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Endemic angiostrongyliasis in the Brazilian Amazon: Natural parasitism of *Angiostrongylus cantonensis* in *Rattus rattus* and *R. norvegicus*, and sympatric giant African land snails, *Achatina fulica*

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ABSTRACT

Angiostrongylus cantonensis, the rat lungworm, is one etiological agent of eosinophilic meningoencephalitis in humans. This zoonosis is frequently found in Asia and, more recently, in North America, Caribbean Island and northeastern of South America. Until now, research of *A. cantonensis* in southern, southeastern and northeastern regions of Brazil has been found natural infections only terrestrial and freshwater intermediate snail hosts (*Achatina fulica*, *Sarasinula marginata*, *Subulina octona*, *Bradybaena similaris* and *Pomacea lineate*). In this study, we examined the occurrence of helminthes in the synantropic rodents *Rattus rattus* and *Rattus norvegicus* in northern Brazil, focusing on the role of these species as vertebrate hosts of *A. cantonensis* and *A. fulica* as intermediate host have found natural. Thirty specimens of *R. rattus* and twelve of *R. norvegicus* were collected in the Guamá and Jurunas neighborhoods of the city of Belém, in the Brazilian state of Pará, of which almost 10% harbored adult worms in their pulmonary arteries. Sympatric *A. fulica* were found to be infected by L_3 larvae, which experimental infection confirmed to be *A. cantonensis*. Natural infection of snails and rodents with *A. cantonensis* was confirmed through morphological and morphometrical analyses of adults and larvae using light microscopy, scanning electron microscopy and molecular sequences of partial *Cytochrome Oxidase subunit I*. Phylogenetic analyses showed that *A. cantonensis* isolated from Pará, Brazil is similar to Japan isolate; once these specimens produced a single haplotype with high bootstrap support with Rio de Janeiro isolate. This study confirms that *A. cantonensis* is now endemic in northern Brazil, and that *R. rattus* and *R. norvegicus* act as natural definitive hosts, and *A. fulica* as the intermediate host of the parasite in this region.

1. Introduction

Angiostrongylus cantonensis Chen, 1935 was first described as a parasite of the pulmonary artery of *Rattus norvegicus* and *Rattus rattus* in Canton, China. This helminth is one etiological agent of eosinophilic meningoencephalitis, a zoonotic disease endemic to certain Asian countries, which recently dispersed to Africa, North America, and the Caribbean Islands (Richards and Merrit, 1967; Prociv et al., 2000; Berenguer, 2006; Jitpimolmard et al., 2007). In humans, infection occurs through the ingestion of third stage larvae (L_3), which are deposited on the leaves of vegetables and fruits with the mucous of the intermediate host, generally a mollusk gastropod, without adequate washing. The larvae may also be ingested through the consumption of the intermediate or paratenic hosts when eaten raw or undercooked (Kim et al., 2002; Toma et al., 2002; Neuhauss et al., 2007). A number of different species of gastropod mollusks are known to be intermediate hosts for the parasite, while freshwater prawns, crabs, amphibians, and flatworms may act as paratenic hosts (Slom and Johnson, 2003).

In Brazil, terrestrial mollusks infected naturally by *A. cantonensis* have been observed in the southeastern region (Caldeira et al., 2007; Maldonado et al., 2010) and the northeastern regions (Thiengo et al., 2010), where human cases of eosinophilic meningoencephalitis have also been recorded (Lima et al., 2009). The giant African land snail, *Achatina fulica* Bowdich, 1822, has been

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identified as the principal vector of the parasite in Brazil (Panha, 1988; Prociv et al., 2000; Vasconcellos and Pile, 2001; Toma et al., 2002), while *R. norvegicus* appears to be responsible for the maintenance of the parasite in the southeast of the country (Simões et al., 2011). The natural infection of commensal synanthropic rodents (*Rattus* spp.) by *A. cantonensis* has been recorded in the United States (Kim et al., 2002), in Cuba (Kim et al., 2002; Contreras et al., 2009) and Puerto Rico (Kim et al., 2002), indicating endemicity of the parasite in these countries.

In the present study, *A. cantonensis* infection in the city of Belém, in the Brazilian state of Pará, was investigated based on the identification of adult specimens of the parasite found in *R. rattus* and *R. norvegicus*, and L_3 larvae from sympatric *A. fulica*. The natural infection of these snails and rodents was confirmed through morphological and morphometric analyses using light microscopy, scanning electron microscopy, and molecular sequences of subunit I of the cytochrome oxidase gene (COI), as well as the experimental infection of *R. norvegicus* with L_3 larvae.

2. Materials and methods

2.1. Study area and collection of specimens

Synanthropic rats, *R. rattus*, *R. norvegicus* and *A. fulica* snails were collected between January 2009 and August 2010, in Jurunas neighborhood, Belém city of the Brazilian state of Pará (01°47′43″S, 48°49′24″W) and Guamá neighborhoods (01°47′24″S, 48°45′71″W), which borders the Guamá River. These slum neighborhoods lack adequate public sanitation system, and are subject to high rates of human leptospirosis, according to the Disease Notification System (SINAN) of Brazilian Ministry of Health.

Traps were set in two transects in areas surrounding private residences. Each transect consisted of two trapping stations 20 m apart. At each station, one Tomahawk (model 201; 40.6 cm × 12.7 cm × 12.7 cm) and one Sherman trap (model XLK; 7.6 cm × 9.5 cm × 30.5 cm) were placed on the ground and under the roof, respectively. Traps were baited with a mixture of peanut butter, banana, oatmeal, bacon and manioc, and were checked daily in the morning.

2.2. Collection of helminthes

The rats were captured and taken to the Laboratory of Cell Biology and Helminthology at the Institute of Biological Sciences of the Federal University of Pará, where they were sedated profoundly with Ketamine chloride (100 mg/kg) associated with Xylazine (40 mg/kg) applied intramuscularly. All animal procedures were carried out according to the guideline for capture, handling and care of mammals of American Society of Mammalogy and the biosecurity procedures of the Brazilian Ministry of Health. They were then sexed and weighed, and the species was identified by morphometric parameters (FUNASA, 2002; WHO, 2008). For analysis, the organs were separated in phosphate buffered saline (PBS), and observed under a stereomicroscope. The thoracic and abdominal cavities were also examined.

2.3. Light and scanning electron microscopy

The helminthes found in the lumen of the pulmonary artery were fixed in a solution of 2% glacial acetic acid, 3% formaldehyde, and 95% ethanol 70° (AFA) heated to 60 °C (Amato et al., 1991). The specimens were then dehydrated and clarified in increasing concentrations of glycerin, for analysis under Olympus BX 41 microscope, and drawn using a coupled camera Lucida. The structures were measured and the values were presented in millimeters.

For analysis by scanning electron microscopy, male and female specimens fixed in AFA were post-fixed in 1% Osmium tetroxide (OsO_4) for 2 h, washed in PBS, pH 7.4, and dehydrated in ethanol series. The specimens were dried in an Emitech K850 critical point dryer, metalized in an Emitech K550, and analyzed in a LEO 1450 VP.

2.4. Processing of the helminthes for genetic sequencing

Molecular sequences were used to confirm the morphological identification of the *Angiostrongylus* specimens. The DNA was extracted from two adult worms recovered from the pulmonary arteries of naturally infected *R. rattus*, and two others from the experimentally infected *R. norvegicus* (one sourced from sympatric *A. fulica* and the other from *R. rattus*) using a QIAGEN DNA purification kit. The Polymerase Chain Reaction (PCR) was conducted using the primers and protocols available for a partial region of the COI gene (Bowles et al., 1993): COI_F 5′ TTTTTTGGGCATCCTGAGGTTTAT 3′ and COI_R 5′ TAAAGAAAGAACATAATGAAAATG 3′. The amplified products were purified using a QIAquick PCR Purification kit (QIAGEN), and sequencing reactions were conducted using an ABI Prism Dye Terminator Cycle Sequencing Core kit (Applied Biosystems, USA). The two 360-bp COI sequences obtained here were deposited in GenBank (GenBank accession no. JQ595406) and aligned with homologous *Angiostrongylus* sequences obtained (GenBank accession nos. HQ440217, GQ398121, and GQ398122). The *Angiostrongylus vasorum* sequences obtained from Brazil (*A. vasorum* 5421, 5641, and 5642, Jefferies et al., 2009) were utilized to phylogenetics comparisons. *Ancylostoma tubaeforme* (GenBank accession no. AJ407940) was used as outgroup. The alignment was obtained using CLUSTAL X (Thompson et al., 1997). The MEGA4 program was used to construct a Neighbor-Joining phylogenetic tree based on Kimura 2-parameter (K2-p) distances (Tamura et al., 2007).

2.5. Recovery of L_3 larvae from A. fulica and experimental infection of R. norvegicus

Specimens of snails collected within the study area were examined in the Malacology Laboratory at the Oswaldo Cruz Institute (IOC) for the presence of L_3 larvae of *A. cantonensis*. For this analysis, the mollusks were minced individually and digested in a 0.7% HCl solution for 6 h. The digested samples were then placed in a Baermann apparatus and allowed to sediment overnight (Caldeira et al., 2007). Part of the sample of L_3 larvae was set aside for morphological characterization while the other part was administered orally to 3-month-old Wistar rats (*R. norvegicus*), with 100 larvae being given to each rat (Bahaibulaya, 1975). Thirty-five days after administration of the larvae, the rodents were euthanized in a CO_2 chamber. Some of the adult worms collected from the pulmonary arteries were frozen at 70 °C for molecular studies, with the rest being washed in physiological solution and fixed in AFA at 60 °C.

3. Results

A total of 42 rats were captured – 23 *R. rattus* (10 males and 12 females from Guamá neighborhood, and one male from Jurunas) and 19 *R. norvegicus* (four males and one female from Guamá, and eight males and six females from Jurunas). Two of the female *R. rattus* collected in Guamá were infected with *A. cantonensis*, as were a female *R. norvegicus* from Guamá and a male from Jurunas (Table 1). Adult helminthes of both sexes were found in the lumen of the pulmonary artery.

Morphological and morphometric analyses of the adult worms under light and scanning electron microscopy revealed a simple

Table 1
Occurrence of *Angiostrongylus cantonensis* in the pulmonary artery of specimens of *Rattus rattus* and *Rattus norvegicus* collected from the Jurunas and Guamá neighborhoods in the Brazilian city of Belém, Pará State, in the Oriental Amazonian.

Species	Neighborhood	Number of hosts	Sex		Occurrence of infection	Prevalence
			♀	♂		
R. rattus	Guamá	22	10	12	2♀	9%
	Jurunas	1	1	–	–	0%
R. norvegicus	Guamá	5	4	1	1♀	20%
	Jurunas	14	8	6	1♂	7%
Total		42	23	19	3♀/1♂	9%

anterior extremity with simple oral opening, labia absent, esophagus short claviform, nerve ring at posterior third of esophagus, excretory pore perpendicular to the longitudinal axis of the body, below the junction of the esophagus with the intestine. The male worms ($n = 7$) are smaller and less robust that the females, with mean body 21.9 ± 0.7 (20.6–22.7) $\times$ 0.33 ± 0.02 (0.30–0.37). Esophagus 0.31 ± 0.08 (0.30–0.32) $\times$ 0.06 ± 0.001 (0.057–0.062), at bulb width. Nerve ring and excretory pore, 0.25 ± 0.01 (0.23–0.25) and

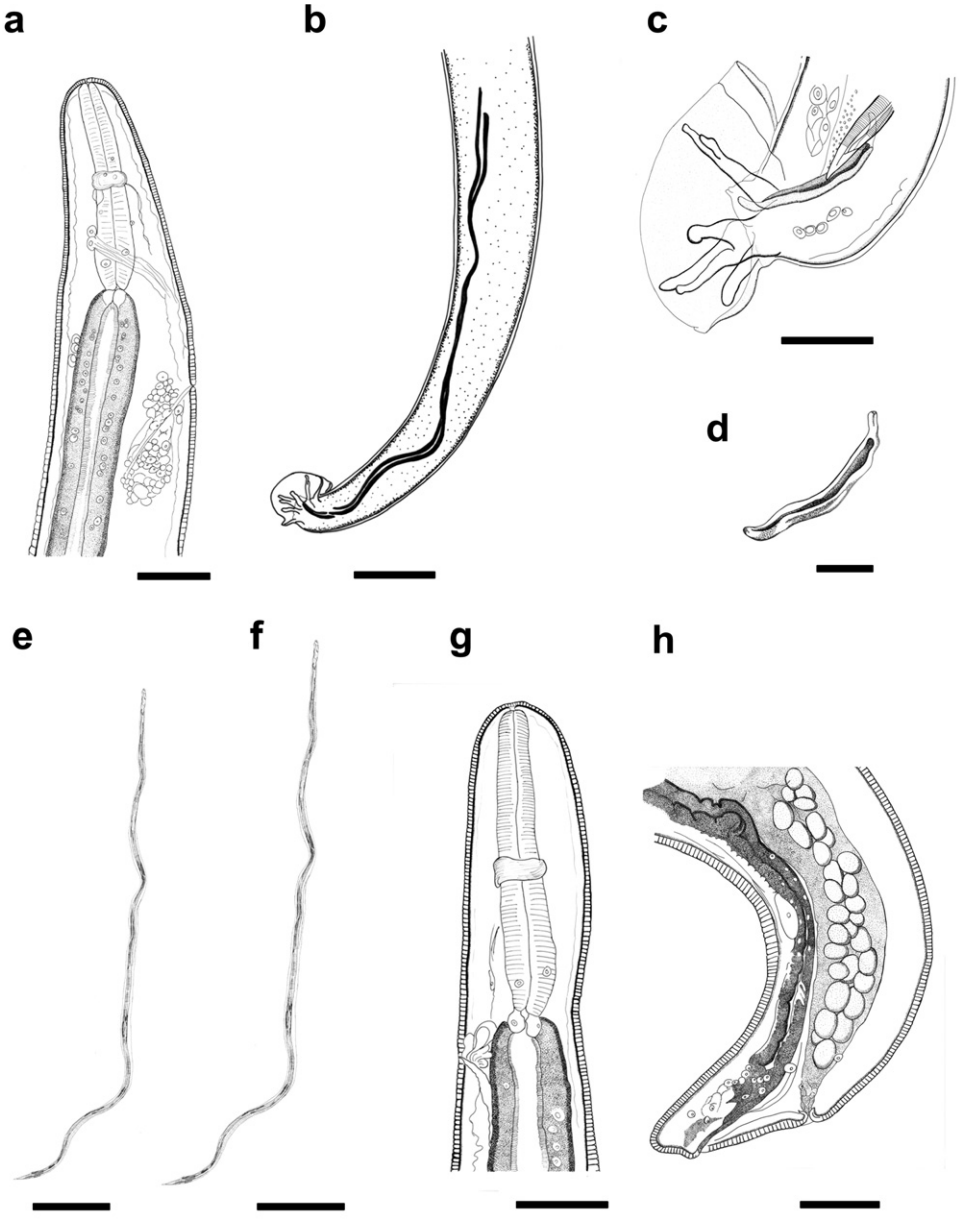

Fig. 1. Light microscopy of adults of *Angiostrongylus cantonensis*. Male anterior end (a). Bar = 100 μm. Lateral view of male posterior end, where copulatory bursa can be observed (b). Bar = 200 μm. Detail of copulatory bursa, in lateral view, showing rays disposition (c). Bar = 100 μm. Gubernaculum (d). Bar = 30 μm. Left spicule (e). Bar = 300 μm. Right espicule (f). Bar = 300 μm. Female anterior end (g). Bar = 100 μm. Female posterior end (h). Bar = 100 μm.

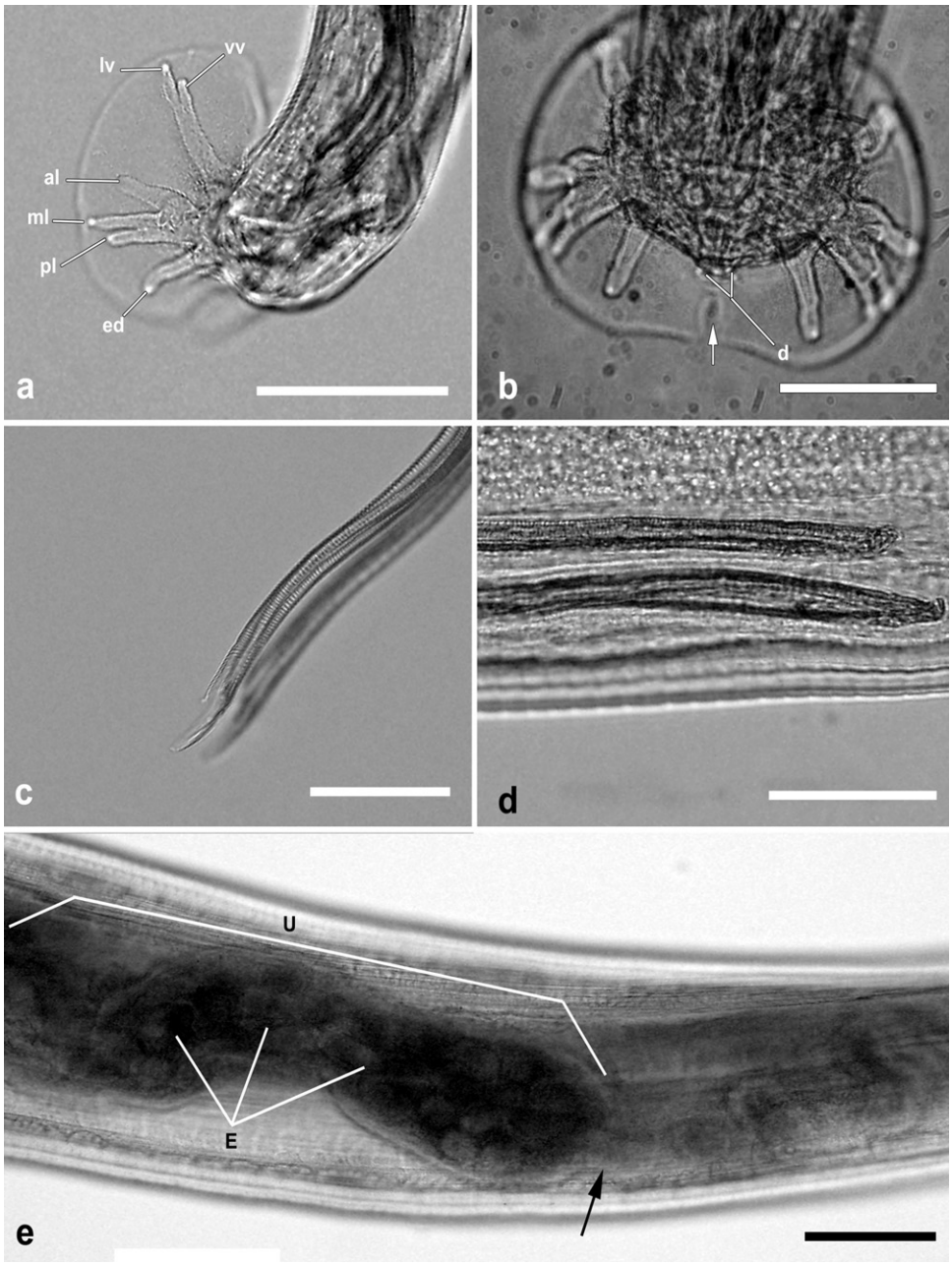

Fig. 2. Light microscopy of adults of *Angiostrongylus cantonensis*. Lateral view of male copulatory bursa, with ventro-ventral rays (vv), latero-ventral ray (lv) arising from the same trunk, lateral rays are divided in antero-lateral (al), median-lateral (ml) and postero-lateral; and external-dorsal ray (ed) (a). Bar = 50 μm. Ventral view of copulatory bursa, showing dorsal rays (d) and the small indentation (arrow) (b). Bar = 50 μm. Detail of posterior end of spicules, that are covered by striated sheet (c). Bar = 50 μm. Detail of anterior, rounded end of spicules (d). Bar = 50 μm. Detail of female body showing, uterus (U) fulfilled of eggs (E) and the ovojector (arrow) (e). Bar = 200 μm.

0.43 ± 0.03 (0.37–0.47) from the anterior extremity, respectively (Fig. 1a). Posterior extremity curved dorsoventrally with a small copulatory bursa (Figs. 1b, c and 2a, b). Gubernaculum well developed, 0.11 ± 0.008 (0.11–0.13) in length (Fig. 1d). Spicules long, fine and subequal (Fig. 1e, f), tape-ring posterior extremity, and delicate coating sheath with transversal striations (Fig. 2c, d). Right spicule, 1.21 ± 0.17 (0.96–1.30) and left spicule 1.19 ± 0.09 (1.00–1.28).

Copulatory bursa bilobed, with small indentation in the posterior extremity (Figs. 2a, b and 4e, f). The rays of the bursa are well-defined, robust and with papiliform structure at their extremity (Fig. 4e, f). They are organized in ventro-ventral and latero-ventral rays that emanate from a common trunk and bifurcate from the distal third. The extremity of the latero-ventral ray is longer than the ventro-ventral ray, and almost reaches the edge of the bursa. Three lateral rays originate from the same trunk. The antero-lateral ray is the smallest and widest, whereas the medio-lateral ray is the longest, reaching the edge of the bursa, although it is joined with the postero-lateral ray along half its length. The externo-dorsal ray is separated from the lateral rays, but is similar in length to the postero-lateral ray (Figs. 1c and 2a, b). The dorsal ray is reduced in length (Fig. 2b).

The female specimens ($n = 7$) were larger and more robust than the males, with a mean 26.9 ± 1.7 (25.2–28.8) $\times$ 0.41 ± 0.02 (0.38–0.44) (Fig. 4a, b). Esophagus short, 0.37 ± 0.02 (0.35–0.41) $\times$ 0.06 ± 0.01 (0.051–0.07), at bulb width. Nerve ring and excretory pore 0.28 ± 0.025 (0.25–0.32) and 0.46 ± 0.02 (0.48–0.49) from the anterior extremity, respectively (Fig. 1g). The posterior extremity with a short and rounded tail (Figs. 1h and 4c),

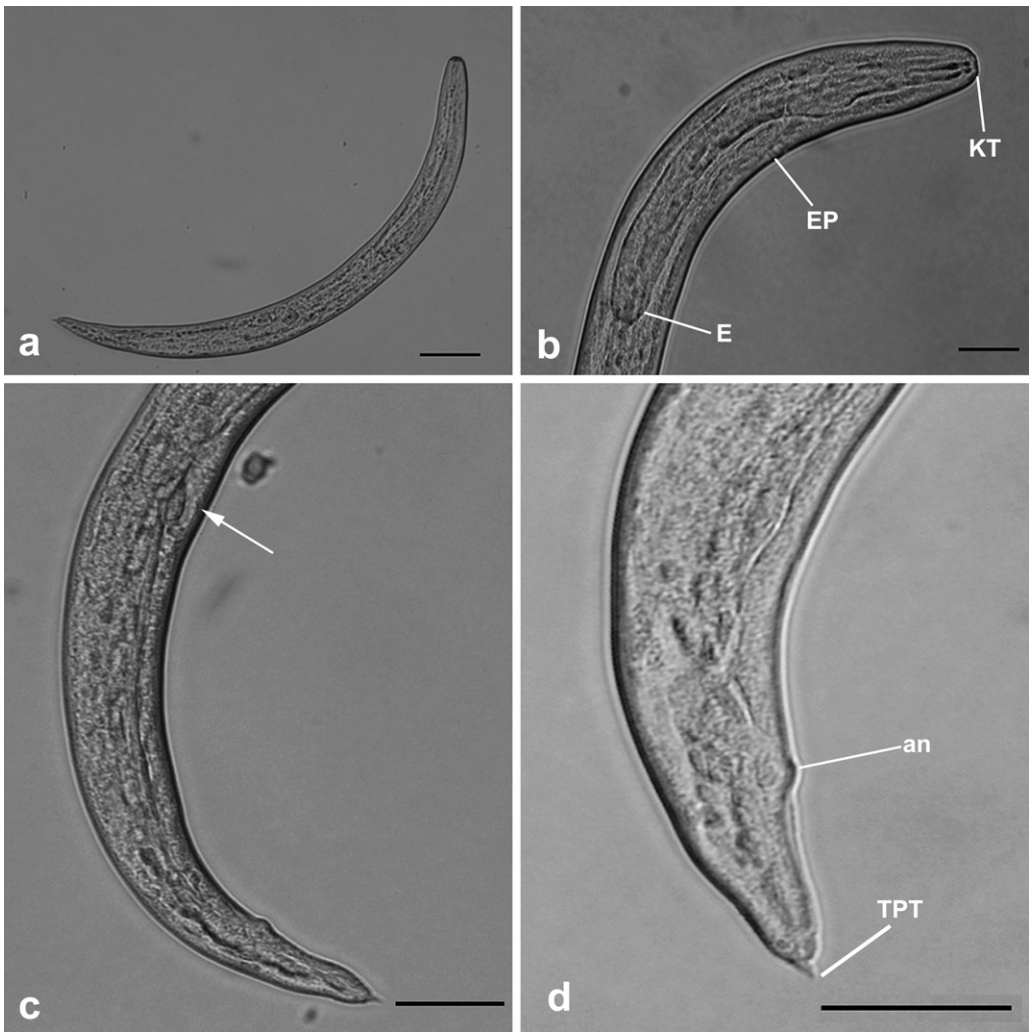

Fig. 3. Light microscopy of third-stage larvae (L_3) of *Angiostrongylus cantonensis*. General view of third-stage larvae (a). Bar = 40 μm. Details of anterior end showing knob-like tips (KT), esophagus (E), excretory pore (EP) (b). Bar = 20 μm. Details of posterior end of larvae showing tail pointed tip (TPT) and anus (an) and genital primordium (arrow) (c and d). Bars = 20 μm.

vulva close to the anus, transversal opening with a slightly elevated region adjacent to the anterior labium 0.20 ± 0.04 (0.14–0.28) (Figs. 1h and 4c, d). Uterus full of eggs, ovojector present (Figs. 1h and 2e). Anal opening subterminal, 0.11 from the posterior extremity of the body (Figs. 1h and 4c). Voucher specimens were deposited at the Helminthological Collection of the Oswaldo Cruz Institute, under catalog number (CHIOC nos: 35738a and 35738b).

All the specimens, including L_3 larvae obtained from digested snails and the feces of rats infected experimentally, as well adult worms retrieved from the pulmonary artery from rodents in the 35–45 day interval, were identified morphologically as *A. cantonensis* (Fig. 3a–d).

Larvae presented body filiform, cuticle with transversal ridges, with anterior extremity rounded, genital primordium with refraction, buccal capsule long, esophagus long with filariform bulb, excretory pore and nerve ring situated in middle region of esophagus (Fig. 3a–d).

Posterior extremity of L_3 larvae is curved dorso-ventrally, tail tip with a marked retreat in dorsal surface (Fig. 3c, d). Genital primordium is well developed in this phase and situated in second third of the body, but their evidence did not aid in sex differentiation (Fig. 3c).

The specimens of *A. cantonensis* found in naturally infected *R. rattus*, adult worms obtained from *R. norvegicus* infected experimentally with L_3 larvae in *A. fulica* from Belém, and *A. cantonensis* from Rio de Janeiro (Brazil), did not present nucleotides differences in COI sequences, and all produced a single haplotype with high bootstrap support (100), which formed a clade with the *A. cantonensis* haplotype from China, with a low genetic distance (K2-p = 0.038), confirming the morphological identification. Comparisons with *A. vasorum* and *A. costaricensis* produced higher genetic distances K2-p = 0.120 and 0.149, respectively (Fig. 5).

4. Discussion

The genus *Angiostrongylus* Kamensky, 1905 belongs to the Angiostrongylidae, family which includes helminthes parasites of the cardiac and pulmonary circulatory system or the mesenteric veins of carnivores, rodents, and marsupials (Urquhart et al., 1996; Vicente et al., 1997; Berenguer, 2006). Dougherty (1946) identified seven *Angiostrongylus* species: *A. vasorum*, *A. raillieti*, *A. tateronae*, *A. ondratae*, *A. cantonensis*, *A. ten*, and *A. gubernaculatus* (Drozdz, 1970; Costa et al., 2003), whereas Skrjabin et al. (1952) divided these species into four distinct genera: *Angiostrongylus*, *Rattostrongylus*, *Angiocaulus*, and *Rodentocaulus* (Costa et al., 2003), a classification

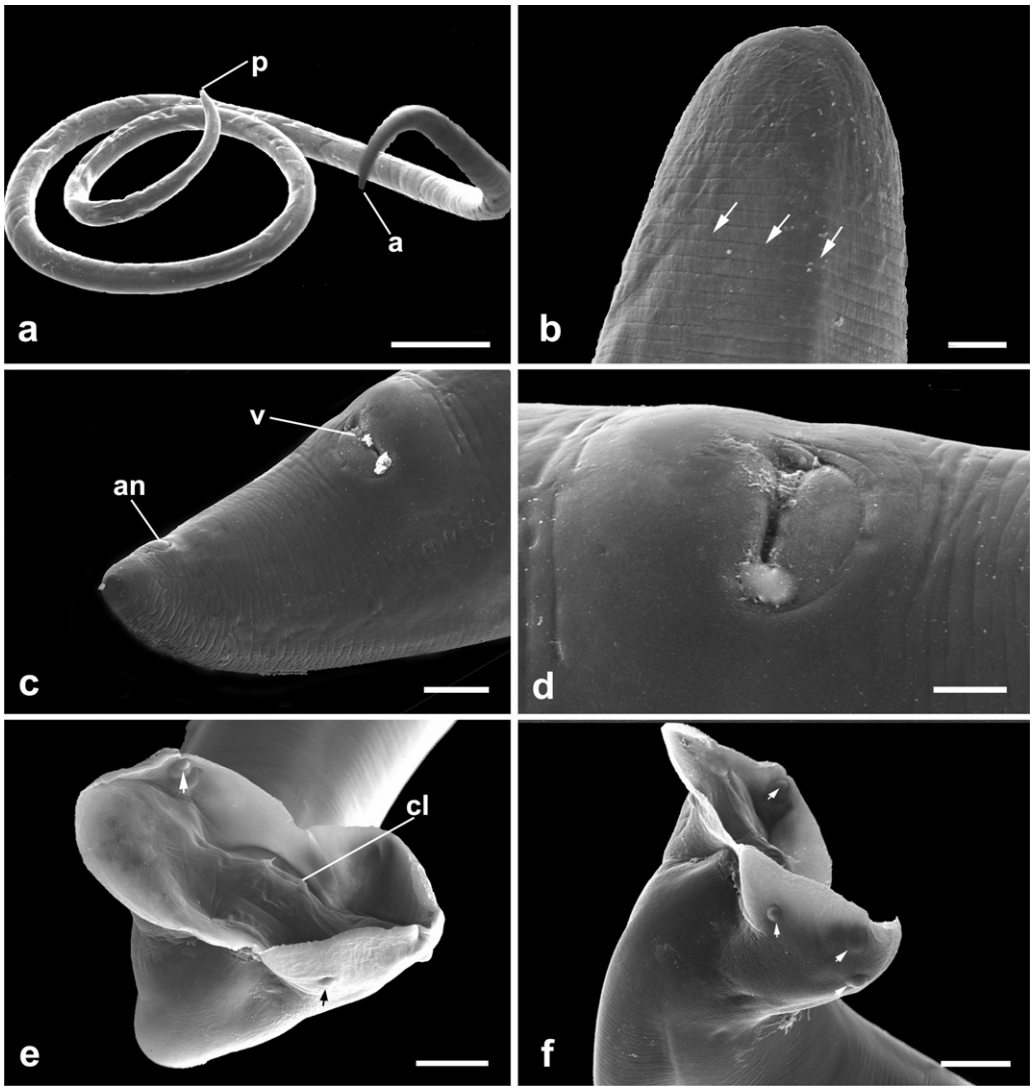

Fig. 4. Scanning electron microscopy of adults of *Angiostrongylus cantonensis*. General view of female, with anterior end (a) and posterior end (p) (a). Bar = 1 mm. Anterior end females where is possible to observe transversal ridges (arrows) (b). Bar = 15 μm. Posterior end of female showing vulva (V) position, close to anus (an) (c). Bar = 25 μm. Detail of vulva opening (d). Bar = 10 μm. Posterior end of male showing a ventral view of copulatory bursa, observe cloaca open (cl) and papiliform structures in terminal portion of rays (arrowheads) (e). Bar = 25 μm. Detail of dorsal view of male copulatory bursa where can be observed papiliform structures in terminal portion of rays (arrowheads) (f). Bar = 25 μm.

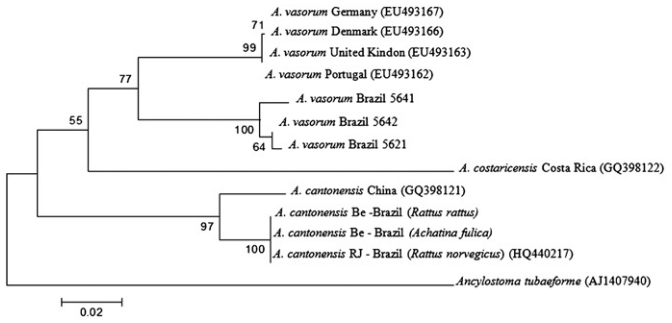

Fig. 5. Phylogenetic tree based in COI sequences of *Angiostrongylus cantonensis*, *Angiostrongylus costaricensis* and *Angiostrongylus vasorum* from different localities, resulted from Neighbor-Joining based on Kimura 2-parameter. Internal nodes show the percentage of genetic relationship between species. The name of species is followed by location and GenBank number access. The scale bar indicates the distance in substitutions per nucleotide.

adopted subsequently by Yamaguti (1961). In 1970, Drozdz divided *Angiostrongylus* into two subgenera, denominated *Angiostrongylus*, which is characterized by the common origin of the lateral rays of the copulatory bursa, and *Parastrongylus*, in which these rays originate from different trunks (Drozdz, 1970; Santos, 1985).

In 1971, Kinsella elaborated an identification key for the genus *Angiostrongylus* using the following parameters for the classification of species: the size of the dorsal ray, length of the spicule, the relative size of the ventro-ventral and latero-ventral rays, the width of the antero-lateral ray relative to that of the other lateral rays, and the location of the anus and the vulva in the females. In 1986, Ubelaker arranged the angiostrongylids in five genera: *Angiostrongylus*, *Parastrongylus*, *Angiocaulus*, *Rodentocaulus*, and *Gallegostrongylus*.

The specimens obtained in the present study were initially identified using Kinsella's (1971) key for *Angiostrongylus*. The morphological and morphometric characteristics of these helminthes, in particular the length of the spicules, the host, and the pulmonary arterial localization of the parasites confirmed the specimens found

as *A. cantonensis*.These same morphometric parameters were in accordance with Chen's (1935) original description of naturally occurring *A. cantonensis* collected from *R. norvegicus* and *R. rattus*, as well as adult specimens obtained from experimental infections in southern (Maldonado et al., 2010) and northeastern Brazil (Thiengo et al., 2010).

Scanning electron microscopy data presented in study show that *A. cantonensis* found in Amazon region have ultraestructural characteristics similar with the descriptions reported previously by Lian-Yin et al. (1984) and Hüttemann et al. (2007).

Previous records of the occurrence of *A. cantonensis* in Brazil (Caldeira et al., 2007; Thiengo et al., 2010) followed reports of cases of eosinophilic meningoencephalitis in humans in the states of Espirito Santo (southeastern) and Pernambuco (northeast). These studies recorded the natural occurrence of the helminth is only in gastropod mollusks. More recently, L_3 larvae were found occurring naturally in *A. fulica* snails in Santa Catarina in the extreme south of the country (Maldonado et al., 2010). These larvae were used to experimentally infect *R. norvegicus*, confirming the role of this species as intermediate host for the parasite under natural conditions. The helminth was subsequently reported from naturally infected *R. norvegicus* in Rio de Janeiro (Simões et al., 2011) confirming its endemicity, given that the rodent can act as the definitive host, while humans are only accidental hosts.

The results of this study represent the first report of natural infection of *R. rattus* and *R. norvegicus* by *A. cantonensis* in northern Brazil, which indicates that the parasite has spread more widely within the country creating new endemic area in this country. In the New World, natural infection of *R. rattus* by *A. cantonensis* has only previously been recorded in Cuba, and this is the first report of participation of this rodent as a definitive host of the parasite in South America. According to Wang et al. (2008), the role of *R. rattus* as a definitive host is based on the prevalence rates recorded in other countries, although its participation in the parasite life cycle may depend on local epidemiological conditions.

The relatively low rates of natural infection observed in both *R. norvegicus* and *R. rattus* from Pará were different from those recorded in Rio de Janeiro by Simões et al. (2011). This suggests that the participation of the two sympatric rodent species in the transmission cycle of *A. cantonensis* may permit greater stability under conditions of environmental stress, given that these rodents have distinct ecological niches.

The recent introduction of *A. cantonensis* into North America has been attributed to the introduction of infected *R. norvegicus* carried in containers being transported by ships during the post-war period (Diaz, 2008). The introduction of *A. cantonensis* and its definitive host into Brazil may date to the colonial period, but its recent dispersal within the country may be related to the introduction and proliferation of the giant African land snail, *A. fulica*, during the 1980s.

Comparison on phylogenetic relationship among Thailand, China, Japan and Hawaii geographical isolates of *A. cantonensis* using a partial sequence of 66 kDa protein gene failure to distinguish geographical isolates, although it have been useful at the specific level (Eamsobhana et al., 2010a). Specific identification was also obtained using complete internal transcribed spacer 2 (ITS-2) and an fragment from small subunit ribosomal RNA (SSU rRNA) nucleotid sequences to *A. cantonensis* isolate from naturally infected *R. rattus* in Canary Island (Foronda et al., 2010).

Recent studies using COI gene to distinguish *A. cantonensis* isolates demonstrated that specimens from three geographical isolates formed two subclades: China and Hawaii isolates and a monophyletic Thailand isolate (Eamsobhana et al., 2010b). Simões et al., 2011 observed that *A. cantonensis* from Rio de Janeiro, Brazil yields a single haplotype which formed a clade with low genetic distance with China isolate. Interestingly, Tokiwa et al. (2012) analyzing others geographical isolate confirmed that Rio de Janeiro isolates are more similar with *A. cantonensis* isolated from Japan. Thus, *A. cantonensis* isolated from Pará, Brazil is similar to Japan isolate, once these specimens produced a single haplotype with high bootstrap support with Rio de Janeiro isolate.

The results of the present study support the hypothesis of a recent *A. cantonensis* introduction, given the lack of difference in the COI sequences of *A. cantonensis* from naturally infected *R. norvegicus* collected in Pará and Rio de Janeiro States. The participation of *A. fulica* in the transmission cycle was also confirmed by the fact that the L_3 larvae obtained from sympatric mollusks were capable of infecting *R. norvegicus* under experimental conditions.

In fact, the snail *A. fulica* has been recently introduced to Brazil as an alternative for *Helix aspersa* providing the escargort for traditional French cuisine (Teles et al., 1997). However, many of these mollusk farms have been abandoned and the snails have been released into natural environment (Neuhauss et al., 2007). They are found in many states across the country, including Amazonic region (Manaus) and Southest region (São Paulo) (Carvalho et al., 2003). These results indicate that the occurrence of *A. cantonensis* in northern Brazil may be the result of a recent dispersal event from *A. fulica* population in southeastern Brazil.

This is also the first record of the natural infection of *A. fulica* by *A. cantonensis* in northern Brazil, specifically in the state of Pará, which represents a large proportion of the Brazilian Amazon basin, and a considerable extension of the area in which this helminth is endemic. This study should also serve as a warning for the public health authorities of the state, given the zoonotic potential of this parasite, the extremely high densities of synanthropic *Rattus* populations, and the occurrence of the exotic snail *A. fulica* throughout most of Brazil.

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