



***Rhinoxenus* (Dactylogyridae) parasitizing piranhas (Serrasalminidae) at its southernmost limit of distribution (Paraná River, Argentina), with the description of two new species**

MARÍA A. ROSSIN¹, PABLO N. DE FRANCESCO², MANUEL M. IRIGOITIA¹, PABLO A. SCARABOTTI³, VERÓNICA TAGLIORETTI¹ and JUAN T. TIMI¹

¹Laboratorio de Ictioparasitología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata/ UNMdP, Instituto de Investigaciones Marinas y Costeras/IIMyC-CONICET, Funes 3350, 7600 Mar del Plata, Argentina

²Laboratorio de Neurofisiología, Instituto Multidisciplinario de Biología Celular/IMBICE-CONICET, Universidad Nacional de La Plata, Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC-PBA), Calle 526 y Cno. Gral. Belgrano, La Plata, Argentina

³Laboratorio de Ictiología, Instituto Nacional de Limnología/INALI-CONICET-UNL, Paraje El Pozo, Ciudad Universitaria UNL, 3000 Santa Fe, Argentina

Manuscript received on June 20, 2019; accepted for publication on September 9, 2019

How to cite: ROSSIN MA, FRANCESCO PN, IRIGOITIA MM, SCARABOTTI PA, TAGLIORETTI V AND TIMI JT. 2019. *Rhinoxenus* (Dactylogyridae) parasitizing piranhas (Serrasalminidae) at its southernmost limit of distribution (Paraná River, Argentina), with the description of two new species. *An Acad Bras Cienc* 91: e20190711. DOI 10.1590/0001-3765201920190711.

Abstract: Three piranha species, *Serrasalmus maculatus*, *S. marginatus* and *Pygocentrus nattereri*, living sympatrically in the lower Paraná River (Argentina) were examined searching for nasal monogeneans to know its diversity and distribution. Four species of monogeneans belonging to *Rhinoxenus* were found parasitizing the nasal cavities. Two new species are described, and new morphological data of 2 previously described species is provided. *Rhinoxenus argentinensis* n. sp. is characterized by having the male copulatory organ (MCO) as a coiled tube with a reel shaped-base; an elongated accessory piece articulated to base of MCO; a sinistral vagina with a sclerotized vestibule, and a sclerotized cap of the ventral anchor laterally modified forming a triangular expansion. *Rhinoxenus paranaensis* n. sp. is characterized by having a MCO as a coiled tube with a reel-shaped base; an accessory piece with an elongate proximal portion, a dilated distal portion with digitiform projections articulated to base of MCO; a sinistral vagina with 5-6 cuticular spine-shaped processes, and a sclerotized cap of the ventral anchor modified laterally forming a bilobate expansion. Additionally, multivariate discriminant analyses showed significant morphometric differences in the ventral anchors among *Rhinoxenus* species parasitizing ‘piranhas’.

Key words: Piranhas, nasal parasites, *Rhinoxenus* spp., South American rivers.

INTRODUCTION

Dactylogyrids of the genus *Rhinoxenus* Kritsky, Thatcher and Boeger, 1988 are parasites of the nasal cavities in characiform fish from the Neotropical

region (Domingues and Boeger 2005). The genus is considered to be monophyletic, and its members have been suggested as suitable markers of the coevolutionary history of their hosts (Boeger et al. 1995, Domingues and Boeger 2005).

The link between *Rhinoxenus* evolutionary history and the historical events in the host

Correspondence to: María Alejandra Rossin
E-mail: mrossin@mdp.edu.ar
ORCID: <https://orcid.org/0000-0002-4006-0951>

clade was reviewed by Domingues and Boeger (2005), who suggested that several processes, such as cospeciation, duplication, dispersion and extinction are required to explain the extant host-parasite associations. These processes apparently have differentially occurred on some *Rhinoxenus* clades, and the authors identified those species from piranhas, *R. piranhus* Kritsky, Boeger and Thatcher, 1988 and *R. euryxenus* Domingues and Boeger, 2005, as examples of cospeciation.

Rhinoxenus piranhus, the type species of the genus (Kritsky et al. 1988), was described from *Pygocentrus nattereri* Kner, 1858 from the Amazonas basin. It was subsequently reported in two additional host species, *Pristobrycon* sp. and *Serrasalmus spilopleura* Kner, 1858, from northern Brazil and French Guiana, and in *S. spilopleura*, *S. marginatus* Valenciennes, 1837 and *P. nattereri* from the Paraná River basin (Domingues and Boeger 2005, Rossin et al. 2017). Moreover, *R. euryxenus* was described from *S. marginatus* from the Paraná River, also reported as *S. spilopleura* in the same river and in the Amazonas basin, parasitizing *S. gouldingi* Fink and Machado-Allison, 1992, *S. rhombeus* (Linnaeus, 1766) and *S. striolatus* (Steindachner, 1908) (= *Pristobrycon striolatus*) (Domingues and Boeger 2005). However, the records of *S. spilopleura* in the Paraná basin are doubtful, since all of them have been synonymized as *S. maculatus*, being *S. spilopleura* restricted to northern basins (Jégu and Dos Santos 2001, Hubert et al. 2007). Consequently, only three species of piranhas inhabit the Paraguay and Paraná Rivers (Freeman et al. 2007): the widely distributed *P. nattereri* and *S. maculatus*, and *S. marginatus*, endemic to this basin (Jégu and Dos Santos 2001, Hubert et al. 2007).

In this work, representatives of the three piranha species, living sympatrically in the lower Paraná River and its tributaries of the Provinces of Santa Fe, Entre Ríos and Buenos Aires, Argentina, were examined in search of nasal monogeneans.

The purpose was to know the diversity of the *Rhinoxenus* genus in the region and provide further evidences on its distribution. As a result, 2 new species are proposed, and supplementary information is provided for 2 previously described species.

The ZooBank Life Science Identifier (LSID) of this publication is: urn:lsid:zoobank.org:pub:025E5890-AB9B-43B2-A002-E37F5DB73484.

MATERIALS AND METHODS

The fish were collected by gill net and fishing rod from different localities in Paraná River, Argentina, during 2015 and 2017, and were examined for nasal monogeneans. A total of 37 specimens of *S. maculatus* were collected in the following sites: Paraná River, Entre Ríos Province (33°2'8"S, 60°33'50"W); La Chancha lagoon, Santa Fe Province (31°49'4"S, 60°42'29"W); Pascualito creek, Santa Fe Province (31°44'57"S, 60°48'9"W); La Curva lagoon, Santa Fe Province (31°45'5"S, 60°48'9"W); Paraná de las Palmas River, Buenos Aires Province (33°57'45"S, 59°12'29"W); 21 specimens of *P. nattereri* were collected in Colastiné River, Santa Fe Province (31°39'1"S, 60°35'38"W); Paraná Viejo River, Santa Fe Province (31°49'29"S, 60°42'37" W); La Curva lagoon, Santa Fe Province (31°45'5"S, 60°48'9"W) and Paraná de las Palmas River, Buenos Aires Province (33°57'45"S, 60°48'9"W); and 14 specimens of *S. marginatus* collected in Colastiné River, Santa Fe Province (31°39'1"S, 60°42'37"W); Paraná Viejo River, Santa Fe Province (31°49'29"S, 60°42'37"W).

Methods of parasites collection, preparation and measurement were carried out as described by Kritsky et al. (1986, 1988) and Rossin et al. (2017). Note that the authors of the new taxa are different from the authors in this paper; see article 50.1 and

Recommendation 50A of the International Code of Zoological Nomenclature.

For the three-dimensional reconstructions of sclerotized structures, examinations, as well as image pre and post processing steps of Z-series, were conducted using the open source Fiji software (Schindelin et al. 2012). Stacks that showed a significant attenuation associated with increasing imaging depth were corrected with a linearly or exponentially increasing factor along the z-axis. Stacks were then deconvolved using the CMLE algorithm with the Huygens software (SVI, Hilversum, Netherlands), which provided a major improvement in the signal-to-noise ratio of the confocal stack, as well as a modest increase in resolution. Afterward, the brightness and contrast of the stacks were adjusted in Fiji, and three-dimensional isosurface reconstructions of the pieces were obtained with the three-dimensional plug-in viewer. A resampling factor of two was always used, and the threshold value was adjusted to obtain the desired level of detail. Finally, these three-dimensional models were processed in the open source software Blender (www.blender.org). The surfaces corresponding to objects of interest were isolated from surrounding debris, and evident artefacts due to fusion with adjacent debris were corrected by editing the model mesh. The location of these edited patches is clearly marked in purple in the supplementary videos. For the final presentation, a smoothing filter (factor: 0.5, repeats: 10) was applied to the meshes, and three-dimensional renders were produced. Full 360° rotation videos of the models were also produced and exported in AVI format.

Measurements are given in micrometers, as range followed by the mean and number of specimens measured in parentheses. Prevalence and mean abundance were calculated according Bush et al. (1997).

Morphometric differences among the ventral anchors of *Rhinoxenus* species found in piranhas

were explored using canonical analysis of principal coordinates (CAP; Anderson and Willis 2003, Anderson et al. 2008). Potential over-parameterization was prevented by choosing the number of PCO axes (m) that maximized a leave-one-out allocation success to groups (Anderson and Robinson 2003). CAP analyses were based on Euclidean distances on 9 morphometric variables of ventral anchors (Table I); these measurements were taken from 6 individuals of each of the four host species (Table I; Figs. 27-30). To test for significant morphometric differences of the ventral anchors among *Rhinoxenus* species, a permutation trace test (sum of squared canonical eigenvalues) was applied; the *P*-value was obtained after 9999 permutations. CAP analyses were performed by PERMANOVA+ for PRIMER package (Anderson et al. 2008).

Type specimens are deposited in the Helminthological Collection of the Museo de La Plata (HCMLP), La Plata, Argentina. Host nomenclature follows Froese and Pauly (2018).

RESULTS

Four species of monogeneans belonging to *Rhinoxenus* were found parasitizing the nasal cavities of serrasalmid fishes, 2 previously described species and 2 new species.

Subclass POLYONCHOINEA Bychowsky, 1937
Order DACTYLOGYRIDEA Bychowsky, 1937
Family DACTYLOGYRIDAE Bychowsky, 1933
***Rhinoxenus* Kritsky, Boeger and Thatcher, 1988**

***Rhinoxenus argentinensis* Rossin and Timi n. sp.**

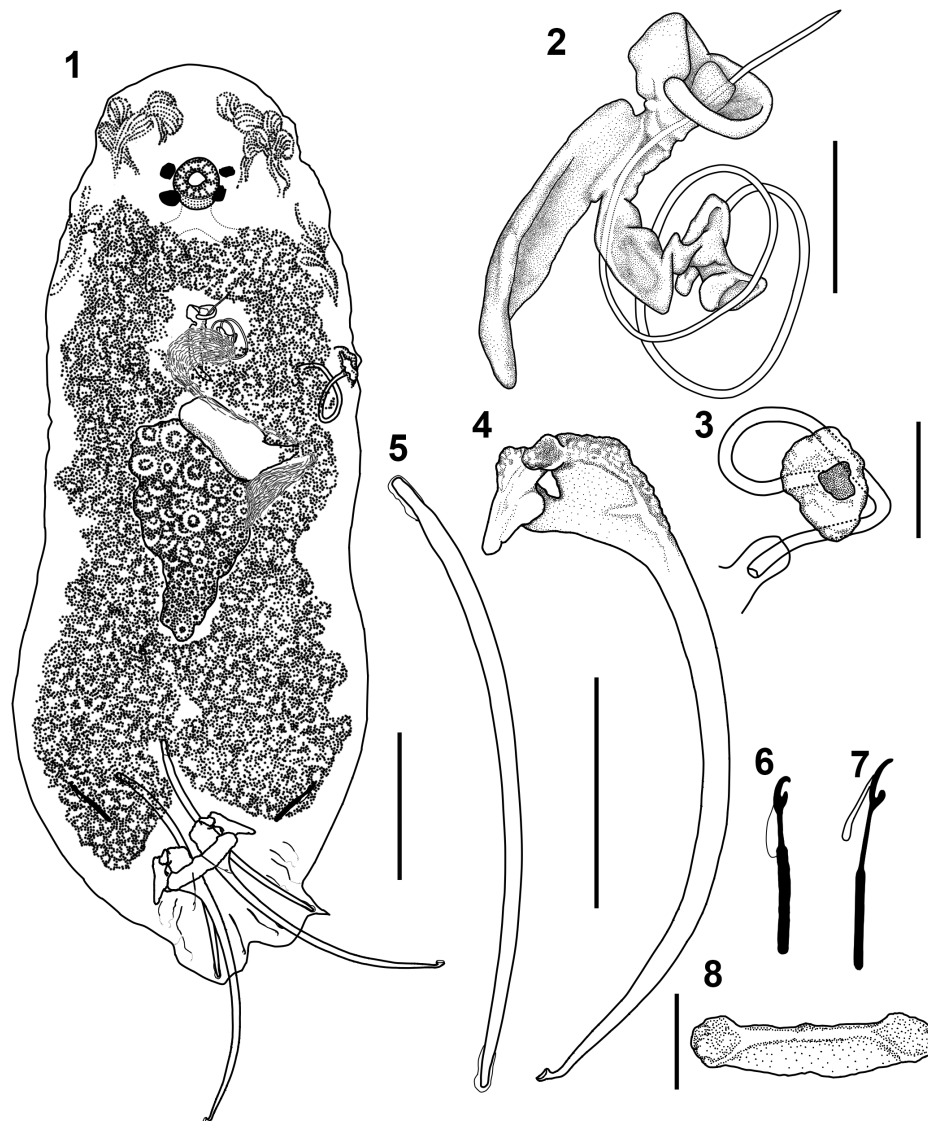
ZooBank registration: the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the International Commission on Zoological Nomenclature (ICZN). The Life Science Identifier (LSID) for *Rhinoxenus argentinensis* n. sp. is: urn:lsid:zoobank.org:act:0830901E-6720-4FB4-B8FB-137FCB58AC5C.

TABLE I
Comparative measurements (in μm) of ventral anchors of 4 *Rhinoxenus* species parasitizing piranhas.

	<i>R. argentinensis</i> n. sp. Host: <i>P. nattereri</i> (Fig. 27)	<i>R. paranaensis</i> n. sp. Host: <i>S. maculatus</i> (Fig. 28)	<i>R. piranhus</i> Host: <i>P. nattereri</i> (Fig. 29)	<i>R. euryxenus</i> Host: <i>S. marginatus</i> (Fig. 30)
N	6	6	6	6
Total length (TL)	136 (124-142)	132 (126-139)	149 (147-153)	136 (134-139)
Shaft length (SL)	116 (107-120)	99 (96-105)	104 (94-111)	109 (100-124)
Base length (BL)	31 (29-34)	42 (40-43)	45 (42-49)	42 (37-46)
Base width (BW)	24.5 (22-26)	31 (30-33)	43 (41-44)	45 (42-47)
Lateral expansion cap length (LECL)	20 (18-25)	15 (13-17)	22 (20-23)	30 (29-32)
Shaft proximal width (SPW)	9 (8-10)	7.7 (7-9)	12 (11-13)	14 (10-16)
Shaft middle width (SMW)	6 (5-7)	6 (5-7)	7.5 (7-8)	9 (9-10)
Shaft end width (SEW)	1.7 (1.5-1.9)	1.6 (1.4 -1.8)	2 (1.9-2.23)	2.8 (2.6-3.2)

Description (based on 10 specimens, Figs. 1-8, Figs. 9-14, Fig. 27): body 384-602 (481; n = 10) long; maximum width 154 -254 (203; n = 10). Cephalic lobes poorly developed; 4 to 5 pairs of cephalic organs; cephalic glands posterolateral to pharynx. Two pairs of eyes, posterior pair slightly larger; eye granules elongate (Fig. 1). Pharynx ovate strongly muscular 20-59 (44; n = 7) long, 22-31 (26; n = 6) maximum width. Haptor small, sub-rectangular, 73-89 (83; n = 10) long, 33-66 (50; n = 10) wide (Fig. 1). Ventral anchor 128-143 (137; n = 10) long, with inconspicuous roots; base 27-33 (31; n = 9) long, 18-22 (20; n = 9) wide; with sclerotized cap modified laterally into triangular expansion for articulation to ventral bar, and with a medial slight concavity where muscle connecting both anchors inserts; base with a triangular proximal hole; shaft moderately curved, pointed with a saucer-like termination. Maximum width of root including sclerotized cap 19-31 (24; n = 9) (Figs. 4, 12-14, 27 - Movie 1). Dorsal anchor straight, with rounded proximal end and distal pointed end with conspicuous sclerotized caps, 127-140 (132; n = 10) long (Fig. 5). Ventral bar with slightly expanded ends and thickened anterior border, 45-51 (49; n = 10) long, 10-14 (12; n = 9) maximum width (Figs. 8, 11). Hook pair 2 with erect thumb and shaft curved,

proximal 3/4 of shank inflated; filamentous hook (FH) loop extended to near the beginning of shank dilation, 23-26 (24; n = 9) long (Fig. 6); remaining hooks with erect thumb, long shaft lightly curved and shank proximally inflated; FH loop does not reach the beginning of shank dilation, 21-29 (24; n = 14) long (Fig. 7). Male copulatory organ (MCO) a coiled tube with 2 rings, base reel shaped, ring diameter 28-32 (30; n = 9), MCO length 140-206 (187; n = 9). Accessory piece elongated, 47-59 (52; n = 10) total length, complex, with laminar concave and elongate distal portion and dilated proximal portion composed of plates arranged in a concentric shape where the MCO emerges. Laminar ligament articulating the accessory piece with base of MCO, serving as guide for the insertion of MCO into the accessory piece (Movie 2-Figs. 2, 9). Testis oval, limits difficult to observe, seminal vesicle fusiform. Ovary elongated, with varying diameter, greatest width 30 -77 (60; n = 4), length 122-144 (134; n = 4). Ootype and uterus not observed (Fig. 1). Vagina sinistral, vaginal vestibule with sclerotized opening as a laminar ring, short sclerotized vaginal canal (Figs. 3, 10). Seminal receptacle elongated, lying diagonally to the left of body's midline. Eggs not observed.



Figures 1-8 - *Rhinoxenus argentinensis* n. sp. **1.** Composite drawing, whole mount, ventral view; **2.** Male copulatory complex (ventral view); **3.** Vagina; **4.** Ventral anchor (ventral view); **5.** Dorsal anchor (ventral view); **6.** Hook pair 2; **7.** Hook; **8.** Ventral bar (ventral view). Scale bars: 1= 100 μ m; 2, 3 and 8 = 20 μ m; 4-7 = 50 μ m.

TAXONOMIC SUMMARY

Type host: *Pygocentrus nattereri* Kner, 1858 (Serrasalminidae).

Type locality: Colastiné River, Colastiné Norte, Santa Fe Province, Argentina.

Other localities: Paraná Viejo River; La Curva lagoon, Sauce Viejo City, Santa Fe Province; Paraná de las Palmas River, Lima, Buenos Aires Province, Argentina.

Site: nasal cavities.

Etymology: The specific name refers to the country from which the host species was collected.

Prevalence: 46.15%.

Mean abundance: 1.53 (1-9).

Type material: Holotype MLP-He 7561 and 3 paratypes MLP-He 7562 were deposited in the Helminthological Collection of the Museo de La Plata, La Plata Argentina.

Comparative measurements and morphology: See Table I and Figs. 1-14, 27.

REMARKS

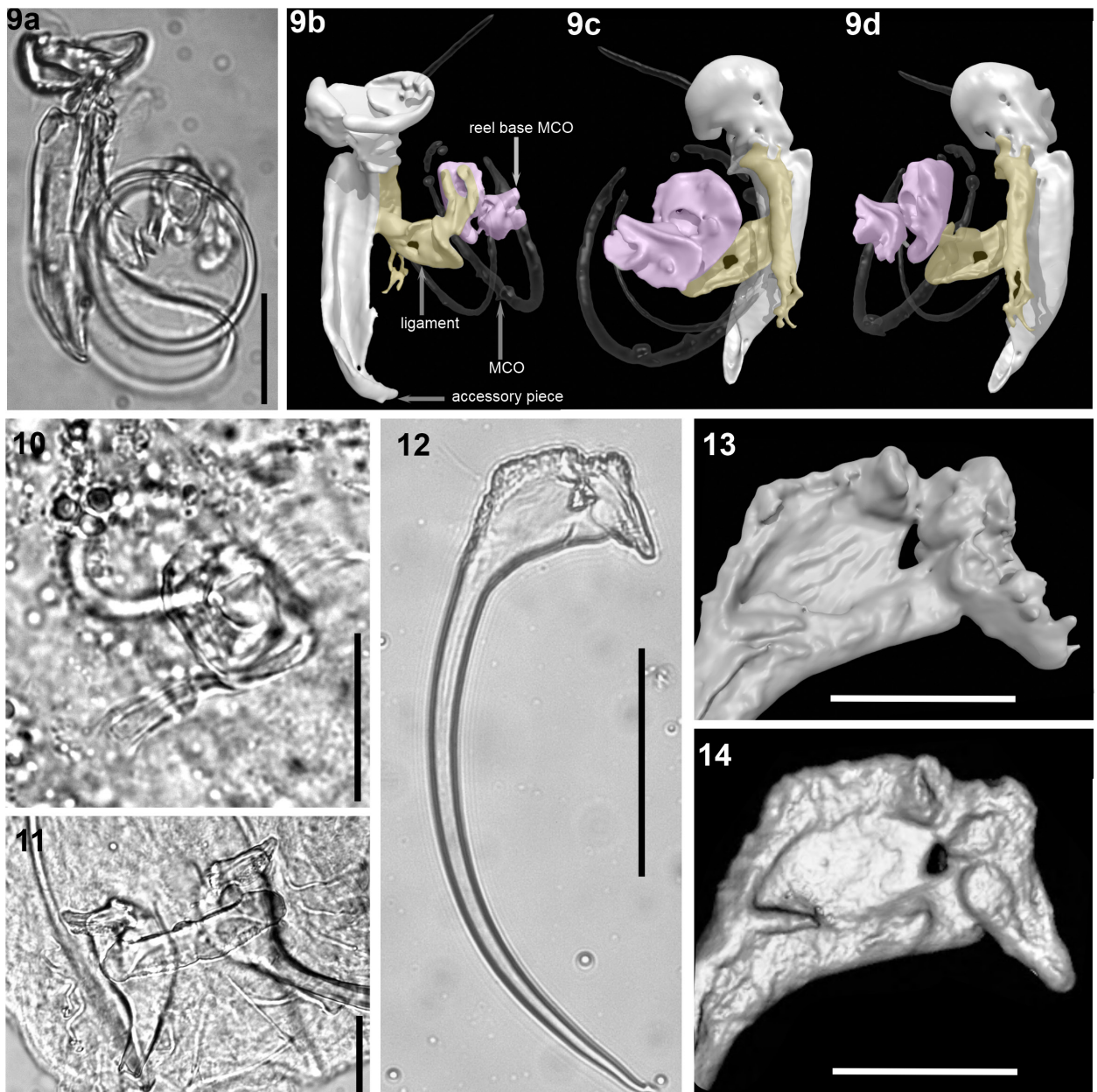
Rhinoxenus argentinensis n. sp. is distinguishable from most of the eight previously described congeners based on the morphology of the ventral anchors. Among the members of this genus, only 2 species, *R. piranhus* and *R. euryxenus*, share a saucer-like distal point in ventral anchors with the new species (Domingues and Boeger 2005). However, *R. argentinensis* n. sp. differs from *R. piranhus* by having a sclerotized cap laterally modified into a triangular expansion, whereas, in *R. piranhus*, the sclerotized cap is bilobate. The area of muscular insertion is also different between both species, being small and slightly pronounced in the new species, while it is larger and more pronounced in *R. piranhus*. The vaginal morphology also differs between both species, with the new species presenting a sclerotized ring in the vaginal opening, while *R. piranhus* has a cup-shaped vestibule (Kritsky et al. 1988, Domingues and Boeger 2005). Furthermore, the morphology of accessory piece in both species is different; *R. piranhus* presents an accessory piece with a broad proximal portion expanding to one side where the ligament joins the accessory piece dorsally, with a maximum width of 25 (Kritsky et al. 1988, Domingues and Boeger 2005, Rossin et al. 2017). Contrarily, the accessory piece of *R. argentinensis* n. sp. is elongated and its proximal portion is cup-shaped, with a maximum width of 19 (Fig. 2). The new species differs from *R. euryxenus* by the curvature of ventral anchor (radius of curvature = 66 vs 43 in *R. argentinensis* n. sp.) and by having the sclerotized cap expansion smaller than that of *R. euryxenus* (20 vs. 30). The area of muscular insertion is also different: *R. euryxenus* presents a big, elongated and pronounced concavity, whereas *R. argentinensis* n. sp. presents a circular and slightly pronounced insertion. The

vaginal morphology is also different in both species; the new species presents a ring-shaped sclerotized vestibule, whereas it is cup-shaped in *R. euryxenus*. Finally, although the morphology of the accessory piece of both species is similar, the proximal laminar portion is relatively longer regarding the cup-shaped distal portion in *R. argentinensis* n. sp.

***Rhinoxenus paranaensis* Rossin and Timi n. sp.**

ZooBank registration: the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the International Commission on Zoological Nomenclature (ICZN). The Life Science Identifier (LSID) for *Rhinoxenus paranaensis* n. sp. is: urn:lsid:zoobank.org:act:EEE4EF50-AFD0-4601-9ED0-FD26E77C96D1

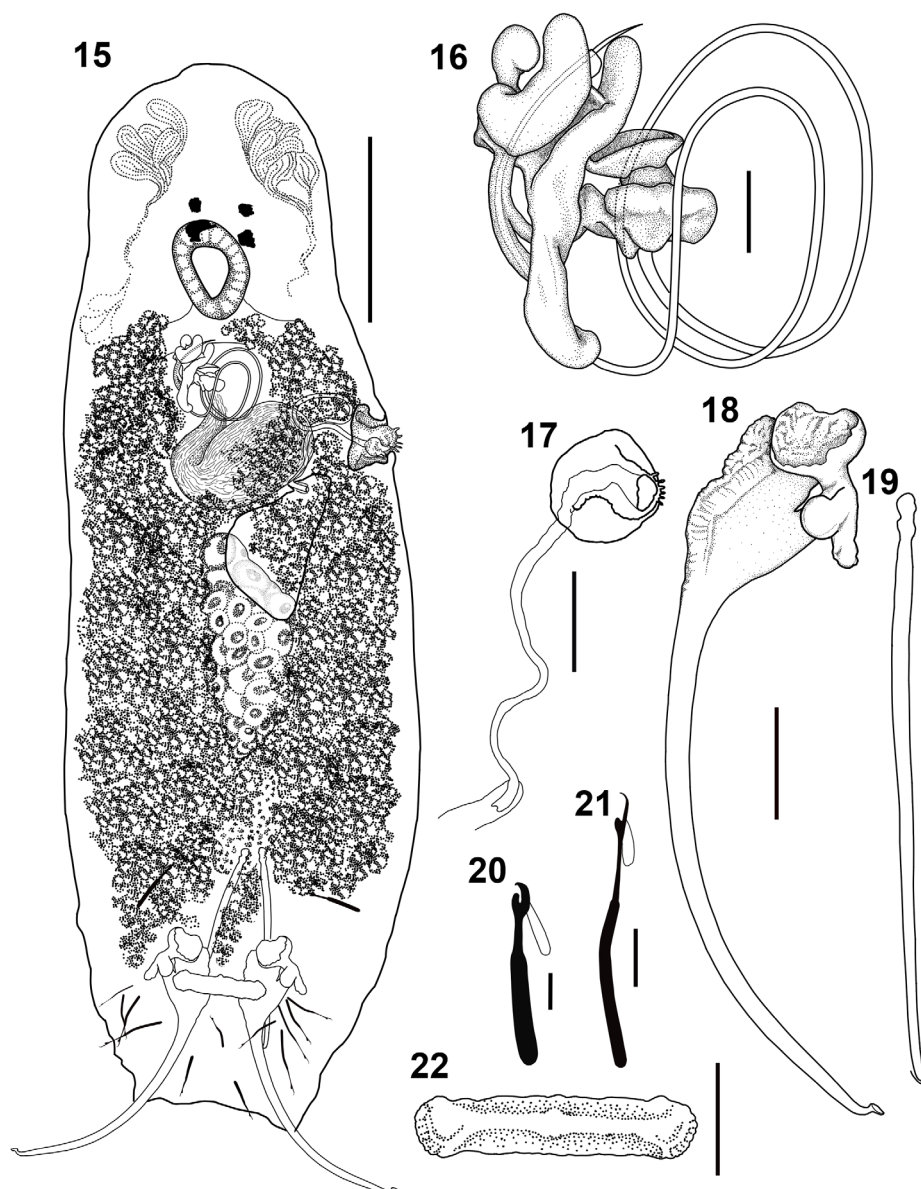
Description (based on 9 specimens, Figs. 15-22, Figs. 23-26, Fig. 28): body 339-573 (420; n = 9) long; maximum width 132-193 (164; n = 9) at body mid-length. Cephalic lobes poorly developed; five pairs of cephalic organs; cephalic glands posterolateral to pharynx. Eyes equidistant, anterior pair smaller than posterior pair (Fig. 15). Pharynx ovate and strongly muscular, 40-52 (46; n = 6) long, 26-32 (31; n = 6) maximum width. Small rectangular haptor, 81-110 (94; n = 9) long, 35-55 (41; n = 9) wide (Fig. 15). Ventral anchor 122-142 (131; n = 10) long, with inconspicuous roots; base 34-39 (36; n = 9) long, 19-22 (20; n = 8) wide; with sclerotized cap laterally modified into a bilobate expansion for articulation to ventral bar, and with a medial large cup-shaped concavity where muscle connecting both anchors inserts; base with a triangular proximal hole; shaft moderately curved, pointed with a saucer-like termination. Maximum width of root including sclerotized cap 24-31 (28; n = 9) (Movie 3-Figs. 18, 26, 28). Dorsal anchor straight with rounded proximal end and distal pointed end with conspicuous sclerotized cap, 101-111 (106; n = 10) long (Fig. 19). Ventral bar



Figures 9-14 - *Rhinoxenus argentinensis* n. sp. **9**. Male copulatory complex, **9a**. Microscope photograph, **9b**. 3D-reconstruction (ventral view), **9c**. 3D-reconstruction (dorsolateral view), **9d**. 3D-reconstruction (dorsal view); **10**. Vagina (ventral view); **11**. Aptoral sclerites (ventral view); **12**. Ventral anchor (ventral view, microscope photograph); **13**. Base of ventral anchor -3D-reconstruction (deconvolved stacks); **14**. Base of ventral anchor -3D-reconstruction (brightness and contrast adjusted). Scale bars: 9-11b-13= 20 μ m; 11a = 50 μ m.

straight with slightly expanded ends and thickened longitudinal axis, 49-54 (51; n = 10) long, 9-12 (11; n = 10) maximum width (Figs. 22-25). Hook pair 2 (Fig. 20) with erect thumb and curved shaft and point, proximal 2/3 of shank inflated, 19-23 (21; n= 9) long; filamentous hook (FH) loop extended

to the level of the beginning of shank's dilation; remaining hooks (Fig. 21) with erect thumb, long and slender shaft, lightly curved point; proximal 2/3 of shank inflated, 21-42 (31) long; FH loop not reaching the shank dilation. Male copulatory organ (MCO) a coiled tube with 1 ½ -2 rings, base reel



Figures 15-22 - *Rhinoxenus paranaensis* n. sp. **15.** Composite drawing, whole mount, ventral view; **16.** Male copulatory complex (ventral view); **17.** Vagina; **18.** Ventral anchor (ventral view); **19.** Dorsal anchor (ventral view); **20.** Hook pair 2; **21.** Hook. **22.** Ventral bar (ventral view). Scale bars: 15 = 100 μ m; 16-17 = 10 μ m; 17-22 = 20 μ m.

shaped, ring diameter 24-30 (28; n = 10), MCO length 134-181 (168, n = 9). Accessory piece elongated, complex, with elongate and dorsally concave proximal portion and a dilated distal portion formed by digitiform projections curved inwards. Tubular ligament articulates accessory piece to base of MCO, joining to a short sheath before connecting accessory piece, sheath serving

as a guide to insert the MCO into accessory piece (Movie 4- Figs. 16-23). Testis oval, limit difficult to observe, seminal vesicle large sacciform. Ovary elongated, with varying diameter, greatest width 30-77 (60; n = 4), length 122-144 (134; n = 4). Ootype and uterus not observed. Vaginal opening sclerotized, with 5-6 cuticular spine-shaped processes, short sclerotized vaginal canal (Figs. 17-

24). Seminal receptacle elongated, lying diagonally to left of body's midline. Eggs not observed.

TAXONOMIC SUMMARY

Type host: *Serrasalmus maculatus* (Kner 1858) (Serrasalmodae).

Type locality: Paraná River, Entre Ríos Province, Argentina (32°58'S, 60°36'W).

Other localities: La Chancha Lagoon, Sauce Viejo, Dpto. La Capital, Santa Fe Province, Argentina (31°49'S, 60°42'W); Lima, Partido de Zárate, Buenos Aires Province, Argentina (33°58'S: 59°12'W) (Table II).

Site: nasal cavities.

Etymology: The specific name refers to the river from which the species was collected.

Prevalence: 41.93%.

Mean abundance: 4.16 (1-30).

Type material: Holotype MLP-He 7563 and 3 paratypes MLP-He 7564 were deposited in the Helminthological Collection of the Museo de la Plata, La Plata, Argentina.

Comparative measurements and morphology: See Table I and Figs. 27-31.

REMARKS

Rhinoxenus paranaensis n. sp. also has a saucer-like distal point of the ventral anchor, sharing this characteristic with *R. piranhus*, *R. euryxenus* and

R. paranaensis n. sp. However, the new species differs from both *R. euryxenus* and *R. paranaensis* n. sp. by having a bilobated expansion of the sclerotized cap of the ventral anchor, rather than a triangular expansion; also the radius of curvature of the ventral anchor is greater in *R. euryxenus* and *R. argentinensis* n. sp. than in *R. paranaensis* n. sp. (Figs. 27-30). Furthermore, the vaginal opening is a laminar sclerotized ring in *R. argentinensis* n. sp. and cup-shaped in *R. euryxenus*, whereas it has 5-6 cuticular spine-shaped processes in *R. paranaensis* n. sp. The new species is similar to *R. piranhus* in relation to the morphology of the ventral anchor and vagina. However, the new species can be readily differentiated from *R. piranhus* by the lightly thinner shaft of ventral anchor and by lacking the small terminal protuberances present in ventral bar (Kristky et al. 1988). Additionally, the male copulatory complex of both species is different. While the accessory piece of *R. piranhus* has a ventrally concave proximal portion, that of the new species is dorsally concave. Furthermore, the digitiform processes present in the distal portion of the accessory piece are absent in *R. piranhus*.

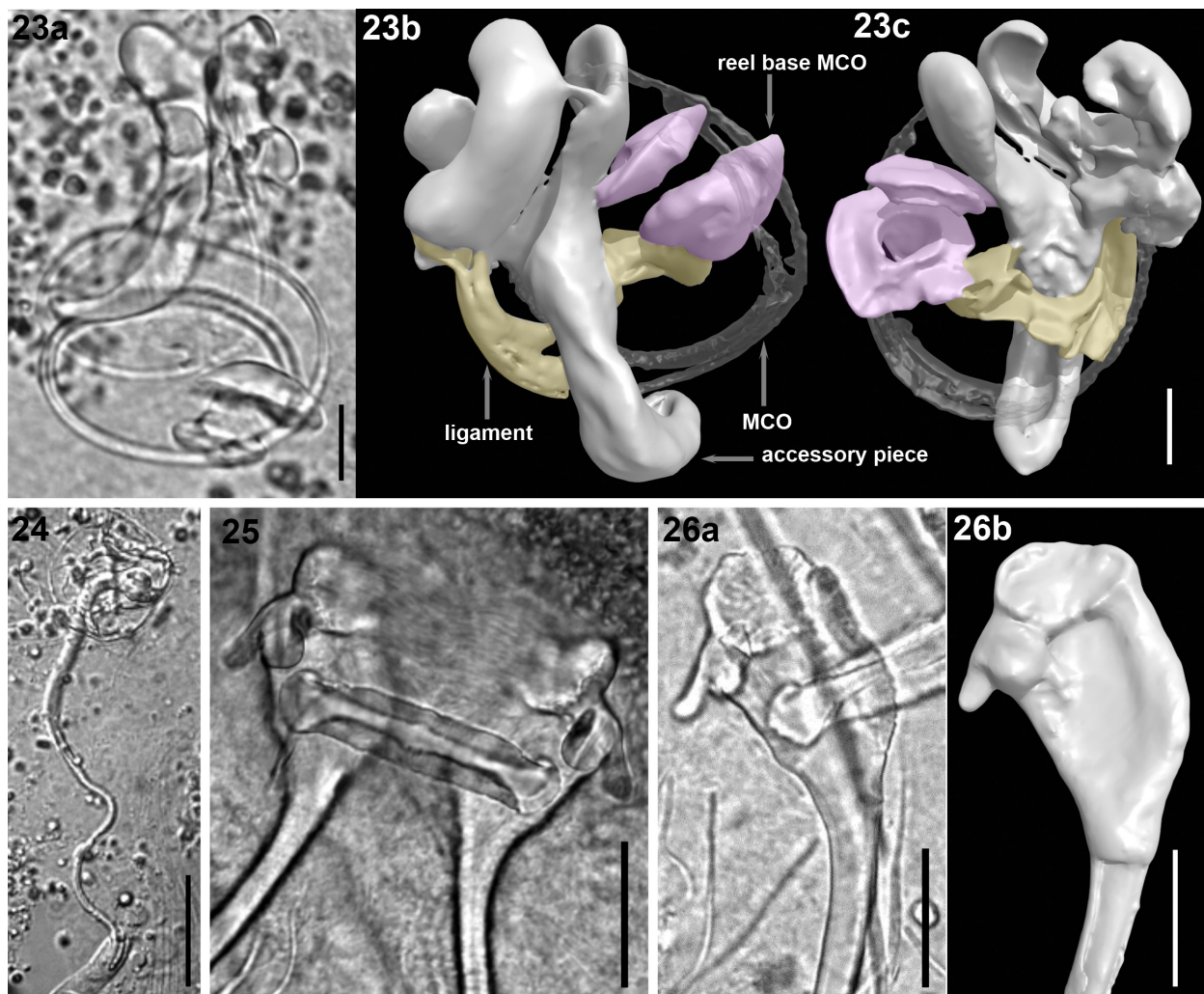
Rhinoxenus piranhus Kritsky, Boeger and Thatcher, 1988

Supplemental observations (based on 10 specimens; Fig. 29, Table I): total body length, 597-1088 (860;

TABLE II
Records of hosts and localities of *Rhinoxenus* Kritsky, Boeger and Thatcher, 1988 species parasitizing piranhas from South America Rivers.

<i>Rhinoxenus</i> species	Paraná River Argentina	Paraná River Brazil	French Guiana + northern Brazil
<i>R. piranhus</i>	<i>P. nattereri</i>	<i>S. marginatus</i> <i>S. maculatus</i> *	<i>P. nattereri</i> <i>Pristobrycon</i> sp. <i>S. spilopleura</i>
<i>R. euryxenus</i>	<i>S. marginatus</i> <i>S. maculatus</i>	<i>S. marginatus</i> <i>S. maculatus</i> *	<i>S. gouldingi</i> <i>S. rhombeus</i> <i>S. striolatus</i>
<i>R. argentinensis</i> n. sp.	<i>P. nattereri</i>	-	-
<i>R. paranaensis</i> n. sp.	<i>S. maculatus</i>	-	-

*As a *Serrasalmus spilopleura* in Domingues and Boeger 2005.



Figures 23-26 - *Rhinoxenus paranaensis* n. sp. **23**. Male copulatory complex, **23a**. Microscope photograph (ventral view), **23b**. 3D-reconstruction (ventral view), **23c**. 3D-reconstruction (dorsal view); **24**. Vagina (ventral view); **25**. Ventral bar; **26a**. Base of ventral anchor (microscope photograph, ventral view); **26b**. Base of ventral anchor 3D-reconstruction (ventral view). Scale bars: 23-24 = 10 μ m; 25-26 = 20 μ m.

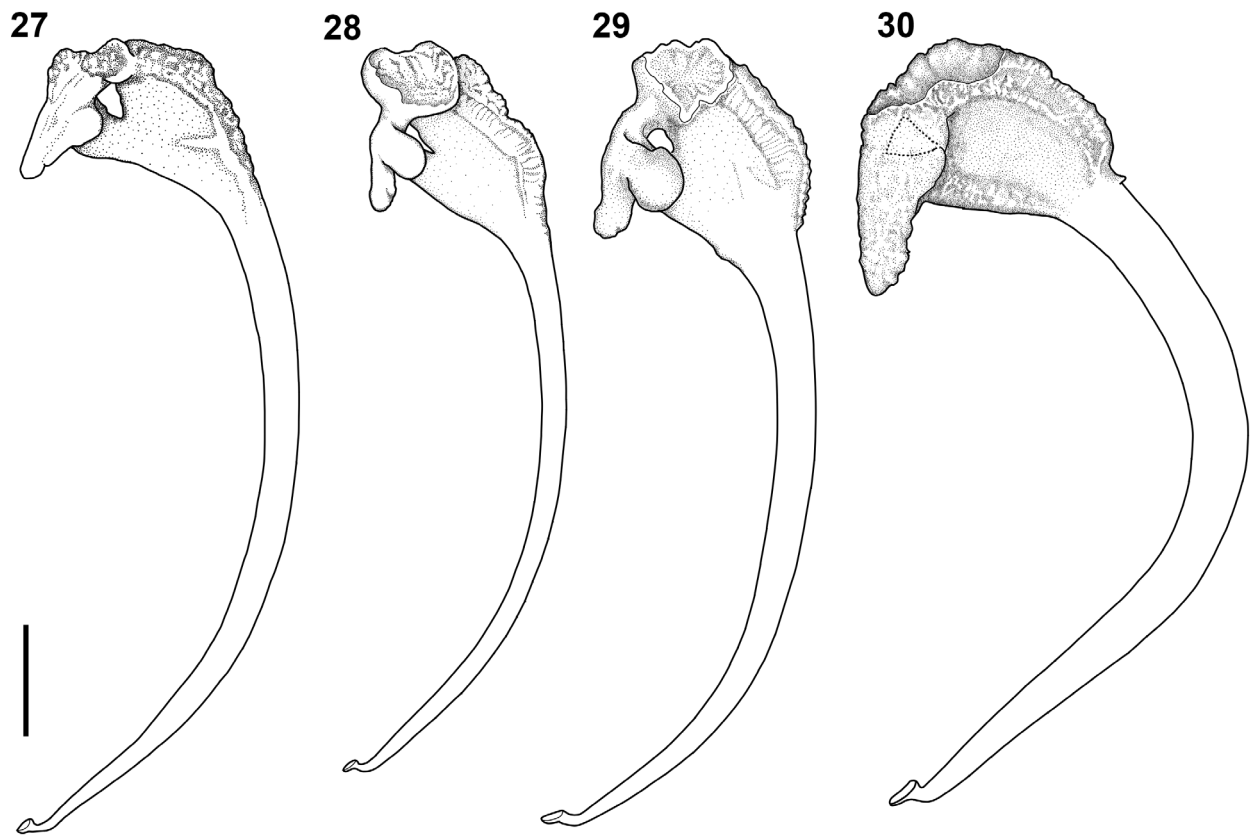
n = 10); maximum width 214-380 (278; n = 9). Ventral anchor 137-157 (150; n = 10) long and 31-55 (39; n = 10) wide (Fig. 29; Table I); with sclerotized cap laterally modified into bilobate expansion for articulation to ventral bar, and with a medial concavity where muscle connecting both anchors inserts; base with a triangular proximal hole; shaft moderately curved, pointed with a saucer-like termination (Fig. 29; Table I). Dorsal anchor straight, 119-157 (133; n = 10) long. Ventral bar 59-73 (66; n = 10) long, 13-21 (17; n = 10) maximum width. Male copulatory organ (MCO) is a coiled tube with two rings, of diameter 30-34 (31;

n = 7), MCO length 158-189 (174; n = 6). Accessory piece, 50-59 (54; n = 9) total length. Hook pair 2, 24-28 (25; n = 4) long; remaining hooks, 27-34 (29; n = 6) long.

TAXONOMIC SUMMARY

Type host and locality: *Pygocentrus nattereri* Kner, 1858 (Serrasalminidae): Ilha Marchantaria, Rio Solimões, near Manaus, Amazonas, Brazil.

Previous records from Brazil: Rio Capucapu, Cachoeira das Garças, near Manaus, Amazonas, from *Pristobrycon* sp. (Serrasalminidae); Río



Figures 27-30 - Comparative morphology of ventral anchor of species that parasitize piranhas. **27.** *Rhinoxenus argentinensis* n. sp. parasite of *Pygocentrus nattereri*; **28.** *Rhinoxenus paranaensis* n. sp. parasite of *Serrasalmus maculatus*; **29.** *Rhinoxenus piranhus* Kritsky, Boeger and Thatcher 1988 parasite of *Pygocentrus nattereri*; **30.** *Rhinoxenus euryxenus* Domingues and Boeger 2005 parasite of *Serrasalmus marginatus*. Scale bars: 27-30 = 20 μ m.

Paraná, near Porto Rico, Paraná, from *Serrasalmus marginatus* and *S. spilopleura*; Rio Uatumã, Lago Tapanã, Santa Anna, Amazonas, from *S. spilopleura* (Serrasalimidae); French Guiana. Lagoa Manga, Igarapé Tapardou, from *P. nattereri* (Serrasalimidae) and *S. spilopleura* (Serrasalimidae) (Table II) (Kritsky et al. 1988, Domingues and Boeger 2005).

Localities from Argentina: Colastiné River, Sauce Viejo, Santa Fe Province, Argentina; Lima, Buenos Aires Province, Argentina (Table II).

Site: nasal cavities.

Prevalence: 24%.

Mean abundance: 0.76 (1-10).

Specimens deposited: three paratypes MLP-He xx were deposited in the Helminthological Collection of the Museo de la Plata, La Plata, Argentina.

Comparative measurements and morphology: See Table I and Fig. 29.

REMARKS

Although *R. piranhus* was adequately described by Kritsky et al. (1988) as the type species of genus, the vaginal vestibule and the male copulatory complex (MCC) were later redrawn by Domingues and Boeger (2005), then Rossin et al. (2017) provided tridimensional morphological data of the MCC. The specimens analyzed here are similar to those described by Kritsky et al. (1988) and the measurements of the sclerotized structures do not differ from those provided in the original description, as well as by Domingues and Boeger (2005) for specimens from different serrasalimids

from Amazonas, Paraná and French Guiana. In this work, additional detail of ventral anchor are provided (morphology of sclerotized cap, presence of a medial concavity and a triangular hole in base of the anchor, enabling an easier discrimination between related species of *Rhinoxenus* with saucer-like termination of the ventral anchor (Figs. 27-30).

Rhinoxenus euryxenus Domingues and Boeger, 2005

Supplementary observations ((based on 10 specimens): Fig. 30, Table I): total body length, 364-786 (510; n = 10); greatest width 115-250 (162; n = 10). Ventral anchor 123-145 (136; n= 10) long, 40-52 (45; n= 8) wide (Fig. 30; Table I); sclerotized cap modified laterally into triangular expansion for articulation to ventral bar, with medial elongated concavity where muscle connecting both anchors inserts; base with triangular proximal hole; shaft curved, pointed with a saucer-like termination (Fig. 30; Table I). Dorsal anchor straight, 112-144 (126; n = 9) long. Ventral bar 58-78 (69; n = 8) long, 11-18 (15; n = 9) maximum width. Male copulatory organ (MCO) a coiled tube with 1.5 to 2 rings, 28-31 (29; n = 10) of diameter, 119-178 (156; n = 10) long. Accessory piece, 41-47 (44; n = 10) total length. Hook pair 2, 24-27 (25; n = 2) long; remaining hooks, 25-30 (27; n = 3) long.

TAXONOMIC SUMMARY

Type host and locality: *S. marginatus* (Serrasalmidae) from Baía da Medalha, Rio Paraná, Matogrosso do Sul, Brazil.

Previous record from Brazil: Rio Uatumã, lago Tapanã, Santa Anna, Amazonas, from *Serrasalmus gouldingi* (Serrasalmidae); Rio Miranda, Passo do Lontra, Mato Grosso do Sul, from *S. marginatus* (Serrasalmidae); Baía da Medalha, Rio Paraná, Mato Grosso do Sul, from *S. marginatus* (Serrasalmidae); Rio Iatapu, lago Maracanã, Manaus, Amazonas, from *S. rhombeus* (Serrasalmidae); Río Paraná, near Porto Rico,

454 Paraná, from *S. spilopleura* (Serrasalmidae); Río Capucapu, near the confluence of the Río Jatapu, Cachoeira das Garças, Amazonas, from *S. striolatus* (Serrasalmidae) and *Leporinus agassizii* (Anostomidae) (Domingues and Boeger, 2005) (Table II).

Hosts and localities from Argentina: *S. marginatus* and *S. maculatus* from Colastiné River, Sauce Viejo, Santa Fe Province, Argentina; Paraná Viejo River, Sauce Viejo, Santa Fe Province, Argentina (Table II).

Site: nasal cavities.

Prevalence: 77.70%.

Mean abundance: 6.90 (1-30).

Specimens deposited: three paratypes MLP-He xx were deposited in the Helminthological Collection of the Museo de la Plata, La Plata, Argentina.

Comparative measurements and morphology: See Table I and Fig. 30.

REMARKS

The specimens analyzed herein are similar to those described by Domingues and Boeger (2005) from serrasalmids from Brazil. All the measurements of the sclerotized structures are similar to those of the original description. Additional observations of ventral anchor are provided in this work, including the morphology of the sclerotized cap with presence of a medial and elongated concavity, presence of a triangular hole in the base of the anchor and a lateral sclerotized triangular cap expansion (Fig. 30). Comparisons of ventral anchor morphology allow to easily differentiate *R. euryxenus* from the rest of its congeners having saucer-like distal point.

COMPARATIVE MORPHOMETRY AND MORPHOLOGY OF VENTRAL ANCHORS

Morphological and morphometric characteristics of anchors of the four parasitic species from piranhas are provided in Figs. 27-30 and Table I. The CAP analysis showed significant morphometric differences in ventral anchors

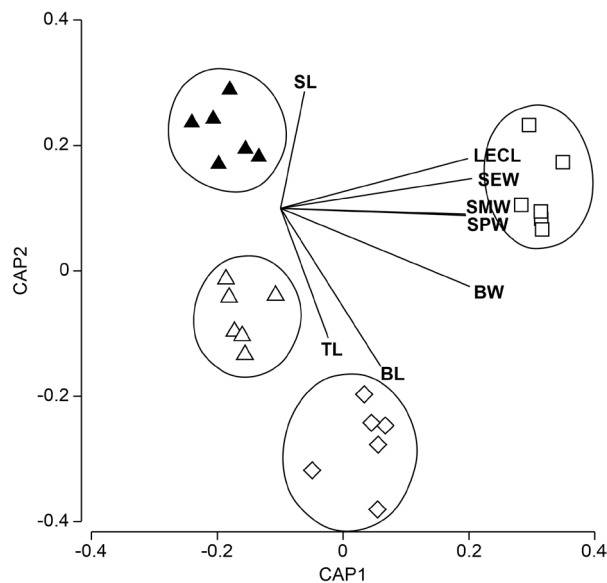


Figure 31 - Biplot of CAP analysis based on Euclidean distances on 9 measurements of ventral anchors. Vectors represent the Spearman correlations of each morphometric measurement to the CAP axes. Black triangle = *Rhinoxenus argentinensis* n. sp.; white triangle = *Rhinoxenus paranaensis* n. sp.; rhombus = *Rhinoxenus piranhus*; square = *Rhinoxenus euryxenus*. Abbreviations see Table I.

among *Rhinoxenus* species ($tr = 2.45$; $P < 0.001$). The selected orthonormal PCO axes ($n = 3$), described 100% of the variation in the data cloud, with a 100% of correct allocations (Fig. 31). When vectors corresponding to the Spearman correlations of morphometric measurements were superimposed the CAP biplot, greater values for most measurements characterized *R. euryxenus*, sharing a longer shaft with *R. argentinensis* n. sp. On the other hand, *R. piranhus* was characterized by larger values of total length and base length, while *R. paranaensis* n. sp. Characterized by the lower values for most measurements.

DISCUSSION

The genus *Rhinoxenus* comprises eight species of parasites from nasal cavities of Neotropical characiform fish, which have been recorded in rivers of Brazil and French Guiana (Kritsky et al. 1988, Boeger et al. 1995, Domingues and Boeger

2005). This is the first report of the *Rhinoxenus* species in Argentina, thus, adding two new species and increasing to ten the species richness of *Rhinoxenus*.

Regarding the phylogeny of *Rhinoxenus*, Domingues and Boeger (2005) proposed the monophyly of this genus. These authors suggest that *R. piranhus* and *R. euryxenus* cluster together supported by the saucer-like point of the ventral anchor as a synapomorphy with a consistency of 100%. The presence of this feature in both new species from piranhas supports that conclusion. Furthermore, the presence of a triangular hole in the base of the ventral anchor is a characteristic present on the 4 species that parasitize the nasal cavities of piranhas.

According to Domingues and Boeger (2005), *R. euryxenus* and *R. piranhus* differ from each other by the morphology of ventral anchors (with shaft strongly recurved near midlength in *R. euryxenus* vs. relatively straight in *R. piranhus*), among other features. The morphology of the ventral anchors is highly variable across the members of this genus (see Domingues and Boeger 2005). However, in the light of the new evidence, these structures are corroborated to be quite homogeneous in shape in those species parasitizing piranhas. The four species exhibit enough differences (in the shape of the sclerotized cap, the size and shape of both the lateral expansion and the medial concavity, and in the curvature and diameter of the shaft) to readily differentiate them from each other.

The presence of *R. piranhus* in *P. nattereri* is reported for the first time in the Paraná basin (Kritsky et al. 1988), but this species was not found in *S. marginatus* or in *S. maculatus* as previously reported along the upper Paraná (*S. maculatus* identified as *S. spiropleura*) (Domingues and Boeger 2005). Similarly, *R. euryxenus* was found mainly in *S. marginatus*, but also in *S. maculatus* (only 1 specimen in 37 examined fish) in the low Paraná River. Except for the finding of one specimen

of *R. euryxenus* in *S. maculatus*, each *Rhinoxenus* species parasitized a single species of piranha in Argentina, despite the sympatry of these hosts. This indicates a high host specificity contrasting to the low specificity observed in piranhas from Brazilian rivers (Table II). Unfortunately, the existing literature only reports the presence of different species, and no quantitative data on prevalence or abundance are available to test the host specificity among these parasites. For instance, the presence of *R. euryxenus* in *Leporinus agassizii* (Anostomidae) was considered accidental by Domingues and Boeger (2005), and other cases of accidental infestations could occur.

According to Jégu (2003), 25 species of piranhas are distributed in the Amazon basin, 16 in the Orinoco, 9 in rivers of the Guyanas, 3 in the Paraguay-Paraná Rivers, and 2 in the São Francisco River, and their phylogeny and phylogeography have been intensely studied (Hubert et al. 2007, Freeman et al. 2007, Ortí et al. 2008). Indeed, this latitudinal gradient in species richness of serrasalmids across South American rivers, along with their known phylogenetic relationships, provide an excellent opportunity for further studies on host-parasite coevolutionary processes, as well as on drivers of parasite specificity.

ACKNOWLEDGMENTS

The authors wish to thank Dra. M. Daniela Villamonte and Lic. Viviana Daniel for the support obtaining images from the Nikon C1 SiR confocal microscope, and Julia Eva Soledad Irigoitia and Eugenia Levy for the revision of the English language. Funding: this work was supported by the Agencia Nacional de Promoción Científica y Tecnológica (grant number ANPCYT-PICT 2015 N° 2013); CONICET (grant number CONICET-PIP N° 11220150100973); and Universidad Nacional de Mar del Plata, Argentina (grant number UNMdP-EXA 915/18).

AUTHOR CONTRIBUTIONS

MARÍA A. ROSSIN worked on parasitological examination of the hosts, quantification, taxonomic determination and description of parasites present, formulation of results and the writing and revision of the manuscript. PABLO N. DE FRANCESCO worked modeling of the hard parts of monogenean creating three-dimensional reconstructions of sclerotized structures, as well as image pre and post processing steps of Z-series. MANUEL M. IRIGOITIA collected and provided samples of the fishes from Paraná River, contributed on the collection of parasites, on the writing of the manuscript and its submission. PABLO A. SCARABOTTI collected and provided samples of the fishes from Paraná River and carried out the taxonomical determination of all hosts. VERÓNICA TAGLIORETTI contributed on the collection and processing of parasites and on the writing of the manuscript. JUAN T. TIMI contributed to writing and structuring the text, realized the statistical analysis (CAP), and revision of the final manuscript submission. We would like to highlight that all authors have read, revised and thoughtfully contributed to the improvement and of this manuscript.

REFERENCES

- ANDERSON MJ, GORLEY RN AND CLARKE KR. 2008. PERMANOVA for PRIMER: guide to statistical Methods. PRIMER-E, Plymouth, p. 240.
- ANDERSON MJ AND ROBINSON J. 2003. Generalized discriminant analysis based on distances. *Aust N Z J Stat* 45: 301-318.
- ANDERSON MJ AND WILLIS TJ. 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* 84: 511-525.
- BOEGER WA, DOMINGUES MV AND PAVANELLI GC. 1995. Neotropical Monogenoidea. 24. *Rhinoxenus bulbovaginatus* n. sp. (Dactylogyridae, Ancyrocephalinae) from the nasal cavity of *Salminus maxillosus* (Osteichthyes, Characidae) from the Rio Paraná, Paraná, Brazil. *Mem Inst Oswaldo Cruz* 90(6): 695-698.

- BUSH AO, LAFFERTY KD, JEFFREY ML AND SHOSTAK AW. 1997. Parasitology meets ecology on its own terms: Margolis et al. Revisited. *J Parasitol* 83: 575-583.
- DOMINGUES MV AND BOEGER WA. 2005. Neotropical Monogonoidea. 47. Phylogeny and coevolution of species of *Rhinoxenus* (Platyhelminthes, Monogonoidea, Dactylogyridae) and their Characiformes hosts (Teleostei, Ostariophysi) with description of four new species. *Zoosystema* 27(3): 441-467.
- FREEMAN B, NICO LG, OSENTOSKI M, JELKS HL AND COLLINS TM. 2007. Molecular systematics of Serrasalmidae: Deciphering the identities of piranha species and unraveling their evolutionary histories. *Zootaxa* 1487: 1-38.
- FROESE R AND PAULY D (Eds). 2018. Fish Base. World Wide Web electronic publication. Available at: www.fishbase.org, version. Accessed on: 02/2018.
- HUBERT N, DUPONCHELLE F, NUÑEZ J, GARCIA-DAVILA C, PAUGY D AND RENNO JF. 2007. Phylogeography of the piranha genera *Serrasalmus* and *Pygocentrus*: implications for the diversification of the Neotropical Ichthyofauna. *Mol Ecol* 16: 2115-2136.
- JÉGU M. 2003. Serrasalminae. In: Reis Re, Kullander SO and Ferraris CJ (Eds), Check List of freshwater fishes of South and Central America. Universidade Católica do Rio Grande do Sul, Porto Alegre (EDIPUCRS), p. 182-196.
- JÉGU M AND DOS SANTOS GM. 2001. Mise au point à propos de *Serrasalmus spilopleura* Kner, 1858 et réhabilitation de *S. maculatus* Kner, 1858 (Characidae: Serrasalminae). *Cybium* 25: 119-143.
- KRITSKY DC, BOEGER WA AND THATCHER VE. 1998. Neotropical Monogenea. 11. *Rhinoxenus*, new genus (Dactylogyridae: Ancyrocephalinae) with descriptions of three new species from the nasal cavities of Amazonian Characoidea *Proc Biol Soc Wash* 101: 87-94.
- KRITSKY DC, THATCHER VE AND BOEGER WA. 1986. Neotropical Monogenea. 8. Revision of *Urocleidoides* (Dactylogyridae, Ancyrocephalinae). *Proc Helminthol Soc Wash* 53: 1-37.
- ORTÍ G, SIVASUNDAR A, DIETZ K AND JÉGU M. 2008. Phylogeny of the Serrasalmidae (Characiformes) based on mitochondrial DNA sequences. *Genet Mol Biol* 31: 343-351.
- ROSSIN MA, DE FRANCESCO PN AND TIMI JT. 2017. Three-dimensional morphology of rigid structures as a tool for taxonomic studies of Dactylogyridae (Monogenea). *Parasitol Res* 116: 2813-2819.
- SCHINDELIN J, ARGANDA-CARRERAS I, FRISE E, KAYNIG V, LONGAIR M AND PIETZSCH T. 2012. Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9(7): 676-682.

SUPPLEMENTARY MATERIAL

Movie 1 - Ventral anchor of *Rhinoxenus argentinensis* n. sp.

Movie 2 - Male copulatory complex of *Rhinoxenus argentinensis* n. sp.

Movie 3 - Ventral anchor of *Rhinoxenus paranaensis* n. sp.

Movie 4 - Male copulatory complex of *Rhinoxenus paranaensis* n. sp.