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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

A NEW SPECIES OF *TRINIBACULUM* (MONOGENEA: DACTYLOGYRIDAE) PARASITE OF THE GILLS OF *ASTYANAX FASCIATUS* (CUVIER, 1819) (CHARACIFORMES: CHARACIDAE) IN A NEOTROPICAL RIVER, SÃO PAULO STATE, BRAZIL

UNA NUEVA ESPECIE DE *TRINIBACULUM* (MONOGENEA: DACTYLOGYRIDAE) PARÁSITO DE LAS BRANQUIAS DE *ASTYANAX FASCIATUS* (CUVIER, 1819) (CHARACIFORMES: CHARACIDAE) EN UN RÍO NEOTROPICAL, ESTADO DE SÃO PAULO, BRASIL

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Abstract

A new species of Monogenea - *Trinibaculum pinctiarum* n. sp. is described from the gills of *Astyanax fasciatus* known as "lambari do rabo vermelho", sampled in the Paranapanema River, Jurumirim reservoir, São Paulo State, Brazil. The new species is allocated to the genus *Trinibaculum* because it has a double ventral bar and dextro-lateral vagina. It is the second species (first species is *Trinibaculum altiparanae*) belonging to this genus that parasitizes the gills of the fish genus *Astyanax*. This monogenean is similar to *T. altiparanae* regarding the haptor structures and the bulbous distal vagina. However, *Trinibaculum pinctiarum* n. sp. differs in having: (1) coiled male copulatory organ, (2) accessory structure shaped as tweezers, (3) absence of post-median process in the ventral bar, and (4) hooks with depressed thumb. The phylogenetic hypothesis of the genus *Trinibaculum* (Consistency index = 87%, Retention index = 80%, length = 16) based on 13 transformation series is (*Trinibaculum rotundus*, *T. brazilensis*, *T. altiparanae*, *T. pinctiarum* n. sp.). Such hypothesis corroborates current phylogenies of characiformes fishes. Moreover, this study includes a new identification key for the species of the genus *Trinibaculum* based in morphological characters.

Keywords: *Astyanax fasciatus* - freshwater fish - identification key - Paranapanema river - phylogeny - taxonomy - *Trinibaculum pinctiarum* n. sp.

Resumen

Una nueva especie de Monogenea *Trinibaculum pinctiarum* n. sp. fue reportada parasitando las branquias de *Astyanax fasciatus*, conocido popularmente como "lambarí de rabo rojo", capturado en el río Paranapanema, reservatorio Jurumirim, São Paulo, Brasil. La nueva especie se asigna al género *Trinibaculum* por la presentación de una barra ventral doble y la apertura vaginal dextro-lateral. Esta es la segunda especie, junto con *T. altiparanae*, perteneciente a este género que parasita las branquias de los peces del género *Astyanax*. Esta especie se parece con *T. altiparanae* en las partes del haptor y la presencia de la vagina distal con el bulbo, pero difiere por tener: (1) un órgano copulador masculino enrollado, (2) pieza accesoria en forma de pinza, (3) proceso posteroventral ausente en la barra dorsal, (4) ganchos con el pulgar deprimido. La filogenia hipotética de especies de *Trinibaculum* (Índice de Consistencia = 87%, Índice de Retención = 80%, longitud = 16) sobre la base de 13 series de transformación es (*Trinibaculum rotundus* (*T. brazilensis* (*T. altiparanae*, *Trinibaculum pinctiarum* n. sp.))). Esta hipótesis se corrobora con la actual filogenia de los peces characiformes. Además en este estudio se presenta una nueva clave de identificación para las especies del género *Trinibaculum* basada en caracteres morfológicos.

Palabras clave: Clave de identificación - Filogenia - Peces de agua dulce - río Paranapanema - Taxonomía - *Trinibaculum pinctiarum* n. sp.

INTRODUCTION

Fishes show a considerable diversity of associate parasite organisms and the class Monogenea Bychowsky, 1937 is an important group of ectoparasites of Neotropical fishes (Eiras *et al*., 2010). The interest in studying monogeneans has been updated considering the amount of publications describing new species or even approaching aspects like pathology, ecology and evolutionary relations.

Evolutionary relations are of great importance for the comprehension of different *taxa* or for relations among hosts (Eiras *et al*., 2010). However, little is known about the relations of monogeneans parasites at the levels of family, sub-family, genus and species (Kritsky & Boeger, 1989; Simková *et al*., 2003).

Currently, approximately 365 monogeneans species are described from Brazil (Cohen, 2013). Although, this number might be underestimated given the remarkable diversity of hosts from the Neotropical region that have not been surveyed yet from a parasitological approach (Reis *et al*., 2003) and also the specificity of these parasites with their hosts or even hosts that are phylogenetically close (Eiras *et al*., 2010).

The Dactylogyridae Bychowsky, 1933 family is composed of species that are common fish gills parasites (Thatcher *et al*., 2006). Within this family, the genus *Trinibaculum* Kritsky, Thatcher & Kayton, 1980 is characterized by showing double dorsal bars, dextro-lateral vagina, confluent intestinal caecum and intercecal gonads, as proposed by Kritsky *et al*. (1980). Species of this genus have been recorded in Characiformes fishes, such as Bryconidae, Anostomidae and Characidae families.

To date, three other species of the genus *Trinibaculum* have been described: *Trinibaculum brazilensis* Kritsky, Thatcher & Kayton, 1980 in *Brycon melanopterus* (Cope, 1872) from Januacá lake, Amazon State (Kritsky *et al*., 1980); *Trinibaculum rotundus* Karling, Lopez, Takemoto & Pavanelli, 2011 in *Schizodon borelli* (Boulenger, 1900) from the Upper Paraná River floodplain, Paraná State

(Karling *et al*., 2011); and *Trinibaculum altiparanae* Abdallah, Azevedo & Silva, 2013 in *Astyanax altiparanae* Garutti & Britski, 2000 from the Peixe River, São Paulo State. Brazil (Abdallah *et al*., 2013).

This study describes a new species *Trinibaculum pinctiarum* n. sp. which was reported parasitizing the gills of *Astyanax fasciatus* (Cuvier, 1819) from the Paranapanema River, Jurumirim reservoir, São Paulo State, Brazil, and presents a phylogenetic hypothesis for the genus *Trinibaculum*. An identification key for the known species of the genus *Trinibaculum* is also provided.

MATERIAL AND METHODS

Sampling, processing and parasites identification

Eighty specimens of *A. fasciatus* were captured from April/2011 to October/2011 with gillnets in the Paranapanema River, Jurumirim reservoir (23°28'20.28"S 48°38'34.40"W - Google Earth), Angatuba municipality, São Paulo State, Brazil. Each fish specimen was placed in separate plastic bags and frozen to perform necropsy subsequently. At the laboratory, the gills were removed and analyzed using a stereomicroscope. The parasites found were stored in flasks with 70% ethanol. Some parasite samples were mounted in Hoyer to visualize the sclerotized structures (Eiras *et al*., 2006). The morphological analyses and photomicrographs of the parasites were obtained using the Differential interference contrast microscopy (Leica DMLB 5000, Leica Microsystems, Wetzlar, Germany). The illustrations were made with the aid of a camera lucida mounted on a Leica DMLS microscope and the image was processed using the program CorelDraw X4. Measurements were obtained using a computerized image analysis system (LAS, Leica Microsystems). The morphometric results are presented as: mean in micrometers ± standard error followed by the amplitude and number of the specimens measured in parentheses. The ecological data of prevalence, mean intensity of infestation and mean abundance for the monogeneans collected on the gills of *A. fasciatus* are in accordance with Bush *et al.* (1997).

Type specimens were deposited in the National Research Institute of Amazon - Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas State, Brazil and in the Helminthological Collection of the Bioscience Institute of Botucatu - Coleção Helmintológica do Instituto de Biociências de Botucatu (CHIBB), São Paulo State, Brazil.

Phylogenetic analyses

The phylogenetic analyses of the species of the genus *Trinibaculum* was carried out using cladistics methods (*sensu* Henning, 1966). The characters used in the phylogenetic analysis were recuperated using the literature and analysis of paratypes for comparison (*T. altiparanae* paratypes: CHIBB 052L, 053L and 054L). The polarization of the characters followed Watrous & Wheeler (1981) using *Apedunculata discoidea* Cugliana, Cordeiro & Luque, 2009, as the out group. The character matrix for the cladistics analyses was constructed and edited using WinClada (Nixon, 1999). The parsimonious analyses executed using the computer phylogenetic analysis program NONA (Goloboff, 1999). The analysis was performed utilizing heuristic analysis with Maximum trees to keep = 1000, Number of replications = 1000, Starting tree per rep = 10, Multiple TBR + TBR, and Bootstrap; Number of replications = 1000, Number of search reps = 10, Starting trees per rep = 10, Max trees = 100, to obtain the most parsimonious cladogram.

Characters used in the phylogenetic analysis

Number in parenthesis representing the character condition, (0) represents plesiomorfic state and (1) represents apormorfic state. The symbol (?) represents lost character or no visualized in the taxon. A total of 13 characters is represented in the polarized matrix of characters showed in Table 1. The characters analyzed are:

A – Eyes disposition. (0) Members of anterior pair of eyes smaller and closer to each other than member of the posterior pair; (1) pairs similar in size, in which members of the posterior pair are

closer to each other than the members of the anterior pair.
B – Cephalic lobes. (0) Poorly developed; (1) Moderately developed.
C - Peduncle. (0) Absent; (1) Present.
D - Vagina opening level. (0) At the level of the male copulatory organ; (1) Below the level of the male copulatory organ.
E – Male copulatory organ. (0) Coiled; (1) Simple tube.
F – Ventral anchor shaft**.** (0) Curved; (1) Straight.
G - Dorsal anchor deep root. (0) Long/Well developed; (1) Short/Poor developed.
H - Ventral bar ends**.** (0) Simple ends, without expansion; (1) Expanded ends faced laterally.
I - Dorsal bar. (0) Single; (1) Double.
J - Vagina with sclerotized distal bulbous. (0) Absent (1) Present.
K - Gonads disposition. (0) Testis dorsolateral to the ovary; (1) Testis post-dorsal to the ovary.
L – Trunk shape. (0) Disc-shape; (1) Fusiform.
M - Ventral anchor roots. (0) Long deep root, both extending laterally to opposite sides from the base; (1) Short deep root, both depressed/extending inferiorly from the base.

RESULTS

Class Monogenoidea Bychowsky, 1937
Subclass Polyonchoinea Bychowsky, 1937
Order Dactylogyridea Bychowsky, 1937
Dactylogyridae Bychowsky, 1933
Ancyrocephalinae Bychowsky, 1937
Trinibaculum Kritsky, Thatcher & Kayton, 1980
Trinibaculum pinctiarum n.sp. (Figs. 1, 2, 3, 4 and 5)

Description (Based on 11 specimens): Body fusiform, divided in cephalic area, trunk, peduncle and haptor, body length 294.1 ± 27.6 (232-416; n=7); maximum width 72.5 ± 3.7 (59.8-83; n=7) usually at the level of the copulatory complex. Three pairs of cephalic lobes moderately developed; three pairs of cephalic organs: two superior and one bilateral; cephalic gland not observed (Fig. 1). Four eyes similar in size: members of the posterior pair of eyes located closer to each other than the members of the anterior pair of eyes (Fig. 1);

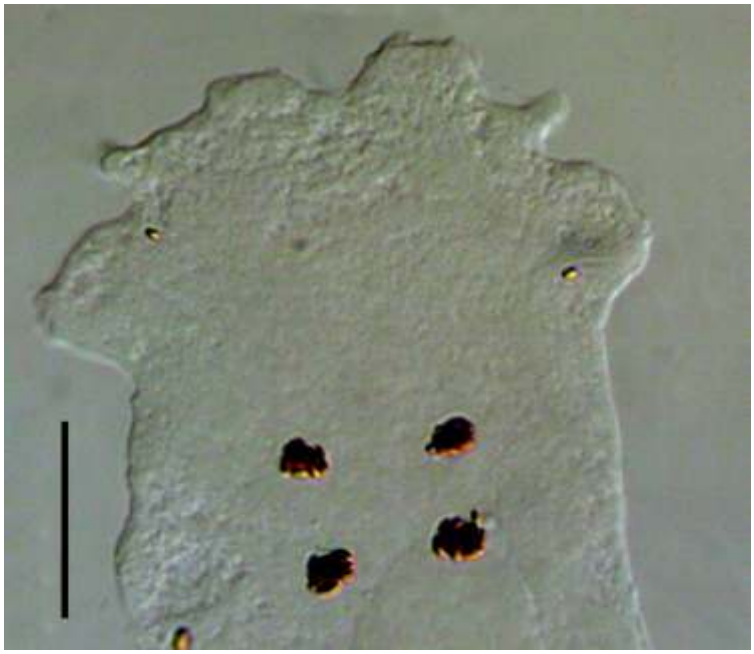

Figure 1. *Trinibaculum pinctiarum* n. sp. Cephalic extremity. Scale bar: 20 µm.

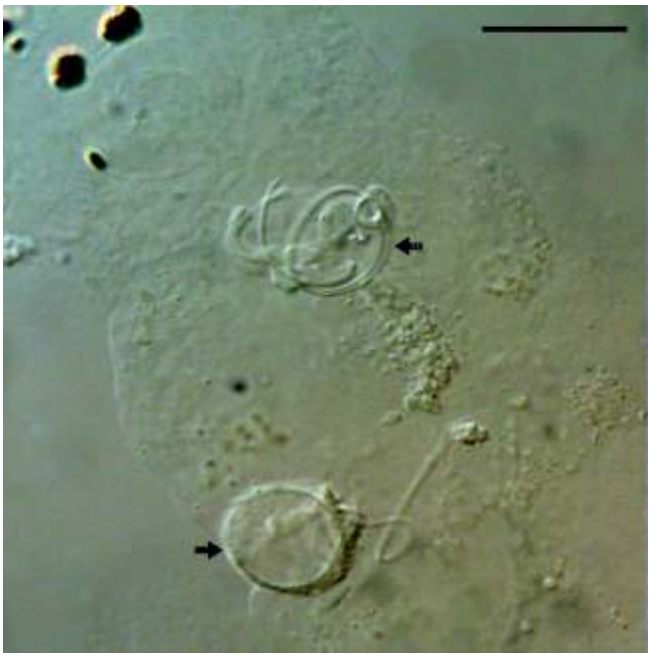

Figure 2. *Trinibaculum pinctiarum* n. sp. Male copulatory organ (segmented arrow) and vagina (full arrow). Scale bar: 20 µm.

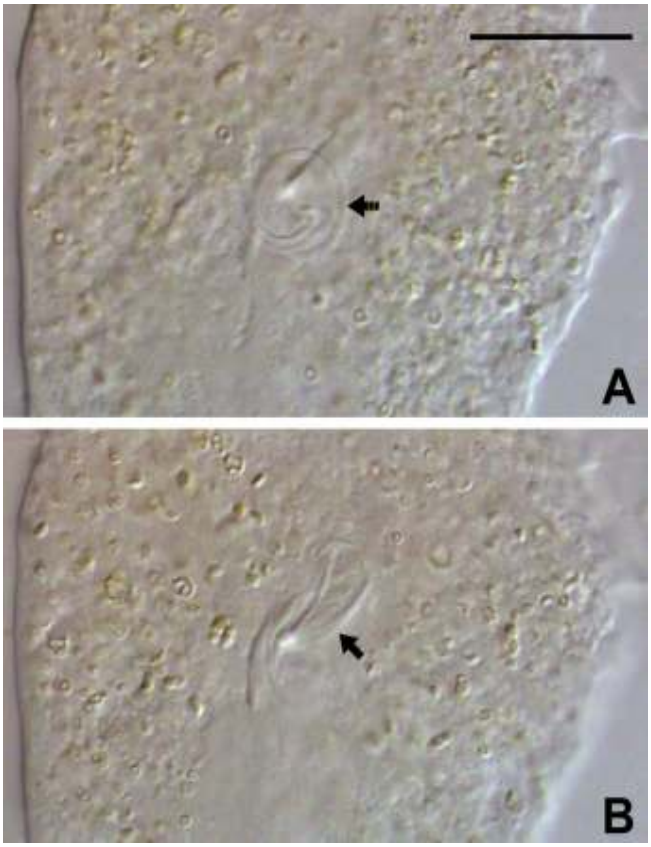

Figure 3. *Trinibaculum pinctiarum* n. sp. Male copulatory organ: (A) Cirrus (segmented arrow) and (B) Acessory piece (full arrow). Scale bar: 20 µm.

accessory granules distributed in the cephalic region and anterior to the trunk. Spherical pharynx, 19.8 ± 1.3 (15.5-25.5; n=7) of diameter. Peduncle present, short. Hexagonal haptor, 46.2 ± 1.8 (41.1-49.8; n=4) long, 54.5 ± 0.9 (51.8-55.6; n=4) wide, comprising a complex of anchors, bars and hooks (Fig. 4). Anchors different in size and shape: bigger ventral anchors. Ventral anchor with well-defined roots, shaft moderately curved, tip recurved, 32.8 ± 0.56 (29.6-35.7; n=14) long, base 12.3 ± 0.5 (9.1-15.6; n=16) wide; anchor filaments extending from shaft to the beginning of the tip in the lateral edge. Dorsal anchor with superficial root well developed and short deep root; shaft and tip curved, 10.3 ± 0.3 (9.4-11,1; n=6) long, base 8.3 ± 0.2 (7.1-9.5; n=13) long. Ventral bar yoked-shaped, 34.6 ± 0.86 (32.1-37.8; n=8) long, 3.9 ± 0.3 (3.1-5.3; n=8) wide; with constriction in the anterior margin and widened lateral ends faced laterally. Dorsal bar double sigmoid, 14 ± 0.89 (12-16; n=6) long. Seven pairs of hooks with Ancyrocephalinae distribution: 10 ventral and 4 dorsal. Hooks different in size and shape; pairs 1 and 5 - 19,2 ± 0,6 (13,8 - 23,4; n=) long, widened in about of 1/2 of the shaft length, depressed thumb, tip and lamina curved; pairs 2, 3, 4, 6 and 7 - 22.1 ± 0.4 (14.6-28.6; n=51) long; widened in about 2/3 of the shaft length, depressed thumb, tip and lamina curved. Hook filament extending up to 1/3 of the stem length. Cirrus coiled with approximately 1 1/2 rings counterclockwise, total length 74.6 ± 3.4 (62.5-85.6; n=6), and diameter of first ring 12.9 ± 0.4 (12.5-13,7; n=3) (Fig. 3). Accessory structure shaped as tweezers, with the anterior portion bifid closing posteriorly, 22 ± 1.3 (18.6-25.1; n=6) long, 8.5 ± 0.5 (7.4-10.4; n=5) wide; no articulated with the cirrus base (Fig. 3). Dextral vagina, sclerotized simple tube with distal bulbous (Fig. 2). Vitellaria distributed throughout the trunk except in the area of the gonads and male copulatory complex. Eggs not observed.

Taxonomic summary
Type-host: *Astyanax fasciatus* (Cuvier, 1819) (Characiformes: Characidae).
Locality: Paranapanema River, Jurumirim reservoir (23°28'20.28"S 48°38'34.40"W - Google Earth), Angatuba municipality, São Paulo State, Brazil.
Infestation site: Gills filaments.
Prevalence: 32.3 %.
Mean Intensity of infestation (Amplitude): 2.1 ± 0.7 (1 - 8).
Mean abundance: 0.7 ± 0.3.
Holotype: INPA 637.
Paratypes: INPA 638; CHIBB 123L, 124L, 125L, 126L, 127L and 128L.
Etymology: The specific epithet refers to the characteristic accessory structure shaped as tweezers from the Latin (*pinctiare* = pinch).

Taxonomic discussion
Trinibaculum pinctiarum n. sp. is similar to *T. altiparanae* due to the following characteristics: cephalic lobes moderately developed, eyes disposition (similar in size with the members of the posterior pair of eyes close to each other), vagina with ending in bulbous, and the morphology of the haptor structures. However, the new species differs from *T. altiparanae* because it shows cirrus comprising about 1 ½ counterclockwise rings (simple tube in *T. altiparanae*), accessory structure shaped as tweezers (considerable curved and basally bifid in *T. altiparanae*), post-median process in the ventral bar absent (present in *T. altiparanae*), hooks with depressed thumb (erected thumb in *T. altiparanae*).

This new species differs from the others of the genus because it presents fusiform body (disc shaped in *T. rotundus*), cephalic lobes moderately developed (incipient in *T. brazilensis* and *T. rotundus*), eyes similar with members of the posterior pair of eyes closer to each other than the members of the anterior pair (members of the posterior pair bigger and more distant in *T. brazilensis* and *T. rotundus*), cirrus comprising 1 ½ counterclockwise rings (simple tube in *T. brazilensis* and 1 ½ clockwise rings in *T. rotundus*), deep root of the dorsal anchor short (long in *T. brazilensis* and *T. rotundus*), dorsal bar with widened ends laterally directed (simple in *T. brazilensis* and *T. rotundus*), hooks with approximately 2/3 of widened shank and depressed thumb (straight thumb in *T. brazilensis*, and slender shaft *T. rotundus*).

An identification key of the genus *Trinibaculum.*

1a - Body disc-shaped; peduncle absent; point of the dorsal anchor well-recurved; dorsal anchor with point extending to the level of the superficial root and superficial root widened; ventral bar straight; hooks shank slender, without widening; cirrus coiled with clockwise rings….....*Trinibaculum rotundus*

1b – Body fusiform; peduncle present; point of the dorsal anchor slight or moderately curved; dorsal anchor with point extending to the next half of the length of the superficial root and no-widened superficial root; ventral bar in U or V shape; hook shank with proximal widening; cirrus as simple tube or coiled with counterclockwise rings…….....2

2a (1b) – Incipient cephalic lobes; four eyes in which the members of the posterior pair of eyes are bigger and more distant to each other than the member of the anterior pair of eyes; deep root of the dorsal anchor long; ventral bar with ends without widening; accessory structure articulated with the base of the cirrus; vagina as simple tube sclerotized….....*Trinibaculum brazilensis*

2b (1b) - Cephalic lobes moderately developed; four eyes similar in size, in which the members of the posterior pair of eyes are closer to each other; deep root of the dorsal anchor short; ventral bar with widened ends faced laterally; accessory structure non-articulated with the base of the cirrus; vagina as simple tube sclerotized with distal bulbous ….....3

3a (2b) - Post-median process prominent in the ventral bar and ends with striae; hooks of different sizes, in which the pairs 1, 2 and 5 are smaller and straight thumb; Cirrus in simple tube; accessory structure well-curved posteriorly bifid and piece flabellate anteriorly…….....*Trinibaculum altiparanae*

3b (2b) - Post-median process in the ventral anchor absent, without striae; hooks pairs 1 an 5 are smaller with depressed thumb; coiled cirrus; accessory structure shaped as tweezers.....*Trinibaculum pinctiarum* n. sp.

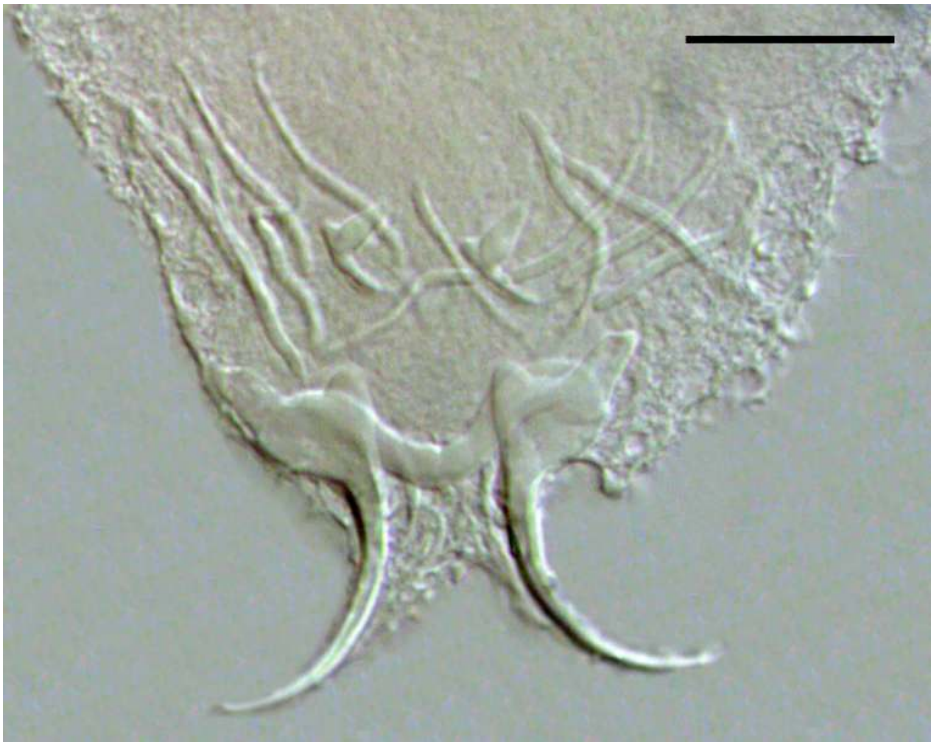

Figure 4. *Trinibaculum pinctiarum* n. sp. Haptor. Scale bar: 20 µm.

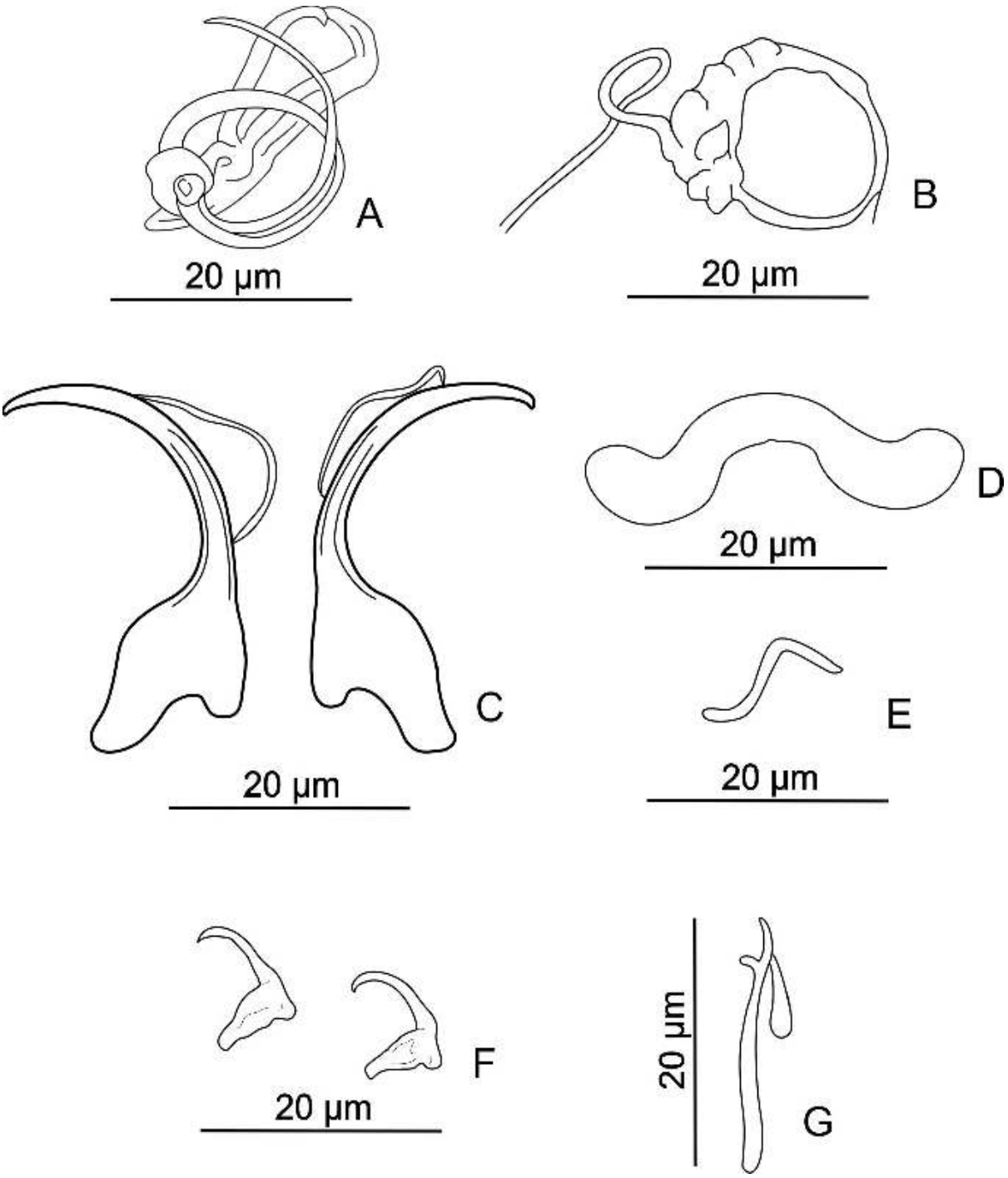

Figure 5. *Trinibaculum pinctiarum* n. sp. (A) Male copulatory organ; (B) Vagina; (C) Ventral anchor; (D) Ventral bar; (E) Dorsal bar; (F) Dorsal anchor; (G) Hook.

Phylogenetic analysis

The most parsimonious cladogram for the relations of the species within *Trinibaculum* genus of cladistics analysis (Consistency index = 87%, Retention index = 80% and length = 16) based on 13 transformation series based on the structures totality and body structures (Table 1) is (*Trinibaculum rotundus* (*T. brazilensis* (*T. altiparanae*, *Trinibaculum pinctiarum* n. sp.))) (Fig. 6). *Trinibaculum pinctiarum* n. sp. is a sister group of *T. altiparanae* supported by five sinapomorphies (A, B, G, H and J) with Consistency index = 100%, corroborating the great similarity between the two fish parasite species of the genus *Astyanax*, however, the character E (male copulatory organ) Consistency index = 50 % in *T. altiparanae* with *T. brazilensis* both present copulatory organ, being characterized as a probable reversion in both *taxa* through the comparison of the character with the outgroup. The character F (dorsal anchor shaft) Consistency index = 50% presents as a probable homoplasy between *T. brazilensis* and *T. rotundus*, given that this last is shown as sister group of (*T. altiparanae* + *Trinibaculum pinctiarum* n. sp.), supported by two sinapomorphies (C and L) IC = 100%.

Table 1. Polarized matrix of characters of species of the genus *Trinibaculum* Kritsky, Thatcher & Kayton, 1980.

	Characters												
Taxon	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**
Apenduculata discoidea	0	0	0	0	0	0	0	0	0	0	0	0	0
Trinibaculum brazilensis	0	0	1	1	1	1	0	0	1	0	1	1	1
Trinibaculum rotundus	0	0	0	1	0	1	0	0	1	0	0	0	1
Trinibaculum altiparanae	1	1	1	1	1	0	1	1	1	1	1	1	1
Trinibaculum pinctiarum n. sp.	1	1	1	1	0	0	1	1	1	1	?	1	1

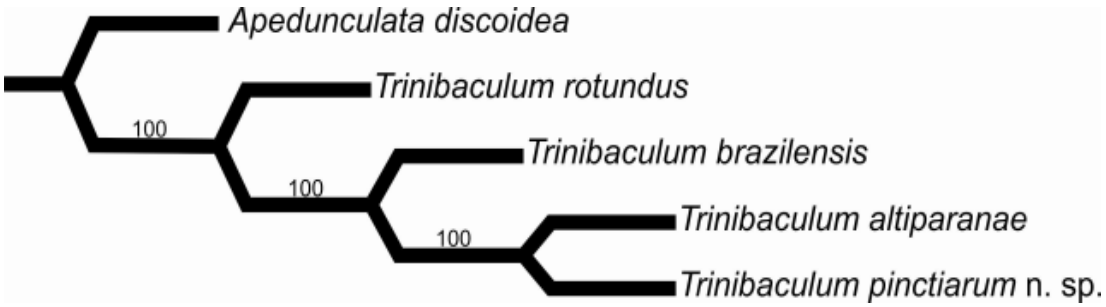

Figure 6. Phylogenetic hypothesis of the four species of *Trinibaculum* Kritsky, Thatcher & Kayton, 1980.

DISCUSSION

The Monogenea are discussed as an ideal parasite taxon for investigations of evolutionary issues because, among other reasons, they are generally host specific (Poulin, 2002). In most of the studies on parasite-host interaction, the specificity is understood to have a genetic basis, however it can also be a result of phenotypic plasticity (Little *et al*., 2006).

Trinibaculum pinctiarum n. sp. is the second species, together with *T. altiparanae*, which parasitizes the gills of fish belonging to the genus *Astyanax*, being found in *A. fasciatus* and *A. altiparanae*, respectively, even though these fish species are widely distributed in several habitats of the upper Paraná River and present quite similar ecological preferences (Bennemann *et al.*, 2005), evidencing high host specificity in morphologically and ecologically similar species.

The phylogenetic relationship among the species of *Trinibaculum* recovered in this study (Fig. 6) is totally congruent with the phylogeny of their hosts as proposed by Oliveira *et al.* (2011) and Mirande (2010). Thus, the host specificity of *Trinibaculum* species seems to be based on non plastic evolutionary events and possibly describes an interesting process of host-parasite coevolution.

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