Article

Hoehnea 47: e742019, 2 tab., 3 fig., 2020 http://dx.doi.org/10.1590/2236-8906-74/2019

Ceratium furcoides (Levander) Langhans: first record in Nova Avanhandava reservoir, Southeast Brazil[1]

Andréa Sampaio Dias[2] and Andréa Tucci[2]

Received: 01.07.2019; accepted: 07.05.2020

How to cite: Dias, A.S. & Tucci, A. 2020. *Ceratium furcoides* (Levander) Langhans: first record in Nova Avanhandava reservoir, Southeast Brazil. Hoehnea 47: e742019. http://dx.doi.org/10.1590/2236-8906-74/2019

ABSTRACT – (*Ceratium furcoides* (Levander) Langhans: first record in Nova Avanhandava reservoir, Southeast Brazil). This is the first record of *Ceratium furcoides* in the Nova Avanhandava reservoir, São Paulo, whose samples were collected in three stations (S1, S2, S3) in March and October of the years 2015 and 2016. Physico-chemical analyzes of the water were performed. The reservoir was classified, according to the TSI, as mesotrophic. The first record of the species occurred in October 2015 at S3 and, the following year, the dispersion for the other stations was verified. The highest density of the species (28 org mL^{-1}) was recorded in October 2016. Similar values were documented in Furnas (MG) and Ilha Solteira (SP). The low density values of *C. furcoides* and the presence of phytoplankton groups with higher densities (Cyanobacteria and Cryptophyceae) suggest that the colonization of the species is at an early stage. Thus, studies about dispersion, dynamics, and interaction of *C. furcoides* with phytoplankton and the potential impacts on aquatic communities are essential to understand the responses of this species to environmental conditions.
Keywords: adaptative strategies, bioinvasion, mesotrophic, net cages

RESUMO – (*Ceratium furcoides* (Levander) Langhans: primeiro registro no reservatório Nova Avanhandava, Sudeste do Brasil). Trata-se do primeiro registro de *Ceratium furcoides* no reservatório Nova Avanhandava, São Paulo, cuja as amostras foram coletadas em três estações (E1, E2, E3) em março e outubro dos anos de 2015 e 2016. Análises físico-químicas da água foram realizadas. O reservatório foi classificado, de acordo com o cálculo do IET como mesotrófico. O primeiro registro da espécie ocorreu em outubro de 2015 na E3 e, no ano seguinte, verificou-se a dispersão para as outras estações. A maior densidade da espécie (28 org mL^{-1}) foi registrada em outubro de 2016. Valores semelhantes foram documentados em Furnas (MG) e Ilha Solteira (SP). Os baixos valores de densidade de *C. furcoides* e a presença de grupos fitoplanctônicos com densidades superiores (Cyanobacteria e Cryptophyceae), sugerem que a colonização da espécie está no início. Assim, estudos sobre a dispersão, dinâmica e interação de *C. furcoides* com o fitoplâncton e os potenciais impactos sobre as comunidades aquáticas são essenciais para entender as respostas desta espécie às condições ambientais.
Palavras-chave: bioinvasão, estratégias adaptativas, mesotrófico, tanques-rede

Introduction

Reservoirs are habitats prone to bioinvasion, especially for ease of dispersion through the flow of water. The invasion of microscopic alien species in these environments is still poorly understood, and it is not possible to accurately estimate what impacts such species would promote to communities, including phytoplankton (Kastovsky *et al.* 2010, Silva *et al.* 2012). However, based on studies of invasion of alien species in other environments, it is possible to predict that the establishment of an invasive species may result in changes in species composition, diversity, and dominance, and in primary production (Elbrächter 1999, Ricciardi & Kipp 2008, Souza *et al.* 2009, Vitule & Prodocimo 2012).

Species of the genus *Ceratium* are considered invasive in continental aquatic environments of South America (Silva *et al.* 2012, Pereira *et al.* 2013, Cavalcante *et al.*

1. Part of the Doctoral Thesis of the first Author
2. Instituto de Botânica, Núcleo de Pesquisa em Ficologia, Avenida Miguel Stéfano, 3687, 04101-902 São Paulo, SP, Brasil
3. Corresponding author: emunah.dias@gmail.com

2016, Crossetti *et al.* 2019). The species *Ceratium furcoides* (Levander) Langhans demonstrates easy adaptation to different temperatures, trophies and physical and chemical conditions of the water thus presenting rapid dispersal and establishment in tropical reservoirs, including Brazilian ones, whose records of the species have been frequent (Santos-Wisniewski *et al.* 2007, Matsumura-Tundisi *et al.* 2010, Oliveira *et al.* 2011, Cavalcante *et al.* 2013, Jati *et al.* 2014, Nishimura *et al.* 2015, Oliveira *et al.* 2016, Rosini *et al.* 2016, Campanelli *et al.* 2017, Silva *et al.* 2018, Roriz *et al.* 2019).

The increase in the density of *C. furcoides* and the formation of blooms in tropical reservoirs is mainly associated with the availability of nutrients (Matsumura-Tundisi *et al.* 2010, Silva *et al.* 2012). In this way, reservoirs that receive external loads of nutrients or where intensive activities are carried out, such as the creation of fish in net cages, become environments conducive to the mass development of the species in question. In this context, the aim of this work was to document the first occurrence of *Ceratium furcoides* in the Nova Avanhandava reservoir, one of the three main centers of the fish farm in State of São Paulo.

Materials and Methods

Ceratium furcoides (Levander) Langhans was identified from phytoplankton sampling carried out during the development of a doctorate degree project in which three reservoirs from the State of São Paulo with fish farms were sampled: Chavantes, Nova Avanhandava, and Ilha Solteira. In this study, we documented the first record of the species in Nova Avanhandava Reservoir.

Study area - The reservoir of the Nova Avanhandava Hydroelectric Plant (21°07'01" S and 50°12'06" W) is located in Buritama city and is in Baixo Tietê Basin (figure 1). It presents an area of 210 km^2, a maximum depth of 30 m and residence time of 46 days. Nova Avanhandava is the penultimate reservoir of a system of consecutive lakes, with the reservoirs of Promissão upstream of it and Três Irmãos downstream.

Samplings were carried out in the subsurface of the water column in March and October of 2015 and of 2016 in three sampling stations (S1, S2 and S3) corresponding to the three different fish farms (f) of creation and their respective upstream areas (u) and downstream (d) (n = 28). S2 is located upstream of S3 and, consequently, only upstream and downstream were sampled for the two stations.

In situ measurements of dissolved oxygen (mg L^{-1}), pH, water temperature (°C) and electrical conductivity (µS cm^{-1}) were carried out by the EXO YSI multi-parameter probe. The water transparency (m) was estimated by the disappearance of the Secchi disk. (Esteves 2011). The concentrations of total phosphorus (µg L^{-1}) and total nitrogen (µg L^{-1})

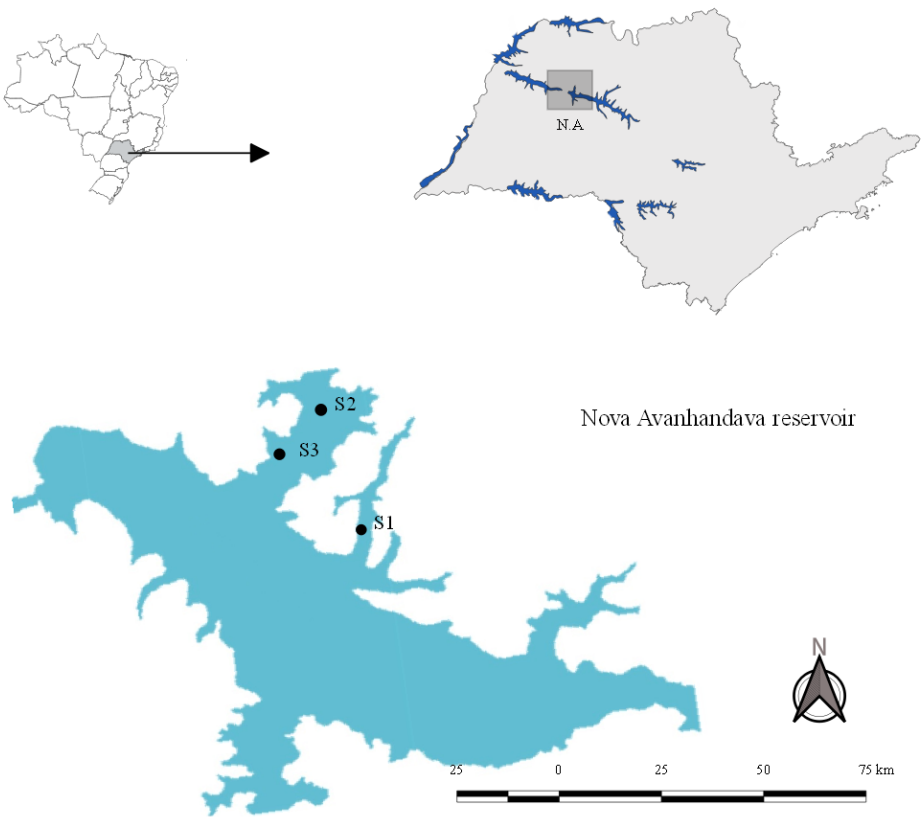

Figure 1. Location of São Paulo State, Brazil and Nova Avanhandava reservoir (N.A.). In detail, the map of Nova Avanhandava reservoir showing the location of the three samplings stations (S1, S2, and S3).

(Valderrama 1981), orthophosphate (μg L^{-1}) (Strickland & Parsons 1960), ammonium ion (μg L^{-1}) (Apha *et al.* 2005), nitrite (μg L^{-1}) e nitrate (μg L^{-1}) (Giné *et al.* 1980) were determined. The Trophic State Index (TSI) was calculated according to Lamparelli (2004).

For the phytoplankton taxonomic study, the samples were obtained with 20 μm mesh plankton net and fixed *in situ* with formaldehyde in the final concentration of 4 to 5%. The phytoplankton community was identified using an optical microscope with a capture camera, specialized bibliography and measurements were obtained with the aid of the Axio Vision 4.8 program. The identification of the *Ceratium furcoides* species (n= 25) was performed according to Santos-Wieniewski *et al.* (2007) and Cavalcante *et al.* (2013).

Samples referring to the first records of *C. furcoides* in the Nova Avanhandava reservoir are deposited in the collection of Herbário Maria Eneyda P. Kauffmann Fidalgo of the Institute de Botânica of São Paulo State.

Samples for quantitative analysis were collected with a Van Dorn bottle and fixed *in situ* with 1% Lugol solution. The count of the phytoplanktonic community and consequently of the populations of *Ceratium furcoides* was carried out by the method of Utermölh (1958). The results of the densities were expressed in org mL^{-1} and the biomass was estimated by calculating the biovolume based on the work of Hillebrand *et al.* (1999) and Fonseca *et al.* (2014).

Descriptive statistics were performed: mean and standard deviation of limnological variables and nutrients.

Results

Nova Avanhandava presented at the sampling stations, high conductivity, neutral pH and temperature ranging between 23°C and 29 °C. As for the trophic level, the seasons were classified as mesotrophic in all samples (mean TSI: 59). The mean values of the physical, chemical and nutrient variables recorded at the sampling stations are presented in Table 1 and 2.

During the study period, we identified 156 taxa distributed in 12 taxonomic goups: Cyanobacteria (33 taxa), Chlorophyceae (33 taxa), Cryptophyceae (nine taxa), Bacillariophyceae (six taxa), Euglenophyceae (6 taxa), Trebouxiophyceae (6 taxa), Coscinodiscophyceae (4 taxa), Chrysophyceae (four taxa), Zygnemophyceae (four taxa), Dinophyceae (two taxa), Choanoflagellata (one taxa), Klebsormidiophyceae (one taxa).

Ceratium furcoides (Levander) Langhans (1925). Published in: Langhans, V.H. (1925). Gemischte Populationen von *Ceratium hirundinella* (O.F.M) schrank und ihre Deutung. Archiv für Protistenkunde 52: 585-602.

Table 1. Mean, minimum and maximum values of the physical. WT: Water Temperature; DO: Dissolved Oxygen; Cond.: Conductivity; Transp.: Transparency; Turb.: Turbidity and chemical variables; Chl α: Chlorophyll-α measured at the three samplings stations.

		WT (°C)	DO (mg L^{-1})	pH	Cond. (μS cm^{-1})	Transp. (m)	Turb. (UNT)	Chl-α (μg L^{-1})
S1	Mean (n = 12)	27	8	8	207	2	4	12
	Min	24	5	8	139	1	3	2
	Max	29	12	9	253	4	7	47
S2	Mean (n = 8)	27	7	8	193	2	2	14
	Min	23	4	7	150	1	1	3
	Max	29	11	9	225	2	4	71
S3	Mean (n = 8)	27	8	8	207	3	2	12
	Min	23	5	7	150	1	1	1
	Max	29	13	9	237	5	3	49

Table 2. Mean values and standard deviation of nutrients concentrations for the samplings stations in Nova Avanhandava reservoir, São Paulo State, Brazil, recorded in March and October 2015, and March and October 2016.

	Total N (μg L^{-1})	Nitrite (μg L^{-1})	Nitrate (μg L^{-1})	Ammonium (μg L^{-1})	Total P (μg L^{-1})	Ortofosfate (μg L^{-1})
S1	1074 ± 550,6	4,2 ± 2,3	99 ± 28,7	292 ± 98,3	27,7 ± 6,9	10,4 ± 2,2
S2	1117,4 ± 638,8	2,9 ± 1,2	101,8 ± 35,8	209,9 ± 98,4	25,9 ± 6,7	10,3 ± 3,0
S3	1325,8 ± 656,1	4,1 ± 1,9	108,9 ± 31,3	360,3 ± 80,0	31,8 ± 9,0	12,9 ± 2,2

Basionym - *C. hirundinella* var. *furcoides* Levander
Figure 2

Cells slightly fusiform, flattened dorsiventrally, 46-58 µm wide, 185-274 µm in length, reticulated plates. The epitheca is conical with a long horn formed above the cingulum, with four apical plates (1',2',3' and 4'). The hypovalve is large and small, with two or three posterior horns of varied sizes formed by antapical plates (1'" and 2'"). Chloroplasts are oval-shaped and numerous. Planktonic habit.

Comments - Some specimens presented morphological variation characterized by the third horn, which makes them similar to *C. hirundinella*, however, Bourrelly (1970) points out that variations in the number and size of horns are linked to environmental changes such as temperature. Thus, the confirmation of the *C. furcoides* populations was carried out observing that the fourth apical plate (4') does not reach the apex of the epitheca.

Material examined - BRAZIL. São Paulo: Zacarias, Nova Avanhandava reservoir, 11-III-2016, A.Dias s.n. (SP470024); *idem*, 03-X-2015, A.Dias s.n. (SP470025); *idem*, 03-X-2015, A.Dias s.n. (SP470026); *idem*, 05-X-2016, A.Dias & A.Tucci s.n. (SP470027); *idem*, 05-X-2016, A.Dias & A.Tucci s.n. (SP470028).

Distribution - They occur from oligotrophic lakes and reservoirs to eutrophic ones. There are records of the species on all continents. (Guiry 2019). In Brazil, there are records in almost all regions, except for the Northern Region (Flora do Brasil 2020 - under construction, Silva *et al.* 2018).

The first occurrence of populations of *C. furcoides* in the Nova Avanhandava reservoir occurred in October 2015 (S2u) and remained in the other samples: March 2016 (S1u) e October 2016 (S1u, S2f1, S3f2). The density and biovolume of *C. furcoides* were low in comparison to the other organisms of the phytoplankton community recorded together, being the highest values (density: 28 org mL^{-1}; biovolume: 0,442 $mm^3 L^{-1}$) for the species registered upstream of S1 (u), in October 2016 (figure 3 a-b).

Discussion

Since its first occurrence of *Ceratium furcoides* in 2007 on Furnas reservoir in Minas Gerais, 13 new studies have been published, whose results document the dispersion of the species to all regions of Brazil, except the Northern Region (Santos-Wisniewski 2007, Matsumura-Tundisi *et al.* 2010, Oliveira *et al.* 2011, Pires *et al.* 2012, Cavalcante *et al.* 2013, Cassol *et al.* 2014, Jati *et al.* 2014, Moreira *et al.* 2015, Nishimura *et al.* 2015, Cavalcante *et al.* 2016, Silva *et al.* 2018, Crossetti *et al.*, 2019, Roriz *et al.* 2019). In the State of São Paulo, the specie has been registered on the reservoirs: Billings (Matsumura-Tundisi *et al.* 2010), Barra Bonita, Itupararanga and Mirinheirinho (Pires *et al.* 2012), Billings and Guarapiranga (Nishimura *et al.*, 2015), Jaguari e Jacareí (Hackbart *et al.*, 2015), Ilha Solteira (Rosini *et al.*, 2016), Lago das Garças (Crossetti *et al.*, 2019). This work is the first record of the occurrence of the species for the Nova Avanhandava reservoir in the State of São Paulo, Southeastern Region.

The records of *C. furcoides* in such distinct aquatic environments demonstrate the great ecophysiological plasticity of the species. In addition, *Ceratium* presents important adaptive strategies for the development and establishment in tropical reservoirs such as encystment (resistance to sedimentation, dispersion capacity, population

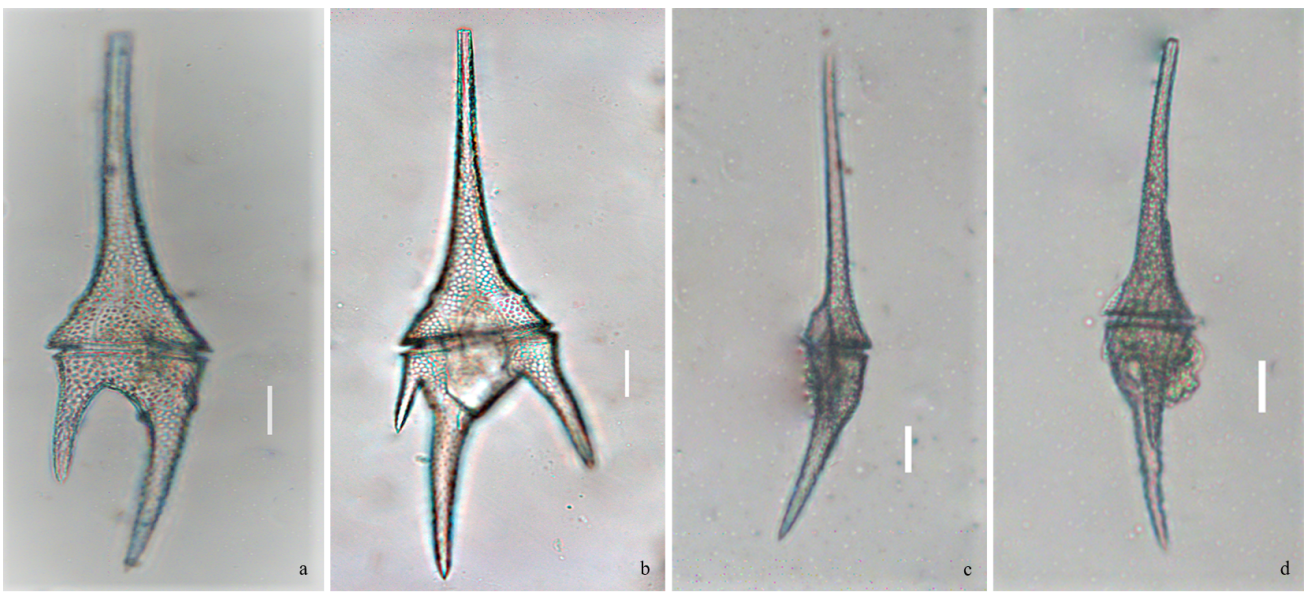

Figure 2. Specimens of *Ceratium furcoides* (Levander) Langhans found in Nova Avanhandava reservoir, São Paulo State, Brazil, in station S2 (SP470024 and SP470025). a. ventral view; b. dorsal view; c-d. lateral views.

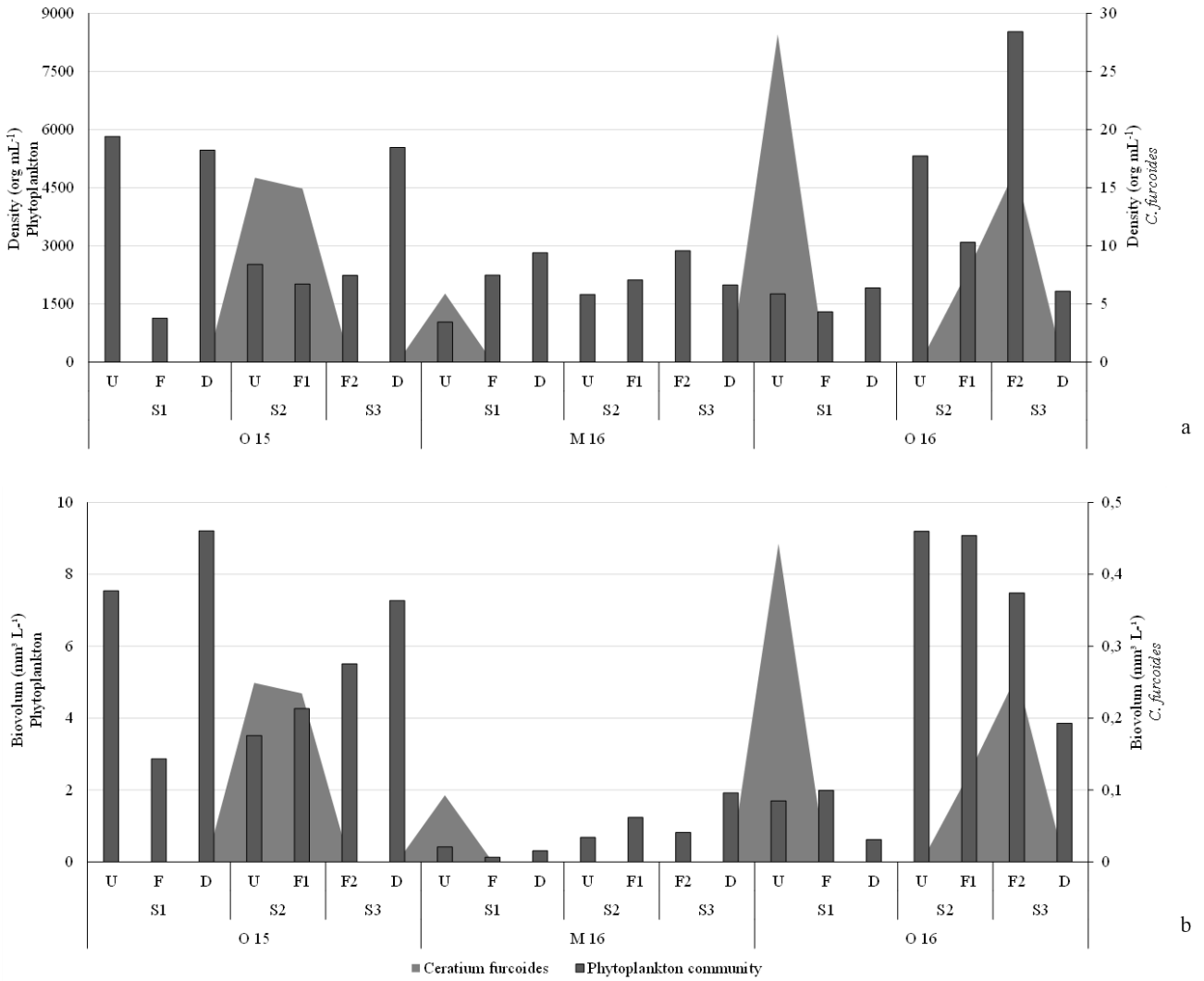

Figure 3. Total density (org mL^{-1}) (a.) and biovolume (mm^3 L^{-1}) (b.) of the *Ceratium furcoides* (Levander) Langhans in compared to phytoplanktonic classes at the six samplings stations (upstream: U, fish farm: F and downstream: D during the period of 2015 and 2016. On the left, scale of the phytoplankton community (bars graph). On the right, scale of the species *Ceratium furcoides* (area graph).

survival), mobility and low predation by zooplankton (Hickel 1988, Pollingher 1988, Xie *et al.* 1998, Cavalcante *et al.* 2016).

We do not have results that indicate concretely how the species was introduced into the reservoir in question, but we considered a passive dispersion of cysts or specimens from Barra Bonita reservoir, whose species had previously been recorded by Pires *et al.* (2012), carried by the water flow of the central body of the reservoir where the endorheic river Tietê flows. However, other forms of dispersal should also be considered: transport of specimens or their form of resistance attached to birds and aquatic insects moving between reservoirs or by anthropic intervention, as mentioned by Kristiansen (1996) and Meichtry *et al.* (2014) or consider *Ceratium* propagules already existing in the sediment, but which only now did they become available for invasion (Reynolds 1996, Rengefors *et al.* 2004, Padisàk 2010).

The literature indicates that some limnological variables are essential in the establishment of populations of *C. furcoides*. Thus, it has been observed the dominance of dinoflagellates, including the *Ceratium* genus in mesotrophic conditions (Periotto *et al.* 2007, Santos-Wisniewskis *et al.* 2007, Silva *et al.* 2018) and development of *C. furcoides* between 10° e 25°C (Cassol *et al.* 2014, Cavalcante *et al.* 2016) and recurrence of the species in environments with high transparency associated with lower stability of the water column and also at high concentrations of ammonia and nitrite.

New Avanhandava is one of the three largest fish farming centers of São Paulo and as well as all economic activity fish farming is likely to cause pollution in the environment. In this case, the continuous and pronounced nutrient discharge from fish feeds and metabolism effectively alter the phytoplankton community, favoring the development of certain groups over

the decrease of others, as well as favoring the establishment of invasive species (Mack *et al.* 2000, Degefu *et al.* 2011).

In this study, the maximum value of density and biovolume of *C. furcoides* was, respectively, 28 org mL^{-1} and 0,442 mm^3 L^{-1} recorded in S1(Oct/16). Density values are extremely low compared to densities in other São Paulo reservoirs recorded by Matsumura-Tundisi *et al.* (2010) (535 ind.mL^{-1}) on the Taquacetuba arm at the Billings dam or by Hackbart *et al.* (2015) on Jaguari dam (131.954,0 ind.mL^{-1}) and low biomass compared to the records of Nishimura *et al.* (2015) on Billings dam (5,7 mm^3 L^{-1}) and on Guarapiranga (2,6 mm^3 L^{-1}), of Rosini *et al.* (2016) on Ilha Solteira (15,698 mm^3 L^{-1}) and of Crossetti *et al.* (2019) on Lago das Garças (12,3 µg L^{-1}).

Based on the limnological and phytoplankton community results obtained and based on reports in the literature of limnological conditions of the others Brazilian environments in which *C. furcoides* has been registered, it can be said that Nova Avanhandava has environmental conditions favorable to the adaptive success of the species since both the maximum density and the peaks above 15 org mL^{-1} of *C. furcoides* recorded in Oct/15 (u and f1 of S1) and Oct/16 (u S1 and f2 S3) were associated with high values of electrical conductivity and at temperatures of 24° C and high dissolved inorganic nitrogen values, principally in the fish farm areas. However, the low density and biovolume values, as well as the absence of *C. furcoides* in other sampled compartments, suggest that the colonization of the species is at an early stage.

At first, were not observed changes of phytoplankton due to the presence of *C. furcoides* on this aquatic environment, it was found that other phytoplankton groups (Cyanobacteria and Cryptophyceae) showed high densities / biovolumes compared to *C. furcoides*, which corroborates the hypothesis that the species is in the process of colonization of the reservoir, establishing competition with the other species for nutrients due to its migration capacity vertical in the water column (Stephaniak *et al.* 2007, Cassol *et al.* 2014, Moreira *et al.* 2015, Almanza *et al.* 2016).

Punctual records in different environments and some possible equivocal identifications of *Ceratium* species may be underestismating the invasion of the species in the Brazilian territory since the species demonstrate a great capacity of dispersion and adaptation to different conditions. Thus, a greater number of studies in aquatic environments with similar limnological conditions and consequently their sediments (to verify the presence of cysts) could indicate if *C. furcoides* is already dispersed but not yet registered. From our results, we can suggest more specific studies in the Nova Avanhandava reservoir that establish the dispersion forms of *Ceratium furcoides* as well as studies that identify the closest aquatic environments that are favorable to the bioinvasion of the species and later studies to understand the relationships with the other groups of phytoplanktonic algae in addition to evaluating the consequences of establishing the species for the ecosystem.

Acknowledgments

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES, for funding the Doctorate degree of the first Author; to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (Grant 2013/50504-5), for funding the IMPCON Thematic Project of which the Doctoral Project was part; and all integrants of IMPCON Group, especially the coordinator Reinaldo José da Silva.

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ISSN da publicação *online* 2236-8906