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Nematode larvae with zoonotic importance found in peacock bass *Cichla monoculus* (Spix & Agassiz, 1831) from floodplain lakes in Central Amazon

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Abstract. This study exhibits data pertaining to the zoonotic potential of larvae of nematode of peacock bass (*Cichla monoculus*). Thirty-eight specimens were collected from Central Amazon floodplain lakes throughout the four seasons of a complete hydrological cycle: rising, high, receding and low water level. Third-stage larvae (L3) of *Anisakis* sp. and *Contracaecum* sp. Nematode larvae were found at prevalences of 13.15%; 7.89%, mean intensity 2.6; 36.67, and mean abundance 0.34; 2.89 respectively. The results indicated there being infection hazard to humans consuming *C. monoculus* from floodplain lakes in Central Amazon.

Keywords: parasite, Anisakidae, zoonosis, fish, tucunaré.

Resumo. Larvas de nematóides de importância zoonótica encontradas em tucunarés *Cichla monoculus* (Spix & Agassiz, 1831) de lagos de várzea da Amazônia Central. No presente artigo dados sobre o potencial zoonótico de nematóides do tucunaré (*Cichla monoculus*) são apresentados. Trinta e oito espécimes com comprimento padrão médio de 22,86 ± 5,62 cm e o peso médio de 249,42 ± 162,38 g foram coletados em lagos de várzea da Amazônia Central ao longo das quatro estações de um ciclo hidrológico completo: enchente, cheia, vazante e seca. Larvas de anisakídeos de terceiro estágio L3 das espécies *Anisakis* sp. e *Contracaecum* sp. foram coletadas, apresentando prevalências de 13,15% e 7,89%, Intensidade média de 2,6 e 36,67 e abundância média de 0,34 e 2,89 respectivamente. Os resultados registraram a presença de nematoides com potencial zoonótico, o que serve de alerta para o risco de infecção em seres humanos na situação do consumo in natura de *C. monoculus*.

Palavras-chave: parasito, Anisakidae, zoonose, peixe, tucunaré.

Fish consumption has become an option for whoever may be looking for a healthier diet. Nevertheless, when eaten raw, undercooked or partially smoked, this type of food may turn into a public health burden, since among the parasitic infections, which are liable to be transmitted by fish, some have shown to be potentially harmful to human health (BATAIER *et al.* 2009).

What happens is that humans act as accidental hosts to a certain number of parasites. Larvae do not complete their development, but they can penetrate the digestive tract and invade the attached organs, causing quite a few unwanted pathological effects (LYMBERY & CHEAH 2007).

Anisakidosis or anisakiasis is human infestation with the larval stage of several nematode species of family Anisakidae. Species of *Anisakis* Dujardin, 1845, *Pseudoterranova* Mozgovoy, 1951, *Hysterothylacium* Ward and Magath, 1917 and *Contracaecum* Railliet and Henry, 1912 are the involved in the emergence of anisakidosis (CRUZ *et al.* 2010; HOCHBERG & HAMER 2010).

The species *Anisakis simplex* (Rudolphi, 1809) and *Pseudoterranova decipiens* (Krabbe, 1878) stand out on account of being responsible for the largest number of reported cases (MERCADO *et al.* 2001), caused by the ingestion of raw or undercooked fish or seafood (CRUZ *et al.* 2010; HOCHBERG & HAMER 2010).

This infestation occurs worldwide, with the highest incidence in coastal areas, with most cases (90%) registered in Japan (CDC 2013; HOCHBERG & HAMER 2010). Estuarine (DIONE *et al.*, 2014) and freshwater fish also get to be parasitized by these species which give rise to diseases in humans as well (MORAIS, 2012; MENEGUETI *et al.*, 2013).

In Brazil, one case of human anisakidosis caused by *Anisakis* sp. was diagnosed through

gastrointestinal endoscopy in a patient who had probably eaten raw shellfish. The exam indicated the presence of encysted third-stage larvae (L3) of *Anisakis* sp. in the stomach and intestine along with injuries caused by them (CRUZ *et al.*, 2010).

The first record on Anisakidae species parasitizing fish in Amazonia occurred when free *Anisakis* sp. third-stage larvae (L3) were found in the intestine of *Pygocentrus nattereri* (Kner, 1958) captured in Solimões River floodplain lakes (MORAIS, 2012). The second were in *Arapaima gigas* (Schinz, 1822) juveniles with 14.5cm ($\pm 2,10$) mean standard length and 32.6g (± 16.40) mean weight in a fish farm in the township of Rio Preto da Eva. The third-stage larvae (L3) of *Hysterothylacium* Ward & Magath, 1917 parasitized the intestine, stomach and pyloric caeca (ANDRADE-PORTO *et al.*, 2016).

Knowing that consuming raw fish has been increasing and that the Amazonian population has been using local fish for Japanese cuisine, among others, served in bars and restaurants in the region. The present study aims to investigate the presence of nematode larvae bearing zoonotic potential in *Cichla monoculus* (SPIX & AGASSIZ, 1831) captured in Central Amazon floodplain lakes.

Thirty-eight (38) specimens of *C. monoculus* collected from six floodplain lakes located in Solimões and Purus rivers during the months of March (rising water), June (high water), September (receding water) and December (low water), 2012, were examined. A set of eight

randomly arranged (30 x 100 mm) waiting for nets was utilized to collect the fish. These nets remained in the water for approximately 10 h in each lake in the daytime with two fish landings being performed.

After being euthanized, the fish were necropsied, their muscle and organs individualized and examined on the search for larvae and adults. Each collected parasite specimen was transferred to a Petri dish holding 0.65% saline solution for cleaning the individuals. Then, they were fixed in 70% ethanol heated at 65 ° C and transferred to vials containing ethanol 70° GL with 5% glycerin, where they were kept up to the preparation of histological slides (AMATO *et al.*, 1991; MORAVEC, 1998).

Nematode third-stage larvae (L3) of *Anisakis* sp. and *Contracaecum* sp. (Table 1) were found at prevalences of 13.15%; 7.89%, mean intensity 2.6; 36.67 and mean abundance 0.34; 2.89 respectively. Specimens of *Anisakis* sp. (Figure 1) were found free in the intestine

and encysted in the liver, while the cysts of *Contracaecum* sp. (Figure 2) were attached to the outer wall of the stomach. All Nematode were deposited in the invertebrate collection at INPA, with number INPA 79 and INPA 80. The presence of Anisakidae Nematoda larvae with low parasitary prevalence and abundance located only in the fish mesentery indicates a low zoonotic potential, with no human infection hazard (LUQUE & ALVES 2001). The same was found in this study, the *C. monoculus* from Central Amazon floodplain lakes represented a low risk for potential infections larvae of Anisakidae.

Although the nematodes are present in the abdominal cavity and the viscera, cannot rule out the risk of ingestion. Since these larvae have migration capability for the fish muscle and especially the immunogenic characteristics of these parasites (FONTENELLE *et al.*, 2013).

That migration can take place while the fish is still alive or even after it has been caught, especially throughout the long waiting periods in

Table 1. Mean values (mm) of *Anisakis* sp and *Contracaecum* sp. larvae parasite of *Cichla monoculus* from floodplain lakes in Central Amazon.

Characters	<i>Anisakis</i> sp.	<i>Contracaecum</i> sp.
Total lenght	14.17 (9.80 - 17.05)	4.28 (3.23 - 4.92)
Width	0.25 (0.19 - 0.35)	0.12 (0.09 - 0.16)
Ventriculus lenght	0.39 (0.28 - 0.47)	0.03 (0.03 - 0.04)
Ventriculus width	0.13 (0.09 - 0.17)	0.02 (0.01 - 0.04)
Nerve ring*	0.20 (0.19 - 0.21)	0.13 (0.12 - 0.15)
Intestinal caecum lenght	-	0.19 (0.15 - 0.21)
Oesophagus lenght	1.13 (0.81 - 1.18)	0.41 (0.36 - 0.52)
Tail	0.11 (0.06 - 0.24)	0.11 (0.10 - 0.13)

* distance from anterior end of body; excretory pore situate near to larval tooth.

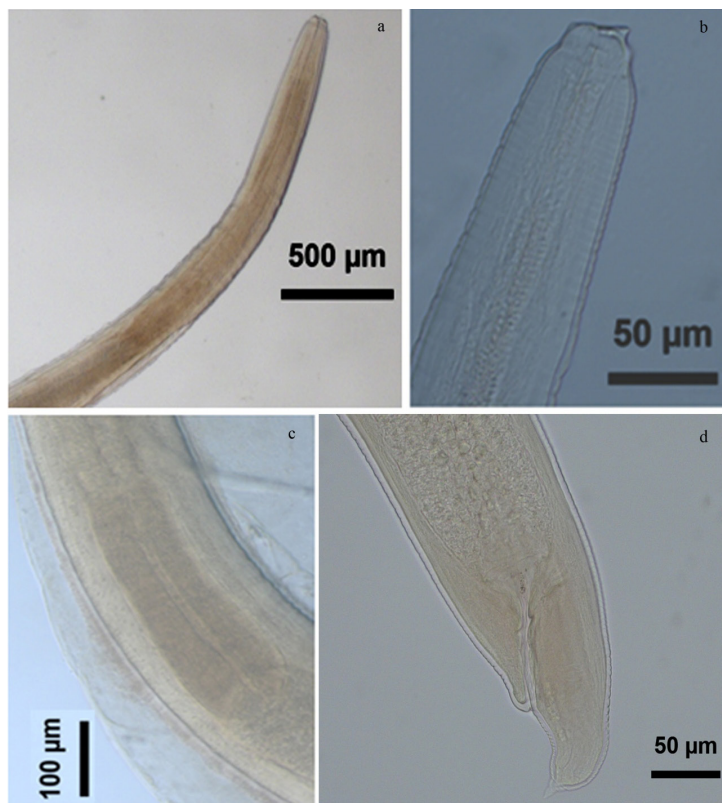


Figure 1. *Anisakis* sp. de *Cichla monoculus*. (a) Anterior end; (b) Larval tooth detail; (c) Ventriculus e (d) Posterior region showing conical tail.

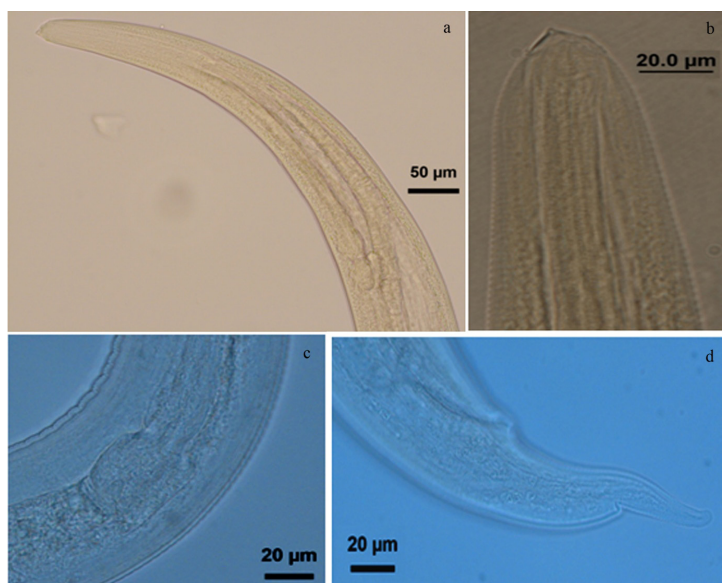


Figure 2. *Contraecaecum* sp. de *Cichla monoculus*. (a) Anterior end; (b) Larval tooth detail; (c) Ventriculus e (d) Posterior region showing tail.

the boats and warehouses where these fish are kept completely still with their viscera. Fish are recommended to be gutted while still on board so as to diminish the larval migration (MORAIS, 2012; RIBEIRO, 2012).

Given a large number of cases of anisakiasis being registered in the world, and with the use of local raw Amazonian fish in increasingly elaborate recipes and sought by the population, more research is needed to know the real risks of the zoonotic potential of larvae L3 of *C. monoculus*. Furthermore, prior education of cooks so they take more care at the time of preparation of the dishes, by selecting the fish that were frozen at the ideal temperature, as well as of consumers for selecting the fish that have been well inspected and properly cooked, minimizes the risk of disease.

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