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Short communication

Intestinal Ciliates of Brazilian Capybara (*Hydrochoerus hydrochaeris* L.)

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Abstract. In our study, we aimed to identify and quantify the intestinal ciliates in a Brazilian capybara and to compare the obtained data with previous studies on the capybara ciliate community in other geographic locations within the American continent. We identified 20 species belonging to four families and ten genera. This is the first study on intestinal ciliates in Brazilian capybara since the last reports for the country in the 1960s. Among the identified species, *Anacharon gracilis*, *A. lepturus*, *Cycloposthium bursa*, *Monoposthium cynodontum*, *Ogimotopsis pumila*, *Paracunhamunizia calocoma*, *Protohallia nana* and *Uropogon urai* were recorded for the first time in Brazil, and the giant ciliate *Muniziella cunhai* was observed for the second time in a symbiotic association with capybaras in the country. The present study highlighted the importance of knowing the gastrointestinal ciliate community associated with wild hosts in order to better understand their geographic distribution and host specificity.

Key words: Capybara, Entodiniomorphida, rodents, symbiotic ciliates, Trichostomatia, Vestibuliferida

INTRODUCTION

Many herbivorous mammals, such as Artiodactyla, Perissodactyla, Proboscidea, Rodentia, Primates, and Marsupialia have established symbiotic associations with ciliated protozoa in their gastrointestinal tract, and these microeukaryotes can digest cellulose and starch, contributing significantly to the digestive metabolism of their hosts (Dehority 1986).

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In rodents, endosymbiotic ciliates were detected in association with Brazilian-guinea-pigs (*Cavia aperea*) (Cunha *et al*. 1914, Neiva *et al*. 1914, Hasselmann 1918), guinea-pigs (*Cavia porcellus*) (Alves *et al*. 2007, Cunha *et al*. 1914, Hasselmann 1918), coypus (*Myocastor coypus*) (Silva *et al*. 2007), gundis (*Ctenodactylus gundi*) (Chatton and Pérard 1919) and capybaras (*Hydrochoerus hydrochaeris*) (Table 2).

The capybara is native to South America, more precisely to the east of the Andes, from Colombia and Venezuela south to Northern Argentina. It is the largest living rodent in the world (106–134 cm head-body length, 50–62 cm shoulder height, and 35–66 kg weight) inhabiting savannas and dense forests, living near bodies of water. It is a highly social species and can be found in groups as large as 100 individuals (Emmons 1997).

Endosymbiotic ciliates in Brazilian capybaras were first recorded by Cunha (1915), which described five species belonging to genera *Cycloposthium* (Entodiniomorphida) and *Paraisotricha* (Vestibuliferida). After this first report, several species have been described within the national territory (Cunha 1915, Cunha and Muniz 1925, Cunha and Muniz 1927a, b, c; Hollande and Batisse 1959; Batisse 1965, 1966), with only a few reports on these ciliates in other geographical locations (McLure 1976; Dehority 1987; Ito and Imai 2000a, b; Rodríguez-Durán *et al*. 2015).

In our study, we aimed to identify and quantify the intestinal ciliates in a Brazilian capybara and to compare the data obtained with previous studies on its ciliate community in different geographical locations.

MATERIALS AND METHODS

We studied a single capybara (*Hydrochoerus hydrochaeris*). The specimen was provided by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), Juiz de Fora, Minas Gerais, Brazil (218470S, 438220W) after being hit by a car. Samples were collected manually from the cecal mass, consisting of 20 cm^3 of cecal content, which was fixed in 18.5% formalin (v/v) (Dehority 1984).

Species identification was based on the descriptions made by Cunha (1915), Hasselmann (1918), Cunha and Muniz (1925, 1927a, b, c), Fonseca (1939), Hollande and Batisse (1959), Batisse (1965, 1966) and Ito and Imai (2000a, b). To identify the ciliates, we used an Olympus BX-51 light microscope (600 X and 1000 X magnification) equipped with an Olympus Evolt E-330 digital camera. We used the Image-Pro Plus 6.0 software to perform morphometry on 20 specimens of each species stained with the Lugol's solution (D'Agosto and Carneiro 1999) and impregnated by silver carbonate (Rossi *et al*. 2016).

The average relative abundance was estimated from a sample of 300 cells (Ito *et al*. 1994, Mishima *et al*. 2009) and the density was determined in a Sedgewick-Rafter counting chamber as in methods of to Dehority (1984) and D'Agosto and Carneiro (1999).

RESULTS AND DISCUSSION

In this study, we identified 20 species of trichostomatid ciliates, distributed over two orders (Entodiniomorphida and Vestibuliferida), four families (Cycloposthiidae, Protocaviellidae, Protohallidae and Pycnotrichidae) and ten genera (*Anacharon*, *Cycloposthium*, *Enterophrya*, *Hydrochoerella*, *Monoposthium*, *Muniziella*, *Ogimotopsis*, *Paracunhamunizia*, *Protohallia* and *Uropogon*) (Table 1, Fig. 1). This is the first study on the Brazilian capybara's intestinal ciliates since the previous reports for the area in the 1960s (Batisse 1965, 1966; Holande and Batisse 1959). The ciliate community inventoried in the present work is similar to that of other capybara check-lists in other geographic locations within the American continent (Table 2). The exception is the ciliate community associated with capybaras sampled at Columbus Zoo in Columbus, USA, which presented ciliates belonging to the family Ophryoscolecidae (*Entodinium* spp., *Elytroplastron bubali* and *Eudiplodinium magii*) (Dehority 1987).

According to Dehority (1987) and Ito and Imai (2000a, b) the capybara's cecal ciliate community is highly specific, and few of their symbiotic ciliates species are found in association with other herbivorous mammals. Outstandingly are the species belonging to the genus *Enterophrya* (*E. elongata* and *E. piriformis*), which are originally described as guinea-pig symbionts (*Cavia porcellus*) (Hasselmann 1918) and ophryoscolecid ciliates, sampled in capybaras in the USA (Dehority 1987).

In fact, ophryoscolecid ciliates colonizing the capybara's gastrointestinal tract is an atypical finding since it has not been previously reported. Since the animals sampled by the author were kept in enclosures shared with other animals in the Columbus Zoo, patagonian cavies (*Dolichotis patagonu*) and llama (*Lama glama*), a transfaunation process could be favored and therefore the capybaras could be harbor ciliates typically found in other mammalian species. The referred author also points out that the examination of the cecal content of capybaras housed at the Luiz de Queiroz School of Agriculture in Piracicaba, Brazil has demonstrated the occurrence of ciliated protozoa similar to those described as typical of capybara, another indication that the ciliate community in Columbus Zoo capybaras was unusual.

Studies on the capybaras gastrointestinal tract physiology show that the pH of the cecal region of these animals remains close to neutrality (6.5 to 7) (Gonzalez-Jimenez 1977, Borges *et al*. 2014), very similar values to those found in the ruminal environment and very favorable to the establishment of ophryroscolecid species (Cedrola *et al*. 2016).

Among the identified species, *Anacharon gracilis*, *A. lepturus*, *Cycloposthium bursa*, *Monoposthium cynodontum*, *Ogimotopsis pumila*, *Paracunhamunizia calocoma*, *Protohallia nana* and *Uropogon urai* were recorded for the first time in Brazil. The species *Muniziella cunhai* was observed for the second time in symbiotic association with capybaras in Brazil.

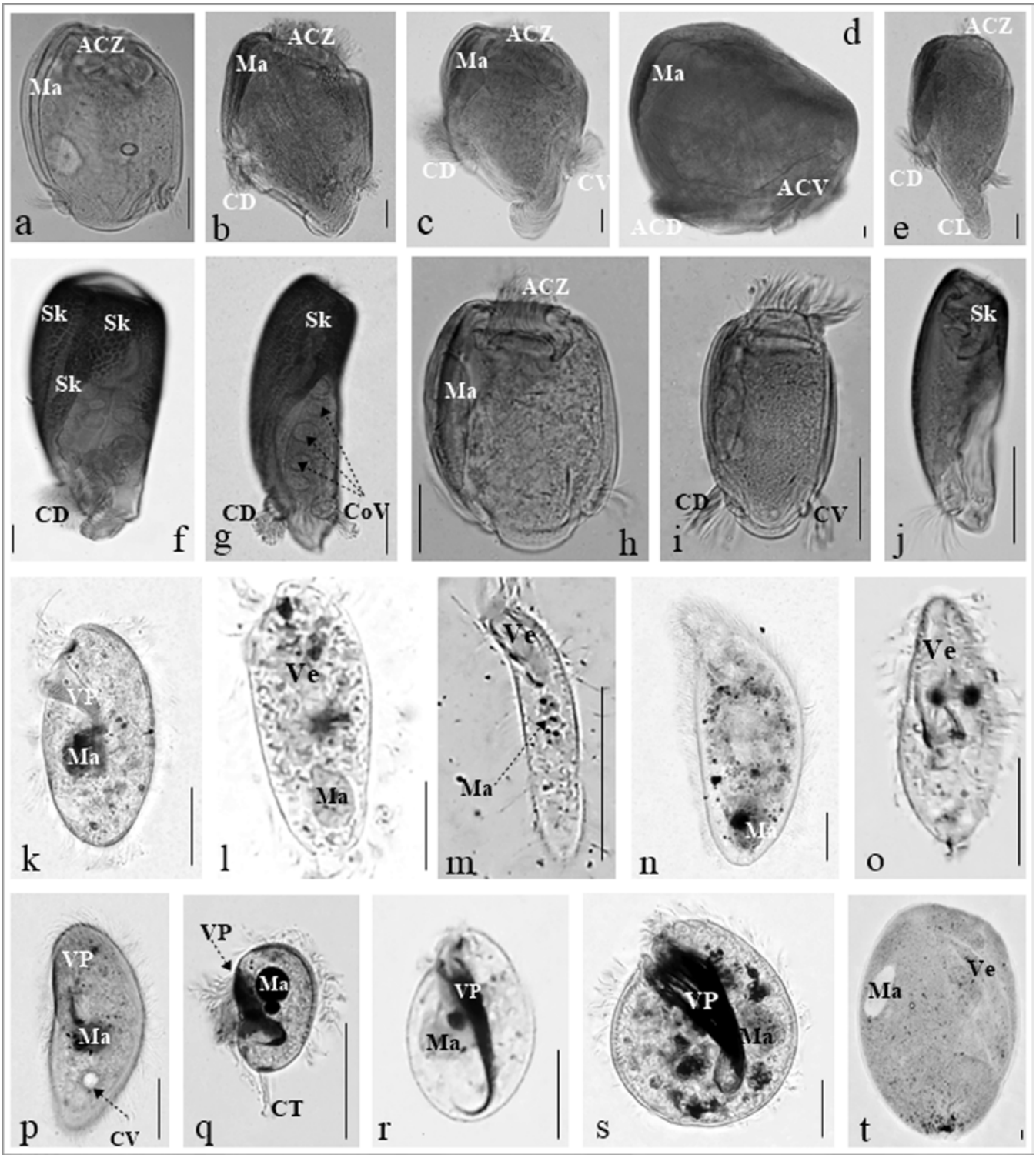

Fig. 1. Intestinal ciliates recorded in Brazilian capybara (*Hydrochoerus hydrochaeris*). **a–j.** family Cycloposthiidae (ciliates after Lugol's solution). **a.** *Cycloposthium bursa*, **b.** *Cycloposthium caudatum*, **c.** *Cycloposthium compressum*, **d.** *Cycloposthium cristatum*, **e.** *Cycloposthium elongatum*, **f.** *Cycloposthium hydrochoeri*, **g.** *Cycloposthium incurvum*, **h.** *Cycloposthium lenticularis*, **i.** *Cycloposthium minutum*, **j.** *Monoposthium cynodontum*. **k–q.** family Protocaviellidae (ciliates after silver carbonate impregnation). **k.** *Anacharon gracilis*, **l.** *Anacharon lepturus*, **m.** *Enterophrya elongata*, **n.** *Hydrochoerella intestinalis*, **o.** *Ogimotopsis pumila*, **p.** *Paracunhamunizia calocoma*, **q.** *Uropogon urai*. **r–s.** family Protohalidae (ciliates after silver carbonate impregnation). **r.** *Protohallia nana*, **s.** *Protohallia uncinata*. **t.** family Pycnotrichidae (live observation), *Muniziella cunhai*. ACZ. Adoral ciliary zone, CD. caudalia dorsal, CV. caudalia ventral, CL. caudal lobe, CoV. contractile vacuole, CT. caudal tail, Ma. macronucleus, Sk. skeletal plate, Ve. Vestibulum, VP. vestibular polybrachykinety. Scale bars: 20 µm.

Table 1. Relative abundance (%) and density (×10^4 ciliates/ ml of cecal contents) of ciliate species recorded in Brazilian capybara (*Hydrochoerus hydrochaeris*)

Order / Family / Species	Relative Abundance	Density
Order Entodiniomorphida Reichenow, 1929		
Family Cycloposthiidae Poche, 1913	58.99	239.1
Cycloposthium bursa Ito and Imai, 2000*	8.33	33.8
Cycloposthium caudatum Cunha and Muniz, 1927	6.66	27
Cycloposthium compressum Cunha, 1915	6	24.3
Cycloposthium cristatum Cunha and Muniz, 1927	6.33	25.7
Cycloposthium elongatum Holande and Batisse, 1959	6	24.3
Cycloposthium hydrochoeri Cunha, 1915	5	20.2
Cycloposthium incurvum Cunha, 1915	5.67	23
Cycloposthium lenticularis Holande and Batisse, 1959	5.67	23
Cycloposthium minutum Cunha and Muniz, 1927	7	28.4
Monoposthium cynodontum Ito and Imai, 2000*	2.33	9.4
Order Vestibuliferida de Puytorac *et al.*, 1974		
Family Protocaviellidae Grain in Corliss, 1979	26.33	120.2
Anacharon gracilis Ito and Imai, 2000*	4.00	16.2
*Anacharon lepturus*Ito and Imai, 2000*	3.33	13.5
Enterophrya elongata Hasselmann, 1918	3.33	13.5
Hydrochoerella intestinalis Cunha and Muniz, 1925	7	28.4
Ogimotopsis pumila Ito and Imai, 2000*	2.67	10.8
Paracunhamunizia calocoma Ito and Imai, 2000*	4.00	16.2
Uropogon urai Ito and Imai, 2000*	5.33	21.6
Family Protohallidae Cunha & Muniz, 1927	10	40.4
Protohallia nana Ito and Imai, 2000*	5	20.2
Protohallia uncinata (Cunha and Muniz, 1927)	5	20.2
Family Pycnotrichidae Poche, 1913	1.33	5.4
Muniziella cunhai Fonseca, 1939	1.33	5.4

* First record in Brazil.

These first records may be related to the use of the silver impregnation technique for the identification of the ciliates. These preparations allowed the visualization of details of the oral and somatic infraciliatures (Fig. 1), essential for the specific identification of these ciliates, especially regarding species of the order Vestibuliferida.

Muniziella cunhai was described by Fonseca (1939) in the cecal contents of Brazilian capybaras. On this occasion, the author described the morphology of the species based on live observation and specimens fixed in 70° GL alcohol. Later, Batisse (1965), re-examining the material obtained by Fonseca (1939), redescribes the species based on information obtained on sections stained with ferric hematoxylin, eosin, and light green. According to both authors (Fonseca 1939 and Batisse 1965), this species is characterized mainly by large body dimensions (> 1 mm), by the body completely covered by cilia and by the presence of a wide body groove where the vestibular opening is located. After these first reports, McLure (1976) and Ito and Imai (2000a) recorded the species in capybaras in Venezuela and Bolivia, respectively. McLure's report (1976) did not involve the morphological characterization of the specimens found, not allowing the comparison of the specimens with those characterized in Brazil. Ito and Imai (2000a), however, pointed out the complexity of describing the morphology of specimens due to the

Table 2. Distribution of intestinal ciliates in symbiotic association with Capybara (*Hydrochoerus hydrochaeris*) around the world

	Geographic locations										
	Brazil (1)	Brazil (2)	Brazil (3)	Brazil (4)	Brazil (5)	Brazil (6)	Ven-ezuela (7)	EUA (8)[+]	Bolívia (9)	Bolívia (10)	Brazil (11)
Order/ Family/ Species											
Order Entodiniomorphida Reichenow in Doflein and Reichenow, 1929											
Family Blepharocorythidae Hsiung, 1929											
Blepharocorys hydrochoeri Cunha and Muniz, 1925		+									
Family Buetschliidae Poche, 1913											
Protolutzia hydrochoeri Cunha and Muniz, 1925		+									
Family Cycloposthiidae Poche, 1913											
Cycloposthium bursa Ito and Imai, 2000										+	+
Cycloposthium caudatum Cunha and Muniz, 1927				+		+	+			+	+
Cycloposthium compressum Cunha, 1915	+		+			+	+			+	+
Cycloposthium cristatum Cunha and Muniz, 1927				+			+			+	+
Cycloposthium elongatum Holande and Batisse, 1959						+	+			+	+
Cycloposthium hydrochoeri Cunha, 1915	+					+	+			+	+
Cycloposthium incurvum Cunha, 1915	+						+			+	+
Cycloposthium lenticularis Holande and Batisse, 1959						+				+	+
Cycloposthium magnum Cunha & Muniz, 1927				+			+				
Cycloposthium minutum Cunha and Muniz, 1927			+			+	+			+	+
Cycloposthium vorax Cunha and Muniz, 1927			+								
Monoposthium cynodontum Ito and Imai, 2000										+	+
Family Ophryoscolecidae Stein, 1959											
Elytroplastron bubali (Dogiel, 1928)								+			
Entodinium bimastus Dogiel, 1927								+			
Entodinium caudatum Stein, 1858								+			
Entodinium dubardi Buisson, 1923								+			
Entodinium longinucleatum Dogiel, 1925								+			
Eudiplodinium maggii (Fiorentini, 1889)								+			
Order Vestibuliferida de Puytorac *et al.*, 1974											
Family Protocaviellidae Grain in Corliss, 1979											
Anacharon gracilis Ito and Imai, 2000									+		+
Anacharon lepturus Ito and Imai, 2000									+		+
Cunhamunizia batissei Ito and Imai, 2000									+		
Enterophrya elongata Hasselmann, 1918		+							+		+
Enterophrya piriformis Hasselmann, 1918		+									
Eriocharon accuminatus Ito and Imai, 2000	+	+							+		+
Hydrochoerella intestinalis Cunha and Muniz, 1925		+					+		+		+
Ogimotoa trichoradiata Ito and Imai, 2000									+		
Ogimotopsis campanulata Ito and Imai, 2000									+		
Ogimotopsis pumila Ito and Imai, 2000									+		+
Paracunhamunizia calocoma Ito and Imai, 2000									+		+
Uropogon urai Ito and Imai, 2000									+		+
Family Paraisotrichidae Cunha, 1917											
Paraisotricha hydrochoeri Cunha, 1915	+	+									
Family Protohallidae Cunha and Muniz, 1927											
Protohallia uncinata (Cunha and Muniz, 1925)		+					+		+		+
Protohallia nana Ito and Imai, 2000									+		+
Family Pycnotrichidae Poche, 1913											
Muniziella cunhai Fonseca, 1939					+		+		+		+

[1]Cunha (1915), [2]Cunha and Muniz (1925), [3]Cunha and Muniz (1927a), [4]Cunha and Muniz (1927b), [5]Fonseca (1939), [6]Holande and Batisse (1959), [7]McLure (1976), [8]Dehority (1987), [9]Ito and Imai (2000a), [10]Ito and Imai (2000b), [11]Present study.

[+]: animal in captivity

difficulty of impregnating them with silver. Ito and Imai (2000a) then presented only the measures of their body dimensions (227.3 ± 34.5 μm). Although Ito and Imai (2000a) provided morphometry for their specimens, they were much lower in body size than those observed by Fonseca (1939) and Batisse (1965). The specimens of *Muniziella cunhai* isolated in the present study are in the process of morphological and molecular characterization and will be, as soon as possible, compare with the species' previous records.

The total ciliate density was 405.9×10^4 ml^{-1} of cecal content. The family Cycloposthiidae dominated the relative abundance and density, followed by the families Protocaviellidae, Protohallidae, and Pycnotrichidae (Table 1). *Cycloposthium* and *Cycloposthium bursa* were respectively the most abundant genus and species in higher density (Table 1). The total ciliate density observed in the present study was lower than the maximum ciliate density found by Ito and Imai (2000a, b), 702.4×10^4 ml^{-1} of cecal contents. Such variations are difficult to explain but are probably related to inherent characteristics of the hosts' individual metabolisms, as well as the type of diet consumed by the animal and geographic and climatic factors, as suggested by Gürelli and Göçmen (2012).

The present study highlights the importance of knowing the gastrointestinal ciliate community associated to wild hosts, in order to obtain more precise information about the geographic distribution and specificity of the ciliate species to certain groups of hosts. In addition, this work may contribute to future studies on the phylogenetic relationships within the subclass Trichostomatia, since none of the symbiotic ciliates species typical to capybaras have molecular descriptions and many of them have not been described based on suitable ciliatological techniques.

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