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Original Article

New species of *Hassalstrongylus* (Trichostrongyloidea: Heligmonellidae) in the large-headed rice rat *Hylaeamys seuanezi*, in the Atlantic Forest of northeast Brazil

Nova espécie de *Hassalstrongylus* (Trichostrongyloidea: Heligmonellidae) no roedor *Hylaeamys seuanezi*, na Mata Atlântica do nordeste do Brasil

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Abstract

A new species of Trichostrongyloidea (Nematoda: Heligmonellidae), *Hassalstrongylus lauroi* n. sp., is described from specimens collected from the small intestine of the rodent *Hylaeamys seuanezi* in the Atlantic Forest of northeastern Brazil (Igrapiúna, state of Bahia). The genus *Hassalstrongylus* includes 17 species, which parasitize rodents occurring in the Neotropical and Nearctic regions. It differs from the genus *Stilestrongylus* through its smaller number of ridges in the synlophe and through the size of the genital cone. The main taxonomic characteristics of this new species are the subsymmetrical caudal bursa of type 2-2-1, ray 8 branching out at the base of the dorsal trunk, right lobe smaller than the left, and rays 4 and 5 of robust nature. In addition, the ornamental ray 5 and the robustness of ray 4 on the male caudal bursa, along with the modification of the ridges of the posterior end of the female, allow us to consider the specimens found to be a new species.

Keywords: Helminths, Nematoda, Rodentia, Cricetidae.

Resumo

Uma nova espécie de Trichostrongyloidea (Nematoda: Heligmonellidae), *Hassalstrongylus lauroi* n. sp., descrita a partir de espécimes coletados do intestino delgado do roedor *Hylaeamys seuanezi* na Mata Atlântica do Nordeste do Brasil (Igrapiúna, estado da Bahia). O gênero *Hassalstrongylus* inclui 17 espécies, que parasitam roedores que ocorrem nas regiões Neotropical e Neártica. Este difere do gênero *Stilestrongylus* por ter menor número de cristas na sínlofe e pelo tamanho do cone genital. As principais características taxonômicas dessa nova espécie são a bolsa caudal subsimétrica do tipo 2-2-1, raio 8, ramificando-se na base do tronco dorsal, lobo direito menor que o esquerdo e os raios 4 e 5 são robustos. Além disso, o raio 5 é ornamentado na bolsa caudal dos machos, e ocorre uma modificação nas cristas da extremidade posterior da fêmea, que permite considerar os espécimes encontrados como uma espécie nova.

Palavras-chave: Helmintos, Nematoda, Rodentia, Cricetidae.

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Introduction

Heligmonellidae (Skrajabin & Schikhobalova, 1952 tribe) Durette-Dessete & Chabaud, 1977 is a large family of small nematodes. It parasitizes the small intestine and, more rarely, the stomach, lungs and liver of terrestrial vertebrates, particularly rodents (Durette-Desset, 1968). Infrafamilial taxonomy is based on characteristics of the bursa, with traits of the synlophe used for the determination of genera (Durette-Desset, 1971a; Vicente et al., 1997). *Hassalstrongylus* Durette-Desset, 1971, includes 17 species with 19 to 24 cuticular longitudinal ridges that vary in body size and body sagittal plane. This genus has a symmetrical or subsymmetrical caudal bursa and a non-hypertrophied genital cone, and mainly parasitizes cricetid rodents (Anderson, 2000; Durette-Desset, 1985). Species of the genus *Hassalstrongylus* share several traits with species of the genus *Stilestrongylus* Freitas, Lent and Almeida, 1937. However, the number of ridges (less than 24 cuticular subequal) in the synlophe, the bursal characters, and the size of the genital cone of the former in relation to the latter are reliable characteristics for separating these genera (Durette-Desset & Digiani, 2010; Suriano & Navone, 1992).

The large-headed rice rat, *Hylaeamys seuanezi* (Weksler, Geise & Cerqueira, 1999), is a sigmodontine rodent endemic to the Brazilian Atlantic Forest. It occurs in forested areas and agroforestry systems in northeastern and southeastern Brazil, in the states of Bahia, Espírito Santo, Minas Gerais and Rio de Janeiro (Brennand et al., 2013; Silva et al., 2020). It has a mean adult body mass of 51 g and mean adult length of 275 mm (Bonvicino et al., 2008). It has nocturnal habits and forages mainly on fruits and seeds (Paglia et al., 2012).

The only study so far to have reported on the helminth fauna of *H. seuanezi* was carried out in agroforestry areas in the municipality of Ilhéus, state of Bahia, northeastern Brazil (Kersul et al., 2019). In that study, occurrences of *Hassalstrongylus* sp. in this rodent were recorded.

Here, we describe a new species of the genus *Hassalstrongylus* that was found parasitizing the rodent *H. seuanezi* in the Brazilian Atlantic Forest, in the municipality of Igrapiúna, also in the state of Bahia.

Materials and Methods

During a comprehensive study of mammal diversity and parasites in the Atlantic Forest, rodents were captured and helminths were recovered for ecological studies. These activities was carried out in preserved areas of the Pratigi Environmental Protection Area (13°51'8" S; 39°16'54.9" W) in the municipality of Igrapiúna, southern Bahia. The area encompasses valleys and plains within the landholding of the Juliana Valley United Farms (Fazendas Reunidas Vale do Juliana). These farms comprise a set of agroforestry systems (rubber, cocoa, clove and peach palm production) and also include patchy areas of dense ombrophilous forest (OCT, 2019).

The animals were collected by means of Sherman and Tomahawk traps. These were placed on the ground along six transects of 15 trapping points. Pitfall traps were also installed using 60-liter buckets along four additional transects. The spacing between the transects was 500 m and between traps, 20 m. All the traps were baited with a mixture of peanut butter, sardines in soybean oil, ripe bananas, cornmeal and oat flakes. Trapping was carried out during ten consecutive nights in August 2014 and March 2015. The host rodents were identified from their external morphology and cranial morphometries. The specimens were preserved by means of taxidermy and were housed as voucher specimens in the "Alexandre Rodrigues Ferreira" Mammal Collection of Santa Cruz State University (CMARF-UESC*).* The animals were caught under authorization from the Brazilian Government's Chico Mendes Institute for Biodiversity and Conservation (ICMBio; license number 17131-4) and from the Ethics Committee for Animal Use (CEUA) of the Oswaldo Cruz Foundation (license number LW-39/14*).* Biosafety techniques and personal safety equipment were used during all the procedures involving animal handling and biological sampling (Lemos & D'Andrea, 2014).

The stomach, intestine, lungs and thoracic and abdominal cavities of the hosts were searched for helminths. For morphological characterization and description of the internal structures, and for observation of the morphological characteristics, the helminths were diaphanized in 0.5% lactophenol or 50% glycerol and were mounted between a slide and cover slip. These specimens were analyzed using a compound microscope (Zeiss Standard 20), and drawings were made with the aid of a camera lucida.

The specimens were also subjected to the scanning electron microscopy (SEM) technique. The nematodes were fixed in a 2.5% glutaraldehyde solution at room temperature for one hour and were then washed in 0.1 M sodium cacodylate buffer with 3.5% sucrose (pH 7.2). They were post-fixed in a 2% osmium tetroxide solution, in 0.2 M sodium cacodylate buffer with 7% sucrose, at room temperature for two hours. After washing, the specimens were

dehydrated in an increasing ethanol series, at each step for one hour. They were then critical-point dried using CO_2, mounted with silver adhesive tape on aluminum stubs and sputter-coated with a 20 nm layer of gold. The samples were observed in a Jeol JSM-6390 LV scanning microscope at a voltage of 15 kV, at the electron microscopy platform of the Oswaldo Cruz Institute.

The synlophe was studied as indicated by Durette-Desset (1985), and the nomenclature referring to the axis of orientation and other characteristics of the synlophe followed the descriptions of Durette-Desset & Digiani (2005) and Durette-Desset et al. (2017). The morphometric data were expressed in micrometers (unless otherwise stated), with minimum, maximum, and average in parentheses. The type species was deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, Brazil.

Hassalstrongylus lauroi n. sp. (Figures 1-2)

Types: holotype (voucher number: CHIOC 38976 a); allotype (voucher number: CHIOC 38976 b); paratype (voucher number: CHIOC 38976 c)

Type host: *Hylaeamys seuanezi* (Weksler, Geise & Cerqueira, 1999) (Rodentia, Sigmodontinae); voucher number CMARF 1488

Other hosts: *Oxymycterus dasytrichus* Fischer, 1814

Site of infection: small intestine

Type locality: Pratigi Environmental Protection Area, Igrapiúna (13° 51' 8" S; 39° 16' 54.9" W), Bahia, Brazil

Abundance: 367 (178 males and 189 females). Mean abundance: *H. seuanezi*, 16.95 (2-82). *O. dasytrichus*, 0.46 (11).

Prevalence: *H. seuanezi*, 42% (21 infected / 50 examined hosts); *O. dasytrichus*, 4.17%, one of 24 examined hosts (eleven worms in a single host).

Etymology: Dedicated to Dr. Lauro Travassos a renowned parasitologist who made substantial contributions to helminthology and the systematics of the Trichostrongyles. For further information about Dr. Travassos, see Ferreira (1989).

Description

General: small nematodes, sexually dimorphic, body coiled along left ventral side in 2-4 spirals in males, more loosely and irregularly coiled in females. Nerve ring just before the excretory pore. Excretory pore 79-98% of esophageal length in males, 60-95% in females. Deirids not observed. Cephalic vesicle present (Figure 1A). In apical view, rounded buccal opening surrounded by very thin ring. Two amphids, six external-labial papillae and four submedian cephalic papillae observed (Figure 2B).

Synlophe (based on 2 males and 3 females): in both sexes there is a cephalic vesicle with ridges appearing posterior to cephalic vesicle, ridges ending immediately anterior to caudal bursa in male and to anus in female (Figures 2A, D). At level of esophagus, males feature 18-20 ridges (Figure 1B) and females, 16-20 ridges (Figure 1E); at middle body, males feature 20-24 ridges (Figure 1C) and females, 19 ridges (Figure 1F). Males feature 22-24 ridges at level of spicules (Figure 1D) and females, 11 ridges at level of vulva (Figure 1G). Ridges slightly unequal in size, with right ventral ridges smaller than left ridges. Right-ventral ridges oriented more perpendicularly to body surface at esophagus-intestinal junction in male, and at middle body in female. Ventrolateral ridges at level of vulva are larger than the other ridges and extend ventrally. Double axis of orientation of ridges; right axis inclined at about 62° and left axis at 75° to sagittal axis in males. In females, right axis inclined at about 51° and left axis at 72° to sagittal axis.

Male: measurements (range and average) based on 11 specimens: 2.8-4.6 (3.6) mm long, 121-179 (144) wide at mid-body. Cephalic vesicle 42-56 long (49), 31-44 wide (37). Nerve ring and excretory pore situated 113-233 (173) and 383-458 (421) from apex, respectively. Esophagus 392-647 long (560) (Figure 1A). Caudal bursa subsymmetrical, with left lobe slightly larger, dorsal lobe with cleft, ray pattern 2-2-1. Ray 2 thin, curved medially. Ray 3 longer than ray 2, straight, reaching bursal margin. Ray 4 robust and slightly longer than ray 5, both distally, ray 4 anteriorly curved, ray 5 slightly posteriorly curved with ornamentation along the structure, similar to a "corrugated". Ray 6 posteriorly curved, not reaching bursal margin (Figure 1H,). Ray 8 arising from the base of the dorsal ray, not reaching bursal margin. Dorsal ray slender, divided at about distal third into two branches, each bifurcating into ray 9 (external branches) and ray 10 (internal branches). Genital cone conical, 58-78 long (71) by 48-65 wide (51)

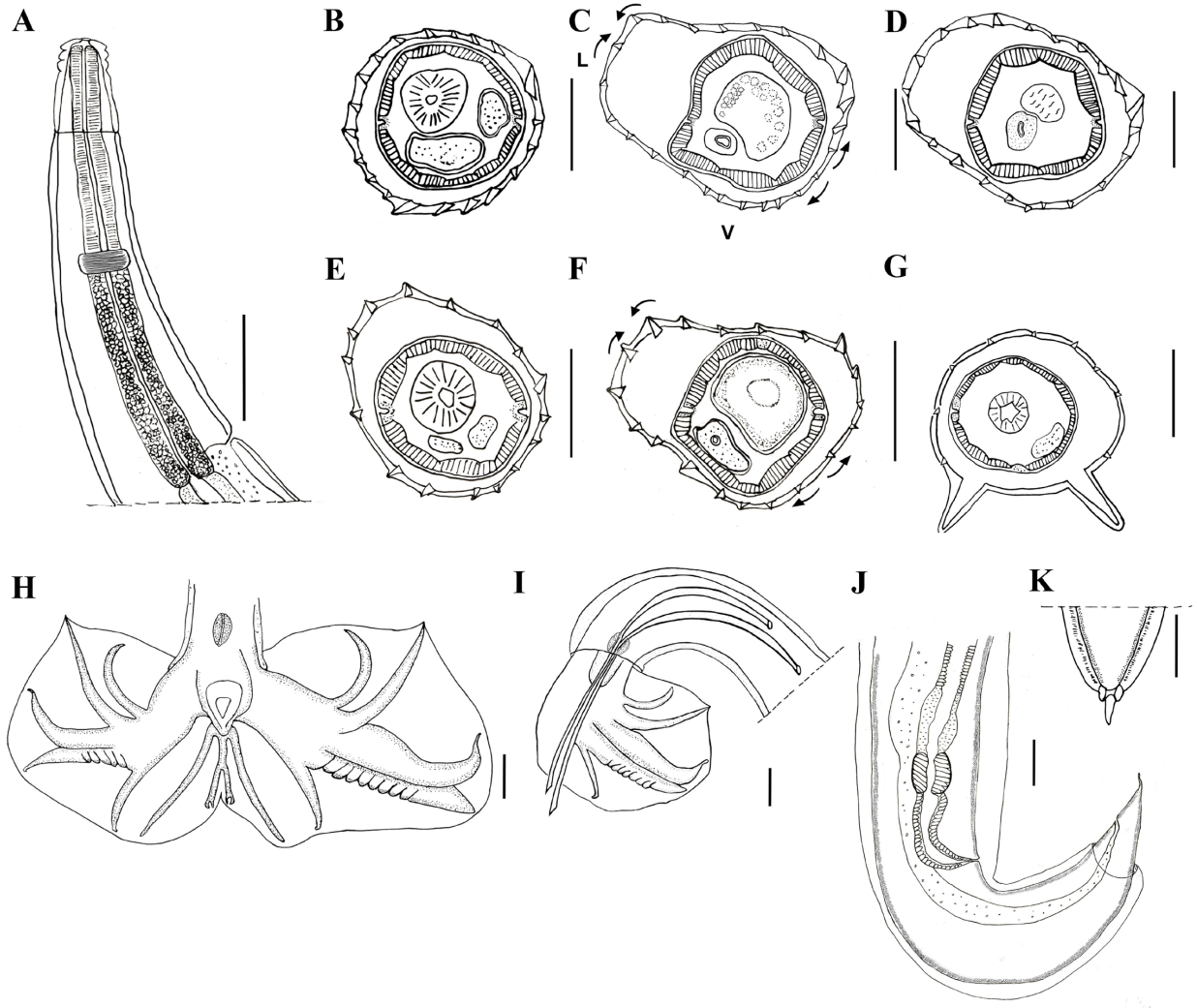

Figure 1. *Hassalstrongylus lauroi* n. sp. (A), ventral view of the anterior end of a male, showing cephalic vesicle, esophagus, nerve ring and excretory pore anterior extremity. Synlophe in sections at different body levels, with section orientation on dorsal side and left side; (B-G), male paratype: (B), at esophago-intestinal junction; (C) at mid-body; (D) at posterior-end third of body length, at level of spicules. E-G, female paratype: E, at esophago-intestinal junction, female; (F), at mid-body; (G) at level of vulva; (H) male, ventral view of the posterior end of a paratype, showing caudal bursa, genital cone and spicules omitted; (I) male, lateral view, showing left lobe and spicules; (J) posterior end of a paratype showing a cuticle expansion around the tail, vulva, anus, infundibulum and tail; (K) ventral view of a female, showing lateral appendix. Symbols: L, left, V, ventral. Scale bars= 50 µm

at base in ventral view. Gubernaculum 38-54 long (46) and 21-28 wide (24) at bas. Equal size spicules 511-674 long (622.03) (Figure 1I). Ratio SpL/BL: 18% (16–20%).

Female: measurements (range and average) based on 10 specimens: 2.9-4.3 mm long (3.6 mm), 121-188 wide (151) at mid-body. Cephalic vesicle 42-54 long (47), 28-39 wide (31). Nerve ring and excretory pore situated 194-267 (246) and 225-347 (279) from apex, respectively. Esophagus 364-556 long (445). Uterus monodelphic. Vulva situated 228-379 (298) from caudal end. Vagina vera 22-26 long (24), vestibule 103-122 long (110), sphincter 40-53 (46) long and 36-44 wide (38), infundibulum 86-106 long (94). Infundibulum folded; proximal end not clearly observed (Figure 1J). Eggs 58-66 long (62) and 26-32 wide (30). Tail ending short and pointed in a lateral appendix on each side (Figures 1K, 2E) and a cuticle expansion around the tail (prepuce) (Figure 2D). Ventrolateral ridges around vulva ventrally projected, forming a concavity (Figures 2C).

Taxonomic discussion

According to Durette-Desset (1971a), the genus *Hassalstrongylus* is characterized by a non-hypertrophied genital cone, a symmetrical or subsymmetrical caudal bursa and a synlophe with a number of ridges ranging from 19 to

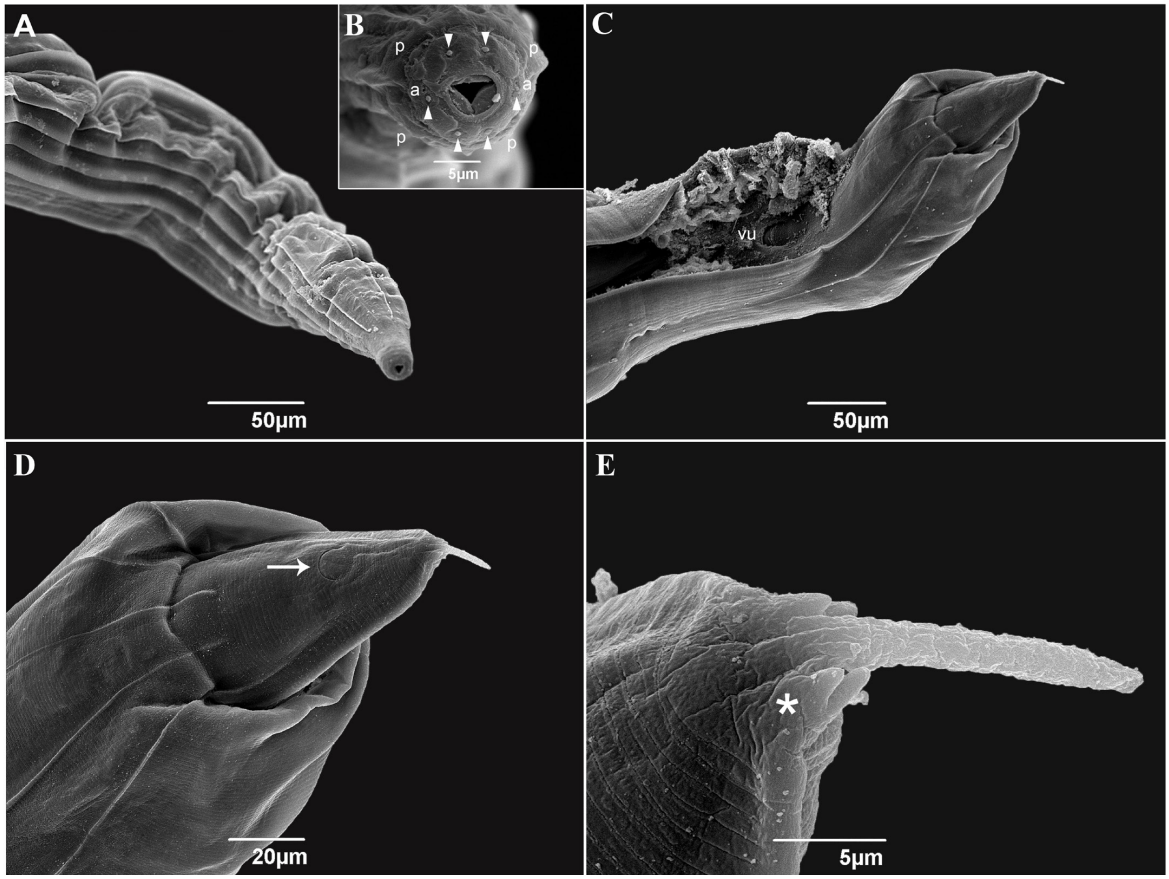

Figure 2. Scanning electron micrographs of *Hassalstrongylus lauroi* n. sp. (A) anterior dorsal view of a female, showing cephalic vesicle and ridges appearing posteriorly to cephalic vesicle; (B) apical view of a female, showing cephalic papillae (p), amphids (a) and external labial papillae (arrowhead ►); (C) posterior end of a female, showing vulva (vu) and modified ridges; (D) tail showing anus (arrow →); (E) tail tip with appendages highlighted (asterisk *).

24, and mainly parasitizes rodents in Cricetidae. The following taxonomic characteristics: (1) number of cuticular ridges, (2) rotation of the orientation axis and (3) elongation of the genital cone of the caudal bursa are essential for identifying *Hassalstrongylus* and separating it from the genera *Carolinensis, Stilestrongylus* and *Guerrerostrongylus*, which belong to the same evolutionary lineage (Durette-Desset & Digiani, 2010; Pérez-Ponce de León et al., 2000; Weirich et al., 2016). Because of the number of ridges (24 in the middle body) and the characteristics of the caudal bursa (subsymmetrical with non-hypertrophied genital cone), the specimens studied here can be included in the genus *Hassalstrongylus*.

The main taxonomic characteristics of this new species comprise a subsymmetrical caudal bursa of type 2-2-1, ray 8 arising from the base of the dorsal trunk, left lobe larger than the right lobe and rays 4 and 5 of robust nature, the latter presenting a corrugated shape in both lobes. Because the new species presents ray 8 longer than the dorsal ray, it can be distinguished from *H. dessetae* Pinto, 1978, *H. epsilon* (Travassos, 1937) Durette-Desset, 1971b, *H. puntanus* Digiani & Durette-Desset, 2003, *H. argentinus* Freitas, Quaresma & Almeida, 1937; *H. hoineffae* Durette-Desset, 1969 *H. beta* (Travassos 1918); *H. echalieri* Diaw, 1976. This character (ray 8 larger than dorsal ray) was observed in all specimens analyzed of *H. lauroi*. For *H. chabaudi* and *H. dollfusi* Diaz-Ungria, 1963, this character could not be clearly observed (Diaw, 1976). The spicules of *H. lauroi* are larger than those of *H. dessettae*, *H. puntanus*, *H. hoineffae*, *H. echalieri* and *H. chabaudi*. The length of the spicules of *H. lauroi* is similar to spicules of *H. dollfusi*, *H. beta* and *H. argentinus*. Concerning *H. dollfusi*, this species has similarities with *H. lauroi* in relation to the caudal bursa, differing only in the size of the lobes, with the left lobe larger than the right one in *H. lauroi* and the opposite in *H. dollfusi*. In addition, *Hassalstrongylus lauroi* n. sp. differs from *H. chabaudi* Diaw, 1976, *H. bocqueti* Denke, 1977, *H. lichtenfelsi* Durette-Desset, 1974, *H. forresteri* Durette-Desset, 1974, *H. musculi* (Dikmans, 1935) Durette-Desset, 1974 and *H. schadi* (Durette-Desset, 1971) in that its rays 4 and 5 are robust. It also differs from *H. mazzai* Freitas, Quaresma & Almeida, 1937, *H. aduncus* (Chandler, 1932) and *H. luquei* Costa, Maldonado, Bóia,

Lucio & Simões, 2014, in that ray 8 is inserted at the base of the dorsal trunk. The specimens differ from *H. schadi* through the presence of a simple genital cone. Thus, the presence of specific caudal bursa characteristics makes it possible to consider the specimens found in *H. seuanezi* to be a new species.

Furthermore, two morphological characteristics described above seem to be specific to our specimens: the ornamentation on corrugated shape ray 5 and the robustness of ray 4 on the male caudal bursa. In addition, the ventrolateral ridges around the vulva, ventrally projected forming a concavity, observed in *H. lauroi,* was also observed only in *H*. *dollfusi* (Diaw, 1976; Travassos, 1939). In *H. aduncus,* ridges were also observed, and these ended between the vulva and the anus. All of these specific characteristics allow us to consider that our specimens are a new species, named here *Hassalstrongylus lauroi* n. sp.

Discussion

Among the 17 species of the genus *Hassalstrongylus*, 14 parasitize sigmodontine rodents and three, murine rodents. This genus has wide geographical distribution and is found in both the Neotropical and the Nearctic regions. In North America, four species have been described in the USA: *H. musculi* (syn. *Longistriata musculi*) parasitizing *Mus musculus* (Linnaeus, 1758); *H. aduncus* parasitizing *Sigmodon hispidus* Say and Ord, 1825; and *H. lichtenfelsi* and *H. forresteri* infecting *Oryzomys palustris* (Harlan, 1837) (Durette-Desset, 1972, 1974). One species has been described in Mexico: *H. bocqueti* infecting *Oryzomys alfaroi* (syn. *Handleyomys alfaroi*, Allen, 1891) (Durette-Desset, 1974, 1985). In South America, 12 species have been registered: *Hassalstrongylus schadi* (syn. *Longistriata schadi*) infecting *Nectomys alfari* (Goldman, 1913) (syn. *Sigmodontomys alfari* J.A. Allen, 1897) in Colombia; *H. dollfusi* parasitizing *Mus musculus* in Venezuela; *H. echalieri* infecting *Oryzomys* sp. in French Guiana; *H. puntanus* infecting *Graomys griseoflavus* (Waterhouse, 1837) in Argentina; and *H. argentinus* and *H. mazzai* (syn. *Heligmonoides mazzai*) Freitas, Lent & Almeida, 1937 parasitizing *Holochilus chacarius* (Thomas, 1906) in Argentina (Diaz-Ungria, 1963; Digiani et al., 2015; Durette-Desset, 1971b). The other six species have been described in Brazil.

In Brazil, *Hassalstrongylus beta* (syn. *Heligmosomum beta*) was described parasitizing the small intestine of murine rodents in Angra dos Reis, state of Rio de Janeiro (Travassos, 1921). *Hassalstrongylus epsilon* (syn. *Longistriata epsilon*) was described by Travassos (1939) in *Nectomys rattus* (reported as *N. squamipes*) in Formosa, state of Goiás. Later on, Durette-Desset (1969) added morphological characteristics to this species. This helminth was reported as parasitizing *N. squamipes* in Sumidouro, state of Rio de Janeiro (Côrrea Gomes & Vicente, 1984; Maldonado et al., 2006). Kuhnen et al. (2012) also reported the occurrence of *Hassalstrongylus* sp. on the water-rat *N. squamipes* in Serra do Tabuleiro State Park, state of Santa Catarina. Kersul et al. (2019) also registered *H. epsilon* in this host in Ilhéus, Bahia. The specimens of *Hassalstrongylus* sp. recovered from *Hyleamys seuanezi* by Kersul et al. (2019) were compared with the specimens of the present study, which confirmed that they are *H. lauroi*.

In a study on the helminths of the rice rat, *Euryoryzomys russatus* (Wagner, 1848), Costa et al. (2014) described *Hassalstrongylus luquei* in Angra dos Reis, state of Rio de Janeiro. *Hassalstrongylus dessetae* was described by Pinto (1978) infecting *Neacomys* sp. in Serra do Navio, state of Amapá. Later on, Durette-Desset & Digiani (2010) added morphological and morphometric data to this species. *Hassalstrongylus hoineffae* (syn. *Longistriata hoineffae*) and *H. chabaudi* collected in Exu, state of Pernambuco, were described parasitizing *Wiedomys pyrrhorhinus* (Wied-Neuwied, 1821) (Diaw, 1976; Durette-Desset, 1969). *Hassalstrongylus hoineffae* was also described parasitizing *Calomys expulsus* (Lund, 1841) (reported as *Calomys callosus* (Rengger, 1830)) and *Oryzomys nigripes* Trouessart, 1897 (syn. *Oligoryzomys nigripes* (Olfers, 1818) (Durette-Desset, 1969).

The new species described here, *Hassalstrongylus lauroi* n. sp., was found in two rodent species, *H. seuanezi* and *Oxymycterus dasytrichus,* in preserved areas of the Atlantic Forest, with higher prevalence in the former. Although both rodent species occur in both forested and open areas (Bonvicino et al., 2008), *O. dasytrichus* prefers open habitats, with lower overstory and understory vegetation density (Delciellos et al., 2016). This is a new record of the genus *Hassalstrongylus* for the host genus *Oxymycterus*.

In the light of the morphological description of the specimens analyzed in this study, i.e., the presence of a specific caudal bursa, the corrugated ornamental ray 5 and the robustness of ray 4 on the male caudal bursa, we can consider these specimens to be a new species, which is named here as *Hassalstrongylus lauroi* n. sp. The morphological data presented in this study, together with the geographical locality and host of this new species, will contribute to better understanding of the diversity and distribution of species of the genus *Hassalstrongylus*.

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