; (B) *B. angustatum* HA4187–MV1; (C) *B. calidum* TH04–Ema; (D) *B. burkei* HA4348–LM4; (E–F) *B. cataractarum* JK04–144; (G) *B. cubense* JOH13, TH06–T13, 00557_00001; (H) *B. cubense* TH05–T14; (I) *B. elongatum*, FM05–MK46, *B. ohuense* JK05–148; (J–L) *B. hortense* SAG 67.81; (M) *B. kauaiense* HA4401–MV4; (N). *B. komarekii* M42–F26I; (O) *B. komarekii* M42–F26I, FM02–MK44, SEY2; (P) *B. lyngbyoides* CCALA175; (Q) *B. lyngbyoides* SAG 40.90; (R) *B. mata–atlanticum* P09–MK13, LBR–MK68; (S) *B. santannae* BLCC–T64, BLCC–T43, P608; (T–U) *B. stuposum* ACOI 889; (V) *B. stuposum* PT5–MK70; (W). *B. tolantongense* ACOI 580, ACOI 572; (X) *B. tolantongense* MX6, BLCC–T49, BLCC–T61; (Y). *B. tolantongense* MX6, BLCC–T51; (Z). *B. villosum* M31–F20BII; (AA) *B. xilitlae* MX3; (BB) *B. xilitlae* MX3, P125C, P217A.

Fig. S8. V3 helices for *Brasilonema Octagenarum* group, all operon types (operon labels above each helix). Variable–length basal clamps illustrated, but 5'–UGU–3' sequence of 5' side of clamp highly conserved and aligned horizontally in this Fig. to assist in comparing upper helix which is fairly conserved in these helices. Species indicated by lines under figures. (A) *B. octagenarum*; (B) *B. amethysteum*; (C) *B. bambusae*; (D) *B. bambusicola*; (E) *B. brancoi*; (F) *B. calcareum*; (G) *B. corcovadense*; (H) *B. elegans*; (I) *B. epiphyllum*; (J) *B. fatamorganum*; (K) *B. fioreae*; (L) *B. geniculatum*; (M) *B. incudis*; (N) *B. lichenoides*; (O) *B. mombasense*; (P) *B. muscicola*; (Q) *B. palmarum*; (R) *B. roberti–lamyi*; (S) *B. verawernerae*.

Fig. S9. V3 helices for *Brasilonema Bromeliae* group, all operon types (operon labels above each helix). Variable–length basal clamps illustrated, but 5'–UGU–3' sequence of 5' side of clamp highly conserved and aligned horizontally in A–M to assist in comparing upper helix which is fairly conserved in these helices; N–R have longer V3 helices and basal clamps very different from all other species. Species indicated by lines under figures. (A) *B. bromeliae*; (B) *B. angustatum*; (C) *B. calidum*; (D) *B. cataractarum*; (E) *B. cubense* (unstable structure, basal clamp of U–A nucleotide pair likely does not form); (F) *B. hortense*; (G) *B. kauaiense*; (H) *B. lyngbyoides*; (I) *B. mata–atlanticum*; (J) *B. santannae*; (K) *B. stuposum*; (L) *B. tolantongense*; (M) *B. villosum*; (N) *B. burkei*; (O) *B. komarekii*; (P) *B. elongatum*; (Q) *B. ohuense*; (R) *B. xilitlae*.

Fig. S10. *Brasilonema octagenarum*, showing pigmentation variation, vacuolization and trichome polarity. (A–C, L) strain HA4186–MV1; (D–E, J–K) strain M27–E1; (F) strain M36–T17; (G) strain RKST–322; (H–I) strain RKST–381. Abbreviations: (fb) false branching, (h) hormogonium, (hpf) heteropolar filament, (ipf) isopolar filament, (n) necridia. Scale bars 20 μm.

Fig. S11. *Brasilonema amethysteum* strain CR10–2F/1, showing pigmentation consistency and false branching. Abbreviations: (dfb) double false branching, (sfb) single false branching, (h) hormogonium, (wsp) purplish water soluble pigment trapped within sheath. Scale bars 20 μm.

Fig. S12. *Brasilonema bambusae*, showing pigment variation, vacuolization and trichome polarity. (A–L) strain CR06–5A/1; (M–P) strain CR06–5F1. Abbreviations: (dfb) double false branching, (h) hormogonia, (hpf) heteropolar filament, (ipf) isopolar filament, (ts) textured sheath. Scale bars 20 μm.

Fig. S13. *Brasilonema bambusicola*, showing pigment variation and branching patterns. (A) strain CR06–5E/2; (B, H) strain CR06–5E/1; (C–D, I–J) strain CR06–5A/2; (E–G) strain CR06––5B1. Abbreviations: (dfb) double false branching, (fb) false branching, (hf) hormogonia formation, hpf heteropolar filament, (pv) prominent vacuolization, (wsp) water soluble pigment trapped in sheath. Scale bars 20 μm.

Fig. S14. *Brasilonema brancoi* strain RKST3291, showing pigment variation and characteristic short hormogonia. Abbreviations: (cg) central granulation, (dfb) double false branching, (sfb) single false branching, (h) hormogonia. Scale bars 20 μm.

Fig. S15. *Brasilonema calcareum*, showing variable pigmentation of cells and sheath and branching patterns. (A–B) strain CR12–6B1; (C–G) strain CR12–6A/2; (H–J) strain CR12–6A/1. Abbreviations: (dfb) double false branching, (sfb) single false branching, (rfb) repeated false branching, (rtf) rapidly tapering trichome, (vf) vacuolized filament. Scale bars 20 μm.

Fig. S16. *Brasilonema corcovadense*, showing repeated false branching and low variability in pigmentation. (A–B, D, J–M) strain CR09–11F/1; (C, H–I) strain CR09–11B/1; (E–G) strain BZ–HDL–007. Abbreviations: (cg) central granulation, (dfb) double false branching, (h) hormogonia, (n) necridia, (rfb) repeated false branching, (wsp) water soluble pigment trapped within sheath. Scale bars 20 μm.

Fig. S17. *Brasilonema elegans*, showing variation in pigmentationand branching patterns. (A, H) strain CR06–4C/1; (B–G) strain CR11–11B/1. Abbreviations: (dfb) double false branching, (rfb) repeated false branching, (sfb) single false branching, (ipf) isopolar filament, (mr) meristematic region, (n) necridia, (rrh) regularly repeating heterocytes. Scale bars 20 μm.

Fig. S18. *Brasilonema epiphyllum* strain P722A, showing consistency in pigmentation and polarity of hormogonia. Abbreviations: (h) hormogonia, (hh) heteropolar hormogonium, (ih) isopolar hormogonium, (dfb) double false branching, (sfb) single false branching, (tf) tapering filament, (vf) vacuolized filament. Scale bars 20 μm.

Fig. S19. *Brasilonema fatamorganum*, showing consistency in pigmentation, cental granulation, and ensheathed hormogonia (i.e. hormocytes). (A–C, E–F) strain FM01–MK42; (D, G–K) strain FM06–MK55. Abbreviations: (cg) central granulation, (ih) isopolar hormogonium, (n) necridia, (sfb) single false branching, (vf) vacuolized filament, (wsp) water soluble pigment trapped in sheath. Scale bars 20 μm.

Fig. S20. *Brasilonema fioreae* strain LIS1–MK92, showing pigmentation, vacuolization and variation in hormogonia. Abbreviations: (bwsp) black water soluble pigment, (dfb) double false branching, (h) hormogonium, (vh) vacuolized hormogonium. Scale bars 20 μm.

Fig. S21. *Brasilonema incudis*, showing variation in pigmentation and repeated false branching. (A–B, E–H) strain P801A; (C–D) strain P715A; (I) strain P715A. Abbreviations: (dfb) double false branching, (rfb) repeated false branching, (sfb) single false branching, (ih) isopolar hormogonium, (vf) vacuolated filament. Scale bars 20 μm.

Fig. S22. *Brasilonema mombasense* strain KEN–MK50, showing pigmentation, enlarged end cell, and necridia. Abbreviations: (dfb) double false branching, (sfb) single false branching, (eec) enlarged end cell, (h) hormogonium, (het) heterocyte, (n) necridia. Scale bars 20 μm.

Fig. S23. *Brasilonema muscicola* strain P811B, showing pigmentation and polarity of hormogonia. Abbreviations: (es) extended sheath, (hh) heteropolar hormogonium, (ih) isopolar hormogonium. Scale bars 20 μm.

Fig. S24. *Brasilonema palmarum* strain CR06–4B/1, showing pigmentation of trichomes and sheath material. Abbreviations: (dfb) double false branching, (sfb) single false branching, (h) hormogonia, (n) necridia, (rsh) regularly spaced intercalary heterocytes, (vf) vacuolated filament. Note: end cells regularly have reduced pigmentation. Scale bars 20 μm.

Fig. S25. *Brasilonema verawernerae* strain FL15–MK94, showing consistency in pigmentation and water soluble pigment trapped in sheath. Abbreviations: (ec) elongated cell, (dfb) double false branching, (h) hormogonium, (n) necridium, (wsp) water soluble pigment. Scale bars 20 μm.

Fig. S26. *Brasilonema bromeliae*, showing variation in pigmentation, cytoplasmic inclusions and water soluble pigment in sheath. (A–B, F–H, J) strain P117A; (C, I) strain RKST321; (D–E, K–L) strain SAG 39.90. Abbreviations: (bs) brown sheath, (h) hormogonium, (sfb) single false branching, (tf) tapering filament, (vf) vacuolated filament, (wsp) water soluble pigment, (ys) yellowish sheath. Scale bars 20 μm.

Fig. S27. *Brasilonema calidum* strain TH04–Ema, showing variation in pigmentation and polarity of hormogonia. Abbreviations: (ac) apical cap, (hh) heteropolar hormogonium, (ih) isopolar hormogonium, (ihet) intercalary heterocyte, (n) necridia, (sfb) single false branching, (th) tapering hormogonium, (vf) vacuolated filament. Scale bars 20 μm.

Fig. S28. *Brasilonema cataractarum* strain JK04–144, showing variation in pigmentation and distinctive refractive apical caps. Abbreviations: (ac) refractive apical cap, (h) hormogonium, (sfb) single false branching, (vf) vacuolated filament. Scale bars 20 μm.

Fig. S29. *Brasilonema cubense*, showing variation in pigmentation and apical caps. (A–E, I–K) strain JOH13; (F–H) strain TH05–T14; (L) strain TH05–T13. Abbreviations: (aac) achlorophyllous apical cells; (ac) apical cap; (dfb) double false branching; (sfb): single false branching; (wsp) water soluble pigment. Scale bar 20 μm, all panels to same scale.

Fig. S30. *Brasilonema elongatum* strain FM05–MK46, showing variation in pigmentation and necridia/water soluble pigment. Abbreviations: (ac) apical cell, (dfb) double false branching, (n) necridia, (ss) structured sheath, (ts) thickened sheath, (wsp) water soluble pigment. Scale 20 μm, all panels to same scale.

Fig. S31. *Brasilonema hortense* strain SAG 67.81, showing consistency in pigmentation, branching patterns and hormogonia. Abbreviations: (dfb) double false branching, (sfb) single false branching, (h) hormogonium, (if) isopolar filament. Scale bars 20 μm.

Fig. S32. *Brasilonema kauaiense* strain HA4401–MV4, showing range in pigmentation and distinctive bulbous cells at trichome apex. Abbreviations: (ac) apical cells, (bc) bulbous apical cells, (dfb) double false branching, (sfb) single false branching, (h) hormogonia, (vf) vacuolated filament, (wsp) water soluble pigment. Scale bars 20 μm.

Fig. S33. *Brasilonema komarekii*, showing variation in pigmentation of trichomes and sheath material, as well as hormogonial release. (A–C, E–M) strain FM02–MK44; (D) strain M42–F26 I. Abbreviations: (ct) constricted trichome, (h) hormogonium, (hr) hormogonial release, (ih) isopolar hormogonia, (n) necridium, (sfb) single false branching, (ss) structured sheath, (ys) yellowish sheath. Scale bars 20 μm.

Fig. S34. *Brasilonema lyngbyoides*, showing wide variation in pigmentation of trichome and sheath material. (A–K) strain SAG 40.90; (L–O) strain CCALA175. Abbreviations: (dfb) double false branching, (hf) heteropolar filament, (hr) hormogonia release, (sfb) single false branching, (vf) vacuolated filament. Scale 20 μm, all panels to same scale.

Fig. S35. *Brasilonema mata–atlanticum*, showing the diagnostic sliding apical caps and marked purple pigmentation. (A–I) strain P09–MK13; (J–L) strain LBR–MK68. Abbreviations: (ac) apical cell, (dfb) double false branching, (h) hormogonia, (sac) slipping apical caps, (vf) vacuolated filament. Scale bars 20 μm.

Fig. S36. *Brasilonema ohuense* strain JK05–148, showing yellowish brown to purplish brown pigmentation. Abbreviations: (ac) apical cap, (dfb) double false branching, (sfb) single false branching, (eh) elongated heterocyte. Scale bars 20 μm.

Fig. S37. *Brasilonema santannae* strain P608A, showing distinctive olive pigmentation. Abbreviations: (ac) apical cap, (bc) bulbous apical cells, (dfb) double false branching, (sfb) single false branching, (h) hormogonium, (wsp) water soluble pigment. Scale bars 20 μm.

Fig. S38. *Brasilonema stuposum*, showing variation in pigmentation and hormocytes. (A, E–F) strain MX2; (B, G–J) strain ACOI 889; (C–D) strain PT5–MK70. Abbreviations: (ac) apical cap, (ct) constricted trichome, (dfb) double false branching, (sfb) single false branching, (hs) hormogonia in series, (ts) thickened sheath, (ut) unconstricted trichomes, (vf) vacuolated trichomes, (wsp) water soluble pigment. Scale bars 20 μm.

Fig. S39. *Brasilonema tolantongense,* showing distinctive variation in pigmentation and hormocytes. (A–B, M–N) strain ACOI 572; (C–H) strain CZE–MK89; (I–L) strain ACOI 580. Abbreviations: (dfb) double false branching, (sfb) single false branching, (h) hormogocyte (ensheathed hormogonium), (hf) hormogocyte formation, (n) necridia, (vf) vacuolated filament. Scale bars 20 μm.

Fig. S40. *Brasilonema villosum*, showing release of hormogonia. (A–H) strain M29–F12; (I–J) strain M31–F20BII. Abbreviations: (dfb) double false branching, (sfb) single false branching, (hr) hormogonia release, (vf) vacuolated filament, (wsp) water soluble pigment. Scale bars 20 μm.

Fig. S41. *Brasilonema xilitlae*, showing wide range of pigmentation in filaments. (A–B, L–S) strain MX3; (C–F) strain P125C; (G–K) strain P217A. Abbreviations: (ct) constricted trichome, (dfb) double false branching, (sfb) single false branching, (h) hormogonium, (ss) structured sheath, (wsp) water soluble pigment. Scale bars 20 μm.

Supplementary Table 1. Metadata for *Brasilonema* strains isolated, morphologically characterized, or sequenced directly by authors of this study.

Supplementary Table 2. Average 16S rRNA percent similarity among species. For species represented by multiple sequences, the within-species PS is shown on the diagonal in yellow shading (NA=not available due to a single sequence). Gray shading represents pairwise species comparisons < 98.7% similarity. For unshaded comparisons, the similarity is ≥ 98.75 and is uninformative for species-level decisions.

Supplementary Table 3. Percent dissimilarity in ITS sequence among the operons lacking tRNA genes. For species which had multiple sequences, averages were calculated and strains are not given. Values > 7.0 are considered strong evidence of lineage separation. Same species generally have values < 3.0%. Operon designation is given before the taxon name (i.e. Ao, Bo, Co). Values for comparisons between different operons should not be used to differentiate species, and appear shaded. However, no taxon had more than one operon lacking tRNA genes, so taxon comparisons in the shaded areas are all different species.

Supplementary Table 4. Percent dissimilarity in ITS sequence among the Bt operons. For species which had multiple sequences, averages were calculated and strains are not given. For strains with two Bt operons averages were also computed, but the strain name is given (i.e. A. amethysteum, *B. brancoi* and *B. verawerneraerae*). Values >7.0 are considered strong evidence of lineage separation. Same species generally have values < 3.0%.

Supplementary Table S5. Percent dissimilarity of aligned operons with shorter D1-D1' helix and containing tRNAIle and tRNAAla genes. Note that all species are separated. Values < 3.0 are found within different strains of the same species.

Supplementary Table S6. Genbank accession numbers for protein coding genes in *Brasilonema* strains.

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)

© Czech Phycological Society (2024)
Received August 11, 2023
Accepted January 5, 2024