



Revista mexicana de biodiversidad

ISSN: 1870-3453

ISSN: 2007-8706

Instituto de Biología

Cárdenas, Melissa Q.; Fernandes, Berenice M. M.;  
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Revista mexicana de biodiversidad, vol. 89, no. 3, 2018, pp. 631-636  
Instituto de Biología

DOI: 10.22201/ib.20078706e.2018.3.2397

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## Taxonomy and systematics

### On two species of *Ichthyouris* (Nematoda: Pharyngodonidae) parasites of *Mesonauta festivus* (Pisces: Cichlidae) in the Brazilian Amazon region

#### *Sobre dos especies de Ichthyouris (Nematoda: Pharyngodonidae) parásitas de Mesonauta festivus (Pisces: Cichlidae) en la Amazonía brasileña*

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Received: 19 July 2017; accepted: 26 February 2018

#### Abstract

Two species of nematodes belonging to the genus *Ichthyouris* Inglis, 1962 (*I. ro* and *I. bursata*), were collected from the cichlid fish *Mesonauta festivus* in the central Brazilian Amazon region. Both species are reported in this locality for the first time. *I. bursata* is reported in a new host, *M. festivus*, and this is the first report of *I. ro* parasitizing its type host in Brazil. We provide a more detailed description for *I. ro*, including morphological aspects that were not mentioned in the original description.

**Keywords:** Biodiversity; Nematodes; Fish; Amazonas State; Brazil

#### Resumen

Se recolectaron 2 especies de nemátodos pertenecientes al género *Ichthyouris* Inglis, 1962, *I. ro* e *I. bursata*, del pez cíclido *Mesonauta festivus* en el centro de la Amazonía brasileña. Ambas especies se reportan por primera vez en esta localidad. *I. bursata* se registra en un nuevo hospedero, *M. festivus*, y éste es el primer informe de *I. ro* parasitando a su hospedero tipo en Brasil. Se da una descripción detallada de *I. ro*, incluyendo aspectos morfológicos que no fueron mencionados en la descripción original.

**Palabras clave:** Biodiversidad; Nemátodos; Peces; Estado Amazonas; Brasil

#### Introduction

*Mesonauta festivus* (Heckel, 1840) (Perciformes: Cichlidae) is a freshwater benthopelagic fish commonly named “acará”. It belongs to the family Cichlidae, which

is the most species-rich non-ostariophysan freshwater fish family worldwide (Kullander, 1998). It is a small fish that can reach up to 10 cm in length. It feeds primarily on filamentous algae that commonly occur with floating vegetation on the water surface (Froese & Pauly, 2016;

Kullander, 2003; Santos et al., 2006). The geographical distribution of this fish species includes freshwaters of South America: Paraná river basin, in the portion drained by the Paraguay river in Brazil and Paraguay; and Amazon river basin, in the portions drained by the following rivers: Guaporé (Brazil and Bolivia), Madre de Dios (Peru), Mamoré (Bolivia) and Jamari and Tapajós (Brazil) (Froese & Pauly, 2016). So far, the only report of helminths in *M. festivus* comes from the original description of *Ichthyouris ro* by Inglis (1962), from Guyana (Amazon region).

To date, the genus *Ichthyouris* Inglis, 1962, includes 6 species, all from South American freshwater fish. The first report of this genus in the Brazilian Amazon region was the species *Ichthyouris ovifilamentosa* Moravec and Thatcher, 2001 parasitizing *Cichlasoma* sp. (Moravec & Thatcher, 2001).

The aims of this paper were to present new morphological data on *I. ro*, considering that the original description by Inglis (1962) lacked detail; and to report *M. festivus* as a new host for *Ichthyouris bursata* Moravec and Prouza, 1995.

## Material and methods

During July 2009, 87 specimens of *M. festivus* (Heckel, 1840) (Perciformes: Cichlidae) from 5 localities along of 400-km stretch of the Solimões river, between the cities of Manaus and Coari, state of Amazonas, Brazil, were examined for helminths. The nematodes collected were washed in physiological saline (0.9% NaCl solution) and fixed in hot AFA (2% glacial acetic acid, 3% formaldehyde and 95% ethanol at 70 °GL).

For light microscopical examination (LM), the nematodes were cleared in phenol. Observations were made using a Zeiss Axioscope 2 light microscope equipped with a camera lucida. Photomicrographs were taken using the same microscope. All measurements were made in millimeters, and the range is presented followed by the mean in parentheses. For scanning electron microscopy (SEM), specimens of *I. ro* were fixed in hot AFA, washed in 0.1 M cacodylate buffer (pH 7.2) and postfixed in a solution containing 1% osmium tetroxide and 0.8% potassium ferricyanide (pH 7.2) for 1 hour and subsequently dehydrated through a graded ethanol series, critical-point dried and sputter-coated with gold. They were examined at the "Plataforma de Microscopia Eletrônica Rudolf Barth" of the "Instituto Oswaldo Cruz" (Fiocruz, Rio de Janeiro), using a JEOL JSM-6390 LV scanning electron microscope at an accelerating voltage of 30 kV. The fish names follow FishBase (Froese & Pauly, 2016).

The prevalence and intensity of infection were estimated for our focal species in relation to the entire

sample of *M. festivus*. Calculations for the infection parameters of prevalence and intensity of infection were based on Bush et al. (1997).

## Results

Family Pharyngodonidae Travassos, 1919

*Ichthyouris ro* Inglis, 1962

Redescription (Figs. 1-5)

Small nematodes with cuticle densely transversely striated. Narrow lateral alae initiating anteriorly near anterior extremity and extending posteriorly to caudal alae in males and at level of anus in females. Oral aperture hexagonal, provided with 6 well-developed oral lamellae (2 dorsal, 2 ventral and 1 lateral on either side), arising from its inner surface. Four rather large spherical cephalic papillae and 1 pair of smaller lateral papilla-like amphids. Oral apparatus differs between sexes: simpler in male than in female. Esophagus formed by long, almost cylindrical corpus with slightly outlined "pharynx", a very short isthmus and well-developed bulb. Nerve ring situated in anterior part of corpus. Excretory pore at some distance posteriorly to esophagus. Tail very long, narrowing posteriorly.

Female (10 specimens). Length of body including caudal spike 2.330-3.425 (2.792), maximum width 0.190-0.275 (0.223). Cuticular striations at middle part of body 0.005-0.007 (0.006) long. Lateral alae very narrow. Anterior end of esophagus forming short, slightly outlined "pharynx", which anterior end is modified as cuticular edges, like a teeth in lateral view. Buccal cavity 0.015-0.032 (0.028) long and 0.015-0.020 (0.017) wide. Entire esophagus 0.470-0.570 (0.510) long; corpus including "pharynx" 0.380-0.630 (0.436) long and 0.050-0.062 (0.053) wide, "pharynx" 0.017-0.030 (0.023) long; isthmus 0.005-0.010 (0.006) long and 0.017-0.027 (0.026) wide; bulb 0.100-0.125 (0.109) long and 0.110-0.150 (0.125) wide. Nerve ring and excretory pore 0.125-0.142 (0.133) and 0.500-0.740 (0.603), respectively, from anterior extremity. Vulva slightly anterior half of the body, 0.780-1.135 (1.047) from anterior end. Uterus prodelphic. Muscular vagina directed anteriorly from vulva. One ovary anterior to level of vulva; other one posterior to it. Uterus containing numerous oval eggs, 0.077-0.092 (0.087) × 0.025-0.032 (0.028). Each egg pole provided with 1 very long filament. Entire tail 0.670-0.940 (0.797) long; length of slender caudal spike 0.520-0.730 (0.628).

Male (10 specimens). Length of body including caudal spike 0.900-1.450 (1.212), maximum width 0.070-0.100 (0.075). Striae at middle of body 0.002 long in all specimens studied. Anterior end of esophagus forming short, slightly outlined "pharynx", without structures like a teeth in

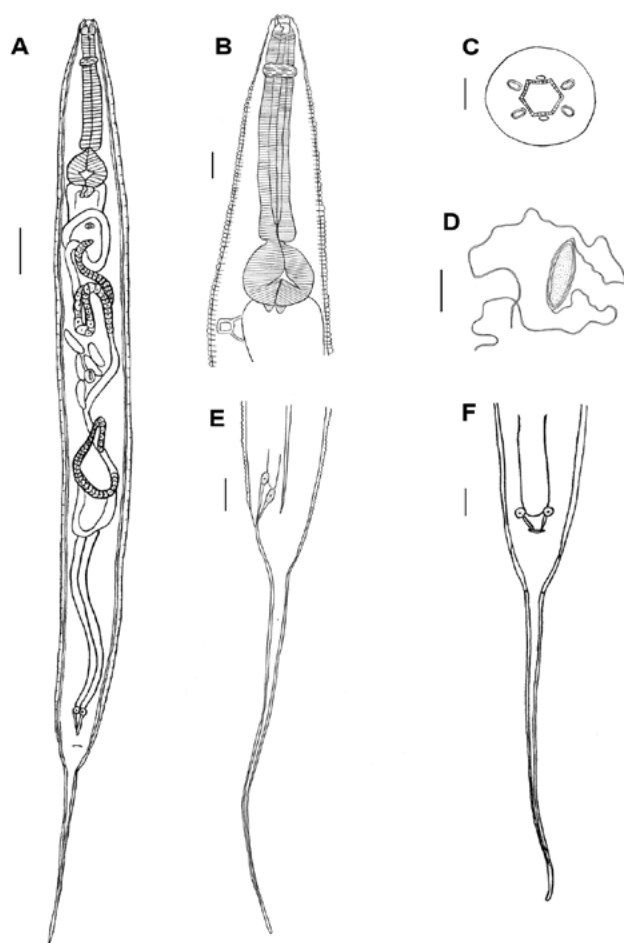


Figure 1. *Ichthyouris ro* Inglis, 1962. A, Female, general view; B, anterior end of female, lateral view; C, cephalic end of female, apical view; D, egg; E, tail of female, lateral view; F, tail of female, ventral view. Scale-bars: A= 1.5 mm; B, E, F= 0.5 mm; C= 0.01 mm; D= 0.05 mm.

lateral view. Entire esophagus 0.245-0.300 (0.280) long; corpus including “pharynx” 0.177-0.222 (0.206) long and 0.020-0.025 (0.022) wide; isthmus 0.070-0.012 (0.010) long and 0.015-0.020 (0.016) wide; bulb 0.055-0.070 (0.063) long and 0.055-0.072 (0.061) wide. Nerve ring and excretory pore 0.087-0.135 (0.108) and 0.350-0.460 (0.393), respectively, from anterior extremity. Genital cone rudimentary. Tail provided with relatively short, wide membranous lateral caudal alae. Preanal papillae: 1 pair of large, elongate, ventral posteriorly oriented papillae just anterior to level of cloacal opening. Postanal papillae: 2 pairs of small ventral papillae oriented anteriorly; 1 pair of subventral elongated papillae oriented posteriorly and 1 pair of small sessile papillae lying just posterior to more posterior pair of long papillae. Caudal alae present from

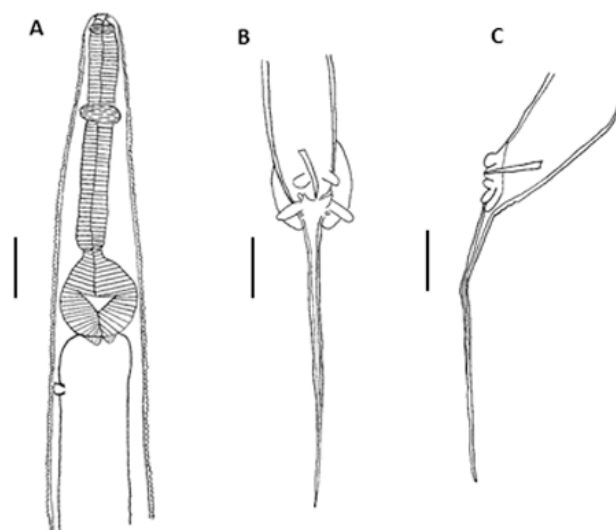


Figure 2. *Ichthyouris ro* Inglis, 1962. A, Anterior end of male, lateral view; B, caudal end of male, ventral view; C, caudal end of male, lateral view. Scale-bars: A, B, C= 0.05 mm.

anterior preanal papillae to posteriorly sessile papillae. Single spicule, well sclerotized 0.040-0.050 (0.046) long and 0.002-0.005 (0.003) wide, its distal tip pointed. Entire tail 0.265-0.300 (0.290) long; its broader anterior part 0.050-0.100 (0.067) long, length of slender caudal spike 0.200-0.225 (0.215). Tail forming 21- 29% (24%) of entire length of body.

#### Taxonomic summary

*Host:* *Mesonauta festivus* (Heckel, 1840) (Perciformes: Cichlidae).

*Localities:* Lake Catalão (03° 09'47" S, 059° 54'29" W), Manaus municipality, Lake of Baixio (03°17'27,2" S, 60°04'29,6" W), Iranduba municipality; Lake Preto (03°21'17,1" S, 60°37'28,6" W), Manacapuru municipality; Lake Ananá (03°53'54,8" S, 61°40'18,4" W), Anori municipality and Lake Maracá (03°50'32,8" S, 62°34'32,4" W), Coari municipality. All localities in Amazonas state, Brazil, occurring in an approximately 400-km long stretch on the Solimões river floodplain, around the municipalities of Manaus and Coari.

*Material deposited:* 7 males and 8 females were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC n° 38504).

*Prevalence:* 15 out of 87 (17.24%).

*Intensity of infection:* 2-5.

*Site of infection:* intestine.

#### *Ichthyouris bursata* Inglis, 1962

*I. bursata* was well described and redescribed by Moravec and Prouza (1995) and Moravec and Laoprasert

(2008), respectively. Therefore, in this study only the main measurements are presented, in order to compare possible morphometric variations according to different hosts, since *M. festivus* represents a new host record for *I. bursata*.

Female (10 gravid females). Length of body including caudal spike 1.120-3.130 (2.305); maximum width 0.140-0.350 (0.236). Cuticular striations at middle part of body 0.005-0.010 (0.006) long. Esophagus including bulb 0.375-0.410 (0.393) long; corpus 0.267-0.292 (0.079) long, 0.035-0.042 (0.039) wide; isthmus 0.007-0.012 (0.009) long 0.020-0.027 (0.021) wide. Bulb 0.090-0.110

(0.103) long, 0.100-0.117 (0.111) wide. Nerve-ring 0.125-0.142 (0.135) from anterior extremity. Excretory pore 0.440-0.630 (0.530) from anterior end of body. Vulva pre-equatorial, 0.760-1.150 (0.810) from anterior end. Vagina directed posteriorly from vulva. Eggs oval, thin-walled, 0.077-0.100 (0.091)  $\times$  0.017-0.040 (0.038); each egg pole provided with 1-2 long filaments. Entire tail 0.375-0.590

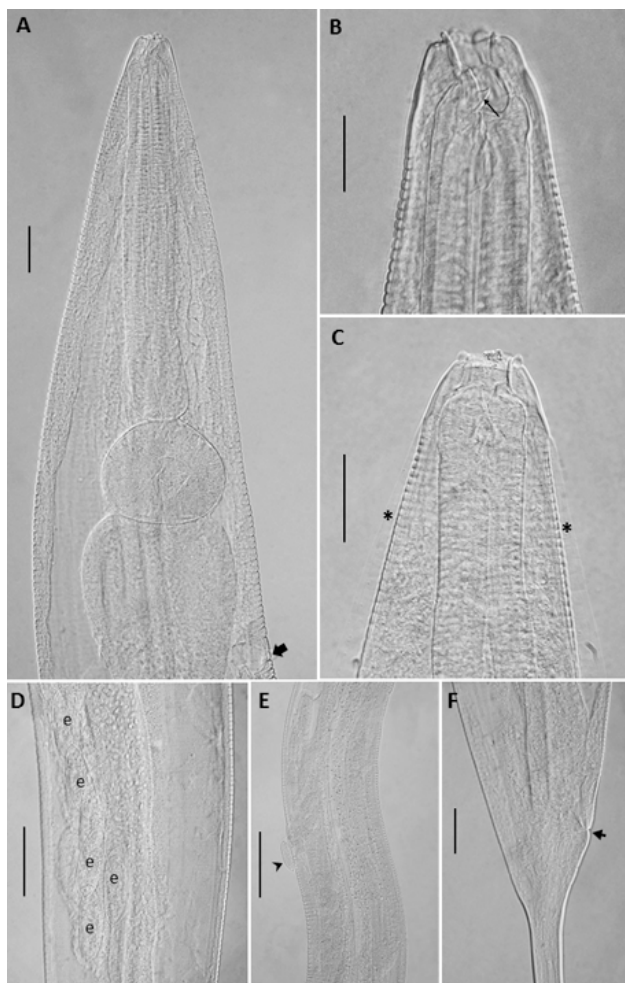


Figure 3. *Ichthyouris ro* Inglis, 1962. LM micrographs (differential interference contrast) of female. A, Anterior region, lateral view (arrow: excretory pore); B, detail of anterior region, lateral view, showing the teeth-like structure (thin arrow); C, anterior region in ventral view (asterisk: lateral alae); D, uterus bearing many eggs (e); E, egg leaving the uterus through vulva (arrow-head); F, posterior region (lateral view) showing anus (arrow). Scale-bars: A, B, C= 0.5 mm; D= 0.085 mm; E= 0.154 mm; F= 0.5 mm.

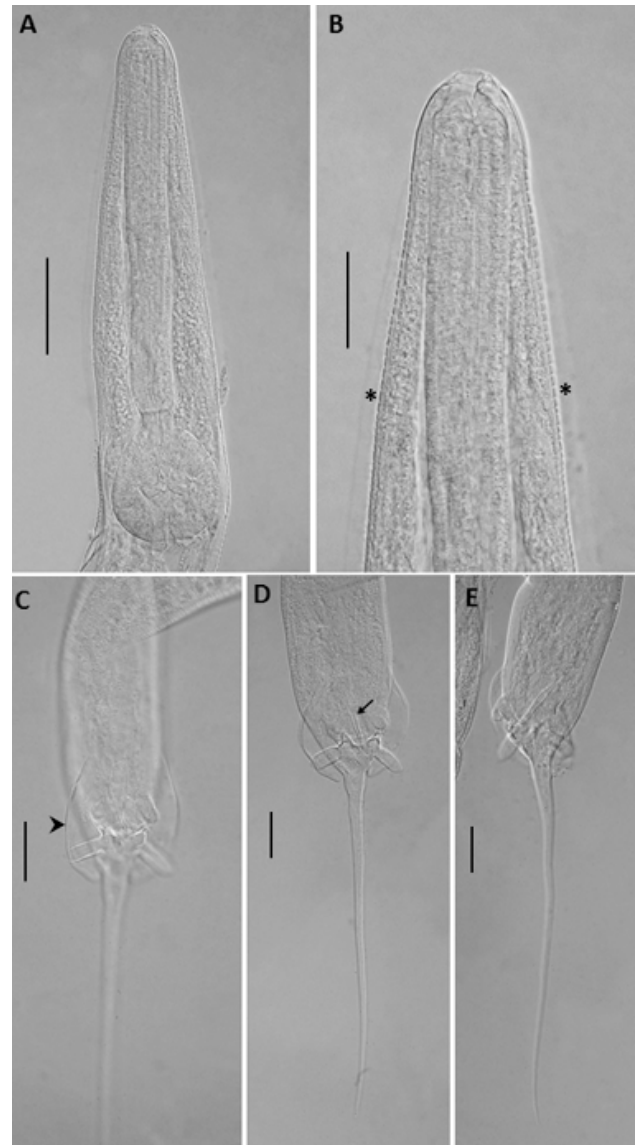


Figure 4. *Ichthyouris ro* Inglis, 1962. LM micrographs (differential interference contrast) of male. A, Anterior region, ventral view; B, detail of anterior region showing the lateral alae (asterisk); C, D, posterior region of male, ventral view (arrowhead: lateral alae; thin arrow: spicule); E, posterior region of male, lateral view. Scale-bars: A= 0.05 mm; B= 0.32 mm; C= 0.03 mm; D= 0.27 mm; E= 0.028 mm.

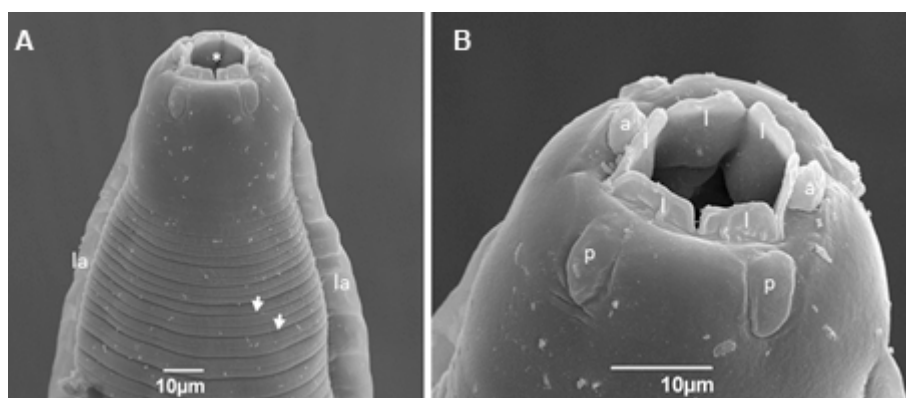


Figure 5. *Ichthyouris ro* Inglis, 1962. SEM micrographs of female. A, Anterior end of body, showing hexagonal oral aperture (asterisk), lateral alae (la) and cuticular striations (arrow); B, cephalic end provided with 6 well-developed oral lamellae (l), cephalic papillae (p) and 1 pair of papilla-like amphid (a). Scale-bars: A, B= 10 µm.

(0.450); length of slender caudal spike 0.200-0.300 (0.240).

Male (10 specimens). Length of body including caudal spike 0.950-1.520 (1.300); maximum width 0.070-0.120 (0.090). Cuticular striations at middle part of body 0.002 long. Esophagus including bulb 0.205-0.245 (0.221); corpus 0.120-0.170 (0.140) long, 0.017-0.027 (0.021) wide; isthmus 0.010-0.017 (0.012) long, 0.010-0.017 (0.014) wide. Bulb 0.055-0.067 (0.061) long, 0.055-0.067 (0.060) wide. Nerve-ring 0.062-0.117 (0.088) from anterior extremity. Excretory pore 0.320-0.480 (0.390) from anterior end of body. Sclerotized spicule 0.050-0.052 (0.052) long. Genital papillae represented by 1 pair of large pre-anal papillae and 3 postanal papillae (1 pair of subventral papillae situated posterior to cloaca, 1 pair of large postanal papillae and 1 pair of papillae-like phasmids). Length of tail, including slender caudal spike, 0.072-0.120 (0.071).

#### Taxonomic summary

*New host:* *Mesonauta festivus* (Heckel, 1840) (Perciformes: Cichlidae).

*Localities:* Lake Catalão (03°09'47" S, 59°54'29" W), Manaus municipality, Lake of Baixio (03°17'27,2" S, 60°04'29,6" W), Iranduba municipality; Lake Preto (03°21'17,1" S, 60°37'28,6" W), Manacapuru municipality; Lake Ananá (03°53'54,8" S, 61°40'18,4" W), Anori municipality and Lake Maracá (03°50'32,8" S, 2°34'32,4" W), Coari municipality. All localities in Amazonas state, Brazil.

*Material deposited:* 7 males and 8 females were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC n° 38503).

*Prevalence:* 23 out of 87 (26.43%).

*Intensity of infection:* 2-7.

*Site of infection:* intestine.

#### Discussion

To date, the genus *Ichthyouris* comprises 6 species. *I. ro*, the type species of the genus, was originally described from the intestine of *M. festivus* (= *Cichlasoma festivum*) from Guyana (formerly British Guiana). Four species have been described from Brazil: *Ichthyouris brasiliensis* Moravec, Kohn and Fernandes, 1992, from the catfish *Megalancistrus aculeatus* (Peters, 1881); *Ichthyouris laterifilamenta* Moravec, Kohn and Fernandes, 1992, from the catfish *Trachydoras paraguayensis* (Eigenmann and Ward, 1907); *Ichthyouris voltagrandensis* Martins, Yoshitoshi and Umekita, 2001, from *Myleus tiete* (Eigenmann and Norris, 1900); and *Ichthyouris ovifilamentosa* Moravec and Thatcher, 2001, from *Cichlasoma* sp. *Ichthyouris bursata* Moravec and Prouza, 1995, was described from the aquarium-reared discus *Symphysodon discus* Heckel, 1840, in Europe (host apparently originally introduced together with fish from South America) (Inglis, 1962; Martins et al., 2001; Moravec et al., 1992a, b; Moravec & Prouza, 1995; Moravec & Thatcher, 2001).

The type material of *I. ro* was described in South America from the intestine of *M. festivus* (= *Cichlasoma festivum*) from Guyana. Subsequently, it was reported in Peru parasitizing *Geophagus steindachneri* Eigenmann and Hildebrand, 1922 (Tantaleán et al., 1985). The present study provides the first report of this species in Brazil parasitizing the type host.

In the original description, Inglis (1962) described the anterior region of the female as having 2 structures similar to teeth, in dorsal view. In the specimens studied here, only 1 tooth was visible in lateral view. Inglis presented measurements of  $0.036 \times 0.011$  mm for a single egg, and no filament was mentioned; while in our material the eggs are larger than in the type material ( $0.087 \times 0.028$ ) and present 1 long filament at each pole. In addition, we also described other important morphological features that were not previously included in the original description, such as buccal cavity and details of the esophagus and pharynx.

After the original description of *I. bursata*, it was redescribed by Moravec and Laoprasert (2008) from *Symphysodon* spp. and hybrids, and from *S. discus* in Thailand. In the same paper, these authors reported *I. bursata* parasitizing *S. aequifasciatus* from an unknown locality in Brazil. In the present paper, we present the first record of *I. bursata* in *M. festivus*, which represents a new host for this nematode. The specimens described here are in agreement with the original description by Moravec and Prouza (1995).

The present study contributes with new morphological data, and it increases knowledge of biodiversity besides expanding the geographic distribution of nematode parasites of fish.

## Acknowledgements

The authors are grateful to the FINEP/PETROBRAS PIATAM project (“Projeto Inteligência Socioambiental Estratégica da Indústria do Petróleo na Amazônia”), FAPEAM (“Fundação de Amparo à Pesquisa do Estado do Amazonas”), INPA/MCT, UNINORTE (Laureate Universities) and FAPERJ (“Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro”). To Dr Suzana Côrte-Real and Roger Magno Macedo-Silva, from “Plataforma de Microscopia Eletrônica Rudolf Barth” (IOC-Fiocruz) for allowing use of the scanning electron microscope.

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