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SHORT COMMUNICATION

Occurrence of phoresy between *Ancistrus multispinis* (Actinopterygii: Siluriformes) and *Ichthyocladius* sp. (Diptera: Chironomidae) in Atlantic forest streams, Southeastern Brazil

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ABSTRACT. Phoretic relationships often bring large advantages to epibionts. By attaching themselves to mobile hosts, epibionts are able to: expand their ranges without spending energy, reduce their risk of being predated, and increase their probability of finding food. We assessed the phoretic relationship between the siluriform fish *Ancistrus multispinis* (Regan, 1912) and the chironomid larva *Ichthyocladius* sp. in three streams of the Atlantic forest in southeastern Brazil. We evaluated changes in epibiont distribution throughout the body regions of the host and among three different aquatic systems. We had predicted that certain body regions are more prone to support epibiont attachment, and that epibiont prevalence increases with increased host size and quality of the aquatic system. Three streams (Santana, São Pedro and D'Ouro), tributaries of the Guandu River, were sampled during 2010 and 2011. A total of 102 specimens of *A. multispinis* were collected and analyzed. Epibionts were found in fourteen of fifteen body regions of the host. Observation from scanning electron microscopy revealed that Chironomidae larvae fix themselves to the spicules through the anal prolegs, not at the skin, as previously reported. The amount of epibionts (degree of infestation) was significantly correlated with fish size in the Santana Stream ($r = 0.6$, $p < 0.01$), and São Pedro Stream ($r = 0.56$, $p < 0.01$), but not in the D'Ouro Stream, the most altered of the three. The presence of epibionts on the body of the fish is directly correlated with the availability of spicules on the fish's body, the largest numbers of infestations being found in structures associated with swimming (caudal and pectoral fins), since the swimming movement can create favorable conditions (e.g., suspension of organic particles, increasing oxygenation) for the epibiont.

KEY WORDS. Commensalism, larval dispersion, midges, stream ecology.

Ichthyocladius (Diptera: Chironomidae: Orthocladiinae) was described by Fittkau (1974). There are records of species from Peru and Ecuador. The larval phase of this Diptera develops on organisms of different orders such as Ephemeroptera, Plecoptera, Megaloptera and siluriform fish (Roque et al. 2004). Studies on the association between Chironomidae and aquatic organisms revealed complex interaction patterns that vary from commensalism, such as phoresy, to parasitism (Steffan 1967, Jacobsen 1995, Tokeshi 1995, Ashe and O'Connor 2002, Roque et al. 2004, Sydow et al. 2008). In Brazil, studies on this subject are scarce (e.g., Roque et al. 2004, Sydow et al. 2008) and have documented the presence of *Ichthyocladius* in association with loricariid fish.

Loricariidae (Actinopterygii: Siluriformes), armored catfish, has ca. 973 recognized species (Eschmeyer et al. 2014), being the most diverse family of Neotropical freshwater fish (Roxo et al. 2012). They inhabit mainly lotic systems and have developed morphological adaptations to explore the consolidate substrate, such as a dorsoventrally flattened body covered with bone plates and a suckermouth.

The term phoresy, meaning "to carry or to transport" was first used by Lesne (1896) to describe what seemed like a temporary natural event, since the epibiont eventually detaches from its host. In this type of relationship, the organism fixes itself to the surface of the host without causing any harm to it and without forming a mutualistic or parasitic relationship (Deegener 1917 apud Houck and Oconnor 1991). Farish and Axtell (1971) addressed these issues, and contributed to standardize the definition of phoresy to include any "phenomenon in which one animal actively seeks out and attaches to the outer surface of another animal for a limited time during which the attached animal (termed the phoretic) disperses from areas unsuited for further development, either of the individual or its progeny". Overall, such associations have remained unexplored in the greatly diverse and complex Neotropical streams, particularly in the Atlantic Forest (Myers et al. 2000, Miranda 2012). The latter is of particular interest due to its high rates of endemism, and also the impact of anthropogenic activities on its streams (Myers et al. 2000).

The Guandu River is the main water supplier for the metropolitan area of the state of Rio de Janeiro. This river has three 4-order tributaries in different states of conservation, and which drain at the Biological Tinguá Reserve. The São Pedro Stream has the best water quality, followed by the Santana Stream, whereas the D'Ouro Stream is the most altered due to anthropogenic activities at its margins (Vettorazzi et al. 2012). The fish species investigated was the armored catfish *Ancistrus multispinis* (Regan, 1912) (Fig. 1), a siluriform species classified in the family Loricariidae, endemic to South America. It feeds mainly on algae (Winemiller and Jepsen 1998, Geerinckx et al. 2008).

Here, we describe the phoretic relationship between *A. multispinis* and the larvae of *Ichthyocladius* sp. at the three tributaries of the Guandu River in Southeastern Brazil. Specifically, our objectives were: (1) to analyze the site of attachment and the spatial distribution of epibionts on the body regions of the host; (2) to determine whether there is a relationship between host body size and the amount of epibiont organisms on it; and (3) to compare the occurrence and intensity of phoresy among the three different aquatic systems. We expected that the distribution of epibionts is clumped in certain, more suitable areas of the host's body, and that the largest individuals have more epibionts. Moreover, we expected that the streams with better water quality would have more cases of phoresy.

Sampling was carried out in the three streams (Santana, São Pedro and D'ouro) during the wet (February 2010 and January 2011) and dry (June 2010 and July 2011) seasons. Two regions were sampled for each stream, one at the middle-upper and the other at the middle-lower reaches. In total, 24 sampling were performed (2 seasons × 2 years × 3 streams × 2 sites). Electrofishing was performed (3.000 W, 220 v generator) on a 90 m stretch of each stream for one hour. Four people carried out the fishing procedures, two carrying the electrodes and the other two collecting the fish. The effort was standardized to compare the occurrence of phoresy among the fixed factors (body regions and streams).

All collected fish were fixed in 10% formalin and after 48 hours they were transferred to 70% ethanol. Vouchers specimens were deposited at the Ichythyological Collection of the Laboratory of Fish Ecology, Universidade Federal Rural do Rio de Janeiro, under numbers LEP-UFRRJ #1917, 1918 and 1919.

Figure 1. *Ancistrus multispinis* (95.8 mm standard length and 9.4 g weight) in three views: lateral, upper and ventral.

Epibiont larvae were identified and counted under a binocular Coleman stereomicroscopic (40×). The epibionts were counted on the following body regions of the host: 1) pectoral fins (right and left) and lower and upper part; 2) dorsal fins (right and left); 3) pelvic fins (right and left); 4) caudal fin; 5) adipose fin; 6) anal fin; 7) opercula; 8) odonthoids (right and left); and 9) body (all body surface excluding those previously referred in the items 1–8).

Each fish was weighted (g) and its total length (mm) was measured with a digital caliper. The association between each fish and the number of epibionts on it was assessed using the non-parametric Spearman correlation ($\alpha = 0.01$). Prevalence (frequency of occurrence), intensity (% numerical of the number of epibiont per body region), and the percent number in relation to the total number of epibionts were calculated for each body region. The total number of epibionts in each body region was compared among the streams using a one-way Analysis of Variance ($\alpha = 0.01$).

A 1-cm^2 sample, containing epibionts, was analyzed under scanning electronic microscopy (Hitachi TM-1000) to enable observation of the details of the epibiont's body and the way these organisms attach to the fish. A total of 102 specimens were examined, 57 from the Santana Stream, 25 from São Pedro

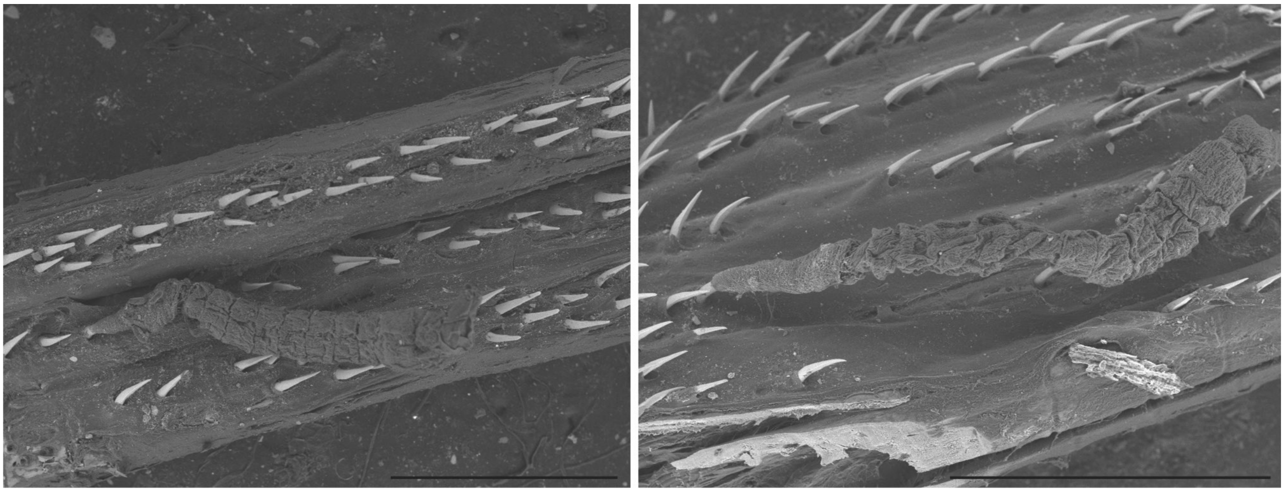

Figure 2. Larvae of *Ichthyocladius* sp. attached to a distal part of the abdomen (proleg) to a spicule on the pectoral fin of *Ancistrus multispinis*. Scale bars: 1 mm.

Stream and 20 from D'Ouro Stream. In the latter, there was only one occurrence of the epibiont *Ichthyocladius* sp. (one larvae attached to the adipose fin and another to the left odontoid) on a single specimen of *A. multispinis* (Total length = 69 mm and weight = 7.4 g). For this reason, this stream was not considered in further analyses.

We observed that the occurrence and attachment of *Ichthyocladius* sp. on the body surface of *A. multispinis* is not random. It happens more often on the spicules, which are structures formed by calcium carbonate and occur over the body and fins of the fish (Fig. 2). Such spicules are more abundant on bone plates and fin soft rays. Epibiont attachment can also occur on the odontoids, another calcified structure of the fish's body. The attachment process occurs through the adherence of the distal part of the epibiont's abdomen (prolegs) to the extremity of the spicules, forming a structure similar to a cocoon, with a single individual adhered at each spicule (Fig. 2). The epibiont head remains free on the other extremity and is free to search for food and to ingest it.

Phoresy by *Ichthyocladius* sp. was found in 72.42% of the 102 fish specimens analyzed. Its frequency was higher in the São Pedro Stream (100%), followed by the Santana Stream (78.95%), whereas only one specimen was positive for phoresy in the D'Ouro Stream (Table 1).

Among the 15 fish body regions examined, only the anal fin was not colonized by epibionts. The highest frequencies of epibionts were found on the caudal fin (47.4 and 72.0%), and at the upper part of the left (47.4 and 52.0%), and the right (47.4 e 56.0%) sides of the pectoral fin in fish from Santana and São Pedro streams, respectively (Table 2).

This study is the first report of a phoretic association between Chironomidae larvae and a fish species in the Guandu River basin. This expands the previous known distribution of this association northward. The studied species (*A. multispinis*) is common in several streams from the Atlantic forest, and their phoresy with *Ichthyocladius* sp. was previously reported only for the Southern Brazil, state of Rio Grande do Sul (Sydow et al. 2008) and in Southeastern Brazil in the state of São Paulo (Sazima et al. 2001). Nessimian et al. (2003) found *Ichthyocladius* in Preto River, Rio de Janeiro, associated with two fish species, the Trichomycteridae *Trichomycterus mirissumba* Costa, 1992 and the Loricariidae *Pareiorhinaru dolphi* (Gosline, 1947). In other studies, a phoretic relationship was described for *Ichthyocladius* and other fish species, such as *Kronichtys* spp., *Harttia* spp., *Hypostomus* cf. *garmani* (Regan, 1904) (Mendes et al., in press apud Roque et al. 2004), *Ancistrus brevipinnis* (Regan, 1904), *Ancistrus bufonius* (Valenciennes, 1840), *Ancistrus triradiatus* Eigenmann, 1918, *Ancistrus cirrhosis* (Valenciennes, 1836), *Plecostomus strigatceps* (Regan, 1908), and *Xenocara gymnorhynchu* Kner, 1854 (Freihofer and Neil 1967, Fittkau 1974).

Table 1. Number of examined fish, size range and occurrences of epibiont in the three streams from Atlantic forest in Southeastern Brazil. (N) Number of fishes, (FO) frequency of occurrence, (TL) total length (in mm).

Streams	N	TL Mean (range)	FO(%)	Total number of epibionts	Total number of infested individuals	Mean epibiont/ fish
Santana	57	63.6 (24.4–112.6)	78.95	325	45	7.2
São Pedro	25	80.1 (40.2–112.2)	100	331	25	13.2
D'ouro	20	78.6 (28.6–101.4)	5	2	1	2.0

Table 2. Number and mean intensity (+SD) of epibiont, frequency of occurrence in the fish body region of *A. multispinis* in the Santana and São Pedro streams. (N) Number of fishes, (FO) frequency of occurrence. The highest intensity and occurrence in bold.

	Santana Stream			São Pedro Stream		
Fishbodyregion	Number and Mean Intensity of epibionts (+SD)	N%	FO	Number and Mean Intensity of epibionts (+SD)	N%	FO
Operculae	6 (1.0 ± 0)	1.85	10.5	1 (1.0 ± 0)	0.30	4
Pectoral fin						
Upper left	**51 (1.9 ± 1.2)**	**15.7**	**47.4**	**33 (2.5 ± 1.0)**	**9.97**	**52**
Lower left	7 (1.0 ± 0)	2.15	12.3	23 (1.7 ± 1.1)	6.95	52
Upper right	**54 (2.0 ± 1.3)**	**16.6**	**47.4**	**32 (2.2 ± 0.9)**	**9.67**	**56**
Lower right	10 (1.0 ± 0)	3.08	17.5	25 (1.3 ± 0.6)	7.55	72
Pelvic fin						
Left	7 (2.3 ± 1.5)	2.15	5.3	4 (1.3 ± 0.5)	1.21	12
Right	6 (1.5 ± 1.0)	1.85	7.0	4 (1.0 ± 0)	1.21	16
Dorsal fin						
Left part	16 (1.1 ± 0.5)	4.92	24.6	10 (1.25 ± 0.7)	3.02	32
Right part	19 (1.4 ± 0.49)	5.85	24.6	6 (1.5 ± 1.0)	1.81	16
Caudal fin	**73 (2.7 ± 2.3)**	**22.5**	**47.4**	**122 (6.7 ± 9.9)**	**36.86**	**72**
Adipose fin	22 (1.2 ± 0.5)	6.77	31.6	18 (1.5 ± 0.6)	5.44	48
Anal fin	–	–	–	–	–	–
Left odonthoid	16 (1.2 ± 0.5)	4.92	22.8	3 (1.0 ± 0)	0.91	12
Right odonthoid	17 (1.2 ± 0.4)	5.23	24.6	6 (1.2 ± 0.4)	1.81	20
Body	21 (1.6 ± 0.6)	6.46	22.8	44 (2.9 ± 2.8)	13.29	60

The presence of spicules seems to be a preponderant factor for larval attachment, since we observed, through the SEM images, that in all cases the epibionts were attached to these calcified structures present in some fins and bony plates. This contrasts with the observations of Fittkau (1974) and Mendes et al. (2004), who reported that the larva attaches to the skin of the fish using its anal prolegs. In our study, the lack of spicules explains the absence of larvae on the anal fin and the ventral part of the fish's body.

Similarly to Sydow et al. (2008), we found that the number of epibionts (prevalence and intensity of infestation) had a significant correlation with fish size and the way that the epibiont colonizes specific parts of the host body. In the study of Sydow et al. (2008), for all three loricariid species a significant and positive correlation was found between the number of epibionts and body size. This is consistent with our findings for two studied streams, the Santana ($r = 0.6$, $p < 0.01$) and the São Pedro ($r = 0.56$, $p < 0.01$). In relation to epibiont spatial distribution throughout the host's body, the caudal fin, followed by the pectoral fin, were the body structure that had the highest prevalence of epibionts. Also, we found a significant increase in the number of epibionts colonizing the lower part of the pectoral fins compared with the upper part ($F = 13.68$, $p < 0.0001$). Moreover, both in our study and in the data of Sydow et al. (2008), no epibionts were found in the anal fin.

Water quality seems to be a relevant factor for the foretic relationship. In our data, 100% of individuals of *A. multispinis* had epibionts on them at the best preserved stream (São Pedro), whereas in the Santana Stream, where the water quality was intermediate, only 78.9% did. Sydow et al. (2008) also found that 100% of *A.* cf. *multispina* individuals sampled in four lotic systems with preserved riparian cover, clean water, rocky substrate and riffle-pool mesohabitats, had epibionts, (Vilella et al. 2004). Consistent with these findings, we conclude that, in the results of our study, suitable water quality determined the occurrence of both, *Ichthyocladius* sp. and *A. multispinis*, which preferably inhabit the main channel of streams, taking advantage of the constant water flow that brings organic matter and detritus (Tokeshi 1993, Sydow et al. 2008). In such conditions, there is plenty of dissolved oxygen available, and shelters that help to conceal the epibionts from predators (Tokeshi 1993, Sydow et al. 2008). The occurrence of only one record of phoresy in the D'ouro Stream is likely to be attributed to the high state of degradation from pollutant discharges, poor riparian cover, habitat degradation and sedimentation (Vettorazzi et al. 2012). Riparian degradation, which increases sedimentation and warming of the water, also contribute to stream degradation (Osborne and Kovacic 1993, Casatti et al. 2006). These changes have deleterious effects on the structure of the Chironomidae community, influencing composition and density (Pinder 1986, Rossaro 1991, Schmid 1992, Sanseverino and Nessimian 1998, Rosin et al. 2009, Rosa et al. 2011).

Being carried away by the host widens the distribution area of the epibiont and its capacity to explore and colonize other microhabitats during the larval phase, increases its protection against environmental disturbance and provides opportunities to search for and to obtain food (Roque et al. 2004, Sydow et al. 2008, Henriques-Oliveira and Nessimian 2009). The interaction between the epibiont and its host can be 'neutral'. This happens when both organisms share a given habitat, have close physical contact, but their effect on one another is neglectable. Conversely, it can be highly beneficial for the epibiont. This study clarifies the foretic relationship between fish and chironomids in Neotropical streams of Southeastern Brazil. Further studies involving the anatomic, physiological and behavioral characteristic of the two species involved should be performed to obtain a more comprehensive understanding of the relationship between them.

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