

## ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

*CLINOSTOMUM MARGINATUM* (BRAUN, 1899) AND *AUSTRODIPLOSTOMUM COMPACTUM* (LUTZ, 1928) METACERCARIAE WITH ZOONOTIC POTENCIAL ON *PYGOCENTRUS NATTERERI* (KNER, 1858) (CHARACIFORMES: SERRASALMIDAE) FROM CENTRAL AMAZON, BRAZIL

*CLINOSTOMUM MARGINATUM* (BRAUN, 1899) Y *AUSTRODIPLOSTOMUM COMPACTUM* (LUTZ, 1928) METACERCARIAS CON POTENCIAL ZONÓTICO EN *PYGOCENTRUS NATTERERI* (KNER, 1858) (CHARACIFORMES: SERRASALMIDAE) DE LA AMAZONÍA CENTRAL, BRASIL

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### Abstract

The present study investigated the presence of helminths in fillets of red piranha, *Pygocentrus nattereri* (Kner, 1858), with a note of a possible zoonotic potential of its consumption *in nature*. We examined 40 fish caught in lakes in the floodplain of the Solimões River, located in the municipalities of Coari and Manaus in central Amazonia, Brazil. All fish examined were adults and had yellow cysts in the musculature. Of the 69 cysts found, 66 were identified as metacercariae of *Clinostomum marginatum* (Braun, 1899) and 3 as *Austrodiplostomum compactum* (Lutz, 1928). The analysis shows a higher susceptibility of *P. nattereri* for metacercariae of *C. marginatum*. The fact that metacercariae were found in fillets of *P. nattereri*, suggests a risk situation, addresses a concern about its use in raw form. It should not be consumed without proper inspection, processing and freezing, since ceviche, is readily consumed, mainly in the border region of the upper and Western Amazonia.

**Key Words:** Brazil - Digenea - fish - metacercariae - *Pygocentrus nattereri* - Zoonoses.

### Resumen

El presente estudio investigó la presencia de helmintos en filetes de la piraña roja, *Pygocentrus nattereri* (Kner, 1858), alertando sobre un posible potencial zoonótico de su consumo en la forma *in natural*. Fueron examinados 40 peces capturados en lagos de varzea del Río Solimões, localizados entre los Municipios de Coari y Manaus en la Amazonía Central, Brasil. Todos los peces analizados fueron adultos y presentaban quiste amarillos en la musculatura del filete. Fueron encontrados 69 quistes e identificadas las metacercarias de las especie *Clinostomum marginatum* (Braun, 1899) (66 quistes) y *Austrodiplostomum compactum* (Lutz, 1928) (3 quistes). El análisis demuestra una mayor susceptibilidad de *P. nattereri* para metacercarias de *C. marginatum*. El hecho de haber sido encontradas metacercarias en filetes de *P. nattereri*, genera una situación de riesgo, remitiendo la preocupación en cuanto a su consumo en la forma cruda, no debiendo ser consumido sin una adecuada inspección, procesamiento y congelamiento, considerando que el cebiche es un alimento bastante consumido, principalmente en la región fronteriza del Alto Solimões en la Amazonía Occidental.

**Palabras claves:** Brasil - Digenea- metacercarias- pez- *Pygocentrus nattereri*- Zoonosis.

## INTRODUCTION

Human cases of parasitism transmitted by fish consumption have been reported in several countries, especially in those where raw fish dishes are part of their staple diet (Hirai *et al.*, 1987; Umegai *et al.*, 1990; Barros *et al.*, 2006;). Human beings are also liable to get intoxicated through the ingestion of dead anisakids larvae (Nematoda) (Audicana *et al.*, 2002), as well as Clinostomidae digenetic trematode metacercariae (Kifume *et al.*, 2000), lying within the fish's musculature.

Fish trematode zoonoses have great medical importance in far eastern and southeastern Asia, where up to 30 million people might be infected. Infection comes through fresh water fish, which makes up these people's main source of protein. The ethological agents are several Digenea species, which cause high morbidity but are seldom the cause of death. Transmission comes through the ingestion of metacercariae lying mainly in fresh water fish muscular and subcutaneous tissue (Santos, 1995; Ferre, 2001).

Most fish parasites which have been reported occurring in man belong to Digenea. Among them, *Clonorchis sinensis* (Looss, 1907), *Diplostomum spathaceum* (Rud, 1819), *Stellantchasmus falcatus* Onji & Nishio, 1915, *Procerovum varium* Onji & Nishio, 1916, *Nanophyetus schickhobalowi* Skrjabin and Podjapolskaja, 1931, *Cryptocotyle lingua* (Creplin, 1825), *Metorchis conjunctus* (Cobbold, 1860), *Echinostomum perfoliatum* (Ratz, 1908), *Echinostoma hortense*, *Clinostomum complanatum* Rudolphi, 1814, *Pseudamphistomum truncatum* (Rudolphi, 1819) and *Isoparorchis hypselobagri* Billet, 1898 (Okumura *et al.*, 1999).

Often infections are rare and occasional, as it happens with *Clinostomum marginatum* (Braun, 1899) and *Austrodiplostomum compactum* (Lutz, 1928), and usually present no pathogenic importance, except when dealing with abundant infections. In the case of *A. compactum*, human parasitose has been

associated to the condition known as parasitic laryngopharyngitis which, in its acute forms, may lead to death through suffocation (Eiras, 1994).

*Pygocentrus nattereri* (Kner, 1958) commonly known as red piranha or caju piranha, belongs to Characiformes order, Characidae family and is common in lentic environments (Santos *et al.*, 2006). It is a medium-sized fish, with a standard length of nearly 25 cm. It occurs in the Amazonas, Paraná-Paraguai and Essequibo River basins, as well as in Brazil's northeastern region Rivers (Jégu, 2003).

Its parasitic fauna is rather diversified, and it is thought to be the Amazonian fish species bearing the largest number of known parasite species, with 35 of them having already been reported as belonging to Monogenoidea, Copepoda, Branchiura, Nematoda and Isopoda Classes (Thatcher, 2006).

*Pygocentrus nattereri* is much consumed by the local population which considers it as a commercial fish (Santos *et al.*, 2006). The lack of sanitary inspection in order to detect pathogens in its fillets favors the transmission of zoonoses, mainly when they are ingested while still raw, or not having been subjected to proper boiling procedures (Barros *et al.*, 2007).

The present study aims to investigate the presence of helminths in *P. nattereri* fillets, calling attention to their zoonose acquiring potential if consumed *in natura*.

## MATERIAL AND METHODS

Fish captures, for parasitological analyses, were undertaken from March to June and from September to December 2008, which cover the high and low water seasons in the central Amazon. We determined the fishing effort standard by using gill nets placed at random in the following five lakes:

Lago do Baixio (S 03°17'27.2"/ W 60°04'29.6") located at Iranduba Township. Lago Iauara (S 03°36'39.2"/ W 61°16'33.0") sited at Manacapuru Municipality. Lago Ananá (S 03°53'54.8"/ W 61°40'18.4") located at Anori County. Lago Araçá (S 03°46'15.8"/ W 62°20'10.3") sited at Codajás Township. Lago Maracá (S 03°50'32.8"/ W 62°34'32.4") localized at Coari Municipality. All of them on a nearly 400 km long stretch on the Solimões River floodplain between Coari County and the Solimões and Negro Rivers merging area in the Manaus neighborhood.

After capture the specimens of *P. nattereri* were weighed, measured, and sex identified. Afterwards, we removed the fillets and immediately examined them for cysts in a light box. We collected the cysts we found by hand, broke them with the aid of thin dissecting needles and observed them under light microscope and stereoscope.

Digenea were cold fixed in AFA (alcohol, formalin and acetic acid) with and without compression. Specimens were stained with Langeron's alcoholic carmine, dehydrated in an ethyl alcohol series, cleared in beechwood creosote and mounted in Canada balsam as permanent slides. Parasitological indices, such as, prevalence (P), intensity (I); infestation mean intensity (IMI) and abundance (A) were calculated and analyzed according to Bush *et al.* (1997). Taxonomical identification is in accordance with Travassos *et al.* (1969) and Kohn *et al.* (1995). Voucher specimens (PL 579, 580, 581 and 582) were deposited in the collection of the Instituto Nacional de Pesquisas da Amazônia (INPA).

## RESULTS

Forty *P. nattereri* were examined for helminths. Mean standard weight and length were  $232.78 \pm 91.92$  g and  $15.98 \pm 1.87$  cm, respectively. All analyzed fish were adults and bore yellow cysts in their fillet musculature and were collected 69 metacercariae, as a whole.

The cysts founded were morphologically identified as metacercariae of *A. compactum* and *C. marginatum* (Figure 1A,B). Sixty-six metacercariae belong to *C. marginatum* and only three were of *A. compactum* (Figure 2).

All sampled fish displayed higher parasitological indices of *C. marginatum* than of *A. compactum* (Table 1). From the analyzed fish 37 (85%) were female and all of them showed to be infested by *C. marginatum*, whereas *A. compactum* was present only in three male hosts (15%). We collected both parasite species during low water season.

Parasitological indices of *C. marginatum* infesting *P. nattereri* were: prevalence (P) 100%; intensity (I) 66 with the least of two or the most of six metacercariae per fish, infestation mean intensity (IMI)  $3.36 \pm 1.56$  and mean abundance (MA)  $3.36 \pm 1.56$ . *A. compactum* parasitological indices were: P=15%; (I)= 3(1-2); IMI=  $1.57 \pm 0.71$  and MA=  $0.15 \pm 0.71$ .

## DISCUSSION

Metacercariae of Clinostomidae use fish as their intermediate hosts. The adult form infests the mouth and esophagus of birds. Size and color are their most significant trait. They are the largest-sized metacercariae and bear bright yellow coloring "yellow worms". They occur in all continents. The two most common species are *C. marginatum*, which occurs likewise in North, Central and South America, and *C. complanatum* in Europe, Asia and Africa (Thatcher, 1981; Bullard & Overstreet, 2008).

Three Amazonian fish species have already been reported as being parasitized by metacercariae of *C. marginatum*: *Cichla monoculus* Spix and Agassiz, 1831 with metacercariae encysted in the gills; *Crenicichla* sp. in skin and fins (Thatcher, 1981) and *Semaprochilodus insignis* (Jardine, 1841) in the musculature (Castelo, 1984).

*Clinostomum marginatum* is cited as parasite mouth of several Amazonian piscivorous bird species: *Ardea cocoi* Linnaeus, 1766; *Jabiru mycteria* (Lichtenstein, 1819); *Egretta caerulea* (Linnaeus, 1758); *Phalacrocorax brasilianus* (Gmelin, 1789); *Anhinga* (Linnaeus, 1766); *Ardea cinerea* Linnaeus, 1758; *Ardea alba* Linnaeus, 1758; *Nycticorax nycticorax* (Linnaeus, 1758) and *Butorides striata* (Linnaeus, 1758) (Travassos et al., 1969).

Species of families Bolbophoridae, Diplostomidae, Neodiplostomidae and Strigeidae, members of the super family Diplostomatoidea (Strigeoidea), were called fish metacercariae in the old literature (Bullard & Overstreet, 2008).

Many metacercariae of the type “diplostomulum” accommodate themselves in the eyes of their hosts, causing the formation of cataracts and sometimes even their death. The most important species presenting this behavior is *Diplostomum sphataceum* Rudolphi, 1819. The disease caused by *Diplostomum* spp. was observed in more than 125 fish species with very wide geographical distribution. Cercariae penetrate into the fish and migrate to the eyes, probably through the blood stream and parasitize mainly crystalline, but may also go to the retina and vitreous humor (Eiras, 1994).

Metacercariae of Strigeoidea were found parasitizing various organs of Amazonian fishes: *Pterygoplichtys* sp.; *Astronotus ocellatus* (Agassiz, 1831); *Oxydoras niger* (Valenciennes, 1821) and *Geophagus brasiliensis* (Quoy & Gaimard, 1824) (Thatcher, 1981). In *Pterygoplichtys pardalis* (Castelnau, 1855) metacercariae of *A. compactum* parasitized the eyes, stomach and gonads (Porto, 2009).

In the present paper *C. marginatum* and *A. compactum* are reported for the first time

*Clinostomum marginatum* presented high parasitological indices whereas just three individual of *A. compactum* were collected.

Parasite species may occur in a distinct form in male and female hosts, a characteristic which has been frequently investigated in fish (Luque et al., 1996; Poulin, 1996; Machado et al., 2005). In the present paper *C. marginatum* only parasitized *P. nattereri* females, while *A. compactum* occurred only in males. The host's sex had an influence on the parasite species, pointing out there being ecological and physiological factors determining this specificity.

Parasites acquire more importance if or when they are able to infecting human beings (Castelo, 1984). Presently, there is more emphasis on diseases originating from contaminations by microbial organisms or their toxins than on zoonoses or diseases acquired by ingesting animals hosting these parasites (Khamboonruang, 1991).

There are 17 confirmed cases of infections by *C. complanatum* in Japan and one in Korea. The main symptom is a queer feeling in the throat a few days after ingesting any raw fish dish. The treatment is carried out by an otorrinolaringologist through the mechanical removal of the metacercaria that lies fixed either on the pharynx mucus wall or on the arytenoids, being later identified (Furukawa & Miyasato, 1987; Chung et al., 1995; Kifume et al., 2000).

Finding metacercariae in *P. nattereri* fillets, warns to there being a risky situation, and stresses the care as to them being consumed while still raw, making it clear they can become a public health problem, once these fillets may be consumed in the region as sushi, sashimi or ceviche.

This paper brings an alert that *P. nattereri* should only be consumed raw, after having gone through prior freezing treatment at -20°C

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for seven days or -30°C for 15 hours, corroborating with Barros *et al.* (2006), and according to ANVISA technical ruling (2009) for the consumption of raw or half boiled fish, thus offering greater assurance to the population.

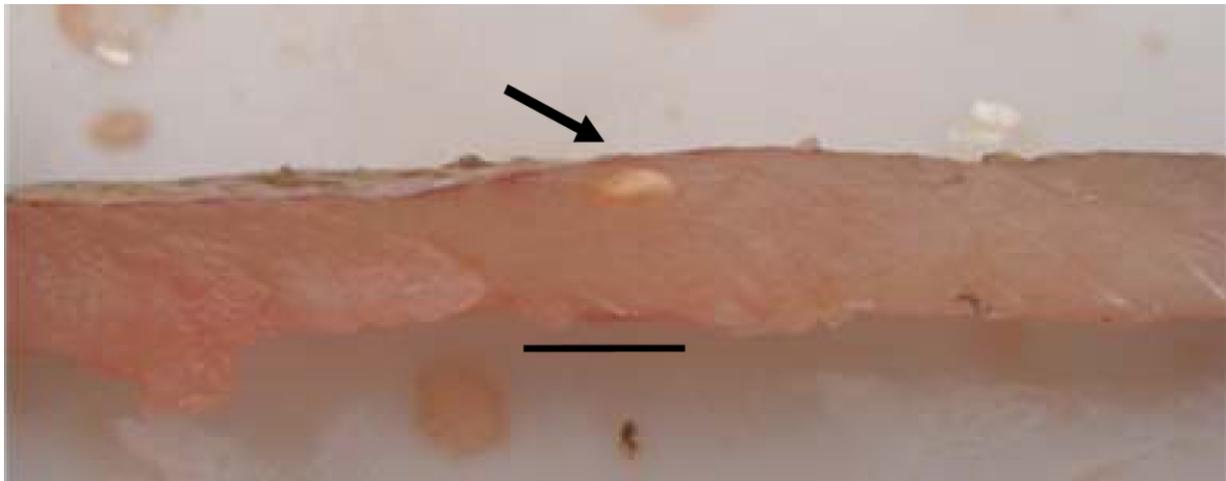
Backing: FINEP, PETROBRAS (PIATAM project), FAPEAM, INPA/MCT.

**Table 1.** *Pygocentrus nattereri*. parasitological indices, Mean fish weight (MW), fish mean standard Length (MSL), prevalence (P), intensity (I), infection mean intensity (IMI) and mean abundance (MA). SE = standard error.

Parasite species	P (%)	I (variation)	IMI±DP	MA±SE	Host Sex	
					♂	♀
<i>Clinostomum marginatum</i>	100	66(2-6)	3,36±1,56	3,3±1,56	0	66
<i>Austrodiplostomum compactum</i>	15	3(1-2)	1,57±0,71	0,15±0,71	3	0



**Figure 1.** *Clinostomum marginatum* (A) Bar = 200 µm. *Austrodiplostomum compactum* (B) Bar = 200 µm.



**Figure 2.** Metacercarie cyst standing out in *Pygocentrus nattereri* musculature fillet (C). Bar = 3 cm.

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