Revista Brasileira de Parasitologia Veterinária
Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv

Original Article

Angiostrongylus minasensis n. sp.: new species found parasitizing coatis (*Nasua nasua*) in an urban protected area in Brazil

Angiostrongylus minasensis n. sp.: nova espécie encontrada parasitando quatis (Nasua nasua) em área de proteção urbana no Brasil

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How to cite: Almeida LR, Souza JGR, Santos HA, Torres EJL, Vilela RV, Cruz OMS, Rodrigues L, Pereira CAJ, Maldonado Junior A, Lima WS. *Angiostrongylus minasensis* n. sp.: new species found parasitizing coatis (*Nasua nasua*) in an urban protected area in Brazil. *Braz J Vet Parasitol* 2020; 29(1): e018119. https://doi.org/10.1590/S1984-29612019103

Abstract

Currently, there are 21 species of *Angiostrongylus* that parasitize the pulmonary or mesenteric arteries of wild and domestic rodents, felids, canids and human. Species of *Angiostrongylus* have cosmopolitan distribution covering tropical, subtropical and temperate regions. The procyonid *Nasua nasua* (coati) is a reservoir host for a wide variety of parasites that may be harmful to its populations or may contain etiological agents with zoonotic potential. In urban areas, coatis are usually found in close association with humans and domestic animals. We morphologically and molecularly characterized a new species of *Angiostrongylus* found in *N. nasua* in a protected area within Belo Horizonte, Brazil. The new species of *Angiostrongylus* differs from other species of the same genus in terms of the length and bifurcation level of the lateral and ventral rays, the length of spicules and female tail morphology. Molecular phylogenetic results based on the mitochondrial cytochrome c oxidase subunit 1 gene suggest that the newly identified species belongs to a genetic lineage that is separate from other species of *Angiostrongylus*. This new species was collected from the mesenteric arteries of *N. nasua*. It was named *Angiostrongylus minasensis* n. sp..

Keywords: *Angiostrongylus minasensis*, taxonomy, morphology, morphometric and phylogenetic analysis, *Nasua nasua*.

Resumo

Existem 21 espécies de *Angiostrongylus* que parasitam as artérias pulmonares ou mesentéricas de roedores silvestres e domésticos, felídeos, canídeos e homem. Espécies de *Angiostrongylus* têm uma distribuição cosmopolita que abrange regiões tropicais, subtropicais e temperadas. O procionídeo *Nasua nasua* (quati) é hospedeiro de vários parasitos que podem ser prejudiciais para suas populações ou conter agentes etiológicos com potencial zoonótico. Nas áreas urbanas, os quatis

Received October 10, 2019. Accepted November 19, 2019,
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podem ser encontrados em estreita associação com seres humanos e animais domésticos. Nós caracterizamos morfológica e molecularmente uma nova espécie de *Angiostrongylus* encontrada em *N. nasua* de uma área protegida na cidade de Belo Horizonte, Brasil. A nova espécie de *Angiostrongylus* difere de outras espécies do mesmo gênero pelo comprimento e nível de bifurcação dos raios lateral e ventral, o comprimento dos espículos e a morfologia da cauda da fêmea. Resultados moleculares e filogenéticos baseados no gene mitocondrial citocromo c oxidase subunidade 1 indicam que a espécie recém-identificada pertence a uma linhagem genética separada de outras espécies de *Angiostrongylus*. O presente relato descreve uma nova espécie de *Angistrongylus* coletada das artérias mesentéricas de *N. nasua*, denominada *Angiostrongylus minasensis* n. sp..

Palavras-chave: *Angiostrongylus minasensis*, taxonomia, morfologia, análise morfométrica e filogenética, *Nasua nasua*.

Introduction

Species of the genus *Angiostrongylus* Kamensky, 1905, have widespread geographical distribution and have been reported in Australia, Americas, Asia, Africa and Europe (Jefferies et al., 2009). Currently, 21 species of *Angiostrongylus* are known the world (Maldonado et al., 2012). Species have been described parasitizing carnivores: *Angiostrongylus vasorum* (Baillet, 1866); *A. gubernaculatus* (Dougherty, 1946); *A. chabaudi* (Biocca, 1957); *A. daskalovi* (Yanchev & Genov, 1988); and *A. felineus* (Vieira et al., 2013). Other species have been described in rodents or occasionally in aberrant hosts (Spratt, 2015): *A. taterone* (Baylis, 1928); *A. cantonensis* (Chen, 1935); *A. sciuri* (Merdevenci, 1964); *A. mackerrasae* (Bhaibulaya, 1968); *A. sandarsae* (Alicata, 1968); *A. petrowi* (TARJYMANOVA & TSCHERTKOVA, 1969); *A. dujardini* (Drozdz & Doby, 1970); *A. schmidti* (Kinsella, 1971); *A. costaricensis* (Morera & Cespedes, 1970); *A. malaysiensis* (Bhaibulaya & Cross, 1971); *Angiocaulus ryjikovi* (Jushkov, 1971); *A. andersoni* (PETTER, 1972); *A. siamensis* (Ohbayashi et al., 1979); *A. morerai* (Robles et al., 2008); and *A. lenzii* (Souza et al., 2009).

The coati, *Nasua nasua* (Procyonidae) Linnaeus, 1766, is common in South America (Decker, 1991). Coatis live preferentially in forested environments, are diurnal and omnivorous. In urban environments, and thus in recreational areas, coatis may reach high population densities and are found in close association with humans and domestic animals (Crespo, 1982; Gompper & Decker, 1998; Alves-Costa et al., 2004).

We describe a new species of *Angiostrongylus* that was found parasitizing the mesenteric arteries of specimens of *N. nasua* that had been collected in a protected area in the state of Minas Gerais, Brazil. We characterized the new species by means of optical and scanning electron microscopy (SEM) and used molecular phylogenetic analysis to determine its relationships within the family Angiostrongylidae. The species was named *Angiostrongylus minasensis* n. sp..

Materials and Methods

Origin of the parasites

Ten road-killed specimens of *Nasua nasua* (four adult females, four adult males and two young females) were collected in the Mangabeiras Municipal Park, an environmental conservation area located in Belo Horizonte, the capital of the state of Minas Gerais, Brazil (latitude 19°56'43" S and longitude 43°54'42" W), between August 2011 and August 2016. The carcasses were sent to the Helminthology Laboratory of the Institute of Biological Sciences, Federal University of Minas Gerais (UFMG). The study was authorized through the system for authorizations and for biodiversity information (SISBIO) of the Chico Mendes Institute for Conservation and Biodiversity [ICMBio/39817-1, ICMBio/54546-1] and by UFMG's ethics committee for use of animals [306/2017].

Nematodes were collected from the mesenteric arteries of the coati specimens. They were washed in 0.85% saline solution and fixed in 10% formalin at 80 °C for morphological and morphometric analyses, as described by Lima et al. (1985), or were placed in 100% ethanol and stored at -20 °C for molecular analysis. Feces were recovered from the rectum of all the necropsied animals, and then first stage (L1) larvae were isolated using the Baermann apparatus (Barçante et al., 2003).

Microscopy analysis

The paratypes (10 males and 10 females), included one holotype (male), and one allotype (female) were cleared in Amann's lactophenol. Measurements were made in millimeters (unless otherwise stated). These measurements are presented as holotype or allotype values, followed by the interval, mean and variation coefficient between parentheses.

Images were captured using a digital camera attached to an optical microscope. Drawings were made with the aid of a camera lucida. The new *Angiostrongylus* species was morphologically identified using the method of Anderson et al. (2009). Nematodes were prepared for conventional SEM as previously described (Lopes et al., 2013) and observed using the Zeiss Auriga Compact equipment at the urogenital research unit of the State University of Rio de Janeiro.

DNA isolation, PCR and sequencing

Genomic DNA was isolated from adult nematode specimens that were recovered from the mesenteric arteries of the coatis using the QIAGEN® QIAamp® DNA mini kit, following the manufacturer's protocol. The polymerase chain reaction (PCR) was carried out using a primer cocktail, as described by Prosser et al. (2013), to produce amplicons of the cytochrome c oxidase subunit 1 gene (MT-CO1), using the Invitrogen™ Platinum™ Taq DNA polymerase.

After checking the PCR products on agarose gel check, they were then purified using the GE Healthcare Illustra™ GFX™ PCR DNA and Gel Band purification kit, following the manufacturer's protocol. Purified amplicons were cycle-sequenced using the Applied Biosystems™ BigDye™ Terminator v3.1 cycle sequencing kit individually using each of the six primers from the above-mentioned cocktail (Prosser et al., 2013). Sequencing was performed using the ABI 3730 DNA analyzer at the DNA sequencing platform of the Oswaldo Cruz Institute (PDTIS/FIOCRUZ). Six overlapping fragments for each sample were assembled into contigs, and ambiguities were edited to generate a consensus sequence for each specimen, using the Geneious 9.1 software package with default parameters (http://www.geneious.com) (Kearse et al., 2012).

Phylogenetic analyses

Consensus sequences were aligned with GenBank sequences of representative members of the family Angiostrongylidae, four species of the metastrongyloid taxa (*Protostrongylus rufescens*, *Metastrongylus pudendotectus*, *M. salmi* and *Parafilaroides normani*) and two unpublished sequences of *Heterostrongylus heterostrongylus*. Two strongylid GenBank sequences were used as outgroups: *Ancylostoma duodenale* and *Necator americanus* (Table 1).

Table 1. Accession numbers of nematode MT-CO1 gene sequences retrieved from GenBank and respective hosts, sites of infection, geographical origin, and references.

Referred species	GenBank accession	Host	Site of infection	Geographical origin	Reference
Necator americanus	AJ417719	Human	Small intestine	China	Hu et al. (2002)
Ancylostoma duodenale	AJ417718	Human	Small intestine	China	Hu et al. (2002)
Aelurostrongylus abstrusus	JX519458	*Felis catus*	Lung	-	Jabbar et al. (2013a)
Heterostrongylus heterostrongylus	XXXXX	*Didelphis aurita*	Lung	Brazil	Costa et al. (2016)
H. heterostrongylus	XXXXX	*Didelphis aurita*	Lung	Brazil	Costa et al. (2016)
Metastrongylus pudendotectus	NC_013813	Pig	Terminal bronchi in the lungs	Estonia	Jex et al. (2010)
Metastrongylus salmi	NC_013815	Pig	Terminal bronchi in the lungs	Estonia	Jex et al. (2010)
Parafilaroides normani	NC_024656	*Arctocephalus pusillus*	Lung	Australia	Jabbar et al. (2014)
Protostrongylus rufescens	NC_023262	Sheep	Lung	Australia	Jabbar et al. (2013b)
Angiostrongylus vasorum	GQ982735	*Canis familiaris*	Heart and pulmonary arteries	United Kingdom	Jefferies et al. (2010)
A. vasorum	GQ982871	*Vulpes vulpes*	Heart and pulmonary arteries	Portugal	Jefferies et al. (2010)
A. chabaudi	KU521521	*Felis silvestris*	Heart and pulmonary arteries	Romania	Gherman et al. (2016)
A. cantonensis	GQ398121	*Rattus norvegicus*	Pulmonary arteries	China	Lv et al. (2012)
A. malaysiensis	KT947979	*Rattus rattus*	Heart and lung	Malaysia	Yong et al. (2016)
A. costaricensis	KX378965	*Nasua narica*	Mesenteric vessels	Costa Rica	Santoro et al. (2016)
A. costaricensis	KR827449	-	Mesenteric vessels	Costa Rica	Yong et al., (2015)
A. costaricensis	GQ398122	-	Mesenteric vessels	Brazil	Lv et al. (2012)
A. minasensis n. sp.	XXXXX	*Nasua nasua*	Mesenteric vessels	Brazil	Present study

(-) data absent in the original article; XXXXX: not published.

The alignment was done as described by Maddison & Maddison (2015). The incidence of substitution saturation was verified using the test suggested by Xia et al. (2003) and Xia (2009). In addition, plots of Jukes & Cantor (1969) pairwise distances against transitions and transversions were used for each codon position. We carried out both tests using the DAMBE software, version 5.6.14 (Xia, 2013).

Phylogenetic reconstructions were carried out using maximum likelihood (ML) and Bayesian inference (BI) as optimality criteria using the software Treefinder version of March 2011 (Jobb, 2011) and MrBayes version 3.2.6 (Ronquist et al., 2012), respectively.

To account for different evolutionary processes at each of the three codon positions, ML and BI analyses were performed with distinct models per codon position.

ML-pairwise distances were computed using the same codon-based partitioned models (Schwarz, 1978). The robustness of nodes in ML was assessed by means of nonparametric bootstrap percentages (BP) after 1,000 pseudo-replicates had been produced, and by means of the expected-likelihood weights, which were applied to local rearrangements of tree topology (LR-ELW) after 1,000 replicates.

In the BI analyses, distinct GTR+I+Γ models were used for each codon position, with unlinking of base frequencies and parameters. Markov chain Monte Carlo (MCMC) sampling was performed for 10,000,000 generations with four simultaneous chains, in two runs. The robustness of nodes in BI was assessed by means of Bayesian posterior probabilities (BPP), which were calculated from trees that were sampled every 100 generations. The adequacy of BI analysis sampling was assessed using the Tracer software, version 1.6 (Rambaut et al., 2014). Presence of more than 100 effectively independent samples was considered sufficient. To save computational time, BI analyses were carried out on XSEDE cluster using the CIPRES Science Gateway (Miller et al., 2010).

Results

Angiostrongylus minasensis n. sp. (Figures. 1-4)

Type host. *Nasua nasua* (Linnaeus, 1766) (Carnivora: Procyonidae); common name: coati.

Type locality. Mangabeiras Park, Belo Horizonte, state of Minas Gerais, Brazil (9°56'43" S 43°54'42" W).

Site in host. Mesenteric arteries (Figure 1).

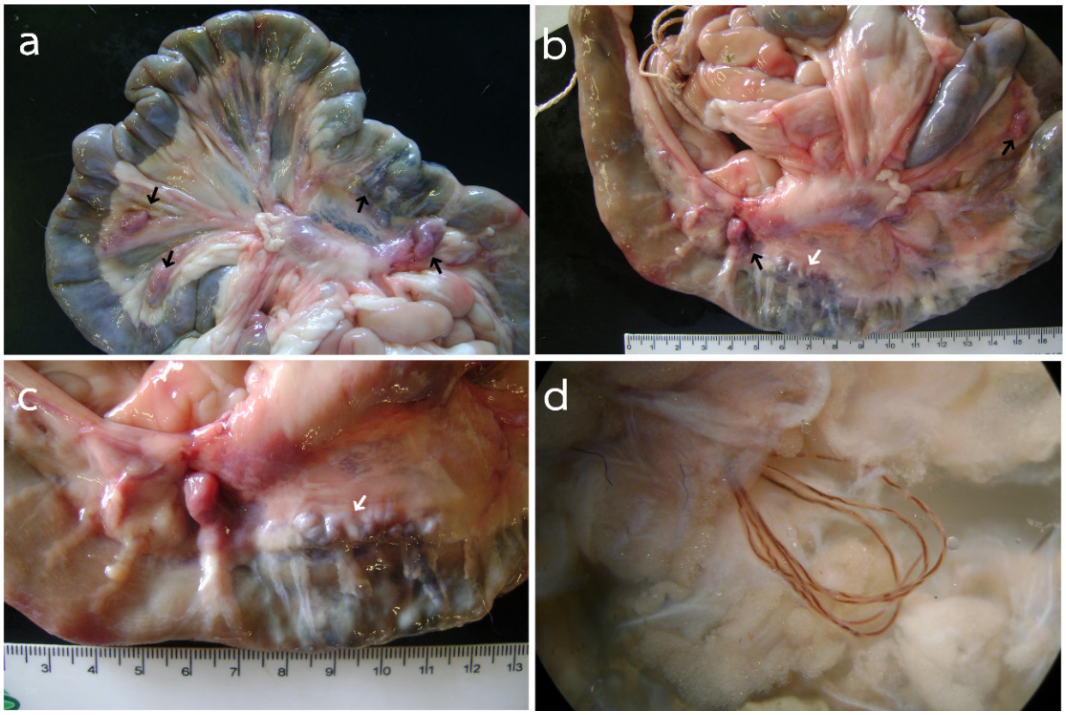

Figure 1. *Angiostrongylus minasensis* on the mesenteric arteries. The black arrows indicate nodules on the mesentery (a, b). The white arrows point to blood vessels (b, c). The parasite recovered from blood vessels (d).

Prevalence. 100% (n = 10).

Type material. Holotype male (CHIOC number 38505a) and allotype female (CHIOC number 38505b). Deposited in the Oswaldo Cruz helminthological collection (CHIOC), Rio de Janeiro, Brazil.

Etymology. The species name refers to the place where the new species was isolated.

Description

General. (Figures 2a and 3a) Body of both sexes filiform with transparent transversally striated cuticle. Oral aperture simple. Esophagus short and claviform. Excretory pore posterior to esophago-intestinal junction. Nerve ring anterior to the middle of esophagus. SEM results showed details of the cuticular topography, including important taxonomical structures. Anterior end of male and female is characterized by the presence of a circular oral opening surrounded by six sensory papillae and two amphids. The papillae are distributed in two dorsal and four lateroventral and amphids on the lateral position of the anterior end (Figure 4a and b). Dorsal side of the mouth presents a structure with a cleft, which was named a tooth-like structure (Figure 4b).

Figure 2. Adult specimen of *Angiostrongylus minasensis*. Anterior extremity showing the excretory pore (a). Female posterior extremity showing the vulva (V), anus (AN) and the tip of the tail (TPT) (b). Ventral view of the copulatory bursa, with the ventro-ventral (VV) and lateroventral (LV) rays emerging from the same trunk; lateral rays divided into anterolateral (AL), mediolateral (ML) and posterolateral (PL); and externodorsal (ED) and dorsal (D) rays (c). Lateral view of the copulatory bursa showing the gubernaculum (GB) and the size of the spicule (SP) (d).

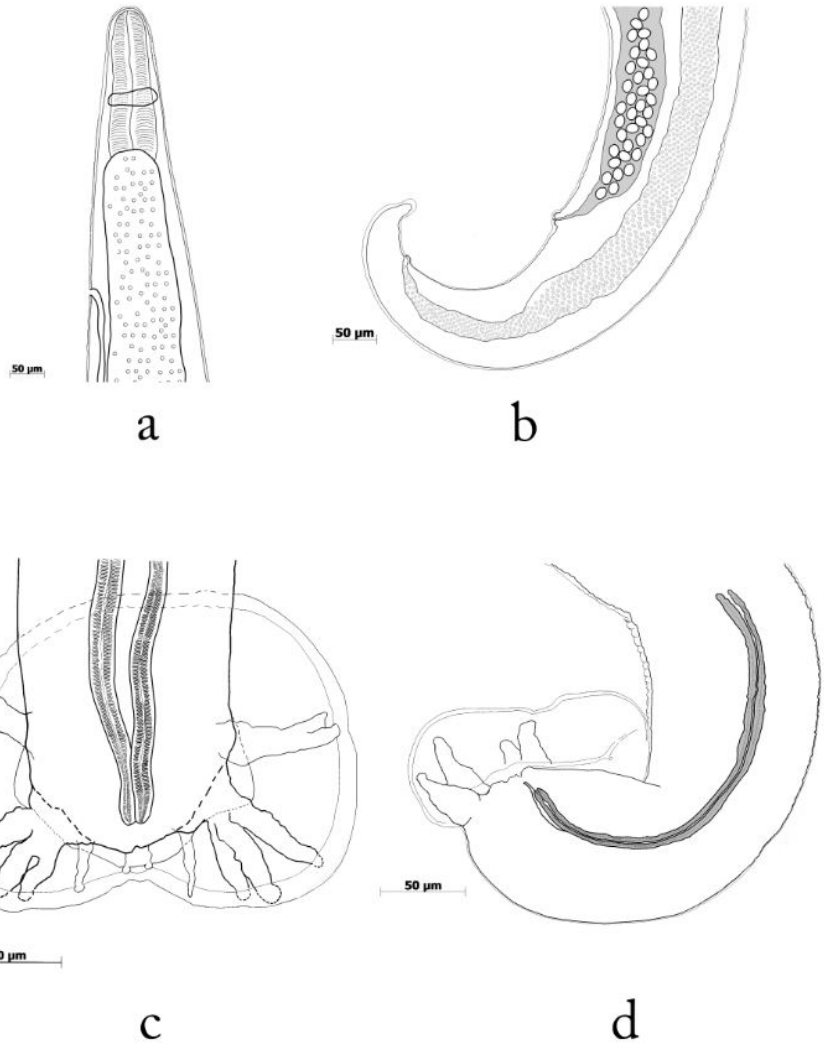

Figure 3. Drawings of *A. minasensis* using optical microscopy in association with a camera lucida. Female anterior extremity (a). Female posterior extremity (b). Ventral view of posterior extremity of male, showing the copulatory bursa (c). Posterior extremity of male in lateral view, showing the spicules and gubernaculum (d).

Male. [Based on one holotype and 10 paratypes; Figure 2c, 2d, 3c and 3d] Body 13.22 mm [12.12-14.1 (13.27 ± 0.67)] long and 0.17 mm [0.19-0.22 (0.2 ± 0.01)] wide. Esophagus 0.25 mm [0.20-0.25 (0.23 ± 0.02)] long and 0.04 mm [0.03-0.04 (0.03 ± 0.005)] wide at its base. Nerve ring and excretory pore 0.073 mm [0.064-0.075 (0.07 ± 0.005)] and 0.37 mm [0.3-0.37 (0.34 ± 0.03)] from anterior end, respectively. Caudal extremity ventrally curved. Copulatory bursa symmetrical and small. Ventral rays have a common origin, bifurcating distally; lateroventral ray is longer and more robust than ventro-ventral ray. Lateral rays emerge from a common trunk and are similar in size; antero-lateral ray is robust and separate from the common trunk close to the base; mediolateral and posterolateral rays are separated close to the extremity. Externo dorsal rays are separate from the dorsal ray and are longer and more slender. The dorsal ray is reduced and bifurcates distally into two small branches. Spicules short, equal in size with a striated sheath, 0.25 mm [0.24-0.27 (0.25 ± 0.012)] length. Gubernaculum 0.039 mm [0.034-0.045 (0.04 ± 0.003)] in length. SEM results showed symmetrical copulatory bursa with quadrangular aspect and two spicules (Figure 4d). On the dorsal face, it was possible to

identify the external lateral and dorsal rays (Figure 4d). Ventral, lateral and dorsal trunks were observed and, in addition, it was possible to differentiate the lateral trunk divided into three rays: anterolateral, mediolateral and posterolateral (Figure 4d and e). At the end of the anterolateral ray on both sides, near to the bursal edge, we identified one papilla (Figure 4d and f). The spicules were everted, trough-shaped, and the surface presented a striated external appearance (Figure 4f).

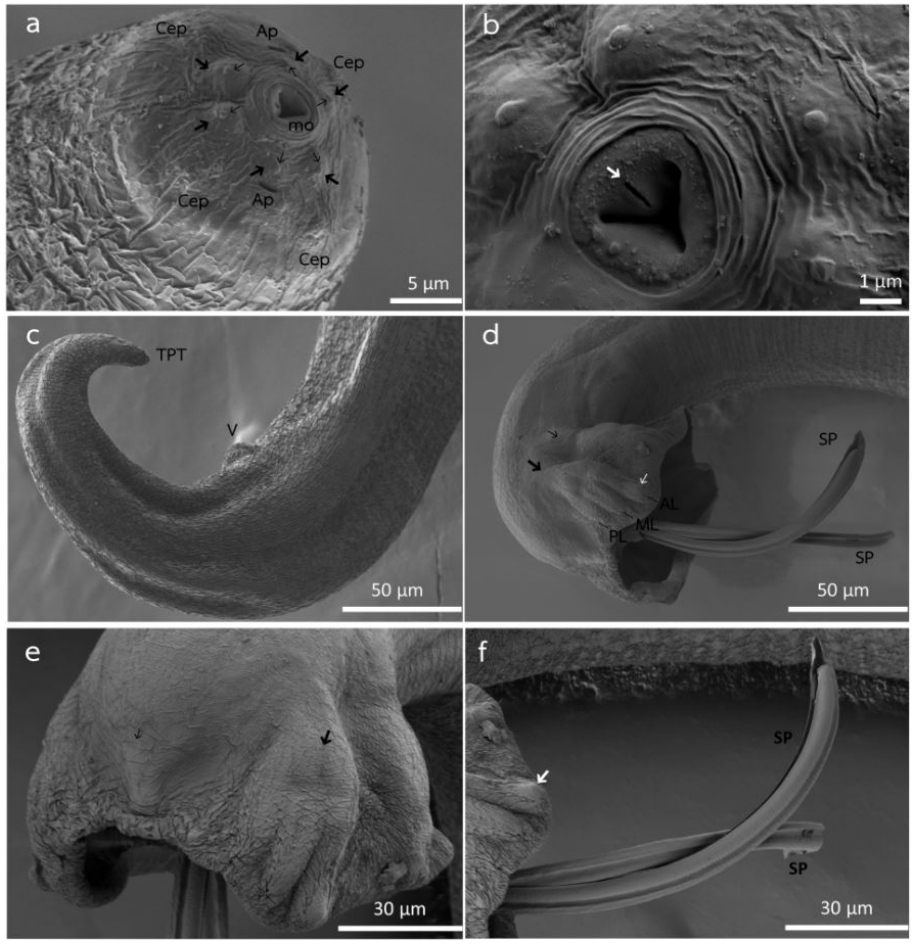

Figure 4. Scanning electron microscopy photomicrographs of *A. minasensis*. Anterior extremity, showing triangular opening of the oral opening (mo); six labial papillae (black thick arrows) with six small protuberances (black fine arrows); amphidial pores (Ap) and four cephalic papillae (CeP) (a). Magnification of the anterior extremity, showing a structure with a slit on the dorsal side of the mouth, which was named "tooth-like" (white thick arrow) (b). Posterior extremity of the female in lateral view, showing the vulva (V) and the long and sharp tail (TPT) (c). Posterior extremity of the male with the copulatory bursa in lateral view, showing: on the dorsal side, the lateral trunk (black thick arrow) with the anterolateral rays (AL) with one papilla (white arrow), mediolateral rays (ML) and posterolateral rays (PL); ventral trunk (black think arrow) and spicules (SP) (d). Copulatory bursa in dorsal view, showing the reduced dorsal ray (thin arrow) and lateral trunk (black thick arrow) (e). Spicules trough-shaped and, showing striated topography (SP); papilla in the anterolateral ray (white arrow) (f).

Female. [Based on one allotype and 10 paratypes; Figures 2b and 3b] Body 18.4 mm [16.25-20.83 (19.13 ± 1.5)] long and 0.22 mm [0.23-0.26 (0.24 ± 0.01)] wide. Esophagus 0.23 mm [0.22-0.25 (0.24 ± 0.01)] long and 0.03mm [0.03-0.05 (0.04 ± 0.005)] wide at its base. Nerve ring and excretory pore 0.069 mm [0.066-0.073 (0.07 ± 0.003)] and 0.035 mm [0.38-0.44 (0.41 ± 0.02)] from anterior end, respectively. Monodelphic. Vulva aperture 0.36

mm [0.31-0.37 (0.34 ± 0.020)] from posterior extremity, with protuberant lips. In live specimens, the uterine tubules can be seen to spiral around the blood-filled intestine, and this can be seen through the transparent cuticle. Anus 0.09 mm [0.08-0.09 (0.09 ± 0.005)] from posterior end. Long tail ventrally curved with a tapered tip. SEM results showed that the vulvar opening was near to the posterior end of the body and the long and sharp tail (Figure 4c).

First stage larvae. [n=7] Using the Baermann apparatus, L1 were recovered from the rectum of all the necropsied animals. Body 290 µm ± 0.01 (260 µm – 290 µm) long and 10 µm ± 0.004 (10 µm – 20 µm) wide. Esophagus is slender, measuring 130 µm ± 0.007 (110 µm – 130 µm) in length. The intestine is a thin tube. Anus is located 24 µm ± 0.002 (23 µm – 27 µm) from the distal end. The tail ends at an unguiform appendix on dorsal surface.

Taxonomic

The copulatory bursa and spicules of *A. minasensis* n. sp. differed from those of the *Angiostrongylus* species previously described. It has a longer externodorsal ray than its dorsal ray, thus differing from *A. raillieti*, *A. felineus*, *A. sandarsae*, *A. dujardini*, *A. lenzii*, *A. siamensis* and *A. morerai*, which have externodorsal rays that are smaller than or equivalent in length to the dorsal ray (Travassos, 1927; Alicata, 1968; Dróźdź & Doby, 1970; Ohbayashi et al., 1979; Robles et al., 2008; Souza et al., 2009; Vieira et al., 2013).

Although eight species of *Angiostrongylus* also have longer externodorsal rays than their dorsal rays, they can be differentiated from *A. minasensis* n. sp. by the lateral rays, the ventral rays and the length of the spicules. *A. minasensis* n. sp. has ventral trunk rays that are more robust, and its spicules are shorter than those of *A. vasorum* and *A. gubernaculatus* (Baillet, 1957; Dougherty, 1946; Duarte et al., 2007). *Angiostrongylus minasensis* n. sp. differs from *A. chabaudi* and *A. costaricensis* because its externodorsal ray is separated from the lateral trunk and its spicules are shorter (Biocca, 1957; Morera & Céspedes, 1970). The new species differ from *A. taterona* because the anterolateral ray bifurcates close to the base of the lateral trunk, while the mediolateral and posterolateral rays bifurcate in the distal third of the trunk. Moreover, the spicules are shorter (spicules of *A. minasensis* n. sp. measured 240-270 µm versus 500-620 µm in *A. taterone*) (Baylis, 1928).

Angiostrongylus minasensis n. sp. differs from *A. cantonensis* and *A. malaysiensis* (Chen, 1935; Bhaibulaya & Cross, 1971) because it possesses a lateral trunk with almost equivalent length of rays. It also differs from *A. mackerrasae* and *A. schmidti* (Bhaibulaya, 1968; Kinsella, 1971), since its lateral trunk rays bifurcate at various levels and its spicules are smaller (spicules of *A. minasensis* n. sp. measured 240-270 µm, versus 420 µm in *A. schmidti*). *Angiostrongylus minasensis* n. sp. differs from *A. ryjikovi* (Jushkov, 1971) through its posterolateral ray that is similar in length to the mediolateral ray. It also differs from *A. daskalovi* (Yanchev & Genov, 1988) with respect to its spicules (spicules of *A. minasensis* n. sp. measured 240-270 µm, versus 336-409 µm in *A. daskalovi*).

The species that was found in *N. nasua* differs morphologically from *A. costaricensis*. The rays of the copulatory bursa of *A. minasensis* n. sp. lack dilations in the distal extremity and have a slender externodorsal ray. The externodorsal ray of *A. costaricensis* is approximately the same thickness as the rays of the lateral trunk, has the same dilation in the distal extremity and presents longer spicules. The posterior extremity of the female of *A. minasensis* n. sp. is longer and sharper than that of *A. costaricensis* and *A. vasorum*, which is roughly conical and slightly curved in both of these species (Morera & Céspedes, 1970; Duarte et al., 2007). The tails of females of *A. siamensis* and *A. minasensis* are of the same length and sharpness, but the copulatory bursa of males of *A. siamensis* is smaller and presents shorter rays (Ohbayashi et al., 1979).

Finally, *A. minasensis* n. sp. is morphometrically different from all other species found at the same site of infection (mesenteric arteries) and different from all other species found in the order Carnivora, as described in Table 2.

Table 2. Measurements (in millimetres) from *A. minasensis* n. sp. (10 males and 10 females) in comparison with five other species of *Angiostrongylus*.

	A. minasensis	***A. costaricensis***	***A. siamensis***	***A. gubernaculatus***	***A. vasorum***	***A. chabaudi***
Reference		Morera & Céspedes (1970)	Ohbayashi et al. (1979)	Dougherty (1946)	Guilhon & Cens (1973)	Biocca (1957)
Host	*Nasua nasua*	Man	*Rattus sabanus, R. berdmorei*	*Taxidea taxus neglecta* Mearns	*Canis lupus familiaris*	*Felis silvestris*
Locality	Brazil	Costa Rica	Thailand	California	France	Central Italy
MALE						
Length	12.12-14.1	15.0-17.9	10	18-19.5	14-15.5	14.6-16.3
Width	0.19-0.22	-	-	0.3	-	0.185-0.225
Oesophagus	0.2-0.25	0.16-0.18	0.23	0.3-0.34	0.22-0.28	0.3-0.345
Nerve ring	0.064-0.075	-	-	-	0.08-0.092	0.185-0.23
Excretory pore	0.3-0.37	-	0.32	-	0.31-0.35	0.335-0.405
Spicule	0.24-0.27	0.267-0.297	0.339	0.52-0.56	0.4-0.48	0.510-0.555
Gubernaculum	0.034-0.045	Present	Present	0.045-0.05	0-04-0.055	Absent
FEMALE						
Length	16.25-20.83	26.9	11-13	22-24	15-20.5	19.8-24.1
Width	0.23-0.26	-	-	0.35	-	0.245-0.298
Oesophagus	0.22-0.26	0.10-0.26	0.23-0.27	0.34-0.35	0.24-0.28	0.345-0.38
Nerve ring	0.066- 0.073	-	-	-	0.08-0.096	0.21-0.24
Excretory pore	0.38-0.44	-	0.32-0.36	-	0.35-0.37	0.395-0.47
Vulva-tail	0.31-0.37	0.175	0.3-0.38	0.21-0.26 (vulva-anus)	0.22-0.315	0.17-0.21
Anus-tail	0.08-0.09	0.053	0.07-0.1	0.075-0.09	0.061-0.1	0.062-0.075

(-) represents lack of data.

Phylogenetic analysis

The aligned sequences resulted in a matrix comprising 18 taxa and 702 characteristics, of which 459 were constant and 190 were variable characteristics that were informative for parsimony. The phylogenies described here were based on the MT-CO1 gene and were inferred using two different optimality criteria (ML and BI), which resulted in similar topologies with little variation in nodes and support values (Figure 5).

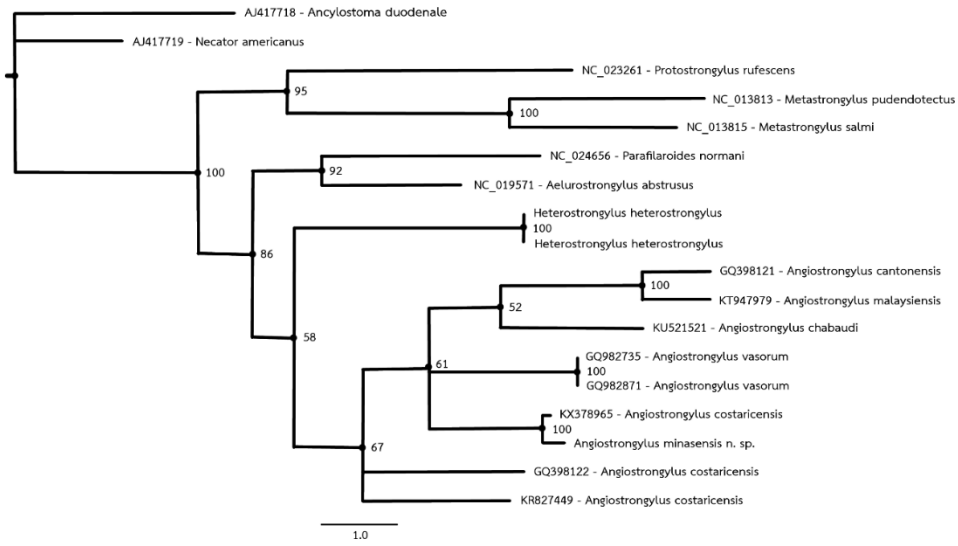

Figure 5. Bayesian phylogenetic reconstruction, based on the MT-CO1 gene. Values at the nodes are the Bayesian posterior probabilities. Scale bar represents branch lengths in substitutions per site.

The ML method resulted in a tree with lnL score = -3642.256. The evolutionary models selected through BIC were as follows: HKY+G with fixed substitution rates (3,1,1,1,1,3); frequency of bases and gamma distribution optimized in the first codon position and independent J1+G with substitution rates; and frequency of bases and proportion of invariable sites optimized in the second and third codon positions.

The BI method resulted in values that were highly significant for all the parameters for the estimated size of the sample (ESS). The genera *Angiostrongylus*, *Heterostrongylus*, *Aelurostrongylus* and *Parafilaroides* formed a monophyletic group in both topologies with support ranging from low to moderate (LR-ELW = 76%, ML-BP < 50%, BPP = 86%). The genus *Angiostrongylus* was monophyletic in the BI topology with low support (BPP = 67%).

Angiostrongylus minasensis n. sp. was a sister to the specimen isolated from *Nasua narica* (Santoro et al., 2016) with strong support in all topologies (LR-ELW = 93%; ML-BP = 99%; BPP = 100%). The genetic lineage formed by *Angiostrongylus* in coatis was connected via basal polytomy to the lineage formed by *A. vasorum* and the lineage formed by *A. cantonensis*, *A. malaysiensis* and *A. chabaudi*, with low support in the BI topology (BPP = 61%).

ML-pairwise genetic distances of the MT-CO1 gene between *Angiostrongylus* species ranged from 6.8% between *A. cantonensis* and *A. malaysiensis* to 27.3% between *A. chabaudi* and *A. costaricensis*. Distances between genera ranged from 16.1% between *Necator* and *Ancylostoma* to 41.4% between *Ancylostoma* and *Angiostrongylus*. Distances between *Aelurostrongylus*, *Heterostrongylus* and *Angiostrongylus* species sequences ranged from 20.8% to 31.1%. The distance within *Angiostrongylus* taxa from coatis was 0.2%, whereas the distance within *A. costaricensis* was 13.3%. The distances between *Angiostrongylus* taxa from coatis and *A. costaricensis* taxa ranged from 16.2% to 18.5% (Table 3).

Table 3. Pairwise ML genetic distances of the MT-CO1 gene between *A. minasensis* n. sp (bold) and other strongylids.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Ancylostoma duodenale (AJ417718)																
2. *Necator americanus* (AJ417719)																
3. *Protostrongylus rufescens* (NC_023262)	37.240															
4. *Metastrongylus pudendotectus* (NC013813)	38.343	35.950														
5. *Metastrongylus salmi* (NC013815)	45.664	42.342	20.002													
6. *Parafilaroides normani* (NC024656)	24.342	30.736	31.055	35.661												
7. *Heterostrongylus heterostrongylus* (Ilha Grande, RJ)	27.033	34.833	32.439	35.547	25.143											
8. *Heterostrongylus heterostrongylus* (PEPB/CFMA, RJ)	26.825	34.858	32.315	35.627	24.855	0.007										
9. *Aelurostrongylus abstrusus* (NC019571)	27.617	28.646	37.879	31.581	20.158	20.917	20.834									
10. *Angiostrongylus cantonensis* (GQ398121)	25.772	36.751	37.191	36.876	27.641	21.953	22.020	23.555								
11. *Angiostrongylus malaysiensis* (KT947979)	31.707	35.441	39.113	38.985	30.821	24.849	24.834	26.821	6.851							
12. *Angiostrongylus chabaudi* (KU521521)	31.619	37.349	43.042	40.547	30.834	31.075	31.107	28.164	20.284	19.502						
13. *Angiostrongylus vasorum* (GQ982735)	30.756	37.879	37.246	38.593	23.388	28.032	27.959	24.266	21.889	23.614	14.273					

Table 3. Continued...

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
14. *Angiostrongylus vasorum* (GQ982871)	30.804	37.872	37.293	38.587	23.386	28.078	28.006	24.315	21.886	23.610	14.326	0.003				
15. *Angiostrongylus costaricensis* (GQ398122)	30.033	37.828	36.428	30.768	26.478	21.172	21.296	20.626	20.013	21.170	21.327	18.523	18.520			
16. *Angiostrongylus costaricensis* (KR827449)	29.261	30.774	33.170	27.778	26.751	23.981	24.094	20.862	19.454	19.216	27.288	21.502	21.499	13.280		
17. *Angiostrongylus costaricensis* (KX378965)	30.746	38.294	37.080	36.371	28.946	23.149	23.103	25.270	17.715	17.172	16.790	16.262	16.258	16.172	17.539	
18. *Angiostrongylus minasensis* n. sp.	**30.828**	**37.776**	**35.207**	**35.380**	**28.988**	**23.065**	**22.832**	**25.307**	**19.866**	**18.284**	**17.989**	**17.007**	**17.003**	**18.439**	**17.466**	**0.233**

Discussion

The finding of helminths harbored in the mesenteric arteries of coatis from the Mangabeiras Park in Belo Horizonte (state of Minas Gerais, Brazil) initially suggested the possibility of infection by *A. costaricensis*, because this species has been reported infecting rodents and *N. narica* coatis in the Americas (Morera, 1973; Monge et al., 1978; Santoro et al., 2016). An alternative explanation was that the animals were infected by *A. siamensis*, which has been described infecting rodents in Thailand (Ohbayashi et al., 1979), since the latter species was also found infecting the mesenteric arteries of the caecum.

Given that *Angiostrongylus* spp. present low specificity to their vertebrate hosts, we examined the possibility that the species described here was *A. vasorum*, because this species has been described parasitizing domestic and wild canids in the state of Minas Gerais (Lima et al., 1985; Lima et al., 1994). However, morphological evaluation of the posterior extremity of the specimens of the present study that were found parasitizing coatis, in comparison with species found at the same infection sites and all the species found parasitizing carnivores, allowed us to conclude that the *Angiostrongylus* sp. described here could not be identified as any of the above mentioned species and is, therefore, a new species.

The ML-pairwise genetic distances of the MT-CO1 gene showed that *A. minasensis* n. sp. is significantly distant from the *A. costaricensis* taxa isolated from rodents in Brazil and Costa Rica. On the other hand, the genetic distance between *A. minasensis* n. sp. recovered from *Nasua nasua* in Brazil and *A. costaricensis* isolated from *N. narica* from Costa Rica is small and is only larger than the distance between the European sequences of *A. vasorum*. Similarly, the distances between the *A. costaricensis* taxa from *N. narica* and rodents, both from Costa Rica, are also significantly large. The phylogenetic analyses presented here suggest that *A. minasensis* n. sp. corresponds to a new taxon.

Interestingly, the new species is closely related to the *A. costaricensis* taxon occurring in *N. narica* in Costa Rica.

Angiostrongylus costaricensis may also have low host specificity, since it parasitizes primates and the marsupial *Didelphis virginiana,* and *Procyon lotor* in Miami (Florida, USA) (Miller et al., 2006). However, L1 larvae isolated from faeces was not reported in these cases (Miller et al., 2006). In the present study, the occurrence of L1 in all the samples of feces from all the coatis that presented the adult parasite suggests that *N. nasua* is the natural host of *A. minasensis* n. sp. In this area, coatis occur at high population densities due to the absence of natural predators and the high abundance of food offered and discarded by humans (Porfírio et al., 2006). The Mangabeiras Park is located on the border between the metropolitan area of Belo Horizonte, which is subjected to anthropic pressure, and a conservation area (Serra do Curral), which could facilitate animal dispersion (Porfírio et al., 2006).

Conclusion

Angiostrongylus minasensis n. sp. is a new species of *Angiostrongylus* found in the mesenteric arteries of *Nasua nasua* in Brazil.

Acknowledgements

The authors would like to thank the Oswaldo Cruz Institute for its help in DNA sequencing; IBAMA's wild animal screening center; and the Projeto Quatis and parks foundation of Prefeitura of Belo Horizonte, Minas Gerais. We also would like to thank Marco Anacleto (Scientific Illustration Laboratory, Instituto de Ciências Biológicas, Federal University of Minas Gerais, Brazil), and Andrea Kauffmann for the translation. We thank the Research Support Foundation of the State of Minas Gerais (Fundação de Amparo à Pesquisa do Estado de Minas Gerais, FAPEMIG) for its financial support (WSL, grant number 18898), and the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq) for its financial support (WSL, grant number 480469/2012-1).

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