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Morphological and molecular characterization of *Buzionema lutgardae* n. sp. (Nematoda: Oxyuridomorpha: Thelastomatidae) from the cockroach *Byrsotria* sp. (Blattaria: Blaberidae) in Cuba

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Abstract

Buzionema lutgardae **n. sp.** (Nematoda: Oxyuridomorpha: Thelastomatidae) is described from the cockroach *Byrsotria* sp. (Blattaria: Blaberidae), endemic to Cuba. Females of *B. lutgardae* **n. sp.** are shorter than those of *B. validum* Kloss, 1966 (1600–2150 µm *vs.* 3131–3378 µm), but the oesophagus is comparatively longer (b = 2.96–3.77 *vs.* 4.65–4.87). The lateral alae of the new species extend from *ca.* the midpoint of the cylindrical part of the procorpus to the level of the anus in contrast to the base of the basal bulb to the level of the anus in *B. validum*. The males of *B. lutgardae* **n. sp.** are shorter than those of *B. validum* (780–940 µm *vs.* 1177–1423 µm) and their lateral alae end at some distance before the cloaca instead the level of the cloaca in *B. validum*. The phylogeny of *B. lutgardae* **n. sp.** is inferred by the D2-D3 domains of the 28S rDNA. *B. lutgardae* **n. sp.** and *B. validum* form a monophyletic clade with strong nodal support, as sister-group of the genus *Leidynema* Schwenck in Travassos, 1929.

Key words: *Buzionema*, new species, phylogeny, SEM, West Indies, 18S rDNA, 28S rDNA

Introduction

The nematode family Thelastomatidae (Oxyuridomorpha: Thelastomatoidea) comprises a large group of monoxenous parasites from arthropods (cockroaches, crickets, beetles and millipedes) and earthworms (Adamson & Van Waerebeke 1992). Among the parasites from cockroaches, the monotypic genus *Buzionema* Kloss, 1966 is quite characteristic in having females with the oral opening surrounded by three prominent lips, a notably fusiform procorpus, a long isthmus and a didelphic genital tract, with the vulva in the posterior half of the body (Kloss 1966; Adamson & Van Waerebeke 1992).

The genus was first described from Brazil, with *B. validum* Kloss, 1966 as its type and only species, in *Eublaberus* sp. (Blattaria: Blaberidae) (Kloss 1966). More recently, García & Coy (1998) found female specimens of a *Buzionema* sp. from the blaberid cockroach *Byrsotria* sp. (Blattaria: Blaberidae), which is a genus endemic to Cuba. The specimens were almost twice as short as *B. validum* but morphologically identical, resulting in the authors assigning them to *B. validum* and considering their differences as intraspecific variations. Carreno & Tuhela (2011) also found nematodes attributable to *B. validum* in the blaberid *Archimandrita tesselata* Rehn, 1903 from Costa Rica and offered the first SEM studies on the species.

In the present work the specimens of *Buzionema* (both sexes) from Cuban *Byrsotria*, were studied using light microscopy and SEM. The morphological and meristic differences with *B. validum* support the proposal of these specimens as a new species. Also, the morphology of the oesophagus of the genus is analyzed and the phylogenetic relationship of both *B. validum* and the new species herein described was inferred by means of partial 28S rDNA sequences.

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Materials and methods

Processing of hosts and nematodes

Specimens of *Byrsotria* sp. were collected by hand from rotting logs in the Reserva Ecológica "Limones-Tuabaquey", Sierra de Cubitas, Camagüey province, Cuba. Cockroaches were maintained alive in plastic jars with moistened wood chips until arrival at the laboratory.

Hosts were killed with vapours of ethyl-acetate and immediately dissected by making longitudinal incisions in both abdominal pleural membranes. Intestines were withdrawn from the body and dissected in Petri dishes with 0.9% NaCl physiological solution. Nematodes found were killed with hot 0.9% NaCl (70ºC) and fixed in 70% ethanol. Specimens for molecular studies were directly fixed in 96% ethanol. For light microscopy studies the nematodes were transferred to anhydrous glycerin via slow evaporation (Seinhorst 1959) and mounted in the same medium. The edges of the coverslips were sealed with wax rings.

The specimens of *B. validum* used for molecular studies were recovered from an *A. tesselata* collected at the Parque Nacional de Santa Rosa, Área de Conservación Guanacaste, Costa Rica.

Studied material has been deposited in the Colección Helmintológica de las Colecciones Zoológicas, Instituto de Ecología y Sistemática (CZACC), Havana, Cuba.

Morphological and morphometric studies

Measurements were taken with the aid of a calibrated eyepiece micrometer. De Man's indices a, b, c and V% were calculated. Variables are shown as the range followed by the mean plus standard deviation in parentheses, and the number of measurements is also given. Micrographs were generated with an AxioCam digital camera attached to a Carl Zeiss Axioskop 2 Plus compound microscope. Line drawings were made on the basis of micrographs using a Wacom Intuos Art drawing tablet with Adobe Illustrator CS6 and Adobe Photoshop CS6. Scale bars of all figures are given in micrometers.

SEM studies

Nematodes were post-fixed overnight with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 6.0) and one hour in 2% osmium tetroxide. They were dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, 95%, 100% × 2, 30 min in each). Prior to freeze drying in an ES-2030 freeze dryer (Hitachi, Japan) they were transferred to a mix of absolute ethanol/t-butanol (1:1, v/v) and then to pure t-butanol. Nematodes were then mounted on double sided aluminum tape on a stage, sputter coated with gold using an E-1030 sputter coater (Hitachi, Japan), and observed with a JSM-6510LA scanning electron microscope (JEOL, Japan) at 15 kV accelerating voltage.

DNA extraction, gene amplification and sequencing

Genomic DNA was extracted from single individuals with the DNeasy® Blood & Tissue (Qiagen, USA) and DNAzol (Molecular Research Center Inc., Cincinnati, Ohio, USA) kits, following manufacturer´s instructions. The D2-D3 segment of the large ribosomal subunit ribosomal RNA gene (D2-D3 28S rDNA) was amplified with the primers D2A (5´-ACA AGT ACC GTG AGG GAA AGT TG-3´) and D3B (5´-TCG GAA GGA ACC AGC TAC TA-3´) (Nunn 1992) for the specimens of the new species and with the primers #391 (5´-AGC GGA GGA AAA GAA ACT AA-3´) and #501 (5´-TCG GAA GGA ACC AGC TAC TA-3´) (Nadler *et al*. 2006) for *B. validum*. The small ribosomal subunit ribosomal RNA gene (18S rDNA) of the new species was amplified with the primer set SSUF07_For (5´-AAA GAT TAA GCC ATG CAT G-3´) and SSUR26_Rev (5´-CAT TCT TGG CAA ATG CTT TCG-3´) (Blaxter *et al.* 1998). In the case of *B. validum*, the 18S rDNA was amplified in two fragments using primers 47 (5´-CCC GAT TGA TTC TGT CGG C-3´) and 112 (5´-GGC TGC TGG CAC CAG ACT TGC-3´) with the overlapping primer set 135 (5´-CGG AGA GGG AGC CTG AGA AAC GGC-3´) and 136 (5´-TGA TCC TTC TGC AGG TTC ACC TAC-3´) (Carreno & Nadler 2003).

PCR reactions for the new species were performed in a total volume of 20 µL with the KOD FX Neo DNA polymerase (Toyobo, Osaka, Japan). PCR cycling parameters consisted of an initial denaturation at 94ºC for 2 min followed by 35 cycles of 98ºC for 10 s, 50ºC for 30 s (for the 28S and 18S rDNA) and 68ºC for 30 s and a final extension step of 68ºC for 5 min. The results of the PCR were checked by agarose gel electrophoresis, visualizing the DNA bands with ethidium bromide. PCR products were excised from the gel and purified with the NucleoSpin Gel and PCR Clean Up kit (Macherey-Nagel, Düren, Germany), following the manufacturer´s protocol. Samples

were submitted to Hokkaido System Science Co., Sapporo, Japan. The original PCR primers were used to sequence both strands.

PCR reactions for *B. validum* from Costa Rica were performed in a total volume of 25 µL with the Finnzymes DyNAzyme EXT polymerase (MJ Research, Watertown, Massachusetts, USA). PCR cycling parameters consisted of an initial denaturation at 94ºC for 3 min followed by 35 cycles of 94ºC for 30 s, 54ºC, 58ºC or 60ºC for 30 s (for the primer sets 391/501, 47/112 and 135/136, respectively) and 72ºC for 1 min and a final extension step of 72ºC for 7 min. PCR products were treated with the ExoSAP-IT kit (USB Products, Affymetrix Inc., Cleveland, Ohio, USA), and sequenced using a 3730 DNA Analyzer from Applied Biosystems Inc. and BigDye Terminator Cycle Sequencing chemistry at the Plant-Microbe Genomics Facility, Ohio State University, Columbus, Ohio.

Raw sequences were manually edited with Sequencher 3.0 and 4.1.4 (http://genecodes.com) and deposited in GenBank NCBI (http://www.ncbi.nlm.nih.gov/genbank/). The accession numbers of the new species of *Buzionema* are MW030186 and MW030190 for the 28S rDNA and the 18S rDNA, respectively. For *B. validum* the accession numbers are MW030187 and MW030191 for the 28S rDNA and the 18S rDNA, respectively.

Phylogenetic analysis

Several sequences of thelastomatoid species (Hystrignathidae, Thelastomatidae and Travassosinematidae) were selected from GenBank for the phylogenetic analyses (accession numbers in the phylogram). *Hystrignathus* sp. (Hystrignathidae), *Cameronia multiovata* (Thelastomatidae) and three species of *Travassosinema* (Travassosinematidae) were used as the outgroup taxa.

Due to a paucity of 18S rDNA sequences for representative species of the Thelastomatoidea, the phylogenetic analyses were carried out using only 28S rDNA sequences. Multiple sequence alignments were made using MUSCLE (Edgar 2004) with the default parameters as implemented in MEGA6 (Tamura *et al.* 2013). Poorly aligned regions and gaps were automatically removed with trimAl (Capella-Gutiérrez *et al.* 2009). MEGA6 was also used to identify the optimal model of evolution for the data set (GTR+G+I) following the Akaike Information Criterion (AIC) and to construct phylogenetic trees based on the Maximum Likelihood (ML) method. Nodal support was inferred by bootstrap analysis using 1,000 iterations. Bayesian Inference analysis (BI) was performed with MrBayes v3.2.6 (Ronquist *et al.* 2012), with 3×10^6 generations, sampling every 100 generations and discarding the first 25% of the sample runs as burn-in. The convergence statistics of the BI process stationarity and the number of burn-in trees were checked using Tracer v1.5 (Rambaut *et al.* 2003).

Systematics

Family Thelastomatidae Travassos, 1920

Buzionema Kloss, 1966

Buzionema lutgardae n. sp.

Fig. 1 A–E, Fig. 2 A–F, Fig. 3 A–H

Type material. Holotype: ♀, Cuba, Camagüey province, Sierra de Cubitas, Reserva Ecológica "Limones-Tuabaquey"; in *Byrsotria* sp.; XII/2015; J. Morffe, N. García coll.; CZACC 11.7339. Paratypes: 11♀♀, same data as the holotype; CZACC 11.7340–11.7350. 11♂♂, same data as the holotype; CZACC 11.7351–11.7361.

Description. Female. Body robust, markedly spindle-shaped, with the maximum body-width at level of the midpoint of the oesophagus. Cervical cuticle unarmed. Cuticle with annuli from the base of the lips to *ca.* level of anus. Annuli visible from base of lips to level of vulva (*ca*. 12 µm); annuli posterior to vulva less marked and slightly wider (*ca*. 15 µm). Lateral alae well-developed from *ca.* the midpoint of cylindrical part of procorpus to level of anus. Oral aperture triangular, with isometric sides, one dorsal and two sub-ventral, lined by a cuticular band. Cuticle at midpoint of band extending and forming a triangular flap. Three large, isomorphic and isometric lips surrounding mouth, arranged as one dorsal and two sub-ventral lips, coinciding with sides of oral opening. Lips sub-triangular, with vertices rounded and edges convex. Amphids lateral in position, pore-like, located in cuticular prominences at distal vertex of each sub-ventral lip. Buccal capsule short, wide, notably cuticularized, its lumen

triradiate. Several drop-like, dark cells surrounding buccal capsule. Oesophagus consisting of a corpus with swollen, fusiform anterior muscular part, and slender cylindrical posterior part, its diameter similar to that of isthmus. Junction of cylindrical part of corpus with isthmus barely evident. Length of anterior part of corpus *ca.* 1.5 posterior parts-length. Basal bulb rounded, valve-plate well developed. Intestine simple, sub-rectilinear, its anterior region dilated. Rectum short. Anus a crescent-like slit, its convex side anteriorly directed. Nerve ring encircling procorpus at level of its anterior part, a short distance behind its junction with buccal capsule. Excretory pore ventral, located at *ca.* last third of cylindrical part of corpus. Vulva a ventro-median transverse slit, displaced to posterior half of body. *Vagina vera* muscular, anteriorly directed. Genital tract didelphic-amphidelphic. Both ovaries reflexed. Oocytes in single rows. Eggs broadly oval, with thin and smooth shell. Tail comparatively long *ca.* one third of the body length, filiform, subulate, ending in fine tip.

Male. Body smaller and less robust than that of females; posterior end ventrally curved. Anterior end bluntly rounded. Lateral alae well-developed from *ca.* base of basal bulb to a distance (*ca.* 150 µm) before posterior end. Cephalic cap *ca.* 35 µm, conical, truncate, with smooth cuticle, without distinctive papillar structures. Cuticle unarmed, with conspicuous, wide annuli from base of cephalic cap to level of first pair of pre-cloacal papillae. Annuli of *ca.* 8 µm at level of first portion of corpus and *ca.* 5 µm at level of first pre-cloacal pair of papillae. Mouth dorso-ventrally orientated, sub-rectangular in shape, with the shorter sides dorsal and ventral and the longer lateral. Each lateral side of mouth presenting a sub-triangular projection, creating a bilobed appearance. Amphids pore-like, located in each lateral projection of mouth. Buccal capsule short. Several drop-like, long dark cells originating near level of buccal capsule and extending to about half of distance between base of buccal capsule and nerve ring. Oesophagus consisting of muscular, sub-cylindrical corpus, slightly diminishing its diameter toward the cylindrical isthmus, its anterior end slightly expanded. Basal bulb rounded, valve-plate well developed. Intestine simple, sub-rectilinear, its anterior region dilated. Nerve ring encircling corpus at *ca.* 60% of its length. Excretory pore ventral, located at level of isthmus. Monorchic. Testis ventral, reflexed at *ca.* 50 µm posterior to basal bulb, distal flexure *ca.* a body-width length. *Vas deferens* divided into three regions: an anterior region filled with rod-like spermatids, a median region with rounded cells and a posterior region with longer polygonal cells, increasing slightly in diameter and then gradually tapering towards its junction with cloaca. Regions immediately anterior and posterior to cloaca with wrinkled cuticle, conferring a rough appearance. Short, posteriorly directed cuticular flap present at anterior lip of cloaca. Two digitiform cuticular projections present just posterior to cloaca, one on each side of cloaca. Spicule absent. Six copulatory papillae present, four pre-cloacal and two post-cloacal. First pre-cloacal pair ventromedian, papillae close to each other located at *ca*. 13 µm from cloaca. Second pair of pre-cloacal papillae formed by larger, rounded and prominent papillae, sub-lateral in position, located at short distance (*ca.* 5 µm) posterior to first pre-cloacal pair. Sensilla of each papilla of second pre-cloacal pair surrounded by eight small protuberances. One pair of post-cloacal papillae present: a ventral pair of minute papillae near tail tip (*ca.* 7 µm). Tail short, conical, ending in sharp tip.

Differential diagnosis. The females of *B. lutgardae* **n. sp.** present the body notably shorter than that of *B. validum* (1600–2150 µm *vs.* 3131–3378 µm), but the oesophagus is comparatively longer (b = 2.96–3.77 *vs.* 4.65–4.87). The lateral alae of *B. validum* extend from the base of the basal bulb to the level of the anus, in contrast to *B. lutgardae* **n. sp.**, with lateral alae from *ca.* the midpoint of the cylindrical part of the procorpus to the level of the anus. The larger diameter of the eggs is bigger in *B. validum* (81–88 µm *vs.* 63–75 µm).

The males of *B. lutgardae* **n. sp.** are shorter than those of *B. validum* (780–940 µm *vs.* 1177–1423 µm). In the new species the origin of the lateral alae near the level of the basal bulb is similar to *B. validum*, but in the case of the latter species they end at the level of the cloaca (Kloss 1966), rather than some distance (*ca*. 150 µm) before the cloaca.

Type locality. Reserva Ecológica "Limones-Tuabaquey", Sierra de Cubitas, Camagüey province, Cuba.

Other localities. Los Pinos beach, Key Paredón Grande, Sabana-Camagüey archipelago, Camagüey province, Cuba. Las Coloradas beach, Key Coco, Sabana-Camagüey archipelago, Ciego de Ávila province, Cuba (García & Coy 1998).

Type host. *Byrsotria* sp. (Insecta: Blattaria: Blaberidae).

Site. Hind gut.

Etymology. Specific epithet dedicated to Dr. Lutgarda González Géigel (1948–2006) eminent Cuban botanist. This is a homage to an excellent human being and professor of the senior and second authors as well as several generations of Cuban biologists.

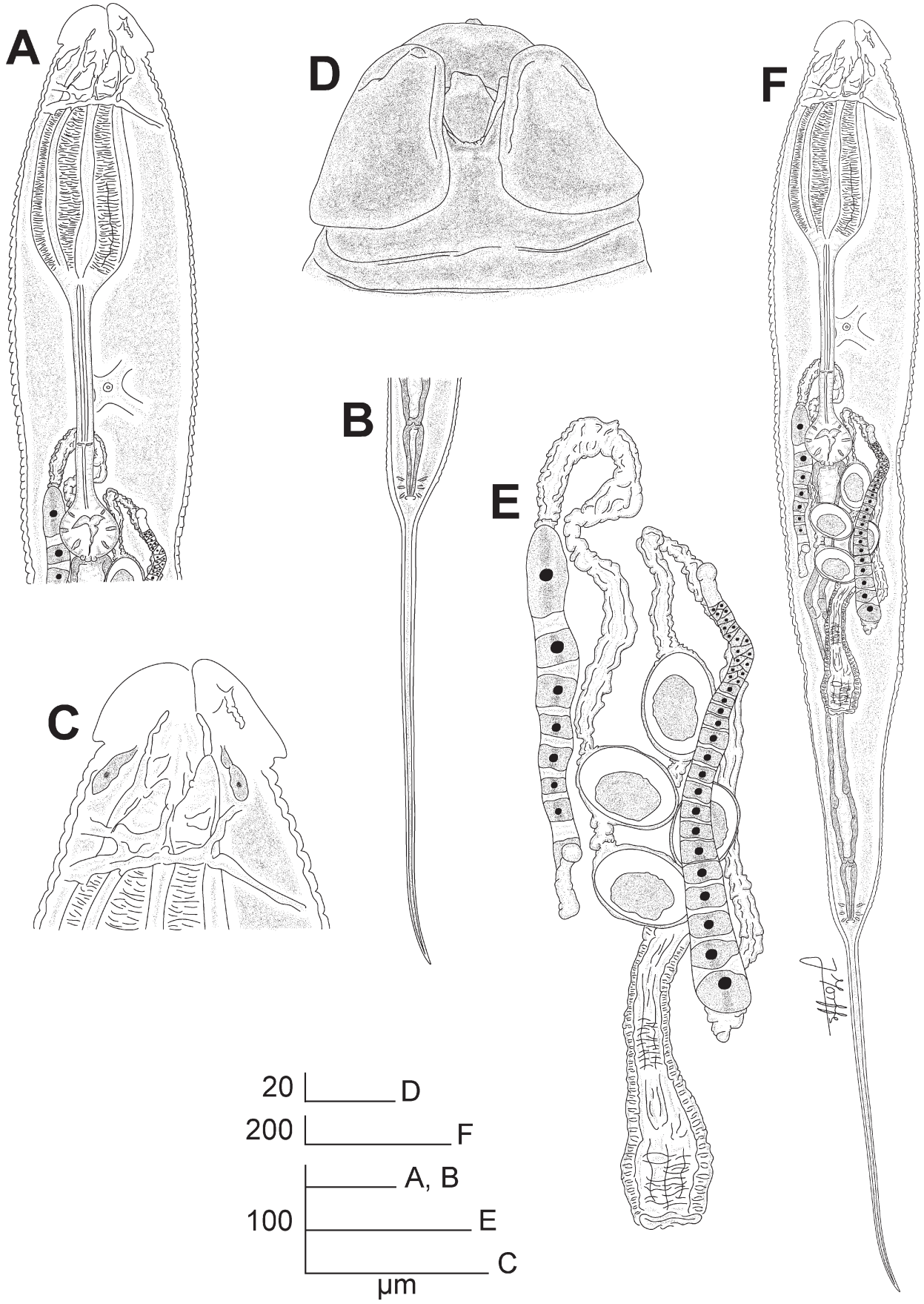

FIGURE 1. *Buzionema lutgardae* **n. sp.** Female. A. Oesophageal region, ental view. B. Tail, ventral view. C. Cephalic end, optical section. D. Cephalic end (reconstructed from SEM images). E. Genital tract, ventral view. F. Habitus, ventral view.

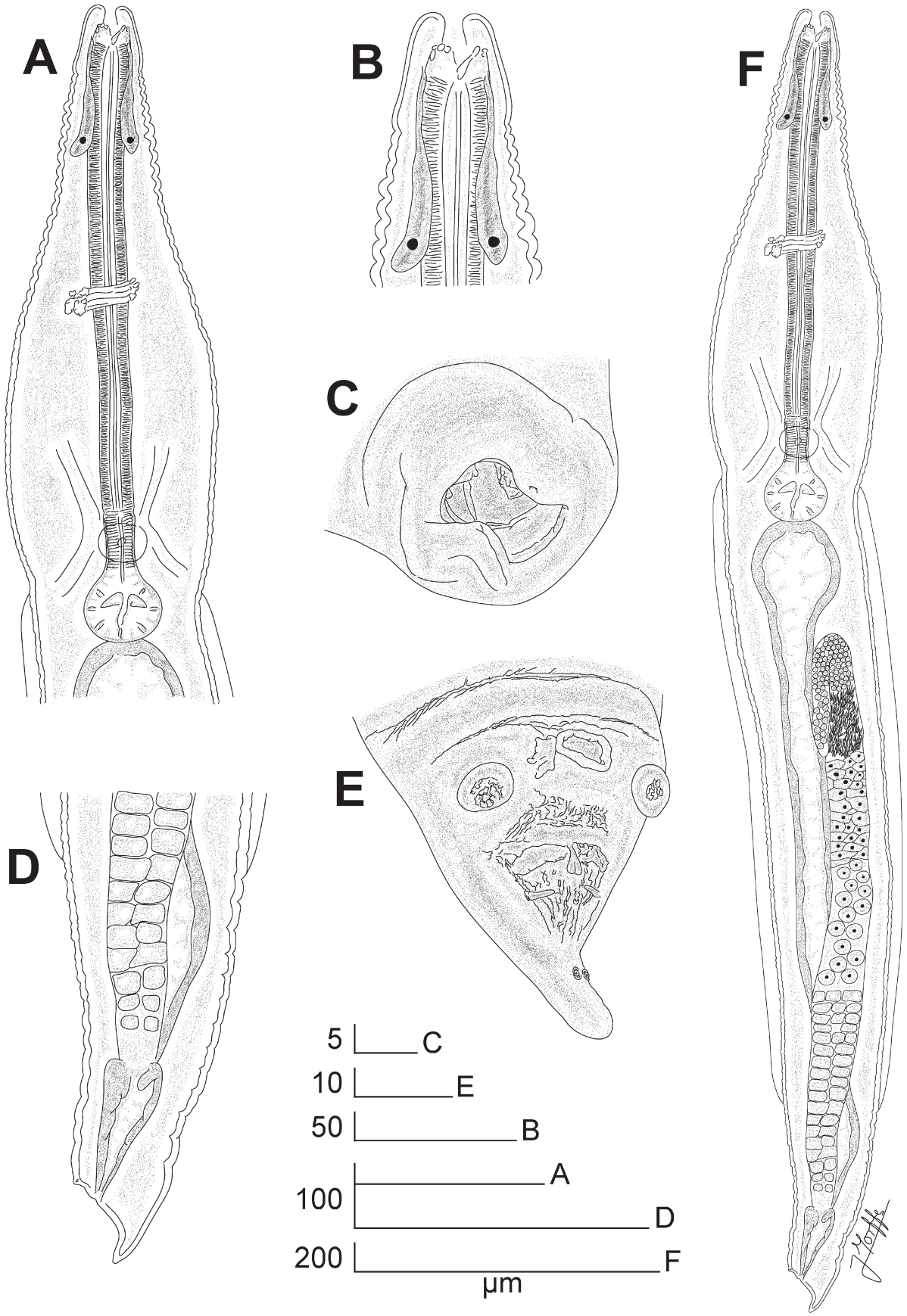

FIGURE 2. *Buzionema lutgardae* **n. sp.** Male. A. Oesophageal region, ventral view. B. Cephalic end, optical section. C. Cephalic end, *en face* view (reconstructed from SEM images). D. Tail, ventro-lateral view. E. Tail end, ventral view (reconstructed from SEM images). F. Habitus, ventral view.

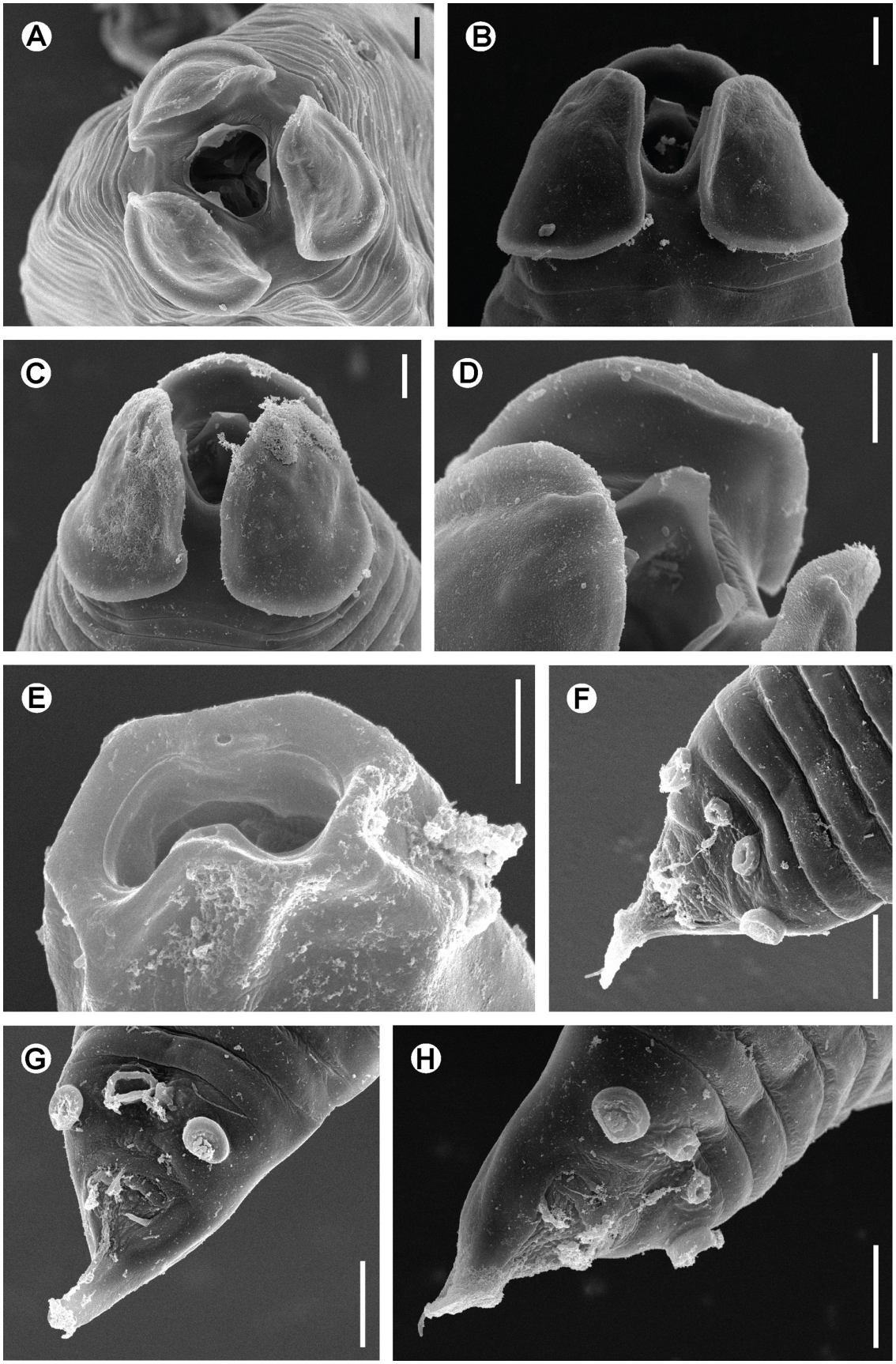

FIGURE 3. *Buzionema lutgardae* **n. sp.**, SEM images. Female. A. Cephalic end, *en face* view. B. Cephalic end, lateral view. C. Cephalic end, ventral view. D. Detail of the cephalic end. Male. E. Cephalic end, *en face* view. F. Tail end, ventral view. G, H. Tail end, ventral view. Scale bars: A, B, C, D, F, G, H. 10 µm. E. 5 µm.

TABLE 1. Morphometrics of *Buzionema lutgardae* **n. sp.** (Nematoda: Oxyuridomorpha: Thelastomatidae) from *Byrsotria* sp. (Insecta: Blattaria: Blaberidae) from Reserva Ecológica "Limones-Tuabaquey", Sierra de Cubitas, Camagüey province, Cuba. All of the measurements are given in micrometers.

Character	Females		Males
	Holotype	Paratypes (n = 11)	Paratypes (n = 11)
a	8.45	7.74–9.61 (8.34 ± 0.51, n = 11)	7.91–12.13 (8.68 ± 1.42, n = 8)
b	3.23	2.96–3.77 (3.53 ± 0.26, n = 11)	2.50–3.48 (2.71 ± 0.32, n = 8)
c	2.91	2.88–3.36 (3.15 ± 0.15, n = 11)	29.09–41.78 (34.44 ± 4.42, n = 10)
V%	51.27	49.88–54.38 (52.29 ± 1.40, n = 11)	–
Total length	1775	1600–2150 (1852 ± 230, n = 11)	780–940 (837 ± 41, n = 11)
Maximum width	210	190–270 (223 ± 30, n = 11)	78–108 (98 ± 10, n = 8)
Buccal cavity length	35	25–43 (34 ± 6, n = 11)	8–13 (10 ± 2, n = 10)
Procorpus length	420	390–450 (418 ± 19, n = 11)	188–255 (237 ± 20, n = 9)
Length of the fusiform part of the procorpus	250	250–280 (255 ± 9, n = 11)	–
Length of the cylindrical part of the procorpus	170	140–190 (163 ± 16, n = 11)	–
Isthmus length	60	60–70 (65 ± 3, n = 11)	33–45 (40 ± 5, n = 8)
Length of the cylindrical part of the procorpus plus the isthmus	220	220–240 (230 ± 8, n = 11)	–
Basal bulb diameter	65	63–80 (69 ± 7, n = 11)	35–40 (38 ± 2, n = 8)
Oesophagus length	550	500–600 (551 ± 31, n = 11)	270–333 (313 ± 20, n = 8)
Nerve ring-anterior end	108	75–118 (93 ± 14, n = 11)	143-158 (150 ± 6, n = 9)
Excretory pore-anterior end	440	375–458 (418 ± 37, n = 7)	230–288 (269 ± 16, n = 9)
Vulva-anterior end	910	840–1130 (967 ± 110, n = 11)	–
Tail length	610	500–680 (588 ± 61, n = 11)	20–28 (25 ± 2, n = 10)
Eggs	65×45 (n = 3)	63–75×43–50 (70 ± 4×47 ± 2, n = 16)	–

DNA studies. Two partial sequences of the D2-D3 region of the 28S rDNA were obtained from females of *B. lutgardae* **n. sp.** and *B. validum* (721 bp and 1029 bp, respectively). Both sequences differ in 79 homologous positions in an alignment of 733 bp. In addition, two partial sequences of the 18S rDNA were obtained from females of *B. lutgardae* **n. sp.** and *B. validum* (815 bp and 1652 bp, respectively). The differences between both sequences are two homologous positions (in an alignment of 809 bp).

In both ML and BI phylograms both species of *Buzionema* form a monophyletic clade with strong nodal support, as sister-group of *Leidynema* Schwenck in Travassos, 1929. The clade of *Buzionema* + *Leidynema* is also well-supported.

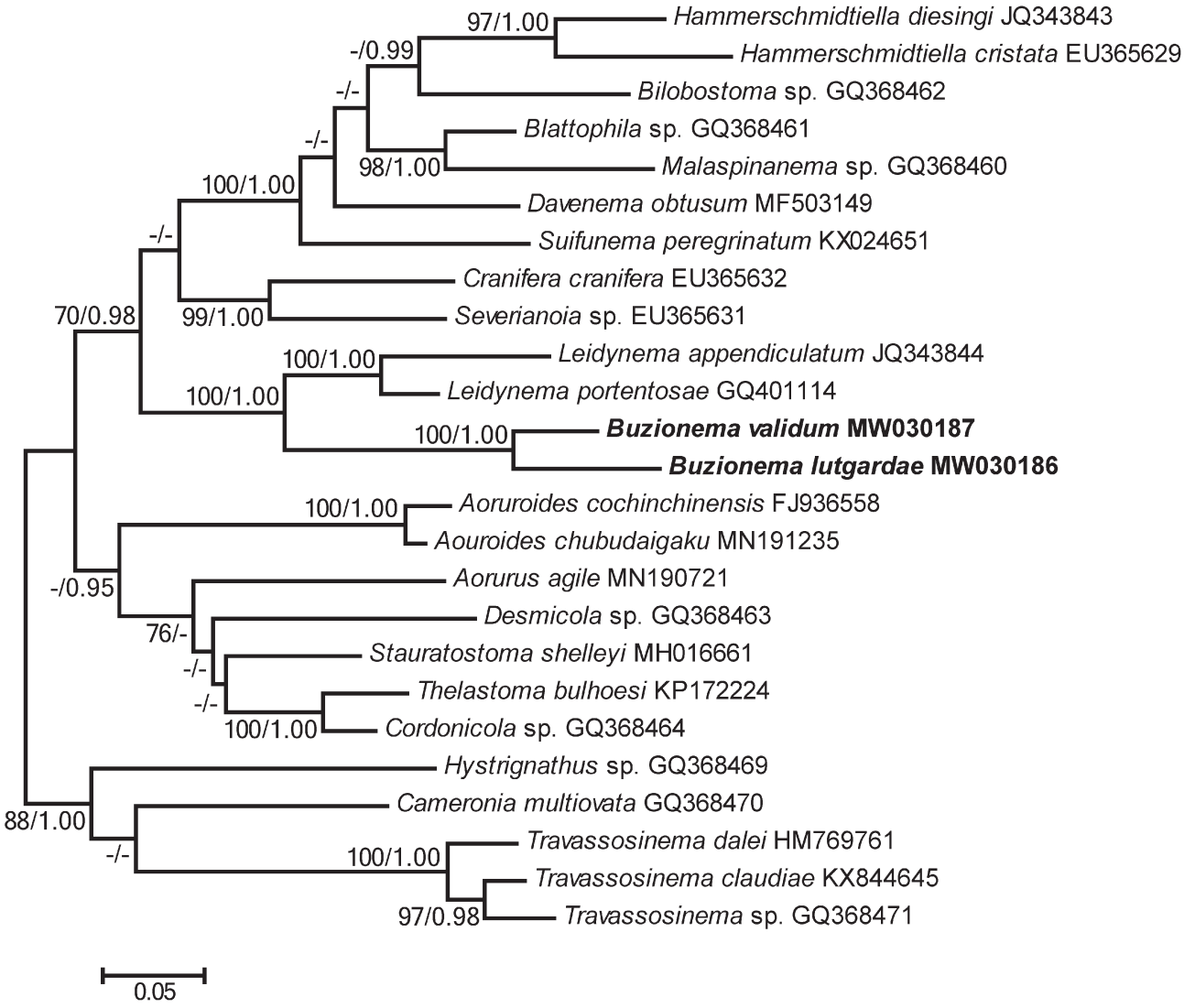

FIGURE 4. Maximum likelihood (ML) tree inferred from the D2-D3 28S rDNA for several species of the superfamily Thelastomatoidea (Nematoda: Oxyuridomorpha). *Hystrignathus* sp. (Hystrignathidae), *Cameronia multiovata* (Thelastomatidae) and three species of *Travassosinema* (Travassosinematidae) were used as outgroup taxa. Values at the nodes correspond to bootstrap resampling (≥70)/posterior probability (≥0.95). Newly obtained sequences in bold.

Discussion

García & Coy (1998) recorded specimens of *Buzionema* parasitizing *Byrsotria* sp. that were also from central Cuba. Those specimens were morphologically identical to *B. validum*, but several of their measurements were almost twice as short as the Brazilian species (*i.e.* body length = 1522–1827 μm *vs.* 3131–3378 μm). Despite these differences, the authors were cautious and considered the Cuban individuals as conspecific with *B. validum*, based on their morphological similarities. The morphology and measurements of the specimens from the present study agree with the ones from the work of García & Coy (1998) and this, together with the same host suggests that nematodes from both localities belong to the same *Buzionema* species. The differences between the material from Cuba and *B. validum* in several meristic variables and the extension of the lateral alae of both sexes, along with the molecular evidence support the proposal of this Cuban *Buzionema* as a new species.

According to Kloss (1966) and Carreno & Tuhela (2011), the female corpus of *Buzionema* is broad and fusiform,

with the isthmus very long. In the species described herein we observed that, in fact, the female corpus consists of two parts, an anterior muscular that is swollen and fusiform (consistent with the descriptions of the aforementioned authors) and a posterior one that is long and slender and similar in diameter to the short isthmus. The joint of the posterior part of the corpus with the isthmus is present, but not conspicuous. This would be the cause of confusion about the real extension of the isthmus and the reasons of the observations of Kloss (1966) and Carreno & Tuhela (2011).

There are also discrepancies about the position of the excretory pore in the genus *Buzionema*. Kloss (1966) did not distinguish this structure in the females of *B. validum* and in the case of the males observed it just posterior the oesophagus. According to the line drawings of Carreno & Tuhela (2011) in both sexes of the Costa Rican *B. validum* the excretory pore is post-bulbar, just past the base of the oesophagus. In *B. lutgardae* **n. sp.** the excretory pore is pre-bulbar at the level of the last third of the cylindrical part of the corpus in the females and at the level of the isthmus in the males. Further examination of material from *B. validum* is necessary in order to clarify the discrepancies in the structure of the oesophagus and the position of the excretory pore recorded in the previous studies.

The arrangement of both species of *Buzionema* as sister-group to *Leidynema* is surprising due to the obvious differences between both genera. The cephalic end of *Buzionema* is quite characteristic in having three lips around the oral opening in the females instead of the eight labiopapillae surrounding the mouth in *Leidynema.* The oesophagus also differs, with a corpus with an anterior part that is swollen, fusiform, and a posterior part that is slender and cylindrical, its diameter very similar to the isthmus in *Buzionema* and a corpus with an anterior part cylindrical and a posterior part broader in *Leidynema.* Additionally, *Leidynema* presents a blind diverticulum in the anterior part of the intestine (which constitutes an autapomorphy of the genus). In *Leidynema* females the lateral alae end in a spine-like projection. Moreover, the males of *Leidynema* present a spicule and a spine-like process at the tail end, both of which are absent in *Buzionema*.

Despite the aforementioned differences both genera present some similarities that could justify the nodal supports, namely the didelphic-amphidelphic female genital tract, the sub-cylindrical corpus of the males and their short and truncate tail. Less than a half of the known thelastomatid genera are available as sequences of the 28S rDNA, the most widely used nuclear DNA locus for this group. Due to this shortage of information, studies on the molecular phylogeny of Thelastomatidae (and on the superfamily Thelastomatoidea in general) are biased. The gradual inclusion of more genera in these analyses may clarify the unclear phylogenetic relationships of several taxa.

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Literature cited

Adamson, M.L. & Van Waerebeke, D. (1992) Revision of the Thelastomatoidea, Oxyurida of invertebrate hosts I. Thelastomatidae. *Systematic Parasitology*, 22, 111–130.
https://doi.org/10.1007/BF00009911

Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M.,

Frisse, L.M., Vida, J.T. & Thomas, W.K. (1998) A molecular evolutionary framework for the phylum Nematoda. *Nature*, 392, 71–75.
https://doi.org/10.1038/32160
Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, 25, 1972–1973.
https://doi.org/10.1093/bioinformatics/btp348
Carreno, R.A. & Nadler, S.A. (2003) Phylogenetic analysis of the Metastrongyloidea (Nematoda: Strongylida) inferred from ribosomal RNA gene sequences. *Journal of Parasitology*, 89, 965–973.
https://doi.org/10.1645/GE-76R
Carreno, R.A. & Tuhela, L. (2011) Thelastomatid Nematodes (Oxyurida: Thelastomatoidea) from the Peppered Cockroach, *Archimandrita tesselata* (Insecta: Blattaria) in Costa Rica. *Comparative Parasitology*, 78 (1), 39–55.
https://doi.org/10.1654/4455.1
Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.
https://doi.org/10.1093/nar/gkh340
García, N. & Coy, A. (1998) Nuevo género, nueva especie y nuevos registros de thelastomátidos (Oxyurida; Thelastomatidae) parásitos de *Byrsotria* sp. (Dictyoptera; Blaberidae). *Avicennia*, 8/9, 41–49.
Kloss, G.R. (1966) Revisão dos nematóides de Blattaria de Brasil. *Papéis Avulsos do Departamento de Zoologia, Sao Paulo*, 18, 147–188.
Nadler, S.A., De Ley, P., Mundo-Ocampo, M., Smythe, A.B., Stock, S.P., Bumbarger, D., Adams, B.J., De Ley, I.T., Holovachov, O. & Baldwin, J.G. (2006) Phylogeny of Cephalobina (Nematoda): molecular evidence for recurrent evolution of probolae and incongruence with traditional classifications. *Molecular Phylogenetics and Evolution*, 40, 696–711.
https://doi.org/10.1016/j.ympev.2006.04.005
Nunn, G.B. (1992) *Nematode molecular evolution.* University of Nottingham, Nottingham, 187 pp.
Rambaut, A., Suchard, M.A., Xie, W. & Drummond, A.J. (2003) Tracer. Version 1.6. Available from: http://beast.bio.ed.ac.uk/Tracer (accessed 12 October 2016)
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Aaron, D., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542.
https://doi.org/10.1093/sysbio/sys029
Seinhorst, J.W. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, 4, 67–69.
https://doi.org/10.1163/187529259X00381
Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.
https://doi.org/10.1093/molbev/mst197