

Characterisation of some plant-parasitic nematodes of the family Hoplolaimidae from Mexico with descriptions of *Hoplolaimus texcocoensis* sp. n. and *Scutellonema montecilloensis* sp. n.

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Summary. Between 2018 and 2025, nematological surveys conducted across several Mexican states resulted in the collection and characterisation of two new species, *Hoplolaimus texcocoensis* sp. n. and *Scutellonema montecilloensis* sp. n. as well as four known species, *Helicotylenchus dihystra*, *Hoplolaimus seinhorsti*, *S. brachyurus*, and *S. bradys*, five unidentified species of *Helicotylenchus*, and three unidentified species of *Rotylenchus*. In this study, species delimitation within the Hoplolaimidae was based on an integrative taxonomic approach combining traditional morphological characters with molecular criteria. The hoplolaimid taxa were characterised molecularly, and their phylogenetic relationships with other representatives of the group were reconstructed using sequences from the D2-D3 expansion segments of 28S rRNA, the ITS rRNA region, and the mitochondrial *COI* gene. In total, 23 new 28S rRNA, 5 new ITS rRNA, and 13 new *COI* gene sequences were generated in this study. Mexican populations of *Helicotylenchus* and nematodes identified as *Rotylenchus*, formed distinct molecular lineages, suggesting possible endemism. These findings support the hypothesis that valleys surrounded by the ridges of the Sierra Madre mountain ranges in Mexico may represent one of the global centers of diversity for hoplolaimid nematodes.

Key words: *Helicotylenchus dihystra*, *Helicotylenchus*, *Hoplolaimus seinhorsti*, new species, phylogeny, *Rotylenchus*, *Scutellonema brachyurus*, *Scutellonema bradys*, spiral nematodes.

The family Hoplolaimidae Filipjev, 1934 currently comprises 12 valid genera and contains two subfamilies Hoplolaiminae Filipjev, 1934 and Rotylenchulinae Husain & Khan, 1967. Adult hoplolaimid females display marked morphological diversity, ranging from vermiform to posteriorly swollen, obese, or reniform shapes. Members of the family are obligate plant-parasitic nematodes that are predominantly root ectoparasites, exhibiting either migratory or sedentary habits. Hoplolaimid nematodes have a cosmopolitan distribution across all major climatic zones, and several species are of considerable economic importance due to their role as destructive pests of cultivated crops (Van den Berg

et al., 2025).

In Mexico, species of the genera *Helicotylenchus*, *Hoplolaimus*, and *Scutellonema* belonging to the subfamily Hoplolaiminae are widely distributed in both agricultural and natural soils, they frequently occur across diverse cropping systems. High population densities of *Helicotylenchus* spp. have been consistently reported in various nematological surveys (Desgarennés *et al.*, 2011; Lara-Posadas *et al.*, 2016; Franco-Navarro & Godínez-Vidal, 2017; Cid del Prado Vera *et al.*, 2018; Martínez-Bolaños *et al.*, 2020; Carrión *et al.*, 2023; Núñez-Sánchez *et al.*, 2024; Salazar-Villa *et al.*, 2025). Despite their frequent detection, most published records identify

hoplolaimid nematodes only to the genus level and lack any morphological descriptions. To date, relatively few representatives of Hoplolaiminae have been formally identified and described from different Mexican states using traditional morphological approaches. These include ten species: *Helicotylenchus craigi* Knobloch & Laughlin, 1973; *H. digonicus* Perry in Perry, Darling & Thorne, 1959; *H. dihystra* (Cobb, 1893) Sher, 1961; *H. erythrinae* (Zimmerman, 1904) Golden, 1956; *H. jojutlensis* Zavaleta-Mejia & Sosa-Moss, 1979; *H. multicinctus* (Cobb, 1893) Golden, 1956; *H. truncatus* Roman, 1965; *H. galeatus* (Cobb, 1913) Thorne, 1935; *H. igualaensis* Cid del Prado Vera, 1994; and *H. maggentii* Cid del Prado Vera, 1994. Four species: *Helicotylenchus craigi*, *H. jojutlensis*, *Hoplolaimus igualaensis* and *H. maggentii* were described from Mexico and could be considered as endemic for this region (Knobloch & Laughlin, 1973; Zavaleta-Mejia & Sosa-Moss, 1979; Cid del Prado Vera, 1994; Lara-Posadas *et al.*, 2016; Cid del Prado Vera *et al.*, 2018).

Molecular characterisation of Mexican representatives of the subfamily Hoplolaiminae remains absent and, so far, only a partial 28S rRNA gene of a single unidentified *Helicotylenchus* species from Puebla, Atlixco has been sequenced and published (Mejía-Madrid, 2018). Consequently, the species diversity and phylogenetic relationships of hoplolaimid nematodes in Mexico remain largely unexplored, highlighting the need for integrative taxonomic studies combining morphological and molecular data.

The present study is focused on morphological and molecular characterisation of some hoplolaimid nematodes collected during nematological surveys in natural and other areas across different Mexican states. The main objectives of the current study were: *i*) to describe two new species, *Hoplolaimus texcocoensis* sp. n. and *Scutellonema montecilloensis* sp. n. and *ii*) to characterise molecularly hoplolaimid nematodes collected in Mexico using the D2-D3 expansion segments of the 28S rRNA, the ITS rRNA and partial *COI* gene sequences.

MATERIALS AND METHODS

Nematode samples. Soil and root samples were collected from different localities across Mexico between 2018 and 2025 (Table 1), encompassing a range of natural ecosystems. This broad geographic sampling was designed to capture representative hoplolaimid diversity across different regions and host plants. Nematodes were extracted from soil using the centrifugal-flotation (sugar flotation) method of Jenkins (1964). Following extraction,

specimens were handpicked under a stereomicroscope. Individuals intended for morphological study were processed and mounted for light microscopy (LM), whereas those selected for molecular analyses were transferred individually to 70% ethanol and stored until DNA extraction. This dual approach ensured the integration of morphological observations with molecular data for accurate species identification and characterisation.

Morphological study. Nematode specimens were killed by heating to 60°C in a microwave oven for 6 s in about 7 ml of water in a 15 ml vial. An equal volume of Golden fixative (distilled water – 89.6%, formalin – 8.4% and glycerin – 2.0%) was added to achieve a final fixative concentration of 4% (Hooper, 1970). The nematodes were stored at room temperature for 10 days then transferred to a covered Petri dish. Fixative was carefully removed by pipetting from the surface, without disturbing the nematodes, until the depth was reduced to ~ 4 mm. The covered Petri dish was placed in a small desiccator over 96% ethanol and incubated at 40°C. After 3 days, when the odour of formalin was no longer detectable, the volume of liquid in the dish was reduced to half by removing liquid with a pipette under a microscope without disturbing the nematodes. Samples were further processed to glycerin using a modification of the methods by Seinhorst (1959) and De Gisse and Choi (1971). An equal volume of Seinhorst A solution (1 part of glycerin, 20 parts of 96% ethanol, 79 parts of water) was added to the dish, and it was incubated at 40°C with the cover slightly open. When the solution level dropped to 1 mm, Seinhorst B solution (95 parts of 96% ethanol, 5 parts of glycerin) was added, and the dish was incubated at 40°C. When the solution level again dropped to 1 mm, Seinhorst C solution (80 parts of 96% ethanol, 20 parts of glycerin) was added and was incubated at 40°C. Three days later, 1 ml of pure glycerin was added to the dish. Selected nematodes were hand-picked from the dish for mounting on glass slides using the paraffin wax ring method (De Maeseneer & d'Herde, 1963). Measurements and drawings were made using a drawing tube mounted on an American Optical compound microscope (MA, USA). Light microscopy photographs were taken with automatic Lumenera Infinity 2 camera (Ontario, Canada) attached to a compound Olympus BX51 microscope (Tokyo, Japan) equipped with Nomarski differential interference contrast.

The specimens for scanning electron microscope (SEM) studies were washed in magnesium buffer solution, pH 6.8 and 0.05 M, for 20 min and dehydrated in an ethanol solution series, from 10 to

Table 1. Plant-parasitic nematodes of the subfamily Hoplolaiminae from Mexico characterised in this study.

Species	Locality	GPS coordinates (Host)	Sample code	GenBank accession number		
				ITS rRNA gene	D2-D3 of 28S rRNA gene	COI gene
<i>Helicotylenchus dihystrera</i>	Queretaro State, oak forest	20.24737; -100.07675 (<i>Cosmos bipinnatus</i>)	CD2829, (sample 29, trip 1)	–	PX995117	–
<i>H. dihystrera</i>	Mexico State, Amecameca, San Diego Huhuecalco	19.05558; -98.44692 (Unknown plants)	CD4221, EJ3	–	PX995118	–
<i>Helicotylenchus</i> sp.1	Mexico State, Amecameca, San Diego Huhuecalco	19.05558; -98.44692 (<i>Solanum dulcamara</i>)	CD2805a, b, c, d (sample 4, trip 1)	–	PX995112- PX995115	PZ023940
<i>Helicotylenchus</i> sp.2	Texcoco State, Campus Montecillo, Colegio de Postgraduados, Nursery	19.46653; -98.89606 (Grasses)	CD2825 (sample 62, trip 1)	–	PX995109	PZ023938
<i>Helicotylenchus</i> sp.3	Queretaro State	20.50528; -99.91865 (Cactaceae)	CD2799 (sample 30, trip 1)	–	PX995110	PZ023939
<i>Helicotylenchus</i> sp.4	Texcoco State, Campus Montecillo, Colegio de Postgraduados, Peach orchard	19.45588; -98.54812 (Grass)	CD2796 (sample 60, trip 1)	–	PX995116	–
<i>Helicotylenchus</i> sp.5	Hidalgo State	20.45408; -98.67885 (Cactaceae)	CD3018 (sample 71, trip 2)	–	PX995111	PZ023937
<i>Hoplolaimus texcocoensis</i> sp. n.	Texcoco State, Campus Montecillo, Colegio de Postgraduados, Nursery	19.46653; -98.89606 (<i>Bambusa vulgaris</i>)	CD2826 (sample 62, trip 1)	PX995131	PX995123	PZ023928
<i>H. seinhorstii</i>	Veracruz State, Ecological Station La Mancha	19.98428; -96.63472 (<i>Musa acuminata</i>)	CD4225	PX995132	PX995122	PZ023929
<i>Rotylenchus</i> sp.1	Mexico State	19.06769; -98.83345 (<i>Quercus</i> sp.)	CD2830 (sample 10, trip 1)	–	PX995119	PZ023935
<i>Rotylenchus</i> sp.1	Mexico State, Amecameca, San Diego Huhuecalco	19.05558; -98.44692 (<i>Solanum dulcamara</i>)	CD2804 (sample 4, trip 1)	–	PX995120	PZ023934
<i>Rotylenchus</i> sp.2	Tlaxcala State, Huamantla, Francisco Villa Tecoaac, Cerro El Benito	19.38753; -97.92972 (<i>Solanum</i> sp.)	CD2801 (sample 43, trip 1)	–	PX995121	–
<i>Rotylenchus</i> sp.3	Tlaxcala State, Huamantla, Francisco Villa Tecoaac, Cerro El Benito	19.38753; -97.92972 (<i>Solanum rostratum</i>)	CD2807 (sample 47, trip 1)	–	PX995124	PZ023936
<i>Scutellonema brachyurus</i>	Puebla State, Jalpan, La Garza	20.409436; -97.873314 (<i>Saintpaulia ionantha</i>)	CD4226, CD4222, EJ1	PX995133, PX995134	PX995128, PX995129	PZ023932
<i>S. bradys</i>	Veracruz State, Ecology Station La Mancha, Dunes	19.99117; -96.64358 (Grass)	CD4227	PX995130	PX995108	PZ023933
<i>S. montecilloensis</i> sp. n.	Texcoco State, Campus Montecillo, Colegio de Postgraduados, Nursery	19.46653; -98.89606 (<i>Bambusa vulgaris</i>)	CD2797 (sample 60, trip 1) CD3606a, c.	–	PX995125, PX995127	PZ023930, PZ023931

100% in 10 stages for 15 min each. Samples were then critical-point dried before coating with gold/palladium (80/20%) for 4 min. Specimens were observed using a Jeol JSM-6390 microscope (Tokyo, Japan) at 10 KV acceleration voltage.

Molecular study. Genomic DNA was extracted from several individuals of each population using a standard proteinase K lysis protocol. Several nematodes were transferred to lysis buffer, mechanically disrupted, and incubated to ensure complete digestion prior to PCR amplification. DNA extraction, PCR amplification and sequencing were performed following the protocols of Subbotin (2021). Multiple primer sets were used to target nuclear and mitochondrial markers commonly applied in nematode systematics: D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') for amplification of the D2-D3 expansion segments of the 28S rRNA gene, TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') for amplification of the ITS1-5.8S-ITS2 rRNA region and Het-coxiF (5'-TAG TTG ATC GTA ATT TTA ATG G-3') and Het-coxiR (5'-CCT AAA ACA TAA TGA AAA TGW GC-3') for partial amplification of the mitochondrial *COI* gene (Subbotin, 2021). PCR products were purified and sequenced by Azenta (CA, USA). Newly generated sequences were deposited in GenBank under the accession numbers PX995108-PX995129 (D2-D3 region of the 28S rRNA gene), PX995130-PX995134 (ITS rRNA gene), and PZ023928-PZ023940 (*COI* gene). Accession numbers of new sequences obtained in this study are given in Table 1 and incorporated into the phylogenetic trees.

Newly obtained sequences were aligned with corresponding published sequences of the D2-D3 expansion segments of the 28S rRNA, ITS rRNA, and *COI* genes (Vovlas *et al.*, 2008; Bae *et al.*, 2009; Subbotin *et al.*, 2011, 2015, 2025; Cantalapiedra-Navarrete *et al.*, 2013; Van den Berg *et al.*, 2013, 2017; Holguin *et al.*, 2015; Kolombia *et al.*, 2017; Mejía-Madrid, 2018; Munawar *et al.*, 2024; Mwamula *et al.*, 2024; Maosa *et al.*, 2025; Mwesige *et al.*, 2025 and others) using ClustalX 1.83 (Chenna *et al.*, 2003) with default settings. Phylogenetic analyses were conducted using Bayesian inference (BI) implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). For each gene dataset, BI analyses were initiated from a random starting tree and run with four Markov chains for 1.0×10^6 generations. Posterior probability (PP) values are indicated on the corresponding clades. Sequence alignment analyses were performed using PAUP*

4b10 (Swofford, 2003). Pairwise genetic divergences among taxa were calculated as absolute distances and as mean percentage distances based on the complete alignments, with adjustments for missing data.

RESULTS

More than two hundred soil samples were analysed, and representatives of the subfamily Hoplolaiminae were detected in the majority of them, indicating their widespread occurrence in the surveyed areas. From this material, specimens from 16 selected soil samples were chosen for detailed taxonomic study. Using traditional morphological taxonomic characters integrated with molecular criteria, we distinguished two new species, *Hoplolaimus texcocoensis* sp. n. and *S. montecilloensis* sp. n., four known species, *Helicotylenchus dihystra*, *Hoplolaimus seinhorsti* Luc, 1958, *S. brachyurus* (Steiner, 1938) Andrassy, 1958, and *S. bradys* (Steiner & LeHew, 1933) Andrassy, 1958, five unidentified *Helicotylenchus* species (sp.1-sp.5) and three unidentified *Rotylenchus* species (sp.1-sp.3). Morphological descriptions are provided for *Hoplolaimus texcocoensis* sp. n., *H. seinhorsti*, *S. montecilloensis* sp. n. and *S. bradys* based on detailed examinations of well-preserved specimens. Due to the limited number of available specimens for the remaining taxa, morphological characterisation was not undertaken. In these cases, species identification relied primarily on molecular results, supplemented by preliminary morphological observations where possible. Additional sampling will be necessary to obtain sufficient material for full morphological analyses and formal descriptions of these taxa.

Hoplolaimus texcocoensis sp. n. (Figs 1, 2A, 3A, 4A, 5)

Measurements: See Table 2.

Female. Body vermiform, cylindrical with slight curved or C-shaped when fixed. Lip region trapezoid shaped, set off from body contour, with massive cephalic framework, bearing small blocks of different size resulting from longitudinal striations. Stylet massive with tulip-shaped basal knobs. Pharyngeal glands with three nuclei. Excretory pore at 10.9-14.0% of body length and located one annulus posterior to the hemizonid. Lateral field with four incisures, completely areolated, although irregularly between the inner incisures and completely areolated at scutellum level. Lateral field of anterior of body, narrowing to two incisures; at ten annuli posterior to basal annulus of the lip region. Both scutella are

posterior to the vulva. Vulva located near mid-body, with deep transverse slit and epiptygma double but inconspicuous. Two ovaries outstretched. Anterior spermatheca 31.3 µm long and 24.3 µm wide and posterior spermatheca 25.9 µm long and 21.6 µm wide on average, containing oval shape sperm. Tail hemispherical, intestine overlapping rectum.

Male. Body cylindroid, vermiform. Body length slightly shorter than females. Lip region set off, less developed than females, bearing five annuli. Basal annulus is striated longitudinally with 38 striations. Stylet knobs tulip-shaped. Excretory pore at 11.9-13.9% of body length, located one annulus posterior to hemizonid. Lateral field with four incisures, areolated, Spicules with delicate velum present and gubernaculum prominent, bursa 60-82 (72.5 ± 6.7) µm long. Titillae absent.

Type locality and host. All the specimens were obtained from soil collected in a nursery of forest bamboo trees in Campus Montecillo Colegio de Postgraduados, Texcoco State, Mexico, around the roots of bamboo, *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Poaceae).

Type material. Type specimens are deposited in the Laboratorio de Helminología del Instituto de Biología, National University of Mexico (UNAM), Mexico. Holotype female: CNHE 11591, allotype male: CNHE 11815, paratype females: CNHE 11816. Other paratype materials (A-116) are deposited in the Colegio de Postgraduados Nematode Collection (CPNC), Mexico and the Nematology Collection, University of California, Davis, CA, USA. LSID urn:lsid:zoobank.org:act:B0DC66C5-D573-47A4-97BE-6825D84E4700.

Etymology. Specific name derived from the type locality, Texcoco County, Mexico State.

Diagnosis and relationships. *Hoplolaimus texcocoensis* sp. n. is similar to *H. californicus* Sher, 1963 in having the hemizonid one annulus anterior to the excretory pore, four incisures in lateral field with outer band completely areolated. It differs, in having blocks of different size in the lip region without distinct annuli vs six lip annuli in *H. californicus*, and in outer and inner band completely areolated vs inner band not areolated. Also, *H. texcocoensis* sp. n. differs from *H. californicus* in the position of the vulva, 51.4-54.2 (53)% vs 52-61%, in the stylet length of females and males, 39-45 (42.4) µm and 40-45 (42) µm long vs 46-53 µm and 42-50 µm long, respectively, the spicule length, 37.6-50 (43.8) µm vs 45-55 µm and titillae absent vs present. *Hoplolaimus texcocoensis* sp. n. is similar at *H. maggentii* in body length of females, and index b; it differs from it in the presence vs absence of males, in the position of anterior phasmid, 51-71 (61.4)% vs 41% and in the

position of excretory pore 147.6-200 (164.1) µm vs 104-139 µm from the anterior end. *Hoplolaimus texcocoensis* sp. n. is similar to *H. igualaensis* in the position of the vulva, and in the position of anterior and posterior scutella. The new species differs from it in female length, 1.13-1.68 (1.36) mm vs 1.06-1.46 (1.25) mm, in the ornamentation of the lip region bearing small blocks of different size without lip annuli vs the basal annulus with 5-6 lip annuli. *Hoplolaimus texcocoensis* sp. n. is clearly differentiated from other sequenced *Hoplolaimus* species by sequences of the 28S rRNA, ITS rRNA and *COI* gene sequences.

Hoplolaimus seinhorsti Luc, 1958 (Fig. 6)

Measurements. See Table 2.

Female. Body open C shape when fixed. Lip region set off from body, rounded to trapezoid shape and, in some specimens, hemispherical, with four irregular annules that are not well defined and six longitudinal incisures forming six blocks between the base of the cephalic annuli and the labial disc. Basal annulus of lip region wider than others, without striations. Stylet knobs with small anterior projections. Pharyngeal glands with 4-5 nuclei. Intestine does not overlap the rectum. Excretory pore anterior to the hemizonid, invisible in some specimens. Lateral field without incisures; anterior scutellum 263-325 µm anterior to the vulva and the posterior scutellum 215-347 µm posterior to the vulva. Scutella not areolated. Epiptygma single, present only in some specimens. Spermatheca empty and poorly developed. Tail rounded with 10 to 15 annuli to posterior end of body.

Male. Not found.

Locality. Soil around banana plant *Musa acuminata* L. (Musaceae) in the Ecology Experimental Station La Mancha of Ecology Institute Veracruz State, Mexico.

Materials. Nematode specimens (CNHE 12154) are deposited in the Helminthology Collection of Biology Institute of National University of Mexico (UNAM) and (A-121) in the Colegio de Postgraduados Nematode Collection.

Scutellonema montecilloensis sp. n. = *Scutellonema* sp. A apud Van den Berg *et al.* 2013 (Figs 2B-D, 3B-D, 7 & 8)

Two populations of unidentified *Scutellonema* sp. A, previously morphologically and molecularly characterised by Van den Berg *et al.* (2013, 2017)

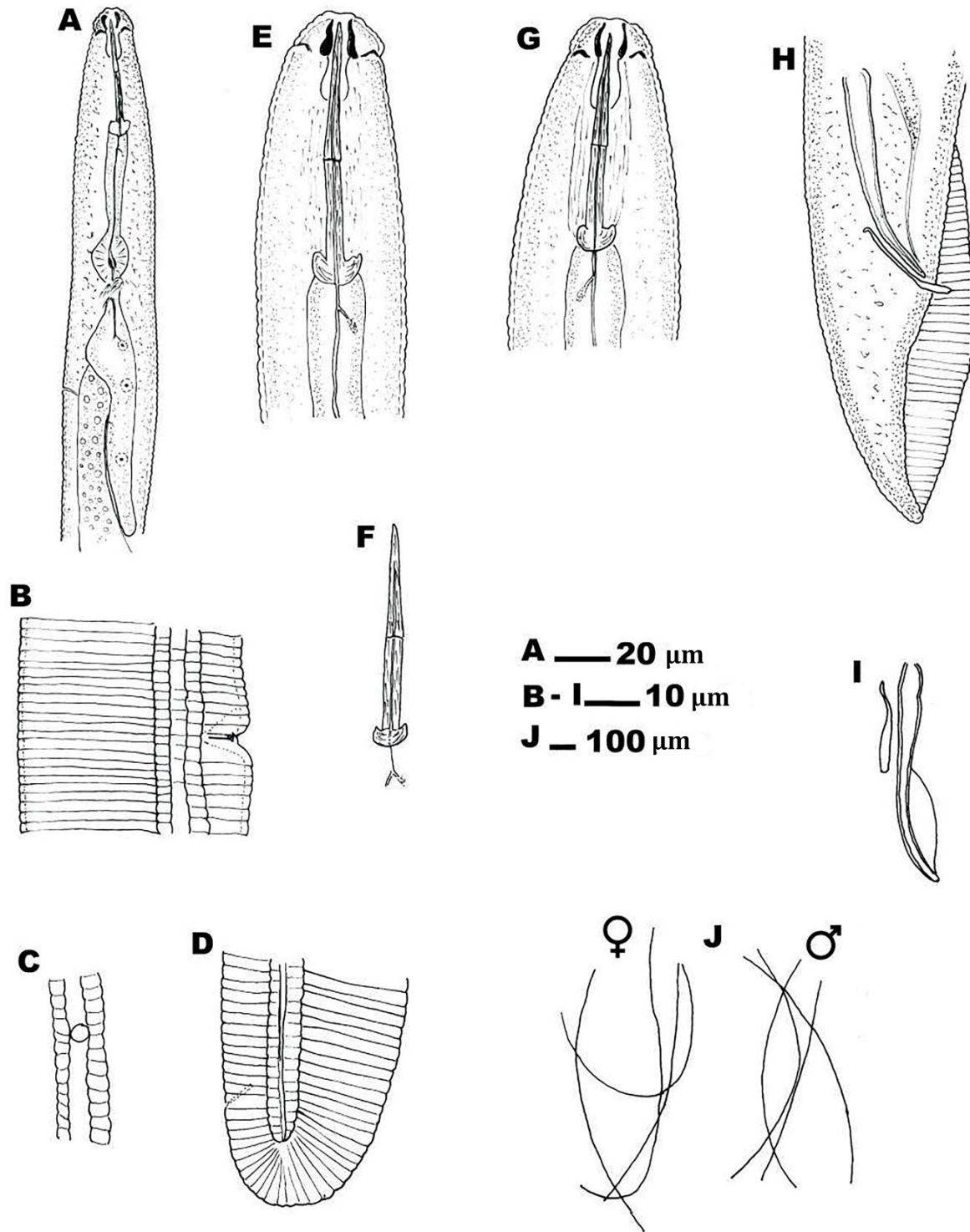


Fig. 1. *Hoplolaimus texcocoensis* sp. n. Female (A-F). A: Cervical region, dorsal view; B: Lateral view at vulva level; C: Anterior scutellum; D: Tail; E: Anterior end body; F: Stylet. Male (G-I). G: Anterior end body; H: Tail; I: Gubernaculum and spicule; J: Body shapes for females and males.

Table 2. Morphometrics of *Hoplolaimus texcocoensis* sp. n. and *H. seinhorsti* analysed in the present study. All measurements are in μm and in the form: mean $\pm$ s.d. (range).

Characters	Species <i>H. texcocoensis</i> sp. n. Holotype female	<i>H. texcocoensis</i> sp. n. Paratype females	<i>H. texcocoensis</i> sp. n. Paratype males	<i>H. seinhorsti</i> Females
n	1	11	18	12
L (mm)	1.27	1.36 $\pm$ 0.2 (1.13-1.68)	1.2 $\pm$ 0.1 (1.0-1.5)	1.3 $\pm$ 0.1 (1.0-1.6)
a	30.1	29.8 $\pm$ 3.5 (23.1-34.4)	29.9 $\pm$ 3.9 (22-37.3)	29.1 $\pm$ 4.6 (18.8-37)
b	8.6	8.9 $\pm$ 1.8 (4.6-10.7)	8.3 $\pm$ 1.1 (6.0-10.3)	11.6 $\pm$ 1.8 (8.8-15.1)
b'	6.7	6.8 $\pm$ 1.5 (4.3-9.6)	—	8.7 $\pm$ 1.4 (6.9-11.0)
c	45.2	53.5 $\pm$ 13.2 (42.8-89.5)	32.9 $\pm$ 5.1 (27.5-48.9)	53.2 $\pm$ 11.3 (34.4-70.7)
c'	0.73	0.7 $\pm$ 0.1 (0.6-0.9)	1.5 $\pm$ 0.4 (0.6-2.0)	0.8 $\pm$ 0.1 (0.7-1.0)
V/T%	53.4	53.2 $\pm$ 1.8 (50.9-56.8)	45.9 $\pm$ 6.7 (34.7-54.9)	55.8 $\pm$ 2.5 (52.8-60)
Lip width	15	16.4 $\pm$ 0.9 (15.0-18.0)	15.1 $\pm$ 1.3 (11.0-17)	15.0 $\pm$ 1.1 (7.5-13.0)
Lip annulus number	5	4.9 $\pm$ 0.3 (4.0-5.0)	5.3 $\pm$ 0.5 (5.0-6.0)	4.0
Stylet	42	43.3 $\pm$ 2.6 (39.5-48.0)	42.0 $\pm$ 1.4 (40-45)	41.5 $\pm$ 2.1 (38-45.0)
o	9.5	9.9 $\pm$ 2.2 (6.6-13.9)	10.1 $\pm$ 1.2 (8.8-12)	15.1 $\pm$ 5.4 (8.2- 25.0)
DGO	9.5	9.9 $\pm$ 2.2 (6.6-11.6)	4-5	6.6 $\pm$ 2.1 (5.0-10.0)
Nerve ring from anterior end	122	119.8 $\pm$ 8.0 (108.1-135)	121.5 $\pm$ 11.4 (90-135)	103.3 $\pm$ 10.7 (90-117)
Excretory pore from anterior end	153	164.1 $\pm$ 16.1 (147.6-200)	153.8 $\pm$ 10.1 (140-178)	117.7 $\pm$ 8.9 (103-131)
Median bulb diam.	17.0	18.5 $\pm$ 2.0 (15.9-21.0)	14.4 $\pm$ 2.1 (11.0-20.0)	16.2 $\pm$ 1.6 (13-18)
Median bulb length	20.0	20.1 $\pm$ 1.0 (18.8-21.6)	19.6 $\pm$ (14.0-20.0)	16.7 $\pm$ 1.8 (14-20)
Pharynx length	148	219 $\pm$ 42.5 (172-285)	188 $\pm$ (150-233)	124.4 $\pm$ 14.3 (102-140)
Anterior scutellum % body	71	61.4 $\pm$ 6.5 (51-71)	63.2 $\pm$ 5.3 (56-72)	29.7 $\pm$ 2.9 (24.5-33.7)
Posterior scutellum % body	85	83.4 $\pm$ 23.7 (77-86)	81.8 $\pm$ 10.5 (58.8-92.6)	79.3 $\pm$ 2.7 (75.7-82.9)
Body diameter at vulva	45	46.4 $\pm$ 5.4 (41-57)	—	45.4 $\pm$ 5.2 (38-54)
Maximum body diameter	42	46.6 $\pm$ 7.7 (32.9-58.3)	39.9 $\pm$ 4.8 (30-49)	46 $\pm$ 5.2 (40-55)
Tail length	28	26.1 $\pm$ 4.4 (18.8-31.9)	36.5 $\pm$ 5.6 (21.0-46)	24.8 $\pm$ 3.5 (20-30)
Tail width	38	34.2 $\pm$ 6.2 (24-46)	26.3 $\pm$ 8.1 (20-49)	30.2 $\pm$ 2.2 (27-34)
Spicules	—	—	43.2 $\pm$ 3.3 (37.6-50)	—
Gubernaculum	—	—	21.7 $\pm$ 3.5 (15.0-26.0)	—

from Southern California, are considered here as representatives of *S. montecilloensis* sp. n.

Measurements: See Table 3.

Female. Body arcuate or spiral shaped with hemispherical-truncate lip region marked with 3-4 annuli. Labial disc slightly distinct, lip region slightly set off from body and divided into six sectors by fine longitudinal striae from the basal annulus to labial disc. Labial framework moderately developed. Anterior cephalid located three annuli behind the base of lip region, posterior not seen. Stylet long,

robust, with knobs rounded posteriorly and slightly indented anteriorly. Pharyngeal gland lobe variable in length 20-50 μm long, with three gland nuclei. Excretory pore opposite posterior of pharyngeal gland lobe. Hemizonid 4-8 μm and two annuli long and visible anterior to excretory pore. Hemizonion not seen. Intestine not extending posterior to anus. Lateral field with four lines and three equal bands not areolated; there is areolation on the two outer bands only in the anterior end of the body and at level of scutellum. Scutella situated at level of anus or

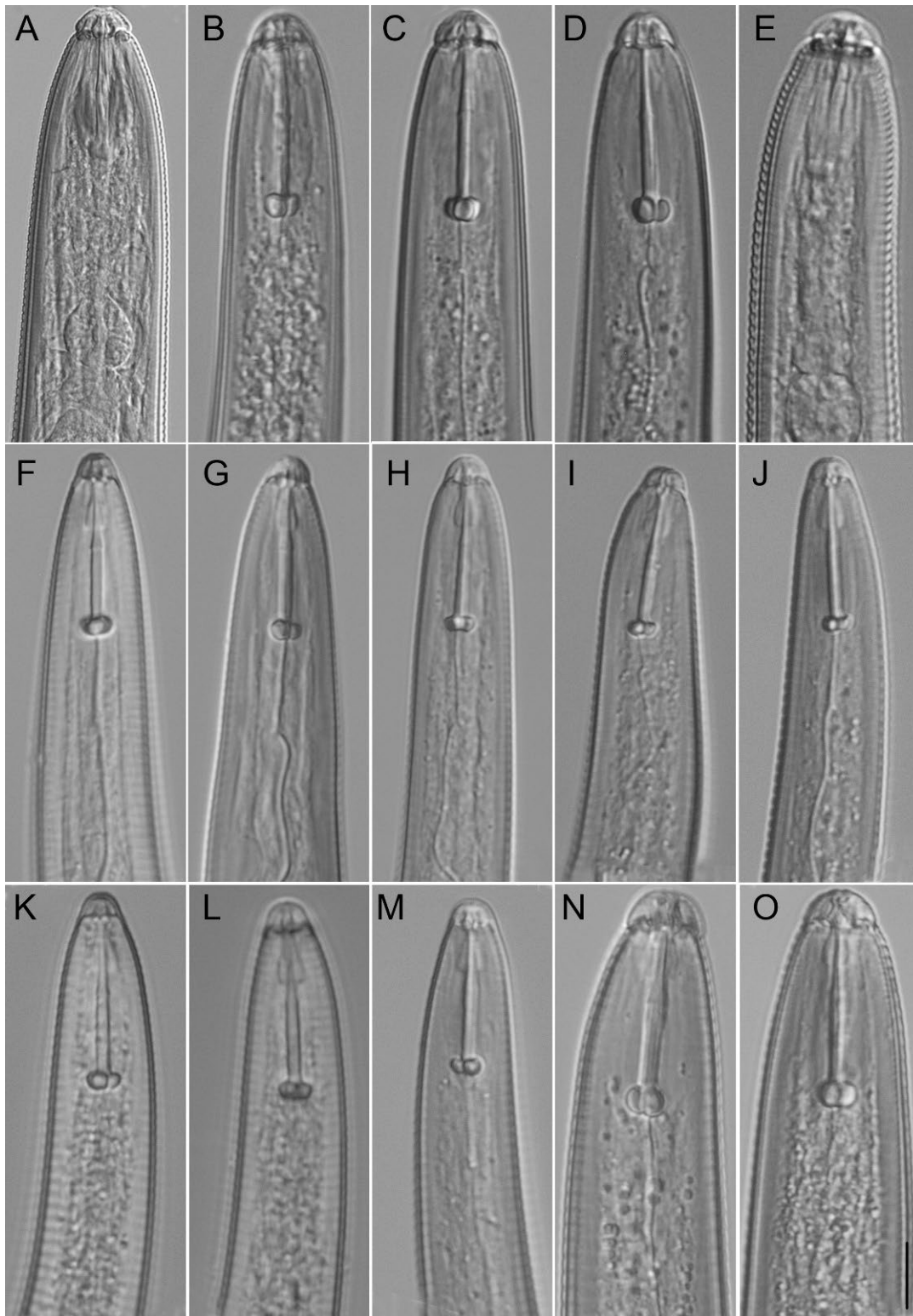


Fig. 2. Anterior regions of females of nematodes from the subfamily Hoplolaiminae. LM photographs. A: *Hoplolaimus texcocoensis* sp. n.; B-D: *Scutellonema montecilloensis* sp. n. (CD2797); E: *S. bradys* (CD4227); F, G: *Helicotylenchus* sp. 5 (CD3018); H: *Helicotylenchus* sp. 3 (CD2799); I, J: *Rotylenchus* sp. 3 (CD2807); K, L: *Helicotylenchus* sp. 2 (CD2825); M: *Helicotylenchus dihystra* (CD2829); N, O: *Rotylenchus* sp. 1 (CD2830). Scale = 10 μ m for B-O and 20 μ m for A.

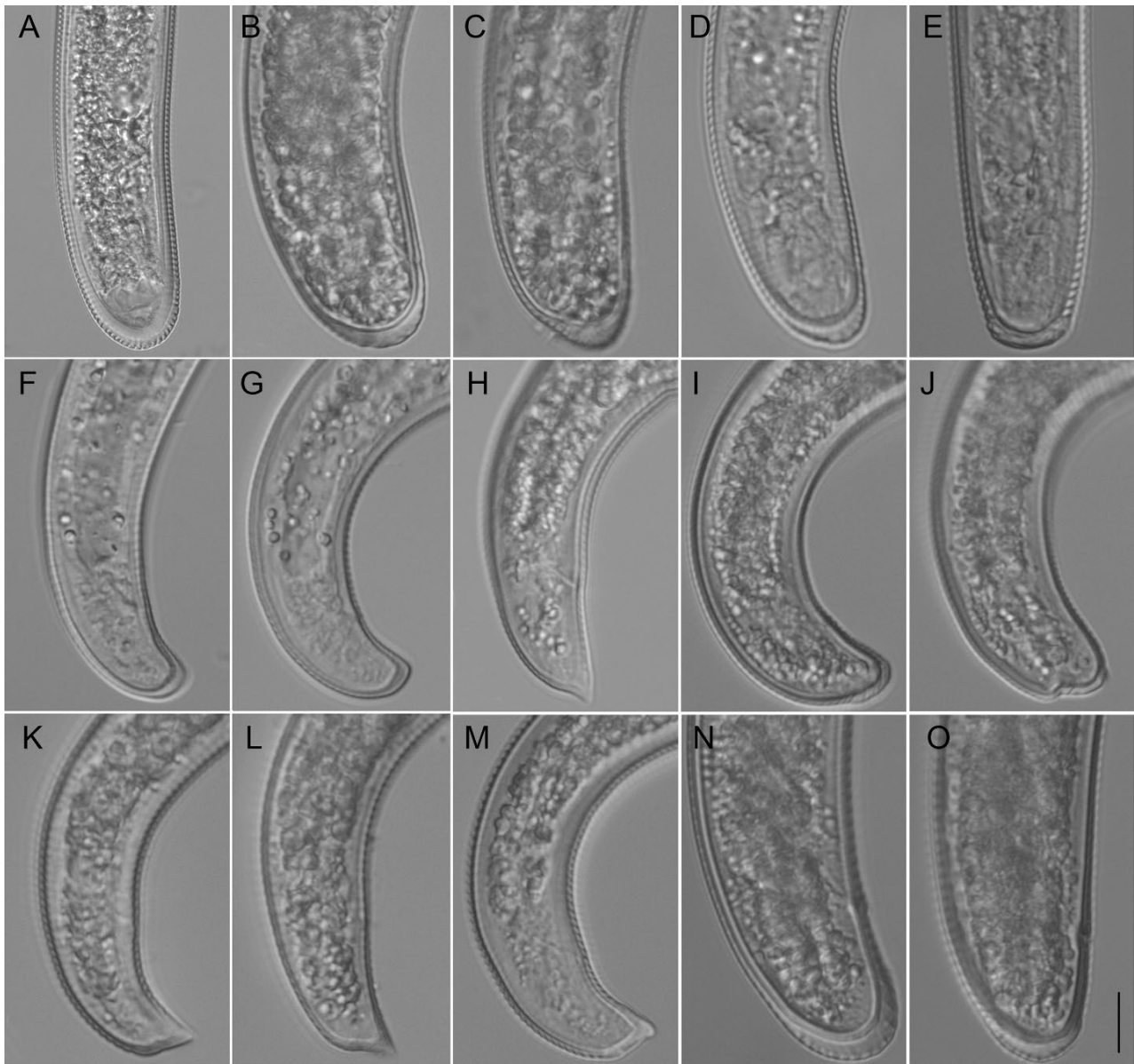


Fig. 3. Posterior regions of females of nematodes from the subfamily Hoplolaiminae. LM photographs. A: *Hoplolaimus texcocoensis* sp. n.; B-D: *Scutellonema montecilloensis* sp. n. (CD2797); E: *S. bradys* (CD4227); F, G: *Helicotylenchus* sp. 5 (CD3018); H: *Helicotylenchus* sp. 3 (CD2799); I, J: *Rotylenchus* sp. 3 (CD2807); K, L: *Helicotylenchus* sp. 2 (CD2825); M: *Helicotylenchus dihystra* (CD2829); N, O: *Rotylenchus* sp. 1 (CD2830). Scale = 10 μ m for B-O and 20 μ m for A.

anterior to it. Spermatheca small, poorly developed and empty, 3-8 μ m long and 5-10 μ m wide. Two small, oblong vaginal glands with conspicuous nuclei. Vulval slit with thickened edges, epiptygma absent. Tail cylindrical with rounded terminus, consisting of 9 to 12 annuli.

Male. Not found.

Type host and locality. Soil from *Bambusa vulgaris* Schrad. ex J.C. Wendl in a nursery of forest trees, in the Campus Montecillo, Colegio de Postgraduados, Texcoco State, Mexico.

Other localities. This species was found in a soil sample from grass roots collected at Fairmount Park, Riverside, CA, USA, and from a soil sample collected from roses at Escondido, CA, USA (Van den Berg *et al.*, 2013).

Type material. Holotype (11605) and paratype (11817) slides are deposited in the Helminthology Collection of Biology Institute of National University of Mexico. Paratypes slide (A-117) is also deposited in the Colegio de Postgraduados Nematode Collection (CPNC) Mexico and the Nematology

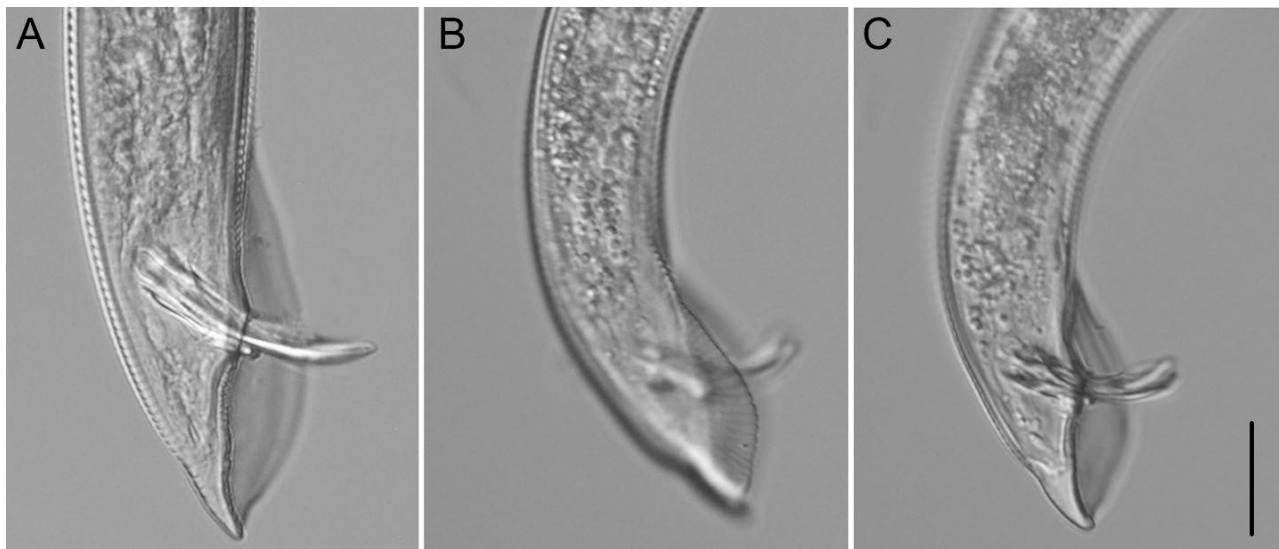


Fig. 4. Posterior regions of males of nematodes from the subfamily Hoplolaiminae. LM photographs. A: *Hoplolaimus texcocoensis* sp. n.; B, C: *Rotylenchus* sp. 1. Scale = 20 μ m for A and 10 μ m for B and C.

Collection, University of California, Davis, CA, USA. LSID urn:lsid:zoobank.org:act:D3F72377-69C4-46A9-A3B2-160C13302F7A.

Etymology. Specific name derived from type locality, Montecillo, Mexico State.

Diagnosis and relationships. *Scutellonema montecilloensis* sp. n. is characterised by females with a hemispherical-truncate lip region, with four annuli and the basal lip annulus with fine longitudinal striations, lip region divided into six sectors by oblique fine longitudinal striae from the basal annuli to labial disc. Males are absent. Lateral field with four incisures, not areolated, scutellum at level of anus or anterior to it. Tail cylindrical with rounded terminus. Stylet 25-30 μ m long. Vaginal glands present with conspicuous nuclei. Spermatheca poorly developed and not containing sperm.

Scutellonema montecilloensis sp. n. is similar to *S. cephalidium* Anderson, Handoo & Townshend, 1984 (Anderson *et al.*, 1984), in the C or spiral shape of the body after fixation, in the size of the body and in the indexes a, and b, in V% and size of the stylet, the lip region having fine longitudinal striae forming irregular sectors, and in distance of excretory pore to anterior end of the body. *Scutellonema montecilloensis* sp. n. differs from *S. cephalidium* in the pharynx length 142-308 (227.7) μ m vs 133-154 (141) μ m, in the index c 47-71 (61.5) vs 62-122 (78), in the shape of the stylet knobs, with anterior projection vs rounded and in the length of the tail, 10-16 (12.1) μ m vs 6.0-13 (10) μ m.

Scutellonema montecilloensis sp. n. is different from *S. clathricaudatum* Whitehead, 1959 in the shape of the stylet knobs, with oval shape to anterior

projections vs rounded, lip region with 3-4 annuli vs 4-9 annuli, shorter tail length 10-16 (12) μ m vs 11.5-22 (18) μ m. *Scutellonema montecilloensis* sp. n. is very similar to *S. brachyurus* and differs from this species by longer average of the stylet length, 28 vs 29-30 μ m, and larger average of index a 25.9-27.7 vs 19-21.5. *Scutellonema montecilloensis* sp. n. is clearly differentiated from other sequenced *Scutellonema* species by the 28S rRNA, ITS rRNA and COI gene sequences.

Scutellonema bradys (Figs 2E, 3E & 9)

Measurements: See Table 3.

Female. Body straight or slightly curved. Lip region rounded, offset from body contour by a slight constriction, with 6 annuli. Longitudinal striations between basal annulus and labial disc are absent. Lateral fields have three bands (four incisures) and the areolation along the body on the external bands is more abundant around and posterior to the scutella; the central band is not areolated and is narrower. Stylet knobs are oval shaped. Hemizonid 7-10 annuli anterior to excretory pore. Spermatheca rounded, filled with sperm, the anterior 16.4 ± 2.1 (13-20) μ m long and 14.3 ± 1.7 (11-17) μ m wide; the posterior 16.4 ± 2.9 (12-20) μ m long by 13.9 ± 1.8 (11-16) μ m wide. Vaginal glands present with evident nuclei. Epiptygma absent. Scutella rounded, located anterior to the anus, areolated and occupying two annules. Tail 10 to 15 annuli long, rounded with striated terminus.

Male. Body sigmoid shaped when fixed. Lip

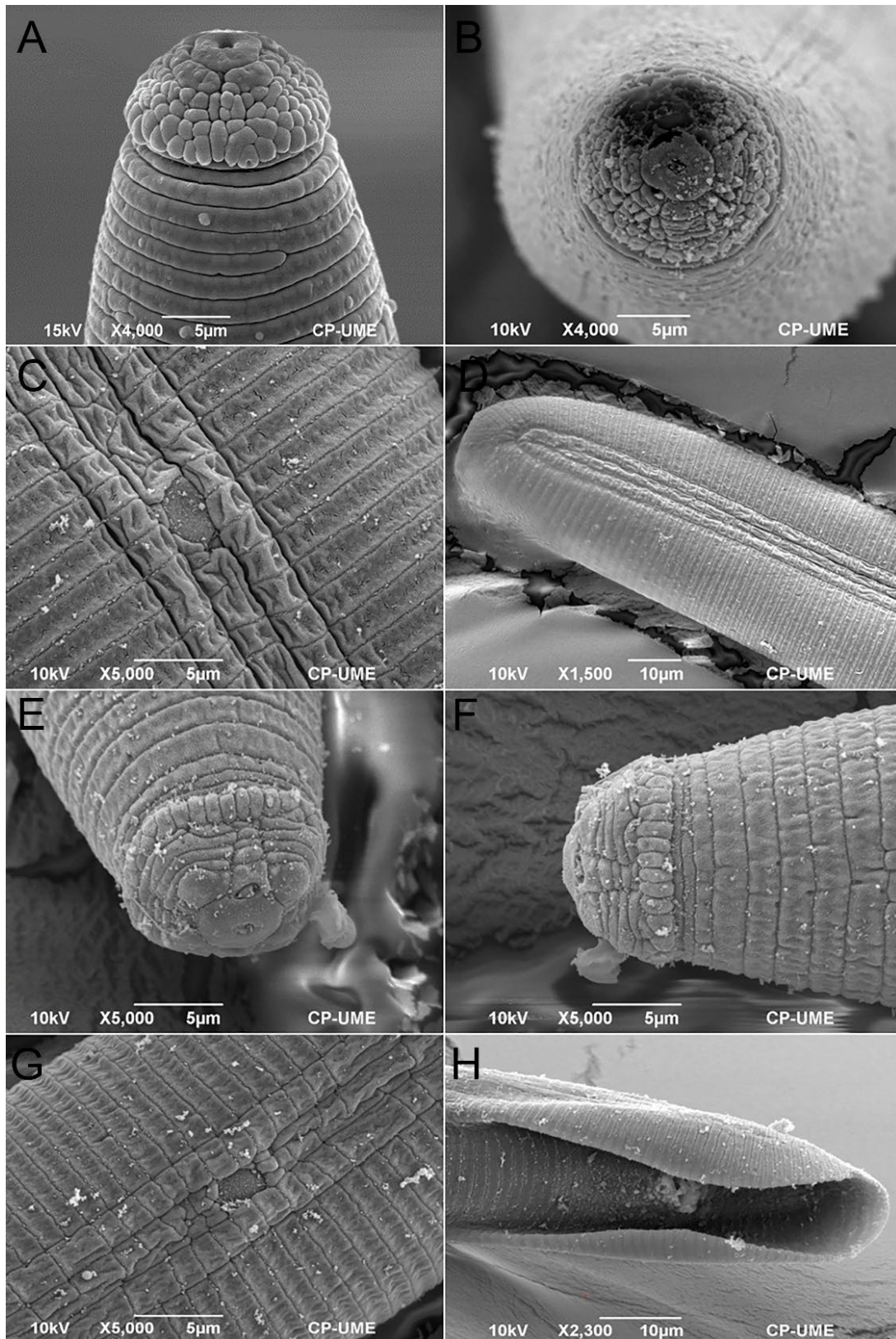


Fig. 5. *Hoplolaimus montecilloensis* sp. n. SEM photographs. Female (A-D). A: Cephalic region, dorsal view; B: Cephalic region, frontal view; C: Anterior scutellum; D: Tail. Male (E-H). E: Cephalic region, frontal view; F: Cephalic region, lateral view; G: Posterior scutellum; H: Tail, ventral view.

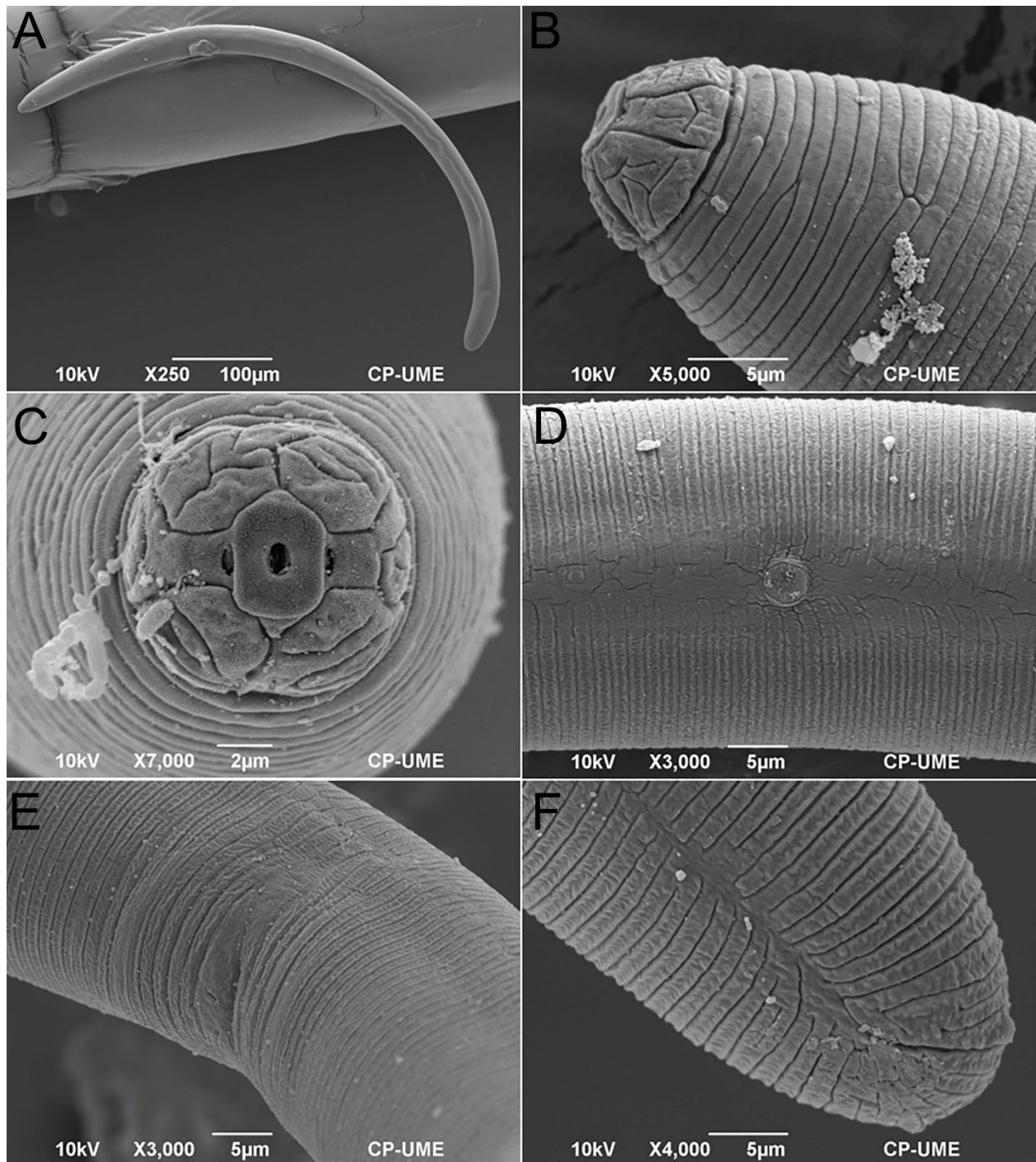


Fig. 6. *Hoplolaimus seinhorsti*. SEM photographs. Female. A: Body shape; B: Cephalic region, lateral view; C: Face view; D: Anterior scutellum; E: Vulva, lateral view; F: Tail.

region rounded, with 5 to 6 annuli, without longitudinal striations. Stylet knobs rounded. Excretory pore situated at level with the mid-length of the pharyngeal glands. Lateral fields not areolated. Gubernaculum without swollen distal end.

Locality. Rhizosphere soil of grass in the dunes of Ecology Station of Institute of Ecology (INECOL)

Veracruz State.

Materials. Slides with specimens (CNHE 12154) are deposited in the Laboratorio de Helmintología del Instituto de Biología, National University of Mexico (UNAM), Mexico. Paratypes slide (A-117) is also deposited in the Colegio de Postgraduados Nematode Collection (CPNC A-120).

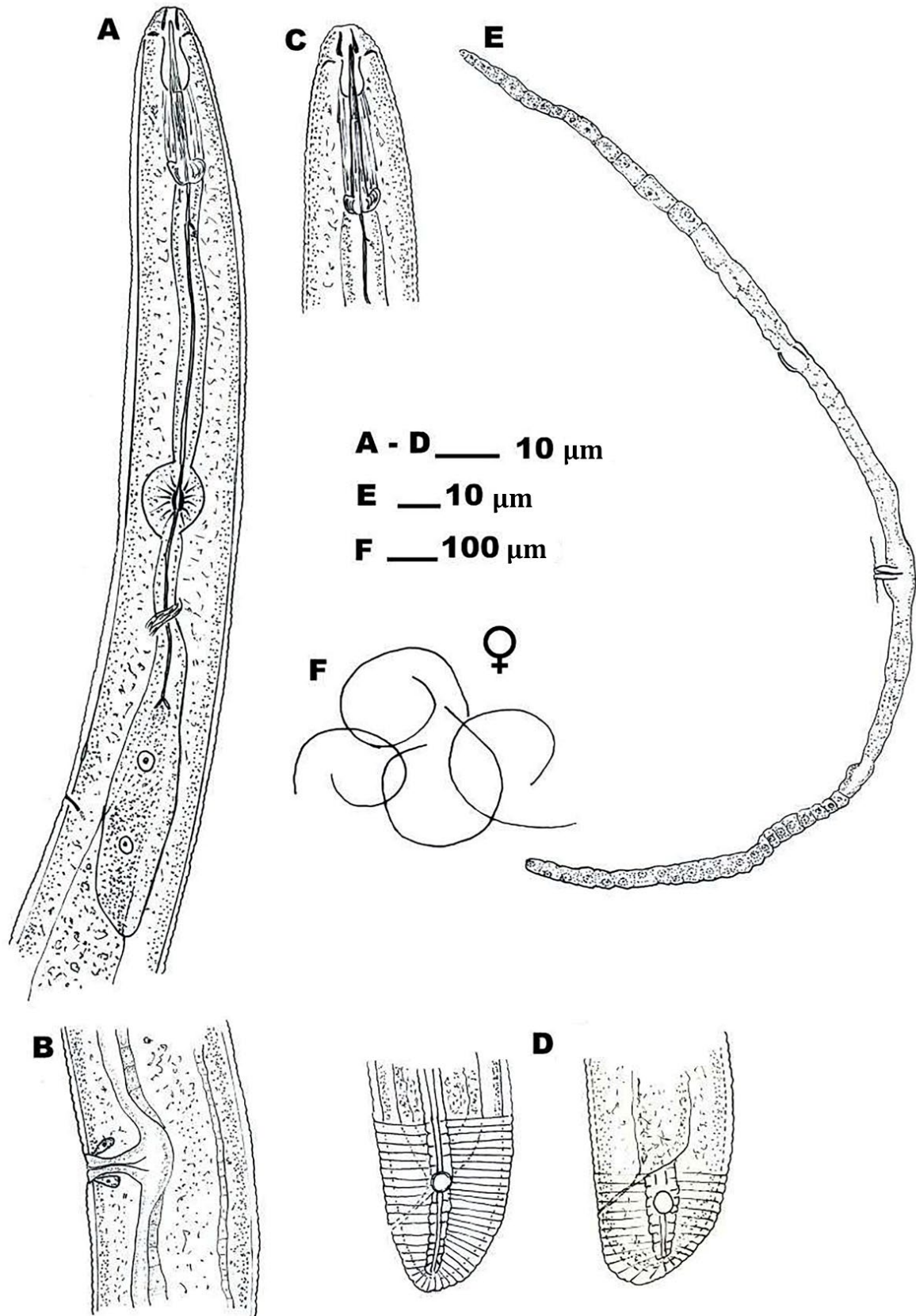


Fig. 7. *Scutellonema montecilloensis* sp. n. Female. A: Anterior body end; B: Lateral view at vulva region; C: Anterior body region; D: Tail and scutellum; E: Anterior and posterior gonads; F: Body shapes.

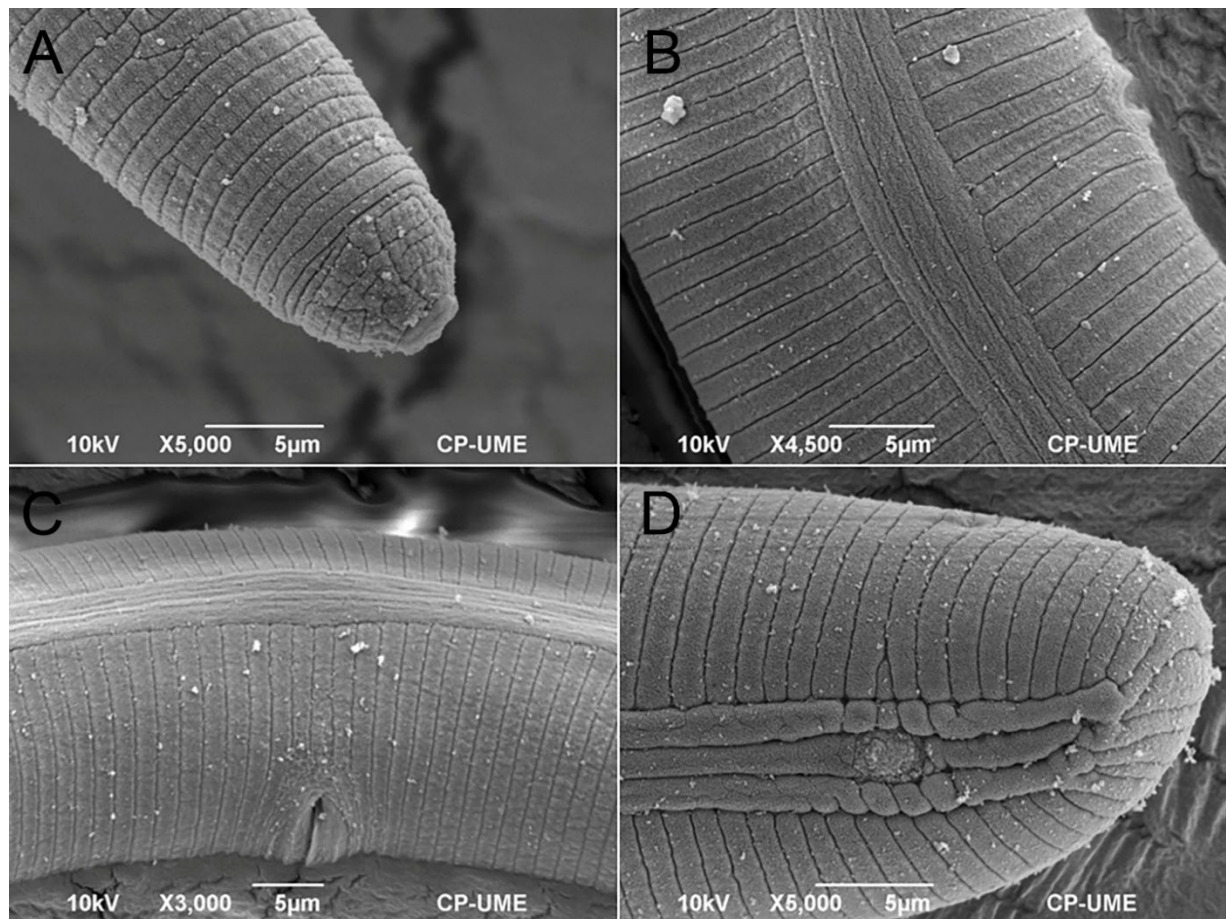


Fig. 8. *Scutellonema montecilloensis* sp. n. SEM photographs. Female. A: Anterior region of body; B, C: Lateral ventral view at vulva region; D: Tail and scutellum.

***Helicotylenchus* and *Rotylenchus* species (Figs 2-4)**

Helicotylenchus dihystra, five unidentified *Helicotylenchus* species (sp.1-sp.5) and three unidentified *Rotylenchus* species (sp.1-sp.3) were recovered during the present survey. Although several of these taxa could not be assigned to described species due to limited material and inconclusive morphological differentiation, their distinct molecular profiles support their recognition as separate lineages pending further study. Light micrographs of females are provided to document key morphological features and facilitate future comparisons. These include *Helicotylenchus dihystra* (Figs 2M & 3M), *Helicotylenchus* sp. 2 (Figs 2K, L; 3K, L), *Helicotylenchus* sp. 3 (Figs 2H & 3H), *Helicotylenchus* sp. 5 (Figs 2F, G; 3F, G), *Rotylenchus* sp. 1 (Figs 2N, O; 3N, O), and *Rotylenchus* sp. 3 (Figs. 2I, J; 3I, J). These images illustrate diagnostic characters such as lip region morphology, stylet structure, and tail shape. Males

were observed only in *Rotylenchus* sp. 1 (Fig. 4B). The presence of males in this population provides additional morphological information, including spicule structure and gubernaculum morphology, which may prove valuable for future taxonomic resolution. Further sampling and integrative analyses will be required to determine whether these unidentified taxa represent previously described species or novel, potentially endemic forms.

Molecular characterisation and phylogenetic relationships.

D2-D3 expansion segments of 28S rRNA gene.

The alignment comprised 97 sequences representing members of the subfamily Hoplolaiminae, along with three outgroup taxa, and had a total length of 765 bp. Twenty-two sequences were newly generated in the present study. The phylogenetic relationships of Hoplolaiminae representatives from Mexico, in comparison with related species worldwide, are shown in Figure 10. In the phylogenetic tree, sequences of *H. dihystra*, *S. brachyurus*, *S. bradys*

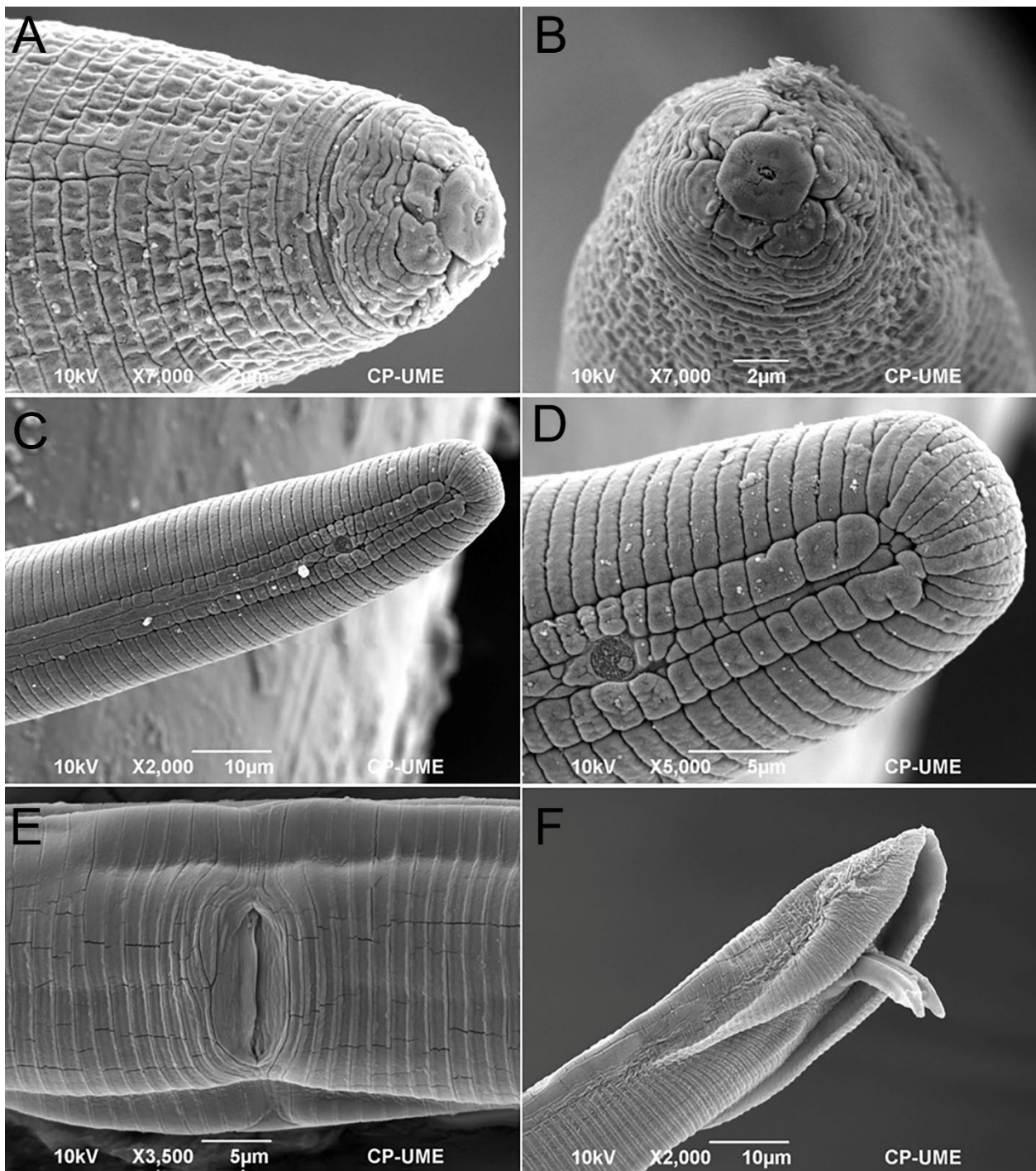


Fig. 9. *Scutellonema bradys*. SEM photographs. Female (A-E). A: Anterior region, lateral view; B: Cephalic region, frontal view; C: Posterior region; D: Tail and scutellum; E: Vulva, ventral view. Male (F). F: Tail with bursa.

and *H. seinhorsti* from Mexico clustered with sequences of the corresponding species from other countries, confirming their conspecificity. Sequences of *Helicotylenchus* spp. from Mexico formed a well-supported clade with *H. labiodiscinus* Sher, 1966 and *H. erythrinae* (Zimmermann, 1904) Golden, 1956. Sequences of three *Rotylenchus* species from Mexico

formed distinct lineages, clearly separated from other *Rotylenchus* species included in the analysis. Sequences of *S. montecilloensis* sp. n. clustered with those previously reported as *Scutellonema* sp. A from California, differing by only 0.7%, and were therefore considered conspecific. By contrast, sequences of *S. montecilloensis* sp. n. differed from

Table 3. Morphometrics of *Scutellonema montecilloensis* sp. n. and *S. bradys* analysed in the present study. All measurements are in μm and in the form: mean $\pm$ s.d. (range).

Species Characters	<i>S. montecilloensis</i> sp. n. Holotype	<i>S. montecilloensis</i> sp. n. Paratype females	<i>S. bradys</i> Females	<i>S. bradys</i> Males
n	1	19	9	4
L (mm)	0.792	0.73 $\pm$ 0.1 (0.62-0.91)	0.77 $\pm$ 0.08 (0.65-0.87)	0.67 $\pm$ 0.05 (0.63-0.73)
a	28.3	25.9 $\pm$ 2.9 (19.9-31.1)	27.1 $\pm$ 2.7 (24-31.8)	29.1 $\pm$ 0.8 (27.9-29.8)
b	6.9	6.8 $\pm$ 1.1 (4.6-8.2)	8.8 $\pm$ 1.9 (5.5-10.9)	8.1 $\pm$ 1.3 (6.6-9.7)
c	60.1	61.5 $\pm$ 6.2 (47-71.1)	39.3 $\pm$ 5.1 (32- 48)	29.4 $\pm$ 1.4 (27.9-31.2)
c'	0.6	0.6 $\pm$ 0.1 (0.6-0.8)	1.4 $\pm$ 0.3 (0.9-1.7)	1.5 $\pm$ 0.2 (1.2-1.7)
V/T%	61.9	59.2 $\pm$ 3.0 (51.4-63.6)	58.2 $\pm$ 3.0 (53.8-63.9)	42.8 $\pm$ 6.3 (36.4-50.8)
Lip width	9.0	8.9 $\pm$ 0.6 (8.0-10)	9.8 $\pm$ 0.7 (9.0-11)	9.0
Lip annulus number	4	4.0	5.9 $\pm$ 0.4 (5.0-6.0)	5-6
Stylet	28	27.8 $\pm$ 1.3 (25-30)	22.9 $\pm$ 1.3 (21-25)	22.0
DGO	5.0	5.2 $\pm$ 1.0 (4.0-8.0)	4.2 $\pm$ 0.8 (3.0-5.0)	3.8 $\pm$ 0.5 (3.0-4.0)
o	17.9	18.7 $\pm$ 4.0 (13.3-28)	18.3 $\pm$ 2.4 (14.3-20.8)	16.7 $\pm$ 2.7 (13.6-18.2)
Body width	28.0	28.2 $\pm$ 2.3 (25-36)	28.3 $\pm$ 1.9 (26-31)	23.5 $\pm$ 1.9 (22-26)
Nerve ring from anterior end	103	95.7 $\pm$ 9.1 (80-120)	74.9 $\pm$ 7.1 (66-87)	77.5 $\pm$ 6.5 (70-85)
Excretory pore from anterior end	133	122.6 $\pm$ 7.1 (107-133)	110 $\pm$ 10.0 (90-122)	111 $\pm$ 8.1 (104-120)
Pharynx length	150	227 $\pm$ 43.1 (142-308)	124 $\pm$ 14.3 (102-140)	120.8 $\pm$ 8.3 (110-130)
Scutellum width	5.0	4.4 $\pm$ 0.6 (3.0-5.0)	4.7 $\pm$ 0.5 (4.0-5.0)	4.0
Tail length	13.0	12.1 $\pm$ 1.5 (10-16)	20.3 $\pm$ 2.9 (17-23)	23.3 $\pm$ 2.1 (21.0-25)
Tail width	21.0	18.6 $\pm$ 1.8 (15-21)	21.3 $\pm$ 1.6 (19-24)	15.0
Tail annuli	11	11	11	12-18

those of *S. brachyurus* by 6.4%. Similarly, sequence of *H. texcocoensis* sp. n. from Mexico formed a distinct clade and differed from that of *Hoplolaimus* sp. A from California by 6.1%, supporting their separation at the species level. Sequence of a spiral nematode identified by Mejía-Madrid (2018) as *Helicotylenchus* sp. from Mexico was different in one nucleotide from that of *H. broadbalkiensis* Yuen, 1964 and, thus, it is considered as representative of this species. The genera *Helicotylenchus*, *Rotylenchus* and *Scutellonema* were non-monophyletic based on this analysis.

ITS rRNA gene. The alignment comprised 35 sequences representing species of *Scutellonema* and *Hoplolaimus*, with two sequences of *Helicotylenchus*

included as outgroup taxa, and had a total length of 948 bp. Five new sequences were generated in the present study. The phylogenetic relationships among species of *Scutellonema* and *Hoplolaimus* are shown in Figure 11. In the resulting phylogenetic tree, sequences of *H. seinhorsti*, *S. bradys* and *S. brachyurus* from Mexico clustered with sequences of the corresponding species from other geographic regions, supporting their species identification. The sequence of *S. bradys* from Mexico formed a distinct subclade with a sequence of the same species from Florida; however, these sequences differed by 6.8%, indicating notable intraspecific genetic divergence. Sequences of *H. texcocoensis* sp. n. from Mexico clustered with those previously reported as

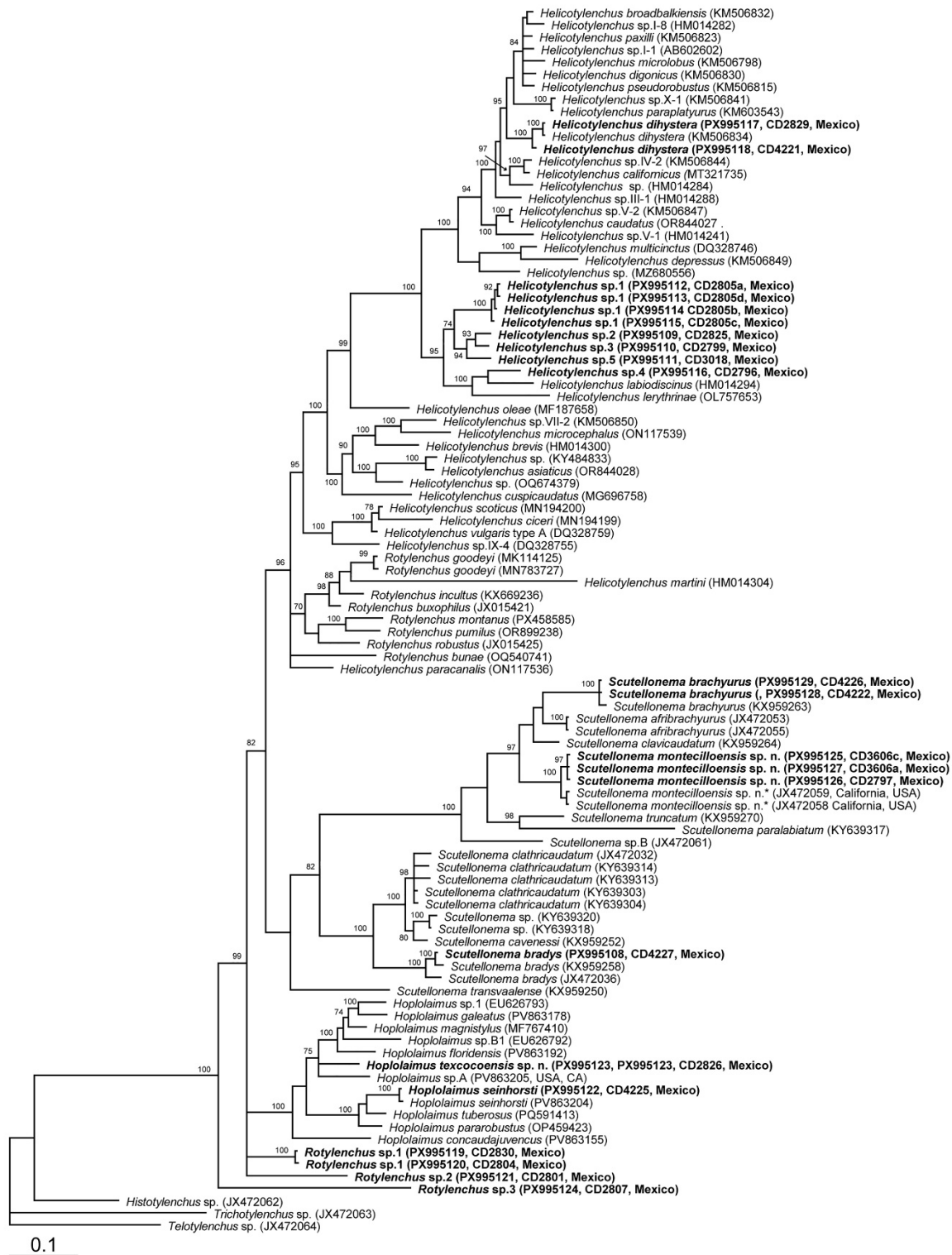


Fig. 10. Phylogenetic relationships of representatives of the subfamily Hoplolaiminae from Mexico with other species as inferred from Bayesian analysis of the D2-D3 of the 28S rRNA gene sequences under the GTR + I + G model. Posterior probabilities equal to or greater than 70% are given for appropriate clades. The new sequences are indicated in bold. * – identified as *Scutellonema* sp. A by Van den Berg *et al.* (2013, 2017); ** – identified as *Helicotylenchus* sp. by Mejía-Madrid (2018).

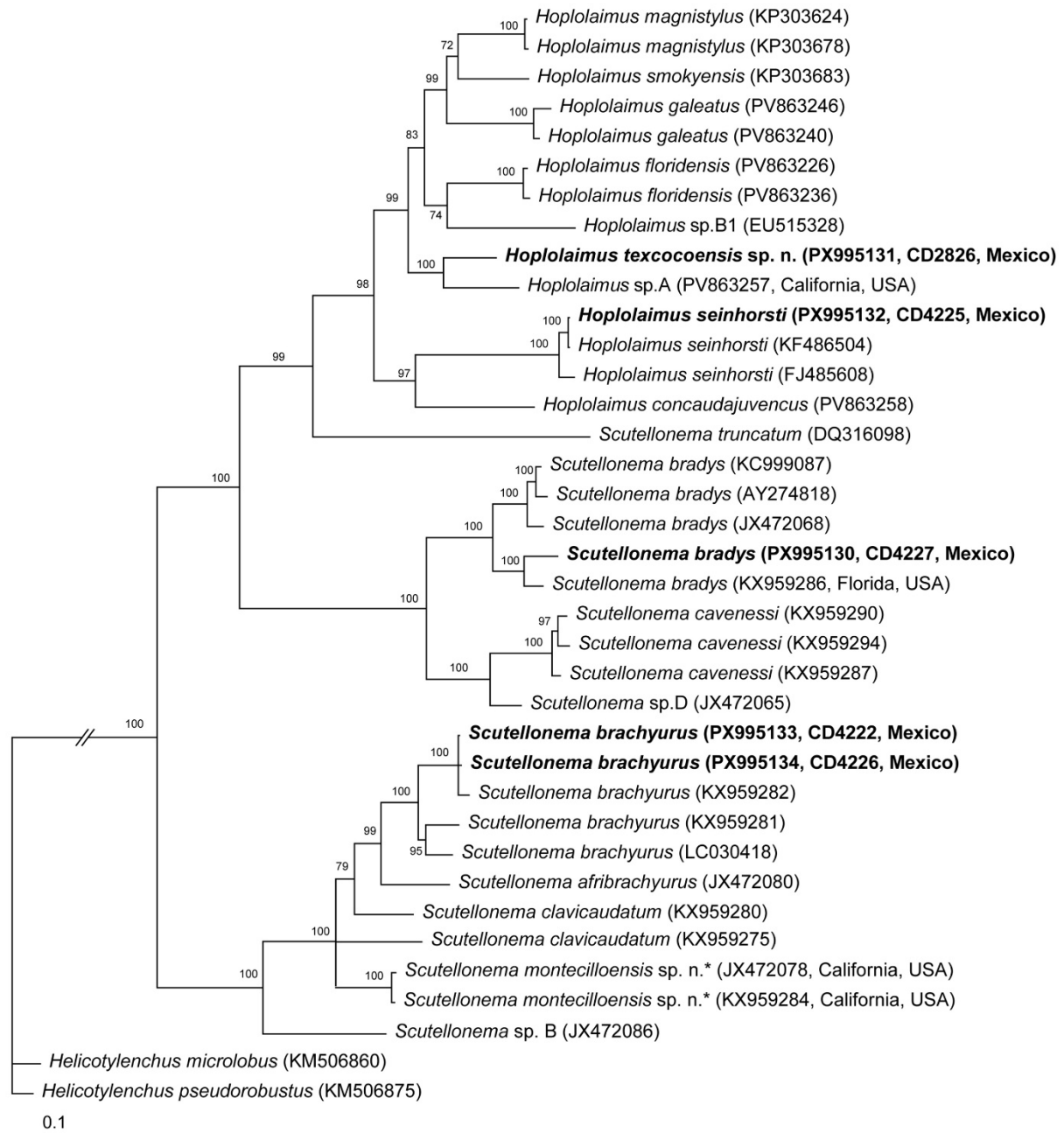


Fig. 11. Phylogenetic relationships of representatives of the genera *Hoplolaimus* and *Scutellonema* from Mexico with other species as inferred from Bayesian analysis of the ITS rRNA gene sequences under the GTR + I + G model. Posterior probabilities equal to or greater than 70% are given for appropriate clades. The new sequences are indicated in bold. * – identified as *Scutellonema* sp. A by Van den Berg *et al.* (2013, 2017).

Hoplolaimus sp. A from California but differed by 11.8%. This level of sequence divergence supports the recognition of *H. texcocoensis* sp. n. as a distinct species.

COI gene. Four *COI* gene sequence alignments were generated, one for each genus. The alignment lengths for *Scutellonema*, *Hoplolaimus*, *Rotylenchus* and *Helicotylenchus* were 391, 308, 377 and 387 bp,

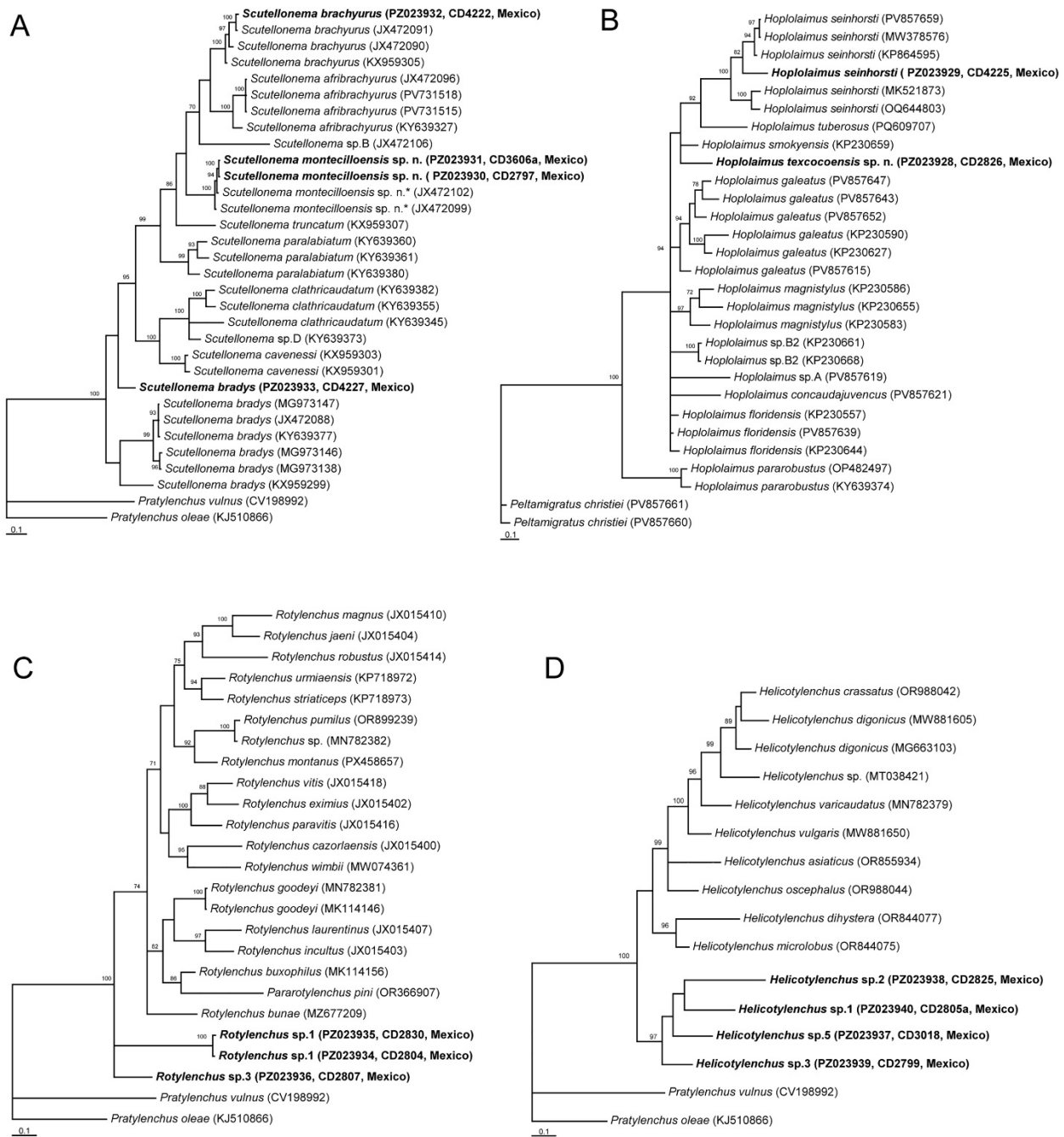


Fig. 12. Phylogenetic relationships of representatives of the genera *Scutellonema* (A), *Hoplolaimus* (B), *Rotylenchus* (C) and *Helicotylenchus* (D) from Mexico with other species as inferred from Bayesian analysis of *COI* gene sequences under the GTR + I + G model. Posterior probabilities equal to or greater than 70% are given for appropriate clades. The new sequences are indicated in bold. * – identified as *Scutellonema* sp. A by Van den Berg *et al.* (2013).

respectively. Thirteen new sequences were obtained in the present study. The phylogenetic relationships among species of these genera based on *COI* gene sequences are shown in Figure 12. In the phylogenetic tree, sequences of *S. brachyurus* and *H.*

seinhorsti from Mexico clustered with sequences of the corresponding species from other geographic regions, supporting their conspecificity. Sequences of *S. montecilloensis* sp. n. clustered with those previously reported as *Scutellonema* sp. A from

California and were therefore considered conspecific. Intraspecific sequence variation for this species was up to 1.3%. By contrast, the *COI* gene sequence of *S. bradys* from Mexico formed a separate lineage and differed by 14.1-19.3% from sequences of this species reported from other countries, indicating substantial genetic divergence. The *COI* gene sequence of *H. texcocoensis* sp. n. from Mexico formed a distinct clade and differed by 15-25% from those of other *Hoplolaimus* species included in the analysis, supporting its recognition as a new species. Sequences of *Helicotylenchus* spp. and *Rotylenchus* spp. from Mexico also showed clear genetic differentiation from previously published sequences, suggesting additional diversity within these genera.

The *COI* genes of several species examined in this study followed a genetic code that deviates from the standard invertebrate mitochondrial code. Analysis of the *COI* amino acid alignments revealed that the codon UAA, which typically functions as a stop codon, is reassigned to encode tyrosine (Tyr) in *S. montecilloensis* sp. n., *S. brachyurus* and *Helicotylenchus* sp. 1, 2 and 5.

DISCUSSION

The present study constitutes the first comprehensive molecular characterisation of hoplolaimid nematodes from Mexico, providing an essential framework for integrative taxonomic and phylogenetic research on this group in the region. With the description of *Hoplolaimus texcocoensis* sp. n., the number of *Hoplolaimus* species recorded from Mexico increases to four: *H. igualaensis*, *H. maggentii*, *H. seinhorsti* and *H. texcocoensis* sp. n. However, the first two species have not yet been characterised using molecular markers. Therefore, DNA-based analyses are still needed to confirm the validity of their species boundaries and delimitation.

To the best of our knowledge, this study also provides the first molecularly verified records of *Scutellonema brachyurus* and *S. bradys* in Mexico. The Carolina spiral nematode, *S. brachyurus*, is a widely distributed plant-parasitic nematode occurring mainly in tropical and subtropical regions, including the southeastern USA. It is a migratory ectoparasite with a very broad host range that includes soybean, cotton, corn, peanut, numerous vegetable crops, turfgrasses, ornamentals and several perennial crops, and it can cause significant yield losses when population densities are high. *Scutellonema bradys*, commonly known as the yam nematode, is responsible for severe damage to yam (*Dioscorea* spp.) crops, particularly in Africa, the Caribbean and parts of South America. In North

America, this species had previously been reported only from Florida (Sher, 1964; Van den Berg *et al.*, 2017) and Arkansas (Wehnt *et al.*, 1989). Our results revealed substantial divergence in the mitochondrial *COI* gene between the Mexican population of *S. bradys* and populations from Africa and Florida, suggesting pronounced geographic structuring and possible cryptic diversity within this species. The confirmation of these economically important species expands their known geographic distribution and has potential implications for agricultural management and plant health monitoring in the region.

Molecular characterisation of other hoplolaimid nematodes further showed that Mexican populations of *Helicotylenchus* formed a distinct clade, potentially representing an undescribed endemic species. Similarly, Mexican nematodes identified here as *Rotylenchus* formed separate molecular lineages, indicating possible endemism. As previously suggested, the isolated valley regions of Mexico, surrounded by the ridges of the Sierra Madre mountain systems, may represent one of the world's centres of species diversity not only for cystoid and cyst nematodes and dagger nematodes (Cid del Prado Vera & Subbotin, 2023; Cid del Prado Vera *et al.*, 2024), but also for members of the family Hoplolaimidae. Thus, our findings highlight Mexico as an underexplored area for hoplolaimid diversity and underscore the importance of combining morphological and molecular approaches to achieve accurate species identification and biodiversity assessment.

Analysis of the *COI* genes from the nematode species examined in this study revealed the use of a non-standard genetic code that deviates from the canonical invertebrate mitochondrial code. Examination of the *COI* amino acid alignments showed that the codon UAA, which normally serves as a stop codon, is consistently reassigned to encode tyrosine in several representatives of Hoplolaimidae. The reassignment of the canonical stop codon UAA to encode tyrosine (UAA → Tyr) has previously been reported in the mitochondrial genomes of species belonging to the genus *Radopholus* (Jacob *et al.*, 2009). This unusual modification of the mitochondrial genetic code is of particular evolutionary interest because molecular phylogenetic analyses subsequently demonstrated that *Radopholus* clusters within the family Hoplolaimidae rather than within its traditional taxonomic placement in Pratylenchidae (Subbotin *et al.*, 2006). The concordance between this rare codon reassignment and phylogenetic relationships suggests that mitochondrial genetic-code alterations may represent

informative synapomorphic character reflecting deeper evolutionary divergence within these lineages. Our observation suggests that this alternative genetic code may be more widespread within plant-parasitic nematodes than previously recognised. The occurrence of this reassignment in multiple genera also indicates importance of applying the appropriate mitochondrial translation table in phylogenetic and barcoding studies of nematodes.

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I. Cid del Prado Vera, H. Ferris, A. Michaud and S.A. Subbotin. Характеристика некоторых фитопаразитических нематод семейства Hoplolaimidae из Мексики с описанием *Hoplolaimus texcocoensis* sp. n. и *Scutellonema montecilloensis* sp. n.

Резюме. В период с 2018 по 2025 гг. в результате нематологических исследований, проведенных в нескольких штатах Мексики, были собраны и охарактеризованы два новых вида, *Hoplolaimus texcocoensis* sp. n. и *Scutellonema montecilloensis* sp. n., а также четыре известных вида: *Helicotylenchus dihystra*, *Hoplolaimus seinhorsti*, *S. brachyurus* и *S. bradys*, пять неопределенных видов *Helicotylenchus* и три неопределенных вида *Rotylenchus*. В этом исследовании, определение видов в семействе Hoplolaimidae основывалось на интегративном таксономическом подходе, сочетающем традиционные морфологические признаки с молекулярными критериями. Таксоны Hoplolaimidae были охарактеризованы молекулярно, а их филогенетические связи с другими представителями группы были реконструированы с использованием последовательностей из сегментов расширения D2-D3 28S рРНК, ITS рРНК и митохондриального *COI* генов. В общей сложности в этом исследовании было получено 23 новые последовательности гена 28S рРНК, 5 новых последовательностей гена ITS рРНК и 13 новых последовательностей гена *COI*. Мексиканские популяции *Helicotylenchus* и нематоды, идентифицированные как *Rotylenchus*, образовали отдельные молекулярные линии, что предполагает их возможный эндемизм. Эти результаты подтверждают гипотезу о том, что долины, окруженные хребтами горного массива Сьерра-Мадре в Мексике, могут представлять собой один из глобальных центров разнообразия нематод семейства Hoplolaimidae.