Research Note

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Detailed morphological description of *Habronema clarki* Foster & Chitwood, 1937, a nematode parasite of capybaras *Hydrochoerus hydrochaeris* (Linnaeus, 1766) in Brazil

Descrição morfológica detalhada de *Habronema clarki* Foster & Chitwood, 1937, um nematódeo parasita de capivaras *Hydrochoerus hydrochaeris* (Linnaeus, 1766) no Brasil

Estevam Guilherme Lux Hoppe[1]*; José Hairton Tebaldi[1]; Daniel Fontana Ferreira Cardia[2]

[1]Departamento de Medicina Veterinária Preventiva e Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista – UNESP, Jaboticabal, SP, Brasil

[2]Programa de Pós-graduação em Medicina Veterinária - Preventiva, Departamento de Medicina Veterinária Preventiva e Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista – UNESP, Jaboticabal, SP, Brasil

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Abstract

The genus *Habronema* has four valid species, of which only two are properly known. The present study aimed to describe in detail the morphology of *Habronema clarki* through optical and scanning electron microscopy analyses. Our results showed that the labial morphology of this parasite is closer to *H. muscae* than to *H. microstoma*. Even so, the characteristic pseudolabia and the slightly convex border of the dorsal and ventral lips are sufficient to distinguish these nematodes. Additional morphological data are presented, thus contributing to the knowledge on this little known nematode. In addition, this study provides new locality records for this species.

Keywords: Nematoda, optical microscopy, scanning electron microscopy, labial morphology.

Resumo

O gênero *Habronema* tem quatro espécies válidas, das quais apenas duas são propriamente conhecidas. O presente estudo visa descrever em detalhes a morfologia de *Habronema clarki* por meio de microscopia eletrônica de varredura e de luz. Os resultados demonstram que a morfologia labial do parasita é mais próxima de *H. muscae* que de *H. microstoma*. Ainda assim, os pseudolábios característicos e a borda discretamente convexa dos lábios dorsal e ventral são suficientes para se diferenciar esses nematódeos. Dados morfológicos adicionais são apresentados, contribuindo para o conhecimento deste nematódeo pouco conhecido. Em adição, este estudo representa um novo registro de localidade para a espécie.

Palavras-chave: Nematoda, microscopia óptica, microscopia eletrônica de varredura, morfologia labial.

Introduction

The Habronematoidea Chitwood & Wehr, 1932, are a highly diverse group of nematode parasites of birds and mammals. Contrary to the International Code of Zoological Nomenclature, the taxon used as the basis for the superfamily is not the oldest, but the most recognized species: *Habronema muscae* (Carter, 1861) Diesing, 1861 (CHABAUD, 2009). Oddly, although there are four valid species in the genus *Habronema* Diesing, 1861, which is the type genus of this group, only *H. muscae* and *Habronema microstoma* (Schneider, 1866) Ramson, 1911 have been properly studied regarding their biology (ANDERSON, 2000) and morphology (NAEM, 2007).

In Brazil, three species of this genus have been reported (VICENTE et al., 1997) and, of these, only *Habronema clarki* Foster & Chitwood, 1937, seems to be native, since *H. muscae* and *H. microstoma* were probably introduced along with their natural host, the domestic horse.

Despite a recent record of this nematode in capybaras *Hydrochoerus hydrochaeris* (Linnaeus, 1766) in Bolivia (CASAS et al., 1995), the morphology of this nematode was only discussed in the original description, based on specimens obtained from *Hydrochoerus isthmius* Goldman, 1912, from Panama (FOSTER; CHITWOOD, 1937). There are no other records relating to the morphology of

*Corresponding author: Estevam Guilherme Lux Hoppe
Rod. Prof. Paulo Donato Castelane, s/n, CEP 14884-900, Jaboticabal, SP, Brazil
e-mail: hoppe@fcav.unesp.br

this nematode, and, particularly, its labial morphology, which is essential for the group (CHABAUD, 1958), is scarcely known.

The present study aimed to describe in detail the morphology of *Habronema clarki*, a nematode parasite of Hydrochoeridae rodents.

Methodology

Specimens

We analyzed 23 specimens deposited in the helminthological collection of the 'Prof. Dr. Orlando Ferrari' Animal Parasitic Diseases Laboratory, Department of Preventive Veterinary Medicine and Animal Reproduction, FCAV/UNESP, Jaboticabal, state of São Paulo, Brazil. These were obtained from a capybara in Bandeirantes, state of Paraná, Brazil. Specific identification of these nematodes was performed prior to this study, based on the original description of this species (FOSTER; CHITWOOD, 1937).

Three specimens were prepared for scanning electron microscopy analysis on the labial morphology and the others were processed using routine procedures for optical microscopy investigation.

Scanning electron microscopy

For scanning electron microscopy (SEM), the nematodes were post-fixed overnight with 2% osmium tetroxide at 23 °C for 12 h. After that, the specimens were dehydrated in a graded acetone series, critical-point dried with liquid CO_2, and then mounted on SEM stubs and coated with gold. This study was conducted using a JEOL JSM-5410 scanning electron microscope.

Optical microscopy

For optical microscopy, the parasites were clarified in 80% acetic acid (v/v) and beechwood creosote. Measurements in millimeters, expressed as the mean ± standard deviation and range in parentheses, were obtained using an Olympus® BX-51 microscope equipped with QColor3® image system. The images obtained were processed using the Image ProPlus® v.5.0 software. All measurements on morphological characteristics were based on data from ten specimens of each sex, unless otherwise stated.

Results and discussion

Habronema clarki Foster & Chitwood, 1937

General. Large, whitish nematodes *in vivo*. The dorsal and ventral lips bear two papillae each, with the anterior border slightly convex. The pseudolabia are deeply trilobed, with amphids at their bases. The outer lobes are a little longer than the inner ones, and all three lobes are rounded at the distal extremity (Figure 1). Bilateral, symmetrical lateral alae may be observed along the body.

Females. Body is 21.276 ± 1.608 (18.853-23.529) in length and 0.336 ± 0.018 (0.296-0.361) in width. The stoma length is 0.090 ± 0.008 (0.074-0,100) and the esophagus length is 2.44 ± 0.301 (1.705-2.824). Nerve ring is situated at 0.269 ± 0.020 (0.241-0.293) from the anterior ending. The cervical papillae and excretory pore (n = 7) are 0.205 ± 0.017 (0.181-0.232) and 0.339 ± 0.020 (0.302-0.367) from the anterior ending, respectively (Figure 2A. Cervical papillae not shown). The vulvar opening is ventral, 4.604 ± 0.677 (3.492-5.326) from the anterior portion. The tail is conical and the anus is 0.245 ± 0.03 (0.194-0.296) from the rounded tail tip (Figure 2B).

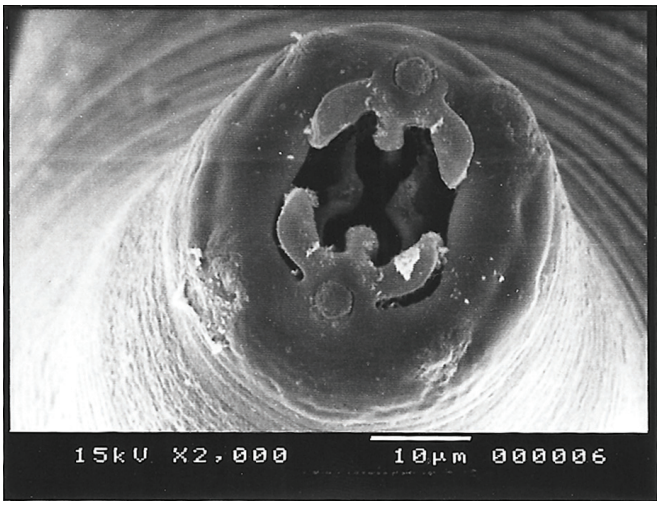

Figure 1. ***Habronema clarki.*** SEM of the anterior end of a male specimen. Note the trifurcate pseudolabia with a round amphid at their base and the simple dorsal and ventral lips. Labial papillae are eroded due to the relatively poor preservation of the specimens studied. **Bar:** 10 µm.

Males. Body is 12.372 ± 1.012 (10.530-14.560) in length and 0.236 ± 0.029 (0.198-0.277) in width. The stoma length is 0.082 ± 0.011 (0.055-0.095) and the esophagus length is 1.88 ± 0.2354 (1.426-2.147). The cervical papillae, nerve ring and excretory pore (n = 6) are 0.185 ± 0.023 (0.146-0.213), 0.2294 ± 0.018 (0.190-0.249) and 0.276 ± 0.027 (0.252-0.328) distant from the anterior ending. The typical spirurid-type male tail has two caudal alae supported by four pairs of pre-cloacal pedunculated papillae and two pairs of asymmetrically arranged post-cloacal pedunculated papillae. Ten small, sessile papillae can be seen at the tail tip. The right spicule is short, measuring 0.381 ± 0.041 (0.333-0.488) in length and the left one is long, twisted in the middle part and 0.752 ± 0.038 (0.695-0.807) in length. The gubernaculum is complex, longer than its width, measuring 0.060 ± 0.005 (0.053-0.069) x 0.050 ± 0.003 (0.046-0.056).

Taxonomic summary

Type host. *Hydrochoerus isthmius* Goldman, 1912
Other host. *Hydrochoerus hydrochaeris* L., 1766
Site of infection. Mucosa of the stomach.
Type locality. Darien Province, Panama.
New locality record. Bandeirantes, state of Paraná, Brazil.
Collection information. Male and female vouchers were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC/Fiocruz), Rio de Janeiro, RJ, Brazil, under collection number CHIOC 35893, as wet mounts. Other

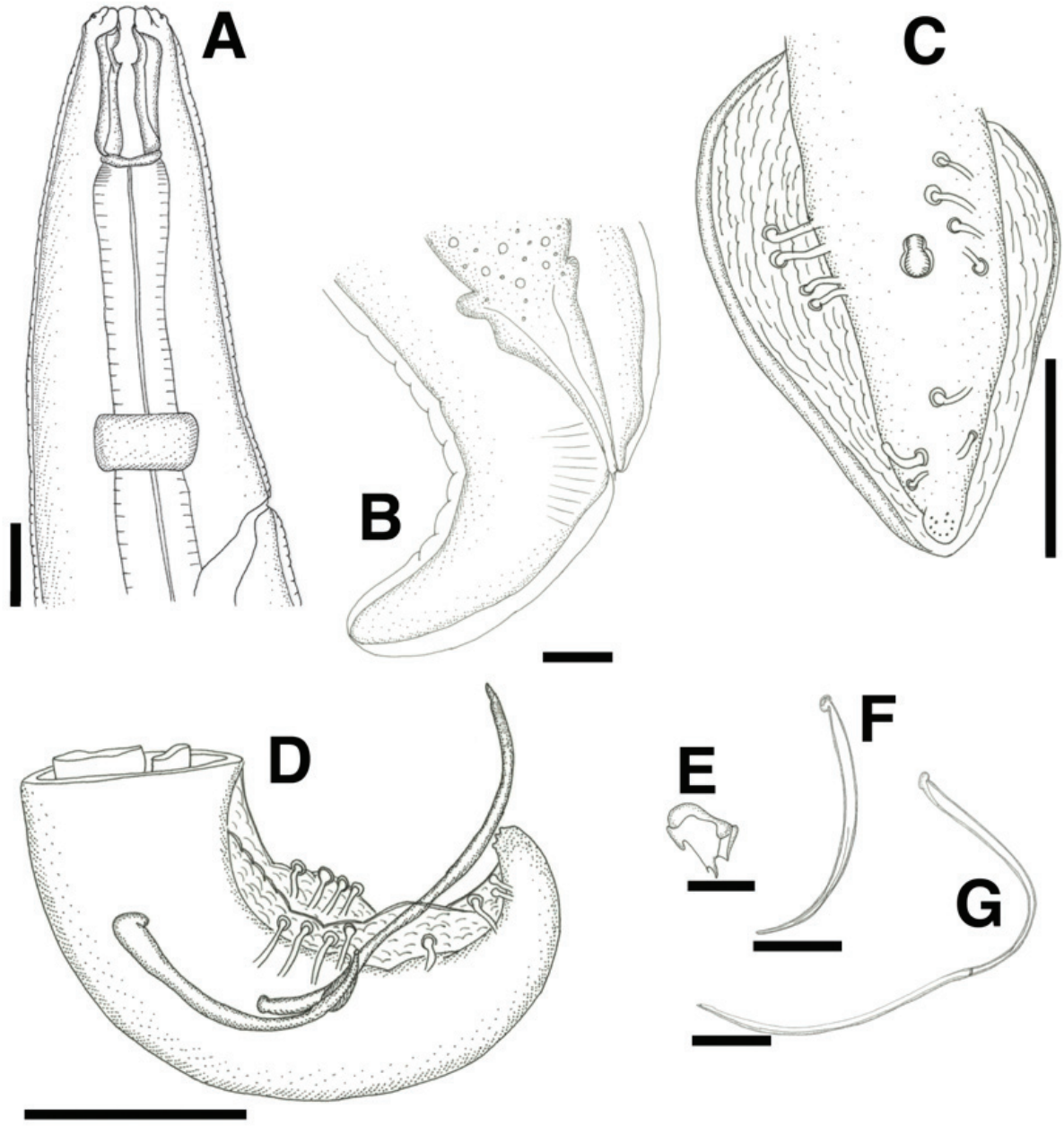

Figure 2. *Habronema clarki*. A. Female, anterior end, lateral view; **B.** Female, posterior end, lateral view; **C.** Male, tail, ventral view (spicules not drawn); **D.** Male, tail, lateral view; **E.** Gubernaculum, lateral view; **F.** Right spicule, left view; **G.** Left spicule, left view. **Bars:** 0.05 mm (Figures A, B, and E); 0.1 mm (Figures F and G); 0.25 mm (Figures C and D).

vouchers were deposited in the helminthological collection of the 'Prof. Dr. Orlando Ferrari' Animal Parasitic Diseases Laboratory, FCAV/UNESP, Jaboticabal, SP, Brazil, also as wet mounts.

Discussion

Among the *Habronema* species, only *H. muscae* shows a morphological pattern similar to *H. clarki*, with well-developed trifurcated pseudolabia, while *H. microstoma* has simple trapezoid pseudolabia (NAEM, 2007). The characteristic pseudolabia and the slightly convex border of the dorsal and ventral lips are sufficient to differentiate *H. clarki* from *H. muscae*. The description of the labial morphology was based on the SEM findings. Nonetheless, analysis of *en face* preparations is sufficient to view the pseudolabia and papillae. Remarkably, the labial morphology of *Habronema zebrae* Theiler, 1923, the fourth species of the *Habronema* genus, is still unknown. Further studies are important in order to evaluate the classification of the latter species within this genus.

In comparison with the other *Habronema* species registered in Brazil, *H. clarki* shows a longer stoma, but the general structure of the caudal portion of the male is identical to *H. muscae* and

H. microstoma. However, the left spicule length, which is slightly less than the size proposed in keys for the *Habronema* species (Chabaud, 1958) and also in the original description (FOSTER; CHITWOOD, 1937), may be used for specific identification.

In the original description, Foster and Chitwood (1937) used the vaginal musculature as characteristic in the specific key that they provided. Moreover, the key proposed by Chabaud (1958) focused specific identification only on male characteristics. In our study, it was found that observation of the vaginal musculature may be very difficult in mature females because of their thickened body. We suggest that the stoma should be used as the identification criterion for females, especially in situations of monosexual infections.

Although this study represents the first official record of *H. clarki* in Brazil, previous studies have reported occurrences of *Habronema* nematodes in Brazilian capybaras in the states of São Paulo and Mato Grosso do Sul (SINKOC et al., 2004; TRAVASSOS, 1945), thus suggesting that this species has widespread distribution.

Conclusion

Based on the present data, the trifurcated pseudolabia could be an important characteristic of this genus and, therefore, should be studied in detail. Also, these characteristics should be included in future taxonomic keys in an attempt to improve the beta-taxonomy of this group. Additionally, new locality data is given in our study.

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