

Research Note

***Plesiochorus cymbiformis* (Rudolphi, 1819) Looss, 1901 (Digenea: Gorgoderidae) in Olive Ridley Sea turtles *Lepidochelys olivacea* (Eschscholtz, 1829) (Testudines: Cheloniidae) from the Brazilian coast: New geographic occurrence and associated injuries**

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Summary

The present study reports the first occurrence of *Plesiochorus cymbiformis* (Digenea: Gorgoderidae), in two Olive Ridley Sea turtles *Lepidochelys olivacea* (Testudines: Cheloniidae), from the states of São Paulo and Sergipe in Brazilian coast. Concerning the Neotropical region, *P. cymbiformis* has been previously reported in green sea turtles (*Chelonia mydas*) from Panama and Brazil, in loggerhead sea turtles (*Caretta caretta*) from Brazil, in hawksbill sea turtles (*Eretmochelys imbricata*) from Puerto Rico, and in Olive Ridley Sea turtles only in Costa Rica. Lesions resulting from the presence of parasites in the hosts' urinary bladders are also presented. This is the second report on endoparasites in Olive Ridley sea turtles from Brazil.

Keywords: Brazil; Cystitis; Gorgoderidae; Helminthfauna; *Lepidochelys olivacea*; Lesion; Pathology; *Plesiochorus cymbiformis*; Olive Ridley sea turtles; Sea turtles

Introduction

The helminth fauna of Olive Ridley sea turtle, *Lepidochelys olivacea* (Eschscholtz, 1829) (Testudines: Cheloniidae) has a gap of knowledge in Brazilian coast (Werneck & Silva 2016), and only *Pyelossomum cochlear* Looss, 1899 (Digenea: Pronocephalidae) has been described for this host so far in this area (Werneck *et al.*, 2015).

The genus *Plesiochorus* Looss, 1901 (Digenea: Gorgoderidae) is a exclusive parasite of sea turtles and only two species are currently accepted, *Plesiochorus cymbiformis* (Rudolphi, 1819) Looss, 1901, described in the green sea turtle *Chelonia mydas* Linnaeus, 1758 (Testudines: Cheloniidae), the loggerhead sea turtle *Caretta caretta* Linnaeus, 1758, the Hawksbill sea turtle *Eretmochelys im-*

bricata (Linnaeus, 1766) and in the Olive Ridley sea turtle (Blair & Limpus, 1982), and *Plesiochorus elongatus* Pigulevsky, 1953, described in loggerhead turtles (Werneck *et al.*, 2019).

Concerning the Neotropical region (see Werneck & Silva, 2016), *P. cymbiformis* has been described in green turtles from Panama (Caballero & Caballero, 1954) and Brazil (Binoti *et al.*, 2016), in hawksbill turtles from Puerto Rico (Fischthal & Acholonu, 1976), in loggerhead sea turtles from Brazil (Werneck *et al.*, 2018) and in Olive Ridley sea turtles from Costa Rica (Santoro & Morales, 2007), while *P. elongatus* has been reported in loggerhead turtles in Brazil (Werneck *et al.*, 2019).

This note describes the first occurrence of *P. cymbiformis* in Brazil, in the urinary bladder of two Olive Ridley sea turtles found off the Brazilian coast, also reporting tissue organ alterations.

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Table 1. Morphometric data of the genus *Plesiochorus cymbiformis* (Rudolphi, 1819) Looss, 1901 (Digenea: Gorgoderidae) from marine turtles (Testudinidae: Cheloniidae). Data are presented as range (mean) and measurements are in millimeters.

Host	Caballero (1954)		Chattopadhyaya (1970)		Blair & Limpus (1982)		Werneck et al. (2018)		Santoro & Morales (2007)		Present report	
	<i>C. mydas</i>	Panamá	<i>E. imbricata</i>	India	<i>C. caretta</i>	Australia	<i>C. caretta</i>	Brazil	<i>L. olivacea</i>	Costa Rica	<i>L. olivacea</i>	Brazil
Locality	UB		Liver		UB		UB		UB		UB	
Site of infection												
Number of parasites	3		1				6		31 (10 mens.)		13 (13 mens.)	
Body length	2.5–3.2		8.5		2.97–7.21 (5.47)		7.91 – 8.86 (8.23)		12.8–15.7 (14)		4.8 – 8.8 (5.12)	
Body width	1.21–1.27		4.7		1.31–3.7 (2.2)		2.73 – 3.04 (2.87)		4.4–5.4 (5.1)		1.8 – 4.0 (2.1)	
Oral sucker length	0.13–0.24		0.74		0.41–0.86 (0.62)		0.51 – 0.62 (0.58)		0.84–1.07 (0.96)		0.61 – 0.95 (0.77)	
Oral sucker width	0.28–0.29		0.86		0.42–0.85 (0.64)		0.75 – 0.85 (0.79)		1.14–1.32 (1.24)		0.61 – 1.0 (0.80)	
Esophagus length	0.038–0.057		0.16		–		0.35 – 0.45 (0.39)		0.31–0.56 (0.44)		0.15 – 0.38 (0.28)	
Esophagus width	0.038–0.046		–		–		0.09 – 0.2 (0.16)		–		0.05 – 0.12 (0.08)	
Pharynx length	0.068–0.076		0.26		0.15–0.3 (0.21)		0.27 – 0.34 (0.29)		0.25–0.37 (0.32)		0.19 – 0.33 (0.25)	
Pharynx width	0.099–0.10		0.36		0.18–0.37 (0.25)		0.29 – 0.34 (0.31)		0.36–0.37 (0.37)		0.25 – 0.33 (0.29)	
Ventral sucker length	0.37–0.39		1.4*		0.5–1.26 (0.95)		0.92 – 1.37 (1.2)		1.51–1.89 (1.72)		0.97 – 1.05 (1.21)	
Ventral sucker width	0.40–0.41				0.62–1.37 (0.95)		1.05 – 1.34 (1.24)		1.57–1.89 (1.73)		0.98 – 1.5 (1.27)	
Right testis length	0.27–0.28		2.4		0.48–1.52 (0.9)		1.39 – 1.6 (1.34)		2.39–3.46 (2.83)		0.210 – 1.47 (1.09)	
Right testis width	0.19–0.2		1.7		0.43–1.15 (0.7)		0.89 – 1.3 (1.08)		1.57–2.14 (1.93)		0.24 – 1.16 (0.78)	
Left testis length	0.26–0.33		2.4		0.48–1.52 (0.9)		1.34 – 1.53 (1.46)		2.4–3.4 (2.9)		0.78 – 1.56 (1.24)	
Left testis width	0.11–0.17		2.2		0.43–1.15 (0.7)		0.83 – 1.38 (1.07)		1.63–2.2 (1.95)		0.54 – 1.16 (0.83)	
Ovary length	0.11–0.14		0.45		0.23–0.37 (0.29)		0.43 – 0.45 (0.43)		0.44–0.63 (0.53)		0.18 – 0.38 (0.28)	
Ovary width	0.084–0.087		0.71		0.21–0.37 (0.29)		0.41 – 0.49 (0.44)		0.37–0.69 (0.57)		0.20 – 0.54 (0.38)	
Mehlis' gland length	0.065–0.095		–		–		0.28 – 0.45 (0.34)		0.18–0.31 (0.28)		0.15 – 0.47 (0.28)	
Mehlis' gland width	0.095–0.152		–		–		0.34 – 0.38 (0.36)		0.18–0.37 (0.28)		0.25 – 0.60 (0.43)	
Right Vit. follicles length	–		–		0.19–0.42 (0.32)		0.48 – 0.52 (0.5)		0.56–0.94 (0.79)		0.27 – 0.68 (0.44)	
Right Vit. follicles width	–		–		0.19–0.5 (0.28)		0.34 – 0.5 (0.43)		0.31–0.63 (0.50)		0.27 – 0.50 (0.36)	
Left Vit. follicles length	–		–		0.19–0.42 (0.32)		0.47 – 0.56 (0.53)		0.56–0.88 (0.73)		0.30 – 0.74 (0.47)	
Left Vit. follicles width	–		–		0.19–0.5 (0.28)		0.37 – 0.46 (0.41)		0.50–0.56 (0.57)		0.21 – 0.41 (0.70)	
Egg length	0.042–0.046		0.0312–0.044		0.03–0.047 (0.038)		0.023 – 0.04 (0.033)		0.032–0.039 (0.034)		0.029 – 0.037 (0.032)	
Egg width	0.027–0.030		0.0310–0.0448		0.022–0.041 (0.031)		0.017 – 0.03 (0.025)		32		0.021 – 0.032 (0.027)	(n=45)

Legend: * = diameter

Case description

The first turtle, an adult female (verified by the size of the tail and confirmed during the necropsy, through gonad visualization) measuring 68 centimeters (cm) of curvilinear carapace length (CCL) and weighing 32.76 kilograms (kg) was found dead in April 2019, on Caçandoca beach, located in the city of Ubatuba (23°33'48.8262" S 45°13'3.0612" W) in the state of São Paulo, Brazil.

Macroscopic necropsy findings comprised the presence of aerated fluid in the trachea, bronchi and lung parenchyma, due to anthropic interactions with fishing and amputation of the right anterior fin.

The liver displayed a nutmeg pattern (chronic passive congestion with evidence of a lobular pattern), brownish in color, with the accumulation of lumpy bile in the gallbladder. Kidneys presented a diffuse yellowish color parenchyma. The urinary bladder displayed a slight thickening of the wall and the presence of parasites attached to the mucosa.

The second specimen, a male, (verified by the size of the tail and confirmed during the necropsy, through gonad visualization), was found in January 2020 at Abaís beach, in the municipality of Estância, in the state of Sergipe (10°28'28.598" S 36°51'40.4706" W), Brazil, measuring 67.8 cm in CCL and weighing 31.4 kg. This

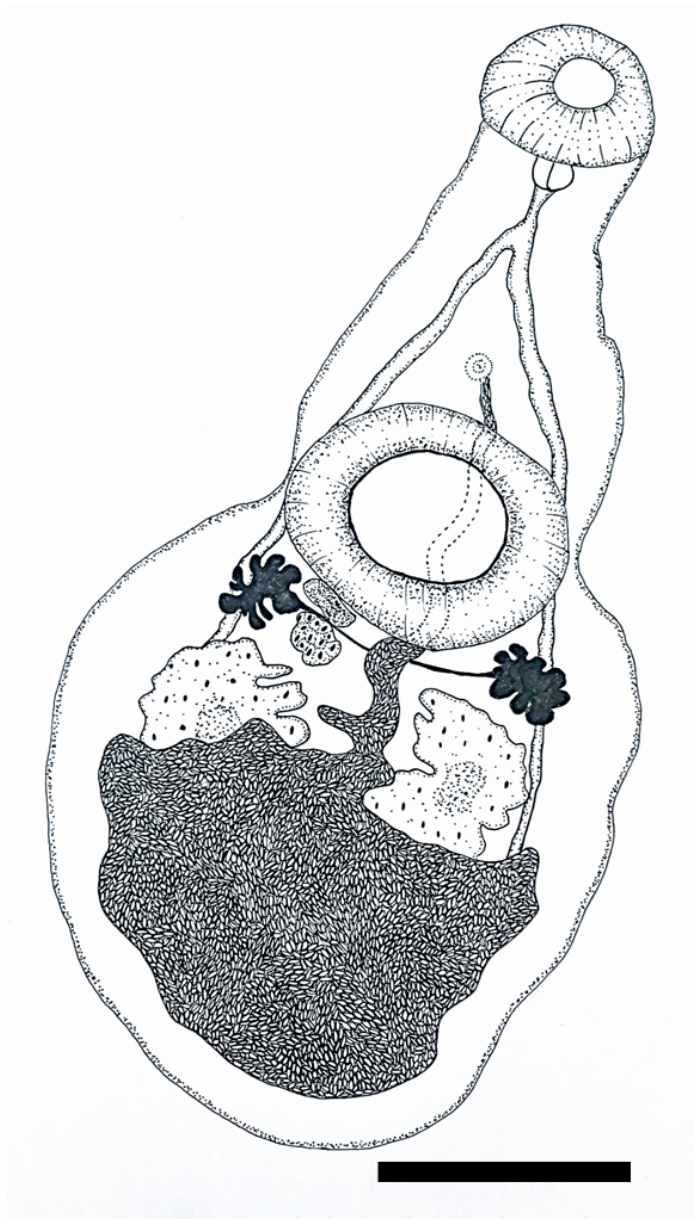


Fig. 1. *Plesiochorus cymbiformis* (Rudolphi, 1819) Looss, 1901 (Digenea: Gorgoderidae) found in *Lepidochelys olivacea* (Eschscholtz, 1829) (Testudines: Cheloniidae) from Brazil. (scale bar=1 mm).

animal was found with no vital signs during regular beach monitoring efforts and sent to the Rehabilitation and Depetrolization Center in Aracaju, Sergipe, for necropsy procedures. A good body score and evident normal mucous membranes were observed during the anatomopathological evaluation. The liver presented an intraparenchymal abscess adhering strongly to the serosa of the adjacent small intestine. The lungs were diffusely reddish and hypocrepitant, with a large amount of foamy liquid flowing to the parenchyma cut. The bladder contained a moderate number of parasites occupying the lumen. No macroscopic changes were observed in other organs.

Samples from the respiratory, digestive and reproductive tract, liver, heart, pancreas, kidneys and urinary bladder were taken, fixed in 10 % neutral buffered formalin, embedded in paraffin wax, sectioned (5 µm), and stained with hematoxylin and eosin for subsequent analyses under an optical microscope.

Thirteen *P. cymbiformis* specimens were recovered in both hosts, seven in the female and six in the male. Parasites were placed in a Petri dish, preserved in 70 % alcohol, stained with carmine, and cleared with eugenol. Morphometric data were collected with

the aid of a Global Optics NO216T microscope (Global Optics) using the S-eyes image analysis program. The helminths were deposited at the Instituto Oswaldo Cruz Helminthological Collection (38934 and 38935), in the state of Rio de Janeiro, Brazil. Parasite analyses were authorized by federal licenses approving activities with scientific purposes (SISBIO 30600).

Morphological and morphometric comparisons among the analyzed parasites were carried out using the genera identification key proposed by Campbell (2008), in addition to the descriptions by Rudolphi (1819), Looss (1901, 1902), Pratt (1914), Caballero y Caballero (1954), Chattopadhyaya (1970), Blair and Limpus (1982), Santoro and Morales (2007) and Werneck *et al.* (2018). The report by Werneck *et al.* (2019) was used for comparisons with other members of the genus (*i.e.*, *P. elongatus*).

Results

Of the 13 *P. cymbiformis* specimens (Fig. 1), all were measured (Table 1). The parasites exhibited a tapered anterior body and a broad posterior body, evident constriction in the acetabulum

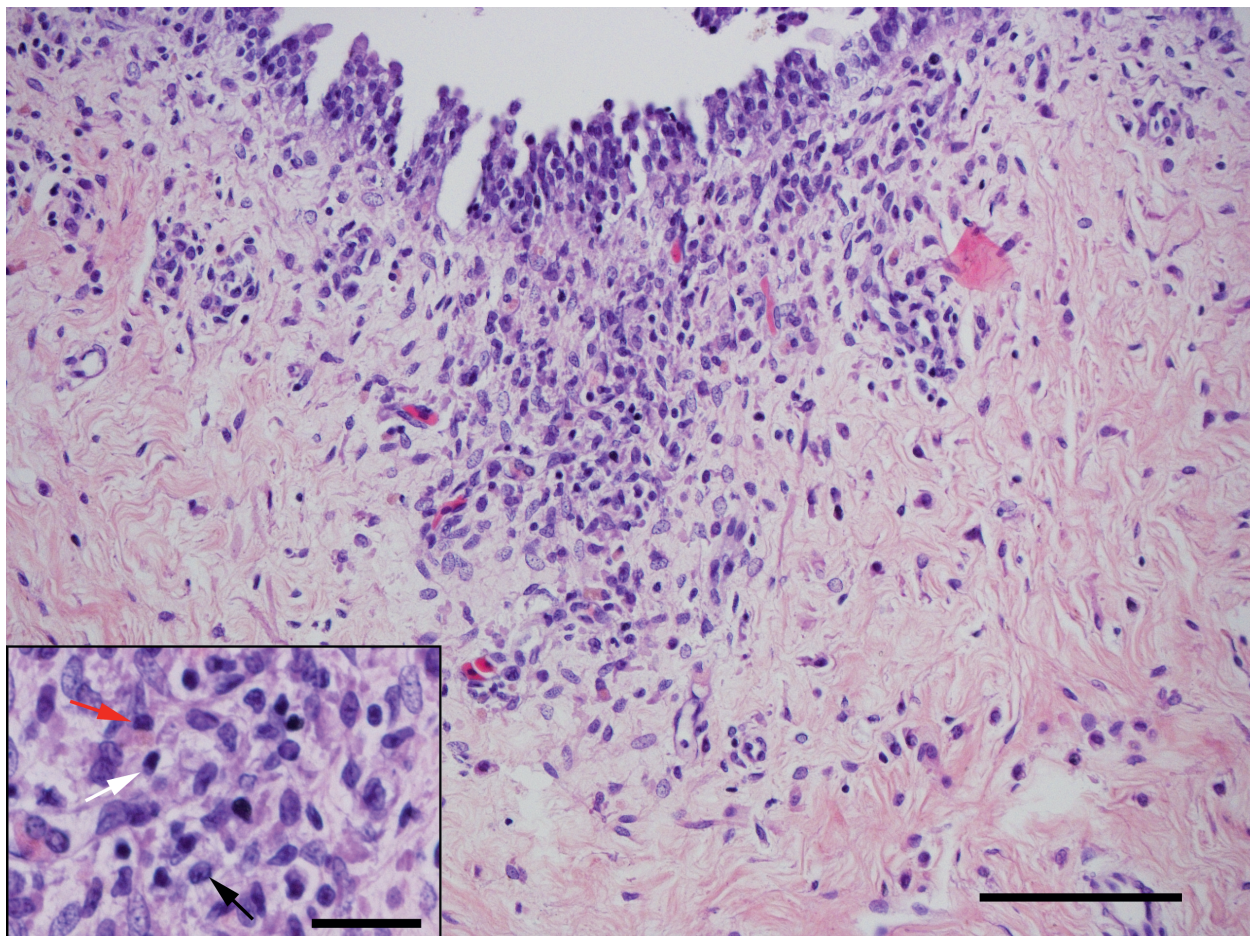


Fig. 2. Marked inflammatory infiltrate in the lamina propria of the urinary vesicle composed of macrophages, lymphocytes and heterophiles (scale bar = 100 µm). Inset: macrophages (black arrow), rare lymphocytes (white arrow) and heterophiles (red arrow) (scale bar = 25 µm).

region, terminal oral suction cup, circular acetabulum, pharynx present, short esophagus, enclosures parallel to the edges of the body, ending in the posterior region of the body and covered by the uterine loops, post acetabular and partly intracecal vitellaria in the shape of flower petals, irregularly shaped and post acetabular ovary and Mehlis gland, two large testicles, irregularly shaped ovarian powders, ventral to the caecae and, in some specimens, covered by the uterine loops, and genital pore between the cell bifurcation and the anterior edge of the acetabulum. Rounded and/or oval eggs, uterine loops occupying a large part of the posterior region of the body, maintaining distance from the edge of the body.

A histopathological analysis of the urinary vesicle of the female host revealed an accentuated and multifocal inflammatory infiltrate in the lamina propria submucosa associated with mild edema and slightly reactive fibrous tissue just below the mucosa. The inflammation was composed of macrophages, lymphocytes and rare

heterophils randomly distributed characterizing chronic cystitis (Fig. 2).

The histopathology of the male host indicated the presence of a moderate mixed inflammatory infiltrate, composed of heterophils, macrophages and lymphocytes, multifocally distributed in the mucous and submucosal layer, with multifocal necrosis areas associated with inflammation, indicating mixed and ulcerative, moderate, multifocal cystitis (Fig. 3).

Discussion

The genus *Plesiochorus* was originally described by Rudolphi (1819) as "*Distoma cymbiforme*", from individuals found in the urinary bladder of *Testudo mydas* (*Chelonia mydas*) specimens from the Adriatic Sea-Italy (Rimini area) (number 20, p. 96). The species was better studied later by Sonsino (1893), Stossich (1895, 1897),

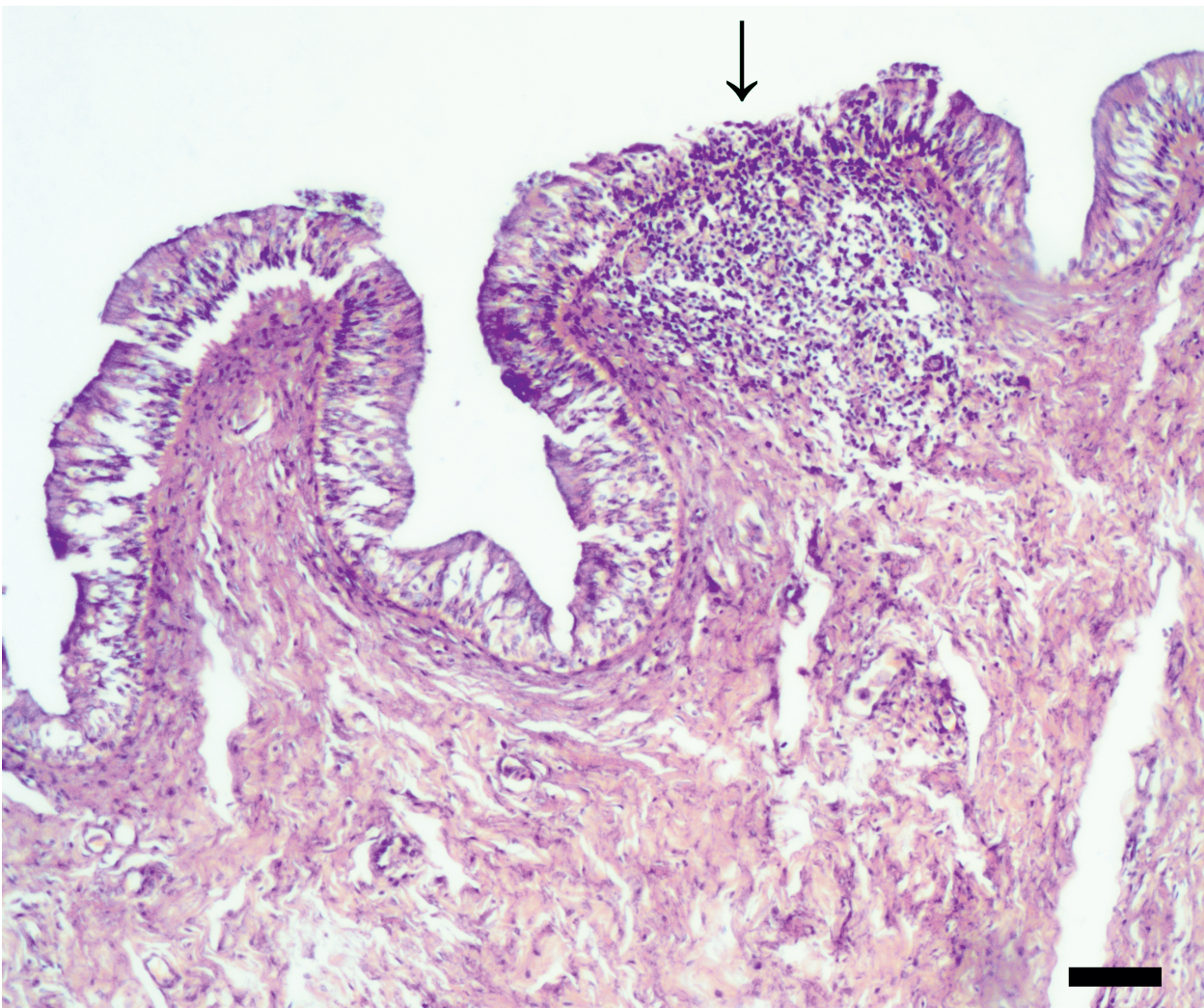


Fig. 3. Focal area of discontinuity of the bladder epithelium (arrow), with replacement of the region by necrotic debris and mixed inflammatory infiltrate (scale bar = 50µm).

Braun (1899), who expanded the descriptions for the species and, later Looss, (1901, 1902), who presented the *Plesiochorus* genus (species type *P. cymbiformis*).

Two species are currently accepted for the genus, the first comprising *P. cymbiformis*, described in green turtles from Egypt, India, Italy, Panama and the USA, in hawksbill turtles from India, Puerto Rico and the USA, in loggerhead turtles from Australia, Brazil, Egypt, Spain, Greece, Italy and the USA, and in Olive Ridley sea turtles from Costa Rica, Japan and Mexico (see Blair & Limpus, 1982), and the second, *Plesiochorus elongatus* Pigulevsky, 1953, described in loggerhead turtles from Egypt and Brazil (Werneck *et al.*, 2019).

The parasites studied herein exhibited a body with a narrow anterior portion and enlarged posterior end, large testicles that overlap the caecum and pharynx displaying characteristics described by Campbell (2008) for the Gorgoderidae Looss 1899 family and Anaporrhutinae Looss, 1901 subfamily. In addition, they present vitellaria in the shape of partially extracecal rose petals and uterine loops on the posterior part of the body ("hindbody"), both intra and extracecal, characteristic for the genus (see Campbell, 2008), in addition to being identical to *P. cymbiformis* descriptions (Looss, 1901, 1902; Platt 1914; Caballero, 1954; Chattopadhyaya, 1970; Blair & Limpus, 1982; Santoro & Morales, 2007 and Werneck *et al.*, 2018). Furthermore, a morphometric analysis did not reveal any difference in the determined measures when compared to the previously published data for the species (Table 1).

Plesiochorus cymbiformis is very similar to *P. elongatus*, as described by Werneck *et al.* (2019), two morphological characteristics are accepted to differentiate these species: *P. elongatus* has a more elongated body and the uterine loops end closer to the body edge in the posttesticular region, there is practically no space between the body edge and the loops uterine. On the other hand, *P. cymbiformis* has a less elongated body and the uterine loops have a space between the sides of the body posterior to the testicles. In this note, the assessed parasites (Fig. 1) had uterine loops that maintained some distance from the edge of the body and did not exhibit an anterior portion of the body as elongated when compared to *P. elongatus*.

The first description of *P. cymbiformis* in Olive Ridley turtles from the Neotropical region was reported by Santoro and Morales (2007) when analyzing three hosts from Costa Rica, reporting 31 specimens of *P. cymbiformis* in only one of the hosts. Later, Santoro *et al.*, (2010) described *P. cymbiformis* in loggerhead turtles from the Valencia region in the Mediterranean Sea, with a prevalence of 4.5 % (0.8 – 15.6).

When analyzing sea turtles from Florida from 1991 to 2006, Greiner (2013) reported the presence of *P. cymbiformis* in 68.21 % of loggerhead turtles (30/44), 5.4 % of green turtles (4/74) and in only one of three analyzed hawksbill turtles.

In Brazil, Binotti *et al.*, (2016) reported the parasitological analysis of 50 *C. mydas* individuals from the state of Espírito Santo, with *P. cymbiformis* observed in only 2 animals (2 %). Werneck *et al.*

(2018) described lesions resulting from *P. cymbiformis* in the urinary bladder of an adult female loggerhead turtle from the state of Rio de Janeiro.

Microscopically, the lesions reported herein are similar to those described by Werneck *et al.* (2018), who described a chronic inflammatory process close to the mucosa associated with edema in a loggerhead turtle. In addition to lymphocytes and heterophiles, as described by Werneck *et al.* (2018). However, this inflammatory process was not associated with the cause of death of the assessed hosts in any of the reported cases.

Unfortunately, the helminthofauna of Olive Ridley turtles in the Brazilian region is still obscure due to the scarcity of published works and collected individuals, and, until now, only *P. cochlear* (Werneck *et al.*, 2015) and *P. cymbiformis* (present note) have been reported in these hosts.

Ethical Approval and/or Informed Consent

For this study formal consent is not required.

Conflict of Interest

Authors state no conflict of interest.

Acknowledgements

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