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Aspectos histopatológicos em *Plagioscion squamosissimus* induzidos por *Neoechinorhynchus veropesoi*, metacestódeos e juvenis da Família Anisakidae

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Abstract

Plagioscion squamosissimus (Heckel 1840), a fish endemic to the Amazon Basin and commonly known as the "silver croaker", plays an important role in the ecology and economy of Pará State, Brazil. Knowledge of host-parasite relationships is important to understanding the role of parasites in the control of natural host populations. This work describes histopathological aspects caused by several common intestinal parasites found during a helminthological survey of fish in northern Brazil. We observed a high prevalence of helminth infection, especially by J3 nematode juveniles of the family Anisakidae and metacestodes of the family Protocephalidae (both with 100% prevalence). An external capsule surrounded each juvenile with numerous juveniles inside sac-like structures formed of connective tissue. Inflammation was observed to be caused by infection of metacestodes, reaching the intestinal muscularis mucosa. *Neoechinorhynchus veropesoi* (38% prevalence) was found in the small intestine of *P. squamosissimus*, invading the mucosa, submucosa, and internal muscularis of the intestine causing intense inflammation. Histopathology of host-parasite relationships in fish has been rare, and the pathology of parasites in *P. squamosissimus* is described herein.

Keywords: Host-parasite relationship, histopathology, *Plagioscion squamosissimus*, *Neoechinorhynchus veropesoi*, metacestodes, Anisakidae.

Resumo

Plagioscion squamosissimus (Heckel, 1840) é uma espécie de peixe endêmico da Bacia Amazônica e comumente conhecido como "pescada branca" e é de extrema importância ambiental e comercial no Estado do Pará. O estudo das relações parasito-hospedeiro são importantes para a compreensão do papel dos parasitas no ambiente. O presente trabalho descreve aspectos histopatológicos causados por helmintos intestinais e cavitários encontrados parasitando *P. squamosissimus* de Belém, PA. As larvas da família Anisakidade e metacestóides (Proteocephalidae) (ambos prevalência de 100%) encontravam-se recobertas por uma cápsula de tecido conjuntivo do hospedeiro, sendo observada fraca reação inflamatória para larvas de Anisakidae, no entanto observou-se que metacestoides embebidos na muscular interna apresentaram um infiltrado inflamatório. *Neoechinorhynchus veropesoi* (prevalência de 38%) encontrava-se embebido na camada muscular interna do intestino, induzindo reação inflamatória mais intensa. Estudos evidenciando as reações

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histopatológicas em peixes são raros, no entanto são de extrema importância para a compreensão das relações parasito-hospedeiro.

Palavras-chave: Relação parasito-hospedeiro, histopatologia, *Plagioscion squamosissimus*, *Neoechinorhynchus veropesoi*, metacestódeos, Anisakidae.

Introduction

Plagioscion squamosissimus (Heckel 1840) is a fish endemic to the Amazon Basin; its diet includes crustaceans, molluscs, and small fishes (CASATTI, 2005). As has been shown in previous research on this species, the diversity of helminth parasites is directly related to what they eat. During development from fry to adult, they ingest large quantities of the intermediate hosts of several groups of parasites (EIRAS, 1994; SARAIVA, 1994; MALTA et al., 2001).

Parasites are very common in fish, especially in natural environments where ecological requirements for intermediate hosts and parasite transmission are met. In wild populations, parasites and diseases can modulate host dynamics, causing rapid population declines (FEIST; LONGSHAW, 2008). In aquaculture, parasites are an important cause of mortality, with considerable impacts on growth, resistance, and susceptibility to predation; in certain cases, they affect commercial interests and impact local economies (THATCHER, 2006; WOO, 2006).

In the Brazilian Amazon, *P. squamosissimus* has an important environmental and economic role. This species is an important component of the food web, and more than 10 tons are produced for sale each year in Belém, Pará State, Brazil (BARTHEM, 1985; BRASIL, 2005).

Host responses to parasite infection are important to provide an understanding how parasites may impact host fitness, the dynamics of host populations, and possible indirect consequences for non-host populations through changes in the intensity of interspecific competition (FEIST; LONGSHAW, 2008).

During a survey of the helminth fauna of *P. squamosissimus* in Guajará Bay, MELO et al. (2013) found a new species of acanthocephalan and a high prevalence of both metacestodes and juveniles of nematodes of Anisakidae. The present paper describes histopathological changes caused by the intestinal parasites common in this species of fish.

Materials and Methods

Between February 2007 and August 2010, 29 specimens of *P. squamosissimus* were obtained from a local fisherman, who caught them in the estuary of the Guamá River and the neighbouring Guajará Bay, adjacent to the city of Belém (1° 15' S-1° 29'S, 48° 32' W-48° 29' W) in the Brazilian state of Pará, in the eastern Amazon basin of Brazil. The fish were transported to the "Laboratorio de Biologia Celular e Helmintologia Profa. Dra. Reinalda Marisa Lanfredi" where necropsies were performed. The worms were rinsed in phosphate-buffered saline (PBS) and fixed in alcohol-formalin acetic acid (AFA). The parasites were identified using standard methods (AMATO et al., 1991).

Sections of the intestines of the fish were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), dehydrated and embedded in paraffin and methacrylate resin (LEICA Historesin, Heidelberg, Baden-Wurttemburg, Germany) (SILVA et al., 2013). Specimens embedded in the paraffin blocks were sectioned at 4-6 μm thickness, placed on glass slides, and stained with hematoxylin and eosin (H&E) and 1% toluidine blue (TB). The slides were then analysed using an Olympus BX53 light microscope equipped with an Olympus DP72 camera and image-acquisition system (Olympus America Inc.).

Results

Of 29 specimens of *P. squamosissimus*, 11 presented acanthocephalans in the intestine (prevalence of 38%), and 100% were infected with metacestodes and Anisakidae juveniles (intensity of infection ranged from 1 to 452 parasites per host).

The anisakid juveniles were found encapsulated in a sac composed by conjunctive-tissue comprised of numerous thin, membranous capsules, ovoid-to-spherical in shape, each containing a single juvenile, in the body cavity surrounding the internal organs of the host (Figure 1A). The cysts were surrounded by aggregates of macrophages and an amorphous material (Figures 1B-D) and the macrophages were occasionally observed between the fibres of the cyst wall. The juveniles removed from the tissues were compared to the morphological features and sizes reported by Moravec (1998), and we determined that they were stage 3 juveniles (J3) of the family Anisakidae; however, the nematodes were not identified. Juveniles were found encapsulated in the peritoneal serosa surrounding the outer surface of the internal organs (Figure 1B). Each juvenile nematode was confined in a cyst with thick walls composed mainly of fibroblasts and collagenous fibres.

The morphological characteristics of the nematodes themselves were studied, having been taken from various organs of the host. The tissues of the intestines of the nematodes were observed to be composed one layer of cells with granular inclusions (Figure 1C). Also evident was the striated cuticle of the juvenile nematode (Figures 1C, D).

Cestode larvae were found to occur in the tissues of the host either alone (in cysts in the peritoneal lining) or with juvenile anisakids embedded near the cyst of the metacestode (Figure 2A). The metacestodes were individually removed from the cysts, and the morphological features of the larvae were compared to the key and descriptions of Rego et al. (1999); the metacestodes were identified as plerocercoids of the family Proteocephalidae. Some larvae were found in the external muscularis mucosa of the small intestine, enveloped by a cyst wall composed of fibroblasts. One or more larvae were observed in each cyst, (Figures 2B-D); each metacestode had an individual cyst membrane with amorphous material between the larval cyst and the cyst wall (Figures 2C, D).
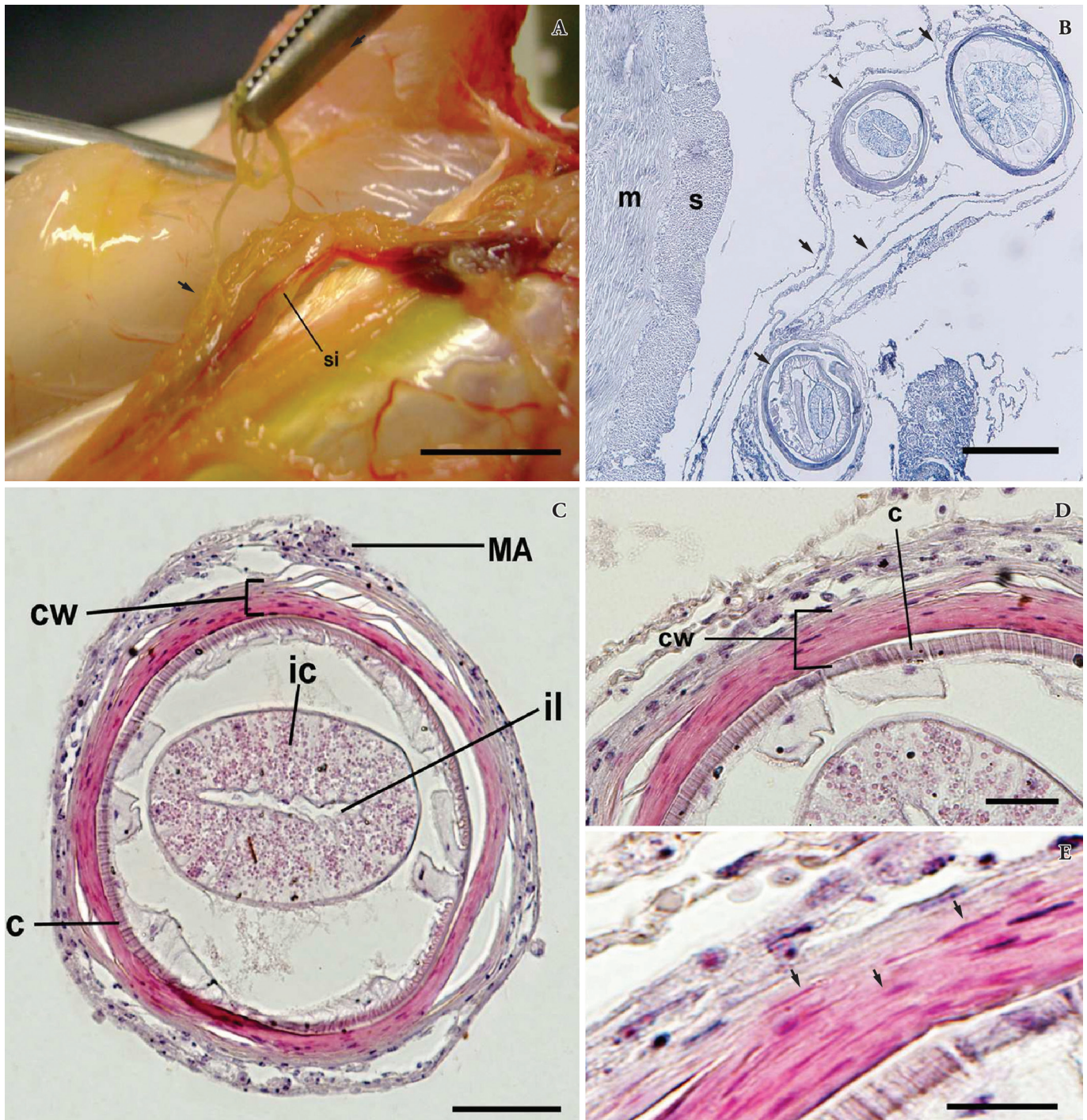
Figure 1. Anatomical view and histopathological aspects of AnisakidaeJ3 juveniles from the mesenteries of *Plagioscion squamosissimus* (paraffin-embedded). **A)** Coelomatic cavity of host infected with Anisakidae J3 juveniles **(arrow)** in the mesenteries, covering the small intestine **(si)**. Bar: 1 cm. **B)** Histological aspects of AnisakidaeJ3 juveniles **(arrows)** in the mesenteries of *Plagioscion squamosissimus;* the muscularis **(m)** and serosa **(s)** layers are visible. The nematode juveniles are not directly attached to the small-intestine serosa and are connected by fibrous host tissue **(arrowheads)**.(TB).Bar: 200 μm. **C)** Detailed view of a helminth in transverse section showing the intestinal lumen **(il)**, the characteristics of the intestinal cells **(ic)**, the nematode cuticle **(c)** and the fibrous wall of the cyst **(cw)** surrounded by macrophage agglomerates **(MA)**. (H&E). Bar: 50 μm. **D)** Detail of the transverse section showing the histological aspects of the nematode cuticle **(c)** and the cyst wall **(cw)**, composed of numerous fibroblasts. (H&E). Bar: 20 μm. **E)** Detail of the cyst wall; numerous fibroblasts are visible **(arrows)**. (H&E). Bar: 10 μm.
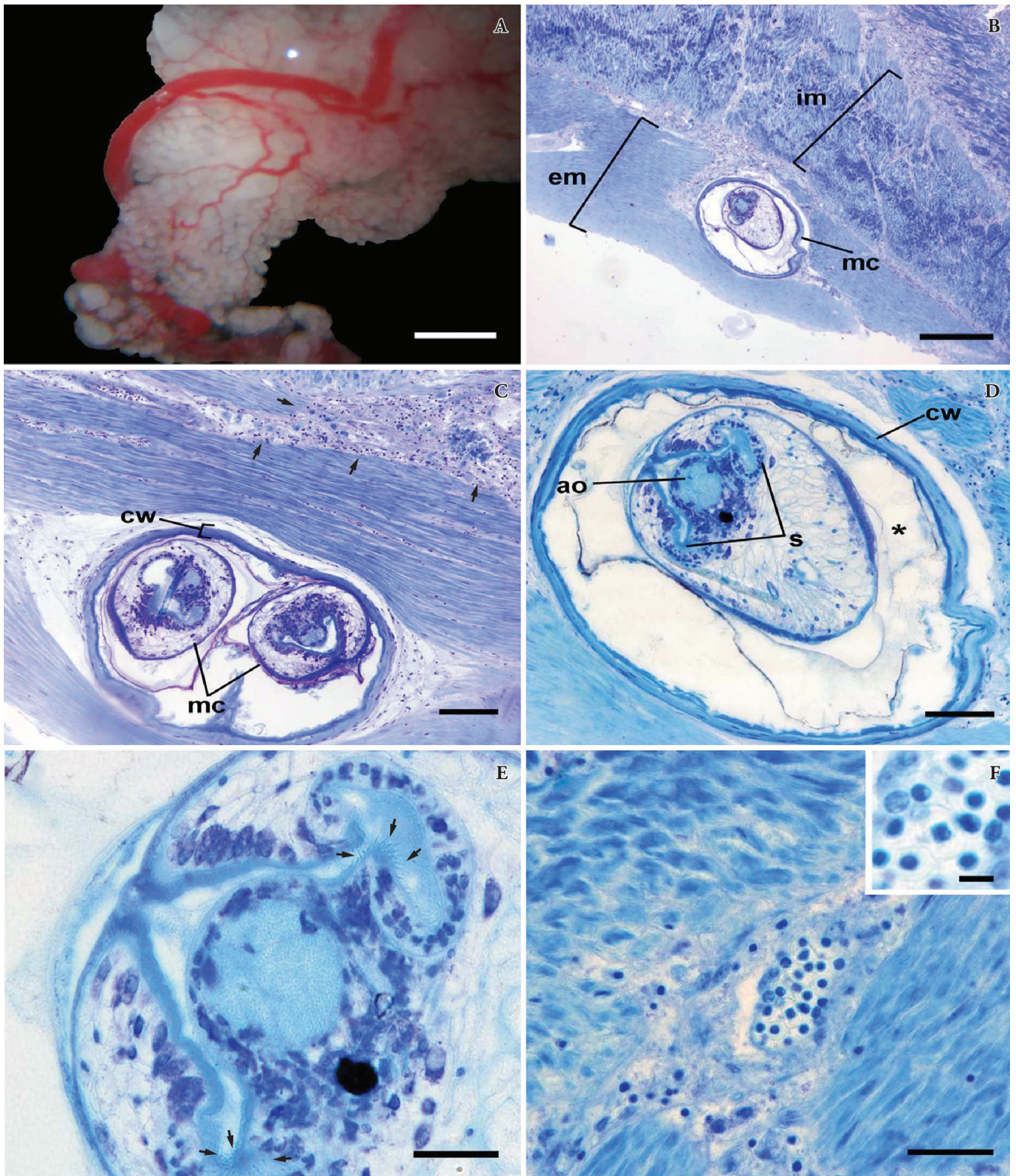
Figure 2. Anatomical view and histopathological aspects of metacestodes parasitising the mesenteries of *P. squamosissimus.* (Historesin-embedded, Toluidine blue 1%). **A)** General view of the connective tissue sacs with numerous rounded metacestode cysts. Bar: 1 cm. **B)** Histological aspects of a metacestode cyst **(mc)** infiltrating the external muscular layers **(em)** of the small intestine. **(im)** internal muscular layer. Bar: 100 μm. **C)** Detailed view of a cyst containing two metacestodes **(mc)**; the cyst wall and the inflammatory reaction, which is characterised by macrophage accumulation **(arrows)**, is shown. Bar: 50 μm. **D)** Histological features of a metacestode cyst. The cyst wall **(cw)**, the amorphous material between the cyst wall and the larva (*), the suckers **(s)** and the apical organ **(ao)** are shown. Bar: 20 μm. **E)** Detailed view of the suckers, showing the spine-like structures **(arrows)**. Bar: 20 μm. **F)** Detailed view of the macrophage agglomeration observed in the external muscular layer. Bar: 20 μm. Inset: hypertrophied cells. Bar: 2 μm.

The morphology of the larvae in histological sections show details of the elliptical larval body, with non invaginated/retracted scolex or primary lacuna, a simple apical organ, and suckers presenting spine-like structures (Figures 2D, E). An inflammatory granulomatous reaction was observed in the external muscular layer close to the cyst; however, the accumulated macrophages were hypertrophied, suggesting the beginning of cell degeneration (Figures 2C, F).

The acanthocephala was identified as *Neoechinorhynchus veropesoi*, recently described by Melo et al. (2013). In the sites of attachment of this worm, the structure of the intestine were damaged; the proboscis of the parasite penetrated the mucosal layer, causing lesions (Figure 3A), with hypertrophy of macrophages, and necrosis (Figures 3B, C). The tissue section close to insertion of the proboscis showed the proboscis-hook insertion point, with marked cellular injury, cell hypertrophy, and necrosis; some cells showed pyknotic or fragmented nuclei and karyolysis with no visible limitations of the cytoplasm.

Discussion

Host reactions to extra-intestinal parasites usually culminate in the formation of a cystic capsule that isolates the helminth from the host tissues (WOO, 1992; HUIZINGA; NADAKAVUKAREN, 1997). The initial response is characterised by a non-specific cellular inflammatory response against the parasite that results in granulomatous encapsulation and fibroblast deposition (WOO, 1992).

An external capsule surrounded each larva, and numerous larvae were present within sac-like structures formed from connective tissue. Such capsules were observed by Margolis (1970) in *Melanogrammus aeglefinus* parasitized by *Anisakis simplex* juveniles, by Miyazaki et al. (1988) in *Polyodon spathula* and by Dezfuli et al. (2007) in *Platichthys flesus*; however, this is the first report of this sac-like structure and the first report of such a high parasite burden in this hosts species.

In studies of salmonids from Chitose River, Urawa (1986) and Sugawara et al., (2004) reported that the mean intensity of infection increased during the years of the study, ranging between 9.5 and 30 parasites per fish. Subsequently, Urawa and Fujisaki (2006) observed a greater range of intensity of infection than was previously recorded (11-393); however, more extensive infections were observed in *P. squamosissimus* (1-452 parasites), and the authors did not report the presence of a sac-like structure.

Macrophage agglomerates (MAs) and amorphous material surrounding the external capsule in anisakid juveniles were also observed in our specimens. The MAs in fish are most likely a result of a chronic inflammation; such responses play an important role in the recruitment of other immune cells and the release of chemical factors responsible for the fibrous tissue that surrounds and isolates the parasite. However, Dezfuli et al. (2007) presented the first report of MAs very close to the juveniles, unlike those observed in *P. squamosissimus* from the Amazon.

An external capsule was also observed in the metacestodes occurring in *P. squamosissimus*; additionally, we observed an intense inflammatory reaction in the external muscular layer of the intestine (muscularis mucosa) close to the cyst wall. Few
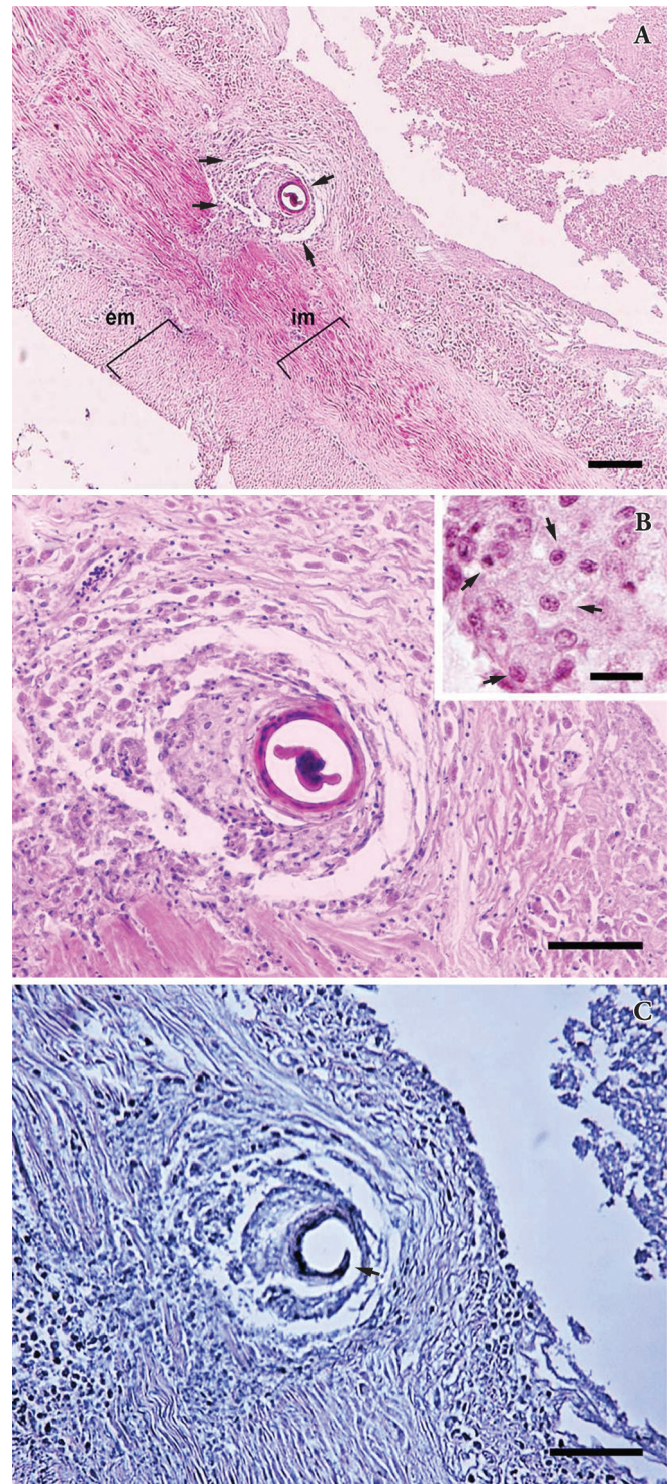
Figure 3. Histological aspects of small intestine tissues of *P. squamosissimus* parasitized by *Neoechinorhynchus veropesoi* (Paraffin embedded). **A)** General view of a parasitized intestine showing *N. veropesoi* **(arrow)** invading the mucosa and reaching the submucosal and internal muscular intestinal layers **(im)**; the inflammatory reaction is also shown (arrowhead). Note **em =** external muscular layer. (H&E). Bar: 100 μm. **B)** Detailed view of a parasite embedded in the intestine and surrounded by macrophages. (H&E). Bar: 50 μm. **C)** Detailed view of the parasite attachment region. Note the anterior proboscis hook **(arrow).** (TB). Bar: 50 μm.

studies have reported on the pathological alterations produced by Proteocephalidae larvae encysted in the tissues of fishes. As in *P. squamosissimus* from Amazonia, larval stages in the intestinal tissues of *Astronotus* sp. induced an intense haemorrhagic reaction (THATCHER, 1981). Similarly, Schäffer et al. (1992) described intense infections of Proteocephallidae larvae in *Pseudoplatystoma corruscans*, *P. fasciatus,* and *Astronotus ocellatus* characterised by numerous nodules in the peritoneum and inside the liver and spleen parenchyma containing encysted larvae and a hepatic inflammatory reaction. These histopathological aspects are similar to our findings in *P. squamosissimus*; however, we observed inflammation in the muscular wall of the intestine.

Infection with *N. veropesoi* caused deep and irreversible mechanical damage to the host tissues due to the attachment of the proboscis. This invasion affected the architecture of the intestinal tissues, leading to cellular changes, degeneration of the intestinal villi, haemorrhagic reactions, and proliferation of granular tissues that affect absorption. These pathological features were similar to those observe in *Piaractus mesopotamicus* by Ferraz de Lima et al. (1990) in *Catostomus columbianus* by Amin and Heckmann (1992) and in *Prochilodus lineatus* by Martins et al. (2001); however, Malta et al. (2001) did not observe such alterations in *Colossoma macropomum*. Thatcher (1981) observed that intestinal occlusion was common in individual fish infected with a large number of *Neoechinorhynchus* sp. (host *Colossoma macropomum*) and *Gorytocephalus elongorchis* (Neoechinorhynchidae; host *Plecostomus carinatus*), although only a weak inflammatory reaction was observed in the intestinal epithelium.

Fish that harbor acanthocephalans are usually asymptomatic, especially when the parasites are attached only to the epithelial mucosa. However, deeply embedded forms, such as those observed here, can cause formation of extensive granulomas and subsequent fibrosis, thereby diminishing the effectiveness of intestinal absorption and significantly affecting the host's growth, immune response, and susceptibility to infection by other pathogens. In some cases, massive infections can cause intestinal obstruction or perforation of the intestinal wall or the proboscis can protrude into the coelom or attach its hooks to a different organ, as previously observed by Thatcher (1981, 2006).

Some studies of host-parasite relationships have used histopathological features to evaluate the host response to different groups of helminths; however, the pathogenesis of parasites in *P. squamosissimus* in the eastern Amazonian lowlands of Brazil was described here for the first time.

Acknowledgements

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