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Taxonomy and systematics

## ***Oslerus (Oslerus) brachyurus* sp. nov. (Metastrongyloidea: Filaroididae) from *Chrysocyon brachyurus* (Carnivora: Canidae) in Brazil**

### ***Oslerus (Oslerus) brachyurus* sp. nov. (Metastrongyloidea: Filaroididae) de *Chrysocyon brachyurus* (Carnivora: Canidae) en Brasil**

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#### **Abstract**

*Oslerus (Oslerus) brachyurus* sp. nov. was collected from the mucosa of the inner surface of the trachea and bronchi of *Chrysocyon brachyurus* from the Atlantic forest area, in the state of Minas Gerais, Brazil. The new species is described based on morphological and metric analyses. *Oslerus (Oslerus) brachyurus* sp. nov. differs morphologically from the only species of the subgenus *O. (Oslerus)*, *O. (O.) osleri*, for not possessing a cuticular collar or a protusible rostrum in anterior region, for having only one well developed excretory gland, not present circumoral lips and papillae, by having 3 pairs of vestigial rays of the bursa as small protuberances, by females being larger than the females of *O. (O.) osleri*, by the oesophagus of males and females of the *O. (O.) brachyurus* sp. nov. have larger ratio oesophagus length/total body length ratio than *O. (O.) osleri*, and the males of *O. (O.) brachyurus* sp. nov. have spicules larger and gubernaculum smaller than *O. (O.) osleri*. The new species described herein is the second species of the subgenus *O. (Oslerus)* and the first recorded and carefully analysed morphologically parasitizing *C. brachyurus* from Brazil.

**Keywords:** Parasitic nematodes; Brazil; Wild carnivore mammals

## Resumen

*Oslerus (Oslerus) brachyurus* sp. nov. se recuperó de la mucosa de la superficie interna de la tráquea y los bronquios de *Chrysocyon brachyurus* del bosque Atlántico, en el estado de Minas Gerais, Brasil. La especie nueva se describe con base en análisis morfológicos y métricos. *Oslerus (Oslerus) brachyurus* sp. nov. difiere morfológicamente de las únicas especie del subgénero *O. (Oslerus)*, *O. (O.) osleri* por no tener un collar cuticular o un rostrum protusible en la región anterior, por tener solo una glándula excretora bien desarrollada, por no tener labios y papilas circumorales, por tener 3 pares de rayos vestigiales de la bolsa como pequeñas protuberancias, las hembras son más grandes que las *O. (O.) osleri*, por el esófago de machos y hembras de *O. (O.) brachyurus* sp. nov. que tienen una mayor proporción longitud del esófago/longitud corporal total de *O. (O.) osleri*, y los machos de *O. (O.) brachyurus* sp. nov. tienen espículas más grandes y el gubernaculum más pequeño que *O. (O.) osleri*. La nueva especie descrita en este documento es la segunda especie del subgénero *O. (Oslerus)* y la primera registrada y morfológicamente analizada cuidadosamente parasitando a *C. brachyurus* de Brasil.

**Palabras clave:** Nemátodos parásitos; Brasil; Mamíferos carnívoros silvestres

## Introduction

The genus *Oslerus* Hall, 1921 (Metastrongyloidea, Filaroididae) was proposed to accommodate *Filaria osleri* described by Cobbold (1879), from specimens collected by Osler (1877) in domestic dogs in Europe. The proposal of division of this genus into 2 subgenera was made by Anderson (1978), who established the 2 monotypic subgenera recognized to date: *Oslerus (Oslerus)* (Hall, 1921) and *O. (Anafilaroides)* (Gerichter, 1949). Nematodes of this genus parasitize the lungs of domestic and wild dogs (*Canis lupus familiaris*) and cats (*Felis catus*) in most of the world (Bowman et al., 2002). However, in South America reports of this genus are scarce, it has been reported in domestic dogs from Chile (Alcaino & Gorman, 1999; Muñoz et al., 2007), and in *Chrysocyon brachyurus* (Illiger, 1815) (Carnivora, Canidae) (Dias et al., 2012; Vieira et al., 2017) and in *Puma yagouaroundi* from Brazil (Corrêa et al., 2019), however, none of these studies made a taxonomic analysis of these nematodes. The current study is aimed to describe a new species of *Oslerus* parasitizing *C. brachyurus* from the state of Minas Gerais, Brazil.

## Materials and methods

Five specimens of *Chrysocyon brachyurus* (Maned Wolf) were necropsied, between June 2002 and January 2010. The hosts were road kills and donated by the Regional Office of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), in the municipality of Juiz de Fora, in the state of Minas Gerais (21°41'20" S, 43°20'40" W). The hosts were identified according to Dietz (1985).

The nematodes collected were fixed in AFA (70% ethanol, 93 parts; 37% formalin, 5 parts; glacial acetic acid, 2 parts) for 48 hr, and preserved in 70% ethanol with 5% glycerin. For light microscopy studies, the nematodes were cleared in Amann's lactophenol (1:1:2:1 phenol: lactic acid: glycerin: water), mounted in temporary slides, in which they were kept during measuring and drawing. Drawings were made with the aid of a drawing tube attached to the Olympus BX41 light microscope (Olympus America Inc., Melville, New York). Measurements are given as ranges in micrometers (µm), with the mean and standard deviation in parentheses. Photomicrographs were made using a compound Olympus BX51 light microscope equipped with Nomarski Differential Interference Contrast (DIC) optics.

For scanning electron microscopy (SEM) studies, some specimens were dehydrated through a graded ethanol series, dried in 1,1,1,3,3,3-Hexamethyldisilazane 97% (HMDS) (Sigma-Aldrich), coated with gold and observed in a JEOL JSM 6390LV microscope (operating at 15 kV), in the Plataforma de Microscopia Eletrônica Rudolf Barth, of the IOC, FIOCRUZ, Rio de Janeiro, Brazil.

Identification and classification of nematodes to the generic level follow Anderson (1978) and Gibbons (2010). Holotype, allotype, paratypes and voucher specimens are deposited in the Instituto Oswaldo Cruz Helminthological Collection (CHIOC), Rio de Janeiro, Brazil.

## Description

*Oslerus (Oslerus) brachyurus* sp. nov. (Figs. 1a-d, 2a-d, 3) <http://zoobank.org/urn:lsid:zoobank.org:act:235C8772-DDD9-4713-BF2C-82BBA5A004C4>

Parasites found inserted under the mucosa of the trachea and bronchi, upon collection. Delicate specimens, with filiform body with pronounced sexual dimorphism. Cuticle with smooth surface, not ornamented and with evident cuticular sheath. Anterior extremity without cuticular collar (Figs. 1a, 2a). Circumoral lips and papillae not seen. Buccal opening directly connected to the oesophagus, with no trace of buccal capsule. Oesophagus claviform (Fig. 1a). Single excretory gland elongated and robust (Fig. 1a).

*Male (based on 10 adult specimens).* Total body length 6.5-9.4 ( $7.6 \pm 1.2$ ) mm. Body width at level of base of the oesophagus 80-100 ( $92 \pm 8.7$ ); maximum body width 120-170 ( $146 \pm 22.6$ ). Oesophagus 160-230 ( $200 \pm 32.4$ ) long. Oesophagus length/body length ratio 40:1. Nerve ring 35-55 ( $45 \pm 9.5$ ), and excretory pore 40-62 ( $51 \pm 10.9$ ) from anterior end. Excretory gland 530-640 ( $564 \pm 48.4$ ) long. Copulatory bursa not evident, with 3 pairs of ventrolateral papillae homologous to bursal rays, not clearly defined (Figs. 1b, c, 2b, c). Two spicules short, robust and with blunted distal end (Figs. 1b, c, 2b, c). Spicules subequal, 88-105 ( $96 \pm 7.9$ ) long. Spicules length/body length ratio 81:1. Gubernaculum well sclerotized and robust, with distal and proximal ends narrow and tapered, 22-35 ( $27 \pm 4.4$ ) long (Figs. 1b, 2b).

*Female (based on 10 adult specimens).* Total body length 15.2-18 ( $16.8 \pm 1.3$ ) mm. Body width at level of base of the oesophagus 140-160 ( $151 \pm 9.7$ ); maximum body width 370-410 ( $392 \pm 12.4$ ). Oesophagus 260-360 ( $309 \pm 35.6$ ). Oesophagus length/body length ratio 54:1. Nerve ring 48-65 ( $57 \pm 5.4$ ), and excretory pore 53-72 ( $63 \pm 6.1$ ) from anterior extremity. Excretory gland 606-739 ( $675 \pm 43.7$ ) long. Vagina and vulva with no distinctive sphincters (Figs. 1d, 2d). Posterior end bluntly rounded with vulva and anus terminal, located at the tip (Figs. 1d, 2d). Ovoviviparous. First-stage larvae into the vagina with 192-258 ( $227 \pm 21.5$ ) long ( $n = 30$ ) and 12-17 ( $16 \pm 1.7$ ) wide ( $n = 30$ ) (Figs. 1d, 2d).

#### Taxonomic summary

*Type host:* *Chrysocyon brachyurus* (Illiger, 1815) (Carnivora, Canidae).

*Site of infection:* inserted in the mucosa of the inner surface of the trachea and bronchi.

*Type locality:* Juiz de Fora, Minas Gerais, Brazil, 21°41'20" S, 43°20'40" W.

*Prevalence:* 40% (5 hosts examined; 2 hosts infected).

*Intensity of infection:* mean 205.5 (range 137-274)

*Type specimens:* holotype male CHIOC No. 35929a, allotype female CHIOC No. 35929b and 6 paratypes (3 males and 3 females) CHIOC No. 35929c (stored in 70% ethanol with 5% glycerine).

*Etymology:* the specific Latin name *brachyurus* is a reference to the specific Latin name of the host.

#### Remarks

The Family Filaroididae Schulz, 1951 (Strongylida, Metastrongyloidea) has 3 genera that are distinguished by the morphology of the vestigial rays in the copulatory bursa in males, and the position of vulva and anus in females (Anderson, 1978). *Filariopsis* van Thiel, 1926 is characterized by having the vestigial lateral and ventral rays clearly defined, and the anus and vulva in females are not in terminal position (Anderson, 1978). The genera *Oslerus* Hall, 1921 and *Filaroides* van Beneden, 1858 have males with the vestigial lateral and ventral rays not clearly defined. However, *Oslerus* differs from *Filaroides* because vulva and anus are terminal in the former, while in *Filaroides*, these structures are never terminal or subterminal (Anderson, 1978). The study of Eamsobhana et al. (2015), with molecular phylogeny of Nematoda, Metastrongyloidea inferred from 18S rDNA sequences, demonstrates that these last 2 genera are also genetically distinct.

A striking characteristic justifies the division of *Oslerus* into 2 subgenera: the presence or absence of sphincters in the vagina of females (Anderson, 1978). The females of *O. (Anafilaroides)*, a lung parasite of domestic cats in North America, Europe, and eastern Asia (Bowman et al. 2002), have a sphincter in the region of the vagina, while this structure does not occur in *O. (Oslerus)* (Anderson, 1978).

*Oslerus (Oslerus)* has only 1 species, *O. (Oslerus) osleri* (Cobbold, 1879), which has been reported from wild and domestic canids in Europe, North America, Africa, Asia and Oceania (Dunsmore & Spratt, 1979; Hare, 1930; Kotani et al., 1995; Olsen & Bracken, 1959; Sillero-Zubiri et al., 2004; Urquhart et al., 1954). The species described in the current study is included in *Oslerus* based on the ovoviviparous females featuring a terminal vulva and anus. The justification for the placement in the subgenus is based on the apparent absence of vaginal sphincters (Anderson, 1978).

*Oslerus (Oslerus) brachyurus* sp. nov. differs morphologically from *O. (O.) osleri* for not possessing a cuticular collar or a protusible rostrum in anterior region, for having only one well developed excretory gland, and absence of circumoral lips and papillae. The visualization in ventral and lateral views of the posterior region of the male of *O. (O.) brachyurus* sp. nov. with DIC microscopy demonstrates the presence of 3 pairs of vestigial rays of the bursa as small protuberances (Figs. 2b, c), in contrast with *O. (O.) osleri* studied by Seneviratna (1959), which has a copulatory bursa with 2 vestigial rays. In the females of *O.*

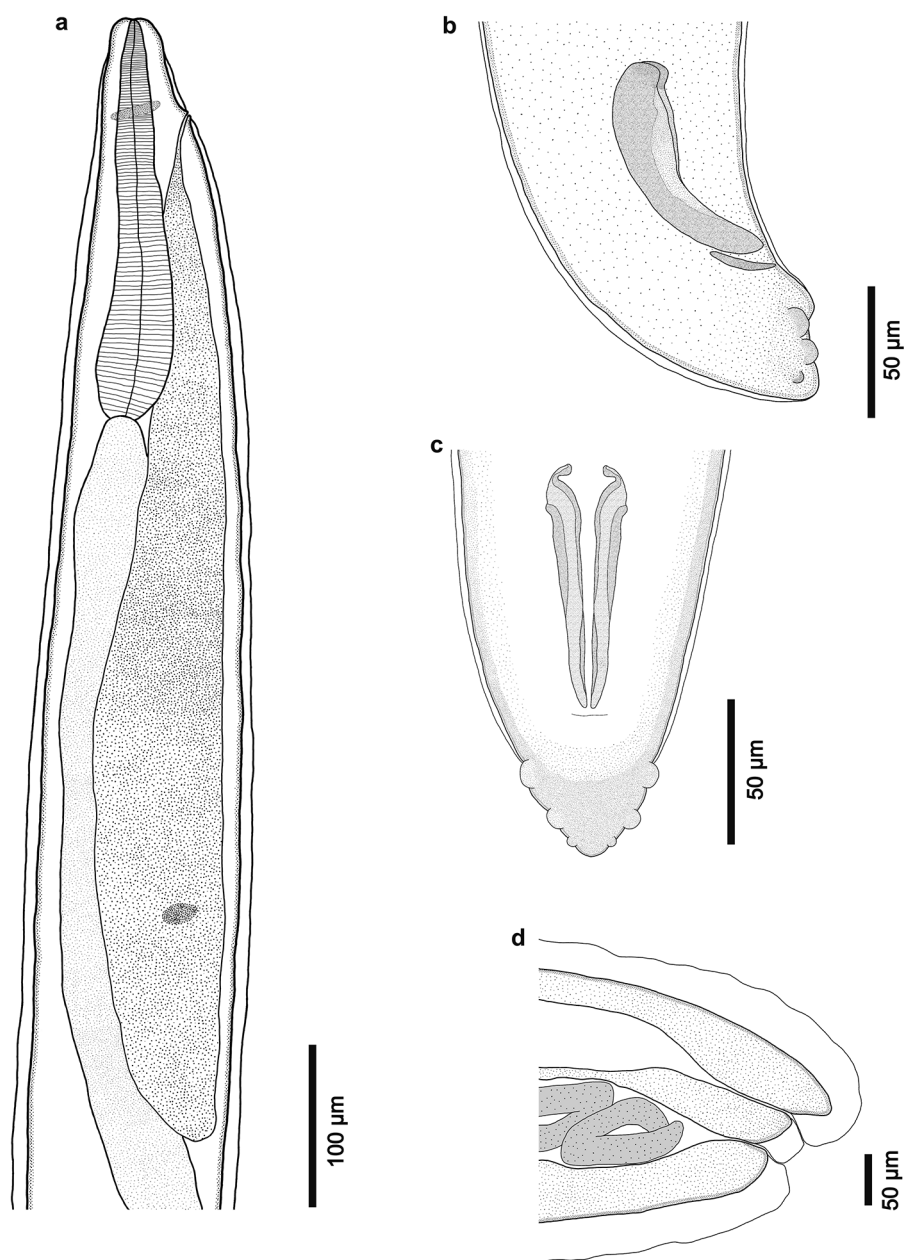


Figure 1. *Oslerus (Oslerus) brachyurus* sp. nov. from *Chrysocyon brachyurus* of Brazil: a) anterior region of male, lateral view; b) posterior region of male, lateral view; c) posterior region of male, ventral view; d) posterior region of female, lateral view.

(*O.*) *brachyurus* sp. nov. studied with DIC microscopy, we determined that vulval and vaginal sphincters are absent (Fig. 2d).

In the comparative morphometric study of the 2 species of *O. (Oslerus)* (Table 1), we observed that the females of *O. (O.) brachyurus* sp. nov. are larger than the females of *O. (O.) osleri* studied by Cobbold (1879) and Urquhart

et al. (1954), that the oesophagus of males and females of the new species have a larger oesophagus length/total body length ratio compared to the specimens studied by Urquhart et al. (1954), and the males of *O. (O.) brachyurus* sp. nov. have larger spicules and a smaller gubernaculum than *O. (O.) osleri* (Table 1).

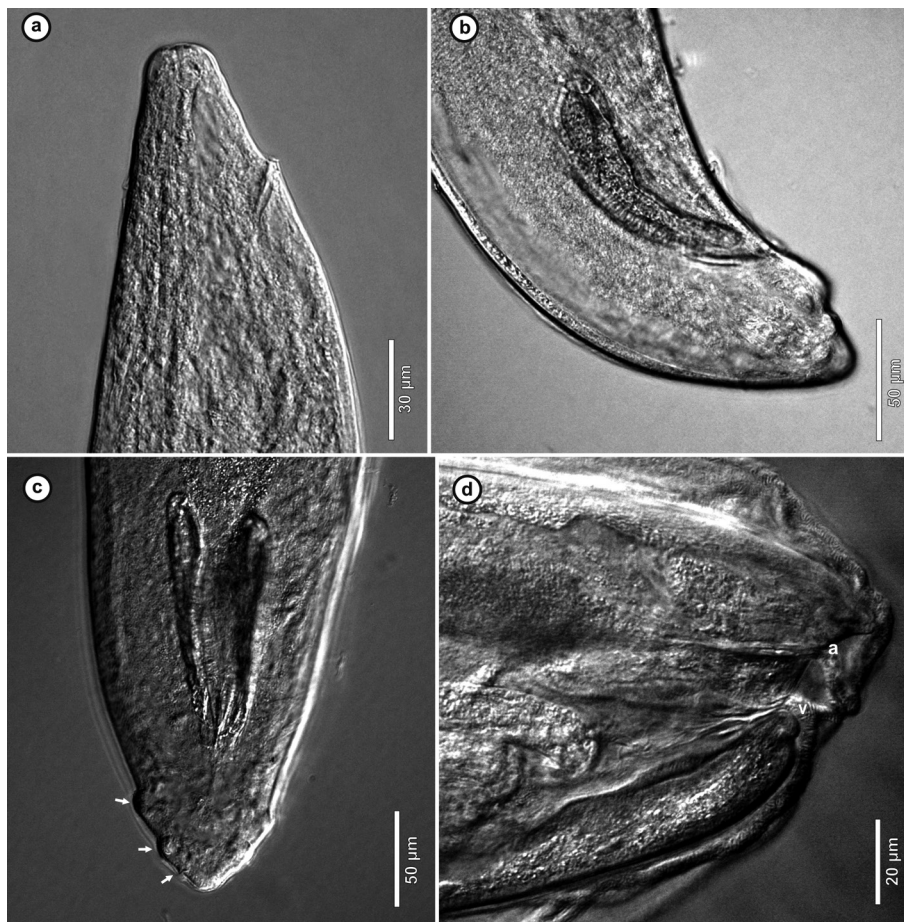


Figure 2. *Oslerus (Oslerus) brachyurus* sp. nov. from *Chrysocyon brachyurus* of Brazil, differential interference contrast light micrographs: a) anterior region of female, lateral view; b) posterior region of male, lateral view; c) posterior region of male, ventral view (white arrows indicates the vestigial rays of the copulatory bursa); d) posterior region of female, lateral view. Abbreviations: a-anus, v-vulva.

## Discussion

In the original description of *O. (Oslerus) osleri* (= *Strongylus canis bronchialis*, *Filaria osleri*) made by Cobbold (1879), the author provided only a few morphological and morphometric data for the species (Table 1). Hall (1921) proposed the genus *Oslerus* to accommodate *F. osleri*. This author claimed not to have a definite opinion about the morphology of the anterior region in this species but stated that it probably had no lips in the buccal opening. Hall (1921) also noted that disagreements existed among other authors about the morphology of the anterior region of this species, some of which claim that it has 2 or 3 lips, and others state the presence of 2 or 3 cephalic papillae.

Hall (1921) did not mention the presence of the cuticular collar in the anterior region and the vestigial

rays of the bursa or caudal papillae in the males of *O. osleri*. The cuticular collar in the cervical region in males and females of this species was first reported by Urquhart et al. (1954). These authors also affirmed that this species had no lips. Seneviratna (1959) reported in *O. osleri* from England a protusible rostrum in the anterior region, 6 small lips, and 3 unequal excretory glands. In the illustrations of *O. osleri* in the study of Seneviratna (1959) the caudal region of the male is represented with 2 pairs of vestigial rays of the copulatory bursa, 1 pair of post-cloacal papillae, and 1 subterminal papilla.

In South America, *O. (O.) osleri* has been reported only in domestic dogs from Chile (Alcaino & Gorman, 1999; Muñoz et al., 2007). The study of Dias et al. (2012) reported *O. (O.) osleri* parasitizing *C. brachyurus* from Brazil, but the authors identified the species of nematode based on clinical and coprological diagnosis of larval

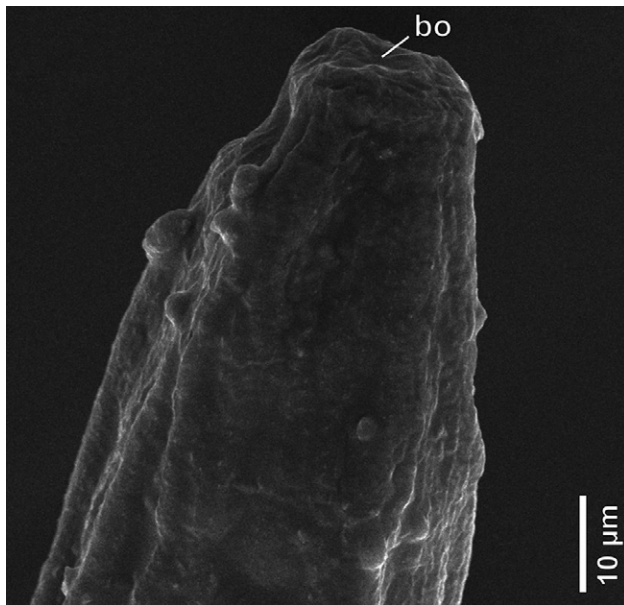


Figure 3. *Oslerus (Oslerus) brachyurus* sp. nov. from *Chrysocyon brachyurus* of Brazil, scanning electron microscopy image. Female anterior region, lateral view. Abbreviation: bo-buccal opening.

parasites, which did not allow a reliable identification of the nematode species from the wild host. The other reports of *Oslerus* spp. in Brazilian hosts do not identify

the parasite to species (Corrêa et al., 2019; Vieira et al., 2017).

In Brazil, 2 species assigned to the genus *Oslerus* were proposed in the first half of the 20<sup>th</sup> Century: *Oslerus barreto* Travassos, 1921 and *Oslerus gordius* Travassos, 1921, both parasitizing primates (Travassos, 1921). In further studies, these species were included in *Filariopsis* van Thiel, 1926 (Metastrongyloidea, Filaroididae) based on the anterior end with 3 evident lips, the presence of vestigial bursal rays clearly defined, and the vulva and anus not terminal in the females (Rego, 1974, 1988; Webster, 1978). Therefore, the new species described herein is the second species of the subgenus *Oslerurs* (*Oslerus*) and the first recorded and carefully analysed morphologically parasitizing *C. brachyurus* from Brazil.

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Table 1

Comparative measurements of *Oslerus (Oslerus) brachyurus* sp. n. and *O. (Oslerus) osleri* (Cobbold, 1879).

References	<i>O. (O.) brachyurus</i> sp. n.		<i>O. (O.) osleri</i>		<i>O. (O.) osleri</i>	
	Current study		Urquhart et al. (1954)		Cobbold (1879)	
	Male	Female	Male	Female	Male	Female
Total body length (mm)	6.5-9.4	15.2-18	6.5-7	10-13.5	4.23	6.35
Maximum body width (μm)	120-170	370-410	-	-	-	-
Body width at level of oesophagus end (μm)	80-100	140-160	-	-	-	-
Oesophagus length (μm)	160-230	260-360	213.9-252.2	226-248	-	-
Oesophagus - body length ratio	40:1	54:1	29:1	49:1	-	-
Nerve ring (μm)	35-55	48-65	-	-	-	-
Excretory pore (μm)	40-62	53-72	-	-	-	-
Number of excretory glands						
Excretory gland (μm)	530-640	606-739	-	-	-	-
Spicules (μm)	88-105	-	99.2-112.8	-	-	-
Gubernaculum (μm)	22-35	-	36.6-51.2	-	-	-
Number of vestigial bursal rays	3	-				
Spicule - body length ratio	81:1	-	63:1	-	-	-

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