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Floristic composition of cyanobacteria in a neotropical, eutrophic reservoir

Hilda Maria Palacio[1] · John Jairo Ramírez[2] · Ricardo Omar Echenique[3] · Jaime Alberto Palacio[1] · Célia Leite Sant'anna[4]

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Abstract In order to know the flora of planktonic cyanobacteria, 24 water samples (May 2010–October 2013) from the Riogrande II Reservoir, Colombia (75°32′30′W–75°26′10′W and 6°33′50′N–6°28′07′N) were studied. The reservoir provides water to 1.4 million inhabitants in Medellin, Colombia (40 % of the total population). Among the cyanobacteria, we identified 11 morphospecies belonging to three families of the order Chroococcales (Merismopediaceae (2 species), Microcystaceae (3) and Synechococcaceae (1)), one of Oscillatoriales (Pseudanabaenaceae, 1 species) and one of Nostocales (Nostocaceae, 4 species). The genera with the highest number of taxa were *Microcystis* Kützing ex Lemmermann and *Dolichospermum* (Ralfs ex Bornet et Flahault) Wacklin, Hoffmann & Komárek, both known to form dense blooms, many of them toxic. That is why the aim of this research is to understand the cyanobacterial species richness in the reservoir.

✉ Hilda Maria Palacio
hildapalacio@gmail.com

[1] Grupo de Investigación en Gestión y Modelación Ambiental, GAIA, Universidad de Antioquia, Cl. 62 No. 52-59, Medellín, Antioquia, Colombia

[2] Grupo de Limnología Básica y Experimental, Biología y Taxonomía Marina, LimnoBase-BiotaMar, Universidad de Antioquia, Medellín, Antioquia, Colombia

[3] División Ficología. Dr. "Sebastián Guarrera", Facultad de Ciencias Naturales y Museo, (UNLP) y CIC-BA, La Plata, Argentina

[4] Núcleo de Pesquisa em Ficologia, Instituto de Botânica, São Paulo, Brasil

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Introduction

The rise in human population promotes increased nutrient loads provoking the degradation of limnetic ecosystem health. This situation favours the proliferation of cyanobacterial blooms which tend to be harmful both to the environment and to humans (O'Neil et al. 2012; Reichwaldt and Ghadouani 2012).

The cyanobacteria are a group of gram-negative, aerobic and photoautotrophic bacteria. Morphologically, they have not changed significantly over evolution (about 2700 million years ago during the Precambrian). Their remarkable adaptability to environmental conditions and their ability to tolerate wide ranges of environmental conditions (Whitton and Potts 2002) have allowed them to colonize many habitats. Even though they are found in many different environments, cyanobacteria are mainly present in aquatic ecosystems as part of the phytoplankton and periphyton, playing an important role as primary producers.

Numerous studies have shown a direct and positive relationship among the increase in the availability of nutrients (eutrophication), global warming and cyanobacterial density (Mur et al. 1999; Oliver and Ganf 2000; Huisman and Hulot 2005; Aubriot et al. 2009; de Oliveira Fernandes et al. 2009 Ferrari et al. 2011; Gonzáles-Piana et al. 2011; Sinha et al. 2012; El-Shehawy et al. 2012; Reichwaldt and Ghadouani 2012). Colombia is no exception to this condition and therefore, the presence of cyanobacteria has disturbingly increased in recent years. However, there are some works on this group in the country, though they have been focused in limnological aspects; except for the publication of Ramírez (1996), we do not know of any other study of taxonomic nature and therefore, the available information on the taxonomy of cyanobacteria in Colombia is sparse. Most studies that report cyanobacteria are theses and dissertations and are ecologically oriented, thus few peer-reviewed publications exist.

In a review for South America, Dörr et al. (2010) note that there are no references on the findings of microcystins in Colombia. However, Bula (1985) described that a bloom of *Microcystis aeruginosa* (Kützing) Lemmermann was associated with the death of fish and other wild and domestic animals in the Grande de Santa Marta Swamp (Santa Marta, Colombia). In the same year, Escobar and Manjarres (1985) reported a bloom of *M. aeruginosa* and *Nostoc commune* Vaucher ex Bornet et Flahault associated to massive fish, domestic and wild animals kills in San Rafael de Buenavista Swamp (Magdalena, Colombia).

Again, in the Grande de Santa Marta Swamp more than 20 tons of fish were killed in 1994 (Mancera and Vidal 1994), possibly caused by a cyanobacterial bloom.

Unpublished events of massive growth of these microorganisms were also mentioned during the early stage of reservoir operation, dominated by the genera *Microcystis* and *Cylindrospermopsis* Seenayya et Subba Raju (Codd et al. 2005a). In the Riogrande II Reservoir, a cyanobacterial bloom composed of *Microcystis aeruginosa*, *M. wesenbergii* (Komárek) Komárek in Kondrateva, *M. protocystis* Crow, *Radiocystis* sp. and *Anabaena* sp. was reported, between 2006 and 2007, after a sharp drop in water levels followed by a rapid rise. The toxicity results with *Daphnia pulex* Linnaeus, mouse and ELISA tests on the water and cyanobacterial extract confirmed microcystins (Correa 2008). Finally, in the old delta of Río Sinu (Córdoba, Colombia), between December 2008 and May 2010, Galeano and Villalobos (2010) found an abundance of cf. *Planktolyngbya limnetica* (Lemmermann) Komárková-Lergerová et Cronberg with positive intracellular toxicity in some samples. Also, associated with the discharge of wastewater into the Río Cesar (César, Colombia), Rivera and Gómez (2010) observed that *Phormidium* sp., *Oscillatoria* sp. and *Pseudanabaena* sp. were dominant.

This study was conducted in Riogrande II Reservoir, which provides water to 1.4 million inhabitants of Medellín (40 % of the total population). The watershed has a high degree of human intervention with significant source of domestic waste and agricultural origin, and the tributaries go into the reservoir. Consequently, the water body is highly eutrophic, thus abundant occurrence of cyanobacteria is expected, that is why the aim of this research is to survey the cyanobacterial species richness in the Riogrande II Reservoir.

Materials and methods

The Riogrande II Reservoir is located between 75°32′30 ‘W–75°26′10′ W and 6°33′50 ′N–6°28′07′ ′N, 2200 m in the central Andes, northeastern Colombia. This water body has a capacity of 253 Mm^3, a maximum depth of 47.2 m in normal operation and floods an area of approximately 1100 ha. It was formed by damming the waters of the Grande and Chico Rivers and Las Ánimas Creek by a dam located 1.7 km downstream of the confluence of these rivers and became operational in 1991 (Fig. 1).

Between March 2010 and November 2013, 24 field campaigns were conducted in eight stations of the reservoir, considering the more salient features of each of the major tributaries and trying to cover all the parts of the reservoir (Fig. 1). At each station, two samples were collected using a plankton net (20 µm mesh), for 5 min. This material was fixed “in situ” with a solution of 4 % formaldehyde.

Qualitative analysis of cyanobacteria, measurements and photographs were undertaken using a binocular light microscope (Zeiss Axioplan-2) equipped with a Zeiss Axiocam MRC digital camera and AxioVision software version 4.6 Carl Zeiss. During the qualitative analysis, morphological and morphometric characteristics of the taxa were observed. Measurements of 30 individuals of taxonomic interest were performed, whenever possible, in order to establish the morphometric variability of the population. Measurements of taxonomic interest are represented by the maximum and minimum limits of morphometric taxa identified. Outliers, as they do not represent important sequence in the maximum and minimum limits, are included in parentheses. India ink was used to observe mucilage and sheath in colonial and filamentous forms, respectively. Intrageneric taxonomic identification was based on standard literature, for each group of cyanobacteria. The classification system adopted follows Hoffmann et al. (2005).

Results

Below we present the dichotomous key for the taxa found in the Riogrande II Reservoir.

(1a) Colonial form……2
(1b) Filamentous form……7
(2a) Aerotopes absent, cells up to 1.2 µm wide……*Aphanocapsa delicatissima*
(2b) Aerotopes present, cells wider than 1.2 µm……3
(3a) Cells oval arranged at the periphery of the colony……*Woronichinia naegeliana*
(3b) Cells spherical distributed throughout the colony……4
(4a) Cells organized from the middle forming more/less uniseriate rays……*Radiocystis fernandoi*
(4b) Cells organized disorderly in the mucilage……5
(5a) Mucilage conspicuous with a visible edge……*Microcystis wesenbergii*
(5b) Mucilage inconspicuous, diffluent……6
(6a) Cells disordered and scattered within the mucilage, sometimes with an individual envelope……*Microcystis protocystis*
(6b) Cells close to each other, concentrated in the centre of the colony……*Microcystis aeruginosa*
(7a) Trichomes homocyted……*Pseudanabaena mucicola*
(7b) Trichomes heterocyted……8
(8a) Trichomes straight or slightly curved……*Dolichospermum* cf. *planctonicum*
(8b) Trichomes coiled……9
(9a) Akinetes spherical beside heterocyte……*Sphaerospermopsis torques-reginae*
(9b) Akinetes kidney-shaped on both sides of the heterocyte……*Dolichospermum lemmermannii*

Order Chroococcales

Aphanocapsa delicatissima W.West and G.S.West 1912

Colonies spherical or irregular, mucilage hyaline. Cells spherical, without aerotopes, 0.8–1.2 µm diameter, irregularly distributed in the mucilage (Fig. 2).

Toxin: Although there is no evidence that this species produces cyanotoxins, other species of the genus *Aphanocapsa* produce microcystins (Brena and Bonilla 2009).

Woronichinia naegeliana (Unger) Elenkin 1933
Basionym: *Coelosphaerium naegelianum* Unger 1854

Colonies spherical or irregular, mucilage colourless, wide. Cells oval, with aerotopos, 4–6 µm long, 2.5–4.2 µm wide, arranged radially at the periphery of the colony, disposed at the end of thin or thick stalks in radial from the centre of the colony (Figs. 3–6).

Remarks: The cell shape differs slightly from the description of Komárek and Anagnostidis (1998) and those described for Brazil (Sant'Anna et al. 2012.). In the material found in the reservoir it is common to find single cells (Fig. 6).

Toxin: This species has been mentioned as microcystins producer (Cirés and del Corral 2011; Echenique and Aguilera 2011; Carvalho et al. 2013).

Family microcystaceae

Microcystis aeruginosa (Kützing) Lemmermann 1907
Basionym: *Micraloa aeruginosa* Kützing 1833

Colonies more or less rounded, sometimes clathrate, mucilage wide, hyaline and diffluent. Cells spherical, 4–5.1 µm wide, with aerotopes, close to each other, concentrated towards the centre of the colony (Figs. 7–10).

Toxin: Known as a microcystin-producer (Sivonen and Jones 1999; Codd et al. 2005b; Chorus 2012).

Remarks: Can be confused with *Radiocystis* by the size and shape of the cells, but the cells never have radial arrangement as in *Radiocystis*.

Microcystis protocystis Crow 1923
Colonies irregular, mucilage hyaline, inconspicuous, diffluent. Cells spherical, 5.3–8.6 µm wide, dispersed throughout, scattered disorderly in the mucilage, sometimes having an individual mucilaginous envelope (Figs. 11– 14).

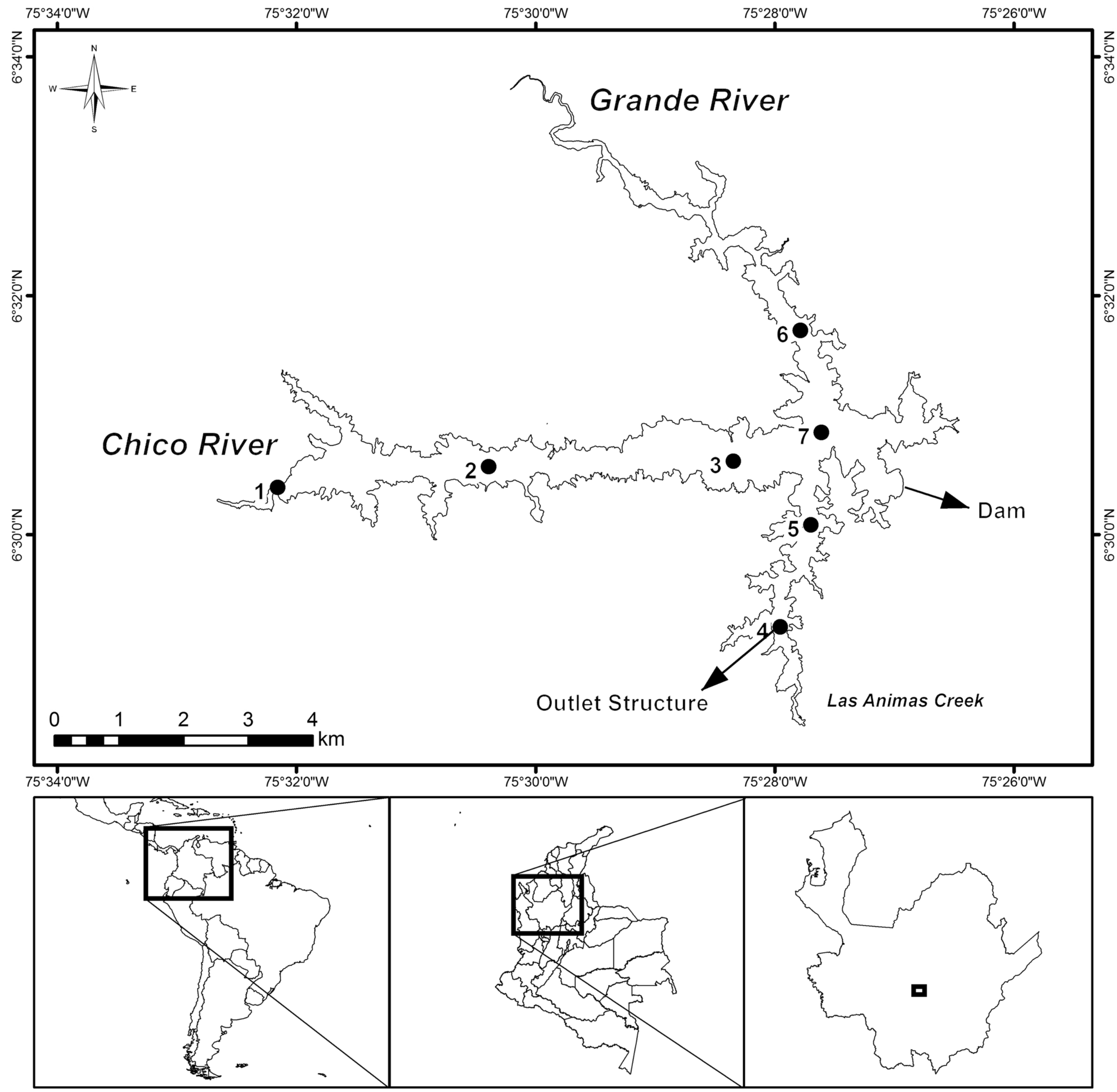


Fig. 1 Location of Riogrande II Reservoir and sampling stations

Toxin: Known to produce microcystins (Vidal et al. 2009).

Microcystis wesenbergii Komárek 1968
Basionym: *Diplocystis wesenbergii* Komárek 1958

Colonies round, elongated or irregular, sometimes composed of sub-colonies. Mucilage colourless, conspicuous outline. Spherical cells, 3.6–7 µm diameter, scattered disorderly in the mucilaginous matrix (Figs. 15–18).

Toxin: This species has been found producing microcystins (Kardinaal and Visser 2005; Vidal et al. 2009).

Family Synechococcaceae
Radiocystis fernandoi Komárek and Komárková-Legnerová 1993

Colonies irregular or round. Mucilage colourless, wide, diffluent. Cells spherical with aerotopes, 6–8.6 µm wide, organized from the centre to radially outward, forming

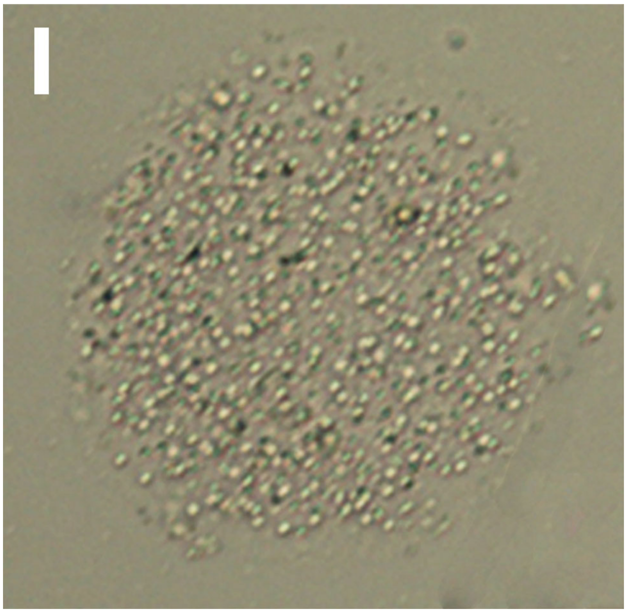


Fig. 2 *Aphanocapsa delicatissima*. General aspect of the colony. *Bar* 10 µm

rows of cells to the periphery of the colony, without going to the extremes of the same (Figs. 19–22).

Toxin: This species has been found producing microcystins (Vieira et al. 2003).

Order Oscillatoriales

Family Pseudanabaenaceae

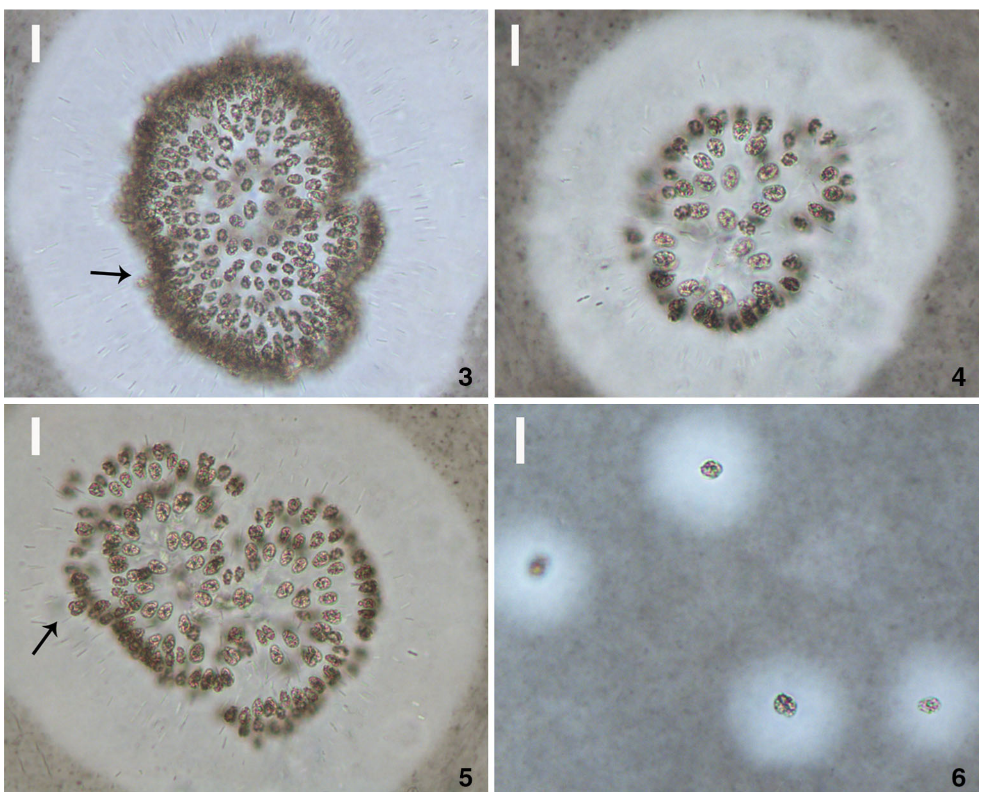
Pseudanabaena mucicola (Naumann and Hubber- Pestalozzi) Bourrelly 1970

Basionym: *Phormidium mucicola* Naumann and Huber-Pestalozzi 1929.

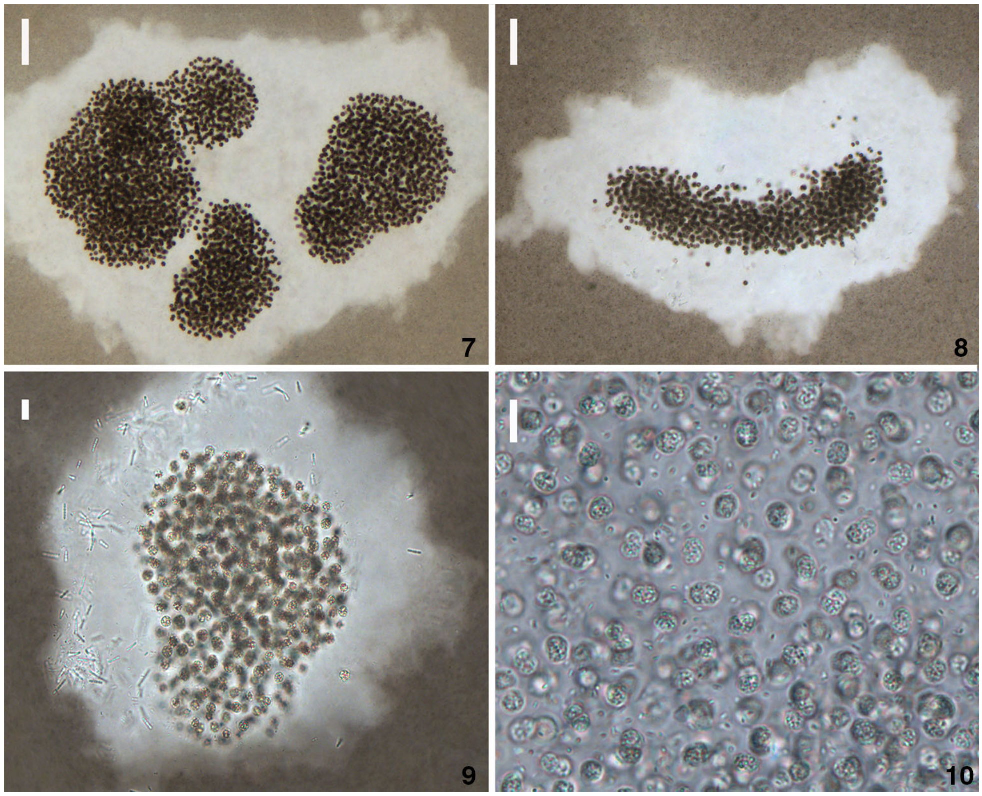
Trichomes solitary, straight or slightly curved and short (3–6 cells), regularly 6.5–13 long, 1.5–1.9 wide. Cells usually isodiametric; apical cell cylindrical with conical ends (Figs. 23–25).

Toxin: This species has been found producing microcystins (Vidal et al. 2009).

Remarks: This species is characterized by growing in the mucilage of other cyanobacteria, and in the reservoir was found in the mucilage of *Microcystis aeruginosa* and *M. wesenbergii*.



Figs. 3–6 *Woronichinia naegeliana*. **3–5** General aspect of the colonies. **3** The *arrow* indicated the radial aspect of the cells **5** A cell leaving the colony (*arrow*). **6** Solitary cells. *Bar* 10 µm



Figs. 7–10 *Microcystis aeruginosa*. **7–9** Morphological variation of colonies with wide mucilage and cell arrangement of in the center. **10** Details cells with aerotopes. *Bar* 50 µm (**7**, **8**); 10 µm (**9**, **10**)

Order: Nostocales
Family: Nostocaceae
Dolichospermum lemmermannii (Richter) Wacklin, Hoffmann and Komárek 2009.
Basionym: *Anabaena lemmermannii* Richter 1903.

Trichomes solitary, irregularly spiral without an apparent mucilage. Cells barrel-shaped from 4.4 to 8.2 µm s long, 4.1–5.8 µm wide. Heterocytes more or less spherical, 4.6–7.6 µm long, 3.9–5.6 µm wide. Akinetes kidney-shaped with heterocytes on both sides of 22–29 (35?) µm long, 6.6–9.2 µm wide (Figs. 26–28).

Toxin: species mentioned as producer of anatoxins (Onodera et al. 1997; Ruge Holte et al. 1998).

Remarks: It is commonly found in phytoplankton of eutrophic reservoirs in temperate zones (Ruge Holte et al. 1998; Olli et al. 2005; Komárek and Zapomělová 2007; Täuscher 2011; Cărăuş 2012). It has not been reported in neotropical regions.

Dolichospermum cf. ***planctonicum*** (Brunnthaler) Walcklin, Hoffmann and Komárek 2009
Basionym: *Anabaena planctonica* Brunnthaler 1903.

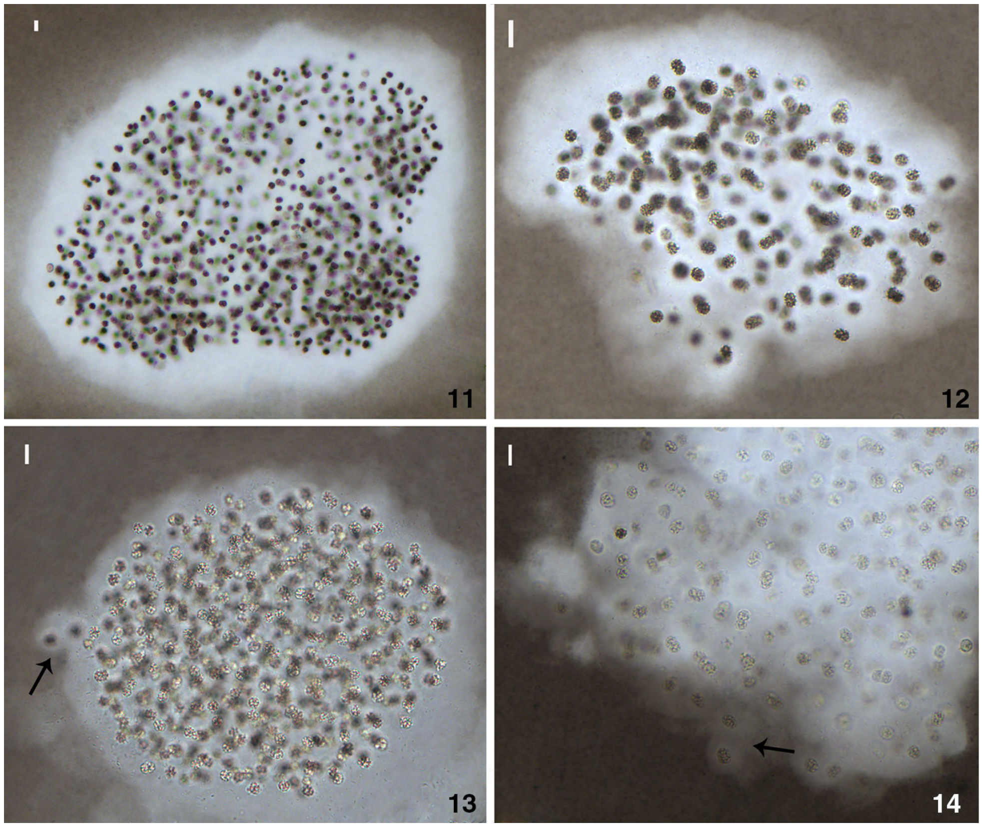
Trichomes solitary, straight or slightly curved, with a indistinct mucilage, mean of 38 µm wide. Cells barrel-shaped, 6.3–10.3 µm long, 8.2–10 µm wide. Heterocytes spherical 7.1-10.5 µm wide. Akinetes oval, 11-33 µm long, 7-15 µm wide, adjacent to heterocytes (Figs. 29–33).

Toxin: anatoxin and cylindrospermopsin (Sivonen & Jones, 1999).

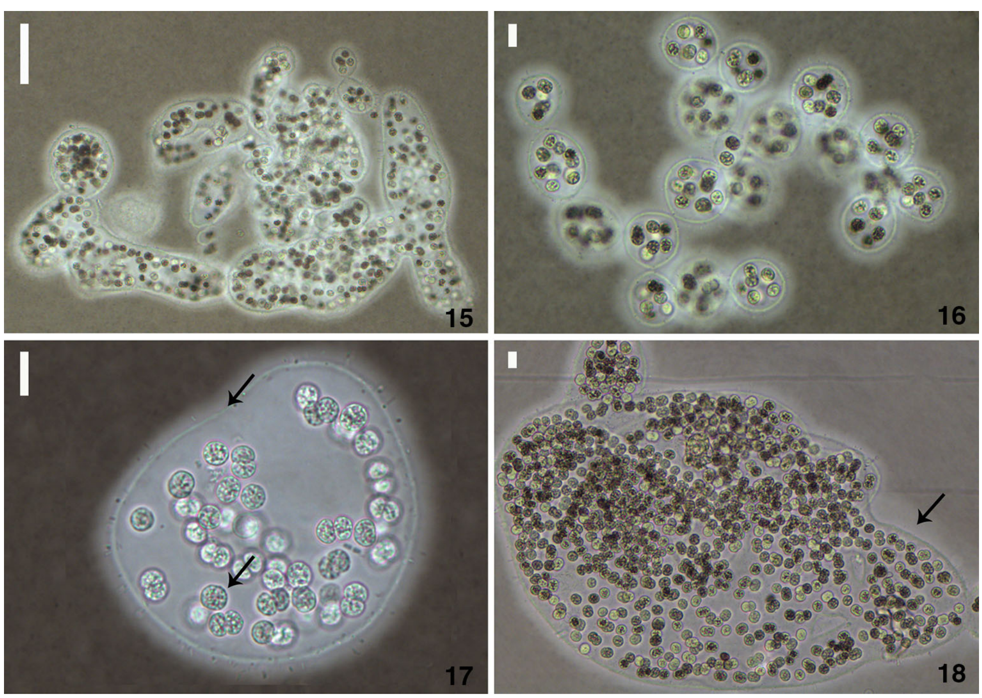
Remarks: *D. planctonicum* has as diagnostic character, the position of akinetes distant from the heterocytes. However, the population found in the reservoir has akinetes beside heterocytes. But it fits within the remaining characters.

Dolichospermum sp
Trichomes solitary or matted, irregularly coiled. Mucilage wide, colourless. Cells barrel-shaped, 4.4–7.6 µm long, 4.3–6.3 µm wide. Heterocytes intercalary, oval, 4.2–5.6 µm long, 4.4–5.1 wide (Figs. 34–37).

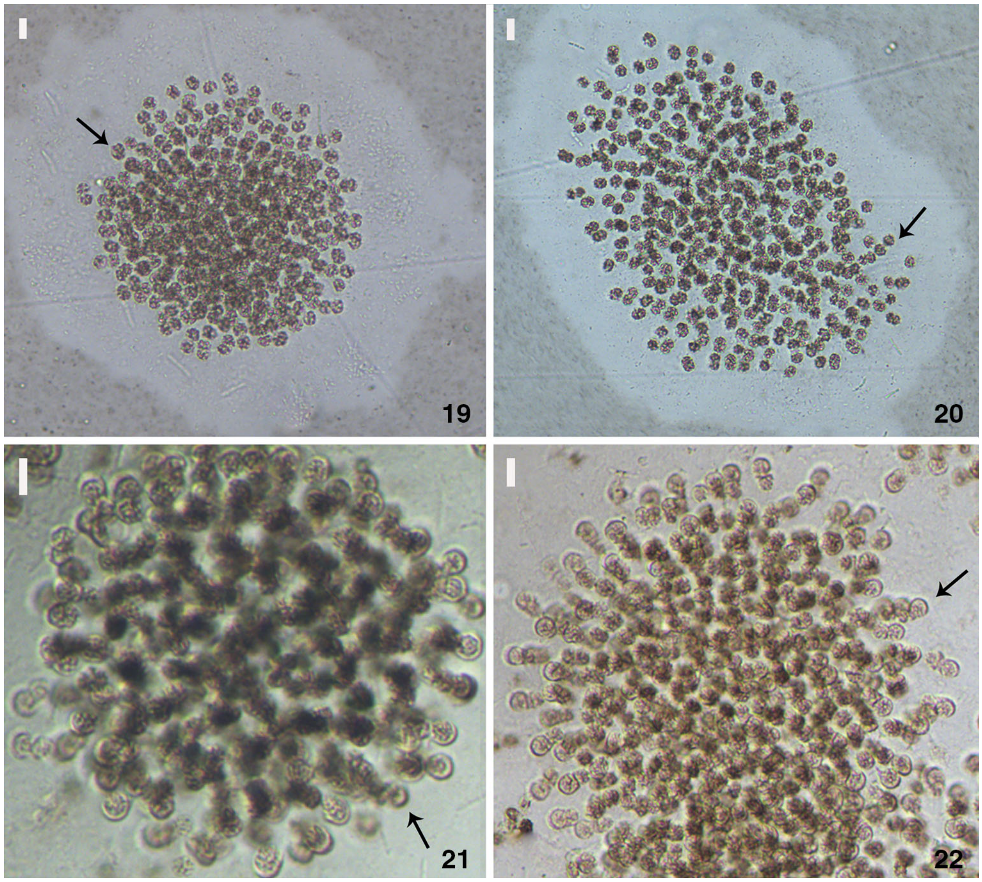
Remarks: Akinetes were not observed in this population, which are an essential character in determining species in the genus *Dolichospermum*. According to the cell size and the spiral of the coils, this population corresponds to *D. flos-aquae* (Brébisson ex Bornet et Flahault) Walcklin, Hoffmann & Komárek. However, according to



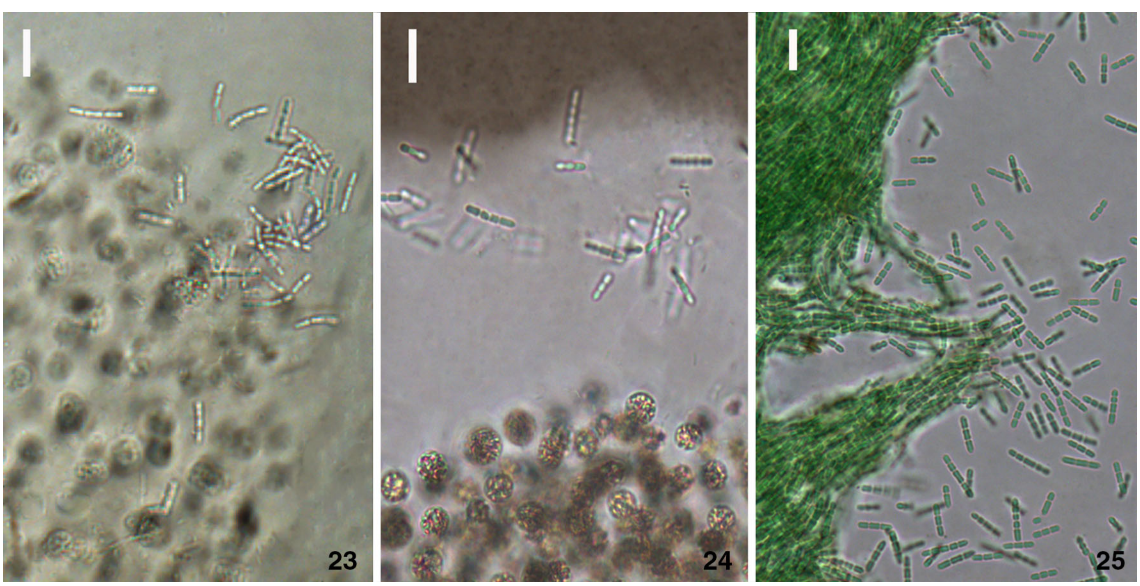
Figs. 11–14 *Microcystis protocystis*. **11**, **12** General aspect of the colonies. **13**, **14** *Arrows* indicate cells with individual mucilaginous envelopes. *Bar* 10μm



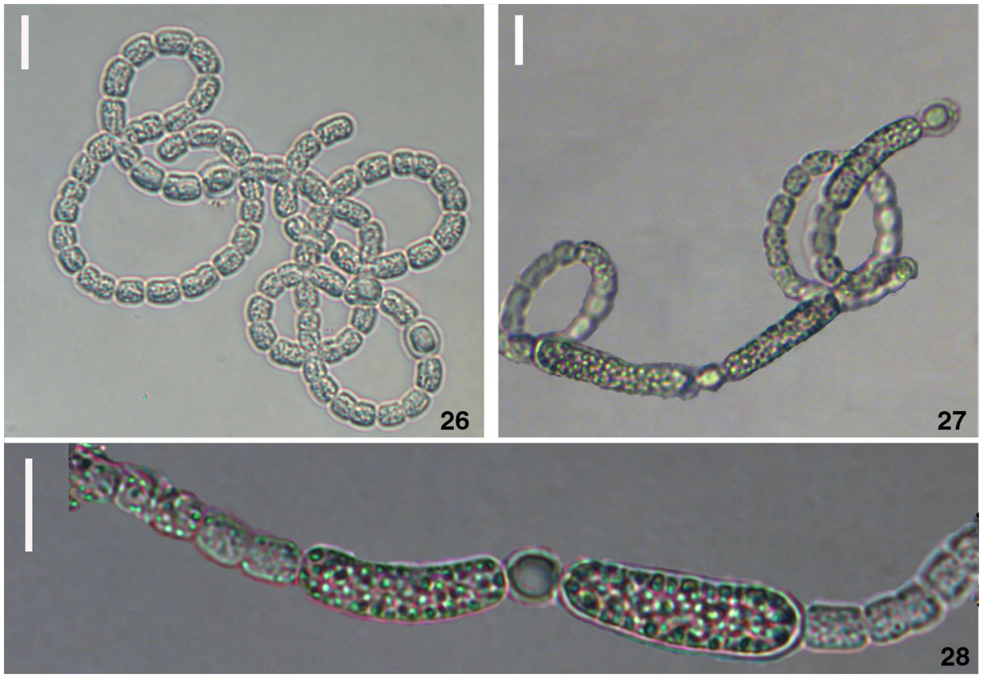
Figs. 15–18 *Microcystis wesenbergii*. **15**–**18** Morphological variation of the colonies. **17** Details of the cells showing the aerotopes. **17**, **18** Firm mucilage around the colony (*arrows*). *Bar* 50 μm (**15**); 10 μm (**16**–**18**)



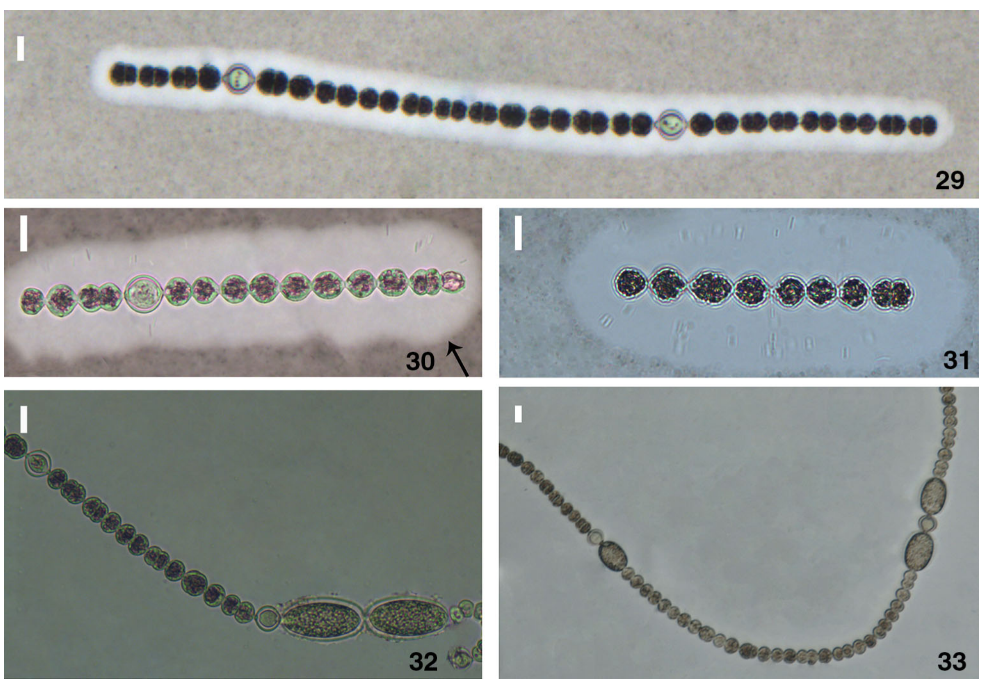
Figs. 19–22 *Radiocystis fernandoi*. **19**, **20** General aspect of the colonies; observe the wide mucilage. **21**, **22** Form and disposition of the cells. The rows of cells are shown (*arrows*). *Bar* 10 µm



Figs. 23–25 *Pseudanabaena mucicola*. **23**, **24** Filaments of *P. mucicola* in the mucilage of *Microcystis aeruginosa*. **25** Individuals in culture. *Bar* 10 µm

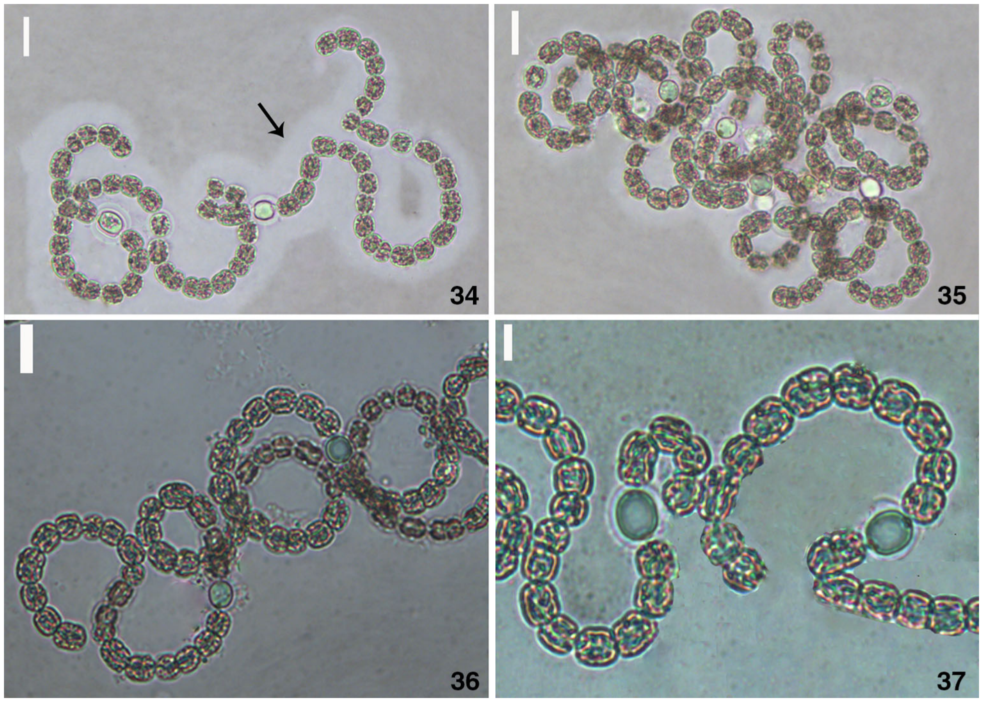


Figs. 26–28 *Dolichospermum lemmermannii*. **26** General aspect of irregularly coiled trichomes. **27** Shape and position of akinetes in the filament. **28** Detail of akinetes on both sides of the heterocyte. *Bar* 10 μm

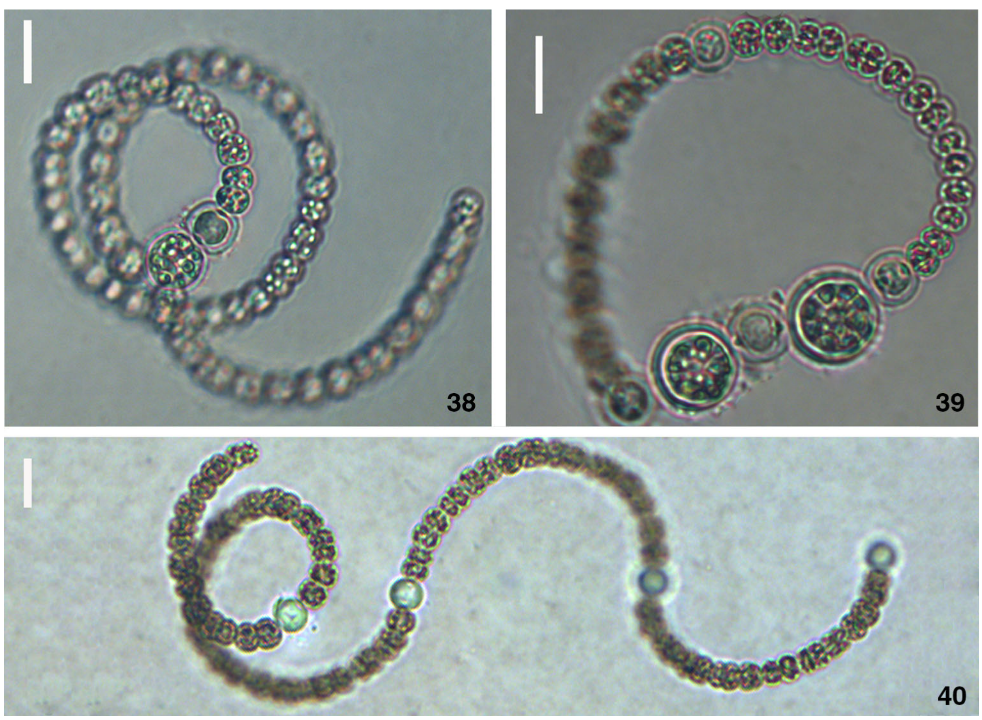


Figs. 29–33 *Dolichospermum* cf. *planctonicum*. **29** General view of a trichome. **30** *Arrow* points to the wide mucilage. **31** Hormogonium. **32** Detail of akinetes in pairs. **33** Location of akinetes adjacent to the heterocyte. *Bar* 10 μm

Komárková-Legnerová and Eloranta (1992), the heterocytes of *D. flos-aquae* are spherical or slightly ellipsoidal and this morphotype has clearly oval heterocytes. The morphotype found is also very similar to *D. spiroides* (Klebahn) Walcklin, Hoffmann & Komárek, but the trichomes of the latter species are always regularly coiled and the vegetative cells are larger (6.5–8 μm in diameter) (Komárková-Legnerová and Eloranta 1992).



Figs. 34–37 *Dolichospermum* sp. **34** The *arrow* indicates the mucilage. **35**, **36** General aspect of the irregularly coiled trichomes. **37** Detail of the heterocytes and cells. *Bar* 10 µm (**34**–**36**); 5 µm (**37**)



Figs. 38–40 *Sphaerospermopsis torques-reginae*. **38** General aspect of a coiled trichome with a spherical akinete adjacent to a heterocyte. **39** Akinetes beside heterocyte. **40** Trichome with heterocyte. *Bar* 10 µm

Sphaerospermopsis torques-reginae (Komárek) Werner, Laughinghouse IV, Fiore & Sant'Anna 2012
Basionym: *Anabaena torques-reginae* Komárek 1984.

Trichomes solitary. Cells rounded, 3.5–6.7 (7.4) µm long, 4.3–7.4 µm wide. Heterocytes spherical, 5.5–9.7 (11) µm in diameter. Akinetes spherical always adjacent to one or both sides of the heterocyte (6.2) 7.0–11.1 μm diameter (Figs. 38–40).

Toxin: anatoxin (Dörr et al. 2010; Werner et al. 2012).

Remarks: The species was originally described as *Anabaena torques-reginae* Komárek (1984) and further reclassified by Werner et al. (2012) based on morphological, molecular and phylogenetic characteristics of South American populations, including one from the municipality of La Loma de Calentura (Cesar, Colombia).

Conclusions

We identified 11 morphospecies in the reservoir corresponding to Cyanobacteria, belonging to the orders: Chroococcales—Merismopediaceae (2), Microcystaceae (3), Synechococcaceae (1); Oscillatoriales—Pseudanabaenaceae (1) and Nostocales—Nostocaceae (4). The genera with the highest number of taxa were *Microcystis* (3) and *Dolichospermum* (3), both known for being responsible for forming dense blooms, many of them toxic. The species recordered in this study are potentially toxic and all the genera identified are reported as including cyanotoxin-producing species.

This is the first time *Dolichospermum lemmermannii* is reported for the neotropics. Based on the available information, *Aphanocapsa delicatissima, Pseudanabaena mucicola* and *Dolichospermum* cf. *planctonicum* are the first records in Colombia. One taxa could not be identified to species, because some of the diacritical features were not observed during our study (*Dolichospermum* sp.). Additionally, some of the taxa collected during the study showed substantial morphological differences from the descriptions in the literature (*Dolichospermum* cf. *planctonica*).

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