

Research Paper

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A new heligmonellid (Nematoda) species of the genus *Stilestrongylus* in *Euryoryzomys russatus* (Rodentia: Sigmodontinae) in the Atlantic Forest, southern Brazil

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Abstract

A new species of Nippostrongylineae (Nematoda: Heligmonellidae), *Stilestrongylus rolandoi* n. sp., is described from specimens collected from the small intestine of the rodent *Euryoryzomys russatus* in the Atlantic Forest (Santo Amaro da Imperatriz, Santa Catarina state, southern Brazil). The genus *Stilestrongylus* includes 23 species, which parasitize rodents occurring in the Neotropical region. *Stilestrongylus aureus* (Durette-Desset & Sutton, 1985) from Argentina, *S. azarai* (Durette-Desset & Sutton, 1985) from Argentina, *S. flavescens* (Sutton & Durette-Desset, 1991) from Uruguay, *S. franciscanus* (Digiani & Durette-Desset, 2002) from Argentina, *S. graciellae* (Digiani & Durette-Desset, 2006) from Argentina, and *S. oryzomysi* (Sutton & Durette-Desset, 1991) from Argentina are closely related to *Stilestrongylus rolandoi* n. sp., all having caudal bursa patterns of types 1–4 in one of the lobes. *Stilestrongylus rolandoi* n. sp. is distinguished from the aforementioned species by its ray 6 being short in relation to rays 4 and 5, which are long and robust, and by having caudal bursa patterns of types 1–4 in both lobes. The new species has 27 ridges in the mid-body in males, and 24 in females, and has one of the highest ratios of spicule length to body length (21–33%) in this genus.

Introduction

The genus *Stilestrongylus* Freitas, Lent & Almeida, 1937, belongs to the family Heligmonellidae and the subfamily Nippostrongylineae (Durette-Desset, 1971). Currently, 23 species are recognized in this genus, 21 of which are parasites of cricetid rodents, one of murids and one of echimyds (Digiani & Durette-Desset, 2007; Digiani *et al.*, 2007; Souza *et al.*, 2009). The main taxonomic characteristics used to identify the genus *Stilestrongylus* are its dissymmetrical caudal bursa, its hypertrophied genital cone, and the number of ridges and the degree of inclination and orientation of the synlophes (Durette-Desset & Digiani, 2005, 2012).

Here we describe a new species of *Stilestrongylus* (Heligmosomoidea: Heligmonellidae) found parasitizing russet rice rats *Euryoryzomys russatus* (Wagner, 1848) (syn. *Oryzomys russatus*) (Rodentia, Sigmodontinae) in the Atlantic Forest in the Serra do Tabuleiro State Park, municipality of Santo Amaro da Imperatriz, Santa Catarina state, southern Brazil. The rice rats have terrestrial habits, feed on seeds, fruits and insects (Emmons & Feer, 1997) and inhabit south-eastern and southern Brazil, north-eastern Argentina and eastern Paraguay (Musser & Carleton, 2005). In Brazil, *E. russatus* occurs in the Atlantic Forest from Bahia to Rio Grande do Sul states, including eastern Minas Gerais state (Patton *et al.*, 2015).

Materials and methods

Collection of nematodes

Twenty-eight specimens of *E. russatus* were collected at the Serra do Tabuleiro State Park (27° 52' 27" S, 48° 49' 26" W), a reserve of the Atlantic Forest of c. 84,130 ha. The rodents were captured in areas of Ombrophilous Dense Forest in October 2014 and May 2015 using Sherman and Tomahawk traps (H.B. Sherman traps, USA; Tomahawk Live Trap, USA) and pitfall traps made of buckets. Biosafety techniques and personal safety equipment were used during all procedures involving animal handling and biological sampling.

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Morphological analysis

The rodents were euthanized and dissected for collection of helminths. The nematodes were collected from the small intestine, washed briefly in NaCl solution and fixed in hot AFA (2% acetic acid, 3% formaldehyde and 95% ethanol). Fifteen male and 10 female nematodes were cleared in lactophenol and examined for their morphological characteristics. Drawings were made using a Zeiss Standard 20 light microscope (Carl Zeiss AG, Germany) equipped with a lucid camera. The synlophe was studied in one male and one female, and the total number of dorsal and ventral ridges was counted according to Durette-Desset (1985) and Durette-Desset & Digiani (2015). The terminology used here relating to the caudal bursa follows Durette-Desset & Digiani (2012) and Durette-Desset *et al.* (2017). Measurements are given in micrometres (unless otherwise stated) for holotype or allotype specimens, followed by the range for paratypes, with the mean in parentheses. Holotype, allotype and paratype specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) at the Oswaldo Cruz Foundation, in Rio de Janeiro state, Brazil.

Results

Systematics

Heligmosomoidea Travassos, 1914

Heligmonellidae Leiper, 1912

Nippostrongylineae Durette-Desset, 1971

Stilestrongylus rolandoi n. sp.

Description

General. Small nematodes tightly coiled. Excretory pore located at posterior end of the oesophagus (fig. 1a). Cephalic vesicle present. Rounded mouth opening in apical view, surrounded by two amphids, six internal labial papillae, four external labial papillae and four submedian cephalic papillae (fig. 1b). Cuticle bearing longitudinal, uninterrupted ridges appearing posterior to cephalic vesicle, ending just anterior to caudal bursa in males and reaching posterior extremity in females. Synlophe with 20 ridges (nine dorsal and 11 ventral) in males (fig. 1c) and 22 (11 dorsal and 11 ventral) in females (fig. 1d) at level of the oesophago-intestinal junction; 27 ridges at mid-body (12/15) in males (fig. 1e) and 24 (10/14) in females (fig. 1f); 22 (11/11) anterior to caudal bursa in males (fig. 1g) and 23 (11/12) anterior to vulva in females (fig. 1h). Ridges at mid-body slightly unequal in size in both males and females, with smaller ridges oriented from ventral right to ventral left and from dorsal right quadrant to dorsal left. Ridges at mid-body positioned in double-axis orientation. In males, right axis inclined at 75° to sagittal axis and left axis at 75°. In females, right axis inclined at 65° to sagittal axis and left axis at 70°.

Male (holotype and 14 paratypes). Length 4.08 [2.82–4.14 (3.42)] mm, n = 15, width at mid-body 90.7 [94.5–149.1 (117.5)], n = 15; cephalic vesicle 74 [68.8–98.5 (85.7)] long, n = 8, and 33.6 [28.4–37.6 (32.1)] wide, n = 8. Nerve ring (n = 5) 156.1 [139.7–162.5 (148.8)] and excretory pore (n = 4) 225.3 [313.6–338.7 (326.9)] from anterior end. Oesophagus 321 [305.1–333.5 (321.5)] long, n = 5. Dissymmetrical caudal bursa, with right lobe more strongly developed than left lobe (CB DS RL+), both of types 1–4. Ray 2 in right lobe shorter than ray 2 in left lobe. Rays 3, 4 and 5 emerging from a common trunk: rays 3 separating

from rays 4 at the middle of the lateral trunk, rays 4 and 5 robust, bifurcating at distal third. Ray 6 small, arising at the same level of bifurcation as rays 3. Rays 8 arising dissymmetrically on dorsal trunk, right ray more distally than left ray. Dorsal ray divided at distal third into two branches, each divided at the extremity into two subequal branches, rays 9 (external) slightly longer than rays 10 (internal). Spicules alate, equal in length, 943.4 [807.7–1080.2 (963.3)] long, n = 13; spicule length to body length ratio (SpL/BL) of 23 (21–33%). Gubernaculum present, 47.7 [42.3–55.8 (49.3)] long and 25.3 [18.1–33.8 (26.6)] wide, n = 9. Genital cone hypertrophied, 56.2 [54–72.2 (62.3)] long and 53.5 [46.5–58 (52.2)] wide, n = 8 (fig. 1i, j).

Female (allotype and nine paratypes). Length 5.38 [4.13–6.17 (5.02)] mm, 128.6 [105.5–126 (116.5)] mm wide at mid-body, n = 10. Cephalic vesicle 85.6 [61.2–111.7 (85.1)] long and 34.7 [30.0–42.1 (34.6)] wide, n = 10. Distance from anterior end to nerve ring and to excretory pore 192.6 [172.8–209.9 (184.9)], n = 6 and 458 [375.3–473.6 (430.7)], n = 3, respectively. Oesophagus 453 [370.4–458.3 (431.2)] long, n = 4 (fig. 1a). Monodelphic, posterior extremity invaginated. Vulva situated at 74.2 [70.4–91.6 (81.7)], n = 6, from caudal extremity, vagina vera 34.1 [32.4–41.1 (37.5)] long, n = 6, vestibule 259.4 [219.6–258.1 (238.3)] long, n = 7, sphincter 50.6 [47.0–57.2 (51.6)] long and 50.7 [50.3–53.9 (52.0)] wide, n = 8, and infundibulum 118.7 [108.3–116.7 (112.1)] long, n = 6. Uterus 1.69 [1.56–1.78 (1.67)] mm long, n = 3. Number of eggs 16 [13–18 (16)], n = 6. Tail 29.6 [30.7–57.7 (45.3)] long, n = 7. Eggs 73.3 [71.3–89.1 (77.9)] long and 33.2 [30.5–43.4 (35.9)] wide, n = 6 (fig. 1k).

Taxonomic summary

Type host. *Euryoryzomys russatus* (Wagner, 1848) (Rodentia, Cricetidae, Sigmodontinae).

Type locality. Serra do Tabuleiro State Park (27°52'27"S, 48°49'26"W), municipality of Santo Amaro da Imperatriz, Santa Catarina state, Brazil.

Site of infection. Small intestine.

Prevalence. 25% (7 rodents infected / 28 rodents collected).

Mean intensity. 25.5 ± 16.9.

Mean abundance. 6.39 ± 13.8.

Type material. Holotype accession number CHIOC no. 38566a (male); allotype accession number CHIOC no. 38566b (female); paratypes' accession number CHIOC no. 38566c (3 males and 3 females).

Etymology. The new species is named in honour of Antônio Rolando Oliveira de Castro.

Discussion

The presence of 24–27 ridges in the synlophe at the mid-body, unequal in size, the dissymmetrical caudal bursa and the prominent genital cone (Durette-Desset, 1971; Durette-Desset & Digiani, 2012) permit the inclusion of the studied specimens in the genus *Stilestrongylus* (Heligmonellidae, Nippostrongylineae).

Among the 23 Neotropical species, *S. aureus* (Durette-Desset & Sutton, 1985) from Argentina, *S. azarai* (Durette-Desset & Sutton, 1985) from Argentina, *S. flavescens* (Sutton & Durette-Desset, 1991) from Uruguay, *S. franciscanus* (Digiani & Durette-Desset, 2002) from Argentina, *S. graciellae* (Digiani &

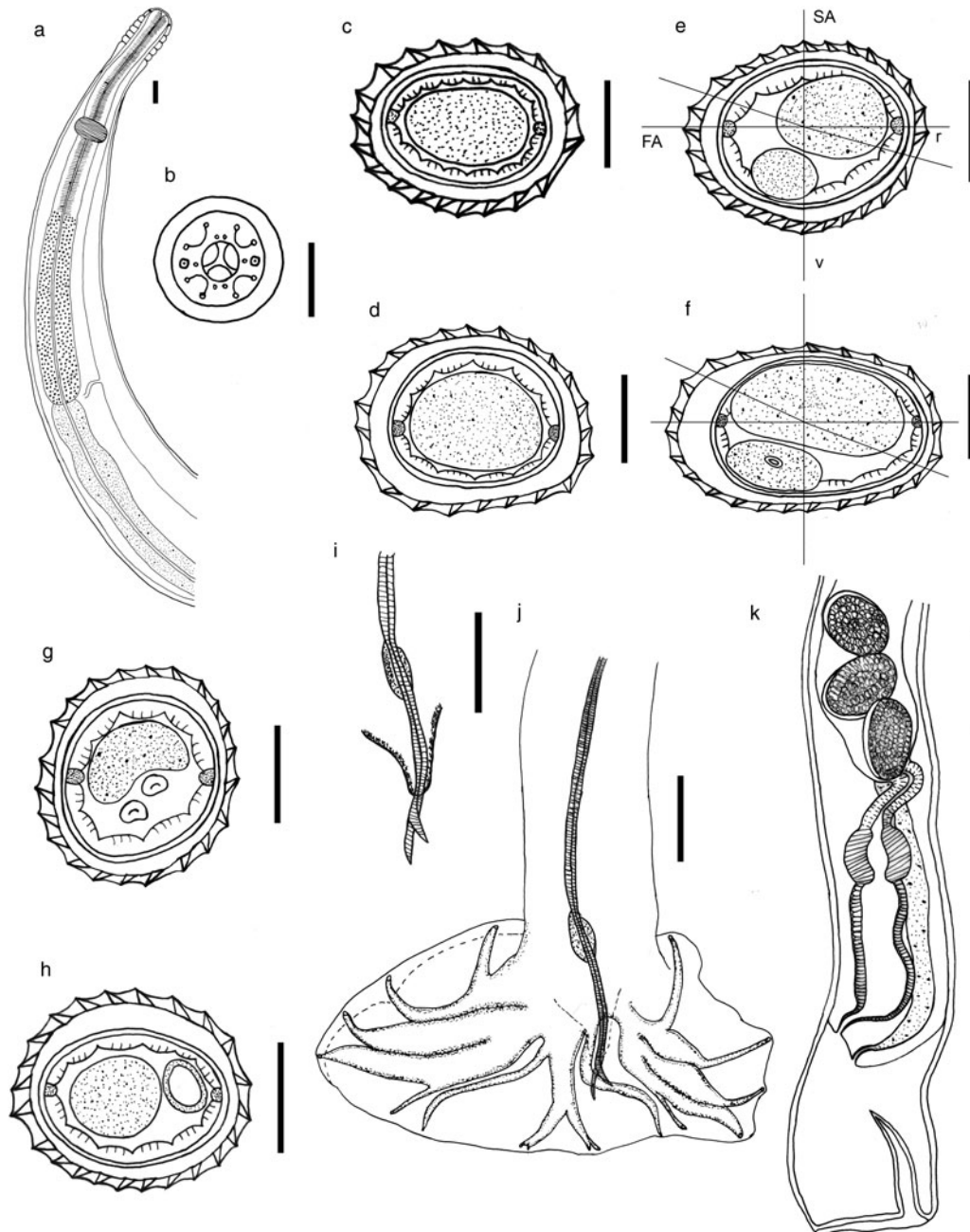


Fig. 1. *Stilestrongylus rolandoi* n. sp. (a) Anterior extremity, right lateral view, female. (b) Female, head, apical view. (c) Section through anterior body, posterior to oesophago-intestinal junction, male. (d) Section through anterior body, posterior to oesophago-intestinal junction, female. (e) Transverse section of body, at mid-body, male. (f) Transverse section of body, at mid-body, female. (g) Transverse section of body, female, just anterior to caudal bursa. (h) Transverse section of body, female, just anterior to vulva. (i) Male, genital cone. (j) Male, caudal bursa, ventral view. (k) Female, posterior extremity, left lateral view. Scale bar 50 μ m. Abbreviations: r, right; v, ventral; SA, sagittal axis; FA, frontal axis.

Durette-Desset, 2006) from Argentina and *S. oryzomysi* (Sutton & Durette-Desset, 1991) from Argentina are closely related to *S. rolandoi* n. sp. The new species has caudal bursa patterns of types 1–4 and hypertrophy of the right lobe, and rays 4 and 5 are of equivalent length and divergent at the extremity. Although *S. aureus* presents caudal bursa pattern 1–4 in the right lobe, the left caudal bursa pattern is of type 2–3, tending to 2-2-1; *S. azarai* differs from *S. rolandoi* n. sp. by having right lobe with rays 4 and 6 divergent at their extremity, and *S. oryzomysi* by having right ray 4 smaller than right ray 5, and rays 4, 5 and 6 diverging at the same level; *S. franciscanus* is distinguished

by rays 3 not reaching the edge of the caudal bursa and by the bifurcation of the dorsal ray at the middle of the trunk; *S. flavescens* is differentiated by its right rays 2 and 3 diverging at 'V'; and *S. graciellae* is distinguished by its long rays 6 and very small spicules (100 μ m). *Stilestrongylus rolandoi* n. sp. has the longest spicules in the genus (SpL/BL 21–33%), exceeding those of *S. lanfrediae* (SpL/BL 20%).

Panisse *et al.* (2017) reported the presence of a new species of *Stilestrongylus* infecting *E. russatus* and *Sooretamys angouya* from the Atlantic Forest in north-eastern Argentina. This species needs to be described in detail, and illustrations should be provided to

clarify whether it constitutes the same species as that described here, as it was found in the same host species and in the same biogeographical region.

The main characteristics of the new species are the pattern of the caudal bursa, ray 2 of the left lobe being longer than ray 2 of the right lobe, the small rays 6, the asymmetry of rays 8, the high number of ridges (27 in males and 24 in females), and the longest spicule length to body length ratio in the genus. All these characteristics provide evidence that the nematodes from *E. russatus* are a new species of *Stilestrongylus*.

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Conflict of interest. None.

Ethical standards. Animals were captured under the authorization of the Brazilian Government's Chico Mendes Institute for Biodiversity and Conservation (ICMBIO, license number 26934-1) and the Environmental Foundation of Santa Catarina State (FATMA, license number 043/2014). All procedures followed the guidelines for capture, handling and care of animals of the Ethical Committee on Animal Use of the Oswaldo Cruz Foundation (CEUA license number LW – 39/14).

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