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Litomosoides silvai (Nematoda: Onchocercidae) parasitizing *Akodon montensis* (Rodentia: Cricetidae) in the southern region of Brazil

Litomosoides silvai (Nematoda: Onchocercidae) parasitando *Akodon montensis* (Rodentia: Cricetidae) na região Sul do Brasil

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Abstract

In the present study, *Litomosoides silvai* parasitizing *Akodon montensis* in the southern region of Brazil is reported for the first time. New morphological information is provided for some structures of this nematode species, such as a flattened cephalic extremity, presence of two dorsal cephalic papillae, female tail with a constriction at its tip, "s" shaped vagina, spicules characteristic of the *carinii* species group and microfilaria tail constricted at the tip. This nematode was found parasitizing the thoracic cavity with a prevalence of 10% (2/20), mean intensity of 4 (6/2), mean abundance of 0.4 (8/20) and range of infection of 2-6 specimens per host, in southern Brazil. This occurrence of *L. silvai* in *A. montensis* is a new geographical record for southern Brazil, in the Upper Paraná Atlantic Forest ecoregion of the northwestern region of Rio Grande do Sul, which is part of the Atlantic Forest biome.

Keywords: Filarioid, Atlantic Forest biome, nematode, rodent, Rio Grande do Sul.

Resumo

No presente estudo é relatado pela primeira vez *Litomosoides silvai* parasitando *Akodon montensis* coletados na região Sul do Brasil. Foram fornecidas novas informações morfológicas para algumas estruturas desta espécie de nematódeo, tais como extremidade cefálica achatada, a presença de duas papilas cefálicas dorsais, cauda das fêmeas com uma constrição na ponta da cauda, vagina em forma de "s", espículas de característica do grupo de espécies de *carinii* e cauda da microfilária com constrição na ponta. Este nematódeo parasitava a cavidade torácica com uma prevalência de 10% (2/20), intensidade média de 4 (8/2) e abundância média de 0,4 (8/20), e intervalo de infecção de 2-6 espécimes por hospedeiro no Sul do Brasil. A ocorrência de *L. silvai* em *A. montensis* é um novo registro geográfico, no sul do Brasil, a noroeste do estado do Rio Grande do Sul, na ecorregião da Mata Atlântica do Alto Paraná, parte do bioma da Mata Atlântica.

Palavras-chave: Filarideo, bioma Mata Atlântica, nematoda, roedor, Rio Grande do Sul.

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Introduction

Turvo State Park (Parque Estadual do Turvo, PST) is a natural reserve located in the northwest of the state of Rio Grande do Sul, southern Brazil, comprising an area of approximately 17,491 hectares. The park is covered almost entirely by subtropical broadleaf or broadleaf deciduous forest. The mammal fauna of the park consists of carnivores such as *Galictis* sp., *Cerdocyon thous*, *Nasua nasua*, *Procyon cancrivorus*, *Eira barbara*, wild cats, herbivorous like deer and tapirs, some species of lagomorphs, primates, didelphimorphia, and xenarthrans, many species of chiropterans and as well as numerous rodents (KASPER et al., 2007; MELO et al., 2011).

One of the genera of Cricetidae rodents that is well represented in Brazil is *Akodon* Meyen, 1833, with 10 species recorded in this country (SILVEIRA et al., 2013). Among these, *Akodon montensis* Thomas, 1913 is the only species of this genus found in this reserve (MELO et al., 2011). This species occurs in Paraguay, northern Argentina and southeastern to southern Brazil, including the states of Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul (MUSSER & CARLETON, 2005).

Filarioids of the genus *Litomosoides* Chandler, 1931, are parasites of the abdominal and thoracic cavities of bats, marsupials and many rodents. Currently, this genus is represented by 43 known species (NOTARNICOLA et al., 2012; NOTARNICOLA & de la SANCHA, 2015; OVIEDO et al., 2016). In Brazil, species of *Litomosoides* have been found parasitizing marsupials (BAIN et al., 1980; MULLER, 1980), bats (LINS DE ALMEIDA, 1936 apud NOTARNICOLA et al., 2010; REGO, 1961; GUERRERO et al., 2002; BAIN et al., 2003) and rodents (TRAVASSOS, 1919 apud PADILHA & FARIA, 1977; PADILHA & FARIA, 1977; BAIN et al., 1989; MORAES-NETO et al., 1997; SIMÕES et al., 2012). Two species, *Litomosoides silvai* (PADILHA & FARIA, 1977) and *Litomosoides chagasfilhoi* (MORAES-NETO et al., 1997) have been recorded in the rodent *Akodon cursor* Winge, 1887, in Itaguaí and Rio Bonito, in the Atlantic Forest region of the state of Rio de Janeiro (PADILHA & FARIA, 1977; MORAES-NETO et al., 1996, 1997; MORAES-NETO & LANFREDI 1997; SIMÕES et al., 2011, 2012).

The aim of the present study was to report on *L. silvai* parasitizing *A. montensis* in the Turvo State Park, state of Rio Grande do Sul, Brazil, which is in the Upper Paraná Atlantic Forest ecoregion, part of Atlantic Forest biome. Furthermore, this study provides new morphological data for some structures, such as the cephalic extremity, tail and vagina, spicules and microfilariae tail; and it presents parasitological indices (prevalence, mean intensity and mean abundance) and data on range and infection site.

Materials and Methods

During a survey carried out to study tick-borne diseases (KRAWCZAK, 2016), 164 specimens of small mammals were caught: 117 *Akodon montensis*, 13 *Brucepattersonius iheringi*, 1 *Cryptonanus guahybae*, 7 *Didelphis aurita*, 1 *Euryoryzomys russatus*, 9 *Oligoryzomys nigripes*, 7 *Oxymycterus judex*, 4 *Sooretamys angouya* and 5 *Thaptomys nigrita*. They were caught in the Turvo State Park (latitude 27°00' S to 27°20' S; longitude 53°40' W to 54°10' W), in the northwest of the state of Rio Grande do Sul, Brazil, between June 2013 and January 2014. However, only some of the rodents, which died due to the procedures for anesthesia and blood collection, were used in the present study: 20 specimens of *Akodon montensis* Thomas, 1913; two *Brucepattersonius iheringi* Thomas, 1896; one *Oxymycterus judex* Thomas, 1909; and one *Oligoryzomys nigripes* Olfers, 1818. Some other rodents that died were preserved in absolute ethanol until arrival at the Parasitic Diseases Laboratory of the School of Veterinary Medicine and Animal Science, Universidade de São Paulo (USP). These were then analyzed for tick presence and were identified in accordance with Bonvicino et al. (2008) and Melo et al. (2011). These animals were deposited in the Rodent Collection of the "Luiz de Queiroz" Higher Agriculture School, USP, state of São Paulo, Brazil.

The animals were caught using a total of 80 live traps (75 Sherman and five Tomahawk) baited with bacon, banana, apple and peanut butter. These were set up for four consecutive nights during each field campaign. Additionally, three pitfall stationary traps each consisting of five buckets of 42.5 cm in diameter and 60 cm in height, which were connected using a plastic fence (at least 30 m long and 50 cm high), were set up for the same period. The animals thus trapped were then anaesthetized using ketamine and xylazine.

Adult nematode specimens were recovered from the thoracic cavity of *A. montensis*, stored in Railliet & Henry liquid, and were sent to the Veterinary Parasitology Laboratory, Universidade Federal de Santa Maria, state of Rio Grande do Sul, Brazil, for identification. To study their morphological characteristics, they were then cleared in lactophenol, temporarily mounted, examined under a bright-field microscope (Olympus BX-51) and photographed using a Zeiss Axiophot. Microfilariae from the uterus of one fixed female were dissected and examined. Illustrations were made with the aid of a drawing tube. Measurements are given in micrometers unless otherwise stated. The parasite terms used are in accordance with Bush et al. (1997).

Nematode specimens were deposited in the Helminth Collection of the Museum of La Plata (MLP-he). Moreover, specimens of *L. silvai* parasitizing *A. cursor,* from Rio Bonito, state of Rio de Janeiro (MORAES-NETO et al., 1996) deposited in the Oswaldo Cruz Institute Helminthological Collection (CHIOC) under number 33518 (one female and one male) were studied to improve the diagnosis.

This study was approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science, USP, under protocol number 2908/2013; by the Chico Mendes Institute for Biodiversity Conservation (ICMBio), under license number SISBIO 38502-1; and by the State Environmental Department of Rio Grande do Sul (SEMA, RS).

Results

Three female filarioid specimens, two anterior extremities and three posterior extremities were recovered from the thoracic cavity of two specimens of *A. montensis.* Morphometric data on the female and microfilarial specimens are showed in Table 1. The species identification was aided through studying male and female specimens deposited in CHIOC (number 33518).

Table 1. Compared measurements of females from *Litomosoides silvai* reported in the literature and the present study.

Host species	*Akodon cursor*	*Akodon cursor*	*Akodon montensis*
Reference	Padilha & Faria (1977)	Moraes-Neto et al. (1996)	Present study
Locality, Country	Serra do Caçador, Itaguaí, RJ.	Catimbau Grande, Rio Bonito, RJ.	Parque Estadual do Turvo, Derrubadas, RS.
Localization	Thoracic cavity	Thoracic cavity	Thoracic cavity
Females	(n = 10)	(n = 10)	(n = 6)
Body length (mm)	46-64	48.9-89	60.88-82.46
Body width	250-290	220-363	211-230
Buccal capsule length	16-25	17-25	16-22
Esophagus length	440-750	491-659	480-1040
Tail lengh	440-800	267-450	380-480
Vulva to apex	1000-1650	1011-3159	1340-2000
Microfilariae length	-	68-77*	87-91*

Measurements are in micrometers (unless otherwise indicated); *Uterine microfilariae.

Litomosoides silvai Padilha and Faria, 1977 (Figures 1-11)

Diagnosis: Adult worms small, males four times smaller than females. Cephalic extremity flattened. In apical view, 4 labial papillae distributed in a stretched dorsoventral rectangle; 2 dorsal cephalic papillae. Buccal capsule slender; anterior segment transparent and posterior chitinous portion with irregular external walls; buccal cavity with 2 short enlargements near the base. Female tail slender, with a constriction at tip of tail. Esophagus muscular, becoming slightly glandular in the posterior portion. Vulva posterior to the esophagus-intestine junction. Vagina "s" shaped; vagina perpendicular to the axis of the vulva with a flattened lumen consisting of a simple "s" shaped tube surrounded by muscular walls. Male tail slender; left spicule with handle longer than blade; blade consisting of a membranous ala that converts into a filament; right spicule is a twisted conduit with a heel that is not strongly cuticularized. Microfilariae slender, with sheath closely applied to the body; nuclei not extending to the tip of tail; tail becomes constricted.

Taxonomic summary

Host: *Akodon montensis* Thomas, 1913.

Locality: Turvo State Park, state of Rio Grande do Sul, Brazil.

Infection site: thoracic cavity.

Parasitological indices: prevalence (P) = 10% (2/20), mean intensity (MI) = 4 (6/2), mean abundance (MA) = 0.4 (8/20) and range of infection of 2-6 specimens per host.

Specimen deposition numbers: MLP-he 7204; MLP-he 7205.

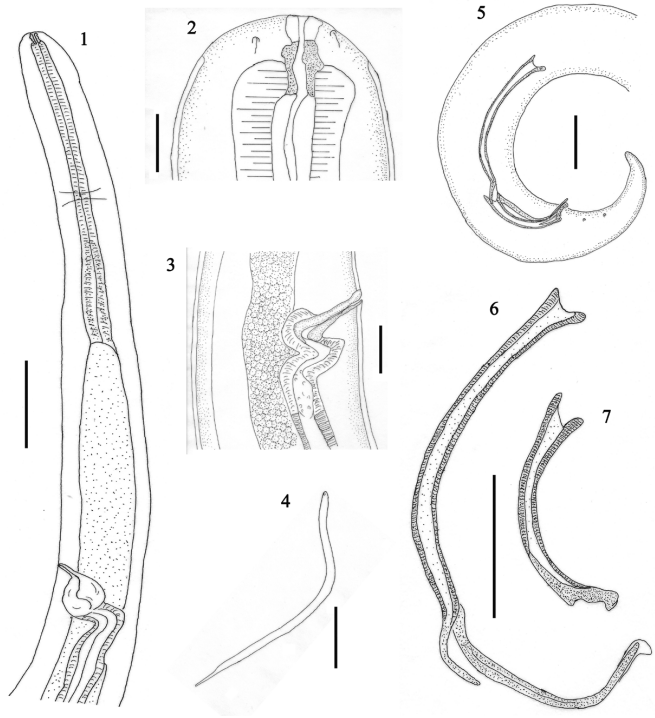

Figure 1-7. 1-4. Female specimens of *Litomosoides silvai* parasitizing *Akodon montensis* from Turvo State Park, state of Rio Grande do Sul. **1.** Anterior region of female, lateral view. **2.** Anterior extremity, lateral view. **3.** Detail of the vulva, lateral view. **4.** Uterine microfilaria. **5-7**. Male specimen of *L. silvai* parasitizing *A. cursor,* from Rio Bonito, state of Rio de Janeiro, CHIOC 33518. **5.** Posterior extremity showing the spicules and the cloaca, lateral view. **6.** Detail of the left spicule, lateral view. **7.** Detail of the right spicule, lateral view. Scale bars: 1 = 200 µm; 2 and 4 = 20 µm; 3, 5-7 = 50 µm.

Remarks

The specimens found in *A. montensis* correspond to the species *L. silvai* and are in accordance with the morphology and morphometrical characteristics given by Padilha & Faria (1977) and Moraes-Neto et al. (1996). The identification was confirmed by the shape of the buccal capsule, flattened anterior extremity, vulva located posteriorly to the esophagus-intestine junction, non-globular shape of the vulva, tail tip with a constriction, shape of the spicules corresponding to the *carinii* species group, and constricted tail tip of the microfilariae. In the present study, female specimens were compared with previous specimens deposited in CHIOC, which confirmed the identity of the species.

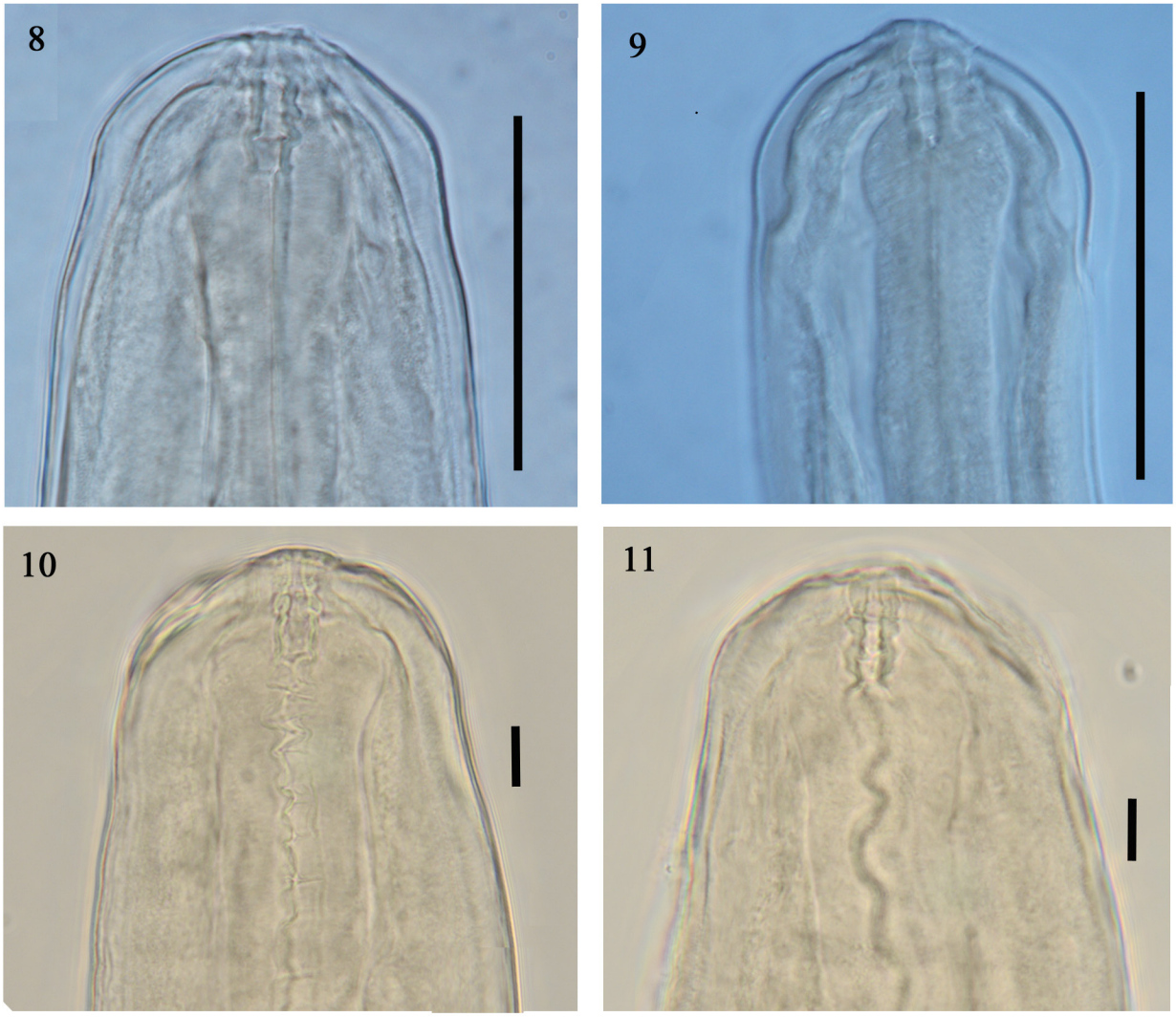

Figure 8-11. Detail of anterior extremities of *Litomosoides silvai*. **8-9.** Female and male specimens, respectively, of *L. silvai* parasitizing *Akodon cursor,* from Rio Bonito, state of Rio de Janeiro, CHIOC 33518. **10-11.** Female specimen of *L. silvai* parasitizing *A. montensis* from Turvo State Park, state of Rio Grande do Sul. Scale bars: 8-9 = 25 µm; 10-11: 10 µm.

Discussion

Litomosoides silvai was originally described by Padilha & Faria (1977), as a parasite of *A. cursor*. Later on, Moraes-Neto et al. (1996) redescribed the species including new morphological data and scanning electron microphotographs. These authors showed the distribution of the head papillae, the rugose area, the tip of the tail and the structure of the cuticle at the vulva. However, the position of the head papillae was not stated.

Here, four labial papillae distributed in a stretched dorsoventral rectangle and two dorsal cephalic papillae were observed; amphids were located ventrally in relation to the cephalic papillae. Additionally, the vulva was "s" shaped and differed from the globular shape of other *Litomosoides* species (NOTARNICOLA et al., 2000; OVIEDO et al., 2016).

Recently, Simões et al. (2011, 2012) reported the presence of *L. silvai* parasitizing the sympatric rodents *A. cursor* and *A. montensis* in Teresópolis, state of Rio de Janeiro (RJ). All previous reports of *L. silvai* are from the Atlantic Forest ecoregion, in the state of Rio de Janeiro. Therefore, in the present study, the occurrence of *L. silvai* in *A. montensis* in southern Brazil is a new geographical record, in the Upper Paraná Atlantic Forest ecoregion of the northwest of the state of Rio Grande do Sul, which is part of the Atlantic Forest biome.

According to Moraes-Neto & Lanfredi (1997), *L. silvai* parasitizing *A. cursor* in Rio Bonito, RJ (Serra do Mar Coastal Atlantic Forest ecoregion), presented P = 20.7% (12/58), MI = 18.6 (223/12) and MA = 3.8 (223/58); while Simões et al. (2011) reported P = 12%, MI = 2 and MA = 0.2, in Teresópolis, RJ, which is a nearby locality. Moreover, these last authors also found *L. silvai* parasitizing the sympatric rodent *A. montensis* (P = 2%, MI = 26 and MA = 0.5). The results from the present study show intermediate values that may be related to a different ecoregion or that may show that environmental factors are involved. Interestingly, in Rio Bonito *A. cursor* was also found to be parasitized with *L. chagasfilhoi*, with a prevalence of 12%, and both species of filarioids were

concurrent in 4 out of the 58 hosts sampled (MORAES-NETO & LANFREDI, 1997).

It seems that *L. silvai* always is located in the thoracic cavity when it parasitizes *A. cursor* or *A. montensis*, while *L. chagasfilhoi* is located in the abdominal cavity. Both species of filarioid are present in Rio Bonito, while only *L. silvai* is present in the other localities (Teresópolis and Turvo State Park). Thus, *L. silvai* is present in both *Akodon* species, but because of the different southern distribution of their hosts, the filarioid only follows *A. montensis*, which is the only species of this genus that has been trapped in the study area (MELO et al., 2011). It is interesting to note that among more than 236 specimens of *A. montensis* that were sampled by one of the present authors (JN, unpublished data) in different localities in Misiones province (Argentina), which is close to Turvo State Park, no filarioid specimens were detected.

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