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Larval *Contracaecum* sp. (Nematoda: Anisakidae) in *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus* (Osteichthyes: Erythrinidae) of economic importance in occidental marshlands of Maranhão, Brazil

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Abstract

This work describes the presence of *Contracaecum* sp. larvae (Nematoda: Anisakidae) found in food fish of economic importance captured in occidental marshlands in the State of Maranhão, Brazil. *Hoplias malabaricus* (Erythrinidae) and *Hoplerythrinus unitaeniatus* (Erythrinidae), commonly called ''traíra'' and ''jejú'', respectively, were examined. All *H. malabaricus* were infected with a mean intensity of 24.6 ± 38.3 (1–137 parasites per host) while eighty percent of *H. unitaeniatus* showed a mean intensity of 10.4 ± 9.2 (2–31) parasites. Nematodes were most similar to larvae of a type 2 described by Moravec, Kohn and Fernandes [Moravec, F., Kohn, A., Fernandes, B.M.M., 1993. Nematode parasites of fishes of the Paraná River, Brazil. Part 2. Seuratoidea, Ascaridoidea, Habronematoidea and Acuarioidea. Folia Parasitol. 40, 115–134], but differed in having a longer intestinal caecum and greater caecum/ventricular appendix ratio. Larval measurements and a description of their Brazilian fish hosts are presented.

Keywords: Nematoda; *Contracaecum*; *Hoplias malabaricus*; *Hoplerythrinus unitaeniatus*; Description; Brazil

1. Introduction

Nematode larvae of the genus *Contracaecum* are widely distributed in freshwater and marine fishes. They have been found in fish from Kotor Bay, Yugoslavia (Peter et al., 1984), the Northeast Baltic

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Sea (Valtonen et al., 1988; Rokicki et al., 1993), Hokkaido, Japan (Moravec et al., 1985), the Thames Estuary (El-Darsh and Whitfield, 1999), Mexico (Moravec et al., 1995; Valles-Rios et al., 2000), Venezuela (Moravec et al., 1997), the Southwest Atlantic (Timi et al., 2001), Nicaragua (Aguirre-Macedo et al., 2001), Northeastern Argentina (Hamann, 1999) and the South Shetland Islands (Zdzitowiecki, 2001). Adult parasites live in the stomach or small intestine of piscivorous birds. Freshwater copepods become infected when they ingest free-swimming second-stage larvae. The life cycle of *Contracaecum multipapillatum* showed that the guppy (*Lebistes reticulatus*) becomes infected when infected copepods are ingested (Huizinga, 1967). Larval stages are commonly found encapsulated in the mesenteries and visceral organs of a variety of marine and freshwater fishes. According to Lymbery et al. (2002) the high prevalence of *Contracaecum* in mullet may have implications for the health of the fish.

In Brazil, encysted larval nematodes of the genus *Contracaecum* have been reported in muscle or viscera in native fish. Little is known about the helminthological fauna of fish from the State of Maranhão. Vicente and Fernandes (1978) related the presence of *Contracaecum* sp. in *Macrodon ancylodon* collected from São Luís Island. Residents who had regularly eaten fish from these inundated fields complained about a massive presence of parasites. The region is composed of 21 counties with a total estimated human population of 480,000, many of whom use fish caught by hook or net as their main source of animal protein. The fishes used in this study are basically predators, eating insects and crustaceans, becoming essentially ichthyophagous in the adult stage (Pereira et al., 1981; Oliveros and Rossi, 1991; Bistoni et al., 1995). *Hoplias malabaricus* behaves as a zoophagous and euryphagic species, with fish constituting its main food at approximately 20 cm standard length. Insects are important for fish smaller than 5 cm. Crustaceans have lesser importance for both size groups, and the relative importance of each prey type varies with predator length (Bistoni et al., 1995). Both species have commercial importance as food fish, principally *H. malabaricus*, which reaches a larger size. However, *Hoplerythrinus unitaeniatus* is commonly used as bait for larger carnivorous fish.

This work describes the presence of *Contracaecum* sp. larvae in food fish captured in the State of Maranhão. Parasite specimens were measured and compared with other larvae of the same genus described from Brazil and elsewhere. Comparative measurements of larvae and a list of the Brazilian hosts are also presented.

2. Material and methods

All fish assessed were caught using nets from an inundated field of occidental marshlands in the State of Maranhão, Brazil. *H. malabaricus* (Erythrinidae) ($n = 10$) of 22.3 ± 4.1 cm total length and 123.5 ± 79.9 g mean weight and *Hoplerythrinus unitaeniatus* (Erythrinidae) ($n = 8$) of 18.1 ± 1.5 cm and 74.1 ± 24.8 g, commonly called ''traíra'' and ''jejú'', respectively, were maintained in ice for examination at the laboratory. The nematodes found in the fish were carefully dissected from the cysts located in muscles or mesentery. They were maintained in a 0.65% saline solution and later fixed in AFA 65 °C and preserved in 70% alcohol with 10% glycerine. After clearing in glycerine, by evaporation of alcohol or Faia creosote, 20 specimens from each host were drawn and measured using a camera lucida. Cephalic papillae were observed by Scanning Electron Microscopy and by light microscopic examination of en face mounts in glycerine gelatine. All measurements are given in millimeters with mean values followed by standard deviation and their variation in parentheses. For SEM, nematodes were fixed at room temperature in a 3% glutaraldehyde solution with a 0.1 M phosphate buffer (pH 7.4), post-fixed in 1% osmium tetroxide, dehydrated by serial concentrations of alcohol, critically point dried and sputter coated with 20 nm gold palladium. A JEOL JSM-5410 scanning electron microscope was used and identification was done according to Hartwich (1974), Moravec (1994) and Vicente and Pinto (1999). The prevalence of infection and mean intensity values were calculated according to Bush et al. (1997).

3. Results

A total of 246 nematodes were recovered from *H. malabaricus* showing a 100% prevalence and mean

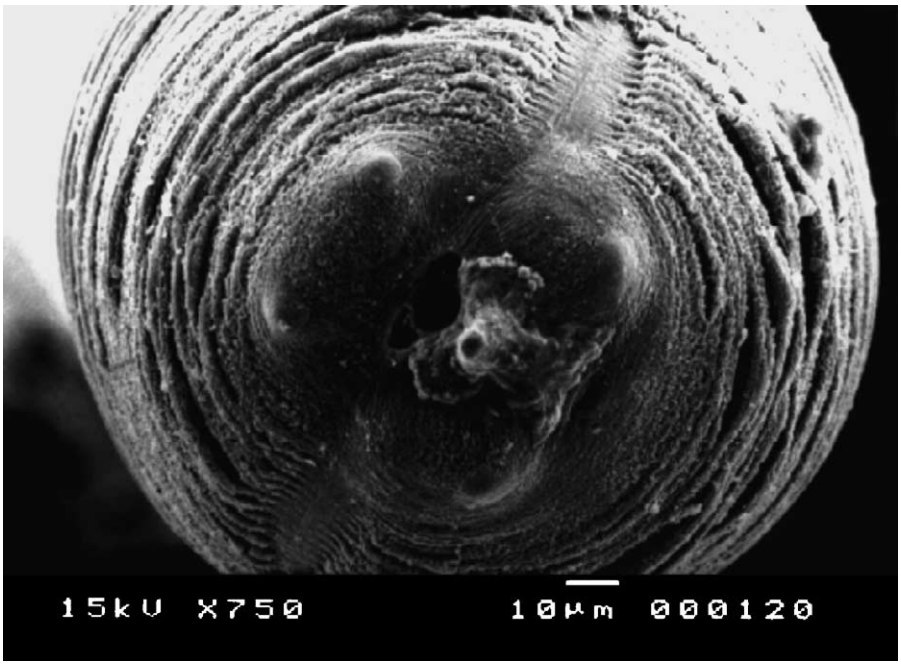

Fig. 1. *Contracaecum* larva from *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus*. SEM of anterior end.

infection intensity of 24.6 ± 38.3 (1–137). From *H. unitaeniatus* specimens, 83 nematodes were found with 80% parasite prevalence and a mean infection intensity of 10.4 ± 9.2 (2–31). All larvae recovered were identified as *Contracaecum* sp. Railliet and Henry, 1912 (Nematoda: Anisakidae), and were embedded in muscles or adhering to host visceral mesentery. Their appearance and dimensions were as follows.

3.1. General description

The body is of whitish colour, cuticle transversely striated, head end rounded. The mouth with three lips. The dorsal lip has two lateral papillae. The two ventro-lateral lips have a small papilla in each. Between these lips is situated a cephalic tooth (Fig. 1). Cervical alae are absent. One pair of deirids is situated at the level of nerve ring. The excretory pore opens at the head end slightly posterior to the larval tooth (Fig. 1). The oesophagus is narrow and longer than the ventricular appendix; the ventriculus is small (narrower than the oesophagus at its widest point); the ventricular appendix is relatively short. The anterior intestinal caecum is long, extending to the level of the nerve ring. The intestinal caecum is significantly longer than the ventricular appendix and slightly shorter than the oesophagus. The nerve ring surrounding the oesophagus is located at the first third of its length. The tail is conical and short (Figs. 2–4).

3.2. Contracaecum sp.

The body is 24.18 ± 4.46 (12.00–34.50) long by 0.87 ± 0.14 (0.60–1.03) wide. The maximum width at the ventriculus level 0.43 ± 0.08 (0.32–0.71). Between the ventro-lateral lips is situated a cephalic tooth 0.02 ± 0.004 (0.01–0.02) long. The cervical papillae and nerve ring are located 0.34 ± 0.04 (0.24–0.41) and 0.29 ± 0.05 (0.18–0.37), respectively, from the anterior end. The oesophagus is narrow, 2.21 ± 0.23 (1.87–2.61) long by 0.09 ± 0.01 (0.06–0.12) wide. The ventriculus is 0.09 ± 0.02 (0.06–0.12) long by 0.09 ± 0.01 (0.06–0.12) wide and separated from the oesophagus by a slight constriction. The ventricular appendix is posteriorly directed, 0.48 ± 0.07 (0.40–0.72) long by 0.09 ± 0.02 (0.06–0.15)

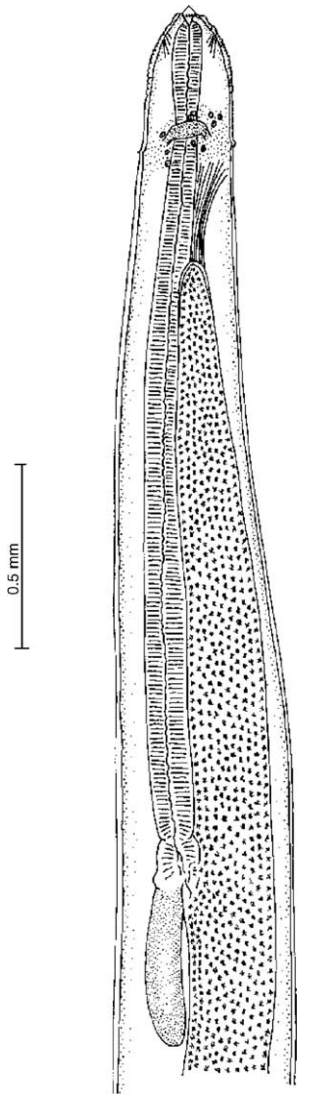

Fig. 2. *Contracaecum* larva from *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus*. Anterior end of body showing the intestinal caecum, ventricular appendix and the position of nerve ring and cephalic papilla.

wide. The intestinal caecum, is 1.61 ± 0.24 (1.20–2.15) long by 0.18 ± 0.03 (0.16–0.28) wide. The end of intestinal caecum is 0.67 ± 0.11 (0.44–0.83) from the anterior extremity. The ratio of the length of intestinal caecum and ventricular appendix is $1{:}0.30 \pm 0.05$ (1:0.22–1:0.41) and of the caecum and oesophagus $1{:}1.38 \pm 0.11$ (1:1.21–1:1.67). The tail is conical with 0.15 ± 0.03 (0.10–0.22).

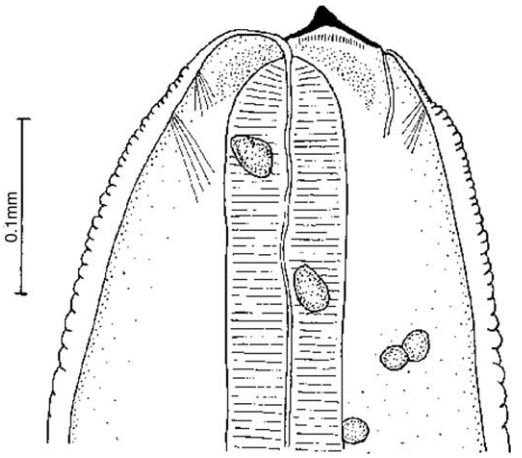

Fig. 3. *Contracaecum* larva from *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus*. Lateral view of cephalic end.

Hosts: *H. malabaricus* Bloch, 1794 and *H. unitaeniatus* Agassiz, 1829
Site of infection: muscles and mesentery
Locality: inundated fields of occidental marshland of Maranhão (5°0′S, 46°0′W)

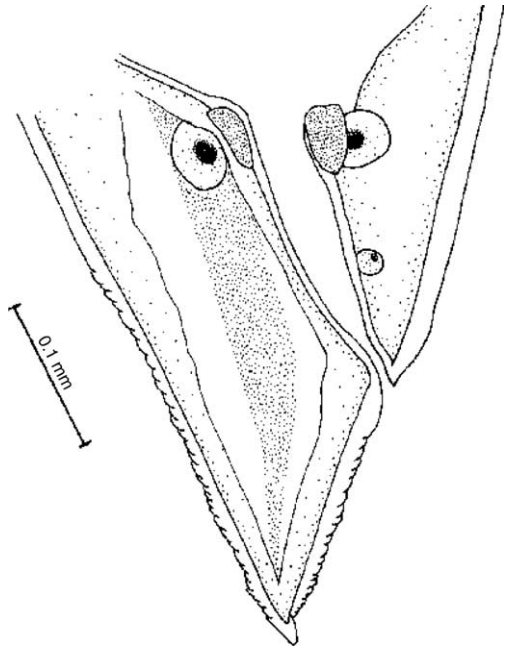

Fig. 4. *Contracaecum* larva from *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus*. Tail.

Specimens deposited at the Helminthological Collection of Oswaldo Cruz Institute (CHIOC), Av. Brasil 4365, 21045-900, Rio de Janeiro, Brazil. CHIOC no. 35107 a–f from *H. malabaricus* and CHIOC no 35108 a–f from *H. unitaeniatus*

4. Remarks

This work attains special importance because of concerns over larval *Pseudoterranova*, *Anisakis* and *Contracaecum* causing a parasitic syndrome in humans. Such larvae which are normally found in the intestinal mesentery or adhered to viscera, may travel to muscles after the host's death. Their preference for parasitizing visceral organs limits their zoonotic potential, but if fish are not frozen or filleted after capture, nematodes may migrate into the flesh, as suggested by Wharton et al. (1999). Thus, considering the potential public health importance of larval anisakids, we emphasize special care in the consump-

Table 1
List of the Brazilian fish parasitized by *Contracaecum* sp., geographic zone and authors

Hosts	Localities	Authors
Pimelodella lateristriga, Acestrorhamphus sp.	Pirassununga, SP	Travassos et al. (1928)
Astyanax bimaculatus, A. fasciatus, A. schubarti	Rio Mogi Guaçú, SP	Kloss (1966)
Scomberomorus cavalla, S. maculates	Mucuripe, Iguape, CE	Klein (1973) [a]
Harengula clupeola	Salvador, BA	Guimarães and Cristofaro (1974) [a]
A. fasciatus, Galeocharax humeralis, S. maxillosus, Hoplias malabaricus, Leporinus lacustris, L. copelandii	Mogi Guaçú River, Emas, SP	Burhnhein (1976)
Macrodon ancylodon	Paço do Lumiar, Vieira Beach, MA	Vicente and Fernandes (1978)
H. malabaricus	Campos, RJ	Fabio (1982)
Pomatomus saltatrix	Rio de Janeiro	Rego et al. (1983)
Acestrorhamphus macrolepis, Bergiaria sp., *Crenicichla lepidota, Geophagus brasiliensis, H. malabaricus, Pimelodus ortmanni*	Basin of Iguaçu River, PR	Kohn et al. (1988)
Rhamdia quelen	Passo Fundo Reservoir, RS	Kohn et al. (1989)
Pseudoplatystoma fasciatum	Várzea Grande, Barão de Melgaço, MT	Eiras and Rego (1989)
Serrasalmus altuvei	Basin of Negro River, Anavilhanas	Leão et al. (1991)
H. malabaricus	Santa Maria, RS	Weiblen and Brandão (1992)
G. humeralis, G. knerii, Rhaphiodon vulpinus, H. malabaricus, Plagioscion squamosissimus, C. lepidota	Paraná River, Foz do Iguaçú, PR	Moravec et al. (1993) [b]
R. vulpinus, Prochilodus scrofa, Pseudoplatystoma corruscans	Paraná River, Foz do Iguaçu, PR	Moravec et al. (1993) [c]
Pseudoplatystoma corruscans	Paraná River, Porto Rico, PR	Machado et al. (1996) [b,c]
Oligoplites palometa, O. saurus	Rio de Janeiro	Takemoto et al. (1996)
Lutjanus purpureos	Northeast Littoral of Brasil	Barros and Cavalcanti (1998)
Pomatomus saltator	Rio de Janeiro	Luque and Chaves (1999)
Cichla monoculus	Paraná River, Porto Rico, PR	Machado et al. (2000)
Caranx latus	Rio de Janeiro	Luque and Alves (2001)
Hexanchus griséus, Heptranchias perlo, Scyliorhinushaeckeli, Mystelus canis, M. schmitti, Galeorhinus vitaminicus, Carcharhinus brachyurus, C. signatus, Sphyrna zygaena, Dipturus trachyderma, Squatina sp.	Littoral of Rio Grande do Sul and Paraná	Knoff et al. (2001)
Selene setapinnis	Rio de Janeiro	Cordeiro and Luque (2002)
Acestrorhynchus lacustris	Paraná River, PR	Carvalho et al. (2002)
Brevoortia aurea	Rio de Janeiro	Tavares et al. (2002)
Geophagus brasiliensis	Vargem, SP	Madi and Ueta (2002)
Genypterus brasiliensis	Rio de Janeiro	Alves et al. (2002)
Ageneiosus brevifilis	Tocantins River, PA	Vasconcelos et al. (2002)
Hemisorubim platyrhynchus	Baía River, MS	Guidelli et al. (2003) [b,c]
Cichla ocellaris, P. squamosissimus, H. malabaricus	Paraná River, Presidente Epitácio, SP	Martins et al. (2003)

[a] *C. fortalezae*.
[b] *Contracaecum* type 1.
[c] *Contracaecum* type 2.

tion of fish from that area. In humans, the presence of larvae (L_3–L_4) in the gastrointestinal tract causes pain, diarrhoea, nausea and vomiting, according to Bouree et al. (1995). The larvae of *Contracaecum* have been shown to be infective to mammals (Vidal-Martinez et al., 1994).

The wide susceptibility of Brazilian fish species to *Contracaecum* larvae, for both freshwater and marine fish species, is shown in Table 1. The prevalence of *Contracaecum* larvae reported in this work was higher than observed in fish from the Thames Estuary (8%) by El-Darsh and Whitfield (1999); from Mexico (30%) by Valles-Rios et al. (2000); from the Coast of Nicaragua (33%) by Aguirre-Macedo et al. (2001) and in Brazilian fish species with reports of 64.4% (Fabio, 1982); 42% (Rego et al., 1983); 66.9% (Weiblen and Brandão, 1992); 78% (Barros, 1994); 8.2% (Machado et al., 1996); 26.2% (Takemoto et al., 1996); 20.7% (Barros and Cavalcanti, 1998); 21.8% (Luque and Chaves, 1999); 18.2% (Luque and Alves, 2001) and

Table 2
Comparative measurements (mm) of larvae of *Contracaecum* from *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus* from Maranhão, Brazil and other descriptions in fish

Characters	Present work	Moravec et al. (1985)	Rokicki et al. (1993)	Moravec et al. (1993), Tipo 1	Moravec et al. (1993), Tipo 2	Moravec et al. (1995), Tipo 1[a]
Total length	12.00–34.50	14.28	11.32–22.53	3.89–4.80	15.70–25.70	4.88–5.49
Maximum width	0.60–1.03	0.408	0.385–0.693	0.150–0.313	0.449–0.843	0.218–0.231
Oesophagus (L)	1.87–2.61	1.320	0.847–1.430	0.537–0.693	1.970–2.110	0.503–0.680
Oesophagus (W)	0.06–0.12	–	0.055–0.088	–	–	–
Nerve ring[b]	0.18–0.37	0.367	0.264–0.361	0.204–0.225	0.313–0.381	0.204–0.245
Ventriculus (L)	0.06–0.12	–	0.050–0.100	0.024–0.033	0.063	0.030–0.036
Ventriculus (W)	0.06–0.12	–	0.044–0.111	0.033–0.036	0.081	0.030–0.036
Ventricular appendix (L)	0.40–0.72	1.250	0.891–1.540	0.510–0.721	0.462–0.503	0.449–0.503
Ventricular appendix (W)	0.06–015	–	0.088–0.154	–	–	–
Intestinal caecum (L)	1.20–2.15	0.750	0.561–0.935	0.310–0.476	1.500–1.580	0.403–0.449
Intestinal caecum (W)	0.16–0.28	–	0.066–0.121	–	–	–
Ratio cacum/appendix	1:0.22–1:0.41	–	1:0.58	1:1.50–1.70	1:0.30	1:1.00–1.18
Ratio caecum/oesophagus	1:1.21–1:1.67	–	–	–	–	–
Tail[c]	0.10–0.22	0.147	0.095–0.275	0.078–0.126	0.095	0.122–0.136

	Moravec et al. (1995), Tipo 1[d]	Moravec et al. (1995), Tipo 2	Moravec et al. (1997), Tipo 1	Moravec et al. (1997), Tipo 2	Moravec et al. (1997), Tipo 3	Timi et al. (2001), Tipo 3
Total length	3.94–4.68	20.40–23.53	3.94	22.03–24.01	9.09	4.06–5.82
Maximum width	0.218–0.258	0.680–0.775	0.150	0.680–0.762	0.354	0.160–0.240
Oesophagus (L)	0.625–0.775	–	0.394	2.260–2.630	0.993	0.400–0.630
Oesophagus (W)	–	–	–	–	–	–
Nerve ring[b]	0.190–0.218	0.299–0.326	0.135	0.326–0.394	0.231	0.160–0.240
Ventriculus (L)	0.030–0.039	0.082–0.095	0.018	0.109	0.068	–
Ventriculus (W)	0.039–0.045	0.082–0.109	0.024	0.503	0.054	–
Ventricular appendix (L)	0.449–0.666	0.476–0.665	0.309	0.490–0.503	0.449	0.300–0.430[e]
Ventricular appendix (W)	–	–	0.042	0.095–0.109	–	–
Intestinal caecum (L)	0.435–0.598	1.720–1.930	0.207	1.560–1.990	0.707	0.210–0.340
Intestinal caecum (W)	–	–	0.045	–	–	–
Ratio caecum/appendix	1:1.03–1.11	1:0.30	1:1.49	1:0.24–0.39	1:0.64	–
Ratio caecum/oesophagus	–	–	–	–	–	–
Tail[c]	0.109–0.136	–	0.108	0.109–0.190	0.136	0.09–0.13

L: total length; W: width.
[a] Host: *Astyanax fasciatus*.
[b] Distance from anterior end.
[c] Distance of anus to posterior end.
[d] Host: *Rhamdia guatemalensis*.
[e] Ventricular appendix plus ventriculus length.

11% (Alves et al., 2002) parasitic prevalence. However, the results found here were similar to those seen by Kohn et al. (1988) in fish from the Iguaçu river; and by Machado et al. (2000) and Martins et al. (2003) in fish from two locations on the Paraná river, County of Presidente Epitácio; by Zdzitowiecki (2001) in fish from South Shetland Island and those reported by Lymbery et al. (2002) in fish from south-western Australia and by Guidelli et al. (2003) in fish from the upper Paraná River floodplain. Different strategies of transmission were discussed when Guidelli et al. (2003) observed negative (in *Contracaecum* type 1) and positive (in *Contracaecum* type 2) correlations between size and prevalence of these larvae in fish. In this work, the two fish species occupy a similar position in the food web, being preyed upon by birds.

Taxonomic characteristics important for identifying anisakid larvae have included appearance of cephalic papillae, location of the excretory pore, the presence of ventricular appendices and intestinal caecum (Rego et al., 1983). Measurements used in identification of anisakid larvae must include total length, presence of cephalic tooth, oesophagus length, presence of cervical papilla and the ratio of the length of the intestinal caecum to the ventricular appendix and oesophagus (Table 2). The presence of a cephalic tooth was in accordance with the *Contracaecum* larvae described by Peter et al. (1984), Rokicki et al. (1993), Moravec (1994), Moravec et al. (1985, 1995, 1997) and Timi et al. (2001). Specimens described here are similar in length to L_3 larvae found by Moravec et al. (1985) and Rokicki et al. (1993); and also by Moravec (1994) in *C. oscullatum* and *C. rudolphii*, although they differ in having a longer oesophagus and caecum, shorter ventricular appendix and lower caecum/appendix ratio. The similarity of the larvae described here to advanced L_3 of *C. rudolphii* (Moravec, 1994) should be noted. In this case, the main differences observed were a greater ventriculus diameter and greater distance of the nerve ring from the anterior end. The specific identification of the *Contracaecum* larvae in the present work was not possible because of the lack of identification of a definitive host.

Finally, when this material was compared to *Contracaecum* larvae type 2 described by Moravec et al., 1993, all measurements were found to be similar except for the presence of cervical papilla and the longer intestinal caecum of *Contracaecum* larvae from inundated fields of Maranhão State, Brazil. Further studies should be carried out to determine the extent of susceptible fish hosts, parasitic seasonality and their presence in birds.

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