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Short notes

Histopathological study of *Corydoras micracanthus* (Siluriformes: Callichthyidae) parasitized by the nematode *Procamallanus* (*Spirocamallanus*) *pintoi* (Camallanidae) from Lesser River, northwestern Argentina, South America

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ABSTRACT. The nematode parasites can cause mechanical injuries such as irritation or atrophy of tissue and occlusions of the digestive tract, blood vessels or other ducts in their hosts. Some species belonging to the family Camallanidae have been the focus of histopathological studies because they lacerate the host´s intestinal wall with their buccal capsule. In this study, we describe the histopathological effects produced by *Procamallanus* (*Spirocamallanus*) *pintoi* in the small fish *Corydoras micracanthus* from Lesser River, in the northwest of Argentina, South America. The samples were processed with routine histology techniques. At light microscopy, specimens of *P.* (*S.*) *pintoi* were observed occupying the lumen of the gut. The histopathological sections revealed damage to the host intestine with the total destruction of the mucosa and submucosa, observed mainly in the region which is surrounding the buccal capsule of parasites. Moreover, an inflammatory reaction was observed with lymphatic accumulations in the affected tissue. This kind of degenerative and necrotic changes in the mucosa could affect negatively the absorption function of the host intestine, which may have adverse effects of the health status of fish. The present study provides the firs data about the histopathological effects of *P.* (*S.*) *pintoi* in *C. micracanthus*.

Keywords: nematode parasite, histopathology, Callichthyidae, Neotropical Region

Introduction

Parasitic infections in fishes are greatly common, especially in wild populations from different aquatic environments where ecological requirements for intermediate hosts and parasite transmission are encountered [1]. The grade of pathogenicity of the different fish endoparasitic helminths vary from one species to other, and depend on different factors such as the intensity of infection, the organ or tissue affected and the presence of other concomitant infections [2]. The range of histopathological responses to these parasites can vary from encapsulation of the parasite by host cells to acute and chronic inflammation and necrosis [1]. In general, the intestinal parasites can cause occlusion of the gut or affect the structure of the intestinal epithelium through attachment or feeding [1].

Camallanidae Railliet and Henry, 1915 is a family of nematodes that has been widely reported in marine and freshwater fish of Australian, Ethiopian, Nearctic, Neotropical, Oriental and Palearctic Regions [3–5]. These parasites are considered a significant problem for fish that are

maintained in closed environs; due to larvae of these nematodes are found in zooplankton and can be introduced into pisciculture when live plankton is used as food for fish [6]. Despite there are many studies dealing the taxonomy, biology and geographical distribution of these nematode parasites, only a few have dealt about histopathological effects caused by the presence of these helminths in the digestive tract of their hosts [7–10]. These parasites can draw or lacerate the host´s intestinal wall with their buccal capsule, causing a localized inflammatory reaction at the attachment site and primary anemia due to blood loss [3,11].

Procamallanus (*Spirocamallanus*) *pintoi* (Kohn and Fernandes, 1988) is a parasite widely recorded in freshwater fish belonging to the family Callichthyidae from the Neotropical Region [12–18]. Despite several authors studied the morphology of this species, its pathogenicity is still unknown. Therefore, the aim of this study is to describe the histopathological effects caused by the infection of *P.* (*S.*) *pintoi* in *Corydoras micracanthus* Regan, 1912 (Callichthyidae) from Lesser river (Bermejo River basin), northwestern Argentina.

Materials and Methods

Nineteen specimens of *Corydoras micracanthus* (standard length 16.13–29.50 mm; weight 0.10–0.80 g) were collected from Lesser River (24°39'43.56"S; 65°28'49.079"W) (Bermejo River basin) with the aid of gill nets during February–May 2017. Fish were kept alive in small water tanks and brought to laboratory prior to helminthological examinations. At necropsy, the digestive tracts were removed and the intestines were opened longitudinally and examined under a stereomicroscope for helminth infection. Histopathology was studied by comparing tissues from four specimens of *C. micracanthus* infected by one specimen of *Procamallanus* (*Spirocamallanus*) *pintoi* to tissues, respectively, from one uninfected fish, this latter using as a control.

Pieces of intestine, measuring up to 1×6 mm, were excised and fixed in 10% formalin for 24 h and then transferred to 70% ethanol for storage. These pieces were dehydrated through a graded series of ethanol and prepared for paraffin embedding. Cut sections (5-6 µm thick) were stained with Hematoxylin–Eosin (HE). Photomicrographs were taken with a Leica (DM 280) and Olympus microscopic cameras. Infection parameters (prevalence and mean intensity) were estimated based on Bush et al. [19].

Results and Discussion

Thirteen of nineteen fish examined (prevalence = 68%; mean intensity (range) =1.2 (1–2) were parasitized by *P.* (*S.*) *pintoi*. Macroscopically, the examined specimens did not present alterations or anomalies. The uninfected specimen of *C. micracanthus* showed an intestinal wall composed by epithelial mucosa, submucosa, circular muscle layer and serosa (Figs. 1A, B).

In the infected fish, some specimens have nematodes attached to the intestinal wall by their buccal capsule (Fig. 1F); and in others, the nematodes were observed moving freely in the lumen of the gut. The specimens of *P.* (*S.*) *pintoi* were usually found in the anterior part of the intestine (Figs. 1C–E). The intestinal cross-sections revealed the total destruction of the mucosa and submucosa, mainly in the region which is surrounding the buccal capsule of the parasites (Fig. 1F). These mechanic damages, mainly the mucosal destruction, modified the structure of the villi (Fig. 1D). Some ulcerations of the intestinal epithelium were also observed with the detachment of the epithelial cells into the intestinal lumen (Figs. 1C, D). Moreover, an inflammatory process was observed with the presence of lymphatic accumulation in the affected tissues (Fig. 1D). In addition, an increment in the number of mucosa cell (globet cell) was observed. No other histopathological damage was detected at circular muscle layer and serosa.

The presence of *P.* (*S.*) *pintoi* affects the intestinal mucosa and submucosa, due to the mechanic-traumatic damage provoked mainly by the attachment of the nematode to the intestinal wall through its buccal capsule. Similar observations were described for specimens of *Camallanus oxycephalus* Ward and Magath, 1916 in *Lepomis cyanellus* Rafinesque, 1819 (Centrarchidae), which penetrated the mucosa tissues and reached the stratum compactum of submucosa [7]. However, in some cases, *C. oxycephalus* also penetrated the muscular layer [7], different from the reported in the present study. Teran et al. [8] reported similar findings for *P.* (*S.*) *hilarii* in *Astyanax fasciatus* (Cuvier, 1819) and *A. abramis* (Jenyns, 1842) (Characidae); these authors observed the destruction of the mucosa, whereas the submucosa was not damaged. Gaines et al. [10] studied the histopathological changes in *Arapaima gigas* (Schinz, 1822)

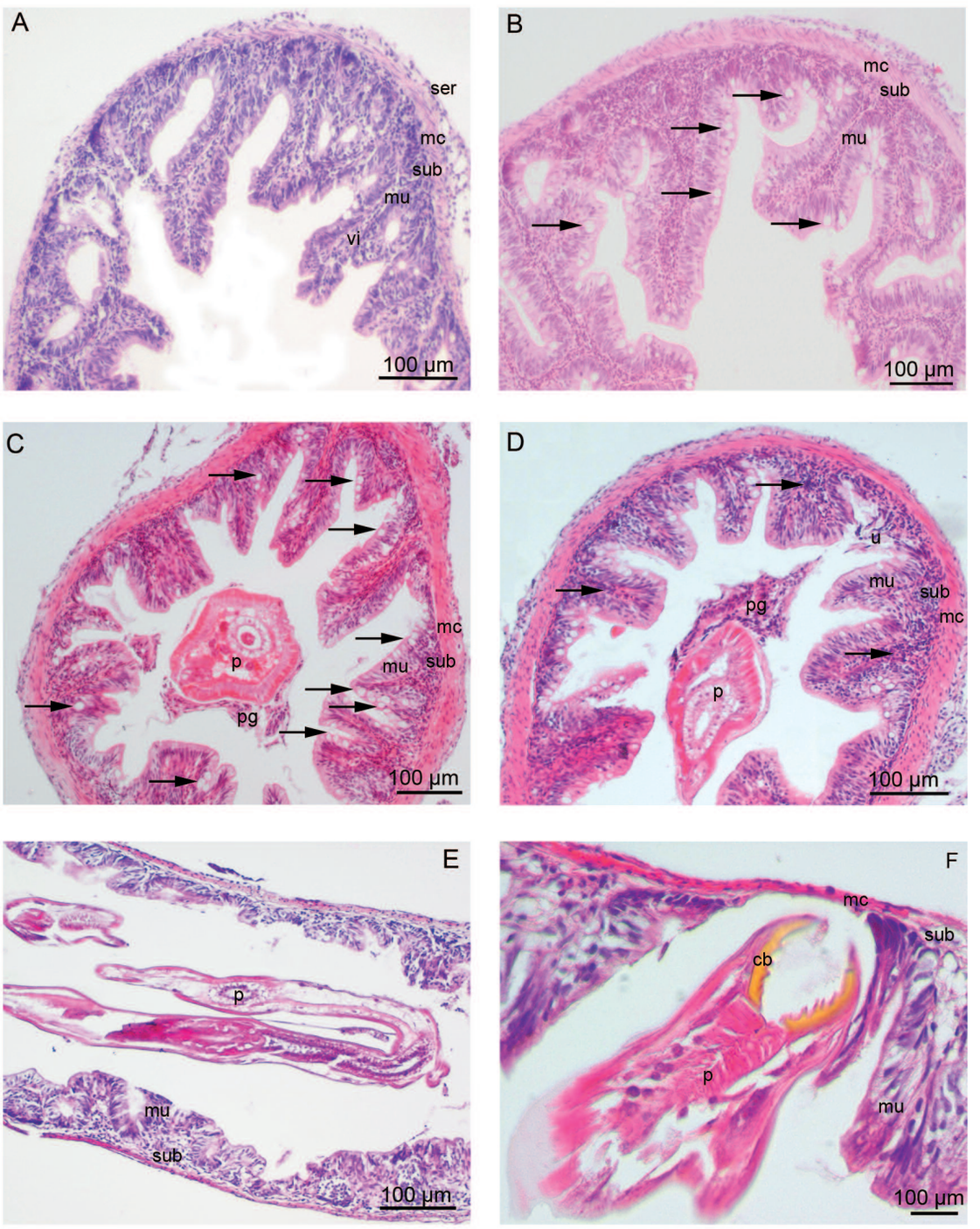

Fig. 1. Histological sections of *Corydoras micracanthus* intestine infected with *Procamallanus* (*Spirocamallanus*) *pintoi.* (A) Cross section, normal structure of intestinal epithelium (stain HE). (B) Cross section, detail of the intestinal mucosa with goblet cells (black arrows) (stain HE). (C) Cross section, the nematode occupies partially the intestinal lumen, detachment of epithelial cells and intestinal mucosa with globet cells (black arrows) (stain HE). (D) Cross section, ulcerations and detachment of epithelial cells in the intestinal lumen, and presence of lymphatic accumulations in the lamina propia of the mucosa (black arrows) (stain HE). (E) Longitudinal section, with the parasite partially obstructing the intestinal lumen (stain HE). (F) Longitudinal section, with parasite attached to intestinal wall through the buccal capsule, with total destruction of mucosa and submucosa (stain HE).
Abbreviations: m, mucosa; sub, submucosa; mc, muscularis; ser, serosa; p, parasite; pg, detachment of epithelial cells; u, ulceration; cb, buccal capsule; vi, villi.

(Arapaimidae) infected by *Procamallanus* (*S.*) *inopinatus* Travassos, Artigas and Pereira, 1928, and reported focal areas of necrosis, desquamation and hemorrhage. The level of damage seems to depend on the type of attachment to the host; in this case *P.* (*S.*) *inopinatus* has two median teeth, different of the case of *P.* (*S.*) *pintoi*, which not present these structures in the buccal capsule; which could influence in the level of damage. On the other hand, Menezes et al. [9] studied the pathology induced by *Camallanus cotti* in *Betta splendens* Regan, 1910 (Osphronemidae) and *Poecilia reticulate* Peters, 1859 (Poeciliidae). These authors observed hemorrhage, congestion, edema, a few glandular elements, and extensive erosion areas in the rectum mucosa, with a marked thickening of the wall and absence of inflammatory infiltrate; different to that type of lesions observed in the present study.

The histopathological changes localized at the region of parasite attachment, affect mainly the structure of villi (Fig. 1D–F). Therefore, the destruction of the villi, along with other degenerative and necrotic changes suffered in the mucosa would affect negatively the absorption function of the host intestine [20].

According to Thatcher [11], some camallanids ingest blood and could cause anemia in their hosts. Ruhela et al. [21] described a macrocytic anemia in *Clarias batrachus* (Linnaeus, 1758) (Clariidae) infected by *Procamallanus* sp.; they reported a marked reduction in the total erythrocyte count and in the haemoglobin content. In the present study, it was not possible to confirm if these nematodes caused anemia in their hosts. Therefore, further hematological studies will be necessary to verify if anemia is caused by the loss of blood or there are other factors that could contribute to these process.

The presence of *P.* (*S.*) *pintoi* in the intestine of *C. micracanthus* caused mainly reactions of the inflammatory kind in the affected tissues, with the presence of lymphatic accumulations; this observation coincides with that reported by Moravec [3], who described an inflammatory process in the attachment site of the parasite. Moreover, the presence of mucosa cell (globet cell) could be related to the secretion of mucus that covers the surface of the intestine [5]. According to Meguid et al. [22] the accumulation of mucus generates a physical barrier for the establishment of endohelminths, or also mucus could contain antibodies against parasites, which could alter the helminth community structure.

The pathological effects of helminths on their hosts and the immune response of fish to infection are two important aspects of the host-parasite interaction [23]. Although some information has been reported, little is known about the pathological effects caused by nematodes belonging to Camallanidae [3]. Therefore, the knowledge about histopathological effects caused by different species of Camallanidae should be studied, mainly taking into account that these nematodes present variations in their buccal capsule morphology [3], which could change the level of damage caused in the intestinal epithelium. Moreover, this is a first data about histopathological effects of *P.* (*S.*) *pintoi* in *C. micracanthus*.

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