Research Note

The genus *Rhytidodoides* 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 *Rhytidodoides intestinalis* and *Rhytidodoides 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: *Rhytidodoides 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; *Rhytidodoides intestinalis*; *Rhytidodoides 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 *Rhytidodoides* 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. *Rhytidodoides 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...
Table 1. Morphometric data of *Rhytidodoides similis*, Price, 1939 (Digenea: Rhytidodidae) from *Chelonia mydas* (Testudines, Cheloniidae). Measurements in micrometers as Range (mean).

| Site                  | Price (1939) | Smith et al. (1941) | Caballero (1954) | Wemeck et al. (2015) | Present Report |
|-----------------------|--------------|---------------------|-------------------|----------------------|---------------|
| Localities            | 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 | 165 – 370    | 170 – 385 (300)     | 289 – 312         | 210 – 220             | 302 – 359 (335)  |
| Ventral sucker width  | 266 – 285    | 210 – 230           | 277 – 354 (303)   | 499 – 856 (638)       |
| 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    | 481 – 515           | 720 – 900         | 809 – 853 (827)       |
| Cirrus sac width      | 160 – 430    | 199 – 216           | 220 – 245         | 295 – 345 (320)       |
| Ovary length          | 63 – 70      | 36                  | 61 – 65           | 37 – 60 (45)          | 47 – 59 (54)    |
| Ovary width           | 37 – 40      | 38 – 42             | 20 – 40 (29)      | 22 – 34 (28)          |
| Distance from (mm)    | –            | –                   | –                | 1.146 – 1.264 (1.22) |
| To intestinal caeca bifurcation to anterior end | – | – | – | 0.810 – 0.916 (0.847) |
| To genital aperture to anterior end | – | – | 747 – 863 | 0.871 – 1.05 (0.955) |
| To ovary to ventral sucker | – | – | – | – |
| Site            | Price (1939) | Caballero (1954) | Present Report |
|----------------|--------------|------------------|----------------|
| Gall bladder   | USA          | Small intestine  | Gall bladder   |
| Locality       |              | Panama           | Brazil         |
| N              | 2            | 1                | 3              |
| Body length (mm) | 2.0 – 2.1   | 3.12             | 2.4 – 2.5      |
| Body width     | 320 – 335    | 448              | 268 – 382      |
| Oral sucker length | 110 – 130*  | 57               | 117 – 142      |
| Oral sucker width  | 190         |                  | 129 – 159      |
| Pharynx length  | 60 – 70      | 68               | 55 – 60        |
| Pharynx width   | 55 – 70      | 76               | 67 – 69        |
| Ventral sucker length | 103 – 115* | 118              | 115 – 140      |
| Ventral sucker width  | 125         |                  | 88 – 126       |
| Esophagus length | 730 – 830   | 912              | 887 – 970      |
| Esophagus width  | –            | 68               | 39 – 49        |
| Anterior Testes length | 130*       | 141              | 123 – 149      |
| Anterior Testes width  |            | 118              | 109 – 137      |
| Posterior Testes length | 130*      | 198              | 107 – 144      |
| Posterior Testes width  |            | 160              | 102 – 151      |
| Cirrus sac length | 110 – 137   | 190              | 149 – 196      |
| Cirrus sac width   | 105 – 115    | 148              | 124 – 151      |
| Seminal vesicle length | –          | 114              | –              |
| Seminal vesicle width  | –           | 061              | –              |
| Ovary length     | 90 – 110*    | 114              | 94 – 104       |
| Ovary width      | 122          |                  | 85 – 144       |
| Mehlis’ gland length | –           | 152              | 72 – 89        |
| Mehlis’ gland width  | –           | 84               | 62 – 126       |
| Eggs length      | 60 – 63      | 57               | 51 – 63        |
| Eggs width       | 45 – 52      | 34 – 38          | 27 – 40        |
| Distance from (mm) |              |                  |                |
| To intestinal caeca bifurcation to anterior end | –          | –                | 1.08 – 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*
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 μm), 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 μm) 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 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
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 spirorchids eggs (type 1 and type 3 eggs) in the spleen, brain, cerebellum, heart, large intestine, salt gland and kidneys. In the gallbladder, a mild papillary 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 eonsinophils 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 spirorchidiosis, 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
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, mucus 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 Rhytidodoides 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 &
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, plasmocytes as well as scarce heterophils and eosinophils was associated with mildly proliferated (mild fibrosis), reactive, loose connective tissue and moderate hydric 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 gland 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.

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