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First Record of Epibiont Ciliates (Ciliophora: Suctoria) Associated with *Helobdella adiastola* Ringuelet, 1972 (Hirudinea: Glossiphoniidae) in Brazil

Lucas Duarte de Lima[1*]
https://orcid.org/0000-0001-6017-8327

Ricardo Massato Takemoto[2]
https://orcid.org/0000-0001-7592-2083

[1]Universidade Estadual de Maringá UEM, Programa de Pós-Graduação em Biologia Comparada, Maringá, Paraná, Brasil; [2]Universidade Estadual de Maringá-UEM, Centro de Pesquisas em Limnologia, Ictiologia e Aquicultura, Maringá, Paraná, Brasil.

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*Correspondence: lucasduartelimax@gmail.com; Tel.:(55) +44 99979-7167 (L.D.L.)

HIGHLIGHTS

- First record of epibiosis among protozoan ciliates (Ciliophora: Suctoria) associated with *Helobdella adiastola* in Brazil.
- The genus *Helobdella* Blanchard 1896 is one of the most diverse in number of species within South America.
- (Ciliophora: Suctoria) attached only to the nuchal scute structure, suggesting that this may be a suitable, and perhaps the only, site where epibionts can attach and settle on leeches

Abstract: Epibiosis is an interaction between two organisms, and it can be a relationship with both positive and negative effects. Protozoa of the subclass Suctoria are common epibionts in freshwater environments. Their preference for attaching to other organisms reflects their biological needs, such as food availability and environmental conditions, as well as the anatomy of their hosts. The study area of this work was the lakes of Ingá Park located in Maringá, Paraná state, Brazil. Leeches were collected in August 2019, through an active search on aquatic vegetation, among submerged trunks and rocks. Subsequently, the specimens were sent to the laboratory. In the laboratory, they were fixed in 5% formaldehyde, and subsequently submitted to two procedures, in which permanent slides of the specimens stained by the hydrochloric carmine method were made and images were obtained by electronic scanning microscopy. For the identification and description of the leech species, morphological data, taxonomic characteristics, and the use of the identification key were obtained. The epibiont ciliated protozoa occurred in Antiga Santa Lake, associated with four specimens of the leech *Helobdella adiastola*. We report the first record of epibiosis among the protozoan epibiont ciliates (Ciliophora: Suctoria) associated with *Helobdella adiastola* in Brazil.

Keywords: Leeches; Epibiosis; Freshwater; Protozoa; Taxonomy.

INTRODUCTION

Association by epibiosis is an interaction between two organisms, one of which is the epibiont and the other the basibiont. The epibionts are organisms that, during the sessile phase of their life cycle, are attached to the surface of a living substrate, while the basibiont is the organism that serves as a support for the colonizing epibiont [1,2,3]. Epibiosis can have positive and negative effects. In cases of positive effects, the epibiont can provide mimetic protection to the basibiont against predators, protect the basibiont against desiccation and in other situations, and the basibiont can benefit by feeding on the epibiont [1,4,5,6].

The negative effects of epibiosis on the basibiont organism can involve a series of consequences. When there is an excess of epibionts, these can cause a decrease in locomotion, fecundity, and feeding capacity, or make the basibiont more susceptible to predation [7,8,2,9]. For the epibiont, one of the advantages is that they are transported by the basibiont to locations that may have greater availability of oxygen and food. Furthermore, epibionts can fix themselves to mobile organisms, which can allow them to avoid predation by zooplankton, since protozoa are easily predated when fixed in localities [7,9].

Protozoa of the subclass Suctoria Claparède and Lachmann, 1858, are a special group of ciliates because they have an asexual mode of reproduction by budding, while most ciliates reproduce through binary fission, [10,11]. Suctoria is one of the most species-rich groups of ciliates, comprising more than 560 species in three orders and including 41 families with approximately 126 genera [11,12, 13]. They are devoid of locomotor organelles, like cilia, in their adult stage. They do not present an oral opening and use tentacle-like haptocysts with which they capture and immobilize their prey; this is an important characteristic for this group [14, 15].

Suctorians are very common in freshwater and marine environments, frequently living as epibionts on a variety of animals [16]. These protozoa fix themselves on their hosts directly and feed mainly on other ciliates; therefore, they are important components of the food chain as predators [11,17].

In freshwater environments, they are observed to be influenced by factors such as chlorophyll, pH, electrical conductivity, and dissolved oxygen content in the occurrence and prevalence of ciliated protozoan infestation [18]. Epibiosis can also be modulated by factors such as temperature, CO_2, pH, salinity, nutrients and type of organism that will serve as basibiont [19].

Several groups of organisms can serve as basibionts for (Ciliophora: Suctoria), with aquatic invertebrates being the most common in this interaction. These include primarily crustaceans from the subclass Copepoda, insects from the Notonectidae family, gastropods from the Ampullaridae family, nematodes from the Desmodoridae family, and oligochaete worms from the phylum Annelida [20, 21, 22, 23, 24].

The family Glossiphoniidae Vaillant, 1890, includes freshwater leeches that present an eversive proboscis. They are also characterized by being the only leeches that have parental care for their offspring, carrying the eggs and juveniles from the stage of hatching eggs until they reach the adult stage [25,26,27].

The genus *Helobdella* Blanchard 1896 is one of the most diverse in number of species among the groups of leeches with predatory feeding habits on aquatic invertebrates such as chironomids, oligochaetes, and mollusks [28,29]. There are more than 50 described *Helobdella* species in South America, and the level of endemism of this genus in South America is around 90% [30,31,32].

They can serve as bioindicators of water quality and biodiversity, and the presence of specific leech species is linked to abiotic and biotic conditions [33,34,35]. The complete distribution of the majority of leeches species is unknown. The lack of knowledge is due to the lack of regional research and the difficulty of species identification. Misidentifications will also confuse geographic distribution of species [36].

The present study aimed to observe and identify the presence of epibiosis among ciliated epibiont protozoa associated with leeches in the lakes of Ingá park, Maringá, Paraná, Brazil, and to verify whether epibionts have a possible preference for certain attachment sites in leeches.

MATERIAL AND METHODS

Study area

The city of Maringá is located in the northwestern part of the State of Paraná. It possesses remnants of the original submontane semidecidual seasonal forest of the Atlantic Forest biome. Ingá Park, a Conservation Unit, is located within the urban perimeter of the city (23° 25' 37.03" S and 51° 55' 47.24" W), at the source of Moscados Creek, in the Pingüim microbasin within the hydrographic basin of the Ivaí River. The total area of the park is 0.473 km², and it contains numerous species of fauna and flora native to the region. Inside this urban forest fragment, there are five artificial lakes, of which small ones lie within the Japanese garden, a medium-sized one is Antiga Santa lake, and the main lake has approximately 487 m in length and 119 m in width. The human population has access to all the lakes [37].

Data collection

Leeches were collected in August 2019, through an active search on aquatic vegetation, among submerged trunks and rocks around the shores of all the lakes in the Ingá Park. The leeches were collected with tweezers and placed in Eppendorf tubes with local water, keeping the leeches alive. Subsequently, the specimens were sent to the laboratory Ictioparasitologia at the State University of Maringá (UEM) for identification.

In the laboratory Ictioparasitologia, leeches were subjected to the relaxation method adapted from Davies and Govedich [27] was applied with carbonated water until the specimens became temporarily inert. Then, they were fixed in 5% formaldehyde and later stained by the hydrochloric carmine method, as indicated by Eiras and coauthors [38]. After fixing, we carried out the photographic record of the specimens, for identification.

In the laboratory of the Research Support Center Complex (COMCAP) at the State University of Maringá (UEM), scanning electron microscopy (SEM) images were obtained, for which the specimens were dehydrated with an ethanol battery (70%, 80 %, 90% and 100%) and submitted to the critical point. Then, metallization with gold was performed, and scanning microscopy images were generated.

The identification of leech species obtained by, taxonomic characteristics, and the use of Thorp & Covich's [36], the South American leech species identification key.

RESULTS

Two species of leeches occurred in Antiga Santa lake: *Helobdella triserialis* Blanchard, 1849 and *Helobdella adiastola* Ringuelet, 1972. The description of *H. triserialis* was based on 10 specimens, which exhibited the following characteristics: one pair of comma-shaped eyes on somite III, well-separated from each other by a pigmented central line; no nuchal scute; absence of skin protuberance in somite VIII; presence of papillae, sensillae, and lines on the dorsal surface; gonopores separated by one annuli; median somites triannulate, ([a1]+[a2]+[a3]); one pair of diffuse salivary glands; six pairs of testisacs; five pairs of digitiform gastric caeca, with the last pair forming postcaeca; and ascending ejaculatory ducts in somite XII/XIII.

The description of *H. adiastola* was based on 16 specimens, which displayed the following characteristics: a dorsoventrally flattened lanceolate body shape, semi-translucent yellowish or greenish coloration, one pair of semi-triangular or semicircular eyespots close to each other, presence of a nuchal scute in somite VIII, absence of skin protrusion in the nuchal region, lack of papillae, sensilla, and lines on the dorsal surface, gonopores separated by one annuli, ascending ejaculatory ducts in somite XI/XIII, median somites triannulate, ([a1]+[a2]+[a3]), presence of one pair of diffuse salivary glands, six pairs of testisacs, and six pairs of digitiform gastric caeca with the latter pair forming post-caeca.

The epibiont ciliate protozoa occurred only in *Helobdella adiastola* associated with four specimens, in the dorsal region in somite VIII adhered to the *Helobdella adiastola* has a nuchal scute (Figure 1). They were identified, according to the identification key as Protozoa, Ciliophora by Foissner and Berger [39], Berger and Foissner [40] belonging to Ciliophora: Suctoria.

Belonging to Ciliophora: Suctoria, the following characteristics were observed: the presence of a lorica, a peduncle, an absence of an oral opening, a ribbon-like macronucleus, and the presence of numerous tentacles with a distal extension (knob).

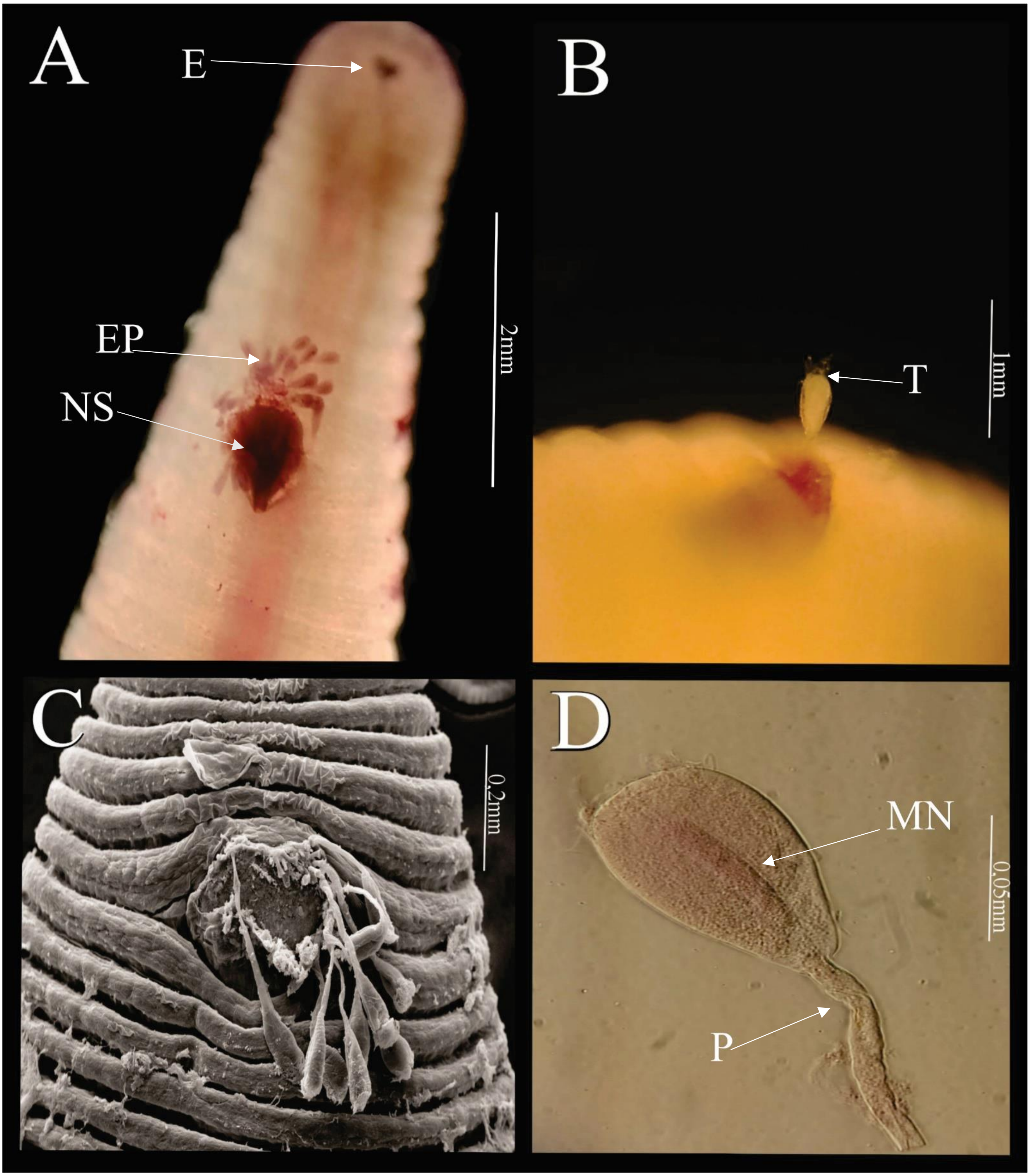

Figure 1. (A): Dorsal view of the anterior region of *Helobdella adiastola*, stained with the hydrochloric carmine method, showing E. eyespots; EP. epibionts; NS. nuchal scute is located on somite VIII; (B). Lateral view of *Helobdella adiastola* showing nuchal scute with epibiont showing T. tentacles; (C). Scanning electron microscopy image focusing on the epibionts; (D). Dorsal view of an epibiont showing MN. Macronucleus; P. peduncle. identified as Ciliophora: Suctoria under a light microscope

DISCUSSION

Only *H. adiastola* and *Helobdella stagnalis* Linnaeus, 1758, have been recorded in Brazil, which have in their dorsal ornamentation an external sclerotized structure called the nuchal scute, located in somite VIII.

According to Saglam and coauthors and Iwama and coauthors [41,32], morphological, molecular and phylogenetic studies have found that many specimens supposedly identified as *Helobdella stagnalis* that have been reported in a wide geographical range, including Europe, Asia, Africa, North America and South America, have morphological differences, such as the position of the gonopores, the shape and size of the nuchal scute, as well as the number of gastric caeca and testisacs, in addition to genetic differences that signal these specimens as supposedly different species. Possibly, specimens identified as *Helobdella stagnalis*, described in South America, is a misidentification and is likely to be *Helobdella adiastola*.

As the nuchal scute is a hard structure and located in a way that possibly avoids friction with the body or the environment, we believe that this could be a suitable, and perhaps the only, structure that allows the attachment and establishment of epibiont organisms in leeches.

The presence of epibionts associated with the nuchal scute of *H. adiastola* should possibly have no negative effect on the leech, since the size of the basibiont is significantly larger compared to the epibiont and they are attached only in a dorsal sclerotized external structure with no apparent function and because of these issues expect these organisms not to interfere with any vital functions of the leech, such as feeding, locomotion or respiration. Furthermore, this interaction is unlikely to make the leech visible and vulnerable to predation by other animals. However, further studies are needed to better understand the effects of this interaction.

The settlement and establishment sites of epibionts are determined by their biological needs, such as food availability, which can also be determined by local oxygenated environmental conditions and with the anatomy of basibionts, which include hard and frictionless regions [42,18]. This preference was reported by Dias and coauthors, Lima and coauthors [43,44], in a study that recorded epibionts in the abdominal tubules of chironomid larvae (Diptera: Chironomidae) and in the gill region of *Dero digitata* Müller, 1773 (Oligochaeta: Naididae), these are places where oxygen availability is increased for the epibiont. However, some regions of the basibionts are avoided, including soft regions or those with more attrition [45,46].

In this study, we obtained the first record of protozoan epibiont ciliates (Ciliophora: Suctoria) associated with *H. adiastola*. In addition, we found that ciliated epibionts attached only to the nuchal scute structure, suggesting that this may be a suitable, and perhaps the only, site where epibionts can attach and settle on leeches, and it likely does not cause any negative effects. However, further study on this interaction is needed to clarify more information. Our results may contribute to future studies on the interaction of ciliated epibionts (Ciliophora: Suctoria) with basibionts and their adhesion sites, providing insights into ideal conditions for ciliated epibiont protozoa.

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Conflicts of Interest: The funders had no role in the study design; in the collection, analysis or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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