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# A NEW GENUS AND TWO NEW SPECIES OF XUSTROSTOMATIDAE HUNT, 2002 (Nematoda: Rhigonematomorpha) from the West Indies

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#### **ABSTRACT**

A new genus and two new species of xustrostomatids (Nematoda: Xustrostomatidae) are described parasitizing diplopods from the West Indies. Trachyglossoides jimenoi gen. nov. sp. nov. from Cuba, is similar to Trachyglossus Hunt, 2002. It differs by having the female genital tract monodelphic, feature unique in the family. This constitutes the second record of the family from the country. Zalophora dominicana sp. nov. from the Dominican Republic resembles Z. repentina García & Morffe, 2010. It can be segregate by having a minor development of the cephalic crests and the cephalic end barely inflated. This constitutes the first record of the family and genus for the Dominican Republic.

KEY-WORDS: Trachyglossoides gen. nov. sp. nov.; Zalophora; Diplopoda; Cuba; Hispaniola.

#### **INTRODUCTION**

The description of the genus *Trachyglossus* Hunt, 2002 determined the establishment of the family Xustrostomatidae Hunt, 2002; including the genera *Xustrostoma* Adamson & Van Waerebeke, 1984; *Obainia* Adamson, 1983 and *Zalophora* Hunt, 1994. These are characterized by having the cephalic end laterally depressed and the oral opening dorsoventrally elongated (Adamson, 1983; Adamson & Van Waerebeke, 1984; Hunt, 1994, 2002). The features mentioned above locate the family in an intermediate position between Rhigonematidae and Ichthyocephalidae (Hunt, 2002). Xustrostomatids are parasitic of the hind gut of spirobolid and spirostreptid millipedes (Diplopoda: Spirobolida, Spirostreptida) from Africa

and Australasia (Hunt, 2002). Zalophora repentina García & Morffe, 2010 is the only species from the West Indies (García & Morffe, 2010).

As a result of the current study, several specimens belonging to a xustrostomatid with characters close to *Trachyglossus* Hunt, 2002 were found. Such characters, *e.g.*, the presence of a tongue-like rasp as part of the jaw apparatus, corpus and isthmus hypertrophied, caudal alae in the males and females with a vulval flap by hypertrophy of the anterior lip but with the genital tract monogonant suggest the presence of a new genus of Xustrostomatidae. This new genus and species from Cuba constitute the second record for the family in The Caribbean.

The genus *Zalophora* Hunt, 1994 comprises four species, including one from Cuba (cited above): *Z. he-*

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pialum Hunt, 1994; Z. nemophila Hunt & Moore, 1999 and Z. deinostoma Hunt & Moore, 1999 (all from Australasia) (Hunt, 1994; Hunt & Moore, 1999). The genus is characterized by the presence of dorsoventral crests surrounding the oral opening, giving a peculiar appearance to the markedly globular cephalic end. The description of a new species in the present paper constitutes the first record of the family and genus for Hispaniola.

#### **MATERIAL AND METHODS**

The hosts were collected by hand under litter or rotting logs. Two specimens of *Spirobollelus* sp. (Diplopoda: Spirobolida: Spirobollelidae) from La Jaula, San José de Las Lajas, Mayabeque province, Cuba were kept alive in a jar with moistened litter until processing. Other specimens of an unidentified Spirobolida from Río de los Negros, Santiago province, Dominican Republic were immediately killed, the guts extracted and fixed in 70% ethanol.

The *Spirobollelus* sp. were killed with ethylether and immediately dissected by cutting the last body segments. The guts were pulled out with the aid of pinzettes and excised in Petri dishes with normal saline. Parasites were killed with hot saline (70°C) and fixed in 70% ethanol. The guts of the millipedes from the Dominican Republic were dissected and the nematodes extracted were stored in 70% ethanol.

The nematodes were transferred to anhydrous glycerine via slow evaporation method and mounted in this medium. The edges of the coverslips were sealed with nail polish. Measurements were taken with a calibrated eyepiece micrometer attached to a compound microscope (± 0.001 mm). Variables measured were 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.

For SEM studies the specimens were dehydrated in a graded ethanol series, critical point-dried in a Balzers CPD 030 critical point drier. They were mounted in aluminum stubs and coated in gold with a Bal-Tec SCD 050 sputter coater. 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 compound microscope) as masters. Scales of all plates are given in millimeters.

Type material is deposited in the Colección Helmintológica de las Colecciones Zoológicas (CZACC), Instituto de Ecología y Sistemática, Havana, Cuba; the Coleçao Helmintologica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil and the Nematode Collection of the Royal Belgian Institute of Natural Sciences (RIT), Brussels, Belgium.

#### **Systematics**

# Family Xustrostomatidae Hunt, 2002 Trachyglossoides García & Morffe gen. nov.

Type species: Trachyglossoides jimenoi gen. nov. sp. nov. (here designated)

Diagnosis: Both sexes similar in the shape of the cephalic end and oesophagus. Cephalic end cuticularized, laterally compressed, with a cephalic cap and a cephalic collar, both sub-terminal in position and ventrally displaced. Microtricha present, from the base of the cephalic collar to ca. the midpoint of body. The microtricha near the junction with the cephalic collar are longer. Oral aperture sub-terminal, ventrally displaced, moderately elongated in a dorsoventral direction, its dorsal part slightly reduced. Jaw-like apparatus modified as rasps bearing rows of spines, the ventral hypertrophied as a tonguelike structure situated between two sub-dorsal rasps. Pharynx with a hypertrophied corpus, muscular and robust. Isthmus sub-cylindrical, almost as wide as the corpus. Basal bulb not hypertrophied, sub-spherical, similar in length to the isthmus. Nerve ring at level of the isthmus. Excretory pore ventral, situated at level of the isthmus. Anterior lip of vulva hypertrophied in a triangular vulval flap. Female genital tract monodelphic-prodelphic, Type 1 after Adamson (1987). Male body shorter and less robust than female. Monorchic. Caudal alae present. Spicules isomorphic and isometric, arcuate, capitulum barely developed. Nineteen copulatory papillae, arranged in five pairs of pre-cloacal papillae, four pairs of postcloacal papillae and a single median pre-cloacal papilla.

Distribution: Cuba.

Etymology: The name of the genus is a combination of *Trachyglossus* and the Latin suffix *oides*: similar to, after the resemblances between both taxa.

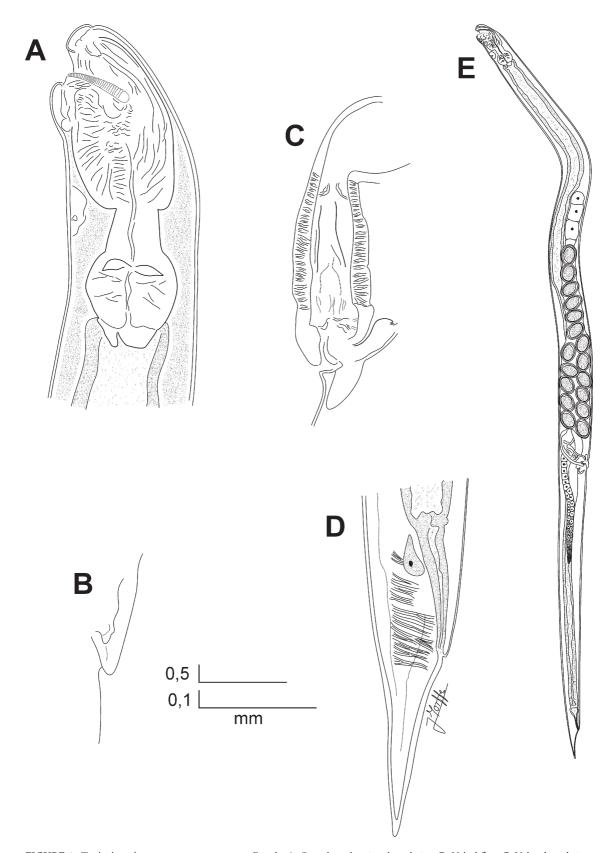


FIGURE 1: *Trachyglossoides jimenoi* gen. nov. sp. nov. Female. A. Oesophageal region, lateral view. B. Vulval flap. C. Vulva, lateral view. D. Tail, lateral view. E. Habitus.

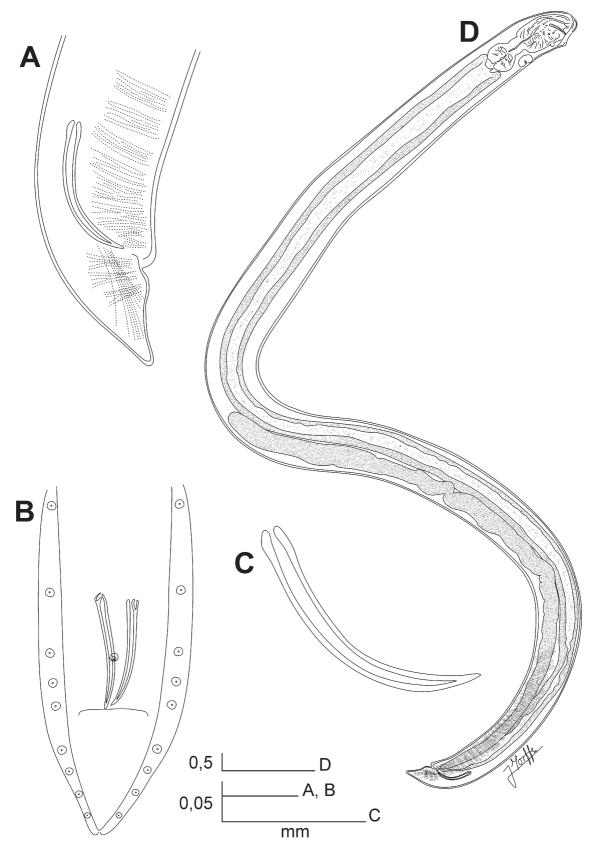
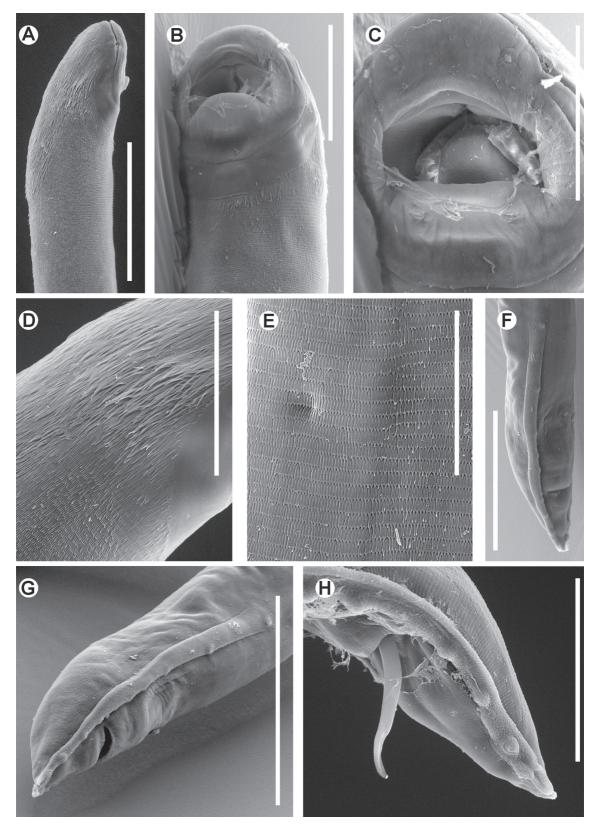


FIGURE 2: Trachyglossoides jimenoi gen. nov. sp. nov. Male. A. Tail, lateral view. B. Tail, ventral view. C. Spicule. D. Habitus.



**FIGURE 3:** *Trachyglossoides jimenoi* gen. nov. sp. nov. SEM images. Male. **A.** Cephalic end, dorsolateral view. **B.** Cephalic end, ventral view. **C.** Oral aperture. **D.** Microtricha at level of the cephalic end. **E.** Microtricha al level of the midbody. **F.** Tail, ventrolateral view. **G.** Tail, lateral view. **H.** Tail tip and spicule. Scale bars: A, G, F = 0.100 mm. B = 0.050 mm. E = 0.020 mm. C, D, H = 0.040 mm.

# Trachyglossoides jimenoi García & Morffe gen. nov. sp. nov. (Fig. 1A-E, Fig. 2A-D, Fig. 3A-H)

Type material: ♀ holotype, Cuba, Mayabeque province, San José de Las Lajas, La Jaula, 82°06′00″N, 23°01′00″W; in *Spirobollelus* sp.; 18/II/2013; J. Morffe, N. García & M.A. Olcha coll.; CZACC 11.4715. Paratypes 8 ♀♀, same data as holotype, CZACC 11.4716-11.4723; 3 ♂♂, same data as holotype, CZACC 11.4724-11.4726; ♀, same data as holotype, CHIOC; ♂, same data as holotype, CHIOC; ♀, same data as holotype, RIT 835; ♂, same data as holotype, RIT 836.

#### Measurements

Holotype (female) a = 22.39, b = 16.12, c = 26.87, V% = 62.03, total length = 4.030, maximum body width = 0.180, corpus length = 0.148, isthmus length = 0.050, diameter of basal bulb = 0.070, total length of oesophagus = 0.250, nerve ring to anterior end = 0.198, excretory pore to anterior end = 0.210, vulva to posterior end = 1.530, vulval flap length = 0.048, tail length = 0.150, eggs = 0.105-0.108 × 0.078  $(0.106 \pm 0.001 \times 0.078, n = 3)$ .

Paratypes (females) (n = 10) a = 21.89-30.75 $(24.18 \pm 2.60, n = 10), b = 13.91-16.77 (15.34 \pm 0.93,$ n = 10, c = 18.94-35.20 (25.08 ± 4.75, n = 9), V% = 59.66-66.57 (63.62 ± 2.19, n = 10), total length  $= 3.270-4.080 (3.690 \pm 0.256, n = 10), maximum$ body width =  $0.120-0.180 (0.154 \pm 0.018, n = 10)$ , corpus length = 0.130-0.163 ( $0.143 \pm 0.009$ , n = 10), isthmus length = 0.030-0.043 (0.037 ± 0.004, n = 10, diameter of basal bulb = 0.070-0.083  $(0.076 \pm 0.004, n = 10)$ , total length of oesophagus  $= 0.225-0.255 (0.241 \pm 0.009, n = 10)$ , nerve ring to anterior end = 0.175 (n = 1), excretory pore to anterior end = 0.175-0.188 ( $0.182 \pm 0.006$ , n = 6), vulva to posterior end =  $1.120-1.490 (1.343 \pm 0.129, n = 10)$ , vulval flap length = 0.038-0.050 (0.044 ± 0.006, n = 6), tail length = 0.100-0.180 (0.151 ± 0.024, n = 9), eggs = 0.080-0.108 × 0.065-0.075  $(0.094 \pm 0.008 \times 0.071 \pm 0.004, n = 19).$ 

Paratypes (males) (n = 5) a = 20.21-23.58 (22.32  $\pm$  1.40, n = 5), b = 12.00-13.73 (12.89  $\pm$  0.63, n = 5), c = 35.61-49.22 (41.77  $\pm$  5.29, n = 5), total length = 2.760-3.020 (2.892  $\pm$  0.120, n = 5), maximum body width = 0.120-0.140 (0.130  $\pm$  0.010, n = 5), corpus length = 0.130-0.140 (0.136  $\pm$  0.004, n = 5), isthmus length = 0.035-0.038 (0.037  $\pm$  0.001, n = 5),

diameter of basal bulb = 0.070-0.075 ( $0.073 \pm 0.003$ , n = 5), total length of oesophagus = 0.215-0.235 ( $0.225 \pm 0.008$ , n = 5), nerve ring to anterior end = 0.168-0.185 ( $0.177 \pm 0.006$ , n = 5), excretory pore to anterior end = 0.165-0.175 ( $0.170 \pm 0.005$ , n = 3), tail length = 0.058-0.078 ( $0.070 \pm 0.008$ , n = 5), spicule length (on chord) = 0.078-0.093 ( $0.085 \pm 0.005$ , n = 6).

#### Description

General: Cephalic end cuticularized, laterally compressed, consisting of a cephalic cap and a cephalic collar, both sub-terminal in position and ventrally displaced. The cephalic cap and cephalic collar are separated by a groove, wider and deeper in the dorsolateral sector and shallower in the ventrolateral sector. Amphids pore-like, dorsolateral, situated in the groove between the cephalic cap and the cephalic collar. Cephalic papillae very short, two sub-dorsal and two subventral. Cuticle finely annulated, covered with fine, dense microtricha, from the end of the cephalic collar to ca. the midpoint of body. The microtricha near the cephalic collar (dorsally, the ones that extends ca. two body widths further down the body and ventrally, the first two or three rows) are notably longer (ca. three times the length of the shorter microtricha located behind them). The shorter microtricha are as longer as the annuli (from ca. 1 µm at the anterior part of body to ca. 0.5 µm at midbody) and are arranged in alternate rows. Oral aperture sub-terminal, ventrally displaced, moderately elongated in a dorsoventral direction, its dorsal part slightly reduced and the ventral part with concave margin. Jaw-like apparatus modified as rasps, covered with very small spines arranged in opposite rows. Ventral rasp hypertrophied, forming a tongue-like structure inserted between two sub-dorsal rasps, not hypertrophied and embedded in the musculature of the oesophageal corpus. Pharynx with a hypertrophied corpus, very muscular and robust. Isthmus well developed, sub-cylindrical, almost as wide as the corpus, similar in length to the basal bulb. Basal bulb not hypertrophied, sub-spherical, cuticularised valve-plate present, cardias wide and short, projected into the intestine lumen. Intestine simple, sub-rectilinear, its fore region barely inflated. Nerve ring not evident, at level of the isthmus. Excretory pore ventrally situated, at level of the isthmus.

Female: Vulva displaced to the posterior half of body. Anterior lip of vulva hypertrophied, forming a short lobe, slightly inflated, triangular in shape. Posterior lip of vulva with recurved margin. Vagina compara-

tively short and muscular. Vaginal diverticulum absent. Genital tract monodelphic-prodelphic, Type 1 after Adamson (1987). Ovary reflexed at *ca.* five body-widths posterior to the basal bulb. Distal flexure of the ovary very long, *ca.* 14 body-widths long. Oöcytes of the distal part of the ovary in two rows, in the rest of the ovary they are arranged in a single row. The ovary continues with a uterus that leads in the vagina. Eggs ellipsoidal in shape, shell smooth and more or less thin. A number of 10-41 eggs at a time in the uterus. Tail comparatively short, attenuate, its tip rounded.

Male: Body shorter and less robust than females. Monorchic. Testis outstretched. Spicules isomorphic, almost isometric. Shafts ventrally arcuate. Capitulum not evident. Cuticle of the shafts smooth, not ornamented. Posterior end of body ventrally curved. Caudal alae well developed, sub-ventral, not lobate, extending from the tail tip to ca. 1.5 body-widths long. The posterior end of the caudal alae ending in a rounded lobe that extends to a short distance posterior to the tail tip. Nineteen copulatory papillae arranged as a median precloacal papilla and nine pairs situated on the caudal alae. Five pre-cloacal pairs, the first near the level of the cloaca; second and third pairs close to the first and equidistant from each other; fourth pair situated at ca. twice of the distance between the second and third pairs; fifth pair located at the end of the caudal alae, equidistant from both precedent pairs. A single pre-cloacal, median papilla located near the level of the third pre-cloacal pair. Post-cloacal papillae arranged in four equidistant pairs. Tail conical, short, its tip rounded, more or less digitiform.

Discussion: Trachyglossoides jimenoi gen. nov. sp. nov. is very close to Trachyglossus by the presence of a cephalic cap laterally compressed, the oral aperture dorsoventrally elongated, the plate of the jaw apparatus hypertrophied as a tongue-like structure, the corpus and isthmus also hypertrophied, the anterior lip of vulva well developed and lobed and the males with caudal alae. It differs from Trachyglossus by the slight compression of the cephalic cap and the comparatively reduced elongation of the oral aperture vs. the more marked of Trachyglossus (Hunt, 2002). The tonguelike structure is ventral in the new genus in opposition to the dorsal of Trachyglossus and shows less development. In addition, the vulval flap of the females is reduced to a short, triangular lobe in Trachyglossoides instead of the more extended lobe of the Congolese genus. The caudal alae are not lobed and more extended vs. lobed and shorter and the number and arrangement of the copulatory papillae also differ: 19 in *Trachyglossoides* and 23 in *Trachyglossus*.

Trachyglossoides jimenoi gen. nov. sp. nov. is the only monogonant xustrostomatid. The degree of the lateral compression of the cephalic cap as well as the dorso-ventral elongation of the oral aperture is less marked than in other genera in the family. It differs from Xustrostoma and Obainia by presenting the oesophageal corpus and the isthmus hypertrophied instead of the bulb as occurs in the latter genera (Adamson, 1983; Adamson & Van Waerebeke, 1984). Also, it can be segregated by having a tongue-like plate in the jaw apparatus, a vulval flap, caudal alae in the males and a different number and arrangement of the copulatory papillae.

Both *Trachyglossoides* gen. nov. and *Zalophora* present the corpus hypertrophied but differ by the presence of an evident isthmus in *Trachyglossoides* gen. nov. in opposition to the corpus amalgamated with the basal bulb (also hypertrophied) in *Zalophora*. *Trachyglossoides* gen. nov. shows a jaw apparatus consisting of a ventral tongue-like rasp with two sub-dorsal rasps. Otherwise, *Zalophora* present three cuticularized plates in the anterior part of the oesophagus, with their margins serrated (Hunt, 1994). The distinctive vulval flap and caudal alae of *Trachyglossoides* gen. nov. are absent in *Zalophora*.

Type locality: La Jaula, San José de Las Lajas, Mayabeque province, Cuba.

*Type host: Spirobollelus* sp. (Diplopoda: Spirobolida: Spirobollelidae).

Site: Hind gut.

Etymology: Species dedicated to the distinguished Cuban naturalist Francisco Jimeno y Fuentes (1825-1891), pioneer of the studies on Natural History in Cuba and founder of the first Cuban collections.

### Genus Zalophora Hunt, 1994

# Zalophora dominicana García & Morffe sp. nov. (Fig. 4A-D, Fig. 5A-D)

*Type material:* ♀ holotype, Dominican Republic, Santiago province, Río de los Negros, 19°07'05.6"N, 71°00'17.9"W; in unidentified Spirobolida; IV/1999; L.F. de Armas coll.; CZACC 11.4727. Paratypes 2♀♀, same data as holotype, CZACC 11.4728-11.4729.

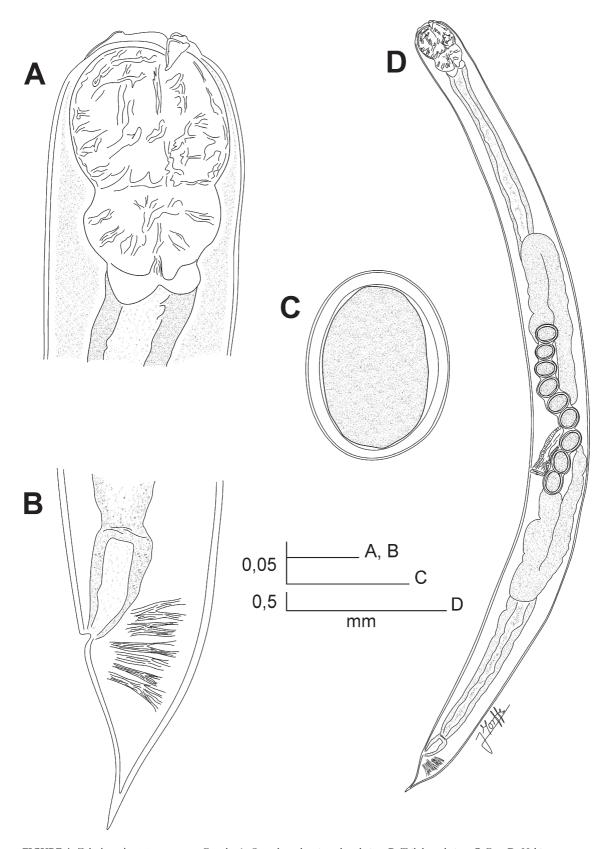
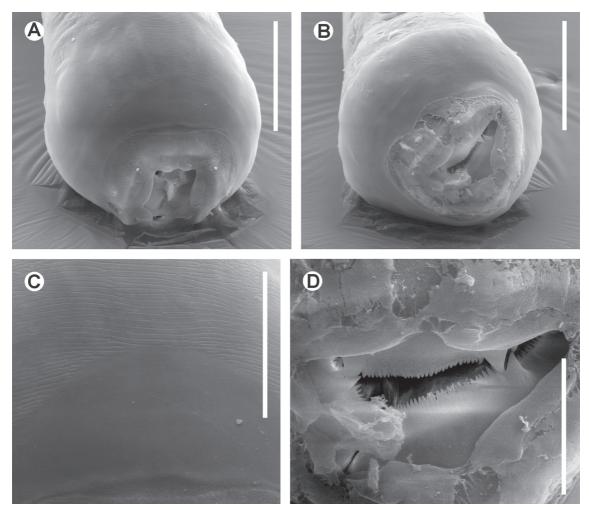


FIGURE 4: Zalophora dominicana sp. nov. Female. A. Oesophageal region, dorsal view. B. Tail, lateral view. C. Egg. D. Habitus, ventro-lateral view.



**FIGURE 5:** *Zalophora dominicana* sp. nov. SEM images. Female. **A.** Cephalic end, frontal view. **B.** Cephalic end, *en face* view. **C.** Cuticular striae in the junction with the cephalic collar. **D.** Oral aperture. Scale bars: A, B = 0.050 mm. C = 0.025 mm. D = 0.020 mm.

#### Measurements

Holotype (female) a = 14.25, b = 16.25, c = 19.62, V% = 58.65, total length = 2.600, maximum body width = 0.183, corpus length = 0.100, diameter of basal bulb = 0.093, total length of oesophagus = 0.160, excretory pore to anterior end = 0.080, vulva to posterior end = 1.075, tail length = 0.133, eggs = 0.070-0.078 × 0.055-0.063  $(0.074 \pm 0.003 \times 0.059 \pm 0.003, n = 4)$ .

Paratypes (females) (n = 2) a = 14.00-14.09 ( $14.05 \pm 0.064$ , n = 2), b = 14.76-15.44 ( $15.10 \pm 0.48$ , n = 2), c = 18.24-21.00 ( $19.62 \pm 1.95$ , n = 2), V% = 50.48-59.14 ( $54.81 \pm 6.13$ , n = 2), total length = 2.325-2.625 ( $2.475 \pm 0.212$ , n = 2), maximum body width = 0.165-0.188 ( $0.176 \pm 0.016$ , n = 2), corpus length = 0.103-0.113 ( $0.108 \pm 0.007$ , n = 2), diameter of basal bulb = 0.093-0.095 ( $0.094 \pm 0.002$ , n = 2), total

length of oesophagus = 0.158-0.170 ( $0.164 \pm 0.009$ , n = 2), excretory pore to anterior end = 0.098 (n = 2), vulva to posterior end = 0.950-1.300 ( $1.125 \pm 0.247$ , n = 2), tail length = 0.125-0.128 ( $0.126 \pm 0.002$ , n = 2), eggs = 0.065- $0.075 \times 0.060$ -0.063 ( $0.071 \pm 0.004 \times 0.061 \pm 0.001$ , n = 5).

### Description

Female body short in length and robust. Anterior end with the globular appearance characteristic of the genus but not too marked. Constriction of the body at the base of oesophagus not evident. Cuticle finely striated from the base of the cephalic collar to the level of the anus. No microtricha or spinelets present. Cephalic cap with four small papillae, two sub-dorsal and two sub-lateral. Oral aperture triangular in shape, dorsoventrally elongated. Two dorsoventral, barely developed cuticular crests present on each side of the

oral aperture. Each crest presents a wide cleavage at its midpoint and two conical papillae, one dorsal and one lateral. Anterior end of pharynx with three cuticularized jaw-like structures, the dorsal shorter than the two sub-ventral, similar in length. Each jaw presents small, pointed and triangular teeth. Near each angle of the jaws there is one teeth notably larger, pointed and triangular. Between the two larger teeth of the sub-ventral jaws there are ca. 31 small teeth. Between the large teeth and the end of the sub-ventral jaws there seems to be ca. 10 small teeth (the crests make the observation difficult). Pharynx with the corpus and the bulb hypertrophied, notably rounded and muscular, inflated. The bulb is amalgamated to the corpus with half of its diameter. Intestine simple, subrectilinear, its fore region not inflated. Rectum short, anus not prominent. Excretory pore at level of the base of corpus. Vulva as a ventral median transverse slit, its lips not prominent, slightly displaced to the posterior half of body. Genital tract didelphic-amphidelphic, Type 1 after Adamson (1987). Eggs spherical, shell thin and smooth. A number of 10-19 eggs at a time in the uterus. Tail comparatively short and subulate. Male unknown.

Etymology: Z. dominicana sp. nov. differs from the other species in the genus by its shorter body and the crests less developed. Is similar in length to Z. repentina from Cuba (2.325-2.625 vs. 2.575-2.875), but differs by having reduced crests and the cephalic end less globular in shape. Z. repentina presents more evident crests and cephalic dilation (García & Morffe, 2010). Moreover, the tail of Z. dominicana sp. nov. is subulate and shorter ( $c = 18.24-21.00 \ vs. 12.88-14.38$ ), lacking the median caudal dilatation characteristic of the Cuban species, which presents a more attenuate and conical tail. Z. dominicana sp. nov. is slender than Z. repentina ( $a = 14.00-14.25 \ vs. a = 12.50-12.88$ ) and the vulva is more anterior (V% = 50.48-59.14 vs. 61.17).

Z. dominicana sp. nov. can be segregated from Z. hepialum and Z. nemophila (Hunt, 1994; Hunt & Moore, 1999) by its crests reduced and not lobate and the shorter body (2.325-2.625 vs. 5.590-6.030 and 7.100-7.590, respectively). Also, it differs in the rest of the measurements, always shorter in Z. dominicana sp. nov. as well as in the shape of the cephalic end, less inflated.

*Z. deinostoma* presents short crests (Hunt & Moore, 1999), as in *Z. dominicana* sp. nov., but differs in the longer tail (c = 8.10-11.10 vs. 18.24-21.00). On the other hand, *Z. deinostoma* (being the shortest of the Australasian species of the genus) is nearly twice

the length of the Dominican species (3.380-4.910 *vs.* 2.325-2.625) as it happens with the rest of the measurements.

*Type locality:* Río de los Negros, Santiago province, Dominican Republic.

Type host: Unidentified Spirobolida (Diplopoda).

Site: Hind gut.

*Etymology:* Specific epithet referred to the Spanish name of the country of the type locality: the República Dominicana.

#### RESUMEN

Se describe un nuevo género y dos nuevas especies de xustrostomátidos (Nematoda: Xustrostomatidae) parásitos de diplópodos antillanos. Trachyglossoides jimenoi gen. nov. sp. nov., es muy similar a Trachyglossus Hunt, 2002, pero difiere del mismo por ser el único xustrostomátido con sistema reproductor femenino monodelfo. Este constituye el segundo registro de la familia para Cuba. Zalophora dominicana sp. nov., de República Dominicana es muy similar a Z. repentina García et Morffe, 2010. Se caracteriza por presentar las crestas cefálicas reducidas y el extremo cefálico poco globoso. Este es el primer registro de la familia y el género para el país.

Palabras-Clave: *Trachyglossoides* gen. nov. sp. nov.; *Zalophora*; Diplopoda; Cuba; Hispaniola.

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