Proposal of *Ancylothrix* gen. nov., a new genus of *Phormidiaceae* (Cyanobacteria, Oscillatoriales) based on a polyphasic approach

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During a study about the diversity of *Phormidioideae (Phormidiaceae*, *Oscillatoriales*) in Brazil, seven strains from southern and southeastern regions were isolated in monospecifc cultures and submitted to polyphasic evaluation (morphological, ecological, cytological and molecular studies). The populations studied were found to be morphologically similar to *Kamptonema* (filaments narrowed and bent at the end) and cytologically different (thylakoids' arrangement - radial distribution in Brazilian strains and parietal distribution in *Kamptonema*). The original habitats were very diverse among the Brazilian strains (freshwater, wet soil and barks of trees). Phylogenetic analysis based on 16S rRNA gene sequences revealed that the 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 *Ancylothrix* Martins et Branco gen. nov. with two species [*Ancylothrix rivularis* gen. nov., sp. nov. (type species) and *Ancylothrix terrestris* sp. nov.], distinguishable by differences in genetic and ecological characteristics and described under the provisions of the International Code of Nomenclature for algae, fungi and plants. Secondary structures of D1-D1′, box-B and V3 regions were conserved in *A. rivularis* gen. nov. sp. nov. and more variable in *A. terrestris* sp. nov.

During the last twenty-five years, the knowledge about evolutionary relationships among micro-organisms increased mainly due to 16S rRNA gene sequence analyses. This approach, initiated by Woese *et al.* (1990), has been widely used and became the basis for the definition of taxa in Cyanobacteria. Since then, many new genera have been described.

The revisions proposed by Komárek & Anagnostidis (2005) reorganized many cyanobacterial genera of the order *Oscillatoriales* based on polyphasic studies (ecological, biochemical and morphological characters such as thylakoid arrangement and type of cell division), although the majority of the genera recognized by morphological features are still not tested by molecular methods.

Abbreviations: ML, maximum-likelihood; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession numbers for the 16S-23S rRNA gene sequences of *Ancyclothrix rivularis* strains 7PC, 8PC and 9PC, and *Ancyclothrix terrestris* strains 10PC, 11PC, 12PC and 13PC are KT819196, KT819197, KT819198, KT819199, KT819200, KT819201 and KT819202, respectively.

Among the *Oscillatoriales, Phormidium* Kützing ex Gomont is considered a taxonomically complex genus and comprises many species of unclear delimitation. It is known to be polyphyletic, as follows from molecular analyses (Casamatta *et al.*, 2005; Taton *et al.*, 2006; Comte *et al.*, 2007; Marquardt & Palinska, 2007; Palinska & Marquardt, 2008; Strunecký *et al.*, 2010, 2011, 2012; Hašler *et al.*, 2012; Sciuto *et al.*, 2012).

Originally, *Phormidium* was composed of 29 species (Gomont, 1892, nomenclatural late starting point), and then Geitler (1932) presented 85 species for the genus. Later, Komárek & Anagnostidis (2005) listed about 160 species, including species originally described in the genera *Lyngbya* Agardh ex Gomont and *Oscillatoria* Vaucher ex Gomont.

Studies of the *Phormidium*-group based on phenotypic and genotypic data, mostly using 16S rRNA gene sequences, have revealed a wide diversity and heterogeneity. As a consequence, several new genera have been described, including *Phormidesmis* (Komárek *et al.*, 2009), *Wilmottia* (Strunecký *et al.*, 2011), *Oxynema* (Chatchawan *et al.*, 2012),

Roseofilum (Casamatta *et al.*, 2012), *Ammassolinea* (Hašler *et al.*, 2014) and *Kamptonema* (Strunecký *et al.*, 2014).

During an investigation about *Phormidioideae* diversity in Brazil, seven strains from very diverse habitats of tropical and subtropical regions with morphological resemblance to the genus *Kamptonema* were collected. The taxonomic position of these strains was characterized using polyphasic evaluation (morphological, ecological, cytological and molecular analyses) and resulted in the proposition of a new genus which includes freshwater and aerophytic taxa.

The seven population samples studied in this work were collected in different localities of southeastern (tropical climate) and southern (subtropical climate) Brazil (Table 1). Samples were collected between March 2010 and January 2015, and the cyanobacterial populations were found inhabiting aquatic and aerophytic habitats such as wet soils, barks of trees and growing on fine sandy substrata in streams.

Morphological variability of populations was evaluated from fresh field material, from cultured samples and from 4 % formaldehyde-fixed material 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 cells for each sample. The strains were identified according to Komárek & Anagnostidis (2005) and related literature.

Each cyanobacterial strain was isolated from a single trichome grown on BG11 medium, adapted from 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 (DMIL LED; Leica). The trichomes were then isolated and inoculated in tubes with BG11 growth medium to the establishment of monospecific cultures. Strains were cultured and maintained under 20 °C ± 1.50 µmol photons m^{-2} s^{-1} irradiance and 14 : 10 h light: dark cycle in the Culture Collection of IBILCE/UNESP.

The samples were fixed by immersion in 3 % glutaraldehyde plus 0.25 % tannic acid solution in Millonig's buffer, pH 7.3, containing 0.54 % glucose for 24 h. After washing with the same buffer, they were post-fixed with 1 % osmium tetroxide for 2 h, washed again, dehydrated in a graded acetone series and embedded in Araldite resin (Cotta-Pereira *et al.*, 1976). Ultrathin sections (50–75 nm) were cut using a diamond knife and contrasted with 2 % uranyl acetate for 30 min (Watson, 1958), followed by 2 % lead citrate in sodium hydroxide solution for 10 min (Venable & Coggeshall, 1965). Samples were evaluated with a LEO-Zeiss 906 (Zeiss) transmission electron microscope operated at 80 kV.

The biomass for DNA extraction was obtained from non-axenic unicyanobacterial cultures by repeated centrifugation, during which the filaments were washed several times with sterile water to remove or reduce mucilage, medium substances and heterotrophic bacteria. DNA was extracted using PowerSoil DNA Isolation kit (MO BIO Laboratories) according to the manufacturer's protocol.

The 16S rRNA gene and 16S–23S ITS region were amplified with primers 16S 27F and 23S30R (Taton *et al.*, 2003) using a Techgene TC-512 thermal cycler (Techne) and 25 µl reaction volume containing 5 µl 10× PCR buffer, 2 µl 50 mM $MgCl_2$, 1 µl 10 mM dNTP mix, 1.25 µl 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 10 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 from electrophoretic runs 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 pGEM-T Easy Vector System I (Promega) according to the supplier's manual. Competent *Escherichia coli* DH5-α 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 BigDye Terminator v3.0 Cycle Sequencing Ready Reaction kit (Applied Biosystems) as per the manufacturer's protocol. M13F and Sp6R primers correspond to the vector sites and the internal primers 357F, 704R, 1114F and 1494R (Neilan *et al.*, 1997) were used for

Table 1. Locality and habitat where the cyanobacterial strains examined in this study were collected

(SP) São Paulo State; (RS) Rio Grande do Sul State; (RJ) Rio de Janeiro State.

Species/strain	Habitat	Locality (State)	Latitude (S)	Longitude (W)
Ancylothrix rivularis gen nov., sp. nov.				
7PC	Benthos	Triunfo Reservoir (RS)	29° 41′ 21″	51° 28′ 01″
8PC	Benthos	Barra Funda stream (SP)	20° 51′ 39″	49° 36′ 54″
9PC	Benthos	Jacaré stream (SP)	20° 51′ 36″	49° 33′ 59″
Ancylothrix terrestris sp. nov.				
10PC	Soil	Deciduous forest (RS)	29° 73′ 41″	52° 41′ 39″
11PC	Tree trunk	Semi-deciduous forest (SP)	20° 44′ 25″	49° 25′ 51″
12PC	Tree trunk	*Araucaria* forest (SP)	22° 40′ 38″	45° 38′ 54″
13PC	Soil	Atlantic rainforest (RJ)	23° 13′ 03″	44° 42′ 43″

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 sequences obtained were compared to previously published sequences 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 ClustalW v1.8 under default parameters (Thompson *et al.*, 1994) in the MEGA 6.06 package (Tamura *et al.*, 2013), and was visually refined. The unicellular *Gloeobacter violaceus* PCC 7421 (GenBank accession number NC005125) was used as evolutionary outgroup.

Phylogenetic analyses were performed based on partial 16S rRNA gene sequences. The appropriate nucleotide substitution models were selected using Bayesian Information Criterion (BIC) in ModelTest 3.06 (Posada & Crandall, 1998). Neighbour-joining (NJ) and maximum-likelihood (ML) inferences were performed using the MEGA 6.06 package (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.

The topology was validated by Bayesian inference (BI) analysis run in MrBayes 3.1.2 software (Huelsenbeck & Ronquist, 2001). 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) was applied. BI analysis comprised two runs of four Markov Chain Monte Carlo (MCMC), each with 10 000 000 generations and sampling every 100 generations, and the initial 10 000 generations were discarded as burn-in. Sequence identity matrix/nucleotide divergence was calculated from the alignment in BioEdit (Hall, 1999) for all positions including gaps.

The 16S-23S ITS regions D1-D1', Box-B and V3 were identified and their secondary structure were determined using Mfold 3.2 (Zuker, 2003) and edited in Macromedia Fireworks 8.0. The tRNA sequences were identified with tRNAscan-SE 1.21 (Lowe & Eddy, 1997).

The populations studied showed a very uniform morphology: trichomes forming bright blue-green or dark blue-green mats, almost always growing without sheaths. Sheaths were only rarely recognizable under the optical microscope in the form of indistinct mucilaginous diffluent, especially in culture. Trichomes are cylindrical, bent and slightly narrowed towards the ends, not constricted or slightly constricted at the cross-walls, 4.0–7.0 μm wide. Cells are isodiametric or slightly shorter than wide, 2.5–6μm long. Apical cells rounded-conical, without calyptra, 3.2–5.2 μm wide, 3.2–5.2 μm long. Cell content is blue-green, mostly heterogeneous, sometimes with evident chromatoplasma and centroplasm, granulated cross-walls (Figs 1 and 2). Reproduction by disintegration of trichomes into hormogonia, with necridic cells.

The seven populations showed morphology corresponding to the species included in *Phormidium*-group III according to Komárek & Anagnostidis (2005) and differ from typical *Phormidium (P. lucidum* Agardh ex Gomont) (Fig. 1).

Although rarely recognizable under the light microscope, the sheath is thin, homogeneous and more or less smooth on the surface (Fig. 2). Thylakoids appear radially

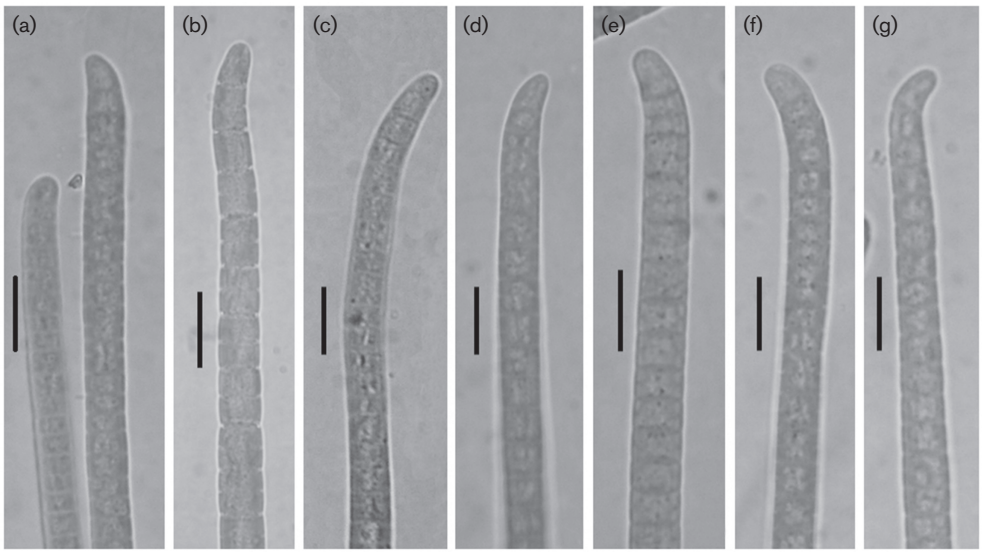

Fig. 1. Photomicrographs of seven strains of *Ancylothrix* gen. nov. (a) *A. rivularis* gen. nov., sp. nov. 7PC. (b) *A. rivularis* gen. nov., sp. nov. 8PC. (c) *A. rivularis* gen. nov., sp. nov. 9PC. (d) *A. terrestris* sp. nov. 10PC. (e) *A. terrestris* sp. nov. 11PC. (f) *A. terrestris* sp. nov. 12PC. (g) *A. terrestris* sp. nov. 13PC. Bars, 10 μm.

distributed in the cells (Fig. 2c); in longitudinal view, they are organized in bundles situated irregularly over the whole protoplast. Different structures were observed in the cytoplasm. Cyanophycin granules are common and can vary in size and form, often located along the cross-walls (Fig. 2d, e, f). Carboxysomes and polyphosphate granules are rare, bigger than the cyanophycin granules and can be found scattered throughout the cell (Fig. 2a). Gas vesicles (aerotopes) are absent.

The comparison of partial 16S rRNA gene sequences of the seven studied strains with sequences from the GenBank database revealed identities below 92 %. The BLAST best match for all Brazilian strains resulted in 91 % identity with *Phormidium animale* CCAP 1459-6 (GenBank accession number EF654087) and with *Phormidium autumnale* SAG 78.79 (EF654084).

The Brazilian strains clustered together in a separate clade, labelled *Ancylothrix* clade (Fig. 3), which was well supported by bootstrap and posterior probability values (99 % and 1, respectively). Similarity scores based on the 16S rRNA gene region were higher than 95.6 % within this cluster and lower than 91 % when compared with other genera, such as *Phormidium*, *Oscillatoria*, *Lyngbya*, *Kamptonema*, *Microcoleus* and *Coleofasciculus*.

The clade *Ancylothrix* is formed by two internal groups (Fig. 3), both well supported, with similarity from 95.5 to 96 %, while the similarity scores within each group ranged from 99.2 to 100 %.

The 16S–23S ITS regions for all seven strains studied ranged from 364 to 516 bp (Table 2) and revealed the presence of two different operons. One operon contains both tRNAs for isoleucine ($tRNA^{Ile}$) and alanine ($tRNA^{Ala}$) and the other lacks tRNA for alanine (Table 2). D1-D1', box-B and V3 regions exhibited six, four and five different patterns, respectively (Fig. 4). D1-D1' helix showed conserved secondary structure in strains 7PC, 8PC and 9PC, being identical for 7PC and 8PC (*A. rivularis* clade) and variable secondary structure in strains 10PC, 11PC, 12PC and 13PC (*A. terrestris* clade). The same was observed for box-B and V3 helices, which were conserved in strains 7PC, 8PC and 9PC and variable in 10PC, 11PC, 12PC and 13PC (Fig. 4).

Many traditional taxa of *Cyanobacteria* are known to be polyphyletic and the genus *Phormidium* is one of them (Casamatta *et al.*, 2005; Marquardt & Palinska, 2007; Palinska & Marquardt, 2008; Sciuto *et al.*, 2012). That is the reason why Komárek & Anagnostidis (2005) proposed *Phormidium* would require a broad taxonomic revision, and it resulted in many studies on the group. As consequence, several genera have been proposed based on species originally described under *Phormidium*, including *Phormidesmis* (Komárek, 2009), *Wilmottia* (Strucnecký *et al.*, 2011), *Oxynema* (Chatchawan *et al.*, 2012), *Roseofilum* (Casamatta *et al.*, 2012), *Ammassolinea* (Hašler *et al.*, 2014) and *Kamptonema* (Strunecký *et al.*, 2014).

The populations studied here correspond morphologically to *Phormidium*-group III (Anagnostidis & Komárek, 2005) and they could be considered members of the genus *Kamptonema* based on morphological analysis, but instead, phylogenetic analyses on 16S rRNA gene sequences showed the populations form a genetically well-defined group that is apart from *Kamptonema*.

The high number of recently described genera based on molecular data reveals the high genetic diversity of Cyanobacteria. Similarly, this study showed *Phormidium*-group III of Komárek & Anagnostidis (2005) as more diverse genetically than morphologically and reinforces the idea that *Phormidium* is not a natural group. Strains of group III were found to be phylogenetically distant from each other, despite the morphological similarity (trichomes attenuated and bent at the apex - *Kamptonema*, *Phormidium* sp. ETS-05 and *Ancylothrix* gen. nov., Fig. 3). The ultrastructure of *Phormidium* species is heterogeneous and both radial and parietal arrangements of thylakoids are observed (Marquardt & Palinska, 2007). Thylakoids of *Ancylothrix rivularis* gen. nov., sp. nov. and *A. terrestris* sp. nov. exhibit radial orientation, as found in *Microcoleus autumnalis* (Strunecký *et al.*, 2013), and, mainly in longitudinal view, it is oriented in diagonal throughout the whole cytoplasm. This is also very similar to *Ammassolinea.*

The separation of the studied strains in two internal groups indicates that *Ancylothrix* gen. nov. is composed of two species genetically and ecologically well-defined. One group represents a species with only freshwater occurrences and the other group, with only aerophytic populations. The species proposed in this study (*A. rivularis* gen. nov., sp. nov. and *A. terrestris* sp. nov.) also could not be differentiated by morphological traits, but they were clearly separated by molecular analysis. The same was described for a number of genera and species recently proposed based on molecular data (Siegesmund *et al.*, 2008; Premanandh *et al.*, 2009; Hašler *et al.*, 2012; Osorio-Santos *et al.*, 2014; Mühlsteinová *et al.*, 2014), confirming the high genetic diversity in Cyanobacteria.

The separation of the two species of *Ancylothrix* gen. nov. is also supported by 16S–23S ITS sequences. In *A. rivularis* gen. nov, sp. nov. they were stable in length and secondary structures (Table 2, Fig. 4). However, *A. terrestris* sp. nov. showed high variation in 16S–23S ITS sequences, which can be related to its occurrence habitat. Aerophytic habitats present more variations in environmental conditions, such as humidity, UV radiation, wind and temperature, than aquatic environments. This condition may select organisms with a variety of mutations, and this genomic variation may confer a higher phenotypic plasticity to survive in such environments. This could be seen in ITS variations in strains isolated herein from aerophytic samples.

Despite the still restricted known distribution of the genus, populations of the group could be found over a wide geographical range in Brazil, from subtropical in the south, to tropical regions in the southeast. More studies, including

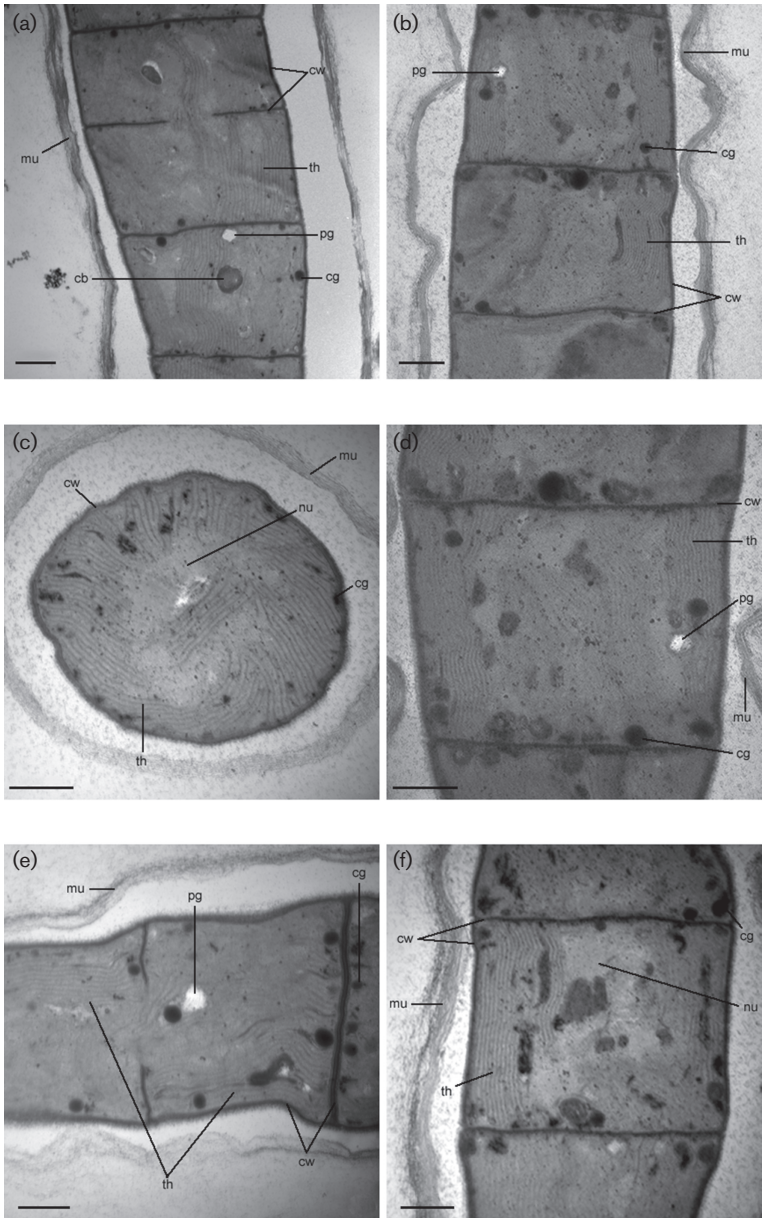

Fig. 2. Ultrastructure of strains of *Ancylothrix* gen. nov. with position of thylakoids (th), cell wall (cw), sheath (mu), nucleoplasm (nu), carboxysome granules (cb), polyphosphate granules (pg) and cyanophycin granules (cg). (a, b, d, e, f) Longitudinal sections; (c) cross-section. Bars, 1 μm.

different areas of Brazil and South America, will probably expand reports of the occurrence of *Ancylothrix* gen. nov. The strains were found in a wide range of habitats, such as streams, wet soils and tree trunks. The phylogenetic data reflected the distinction of the ecological occurrences between the two species placing the freshwater strains in a distinct clade grouping from the aerophytic forms. Although genetically distinct, the two groupings are morphologically very close, making the phenotypic differentiation almost impossible, and the ecological data became extremely important for species distinction. The results of phylogenetic studies make it possible to argue the two species could have evolved from a common ancestor with significant physiological plasticity allowing descents to cope with different environmental pressures even though there are no morphological changes.

The autapomorphic characters of *Kamptonema*, presented by Strunecký *et al.* (2014), were also observed in *Ancylothrix* gen. nov., making the morphological separation of the two genera very difficult. However, the genetic distance observed between these groups strongly suggests they have to

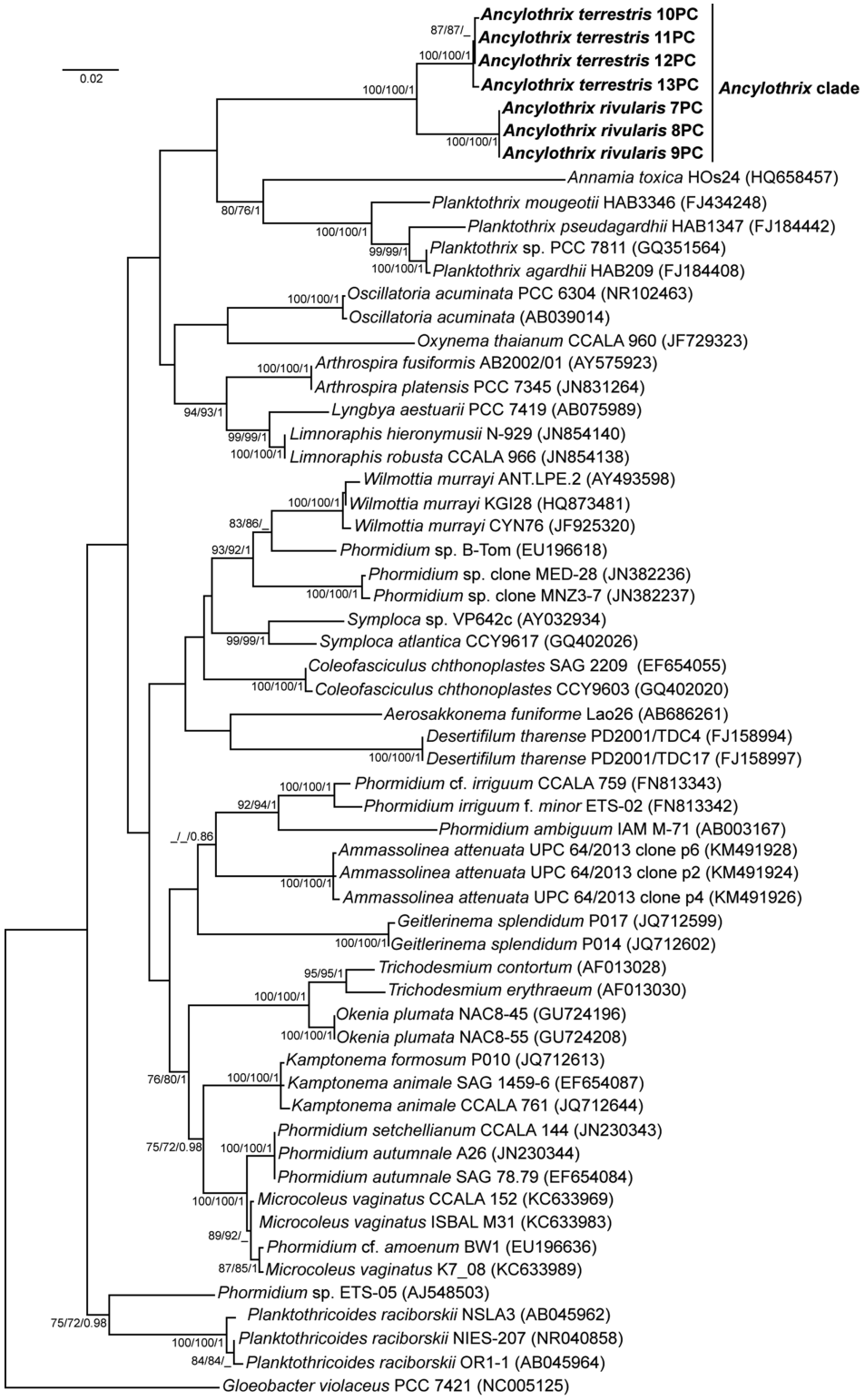

Fig. 3. Maximum-likelihood phylogenetic tree based on the 16S rRNA gene sequences (1515 bp) of oscillatoriacean cyanobacteria. Strains of *Ancylothrix* gen. nov. are shown in bold. A bootstrap test involving 1000 resamplings was performed. Bootstrap values (>70 %) and probabilities (>0.7) obtained from ML/NJ/ Bayesian methods, respectively, are displayed at the relevant nodes. GenBank accession numbers are shown in parentheses. Bar, 0.02 substitutions per nucleotide position.

Table 2. Lengths of 16S–23S ITS regions (number of nucleotides) in the analysed strains of *Ancylothrix* gen. nov.

Strain	Complete ITS	Leader	D1-D1' helix	D2 with spacer	D3 with spacer	tRNA^{Ile} gene	V2	tRNA^{Ala} gene	Pre-Box-B spacer	Box-B helix	Post-Box-B spacer	Box-A	D4	V3 with spacer	D5
Ancylothrix rivularis gen. nov., sp. nov.															
7PC	437	6	57	29	17	74	16	73	18	36	17	12	7	56	19
8PC	438	6	57	29	17	74	16	73	19	36	17	12	7	56	19
9PC	436	6	56	29	17	74	16	73	18	36	17	12	7	56	19
Ancylothrix terrestris sp. nov.															
10PC	515	8	54	29	19	74	24	73	78	34	16	12	7	65	22
11PC	364	7	63	28	37	74	–	–	41	37	23	12	7	18	17
12PC	409	7	68	29	18	74	–	–	36	33	16	11	8	83	26
13PC	516	8	54	29	19	74	24	73	80	34	16	12	7	65	21

be considered as separate genera. In addition to the 16S rRNA sequences, *Ancylothrix* gen. nov. can be differentiated from *Kamptonema* by the thylakoids arrangement that is radial in the first and parietal in the second. Furthermore, Strunecký *et al.* (2014) comment that *Kamptonema* strains, when kept under certain cultivation conditions, like dark for a few days, can change their thylakoidal structure, showing a limited tendency to keritomy, which was not observed in *Ancylothrix* gen. nov. strains. The relationship between *Ancylothrix* gen. nov. and other groups is unclear as the outermost branches were not strongly supported. However, the separation between *Ancylothrix* and *Phormidium* is clear.

It is evident that morphological characters traditionally used to separate genera and species are not properly related to phylogenetic patterns, since differentiation between *Ancylothrix* gen. nov. and *Kamptonema* is not possible based on these characters. As these genera are indistinguishable by morphological or ecological (both comprise aerophytic and aquatic species) markers and can only be separated by ultrastructure and molecular data, they can be considered cryptogenera, according to Komárek *et al.* (2014). These results support other studies showing that the phylogeny of *Oscillatoriales* is not well defined, mainly due to the relatively simple morphology of these organisms, and more studies are needed to better understand the evolutionary history of the group.

Description of the genus and species under the provisions of the International Code of Nomenclature for algae, fungi and plants

Ancylothrix Martins et Branco gen. nov.

Ancylothrix (An.cy'lo.thrix. Gr. adj. *ankylos* curved; Gr. fem. n. *thrix*, *trichos* hair; N.L. fem. n. *Ancylothrix*, curved hair).

Diagnosis: Filamentous cyanobacteria, forming bright green compact biofilms on aerophytic substrata or dark green mats on submerged substrata. Sheaths very thin, rare, colourless, 4.5–7.0 µm wide. Trichomes cylindrical, uniseriate, not or slightly constricted at the cross-walls, attenuated and bent at the ends. Cells cylindrical, shorter than wide to isodiametric, without aerotopes. Cell content slightly granular, blue–green. Apical cells conical-rounded and narrowed, calyptra absent. Thylakoids radially distributed in the cells; in the lateral view, organized in bundles situated irregularly over the whole protoplast. All cells are capable of division. Reproduction by fragmentation of trichomes by necridic cells. Heterocytes and akinetes missing.

Habitat: Stream benthos, shadowed soils and bark of trees.

Type species: Ancylothrix rivularis Martins et Branco sp. nov.

Ancylothrix rivularis Martins et Branco sp. nov.

Ancylothrix rivularis (*ri.vu.la'ris.N. L. fem. adj. rivularis belonging to a stream*).

Fig. 1a, b, c.

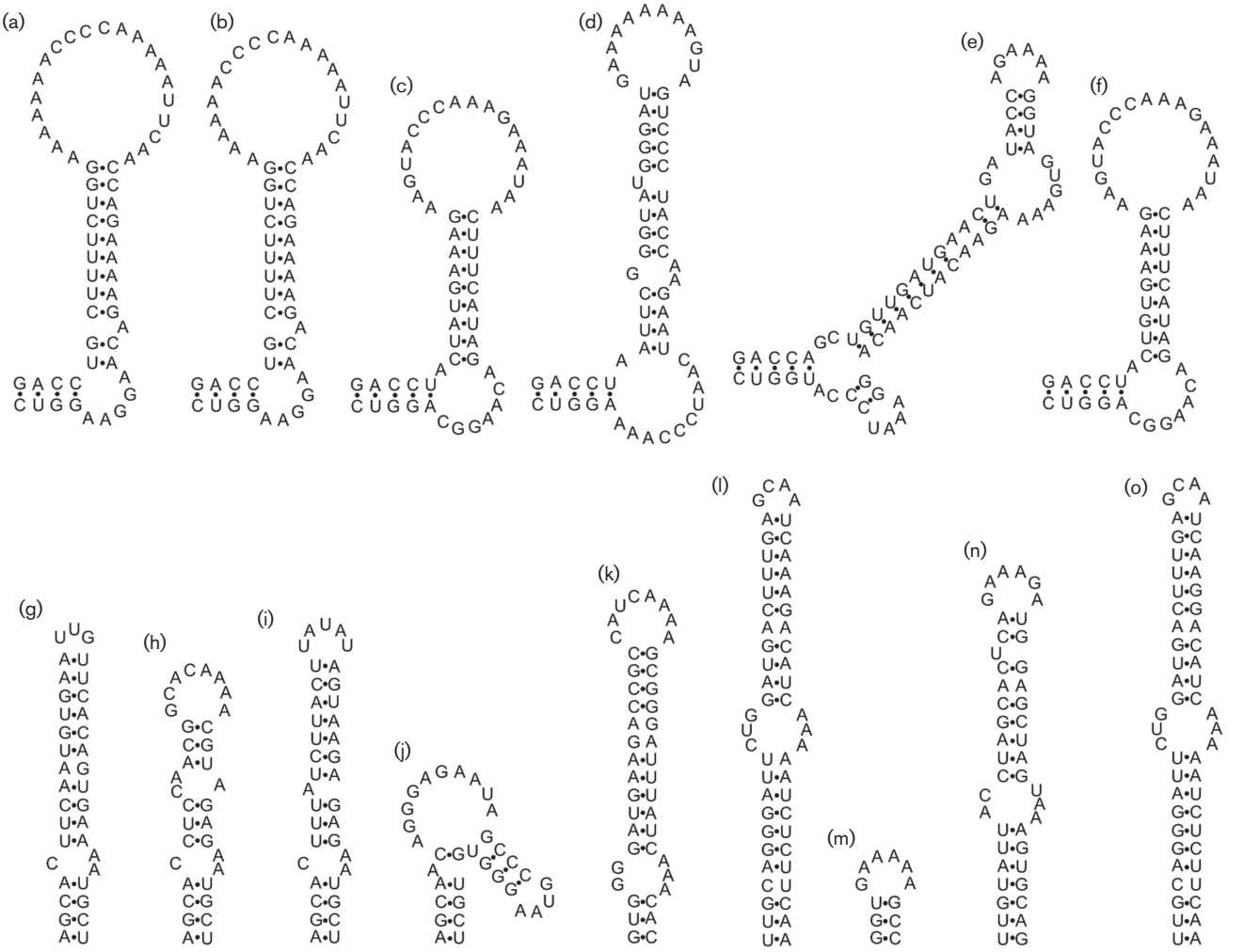

Fig. 4. Secondary structure of conserved regions of 16S–23S ITS of studied strains of *Ancylothrix* gen. nov. (a–f) D1-D1' helices: (a) 7PC and 8PC; (b) 9PC; (c) 10PC; (d) 11PC; (e) 12PC; (f) 13PC. (g–j) Box-B helices: (g) 7PC, 8PC and 9PC; (h) 10PC and 13PC; (i) 11PC; (j) 12PC. (k–o) V3 helices: (k) 7PC, 8PC and 9PC; (l) 10PC; (m) 11PC; (n) 12PC; (o) 13PC.

Diagnosis: Filaments forming dark green thallus. Sheaths rare, thin, homogenous, colourless, 4.5–7.0 μm wide. Trichomes cylindrical, not or slightly constricted at the cross-walls, attenuated and bent at the ends, 5.5–7.0 μm wide. Cells cylindrical, shorter than wide to isodiametric, 2.5–6.0 μm long. Apical cells conical-rounded and narrowed, without calyptra, 3.2–4.8 μm wide, 3.2–4.8 μm long.

Habitat: Stream benthos.

Holotype here designated: Formaldehyde-fixed sample of strain 8PC deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, voucher number SJRP 31538.

Type locality: Barra Funda stream, municipality of Neves Paulista, São Paulo State, Brazil (20° 51′ 39 S 49° 36′ 54″ W).

Ancylothrix terrestris Martins et Branco sp. nov.

Ancylothrix terrestris (ter.res′tris. L. fem. adj. *terrestris* terrestrial, referring to the habitat of the populations pertaining to this species).

Fig. 1d, e, f, g.

Diagnosis: Filaments forming bright green compact biofilms, entangled. Sheaths rare, thin, homogenous, colourless, 4.5–6.5 μm wide. Trichomes cylindrical, not or slightly constricted at the cross-walls, attenuated and bent at the ends, 4.0–6.5 μm wide. Cells cylindrical, shorter than wide to isodiametric, 2.5–5.5 μm long. Apical cells conical-rounded and narrowed, without calyptra, 3.2–4.8 μm wide, 2.4–4.8 μm long.

Habitat: Soil and bark of trees.

Holotype here designated: Formaldehyde-fixed sample of strain 10PC deposited in Herbarium SJRP (IBILCE/UNESP), Brazil, voucher number SJRP 31539.

Type locality: Soil of decidual forest, municipality of Cachoeira do Sul, Rio Grande do Sul State, Brazil (29° 73′ 41″ S 52° 41′ 39″ W).

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