POLYPHASIC APPROACH USING MULTILOCUS ANALYSES SUPPORTS THE ESTABLISHMENT OF THE NEW AEROPHYTIC CYANOBACTERIAL GENUS PYCNACRONEMA (COLEOFASCICULACEAE, OSCILLATORIALES)¹

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A new Phormidium-like genus was found during an investigation of Oscillatoriales diversity in Brazil. Eight aerophytic populations from south and southeastern regions were isolated in monospecific cultures and submitted to polyphasic evaluation. The populations presented homogeneous morphology with straight trichomes, not attenuated, and apical cell with thickened cell wall. Phylogenetic analyses based on 16S rRNA gene sequences showed that these populations, plus the Brazilian strain Phomidium sp. B-Tom from GenBank, formed a highly supported and distinctive clade, which corresponds to the new genus Pycnacronema, comprising six new species: P. brasiliensis (type species), P. arboriculum, P. conicum, P. marmoreum, P. rubrum, and P. savannensis. These results were confirmed and supported by rpoC1 and rbcL genes evaluated independently and by the concatenated analysis of 16S rRNA, rpoC1 and rbcL genes (for all species but P. savannensis). Secondary structures of the D1-D1', box-B, and V3 regions of the internal transcribed spacer were informative at specific level, being conserved in P. brasiliensis and variable among the other strains, also confirming the phylogenetic analyses. The generic name and specific epithets of the new taxa are proposed under the provisions of the International Code of Nomenclature of algae, fungi, and plants.

Abbreviations: BI, Bayesian inference; ML, maximum likelihood

Over the last 20 years, taxonomic studies of cyanobacteria based on a polyphasic approach have revealed a much greater generic diversity than previously suspected, and many recently described genera would have been mistakenly identified as members of traditional genera (e.g., Phormidium, Lyngbya, and Leptolyngbya) if only evaluated on their morpho-anatomical features. DNA sequences and their secondary structure play a key role in identifying cyanobacterial taxa and resolving their phylogenetic relationships.

The 16S rRNA gene is the marker universally used to assess evolutionary relationships among prokaryotes, and is the basis for recognizing new taxa from subclasses through to even species and reorganizing the entire classification system of cyanobacteria (Komárek et al. 2014). However, this gene alone is insufficient to resolve species (Boyer et al. 2002, Engene et al. 2010, Kim et al. 2014). Thus, the secondary structures of the conserved domains D1-D1', box-B, and V3 of the 16S-23S internal transcribed spacer (ITS; Iteman et al. 2000) have been used to assess the infrageneric diversity (Johansen et al. 2011, Osorio-Santos et al. 2014, Martins and Branco 2016). Alternatively, better phylogenetic resolution, especially at infrageneric ranks, can be obtained by using protein-coding genes due to their slow and constant rate of evolution (Glæsner and Kämpfer 2015). However, studies have shown that single protein-coding genes may not reflect the true evolutionary relationships among species (Jordan et al. 2002, Zeigler 2003) and that multiple gene-based phylogenies are better for resolving these relationships (Stackebrandt et al. 2002, Tănabei et al. 2007, Mazard et al.
2012, Sciuto et al. 2012, Malone et al. 2015). Two genes that have been used in multilocus sequence analysis are rpoC1 and rbcL, since they code for subunits of ubiquitous enzymes, the γ-subunit of RNA polymerase (rpoC1), and the large subunit of ribulose-1,5-biphosphate carboxylase/oxidase (rbcL), respectively.

Genetic markers are indispensable in taxonomic and phylogenetic studies and should form their basis, but morphology and ecology are still very important characters and must be considered in cyanobacterial diversity (Komárek 2016). Herein, we studied eight cyanobacteria populations morphologically resembling Phormidium sensu lato. Komárek and Anagnostidis (2005) recognized the heterogeneity of the genus and divided it into eight non-taxonomic clusters based on morphological characteristics of trichome tips. The type species (lectotype) is P. lucidum, known only from the original description and illustration and placed in Komárek and Anagnostidis’ group VIII, which includes the species with trichomes shortly attenuated toward the apices and apical cells with calyptra or with thickened outer cell walls. Sciuto et al. (2012) argued that, as the P. lucidum type is known only by illustration, the species P. irriguum (strain CCALA 759) could represent the type species since both are members of group VIII (Komárek and Anagnostidis 2005). This proposal has been largely accepted, and P. irriguum has been used to represent the genus in studies where several new genera related to Phormidium have been erected (e.g., Strunečková et al. 2014, Engene et al. 2015, Malone et al. 2015, Brito et al. 2017, Buch et al. 2017). We used multilocus sequence analysis (16S rRNA, rpoC1, and rbcL genes) along with primary and secondary structures of 16S-23S ITS, morphological traits and ecological data to propose Pycnacronema gen. nov. comprising six new species.

**MATERIAL AND METHODS**

**Origin of strains and culturing.** Eight environmental samples, growing on aerophytic habitats, such as tree bark, rocks, and soil, were collected in different localities of southeastern (tropical) and southern (subtropical) Brazil (Table 1). Each cyanobacterial strain was isolated from a single trichome grown on BG11 medium (Rippka et al. 1979). Trichomes from 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, Leica Microsystems GmbH, Wetzlar, Germany). The single trichomes were placed in tubes and inoculated with BG11 growth medium to establish unicellular cyanobacterial cultures. Strains were cultured and maintained under 20°C ± 1°C, 50 μmol photons m⁻² s⁻¹ irradiance and a 14:10 h light:dark cycle in the Culture Collection of IBILCE/UNESP.

**Morphological characterization and identification.** Morphological variability in populations was evaluated from fresh field material and from cultured samples using an Olympus BH2 (Olympus Optical Co., Ltd., Tokyo, Japan) microscope.

Taxonomic features, such as cell width, cell length, attenuation of trichomes, and apical cell shape and dimensions, were analyzed from a minimum of 30 trichomes from each sample. Representatives of each species here studied were deposited in Herbarium SJR (IBILCE/UNESP), Brazil.

**Molecular methods and sequencing.** The biomass for DNA extraction was obtained from non-axenic unicellular cyanobacterial cultures by repeated centrifugation. During the centrifugation process, filaments were washed several times with sterile deionized water to remove or reduce mucilage and suspensions of medium substances. DNA was extracted using the PowerSoil DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer’s protocol.

The 16S rRNA gene and 16S-23S ITS were amplified using polymerase chain reaction (PCR) using primers 16S27F and 23S50R (Taton et al. 2003) and performed in a Techgene TC-512 thermal cycler (Techne Inc., Burlington, NJ, USA) using 25 μL reaction volumes containing 5 μL of 10× PCR buffer, 2 μL of 50 mM MgCl₂, 1 μL of 10 mM dNTP mix, 1.25 μL of each primer (5 pmol), 14.2 μL of Milli-Q water, 1.5 U Platinum Taq DNA polymerase (Life Technologies, Carlsbad, CA, USA), and 10 ng of genomic DNA. Thermal cycling was performed at 94°C for 3 min, followed by 10 cycles of 94°C, 45 s; 57°C, 45 s; 72°C, 2 min; 25 cycles of 92°C, 45 s; 54°C, 45 s; and one final step of 72°C, 7 min.

The rbcL and rpoC1 loci were amplified as described by Fewer et al. (2007) and Malone et al. (2015), respectively. The PCR products were analyzed on 1% agarose gels stained with GelRed 0.6X (Biotium, Fremont, CA, USA) and viewed on a “Mini Bis Pro” transilluminator (Micro Photonics, Allen-town, PA, USA).

The positive products of 16S rRNA gene and 16S-23S ITS fragments were cloned using pGEM-T Easy Vector System I (Promega, Madison, WI, USA), 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, Waltham, MA, USA). The purified (NucleoSpin Gel and PCR Clean-up kit; Macherey-Nagel, Düren, Germany) PCR products of rbcL and rpoC1 genes were sequenced.

Sequencing was performed on an ABI 3130 sequencer, using “BigDye Terminator v3.0 Cycle Sequencing Ready Reaction” kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer’s protocol. M13F and Sp6R primers corresponded to the vector sites and the internal primers 357F, 704R, 114F, and 1494R (Neilan et al.1997) were used for sequencing of 16S rRNA gene and 16S-23S ITS sequences. Primers rbcLF and rbcLR (Fewer et al. 2007) were used for sequencing of rbcL gene and the primers rpoC3 and rpoC4 (Głowacka et al. 2011), for rpoC1 gene. DNA fragments were assembled into contigs using the Phred/Phrap/Consed software (Ewing and Green 1998, Ewing et al. 1998, Gordon et al. 1998), and only bases with quality higher than 20 were considered.

**Alignment and phylogenetic analyses.** Sequences were aligned using the software ClustalW v1.8 (Thompson et al. 1994) in MEGA 6.06 (Tamura et al. 2013) and inspected and refined manually. They were compared to 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 sequences were added to the obtained sequences for the analyses. Phylogenetic analyses were performed for separate loci and concatenated sequences. The appropriate nucleotide substitution model for each gene was selected in jModelTest 2.1.1 (Darriba et al. 2012). Gloeobacter violaceus PCC 7421 (GenBank accession number NC005125) was used as the outgroup. The Bayesian (BI) analysis was performed in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001), with GTR+G+I model

<table>
<thead>
<tr>
<th>Strain</th>
<th>Name</th>
<th>Locality</th>
<th>Substrate</th>
<th>Latitude (S)</th>
<th>Longitude (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41PC</td>
<td><em>Pycnacronema arboriculum</em></td>
<td>Region of Campos do Jordão/SP</td>
<td>Tree bark</td>
<td>22°40'38&quot;</td>
<td>45°38'54&quot;</td>
</tr>
<tr>
<td>44PC</td>
<td><em>P. brasiliensis</em></td>
<td>UNESP campus - SJRP/SP</td>
<td>Tree bark</td>
<td>20°47'07&quot;</td>
<td>49°21'37&quot;</td>
</tr>
<tr>
<td>45PC</td>
<td><em>P. brasiliensis</em></td>
<td>UNESP campus - SJRP/SP</td>
<td>Tree bark</td>
<td>20°47'04&quot;</td>
<td>49°21'33&quot;</td>
</tr>
<tr>
<td>46PC</td>
<td><em>P. brasiliensis</em></td>
<td>Forest reminiscent - Macaúbal/SP</td>
<td>Tree bark</td>
<td>20°44'25&quot;</td>
<td>49°25'51&quot;</td>
</tr>
<tr>
<td>7PC</td>
<td><em>P. conicum</em></td>
<td>Historic Center - Parati/RJ</td>
<td>Tree bark</td>
<td>23°13'05&quot;</td>
<td>44°43'11&quot;</td>
</tr>
<tr>
<td>42PC</td>
<td><em>P. marmoratum</em></td>
<td>UNESP campus - SJRP/SP</td>
<td>Tree bark</td>
<td>20°47'07&quot;</td>
<td>49°21'37&quot;</td>
</tr>
<tr>
<td>43PC</td>
<td><em>P. rubrum</em></td>
<td>PUCRS campus/RS</td>
<td>Tree bark</td>
<td>30°03'30&quot;</td>
<td>51°10'17&quot;</td>
</tr>
<tr>
<td>40PC</td>
<td><em>P. savannensis</em></td>
<td>Casca D’Anta area - Canastra National Park/MG</td>
<td>Soil crust</td>
<td>20°21&quot;</td>
<td>46°38&quot;</td>
</tr>
<tr>
<td>B-Tom</td>
<td><em>Phormidium</em> sp.</td>
<td>Toninhas beach - Ubatuba/SP</td>
<td>Wet rock</td>
<td>23°28'51&quot;</td>
<td>45°04'15&quot;</td>
</tr>
</tbody>
</table>

Results

Morphology and ecology. The eight populations studied were found growing as dark green or brown macroscopic mats on different types of aephytic substrate (Table 1). In addition, one population described in the literature, recorded in GenBank as *Phormidium* sp. B-Tom strain and studied by Lokmer (2007) from material collected in Brazil (on stones near the seashore, probably at Toninhas’ Beach, Ubatuba Municipality), was included in the analyses.

All populations presented a uniform morphology corresponding to species included in *Phormidium*-group V, characterized by Komárek and Anagnostidis (2005) as having cylindrical trichomes with widely rounded or obtuse apical cells without calyptra and belonging to three different morphotypes (Fig. 1). All of the group V morphotypes morphologically differ from *P. cf. irriguum* CCALA 759 that Sciuto et al. (2012) proposed to represent the genus *Phormidium*, and which presents trichomes cylindrical with cells distinctly shorter than wide.

One morphotype (strains 7PC, 41PC, 44PC, 45PC, 46PC, 49PC, and *Phormidium* B-Tom) presented filaments with thin and colorless sheaths; trichomes not constricted to slightly constricted at the ungranulated cross walls; cells isodiametric; cell content blue-green; apical cell cylindrical, with rounded or conical-rounded apex, with thickened outer cell wall (Fig. 1, a–h, r–t; Table 2). The second morphotype, represented by strain 42PC, presented filaments with thin and colorless sheaths; trichomes not constricted to slightly constricted at the ungranulated or granulated cross walls; cells isodiametric; cell content red-brownish with keratinized chromatoplasm; apical cell rounded, with thickened outer cell wall (Fig. 1, i–k; Table 2). The third morphotype (strain 43PC) presented filaments with thin and colorless sheaths; trichomes distinctly constricted at the cross walls; cells isodiametric; cell content red-brownish, homogeneous, or with large prominent granules; apical cell rounded, with thickened outer cell wall (Fig. 1, l–p; Table 2).

Phylogenetic analyses – 16S rRNA, rbcL, and rpoCl. Comparing the 16S rRNA gene partial sequences (1,164–1,485 bp) of the eight studied strains with sequences in GenBank revealed similarities above 97% with *Phormidium* sp. B-Tom (EU196618) and below 95% with the other strains’ sequences. The 16S rRNA sequences of the studied populations and *Phormidium* sp. B-Tom were clustered in a separate clade (hereafter named *Pycnacronema* clade; Fig. 2) and were clearly apart from the other strains, including the *Phormidium* clade, represented by *Phormidium* cf. *irriguum* CCALA 759 and *P. irriguum* f. minor ETS-02 strains, according to Sciuto et al. (2012).

The tree obtained with BI analysis is the tree reported herein (Fig. 2). Phylogenetic analyses showed that the *Pycnacronema* clade is highly supported by ML and BI analyses (98% and 1, respectively; Fig. 2) and the similarity scores of the sequences within this cluster were higher than 96.5% (Table S1 in the Supporting Information). Members of this clade are also positioned in a statistically well supported group together with
FIG. 1. Photomicrographs of Pycnacronema species: (a–c) *P. brasiliensis*; (d–f) *P. arboriculum*; (g–h) *P. conicum*; (i–k) *P. marmoreum*; (l–p) *P. rubrum*; (r–t) *P. savannensis*. Bars, 10 μm.
were unable to sequence these genes available for cyanobacteria, including genes (Figs. 3 and 4 respectively) contained fewer strongly supported clade (in both phylogenies 100- that strains 44PC, 45PC, and 46PC formed a supported the results of the 16S rRNA gene tree Nevertheless, the phylogenetic analyses of both genes were lower than 94.0% (Table S2).

P. marmoreum 42PC - Brown-reddish P. conicum 46PC - Blue-green P. brasiliensis 49PC - Blue-green P. rubrum 43PC, 45PC, and 46PC formed a clade, positioned sister to all remaining strains; strains 7PC and 41PC formed a highly supported internal clade (97%-ML and 1-BI); strain 43PC was distinct; strains 44PC, 45PC, and 46PC were in a highly supported clade (100%-ML and 1-BI), as also observed in all single gene analyses.

Molecular analyses – 16S-23S ITS. The analysis of the 16S-23S ITS region for all Pycnacronema strains was informative and supported other DNA sequence data, mainly the 16S rRNA gene. All strains had operons only with the tRNAAla gene, and the length of the spacer ranged from 364 to 433 bp (Table 3). Only three out of 12 ITS regions had the same length in all strains with the spacer preceding box-B helix presenting the greatest variation in length. The V2 region was not identified, as it is located between tRNAAla and tRNALeu genes, the latter absent in the strains studied (Table 3). The basal sequences in the helices were, in most of the strains, conserved and, thus, secondary structure could be determined (Fig. 6).

D1-D1′, box-B, and V3 regions were highly variable in primary structure and exhibited six different patterns of secondary structure among the strains.

**Table 2. Morphological comparison among Pycnacronema species.** Dimensions (µm) represent full ranges observed. L/W, cell length/cell width ratio; -, absence; +, presence.

<table>
<thead>
<tr>
<th>Species/strain</th>
<th>Granules</th>
<th>Cell content</th>
<th>Apical cell</th>
<th>Filament width (n = 30)</th>
<th>Trichome width (n = 30)</th>
<th>Cell length (n = 150)</th>
<th>Apical cell width (n = 30)</th>
<th>Apical cell length (n = 30)</th>
<th>L/W</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. marmoreum</em></td>
<td>-</td>
<td>Blue-green</td>
<td>Conical-rounded</td>
<td>7.8-8</td>
<td>6.5-7.5</td>
<td>4.7-5</td>
<td>4.5-7.2</td>
<td>0.6-1.1</td>
<td></td>
</tr>
<tr>
<td><em>P. brasiliensis</em></td>
<td>41PC</td>
<td>homogeneous</td>
<td>Conical-rounded</td>
<td>5.5-8</td>
<td>4.8-7.2</td>
<td>3.6-7</td>
<td>5.5-6.8</td>
<td>4.6-8</td>
<td>0.6-1.2</td>
</tr>
<tr>
<td><em>P. brasiliensis</em></td>
<td>45PC</td>
<td>homogeneous</td>
<td>Conical-rounded</td>
<td>6-8</td>
<td>5.2-7.2</td>
<td>3.2-6.8</td>
<td>4.8-7.2</td>
<td>3.6-7</td>
<td>0.6-1.1</td>
</tr>
<tr>
<td><em>P. brasiliensis</em></td>
<td>46PC</td>
<td>homogeneous</td>
<td>Conical-rounded</td>
<td>5.5-7.2</td>
<td>5.6-7.2</td>
<td>3.2-6.8</td>
<td>5.5-7.2</td>
<td>4.7-2</td>
<td>0.5-1.2</td>
</tr>
<tr>
<td><em>P. conicum</em></td>
<td>7PC</td>
<td>homogeneous</td>
<td>Conical-rounded</td>
<td>7.2-8.8</td>
<td>6.4-7.6</td>
<td>4.9-6</td>
<td>4.6-7.4</td>
<td>4.8-9.2</td>
<td>0.6-1.2</td>
</tr>
<tr>
<td><em>P. marmoreum</em></td>
<td>42PC</td>
<td>Brown-reddish</td>
<td>Cylindrical-rounded</td>
<td>7.2-9.2</td>
<td>6.4-8</td>
<td>4-10.5</td>
<td>6.4-8</td>
<td>6.2-10.4</td>
<td>0.5-1.2</td>
</tr>
<tr>
<td><em>P. rubrum</em></td>
<td>43PC</td>
<td>+, -</td>
<td>Cylindrical-rounded</td>
<td>6-8.8</td>
<td>6-8</td>
<td>3.2-9.6</td>
<td>5.6-8</td>
<td>5.6-8.4</td>
<td>0.5-1.3</td>
</tr>
<tr>
<td><em>P. savannensis</em></td>
<td>49PC</td>
<td>+</td>
<td>Cylindrical-rounded</td>
<td>5-7</td>
<td>4-7</td>
<td>3.5-8</td>
<td>4-7</td>
<td>5.5-8</td>
<td>0.5-1.5</td>
</tr>
<tr>
<td>B-Tom a</td>
<td>NA</td>
<td>Blue-green</td>
<td>Conical-rounded</td>
<td>5.7-7.3</td>
<td>5.5-6.3</td>
<td>4.7-8</td>
<td>3.2-7.2</td>
<td>5.5-8.2</td>
<td>0.5-1.7</td>
</tr>
</tbody>
</table>

aAccording to Lokmer (2007) (filament width, cell length, and apical cell length were taken from figure 7). NA, data not available.

Potamolinea and Wilmottia strains, suggesting phylogenetic proximity among those genera, but the divergence of 16S rRNA gene sequences among them are higher than 4.9%.

Sequences of *Pycnacronema* strains formed a polytomy of four internal branches (A, B, C, and D; Fig. 2). However, only clade A, containing sequences of the strains 44PC, 45PC, 46PC, and *Phormidium* B-Tom, was highly supported (99%-ML, 1-BI; Fig. 2). Similarity scores among the 16S rRNA gene (Table S1) within clade A ranged from 99.2% to 99.9%, while between strains of group C (42PC and 43PC) and between strains of group D (7PC and 41PC) the scores were 97.1% and 98.3%, respectively. The similarity of 49PC (clade B) strain with the other strains of *Pycnacronema* ranged from 98.2% to 98.7%. Comparison of the *rpoC1* and *rbcL* partial sequences among the eight studied strains ranged from 82.2% to 100% and from 86.5% to 100%, respectively (Tables S2 and S3 in the Supporting Information). 16S rRNA gene similarity scores of sequences of *Pycnacronema* species with sequences of other genera, such as *Phormidium*, *Symphloea*, *Coleofasciculus*, *Kamptonema*, and *Microcoleus*, were lower than 94.0% (Table S2).

The phylogenetic trees based on *rpoC1* and *rbcL* genes (Figs. 3 and 4 respectively) contained fewer strains, because there are very few sequences of these genes available for cyanobacteria, including *Phormidium* sp. B-Tom. Despite several attempts, we were unable to sequence *rpoC1* for strain 49PC. Nevertheless, the phylogenetic analyses of both genes supported the results of the 16S rRNA gene tree that strains 44PC, 45PC, and 46PC formed a strongly supported clade (in both phylogenies 100-ML and 1-BI). Group D in the 16S rRNA gene tree (strains 7PC and 41PC; Fig. 2) had high support in *rpoC1* gene tree (Fig. 3, 100-ML and 1-BI), but those strains were distantly placed from each other in the *rbcL*-based tree (Fig. 4).

Molecular analyses and phylogeny – concatenated analyses. The topology of the multilocus-based (16S rRNA, *rpoC1*, and *rbcL* genes) phylogenetic tree (Fig. 5) was the same as the *rpoC1* gene analysis, with four internal clades. Strain 42PC diverged first in the *Pycnacronema* clade, positioned sister to all remaining strains; strains 7PC and 41PC formed a highly supported internal clade (97%-ML and 1-BI); strain 43PC was distinct; strains 44PC, 45PC, and 46PC were in a highly supported clade (100%-ML and 1-BI), as also observed in all single gene analyses.

Molecular analyses – 16S-23S ITS. The analysis of the 16S-23S ITS region for all *Pycnacronema* strains was informative and supported other DNA sequence data, mainly the 16S rRNA gene. All strains had operons only with the tRNAAla gene, and the length of the spacer ranged from 364 to 433 bp (Table 3). Only three out of 12 ITS regions had the same length in all strains with the spacer preceding box-B helix presenting the greatest variation in length. The V2 region was not identified, as it is located between tRNAAla and tRNAAsp genes, the latter absent in the strains studied (Table 3). The basal sequences in the helices were, in most of the strains, conserved and, thus, secondary structure could be determined (Fig. 6).

D1-D1’, box-B, and V3 regions were highly variable in primary structure and exhibited six different patterns of secondary structure among the strains.
FIG. 2. Bayesian Inference tree based on 16S rRNA gene sequences of oscillatorian cyanobacteria. Clade comprising *Pycnacronema* gen. nov., consisting of strains studied and *Phormidium* sp. B-Tom strain, is indicated. Names in boldface are genera erected from species of "traditional *Phormidium" or are genera morphologically related to *Phormidium." "True *Phormidium" clade, according to Sciuto et al. (2012), was also indicated. Bootstrap values (ML >70%) and probabilities (BI >0.7) shown at nodes. GenBank accession numbers in parentheses. Bar represents 0.1 substitutions per nucleotide position.

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D1-D1’ helix showed conserved primary and secondary structures in 44PC, 45PC, 46PC, and Phormidium B-Tom, whereas it was variable in strains 7PC, 41PC, 42PC, 43PC, and 49PC (Fig. 6, a–f; Table S4). The same pattern was observed for box-B (Fig. 6, g–m), except for a low variation in primary structure of strain Phormidium B-Tom compared to 44PC, 45PC, and 46PC (two divergent nucleotides, Table S4). V3 helices (Fig. 6, n–s) had primary and secondary structures conserved in strains 44PC, 45PC, 46PC, and Phormidium B-Tom and variable in strains 7PC, 41PC, 42PC, 43PC, and 49PC.

On the basis of the results of the phylogenetic analyses, we propose the following taxonomic novelties:

**Pycnacronema** M.D.Martins et Branco gen. nov.

**Description:** Mats attached to the substrate. Filaments densely entangled, trichome motility present. Sheaths facultative, firm, thin, colorless, hyaline. Trichomes isopolar, cylindrical along their whole length, not constricted or constricted at the cross walls, not attenuated toward the apex, 4.8–8 μm wide. Cells isodiametric or shorter or longer than wide. Cell content finely granular or with scattered larger granules. Apical cell rounded or conical-rounded, without calyptra, with thickened cell wall. Heterocytes and akinetes absent. Reproduction by disintegration of trichomes into hormogonia.

**Etymology:** *Pycnacronema* (Pyc.na.cro.ne’ma. Gr. adj. *pyknos* = thick; Gr. neut. n. *akro* = tip; Gr. neut. n. *nema* = filament; N.L. neut. n. *Pycnacronema*, a filament with thickened outer membrane at the apical cell).

*Type species:* *Pycnacronema brasiliensis* M.D.Martins et Branco sp. nov.

**Pycnacronema brasiliensis** M.D.Martins et Branco sp. nov.

**Description:** Thallus dark blue-green. Filaments entangled, 5.5–8 μm wide. Sheaths firm, thin,
homogenous, colorless. Trichomes motile, cylindrical, not attenuated, slightly constricted at the ungranulated cross walls, 4.8–7.2 μm wide. Cells cylindrical, shorter to longer than wide, 3.2–7.2 μm long. Cell content blue-green, homogeneous. Apical cells slightly conical-rounded, with thickened cell wall, 6.5–7.5 μm wide, 4.5–7.2 μm long.

Holotype: formaldehyde-fixed sample of strain 45PC deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, voucher number SJRP 31641, collected by Mariëllen D. Martins on December 7, 2012.

Pycnacronema arboriculum M.D.Martins et Branco sp. nov.

Description: Thallus dark blue-green. Filaments entangled, 7–8 μm wide. Sheaths firm, thin, homogenous, colorless. Trichomes motile, cylindrical, not attenuated, slightly constricted at the ungranulated cross walls, 6.5–7.5 μm wide. Cells cylindrical, shorter to longer than wide, 4–7.5 μm long. Cell content blue-green, homogeneous. Apical cells slightly conical-rounded, with thickened cell wall, 6.5–7.5 μm wide, 4.5–7.2 μm long.

Fig. 5. Maximum Likelihood tree based on a multiple alignment of 16S rRNA gene + rpoC1 + rbcL sequences. The clade comprising Pycnacronema gen. nov. strains is indicated. Bootstrap values (>85%) and probabilities (>0.85) obtained from ML/BI methods, respectively, are displayed at the relevant nodes. GenBank accession numbers are shown in parentheses. Bar represents 0.05 substitutions per nucleotide position.

Table 3. Lengths of 16S-23S ITS regions (number of nucleotides) in the analyzed Pycnacronema strains.

<table>
<thead>
<tr>
<th>Species/strain</th>
<th>Complete ITS</th>
<th>Leader</th>
<th>D1-D1′ helix</th>
<th>D2 with spacer</th>
<th>D3 with spacer</th>
<th>tRNA^Lys gene</th>
<th>Pre-Box-B spacer</th>
<th>Box-B helix</th>
<th>Post-Box-B spacer</th>
<th>Box-A</th>
<th>D4</th>
<th>V3 with spacer</th>
<th>D5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pycnacronema arboriculum 41PC</td>
<td>424</td>
<td>7</td>
<td>59</td>
<td>31</td>
<td>18</td>
<td>74</td>
<td>76</td>
<td>36</td>
<td>18</td>
<td>12</td>
<td>7</td>
<td>65</td>
<td>21</td>
</tr>
<tr>
<td>P. brasiliensis 44PC</td>
<td>433</td>
<td>7</td>
<td>58</td>
<td>31</td>
<td>18</td>
<td>74</td>
<td>84</td>
<td>40</td>
<td>18</td>
<td>12</td>
<td>7</td>
<td>63</td>
<td>21</td>
</tr>
<tr>
<td>P. brasiliensis 45PC</td>
<td>433</td>
<td>7</td>
<td>58</td>
<td>30</td>
<td>19</td>
<td>74</td>
<td>84</td>
<td>40</td>
<td>18</td>
<td>12</td>
<td>7</td>
<td>63</td>
<td>21</td>
</tr>
<tr>
<td>P. brasiliensis 46PC</td>
<td>432</td>
<td>6</td>
<td>58</td>
<td>31</td>
<td>18</td>
<td>74</td>
<td>84</td>
<td>40</td>
<td>18</td>
<td>12</td>
<td>7</td>
<td>63</td>
<td>21</td>
</tr>
<tr>
<td>P. conicum 7PC</td>
<td>364</td>
<td>7</td>
<td>63</td>
<td>28</td>
<td>37</td>
<td>74</td>
<td>41</td>
<td>37</td>
<td>23</td>
<td>12</td>
<td>7</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>P. marmoreum 42PC</td>
<td>375</td>
<td>7</td>
<td>62</td>
<td>34</td>
<td>19</td>
<td>74</td>
<td>31</td>
<td>36</td>
<td>18</td>
<td>12</td>
<td>7</td>
<td>63</td>
<td>12</td>
</tr>
<tr>
<td>P. rubrum 43PC</td>
<td>429</td>
<td>7</td>
<td>58</td>
<td>31</td>
<td>18</td>
<td>74</td>
<td>88</td>
<td>39</td>
<td>18</td>
<td>12</td>
<td>7</td>
<td>59</td>
<td>18</td>
</tr>
<tr>
<td>P. savannensis 49PC</td>
<td>422</td>
<td>6</td>
<td>57</td>
<td>32</td>
<td>18</td>
<td>74</td>
<td>80</td>
<td>36</td>
<td>18</td>
<td>12</td>
<td>7</td>
<td>64</td>
<td>18</td>
</tr>
<tr>
<td>B-Tom</td>
<td>429</td>
<td>7</td>
<td>58</td>
<td>31</td>
<td>18</td>
<td>74</td>
<td>80</td>
<td>40</td>
<td>18</td>
<td>12</td>
<td>7</td>
<td>63</td>
<td>21</td>
</tr>
</tbody>
</table>
FIG. 6. Secondary structure of conserved regions of 16S-23S ITS of Pycnacronema strains. (a-f) D1-D1' helices. (g-m) Box-B helices. (n-s) V3 helices.
Representative sequences (GenBank codes – *Pycnacronema arboriculum*, strain 41PC): MF581657 – 16S rRNA gene; MF566084 – rpoC1; MF566091 – rbcL.

Etymology: *arboriculum* (L. adj. n. ar.bo.ri’.cu.lum, from bark of trees)

Type locality: Campos do Jordão State Park surroundings, municipality of Campos do Jordão, São Paulo State, Brazil (22°40’38” S, 45°38’54” W).

Habitat: on barks of trees.

Holotype: formaldehyde-fixed sample of strain 41PC deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, voucher number SJRP 31885, collected by Mariéllei D. Martins on March 24, 2012.

*Pycnacronema conicum* M.D. Martins et Branco *sp. nov.*

Figure 1, g and h

*Description:* Thallus dark blue-green. Filaments entangled, 7.2–8.8 μm wide. Sheaths firm, thin, homogenous, colorless. Trichomes motile, cylindrical, not attenuated, not or slightly constricted at the ungranulated cross walls, 6.4–7.6 μm wide. Cells cylindrical, shorter to longer than wide, 4.9–6 μm long. Cell content blue-green, homogenous. Apical cells conical-rounded, with thickened cell wall, 6.4–7.6 μm wide, 4.8–9.2 μm long. Representative sequences (GenBank codes – *Pycnacronema conicum*, strain 7PC): MF581656 – 16S rRNA gene; MF566083 – rpoC1; MF566090 – rbcL.

Etymology: *conicum* (L. adj. n. co’ni.cum, refers to the conical shape of the apical cell)

Type locality: Historic Center of Paraty, municipality of Paraty, Rio de Janeiro State, Brazil (23°13’03” S, 44°43’11” W)

Habitat: on barks of trees.

Holotype: formaldehyde-fixed sample of strain 7PC deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, voucher number SJRP 31652, collected by Mariéllei D. Martins on July 19, 2010.

*Pycnacronema marmoreum* M.D. Martins et Branco *sp. nov.*

Figure 1, i–k


Etymology: *marmoreum* (L. adj. n. mar’mo.re.um, keratinized aspect of chromatoplasm)

Type locality: IBILCE/São Paulo State University campus, municipality of São José do Rio Preto, São Paulo State, Brazil (20°21’07” S, 49°21’37” W).

Habitat: on barks of trees.

Holotype: formaldehyde-fixed sample of strain 42PC deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, voucher number SJRP 31651, collected by Mariéllei D. Martins on December 7, 2012.

*Pycnacronema rubrum* M.D. Martins et Branco *sp. nov.*

Figure 1, l–p

*Description:* Thallus dark blue-green. Filaments entangled, 6–8.8 μm wide. Sheaths firm, thin, homogenous, colorless. Trichomes motile, cylindrical, not attenuated, constricted at the granulated or ungranulated cross walls, 6–8 μm wide. Cells cylindrical, shorter to longer than wide, 3.2–9.6 μm long. Cell content brown-reddish, granulated. Apical cells rounded, with thickened cell wall, 5.6–8 μm wide, 5.6–8.4 μm long. Representative sequences (GenBank codes – *Pycnacronema rubrum*, strain 43PC): MF581657 – 16S rRNA gene; MF566086 – rpoC1; MF566093 – rbcL.

Etymology: *rubrum* (L. adj. n. ru’brum, reddish cell content)

Type locality: Pontifical Catholic University of Rio Grande do Sul State, municipality of Porto Alegre, Rio Grande do Sul State, Brazil (30°03’30” S, 51°10’17” W).

Habitat: bark of trees.

Holotype: formaldehyde-fixed sample of strain 43PC deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, voucher number SJRP 31640, collected by Mariéllei D. Martins on January 12, 2012.

*Pycnacronema savannensis* M.D. Martins, N.M. Machado-de-Lima et Branco *sp. nov.*

Figure 1, q–t

*Description:* Filaments entangled, 5–7 μm wide. Sheaths firm, thin, homogenous, colorless. Trichomes motile, cylindrical, not attenuated, not slightly constricted at the ungranulated cross walls, 4–7 μm wide. Cells cylindrical, shorter to longer than wide, 3.5–8 μm long. Cell content blue-green, homogenous. Apical cells rounded, with thickened cell wall, 4–7 μm wide, 5.5–8 μm long. Representative sequences (GenBank codes – *Pycnacronema savannensis*, strain 49PC): MF581663 – 16S rRNA gene; MF566091 – rbcL.

Etymology: *savannensis* (L. adj. fem. sa.van.nen’sis, from Brazilian savannah)

Type locality: Casca d’Anta waterfall, Serra da Canastra National Park, Minas Gerais State, Brazil (20°21’S, 46°38’W).

Habitat: soil crust.

Holotype: formaldehyde-fixed sample of strain 49PC deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, voucher number SJRP 31884, collected by Branco, L.H.Z. on May 23, 2013.

**DISCUSSION**

Phormidium, like several other traditional genera of cyanobacteria (e.g., Lyngbya, Oscillatoria, Microcoleus, Anabaena, Nostoc, and Scytomena), is
polyphyletic (Casamatta et al. 2005, Palinska and Marquardt 2008, Sciuto et al. 2012), and a broad revision has been recommended (Komárek and Anagnostidou 2005). Since the advent of DNA-based phylogenetic studies, mainly using the 16S rRNA gene, many new cyanobacterial taxa, from orders to genera and species, have been described, revealing that the genetic diversity is higher than the morphological diversity. In the last 15 years, more than 40 new genera of cyanobacteria were described mainly based on molecular data. This new source of information resulted in significant changes in cyanobacterial systematics by the reinterpretation of phylogenetic relationships and by the establishment of a new and continuously updated classification system. Although the proposal of Sciuto et al. (2012), to use Phormidium cf. iriguum to represent P. lucidum, the generitype species, has allowed taxonomic studies within the complex, it has to be further evaluated. Several studies have shown that morphological similarity does not guarantee genetic proximity in cyanobacteria, and this “substitution” is not recognized by the “International Code of Nomenclature for algae, fungi, and plants” (Turland et al. 2018). In addition, the designation of P. lucidum as lectotype of the genus itself is debatable. Droz (1968) indicated that the type species is P. inundatum, and Komárek and Anagnostidou (2005) argued that Phormidium should be proposed as nomen conservandum and re-typified. As some genera were proposed by considering Phormidium cf. iriguum as the generitype and a genetic reference of Phormidium, we used the same concept in the present work. However, the type species of Phormidium needs to be resolved and an epitype designated in accordance with the ICN (Turland et al. 2018), after which, the recently proposed genera erected from Phormidium need to be taxonomically reevaluated.

This study, based on eight aerophytic oscillato- rian populations from Brazil, proposes a new genus of cyanobacteria, Pycnacronema, strongly supported by phylogenetic analyses of three genes (16S rRNA, rpoC1, and rbcL) and by the concatenated dataset. Despite morphological (cylindrical homocystous trichomes, intermediate cell width with 4–10 μm) and ecological (occurrence in aquatic and aerophytic habitats) similarities, Pycnacronema species are genetically distantly positioned from Phormidium (as defined by Sciuto et al. 2012) and from related genera, such as Potamolinea and Wilmottia. The high similarity of the 16S rRNA gene sequences among the Pycnacronema strains (96.5%–99.9%) and the low similarity of Pycnacronema with sequences of other genera (<94.1%) corroborate the studied material belong to a consistent and distinct generic entity.

The strains of Pycnacronema are distributed in four internal clades (Fig. 2), corresponding to six distinct species. Pycnacronema brasiliensis (strains 44PC, 45PC, 46PC, and Phormidium B-Tom) was well supported by all the phylogenetic analyses, with high similarity scores for 16S rRNA gene among its strains (99.2%–99.9%). The other five species correspond to strains 7PC (P. conicum), 41PC (P. arbori culum), 42PC (P. marmoreum), 43PC (P. rubrum), and 49PC (P. savannensis). Although P. savannensis strain presented high similarity scores for 16S rRNA gene with other Pycnacronema strains (98.2%–98.7%), it was positioned in a solitary branch (clade B, Fig. 2) in all phylogenetic methods performed based on 16S rRNA gene (ML and BI).

Pycnacronema marmoreum and P. rubrum (clade C) presented a 97.1% similarity score for 16S rRNA gene sequences, indicating they belong to different species, according to the limits proposed by Kim et al. (2014) (98.65% minimum similarity threshold of 16S rRNA gene to two sequences are considered the same species, since both strands are sequenced and 16S rRNA gene sequences are complete). The same was observed for P. conicum and P. arboriculum (clade D), with 98.3% of similarity score between them.

The lack of support for the internal clades, except for clade A (Fig. 2), likely is a consequence of the high similarity in 16S rRNA gene sequences (96.5%–99.9%). The relatively low divergence in this gene may indicate recent speciation events, that are not indicated in the conserved 16S rRNA sequences.

The use of additional molecular markers was necessary to establish the distinction of the related strains. Although the phylogenetic trees based on rpoC1 and rbcL genes (Figs. 3 and 4) hypothesized different relationships among the Pycnacronema species from the 16S rRNA gene, they support the same number of species indicated by 16S rRNA gene analysis. The strains 44PC, 45PC, and 46PC of P. brasiliensis were again placed in a common clade, highly supported by bootstrap and posterior probabilities for both genes analyses. Pycnacronema marmoreum positioned as sister to the other Pycnacronema species and P. rubrum, as sister to P. brasiliensis was also revealed by 16S rRNA gene-based analysis. The analysis based on the rpoC1 gene revealed a sister taxon relationship between P. conicum and P. arboriculum that was not confirmed by the rbcL analysis. 16S rRNA similarity score (98.3%) between these two strains indicates, according to Kim et al. (2014), they belong to different species, corroborating the data from rbcL analysis. The number of available sequences for rpoC1 and rbcL is limited; the addition of new sequences will make phylogenetic hypotheses more robust.

The 16S rRNA and rpoC1 genes showed that Pycnacronema conicum and P. arboriculum are related, but the evaluation of D1-D1′, Box-B, and V3 domains of ITS revealed differences in their secondary structures. V3 helix showed higher distinction between these strains, whereas D1-D1′ and Box-B helices were similar, but they have different
primary structures (Fig. 6), reinforcing that the two species are really distinct.

The concatenated analysis performed in this study confirmed the general results of the analyses using each gene independently and, based on the strains studied, supported the existence of five species in *Pycnacronema* (rpoC1 could not be sequenced for *P. savannensis*). Nevertheless, the multilocus analysis more clearly supported the distinctions among the *Pycnacronema* species, resolving most of the polytomy in the 16S rRNA gene tree (Fig. 1). That result corroborates other studies that have pointed out that the use of more than one molecular marker (multilocus analyses) is a more appropriate way to reconstruct phylogenetic history and to reveal the real infrageneric diversity (Tanabe et al. 2007, Wu et al. 2011, Mazard et al. 2012, Li et al. 2014, Glaeser and Kämper 2015). The 16S rRNA gene due to its conservation is useful for generic placement in cyanobacteria (Boyer et al. 2001, Halinen et al. 2008, Wu et al. 2011), but, in some cases, does not distinguish species.

The rpoC1 and rbcL gene sequences showed greater variation than 16S rRNA (Tables S1, S3 and S5 in the Supporting Information) and, consequently, increased the resolution and robustness of the *Pycnacronema* branches (Figs. 2–4). The concatenation of these genes allowed the discrimination of *Pycnacronema* strains at the species level. The average genetic distance among species from these concatenated sequences was 6.2%, against 2.2% with the 16S rRNA gene alone, which indicates that phylogenetic analyses based on concatenated genes provides more resolution to distinguish cyanobacterial species.

16S-23S ITS sequences were considered informative for species distinction in *Pycnacronema*. The secondary structures of D1-D1’, Box-B, and V3 regions were conserved in the strains of *P. brasiliensis* (44PC, 45PC, 46PC, and *Phormidium* sp. B-Tom) and variable among the other species and strains (Fig. 6). These results showed that ITS can be a good molecular marker for species distinction in *Pycnacronema*, corroborating the same observation for other genera such as *Microcoleus* (Boyer et al. 2002), *Tabinothrix* (Bohunická et al. 2011), *Leptolyngbya* (Johansen et al. 2011), and *Potamolinea* (Martins and Branco 2016).

The arrangement of genera within families in cyanobacteria is still in flux. The reduced number of sequenced genera do not allow to address robust conclusions about the familial arrangement of the group. Nevertheless, the proximity among *Pycnacronema*, *Potamolinea*, *Wilmottia*, *Desertifilum*, and *Coleofasciculus* and the high posterior probability support values of the clade formed by these genera suggest that *Pycnacronema* is more closely related to the family Coleofasciculaceae than to Microcoleaceae, following the classification proposed by Komárek et al. (2014).

*Phormidium* sp. B-Tom has been included in other phylogenetic studies, and it has been interpreted to be related to the genus *Wilmottia* (Lokmer 2007, Strunecký et al. 2011, Martins and Branco 2016, Martins et al. 2016, Machado-de-Lima et al. 2017). However, its real identity has been debated. According to Lokmer (2007), this aerophytic strain would belong to *Phormidium*, while Strunecký et al. (2011) suggest it to pertain to the genus *Wilmottia*. Based on the morphological analysis performed by Lokmer (2007), it can be concluded that *Phormidium* sp. B-Tom is very similar to *Pycnacronema* species considering metric (trichome width, cell length, cell length/width ratio), apical cell form, cylindrical/attenuate the trichomes, and constriction (Table 2), whereas it can be morphologically distinguished of *Wilmottia murrayai* (described according to Komárek and Anagnostidis 2005 - as *Phormidium murrayi*) that develops narrower trichomes (3.1–3.3 µm). Although there are no rpoC1 and rbcL sequences available, the 16S rRNA gene and 16S-23S ITS sequences show that *Phormidium* sp. B-Tom and the strains 44PC, 45PC, and 46PC (*P. brasiliensis*) belong to the same genus. Thus, the DNA sequences, morphological and ecological data clearly indicate that it is a species of *Pycnacronema*. The ITS secondary structure analysis was also decisive to confirm that *Phormidium* sp. B-Tom is a strain of the species of *P. brasiliensis*.

Morphological characteristics of *Pycnacronema* populations were not totally congruent with analyses of DNA sequences. At the genus level, *Pycnacronema* can be distinguished from morphologically similar genera (e.g., *Potamolinea*, *Wilmottia*, and *Coleofasciculus*), by the presence of a thickened outer membrane at the apical cell of trichomes. However, whereas the DNA sequence data indicate the existence of six distinct species among the eight populations studied, the morphological traits differentiate just three morphotypes. *Pycnacronema marmoreum* and *P. rubrum* are distinct from other morphotypes by their consistent red-brownish cell content. Although they have overlapping filament and trichome dimensions (Table 2) and cylindrical-rounded apical cells, both strains can be separate by the cell content, that is keritomized in *P. marmoreum* and granulated in *P. rubrum*. Additionally, the constriction at the cross wall is inconspicuous in the first and conspicuous in the latter species (Fig. 1, i–p). The other four species, *P. conicum*, *P. arboriculum*, *P. brasiliensis*, and *P. savannensis*, cannot be distinguished by morphological characteristics, and are considered cryptic species. The detection of these four cryptic species showed that *Pycnacronema* is more diverse genetically than morphologically, which was also observed for other cyanobacterial genera, such as *Leptolyngbya* (Johansen et al. 2011), *Oculatella* (Osorio-Santos et al. 2014), *Ancylothrix* (Martins et al. 2016), and *Wilmottia* (Machado-de-Lima et al. 2017).
It is clear that morphological data traditionally used to distinguish genera and species are not properly related. The only morphological character observed in *Pycnacronema* that was not observed in its more related genus *Potamolinea* is the presence of a thickened outer membrane at the apical cell. However, these genera can also be distinguished by their ecology, as *Potamolinea* comprises the aquatic species and *Pycnacronema* includes aerophytic species. Although genetically distinct, *Pycnacronema* and *Potamolinea* are morphologically very close and the ecological data became very important for distinction of genera. The phylogeny based on the 16S rRNA gene suggest that these two genera have evolved from a common ancestor with significant physiological plasticity allowing descendants to cope with different environmental factors. Considering morphological and ecological features, it is reasonable to consider that other species of *Phormidium* with similar morphological characteristics and that are found in aerophytic habitats such as soils and bark of trees (e.g., *Phormidium kuetzingianum* and *Phormidium crouanii*) belong to *Pycnacronema*.

Thus, based on polyphasic approach, *Pycnacronema* is defined and differentiated from its closest genera essentially by DNA sequence data from the 16S RNA, *rpoC1*, and *rbcL* genes and the ITS marker. Morphological (presence of a thickened outer membrane) and ecological (aerophytic habitat) traits, although not exclusive to the genus, are complementary for its definition.

The use of DNA sequence data in biodiversity studies, particularly the analysis of 16S rRNA gene sequences, has been deeply modifying the cyanobacterial taxonomy, but even this genetic marker shows limitations due to its conservative nature. Thus, the inclusion of other genes in phylogenetic analyses allows for a better understanding of the complexity of evolution within cyanobacterial genera. These results argue for multilocus sequence analyses in order to refine the phylogeny and build a classification that reflects the evolutionary history of cyanobacteria, along with morphological and ecological data, which contribute to our understanding of these eubacterial photosynthesizers.

We thank the São Paulo Research Foundation (FAPESP 2010/09686-4, 2012/19468-0, 2013/08207-3) for financial support.


**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

**Table S1.** Similarity (%) of partial 16S rRNA gene sequences among *Pycnacronema* species/strains.

**Table S2.** Similarity of 16S rRNA gene of the *Pycnacronema* type species with strains of related genera.

**Table S3.** Similarity (%) of partial *rpoC1* gene sequences among *Pycnacronema* species/strains.

**Table S4.** Similarities (%) of each 16S-23S ITS domain (inferior part) and nucleotide divergence (number of nucleotides - upper part) among *Pycnacronema* species/strains.

**Table S5.** Similarity (%) of partial *rbcL* gene sequences among *Pycnacronema* species/strains.