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Checklist of ciliated protozoa from surface water and sediment samples of Atibaia River, Campinas, São Paulo (Southeast Brazil).

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Abstract. The knowledge of the diversity of ciliated protozoa in an aquatic ecosystem is an important information that can serve as a tool in the environmental quality management. A survey of the ciliated protozoa of the Atibaia River, Campinas, São Paulo was done during 24 months. Surface water and sediment samples were collected monthly from two sampling points. Qualitative analyses of ciliates were performed *in vivo* and by protargol staining. A total of 66 taxa belonging to 55 genera were identified. This is the first study in Atibaia River and, as this is an impacted water body, these informations can be very useful for conservation.

Key words: biodiversity, Ciliophora, freshwater, sediment, survey.

Introduction

Ciliated protozoa successfully colonized most of the world's habitats, occurring as free-living forms in fresh, brackish and marine water environments, including the deep sea, polar and desert regions, soils, associated with bromeliads, mosses and other tanks plants (Lynn, 2008). The ciliates are considered important planktonic and benthic components of freshwater environments as they are responsible for the energy transfer from primary producers to higher trophic levels in the microbial loop (Zingel *et al*., 2007; Almeida *et al*., 2009; Jiang *et al*., 2013).

It is estimated that about 83% to 89% of the diversity of ciliates are still unknown to science (Foissner & Hawksworth, 2009) which, according to Cotterill *et al*. (2008), reflects the fact that protists in general are rarely considered in conservation and biodiversity management programs, although the importance of their roles in ecosystems has been thoroughly emphasized in the literature (Fenchel 1987; Corliss, 2004; Foissner, 2006; Dopheide *et al*., 2009).

The taxonomic survey of the ciliates species is very important due to the rapid responses of these organisms to human activities such as the discharge of organic matter in water bodies. Also, the importance of biodiversity knowledge is also related to the great biotechnological potential they present. According to Gutierrez *et al*. (2011), ciliated protozoa are capable of accumulating metal ions to bind proteins, such as zinc, cadmium and copper and may be used in bioremediation processes. In addition, the ciliated protozoa have been considered as a new source of secondary metabolites, since such molecules may have antiviral and antimicrobial activities (Petrelli *et al*., 2012).

Ciliated protozoa play a vital role in the indication of pollution degree in freshwater environments (RAJABUNIZAL & RAMANIBAI, 2013). Then, these organisms can be used in the monitoring of water courses because they play a significant role in the decomposition process through the consumption of bacteria and promptly react to a variety of factors, such as increased organic matter content (TIRJAKOVÁ & VDACNY, 2013). Additionally, the abundance ratios of ciliates may provide a comparatively reliable picture about the actual intensity of trophic conditions and processes of self-purification in freshwater environments (TIRJAKOVÁ & VDACNY, 2013).

The aim of the present work was to do a taxonomic survey of ciliated protozoa from surface water and sediment samples in order to suggest a checklist of these organisms from the Atibaia River, which is the major water supply of the Campinas Metropolitan Area (Southeast Brazil). This is the first record of ciliated protozoa in this water body that is already impacted by pollution.

MATERIAL AND METHODS

Study area

The study was conducted at the Atibaia River that is currently responsible for 95% of the water supply of Campinas but its water quality is unsatisfactory, so modern treatment techniques are required (SANASA, 2013). The Atibaia River is part of Piracicaba, Capivari and Jundiaí Basin, and also provides water to the Cantareira reservoir that supplies 52% of the Metropolitan Region of São Paulo (SANASA, 2013). This river receives domestic and industrial sewage directly and indirectly through its tributaries as the Pinheiros Stream (CETESB, 2012).

In this study, it was considered the possible effects of dewatering waters of the Pinheiros Stream and, for this, two points were selected: the first (P1) is located upstream of the Pinheiros Stream and the second (P2) is downstream of this water body (Figure 1).

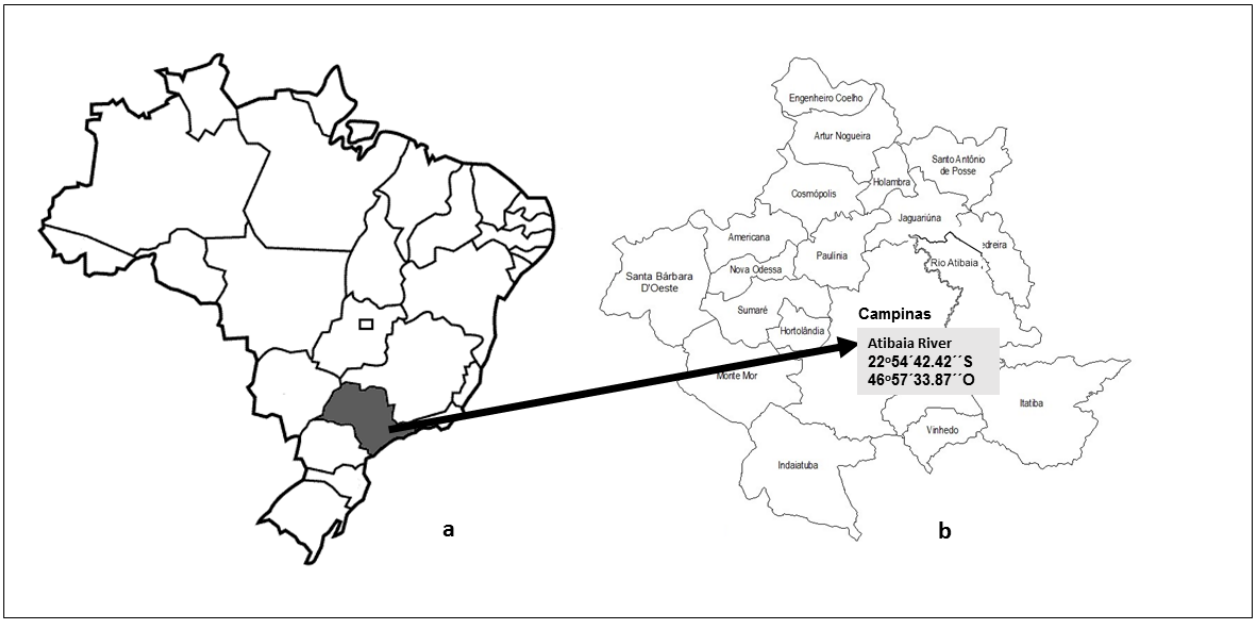

Figure 1. Collection samples site: a) Brazil; b) metropolitan region of Campinas and the collection site at Atibaia River.

Sampling

During the period from April 2012 to March 2014, twenty four samples of surface water and sediment were collected in the sampling points (P1 and P2) of the Atibaia River, Campinas, SP. In each sampling point, surface water and sediment samples were collected separately.

First, the surface water samples (500 mL) were collected with the aid of a large aperture collector. The sediment samples were obtained with an Ekman dredge, taking precautions to avoid waves which could lead to resuspension of surface sediment. This equipment has the advantage of preserving the sediment-water interface, referring to about 5 cm in the sedimentary column (Figueiredo & Brehme, 2000). The samplers were washed between collections of points P1 and P2. Surface water samples and sediment samples from each point were placed in plastic bottles, previously identified.

Methodology for ciliatofauna study

To evaluate the ciliatofauna of Atibaia River, the fresh samples, *in natura*, were analyzed until six hours after collection under a differential interference contrast (DIC) microscopy in order to make preliminary identifications. Slides were done and visualized under DIC microscopy at 40x magnification to 100x.

In the laboratory, 500 ml of each sample collected was manually homogenized and a sub-sample of 200 ml was placed in a beaker to promote material settling. Surface water samples were analyzed after the disposal on the beaker and sediment samples were analyzed only after about sixty minutes, in order to create the sediment-water interface (Bradley *et al*., 2010). When it was necessary, sediment sample was diluted with mineral water for better observation of ciliates.

Cultures of each sample were prepared in order to observe the encysted diversity and to carry out the identification techniques. Aliquots of 20 mL of each material were placed in a Petri dish with addition of crushed rice grains and were observed once a week, during four weeks, under a stereoscopic microscope and ciliated protozoa were isolated with glass micropipettes. Morphological aspects of ciliates *in vivo* were observed and photo-documented by scientific digital color camera, model Axiocam MRC - Zeiss Axio Imager attached to the microscope. The observation of ciliates *in vivo* is of great importance in the taxonomic classification of the genera and species (Foissner & Berger, 1996; Berger, 2011).

In our study, details of the ciliature organization, macro and micronucleus were revealed by protargol impregnation technique proposed by Dieckman (1995) and by scanning electron microscopy according Silva-Neto *et al*. (2012).

Results and Discussion

In the present study, it was recorded 67 taxa belonging to 56 genera. The highest number of taxa was found in the sediment (SD) and surface water (SW) of point 2, being respectively 52 and 39 morphospecies identified (Table 1 and 2). In the sediment samples and surface water point 1, were recorded 40 and 17 morphospecies, respectively. Some ciliates registered in this study are presented in Figure 2 and 3.

The identified morphospecies are distributed in the classes **Armophorea** Lynn, 2004; **Colpodea** Small & Lynn, 1981; **Heterotrichea** Stein, 1859; **Karyorelictea** Corliss, 1974; **Litostomatea**

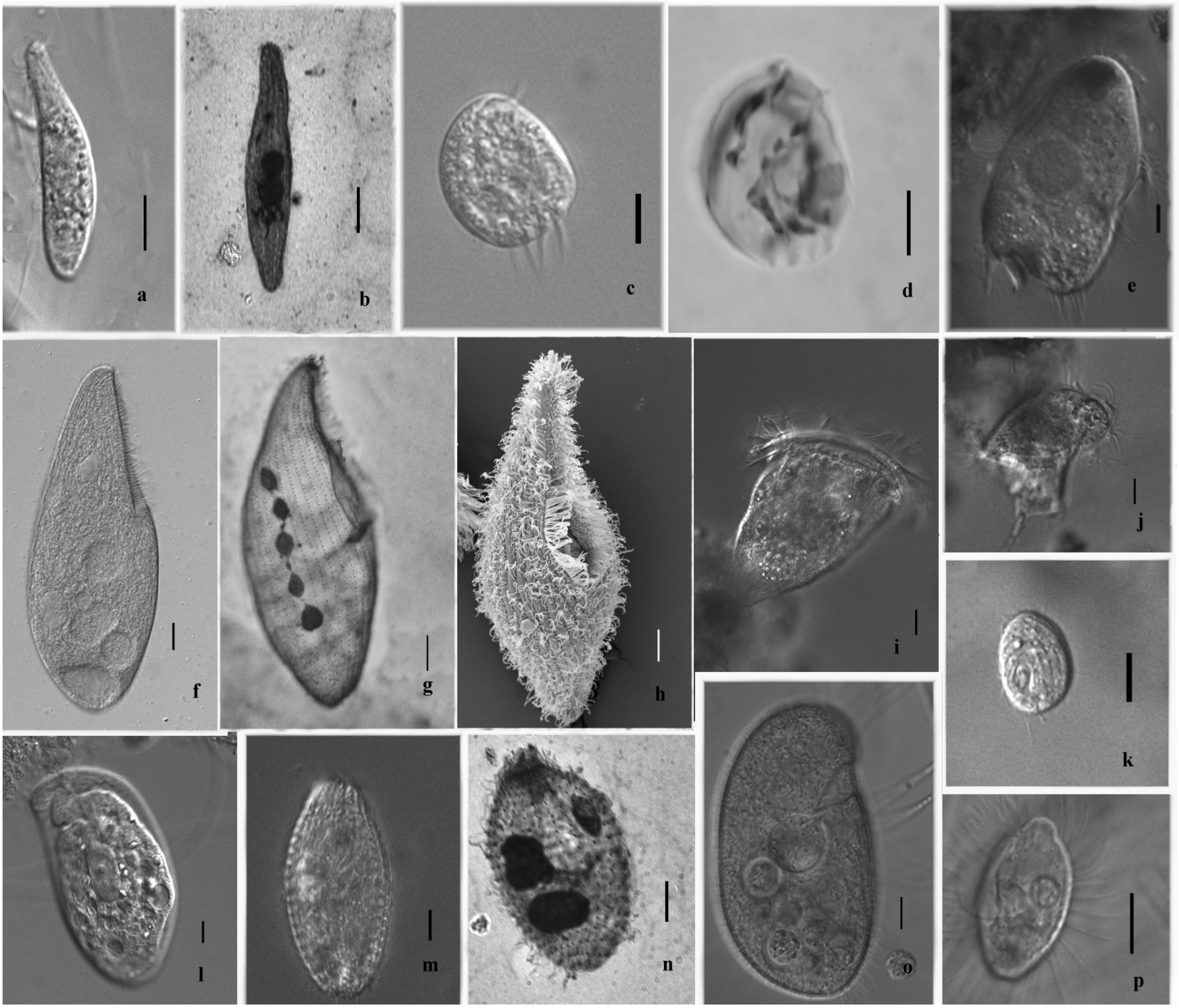

Figure 2. Some ciliated protozoa found in surface water and sediment samples from Atibaia River, Campinas, São Paulo. *In vivo* organisms (a; c; e; f; i; j; k; l; m; o; p), Protargol stained (b; d; g; n) and electromicrography (h). a-b: *Acineria uncinata*; c-d: *Aspidisca cicada*; e: *Brachonella spiralis*; f-h: *Blepharisma sinuosum*; i: *Campanella umbellaria*; j: *Caenomorpha* sp.; k: *Cinetochilum margaritaceum*; l: *Chilodonella uncinata*; m-n: *Coleps hirtus*; o: *Colpidium colpoda*; p*: Cyclidium glaucoma*. Bars = 10 µm.

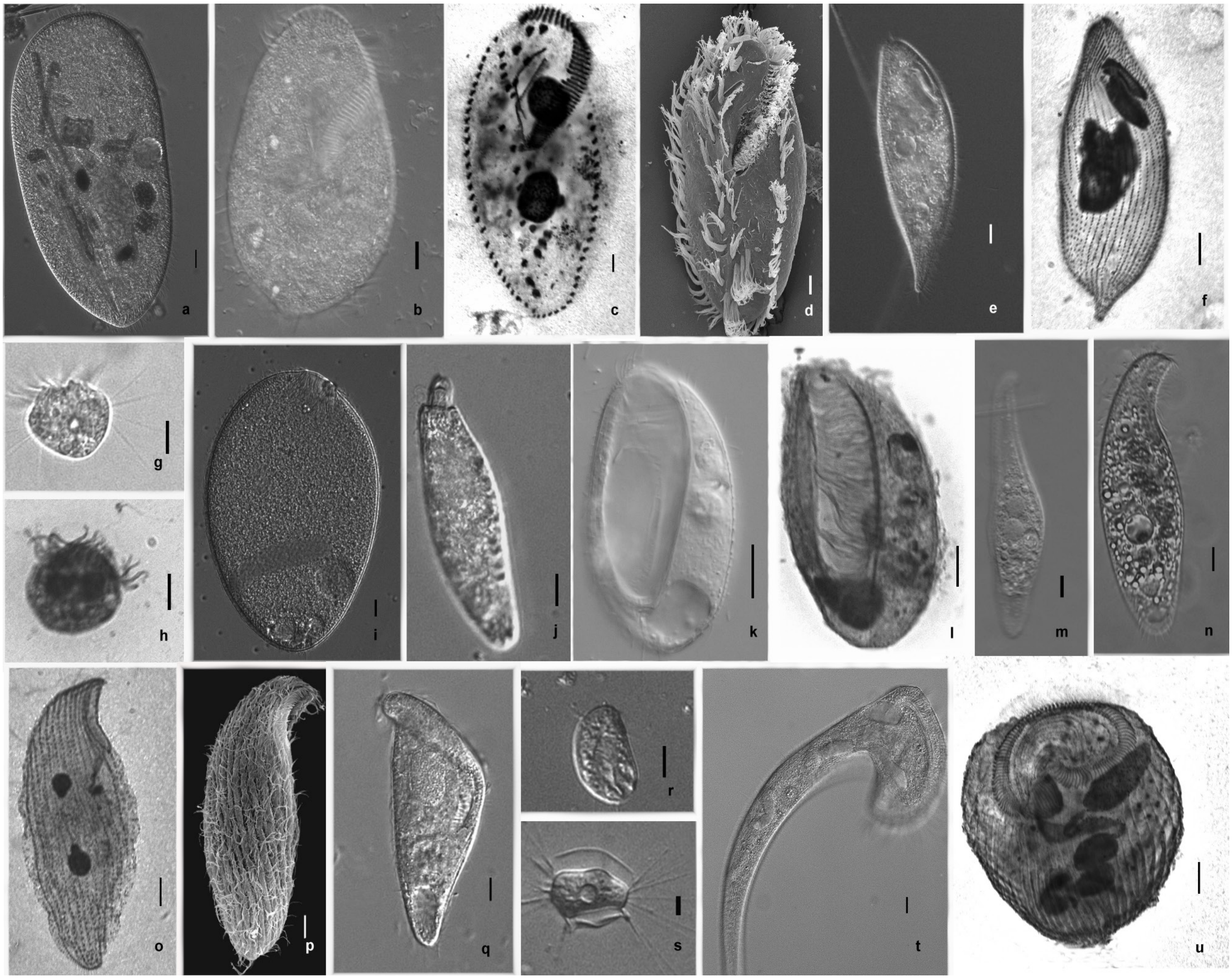

Figure 3. Some ciliated protozoa found in surface water and sediment samples from Atibaia River, Campinas, São Paulo. *In vivo* organisms (a; b; e; g; I; j; k; m; n; q; r; s; t), Protargol stained (c; f; h; l; o; u) and electromicrography (d; p). a: *Frontonia leucas*; b-d: *Gastrostyla setifera*; e-f: *Glaucoma frontata*; g-h: *Halteria grandinella*; i: *Holophrya* sp.; j: *Lagynus elegans*; k-l: *Lembadion lucens*; m: *Litonotus* sp.; n-p: *Loxodes striatus*; q: *Metopus* sp.; r: *Microthorax pusillus*; s: *Metacineta* sp.; t-u: *Stentor coeruleus*. Bars = 10 µm.

Table 1. Ciliated protozoa (Phylum Ciliophora) species found in surface water samples collected from point 1 (P1) and point 2 (P2) of Atibaia River, Campinas, São Paulo.

Subphylum	Class	Order	Specie	Occurrence
Postciliodesmatophora Gerassimova & Seravin, 1976	**Karyorelictea** Corliss, 1974	Loxodida Jankowski, 1980	*Loxodes striatus* (Englemann, 1862) Penard, 1917	P2
Intramacronucleata Lynn, 1996	**Armophorea** Lynn, 2004	Armophorida Jankowski, 1964	*Metopus* sp.	P1/P2
	Litostomatea Small and Lynn, 1981	Pleurostomatida Schewiakoff, 1896	*Acineria incurvata* Dujardin 1841 *Enchelys* sp. *Litonotus* sp. *Trachelophyllum* sp.	P2 P2 P1/P2 P2
	Nassophorea Small e Lynn, 1981	Nassulida Jankowski, 1968	*Microthorax pusillus* Engelmann, 1862	P1/P2
	Oligohymenophorea Puytorac *et al*., 1974	Peniculida Fauré-Fremiet in Corliss, 1956	*Lembadion lucens* (Maskell, 1887) Kahl, 1931 *Paramecium aurelia*-complex *Paramecium caudatum* Ehrenberg, 1833	P1/P2 P1/P2 P2
		Philasterida Small, 1967	*Uronema nigricans* (Müller, 1786) Florentin, 1901	P1/P2
		Pleuronematida Fauré-Fremiet in Corliss, 1956	*Cyclidium glaucoma* O.F.M., 1786	P1/P2
		Sessilida Kahl, 1933	*Carchesium polypinum* Complex *Epistylis plicatilis* -complex *Vorticella aquadulcis* -complex *Vorticella convalaria* -complex *Vorticella* sp.	P2 P2 P2 P2 P1/P2
		Tetrahymenida Fauré-Fremiet in Corliss, 1956	*Dexiostoma campylum* (Stokes, 1886) Jankowski, 1967 *Glaucoma frontata* (Stokes, 1886) Kahl, 1931 *Glaucoma scintillans* Ehrenberg, 1830 *Tetrahymena pyriformis* -complex	P1/P2 P2 P1/P2 P2
		Urocentrida Jankowski, 1980	*Urocentrum turbo* (Mueller, 1786) Furgason, 1940	P2
	Phyllopharyngea Puytorac *et al*., 1974	Chlamydodontida Deroux, 1976	*Chilodonella uncinata* Ehrenberg, 1838) Strand,1928 *Trithigmostoma cucullulus*	P2 P1/P2
		Evaginogenida Jankowski, 1978	*Heliophrya* sp.	P2
		Exogenida Collin, 1912	*Metacineta* sp.	P2
		Philasterida Small, 1967	*Cinetochilum margaritaceum* (Ehrenberg, 1831) Perty, 1849	P2
	Prostomatea Schewiakoff, 1896	Prorodontida Corliss, 1974	*Coleps hirtus* (Mueller, 1786) Nitzsch, 1827	P1/P2

Table 1. Continuation.

	Spirotrichea Bütschli, 1889	Choreotrichida Small & Lynn, 1985	*Strobilidium caudatum (*Fromentel, 1876) Foissner, 1987	P1/P2
		Euplotida Small & Lynn, 1985	*Aspidisca cicada* (Müller, 1786) Claparede & Lachmann, 1858 *Euplotes aediculatus* Pierson, 1943 *Euplotes eurystomus* (Wrzesniowski, 1870) Kahl, 1932	P1/P2 P1/P2 P2
		Stichotrichida Fauré-Fremiet, 1961	*Gastrostyla setifera* (Engelmann, 1862) Kent, 1882	P1/P2
		Sporadotrichida Fauré-Fremiet, 1961	*Halteria grandinella* (Müller, 1773) Dujardin, 1841 *Oxytricha chlorelligera* Kahl, 1932 *Oxytricha* sp. *Tetmemena pustulata* (Müller, 1786) Eigner, 1999	P2 P2 P1/P2 P2
		Strombidiida Petz & Foissner, 1992	*Strombidium* sp.	P2

Table 2 Ciliated protozoa (Phylum Ciliophora) found in sediment samples collected fromLe point 1 (P1) and point 2 (P2) of Atibaia River, Campinas, São Paulo.

Subphylum	Class	Order	Specie	Occurrence
Postciliodesmatophora Gerassimova & Seravin, 1976	**Karyorelictea** Corliss, 1974	Loxodida Jankowski, 1980	*Loxodes striatus* (Englemann, 1862) Penard, 1917 *Loxodes* sp.	P1/P2 P2
	Heterotrichea Stein, 1859	Heterotrichida Stein, 1859	*Blepharisma sinuosum* Sawaya, 1940 *Spirostomum minus* Roux, 1901 *Spirostomum teres* Claparède & Lachmann, 1858 *Stentor coeruleus* Ehrenberg, 1830	P1 P1/P2 P1/P2 P2
Intramacronucleata Lynn, 1996	**Armophorea** Lynn, 2004	Armophorida Jankowski, 1964	*Brachonella spiralis* (Smith, 1897) Jankowski, 1964 *Caenomorpha* sp. *Metopus* sp.	P2 P1/P2 P1/P2
	Colpodea Small and Lynn, 1981	Colpodida de Puytorac *et al.*, 1974	*Colpoda* sp.	P2
	Litostomatea Small and Lynn, 1981	Pleurostomatida Schewiakoff, 1896	*Acineria incurvata* Dujardin 1841 *Amphileptus* sp. *Litonotus* sp.	P1/P2 P2 P1/P2
		Haptorida Corliss, 1974	*Perispira* sp.	P2
	Nassophorea Small and Lynn, 1981	Nassulida Jankowski, 1968	*Drepanomonas revoluta* Penard 1922 *Microthorax pusillus* Engelmann, 1862	P2 P1/P2
	Oligohymenophorea de Puytorac *et al.*, 1974	Peniculida Fauré-Fremiet in Corliss, 1956	*Frontonia leucas* (Ehrenberg, 1833) Ehrenberg, 1838 *Lembadion lucens* (Maskell, 1887) Kahl, 1931 *Paramecium aurelia* -complex *Paramecium caudatum* Ehrenberg, 1833	P1/P2 P1 P1/P2 P1/P2

Table 2. Continuation.

		Philasterida Small, 1967	*Uronema nigricans* (Müller, 1786) Florentin, 1901	P1/P2
		Pleuronematida Fauré-Fremiet in Corliss, 1956	*Cyclidium glaucoma* O.F.M., 1786	P1/P2
		Sessilida Kahl, 1933	*Campanella umbellaria* (Linnaeus, 1758) Goldfuss, 1820 *Carchesium polypinum*-complex *Epistylis plicatilis*-complex *Pseudovorticella* sp. *Vorticella aquadulcis*-complex *Vorticella* sp.	P1/P2 P2 P1/P2 P1 P1/P2 P1/P2
		Tetrahymenida Fauré-Fremiet in Corliss, 1956	*Colpidium colpoda* (Losana, 1829) Stein, 1860 *Dexiostoma campylum* (Stokes, 1886) Jankowski, 1967 *Glaucoma frontata* (Stokes, 1886) Kahl, 1931 *Glaucoma scintillans* Ehrenberg, 1830 *Tetrahymena pyriformis* -complex	P2 P1/P2 P1/P2 P1/P2 P1/P2
		Urocentrida Jankowski, 1980	*Urocentrum turbo* (Mueller, 1786) Nitzsch, 1827	P1/P2
	Prostomatea Schewiakoff, 1896	Prorodontida Corliss, 1974	*Coleps hirtus* (Mueller, 1786) Nitzsch, 1827 *Holophrya* sp. *Lagynus elegans* (Engelmann, 1862) Quennerstedt, 1867 *Prorodon* sp.	P1/P2 P1 P1/P2 P1
	Phyllopharyngea Puytorac *et al*., 1974	Chlamydodontida Deroux, 1976	*Chilodonella uncinata* Ehrenberg, 1838) Strand,1928 *Trithigmostoma cucullulus* Jankowksi, 1967	P1/P2 P2
		Endogenida Collin, 1912	*Acineta* sp. *Tokophrya* sp.	P2 P1
		Exogenida Collin, 1912	*Metacineta* sp. *Podophrya fixa* Müller, 1786	P2 P1/P2
		Philasterida Small, 1967	*Cinetochilum margaritaceum* (Ehrenberg, 1831) Perty, 1849	P1/P2
	Spirotrichea Bütschli, 1889	Choreotrichida Small & Lynn, 1985	*Strobilidium caudatum* (Fromentel, 1876) Foissner, 1987	P1/P2
		Euplotida Small & Lynn, 1985	*Aspidisca cicada* (Müller, 1786) Claparede & Lachmann, 1858 *Aspidisca lynceus* O.F. Müller, 1773 *Euplotes aediculatus* Pierson, 1943 *Euplotes eurystomus* (Wrzesniowski, 1870) Kahl, 1932 *Euplotes woodruffi* Gaw 1939	P1/P2 P1/P2 P1/P2 P2 P1/P2

Table 2. Continuation.

	Sporadotrichida Fauré-Fremiet, 1961	*Halteria grandinella* (Müller, 1773) Dujardin, 1841	P2
		Oxytricha granulifera Foissner and Adam, 1983	P1/P2
		Oxytricha sp.	P1/P2
		Pleurotricha lanceolata (Ehrenberg, 1838) Stein, 1859	P1
		Sterkiella histriomuscorum -complex	P2
		Stylonychia bifaria (Stokes, 1887) Berger, 1999	P1/P2
		Tetmemena pustulata (Müller, 1786) Eigner, 1999	P1/P2
	Strombidiida Petz & Foissner, 1992	*Strombidium* sp.	P2
	Urostylida Jankowski, 1979	*Pseudourostyla nova* Wiackowski, 1988	P2

Small & Lynn, 1981 **Nassophorea** Small & Lynn, 1981; **Oligohymenophorea** Puytorac *et al*., 1974; **Prostomatea** Schewiakoff, 1896; **Phyllopharyngea** Puytorac *et al*., 1974 and **Spirotrichea** Bütschli, 1889, these classified according Lynn (2008) (Table 1 and 2).

Ciliates are found in diverse aquatic habitats primarily in free-living form (Foissner, 2006; Foissner *et al*., 2008), and their composition, density and distribution reflect the physical, chemical and biotic aspects of the environment (Zhou *et al*., 2008; Almeida *et al*., 2009; Bagatini *et al*., 2013; Nawrot & Mieczan, 2014). The spatial and seasonal distribution of density and biomass of ciliates generally follows the fluctuations of bacteria and phytoplankton production (Santoferrara & Alder, 2009).

The ciliates communities recorded in sediment samples from both points (P1 and P2), compared to surface water, were more diverse and this occurred probably due to the retention of organic matter and higher bacterial biomass as point 2 is downstream Pinheiros Stream and had illegal sewer discharges. Many ciliated protozoa are able to form cysts (Foissner, 1987), allowing them to persist in the sediment, and subsequently colonize the body of water through excystment (Küppers *et al*., 2009).

In the literature there are revised and expanded surveys of ciliated protozoa species that are used as indicator organisms for environmental quality (Sladecek, 1973; Foissner, 1988; Foissner *et al*., 1995; Madoni & Zangrossi, 2005). Most species of ciliates found in surface water and sediment samples in the Atibaia River are included in the saprobic system, and are considered bioindicators. The saprobic index (Sladecek, 1973; Foissner 1996) is based on the degree of tolerance of the species of ciliates to organic pollution.

From the species listed in the present study, 57 of them are included in saprobic system and are considered indicators of the following environments: polisaprobic (n = 14), alphamesosaprobic **(**n = 16), alpha-betamesosaprobic (n = 14), betamesosaprobic (n = 12) and oligosaprobic (n = 1). Several studies carried in rivers from France (Sparagano & Grolière, 1991), Spain (Sola *et al*., 1996), Italy (Madoni & Ghetti, 1981; Madoni, 1993; Madoni & Bassanini, 1999; Madoni, 2005; Madoni & Braghiroli, 2007) have demonstrated an increase in the proportion of polysaprobic and alphamesosaprobic species and a decrease in betamesosaprobic and oligosaprobic with the rise of pollution load caused by antropic impacts. Anthropogenic pressures that impact water bodies, such as organic matter, toxic pollution or eutrophication may alter the structure of ciliated protozoa community (Madoni, 2000; Madoni & Bassanini, 1999; Tirjaková & Vdacny, 2013; Xu *et al*., 2005).

Species like *Enchelys* sp., *Gastrostyla setifera*, *Heliophrya* sp., *Oxytricha chlorelligera*, *Trachelophyllum* sp. and *Vorticella convalaria*-complex were registered only in surface water samples. Only the morphospecies *Gastrostyla setifera* occurred in point 1 and 2, the others occurred only in point 2.

The ciliate *G. setifera* was already registered by Dias *et al*. (2007) in water and sediment samples from an urban freshwater stream in the city of Juiz de Fora, Minas Gerais, Brazil. Madoni & Zangrossi (2005) collected at two stations *O. chlorelligera* from water-sediment interface of a branch of the River Taro, Italy.

The following species were only found in sediment samples: *Aspidisca lynceus*, *Blepharisma sinuosum*, *Brachonella spiralis*, *Caenomorpha* sp., *Campanella umbellaria*, *Colpoda* sp., *Euplotes woodruffi*, *Frontonia leucas*, *Holophrya* sp., *Lagynus elegans*, *Oxytricha granulifera*, *Perispira* sp., *Pleurotricha lanceolata*, *Podophrya fixa*, *Prorodon* sp., *Pseudourostyla nova*, *Pseudovorticella* sp., *Spirostomum teres*, *Sterkiella histriomuscorum*-complex, *Stylonychia bifaria* and *Tokophrya* sp.

Some morphospecies were rarely described in freshwater environments. *Acineria incurvata* was found in surface water of point 2. Previous studies found this ciliated protozoan as the survey done in Slovakia freshwater and marine environments (Vdacny & Rajter, 2014) and also, by Jiang & Shen (2005) in an urban water system in China. This ciliated protozoan is most commonly found in wastewater treatment plants as reported by Pérez-Uz *et al*. (2010) and Salvadó *et al*. (2004) and is associated to polysaprobic environments (Foissner, 1996).

In Brazil there are some studies done in freshwater environments with the aim to survey ciliated protozoa species. Regali-Seleghim *et al*. (2011) collected samples from 75 freshwater environments in São Paulo State and found 218 genera and 304 species of ciliated protozoa. Paiva & Silva Neto (2004) found 34 species in Cabiúnas Lagoon in Rio de Janeiro. Pauleto *et al*. (2009) surveyed 61 species of ciliated protozoa at the floodplain of the Paraná River; Velho *et al*. (2013) detected 35 ciliated species in the lake of the Ingá Park, a permanent preservation area of Maringá city in Paraná State; Mendonça (2012) identified 45 planktonic ciliated protozoa species and 32 epibiontic ciliated in Rio das Velhas, Minas Gerais.

There is a need for studies to survey ciliated protozoa biodiversity as they have a great potential to be used as bioindicators and also for biotechnology purposes. According Cotterill *et al*. (2008), there is a lack of local and global research about these diversity organisms and this is the reason for their exclusion from environmental management programs and biodiversity conservation. The employment of impregnation techniques using silver proteinate are very valuable for ciliated protozoa identification as they show the most important morphological characteristics and complement the *in vivo* information, which makes such procedures needed in studies of biodiversity of ciliates. The survey of ciliated protozoa found in Atibaia River extends the knowledge and the occurrence of species present in brazilian lotic systems.

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