Diversity and Distribution of Peritrich Ciliates on the Snail *Physa acuta* Draparnaud, 1805 (Gastropoda: Physidae) in a Eutrophic Lotic System

Bianca Sartini[1], Roberto Marchesini[1], Sthefane D´ávila[2], Marta D'Agosto[1], and Roberto Júnio Pedroso Dias[1,*]

[1]*Laboratório de Protozoologia, Programa de Pós-graduação em Ciências Biológicas (Zoologia), ICB, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, 36036-900, Brazil*
[2]*Museu de Malacologia Prof. Maury Pinto de Oliveira, ICB, Universidade Federal de Juiz de Fora, Minas Gerais, 36036-900, Brazil*

Bianca Sartini, Roberto Marchesini, Sthefane D´ávila, Marta D'Agosto, and Roberto Júnio Pedroso Dias (2018) Freshwater gastropods represent good models for the investigation of epibiotic relationships because their shells act as hard substrates, offering a range of microhabitats that peritrich ciliates can occupy. In the present study we analyzed the community composition and structure of peritrich epibionts on the basibiont freshwater gastropod *Physa acuta*. We also investigated the spatial distribution of these ciliates on the shells of the basibionts, assuming the premise that the shell is a topologically complex substrate. Among the 140 analyzed snails, 60.7% were colonized by peritrichs. We observed seven peritrich species: *Epistylis plicatilis* and *Epistylis* sp. (Epistylididae); *Opercularia articulata* (Operculariidae); C*archesium polypinum*, *Vorticella campanula* and *Vorticella* sp. (Vorticellidae) and *Thuricola kellicottiana* (Vaginicollidae). We observed a high prevalence of epibiosis (> 60%) when all species of ciliates were considered conjunctly. However, the prevalence was low (1-58%) when each species was considered separately, reflecting their aggregate distribution pattern. The most prevalent species were *Epistylis* sp. (58.60%), *Vorticella* sp. (14.30%) and *O. articulata* (13.60%). Although the epibionts were distributed through the shell's entire extension, we observed greater values of abundance, density, diversity and dominance on the dorsal surface. Only *Epistylis* sp. was widely distributed on the ventral surface. We also observed that the peritrichs predominantly occupied the areas of the shell delimited by the sutures. We interpreted these distribution patterns considering that the peritrichs

select their attachment sites under pressures related to basibiont behavior, physical forces that may dislocate them and protective characteristics of the shell's areas.

Key words: Brazil, Peritrichia, *Physa acuta*, Protozoan epibionts, Site preference.

*Correspondence: Tel: +55 32 21023223. E-mail: rjuniodias@hotmail.com

BACKGROUND

Epibiosis is a facultative association, widespread in aquatic environments, which involves the epibionts, organisms that remain attached to the surface of living substrates, the basibionts, during the sessile phase of their life cycles (Wahl 1989). Epibiosis not only affects the organisms involved, but also promotes changes at the community and the microenvironment levels (Fernandez-Leborans and Cárdenas 2009). Epibiosis involving ciliates is relatively well documented in a number of studies addressing the composition of epibiotic communities (Fernandez-Leborans and Tato-Porto 2000; Chatterjee et al. 2013; Bielecka and Boehnke 2014), spatial and temporal distribution of epibionts in marine and limnetic ecosystems (Hanamura 2000; Utz and Coats 2005; Dias et al. 2008; Cabral et al. 2010; Cabral et al. 2017), negative impacts on basibionts (Visse 2007; Souissi et al. 2013; Burris and Dam 2014), advantages and adaptations of epibionts (Delgery et al. 2006; Fernandez-Leborans and Rintelen 2010), spatial colonization patterns of epibionts on basibionts (Fernandez-Leborans et al. 2006; Marinõ-Perez and Mayén-Estrada 2009; Fernandez-Leborans et al. 2013) and epibionts biology (Gilbert and Schröder 2003; Utz 2008; Bickel et al. 2012).

Epibiont ciliates are found in several groups: apostomatids, chonotrichids, heterotrichs, peritrichs and suctorians (Corliss 1979; Fernandez-Leborans et al. 2006). Among these, greater diversity of epibiont ciliates are found in the subclass Peritrichia, Stein 1859, whose life cycles contain a sessile stage fixed by a stalk to an inert or living substrate as well as by a dispersal stage represented by free-swimming forms that seek new substrates (Gilbert and Schröder 2003). They can live as epibionts on several metazoans as cnidarians, ctenophorans, rotifers, tardigrades, platyhelminthes, crustaceans, annelids, insect larvae, mollusks, fishes, tadpoles and turtles (Kahl 1935; Corliss 1979; Foissner et al. 1992; Moss et al. 2001; Mayén-Estrada and Aladro-Lubel 2002; Gilbert and Schröder 2003; Dias et al. 2008, 2009). Although the majority of peritrich species are able to colonize both inert and living substrates evenly, the colonization of basibionts is not a random phenomenon. There is experimental evidence that suggests that these ciliates actively select the colonized species,

as well as the attachment sites on the basibionts (Cook et al. 1998; Mayén-Estrada and Aladro-Lubel 2001; Gilbert and Schröder 2003; Fernandez-Leborans et al. 2006).

The advantages of colonizing a living substrate have been mostly associated to nutrient acquisition through the water currents generated by the basibionts (Wahl 1989; Threlkeld 1993; Fernandez-Leborans et al. 2006), or from the bacteria-rich periphyton that accumulate on their exoskeletons (Bernot et al. 2005). Another important aspect is the fact that the basibionts are mobile, moving away from unfavorable environments and thus promoting the survival of the epibionts (Dias et al. 2008). Also, the basibiont may potentially promote epibiont dispersal during their own migrations, contributing to the gene flow between populations (Wahl 1989).

Freshwater gastropods are good models for investigating epibiotic relationships because their shells act as hard substrates that are complex in form and ornamentation and therefore offer a range of microhabitats that can be occupied by peritrich ciliates. The shell is not discarded during the development of the snails, like it is with the exoskeletons of arthropods (Hanamura 2000; Delgery et al. 2006; Vicente et al. 2008), allowing the continuous colonization by ciliate species and leading to complex interactions between ciliate species in space and time (Dias et al. 2008).

As a result, freshwater snails can be regarded as a source of biodiversity in limnic environments (Voight and Walker 1995; Gutiérrez et al. 2003). However, most studies on the matter are restricted to marine species bearing communities composed of polychaetes and anemones (Wahl and Sönnichsen 1992; Warner 1997; Vasconcelos et al. 2007; Mercier et al. 2011). Studies aiming to investigate epibiotic interactions between peritrich ciliates and freshwater gastropods are scarce, more particularly in neotropical ecosystems (López-Ochoterena 1964; Dias et al. 2006, 2008, 2010; Utz 2007).

Physa acuta Draparnaud, 1805 (recent synonym *Haitia acuta*) (Pulmonata, Physidae) is an invasive freshwater snail originating from North America, presently found in all continents (Dillon et al. 2002; Van Leeuwen et al. 2012). These snails exploit several dispersal vectors that may have contributed to its wide geographical range (Van Leeuwen et al. 2012), and there is genetic and demographic evidence that these snails recurrently recolonize habitats (Bousset et al. 2004; Chlyeh et al. 2006). They show tolerance to a wide range of environmental conditions, including organic pollution, and were recently used as biological models to assess toxicological effects of nanoparticles in freshwater environments (Fahmy et al., 2014). They are commonly found in eutrophic lakes and rivers in Europe, Asia, Africa, Australia and North and South America (Paraense and Pontier 2003; Bernot et al. 2005; Albrecht et al. 2009; Tietze and De Francesco 2010).

Herein we analyzed the community composition and structure of peritrich epibionts on *P. acuta*. We also investigated the spatial distribution of these ciliates on the shells of basibionts,

assuming that the shell is a topologically complex substrate. We considered that the different parts of the shell represent distinct microhabitats and some of its areas may be more favorable for the establishment of epibionts. We interpreted the observed distribution patterns, considering that the peritrichs select their attachment sites under pressures related to the basibiont behavior, physical forces that may dislocate them and protective characteristics of the shell's areas.

MATERIALS AND METHODS

Snail sampling

We collected 140 *Physa acuta* specimens in a eutrophic stream (total nitrogen = 20.05 mg/ L^{-1}, total phosphor = 0.29 mg/ L^{-1}, electric conductivity = 60.91 µS.cm^{-1} and dissolved oxygen = 5.29 mg.L^{-1}) in June 2010. This stream is located in the urban area of the Juiz de Fora municipality, Minas Gerais state, southeastern Brazil (21°43'55.5"S. 43°19'35.1"W). The snails were taken from the aquatic vegetation with a collecting net. After collecting the snails, each specimen was transferred to individual plastic bags containing water obtained from the collecting site previously passed through a filter paper (15µm pore size), then transported to the laboratory. The snail's species identification was based on the study of shell morphology and anatomy of the renal and reproductive systems, according to the description provided by Paraense and Pointier (2003). The shell length was measured with a caliper rule and the surface area of each region delimited by the sutures was calculated by the software Image Pro-plus 5.0 Olympus$^{®}$ from digital images of the shell, taken by a digital camera (Olympus DP73$^{®}$) coupled to a stereoscopic microscope.

Spatial distribution analysis

The snails were individually placed in Petri dishes and observed for the presence of epibionts under a stereoscopic microscope within 8 hours after collection. To determine the attachment sites of the epibionts, five dorsal and four ventral regions were defined and the position of each colony was annotated in schematic drawings of *P. acuta*'s shell. Subsequently, each specimen was isolated with micropipettes and observed under an Olympus$^{®}$ differential interference contrast microscope aiming to quantify the zooids and to perform specific identification according to Kahl (1935) and Foissner et al. (1992). *in vivo* morphometric data and the shape of the nuclear apparatus (semipermanent preparations with methyl green-based dyes) were used for species-level identification.

Epibiotic community structure

To access epibiotic community structure, we analyzed the data on prevalence, mean intensity, mean abundance and the discrepancy index (Poulin 1993) for each epibiont species with the Quantitative Parasitology 3.0 software (Rosza et al. 2000). In all analyses, colonial peritrichs were quantified considering each zooid as an individual. The Shannon diversity index and dominance index of Berger-Parker (Magurran 1988) were calculated for each region of the shell with the PAST 2.10 software (Hammer et al. 2001). The differences in abundance (number of zooids), density (number of zooids/shell area region), diversity and dominancy between the ventral and dorsal surfaces of the shell were assessed through the Wilcoxon test (paired samples) and Kruskal-Wallis test for nine regions (A-I). The correlations between epibiont abundance, host size and richness were assessed using the Spearman Rank Correlation test with the SPSS 17.0 software. A non-metric multidimensional scaling (NMDS) was used to observe differences in species abundance in the shell regions. The NMDS was performed using the Bray-Curtis distance and with 50 random starts to determine the values of the lowest stress (Standardized Residual Sum of Squares). Stress values lower than 0.2 were considered good representations of the data.

RESULTS

Epibiotic community

We registered seven peritrich species belonging to four families: *Epistylis plicatilis* Ehrenberg, 1831 and *Epistylis* sp. (Epistylididae); *Opercularia articulata* Goldfuss, 1820 (Operculariidae); C*archesium polypinum* (Linneaus, 1758); *Vorticella campanula* Ehrenberg, 1831 and *Vorticella* sp. (Vorticellidae) and *Thuricola kellicottiana* (Stokes 1887) (Vaginicolidae) (Fig. 1; Table 1).
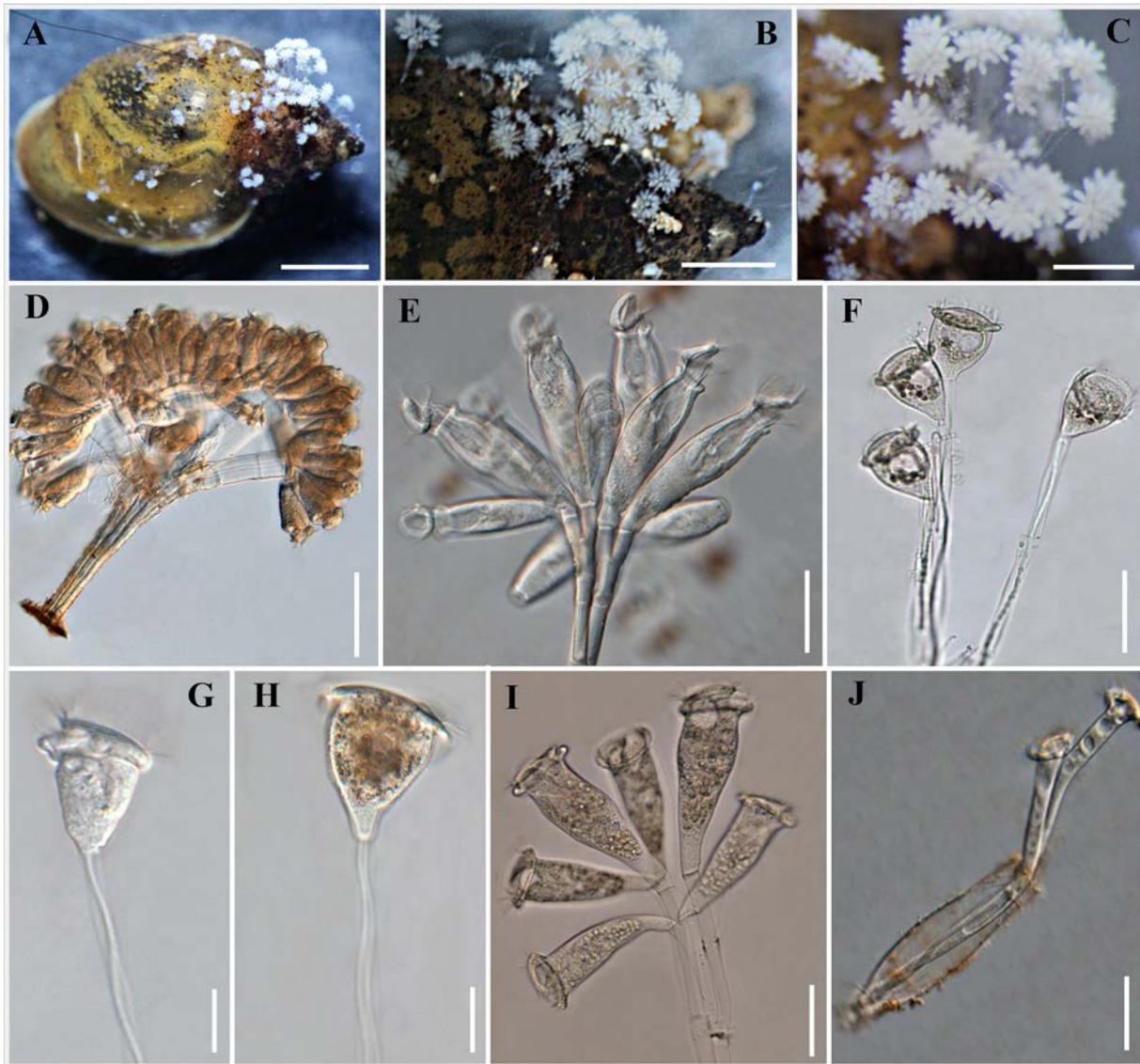
Fig. 1. *in vivo* photomicrographics of peritrich ciliates species on *Physa acuta*. (A-C) Peritrich ciliates attached to the shell. (D) *Epistylis* sp. (E) *Opercularia articulata*. (F) *Carchesium polypinum*. (G) *Vorticella* sp. (H) *Vorticella campanula*. (I) *Epistylis plicatilis*. (J) *Thuricola kellicottiana*. Scale bars: A = 0.15 cm; B = 1.5 mm; C = 0.5 mm; D-L = 25 µm.

Table 1. Community structures of peritrich epibiont species on *Physa acuta* in a lotic eutrophic system, Brazil

Species	Prevalence (%)	Intensity	Mean intensity	Mean Abundance	Discrepancy index
Epistylis sp.	58.60	1-2358	114.8 ± 337.60	67.25 ± 332.10	0.89
Epistylis plicatilis	7.10	2-49	13.4 ± 14.15	0.96 ± 6.35	0.96
Vorticella campanula	7.90	1-5	1.9 ± 1.57	0.15 ± 0.84	0.94
Vorticella sp.	14.30	1-10	2.1 ± 2.15	0.31 ± 1.38	0.90
Carchesium polypinum	5.00	1-2031	293.7 ± 766.07	14.69 ± 220.26	0.99
Opercularia articulata	13.60	2-623	89.3 ± 145.19	12.13 ± 76.94	0.94
Thuricola kellicottiana	1.40	1-3	2.0 ± 1.41	0.03 ± 0.34	0.98

Of the 140 analyzed snails, 60.7% ($n = 85$) were colonized by peritrich ciliates, with a mean intensity of 157 ($\pm$ 429.5) epibionts per snail, varying from 1 to 2390 epibionts per snail. We observed weak correlation between snail size and epibiont abundance ($r_s = 0.350$, $p = 0.001$) and snail size and epibiont richness ($r_s = 0.217$, $p = 0.046$).

The most prevalent species were *Epistylis* sp. (58.60%), *Vorticella* sp. (14.30%) and *O. articulata* (13.60%). The greatest values of mean intensity were presented by *C. polypinum* (293.7), *Epistylis* sp. (114.8) and *O. articulata* (89.3). All species presented an aggregate pattern of distribution, as demonstrated by the discrepancy index values nearing 1 (Table 1).

Epibiont distribution

We observed that the epibionts were distributed through the entire extension of the shell's dorsal and ventral surfaces when the distributions of all peritrich species were plotted conjunctly (Fig. 2). However, the comparison between the dorsal and ventral surfaces showed that the ciliates occupied mostly the dorsal surface, for which we observed the greater values of abundance (T = 385, $p < 0.001$), density (T = 738, $p < 0.001$), diversity (T = 101, $p = 0.02$) and dominance (T = 381, $p < 0.0001$) (Fig. 3). When the species distribution was analyzed separately, we observed that *Epistylis* sp. was the only species to be widely distributed on the ventral surface, while *Thuricola kellicottiana* occupied exclusively the dorsal surface and the other species were more abundant (*C. polypinum*) or occurred exclusively on the superior third of the dorsal surface (*O. articulata*, *E. plicatilis* and *Vorticella* sp.) (Fig. 2).

With the exception of *Carchesium polypinum* and *T. kellicottiana*, all species were significantly more abundant ($p < 0.005$) on the dorsal surface than the ventral one. The highest density values were observed on the dorsal surface for *Epistylis* sp., *O. articulata* and *C. polypinum* (Fig. 4).
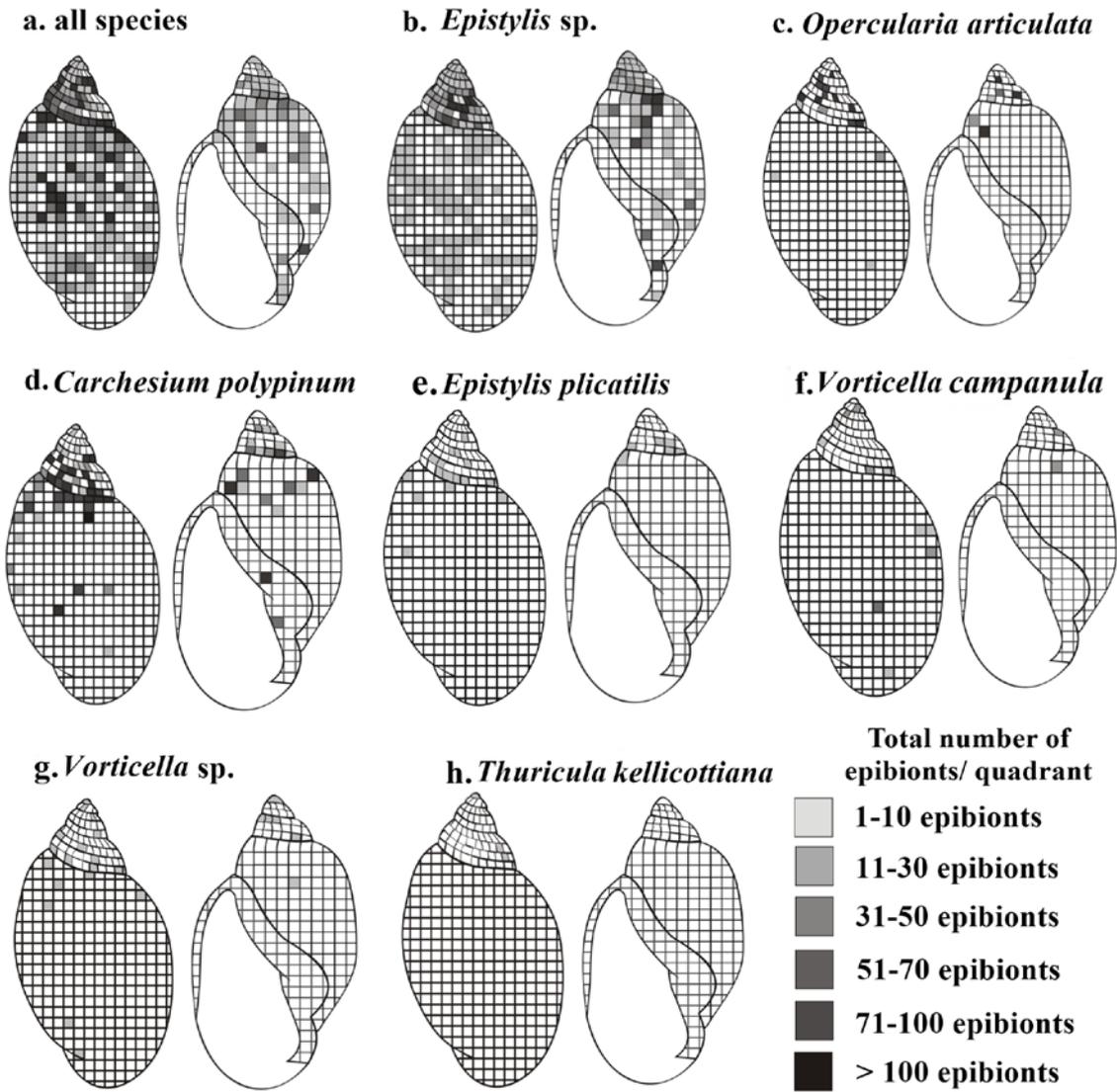
a. all species

b. *Epistylis* sp.

c. *Opercularia articulata*

d. *Carchesium polypinum*

e. *Epistylis plicatilis*

f. *Vorticella campanula*

g. *Vorticella* sp.

h. *Thuricula kellicottiana*

Total number of
epibionts/ quadrant

1-10 epibionts

11-30 epibionts

31-50 epibionts

51-70 epibionts

71-100 epibionts

> 100 epibionts

Fig. 2. Schematic representation of the distribution of peritrichs ciliates species on the *Physa acuta* shell.
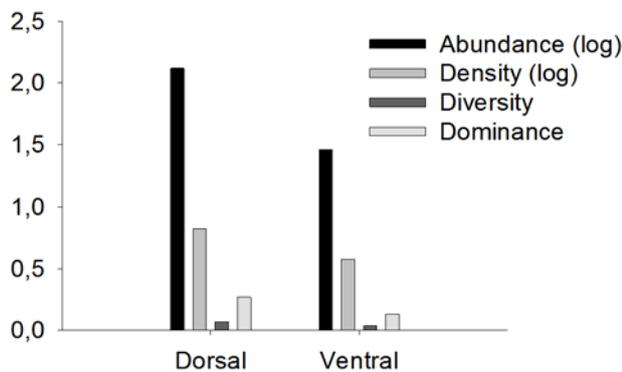
Fig. 3. Abundance, density, diversity and dominance of peritrich ciliates on surface of the *Physa acuta* shell. Different letters indicate statistical differences ($p < 0.05$).
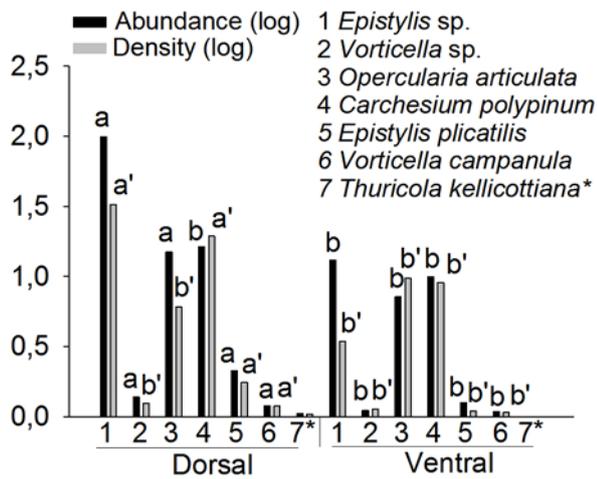
Fig. 4. Abundance and density of peritrich species on the surface on the *Physa acuta* shell. Different letters indicate statistical differences (*p* < 0.05) and * indicates species present exclusively on the dorsal surface.

We observed significant differences in the abundance (H = 124.8, *p* < 0.001) and density (H = 157.2, *p* < 0.001) of peritrich epibionts between shell regions (A-I) (Fig. 5). The areas with greater abundance were the regions delimited by the sutures in the spire whorls (Fig. 2). In figure 6, we observed significant differences in the abundance, density, diversity and dominance of peritrichs epibionts between shell regions and the ventral and dorsal surfaces.

We also observed differences between the species regarding the extent to which they occupied different shell regions. The peritrichs *Epistylis* sp., *O. articulata* and *C. polypinum* were more broadly distributed through the shell regions compared to the others species (Fig. 2). The two first principal components of the PCA - performed using the mean number of epibiont species on the regions of the shell - showed three species' groups with different colonization patterns (Fig. 7). The group *Epistylis* sp. and *C. polypinum* had the highest mean intensities and were the most widely distributed through the regions of the shells (see Fig. 2 and Table 1). Another group - *E. plicatilis*, *V. campanula*, *Vorticella* sp. and *T. kellicottiana* - integrated the species with low mean intensity and with more restricted distributions through the shell. The species *O. articulata* had intermediate values of mean intensity, which put them apart from these groups.

We also observed differences between the species regarding the extent to which they occupied different shell regions. The peritrichs *Epistylis* sp., *O. articulata* and *C. polypinum* showed broader distribution through the shell regions compared to the others species (Fig. 2). The NMDS analysis showed three species groups with different colonization patterns (Fig. 7). One group included *Epistylis* sp., *C. polypinum* and *O. articulata* which were the species with the highest mean intensities and most widely distributed through the regions of the shells (see Fig. 2 and Table 1). Another group-*V. campanula*, *Vorticella* sp. and *T. kellicottiana*-integrated the species with low

mean intensity and had more restricted distributions throughout the shell. The species *E. plicatilis* appeared apart from these groups by presenting intermediate values of mean intensity.
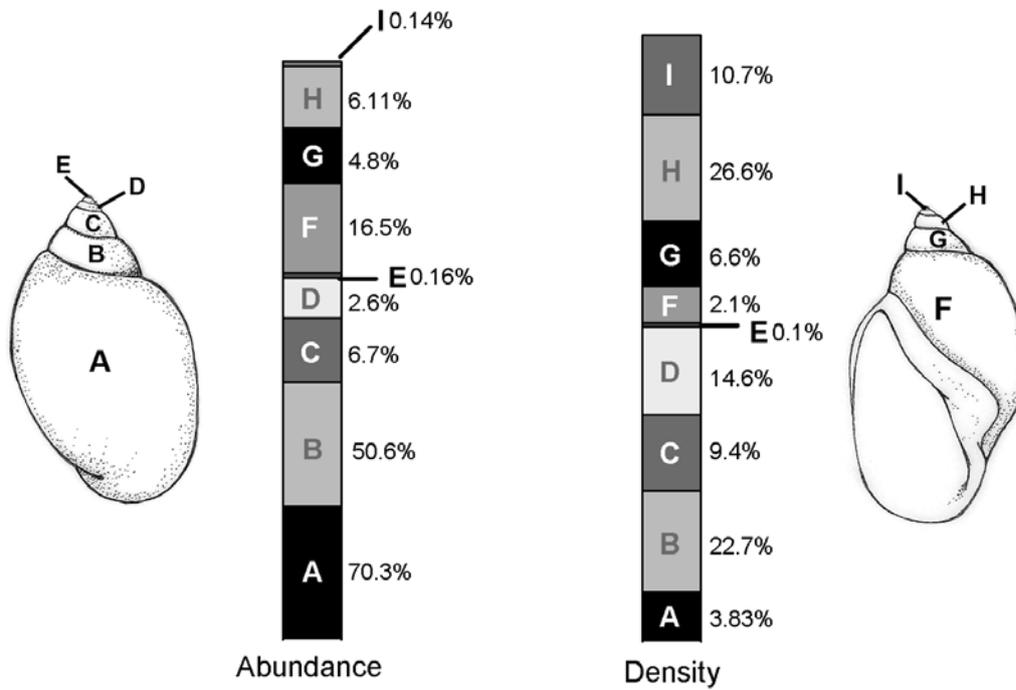

Fig. 5. Abundance and density of peritrich ciliates on the sites of the *Physa acuta* shell. Distribution of the total number of epibionts throughout the antero-posterior axis of the shell.
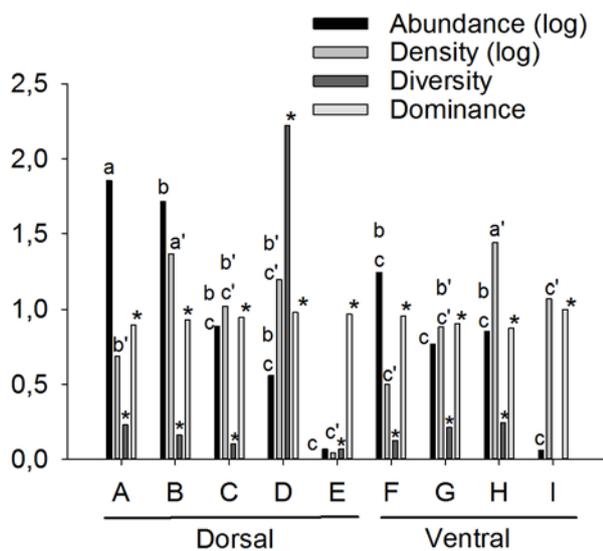

Fig. 6. Abundance, density, diversity and dominance of peritrich epibionts on sites of the *Physa acuta* shell. Different letters indicate statistical differences $p < 0.05$ and the symbol *indicate $p > 0.05$.
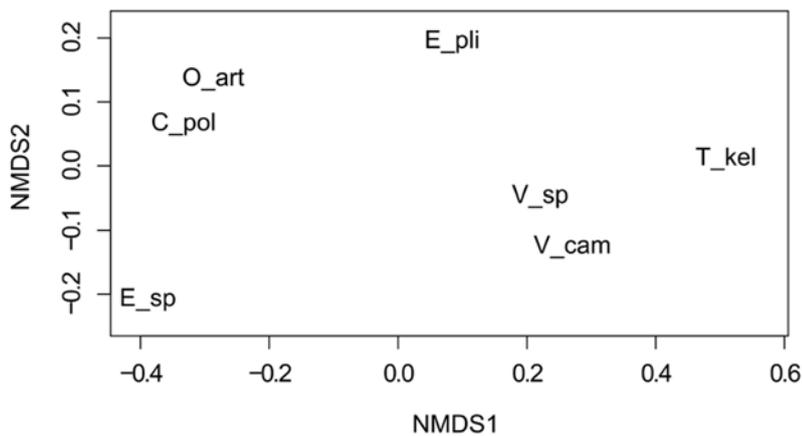
Fig. 7. Non-metric multidimensional scaling (NMDS) for peritrichs species abundance on *Physa acuta* shell. Stress = 0. C_pol = *Carchesium polypinum*, E_plic = *Epistylis plicatilis*, E_sp = *Epistylis* sp., O_art = *Opercularia articulata*, T_kel = *Thuricola kellicottiana*, V_cam = *Vorticella campanula*, V_sp = *Vorticella* sp.

DISCUSSION

Occurrence

The present study constitutes the first record of epibiosis by peritrich ciliates on *Physa acuta* on neotropics. The epibiotic relationship among ciliates and freshwater mollusks has been scarcely studied, particularly in the neotropics (Lópes-Ochoterena 1964; Dias et al. 2006, 2008; Utz 2007). The composition of the peritrich ciliates community described in the present study is similar to the results presented by other authors for freshwater snails of the genera *Pomacea*, *Lymnaea* and *Physa* in eutrophic environments (Lópes-Ochoterena 1964; Dias et al. 2006, 2008, 2010; Utz 2007). The most frequently found genera are *Carchesium*, *Epistylis*, *Vorticella*, *Opercularia* and *Vaginicola*. *Epistylis plicatilis* was recorded on *Lymnaea atenuata* Say, 1829 and *Physa osculan* Haldeman, 1841 in a lake in Mexico (Lópes-Ochoterena 1964). Dias et al. (2006) recorded peritrichids of the genus *Carchesium*, *Epistylis*, *Vorticella*, *Opercularia* e *Vaginicola* on *Pomacea lineata* (Spix, 1827), and Dias et al. (2008) recorded *C. polypinum*, *V. microstoma*, *V. campanula*, *E. plicatilis*, *Opercularia* sp. and *Epistylis* sp. on *Pomacea figulina* (Spix, 1827). Utz (2007) recorded *E. plicatilis* on *Pomacea canaliculata* (Lamarck, 1819) in southern Brazil.

All species recorded on *P. acuta* in the present study are facultative epibionts since they were previously found colonizing inert substrates (Kahl 1935; Foissner et al.1992). *Carchesium*

polypinum, *E. plicatilis*, *V. campanula* and *T. kellicottiana* were formerly recorded for non-molluscan basibionts (Kahl 1935; Laird 1959; Foissner et al. 1992; Cook et al. 1998; Mayén-Estrada and Aladro-Lubel 2002; Szlauer-Lukaszewska 2007; Utz 2007; Risse-Buhl and Küssel 2009). However, even if the epibiotic relationship between *P. acuta* and the peritrich species found in the present study is of a facultative nature, the colonization of living substrates may be an adaptation for these ciliates. The basibiont-epibionts system *P. acuta* and peritrich ciliates would be an interesting future model to investigate the ability of peritrichs to be carried by basibionts since these snails can efficiently disperse via water and its ability to disperse may be enhanced by zoochory.

Prevalence and aggregate distribution

Among the factors that influence the prevalence of epibiosis, the following are critical: the abundance of epibiont populations (Cook et al. 1998), the frequency of contact between the free-swimming forms and the basibionts, the basibiont behavior and the efficiency of their anti-colonization mechanisms (Wahl and Sönnichsen 1992; Xu 1992) as well as the organic pollution levels and nutrients availability (Olsen and Towsend 2003; Utz and Coats 2005).

The present study observed high prevalence of epibiosis when all species of ciliates were considered conjunctly. Similar results were obtained for the ampullariids *P. lineata* (100%; $n = 50$) (Dias et al. 2006) and *P. figulina* (82%; $n = 23$) (Dias et al. 2008). However, the prevalence obtained was low (1-58%) when each species was considered separately, which reflects the aggregate distribution pattern of the species.

Peritrichs are commonly found covering the surface of inert and living substrates in lotic environments. These ciliates show higher abundance and prevalence at locations with high levels of organic load (Laird 1959; Henebry and Ridgeway 1979; Xu 1992; Cabral et al. 2010). We observed domestic sewage discharge at several sites of the studied stream. The process of eutrophication creates favorable conditions for the development of bacterivore ciliate communities (Madoni 2005) and for the establishment of *P. acuta* populations as well (Bernot et al. 2005; Kefford and Nugegoda 2005). The similarities between snail and ciliate autoecology create the opportunity to the colonization of these basibionts by peritrichs. Thus, in the present study, the high prevalence of epibiosis is better explained by the expressive levels of organic pollution in the environment and not by the absence of defense mechanisms of the basibiont. Also, the establishment of the epibionts by transference of telotrochs between basibionts may be favored by a trend to form aggregates presented by several molluscan species, including *P. acuta* (Fratini et al. 2001; Ohbayashi-Hodoki et al. 2004; D'ávila et al. 2006; Dias et al. 2006).

In the present study, the peritrich populations presented an aggregate distribution, which may be evidence of interespecific interaction, although laboratory trials are required to prove this. The peritrich telotrochs tend to attach themselves to substrates previously colonized by congeners (Taylor 1983; Langlois 1975). This behavior is probably mediated by the perception of chemical signs, given all the evidences that ciliates use elaborate chemical communication. This trend to colonize substrates with the presence of congeners, combined with the peritrichs' high binary fission rate (Taylor, 1983) may have contributed to the aggregate distribution observed in the present study.

Distribution of epibionts on the shell surface

The gastropod shell is a long-lasting substrate, growing throughout the snail's development. We observed positive correlation between snail size and the abundance and richness of peritrichs, which indicates that larger individuals present a greater surface to be colonized. Moreover, bigger shells indicate that the snails are older, being exposed to peritrich colonization for a longer time and thus accumulating a greater abundance and diversity of epibiont species (Creed 2000; Gutiérrez et al. 2003; Vasconcelos et al. 2007).

It is common for epibiont organisms to colonize specific sites on the basibiont, although the degree of specificity varies between species (Mayén-Estrada and Aladro-Lubel 2002). Peritrich ciliates more frequently tend to colonize areas that are protected against the grooming behavior of the basibiont (Baldock 1986; Cook et al. 1998; Fernandez-Leborans et al. 2006) or that are favorable to adhesion and provide oxygen and nutrients (Mayén-Estrada and Aladro-Lubel 2002; Dias et al. 2009), showing low levels of turbulence and mechanical disturbance during the basibiont locomotion (Utz and Coats 2005).

The structural characteristics of the shell components-such as form, size, ornamentation and texture-are factors that make the shell a complex substrate, influencing the distribution pattern of the epibionts (Creed 2000). Some areas of the shell may be more favorable to the attachment of the epibionts by offering less mechanical disturbance and a consequent lesser possibility of detachment of the ciliates (*i.e.*: dorsal compared to ventral surface), greater protection against water turbulence (*i.e.*: the sutures area compared with the more exposed areas of the shell) and greater protection against the grooming behavior of the basibiont (*i.e.*: the spire compared with the body whorl).

In the present study, the low colonization by peritrichs on the ventral surface of the shell may be related to the disturbance produced by the cephalopodal mass during snail locomotion. Another factor to be considered is the intense production of mucus by the pedal gland, which may prevent the adhesion of epibionts or inhibit the cilia battement during filtration (Wahl 1989; Wahl and

Sönnichsen 1992). *Epistylis* sp. was the only species to extensively occupy the ventral surface of the shell. All other species occurred exclusively or preferentially on the dorsal surface. This is probably related to the more robust constitution (among colonial peritrichs) of the *Epistylis* sp.'s stalk (Fig. 1A-D), which allows a better adhesion to surfaces constantly exposed to attrition.

Lom and Corliss (1968) observed a wide variation in the structure of the scopula in species of the genus *Scyphidia* (Scyphidiidae). The differences observed concerns ultrastructural aspects, but also the mode of attachment to the host. The variability in the structure of scopula and stalk is not well known for free-living peritrichs. However, since these structures have a great adaptive significance to these ciliates, we can expect that they are as functionally and structurally variable, as observed for ectocomensal peritrichs. These variations may represent adaptations to some specific conditions prevailing on the surface of living substrates or preconditions for attachment to certain sites (Rhode 1994). Thus, the fixation on certain sites on the basibiont would not be simply the result of the offered advantages (oxygenation, nutrients), but also a consequence of the species' ability to remain fixed on that site.

We observed that the peritrichs predominantly occupied the areas of the shell delimited by the sutures. Although the shell of *P. acuta* has no evident ornamentation, the areas delimited by the sutures in the spire whorls have irregular surfaces because the growth lines are more pronounced at these areas, creating a favorable microenvironment for peritrichs to attach (Warner 1997; Olabarria 2000). In addition, the angle formed between the successive whorls makes the area near the suture more protected. Assuming that the regions near the sutures are more suitable for epibionts to attach to, the greater abundance of *Epistylis* sp., *O. articulata* and *C. polypinum* at this region may be evidence of competitive exclusion. These species have more robust stalks, which support a great number of zooids. Once the colonies of these species are established, this probably makes it difficult for other species to colonize. Other authors have documented interespecific competition involving peritrich ciliates, such as the ability to compete for space being related to morphological features (contractile vs. non-contractile stalks) as well as to the number of zooids (solitary vs. colonial peritrichs) (Ebert et al. 2001; Utz and Coats 2005). The greater range of intensity presented by *Epistylis* sp., *O. articulata* and *C. polypinum* compared to the others may be an evidence of competition between these species.

We observed greater abundances of peritrichs in the regions located at the spire than the corporal whorl. Epibionts choose attachment sites protected against the mechanic action of the basibionts, particularly grooming behavior, which could dislocate them (Wahl and Sönnichsen 1992). Gastropods perform grooming behavior by moving the mouth and radula around the shell border. In the present study, the distribution of epibionts was more concentrated at the spire and

farther from the shell aperture border; this is in agreement with the hypothesis that epibionts occupy areas where they are protected against basibiont grooming behavior.

The data on ciliate distribution on mollusks shown in this study confirm that the shell represents a complex and heterogeneous substrate for epibionts and calls out for new laboratory studies aiming to better understand if the peritrich ciliates make up an interactive community with interspecific competition.

CONCLUSIONS

This is the first study to investigate the structure and distribution of epibiont peritrich ciliate communities on mollusk shells. We can conclude that the high prevalence of epibionts recorded on *Physa acuta* is related to the high level of organic pollution in the studied lotic system; that the seven peritrich species found show aggregate distribution; and that ciliates mainly colonize the sutures and the dorsal region of the shell, which confirms the hypothesis that the shell makes up a complex and heterogeneous environment for peritrich colonization. These new findings highlight the need for future studies on the epibiotic relationships between peritrich ciliates and aquatic metazoans for understanding the dispersion of peritrichs in lotic systems. Furthermore, they point out the need for controlled studies in laboratories in order to confirm the occurrence of interespecific competition between these ciliates.

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