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Original Article

Cotylophoron marajoensis n. sp. (Digenea: Paramphistomidae) a parasite of *Bubalus bubalis* on Marajó Island, Pará, Brazilian Amazon

Cotylophoron marajoensis n. sp. (Digenea: Paramphistomidae) parasito de *Bubalus bubalis* na Ilha de Marajó, Pará, Amazônia Brasileira

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Abstract

The genus *Cotylophoron* belongs to the Paramphistomidae family and its definitive hosts are ruminants in general. This work describes the presence of a new species of the gender, a parasite in the rumen and reticulum of *Bubalus bubalis*, on Marajó Island in the Eastern Brazilian Amazon, using of light microscopy, scanning electronic microscopy and molecular biology techniques. One hundred and ten animals were analyzed, of which 4.54% were parasitized by flukes in their adult forms. The helminths were found fixed to the ruminal mucosa and present *Liorchis*-type pharynx, *Cotylophoron*-type genital sucker, oblique testicles larger than the ovary, uterus in rings full of eggs and *Cotylophoron*-type acetabulum. These morphologic characters do not fit into any previously described species. Thus, it is proposed that this is a new species in the genus *Cotylophoron*. The present work expands the record of parasitism by helminths in *Bubalus bubalis*, this being the first record of trematoda from the genus *Cotylophoron* for this host in the Brazilian Amazon.

Keywords: Taxonomy, parasite, Trematoda, buffalo, Brazil.

Resumo

O gênero *Cotylophoron* pertence à família Paramphistomidae e possui como hospedeiros definitivos ruminantes em geral. Este trabalho descreve a presença de uma espécie nova do gênero, parasito do rúmen e retículo de *Bubalus bubalis*, na Ilha de Marajó, Amazônia oriental brasileira, a partir das técnicas de microscopia de luz, microscopia eletrônica de varredura e biologia molecular. Foram analisados 110 animais, dos quais 4,54% estavam parasitados por trematódeos na sua forma adulta. Os helmintos foram encontrados fixados à mucosa ruminal, apresentando faringe do tipo *Liorchis*, ventosa genital do tipo *Cotylophoron*, testículos oblíquos maiores que o ovário, útero em alças repleto de ovos, e acetábulo do tipo *Cotylophoron*. Estes caracteres morfológicos não se enquadram em nenhuma espécie previamente descrita. Assim, propõe-se uma nova espécie ao gênero *Cotylophoron*. O presente trabalho amplia o registro do parasitismo por helmintos em *Bubalus bubalis*, sendo este o primeiro registro de trematódeos do gênero *Cotylophoron* nesse hospedeiro para a Amazônia brasileira.

Palavras-chave: Taxonomia, parasito, Trematoda, búfalo, Brasil.

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Introduction

The *Bubalus bubalis* species is known as the "buffalo" throughout Brazil and is bred in several regions of the world because of its sturdiness, milk and dairy products and good-quality meat, besides being used for work (Damasceno et al., 2010). In Brazil, buffalos have been imported from different countries such as Australia, Egypt, India, Italy, and Southwest Asia and were first introduced in the continent on Marajó Island in 1895, where the natural conditions of this region were excellent for their development (Marques, 2000). And although it is a domestic and economically important animal, little is known about its parasitic interactions.

The genus *Cotylophoron* (Stiles & Goldberger, 1910) belongs to the Paramphistomidae family (Fischoeder 1901). In general, they have ruminants as definite hosts, the rumen and reticulum being their sites of infection when adults. The immature forms inhabit the small intestine, where they perform the backward movement in their development process in the digestive tract, traveling from the small intestine to the rumen/reticulum (Forlano et al., 2001). This behavior is due to parasitic ability of genetic expression that allows migration through host's tissues while triggering it's immune responses (Pérez-Ponce de León & Hernández-Mena, 2019).

Currently, the genus *Cotylophoron* comprises eight species: *Cotylophoron cotylophorum* (Fischoeder 1901); *Cotylophoron jacksoni* Nasmark 1937; *Cotylophoron fulleborni* Nasmark, 1938; *Cotylophoron panamensis* Prince & McIntosh 1953; *Cotylophoron bareilliense* Mukherjee & Chauhan 1965; *Cotylophoron macrosphinetris* Sey & Graber 1979; *Cotylohoron xiangjiangense* Wang 1979; and *Cotylophoron travassosi* (Martins & Pezzi, 1992).

This genus includes trematode parasites of rumen and reticulum in ruminants worldwide (Martínez & Velásquez, 2012). Alarcón & Velásquez (2009) described the *C. cotylophorum* species as also present in the rumen of *Bos taurus* in the region of Rio Negro, Colombia. Sánchez et al. (2009) described the same species as parasitizing the rumen of bovines in Loreto in Peru. Morales et al. (2015) reported the presence of *C. fulleborni* in the ruminal mucosa of bovines in Venezuela.

In Brazil, the genus *Cotylophoron* is reported as parasitizing different ruminants in different locations. Costa & Guimarães (1990) mention the first occurrence of *C. bareilliensis* parasitizing the rumen of sheep in the state of Pará and Costa & Guimarães (1992) describe the *C. travassosi* species in bovines in the state of Maranhão. Miranda & Costa (1999) report the incidence of *C. panamensis* parasitizing bovines of the state of Rondônia and *C. fulleborni* in bovines and goats in the states of Roraima, Pará and Maranhão and *C. jacksoni* also in bovines in the states of Pará, Roraima and Rondônia.

With this in mind, the present work describes the morphology, prevalence and molecular data of new species of Paramphistomidae that was found as a parasite of *B. bubalis* on Marajó Island, Brazil.

Material and Methods

Study area and collection

The sampling was composed of hosts deriving from the island of Marajó, from the municipality of Soure (00°4'00"S, 48°31'24"W), Breves (01°40'56"S, 50°28'49"W) and Cachoeira do Arari (01°00'41"S, 48°57'48"W), totaling 110, all rumens and reticulums of the examined buffalos obtained from slaughter in the Abatedouro Frigorífico, in Tapanã, at Cooperativa da Indústria Agropecuária do Pará (SOCIPE), in Belém and in the Matadouro Frigorífico Municipal de Soure, Pará. Each animal had its locality confirmed through its Animal Transport Guide (GTA). Fragments of rumen and reticulum were collected and transported cooled to the Laboratório de Histologia e Embriologia Animal (LHEA), Universidade Federal Rural da Amazônia (UFRA).

Preparation of parasites for light microscopy

The trematodes found were fixed and processed according to Giese et al. (2015). Ten trematodes were used for morphologic and morphometric analysis. The measures were obtained in millimeters and are presented in the form of average and amplitude between parentheses. The images in light microscopy were obtained by photomicroscope with connected clear vision camera (LEICA DM2500). For scanning electron microscopy, the helminths specimens were washed in distilled water, post-fixed in 1% osmium tetroxide, dehydrated to the critical point of CO_2, metallized with gold+palladium, and analyzed using a TESCAN scanning electron microscope (VEGA 3) in the Scanning Electron Microscopy Laboratory, Universidade Federal Rural da Amazônia.

Molecular analysis

Molecular characterization was based on partial 18S small subunit ribosomal rRNA gene (18S SSU), which after DNA extraction through the Invisorb Spin Tissue Mini Kit (Stratec Molecular), was amplified using the primers cc18sf (5'- cggtgaaaccgcgaatggctc - 3') and cc18sr (5'- gacgggcggtgtgtacaaagg - 3'). The polymerase chain reactions (PCRs) were carried out in 25 µL final volume, containing 5-10 ng of DNA, 50 mM KCl, 2 mM $MgCl_2$, 10 mM Tris-HCl, 50 µM of each DNTP, 0.5 µM of each oligonucleotide and one unit of Taq DNA polymerase (Invitrogen). The amplification reaction consisted of 35 cycles of 1 min at 95 °C, 1 min at 67 °C, and 1 min 30 sec at 72 °C, preceded by 5 min at 95 °C and followed by 10 min at 72 °C. Amplicons were enzymatically purified with Illustra ExoProStar (GE Healthcare). Nucleotide sequencing was performed in an ABI 3500 xL Genetic Analyzer (Thermo Fisher Scientific), according to the manufacturer's specifications. BioEdit software (Hall, 1999) was used to align forward and reverse sequences.

Results

Paramphistomidae (Fischoeder 1901)

Genus *Cotylophoron* (Stiles & Goldberger 1910)

Cotylophoron marajoensis n. sp.

Description (Figures 1, 2)

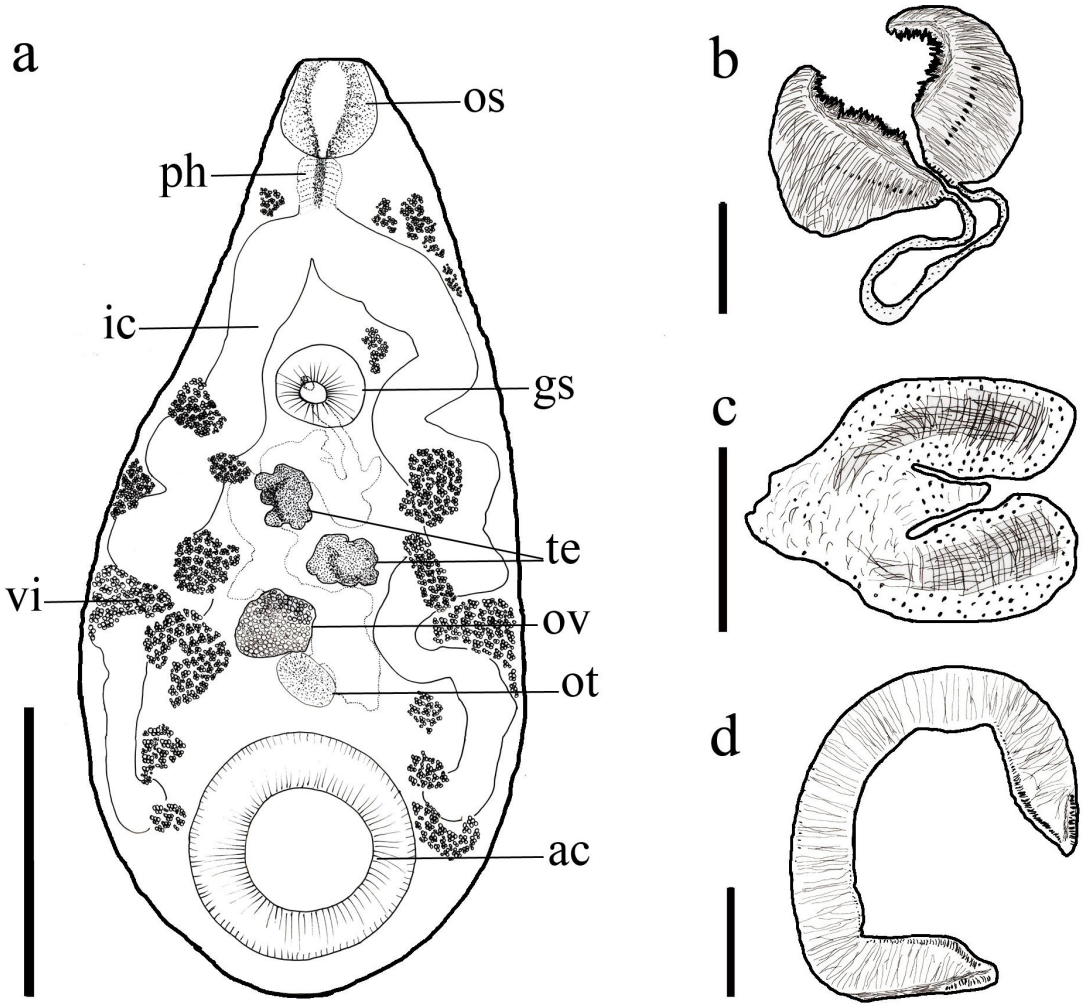

Figure 1. Morphology of *Cotylophoron marajoensis* n. sp.: (a) entire helminth, ventral view with oral sucker (os), pharynx (ph) of *Liorchis* type, intestinal cecum (ic), genital sucker (gs); ootype (ot), acetabulum (ac), ovary (ov), testicles (te) and vitellaria (vi); (b) pharynx of *Liorchis* type; (c) genital pore of *Cotylophoron* type; (d) acetabulum of *Cotylophoron* type. *Scale-bars*: **A** 200 µm, **B** 50 µm, **C** 100 µm, **D** 50 µm.

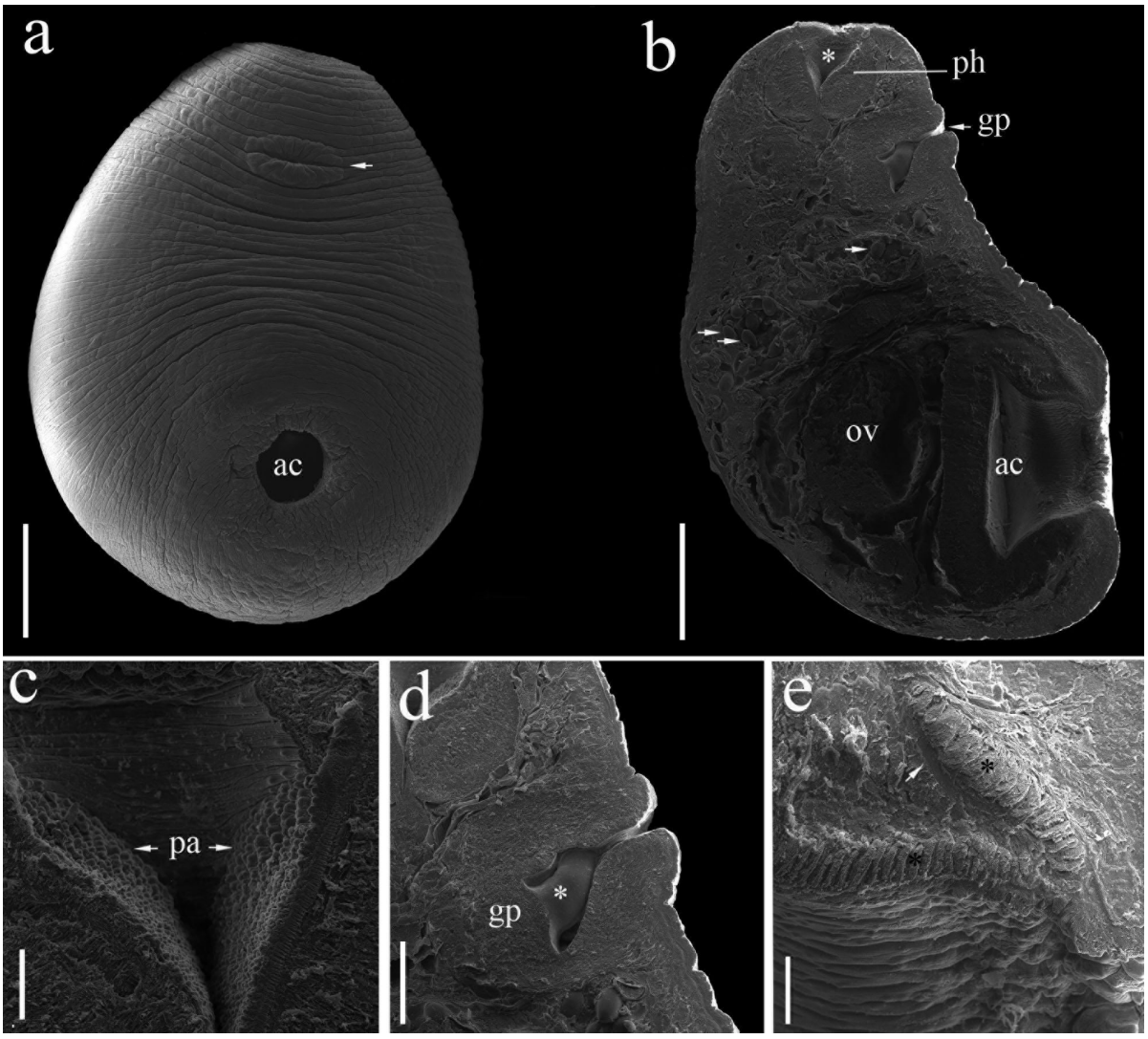

Figure 2. Scanning electron micrographs of *Cotylophoron marajoensis* n. sp.: (a**)** external morphology with genital pore (arrow) in the anterior portion of the helminth and acetabulum (ac) in the posterior portion of the helminth; (b) internal morphology with pharynx (ph) along with oral sucker (*), genital papillae along with genital pore (gp), uterus in handles full of eggs (arrow), and ovary (ov) central, acetabulum (ac) in the posterior portion of the helminth; (c) detail of the pharynx's papillae (pa); (d) detail of the genital papillae (*) around the genital pore (gp); (e) detail of the muscular bands from acetabulum (*) and oblique muscular band (arrow). *Scale-bars*: **A** 500 µm, **B** 500 µm, **C** 50 µm, **D** 200 µm, **E** 50 µm.

[Based on 10 adult specimens: metrical data in Table 1**].** Pear-shaped, smooth body, slightly recurved in the ventral direction, measuring 6.30 (3.42-754) × 2.98 (2.62-3.42) mm, maximum width to the testis level. Oral sucker, adorned with innumerable ciliate papillae in the anterior extremity of the trematode. Pharynx with well-defined muscular portion and evidencing papillae of varied sizes in its mucosa, characteristic of pharynx of *Liorchis* genus, measuring 0.56 (0.43-0.65) × 0.63 (0.33-0.80) mm. Muscular oesophagus 0.58 (0.42-0.79) × 0.68 (0.15-0.45) mm, with muscular streaks in an uniformly thin layer, absent bulb. Caeca with undulations, finishing to the level of the anterior edge of the acetabulum. Acetabulum in the posterior fraction of the body, subterminal adorned with numerous ciliate papillae in a well-developed concentric arrangement, demonstrating definite transversal muscular bands, as well as external oblique muscular band, characterizing it as a *Cotylophoron* acetabulum, measuring 1.40 (0.74-1.77) × 1.15 (0.43-1.67) mm. Genital sucker, located in the anterior fraction of the body, the genital papillae and sucker below the oral sucker, with conspicuous genital papilla, characteristic of *Cotylophoron*, measuring 0.59 (0.43-0.78) × 0.74 (0.46-1.66) mm. Oblique, lobe-segmented testes, smaller in relation to the ovary, located in the middle fraction of the body, anterior measuring 0.38 (0.29-0.56) × 0.39 (0.32-0.55), posterior measuring 0.36 (0.26-0.50) × 0.42 (0.31-0.74) mm, Ovary located after the testes in the middle fraction of the body with 0.76 (0.32-0.95) × 0.49 (0.37-0.63) mm. Uterine loops packed with ascending eggs, located in the middle fraction, in the middle of

the parasite. Mehlis' gland (Ootype) measuring 0.46 (0.33-0.59) × 0.38 (0.27-0.43) mm. Vitellogenic glands, situated in the lateral parts from the anterior fraction going until the posterior fraction, sometimes invade the intracaecal area. Eggs operculate 0.130 (0.08-0.14) × 0.05 (0.04-0.06) mm.

Excluding primers, 1712 base pairs were obtained for 18S SSU rRNA gene (Genbank accession number: SUB8164216 Cotylophoron MW024900). In comparison to *Cotylophoron cotylophorum* (JX678230) a BLAST search resulted in maximum score = 3068, query coverage = 95% and percent identity = 99.88%.

Table 1. Morphological and morphometric comparison of *Cotylophoron marajoensis* n. sp. collected from *Bubalus bubalis* commercialized in the state of Pará and compared with other authors.

Character	*Cotylophoron* spp.				
	***Cotylophoron marajoensis* n. sp.**	***C. cotylophorum***	***C. panamensis***	***C. macrosphinctris***	***C. xiangjiangense***
Host **Locality** **Author**	***Bubalus bubalis*** **Brazil**	*Bos taurus* Colombia (Fischoeder, 1901)	*Bos indicus* Colombia Price & McIntosh, 1953	*Syncerus caffer* Uganda Sey & Graber, 1979	*Bubalus bubalis* China Wang, 1979
Length	**3.43-7.54**	5.58-7.31	6.63-8.84	5.41-7.82	3.85-5.60
Width	**2.63-3.43**	2.01-2.19	2.31-3.25	2.30-3.05	1.93-3.05
Type of pharynx	***Liorchis***	*Calicophoron*	*Calicophoron*	*Calicophoron*	*Liorchis*
Pharynx ^{L}	**0.43-0.65**	0.23-0.62	0.72-0.96	0.78-1.23	0.43-0.78
Pharynx ^{W}	**0.33-0.80**	0.43-0.73	0.64-0.86	0.57-1.03	0.56-0.78
Esophageal bulb	**Absent**	Present	Absent	Present	Present
Esophagus ^{L}	**0.42-0.79**	0.70-0.80	0.42-0.86	1.15-1.52	-
Esophagus ^{W}	**0.15-0.45**	0.20-0.30	0.18-0.28	-	-
Type of Ventosa Genital	***Cotylophoron***	*Cotylophoron*	*Cotylophoron*	*Schistocotyle*	*Cotylophoron*
Ventosa Genital ^{L}	**0.43-0.78**	0.50-0.70	0.74-1.02	-	0.52-0.61
Ventosa Genital ^{W}	**0.46-1.66**	0.30-0.60	0.58-0.98	-	0.52-0.64
Ovary ^{L}	**0.32-0.95**	0.34-0.60	0.56-0.80	0.28-0.68	0.37-0.40
Ovary ^{W}	**0.37-0.63**	0.46-0.80	0.54-0.92	0.76-0.96	-
Ootype ^{L}	**0.33-0.59**	0.18-0.32	0.40-0.70	0.57-0.66	-
Ootype ^{W}	**0.27-0.43**	0.20-0.40	0.44-0.98	0.50-0.55	-
Anterior testis ^{L}	**0.29-0,56**	1.24-2.24	0.70-1.43	0.41-1.82	-
Anterior testis ^{W}	**0.32-0.55**	2.55-1.38	1.63-2.63	1.23-1.62	-
Posterior testis ^{L}	**0.26-0.50**	1.30-2.34	0.94-1.75	0.52-1.85	-
Posterior testis ^{W}	**0.31-0.74**	1.74-2.55	0.94-2.27	1.10-1.92	-
Type of Acetabulum	***Cotylophoron***	*Cotylophoron*	*Cotylophoron*	*Cotylophoron*	*Paramphistomum*
Acetabulum ^{L}	**0.74-1.77**	1.10-1.60	1.37-2.25	-	1.23-1.72
Acetabulum ^{W}	**0.43-1.67**	1.35-1.80	1.22-1.87	-	1.23-1.58
Egg ^{L}	**0.08-0.14**	0.102-0.108	0.057-0.083	0.100-0.151	0.115-0.040
Egg ^{W}	**0.04-0.06**	0.062-0.070	0.057-0.083	0.060-0.073	0.063-0.080
References	**From this study**	Alarcón & Velásquez (2009)	Martínez & Velásquez (2012)	Eduardo (1985)	Eduardo (1985)

Abbreviations: L= length, W= width.

Table 1. Continued...

Character	*Cotylophoron* spp.				
	***Cotylophoron marajoensis* n. sp.**	***C. jacksoni***	***C. fuelleborni***	***C. bareilliensi***	***C. travassosi***
Host Locality Author	***Bubalus bubalis* Brazil**	*Bos indicus* Brazil Nasmark,1937	*Bos indicus* and *Capra hircus* Brazil Nasmark, 1937	*Capra hircus* Brazil Mukherjee & Chauhan, 1965	*Bos indicus* Brazil (Costa & Guimarães, 1992)
Length	**3.43-7.54**	6.04-7.50	5.10-6.22	5.26-7.38	4.70-5.51
Width	**2.63-3.43**	2.13-3.14	1.90-2.43	1.90-2.43	2.67-3.30
Type of pharynx	***Liorchis***	*Calicophoron*	*Calicophoron*	*Calicophoron*	*Calicophoron*
Pharynx ^{L}	**0.43-0.65**	0.76-1.56	0.49-0.69	0.64-1.02	0.57-0.74
pharynx ^{W}	**0.33-0.80**	0.76-0.96	0.61-0.69	0.63-0.91	0.77-0.88
Esophageal bulb	**Absent**	Absent	Absent	Absent	Absent
Esophagus ^{L}	**0.42-0.79**	0.40-0.70	0.47-0.66	0.25-0.30	0.40-0.72
Esophagus ^{W}	**0.15-0.45**	0.24-0.34	0.18-0.23	0.17-0.31	0.27-0.36
Type of Ventosa Genital	***Cotylophoron***	*Cotylophoron*	*Cotylophoron*	*Cotylophoron*	*Cotylophoron*
Ventosa Genital ^{L}	**0.43-0.78**	0.27-0.39	0.46-0.69	0.24-0.38	0.60-0.93
Ventosa Genital ^{W}	**0.46-1.66**	-	0.49-0.62	-	0.75-0.92
Ovary ^{L}	**0.32-0.95**	0.70-0.93	0.33-0.47	0.61-0.86	0.73-1.05
Ovary ^{W}	**0.37-0.63**	0.56-0.79	0.36-0.52	0.49-0.88	0.88-1.14
Ootype ^{L}	**0.33-0.59**	0.44-0.64	0.19-0.47	0.44-0.58	0.40-0.64
Ootype ^{W}	**0.27-0.43**	0.40-0.78	0.23-0.52	0.47-0.72	0.51-0.76
Anterior testis ^{L}	**0.29-0,56**	0.46-1.00	0.64-0.77	0.32-0.51	0.48-0.59
Anterior testis ^{W}	**0.32-0.55**	0.70-1.08	0.61-0.84	0.45-0.71	0.64-0,90
Posterior testis ^{L}	**0.26-0.50**	0.48-1.18	0.53-0.81	0.33-0.53	0.35-0.63
Posterior testis ^{W}	**0.31-0.74**	0.54-1.22	0.56-0.87	0.51-0.76	0.73-0.94
Type of Acetabulum	***Cotylophoron***	*Cotylophoron*	*Cotylophoron*	*Cotylophoron*	*Cotylophoron*
Acetabulum ^{L}	**0.74-1.77**	1.39-1.90	1.18-1.36	1.39-1.84	1.29-1.53
Acetabulum ^{W}	**0.43-1.67**	1.17-1.88	1.16-1.33	1.42-1.93	1.70-1.90
Egg ^{L}	**0.08-0.14**	0.120-0.140	0.130-0.150	0.130-0.161	0.120-0.150
Egg ^{W}	**0.04-0.06**	0.070-0.080	0.060-0.070	0.061-0.081	0.060-0.080
References	**From this study**	Miranda & Costa (1999)	Miranda & Costa (1999)	Miranda & Costa (1999)	Miranda & Costa (1999)

Abbreviations: L= length, W= width.

Taxonomy summary

Cotylophoron marajoensis n. sp.

Type-host: *Bubalus bubalis* (Linnaeus 1758). Common name: Water Bufallo

Site of infection: rumen and reticulum.

Location type: Soure (00°4'00"S, 48°31'24"W), Breves (01°40'56"S, 50°28'49"W) and Cachoeira do Arari (01° 00' 41" S, 48° 57' 48" W), Marajó Island, Pará, Brazil.

Prevalence: 4.54% (5 infected hosts of 110 analyzed).

ZooBank registration: SUB8164216 Cotylophoron MW024900

Etymology: The specific name *marajoensis* refers to the geographical region of the distribution (i.e., Marajó Island, state of Pará, Amazon, Brazil).

Deposit of Specimens: Holotype MPE 000261 and paratype MPEG 00262 a 00265, were deposited in an invertebrate collection at Museu Paranse Emílio Goeldi (MPEG), Belém, Pará, Brazil.

Discussion

Morphological delimitation

The characteristics presented for the taxon studied here such as pear-shaped body, smooth tegument without papillae, oral sucker in the anterior extremity of the body, genital sucker with genital papilla in the anterior fraction of the body and sub-ventral acetabulum of average size, fit it into the *Cotylophoron* genus in accordance with the keys and descriptions of the genus (Eduardo, 1985). The morphologic characteristics of *Cotylophoron*, in the present work, are similar to those of the of genus *Paramphistomum* (Fischoeder 1901) and genus *Balanorchis* (Fischoeder 1901), but they are distinguished mainly in relation to the *Paramphistomum* because of the presence of genital papilla and, in relation to the *Balanorchis*, because of the absence of long papillae in the oral sucker (Jones et al., 2005).

Cotylophoron marajoensis n. sp. differs from *C. cotylophorum* in possessing a *Liorchis* pharynx, whereas in *C. cotylophorum* it is *Calicophoron*; still, absence of esophageal bulb in *C. cotylophorum*. Oblique and smaller testes in relation to the ovary presented by *Cotylophoron marajoensis* n. sp. distinguish it from *C. cotylophorum,* which presents larger in tandem testes in relation to the ovary (Alarcón & Velásquez, 2009). Associated to these morphologic characteristics, *C. cotylophorum* has not been found in *B. bubalis* until now, nor in Brazilian territory.

In Brazil, *C. fulleborni* e *C. jacksoni* had been only described in *Bos indicus* e *Capra hircus* (Miranda & Costa, 1999), without occurrence in *B. bubalis*. These species differ from *Cotylophoron marajoensis* n. sp. in possessing *Calicophoron* pharynx and larger testes in relation to the ovary (Morales et al., 2015). Around the world, *C. panamensis* has been described only in *Bos indicus*. It is shorter in length than *Cotylophoron marajoensis* n. sp., although larger in width. It possesses *Calicophoron* pharynx and, differently from *Cotylophoron marajoensis* n. sp. (whose testes are oblique and smaller than the ovary), it possesses horizontally parallel and larger testes in relation to the ovary, with its vitellarium exceeding the edge of the caeca, also differing from *Cotylophoron marajoensis* n. sp. because these do not exceed the caecal edges (Martínez & Velásquez, 2012).

The species *Cotylophoron bareilliense* occurs in *B. bubalis* in the Phillipines. In Brazil, however, it is mentioned only as parasitizing *Bos indicus*, *Capra hircus* and *Ovis aries* (Miranda & Costa, 1999). Moreover, it is longer in length than *Cotylophoron marajoensis* n. sp., and it possesses a *Calicophoron* pharynx (Eduardo, 1985), differing from the *Liorchis* pharynx of the new species here being described.

Cotylophoron macrosphinctris is parasitic on buffalo, but of the species *Syncerus caffer*, the African buffalo; it has not been described in *B. bubalis*, nor in the Brazilian territory it is differentiated from the *Cotylophoron marajoensis* n. sp. by its *Calicophoron* pharynx, larger esophageal bulb and testicles in relation to the ovary. Furthermore, it possesses a *Schistocotyle* genital sucker, whereas *Cotylophoron marajoensis* n. sp. possesses a *Cotylophoron* genital sucker (Eduardo, 1985).

Besides having *B. bubalis* as host and the same type of pharynx (*Liorchis*) of the *C. xiangjiangense* species, *Cotylophoron marajoensis* n. sp. differs by the description of its biome, found in Amazon, while for *C. xiangjiangense* has only been described as occurring in China. In addition, *Cotylophoron marajoensis* n. sp. is longer in length and it does not possess an esophageal bulb, which present in the *C. xiangjiangense*. To strengthen this difference, the testis of *Cotylophoron marajoensis* n. sp. has a lobe format, whereas *C. xiangjiangense* possesses an oval testis format (Eduardo, 1985).

The *Cotylophoron travassosi* species has not been described in *B. bubalis* up until now, being found, in Brazil, only in *Bos indicus*, *Capra hircus* and *Ovis aries*. It is shorter in length than *Cotylophoron marajoensis* n. sp., and it possesses a *Paramphistomum* pharynx (Miranda & Costa, 1999). Additional morphometric comparisons between *Cotylophoron marajoensis* n. sp. and the other species of the genus *Cotylophoron* are presented in Table 1.

When analyzed by the SEM, the external morphology of *Cotylophoron marajoensis* n. sp. disclosed a smooth body, presence of genital papilla, oral sucker and acetabulum, these structures being common to the *Cotylophoron*

(Miranda & Costa, 1999). The internal morphology visualized by SEM has supplied data that have guaranteed diagnosis of the species, disclosing more details about the structures for type of pharynx and acetabulum.

Conclusion

In view of the above, the morphological evidence presented here for the new species falls within the genus *Cotylophoron* and the absence of all its valid species. Thus, we have added a species to this genus, named *Cotylophoron marajoensis* n. sp. Additionally we present its partial 18S small subunit ribosomal rRNA gene sequence which may contribute to future phylogenetics studies.

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