Brazilian Journal of Biology
ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

https://doi.org/10.1590/1519-6984.187301
Notes and Comments

Ciliated microeukaryotes (Alveolata: Ciliophora) of a lotic urban system located in Minas Gerais - Brazil

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Received: October 31, 2017 – Accepted: January 2, 2018 – Distributed: August 31, 2019
(With 1 figure)

The phylum Ciliophora is organized in 14 classes with ~8,000 described species (Gao et al., 2016). Among unicellular microeukaryotes, ciliates are the most specialized, diversified and with the highest complexity in terms of cellular organization (Puytorac, 1994). They typically occupy basal trophic levels and display a wide geographical distribution occurring in almost all environments such as marine, fresh and brackish waters, and also in edaphic systems like soils, mosses and lichens (Lynn, 2008).

Their diversity in lotic systems have been extensively studied in the northern hemisphere because of their potential use as water quality bioindicators (Wiackowski, 1981; Primc, 1988; Groliére et al., 1990; Madoni, 2005). However, scarce information is available in literature about their diversity in aquatic ecosystems in Brazil, even though its privileged hydrological condition. The access to ciliate diversity and biogeography is a challenge tasks because they are diminute organisms, difficult to identify (Finlay and Fenchel, 1999), there is a lack of experienced specialists (Foissner, 2006) and a number of species stay encysted for most of their life cycles (Foissner, 2004). In Brazil, significant works were performed with this emphasis such as conducted in Rio Grande do Sul (Safi et al., 2014), Paraná (Pauleto et al., 2009; Buosi et al., 2011; Velho et al., 2005, 2013), São Paulo (Bagantini et al., 2013; Regali-Seleghim et al., 2011), Pará (Castro et al., 2014), Minas Gerais (Dias et al., 2008, 2010) and Rio de Janeiro (Paiva and Silva-Neto, 2004a, b) states. Inventory studies constitute the first step for development of applied biotechnological usage of ciliates. Regali-Seleghim et al. (2011) highlight the importance of more works surveying the diversity of ciliates in less studied regions of Brazil given their ecological importance. Moreover, establishment of *in vitro* cultures will contribute with information to biomonitoring programs (Madoni and Romeo, 2006; Shi et al., 2012) and for better evaluation of the biotechnological potential of these organisms (Mansano et al., 2016). This present work aimed to survey the diversity of the species of ciliated protists in a neotropical lotic urban system located in Southern region of Minas Gerais state, Brazil.

The samples were taken from José Pereira stream (45°27'31" and 45°20' 57W, 22°23'18" and 22°26'57"S) a highly impacted watercourse with *in natura* disposal of sewage (Thomaz da Silva, 2015) located in the municipality of Itajubá, Minas Gerais, Brazil. A Van Veen dredge was used to collect sediment monthly for over a year (October 2014 to October 2015). The sediment samples were readily transferred to 500 mL plastic containers and moved to the laboratory to be processed. Each sample were then divided (~20 mL) into three petri dishes and screened using glass micropipettes. Each Petri dish was analyzed in the day of collecting and weekly for up to 4 weeks. For *in vitro* cultures, ciliates were transferred to new Petri dishes filled with mineral water where rice grains with shells were added to served as carbon source for bacterial growth that would act to sustain the propagation of the tested ciliates. The ciliates were identified according to Foissner and Berger (1996). The photographic records of *in vivo* specimens were carried out with the aid of a camera attached to a microscope Olympus BX 51. The main features used in the identification of ciliates were: body shape, position and number of contractile vacuoles, oral and somatic ciliatures, position of macronucleus and shape of inclusions and color and the cytoplasm. Eventually, DAPI, a DNA specific staining method (Kapuscinski, 1995), protargol (Dieckmann, 1995) and dry silver nitrate (Klein, 1958) were used for species confirmation.

We recorded 48 ciliate morphospecies from the sediment samples taken from José Pereira stream (Figure 1). These microorganisms were classified according to Lynn (2008) and distributed into the classes Karyorelictea (n=1), Heterotrichea (n=6), Spirotrichea (n=15), Litostomatea (n=2), Phyllopharyngea (n=2), Colpodea (n=1), Prostomatea (n=1), Oligohymenophorea (n=20) (Table 1). The class Oligohymenophorea were the most abundant in species number being distributed into the sub-classes: Peniculia (n=6), Hymenostomatia (n=4) and Peritrichia (n=10).

Among all these morphospecies, the species *Euplotes aediculatus*, *Euplotes eurystomus*, *Spirostomum minus* and *Spirostomum teres*, and *Paramecium bursaria*, *Paramecium caudatum* and *Tetmemena pustulata* were

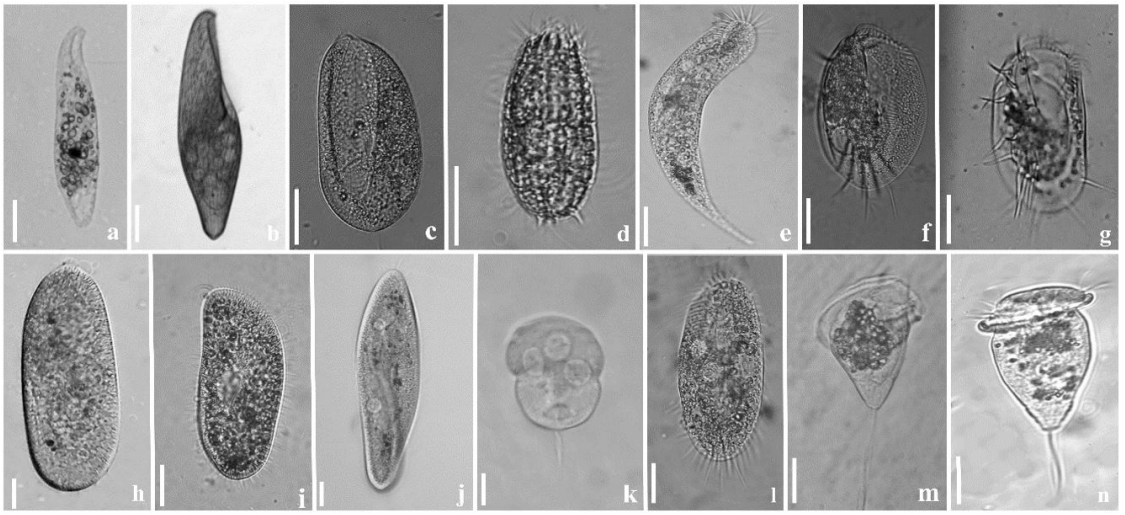

Figure 1. Representatives of ciliated found in José Pereira. (a) *Loxodes striatus*; (b) *Blepharisma sinuosum*; (c) *Lembadium lucens*; (d) *Coleps hirtus*; (e) Spirotrichea (morphospecies 1); (f) *Euplotes aediculatus*; (g) *Euplotes eurystomus*; (h) *Frontonia leucas*; (i) *Paramecium bursaria*; (j) *Paramecium caudatum*; (k) *Urocentrum turbo*; (l) *Tetmemena pustulata*; (m) *Vorticella campanula*; (n) *Vorticella convallaria*-complex. Barras: 20 μm.

the ones that best adapted to the in vitro growth conditions (up to several months) using mineral water and rice grains and Cerophyl medium (Sonneborn, 1957), respectively (data not shown).

This study contributed to the understanding of the diversity of ciliated protists in Brazil, since this is the first work in the Southern region of Minas Gerais. Still, such studies can be useful to a better comprehension of the trophic relationships in aquatic environments, can support biomonitoring programs that assess the quality of water as well as the maintenance and conservation of the species with biotechnological potential (Madoni and Romeo, 2006; Regali-Seleghim et al., 2011; Gutièrrez et al., 2011).

In Brazil, the first work on ciliates from freshwater environments were carried out by Cunha in the early twentieth century (Cunha, 1913; Faria and Cunha, 1917; Cunha and Fonseca, 1918) with few recent studies on inventory of these micro-organisms in inland waters (Paiva and Silva-Neto, 2004a, b; Dias, 2007; Dias et al., 2008; Regali-Seleghim et al., 2011; Safi et al., 2014; Sartini, 2012; Mendonça, 2012; Castro et al., 2014; Kuhner et al., 2016). Cotterill et al. (2008) estimated that there are about 40,000 species of free-living ciliates, where only 4,500 species (~ 11%) have been described so far. Recent studies emphasize the need to increase sampling effort in South America for a better understanding of ciliates diversity in this region (Fenchel and Finlay, 2004; Foissner, 2006; Foissner and Hawksworth, 2009), as there are a large number of unexplored environments and potential implication to biomonitoring and conservation of these ecosystems (Mitchell and Meisterfeld, 2005; Cotterill et al., 2008).

The saprobic system for water quality evaluation, and more specifically organic pollution, developed by Kolkwitz and Marsson (1908, 1909), is widely used in biological classification of running water. The original list of indicator species, including ciliates, was revised and expanded (Foissner, 1988). Among the 48 morphospecies found in the stream José Pereira, 23 are included in the saprobic system and are considered biomarkers (Table 1), in which the vast majority were indicative of organically enriched environments (polluted or extremely polluted water), such as *Loxodes striatus*, *Spirostomum teres*, *Paramecium caudatum*, *Euplotes aediculatus*, *Euplotes eurystomus*, *Tokophrya lemnarum*, *Cyclidium* cf. *glaucoma*, *Carchesium polypinum*, *Vorticella convallaria-complex*, *Spirostomum minus*, *Stentor polymorphus*, *Stentor roeselii*, *Aspidisca* and *Coleps hirtus*. This observation is corroborated by a recent study (Thomaz da Silva, 2015) focusing in the quality of the water in this same sampling station, using physical and chemical parameters to classify this lotic system as Class III (highly polluted water) (Brasil, 2005), and highlighted the high levels of electrical conductivity, total coliforms, phosphorus, total nitrogen, ammonia and chlorophyll.

Moreover, we were able to stably maintain the *in vitro* growth of seven species of ciliates: *Euplotes aediculatus*, *Euplotes eurystomus*, *Paramecium bursaria*, *Paramecium caudatum*, *Spirostomum minus*, *Spirostomum teres* and *Tetmemena pustulata*. The ability to grow these organisms *in vitro* using cerophyl medium (Sonneborn, 1957) expands the possibilities of future applied studies such as acute trials (ecotoxicology), detection, characterization and isolation of secondary metabolites, characterization of molecules with antimicrobial activity, contributing to neotropical water monitoring programs (Madoni and Romeo, 2006; Petrelli et al., 2012; Mansano et al., 2016). This present study contributes to a better comprehension about the diversity of ciliated protists in limnic ecosystems in Brazil and emphasizes the importance of development

Table 1. Ciliated protist species found in José Pereira stream, Itajubá, Minas Gerais, Brazil.

Ciliates from Brazilian freshwater ecosystems	D	1st	2nd	3rd	4th	S
Class Karyorelictea	**D**	**1st**	**2nd**	**3rd**	**4th**	**S**
Order Loxodida						
Loxodes striatus	+	–	–	–	–	p
Class Heterotrichea						
Order Heterotrichida						
Blepahrisma sinuosum	+	+	–	–	–	**
Spirostomum minus	+	+	+	+	+	**a-b**
Spirostomum teres	+	+	+	+	+	**p**
Stentor polymorphus	+	+	–	–	–	a-b
Stentor roeselii	+	+	–	–	–	a-b
Class Spirotrichea						
Sub-class Hypotrichia						
Aspidisca cicada	+	+	–	–	–	a-b
Euplotes aediculatus	+	+	+	+	+	a
Euplotes eurystomus	+	+	+	+	+	a
Sub-class Stichotrichia						
Tetmemena pustulata	+	+	+	+	+	**b**
Stichotrichia (morphospecies 1-9)	+	+	–	–	–	**
Sub-class Oligotrichia						
Halteria cf. *grandinella*	+	+	–	–	–	b-a
Class Litostomatea						
Order Haptorida						
Dipleptus sp.	+	+	+	–	–	**
Order Pleutostomatida						
Litonotus sp.	+	+	–	–	–	**
Class Phyllopharyngea	**D**	**1st**	**2nd**	**3rd**	**4th**	**S**
Sub-class Suctoria						
Order Endogenida						
Tokophrya lemnarum	+	+	–	–	–	a
Suctoria (morphospecies 1)	+	+	–	–	–	**
Class Prostomatea						
Coleps hirtus	+	+	–	–	–	a-b
Class Oligohymenophorea						
Sub-class Peniculia						
Frontonia leucas	+	+	+	–	–	b
Lembadium lucens	+	+	+	–	–	ba
Paramecium aurelia-complex	+	+	+	+	–	b-a
Paramecium bursaria*	+	+	+	+	+	**b**
Paramecium caudatum	+	+	+	+	–	p-a
Urocentrum turbo	+	+	–	–	–	b
Sub-class Hymenostomatia						
Glaucoma frontata	+	+	–	–	–	**
Hymenostomatia (morphospecies 1-2)	+	+	–	–	–	**
Order Scuticociliatida						
Cyclidium cf. *glaucoma*	+	+	–	–	–	a
Sub-class Peritrichia						
Carchesium polypinum	+	+	–	–	–	a
Epistylis sp.	+	+	–	–	–	**
Opercularia sp.	+	+	–	–	–	**
Vorticella convallaria-complex	+	+	+	–	–	a
Vorticella campanula	+	+	–	–	–	b
Vorticella (morphospecies 1-4)	+	+	–	–	–	**

D = appeared in the same day of assay; 1st = appeared in the first week of assay; 2nd = in the second week; 3rd = in the third week; 4th = in the fourth; S = saprobicity (Foissner & Berger, 1996); p = polysaprobic; a = alpha-mesosaprobic; b = beta-mesosaprobic; **= not classified; + = occurred; - = absent. Dark names represent species that have been successfully *in vitro* cultivated in mineral water supplemented with crushed rice with shells; *First record in Minas Gerais state.

of new and efficient growth methods for *in vitro* culture of these microorganisms aiming future biotechnological end environmental monitoring studies.

Acknowledgements

This work was partially supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Edital Universal 2016, FAPEMIG). The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provided research grant to Roberto Júnio P. Dias (Bolsa de Produtividade PQ) and CAPES to Marcus Vinicius Xavier. We thank Marcelo José de Carvalho for help in laboratory work.

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