Phytotaxa 164 (4): 255–264
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.164.4.4

Morphological and Molecular Characterization of *Brasilonema roberti-lamii* (Cyanophyceae, Nostocales, Scytonemataceae), from Central Mexico

BEATRIZ RODARTE[1†], ITZEL BECERRA-ABSALÓN[2*], GUSTAVO A. MONTEJANO[2], KARINA OSORIO-SANTOS[2], LUISA ALBA-LOIS[1], HILDA LEÓN-TEJERA[2] & CLAUDIA SEGAL-KISCHINEVZKY[1]

[1] *Departamento de Biología Celular.*

[2]*Departamento de Biología Comparada. Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM). Colonia Coyoacán, Código Postal 04510, P.O. Box 70-474, México, Distrito Federal, México. *e-mail: iba@ciencias.unam.mx*

Abstract

This paper is a contribution to the morphological and molecular characterization of the cyanobacterium *Brasilonema roberti-lamii* from populations found in Central Mexico. The general growth form and the morphological, morphometric and ecological characteristics of the populations studied clearly correspond to those described for *Brasilonema roberti-lamii* (basionym: *Tolypothrix roberti-lamii*) from the French Antilles. Based on molecular data from DNA sequencing of the16S rRNA gene and the IGS of the *cpc*B-*cpc*A phycocyanin operon (*cpc*BA-IGS), we propose that the populations that we studied are closely related to those of other *Brasilonema* species, including *B. octagenarum* UFV-OR1, UFV-E1 and HA4187-MV1-p1F, *Brasilonema sennae* CENA 114, *B. tolantongensis*, *B. terrestre* CENA 116, *B. angustatum* HA4187-MV1-B2+p1F and HA4187-MV1-B2+p1H and *B. bromeliae* SPC951. Our findings support the transference of *Tolypothrix roberti-lamii*, which was made based exclusively on morphological criteria, to *Brasilonema*. The use of molecular analyses in addition to traditional morphological and ecological criteria, known as polyphasic approach, is a good alternative to describe taxa of cyanobacteria, mainly at the genus and species levels.

Keywords: *cpc*BA-IGS gene, Cyanobacteria, 16S rRNA gene, *Tolypothrix roberti-lamii*, polyphasic approach, systematics, taxonomy

Introduction

The use of several criteria, in addition to the morphological, such as molecular, ultrastructural, etc. (known as the "polyphasic approach") has been proven to be adequate for the recognition and characterization of different taxa of cyanobacteria. The term "polyphasic" has been used in this sense by several researches (Flechtner *et al*. 2002, González-Resendiz *et al*. 2013, Komárek 2010, Vaccarino & Johansen 2011, 2012). The results reported by several authors (Wilmotte & Golubic 1991, Komárek 2006, 2010) show a clear relationship between the morphology and the DNA sequences of different genes within cyanobacteria, mainly the 16S rRNA. Several traditional cyanobacterial genera in the classification proposed by Geitler (1932) have been supported by DNA sequencing. Some recently described cyanobacteria have been separated from traditional genera to establish new genera such as *Brasilonema* Fiore, Sant'Anna, Azevedo, Komárek, Kaštovský, Sulek & Lorenzi (2007: 794), *Coleofasciculus* Siegesmund, Johansen & Friedl in Siegesmund *et al*. (2008: 1575), *Geminocystis* Korelusová, Kaštovský & Komárek (2009: 933) or *Phormidesmis* Turicchia, Ventura, Komárková & Komárek (2009: 179). Some studies of extreme habitats have also led to the discovery of new genetically and morphologically well-defined genera, i.e. *Spirirestis* Fletchtner & Johansen in Fletcher *et al*. (2002: 6) or *Rexia* Cassamata, Gomez & Johansen (2006: 23).

The sequencing of the 16S rRNA gene has been used as a standard genetic approach to delimitate cyanobacterial genera. However, There are cases where some morphological and ecological different species. show a high percentage of DNA similarity, whereas other populations with similar morphology appear in different clades when their 16S rRNA gene sequences are compared (the so-called "cryptic species", Komárek 2005, 2006, 2010).

The genus *Brasilomena* was erected based on both its morphological characters and DNA sequences, analyzed from the 16S rRNA gene and the IGS of the *cpc*B-*cpc*A phycocyanin operon sequences. At present, nine species

Accepted by Saúl Blanco Lanza: 5 Mar. 2014; published: 11 Apr. 2014

are included in this genus, six of them have been erected based both on morphological and molecular criteria: *Brasilonema bromeliae* Fiore, Sant'Anna, Azevedo, Komárek, Kaštovský, Sulek & Lorenzi (2007: 796), *B. octagenarum* Aguiar, Fiore, Franco, Ventrella, Lorenzi, Vanetti & Alfenas (2008: 1325), *B. sennae* (Komárek 2003: 225) Sant'Anna & Komárek in Sant'Anna *et al*. (2011: 57), *B. terrestre* Sant'Anna & Komárek in Sant'Anna *et al*. (2011: 59), *B. angustatum* Vaccarino & Johansen (2012: 3) and *B. tolantongensis* Becerra-Absalón & Montejano in Becerra-Absalón *et al*. (2013: 27); and three other species based only on morphological characters: *Brasilonema ornatum* Sant'Anna & Komárek in Sant'Anna *et al*. (2011: 54), *B. epidendron* Sant'Anna & Komárek in Sant'Anna *et al*. (2011: 52) and *B. roberti-lamii* (Bourrelly) Sant'Anna & Komárek in Sant'Anna *et al*. (2011: 56).

During the study of the cyanobacterial biota from Mexico's central region, we found a population corresponding in morphology and ecology to *B. roberti-lamii*. This species was originally described from the French Antilles as *Tolypothrix roberti-lamii* Bourrelly & Manguin (1952: 151). Recently, this taxon has been moved to the genus *Brasilonema* based on its morphology and distinctive life cycle (Sant'Anna *et al*. 2011). Given our material and the taxonomic situation of the genus *Brasilonema*, we decided to perform a medium-term study (four years) of these cyanobacteria. The specific aim of this paper is to study the molecular, morphological and ecological correspondence (polyphasic approach) between our populations and those of other *Brasilonema* species, in order to corroborate the transfer from *Tolypothrix* Kützing ex Bornet & Flahault (1886–1888: 118) to *Brasilonema roberti-lamii*, as proposed by Sant'Anna *et al*. (2011).

Materials and Methods

Site description, sample collection and morphological analyses:—Samples were collected during the 2006–2010 period in the locality of Los Manantiales, Morelos, in Central Mexico (18° 55' 39" N, 96° 00' 37" W; 800 m a.s.l.). The climate of the locality is warm, sub-humid, with summer rain (Aw0 (w), Köppen modified by García (1988)) and the vegetation is a tropical deciduous forest. The populations were growing aerophytically on rocks and walls. Samples were transported dry to the laboratory. In the populations studied the following characteristics were analyzed: filament and trichome diameter, thallus and sheath morphology and coloration, cell color, length and width of heterocytes, branching, and young trichome form. The observations were carried out on an Olympus BX 51 microscope equipped with DIC (differential interferential contrast) and a DP 12 digital camera. The characteristics of our populations were compared to the information on *Brasilonema* populations reported in Fiore *et al*. (2007), Aguiar *et al*. (2008), Sant'Anna *et al*. (2011), Vaccarino & Johansen (2012), and Becerra-Absalón *et al*. (2013).

Genomic DNA extraction:—DNA was extracted according to Neilan *et al*. (1995) from ~30 mg of fresh field material, which was studied and selected under an Olympus SZ2-ILST stereoscopic microscope to check for possible contaminations.

PCR amplification and sequencing:—PCR amplification was done using the primer set reported by Neilan *et al*. (1995): PCßF and PCαR for the intergenic sequence of the c*pc*B and *cpc*A of the phycocyanin operon (*cpc*BA-IGS), and 27 F1 and 1494Rc for the 16S rRNA (Neilan *et al*. 1997). Amplification was performed in a Mastercycler Eppendorf. The reaction volume was 50 µL and contained 1X reaction buffer, 2.5 mM $MgCl_2$, 0.3 mM of each deoxynucleotide triphosphate (dNTPs), 0.5 µM of each primer and 1U of DNA polymerase Amplificasa (Biogénica, Mexico). The PCR program was the same for both amplifications and consisted of an initial denaturation at 94 °C for 7 minutes; 30 cycles at 94 °C for 30 seconds, 63 °C for 1 minute, 72 °C for 1 minute, and a final extension at 72 °C for 7 minutes. The amplification products were visualized on a 1.5% agarose electrophoresis gel and the amplification products of 16S rRNA were cloned into the pJet 1.2/blunt vector of the Clone Jet PCR Cloning Kit (Fermentas, Maryland, USA). The DNA from the positive samples were purified using GeneClean®III Kit (Bio 101 Systems, California, USA) as recommended by the manufacturer. The obtained DNAs were submitted to "Unidad de Biología Molecular, Instituto de Fisiología Celular, UNAM (D.F., Mexico)", to be sequenced. Sequences of both DNA strands were determined by using the same primers as those used for amplification. Fragments were assembled using the software Bioedit version 7.0.9.0 (Hall 1999). The GenBank/EMBL/DDBJ accession number for the *cpc*BA-IGS sequence determined in this study is EF676035, while the accession number for the 16S rRNA sequence determined in this study is GQ443308.1.

Sequence alignment and phylogenetic analyses:—Phylogenetic analyses were based on two DNA sequences: the 16S rRNA gene (bp 106–1494), and the *cpc*BA-IGS region of the phycocyanin gene. The taxa used for 16S rRNA and *cpc*BA sequence analyses included a total of 124 and 25 OTUs, respectively, both with one novel sequence of *B. roberti-lamii* from Los Manantiales Morelos, Mexico, and OTUs from GenBank. We made a BLAST search to select the sequences most closely related to these of *B. roberti-lamii*. We also selected sequences based on morphological classification criteria, so we included sequences from all the Nostocalean families: Scytonemataceae, Microchaetaceae, Nostocaceae, Rivulariaceae, Symphyonemataceae, Hapalosiphonaceae and Stigonemataceae. In both analyses the external group was *Gloeobacter violaceaus* Rippka, Waterbury & Cohen-Bazire (1974: 436). The initial alignment was constructed using ClustalW (Larkin *et al*. 2007), and the sequences were manually reviewed as well using the program PhyDE-1 version 0.9971 (Müller *et al*. 2010). The trees were constructed using parsimony and maximum likelihood analyses in MEGA version 5 (Tamura *et al*. 2011). Parsimony analysis was performed using the Subtree Pruning Regrafting (SPR) search method with 10 random additions and 100 trees retain. Bootstrapping of 500 replicates was used. The best models for analysis with maximum likelihood were found with MEGA version 5. Maximum likelihood was performed using the GTR+I+G likelihood model for the 16S rRNA and Tamura 3-parameter likelihood model for the *cpc*BA-IGS. The heuristic search followed the Nearest Neighbor Interchange (NNI) method. Bootstrapping was conducted with 500 pseudoreplicates for the 16S rRNA and 1000 pseudoreplicates for the *cpc*BA-IGS.

Results

Morphological analysis:—Our material of *B. roberti-lamii* clearly belongs to that described for the Antilles Island by Bourrelly and Manguin (1952). The habit is heterotrichous with a postrated and erect portion (Fig. 1, a–e); this last one is organized in fascicles with parallel trichomes (Fig. 1, d–e, j). Filaments have a distinctive "C" or "J" form (Fig. 1, f–h), and the morphometry and ecology also correspond to the description of *B. roberti-lamii* (Table 1). *Brasilonema roberti-lamii* is quite different from other *Brasilonema* species: The filaments of *Brasilonema bromeliae*, *B. ornatum*, *B. sennae* and *B. tolantongensis* are wider than *B. roberti-lamii*, *B. epidendron* and *B. ornatum* have ornamented sheaths which *B. roberti-lammi* lacks, *B. epidendron* has bright blue-green cells (whereas those of *B. roberti-lamii* are violet), and finally *B. angustatum* is tapering while *B. roberti-lamii* is not (Table 1).

Brasilonema roberti-lammi (Bourrelly & Manguin) Sant'Anna & Komárek

Thallus in erect fascicles, forming extended carpets 2–3 mm high, brownish and blackish in color (Fig. 1, a–e). Filaments parallel, closely packed, organized in fascicles, 11–14 µm in diameter (Table 1). Occasional false single branching, sometimes from hormogonia. Sheath thin, colorless or yellow brownish (Fig. 1, e). Trichomes isopolar, cylindrical, not constricted at the cell walls (Fig. 1, e–h), not attenuated toward the ends and rounded at the apex, ends 9.5–11.5 µm in diameter. Young trichomes with a characteristically "C" or "J" shape (Fig. 1, f–h). Cells isodiametric or slightly longer than wide, except in meristematic zones where they are disk-shaped. Cells present "vacuolization" frequently. Heterocytes intercalary, 11.0–13.5 µm wide × 10.5–13.0 µm long. Hormogonia develop in the apical extremes of filaments by formation of necridic cells (Fig. 1, i–l).

Sequences alignments and phylogenetic analyses:—The Maximum Likelihood (ML) and Maximum Parsimony (MP) algorithms produced similar tree topologies in the 16S rRNA and *cpc*BA-IGS genes analyses. Only ML trees are shown in figures 2 and 3. The phylogenetic tree resulting from the 16S rRNA gene analysis (Fig. 2) showed that *B. roberti-lamii* clusters with the other six recently reported *Brasilonema* taxa, with a high bootstrap value (92%). In this tree, *B. roberti-lamii* is a sister species of *B. octagenarum* and the cluster formed with both species is a sister with *B. angustatum*. The sister cluster of the *Brasilonema* group is formed with *Symphyonemopsis* Tiwari & Mitra (1969: 93) sequences and the next branch related to both clusters is formed with several sequences of *Scytonema* Agardh ex Bornet & Flahault (1886: 85). We determined 1379 bp from the 16S rRNA gene sequence from *Brasilonema roberti-lamii* strain, this sequence was analyzed by the BLAST algorithm and the result showed that the percentage of similarity between *B. roberti-lamii*, *B. octagenarum* UFV-ORI, *B. octagenarum* UFV-E1 and *B.*

angustatum was 99%, the next similar strains were *B. tolantongensis* with 98%, and lastly *B. terrestre*, *B. sennae* CENA 114 and *B. bromeliae* SPC951 with 97% similarity. The other taxa presented in Table 2 were less than 96% similar to *B. roberti-lamii*. The phylogenetic tree resulting from the cpcBA-IGS gene analysis (Fig. 3) showed that all six *Brasilonema* sequences were clustered together in one defined clade with high bootstrap values, the nearest (but in a different branch) was *Tolypothrix sp*, while *Nostoc linckia* Bornet ex Bornet & Flahault (1888: 192), *Nostoc* sp. and *Scytonema hofmannii* Agardh ex Bornet & Flahault (1887: 97) were found in a sister cluster. Using the phycocyanin *cpc*BA-IGS to determine the phylogenetic placement of our populations results in *B. roberti-lamii* being a member of the *Brasilonema* genus and indeed constituting a distinctive species.

FIGURE 1. *Brasilonema roberti-lamii*. A. According to Bourrelly and Manguin (1952). B–L. *Brasilonema roberti-lamii* from Los Manantiales. B–D. Habit. E. Detail of filament organization in fascicles. F–H. typical "C" or "J" shape of young trichomes with heterocytes. I, K, L. Different stages of hormogonia development. Arrow: proheterocyte. J. Parallel organization of filaments. Scale bars: C = 0.5 mm, D = 50 µm, E–J, L = 20 µm, K = 7 µm.

TABLE 1. Morphological characters of *Brasilonema* species. In bold, "Los Manantiales" population, Mexico. The information about the other species was obtained from Fiore *et al*. (2007), Aguiar *et al*. (2008), Santa'Anna *et al*. (2011), Vaccarino & Johansen (2012) and Becerra-Absalón *et al*. (2013).

Character	*B. bromeliae* (Fiore *et al*. 2007)	*B. epidendron* (Sant'Anna *et al*. 2011)	*B. octogenarum* (Aguiar *et al*. 2008)	*B. ornatum* (Sant'Anna *et al*. 2011)	*B. angustatum* (Vaccarino & Johansen 2012)	*B. sennae* (Sant'Anna *et al*. 2011)	*B. terrestre* (Sant'Anna *et al*. 2011)	*B. tolantongensis* (Becerra-Absalón *et al*. 2013)	*B. roberti-lamii* (Sant'Anna *et al*. 2011)	*B. roberti-lamii* (Present study)
Filament diameter (µm)	10–14.8–21	(7)10.9–12(14)	9.8–16–18.5	20–23	9.8–17.8	10–20	12–17	17–25	12–15–18	**11.5–13**
Trichome diameter (µm)	8–13.2–18	(5.5)8.2–10(11)	9.5–14.9–18.4	17–18	(5.5)9.8–17.0	6–12.5	9–15	12.5–20	8	**8.5–11.5**
Sheath morphology	Thin, firm	Thin, firm	Thin, firm, later lamellated	Thick, lamellated, regularly ornamented	Thin, smooth	Thin, firm, later lamellated	Thin, firm, ornamented	Thin, firm, smooth	Thin firm sometimes lamellated	**Thin, firm**
Cell color	Grayish blue or brownish, olive-green, or violet	Bright blue-green	Brownish, olive-green, or rarely violet	Dark blue-green	Brownish or purplish gray	Blue-green or olive-green	Greyish-green or green-blue	Violet or brown	-	**Violet**
Thallus morphology	Free fascicles	Irregular erect fascicles	Mats of irregular fascicles, creeping fascicles	Irregular fine mats with creeping, partly fasciculated filaments	Free, erect moderately long filaments	Regular erect fascicles	Forming mats or irregular fascicles	Creeping fascicles	Irregular fascicles, parallel filaments	**Erect fascicles, parallel filaments**
Thallus color	Blackish-green to blackish violet	Dark green to blackish	Dirty-green, brownish, or blackish-green	Greyish green	Brown	Dirty-green, brownish, or blackish-green	Dirty green	Blackish-violet	Violet	**Brownish blackish**
Heterocytes (µm)	±Cylindrical 4–19 long × 15–16.8 wide	Barrel-shaped to cylindrical (7)8–10(11.5) long × 7–9 wide	Cylindrical 5.4–15.6 × 10–17.6	Rounded, 17–18 long × 3–6 wide	Intercalary, flattened or elongated 10–17 wide × 2.8–17.4 long	Cylindrical 6.8–15.4 long × 10.2–11.2 wide	Cylindrical-barrel shaped 6–17 long × 13–14 wide	7–15.5 wide × 12.5–16.5 long	Rectangular	**Cylindrical, isodiametrical**
Branching type	Double or single	Simple	Double or single	-	Double or single	Double or single	-	Double, single or from hormogonia	Single (like *Tolypothrix* ?)	**Double or single from hormogonia**
Shape of young trichomes	"J"- and "C"-shaped	-	Lightly curved	-	Curved, with heterocytes at one end	-	-	Curved, attenuated at one or both ends.	"J"- and "C"-shaped	**"J"- and "C"-shaped**
Hormogonia development	-	Short cells, slightly constricted at cross walls, granulated	-	-	Crescent-shaped, isopolar when first released	-	-	Initially with one or two intercalary, asymmetric heterocytes, after fragmentation, with basal heterocytes	Medium heterocyst	**Initially with one or two intercalary, asymmetric heterocytes, after fragmentation, with basal heterocytes**
Ecology	Subaerial, epiphytic on living and dead leaves of bromeliads (inside of leaf rosettes)	Subaerial, corticolous or on old wooden substrates in Atlantic Forest.	Epiphytic on living and dead leaves, stem, and buds of *Eucalyptus grandis*	Subaerial, on bark of trees among mosses and lichens	Moss bank along Moleka Trail	Subaerial, edge of springs on wet wooden stony and iron substrates	Subaerial, on concrete	Subaerial, on walls near runoffs	Subaerial, on rocks and roofs	**Subaerial, on rocks and walls**

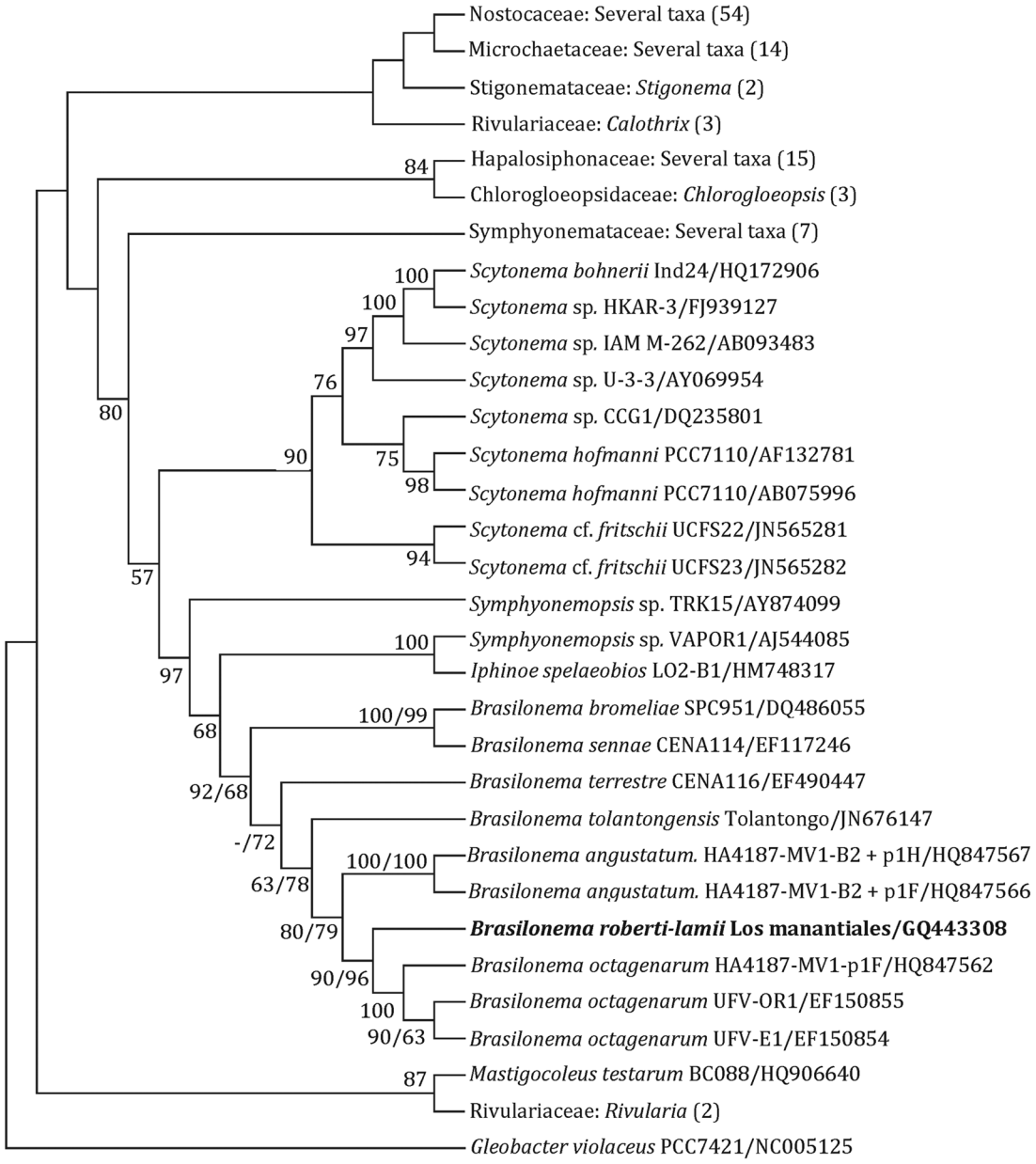

FIGURE 2. Maximum Likelihood tree based on sequences of the 16S rRNA gene. The phylogenetic analysis showed the relationships between *Brasilonema* species and Cyanobacteria of the same order (Nostocales), using *Gloeobacter violaceus* as the external functional group. Bootstrap values at supported nodes (> 50%) are given for Maximum Likelihood, and Maximum Parsimony values only in the cluster with *Brasilonema*.

Discussion

Our populations showed the morphological diagnostic features of *Brasilonema roberti-lamii* described for the French Antilles populations (Bourrelly & Manguin 1952), including habit, morphology, morphometry and ecology, therefore we conclude that, according to our analyses, they correspond to this species (Table 1, Fig. 1, a–e). This species has the distinctive morphological and molecular characteristics of *Brasilonema*: growth in fascicles with parallel filaments; cylindrical trichomes with vacuolization; thin, colorless or yellow-brown sheaths, roundly closed at the ends; cells with the typical violet color; solitary, intercalary heterocytes lacking akinetes (Fiore *et al*. 2007) and hormogonia with asymmetric development (Becerra-Absalón *et al*. 2013. Table 1. Fig. 1, e–l). At the molecular (DNA sequence) level, the 16S RNA (Fig. 2) and *cpc*B-IGS (Fig. 3) analyses showed that the sequences

of *B*. *roberti-lamii* belong to the same clade as the other *Brasilonema* species. Therefore, our results support the transfer of *Tolypothrix roberti-lamii* to *Brasilonema*, as Sant'Anna *et al*. (2011) proposed.

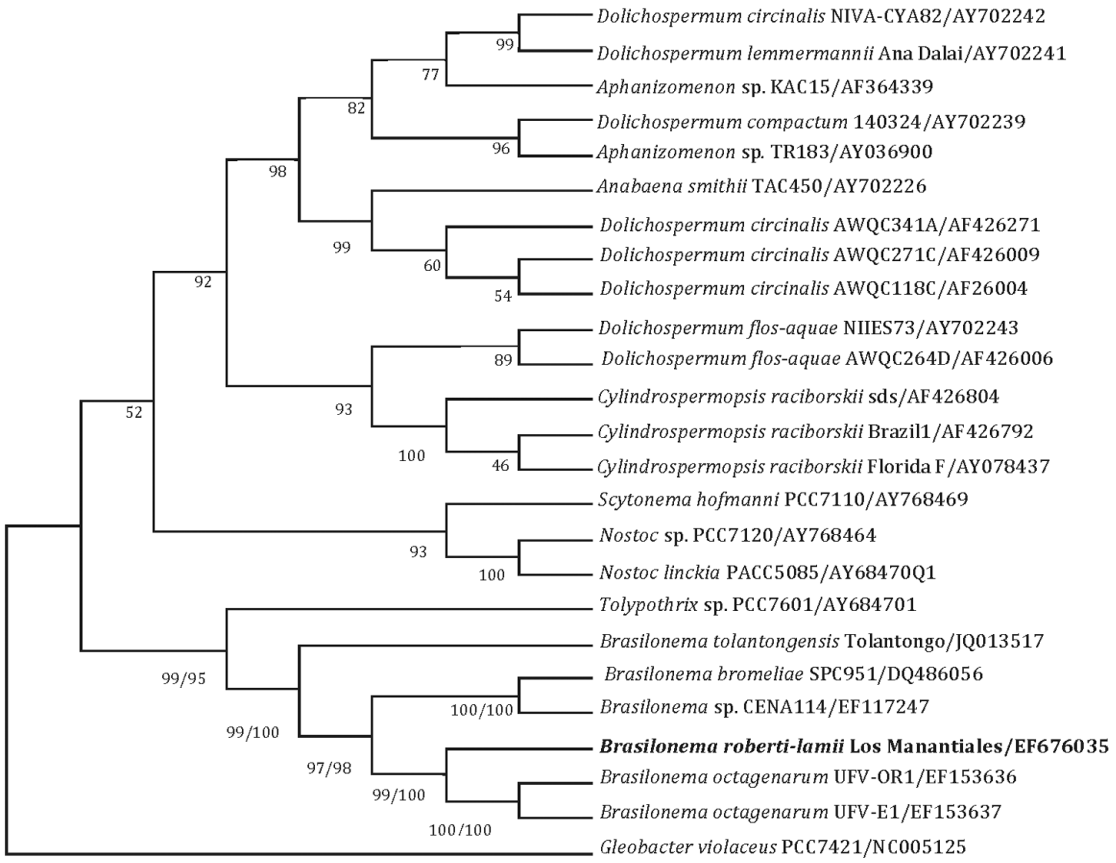

FIGURE 3. Maximum Likelihood tree based on sequences of the *cpc*BA-IGS gene. The analysis showed the relationships among *Brasilonema* species and Cyanobacteria of the same order (Nostocales), using *Gloeobacter violaceus* as the external functional group. Bootstrap values at supported nodes (> 50%) are given for Maximum Likelihood, and Maximum Parsimony values only in the cluster with *Brasilonema*.

In the 16S rRNA analysis, the *Brasilonema* clade is separated from the most morphologically similar genus *Scytonema*. *Brasilonema* appeared also as a sister group to *Symphyonemopsis* (Fig. 2).

At the species level, *B. roberti-lamii* exhibits a high percentage of similarity (99%) to both *B. octagenarum* and *B. angustatum* (Table 2). Indeed, in the phylogenetic tree these three species are part of a single tight cluster. At this level it is difficult to decide, based exclusively on differences among the 16S rRNA sequences, whether these three populations belong to the same or different species, although the differences in morphology and ecology (Table 1) allow us to consider that *B*. *roberti-lamii* is actually a separate species from *B*. *octagenarum* and *B*. *angustatum*. The morphologically closest species (not coincident with the genetic closeness) is *B*. *bromeliae*, similar in habit and morphology with the young trichomes of *B*. *roberti-lamii*, and sharing the "C" and "J" shapes (Table 1). However, this latter species exhibits double branches and is epiphytic, whereas *B*. *roberti lamii* has single branches and is epilithic (Table 1).

In summary, our results support the proposal of Sant'Anna *et al*. (2011) to transfer *Tolypothrix roberti-lamii* to the genus *Brasilonema* since this species presents the diagnostic characteristics of the genus (Fiore *et al*. 2007) and belongs to the *Brasilonema* cluster. Additionally, *B*. *roberti-lamii* can be regarded as a species different from other taxa of the genus when applying morphological, genetic and ecological criteria. It was evident that, in the case of *Brasilonema* species, the 16S rRNA gene analysis (phylogeny and percentage of similarity) is not enough for species recognition and delimitation, thus stressing the necessity of a polyphasic approach with morphological, molecular and ecological data.

TABLE 2. Similarity matrix (percentages) for 16 strains comparing partial sequences of the 16S rRNA gene. Strain access numbers: (1) GQ443308, (2) HQ847567, (3) EF150855, (4) HQ847562, (5) JN676147, (6) EF490447, (7) DQ486055, (8) EF117246, (9) AJ544085, (10) AJ544079, (11) AJ544084, (12) AJ544083, (13) AF334700, (14)AB075996, (15) AY069954, (16) AB093483.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. ***Brasilonema roberti-lamii***	**99**	**99**	**99**	**98**	**97**	**97**	**97**	**96**	**94**	**94**	**95**	**95**	**93**	**93**	**93**
2. *B. angustatum* HA4187-MV1		99	99	98	98	97	97	96	95	94	95	95	93	93	93
3. *B. octagenarum* UFV-OR1			100	99	98	97	97	96	95	94	95	95	93	93	93
4. *B. octagenarum* HA4186-MV1				99	98	97	97	96	95	94	95	95	93	93	93
5. *B. tolantongensis*					98	98	98	97	95	94	95	94	93	93	93
6. *B. terrestre* CENA116						97	97	97	95	94	95	94	93	93	93
7. *B. bromeliae* SPC951							99	96	95	94	95	94	93	92	92
8. *B. sennae* CENA114								96	95	95	95	94	93	92	93
9. *Symphyonemopsis* VAPOR 1									95	94	94	94	93	93	93
10. *Mastigocladopsis repens* MORA										96	96	95	93	92	92
11. *Symphyonema* sp. 1517											99	97	93	92	92
12. *Symphyonema* sp. 1269-1												96	93	92	92
13. *Scytonema hyalinum*													93	92	91
14. *Scytonema hoffmannii* PCC7110														95	95
15. *Scytonema* sp. U-3-3															98
16. *Scytonema* sp. IAM M-262															100

Conclusions

The taxonomy of Cyanobacteria has changed considerably in recent years. The incorporation of molecular techniques has been of great help to delimit and characterize genera and higher-level taxa, allowing to understand the diversification processes within cyanobacteria, which in turn has affected their classification. At the species level, however, the use of the 16S rRNA gene is not generally sufficient by itself for species delimitation because the similarity values are often high (above 98%) and phylogenetic analyses allow already the recognition of significant differences. Nevertheless, the interpretation of such differences requires the use of other criteria (such as morphological and ecological) to provide sufficient data for species delimitation. For this reason, the best approach available to date is the use of complementary criteria (polyphasic approach).

Acknowledgements

The authors would like to thank "Programa de Apoyo a Proyectos de Investigación e Inovación Tecnológica (PAPIIT)" for supporting project No IN207709, to Dr. Michele Gold Morgan for her critical review and English revision and to the anonymous Reviewers for reviewing and commenting on the manuscript.

References

Aguiar, R., Fiore, M.F., Franco, M.W., Ventrella, M.C., Lorenzi, A.S., Vanetti, C. & Alfenas, A.C. (2008) A novel epiphytic cyanobacterial species from the genus *Brasilonema* causing damage to *Eucalyptus* leaves. *Journal of Phycology* 44: 1322–1334.
http://dx.doi.org/10.1111/j.1529-8817.2008.00584.x

Becerra-Absalón, I., Rodarte, B., Osorio, K., Alba-Lois, L., Segal-Kischinevzky, C. & Montejano, G. (2013) A new species of *Brasilonema* (Scytonemataceae, Cyanoprokaryota) from Tolantongo, Hidalgo, Central Mexico. *Fottea*, *Olomouc* 13: 25–38.

Bornet, É. & Flahault, C. (1886–1888) Revision des Nostocacées hétérocystées contenues dans les principaux herbiers de France. *Annales des Sciences Naturelles*, *Botanique*, *Trosième Série* 7: 51–129.

Bourrelly, P. & Manguin, E. (1952) *Algues d'eau douce de la Guadeloupe et dépendances. Recueillies par la Mission P. Allorge en 1936*. Société d'Edition d'Enseignement Supérieur, Paris, 281 pp.
http://dx.doi.org/10.2307/2257052

Casamatta, D.A., Gomez, S.R & Johansen, J.R. (2006) *Rexia erecta* gen. et sp. nov. and *Capsosira lowei* sp. nov., two newly described cyanobacterial taxa from the Great Smoky Mountains National Park (USA). *Hydrobiologia* 561: 13–26.
http://dx.doi.org/10.1007/s10750-005-1602-6

Fiore, M.F., Sant'Anna, C.L., Azevedo, M.T.P., Komarék, J., Kástowský, J., Sulek, J. & Lorenzi, A.S. (2007) The cyanobacterial genus *Brasilonema*, gen. nov., a molecular and phenotypic evaluation. *Journal of Phycology* 43: 789–798.
http://dx.doi.org/10.1111/j.1529-8817.2007.00376.x

Flechtner, V.R., Boyer, S.L., Johansen, J.R. & DeNoble, M.L. (2002) *Spirirestis rafaelensis* gen. et sp. nov. (Cyanophyceae), a new cyanobacterial genus from arid soils. *Nova Hedwigia* 74: 1–24.
http://dx.doi.org/10.1127/0029-5035/2002/0074-0001

García, E. (1988) Modificaciones al sistema de clasificación climático de *Köppen*. Adaptación a las condiciones de la República Mexicana. Cuarta Edición. Instituto de Geografía, UNAM,. México, D.F, 246 pp.

Geitler, L. (1932) *Cyanophyceae*. Akademische Verlagsgesellschaft, Leipzing, 1196 pp.
http://dx.doi.org/10.1038/130292a0

González-Resendiz, L., León-Tejera H., Díaz-Larrea, J., Alba-Lois, L. & Segal-Kischinevzky, C. (2013) *Hassallia littoralis sp. nov.* (Cyanobacteria, Microchaetaceae) from Mexico's marine supralittoral based on morphological and molecular evidence. *Phytotaxa* 137(1): 35–47.
http://dx.doi.org/10.11646/phytotaxa.137.1.4

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids*. *Symposium Series* 41: 95–98.

Komárek, J. (2003) Two *Camptylonemopsis* species (cyanoprokaryotes) from "Mata Atlantica" in coastal Brazil. *Preslia, Praha* 75: 223–232.

Komárek, J. (2005) The modern classification of cyanoprokaryotes (Cyanobacteria). *Oceanological and Hydrobiological studies* 34: 5–17.

Komárek, J. (2006) Cyanobacterial taxonomy: Current Problems and prospects for the integration of traditional and molecular approaches. *Algae* 21(4): 349–375.

Komárek, J. (2010) Recent changes (2008) in cyanobacteria taxonomy based on a combination of molecular background with phenotype and ecological consequences (genus and species concept). *Hydrobiologia* 639: 245–259.
http://dx.doi.org/10.1007/s10750-009-0031-3

Korelusová, J., Kaštovský, J. & Komárek, J. (2009) Heterogeneity of the cyanobacterial genus *Synechocystis* and description of a new genus, *Geminocystis*. *Journal of Phycology* 45: 928–937.
http://dx.doi.org/10.1111/j.1529-8817.2009.00701.x

Larkin, M.A., Blackshields, G., Brown. N.P., Chenna. R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. & Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947-2948.

Müller, J., Müller, K., Neinhuis, C. & Quandt, D. (2010) *PhyDE-Phylogenetic Data Editor*. Available from: http://www.Phyde.de (accessed 30/1/2014).

Neilan, B.A., Jacobs, D. & Goodman, A.E. (1995) Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the phycocyanin locus. *Applied and Environmental Microbiology* 61: 3875–3883.

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.
http://dx.doi.org/10.1099/00207713-47-3-693

Rippka, R., Waterbury, J. & Cohen-Bazire, G. (1974) A cyanobacterium which lacks thylakoids. *Archives for Microbiology* 100: 419–436.

Sant'Anna, C.L., Azevedo, M.T.P., Fiore, M.F., Lorenzi, A.S., Kaštovský, J. & Komárek, J. (2011) Subgeneric diversity of *Brasilonema* (Cyanobacteria, Scytonemataceae). *Revista Brasileira de Botânica* 34: 51–62.

Siegesmund, M.A., Johansen, J.R., Karsten, U. & Friedl, T. (2008) *Coleofasciculus* gen. nov. (Cyanobacteria): morphological and molecular criteria for revision of the genus *Microcoleus* Gomont. *Journal of Phycology* 44: 1572–1585.
http://dx.doi.org/10.1111/j.1529-8817.2008.00604.x

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731–2739.
http://dx.doi.org/10.1093/molbev/msr121

Tiwari, G.L., & Mitra, A.K. (1969) *Symphyonemopsis katniensis* gen. et sp. nov., a new member of the Myxophyceae. *Phykos* 7: 186–194.

Turicchia, S., Ventura, S., Komárkova, J. & Komárek, J. (2009) Taxonomic evaluation of cyanobacterial microflora from alkaline marshes of northern Belize. 2. Molecular and phenotype diversity of oscillatorialean genera. *Nova Hedwigia* 89: 165–200.

http://dx.doi.org/10.1127/0029-5035/2009/0089-0165
Vaccarino, M.A. & Johansen, J.R. (2011) *Scytonematopsis contorta* sp. nov. (Nostocales), a new species from the Hawaiian Islands. *Fottea* 11: 149–161.
Vaccarino, M.A. & Johansen, J.R. (2012) *Brasilonema angustatum* sp. nov. (Nostocales), a new filamentous cyanobacterial species from the Hawaiian Islands. *Journal of Phycology* 48: 1178–1186.
http://dx.doi.org/10.1111/j.1529-8817.2012.01203.x
Wilmotte A. & Golubić S. (1991) Morphological and genetic criteria in the taxonomy of Cyanophyta/Cyanobacteria. *Algological Studies* 64: 1–24.