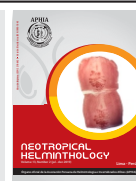




## Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

**PARVAPROSTATUM SYNBRANCHI N. GEN. N. SP. (TREMATODA: DIPLOSTOMIDA), PARASITE OF SWAMP-EEL SYNBRANCHUS MARMORATUS BLOCH, 1795 IN URUGUAY**

**PARVAPROSTATUM SYNBRANCHI N. GEN. N. SP. (TREMATODA: DIPLOSTOMIDA), PARÁSITO DE LA ANGIULA SYNBRANCHUS MARMORATUS BLOCH, 1795 EN URUGUAY**

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### ABSTRACT

The family Synbranchidae has a Neotropical distribution with parasite reports in Mexico, Brazil and Argentina, evidencing the lack of studies regarding the parasitofauna of swamp-eels. Parasitological examination through classic methods done on five swamp-eels (*Synbranchus marmoratus* Bloch, 1795) captured in Sauce Lagoon in Uruguay, showed the presence of Massoprostatinae parasites, which were stained and mounted through classical procedure, with Langeron's Carmine and Entellan, respectively. The Subfamily Massoprostatinae is monotypic with *Massoprostatum* Yamaguti, 1958 as the only genus and *M. longum* Caballero, 1948 as only species. To-date, it is only reported in crocodilians living in Mexico and Columbia. Morphological and biometric comparisons of relevant characteristics for the taxonomy of the family such as paraprostata size, hindbody and forebody ratio and vitellaria distribution, show their correspondence to a new genus and species, hereby named *Parvaprostatum synbranchi*. The lack of reports makes it difficult to conclude if Massoprostatinae is truly specific to crocodilians and thus, we cannot ascertain if the present report in eels implies host-switching. Despite the possibility of the parasites being progenetic metacercariae, conspicuous development of structures such as the tribocytic and reproductive organs, the presence of food content and the fact that they were un-encysted and gravid, make the specimens morphologically reliable for species identification. This new fish host and type locality report emphasizes the need for further studies to understand the underlying lifecycle of Massoprostatinae species.

**Keywords:** Massoprostatinae – *Massoprostatum* – metacercaria – *Parvaprostatum* – parasite – progenetic – Sauce Lagoon – swamp-eel – Uruguay

## RESUMEN

La familia Synbranchidae presenta una distribución Neotropical con reportes parasitarios en Méjico, Brasil y Argentina, lo cual evidencia la falta de estudios en cuanto a la parasitofauna de estas anguilas. La inspección parasitológica clásica realizada en cinco anguilas (*Synbranchus marmoratus* Bloch, 1795) capturadas en Laguna del Sauce en Uruguay, reveló la presencia de parásitos de la subfamilia Massoprostatinae, los cuales fueron teñidos y montados mediante procedimientos convencionales, con Carmín de Langeron y Entellan, respectivamente. La subfamilia Massoprostatinae es monotípica, siendo *Massoprostatum* Yamaguti, 1958 su único género y *M. longum* Caballero, 1948 su única especie. A la fecha, esta especie solo se ha reportado en cocodrilos en Méjico y Colombia. Comparaciones morfológicas y biométricas de caracteres con relevancia taxonómica para la familia tales como el tamaño de la paraprostata, relación del tramo anterior y posterior del cuerpo y la distribución de las glándulas vitelarias, muestra que los ejemplares corresponden a un nuevo género y especie: *Parvaprostatum synbranchi*. La falta de reportes dificulta concluir si Massoprostatinae es realmente específica para cocodrilos y por lo tanto, no se puede aseverar si el presente reporte en anguilas implica un cambio de hospedador. Mas allá de la posibilidad de que los parásitos correspondan a metacercarias progenéticas, el desarrollo conspicuo de estructuras como el órgano tribocítico y los órganos reproductores, la presencia de contenido alimenticio en los ciegos intestinales y el hecho de que se hallaron desenquistados y grávidos, hace que los especímenes sean morfológicamente confiables para la identificación a nivel específico. El reporte de un nuevo hospedador y una nueva localidad enfatizan la necesidad de estudios que permitan entender los ciclos de vida subyacentes en especies de la subfamilia Massoprostatinae.

**Palabras clave:** Massoprostatinae – *Massoprostatum* – metacercaria – *Parvaprostatum* – parásito – progenético – Laguna del Sauce – anguila – Uruguay

## INTRODUCTION

The swamp-eels (Synbranchidae) present a Neotropical distribution, living in fresh-water, lacustrine environments (Nelson *et al.*, 2016). In regards to the family's parasitological fauna, reports exist for Brazil: *Cyrlia gomesi* (Neiva & Pinto, 1926) Lainson, 1981 (Lainson & Canning, 1981), *Trypanosoma bourouli* (Neiva & Pinto, 1926) Lainson, 1981 (Lainson & Canning, 1981), *Cyrlia lignieresi* Laveran, 1906 (Diniz *et al.*, 2002), *Clinostomum* sp. Leidy, 1856 (Erias *et al.*, 2010) and *C. detruncatum* Braun, 1899 (Locke *et al.*, 2015; Acosta *et al.*, 2016) in *Synbranchus marmoratus* Bloch, 1795; Mexico (Salgado-Maldonado, 2008): *Genarchella isabellae* Lamothe-Argumedo, 1977 (Kohn *et al.*, 1990), *Pseudocapillaria ophisterni* Moravec, Salgado-Maldonado & Jimenez-Garcia, 2000 (Moravec *et al.*, 2000), *Monticellia ophisterni* de Chambrier & Salgado-Maldonado, 2001 (Scholz *et al.*, 2001), *Philometra ophisterni* Moravec, Salgado-

Maldonado & Aguilar-Aguilar, 2002 (Moravec *et al.*, 2002a), *Gibsonnema ophisterni* Moravec, Salgado-Maldonado & Aguilar-Aguilar, 2002 (Moravec *et al.*, 2002b) in *Ophisternon aenigmaticum* Rosen & Greenwood, 1976; and Argentina: *Synbranchiella mabelae* Arredondo, Viera Alves & de Pertierra, 2017 (Arredondo *et al.*, 2017) in *S. marmoratus*. Considering the wide Neotropical distribution of the swamp-eels, knowledge of their parasitological fauna is evidently scarce, given there is a lack of knowledge from several countries in South America, one of them being Uruguay.

We describe a new parasite genus and new species of the family Proterodiplostomidae, found in the marble swamp-eel *Synbranchus marmoratus* Bloch, 1795 from a coastal lagoon in Uruguay. Additionally, we propose slight changes to the diagnosis of the subfamily Massoprostatinae, considering that, in the light of our finding, the rigidity of its description likely comes from a lack of known diversity.

## MATERIALS AND METHODS

Sampling of five (5) swamp-eels *S. marmoratus* took place in Sauce Lagoon, Maldonado, Uruguay, in May of 2019. The eels were taken to the laboratory and euthanized with eugenol overdose before a cervical and cephalic incision, under the approval of the institutional Ethics Council of Animal Experimentation, in compliance with the AVMA international guidelines for fish euthanasia (Leary *et al.*, 2013). Autopsy on the specimens was performed immediately after, removing the visceral organs for parasitological inspection. The parasites were removed manually and observed in fresh condition to better describe body shape. Afterwards, holotype and paratypes were fixated under pressure with heat and formalin 10% and stored in ethanol 70%. They were then stained with Langeron's Carmine solution for 10-15 min (following staining process through stereoscopic microscope Nikon C-LEDS), dehydrated with ethanol bathes of increasing concentration (10 minutes ethanol 70%, 10 minutes ethanol 80%, 10 minutes ethanol 95%, 10 minutes ethanol 100%) and then permanently mounted in Entellan medium between slide and coverslip.

The specimens were observed in Light Microscope (Olympus BX50) and photos were taken with adapted 318CU 3.2M CMOS camera and Micrometrics SE Premium program. Measurements were taken over the drawings with regular rulers and then converted to micrometers ( $\mu\text{m}$ ), or alternatively with ruler grill installed into the ocular of the microscope. Images taken with Micrometrics SE Premium were also used for measuring through the program ImageJ. Digital drawings were performed with open software GIMP2, based on the pictures taken or as a summary of the structures seen in different specimens. Exact Binomial Confidence Interval (CI) and Bootstrap Bias-corrected and Accelerated Confidence Interval (BCa) were calculated for the Prevalence and the Mean Intensity respectively, were using R Program packages Binom and BootBCa correspondingly.

Holotype and paratypes were stored as an annex to the Ichthyological Collection of the National Museum of Natural History, Montevideo, Uruguay, with access numbers MNHN 4213 and

MNHN 4214 (lot of 4 paratypes), respectively.

### Ethic aspects

The authors point out that they fulfilled all national and international ethical aspects.

## RESULTS

Three (3) of the five (5) marble swamp-eels analyzed were infected, with number of specimens ranging from 5 to 62. The parasites were found across the entirety of the celom, under and above the mesenteries, un-encysted, associated with the liver, the gonads and the outside of the gut. In one case, parasite eggs were observed within the ovary of the host.

### Subclass Digenea

#### Superfamily Diplostomoidea

#### Family Proterodiplostomidae, Dubois 1936

#### Subfamily Massoprostatinae, Yamaguti 1958

#### Genero *Parvaprostatum* n. gen.

Body type elongate, bipartite without notable constriction (Figure 1). Forebody length less than four times the size of hindbody. Anterior end without accessory suckers or other adhesive structures. Posterior end without copulatory bursa. Vitelline follicles small, lateral, posterior to tribocytic organ, anterior to ovary. Uterus short, intercaecal, posterior to tribocytic organ. Paraprostate short. Uterus, Ejaculatory duct and Paraprostate opening separately into small genital atrium. Neotropical. Type and only species *P. synbranchi*.

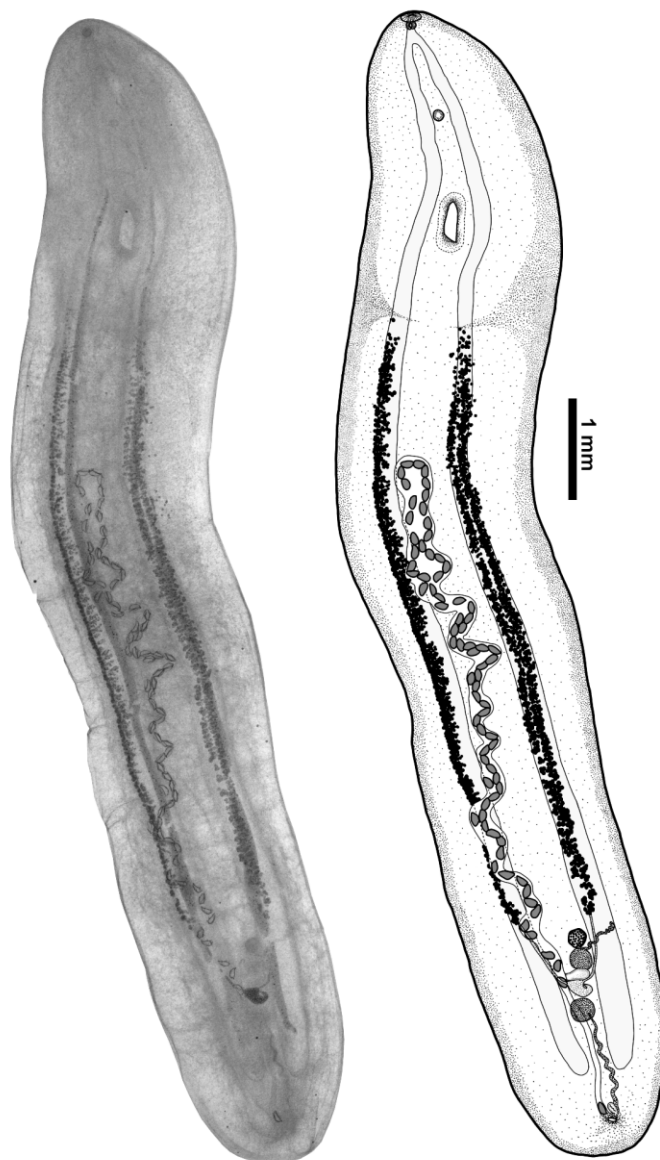
### Species *Parvaprostatum synbranchi* n. sp.

Description based in 5 specimens (holotype and paratypes) unless indicated otherwise. Measurements expressed in micrometers ( $\mu\text{m}$ ) unless indicated otherwise, under the form Average (Minimum-Maximum) and Length x Width, when corresponding.

Body type holostome, elongate, bipartite without notable constriction (Figure 1); total large, 11.49 mm (9.88-13.63), maximum width/total large ratio, 1:0.14 (0.11-0.17); forebody flattened, slightly convex ventrally 3.09 mm (3-3.40) x 1.6 mm (1.38-1.86), hindbody cylindrical with round

end, 8.4 mm (6.86-11.78); forebody/hindbody ratio, 1:2.35 (1.93-2.67). Paranephridial plexus present in fresh specimens, following 'Diplostomulum' pattern; protonephridia not visible. Anterior end without accessory suckers or other adhesive structures. Oral sucker terminal or subterminal, small, circular 131 (120-150). Prepharynx not noticeable; pharynx small, spherical, bulbous biradiated, 73 (60-80); esophagus very short, 237 (130-285); intestinal ceca bifurcation anterior to acetabulum, ceca

ending near posterior end, 10.31 mm (9.30-12.50) x 145 (122-150), ceca length/total length ratio, 1:0.9 (0.74-0.97). Ceca with food content. Acetabulum vestigial, circular or oval, 95 (70-135) x 80 (70-100), ventrally medial, between ceca bifurcation and tribocytic organ. Tribocytic organ oval longitudinally, pre-equatorial, 572 (480-660) x 338 (300-370), distance to anterior end, 1.97 mm (1.63-2.40). Papilla in the medial line of tribocytic organ and proteolytic glands not visible.

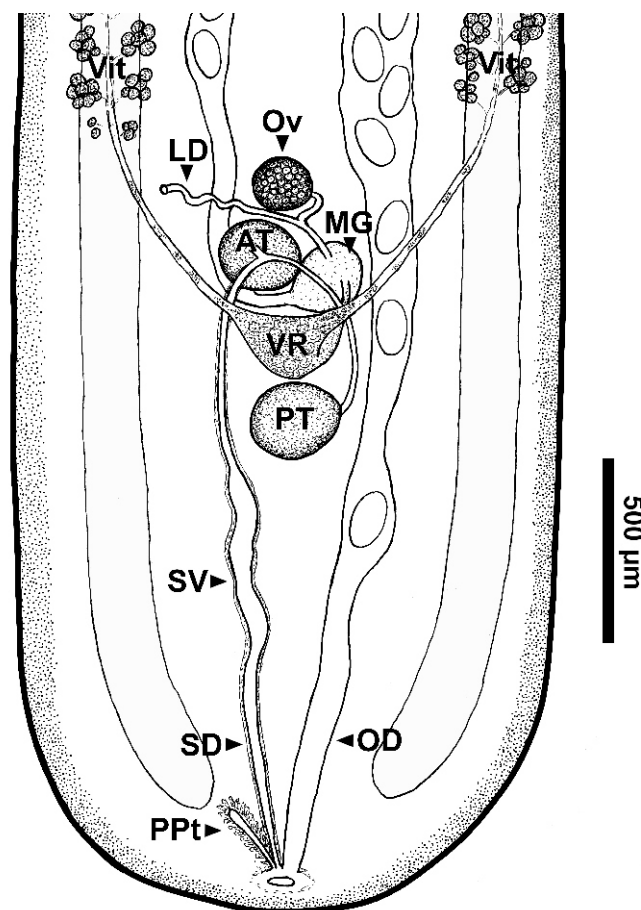


**Figure 1.** *Parvaprostatum synbranchi* n. gen. n sp. *In toto* photograph (left) with corresponding descriptive linear schematization (right). Note the uterus not visibly reflecting antero-posteriorly due to superposition of the structure.

Vitelline follicles small, lateral, coinciding with intestinal ceca, posterior to tribocytic organ, anterior to ovary. Ovary in posterior zone of hindbody, spherical or ovoid, pretesticular, in contact with anterior testis, 120 (100-145) x 122 (90-145). Oviduct emerges posteriorly to ovary sinuously connecting to intertesticular Mehlis' gland, associated to Ootype. Laurer's canal emerges obliquely from oviduct near ovary (Figure 2) opening into gonopore. Vitelline reservoir triangular or irregular, intertesticular, 150 (120-170) x 174 (150-210), with two efferent vitelline ducts and one deferent ootype duct. Ootype intertesticular, anterior to vitelline reservoir, 168 (133-200) x 58 (38-75) (n=4). Uterus short, intercaecal, projecting anteriorly to posterior end of tribocytic organ, then descending into genital

atrium; holding 60 (43-76) eggs. Testis subequal, in tandem, spherical or ovoid, posterior to ovary, anterior testis 167 (125-210) x 161 (115-190), posterior testis 170 (135-230) x 183 (160-220). Efferent ducts of testis conjoin forming a single duct directed posteriorly which widens forming sinuous seminal vesicle, 376 (270-480) x 22 (20-28); seminal vesicle thins posteriorly, becoming spermiduct (Figure 2) which opens to genital atrium. Paraprostate small, 148 (120-180) x 36 (30-50), opening separately from spermiduct and oviduct, into genital atrium. Excretory pore in posterior end, terminal or subterminal.

Eggs measured *in vivo*, 120 (110-130) x 62 (60-70) (n=10).



**Figure 2.** Detail of the genitalia of *Parvaprostatum synbranchi*. Reconstruction based on the study of all specimens. Note small paraprostate opening separately from spermiduct and uterus into small genital atrium. References: AT) Anterior Testis; LD) Lourer's Canal; MG) Mehlis' Gland; OD) Oviduct; Ov) Ovary; PPt) Paraprostate; PT) Posterior Testis; SD) Spermiduct; SV) Seminal Vesicle; Vit) Vitellaria; VR) Vitellaria Reservoir.

Taxonomic summary

Type host: *Synbranchus marmoratus* Bloch, 1795  
 Type locality: Sauce Lagoon, Maldonado, Uruguay.  
 Site of Infection: body cavity under mesenteries, liver, gonads.  
 Prevalence (CI): 60 (15-95)%  
 Mean Intensity (BCa): 26 (5-45)  
 Type species: *Parvaprostatum synbranchi*

Commentary

New genus name *Parvaprostatum*, comes from Latin *parva*- small, *prostatum*- prostate, in reference to the small size of the paraprostate. Specific epithet *synbranchi* comes from the host genus, *Synbranchus*.

**Table 1.** Biometric comparison of the descriptions of *Massoprostatum* Caballero, 1948 previously reported versus *Parvaprostatum synbranchi*. All measurements expressed in micrometers ( $\mu\text{m}$ ) in the format Average (Minimum Value-Maximum Value) when the data was available. Data from present work taken from 5 individuals except Egg size which was taken from 10 fresh individuals. Reference: AT) Anterior Testis; FB) Forebody; HB) Hindbody; OS) Oral Sucker; Ov) Ovary; PT) Posterior Testis; SV) Seminal Vesicle; TL) Total Length; TO) Tribocytic Organ; VR) Vitellaria Reservoir; VS) Ventral Sucker (Acetabula).

	<i>M. longum</i> (Caballero, 1947)	<i>M. longum</i> (Alvarez <i>et al.</i> , 2005)	<i>Parvaprostatum synbranchi</i> (present work)
TL	10500 (10072-10544)	1730 (1400-2060)	11489 (9875-13650)
Forebody	873-1183 x 336-673	280	3090 (3000-3400) x 1610 (1375-1875)
Hindbody	9454-9635 x 673-763	1600	8400 (6875-11775) x 1250
HB/FB	8.15-10.8	5.71	2.35 (1.93-2.67)
OS	46-50 x 58	44 (39-49)	131 (120-150)
Pharynx	46-54 x 25-37	35 (31-39)	73 (60-80) x 77 (70-85)
Esophagus	104-121	200	237 (130-285)
VS	71 x 112-116	39 (23-55)	95 (70-135) x 80 (70-100)
TO	283-291 x 250-270	100	572 (480-660) x 381 (370-400)
Ceca	Width 12-21; Smooth, extends up to posterior end	Smooth; extends up to posterior end	Width 145 (122-15); Smooth, extends up to posterior end
Ovary	162-175 x 216-229	97 (85-109) x 111 (103-109)	120 (100-145) x 121 (90-145)
AT	354-364 x 381-382	170 (140-180) x 220 (200-250)	147 (100-210) x 161 (115-190)
PT	364-366 x 361-382	180 (141-230) x 210 (180-250)	170 (140-230) x 183 (160-220)
Vitellaria	From posterior end of holdfast to posterior end of body	From anterior end of holdfast to posterior end of body	From anterior end of holdfast not reaching anterior end of ovary
VR	50-180 x 156-225	–	150 (120-170) x 174 (150-210)
Uterus	Intercaecal; anterior to ovary	Intercaecal; anterior to ovary	Intercaecal; anterior to ovary
Egg size	79-112 x 46-62	96 (87-110) x 69 (59-79)	120 (110-130) x 62 (60-70)
Paraprostate	1684-1839 x 42-46	–	148 (120-180) x 36 (30-50)
SV	1539-2271 x 58-79	–	376 (270-480) x 22 (20-30)
Spermiduct	308-1040 x 12-21	–	472 (390-650) x 13 (12-15)

## DISCUSSION

The family Proterodiplostomidae in South America, has been reported for Argentina, Brazil, Colombia, Paraguay, Venezuela and Uruguay, summing a total of 11 genus and 18 species in the continent (Mañé-Garzón & Holcman-Spector, 1969; Eiras *et al.*, 2010; Das *et al.*, 2011; Kohn & Fernández, 2014; Palumbo & Diaz, 2018). In particular, the subfamily Massoprostatinae Yamaguti, 1958, possesses only one genus, *Massoprostatum* Caballero, 1948, and one species, *Massoprostatum longum* Caballero, 1948, which is defined by characteristics of the subfamily, notably: body bipartite; forebody small, spoon-shaped; hindbody cylindrical, very long; pseudosuckers absent; oral and ventral suckers small; holdfast organ small, round, with papillate margins of aperture; pharynx small; oesophagus short; caeca reaching close to posterior end of body; testes tandem but slightly diagonal, near posterior extremity; ovary pretesticular; vitellarium extends full length of hindbody; paraprostate long, sinuous, opening beside ejaculatory duct at tip of small genital cone; uterus opens separately; copulatory bursa small, with terminal aperture. Host type and distribution are also considered diagnostic characters, which are crocodilians hosts and Neotropical distribution (Gibson *et al.*, 2002). According to the species checklist of Tellez (2014), *M. longum* has been found in *Crocodylus moreletii* Duméril & Bibron, 1851 (Caballero, 1947) in Mexico and *Caiman crocodylus fuscus* Cope, 1868 (Alvarez *et al.*, 2005) in Colombia, which circumscribes the distribution of the species, and by transitive, of the subfamily, strictly to southern Central America and northern South America. Consequently, the taxonomic key of Niewiadomska (Key to Trematoda, Gibson *et al.*, 2002), places the specimens hereby found under the subfamily Massoprostatinae due to the disposition of the gonads and the defining character of vitellaria not reaching the forebody. Furthermore, general characteristics already mentioned, such as body bipartite, forebody more than two times smaller than hindbody, lack of pseudo-suckers, esophagus, pharynx, acetabulum and tribocytic organ small, ceca very long reaching up to posterior end of body and uterus and spermiduct opening into the same genital atrium with a common excretory pore, all

strengthen this classification.

Comparing the two published descriptions of *M. longum* (Table 1) with the specimens, the latter present important morphological and biometrical distinctions: larger body size, no notable differences between width of forebody and hindbody, notably smaller hindbody/forebody ratio; differences in size and form to tribocytic organ; vitellaria posterior to tribocytic organ and anterior to ovary; testis and ovary similar in size, testis small in relation to total length; notably smaller seminal vesicle; paraprostate very small. The contrasting size of the paraprostate is of utmost relevancy given it is a key trait in the classification of the family, subfamily, genus and species. Additionally, distribution area and host are novel for Massoprostatinae as a whole. The dissimilarities with the subfamily lead us to believe that, perhaps, certain features which define this hierarchy are more related to the species level. We propose the following description for the subfamily Massoprostatinae to fit the inclusion of the new genus, modified from Key to Trematoda Vol. 1 (Gibson *et al.*, 2002):

Body bipartite; forebody small, spoon-shaped; hindbody cylindrical, more than two times longer than forebody. Pseudosuckers absent; oral and ventral suckers small; holdfast organ small, round or oval, with or without papillate margins of aperture. Pharynx small; oesophagus short; caeca reaching close to posterior end of body. Testes tandem, slightly diagonal, near posterior extremity. Ovary pretesticular. Vitellarium confined to hindbody. Paraprostate present, opening beside ejaculatory duct at tip of small genital cone. Copulatory bursa small with terminal aperture, or absent. In crocodilians and eels. Neotropical. Type genus *Massoprostatum* Caballero, 1948.

The key to genera should then depend mainly on the size of the paraprostate, with some supporting characters to reinforce the classification:

Paraprostate large; forebody length more than four times the size of hindbody; vitellaria reaching posterior end of body.....  
*Massoprostatum*, Caballero 1948

Paraprostate small; forebody length less than four times the size of hindbody; vitellaria not surpassing

anterior end of ovary.....  
*Parvaprostatum*, present work.

The lack of reports makes it difficult to conclude if Massoprostatinae is specific to crocodylians and thus, we cannot ascertain if our report in eels imply either host-switching or life cycle shortening. If the latter case is true, the specimens should then be progenetic metacercaria (metacercarial development of somatic and sexual characteristics like those of an adult while in an intermediary host, shortening the lifecycle (Poulin & Cribb, 2002; Lefebvre & Poulin, 2005). The family Proterodiplostomidae has reports of progenetic metacercaria (*Crocodylicola pseudostoma* (Willemoes-Suhm, 1870) Poche, 1925, in body cavity of *Rhamdia guatemalensis* (Günther, 1864) (Pérez-Ponce de León *et al.*, 1992); *C. pseudostoma* in body cavity of *Hemisorubim platyrhynchos* Cuvier & Valenciennes, 1840 (Guidelli *et al.*, 2003), therefore making it probable that the proposed species *Parvaprostatum synbranchi* is progenetic as well. While it could be controversial to use them as basis for the report of a new species, the conspicuous development of structures such as the tribocytic and reproductive organs, the finding of food content in the ceca, and the fact that they were found un-encysted and gravid, lead us to consider the specimens adequate and morphologically reliable. This same argumentation has already been applied by other authors (Ibañez & Jara, 1999; Arredondo, 2013) that describe new species based on metacercaria. It is worth mentioning that although they are referred to as progenetic metacercaria, this is only to emphasize their sexual development in a secondary intermediate host, but they are not neotenic (Lefebvre & Poulin, 2005).

In Uruguay, the only known crocodylian species is *Caiman latirostris* Daudin, 1801, distributed in the west (Rio Uruguay basin) and east (Merin Lagoon basin) of the country, with no reports in coastal lagoons such as Sauce Lagoon (Borteiro *et al.*, 2006). It becomes difficult to theorize a scenery of host-switching with the current state of knowledge. Another explanation would be that the assumed host specificity might be a consequential bias resulting from the scarcity of studies regarding this parasitic family, as hinted by the description of a proterodiplostomid species (*Paradiplostomum spatulatum* Dharejo, Bilqees & Khan, 2011)

parasitizing a piscivore avian (*Egretta garzetta* Linnaeus, 1766) (Dharejo *et al.*, 2011).

Future works should focus on completing the lifecycle of this new species, to better understand the oddity of its type host and locality, as well as include comparative genetic data to shed more light into the subjacent evolutionary trends.

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