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Anatomopathological study of parrot pufferfish *Colomesus psittacus* parasitized by the aspidogastrean *Rohdella* sp.

Estudo anatomopatológico do peixe baiacu papagaio *Colomesus psittacus* parasitado pelo aspidogastrea *Rohdella* sp.

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Abstract

Aspidogastrea are globally-distributed parasites of the class Trematoda, which have been described as pathogens of a range of aquatic organisms, in marine and freshwater environments. The principal morphological characteristic of the group is an adhesive ventral disc, which is responsible for fixing the parasite to the host organism. In this study, 112 specimens of *Colomesus psittacus* from the municipality of Cametá, in the state of Pará (Brazil), were necropsied. Platyhelminthes of the genus *Rohdella* attached to the mucous membrane of the fish's intestine by the adhesive disc were observed. Fragments of parasitized tissue were fixed in Davidson solution and then processed and stained with hematoxylin-eosin. Other fragments were fixed in glutaraldehyde, processed and observed under a scanning electron microscope. The prevalence of the parasite was 76.4%, mean intensity of infection was 8.0 and mean abundance was 6.2. The parasitism provoked chronic enteritis with diffused inflammatory infiltration. The adherence of the parasite to the mucous membrane of the intestine resulted in strangulation and hyperplasia of the region, as well as causing hypertrophy of the muscle of the mucous membrane. The present study describes the anatomopathological and ultrastructural aspects of the parasitism of the intestine of *C. psittacus* by *Rohdella* sp.

Keywords: Amazonia, parrot puffer fish, intestine, parasite, histopathology, *Rohdella* sp.

Resumo

Os Aspidogastreas são parasitos da classe Trematoda, distribuídos globalmente e têm sido descritos como patógenos em uma gama de organismos aquáticos de ambientes marinhos e de água doce. A principal característica morfológica do grupo é um disco adesivo na região ventral responsável pela fixação do parasito no organismo hospedeiro. Neste estudo, 112 espécimes de *Colomesus psittacus* provenientes do município de Cametá, no estado do Pará (Brasil), foram necropsiados. Foram observados platelmintos do gênero *Rohdella* aderidos à mucosa intestinal através do disco adesivo. Fragmentos de tecido com parasito foram fixados em solução de Davidson e processados e corados em Hematoxilina-Eosina. Outros fragmentos foram fixados em glutaraldeído, processados e observados em microscopia eletrônica de varredura. A prevalência parasitária foi de 76, 4%, intensidade média de infecção de 8,0 e abundância média de 6,2. O parasitismo ocasionou uma enterite crônica com difuso infiltrado inflamatório. A fixação do parasito na mucosa intestinal provocou estrangulamento e hiperplasia da região, bem como hipertrofia da muscular da mucosa. O presente trabalho descreve os aspectos anatomopatológicos e ultra-estruturais da ação parasitária por *Rohdella* sp. no trato intestinal de *C. psittacus.*

Palavras-chave: Amazônia, baiacu papagaio, intestino, parasito, histopatologia, *Rohdella* sp.

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Introduction

The genus *Rohdella* Gibson & Chinabut, 1984, belongs to the subclass Aspidogastrea Faust & Tang, 1936 (OLSON; TKACH, 2005), which contains only four families, 12 genera and approximately 80 species (ROHDE, 2001a; SCHLUDERMANN et al., 2005; MING-XIU et al., 2010). Aspidogastreans are distributed worldwide, and have been described as pathogens in different aquatic organisms, such as mollusks, fish, and turtles, including both marine and freshwater species (SNYDER; TKACH, 2007; SCHLUDERMANN et al., 2005).

The most marked characteristic of aspidogastreans is the adhesive disc on the ventral surface, which is subdivided into longitudinal lines, or alveoli (GRIZZLE; BRUNNER, 2007; TOLSTENKOV et al., 2010). The principal function of this organ is to fix the parasite to its host, but it may also have a sensorial function or play a role in external digestion (GRIZZLE; BRUNNER, 2007). The life cycle of these organisms generally involves a mollusk as intermediate host and a vertebrate definitive host, although in some cases, the mollusk is the definitive host (ROHDE, 2001b; ZAMPARO; BROOKS, 2003). In the case of fish hosts, the parasites are found in the intestinal tract (ABOUL-DAHAB et al., 1993).

The presence of parasites in the host organism, especially in the intestine, may result in both structural and metabolic alterations, which may be reflected in a variety of pathological processes, and may even result in the death of the host in some cases (ROCHA et al., 2010; PEÑA-REHBEIN; RÍOS-ESCALANTE, 2012). The present study describes the anatomopathological and ultra-structural features of parasitic infestation of the intestinal tract of the parrot pufferfish (*Colomesus psittacus*) by *Rohdella* sp.

Materials and Methods

1. Hosts

A total of 112 specimens of the euryhaline parrot pufferfish, *Colomesus psittacus* Bloch and Schneider, 1801, were obtained from the municipality of Cametá (02º 14' S and 49º 49' W) in the Brazilian state of Pará, between January 2009, and April 2010. The specimens had a mean body length of 12 ± 3 cm and weight of 5 ± 2 g, and were transported live to the Carlos Azevedo Research Laboratory (UFRA), where they were anesthetized with MS 222 (Sandoz Laboratories), prior to necropsy.

2. Necropsy and optical microscopy

The necropsies on the hosts began with opening the abdominal cavity using a pair of anatomical scissors, in order to gain access to the viscera. The organs were examined under a stereomicroscope. The parasites were found adhering to the intestinal mucous membrane of the hosts, and data on prevalence, mean intensity of infection and mean abundance were calculated as described by Serra-Freire (2002). The parasitized intestines were then removed. For examination using optical microscopy, the organ was pre-fixed for 2 hours in order to maintain the cylindrical shape. The intestine was fragmented into 0.5 cm-thick segments. These fragments were fixed in Davidson solution (formaldehyde, acetic acid, 95% ethanol and distilled water) at room temperature for 24 hours, and were then processed and stained with hematoxylin-eosin. The stained sections were documented using a Zeiss Primo Star microscope with a Canon A610/A620 52 mm adaptor. Adult specimens of the parasites were collected, rinsed in saline solution, fixed in AFA (acetic acid, formaldehyde and 80% ethanol) at room temperature and set between a slide and coverslip, with light pressure. Finally, the specimens were stained in alcoholic carmine and mounted in Canada balsam, as described by Amato (1985).

3. Electron microscopy

For scanning electron microscopy (SEM), small fragments (1.0 cm) of the intestinal tissue containing parasites were fixed in 5% glutaraldehyde, buffered with sodium cacodylate (pH 7.2), for 12 hours at 4 °C. They were then rinsed overnight in the same buffer and post-fixed in 2% OsO_4, buffered in the same solution for 3 hours at the same temperature. The specimens were then dehydrated in an ascending ethanol series. The fragments were dried to the critical point, metalized with a fine (20 nm) layer of gold, and photographed under the LEO 1459 VP SEM, operated at 80 kV.

Results

The fish did not present clinical symptoms of parasitosis, but in the infected specimens, reddish areas could be observed in the interior of the intestine (through the transparent wall), after opening the abdominal cavity (Figure 1a). When the intestine was opened, it was possible to confirm that these reddish areas corresponded to the presence of platyhelminth parasites, of the genus *Rohdella*, which were adhering to the intestinal wall by means of their ventral region (Figure 1b, c). The parasites were identified through the descriptions of Gibson and Chinabut (1984). They could be observed either in isolation or in small clumps dispersed throughout the organ.

The prevalence of *Rohdella* sp. was 76.4%, with a mean intensity of infection of 8.0 and a mean abundance index of 6.2 (Table 1). Overall, 683 worms were recorded, and these trematodes were observed during all sampling periods (Table 1).

The microscopic lesions corresponded to chronic congested enteritis with discreet and diffuse inflammatory infiltration of the mononuclear cells. Trematodes were observed in the lumen of the viscera, fixed to the mucous membrane by means of adhesive discs. Hypertrophy of the muscle of the mucous membrane adjacent to the parasites could be seen (Figure 1d). The adhesive disk caused compression of the epithelium with its projections (Figure 1e). Strangling and hyperplasia of the intestinal mucous membrane were observed, reflecting the disorganization of the tissue (Figure 1f). The mucous surface of the intestine presented ovoid or discoid imprints corresponding to the shape of the parasite's adhesive disk (Figure 2a, b).

Discussion

Aspidogastreans of the genus *Rohdella* are reported here for the first time from the Amazonian freshwater parrot pufferfish (*Colomesus psittacus*). The infection levels of *Rohdella* sp. were compared with those of other parasites belonging to the subclass Aspidogastrea and parasitizing other hosts. The total prevalence (Table 2) of these parasites (76.4%) was much higher than the values recorded for *Barbus barbus* from the Fischa River in Australia parasitized by *Aspidogaster limacoides* (SCHLUDERMANN et al., 2005); for *Cyprinus carpio* and *Mylopharyngodon piceus*, parasitized by *Aspidogaster ijimai* and *A. conchicola*, respectively, in a floodplain lake in the middle Yangtze River region (GAO et al., 2003); and for *Trachinotus marginatus*,

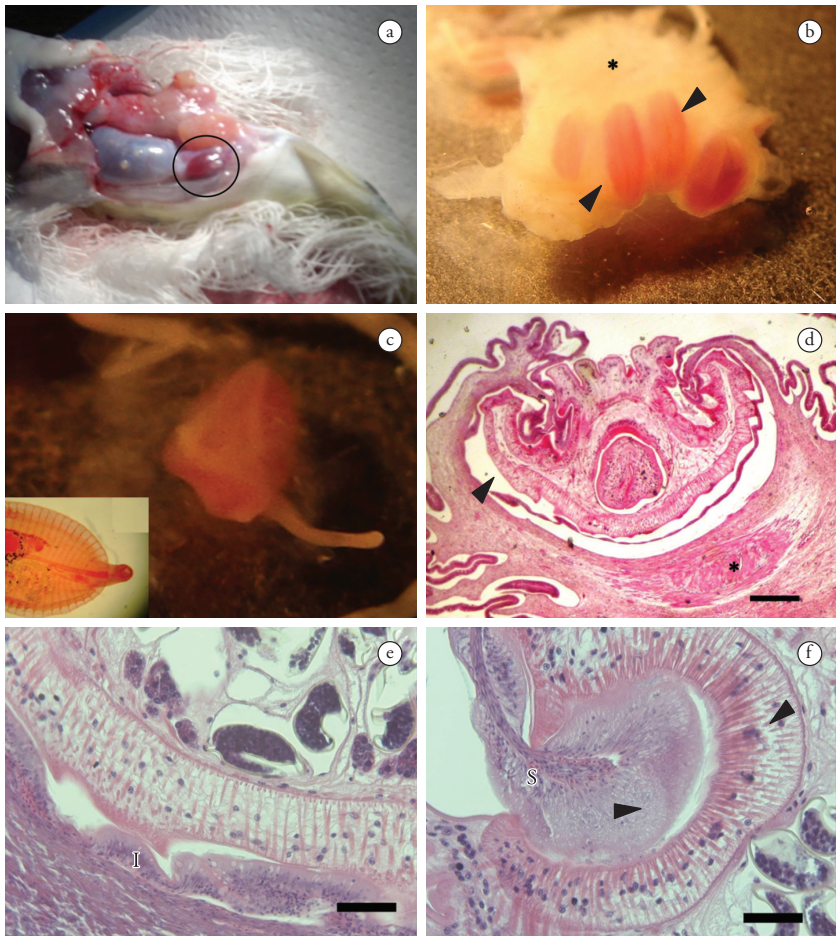

Figure 1. a) *Colomesus psittacus.* Intestine. *Rohdella* sp. Parasitism by *Rohdella* sp. Note the presence of a reddish mark on the intestine (circled); b) *C. psittacus.* Intestine. *Rohdella* sp. Fresh parasite (arrows) adhering to the mucous membrane of the intestine (*); c) *Rohdella* sp. Fresh parasite, with retractable anterior region exposed. Detail - *Rohdella* sp. Parasite between slide and coverslip stained with alcoholic carmine. Magnification 4×; d) *C. psittacus*. Intestine. *Rohdella* sp. Hypertrophy of the muscular mucosa (*) adjacent to the parasite (arrows) in the intestinal tract. H.E. Magnification 4×. Scale bar: 200 µm; e) *C. psittacus*. Intestine. *Rohdella* sp. Detail of the insertion point of the projections of the parasite's ventral disc marking the mucous membrane of the intestine (I). H.E. Magnification 40×. Scale bar: 40 µm; f) *C. psittacus*. Intestine. *Rohdella* sp. Parasite fixed to the mucous membrane by the adhesive disc (arrow). Note the strangulation (S) and hyperplasia of the tissue, which has become disarranged (arrow head). H.E. Magnification 40×. Scale bar: 40 µm.

Table 1. Prevalence (%), mean intensity of infection and mean abundance of infection by *Rohdella* sp. parasites in the intestine of *Colomesus psittacus* between January 2009 and April 2010, from the municipality of Cametá (Pará).

Month	Prevalence (%)	Mean intensity ± SD (range)	Abundance ± SD (range)
Jan. 09	81.8	9.2 ± 3.9 (0-14)	7.5 ± 3.0 (5-14)
Apr. 09	94.4	9.3 ± 5.1 (0-22)	8.7 ± 4.7 (4-22)
June 09	75.0	7.9 ± 4.1 (0-11)	5.9 ± 2.0 (5-11)
Sept. 09	76.5	5.8 ± 3.1 (0-10)	4.5 ± 2.4 (2-10)
Nov. 09	76.5	8.4 ± 4.6 (0-15)	6.4 ± 3.4 (4-15)
Jan. 10	70.6	8.6 ± 4.2 (0-12)	6.1 ± 2.4 (5-12)
Apr. 10	60.0	6.8 ± 4.1 (0-11)	4.1 ± 3.1 (2-11)

% - percentage, SD - Standard desviation.

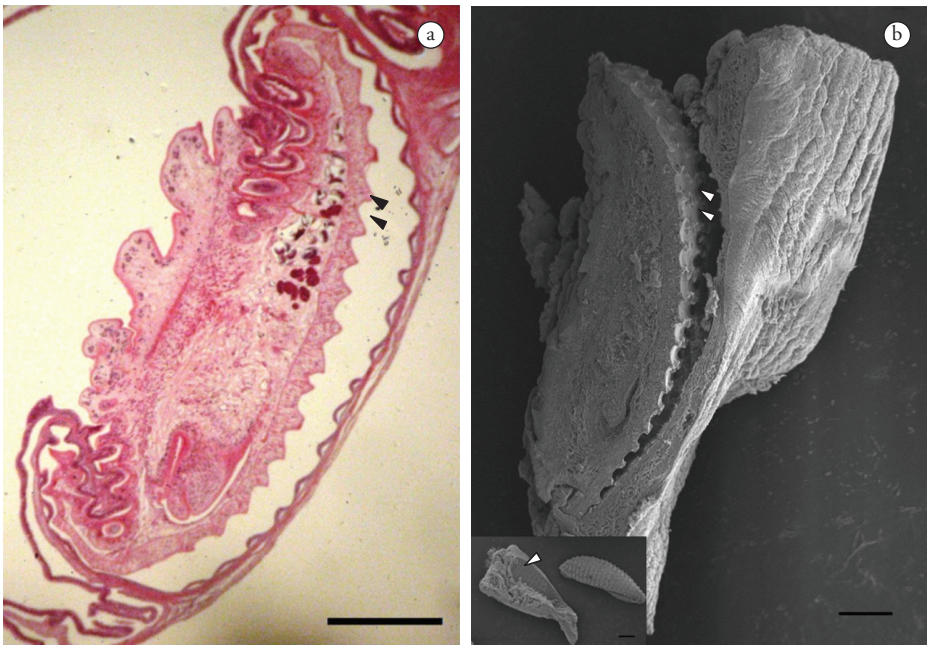

Figure 2. a) *Colomesus psittacus.* Intestine. *Rohdella* sp. Longitudinal section showing the parasite's adhesive disc (double arrow) adjacent to the mucous membrane of the intestine. H.E. Magnification 10×. Scale bar: 400 µm; b) *Rohdella* sp. SEM image of Figure 2a, showing the adhesive disc (double arrow). Scale bar: 200 µm. Detail: ovoid or discoid indentation of the mucous membrane of the intestine corresponding to the mark left by the parasite's adhesive disc (arrow). Scale bar: 300 µm.

Table 2. Parasitic indices recorded for different aspidogastrean parasites in fish from Brazil, Australia and China.

Parasite	Host	Prevalence (%)	Mean intensity ± SD	Abundance ±SD	Reference
Aspidogaster limacoides	*Barbus barbus*	11.75	10.0 ± 7.8	0.95 ± 0.8	Schludermann et al. (2005)
Aspidogaster ijimai	*Cyprinus carpio*	8.37	16.7 ± 15.7	-	Gao et al. (2003)
Aspidogaster conchicola	*Mylopharyngodon piceus*	50.00	31.9 ± 16.2	-	Gao et al. (2003)
Labatostoma hanumanthai	*Trachinotus marginatus*	51.10	38.2	29.80	Pereira Junior et al. (2004)
Labatostoma kemostoma	*T. marginatus*	26.97	7.0	2.01	Pereira Junior et al. (2004)
Rohdella sp.	*Colomesus psittacus*	76.40	8.0	6.17	Present study

% - percentage, SD - Standard desviation.

parasitized by *Lobatostoma hanumanthai* and *L. kemostoma* on the coast of southern Brazil (PEREIRA JUNIOR et al., 2004). However, the latter authors recorded a much higher mean intensity for *L. kemostoma* than what was recorded in the present study. In this study, higher abundance values were observed, in comparison with the values recorded for other species (Table 2).

Mollusks are known to be intermediate hosts in the life cycle of many aspidogastreans (ROHDE, 2001b), and are an important part of the diet of *C. psittacus* (CAMARGO; MAIA, 2008). Given this, and the prevalence of Aspidogastrea of the genus *Rohdella* in the specimens collected in the present study, *C. psittacus* appears to be a common host of this parasite in the wild.

A high parasite load, such as what was recorded in the present study (which reached its peak in April 2009 – see Table 1), may favor obstruction of the intestinal tract, especially in small fish such as *C. psittacus*. This may affect the metabolic and reproductive development of the host and, in cases of extreme infection, cause its death (THATCHER, 2006; COSTA, CAMARGO, 2009).

When fixed to the mucous membrane of the intestine, the adhesive disc of aspidogastreans causes strangulation and hyperplasia of the area, with hypertrophy of the muscle, as described by Grizzle and Brunner (2007) and Tolstenkov et al. (2010). These reactions, according to Korting (1977), are the most frequent manifestations of the process of parasitic invasion.

The anatomopathological features observed in the present study indicate that each parasite tends to provoke relatively small and localized impacts. The most important impact for the host would appear to be nutritional deficiency resulting from alterations caused to the intestinal epithelium. Under intense infestation, however, there is a possibility of intestinal obstruction, especially in hosts of small size, as suggested by Pavanelli et al. (1997) for a digenetic trematode in a siluriform fish. We were unable to determine the species of parasite: this would require further investigation using electron microscopy and molecular analysis, with possible determination of a new species. This is the first report of parasitism by an aspidogastrean trematode in the intestine of *C. psittacus* from eastern Amazonia.

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