Potamolinea gen. nov. (*Oscillatoriales*, *Cyanobacteria*): a phylogenetically and ecologically coherent cyanobacterial genus

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Phormidium Kützing ex Gomont, a common genus of the *Cyanobacteria*, is widely known as a problematic group. Its simple morphology is not congruent with its genetic heterogeneity and several new generic entities have been described based on 16S rRNA gene sequence analyses from populations with similar morphology. During a study of the diversity of *Phormidioideae* (*Phormidiaceae*, *Oscillatoriales*) in Brazil, ten *Phormidium*-like strains from south-eastern and mid-western regions were isolated in monospecific cultures and submitted to polyphasic evaluation (morphological, ecological and molecular studies). The populations studied presented homogeneous morphology (trichomes straight, not attenuated and apical cell rounded or obtuse), differing mainly in cell length from the type species of the genus *Phormidium* (*Phormidium lucidum* Agardh ex Gomont) and occurring as three morphotypes. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the populations studied, with European *Phormidium aerugineo-caeruleum* (Gomont) Anagnostidis & Komárek strains, were placed together in a very distinctive and highly supported clade. Thus, the set of characteristics of the strains resulted in the recognition of the new genus *Potamolinea* Martins et Branco with two species: *Potamolinea magna* as the type species (strains 47PC and 48PC) and *Potamolinea aerugineo-caerulea* (Gomont) Martins et Branco (strains 1PC, 2PC and 38PC). These two species plus one still undetermined lineage, *Potamolinea* sp., are morphologically and genetically distinguishable, whereas the secondary structures of the D1-D1′, box-B and V3 regions were conserved within each one. The generic name and specific epithets of the new taxa are proposed under the provisions of the International Code of Nomenclature for algae, fungi and plants.

Introduction

To build a classification system that reflects the evolutionary history of *Cyanobacteria*, many changes have been occurring in the delimitation of orders, families, genera and species. The classification system recently proposed by Komárek *et al.* (2014), which is based on 16S rRNA gene sequence analyses, has brought considerable changes and new taxonomic groups have been described. Taxonomic studies based on polyphasic approach are changing the arrangement of *Cyanobacteria* and the value of characters used in the reconstruction of its phylogeny.

In the order *Oscillatoriales*, the heterogeneous nature of which has been emphasized by many authors (Teneva *et al.*, 2005; Casamatta *et al.*, 2012; Engene *et al.*, 2012, 2013; Komárek *et al.*, 2013; McGregor & Sendall, 2015; Martins *et al.*, 2016), *Phormidium* is considered a taxonomically complex genus, due to its morphological simplicity and large number of described species (about 200, according to Komárek & Anagnostidis, 2005), and is widely known to be polyphyletic. Several genera have been recently described from species previously classified under the genus *Phormidium*, such as *Phormidesmis* (Turicchia *et al.*, 2009), *Wilmottia* (Strunecký *et al.*, 2011), *Roseofilum* (Casamatta *et al.*, 2012), *Ammassolinea* (Hašler *et al.*, 2014), *Kamptonema* (Strunecký *et al.*, 2014), *Cephalothrix* (Malone *et al.*, 2015) and *Ancylothrix* (Martins *et al.*, 2016).

Abbreviations: BI, Bayesian inference; ITS, internal transcribed spacer; ML, maximum-likelihood; NJ, neighbour-joining.

The GenBank/EMBL/DDJB accession numbers for the 16S–23S rRNA gene sequences of the strains reported in this study are KX001786, KX001787, KX001788, KX001789, KX001790, KX001791, KX001792, KX001793, KX0017943 and KX001795.

Two supplementary tables are available with the online Supplementary Material.

Morphologically, the genus *Phormidium* is characterized by uniseriate, cylindrical, isopolar, non-branched trichomes, without differentiated cells. The apical part of trichomes can present wide morphological variation and Komárek & Anagnostidis (2005) used that criterion to organize the *Phormidium* species into eight groups. According to these authors, group V is formed by species with cylindrical trichomes along their whole length and the apical cell is widely rounded; this group comprises most of *Phormidium*species.

During an investigation about *Phormidioideae* diversity in Brazil, ten *Phormidium*-like strains, isolated from nine Brazilian streams and morphologically belonging to the above mentioned group V, were investigated. The taxonomic positioning of these and European strains, also isolated from streams, was defined based on a polyphasic evaluation (morphological, ecological and molecular analyses) and resulted in the proposition of a new genus, *Potamolinea* gen. nov.

Methods

Origin of the strains and cultivation. Ten environmental samples, growing in nine stream bottoms in Brazil, were collected (Table 1). Each cyanobacterial strain was isolated from a single trichome grown on BG11 medium (Rippka *et al*., 1979). Trichomes of each mat were repeatedly separated from others and successively transferred to clean drops of deionized water using a Pasteur pipette under an inverted light microscope (Leica DMIL LED). The sole trichomes were then inoculated in tubes with BG11 growth medium for the establishment of unicyanobacterial cultures. Strains were cultured and maintained under 20±1 °C, 50 µmol photons m^{-2} s^{-1} of irradiance and a 14 : 10 h light–dark cycle in the culture collection of IBILCE/UNESP.

Morphological characterization and identification. Morphological variability of populations was evaluated from fresh field material and from cultured samples by using an Olympus BH2 microscope. Taxonomic features, such as cell width, cell length, attenuation of trichomes and apical cell shape and dimensions, were analysed in at least 30 trichomes for each sample. Representatives of each species studied were deposited in Herbarium SJRP (IBILCE/UNESP), Brazil.

Molecular analyses. Biomass for DNA extraction was obtained from non-axenic unicyanobacterial cultures by repeated centrifugations. During the centrifugation process, the filaments were washed several times with sterile deionized water to remove or reduce mucilage and growth medium substances. DNA was extracted using the PowerSoil DNA Isolation kit from MO BIO Laboratories according to the manufacturer's protocol.

The 16S rRNA gene and 16S–23S internal transcribed spacer (ITS) markers were amplified by polymerase chain reaction (PCR) using the primers 16S27F and 23S30R (Taton *et al*., 2003), which was performed in a Techgene TC-512 thermal cycler using 25 µl reaction volumes containing 5 µl 10× PCR buffer, 2 µl 50 mM $MgCl_2$, 1 µl 10 mM dNTP mix, 1.25 µl of each primer (5 pmol), 14.2 µl Milli-Q water, 1.5 U Platinum *Taq* DNA polymerase (Life Technologies) and 10 ng genomic DNA. Thermal cycling was 94 °C for 5 min, followed by ten cycles of 94 °C for 45 s, 57 °C for 45 s and 72 °C for 2 min; 25 cycles of 92 °C for 45 s and 54 °C for 45 s; and one final cycle of 72 °C for 7 min. The PCR products were analysed on 1 % agarose gels stained with GelRed 0.6× (Biotium) and viewed on a 'Mini Bis Pro' transilluminator (Micro Photonics). The positive products were cloned using the pGEM-T Easy Vector System I (Promega) according to the supplier's manual. Competent *Escherichia coli* DH5α cells were transformed by heat shock and recombinant plasmids were isolated using the 'GeneJET Plasmid Miniprep' kit (Thermo Fisher Scientific). Sequencing was performed on an ABI 3130 sequencer, using a 'BigDye Terminator v3.0 Cycle Sequencing Ready Reaction' kit (Applied Biosystems) as the manufacturer's protocol. Primers M13F and Sp6R correspond to the vector sites and the internal primers 357F, 704R, 1114F and 1494R (Neilan *et al*., 1997) were used for sequencing. The DNA fragments were assembled into contigs using the Phred/Phrap/Consed software (Ewing & Green, 1998; Ewing *et al*., 1998; Gordon *et al*., 1998), and only bases with quality higher than 20 were considered. The nucleotide sequences obtained in this study have been deposited in the GenBank database under accession numbers: KX001786, KX001787, KX001788, KX001789, KX001790, KX001791, KX001792, KX001793, KX0017943 and KX001795.

Alignment and phylogenetic analyses The sequences obtained were compared with sequences previously published in NCBI (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/) by BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and the closest related were selected to compose the database. The alignment was carried out using the software CLUSTAL W v1.8 (Thompson *et al*., 1994) in MEGA 6.06 (Tamura *et al*., 2013) and inspected and refined manually. The unicellular cyanobacteria *Gloeobacter violaceus* PCC 7421 and *G. violaceus* PCC 8105 (GenBank accession numbers NC005125 and AF132791, respectively) were designed as the evolutionary outgroup.

Phylogenetic analyses were performed based on partial 16S rRNA gene sequences. The appropriate nucleotide substitution models were selected in jModelTest 2.1.1 (Darriba *et al*., 2012). Bayesian inference (BI) analysis was performed in MrBayes 3.1.2 software (Huelsenbeck & Ronquist, 2001), run with GTR+G+I model (rate matrix with six different substitution types, number of rate categories = 4, and with the nucleotide frequencies, shape parameter and pINVAR estimated from the data). BI analysis comprised two runs of four Monte Carlo Markov chains, each with 10 000 000 generations and sampling every 100 generations. The initial 10 000 generations were discarded as burn-in. Neighbour-joining (NJ) and maximum-likelihood (ML) inferences were performed using MEGA 6.06 (Tamura *et al*., 2013) and the GTR model was applied in the last method assuming heterogeneous substitution rate and gamma substitution of variable sites. Bootstrap resampling was performed on 1000 replicates. Sequence similarity matrix/nucleotide divergence was calculated from the alignment in BioEdit (Hall, 1999) from all positions including gaps.

Alignment of the 16S–23S ITS region was made using a combination of CLUSTAL_W v1.8 (Thompson *et al*., 1994) in MEGA6.06 (Tamura *et al*., 2013) and manual alignment utilizing secondary structure of conserved domains. The tRNA sequences were identified with tRNAscan-SE 1.21 (Lowe & Eddy, 1997). D1-D1′, box-B and V3 ITS regions were identified and their secondary structures were determined using Mfold 3.2 (Zuker, 2003) and re-drawn in Macromedia Fireworks 8.0.

Results

Morphological evaluation

The ten populations were observed forming benthic, macroscopic, dark green mats in streams. All the strains, belonging to three different morphotypes (*Potamolinea magna*, *P. aerugineo-caerulea* and *Potamolinea* sp.), presented a morphology corresponding to species included in *Phormidium* group V, according to Komárek & Anagnostidis (2005), but different from the type species of the genus *Phormidium* (*P. lucidum* Agardh ex Gomont).

Table 1. Sampling sites for the *Potamolinea* strains studied

Strains: 1PC, 2PC and 38PC, *P. aerugineo-caerulea*; 32PC, 33PC, 34PC, 35PC and 36PC, *Potamolinea* sp.; 47PC and 48PC, *P. magna*. MT, Mato Grosso State; SP, São Paulo State.

Strain	Locality	Latitude (S)	Longitude (W)	Habitat
1PC	Guarantã do Norte stream/MT	9° 46′ 07″	54° 39′ 09″	On rocky bottom of an oligotrophic, partially shaded stream in a pasture with remnant marginal vegetation at a disturbed Brazilian Amazon area
2PC	Santos Reis stream/MT	9° 45′ 23″	54° 34′ 46″	On rocky bottom of an oligotrophic, partially shaded stream in a pasture with remnant marginal vegetation at a disturbed Brazilian Amazon area
32PC	Felicidade stream/SP	20° 47′ 55″	49° 18′ 41″	On clay bottom of an oligotrophic, open stream in a pasture at a disturbed semi-deciduous seasonal forest area
33PC	Picinguaba Station - Serra do Mar State Park/SP	23° 22′ 16″	44° 49′ 50″	On sandy-clay bottom of an oligotrophic, shaded stream in a preserved Atlantic Rainforest area
34PC	Picinguaba Station - Serra do Mar State Park/SP	23° 22′ 47″	44° 49′ 21″	On sandy-clay bottom of an oligotrophic, shaded stream in a preserved Atlantic Rainforest area
35PC & 47PC	Jacaré stream/SP	20° 51′ 39″	49° 36′ 54″	On sandy bottom of a partially shaded stream in a pasture area with remnant marginal vegetation at a disturbed semi-deciduous seasonal forest area
36PC	Barra Funda stream/SP	20° 38′ 45″	49° 24′ 13″	On sandy-clay bottom of an oligotrophic, partially shaded stream in a pasture area with remnant marginal vegetation at a disturbed semi-deciduous seasonal forest area
38PC	Euclides Brentino stream - Furnas Bom Jesus State Park/SP	20° 15′ 15″	47° 27′ 23″	On sandy bottom of an oligotrophic, shaded stream in a preserved Brazilian savanna area
48PC	Preto river at São Roberto waterfall/SP	20° 11′ 10″	49° 41′ 06″	On sandy-clay bottom of an oligotrophic partially shaded stream in a pasture area

One morphotype (strains 47PC and 48PC) presented densely entangled filaments, 13.2–16.8 μm wide; sheaths facultative, attached to the trichomes, firm, thin, colourless; trichomes cylindrical, not attenuated, not constricted to slightly constricted at the ungranulated or slightly granulated cross-walls, 9.6–16.8 μm wide; cells 0.4–0.9 time longer than wide, 4–12.8 μm long; cell content blue–green, homogeneous to finely granulated; apical cell rounded, without calyptra (Fig. 1a–d, Table 2). Strain 47PC was selected as representative of the populations studied and was deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, under voucher number SJRP 31642.

Table 2. Morphological comparison among *Potamolinea* strains

Dimensions (μm) represent full ranges observed. L/W, cell length/width ratio; −, absence; +, presence. Strains: 1PC, 2PC and 38PC, *P. aerugineo-caerulea*; 32PC, 33PC, 34PC, 35PC and 36PC, *Potamolinea* sp.; 47PC and 48PC, *P. magna*.

Strain	Granuled cross-walls	Filament width*	Trichome width*	Cell length†	Apical cell width*	Apical cell length*	L/W
1PC	−	6–8.4	5–7.2	5.6–7.6	6–7.2	7.6–9.6	0.7–1.1
2PC	−	6–9	5.4–7.5	5–7.2	6–7.5	6.8–10.4	0.7–1.1
32PC	−	10.4–13.6	9.6–13.6	5.6–14.4	9.6–13.6	8–16.8	0.4–1.5
33PC	−	6.8–13.6	6.4–13.6	4.8–13.6	6.4–13.6	7.2–14.4	0.5–1.5
34PC	−	7.2–10.4	6–10.4	4.8–14.4	6–10.4	6.8–15.2	0.4–1.5
35PC	−	−	7–11.2	5–13.6	7–11.5	8–12.8	0.5–1.3
36PC	−	−	9.6–10.8	4.8–12	9.6–10.8	7.2–12	0.4–1.2
38PC	+	−	5.4–7.2	3.2–6.4	6.4–8	4–6	0.4–0.9
47PC	+/−	13.2–16.8	9.6–16.8	4–12.8	9.2–16.8	6.4–15.2	0.4–0.8
48PC	+/−	13.6–17.6	12.8–16	5.6–12	12.4–16	8–11.2	0.4–0.9

*n=30.
†n=300.

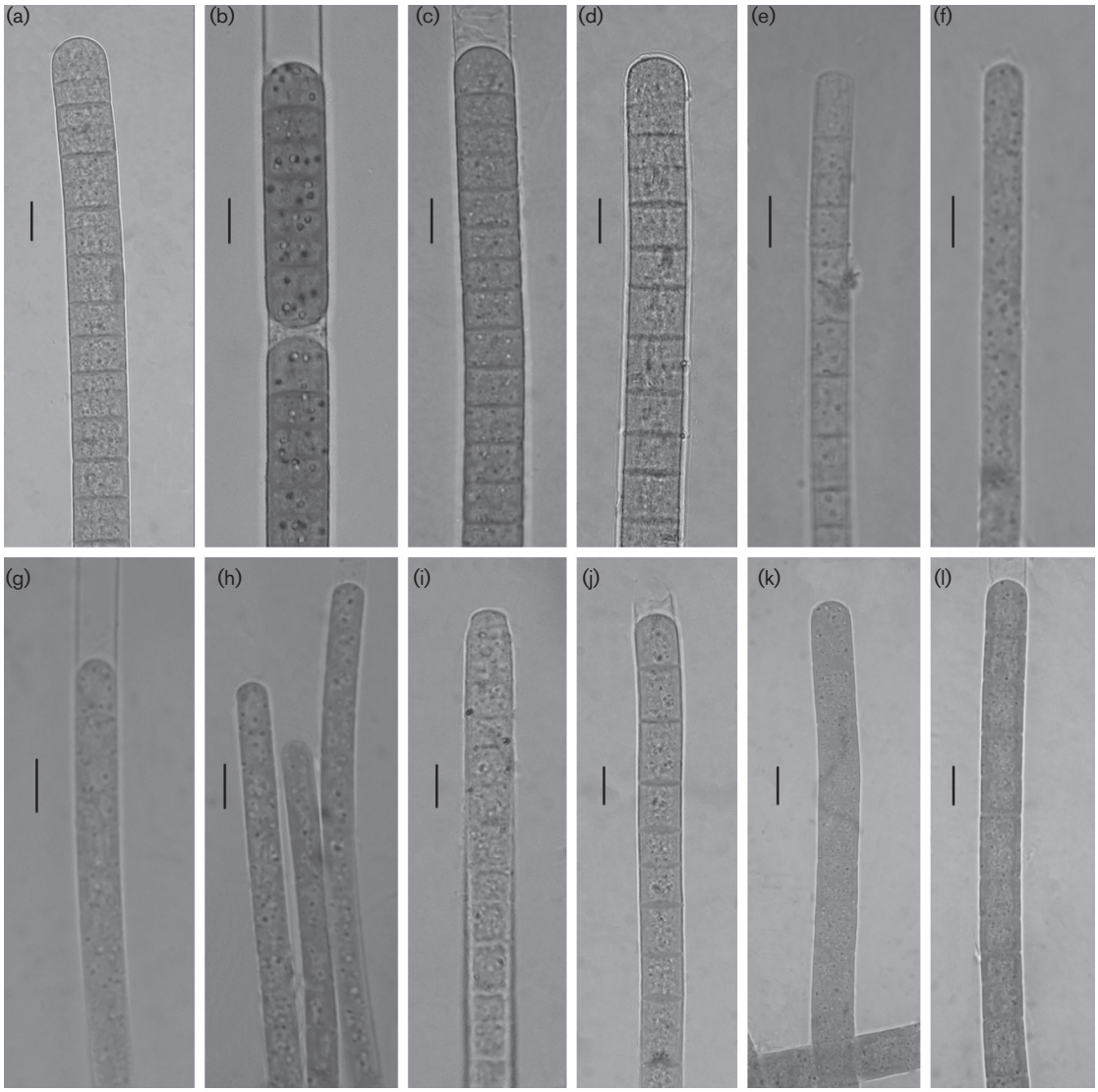

Fig. 1. Photomicrographs of Brazilian strains of *Potamolinea*: (a–d) *P. magna*; (e–h) *P. aerugineo-caerulea*; (i–l) *Potamolinea* sp. Bars, 10 μm.

The second morphotype, represented by strains 1PC, 2PC and 38PC, presented filaments densely entangled, 6–9 μm wide; sheaths facultative, attached to the trichomes, firm, thin, colourless; trichomes cylindrical, not attenuated, not constricted at the ungranulated cross-walls, 5–7.5 μm wide; cells 0.7–1.1 time longer than wide, 5–7.6 μm long; cell content blue–green, homogeneous to finely granulated, sometimes with scattered larger granules; apical cell rounded, without calyptra (Fig. 1e–h, Table 2). Strain 1PC was selected as representative of the populations studied and it was deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, under voucher number SJRP 31634.

The third morphotype, represented by strains 32PC, 33PC, 34PC, 35PC and 36PC, presented filaments densely entangled, 6.8–13.6 μm wide; sheaths facultative, attached to trichomes, firm, thin, colourless; trichomes cylindrical, not attenuated, not constricted to slightly constricted at the ungranulated cross-walls, 6–13.6 μm wide; cells 0.4–1.5 time longer than wide, 4.8–14.4 μm long; cell content blue–green, homogeneous to finely granulated; apical cell rounded or truncate, without calyptra (Fig. 1i–l, Table 2). Strain 35PC was chosen as representative of the populations studied and it was deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, under voucher number SJRP 31636.

Molecular analyses and phylogeny – 16S rRNA gene

The comparison among partial 16S rRNA gene sequences of the ten studied strains and sequences from GenBank showed similarities above 95.3 % with six Spanish

Phormidium aerugineo-caeruleum strains (MNZ-37, MNZ-32, MNZ-31, MED-28, MED-31, MED-17; Loza *et al.*, 2013) and below 94 % with other strains.

The tree obtained with BI analysis contained more well-supported nodes than those obtained with ML and NJ analyses and it is consequently the tree topology reported herein. Analyses of the 16S rRNA gene sequences showed a clear separation of the group comprising the studied populations and Spanish *Phormidium aerugineo-caeruleum* strains from the remaining strains (clade I; Fig. 2), and especially from the true *Phormidium* clade that is, according to Sciuto *et al.* (2012), represented by *Phormidium* cf. *irriguum* CALA 759 and *P. irriguum* f. *minor* ETS-02.

Similarity scores based on the comparison of 16S rRNA gene sequences of strains of clade I with sequences of strains pertaining to other genera, such as *Phormidium*, *Oscillatoria*, *Lyngbya*, *Kamptonema*, *Microcoleus* and *Coleofasciculus*, were lower than 93.8 %. The tree topology showed a close phylogenetic relationship among members of clade I with *Wilmottia* and *Phormidium* sp. B-Tom, highly supported by posterior probabilities, and the divergence among the three groups was higher than 5.6 %. The clade I was highly supported in ML, NJ and BI analyses (99 %, 100 % and 1, respectively; Fig. 2) and the similarity scores based on 16S rRNA gene sequences within this cluster were higher than 95.3 % (Table S1, available in the online Supplementary Material).

The strains of clade I clustered into three subgroups, labelled A, B and C, which were well supported by posterior probabilities (Fig. 2) and each of the subgroups corresponded to one of the three morphotypes previously cited. Similarity scores within each subgroup were from 97.3 to 99.9 % in subgroup A, 99.4 to 100 % in subgroup B and 99.5 % in subgroup C (Table S1).

Molecular analyses – 16S–23S ITS

Analysis of the 16S–23S ITS region for all ten strains studied was informative and supported both morphological and molecular (16S rRNA gene sequences) data. All strains had operons only with the $tRNA^{Ile}$ gene and the length of the 16S–23S ITS region ranged from 372 to 406 bp (Table 3). Six of the 12 ITS regions had the same length in all strains and the more variable-length regions among the strains were the spacers preceding box-B and V3 helices. The V2 region was not identified, as it is located between $tRNA^{Ile}$ and $tRNA^{Ala}$ and this latter gene was absent in the studied strains. The basal sequences of the helices were mostly conserved and the secondary structure could be determined (Fig. 3).

Similarity scores based on 16S–23S ITS sequences within each subgroup were higher than 98.6 % (Table S2) but lower than 74 % when compared among subgroups of the genus.

The D1-D1′ helix presented small differences in nucleotide sequences among the strains (Fig. 3a–d). Strains 1PC and 2PC presented identical nucleotide sequences and, consequently, the same secondary structure. The D1-D1′ sequence and secondary structure of strain 38PC were very similar to those of strains 1PC and 2PC, but not identical (Fig. 3a, b). Strains 32PC, 33PC, 34PC, 35PC and 36PC had identical nucleotide sequence and secondary structure for the D1-D1′ helix, differing from the others (Fig. 3c). Strains 47PC and 48PC also showed identical D1-D1′ nucleotide sequence and secondary structure, but distinct from other strains (Fig. 3d).

The studied strains presented four different box-B helix secondary structures (Fig. 3e–i). Although strains 1PC and 2PC had box-B sequences distinct in one nucleotide, they presented the same secondary structure (Fig. 3e, f). Strain 38PC also presented box-B nucleotide sequence very similar to those of strains 1PC and 2PC, differing in only one

Table 3. Lengths of 16S–23S ITS regions (number of nucleotides) in the analysed *Potamolinea* strains

Strains: 1PC, 2PC and 38PC, *P. aerugineo-caerulea*; 32PC, 33PC, 34PC, 35PC and 36PC, *Potamolinea* sp.; 47PC and 48PC, *P. magna.*

Strain	Complete ITS	Leader	D1–D1′ helix	D2 with spacer	D3 with spacer	$tRNA^{Ile}$ gene	Pre-box-B spacer	Box-B helix	Post-box-B spacer	Box-A	D4	V3 with spacer	D5
1PC	372	6	63	33	19	74	17	49	19	12	7	51	22
2PC	372	6	63	33	19	74	17	49	19	12	7	51	22
32PC	384	6	63	33	18	74	17	41	19	12	7	73	21
33PC	384	6	63	33	18	74	17	41	19	12	7	73	21
34PC	384	6	63	33	18	74	17	41	19	12	7	73	21
35PC	384	6	63	33	18	74	17	41	19	12	7	73	21
36PC	384	6	63	33	18	74	17	41	19	12	7	73	21
38PC	372	6	63	33	19	74	17	48	19	12	7	51	22
47PC	407	6	63	33	19	74	33	48	18	12	7	72	22
48PC	407	6	63	33	19	74	33	48	18	12	7	72	22

Fig. 2. BI tree based on the 16S rRNA gene sequences of oscillatorialean cyanobacteria. The clade comprising *Potamolinea* gen. nov., consisting of Brazilian (in bold) and European strains, is highlighted. A bootstrap test involving 1000 resamplings was performed. Bootstrap values (>70 %) and probabilities (>0.7) obtained from ML/NJ/BI methods, respectively, are displayed at the relevant nodes. GenBank accession numbers are shown in parentheses. Bar, 0.02 substitutions per nucleotide position.

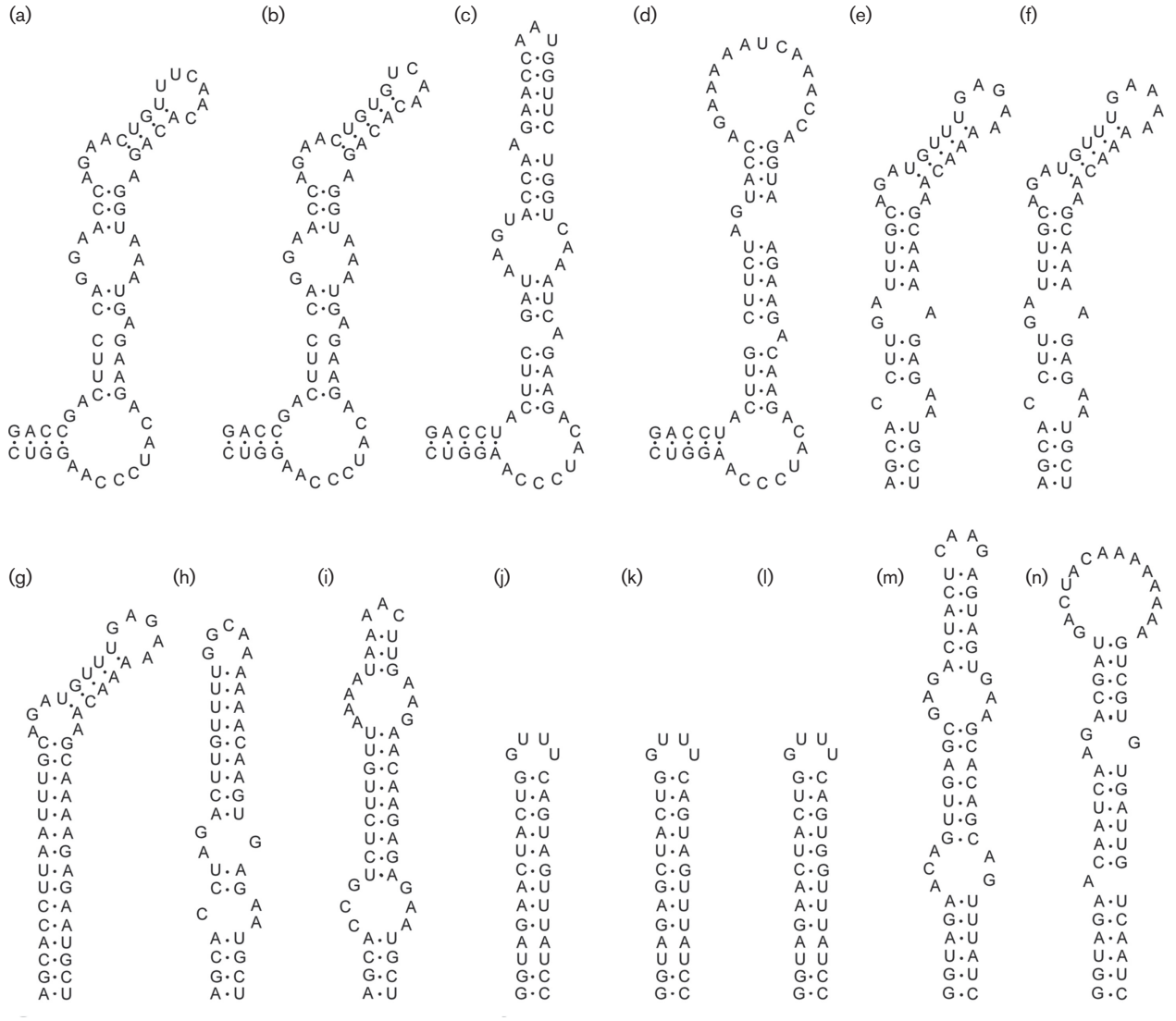

Fig. 3. Secondary structure of conserved regions of 16S–23S ITS of *Potamolinea* strains studied. (a–d) D1-D1′ helices: (a) 1PC and 2PC; (b) 38PC; (c) 32PC, 33PC, 34PC, 35PC and 36PC; (d) 47PC and 48PC. (e–i) Box-B helices: (e) 1PC; (f) 2PC; (g) 38PC; (h) 32PC, 33PC, 34PC, 35PC and 36PC; (i) 47PC and 48PC. (j–n) V3 helices: (j) 1PC; (k) 2PC; (l) 38PC; (m) 32PC, 33PC, 34PC, 35PC and 36PC; (n) 47PC and 48PC.

nucleotide, and its secondary structure of box-B helix also was distinct (Fig. 3g). Box-B helix sequences and secondary structures of strains 32PC, 33PC, 34PC, 35PC and 36PC were identical (Fig. 3h). Box-B helix sequence and secondary structure of strains 47PC and 48PC were identical between them and different from other strains (Fig. 3i).

Finally, the V3 helix of studied strains revealed three different secondary structures (Fig. 3j–n). Strains 1PC, 2PC and 38PC had identical secondary structure (Fig. 3j–l), although they had two to three divergent nucleotides. Strains 32PC, 33PC, 34PC, 35PC and 36PC presented the same nucleotide sequence and secondary structure (Fig. 3m). Strains 47PC and 48PC showed the same sequences and secondary structures, but these were different from the other strains (Fig. 3n).

Discussion

Morphology has been the basis for the taxonomic identification and traditional classification systems of cyanobacteria, such as by Gomont (1892), Geitler (1932) and Komárek & Anagnostidis (2005). However, polyphasic approach, mainly after the inclusion of molecular data, has revealed that morphological characters are insufficient to delimit species and understand the evolutionary history of *Cyanobacteria*. Recent taxonomic studies of members of the *Oscillatoriales* have shown that phenotypically similar organisms can exhibit considerable genotypic diversity (Siegesmund *et al.*, 2008; Komárek *et al.*, 2013; Hašler *et al.*, 2014; Strunecký *et al.*, 2014; McGregor & Sendall, 2015; Martins *et al.*, 2016). The failure of morphological characters to reflect

biodiversity has been increasingly evident with the many new taxonomic entities that have been described based mainly on molecular data (16S rRNA gene and 16S–23S ITS) in the last few years. Many of these were found in less well-studied geographical locations, such as tropical habitats (Fiore *et al.*, 2007; Genuário *et al.*, 2015; Malone *et al.*, 2015; Vaz *et al.*, 2015; Martins *et al.*, 2016).

The polyphyly of *Phormidium*, previously reported by many authors (Turicchia *et al.*, 2009; Strunecký *et al.*, 2011, 2014; Casamatta *et al.*, 2012; Malone *et al.*, 2015), was also corroborated in this study, indicating the heterogeneity of the genus and reinforcing questions about the fragility of the morphological data in delimitation of taxa.

The phylogenetic analyses presented indicate that the group comprising Brazilian and European strains (*Phormidium aerugineo-caeruleum* MNZ-37, MNZ-32, MNZ-31, MED-28, MED-31, MED-17) defines a new genus, hereafter named *Potamolinea*, with strong support (99 % ML, 100 % NJ and 1 BI) and distant from 'true *Phormidium*', according to Sciuto *et al.* (2012). The high similarity of 16S rRNA gene sequences among *Potamolinea* strains (95.3–99.9 %) and low similarity between *Potamolinea* and other genera (<93.8 %) corroborate that they belong to a consistent and distinct generic entity. Morphological and ecological data also confirm *Potamolinea* as a well-delimited genus, as strains that comprise it have similar morphology of apex of trichomes (not attenuated, with rounded or obtuse apical cells) that corresponds to *Phormidium* group V (Komárek & Anagnostidis, 2005) and were found in freshwater benthos of lotic ecosystems.

Considering morphological and ecological characteristics, it is reasonable to consider that other species of *Phormidium* group V of Komárek & Anagnostidis (2005) that are found in the same type of environment belong to this genus: *Phormidium granulatum* (Gardner) Anagnostidis, *P. retzii* (Agardh) Gomont ex Gomont, *P. taylorii* (Drouet et Strickland) Anagnostidis and *P. tergestinum* (Kützing) Anagnostidis et Komárek. However, this hypothesis will need to be supported by molecular data, and these are missing for the cited species.

Similarity scores of 16S rRNA gene sequences among strains of the same subgroup were high, indicating that this analysis was sufficient to separate the different subgroups and each corresponds to a species of *Potamolinea*: A, *P. aerugineo-caerulea* comb. nov; B, *Potamolinea* sp.; and C, *P. magna* sp. nov. *Potamolinea aerugineo-caerulea* showed greater variation in the 16S rRNA gene, but this is probably related to its geographical distribution, as European strains were closer to each other (98.5–99.7 % similarity) than to Brazilian lineages.

The ITS sequences presented little infraspecific variation and significant distinction among species, corroborating analyses based on 16S rRNA gene sequences. The secondary structures of D1-D1′, box-B and V3 regions were conserved within each species, with small variations in D1-D1′ and box-B of *Potamolinea aerugineo-caerulea* strains, which were not sufficient to separate them into different species. Thus, the results confirm ITS as an important marker, adequate for species-level recognition/distinction, as observed by Boyer *et al.* (2001, 2002), Bohunická *et al.* (2011), Johansen *et al.* (2011), Perkerson *et al.* (2011) and Malone *et al.* (2015).

Although morphological data are not considered to be phylogenetically informative (Casamatta *et al.*, 2003; Muhlsteinová *et al.*, 2014; Osorio-Santos *et al.*, 2014; Martins *et al.*, 2016), our results showed that they are important for species identification in *Potamolinea*. The species can be distinguished based on cell content (granular in *Potamolinea aerugineo-caerulea* and homogeneous in the others), characteristics of cross-walls (inconspicuous in *P. aerugineo-caerulea* and conspicuous in the others, granulated only in *P. magna*) and morphology of the apical cell (truncate in *Potamolinea* sp. and rounded in the other species). Although overlapping in *P. aerugineo-caerulea* and *Potamolinea* sp., filament and trichome width are clearly wider in *P. magna*. Sheaths are facultative in all populations studied and cell length showed a wide and overlapping variation and therefore these characters are not relevant for species distinction.

Brazilian populations of *Potamolinea aerugineo-caerulea* are morphologically very similar to populations of *Phormidium aerugineo-caeruleum* described in several classic taxonomic studies (Gomont, 1892; Geitler, 1932; Komárek & Anagnostidis, 2005) and to Spanish populations described by Loza *et al.* (2013). They are also similar according to their occurrence habitat: lotic ecosystems. The 16S rRNA gene sequences of Spanish populations allowed to compare them at the genetic level to Brazilian material, which revealed a very close relationship. Although the two geographical/climatic regions are distinct, a number of convergent characteristics suggest that all populations pertain to the same species. It is possible and reasonable to consider that some genotypes enable individuals of the same species to adapt to a wide range of environmental conditions (eurytolerant) if these are not extreme. The similarities found are robust in different markers, justifying the transfer of *Phormidium aerugineo-caeruleum* to the genus *Potamolinea*.

Potamolinea sp. strains presented morphological and ecological characteristics that are in agreement with those cited for *Phormidium retzii* by Gomont (1892), Geitler (1932) and Komárek & Anagnostidis (2005). Besides all morphometric characters, these included the formation of truncate apical cells and the occurrence in flowing water for all the populations studied. However, they were not identified as *Phormidium retzii* because they were collected in streams of a tropical region that can be considered very different from the type locality (streams of southern Sweden). Despite the high number of occurrences of this species reported around the world (Sheath & Cole, 1992; Branco *et al.*, 1999; Komárek & Anagnostidis, 2005; McGregor, 2007), there are no 16S rRNA gene sequences available in GenBank,

which should be an imperative to confirm the identification (or not) of *Potamolinea* sp. as *Phormidium retzii* and to effectively proceed to transfer it to the new genus. Nevertheless, based on the results of the present study for *Potamolinea aerugineo-caerulea*, this is highly likely when molecular data of European strains of *Phormidium retzii* become available.

The phylogenetic relationships of cyanobacteria based on analyses of molecular markers have been accepted as the decisive criteria for their classification (Komárek *et al.*, 2014). The 16S rRNA gene is most frequently used for generic definition and a combined analysis with ITS is used to achieve a higher taxonomic resolution. However, other characters must also be considered, mainly in species identification. Thereby, *Potamolinea* is shown to be very similar based on morphology, ecology and molecular data, and is probably widely distributed, occurring in similar environmental conditions around the world.

Description of the genus and species

Potamolinea **Martins et Branco gen. nov.** Diagnosis: Thallus gelatinous, mucilaginous, attached to the substrate, dark blue–green. Filaments densely entangled, trichome motility present. Sheaths facultative, attached to trichomes, firm, thin, colourless, hyaline. Trichomes isopolar, cylindrical along their whole length, not constricted or slightly constricted at the cross-walls, not attenuated toward the apex, 6–16.8 μm wide. Cells isodiametric or shorter or longer than wide. Cell content finely granular or with scattered larger granules. Apical cell rounded, without calyptra. Heterocytes and akinetes missing. Reproduction by disintegration of trichomes by necridic cells into hormogonia.

Etymology: *Potamolinea* (Po.ta.mo.li′ne.a. Gr. n. *potamos* river; L. n. *linea* line; N.L. fem. n. *Potamolinea* filament from a river).

Type species: *Potamolinea magna* Martins & Branco sp. nov.

Potamolinea magna **Martins & Branco sp. nov.** Thallus mucilaginous, dark blue–green. Filaments densely entangled, 13.2–16.8 μm wide. Sheaths facultative, attached to trichomes, firm, thin, colourless. Trichomes motile, cylindrical, not attenuated, not constricted to slightly constricted at the ungranulated or slightly granulated cross-walls, 9.6–16.8 μm wide. Cells 0.4–0.9 time longer than wide, 4–12.8 μm long. Cell content blue–green, homogeneous to finely granulated. Apical cell rounded, without calyptra.

Etymology: *magna* (mag′na. L. fem. adj. *magna* large, referring to the greater diameter of trichomes).

Type locality: Jacaré stream. Municipality of Neves Paulista, São Paulo State, Brazil; 20° 51′ 39″ S 49° 36′ 54″ W.

Habitat: stream bottoms.

Holotype: formaldehyde-fixed sample of strain 47PC deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, voucher number SJRP 31642.

Potamolinea aerugineo-caerulea **(Gomont) comb. nov.** Basionym: *Lyngbya aerugineo-caerulea* Gomont, Ann. Sci. nat. 7, Bot. 16 : 146, 1892.

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