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A new species of *Carnoya* Gilson, 1898 (Rhigonematida: Carnoyidae) parasite of a spirobolid (Diplopoda: Spirobolida) from Cuba

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Abstract

Carnoya isabelica n. sp. is described parasitizing *Nesobolus piedra* from Eastern Cuba. The new species is characterized by the presence of 13 copulatory papillae in males (the post-cloacal arranged in two trios, with two lateral papillae and one median papilla that can be laterally displaced), the presence of collars of spines in the both sexes and females with 23–25 collars of spines, cephalic end not swollen and lateral alae extending from the end of spines to the base of the tail. SEM images of both sexes are given.

Key words: Carnoyidae, *Carnoya*, Cuba, new species, taxonomy, SEM

Introduction

The family Carnoyidae comprises 16 genera of rhigonematids, parasites of millipedes in the tropical and subtropical regions. *Carnoya* Gilson, 1898, includes ca 22 species from South America, West Indies, Australasia and the Pacific. This genus is characterized by its remarkable appearance, notable sexual dimorphism and apparent affinity for millipedes of the family Rhinocricidae (Hunt and Moore 1998).

Five species have been recorded from Cuba: *C. pyramboia* Artigas, 1926, the only rhigonematid shared with South America; *Carnoya guantanamera* Spiridonov, 1989; *Carnoya ariguanabo* (Coy, García & Álvarez 1993), previously described as *Clementeia ariguanabo*; *Carnoya abeli* García, 1997 and *Carnoya cubitas* García, Coy & Ventosa, 1998 (Spiridonov 1989, Coy *et al.* 1993, García 1997, García *et al.* 1998, García and Ventosa 2009).

The aim of this paper is to describe a new species of *Carnoya* parasitizing a species of Spirobolida from Cuba.

Material and methods

Five specimens of *Nesobolus piedra* Pérez-Asso, 1996 (Diplopoda: Rhinocricidae) were collected in the surroundings of the coffee plantation “La Isabelica”, La Gran Piedra, Santiago de Cuba province, Cuba, at 1000 m a.s.l. The millipedes were collected by hand from litter or rotting logs and kept alive in plastic jars with moistened litter until arriving to the lab.

The hosts were killed by immersion in 70% ethanol and immediately dissected by cutting the last segments of the body. Intestines were extracted and excised in normal saline. The nematodes found were killed with hot water (60–70°C) and fixed in 70% ethanol.

Nematodes were transferred to anhydrous glycerine via slow evaporation method and then mounted in the same medium. The edges of the coverslips were sealed with nail varnish in order to avoid the hydration of the glycerine. Measurements were made after Hunt and Moore (1998) using a calibrated eyepiece micrometer attached to a compound microscope and are given in millimeters. De Manian indexes a, b, c and V were calculated. Variables are shown as range followed by the mean plus standard deviation in parentheses. The number of measurements is also given.

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

Some specimens were used for SEM. They were dehydrated in a graded ethanol series, critical point-dried, mounted in aluminum stubs and coated with gold. SEM micrographs were taken at an average acceleration voltage of 22 kV.

The type material is deposited in the Colección Helminológica de las Colecciones Zoológicas (CZACC), Instituto de Ecología y Sistemática, Havana, Cuba and the Coleção Helminologica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil.

Results

Family Carnoyidae

Carnoya isabelica n. sp.

Fig. 1 A–G, Fig. 2 A–F, Fig. 3 A–D, Fig. 4 A–D.

Type material. ♀ holotype, Cuba, Santiago de Cuba province, La Gran Piedra, La Isabelica, 75°37'18.8" N, 20°00'32.68" W; in *Nesobolus piedra*; IV/2009; J. Morffe coll.; CZACC 11.4538. Paratypes 9♀♀, same data as holotype, CZACC 11.4540–11.4548; 7♂♂, same data as holotype, CZACC 11.4539, 11.4552–11.4557; 3♀♀, same data as holotype, CHIOC; 2♂♂, same data as holotype, CHIOC.

Measurements. Holotype (female) a = 10.65, b = 6.24, c = 5.48, V = 43.65, total length = 1.810, maximum body width = 0.170, buccal cavity length = 0.090, procorpus length = 0.113, isthmus length = 0.103, diameter of basal bulb = 0.070, total length of oesophagus = 0.290, nerve ring to anterior end = 0.088, excretory pore to anterior end = 0.180, vulva to posterior end = 1.020, tail length = 0.330, eggs = 0.165–0.185×0.095–0.100 (0.175 ± 0.014×0.098 ± 0.004 n = 2).

Paratypes (females) (n = 12) a = 8.95–13.33 (10.48 ± 1.15 n = 12), b = 5.86–7.06 (6.29 ± 0.35 n = 11), c = 4.81–6.21 (5.61 ± 0.38 n = 11), V = 39.38–46.20 (43.86 ± 1.86 n = 10), total length = 1.840–2.325 (2.100 ± 0.169 n = 12), maximum body width = 0.170–0.253 (0.203 ± 0.027 n = 12), buccal cavity length = 0.085–0.113 (0.099 ± 0.008 n = 12), procorpus length = 0.110–0.140 (0.129 ± 0.010 n = 12), isthmus length = 0.105–0.150 (0.124 ± 0.016 n = 12), diameter of basal bulb = 0.068–0.090 (0.082 ± 0.007 n = 12), total length of oesophagus = 0.280–0.370 (0.333 ± 0.027 n = 11), nerve ring to anterior end = 0.085–0.103 (0.094 ± 0.005 n = 12), excretory pore to anterior end = 0.180–0.218 (0.192 ± 0.013 n = 7), vulva to posterior end = 0.990–1.370 (1.170 ± 0.112 n = 10), tail length = 0.330–0.470 (0.380 ± 0.040 n = 11), eggs = 0.173–0.210×0.083–0.115 (0.187 ± 0.011×0.098 ± 0.008 n = 12).

Paratypes (males) (n = 9) a = 9.42–12.24 (11.11 ± 0.90 n = 9), b = 3.66–4.13 (3.94 ± 0.17 n = 8), c = 5.03–5.88 (5.50 ± 0.31 n = 9), total length = 1.490–1.770 (1.588 ± 0.094 n = 9), maximum body width = 0.128–0.173 (0.144 ± 0.016 n = 9), buccal cavity length = 0.063–0.075 (0.067 ± 0.004 n = 9), procorpus length = 0.248–0.325 (0.270 ± 0.026 n = 9), isthmus length = 0.063–0.080 (0.073 ± 0.005 n = 9), diameter of basal bulb = 0.058–0.068 (0.062 ± 0.004 n = 9), total length of oesophagus = 0.370–0.450 (0.400 ± 0.030 n = 8), nerve ring to anterior end = 0.103–0.133 (0.117 ± 0.012 n = 5), excretory pore to anterior end = 0.158–0.188 (0.176 ± 0.016 n = 3), tail length = 0.260–0.340 (0.290 ± 0.032 n = 9), spicule length = 0.075–0.115 (0.096 ± 0.011 n = 9), gubernaculum length = 0.053–0.075 (0.066 ± 0.006 n = 9).

Description. *Female.* Body fusiform, reaching maximum diameter at mid-body. Cephalic end formed by a wide oral annule, followed by three well marked, narrower annuli. Next to these, arise three wider annuli, their margins straight; the last of them bearing the first row of spines. Spines arranged in ca 23–25 collars, extending to the level of the basal bulb. First collar with about 60 short, fine spines becoming larger and wider backwardly, then reduce their size through the last collars. Spines about 50 in the collars of midpoint of spiny region. The last two or three annuli of spines are interrupted laterally. Spines with wide base and pointed tips (some spines with two or three tips). Cuticle thin, finely annulated from the end of the spiny region to the level of the tail. Lateral alae wide, commencing at level of the last discontinuous annuli of spines and extending to the base of the tail. Oral aperture hexagonal in shape, surrounded by three lips, one dorsal and two sub-ventral. Four rounded, large papillae around

the mouth. Amphids lateral, similar in diameter to papillae. Cuticle around the oral aperture, papillae and amphids notably wrinkled. Buccal cavity consisting of an initial short, thick-walled capsule followed by a long, narrow, cylindrical portion, its base embedded in the initial oesophageal tissue. Initial part of the buccal cavity bearing six thin, cuticularised pieces arranged as one ventral pair and two sub-dorsal pairs, located immediately posterior to the oral aperture. The margins of these pieces are serrated. Three petal-like teeth, arranged as one dorsal and two sub-ventral located posterior to the serrated pieces. Oesophagus narrowed anteriorly, surrounding the posterior part of the buccal cavity, then widening to a cylindroid, thick walled corpus. Isthmus long. Basal bulb spherical, muscular, valve-plate well developed. Intestine simple, sub-rectilinear, its fore region slightly inflated. Nerve ring located at the base of the buccal cavity, at the point of its joint with the oesophagus. Excretory pore displaced to the posterior half of the oesophageal corpus. Vulva slightly displaced to the anterior half of body. Vagina comparatively long, muscular, posteriorly directed. Genital tract didelphic-prodelphic, ovaries extending to level of the bulb, sometimes surrounding the base of the isthmus. Eggs comparatively large, their shell smooth and thin. A maximum number of two or three eggs at a time in uterus. Tail conical, subulate, ending in a fine tip.

Male. Posterior region of body ventrally curved. Cephalic end consisting of a simple oral annule followed by three narrow, well marked annuli, the first and second similar in width, the third wider, *ca* two widths of the latter. Next to these, extend three wide annuli, concave and slightly inflated, followed by a fourth annuli with straight margins, bearing the first collar of spines at its base (with *ca* 44 elements). Spines very fine, filiform, arranged in about 23 collars extending to the middle of corpus. At the midpoint of the spiny region, collars with *ca* 70 spines. Cuticle finely striated from the end of spines to the base of the tail. Lateral alae extending from a distance posterior to the last collar of spines (*ca* seven annuli) to the base of tail (at level of the last pairs of post-cloacal papillae). Mouth trirradiate with three lips: one dorsal and two sub-ventral, with rounded projections. Four rounded, less marked papillae surrounding the mouth. Amphids lateral, their diameter smaller than in papillae. Cuticle around the mouth, papillae and amphids notably wrinkled. Buccal cavity cylindrical. Oesophageal corpus fusiform, well differentiated from the isthmus. Basal bulb spherical, valve-plate well developed. At least three pairs of brown, drop-like oesophageal glands in the anterior region of corpus. Nerve ring encircling corpus at its first third. Excretory pore located near the midpoint of the corpus. Monorchic. Spicules boat-shaped, isomorphic and isometric. Gubernaculum robust. Copulatory papillae 13, one single, larger ventromedian papilla located on the anterior lip of the cloacal aperture; a first ventral pre-cloacal pair, about 20–22 annuli near the cloacal aperture, individual papillae sometimes in different annuli; two pairs sub-ventral, at both sides of the median single papilla: the outer slightly displaced forward. Six post-cloacal papillae arranged in two trios: the first located at 28–30 annuli from cloacal aperture, the second at 66 annuli posterior to the cloacal aperture. The trios consist of two sub-ventral papillae and a median papilla. In some specimens the median papilla of the first trio can be laterally displaced to the right side, near the right sub-ventral papilla. In the second trio the median papilla can be also laterally displaced to the right side, almost touching the right sub-ventral papilla. Such median papilla can be slightly displaced forward or can be in line with the other elements of the trio. Tail long, conical, subulate, ending in a fine tip.

Differential diagnosis. By having the males with cervical spines, *C. isabelica* **n. sp.** differs from *C. pyramboia*, *C. dollfusi* Adamson, 1984; *C. strobilina* Hunt & Sutherland, 1984; *C. mackintoshae* Adamson, 1985; *C. kermarreci* Adamson & Van Waerebeke, 1985; *C. abeli*, *C. wallacei* Hunt, 1997; *C. caputbulla* Hunt & Moore, 1998; *C. janiceae* Hunt & Moore, 1998 and *C. borinquena* García & Fontenla, 2004 the males of which present the cervical cuticle unarmed (Artigas 1926, Adamson 1984, 1985, Hunt and Sutherland 1984, Adamson and Van Waerebeke 1985, García 1997, Hunt 1997, Hunt and Moore 1998, García and Fontenla 2004). This feature also contributes to segregate *C. isabelica* **n. sp.** from *C. fimbriata* Hunt & Sutherland, 1984; *C. perbella* Hunt & Sutherland, 1984 and *C. posteroovulva* Hunt & Moore, 1998 the males of which present a single collar of spines.

C. ariguanabo differs from the new species by lacking the females of cervical spines. *C. vitiensis* Gilson, 1898 and *C. dubia* Dollfus, 1952 can be differentiate by the abundance and extension of cervical spines in the females: one or two collars in the first species and more than 30 in the second (Gilson 1898, Dollfus 1952). Whereas, *C. isabelica* **n. sp.** has about 23–25 collars of spines. *C. paradubia* Adamson, 1984; *C. martiniquensis* Adamson, 1984; *C. venezuelensis* Adamson, 1984 and *C. cubitas* have the cephalic end knob-like, instead of *C. isabelica* **n. sp.** which lacks the swollen head (Adamson 1984, García *et al.* 1998). On the other hand, *C. cubitas* together with *C. haiti* García, Coy & Ventosa, 2001 lack lateral alae (García *et al.* 1998, 2001) and are easily differentiated from *C. isabelica* **n. sp.**

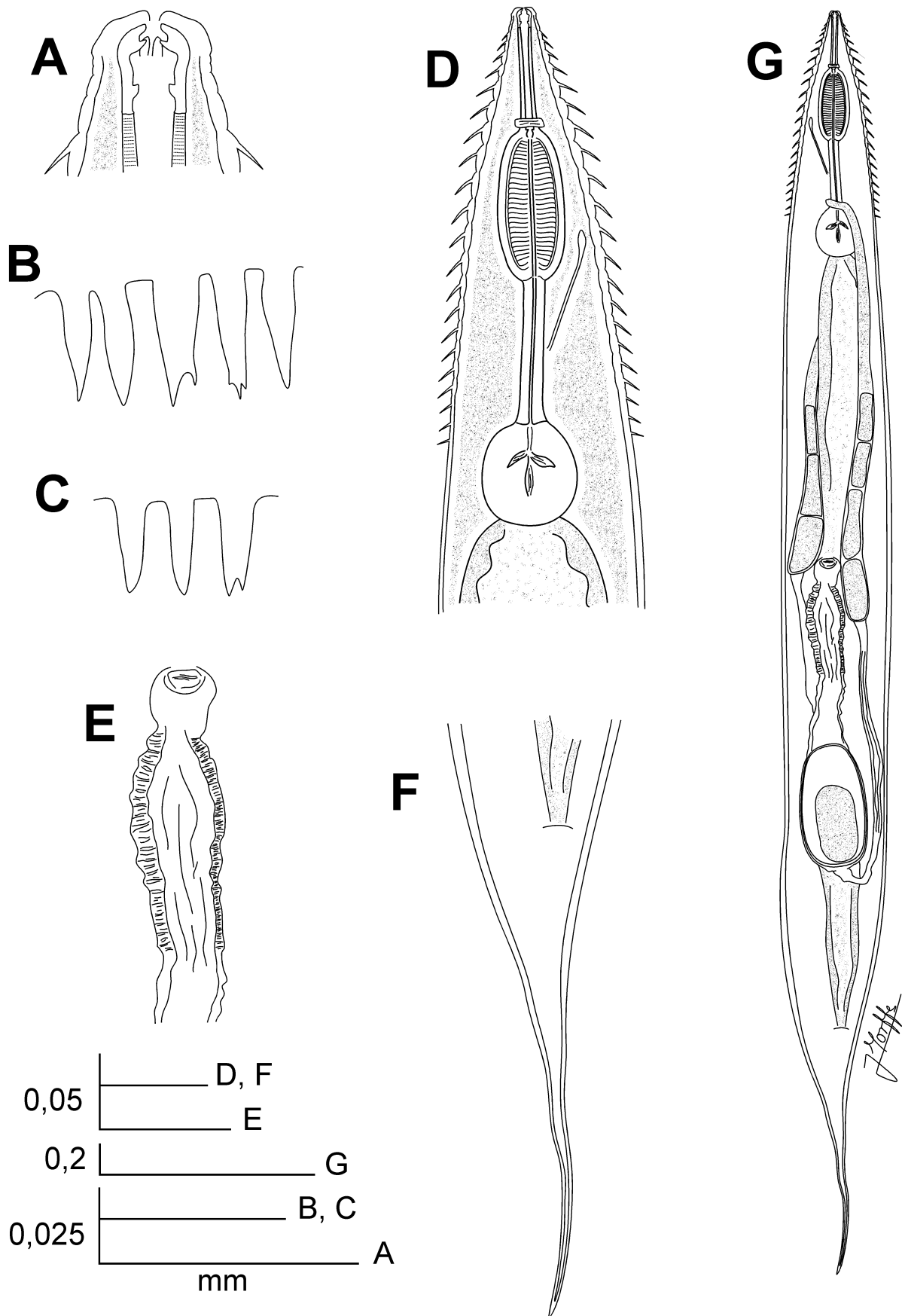


FIGURE 1. *Carnoya isabelica* n. sp. Female. A. Cephalic end, optical section. B. Spines of the midpoint of the spiny region. C. Spines of the last rows. D. Oesophageal region. E. Vulva, ventral view. F. Tail, ventral view. G. Habitus.

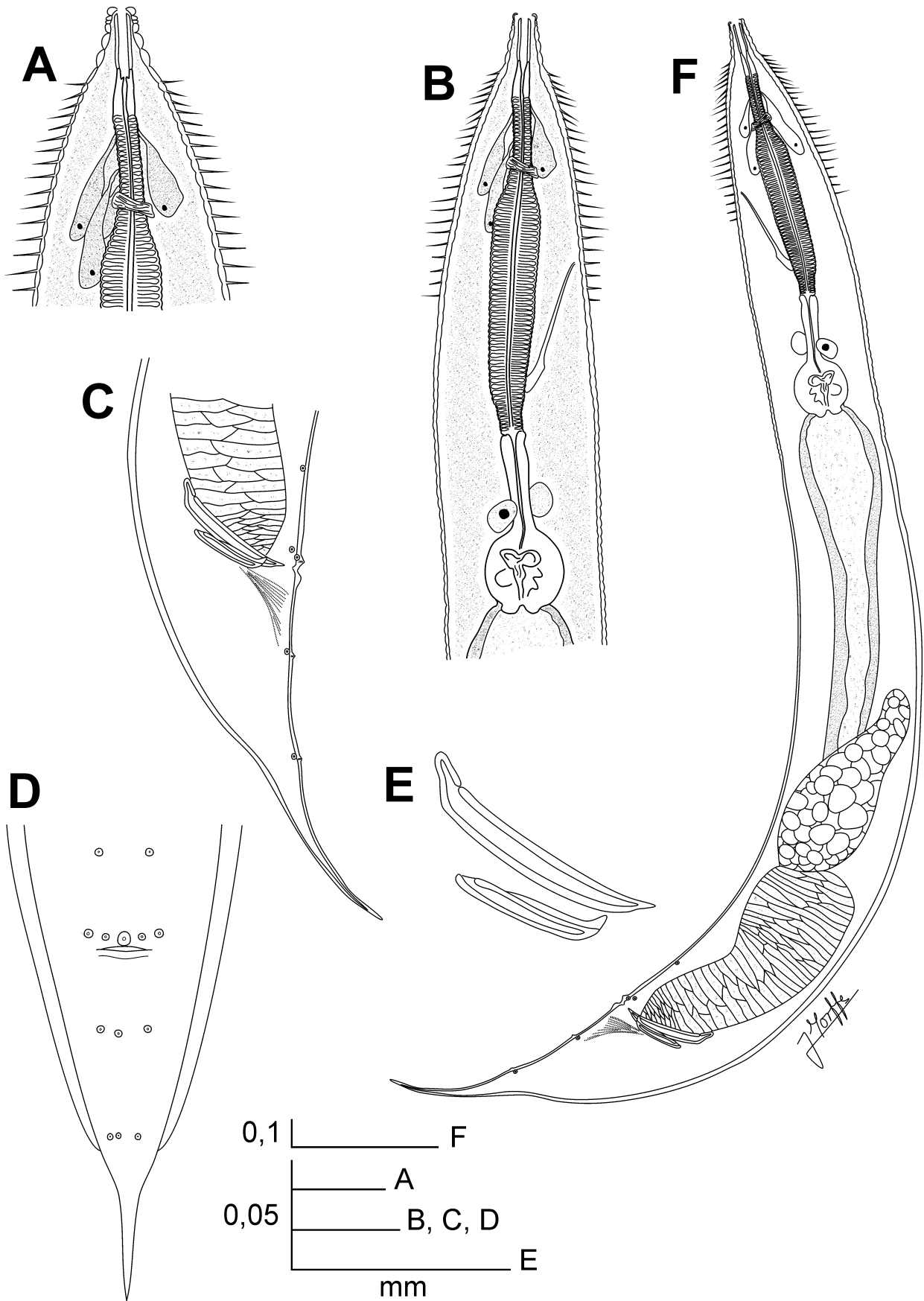


FIGURE 2. *Carnoya isabelica* n. sp. Male. A. Cephalic end, optical section. B. Oesophageal region. C. Tail, lateral view. D. Tail, ventral view. E. Spicule and gubernaculum. F. Habitus.

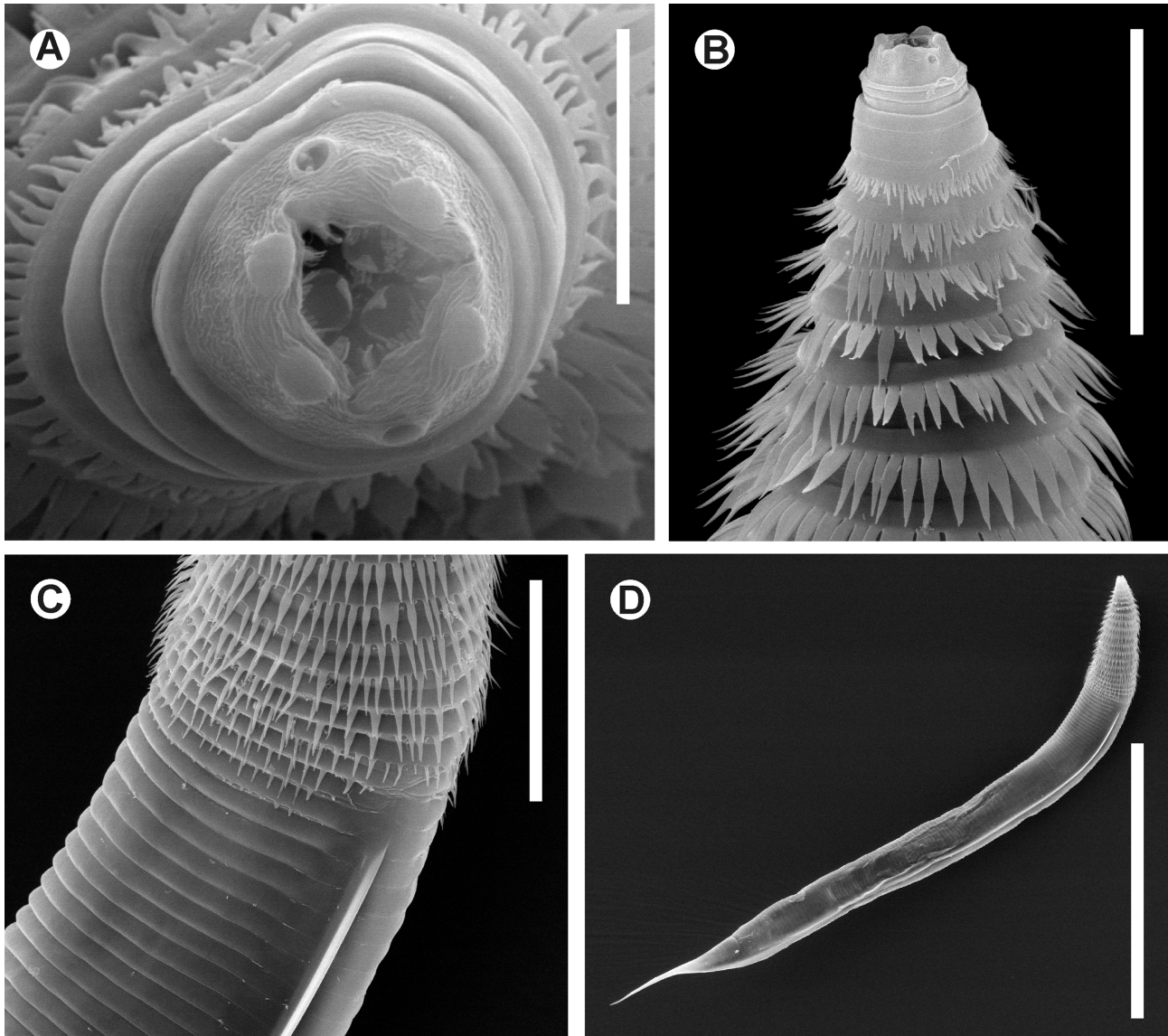


FIGURE 3. *Carnoya isabelica* n. sp. Female. SEM images. A. Cephalic end, *en face* view. B. Cervical region. C. End of spines and beginning of lateral alae, ventrolateral view. D. Habitus, ventrolateral view. Scale lines: A. 0.01 mm, B. 0.04 mm, C. 0.05 mm, D. 0.4 mm.

The presence of two trios of post-cloacal copulatory papillae in males constitutes an unusual feature among genus, shared only with the also Cuban species *C. guantanamera*. From the latter, *C. isabelica* n. sp. can be differentiated by the irregular arrangement of the post-cloacal papillae, with the median papilla of the trios that can be laterally displaced instead of *C. guantanamera* which present the trios arranged as two lateral and one central papillae (according with the line drawings). The lateral alae of *C. isabelica* n. sp. extend until the base of the tail vs the midbody in *C. guantanamera*. Both sexes of *C. isabelica* n. sp. are shorter (♀ 1.810–2.325 vs 2.450–2.720; ♂ 1.490–1.770 vs 1.700–2.200), with the tail, in proportion, also shorter (♀ c = 4.81–6.21 vs 3.10–3.60; ♂ c = 5.03–5.88 vs 3.90–4.60). Moreover, the vulva is more posterior in the females of *C. isabelica* n. sp. (V = 39.38–46.20 vs 28.00–33.00). In the males of *C. isabelica* n. sp. the spines commence at level of the seventh ring, instead of the eleventh in *C. guantanamera* (Spiridonov 1989).

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

Site of infestation. Hind gut.

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

Etymology. Specific epithet in apposition refers to the type locality of the taxon: La Isabelica.

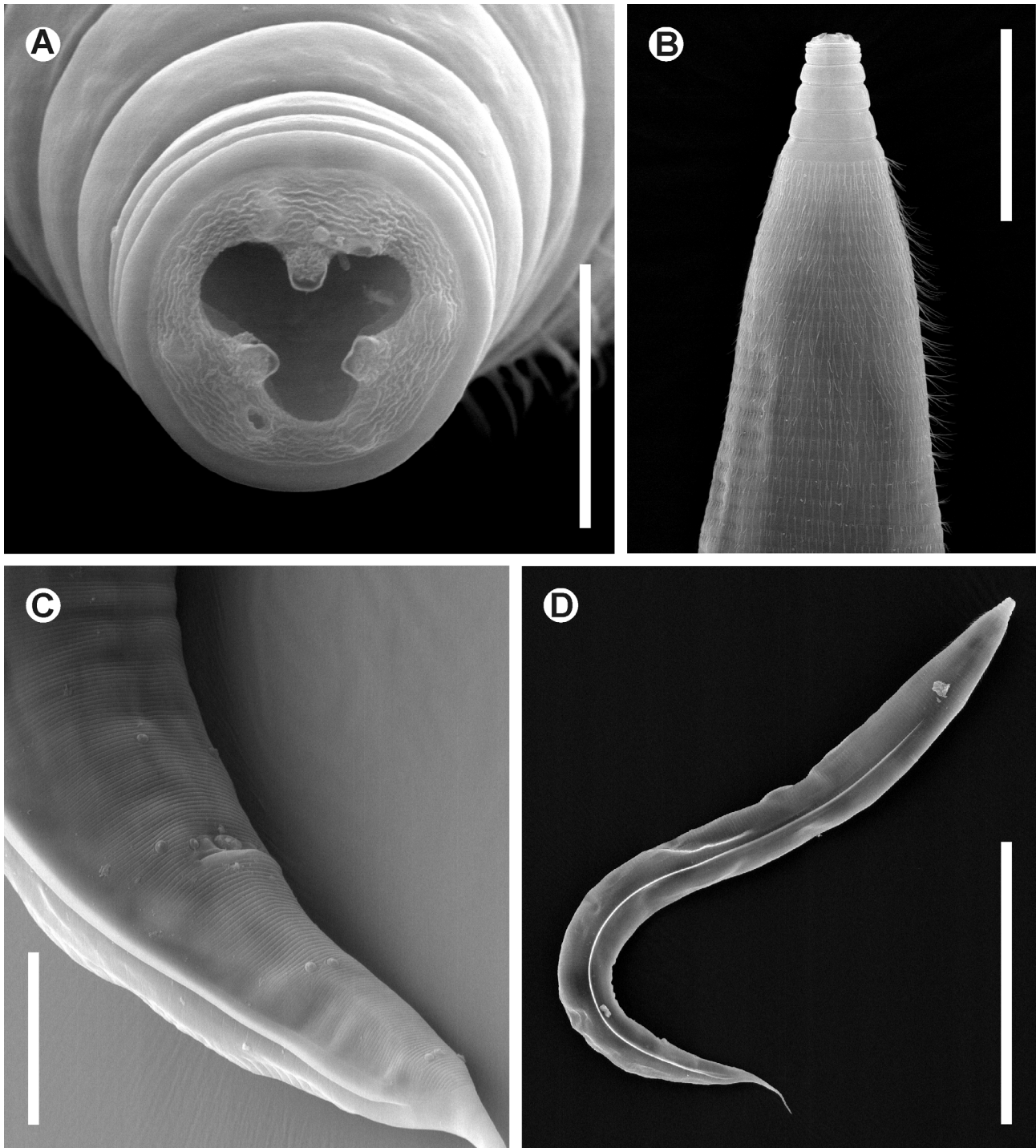


FIGURE 4. *Carnoya isabelica* n. sp. Male. SEM images. A. Cephalic end, *en face* view. B. Cervical region. C. Caudal region, ventrolateral view. D. Habitus, lateral view. Scale lines: A. 0.01 mm, B, C. 0.05 mm, D. 0.4 mm.

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