

# First Record in Brazil of *Epistylis* sp. (Ciliophora) Adhered to *Argulus* sp. (Argulidae), a Parasite of *Hoplias aimara* (Eritrhinidae)

Lincoln Lima Corrêa<sup>1\*</sup>, Marcos Sidney Brito Oliveira<sup>2</sup>, Luiza Prestes<sup>3</sup>,  
Marcos Tavares-Dias<sup>4</sup>

<sup>1</sup>Universidade Federal do Oeste do Pará (UFOPA), Instituto de Ciências e Tecnologia das Águas (ICTA), Santarém, Brasil

<sup>2</sup>Programa de Pós-Graduação em Recursos Aquáticos Continentais Amazônicos (PPGRACAM), Universidade Federal do Oeste do Pará (UFOPA), Instituto de Ciências e Tecnologia das Águas (ICTA), Santarém, Brasil

<sup>3</sup>Programa de Pós-Graduação em Ecologia Aquática e Pesca (PPGEAP), Universidade Federal do Pará (UFPA), Belém, Brasil

<sup>4</sup>Embrapa Amapá, Rodovia Juscelino Kubitschek, Macapá, Brasil

Email: [lincorre@gmail.com](mailto:lincorre@gmail.com)

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## Abstract

This paper records the first occurrence of *Epistylis* sp. on the body surface of *Argulus* sp. parasitizing *Hoplias aimara* from the upper Araguari River, in the eastern Amazon region, in the north of Brazil. Of the 16 specimens of *H. aimara* examined, 93.7% had their pelvic, caudal and pectoral fins and tegument infested by *Argulus* sp. (n = 73), which in turn were infested by *Epistylis* sp. The specimens of *Epistylis* sp. from the body surface of *Argulus* sp. were analyzed using Scanning Electron Microscopy (SEM). The present study also identified a widening of the geographic distribution of these two species of ectoparasites to the eastern Amazon region of Brazil.

## Keywords

Parasite Ecology, Opportunistic Parasite, Rio Araguari, Eastern Amazon

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\*Corresponding author.

## 1. Introduction

*Hoplias aimara* Valenciennes, 1847 is a Characiforme from the Erythrinidae family which is distributed from Central America to Argentina, and is popularly known as giant lungfish, lungfish or Aymara. In Brazil, this species is distributed in the Trombeta, Xingu, Tapajós, Jari, Tocantins and Araguari Rivers, as well as the coastal rivers of Surinam and French Guyana [1] [2]. This fish lives in a variety of habitats, from lotic environments such as large rivers and waterfalls to lentic environments such as lakes and reservoirs [3]. However, little is known about the parasites that the fish hosts.

Among Branchiura Thorell, 1818 crustaceans, species of the genus *Argulus* Müller, 1785 are highly adapted to ectoparasitism in different fish species [4]-[6]. Some *Argulus* spp. are endemically distributed in the region of the Brazilian Amazon [6]. These ectoparasite crustaceans can reduce the weight, growth rates, breathing capacity, and muscle and fat percentage of fish, and lead to tissue traumatization. The severity of parasitic infection mainly depends on the infestation intensity. When it is high, there may be fish mortality, causing damage to fishing and fish farming [4] [7]-[9].

Species of *Epistylis* Ehrenberg, 1830 are colony forming ciliates of whom several species are free-living (freshwater and marine) or live in epibiosis, colonizing the body surface of insects and aquatic plants, mollusks, amphibians, reptiles, rotifers and fish. In fish, the main organ colonized is the gills and in Brazil, *Epistylis* sp. is an emerging parasite [10] [11]. The death of thousands of Centrarchidae in the USA has been attributed to heavy infestation by *Epistylis* sp., which coincide with ulcers and lesions. As this is not an obligate parasite, these injuries can be gateways to secondary infections, so these mortalities are most often associated with secondary infections caused by bacteria [12] [13]. *Epistylis* spp. commonly colonizes planktonic copepods [14]-[17]. However, colonization studies of ectoparasite crustaceans are rare [11].

This study describes the first record of *Epistylis* sp. in *Argulus* sp. collected from *Hoplias aimara*, a fish from the upper Araguari River, in the state of Amapá, in the north of Brazil.

## 2. Materials and Methods

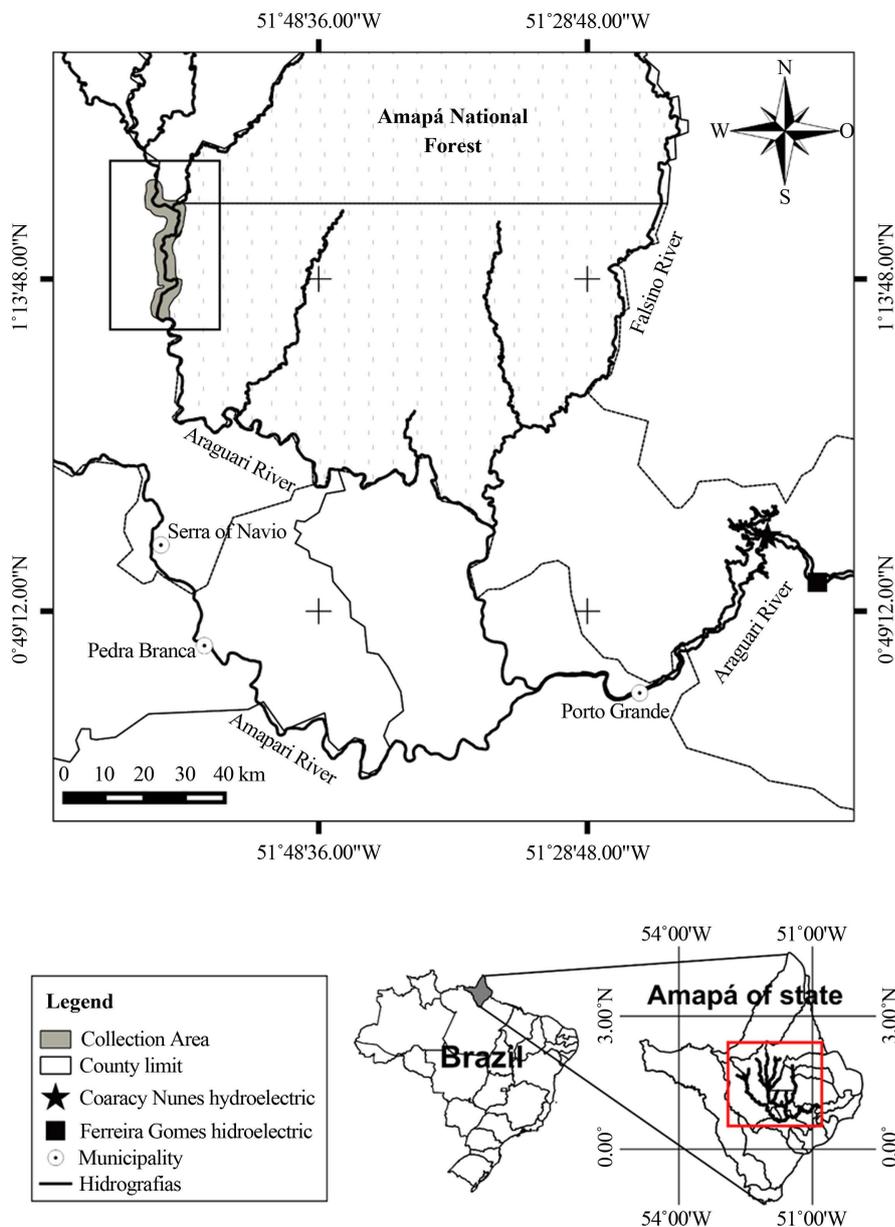
A total of 16 specimens of *H. aimara* were collected from a stretch of the Araguari River, in the state of Amapá, Brazil (Figure 1), which is the largest river to be truly located in Amapá, being 617 km long. Its source is found in the Parque Nacional Montanhas do Tumucumaque (Tumucumaque Mountains National Park), in the Guiana Shield [18]. It passes through the municipalities of Porto Grande and Ferreira Gomes, in the Floresta Nacional do Amapá (Amapá National Forest) (FLONA), the Floresta Estadual do Amapá (Amapá State Forest) (FLOTA) and the Reserva Biológica do Lago Piratuba (Lake Piratuba Biological Reserve) (REBIO), and there are two hydroelectric power plants along its course.

The collection of *H. aimara* took place in July 2014, using a hand line and gillnet with different mesh sizes. Immediately after capture, the fish were macroscopically examined for the presence of ectoparasite crustaceans. Then, each fish was weighed (g) and its standard length measured (cm). The crustacean specimens collected were fixed in 70% ethanol before being transferred to 70% glycerinated alcohol after 48 hours (10%) and identified in accordance with [8]. The prevalence values, mean abundance and mean intensity were calculated in accordance with [19]. These procedures were carried out in accordance with the principles of the Colégio Brasileiro de Experimento Animal (the Brazilian College of Animal Experimentation) (COBEA).

For analysis with a scanning electron microscope (SEM), five specimens of *Argulus* sp. previously fixed in 70% ethanol were transferred to a 2.5% glutaraldehyde solution in 0.15 M phosphate buffer (pH 7.3) for 24 h, and post-fixed in 1% osmium tetroxide in the same buffer for 2 h. Dehydration was performed with an increasing sequence of ethanol solutions, and critical point drying was carried out with a CPD 020 (Union Balzer) with liquid CO<sub>2</sub>. The specimens were coated with a 20 nm layer of gold using the MED 010 apparatus (Balzer Union). Analysis was performed by SEM (SEM 515 Philips).

## 3. Results

A total of 16 *H. aimara* fish with a body weight of  $1619.9 \pm 1054.7$  g and a standard length of  $40.7 \pm 9.3$  cm were collected. From these hosts ( $n = 15$ ) a total of 73 specimens of *Argulus* sp. were collected from the pelvic, caudal and pectoral fins and tegument, with a prevalence of 93.7%, average intensity of 4.9 host and mean abundance of 4.6. Of these specimens of *Argulus* sp. five were infested by *Epistylis* sp., which were adhered in large quantity to the ventral region of the argulidae (Figure 2).

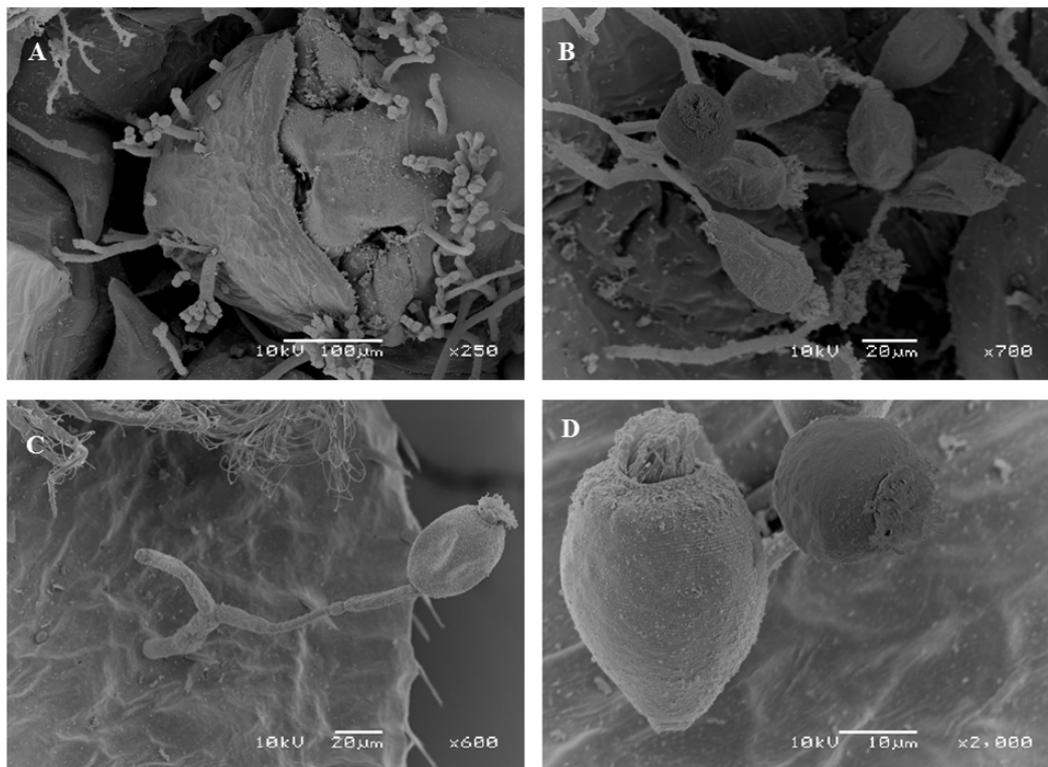


**Figure 1.** Geographic location of *Hoplias aimara* collection area in a stretch of the upper Araguari River, in the state of Amapá, in the north of Brazil.

The specimens of *Epistylis* sp. established themselves on *Argulus* sp., which in turn serves as a substrate and means of transport, with no lysis or mechanical injury to the infested *Argulus* sp. observed. The *Argulus* sp. were infested by *Epistylis* sp. in various parts of the bodies, with a stalk bearing several zooids (**Figure 2A** and **Figure 2B**). This stalk is relatively long and may be divided to form colonies which can be either longer or shorter or more or less ramified (**Figure 2B**). The vaulted epistomial disc is slightly elevated above the peristomial lips and slanted. The macronuclei are long (**Figure 2D**).

#### 4. Discussion

*Hoplias aimara* was infested by *Argulus* sp., which is a new species found parasitizing this host from the Araguari River basin in the state of Amapá (eastern Amazon region, Brazil). Different species of *Argulus* have been found infesting Brazilian fish, especially species of Anostomidae, Pimelodidae, Cichlidae and Serrasalmididae [6].



**Figure 2.** A. Scanning Electron Microscopy (SEM) detailing *Epistylis* sp. adhered to the anterior region of *Argulus* sp. B. Extensive and ramified colonies of *Epistylis* sp. adhered to *Argulus* sp. C. *Epistylis* sp. D. Vaulted epistomial disc, slightly elevated above peristomial lips.

These ectoparasites are often found parasitizing the lentic fish environment, as natant-free forms can find their host more easily in such an environment [4] [7] [20]. However, in *H. aimara* we found a high prevalence of *Argulus* sp., indicating that the infestations also occur in fish from lotic environments, as this host fish also inhabits rapids environments [3], such as the Rio Araguari, the basin of this study. Additionally, the levels of infestation of *Argulus* sp. in *H. aimara* were similar to those of *Argulus foliaceus* described for *Cyprinus carpio* and *Abramis brama* [4] and greater than *Argulus pestifer* in *Pseudoplatystoma faciatum* [7].

Species of *Epistylis* are not obligate ectoparasites, but live in epibiosis with shellfish, aquatic plants, rotifers, aquatic insects, amphibians, reptiles, free crustaceans and parasitic crustaceans [10] [11] as has been described for the occurrence of *Epistylis* sp. in *Ergasilus chelanguatus* from *Pimelodus maculatus* [11]. In this manner, the results of the present study suggest that *Epistylis* sp. also colonizes *H. aimara*, the fish from which specimens of *Argulus* sp. were collected. Species of *Epistylis* have been reported in different species of freshwater fish in Brazil, such as *Colisa lalia*, *Ctenopharyngodon idella*, *Oreochromis niloticus*, *Piaractus mesopotamicus*, *Pseudoplatystoma corruscans*, *Pseudoplatystoma reticulatum*, *Leiarius marmoratus*, *Hemisorubim platyrhynchus* and *Zungaro jahu* [10] [21] [22], which were possibly also infected by crustacean species, suggesting that crustaceans are important for the development and maintenance of the life of *Epistylis* sp. [11] [14]. Furthermore, it is suggested that the infective forms seek new hosts for the fixation and development of new colonies, such as zooplankton copepods, which can act as vectors or host reservoirs of *Epistylis* spp., as it is common to find these ciliates fixed on the body surface of copepods [14]-[16] thereby increasing the dissemination of this ciliate.

While *Epistylis* spp. are considered commensal, this protozoan causes damage such as bodily injury, locomotor disorders, low fertility, reduced competitiveness, increased susceptibility to predation, increased energy demand, faster sinking rates and a decrease in the survival rate to hosts, as well as creating the possibility of bacterial infections [12]-[14] [16]. However, in *Argulus* sp. parasitizing *H. Aymara* no injury was found. At the same time, the fixation of *Epistylis* spp. to fish can result in the proliferation of the parasite, causing depigmentation and injuries to the integument with bleeding issues and the excessive production of mucus, triggering anorexia and itching [21]. Thus, it is suggested that *Epistylis* spp. is an opportunistic parasite that uses *Argulus*

sp. as a substrate and a means of infecting other animals such as *H. aimara*, which have a greater area for their proliferation.

This first report of *Epistylis* sp. on the body surface of *Argulus* sp., in addition to being the first record of *Argulus* sp. parasitizing *H. aimara*, extends the geographical distribution of these two species of ectoparasites to the eastern Amazon region in the extreme north of Brazil.

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