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Taxonomic updates in the family Leptolyngbyaceae (Leptolyngbyales, Cyanobacteria): the description of *Pseudoleptolyngbya* gen. nov, *Leptolyngbyopsis* gen. nov., and the replacement of *Arthronema*

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ABSTRACT

In this paper, we used the polyphasic approach combining 16S rRNA gene phylogenies, identity (p-distance), 16S-23S ITS secondary structures, and morphological and habitat analyses to describe two new genera of Leptolyngbyaceae, *Pseudoleptolynbgya* G.S. Hentschke gen. nov. (based on LEGE 16533 and LEGE 16651) and *Leptolyngbyopsis* G.S. Hentschke gen. nov. (based on LEGE 16524). These strains were sampled from freshwater environments at Figueira da Foz, Monchique and Coimbra, Portugal. Both genera are morphologically and ecologically identical to *Leptolyngbya* and can only be distinguished from it by molecular analyses. Based on morphological and ITS secondary structure analyses we describe two species of *Pseudoleptolyngbya, P. figueirensis* and *P. monchiquensis. P. figueirensis* differs from *P. monchiquensis* by the denser arrangement of the trichomes, the presence of firm sheaths, the common formation of shorter trichomes (up to 30 cells), and the olive or yellowish-green cell content, which is darker in *P. monchiquensis*. Moreover, the macroscopic appearance of *P. figueirensis* is a dense brownish mat, while *P. monchiquensis* is less dense and reddish. 16S rRNA gene sequences of *Arthronema africanum* CCALA020 are > 99% identical to *Leptolyngbya boryana* PCC6306, so we transfer *A. africanum* to *Leptolyngbya*, as *L. africanoidea*.

HIGHLIGHTS

- *Pseudoleptolyngbya* gen. nov. and *Leptolyngbyopsis* gen. nov. are described.
- *Arthronema* is placed in synonymy with *Leptolyngbya*.

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Introduction

Cyanobacteria, also known as blue-green algae, are the only Gram-negative bacteria capable of performing oxygenic photosynthesis. As primary oxygen producers, they have had a profound impact on the evolution of life on Earth. Cyanobacteria inhabit various ecosystems, ranging from terrestrial to freshwater, brackish, marine and extreme environments. They can exist either independently or in symbiotic relationships with other organisms, such as marine invertebrates and higher plants, or as part of cyanolichens. Their remarkable ability to adapt and thrive in diverse environments enables them to biosynthesize a wide array of secondary metabolites with diverse biological activities. In addition to their toxin production capability, cyanobacteria also produce numerous structurally unique compounds with antibacterial, antifungal, cytotoxic, antitumour, anti-inflammatory and protease inhibitory properties. These compounds hold great potential for biotechnological applications in pharmaceuticals and cosmeceuticals (Whitton & Potts, 2002; Lopes *et al.*, 2022).

Among cyanobacteria, the Leptolyngbyaceae are characterized by thin trichomes (about 1.5–2.5 (4.5) µm wide), facultatively constricted at cross-walls, and with cylindrical cells (Strunecký *et al.*, 2023). In this family, based on the polyphasic approach, several new genera morphologically very similar to *Leptolyngbya boryana* Anagnostidis & Komárek have been described, primarily based on molecular and ecological features. These include *Pantanalinema* Vaz *et al.* and *Alkalinema* Vaz *et al.*, which were described from alkaline and saline wetlands in the Pantanal, Brazil (Vaz *et al.*, 2015). However, recent studies have demonstrated that *Alkalinema* is very closely related to *Planktolyngbya* Anagnostidis & Komárek (Mai *et al.*, 2018; Hentschke *et al.*, 2024) and thus we think that it requires re-evaluation. Similarly, the traditional Leptolyngbyacean genus *Arthronema* Komárek & Lukavský (1988), initially described solely on morphological traits, also needs to be reassessed. In fact, the only currently accepted species, *Arthronema africanum*

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Komárek & Lukavsky, shares > 99% 16S rRNA gene sequence identity with genuine members of the genus *Leptolyngbya* (Strunecký *et al*., 2023).

The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC), at CIIMAR (University of Porto), Portugal, holds a great diversity of Leptolyngbyaceae isolates, in addition to the type strain of *Romeriopsis navalis* Hentschke *et al.* (2022). During our work on identification of these isolates, we found that the strains LEGE 16533, LEGE 16651 and LEGE 16524 do not fit the circumscription of any cyanobacterial genera. Therefore, we describe here the new taxa *Pseudoleptolyngbya* gen. nov., including *P. figueirensis* sp. nov (LEGE 16533) and *P. monchiquensis* sp. nov. (LEGE 16651) and *Leptolyngbyopsis coimbrensis* gen. et sp. nov. (LEGE 16524). Additionally, our results indicate that *Arthronema* species should be transferred to *Leptolyngbya*.

Material and methods

Sampling, isolation of strains and morphological analysis

The samples were collected from freshwater environments at Figueira da Foz (40°09′03.5”N, 8° 51′27.1”W), Malhada Quente (Monchique) (37° 19′44.8”N, 8°31′33.3”W) and Coimbra (40° 12′28.8”N, 8°25′03.6”W), Portugal. From these samples, the studied strains were isolated manually, using Pasteur pipettes, in Z8 liquid medium (Kotai, 1972), at the Doctor Maria de Fátima Santos personal culture collection (Alga2O, Coimbra). Then, in 2018, they were acquired by LEGE-CC, where they are currently maintained under the following conditions: 19°C, 12:12 hours light/dark cycle (25 μmol photons m^{-2} s^{-1}). The strains were observed and photographed under a Leica DMLB microscope. For each strain, measurements of 40 cells were obtained using dedicated software (Leica LAS EZ; Leica Microsystems), and some biomass was lyophilized and preserved in the University of Porto Herbarium.

DNA extraction, PCR amplification and sequencing

Genomic DNA isolation was performed using the NZY Microbial gDNA Isolation Kit (NzyTech), following the manual's specifications. The 16S rRNA gene and 16S-23S ITS gene amplifications were performed using the primers 27 F and 23SR (Neilan *et al*., 1997; Nübel *et al*., 1997), under the following conditions: initial denaturation step at 95°C for 5 min; 10 cycles of denaturation at 94°C for 45s, primers annealing at 57°C for 45s, strand extension at 72°C for 2 min; 25 cycles of denaturation at 92°C for 45 s, primers annealing at 54°C for 45 s, strand extension at 72°C for 2 min and a final elongation step at 72°C for 7 min. The PCR products were purified using the NZYGelpure kit (NZYTech), according to the instructions, and sequenced using the primers 27F, 359F, 781R, 1114F and 23SR (Neilan *et al*., 1997; Nübel *et al*., 1997; Iteman *et al*., 2000). These sequences were deposited in GenBank (NCBI) and the codes are reported in the phylogenetic trees.

Phylogenetic analysis

To determine the position of our strains among Cyanobacteria, we aligned the 16S rRNA nucleic acid sequences obtained in this study with those of reference strains of Pseudanabaenales, Nodosilineales, Oculatellales and Leptolyngbyales. Also, other sequences were retrieved from GenBank (NCBI) using BLAST. The outgroup used was *Gloeobacter violaceus* PCC8105 (AF132791). The sequences were aligned using ClustalW, in MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura *et al*., 2021), and the final dataset contained 120 sequences with 939 aligned positions. The phylogenetic trees were built using Maximum Likelihood analysis in IQ-Tree online version v1.6.12 (Trifinopoulos *et al*., 2016), and Bayesian Inference analysis with MrBayes (Ronquist *et al*., 2012) in Cipres Gateway (Miller *et al*., 2010). The GTR+G + I evolutionary model was selected by MEGA 11. The robustness of ML trees was estimated by the bootstrap test (Felsenstein, 1985), using 1000 replications. Bayesian trees were constructed in two independent runs, with four chains each, for 5×10^6 generations, burn-in fraction set to 0.25, sample frequency set to 1000. The analyses were continued until the value of the standard deviation of split frequencies was below 0.01. Also, an identity (p-distance) matrix was generated. The 16S-23S ITS secondary structures of D1-D1’, V2, Box B and V3 helices were folded using MFold (Zuker, 2003), according to Lukesova *et al.* (2009).

Transmission electron microscopy (TEM)

The strains were fixed with 2.5% glutaraldehyde and 2% paraformaldehyde in 50 mM sodium cacodylate buffer (pH 7.2) for 72 h and postfixed overnight with 2% osmium tetroxide in 50 mM sodium cacodylate buffer (pH 7.2). Subsequently, the cells were washed three times in sodium cacodylate buffer and stained *en bloc* overnight with aqueous 2% uranyl acetate solution overnight, dehydrated and embedded in Embed-812 resin (#14120; Electron Microscopy sciences). Ultra-thin sections (50 nm thickness) were cut on an RMC Ultramicrotome (PowerTome, USA) using Diatome diamond knives, mounted on mesh copper grids (Electron Microscopy Sciences), and stained with uranyl acetate substitute (#11000; Electron Microscopy Sciences) and lead citrate (#11300; Electron Microscopy Sciences) for 5 min each. Samples were visualized on a JEOL JEM 1400 transmission electron microscope (JEOL, Tokyo, Japan) operating at 80 kV, and images were digitally recorded using a CCD digital camera Orius 1100 W (Tokyo, Japan).

Results

The phylogenetic tree based on ML and BI analyses (Fig. 1) separates the orders Pseudanabaenales, Nodosilineales, Oculatellales and Leptolyngbyales into strongly supported monophyletic groups.

In the Leptolyngbyaceae all the genera proved to be monophyletic. The strains LEGE 16533 and LEGE 16651 form a separate clade, and are described here as *Pseudoleptolyngbya*, which is characterized by the presence of homocytous, thin, cylindrical, and constricted trichomes, with thin and colourless sheaths. These features make *Pseudoleptolyngbya* morphologically indistinguishable from *Leptolyngbya, Planktolyngbya*, and *Alkalinema*. In terms of habitat, *Pseudoleptolyngbya, Leptolyngbya* and *Planktolyngbya* are typically found in freshwater environments, while *Alkalinema* is associated with alkaline and saline environments. Despite these morphological and ecological similarities, the 16S rRNA gene phylogenetic analyses (Fig. 1) clearly differentiate those genera as distinct cryptic taxa. A comparison of morphology and habitats between *Pseudoleptolyngbya* and its most closely related genera is presented in Table 1 and further elaborated upon in the Discussion.

Pseudoleptolyngbya is a distinct monophyletic taxon (ML = 100, BI = 1), and is closely related to *Romeriopsis*, a very unusual marine genus characterized by few-celled trichomes, which was described from a population growing on rocks, at a depth of 13 m. *Leptolyngbya* (ML = 100, BI = 0.9) is phylogenetically distant from the *Pseudoleptolyngbya/ Romeriopsis* cluster and is more closely related to *Tapinothrix* in the crown of the tree.

The 16S rRNA gene identity (p-distance) analysis (Supplementary table S1), also indicates that *Pseudoleptolyngbya* is a distinct genus within the family Leptolyngbyaceae. Intrageneric identity values range from 99.15% to 100%. *Romeriopsis* is the closest genus, but the similarity values are low, 91.4–92.5%. Compared with *Alkalinema*, *Leptolyngbya* and *Planktolyngbya*, the morphologically most similar genera, the 16S rRNA gene identity values are also low, ranging from 91% (vs *Leptolyngbya*) to 92.4% (vs *Leptolyngbya*).

The 16S-23S ITS sequences of *Pseudoleptolyngbya* include both tRNA^{Ile} and tRNA^{Ala}, along with the D1-D1', V2, Box B and V3 helices. The D1-D1' helix of *Pseudoleptolyngbya* is unique among these genera, with a stem (5′GAGGUCACUC3') originating from the first lateral bulge and opposing a sequence of five free residues (5′AGCCC3') (Fig. 2). Furthermore, *Pseudoleptolyngbya* D1-D1' helices differ from the other genera in sequence, length and structure. The V2, Box B and V3 helices of *Pseudoleptolyngbya* are also different from the other genera in sequence, length and structure (Figs 3–5). There are no available 16S-23S ITS sequences of *Planktolyngbya limnetica* in GenBank (NCBI), for comparison with *Pseudoleptolyngbya*, but the genera occupy very divergent phylogenetic positions and exhibit low 16S rRNA similarity values. Based on these findings, the ITS secondary structures analyses are in agreement with the phylogenetic analyses, and reinforce the separation of *Pseudoleptolyngbya* as a distinct genus.

Within the *Pseudoleptolyngbya* clade, the type strain LEGE 16533 clusters with LEGE 15546. Both strains exhibit identical morphology and share 100% 16S rRNA identity. Based on this, we consider both strains to be within the circumscription of the species here named *P. figueirensis*. Strain LEGE 16651 is more distant from the type strain LEGE 16533, and differs morphologically in some aspects: LEGE 16533 has a denser arrangement of trichomes, firmer sheaths, commonly forms shorter trichomes (up to 30 cells), and has an olive or yellowish-green cell content, which is darker or brownish in LEGE 15546. The macroscopic aspect of LEGE 16533 is denser and brownish, while 16651 is less dense and reddish. The ITS secondary structures of these two strains differ. While both strains have the generic diagnostic stem in the first lateral bulge of the D1-D1' helix, they differ in the terminal portion of this helix. Furthermore, the V2, Box B and V3 helices of these two strains clearly differ in sequence, length and structure (Figs 2–5). These findings, based on morphological, phylogenetic, and ITS analyses, support the recognition of LEGE 16533 (*P. figueirensis*) and LEGE 16651 (*P. monchiquensis*) as two distinct species.

Considering the above findings, the polyphasic analyses encompassing 16S rRNA gene phylogenies, identity, morphology, habitat and 16S-23S ITS secondary structures are in agreement, and support the description of *Pseudoleptolyngbya*, as well as the species *P. figueirensis* and *P. monchiquensis.*

The phylogenetic analyses also show that the strain LEGE 16524 is not related to any known genus of the Leptolyngbyaceae, and consequently is described here as *Leptolyngbyopsis*, which is very similar to *Pseudoleptolyngbya* and is also characterized by the

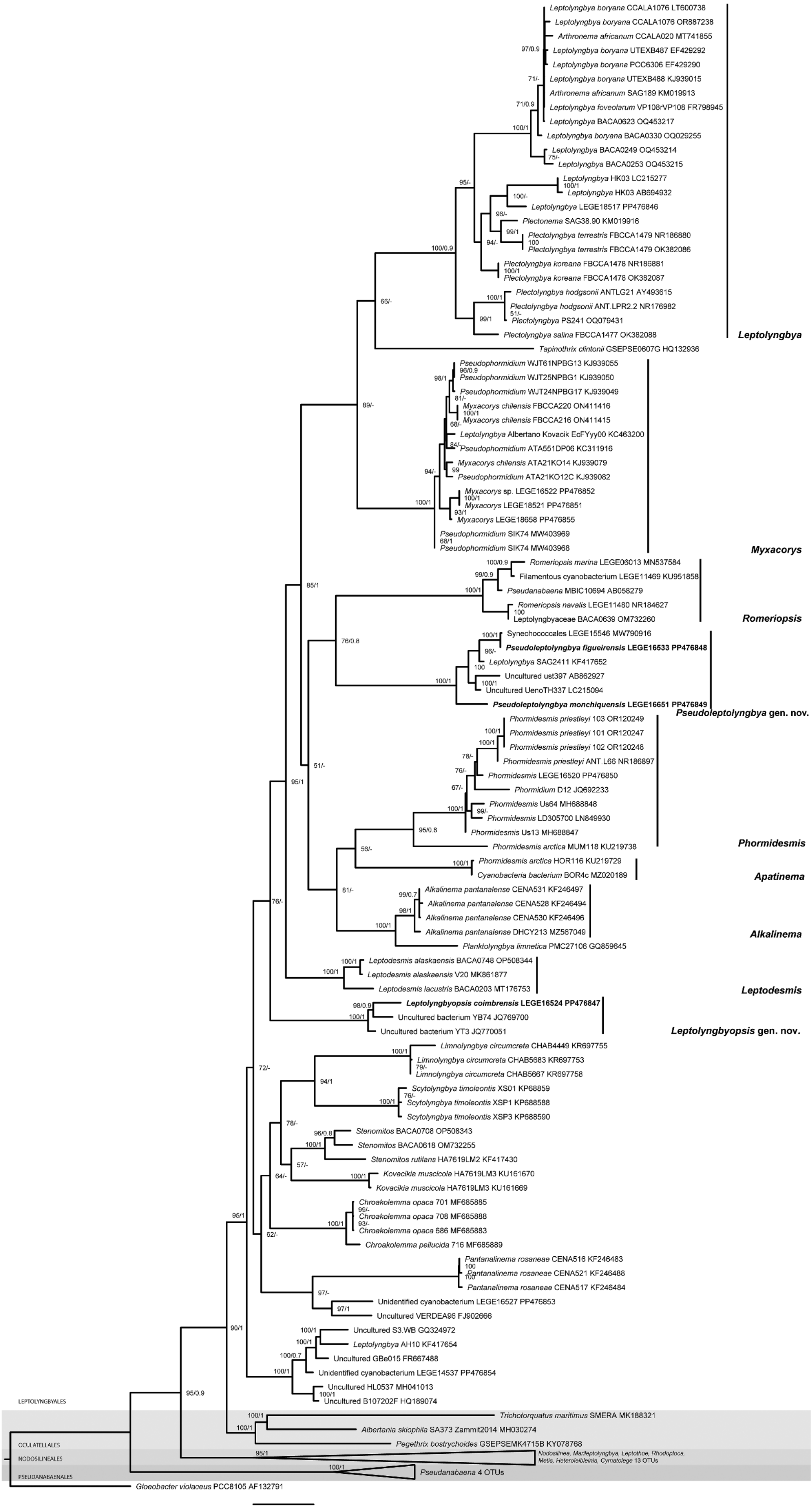


Fig. 1. ML phylogenetic tree of *Pseudoleptolyngbya* gen. nov., *Leptolyngbyopsis* gen. nov. and related genera in the Leptolyngbyaceae. Numbers at nodes represent ML values and BI posterior probabilities. The strains isolated for this work are in bold.

Table 1. Morphological and habitats comparisons between *Pseudoleptolyngbya, Leptolyngbyopsis* and their phylogenetically most closely related genera.

Genus (reference)	*Leptolyngbya* (Komárek & Anagnostidis, 2005)	*Myxacorys* (Pietrasiak *et al.*, 2019)	*Romeriopsis* (Hentschke & Gama-Júnior, 2022)	*Phormidesmis* (Komárek & Anagnostidis, 2005)	*Alkalinema* (Vaz *et al.*, 2015)	*Leptodesmis* (Raabová *et al.*, 2019)	*Planktolyngbya* (Komárek & Anagnostidis, 2005)	*Pseudoleptolyngbya* gen. nov.	*Leptolyngbyopsis* gen. nov.
Filament organization	Long, solitary or in clusters	Long, wavy, in colonies, sometimes rope-like	Short, in irregular clusters	Long, solitary or in irregular clusters, sometimes densely entangled and ± parallel	Long, solitary or interwoven	Long, solitary, straight or flexuous	Long, solitary, straight, flexuous or coiled	Short or long, solitary or forming entangled clusters, flexuous	Short or long, solitary or forming entangled clusters, flexuous
Trichomes	Cylindrical or slightly attenuated. One trichome per sheath. Constricted or not	Cylindrical. One or two trichomes per sheath. Sometimes twisted within the sheath. Slightly constricted	Cylindrical. Usually few celled or more rarely >50 cells. Constricted	Cylindrical, usually constricted at the cross-walls. Sometimes 2–3 trichomes per sheath.	Cylindrical, constricted	Slightly widened at the apex. Constricted. One filament per sheath.	Cylindrical, constricted or not	Cylindrical, constricted, with terminal cells rounded at the ends	Cylindrical, constricted, with terminal cells rounded at the ends
Cells	Isodiametric or longer than wide	Shorter than wide or isodiametric. Occasionally longer than wide	Longer than wide or isodiametric	Cells isodiametric, slightly shorter or longer than wide	Isodiametric or longer than wide	Barrel-shaped	Isodiametric or usually longer than wide	Mainly longer than wide, rarely isodiametric	Mainly, isodiametric, rarely longer or shorter than wide
Cell content	Homogeneous. Pale blue-green to reddish	Homogeneous or granulated	Homogeneous. Olive-green to reddish	Central granules	Homogeneous. Olive to brownish-green	Central granules	Homogeneous	Homogeneous, olive to browinish-green	Homogeneous, pale green
Cells measurements (µm)	1–4.5 long × 0.5–3.5 wide	1–3 long, 0.7–3 wide	1.6–3 long × 1.5–2 wide	1–3.8 long × 1–4 wide	1.5–4.1 long × 1.1–2.2 wide	1–2 long × 1–2 wide	1.5–16 long × 1–5 wide	2.3–3.4 long × 1.6–2 wide	1.6–2.5 long × 1.7–2 wide
Hormogonia	Cylindrical	Cylindrical or widened or attenuated at the ends	Cylindrical	Not described	Not described	Not described	Cylindrical	Cylindrical	Cylindrical
Sheaths	Thin, joined to the trichomes	Thin, firm, sometimes forming a cap at the ends of the trichomes. Colourless	Thin, soft. Colourless	Facultative. Thin or thick, firm, homogeneous or lamellate, colourless, slightly yellowish-brown or black	Soft	Thick, firm, colourless	Thin, firm, colourless	Facultative, very thin, firm, joined to the trichome, colourless	Very thin, firm, joined to the trichome, colourless
Habitat	Mats in fresh water, marine, terrestrial and thermal spring environments	Desert soil or on limestone rocks	Marine. On rocky substrates at 13 m depth or intertidal	Terrestrial	Alkaline and saline wetlands	Mud of littoral pools in Antarctica	Planktonic in fresh water	Planktonic in fresh water	Planktonic in fresh water

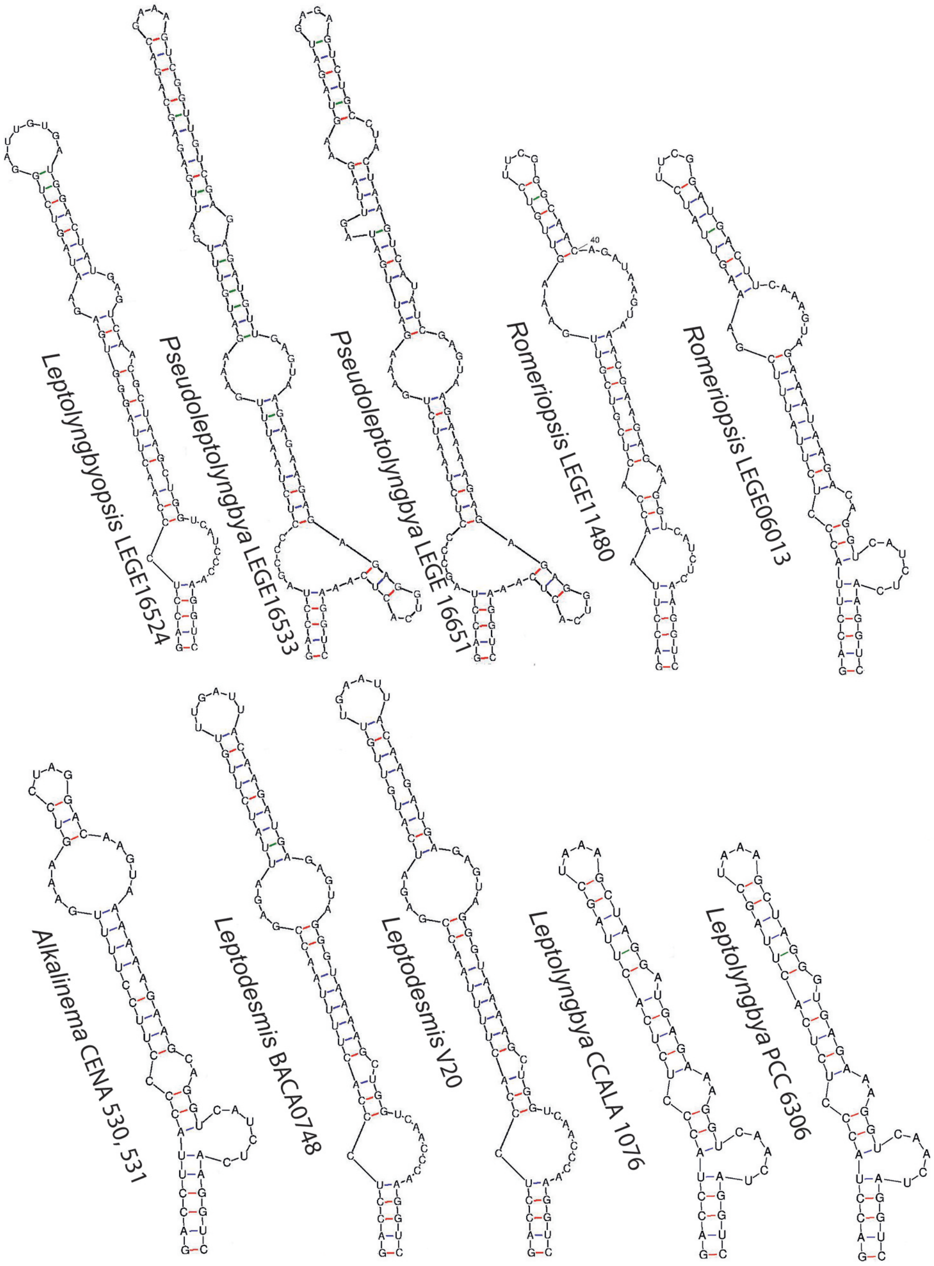


Fig. 2. 16S–23S ITS D1-D1’ helices of *Pseudoleptolyngbya* gen. nov., *Leptolyngbyopsis* gen. nov., and related genera in the Leptolyngbyaceae.

presence of homocytous, thin, cylindrical and constricted trichomes, with thin and colourless sheaths. The only difference between these genera is that *Leptolyngbyopsis* cells are usually isodiametric and rarely longer or shorter than wide, while *Pseudoleptolyngbya* cells are usually longer than wide and rarely isodiametric. Nevertheless, as demonstrated for *Pseudoleptolyngbya*, based on these characters, it is impossible to distinguish *Leptolyngbyopsis* from *Leptolyngbya*, *Planktolyngbya* and *Alkalinema* (Table 1).

Despite these similarities, the 16S rRNA gene tree places *Leptolyngbyopsis* in a strongly supported clade (ML = 100, BI = 1) at the base of a cluster containing *Leptodesmis* Raabová *et al.*, *Planktolyngbya*, *Alkalinema*, *Phormidesmis*, *Apatinema* Davydov, *Pseudoleptolyngbya*, *Romeriopsis,* *Myxacorys* Pietrasiák & Johansen and *Leptolyngbya*, but it is not closely related to any of them. This phylogenetic position indicates that *Leptolyngbyopsis* constitutes a distinct cyanobacterial lineage, warranting its description as a new genus.

The 16S rRNA gene identity (p-distance) analysis (Supplementary table S1) is in agreement with the phylogenetic results and also indicates that *Leptolyngbyopsis* is a distinct genus within the family Leptolyngbyaceae. The intrageneric identity ranges from 98.3% to 98.6%. *Leptolyngbyopsis* is < 95%

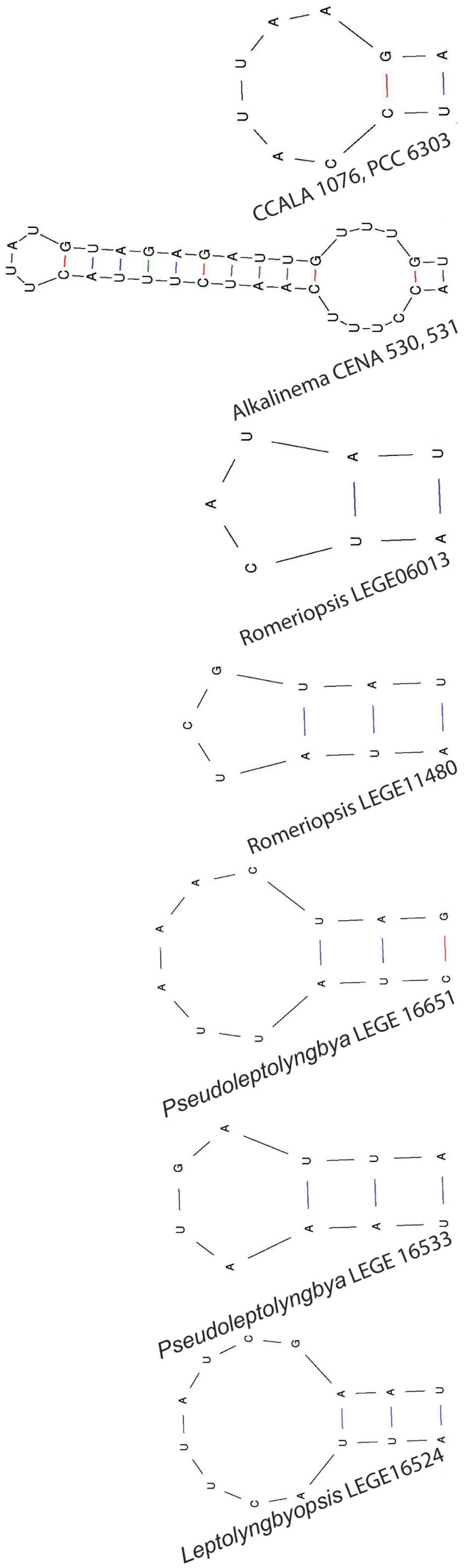


Fig. 3. 16S–23S ITS V2 helices of *Pseudoleptolyngbya* gen. nov., *Leptolyngbyopsis* gen. nov., and related genera in the Leptolyngbyaceae.

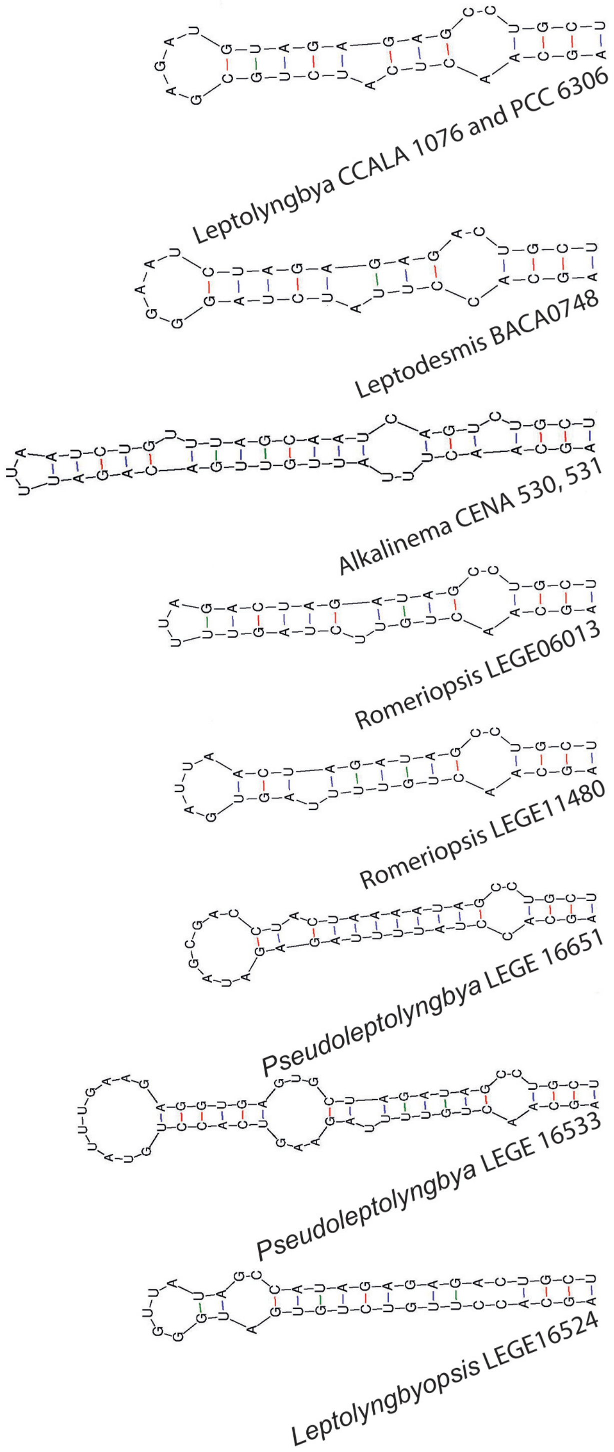


Fig. 4. 16S–23S ITS Box B helices of *Pseudoleptolyngbya* gen. nov., *Leptolyngbyopsis* gen. nov., and related genera in the Leptolyngbyaceae.

similar to the closest related taxa. The greatest similarity is 95.2%, with *Kovacikia* Miscoe *et al.*, which is more closely related to *Stenomitos* Miscoe & Johansen, *Scytolyngbya* Song & Li and *Limnolyngbya* Li & Li.

The structure of the basal stem and the first lateral bulge of the D1-D1' helix in *Leptolyngbyopsis* resembles that of *Leptodesmis*, but they differ in their sequences (Fig. 2). Furthermore, the final portion of this helix differs

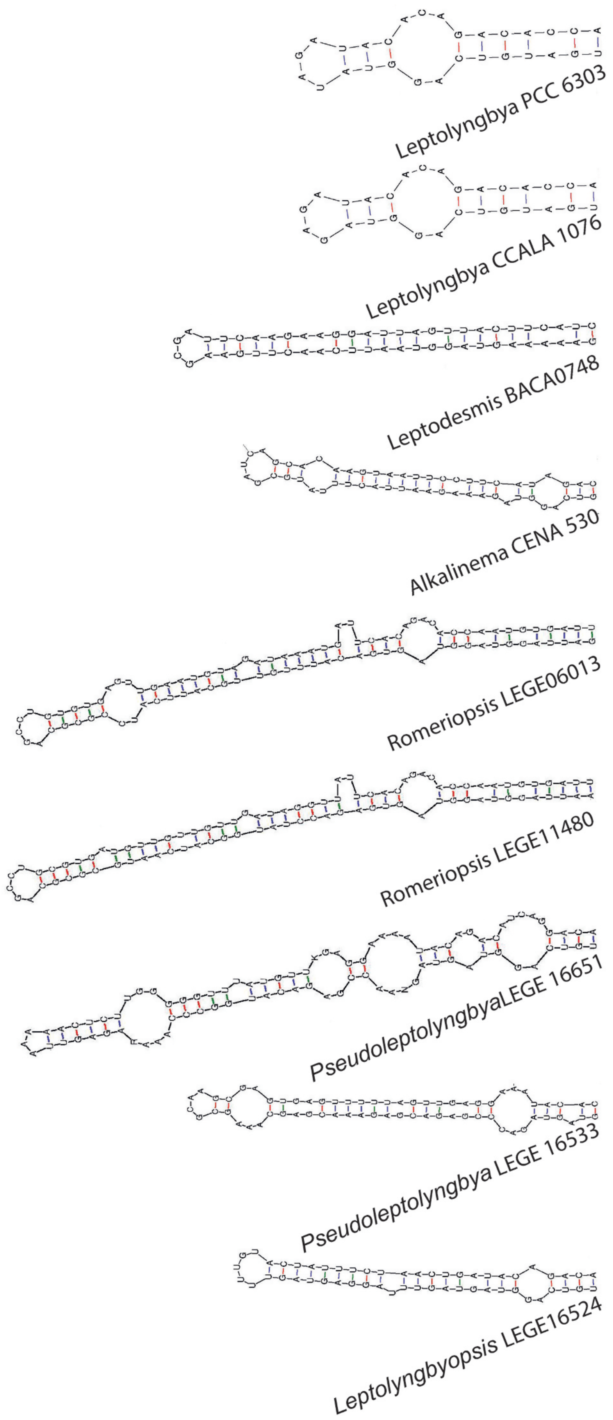


Fig. 5. 16S–23S ITS V3 helices of *Pseudoleptolyngbya* gen. nov., *Leptolyngbyopsis* gen. nov., and related genera in the Leptolyngbyaceae.

in sequence, length and structure between genera. Another difference between both genera is that *Leptodesmis* 16S-23S rRNA ITS sequences present both tRNAs, but in the V2 helix region, this genus has a short sequence (5′ACCTA3’), that cannot be folded. The V2, Box B and V3 helices were more variable among the studied genera, and those of *Leptolyngbyopsis* also differ from the others in sequence, length and structure.

The polyphasic analyses considering 16S rRNA phylogenies, identity, morphology, habitat and ITS secondary structures are in agreement, and support the description of *Leptolyngbyopsis*.

Another important finding in our phylogenies (Fig. 1) is that the *Arthronema* type strain *Arthronema africanum* CCALA020 is clustered with strong phylogenetic support (ML = 100, BI = 1) with *Leptolyngbya boryana* PCC6306, one of the representative strains of the genus *Leptolyngbya*. The 16S rRNA gene identity (p-distance) between these two strains is 99.8% (Supplementary table S1). These results indicate that *Arthronema africanum* must be combined into the older genus *Leptolyngbya*, as *L. africanoidea* comb. nov. et nom. nov.

Discussion

The general overview of the tree permits the identification and the separation of *Pseudoleptolyngbya* and *Leptolyngbyopsis* from the other genera of the Leptolyngbyaceae. Our results show the close relationships between *Leptolyngbya* and ‘*Arthronema*’ and between *Planktolyngbya* and *Alkalinema*.

Pseudoleptolyngbya is a cryptic genus, morphologically and ecologically indistinguishable from *Leptolyngbya* and *Planktolyngbya* (Komárek & Anagnostidis, 2005; Raabová *et al.*, 2019). As shown in Table 1, all three genera share identical trichome structure: cylindrical, constricted and with thin sheaths. They grow in similar freshwater habitats, and can be differentiated only by molecular analysis.

Despite these similarities, our 16S rRNA gene phylogenies confirm previous studies (Mai *et al.*, 2018), separating *Leptolyngbya* and *Planktolyngbya* as distinct monophyletic taxa. *Planktolyngbya* is clustered with *Phormidesmis*, *Apatinema* and *Alkalinema*. *Leptolyngbya* (including ‘*Arthronema*’) is in the crown of the tree, close to *Tapinothrix*. *Pseudoleptolyngbya* is phylogenetically distant from them, and is more related to the marine genus *Romeriopsis*, which presents a *Romeria*-like structure, with short trichomes and lacks mucilaginous sheaths (Hentschke *et al.*, 2022).

The identity values comparing *Pseudoleptolynbgya* with the other Leptolyngbyaceae genera are low (Supplementary table S1), with a maximum of 95.4% with *Phormidesmis*. However, *Pseudoleptolyngbya* and *Phormidesmis* are phylogenetically divergent and morphologically different.

The 16S-23S rRNA ITS secondary structures are also in agreement with the separation of *Pseudoleptolyngbya* from the other Leptolyngbyaceae (Figs 2–5). The stem originating from the first lateral bulge in the D1-D1’ helix is unique among the studied genera and can be used as a marker for the genus, considering that it is present in both species *P. figueirensis* and *P. monchiquensis*.

The other described taxon *Leptolyngbyopsis coimbrensis* is in a strongly supported monophyletic clade (ML = 100, BI = 1) phylogenetically distant from all the other cyanobacterial genera. The 16S rRNA gene identity analysis and the ITS secondary structures corroborate the description of this taxon. Like *Pseudoleptolyngbya, Leptolyngbyopsis* is a cryptic genus in the family Leptolyngbyaceae.

Finally, including '*Arthronema*' as a synonym of the genus *Leptolyngbya* is based on phylogenetic ML and BI analyses, as well as 16S rRNA gene identity. The type species *Arthronema africanum* was described based only on morphological analysis (Komárek & Lukavský, 1988) and was classified among the Pseudanabaenales. It was considered to differ from *Leptolyngbya* primarily by the presence of irregularly swollen cells, which were not described for *Leptolyngbya* (Komárek & Anagnostidis, 2005). Molecular analyses show the close relationship between both genera (Fig. 1). The *Leptolyngbya* representative strains (*L. boryana* CCALA1076 and PCC6306) are identical to those of *Arthronema africanum* (SAG 1.89 and CCALA020), as reported by Strunecký *et al.* (2023). This is not the first time that a genus has been combined into *Leptolyngbya*. *Plectolyngbya* was previously merged with *Leptolyngbya*, presenting less than 97% of identity with this genus (Kastovský *et al.*, 2023). Based on these findings, we transfer *Arthronema africanum* into the circumscription of *Leptolyngbya* and present an emended description of this genus, following the work of Kastovský *et al.* (2023). However, the specific epithet '*africana*' cannot be used, because *L. africana* Anagnostidis & Komárek already exists. Consequently, we used the new name '*africanoidea*'. The species *Arthronema gygaxiana* Casamatta *et al.* (2005) is more closely related to *Limnothrix* (Casamatta *et al.*, 2005) and shows only 88% 16S rRNA gene identity with the type strain of *A. africanum*. For that, reason, we excluded it from our analysis.

Description of taxa

Pseudoleptolyngbya G.S.Hentschke gen. nov.

In culture, brownish or reddish macroscopic mats. Adult filaments short or long, flexuous, solitary or forming entangled clusters, not orientated in one direction. Trichomes cylindrical, constricted, with terminal cells rounded at the ends; not branched and not attenuated. Sheaths facultative, very thin, joined to the trichome, firm, hyaline. Cells mainly longer than wide, rarely isodiametric, with homogeneous content, olive to dark-green. Cell measurements: 2.3–3.4 µm long × 1.6–2.0 µm wide.

DIAGNOSIS: Sister clade to *Romeriopsis*. The 16S rRNA gene D1-D1' helix presents a stem (5′GAGGUCACUC3') originating from the first lateral bulge and opposing the free residues 5′AGCCC3'.×

ETYMOLOGY: '*Pseudo*' is derived from ancient Greek '*Pseudes*' and means 'false'. '*Pseudoleptolyngbya*' refers to 'false' *Leptolyngbya*, due to the identical morphology of these genera.

TYPE SPECIES: *Pseudoleptolyngbya figueirensis* G.S. Hentschke sp. nov.

Pseudoleptolyngbya figueirensis G.S.Hentschke sp. nov. Figs 6–11

In culture forming dense brownish macroscopic mats. Filaments solitary or densely entangled. Trichomes long, or often short (up to 30 cells). Sheaths very thin, joined to the trichome, firm, colourless. Cell content olive-green. Cell measurements: 2.3–3.3 µm long x 1.6–2 µm wide.

DIAGNOSIS: Differs from *P. monchiquensis* G.S. Hentschke, by the presence of firm sheaths, the denser arrangement of the trichomes, the common formation of shorter trichomes (up to 30 cells); the olive or yellowish-green cell content, which is darker in *P. monchiquensis*; and the formation of dense brownish mats in culture, which are less densely aggregated and reddish in *P. monchiquensis*.

ETYMOLOGY: '*figueirensis*' refers to Figueira da Foz, Portugal, from where the species was sampled.

HOLOTYPE: Collected from an artificial freshwater fountain, at Figueira da Foz (40°09′03.5"N, 8° 51′27.1"W), Portugal in 2016, by Maria de Fátima Santos. Deposited in a metabolically inactive state (lyophilized) in the University of Porto herbarium under the code PO-T4789.

TYPE STRAIN: LEGE 16533 (PP476848)

Pseudoleptolyngbya monchiquensis G.S.Hentschke sp. nov Figs 12–17

In culture, forming loose reddish macroscopic mats. Filaments solitary or entangled, Trichomes long, flexuous. Mucilage diffluent, very thin. Cell content olive-green to brownish. Cell measurements: 2.4–3.4 µm long, 1.6–1.9 µm wide.

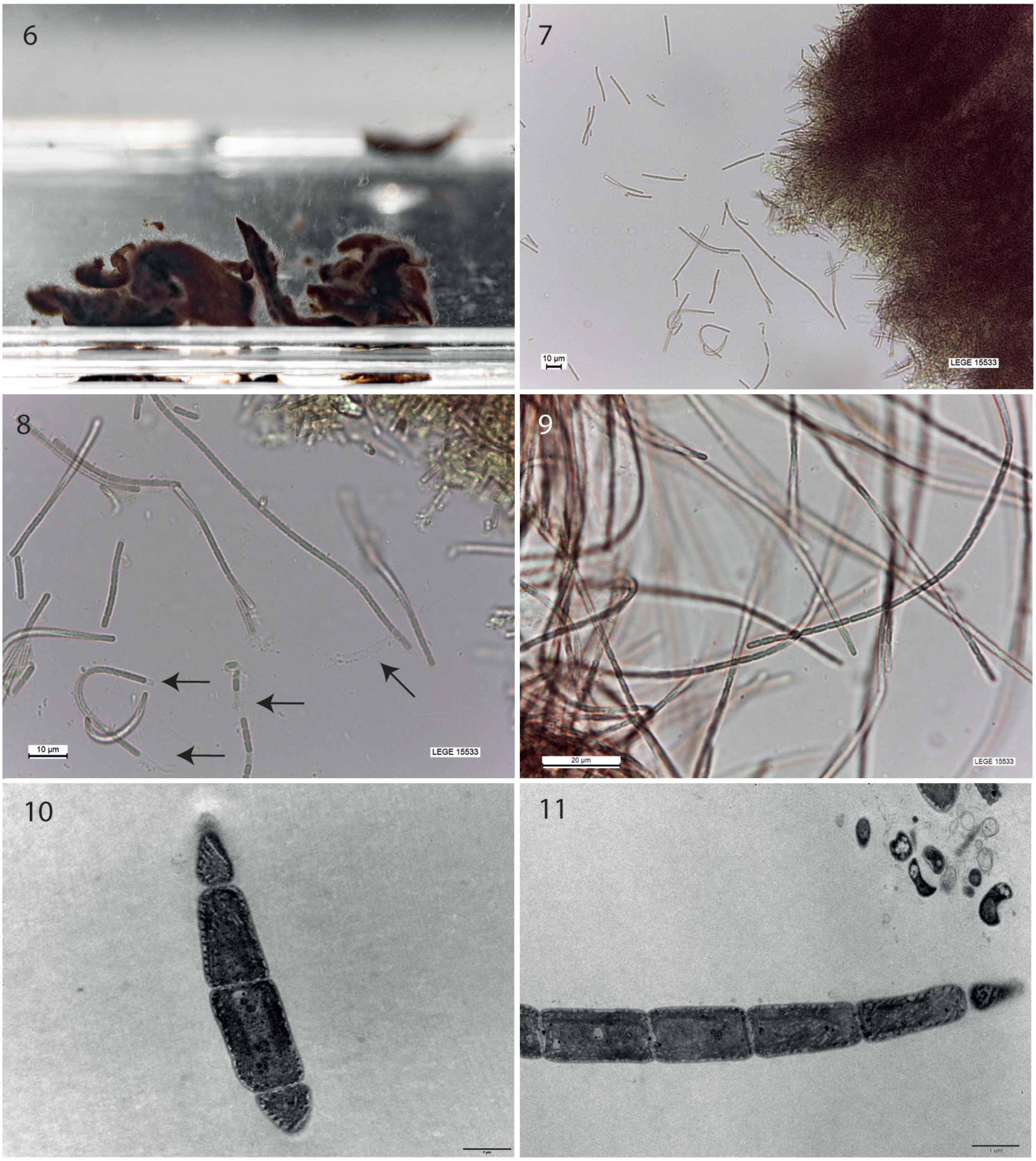
ETYMOLOGY: '*monchiquensis*' refers to Monchique, Portugal, from where the species was sampled.

HOLOTYPE: Collected from an artificial freshwater tank, at Malhada Quente (Monchique) (37°19′44.8"N, 8°31′33.3"W), Portugal in 2016, by Maria de Fátima Santos. Deposited in a metabolically inactive state (lyophilized) in the University of Porto herbarium under the code PO-T4790.

TYPE STRAIN: LEGE 16651 (PP476849)

Leptolyngbyopsis G.S.Hentschke gen. nov.

Features as for the type species, *Leptolyngbyopsis coimbrensis* sp. nov.



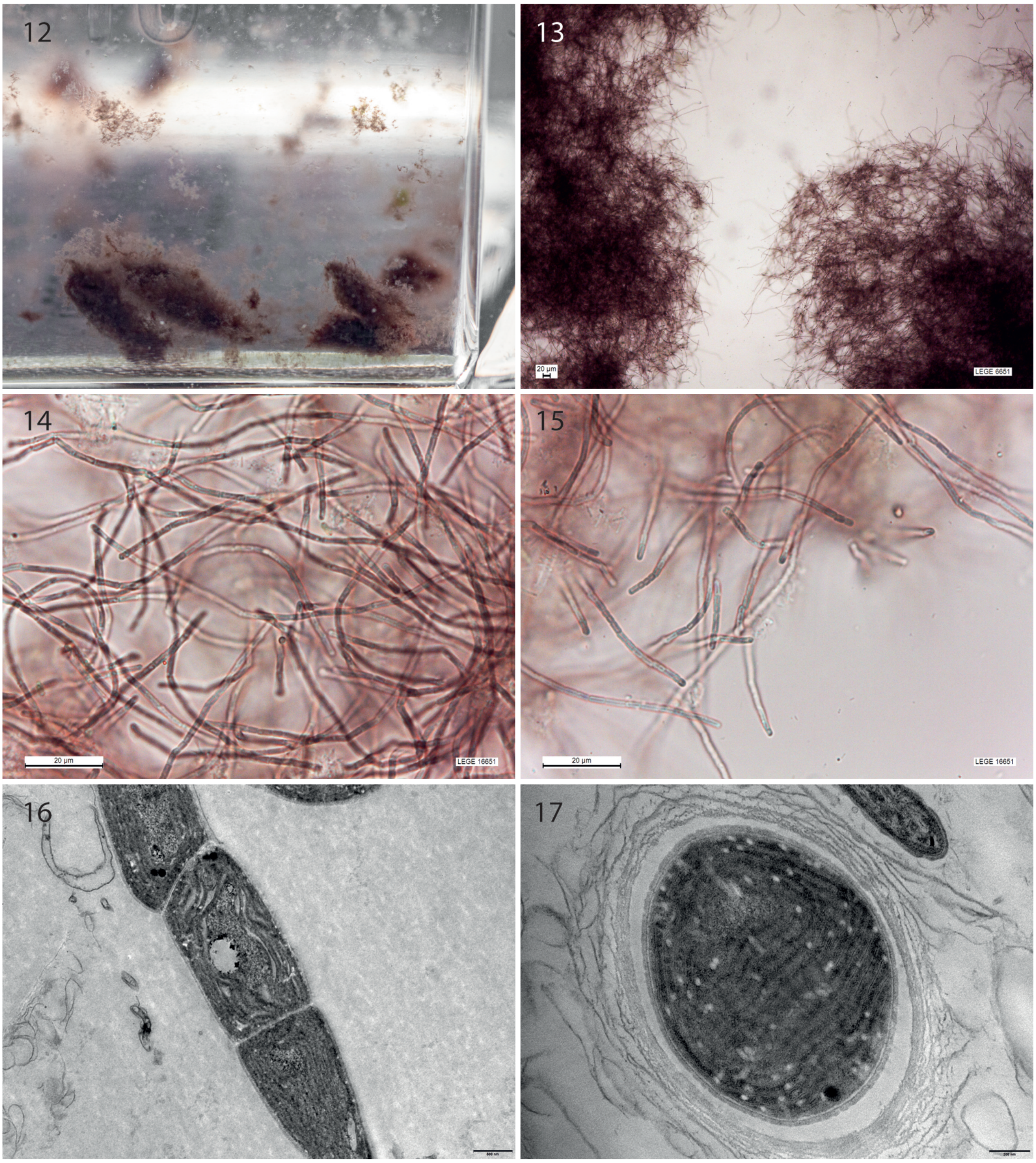
Figs 6–11. *Pseudoleptolyngbya figueirensis* LEGE 16533. **Fig. 6.** Macroscopic mat growing in culture flask. **Fig. 7.** General aspect of the colonies. **Fig. 8.** Details of trichomes and sheaths. **Fig. 9.** Details of the cylindrical and constricted trichomes with rounded ends. **Figs 10, 11.** Ultrastructure (TEM). The arrows point to the sheaths.

Leptolyngbyopsis coimbrensis **G.S.Hentschke sp. nov.** Figs 18–23

In culture, forming green mats attached to the flask walls. Adult filaments short or long, flexuous, solitary or forming entangled clusters, not orientated in one direction. Trichomes cylindrical, constricted, with terminal cells rounded at the ends; not branched and not attenuated. Sheaths, thin, joined to the trichome, firm, hyaline. Cells mainly isodiametric, rarely longer or shorter than wide, with homogeneous content, pale green. Cell measurements: 1.6–2.5 long × 1.7–2 wide.

DIAGNOSIS: Phylogenetically related to *Leptodesmis, Alkalinema, Phormidesmis, Apatinema, Pseudoleptolygbya, Romeriopsis, Myxacorys and Leptolygnbya*. The 16S rRNA D1-D1' helix has the sequence 5′UCAUCCCA3' in the first lateral bulge.

ETYMOLOGY: '*opsis*' is derived from Greek and means 'resembling'. '*Leptolyngbyopsis*' refers to



Figs 12–17. *Pseudoleptolyngbya monchiquensis*. **Fig. 12.** Macroscopic mat growing on culture flask. **Fig. 13.** General aspect of the colony. **Figs 14, 15.** Details of the cylindrical constricted trichomes with rounded ends. **Figs 16, 17.** Ultrastructure (TEM).

resembling *Leptolyngbya*, due to the identical morphology of these genera. '*coimbrensis*' is for Coimbra, Portugal, from where the species was sampled.

HOLOTYPE: Collected from a natural freshwater ditch, at Coimbra (40°12′28.8"N, 8°25′03.6"W), Portugal, in 2016, by Maria de Fátima Santos. Deposited in the University of Porto herbarium under the code PO-T4791.

TYPE STRAIN: LEGE 16524 (PP476847)

***Leptolyngbya* Anagnostidis & Komárek 1988**

SYNONYM: *Plectolyngbya* A.Taton, A.Wilmotte, J. Smarda, J.Elster & Komárek 2011

SYNONYM: *Arthronema* Komárek et Lukavský 1988

TYPE SPECIES: *Leptolyngbya boryana* (Gomont) Anagnostidis & Komárek

Leptolyngbya africanoidea G.S.Hentschke nom. nov.

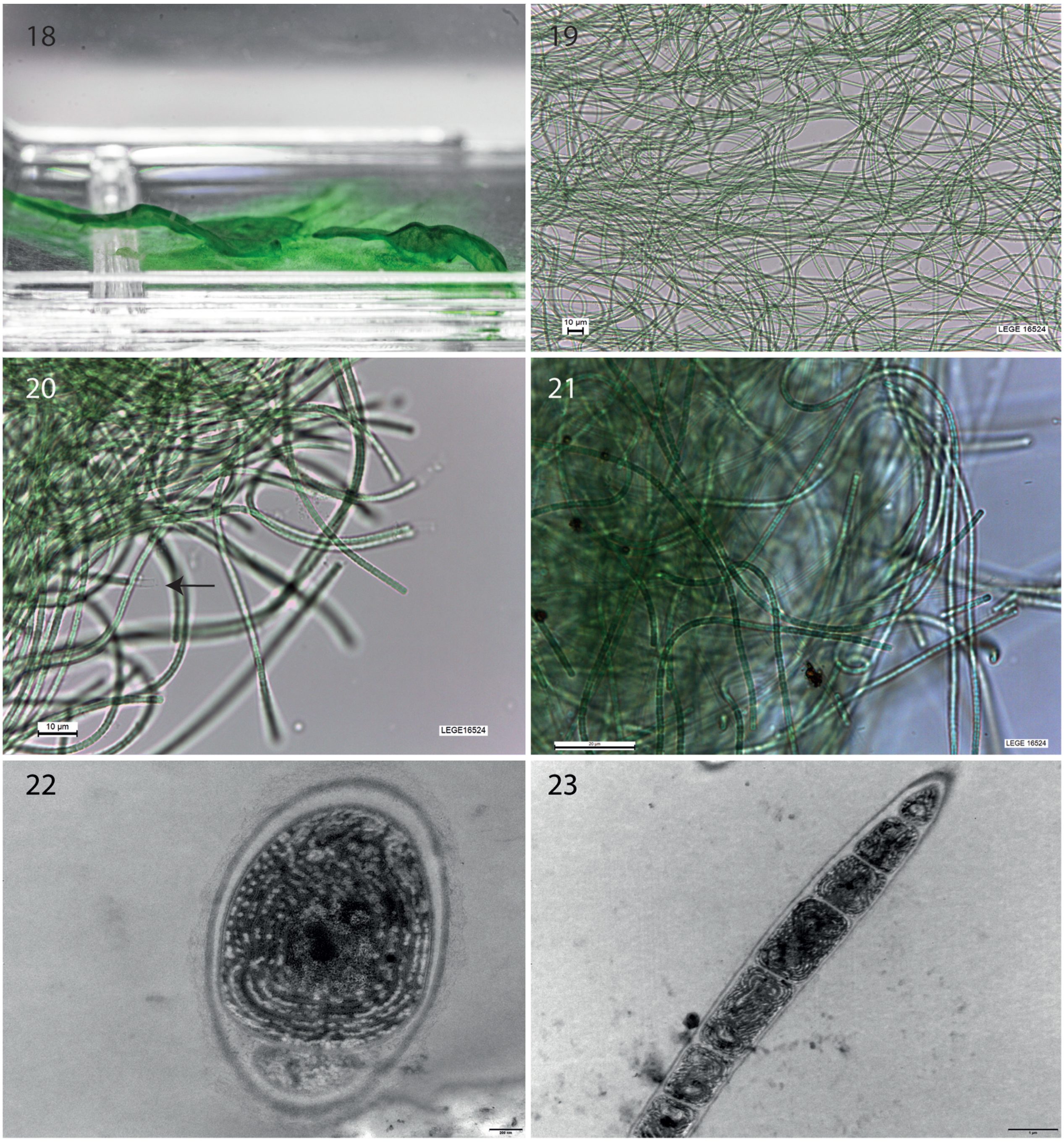


Fig. 18–23. *Leptolyngbyopsis coimbrensis*. **Fig. 18.** Macroscopic mat growing on culture flask. **Fig. 19.** General aspect of the colony. **Fig. 20.** Details of trichomes and sheaths. **Fig. 21.** Details of the cylindrical and constricted trichomes with rounded ends. **Figs 22, 23.** Ultrastructure (TEM). The arrow points to the sheaths.

SYNONYM: *Arthronema africanum* Komárek & Lukavský 1988: 266
BASIONYM: *Pseudanabaena africana* Schwabe & Simonsen 1961: 259

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Supplementary material

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at https://doi.org/10.1080/09670262.2024.2404887

Supplementary table S1. 16S rRNA gene identity (p-distance) among Leptolyngbyaceae genera.

Author contributions

G. S. Hentschke: original concept, drafted and edited manuscript and figures; F. Oliveira: drafted the manuscript, morphological analysis and obtained molecular data from the strains; J. Morais, R. Silva and P. Cruz: drafted the manuscript, isolation and cultivation of strains, retrieved the strains' 16S rRNA gene; V. Vasconcelos: advisor, review of the final version and funding.

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