ISNN 1517-6770

First report of an epibiotic relationship between ciliates and planktonic copepods in a Brazilian floodplain.

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Abstract. The peritrich ciliates are a widely distributed group of protozoa and include stalked and sedentary organisms, which may be solitary or colonial. These organisms are often observed as part of a relationship entitled epibiosis. This relationship is a facultative association between two organisms: the epibiont, which colonizes the surface of living substrates, and the basibiont, which hosts the epibionts. During a survey on the epibiotic relationship between ciliates e copepods in upper Paraná River floodplain-PR/MS Brazil, samples were taken in different types of environments (three rivers, three canals, 16 connected and 14 unconnected lakes), in a total of 36 sampling sites. Among these environments, 22 showed copepods infested by epibiont ciliates. The copepods *Thermocyclops decipiens, Thermocyclops minutus* and *Notodiaptomus amazonicus* where colonized by epibiont ciliates from the genus *Epistylis.* In this study, we reported for the first time, epibiosis in a Brazilian floodplain.

Keywords: Ciliophora, epibiosis, lake, Neotropics, zooplankton.

Introduction

Peritrich ciliates are known for more than 300 years as a distinct group, by presenting a prominent oral ciliary apparatus and somatic ciliature reduced to a throcal band (Miao *et al*., 2001). The Subclass Peritrichia (Ciliophora) encompasses ciliates, usually pedunculated, which adhere to different substrates, such as rocks, algae and/or animals, and could live as epibionts on a large number of species of aquatic metazoa, including several groups of invertebrates and vertebrates (Regali-Seleghim & Godinho, 2004; Utz & Coats, 2005; Dias *et al*., 2008, 2009; Cabral *et al.,* 2010; Fernandez-Leborans & von Rintelen, 2010; Fard *et al*., 2011; Chatterjee *et al*., 2013). According to Fenchel (1987), it is likely that virtually all the aquatic metazoans are potential hosts for epibiont ciliates.

The term epibiont includes organisms that colonize the surface of a living substrate during the sessile portion of its life cycle (Wahl, 1989). This relationship involves advantages and disadvantages for both the epibiont and host or it also could be neutral, depending on the ecological context (Wahl, 1989; Fernandez-Leborans & Tato-Porto, 2002; Wahl, 2008). Despite its widespread occurrence, most studies have emphasized morphological and taxonomic aspects of epibiont, with a few studies focusing on ecological aspects of this relationship and studies emphasizing the influence of the environment in epibiont / basibiont relationship are still incipient (Hanamura, 2000; Utz & Coats 2005; Dias *et al.,* 2008; 2009; Cabral *et al.,* 2010).

On the other hand floodplains, are important sites for studying the biodiversity of protists, due to its high spatial and temporal heterogeneity, which gives them the title of hot spots of biological diversity. Due to its river dynamics, a floodplain is characterized by the presence of many aquatic species with a wide range of lotic and lentic environments, and should be fully analyzed as a unit called system-river-floodplain (Junk *et al*., 1989).

During a survey of ciliates epibionts on copepods in the Parana River Floodplain, we observed ciliates from genus *Epistylis* Ehrenberg, 1830 attached to *Thermocyclops minutus* Lowndes 1934, *Thermocyclops decipiens* Kiefer, 1929 and *Notodiaptomus amazonicus* S. Wright 1935. Here we report for the first time, the occurrence of epibiosis involving protozoan ciliates and copepods in a Brazilian floodplain and present data on infestation prevalence of this epibiont relationship.

Material and Methods

The Paraná River has a broad channel, with an extensive floodplain, reaching up to 20 km wide, with numerous side channels, ponds and tributaries including Ivinhema and Baia Rivers (Agostinho & Zalewski, 1996). The study area covers a variety of lakes with different degrees of connectivity with the main River, as well as morphological and morphometric differences. Three rivers, three channels, 16 connected lakes and 14 unconnected lakes were sampled, totalizing 36 environments. The stretch of the floodplain studied here is located between the coordinates 22° 26’, 22° 56’ S and 53° 10’, 53° 40’ W. For the coordinates of each sampling station, see supplementary material (S1).

Two samples, with a final concentration of 300 mL, were collected from each sampling station using 100-µm plankton net, vertically positioned. One of them was fixed at the moment of sampling with Bouin fixative, and the other one was kept without fixation for in vivo observation using brightfield, differential interference contrast (DIC) and scanning electron microscopy procedure (Silva-Neto *et al*., 2012).

Ciliate epibionts as well their copepod hosts were identified using specialized taxonomic literature (Sendacz & Kubo, 1982; Warren, 1986; Reid, 1985; Foissner *et al*., 1992). Fixed samples were concentrated to a final volume of 100 ml and 10 ml (about 500-1500 individual), and the host density and infestation prevalence of epibionts were estimated using a Sedgewick-Rafter counting chamber. The prevalence was calculated by dividing the number of infested individuals by the total number of individuals in the sample, as proposed by Bush *et al.* (1997).

Results

Among the 36 environments analyzed, 22 of them presented copepods infested by ciliate epibionts. From a total of 13 species of copepods observed in the samples, only three (*Thermocyclops decipiens*, *Thermocyclops minutus* and *Notodiaptomus amazonicus*) harbored epibiont ciliates of the genus *Epistylis.*

The two genera of copepods were colonized by different species of *Epistylis* (Figure 1). At the same way, each genus of host was always infested by the same species of epibiont, evidencing

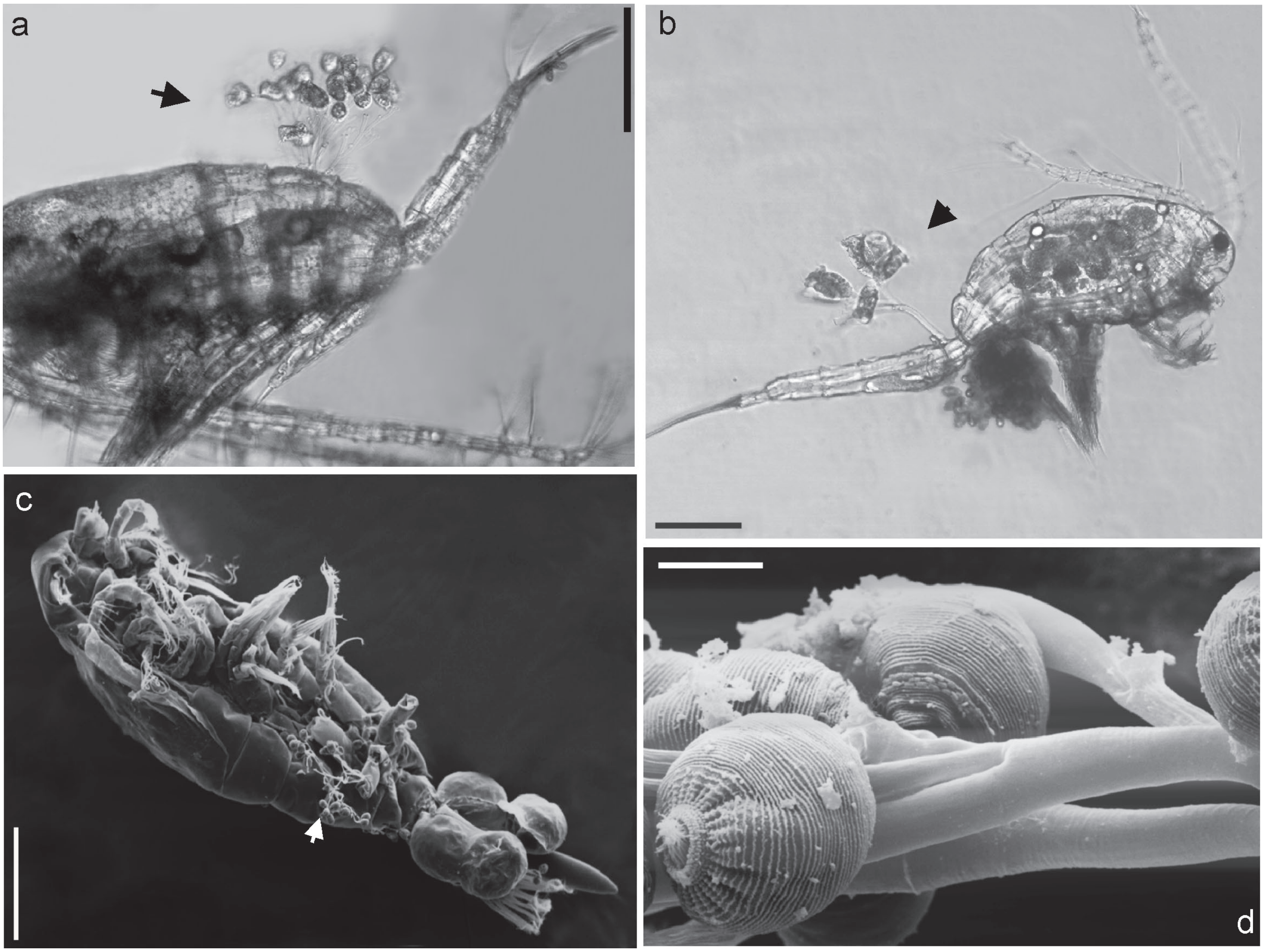

Figure 1. *Epistylis* sp. attached to planktonic Copepods, *Thermocyclops minutus* and *Notodiaptomus amazonicus,* observed *in vivo* through differential interferential contrast (DIC) and scanning electron microscopy. **a.** The posterior region of *N. amazonicus* showing a colony of *Epistylis* sp*.* attached to the host (arrow). **b.** *T. minutus*, exhibiting epibionts on posterior region (arrow). **c.** *N. amazonicus* showing a colony of *Epistylis* sp*.* (arrow). **d.** Detail of the ciliate epibiont of *T. minutus*, after the SEM procedure. Bars: a,c: 100 μm. b: 200μm. d: 5μm.

some specificity of this epibiotic relationship. We observed the ciliates attached to adults and juveniles (copepodites), but we did not detect epibionts in the larvae (nauplii) stages.

Although the ciliates were found attached to juvenile and adult stages of copepods in the present study, the mean infestation prevalence was significantly higher on adults 19.85% (±4.86) in comparison with copepodites 13,49% (± 11.25). When hosts were separated by Order (Calanoida and Cyclopoida) the same pattern was observed. Adults of *N. amazonicus* had a higher mean prevalence of infestation in comparison to copepodites. The same pattern was observed for *T. minutus*. The epibiotic relashionship of *T. decipiens* occurred only in one lake and the mean prevalence was 0.46% (± 1.59). Mean prevalence of infestation and number of hosts for each copepod species are shown in Table 1.

Table 1. Absolute numbers of observed hosts as well as the mean prevalence for the total of hosts and separated by each order and species observed in Paraná River Floodplain.

	Total Copepods	Total Calanoid	Total Cyclopoid	Adults (*N. amazonicus*)	Adults (*T. minutus*)	Juveniles (Calanoid)	Juveniles (Cyclopoid)
Number of Hosts	15877	8997	6882	2293	1985	5998	4129
Prevalence (mean)	4.85% (±4.86)	7.42% (±13.25)	9.43% (±12.58)	14.71% (± 7.03)	23.69% (± 12.58)	9.94% (±7.72)	15.43% (± 12.58)

Discussion

Some epibionts are known to present degrees of specificity per basibiont varying from individuals that colonize a wide range of substrate being non-restricted to epibiotic way of life, to obligate epibiont individuals with degree of specificity at levels of family, genus or even species (Cook *et al*., 1998; Nenninger, 1948). In the present study, each genus of host was always colonized by the same species of epibiont. According to Görtz (1996), specific relationships present low prevalence and intensity of infestation, as we observed in the present study. Wahl & Mark, 1999 postulated that the development of a highly specified epibiotic relationship should be tolerable or even benefic to the host. On an experiment with the copepod *Eurytemora affinis* infested by the ciliate epibiont *Zoothamnium* sp., Souissi *et al.* (2013) observed that the copepods presenting very low levels of infestation revealed regular swimming behavior, having their mating improved in relation to non-infested copepods.

Our results are similar to the literature, since the three most abundant copepod species in the upper Paraná River floodplain (Lima *et al*., 1998; Lansac-Toha *et al*., 2004) were infested by epibionts, suggesting that no severe damage ware made by epibiont on the life cycle of the host. This result confirms those observed by Gaevskii *et al.* (2004) that found no significant correlation between the epibiotic relationship and proportion of dead crustaceans, characterizing their natural mortality.

The fact that epibionts were only observed attached to adults and juveniles (copepodites), but never observed colonizing the naupliar stages, could be explained by the higher molt rates found in the early stages of the copepod life cycle (Utz & Coats, 2005). In general, the epibiont is shed with the exuvium and needs to find another place to attach. In sessile organisms, this includes the formation of a free-swimming stage, which could be onerous for the organism. Thus, colonizing more stable substrates would be advantageous for the epibiont. The same pattern has been observed in other studies. Chiavelli & Mills (1993) observed *Colacium vesiculosum* Ehrenberg 1853 (Protozoa – euglenidae) as epibiont on zooplankton communities and noted that the prevalence was highly on adult cyclopoids than on calanoids or naupliar stages. Cabral *et al.* (2014) observed that flagellate colonization was higher on adult forms than on larval and juvenile forms (nauplii and copepodites) of *N. amazonicus* and *T. minutus.*

This study constitutes the first investigation of the occurrence of epibiotic relationship between copepods and ciliates in a Brazilian floodplain, it is also the first record of the epibiotic relationship between ciliates of genus *Epistylis* and the planktonic copepods *Thermocyclops minutus, Thermocyclops decipiens*, and *Notodiaptomus amazonicus* in Brazil. We have shown that infestation rates were higher on adult forms than on larval and juvenile forms (nauplii and copepodites), which could be explained by the fact that adult copepods provide a more stable substrate for epibionts, corroborating other similar studies. Nevertheless more in depth analyses are necessary to better understand different aspects of this epibiotic relationship between zooplanktonic crustaceans and ciliates.

Acknowledgments

We thank NUPELIA and the Programa de Pós-Graduação em Ambientes Aquáticos Continentais for providing logistical and financial support (PROEX/Capes). We also thank CNPq (National Counsel of Technological and Scientific Development) for providing financial support, a postdoctoral fellowship and a Research Productivity scholarship.

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Supplementary Material.
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Coordinates of each sampling station in the Paraná River floodplain.

	Sampling station name	Latitude (S)	Longitude (W)
1	*Lagoa Finado Raimundo*	-22.79933333	-53.54143333
2	*Lagoa Ventura*	-22.85658333	-53.60028333
3	*Lagoa Jacaré*	-22.78390000	-53.49696667
4	*Lagoa do Cervo*	-22.77488333	-53.49638333
5	*Lagoa Sumida*	-22.78188333	-53.48950000
6	*Lagoa Capivara*	-22.79903333	-53.53483333
7	*Lagoa Peroba*	-22.90841667	-53.64008333
8	*Lagoa Zé do Paco*	-22.83436667	-53.57166667
9	*Lagoa Boca do Ipoitã*	-22.83553333	-53.56538333
10	*Lagoa dos Patos*	-22.82601667	-53.55275000
11	*Canal do Ipoitã*	-22.83543333	-53.56208333
12	*Rio Ivinhema*	-22.79990000	-53.53925000
13	*Lagoa Fechada*	-22.71053333	-53.27585000
14	*Lagoa do Aurélio*	-22.69296667	-53.23071667
15	*Lagoa da Onça*	-22.66345000	-53.20045000
16	*Lagoa do Gavião*	-22.67998333	-53.23151667
17	*Lagoa Traíra*	-22.74600000	-53.33935000
18	*Lagoa do Guaraná*	-22.72130000	-53.30256667
19	*Lagoa Maria Luiza*	-22.67505000	-53.21976667
20	*Lagoa dos Porcos*	-22.70123333	-53.24446667
21	*Lagoa Pousada das Garças*	-22.70031667	-53.25653333
22	*Canal Baía*	-22.69081667	-53.22481667
23	*Canal Curutuba*	-22.75080000	-53.35895000
24	*Rio Baía*	-22.72310000	-53.29041667
25	*Lagoa do Osmar*	-22.77406667	-53.33226667
26	*Lagoa Genipapo*	-22.75923333	-53.26831667
27	*Lagoa Clara*	-22.75486667	-53.25795000
28	*Lagoa Pousada*	-22.74493333	-53.23536667
29	*Lagoa das Pombas*	-22.79886667	-53.35905000
30	*Ressaco do Manezinho*	-22.77908333	-53.34910000
31	*Ressaco do Bilé*	-22.75376667	-53.28596667
32	*Lagoa das Garças*	-22.72421667	-53.21793333
33	*Canal Cortado*	-22.81206667	-53.37803333
34	*Rio Paraná*	-22.76110000	-53.25206667
35	*Ressaco do Pau Véio*	-22.74743333	-53.25310000
36	*Ressaco do Leopoldo*	-22.75666667	-53.26888333