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PRESENCE OF METACERCARIAE OF Opisthorchis sp. BLANCHARD, 1895 (TREMATODA: OPISTHORCHIIDAE) IN NEW HOST Macrobrachium amazonicum (HELLER, 1862), FROM THE NORTHERN BRAZIL: AN EMERGING RISK TO HUMANS BY THE SHRIMP CONSUMPTION

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Abstract

Herein, we report the first observation of the metacercaria *Opisthorchis* sp. Blanchard, 1895 recovered from a new intermediate host *Macrobrachium amazonicum* (Heller, 1862), an important food resource from northern Brazil, emphasising the risk of contamination from inappropriate human consumption. The shrimp specimens of *M. amazonicum* were collected during June 2023, using a local trap called matapi in the Guamá River (01°27'54.2"S; 048°26'02.6"W), which supplies the city of Belém, showing a characteristic of an eutrophicated river. The collected specimens were sexed, measured, weighted and dissected in the carapace region, to observe the presence of parasites in gills, hepatopancreas and gonads. We observed 56 specimens of *M. amazonicum* (20 males, 32 females and 4 ovigerous females), of which thirteen individuals (23.21% of total sampled) showed encysted metacercariae of *Opisthorchis* sp. only in gonadal tissues, with high preference in females (66.6%), when compared with males (33.4%). The observation of metacercaria in Amazon river prawn is a risk to human health, due to the parasite's life cycle being completed in humans, leading to several health problems such as hepatic and biliary degradation, diarrhoea, vomiting and respiratory problems. Additionally, the eutrophication process observed in the Guamá River may favour parasite proliferation, and dissemination to other hosts and regions, possibly increasing the cases of people infected with parasites in Belém metropolitan area. Based on that, this work is an alert to the authorities for the risk of contamination of the population due to inadequate consumption of (molluscs, crustaceans and fishes) from the eutrophic waters of the Guamá River and adjacent waters.

Keywords: Trematode parasite. New host association. Risk to human health.Fish-borne trematodes.

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1 Introduction

The biodiversity in aquatic environments is correlated to abiotic (e.g. temperature, salinity, pH and dissolved oxygen); and biotic factors (e.g input of organic matter, photosynthesis (primary production) and energy flow in food chain), with the species playing a wide range of biological interactions such as: symbiosis, mutualism, competition, predation, commensalism and parasitism; this latter can be associated with the health of the environment and indications of environmental impacts (EMPARANZA et al., 2011; GÓNGORA-GÓMEZ et al., 2015).

The transmission of diseases through the ingestion of contaminated water or food is a public health problem in underdeveloped and developing countries, including transmission of parasites between aquatic animals and humans (BELIZARIO et al., 2001; HOP et al., 2007; PUMIDONMING et al., 2018).

The fish-borne trematodes (FBT) are one of the main parasitic diseases transmitted between aquatic organisms (molluscs and fishes) and birds, domestic animals and humans (SITHITHAWORN; HASWELL-ELKINS, 2003; LOVIS et al., 2009; CHAI et al., 2017).

Currently in literature, the trematodes use snails as first intermediate host (metacercaria stage), freshwater fishes as second intermediate host; these are species consumed by fish-eating birds and mammals (including humans), which become definitive hosts (TOUCH et al., 2009; DAO et al., 2017; PHYO MYINT et al., 2020).

Up to date, more than 20 million people are contaminated with trematodes, especially in Asian and Middle Eastern countries, where the degradation of water bodies has been intensified in the last years, causing an increase in the number of cases by the consumption of raw fish and molluscs (PETNEY et al., 2013; PHYO MYINT et al., 2020).

The trematode family Opisthorchiidae Looss, 1899 is composed of 53 genera distributed worldwide, occurring as obligate parasites in a wide range of hosts. One of the most diverse genus into Opisthorchiidae is *Opisthorchis* Blanchard, 1895, covering 47 species (CHAI, 2007; JEON et al., 2012; NOUDEH; PAZOOKI, 2019; WORMS, 2023).

Its proliferation around the world is caused by the inadequate human consumption of aquatic organisms; the parasite can reach great distances inhabiting the human intestines, additionally observed in migratory birds, fishes and by the aquarium trade (LEMOS et al., 2007).

In Brazil, the presence of these trematodes can be associated with water quality, the tourism flows in parallel with the lack of basic sanitation in the some regions (THATCHER, 1993; PINTO; MELO, 2012; MATTOS; VERICIMO; SÃO CLEMENTE, 2013); aquarium trade process and the transport of these parasites through the introduction of exotic species, as observed by Pinto; Melo (2010) through the introduction of snail *Melanoides tuberculata* (Müller, 1774), an intermediate host of trematodes *Centrocestus formosanus* (Nishigori, 1924).

Despite being widely reported in the world, in Brazil, studies with trematodes are poorly documented, especially in the northern region; crustaceans are usually studied for growth, reproduction and fishery parameters, but not studied as parasite hosts. This study is the first observation of metacercariae of *Opisthorchis* sp. Blanchard, 1895 recovered from a new intermediate host *Macrobrachium amazonicum* (Heller, 1862), an important food resource from northern Brazil, emphasising the risk of contamination from inappropriate human consumption.

2 Material and Methods

Study Area

The prawn specimens of *M. amazonicum* were collected in the Guamá River (01°27'54.2"S; 048°26'02.6"W) (Figure 1), one of the main rivers that crosses the city of Belém, in the State of Pará. The river is highly eutrophic, with muddy and turbid waters, with a strong presence of clay particles in suspension, receiving domestic waste from the Belém metropolitan area, as well as chemical elements from the factories located along the Guamá River (COSTA et al., 2022). This region presents a tropical humid climate (Köppen classification Af), with high temperatures all over the year (always above 18°C) and annual precipitation between 2250 and 3000 mm, with the characteristic rainy period between December and May and the dry period between June and November. The samples occurred in June 2023, using a local trap called "matapi" placed at night time and recovered in the next morning.

Laboratory Procedures

The collected individuals were transported alive to the carcinology laboratory (LABCRUS) of the Federal Rural University of the Amazon (UFRA), where the individuals were identified, sexed and measured with a calliper (0.01 mm) in Total Length (TL), Carapace Length (CL) and Total Weight (TW). Afterwards, the specimens were dissected in the carapace region for parasites in gills, hepatopancreas and gonads under a stereomicroscope.

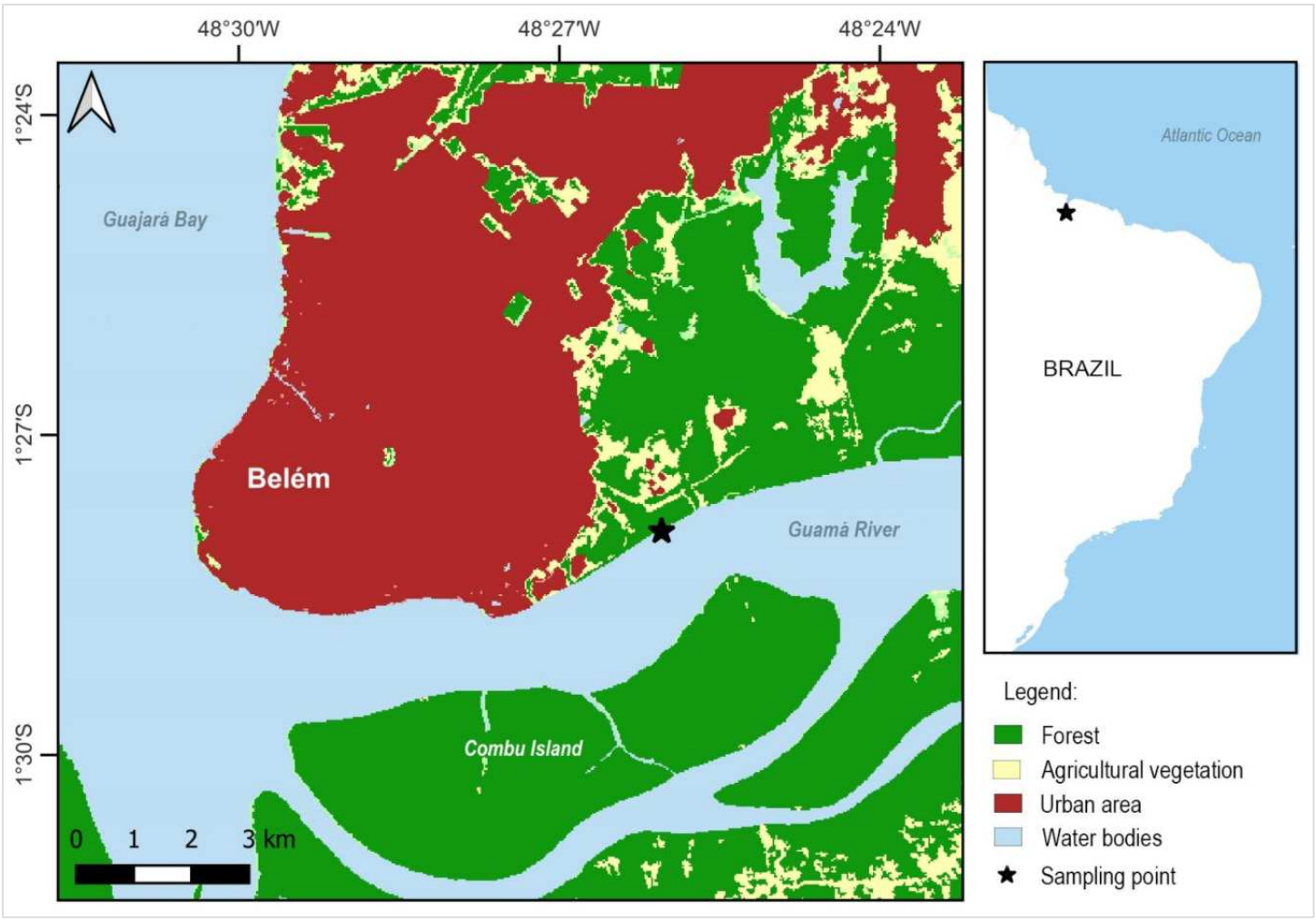

Figure 1. Map of the study area indicating the urbanised area, corresponding to the metropolitan region of Belém- PA. Black star = sampling point.

In specimens that showed trematodes, the tissues were removed, transferred to petri dishes containing saline solution (NaCl 0.85%) and then analysed by means of optical microscopy to determine whether metacercariae were present for counting and photographing using the camera Motic Moticam5. For the fixation of metacercariae, we used AFA (Acetone - Formaldehyde - Alcohol).

For the identification of metacercariae, we followed the descriptions provided by Vajrasthira; Harinasuta; komiya (1961) and Scholz; Ditrich; Giboda (1992), for individuals of the genus: cyst in elliptical form, with equal size of oral sucker and ventral sucker, brownish pigment granules scattered within the body and an O-shaped excretory bladder occupying the greater part of the posterior body.

3 Results and Discussion

We collected 56 specimens of *M. amazonicum* (Figure 2A), being 20 males, 32 females and 4 ovigerous females, measuring: TL (min.: 6.18; max.: 9.85 cm; mean: 6.18 ± 1.12), CL (min.: 0.92; max.: 2.44 cm: mean: 1.45 ± 0.326) and TW (min.: 0.34; max.: 6.83 g; mean: 1.80 ± 1.04), which 13 individuals (23.21% of total sampled) showed the metacercariae of *Opisthorchis* sp. between the gonadal tissue (Figure 2B, C, D).

There were 53 metacercariae (covering between 3 and 5 metacercariae by prawn), with these parasites showing sizes between 288 and 725 µm (mean: 400 µm± 121). The highest preference of *Opisthorchis* sp. was observed in female specimens of *M. amazonicum* (66.6%), when compared with males (33.4%).

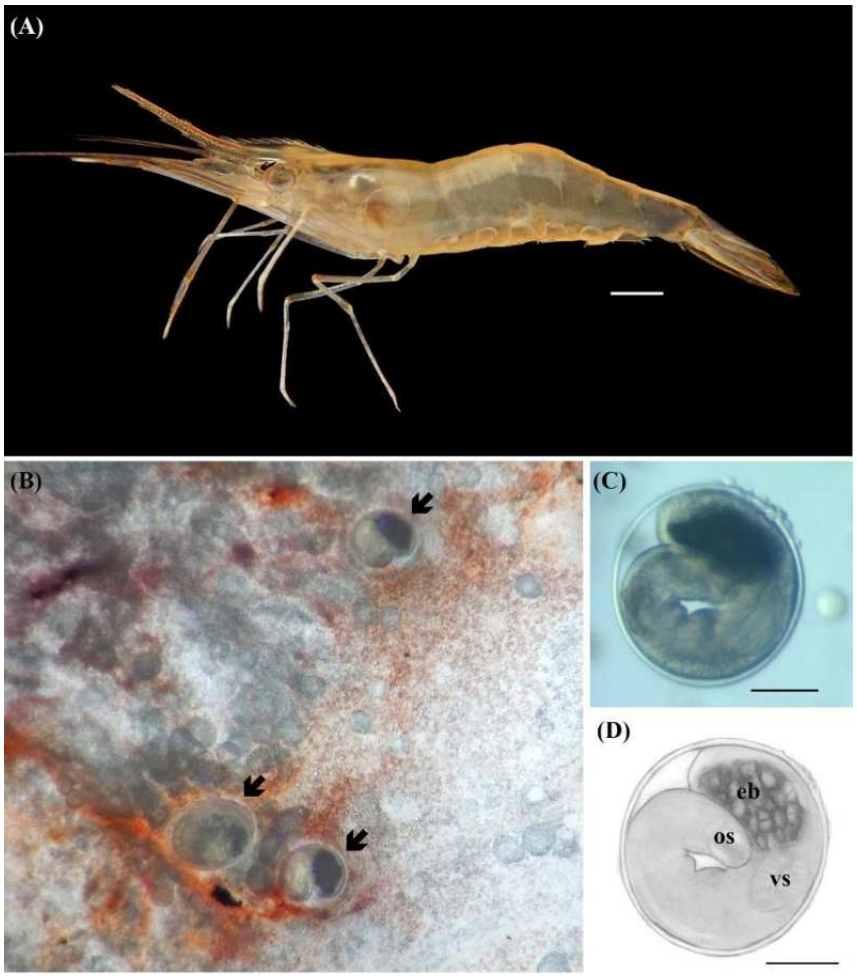

Figure 2. (A) *Macrobrachium amazonicum* (Heller, 1862) parasitised by metacercariae of *Opisthorchis* sp. Blanchard, 1895; (B) Histological view of metacercariae of *Opisthorchis* sp. adhered on the gonads of *M. amazonicum*; (C) Metacercariae of *Opisthorchis* sp. in highlighted view; (D) Schematic drawing of the metacercaria of *Opisthorchis* sp. indicating the oral sucker (OS), ventral sucker (VS) and brownish pigment granules and an O-shaped excretory bladder (EB). Scale bars (A) = 0.5 cm; (C, D) = 100 µm.

From North America, many studies reported the presence of trematodes in impacted environments; it indicates the transmission through different routes such as intestinal transport due to tourist flow, fresh oysters exports for human consumption, aquarium trade with parasitised fishes, introduction of exotic molluscs in subtropical and tropical areas (e.g. *Lissachatina fulica* (Bowdich, 1822) and *M. tuberculata*) and climate changes setting new migratory routes of birds and fishes transporting the parasites into new regions (MURRAY; HAINES, 1969; NOLLEN; MURRAY, 1978; ISMAIL; ARIF, 1991; SCHOLZ; SALGADO-MALDONADO, 2000; DÍAZ; HERNANDEZ; BASHIRULLAH, 2002; TOLLEY-JORDAN; OWEN, 2008; PINTO; MELO, 2012).

In Brazil, the three main trematode parasite into Opisthorchiidae observed for the central and southeastern regions are *Centrocestus formosanus*, *Opisthorchis felineus* (Rivolta, 1884) and *Opisthorchis viverrini* (Poirier, 1886), with reports as endoparasites in snails (intermediate host), cats and potentially in humans (definitive hosts), as observed in case studies reported from Asian regions (OLIVEIRA et al., 2005).

Additionally, in Brazil, the trematode species of the family Heterophyidae are widely reported as intermediate hosts of the exotic species *M. tuberculata* (in metacercariae stages), with reports of parasitism in the states of Rio de Janeiro by Thiengo et al. (2001), Boaventura et al. (2002) and Bogéa; Cordeiro; Gouveia (2005); Bahia by Lemos et al. (2007); Minas Gerais by Carneiro et al. (2004) and Pinto et al. (2005); and in Brasília by Andrade et al. (2008). Additionally, the pleurolofocerca stage was reported as an endoparasite in birds, fishes and in the biliary system of humans (PINTO; MATI; MELO, 2013).

Some authors indicate the mollusc *M. tuberculata* as specific host of the families Philophthalmidae Looss, 1899 and Echinochasmidae Odhner, 1910 from Asian region (SUKONTASON et al., 2005; SAYASONE et al., 2009). However, these studies report only molluscs as the main intermediate hosts, with no observations in other aquatic invertebrates such as crustaceans, as observed herein.

In Brazilian clinical cases of trematodes as human parasites (definitive host), there are reports of *Fasciola hepatica* Linnaeus, 1758 from states of Rio de Janeiro, São Paulo and Minas Gerais, with parasites occupying the hepatic and biliary areas, acquired by eating fish from contaminated regions; additionally, Chieffi et al. (1990) observed a human case of parasitism of *Ascocotyle* sp. Looss, 1899 (in a 31-year-old woman) due to the ingestion of uncooked fish. Dias et al. (1992) observed the presence of *Clonorchis sinensis* (Cobbold, 1875) Looss, 1907 in Asian immigrants in Brazilian territory.

An infestation by *Paragonimus westermani* (Kerbert, 1878) Braun, 1899 was observed in the state of Bahia by Lemos et al. (2007), in one adult man, showing pulmonary infection, probably caused by consumption of uncooked molluscs and shrimps. Another potential trematodes transmitter are the molluscs of the genus *Biomphalaria* Preston, 1910 (intermediate host), which are widely reported in degraded aquatic environments, and used as intermediate hosts of a wide range of parasites (GIOVANELLI; VIEIRA; SILVA, 2003).

On other hand, another route of trematode contamination in humans, is the direct contact with the faeces of domestic animals (dogs and cats), once these domestic animals in the process of hunting, may consume fishes, birds, mice and other parasitised mammals in urbanised regions. Humans, in contact with the faeces of domestic animals, become definitive hosts.

The proliferation of trematode parasites in humans causes abdominal pain, hepatic and biliary degradation (in severe cases, bladder and bile cancer - Biliary Tract Carcinoma), diarrhoea, vomiting and respiratory problems; due to the absence of basic sanitation in many regions, especially in northern Brazil, these parasites are introduced into the aquatic environment, where they complete their cycle, spreading to other regions, seeking other intermediate / definitive hosts (COSTA; LIMA; COSTA, 1984).

In an experiment performed by Fan (1998), it was observed the high resistance of the trematode *C. sinensis* in metacercariae stages, recovered as parasite of freshwater fish *Pseudorasbora pamu* (Temminck & Schlegel, 1846): the fish was frozen for 7 days at a temperature of - 20 °C, however, the metacercariae remained alive and with high parasitic potential. Additionally, the same author analysed the resistance of *C. sinensis* in fish salting process, finding that the parasite remains active for up to 7 days in high salt concentration (above 10 grams of salt by cm^2 of fish body).

In observations made by Phyo Myint et al. (2020) in public supermarket from Tachileik (Myanmar), eight popular fish species parasitised by four metacercariae species were found: *O. viverrini*, *Haplorchis taichui* (Nishigori, 1924); *Haplorchis pumilio* (Looss, 1896) and the genus *Haplorchoides* Chen, 1949; it indicates the high resistance in uncooked dishes consumed by the population (above 48 hours), emphasising the high risk of contamination of the population through the consumption of contaminated fish.

According to Fan (1998) and Aukkanimart et al. (2017) to prevent the presence of the parasites in humans, it is recommended the consumption of well-cooked fishery products, additionally being effective against the parasite, the consumption in salted and marinated forms or kept frozen below - 20°C for long periods.

In the state of Pará, the Amazon River prawn *M. amazonicum* is commonly sold fresh and dry salted in open-air markets and supermarkets and largely used in the local cuisine. The artisanal production process of dry salted prawn involves cooking in brine followed by natural drying, which can be an alternative for inactivating the parasite and should be studied more carefully in future works. The consumption of fresh shrimp may favour the occurrence of trematodes, in particular, *Opisthorchis* sp. in humans.

The high urbanisation process as observed at Belém metropolitan area (see Figure 1), associated to regions without basic sanitation and the eutrophication process of the Guamá River (one of the fishing locations of the Amazon River prawn consumed in the area), makes us alert the presence of the trematode *Opisthorchis* sp. parasitising a new intermediate host species and the potential risk of parasitism in humans.

4 Conclusions

In conclusion, we would like to alert the authorities and the population about the risk of contamination by metacercariae in humans (definitive host), due to inadequate consumption (for example, not well cooked in dishes, such as sushi, steamed or in salads) of aquatic organisms such as molluscs, crustaceans and fishes, collected in the Guamá River and adjacent waters, which may become a serious health problem in the region.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

DEGM and FAAJ conceived the research ideas, designed the study and writing the manuscript; MGM and IHAC performed the first draft of this manuscript and revisions along the main text.

DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence this study.

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