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Short communication

First Report of *Colacium vesiculosum* Ehrenberg 1853 (Euglenophyceae), as Epibiont on Planktonic Copepods (Crustacea, Copepoda), in a Brazilian Floodplain Lake

Adalgisa Fernanda CABRAL[1], Bárbara DUNCK[2], Fernando Miranda LANSAC-TOHA[2], Liliana RODRIGUES[2], Laura Roberta PINTO UTZ[3], Luiz Felipe MACHADOVELHO[2]

[1]Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Biologia Geral. Rodovia Goiânia-Nerópolis, Itatiaia, Goiânia, GO – Brasil; [2]Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais, Universidade Estadual de Maringá, Maringá, Paraná, Brasil; [3]Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul.

Abstract. Epibiosis is a facultative association between two organisms: the epibiont, which colonizes the surface of living substrates, and the basibiont, which hosts the epibionts. Among protists, while numerous accounts exist for ciliates as epibionts of zooplankton, little information is available about the occurrence of this relationship between flagellates and microcrustaceans in the neotropics. During a survey on the epibiotic relationship between ciliated protists and planktonic copepods in a tropical floodplain, we reported for the first time the occurrence of *Colacium vesiculosum* as an epibiont on *Thermocyclops minutus* and *Notodiaptomus amazonicus* from southern Brazil. The mean infestation prevalence was significantly higher on adults 30.53% (± 2.85) in comparison with copepodites 0.87% (± 0.55). When hosts were separated by order (Calanoida and Cyclopoida) copepodites presented a mean infestation prevalence of 0.92% (± 0.85) and 0.83% (± 0.60) respectively, while adults had a mean prevalence of 29.55% (± 6.8) and 30.13% (± 5.83), which could be explained by the fact that adult copepods provide a more stable substrate for epibionts.

Key words: Epibiosis, euglenoid, flagellates, neotropics.

INTRODUCTION

The term epibiont includes organisms that colonize the surface of a living substrate during the sessile portion of its life cycle, involving advantages and disadvantages for both the epibiont and the host (Fernandez-Leborans and Tato-Porto 2002, Wahl 1989). This relationship could have a negative or positive effect for both the host and epibiont, or they also can be neutral, depending on the ecological context (Wahl 2008). To the host, the epibionts may negatively: decrease their survival time (Gilbert and Schroder 2003), altering the physiology and handling (Rothäusler *et al.* 2011), may also make them more susceptible to predation (Willey

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Address for correspondence: Adalgisa Fernanda Cabral, Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Biologia Geral. Rodovia Goiânia-Nerópolis, km 5, Campus 2, Itatiaia, 74001970 – Goiânia, GO – Brasil; Phone (+55-62-35211480); E-mail: adalgisacabral@gmail.com

and Threlkeld 1993). However, the epibiont can provide protection for basibiont against predators (Wahl 1989), and also against drying (Wahl and Mark 1999).

Moreover, epibiont populations may play relevant roles at the ecosystem level. For example, when abundant, epibionts may even help to fuel higher trophic levels. Interestingly, the algal epibiont *Korshicoviella gracilipes* on *Daphnia pulicaria* forms free-swimming, dispersal, and unattached overwintering stages that can represent an important food source for the host when more typical planktonic prey is at low abundance levels (Barea-Arco *et al.* 2001). Henebry and Ridgeway (1979) reported that the prevalence of peritrich and suctorian epibionts on crustacean zooplankton could reflect the eutrophic condition of an environment and could be used as an indicator of water pollution.

Sessile peritrich ciliates in the genera *Zoothamnium*, *Epistylis* and *Vorticella* (Mayén-Estrada and Aladro-Lubel 2001, Gilbert and Schroder 2003, Regali-Seleghim and Godinho 2004, Utz and Coats 2005), suctorians (Dias *et al*. 2006), and euglenoid flagellates (Utz and Coats 2005) have been observed on the external surfaces of planktonic crustaceans (Henebry and Ridgeway 1979, Sleigh 1988, Xu and Burns 1991) but there is no much information about this relationship evolving flagellates.

The genus *Colacium* Ehrenberg 1853 belongs to the class Euglenoidea and to the order Euglenida Bütschi 1884. The dominant vegetative phase of the organisms is sessile, forming arborescent colonies (Bicudo and Menezes 2006). The free living phase is flagellated, typically euglenoid being structurally identical to the genus *Euglena*. These euglenids secrete a mucilaginous substance and fix themselves by the anterior cytopharynx (flagellar region). Formation of a stalk is facultative in the genus (Tell and Conforti 1986). These organisms were found attached to other living substrates such as planktonic invertebrates (Tell and Conforti 1986) and zooplanctonic organisms (Zalocar *et al*. 2011).

During a survey of ciliates epibionts on copepods in the Parana River Floodplain, we observed *Colacium vesiculosum* Ehrenberg, 1838, attached to *Thermocyclops minutus* Lowndes 1934 and *Notodiaptomus amazonicus* S. Wright 1935. Here we report the occurrence of this euglenoid and present data on infestation prevalence of the epibiont.

MATERIALS AND METHODS

Water samples were taken on June 14th 2012 at Pousada das Garças Lake. This lake (22°42′1.14″S; 53°15′23.52″W) is a tributary of Baía River, which belongs to the river-floodplain system of the Paraná river and is located on the left bank of the river. It is a small pond (3.8 ha in area, elongated and average depth of 2.3 m), with a marginal vegetation composed mainly by the *Cyrpus* sp. sedges (40%), grasses (50%) and *Polygnum* sp. (10%). The pond presents an assemblage of fish with considerable diversity (about 26 species) (Petry *et al*. 2003), including *Serrasalmus marginatus*, *Loricariichthys platymetopon*, *Moenkhausia intermedia* and *Parauchenipterus galeatus*. Environmental parameters such as water temperature, dissolved oxygen, pH, conductivity, and transparency were measured in the field.

The samples were collected with a 100 µm plankton net, vertically positioned. About 700 L of water were filtered on each sample, and this volume was calculated by multiplying the diameter of the net by the lake depth, so several drags were necessary to obtain this volume. A total of four samples, with a final concentration of 300 mL, were collected from different points of the lake: three were fixed at the moment of sampling with 100% alcohol to a final concentration of 50%, and one was kept without fixation for *in vivo* observation using brightfield, and differential interference contrast (DIC). The sample that was kept alive was fixed later for scanning electron microscopy procedure that followed the method described by Silva-Neto *et al.* (2012). The euglenid epibiont as well its copepod hosts were identified using specialized taxonomic literature (Sendacz and Kubo 1982, Willey 1982, Reid 1985). The three fixed samples were concentrated to a final volume of 100 ml and 10 ml (about 500–1500 individuals) that were counted using a Sedgewick-Rafter chamber to quantify the host density and infestation prevalence of the epibionts. The prevalence was calculated by dividing the number of individuals infected by the total number of individuals in the sample, as proposed by Bush *et al.* (1997).

RESULTS AND DISCUSSION

The present study recorded for the first time *Colacium vesiculosum* as epibiont on two copepod species *Thermocyclops minutus* (Figs 1a, f and h) and *Notodiaptomus amazonicus* (Figs 1b, e and g) in a Brazilian floodplain lake. In the neotropics, *C. vesiculosum* (Figs 1c, h–j) has been recorded attached to *N. spiniger* (Calanoid) and *T. minutus* (Cyclopoid), in a shallow lake in Argentina (Zalocar *et al*. 2011). In Brazil, the genus was observed attached to zooplanktonic organisms collected in a shallow eutrophic artificial reservoir, in São Paulo (Regali-Seleghim and Godinho 2004).

Colacium vesiculosum was found attached to adult and copepodite stages of *N. amazonicus* and *T. minutus*, but was never observed in the naupliar stages. This could be explained by the higher molt rates found in

Table 1. Absolute numbers of observed copepods for each order, and the number of copepods colonized by *C. vesiculosum*.

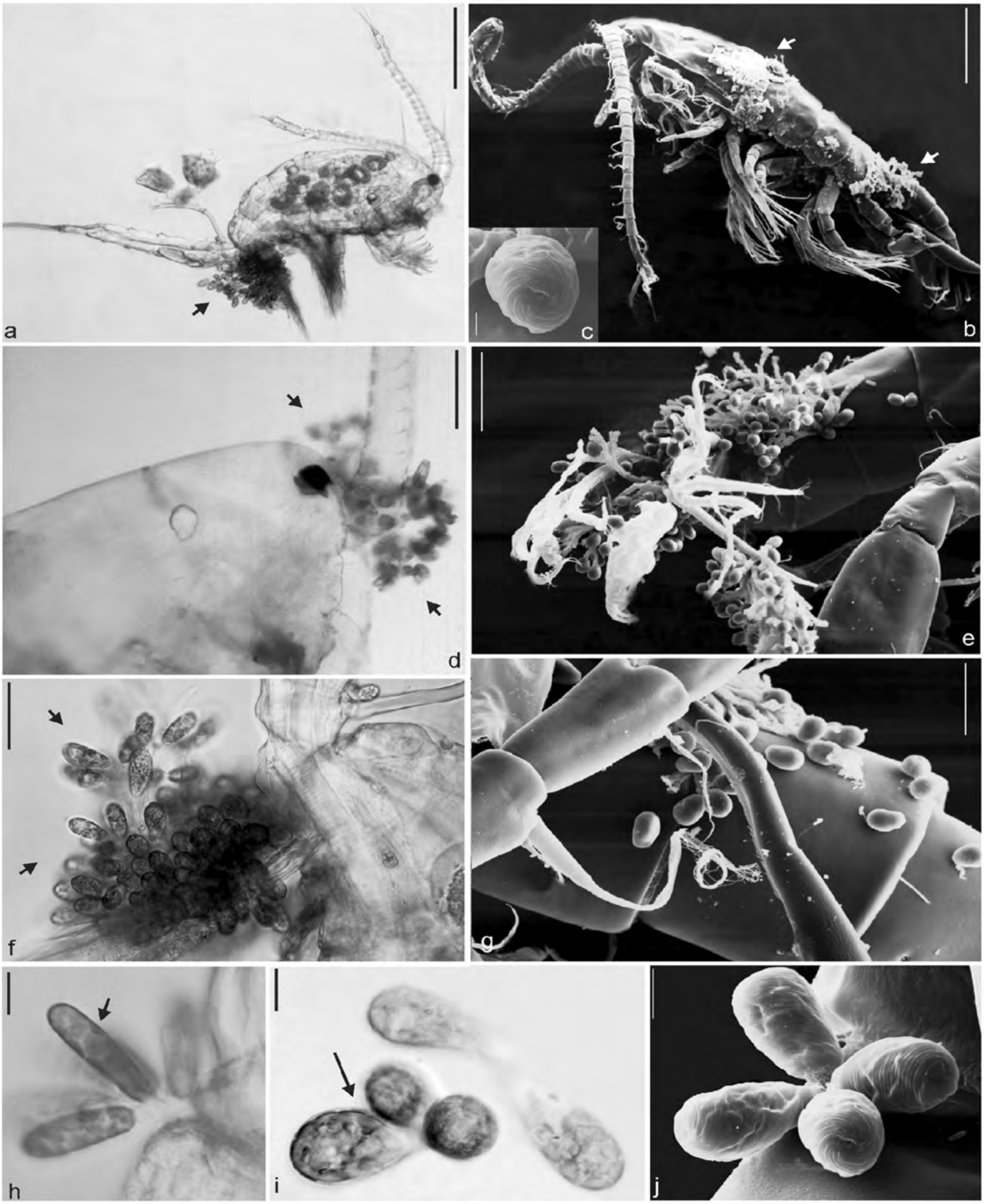
	Total of copepods	Total of copepods colonized	Juveniles	Juveniles colonized	*T. minutus*	*T. minutus* colonized	*N. amazonicus*	*N. amazonicus* colonized
Cyclopoid	2189	343	1453	122	736	221	0	0
Calanoid	1112	183	727	68	0	0	385	115

these early stages in comparison with juvenile stages (Utz and Coatz 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. Although *C. vesiculosum* was found attached to juvenile and adult stages of copepods in the present study, the mean infestation prevalence was significantly higher on adults 30.53% (± 2.85) in comparison with copepodites 0.87% (± 0.55). This is explained by the fact that adult copepods do not molt, thus not being able to shed their epibionts. When hosts were separated by order (Calanoida and Cyclopoida) the same pattern was observed. Calanoida copepodites presented a mean infestation prevalence of 0.92% (± 0.85), while adults of *N. amazonicus* had a mean prevalence of 29.55% (± 6.8) (Fig. 2A). Epibionts showed a mean infestation prevalence of 0.83% (± 0.60) in copepodites of Cyclopoida, while 30.13% (± 5.83) of the adults of *T. minutus* were infested (Fig. 2B). The same pattern has been observed in other studies. Chiavelli and Mills (1993) observed *C. vesiculosum* as epibiont on zooplankton communities and noted that the prevalence was highly on adult cyclopoids than on calanoids or naupliar stages. Zalocar *et al.* (2011) also found that algal colonization was higher on adult forms than on larval and juvenile forms (nauplii and copepodites) of *N. spiniger*. The absolute numbers of observed copepods for each species, and the number of colonized copepods are shown on Table 1. This absence of epibiont on larvae can be explained by the fact that adult copepods provide a more stable substrate for epibionts, since they do not suffer more and seedlings can accumulate a higher density of epibionts (Dubovskaya *et al.* 2005).

Epibionts, in general, could present different degrees of specificity for the host (Willey and Threlkeld 1993). Some organisms are obligate epibionts, colonizing only living hosts, while others are opportunistic, found attached to living and non-living substrates (Utz and Coats 2008). This flagellate have been observed as a free-living flagellate stage, as well as part of the periphytic community (Dogadina *et al.* 1999, Totti *et al.* 2011, Castillo-Escrivà *et al.* 2013). Dubovskaya and coworkers (2005) found *C. vesiculosum* attached to other zooplanktonic organisms, such as, *Daphnia longispina*, *Cyclops vicinus* and *Mesocyclops leuckarti*, while Camargo and Velho (2010) found *C. vesiculosum* as a free swimming flagellate at lakes from the same floodplain where the present study was performed. According to the results presented in this study and those reported in the literature, this euglenid shows no specificity to the host, being opportunistic and able to attach to living or non-living substrates.

Some studies point out that may exist a positive cost-benefit balance for both epibiont and host in such a way that a mutualistic relationship can be suggested (Barea-Arco *et al.* 2001). High infestation rates of *C. vesiculosum* on *N. spiniger* observed by Zalocar and co-workers (2011) was in accordance with the high abundance of this calanoid species in a subtropical shallow lake in Argentina. Our results are similar to the literature, since the two most abundant copepod species in the floodplain of the upper Paraná River (Lima *et al.* 1998, Lansac-Toha *et al.* 2004) were infested by *C. vesiculosum*, suggesting that the choice of this opportunistic epibiont is based on substrate availability. This result is also similar to those observed to Gaevskii *et al.* (2004) that found no significant correlation between the proportion of dead crustaceans and the epibiotic relationship, characterizing their natural mortality, they also proposed that phototrophic epibionts are not a potential cause of natural mortality of copepods and cladocerans since mortality rates are unrelated to predation in that system.

This study constitutes the first investigation of the occurrence of epibiotic relationship between copepods and euglenid flagellates in a floodplain, it is also the first record of the epibiotic relationship between *Colacium vesiculosum* and the planktonic copepods *Thermocyclops minutus* and *Notodiaptomus amazonicus* in Bra-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zil. 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. Overall, the majority of the research focusing on euglenids as epibionts has been carried out in temperate lakes, with few data being reported from subtropical and neotropical lakes (e.g. López *et al*. 1998, Regali-Seleghim and Godinho 2004, Zalocar *et al*. 2011), thus more in depth analyses are necessary to better understand different aspects of these epibiotic relationship between zooplanktonic crustaceans and euglenids.

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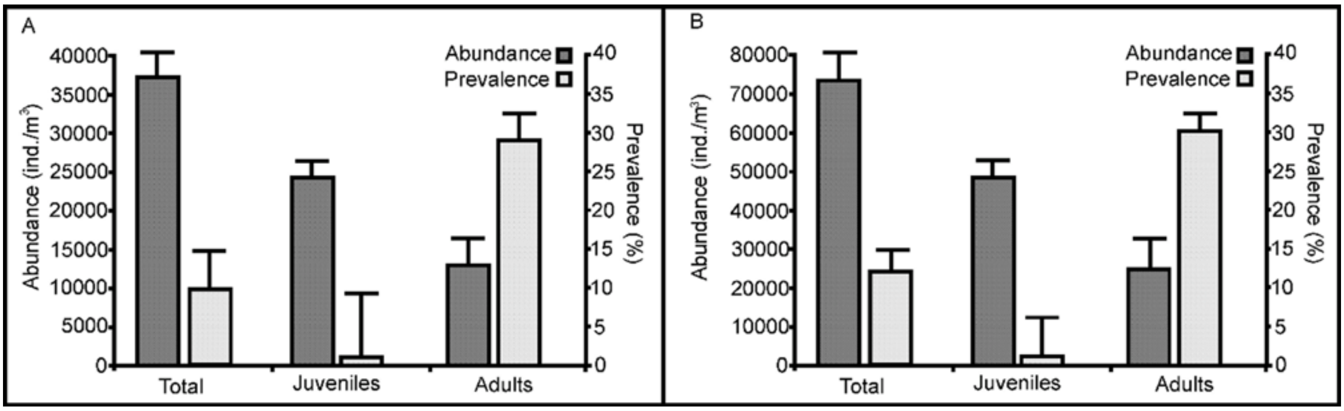

Fig. 2. Mean abundance of hosts and mean infestation prevalence on the total or on each live stage of the copepods. **A** represents those aspects of the epibiotic relationship on *N. amazonicus* and on **B**, those on *T. minutus.*

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Figure 1 (a-j). Euglenid *Colacium vesiculosum* attached to planktonic Copepods, *Thermocyclops minutus* and *Notodiaptomus amazonicus*. **Figures a, d, f, h and i**. Euglenid observed *in vivo* using differential interferential contrast (DIC). **a**. *T. minutus* showing euglenids in a posterior region (arrows), this copepod was infested by peritrichs ciliates too. **d and f**. The anterior and posterior region of N. *amazonicus* respectively, with a colony of *C. vesiculosum* (arrows). **h and i.** Detail of *C. vesiculosum* showing respectively the chloroplasts and the stigma (arrows). **Figures b, c, e, g, and j**. Euglenids observed by scanning electron microscopy procedure. **b**. *C. vesiculosum* attached to *N. amazonicus* (arrows). **c and j**. Detail of the euglenid showing the striations. **d**. Detail of the posterior region of the copepods with the euglenid attached. Bars: 100 (a); 200 µm (b); 2 µm (c) 50 µm (d); 20 µm (e, f); 50 µm (g);10 µm (h,i); 5 µm (j).

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