Zootaxa 4933 (3): 391–402
https://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4933.3.6
http://zoobank.org/urn:lsid:zoobank.org:pub:BFA44668-69F6-435B-871A-5DB85D490710

Morphological and molecular characterization of *Klossnema viguerasi* n. sp. (Nematoda: Oxyuridomorpha: Hystrignathidae) from a Cuban passalid beetle (Coleoptera: Passalidae), first record of the genus for Cuba

JANS MORFFE[1,3*], NAYLA GARCÍA[1,2], KOICHI HASEGAWA[3] & KARIN BREUGELMANS[4]

[1]*Instituto de Ecología y Sistemática, Carretera Varona 11835 e/ Oriente y Lindero, La Habana 19, CP 11900, Calabazar, Boyeros, La Habana, Cuba.*

[2] *nayla@ecologia.cu; https://orcid.org/0000-0002-3979-8086*

[3]*Department of Environmental Biology, College of Bioscience & Biotechnology, Chubu University, 1200 Matsumoto, Kasugai, Aichi 487–8501, Japan. koichihasegawa@isc.chubu.ac.jp; https://orcid.org/0000-0002-9968-8129*

[4]*Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000, Brussels, Belgium.*
karin.breugelmans@naturalsciences.be; https://orcid.org/0000-0002-1236-7403

[*]*Corresponding author. jans@ecologia.cu; https://orcid.org/0000-0001-6105-2697*

Abstract

Klossnema viguerasi **n. sp.** (Nematoda: Oxyuridomorpha: Hystrignathidae) is described from the passalid beetle *Antillanax pertyi* (Kaup, 1869), endemic to Cuba. The females of *K. viguerasi* **n. sp.** are morphologically similar but slightly longer than *K. repentina* Cordeiro & Artigas, 1983 (1.143 mm *vs.* 1.000 mm). Both species differ in that *K. viguerasi* **n. sp.** has a longer procorpus (139 µm *vs.* 110 µm), isthmus (39 µm *vs.* 24 µm), and tail length (28 µm *vs.* 21 µm). The distance from the vulva to the anterior end is also longer in the new species (0.748 mm *vs.* 0.650 mm). The males of *K. viguerasi* **n. sp.** are larger than *K. repentina* (0.980 mm *vs.* 0.800 mm), but their isthmus is shorter (38 µm *vs.* 48 µm). New features of the cephalic end of both sexes, and copulatory papillae pattern of the males were observed by SEM and the generic diagnosis is emended in order to include such features. The phylogeny of *K. viguerasi* **n. sp.** is inferred by the analysis of the D2–D3 domains of the 28S rDNA and the 18S rDNA. This constitutes the first record of the genus *Klossnema* for the Cuban archipelago and the West Indies.

Key words: *Klossnema*, new species, SEM, phylogeny, Cuba, 28S rDNA, 18S rDNA

Introduction

Cordeiro & Artigas (1983) described the monotypic genus *Klossnema* Cordeiro & Artigas, 1983 on the basis of *K. repentina* Cordeiro & Artigas, 1983 from several species of Brazilian passalid beetles (Coleoptera: Passalidae). The same authors erected the subfamily Klossnematinae in order to accommodate the new genus and species.

Klossnema is quite characteristic in Hystrignathidae, since females and males present an unarmed cervical cuticle. The females have a clavate procorpus, didelphic-amphidelphic genital tract, vulva in the posterior quarter of the body and a short, digitiform tail. On the other hand, the males lack a spicule, presenting instead a thickened dorsal cuticle of the tail, two pre-cloacal papillae and three or four minute sub-terminal papillae (Cordeiro & Artigas 1983; Adamson & Van Waerebeke 1992).

In the current work a new species of *Klossnema* is described on the basis of light microscopy and Scanning Electron Microscopy (SEM) studies. Specimens were collected from the gut of the the passalid beetle *Antillanax pertyi* (Kaup, 1869), endemic to Cuba. The generic diagnosis is amended with new observed features. The phylogenetic position of the species is discussed on the basis of the analysis of the nuclear D2–D3 domains of the 28S rDNA and the 18S rDNA.

Accepted by R. Carreno: 25 Jan. 2021; published: 22 Feb. 2021

Materials and methods

Processing of the hosts and nematodes

Specimens of *A. pertyi* were collected by hand from rotting logs in several localities from Cuba. 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 practicing 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 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 (Seinhorst 1959) and mounted in the same medium. The edges of the coverslips were sealed with paraffin wax.

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

Morphological and morphometric studies

Measurements were taken with the aid of a calibrated eyepiece micrometer. Indices a, b, c and V% (De Man 1884) 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 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 with 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, Tokyo, 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, Tokyo, Japan), and observed with a JSM-6510LA scanning electron microscope (JEOL, Tokyo, Japan) at 15 kV accelerating voltage.

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, Maryland, 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). The small ribosomal subunit ribosomal RNA gene (18S 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).

The PCR reactions for the 28S rDNA were performed in a total volume of 11 µL, containing 1 µL of DNA extract, 1 µL of each primer (2 µM), 1 µL of the deoxinucleoside triphosphates (2 mM of each nucleotide), 0.1 µL of Taq DNA Polymerase (Qiagen®, 5 U/µL), 1 µL 10x Taq buffer (Qiagen®, containing 15 mM $MgCl_2$), 0.6 µL $MgCl_2$ (25 mM) and 5.3 µL dd H_2O. PCR cycling parameters were as follows: an initial denaturation 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, Massachusetts, USA). Bidirectional sequences were obtained using Big Dye Terminator chemistry (Applied Biosystems, Massachusetts, USA) using the same primers as their respective PCR reactions.

The PCR reactions for the 18S rDNA were performed in a total volume of 20 µL, containing 2 µL of DNA extract, 0.6 µL of each primer (10 pmol), 4 µL of the deoxinucleoside triphosphates (2 mM of each nucleotide), 0.4

μL of KOD FX Neo DNA polymerase (Toyobo, Osaka, Japan, 1 U/μL), 10 μL of 2x PCR Buffer for KOD FX Neo and 2.4 μL of dd H_2O. 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 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. PCR products were submitted to Hokkaido System Science Co., Sapporo, Japan for sequencing. 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 taxon are provided in Table 1.

Phylogenetic analysis

Sequences of species of Thelastomatoidea (Hystrignathidae and Travassosinematidae) were selected from GenBank for the phylogenetic analyses (accession numbers in Table 1). One species of *Travassosinema* (Travassosinematidae) was used as the outgroup taxon, since this genus is the sister-group of Hystrignathidae according to previous studies (Spiridonov & Guzeeva 2009).

Multiple sequence alignments were made for both 28S rDNA and 18S rDNA datasets using the MUSCLE algorithm (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). Phylogenetic analyses were performed for a concatenated dataset of both genes. MEGA6 was also used to identify the optimal model of evolution (GTR+G+I) following the Akaike Information Criterion (AIC), as well as to construct a phylogenetic tree based on Maximum Likelihood (ML). Nodal support was estimated 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 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) and ascaridoid nematodes (Ascaridomorpha) used in the present study. Newly obtained sequences in bold.

Species	Country	28S rDNA	18S rDNA
Hystrignathidae			
Coynema poeyi	Cuba	MH244508	MH577322
Hystrignathus rigidus	USA	MH411129	MH411156
Klossnema viguerasi **n. sp.**	Cuba	**MW030185**	**MW030189**
Lepidonema magnum	Cuba	MH569782	MH577324
Longior longior	Cuba	KX427524	MH411158
L. similis	Cuba	KX427528	MH411157
Triumphalisnema zuuei	Mexico	MN628599	MH220047
Tuhmai garciaprietoi	Mexico	MT070420	MT069968
Urbanonema osorioi	Mexico	MN578047	MN578051
Xyo pseudohystrix	USA	MH569779	MH577323
Travassosinematidae			
Travassosinema claudiae	Japan	KX844645	KX844644

Systematics

Family Hystrignathidae Travassos, 1920

Klossnema Cordeiro & Artigas, 1983

Emended diagnosis. General. Small and slender nematodes. Cephalic capsule smooth, dorsoventrally compressed.

Mouth hexagonal, laterally orientated, surrounded by six labia, each of them coinciding with one of its sides. Labia set-off from each other by short cleavages coinciding with mouth edges. Four flat, elongated cephalic papillae present, arranged as one dorsal pair and one ventral. Papillae of each pair touch dorsally and ventrally, respectively, forming obtuse angles. Digitiform structure present close to each lateral edge of the mouth. Cuticle unarmed and finely annulated from the base of the cephalic capsule to the posterior region. Lateral alae absent. Oesophagus with a muscular, sub-cylindrical procorpus, its base slightly dilated. Isthmus comparatively long. Basal bulb pyriform, valve plate well developed. Intestine simple, sub-rectilinear, anterior portion barely dilated. Nerve ring encircling procorpus at its posterior half. Excretory pore ventral, post-bulbar.

Female. Reproductive system monodelphic-prodelphic. Eggs comparatively large, ovoid, smooth-shelled. Tail very short, subulate and curved, with a hook-like appearance.

Male. Body smaller and slightly less robust than females, posterior end ventrally curved. Monorchic. Spicule absent. Tail very short, conoid, its tip sharp. Dorsal cuticle of the tail end thickened and smooth. Six copulatory papillae present, four pre-cloacal and two post-cloacal. First pre-cloacal pair consists of a ventromedian duplex papilla on a protuberance, the sensilla of each papilla of this pair surrounded by peg-like prominences, arranged in more or less concentric circles. Second pair of pre-cloacal papillae formed by large peg-like papillae, lateral in position, located at a short distance before the level of the cloaca. The pair of post-cloacal papillae consists of sub-lateral minute papillae near the tail tip. Phasmids pore-like, located at the tail tip.

***Klossnema viguerasi* n. sp.**

Fig. 1 A–E, Fig. 2 A–F, Fig. 3 A–H

Type material. Holotype: ♀, Cuba, Artemisa province, Sierra del Rosario, Soroa; 22°48′00″N, 83°01′00″W; in *Antillanax pertyi*; II/2018; M. Iturriaga coll.; CZACC 11.7283. Paratypes: 9♀♀, same data as the holotype; CZACC 11.7284–11.7292. 12♂♂, same data as the holotype; CZACC 11.7293–11.7304.

Other examined material. Vouchers: 9♀♀, Cuba, Sancti Spíritus province, Trinidad, Topes de Collantes, path to the Caburní River; 21º53′41″N, 79º54′20″W; in *Antillanax pertyi*; 12/X/2014; J. Morffe, N. García coll.; CZACC 11.7309–11.7317. 8♂♂, same data as the latter; CZACC 11.7318–11.7325.

Vouchers: 7♀♀, Cuba, Guantánamo province, El Salvador, Limonar; 20°12′34″N, 75°13′23″W; in *Antillanax pertyi*; VI/2013; J. Morffe, N. García, M. Olcha coll.; CZACC 11.7326–11.7332. 6♂♂, same data as the latter; CZACC 11.7333–11.7338.

Measurements. See Table 2.

Description. General. Small nematodes, body comparatively slender, ventrally curved in heat-fixed specimens. Cephalic end bluntly rounded, followed by the body diameter slightly increasing, keeping almost constant diameter towards the body length and gradually decreasing near tail level towards posterior end. Cephalic capsule smooth, dorsoventrally compressed. Mouth hexagonal, laterally orientated, with sides arranged as one dorsal, one ventral, two sub-dorsal and two sub-ventral. Mouth surrounded by six labia, each of them coinciding with one of its sides. Labia set-off from each other by short cleavages coinciding with the mouth edges. Four flat, elongated cephalic papillae present, arranged as one dorsal pair and one ventral. Papillae of each pair touching dorsally and ventrally, respectively, forming obtuse angles. Digitiform structure *ca.* 2 µm in length present close to each lateral edge of the mouth. Cuticle unarmed, finely annulated (annuli *ca.* 0.5 µm) from the base of the cephalic capsule to the base of the tail. Lateral alae absent. Oesophagus with a muscular, sub-cylindrical procorpus, its base slightly dilated. Isthmus comparatively long, *ca.* one third of the procorpus length. Basal bulb pyriform, valve plate well-developed. Intestine simple, sub-rectilinear, anterior portion barely dilated. Nerve ring encircling procorpus at its posterior half, *ca.* 60% of its length. Excretory pore ventral.

Female. Cuticle finely annulated from base of the cephalic capsule to level of anus. Rectum short, anus slightly prominent. Excretory pore ventral, located at *ca.* a body-width posterior the basal bulb. Vulva a ventro-median transverse slit, displaced to the posterior half of body, at *ca.* 60% of the body-length, lips slightly prominent. Genital tract monodelphic-prodelphic. Ovary unreflexed, its distal tip located at *ca.* 1–3 body-widths posterior to the excretory pore. Oocytes in single rows. Eggs ellipsoidal in shape, smooth-shelled. Gravid females with a single egg in the uterus, rarely two or three. Tail very short and subulate, sometimes curved in a hook-like appearance, ending in a sharp tip.

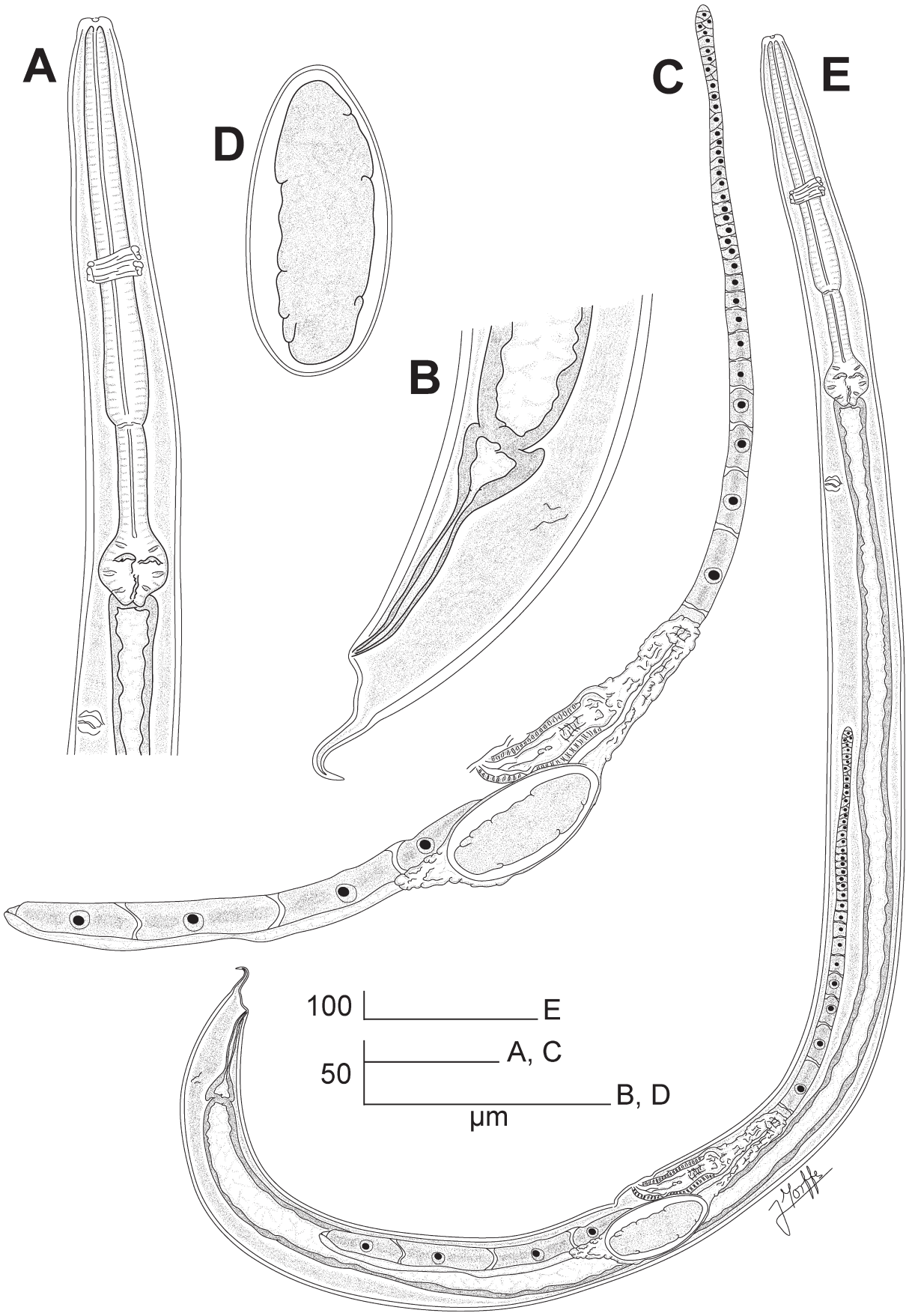

FIGURE 1. *Klossnema viguerasi* **n. sp.** Female. A. Oesophageal region, lateral view. B. Tail, lateral view. C. Genital tract, lateral view. D. Egg. E. Habitus, lateral view.

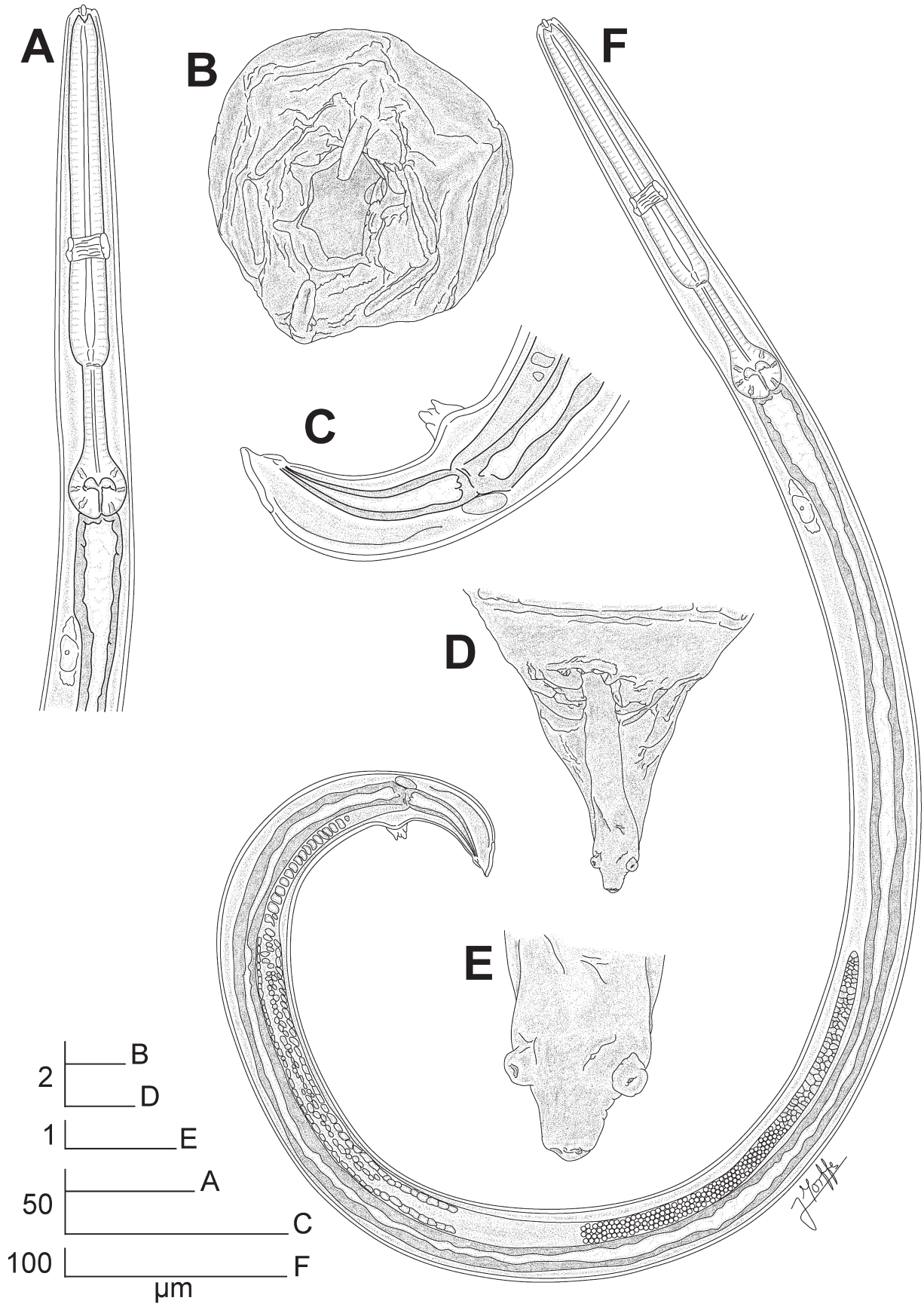

FIGURE 2. *Klossnema viguerasi* **n. sp.** Male. A. Oesophageal region, lateral view. B. Cephalic end, *en face* view (reconstructed from SEM images). C. Tail, lateral view. D. Tail end, ventral view (reconstructed from SEM images). E. Detail of the tail tip, ventral view (reconstructed from SEM images). F. Habitus, lateral view.

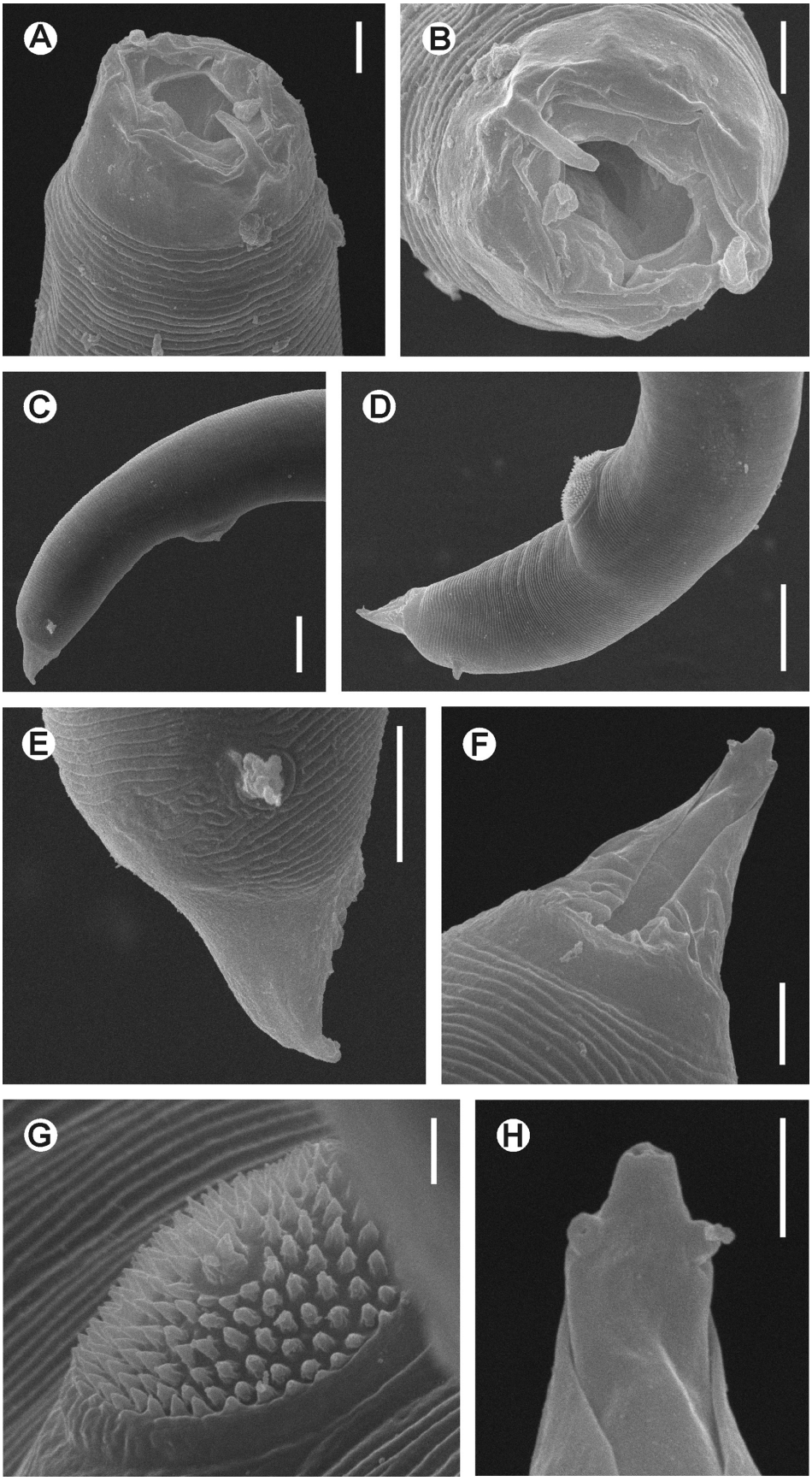

FIGURE 3. *Klossnema viguerasi* **n. sp.**, SEM images. Male. A. Cephalic end. B. Cephalic end, *en face* view. C. Tail, lateral view. D. Tail, ventro-lateral view. E. Detail of the tail, lateral view. F. Detail of the tail, ventral view. G. Ventromedian papilla, ventral view. H. Tail tip, ventral view. Scale bars: A, B, F. 2 µm. C, D. 10 µm. E. 5 µm. G, H. 1 µm.

Male. Body smaller and slightly less robust than females, posterior end ventrally curved. Cuticle finely annulated from base of cephalic capsule to beginning of dorsal cuticular thickening of posterior end. Excretory pore ventral, located at *ca.* 1.5 body-widths posterior the basal bulb. Monorchic. Testis ventral, outstretched, commencing at a distance of *ca.* seven body-widths posterior to the excretory pore. *Vas deferens* with three distinguishable regions: an anterior region (occupying *ca.* 40% of the testis length) slender, with granular content and swollen in its joint with a median region (occupying *ca.* one third of the testis length) with large, rounded cells, and a posterior region that diminishes its diameter through the cloaca. Spicule absent. Tail very short, conoid, its tip sharp. Dorsal cuticle of tail end thickened and smooth, from *ca.* half of the level of the lateral pre-cloacal papillae to the tail tip. Six copulatory papillae, four pre-cloacal and two post-cloacal. First pre-cloacal pair consists of a ventromedian duplex papillae, very close to each other on a protuberance (appearing to be a single papilla in lateral view) located at *ca*. 40 μm from the cloaca. Sensilla of each papilla of this pair surrounded by peg-like prominences, arranged in *ca.* five circles more or less concentric. Second pair of pre-cloacal papillae formed by large peg-like papillae, lateral in position, located at a short distance (*ca.* 5 μm) before the level of the cloaca. One pair of post-cloacal papillae: a sub-lateral pair of minute papillae sub-terminal, near tail tip (*ca.* 1 μm). Phasmids pore-like, located at tail tip, very close to each other.

Differential diagnosis. Cordeiro & Artigas (1983) only offered the mean of the measurements of *K. repentina.* Therefore, we use these values for comparison with the new species. The females of *K. viguerasi* **n. sp.** are morphologically quite similar and only slightly longer than *K. repentina* (1.143 mm *vs.* 1.000 mm). However, several meristic variables are higher in *K. viguerasi* **n. sp.**, namely the length of the procorpus (139 μm *vs.* 110 μm), the length of the isthmus (39 μm *vs.* 24 μm), the distance from the vulva to the anterior end (0.748 mm *vs.* 0.650 mm) and the tail length (28 μm *vs.* 21 μm). The males of *K. viguerasi* **n. sp.** are also larger than *K. repentina* (0.980 mm *vs.* 0.800 mm), but their isthmus is shorter (38 μm *vs.* 48 μm).

Type locality. Soroa, Sierra del Rosario, Candelaria municipality, Artemisa province, Cuba.

Other localities. Path to the Caburní river, Gran Parque Natural Topes de Collantes, Trinidad municipality, Sancti Spíritus province, Cuba; Limonar, El Salvador municipality, Guantánamo province, Cuba.

Type host. *Antillanax pertyi* (Kaup, 1869) (Coleoptera: Passalidae).

Site. Hind gut.

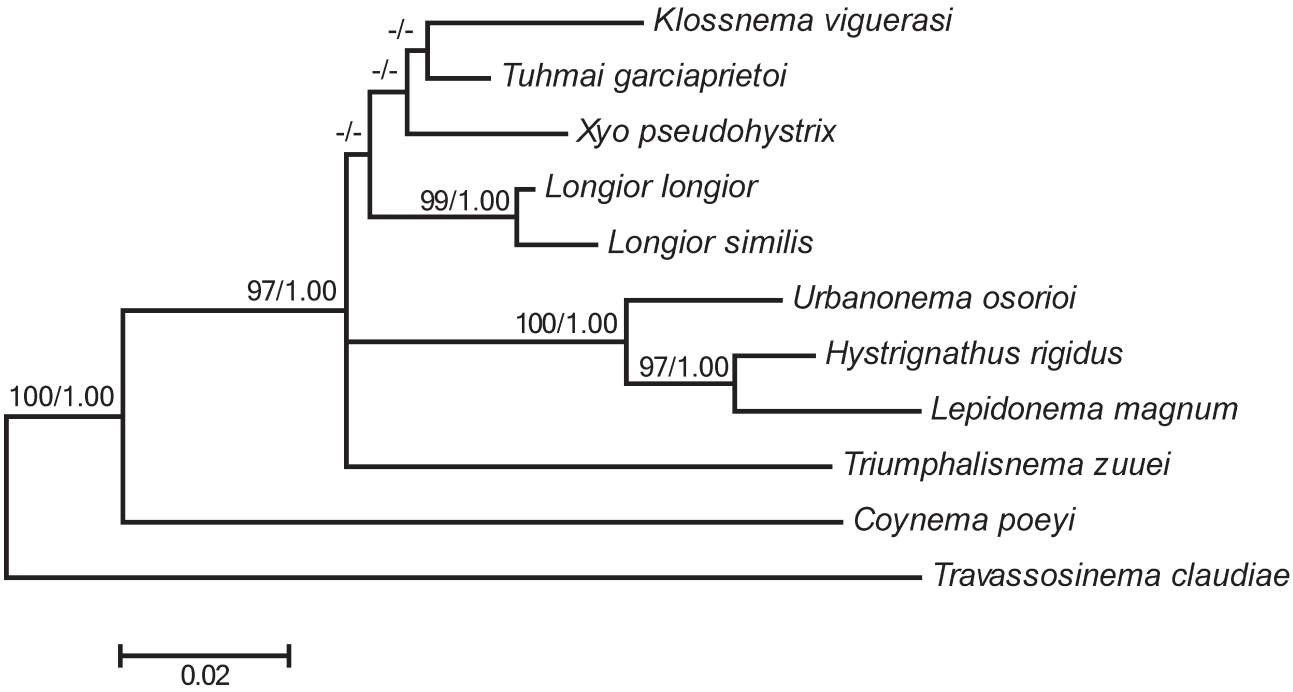

FIGURE 4. Maximum likelihood (ML) tree inferred from a concatenated dataset of the D2–D3 28S rDNA and the 18S rDNA for several species of the family Hystrignathidae (Nematoda: Oxyuridomorpha: Thelastomatoidea). One species of *Travassosinema* (Travassosinematidae) was used as outgroup taxon. Values at the nodes correspond to ML bootstrap resampling (≥70)/ Bayesian posterior probability (≥0.90).

Etymology. Specific epithet dedicated to Ildefonso Pérez Vigueras (1892–1959) eminent Cuban veterinarian and parasitologist, and pioneer in studies of parasitic helminths from Cuban fauna, including invertebrates.

DNA studies. One partial sequence (806 bp) of the D2–D3 region of the 28S rDNA and one partial sequence (813 bp) of the 18S rDNA were obtained from females of *K. viguerasi* **n. sp.** The length of the 28S rDNA and the 18S rDNA datasets, once the poorly aligned regions and gaps were removed was of 738 bp and 725 bp, respectively. The concatenated dataset of both markers resulted in a 1463 bp aligment.

ML and BI phylograms of the concatenated dataset depict *Klossnema viguerasi* **n. sp.** as sister taxon to *Tuhmai garciaprietoi* Garduño-Montes de Oca & Oceguera-Figueroa, 2020, but with low nodal support. The aforementioned clade of *Klossnema* + *Tuhmai* is part of a larger clade containing *Xyo* and *Longior* (Fig. 4).

Discussion

The morphology of the cephalic end of *Klossnema* was not detailed in previous studies (*i.e.* Cordeiro & Artigas 1983; Adamson & Van Waerebeke 1992). The shape of its dorsoventrally compressed cephalic capsule, and the absence of an evident first cephalic annule is not common in Hystrignathidae, especially in the females. These features appear to be more frequent in male specimens, such as the ones described as "type A" (that were unable to be assigned to their proper females) by Van Waerebeke (1973) and Hunt (1981). Additionally, the presence of only four cephalic papillae in *Klossnema* is unusual, eight being the more frequent number in the females of the family.

Cordeiro & Artigas (1983) noticed some sexual dimorphism in the shape of the oesophagus of *K. repentina*: the presence of a stoma, a divided procorpus and an undifferentiated isthmus in the males. In the present study we observed that the shape of the oesophagus is very similar in both sexes, which does not coincide with the original description of the Brazilian species. Both females and males of *K. viguerasi* **n. sp.** lack of a conspicuous stoma, their procorpus is not divided and the isthmus is well defined.

The arrangement of the copulatory papillae described by Cordeiro & Artigas (1983) differs from the one observed in the present study. These authors mentioned the presence of two pre-cloacal papillae, one of them adanal and a number of three or four minute post-cloacal papillae, sub-terminal in position. Herein, by means of SEM the papillary pattern is amended, with two pre-cloacal pairs of papillae instead of only two papillae and a single pair of post-cloacal papillae instead of three or four papillae. These post-cloacal papillae are sub-terminal, as observed by Cordeiro & Artigas (1983). The shape of the anteriormost pair of pre-cloacal papillae, with the sensilla of each papillae surrounded by peg-like prominences is quite characteristic and so far, not observed in males of Hystrignathidae, appearing to be a autapomorphy of the genus.

The original description of *K. repentina* is based on a syntype series formed by a mix of specimens from four host species, namely *Passalus inundulifrons* (Kuwert, 1898), *P. morio* Percheron, 1835; *P. punctatostriatus* Percheron, 1835 and *P. rusticus* Percheron, 1835 from two localities from Sao Paulo, Brazil (Cordeiro & Artigas 1983). Therefore, it is possible that such syntype series could consist of several *Klossnema* species from the different hosts. This fact is supported by the apparent morphological homogeneity of the genus, with the interspecific differences based mostly on meristic variables. Further morphological and molecular studies are needed in order to separately examine *Klossnema* material from the aforementioned passalids in order to clarify if, in fact, we are dealing with the single species *K. repentina* or with more than one.

Phylogenetic analysis of the concatenated dataset showed that *Klossnema* forms a clade with *Tuhmai*. This arrangement is supported by several morphological similarities among both genera, namely the unarmed cervical cuticle, the sub-cylindrical procorpus and the monodelphic-prodelphic female genital tract, with the vulva located in the posterior half of the body. However, *Klossnema* differs from *Tuhmai* by the absence of a first cephalic annule and lateral alae, which are conspicuous in *Tuhmai*. Alternatively, the body shape of *Klossnema* tends to be ventrally curved, whereas, like most hystrignathids, *Tuhmai*, has a straight body. The cephalic end of *Tuhmai* is typical of several genera of Hystrignathidae, with eight rounded, flattened, paired cephalic papillae, and with a triangular mouth (Garduño-Montes de Oca & Oceguera-Figueroa 2020) in contrast to the characteristic cephalic end of *Klossnema*. These evident differences could be reflected in the low support values for the *Klossnema* + *Tuhmai* clade.

Despite the arrangement of *Xyo pseudohystrix* (a spiny species with a didelphic-amphidelphic genital tract) in the clade formed by *Klossnema* + *Tuhmai* + *Xyo* + *Longior*, in the phylogenies *Klossnema* is more related to *Longior* (both genera share its monodelphic female genital tract, unarmed cervical cuticle and elongated body) than to other

TABLE 2. Morphometrics of *Klossnema viguerasi* **n. sp.** (Nematoda: Oxyuridomorpha: Hystrignathidae) from several localities from Cuba. All the measurements are given in micrometers unless otherwise indicated.

Character	Soroa, Artemisa province (type locality)			Path to the Caburní river, Sancti Spíritus province		Limonar, Guantánamo province	
	Females		Males	Females (n = 9)	Males (n = 8)	Females (n = 7)	Males (n = 6)
	Holotype	Paratypes (n = 9)	Paratypes (n = 12)	Vouchers		Vouchers	
a	33.85	30.40–34.86 (32.32 ± 1.51, n = 9)	32.33–41.20 (35.78 ± 2.75, n = 12)	26.82–32.86 (29.75 ± 2.04, n = 9)	34.55–43.50 (38.16 ± 3.04, n = 8)	27.06–31.25 (28.62 ± 1.44, n = 7)	32.67–40.40 (37.94 ± 2.92, n = 6)
b	5.43	4.93–5.95 (5.62 ± 0.29, n = 9)	5.13–5.70 (5.42 ± 0.17, n = 12)	5.04–5.98 (5.44 ± 0.28, n = 9)	4.91–5.58 (5.15 ± 0.21, n = 8)	5.05–5.72 (5.37 ± 0.29, n = 7)	5.00–5.59 (5.22 ± 0.22, n = 6)
c	40.00	36.73–47.20 (41.85 ± 3.05, n = 9)	103.00–208.00 (146.00 ± 34.94, n = 12)	32.29–41.45 (36.90 ± 3.67, n = 9)	87.00–133.33 (114.92 ± 20.08, n = 8)	35.38–44.73 (39.75 ± 3.67, n = 7)	100.00–202.00 (136.06 ± 35.85, n = 6)
V%	65.45	63.66–67.21 (65.43 ± 0.97, n = 9)	–	63.91–68.18 (66.61 ± 1.21, n = 9)	–	64.17–68.70 (66.36 ± 1.88, n = 7)	–
Total length (in mm)	1.100	1.010–1.220 (1.148 ± 0.069, n = 9)	0.860–1.040 (0.980 ± 0.055, n = 12)	1.020–1.330 (1.152 ± 0.095, n = 9)	0.870–1.000 (0.948 ± 0.047, n = 8)	1.150–1.330 (1.216 ± 0.061, n = 7)	0.980–1.090 (1.023 ± 0.040, n = 6)
Maximum width	33	33–38 (36 ± 2, n = 9)	25–30 (28 ± 2, n = 12)	35–48 (39 ± 4, n = 9)	20–28 (25 ± 3, n = 8)	40–45 (43 ± 1, n = 7)	25–30 (27 ± 2, n = 6)
Procorpus length	138	128–143 (139 ± 5, n = 9)	115–125 (121 ± 3, n = 11)	128–155 (145 ± 9, n = 9)	113–133 (125 ± 6, n = 8)	145–165 (156 ± 7, n = 7)	130–135 (133 ± 2, n = 6)
Isthmus length	40	33–43 (39 ± 3, n = 9)	33–40 (38 ± 2, n = 11)	33–43 (39 ± 4, n = 9)	30–45 (37 ± 4, n = 8)	38–48 (44 ± 3, n = 7)	40–43 (41 ± 1, n = 6)
Basal bulb diameter	25	25 (n = 9)	18–23 (21 ± 2, n = 12)	25–30 (28 ± 1, n = 9)	23–25 (23 ± 1, n = 8)	25–28 (27 ± 1, n = 7)	20–25 (23 ± 2, n = 6)
Oesophagus length	203	188–213 (204 ± 8, n = 9)	168–188 (181 ± 6, n = 12)	188–223 (212 ± 12, n = 9)	170–195 (184 ± 9, n = 8)	208–240 (227 ± 12, n = 7)	195–200 (196 ± 2, n = 6)
Nerve ring-anterior end	93	88–100 (96 ± 4, n = 9)	78–88 (83 ± 4, n = 12)	90–105 (99 ± 5, n = 9)	80–90 (85 ± 4, n = 8)	98–113 (105 ± 5, n = 7)	88–90 (89 ± 1, n = 6)
Excretory pore-anterior end	243	230–263 (249 ± 10, n = 9)	208–255 (234 ± 12, n = 12)	243–305 (276 ± 22, n = 9)	228–270 (242 ± 15, n = 8)	258–300 (288 ± 15, n = 7)	250–265 (258 ± 6, n = 6)
Vulva-anterior end (in mm)	0.720	0.670–0.820 (0.751 ± 0.046, n = 9)	–	0.690–0.850 (0.767 ± 0.052, n = 9)	–	0.760–0.910 (0.807 ± 0.055, n = 7)	–
Tail length	28	25–30 (28 ± 2, n = 9)	5–10 (7 ± 2, n = 12)	28–35 (31 ± 3, n = 9)	8–10 (8 ± 1, n = 8)	28–33 (31 ± 2, n = 7)	5–10 (8 ± 2, n = 6)
Eggs	65×23 (n = 1)	60–68×23 (63 ± 3×23, n = 5)	–	60–68×23–25 (64 ± 3×23 ± 1, n = 4)	–	65–68×20–23 (67 ± 1×22 ± 1, n = 3)	–

digonant and spiny genera such as *Hystrignathus*, *Lepidonema* and *Urbanonema*. Morffe *et al*. (2019) obtained similar results in the form of a monophyletic clade formed by two quite different genera: *Xyo* and *Longior*. These authors recommended the inclusion of more molecular data in the phylogenetic analyses of Hystrignathidae, as well as a better characterization of the morphology of the taxa (including the males) in order to obtain more robust support of evolutionary relationships.

Klossnema viguerasi **n. sp.** is present in a locality from western Cuba, namely Soroa (type locality), Artemisa province as well as Caburní, Sancti Spíritus province and Limonar, Guantánamo province from Central and Eastern Cuba, respectively. The individuals from the aforementioned localities coincide morphologically and morphometrically (Table 2).

Acknowledgements

We are very grateful to Manuel Iturriaga (Instituto de Ecología y Sistemática) for collecting some of the hosts examined in this study. We thank the hospitality and help afforded by the staff of the Facultad Agropecuaria de Montaña "Escambray" (FAME), Topes de Collantes and Órgano de Montaña, Limonar, Guantánamo. We thank MSc. Eduardo Furrazola (Instituto de Ecología y Sistemática) for his help with the micrographs. The Belgian Development Cooperation, through the Belgian Focal Point of the Global Taxonomy Initiative (GTI; 2013 call) supported access by the senior author to molecular techniques at the Royal Belgian Institute of Natural Sciences. Access to molecular techniques and SEM at Chubu University was possible with the funds from the Japanese Society for the Promotion of Science (JSPS) Long Term Fellowship (ID No. L16566). This research was also supported by the project "Colecciones Zoológicas, su conservación y manejo III", Ministerio de Ciencia, Tecnología y Medio Ambiente, Cuba, and the Research Institute for Biological Function, Chubu University.

Literature cited

Adamson, M.L. & Van Waerebeke, D. (1992) Revision of the Thelastomatoidea, Oxyurida of invertebrate hosts III. Hystrignathidae. *Systematic Parasitology*, 22, 111–130.
https://doi.org/10.1007/BF00009604

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

Cordeiro, N.S. & Artigas, P.T. (1983) Nematóides de Passalidae (Coleoptera). Descrição de *Klossnema repentina* n. gên., n. sp. (Nematoda: Hystrignathidae). *Memorias do Instituto Butantan*, 47/48, 107–111.

De Man, J.G. (1884) *Dei frei der reine Erden und in sussen Wasser Lebenden nematoden neiderlanddischen fauna, Eine Systematische Faunistische Monographie*. E.J. Brill, Leiden, 206 pp.
https://doi.org/10.5962/bhl.title.46884

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

Garduño-Montes de Oca, U. & Oceguera-Figueroa, A. (2020) Molecular Phylogeny of Thelastomatoidea (Nematoda) with the Description of a New Genus and Two New Species of Hystrignathidae Associated with Bess Beetles (Coleoptera: Passalidae) from Oaxaca, Mexico. *Journal of Parasitology*, 106 (5), 679–688.
https://doi.org/10.1645/20-40

Hunt, D.J. (1981) On *Artigasia horridospina* n.sp., *Longior semialata* n.sp., *Mentecle magnifica* n.sp., *Paraxyo ensicrinatus* n.sp. (Oxyurida: Hystrignathidae) and *Pulchrocephala* ? *pulchrocephala* Travassos, 1925 (Oxyurida: Pulchrocephalidae). *Systematic Parasitology*, 3, 33–52.
https://doi.org/10.1007/BF00012238

Morffe, J., García, N., Davis, A.K., Hasegawa, K. & Carreno, R.A. (2019) Morphological and molecular characterization of *Xyo pseudohystrix* Travassos & Kloss, 1958 (Nematoda: Oxyuridomorpha: Hystrignathidae) from *Odontotaenius disjunctus* (Illiger, 1800) (Coleoptera: Passalidae) from USA and discussion on its taxonomic status. *Zootaxa*, 4619 (2), 391–400.
https://doi.org/10.11646/zootaxa.4619.2.13

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 v1.6. 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
Spiridonov, S.E. & Guzeeva, E.A. (2009) Phylogeny of nematodes of the superfamily Thelastomatoidea (Oxyurida) inferred from LSU rDNA sequence. *Russian Journal of Nematology*, 17, 127–134.
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
Van Waerebeke, D. (1973) Les oxyuroïdes associés aux Passalidae à Madagascar. *Cahiers ORSTOM*, Série Biologie, 18, 3–43.