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Morphological and molecular characterization of cyanobacterial isolates from the mouth of the Amazon River

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Abstract

The Amazon region contains a great diversity of species, and the Amazon River basin accounts for almost 20% of all the freshwater in the world. Despite the favorable environmental conditions in this region, little is known about the cyanobacterial diversity of this waterbody, especially at the mouth of the river. In this paper, we used the polyphasic approach to identify 14 cyanobacterial strains isolated in the Amazon River on the inlet site from a drinking water supply located close to the river mouth. The isolated strains were characterized based on morphology, behavior in culture, 16S rRNA gene sequencing, phylogenetic analysis and potential for toxin production. The isolated strains belong to seven different genera, namely, *Alkalinema, Cephalothrix, Limnothrix, Leptolyngbya, Phormidium, Pseudanabaena* and an unidentified Nostocales taxa that may represent a new genus. Strikingly, there were no new species, nor detection of gene clusters associated with cyanotoxin production. However, the phylogenetic placements of the Amazonian strains of *Limnothrix* and *Pseudanabaena* provide new insight into the taxonomy of these genera, reinforcing the need for taxonomic revision.

Keywords: Amazonia, *Limnothrix*, Polyphasic approach, *Pseudanabaena*, 16S rRNA phylogeny

Introduction

Cyanobacteria are among the most important organisms on Earth. In the past, the photosynthetic ability of cyanobacteria altered the atmosphere from reduced to oxygenic, helping the development of aerobic respiration and multicellular life (Soo *et al*. 2017). Cyanobacteria inhabit a diverse range of terrestrial and aquatic habitats, including extreme environments with high salinity and high levels of ultraviolet radiation (Whiton & Potts, 2012), and cyanobacterial biomass increases in warmer climates (Kosten *et al*. 2012) due to the high optimal temperature for the growth of many cyanobacteria (Whiton & Potts, 2012).

Currently, cyanobacteria have many useful utilizations, such as in cosmetics, human and animal food, in wastewater treatment, industrial CO_2 fixation, and soil fertilization (Grewe & Pulz, 2012). In addition, some cyanobacterial applications that are currently being studied are not yet economically feasible. Cyanobacteria are a good source of new products (e.g. Grewe & Pulz, 2012, Ramos *et al*. 2018), but to explore its full biotechnological potential, it is essential to better know their diversity, richness and distribution, which Grewe & Pulz (2012) consider to be understudied.

The Amazon region and its watershed form an ecosystem with a megadiversity of plants and animals (Tundisi *et al*. 2014). In addition, the species richness tends to increase toward the Equator line, where the study area from this work is located (Gaston, 2000). Despite this biodiversity, knowledge of cyanobacteria from this region is scarce compared to that from other regions in Brazil and countries of the northern hemisphere (Menezes *et al.* 2015, Genuário *et al*. 2017, Rigonato *et al.* 2017).

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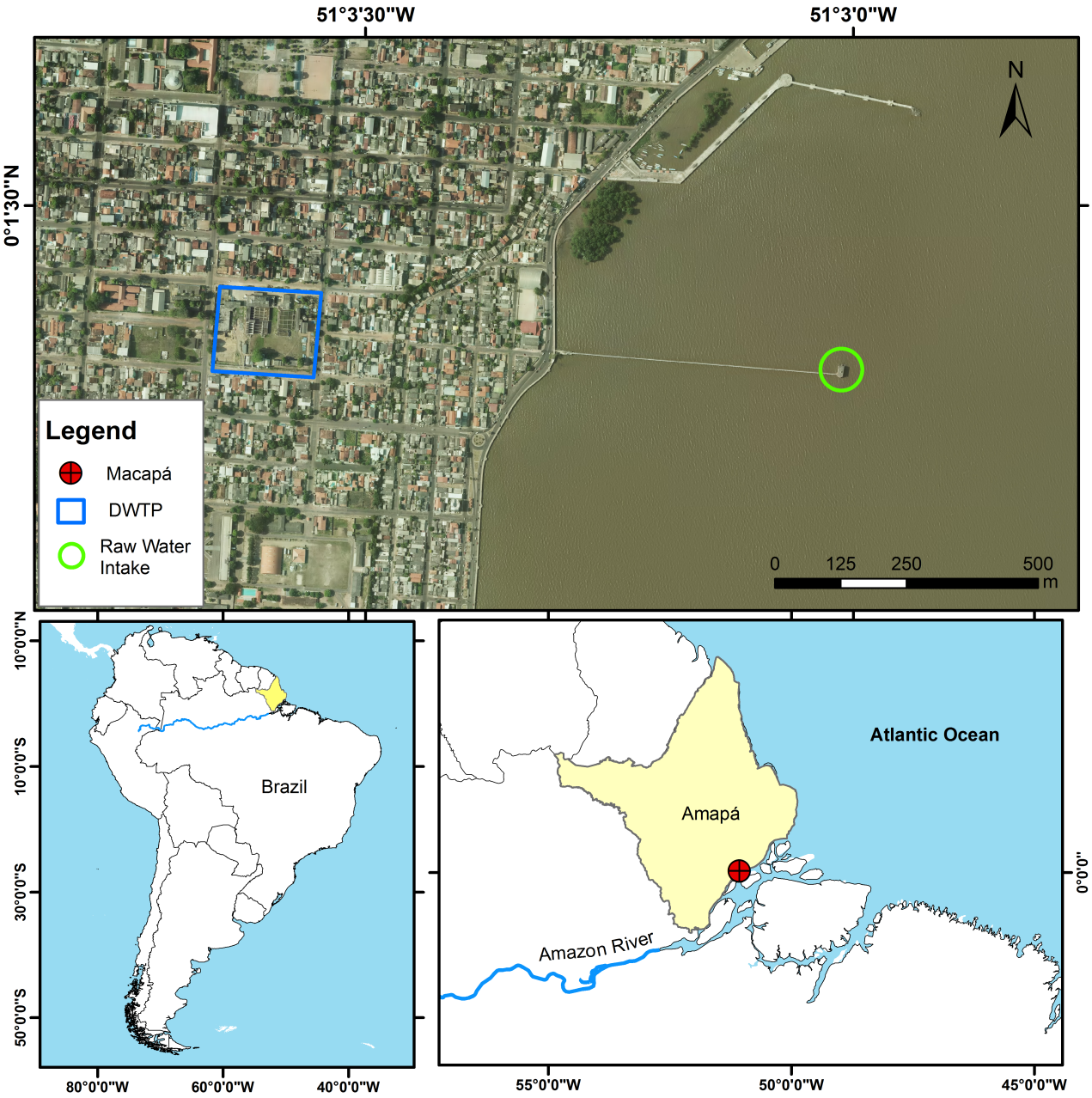

FIGURE 1. Map of the study area with an aerial view of the sampling sites: raw and treated water. *Coordinate System:* UTM zone 22N, SIRGAS 2000.

The search for nitrogen-fixing strains from the sediments of the Amazon and Solimões rivers, and from tributaries in Western Amazon was one of the first investigations on Amazon cyanobacteria (Fiori *et al.* 2005). In that work, several strains from the genera *Nostoc, Calothrix, Cylindrospermum, Fischerella* and *Anabaena* were isolated. Likewise, Vieira *et al*. (2005) isolated strains from the genera *Aphanizomenon, Aphanocapsa, Hapalosiphon, Microcystis, Nostoc, Oscillatoria, Planktothrix, Radiocystis* from two drinking water reservoirs in Pará, Eastern Amazon. Later, Dall'Agnol *et al.* (2012) used a culture-independent approach to study the diversity of *Synechococcus* in the Tucuruí Hydroeletric Power Station Reservoir, in Pará. More recently, by using the same approach, Doherty *et al.* (2017) studied the biogeography of Bacteria in the Amazon River mainstream and tributaries. They detected that cyanobacteria were mainly represented by *Synechococcus* and *Merismopedia* species, which showed higher proportion during low discharges. In turn, Satinsky *et al.* (2017) used culture-independent methods to study the Bacteria diversity in Amazon waters. They detected the dominance of *Synechococcus* at the Tapajós River, and of *Microcystis aeruginosa* in Belém (Pará). In another survey through the Amazon River and tributaries, 11 homocytous strains were isolated, belonging to the genera *Alkalinema, Cephalothrix, Pantalinema, Planktothrix* and *Pseudanabaena* (Genuário *et al.* 2017). Only

recently novel cyanobacteria species were described in the Amazon region: *Cronbergia amazonensis* from the Solimões River (Genuário *et al.* 2018a), and *Amazoninema brasiliense* from the Amazon and Solimões River (Genuário *et al.* 2018b).

The cyanobacteria diversity of the Amazon region is also being described through the use of morphological-based studies (Aprile *et al.* 2013, Cunha *et al.* 2013, Sena *et al.* 2015, Silva *et al.* 2018, Silveira-Júnior, 2012). However, it is nowadays recognized that morphological-based data are not sufficient for the proper identification of cyanobacteria taxa (Komárek, 2016). Currently, the recommended methodology for cyanobacteria studies is the polyphasic approach (Sciuto *et al.* 2012, Komárek, 2016). This approach consists of combining data derived from molecular sequencing and phylogenetic analysis with those obtained using other methods such as morphological, ecological and ecophysiological analyses (Komárek, 2016). In the last few years, many studies have used the polyphasic approach to elucidate the taxonomy and phylogenetic relationships of cyanobacteria (Furtado *et al*. 2009, Sciuto *et al*. 2012, Andreote *et al*. 2014, Yu *et al*. 2015, Bravakos *et al*. 2016, Genuário *et al*. 2017). In the present study, we isolated cyanobacterial strains from the Amazon River, near the inlet site from a drinking water supply located close to the river mouth (in front of Macapá city—Fig.1), and characterized them by studying their morphology, behavior in culture, 16S rRNA gene sequences, phylogenetic placement and their potential for toxin production. The phylogenetic relationship between the isolates from this study with other species of similar genera was also discussed.

Materials and methods

Study area and sampling:—The Amazon River is the main source of drinking water for the city of Macapá. The incoming raw water is extracted at 500 m from the river margin in the city downtown, a densely populated and commercial area where it is possible to see the punctual and diffuse discharges of sewage directly into the river. The study was conducted at the drinking water treatment plant (DWTP) of Macapá (Fig. 1). Samples were taken from raw and treated water from April 2015 to April 2016, as part of a larger project to study the cyanobacteria composition and population dynamics, and their relation to environmental data. Thus, a comprehensive information on data from these samples should be made available in a subsequent publication. In this work, some subsamples were taken in order to isolate cyanobacterial strains and characterize them, which is the focus of this paper.

Environmental characterization:—The physical and chemical parameters of the study sites were analyzed. Water temperature, pH, conductivity, dissolved oxygen and total dissolved solids were measured *in situ* using a YSI multiparameter probe. Samples were collected in amber glass bottles and stored in the dark for laboratory analysis of chemicals and metals (NH^{3-}, NO^{3-}, PO_3^{3-}, SO_4^{2-}, Cl^-, Al^{3+}, Fe^{2+}) by spectrophotometry with a DR 3900 spectrophotometer (Hach, Colorado, USA). Turbidity was measured with a AP2000iR turbidimeter (PoliControl, Brazil) as recommended by the American Public Health Association (APHA) (2005), and water transparency was measured in the field with a Secchi disk. Solar irradiation and insolation data for Macapá were provided by the National Institute of Space Research (CPTEC/INPE, 2016), while precipitation and air temperature data were provided by the National Institute of Meteorology (INMET).

Isolation and morphology:—Water samples for bacterial isolation were collected in sterilized 500 ml amber glass bottles to simulate the dark color of the Amazon River. Two main strategies were adopted to isolate the strains: the streak plate method (Rippka *et al*. 1981) and the separation of individual trichomes by micromanipulation (Waterbury, 2006). In the latter method, a drop of each sample was placed on a microscope slide and examined under a microscope. Several trichomes were selected, extracted with the help of drawn-out Pasteur pipettes (Jacinavicius *et al*. 2012) and transferred to fresh liquid medium. The culture media used for the isolation procedures and for growth were BG11, $BG11_0$ (Rippka *et al*. 1979) or Z8 (Kotai, 1972). All the procedures were performed aseptically, using sterile materials. Cultures were maintained at 23ºC in constant light exposure. The isolates were deposited in the LEGE Culture Collection at CIIMAR, Portugal (Ramos *et al*. 2018), under the codes LEGE 15484–LEGE 15497.

The morphological identification of cyanobacterial isolates was conducted by standard light microscopy and with the aid of a specialized bibliography, which included but was not limited to Komárek & Anagnostidis (2005), Komárek (2013), Sant' Anna *et al*. (2006; 2012), and Lamparelli *et al*. (2014). Some recent taxonomic literature describing new genera was also consulted (e.g., Malone *et al*. 2015, Vaz *et al*. 2015).

DNA extraction, PCR amplification of the 16S rRNA gene, and sequencing:—Genomic DNA (gDNA) was extracted with the PureLink Genomic DNA Kit from Invitrogen using the lysis protocol for gram-negative bacteria, following the manufacturer's instructions. Then, the genomic DNA was amplified by PCR with a Biometra TProfessional gradient thermocycler (Biometra, Göttingen, Germany).

TABLE 1. Primers used to amplify the 16S rRNA gene and the genes involved in the production of cyanotoxins.

Target	Primer set	Primer sequence 5'– 3'	Expected size (bp)	Reference
16S rRNA	CYA106F CYA781R	CGGACGGGTGAGTAACGCGTGA GACTACTGGGGTATCTAATCCCATT	675	Nübel *et al*. (1997)
16S rRNA	CYA359F CYA1494R	GGGGAATYTTCCGCAATGGG TACGGCTACCTTGTTACGAC	1135	Nübel *et al*. (1997) Neilan *et al*. (1997)
mcyA	CD1F CD1R	AAAATTAAAAGCCGTATCAAA AAAAGTGTTTTATTAGCGGCTCAT	297	Hisbergues *et al*. (2003)
mcyE	HEPF HEPR	TTTGGGGTTAACTTTTTTGGGCATAGTC AATTCTTGAGGCTGTAAATCGGGTTT	472	Jungblut; Neilan (2006)
sxtI	SXTI682F SXTI877R	GGATCTCAAAGAAGATGGCA GCCAAACGCAGTACCACTT	100	Lopes *et al*. (2012)
anaC	anaCF anaCR	TCTGGTATTCAGTCCCCTCTAT CCCAATAGCCTGTCATCAA	366	Rantala-Ylinen *et al*. (2011)
PKS[a]	M4 M5	GAAGCTCTGGAATCCGGTAA AATCCTTACGGGATCCGGTGC	535/540	Schembri *et al*. (2001)
PS[a]	M13 M14	GGCAAATTGTGATAGCCACGAGC GATGGAACATCGCTCACTGGTG	511/534	Schembri *et al*. (2001)

[a] PKS and PS are genes directly associated with the ability to produce cylindrospermopsin (Schembri *et al*. 2001).

PCRs were performed in a final volume of 20 µl. The reaction mixture contained 5 × Green GoTaq® Flexi buffer, 25 mM $MgCl_2$, 10 pmol of forward and reverse primers (Table 1), 187.5 µM of dNTP mix, bovine serum albumin (BSA) and 0.5U of GoTaq® DNA polymerase. PCR conditions for the amplification of 16S rRNA gene fragments (Table 1) were: an initial denaturation step for 2 min at 95°C, followed by 35 cycles, each consisting of a denaturation step of 1 min at 95°C, an annealing step at 55°C for 45s, and an extension step for 1 min at 72°C. PCR products were examined by agarose gel electrophoresis in 1.5% agarose gels stained with SYBR® Safe. The PCR products were then purified with a NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. Purified products were sequenced bidirectionally by Sanger sequencing by GATC Biotech (Germany). The 16S rRNA gene sequences from each isolate were then visually inspected and assembled using Geneious (version 8.1.8). The sequences obtained in this study were compared with those deposited in GenBank by using the BLASTN algorithm, and the best hits obtained for each one of our sequences were recorded. Sequences were deposited in NCBI GenBank under the accession numbers MF629801–MF629814.

Sequence similarities and phylogenetic analysis:—Sequences from the 14 strains obtained in this study, from a selection of strains that comprised reference strains included in the online database CyanoType (Ramos *et al*. 2017), and from the best hits retrieved from BLASTN searches were aligned with the ClustalW algorithm, by using the MEGA7 package (Kumar *et al*. 2016). The outgroup *Chloroflexus aurantiacus* J-10-fl (NR_074263) was also added to the alignment, which totaled 95 sequences. A subset of sequences in the alignment were then selected in order to generate a similarity matrix of 16S rRNA gene sequences. Three different methods were employed to reconstruct tree topologies, using the total alignment: Maximum Likelihood (ML), Bayesian Inference (BI) and Maximum Parsimony (MP). Alignment sites with ambiguities, missing data or gaps were excluded from phylogenetic analyses, which led to a final dataset composed of 871 positions in total. The MrBayes (version 3.2.6) plugin in Geneious (version 8.1.8; Biomatters) was used to compute the BI tree, while ML and MP trees were computed in MEGA7 (Kumar *et al*. 2016), the same software that allowed us to choose the general time-reversible model (GTR+G+I) as the best model of evolution according to the corrected Akaike information criterion. In this way, a discrete Gamma distribution was used to model evolutionary rate differences among sites (4 categories (+G, parameter = 0.3133)), with some sites allowed to be evolutionarily invariable ([+I], 29.62% sites). For the ML and MP trees, the robustness of the nodes was assessed by 1000 bootstrap resamplings. The subtree-prune and regraft (SPR) tree rearrangement operation was applied for MP tree searches. For the BI tree, a Markov Chain Monte Carlo (MCMC) analysis was ran with the following settings: bayesian searches were performed using a random starting tree, with one cold and seven incrementally heated chains

(temperature = 0.2), the number of cycles for the MCMC algorithm was set to 1,100,000 generation, with a burn-in length of 100,000, and with a tree sampling frequency of 200.

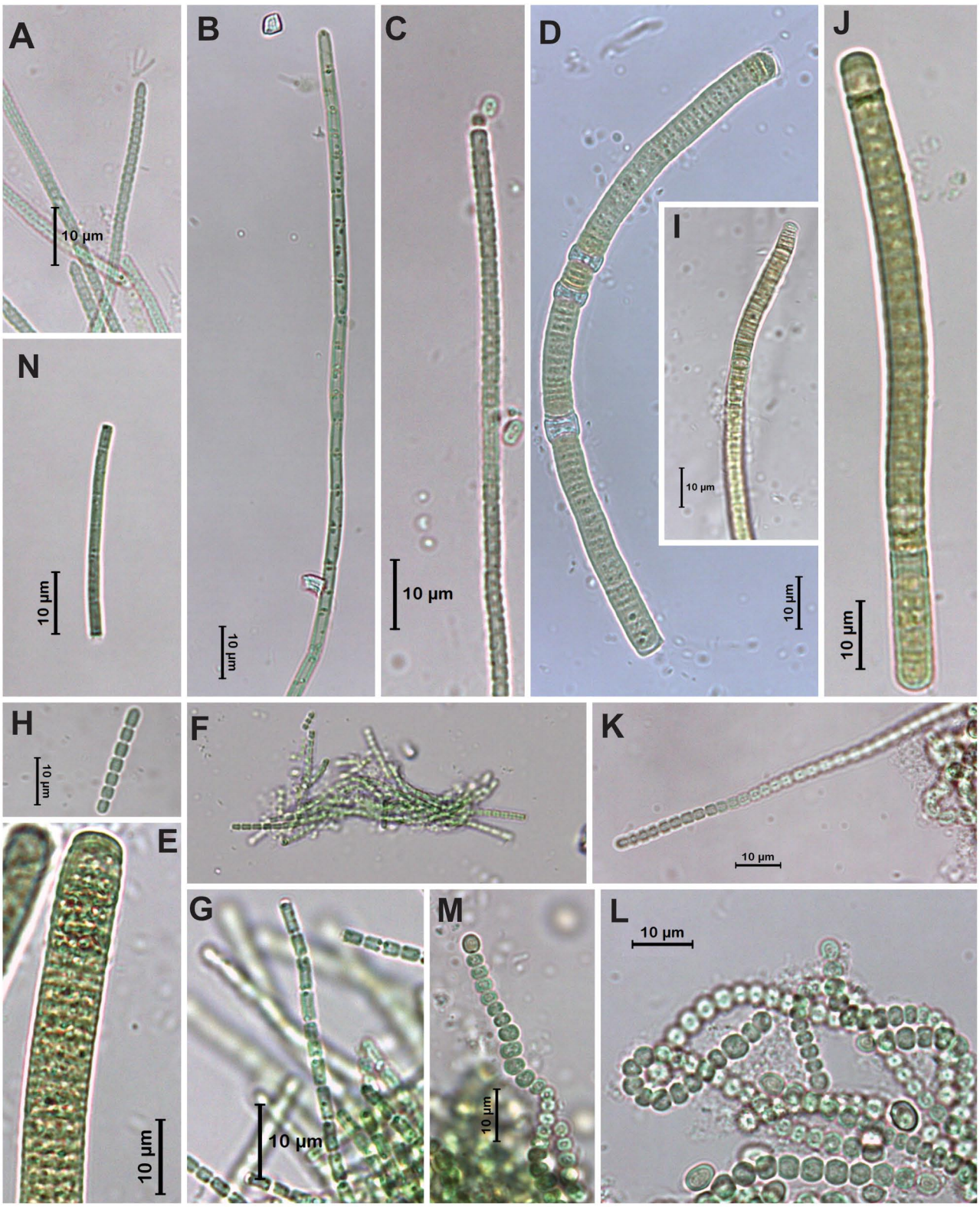

FIGURE 2 Microphotographs of the Amazonian cyanobacterial strains: A) *Alkalinema* aff. *pantanalense* LEGE 15484, B) *Limnothrix* cf. *planctonica* LEGE 15485, C) *Leptolyngbya boryana* LEGE 15486, D) *Cephalothrix lacustris* LEGE 15487, E) *Phormidium* sp. LEGE 15488, F) *Pseudanabaena* cf. *galeata* LEGE 15489, G) *P.* cf. *galeata* LEGE 15490, H) *P.* cf. *galeata* LEGE 15491, I) *Cephalothrix lacustris* LEGE 15492, J) *C. lacustris* LEGE 15493; K) unidentified Nostocales LEGE 15494, L) unidentified Nostocales LEGE 15495, M) unidentified Nostocales LEGE 15496, and N) *Limnothrix* cf. *planctonica* LEGE 15497.

Potential for cyanotoxin production:—The toxic potential was studied by using primer sets targeting the gene clusters involved in the production of microcystin, saxitoxin, anatoxin, and cylindrospermopsin (Moreira *et al*. 2014), as indicated in Table 1. PCR conditions for the amplification of the *mcyA* gene were: the first step was at 95°C for 2 min, followed by 35 cycles consisting of 95°C for 1 min 30s, 56°C for 30s, and 72°C for 50s. For the *mcyE* gene, the first step was at 92°C for 2 min, followed by 35 cycles consisting of 92°C for 20s, 52°C for 30s, and 72°C for 1 min. The strain *Microcystis aeruginosa* LEGE 91339 was used as positive control for the *mcyA* and *mcyE* gene amplification. For the amplification of the *sxtI* gene, the first step was at 94°C for 3 min, followed by 35 cycles consisting of 94°C for 10s, 60°C for 20s, and 72°C for 1 min. The strain *Aphanizomenon gracile* LMECYA 040 was used as positive control for the *sxtI* gene amplification. For the amplification of the *anaC* gene, the first step was at 94°C for 2 min, followed by 35 cycles consisting of 94°C for 30s, 58°C for 30s, and 72°C for 30s. The strain *Anabaena* sp. LEGE X-002 was used as positive control for the *anaC* gene amplification. Lastly, the first step for the PS and PKS genes was at 95°C for 2 min, followed by 35 cycles consisting of 95°C for 1min 30s, 55°C for 30s, and 72°C for 50s. The strain *Cylindrospermopsis raciborskii* LEGE 97047 was used as positive control for this amplification.

Results

Environmental characterization:—The study site parameters are described in Table 2. The Amazon River water at Macapá is turbid (19.8–122 NTU), the Secchi depth ranges from 12–36.5 cm, and the pH is approximately 7. There is a high concentration of dissolved solids (20–30 ppm) in the water, and the electrical conductivity (EC) mean is approximately 50 μδ cm^{-1}. Moreover, with regard to the climate, the air temperatures range from 22.7 to 35.3ºC, and there is a high amount of monthly rainfall (mean 205.9 mm) distributed over rainy and dry seasons.

Isolated strains—morphology and behaviour in culture:—Fourteen strains were isolated (Fig. 2), thirteen of which were from the Amazon raw water, at the drinking water inlet, and one was from the treated water. All the strains isolated in this study were filamentous cyanobacteria, and most of these strains were non-heterocytous. The polyphasic examination demonstrates that they belong to the orders Synechococcales, Oscillatoriales, and Nostocales (Table 3).

TABLE 2. Physical and chemical parameters of the study sites.

Parameters	Raw water			Treated water		
	Medium	Minimum	Maximum	Medium	Minimum	Maximum
Secchi depth (cm)	25.11	12	36.5	-	-	-
Electrical conductivity (μδ cm^{-1})	50.35	40	60	68.3	50.0	80.0
Total dissolved solids (ppm)	24.00	20	30	31.1	20.0	40.0
Dissolved oxygen (mg l^{-1})	5.97	4.86	6.8	4.4	3.9	6.3
Water temperature (°C)	28.82	25	30.3	28.6	24.4	30.7
pH	6.58	6	7.2	6.0	5.4	6.7
Turbidity (NTU)	54.70	19.8	122	7.5	1.4	20.4
Cl^- (mg l^{-1})	8.72	1.6	25.8	3.8	1.8	6.8
Fe^{2+} (mg l^{-1})	2.18	0.89	5.46	0.7	0.1	3.9
Al^{3+} (mg l^{-1})	0.10	0.03	0.31	0.1	0	0.2
PO_3^{3-} (mg l^{-1})	0.30	0.02	1.66	0.1	0.1	0.4
NH_3^- (mg l^{-1})	0.65	0.05	1.67	0.1	0	0.2
NO_3^- (mg l^{-1})	0.43	0	2.5	0.9	0	4.5
SO_4^{2-} (mg l^{-1})	1.75	0	4	14	9	21
Monthly Precipitation (mm)	205.9	0	528.2	205.9	0	528.2
Maximum air temperature (°C)	33.13	28.9	35.3	33.13	28.9	35.3
Minimum air temperature (°C)	24.3	22.7	25.7	24.3	22.7	25.7
Insolation (h)	7.44	1.3	10.6	7.44	1.3	10.6
Irradiation day (W m^{-2})	238.65	86.6	312.3	238.65	86.6	312.3

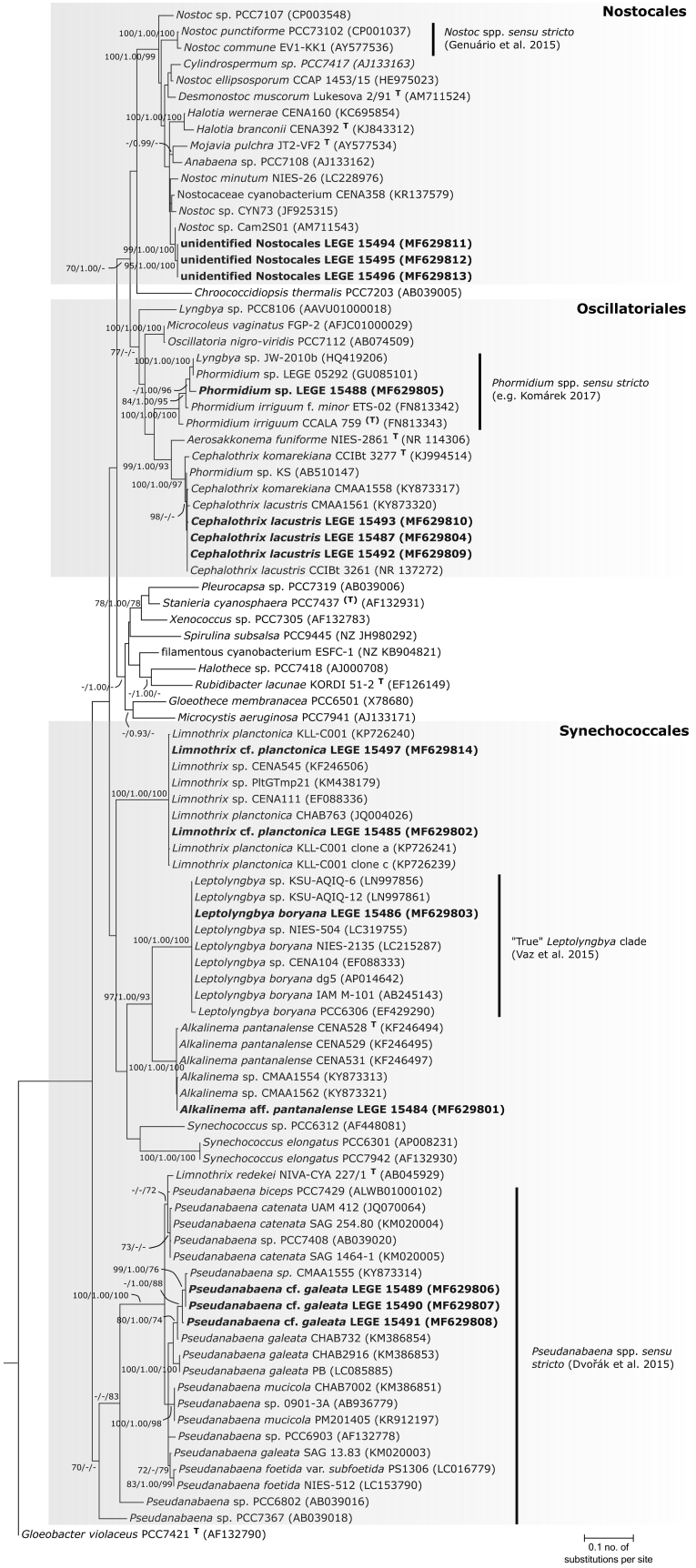

FIGURE 3. ML phylogenetic tree based on 16S rRNA gene sequences showing the placement of the Amazonian cyanobacterial strains (in bold). Bootstrap values >70% or Posterior Probabilities >0.90 are indicated below the clade nodes (ML/BI/MP). Each strain sequence is identified by its accession number. The tree was rooted with the outgroup *Chloroflexus aurantiacus* J-10-fl (NR_074263), which was pruned from the tree for clarity. (**T**) refers to type strains according to CyanoType (Ramos *et al*. 2017).

TABLE 3. Cyanobacterial isolates from the Amazon River obtained in this study.

Order	Strain	Site	Accession number	Culture media
Synechococcales	*Alkalinema* aff. *pantanalense* LEGE 15484	raw water	MF629801	BG11, Z8
	Limnothrix cf. *planctonica* LEGE 15497	raw water	MF629814	BG11, Z8
	Limnothrix cf. *planctonica* LEGE 15485	raw water	MF629802	BG11, Z8
	Leptolyngbya boryana LEGE 15486	treated water	MF629803	BG11, Z8
	Pseudanabaena cf. *galeata* LEGE 15489	raw water	MF629806	BG11, Z8
	Pseudanabaena cf. *galeata* LEGE 15490	raw water	MF629807	BG11, Z8
	Pseudanabaena cf. *galeata* LEGE 15491	raw water	MF629808	BG11, Z8
Oscillatoriales	*Cephalothrix lacustris* LEGE 15487	raw water	MF629804	BG11, Z8
	Cephalothrix lacustris LEGE 15492	raw water	MF629809	BG11, Z8
	Cephalothrix lacustris LEGE 15493	raw water	MF629810	BG11, Z8
	Phormidium sp. LEGE 15488	raw water	MF629805	BG11, Z8
Nostocales	unidentified Nostocales LEGE 15494	raw water	MF629811	$BG11_0$
	unidentified Nostocales LEGE 15495	raw water	MF629812	$BG11_0$
	unidentified Nostocales LEGE 15496	raw water	MF629813	$BG11_0$

Alkalinema* aff. *pantanalense Vaz *et al.* (2015: 305) (Fig 2A)

Strains:—LEGE 15484 (MF629801)

Description:—The cultures are pale to bright blue-green, composed either of free-floating trichomes or of mats that float in the culture flask. Trichomes are short, straight, immotile, deeply constricted with a hyaline, thin, diffluent mucilage and without necridia. The cells are longer (1.2–4.0 μm) than wide (1.7–2.9 μm). Trichomes with round or narrow apical cells. The cell content is homogeneous and pale blue-green and lacks gas vesicles.

Phylogeny and polyphasic identification:—The 16S rRNA gene sequence of the isolate LEGE 15484 shares more than 99% identity with sequences from four *Alkalinema* spp. Vaz *et al*. (2015: 302) strains, including *A. pantanalense* CENA 531 and CENA 529 (Table 4 and Table S1). Indeed, as illustrated in Table 4, the sequence of *A. pantanalense* LEGE 15484 shares a very high similarity value with that of the type strain *A. pantanalense* CENA 528 (99.6%) and with *Alkalinema* sp. CMAA 1554 (99.8%). All those strains form a strong cluster (Fig. 3), with node support values of 100% (ML and MP) or 1.00 (BI). Even though LEGE 15484 seems to be more closely related to two *Alkalinema* sp. CMAA strains, the phylogenetic distance between all the strains constituting the clade is minimal (Fig. 3). The polyphasic evaluation of LEGE 15484 allowed the identification of this strain as *Alkalinema* aff. *pantanalense* (Vaz *et al*. 2015).

Limnothrix* cf. *planctonica (Woloszynska) Meffert (1988: 269) (Figs 2B, 2N)

Strains:—LEGE 15485 (MF629802) and LEGE 15497 (MF629814).

Description:—In culture, groups of trichomes might float, indicating gas-vesicle-driven buoyancy. Trichomes are arranged in fascicles, which are bright blue-green in color and may form thin to thick biofilms at the air-medium interface on the walls and at the bottom of the culture flask. If detached from the bottom, the culture becomes a floating mass. Long trichomes, almost-straight, non-constricted and non-attenuated with facultative, fine, diffluent mucilage-visible in the apical cells (Fig. 2B, 2N). The trichomes exhibited a slow gliding motility. The cells are cylindrical, pale blue-green, 1.6–2.6 μm wide and 2.9–8.6 μm long with small facultative gas vesicles localized to the cell poles and with facultative granules. The morphology of these strains resembles those of *Limnothrix redekei* (Goor 1918: 258) Meffert (1988: 274) and *Limnothrix planctonica* (Woloszynska 1912: 530) Meffert 1988: 269. However, the LEGE strains share more morphological attributes with the latter than with the former (Komárek & Anagnostidis, 2005).

Phylogeny and polyphasic identification:—The 16S rRNA gene sequences of the *Limnothrix* Meffert (1988: 274) LEGE isolates share more than 99% of identity with the sequences from *Limnothrix* sp. CENA545 and from other strains identified as *L. planctonica* or *Limnothrix* sp. (Table 4 and Table S1). These sequences form a clade highly supported by ML and BI methods, and exclusively composed by strains from the *Limnothrix* genus (Fig. 3). However, this clade is placed distantly from that of the type strain *L. redekei* NIVA-CYA 227/1 (Suda *et al*. 2002), which shows to be phylogenetically more related to *Pseudanabaena* Lauterborn (1915: 437) strains (Fig. 3). Based on all the data, the strains LEGE 15497 and LEGE 15485 were identified as *Limnothrix* cf. *planctonica* (Meffert, 1988; Komárek & Anagnostidis, 2005).

TABLE 4. Similarity matrix of cyanobacterial 16S rRNA gene sequences from the LEGE strains obtained in this study (in bold), from the BLASTN best hits*, and from Type (T) or Reference (R) strains as in Ramos *et al*. (2017).

	CCALA 759	JW-2010b	**LEGE 15488**	CCIBt 3277	CMAA1561	**LEGE 15487**	**LEGE 15492**	**LEGE 15493**	CCIBt 3261	**LEGE 15494**	**LEGE 15496**	**LEGE 15495**	Cam2S01	CENA358	PCC 7108	**LEGE 15485**	CENA545	**LEGE 15497**	**LEGE 15484**	CENA528	CMAA1554	NIES-504	**LEGE 15486**	PCC 6306	PCC 7429	CMAA1555	**LEGE 15490**	**LEGE 15489**	**LEGE 15491**	CHAB7002	LEGE 05292	CENA392	JT2-VF2	Lukesova 2/91	EV1-KK1	PCC 73102	CHAB732	NIES-512	SAG 254.80	NIVA-CYA 227/1
Phormidium irriguum CCALA 759 (T)																																							
Lyngbya sp. JW-2010b *	97,2																																						
***Phormidium* sp. LEGE 15488**	96,9	98,1																																					
Cephalothrix komarekiana CCIBt 3277 T	91,1	91,3	91,7																																				
Cephalothrix lacustris CMAA1561 *	90,9	90,3	90,7	98,5																																			
***Cephalothrix lacustris* LEGE 15487**	91,6	90,7	91,2	98,4	99,5																																		
***Cephalothrix lacustris* LEGE 15492**	90,9	90,3	91,1	98,0	98,9	99,9																																	
***Cephalothrix lacustris* LEGE 15493**	91,6	90,7	91,0	98,6	99,5	99,9	99,9																																
Cephalothrix lacustris CCIBt 3261 *	91,1	90,9	90,9	99,0	99,4	99,5	98,8	99,4																															
unidentified Nostocales LEGE 15494	90,3	90,1	90,9	90,4	89,1	89,3	89,5	89,5	89,7																														
unidentified Nostocales LEGE 15496	90,3	90,1	90,9	90,4	89,2	89,3	89,5	89,6	89,7	100																													
unidentified Nostocales LEGE 15495	90,1	89,9	90,8	90,3	89,0	89,4	89,4	89,4	89,5	100	100																												
Nostoc sp. Cam2S01 *	89,9	90,1	90,6	89,5	88,7	89,3	88,9	89,6	89,2	99,6	99,6	99,5																											
Nostocaceae cyanobacterium CENA358 *	89,7	90,1	90,3	89,5	88,8	89,4	89,0	89,8	89,0	97,4	97,5	97,3	97,2																										
Anabaena sp. PCC 7108 R	89,4	90,0	89,9	88,7	87,7	88,8	88,3	89,0	88,0	96,8	96,6	96,7	95,5	95,2																									
***Limnothrix* cf. *planctonica* LEGE 15485**	88,7	88,3	88,4	86,7	86,3	86,4	86,7	86,7	86,5	89,1	89,1	89,0	89,0	89,1	88,0																									
Limnothrix sp. CENA545 *	88,5	88,3	88,5	86,5	86,1	86,3	86,4	86,9	86,3	89,1	89,0	89,0	88,5	88,7	87,4	99,7																							
***Limnothrix* cf. *planctonica* LEGE 15497**	88,8	88,4	88,5	86,9	86,5	86,3	86,8	86,9	86,7	89,1	89,0	89,0	89,0	89,1	88,0	99,7	100																						
***Alkalinema* aff. *pantanalense* LEGE 15484**	89,5	89,8	89,5	88,3	88,0	87,6	87,8	88,2	87,9	88,2	88,3	88,0	88,0	88,2	87,4	89,1	89,1	89,1																					
Alkalinema pantanalense CENA528 T	89,2	89,7	89,4	88,0	87,9	87,3	87,5	88,1	87,8	88,0	88,2	87,8	87,8	87,9	86,8	89,0	89,0	89,2	99,6																				
Alkalinema sp. CMAA1554 *	89,2	90,0	89,7	88,2	87,6	87,5	87,7	88,2	88,0	88,2	88,3	88,0	87,9	88,1	86,9	89,1	89,0	89,1	99,8	99,6																			
Leptolyngbya sp. NIES-504 *	88,2	88,9	88,5	86,7	86,5	86,4	86,5	87,3	86,4	87,7	87,8	87,5	86,8	87,6	86,4	87,7	87,4	87,9	92,1	91,7	91,8																		
***Leptolyngbya boryana* LEGE 15486**	88,8	88,9	88,5	87,1	86,9	86,5	87,1	87,3	86,9	87,7	87,7	87,6	87,8	87,9	87,3	87,8	87,9	87,9	92,0	91,9	92,0	100																	
Leptolyngbya boryana PCC 6306 R	88,9	89,8	89,1	88,8	88,2	88,8	88,3	89,3	88,9	89,0	89,0	88,9	88,3	88,7	87,8	89,2	88,6	89,3	92,5	92,4	92,2	99,5	99,4																
Pseudanabaena biceps PCC 7429	88,0	87,7	87,6	86,1	86,0	85,2	85,4	85,9	85,9	88,1	88,3	87,9	88,0	87,6	87,4	88,2	88,0	88,4	87,7	87,7	87,9	87,0	87,8	87,8															
Pseudanabaena sp. CMAA1555 *	87,4	87,3	87,1	85,7	85,0	84,7	85,1	85,5	85,5	87,1	87,4	86,9	87,2	87,1	87,1	87,9	87,6	87,9	87,8	88,0	88,1	87,5	88,3	87,5	96,7														
***Pseudanabaena* cf. *galeata* LEGE 15490**	87,3	86,8	86,7	84,8	84,4	85,0	84,8	84,8	84,5	86,8	86,8	86,9	87,1	86,9	87,0	87,5	87,4	87,4	87,2	87,0	87,2	87,4	87,4	87,1	96,6	99,7													
***Pseudanabaena* cf. *galeata* LEGE 15489**	88,0	87,6	87,3	85,7	85,4	85,0	85,7	85,8	85,4	87,4	87,6	87,2	87,8	87,6	87,7	88,2	88,2	88,2	88,2	88,1	88,2	88,6	88,5	88,5	97,0	99,8	100												
***Pseudanabaena* cf. *galeata* LEGE 15491**	87,5	87,2	87,2	85,9	85,4	85,0	85,7	85,8	85,5	87,0	87,3	86,9	87,3	87,3	87,1	88,2	88,2	88,2	87,9	87,7	87,9	88,9	88,9	89,0	96,6	99,0	99,1	99,2											
Pseudanabaena mucicola CHAB7002	88,5	88,0	87,8	86,3	85,6	85,5	85,7	86,2	86,1	87,6	87,8	87,4	87,7	87,1	87,1	87,8	87,6	88,0	88,0	87,9	88,0	87,6	88,5	85,0	97,5	98,2	98,5	98,7	97,9										
Phormidium sp. LEGE 05292 *	97,2	99,9	98,2	90,5	89,6	90,8	90,1	90,8	90,2	90,1	90,2	89,9	89,1	89,2	88,8	88,4	88,0	88,4	89,9	89,0	89,2	88,3	89,0	89,0	87,3	86,9	86,9	87,7	87,3	87,7									
Halotia branconii CENA392 T	88,9	89,5	89,3	88,2	88,3	88,2	88,1	88,6	88,2	94,5	94,5	94,4	94,3	95,7	93,4	87,1	87,0	87,2	88,2	88,2	88,5	86,9	87,4	88,3	86,5	86,7	86,0	86,7	86,2	86,7	88,6								
Mojavia pulchra JT2-VF2 T	89,1	89,5	89,9	89,9	89,2	90,0	89,6	90,3	89,4	96,4	96,2	96,3	95,8	96,1	94,8	87,5	87,3	87,7	87,8	87,3	87,5	87,2	88,0	87,0	87,9	88,1	88,2	88,8	88,0	87,9	88,5	94,7							
Desmonostoc muscorum Lukesova 2/91 T	90,7	90,9	90,9	89,9	89,3	89,6	89,2	89,8	89,5	96,7	96,8	96,6	96,6	97,1	94,6	88,8	88,4	88,8	88,4	88,5	88,6	87,3	87,6	88,7	87,7	87,3	87,0	87,6	87,2	87,4	89,9	94,7	95,1						
Nostoc commune EV1-KK1	88,8	88,6	89,2	90,7	90,2	91,3	90,5	91,3	90,4	96,1	95,9	96,0	95,1	95,4	93,6	88,6	88,4	88,7	87,9	87,6	87,8	87,7	88,3	87,5	87,9	87,9	88,0	88,5	88,2	87,8	87,6	94,3	95,4	95,7					
Nostoc punctiforme PCC 73102 R	88,8	88,5	89,1	89,9	89,2	89,4	89,1	89,7	89,5	96,1	95,9	95,9	95,5	95,3	94,1	88,9	88,8	89,1	87,8	87,9	88,0	86,6	87,0	87,8	87,4	86,8	86,2	86,9	86,6	86,8	87,8	93,9	95,8	95,4	99,2				
Pseudanabaena galeata CHAB732	87,9	87,3	87,0	86,5	86,4	85,8	86,6	86,5	86,3	87,6	87,8	87,4	88,1	87,9	87,8	88,2	88,6	88,6	88,1	87,9	88,1	88,9	88,9	88,6	97,3	98,8	98,8	98,9	98,8	98,8	87,3	87,1	88,0	88,1	88,1	87,3			
Pseudanabaena foetida NIES-512	87,5	87,0	87,0	85,6	85,1	84,7	85,0	85,4	85,4	87,6	87,8	87,4	87,6	87,2	87,3	87,9	87,7	88,1	87,6	87,6	87,7	87,5	88,3	87,1	98,3	97,3	97,2	97,5	97,1	97,5	86,7	86,5	87,9	87,5	88,0	87,1	97,9		
Pseudanabaena catenata SAG 254.80	88,4	87,9	87,8	86,4	86,2	85,5	85,7	86,2	86,2	88,3	88,5	88,1	88,3	87,7	88,0	88,8	88,6	89,0	87,7	87,7	87,8	86,8	87,6	87,9	99,1	95,9	95,7	96,1	95,8	96,8	87,6	86,7	87,6	88,0	87,9	87,7	96,8	97,6	
Limnothrix redekei NIVA-CYA 227/1 T	87,5	87,5	87,3	85,4	85,1	84,9	85,2	85,6	85,1	87,9	88,1	87,7	87,5	87,4	87,2	88,0	87,8	88,3	87,6	87,1	87,2	86,9	87,9	87,2	98,9	95,7	95,7	96,1	95,8	96,6	87,2	86,0	87,3	87,3	87,7	87,0	96,7	97,3	98,7

Leptolyngbya boryana (Gomont 1899: 36) Anagnostidis et Komárek (1988: 391) (Fig 2C)
Strains:—LEGE 15486 (MF629803)

Description:—The filaments are entangled, forming a dark-green biofilm attached to the culture flask. In aged cultures, a characteristic ferruginous color can be observed for this strain. Trichomes are flexuous, immotile and non-attenuated. They are constricted at the cross-walls and have necridic cells and thin hyaline sheaths (Fig. 2C). The cells are subquadratic, i.e., the cells are longer than wide (1.6–2.9 μm long and 1.9–2.6 μm wide), and pale blue-green in color.

Phylogeny and polyphasic identification:—The strain LEGE 15486 shares 100% identity with the sequences from four strains of *Leptolyngbya* Anagnostidis & Komárek (1988: 390), namely *Leptolyngbya* sp. NIES-504; *Leptolyngbya* sp. KSU-AQIQ-12 and KSU-AQIQ-6; and *Leptolyngbya* sp. CENA104 (Table S1). The 16S rRNA gene sequence of LEGE 15486 shares 99.4% similarity with *L. boryana* PCC 6306 (Table 4), a reference strain according to Bergey's Manual (Castenholz *et al*. 2015). These strains along with other *Leptolyngbya boryana* and *Leptolyngbya* sp. strains clustered in a monophyletic clade with very good node support (ML/BI/MP = 100/1.00/100) (Fig. 3). Based on the polyphasic evaluation of LEGE 15486, this strain was identified as *L. boryana* (Komárek & Anagnostidis, 2005).

Cephalothrix lacustris Malone *et al*. (2015: 3004) (Figs 2D, 2I, 2J)
Strains:—LEGE 15487 (MF629804), LEGE 15492 (MF629809) and LEGE 15493 (MF629810).

Description:—In culture, the trichomes start growing at the bottom of the culture flask, forming a thin biofilm towards the top, at the air-medium interface. Groups of filaments may float on the surface of the culture medium or may stay attached to air bubbles at the bottom of the flask. Trichomes are straight or flexuous, slightly constricted at the cell-cell joints and exhibit gliding motility and biconcave necridia. The trichomes are pale to olive-green and are solitary or grouped in mats. In addition, the trichomes have facultative hyaline and firm sheaths that are either attached to the trichome or are at a slight distance (Fig. 2D, 2I, 2J). Cells are wider (4.2–7.5 μm) than long (1.4–3.8 μm) and have facultative gas vesicles. Apical cells might be rounded or capitate and have facultative calyptra.

Phylogeny and polyphasic identification:—The 16S rRNA gene sequences of LEGE 15487, LEGE 15493 and LEGE 15492 showed similarity values equal or higher than 98.8% (Table 4) with the sequences from *Cephalothrix lacustris* Malone *et al*. (2015: 3004) CCIBt 3261 (98.8–99.5%) and *C. lacustris* CMAA 1561 (98.9–99.5%). Concerning the phylogenetic tree, only the ML method was able to give support to a subgroup formed by the abovementioned strains plus *C. komarekiana* Malone *et al*. (2015: 3003) CMAA 1558 and *Phormidium* sp. KS (Fig. 3, see also Fig. S1 and Fig. S2). Still, the three inference methods support a major clade (ML/BI/MP = 100/1.00/97) that encloses the abovementioned group of sequences and also the sequence of *Cephalothrix komarekiana* CCIBt 3277, the type strain of the genus (Fig. 3). Based on the morphology, ecology and molecular data, these LEGE strains can be identified as *Cephalothrix lacustris*.

Phormidium (Kützing 1843: 190) Gomont (1892: 156) (Fig. 2E)
Strains:—LEGE 15488 (MF629805)

Description:—In culture, dark-green groups of filaments grow in fascicles all over the flask walls and at the interface between the air and the culture medium. The strain produces air bubbles that are visible at the bottoms of culture flasks. Trichomes are long, olive-green to dark-green. The trichomes are isopolar, straight to curved or even coiled, immotile, non-constricted or slightly constricted at the cross-walls, non-attenuated at their ends, and have necridia and facultative sheaths (Fig. 2E). The cells are wider (5.6–8.8 μm) than long (1.7–3.8 μm) and have granules.

Phylogeny and polyphasic identification:—The 16S rRNA gene sequence of LEGE 15488 shares 98% identity with the sequences of three strains identified as *Phormidium*, *Lyngbya* Agardh ex Gomont (1892: 118) and with an unidentified cyanobacterium (Table S1). The sequence of LEGE 15488 (Table 4) shows higher similarity values with the sequences from the strains *Phormidium* sp. LEGE 05292 (98.2%) and *Lyngbya* JW-2010b (98.1%), and to a less degree with that of *Phormidium irriguum* (Kützing ex Gomont) Anagnostidis & Komárek (1988: 405) CCALA 759 (96.9%). As shown in Fig. 3, LEGE 15488 is placed in a robust clade (ML/BI/MP = 100/1.00/100) with the strain *P. irriguum* CCALA 759, at the base of the clade, which is an isolate that was proposed as epitype for this species and also for the genus (Sciuto *et al*. 2012). Based on the polyphasic evaluation of LEGE 15488, this strain was identified as *Phormidium* sp. (Komárek & Anagnostidis, 2005, Sciuto *et al*. 2012).

Pseudanabaena* cf. *galeata (Figs 2F, 2G, 2H)

Strains:—LEGE 15489 (MF629806), LEGE 15490 (MF629807) and LEGE 15491 (MF629808).

Description:—Planktonic cyanobacteria with trichomes that are almost straight and are either solitary or form mats in culture. Thin emerald-green mats float at the air-medium interface in the culture flask and grow on the walls and bottom of the flask in clusters. The trichomes are deeply constricted at the cross-walls, are non-attenuated and have facultative granules (Fig. 2F, 2G, 2H). The cells are pale blue-green or bright blue-green in color, 1.3–2.8 μm wide, 1.4–5.7 μm long and cylindrical and are connected via hyaline bridges. The apical cells exhibit polar gas vesicles. The strain LEGE 15491 exhibited a space between the cells similar to gas vesicles. The trichomes move forward slowly via trembling motility. These strains are longer than *Pseudanabaena mucicola* (Naumann et Huber-Pestalozzi) Schwabe (1964: 32) and exhibit a morphology that matches that of *P. galeata* Böcher (1949: 13) (Komárek & Anagnostidis, 2005).

Phylogeny and polyphasic identification:—The BLAST search results for the sequences from strains LEGE 15489 and LEGE 15490 were very similar, while the BLAST results for the sequence from LEGE 15491 was comparable, although slightly different (Table S1). Despite this, *Pseudanabaena* LEGE strains share high similarity values between themselves (99.1–100%), and between themselves and the strains retrieved from the BLAST searches: *Pseudanabaena* sp. CMAA1555 (99–99.8%) and *P. galeata* CHAB 732 (98.8–99.9%) (Table 4). LEGE 15489 and LEGE 15490 are clustered with *Pseudanabaena* sp. CMAA1555 in a clade with good support values (ML/BI/MP = 99/1.00/76), as illustrated in Fig. 3. On the other hand, these strains form a larger clade together with LEGE 15491 and *P. galeata* CHAB732, even though in this case it is better supported by BI (1.00) than by ML (80%) and MP (74%) methods. The closest branch of this clade is a cluster that includes *P. galeata* CHAB 2916 and *P. galeata* PB (Fig. 3). Based on the morphological description, behavior in culture and phylogenetic information, the three LEGE strains were identified as *Pseudanabaena* cf. *galeata* (see also Komárek & Anagnostidis, 2005).

Unidentified Nostocales (Figs 2K, 2L, 2M)

Strains:—LEGE 15494 (MF629811), LEGE 15495 (MF629812) and LEGE 15496 (MF629813).

Description:—Heterocytous cyanobacteria, typically belonging to the order Nostocales (Komárek, 2013). The filaments grow at the bottom and walls of the culture flask and may produce air bubbles. These bacteria show flexuous or coiled filaments with tight, diffluent, mucilaginous envelopes (Fig. 2K, 2L, 2M). Hormogonia are present and exhibit gliding motility. The vegetative cells are oval or highly spherical, 2.8–4.6 μm wide, and 2.0–4.0 μm long. The terminal heterocysts are 3.2 ± 0.4 μm wide and 3.3 ± 0.4 μm long. The colonies are olive-green and rounded, flattened or irregular in form.

Phylogeny and polyphasic identification:—BLAST results show that sequences from the filamentous, heterocytous LEGE strains have more than 98% identity with sequences from several strains identified as *Nostoc* Vaucher ex Bornet et Flahault (1888: 181) and with a sequence from an unidentified Nostocaceae cyanobacterium (Table S1). Table 4 shows that the LEGE sequences have very high similarities to the sequence of *Nostoc* sp. Cam2S01 (ranging from 99.5% to 99.6%). Unlikely, LEGE strains sequences present lower similarity values when compared with that of the reference strain *Nostoc punctiforme* PCC 73102 (95.9–96.1%) (Table 4). In fact, the Nostocales LEGE strains are phylogenetically apart from the *Nostoc* clade *sensu stricto* (Genuário *et al*. 2015), which includes *N. punctiforme* PCC 73102 (Fig. 3). Data from morphological, ecological, and phylogenetic analyses were not sufficient to properly identify these strains at the species or genus level. However, these strains can be classified as belonging to the order Nostocales.

Screening for cyanotoxins:—None of the genes associated with cyanotoxin production could be amplified.

Discussion

The chemical and physical characteristics observed in this study for the Amazon River are typical of whitewater rivers: turbid, showing high levels of total dissolved solids, low Secchi depth, warm and near-neutral pH (Junk *et al*. 2011, Ward *et al*. 2015). The climate was also typical of the Amazon forest with high air temperatures, precipitation and insolation (Junk *et al*., 2011; Cunha & Sternberg, 2018). Some of the taxa identified in this study have been previously recorded in the Amazon region (Cunha *et al.* 2013; Genuário *et al.* 2017). Some other represent first records for the Amazon region and even for Brazil. These taxa include the species *Alkalinema pantanalense* and *Limnothrix planctonica*, which to our knowledge, have been recorded in the Amazon region for the first time, considering modern

taxonomic methods. Indeed, the "List of species from the Brazilian Flora (Cyanophyceae)" has no records of these taxa for this region (Werner *et al*. 2015). Furthermore, *Leptolyngbya boryana* has never been reported previously from Brazil (Werner *et al*. 2015).

With regard to *Alkalinema* aff. *pantanalense* LEGE 15484, the cell content of this strain is pale to bright blue-green in color, while the color of the reference strain *Alkalinema pantanalense* CENA 528 is reddish to brownish (Vaz *et al*. 2015). The type strain was isolated from a saline-alkaline lake in the Brazilian Pantanal wetlands (in the central-west region of Brazil) from a water sample with unusual values for certain parameters, such as EC (ranging from 1.1–4.7 μδ cm^{-1}) and pH (ranging from 8.5 to 8.8) (Andreote *et al*. 2014; Vaz *et al*. 2015). *A.* aff. *pantanalense* LEGE 15484 was also collected in Brazil but in the eastern part of the Amazon River basin, from water with pH values ranging from 6 to 7.2 and EC values ranging from 40 to 60 μδ cm^{-1}. Despite the differences in color and ecology, the morphological characteristics of the two strains are very similar. Therefore, the Amazonian strain isolated in this work was identified as *Alkalinema* aff. *pantanalense* LEGE 15484. Three additional *Alkalinema* strains have been recently isolated from other regions of the Brazilian Amazon basin: *Alkalinema* sp. CACIAM 70d (Lima *et al*. 2017), *Alkalinema* sp. CMAA1554 and CMAA1562 (Genuário *et al*. 2017). *Alkalinema* sp. CACIAM 70d was also isolated in the eastern Amazon, from the Tucuruí hydroelectric power station reservoir in Pará, a state in northern Brazil. The genome of this strain was recently sequenced, and it is the first publicly available whole-genome sequence of this genus (Lima *et al*. 2017). On the other hand, additional morphological or ecological information about this strain is not yet available in the literature. *Alkalinema* sp. CMAA1554 is brownish and has conical/rounded apical cells, and the cell dimensions are 1.0–2.3 μm (length) and 2.5–3.1 μm (width) (Genuário *et al*. 2017). Thus, this strain exhibits some differences from *A.* aff. *pantanalense* LEGE 15484 in terms of color and cell size (Fig. 2A). However, these morphological differences are not reflected in the phylogenetic analysis, where the CMAA strains were seen to be the closest relatives of *A.* cf. *pantanalense* LEGE 15484 (Fig. 3). The *Alkalinema* strains CMAA 1554 and 1562 were isolated from the Amazon and Solimões rivers, in the western part of the Brazilian Amazon basin (Genuário *et al*. 2017), under similar ecological conditions as those observed in Macapá. The genus *Alkalinema* was recently identified in Amapá State in a morphological-based study of the phytoplankton of the Araguari River (Silva *et al.* 2018). *Alkalinema pantanalense*-like cyanobacteria are apparently widely distributed along the waters of the Brazilian Amazon basin (which are normally acidic), although the name of the genus refers to the alkaline lakes from where it was first isolated (Vaz *et al*. 2015). This finding demonstrates that this species exhibits excellent tolerance to a range of pH values in the natural environment; this tolerance range has previously been demonstrated in laboratory experiments (Vaz *et al*. 2015; Genuário *et al*. 2017). Notably, as far as we know, the geographic distribution of *Alkalinema* is restricted to Brazil.

The morphologies of the *Limnothrix* isolates LEGE 15497 and LEGE 15485 (Fig. 2B, 2N) are more similar to that of *L. planctonica* than to that of *L. redekei*. The morphologies of these strains differ from that of *L. planctonica* only in that the gas vesicles occur solely at the cell poles and not in the middle of the cell (Komárek & Anagnostidis, 2005). However, LEGE strains show motility, while *L. redekei* trichomes are immotile. The LEGE cells are shorter and form smaller gas vesicles, occupying less than 10% of the cell content compared with the maximum size of aerotopes observed in *L. redekei*, which represent 10 to >50% of cell content (Komárek & Anagnostidis, 2005). The molecular-based results are consistent with the morphological characterization; the 16S rRNA gene sequences of the two LEGE strains were shown to be phylogenetically related to those from several *L. planctonica* and *Limnothrix* sp. strains but not with the sequence from the type strain *L. redekei* NIVA-CYA 227/1 (Fig. 3, Table 4, Suda *et al*. 2002). *L. redekei* NIVA-CYA 227/1 is one of the strains that was used to describe the type species, i.e. the holotype for *Limnothrix* (Meffert 1988). However, this strain grouped with *Pseudanabaena* spp. within the *Pseudanabaena* clade (Fig. 3), which has also been observed in several previous studies (e.g., Suda *et al*. 2002, Gkelis *et al*. 2005, Willame *et al*. 2006, Zhu *et al*. 2012, Andreote *et al*. 2014, Yu *et al*. 2015). Moreover, some of these studies (e.g., Zhu *et al*. 2012, Andreote *et al*. 2014, Yu *et al*. 2015) have shown that the same clade that contains two strains isolated in this study, *L.* cf. *planctonica* LEGE 15497 and LEGE 15485 (Fig. 3), is composed exclusively of *Limnothrix* spp. strains and is phylogenetically distant from *L. redekei* NIVA-CYA 227/1. Willame *et al.* (2006) had already reported that *Pseudanabaena* spp. sequences were closely related to the *Limnothrix* "Meffert sequences", highlighting the high genotypic relationship of both genera and their unnoticeable differences in terms of phenotype. Therefore, Yu *et al*. (2015) suggest that strain *L. redekei* NIVA-CYA 227/1 should be transferred to the genus *Pseudanabaena*, since this genus was described before *Limnothrix* (Meffert, 1988, Komárek & Anagnostidis, 2005), and the abovementioned clade should remain under the genus *Limnothrix*. Andreote *et al*. (2014) also agree with this taxonomic reevaluation of *Limnothrix* and its holotype, an opinion that we share. Other authors (Gkelis *et al*. 2005, Zhu *et al*. 2012) believe that the clade with the type strain should remain under the genus *Limnothrix*, while the well-supported clade of other

Limnothrix spp. should be taxonomically revised and established as a novel genus. To make it even more intricate, Fig. 3 shows that *Limnothrix redekei* NIVA-CYA 227/1 is in the same clade as several strains assigned to the type species of the genus *Pseudanabaena*, which is *P. catenata*. However, some authors (e.g., Yu *et al*. 2015) consider that polyphasic studies on strains of *P. catenata* are still lacking and must be performed to re-evaluate the genus *Pseudanabaena* under modern taxonomy. Earlier, when it was suggested that *Limnothrix* species constitute a polyphyletic group, there were few strains available for phylogenetic studies. Currently, there are several available strains from Africa, America, Asia, Europe, and Oceania (Bernard *et al*. 2011, Zhu *et al*. 2012) that can help elucidate the phylogenetic relationships within this genus. Considering the threshold values of 98.7–99% for species delimitation when using 16S rRNA gene sequences (Stackebrandt & Ebers 2006, see also Kim *et al.* 2014), the two *Limnothrix* strains from this study and *Limnothrix* sp. CENA 545 (99.7–100%, Table 4) should belong to the same species, in this case *L. planctonica*. CENA 545 was also collected in Brazil, from a saline-alkaline lake in the state of Mato Grosso do Sul (in the Central-West region of Brazil). Similarly as it was observed for *Alkalinema* aff. *pantanalense*, this result seems to indicate the ecological versatility of *Limnothrix* cf. *planctonica*.

Leptolyngbya boryana LEGE 15486 is the only strain from this study that could be isolated from the treated water stored in the reservoir. For instance, *Leptolyngbya* sp. CENA 104, one of the strains included in the same clade as *L. boryana* LEGE 15486, was isolated from a chlorination baffle tank at the exit area of a waste stabilization pond system during a no-chlorination operation (Furtado *et al*. 2009). Interestingly, both systems are closed, artificial (i.e., human-made) environments where the presence of some chlorine in the water is expected. The morphological characteristics of CENA 104, which was originally identified at only the genus level (Furtado *et al*. 2009), are similar to those of *L. boryana* LEGE 15486. The trichomes of these strains are arranged in dense clusters and are attached to the glass walls of the culture flasks. The color and size of the cells and other morphological traits of the two strains are also very similar (Furtado *et al*. 2009). These two strains, which share common features and were collected from relatively close geographic regions, are thus far the sole reported *L. boryana* strains from Brazil. Another characteristic that might be common to strains from this species is the ability to grow in low light. For instance, in addition to the two abovementioned strains (obtained from closed systems), *L. boryana* dg5 is a dark-adapted variant of IAM M-101 that can grow heterotrophically in the dark (Fujita *et al*. 1996; Hiraide *et al*. 2015). Both strain dg5 and strain IAM M-101 are included in the *Leptolyngbya* true clade shown in Fig. 3.

Isolates from the genus *Cephalothrix* are morphologically very similar to the strains described in Malone *et al*. (2015). Additionally, the ecology and life cycle of the isolates are comparable. During the isolation process, we observed the existence of planktonic and benthic phases for the *C. lacustris* LEGE strains, which was also described by Malone *et al*. (2015). The culture is sometimes attached to the bottom of the culture flask, while at other times, a mass of trichomes may be found floating at the air-medium interface. Phylogenetically, the LEGE strains were grouped with *Cephalothrix lacustris* CCIBt 3261 (Fig. 3)—the strain used to describe this species (Malone *et al*. 2015)—, and with other *C. lacustris* strains and one *C. komarekiana* strain. However, the high 16S rRNA gene sequences similarity values (≥98.8%, see Table 4) of the LEGE isolates when compared to the type strain CCIBt 3261, along with the morphological data, indicates that they belong to *C. lacustri*s. Moreover, the similarity values in relation to *C. komarekiana* CCIBt 3277 (98.0–98.6%) are always below the threshold value for being considered one single species (Stackebrandt & Ebers, 2006, Kim *et al.* 2014). *Phormidium* sp. KS, that is included in the same clade (Fig. 3), most probably belongs to *C. lacustris*, something that was already suggested by Malone *et al*. (2015). Indeed, the morphological characteristics and behavior in culture of the three *C. lacustris* LEGE strain isolates and the strain *Phormidium* sp. KS are also very similar. At the macroscopic level, these strains all have similar colors, form spirals on agar plates, and form air bubbles (Sato *et al*. 2014). To date, isolation of the recently established species *C. lacustris* has been reported only from Brazil (Malone *et al*. 2015, Genuário *et al*. 2017) and most likely from Japan (Sato *et al*. 2014). Thus, the strains *C. lacustris* LEGE 15493, 15487, and 15492 isolated in this study might help reveal the global distribution of this taxon. *C. lacustris* was first isolated from a freshwater lake, Lago das Garças (pH 7–8), in southeastern Brazil (Malone *et al*. 2015). Genuário *et al*. (2017) isolated the strain *C. lacustris* CMAA1561 from a sample collected in the western Brazilian Amazon region. Therefore, the LEGE strains represent the second record of this taxon from the Amazon basin, this time from the Amapá State, in the eastern part of the Amazon.

The strain *Phormidium* sp. LEGE 15488 showed 98% identity with *Phormidium irriguum* f. *minor* ETS-02, *Phormidium* sp. LEGE 05292 (Dias *et al*. 2017), and *Lyngbya* sp. JW-2010b (Table S1). According to Komárek (2017), all these strains should be considered to be *Phormidium* spp. *sensu stricto* since they clustered together in a well-defined clade that includes the strain *P. irriguum* CCALA 759 (Fig. 3). This strain was proposed by Sciuto *et al*. (2012) to serve as an epitype of this species. Additionally, according to the authors, *P. irriguum* CCALA 759 should be considered the holotype for the genus *Phormidium*, which is consistent with the new delimitation of this genus proposed by

Komárek (2017). With regard to the behavior in culture, the strain *Phormidium* sp. LEGE 05292, previously isolated from a freshwater aquarium wall in Porto, Portugal (Leão *et al*. 2009), is very similar to *Phormidium* sp. LEGE 15488, which was isolated from the Amazon River. Furthermore, LEGE 05292 is the strain that shows the higher sequence similarity value (98.2%, see Table 4) with respect to the *Phormidium* strain isolated in this study. Both strains grow while attached to the surface of the culture flask, forming a dense mat that is very resistant, and strong agitation is not enough to detach the cyanobacterial trichomes (Leão *et al*. 2010). The mat of the Amazonian strain remains above the culture media like a spider-net when the level of the liquid decreases. With regard to other closely phylogenetically related strains (Fig. 3), *P. irriguum* f. *minor* ETS-02 was isolated from a thermal mud surface in Italy, from water at a temperature of 45°C and with a pH of 6.8; and *P. irriguum* CCALA 759 was isolated from a lake in Bratislava, Slovakia (Sciuto *et al*. 2012). In Amazonia, *Phormidium* sp. was recorded in the Amazonas, Pará, Roraima (Werner *et al*. 2015) and Amapá states (Cunha *et al*. 2013). These findings reinforce the idea that the revised genus *Phormidium* (Sciuto *et al*. 2012; Komárek, 2017) is cosmopolitan.

The features of the cells and trichomes, the behavior in culture and the ecology of the strains *Pseudanabaena galeata* LEGE 15489, LEGE 15490, and LEGE 15491 (Fig. 2F, 2G, 2H) match the description of *Pseudanabaena galeata* (Komárek & Anagnostidis, 2005). This genus has been recorded in Amazonas and Tocantins states (Werner *et al*. 2015). Moreover, *P. galeata* was identified by morphological observations in the Araguari River, in the Amapá State (Cunha *et al*. 2013). The phylogeny of the LEGE *Pseudanabaena* strains (Fig. 3) supports their identification, once these strains are clustered together in a large clade with several *Pseudanabaena* species. The most closely phylogenetically related strain is *Pseudanabaena* sp. CMAA1555. This strain is also the most closely related in terms of geographic origin; this strain was also collected from the Brazilian part of the Amazon River (Genuário *et al*. 2017). Unfortunately, the culture was lost, and there is no detailed morphological description of this strain (Genuário *et al*. 2017). Other closely related strains are *P. galeata* CHAB732, *P. galeata* CHAB2916, and *P. galeata* PB. The first two strains were isolated in China from a pond and a lake, respectively, and these strains are morphologically similar to *P. galeata* LEGE 15491, exhibiting a bubble-shaped gap between the walls (Yu *et al*. 2015). *P. galeata* PB was isolated at the Serikawa Dam in Japan and showed high ability to produce the odoriferous compound 2-methylisoborneol (2-MIB) (Takahashi *et al*. 2016). This compound imparts a musty, earthy odor and flavor to the water (Takahashi *et al*. 2016).

The clade with the *Pseudanabaena* LEGE strains is a sister of a clade that includes *P. galeata* SAG 13.83, *P. subfoetida* PS1306, and *P. foetida* NIES-512. The two latter strains were collected in Japan and are both 2-MIB producers (Niiyama *et al*. 2016, Tuji & Niiyama, 2016). *P. foetida* NIES-512, previously identified as *P. galeata*, is considered a model strain for studying 2-MIB-producing cyanobacteria (Tuji & Niiyama, 2016). According to Niiyama *et al*. (2016), the production of 2-MIB should be considered a taxonomic characteristic of the genus *Pseudanabaena*. Given these observations, the *Pseudanabaena* strains present in the incoming raw water of the Amazon River destined for drinking purposes might be 2-MIB producers; this hypothesis warrants further examination using the *P. galeata* LEGE strains. In fact, citizens of Macapá frequently complain about an earthy taste in the drinking water; this taste might be due to the presence of this odoriferous compound or due to sediment that has not been entirely removed during drinking water treatment. Notably, the Amazon River contains a considerable amount of sediment, which is characteristic of whitewater rivers (Junk *et al*. 2011, Ward *et al*. 2015).

The heterocytous, filamentous strains from the order Nostocales isolated in this study were grouped in a well-supported clade together with *Nostoc* sp. Cam2S01 (Fig. 3), which was isolated from soil of volcanic origin in Cameroon (Papaefthimiou *et al*. 2008). All these strains shows a high sequence similarity value among them (>98.7, Table 4), however they fell outside the '*Nostoc sensu stricto'* clade (Hrouzek *et al*. 2013, Genuário *et al*. 2015), as can be observed in Fig. 3. This clade is currently used to phylogenetically define the genus *Nostoc*, and to better delimit typical species such as *Nostoc commune* Vaucher ex Bornet & Flahault (1888: 203), *N. calcicola*, *N. edaphicum* and *N. punctiforme* (Rajaniemi *et al*. 2005, Papaefthimiou *et al*. 2008, Hrouzek *et al*. 2013, Genuário *et al*. 2015) and other strains related to *N. commune* (Papaefthimiou *et al*. 2008). According to Komárek (2013), cyanobacteria fitting the description of *Nostoc* likely include specimens that produce macroscopic colonies with distinct morphologies, as observed in the type species *Nostoc commune*. So, despite being placed in a clade with a strain identified as *Nostoc* sp., the Amazonian isolates clearly do not belong to this redefinition of the genus *Nostoc*; however, these strains possess enough morphological and molecular biological traits to be unequivocally assigned to the order Nostocales. Also, the LEGE Nostocales strains and Cam2S01 enclosed in such distinct, well-supported clade (Fig. 3) should deserve a more exhaustive taxonomic study in the future, since they may represent a new genus.

None of the strains isolated in the present study were shown to possess genes associated with the production of common cyanotoxins, indicating that these isolates may not have the potential to produce microcystin, saxitoxin,

anatoxin, or cylindrospermopsin. This finding is noteworthy given that the water from the study area is used for drinking purposes. However, other cyanobacteria that may be present at the sampling sites but could not be detected in this study could still represent a risk.

Conclusion

This study has provided novel data on the cyanobacterial biodiversity of the Amazon region, especially at the mouth of the Amazon River, places with few available studies about cyanobacteria. This study has additional importance as a local basis for the development of monitoring and evaluation tools for multiple purposes, such as for sanitary and public health risk assessments (e.g., studies on potentially toxic cyanobacteria and species from ballast water), for drinking water for human consumption (e.g., studies on cyanobacteria that produce 2-MIB and/or geosmin) and for river conservation (e.g., studies on invasive alien cyanobacteria, namely, bloom-forming species). The present study is the first to use a polyphasic approach to study cyanobacteria in the eastern Amazon, combining morphological and molecular characterizations of the isolates, phylogenetic analyses, examination of their behavior in culture, toxicity evaluation, and ecological data. Most of the cyanobacterial strains obtained represent first records for the eastern Amazon, particularly for the Amapá State, thus expanding the knowledge on the geographical distribution of the species identified herein. Additionally, the results from this study provide new insight into the taxonomy of the genera *Limnothrix* and *Pseudanabaena*, hence contributing to the general taxonomic revision of the group, a process that is ongoing (Komárek, 2016). The fourteen cyanobacterial strains from the present study represent a small portion of the culturable cyanobacterial biodiversity. Therefore, continued efforts to improve the knowledge about the cyanobacterial biodiversity at the mouth of the Amazon River, either by obtaining new cyanobacterial strains or by employing culture-independent techniques (e.g., high-throughput sequencing) are important.

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Compliance with ethical standards

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

Anagnostidis, K. & Komárek, J. (1988) Modern approach to the classification system of cyanophytes. 3. Oscillatoriales. *Archiv für Hydrobiologie, Supplement* 80: 327–472.

Andreote, A.P.D., Vaz, M.G.M.V., Genuário, D.B., Barbiero, L., Rezende-Filho, A.T. & Fiore, M.F. (2014) Nonheterocytous cyanobacteria from Brazilian saline-alkaline lakes. *Journal of Phycology* 50: 675–684. https://doi.org/10.1111/jpy.12192

APHA-American Public Health Association (2005) *Standard methods for the examination and wastewater*, 21th ed. APHA, Washington, DC.

Aprile, F., Darwisch, A.J., Mera, P.A.S., Robertson, B.A., Marshall, B.G. & Siqueira, W. (2013) On the occurrence of cyanobacteria in the Madeira River in the Brazilian Amazon. *Global Journal of Biology, Agriculture & Health Sciences* 2: 125–133.

Bernard, C., Froscio, S., Campbell, R., Monis, P., Humpage, A. & Fabbro, L. (2011) Novel toxic effects associated with a tropical *Limnothrix/Geitlerinema*-like cyanobacterium. *Environmental Toxicology* 26: 260–270. https://doi.org/10.1002/tox.20552

Böcher, T.W. (1949) Studies on the sapropelic flora of the lake Flyndersø with special reference to the Oscillatoriaceae. Kongelige Danske Videnskabernes Selskab *Biologiske Meddelelser* 21: 1–46.

Bornet, É. & Flahault, C. (1888) Revision des Nostocacées hétérocystées contenues dans les principaux herbiers de France (quatrième et dernier fragment). *Annales des Sciences Naturelles, Botanique, Septième Série* 7: 177–262.

Bravakos, P., Kotoulas, G., Skaraki, K., Pantazidou, A. & Economou-Amilli, A. (2016) A polyphasic taxonomic approach in isolated strains of cyanobacteria from thermal springs of Greece. *Molecular Phylogenetics and Evolution* 98: 147–160. https://doi.org/10.1016/j.ympev.2016.02.009

Castenholz, R.W., Rippka, R., Herdman, M., Wilmotte, A., Castenholz, R.W., Rippka, R., Herdman, M. & Wilmotte, A. (2015) Form-*Leptolyngbya*. *In*: Whitman, W.B., Rainey, F., Kämpfer, P., Trujillo, M., Chun, J., DeVos, P., Hedlund, B. & Dedysh, S. (Eds.) *Bergey's manual of systematics of Archaea and Bacteria*. John Wiley & Sons Ltd, Chichester, UK, pp. 1–3. https://doi.org/10.1002/9781118960608.gbm00437

CPTEC/INPE (2016) *Divisão de satélites e sistemas ambientais.* Available from: http://satelite.cptec.inpe.br/radiacao/ (accessed 29 April 2016)

Cunha, A.C. da & Sternberg, L. da S.L. (2018) Using stable isotopes ^{18}O and ^{2}H of lake water and biogeochemical analysis to identify factors affecting water quality in four estuarine Amazonian shallow lakes. *Hydrological Processes* 32: 1188–1201. https://doi.org/10.1002/hyp.11462

Cunha, E.D.S., da Cunha, A.C., da Silveira Junior, A.M. & Faustino, S.M.M. (2013) Phytoplankton of two rivers in the Eastern Amazon: characterization of biodiversity and new occurrences. *Acta Botanica Brasilica* 27: 364–377. https://doi.org/10.1590/S0102-33062013000200011

Dall'Agnol, L.T., Ghilardi-Junior, R., McCulloch, J.A., Schneider, H., Schneider, M.P.C. & Silva, A. (2012) Phylogenetic and gene trees of *Synechococcus*: choice of the right marker to evaluate the population diversity in the Tucuruí hydroelectric power station reservoir in Brazilian Amazonia. *Journal of Plankton Research* 34: 245–257. https://doi.org/10.1093/plankt/fbr109

Dias, F., Antunes, J.T., Ribeiro, T., Azevedo, J., Vasconcelos, V. & Leão, P.N. (2017) Cyanobacterial allelochemicals but not cyanobacterial cells markedly reduce microbial community diversity. *Frontiers in Microbiology* 8: 1495. https://doi.org/10.3389/fmicb.2017.01495

Doherty, M., Yager, P.L., Moran, M.A., Coles, V.J., Fortunato, C.S., Krusche, A.V., Medeiros, P.M., Payet, J.P., Richey, J.E., Satinsky, B.M., Sawakuchi, H.O., Ward, N.D. & Crump, B.C. (2017) Bacterial biogeography across the Amazon River-ocean continuum. *Frontiers in Microbiology* 8: 882. https://doi.org/10.3389/fmicb.2017.00882

Dvořák, P., Jahodářová, E., Hašler, P., Gusev, E. & Poulíčková, A. (2015) A new tropical cyanobacterium *Pinocchia polymorpha gen. et sp. nov.* derived from the genus *Pseudanabaena*. *Fottea* 15: 113–120. https://doi.org/10.5507/fot.2015.010

Fiore, M. de F., Neilan, B.A., Copp, J.N., Rodrigues, J.L.M., Tsai, S.M., Lee, H. & Trevors, J.T. (2005) Characterization of nitrogen-fixing cyanobacteria in the Brazilian Amazon floodplain. *Water Research* 39: 5017–5026. https://doi.org/10.1016/j.watres.2005.10.002

Fujita, Y., Takagi, H. & Hase, T. (1996) Identification of the chlB gene and the gene product essential for the light-independent chlorophyll biosynthesis in the cyanobacterium *Plectonema boryanum*. *Plant and Cell Physiology* 37: 313–323. https://doi.org/10.1093/oxfordjournals.pcp.a028948

Furtado, A.L.F.F., Calijuri, M.D.C., Lorenzi, A.S., Honda, R.Y., Genuário, D.B. & Fiore, M.F. (2009) Morphological and molecular characterization of cyanobacteria from a Brazilian facultative wastewater stabilization pond and evaluation of microcystin production. *Hydrobiologia* 627: 195–209. https://doi.org/10.1007/s10750-009-9728-6

Gaston, K.J. (2000) Global patterns in biodiversity. *Nature* 405: 220–227.

https://doi.org/10.1038/35012228

Genuário, D.B., Sant'Anna, C.L. & Melo, I.S. (2018a) Elucidating the *Cronbergia* (Cyanobacteria) dilemma with the description of *Cronbergia amazonensis sp. nov.* isolated from Solimões river (Amazonia, Brazil). *Algal Research* 29: 233–241. https://doi.org/10.1016/j.algal.2017.11.034

Genuário, D.B., Souza, W.R., Monteiro, R.T.R., Sant' Anna, C.L. & Melo, I.S. (2018b) *Amazoninema gen. nov.*, (Synechococcales, Pseudanabaenaceae) a novel cyanobacteria genus from Brazilian Amazonian rivers. *International Journal of Systematic and Evolutionary Microbiology* 68: 2249–2257. https://doi.org/10.1099/ijsem.0.002821

Genuário, D.B., Vaz, M.G.M.V. & Melo, I.S. de (2017) Phylogenetic insights into the diversity of homocytous cyanobacteria from Amazonian rivers. *Molecular Phylogenetics and Evolution* 116: 120–135. https://doi.org/10.1016/j.ympev.2017.08.010

Genuário, D.B., Vieira Vaz, M.G.M., Hentschke, G.S., Sant ' Anna, C.L. & Fiore, M.F. (2015) *Halotia gen. nov.*, a phylogenetically and physiologically coherent cyanobacterial genus isolated from marine coastal environments. *International Journal of Systematic and Evolutionary Microbiology* 65: 633–675. https://doi.org/10.1099/ijs.0.070078-0

Gkelis, S., Rajaniemi, P., Vardaka, E., Moustaka-Gouni, M., Lanaras, T. & Sivonen, K. (2005) *Limnothrix redekei* (Van Goor) Meffert (Cyanobacteria) strains from Lake Kastoria, Greece form a separate phylogenetic group. *Microbial Ecology* 49: 176–182. https://doi.org/10.1007/s00248-003-2030-7

Gomont, M. (1892) Monographie des Oscillariées (Nostocacées Homocystées). Deuxième partie. - Lyngbyées. *Annales des Sciences Naturelles, Botanique* Série 7, 16: 91–264.

Gomont, M. (1899) Sur quelques Oscillariées nouvelles. *Bulletin de la Société Botanique de France* 46: 25–41. https://doi.org/10.1080/00378941.1899.10831710

Goor, A.C.J. van (1918) Zur Kenntnis der Oscillatoriaceen. *Reçueil des Travaux Botaniques Néerlandais* 15: 255–262.

Grewe, C.B. & Pulz, O. (2012) The biotechnology of cyanobacteria. *In*: Whitton, B.A. (Ed.) *Ecology of Cyanobacteria II*. Springer Netherlands, Dordrecht, pp. 707–739. https://doi.org/10.1007/978-94-007-3855-3_26

Hiraide, Y., Oshima, K., Fujisawa, T., Uesaka, K., Hirose, Y., Tsujimoto, R., Yamamoto, H., Okamoto, S., Nakamura, Y., Terauchi, K., Omata, T., Ihara, K., Hattori, M. & Fujita, Y. (2015) Loss of cytochrome c M stimulates cyanobacterial heterotrophic growth in the dark. *Plant and Cell Physiology* 56: 334–345. https://doi.org/10.1093/pcp/pcu165

Hisbergues, M., Christiansen, G., Rouhiainen, L., Sivonen, K. & Börner, T. (2003) PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. *Archives of Microbiology* 180: 402–410. https://doi.org/10.1007/s00203-003-0605-9

Hrouzek, P., Lukešová, A., Mareš, J. & Ventura, S. (2013) Description of the cyanobacterial genus *Desmonostoc gen. nov.* including *D. muscorum comb. nov.* as a distinct, phylogenetically coherent taxon related to the genus *Nostoc*. *Fottea* 13: 201–213. https://doi.org/10.5507/fot.2013.016

Jacinavicius, F.R., Gama Júnior, W.A., Azevedo, M.T. de P. & Sant'Anna, C.L. (2012) *Manual para cultivo de cianobactérias*. 32 pp.

Jungblut, A.D. & Neilan, B.A. (2006) Molecular identification and evolution of the cyclic peptide hepatotoxins, microcystin and nodularin, synthetase genes in three orders of cyanobacteria. *Archives of Microbiology* 185: 107–114. https://doi.org/10.1007/s00203-005-0073-5

Junk, W.J., Piedade, M.T.F., Schöngart, J., Cohn-Haft, M., Adeney, J.M. & Wittmann, F. (2011) A classification of major naturally-occurring Amazonian lowland wetlands. *Wetlands* 31: 623–640. https://doi.org/10.1007/s13157-011-0190-7

Kim, M., Oh, H.-S., Park, S.-C. & Chun, J. (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *International Journal of Systematic Evolutionary Microbiology* 64: 346–51. https://doi.org/10.1099/ijs.0.059774-0

Komárek, J. (2013) Cyanoprokaryota. 3. Teil: Heterocytous Genera. *In:* Büdel, B., Gärtner, G., Krienitz, L. & Schagerl, M. (Eds.) *Süßwasserflora von Mitteleuropa*, *vol. 19/3*. Springer Spektrum, Berlin, Heidelberg, pp. 1–1131.

Komárek, J. (2016) A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. *European Journal of Phycology* 51: 346–353. https://doi.org/10.1080/09670262.2016.1163738

Komárek, J. (2017) Delimitation of the family Oscillatoriaceae (Cyanobacteria) according to the modern polyphasic approach (introductory review). *Brazilian Journal of Botany*.

https://doi.org/10.1007/s40415-017-0415-y

Komárek, J. & Anagnostidis, K. (2005) Cyanoprokaryota. 2. Teil: Oscillatoriales. *In:* Büdel, B., Gärdner, G., Krienitz, L. & Schagerl, M. (Eds.) *Süsswasserflora von Mitteleuropa, vol. 19/2*. Elsevier, München, pp. 1–759.

Kosten, S., Huszar, V.L.M., Bécares, E., Costa, L.S., van Donk, E., Hansson, L.A., Jeppesen, E., Kruk, C., Lacerot, G., Mazzeo, N., De Meester, L., Moss, B., Lürling, M., Nõges, T., Romo, S. & Scheffer, M. (2012) Warmer climates boost cyanobacterial dominance in shallow lakes. *Global Change Biology* 18: 118–126.
https://doi.org/10.1111/j.1365-2486.2011.02488.x

Kotai, J. (1972) *Instructions for preparation of modified nutrient solution Z8 for algae*. Norwegian Institute for Water Research, Blindern, Oslo, Norway.

Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.
https://doi.org/10.1093/molbev/msw054

Kützing, F.T. (1843) *Phycologia generalis: oder, Anatomie, Physiologie und Systemkunde der Tange*. F.A. Brockhaus, Leipzig, 459 pp.

Lamparelli, M.C., Tucci, A., Sant'Anna, C.L., Pires, D.A., Lerche, L.H.M., Carvalho, M.C. & Rosal, C. (2014) *Atlas de cianobactérias da bacia do Alto Tietê*. CETESB, São Paulo, pp. 1–76.

Lauterborn, R. (1915) Die sapropelische Lebewelt. Ein Beitrag zur Biologie des Faulschlammes natürlicher Gewässer. *Verhandlungen des Naturhistorisch-Medizinischen Vereins zu Heidelberg* Ser. 2, 13: 395–481.

Leao, P.N., Pereira, A.R., Liu, W.-T., Ng, J., Pevzner, P.A., Dorrestein, P.C., Konig, G.M., Vasconcelos, V.M. & Gerwick, W.H. (2010) Synergistic allelochemicals from a freshwater cyanobacterium. *Proceedings of the National Academy of Sciences* 107: 11183–11188.
https://doi.org/10.1073/pnas.0914343107

Leão, P.N., Vasconcelos, M.T.S.D. & Vasconcelos, V.M. (2009) Allelopathic activity of cyanobacteria on green microalgae at low cell densities. *European Journal of Phycology* 44: 347–355.
https://doi.org/10.1080/09670260802652156

Lima, A.R.J., Castro, W. de O., Moraes, P.H.G., Siqueira, A.S., Aguiar, D.C.F., de Lima, C.P.S., Vianez-Júnior, J.L.S.G., Nunes, M.R.T., Dall'Agnol, L.T. & Gonçalves, E.C. (2017) Draft genome sequence of *Alkalinema* sp. strain CACIAM 70d, a cyanobacterium isolated from an Amazonian freshwater environment. *Genome Announcements* 5: e00635-17.
https://doi.org/10.1128/genomeA.00635-17

Lopes, V.R., Ramos, V., Martins, A., Sousa, M., Welker, M., Antunes, A. & Vasconcelos, V.M. (2012) Phylogenetic, chemical and morphological diversity of cyanobacteria from Portuguese temperate estuaries. *Marine Environmental Research* 73: 7–16.
https://doi.org/10.1016/j.marenvres.2011.10.005

Malone, C.F. da S., Rigonato, J., Laughinghouse, H.D., Schmidt, É.C., Bouzon, Z.L., Wilmotte, A., Fiore, M.F. & Sant'Anna, C.L. (2015) *Cephalothrix gen. nov.* (Cyanobacteria): towards an intraspecific phylogenetic evaluation by multilocus analyses. *International Journal of Systematic and Evolutionary Microbiology* 65: 2993–3007.
https://doi.org/10.1099/ijs.0.000369

Meffert, M.E. (1988) *Limnothrix Meffert nov. gen.*- the unsheathed planktic cyanophycean filaments with polar and central gas vacuoles. *Algological Studies/Archiv für Hydrobiologie, Supplement Volumes*: 269–276.

Menezes, M., Bicudo, C.E.M. & Moura, C.W.N. (2015) Update of the Brazilian floristic list of algae and cyanobacteria. *Rodriguesia* 66: 1047–1062.
https://doi.org/10.1590/2175-7860201566408

Moreira, C., Ramos, V., Azevedo, J. & Vasconcelos, V. (2014) Methods to detect cyanobacteria and their toxins in the environment. *Applied Microbiology and Biotechnology* 98: 8073–8082.
https://doi.org/10.1007/s00253-014-5951-9

Neilan, B.A., Jacobs, D., Del Dot, T., Blackall, L.L., Hawkins, P.R., Cox, P.T. & Goodman, A.E. (1997) rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. *International Journal of Systematic Bacteriology* 47: 693–697.
https://doi.org/10.1099/00207713-47-3-693

Niiyama, Y., Tuji, A., Takemoto, K. & Ichise, S. (2016) *Pseudanabaena foetida sp. nov.* and *P. subfoetida sp. nov.* (Cyanophyta/Cyanobacteria) producing 2–methylisoborneol from Japan. *Fottea* 16: 1–11.
https://doi.org/10.5507/fot.2016.006

Nubel, U., Muyzer, G., Garcia-Pichel, F. & Muyzer, G. (1997) PCR primers to amplify 16S rRNA genes from cyanobacteria. *Microbiology* 63: 3327–3332.

Papaefthimiou, D., Hrouzek, P., Mugnai, M.A., Lukesova, A., Turicchia, S., Rasmussen, U. & Ventura, S. (2008) Differential patterns of evolution and distribution of the symbiotic behaviour in nostocacean cyanobacteria. *International Journal of Systematic and*

Evolutionary Microbiology 58: 553–564.
https://doi.org/10.1099/ijs.0.65312-0

Rajaniemi, P., Hrouzek, P., Kaštovská, K., Willame, R., Rantala, A., Hoffmann, L., Komárek, J. & Sivonen, K. (2005) Phylogenetic and morphological evaluation of the genera *Anabaena, Aphanizomenon, Trichormus and Nostoc* (Nostocales, Cyanobacteria). *International Journal of Systematic and Evolutionary Microbiology* 55: 11–26.
https://doi.org/10.1099/ijs.0.63276-0

Ramos, V., Morais, J., Castelo-Branco, R., Pinheiro, Â., Martins, J., Regueiras, A., Pereira, A.L., Lopes, V.R., Frazão, B., Gomes, D., Moreira, C., Costa, M.S., Brûle, S., Faustino, S., Martins, R., Saker, M., Osswald, J., Leão, P.N. & Vasconcelos, V.M. (2018) Cyanobacterial diversity held in microbial biological resource centers as a biotechnological asset: the case study of the newly established LEGE culture collection. *Journal of Applied Phycology* 30: 1437–1451.
https://doi.org/10.1007/s10811-017-1369-y

Ramos, V., Morais, J. & Vasconcelos, V.M. (2017) A curated database of cyanobacterial strains relevant for modern taxonomy and phylogenetic studies. *Scientific Data* 4: 1–8.
https://doi.org/10.1038/sdata.2017.54

Rantala-Ylinen, A., Känä, S., Wang, H., Rouhiainen, L., Wahlsten, M., Rizzi, E., Berg, K., Gugger, M. & Sivonen, K. (2011) Anatoxin-a synthetase gene cluster of the cyanobacterium *Anabaena* sp. strain 37 and molecular methods to detect potential producers. *Applied and Environmental Microbiology* 77: 7271–7278.
https://doi.org/10.1128/AEM.06022-11

Rigonato, J., Alvarenga, D.O. & Fiore, M.F. (2017) Tropical cyanobacteria and their biotechnological applications. *In*: de Azevedo, J.L. & Quecine, M.C. (Eds.) *Diversity and Benefits of Microorganisms from the Tropics*. Springer, Cham, pp. 139–167.
https://doi.org/10.1007/978-3-319-55804-2_7

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. & Stanier, R.Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology* 111: 1–61.
https://doi.org/10.1099/00221287-111-1-1

Rippka, R., Waterbury, J.B. & Stanier, R.Y. (1981) Isolation and purification of cyanobacteria: some general principles. *In*: Starr, M.P., Stolp, H., Trüper, H.G., Balows, A. & Schlegel, H.G. (Eds.) *The prokaryotes*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 212–220.
https://doi.org/10.1007/978-3-662-13187-9_8

Sant' Anna, C.L., Azevedo, M.T.P., Agujaro, L.F., Carvalho, M.C., Carvalho, L.R. & Souza, R.C.R. (2006) *Manual ilustrado para identificação e contagem de cianobactérias planctônicas de águas continentais brasileiras*. Rima, Rio de Janeiro, 58 pp.

Sant'Anna, C.L., Tucci, A., Azevedo, M.T.P., Melcher, S.S., Werner, V.R., Malone, C.F.S., Rossini, E.F., Jacinavicius, F.R., Hentschke, G.S., Osti, J.A.S., Santos, K.R.S., Gama-Júnior, W.A., Rosal, C. & Adame, G. (2012) *Atlas de cianobactérias e microalgas de águas continentais brasileiras*. Instituto de Botânica, São Paulo, 175 pp.

Satinsky, B.M., Smith, C.B., Sharma, S., Ward, N.D., Krusche, A.V., Richey, J.E., Yager, P.L., Crump, B.C. & Moran, M.A. (2017) Patterns of bacterial and archaeal gene expression through the Lower Amazon River. *Frontiers in Marine Science* 4: 1–15.
https://doi.org/10.3389/fmars.2017.00253

Sato, N., Katsumata, Y., Sato, K. & Tajima, N. (2014) Cellular dynamics drives the emergence of supracellular structure in the cyanobacterium, *Phormidium* sp. KS. *Life (Basel, Switzerland)* 4: 819–36.
https://doi.org/10.3390/life4040819

Schembri, M.A., Neilan, B.A. & Saint, C.P. (2001) Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermopsis raciborskii*. *Environmental Toxicology* 16: 413–421.
https://doi.org/10.1002/tox.1051

Schwabe, G.H. (1964) Grundprobleme der Cyanophytentaxonomie. *Gewässer und Abwässer* 36: 7–39.

Sciuto, K., Andreoli, C., Rascio, N., La Rocca, N. & Moro, I. (2012) Polyphasic approach and typification of selected *Phormidium* strains (Cyanobacteria). *Cladistics* 28: 357–374.
https://doi.org/10.1111/j.1096-0031.2011.00386.x

Sena, B.A., Costa, V.B., Nakayama, L. & Rocha, R.M. (2015) Composition of microphytoplankton of an estuarine Amazon River, Pará, Brazil. *Biota Amazonia* 5: 1–9.
https://doi.org/10.18561/2179-5746/biotaamazonia.v5n2p1-9

Silva, N.B., Lopes, J.O., Brito, J.D.M., Santos, S.K., Takiyama, L.R., Montagner, A.E.A.D. & Oliveira, E.D.C. de. (2018) Water quality of areas used for buffalo farming - Lower Araguari River, Amapa, Brazil. *Biota Amazonia* 8: 22–28. Available from: https://periodicos.unifap.br/index.php/biota/article/view/3419 (Accessed 11 Jan. 2019)

Silveira-Júnior, A.M. (2012) Composição e biomassa microfitoplanctônica associadas a variáveis físico e químicas em dois transectos da zona estuarina do Rio Amazonas (Amapá, Amazônia, Brasil). Unifap, Macapá, 93 pp.

Soo, R.M., Hemp, J., Parks, D.H., Fischer, W.W. & Hugenholtz, P. (2017) On the origins of oxygenic photosynthesis and aerobic respiration in cyanobacteria. *Science* 355: 1436–1440.
https://doi.org/10.1126/science.aal3794

Suda, S., Watanabe, M.M., Otsuka, S., Mahakahant, A., Yongmanitchai, W., Nopartnaraporn, N., Liu, Y. & Day, J.G. (2002) Taxonomic revision of water-bloom- forming species of oscillatorioid cyanobacteria. *International Journal of Systematic and Evolutionary Microbiology* 52: 1577–1595.
https://doi.org/10.1099/00207713-52-5-1577

Stackebrandt, E. & Ebers, J. (2006) Taxonomic parameters revisited: tarnished gold standards. *Microbiology Today* 33: 152–155.

Takahashi, T., Takase, K. Takeshita, K., Kawano, H., Mamizuka, M. & Kizu, H. (2016) State transition of musty-odor-producing cyanobacteria and 2-methylisoborneol in the Serikawa Dam reservoir. *Journal of Japan Society on Water Environment* 39: 51–62.
https://doi.org/10.2965/jswe.39.51

Tuji, A. & Niiyama, Y. (2016) The identity and phylogeny of *Pseudanabaena* strain, NIES-512, producing 2-methylisoborneol (2-MIB). *Bulletin of the National Museum of Nature and Science* 42: 83–89.

Tundisi, J.G., Goldemberg, J., Matsumura-Tundisi, T. & Saraiva, A.C.F. (2014) How many more dams in the Amazon. *Energy Policy* 74: 703–708.
https://doi.org/10.1016/j.enpol.2014.07.013

Vaz, M.G.M.V., Genuário, D.B., Andreote, A.P.D., Malone, C.F.S., Sant'Anna, C.L., Barbiero, L. & Fiore, M.F. (2015) *Pantanalinema gen. nov.* and *Alkalinema gen. nov.*: novel pseudanabaenacean genera (Cyanobacteria) isolated from saline–alkaline lakes. *International Journal of Systematic and Evolutionary Microbiology* 65: 298–308.
https://doi.org/10.1099/ijs.0.070110-0

Vieira, J.M.D.S., Azevedo, M.T.D.P., De Oliveira Azevedo, S.M.F., Honda, R.Y. & Corrêa, B. (2005) Toxic cyanobacteria and microcystin concentrations in a public water supply reservoir in the Brazilian Amazonia region. *Toxicon : Official Journal of the International Society on Toxinology* 45: 901–9.
https://doi.org/10.1016/j.toxicon.2005.02.008

Ward, N.D., Krusche, A.V., Sawakuchi, H.O., Brito, D.C., Cunha, A.C., Moura, J.M.S., da Silva, R., Yager, P.L., Keil, R.G. & Richey, J.E. (2015) The compositional evolution of dissolved and particulate organic matter along the Lower Amazon River-Óbidos to the ocean. *Marine Chemistry* 177: 244–256.
https://doi.org/10.1016/j.marchem.2015.06.013

Waterbury, J.B. (2006) The Cyanobacteria—Isolation, Purification and Identification. *In:* Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. & Stackebrandt, E. (Eds.) *The Prokaryotes*. Springer US, New York, NY, pp. 1053–1073.
https://doi.org/10.1007/0-387-30744-3_38

Werner, V.R., Cabezudo, M.M., Neuhaus, E.B., Caires, T.A., Sant'Anna, C.L., Azevedo, M.T.P., Malone, C., Gama Jr., W.A., Santos, K.R.S. & Menezes, M. (2015) Cyanophyceae. *In:* Forzza, R.C. (Ed.) *Lista de espécies da flora do Brasil*. Rio de Janeiro, Brazil. Available from: http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB116809 (accessed 29 April 2016)

Whitton, B.A. & Potts, M. (2012) Introduction to the Cyanobacteria. *In*: Whitton, B.A. (Ed.) *Ecology of Cyanobacteria II*. Springer Netherlands, Dordrecht, pp. 1–3.
https://doi.org/10.1007/978-94-007-3855-3_1

Willame, R., Boutte, C., Grubisic, S., Wilmotte, A., Komárek, J. & Hoffmann, L. (2006) Morphological and molecular characterization of planktonic cyanobacteria from Belgium and Luxembourg. *Journal of Phycology* 42: 1312–1332.
https://doi.org/10.1111/j.1529-8817.2006.00284.x

Yu, G., Zhu, M., Chen, Y., Pan, Q., Chai, W. & Li, R. (2015) Polyphasic characterization of four species of *Pseudanabaena* (Oscillatoriales, Cyanobacteria) from China and insights into polyphyletic divergence within the *Pseudanabaena* genus. *Phytotaxa* 192: 1–12.
https://doi.org/10.11646/phytotaxa.192.1.1

Zhu, M., Yu, G., Li, X., Tan, W. & Li, R. (2012) Taxonomic and phylogenetic evaluation of *Limnothrix* strains (Oscillatoriales, Cyanobacteria) by adding *Limnothrix planktonica* strains isolated from central China. *Hydrobiologia* 698: 367–374.
https://doi.org/10.1007/s10750-012-1127-8