Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Morphology and morphometry of *Paratanaisia bragai* (Santos, 1934) Freitas, 1959 (Digenea, Eucotylidae) through light and scanning electron microscopy

Morfologia e morfometria de *Paratanaisia bragai* (Santos, 1934) Freitas, 1959 (Digenea, Eucotylidae) através da microscopia de luz e microscopiaeletrônica de varredura

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Abstract

Paratanaisia bragai is a digenetic trematode that reaches sexual maturity in the kidney collecting ducts of domestic and wild birds, while the snails *Subulina octona* and *Leptinaria unilamellata* serve as its intermediate hosts in Brazil. The present study analyzed the morphology and morphometry of *P. bragai*. Adult specimens of the parasite were collected from naturally infected *Columba livia* kidneys, fixed and prepared for observation via bright field and differential interference contrast light microscopy and scanning electron microscopy. The parasite has an elongated and flattened body, with a subterminal oral sucker located at the anterior end of the body, as observed by all techniques used. Staining the parasite with hematoxylin-eosin enabled observation of the pharynx, located posteriorly to the oral sucker, the vitelline glands, which are extra-cecal and extend anteriorly to the pre-ovarian region and later to the median region of the body, and intestinal caeca parallel to the vitelline glands. The presence and functionality of the acetabulum are controversial points in the literature, but it was observed in all specimens analyzed by scanning electron microscopy, with a major diameter of 38.36 ± 6.96 (28.77 – 45.39) and minor diameter of 31.59 ± 7.04 (21.75 - 38.16). Close to the acetabulum, scales were observed in the integument of the parasite. Scales with (1 - 5) blade divisions were identified. In the genital pore, it was possible to see the everted cirrus with rosette shape. The excretory pore (first morphometric record) is dorsal and subterminal, with major diameter of 12.27 ± 9.16 (5.79 - 18.75) and minor diameter of 3.95 ± 1.49 (2.89 - 5.00).

Keywords: *Paratanaisia bragai*, morphology, morphometry, light microscopy, scanning electron microscopy.

Resumo

Paratanaisia bragai é um trematódeo digenético que atinge a maturidade sexual nos ductos coletores de aves domésticas e silvestres, enquanto os moluscos *Subulina octona* e *Leptinaria unilamellata* atuam como seus hospedeiros intermediários no Brasil. O presente estudo analisou a morfologia e morfometria de *P. bragai*. Amostras adultas do parasito foram coletadas de rins de *Columba livia* naturalmente infectada, fixadas e preparadas para observação na microscopia de campo claro e microscopia de luz de contraste de interferência diferencial e microscopia eletrônica de varredura. O parasite possui corpo alongado e achatado, com uma ventosa oral subterminal localizada na extremidade anterior do corpo, conforme observado por todas as técnicas utilizadas. A coloração do parasito com hematoxilina-eosina permitiu observar a faringe, localizada posteriormente à ventosa oral, as glândulas vitelogênicas, que são extracecais e estendem-se anteriormente à região pré-ovariana e posteriormente à região mediana do corpo, e os cecos intestinais paralelos às glândulas vitelinas. A presença e funcionalidade do acetábulo são pontos controversos na literatura, mas foi observado em todos os espécimes analisados por microscopia eletrônica de varredura, com diâmetro maior de 38.36 ± 6.96 (28.77 – 45.39) e diâmetro menor de 31.59 ± 7,04

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Received: January 28, 2023 – Accepted: April 20, 2023

(21.75 – 38.16). Próximo ao acetábulo foram observadas escamas no tegumento do parasito. Escamas com (1 - 5) divisões de lâmina foram identificadas. No poro genital, foi possível visualizar o cirro evertido com formato de roseta. O poro excretor (primeiro registro morfométrico) é dorsal e subterminal, com diâmetro maior de 12.27 ± 9.16 (5.79 – 18.75) e diâmetro menor de 3.95 ± 1.49 (2.89 – 5.00).

Palavras-chave: *Paratanaisia bragai*, morfologia, morfometria, microscopia de luz, microscopia eletrônica de varredura.

1. Introduction

Paratanaisia bragai (Santos, 1934) Freitas, 1959 is a digenetic trematode that reaches sexual maturity in the kidney collecting ducts of domestic and wild birds as its definitive hosts, with the snails *Subulina octona* (Bruguière, 1798) and *Leptinaria unilamellata* (d'Orbigny, 1837) (Mollusca, Gastropoda, Pulmonata) serving as its intermediate hosts, in which larval development occurs, the latter being considered the intermediate host in Brazil (Brandolini et al., 1997; Bonfim et al., 2021).

Passive infection is established in snails by the ingestion of embryonated eggs of *P. bragai* eliminated with the excretion products of the definitive host. After the miracidia hatch, two generations of sporocysts, cercariae and metacercariae, develop inside the snail. The definitive host becomes infected by ingesting the parasitized snail (Maldonado, 1945; Keller and Araujo, 1992; Brandolini and Amato, 2006).

The occurrence of birds parasitized by *P. bragai* has been reported in several regions of the world, such as North, Central and South America, Asia and Europe (Byrd and Denton, 1950; Arnizaut et al., 1992; Kumar et al., 2018; Unwin et al., 2012; D'Ávila et al., 2017; Bolfa et al., 2019), evidencing its wide geographical distribution. *Paratanaisia bragai* has also been reported in wild and zoo-housed birds, as well as in pet birds, of the orders Columbiformes (Carneiro et al., 1975; Pinto et al., 2004; Brandolini and Amato, 2006; Unwin et al., 2012; Xavier et al., 2015), Galliformes (Keller and Araújo, 1992; Gomes et al., 2005; Menezes et al., 2001; Xavier et al., 2015), Anseriformes (Fedynich et al., 1996), Psittaciformes (Luppi et al., 2007; Silva et al., 2016), and Passeriformes (Tavela et al., 2016; Unwin et al., 2012). This characteristic suggests low specificity regarding the definitive host.

Wild birds infected with *P. bragai* probably act as reservoirs, because they often come into proximity by eating the same food as chickens kept outdoors (Gomes et al., 2005). Migration of wild birds infected by trematodes can also spread helminths to new areas, posing a high risk of native hosts being exposed to infection (Huffman, 2008). These factors give veterinary importance to *P. bragai* because infection by this parasite can cause economic losses when it results in serious lesions or death of the host.

Many studies have been published on the morphology and morphometry the adult stage of *P. bragai* (Tubangui and Masiluñgan, 1941; Maldonado, 1945; Stunkard, 1945; Freitas, 1951; Franco, 1965; D'Ávila et al., 2017). Using scanning electron microscopy, Brandolini and Amato (2007) analyzed the external morphology *P. bragai* adults and found an irregular and highly wrinkled tegument, with the presence of simple and bifid scales.

This digenetic trematode species has a very confused taxonomy. For this reason, better knowledge of the morphology is important to clarify the correct taxonomic position of *P. bragai*, not only allowing more accurate diagnoses, but also better clinical management of parasitosis caused by this parasite in its vertebrate host. So, because of the dearth of morphological studies of *P. bragai* adults, especially using advanced electron microscopy techniques, the present study aims to describe the morphology and morphometry the adult stage of *P. bragai* using light microscopy and scanning electron microscopy.

2. Materials and Methods

Adult *C. livia* pigeons were caught in the Irajá district of the city of Rio de Janeiro (22°49'51"S and 43°20'17"W). The birds were transported to the Helminth Biology and Ecology Laboratory of the Animal Biology Department of Rio de Janeiro Federal Rural University (UFRRJ), in the city of Seropédica, where they were examined according to Ritchie's fecal sedimentation method (De Carli, 1994).

Two infected birds were euthanized in a CO_2 chamber and necropsied to remove the kidneys. The organs were placed in Petri dishes containing a 0.85% physiological saline solution (0.85% NaCl) and then sectioned with a scalpel. Adult helminths were recovered and transferred to another Petri dish containing the same saline solution.

The adult helminths were fixed in 2.5% glutaraldehyde and cacodylate buffer (0.1 M, pH 7.4) at 4ºC for 24 hours (Pinheiro et al., 2004).

Light microscopy (LM) - The fixed adult helminths were mounted between slides and cover slips using the fixing agent as mounting medium and observed under an Olympus BX51 light microscope coupled to an Olympus DP12 digital camera. The images were obtained and processed using the iTEM image capture system, under bright field and differential interference contrast (DIC).

The captured images of adult helminths were used to obtain the measures, which are presented in micrometers (µm), expressed as mean ± standard deviation, with minimum and maximum values within parentheses.

Staining – The adult helminths fixed as previously described were stained with Delafield's hematoxylin, according to the regressive technique proposed by Amato and Amato (2010) and mounted on glass slides. Images of the tissues were obtained using an Olympus DP12 digital image system coupled to an Olympus BX51 light microscope.

Scanning electron microscopy (SEM) - The fixed adult helminths were washed with cacodylate buffer (0.1 M, pH 7.4), post-fixed in 1.0% osmium tetroxide and 0.8%

potassium ferrocyanide and washed again in the buffer solution. The specimens were then dehydrated in an ascending ethanol series (30% to 100%) for 1 hour and dried in a critical point chamber using CO_2 (Baltec CPD, Balzers Union). The processed adult helminths were then mounted on metal bases and gold sputtered for observation under an FEI Quanta 250 scanning electron microscope operating at 20kV (Pinheiro et al., 2004).

3. Results

3.1. Light microscopy

Differential interference contrast (DIC) light microscopy revealed that the adult *P. bragai* trematode has an elongated and dorsoventrally flattened body, with the presence of a subterminal oral sucker at the anterior end of the body, followed by the pharynx, along with the presence or vitellogenic glands on both sides of the parasite's body (see Figures 1A and 1C).

The average body measurements of the adult parasite obtained by light microscopy were length of 1,712.00 ± 86.43 µm (1600 – 1840), anterior region width of 470.00 ± 56.12 µm (410 – 540), width of the acetabulum region of 490.00 ± 48.48 µm (430 – 540) and width of the posterior region of 452.00 ± 38.34µm (410 – 500).

The oral sucker (see Figures 1A-1C) had major diameter of 181.86 ± 13.88 µm (164.21 – 200.00) and minor diameter of 156.36 ± 9.07 µm (140 – 170). The acetabulum, however, could not be visualized by light microscopy.

The pharynx (see Figures 1A-1C) presented major diameter of 87.55 ± 15.53 µm (65.26 – 102.94) and minor diameter of 65.36 ± 9.79 µm (54.74 – 78.57). The esophagus was not observed in any specimen in this study.

The specimens stained with hematoxylin-eosin (see Figure 1B), were observed by bright-field light microscopy, showing more details of the vitellogenic glands, which are outside the cecum and extend to the pre-ovarian region.

The intestinal ceca (see Figure 1B) are parallel to the vitellogenic glands, on both sides of the body, with sinuous shape, located 230.00 µm from the posterior region of the body.

The morphometric data are compiled (see Table 1), which also presents a comparison between the morphometric parameters observed in this study with those reported by other authors.

Scanning electron microscopy: The adult specimens of *P. bragai* trematode presented average length of 1,295.69 ± 232.77 µm (1,028.17 – 1,451.92), average width in the anterior region of 250.68 ± 32.96 µm (230.76 – 288.73), average width in the acetabulum region of 289.82 ± 46.19 µm (244.18 – 336.54) and average width in the posterior region of 280.65 ± 32.11 µm (255.81 – 316.90).

The oral sucker presented major diameter of 115.11 ± 11.59 µm (103.22 – 126.37) and minor diameter of 98.14 ± 21.19 µm (77.42 – 119.78).

Through scanning electron microscopy, it was possible to gain an overview of the parasite, which presented an elongated and dorsoventrally flattened body, with the presence of a subterminal oral sucker at the anterior end of the body, as well as an oral opening and acetabulum (see Figure 2A). Detailed examination of the oral sucker revealed the presence of external papillae around the suction cup, arranged irregularly, along with internal papillae (see Figures 2B – 2D). We observed a wall with musculature in parallel rows inside the oral cavity (see Figures 2E – 2F). The external papillae had major diameter of 4.52 ± 1.20 µm (2.42 – 8.33) and minor diameter of

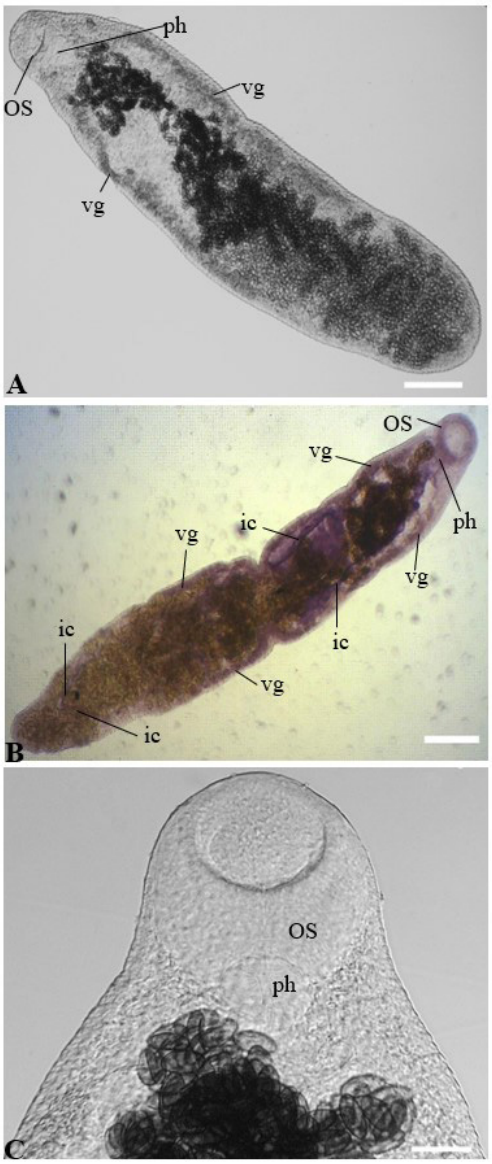

Figure 1. Adult stage of *Paratanaisia bragai* observed by light microscopy. A. Overview of the helminth, with highlight on the oral sucker (os), pharynx (ph) and vitellogenic glands (vg). Differential interference contrast microscopy (DIC. Scale bar = 200 µm. B. Overview of the helminth, stained with hematoxylin-eosin, with highlight on the oral sucker (os), pharynx (ph), intestinal ceca (ic) and vitellogenic glands (vg). Bright-field microscopy. Scale bar = 200 µm. C. Detail of the oral sucker (os) and pharynx (ph). Differential interference contrast microscopy (DIC). Scale bar = 40 µm.

Table 1. Comparative morphometry of the adult stage of *Paratanaisia bragai*.

Measures (μm)	Present study Light microscopy	Present study SEM	Santos (1934)	Stunkard (1945)	Byrd and Denton (1950)	Freitas (1951)	Franco (1965)	Brandolini and Amato (2007)	D'Àvila et al. (2017) post-ovigerous
	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD
Length	1,712.00 ± 86.43 (1,600 – 1,840)	1,295.69 ± 232.77 (1,028.17 – 1,451.92)	- Up to 3,000	- (1,200 – 3,000)	1,990 ± - (1,620 – 2,550)	- (1,670 – 3,950)	- (3,490 – 4,260)	1,735 ± - (1,674.80 – 1,756.34)	1,879 ± 186 (1,675 – 2,250)
Anterior region width	470.00 ± 56.12 (410 – 540)	250.68 ± 32.96 (230.76 – 288.73)	- Up to 660	- (200 – 450)	420 ± - (320 – 530)	- (450 – 700)	- (910 – 1,030)	-	
Acetabulum region width	490.00 ± 48.48 (430 – 540)	289.82 ± 46.19 (244.18 – 336.54)							275.70 ± 49.00 (191.60 – 350)
Posterior region width	452.00 ± 38.34 (410 – 500)	280.65 ± 32.11 (255.81 – 316.90)							
Oral sucker major diameter	181.86 ± 13.88 (164.21 – 200.00)	115.11 ± 11.59 (103.22 – 126.37)	- 500	- (100 – 145)	190 ± - (140 – 230)	- (180 – 300)	- (270 – 310)	133.47 ± - (128.44 – 140.07)	145.70 ± 30.80 (77.50 – 190)
Oral sucker minor diameter	156.36 ± 9.07 (140 – 170)	98.14 ± 21.19 (77.42 – 119.78)	- 410		170 ± - (130 – 200)	- (150 – 270)	- (230 – 320)	102.07 ± - (98.61 – 103.34)	137.00 ± 23.59 (75 – 155)
Pharynx major diameter	87.55 ± 15.53 (65.26 – 102.94)	-	- 160	- (45 – 65)	80 ± - (60 – 90)	- (50 – 110)	- (120 – 130)	-	71 ± 11 (55 – 85)
Pharynx minor diameter	65.36 ± 9.79 (54.74 – 78.57)	-			60 ± - (40 – 80)	- (40 – 100)	- (90 – 130)	-	57.70 ± 11.30 (40 – 75)
Acetabulum major diameter	-	38.36 ± 6.96 (28.77 – 45.39)	-	- (40 – 50)	-	- (30 – 100)	-	40.83 ± - (37.70 – 43.96)	-
Acetabulum minor diameter	-	31.59 ± 7.04 (21.75 – 38.16)	-		-	- (30 – 80)	-	20.41 ± - (18.30 – 22.52)	-
Excretory pore major diameter	-	12.27 ± 9.16 (5.79 – 18.75)	-	-	-	-	-	-	-
Excretory pore minor diameter	-	3.95 ± 1.49 (2.89 – 5.00)	-	-	-	-	-	-	-

( ) Numbers in parentheses represent the observed minimum and maximum values. X ± SD= mean ± standard deviation. SEM= Scanning electron microscopy. - = Data not provided by the authors. **Observation:** The nomenclature larger diameter and smaller diameter was used in this study for structures with rounded appearance, such as oral cups, pharynx and acetabulum, the same nomenclature used by Brandolini and Amato (2007) in measuring cups. The other cited authors used the nomenclature width and length. Thus, when filling out the table, the largest values were inserted in the longest length and the smallest values in the shortest length, regardless of whether width or length was considered by the other authors.

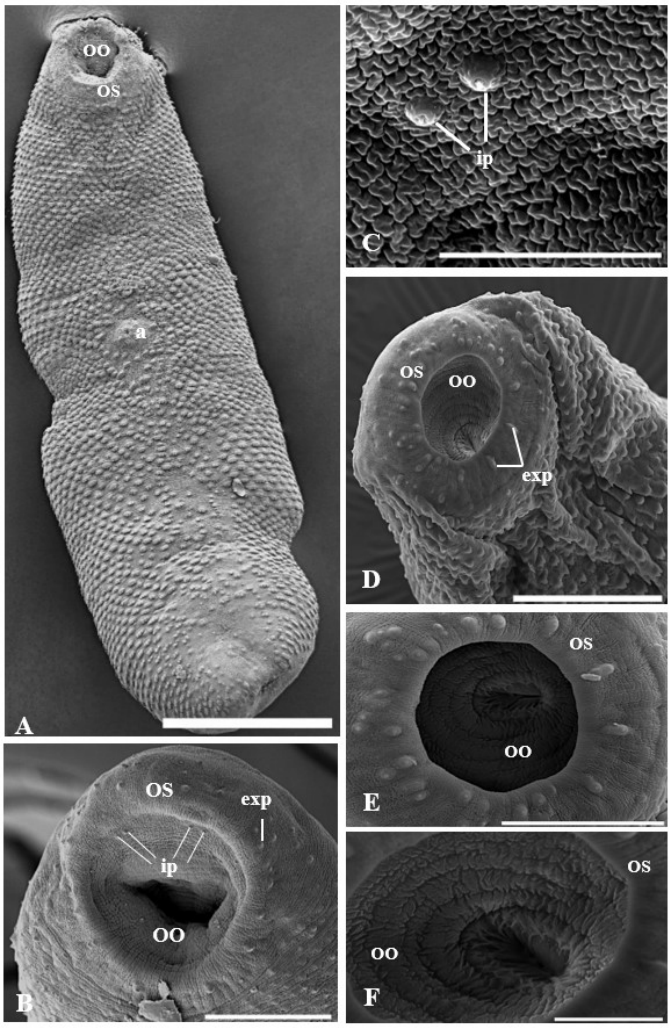

Figure 2. Adult stage of *Paratanaisia bragai* observed by scanning electron microscopy. A. Overview to the helminth with highlight on the oral sucker (os), oral opening (oo) and acetabulum (a). Scale bar = 250 µm. B. Detail of the oral sucker (os), with highlight on the presence of external papillae (exp) surrounding the suction cup and internal papillae (ip), inside the oral opening (oo). Scale bar = 50 µm. C. Detail of the internal papillae (ip) in the region of the oral sucker. Scale bar = 10 µm. D. Oral sucker (os) with external papillae (exp) surrounding it and oral opening (oo). Scale bar = 100 µm. E and F. Detail of the internal region of the oral opening (oo) of the oral sucker (os), with visualization of the lateral wall with musculature in parallel rows. Scale bar = 50 µm and 20µm, respectively.

3.62 ± 0.83 µm (2.35 – 5.55), while the internal papillae were smaller, with major diameter of 2.24 ± 0.66 µm (1.65 – 3.53) and minor diameter of 1.68 ± 0.29 µm (1.43 – 2.09).

The acetabulum had major diameter equal to 38.36 ± 6.96 µm (28.77 – 45.39) and minor diameter of 31.59 ± 7.04 µm (21.75 – 38.16). Besides this, for the first time we measured the distance from the oral sucker to the acetabulum, finding 490.82 ± 54.71 µm (429.58 – 534.88), while the distance from the acetabulum to the most apical part of the posterior end was 746.54 ± 160.19 µm (563.38 – 860.46). The papillae present in the acetabulum region had major diameter of 1.79 ± 0.41 µm (1.40 – 2.65) and minor diameter of 1.51 ± 0.52 µm (0.88 – 2.35) and were arranged irregularly around the border. Finally, we observed the absence of scales around the acetabulum (see Figures 3A– 3C).

Only one genital pore was observed, ventrally and to 109,89 µm anterior to the acetabulum with major diameter of30.59 µm and minor diameter of 26.18 µm (see Figures 4A – 4B). The cirrus was everted, projecting through the genital pore (see Figure 4C), with rosette ornamentations.

The excretory pore was observed at the posterior end, subterminal, in the dorsal region of the body, with major diameter of 12.27 ± 9.16 µm (5.79 – 18.75) and minor diameter of 3.95 ± 1.49 µm (2.89 – 5.00) (see Figures 4D and 4E).

Scales were observed when examining the details of the region of the acetabulum and genital pore, arranged in 1 to 3 divisions (see Figures 3A, 3B and 4C).

The morphometric data are compiled (see Table 1), which also presents comparisons with the morphometric parameters reported by other authors.

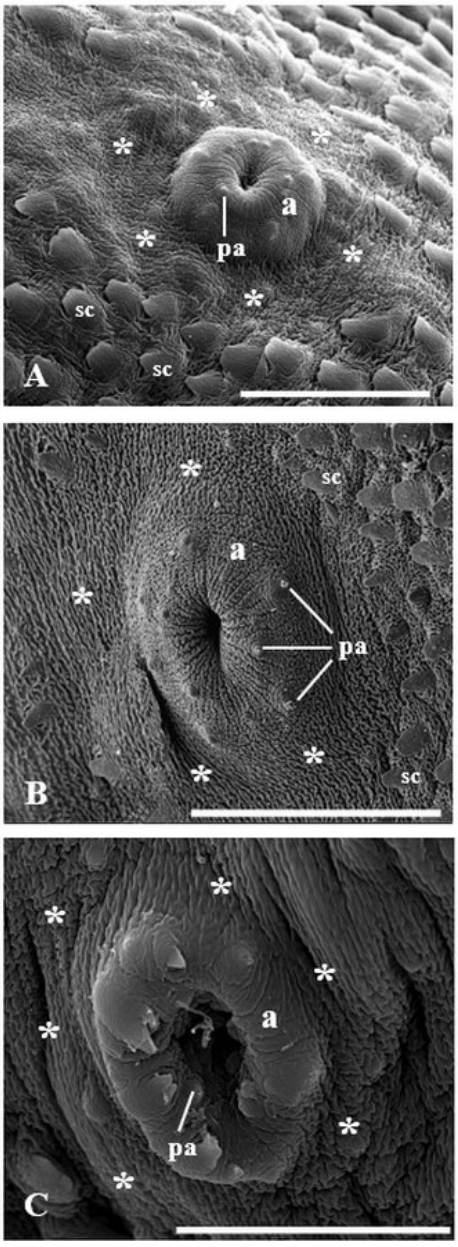

Figure 3. Middle third region of the adult stage of *Paratanaisia bragai* observed by scanning electron microscopy. A, B and C. Different views of the acetabulum (a), indicating the presence of papillae (pa) surrounding it and an adjacent peripheral area (*) without the presence of scales. Scale bar = 50 µm, 30 µm and 40µm, respectively.

4. Discussion

The characteristics of the adult stage of *P. bragai* observed in this study, such as elongated and flattened body, presence of a subterminal oral sucker at the anterior end of the body, followed by the pharynx and vitellogenic glands on both sides of the body, corroborates the observations described by various authors (Santos, 1934; Stunkard, 1945; Byrd and Denton, 1950; Freitas, 1951; Franco, 1965), besides the presence of the acetabulum, also reported by Maldonado (1945), Stunkard (1945), Freitas (1951) and Brandolini and Amato (2007).

In this study, the measurements of the adult parasite obtained by light microscopy were larger than those obtained by scanning electron microscopy for the average length, average width of the anterior region, average width of the acetabulum region and average width of the posterior region.

The literature presents variations of the size of adult specimens of *P. bragai* regarding length and width. Santos (1934) found that the width of adults reached up to 3,000 µm and the width up to 600 µm, but did not differentiate the body regions, i.e., anterior, acetabular and posterior. The variation observed by Santos (1934) for length was very broad but fits in the range of measures obtained in this study, both under light microscopy and scanning electron microscopy. With respect to the width, the measures presented by that author are only in accordance with those obtained by us through light microscopy. Stunkard (1945) stated that the gravid parasite's length ranged from 1,200 to 3,000 µm and width from 200 to 450 µm, similar to the ranges observed by us according to both microscopic techniques.

The measures reported by Byrd and Denton (1950), Freitas (1951) and Brandolini and Amato (2007) are similar to those found by in this study by light microscopy. Franco (1965), however, observed much larger parasites than those reported by the other authors, with length varying between 3,490 and 4,260. D'Ávila et al. (2017) stated that the length of the post-ovigerous adult stage varied from 1,675 to 2,250 µm, a range that fits with our measurements by light microscopy, and width varying from 191.60 to 350.00 µm, similar to our results obtained by scanning electron microscopy.

Maldonado (1945) observed that *P. bragai* with four days adult development had length of 600 µm, as well as Stunkard (1945) who verified the same measurement for the length of the immature specimen, while specimens at eight days had length of 950 µm and the adult stage, with fully developed and functional reproductive system (between 11 and 15 days of development) had length of 1250 µm. This last value is within the variation of the specimens we observed by scanning electron microscopy, but we did not distinguish between the stages before and after oviposition. In turn, D'ávila et al. (2017) reported length ranging from 530 and 1,000 µm for adults before egg laying and between 1,675 and 2,250 µm for specimens afterward. These studies are the only ones to report comparisons of adults before and after oviposition, and they observed differences in the amplitude of variation.

The variations found in the literature in comparison with those found in this study with respect to the body dimensions of *P. bragai* adults are related to the different development stages of the helminth analyzed (before and after oviposition), and within these stages the number of days of development. They can also be related to the contraction of the parasite during the processing for the different microscopic techniques employed.

In the present study, the measures of the oral sucker by light microscopy are similar to those reported by

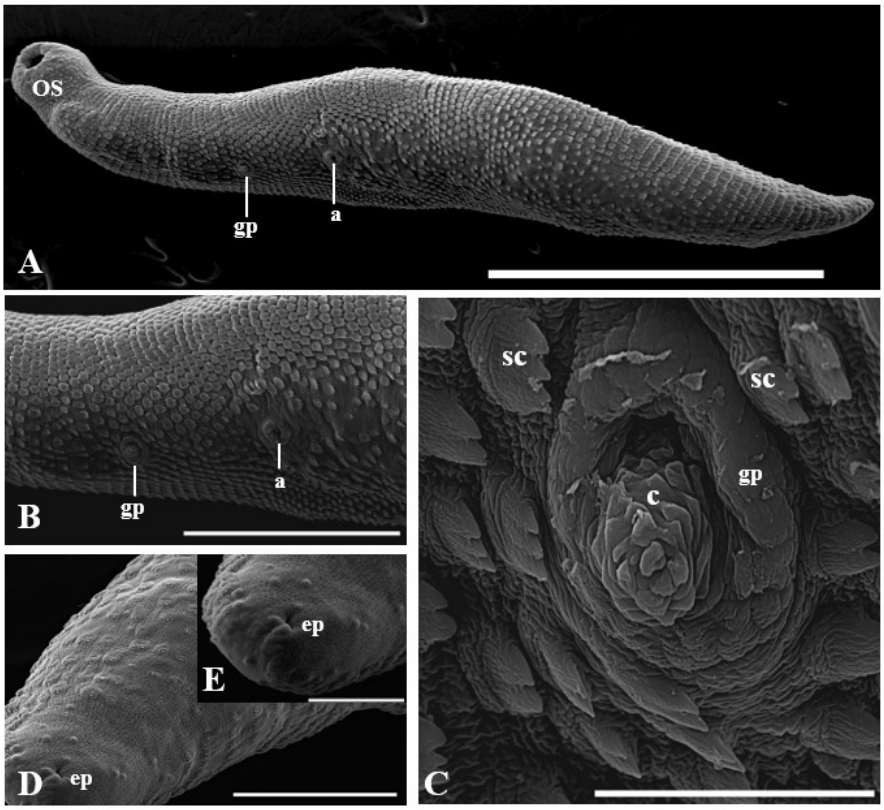

Figure 4. Adult stage of *Paratanaisia bragai* observed by scanning electron microscopy. A. Overview of the helminth, in the ventral region, indicating presence of the oral sucker (os), location of the genital pore (gp) and acetabulum (a). Scale bar = 500 µm. B. Detail of the genital pore (gp) and acetabulum (a). Scale bar = 200 µm. C. Detail of the genital pore (pg) with presence of the everted cirrus (c). Scale bar = 30 µm. D. Excretory pore (ep) in the posterior dorsal region. Scale bar= 100 µm. E. Detail of the posterior dorsal region indicating the excretory pore. Scale bar= 50 µm.

Byrd and Denton (1950) and Freitas (1951), while those obtained by scanning electron microscopy are similar to those observed by Stunkard (1945) and Brandolini and Amato (2007). By the two microscopic methods used by us, the values are in accordance with those reported by D'Ávila et al. (2017) while the measures reported by Santos (1934) and Franco (1965) are much greater than those found by the other authors.

Through detailed analysis of the oral sucker, we observed external papillae around the suction cup, arranged irregularly, as also observed by Brandolini and Amato (2007). Besides this, we observed internal papillae. According to Hockley (1973), who examined the oral sucker of specimens of both sexes of *Schistosoma mansoni* Sambon, 1907, the internal surface of the oral sucker of both sexes contained small spines pointing toward the mouth, but these were absent on the external surface of the suction cup.

The pharynx, only visualized by light microscopy, presented amplitude of variation similar to the reports of Stunkard (1945), Byrd and Denton (1950), Freitas (1951) and D'Ávila et al. (2017), but smaller than observed by Franco (1965) and Santos (1934).

The esophagus was not visualized in any specimen examined in this study, as also occurred in the study by Santos (1934). D'Ávila et al. (2017) did not refer to the esophagus, and according to the figures presented, this organ was not observed by them. In contrast, Stunkard (1945) found the presence of a short esophagus, visualized only when the posterior end of the parasite was extended. Byrd and Denton (1950) described an esophagus variable, as did Franco (1965) and Freitas (1951), who reported variation of the organ in the specimens examined, long esophagus, with and without dilation, as well as absence in some cases.

Through DIC and staining it was possible to observe the vitellogenic glands in more detail. They are located outside the cecum and extend to the pre-ovarian region, as also observed by Freitas (1951) and Byrd and Denton (1950). Stunkard (1945) found that the ovary is situated about a third of the body length of the anterior end. The vitellogenic glands visualized in the present study are located beyond this region, that is, they are pre-ovarian. The dimensions of the vitellogenic glands observed in this study are within the variation reported D'Ávila et al. (2017). Other authors mentioned here use the ovary as a reference for positioning the vitellogenic glands.

The location of the vitellogenic glands is used to distinguish the genera of the subfamily Tanaisiinae.

The species *Tanaisia bragai* (Santos, 1934), *Tanaisia confuse* Freitas, 1951 and *Tanaisia robusta* Freitas, 1951 have vitellogenic glands that extend anteriorly beyond the ovarian zone, a trait that differs from the other species of the genus (Freitas, 1951). Due to this particularity, Freitas (1959) described the genus *Paratanaisia* as including the three species, with *P. bragai* being the type species. The genus *Paratanaisia* was maintained by Kanev et al. (2002), where in the taxonomic key it uses as a characteristic that the vitellogenic fields extend pre and post testicular. According to Freitas (1959) the genus *Tanaisia* (*Paratanaisia*) has a pre-testicular ovary, which confirms the anterior position of the ovarian zone. Therefore, the study of the morphology of the adult stage of *P. bragai* is very important to establish its taxonomy, because besides the use of vitellogenic glands as a specific trait, the tegument can also be utilized, since according to Freitas (1951), the three species mentioned above each presents peculiarities: *T. confuse* has spines in its tegument, unlike *T. robusta* and *T. bragai*, which have scales and differ from each other by the appearance of these scales. According to the illustrations by Freitas (1951) *P. robusta* has scales divided up to the base of attachment to the tegument, while *P. bragai* the scales have the part attached to the tegument, not divided, and a free part that has divisions, as well as, visualized in this study.

We observed that the intestinal ceca run parallel to the vitellogenic glands, on both sides of the body, in sinuous form, and fuse at the posterior end, corroborating the observation of Santos (1934). D'Ávila et al. (2017) verified that in post-ovigerous adults the distance between the intestinal ceca and posterior region of the body had amplitude of variation of 200 – 360 µm, which includes the values found in this study. The results of Freitas (1951) are also corroborated by our findings, since he described the presence of the intestinal ceca dorsal to the gonads more or less sinuous and fused, located100 to 450 µm from the posterior end of the body. Byrd and Denton (1950) also reported the ceca running parallel at the edges of the body, fusing near the body's posterior end, at a distance from that end of one-fifth to one-eighth of the length of the parasite, a relation similar to that found by us, as well as by Freitas (1951) and D'Ávila et al. (2017). Franco (1965) also observed that the intestinal ceca fuse at a distance of 580 to 610 µm from the posterior end of the body. These are very large measures compared to those observed by us. However, these lengths fit in the range described by Byrd and Denton (1950). The difference between the values found in this study and those presented by Franco (1965) can be attributed to the fact he analyzed larger specimens (measuring 3,490 – 4,260 µm) than those analyzed in the present study.

The visualization of the acetabulum by differential interference contrast microscopy was very difficult due to its small size and by the fact that all the specimens observed in this study were in the post-ovigerous stage, in which numerous eggs were observed. The presence of the acetabulum was confirmed by scanning electron microscopy in all the specimens analyzed. Some scientific works, however, do not mention the presence of the acetabulum and others state that it was not observed in the adult parasite (Santos, 1934; Byrd and Denton, 1950; Franco, 1965).

The difficulty of observing the acetabulum in post-ovigerous adults of *P. bragai* was also reported by D'Ávila et al. (2017), because they were unable to visualize the acetabulum of such specimens of *P. bragai* using light microscopy, only doing so by confocal laser scanning microscopy. In contrast, those authors were able to visualize the acetabulum of pre-ovigerous adults by light microscopy. Therefore, it is important associate the stage of the adult specimen (pre-ovigerous or post-ovigerous) with the microscopic technique so as not to err in describing the morphology of the species. Maldonado (1945) described for the first time the presence of the acetabulum in adults with eight days of development, but when the specimens reached sexual maturity, the organ was atrophied, less than 40 µm in diameter or even absent. In the present study, however, analysis of the acetabulum morphology by scanning electron microscopy indicated that although small, the acetabulum was not atrophied, and instead was still functional, as also reported by Brandolini and Amato (2007) and D'Ávila et al. (2017).

The dimensions of the acetabulum measured in the present study are in accordance with the range of variation reported by Freitas (1951), with length of 30 – 100 µm and width of 30 – 80 µm; Stunkard (1945), with diameter of 40.00 – 50.00 µm; Brandolini and Amato (2007), with average of 40.83 µm (37.70 – 43.96) for the major diameter and 20.41 µm (18.30 – 22.52) for the minor diameter; and D'Ávila et al. (2017), with average length of 39.91 µm (25– 55 µm) and width of 36.70 µm (25–50 µm). The last authors carried out measurements of pre-ovigerous adults but did not report measurements of the acetabulum in post-ovigerous adults, although affirming that the acetabulum of post-ovigerous adults was smaller than that in pre-ovigerous adults. The reduction of size of the acetabulum described by D'Ávila et al. (2017) can be seen in the figures of that work, which show that the opening of the acetabulum cavity is smaller in post-ovigerous adults. However, the acetabulum cavity's size might have changed due to contraction of the parasite, as mentioned by Maldonado (1945) for development of the acetabulum of *P. bragai* cercariae.

Maldonado (1943) previously found that during the larval development, *P. bragai* cercariae had a well-developed acetabulum, but it then atrophied with development of the parasite. In later work Maldonado (1945) verified that in adult specimens in the eighth day of development, the acetabulum had grown, although the enlargement was not in the same proportion as the entire body, and that the acetabulum had atrophied when the specimens reached the adult stage. So, it is important to conduct further studies regarding the size of the acetabulum of *P. bragai* during its development to elucidate these questions. However, the presence of this suction cup in the adult stage of *P. bragai* is unquestionable.

The measures of the distances between the oral sucker and acetabulum and between it and the apical part of the posterior end carried out in this study corroborate the observations of Stunkard (1945) and Brandolini and Amato (2007), that the acetabulum is located in the middle

third of the body. Our finding of the presence of papillae arranged irregularly around the acetabulum corroborates the observation of Brandolini and Amato (2007), who also reported the presence of protuberances in the tegument that surrounds the acetabulum. That structure was observed in the present study in other regions of the parasite's tegument, denominated papillae. Hockley (1973) also reported the presence of sensory papillae along with spines, but only on the internal surface of the acetabulum, in *S. mansoni* of both sexes, with spines being absent on the external surface of the suction cups. The absence of scales near the region of the acetabulum found in the present study is in accordance with the images presented by Brandolini and Amato (2007). However, those authors did not discuss this fact in the text.

The types of scales observed in this study, with variation of 1 to 3 divisions, are in accordance with the findings of Brandolini and Amato (2007), who reported single and bifid scales, i.e., scales divided into two elements with a free end. Stunkard (1945) reported the presence of three to eight fused elements. In turn, D'ávila et al. (2010) reported that the scales had grooves that delineated two to four teeth. How the teeth develop is controversial, since the literature reports different terms to classify them, such as fused elements as described by Stunkard (1945), and division of scales as expressed by Brandolini and Amato (2007). Therefore, new studies are necessary to clarify the ontogenesis of these structures, due to the importance of the scales as a distinctive trait of species of the family Eucotylidae, as noted by Freitas (1951).

With application of scanning electron microscopy, we noted the presence of the genital pore, located above the acetabulum. Its location in the middle and ventral region of the body is corroborated by Stunkard (1945), who observed it below the anterior region of the ovary, ventral in the middle region of the body. Byrd and Denton (1950) observed that besides the ventral and median position, the genital pore is located below the anterior end of the ovary or at the same level as the cephalic margin of the ovary, and that the genital atrium is common to the male and female genital apparatus. Freitas (1951) stated that the genital pore is median, as also reported by Franco (1965) and Brandolini (2000).

Furthermore, we observed the presence of an everted cirrus with rosette ornamentations projecting through the genital pore. Byrd and Denton (1950) stated that the cirrus of *T. bragai* is short and robust (corroborated by our observation), and is contained in the cirrus pocket, located posterior to the genital pore.

D'ávila et al. (2010) reported that the cirrus of *Tanaisia inopina* Freitas, 1951 is highly muscular and cylindrical, and that the reproductive system of *T. bragai* is normally similar to that described in *T. inopina*. Besides this, they observed that both the cirrus of *T. inopina* and the distal part of the uterus open into a genital atrium that leads to a common gonopore. Besides this, the cirrus is the copulatory organ of digenetic trematodes, which may or may not be contained in the cirrus pocket and is a muscular tube that everts during copulation and projects outward (Rey, 2008). However, D'Ávila et al. (2017) did not report any ornamentation in the cirrus, as observed in the present study, which can be related to the species analyzed, *T. inopina*, demonstrating that with respect to these reproductive elements, *T. bragai* and *T. inopina* are not similar.

The presence of ornamentations in the cirrus of *P. bragai* is in accordance with Dawes (1968), who stated that the cirrus of digenetic trematodes generally has a wall with circular and longitudinal musculature along with spines. The presence of spines was verified in *Fasciola gigantic* Cobbold, 1855 by Srimuzipo et al. (2000). They visualized the cirrus in the shape of a sausage with spines of varying lengths and sharpness. Naem et al. (2012) observed that the cirrus of *Fascioloides magna* Bassi, 1875 has a smooth surface, with small pores in the organ's dorsal region, and that certain zones between the cirrus folds contain small pores and groups of small spines.

The excretory pore is located in the posterior end, in a subterminal position in the dorsal region of the body, as described by Brandolini (2000), but unlike observed by Stunkard (1945), who classified the excretory pore as terminal. In contrast, D'Ávila et al. (2017) described the presence of an excretory canal and measured its length in pre-ovigerous specimens of *P. bragai*, so our study contains the first morphometric record of the excretory pore of adult specimens of *P. bragai.*

The use of electron microscopy has enabled considerable advances in knowledge about the morphology of trematode species, not only regarding taxonomy, but also biological and immunological aspects, as well as physiological and biochemical processes of the host-parasite relationship. New morphological traits canal so be elucidated, including their functional roles, thus allowing the inclusion of these traits in the taxonomy of trematode groups.

The results presented in our study shed light on some important aspects of the morphology and taxonomy of *P. bragai*. Contrary to what some parasitologists claim, *P. bragai* is not a parasite that only poses a regional problem. Brazil is one of the main countries from which smugglers obtain wild birds for sale worldwide. This illegal activity, by circumventing health inspection, contributes to the dissemination of not only *P. bragai*, but several other species of parasites that are hosted in these birds. In the literature, there are records of the occurrence of *P. bragai* in birds from various countries, which is only discovered by postmortem diagnosis, indicating the lethality of infection by this trematode and the difficulty of identifying infection before death of the vertebrate host. In addition, *P. bragai* has been found parasitizing pet birds, causing their death.

In spite of this great veterinary, economic, and public health importance, there are few studies about this parasite. Additionally, its controversial taxonomy further hampers the identification and correct diagnosis of parasitosis. For this reason, the present study updates previous findings about the *P. bragai* taxonomy and adds new information about the morphology of this parasite, enriching the morphological tools for better identification of the parasites of this species.

Acknowledgements

To the Otto Wucherer Helminth Biology Laboratory, Cell Biology and Parasitology Program, Carlos Chagas Filho

Institute of Biophysics, Health Sciences Center, UFRJ, Rio de Janeiro, RJ, Brazil, for support in the procedures and microscopic analysis.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – process number 303248/2018-1, and the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ – process number E-26/203.004/2016).

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