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Intestinal endoparasitism in *Corydoras multiradiatus* (Siluriformes: Callichthyidae) in Iquitos, Loreto-Peru

Endoparasitismo intestinal em Corydoras multiradiatus (Siluriformes: Callichthyidae) em Iquitos, Loreto-Peru

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ABSTRACT

Corydoras multiradiatus (Orcés V.1960), commonly called “hog-nosed brochis” and popularly known in Peru as “coridora jumbo” is a species of commercial importance among the Amazonian ornamental fish. It is known that the integrity of the health of aquatic organisms positively increases the production and trade of quality products, however, the opposite negatively affects financial losses and production costs. Among the possible diseases, parasitism stands out, and there is still a lack of information, mainly about metazoans, parasitic species that infect *C. multiradiatus*. Therefore, this study aimed to identify the species of endoparasite metazoans in *C. multiradiatus* and evaluate the histological lesions present to provide information to ornamental fish producers about these pathologies. Thirty specimens of *C. multiradiatus* were collected by fishermen in the municipality of “Belén” in Iquitos, Peru. The results revealed the presence of *Tylodelphys* sp. and nematode larvae in the intestinal wall and *Procamallanus (Spirocamallanus) pintoi* (Kohn & Fernandes 1988) present in the intestine. The histopathological analysis of the intestine showed several lesions: necrosis; cell desquamation; infiltration of lymphocytes in the mucosa; transepithelial infiltration of lymphocytes; enterocyte hyperplasia; goblet cell hyperplasia and enterocyte desquamation. The study revealed the presence of endoparasites in *C. multiradiatus* collected in Iquitos, Loreto-Peru, and a moderate degree of damage caused by histological lesions in the intestine of infected fish. The results of this work will serve the fishermen, collectors and aquarists of the city of Iquitos, who, knowing the endoparasites present in *C. multiradiatus* and the damage they can cause to the fish, will be able to adopt prophylactic measures in order to guarantee the good condition of the fish for its consequent commercialization at a local, national and international level.

KEYWORDS: endoparasites; Peruvian Amazonia; Nematoda; *Procamallanus (Spirocamallanus) pintoi*; *Tylodelphys* sp.

RESUMO

Corydoras multiradiatus (Orcés V. 1960), comumente chamada no Peru como “coridora jumbo” é uma espécie de importância comercial entre os peixes ornamentais amazônicos. Sabe-se que a integridade da saúde dos organismos aquáticos aumenta positivamente a produção e comercialização de produtos de qualidade, porém, o contrário afeta negativamente as perdas financeiras e os custos de produção. Dentre as possíveis doenças, destaca-se o parasitismo, havendo ainda carência de informações, principalmente sobre metazoários, espécies parasitárias que infectam *C. multiradiatus*. Portanto, este estudo teve como objetivo identificar as espécies de metazoários endoparasitas em *C. multiradiatus* e avaliar as lesões histológicas presentes para fornecer informações aos produtores de peixes ornamentais sobre essas patologias. Trinta exemplares de *C. multiradiatus* foram coletados por pescadores no município de “Belén” em Iquitos, Peru. Os resultados revelaram a presença de *Tylodelphys* sp. e larvas de nematóides na parede intestinal e *Procamallanus (Spirocamallanus) pintoi* (Kohn & Fernandes 1988) presentes no intestino. A análise histopatológica do intestino mostrou diversas lesões: necrose; descamação celular; infiltração de linfócitos na mucosa; infiltração transepitelial de linfócitos; hiperplasia de enterócitos; hiperplasia de células caliciformes e descamação de enterócitos. O estudo revelou a presença de endoparasitas em *C. multiradiatus* coletados em Iquitos, Loreto-Peru, e um grau moderado de dano

causado por lesões histológicas no intestino de peixes infectados. Os resultados deste trabalho servirão aos pescadores, coletores e aquaristas da cidade de Iquitos, que conhecendo os endoparasitas presentes em *C. multiradiatus* e os danos que podem causar aos peixes, poderão adotar medidas profiláticas para garantir o bom estado dos peixes para a sua consequente comercialização a nível local, nacional e internacional.

PALAVRAS-CHAVE: endoparasitas; Amazônia peruana; Nematoda; *Procamallanus (Spirocamallanus) pintoi*; *Tylodelphys* sp.

INTRODUCTION

The ornamental fish trade has grown significantly in recent decades, with an estimated value of US$ 15 billion and more than 2 billion ornamental fish traded (SATAM et al. 2018). The main producers and suppliers in the world are developing countries such as Peru, Brazil, and Colombia, which in turn supply more than 60% of the ornamental fish imported worldwide (RAJA et al. 2019). In Peru, Loreto concentrates 94.9% of the national export of ornamental fish, making it one of the largest exporters (PRODUCE 2018). PRODUCE also indicates that exports of ornamental fish have maintained a record of constant currencies and above 2.5 million USD-FOB/year.

Among the ornamental fish with the greatest demand in international markets, species of Siluriformes belonging to the Callichthyidae stand out (MOREY 2018). These fish are susceptible to different infectious agents, being parasites the main organisms that cause high mortalities, leading to significant economic losses in the ornamental fish trade market (THILAKARATNE et al. 2003).

Among the Amazonian callichthyids, *Corydoras multiradiatus* (Orcés V. 1960), commonly named “hog-nosed brochis” and popularly known in Peru as “coridora jumbo”, is an omnivorous species that can measure up to 9 cm in length and inhabits the Mamoré river basin in Bolivia (CHERNOFF et al. 2000), the Amazon basin in Peru (ORTEGA et al. 2012) and the Morona de Santiago - Santiago basin and the Napo river in Ecuador (Orcés, 1960). This species is quite popular and highly demanded in the Chinese ornamental fish market (XU et al. 2020), being exported by several companies to different continents (GARCÍA-DÁVILA et al. 2021).

Regarding reports of endoparasites in callichthyids, MOREY (2018) reported *Procamallanus (Spirocamallanus) pintoi* (Kohn & Fernandes, 1988) in species of *Corydoras*, and registering for the first time the presence of this nematode in *C. multiradiatus*, (MOREY & FLORINDEZ 2018). Currently, studies on metazoan parasites that infect *C. multiradiatus* are very scarce, so this work aimed to identify the endoparasitic metazoan species present in this species and the evaluation of their histological lesions.

MATERIAL AND METHODS

Collection of fish specimens

Thirty specimens of *C. multiradiatus* were collected between May and June 2021 from fishermen of the Municipality of “Belén” in Iquitos, Peru. According to the fishermen, the specimens were fished on “Sapo Playa” island close to the Amazon River (3°27’1.02’’S 72°48’18.36’’W). Fish were transported in plastic bags with water to the “Laboratorio de Parasitologia y Sanidad Acuícola” from “Instituto de Investigaciones de la Amazonía Peruana” (IIAP) in Loreto – Peru.

Statement on ethical approval from an ethics committee and license for working with fish species were followed according to the following resolutions: Resolution No132-2014-GRL-DIREPRO; Resolution No21-2016 GRL-DIREPRO; and PTH-068-16-PEC-SANIPES.

Parasites processing

In the laboratory, fish were euthanized with the use of Eugenol (0.4 ml $^{L-1}$) and then processed by making a cut in the abdominal region, extracting internal organs. Organs were placed individually in Petri dishes with distilled water and observed under a stereoscope Leica EZ4 with magnification between 8x to 35x. For a morphological study of the parasites, some nematodes found were placed in Petri dishes with heated 70% ethanol to induce the parasites' death with the body extended and then preserved in 70% ethanol for posterior analyses. For the identification of nematodes, they were placed into glass slides with Lactic Acid, which is used for the clarification of parasite tissue and consequent visualization of external structures and internal organs (MOREY 2019).

Trematodes were removed from their cysts using stylets and then fixed in FAA solution (10% Formalin, 5% Glacial Acetic Acid, 50% of 70% ethanol, 35% DI water). After 24 hours, trematodes were transferred to 70% ethanol for preservation. For identification, these parasites were stained in Langeron’s alcoholic carmine (MOREY 2019).

For taxonomic identification of the parasites, slides with nematodes and/or trematodes were observed under an optic microscope Leica DM750. Photomicrographs were taken with a digital camera Leica ICC50 and data was processed with the software LAS EZ. The parasites were identified based on the morphology of external structures and internal organs according to BLASCO-COSTA et al. (2017), MOREY (2018), MOREY & FLORINDEZ (2018).

Histological analyses

Samples of the intestine with the parasites were preserved in formalin 10% with Calcium Bicarbonate. Subsequently, they were processed according to routine techniques using the conventional histological protocol for fixed tissues, they were dehydrated in concentrations of ethyl alcohol, soaked in paraffin, clarified, and sectioned in 5 µm. thickness and stained with hematoxylin-eosin (HE). The lesions were classified according to the degree of affection due to the extension of the sample, presented on a scale of degrees (SANTOS et al. 2019).

Scarce: Presence of the lesion, up to 25% of the surface of the entire sample

Mild: Presence of the lesion in > 25% but < 50% of the surface of the entire sample.

Moderate: Presence of the lesion in > 50% but < 75% of the surface of the entire sample

Severe: Presence of the lesion in > 75% and even reaching 100% of the surface of the entire sample.

RESULTS

The analyses of internal organs of *C. multiradiatus* revealed the presence of 700 metacercariae of *Tylodelphys* sp. within a membrane containing two or multiple numbers of individuals (Figs. 1A-C) and 81 nematode larvae on the intestinal wall. Additionally, 30 individuals of *Procamallanus (Spirocamallanus) pintoi* were found inside the intestine.

Tylodelphys sp. presented a piriform body, which consists of an oval-shaped fore body and bluntly pointed hind body; pseudo-suckers indistinct; oval pharynx; intestinal caeca extending up to the posterior region of the body and excretory pore present at the terminal portion of the body; vitellaria distributed throughout the body (Fig. 1D).

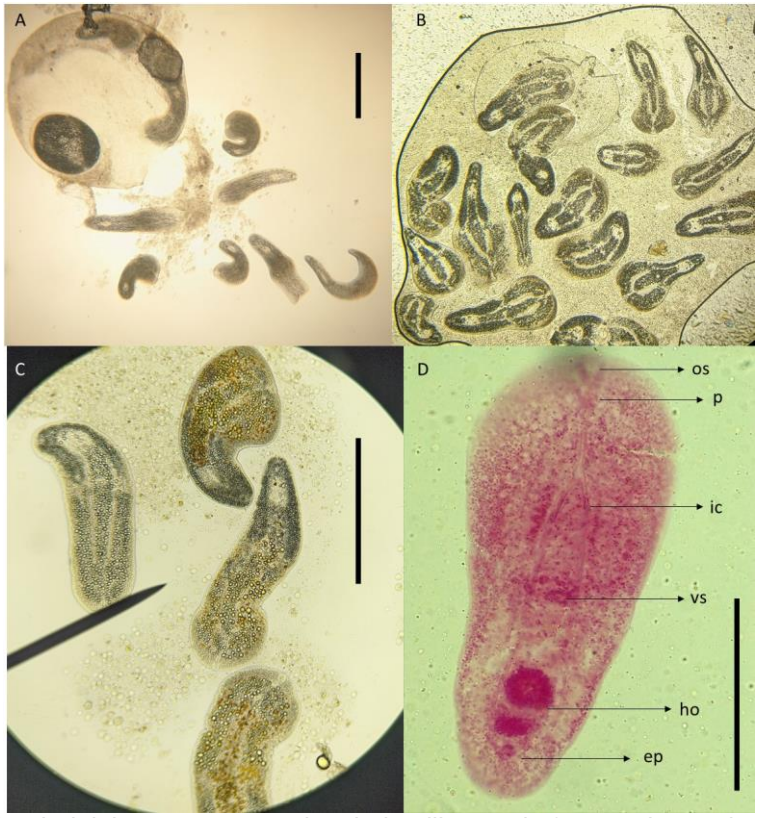

Figure 1. Metacercariae of *Tylodelphys* sp. A. Parasites being liberated of a membrane (magnification 4x). B, C. Multiple parasites inside a membrane (magnification 4x). D. Parasite stained with Carmin (magnification 10x). os = oral sucker, p = pharynx, ic = intestinal caecum, vs = ventral sucker, ho = holdfast organ, ep = excretor pore. Scale bar: 500 µm.

Procamallanus (Spirocamallanus) pintoi presented some important characteristics: body with reddish coloration (Fig. 2), chitinized buccal capsule with helical thickenings (Fig. 3A-B). Esophagus is formed of two parts: muscular and glandular (Fig. 3A). Tapered tail in male specimens, presenting short and sclerotized arrow-shaped spicules, caudal papillae (four preanal pairs, and two post-anal pairs) (Fig.3C). Tail of female specimens with a conical and finger-like termination (Fig.3D).

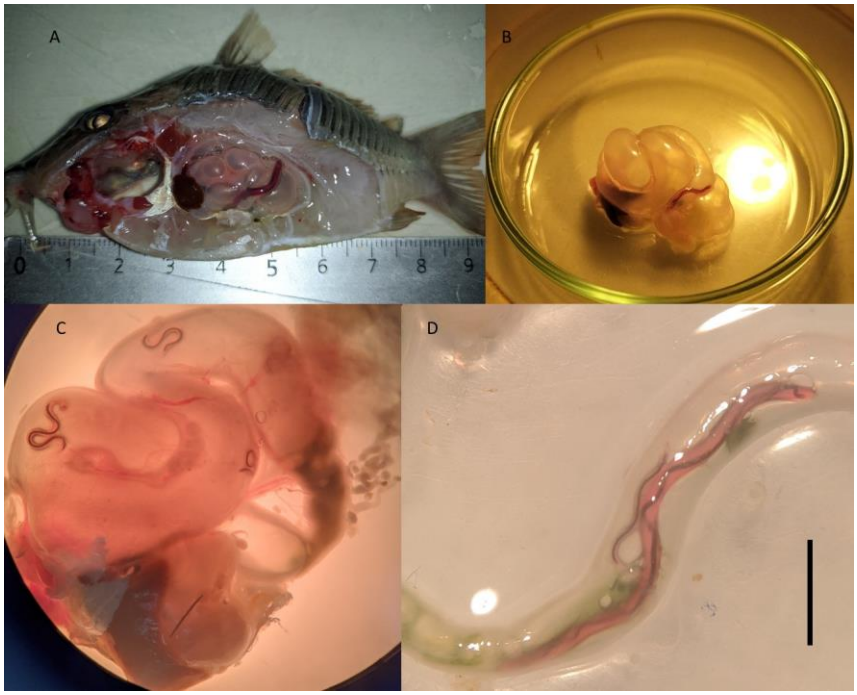

Figure 2. A. Specimen of *Corydoras multiradiatus* parasitized by *Procamallanus (Spirocamallanus) pintoi.* B. Intestine of *B. multiradiatus* with a *P. (S.) pintoi* specimen. C. Multiple specimens of *P. (S.) pintoi* inside the intestine of *C. multiradiatus*. D. Visualization of male and female specimens of *P. (S.) pintoi* inside the intestine of *C. multiradiatus*. Scale bar = 10 mm.

Figure 3. Photomicrography of *Procamallanus (Spirocamallanus) pintoi*. A. Ventral view of the male specimen (magnification 4x). B. Anterior part showing the buccal capsule (magnification 20x). C. Posterior part of the male specimen (magnification 10x). D. Tail of a female specimen (magnification 20x). bc = buccal capsule, me = muscular esophagus, ge = glandular esophagus, es = spicules, prap = pre-anal papillae, psap = post-anal papillae. Scale bar: A = 0.2 mm, B = 0.05 mm, C, D = 0.1 mm

Parasitological indices of the endoparasites found on *C. multiradiatus* revealed high prevalence for *Tylodelphys* sp. with values higher than 90%, followed by larvae of Nematoda with more than 60% and finally *P. (S.) pintoi* with 30%. The same tendency was observed for values of mean intensity and mean abundance (Table 1).

Table 1. Parasitological indices of the endoparasites found in Corydoras multiradiatus

Parasites	Number of infected fish	P%	mI	mA
Metacercariae of *Tylodelphys* sp.	28	93.3	25	23.3
Larvae of Nematoda	19	63.3	4.26	2.7
Procamallanus (Spirocamallanus) pintoi	9	30	3.33	1

Legend: P% = prevalence, mI = mean intensity of infection, mA = mean abundance of infection.

The histopathological analysis of the intestine revealed necrosis in 46.6% (14/30) of fish; cell desquamation in 86.6% (26/30) (Fig. 4A); lymphocyte infiltrates at the mucosal level (Fig. 4B); transepithelial lymphocyte infiltration in 83.3% (25/30) (Fig. 4C); enterocyte hyperplasia in 60% (18/30) (Fig. 4D); goblet cell hyperplasia in 40% (12/30) (Fig. 4E), desquamation of enterocytes (Fig. 4F) (Table 2).

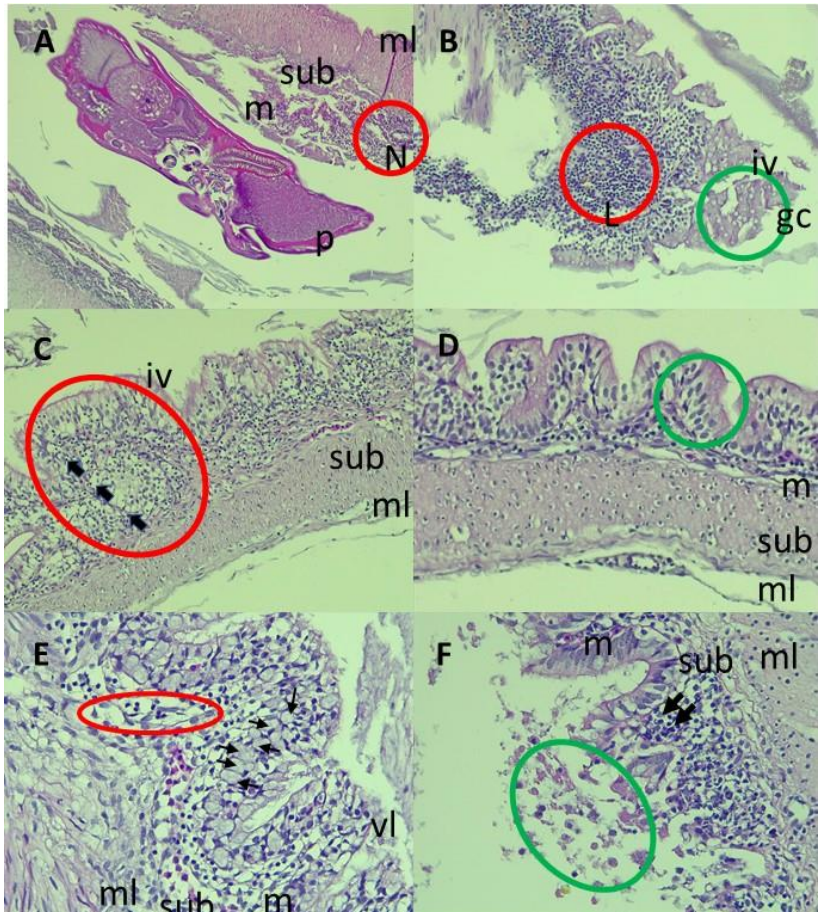

Figure 4. Histological section of the intestine of *C. multiradiatus* infected with *Procamallanus (Spirocamallanus) pintoi*. A. An area of necrosis is observed with an increase in lymphocytes at the level of the intestinal mucosa and submucosa (red circle) (magnification 10x). B. Lymphocyte infiltrate at the mucosal level (red circle) and goblet cell hyperplasia in the region of the intestinal villi (green circle). Acute diffuse lymphocytic enteritis (magnification 10x). C. Transepithelial infiltration of lymphocytes (red circle) in the lamina propria of the intestinal mucosa of *C. multiradiatus*. The migration of lymphocytes from the submucosal layer to the apical zone of the villi can be seen (black arrows) (magnification 10x). D. Slight hyperplasia of enterocytes (green circle) of the intestinal villi (magnification 10x). E. Intestinal villi of *C. multiradiatus* with goblet cell hyperplasia (black arrow) and transepithelial infiltration of lymphocytes (red circle) (magnification 10x). F. Desquamation of enterocytes (green circle) and transepithelial infiltration of lymphocytes (black arrows). m = mucosa, sub = submucosa, ml = muscular layer, p = parasite, N = necrosis. L = linfocytes, iv = intestinal villi, cc = globet cells (magnification 10x).

Table 2. Degree of affection of the lesions presented in *Corydoras multiradiatus* according to histological analyses conducted.

	Degree of affection						
Type of lesions	**Normal**	**Scarce (I)**	**Mild (II)**	**Moderate (III)**	**Severe (IV)**	**Relation**	**Alteration (%)**
INFLAMMATORY							
Lymphocyte transepithelial infiltration	5	4	10	10	1	25/30	83.3
lymphocyte infiltration	10	7	8	5	0	20/30	66.6
Inflammatory cell infiltration	15	8	5	2	0	15/30	50
ADAPTATION							
goblet cell hyperplasia	8	6	7	5	4	22/30	73.3
enterocyte hyperplasia	4	8	12	5	1	26/30	86.6
DEGENERATIVE							
necrosis	9	6	6	7	2	21/30	70
desquamation of epithelial cells	4	11	5	8	2	26/30	86.6

Fish specimens presented different degrees of histopathological lesions, being the moderate degree the most predominant. In addition, *P. (S.) pintoi* was found inside the intestines of some *C. multiradiatus* (Fig. 5A), destroying the intestinal mucosa and submucosa and goblet cell hyperplasia (Fig. 5B, 5C).

Figure 5. A. Procamallanus (Spirocamallanus) pintoi inside the intestine of C. multiradiatus (magnification 4x). B. Anterior part of the nematode adhered to the intestinal wall destroying the intestinal mucosa and submucosa (red circle). Goblet cell hyperplasia (asterisk) (magnification 40x). C. The nematode is enveloped in a dense basophilic mucus (black arrow) surrounded by eosinophilic granule cells, destroying the intestinal mucosa (green square). m = mucosa, sub = submucosa, ml = muscular layer, P = parasite (magnification 10x).

The histological analyses also revealed granuloma in the intestinal serosa, with the presence of lymphocytes and macrophages (Fig. 6A, 6B), granuloma inserted in the muscular layer of the intestine of *C. multiradiatus* (Fig. 6C), granuloma with a parasite developed in the serosa of the intestine (Fig. 6D).

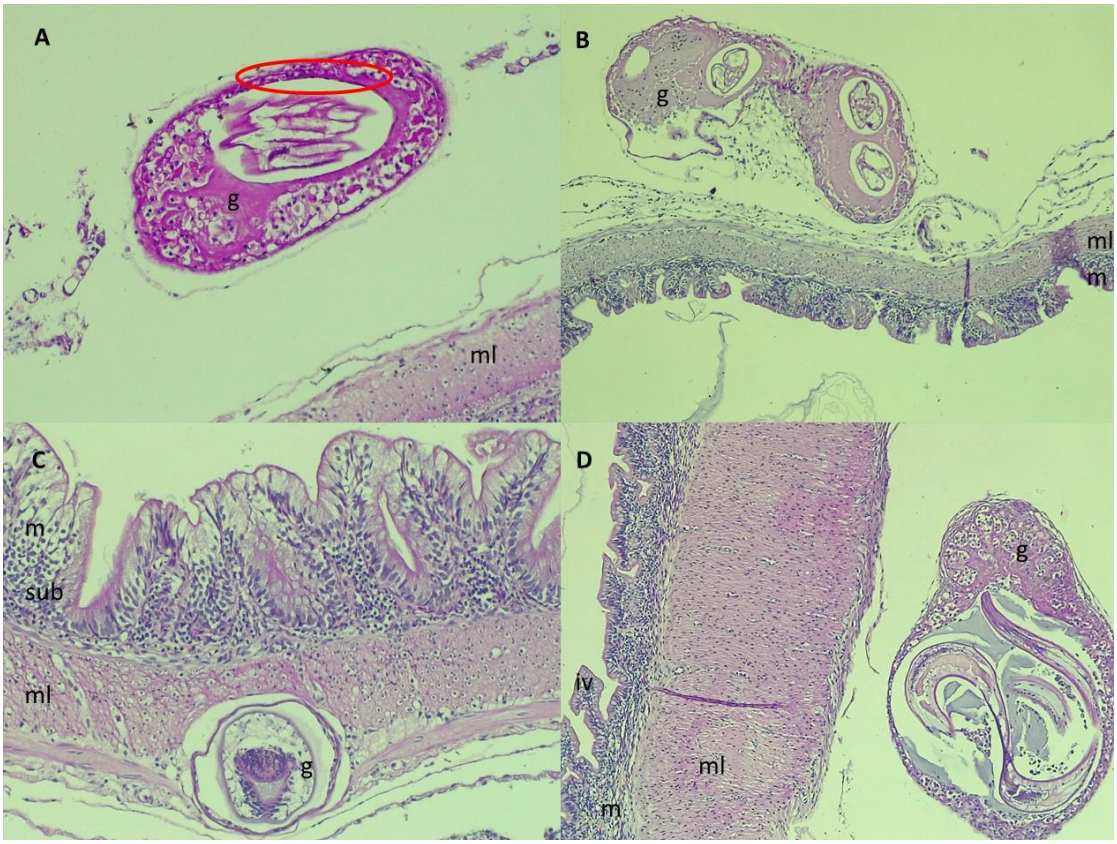

Figure 6. A. Parasitic granuloma in the intestinal serosa of *Corydoras multiradiatus*, in its periphery fibroblasts lymphocytes, and macrophages can be observed (red circle) (magnification 20x). B. Parasitic granuloma in the intestinal serosa of *C. multiradiatus* is seen surrounded by macrophages, eosinophils, and lymphocytes (magnification 10x). C. Granuloma inserted in the muscular layer of the intestine of *C. multiradiatus*, fibroblasts can be observed in the outer layer of the granule, inside lymphocytes, and eosinophils are present (magnification 20x). D. Granuloma with a parasite developed in the serosa of the intestine, inside, lymphocytes, eosinophils, and macrophages can be observed. m = mucosa, sub = submucosa, ml = muscular layer, iv = intestinal villi, g = granuloma (magnification 10x).

DISCUSSION

Metacercariae of *Tylodelphys* sp. was reported in the vitreous humor and cranial cavity of different fish species (MUZZALL & KILROY 2007, OTACHI et al. 2015, BLASCO-COSTA et al. 2017). In Brazil, these metacercariae were reported in the swim bladder of *Potamorhina altamazonica* (Cope, 1878), *P. latior* (Spix & Agassiz, 1829), *P. pristigaster* (Steindachner, 1876) and *Chalceus erythrurus* (Cope, 1870) (VITAL et al. 2018). In the present study, *Tylodelphys* sp. was recorded in the intestine of infected fish. Our results are in concordance with the study carried out in *C. splendens* (Castelnau 1855) from the Tapiche River, in Loreto-Peru (MOREY 2018). Additionally, *Tylodelphys* sp. is recorded for the second time in *C. multiradiatus*, expanding the number of records of this parasite in the Peruvian Amazonia.

The present study recorded a prevalence of 93% and mean intensity of infection of 25 *Tylodelphys* sp. These values were higher than the ones obtained by MOREY (2018), who recorded a prevalence of 40% and a mean intensity of 20. Parasitological indices of *Tylodelphys* sp. infecting *P. latior, P. altamazonica* and *P. pristigaster* recorded values of 93%, 91%, and 93% of prevalence, and mean intensities of infection of 30, 29, and 29 respectively. These differences may be because the fish were sampled in different places and times of the year. MOREY (2019) commented that precipitation and turbidity can influence parasite infection also commenting that temperature greatly influences the life cycle of metacercaria.

Trematode metacercariae usually encyst in rocks, vegetation, or on the surface of the first intermediate host: aquatic snails, to later be ingested by the second intermediate host, fish species

(GOATER et al. 2014). In the present study, the presence of *Tylodelphys* sp. in *C. multiradiatus* indicates that this fish species acts as a second intermediate host of this parasite.

MOREY & FLORINDEZ (2018) recorded for the first time *P. (S.) pintoi* parasitizing *C. multiradiatus* with values in the parasitological indices similar to the ones recorded in the present study. Different values of infection by *P. (S.) pintoi* were recorded in species of *Corydoras*, such as *C. blochi* Nijssen, 1971*, C. paleatus* (Jenys 1842)*, C. micracanthus* Regan, 1912*, C. leopardus* Myers, 1933*, C. reticulatus* Fraser-Brunner, 1939*, C. virginiae* Burgess, 1993*, C. metae* Eigenmann, 1914 (PIÑEROS et al. 2017, AILÁN-CHOKE et al. 2018, MOREY & FLORINDEZ 2018). The differences registered in the indices may be due to inherent characteristics of the species, presenting greater resistance to infections or to the different food items that they may consume, directly influencing the abundance of these nematodes.

In the intestinal section close to the buccal capsule of *P. (S.) pintoi* was observed desquamation of epithelial cells as destruction of the mucosa. These results were similar to the ones of AILÁN-CHOKE et al. (2019) who found epithelial necrosis, and destruction of the total mucosa and submucosa in the intestine of *C. micracanthus* infected by *P. (S.) pintoi*. These same lesions were also observed in a study carried out on *Parachanna obscura* (Günther 1861) infected with *P. (S.) aspiralis* Baylis, 1923 (AKINSANYA et al. 2016). Enterocyte hyperplasia and necrosis may be related to the damage caused by the adhesion of the nematode to the intestinal wall through the buccal capsule (AILÁN - CHOKE et al. 2019), the level of this damage will depend on the type of parasite, for example, in the present work and that of AILÁN - CHOKE et al. (2019) the damage only affected the mucosa layer and the submucosa, unlike the work of AKINSANYA et al. (2016), which found thickening in the muscle layer.

The increase in goblet cells and their hyperplasia may be related to the increased secretion of the mucus that covers the surface of the intestine. This acts as a physical barrier and contains different components (immunoglobulins, lectins, lysozyme, etc.) that are involved in the host-parasite interaction (MOKHTAR 2017, AILÁN - CHOKE et al. 2019). The increase in lymphocytes may be due to the immune response induced by the fish in the presence of flukes and nematodes since they have an antigenic structure that can provoke these responses (MOREY 2018). In the present investigation lesions such us: transepithelial infiltration of lymphocytes at 83.30% (25/30), hyperplasia of goblet cells at 73.30% (22/30), and an increase in lymphocytes at 66.60% (20/30) were recorded.

In the present study, several granulomas were observed, in some of them a nematode larva or a trematode could be observed inside. Within the granulomas, eosinophils, neutrophils, and monocytes were observed, leading to the conclusion that the host tries to encapsulate and isolate the parasites as a defense mechanism (AILÁN -CHOKE et al. 2019). Some *P. (S.) pintoi* were found attached to the lumen of the intestine, others moving freely. In the case of the *Tylodelphys* sp., they were found on the entire outer surface of the intestine. This can affect the complementary respiratory function of the fish, since Callichthyidae species use the intestine as an accessory respiratory organ, also, the presence of these parasites in the intestine can cause nutritional problems by affecting the absorption of food (SANTANA et al. 2017, MOREY 2018).

CONCLUSION

The endoparasites registered in *C. multiradiatus* corresponded to metacercariae of *Tylodelphys* sp, larva of Nematoda and adult specimens of *P. (S.) pintoi*.

Highest parasitological indices were recorded for metacercaria of *Tylodelphys* sp. parasitizing the intestinal wall of *C. multiradiatus.*

The histopathological analysis of the intestine of *C. multiradiatus* revealed several histopathological damages with different degrees of lesions that can compromise the health of the fish.

The presence of *P. (S.) pintoi* inside the intestine of *C. multiradiatus* can destroy the intestinal mucosa and submucosa causing goblet cell hyperplasia.

It is necessary to seek prevention and control mechanisms against endoparasites in *C. multiradiatus*, in order to guarantee their good condition and consequent commercial success through exportation of ornamental fish from the Peruvian Amazon.

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