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Two new species of sedentary nematodes of the genus *Meloidodera* Chitwood, Hannon & Esser, 1956 (Tylenchida: Heteroderidae) from Mexico

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Summary – Two new species of the genus *Meloidodera* collected in Mexico are described here: *M. ferrisi* sp. n. parasitising roots of an oak tree in the State of Mexico and *M. tecoacensis* sp. n. parasitising roots of buffalo bur nightshade in the Tlaxcala State. *Meloidodera ferrisi* sp. n. is characterised by a spherical female body covered completely by a dark thick cuticular layer, length/width of the female body = 0.8-1.6, stylet = 35-43 μm and second-stage juvenile with average body = 340 μm and average tail length = 35.6 μm. *Meloidodera tecoacensis* sp. n. is characterised by the female having a spherical body covered with a yellow transparent material, length/width of the female body = 1.1-2.8, stylet = 20-33 μm and second-stage juvenile with average body = 340 μm and average tail length = 29.8 μm. These two species were molecularly characterised using the D2-D3 expansion segments of 28S rRNA, ITS rRNA and *COI* gene sequences. Phylogenetic analysis revealed that the two new species represent a separate evolutionary lineage within the subfamily Meloidoderinae. An identification key for 12 *Meloidodera* species is provided.

Keywords – *COI*, identification key, Meloidoderinae, morphology, morphometrics, phylogeny, rDNA, taxonomy.

In 2012 and 2018-2022, during nematological surveys in Mexico, white females attached to plant roots, second-stage juveniles and males were found in root and soil samples collected from oak tree and buffalo bur nightshade near Cuijingo, State of Mexico, and near Huamantla, Tlaxcala State, respectively. The nematodes were identified as two representatives of the genus *Meloidodera* Chitwood, Hannon & Esser, 1956. Detailed morphological and molecular examination revealed significant differences of these two species from other known *Meloidodera* species.

This genus *Meloidodera* is characterised by mature females having an annulated cuticle, except near the vulva, the absence of a D-layer, a median to submedian vulva position, a ventrally subterminal or terminal anus and the absence of a terminal cone. No cyst stage is present and eggs are laid, or some retained in the body. Males have a long and slender body with a stylet generally 20-25 μm long and without bursa. Second-stage juveniles (J2) are under 0.6 mm long with four incisures and a stylet 24-35 mm long. Phasmids have a lens-like structure under the muscle layer. Feeding induces a single nurse cell (Siddiqi, 2000). Up to now, the genus *Meloidodera* contained ten valid species and three of them: *M. astonei* Cid del Prado Vera & Rowe, 2000, *M. mexicana* Cid del Prado Vera, 1991 and *M. zacanensis* Cid del Prado Vera, 1997, were described from Mexico (Ghaderi, 2019a, b). Only four of the ten species: *M. astonei*, *M. floridensis* Chitwood, Hannon & Esser, 1956, *M. mexicana* and

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M. sikhotealiniensis Eroshenko 1978, were molecularly characterised (Subbotin *et al*. 2017).

In this paper, we morphologically described and molecularly characterised the two new cystoid species, *Meloidodera ferrisi* sp. n. and *M. tecoacensis* sp. n. from Mexico.

Materials and methods

Nematode samples

Several soil and root samples were collected near the road to the town of Cuijingo, State of Mexico, and from the type locality of *Globodera mexicana*, Francisco Villa-Tecoac, near Huamantla, Tlaxcala State, in 2012 and 2018-2022. Females, males and J2 were extracted from soil samples using a decanting and sieving and sugar flotation method (Jenkins, 1964). Females were individually dissected from plant roots.

Morphological study

Females, males and J2 were killed by heating and then fixed with 8% formalin, processed to glycerin, and mounted on slides using a modification of the Seinhorst (1959) method as described by Cid Del Prado Vera & Subbotin (2012). Measurements and drawings were made using an American Optical compound microscope and a drawing tube. For scanning electron microscopy, some specimens were treated in phosphate buffer for 15 min and dehydrated in an alcohol series (10-100%) for 15 min at each concentration (Cid Del Prado Vera *et al*., 2012). The specimens were critical point-dried and coated with gold-palladium before observation under a Jeol JSM-6390 scanning electron microscope at 10 kV.

Molecular study

DNA was extracted from several J2 specimens using the proteinase K protocol. DNA extraction and PCR protocols were conducted according to Subbotin (2021a). The following primer sets were used in this study: *i*) D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) amplifying the D2-D3 expansion segments of 28S rRNA gene; *ii*) TW81 (5′-GTT TCC GTA GGT GAA CCT GC-3′) and AB28 (5′-ATA TGC TTA AGT TCA GCG GGT-3′) amplifying the ITS rRNA gene; and *iii*) 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′) amplifying the partial *COI* gene of mtDNA (Subbotin, 2021a). The successfully amplified fragments were purified and then sequenced by Azenta with the primer pairs used in PCR.

New sequences were aligned using ClustalX 1.83 with corresponding selected and published gene sequences of Meloidoderinae (Vovlas *et al*., 2013; Subbotin *et al*., 2017, 2018; Kang *et al*., 2019; Powers *et al*., 2019; Gu *et al*., 2020; Singh *et al*., 2022). Poorly aligned positions were removed from the ITS rRNA gene sequence alignment with Gblocks (Talavera & Castresana, 2007) to create the culled ITS alignment. Sequence datasets were analysed with Bayesian inference (BI) using MrBayes 3.1.2 and PAUP* 4.0 and maximum likelihood (ML) using PAUP* (Swofford, 2003) as described by Subbotin (2021b). Pairwise divergences between taxa were computed as absolute distance values and as percentage mean distance values based on whole alignment, with adjustment for missing data. For testing of alternative topologies in ML, the Shimodaira-Hasegawa (SH) test was applied as implemented in PAUP*. The new sequences were submitted to the GenBank database under accession numbers: OP857474, OP857475 (D2-D3 of 28S rRNA gene), OP860550-OP860552 (ITS rRNA gene), OP855689-OP855692 (*COI* gene).

Results

Meloidodera ferrisi sp. n. (Figs 1-3)

Measurements

See Tables 1 and 2.

Description

Female

Body spherical with a conspicuous dark cover over the cuticle. The cover appears separate from the original cuticle rather than the result of an aging process. Females are pearly-white in colour. The body protrudes from the root surface, sometimes isolated but other females close together. Cuticle 10-12 (10.3) μm thick with fine striation in whole body. The neck is short in a few females and covered by a dark material. Cephalic region with one annulus and labial disc present. Stylet cone thin and curved. Excretory pore at the base of neck. Median bulb spherical with conspicuous valve. Vulva in post-equatorial

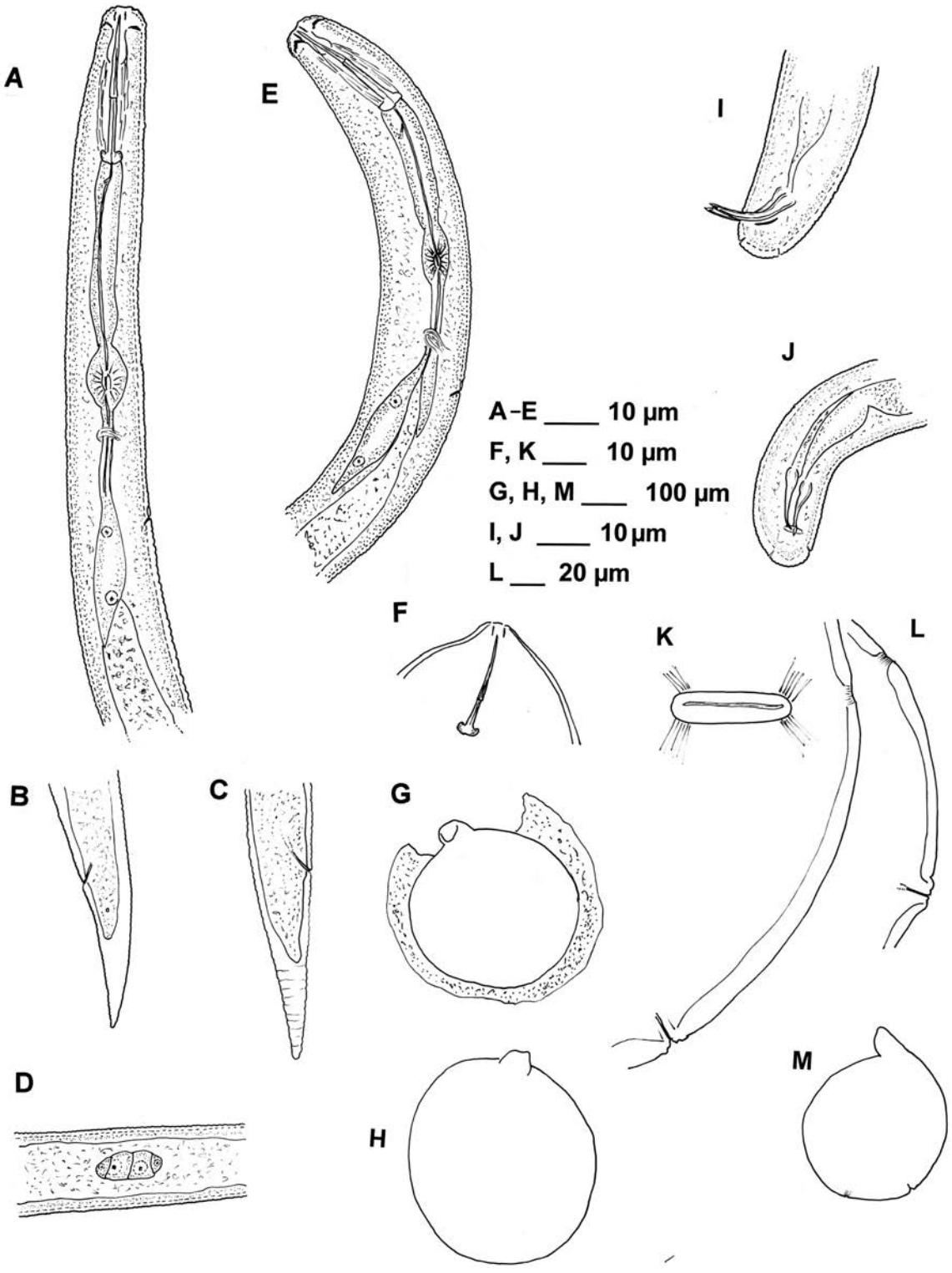

Fig. 1. *Meloidodera ferrisi* sp. n. A-D: Second-stage juvenile. A: Anterior end; B, C: Posterior end; D: Mid-body; Female. F: Anterior end; G, H, M: Outlines of females; K: Vulval slit; L: Vulva-anus region; Male. E: Anterior end. I, J: Posterior end body.

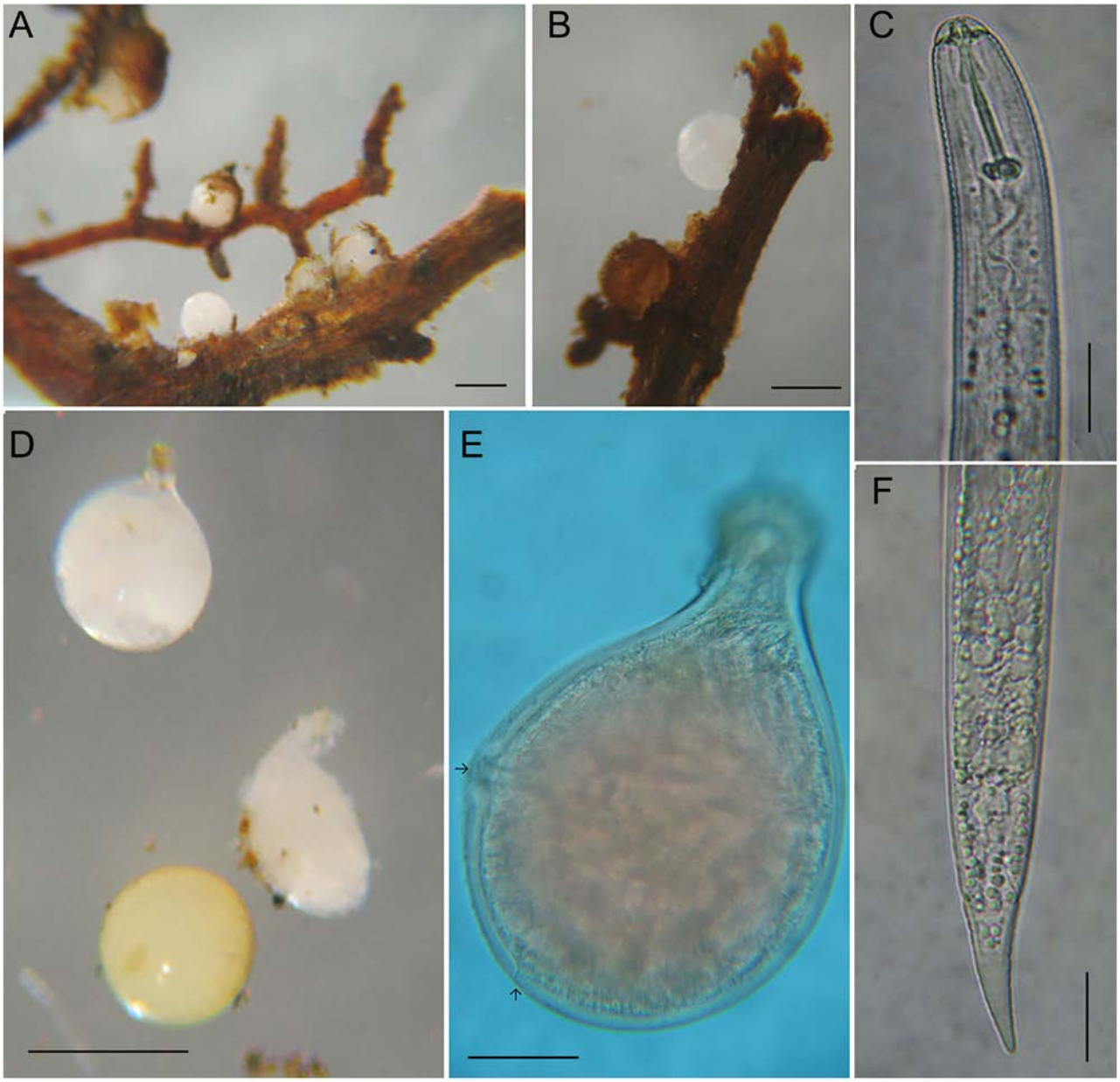

Fig. 2. Light microscopy photos of *Meloidodera ferrisi* sp. n. A, B: Females on roots; C: Anterior end of second-stage juvenile; D, E: Females; F: Posterior end of second-stage juvenile. (Scale bars: A, B, D = 400 μm; C, F = 15 μm; E = 70 μm.)

position and with slightly protruding lips. Anus terminal in a slight depression.

Male

Body vermiform, slightly curved ventrally or twisted. Lip region slightly separated from the rest of the body and comprised of five fine annuli; basal annulus of lip region not striated but appears as rectangular blocks. Stylet slender with dorsal knobs rounded and lateral, one with slight anterior projection. Median bulb oval shaped with valve reduced in size. Pharyngeal glands dorsally overlapping the intestine. Excretory pore at level of posterior end of dorsal gland and one annulus behind of hemizonid. Lateral field with four incisures. Spicules

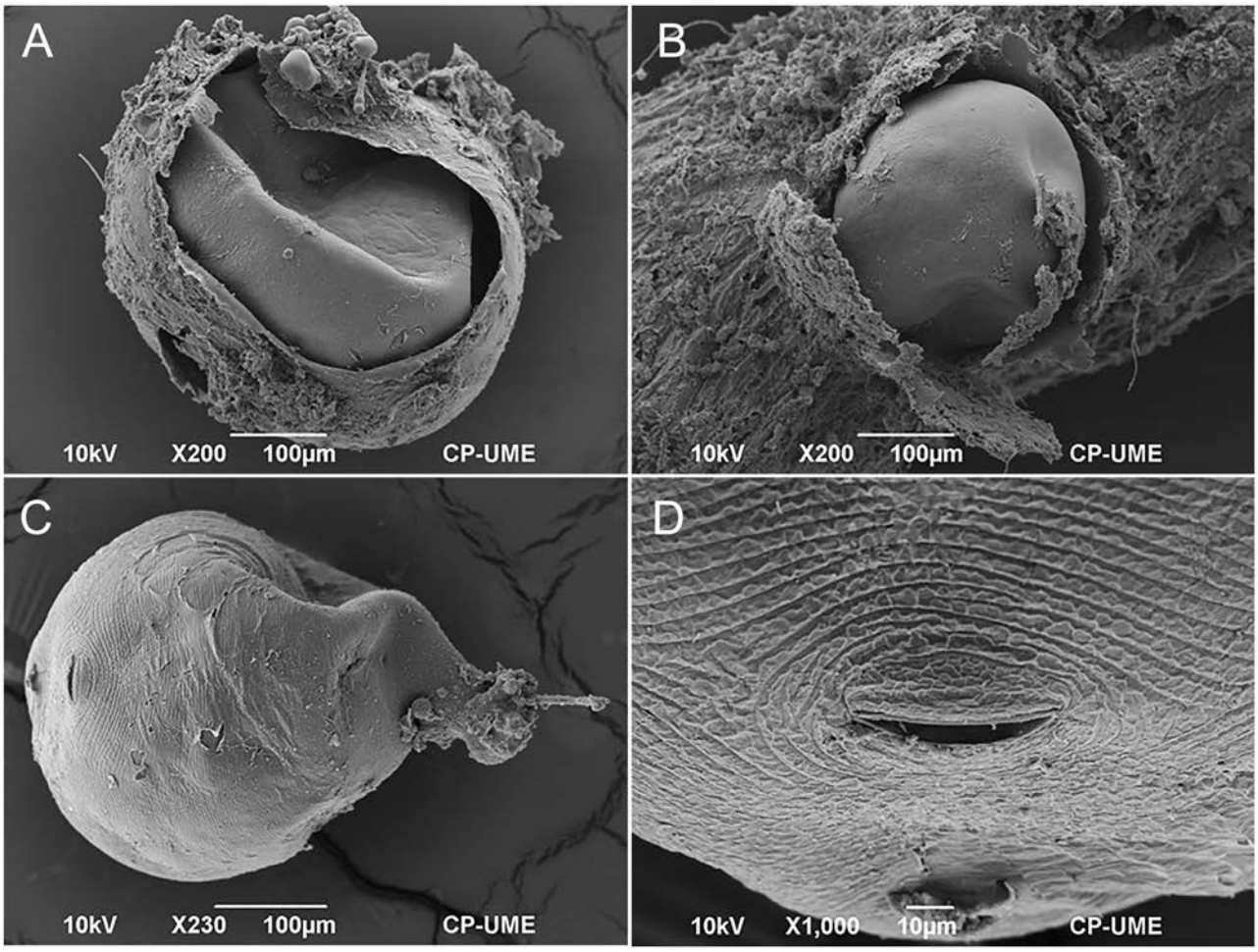

Fig. 3. SEM photos of *Meloidodera ferrisi* sp. n. A-C: Females; D: Vulval area.

slightly curved. Phasmids located subterminal. Tail short and with rounded terminus.

Second-stage juvenile

Body vermiform, slightly curved ventrally. Lip region offset by a slight constriction and comprised of five annuli. Stylet robust, knobs with anterior projection. Median bulb oval shaped with small valve. Pharyngeal glands overlapping the intestine dorsally. Excretory pore at the first third of pharyngeal glands and, in most specimens, one annulus posterior to the hemizonid. Lateral field with four incisures completely areolated. Phasmid lens like and 2-9 (5 ± 1.8) annuli behind anus. Tail conoid, tapering to a finally rounded terminus.

TYPE LOCALITY AND HOST

Meloidodera ferrisi sp. n. was collected from roots and rhizosphere soil of an oak tree, *Quercus rugosa* Née near the road to the town of Cuijingo Mexico, State, Mexico. GPS: 19°4′4.44″ N, 98°50′0.42′ W. Altitude: 2420 m a.s.l.

ETYMOLOGY

This new species is named in honour of Prof. Howard Ferris for his outstanding scientific contributions in nematode ecology.

TYPE MATERIALS

Type specimens are deposited in the Laboratorio de Helmintología del Instituto de Biología, UNAM, México:

Table 1. Morphometrics of females and second-stage juveniles of the genus *Meloidodera* described in this study. All measurements are in μm and in the form: mean ± s.d. (range).

Character	*M. ferrisi* sp. n.		*M. tecoacensis* sp. n.	
	Holotype	Paratypes	Holotype	Paratypes
Females				
n	1	17	1	12
L	385	442 ± 93.5 (280-612)	368	340 ± 0.03 (290-370)
W	368	394 ± 117 (180-570)	168	176 ± 42 (116-250)
L/W	1.04	1.1 ± 0.2 (0.78-1.6)	2.2	2.2 ± 0.5 (1.1-2.8)
Neck length	70	83.3 ± 23.6 (60-137.5)	176	109 ± 27 (80-176)
Stylet	–	38.8 ± 4.8 (35-42.5)	20	27.1 ± 4.8 (20-33)
Median bulb length	–	30.5 ± 8.2 (22-37.5)	20	20.6 ± 4.0 (15-25)
Median bulb width	–	27.5 ± 6.5 (20-35)	15	19.5 ± 5.6 (11-30)
Excretory pore	–	112 ± 36.6 (50.5-145)	-	97.5 ± 3.5 (95-100)
V%	71.6	67.4 ± 4.4 (62-75)	71.5	70 ± 7.5 (57.7-81.5)
Vulva-anus distance	218	143 ± 48.3 (70-230)	105	135 ± 44 (80-190)
Second-stage juveniles				
n	–	25	–	5
L	–	340 ± 40 (230-400)	–	340 ± 20 (330-370)
a	–	21.3 ± 2.6 (15.3-25.2)	–	20.4 ± 3.0 (17.8-25.6)
b	–	3.7 ± 0.6 (2.7-4.6)	–	5.0
b’	–	2.9 ± 0.7 (1.5-4.9)	–	3.5 ± 0.2 (3.3-3.7)
c	–	9.8 ± 1.7 (6.5-13.4)	–	11.7 ± 1.5 (9.9-13.1)
c’	–	3.1 ± 0.5 (1.7-4.0)	–	2.3 ± 0.1 (2.3-2.4)
Stylet	–	28.5 ± 2.2 (24-31)	–	24.4 ± 0.6 (24-25)
Stylet knob height	–	3.0 ± 0.5 (2.0-4.0)	–	2.4 ± 0.6 (2.0-3.0)
Stylet knob width	–	4.7 ± 0.5 (4.0-5.0)	–	4.4 ± 0.6 (4.0-5.0)
DGO	–	4.1 ± 0.5 (3.0-5.0)	–	4.0
Median bulb length	–	11.1 ± 1.0 (9-13)	–	8.4 ± 1.3 (7-10)
Median bulb width	–	8.7 ± 0.9 (7-10)	–	7.2 ± 1.1 (6-9)
Pharynx	–	125.6 ± 19.8 (97-163)	–	99 ± 9.6 (92-110)
Pharyngeal gland length	–	36.7 ± 15.3 (15-64)	–	24
Excretory pore	–	93.5 ± 7.5 (80-105)	–	80.5 ± 15 (70-91)
Tail	–	35.6 ± 4.4 (26-40)	–	29.7 ± 3.1 (27-34)
Hyaline part of tail	–	18.3 ± 1.9 (15-21)	–	14.2 ± 2.3 (11-17)

holotype female CNHE 11597, allotype male CNHE 11 and four paratype females CNHE 11599. Other paratype materials were deposited in the University of California Riverside Nematode Collection (UCRNC): two females and the Colegio de Postgraduados Nematode Collection: six females and three males (CPNC A-113).

Differential diagnosis

Meloidodera ferrisi sp. n. differs from *M. tecoacensis* sp. n. in the longer average female body (442 (280-612) *vs* 340 (290-370) μm), longer average female stylet (38.8 (35-42.5) *vs* 27.1 (20-33) μm), longer average J2 stylet (28.5 (24-31) *vs* 24.4 (24-25) μm) and longer average hyaline part of the J2 tail (18.3 (15-21) *vs* 14.2 (11-17) μm); from *M. astonei* by longer average female stylet (38.8 (35-42.5) *vs* 24 μm), longer average J2 stylet (28.5 (24-31) *vs* 21 (13-25) μm) and longer average hyaline part of J2 tail (18.3 (15-21) *vs* 16.7 (14-26) μm); from *M. mexicana* by shorter average female body (442 (280-612) *vs* 669 (480-800) μm), longer average female stylet (38.8 (35-42.5) *vs* 30 (28-32) μm) and longer average J2 stylet (28.5 (24-31) *vs* 22 (19-24.5) μm); from *M. zacanensis* by shorter average J2 body (340 (230-400) *vs* 384 (318-450) μm) and shorter average J2 tail (35.6 (26-40) *vs* 43 (37-49) μm); and from *M. belli* Wouts 1973 by longer average J2 stylet (28.5 (24-31) *vs* 23.7 μm) and longer average J2 tail (35.6 (26-40) *vs* 29.8 μm).

Table 2. Morphometrics of males of *Meloidodera ferrisi* sp. n. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Paratypes
n	4
L	560 ± 50 (480-610)
W	19.4 ± 0.6 (19-20)
a	29 ± 2.65 (25-32)
b	5.8 ± 0.4 (5.5-6.2)
b'	4.7 ± 0.4 (4.3-5.1)
c	74 ± 12 (59-86)
c'	0.6 ± 0.07 (0.5-0.7)
Stylet	30.7 ± 1.3 (29-32)
Stylet knob height	2.4 ± 0.6 (2-3)
Stylet knob width	5 ± 0.7 (4-6)
DGO	4.1 ± 0.5 (3.0-5.0)
Median bulb length	11.3 ± 1.9 (10-14)
Median bulb width	6.8 ± 1.3 (5-8)
Pharynx	112.2 ± 14.3 (98-131)
Excretory pore	105.5 ± 28 (86-125)
Testis	251 ± 25 (233-280)
Spicules	14 ± 2.2 (11-16)

Meloidogyne tecoacensis **sp. n.**
(Figs 4-7)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

Body a spherical shape attached to the root; some are covered by a yellow gelatin-like capsule, and others are completely covered by material of unknown origin, and all protrude from the roots. When out of root and without capsule, the body has a pearly white colour. Cuticle annulated except for vulva lips and 8.0-10.0 (9.0 ± 0.85) μm in thickness. Neck in most females, short, 87-90 μm long, but in some females more than 100 μm long and in one specimen 176 μm long. Indistinct lip region. Stylet cone thin and curved and stylet knobs with slight anterior projection. Median bulb spherical located in the base of neck. Vulva in post-equatorial position and with lips slightly protruding out of body contour. Anus subterminal in a slight depression.

Male

Not found.

Second-stage juvenile

Body straight or slightly curved ventrally. Lip region of four annuli, offset by a slight constriction and with an indistinct labial disc. Stylet knobs pointing anteriorly. Pharyngeal glands not filling internal body cavity. Excretory pore at level of half of isthmus, hemizonid located one annulus anterior to excretory pore. Lateral field with four incisures, areolated. Phasmids not conspicuous. Tail conical tapering to narrow and rounded terminus.

TYPE LOCALITY AND HOST

Meloidodera tecoacensis sp. n. was collected from roots and rhizosphere soil of Buffalo bur, *Solanum rostratum* Dunal, in Francisco Villa-Tecoac, Huamantla, Tlaxcala State, Mexico. GPS: 19°23′4.38″ N, 97°55′42.78″ W. Altitude: 2440 m a.s.l.

ETYMOLOGY

This species is named after the type locality, Tecoac, Huamantla County, Tlaxcala State, Mexico.

TYPE MATERIALS

Type specimens were deposited in the Laboratorio de Helmintología del Instituto de Biología, UNAM, México: holotype female CNHE 11600 and four paratype females CNHE 11601. Other paratype materials were deposited in the University of California Riverside Nematode Collection (UCRNC): two females, and the Colegio de Postgraduados Nematode Collection: four females (CPNC A-114).

DIFFERENTIAL DIAGNOSIS

Meloidodera tecoacensis sp. n. differs from *M. ferrisi* sp. n. by the shorter average female body (340 (290-370) *vs* 442 (280-612) μm), shorter average female stylet (27.1 (20-33) *vs* 38.8 (35-42.5) μm), shorter average J2 stylet J2 (24.4 (24-25) *vs* 28.5 (24-31) μm) and shorter average hyaline part of the J2 tail (14.2 (11-17) *vs* 18.3 (15-21) μm); from *M. astonei* by the shorter average female body (340 (290-370) *vs* 522 (328-448) μm), longer average J2 stylet (24.4 (24-25) *vs* 21 (13-25) μm) and shorter average J2 tail (29.7 (27-34) *vs* 37.6 (32.4-49.6) μm); from *M. mexicana* by shorter average female body (340 (290-370) *vs* 669 (480-800) μm) and shorter average J2 tail (29.7 (27-34) *vs* 33 (27-39) μm); and from *M. belli* by shorter average female body (340 (290-370) *vs* 400 μm)

Table 3. Results of the Shimodaira-Hasegawa test of tree topologies and alternative phylogenetic hypotheses of the subfamily Meloidoderinae.

Hypothesis	D2 and D3 of 28S rRNA gene			ITS rRNA gene (culled alignment)		
	− ln L	Δ ln L	P	− ln L	Δ ln L	P
ML tree	4476.174	best	-	4688.736	Best	-
Meloidodera is monophyletic	4488.820	12.646	0.151	4729.991	41.254	0.002*
Cryphodera is monophyletic	4483.534	7.360	0.286	4698.703	9.967	0.254

* Tree significantly worse than the best tree at $P < 0.05$.

and longer average J2 hyaline part of tail (14.2 (11-17) *vs* 10.9 μm).

Molecular characterisation and phylogenetic relationships

D2-D3 expansion segments of 28S rRNA gene

The alignment contained 51 sequences of the representatives from Meloidoderinae and two sequences of the outgroup taxa and was 690 bp long. Two new sequences were obtained in this study. Phylogenetic relationships of two new *Meloidodera* species with other Meloidoderinae are given in Figure 8. The D2-D3 of 28S rRNA gene sequence of two new *Meloidodera* species, formed a clade with PP = 100% and differed from each other in 5.4% (36 bp).

ITS rRNA gene

The culled alignment, which contained 50 sequences of the representatives from Meloidoderinae and two sequences from the outgroup taxa, was 598 bp long. Three new sequences were obtained in this study. Phylogenetic relationships of two new *Meloidodera* species with other Meloidoderinae are given in Figure 9. The ITS rRNA gene sequences of the two new *Meloidodera* species formed a basal clade with PP = 100% on the phylogenetic tree. The ITS sequences differed from each other in 14.3-14.8% (111-115 bp).

COI *gene*

The alignment contained 40 sequences of the representatives from Meloidoderinae and two sequences of the outgroup taxa and was 437 bp long. Four new sequences were obtained in this study. Phylogenetic relationships of two new *Meloidodera* species with other Meloidoderinae are given in Figure 10. The *COI* gene sequences of two new *Meloidodera* species formed a clade with PP = 100% and differed from each other in 12.8-13.0% (56-57 bp).

Maximum likelihood testing

The D2-D3 of 28S rRNA gene and ITS rRNA gene alignments containing 31 and 30 sequences, respectively, were used for this testing. Results of the Shimodaira-Hasegawa test of tree topologies and alternative phylogenetic hypotheses of Meloidoderinae are given in Table 3. The SH testing of an alternative topology with the ITS rRNA gene alignment rejected the monophyly for *Meloidodera*. The monophyly of the genus *Cryphodera* was supported as alternative topology in both alignments.

Species identification key for the genus *Meloidodera*

Identification keys for *Meloidodera* species were given by Ivanova & Krall (1985), Wouts (1985), Cid del Prado Vera (1991) and Ghaderi (2019a). An updated key for 12 valid species is presented below.

1. Average tail length of J2 $\geqslant$ 58 μm 2
 - Average tail length of J2 < 58 μm 5
2. Excretory pore at level of median bulb in female .... *M. hissarica*
 - Excretory pore behind median bulb in female 3
3. Body length of J2 > 550 μm ..... *M. eurytyla*
 - Body length of J2 $\leqslant$ 550 μm 4
4. Distance from anterior end to excretory pore of female < 130 μm ..... *M. sikhotealiniensis*
 - Distance from anterior end to excretory pore of female $\geqslant$ 130 μm ..... *M. tianschanica*
5. Average body length of J2 $\leqslant$ 500 μm 6
 - Average body length of J2 > 500 μm .... *M. floridensis*
6. Average stylet length of J2 < 27 μm 7
 - Average stylet length of J2 $\geqslant$ 27 μm 8
7. Average tail length of J2 $\geqslant$ 40 μm .... *M. zacanensis*
 - Average tail length of J2 < 40 μm ... *M. ferrisi* sp. n
8. Average hyaline part of tail length of J2 > 11 μm .. 9

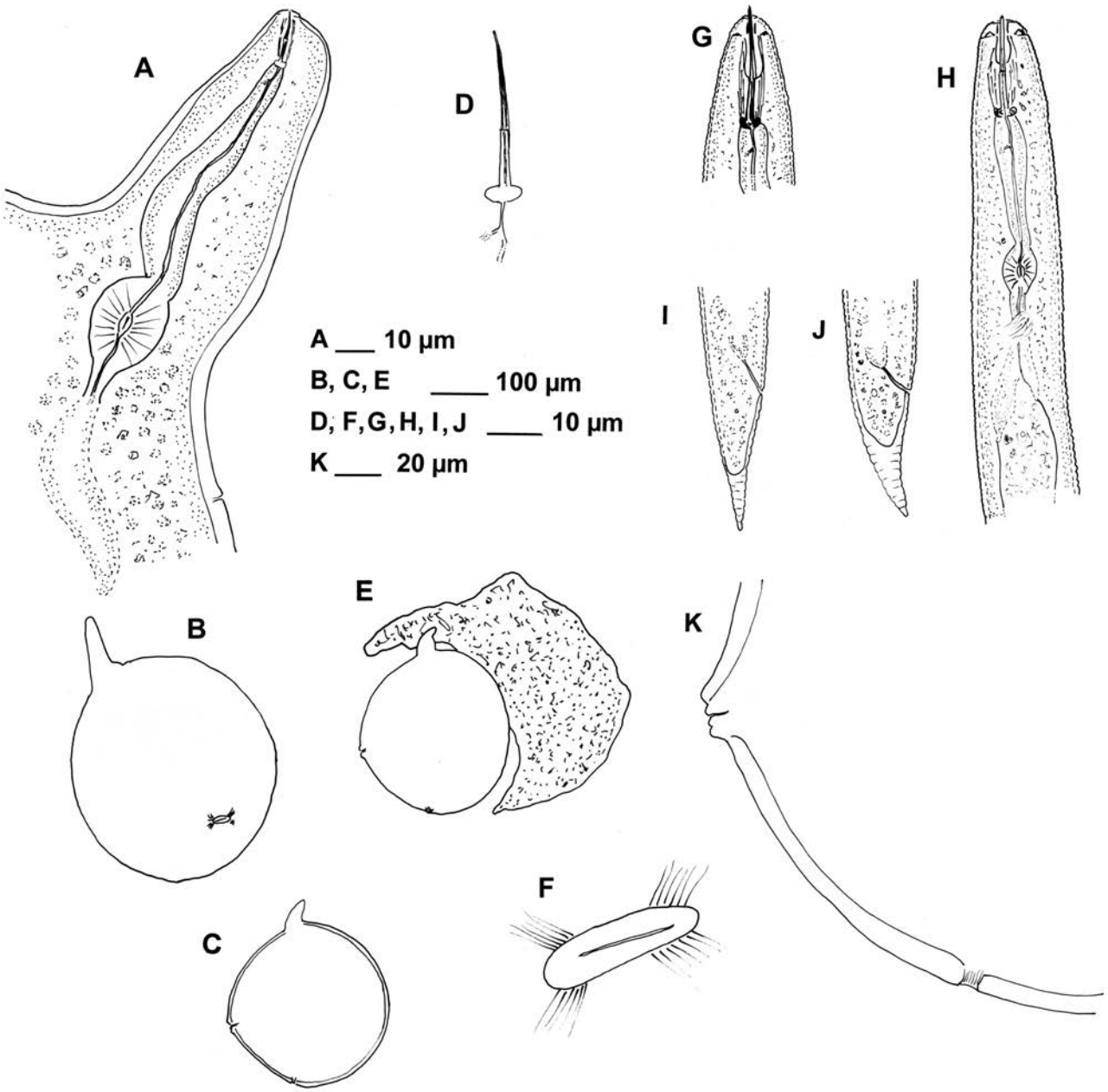

Fig. 4. *Meloidodera tecoacensis* sp. n. A: Anterior end of female; B, C, E: Outlines of females; D: Stylet of female; F: Vulval slit; K: Vulva-anus region of female. G, H: Anterior ends of second-stage juveniles I, J: Posterior end of second-stage juvenile.

- Average hyaline part of tail length of J2 $\leqslant$ 11 μm... ..... *M. belli*

9. Average tail length of J2 $\geqslant$ 40 μm ..... *M. charis*
- Average tail length of J2 $<$ 40 μm 10

10. Average stylet length of female $\leqslant$ 26 μm .... *M. astonei*
- Average stylet length of female $>$ 26 μm 11

11. Average body length of female $\leqslant$ 370 μm..... ..... *M. tecoacensis* sp. n.

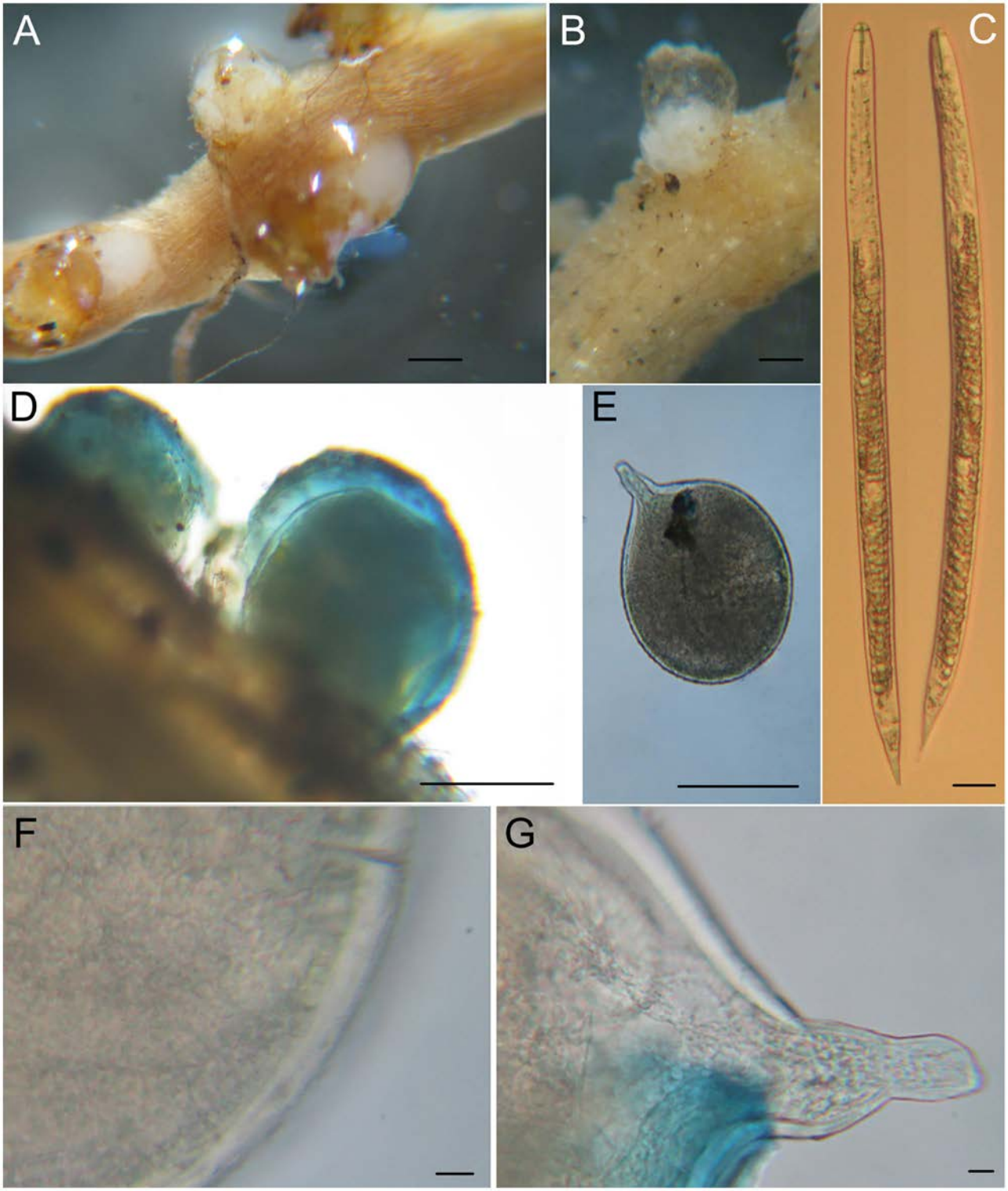

Fig. 5. Light microscopy photos of *Meloidodera tecoacensis* sp. n. A, B, D: Females on roots; C: Second-stage juveniles; E: Female; F: Vulval-anus area of female; G: Anterior end of female; (Scale bars: A, B, D, E = 200 μm; C = 20 μm; F, G = 10 μm.)

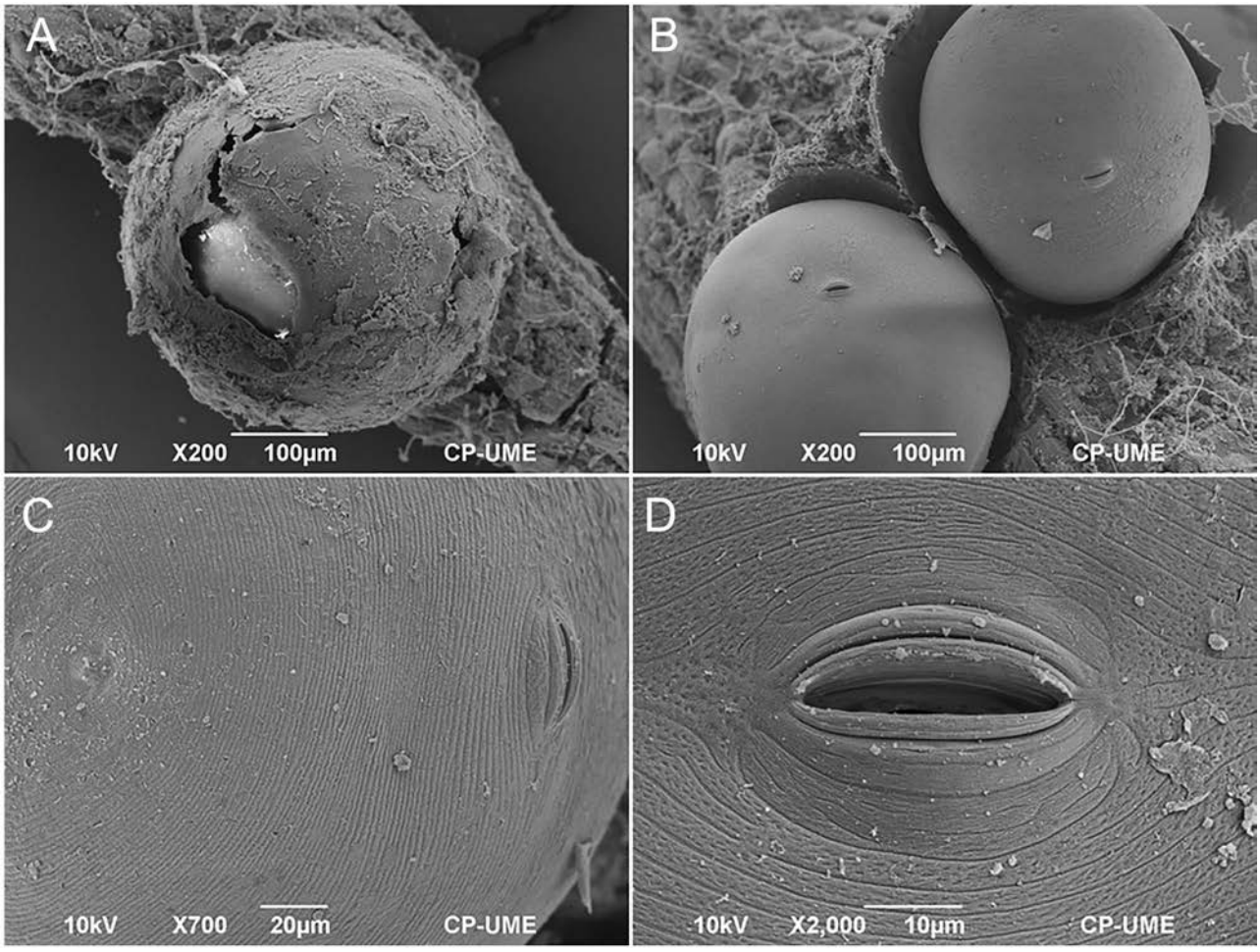

Fig. 6. SEM photos of *Meloidodera tecoacensis* sp. n. A, B: Females; C, D: Vulval area.

- Average body length of female > 370 μm..... ..... *M. mexicana*

Species inquirendae: *Meloidodera armeniaca* Poghossian, 1960; *M. tadzhikistanica* Kirjanova & Ivanova, 1966 (Wouts & Sher, 1971; Wouts, 1985; Siddiqi, 2000).

Discussion

Analysis of morphological characters for *Meloidodera* representatives showed that they are rather variable and overlap between species. Two new species were described in this study, and although they are morphologically similar, molecular analysis clearly separates them from each other and other known *Meloidodera* species. Integrating traditional morphological taxonomic characters and molecular criteria should be applied to differentiate species of this genus from each other (Subbotin *et al*., 2017).

Phylogenetic analysis revealed that the genus *Meloidodera* clustered with representatives of the genera *Cryphodera* (Subbotin *et al*. 2017, 2018). These two genera should be considered with the subfamily Meloidoderinae as has been already suggested by Wouts (1973). Wouts & Sher (1971) proposed that *Cryphodera* probably evolved from *Meloidodera* by a shift of the vulva to a terminal position, thereby distorting the area between vulva and anus with the anus becoming located on a separate elevation slightly outside the body contour of the female, which enables the female to lay eggs outside the roots, even when her body is still partially embedded in the root. Cystoid nematodes of the genera *Meloidodera*

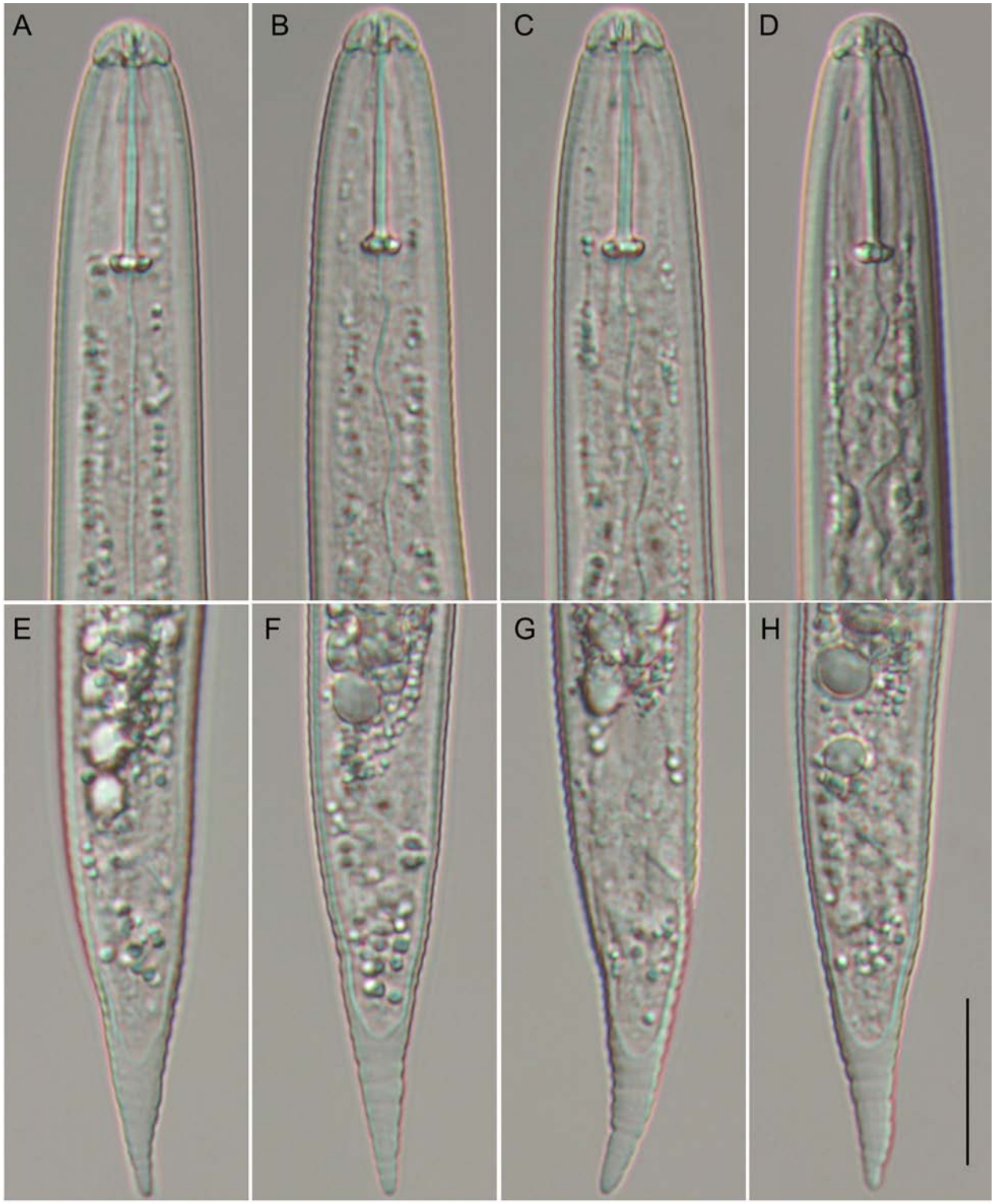

Fig. 7. Photomicrographs of anterior (A-D) and posterior (E-H) regions of second-stage juveniles of *Meloidodera tecoacensis* sp. n. (Scale bar = 20 μm.)

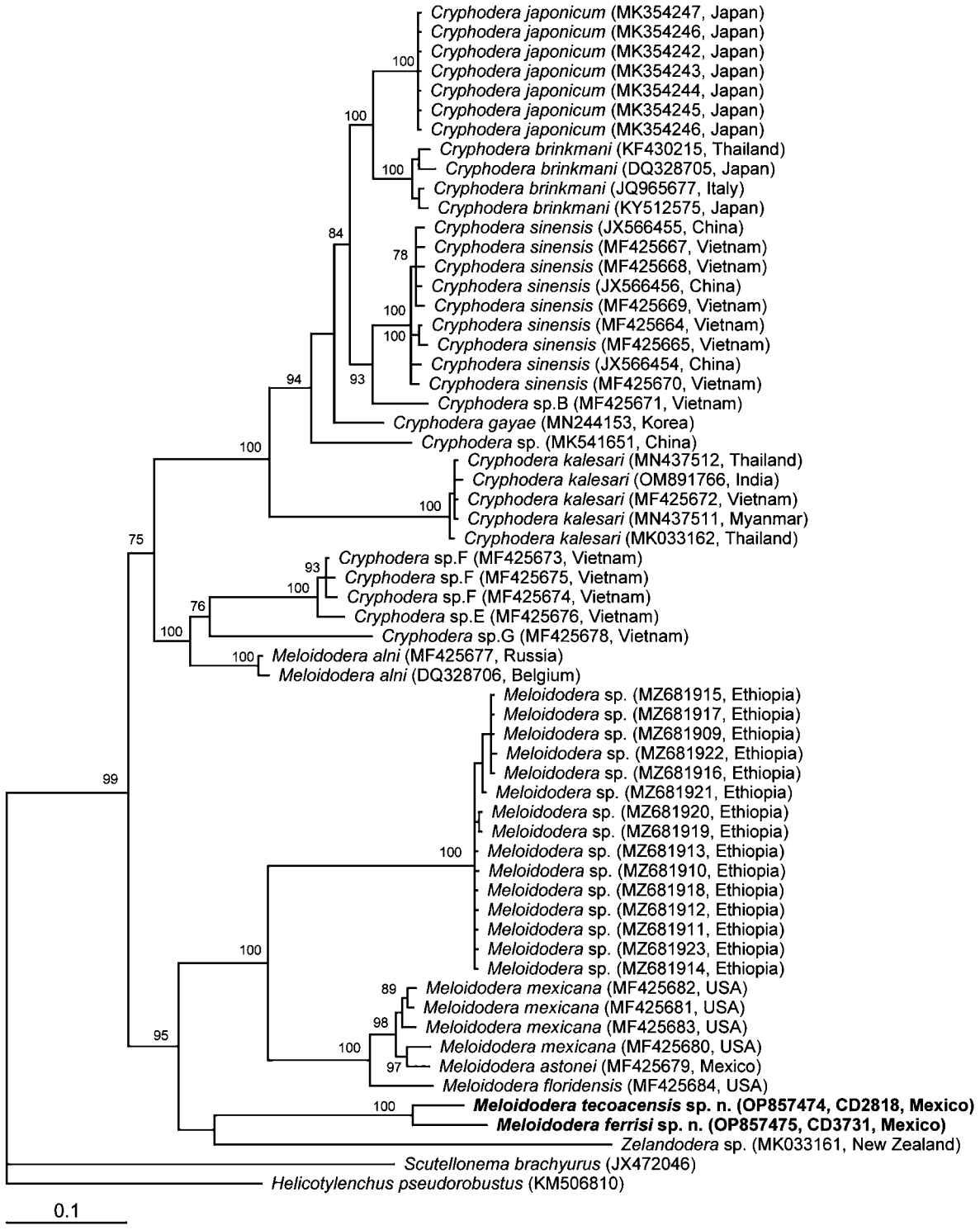

Fig. 8. Phylogenetic relationships within the subfamily Meloidoderinae: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the D2-D3 of 28S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than, 70% are given for appropriate clades. New sequences are indicated in boldface.

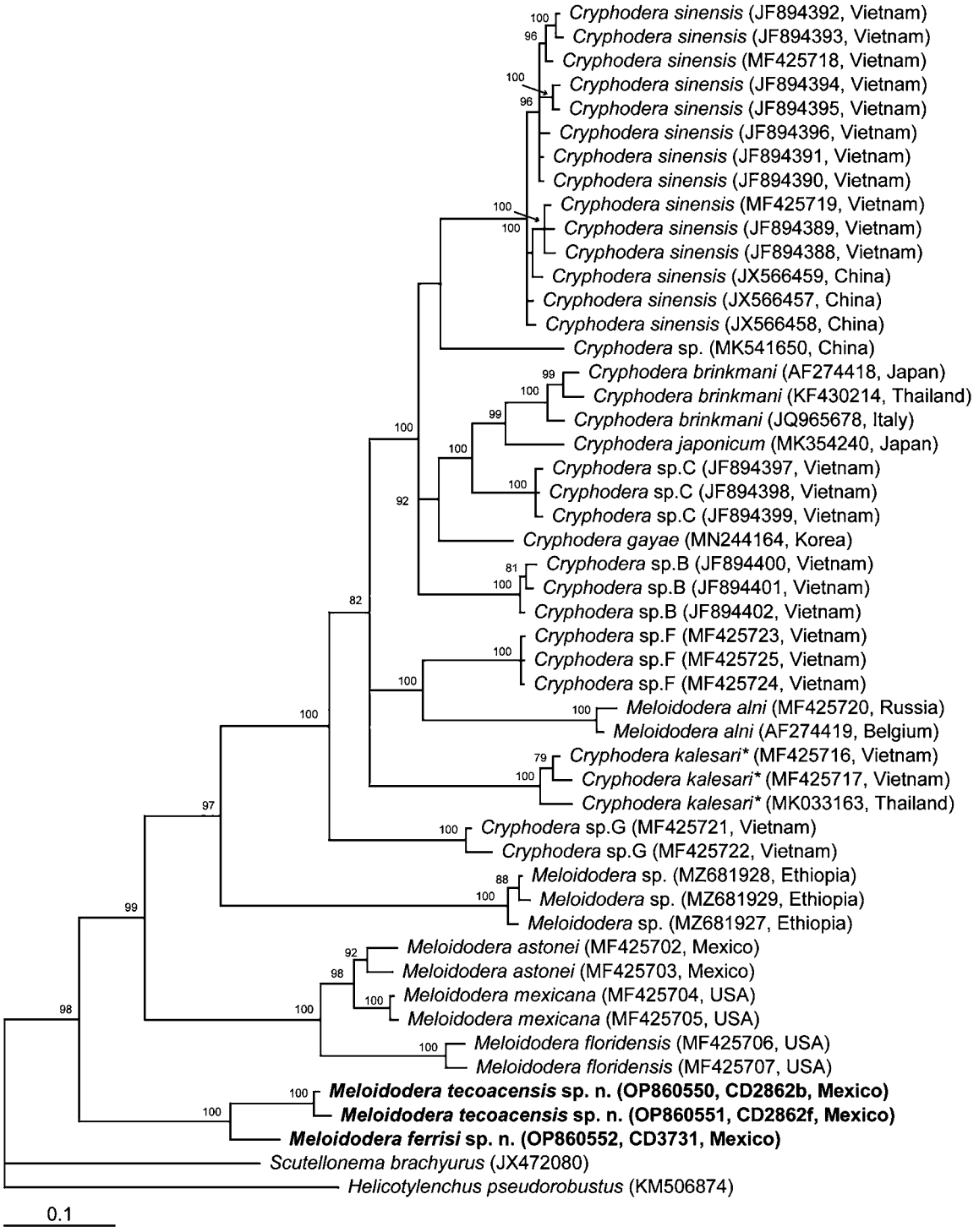

Fig. 9. Phylogenetic relationships within the subfamily Meloidoderinae: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the culled ITS rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than, 70% are given for appropriate clades. New sequences are indicated in boldface. * – originally identified as *Cryphodera* sp. D (Subbotin *et al*., 2018).

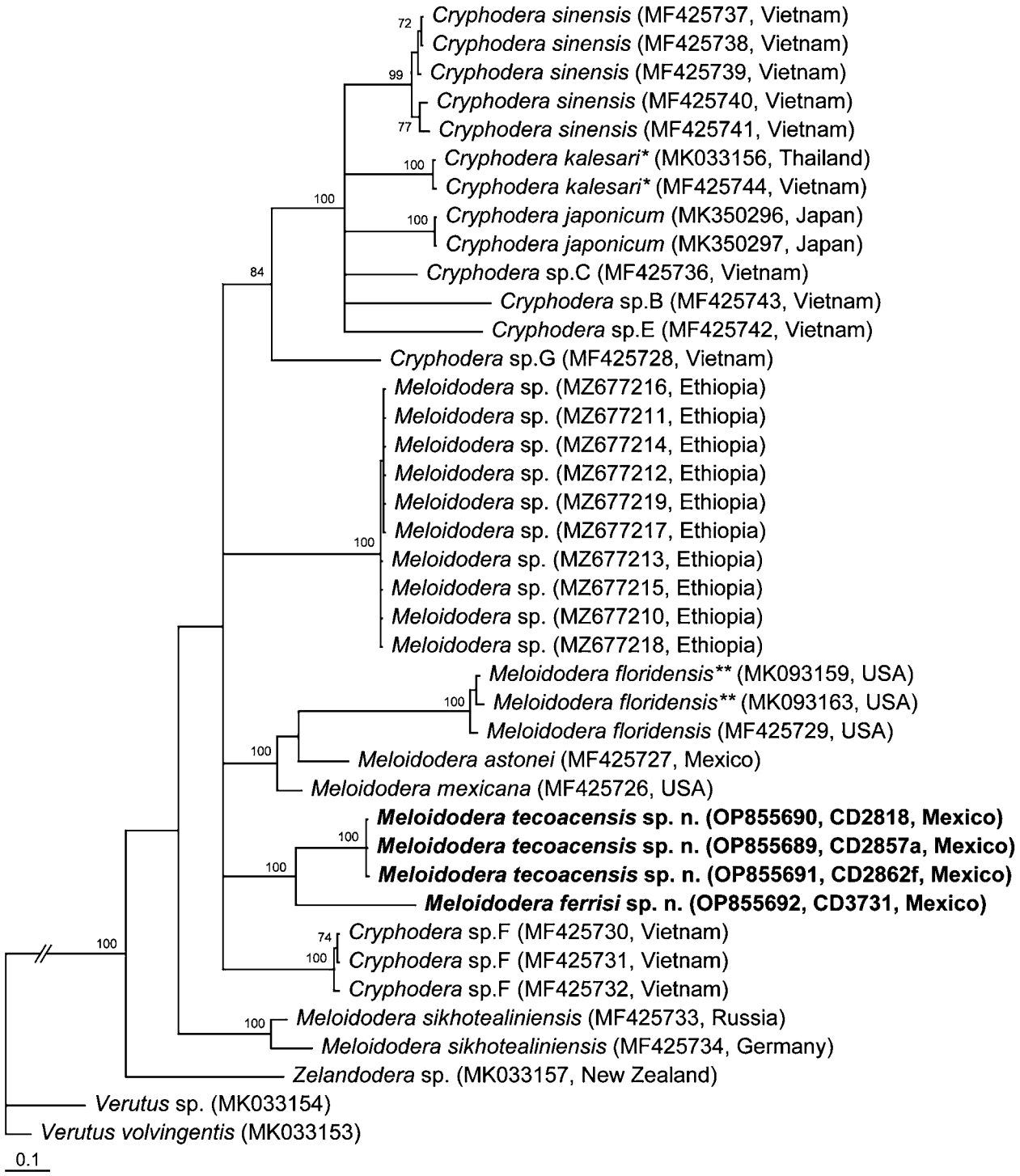

Fig. 10. Phylogenetic relationships within the subfamily Meloidoderinae: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the *COI* gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than, 70% are given for appropriate clades. New sequences are indicated in boldface. * Originally identified as *Cryphodera* sp. D (Subbotin *et al*., 2018). ** Originally identified as *Meloidodera* sp. (Powers *et al*., 2019).

and *Cryphodera* shared a common feature, both genera having females with finely annulated cuticle. However, they differ by vulva position (median to submedian in *Meloidodera* and terminal in *Cryphodera*) and numbers of incisures of J2 (four incisures in *Meloidodera* and three incisures in *Cryphodera*).

Our present and other studies (Subbotin *et al*., 2017, 2018) showed that the genus *Meloidodera* was non-monophyletic in the phylogenetic trees reconstructed from rRNA and *COI* gene sequence datasets and that their representatives were distributed among several clades with *Cryphodera* species. The ML testing done by Subbotin *et al*. (2018) revealed that the trees with an enforced monophyly of the genus *Meloidodera* were not significantly worse than the best ML tree, and thus concluded that a monophyly of the genus could not be excluded from the molecular datasets. However, in the present study, after adding sequences of one African and two new Mexican *Meloidodera* species to the ITS rRNA gene alignment, the ML test rejected a monophyly of this genus. For future studies, additional species of cystoid nematodes should be molecularly analysed to confirm monophylies of the genera *Meloidodera*, *Cryphodera* and *Zelandodera*. It will be also important to evaluate all morphological characters presently used in the taxonomy of this group. It cannot be excluded that representatives of *Cryphodera* could be transferred to the genus *Meloidodera* to obtain in an agreement between phylogenetic patterns and classification of this subfamily.

Based on analysis of species and genetic diversity of the genus *Meloidodera*, Subbotin *et al*. (2017) suggested that a local primary centre of diversity and origin for this genus coincided with one of primary biodiversity hotspots of the world, namely Mesoamerica and the Sierra Madre Mountains in Mexico. This hypothesis was supported by the present study where the two new Mexican species formed a basal clade in the phylogenetic Meloidoderinae tree derived from ITS rRNA gene sequences.

After description of the two new species, the genus *Meloidodera* now comprises 12 valid species. All *Meloidodera* species can be biogeographically divided into three groups according to Ghaderi (2019b): *i*) Northern American, Mexican species — *M. astonei*, *M. mexicana*, *M. ferrisi* sp. n., *M. tecoacensis* sp. n. and *M. zacanensis*; *ii*) Northern American, USA species — *M. belli*, *M. charis* Hopper, 1960, *M. eurytyla* Bernard, 1981 and *M. floridensis*; and *iii*) Eurasian species — *M. hissarica* Krall & Ivanova, 1992, *M. sikhotealiniensis* and *M. tianschanica* Ivanova & Krall, 1985. After including sequences deposited in the GenBank by Singh *et al*. (unpubl.) in our dataset, the phylogenetic analysis might suggest a fourth *Meloidodera* group from Africa. Sturhan (2018) already reported two unidentified African cystoid nematode species and tentatively attributed them to the genus *Meloidodera*; the first one was found from the rhizosphere of coffee plants in Ethiopia and the second one was collected from a rainforest in Cameroon. Recently, Singh *et al*. (unpubl.) deposited sequences of an unidentified *Meloidodera* species from coffee plants in Ethiopia in the GenBank; however, later, in the published article (Singh *et al*. 2022), these authors named these sequences as *Cryphodera* sp. In the 28S rRNA gene phylogenetic tree obtained in our study, these sequences clustered with those of *Meloidodera* species from North America, whereas in the 28S rRNA gene tree reconstructed by Singh *et al*. (2022), the Ethiopian sequences formed a clade with a *Zelandodera* sequence from New Zealand. The different positions of Ethiopian samples in these trees could be in results of influence of alignment parameters or species composition on phylogenetic inference. New studies with additional genetic markers and species could provide a more distinct picture of phylogenetic relationships and phylogeography of the subfamily Meloidoderinae and clarify taxonomic boundaries of its genera.

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