Phoresy and commensalism of Chironomidae larvae (Insecta: Diptera) in the state of Rio de Janeiro, Brazil

Ana Lucia Henriques-Oliveira^{1,2} & Jorge Luiz Nessimian¹

Lab. de Entomologia, Departamento de Zoologia, Instituto de Biologia, CCS, Universidade Federal do Rio de Janeiro, Ilha do Fundão, CP 68044, CEP 21944-970 Rio de Janeiro, RJ. 2 – anahenri@biologia.ufrj.br

Abstract:

Chironomid larvae are frequently found living in phoretic or commensal association with aquatic animals in all regions of the world. In Brazil, new records have been available in the literature in the last years mainly for the state of São Paulo. In collects performed in several streams and rivers in the state of Rio de Janeiro and bordering areas, chironomid larvae were found living in association with Corydalidae (Megaloptera); Perlidae (Plecoptera); Leptophlebiidae (Ephemeroptera); Aeshnidae and Libellulidae (Odonata); Sericostomatidae (Trichoptera); Elmidae (Coleoptera) and catfishes of the families Trichomicteridae and Loricariidae (Pisces). These new records are presented in the present study.

Keywords: Chironomidae, Nanocladius (Plecopteracoluthus), Phoresy, Rheotanytarsus, symbiotic associations.

Introduction

Associations between two organisms may range from simple phoresy to commensalism and parasitism (Giberson et al., 1996). Phoresy is the interrelationship between two organisms in which one is carried on the body of the other, does not necessarily implying interdependency between both (Epler & De La Rosa, 1995). In parasitism, one observes an apparent benefit to the parasite in form of resources being diverted from the host body, while in commensalism the nature of the benefit to a commensal species is less clear. However, there is a logical difficulty in assuming that commensals receive no significant benefit from association with the host (Tokeshi, 1993).

Differently from other groups of aquatics insects, phoretic representatives of Chironomidae and Simuliidae (Diptera) might be widely distributed. Phoretic simuliid larvae are known from Central Asia and Tropical Africa (Moor, 1999). Chironomid larvae frequently live in association with aquatic animals in several parts of the world. Chironomid associations were recorded mainly in Europe, Asia, and Central and North America, with larvae living on Ephemeroptera (e.g. Svensson, 1980; Jacobsen, 1998), Plecoptera (e.g. Steffan, 1965; Dosdall & Mason, 1981; Giberson et al., 1996), Odonata (e.g. White & Fox, 1979; White et al., 1980, Dosdall & Parker, 1998), Hemiptera (e.g. Roback, 1977), Megaloptera (e.g. Tracy & Hazelwood, 1983; De La Rosa, 1992; Epler & De La Rosa, 1995; Hayashi, 1998; Pennuto et al, 2002), Trichoptera (e.g. Gallepp, 1974; Kobayashi et al., 2003) and Gastropoda (e.g. Mancini, 1979).

Received: 25-VI-08 Accepted: 10-I-09 Distributed: 13-IX-11

In Brazil, although still limited, new records have been published in the last years: Ichthyocladius sp. (Orthocladiinae) on fishes of the families Astroblepidae and Loricariidae (Freehofer & Neil, 1967; Fittkau, 1974; Mendes et al., 2004, 2007; Sydow et al., 2008); Nanocladius (Plecopteracoluthus) sp. on Plecoptera (Dorvillé et al., 2000) and Ephemeroptera (Callisto & Goulart, 2000; Pepinelli et al., 2009); Thienemanniella on Megaloptera (Callisto et al., 2006); Rheotanytarsus on Odonata (Ferreira-Peruquette & Trivinho-Strixino, 2003; Rosa et al., 2009), and Coleoptera (Segura et al., 2007). Roque et al. (2004) listed new occurrences of phoretic Chironomidae-larvae living on different aquatic animals, mainly for the state of São Paulo. In the state of Rio de Janeiro, only the association of Nanocladius (Plecopteracoluthus) sp. and Kempnyia tijucana Dorvillé & Froehlich, 1997 (Perlidae) was recorded (Dorvillé et al., 2000).

In collects carried out in the state of Rio de Janeiro and adjacent areas, chironomid larvae were found living on aquatic insects (Ephemeroptera, Odonata, Plecoptera, Megalotera, Coleoptera and Trichoptera) and fishes of the families Trichomicteridae and Loricariidae. Here, these phoretic and/or commensal relationships are described as a contribution to the knowledge of the associations between Chironomidae and their hosts.

Material and methods

The studied material derived from collects carried out in several rivers and streams in the state of Rio de Janeiro (RJ) and adjacent areas in the states of Minas Gerais (MG) and São Paulo (SP), between 1996 and 2006 (Table I). For identification, the Chironomidae larvae were removed from their hosts and mounted on permanent slides using Euparal® as the mounting medium. The larvae were identified under an optical microscope

(1000 X), using the keys by Cranston et al. (1983) and Epler (1995), and taxonomic descriptions by Steffan (1965), Epler (1986), Hayashi (1998) and Mendes et al. (2004). The specimens identified are deposited in the Coleção Entomológica Prof. José Alfredo Pinheiro Dutra, Departamento de Zoologia, Instituto de Biologia at the Universidade Federal do Rio de Janeiro (RJ).

Results and Discussion

The associations involving chironomid larvae presented in this study (listed in Table I) are discussed below:

Odonata: Chironomid larvae were found in association with Rhionaeschna punctata (Martín, 1908) (Aeshnidae), Elasmothemis cannacriodes (Calvert, 1906) and Brechmorhoga Kirby, 1894 (Libellulidae). Two nymphs of R. punctata (Aeshnidae) collected in an Aiuruoca River first order tributary in the Itatiaia National Park (in the states of Rio de Janeiro and Minas Gerais) contained, each one, a tube with a larva of Rheotanytarsus Thienemann et Bause, 1913 (Chironominae: Tanytarsini). The stream was approximately 50 cm wide, running adjacent to a forested area at 1500 m a.s.l. There, the dominant aquatic plants are Senecio icoglossus DC. (Compositae) and Cyperaceae. The larval tubes of Rheotanytarsus were attached to the ventral region, longitudinally to the external area from the prosternal region to the first abdominal segment (Fig. 1-A). The larvae had their heads and thoraxes oriented laterad in the tube, allowing food capture, as Rheotanytarsus larvae are primarily filterers of organic matter in suspension in the water column. Similar observations were reported by Rosa et al. (2009) in nymphs of Calopterygidae and *Heteragrion* in Minas Gerais state.

Tubes of Rheotanytarsus larvae, some of which empty, were observed on nymphs of E. cannacriodes and Brechmorhoga sp. (Libellulidae) collected in the Ubatiba river, Maricá (RJ) (potamal section) at 30 m a.s.l. This river section was about 2 m wide and its riparian vegetation was strongly modified, being characterized by pastures and some shrubs. The nymphs were collected in riffles (gravel and stones) and in pools. Rheotanytarsus tubes were attached to their hosts on the legs, near the head, and between the wingpads, suggesting the lack of a preferential attachment place. White et al. (1980) observed that the head, thorax and legs were the main regions of Macromiidae nymphs used for fixation by chironomid larvae. Ferreira-Piruquette & Trivinho-Strixino (2003) also found E. cannacrioides nymphs with Rheotanytarsus tubes attached to the same regions of the host body. These authors related this fact to the life style of E. cannacrioides, whose nymphs cling on the substrate, and which abdominal expansion-contraction movements make fixation difficult for chironomid larva. Among the hosts observed here, only nymphs of R. punctata presented chironomid tubes attached to the ventral region of their bodies.

Ephemeroptera: One association with *Thraulodes* Ulmer, 1920 (Leptophlebiidae) was found. Nymphs of *Thraulodes* are abundant in rocky-bedded streams in the Bocaina mountain range. However, among several observed nymphs, only one, collected in riffle litter in the Mambucaba River, in the Serra da Bocaina National Park, showed association with *Nanocladius* (*Plecopteracoluthus*) sp. Steffan, 1965 (Orthocladiinae) (Fig. 1-B). The association between species of these genera was only recently recorded in Brazil (Callisto & Goulart, 2000). The larva of

Nanocladius recorded here was attached to the dorsolateral area of the abdomen, above the gills of its host, with its head near the host wingpads, and was involved in a silk layer with small particles of organic matter adhered. Jacobsen (1998) found evidences of parasitism in the association of *Nanocladius* with *Thraulodes*. He observed the presence of haemolymph in the gut contents of the larvae and scars on the host tegument where the larva was attached. However, this was not observed in the present study.

Plecoptera: Phoretic associations involving species of Anacroneuria Klapálek, 1909 and Kempnyia Klapálek, 1914 (Perlidae) were recorded. Nymphs of Anacroneuria (n = 3) and Kempnyia (n = 18) were found in phoretic association with larvae of Nanocladius (Plecopteracoluthus) (Orthocladiinae). The associations were observed in nymphs of Kempnyia colossica Navás, 1934, ranging between 19 mm and 35 mm in length, and Kempnyia sp., between 10 mm and 16 mm in length. The nymphs of Kempnyia were collected in the Aiuruoca River (3rd order, 1860 m a.s.l), in a second-order tributary of this same river at the Itatiaia massif, in the Mantiqueira moutain range (2200 m a.s.l.), between the municipalities of Itamonte (MG) and Itatiaia (RJ), and in the Cascatinha River, Nova Friburgo (RJ) (3rd order ,1300 m a.s.l.). In all of these rivers the nymphs were collected in riffle areas (litter and stones). Anacroneuria nymphs 10 mm to 13 mm in length, each with a single Nanocladius (Plecopteracoluthus) larva, were collected in riffle litter in the Mambucaba River and in the Boqueirão stream, both in the Serra da Bocaina National Park (RJ and SP).

About 75% of the *Nanocladius (Plecopteracoluthus)* sp. larvae observed were close to the thoracic gills of the host nymphs, and approximately 38% of the *Kempnyia* nymphs had two or three individuals attached to them (Fig. 1-C). Two morphospecies of *N. (Plecopteracoluthus)* were observed living in association with stoneflies. *Nanocladius (Plecopteracoluthus)* sp.1 were found living all their larval life among the tracheal gills of the host, producing at the end of the fourth instar a fine silk bag among the gills for pupation. Two pupae and two prepupae were observed in these bags. One pair of small sclerotized hooks occurs in the abdominal sternites I-V of the larvae of this morphospecies. These larvae appear to hold themselves to the host tracheal gills by their posterior parapods; no bag or tube for fixation was observed in the larval stage. This morphospecies was observed on both *Anacroneuria* and *Kempnyia* nymphs.

The larvae of *N.* (*Plecopteracoluthus*) sp.2 were found only in Itatiaia, in two species of *Kempnyia*. These are big larvae and were found under the host wingpads, during the first instars. Differently from *N.* (*Plecopteracoluthus*) sp.1, this morphospecies do not possess small hooks on its abdominal segments. Larvae of the fourth instar were observed only on the ventrolateral surface of the host thorax, suggesting that during this instar, the larva migrates to the ventral region for pupation. It first adopts an inverted-J shape, thus positioning itself along the longitudinal axis of the host body (Fig. 1-D). Dorvillé et al. (2000) recorded the same habit in a species of *N.* (*Plecopteracoluthus*) on *K. tijucana*. Two individuals of *K. colossica* were also collected bearing larvae of both morphospecies of *Nanocladius* (Fig. 1-C).

Megaloptera: Associations with *Corydalus* Latreille, 1902 (Corydalidae) were observed. Phoretic or commensal associations between Chironomidae and Megaloptera (dobsonfly and fishfly) are known from Central and North America (Epler

Table 1 - New occurrences of Chironomidae larvae in phoresy in the state of Rio de Janeiro and localities in bordering states. I- Chironomidae larva of first and second instars; M –Chironomidae mature larva; P – pupa. P.N. = National Park.

Host	Ind. collected	Chironomidae	Instar	Ind./host	Host position	Locality
R. punctata	2	Rheotanytarsus	I, M	1	Tube on the ventral region between the forelegs and first abdominal segment.	Aiuruoca River tributary, Itamonte, MG
E. cannacrioides	3	Rheotanytarsus	-	1	Empty tube in abdominal dorsal region.	Ubatiba River, Maricá, RJ
Brechmorrhoga	1	Rheotanytarsus	I	1	Tube between wingpads.	
Thraulodes sp.	1	Nanocladius (P.) sp.3	M	1	On the abdominal dorsal region up the tracheal gills.	Mambucaba River, S.José do Barreiro, SP
Anacroneuria	1	Nanocladius (P.) sp.2	Ι	1	Individuals in thoracic gills filaments	Boqueirão Stream, S.José do Barreiro, SP
Anacroneuria	2	Nanocladius (P.) sp.2	I	1	In thoracic gills filaments	Mambucaba River, S.José do Barreiro, SP
K. colossica	5	Nanocladius (P.) sp.1	I	1 - 2	In thoracic gills filaments	Aiuruoca River, –Itamonte, MG
Kempnyia sp.	2	Nanocladius (P.) sp.2	M	1	Under wingpad and gills of middle legs	
K. colossica	5	Nanocladius (P.) sp.1	I, M, P	1 - 3	Thoracic gills	Aiuruoca River tributary, Itamonte, MG
		Nanocladius (P.) sp.2	I, M	1	Ventral region of the thorax, one larva in 'U' position	
Kempnyia sp.	6	Nanocladius (P.) sp.1	I, M	1 - 8	Individuals in thoracic gills filaments	Cascatinha River, Nova Friburgo, RJ
Corydalus	3	Corynoneura	I, M	2 - 4	In tracheal gills filaments in abdomen	
Corydalus	2	Corynoneura	I	1	In tracheal gills filaments in abdomen	Preto River tributary, Itatiaia, RJ
G. grumicha	4	Rheotanytarsus	Ι	1	Tube attached on caddisflies case	Sertão River, Parati, RJ
M. granosa	3	Rheotanytarsus	M, P	1	Tube attached in ventral region from prothorax to abdominal segments.	Stream in Barreirinha farm, P.N. Serra da Bocaina, S.José do Barreiro, SP
Cylloepus	3	Rheotanytarsus	M, P	1	Ventral region between prothorax and abdominal segments, and one on elytra	Barra Branca Stream, S.José do Barreiro, SP
Heterelmis	2	Rheotanytarsus	-	1	Tube attached on elytra.	Barra Branca Stream, S.José do Barreiro,, SP.
P. rudolphi	32	Ichthyocladius lilianae	I, M	1 - 4	Skin and on fins	Preto River, Maromba, Itatiaia, RJ
T. mirissumba	-	Ichthyocladius lilianae	I, M	1-3	Fins and odontoid plate in opercula	River in Visconde de Mauá, Resende, RJ

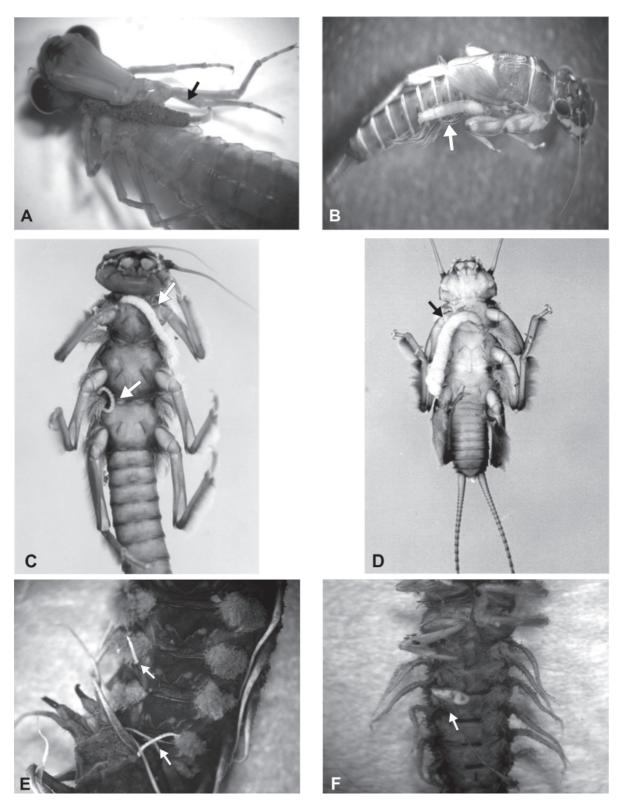


Figure 1 - A) Rhionaeschna punctata nymph with Rheotanytarsus larva attached on ventral region; B) Thraulodes sp. nymph with Nanocladius (Plecopteracoluthus) sp. larva on laterodorsal region; C) Kempnyia colossica nymph with two larvae of different morphospecies of Nanocladius (P.) on ventral region; D) Kempnyia sp. nymph with Nanocladius (P.) sp.2 larva on ventral region; E) Corydalus sp. larva with Corynoneura larvae attached to tracheal gills filaments; F) Corydalus sp. larva with last instar Corynoneura larvae inside silk cocoon.

& De La Rosa, 1995; Pennuto et al., 2002; Pennuto, 2003), Asia (Hayashi, 1998) and from the Brazilian states of São Paulo, Minas Gerais and Amazonas (Roque et al., 2004; Callisto et al., 2006). Corydalus larvae carrying Chironomidae were collected in riffles in a 2nd order tributary of the Cascatinha River, in Nova Friburgo (RJ), at 1300 m a.s.l. (three individuals) and in a tributary of the Preto River, Itatiaia (RJ), at 1600 m a.s.l. (two individuals). The Corydalus larvae were approximately 19 mm long, and carried two to four small Corynoneura (Orthocladiinae) larvae under their tracheal-gill filaments (Figs. 1-E and 1-F). These larvae probably were in the first or second developmental instars. Corynoneura larvae were observed with part of their abdomens under the abdominal gills of the host, with the thorax and head free. The associations recorded here are in accordance with the observations made by Tracy & Hazelwood (1983) that most of the chironomid larvae on corydalid larvae are found on the abdominal region, close to the host gills. Epler & De La Rosa (1995), studying associations between Corydalus and Tempisquitoneura (Corynoneurinigroup) larvae, verified that small phoretic larvae were usually found attached among the abdominal gills of the host, while larger larvae preferred the thorax and first abdominal segments. The Corydalus gills seem to be a safe site for Corynoneura development, hidden from predators (Callisto et al., 2006). These latter authors found Corydalus larvae carrying one up to 33 Corynoneura larvae attached mainly to abdominal segments, under gills. According to Tracy & Hazelwood (1983), other factor favoring this positioning could be the rhythmic movement of the gill tufts, which could provide a constant source of oxygen-rich water to the chironomid larvae.

Coleoptera: Associations were found of Tanytarsini larvae with Cylloepus Erichson, 1847, Heterelmis Sharp, 1882 and Macrelmis granosa (Grouvelle, 1896) (Elmidae). Eight adult individuals of Elmidae were collected in streams at the Bocaina Mountain Range, between 1000 m a.s.l. and 1200 m a.s.l., carrying tubes of Rheotanytarsus: Three individuals of Macrelmis granosa, three Cylloepus sp. and two Heterelmis sp. The specimens of M. granosa had Rheotanytarsus tubes attached on their ventrolateral region, between the prosternum and the abdominal segments I-IV (Fig. 2-A). One individual of Cylloepus had two tubes attached: One of them was empty on the elytra, and the other, with one larva, was attached to the ventrolateral region of the thorax with the opening near the elmid head (Fig. 2-B). A small percentage of adult individuals of Heterelmis were found carrying Tanytarsus larvae. Among 74 specimens collected in a stream, only two possessed Rheotanytarsus tubes. Each one of those specimens had an empty, "U"-shaped tube attached to the pronotum and elytra.

Roque et al. (2004) pointed out that the smooth tegument of host Coleoptera would make it difficult for phoretic chironomids to attach. This fact may be contributing to the absence of phoretic associations with specimens of other families of Coleoptera. The rugosity and punctures of the elmid pronotum, elytra and venter, associated to the occurrence of these beetles in stream riffles, may contribute to the attachment of *Rheotanytarsus* tubes. Moreover, the mobility of the elmid adults facilitates filtering by *Rheotanytarsus* larvae. Elmid adults live preferentially on rock or litter substrates in riffles, the same habitat inhabited by *Rheotanytarsus* larvae. At the moment, the Elmidae is the only known representative of Coleoptera

presenting commensal or phoretic relationships with Chironomidae.

Trichoptera: Chironomid larvae were found in association with Grumicha grumicha (Vallot, 1855) (Sericostomatidae). Five specimens of G. grumicha were found carrying, each, one larval tube of Rheotanytarsus. The larvae of G. grumicha were collected in the Rio do Sertão, a stream in the Bocaina Mountain Range, Parati (RJ), at 943 m a.s.l. (Fig. 2-D). Three tubes had larvae of Rheotanytarsus and two were empty. Rheotanytarsus tubes were attached with their openings directed towards the anterior opening of the host case. Grumicha individuals are scrapers and normally are found on stones or litter in riffles, moving on the substrate in search of food. This habit may favor the Rheotanytarsus larval mode of life, facilitating their positioning in the river current and promoting a more efficient filtration. The Chironomidae larvae could feed on the suspended biofilm resulting from the scraping of stone surface by Grumicha larvae. Ashe et al. (2000) presented a summary of chironomidtrichopteran associations (phoretic, commensal or ectoparasitic), but there were no records for the Neotropical region.

Pisces: Associations of Chironomidae with *Trichomycterus* (Trichomycteridae) and Pareiorhina (Loricariidae) were observed. Individuals of T. mirissumba Costa, 1992 (Trichomycteridae) and P. rudolphi (Ribeiro, 1911) (Loricariidae) were observed carrying larvae of Ichthyocladius lilianae Mendes, Andersen & Saether, 2004 (Orthocladiinae) (Fig. 2-E e F), in the Preto river located at 1600 m a.s.l, in Visconde de Mauá (municipality of Resende, RJ). Larvae of I. lilianae were found on T. mirissumba inside a small gelatinous case composed by silk and small particles of organic matter attached on the odontoid plate of the fish opercula. Frequently, more than one larva were found living in the same specimen of T. mirissumba. In P. rudolphi (Loricariidae), one to five larvae of I. lilianae were found living on several parts of the body of the same individual, mainly on fins. According to Fittkau (1974), chironomid larvae attach to fish skin by their anal parapods and, by the end of the fourth instar, they build up a pupal case, usually attached to the fin or to the interopercular bristle, where they complete their development. Sydow et al. (2008), studying associations between loricariid fishes and I. *lilianae* found a major preference by the later for the fin. According to them, this fact would be associated to the fin movements, which create a favorable microhabitat for the Ichthyocladius larvae, that are filterer-collectors, feeding mainly of algae. Another factor pointed out by the same authors, is that loricariid fishes move quickly in lotic ecosystems, fixing themselves to small and medium-sized rocks where they feed on the peryphitic biofilm by grazing rock surfaces. The suspending biofilm may be helpful for I. lilianae feeding. In the Neotropical region, species of Ichthyocladius were recorded in Venezuela, Guyana, Bolivia, Peru, and in the Brazilian states of Amazonas, Mato Grosso, Minas Gerais, São Paulo and Rio Grande do Sul (Fittkau, 1974; Roque et al., 2004, Sydow et al., 2008).

Obligate or casual relationships: Tokeshi (1993) argued that, in the absence of detailed ecological information, the distinction between obligate and accidental associations is often difficult to make and some relations may be trivial. The exact relationship between chironomid larvae and their hosts is

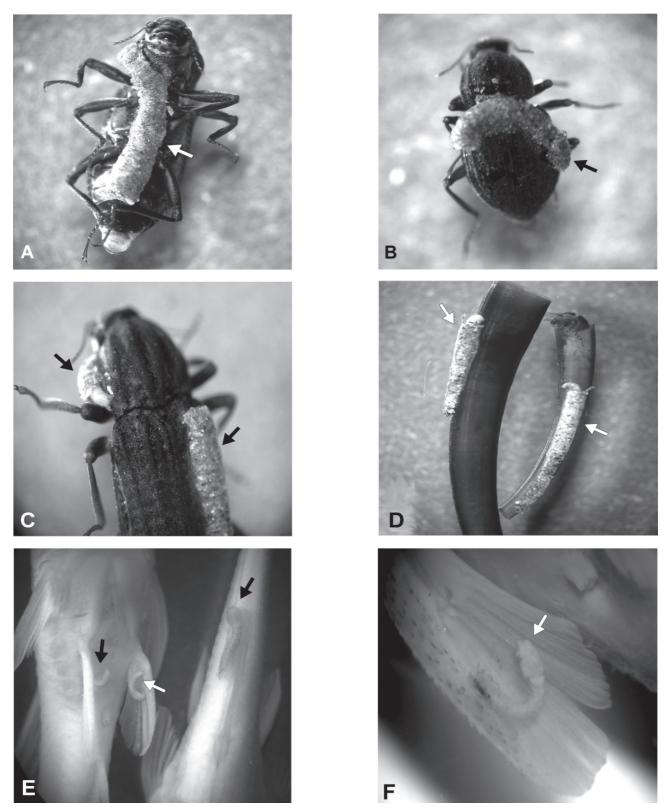


Figure 2 - A) Adult of *Macrelmis granosa* with *Rheotanytarsus* tube on ventral region; B) Adult of *Heterelmis* sp. with *Rheotanytarsus* tube on elytra; C) Adult of *Cylloepus* sp. with two *Rheotanytarsus* tubes, one on dorsal region and other on ventral region. D) *Grumicha grumicha* case with *Rheotanytarsus* tube; E & F) Individuals of *Pareiorhina rudolphi* with *Ichthyocladius lilianae* larvae on the fin and skin.

uncertain and still little known, needing specific studies. Obligate relationships, such as that between *Nanocladius* (*Plecopteracoluthus*) and their hosts, need a tight synchronization between host and commensal, since the pupation or adult emergence of the host may cause the death of its guests (Pennuto, 2003). In the literature, *N.* (*Plecopteracoluthus*) larvae are considered relatively 'generalist' commensals, which maintain associations with Plecoptera, Ephemeroptera and Megaloptera. According to Fittkau (1974), species of *Ichthyocladius* live obligatorily associated with vertebrate hosts. Until now, there are no records in the literature of *Ichthyocladius* larvae living free on other substrate.

Obligatory associations were not observed for Rheotanytarsus larvae, which can be observed living on riverbed substrates or as phoretic or commensals associated with several groups of aquatic insects. Their hosts, elmid beetles (adults and larvae) and sericostomatid caddisflies (larvae), live in riffle areas (rock or litter), have scraper habit, feed on periphyton (Merritt & Cummins, 1996) and their movements to scrape would suspend particles that could be used as food by Rheotanytarsus larvae. These larvae are frequently found living in substrates as rocks in riffle areas, in accordance with the comments of Tokeshi (1993) and Hayashi (1998). According to Tokeshi (1993), the main factor governing the host choice by species of *Rheotanytarsus* would be the availability of a large body, with an exposed surface to attach their filter-feeding tubes and also able to promote protection against predators. In some cases, as verified for nymphs of Brechmorhoga, the association can be accidental, therefore the chironomid larvae were found on individuals with adhered organic matter and little mobility. Hawking & Watson (1990) pointed out that some chironomid larvae (Rheotanytarsus) need a steady platform for support and access to a constant and abundant source of food, besides organic matter for tube construction.

The exact factors guiding host choice are unknown for the associations between Corydalus and the Corynoneura-group larvae. Hayashi (1988) pointed out that the large bodies of the Megaloptera used as hosts by chironomid larvae would decrease their risk of being preyed. Although it is not known how chironomid larvae find their hosts and colonize them, the searching process may limit the complexity of commensal life cycle, being dependent no host density (Hayashi, 1998). Some authors (Tokeshi, 1993; Roque et al., 2004) pointed out that these interactions may benefit the Chironomidae by decreasing predation risks, increasing mobility, improving protection from disturbances, providing greater opportunities for feeding, and also eliminating metabolic waste. Ecologically, commensal life may still represent a largely vacant niche where the possibility of interspecific competition has been low (Tokeshi, 1993). Although, studies on the association between Chironomidae and other aquatic organisms have increased in the Neotropical Region, mainly in Brazil, some questions still remain unanswered. More specific and detailed studies on these associations are necessary. In the present study, we described some of these associations. Some interactions are recorded for the first time for the state of Rio de Janeiro, such as those between Aeshna punctata and Brechmorrhoga (Odonata), Corydalus (Megaloptera), Grumicha grumicha (Trichoptera), and Trichomycterus mirissumba and Pareiorhina rudolphi (Pisces). These results indicate that more detailed investigations might reveal new associations.

Acknowledgments

The authors are grateful to ICMBio, Parque Nacional da Serra da Bocaina and Parque Nacional de Itatiaia for collect permits; to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for the financial support, and to the Laboratório de Ictiologia Geral e Aplicada of the Departamento de Zoologia, Instituto de Biologia of the Universidade Federal do Rio de Janeiro, for donation of specimens of *Ichthyocladius* for identification and study; to Dr. Maria Inês da Silva dos Passos for Elmidae identification; to Dr. Angela Mansolillo Sanseverino and the anonymous referee for corrections and suggestions that improved the manuscript.

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