

A new species of *Lemmermanniella* (Cyanobacteria) from the Atlantic Rainforest, Brazil¹

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ABSTRACT – (A new species of *Lemmermanniella* (Cyanobacteria) from the Atlantic Rainforest, Brazil). The Brazilian Atlantic Rainforest is a highly heterogeneous ecosystem comprising large numbers of tropical and subtropical habitats favorable to the development of cyanobacteria. Studies on cyanobacteria in this ecosystem are still rare, however, especially those involving unicellular and colonial types. The high biodiversity and endemism of this biome has been extremely impacted and fragmented, and less than 10% of its original vegetation cover remains today. We describe here a new species of a colonial cyanobacteria, *Lemmermanniella terrestris*, found on dry soils in a subtropical region of the Atlantic Rainforest in the municipality of Cananéia in southern São Paulo State, Brazil. This new taxon demonstrated all of the diacritical features of the genus *Lemmermanniella* but, unlike the other species of the genus, it was growing on the soil surface and not in an aquatic environment. A set of morphological features, including colonies composed of subcolonies, and cell dimensions, shapes and contents distinguish it from other species of the genus. Considering that species of *Lemmermanniella* are found in very distinct habitats (such as thermal and brackish waters) and that they maintain the same life cycle described for the genus in all of those environments, the morphological structures of the colonies can be used as reliable markers for identifying the genus, and its species differ primarily in relation to the habitats they occupy.

Key words - Chroococcales, *Lemmermanniella terrestris*, terrestrial habitat

INTRODUCTION

The Atlantic Rainforest originally covered most of the tropical and subtropical Brazilian coast, but is now restricted to a matrix of fragments, mainly in southern and southeastern Brazil (Rizzini 1997). This biome comprises many habitat types, each with its own distinct microclimate (Mittermeier et al. 1999). The Atlantic Rain Forest is considered as one of the five most important global hotspots for conservation due to its huge biodiversity, elevated endemism, and high degree of fragmentation (Myers et al. 2000). The Atlantic Rainforest originally covered 68% of São Paulo State, but is currently restricted to only 10.7% of its original area (SOS Mata Atlântica & Inpe 2011).

Many investigators have examined Cyanobacteria diversity in the Atlantic Rainforest, mostly in terrestrial habitats. Three new genera (Fiore et al. 2007, Sant'Anna

et al. 2010) and 27 new species (Sant'Anna et al. 1991a,b, 2007, 2010, 2011a,b, Azevedo & Sant'Anna 1994a,b, Branco et al. 1994, 2006, Azevedo & Kováčik 1996, Komárek 2003, Fiore et al. 2007, Lemes-da-Silva et al. 2010) have recently been described in different terrestrial habitats of the Atlantic Rainforest, with all three new genera and 55% of the new species belonging to Nostocales. These studies are not yet sufficient, however, to determine the full extent of cyanobacteria diversity in this biome, and there are still environments and groups that have not yet been intensively investigated.

One of the least studied groups of Cyanobacteria is composed of unicellular and colonial organisms – traditionally treated as coccoid morphotypes. Their simple cell shapes make their morphological differentiation and specific determinations very difficult and little is known about these organisms in terms of their diversity and distribution, making it impossible to construct their biogeographical profiles (Hoffmann 1996), lending importance to studies of coccoid Cyanobacteria, especially those inhabiting terrestrial environments.

The coccoid Cyanobacteria genus *Lemmermanniella* was first proposed by Geitler (1942). After its original description, however, additional representatives of *Lemmermanniella* were rarely found, and the few published records were restricted to temperate zones; populations from tropical (Azevedo et al. 1999, Komárek

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& Komárková-Legnerová 2007) and subtropical (Juárez & Wenzel 2003) sites have only recently been reported. Two species were originally described from tropical areas: *L. obesa* M.T.P.Azevedo et al., from a Brazilian brackish lagoon, and *L. uliginosa* Komárek & Komárková-Legnerová, from marshes in Belize.

In the present study a new species of *Lemmermanniella* is described found growing on soils in a subtropical area of the Atlantic Rainforest.

MATERIAL AND METHODS

The material examined was encountered on dry soil crusts (mixed with gravel) exposed to full sunlight (figure 1) in the municipality of Cananéia, São Paulo State, Brazil (25°01'15" S; 47°55'32" W). The study area was located at sea level and was surrounded by a preserved fragment of Atlantic Rainforest. The regional climate is characterized by high temperature and humidity levels, with rainy summers but no well-defined dry season (Funari et al. 1987).



Figure 1. General aspect of the soil crusts where *Lemmermanniella terrestris* sp. nov. was found. Bar = 10 cm.

The samples were gathered with a spatula, with one portion being kept dry while another was fixed in 4% formaldehyde. The dry material was rehydrated in the laboratory with distilled water (in Petri dishes) for 24 hours before being examined under a light microscope. We analyzed 20 distinct colonies and measured 10 cells from each to describe the morphological and metric features of the population. Epifluorescence microscopy was used to test for the presence of phycocyanin and chlorophyll, pigments not found in other groups of bacteria. A 1% methylene blue solution was used to stain the mucilage envelope.

RESULTS

Lemmermanniella terrestris W.A. Gama-Jr., sp. nov.
Figures 2-8

Colonies spherical to elongated, 43.7-243.7 μm in diameter, subcolonies frequently present, 10.2-16.9 μm in diameter. Mucilage inconspicuous, hyaline. Cells ellipsoidal, with pointed to rounded (rarely) apices, 2.5-7.1(8.8) μm in diameter \times 1.4-2.5 μm long, 1.7-2.8 times longer than wide, loosely arranged under the mucilage surface, rarely forming dense groups. Pseudofilaments rare, 3-5 cells long. Cell contents granulated, pale blue-green to grey. Reproduction by disintegration of the colonies or by separation of small clusters of cells from the mother colony.

Habitat: found together with other cyanobacteria (*Nostoc* – dominant, *Scytonema*, *Chroococcus*) on dry soil crusts mixed with gravel and exposed to direct sunlight.

Type locality: Brazil, municipality of Cananéia, São Paulo State (25°01'15" S; 47°55'32" W).

Diagnosis: Coloniae sphaericae ad elongatas, 43.7-243.7 μm in diam., sub-coloniae frequenter presentes, 10.2-16.9 μm diam. Mucus inconspicuus, hyalinus. Cellulae ellipsoideae, apicibus punctiformibus rare rotundatis, 2.5-7.1(8.8) \times 1.4-2.5 μm , 1.7-2.8-plo longiores quam latiores, laxe dispersae in involucri mucoso, rare densas catervas formantes. Pseudofilamenta rara, 3-5 cellululis praedita. Contentum cellulare granulare, pallide glaucum ad cinereum. Multiplication a coloniis ruptis vel a parvis fasciculis mucosis cellularibus ex colonia matrice.

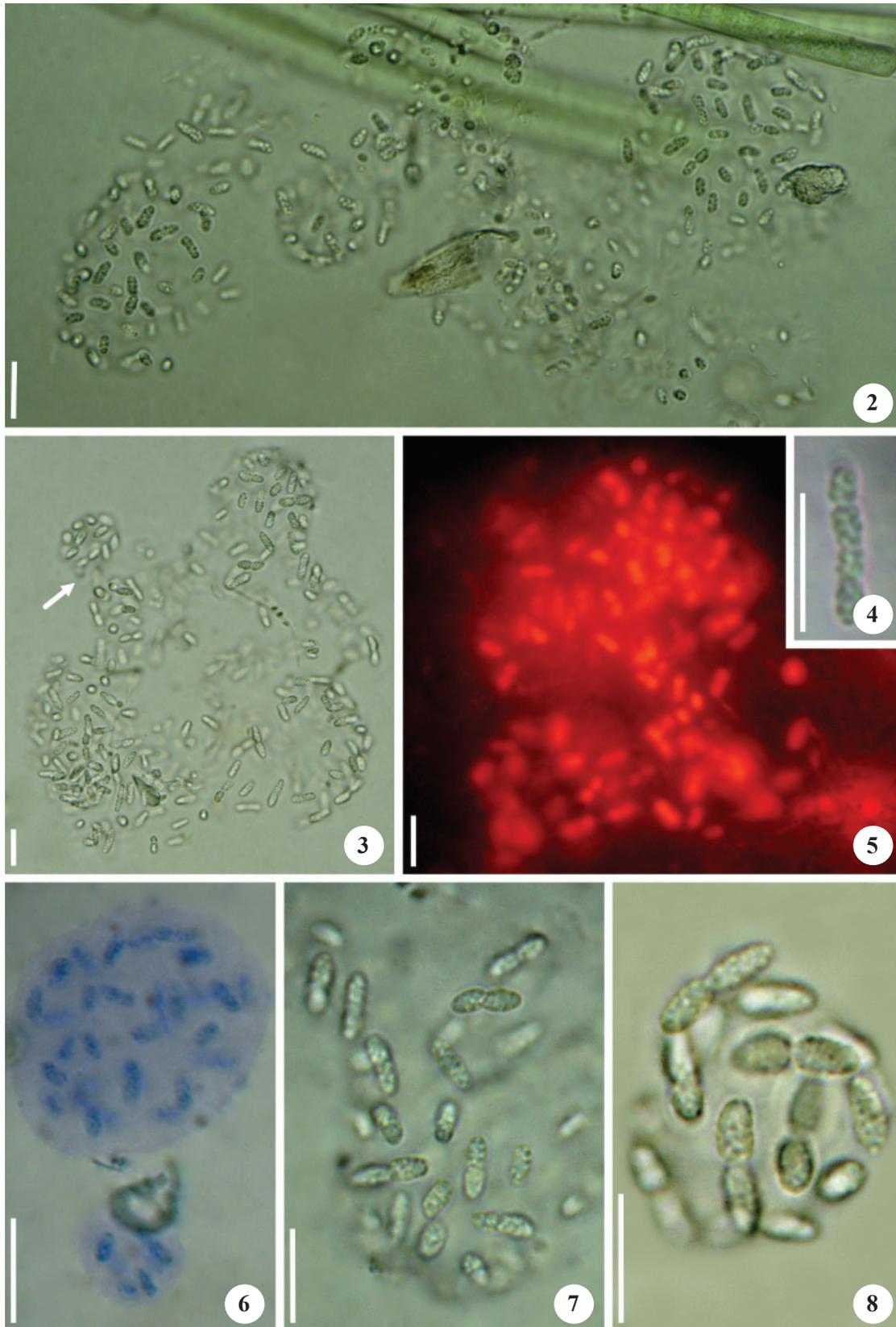
Typus (Holotypus): Exsiccatum SP 401446; dep. in herbarium (SP) Instituti Botanicae, SP, Brazil; icona typica figurae nostrae 2-3.

Habitatio: crustae soli seci glarea mixtae directe sub luce solari expositae.

Locus classicus: Brasilia, provincia São Paulo, in comitatu Cananéia (25°01'15" S; 47°55'32" W).

DISCUSSION

The genus *Lemmermanniella* has been problematic ever since first being proposed by Geitler (1942). Due to the lack of precision in the descriptions of the type species and nomenclatural conflicts, Hindák (1985) undertook a revision of this genus and reestablished its diacritical morphological features: 1) hollow colonies, and 2) cells with their long axes parallel to the colony surface. These characteristics make this genus distinct from *Aphanothece*, and *Epigloeosphaera*, respectively, and match those found in the population studied here.



Figures 2-8. *Lemmermanniella terrestris* sp. nov. 2. General aspect of a colony, showing subcolonies. 3. Mature colony liberating a young colony (arrow). 4. Detail of a pseudofilament. 5. Colony aspect under epifluorescence microscopy. 6. Colony stained by methylene blue. 7. Detail of disintegrating colony. 8. Young colony. Bar = 10 μm.

Lemmermanniella is also similar to the genus *Rhabdogloea* in terms of cell shape, cell division in one plane, and the presence of pseudofilaments. However, the arrangements of their cells in the mucilage matrices are completely different (Komárek & Anagnostidis 1998), as the cells of *Rhabdogloea* species are distributed throughout the mucilaginous colony while *Lemmermanniella* species always have their cells arranged in a single layer beneath the mucilage surface. Hindák (1985) also highlighted the fact that *Lemmermanniella* species always show cells in a single layer at the periphery of the colonial mucilage, with pseudofilaments rarely been observed – characteristics that correspond perfectly to the material gathered in the Atlantic Rainforest.

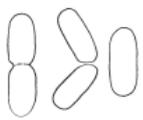
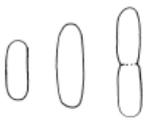
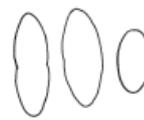
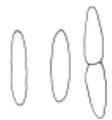
The newly described morphotype from the Atlantic Rainforest is distinguishable from other closely related species (table 1) mainly by the following features: 1) occurrence in a very specific habitat (soil) distinct from the aquatic habitats of all of the other species; 2) new colonies constantly being formed from subcolonies, which is only observed in the other species when they are reproducing; 3) presence of pseudofilaments; and 4) their cell shapes, dimensions and contents.

Difficulties encountered in obtaining successful cultures of many cyanobacteria have made molecular

studies of this group to be very difficult. Johansen & Casamatta (2005) suggested that new taxa could be described based solely on their morphological features if the new morphotypes were distinguishable from existing species in terms of specific ecological and morphological characteristics. The validity of these criteria was supported by a number of recent works using molecular techniques that showed clear differentiations of ecologically distinct cyanobacterial species (Flechtner et al. 2002, Casamatta et al. 2006, Řeháková et al. 2007, Taton et al. 2010). Likewise Gaylarde et al. (2005) demonstrated that species from aerial habitats are genetically distinct from aquatic species of the same genus. Based on these examples, we can conclude that the features presented by *Lemmermanniella terrestris* sp. nov. are sufficient to distinguish it from other *Lemmermanniella* species.

All species of *Lemmermanniella* share the distinct morphology described for the type species, in spite of their occurrence in very different habitats such as marshes (*L. uliginosa*), brackish waters (*L. obesa*), pools with thermal water inflows (*L. flexa* Hindák), and terrestrial sites in the Atlantic Rainforest (*L. terrestris*). These results show that colony structure is an important diacritical feature at the generic level, and that species differ mainly in relation to their habitats.

Table 1. Comparison of *Lemmermanniella terrestris* sp. nov. with its most closely related species.

	<i>L. pallida</i> Lemmermann & Geitler (Komárek & Anagnostidis 1998)	<i>L. uliginosa</i> Komárek & Komárková-Legnerová (Komárek & Komárková-Legnerová 2007)	<i>L. obesa</i> Azevedo et al. (Azevedo et al. 1999)	<i>L. terrestris</i> sp. nov.
Cell arrangements	irregular, more or less densely distributed in the surface layer	irregular in one subsurface layer	irregular in one layer (sometimes two layers)	irregular, loosely dispersed under the mucilage envelope
Sub-colonies	present during reproduction	Present during reproduction	present during reproduction	constantly present
Pseudofilaments	absent	absent	absent	present
Cell diameter (µm)	0.5-1.6	(2.5)3.0-3.4	2.6-3.9	1.4-2.5
Cell length (µm)	(0.7)1-3.7(4.7)	(4.6)6.2-10.0(12.4)	5-6.8	2.5-7.1(8.8)
Length/Diameter	–	–	1.7	2.4-3.1
Cell contents	homogeneous, pale blue-green	finely granular, pale blue-green	homogeneous, dark blue-green	granular, pale blue-green to grey
Habitat	plankton in lakes	benthic in alkaline marshes	plankton in brackish waters	terrestrial on dry soil crusts
Occurrence	Baltic area	Belize	Brazil	Brazil
Cell forms				

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