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VERSITA

The occurrence of epibionts of Gordiida (Nematomorpha) in Catamarca, Argentina

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Abstract

The presence of larvae and pupae of the genus *Metrichia* (Trichoptera, Hydroptilidae), larvae of the genus *Macrelmis* and *Phanocerus* (Coleoptera, Elmidae), three diatom genera *Achnanthes*, *Cocconeis*, *Gomphonema*, and some specimens of very small diatoms that could not be determined to species or genus level, were recorded for the first time as epibionts for *Chordodes brasiliensis* (Gordiida, Nematomorpha). Such epibionts were found on the body surface of this species of hairworms, captured from El Tala stream, Catamarca, Argentina.

Keywords

Gordiida, epibionts, diatoms, Trichoptera, Elmidae

Introduction

Gordiida, known as hairworms are worm-like organisms free-living in freshwater environments as adults where they copulate and lay their eggs. Their minute larvae are obligate parasites from arthropods.

Although many gordiids have been studied for their taxonomic position and life cycle (Schmidt-Rhaesa 2002, Hanelt and Janovy 2004, De Villalobos *et al*. 2005, Schmidt-Rhaesa *et al*. 2005, Zanca *et al*. 2007) nothing is known about their interactions with their epibionts.

Epibiosis is a widespread relationship in aquatic environments that includes two organisms: the epibiont and the basibiont (Wahl and Mark 1999). While epibiont includes organisms that are attached to the surface of a living substratum, the basibiont lodges and constitutes a support for the epibiont (Threlkeld *et al*. 1993). During our investigations of gordiids, it was noted that they are basibionts for several epibionts that inhabit their body surfaces. Gordiids are mobile, providing constant flow of water and nutrients across the cuticle and thereby supplying an optimal habitat for epibionts.

Although after years of collecting different species of gordiids, the presence of epibionts on their body surfaces had been already detected in our studies, in a recent collection the abundant occurrence of epibionts on *Chordodes brasiliensis* has been most unusual.

Consequently, the aim of this study was to analyze the occurrence, for the first time, of the epibiont communities associated with gordiids of the species *Chordodes brasiliensis* collected from Catamarca, Argentina.

Materials and methods

The study was carried out in October 2008. Ninety-eight male specimens of *Chordodes brasiliensis* were collected from El Tala River (28°27′S, 65°50′W). El Tala River belongs to the Central region, originating in the mountain range of the Ambato, Catamarca province, Argentina.

The sampling area covered 500 m along the stream from bank to bank (7–9 m wide). Horsehair worms were detected by visual inspection and collected by hand. Gordiids were maintained alive and placed in individual 300 ml plastic containers with stream water.

As soon as possible in the lab, the distribution of each species of macroepibionts over the body, as well as the occurrence, were recorded and photographed under stereomicroscope for each gordiids studied. In order to examine macroepibionts in detail, *C. brasiliensis* surface was scraped with a scalpel. The extracted material was analyzed *in vivo* using bright field and light microscopy. Also one pupa of *Metrichia* spp. was photographed under SEM. Prevalence and mean intensity were calculated according to (Begon *et al*.

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1996). Coleoptera and Trichoptera were identified according to Manzo (2007) and Trémouilles *et al*. (1995).

Among these epibionts, three unidentified species belonging to three genera of diatoms and some specimens of very small diatoms (less than 10 µm) that could not be determined were observed by SEM on seven specimens of *C. brasiliensis*.

For scanning electron microscopy (SEM) of the diatoms, fragments of the central region of the body of *C. brasiliensis* (about 5 mm in length) were fixed in 95% ethanol and dehydrated in 100% ethanol for 30 min. Afterwards, they were critical-point dried, mounted on aluminium specimen stubs with standard adhesive pads and coated with gold-palladium. Pictures were taken on a JEOL JSM 6360 LV Scanning Electron Microscope. Based on counts of diatoms in SEM photographs we could quantify their density analyzing three randomly selected areas of each sample of 100 µm high and 150 µm wide for each individual studied. Also we calculated the relative frequency (RF) of each genus of diatoms.

Diatoms were identified according to Round (1990), Kramer and Lange Bertalot (1986a, b).

After completion of the afore mentioned studies the gordiids specimens as well as their epibionts were fixed in alcohol 70% and were deposited in the Invertebrate Collection of Natural Science Museum of the University of La Plata (Argentina) under the accession numbers MLP 6066 (*Chordodes brasiliensis*) and MLP 6066a (epibionts).

Results

During the observation of the 98 specimens of horsehairs collected 54 (52.92%) show epibionts on their surface. One genus of Trichoptera, Hydroptilidae (*Metrichia* spp.), two genera of Coleoptera, Elmidae (*Phanocerus* spp. and *Macrelmis* spp.) (Table I) and three genera of diatoms (*Achnanthes* spp., *Cocconeis* spp. and *Gomphonema* spp.) (Fig. 3A, B), were identified as epibionts (Table II, III).

Metrichia Ross, 1938 (Trichoptera, Hydroptilidae)

Pupae (n = 100): Caddisfly pupae of *Metrichia* spp. was the most widespread genera of macroepibionts (47.6%). These epibionts, located along the entire body showed a decrease towards the posterior end on the surface of 53 specimens of *C. brasiliensis* (Figs 1A, C and 2A, B). Only one caddisfly larva of *Metrichia* spp. (0.47%) was observed on the midbody of *C. brasiliensis* (Fig. 1B; Table I).

Macrelmis Motschulsky, 1859 (Coleoptera, Elmidae)

Sixty-eight specimens of *Macrelmis* spp. larvae (32.38%) colonized the entire body surface, but were most numerous on the anterior body part of forty-six specimens of *C. brasiliensis* (Figs 1A, C; Table I).

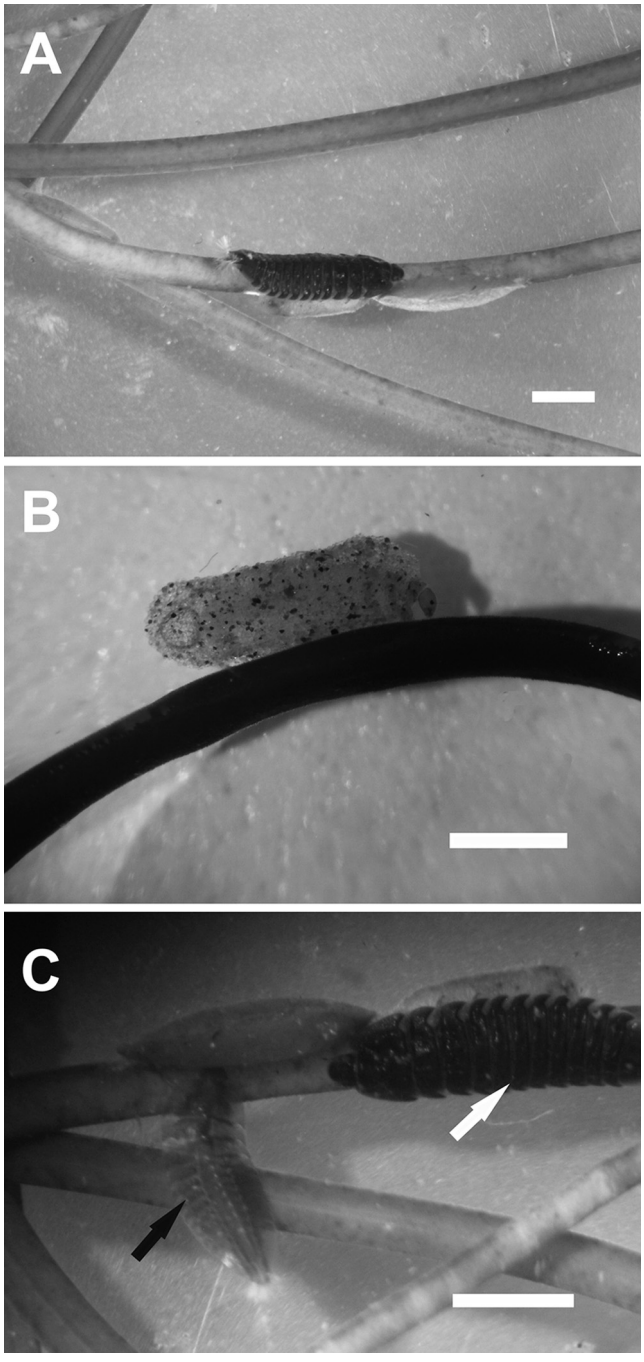

Fig. 1. Stereomicroscope. **A.** Two pupae of *Metrichia* spp. and one larva of *Macrelmis* on the body surface of *Chordodes brasiliensis*. **B.** Larva of *Metrichia* within a larval case. **C.** One larva of *Phanocerus* (black arrow), larva of *Macrelmis* (white arrow) and pupa of *Metrichia* on the body surface of *C. brasiliensis*. Scale bars = 2 mm (A), 1 mm (B), 2 mm (C)

Phanocerus Sharp, 1882 (Coleoptera, Elmidae)

Forty one riffle beetles larvae (19.52%) were found on the midbody surface of 37 specimens of *C. brasiliensis* (Fig. 1C; Table I).

Table I. Prevalence and intensity of macroepibionts of *Chordodes brasiliensis*

	Prevalence (%)	Intensity	
		range	mean
Metrichia spp. pupae	54.08	0–9	1.04
Phanocerus spp. larvae	37.75	0–2	0.43
Macrelmis spp. larvae	46.93	0–2	0.47
Metrichia spp. larvae	1.02	0–1	0.01

Diatoms

All the specimens of *C. brasiliensis* examined by SEM (n = 7) presented diatoms on the cuticle (Figs 3A, B). Diatoms interact with the substratum by releasing adhesive exopolymers (mucilage) through a distinct slit in the frustule called the raphe (Hoagland *et al*. 1993) (Fig. 3A).

The density variation range of total diatoms was 1288–12213 diatoms/mm^2 ($\bar{x}$ = 6155). The highest mean values of diatoms density found were *Gomphonema* spp. ($\bar{x}$ = 1342 specimens per mm^2) and *Achnanthes* spp. ($\bar{x}$ = 854 specimens per mm^2), *Cocconeis* spp. ($\bar{x}$ = 218 specimens per mm^2), while the density of smaller diatoms (<10 µm) was $\bar{x}$ = 3741 individuals per mm^2 (Table II). The highest relative frequency is given by the smaller diatoms (<10 µm) ($\bar{x}$ = 51.2) followed by *Gomphonema* spp. ($\bar{x}$ = 31.0), *Achnanthes* spp. ($\bar{x}$ = 12.7) and *Cocconeis* spp. ($\bar{x}$ = 5.1) (Table III).

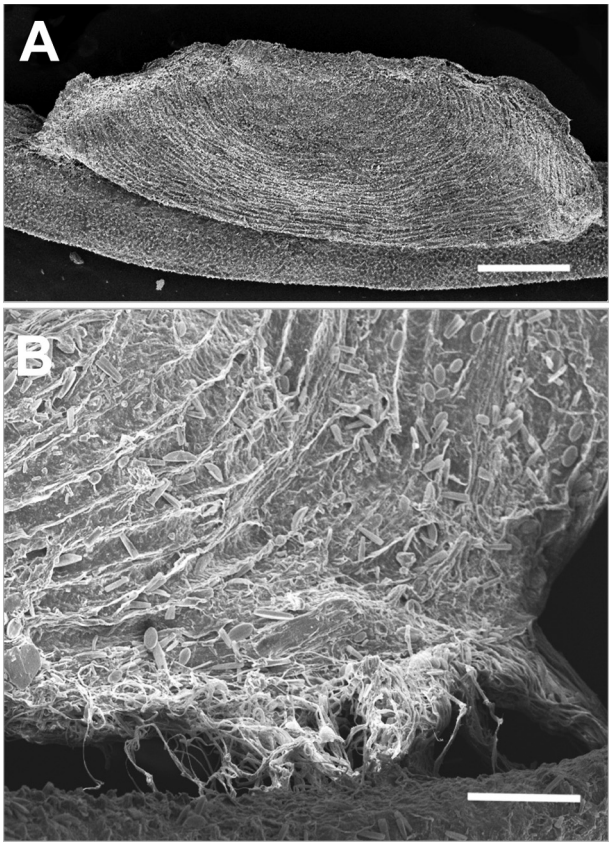

Fig. 2. SEM. **A.** General view of pupa of *Metrichia*. **B.** Detail of the pupa case showing silk filaments that allows the attachment to the body surface of *C. brasiliensis*. Scale bars = 500 µm (A), 100 µm (B)

Table II. Density of diatoms as epibionts of *Chordodes brasiliensis*

	Density of diatoms per mm^2			
Samples	*Gomphonema* spp.	*Cocconeis* spp.	*Achnanthes* spp.	diatoms <10 µm
1	995	559	870	4786
2	701	70	2103	6308
3	814	68	1968	9363
4	823	137	754	3223
5	573	143	286	286
6	3897	390	0	390
7	1591	159	0	1829
Average	1342	218	854	3741

Table III. Relative frequency of microepibionts of *Chordodes brasiliensis*

	Relative frequency (%) of diatoms			
Samples	*Gomphonema* spp.	*Cocconeis* spp.	*Achnanthes* spp.	diatoms <10 µm
1	13.8	7.8	12.1	66.4
2	7.6	0.8	22.9	68.7
3	6.7	0.6	16.1	76.7
4	16.7	2.8	15.3	65.3
5	44.4	11.1	22.2	22.2
6	83.3	8.3	0.0	8.3
7	44.4	4.4	0.0	51.1
Average	31.0	5.1	12.7	51.2

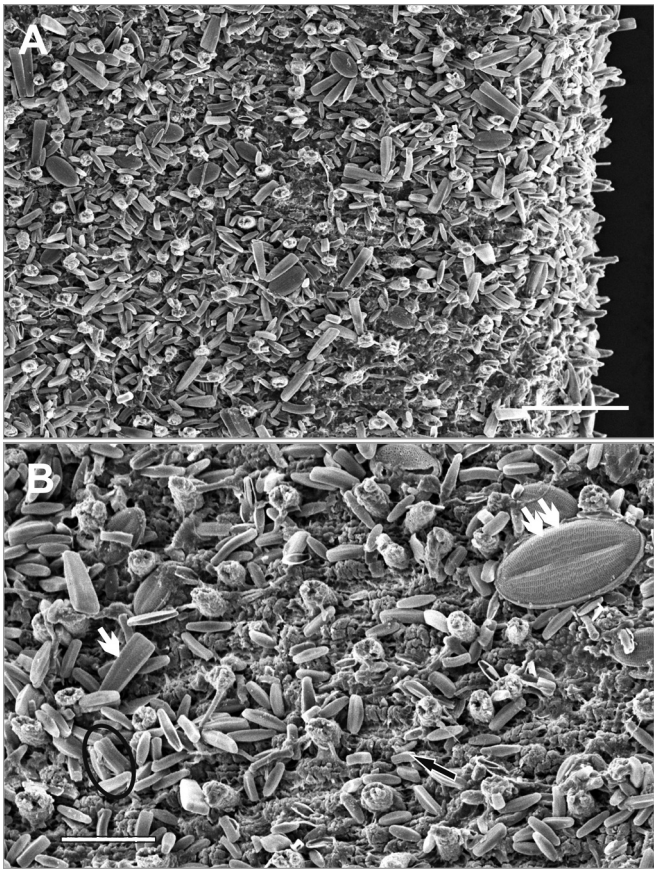

Fig. 3. SEM. **A.** General view of diatoms adhered to the cuticle of *C. brasiliensis* by a mucilage stalk. **B.** Three of main genera of diatoms identified upon cuticle of *C. brasiliensis*. Double white arrows show *Cocconeis* spp.; simple white arrow shows *Gomphonema* spp., black circle shows two cells of the *Achnanthes* spp. forming short bands and black arrow shows indeterminate diatoms (<10 µm). Scale bars = 50 µm (A), 20 µm (B)

Discussion

We found 6 epizoic taxa on the surface of *Chordodes brasiliensis* from El Tala River of Catamarca. The most abundant epibionts were diatoms and caddisfly pupae. Additional organisms such as the Elmidae larvae, *Macrelmis* spp. and *Phanocerus* spp. occurred in less proportion.

Caddisfly larvae and pupae are aquatic; they are sensitive to pollution and habitat degradation and are biological indicators of water quality (Merritt and Cummins1996). Also *Phanocerus* larvae are often good water quality indicators and have been used in several water quality experiments (Miserendino and Archangelsky 2006).

The abundance of epibionts found in *C. brasiliensis* could be explained on the basis of two aspects (1) environmental: El Tala River is not influenced by anthropic activity along its course, having an excellent water quality (Domínguez and Fernández 1998, Paez Zanini *et al*. 2006) and a great diversity of flora and fauna specially invertebrates (Salas 2003, 2005) and (2) characteristics of the basibiont: *C. brasiliensis* presents a very structured cuticular surface (De Villalobos *et al*. 2005) providing a better substrate for these epibionts.

A number of effects are related to epibiosis (Wahl and Mark 1999). These include advantages for the epibionts (diatoms, Trichoptera and Elmidae larvae) such as a suitable substratum, dispersal, access to light and nutrients. Also caddisfly larvae and Elmidae larvae have well developed mandibulate mouthparts which allow them to feed on diatoms and clean up the basibiont surface. Conversely, epibiosis may have the disadvantage of restricting the mobility of the basibiont (Chiavelli *et al*. 1993). Despite this, it is unlikely that diatom epibionts on gordiids surface were harmful. The short stature of the algal assemblage would have minimal physical effects, the diatoms apparently provided oxygen for the gordiids, which can be significant in low oxygen conditions, such as at the bottom of freshwater environments.

Caddisfly larvae can be found on the rocks, plants, mud or, as shown in this study, as epibionts of gordiids. Taking into account the existence of numerous studies that show a host-parasite association between Trichoptera larvae and the developing stages of various horsehair worms (White 1966, Cappuci 1976, Scari *et al*. 1986, Valvassori *et al*. 1988, Schmidt-Rhaesa and Kristensen 2006), it is possible that the caddisfly larvae, as epibionts when moving along the body surface of gordiids could contact rocks or plants where a female had previously deposited eggs and feed from her developed eggs or larvae (eggs develop over 20–35 days see Hanelt and Janovy 1999; De Villalobos *et al*. 2003, 2006; Zanca *et al*. 2007; Achiorno *et al*. 2008, 2009).

Despite its wide occurrence, epibiosis is still not very well known with respect to its consequences for both basibionts and epibionts (Wahl and Mark 1999). Opportunism practiced by free-living trichopteran larvae, coleopteran larvae and diatoms should be investigated in future studies related to gordiids health problems.

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