Protozoan ciliate epibionts on the freshwater apple snail *Pomacea figulina* (Spix, 1827) (Gastropoda, Ampullariidae) in an urban stream of south-east Brazil

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Protozoan ciliate epibionts on the freshwater apple snail *Pomacea figulina* (Spix, 1827) (Gastropoda, Ampullariidae) in an urban stream of south-east Brazil

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The objective of this study was to record and analyze the prevalence of ciliated protozoa associated with prosobranchian snails of the species *Pomacea figulina* collected from an urban stream in south-eastern Brazil. Four collections were carried out between December, 2005, and March, 2006, from which 23 snails and 10 'empty' shells were obtained. The shells and opercula were scraped over Petri dishes and the ciliates were observed using bright field and phase contrast microscopy. Seven species of ciliates were recorded on the snails of *P. figulina*. Of the 23 snails analyzed, 82.60% (*n* = 19) were infested with at least one species of ciliate. No ciliates were found on the 'empty' shells and opercula. The results are discussed in terms of ecological aspects involved in this association.

**Keywords:** Ciliophora; mollusc; peritrichs; prosobranchian snails; suctorian

Introduction

Epibiosis is a facultative association of two organisms: the epibiont, which colonizes the surface of live substrates, and the basibiont, which hosts the epibionts (Wahl 1989; Fernandez-Leborans and Gabilondo 2006).

Historically, epibiosis was understood as a commensal relationship between two or more organisms. However, some studies have shown that epibionts can cause deleterious effects to their hosts (Green 1974; Henebry and Ridgeway 1979; Xu 1992; Puckett and Carman 2002). According to Cook et al. (1998) this ecological relationship has two extremes. At one extreme, it can be of an opportunistic, facultative, and non-specialized nature, with the epibionts colonizing inert substrates, animals and plants. At the other extreme are the obligatory and highly specific associations, with morphological and behavioural adaptations between epibiont and basibiont that provide the conditions for this way of life.

According to Fenchel (1987), it is probable that almost all aquatic metazoans host symbiotic protozoa. Peritrich ciliated protozoa live as epibionts on a variety of species of aquatic metazoans, including various groups of invertebrates: sponges, cnidarians, ctenophores, rotifers, tardigrades, freshwater planarians, crustaceans, annelid worms, insect larvae, molluscs; and groups of vertebrates: fishes, tadpoles, and freshwater turtles (Kahl 1935; Goodrich and Jahn 1943; Laird 1959; Baldock

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Epibionts constitute a significant component of aquatic ecosystems and play an important role in the food chain (Pratt and Cairns 1985; Baldock 1986). However, most studies are of morphological and taxonomic nature, and few have taken ecological aspects into consideration (Baldock 1986; Xu 1992; Fernandez-Leborans et al. 1997; Cook et al. 1998; Hanamura 2000; Utz and Coats 2005). Although studies of records of epibiont ciliates are abundant in the literature, little is known regarding ecological aspects of this relationship in tropical and sub-tropical ecosystems, with the studies by Lopez et al. (1998) and Regali-Seleghim and Godinho (2004) deserving mention.

Prosobranchian snails of the Ampullariidae family are freshwater snails that prefer slow waters in rivers and creeks and are common in various tropical and sub-tropical areas (Thiengo 1995).

The objective of this study was to record and analyze the prevalence of ciliated protozoa associated with prosobranchian snails of the species *Pomacea figulina* (Spix, 1827) (Mollusca, Ampullariidae) collected in an urban stream in south-eastern Brazil.

**Materials and methods**

To investigate the occurrence of ciliated epibionts, 23 snails of the species *P. figulina* (Gastropoda, Ampullariidae) were analyzed. The prosobranchs were collected from a point in Sáo Pedro stream (43°23′23.94″S, 21°46′33.80″W), located in an urban area of the municipality of Juiz de Fora, Minas Gerais, Brazil. Four collections were carried out at approximately 30-day intervals during the rainy season (Latuf 2004), from December 2005 to March 2006.

The snails were collected with the aid of a net, conditioned in plastic jars containing water from the creek that had been previously filtered with filter paper, and transferred to the Protozoology Laboratory at the Federal University of Juiz de Fora, approximately 2 km away. Ten empty shells, without the cephalopodal mass, were also collected. The shell and the operculum were individually scraped, with the aid of a scalpel, over Petri dishes containing previously-filtered creek water. To verify the presence of epibionts, the Petri dishes were observed with a stereoscopic microscope, and the ciliates were isolated using micropipettes and observed with bright field and phase contrast microscopy. The morphometry of the organisms in vivo was done with the aid of an ocular micrometer.

Identification of the ciliated protozoon epibionts was done using the identification keys of Kahl (1935), Warren (1986), Foissner et al. (1992, 1999), Foissner and Berger (1996) and the work of López-Ochoterena (1964). Following analysis, the snails collected were sent to the Malacology Department of the Oswaldo Cruz Institute for identification.

The ecological terms ‘prevalence’ and ‘community status’ used in this article were proposed by Margolis et al. (1982), Bush and Holmes (1986) and Bush et al. (1997) for parasitological studies and have been applied in studies of epibiotic relationships.
The taxocenoses components of ciliated protists were classified according to Bush and Holmes (1986) into central species (present on more than two-thirds of the hosts), secondary species (present on one-third to two-thirds of the hosts) and satellite species (present on fewer than one-third of the hosts). The specificity of ciliates in relation to the host was determined according to Nenninger (1948) and Cook et al. (1998).

Results

The species of ciliated protozoan epibionts found on *P. figulina* are presented in Table 1 and illustrated in Figure 1. This is the first record of ciliated protozoa on shells of *P. figulina*. We recorded seven species of ciliates on *P. figulina*, with six belonging to the Class Oligohymenophorea, Sub-class Peritrichia: *Vorticella campanula* Ehrenberg, 1831 (Figures 1A–B), *Epistylis plicatilis* Ehrenberg, 1831 (Figures 1C–D), *Opercularia* sp. (Figures 1E–F), *Vorticella microstoma*-complex (Ehrenberg, 1830) (Figure 1G), *Epistylis* sp. (Figures 1H–J), *Carchesium polypinum* (Linnaeus, 1758) (Figures 1K–N), and one species to the Class Phyllopharyngea, Sub-class Suctoria: *Tokophrya fasciculata* (López-Ochoterena, 1964) (Figures 1N–Q).

Of the 23 snails of the species *P. figulina* analysed, 82.60% (*n* = 19) were infested with at least one species of ciliate. No ciliates were observed on the opercula of the snails analyzed nor on the empty shells of the ampullariids.

Regarding the prevalence of infestation on *P. figulina*, the ciliate species were classified according to their status within the taxocenoses into central species: *Carchesium polypinum* (78.26%), secondary species: *Vorticella microstoma*-complex (47.82%), *V. campanula* (39.16%), and satellite species: *Epistylis plicatilis* (30.43%), *Opercularia* sp. (17.39%), *Epistylis* sp. (8.70%), *Tokophrya fasciculata* (8.70%) (Table 1).

Among the species of ciliates recorded on the shells of *P. figulina*, *C. polypinum*, *V. microstoma*-complex, *V. campanula* and *E. plicatilis* can be classified in Group O and I, as they showed no substrate specificity and have previously been recorded

<table>
<thead>
<tr>
<th>Protist ciliates</th>
<th>Prevalence (%)</th>
<th>Community status</th>
<th>Specificity</th>
</tr>
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<tbody>
<tr>
<td><em>Carchesium polypinum</em></td>
<td>78.26</td>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td><em>Vorticella microstoma</em>-complex</td>
<td>47.82</td>
<td>S</td>
<td>0</td>
</tr>
<tr>
<td><em>Vorticella campanula</em></td>
<td>39.16</td>
<td>S</td>
<td>0</td>
</tr>
<tr>
<td><em>Epistylis plicatilis</em></td>
<td>30.43</td>
<td>Sa</td>
<td>0</td>
</tr>
<tr>
<td><em>Opercularia</em> sp.</td>
<td>17.39</td>
<td>Sa</td>
<td>–</td>
</tr>
<tr>
<td><em>Epistylis</em> sp.</td>
<td>8.70</td>
<td>Sa</td>
<td>–</td>
</tr>
<tr>
<td><em>Tokophrya fasciculata</em></td>
<td>8.70</td>
<td>Sa</td>
<td>II</td>
</tr>
</tbody>
</table>

Community status: C=central species; S=secondary species; Sa=satellite species.

Specificity: Group 0=individuals colonize a wide range of substrates, including dead, inert, plant and animal substrata, and are not restricted to an epibiotic existence; Group II=partially specialized and restricted to hosts at the phylum, class, or order level.
living on algae, geophytes, and root-floating leaved plants, and colonizing diverse
groups of aquatic invertebrates. The species *T. fasciculata* fits in Group II, and has
been recorded only on stalked epibiont ciliates.

**Discussion**

**Occurrence of ciliated protozoa**

Records of ciliates on ampullariid snails are rare. Dias et al. (2006) recorded, for the first
time, seven genera of sessile ciliates – five peritrichs *Carchesium*, *Epistylis*, *Opercularia*,
*Vaginicola* and *Vorticella* and two suctorians *Acineta* and *Tokophyra* – living as epibionts
on ampullariid snails of the species *Pomacea lineata* (Spix, 1827) in irrigation ditches in
the municipality of Juiz de Fora, Minas Gerais, Brazil. Utz (2007) recorded the species *E.
plicatilis* (Peritrichia, Epistylididae) associated with the shell of *Pomacea canaliculata*
(Lamarck, 1819) in southern Brazil, this being the first record of epibiont ciliates on this
species of snail. The species *T. fasciculata* (Suctoria, Acinetidae) recorded in this study,
when it was described, was found on colonies of *E. plicatilis*, which were in turn
colonizing snails of the species *Lymnaea attenuata* Say, 1829 and *Physa osculans*
Haldeman, 1841 in the Chapultepec Lake, in Mexico City (López-Ochoterena 1964).

The operculum of ampullariid gastropods is a corneal structure of a permanent
nature that has the function of opening and closing the shell when the snail retracts
the cephalopodal mass (Thiengo 1995). The movement of the operculum towards the
opening of the shell when the snail retracts can disturb the epibionts, which may
explain the absence of ciliated protists in this region.

Some authors have observed an absence or lower abundance of ciliates in regions
of the host that have a high incidence of friction (Green 1974; Henebry and
Ridgeway 1979; Cook et al. 1998; Roberts and Chubb 1998; Regali-Seleghim and
Godinho 2004). According to Green (1974), the frequent disturbance caused by the
movement of the antenna of cladoceran hosts interrupts the feeding of the peritrich
colonies by provoking the contraction of the zooids. Henebry and Ridgeway (1979)
and Regali-Seleghim and Godinho (2004) associated the friction caused by the
movement of these hosts with the absence of epibiont ciliates on crustaceans.
According to Roberts and Chubb (1998), the increased water movement along the
edges of the pleopods of the isopod *Asellus aquaticus* (Linneaus, 1758) is one of the
factors that restricts the distribution of the peritrich *Lagenophrys asellii* (Plate, 1889)
to the central region of this appendage.

Many ciliated protozoa can live as epibionts on inert, non-living substrates
(Kudo 1966; Foissner et al. 1992). Nevertheless, the fact that no species of ciliate has
been found on empty shells suggests the existence of benefits to the epibionts derived
from the biological activity and behaviour of the snails. Henebry and Ridgeway
(1979), studying epibiont ciliates of copepods and cladocerans in Ashmore Lake,
Illinois, USA, inserted glass slides into the lake to the same depth at which
planktonic crustaceans live and observed that none of the four species of peritrichs
recorded on these hosts was found colonizing a glass slide.

**Prevalence and community status**

In the present study, a high prevalence of ciliate infestation was recorded on *P.
figulina* (82.60%). Dias et al. (2006) reported a 100% prevalence of epibiont ciliates
on *P. lineata*. Other studies have reported high prevalences of infestation by ciliated protists on different species of crustaceans. Henery and Ridgeway (1979) observed a prevalence of infestation by *V. microstoma* on cladocerans of the species *Daphnia longispina* Müller, 1785 ranging from 92.9 to 100%; Xu and Burns (1991) reported that 92.4% of the copepods *Boeckella triarticulata* Thomson, 1883 analyzed were infested by *Epistyliis daphniae* (Faure-Fremiet, 1905); Xu (1992) found the peritrich *E. daphniae* on 92.4% of cladocerans of the species *Moina macrocopa* (Strauss, 1820) analyzed; Jayasree et al. (2001) recorded a prevalence of ciliated peritrichs and suctorians of 43.1 and 71.8% on *Macrobrachium rosenbergii* (De Man, 1879) and *Metapenaeus dobsoni* (Miers, 1878) respectively; Fernandez-Leborans and Tato-Porto (2002) observed that 63.6% of crustaceans of the species *Schistomysis mysidacii* (Norman, 1892) were infected with the suctorian *Ophrydendron mysidacii* Fernandez-Leborans et al. 1996; Mayén-Estrada and Aladro-Lubel (2002), studying the distribution of 15 species of ciliates on *Cambarellus patzcuarensis* Villalobos, 1943, recorded epibiont ciliates on 73 and 78% of the crustaceans analyzed at two points in Pátzcuaro Lake, in Michoacán, Mexico.

The high prevalences of infestation recorded for *P. figulina* in this study and for *P. lineata* (Dias et al. 2006) suggest that ampullariid snails lack efficient strategies to impede the colonization of their shells by ciliated protists. Xu (1992) concluded that the high percentage of infestation by ciliates on *M. macrocopa* could indicate that these crustaceans have few antifouling mechanisms against infestation by these protists.

Cook et al. (1998), in a study about the ecology of epibiont ciliates of the isopod *A. aquaticus*, found that of the nine species of peritrichs and suctorians recorded, one occurred on more than 66.7% of the hosts (central species), four occurred on 33.3 to 66.67% of the crustaceans (secondary species), and four occurred on fewer than 33.3% of the basibionts (satellite species).

According to Mayén-Estrada and Aladro-Lubel (2002), ciliated protists have the opportunity to colonize their hosts at the same time; however, if other species of ciliates are already present on the surface of the basibiont, the new epibionts occupy the free areas. Competition among the epibiont ciliates is habitual, as observed by Mayén-Estrada and Aladro-Lubel (1998) among three species of suctorians, *Acinetia tuberosa* Ehrenberg, 1838, *Podophrya sandi* Collin, 1911 and *Tokophrya quadripartita* Bütschli, 1899 and also this type of interaction was reported by Roberts and Chubb (1998) among *L. aselli* and *L. platei* (Wallengren 1990). Peritrich ciliates of different species occupy different sites on the host due to their peculiarities with respect to growth rate, organization and shape of the zooids, size of the peduncle, and presence or absence of lorica. Peritrichs with larger colonies can occupy large areas of the surface of the host, thus preventing colonization by other species (Mayén-Estrada and Aladro-Lubel 2002). Colonies of *C. polypinum* can have numerous zooids of medium size ranging from 80–140 μm, which may explain their status as a central species (78.26%) in this study. However, the lower prevalences of colonizing ciliates *E. plicatilis* (30.43%), *Oercularia* sp. (17.39%), and *Epistyliis* sp. (8.70%) on *P. figulina*, compared with the solitary species *V. microstoma*-complex (47.82%) and *V. campanula* (39.16%) cannot be explained by the size of the colonies and is probably due to peculiarities of the ecology of these species.

The largest prevalences of *C. polypinum*, *V. microstoma*-complex and *V. campanula* observed in this study may be related to their preference for eutrophic
environments, as is the case of São Pedro stream. The direct dumping of domestic sewage strongly influences the composition and structure of ciliate communities (Primc 1988; Primc-Habdija et al. 1998; Madoni and Bassanini 1999). Organic pollutants cause an increase in phosphate and other nutrients, which alter the bacterial communities and induce changes in the composition of the ciliate population which depend directly on these bacteria for food (Primc 1988). According to Laird (1959) and Henebry and Ridgeway (1979), the occurrence and prevalence of peritrich epibionts indicate the degree of organic pollution in an aquatic ecosystem. The species *C. polypinum*, *V. microstoma*, and *V. campanula* have been used as indicators of water quality in various biomonitoring studies of rivers and streams in urban areas (Czapik 1982; Kusuoka and Watanabe 1987; Stössel 1987; Madoni 1993, 2005; Sola et al. 1996; Madoni and Bassanini 1999). Snails of the genus *Pomacea* are also tolerant of organic pollution (Thiengo 1995), and have been cited as promising indicators of water quality (Coler et al. 2005). This characteristic increases the ecological opportunity for colonization by ciliated protozoa epibionts adapted to eutrophic environments.

**Substrate specificity**

Epibionts show varying degrees of host specificity. Peritrich epibionts have attributes that reflect the degree of specialization for a given habitat. Nenninger (1948) and Cook et al. (1998) propose three distinct ecological groups of peritrichs classified according to their host specificity. Group 0 and I individuals colonize a wide range of substrates, including dead, inert, plant and animal substrata, and are not restricted to an epibiotic existence. Organisms classified in Group II are partially specialized and restricted to hosts at the phylum, class, or order level. In Group III, the ciliates are obligate epibionts with a higher degree of host specificity, at the family, genus or even species level.

The ciliate species *C. polypinum*, *V. microstoma*-complex, *V. campanula*, and *E. plicatilis*, classified as belonging to Group O, have been recorded living on algae, geophytes and root-floating leaved plants, and diverse groups of aquatic invertebrates (López-Ochoterena 1964; Henebry and Ridgeway 1979; Foissner et al. 1992; Cook et al. 1998; Aladro-Lubel and Martínez-Murillo 1999). In addition to colonizing live substrates, these species also live associated with inert substrates, and have been reported in various lotic systems associated with sediment (Madoni and Ghetti 1981; Hul 1987; Kusuoka and Watanabe 1987; Gracia et al. 1989; Madoni 1993; Sola et al. 1996; Madoni and Bassanini 1999). Although the species *T. fasciculata* have been observed living in hyperepibiosis on algae (Aladro-Lubel et al. 2006) and on snails of the species *L. attenuata*, *P. osculans* (López-Ochoterena 1964) and *P. figulina* (present study), it was classified as Class II because it has been reported living only on stalked epibiont ciliates.

**Ampullariid Basibionts**

Epibiosis can be advantageous for epibionts due to certain biological aspects and behaviours of the basibiont. Basibionts of the genus *Pomacea* can select microhabitats with favourable characteristics, transporting the epibionts to more oxygenated places or away from sources of pollution, or even modifying the conditions of the environment (Milward-de-Andrade et al. 1975). These snails can
even facilitate the access of the ciliates to food by provoking the suspension of periphyton associated with macrophytes when they feed (Thiengo 1995), thus making food available for the peritrichs, which are bacterivores and algivores, as well as the suctorians, which feed on free-swimming ciliates and on sedentary forms. The aggregative behaviour of the snails (Dias et al. 2006) and mating (Guimarães 1981) facilitates contact between the individuals and favours the transfer of the dispersion forms of the epibionts (Fernandez-Leborans and Tato-Porto 2002; Dias et al. 2006). The shell functions as a permanent substrate, not needing to be substituted as the snail grows, which is an inherent advantage of using snails as basibionts. Finally, their longevity (Guimarães 1981) and capacity for survival in unfavourable conditions (Guimarães 1981; Santos et al. 1987) are probably responsible for the relative abundance and stability of populations of *Pomacea*, favouring epibiont communities.

The disadvantages for epibions are mainly related to certain behaviours of the hosts, such as their habit of burying into the substrate at certain times of the year and in unfavourable conditions (Fernandez-Leborans et al. 1997) and remaining outside the water (Santos and Mendes 1981; Santos et al. 1987; Thiengo 1995). During aestivation, species of *Pomacea* bury themselves in the soil, emerging again when the rains begin. These animals are able to remain for long periods outside of the water when they aestivate and during mating and egg laying (Guimarães 1981), and this amphibious behaviour can be harmful to the epibionts.

Snails of the species *P. figulina* therefore constitute a relatively protected micro-environment with food supply favourable for colonization by ciliated protists. Existing studies of epibiotic associations between ciliated protists and aquatic invertebrates mainly consist of new records or address morphological aspects. Therefore, more studies are needed on the ecological relations between these organisms, as epibiont protists are well represented in aquatic ecosystems.

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