



Zoonotic Parasites in *Hoplosternum littorale* Acquired in the Market of Santa Clara, in the City of Iquitos, Loreto-Peru

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Abstract

Purpose The present study aims to report zoonotic parasites in *Hoplosternum littorale* (Hancock, 1828), acquired in a fish market in the Peruvian Amazon.

Methods Forty specimens of *H. littorale* with an average size of 12.3 ± 2.5 cm and weight 58.3 ± 10 g were acquired in the “Santa Clara” Market, in the city of Iquitos, Loreto-Peru. Fish samples were taken alive, placed in plastic bags with water, and transported to the laboratory of “Parasitología y Sanidad Acuicola” of “Instituto de Investigaciones de la Amazonia Peruana” in Iquitos, Peru for analyses.

Results Two groups of endoparasites were identified: Trematoda, with specimens of *Clinostomum* sp. parasitizing the musculature and skin, and Nematoda, with specimens of *Eustrongylides* sp. parasitizing the intestine.

Conclusions The findings of the present study constitute a health risk in the event of accidental ingestion of any of these parasites. Socialization campaigns on the dangers of raw or undercooked fish consumption should be implemented by the competent authorities to guarantee the health of the Amazonian population.

Keywords Brown hoplo catfish · Endoparasites · Nematoda · Peruvian amazon · Trematoda

Introduction

In the Peruvian Amazon, we found 79 species of fish that are being traded in the markets of the Loreto, Ucayali, and Madre de Dios regions as meat for human consumption. In

the Loreto region, fish is marketed in 3 different preservation states: fresh, salted, and dried-salted [1]. Among the 79 fish species recorded as consumption fish, *Hoplosternum littorale* (Hancock, 1828), is a fish species from the Siluriformes: Callychthidae, commonly named as Brown Hoplo Catfish, and popularly known in the Peruvian Amazon as “portol” or “shirui”. This armored catfish is acquired by the local population due to the taste of its meat and its accessible price [1].

Fish are considered to be the animals that are in contact for the longest time with parasites [2]. These parasites spread through trophic chains by interactions between host, predator, prey, and competitor populations [3]. Fish diet strongly influences the composition of the fish parasitic fauna, because it is the pathway by which a fish can become infected by endoparasites, which can be detrimental to the health of the consumer by accidentally ingesting fish flesh contaminated with any group with zoonotic potential [4].

In the Peruvian Amazon, parasite species with zoonotic potential have been reported in samples of 27 species of fish traded in markets in the city of Iquitos [5]. In the case of

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parasites with zoonotic potential, they can enter humans by consuming infected fish meat. The transmission of pathogens to humans can constitute a major public health problem, due to the negative health consequences that can result from the accidental ingestion of zoonotic parasites [6]. Within this large group of zoonotic parasites, parasites of the trematode and nematode groups stand out, which can be ingested in gastronomic dishes widely consumed in the Peruvian Amazon, such as sushi and ceviche, or in other preparations that use raw or undercooked fish meat [7; 6].

Some species of the genus *Clinostomum* (Trematoda) can parasitize humans and are responsible for Halzoun syndrome, a rare condition transmitted by consuming raw fish infected with metacercariae [8], causing laryngopharyngitis; cases have been reported in countries such as Korea and Japan where raw fish are consumed [9]. In Peru, no cases of human clinostomiasis have been reported; however [10], have pointed out that clinostomiasis in South America is latent and to prevent it, it is recommended to avoid the consumption of raw and fresh fish.

The toxicity and transmission potential of *Eustrongylides* spp. (Nematoda) are what cause zoonotic diseases [11]. The presence of *Eustrongylides* larvae in the muscles and viscera of several fish species is significant for public health [6]. By negatively affecting their marketability, the presence of these parasites in fish hosts might result in significant harm that can impair their health and render fish products unfit for human consumption [12].

Given the importance of *H. littorale* as a fish for consumption in the Peruvian Amazon and the lack of records in this part of Peru, the present study aimed to report and identify the zoonotic parasites present in this armored catfish acquired from the main market of Santa Clara, in Loreto-Peru.

Materials and Methods

The interest in conducting this research is due to an anecdote experienced by one of the authors of this study, who prepared a traditional dish using raw individuals of *H. littorale* specimens; while eating, he noticed worms coming out of the body of the fish, which caused his curiosity to see what they were. So, he collected those worms and took them to the Laboratorio de Parasitología y Sanidad Acuicola (LAPYSA) from the Instituto de Investigaciones de la Amazonía Peruana (IIAP), in Iquitos Peru where it was determined that they were metacercariae of *Clinostomum* sp. Immediately, we went to the place where the fish were purchased (Santa Clara market) and acquired the fish for the respective analyses.

Forty specimens of *Hoplosternum littorale* with an average size of 12.3 ± 2.5 cm and weight 58.3 ± 10 g were acquired in the Santa Clara Market ($3^{\circ}47'00.1''\text{S}$ $73^{\circ}20'26.3''\text{W}$), in the city of Iquitos, Loreto-Peru. Fish samples were taken alive, placed in plastic bags with water, and transported to the laboratory of Parasitología y Sanidad Acuicola of Instituto de Investigaciones de la Amazonía Peruana in Iquitos, Peru for analyses. In the laboratory, fish were euthanized with the use of Eugenol (0.4 ml L^{-1}) [13] and then processed. The lateral musculature of the fish was analyzed by making fine cuts, which were carefully observed. Internal organs were removed 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 Zeiss Stemi 508 with magnification between 8x to 35x.

Trematodes were removed from their cysts using stylets and then fixed in FAA solution (Formalin, Glacial Acetic Acid, and 70% ethanol). After 24 h, trematodes were transferred to 70% ethanol for preservation. For identification, these parasites were stained in Langeron's alcoholic carmine [13]. Nematodes were placed in Petri dishes with heated (70°C) water to induce the parasites' death with the body extended and then preserved in 70% ethanol for posterior analyses. For the identification, parasites 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 [13].

For taxonomic identification of the parasites, slides with trematodes and/or nematodes 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 [6; 14; 15; 16; 17]. Parasitological indices followed those by [18].

Voucher specimens were deposited in the parasite collection of the Laboratorio de Parasitología y Sanidad Acuicola (LAPYSA) from Instituto de Investigaciones de la Amazonía Peruana (IIAP).

Results

Two groups of endoparasites were identified: Trematoda, with specimens of *Clinostomum* sp. parasitizing the musculature and skin (Fig. 1), and Nematoda, with specimens of *Eustrongylides* sp. parasitizing the intestine (Fig. 2). Parasitic indices are presented in Table 1. Voucher specimens of *Clinostomum* sp. corresponded to LAPYSA-T-52a-o; and, voucher specimens of *Eustrongylides* sp. corresponded to LAPYSA-N-15a-o.

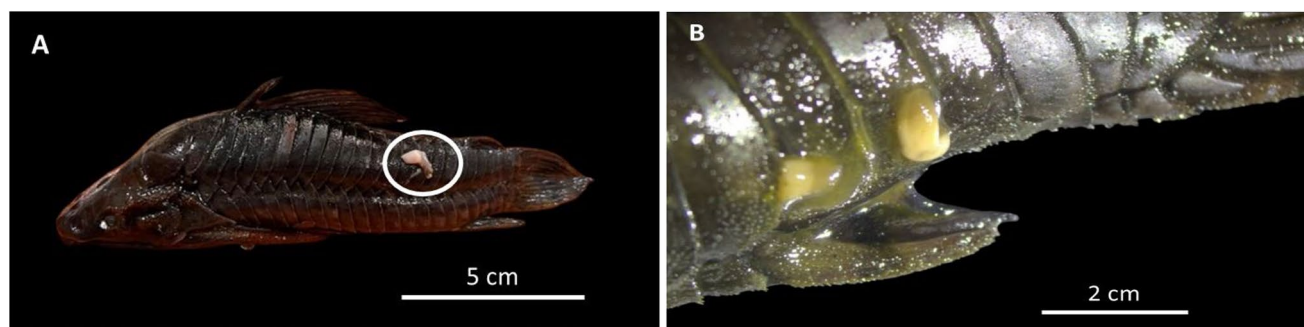


Fig. 1 A. *Hoplosternum littorale* (Hancock, 1828) with metacercariae of *Clinostomum* sp. B. Lateral view of *H. littorale* showing metacercariae encysted in the musculature. With circles indicate the presence of metacercaria of *Clinostomum* sp

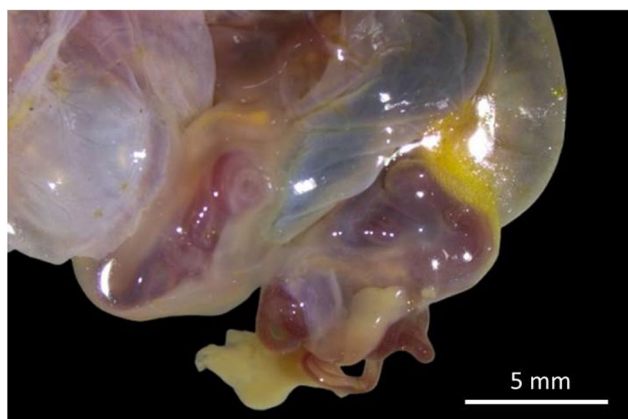


Fig. 2 Intestine of *Hoplosternum littorale* (Hancock, 1828) with *Eustrongylides* sp. inside

Table 1 Parasitic indices of endoparasites recorded in *Hoplosternum littorale* (Hancock, 1828) collected in the market Santa Clara, Loreto-Peru

Species	P%	I	RI	MI	MA
<i>Clinostomum</i> sp.	50	306	8–35	15.3	7.6
<i>Eustrongylides</i> sp.	20	21	1–7	2.6	0.5

Table note. P% = prevalence; I = intensity of infection; RI = range of intensity; MI = mean intensity; MA = mean abundance

Clinostomum sp. (Fig. 3) ($n=15$) presented a small linguliform body, dorsally convex and ventrally concave, with 5.32 mm average length and 1.66 width. Oral sucker with 0.28 mm length and 0.31 width; ventral sucker well-developed, with 0.75 mm length and 0.78 width. The distance from the oral sucker to the ventral sucker is 0.72 mm and the distance from the anterior testis to the posterior testis is 0.31 mm. The caeca are long and simple, with a fairly sinuous wall, particularly pronounced in the anterior half of the body, but devoid of long lateral branches or diverticula. Testes tandem, with irregular shape, located in the posterior half of the body. Anterior testis with 0.24 mm length and 0.45 width, posterior testis with 0.25 mm length and 0.42 width. Ovary intratesticular.

Eustrongylides sp. (Fig. 4) ($n=15$) larvae L4 with 128 mm in average total length, 0.5 mm in width, with reddish coloration when alive, cephalic extremity features a small oral cavity surrounded by 12 cephalic papillae of similar size arranged in two concentric rings. Sinuous oesophagus with 10 mm length. Genital primordia are located in the posterior part of the body.

Discussion

Studies on endoparasites in *H. littorale* have been conducted mainly in Brazil, with reports of *Clinostomum* spp. Parasitizing the intestine of fish collected in the Guamá-Pará River [20], in the digestive tract and eyes of fish from the Paraná-Paraná River [21, 22]; in Argentina, metacercariae were reported in the body cavity of fish caught in the province of Salta [23]. In the present study, metacercariae of *Clinostomum* sp. were identified in the musculature of fish, a site of infection different from the reports of the previously cited authors. According to ([24]) who reported the presence of trematodes in the musculature of *Oreochromis niloticus* (Linnaeus, 1758), state that infected fish showed signs of weakness or deterioration, attributed to the act of encystment of the parasites that acts as a stress factor, causing disturbances in muscle functions and interfering in the movement or swimming of the fish. This could be related to the stage of the parasites found (metacercariae) that need a final host to complete their life cycle. In this sense, the metacercariae present in the muscle could be making it difficult for the fish to escape in the presence of potential predators, making them more susceptible to predation.

[25] analyzed specimens of *H. littorale* from the Batalha River in the municipalities of Piratininga and Reginópolis, State of São Paulo, Brazil, identifying six species of Nematoda, highlighting the presence of *Diectophyme renale* (Diectophymatidae) in the intestine. In the present study, we report the presence of *Eustrongylides* sp. a nematode also belonging to the family Diectophymatidae, which is

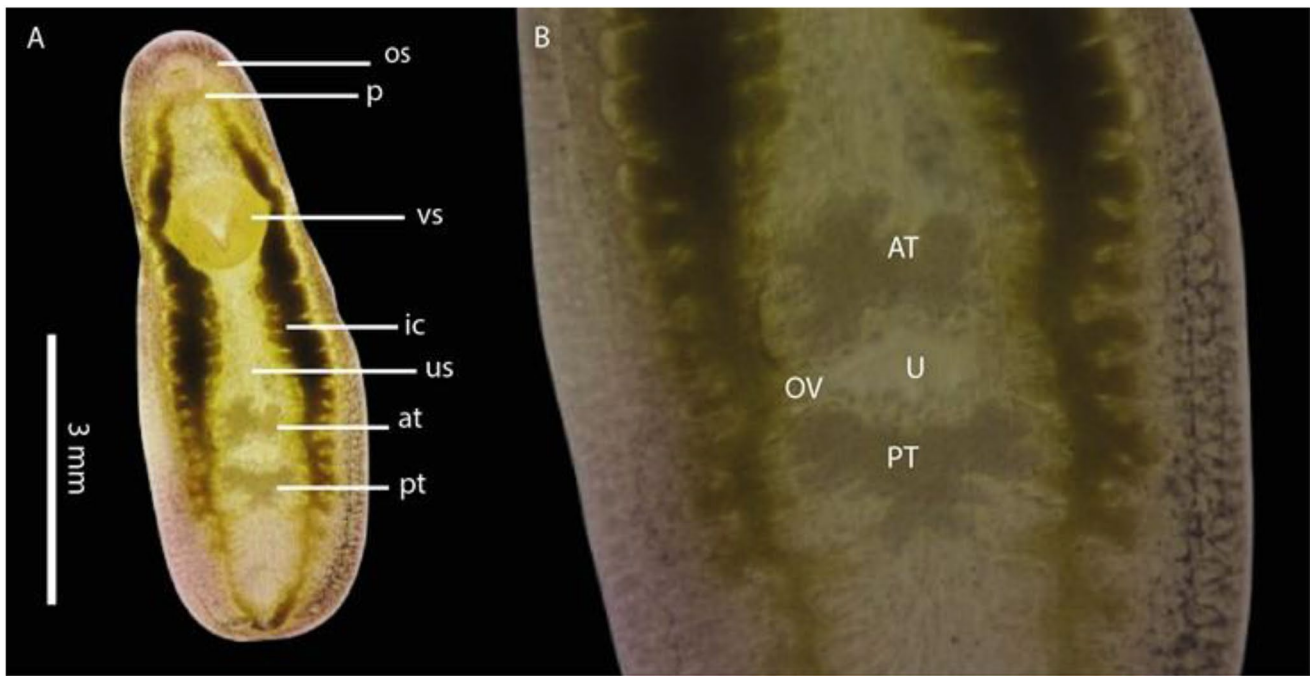


Fig. 3 Metacercariae of *Clinostomum* sp. of *Hoplosternum littorale* (Hancock, 1828). **A.** Ventral view of whole body. **B.** Enlarged view of the reproductive organs of *Clinostomum* sp. os=oral sucker, p=phar-

ynx, vs.=ventral sucker, ic=intestinal caecum, us=uterus sac, at=anterior testis, pt=posterior testis, OV=ovary, U=uterus

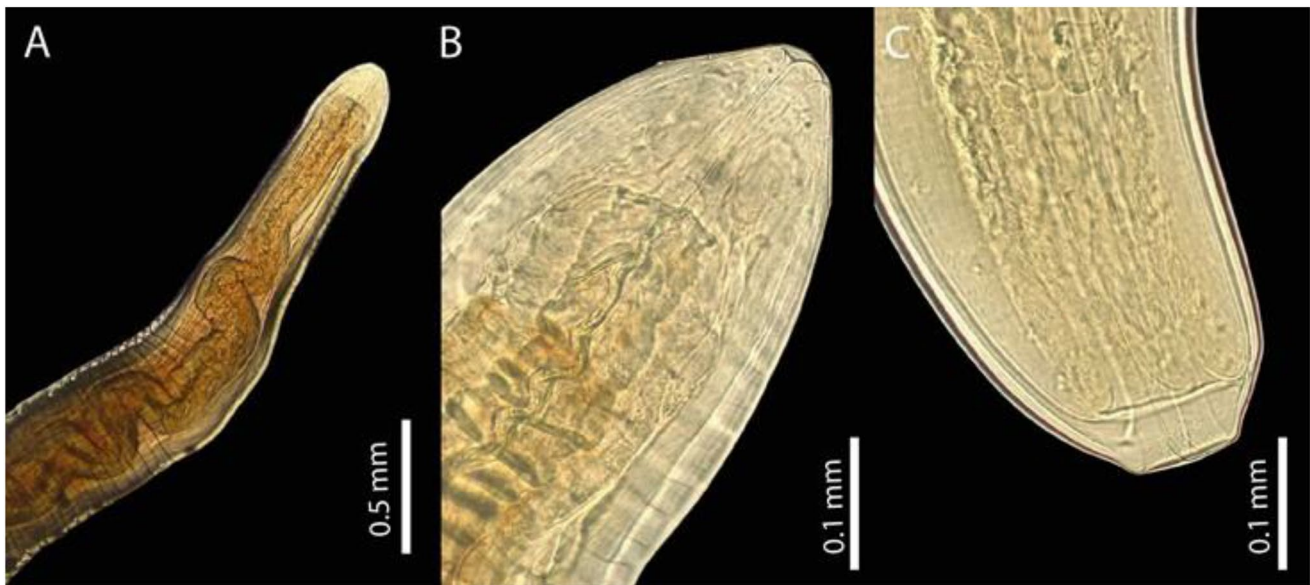


Fig. 4 *Eustrongylides* sp. **A.** Ventral view of the anterior part showing the esophagus, **B.** Anterior part of the body showing cephalic spines, **C.** Posterior part of the body

a parasite of the intestine of *H. littorale*. Like *D. renale*, *Eustrongylides* sp. is a parasite with zoonotic potential that can cause harm to humans when accidentally ingested.

Eustrongylides nematode development cycle starts when eggs reach the organism of the first intermediate host (oligochaete) reaching the first and second stages. The second intermediate host (fish) is infected during swallowing the

infected oligochaetes. Larvae that enter the gastrointestinal tract of fish perforate the intestinal wall and enter the extraintestinal space. Then the *Eustrongylides* nematode larvae migrate along the body. The migrated larvae can locate in the muscular tissue, hepatopancreas and gonads of the fish. As larvae stay in the body of the fish, they shed twice, reaching the 3rd and the 4th stages of development (L3 and

L4). Definitive hosts (fish-eating birds) are infected as they eat fish infected with the *Eustrongylides* nematode larvae [26]. Although the life cycle of *Eustrongylides* sp. in Amazonian fish is not known, it is assumed that oligochaetes are the first intermediate hosts, fish are the second intermediate hosts and piscivorous birds are the final hosts. These organisms must be in constant contact through predator-prey relationships, actively contributing to the fulfillment of the parasite's life cycle and consequent infection of *H. littorale*.

The suborder Digenea has a number of trematodes that parasitize fish and can infect humans. Like clinostomiasis, which is brought on by *Clinostomum complanatum*, these illnesses can be uncommon and sporadic [27]. This trematode merits special attention due to its zoonotic potential. After consumption of raw or undercooked parasitized fish, the metacercariae are freed in the stomach and the parasite migrates towards the esophagus or the oral cavity, usually causing acute pharyngitis or laryngitis [28]. Since *Eustrongylides* species are zoonotic parasites, they pose a threat to people. Humans who eat fish or fish products that are not sufficiently heated can contract eustrongylidosis. According to [29], these infections are typified by gastritis and perforations of the gastrointestinal tract organs. 24 h after the parasites entered the gastrointestinal tract, the patient experienced escalating spastic sensations in the stomach area [29]. The findings of metacercariae of *Clinostomum* sp. in the muscle and intestine of *H. littorale*, together with the presence of *Eustrongylides* sp. in the intestine represents an alert to the local population due to the alimentary habits for consuming raw fish and in some cases consuming the fish guts.

Conclusions

Specimens belonging to *Clinostomum* sp. and *Eustrongylides* sp. are known for their proven zoonotic potential, although, there are no reports in the literature on human contamination by metacercariae of *Clinostomum* spp. and larvae of *Eustrongylides* sp. in Peru, the habit of the Amazonian population to consume *H. littorale*, together with the findings of the present study, constitute a health risk in the event of accidental ingestion of any of these parasites. Socialization campaigns on the risks of raw or undercooked fish consumption should be implemented by the competent authorities to guarantee the health of the Amazonian population.

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Author Contributions GAMM: data curation, formal analysis, and writing—original draft. SGRL, LARC, ACTD, RFCS: formal analysis and data curation. LAGS, EYLC: resources and acquisition of samples.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

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