

Ichthyocladius (Diptera, Chironomidae) on loricariid fishes in Atlantic Forest streams: influence of host size and corporal region on larval attachment

Ichthyocladius (Diptera, Chironomidae) em peixes loricariídeos em riachos da Mata Atlântica: influência do tamanho e da região corporal do hospedeiro na fixação da larva

Sydow, VG.¹, Vilella, FS.¹, Hartz, SM.¹ and Rodrigues, GG.^{1,2}

¹Programa de Pós-Graduação em Ecologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul – UFRGS, Av. Bento Gonçalves, 9.500, Prédio 43422, CEP 91540-000, Porto Alegre, RS, Brazil e-mail: gilberto.rodrigues@ufrgs.br

²Departamento de Botânica, Instituto de Biologia, Universidade Federal da Bahia – UFBA, Campus Universitário de Ondina, Rua Barão de Jeremoabo, s/n, CEP 40170-115, Salvador, BA, Brazil e-mail: biol.gilbertorodrigues@gmail.com

Abstract: Specimens of *Pareiorhaphis hypselurus* (n = 58), *P. nudulus* (n = 21) and *Ancistrus* cff. *multispina* (n = 19) (Pisces: Loricariidae) obtained from field collections in the Maquiné River (Brazil) between 1996 and 2004 were processed in laboratory aiming to verify the presence of *Ichthyocladius lilianae*. We investigated the relationship between fish size and the number of epibionts and also analyzed the occurrence of predominant regions for larva attachment on the fish body. Frequency of attachment was 75.9% on *P. hypselurus*, 42.9% on *P. nudulus* and 100% on *Ancistrus* cff. *multispina*. Spearman correlation between fish size and the number of epibionts was significant for *P. hypselurus* (r = 0.544; P < 0.0001) and *Ancistrus* cff. *multispina* (r = 0.557; P = 0.0133) but not for *P. nudulus* (r = -0.363; P = 0.1060). Fins were the preferential sites for attachment, with the following mean number of epibionts: 2.9 ± 2.96 for pectoral, 1.2 ± 2.16 for caudal; 1.0 ± 1.65 for pelvic and 0.6 ± 1.28 for dorsal. This may be associated to fish behavior as body movement creates favorable conditions (e.g., high oxygenation) for *I. lilianae*.

Keywords: aquatic insects, commensalism, epibiont, stream ecology.

Resumo: Espécimes de *Pareiorhaphis hypselurus* (n = 58), *P. nudulus* (n = 21) e *Ancistrus* cff. *multispina* (n = 19) (Pisces: Loricariidae) coletados no Rio Maquiné (Brasil) entre 1996 e 2004 foram processados em laboratório com o objetivo de verificar a presença de *Ichthyocladius lilianae*. Investigamos a relação entre o tamanho do peixe e o número de epibiontes e analisamos preferência para fixação da larva no corpo do peixe. A frequência de *I. lilianae* foi de 75,9% em *P. hypselurus*, 42,9% em *P. nudulus* e 100% em *Ancistrus* cff. *multispina*. A correlação de Spearman entre tamanho do peixe e número de simbiosites foi significativa para *H. hypselurus* (r = 0,544; P < 0,0001) e *Ancistrus* cff. *multispina* (r = 0,557; P = 0,0133), mas não para *H. nudulus* (r = -0,363; P = 0,1060). As nadadeiras foram as regiões preferenciais para fixação, com os seguintes números de epibiontes: 2,9 ± 2,96 na peitoral; 1,2 ± 2,16 na caudal, 1,0 ± 1,65 na pélvica e 0,6 ± 1,28 indivíduos na dorsal. Isto pode estar associado ao comportamento do peixe, uma vez que o movimento corporal cria condições favoráveis (ex., alta oxigenação) para *I. lilianae*.

Palavras-chave: insetos aquáticos, comensalismo, epibionte, ecologia de riachos.

1. Introduction

Studies on the associations between Chironomidae and aquatic organisms, including vertebrates, have revealed complex patterns of interactions ranging from commensalism and phoresis to parasitism (Steffan, 1967; White et al., 1980; Tokeshi, 1993; Jacobsen, 1995; Tokeshi, 1995; Ashe and O'Connor, 2002; Roque et al., 2004). The interaction between Chironomidae and fishes of the families Astroblepidae and Loricariidae was first described by Freihofer and Neil (1967). However, the multifaceted complexity of Chironomid-fish association has been poorly investigated in the Neotropical region.

The genus *Ichthyocladius* (Diptera: Chironomidae: Orthoclaadiinae) was described in South America by Fittkau in 1974. The larva attaches to the fish skin by its anal prolegs, and by the end of the fourth instar, it builds up a pupal case which is attached to the fin or to the interopercular bristle where it develops (Fittkau, 1974). The presence of *Ichthyocladius* on fishes of the family Loricariidae in Southern Brazil has been little documented (Vilella, 2002; Roque et al., 2004).

In 2002, an association between *Ichthyocladius* species and Loricariidae fishes of the genus *Pareiorhaphis* Miranda

Ribeiro, 1918 and *Ancistrus* (Kner, 1854) was verified for the first time in the Maquiné River basin, Southern Brazil (Vilella, 2002). Recently, Mendes et al. (2004) reviewed the genus *Ichthyocladius* Fitkka and described two new species of *Ichthyocladius* as member of the *Corynoneura*-group (Diptera: Chironomidae: Orthoclaadiinae), including *Ichthyocladius lilianae* (Mendes et al., 2004). This species was found in the State of Rio de Janeiro (Iguassu River) and São Paulo (Mogi-Guassu River) (Mendes et al., 2004).

The nature of the fish-invertebrate association – whether commensalism or parasitism – is a reflex of anatomical, physiological, behavioral and ecological features of both organisms involved (Freihofer and Neil, 1967). Thus, it is important to understand how the invertebrate occurrence on fishes can be influenced by host parameters (e.g., size, part of the body) and environmental characteristics. This study aimed to investigate the occurrence of *I. lilianae* on loricariids in a tropical basin addressing the following questions: 1) does *I. lilianae* occur on three loricariid species from Southern Brazil? 2) is there a pattern for the spatial distribution of the chironomid larvae along the fish body? and 3) is there a relationship between fish size and the quantity of chironomids associated?

2. Material and Methods

2.1. Study site

Fishes were collected on Garapiá, Carvão and Forqueta streams, on the Maquiné River basin, in September and October/1996, November/1999, February, April and November/2000, March and October/2001 and October/2004. Maquiné River (29° 30' S and 49° 50' W) belongs to the Maquiné River basin (Figure 1). Its headwaters are located on the hills of Serra Geral (Rio Grande do Sul State, Brazil) and its mouth is located in Lagoa dos Quadros, on the coastal plain. From its headwaters to its

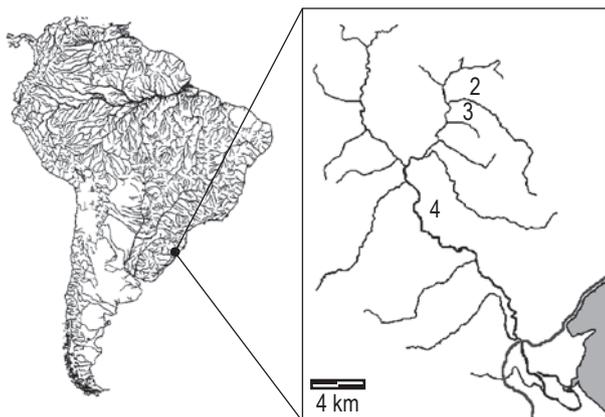


Figure 1. Hydrological map of South America (left), and map of Maquiné River basin, Rio Grande do Sul State, Brazil (right) 1: Garapiá Creek; 2 Forqueta Creek; 3: Carvão Creek; 4: Maquiné River.

downstream mouth, Maquiné River passes through different phytophysiognomies of Coastal Rain Forest: on the hill tops, there is *Araucaria* [*Araucaria angustifolia* (Bert.) Kuntze] Forest and grasslands; the catchment's middle section is surrounded by Coastal Rain Forest (*Mata Atlântica strictu sensu*) and on the mouth of the river there is coastal plain vegetation. Due to its great environmental heterogeneity and the historical aspects of its occupation this basin presents a great richness of fish species, many of which have not yet been described (Becker et al., 2004).

Studied sections are related to streams from first to third order, according to Strahler classification (1963); characterized by the presence of riparian vegetation, clean water, rocky substrate and riffle-pool changes. Limnological parameters of the sites are described elsewhere (Becker, 2002; Vilella, 2002; Vilella et al., 2004).

2.2. Fauna

Pareiorhaphis hypselurus Pereira and Reis, 2002, *P. nudulus* Reis and Pereira, 1999 and *Ancistrus* cff. *multispina* samples were fixed on 10% formaldehyde and then transferred to 70% ethanol. To assess for the spatial occupation of *I. lilianae* over the fish body, this was divided into 17 sections, including body, operculum, fins, and odontodes. The body sections were analyzed and named after its location and characteristics (twofold fins were divided in right and left, upside and downside site, and so on).

Fish total length (FTL) was measured and the number of total chironomids on each site, as well as its developmental stage was registered. Specimens of *I. lilianae* removed from the fish body were preserved on ethanol 70%. The relation between FTL and number of attaching larvae was analyzed by Spearman correlation test ($\alpha = 0.05$).

3. Results

Association between *I. lilianae* larvae and *P. hypselurus*, *P. nudulus* and *Ancistrus* cff. *multispina* was verified in the three streams (Garapiá Creek, Forqueta Creek and Carvão Creek) and in the Maquiné River from the Maquiné hydrographic basin (Figure 1).

A total of 98 fish individuals were observed of which an average 73.5% were positive for *I. lilianae* larvae presence (Table 1). Frequency of occurrence was generally high among the three species, reaching 100% for *Ancistrus* cff. *multispina*. The amount of chironomid larvae was variable among the individuals and also among species, as the mean number of larvae per fish ranged from 2.0 for *P. nudulus* to 12.21 for *Ancistrus* cff. *multispina* (Table 1).

The number of midges attached to the specimens was significantly correlated to fish body length for *P. hypselurus* ($r = 0.5438$; $P < 0.0001$) and for *Ancistrus* cff. *multispina* ($r = 0.5568$; $P = 0.0133$), but not for *P. nudulus* ($r = -0.3628$; $P = 0.1060$).

All of the 17 fish body parts were occupied by *I. lilianae*. There was great variation on the midge number for each site (Table 2). Because there was no difference in the spatial pattern of occupation among the three fish species, the mean numbers of chironomids at each body part were combined. The data showed that pectoral fins (2.94 ± 2.96 organisms), caudal fin (1.21 ± 2.16 organisms), pelvic fin (1.03 ± 1.65 organisms) and dorsal fin (0.65 ± 1.28 organisms) were the sites with the highest numbers of *I. lilianae*.

4. Discussion

Commensalism between chironomids attached to loricariid species has been registered by Freihofer and Neil (1967) for the Amazonian basin. We report here a previously unknown chironomid-fish association for the Subtropical region of Brazil, involving three species commonly found in Brazilian catchments. The high prevalence of chironomids, especially among *Ancistrus* cff. *multispina* and *P. hypselurus*, suggests that this intimate association may be a common feature in the wild.

This study also shows that despite great variation on larva numbers for each host, chironomid density depends on the size for *P. hypselurus* and *Ancistrus* cff. *multispina*, but not for *P. nudulus*. This is not surprising given that *P. nudulus* is a smaller species when compared to the others (for comparison, its mean length is nearly a third of *Ancistrus* cff. *multispina*). Size is one of the most important organism traits and it constrains many life-history and ecological traits of a species (e.g., metabolic rate, generation time, reproductive rate, predators, and perception of heterogeneity) (Peters, 1983). So, the high number of chironomid in larger fishes may be a simple relationship of density-area, as bigger organisms can support more hosts. However, it cannot be neglected the fact that this phenomenon may be related to other factors, such as fish behavior. Larger individuals tend to dislocate more water while feeding and this by its turn, tend to increase the oxygenation and release the food source for this chironomid species. A preliminary histophysiological study has revealed that diatomaceous algae (Baccilariophyta: Pennatibaccilariophyceae) are among

Table 1. Frequency (%) of *Ichthyocladius lilianae* (Chironomidae, Diptera) and mean number of midges at different development stages on loricariid fishes (*Pareiorhaphis hypselurus*, *P. nudulus* and *Ancistrus* cff. *multispina*) collected in the Maquiné River Basin, Brazil, between 1996 and 2004.

Species	Fish (N)	Mean fish total length (cm) ± SD	Frequency (%) of midge occurrence	Mean no. of larvae/fish ± SD	Mean no. of larvae with pupae case ± SD	Mean no. of pupae/fish ± SD	Mean no. of empty pupa case/fish ± SD
<i>P. hypselurus</i>	58	6.25 ± 1.47	75.9	2.57 ± 2.56	0.29 ± 0.96	0.55 ± 0.29	0.10 ± 0.36
<i>P. nudulus</i>	21	3.76 ± 0.71	42.9	2.00 ± 3.29	0.33 ± 1.11	0.05 ± 0.22	0.14 ± 0.36
<i>A. cff. multispina</i>	19	10.42 ± 1.84	100	12.21 ± 5.85	2.21 ± 2.28	0.11 ± 0.46	1.63 ± 2.09

Table 2. Total number of *Ichthyocladius lilianae* (N = 535) attached to loricariids according to their body parts and mean number of midges per fish (*Pareiorhaphis hypselurus*, *P. nudulus* and *Ancistrus* cff. *multispina*).

Fish Body region	<i>P. hypselurus</i> (N = 44)		<i>P. nudulus</i> (N = 9)		<i>A. cff. multispina</i> (N = 19)	
	Total of midges	Mean no. of midges/fish ± s.d.	Total of midges	Mean no. of midges/fish ± s.d.	Total of midges	Mean no. of midges/fish ± s.d.
Body	14	3.50 ± 0.82	7	2.33 ± 1.16	15	1.67 ± 1.00
Operculum	6	2.00 ± 0.45	-	-	6	3.00 ± 1.40
Pectoral fin left superior	28	2.67 ± 0.60	7	1.40 ± 0.89	44	2.75 ± 1.57
Pectoral fin right superior	32	2.46 ± 0.54	5	1.67 ± 0.58	43	2.87 ± 1.51
Pectoral fin left inferior	10	2.22 ± 0.46	6	1.20 ± 0.45	14	2.33 ± 2.42
Pectoral fin right inferior	8	2.00 ± 0.38	7	1.00 ± 0	8	1.14 ± 0.38
Pelvic fin left superior	9	3.00 ± 1.10	2	1.00 ± 0	5	1.25 ± 0.50
Pelvic fin right superior	11	2.44 ± 0.52	2	1.00 ± 0	13	1.44 ± 0.73
Pelvic fin left inferior	3	1.50 ± 0	1	-	15	1.50 ± 0.71
Pelvic fin right inferior	-	-	1	-	12	1.71 ± 1.11
Dorsal fin	21	3.82 ± 0.99	1	-	25	2.27 ± 1.90
Caudal fin	14	2.33 ± 0.47	3	1.00 ± 0	70	4.38 ± 2.66
Anal fin	-	-	1	-	-	-
Adipose fin	13	2.00 ± 0.29	-	-	17	1.55 ± 0.52
Lip	-	-	8	1.60 ± 0.55	4	1.33 ± 0.58
Odontodes left	5	2.50 ± 0.58	2	1.00 ± 0	8	1.14 ± 0.38
Odontodes right	14	3.50 ± 0.82	7	2.33 ± 1.16	8	1.14 ± 0.38

the main food sources for *I. lilianae* (unpublished data). Diatomaceous algae stand out among the periphyton-based species in streams in terms of their richness and abundance (Allan, 2001) and comprise a considerable part of the epilithon in lotic systems (Schneck et al., 2007).

Among the fish body parts where *I. lilianae* was found, fins (pelvic, pectoral, caudal, dorsal) seem to concentrate higher numbers of chironomids. This supposed preference may be associated to the movements performed by pectoral fins that contribute to creating a favorable microhabitat for midge attachment. Besides, bilateral symmetry on larvae localization was observed for *I. lilianae* larvae, larvae plus initial pupae case and pupae case on all different attaching sites on fish's body.

Loricariids are found attached on stones which feed on epilithic diatoms (Vilella et al., 2004). Since *I. lilianae* feeds preferably on diatomaceous algae and loricariids move quickly in lotic ecosystems, fixing themselves to small and medium size rocks where they feed on periphytic biofilm by grazing rocks surface, the suspending biofilm may be helpful for *I. lilianae* feeding. Besides, other advantages of being attached to the fish body consist in moving easily with less energy loss, to obtain a more favorable habitat condition (well oxygenated and properly illuminated to its occurrence) and being protected from disturbances of the lotic ecosystem and against predation risk (Tokeshi, 1993).

Inter-specific interaction such as the one involving *Ichthyocladius lilianae* and loricariid fishes triggers important questions about their co-evolution and are far from being elucidated. For example, it is possible that the chironomids are mere phoretic organisms that cause no harm to the host. Arnal et al. (2001) assessed the relative importance of client ectoparasite load and mucus characteristics for the behavior of cleaning fishes, and their fish clients. Although it is frequently assumed that the chironomid larvae are benefited by decreasing predation risk, enhanced mobility and increase in the available food (Saffo, 1992; Tokeshi, 1993; Roque et al., 2004), a "neutral" interaction can take place, in which both organisms share the environment in close physical contact with minimal effect on each other. Further studies should address these issues in order to assess the real nature of this relationship.

Due to its importance in supporting several endemic species and to its localization as the southernmost limit of Costal Rain Forest, the Maquiné River Basin should rank among the priorities for conservation, especially considering the high habitat loss and severe anthropic pressure it suffers. More thorough knowledge on its biotic components and their interactions is imperative in order to allow for a more accurate evaluation of its real conservation state.

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