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A NEW SPECIES OF *RHIGONEMA* COBB, 1898 (NEMATODA: RHIGONEMATIDAE) FROM A CUBAN SPIROBOLID MILLIPEDE (DIPLOPODA: SPIROBOLIDA)

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ABSTRACT

Rhigonema nesoboli sp. nov. (Nematoda: Rhigonematidae) is described from the gut of *Nesobolus piedra* (Diplopoda: Rhinocricidae) from Eastern Cuba. The new species is characterized by: the body length of both sexes; the cephalic collar umbelliform; the microtricha large, fine, backwardly directed, extending until the first portion of the intestine; the tail comparatively long and subulate in the female and elbowed in the male; the genital tract of Type 4; a number of 19-21 copulatory papillae arranged as 6-7 pre-cloacal pairs with a single median adanal papillae and three post-cloacal pairs; and the spicules arcuate and comparatively slender. It is closer to *R. villosum* Hunt, 1995, but differs by having a shorter body in both sexes and less copulatory papillae: 19-21 vs. 27-31. This is the first *Rhigonema* recorded for Cuba with a genital tract of Type 4.

KEY-WORDS: *Rhigonema*; Diplopoda; Cuba; New species.

INTRODUCTION

The genus *Rhigonema* Cobb, 1898 is the most diverse and widespread in the family Rhigonematidae. Its members in Africa, Asia, Australasia and the Americas are parasites of several species of spirobolid, spirostreptid and polydesmid millipedes (Diplopoda: Spirobolida, Spirotreptida, Polydesmida) (Hunt, 1996, 1998, 1999a, b, 2002a, b). Only two species have been recorded from Cuba: *R. cubanum* (Barus, 1969), frequent in the intestine of *Orthomorpha coarctata* (Diplopoda: Polydesmida), *Trigoniulus lumbricinus* and *Rhinocricus duvernoyi* (Diplopoda:

Spirobolida), and *Rhigonema piedralavela* García, Coy & Ventosa, 1998, found in *Amphelictogon* sp. (Diplopoda: Polydesmida) (Barus, 1969; Coy *et al.*, 1993; García, *et al.*, 1998). *Rhigonema cubanum* was redescribed by García & Morffe (2011).

With the exception of *R. anawakae* Hunt, 1999 and *R. caribae* Hunt, 1999; *R. lanceacauda* Hunt, 1981 and *R. pinguilabellum* Hunt, 1981 all the species described from the West Indies (including those from Cuba) present the reproductive system of Type 1 (Adams 1987; Hunt, 1981, 1996, 1999a). The current new species constitutes the first record for Cuba of a *Rhigonema* with the reproductive system of Type 4.

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MATERIAL AND METHODS

Five specimens of *Nesobolus piedra* Pérez-Asso, 1996 (Diplopoda: Spirobolida: Rhinocricidae) were collected by hand in litter and rotting logs in the former coffee plantation "La Isabelica", La Gran Piedra, Santiago de Cuba province, Cuba (April, 2009). The millipedes were maintained alive in plastic jars with moistened litter until processing.

These hosts were killed by immersion in 70% ethanol and immediately dissected by cutting the last segments. The guts were extracted and excised in Petri dishes with normal saline. Parasites were killed with hot water (70°C) and fixed in 70% ethanol.

The nematodes were transferred to anhydrous glycerine and mounted in the same medium. The edges of the coverslips were sealed with nail varnish in order to prevent hydration of glycerine. Measurements were taken with a calibrated eyepiece micrometer attached to a compound microscope (± 0.001 mm). Variables measured were those proposed by Hunt (2002a, b), expressed in millimeters. De Man's indexes a, b, c and V% were calculated. Each variable is shown as the range followed by the mean plus standard deviation in parentheses; number of measurements is also given.

Some specimens were prepared for SEM as follows: they were dehydrated in a graded ethanol series, critical point-dried, mounted in aluminum stubs and coated in gold. SEM micrographs were taken at an acceleration voltage of 22-25 kV.

Line drawings were made with the softwares CorelDRAW X3 and Adobe Photoshop CS2 using micrographs (taken with an AxioCam digital camera attached to a Carl Zeiss AxiosKop 2 Plus microscope) as templates. Scales of all plates are given in millimeters.

Type material is deposited in the Colección Helminológica de las Colecciones Zoológicas (CZACC), Instituto de Ecología y Sistemática, Havana, Cuba.

RESULTS

Family Rhigonematidae

Rhigonema Cobb, 1898

Rhigonema nesoboli sp. nov.

(Fig. 1 A-E, Fig. 2 A-D, Fig. 3 A-D)

Type material: ♀ holotype, Cuba, Santiago de Cuba province, La Gran Piedra, La Isabelica, 75°37'18.80"N, 20°00'32.68"W; in *Nesobolus piedra*; IV/2009; J. Morffe coll.; CZACC 11.4580.

Paratypes 7 ♀♀, same data as holotype, CZACC 11.4581-11.4587; 6 ♂♂, same data as holotype, CZACC 11.4588-11.4593.

Measurements

Holotype (female) a = 13.03, b = 8.53, c = 5.27, V% = 50.51, total length = 2.475, maximum body width = 0.190, corpus length = 0.220, diameter of basal bulb = 0.083, total length of oesophagus = 0.290, nerve ring to anterior end = 0.133, excretory pore to anterior end = 0.243, vulva to posterior end = 1.225, tail length = 0.470, eggs = 0.100-0.110 \times 0.080-0.085 (0.105 \pm 0.005 \times 0.083 \pm 0.003 n = 3).

Paratypes (females) (n = 7) a = 11.35-14.12 (12.40 \pm 0.89 n = 7), b = 8.89-12.50 (10.39 \pm 1.65 n = 4), c = 4.80-7.99 (5.83 \pm 1.07 n = 7), V% = 44.35-50.46 (48.43 \pm 2.20 n = 7), total length = 2.400-2.875 (2.754 \pm 0.169 n = 7), maximum body width = 0.170-0.240 (0.224 \pm 0.025 n = 7), corpus length = 0.180-0.240 (0.205 \pm 0.026 n = 4), diameter of basal bulb = 0.078-0.103 (0.091 \pm 0.009 n = 7), total length of oesophagus = 0.230-0.310 (0.265 \pm 0.034 n = 4), nerve ring to anterior end = 0.103-0.135 (0.114 \pm 0.015 n = 4), excretory pore to anterior end = 0.158-0.270 (0.200 \pm 0.041 n = 6), vulva to posterior end = 1.225-1.600 (1.413 \pm 0.123 n = 6), tail length = 0.360-0.550 (0.481 \pm 0.062 n = 7), eggs = 0.078-0.105 \times 0.070-0.088 (0.097 \pm 0.007 \times 0.076 \pm 0.004 n = 12).

Paratypes (males) (n = 6) a = 10.62-12.05 (11.02 \pm 0.53 n = 6), b = 8.53-9.37 (8.92 \pm 0.36 n = 4), c = 5.74-7.60 (6.47 \pm 0.84 n = 6), total length = 2.160-2.530 (2.315 \pm 0.143 n = 6), maximum body width = 0.200-0.220 (0.210 \pm 0.006 n = 6), corpus length = 0.195-0.213 (0.203 \pm 0.008 n = 4), diameter of basal bulb = 0.073-0.090 (0.081 \pm 0.006 n = 6), total length of oesophagus = 0.240-0.270 (0.258 \pm 0.013 n = 4), nerve ring to anterior end = 0.100-0.125 (0.118 \pm 0.012 n = 4), excretory pore to anterior end = 0.190-0.213 (0.201 \pm 0.011 n = 5), tail length = 0.290-0.430 (0.363 \pm 0.056 n = 6), spicule length (on chord) = 0.133-0.183 (0.159 \pm 0.017 n = 6).

Description

General: Cephalic extremity formed by a cephalic cap followed by a cephalic collar slightly umbelliform. Cephalic cap circular in *en face* view, bearing four mammiform papillae, two sub-ventral and two sub-dorsal.

Amphids sub-dorsal, located in the junction of cap and cephalic collar, their diameter *ca.* 1.5 times larger than the papillae. Oral opening triangular, dorsal and

ventral sectors equally developed, each bearing a median, rounded, cuticular projection. Cuticle with fine transverse striations, *ca.* 8 μm wide from the cephalic

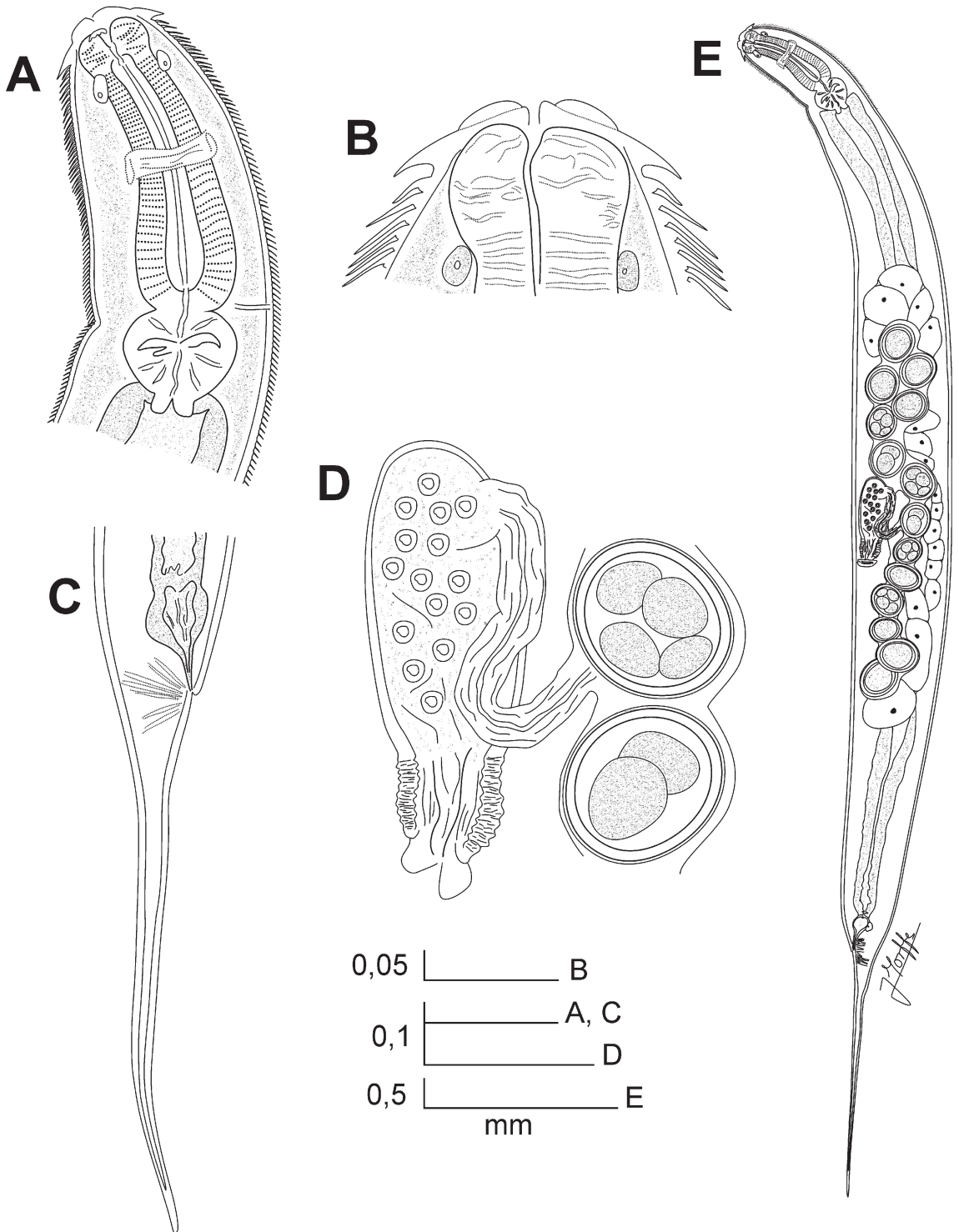


FIGURE 1: *Rhigonema nesoboli* sp. nov. Female. A. Oesophageal region, lateral view. B. Cephalic end, internal view. C. Tail, lateral view. D. Vaginal region and diverticulum. E. Habitus, ventrolateral view.

region to the base of tail; the striae are interrupted on both sides of body. Cervical region with large, fine, numerous microtricha, from the base of the cephalic cap to the first portion of the intestine. Microtricha near the cephalic collar *ca.* 30 μm length. Anterior

part of oesophagus with three triangular jaw-like cuticular plates, one dorsal and two sub-ventral. Cuticular plate margins softly serrated, bearing six large triangular teeth. Teeth of opposite plates interlocking. Oesophagus with a muscular, clavate, comparatively

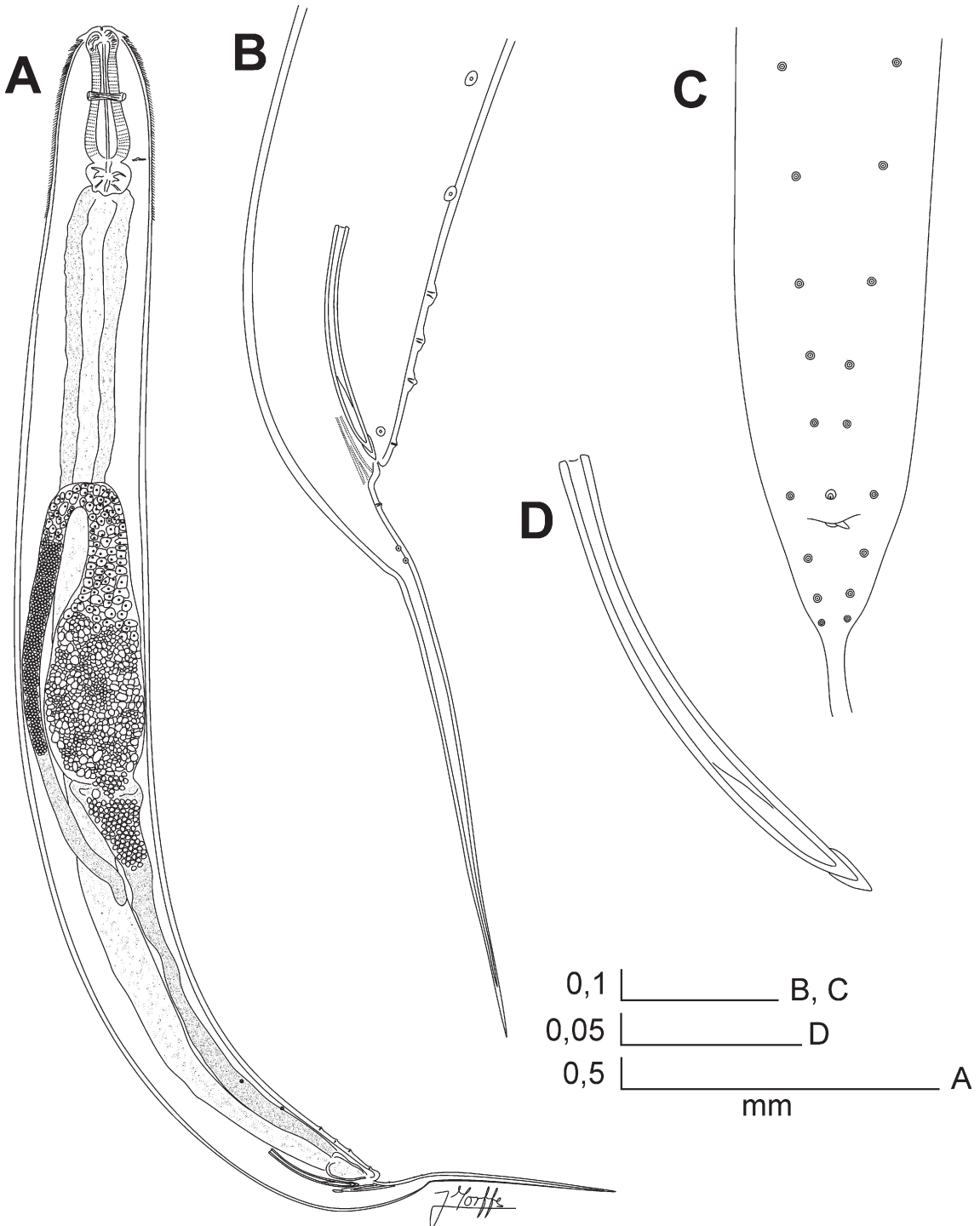


FIGURE 2: *Rhigonema nesoboli* sp. nov. Male. A. Habitus, lateral view. B. Tail, lateral view. C. Tail, ventral view. D. Spicule

short corpus with the anterior end expanded. A row of rounded, brown cells surrounding the corpus at base of its anterior expansion. Isthmus only appreciable as a constriction between the corpus and basal bulb. Basal bulb muscular, sub-spherical, anchored to body wall by muscles attached to its equatorial region, valve plate well developed. Intestine simple, sub-rectilinear. Nerve ring in the midpoint of the corpus. Excretory pore located in the base of corpus, near the junction of the basal bulb. Three short, rounded cardia extending from the base of basal bulb into the intestine.

Female: Type 4 genital tract after Adamson, 1987. Vulva not prominent, slightly displaced to posterior half of body. Vagina short, anteriorly directed, leading into a well developed, comparatively short and fine-walled diverticulum. Two ovaries reflexed, arranged as a didelphic-amphidelphic genital system. Both uteri converge in a common duct that leads into the distal part of diverticulum. Eggs comparatively large, ellipsoidal, shell fine and smooth. A number of 4-18 eggs at a time in the uteri. Tail conical, subulate, comparatively long, ending in a fine tip.

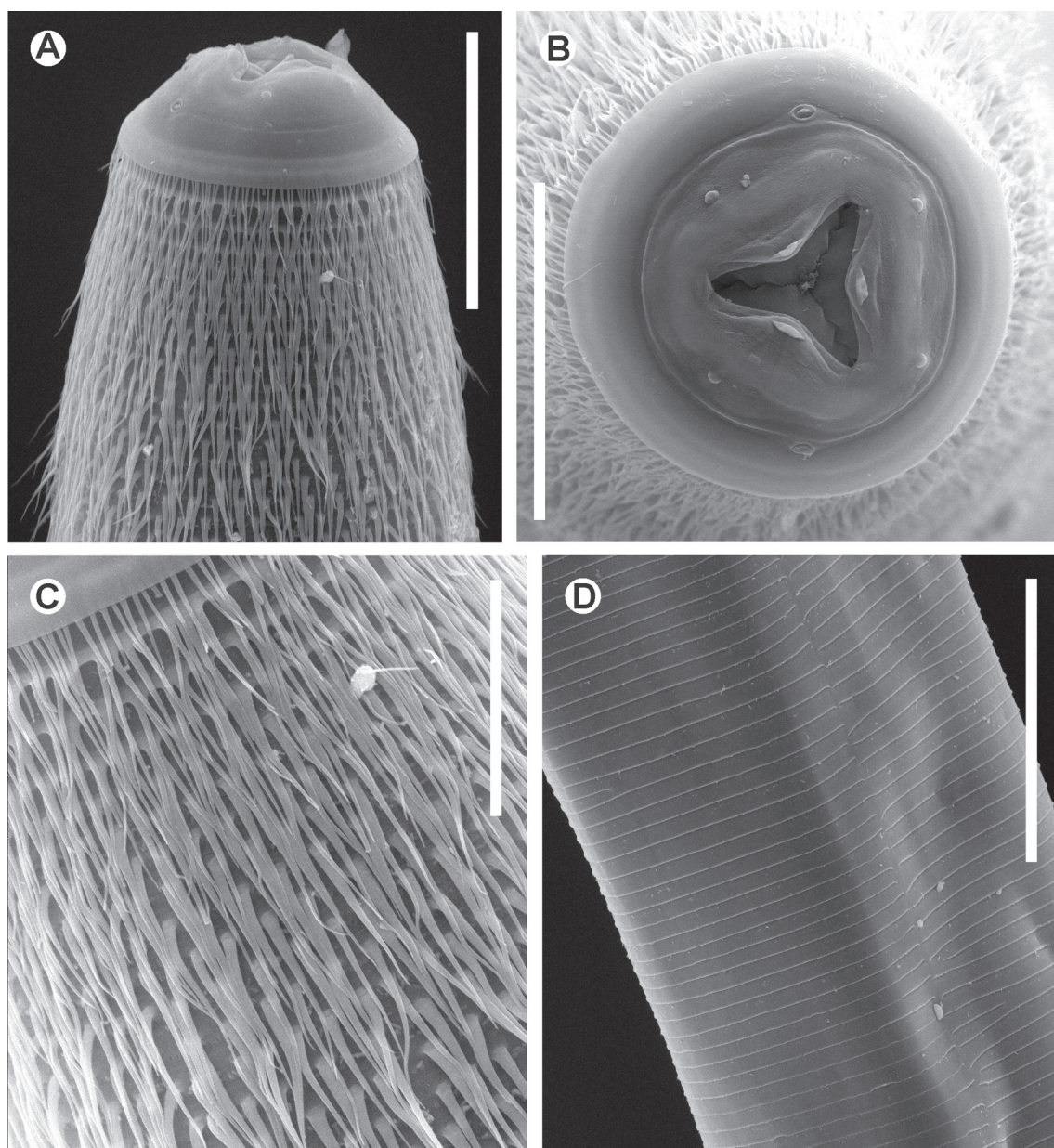


FIGURE 3: *Rhigonema nesoboli* sp. nov. Female. SEM images. **A.** Cephalic end, dorsolateral view. **B.** Cephalic end, *en face* view. **C.** Microtricha. **D.** Cuticular striae. Scale bars: A. 0.04 mm; B. 0.02 mm; C. 0.05 mm; D. 0.1 mm.

Male: Body length slightly shorter than female. Monorchic. Spicules moderately arcuate, slender, isomorphic and isometric. Spicules capitulum barely developed, distal tips pointed, covered by cuticular velae, shafts sculptured with irregular punctures on their surfaces. Posterior end of body ventrally curved. Tail base conical, becoming finer towards its apical pointed tip. Tail dorsally elbowed at its narrowing point. Nineteen copulatory papillae. Pre-cloacal papillae arranged as a single median papilla near the anterior lip of cloaca, flanked by a pair of sub-ventral papillae at same level; three pairs equidistant (the distance between them similar to the distance between the median papilla and the first of such pairs); two pairs of papillae becoming more sub-lateral towards the anteriormost part. Some specimens present a seventh pre-cloacal pair of papillae more lateral and slightly equidistant from the sixth and fifth pairs. Post-cloacal papillae sub-ventral, comprising a first pair near the posterior lip of cloaca (at a distance of cloaca similar to the single median papilla) and two pairs just before the tail elbowing.

Type locality: La Isabelica, La Gran Piedra, Santiago de Cuba province, Cuba.

Type host: *Nesobolus piedra* Pérez-Asso, 1996 (Diplopoda: Spirobolida: Rhinocricidae).

Site: Hind gut.

Etymology: Specific epithet refers to the genus of the type host: *Nesobolus*.

Differential diagnosis

Rhigonema nesoboli sp. nov. is characterized by the body length of both sexes; the shape of the cephalic end; the form and extension of the microtricha; the form of the tail in both sexes; the genital tract of Type 4 (first Cuban species of the genus with such type of genital system); the number and arrangement of copulatory papillae and the form of the spicules.

Rhigonema villosum Hunt, 1995 is the closer species in the genus by having similar shape of the cephalic end, form and extension of the microtricha and shape of spicules. Differs from *R. nesoboli* sp. nov. by the number and arrangement of the copulatory papillae: 27 to 31 arranged as nine to eleven pre-cloacal pairs and four post-cloacal, plus a single median pre-cloacal papilla. The new species present 19 to 21 papillae: six or seven pre-cloacal pairs, three post-cloacal, and the single median pre-cloacal papilla. Both

sexes of *R. villosum* are larger (total length of females 3.610-5.850 *vs.* 2.400-2.875; total length of males 2.570-4.410 *vs.* 2.160-2.530) (Hunt, 1995).

Rhigonema licinacanthum Hunt & Moore, 1999 resembles *R. nesoboli* sp. nov. by the shape of the tail in both sexes and the spicules in males. It can be segregated by the upwardly bent microtricha *vs.* that backwardly directed of *R. nesoboli* sp. nov. and the number of copulatory papillae in males: 23 to 27 *vs.* 19 to 21. In *R. licinacanthum*, the duct joint to vaginal diverticulum is at its basal part, near the junction with the vagina (Hunt & Moore, 1999). On the other hand, in *R. nesoboli* sp. nov. the duct joint to diverticulum is at its distal part. Females and males of *R. licinacanthum* are larger (total length of females 5.340-6.160 *vs.* 2.400-2.875; total length of males 4.420-5.340 *vs.* 2.160-2.530) and the tails are comparatively longer (c of females = 3.50-4.20 *vs.* 4.80-7.99; c of males = 4.20-4.90 *vs.* 5.74-7.60).

Rhigonema thysanophora Crites, 1965 presents a similar tail shape and form, as well as extension of microtricha (long, fine and abundant, extending to the first portion of intestine) to those of *R. nesoboli* sp. nov. It differs by the shape of spicules, robust *vs.* slender in *R. nesoboli* sp. nov. The females of *R. thysanophora* are longer (3.010-4.040 *vs.* 2.400-2.875), but their tails are comparatively shorter (c = 13.68-16.83 *vs.* 4.80-7.99). The vulva is slightly displaced forward in *R. nesoboli* sp. nov. (V% = 44.35-50.46 *vs.* 53.00-56.00). Despite its longer body, the eggs of *R. thysanophora* are shorter than those of *R. nesoboli* sp. nov. (0.060-0.070 × 0.048-0.053 *vs.* 0.078-0.105 × 0.070-0.088) (Crites, 1965).

The males of *R. longicaudatum* Dollfus, 1952 present an elbowing in the tail, as in *R. nesoboli* sp. nov. They differ by having very short microtricha, not surpassing the level of the oesophagus (Dollfus, 1952). The spicules of *R. longicaudatum* are more curved and robust than in *R. nesoboli* sp. nov. Moreover, females and males of *R. longicaudatum* are longer (3.500-3.800 *vs.* 2.400-2.875) and present a notably shorter tail (c = 112.67-14.00 *vs.* 4.80-7.99). As occurs in females, males are also longer (2.900-3.000 *vs.* 2.160-2.530), with shorter tail (c = 15.00-15.68 *vs.* 5.74-7.60).

Rhigonema nesoboli sp. nov. differs from *R. critesi* Ramírez, 1974; *R. nigella* Dollfus, 1964; *R. alvarengai* Travassos & Kloss, 1960; *R. carlosi* Adamson, 1987; *R. infecta* (Leidy, 1849) and *R. truncatum* Artigas, 1926 by the shape of the cephalic collar, umbelliform in the Cuban species *vs.* rounded in the others. The tail of *R. nesoboli* sp. nov. is long and filiform in opposition to the very short, conical tail of the taxa men-

tioned above. Spicules of males of the present species are comparatively slender and less arcuate *vs.* the arcuate of *R. critesi*, *R. nigella* and *R. alvarengai* (Travassos & Kloss, 1960; Dollfus, 1964; Ramírez, 1974), and the robust of *R. carlosi*, *R. infecta* and *R. truncatum* (Artigas, 1926; Adamson, 1987). In addition, *R. nesoboli* sp. nov. can be segregated by the shape and extension of the microtricha.

Rhigonema nesoboli sp. nov. can be differentiated from *R. pinguilabellum* Hunt, 1981; *R. pilosum* Hunt, 1998; *R. arawakae* Hunt, 1999; *R. caribae* Hunt, 1999 and *R. selvatica* Tantalean & Altmamm, 1987 by the shape and length of the tail in males: longer and elbowed *vs.* short, from conical to slightly subulate in those species (Hunt, 1981; Tantalean & Altmamm, 1987; Hunt, 1998, 1999a). *Rhigonema nesoboli* sp. nov. also differs from *R. pilosum* and *R. selvatica* by the shape of spicules of males: slender *vs.* robust.

Males of *R. lanceacauda* Hunt, 1981; *R. flabellifer* Hunt, 1999; *R. tomentosum* Hunt, 1995 and *R. golovatchi* Hunt, 1999 present the tail long, subulate and lacking in the characteristic elbowing of *R. nesoboli* sp. nov. (Hunt, 1981, 1995, 1999b) They also differ by the shape of spicules, slender, distally pointed and less curved in *R. nesoboli* sp. nov., in opposition to arcuate and robust in *R. lanceacauda* and *R. flabellifer*. Spicules of *R. tomentosum* are also slender, but notably arcuate. The microtricha of *R. nesoboli* sp. nov. are well developed, long, fine, their tips backwardly directed; unlike *R. lanceacauda*, *R. flabellifer* and *R. tomentosum* with densely distributed microtricha, their tips reflexed. Moreover, *R. golovatchi* possesses microtricha with a woolly appearance: quite dense and minute (Hunt, 1999b).

Rhigonema acrucanthia Hunt, 1995 resembles the new species by the shape of the tail and spicules, but differs by presenting curved, well developed spines, from the cephalic end to the tail (Hunt, 1995). Such feature is unique among the species of this genus.

The other species with reproductive system of Type 4 are *R. africanum* Dollfus, 1964, considered *specie inquirendae* and *R. subtruncatum* Dollfus, 1964, synonym of *R. truncatum* (Van Waerebeke, 1984; Hunt, 1996).

RESUMEN

Se describe a *Rhigonema nesoboli* sp. nov. (Nematoda: Rhigonematidae) parasitando el intestino de *Nesobolus piedra* (Diplopoda: Rhinocricidae) de Cuba Oriental. La nueva especie se caracteriza por la longitud del cuerpo

en ambos sexos, el collar cefálico umbeliforme, microtricos largos y finos, dirigidos hacia atrás, que se extienden hasta la primera porción del intestino; la cola relativamente larga, subulada en la hembra y acodada en el macho; sistema reproductor femenino de Tipo 4; de 19 a 21 papilas caudales dispuestas en 6-7 pares precloacales con una papila media individual en posición adanal y tres pares postcloacales; las espículas arqueadas y relativamente delgadas. *Rhigonema nesoboli* sp. nov. es similar a *R. villosum* Hunt, 1995, pero difiere por tener ambos sexos una menor talla y poseer los machos un número menor de papilas: 19-21 *vs.* 27-31. Esta es la primera especie del género *Rhigonema* registrada para Cuba con sistema reproductor de Tipo 4.

PALABRAS-CLAVE: *Rhigonema*; Diplopoda; Cuba; Especie nueva.

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