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Original Article

A new species of *Tereancistrum* (Monogenea: Dactylogyridae), parasite of *Prochilodus lineatus* (Characiformes: Prochilodontidae) from southeast Brazil

Uma nova espécie de *Tereancistrum* (Monogenea: Dactylogyridae), parasito de *Prochilodus lineatus* (Characiformes: Prochilodontidae) do sudeste do Brasil

Lucas Aparecido Rosa Leite[1]; Larissa Sbeghen Pelegrini[1]*; Rodney Kozlowiski de Azevedo[2]; Vanessa Doro Abdallah[2]

[1] Instituto de Biociências de Botucatu, Universidade Estadual Paulista – UNESP, Botucatu, SP, Brasil
[2] Programa de Pós-graduação em Análise de Sistemas Ambientais, Centro Universitário CESMAC, Maceió, AL, Brasil

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Abstract

A new species of *Tereancistrum* Kritsky, Thatcher & Kayton, 1980 collected of *Prochilodus lineatus* gills from the Batalha River, Tietê-Batalha basin, São Paulo State, Brazil is described. The new species can be distinguished from its congeners mainly by the configuration of the ventral bar, which has an anvil-shaped characteristic with corrugated anterior projection. *Tereancistrum takemotoi* n. sp. is morphologically similar to *T. toksonum* Lizama, Takemoto & Pavanelli, 2004 in terms of their dorsal bars (Y-shaped), their dorsal anchors with divergent roots (superficial and deep) wherein their deep root rather elongated, and by the fact that they both have the male copulatory organ counterclockwise. However, only *T. takemotoi* n. sp. presents the male copulatory organ with 2¼ rings and shows undulations in the anterior margin of the dorsal bar. These undulations are absent in *T. toksonum* (which only has 1¼ rings). This is the fourth *Tereancistrum* species described for *P. lineatus* and the first described for the region from the Tietê-Batalha basin.

Keywords: Freshwater fish, gills parasite, taxonomy, Dactylogyridea, Platyhelminthes, Batalha river.

Resumo

É descrita uma nova espécie de *Tereancistrum* Kritsky, Thatcher & Kayton, 1980, coletada das brânquias de *Prochilodus lineatus* do rio Batalha, bacia do Tietê-Batalha, estado de São Paulo, Brasil. A nova espécie pode ser diferenciada de seus congêneres, principalmente pela configuração da barra ventral, que tem o formato de bigorna e apresenta uma projeção anterior com ondulações. *Tereancistrum takemotoi* n. sp. é morfologicamente semelhante a *T. toksonum* Lizama, Takemoto & Pavanelli, 2004, em termos de suas barras dorsais (em forma de Y), suas âncoras dorsais com raízes divergentes (superficiais e profundas) sendo que a raiz profunda é bastante alongada, e pelo fato de ambos terem o órgão copulatório masculino no sentido anti-horário. No entanto, apenas *T. takemotoi* n. sp. apresenta o órgão copulatório masculino com 2¼ anéis e também exibe ondulações na margem anterior da barra dorsal, diferentemente do *T. toksonum* (que possui apenas 1¼ anel em seu órgão copulatório masculino). Essa é a quarta espécie de *Tereancistrum* descrita para *P. lineatus* e a primeira descrita para a região da bacia do Tietê-Batalha.

Palavras-chave: Peixes de água doce, parasitos branquiais, taxonomia, Dactylogyridea, Platyhelminthes, rio Batalha.

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*Corresponding author: Larissa Sbeghen Pelegrini. E-mail: larapelegrini@yahoo.com.br

Introduction

Prochilodus lineatus Valenciennes, 1837, known in Brazil as curimba, curimbatá, or curimatã, is one of the most abundant native fish species in the floodplains of the upper Paraná River, although it is distributed across the country. It has substantial commercial appeal and is considered a medium to large species that engages in extensive migrations for food and reproduction (Agostinho et al., 1997). Due to anthropogenic interferences in aquatic environments (such as dams), its natural stocks have been declining, a process that has attracted several ecological, parasitological, and molecular studies on the species (Lizama et al., 2005, 2006; Rosa & Lima, 2008; Oyakawa et al., 2009).

The parasitic fauna of *P. lineatus* is relatively well known in the upper Paraná basin, and many studies have considered taxonomic and ecological aspects of this host. Monogenean ectoparasites are particularly common, with several species having been recorded (Lizama et al., 2004; Leite et al., 2018). The species of the family Dactylogyridae Bychowsky, 1933 are the most know gill parasites in the Neotropical region and are generally not highly pathogenic (Boeger & Vianna, 2006). This family of parasites includes *Tereancistrum* Kritsky, Thatcher & Kayton, 1980 whose main morphological characteristic is the presence of sclerites associated with ventral anchors (Kritsky et al., 1980).

In this study, a new species of *Tereancistrum* was found in the gills of *P. lineatus* from the Tietê-Batalha River basin, São Paulo State, Brazil during an investigation of the parasitic fauna of this fish. We provide a morphological description and illustrations pertinent to its identification.

Material and Methods

Fifty specimens of *P. lineatus* were collected at two sites on the Batalha River, part of the Tietê-Batalha basin, in São Paulo State, Brazil. This river is located in the municipalities of Reginópolis (21°53'17"S, 49°13'31"W) and Piratininga (22°24'46"S, 49°05'05"W). Fish samplings were performed between June 2015 and June 2016. The hosts presented mean standard length and mean weight of 28.45 ± 6.24 cm and 671.48 ± 542.74 g, respectively.

Hosts were collected using nylon monofilament gillnets with different mesh sizes (with a distance of 2 to 10 cm between nodes) placed at varying heights. At each of the sampling points, the nets were installed perpendicularly and in a half-moon shape at sunset (around 5:00 p.m.), and the fish were collected at sunrise (around 5:00 a.m.). After being collected, fish that were still alive were anesthetized with a eugenol-based solution (clove oil) (65 mg/L) and euthanized through the physical method of medullary collapse. Collected fish were individually packed in plastic bags and frozen for two days until necropsy.

During the necropsy, the gills were removed, and the gill arches were separated and placed in a glass jar with water. It was shaken so that the parasites detached from the filaments. Glass content was filtered through at 53 µm mesh sieve, and the contents retained in the sieve were analyzed under a stereomicroscope. The parasites were collected and stored in 70% ethanol solution. For identification, the parasites were cleared and mounted in Gray & Wess's (1950) medium, or stained with Gomori's trichrome and mounted on Canada balsam for the analysis of the internal organs (Gomori, 1950).

The illustrations were prepared with the aid of a camera lucida attached to a Leica DMLS microscope. The identifications and morphological analysis were performed using the Trinocular Nikon E200 microscope and the Motic computerized image analysis system (Moticam 5.0MP). Measurements (in micrometers) were expressed as the mean followed by the standard deviation (SD) and the range in parentheses.

The quantitative descriptors were obtained based on Bush et al. (1997). The terminology related to the sclerites followed the recommendations of Kritsky & Mizelle (1968) and the coils description of the male copulatory organs follows that of Kritsky et al. (1985).

Voucher specimens from the analyzed hosts were deposited in the Fish Collection of the Fish Biology and Genetics Laboratory of the Department of Biosciences of Botucatu at São Paulo State University, Botucatu (UNESP Botucatu) in Botucatu, São Paulo, Brazil (reference number IBB 22918). Parasite holotypes and paratypes were deposited in the Zoology Collection of the National Institute of Amazonian Research (INPA) (holotype nº INPA 809, paratype nº 810) in Manaus, Amazonas, Brazil and in the Helminthological Collection of the Department of Biosciences at UNESP (paratypes nº 578L, 579L, 580L) in Botucatu.

All the procedures followed the guidelines and standards of the Brazilian Biodiversity Information and Authorization System (SISBIO) (authorization nº: 40998-2); in addition, the anesthesia and euthanasia methodologies used on the fish followed the guidelines of the National Council for the Control of Animal Experimentation (CONCEA),

and the research project was submitted to the Research Ethics Committee for Animal Experimentation of the Universidade do Sagrado Coração (authorization nº 3295230615) in the municipality of Bauru, São Paulo State, before it could be carried out.

Results

Monogenea Carus, 1863

Monopisthocotylea Odhner, 1912

Dactylogyridae Bychowsky, 1933

Tereancistrum Kritsty, Thatcher and Kayton, 1980

Tereancistrum takemotoi n. sp.

DESCRIPTION (based on 17 specimens) (Figure 1): Body elongated and fusiform, 539.2 ± 69.5 (359.7-684.1) long; greatest width 130.1 ± 27.6 (95.1-200) at the level of the gonads. Tegument smooth. Cephalic lobes developed; head organs well developed. Eyespots 2 pairs; component granules subspherical. Pharynx ovate, 31.3 ± 3.5 (27.5-36.5) wide, 43.3 ± 5.8 (33.9-52.5) long; esophagus long; intestinal ceca confluent posterior to testis. Peduncle elongate, moderate in width; haptor globose to subhexagonal, 94.5 ± 10.8 (78-109) wide, 62.4 ± 6.2 (54-77.2) long. Hooks 12 ± 1.6 (10.2-14.6) long, with erect thumb, slightly curved shaft and point, and small proximal enlargement of shank; FH loop approximately one half of shank length. Ventral anchor robust, 32.3 ± 2.1 (27.1-35.1) long, with deep root well-developed and superficial root with uneven protuberance, short and straight shaft and recurved point; base 13.7 ± 2.4 (9.5-16.7) wide. Accessory anchor sclerite 30.7 ± 2.1 (28.3-34.7) long, robust, with spatulate

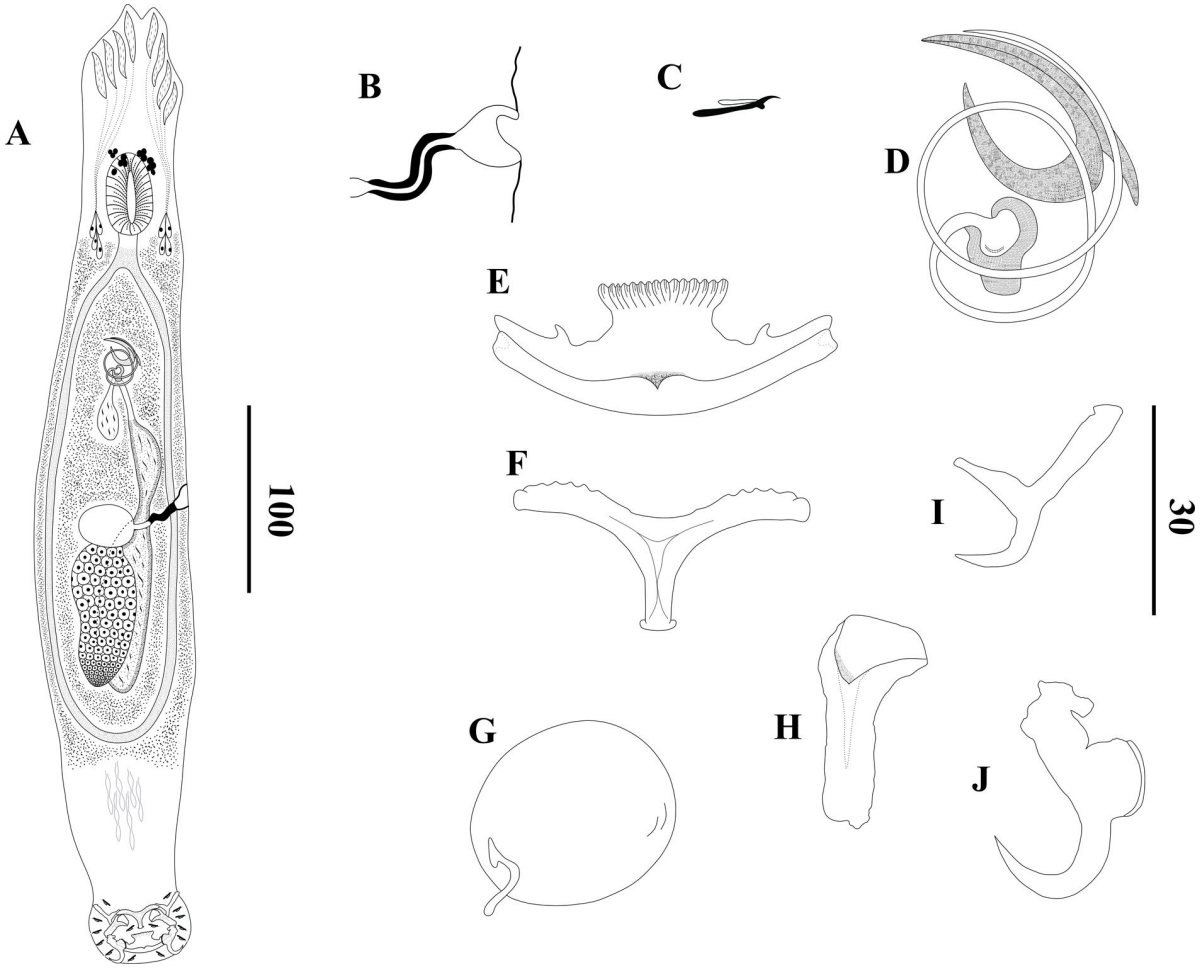

Figure 1. *Tereancistrum takemotoi* n. sp. (ventral view). (A) Holotype (ventral view); (B) Vagina; (C) Hook; (D) Copulatory complex; (E) Ventral bar; (F) Dorsal bar; (G) Egg; (H) Accessory anchor sclerite; (I) Dorsal anchor; (J) Ventral anchor.

end. Dorsal anchor 31.7 ± 1.6 (27.9-33.4) long, with widely divergent roots, short shaft and straight point; base 23.1 ± 2.6 (17.4-26.3) wide. Ventral bar 50.4 ± 5.7 (42.7-58.7) long, anvil-shaped and with corrugated anterior projection. Dorsal bar 38.9 v± 3.9 (30.8-45.3) long, Y-shaped, with internal groove and ends corrugated. Gonads overlapping. Male copulatory organ tapered, tubular, coiled, with 2 1/4 counterclockwise rings; ring diameter 19.4 ± 2.2 (14.8-21.6). Accessory piece 23.2 ± 2.4 (20-27.1) long, variable, not articulated with the base of male copulatory organ. Vagina sinistral, forming a sclerotized sinuous tube. Vitelline follicles random throughout trunk but absent in regions of gonads and copulatory complex.

Taxonomic Summary

Type-host: Prochilodus lineatus Valenciennes, 1837 (Characiformes: Prochilodontidae)

Location: Batalha River, Tietê-Batalha River basin, São Paulo State, Brazil (21°53'17"S; 49°13'31"W)

Site of infestation: Gills

Infestation: Prevalence: 29.3%, Mean intensity: 13.2 ± 28.3

Types: holotype n° INPA 809, paratypes nº INPA 810, 578L, 579L, 580L

Etymology: The specific epipet *takemotoi* is a tribute to Dr. Ricardo Massato Takemoto for his significant contributions to the study of the monogenean c fish parasites, including those in *Tereancistrum.*

Remarks and Discussion

Nine species of *Tereancistrum* have been described after being found in Neotropical species of Characiformes. Except by *Tereancistrum ornatus* Kritsky, Thatcher & Kayton, 1980 reported in gills of *Prochilodus reticulatus* Valenciennes, 1850 from the Colombia, the other eight species were found in Brazil: *Tereancistrum kerri* Kritsky, Thatcher & Kayton, 1980 in *Brycon melanopterus* Cope, 1872; *Tereancistrum parvus* Kritsky, Thatcher & Kayton, 1980 in *Leporinus fasciatus* Bloch, 1794; *Tereancistrum toksonum* Lizama, Takemoto & Pavanelli, 2004; *Tereancistrum curimba* Lizama, Takemoto & Pavanelli, 2004; *Tereancistrum pirassununguensis* Cepeda, Ceccarelli & Luque, 2012 all in *P. lineatus*; *Tereancistrum arcuatus* Cohen, Kohn & Boeger, 2012 in *Salminus brasiliensis* Cuvier, 1816; *Tereancistrum paranaensis* Karling, Lopes, Takemoto & Pavanelli, 2014 in *Schizodon borellii* Boulenger, 1900, and *Tereancistrum flabellum* Zago, Yamada, Franceschini et al., 2017 in *Leporinus* spp. (Kritsky et al., 1980; Lizama et al., 2004; Cepeda et al., 2012; Cohen et al., 2012; Karling et al., 2014; Zago et al., 2017).

The new species is assigned to *Tereancistrum* genus due to the presence of distinctly spatulate accessory sclerites articulated to the tip of the superficial root of the ventral anchors, its two pairs of eyes, its overlapping gonads, and similar hooks (Kritsky et al., 1980; Kritsky & Boeger, 1989). The main characteristic that distinguishes this species from its congeners is the configuration of the ventral bar, which has an anvil-shaped and anterior projection with undulations and striations.

Regarding the male copulatory organ, *Tereancistrum takemotoi* n. sp. differs from *T. kerri* and *T. arcuatus* because these two species have a simple male copulatory organ, with a simple and unrolled conical tube, unlike *Tereancistrum takemotoi* n. sp. which exhibits a male copulatory organ in rings. The other species of the genus that also have a male copulatory organ arranged in rings, but with different number and direction of rings are: *T. parvus* with 3 ½ rings counterclockwise, *T. ornatus* with with 1 ¼ rings counterclockwise, *T. paranaensis* with 2 ½ rings clockwise, *T. flabellum* with 3 ½ ring clockwise, and *T. curimba* with 1 ¼ rings counterclockwise, while *Tereancistrum takemotoi* n. sp. has 2 ¼ rings arranged in counterclockwise.

Tereancistrum kerri and *T. ornatus* present an accessory piece articulated with the base of the male copulatory organ, while *Tereancistrum takemotoi* n. sp. and the other congeners exhibit an accessory piece not articulated with the base of the male copulatory organ.

Tereancistrum kerri and *T. flabellum* have a dextral dorsal vagina, while *Tereancistrum takemotoi* n. sp. and the other species of the genus have a sinistral vagina. In addition, for *Tereancistrum takemotoi* n. sp. the vagina is characterized as a sinuous and strongly sclerotic tube, unlike *T. ornatus* and *T. pirassununguensis* that have a slightly sclerotic vagina with a thin tube without sinuosity; *T. parvus* and *T. flabellum* which have a cone-shaped sclerotic vagina; and *T. paranaensis* and *T. curimba* that present sclerotized vagina forming a simple tube without coils.

Regarding haptor structures, *Tereancistrum takemotoi* n. sp. has a Y-shaped dorsal bar and undulations in the anterior portion, while *T. kerri* has a U-shaped dorsal bar and *T. parvus* a V-shaped one. *T. paranaensis*, *T. arcuatus*, and *T. flabellum exhibits* a straight dorsal bar with slightly enlarged and rounded ends.

Tereancistrum takemotoi n. sp. exhibits a robust accessory sclerite with a spatulated end and a small deep groove opening at one end, unlike *T. arcuatus*, which has a thin accessory sclerite, with a longitudinally present groove on the entire surface; and *T. pirassununguensis*, which present a narrow accessory sclerite, without expansions in the extremities. Moreover, *T. parvus*, *T. paranaensis*, *T. flabellum* and *T. curimba* have and intermuscular structure between sclerites, while *Tereancistrum takemotoi* n. sp. and the other congeners do not have this structure.

Tereancistrum kerri, *T. parvus*, *T. ornatus*, *T. paranaensis* and *T. curimba* have filaments in the ventral and dorsal anchors, while *Tereancistrum takemotoi* n. sp. and the other species of *Tereancistrum* do not have filaments in either anchor. Regarding the hooks, *T. kerri* and *T. arcuatus*, have hooks of unequal sizes, while *Tereancistrum takemotoi* n. sp. and the other species of the genus have hooks of the same size.

Tereancistrum toksonum is the morphologically closest species to *Tereancistrum takemotoi* n. sp. In addition to parasitizing the same host species, they are similar due to the morphology of their dorsal bars (Y-shaped), their dorsal anchors with roots (superficial and deep) very distant from each other, and they both have an elongated deep root; and also, they both exhibit thickening in the posterior margin of the ventral bar, resulting in a bent angle in this bar portion; the accessory piece is not articulated with the male copulatory organ, and they both exhibit a strongly sclerotized sinistral vagina forming a sinuous and evident tube. However, these species differ in that *T. toksonum* exhibits 1¼ rings of the male copulatory organ running counterclockwise (in *T. takemotoi* is 2¼ rings). Although the two species' dorsal bars have the same Y-shape, the dorsal bar in *T. takemotoi* have undulations in the anterior margin. The species also exhibit differences in the ventral bars: it is anvil-shaped in *T. takemotoi* and arched in *T. toksonum*.

This study presents the fourth species of the *Tereancistrum* described as a parasite of *P. lineatus*. The data obtained in this work broaden the geographic distribution of the genus and increase the knowledge on the parasitic diversity of Brazilian fish in freshwater environments.

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