

Gnathostomatidae nematode parasite of *Colomesus psittacus* (Osteichthyes, Tetraodontiformes) in the Ilha de Marajó, Brazilian Amazon

Nematoide Gnathostomatidae parasito de *Colomesus psittacus* (Osteichthyes, Tetraodontiformes) na Ilha de Marajó, Amazônia Brasileira

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Abstract

The genus *Gnathostoma* comprises 17 species, whose adult specimens are found in the stomach serosa of animals that consume raw fish; some species of the genus are zoonotic agents. The present study describes the presence of a nematode (Gnathostomatidae) parasitizing the digestive tract of *Colomesus psittacus* in the Ilha de Marajó in the eastern Brazilian Amazon. Thirty specimens of *C. psittacus* were collected in the municipality of Soure, Ilha de Marajó, state of Pará, Brazil, transported to the laboratory, necropsied and the helminths were collected and fixed. Of the 30 fish that were studied, 16.67% were parasitized with nematodes. The nematode larvae found encysted in the intestinal serosa have anterior region with two lips, each with a pair of papillae; a cephalic bulb armed with six rows of discontinuous spines; four cervical sacs; a claviform esophagus; cuticular striations along the body; a simple excretory pore; and a short tail ending in a mucron. These morphological structures are diagnostic characters of the genus *Gnathostoma*, whose adults parasitize the stomach of carnivorous mammals and, rarely, the stomach of fish. However, fish, amphibians, reptiles, and birds are intermediate hosts of the third-stage larvae (L3), and humans may act as accidental hosts.

Keywords: *Gnathostoma*, parasitism, estuary, puffer fish.

Resumo

O gênero *Gnathostoma* é composto por 17 espécies, sendo reportado o caráter zoonótico para algumas. Este trabalho descreve a presença de nematoide da família Gnathostomatidae, parasito do trato digestório de *Colomesus psittacus*, na Ilha de Marajó, Amazônia oriental brasileira, transportados para o laboratório, necropsiados os helmintos colhidos e fixados. Dos 30 animais analisadas 16,67% estavam parasitados por nematoides. As larvas de nematoides foram encontradas encistadas na serosa intestinal, apresentam na região anterior dois lábios com duas papilas cada, bulbo cefálico armado de 6 fileiras de espinhos descontínuos, quatro sacos cervicais, esôfago claviforme, estrias cuticulares ao longo do corpo, abertura anal simples, cauda curta, terminando em um mucron. A junção de todos esses caracteres morfológicos, são características diagnósticas para gênero *Gnathostoma*. Os indivíduos adultos são parasitos de estômago de mamíferos carnívoros e raramente de peixes, porém peixes, anfíbios, répteis e aves atuam como hospedeiros intermediários das larvas (L3) e o homem pode atuar como hospedeiro acidental.

Palavras-chave: *Gnathostoma*, parasitismo, estuário, baiacu.

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Introduction

Currently, the genus *Gnathostoma* (OWEN, 1836) comprises 17 species: *G. spinigerum* Owen, 1836; *G. gracile* Diesing, 1839; *G. socialis* Leidy, 1858; *G. hispidum* Fedtschenko, 1872; *G. turgidum* Stossich, 1902; *G. doloresi* Tubangui, 1925; *G. americanum* Travassos, 1925; *G. didelphis* Chandler, 1932; *G. procyonis* Chandler, 1932; *G. nipponicum* Yamaguti, 1941; *G. minutum* Schuurmans-Stekhoven, 1943; *G. brasiliense* Ruiz, 1952; *G. miyazakii* Anderson, 1964; *G. vietnamicum* Le-Van, 1965; *G. malaysiae* Miyazaki & Dunn, 1965; *G. binucleatum* Almeyda-Artigas, 1991; and *G. lamothiei* Bertoni-Ruiz, et al., 2005.

Although feral dogs and cats are the most commonly identified definitive hosts of *Gnathostoma* species, adult parasites have also been identified in tigers, leopards, lions, martens, otters, opossums, and raccoons. Frogs, snakes, birds, snails, and pigs can serve as intermediate hosts. Humans may accidentally be part of the life cycle of this nematode; however, the parasite fails to reach maturity in the human host (MCCARTHY & MOORE, 2000).

Colomesus psittacus (Bloch & Schneider, 1801) is a demersal fish found in marine-estuarine waters up to 40 m deep as well as in freshwater. It is distributed along the Gulf of Paria (Venezuela), extending to the eastern Brazilian Amazon (CERVIGÓN et al., 1992), and is found in the Amazon estuary in Brazil (CAMARGO & MAIA, 2008).

The study of fish pathogenic agents, especially zoonotic agents, is of great importance due to the high mortality they cause, thereby reducing the fish populations and commercial values of natural stocks (PAVANELLI et al., 2008). The present study describes the presence of nematodes (Gnathostomatidae) in the coelomic cavity of *C. psittacus* collected at the Ilha de Marajó, eastern Brazilian Amazon.

Materials and Methods

Thirty specimens of *C. psittacus* were caught by fishermen in the municipality of Soure (0°53'39"S, 48°7'39"W), Ilha de Marajó, state of Pará, Brazil. Fish were refrigerated and transported to the Laboratório de Histologia e Embriologia Animal - Instituto da Saúde e Produção Animal, Universidade Federal Rural da Amazônia (UFRA), campus Belém, where they were weighed and measured (total length in cm). After the biometric analysis, the animals were necropsied, and the helminths were collected. The fish organs were removed, placed in Petri dishes with saline solution, and examined using a LEICA ES2 stereomicroscope. The recovered helminths were fixed in a solution of AFA (93 parts 70% ethyl alcohol, 5 parts formaldehyde, and 2 parts glacial acetic acid). For morphological and morphometric analysis the nematodes were dehydrated in an ethanol series, clarified with lactophenol, placed on a microscope slide under a coverslip as a temporary mount, observed using a light microscope, and photographed using a LEICA DM2500 microscope with an imaging capture system. Measurements are shown in micrometers as the mean followed by the range, or as otherwise indicated. For scanning electron microscopy, the helminths were washed in phosphate-buffered saline (pH 7.0), post-fixed in 1% osmium tetroxide, dehydrated to the critical point of CO₂, metalized with gold-palladium, and analyzed with the scanning electron microscope VEGA 3 LMU/TESCAN in

the Laboratório de Microscopia Eletrônica de Varredura, Instituto da Saúde e Produção Animal - Universidade Federal Rural da Amazônia - UFRA, campus Belém, state of Pará, Brazil.

Results

Of the 30 specimens of *C. psittacus* that were collected, 16.67% (5/30) were parasitized by third-stage larvae (L3) of a nematode encysted in the intestinal serosa; these larvae presented characteristics of species of the genus *Gnathostoma*.

Gnathostoma sp. (third-stage larvae) (Figures 1 and 2)

(Description based on five larvae)

Medium-sized nematodes, yellow when alive and whitish when fixed, with fine transversal cuticular striations. Cylindrical body, measuring 12 mm (10 mm-14 mm) × 415 (333-480) (width at level of esophagus-intestine junction) and no cuticular spines on the trunk. Circular oral cavity surrounded by two lateral lips with a pair of papillae on each and one amphid (Figures 2a, b). Three pairs of double spines which increase in size from anterior to posterior, situated dorsally and ventrally between the lips and the first circle of spines of the cephalic bulb (Figures 2a, c). Cephalic bulb 313 (277-387) × 340 (293-386), six discontinuous circles of spines containing I: 35, II: 39, III: 40, IV: 37, V: 35, and VI: 33 spines, respectively, which increase in size with distance from the oral cavity (Figures 1a, 2a). Nerve ring located at level of cephalic bulb, 260 (167-337) from the anterior end. Excretory pore not observed. Deirids not visible in whole mounts but visible using SEM. Claviform muscular esophagus 3 mm (2.67-3.66 mm) × 340 (293-386), occupying 25% of the body length. Two pairs of fully developed transparent contractile cervical sacs (Figure 1a) at the anterior half of the body, one on each side of esophagus, measuring 1 mm (1.18-1.84 mm), occupying 33.3% of the length of the esophagus. Short, thin tail, including terminal mucron, measuring 175 (100-216) (Figures 1b, 2e).

Taxonomic summary

Gnathostomatoidea Railliet, 1895

Gnathostomatidae Railliet, 1895

Gnathostoma Owen, 1836

Gnathostoma sp. (L3 Larvae)

Host: *Colomesus psittacus* (Osteichthyes, Tetraodontiformes); length and average weight of hosts: 35.5 cm and 365.5 g, respectively.

Infection site: Encysted in the intestinal serosa.

Location: Soure, state of Pará, Eastern Amazon, Brazil (0°53'39"S, 48°7'39"W).

Biome: Amazon

Prevalence: 16.67% (5 hosts infected of 30 analyzed)

Range of infection: 1-8

Deposit of Specimens: MPEG. NEM 93; MPEG. NEM 94 and MPEG. NEM 95: Coleção de Invertebrados of the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil.

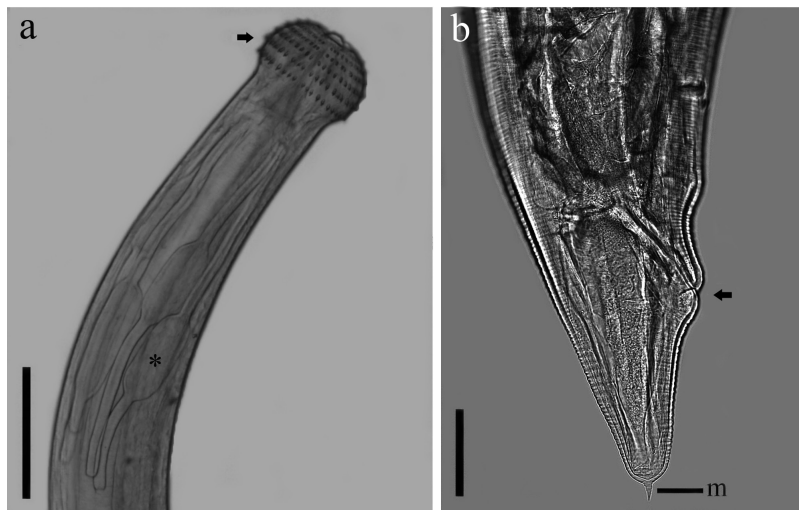


Figure 1. Light microscopy of *Gnathostoma* sp. (L3 larvae) parasite of *C. psittacus*: (a) Lateral view of cephalic region showing the cephalic bulb and the six circles of spines (arrow) and four cervical sacs (*). Bar = 500 µm; (b) Caudal region showing the anus (arrow) and mucron (m). Bar = 100 µm.

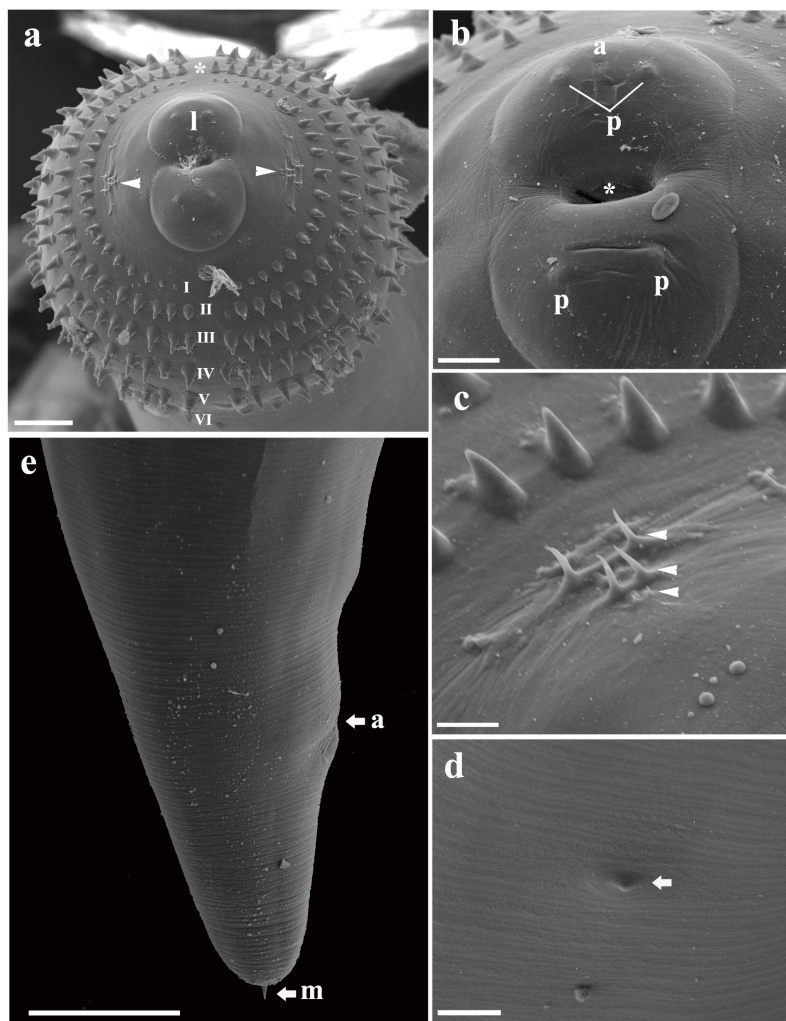


Figure 2. Scanning electron microscopy of *Gnathostoma* sp. (L3 Larvae) parasite of *C. psittacus*. (a) Apical view of the cephalic region showing six circles of discontinuous spines (*), lips (l) and three pairs of dorsal and ventral spines in the cephalic bulb (arrow). Bar = 50 µm; (b) Frontal view of the oral opening (*), cephalic papillae (p), amphid (a). Bar = 20 µm; (c) View of the three pairs of dorsal and ventral spines in the cephalic bulb (arrow). Bar = 10 µm; (d) Deirid (arrow). Bar = 20 µm; (e) Side view of the caudal region, anus (a) and mucron (m). Bar = 100 µm.

Discussion

The nematodes found encysted in the intestinal serosa of *C. psittacus*, collected in the municipality of Soure, state of Pará, eastern Amazonia, have similar characteristics to those of other species of *Gnathostoma*, including the presence of a cephalic bulb armed with spines, a circular oral cavity surrounded by two lateral lips with a pair of papillae on each, an amphid, and four internal cervical sacs. Vicente et al. (1985) and Moravec (1998) identify these as important characters for the identification of nematodes of the family Gnathostomatidae (RAILLIET, 1985) and of *Gnathostoma* (OWEN, 1836). We designated these larvae as being third-stage larvae because this usually is the stage found in fish. However, third-stage larvae usually are smaller than 10 mm in length; the ones reported herein are 10-14 mm in length, a size more typical of fourth-stage larvae. Fourth-stage larvae also can have more than four rows of transverse hooks on cephalic bulb, as do these larvae. However, larvae of this size and with more than four rows of transverse hooks usually are found in mammals and not in fish.

There are few reports of larvae of *Gnathostoma* naturally parasitizing fish in South America. The present study is the first to report of *C. psittacus* as an intermediate/paratenic host of *Gnathostoma*. Among the vertebrate hosts that participate in the life cycle of members of this genus, fish of different families are cited as intermediate and paratenic hosts.

Previous reports of natural hosts of *Gnathostoma* include *Channa argus* (Cantor, 1842) from Japan (Miyazaki, 1954); *Oreochromis aureus* (Steindachner, 1864), *O. mossambicus* (Peters, 1852), *O. niloticus* (Linnaeus, 1758), *Paraneetroplus fenestratus* (Günther, 1860), *Cichlasoma urophthalmus* (Günther, 1862), *Petenia splendida* (Günther, 1862), *Gobiomorus dormitor* Lacepède, 1800, *Cathorops fuerthii* (Steindachner, 1876), *Pomadasys macracanthus* (Günther, 1864), *Mugil curema* Valenciennes, 1836, *Dormitator latifrons* (Richardson, 1844), *Chanos chanos* (Forsskal, 1775), *Ariopsis guatemalensis* (Günther, 1864), *Ariopsis seemanni* (Günther, 1864), *Bagre panamensis* (Gill, 1863) and *Millerichthys robustus* (Miller & Hubbs, 1974) from México (ALMEYDA-ARTIGAS, 1991; ÁLVAREZ-GUERRERO & LAMOTHE-ARGUMEDO, 2000; GUERRERO & ALBA-HURTADO, 2007; MOSQUEDA-CABRERA & OCAMPO-JAIMES, 2009 and GARCÍA-MÁRQUEZ et al., 2009); *Misgurnus anguillicaudatus* (Cantor, 1842) from Korea (SOHN & LEE, 1998); *Channa striata* (Bloch, 1793) from Myanmar (JUNG et al., 2008) and *Poecilia* sp. and *Monopterus* spp. from the United States (ASH, 1960, 1962; COLE et al., 2014).

Natural infections of fish by advanced third-stage larvae of *Gnathostoma* also have been reported, such as those described by Miyazaki (1954), who reported an infection by *G. spinigerum* in *Channa argus* in Japan, Almeyda-Artigas (1991), who reported the occurrence of *G. binucleatum* as a parasite of *O. aureus*, and Álvarez-Guerrero & Lamothe-Argumedo (2000), who recorded the presence of *Gnathostoma* spp. larvae in *Cathorops fuerthii* and *Dormitator latifrons* in Mexico. Cole et al. (2014) described the parasitism of live Asian swamp eels (*Monopterus* spp.) by *Gnathostoma spinigerum*.

Species of *Gnathostoma* have been studied frequently in experimental infections. Ash (1962) performed experimental infections in fish of the genus *Poecilia* Bloch & Schneider, 1801 and in the water

snake *Natrix rhombifera* (Hallowell, 1852) and also reported the development of third-stage larvae in *G. procyonis*. In Mexico, Gaspar-Navarro et al. (2013) obtained larvae of *G. lamothei* from *Poeciliopsis gracilis* (Heckel, 1848) by experimental infection.

Miyazaki (1954) reported that the number, shape, and size of the spines; the shape of the cephalic bulb; and the location of the cephalic papillae and excretory pore are specific characteristics that distinguish different species of *Gnathostoma*. The morphological characterization and morphometry of the cephalic bulb are some of the main characteristics used for the differentiation of members of Gnathostomatidae (MIYAZAKI, 1960; MOSQUEDA-CABRERA & OCAMPO-JAIMES, 2009). The larvae collected from *C. psittacus* in this study were morphologically different from other species that had been previously described as parasitizing fish by size, absence of spines along the body, length and number of circles of spine in the cephalic bulb, size of the esophagus, presence of three pairs of dorsal and ventral spines situated in the cephalic bulb, presence of phasmids, and a tail with a terminal mucron. The specimens found in the present study had a larger cephalic bulb (313 × 340) compared to that reported for the third-stage larvae of *G. binucleatum* (120 × 240), *G. turgidum* (46.6 × 110.3), *G. spinigerum* (93 × 221), *G. procyonis* (180 × 200), and *G. lamothei* (84 × 188).

Chai et al. (2015) suggested that the differentiation of larvae of *Gnathostoma* depends mainly on the number and distribution of spines on the cephalic bulb. The number of spines in each circle of the larvae in the present study ranged from 36 to 40, but the number of spines in the proximal and distal circles was constant and not, as observed by Miyazaki (1991) variable in number.

The number of circles and the average number of spines in the cephalic bulb differentiate the specimens in our study from other species of *Gnathostoma* because those described herein had six discontinuous circles of spines (I: 35, II: 39, III: 40, IV: 37, V: 35, and VI: 33), which increase in size with distance from the oral cavity. In contrast, third-stage larvae of *G. spinigerum*, *G. binucleatum*, *G. turgidum*, *G. procyonis*, and *G. lamothei* each have four circles of spines although.

The larvae of *Gnathostoma* collected from *C. psittacus* did not have spines transversally distributed along the body as shown in photographs taken using the scanning electron microscope. However, this characteristic has been reported in different larvae of different species of the genus. Morphological and morphometric variations in the number of spines on the body also can be used to differentiate species of the genus *Gnathostoma*. Therefore, the specimens found in the present study differ from *G. binucleatum* (242-285), *G. turgidum* (182-202), *G. spinigerum* (225-256), and *G. lamothei* (137-258) because these species have transverse rows of spines along their bodies. Ash (1962), recovered larvae of *G. procyonis* from *Poecilia* spp. and *Natrix rhombifera* and reported the presence of small spines distributed over the body of the parasites, but the author did not specify the number of spines. Further morphometric comparisons among the larvae of *Gnathostoma* species are shown in Table 1.

For the first time in Brazil, *Gnathostoma* sp. is reported as a natural parasite of *C. psittacus*. Some of the morphological characteristics, including the three pairs of spines surrounding the lips, the six discontinuous circles of spines on the cephalic bulb, and the tail with a terminal mucron, are morphological data reported for the first time for larvae of this genus. Knowledge

Table 1. Morphometric comparison of advanced third-stage larvae of *Gnathostoma* spp. and *Gnathostoma* sp. larvae collected from *C. psittacus* in this study.

| Character | Third-stage larvae of <i>Gnathostoma</i> spp. | | | |
|------------------------|---|-------------------------------|--------------------------------|----------------------------------|
| | <i>Gnathostoma</i> sp. | <i>G. binucleatum</i> | <i>G. turgidum</i> | <i>G. spinigerum</i> |
| Hosts—larvae | <i>Colomesus psittacus</i> | <i>Oreochromis aureus</i> | <i>Lithobates zuewilei</i> | <i>Channa argus</i> |
| Hosts—adults | - | <i>Felis pardalis</i> | <i>Didelphis</i> spp. | <i>Natrix rhombifera</i> |
| Type locality | Pará, Brazil | México | USA | <i>Procyon l. lotor</i> USA |
| Author | - | Almeyda-Artigas (1991) | Mosqueda-Cabrera et al. (2009) | Ash (1960, 1962) |
| Length (mm)* | 12 (10-14) | 4.3 (2.6-5.9) | 1.67 (1.53-2) | 5 (4.6-5.5) |
| Width | 415 (333-480) | 320 (310-320) ^a | 140 (134.6-160.4) | 366 (250-425) ^b |
| Cephalic bulb (L)** | 313 (277-387) | 120 (110-130) ^a | 46.6 (36.7-65) | 93 (75-115) ^b |
| Cephalic bulb (W)** | 340 (293-386) | 240 (230-250) ^a | 110 (97.9-118.3) | 221 (165-250) ^b |
| I row | 35 (30-40) | 39 (35-44) | 30.8 (26-34) | 44 (39-49) |
| II row | 39 (36-42) | 42 (38-47) | 34 (29-38) | 47 (42-54) |
| III row | 40 (36-42) | 45 (40-49) | 36.7 (29-43) | 50 (45-56) |
| IV row | 37 (32-40) | 48 (43-52) | 39.6 (33-42) | 52 (45-58) |
| V row | 35 (30-38) | - | - | - |
| VI row | 33 (26-38) | - | - | - |
| VI-I row | 2 | - | - | - |
| Rows in the body | - | - | - | - |
| I Cervical sac (mm)* | - | 271 (242-285) | 188.6 (182-202) | 244 (225-256) ^d |
| II Cervical sac (mm)* | - | - | - | - |
| III Cervical sac (mm)* | 1 (1.18-1.84) | 0.7 (0.6-0.8) ^a | 0.350 (0.302-0.435) | 0.574 (0.330-0.750) ^b |
| IV Cervical sac (mm)* | - | - | - | 0.43 ^c |
| Deirid*** | - | 14 ^a | 11.7 (10-14) | 14 (11-16) ^d |
| Excretory pore*** | - | 30 ^a | 19.7 (15-22) | 25 (22-28) ^d |
| Nerve ring | 260 (167-337) | - | - | - |
| Muscular oc. (L)** | 313 (277-387) | 1.22 (1.22-1.23) ^a | 648 (579.4-722.2) | 1,200 (1,000-1,400) |
| Muscular oc. (W)** | 340 (293-386) | 200 (190-210) ^a | 80.8 (69.4-85.7) | 124 (109-142) |
| Tail | 175 (100-216) | - | 29.2 (24.4-40.8) | 70 ^c |
| Reference | From this study | Almeyda-Artigas (1991) | Stossich (1902) | Chandler (1942) |

*Measurements in micrometers unless indicated. The parameter number of buds is given in amplitude; **Abbreviations: L: length, W: width, Oe: esophagus; ***Location of deirid and excretory pore in relation to transverse rows of corporal spines; ^aFrom García-Márquez et al. (2009), based on measurements of three AdvL3 of *Gnathostoma* found in the skeletal muscle of one specimen of *Dormitator latifrons* and five found in one specimen of *Sciades guatemalensis*; both fish specimens were from the Cuyutlán Lagoon; ^bFrom Chai et al. (2015), based on measurements of 401 advanced third-stage larvae in Asian swamp eels (*Monopterus albus*) purchased from central Myanmar; ^cFrom Jung et al. (2008) based on measurements of an advanced third-stage larva of *G. spinigerum* recovered from the muscle of a snakehead fish; ^dFrom Koga et al. (1994) based on measurements of eight advanced third-stage larvae from naturally infected eels, *Fluta alba*, from Thailand; ^eFrom Ash (1962) based on measurements of 15 fully developed third-stage larvae collected from the muscles of *Natrix rhombifera* in Louisiana, USA.

of the occurrence of larvae of *Gnathostoma* in fish in Brazil is relatively uncommon.

Fish have been reported as intermediate hosts of larvae of *Gnathostoma* (see MORAVEC, 1998). However, few reports exist of these parasites in Brazilian fauna. Among the species reported in Brazil are *G. gracile*, an intestinal parasite of *Arapaima gigas* (fish) in the Amazon River Basin; *G. americanum*, which parasitizes the stomach of *Leopardus tigrinus* (mammal) in the state of Rio de Janeiro; and *G. brasiliense*, a parasite of the liver of *Lutreolina crassicaudata* (mammal).

There remains much to be studied in the ecology and systematics of *Gnathostoma* in Brazil. The species *G. gracile* was described by Diesing (1839) and redescribed from original material by Drasche (1884) as a parasite of the intestine of *Arapaima gigas* (Schinz, 1822) in Brazil. According to Moravec (1998), the morphology of this parasite is still little known because it has been found only once, in an unusual host for parasites that are usually observed in the stomach of carnivores; thus, the species needs to be redescribed. Additionally, Miyazaki (1960, 1991), and Bertoni-Ruiz et al. (2011) consider the species *G. brasiliense* and *G. didelphis* to be synonyms of *G. turgidum*.

At this time, there is no evidence leading to a hypothesis on the introduction of this species to Ilha de Marajó. Bufalos (*Bubalus bubalis* Linnaeus, 1758), pigs (*Sus scrofa* Linnaeus 1758), dogs (*Canis lupus familiaris* Linnaeus 1758), and domestic cats (*Felis catus* Linnaeus 1758) have been introduced to the island. Migratory birds such as *Egretta thula* (Molina, 1782), *Phalacrocorax brasilianus* (Gmelin, 1789) also can serve as paratenic hosts of gnathostomids (NAWA & NAKAMURA-UCHIYAMA, 2004). In this island, these migratory birds are common predators of *C. psittacus* so it is possible that they are part of the life cycle of this helminth. However, preliminary studies of the helminths of the animals mentioned above were negative for this parasite. Almeyda-Artigas et al. (2010) reported finding individuals of *Philander opossum* Linnaeus 1758, a species found on the island, infected with *G. turgidum* in Mexico, but no studies of this possible host have been made.

In the family Gnathostomatidae, Luque et al. (2011) reported the presence of *Echinocephalus daileyi* (DEARDORFF et al., 1981), a parasite of the intestine of *Potamotrygon hystrix* (Müller & Henle, 1841) and *P. motoro* (Müller & Henle, 1841); *Echinocephalus* sp. (parasite of spiral valve of *Potamotrygon falkneri* Castex & Maciel, 1963); and *G. gracile* (Diesing, 1839), parasite of the intestine of *A. gigas* in the Brazilian Amazon.

Gnathostomosis in humans is a food-borne zoonosis caused by the ingestion of raw fish parasitized with third-stage larvae of nematodes of the genus *Gnathostoma*. Only six species have been reported parasitizing humans; these include *G. spinigerum*, mainly in Thailand and Mexico, and *G. dorolei*, *G. hispidum*, *G. malaysiae*, *G. nipponicum*, and *G. binucleatum* in other parts of the world (CHAICUMPA, 2010). Chaicumpa (2010) also reported that humans become infected by ingesting raw or undercooked fish, which can include intermediate or paratenic host of the parasite.

Finally, several species of the genus *Gnathostoma* have been described or redescribed with the aid of SEM during recent decades. Deardorff & Ko (1983), Beveridge (1985, 1987), Hoberg et al. (1998), and Moravec & Justine (2006) stated that

SEM is a very useful method for this type of study, especially to observe morphological structures such as pseudolips and to observe the morphology and distribution of spines present in the cephalic bulb and body, as well as to observe the genital papillae. Moravec & Justine (2006) suggested that the number and arrangement of the spines present in the cephalic bulb and throughout the body may be a very reliable taxonomic criterion, but their identification depends on the use of SEM.

The use of SEM in the present study aided in the morphological characterization of the larvae found parasitizing specimens of *C. psittacus* because it showed clearly the presence of six discontinuous circles of spines, the circular oral cavity surrounded by two lips with a pair of papillae on each, the three pairs of ventral and dorsal spines, the deirid just below the cephalic bulb, and the tail with a terminal mucron. These are features not discerned easily using only a light microscope. Among the most recent studies of the genus *Gnathostoma*, Bertoni-Ruiz et al. (2011) used SEM to characterize morphologically the spines present along the body and caudal papillae in males of *G. miyazakii*, *G. procyonis*, *G. turgidum*, *G. lamothei*, and *G. socialis*, demonstrating that SEM is a crucial technique for the diagnoses of new species or for future redescrptions.

Conclusion

The morphological data of the parasites studied herein are of importance because the present study reports the finding of three pairs of dorsal and ventral spines in the cephalic bulb, an absence of spines along the body, and a tail with a terminal mucron for the first time in larvae of *Gnathostoma*. Additionally, this is the first report of the genus parasitizing fish of the family Tetraodontidae in northern Brazil.

The species *C. psittacus* is not part of the food base of Amazonians; however, large vertebrates that may serve as paratenic or definitive hosts of this parasite are used as food and are consumed by indigenous populations and traditional communities that practice hunting and fishing. The information provided in this study provides important additions to the knowledge of pathogens that may affect aquatic hosts in the Amazon. Further studies are needed to identify the parasite that is described in the present study taxonomically at the species level as well as to elucidate its life cycle and potential risks to local inhabitants.

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