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Distribution and morphological variation of *Aphanizomenon platensis* (Nostocales, Cyanobacteria) from Corrientes Province (Argentina)

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Abstract – During the last ten years, water blooms of *Aphanizomenon* have been observed in aquatic environments of the subtropical region of Argentina. These blooms have been observed to present taxonomic characteristics similar to those of *A. platensis*, a species originally described by Seckt in the Delta of Paraná River, a region of temperate climate, in 1921. Water blooms of *Aphanizomenon* have been observed in aquatic environments of Corrientes Province, a region of Argentina with subtropical climate, during the last ten years. Considering these aspects, we analysed the morphological variation of *A. platensis* in two water bodies located in the northwest of Corrientes province (27° 29' S; 58° 45' W). Our description includes characteristics of the akinetes, which were not originally found by Seckt. The revision of this taxon is important because the species of *Aphanizomenon* are potentially toxic, invasive, and bloom formers. Our study confirms the existence of this morphospecies almost 90 years after it was first described. We further describe the whole species and extend its distribution to the subtropical region, in environments related to the hydrographic net of the Rio de la Plata Basin. Taking into account the diacritic characters analyzed, we may conclude that *A. platensis* is a polymorphic species with highly variable cell dimensions, probably due to variations in the environmental conditions.

***Aphanizomenon* / Cyanobacteria-Cyanophyta / morphological variation / northeast of Argentina / Rio de la Plata Basin / water-bloom**

Résumé – Distribution et variation morphologique d'*Aphanizomenon platensis* (Nostocales, Cyanobactéries) de la province de Corrientes (Argentine). Au cours des dix dernières années, des fleurs d'eau d'*Aphanizomenon* ont été observées dans les milieux aquatiques de la région subtropicale de l'Argentine, présentant les caractéristiques taxonomiques du taxon décrit par Seckt (1921) comme *A. platensis.* Cette espèce a été observée dans le delta du fleuve Paraná, une région de climat tempéré. Compte tenu de ces aspects, dans cette étude, nous avons analysé la variation morphologique de *A. platensis* dans deux masses d'eau situées dans le nord-ouest de la province de Corrientes (27° 29' S et 58° 45' O). La description comprend les caractéristiques de akinètes, qui n'avaient pas initialement été trouvées par Seckt. La révision de ce taxon est importante, car les espèces d'*Aphanizomenon* sont potentiellement toxiques, envahissantes, et peuvent former des fleurs d'eau. Notre étude confirme l'existence de cette espèce morphologique après presque 90 ans. Nous avons en

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outre pu décrire cette espèce et étendre sa distribution à la région subtropicale, dans des environnements liés au réseau hydrographique du bassin du Rio de la Plata. Prenant en compte les caractères diacritiques analysés, on peut conclure que *A. platensis* est une espèce polymorphe avec des dimensions de maille très variables, probablement en raison de variations dans les conditions environnementales.

***Aphanizomenon* / Cyanobactéries-Cyanophytes / variation morphologique / Nord-Est de l'Argentine / bassin du Rio de la Plata / fleurs d'eau**

INTRODUCTION

Cyanobacteria are typical representatives of the phytoplankton community in subtropical and temperate aquatic environments. In Argentina, the genus *Aphanizomenon* Morr. *ex* Born. *et* Flah. has had a discontinuous record since 1921, when it was first cited by Seckt (1921). This author described "*Aphanizomenon flos-aquae* (L.) Ralfs *ex* Born. *et* Flah." and a new species, *A. platensis* Seckt from the Delta of the Paraná River (at approximately 34º S) in the Rio de la Plata Basin, a temperate region of Argentina. Later on, several other types of the genus *Aphanizomenon* have been recorded in different regions of the country, at low density and without formation of water-blooms (Pizzolon *et al.*, 1999).

After the description of Seckt, *Aphanizomenon* water blooms with taxonomic characters similar to those described for *A. platensis* have been observed only in aquatic environments of the northeast of Argentina, in the last decade. Based on these data, the present study analyses the morphological variation of *A. platensis* in two water bodies of the northwest of Corrientes province. The description of this species is herein extended, including diacritic characters of reproductive cells (akinetes) not reported originally by Seckt (1921). Our study confirms the existence of this morphospecies almost 90 years later. It is interesting to point out that it does not occur in other localities.

The genus *Aphanizomenon* (Geitler, 1932 and Huber-Pestalozzi, 1938, among others) is heterogeneous and, according to its genetic background, must be divided in several separated generic entities (Rajaniemi *et al.,* 2005; Stüken *et al.,* 2009; etc.). Although *A. platensis* has not yet been sequenced or evaluated according to molecular criteria, its morphological markers indicate that it belongs to the cluster of typical *Aphanizomenon* species (cf. Komárek & Komárková, 2006).

The revision of *Aphanizomenon platensis* is significant since species of the genus *Aphanizomenon* are potentially toxic, invasive and bloom formers.

MATERIALS AND METHODS

Study sites

The populations studied were collected from two water bodies in the northwest in Corrientes province, 3 and 5 km away from the city of Corrientes

(27° 29' S; 58° 45' W) respectively. One is an artificial pond (Site A), whereas the other is a natural shallow lake (Site B). Sampling sites are 2 km away from each other.

The first site (A) is small (2 × 8 m, 2.50 m in depth) and has no submerged vegetation. The second site (B), the Aeroclub lake, has a surface of 26 ha ($Z_{max.}$ = 4.5 m) and presents submerged vegetation (*Potamogeton illinoensis* Morong, *Nitella* sp. and *Egeria* sp.).

The climate of the region is transition subtropical with predominant maritime influence, with annual mean temperatures between 20 and 22 ºC and annual mean precipitations between 1,100 and 1,300 mm (Bruniard, 1981).

Sampling

Monthly samples were collected in duplicate with a 25-µm plankton net between March 2004 and March 2005. One sample was fixed *in situ* with 4% formaldehyde and the other was kept alive and carried to the laboratory to be observed with optical microscopy.

The environmental variables such as water temperature (ºC), transparency (Secchi disk), pH, conductivity (YSI 33 SCT conductometer) and dissolved oxygen (YSI 54 A oxygen meter) were determined *in situ.* Nutrient analysis (Nitrites +Nitrates, ammonium and orthophosphates) was performed by the staff of the Chemical Laboratory of the CECOAL, following APHA techniques (1995). Precipitations and solar radiation were recorded with a LICOR (LI-12005) data logger.

Twenty four samples of *A. platensis* were analyzed. Cell dimensions (length and width) of trichomes, vegetative cells, akinetes, heterocytes, and terminal cells were measured. In addition, the number of cells per trichome, the characteristics of the sheath, the presence or absence of aerotopes, and the position of heterocytes and akinetes were also studied.

Taxonomic identification

The taxonomic description of each population includes both the results obtained from the two sites studied in this work and the data published by Seckt (1921).

For the taxonomic determination, we took into account the study of Komárek & Anagnostidis (1989) and the description carried out by Komárek & Komárková (2006), who presented the characters of the genus *Aphanizomenon* and the different species that compose it.

Statistical analysis

One-way ANOVA was used to compare the two populations of *A. platensis* after data were normalized (log n+1). Akinete dimensions were not included due to the small number of records.

Table 1. Physical and chemical variables of sampling sites. R: range, M: arithmetic mean, SD: standard deviation, Nd: not detectable, *n*: number of samples.

	Site A *(n= 12)*		***Site B*** *(n= 12)*	
	R	***M (±SD)***	***R***	***M (±SD)***
Temperature (ºC)	15-31	23±5.16	15.5- 30	23.7±5.3
Secchi disk (m)	0.45-1.50	0.71±0.27	0.50-2.17	1.32±0.47
pH	6.7-7.5	7.3±0.3	7.1-7.4	7.3±0.1
Dissolved oxygen ($mg.L^{-1}$)	1.5-15	8.31± 2.8	6.1-10.3	9.48±8.4
Conductivity ($\mu S.cm^{-1}$)	110-202	169±28	40-70	69±54.8
Nitrites+nitrates ($\mu g.L^{-1}$)	Nd-30	17±10	Nd-14	10±4.2
Orthophosphates ($\mu g.L^{-1}$)	Nd-65	18±7.3	Nd-9	5±2.1

RESULTS

The study sites are subjected to similar values of precipitations (average monthly values: 41-98 mm) and solar radiation (average monthly values: 10-27 $MJ.m^{-2}$). As regards their physical and chemical characteristics, there were some variations in conductivity and concentration of nutrients (mainly orthophosphates), which were slightly higher in Site A (Table 1).

Site A

During the study, there was a low density of these cyanobacteria and the phytoplankton was dominated by several *Cryptomonas* species. A bloom of *A. platensis* occurred in spring, from the beginning of October 2004, as an isolated event, which lasted only for a week, forming a blue-green surface scum. Concurrently with the bloom of *A. platensis*, other cyanobacteria were observed: *Microcystis aeruginosa* (Kütz.) Kütz., *M. wesenbergii* (Kom.) Kom. and *Planktolyngbya limnetica* (Lemm.) Kom.-Legn. *et* Cronberg.

Taxonomic characters

Trichomes are slightly curved and aggregated, with a very fine slime, 270-620 µm long. Vegetative cells contain aerotopes and are cylindrical or barrel-shaped, 2-3.5 (5.5-6) µm wide and 3-12 (7-9) µm long. Cells close to the heterocyte are generally longer. Cells located in the middle of the trichome are quadrangular and small (3 × 3 µm). Terminal cells are: 2-16 × 1-3 µm and are separated from the rest by a strong constriction at cross-walls. They are hyaline and have rounded

ends. There are usually 2-4 small and quadrangular subterminal cells, or a long hyaline cell expanded in the base.

There are 1-2 intercalary heterocytes with sub-symmetrical disposition in each trichome. They are cylindrical or barrel-shaped, 2.5-3 (7) µm wide and 5-10 (12) µm long. Akinetes are intercalary, solitary and distant from the heterocytes. There is only one akinete per trichome. They are cylindrical, with thick, smooth and colorless walls. Cell dimensions are 12-19 × 5-5.5 µm (Figs 1, 3a-c, 4).

Site B

Aphanizomenon platensis was dominant in the phytoplankton, in the spring of 2005, but without bloom formation. During the rest of the year, *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya *et* Subba Raju and *Microcystis aeruginosa* were observed in high density and biovolume, respectively.

Taxonomic characters

Trichomes are straight, solitary, with apparent sheath, 80-405 µm long. Vegetative cells are cylindrical or barrel-shaped and contain aerotopes which are 3-4.5 (5.5-6) µm wide and 3-12 (7-9) µm long.

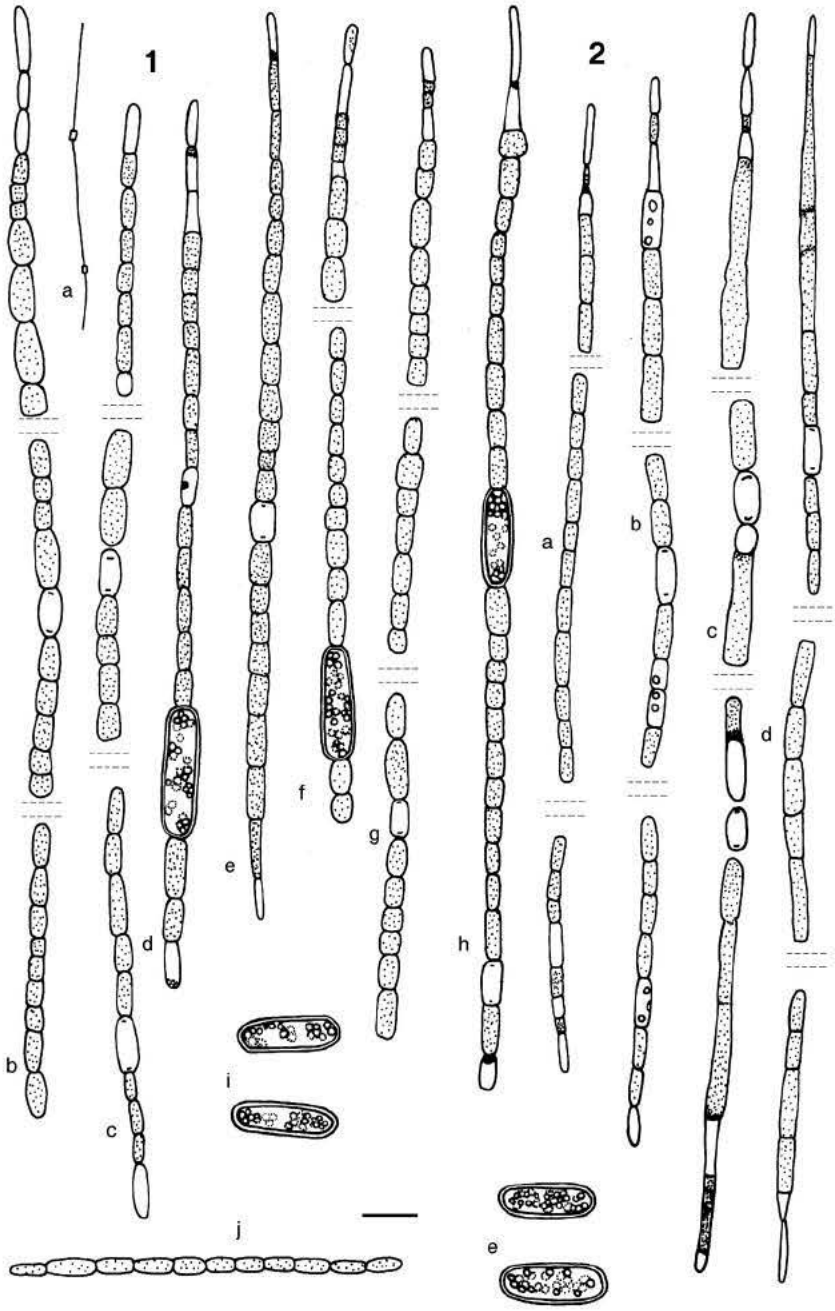
Terminal cells are hyaline and exhibit a clear constriction at cross-walls in the base, which separates them from subterminal cells. These are long or cylindrical, hyaline or with no cell content (1-3 µm wide, and 3-22 µm long), small and quadrangular (3 × 3 µm), or a single long hyaline cell expanded in the base. Heterocytes are cylindrical, 6-9 × 3-5 µm (12 × 7 µm), intercalary, and show sub-symmetrical disposition. Akinetes are intercalary, ellipsoidal cells, 11-15 × 4.5-5 µm, and have characteristics similar to those observed in Site A (Figs 2, 3d, 5).

Statistical analysis

The statistical analysis of the variability of cell dimensions is shown in Table 2. Trichomes from Site A were longer and thinner than those from Site B, and showed statistically significant differences between both populations. The size of vegetative cells was higher in Site B than in Site A, as the width of heterocytes. These were also slightly longer in Site B (M= 7±0.83 µm) than in Site A (M= 6.81±1.76 µm), but showed no significant differences between both populations.

DISCUSSION

Although both populations showed similar morphological characteristics, differences in the size of the trichomes and in the number and shape of terminal cells were observed. These differences are probably related to the limnological characteristics of each environment. Site A, an artificial pond of small dimensions (16 m^2), showed a higher annual concentration of nutrients, mainly orthophosphates. During the bloom of *A. platensis*, however, no nitrogen or phosphorus concentrations were detected in the water. The other physical and chemical variables were similar in both sites. Samples of *A. platensis* collected in this area are slightly different morphologically from those described by Seckt (1921). While this author did not include abiotic data, the lentic environments



Figs 1-2. *Aphanizomenon platensis* **1**: Trichomes from Site A: **a** = schematic position of heterocytes in the solitary trichome; **b, c, e, g** = fragments of trichomes without akinetes; **d, f, h** = trichomes with akinetes; **i** = liberated akinetes; **j** = germinating hormogonium. **2**: Trichomes from Site B: **a-d** = fragments of trichomes without akinetes; **e** = liberated akinetes. Scale bar: 10 µm.

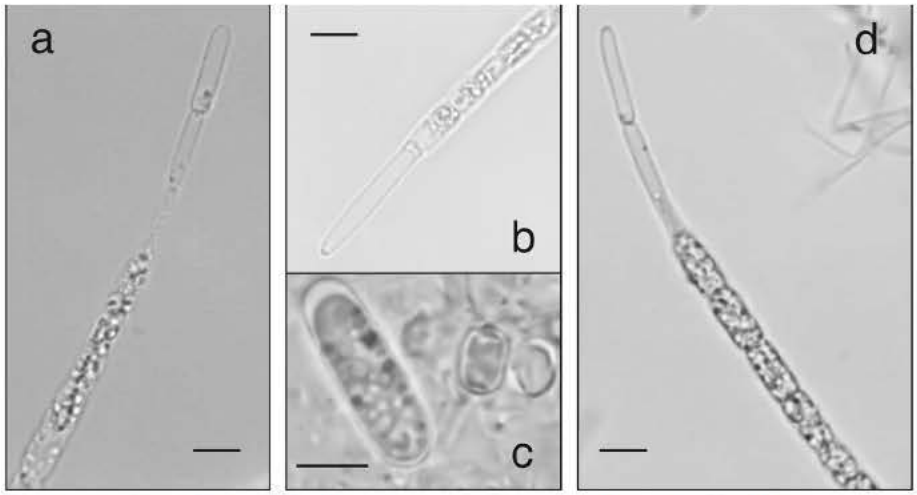


Fig. 3. *Aphanizomenon platensis* **a, b, d** = Morphological variability of terminal cells; **c** = akinete and a liberated heterocyte. Scale bar: 5 µm.

Table 2. ANOVA test between populations of *Aphanizomenon platensis* of Sites A and B. *df*: degrees of freedom; (*): statistically significant differences, *n*: number of observations

	df	*F*	*p*
Length of trichomes (*n*=24)	23	11.33	0.0028*
Dimensions of vegetative cells (*n*=134)			
Width (µm)	133	88.72	<0.0001*
Length (µm)	133	5.32	<0.0226*
Heterocytes (*n*=24)			
Width (µm)	23	18.99	0.0003*
Length (µm)	23	0.16	0.6959

from which samples were collected probably had ecological differences, which may account for the morphological variability of this species.

When we compared our populations with the samples described by Seckt (1921), we also observed some differences in the size and morphology of cells (Table 3, Fig. 6). Although the number of samples studied by Seckt (1921) is unknown, vegetative cells and heterocytes were slightly wider than in our samples. However, the rest of the morphology was identical to our material, in which only dimensions were variable. Besides, Seckt did not describe the morphological variability of terminal cells, and did not report the presence of akinetes, which are described for the first time in our study. However, "*Aphanizomenon flos-aquae*", cited in Seckt (1921, fig. 40), is surely different from the typical *Aphanizomenon flos-aquae* from temperate zones (cf., e.g., figs 5 and 6 in Komárek & Komárková 2006) and belongs evidently to the variation range of *A. platensis* according to

Table 3. Comparison of morphometric characteristics of *Aphanizomenon platensis* between our results and those published by Seckt (1921). R: range of variation, M: arithmetic mean, SD: standard deviation, wd: without data.

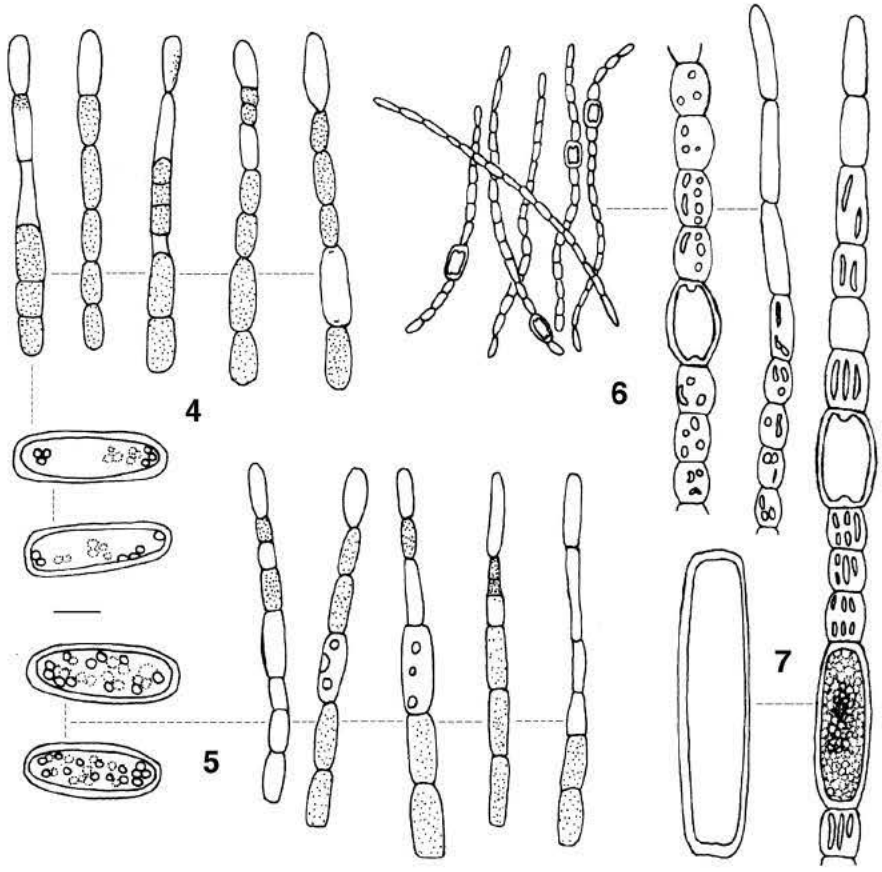
	Sites A+B		***Seckt 1921***	
	R	***M (±SD)***	***A. platensis***	***"A. flos-aquae"***
Length of trichomes (μm) **(*n*=24)**	620 - 680	350 ± 16.9	wd	wd
Dimensions of vegetative cells (*n*=134)				
Width (μm)	2 - 4.5	2.92 ± 0.02	5.5-6	4.5-5
Length (μm)	3 - 12	6.35 ± 0.31	7-9	4.5-5
Dimensions of heterocytes (*n*=24)				
Width (μm)	2.5 - 5	3.43 ± 0.43	7	5-7
Length (μm)	5 - 10	6.9 ± 0.29	12	10-15
Dimensions of akinetes (*n*=6)				
Width (μm)	5	5 ± 1.8	wd	5-7
Length (μm)	12 - 19	15.3 ± 2.3		12-20 (30)

morphological characters. It is also reported for the same localities (small environments in the Lower Paraná River floodplain or Delta). Therefore, this type was also included in the review in our Table 3 and Figure 7.

A. platensis clearly differs from other *Aphanizomenon* species by the morphology of trichomes, particularly of their terminal parts, and by the morphology of cells and akinetes. The most similar *A. slovenicum* Rekar *et* Hindák (2002), described from the European lake Bled (northwest of Slovenia), differs particularly by the morphology of the ends of trichomes and by its ecology.

Other *Aphanizomenon*-like species in Argentina have been recorded in temperate (Pizzolon *et al.*, 1999) and subtropical areas (Matveev *et al.*, 1992; Zalocar de Domitrovic *et al.*, 1998; Asselborn *et al.*, 1998; Pizzolon *et al.*, 1999). *A. flos-aquae* (O´Farrel *et al.*, 1994; Meichtry de Zaburlín, 2002), *Aphanizomenon* sp. (Zalocar de Domitrovic, 2003), *A. issatschenkoi* = *Cuspidothrix issatschenkoi (*Usač.) Rajan. (Zalocar de Domitrovic, 1999) and *A. aphanizomenoides* (Forti) Horecká *et* Komárek (Devercelli, 2008), which were found in the hydrographic sub-basin of the Paraná River belong to different clusters from the typical *Aphanizomenon*. However, the presence of other *Aphanizomenon*-like species has been sporadically recorded in our localities, in low density and without bloom formation.

Up to now, species from the genus *Aphanizomenon* (in its modern revised form) have been observed only in temperate areas and never in the tropics (cf. Anagnostidis *et al.*, 1988; Komárek, 2010). However, *A. platensis* can be found in water bodies from the northeast of Argentina, an area with subtropical climate, whose south limit is the parallel 31ºS, on the Paraná River. It seems from the updated data that the distribution of *A. platensis* is restricted only to the Mesopotamia of Argentina, in the hydrographic sub-basins of the Paraná and Uruguay rivers, since it has not yet been found in any other locality and similar morphotypes have been recently studied only in this region. It is interesting to point out that similar types have also been recorded scarcely (in solitary trichomes) also along the northern shore of the Río de la Plata estuary up to



Figs 4-7: Morphological variability of terminal cells and liberated akinetes of *Aphanizomenon platensis* from Sites A (**4**) and B (**5**). **6**: *Aphanizomenon platensis* redrawn from Seckt (1921). **7**: "*Aphanizomenon flos-aquae*" redrawn from Seckt (1921). Scale bar: 5 µm.

Montevideo, in Uruguay, in the summer season of February 1989 (in litt.) and in Uruguay River (between Argentina and Brazil) in March 2010 (summer). This species may be transported into the Río de la Plata estuary by both the Paraná and Uruguay rivers.

Taking into account the diacritic characters analyzed, we may conclude that *A. platensis* is a polymorphic species restricted to a relatively small area of Argentina, with highly variable cell dimensions, probably due to variations in the environmental conditions.

The summarized and revised description of *Aphanizomenon platensis* is as follows:

Filaments are planktic, solitary or agglomerated in mucilaginous clusters, up to 620 µm long, uniseriate, with or without apparent sheaths, ± straight or indistinctly coiled, slightly narrowed to both ends, distinctly constricted at cross-walls. One or two heterocytes and occasionally single akinetes, subsymmetricaly localized, occur in old trichomes. Vegetative cells are cylindrical or barrel-shaped, 3-12 × 2-6 µm and contain gas vesicles joined to spherical to elongated aerotopes,

sometimes situated parallel to each other. End cells are narrowed (1-3 μm) and longer than vegetative cells (22 μm long), ± cylindrical or slightly narrowed, hyaline, rounded at the ends; sometimes several terminal cells (up to 3) are hyaline and elongated. Cells close to the heterocytes are generally longer, localized in the middle of the trichome, usually quadrangular and relatively small. Heterocytes are cylindrical to ellipsoidal or barrel-shaped, 1-2 in one trichome and in a subsymmetrical position, 5-12(15) × 2.5-7 μm. Akinetes are intercalary, solitary, single in trichomes, distant from heterocytes. They are cylindrical up to elongated-ellipsoid, with thick, smooth and olorless cell walls, 11-20(30) × 4.5-5.5(7) μm.

This is the first report of *A. platensis* after the study carried out by Seckt (1921) almost ninety years ago, in which this species was recorded in the Delta of Paraná River, a region of temperate climate of Argentina. Our study further describes the species and extends its distribution to the northeast of Argentina, a region of subtropical climate, in lentic environments related to the hydrographic sub-basin of the Paraná River, in the Rio de la Plata Basin.

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