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Ceratium furcoides (Levander) Langhans in reservoirs at the Ebro watershed, Spain and Sao Paulo state, Brazil

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ABSTRACT

Ceratium furcoides **(Levander) Langhans in reservoirs at the Ebro watershed, Spain, and Sao Paulo state, Brazil**

This study compares the morphological characteristics of the dinoflagellate *Ceratium furcoides* from three Ebro basin reservoirs (Spain) and from six reservoirs at São Paulo state (Brazil) with the aim to identify the distinct morphotypes of *Ceratium furcoides* in Spain and Brazil. We studied the physical and chemical variables to determine the trophic state of each reservoir. Unlike *Ceratium hirundinella*, *Ceratium furcoides* is not a frequent species within the plankton community at Ebro Basin reservoirs, however, this species has been responsible of one monospecific bloom at El Val reservoir. Nevertheless, both *Ceratium* species are considered invasive in South America. *Ceratium furcoides* has displaced *C. hirundinella* at São Paulo state reservoirs, reaching high densities, to the extent of forming monospecific blooms. Morphometric analysis was performed by optic and electronic microscopy (DIC and SEM respectively). The analysis shows significant differences in: i) total cell length, ii) horns length and iii) presence or absence of a fourth horn. While the microscopic study allows differentiating morphotypes according to the reservoir typology, a deeper study using molecular genetic techniques on the different populations of *C. furcoides* would allow to know better the relationship between the dinoflagellate dynamics and the reservoirs trophic states.

Key words: *Ceratium furcoides*, morphotypes, reservoirs, algal blooms

RESUMEN

Ceratium furcoides *(Levander) Langhans en embalses de la cuenca del Ebro en España y embalses del estado de Sao Paulo en Brasil*

El presente estudio compara las características morfológicas del dinoflagelado Ceratium furcoides *procedente de tres embalses de la cuenca del río Ebro y de seis del estado de Sao Paulo con la finalidad de identificar los distintos morfotipos de* Ceratium furcoides *de España y Brasil. Se estudiaron las características físico-químicas para determinar el estado trófico de cada embalse. Contrariamente a* Ceratium hirundinella*,* Ceratium furcoides *es una especie poco común en la comunidad fitoplanctónica de los embalses de la cuenca del río Ebro, si bien en fechas recientes ha sido el responsable de un bloom monoespecífico en el embalse de El Val. Sin embargo, consideradas estas dos como especies invasoras en América del Sur,* Ceratium furcoides *ha desplazado a* Ceratium hirundinella *en los embalses del estado de Sao Paulo, alcanzando grandes densidades y llegando a formar en algunos casos blooms monoespecíficos. El análisis morfométrico, realizado mediante microscopia óptica y electrónica (DIC y SEM, respectivamente), muestra diferencias significativas en: i) la longitud total de la célula, ii) la longitud de los cuernos y iii) la presencia o no de un cuarto cuerno. Si bien el estudio microscópico permitió determinar diferentes morfotipos en función de la tipología de los embalses, un estudio utilizando técnicas de genética molecular en las diferentes poblaciones de* C. furcoides *permitiría una mejor comprensión de su dinámica en relación al estado trófico del embalse.*

Palabras clave: Ceratium furcoides*, morfotipos, embalses, blooms de algas*

INTRODUCTION

The *Ceratium* genera is known to occur in many temperate and subtropical areas of the world (Meichtry et al., 2016); it is a characteristic summer inhabitant at temperate stratified lakes with low epilimnetic nutrient concentrations (Dokulil & Teubner, 2003, Grigorszky et al., 2003 a, Grigorsky et al., 2003 b, Grigorsky et al., 2019). Inorganic nutrients are often cited as factors that trigger blooms of *Ceratium* (Whittington et al., 2000). According to Bustamante-Gil et al. (2012) *Ceratium hirundinella* (O. F. Muller) Dujardin and *Ceratium furcoides* (Levander) Langhans are ecologically similar.

Ceratium presents different morphologies associated with its life cycle, cyclomorphosis, and variations in certain environmental factors (Hickel, 1988; Lindstrom, 1992; Popovský & Pfiester, 1990; Bustamante Gil et al., 2012; Almanza et al., 2016).

In terms of morphology, *Ceratium furcoides* is a relatively large dinoflagellate with a body length between 162 and 322 µm, and a width between 28 and 42(56) µm (Popovský & Pfiester, 1990). Cells are fusiform, dorsoventrally flattened, with ventral area strongly concave. It has a narrowly conical epi-valve with a large horn and two, rarely three, horns in the hypo-valve (Hickel, 1988; Bustamante Gil et al., 2012). According to the literature, the epivalve of *Ceratium furcoides* is conical whereas in *Ceratium hirundinella* it is helmet-shaped. Nevertheless, the main difference between the two species is the apical plates disposition. *Ceratium hirundinella* has the four apical plates reaching the apex while in *Ceratium furcoides* only the first three apical plates (1′, 2′ y 3′) reach the apex and the fourth does not reach the apex and is shorter.

In South America the first record of *C. hirundinella* was made by Thomasson in 1963 from the Andean Patagonian lakes of Argentina. *C. hirundinella* began to colonize inland water bodies of Argentina, Chile, Bolivia, Brazil, in northward direction (Thomasson, 1963; Parra, 1998; Donagh et al., 2005; Ferrareze & Nogueria, 2006). But the invasion of *C. furcoides* is more recent (Santos-Wisniewski et al., 2007; Meitchtry et al., 2014) and appears to occur in the opposite direction; first through the colonization of those areas not occupied by the genus and subsequently invading environments inhabited by *C. hirundinella*, where was observed the species replacement in many cases (Meichtry et al., 2016).

Ceratium furcoides has been detected as invader in different aquatic ecosystems (Silva et al., 2012), showing advantages on resources competition against any native plankton species. (Sukenik et al., 2012) such as bigger size, selective predation from zooplankton community, better swim and migration in the water column, also, it can form blooms with high densities (Almanza et al., 2016; Cavalcante et al., 2016). In adverse environmental conditions *Ceratium furcoides*, like all the *Ceratium* species, is also cysts forming, being deposited in the sediment toremain in the aquatic ecosystem (Bellinger & Sigee, 2010; Reynolds, 2006). During the water mixing periods they can germinate (Cavalcante, et al., 2013) or remain encysted until the physical and chemical conditions of the water return to their optimum, thus being able to develop and form new blooms.

Ceratium species are tolerant to the stress, due their swim skills that allow vertical daytime migrations to several microhabitats where light and nutriments are more favorable (Cavalcante et al., 2013), besides they can tolerate different temperature and transparency conditions, which guarantees their ability to occur during all the year (Silva et al., 2012).

In addition to the resources expended for algal growth, at the decomposition layers a big amount of oxygen is consumed that it can reach anoxic conditions in the environment (Wetzel, 2001) leading the mortality of algae consumer fishes (Silva et al., 2012). If there are a high load of nutrients in the waterbody, this also favors the development of *C. furcoides* (Santos-Wisniewski et al., 2007; Matsumura-Tundisi et al., 2010; Bustamante-Gil et al., 2012), emphasizing that the eutrophication process can intensify blooms of this dinoflagellate (Almanza et al., 2016).

Although *Ceratium* blooms are not considered to be toxic, it causes several impacts to continental aquatic ecosystems, affecting aesthetics and damaging the water supply system (Almanza et al., 2016), perhaps the better control of *C. furcoides* is the depletion of their resources (Santos-Wisniewski et al., 2007), generating competition between other phytoplankton organisms. Blooms of *C. furcoides* can be present in lakes and reservoirs with a trophic degree varying from mesotrophic to hypereutrophic (Bustamante-Gil et al., 2012; Silva et al., 2012; Meichtry et al., 2014).

Despite the blooms occurrence of this species does not present toxicity, this bio-invader characteristic has the capacity to perform other negative impacts to waterbody, such as: color and water taste alteration, obstruction of filters used in water treatment, besides, the damages to other organisms (Nicholls et al., 1980; Santos-Wisniewski et al., 2007; Silva et al., 2012; Meichtry et al., 2014).

In South America, *C. furcoides* has been recorded in the reservoir La Angostura (Bolivia) (Morales, 2016); also, in the lakes; Lobo (Argentina) (Bordet et al., 2017), Yacyretá (Argentina-Paraguay) (Meichtry et al., 2014), Río Grande II (Colombia) (Bustamante-Gil et al., 2012), and Lo Méndez (Chile) (Almanza et al., 2016). The dispersion and development in several Brazilian basins indicate that this species is in a process of expansion (Jati, et al., 2014). In Brazil the first record of the genera *Ceratium furcoides* was by Santos-Wisniewski et al. (2007) at the hydroelectric dam Furnas (Minas Gerais). In the last years, research in different areas of the country began to record it in some freshwater ecosystems (Silva et al., 2012; Meichtry et al., 2014), for example, at the state of Minas Gerais (Moreira et al., 2015), Billings (Matsumura-Tundisi et al., 2010), Guarapiranga (Nishimura, 2012, Nishimura et al., 2015), Jaguari, Jacareí (Hackbart et al., 2015) and Paiva Castro (Matta, 2016) reservoirs at São Paulo. At the high course of the Paraná river, Paraná st (Jati et al., 2014), Chopim, Capivari and São Jorge dams, at Rio Grande do Sul st, (Cavalcante et al., 2013), at the Itaúba dam (Cassol et al., 2014), Itá and Maia Filho reservoirs, Jacuí and Uruguai rivers (Cavalcante et al., 2013), Jacozinho and Toritama reservoirs in Pernambuco in the semiarid zones (Oliveira et al., 2016), Contas (Bahia) and Moxotó (Alagoas-Pernambuco) rivers, Sobradinho, Paulo Afonso (Bahia), Xingó (Alagoas-Sergipe) and Itaparica (Pernambuco-Bahia) reservoirs (Oliveira et al., 2011), Faxinal and Maestra reservoirs (Caixas do Sul, southern Brasil) (Cavalcante et al., 2016) besides, last records at pisciculture pools in São Carlos, São Paulost (Campanelli et al., 2016).

In Spain in general, contrary to *Ceratium hirundinella*, *Ceratium furcoides* is a not often specie. In the plankton community from the Ebro watershed, however, this specie has been the responsible of one monospecific bloom at El Val reservoir (Vicente et al., 2018), in the same way that in many Brazilian water bodies.

The main goal of this work is to compare the morphotypes of *C. furcoides* from Brazilian (southeast region) and Spanish (Ebro Basin) reservoirs with different trophic state with the aim to identify the distinct morphotypes of *Ceratium furcoides* in Spain and Brazil.

MATERIAL AND METHODS

Study Area

In Brazil, samplings were carried out during July 2015 at six reservoirs in São Paulo state: Atibainha (ATB), Barra Bonita (BB), Broa (BR), Igaratá (IGA), Itupararanga (ITUP) and Salto Grande (SG) (Fig. 1A). The Barra Bonita reservoir is the first of six waterbodies from a waterfall system of the Tietê River (Watanabe et al., 2016), together with the Salto Grande reservoir are in the most industrialized and populated area of the state (Rietzler et al., 2016). Itupararanga reservoir is located at the high course of the Sorocaba River that is the main tributary of Tietê River (Frascareli et al., 2015). Carlos Botelho (Lobo-Broa) is a eutrophic reservoir (maximum deep: 12 meters), located at Itirapina, (Tundisi et al., 2004; 2015), where agriculture is the permanent activity. The Atibainha reservoir, located near the

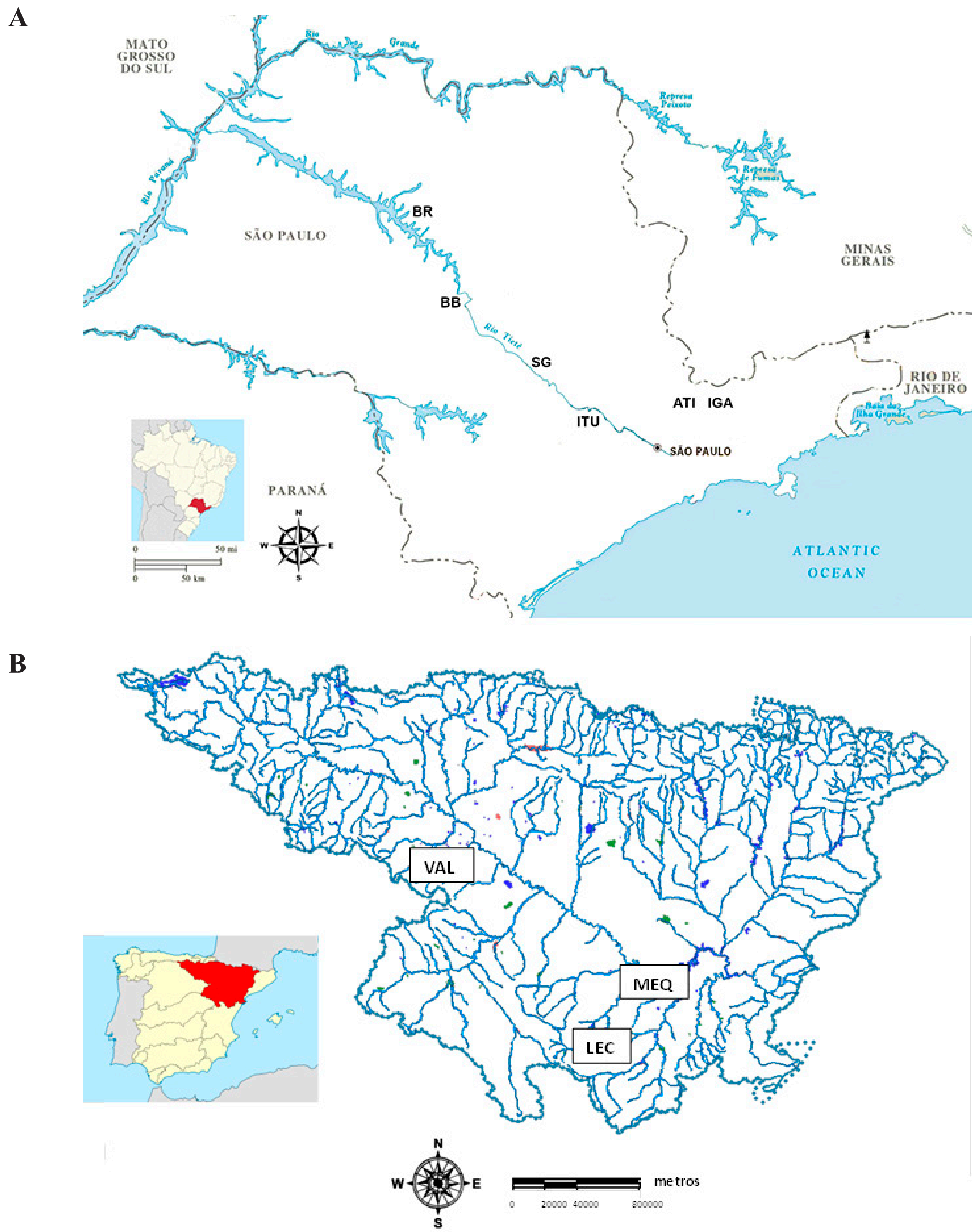

Figure 1. A) Location of the six studied reservoirs at São Paulo state, Brazil, identified by acronyms in the legend: BB: Barra Bonita, BR: Broa, SG: Salto Grande, ITU: Itupararanga, ATI: Atibainha. IGA: Igaratá. Source: Adapted from Frascarelli (2016). B) Location of the three studied reservoirs at the Ebro River watershed in Spain. LEC: Lechago, MEQ: Mequinenza and VAL: El Val. (Adapted from Ebro Basin Authority). *A) Localización de los seis embalses estudiados en el estado de São Paulo, Brasil, identificados por siglas en la leyenda: BB: Barra Bonita, BR: Broa, SG: Salto Grande, ITU: Itupararanga, ATI: Atibainha. IGA: Igaratá. Fuente: Adaptado de Frascarelli (2016). B) Localización espacial de los tres embalses estudiados en la cuenca del río Ebro, España. LEC: Lechago, MEQ: Mequinenza y VAL: El Val. (Adaptado de Confederación Hidrográfica del Ebro).*

city of Narazé Paulista, is part of the Cantareira System and is destined to drinking water supply for the metropolitan region of São Paulo (Carvalho, 2003). The Igaratá reservoir is located at Paraíba do Sul River basin near the city of Igaratá (state of São Paulo) (Frascareli, 2016).

In Spain, the samplings were carried out during July 2015 in three reservoirs of Ebro River watershed (northeast of Iberian Peninsula): Lechago (LEC), El Val (VAL) and Mequinenza (MEQ) (Fig. 1 B). Lechago reservoir is located at the eastern end of the Almazán basin, in the municipality of Calamocha, province of Teruel. The reservoir has a total capacity of 18.16 hm^3 and was built for water regulation of Pancrudo River. It is a monomictic reservoir, of calcareous geology, located in a non-humid zone belonging to headwaters and upper reaches section, with an average annual temperature less than 15 ºC. The Lechago reservoir is part of the Registry of Protected Areas elaborated by the Hydrographic Confederation of the Ebro, in response to Article 6 of Water Framework Directive, in the category of areas for water extraction for human consumption.

El Val reservoir is at Los Fayos municipality near Moncayo mountain range (province of Zaragoza). It is located on El Val River, tributary of the Ebro from the right bank. It regulates the waters of El Val River and receives some water contribution, arriving directly to the reservoir, by a pipeline from Queiles River. The reservoir has a total capacity of 25 hm^3. It is monomictic, calcareous, humid zone, belonging to riverheads and upper reaches section, with annual average temperature less than 15 ºC.

El Val reservoir is part of the Registry of Protected Areas prepared by the Hydrographic Confederation of the Ebro, in response to Article 6 of the Water Framework Directive, within the categories of areas of water extraction for human consumption and areas of habitats or species protection, ZEPA "Sierra de Moncayo - Los Fayos - Sierra de Armas (Natura 2000 Network, code: ES0000297).

Mequinenza reservoir dam is located at the municipality of Mequinenza, in the province of Zaragoza and regulates Ebro River waters. It is a large reservoir with a total capacity of 1534 hm^3. It is monomictic, calcareous geology, located in a non-humid zone and belonging to a low section of the Ebro main river axis. The Mequinenza reservoir is part of the Registry of Protected Areas prepared by the Hydrographic Confederation of the Ebro, in response to Article 6 of the Water Framework Directive, within the following categories: areas of water extraction for human consumption, sensitive areas under the framework of Directive 91/271 / EEC and habitat or species protection zones (Natura 2000 Network Point: SPA, ES0000182 "Valcuerna, Serreta Negra and Liberola") and its waters are mainly used for hydroelectric production and water supply to population.

In Spain, the reservoir sampling stations were stablished in the deepest part of each reservoir, at 300-500 m from the dam, during the summer months. A single integrated sample was taken in each reservoir, representative of the photic area, corresponding to water column until the depth arriving 1 % of surface light intensity (PAR). The integrated sample was carried out by means of a transparent reinforced wall plastic tube with inner light of 25 mm, weighted at one of its ends, which integrated the water column corresponds to the photic zone and whose content subsequently was mixed in a PET container. Then, from the integrated sample, different quantities of water were taken for in-situ processing, making additional measurements of physical, chemical and biological variables along the vertical profile by means of a 12 probes profiler (Sea-Bird 19 plus V2 (SBE19). After sampling, we proceed to water filtration for pigments extractions (Shoaf & Lium, 1976; Jeffrey & Humphrey, 1975) and laboratory analysis of other physical and chemical variables (Ammonium, total Phosphorus, and total Nitrogen) (Verdow et al., 1978; APHA, 1998; Ferree & Shannon, 2001) and phytoplankton variables (species identification, density and biovolume (MFIT-2013, version 1).

In Brazil the samples were collected at the river inflow zone, central and dam areas of each reservoir. A single integrated water column sample was collected, considering 2.7 times the depth of Secchi disc (m) (Cole, 1994). Water samples was analyzed at laboratory for the following variables: total phosphorus (Valderrama, 1981) and chlorophyll-*a* corrected for phaeophytin using 90 % acetone extraction (Lorenzen, 1967; Wetzel & Likens, 1991).

The Trophic state index (TSI) was determinate according to Lamparelli (2004) based in total phosphorus and chlorophyll-*a*. The limits used were: ultraoligotrophic (IET ≤ 47); oligotrophic (47 < IET ≤ 52); mesotrophic (52 < IET ≤ 59), eutrophic (59 < IET ≤ 63); supereutrophic (63 < IET ≤ 67) e hipereutrophic (IET > 67).

For phytoplankton analysis composition, as in the Spanish reservoirs, was used the methodology described in the Analysis Protocol of phytoplankton calculation and metrics in lakes and reservoirs (MFIT-2013, version 1) published by the Ministry of Agriculture, Food and Environment, Spain.

In both, Spain and Brazil, taxonomy and counting of algal individuals were made according Utermöhl method with a Nikon-ECLIPSE TE-2000S optical microscope and for fine morphological and morphometric characterization a Hitachi S-4800 electron microscope (SEM). The taxonomical analysis was based on Popovský and Pfiester (1990).

Significant difference between populations of *Ceratium furcoides* from the different reservoirs according to total length, width, apical horn length, antapical horn length, post-equatorial horn and fourth horn, were performed by statistical treatment (descriptive statistics, one-way ANOVA analysis ($P < 0.05$) and Tukey tests (Martínez-González et al., 2006)). Calculation of Pearson coefficient was used to find *Ceratium furcoides* relationships between different morphometric measurements in each reservoir (total length, width, apical horn length, antapical horn length, post-equatorial horn and fourth horn).

RESULTS

Water bodies located in the southeastern region of Sao Paulo state (Brazil) and the Ebro watershed in (Spain) presented different trophic states, from oligotrophic to hypereutrophic. The Igaratá, Atibainha, and Itupararanga reservoirs (Sao

Table 1. TSI of the different studied reservoirs along with the density and biovolume of *Ceratium furcoides* at each one of them. *TSI de los distintos embalses estudiados junto con la densidad y el biovolumen de* Ceratium furcoides *en cada uno de ellos.*

Reservoir	Sampling area	Total algae (Cel/ml)	*Ceratium* (Cel/ml)	*Ceratium* (%)	Total biovol (mm^3/L)	*Ceratium* (mm^3/L)	*Ceratium* (%)	TSI
Mequinenza	Dam Int 13 m	6867	1.20	0.02	0.78	0.13	16.60	Oligotrophic
Mequinenza	Dam 6 m	8240	2.00	0.03	1.20	0.26	21.70	Oligotrophic
Mequinenza	Dam 9 m	6525	2.00	0.04	1.07	0.26	24.40	Oligotrophic
Igarata	River	4592	467	10.17	56.98	52.47	92.07	Mesotrophic
Igarata	Center	5620	497	8.84	60.44	55.77	92.28	Mesotrophic
Igarata	Dam	4847	3241	66.86	365.70	363.83	99.49	Mesotrophic
Atibainha	River	198485	3209	1.62	392.61	360.31	91.77	Mesotrophic
Atibainha	Center	156078	2028	1.3	240.59	227.69	94.64	Mesotrophic
Atibainha	Dam	209157	1077	0.51	141.82	120.88	85.24	Mesotrophic
Lechago	Dam Int 7 m	25521	0.33	0.001	0.38	0.016	4.30	Mesotrophic
Itupararanga	River	464807	4903	1.05	673.72	550.44	81.70	Eutrophic
Itupararanga	Center	142837	366	0.26	106.03	41.08	38.74	Mesotrophic
Itupararanga	Dam	346307	146	0.04	71.43	16.43	23.00	Eutrophic
El Val	Dam Int 6 m	1588	382	24.1	43.54	43.44	99.80	Eutrophic
El Val	Dam 3 m	1140	842	73.9	95.75	95.73	99.98	Eutrophic
El Val	Dam 7 m	548	359	65.6	40.85	40.83	99.96	Eutrophic
Broa	River	1378414	94	0.007	263.58	10.56	4.01	Supereutrophic
Broa	Center	955358	0	0.000	345.91	0.00	0.00	Supereutrophic
Broa	Dam	599659	115	0.019	476.29	12.91	2.71	Supereutrophic
Barra Bonita	River	842075	7422	0.88	941.43	833.28	88.51	Hipereutrophic
Barra Bonita	Center	4998526	3146	0.06	969.33	353.26	36.44	Supereutrophic
Barra Bonita	Dam	3029142	648	0.02	523.98	72.77	13.89	Eutrophic
Salto Grande	River	1624999	355	0.02	266.28	39.90	14.99	Hipereutrophic
Salto Grande	Center	2072207	387	0.02	298.84	43.42	14.53	Supereutrophic
Salto Grande	Dam	146201	94	0.06	35.96	10.56	29.37	Supereutrophic

Paulo) and Lechago (Spain), were classified as mesotrophic, while Broa, Barra Bonita and Salto Grande reservoirs (Sao Paulo) and El Val reservoir (Spain) were the most eutrophics (supereutrophic to hypereutrophic) (Table1).

Morphotypes of *C. furcoides* in Brazilian and Spanish reservoirs showed differences in size, biovolume and number of horns (Fig. 2A, 2B). Organisms with three or four horns were observed in Mequinenza, Itupararanga and Barra Bonita, while in Igarata, Atibainha, Broa, El Val and Salto Grande the individuals do not present a fourth horn. However, only in Lechago all individuals present the fourth horn (Fig. 2A).

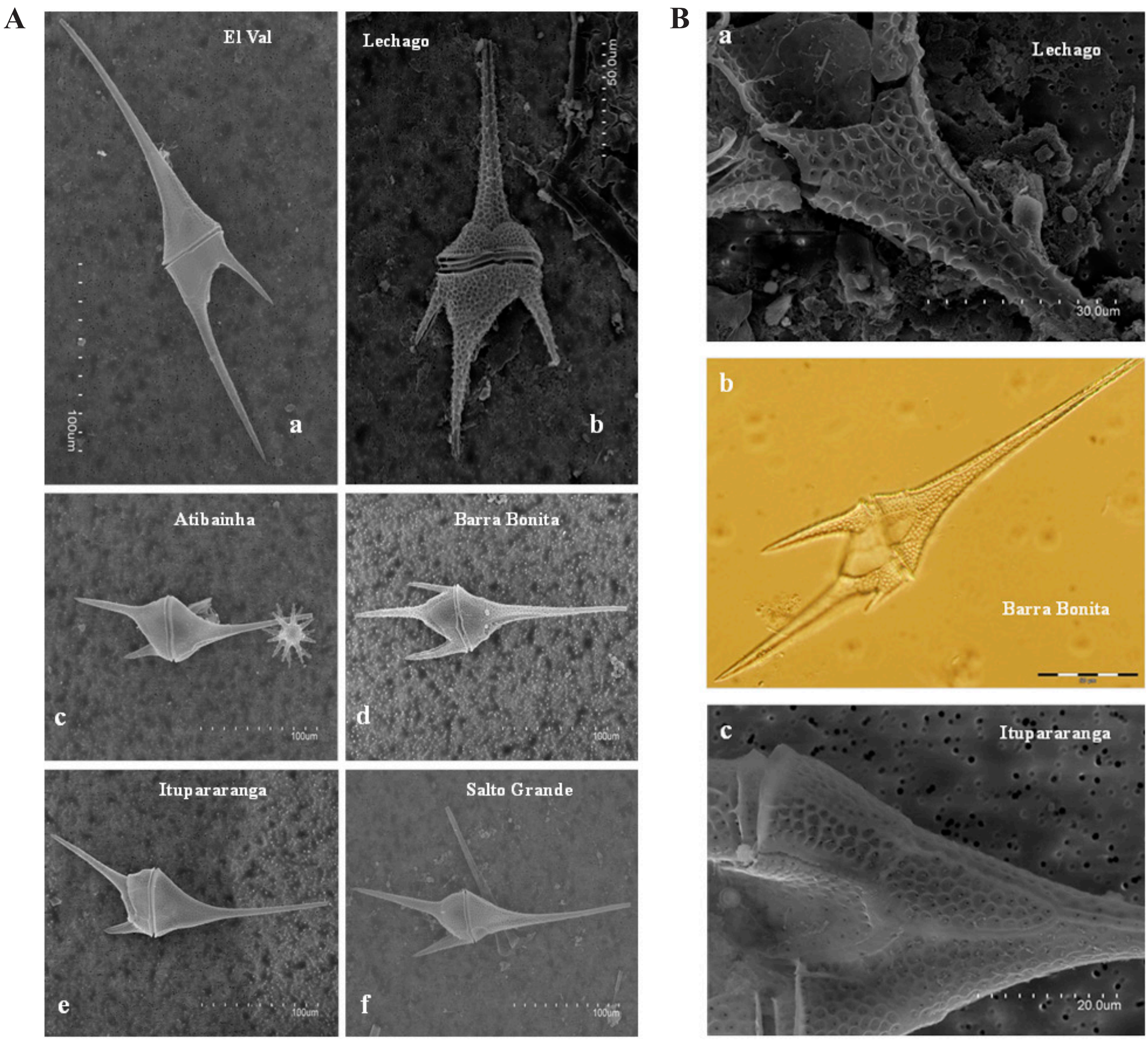

Figure 2. A) SEM images of *Ceratium furcoides* from several reservoirs where its morphological variability can be appreciated. a) El Val reservoir. b) Lechago reservoir. c) Atibainha reservoir. d) Barra Bonita reservoir. e) Itupararanga reservoir. f) Salto Grande reservoir. B). SEM images showing the characteristic plate 4' of *Ceratium furcoides* from the reservoirs: a) Lechago, b) Barra Bonita and c) Itupararanga. *A) Imágenes SEM de* Ceratium furcoides *de distintos embalses en las que se aprecia su variabilidad morfológica. a) Embalse de El Val. b) Embalse de Lechago. c) Embalse de Atibainha. d) Embalse de Barra Bonita. e) Embalse de Itupararanga. f) Embalse de Salto Grande. B) Imágenes SEM donde se puede apreciar la característica placa 4' de* Ceratium furcoides *de los embalses de: a) Lechago, b) Barra Bonita y c) Itupararanga.*

The morphometric study shows that the longer length individuals were present in El Val, with an average of 297 µm, followed by those in Barra Bonita (258 µm), whereas that the less length were in Lechago (185 µm) and Broa (156 µm) (Fig. 3a). The Tukey-test shows significative differences between the populations of El Val (Spain) and the rest of reservoirs, on the other hand the popula-

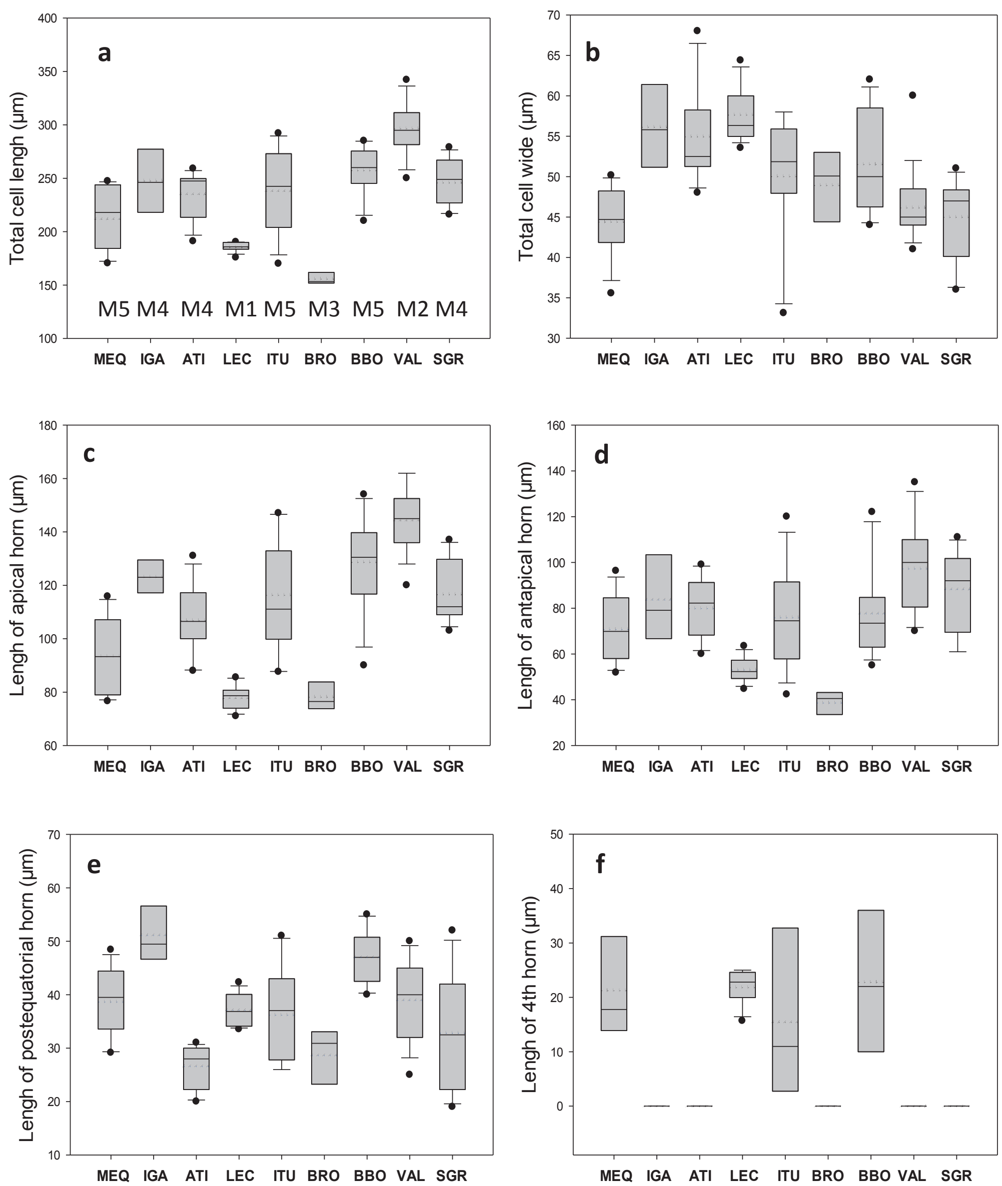

Figure 3. Morphometric measurements of *Ceratium furcoides* from the studied reservoirs (µm) with indication of the different morphotypes. a) Total length of the cell. Morphotype Types: morphotype 1 (M1), morphotype 2 (M2) morphotype 3 (M3) morphotype 4 (M4) and morphotype 5 (M5). b) Total width of the cell (cingulum). c) Length of the apical horn. d) Length of the antapical horn. e) Post-equatorial horn length. f) Length of the fourth horn. *Medidas morfométricas de* Ceratium furcoides *de los embalses estudiados (µm) con indicación de los distintos morfotipos. a) Longitud total de la célula. Morfotipos: morfotipo 1 (M1), morfotipo 2 (M2) morfotipo 3 (M3) morfotipo 4 (M4) y morfotipo 5 (M5). b) Ancho total de la célula (cíngulo). c) Longitud del cuerno apical. d) Longitud del cuerno antapical. e) Longitud del cuerno post-ecuatorial. f) Longitud del cuarto cuerno.*

Table 2. Significative morphometric differences among the measurements of *Ceratium furcoides* from the different reservoirs, according to the Tukey test. *Diferencias morfométricas significativas entre las medidas de* Ceratium furcoides *de los distintos embalses según la prueba de Tukey.*

Different populations (*) depending on Tukey's Test

	MEQ	IGA	ATI	LEC		BRO	BBO	VAL	SGR
MEQ	0					*		*	
IGA		0		*		*		*	
ATI			0			*		*	
LEC				0	*		*	*	*
ITU					0	*		*	
BRO						0	*	*	*
BBO							0	*	
VAL								0	*
SGR									0

According to total length (µm)

	MEQ	IGA	ATI	LEC	ITU	BRO	BBO	VAL	SGR
MEQ	0		*	*					
IGA		0							*
ATI			0					*	*
LEC				0					*
ITU					0				
BRO						0			
BBO							0		
VAL									
SGR									0

According to width (µm)

	MEQ	IGA	ATI	LEC	ITU	BRO	BBO	VAL	SGR
MEQ	0	*			*		*	*	
IGA		0		*		*		*	
ATI			0	*		*	*	*	
LEC				0	*		*	*	*
ITU					0	*		*	
BRO						0	*	*	*
BBO							0		
VAL								0	*
SGR									0

According to the AP horn (µm)

	MEQ	IGA	ATI	LEC	ITU	BRO	BBO	VAL	SGR
MEQ	0					*			
IGA		0				*			
ATI			0			*			
LEC				0				*	*
ITU					0	*			
BRO						0	*	*	*
BBO							0		
VAL								0	
SGR									0

According to the AN horn (µm)

	MEQ	IGA	ATI	LEC	ITU	BRO	BBO	VAL	SGR
MEQ	0		*						
IGA		0	*	*	*	*		*	*
ATI			0				*		
LEC				0					
ITU					0		*		
BRO						0	*		
BBO							0	*	*
VAL								0	
SGR									0

According to the PE horn (µm)

	MEQ	LEC	ITU	BBO
MEQ	0			
LEC		0		
ITU			0	
BBO				0

According to the 4th horn (µm)

tions of Lechago (Spain) and Broa (Brazil) do not have significant differences between them but are different against the rest of reservoirs (Table 2 and Fig. 3a). The rest of reservoirs: 5 from Brazil (Igarata, Atibainha, Salto Grande, Itupararanga and Barra Bonita) and 1 from Spain (Mequinenza) does not show any significant difference among their populations (Table 2 and Fig. 3a).

Regarding the individuals width (cingulum), the highest average was present in Lechago population with, 58 µm, and the lowest, width average 44.5 µm, at Mequinenza (Fig. 3b). Width mean measurement of *C. furcoides* populations presents the lowest significant differences among them (Table 2).

The major length of the apical horn was present in individuals from El Val, with an average of 144 µm, and the less length was in Lechago, average of 78 µm (Fig. 3c). The major length of the antapical horn as well was present in El Val (98 µm), nevertheless, the minor average length was in the individuals from Broa (39 µm) (Fig. 3d).

The average length of the post-equatorial horn varies from 27 µm (Atibainha) to 51 µm (Igaratá) (Fig. 3e). From all morphometric measurements is the length of the fourth horn which presents the major dispersion and do not shows significant difference between the populations (Table 2).

Correlation coefficients were performed with these different measurements and the major positive correlation was between the total length and length of the antapical horn, followed by the correlation of total length and the apical horn, except in Broa and Lechago reservoirs (Table 3) that in these waterbodies *Ceratium furcoides* shows the greatest correlation between the total length and the length of the apical horn (Table 3).

While measurement of the width (cingulum) is only significantly correlated in the reservoirs of Igaratá and Broa, in Itupararanga reservoir the correlation (r = 0.56) is with the apical horn. In Igaratá reservoir the significant correlation is with the apical horn (r = 0.87) as well as with the antapical (r = 0.92). Broa reservoir presents the highest correlation (r = 0.92) between the measurement of the cingulum and the length of the post-equatorial horn (Table 3).

The post-equatorial horn does not show any significant correlation among Mequinenza, Lechago and Barra Bonita reservoirs. However, El Val presents a positive correlation with the antapical horn. Igaratá and Atibainha reservoirs shows a positive correlation with the total length of the cell, while in Itupararanga, Broa and Salto Grande, beside their positive correlation with the total length, shows a correlation as well with the length of the antapical horn, cingulum length and length of the apical horn, respectively (Table 3).

In Lechago and Barra Bonita reservoirs the fourth horn does not show a positive significant

Table 3. Correlation coefficients (Pearson r) among the different *C. furcoides* morphometric measurements from each of the studied reservoirs. *Coeficientes de correlación (r de Pearson) entre las distintas medidas morfométricas de* C. furcoides *de cada uno de los embalses estudiados.*

Correlation Coefficients (r of Pearson) between the different measures in each reservoir

MEQ	L	B	AP	AN	PE	4ª
L	1.00					
B	0.20	1.00				
AP	**0.66**	-0.10	1.00			
AN	**0.89**	0.30	0.29	1.00		
PE	0.15	-0.19	-0.25	0.40	1.00	
4ª	**0.78**	-0.66	**0.59**	**0.52**	0.12	1.00

IGA	L	B	AP	AN	PE
L	1.00				
B	**0.93**	1.00			
AP	**0.86**	**0.87**	1.00		
AN	**0.95**	**0.92**	**0.90**	1.00	
PE	**0.56**	0.27	0.22	0.32	1.00

ATI	L	B	AP	AN	PE
L	1.00				
B	0.05	1.00			
AP	**0.77**	-0.27	1.00		
AN	**0.80**	0.11	0.42	1.00	
PE	**0.52**	0.33	0.20	0.42	1.00

LEC	L	B	AP	AN	PE	4ª
L	1.00					
B	0.39	1.00				
AP	**0.69**	0.18	1.00			
AN	0.18	-0.03	-0.28	1.00		
PE	0.46	0.41	0.22	0.29	1.00	
4ª	-0.04	0.13	-0.11	0.03	-0.30	1.00

ITU	L	B	AP	AN	PE	4ª
L	1.00					
B	**0.65**	1.00				
AP	**0.78**	**0.56**	1.00			
AN	**0.80**	0.43	0.33	1.00		
PE	**0.62**	0.31	0.46	**0.71**	1.00	
4ª	**0.69**	-0.52	0.38	0.07	**0.57**	1.00

BRO	L	B	AP	AN	PE
L	1.00				
B	0.41	1.00			
AP	**0.98**	0.31	1.00		
AN	**0.54**	0.22	0.42	1.00	
PE	**0.54**	**0.97**	0.45	0.40	1.00

BBO	L	B	AP	AN	PE	4ª
L	1.00					
B	0.32	1.00				
AP	0.30	-0.07	1.00			
AN	**0.51**	-0.34	0.20	1.00		
PE	0.13	-0.23	0.43	0.30	1.00	
4ª	-0.89	-0.18	-0.61	0.04	0.32	1.00

VAL	L	B	AP	AN	PE
L	1.00				
B	0.34	1.00			
AP	**0.68**	0.24	1.00		
AN	**0.74**	0.27	0.21	1.00	
PE	0.50	0.37	0.13	**0.63**	1.00

SGR	L	B	AP	AN	PE
L	1.00				
B	0.18	1.00			
AP	0.42	0.11	1.00		
AN	**0.65**	-0.37	-0.01	1.00	
PE	**0.75**	0.27	**0.76**	0.44	1.00

correlation with any other morphometric measurement, however in Mequinenza this correlation is present with the total length, apical and antapical horn length. Nevertheless, in Itupararanga the positive significant correlation of the fourth horn is with the total length and the post-equatorial horn length (Table 3).

Ceratium furcoides population from Lechago reservoir can be defined as a morphotype (M1), since all the organisms observed have four horns. This population has significant differences compared against the rest of reservoir populations, in addition to, presents the lowest variability among their individuals so, is the most homogenous population of the study.

The second morphotype (M2), can be the distinctive of El Val reservoir, where all the individual has three horns and the biggest size. These individuals are the most stylized, because they present the largest horns (apical and antapical), also, this population produce the only bloom observed in the Spanish reservoirs.

A third morphotype (M3) was found in the population of Broa, in this reservoir all the individuals have three horns and the great difference compared with the rest is their body size, since they are the smallest.

Finally, are two more morphotypes, the fourth (M4), where all the organisms present three horns (Igaratá, Atibainha and Salto Grande). The fifth (M5) with populations whose individuals have three or four horns, (Mequinenza, Itupararanga and Barra Bonita). The principal characteristic of this morphotype is the great dispersion of the length of the fourth horn compared against the other populations.

DISCUSSION

The morphological variability of *Ceratium furcoides* has been studied by important authors associating it mainly to several physico-chemical and environmental factors and to its life cycle during the seasonal succession at its water body (Almanza et al., 2016; Cavalcante et al., 2013; Lindström, 1992). In contrast, in the present work the morphological variability of *Ceratium furcoides* between several reservoirs has been studied at a given time of the succession.

Previous works have described how most individuals present two or three antapical horns depending on the advance of the succession, as in the case of Lake Lo Méndez (Almanza et al., 2016) or in the case of the reservoirs of Caxias do Sul (Cavalcante et al., 2013) or all individuals presented two antapical horns, as in Lake Erken (Lindström, 1992). In the different water bodies described at that time, individuals with two and three antapical horns coexisted or, on the other hand, all had two antapical horns. In our case, in the studied reservoirs all the possibilities are given, identifying three groups: 1) all the individuals presented three antapical horns, case of the Lechago reservoir, 2) individuals with two and three horns coexist, Mequinenza, Itupararanga and Barra Bonita and 3) all the individuals presented two antapical horns, Igarata, Atibainha, Broa, El Val and Salto Grande. Therefore, it is evident the high morphological variability of *Ceratium furcoides* and the need to deepen in the typical seasonal succession of each water body to be able to know if these groups remain along all the year and to associate this variability to the different physico-chemical and environmental conditions.

Ceratium abundance was associated with nutrient availability, especially phosphate and nitrate (Cavalcante et al., 2013). Similar results were found by Grigorszky et al. (2003), for dinoflagellates in Hungarian water bodies, and Cardoso et al. (2010), in subtropical reservoirs of Caxias do Sul.

In the urban lake Lo Mendez, Chile, the presence of *C. furcoides* was favored by the high nutrients concentration (Almanza et al., 2016) in concordance with previous research (Matsumura-Tundisi et al., 2010; Bustamante et al., 2012; Silva et al., 2012). In Fetsui Reservoir, Taiwan, the abundance of *Ceratium furcoides* was positively correlated with phosphorus, total organic carbon, bacterial numbers and the biochemical oxygen demand in the water (Wu & Chou, 1998). In Furnas reservoir, Brasil, Silva et al. (2012) related species abundance of *C. furcoides* with low temperatures and high nutrient concentrations (nitrate and nitrite) and in Colombia has been positively related to ammonium concentrations being considered a highly variable species at both temporal and spatial scales (Bustamante-Gil et al., 2012).

Reservoirs where high densities of *C. furcoides* were found in all mesotrophic, eutrophic or hypereutrophic conditions (Wu & Chou, 1998; Matsumura-Tundisi et al., 2010; Bustamante-Gil et al., 2012). Oliveira et al. (2016) also accepted the hypothesis that *C. furcoides* invasion and establishment in two reservoirs of the Capibaribe watershed was related to eutrophication. However, these studies differ from studies carried out by Ginkel et al. (2001), Koenig & Lira (2005) and Moreira et al. (2015) that indicated the presence of *C. furcoides* in ecosystems with low nutrients concentrations and also, the work of Donald et al. (2013) detected no significant influence of the ammonia and nitrate concentrations over the growth of *C. furcoides*. In our case, only one reservoir was classified as oligotrophic, Mequinenza, and the rest of the reservoirs studied were mesotrophic, eutrophic or hypereutrophic and even one of them was classified in its entirety as supereutrophic, the Broa reservoir, just the reservoir where the length of *C. furcoides* was the shortest of all those studied reservoirs.

The individuals of *C. furcoides* found in the samples measured 150.5-297 µm in length (L) and the width (W) varies from 33.1 µm to 68 µm. This morphometric and morphological measurements are consisting with the previous registers for this specie (Hansen & Flaim, 2007), Trentino Province, Italy, L: 165-260 µm, W: 30-50 µm; Oliveira et al. (2011), in semiarid region of Brasil, L: 194-228 µm, W: 43-60 µm; Pandeirada et al. (2013), Portugal, L: 255 µm, W: 44 µm; Almanza et al. (2016), Central Chile, L: 109.9-311.5 µm, W: 36.4-63.1 µm). The individuals described by Pandeirada et al. (2013) in Portugal by their measures and the stylized figure would remain our morphotype M2 together with the individuals described at El Val reservoir in Spain. Nevertheless, according to Nishimura et al. (2015) *C. furcoides* sampled at Billings and Guarapiranga reservoirs (São Paulo state, Brazil), presented a narrowly spindle-shaped, strongly dorsoventrally flattened, 114–154 µm long, 42–54 µm wide; epitheca formed into a narrow horn without shoulders; hypotheca broad and short, drawn out into two posterior horns of different lengths; plates smooth and with shallow net-like ornamentation. The length of those individuals is lower than the average length in all the reservoirs of the present study. On the other hand, the individuals found by Cavalcante et al. (2017) in the State of Paraná, Southern Brazil (L: 86–190 µm; W: 26–57.8 µm); Moreira et al. (2015), Southeast Brazil (L: 180-209 µm; W: 51-60 µm); Meichtry et al. (2014), Paraná river, Argentina-Paraguay (L: 146-212 µm; W: 37-45 µm); Jati et al. (2014), Upper Paraná, Brazil (L: 147.6-184.5 µm; W: 36.9-49.2 µm) and Cassol et al. (2014), Rio Grande do Sul, Brasil (L: 150-173 µm; W: 39-60 µm), would remain by their measurements between M1 and M3 morphotypes, the shortest ones. In absence of further data, the belonging of one or another morphotype would depend on the possession by all observed individuals of only three or four horns.

In the cases of very small and robust individuals, there would be a doubt and then, the same comment made by Cavalcante et al. (2017), they could be interpreted as *Ceratium rhomvoides* Hickel, a species closely related to *C. furcoides* (Hickel 1988) also described in Portugal by Pandeirada et al. (2013).

The contrasting behavior of *Ceratium* species in divergent lake types (with different climatic, morphometric, geological, hydrological, and trophic features) explains the existence of ecotypes of these species adapted to diverse environmental conditions and exhibiting high intra- and interpopulation morphological variability (Salmaso, 2003). Bearing in mind the rapid dispersion of this species in South America and its capacity to form blooms with the negative consequences that this entails it is demonstrated the need to acquire a deeper knowledge of these species.

CONCLUSIONS

This study has constituted the first step identifying the distinct morphotypes of *Ceratium furcoides* in Spain and Brazil and corroborates the different morphologies associated with its life cycle and its relationship with physico-chemical and environmental factors.

Have been found significant differences in the morphometric analysis of the individuals of *C. furcoides* in some reservoirs: i) Total length; ii)

Length of the horns; iii) Presence or absence of the fourth horn, which have led to define the five different morphotypes.

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