










RESEARCH ARTICLE

Description of four new filamentous cyanobacterial taxa from freshwater habitats in the Azores Archipelago

Rúben Luz^{1,2}  | Rita Cordeiro^{1,2}  | Jan Kaštovský³  | Jeffrey R. Johansen^{3,4}  |
Elisabete Dias²  | Amélia Fonseca^{1,2}  | Ralph Urbatzka⁵  | Vitor Vasconcelos^{5,6}  |
Vítor Gonçalves^{1,2} 

¹Faculdade de Ciências e Tecnologia da Universidade dos Açores, Ponta Delgada, Portugal

²CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, BIOPOLIS Program in Genomics, Biodiversity and Land Planning; UNESCO Chair – Land Within Sea: Biodiversity & Sustainability in Atlantic Islands, Universidade dos Açores, Ponta Delgada, Portugal

³Department of Botany, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

⁴Department of Biology, John Carroll University, University Heights, Ohio, USA

⁵Interdisciplinary Centre of Marine and Environmental Research - CIMAR/ CIMAR, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, Matosinhos, Portugal

⁶Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

Correspondence

Rúben Luz, Faculdade de Ciências e Tecnologia da Universidade dos Açores, Ponta Delgada 9501-801, Portugal.
Email: ruben.fs.luz@uac.pt

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Abstract

Simple filamentous cyanobacteria comprise a diverse and polyphyletic group of species, primarily in the orders Leptolyngbyales and Oscillatoriales, that need more sampling to improve their taxonomy. Oceanic islands, such as the Azores archipelago, present unique habitats and biogeographic conditions that harbor an unknown range of diversity of microorganisms. Filamentous cyanobacteria isolated from aquatic habitats in the Azores and maintained in the BACA culture collection were described using morphology, both light and transmission electron microscopy, ecology, and genetic data of the 16S rRNA gene sequences and 16S–23S Internal Transcribed Spacer (ITS) rRNA region secondary structure. Our analyses revealed two new monophyletic genera: *Tumidithrix elongata* gen. sp. nov. (Pseudanabaenaceae) and *Radiculonema aquaticum* gen. sp. nov. (Leptolyngbyaceae). In addition, two new species *Leptodesmis lacustris* sp. nov. (Leptolyngbyaceae) and *Pycnacronema lacustrum* sp. nov. (Wilmottiaceae) are reported as the first aquatic species for these genera. The description of these new taxa and the genetic study of an isolate of *Leptodesmis alaskaensis* from the Azores followed the polyphasic approach, identifying diacritical features. Our results reinforce the need for taxonomic studies on cyanobacteria from less-studied habits and geographic regions, which have a potential for new taxa description.

KEYWORDS

16S rRNA gene, lakes, Leptolyngbyales, Oscillatoriales, phylogeny, Polyphasic approach, streams

INTRODUCTION

The taxonomy of cyanobacteria has been rapidly changing, with many newly published taxa in the last few years based on morphologic, genetic, and ecological data (Cai et al., 2022; Lima & Branco, 2020; Hedlund et al., 2022; Johansen et al., 2021; Komárek et al., 2020; Moro et al., 2021; Soares et al., 2020; Strunecký et al., 2022; Zimba et al., 2020). The use of this combined set of data is important, as many traditional genera are considered polyphyletic, such as the character-poor genus *Leptolyngbya* (Johansen et al., 2011; Komárek et al., 2014). The polyphasic approach has been crucial in some of the most recently described taxa, such as *Leptodesmis* (Raabová et al., 2019) for which molecular methods (e.g., the 16S rRNA gene phylogeny and 16S–23S ITS rRNA region) were important for taxon description. The study of other genera such as *Plectolyngbya* (Taton et al., 2011), *Kamptonema* (Strunecký et al., 2014), *Pegethrix* (Mai et al., 2018), *Pycnacronema* (Martins et al., 2019), and *Tenebriella* (Hauerová et al., 2021), among many others, has been aided by the use of molecular methods for the description of new genera or species with dubious morphologic identification.

For cyanobacteria, habitat has always been important for genus or species description and identification. Several studies of polyphyletic taxa show a clear ecological restriction among morphologically similar cyanobacteria from marine and freshwater habitats (Engene et al., 2012; González-Resendiz et al., 2018). Within the freshwater taxa, cyanobacteria that inhabit thermal habitats represent a special cluster of understudied taxa with a clear phylogenetic separation (Cordeiro et al., 2020). The same can be seen between aquatic and terrestrial cyanobacteria (Komárek & Zapomilová, 2007; Wacklin et al., 2009), with recent studies having identified clear genome speciation related to habitat origin (Chen et al., 2021). Thus, the use of a polyphasic approach is of great importance in the study of simple filamentous cyanobacteria that comprise a diverse and polyphyletic group of species, primarily in the orders Leptolyngbyales and Oscillatoriales, with few morphologic diacritical characters (Strunecký et al., 2017). Furthermore, it has been recognized that this group of cyanobacteria needs more sampling to improve its taxonomy (Komárek et al., 2014).

The Azores is an oceanic archipelago of nine volcanic islands located in the North Atlantic Ocean, roughly 1500 km from Europe and 1900 km from North America. Despite their geographic proximity, each island is unique in habitat diversity, such as in having freshwater and saline lakes, thermal springs, and several permanent or temporary streams (Luz et al., 2022). Regardless of this broad array of suitable habitats for cyanobacteria, their diversity is still poorly known (Luz et al., 2022). The high diversity of cyanobacteria in the

Bank of Algae and Cyanobacteria of the Azores (BACA) culture collection (Cordeiro et al., 2020), combined with the polyphasic approach, can contribute to the taxonomic knowledge of cyanobacteria.

In this work, we describe four new taxa from aquatic habitats, including two new monospecific genera, *Tumidithrix elongata* gen. sp. nov., a morphologically similar genus to *Arthronema* but with an evident distinct ecology, and *Radiculonema aquaticum* gen. sp. nov., a new genus with false branching and distinct morphologic characters. The other two taxa are *Leptodesmis lacustris* sp. nov. and *Pycnacronema lacustum* sp. nov., isolated from aquatic habitats, both with unique morphology and phylogenetic placement relative to previously described species. In the *Leptodesmis* clade, a newly isolated strain of *Leptodesmis alaskaensis* from the entrance of a cave in Pico Island was also identified. This strain has been fully characterized, contributing to the full 16S–23S ITS rRNA region description, which had been missing from some published *Leptodesmis* species.

MATERIALS AND METHODS

Seven filamentous cyanobacterial strains isolated from samples collected in different islands of the Azores were retrieved from BACA culture collection for genetic and morphologic characterization (Table 1). These strains are maintained in the BACA collection with a 14:10 light:dark cycle (under 10–40 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) photoperiod at 19°C.

Strains were morphologically characterized using a Leica DM4 B microscope with Digital Camera Leica MC 190 HD (Leica, Germany) using bright field and differential interference contrast. At least 25 trichomes and 50 cells were examined for each species to obtain cell dimensions and descriptions. The cultures were observed at different phases of growth for morphologic description. Holotypes were deposited in the Herbário Ruy Telles Palhinha (AZB), University of Azores, Portugal (Thiers, 2023).

For DNA extraction, fresh cultures were extracted using the PureLink® Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's protocol for Gram-negative bacteria. For the 16S rRNA gene and 16S–23S ITS rRNA region amplification, primers 27F (Neilan et al., 1997) and 23S30R (Lepère et al., 2000) were used in a polymerase chain reaction (PCR) with a total volume of 25 μL containing 1 \times PCR Buffer, 2 mM MgCl_2 , 250 μM of each deoxynucleotide triphosphate (Thermo Fisher, Waltham, MA, USA), 0.5 pmol of each primer, 5–10 ng of DNA, and 1.5 U of Supreme NZYtaq II DNA polymerase (Nzytech, Portugal). Polymerase chain reaction conditions followed (Taton et al., 2003), and thermal cycling was carried out in a ProFlex™ 3 \times 32-well PCR System (Thermo Fisher, USA). Polymerase chain reaction amplification

TABLE 1 Strain sampling location in the Azores archipelago, Portugal, and GenBank accession codes.

Strain	Taxonomy	Locality	Sampling date	GPS-coordinates	GenBank
BACA0078	<i>Pycnacronema lacustrum</i>	Capitão Lake, Pico Island	01-06-2017	38°29'12.8" N 28°19'05.7" W	OM732220
BACA0141	<i>Tumidithrix elongata</i>	Paul Lake, Pico Island	20-07-2016	38°25'43.7" N 28°13'56.2" W	MT176747
BACA0202	<i>Leptodesmis lacustris</i>	Caldeirão Pequeno Sul Lake, São Miguel Island	01-06-2013	37°49'23.5" N 25°45'01.9" W	OM732227
BACA0203	<i>Leptodesmis lacustris</i>	Peixe Lake, São Miguel Island	01-06-2013	37°49'07.3" N 25°44'10.9" W	MT176753
BACA0204	<i>Leptodesmis lacustris</i>	São Brás Lake, São Miguel Island	01-06-2013	37°47'35.0" N 25°24'36.6" W	OM732228
BACA0748	<i>Leptodesmis alaskaensis</i>	Torres Cave, Pico Island	25-06-2019	38°29'39.6" N 28°30'08.5" W	OP508344
BACA0731	<i>Radiculonema aquaticum</i>	Salto Stream, Santa Maria Island	30-10-2019	36°58'54.4" N 25°03'09.2" W	OM732264

products were visualized by electrophoresis in 0.8% agarose gel and in 0.5×TBE (Tris-Borate-EDTA) buffer, and stained with SYBR™ SAFE. Amplified bands were removed from the gel using a clean scalpel and then purified using NZYGelpure (Nzytech, Portugal). Strains that produced one band were sent directly for sequencing. Strains with more than one band amplified had the PCR product cloned using the NZY-A PCR cloning kit (Nzytech, Portugal), following the manufacturer's protocol. Sequencing of the 16S rRNA gene and 16S–23S ITS rRNA region was conducted using the 27F (Neilan et al., 1997), 781F (Cordeiro et al., 2021), 781R (Nübel et al., 1997), CSIF (Janse et al., 2004), and 23S30R primers (Lepère et al., 2000).

The sequences of the studied strains were aligned with 281 sequences retrieved from GenBank using BLAST and reference strains of cyanobacteria species from previously published papers. The retrieved sequences represented most of the genetically and morphologically well-classified filamentous genera from the Leptolyngbyales and Oscillatoriales. The sequences were aligned in MAFFT v7.490 (Katoh & Standley, 2013) using the G-INS-i algorithm, with the final alignment containing 1042 columns. The best-fit nucleotide model was assessed using ModelTest-NG (Darriba et al., 2020) in the raxmlGUI (Edler et al., 2021), with the selection of the TIM+G4+I evolution model. Phylogenetic trees were constructed using Bayesian inference (BI) with MrBayes v3.2.7a (Ronquist et al., 2012) on XSEDE through the CIPRES Science Gateway and maximum likelihood (ML) with the IQ-Tree online version v1.6.12 (Trifinopoulos et al., 2016), using *Gloeobacter violaceus* PCC 8105 as an outgroup. The BI was carried out with 5.0×10^6 generations, with two runs of four Markov chains, with custom parameters (nswaps=4; temp=0.01), sampling every 1000 generations, with a 0.25 burn-in rate (the final average standard deviation of split frequencies was less than 0.05) with the GTR+GAMMA+I model. The ML analysis was conducted using the TIM+G4+I model with 1000 ultrafast bootstrap replicates (Hoang et al., 2017). Trees were visualized using FigTree v1.4.4 (Rambaut, 2012), and the final composite trees from maximum likelihood with bootstrap values for BI were re-drawn using Inkscape v1.2.

For the 16S rRNA gene *p*-distance, genetic information from phylogenetically relevant or morphologically relevant strains was selected. Sequences were aligned using MAFFT v7.490 with the G-INS-i method (Katoh & Standley, 2013), and *p*-distance was calculated using MEGA 11.0.13 (Tamura et al., 2021). Values were transformed into percentages for easier reading (*p*-distance*100).

The 16S–23S ITS rRNA region secondary structures of the D1–D1', Box-B, and V3 helices were identified (Iteaman et al., 2000), and secondary structures were predicted using M-fold (Zuker, 2003) and re-drawn in Inkscape v1.2.

RESULTS

The taxonomic analysis combining genetic, morphologic, and ecological data provided for the description of two new genera and four new species, all with strong genetic support either phylogenetically (Figure 1) or by *p*-distance (Table S1). Two new monospecific genera, *Tumidithrix elongata* gen. sp. nov. (Pseudanabaenaceae) and *Radiculonema aquaticum* gen. sp. nov. (Leptolyngbyaceae), and two new species,

Leptodesmis lacustris sp. nov. (Leptolyngbyaceae) and *Pycnacronema lacustrum* sp. nov. (Wilmottiaceae), were identified.

Within the Pseudanabaenaceae (Figure 1), *Tumidithrix elongata* gen. sp. nov. BACA0141 (Figure 2) was in a clade with *Pseudanabaena* sp. PCC 7403 (AB039019) and *Phormidium mucicola* IAM M-221 (AB003165). The morphologic differences with the enlarged and elongated cells present in *Tumidithrix elongata* corroborated the new genus description

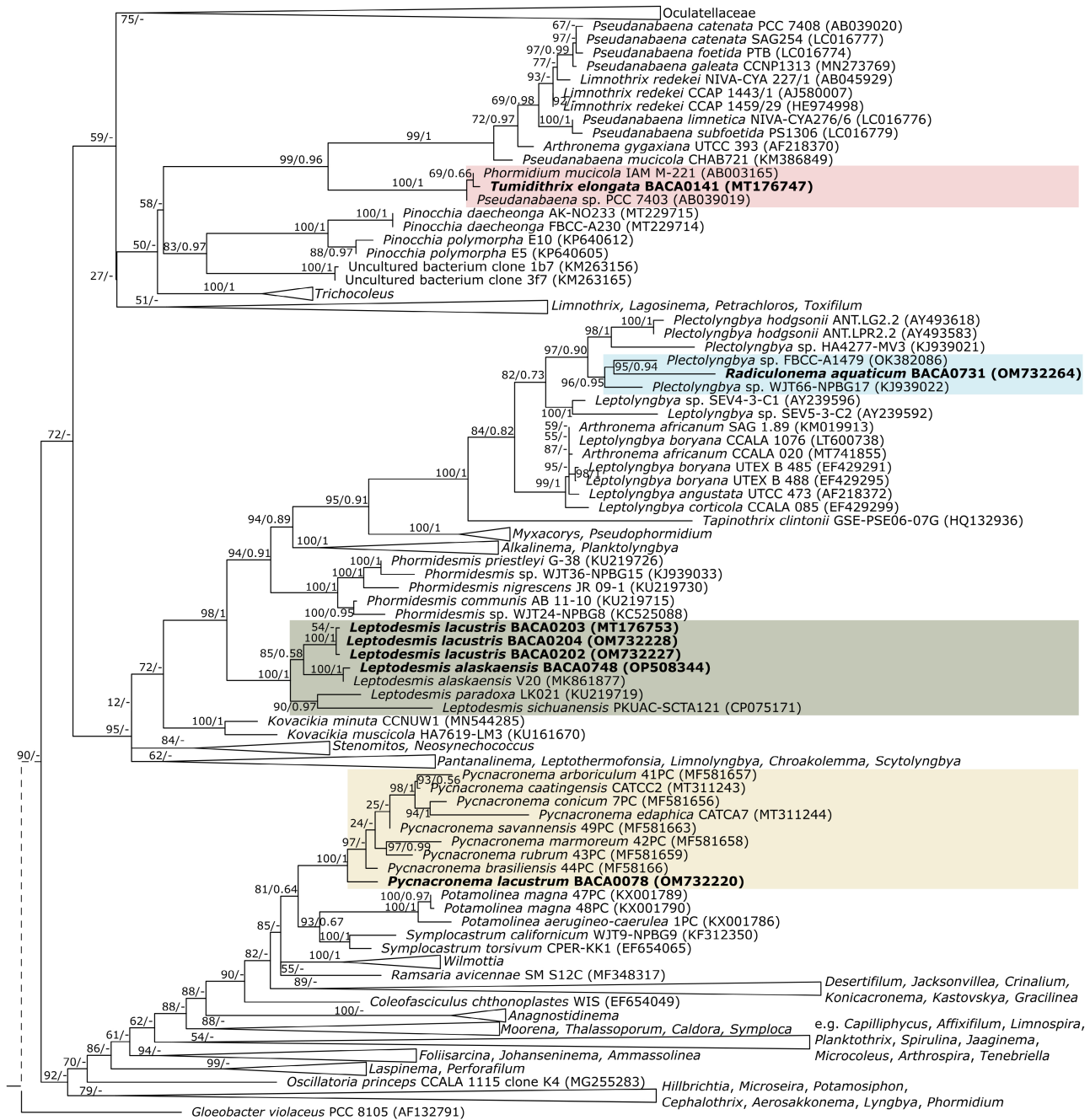


FIGURE 1 Partial maximum likelihood (ML) phylogenetic tree based on 16S rRNA genes. Bootstrap values for maximum likelihood and posterior probabilities for Bayesian inference are indicated on the tree. The studied strains are in bold font.

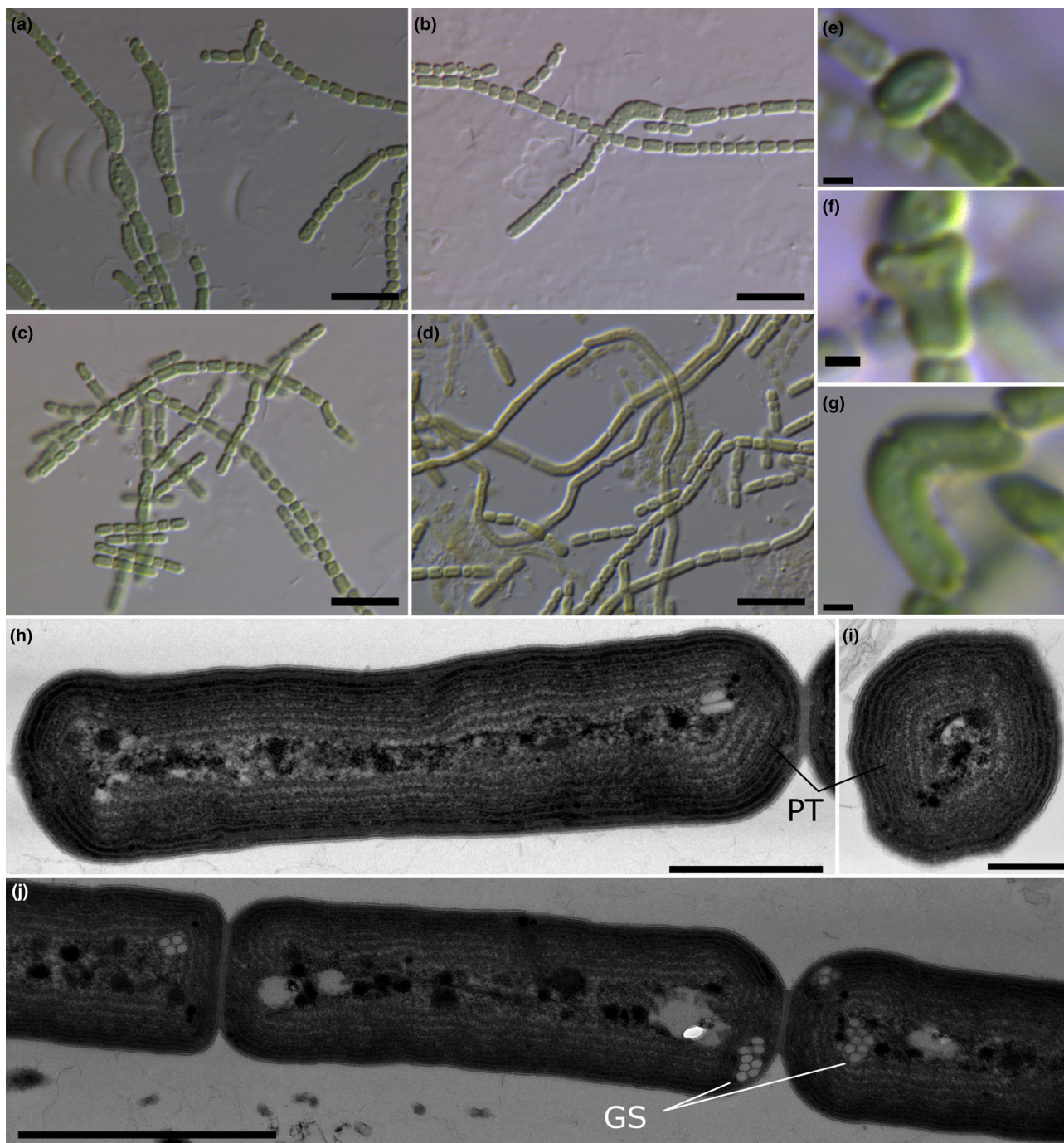


FIGURE 2 *Tumidithrix elongata* BACA0141 morphologic features in light microscopy (a–g) and TEM (h–j). (a, b) Swollen/involution cells; (c) Hormogonia; (d) Elongated cells; (e, f) different cell morphologies found in culture; (h, j) Longitudinal cut with visible gas vesicles; (i) Transversal cut; Scale for (a–d) 10 μm ; scale for (e–h) 1 μm ; scale for (i) 500 nm; scale for (j) 2 μm . GS, gas vesicles; PT, parietal thylakoids.

when compared to the closest phylogenetic genus *Pseudanabaena*. The phylogeny positioned the new genus in a separate and unique clade (Figure 1), and the *p*-distance analysis strongly supported the new genus with a high value (Table S1 in the Supporting Information; >7.9%) along with the 16S–23S ITS rRNA region secondary structure that showed several differences in both sequence and structure (Figure 3).

In the phylogenetic tree (Figure 1), *Radiculonema aquaticum* gen. sp. nov. BACA0731 (Figure 4) was within the Leptolyngbyaceae and closely related to *Leptolyngbya* sensu stricto and *Plectolyngbya hodgsonii* ANT.LPR2.2 (AY493583). In terms of morphology, *Plectolyngbya* and *Radiculonema* shared the trait of both tolypotrichoid and scytonematoid types of false branching. However, *Radiculonema* differed

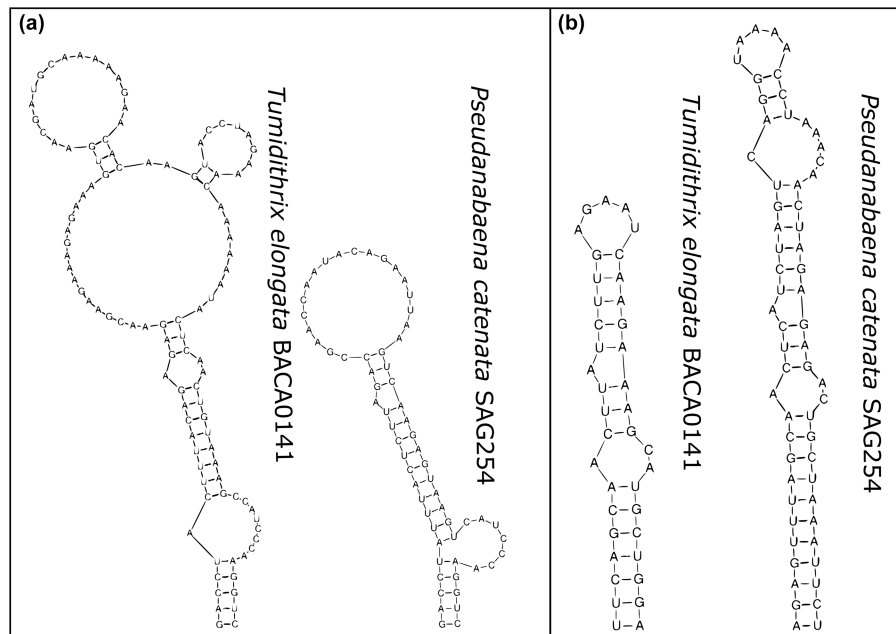


FIGURE 3 Secondary structure of the 16S–23S ITS rRNA region D1–D1' helix (a) and Box-B helix (b) in *Tumidithrix elongata* and *Pseudanabaena* type species.

from *Plectolyngbya* in the formation of hemispherical colonies and divaricated trichomes and rounded wide apical cells. The *p*-distance analysis with the closest genera was superior to 4.4% (Table S1), and the phylogeny positioned the new genus in a separated clade with strong support (ML, 97; BI: 0.90). The 16S–23S ITS rRNA region analysis of *Radiculonema*, *Leptolyngbya*, and *Plectolyngbya* revealed some structural similarities, but their sequences were quite different (Figure 5).

In the 16S rRNA gene phylogeny of the genus *Leptodesmis* (Figure 1), three BACA strains formed a clade that was distinct from the other described species such that *L. lacustris* sp. nov. was identified (Figure 6). This species differed morphologically from the remaining *Leptodesmis* species by cell size and genetically by its 16S rRNA gene *p*-distance (Table S1), phylogenetic placement, and different 16S–23S ITS rRNA region secondary structures (Figure 7). In the *Pycnacronema* clade (Figure 1), the new species *P. lacustrum* sp. nov. (Figure 8) was distinguished from the remaining species by its wider filaments and trichomes, genetic difference (Table S1), and different 16S–23S ITS rRNA region secondary structures (Figures 9–11).

A full comparison of described taxa and close phylogenetic and morphologically similar taxa is shown in Table S2 in the Supporting Information.

Taxonomic descriptions

Tumidithrix elongata R.F.S.Luz, Kaštovský, J.R.Johans., V.Gonçalves gen. sp. nov. (Figure 2).

Diagnosis: Phylogenetically distinct and placed in the Pseudanabaenaceae with distinct swollen or elongated cells, differentiating it from morphologically similar genera like *Pseudanabaena* and *Arthronema*.

Description: Colony dark blue-green. Trichomes uniseriate, straight, slightly irregularly waived or bent, without sheaths. Hormogonia few celled, from two to eight cells. Cells cylindrical or barrel-shaped, constricted at cross walls normally with one or two polar granules, with small clusters of gas vesicles near the cross walls and parietal thylakoids. Cells mostly longer than wide, 1.2–2.0 μm wide (mean = 1.47 μm) and 1.3–6.3 μm long (mean = 3.00 μm), with a length/width ratio of 0.8–4.6 (mean = 2.07). Cells sometimes irregularly swollen, straight to deeply bent, 1.75–3.77 μm wide and 4.0–21.0 μm long, elongated cells up to 30 μm , without necridia. End cells rounded.

Holotype: AZB 3908, type strain-dried material preserved in a permanently inactive state.

Type locality: Lagoa do Paul, Pico Island (Azores), Portugal (38°25'43.7" N 28°13'56.2" W). Collected by the MONITAIA project team on July 20, 2016, in a shallow freshwater lake.

Etymology: *Tumidithrix* – *Tumidus* (swollen) + *thrix* (hair), for its swollen cells, feminine; *elongata*—for its characteristic elongated cells.

Type strain: BACA0141 (Bank of Algae and Cyanobacteria of the Azores, Azores, Portugal).

Gene Sequences: GenBank accession number MT176747 for the 16S rRNA gene and 16S–23S ITS rRNA region.

Radiculonema aquaticum R.F.S.Luz, Kaštovský, J.R.Johans., V.Gonçalves gen. sp. nov. (Figure 4).

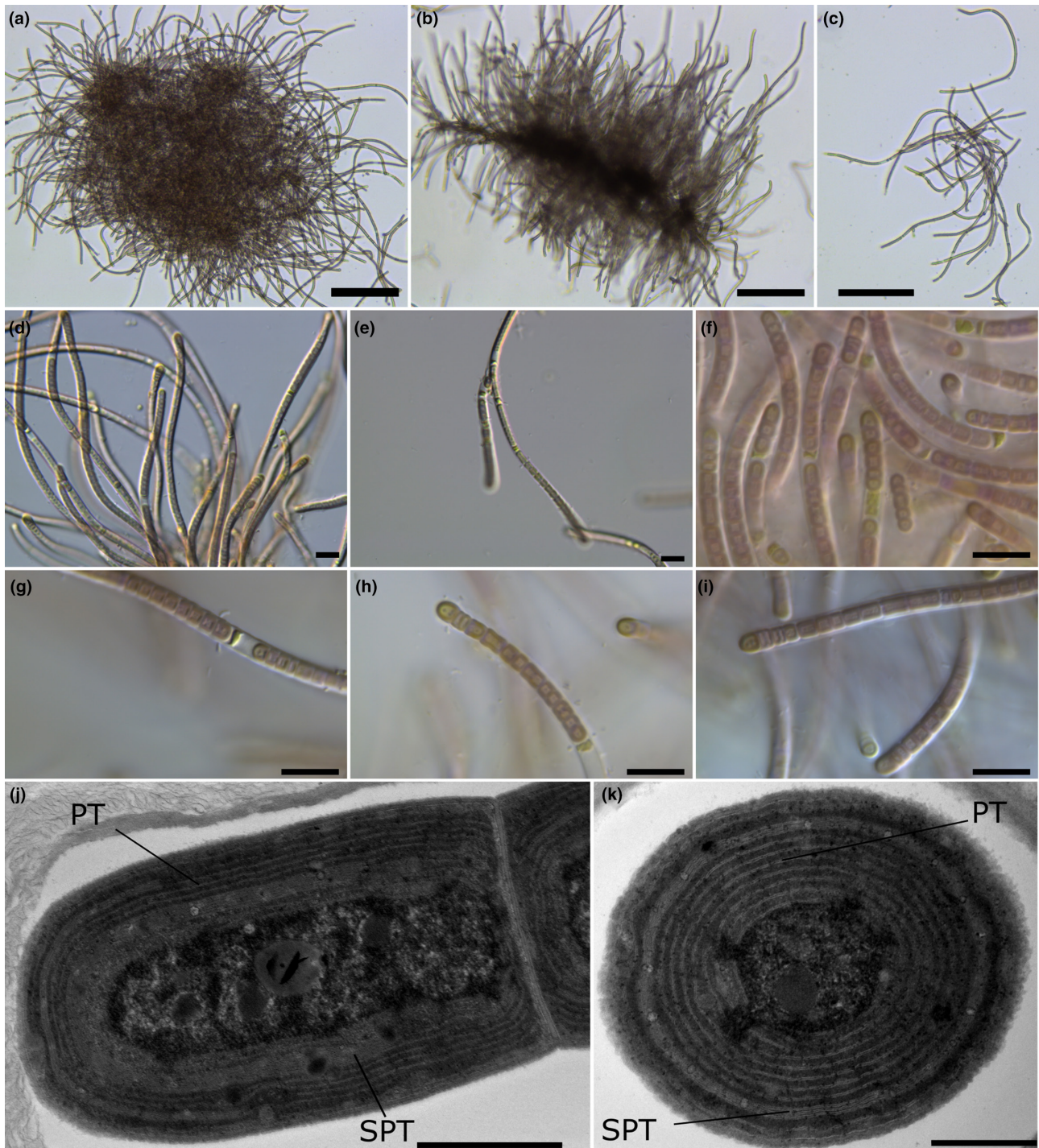


FIGURE 4 *Radicolonema aquaticum* BACA0731 morphologic features under light microscopy (a–i) and TEM (j–k). (a–c) Formation of colonies; (d) Divaricated trichomes; (e) Trichome branching of tolypotrichoid type; (f–h) Necridia and fragmented trichomes; (h, i) Terminal cells; (j) Longitudinal cut; (k) Transversal cut. Scale for (a–c) 100 μm ; scale for (d–i) 10 μm , scale for (j) 1 μm ; scale for (k) 500 nm. PT, parietal thylakoids; SPT, subperipheral layer of parietal thylakoids.

Diagnosis: Phylogenetically distinct and placed in the Leptolyngbyaceae by the 16S rRNA gene. Morphologically different from *Leptolyngbya* in branching and from *Plectolyngbya* in the formation of colonies and phylogenetic placement.

Description: Hemispherical colony greenish-brown, brown to purplish, growing as the culture ages with

deeply entangled filaments. Filaments flexuous, false branched, 3.3–5.7 μm wide (mean = 4.10 μm). Sheath firm, colorless, attached to the trichome. Trichomes are untapered, constricted at cross walls. Hormogonia few celled. Cells shorter than wide, isodiametric, or longer than wide, 2.5–3.8 μm wide (mean = 3.05 μm) and 1.2–7.4 μm long (mean = 3.02 μm), with a length/width

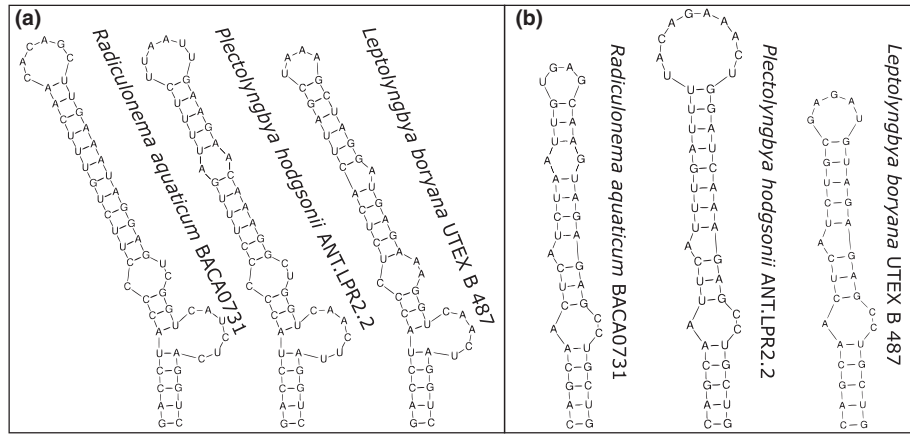


FIGURE 5 Secondary structure of the 16S–23S ITS rRNA region D1–D1' helix (a) and Box-B helix (b) in *Radiculonema aquaticum*, *Plectolyngbya*, and *Leptolyngbya* type species.

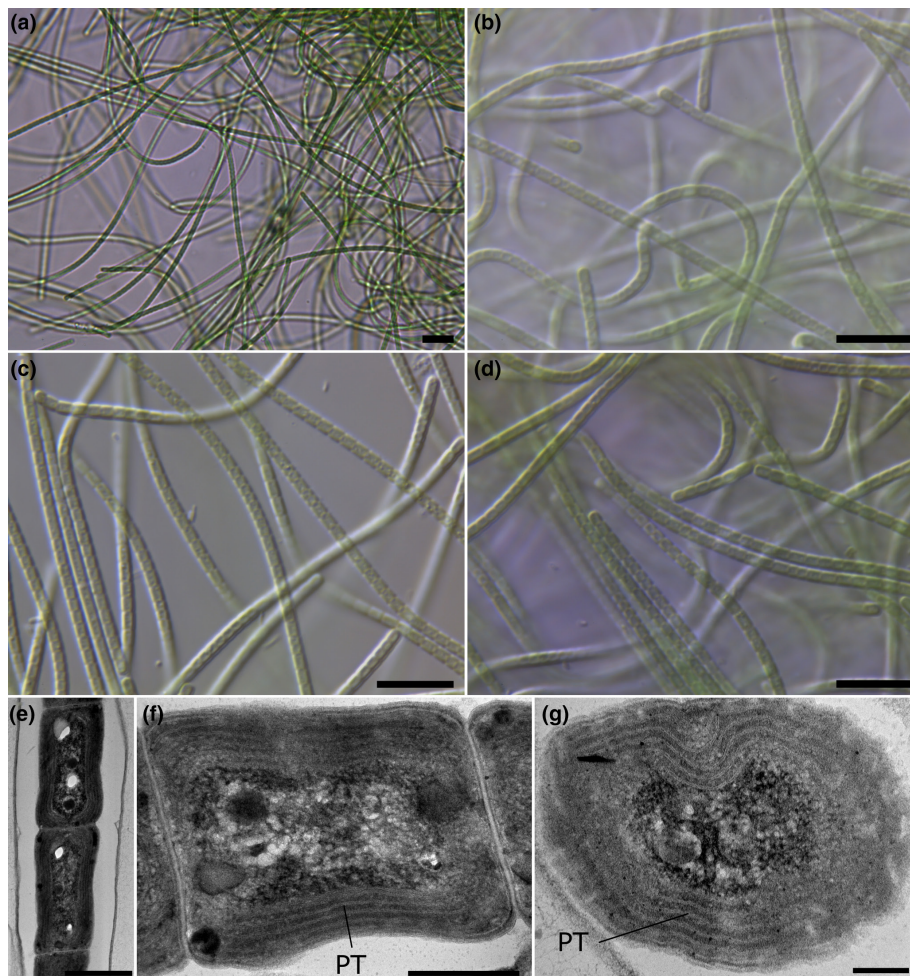


FIGURE 6 *Leptodesmis lacustris* morphologic features. (a, b) Randomly entangled trichomes; (c, d) Cell morphology and parallel arranged trichomes. (d, e) Longitudinal cut; (g) Transversal cut. Scale for (a) 100 μm , scale for (b–d) 10 μm , scale for (e) 1 μm , scale for (f) 500 nm, scale for (g) 200 nm. PT, parietal thylakoids.

ratio of 0.4–2.3 (mean = 0.99). Thylakoids are parietal, somewhat visible in bright-field microscope, stacked in some cells, probably without phycobilisomes, creating a clear thickened layer of thylakoids when observed

under TEM. Terminal cells are normally rounded wide. Necridia are common along the trichome.

Holotype: AZB 4485 type strain-dried material preserved in a permanently inactive state.

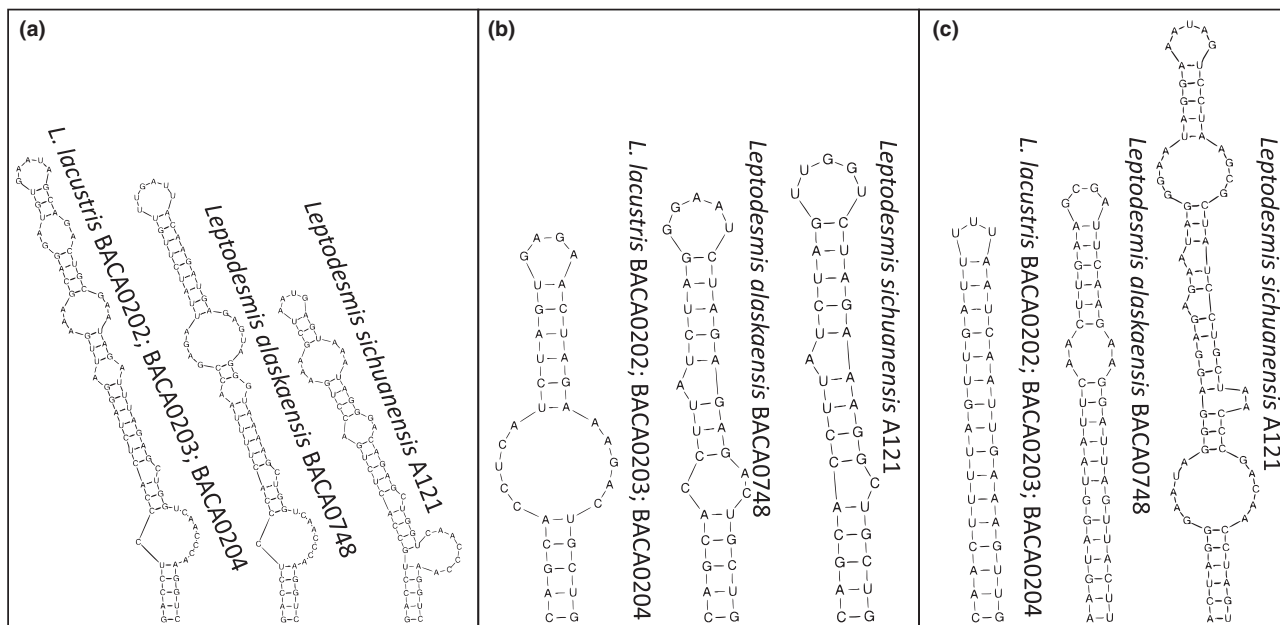


FIGURE 7 Secondary structure of the 16S–23S ITS rRNA region D1–D1' helix (a), Box-B helix (b), and V3 helix (c) of *Leptodesmis* spp.

Type locality: Ribeira do Salto, Santa Maria Island (Azores), Portugal (36°58'54.4" N 25°03'09.2" W). Collected by Rúben Luz and Rita Cordeiro on October 30, 2019, attached to a submerged rock in a freshwater stream.

Etymology: Radiculonema – Radicula (small root) + nema (filament), for the grown shape of the colony, neutral gender; aquaticum—from an aquatic environment.

Type strain: BACA0731 (Bank of Algae and Cyanobacteria of the Azores, Azores, Portugal).

Gene Sequences: GenBank accession number OM732264 for the 16S rRNA gene and 16S–23S ITS rRNA region.

Leptodesmis lacustris R.F.S.Luz, Kaštovský, J.R.Johans., V.Gonçalves. sp. nov. (Figure 6).

Diagnosis: It differs from *Leptodesmis paradoxa* by its narrower cells and from *L. alaskaensis* and *L. sichuanensis* mostly by its longer than wider cells (8-week cultures). *Leptodesmis lacustris* is also distinguished by its phylogenetic position.

Description: Colony blue-green to greenish, flat, with deeply entangled filaments. Filaments straight or flexuous, rarely coiled, sometimes parallel arranged, 1.8–2.5 μm wide (mean = 2.15 μm). Sheath firm, colorless, attached to the trichome. Trichomes are untapered, not or slightly constricted at the visible cross walls, flexuous, without motility. Hormogonia few celled. Cells mostly longer than wide, even when in older cultures, commonly granulated, 1.1–2.1 μm wide (mean = 1.56 μm) and 1.2–4.3 μm long (mean = 2.10 μm), with a length/width ratio of 0.8–2.4 (mean = 1.35) with parietal thylakoids. Necridia are present occasionally. End cells rounded.

Holotype: AZB 3969, type strain-dried material preserved in a permanently inactive state.

Type locality: Lagoa do Peixe, São Miguel Island (Azores), Portugal (37°49'07.3" N 25°44'10.9" W). Collected by Emanuel Xavier on June 1, 2013, in freshwater lakes.

Etymology: lacustris—as all strains were isolated from lakes.

Type strain: BACA0203 (Bank of Algae and Cyanobacteria of the Azores, Azores, Portugal).

Gene Sequences: GenBank accession number MT176753 for the 16S rRNA gene and 16S–23S ITS rRNA region.

Pycnacronema lacustrum R.F.S.Luz, Kaštovský, J.R.Johans., V.Gonçalves sp. nov. (Figure 8).

Diagnosis: Distinguished by its wider filaments and trichomes and by the presence of stratified sheath in older filaments. The 16S rRNA gene phylogeny and 16S–23S ITS rRNA region results also strongly supported this new taxon.

Description: Colony blue-green tufted mat. Filaments long straight, sometimes slightly bent, entangled, 11.0–17.0 μm wide (mean = 14.12 μm). Sheaths normally firm, colorless, thin, or stratified up to 3 μm in older filaments. Trichomes untapered, slightly to distinctly constrict at cross walls, without motility. Cell content is blue-green, sometimes granulated. Cells are mostly shorter than wide, 6.6–14.4 μm wide (mean = 11.27 μm) and 2.5–12.2 μm long (mean = 7.05 μm), with a length/width ratio of 0.2–1.4 (mean = 0.66), with parietal and fascicular thylakoids. Hormogonia not observed. Apical cells rounded, isodiametric, sometimes slightly longer than wide. Necridia present.

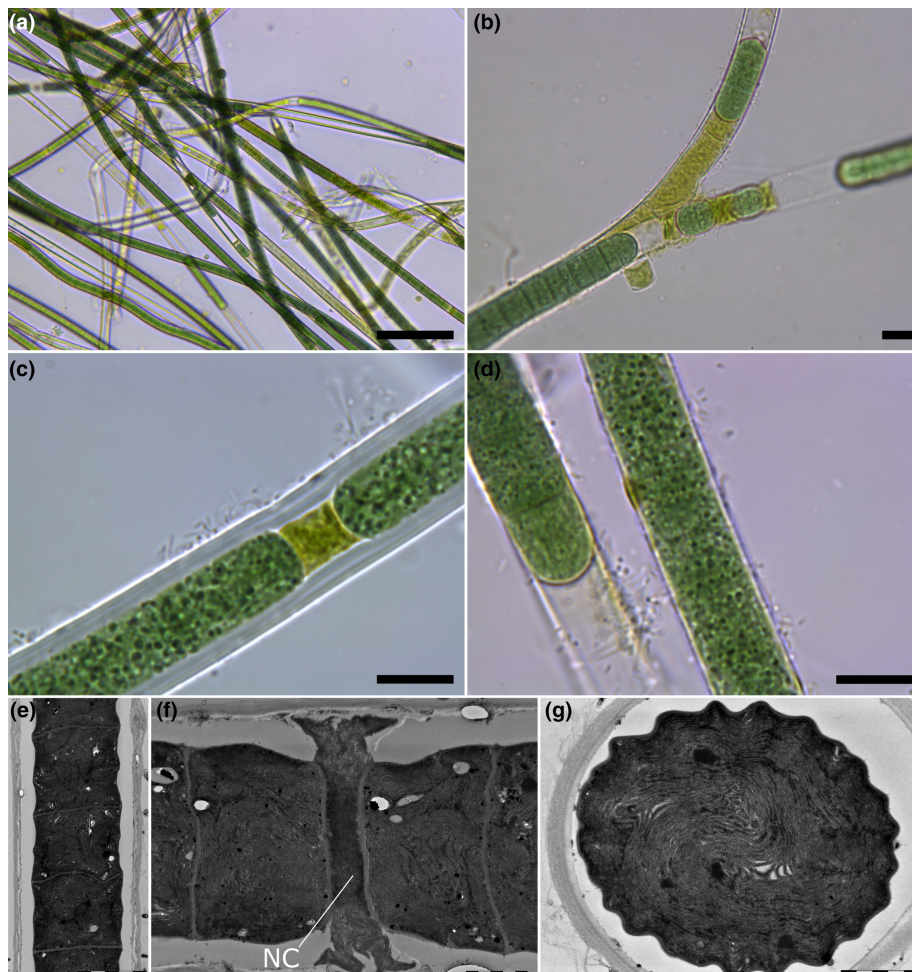


FIGURE 8 *Pycnacronema lacustrum* morphologic features. (a) Entangled trichomes forming a mat; (b) Fragment trichomes with false branching; (c) Necridia and thick sheath; (d) Rounded terminal cell. (e, f) Longitudinal cut; (g) Transversal cut; scale for (a) 100 μm , scale for (b–d) 10 μm , scale for (e) 5 μm , scale for (f, g) 2 μm . NC, Necridia.

Holotype: AZB 3845, type strain-dried material preserved in a permanently inactive state.

Type locality: Lagoa do Capitão, Pico Island (Azores), Portugal (38°29'12.8" N 28°19'05.7" W). Collected by the MONITAIA project team on June 1, 2017, in a freshwater eutrophic lake.

Etymology: lacustrum—as the strain was isolated from a lake.

Type strain: BACA0078 (Bank of Algae and Cyanobacteria of the Azores, Azores, Portugal).

Gene Sequences: GenBank accession number OM732220 for the 16S rRNA gene and 16S–23S ITS rRNA region.

DISCUSSION

The Bank of Algae and Cyanobacteria of the Azores has a diverse culture collection of cyanobacteria isolated from several terrestrial and aquatic habitats (Cordeiro et al., 2020). Following a taxonomic characterization using morphology, molecular data, and

ecology of selected strains enabled us to describe two new genera and four new species of filamentous cyanobacteria. The new taxa are phylogenetically well supported by 16S rRNA gene sequence data. The threshold used for species separation, 98.7% similarity in the 16S rRNA gene (Yarza et al., 2014), is a well-supported value for all the new species (Table S1). For the distinction of new genera, the 16S rRNA gene 94.5% similarity threshold, suggested by Komárek et al. (2014) and Yarza et al. (2014), was surpassed, with a similarity to the closest phylogenetic and morphologic genera of *Tumidithrix* below 94.5%. This is not the case for *Radiculonema*, as the similarity values are close to the generic threshold with *Leptolyngbya* and *Plectolyngbya*. Nevertheless, the similarity threshold values cannot be seen as a rule but only as an indication when clear discontinuities in morphology and ecology are identifiable (Komárek et al., 2014; Yarza et al., 2014).

The closest phylogenetic clade to *Tumidithrix* is *Pseudanabaena*, but *Tumidithrix* show a distinct phylogenetic placement within the Pseudanabaenaceae,

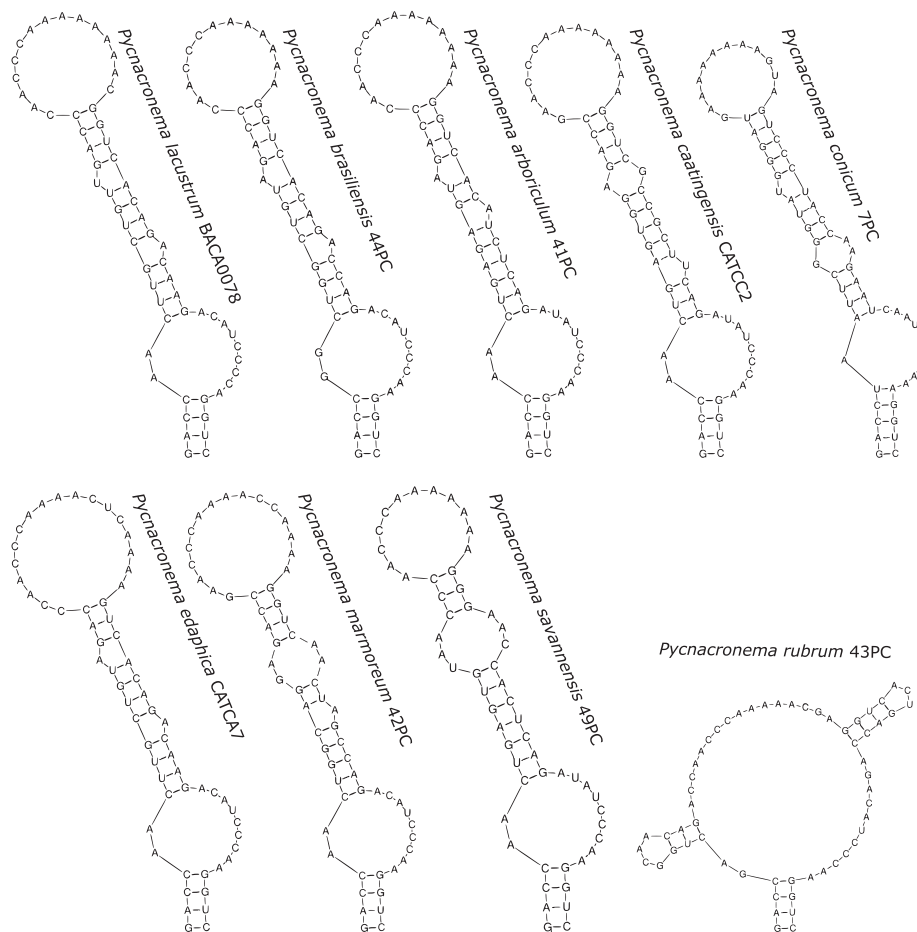


FIGURE 9 Secondary structure of the 16S–23S ITS rRNA region D1–D1' helix in *Pycnacronema* species.

and the 16S–23S ITS rRNA region structures revealed distinctive folding. Morphologically, *Tumidithrix* differs greatly from *Pseudanabaena* by the presence of elongated or swollen cells and gas vesicles. The strains *Pseudanabaena* sp. PCC 7403 and *Phormidium mucicola* IAM M-221, which are phylogenetically positioned close to *Tumidithrix elongata*, probably belong to *Tumidithrix*. However, as key information (e.g., 16S–23S ITS rRNA region and morphology) is missing, they should be treated as unknown species of *Tumidithrix* until a more detailed analysis is possible.

Tumidithrix elongata gen. sp. nov. morphologically resembles *Arthronema africanum* (Komárek & Lukavský, 1988). However, these species have distinct geographic distribution and ecological preferences. *Arthronema africanum* was initially reported by Schwabe and Simonsen (1961) from the Wau en-Namus brackish volcano lake (Libya); the CCALA 020 strain was isolated from within the sand of a dry lake (Kuwait), where crystalline salts could be found, whereas *Tumidithrix elongata* comes from a shallow freshwater lake in Pico Island (Azores) in the North Atlantic region. Furthermore, the new genus is phylogenetically distant from the strain used to describe the genus *Arthronema* (CCALA 020). The phylogenetic position of known *Arthronema* strains,

the closest morphologic genus, presents some challenges: (1) *A. africanum* SAG 12.89 formed a separate and distinct clade in the Leptolyngbyaceae; (2) *A. gygaxiana*, described in Casamatta et al. (2005) depicting the characteristic involution cells, was positioned within the *Pseudanabaena* spp. clade; and (3) *A. africanum* SAG 1.89 and *A. africanum* CCALA 020 were placed in the *Leptolyngbya* sensu stricto clade, a well-defined and recognized clade containing the type species *L. boryana*. This raises some concerns, as *Arthronema* was described taking into account the description of *Pseudanabaena africana* but based on the strain *A. africanum* LUKAVSKY 1980/1 by Komárek and Lukavský (1988) later maintained in the CCALA collection as CCALA 020. Since *A. africanum* CCALA 020 is phylogenetically positioned in the *Leptolyngbya* sensu stricto clade, the current validity of the genus is doubtful.

In the simple filamentous cyanobacteria, there are several examples of genera depicting false branching, such as *Plectonema* from the Oscillatoriales and, more recently, *Plectolyngbya* (Taton et al., 2011) from the Leptolyngbyales. *Radiculonema aquaticum* has false branching and forms compact hemispherical colonies. This branching feature, already seen by Taton et al. (2011) in *Plectolyngbya*, is a crucial

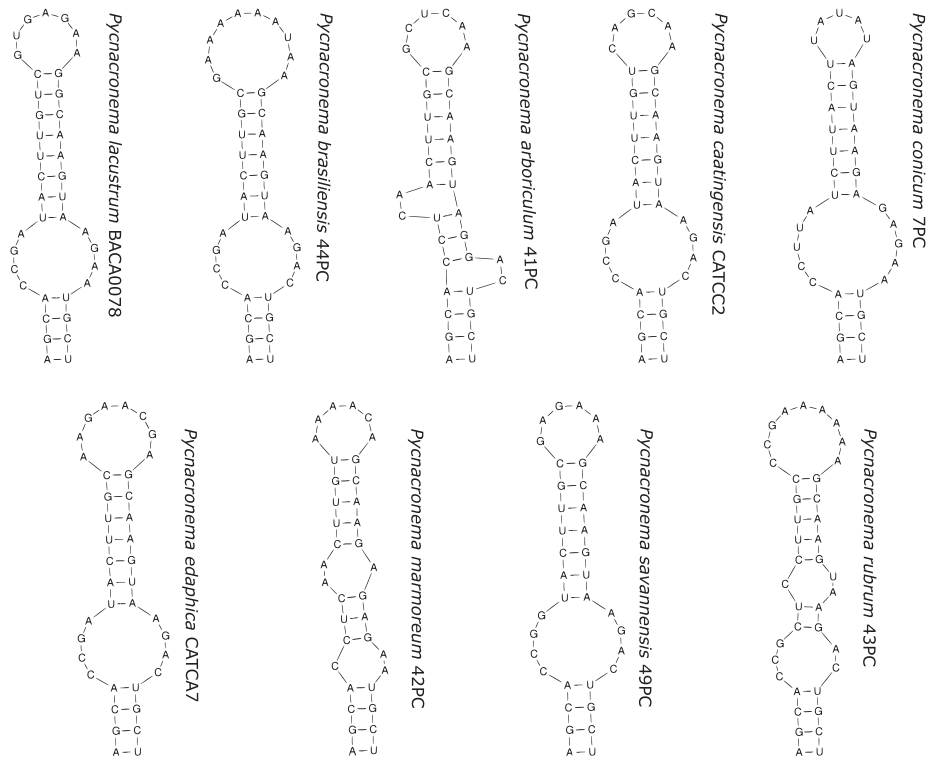


FIGURE 10 Secondary structure of the Box-B helix in *Pycnacronema* species.

characteristic for distinguishing *Radiculonema* from *Leptolyngbya* sensu stricto. Despite their similar branching, *Plectolyngbya* and *Radiculonema* can clearly be distinguished by the formation of hemispherical colonies, divaricated trichomes and the presence of wide rounded apical cells in *Radiculonema*. The thylakoids in the cells of *R. aquaticum* present a parietal distribution, with the absence of phycobilisomes in some of the thylakoids, or at least a modification in the phycobilisomes position, that allows a reduction in the space between thylakoids. This particular feature had already been observed in *Spirulina major* PCC 6313 (Mareš et al., 2019). The phylogenetic placement of this genus is clearly in the Leptolyngbyaceae, close also to the morphologically similar genera *Plectolyngbya* and *Leptolyngbya* sensu stricto. The 16S–23S ITS rRNA region secondary structures of the D1–D1' helix are somehow conserved in their secondary structure but with a different genetic sequence.

The description of *Leptodesmis* follows the review of *Phormidesmis* and is considered a cryptic genus to both *Phormidesmis* and *Leptolyngbya*, from which it is distinguished mostly by its 16S rRNA gene (Raabová et al., 2019). *Leptodesmis lacustris* sp. nov. is identified by its phylogenetic position in the *Leptodesmis* clade but with different cell morphology and the presence of necridia. The complete 16S–23S ITS rRNA region sequence is not available from the type strain, and only a small part is available from *L. alaskaensis* (Strunecky et al., 2019). The full 16S–23S ITS rRNA region

sequence presented here for *L. alaskaensis* BACA0748 allowed the folding of all the conserved 16S–23S ITS rRNA region structures, increasing the genetic knowledge of the genus. In the 16S–23S ITS rRNA region analysis, *L. lacustris* is more similar to *L. alaskaensis* in secondary structures but quite different from *L. sichuanensis*, which is not surprising due to the high phylogenetic distance observed in the 16S rRNA gene phylogeny.

Pycnacronema is a recent genus with eight described species, all from terrestrial habitats in Brazil (Lima & Branco, 2020; Martins et al., 2019). *Pycnacronema lacustrum* sp. nov. is easily distinguishable morphologically from the other species of the genus by its wider trichomes and filaments. Phylogenetically, the new species is distinguished by the position at the base of the *Pycnacronema* clade in the 16S rRNA gene tree. When comparing the 16S–23S ITS rRNA region secondary structures among all *Pycnacronema* species, *P. lacustrum* shows the same type of structures and loop, though it is different in the genetic sequence.

CONCLUSIONS

This work contributes to clarifying the current taxonomic status of cyanobacteria with the description of the morphologically and genetically well-defined new genera *Tumidithrix* and *Radiculonema*. The description of these new taxa will help to improve the cyanobacteria

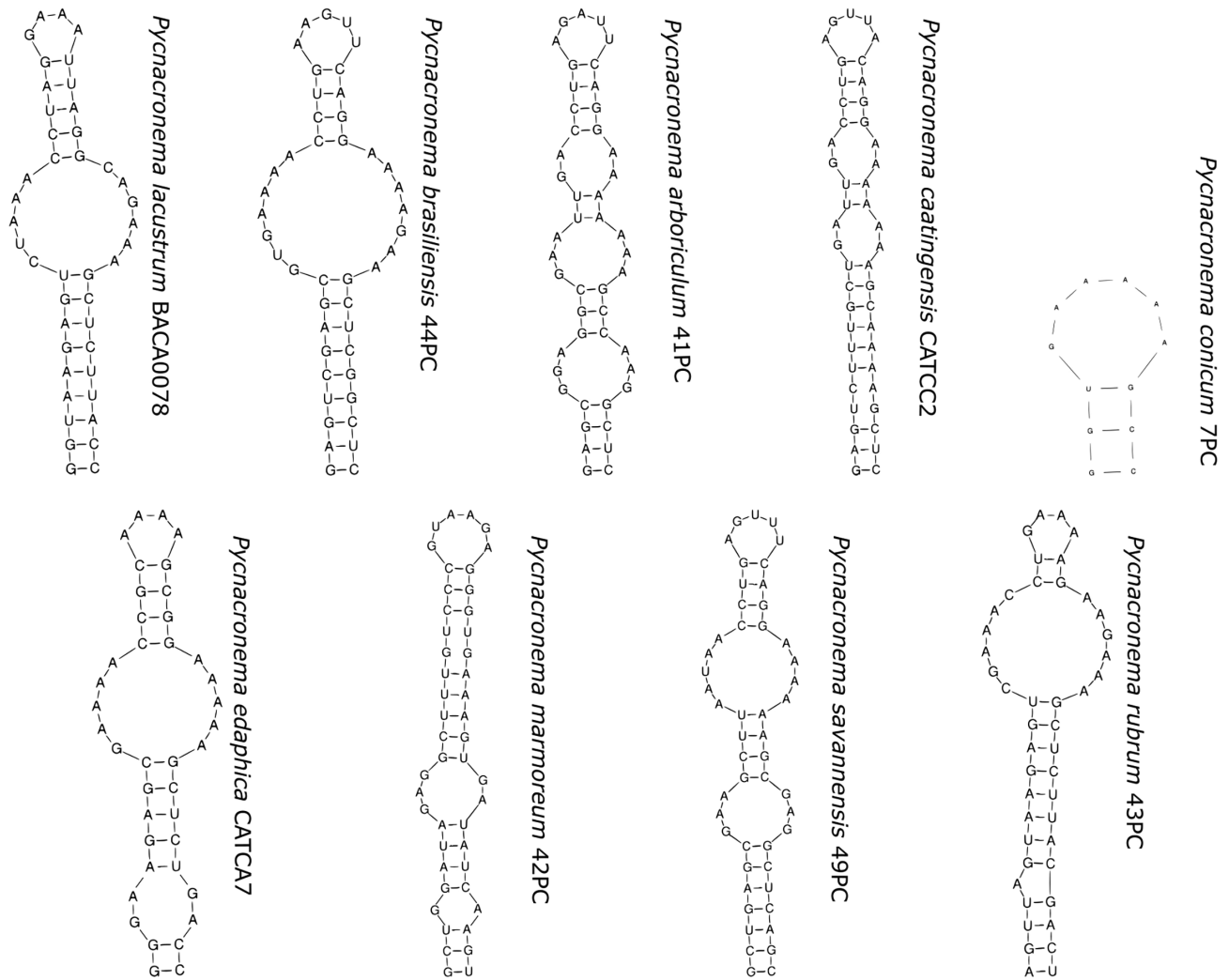


FIGURE 11 Secondary structure of the V3 helix in *Pycnacronema* species.

taxonomy with its identification of diacritical morphologic attributes and new genetic information. Our study also suggests there are many species still to uncover globally and in the Azores. The discovery of two species of *Leptodesmis* in the Azores has increased the biogeographic distribution of this genus, and the enlargement of the ecological distribution of *Pycnacronema* to aquatic habitats confirms the plasticity of cyanobacteria and their ability to adapt to very different habitats and to “travel” across the globe. Further genomic work must be done, especially on such well-characterized strains, that can serve as base for further studies with more robust genetic analyses. In this case, a genomic approach, through ANI and phylogenomics, would help to clarify genera and species delimitation and should soon become the standard for new taxa description.

AUTHOR CONTRIBUTIONS

Rúben Luz: Conceptualization (equal); formal analysis (lead); investigation (lead); writing – original draft (lead).

Rita Cordeiro: Investigation (supporting); writing – review and editing (supporting). **Jan Kaštovský:** Formal analysis (supporting); funding acquisition (supporting); investigation (supporting); writing – review and editing (supporting). **Jeffrey R Johansen:** Investigation (supporting); writing – review and editing (supporting). **Elisabete Dias:** Investigation (supporting); writing – review and editing (supporting). **Amélia Fonseca:** Investigation (supporting); writing – review and editing (supporting). **Ralph Urbatzka:** Conceptualization (equal); writing – review and editing (supporting). **Vitor Vasconcelos:** Conceptualization (equal); writing – review and editing (supporting). **Vitor Gonçalves:** Conceptualization (equal); funding acquisition (lead); writing – review and editing (equal).

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ORCID


Rúben Luz  <https://orcid.org/0000-0001-8223-5943>

Rita Cordeiro  <https://orcid.org/0000-0001-8713-6370>

Jan Kaštovský  <https://orcid.org/0000-0003-4523-3468>

Jeffrey R. Johansen  <https://orcid.org/0000-0002-0794-9417>

Elisabete Dias  <https://orcid.org/0000-0003-0259-3594>

Amélia Fonseca  <https://orcid.org/0000-0002-1668-2363>

Ralph Urbatzka  <https://orcid.org/0000-0001-7476-9195>

Vitor Vasconcelos  <https://orcid.org/0000-0003-3585-2417>

Vitor Gonçalves  <https://orcid.org/0000-0002-5737-296X>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. 16S rRNA gene p -distance of all studied strains and closest taxa. In red is a comparison between new genera and the type species of the closest genera. In green shade is highlighted the 16S rRNA gene p -distance between the type strains of the newly described species and related taxa.

Table S2. Morphological comparison of *Tumidithrix*, *Radiculonema*, *Pycnacronema*, *Leptodesmis* and close phylogenetic and morphological genera.

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