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Morphological and molecular characterization of *Lepidonema magnum* Morffe & García, 2010 (Nematoda: Oxyuridomorpha: Hystrignathidae) from *Passalus interstitialis* Eschscholtz, 1829 (Coleoptera: Passalidae) from Cuba and new locality records for the species

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Abstract

Lepidonema magnum Morffe & García, 2010 (Nematoda: Oxyuridomorpha: Hystrignathidae) is redescribed and illustrated with the aid of SEM. New features of the cephalic end and genital tract of the females were observed. New locality records are given. The phylogenetic position of the species is inferred on the basis of the D2-D3 segment of the 28S LSU rDNA and 18S SSU rDNA. *L. magnum* forms a monophyletic clade formed by other hystrignathids: *Coynema poeyi* (Coy, García & Álvarez, 1993), two species of *Longior* Travassos & Kloss, 1958 and two *Hystrignathus* Leidy, 1850.

Key words: *Lepidonema*, redescription, SEM, 28S LSU rDNA, 18S SSU rDNA, phylogeny, Cuba

Resumen

Se redescribe e ilustra a *Lepidonema magnum* Morffe *et* García, 2010 (Nematoda: Oxyuridomorpha: Hystrignathidae) con el empleo de Microscopía Electrónica de Barrido. Se observaron nuevos caracteres de la región cefálica y el sistema reproductor de las hembras. Se ofrecen nuevos registros de localidad para la especie. La filogenia de *L. magnum* es inferida mediante los marcadores moleculares D2-D3 del 28S LSU rDNA y 18S SSU rDNA. Como resultado, el taxón forma un clado monofilético con otros histrignátidos: *Coynema poeyi* (Coy, García *et* Álvarez, 1993), dos especies del género *Longior* Travassos *et* Kloss, 1958 y dos de *Hystrignathus* Leidy, 1850.

Palabras clave: *Lepidonema*, redescripción, SEM, 28S LSU rDNA, 18S SSU rDNA, filogenia, Cuba

Introduction

The genus *Lepidonema* Cobb, 1898 (Nematoda: Oxyuridomorpha: Hystrignathidae) comprises a small number of nematode species distinguishable by the females having opposite rows of scale-like spines in the cervical cuticle, the procorpus sub-cylindrical and the genital tract didelphic-amphidelphic. The males, on the other hand present a single spicule (Adamson & Van Waerebeke 1992).

The type species of the genus, *L. bifurcata* Cobb, 1898 is unusual by being described from the gut of an Australian beetle larva instead of an adult passalid beetle as is common among the family Hystrignathidae. With the exception of the latter, the rest of the nominal species of *Lepidonema* have been described from the Neotropics

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(Brazil and Cuba). The Brazilian taxa are two, namely *L. brasiliense* Travassos & Kloss, 1957 and *L. caracae* Kloss, 1962 (Travassos & Kloss 1957; Kloss 1962). Two species also occur in Cuba: *L. teresae* García, Ventosa & Morffe, 2009 from Isla de la Juventud and *L. magnum* Morffe & García, 2010 from Western Cuba (García *et al.* 2009; Morffe & García 2010).

In the present work *L. magnum* is redescribed on the basis of light microscopy and SEM studies. The phylogenetic position of the species is inferred on the basis of the D2-D3 domains of the 28S LSU rDNA and the 18S SSU rDNA. New locality records are also provided.

Materials and methods

Processing of the hosts and nematodes. Specimens of *Passalus interstitialis* Eschscholtz, 1829, *Antillanax pertyi* (Kaup, 1869) and *Odontotaenius disjunctus* (Illiger, 1800) (Coleoptera: Passalidae) were collected by hand from rotting logs in several localities from Cuba and USA. Beetles were maintained alive in plastic jars with moistened wood chips as food and humidity source until arrival at the laboratory.

Hosts were killed with vapours of ethyl-ether or 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, TAF or 4% phosphate buffered formalin. Specimens for molecular studies were directly fixed in 96% ethanol. For light microscopy studies the nematodes were transferred to anhydrous glycerine via slow evaporation method (Seinhorst 1959) and mounted in the same medium. The edges of the coverslips were sealed with nail polish.

Studied material is deposited in the Colección Helmintológica de las Colecciones Zoológicas (CZACC), Instituto de Ecología y Sistemática, Havana, Cuba and the Museo Nacional de Historia Natural "Prof. Eugenio de Jesús Marcano" (MNHNSD), Santo Domingo, Dominican Republic.

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, the number of measurements is also given. Micrographs were generated with an AxioCam digital camera attached to a Carl Zeiss AxioScop 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.

For SEM studies the specimens were dehydrated in a graded ethanol series and critical point-dried in a Balzers CPD 030 critical point dryer (BAL-TEC AG, Florida, USA). They were then mounted onto aluminum stubs and coated in gold with a Bal-Tec SCD 050 sputter coater. Micrographs were taken with the aid of a Quanta 200 SEM (Thermo-Fisher Scientific, Hillsborogh, USA).

DNA extraction, gene amplification and sequencing. Genomic DNA was extracted from single individuals with the NucleoSpin® Tissue (Machery-Nagel, Düren, Germany) and DNeasy® Blood & Tissue (Qiagen, USA) kits, following manufacturer´s instructions. The D2-D3 segment of the large ribosomal subunit ribosomal RNA gene (D2-D3 28S LSU 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). The small ribosomal subunit ribosomal RNA gene (18S SSU rDNA) 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).

For the specimen of *L. magnum* the PCR reactions for the 28S LSU rDNA were performed in a total volume of 11 µL with the Taq DNA Polymerase (Qiagen®, 5 U/µL). PCR cycling parameters were as follows: an initial denaturalization at 94ºC for 5 min, followed by 35 cycles of 45 s at 94ºC, 1 min at 50ºC and 1 min at 72ºC, and a final extension step of 10 min at 72ºC. The results of the PCR reactions were checked by agarose gel electrophoresis, visualizing the DNA bands with GelRed™. PCR products were cleaned with ExoSAP-IT (Thermo Fisher, USA). Bidirectional sequences were obtained using Big Dye Terminator chemistry (Applied Biosystems, USA) using the same primers as their respective PCR reactions.

The PCR reactions for the 18S SSU rDNA as well as the 28S LSU rDNA of *H. rigidus* 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 denaturalization at 94ºC for 2 min followed by 35 cycles of 98ºC for 10 s, 50ºC for 30 s 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.

Raw sequences were manually edited with Sequencher 4.1.4 (http://genecodes.com) and deposited in GenBank NCBI (http://www.ncbi.nlm.nih.gov/genbank/). The accession numbers for each sequence are provided in Table 1.

Phylogenetic analysis. Several sequences of thelastomatoid species (Hystrignathidae, Travassosinematidae and Thelastomatidae) were selected from GenBank for the phylogenetic analyses. *Cameronia multiovata* (Thelastomatidae) and species of *Travassosinema* (Travassosinematidae) were used as the outgroup taxa.

Phylogenetic analyses were performed for both D2-D3 28S LSU rDNA and 18S SSU rDNA as well as a concatenated dataset of both genes. Multiple sequence alignments were made using the Muscle algorithm with the default parameters as implemented in MEGA 6 (Tamura *et al.* 2013). Poorly aligned regions and gaps were automatically removed with trimAl (Capella-Gutiérrez *et al.* 2009). MEGA 6 was also used to identify the optimal model of evolution for the datasets (GTR+G) following the Akaike Information Criterion (AIC) and to construct phylogenetic trees based on Maximum Likelihood (ML). Nodal support was inferred by bootstrap resampling 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).

TABLE 1. GenBank accession numbers of the sequences of thelastomatoid nematodes (Oxyuridomorpha: Thelastomatoidea) used in the present study. Newly obtained sequences in bold.

Species	Country	D2-D3 28S LSU rDNA	18S SSU rDNA
Hystrignathidae			
Coynema poeyi	Cuba	MH244508	**MH577322**
Hystrignathus rigidus	USA	**MH411129**	**MH411156**
Hystrignathus sp.	Vietnam	GQ368469	–
Lepidonema magnum	Cuba	**MH569782**	**MH577324**
Longior longior	Cuba	KX427524	**MH411158**
Longior similis	Cuba	KX427528	**MH411157**
Thelastomatidae			
Cameronia multiovata	Russia	GQ368470	–
Travassosinematidae			
Travassossinema claudiae	Japan	KX844645	KX844644
Travassosinema sp.	Vietnam	GQ368471	–

Systematics

Family Hystrignathidae Travassos, 1920

Lepidonema Cobb, 1898

Lepidonema magnum Morffe & García, 2010

Fig. 1 A–H, Fig. 2 A–D

Lepidonema magnum Morffe & García, 2010: 6–9, fig. 3 A–I, fig. 4 A–G

Material examined. Vouchers: 6♀♀, Cuba, Pinar del Río province, Viñales, Sendero “Maravillas de Viñales”; in

Passalus interstitialis; IV/2018; M. Iturriaga, C. Hernández coll.; CZACC 11.7115–11.7120. 2♀♀, same data as the latter; MNHNSD 05.0020–05.0021.

Vouchers: 3♀♀, Cuba, Artemisa province, Sierra del Rosario, El Taburete; in *Passalus interstitialis*; 8/IV/2012; E. Pardo coll.; CZACC 11.7121–11.7123.

Vouchers: 4♀♀, Cuba, Isla de la Juventud, Sierra de Casas; in *Passalus interstitialis*; IV/2014; J. Morffe, N. García, M. Olcha coll.; CZACC 11.7124–7127.

Vouchers: 3♀♀, Cuba, Sancti Spíritus province, Trinidad, Topes de Collantes, Hotel "Los Helechos"; in *Passalus interstitialis*; V/2013; E. Fonseca coll.; CZACC 11.7128–11.7130.

Vouchers: 4♀♀, Cuba, Camagüey province, Sierra de Cubitas, Reserva Ecológica "Limones-Tuabaquey"; in *Passalus interstitialis*; XII/2015; J. Morffe, N. García coll.; CZACC 11.7131–7134.

Redescription. Female. Body comparatively robust, widening gradually posterior to head, reaching its maximum width at level of the vulva, then narrowing gradually towards tail. Sub-cuticular striae present. Cephalic end bluntly rounded. Cervical cuticle armed with *ca.* 67 opposite rows of spines, from the base of the first cephalic annule to the midpoint of the basal bulb in some specimens or a short distance further down its base in other specimens. First row of spines with 16 scale-like elements, their distal ends rounded, *ca.* 5 µm in length. Spines remain scale-like, with rounded tips until *ca.* the level of the 12th row, where they become pointed, in that range of rows the spines increase their length to *ca.* 10 µm. At *ca.* the level of the 14th row some columns of spines duplicate, increasing their number to *ca.* 20 rows at level of the end of the spiny region. Spines from the last rows are shorter (*ca.* 4 µm) and pointed. Cuticle markedly annulated in the spiny region (annuli *ca.* 5 µm in length), further down this region the annuli become shorter (*ca.* 1 µm) and less marked. Lateral alae well-developed, extend from a short distance after the end of the spiny region (*ca.* 30 µm) to the level of the vulva. One cuticular, slightly curled, barely prominent lip surrounding the triradiate oral opening. Head bearing eight slightly ellipsoidal cephalic papillae (larger diameter/shorter diameter ratio *ca.* 1.3) arising from the external edge of the lip. Cephalic papillae are almost equidistant, separated from each other by a distance of *ca.* 4 µm. Amphids lateral, their opening reniform and located at a small cuticular protuberance at level of the external edge of the lip. First cephalic annule comparatively long, truncate, with convex margins and set-off from the head by a marked groove. Stoma short and wide, surrounded by an oesophageal collar, barely extending beyond the level of the first cephalic annule. Oesophagus consists of a muscular, sub-cylindrical procorpus, its base slightly expanded (*ca.* twice as wide as the anterior region), well differentiated from the short, cylindrical isthmus. Basal bulb rounded, valve-plate well-developed. Intestine simple, sub-rectilinear, its fore region slightly dilated, formed by a single layer of large, polygonal cells. Rectum comparatively long. Anus not prominent. Nerve ring encircling procorpus at *ca.* 45% of its length. Excretory pore ventral, located at *ca.* 0.7 body-widths posterior to the basal bulb. Vulva a median transverse slit located near the midbody, its lips slightly prominent. Vagina muscular, forwardly directed. Genital tract didelphic-amphidelphic, both ovaries reflexed. Distal end of the anterior ovary reflexed posterior to the excretory pore, distal flexure *ca.* 1.5 body-widths long. Distal end of the posterior ovary reflexed at *ca*. 2.5–3 body-widths before the level of the anus, distal flexure *ca.* 1–1.5 body-widths long. Oocytes in single rows. A fusiform spermatheca present in the posterior uterus, near the level of the basal end of the anterior ovary. Such spermatheca is absent in the anterior branch of the genital tract. Eggs ellipsoidal, smooth-shelled. Gravid females with 1–6 eggs at a time in the uterus (more frequently two eggs). Tail conical, subulate, ending in a fine tip.

Male. Unknown.

Remarks. Morffe & García (2010) described *L. magnum* based on light microscopy observations. In their description the authors mentioned the presence of eight small, paired cephalic papillae in the head. The current SEM studies show that the cephalic papillae are not paired but almost equidistant. Such arrangement is a feature quite uncommon in Hystrignathidae since most of the taxa studied by SEM or with light microscopy that included *en face* views show paired papillae (*i.e.* Hunt 1981, 1982; Van Waerebeke & Remillet 1982; Morffe & García 2013a, b; Morffe *et al*. 2015; 2018a, b). Line drawings of Van Waerebeke (1973) show Malagasian species of *Artigasia* Christie, 1934 with the cephalic papillae widely spaced, giving an equidistant appearance: *i.e. A. andringitrae* Van Waerebeke, 1973; A. *dispar* Van Waerebeke, 1973; *A. lata* Van Waerebeke, 1973 and *A. semialata* Van Waerebeke, 1973.

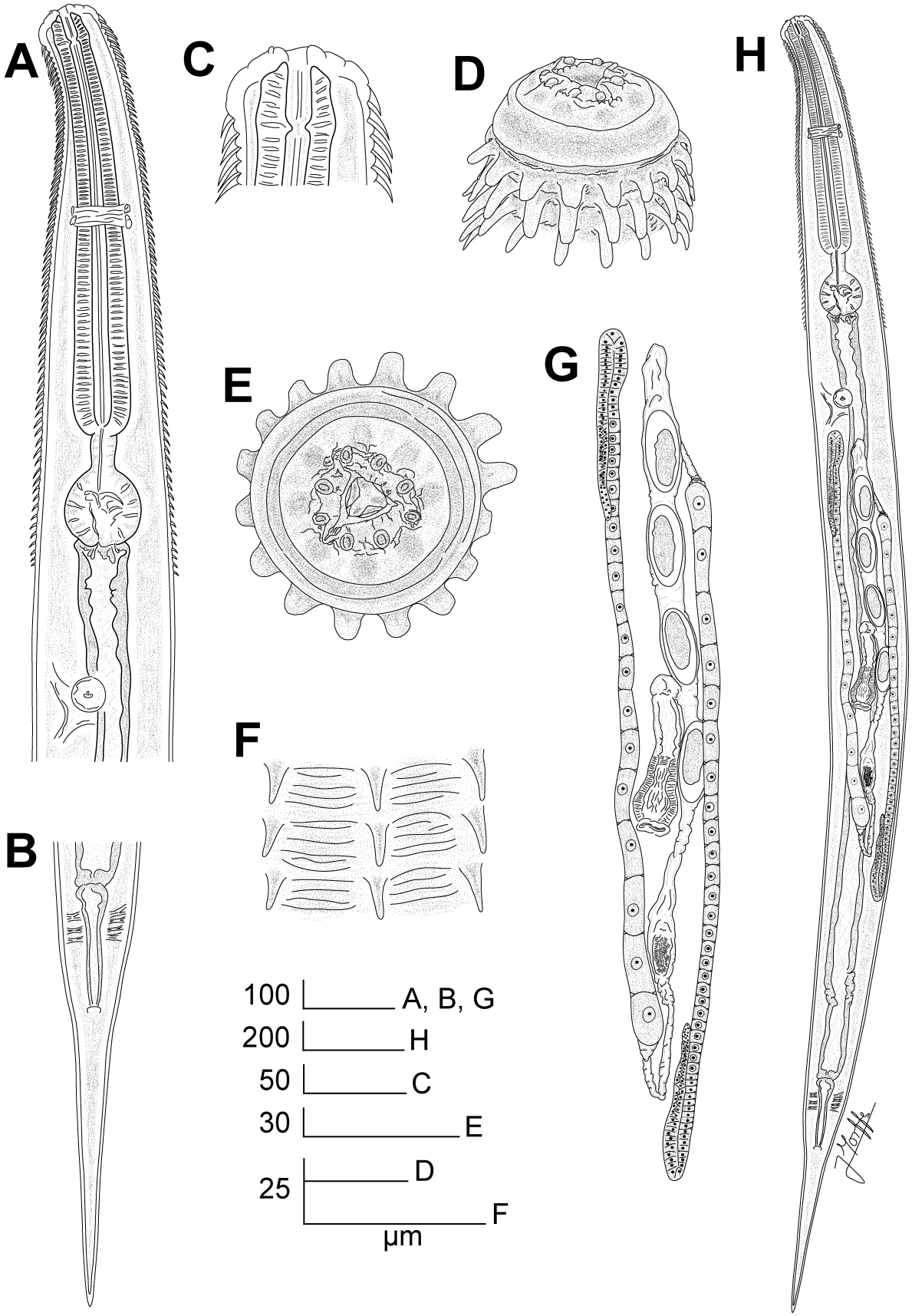

FIGURE 1. *Lepidonema magnum* Morffe & García, 2010. Female. A. Oesophageal region, ventral view. B. Tail, ventral view. C. Cephalic end, optical section. D. Cephalic end, external view (reconstructed from SEM images). E. Cephalic end, *en face* view (reconstructed from SEM images). F. Spines near the end of the spiny region. G. Genital tract, ventral view. H. Habitus, ventral view.

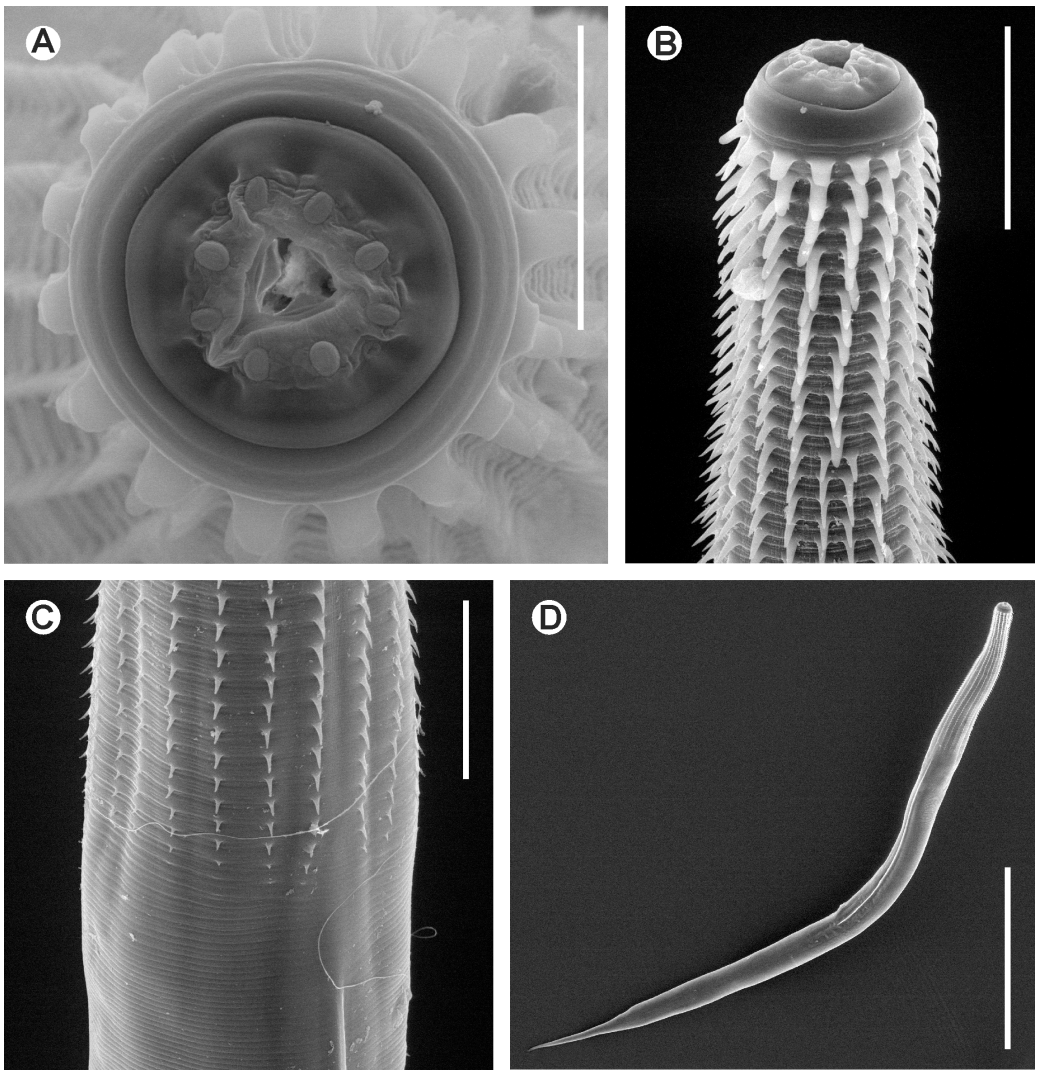

FIGURE 2. *Lepidonema magnum* Morffe & García, 2010. Female. SEM images. A. Cephalic end, *en face* view. B. Cervical region. C. End of the spiny region and beginning of the lateral alae. D. Habitus, lateral view. Scale bars: A. 30 µm. B, C. 50 µm. D. 500 µm.

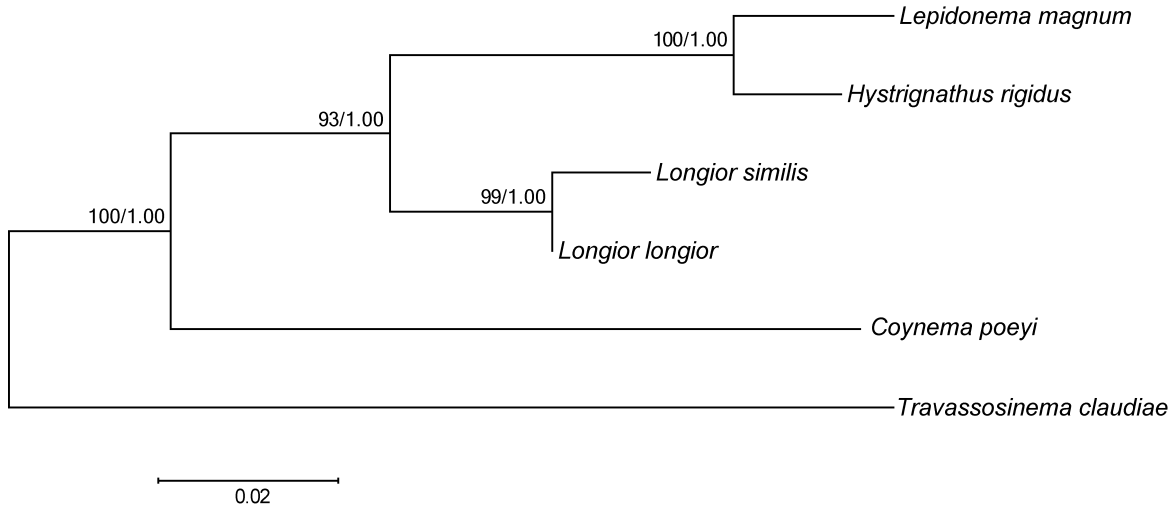

FIGURE 3. Maximum likelihood (ML) tree inferred from the concatenated dataset of the D2-D3 28S LSU rDNA and 18S SSU rDNA for several species of the family Hystrignathidae (Nematoda: Oxyuridomorpha). *Travassosinema claudiae* (Travassosinematidae) was used as outgroup taxon. Values at the nodes correspond to bootstrap resampling/posterior probability.

The presence of a spermatheca in the posterior uterus was not observed in the original description of the species (Morffe & García 2010). This structure has not been previously recorded in Hystrignathidae but it has been noted in species from the Thelastomatidae and Travassosinematidae, such as *Desmicola skrjabini* Adamson, 1984 (Thelastomatidae) and *Travassosinema claudiae* Morffe & Hasegawa, 2017 (Adamson 1984; Morffe & Hasegawa 2017). These authors recorded the presence of a sac-like seminal receptacle in the posterior uterus of both species. The pinworm nematodes (Oxyuridomorpha) are quite unique by their haplodiploid reproduction, with diploid females that originate from fertilized eggs and haploid males derived from unfertilized eggs (Adamson 1994). Adamson (1984) attributed the existence of a single spermatheca as a mechanism for regulating sex ratios, since presumably, the posterior branch of the genital tract (with the spermatheca) could produce fertilized eggs and the anterior branch (without spermatheca) could only produce unfertilized eggs. This could assure the presence of both, females and males among the progeny.

At present, *L. magnum* is only known from its type locality: Escaleras de Jaruco, Mayabeque province in Western Cuba (Morffe & García 2010). Therefore, all the populations currently studied constitute new locality records. Viñales, Pinar del Río province is the westernmost record. The population from Sierra de Casas, Isla de la Juventud is the only known location outside of the main island of the Cuban archipelago. The records from Topes de Collantes, Sancti Spíritus province and Sierra de Cubitas, Camagüey province extends the distribution of the species to Central Cuba, the latter being the easternmost. The individuals from the aforementioned new localities coincide morphologically and morphometrically (Table 2) with the type specimens of the species.

DNA studies. ML and BI analyses were performed for the D2-D3 LSU rDNA, the 18S SSU rDNA and a concatenated dataset of both markers. The trees of the latter dataset were the ones with the highest values of bootstrap support. Since the topology of both ML and BI trees was identical only the former is shown (Fig. 3).

L. magnum is sister taxon to *Hystrignathus rigidus* Leidy, 1850 in a strongly supported monophyletic clade. Such an arrangement is also supported by the morphological similarities between these species. Both, *L. magnum* and *H. rigidus* present opposite rows of spines that are scale-like and pointed, respectively. Also, the female genital tract is didelphic-amphidelphic in these taxa.

The clade formed by the two *Longior* Travassos & Kloss, 1958 species is situated basally to the clade of *Lepidonema* + *Hystrignathus*. This arrangement is supported by the morphological differences between *Longior* and the two latter genera: unarmed *vs.* spiny cervical cuticle, digitiform *vs.* rounded cephalic papillae and monodelphic-prodelphic *vs.* didelphic-amphidelphic genital tract. Additionally, the procorpus of *Longior* is cylindrical and elongated whereas it is claviform in *Hystrignathus* Leidy, 1850 and sub-cylindrical in *Lepidonema.*

Coynema poeyi (Coy, García & Álvarez, 1993) is located basal to the clade formed by the aforementioned hystrignathid species. This result is consistent with Morffe *et al.* (2018b) for the D2-D3 domains of the 28S LSU rDNA. As discussed by Morffe *et al.* (2018b), *Coynema* presents several characteristic features that differentiated this taxon from the rest of the genera included in the analysis, namely paired and elliptic cephalic papillae arranged in a V-like pattern, the sub-cylindrical procorpus with a basal dilation and the anterior region of the intestine inflated, forming a sac-like structure.

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TABLE 2. Morphometrics of *Lepidonema magnum* Morffe & García, 2010 (Nematoda: Oxyuridomorpha: Hystrignathidae) females from several localities from Cuba. All the measurements are given in micrometers unless otherwise indicated.

Locality	Viñales, Pinar del Río province	El Taburete, Artemisa province	Sierra de Casas, Isla de la Juventud	Topes de Collantes, Sancti Spíritus province	Sierra de Cubitas, Camagüey province
Character					
a	13.68–16.56 (15.38 ± 0.94, n = 8)	12.50–14.17 (13.61 ± 0.96, n = 3)	14.86–16.09 (15.24 ± 0.58, n = 4)	15.00–16.62 (15.72 ± 0.82, n = 3)	15.18–17.67 (16.34 ± 1.11, n = 4)
b	4.44–4.95 (4.72 ± 0.17, n = 8)	4.09–4.90 (4.60 ± 0.45, n = 3)	4.77–4.86 (4.80 ± 0.04, n = 4)	4.96–5.00 (4.99 ± 0.03, n = 3)	4.34–5.05 (4.68 ± 0.31, n = 4)
c	6.94–7.80 (7.35 ± 0.28, n = 8)	5.90–7.29 (6.76 ± 0.75, n = 3)	6.96–7.57 (7.30 ± 0.27, n = 4)	7.18–8.33 (7.65 ± 0.61, n = 3)	5.74–7.36 (6.81 ± 0.74, n = 4)
V%	44.00–54.37 (51.99 ± 3.60, n = 8)	53.92–56.47 (54.77 ± 1.47, n = 3)	53.27–54.37 (53.58 ± 0.53, n = 4)	52.68–53.33 (53.04 ± 0.33, n = 3)	52.34–55.66 (54.12 ± 1.61, n = 4)
Total length (mm)	2.500–2.725 (2.606 ± 0.065, n = 8)	2.125–2.550 (2.408 ± 0.245, n = 3)	2.575–2.675 (2.625 ± 0.058, n = 4)	2.800–3.000 (2.875 ± 0.109, n = 3)	2.125–2.675 (2.450 ± 0.262, n = 4)
Maximum width	160–190 (170 ± 11, n = 8)	170–180 (177 ± 6, n = 3)	160–180 (173 ± 10, n = 4)	170–200 (183 ± 15, n = 3)	140–170 (150 ± 14, n = 4)
First cephalic annule (length×width)	15–25×68–83 (19 ± 3×74 ± 5, n = 8)	18×63–68 (18 ± 0×64 ± 3, n = 3)	13–18×58–68 (15 ± 2×62 ± 4, n = 4)	15–20×65–73 (18 ± 3×68 ± 4, n = 3)	15–20×55–63 (17 ± 2×59 ± 3, n = 4)
Stoma length	25–33 (31 ± 3, n = 8)	28–33 (31 ± 3, n = 3)	28–33 (30 ± 2, n = 4)	30–35 (33 ± 3, n = 3)	25–30 (29 ± 3, n = 4)
Procorpus length	410–450 (430 ± 14, n = 8)	400–410 (407 ± 6, n = 3)	410–440 (428 ± 15, n = 4)	430–470 (447 ± 21, n = 3)	370–460 (413 ± 37, n = 4)
Isthmus length	30–40 (36 ± 3, n = 8)	33–38 (36 ± 3, n = 3)	38–40 (38 ± 1, n = 4)	43–45 (43 ± 1, n = 3)	35–43 (38 ± 4, n = 3)
Basal bulb diameter	85–90 (89 ± 2, n = 8)	80–85 (83 ± 3, n = 3)	80–85 (83 ± 2, n = 4)	80 (80 ± 0, n = 3)	75–83 (79 ± 3, n = 4)
Oesophagus length	520–580 (553 ± 18, n = 8)	520–530 (523 ± 6, n = 3)	530–560 (548 ± 15, n = 4)	560–600 (577 ± 21, n = 3)	490–550 (523 ± 25, n = 4)
Nerve ring-anterior end	210–300 (251 ± 26, n = 8)	240–270 (260 ± 17, n = 3)	230–250 (240 ± 8, n = 4)	250 (250 ± 0, n = 3)	200–220 (210 ± 10, n = 3)
Excretory pore-anterior end	750–850 (774 ± 32, n = 8)	660–770 (733 ± 64, n = 3)	740–780 (757 ± 21, n = 3)	800–850 (820 ± 27, n = 3)	630–810 (695 ± 83, n = 4)
Vulva-anterior end (mm)	1.100–1.475 (1.356 ± 0.114, n = 8)	1.200–1.275 (1.317 ± 0.101, n = 3)	1.375–1.425 (1.406 ± 0.024, n = 4)	1.475–1.600 (1.525 ± 0.066, n = 3)	1.175–1.475 (1.325 ± 0.137, n = 4)
Tail length	330–370 (355 ± 12, n = 8)	350–360 (357 ± 6, n = 3)	340–370 (360 ± 14, n = 4)	360–390 (377 ± 15, n = 3)	340–370 (360 ± 14, n = 4)
Eggs	88–95×35–50 (91 ± 3×41 ± 4, n = 10)	88×38 (n = 1)	–	–	–

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