

## Research Note

# The genus *Rhytidodoidea* Price, 1939 (Digenea: Rhytidodidae) in Brazil: New geographic occurrence and report of pathology in the gallbladder

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### Summary

The present note describes the occurrence of *Rhytidodoidea intestinalis* and *Rhytidodoidea similis* (Digenea: Rhytidodidae) in the gallbladder of two juvenile green turtles (*Chelonia mydas* - Testudines, Cheloniidae) found on the coast of Brazil. Both were detected in gallbladder and intestine of green turtles: *Rhytidodoidea similis* (United States, Panama, Costa Rica and Brazil) and *R. intestinalis* (United States, Panama and Costa Rica). This note is the first report of *R. intestinalis* in Brazil and South-West Atlantic Ocean. Also the histological lesions caused by the parasites in one gallbladder are described.

**Keywords:** Brazil; *Chelonia mydas*; Digenea; green turtles; *Rhytidodoidea intestinalis*; *Rhytidodoidea similis*; South-West Atlantic Ocean

### Introduction

Parasites of the family Rhytidodidae Odner, 1926, are found in the intestine and gallbladder of sea turtles. Only two genera are currently accepted for the family: *Rhytidodes* Looss, 1901 [*R. gelatinosus* (Rudolphi, 1819) Looss, 1901] and *Rhytidodoidea* Price, 1939 (*R. intestinalis* Price, 1939, *R. similis* 1939 and *R. pricei* Mehrotra & Gupta, 1978) (Price, 1939; Mehrotra & Gupta, 1978; Blair, 2005).

In the Neotropical region (Central and South America), *R. gelatinosus* has been reported in the loggerhead turtle (*Caretta caretta*) in Brazil, the green turtle (*Chelonia mydas*) in Panama and Brazil and the hawksbill turtle (*Eretmochelys imbricata*) in Puerto Rico and Cuba. *Rhytidodoidea similis* has been reported in the green turtle in Panama and Brazil. *R. intestinalis* has been reported in the green turtle in Panama and Costa Rica (Werneck and Silva, 2016). This note describes the occurrence of *R. similis* and *R. intestinalis*

in green turtles found on the coast of Brazil. The injuries attributed to these parasites in the gallbladder of one host are also described.

### Material and Methods

In October 2017, a green turtle was found stranded on Rasa Beach (22°43'39.324"S; 41° 58' 40.476"W) in the municipality of Búzios in the State of Rio de Janeiro, Brazil, and was taken to a rehabilitation center. The turtle (83 cm in curvilinear carapace length-CCL and weighing 28.45 kg) was thin (with bones evident in the plastron region) and lethargic. Treatment involved rehydration with saline solution, preventive antibiotic therapy (enrofloxacin) and dietary supplementation through an esophageal tube. The animal died on the eleventh day of rehabilitation and was immediately submitted to necropsy.

The examination revealed atrophy of the pectoral muscles, which had a gelatinous appearance. Moreover, the entire carcass was

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Table 1. Morphometric data of *Rhytidodoides similis*, Price, 1939 (Digenea: Rhytidoididae) from *Chelonia mydas* (Testudinidae, Cheloniidae). Measurements in micrometers as Range (mean).

Site	Price (1939)	Smith et al. (1941)	Caballero (1954)	Werneck et al. (2015)	Present Report
	Gall bladder	Gall bladder	Biliary ducts	Small intestine and Gall bladder	Gall bladder
Locality	USA	USA	Panama	Brazil	Brazil
N	2	?	16	2	4
Body length (mm)	1.3–3.8	1.2–4.4	2.573–2.789	3.55–3.7	3.93–7.8 (5.0)
Body width (mm)	0.51–1.4	0.42–1.8	0.747–0.847	1.54–1.6	1.19–1.28 (1.2)
Oral sucker length	150–320*	140–342* (293)	103–114	280–330	248–308 (277)
Oral sucker width			243–247	240–370	297–326 (314)
Pharynx length	74–165	80–179	125–144	160–200	153–174 (163)
Pharynx width	63–160	50–170	110	210	132–143 (138)
Ventral sucker length			289–312	210–220	302–359 (335)
Ventral sucker width	165–370	170–385 (300)	266–285	210–230	277–354 (303)
Esophagus length	220–720	–	391–448	620–700	499–856 (638)
Esophagus width	–	–	76–95	110	103–176 (154)
Anterior Testes length	185–400*	102–420	232–249	330–440	367–488 (411)
Anterior Testes width		250–456	216–282	350–410	321–566 (425)
Posterior Testes length	220–480	152–532*	299–332	420–530	381–564 (473)
Posterior Testes width	135–400		199–216	390–490	309–516 (403)
Cirrus sac length	200–800	150–700	481–515	720–900	809–853 (827)
Cirrus sac width	160–430	150–456	199–216	220–450	295–345 (320)
Ovary length	90–300*	91–280 (250)*	129–133	200–260	137–220 (180)
Ovary width			129–137	200–210	142–240 (194)
Mehlis' gland length	–	–	133–152	110–140	94–211 (134)
Mehlis' gland width	–	–	175–190	120–130	110–196 (141)
Eggs length	63–70	36	61–65	37–60 (45)	47–59 (54)
Eggs width	37–40	72	38–42	20–40 (29)	22–34 (28)
Distance from (mm)					
To intestinal caeca bifurcation to anterior end	–	–	–	–	1.146–1.264 (1.22)
To genital aperture to anterior end	–	–	747–863	–	0.871–1.05 (0.955)
To ovary to ventral sucker	–	–	–	–	0.810–0.916 (0.847)

\*Diameter

Table 2. Morphometric data of *Rhytidoides intestinalis* Price, 1939 (Digenea: Rhytidoididae) from *Chelonia mydas* (Testudines, Cheloniidae). Measurements in micrometers as range (mean).

Site	Price (1939)		Caballero (1954)		Present Report
	Gall bladder	USA	Small intestine	Panama	
Locality					Gall bladder
N		2		1	Brazil
Body length (mm)		2.0 – 2.1		3.12	3
Body width		320 – 335		448	2.4 – 2.5 (2.4)
Oral sucker length				57	268 – 382 (339)
Oral sucker width		110 – 130*		190	117 – 142 (131)
Pharynx length		60 – 70		68	129 – 159 (148)
Pharynx width		55 – 70		76	55 – 60 (57)
Ventral sucker length				118	67 – 69 (67)
Ventral sucker width		103 – 115*		125	115 – 140 (124)
Esophagus length		730 – 830		912	88 – 126 (105)
Esophagus width		–		68	887 – 970 (939)
Anterior Testes length		130*		141	39 – 49 (44)
Anterior Testes width				118	123 – 149 (137)
Posterior Testes length		130*		198	109 – 137 (127)
Posterior Testes width				160	107 – 144 (130)
Cirrus sac length		110 – 137		190	102 – 151 (132)
Cirrus sac width		105 – 115		148	149 – 196 (174)
Seminal vesicle length		–		114	124 – 151 (133)
Seminal vesicle width		–		061	–
Ovary length		90 – 110*		114	–
Ovary width				122	94 – 104 (100)
Mehlis' gland length		–		152	85 – 144 (114)
Mehlis' gland width		–		84	72 – 89 (80)
Eggs length		60 – 63		57	62 – 126 (84)
Eggs width		45 – 52		34 – 38	51 – 63 (55)
Distance from (mm)					27 – 40 (31)
To intestinal caeca bifurcation to anterior end		–		–	1.08 – 1.11 (1.11)
To genital aperture to anterior end		–		1.461	1.1 (only in one)
To ovary to ventral sucker		–		–	520 (only in one)

\*Diameter

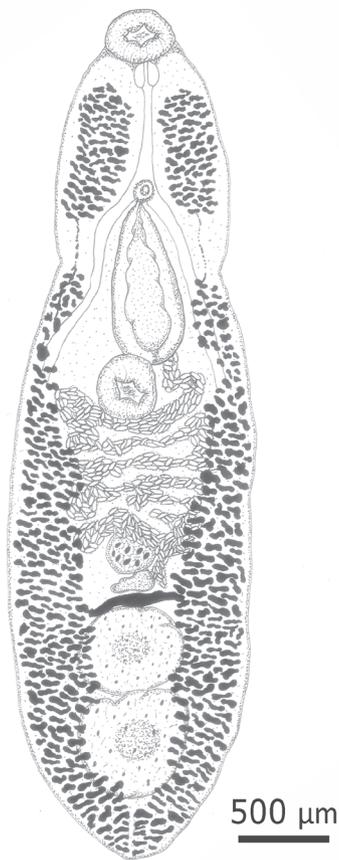


Fig. 1. *Rhytidodoides similis* Price 1939 (Digenea: Rhytidodidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines: Cheloniadae) from Brazil. Ventral view.

pale in color. Samples were taken of the spleen, brain, cerebellum, heart, digestive tract, liver, ocular globe, salt gland, testis, pancreas, respiratory tract, kidneys, urinary tract and gallbladder.

The samples were fixed in 10% formalin solution, embedding in paraffin, sliced (thickness: 5  $\mu\text{m}$ ), stained with hematoxylin and eosin (HE) and analyzed with a light microscope.

The second host was found dead in July 2018 caught in a fishing net near Ilha Grande (23°7'12.549"S; 44° 8' 3.124"W) in the municipality of Angra dos Reis in the State of Rio de Janeiro, Brazil. The turtle had 68.1 cm CCL and weighing 24.6 kg, tissue samples in this host were not histologically analyzed due to the autolytic state. During the analysis of the gallbladders, seven specimens of the family Rhytidodidae [three specimens of *R. intestinalis* and four specimens of *R. similis* were found in the first host and only eight *R. similis* were found in the second host]. The parasites were fixed in 70% alcohol, stained with hydrochloric carmine and cleared in a eugenol solution. The specimens were photographed and measured (data expressed as minimum-maximum values in  $\mu\text{m}$ ) under a microscope (Nikon Eclipse 80i, Kurobane Nikon Co., Ltd., Otawara, Tochigi, Japan) with the aid of the NIS-Elements BR software. Analyses of the parasites were authorized by federal licenses for activities with scientific purposes (SISBIO 30600-1 and 9329-1). The helminths were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (*R. similis* = CHIOC 38590; *R. intestinalis* = CHIOC 38589) in the State of Rio de Janeiro, Brazil.

The identification of the parasites was performed using the genus taxonomic key proposed by Blair (2005), the original description by Price (1939) as well as descriptions by Smith et al. (1941), Caballero 1954, Mehrotra & Gupta (1978) and Werneck et al. (2015). Descriptions by Price (1939), Smith et al. (1941), Caballero (1954) and Werneck et al. (2015) were used for the morphometric comparisons of *R. similis* (Table 1) and descriptions by Price (1939), Caballero (1954) and Mehrotra & Gupta (1978) were used for the comparison of *R. intestinalis* (Table 2).

Description of parasites:

*Rhytidodoides similis* (Figs. 1 and 2; Table 1): body robust, anterior

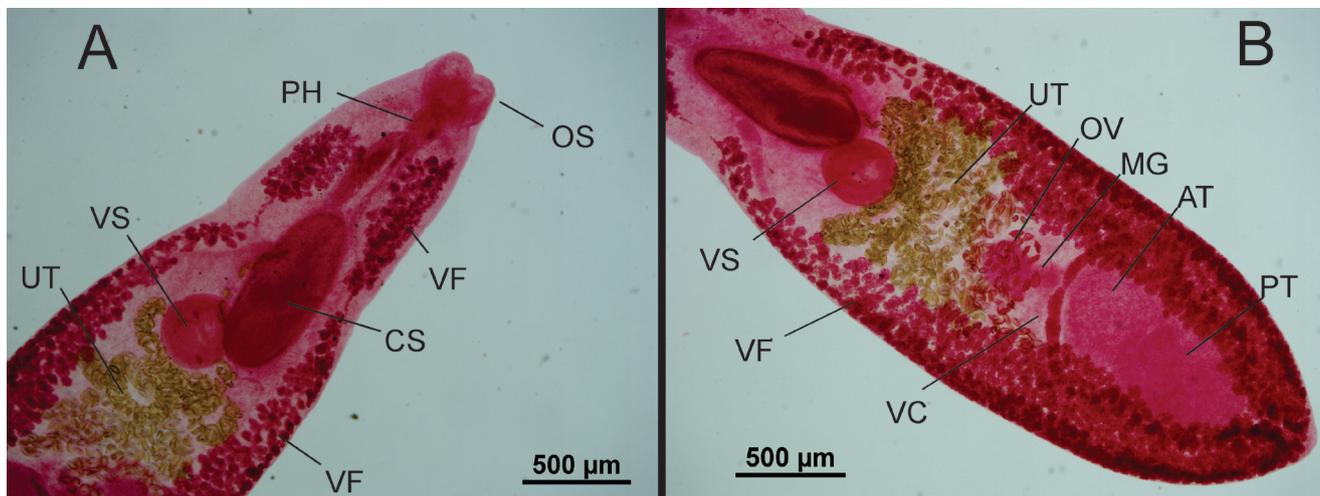


Fig. 2. (A-B) *Rhytidodoides similis* Price 1939 (Digenea: Rhytidodidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines: Cheloniadae) from Brazil. Legend: AT- anterior testes; CS- cirrus sac; MG- Mehlis gland; OS- oral sucker; OV- ovary; PH- pharynx; PT- posterior testes; UT- Uterus; VC- vitelline conduct; VF- vitelline follicles; VS- ventral sucker.

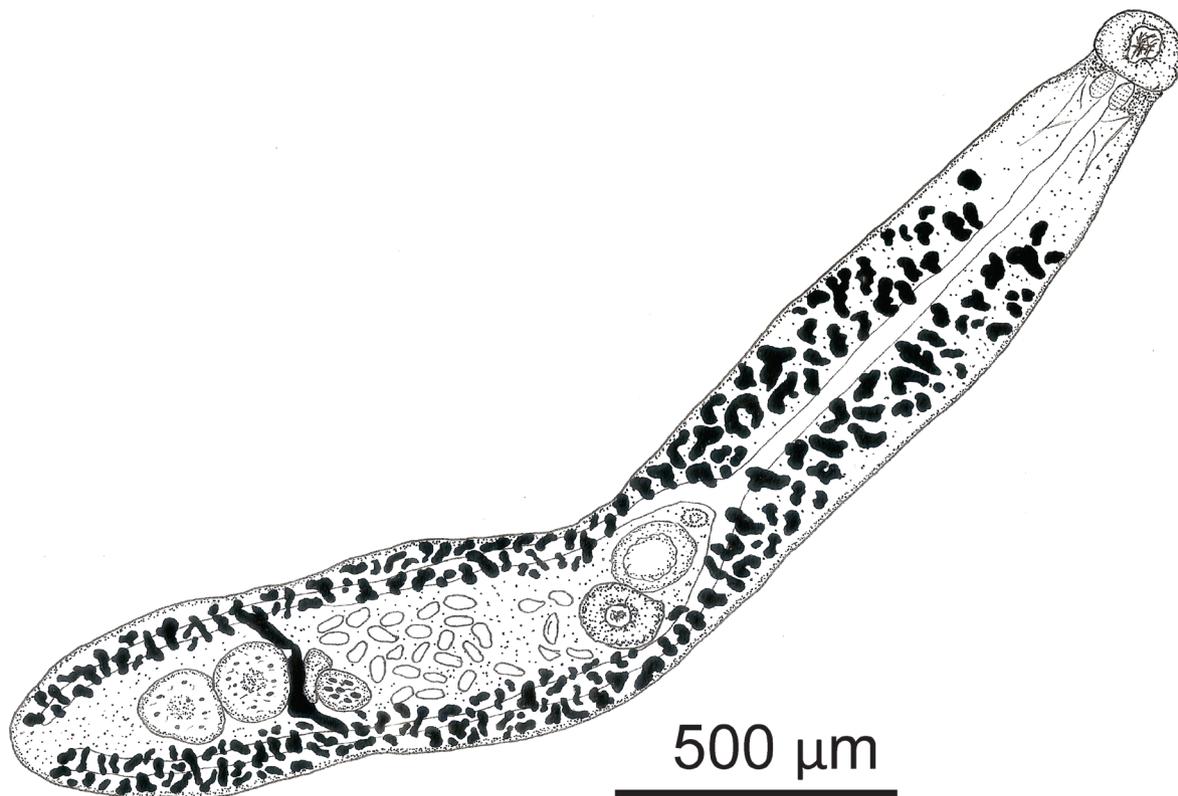


Fig. 3. *Rhytidodoides intestinalis* Price 1939 (Digenea: Rhytidodidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines: Cheloniidae) from Brazil. Ventral view.

tapered, posterior rounded, with constriction in anterior third; terminal oral sucker; pharynx present; acetabulum rounded, posterior to constriction of body; esophagus rectilinear in anterior region of body; two testes in tandem, rounded, located in posterior region of body; cirrus sac voluminous, at level of constriction of body; ovary rounded; Mehlis gland post-ovarian; Vitellaria formed by four groups of follicles, two on each side of the body, two in the anterior region between the pharynx and the anterior portion of the cirrus pouch, these groups connect with the corresponding in the posterior region of the body by a conduit, the second group begin near the region of the half of the cirrus pouch, following in the posterior direction of the body; eggs (18 measured) oval shaped, without polar processes.

*Rhytidodoides intestinalis* (Figs. 3 and 4; Table 2): body small, thin; oral sucker terminal; pharynx present; esophagus long, rectilinear; testes in tandem, rounded, located in posterior region of body; cirrus sac rounded, near cecal bifurcation; ovary spherical; Vitellaria formed by two groups of follicles, one on each side of the body, from the initial third of the esophagus to the posterior extremity of the body; Mehlis gland posterior to ovary eggs (18 measured) large, elliptical.

Histological description: The histological analysis revealed a giant-cell inflammatory response due to spirorchiids eggs (type 1 and type 3 eggs) in the spleen, brain, cerebellum, heart, large intestine, salt gland and kidneys. In the gallbladder, a mild papil-

lary pattern was found, with multifocal inflammatory infiltrate that reached the submucosal lamina propria and was limited to the basal membrane of the mucosa and bile ducts. The inflammation was composed of lymphocytes, plasmocytes as well as scarce heterophils and eosinophils and was associated with mildly proliferated (mild fibrosis), reactive, loose connective tissue and moderate hydropic accumulation. The epithelial cells of the mucosa were moderately degenerated, with evident nucleoli and randomly pyknotic nuclei. An amorphous eosinophilic material covered the mucous layer, with cell debris formed by an increase in mucus secretion and necrotic epithelial cells (Fig. 5).

The microscopic diagnosis was moderate, disseminated spirorchiidiosis, moderate necrotizing cholecystitis, edema, fibrosis, hydropic degeneration in the gallbladder.

#### Ethical Approval and/or Informed Consent

For this study formal concern is not required

#### Remarks

The genus *Rhytidodoides* was created by Price (1939) to group two species: *R. intestinalis* (from the small intestine) and *R. similis* (from the gallbladder). The two species were described based on two specimens collected from a green sea turtle in captivity in the

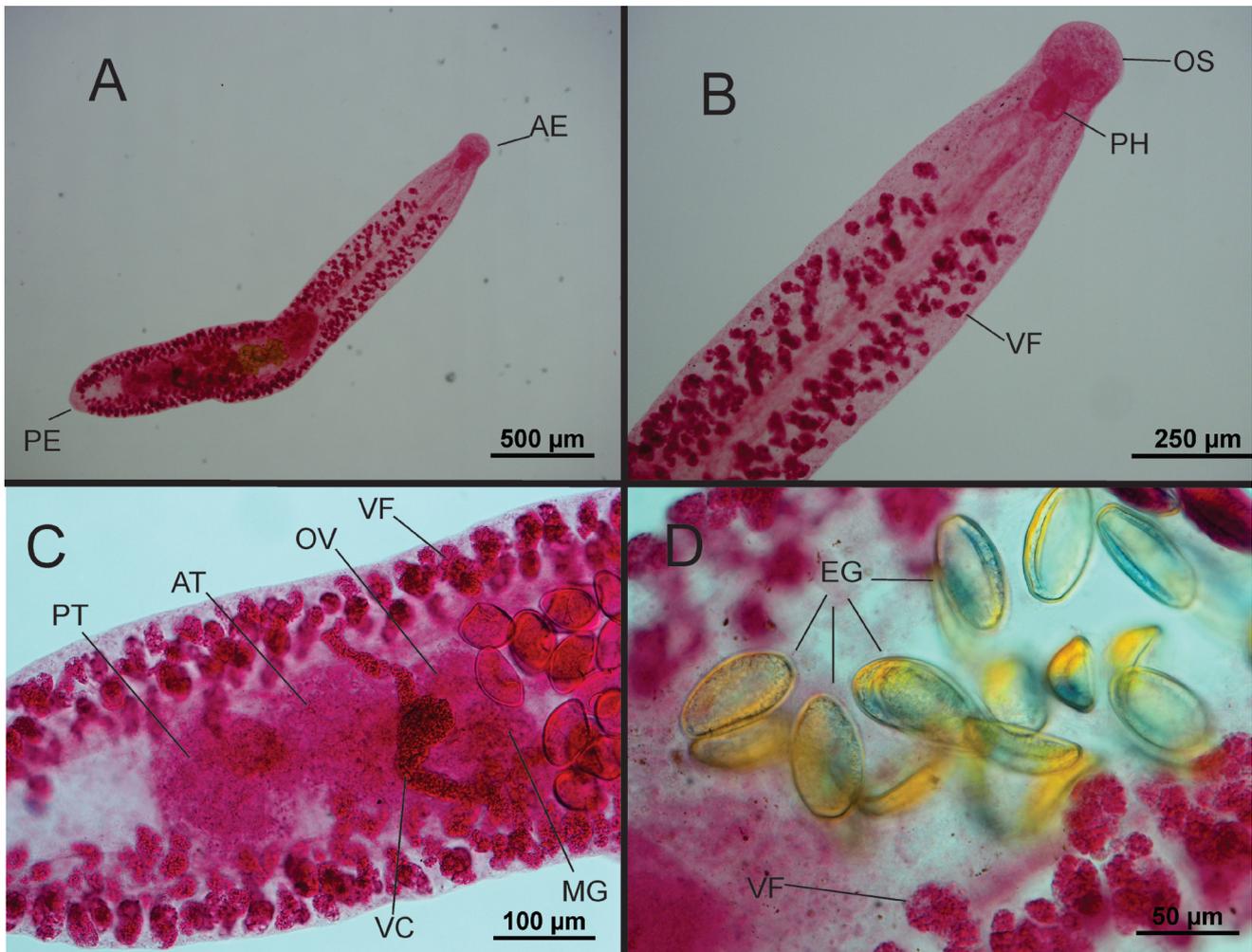


Fig. 4. (A-D) *Rhytidodooides intestinalis* Price 1939 (Digenea: Rhytidodidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines: Cheloniadae) from Brazil. Ventral view. Legend: AE- Anterior end; AT- anterior testes; Eg- eggs; MG- Mehlis gland; OS- oral sucker; OV- ovary; PE- Posterior end; PH- pharynx; PT- posterior testis; VC- vitelline conduct; VF- vitelline follicles.

National Zoological Park, Washington, DC, USA.

Nigrelli (1940) reported the occurrence of *R. similis* collected from the gallbladder of green turtles kept at the New York Aquarium, NY, USA. The author offered few morphometric data (i.e. mean body measuring 2.2 mm in length by 1.97 mm in width among 50 specimens analyzed) and some morphological data: "In larger specimens the vitellaria are definitely separated into two distinct follicular groupings at level of esophagus and two larger masses extending from level of cecal bifurcation to the extreme posterior end, where they unite".

Smith et al. (1941) described papillomatous disease in the gallbladder of green turtles associated with the presence of *R. similis*, reporting papillary hyperplasia of the mucosa, fibroplasia, lymphocytic inflammatory infiltrate, dilation of blood and lymphatic vessels, mucous exudate and the presence of trematode eggs enveloped by multinucleated giant cells in the liver. However, the authors did not describe the eggs.

Nigrelli (1941) described the parasitological analysis of 50 green

turtles, which revealed 17 species distributed among seven families of trematodes, among which the authors included *R. intestinalis*, *R. similis* and "...a new species of *Rhytidodooides* Price" – all found in the gallbladder. The prevalence of *R. similis* was 65% and papillomatous disease associated with infection by *R. similis* was found in four gallbladders.

Caballero (1954) examined 12 green turtles from Chepillo Island in the Panama Bay and reported the occurrence of nine species of trematodes, including one specimen of *R. intestinalis* collected from the duodenum as well as 17 specimens of *R. similis* collected from the liver. The author described papilloma formation in the bile ducts, leukocytic infiltrate and the proliferation of connective tissue (findings similar to Smith et al. in 1941) as well as the occurrence of inflammatory infiltrate composed of plasmocytes and eosinophils.

Mehrotra & Gupta (1978) described *R. pricei* and listed the differences that established the new species (based mainly on the relationship between the oral sucker and acetabulum). Mehrotra

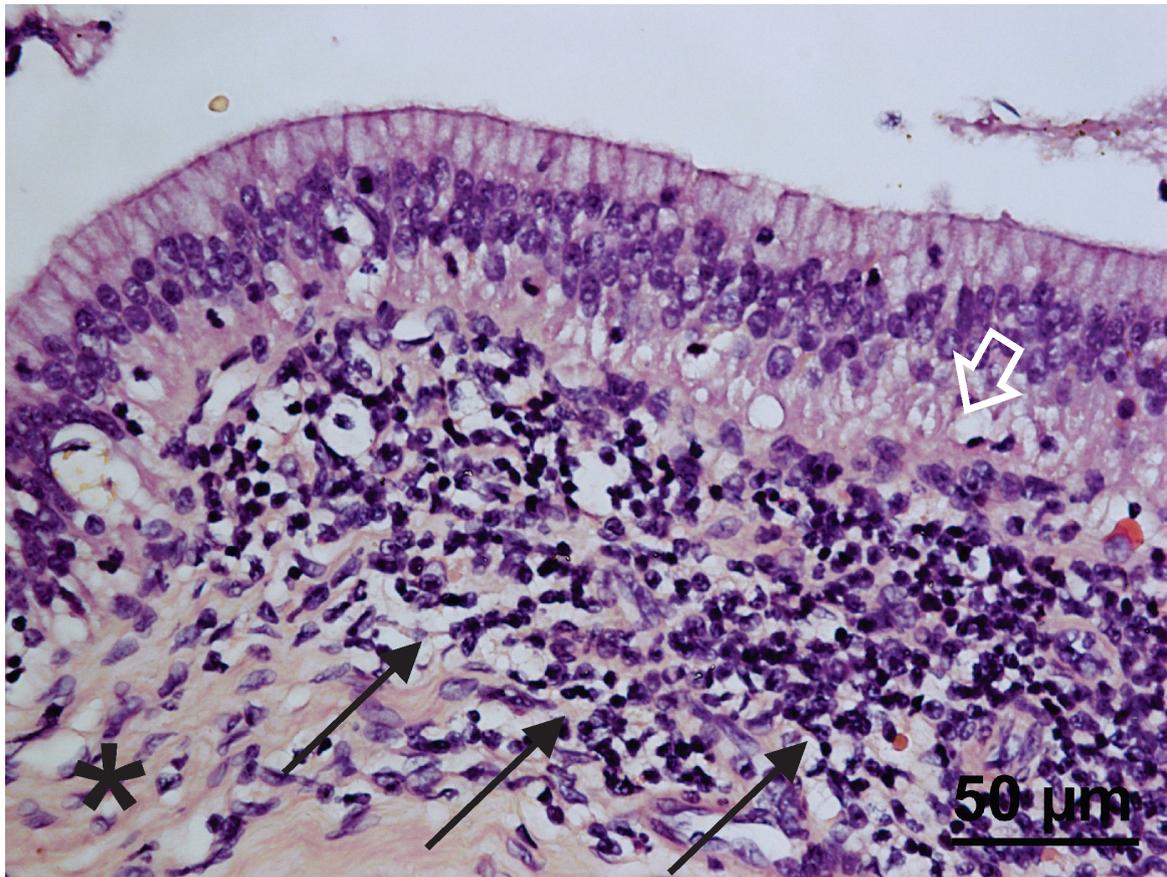


Fig. 5. Histological description of gallbladder: moderate lymphoplasmocytic inflammatory infiltrate, scarce eosinophils and heterophils (black arrows) associated with reactive, proliferated fibrous tissue (\*) and epithelial cells from degenerated mucous layer (white arrow).

& Gupta also stated that the specimen found by Caballero (1954) (identified by Caballero as *R. intestinalis*) was actually *R. pricei*. Unfortunately, the authors did not perform a comparative analysis of the specimens collected by Price (1939) and Caballero (1954) and did not state where the parasite had been deposited. Thus *P. pricei* is a synonym of *R. intestinalis* in our opinion.

Santoro et al. (2006) analyzed 40 adult green turtles (all females) in the Tortuguero National Park in Costa Rica and found *R. similis* (in the gallbladder and liver) in 25% of the hosts examined [mean abundance:  $2.6 \pm 7.1$ ; mean intensity:  $10.6 \pm 11.3$  (range: 1 to 34)] and *R. intestinalis* (in the gallbladder) in 15% of the hosts [mean abundance:  $0.2 \pm 0.4$ ; mean intensity:  $1.2 \pm 0.4$  (range: 1 to 2)]. Subsequently, Santoro et al. (2007) reported cholecystitis and ectasia of the mucosal glands, with the retention of material and small numbers of leucocytes in one turtle associated with 14 flukes identified as *R. similis*.

In an extensive summary, Greiner (2013) described the parasitological analysis of 44 loggerhead sea turtles, 74 green turtles, four hawksbill turtles, two leatherback turtles (*Dermochelys coriacea*), four olive ridley turtles (*Lepidochelys olivacea*) and four Kemp's ridley turtles (*Lepidochelys kempii*) – all from Florida (USA) between 1991 and 2006. The author reported the occurrence of *R.*

*similis* in the liver and gallbladder of 16 green turtles [prevalence: 21.6%; mean intensity: 19.0 (range: 1 to 98)].

More recently, Werneck et al. (2015) described the occurrence of four specimens of *R. similis* in a juvenile green turtle on the coast of Brazil. However, no histological analysis was performed on the gallbladder.

Both *R. similis* and *R. intestinalis* can be considered specialists (see Santoro et al., 2006), as these parasites have only been found in green turtles. *Rhytidodoides similis* has been reported in the United States (Price, 1939; Nigrelli, 1940, 1941; Smith et al., 1941; Greiner 2013), Panama (Caballero, 1954), Costa Rica (Santoro et al., 2006; Santoro et al., 2007) and Brazil (Werneck et al., 2015 and present report). In addition, *R. intestinalis* has been reported in the United States (Price, 1939), Panama (Caballero, 1954), Costa Rica (Santoro et al., 2006) and Brazil (present report).

In the present study, the gallbladder exhibited a mild papillary pattern, multifocal accentuated inflammatory infiltrate that reached the submucosal lamina propria and was limited to the basal membrane of the mucosa. The moderate inflammation composed of lymphocytes, plasmacytes as well as scarce heterophils and eosinophils was associated with mildly proliferated (mild fibrosis), reactive, loose connective tissue and moderate hydropic accu-

mulation. The epithelial cells of the mucosa were moderately degenerated, with evident nucleoli and randomly pyknotic nuclei. An amorphous eosinophilic material covered the mucous layer, with cellular debris formed by an increase in mucus secretion and necrotic epithelial cells (FIG. 5).

The microscopic findings associated with infection by *R. similis* are papillomatous formation, plasmocytic and eosinophilic inflammatory infiltrate, fibroplasia and mucous exudate (Smith et al., 1941; Nigrelli, 1941; Caballero, 1954; Santoro et al., 2006). All these findings are compatible with the results described herein. However, the present report also describes edema in the submucosal lamina propria, the degeneration of epithelial cells of the mucosa, evident nucleoli and mild necrosis of epithelial cells of the mucosa. The edema around the ducts and hydropic degeneration of the mucosa can diminish the lumen and cause biliary stasis. The mild necrosis of the mucosa facilitates infection by infectious agents and makes the bile thicker, which hinders its excretion. Evident nucleoli occurred due to the aggression caused by the inflammation. The morphological findings of the specimens were compatible with data previously described by Price (1939), Caballero (1954) and Blair (2005). The morphometric analysis of the specimens of *R. similis* revealed wider ranges for total body length, acetabulum width, esophagus length and width as well as larger testes and Mehlis glands in comparison to previously published data (Table 1). The analysis of *R. intestinalis* revealed wider ranges regarding the length and width of the oral sucker, acetabulum, anterior testis and cirrus sac as well as larger widths of the ovary and Mehlis gland in comparison to previously published data, whereas the length of the Mehlis gland was smaller compared to data published by other authors (Table 2). Although the morphometrics of both species demonstrated differences for some organs, these differences are believed to be due to the natural variation among individuals and do not compromise the correct identification of the species.

The helminth fauna of green turtle of the coast of Brazil is composed of 36 species of trematodes distributed among ten families and three species of nematodes distributed between two families (Werneck and Silva, 2016, present report).

### Conflict of Interest

Authors state no conflict of interest.

### Acknowledgments

Biological samples were obtained through the beach monitoring project in the state of Rio de Janeiro, Brazil, which is a requirement established by the federal environmental licensing division of the Brazilian environmental agency (IBAMA), for the exploration of oil and gas by Petrobras at the Santos Basin pre-salt province. In the Espírito Santo State the beach monitoring project is a require-

ment established by the federal environmental licensing division of the Brazilian environmental agency (IBAMA) process number N°02022.001407/10

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