



## ***Cylindrospermopsis raciborskii* (Cyanobacteria) extends its distribution to Latitude 34°53'S: taxonomical and ecological features in Uruguayan eutrophic lakes**

LETICIA VIDAL<sup>1\*</sup> & CARLA KRUK<sup>1</sup>

<sup>1</sup>*Limnology Section, Faculty of Sciences. Universidad de la República. Iguá 4225. Montevideo, CP 11400, Uruguay.*  
*\*leti@fcien.edu.uy*

**Abstract:** *Cylindrospermopsis raciborskii*, a potentially toxic tropical cyanobacterium, has recently gained scientific attention because of its invasive behaviour. Many hypotheses have been proposed to explain its expansion towards cooler regions. However, its morphological variability and its southern distribution in South America are still not clear. Here we analyse the spatial occurrence and relative frequency of this species over 47 lakes in southern Uruguay (34°48'-53'S). This is the first report of *C. raciborskii* from Uruguay and the southernmost record in the Americas. We explored the morphological features and described the environmental conditions where the species was detected. Also, we compared our morphotypes with others from temperate and tropical regions. *Cylindrospermopsis raciborskii* was only observed in four of 47 freshwater lakes. These sites were characterized by high water temperature and nutrient concentrations, low light availability and well-mixed waters. There was a high degree of variation in morphotypes in samples from Uruguay and elsewhere, with no clear association to their geographical origin. We argue that in-lake environmental factors (such as light in the water column) are more important than global factors for the establishment and the development of morphological characteristics of each population of *C. raciborskii*. We suggest that a more confident taxonomic resolution and the analysis of the '*Cylindrospermopsis* complex' life cycle, along with genetic studies of the populations are needed to successfully test the causes of the spread of this species.

**Keywords:** Morphotypes, spatial distribution, subtropical lakes, South America.

### **Resumen. Extensión de la distribución de *Cylindrospermopsis raciborskii* (Cyanobacteria) hacia la latitud 34°53'S: características taxonómicas y ecológicas en lagos eutróficos de Uruguay.**

*Cylindrospermopsis raciborskii* es una cianobacteria tropical potencialmente tóxica. Recientemente, esta especie ha recibido mayor atención científica debido a su comportamiento invasor. Se han desarrollado varias hipótesis que intentan explicar su expansión hacia regiones más frías. Sin embargo, su variabilidad morfológica y su distribución en América del Sur aún no son claras. En este trabajo analizamos su distribución espacial y frecuencia de ocurrencia en 47 lagos del área sur de Uruguay (34°53'S). A su vez se presenta el primer registro de esta especie para Uruguay, así como el más al sur para el continente Americano. Se exploraron las características morfológicas y se describieron las condiciones ambientales donde la especie fue observada. Además, se compararon los morfotipos encontrados con otros de regiones templadas y tropicales. *C. raciborskii* solo fue observada en 4 sistemas dulceacuícolas, los cuales se caracterizaron por tener una alta temperatura y concentración de nutrientes, conjuntamente con baja disponibilidad de luz en la columna de agua y condiciones de mezcla. Los morfotipos uruguayos y extranjeros variaron en gran medida, sin una clara asociación con su origen geográfico. Nuestro argumento es que para el establecimiento y el desarrollo de las características morfológicas de cada población de *C. raciborskii*, las características ambientales de cada lago son más importantes que los factores globales. Para poner a prueba en forma exitosa las causas de la dispersión de esta especie sería necesario mejorar la identificación taxonómica e incluir análisis de los cambios durante el ciclo de vida del "complejo *Cylindrospermopsis*", conjuntamente con el desarrollo de estudios genéticos.

**Palabras clave:** Morfotipos, distribución espacial, lagos subtropicales, América del Sur.

## Introduction

The global distribution patterns of phytoplankton species are still poorly known. However, *Cylindrospermopsis raciborskii* (Woloszynka) Seenaya & Subba Raju (1972) is an exception to this rule. This species has recently gained scientific attention due to its invasive behaviour in diverse continental waters of the world. *Cylindrospermopsis raciborskii* is a bloom forming cyanobacteria (order Nostocales) with high potential toxicity and remarkable negative consequences for human health.

*Cylindrospermopsis raciborskii* was first recorded in Java, Indonesia (Woloszynka 1912) and classified as a tropical species optimally growing at 25 °C (Briand *et al.* 2004). In the last 10 years the frequency of *Cylindrospermopsis* blooms has increased in the tropics (Branco & Senna 1994, Fabbro & Duivenvoorden 1996). The species has also been recently observed in subtropical (Jones & Sauter 2005) and temperate regions (Tóth & Pádisák 1986, Fastner *et al.* 2003). Its current distribution reaches as far as northern Europe (Germany, 54° 25' N; Wiedner *et al.* 2002), New Zealand (37° 56' S; Ryan *et al.* 2003), South Africa (33°55' S; Harding 1996) and North America (45°04' S; Hill 1970). In South America the species is well known in tropical waters of Brazil (Huszar *et al.* 2000), but the information about its distribution in southern latitudes is disperse and incomplete (Komárek 2002, Zalocar & Forastier 2005). Prior to this study, the southernmost latitudinal record of bloom forming *C. raciborskii* in South America was from Brazil (32°07'; Torgan & Garcia 1989), while as a non-dominant species it was reported from subtropical Argentina (34° 14', De Tezanos-Pinto *et al.* 2006).

A number of hypotheses have been suggested to explain the successful spread and invasive behaviour of *C. raciborskii* (Komárek 2002). Some authors have proposed that high physiological tolerance to light and temperature (Pádisák 1997) coupled with higher temperatures promoted by climate warming may explain the expansion of this species to new regions (Briand *et al.* 2004). Others suggested that the spread of *C. raciborskii* represents a species radiation after the last glaciation (Gugger *et al.* 2005). Low edibility and allelopathy have also been hypothesized to contribute to increased bloom occurrence and geographic expansion (Branco & Senna 1994, Figueredo *et al.* 2007).

Taxonomic misidentification has obscured the testing of these hypotheses (Komárek 2002). Cyanobacteria as a group, and especially *C.*

*raciborskii*, display large morphological plasticity (Mc Gregor & Fabbro 2000). The variability of the morphological features used for taxonomic identification, caused by genetic or environmental factors (Singh 1962, Hawkins *et al.* 1997, Neilan *et al.* 2003) complicates their differentiation from other Nostocales species. For instance, *C. raciborskii* has been previously misidentified as *Anabaenopsis*, *Raphidiopsis*, and *Cylindrospermum* (Hawkins *et al.* 1997). Clearly, a better understanding of its range of morphological variability and ecological preferences is urgently required (Komárek, 2002) especially in areas with scarcity of studies.

The aim of this study is to analyse for the first time the spatial distribution and relative frequency of *C. raciborskii* close to its southernmost distribution. We sampled subtropical lakes along the coast of Uruguay, including systems of different origin, trophic state and anthropogenic impact. In those systems where the species was found, we analysed the morphological diacritical features and the environmental conditions. We further discuss the classification of *C. raciborskii* into different morphotypes and compare the local morphotypes with others described from temperate and tropical areas.

## Materials and Methods

The study area is located in the coastal region of Uruguay and it extends from 34°50'-S 53°40'W to 34°53'S-56°15'W, an area that includes ca. 200 aquatic ecosystems. From these systems we analysed a database of 47 lakes, including natural and man-made, shallow and deeper sites, as well as freshwater and brackish systems with different trophic states (from oligotrophic to hypertrophic). Most of these systems were sampled since 2003, at least once in summer, with the exception of nine that were sampled seasonally. The available information included lake morphology, water physico-chemistry and phytoplankton characteristics. In the present analysis we included only systems where the genus *Cylindrospermopsis* appeared. Of those lakes that were sampled more than once, the data from the sampling date where the genus was most abundant were included in the analysis.

Specific conductivity, pH, temperature, dissolved oxygen (Horiba sensor) and transparency (Secchi disk) were measured *in situ*. Temperature and dissolved oxygen profiles were measured every 20 cm in the deepest point of the lakes (Horiba sensor), and used to estimate the water column mixing depth. The euphotic zone depth

(1% of incident light) was determined from profiles of photosynthetically available radiation (Licor Li-250/2 $\pi$ ). Integrated water column samples (Ruttner bottle) for nutrients and phytoplankton analysis were collected simultaneously at mid-day. Total nitrogen and total phosphorus were analysed according to Valderrama (1981). Phytoplankton net tows (25- $\mu$ m mesh size) were collected to obtain samples for qualitative analysis. Counts of the whole phytoplankton community were made from quantitative samples (Utermöhl 1958), with taxonomic richness being estimated thereafter.

We followed the description of *C. raciborskii* by Komárek & Komárková (2003) to select the diacritical features. In each sample, we randomly selected at least 50 trichomes and 30 vegetative and differentiated cells for the analysis. We examined the trichomes (length, number of cells, shape, number and location of differentiated cells and presence of constrictions) as well as the shape, length and width of apical cells, vegetative cells, akinetes and heterocytes, under a light microscope with 1000X magnification. We calculated the biovolume according to Hillebrand *et al.* (1999). To test whether these features were significantly different between lake populations we used non-parametric analyses of variance (Kruskall-Wallis) and non-parametric correlation tests (Spearman) to analyse the relationship between morphological features. Using principal components analysis (PCA) performed with the software CANOCO 4.52 (ter Braak and Smilauer 2003), we analysed the importance of the morphological variables in explaining morphotype variability and compared the mean values of the Uruguayan populations with morphotypes described in temperate and tropical systems (References in Table I). A total of 30 field morphotypes were included. When ranges of values were offered in the literature the mean of each morphological feature was used. Two descriptions of *C. raciborskii* (Seenayya & Subba Raju 1972, Komárek & Komárková 2003) and descriptions of three other species (*C. philipinensis*; Taylor, Komárek 1984, *C. cuspis*; Komárek & Kling 1991 and *C. africana*; Komárek & Kling 1991) were also included as references for comparison. The presence of constrictions and the shape of the apical cells were included as nominal (dummy) variables. Trichome shape was incorporated as a fuzzy variable between straight and coiled. When necessary, data were log-transformed.

## Results

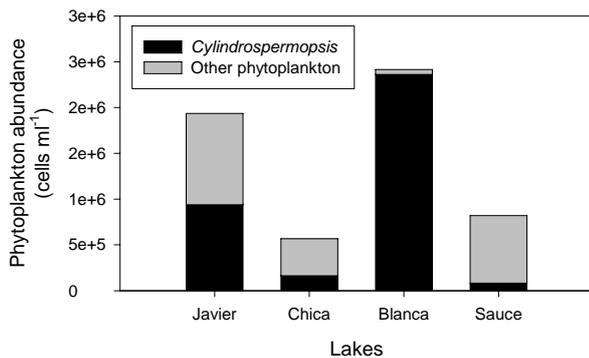
### Ecological features

We found *Cylindrospermopsis* in only four out of the 47 lakes that formed our database. Those systems were: Laguna Blanca (Maldonado), Lago Javier (Canelones), Laguna Chica (Canelones) and Laguna del Sauce (Maldonado). Laguna del Sauce and Laguna Blanca are natural systems used as drinking water sources, while Lago Javier and Laguna Chica are man-made lakes used for recreation. Laguna Blanca has been sampled since 2000 and *Cylindrospermopsis* was found from 2004 until 2007, mainly in summer. Lago Javier and Laguna Chica were sampled in summer 2005 and 2007, and on both occasions *Cylindrospermopsis* was present. Laguna del Sauce has been sampled from 2002 to 2007, and *Cylindrospermopsis* occurred only during the summer of 2004 and 2005. All these systems are polymictic, small to large-sized (0.5 - 4550 ha) and shallow to medium depth (2.6 - 9.8 m maximum depth). Specific conductivity varied between 279 and 421  $\mu$ S  $\text{cm}^{-1}$  and water temperature fluctuated around 24 °C. Secchi depth was low to mean (0.4 - 1.3 m), while the ratio of mixing / euphotic depth varied between 1.27 and 3.06. Total nutrients were generally high (phosphorous = 55.7 - 223.0  $\mu$ g  $\text{L}^{-1}$ , nitrogen = 30.0 - 2079  $\mu$ g  $\text{L}^{-1}$ ), while pH was alkaline (8.9 - 9.1) (Table II). The colour of the water was olive-green in all lakes and surface scum was only observed in Laguna Blanca.

In Laguna Blanca, *C. raciborskii* became the dominant species with an average abundance of  $2.3 \times 10^6$  cells  $\text{ml}^{-1}$  ( $1.0 \times 10^8$  trichomes  $\text{L}^{-1}$ ). In this system, the total phytoplankton abundance was high ( $2.4 \times 10^6$  cells  $\text{ml}^{-1}$ ) but taxonomic richness was low (8 species). In Lago Javier, *C. raciborskii* mean abundance was  $9.4 \times 10^5$  cells  $\text{ml}^{-1}$  ( $6.3 \times 10^7$  trichomes  $\text{L}^{-1}$ ), representing ca. 50% of the total phytoplankton abundance. Other cyanobacteria were co-dominant (*Planktolyngbya* spp.) and total richness was 21 species. Laguna Chica had  $1.6 \times 10^5$  cells  $\text{ml}^{-1}$  *C. raciborskii* ( $6.3 \times 10^6$  trichomes  $\text{L}^{-1}$ ), representing 29% of the total phytoplankton density. Richness was 40 species, and co-dominant species included *Ceratium hirudinella*, chlorophytes and cryptophytes. The lowest abundance of *C. raciborskii* was found in Laguna del Sauce ( $4.6 \times 10^3$  cells  $\text{ml}^{-1}$ ;  $3.2 \times 10^6$  trichomes  $\text{L}^{-1}$ ), where it represented only 9.9% of the total phytoplankton density. *Planktolyngbya limnetica* and *Aphanizomenon issatschenkoi* were dominant and total richness was 29 species in this system. *C. raciborskii* population biovolume varied from 7.9  $\text{mm}^3 \text{L}^{-1}$  in Laguna del Sauce to 312.7  $\text{mm}^3 \text{L}^{-1}$  in Laguna Blanca (Fig. 1).

**Table I.** Morphological features of *Cylindrospermopsis* morphotypes. Morphotypes found in Uruguay and in other regions obtained from literature are included, together with data about authors, location, climatic region, species, type of trichome (straight: Ts and coiled: Tc), length (L) and width (W) of vegetative cells (VC), heterocytes (H), and akynetes (A), and shape of apical cells (AC, attenuated: 1 or not: 0).

Author	Location	Label	Climatic area	Species	Tr	Tc	VCL	VCW	HL	HW	AL	AW	AC
Seenayya & Subba Raju 1972	Description	Desc 72	Description	<i>C. raciborskii</i>	0.5	0.5	9.0	2.5	11.5	4.0	14.5	4.5	
Komárek & Komárková 2003	Description	Desc 03	Description	<i>C. raciborskii</i>	0.5	0.5	10.2	2.6			13.9	4.0	0
Hill 1970	USA Minnesota (Cashaca puddle)	USA r3	Temp	<i>C. raciborskii</i>			10.0	3.2	10.0	3.5	14.4	4.0	
Hill 1970	USA Minnesota (Starring lake)	USA r4	Temp	<i>C. raciborskii</i>			8.5	2.9	10.5	2.9	16.5	4.9	1
Horecká & Komárek 1979	Slovakia	Hun r	Temp	<i>C. raciborskii</i>	1	0	9.3	2.6	8.7	2.9	13.3	4.0	
Hindák 1988	Slovakia (Peri Lagoon)	Eslo r	Temp	<i>C. raciborskii</i>	0.5	0.5	7.0	2.0	10.4	2.9	12.2	3.7	
Padisák 1990-1991	Hungary	Hun r2	Temp	<i>C. raciborskii</i>	0.8	0.2	7.8	2.0	6.5	2.4			0
Couté <i>et al.</i> 1997	France	Fran r2	Temp	<i>C. raciborskii</i>			11.5	1.8	5.0	1.8	12.5	4.0	1
McGregor & Fabbro 2000	Australia	Aust r5	Temp	<i>C. raciborskii</i>	0.5	0.5	5.9	2.2					
Briand <i>et al.</i> 2002	France	Fran r1	Temp	<i>C. raciborskii</i>	1	0	11.0	1.9	5.0	1.8	12.5	3.5	
Moore <i>et al.</i> 2005	Australia	Aust r2	Temp	<i>C. raciborskii</i>	1	0					15.5	4.4	1
Hamilton <i>et al.</i> 2005	Canada	Can r	Temp	<i>C. raciborskii</i>	1	0	2.0	1.8	2.0	1.8	1.8	1.3	1
Hong <i>et al.</i> 2006	USA Michigan (Mona Lake)	USA r	Temp	<i>C. raciborskii</i>	1	0		3.0	8.1	6.1	12.2	3.5	
Baker & Fabbro 1999	Australia	Aust r6	Subt	<i>C. raciborskii</i>	0.7	0.3		7.0	7.0	3.3	11.8	4.0	1
Jones & Sauter 2005	USA Indiana	Usa r2	Subt	<i>C. raciborskii</i>	0.5	0.5	7.6						
This study	Uruguay (Blanca)	Blanca	Subt	<i>C. cuspis</i>	1	0	9.4	1.9	7.0	1.9	11.1	3.2	
This study	Uruguay (Chica)	Chica	Subt	<i>C. cuspis</i>	1	0	8.1	2.9	7.4	1.9	13.5	3.9	1
This study	Uruguay (Javier)	Javier	Subt	<i>C. cuspis</i>	1	0	12.0	1.9	4.0	2.0	4.2	4.0	0
This study	Uruguay (Sauce)	Sauce	Subt	<i>C. cuspis</i>	1	0	8.1	3.0	7.8	2.4	9.5	3.0	1
Singh 1962	India	India r	Trop	<i>C. raciborskii</i>	0.5	0.5		2.8	4.0	2.4	5.5	3.3	0
Komárek 1984	Philippinas. Indonesia and Cuba	Phi Cuba	Trop	<i>C. philippinensis</i>	0	1	16.7	2.6	10.4	3.2	14.6	3.6	1
Komárek 1984	Cuba	Cuba r	Trop	<i>C. raciborskii</i>	1	0		2.8	8.0	2.5	8.4	3.7	1
Komárek & Kling 1991	East Africa	Afr Ep	Trop	<i>C. philippinensis</i>	0	1	4.0	2.6	10.4	3.2	14.6	3.6	
Branco & Senna 1991	Brazil (Paranoa Reservoir)	Bra r2	Trop	<i>C. raciborskii</i>	0.8	0.2		2.4	6.0	2.4	12.5	3.0	1
Komárek & Kling 1991	East Africa (Victoria Lake)	Afr Ea	Trop	<i>C. africana</i>	0.5	0.5	13.3	1.8	7.6	2.6	13.3	1.8	
Komárek & Kling 1991	East Africa	Afr Er	Trop	<i>C. raciborskii</i>	0.5	0.5	10.2	2.6	7.5	2.8	10.0	2.5	1
Komárek & Kling 1991	East Africa (Victoria Lake)	Afr Ec	Trop	<i>C. cuspis</i>	1	0	11.9	1.0	8.7	1.2	11.9	1.0	
Baker 1991	Australia	Aust r1	Trop	<i>C. raciborskii</i>	1	0					11.8	4.0	
Saker <i>et al.</i> 1999	Australia (Solomon dam)	Aust r3	Trop	<i>C. raciborskii</i>	0	1	6.0	3.0	5.3	2.7	9.0	3.9	
Saker <i>et al.</i> 1999	Australia (Solomon dam)	Aust r4	Trop	<i>C. raciborskii</i>	1	0	6.5	2.9	5.8	2.6	11.4	3.9	1
Komárková <i>et al.</i> 1999	Brazil	Bra r1	Trop	<i>C. raciborskii</i>	1	0					12.2	3.7	
Komárek & Komárková 2002	Mexico (Pond central Mexico)	Mex t	Trop	<i>C. taverae</i>	0.5	0.5	4.7	3.1	4.9				1
Chonudomkul <i>et al.</i> 2004	Thailand & Japan	Ta Ja r	Trop	<i>C. raciborskii</i>	0.5	0.5	10.8	2.1			13.2	4.9	1



**Figure 1.** Abundance (cells ml<sup>-1</sup>) and relative (% = length of stacks) abundance of *Cyindrospermopsis raciborskii* and other coexisting taxa (other).

### *Cyindrospermopsis* morphological features

All *Cyindrospermopsis* trichomes were straight, displayed aerotopes and lacked granules (Fig. 2). Filaments with and without differentiated cells were found in all lakes. The number of differentiated cells per trichome varied between 1 and 2 heterocytes and 1 to 3 akinetes. Mean trichome length was 125  $\mu$ m (range 12 - 300  $\mu$ m) and the mean number of cells per trichome was 17 (4 - 41). Slight constrictions at the cross walls were observed only in Laguna Chica. Apical cells were generally attenuated and varied between 4-20  $\mu$ m and 1-3  $\mu$ m in length and width, respectively. Vegetative cells were generally cylindrical, and their size varied from 5 to 20  $\mu$ m in length and from 1 to 4.8  $\mu$ m in width. Heterocytes were generally drop-pointed, with or without calyptra, and were situated always at the end of the trichome. Heterocytes varied between 2 - 12  $\mu$ m in length and 1.4 - 3.0  $\mu$ m in width. Akinetes were ellipsoid (L. Blanca, Chica



**Figure 2.** Image under light microscope of *Cyindrospermopsis raciborskii* from Laguna Chica.

and Javier) and barrel-shaped (L. Sauce). When more than one akinete was found in one trichome, they were always located together. Their size varied from 2.5 to 18  $\mu$ m in length and from 2 to 4.8  $\mu$ m in width (Fig. 3).

All measured characteristics except apical cell length and akinete width varied significantly among the populations found in the four lakes (Kruskall-Wallis  $p < 0.05$ ). However, we observed a high degree of overlap in the range of variation of all the features considered, with the exception of the apical cells (Fig. 3).

Significant relationships were observed between some of the morphological features. The number of heterocytes and akinetes were negatively related ( $r_s = -0.405$ ,  $p < 0.001$ ). Also, the number of akinetes decreased with increasing number of cells per trichome ( $r_s = -0.410$ ,  $p < 0.05$ ), while the number of heterocytes increased with trichome length ( $r_s = 0.332$ ,  $p < 0.001$ ). On the other hand, the vegetative cells width and heterocyte width were positively correlated ( $r_s = 0.529$ ,  $p < 0.000$ ).

### Comparison between Uruguayan and other morphotypes

We compared the local morphotypes with others described in different climatic zones and continents using PCA (Table I, Fig. 4). We used only diacritical features, thus leaving trichome length and number of heterocytes and akinetes out of the PCA analysis. The distribution of samples was successfully explained by the selected variables, with the first four axes accounting for 91.8% of the total variance. While Uruguayan morphotypes occurred close to each other, other samples were not clustered in the PCA plot according to climatic origin, continent or country. For example, Australian morphotypes showed a disperse distribution. The descriptions of *C. raciborskii* of Seenayya & Subba Raju (1972) and Komárek & Komárková (2003) appeared far from each other, the second occurring in a central position. Finally, the descriptions of *C. philippinensis*, *C. cuspidis* and *C. africana* appeared mixed with *C. raciborskii* morphotypes.

### Discussion

In this study we report the southernmost occurrence of *Cyindrospermopsis raciborskii* in America and the first record from Uruguay (34°53'S). With this finding, we contribute to the description of the spatial pattern of this species and confirm its extended geographic distribution. Nevertheless, it should be noted that some species that can be confused with *C. raciborskii* have been recorded in higher latitudes, as in the case of

**Table II.** Main abiotic variables and percentage of *Cylindrospermopsis raciborskii* within phytoplankton density of the four systems in Uruguay where the species occurred. Latitude, percentage of *C. raciborskii* cells, lake area, maximum depth (Zmax), temperature (T), Secchi depth (SD), ratio of mixing depth / depth of euphotic zone (Zmix/Zeul), alkalinity, pH, total nitrogen (TN) and total phosphorus (TP).

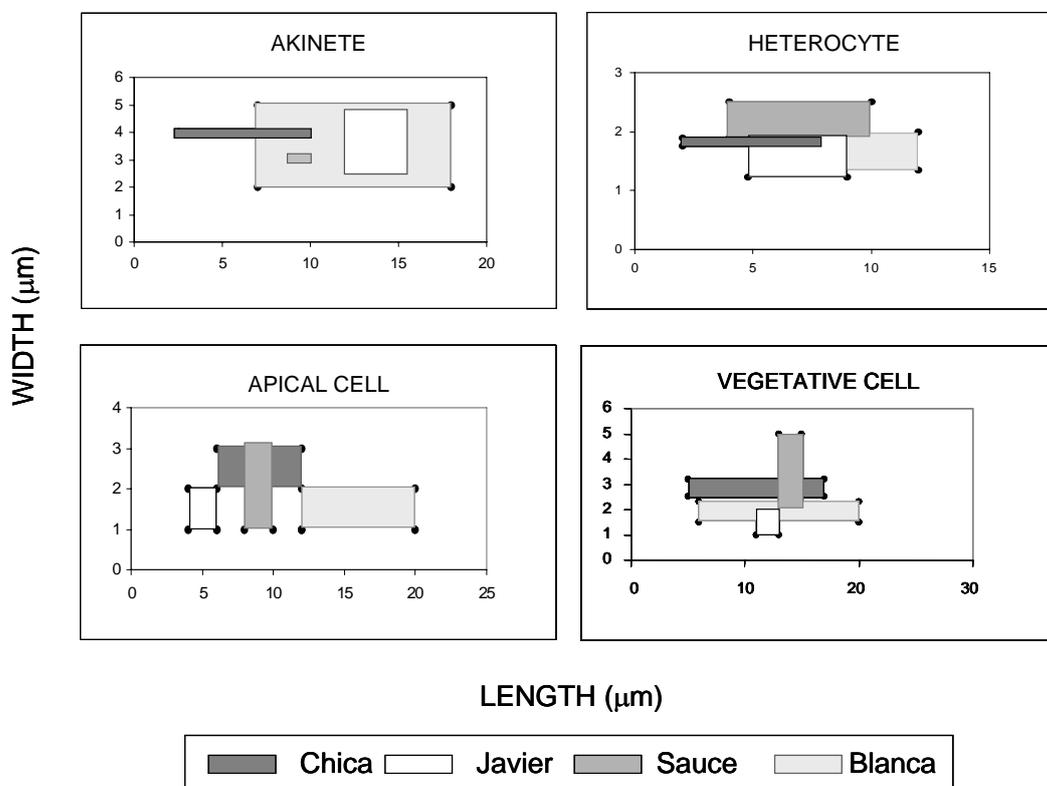
	Coordinates	% <i>C. raciborskii</i> cells	Area (ha)	Z max (m)	T (°C)	SD (m)	Zmix/Zeul	Alkalinity (mg CaCO3 ml <sup>-1</sup> )	pH	TN (mg L <sup>-1</sup> )	TP (mg L <sup>-1</sup> )
Blanca	34° 53'S 54°20'W	92.8	40.5	2.6	25.0	0.5	3.06	91.5	8.9	1694	85.5
Javier	34°51'S 56°02'W	48.6	10	9.8	25.8	0.4	2.35	2.7	9.1	2079	61.2
Chica	34°51'S 50°02'W	28.7	0.5	4.5	26.4	1.3	1.27	2.6	8.9	927	55.7
Sauce	34°48'S 55°03'W	9.9	4550	4.1	23.0			35.8			223

*Raphidiopsis mediterranea* (Argentina, 35°30'S; Izaguirre & Vinocur 1994).

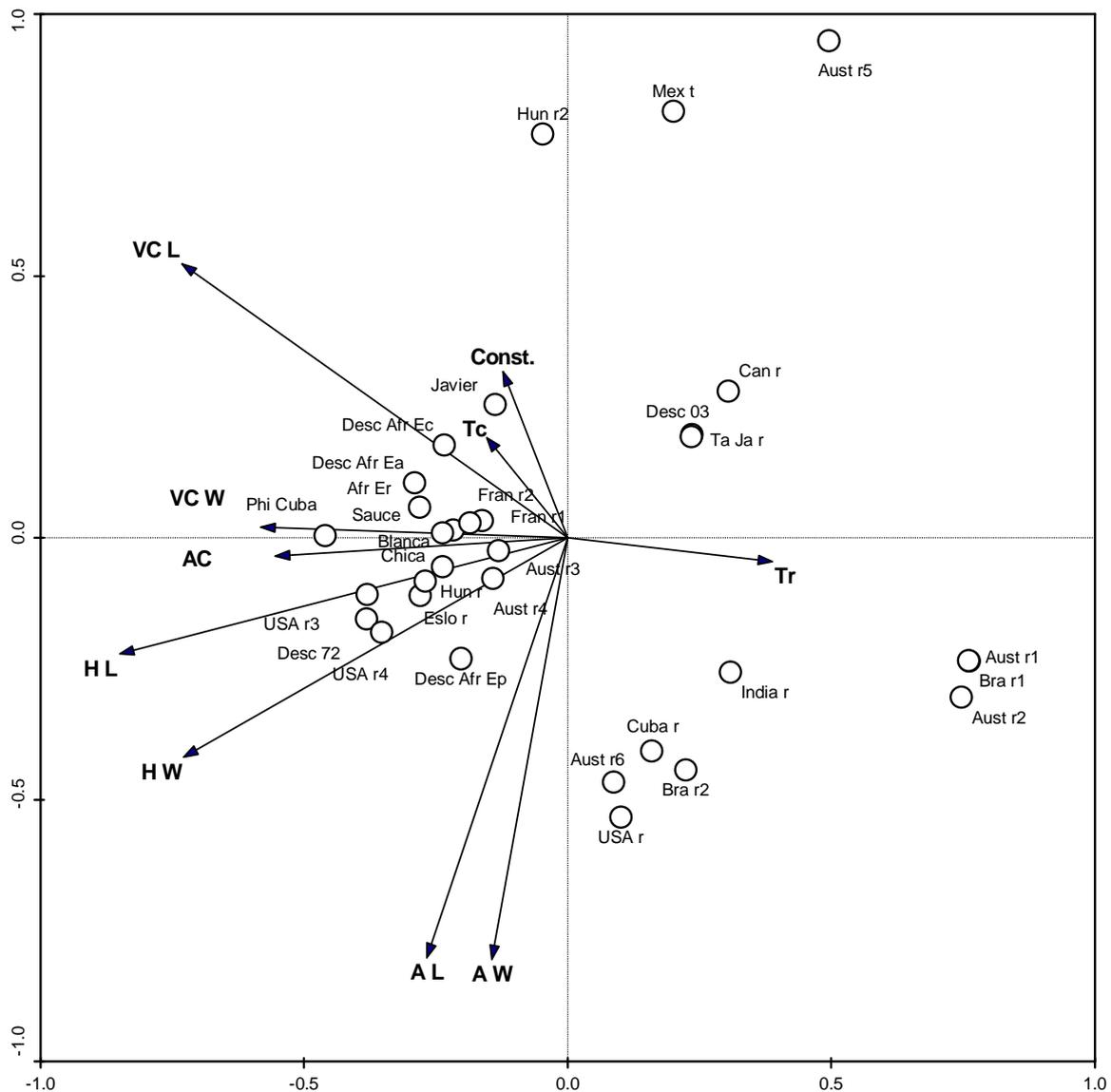
As a result of eutrophication, a substantial increase in the frequency of cyanobacterial blooms has been found in Uruguay. The systems affected are mainly those with anthropogenic impact and include some used for drinking water supply and recreation. Most of the blooms recorded so far correspond to *Microcystis* or *Anabaena* species (Bonilla *et al.* 1995, De León & Yunes 2001). In rare instances, species like *Nodularia baltica* and *Planktothrix agardhii* have been reported as bloom forming (Pérez *et al.* 1999, Sommaruga 1995). Recently, new species of Nostocales have increased their frequency in shallow lakes of this region, also in well-studied

systems. These include species such as *Aphanizomenon* spp. and *Raphidiopsis mediterranea* (Kruk *et al.* 2002, Mazzeo *et al.* 2003). However, these species were found in greater abundances and in more systems than *C. raciborskii*. Our observations of a shift of the bloom forming cyanobacteria from *Microcystis* to Nostocales species, including *Cylindrospermopsis* dominance, are in agreement with other studies from temperate regions (Padisák 1990-1991, Saker & Griffiths 2001) and the description of *C. raciborskii* by Padisák (1997) as an "expanding" species.

In this study we found populations with morphological diacritical features corresponding to field populations of *C. raciborskii* described in the



**Figure 3.** Diagrams showing the range of length and width of different cells of *Cylindrospermopsis raciborskii* from the four studied systems in Uruguay.



**Figure 4.** Species-samples biplot for the first two PCA axis. Uruguayan morphotypes described here are included along with morphotypes from other climatic regions and taxonomic descriptions of species, labels according to Table I. Abbreviations: L for length and W for width, VC: vegetative cells, AC: apical cells with or without narrow ends, const.: with or without constrictions, H: heterocytes, A: akinetes, trichome shape: straight (Tr) and coiled (Tc).

literature. However, we found a high variability in most morphological characteristics within and among lakes. Moreover, it was difficult to clearly separate this species from other *Cylindrospermopsis* taxa, including *C. cuspis* and *C. africana*, due to the high overlap of cell sizes and shapes. Although the identification of these organisms at the species level was not trivial, Uruguayan morphotypes occurred together in the PCA analysis when compared with those of other regions. Based on this exercise, we suggest that more work is needed to clarify the taxonomical aspects as well as their changes during the life cycle of different species of the genera (Komárek 2002).

Morphological variation in *C. raciborskii* was not associated with latitudinal, continental or even country distribution. Moreover, our results showed that morphotypes belonging to the same country could differ as much as those from different continents. Despite the definition of the species as tropical (Padišák 1997), morphological characters were independent of climatic origin. This suggests that local factors are more relevant determining morphology than factors at a global scale. In nature, *C. raciborskii* displays a high morphological variability that is not completely explained by genetic distances (Saker *et al.* 1999). Furthermore, Gugger *et al.* (2005) showed that morphotypes from different continents might be genetically closer than

those from the same region. These authors also detected higher similarities among different species or even genera from the same continent than between *C. raciborskii* of different continents. Our results showing the overlap of *C. raciborskii* with other *Cylindrospermopsis* species gives further evidence for these conclusions. As morphological and genetic differences are not linked, at least at this level, we expect that local environmental conditions might explain this variation (Reynolds 1984, Neilan *et al.* 2003). This is the case for the frequency of akinetes, which increases markedly in cooler regions (Padisák 1997).

*Cylindrospermopsis raciborskii* is commonly observed over a wide range of ecological conditions (Padisák 1997). Most of what it is known about the environmental preferences of *C. raciborskii* is based upon tropical water bodies. However, its expansion towards temperate climates suggests that different ecological trends are emerging. For example, while *C. raciborskii* typically occurs in deep (>50 m) stratified tropical lakes, it seems to prefer shallower waters in temperate zones (i.e. <10 m) (Padisák 1997). However, experimental temperate and tropical isolates have similar light and temperature requirements (Briand *et al.* 2004, Chonodunkul *et al.* 2004). In this study, *Cylindrospermopsis* appeared in densities ranging from low abundances up to bloom conditions (maximum observed densities  $10^8$ – $10^9$  trichomes L<sup>-1</sup> according to Padisák 1997) in newly built reservoirs or natural lakes under current anthropogenic impacts. In the analysed database from Uruguayan subtropical lakes, we observed higher occurrence in shallow, well-mixed systems. Most of the environmental conditions found reflect expectations based on the literature (Padisák 1997). A further refinement of this study might involve the analysis of how morphological organism's attributes change with environmental conditions

The recent appearance and the low frequency of occurrence in southern latitudes of *C. raciborskii* might reflect the effects of climate warming or its spreading from tropical areas. As suggested by Padisák (1997), morphological variations during the *C. raciborskii* life cycle render difficult to clarify the association between morphological and environmental changes. Analysis of the changes in morphological characteristics of different population under different local environmental conditions along with genetic studies might help unravel these questions. In this sense, this working group emphasizes the necessity to analyse the morphological variability within the "*Cylindrospermopsis* complex" during a whole annual cycle.

## Acknowledgements

We are grateful to S. Bonilla, D. Conde and M. Meerhoff for their kind criticism and suggestions on the manuscript. We are thankful to Dermot Antoniades for the English review. We also thank N. Mazzeo, AGUAS DE LA COSTA S.A and URAGUA for the provision of data. This study was partially funded by AGUAS DE LA COSTA S.A., URAGUA and WOTRO foundation for the advance of tropical research.

## References

- Bonilla, S., Pérez M. C. & De León, L. 1995. Cyanophyceae plactónicas del lago Ton-ton, Canelones, Uruguay. **Hoehnea**, 22: 183-190.
- Branco, C. W. C. & Senna, P. A. C. 1991. The taxonomic elucidation of the Paranoá Lake (Brasilia, Brazil) problem: *Cylindrospermopsis raciborskii*. **Bulletin du Jardin Botanique National de Belgique**, 61: 85-91.
- Branco, C. W. C. & Senna, P. A. C. 1994. Factors influencing the development of *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* in the Paranoá reservoir, Brasília, Brazil. **Algological Studies**, 75: 85-96.
- Briand, J. F., Robillot C., Quiblier-Lloberas, C., Humbert, J.-F., Coute, A. & Bernard, C. 2002. Environmental context of *Cylindrospermopsis raciborskii* (Cyanobacteria) blooms in a shallow pond in France. **Water Research**, 36: 3183-92.
- Briand, J. F., Leboulanger, C., Humbert, J. F., Bernard, C. & Dufour, P. 2004. *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: selection, wide physiological tolerance, or global warming? **Journal of Phycology**, 40: 231-238.
- Chonodomkul, D., Yongmanitchai, W., Theeragool, G., Kawachi, M., Kasai, F., Kaya, K. & Watanabe, M. M. 2004. Morphology, genetic diversity, temperature tolerance and toxicity of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) strains from Thailand and Japan. **FEMS Microbiology Ecology**, 48: 345-355.
- De León, L. & Yunes, J. S. 2001. Short Communication. First report of a microcystin-containing bloom of the cyanobacterium *Microcystis aeruginosa* in the La Plata River, South America. **Environmental Toxicology**, 16: 110-112.
- De Tezanos-Pinto, P., Allende, L. & O'Farrell, I. 2006. Influence of free-floating plants on the structure of a natural phytoplankton assemblage: an experimental approach.

- Journal of Plankton Research**, 28: 1-10.
- Fabbro, L. D. & Duivenvoorden, L. J. 1996. Profile of a bloom of the cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju in the Fitzroy River in tropical central Queensland. **Marine & Freshwater Research**, 47: 685-94.
- Fastner, J., Heinze, R., Humpage, A. R., Mischke, U., Eaglesham, G. K. & Chorus, I. 2003. *Cylindrospermopsis* occurrence in two German lakes and preliminary assessment of toxicity and toxin production of *Cylindrospermopsis raciborskii* (Cyanobacteria) isolates. **Toxicon**, 42: 313-21.
- Figueredo, C. C., Gianni, A. & Bird, D. F. 2007. Does allelopathy contribute to *Cylindrospermopsis raciborskii* (Cyanobacteria) bloom occurrence and geographic expansion? **Journal of Phycology**, 43: 256-265.
- Gugger, M., Molica, R., Le Berre, B., Dufour, P., Bernard, C. & Humbert, J-F. 2005. Genetic Diversity of *Cylindrospermopsis* Strains (Cyanobacteria) Isolated from Four Continents. **Applied Environmental Microbiology**, 71: 1097-1100.
- Harding, W. R. 1996. The phytoplankton ecology of a hypertrophic shallow lake, with particular reference to primary production, periodicity and diversity. **PhD Thesis**. University of Cape Town, South Africa.
- Hawkins, P. R., Chandrasena, N. R., Jones, G. J., Humpage, A. R. & Falconer, I. R. 1997. Isolation and purification of *Cylindrospermopsis raciborskii* from an ornamental lake. **Toxicon**, 35: 341-346.
- Hill, H. 1970. *Anabaenopsis raciborskii* Woloszynska in Minnesota lakes. **Journal of Minnesota Academy of Science**, 36: 80-82.
- Hillebrand, H., Dürselen, C-D., Kirschtel, D., Pollinger, U. & Zohary, T. 1999. Biovolume calculation for pelagic and benthic microalgae. **Journal of Phycology**, 35: 403-424.
- Huszar, V. L. M., Silva, L. H. S., Marinho, M., Domingos, P. & Anna, C. L. S. 2000. Cyanoprokaryote assemblages in eight productive tropical Brazilian waters. **Hydrobiologia**, 424: 67-77.
- Izaguirre, I. & Vinocur, A. 1994. Typology of shallow lakes of the Salado River basin (Argentina), based on phytoplankton communities. **Hydrobiologia**, 277: 49-62.
- Jones W. W. & Sauter, S. 2005. **Office of Water Quality. Indiana Department of Environmental Management**. Indianapolis, In: School of Public and Environmental Affairs. Indiana University, Bloomington, IN. 41pp.
- Komárek, J. 1984. Sobre las cianofíceas de Cuba. (3) Especies planctónicas que forman florecimientos de las aguas. **Acta Botánica Cubana**, 19: 1-30.
- Komárek, J. 2002. **Proceedings of Freshwater Harmful algal blooms: health risk and control management**. Istituto Superiore di Sanità. Serena Melchiorre, Emanuela Viaggi and Milena Bruno (Eds). Rapporti ISTISAN 02/9. 103 p.
- Komárek, J. & Kling, H. 1991. Variation in six planktonic genera in lake Victoria (East Africa). **Algological Studies**, 61: 21-45.
- Komárek, J. & Komárková, J. 2003. Phenotype diversity of the cyanoprokaryotic genus *Cylindrospermopsis* (Nostocales); review 2002. **Czech Phycology**, 3: 1-30.
- Kruk, C., Mazzeo, N., Lacerot, G. & Reynolds, C. S. 2002. Classification schemes for phytoplankton: a local validation of a functional approach to the analysis of species temporal replacement. **Journal of Plankton Research**, 24: 1191-1216.
- Mazzeo, N., Rodríguez-Gallego, L., Kruk, C., Meerhoff, M., Gorga, J., Lacerot, G., Quintans, F., Loureiro, M., Larrea, D. & García-Rodríguez, F. 2003. Effects of *Egeria densa* Planch. beds on a shallow lake without piscivorous fish. **Hydrobiologia**, 506-509: 591-602.
- McGregor, G. B. & Fabbro, L. D. 2000. Dominance of *Cylindrospermopsis raciborskii* (Nostocales, Cyanoprokaryota) in Queensland tropical and subtropical reservoirs: Implications for monitoring and management. **Lakes & Reservoirs: Research and Management**, 5: 195-205.
- Neilan, B. A., Saker, M. L., Fastner, J., Törökne, A. & Burns, B. P. 2003. Phylogeography of the invasive cyanobacterium *Cylindrospermopsis raciborskii*. **Molecular Ecology**, 12: 133-140.
- Padisák, J. 1990-91. Occurrence of *Anabaenopsis raciborskii* Wolosz. in the pond Tómalom near Sopron, Hungary. **Acta Botanica Hungarica**, 36: 163-165.
- Padisák, J. 1997. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptative cyanobacterium: worldwide distribution and review of its ecology. **Archiv für Hydrobiologie. Supplementband. Monographic studies**, 107: 563-93.
- Pérez M. C., Bonilla, S., De León, L., Smarda, J. &

- Komárek, J. 1999. A bloom of *Nodularia baltica-spumigena* group (Cyanobacteria) in a shallow coastal lagoon of Uruguay, South America. **Algological Studies**, 93: 91-101.
- Reynolds, C. S. 1984. **The ecology of freshwater phytoplankton**. Cambridge University, London.
- Ryan, E. F., Hamilton, D. P. & Barnes, G. 2003. Recent occurrence of *Cylindropermopsis raciborskii* in Waikato lakes of New Zealand. **New Zealand Journal of Marine and Freshwater Research**, 37: 829-836.
- Saker, M. L., Thomas, A. D. & Norton, J. H. 1999. Cattle mortality attributed to the toxic cyanobacterium *Cylindropermopsis raciborskii* in an outback region of north Queensland. **Environmental Toxicology**, 14: 179-182.
- Saker, M. L. & Griffiths, D. L. 2001. Occurrence of blooms of the cyanobacterium *Cylindropermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju in a north Queensland domestic water supply. **Marine & Freshwater Research**, 52: 907-15.
- Seenayya, G. & Subba Raju, N. S. S. 1972. On the ecology and systematic position of the alga known as *Anabaenopsis raciborskii* (Wolosz.) Elenk. and a critical evaluation of the forms described under the genus *Anabaenopsis*. In: Desikachary, T. V. (Ed.) **Taxonomy and Biology of Blue-green Algae**. University of Madras, Madras, India, pp. 52-57.
- Singh, R. N. 1962. Seasonal variants of *Anabaenopsis raciborskii* Wolosz. **Hydrobiología**, 20: 87-91.
- Sommaruga, R. 1995. Microbial and classical food webs; visit to a hypertrophic lake. **FEMS Microbiology Ecology**, 17: 257-270.
- ter Braak, C. J. F. & Smilauer, P. 2003. **CANOCO for windows version 4.52**. Biometrics-Plant Research International, Wageningen, The Netherlands.
- Torgan, L. & García, M. 1989. Novas ocorrências (Cyanophytas e Chlorophyta) para a ficoflora planctônica do Rio Grande do Sul, Brasil. **Hoehnia**, 16: 57-64.
- Tóth, L. G. & Padišák, J. 1986. Meteorological factors affecting the bloom of *Anabaenopsis raciborskii* Wolosz. (Cyanophyta: Hormogonales) in the shallow Lake Balaton, Hungary. **Journal of Plankton Research**, 8: 353-63.
- Utermöhl H. 1958. Zur Vervollkommung der quantitativen Phytoplankton-Methodik. **Mitteilungen. Internationale Vereinigung fuer theoretische und angewandte Limnologie**, 9: 1-38.
- Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. **Marine Chemistry**, 10: 109-122.
- Wiedner, C., Nixdorf, B., Heinze, R., Wirsing, B., Neumann, U. & Weckesser, J. 2002. Regulation of cyanobacteria and microcystin dynamics in polymictic shallow lakes. **Archiv fuer Hydrobiologie**, 155: 383-400.
- Woloszynka, J. 1912. Das Phytoplankton einiger Javanian Seen mit Berücksichtigung des Sawa-Planktons. **Bulletin of the International Academy of Science Cracoviae**, 6: 649-709.
- Zalocar, Y. & Forastier, M. 2005. Biodiversidad de Cyanophyceae (Cyanobacteria) y especies toxigénicas del litoral fluvial argentino. **Temas de la Biodiversidad del Litoral fluvial argentino II INSUGEO, Miscelánea**, 14: 213-228.

Received June 2007

Accepted July 2008

Published online July 2008