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Expansion of invasive *Ceratium furcoides* (Dinophyta) toward north-central Brazil: new records in tropical environments

Avanço da invasão de sistemas aquáticos por *Ceratium furcoides* (Dinophyta) em direção ao centro-norte brasileiro: novos registros em ambiente tropicais

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Abstract: Aim: We record new occurrences of the invasive species *Ceratium furcoides* in reservoirs and their affluents in the Paraná River basin, State of Goiás (GO), central-western Brazil, and in some localities in the São Francisco River basin, northeastern region. **Methods:** Qualitative and quantitative phytoplankton samples were collected from Corumbá Reservoir and Cascatinha Falls, Caldas Novas, GO, and João Leite Reservoir, Goiânia, GO, both in the Paraná River basin, and samples from the São Franscisco River basin. Specimens of *C. furcoides* were observed with optical, epifluorescence and scanning electron microscopy. **Results:** The individuals of *C. furcoides* from these environments agreed morphologically with populations in other reservoirs in Brazil and other locations, especially concerning the tabulation and the shape of the fourth apical plate. These environments ranged from oligotrophic to eutrophic conditions. Physical and chemical variables of these waterbodies, compared to other environments where this species was found, demonstrate that *C. furcoides* is a highly eurytopic species. The dispersal pattern of *C. furcoides* seems to be more complex than upstream-downstream regulation, since the species occurs in high-altitude environments and systems upstream from previously recorded locations. **Conclusion:** An analysis based on the areas of occurrence and the chronology of the records demonstrated that *C. furcoides* has spread toward northern Brazil. Studies of the relationships among populations recorded in other parts of Brazil and South America are required in order to develop accurate models of dispersal for this invasive species, and will facilitate the development of management policies for aquatic systems in Brazil.

Keywords: invasive species; São Francisco River basin; Corumbá Reservoir; João Leite Reservoir; central-western Brazil; dinoflagellate.

Resumo: Objetivo: O estudo tem como objetivo o registro de ocorrência de *C. furcoides* em reservatórios das bacias do rio Paraná e seus afluentes no estado de Goiás, Centro-oeste brasileiro, e em algumas localidades da Bacia do rio São Francisco, região Nordeste. **Métodos:** Amostras fitoplanctônicas qualitativas e quantitativas do Reservatório de Corumbá e Cascatinha, Caldas Novas, GO, e Reservatório João Leite, Goiânia, GO, ambos da bacia do rio Paraná foram coletados. Representantes de *Ceratium furcoides* foram observados em microscopia óptica, de epifluorescência e de eletrônica de varredura. **Resultados:** Os indivíduos de *C. furcoides* observados nesses ambientes concordaram morfologicamente com aqueles de outras populações encontradas em outros reservatórios do Brasil e outras localidades, especialmente com relação à tabulação e ao formato da quarta placa apical. Esses ambientes variaram de condições oligotróficas a eutróficas. Variáveis físicas e químicas desses ambientes, comparada com outros sistemas onde essa espécie foi registrada, demonstraram que as condições ótimas para o desenvolvimento de *C. furcoides* são ainda ambíguas para essa espécie. O padrão dispersivo de *C. furcoides* parece ser mais complexo do que aquele regulado pelo sentido montante-jusante, já que a espécie já foi registrada em ambientes de grandes altitudes e à montante de locais previamente registrados. **Conclusão:** Uma análise com base nas regiões de ocorrência e na cronologia dos registros demonstrou que *C. furcoides* tem avançado em direção ao norte do Brasil. Estudos que forneçam uma melhor compreensão das relações entre as populações registradas no Brasil e em outras partes da América do Sul são requeridos a fim de traçar modelos de dispersão acurados para essa espécie, os quais facilitarão políticas de gerenciamento para os ambientes aquáticos no Brasil.

Palavras-chave: espécies invasoras; bacia do rio São Francisco; bacia do rio Corumbá; Centro-Oeste brasileiro; dinoflagelado.

1. Introduction

An invasive species is defined as a "non-native species that, once established, can spread and rapidly dominate over native species" (Kernan, 2015). The establishment of an invasive species can be directly or indirectly associated with an anthropogenic event, and its dominance can generate negative effects on the biotic community and environment (Alpert et al., 2000; Kernan, 2015). These effects may result in profound ecological, evolutionary and economic impacts (Epanchin-Niell & Wilen, 2012; Kernan, 2015). Consequently, means of controlling biological invasions are important for ecosystem health. Rapid detection of invasive species and mapping areas with a potential risk of invasions are some of the main strategies for controlling invasions (Lennox et al., 2015; Mazzamuto et al., 2016).

Members of the genus *Ceratium* Schrank have invaded continental aquatic systems worldwide (Meichtry de Zaburlín et al., 2016). This genus of phytoplanktonic dinoflagellates occurs exclusively in freshwater environments (Gómez et al., 2010), such as in stratified lakes with low nutrient concentrations (Grigorszky et al., 2003). The genus is characterized by thecae composed of four apical (4'), six pre-cingular (6"), six cingular (6c), six post-cingular (6"') and two antapical (2"") plates plus 2 or more sulcal platelets (Gómez et al., 2010).

Currently, *Ceratium* encompasses about 10 species (Guiry & Guiry, 2016), of which *C. hirundinella* (O.F.Müller) Dujardin and *C. furcoides* (Levander) Langhans are the most common. In *C. hirundinella* all four apical plates reach the apex of the epitheca, whereas in *C. furcoides* only three apical plates reach this point (Hickel, 1988; Calado & Larsen, 1997).

Ceratium furcoides was first described as *C. hirundinella* var. *furcoides* Levander, based on phytoplankton material from Finland, and later raised to species level by Langhans (1925). The species is common in temperate regions in European countries (Dokulil & Teubner, 2003), but is also reported as an invasive species in other temperate, subtropical and tropical areas around the world. *Ceratium furcoides* is currently known from systems in Egypt (El-Otify et al., 2003) and Turkey (Çelekli et al., 2007), China (Chu et al., 2008), India (Khondker et al., 2009; Keshri et al., 2013), Iran (Darki, 2014), Korea (Li et al., 2015) and Taiwan (Wu & Chou, 1998), Australia (Ling & Tyler, 2000) and New Zealand (Thomasson, 1974; Jolly & Chapman, 1977; Cassie, 1978; Simmonds et al., 2015). In American continent, this species it was found in Argentina (Mac Donagh et al., 2005; Daga & Pierotto, 2014; Meichtry de Zaburlin et al., 2014; Salusso & Moraña, 2014, 2015), Bolivia (Morales, 2016),, Canada (Dermott et al.,2007) Chile (D. Soto and G. Lembeye, unpublished data; Caputo Galarce et al., 2013; Almanza et al., 2016), Colombia (Ramirez-R et al., 2005; Gil et al., 2012; Villabona-González et al., 2014), Cuba (Comas 2009), Paraguay (Meichtry de Zaburlín et al., 2013), Philippines (Rott et al., 2008; Papa & Mamaril Sr., 2011), Uruguay (Meichtry de Zaburlín et al., 2016, Bordet et al., 2017) and the USA (Daily, 1960).

Species of *Ceratium* were not observed in Brazilian freshwater systems before this century (Cavalcante et al., 2013). In 2007, Santos-Wisniewski et al. (2007) and Silva et al. (2012) recorded *C. furcoides* in the Rio Grande basin, State of Minas Gerais, in March and December, respectively. In 2008, *C. furcoides* was also found in Billings Reservoir, State of São Paulo (Matsumura-Tundisi et al., 2010). Oliveira et al. (2011) recorded *C. furcoides* in reservoirs in the states of Alagoas, Bahia, Pernambuco and Sergipe in April 2009, and Nishimura et al. (2015) found this species in Billings Reservoir and Guarapiranga Reservoir, São Paulo, in September 2009. In 2010, Moreira et al. (2015) observed *C. furcoides* in a temporary lagoon at high altitude in Minas Gerais.

In 2011, Rosini et al. (2016) recorded *C. furcoides* in Ilha Solteira Reservoir, São Paulo and Cavalcante et al. (2017) observed this species in aquatic systems from Paraná, and in 2012 this species was observed in several waterbodies in the states of Paraná and Rio Grande do Sul (Cavalcante et al., 2013, 2016; Cassol et al., 2014, 2017; Jati et al., 2014). In 2013, Bressane et al. (2013) and Hackbart et al. (2015) recorded it in Barranco Alto, Minas Gerais, and in Jaguari and Jacareí reservoirs, São Paulo, respectively. In 2014, Oliveira et al. (2016) recorded *C. furcoides* in Jucazinho and Toritama reservoirs, Pernambuco, Jati et al. (2017) observed it in Paraná, and Crossetti et al. (2018) noted the presence of this species in Garças Reservoir, São Paulo. Ferreira & Azevedo (2017) recorded *C. furcoides* in Água Azul lagoon, Guarulhos São Paulo, in 2015. In 2016, Campanelli et al. (2017) reported it from a fish-farm lake in São Carlos, São Paulo, and Almeida et al. (2016) observed it in Maestra Reservoir, Caxias do Sul, Rio Grande do Sul, as previously recorded by Cavalcante et al. (2016, see above). Thus, *C. furcoides* has been found in Brazilian subtropical and tropical environments, including in high-altitude systems.

The establishment and rapid development of invasive species such as *C. furcoides* in aquatic systems can affect the native communities and the water quality, producing various colors and unpleasant tastes and odors (Berthon, 2015; Meichtry de Zaburlín et al., 2016; Napiórkowska-Krzebietke et al., 2017).Species of this genus have been associated to fish-kill in temperate lakes because the oxygen depletion caused by blooms (Nicholls et al., 1980). Therefore, records of the occurrence of *C. furcoides* are important to provide information about its distribution, dispersion patterns, and contributing to the management of Brazilian aquatic systems.

We here record the occurrence of *C. furcoides* in reservoirs in the Paraná River basin and its sources in Goiás, central-western Brazil, and in some localities of the São Francisco River basin, northeastern Brazil.

2. Material and Methods

Cascatinha Falls is located in the eastern area of the Serra de Caldas State Park (PESCaN), a preserved area near Caldas Novas, Goiás, at 750 m a.s.l. Its waters flow along Saia Velha Stream, Caldas Stream and Pirapitinga River until they enter the north end of the Corumbá Reservoir (17°44’53.23”S; 48°33’38.77”W) (Ramos & Carneiro, 2010). Corumbá Reservoir is located 30 km from Caldas Novas, at 668 m a.s.l. The reservoir has an area of 65 km^2 and a total volume of 1.5 km^3, and was formed by damming the Corumbá River in the Paraná Hydrographic Region (Furnas, 2015).

The João Leite Reservoir is located close to Goiânia, at 730 m a.s.l., and was formed by damming João Leite Stream (16°33’55.16”S; 49°12’39.66”W). The reservoir has an area of 10.4 km^2 and supplies drinking water to about 2 million inhabitants of the Metropolitan Region of Goiânia (Gusmão & Valsecchi, 2009).

The São Francisco River is the main aquatic system of the São Francisco Hydrographic Region. It is the longest Brazilian river, reaching 2,914 km from its sourse in the Canastra Mountain Range, State of Minas Gerais, to its mouth in the Atlantic Ocean on the border of the states of Alagoas and Sergipe. Along its course, the river is dammed five times, forming the reservoirs Três Marias, Sobradinho, Luiz Gonzaga, Paulo Afonso and Xingó (Brasil, 2006).

Qualitative samples were taken at Cascatinha Falls, Corumbá Reservoir, and at ten points in the São Francisco River, using a plankton net of 25 µm mesh (Table 1). The samples were fixed with Transeau’s solution (Bicudo & Menezes, 2006) and deposited in the Herbarium of the Federal University of Goiás and the Herbarium of the State University of Londrina, respectively (Table 1). Quantitative samples were composed of 100 mL of water collected from the subsurface at each site described in Table 1, except Cascatinha Falls (GO-Casc) and the margin of the Corumbá Reservoir (GO-Cor-MARG). These samples were

Table 1. Sites of occurrence of *Ceratium furcoides* and sample codes.

Sample Code	Description	Altitude (m a.s.l.)	Coordinates	Herbarium number
GO-Cor-MID	GOIÁS, Caldas Novas, Corumbá Reservoir, middle, lentic, 10.05.2015 [1]	750	17°47'36.70"S; 48°32'32.17"W	UFG 49802
GO-Cor-MARG	GOIÁS, Caldas Novas, Corumbá Reservoir, margin, lentic, 10.05.2015[1]	668	17°46'39.32"S; 48°34'12.02"W	UFG 49804
GO-Casc	GOIÁS, Caldas Novas, Cascatinha, lotic, 10.05.2018[1]	668	17°46'13.83"S; 48°39'29.21"W	UFG 49801
GO-JL-15/03	GOIÁS, Goiânia, João Leite Stream, João Leite Reservoir, catchment, lentic, 15.03.2016[2]	730	16°33'55.16"S; 49°12'39.66"W	-
GO-JL-24/05	GOIÁS, Goiânia, João Leite Stream, João Leite Reservoir, catchment, lentic, 24.05.2016[2]	730	16°33'55.16"S; 49°12'39.66"W	-
AL1-06/05	ALAGOAS, Delmiro Gouveia, São Francisco River, between dam I, II, III and dam IV of Paulo Afonso Reservoir, downstream, lotic, 06.05.2015[3]	167	9°24'36.37"S; 38°12'13.52"W	FUEL 58037
AL2-06/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, gorge, upstream of Sal River, lentic, 06.05.2015[3]	149	9°25'58.00"S; 38°10'09.08"W	FUEL 58038
AL3-06/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, downstream from Sal River, upstream from curve of gorge, from left margin, lentic, 06.05.2015[3]	144	9°26'25.10"S; 38°07'18.12"W	FUEL 58039
AL4-06/05	BAHIA, Paulo Afonso, Xingó Reservoir, downstream Sal River, upstream from curve of gorge, from right margin, lentic, 06.05.2015[3]	144	9°26'31.99"S; 38°07'17.57"W	FUEL 58040
AL5-06/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, downstream of Sal River, downstream from curve of gorge, middle, lentic, 06.05.2015[3]	140	9°26'17.85"S; 38°06'54.63"W	FUEL 58041
AL6-06/05	BAHIA, Paulo Afonso, Xingó Reservoir, downstream Sal River, downstream from curve of gorge, middle, lentic, 06.05.2015[3]	140	9°26'20.77"S; 38°05'17.28"S	FUEL 58042
AL7-06/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, reentrance of the temporary Salgado River, water intake for city of Delmiro Gouveia, lentic, 06.05.2015[3]	140	9°27'32.59"S; 38°01'59.50"W	FUEL 58043
AL8-06/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, reentrance of temporary river, point of sewage oputlet of city of Delmiro Gouveia, lentic, 06.05.2015[3]	140	9°27'31.30"S; 38°01'38.43"W	FUEL 58044
AL9-06/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, next to Bode Island, middle, lentic, 06.05.2015[3]	140	9°31'46.23"S; 37°58'30.73"W	FUEL 58045
AL10-06/05	ALAGOAS, Piranhas, Xingó Reservoir, next to dam, upstream, lentic, 06.05.2015[3]	140	9°36'27.35"S; 37°48'31.82"W	FUEL 58046
AL1-18/05	ALAGOAS, Delmiro Gouveia, São Francisco River, between dam I, II, III and dam IV of Paulo Afonso Reservoir, downstream, lotic, 18.05.2015[3]	167	9°24'36.37"S; 38°12'13.52"W	FUEL 58047
AL2-18/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, gorge, upstream of Sal River, lentic, 18.05.2015[3]	149	9°25'58.00"S; 38°10'09.08"W	FUEL 58048
AL3-18/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, downstream from Sal River, upstream from curve of gorge, to the left margin, lentic, 18.05.2015[3]	144	9°26'25.10"S; 38°07'18.12"W	FUEL 58049
AL4-18/05	BAHIA, Paulo Afonso, Xingó Reservoir, downstream Sal River, upstream from curve of gorge, at right margin, lentic, 18.05.2015[3]	144	9°26'31.99"S; 38°07'17.57"W	FUEL 58050
AL5-18/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, downstream from Sal River, downstream from curve of gorge, middle, lentic, 18.05.2015[3]	140	9°26'17.85"S; 38°06'54.63"W	FUEL 58051
AL6-18/05	BAHIA, Paulo Afonso, Xingó Reservoir, downstream Sal River, downstream from curve of gorge, middle, lentic, 18.05.2015[3]	140	9°26'20.77"S; 38°05'17.28"S	FUEL 58052
AL7-18/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, reentrance of temporary Salgado River, water intake of city of Delmiro Gouveia, lentic, 18.05.2015[3]	140	9°27'32.59"S; 38°01'59.50"W	FUEL 58053

1 Collectors: I.S. Nogueira, S.M. Pessoa, S.H.M. Benício and L.C.P. Prado; 2 Collector: S.M. Santos; 3 Collector: E.M.M. Magalhães.

Table 1. Continued...

Sample Code	Description	Altitude (m a.s.l.)	Coordinates	Herbarium number
AL8-18/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, reentrance of temporary river, point of sewage outlet of city of Delmiro Gouveia, lentic, 18.05.2015[3]	140	9°27'31.30"S; 38°01'38.43"W	FUEL 58054
AL9-18/05	ALAGOAS, Delmiro Gouveia, Xingó Reservoir, next to Bode Island, middle, lentic, 18.05.2015[3]	140	9°31'46.23"S; 37°58'30.73"W	FUEL 58055
AL10-18/05	ALAGOAS, Piranhas, Xingó Reservoir, next to dam, upstream, lentic, 18.05.2015[3]	140	9°36'27.35"S; 37°48'31.82"W	FUEL 58056

1 Collectors: I.S. Nogueira, S.M. Pessoa, S.H.M. Benício and L.C.P. Prado; 2 Collector: S.M. Santos; 3 Collector: E.M.M. Magalhães.

fixed with Lugol's solution (Bicudo & Menezes, 2006).

Morphological analyses were performed with a Zeiss Axioscope 40 optical microscope (LM) equipped with an Axiocam HRc digital camera (Carl Zeiss Microscopy GmbH, Germany). Thecal plates were dyed with 1% calcofluor white (Fritz & Triemer, 1985) and observed in an Olympus BX51 epifluorescence microscope (EM, Olympus, Tokyo, Japan) with a QCapture Suite image system, version 2.68 (QImaging, Bethesda, MD, USA). The metric features were obtained using the AxioVision Rel 4.8 image software (Carl Zeiss Microscopy GmbH, Germany).

For scanning electron microscopy (SEM) analyses, small probes of each material were fixed with glutaraldehyde for 1 h and dehydrated in an acetyl alcohol series series of 20, 40, 60, 80 and 100%, for 150 min. Part of the dehydrated material was deposited under cover slips and dried in desiccators using silica. The cover slips were fixed on aluminum stubs, using carbon-conductive ink. The material was sputtered with a layer of gold in a Desk V sputter coater (Denton Vacuum, LLC). The preparations were analyzed in a Jeol JSM-6610 scanning electron microscope (Jeol, USA), with an electrical potential of 6 kV, spotsize 25–40.

Individuals of *C. furcoides* were quantified with the Utermöhl method (Utermöhl, 1958) using a Zeiss Axiovert 25 microscope (Carl Zeiss Microscopy GmbH, Germany), and were expressed in cell density (cells.mL^{-1}). The species was identified according to Hickel (1988), Popovský & Pfiester (1990), and Matsumura-Tundisi et al. (2010). The phytoplankton biovolume was obtained by multiplying the population density by the volume of individuals, based on the stereometric formula proposed by Cabeçadas (2011).

In the Corumbá and João Leite river basins, physical and chemical variables including water temperature, pH, oxidation-reduction potential (ORP), electrical conductivity, turbidity, dissolved oxygen (DO), and total dissolved solids (TDS) were obtained using a Horiba U21 multiparameter water quality meter. In the João Leite River basin, concentrations of nitrate and total phosphorus (TP) were measured according to APHA (2005). The data for these variables from the São Francisco River Basin were obtained from Technical Report DILAB 003/2015 (http://cbhsaofrancisco.org.br/?wpfb_dl=1980).

3. Results

Ceratium furcoides (Levander) Langhans (1925, p. 597) (Figures 1-7, 8-10).

Basionym: *C. hirundinella* var. *furcoides* Levander

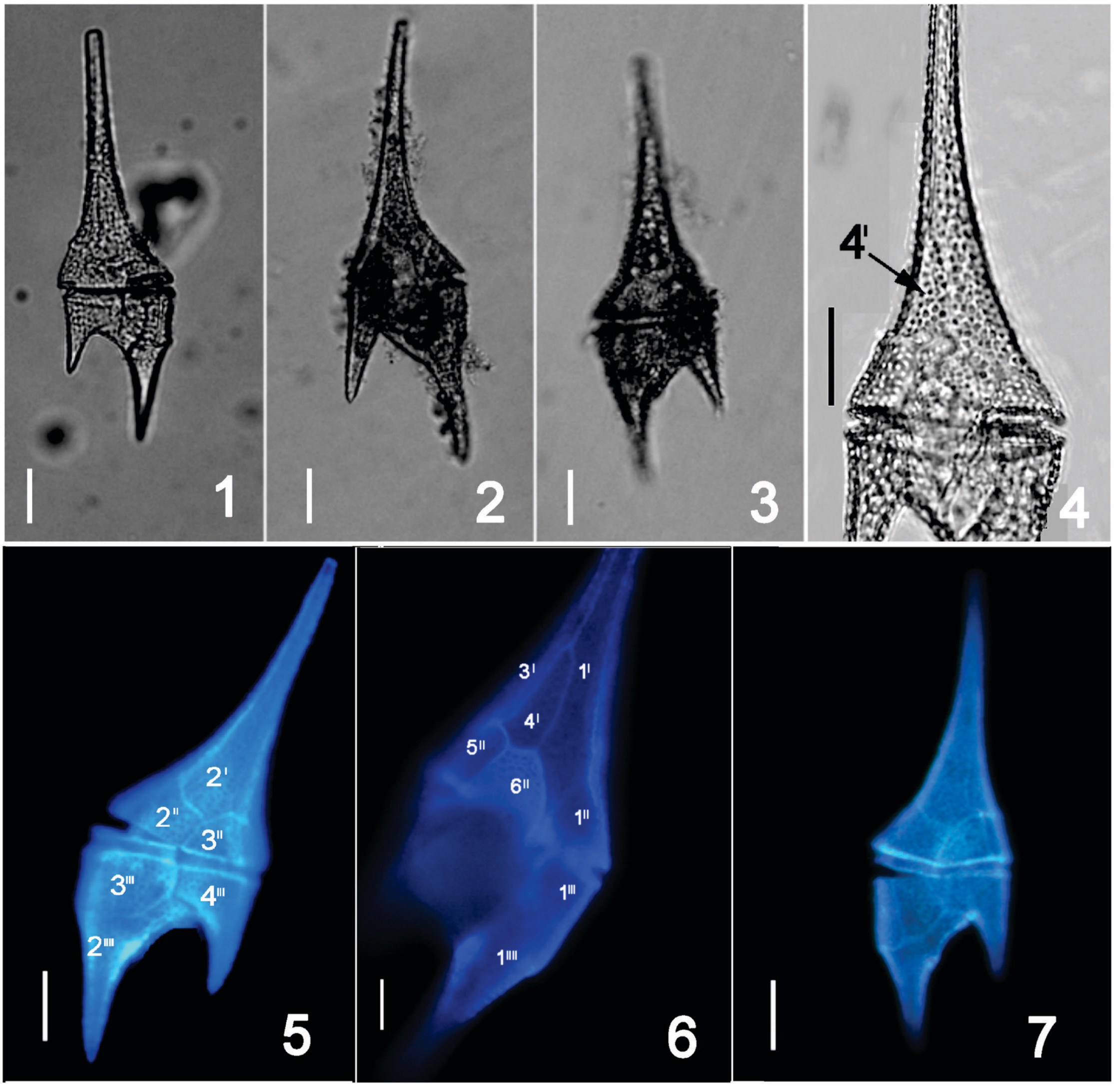
Remarks: Individuals of *Ceratium* showed tabulation formula 4' (one does not reach the apex), 6", 6c, 6"', 2"". Specimens from the Corumbá River basin, including in Cascatinha Falls, were characterized by length 148–178 µm, breadth 41–55, and thickness 27–29 µm; in the São Francisco River Basin, the individuals showed length 106–141 µm, breadth 31-42 µm, and thickness 10–14 µm.

C. furcoides reached 2,050 cells.mL^{-1} and a biovolume of 112.51 mm^3.L^{-1} in Corumbá Reservoir (Table 2). In João Leite Reservoir, the density varied from 39 to 137 cells.mL^{-1}, and the biovolume from 2.14 to 7.51 mm^3.L^{-1}. In the São Francisco River, the density varied from 2.0 to 5,600 cells.mL^{-1} and the biovolume from 0.04 to 112.80 mm^3.L^{-1}.

The physical and chemical characteristics of the environments in which *C. furcoides* was recorded were variable (Table 3).

4. Discussion

Two species of *Ceratium* have been recorded in Brazilian aquatic systems: *C. furcoides* and *C. hirundinella* (Cavalcante et al., 2013). The main



Figures 1-7. Ceratium furcoides. Figures 1-4: light microscopy. Figures 5-7: epifluorescence microscope. Figures 1-4, 7: Population from Corumbá Reservoir, Goiás. Figure 4: Detail of fourth apical plate that does not reach the apex. Figures 5-6: Populations from São Francisco River. Figure 5: Cell in dorsal view showing the apical (2’), precingular (2” and 3”), postcingular (3”’and 4”’) and antapical (2””) plates. Figure 6. Cell in ventral view showing the apical (1’, 3’ and 4’), precingular (1”, 5” and 6”), postcingular (1”’) and antapical (1””) plates; note the fourth apical plate (4’) not reaching the apex. Scale bars: 20 µm.

differences in these species are the shape and size of the plate 4”, which does not reach the apex in *C. furcoides* (Matsumura-Tundisi et al., 2010). This characteristic was observed in specimens found in the São Francisco River, João Leite and Corumbá Reservoirs, confirming the identity of this taxon as *C. furcoides* (Figures 4, 6, 9, 10).

The dimensions of *C. furcoides* were not provided in the original description (Levander, 1894). However, subsequent descriptions reported lengths from 114 to 322 µm and widths from 28 to 54 µm (Daily, 1960; Hickel, 1988; Moreira et al., 2015; Nishimura et al., 2015; Campanelli et al., 2017). The only values for thickness were provided by Hickel (1988), from 20 to 25 µm. The dimensions of the material from João Leite, Corumbá and São Francisco River basins agree with these data, and smaller specimens were observed in populations from the São Francisco River basin (106-141 vs. 114-322 µm).

There is no consensus about the environmental conditions that contribute to the establishment of *C. furcoides*. Species of *Ceratium* have been observed in deep and shallow environments, oligo- to eutrophic, and medium to large sizes (Padisák et al., 2009). More specifically, *C. furcoides* has been positively related to ammonium concentrations and negatively related to alkalinity, nitrate concentration,

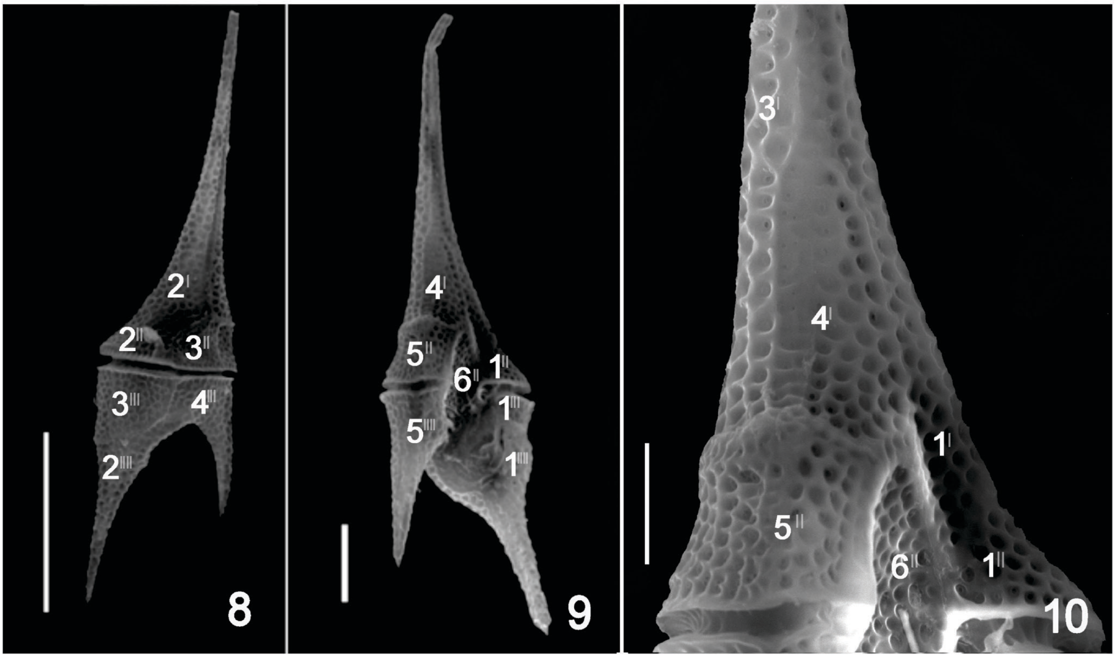
Table 2. Density and biovolume of *Ceratium furcoides* in Corumbá Reservoir and São Francisco River (for sites codes, see Table 1).

Sites	Type of environment	Density (cells.mL^{-1})	Biovolume (µm^3.L^{-1})
GO-Cor-MID	Lentic	2,050	112.51
GO-JL-15/03	Lentic	39	2.14
GO-JL-24/05	Lentic	137	7.51
AL1-06/05	Lotic	20	0.40
AL1-18/05	Lotic	2	0.04
AL2-06/05	Lentic	100	2.01
AL2-18/05	Lentic	3	0.06
AL3-06/05	Lentic	780	15.71
AL3-18/05	Lentic	3	0.06
AL4-06/05	Lentic	120	2.42
AL4-18/05	Lentic	5,600	112.80
AL5-06/05	Lentic	3,800	76.54
AL5-18/05	Lentic	3,720	74.93
AL6-06/05	Lentic	4,660	93.87
AL6-18/05	Lentic	2,070	41.70
AL7-06/05	Lentic	5,280	106.36
AL7-18/05	Lentic	760	15.31
AL8-06/05	Lentic	1,880	37.87
AL8-18/05	Lentic	3,400	68.49
AL9-06/05	Lentic	140	2.82
AL9-18/05	Lentic	510	10.27
AL10-06/05	Lentic	180	3.63
AL10-18/05	Lentic	100	2.01

Table 3. Mean values of physical and chemical variables analyzed in reservoirs and rivers in the Corumbá River basin, Goiás, and the São Francisco River basin, Alagoas (ORP: oxidation-reduction potential; DO: dissolved oxygen; TDS: total dissolved solids).

Variables	Sites			
	Cascatinha Falls (n = 2)	Corumbá Reservoir (n = 4)	João Leite Reservoir (n = 7)	São Francisco River (n = 4)
Water temperature (°C)	22.89	26.57	26.64	29.89
pH	8.14	6.79	7.34	6.42
Conductivity (µS.cm^{-1})	10.00	39.00	113.00	75.00
Turbidity (NTU)	0.86	38.98	9.70	15.3
DO (mg.L^{-1})	26.63	18.23	6.46	11.69
TDS (mg.L^{-1})	6.00	25.00	–	49.00
Nitrate (mg.L^{-1})	–	–	0.25	0.81
Total phosphorus (mg.L^{-1})	–	–	0.02	0.86

DO, silicates, and light availability in environments in Colombia (Gil et al., 2012). In Brazilian environments, this species has shown better development in environments with higher nitrite and nitrate concentrations with deep euphotic zones, as in Billings Reservoir (Nishimura et al., 2015), or in mesotrophic environments, with warmer temperatures and higher electrical conductivity (Silva et al., 2012). Cavalcante et al. (2016) found that high organic-matter content, nutrient availability, well-oxygenated water and temperatures from 15 to 25 °C are the main environmental factors that contribute to the establishment of *C. furcoides* in reservoirs in southern Brazil. In this study, the João Leite, Corumbá and São Francisco systems were acid to slightly alkaline, and the water temperatures were above 25 °C. Moreover, it is not clear if well-oxygenated water is a favorable condition for the development of this species or is a result of its development, since oxygen is a product of photosynthesis Thus, this species has occurred in environments with different DO concentrations;



Figures 8-10. C. furcoides, population from Corumbá Reservoir, Goiás, in SEM. Figure 8: Cell in dorsal view showing the apical (2'), precingular (1" and 5"), postcingular (4"' and 3"') and antapical (2"") plates. Figure 9: Cell in ventral view showing the apical (4 '), precingular (1", 5" and 6"), postcingular (1"' and 5"') and antapical (1"") plates; note the fourth apical plate (4') not reaching the apex. Figure 10: Detail of epitheca in dorsal view. Scale bars: Figure 8 = 50 µm; Figure 9 = 20 µm; Figure 10 = 10 µm.

e.g., in João Leite DO concentrations were low, in contrast to the Corumbá and São Francisco systems. Organic matter can also be influenced by the high biovolumes of this species. Despite the values of nutrient concentrations are sparse in the environments studied here, these resources seem facilitate the establishment and development of the species. Crossetti et al. (2018) observed that the recurrence of *C. furcoides* in Garças reservoir was associated with higher transparency, lower water stability, higher dissolved inorganic nitrogen and lower TP values.

C. furcoides is hitherto known in Brazil in aquatic environments from the Paraná River, São Francisco River, Atlantic (East/South regions), and Uruguay River basins. No records have been reported from the Amazon River, Tocantins-Araguaia River or Atlantic (North/Northeast) basins, nor in some other parts of the world (Figures 11 and 12). The establishment of *C. furcoides* in Brazilian aquatic systems is a recent event (Santos-Wisniewski et al., 2007; Cavalcante et al., 2013). Based on the dates of occurrence, Cavalcante et al. (2013) hypothesized that the dispersal of *C. furcoides* seems to be radial, i.e., northward and southward from the initial appearance in the southeastern region. However, the relationships among the populations in the different Brazilian aquatic systems have not been studied.

In several cases, *C. furcoides* was observed in very low densities and did not appear in quantitative samples, such as at Cascatinha Falls. This suggests that the species may be present in some environments but not in sufficient numbers to be recorded. The failure to find it in some samples may also indicate methodological problems during sampling, since species of *Ceratium* can migrate vertically. Almost all samples taken in Brazilian waterbodies are from the subsurface. However, in some cases, high densities of species of *Ceratium* can be found down to 7 m, where light conditions are more suitable or due to stratification (Harris et al., 1979). In some aquatic systems in Brazil where *C. furcoides* was found, no temporal records exist, which makes the time of its arrival uncertain. These problems add to the uncertainty regarding the actual localities where this species occurs and its distribution patterns.

For some environments in Brazil, it is possible to infer an approximate date of the invasion because of long-term historical floristic studies, such as in Billings Reservoir (Matsumura-Tundisi et al., 2010; Nishimura et al., 2015). Because the João

Figure 11. Occurrence of *Ceratium furcoides* in Brazilian aquatic systems, showing previous records (purple dots) and new records from central-western Brazil and the northeast (red dots).

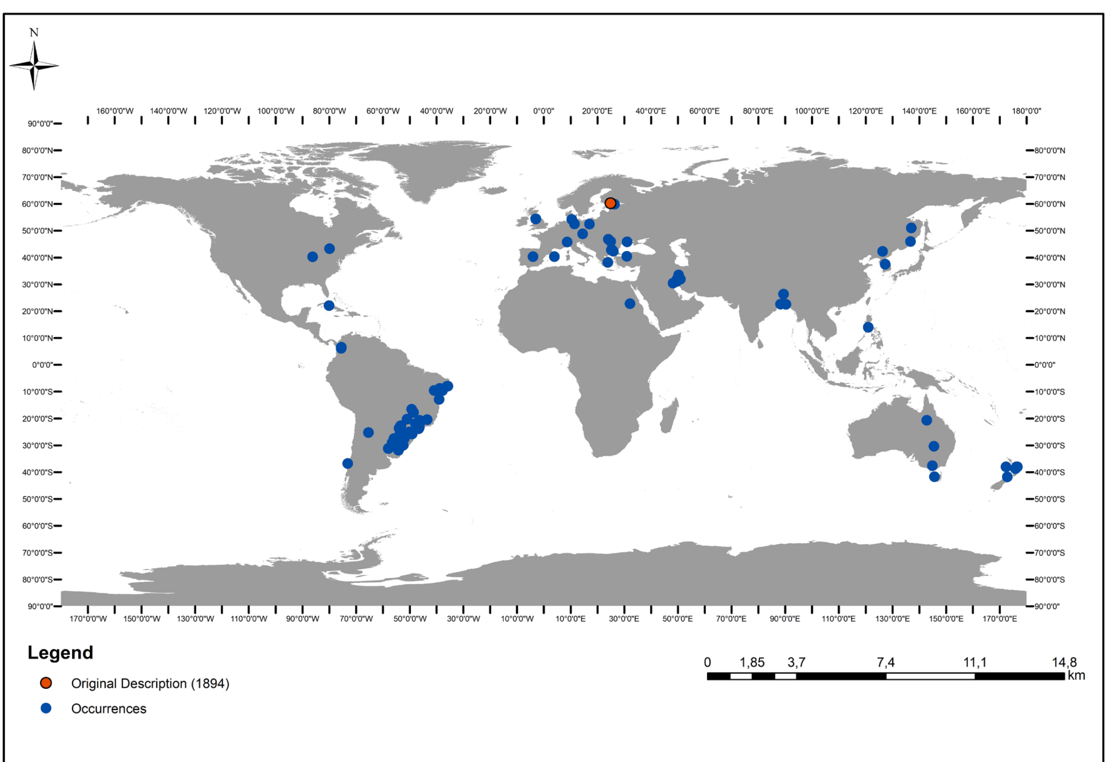


Figure 12. Occurrence of *Ceratium furcoides* in the world.

Leite River basin is the water source for the metropolitan region of Goiânia, the biotic and abiotic conditions of this system have been long exhaustively monitored, mainly after the creation of the reservoir in 2009 (Cunha & Borges, 2015). Thus, *C. furcoides* certainly did not occur in this environment before March 2016. Historical studies of the phytoplankton composition in Corumbá Reservoir are scarce, but the existing studies support the hypothesis that *C. furcoides* did not occur there before 2011 (Silva et al., 2001; I. S. Nogueira, pers. observ.).

While the answer for "when" *C. furcoides* arrives in Brazilian waterbodies seems to be easier to propose, "how" it occur and is occurring is still not possible to verify. Some researchers suggest by boats or migratory birds as some of the main dispersive ways for phytoplanktonic species (Padisák et al., 2016). From that, the establishment of this species in Brazilian waters and its spread can be associated to adequate environmental conditions and drivers upstream-downstream or downstream-upstream. Until 2011, *C. furcoides* was observed in only two hydrographic regions, Paraná and São Francisco. The existence of cascades of reservoirs in large rivers is presumed to contribute considerably to its spread (Padisák et al., 2016). This seems to be a reaseonable explanation for invasions at the upstream-downstream direction. Corumbá and João Leite reservoirs are in the Paraná Hydrographic Region, upstream of the systems where *C. furcoides* was previously recorded.

This sugest the progression of the invasion of this species in aquatic systems toward the central-northern region.

The continuous progression of this species, reaching aquatic systems in the state of Goiás, should be considered and studied. This state contains a watershed divide between tributaries of three of the main river basins of the country. Although these river basins are mostly separated by high-altitude formations, these seem not to limit the movement of *C. furcoides*, since the species occurs in high-altitude systems (Moreira et al., 2015). This suggests that the distribution pattern is more complex than can be explained by the connectivity of aquatic systems. However, this factor may facilate its spread, as in the case of reservoir cascades in large rivers, which are presumed to contribute considerably to the establishment of *C. furcoides* (Padisák et al., 2016).

The harmful of *C. furcoides* is few known. Despite this species is not toxic, it reach great density during some phases because the low preference by grazers and (Santer, 1996) and good enviromental conditions (Crossetti et al., 2018). Similar blooms have contributed to high depletion of oxygen in water column and consequently fish-kill in some lakes and problems in water treatment (Nicholls et al., 1980). Understanding the occurrences and distribution patterns of *C. furcoides* is essential to the development of control policies, which should be used to guarantee the future water quality of Brazilian aquatic systems for different uses in a period when drinking water is becoming scarce. Promptly detecting the occurrence of an invasive species such as *C. furcoides* is essential to understand the ecological factors that can facilitate the establishment of this species and to develop models to prevent its spread (Lennox et al., 2015; Mazzamuto et al., 2016). Future studies should analyze the relationships among populations of *C. furcoides* in Brazil, and make efforts to discover the mechanisms related to the expansion success of this species.

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