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Description of *Xiphinema ficusi* sp. n. (Nematoda: Longidoridae) and molecular characterisation of some *Xiphinema* species from Mexico

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Summary – In 2018-2022, during nematological surveys in several Mexican states, a new species, *Xiphinema ficusi* sp. n., two known species, *X. basiri* and *X. luci*, and four unidentified *Xiphinema* species, *Xiphinema* sp. A, sp. B, sp. C, and sp. D, were collected and characterised. *Xiphinema ficusi* sp. n. was found in a tropical forest in the La Mancha Ecological Reserve of the Ecology Institute of Jalapa, Veracruz state. The new species is characterised by a body length of 2.82-3.79 mm, slightly offset lip region, odontostyle 93-105 μm long, guiding ring at 51-96 μm from anterior end, an elongate conical tail 41-50 μm long with digitate terminus, didelphic-amphidelphic reproductive system, with both gonads equally developed without Z differentiation in females. Males have one pair of adanal and four ventromedial supplements. The seven *Xiphinema* species were molecularly characterised and the phylogenetic relationships of these species with other representatives of this genus were reconstructed using the D2-D3 expansion segments of 28S rRNA and *COI* gene sequences. Molecular analysis revealed the presence of a parasitic oomycete of the genus *Lagenidium* in *X. ficusi* sp. n. and endosymbiotic bacteria of the genus *Candidatus Xiphinematobacter* in *X. luci* and *Xiphinema* sp. D. belonging to the *Xiphinema americanum* group. It has been suggested that valleys surrounded by ridges of Sierra Madre Mountains in Mexico might be one of the world centres of diversity for the genus *Xiphinema*.

Keywords – *Candidatus Xiphinematobacter*, dagger nematode, endosymbiotic bacteria, *ficus* tree, *Lagenidium* sp., morphology, new species, oomycete, taxonomy, *Xiphinema basiri*, *Xiphinema luci*, *Xiphinema* spp.

The nematode genus *Xiphinema* Cobb, 1913 is a very large, diverse and complex nematode taxon. These nematodes are distributed worldwide and some have been shown to damage a wide range of cultivated plants. Some species of the genus are known to transmit nepoviruses in fruit and vegetable crops (Taylor & Brown, 1997). The genus *Xiphinema* has been divided into two species groups: *i*) the *Xiphinema americanum* group of more than 60 species; and *ii*) the *Xiphinema* non-*americanum* group comprising more than 220 species (Coomans *et al.*, 2001; Archidona-Yuste *et al.*, 2016a, b; Peraza-Padilla *et al.*, 2018; Naghavi *et al.*, 2022).

Nematodes of the genus *Xiphinema* have been reported in association with different plants in almost all Mexican states (Cid del Prado-Vera *et al.*, 2018). However, until now, only five species of the genus have been identified from Mexico: *Xiphinema americanum sensu lato* Cobb, 1913 from grapevines in several states (Teliz & Goheen, 1968; Cid del Prado-Vera *et al.*, 2018); *X. basiri* Siddiqi, 1959 in the Campeche, Yucatan and Baja California Sur states (Cohn & Sher, 1972; Norton *et al.*, 1984); *X. californicum* Lamberti & Bleve-Zacheo, 1979 in central Mexico (Lamberti & Golden, 1986); *X. index* Thorne & Allen, 1950 from grapevines in several states (Nor-

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ton *et al.*, 1984; Cid del Prado-Vera *et al.*, 2018), and *X. tropicale* Zullini, 1973 described from a tropical rainforest in Chiapas state (Zullini, 1973). The reports of *X. index* Thorne & Allen, 1950 from cave mud in Chiapas state by Zullini (1973) and *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939 in Sonora state by Norton *et al.* (1984) require confirmation. Robbins & Brown (1991) noticed that it is likely that many of the earliest reports of *X. diversicaudatum* in North America referred to other indigenous species.

In 2018-2022, during nematological surveys in several Mexican states, a new species, two known and several unidentified species of the genus *Xiphinema* were found in natural ecosystems. Several specimens of the new species were collected from the rhizosphere of a *Ficus benghalensis* L. tree in the La Mancha Ecological Reserve in Veracruz state. This nematode is described herein as *Xiphinema ficusi* sp. n. Sporangia of a parasitic oomycete (Oomycota) were observed in bodies of several specimens of this species.

Species of the *Xiphinema americanum* group are known to carry gram-negative rod-shaped verrucomicrobial endosymbionts, *Candidatus Xiphinematobacter* spp., which show strong co-speciation with their nematode hosts (Orlando *et al.*, 2016; Palomares-Rius *et al.*, 2016). Two of the *Xiphinema* species found in Mexico during the present study belonged to the *X. americanum* group and contained these bacteria.

The main objectives of the current study are: *i*) to describe a new species, *Xiphinema ficusi* sp. n., from Veracruz state; *ii*) to characterise molecularly *Xiphinema* spp. collected in Mexico using the D2-D3 expansion segments of the 28S rRNA and partial *COI* gene sequences; and *iii*) to characterise molecularly several bacterial endosymbionts from the genus *Ca. Xiphinematobacter* and a nematoparasitic oomycete (*Lageinidium* sp.) found in *Xiphinema* species.

Materials and methods

Nematode samples

More than 160 soil samples were collected from different plants in several Mexican states. Nematodes were extracted from soil using a sieving and decanting method (Brown & Boag, 1988) or centrifugal-flotation (Coolen, 1979) method and picked up under a stereomicroscope and used for microscopic study or transferred to 70% ethanol for molecular analysis.

Morphological study

The nematodes were killed by heating under an alcohol lamp in 1-2 drops of tap water on a glass microscope slide and then placed in glass vials containing Golden fixative (Hooper, 1970). Nematodes were stored at room temperature for 10 days and then transferred to a small Petri dish placed in a small desiccator containing 95% ethanol and incubated at 40°C for 1-2 days, processed to glycerin using a modification of the Seinhorst (1959), method as described by Cid del Prado-Vera & Subbotin (2012), and then mounted on glass slides using the paraffin wax ring method (De Maeseneer & d'Herde, 1963) in anhydrous glycerin containing 1-2 drops of fast green stain. Measurements and drawings were made using a drawing tube and an American Optical compound microscope. Light photomicrographs of nematodes were taken with an automatic Infinity 2 camera attached to a compound Olympus BX51 microscope equipped with Nomarski interference contrast. 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 different concentration. The specimens were critical point-dried and coated with gold-palladium before observation under a Jeol JSM-6390 scanning electron microscope at 10 kV (Cid del Prado Vera & Subbotin, 2012).

Molecular study

DNA was extracted from single *Xiphinema* specimens using proteinase K. DNA extraction and PCR protocols were conducted according to Subbotin (2021a). Two primer sets were used for amplification of nematode genes: *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; and *ii*) Long-COIFmod (5′-G ATT YTT TGG DCA CCC NGA RGT-3′) and Long-COIRmod (5′-GCH ACY ACR TAR TAR GTR TCR TG-3′), amplifying the *COI* gene. The primer set: 16SF_univ_bact (5′-AGA GTT TGA TCC TGG CTC AG-3′) and Xi_bacter_16SR1 (5′-AGC TCY GAG ATT TCA CAC TTG-3′) was used for amplification of partial 16S rRNA gene for *Ca. Xiphinematobacter* as described by Orlando *et al.* (2016). Two primer sets were used for amplification of oomycete genes from infected nematode specimens: *i*) 18S (5′-TTG ATT ACG TCC CTG CCC TTT-3′) and AB28 (5′-ATA TGC TTA AGT TCA GCG GGT-3′), amplifying the ITS rRNA; and *ii*) D2A and D3B, amplifying the

D2-D3 of 28S rRNA gene. The PCR products were purified using a QIAquick PCR extraction kit (Qiagen) and then submitted for direct sequencing. Sequencing was conducted at Azenta.

New sequences were aligned using ClustalX 1.83 with corresponding selected and published gene sequences for nematodes (He *et al.*, 2005; Lazarova *et al.*, 2006; Gutiérrez-Gutiérrez *et al.*, 2012; Archidona-Yuste *et al.*, 2016a,b, 2020; Palomares-Rius *et al.*, 2017), bacteria (Lazarova *et al.*, 2016; Orlando *et al.*, 2016; Palomares-Rius *et al.*, 2016; Peraza-Padilla *et al.*, 2018; Mobasseri *et al.*, 2019; Vandekerckhove *et al.*, 2000; Myers *et al.*, 2021) and oomycetes (Spies *et al.*, 2016). Several alignments were created: *i*) the D2-D3 of 28S rRNA gene sequence alignment of selected species of the *Xiphinema* non-*americanum* group; *ii*) the D2-D3 of 28S rRNA gene sequence alignment of selected species of the *X. americanum* group; *iii*) the *COI* gene sequence alignment of selected species of the *Xiphinema* non-*americanum* group; *iv*) the *COI* gene sequence alignment of selected species of the *X. americanum* group; *v*) the partial 16S rRNA gene sequence alignment of *Ca. Xiphinematobacter* species; and *vi*) the ITS rRNA and D2-D3 of 28S rRNA gene sequence alignment of selected species of the genera *Lagenidum* and *Myzocytiopsis*. Sequence alignments were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) as described by Subbotin (2021b). The new sequences were submitted to the GenBank database under accession numbers: OR502905-OR502912 (D2-D3 of 28S rRNA gene of *Xiphinema* spp.), OR498812-OR498830 (*COI* gene of *Xiphinema* spp.), OR515752-OR515760 (16S rRNA gene of *Ca. Xiphinematobacter*), OR50514 (ITS rRNA gene of *Lagenidium* sp.) and OR502383 (D2-D3 of 28S rRNA gene of *Lagenidium* sp.).

Results and discussion

Xiphinema specimens were found in ten of 160 collected soil samples. Using traditional morphological taxonomic characters integrated with molecular criteria, we distinguished a new species, *X. ficusi* sp. n., two known species, *Xiphinema basiri* and *X. luci* Lamberti & Bleve-Zacheo, 1979, and three unidentified *Xiphinema* species: *Xiphinema* sp. A, sp. B, sp. C from the *Xiphinema* non-*americanum* group, and one unidentified *Xiphinema* sp. D from the *X. americanum* group (Table 1). Morphological description is given for the new species only; lack of sufficient specimen numbers did not allow giving morphological characterisation of other *Xiphinema*.

Xiphinema ficusi **sp. n.**
(Figs 1-3)

MEASUREMENTS

See Table 2.

DESCRIPTION

Female

Body curved ventrad, cylindrical, tapering at both ends, more so posteriorly. Cuticle smooth, with fine annulation visible on SEM photos, 1.6-2.3 μm thick at neck region, 2.3-3.1 μm at mid-body and 3.0-5.0 μm in the tail region. Lip region rounded, separated by a slight depression. Amphid aperture goblet-shaped. Odontostyle furcated at base. Odontophore well developed, with flanges. Pharyngeal cylindrus occupying 33-49.7 (41.3 $\pm$ 5.5)% of pharynx length. Cardia inconspicuous, small and conoid, 6.9 $\pm$ 1.2 (5.0-8.0) μm long. Nerve ring encircling slender anterior region of pharynx. Reproductive system didelphic-amphidelphic, gonads almost equally developed and both functional, ovaries reflexed. Uteri of uniform diam. Z differentiation not observed. Vulva a transverse slit, vagina with thickened cuticular lining, 12-15 (14.2 $\pm$ 0.9) μm long, about 34-46.9 (39.7 $\pm$ 4.5)% of body width, appearing oblique in a few specimens. Tail conoid with digitate terminus, its length 1.7-2.1 times anal body diam. Caudal papillae absent.

Male

Body slightly curved at posterior end, lip region similar to that of females. Testis monorchic, 52-86% of body length. Dorylaimid spicules with lateral guiding pieces 22 μm long. Five supplements, one of adanal and four ventromedial, anterior to the cloacal aperture: the first at 12-15 (14.3 $\pm$ 1.3) μm, the second at 31-40 (37.4 $\pm$ 3.3) μm, the third at 49-72 (59.6 $\pm$ 9.7) μm, the fourth at 83-95 (88.1 $\pm$ 4.0) μm and the fifth at 108-130 (117.9 $\pm$ 8.3) μm.

Juveniles

Several juvenile specimens were found. Body shape and tail similar to adults; only the third and four stages have been identified.

Table 1. *Xiphinema* species found in this study.

Species	Locality	GPS coordinates (Host)	Sample code	GenBank accession number	
				D2-D3 of 28S rRNA gene	*COI* gene
Xiphinema basiri	San Luis Potosí state, Municipio Axtla de Terrazas	Lat. 21.43038; Long. −98.89461; Alt. 830 m (Unknow trees)	CD3004 (sample 47, trip 2)	OR502908	OR498822-OR498824
X. basiri	Hidalgo state, Municipio de Huejutla de Reyes	Lat. 21.02428; Long. −98.61801; Alt. 1260 m (Unknow trees)	CD3005 (sample 57, trip 2)	OR502907	OR498825
X. ficusi sp. n.	Veracruz state, Municipio de Actopan	19.59316; Long −96.37925; Alt. 2 m (*Ficus benghalensis*)	CD3965, CD4012	OR502909	OR498827
X. luci	Hidalgo state, Municipio de Huejutla de Reyes	Lat. 21.07832; Long. −98.48232; Alt. 294 m (*Solanum* sp.)	CD3002 (sample 48, trip 2)	OR502906	OR498821
X. luci	Hidalgo state, Municipio de Huejutla de Reyes	Lat. 21.07832; Long. −98.48232; Alt. 294 m (*Solanum* sp.)	CD3000 (sample 53, trip 2)	–	OR498817-OR498819
Xiphinema sp. A	Hidalgo state, Municipio de Singuilucan	Lat. 20.09196; Long. −98.58680; Alt. 2850 m (*Senecio* sp.)	CD3008 (sample 81, trip 2)	OR502912	OR498829-OR498830
Xiphinema sp. B	Querétaro state, Municipio Pinal de Amoles	Lat. 21.12250; Long. −99.67141; Alt. 2590 m (Oak tree)	CD3003 (sample 27, trip 2)	OR502910	OR498826, OR498828
Xiphinema sp. C	Hidalgo state, Municiopio de Tlanchinol	Lat. 21.02147; Long. −98.64607; Alt. 1580 m (*Solanum* sp.)	CD3001 (sample 60, trip 2)	OR502911	OR498820
Xiphinema sp. D	Querétaro state, Municipio de Cadereyta de Montes	Lat. 20.73757; Long. −99.71244; Alt. 2190 m (Unknown plants)	CD2999a (sample 11, trip 2)	–	OR498815-OR498816
Xiphinema sp. D	Querétaro state, Municipio de Cadereyta de Montes	Lat. 20.73757; Long. −99.71244; Alt. 2190 m (Unknown plants)	CD2998a (sample 9, trip 2)	OR502905	OR498812, OR498814

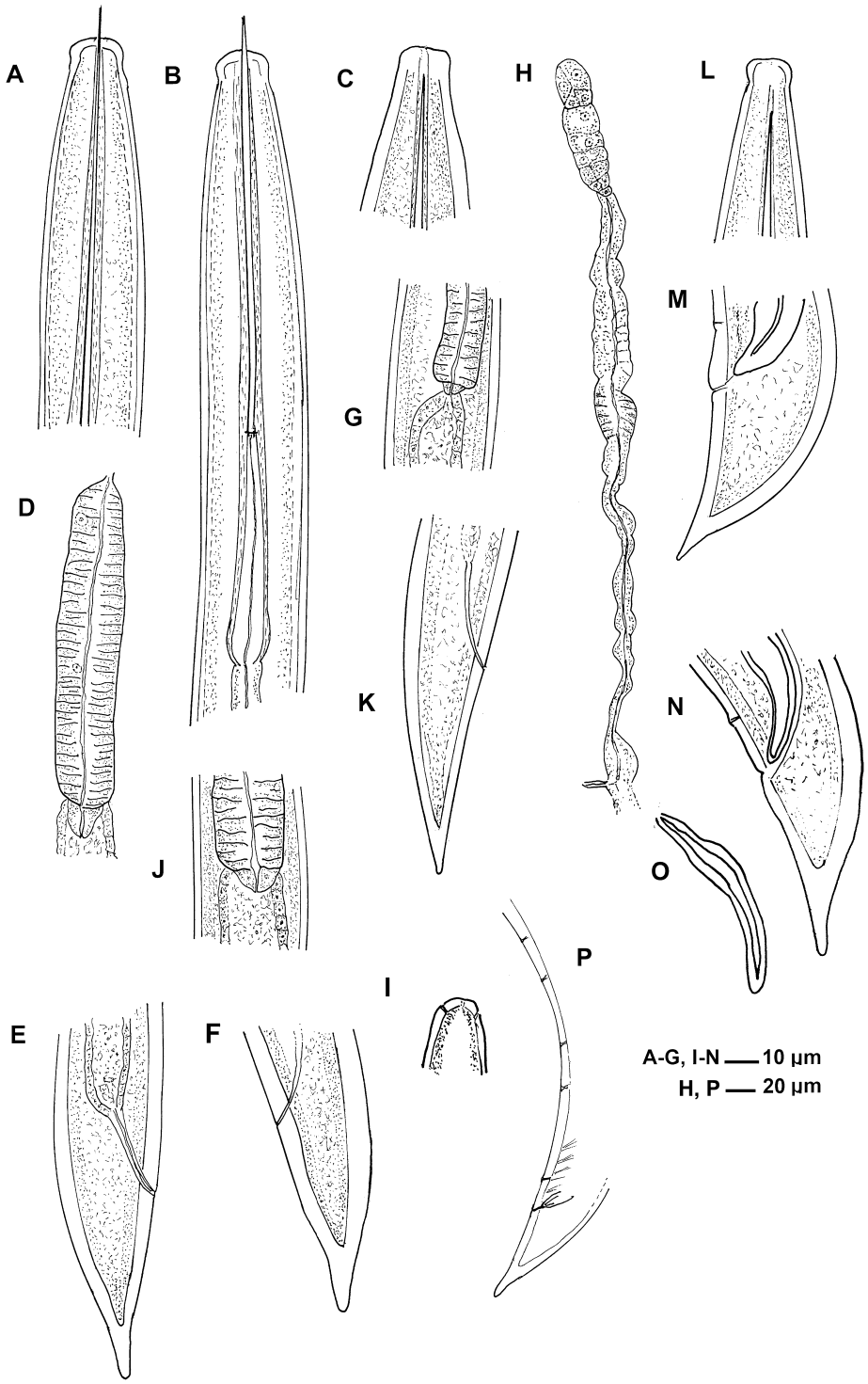

Fig. 1. *Xiphinema ficusi* sp. n. Female. A, B: Anterior region; C: Lip region; D: Pharynx; E, F: Tails; G: Basal part of pharynx; H: Anterior genital branch. Juvenile. I: Lip region; J: Pharyngeal cardia; K: Tail. Male. L: Lip region; M, N: Tails; O: Spicule; P: Posterior body region with supplements.

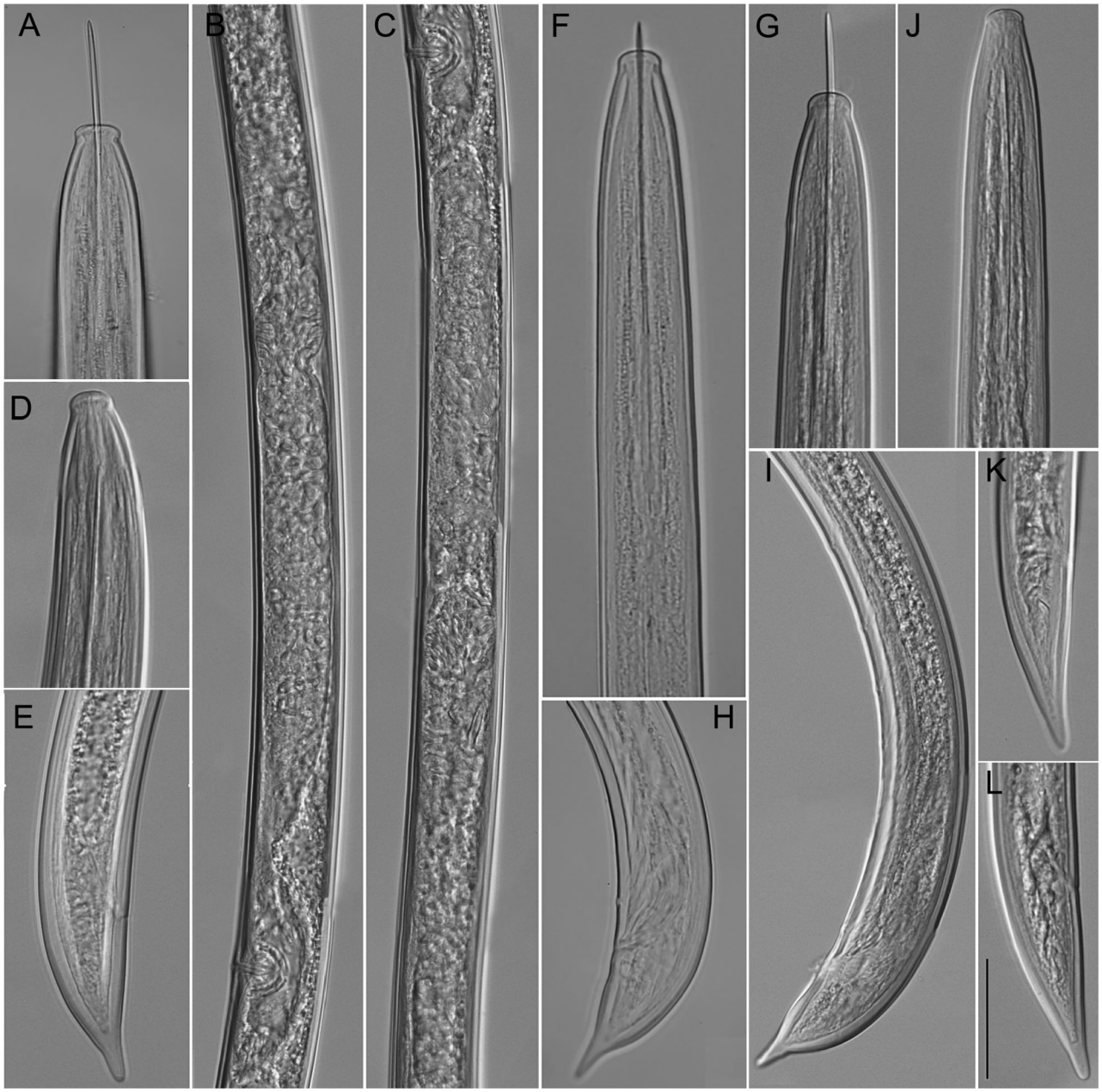

Fig. 2. Light photomicrographs of *Xiphinema ficusi* sp. n. Female. A, D: Anterior region; B: Fragment of anterior genital branch; C: Fragment of posterior genital branch; E: Posterior region. Male. F, G: Anterior region; H, I: Posterior region. Juvenile. J: Anterior region of 4th stage juvenile; K: Tail of third-stage juvenile (J3); L: Tail of fourth-stage juvenile (J4). (Scale bars: A, D-L: = 30 μm; B, C: = 40 μm.)

DIFFERENTIAL DIAGNOSIS

Xiphinema ficusi sp. n. is characterised by body curving ventrad, the lip region having a weak depression, the female didelphic-amphidelphic with equal development of both genital branches, Z differentiation absent, the tail conical, somewhat digitate. Males having five ventromedial supplements.

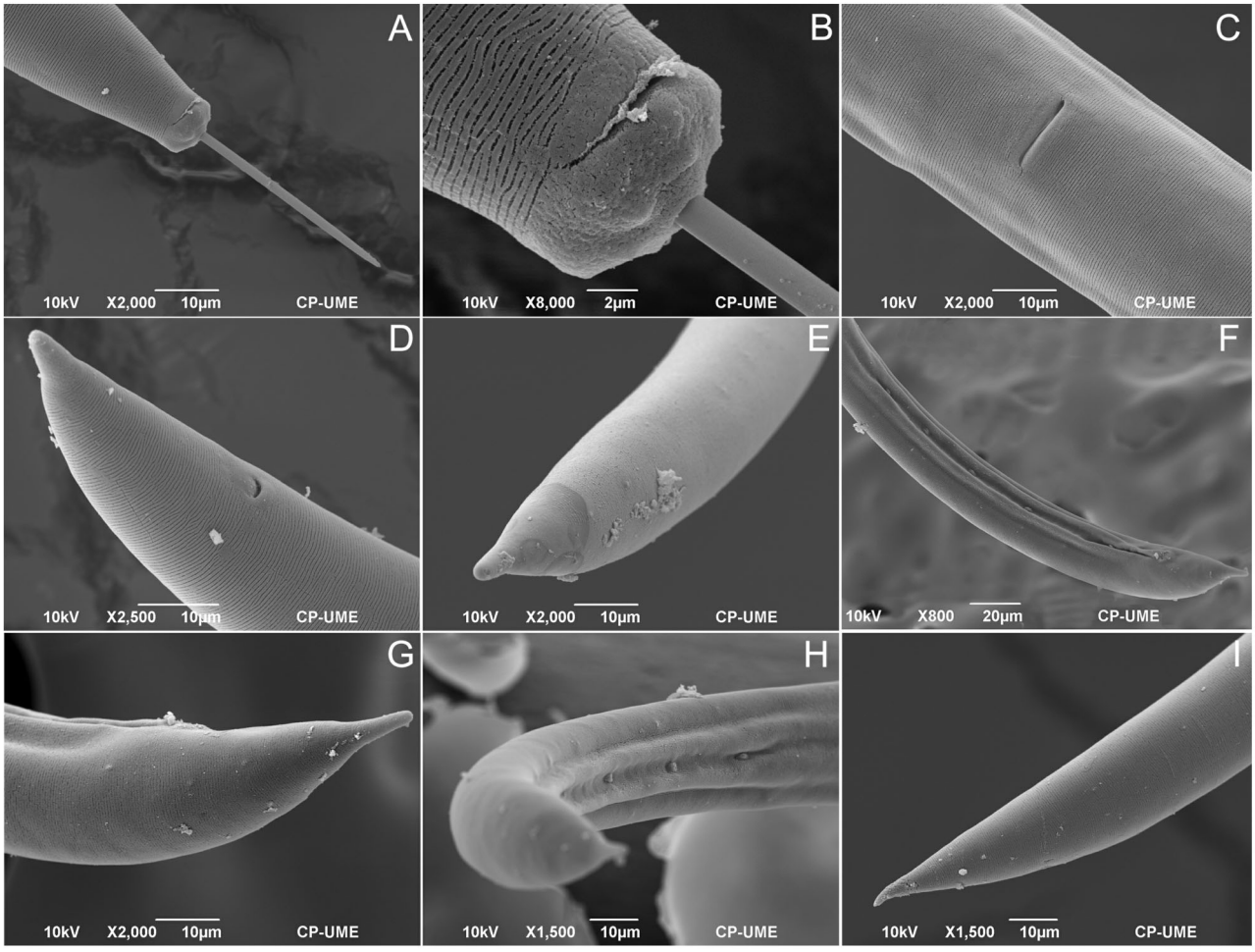

Fig. 3. Scanning electron photomicrographs of *Xiphinema ficusi* sp. n. Female. A: Anterior region; B: Lateral view of lip region; C: Vulva region; D, E: Posterior region. Male. F-H: Posterior region. I: Tail of third-stage juvenile (J3).

According to the polytomous key of Loof & Luc (1990), *Xiphinema ficusi* sp. n. has the following codes: A4, B4, C34, D4, E456, F34, G12, H2, I3, J34, K34, L2 and belongs to group 7.

Xiphinema ficusi sp. n. is morphologically similar to *X. algeriense* Luc & Kostadinov, 1982, *X. ifacolum* Luc & de Guiran, 1960, *X. tropicale* Zullini, 1973, *X. macroacanthum* Lamberti, Roca & Agostinelli, 1989 and *X. israeliae* Luc, Brown & Cohn, 1982.

Xiphinema ficusi sp. n. differs from *X. algeriense* (Luc & Kostadinov, 1982) by shorter body length (2.8-3.8 *vs* 3.9-4.9 mm), lip region separated by a weak depression *vs* conspicuous constriction, smaller index a (80.5-108 *vs* 98-118), and shorter odontostyle length (93-105 *vs* 106-125 μm); from *X. ifacolum* (Luc, 1961; Susulovska *et al.*, 2018) in shorter female odontostyle length (93-105 *vs* 119-127 μm), shorter spicule length (40-48 *vs* 53 μm), heat-relaxed body shape curved ventrad *vs* hook-shaped; from *X. tropicale* (Zullini, 1973) in longer body length (2.8-3.8 *vs* 2.2-2.6 mm), more posterior vulva (V = 44-60 *vs* 37-39%), shorter odontostyle length (93-105 *vs* 110-122 μm) and conical *vs* rounded tail of females; from *X. macroacanthum* (Lamberti *et al.*, 1989) in shorter body length (2.8-3.8 *vs* 4.3-5.7 mm), larger index a (80.5-108.3 *vs* 63.2-81.4), shorter odontostyle length (93-105 *vs* 145.8-169 μm), longer tail (41-50 *vs* 29-43.5 μm) in females and shorter body length (2.1-3.7 *vs* 4.2-5.7 mm), and shorter odontostyle length (95-105 *vs* 145.8-169 μm) in males; from *X. israeliae* (Luc *et al.*, 1982; Tzortzakakis *et al.*, 2014) in shorter odontostyle length (93-105 *vs* 113-

Table 2. Morphometric measurements of *Xiphinema ficusi* sp. n. All measurements are in μm (except L in mm) and presented as mean ± standard deviation (range).

Character	Holotype female	Paratype females	Allotype male	Paratype males	Juveniles, 3rd stage	Juveniles, 4th stage
n	1	11	1	6	2	3
L (mm)	3.37	3.25 ± 0.28 (2.82-3.79)	3.74	3.1 ± 0.62 (2.1-3.7)	1.75, 2.51	2.4 ± 0.12 (2.26-2.52)
a	96.1	95.2 ± 7.4 (80.5-108.3)	124.7	98.8 ± 17.8 (71-125)	55, 66	83 ± 4.3 (78.6-87)
b	15.3	18.5 ± 6.3 (10.8-32.0)	16.3	11.3 ± 4.1 (6.6-16.7)	9.7, 11	11.9 ± 1.3 (10.5-12.8)
c	82.1	71.0 ± 9.2(61.3-90.0)	93.5	68.8 ± 14.2 (47.3-93.5)	30.8, 33	47 ± 7.9 (40.5-56)
c′	1.7	1.9 ± 0.1 (1.7-2.1)	1.4	1.6 ± 0.2 (1.4-1.9)	2.7, 2.8	2.4 ± 0.5 (1.9-2.8)
V%/T%	45.9	49.1 ± 4.4 (44-60)	82.9	61.5 ± 13.7 (50.7-86.7)	–	–
Lip width	12	12.2 ± 0.5 (12-13)	12	12.3 ± 0.5 (12-13)	10, 12	11 ± 0.6 (11-12)
Lip height	4	5.3 ± 0.6 (4-6)	6.0	5.4 ± 0.5 (5.0-6.0)	–	–
Odontostyle	97	100.5 ± 3.3 (93-105)	100	100.0 ± 2.3 (95-103)	69, 72	82.7 ± 0.6 (82-83)
Odontophore	62	57.9 ± 3.1 (52-62)	58	55.3 ± 6.5 (40-61)	44, 45	52.7 ± 2.5 (50-55)
Spear	159	156 ± 6.2 (139-162)	158	156 ± 6.9 (140-162)	113, 117	135 ± 2.1 (133-137)
Pharynx	220	190.6 ± 28.5 (135-221)	229	277 ± 62.6 (187-350)	147, 180	200 ± 13.8 (188-215)
Anterior end to guide ring	78	80.3 ± 13.9 (51-96)	61	81.0 ± 17.2 (57-95)	55, 65	69.0
Anterior end to nerve ring	–	183.7 ± 17.6 (158-200)	196	184 ± 16.5 (156-196)	–	–
Anterior gonad	291	276.8 ± 55.5 (182-317)	–	–	–	–
Posterior gonad	292	261.5 ± 42.8 (220-306)	–	–	–	–
Tail	41	46.4 ± 2.8 (41-50)	40	44.3 ± 3.9 (40-50)	53, 56	51 ± 7.8 (42-56)
Rectum	23	24 ± 2.8 (20-30)	–	–	–	–
Spicules	–	–	40	44.3 ± 3.1 (40-48)	–	–
Hyaline part of tail length	7	12.5 ± 3.1 (7.0-16)	15	14.5 ± 1.9 (13-15)	10, 14	13.7 ± 1.5 (12-15)
Maximal body width	35	34 ± 1.6 (32-37)	30	30.9 ± 1.4 (29-33)	26, 32	28.7 ± 2.3 (26-30)
Body width at level of guide ring	29	27.3 ± 1.4 (25-30)	26	27.0 ± 0.8 (26-28)	–	–
Body width at base of pharynx	32	31.0 ± 1.0 (30-33)	28	30.0 ± 1.6 (27-32)	–	–
Body width at level of anus	24	24.1 ± 0.8 (23-25)	28	27.0 ± 1.6 (24-29)	–	–

139 μm) and shorter distance from anterior to guide ring (51-96 *vs* 96-124 μm) in females and shorter spicules (40-48 *vs* 46-68 μm) in males.

Type locality and host

Sandy soil around roots of a banyan fig, *Ficus benghalensis* L., in the La Mancha Ecological Reserve of the Ecology Institute of Jalapa, Veracruz state, Mexico. Global coordinates: Lat. 19.59316 N; Long. 96.37925 W, 2 m a.s.l.

Etymology

The specific epithet refers to the scientific genus name of the host.

Type specimens

Slides with holotype female (CNHE 12805), allotype male (CNHE 12806) and paratype females (CNHE 12807) were deposited in the Laboratorio de Helmintología del Instituto de Biología, UNAM, Mexico. Other paratype materials were deposited in the University of California, Riverside, Nematode Collection, the Colegio de Postgraduados Nematode Collection (A-119), and the United States Department of Agriculture Nematode Collection, Beltsville, MD, USA.

Xiphinema basiri Siddiqi, 1959
(Fig. 4A, B, J, G, K)

Xiphinema basiri was originally described in association with *Citrus* x *sinensis* (L.) Osbeck. in Aligarh (U.P.),

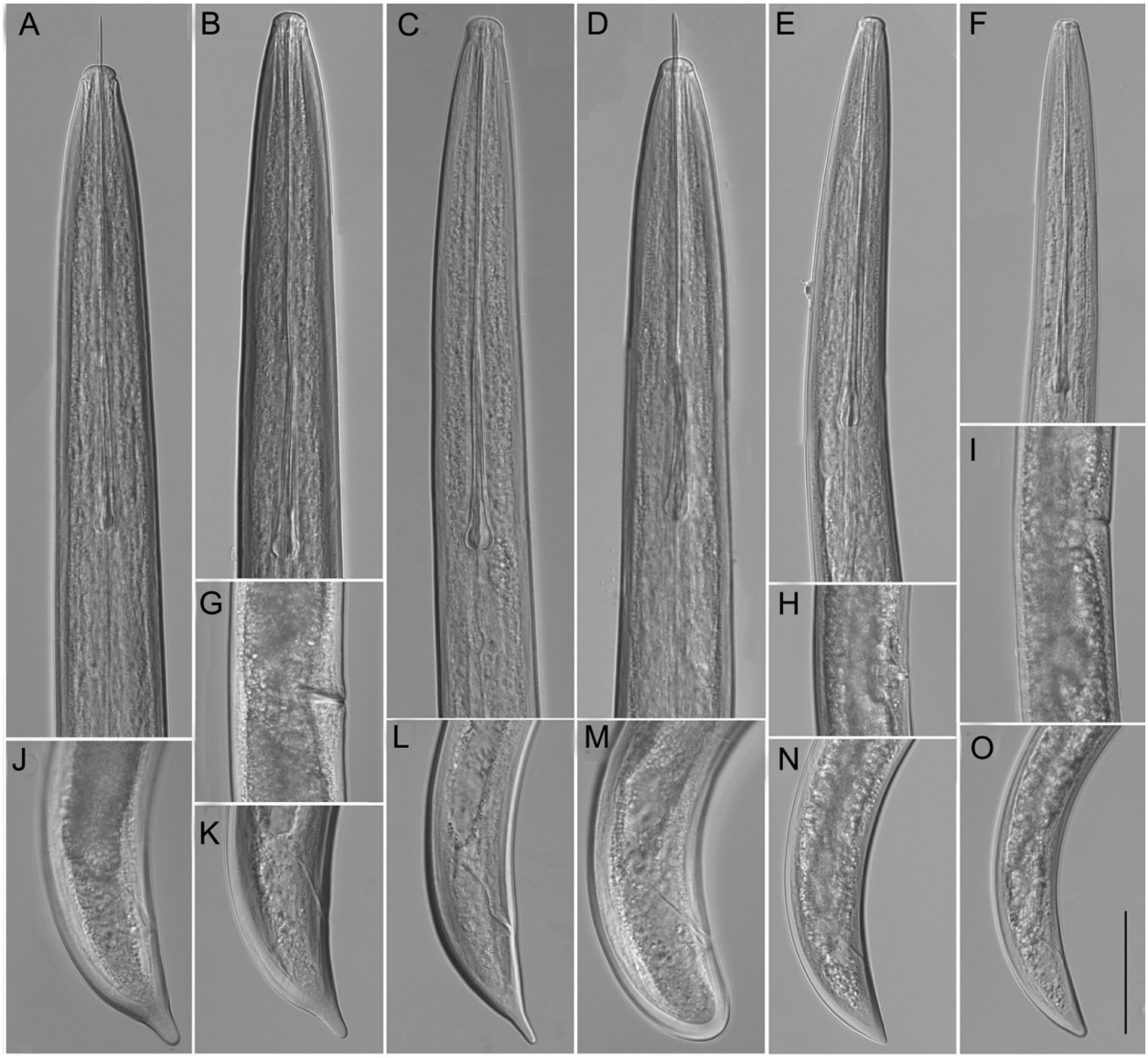

Fig. 4. Light photomicrographs of *Xiphinema* spp. found in Mexico. A, B, G, J, K: *X. basiri*; C, L: *Xiphinema* sp. A; D, M: *Xiphinema* sp. C; E, H, N: *X. luci*; F, I, O: *Xiphinema* sp. D. A-F: Anterior region; G, H, I: Vulva region; J-O: Posterior region. (Scale bar = 30 μm.)

India (Siddiqi, 1959), and was later found in the rhizospheres of various crop plants (Roy, 1973). This species has also been reported from Cuba, Martinique, Mexico, Nigeria, Pakistan, Puerto Rico, Sri Lanka, Sudan, USA (Florida) and Zimbabwe (Cohn & Sher, 1972; Swart & Quénéhervé, 1998; Antúnez & Basterrechea, 2011; Gill & Firoza, 2014).

Xiphinema basiri causes discoloured, swollen and stubby roots on bitter lemon and mango (Yassin, 1974). In experimental tests, *X. basiri* significantly affected the growth of tomato and eggplant (Babu & Murthukrishnan, 1990).

In Mexico, *X. basiri* has been found in Campeche and Yucatan states (Knobloch & Laughlin, 1973; Norton *et al.*, 1984) and in La Paz, Baja California Sur state (Cohn & Sher, 1972). Morphometrics of the Mexican populations of this species were reported by Knobloch & Laughlin (1973) and Cohn & Sher (1972). In this study, the presence of *X. basiri* in Hidalgo state is confirmed by molecular methods. Sequences of the D2-D3 of 28S

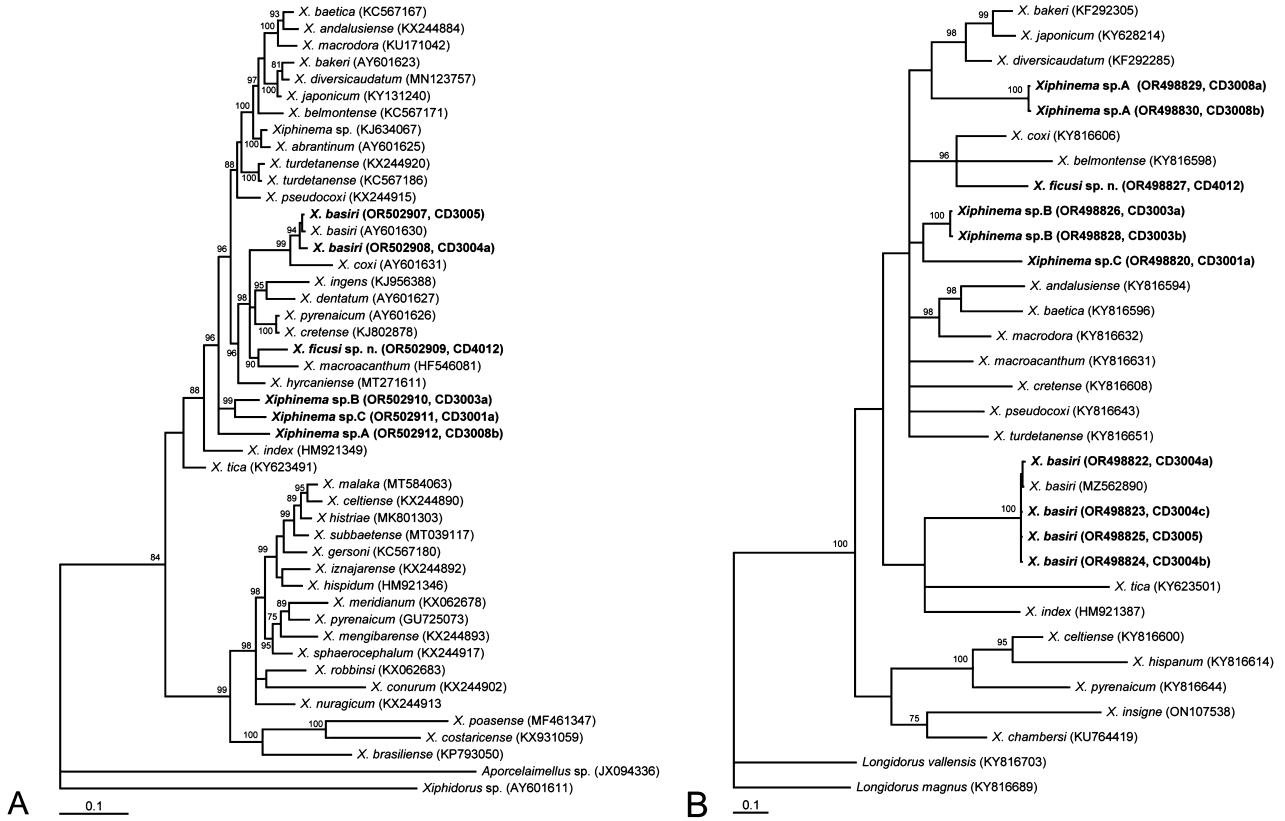

Fig. 5. Phylogenetic relationships within some species of the *Xiphinema* non-*americanum* group. Bayesian 50% majority rule consensus tree as inferred from the analysis of the D2-D3 of 28S rRNA (A) and partial *COI* (B) gene sequence alignments. Posterior probabilities more than 70% are given for appropriate clades. New sequences obtained in this study are in boldface.

rRNA and *COI* genes of the Mexican *X. basiri* clustered with those of *X. basiri* from Cuba (He *et al.*, 2005) and India (Deshwal *et al.*, data unpubl.) (Fig. 5).

Xiphinema luci Lamberti & Bleve-Zacheo, 1979
(Fig. 4E, H, N)

Xiphinema luci was described by Lamberti & Bleve-Zacheo (1979) from Senegal. This species was also reported from Florida, USA (MacGowan, 1988), but Robbins & Brown (1991) indicated that the Floridian *X. luci* report requires confirmation. This species was also found in Spain; it was described and molecularly characterised using *COI*, partial 18S rRNA and 28S rRNA gene sequences (Archidona-Yuste *et al.*, 2016b; Palomares-Rius *et al.*, 2017). Recently, Myers *et al.* (2021) published nematode and endosymbiotic bacterium sequences and indicated that *X. luci* also occurred in Texas and New Mexico, USA.

In the present study, *X. luci* was identified from two samples collected at the same location in Hidalgo state. The presence of *X. luci* in Mexico was herein reported for the first time. Sequences of the *COI* gene of the Mexican *X. luci* clustered with that of *X. luci* from Spain (Palomares-Rius *et al.*, 2017) (Fig. 6A).

Xiphinema spp.

Three unidentified *Xiphinema* species: *Xiphinema* sp. A, sp. B, and sp. C from the *Xiphinema* non-*americanum* group, were found in this study. We present photomicrographs of females of *Xiphinema* sp. A (Fig. 4C, L), females of *Xiphinema* sp. C (Fig. 4D, M) and females of *Xiphinema* sp. D (Fig. 4F, I, O). Adults were not found for *Xiphinema* sp. B.

Xiphinema sp. D was found in two soil samples collected at the same location in Querétaro state. This species belongs to the *X. americanum* group and morphologically

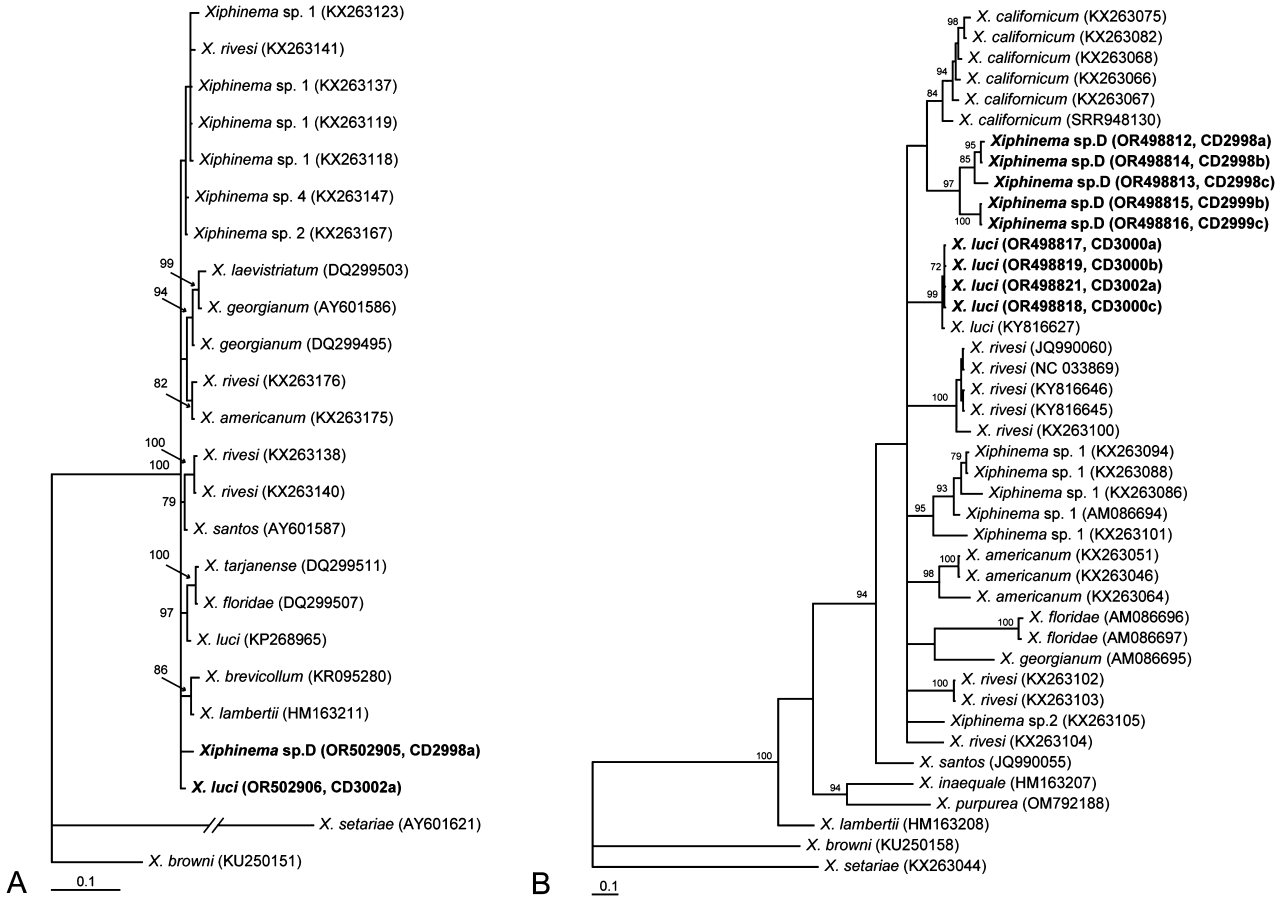

Fig. 6. Phylogenetic relationships within some species of the *Xiphinema americanum* group. Bayesian 50% majority rule consensus tree as inferred from the analysis of the D2-D3 of 28S rRNA (A) and partial *COI* (B) gene sequence alignments. Posterior probabilities more than 70% are given for appropriate clades. New sequences obtained in this study are in boldface.

and molecularly is similar to *X. californicum*. We cannot exclude the possibility that previously published reports of *X. californicum* in Mexico (Lamberti & Golden, 1986) might actually refer to this undescribed species.

Molecular characterisation of *Xiphinema ficusi* sp. n. and other *Xiphinema* spp. from Mexico

D2-D3 of 28S rRNA gene of the Xiphinema *non*-americanum *group*

The sequence alignment contained 45 sequences of 43 species of *Xiphinema* non-*americanum* group and two outgroup taxa and was 807 bp in length. The sequence of *X. ficusi* sp. n. formed a clade with that of *X. macroacanthum* from southern Italy and differed from it by 5.3% (41 bp). Phylogenetic relationships of *X. ficusi* sp. n. with selected species of *Xiphinema* non-*americanum* group are given in Figure 5A. Intraspecific sequence diversity for *X. basiri* varied from 0.1 to 0.8%. Phylogenetic positions of three unidentified *Xiphinema* spp. (sp. A., sp. B, sp. C) and *X. basiri* from Mexico are presented in Figure 5A. All *Xiphinema* non-*americanum* group species found in Mexico belonged to the clade ii *sensu* Peraza-Padilla *et al.* (2018).

D2-D3 of 28S rRNA gene of the Xiphinema americanum *group*

The sequence alignment contained 24 sequences of 14 species of *X. americanum* group from clade I *sensu* Orlando *et al.* (2016) and two outgroup taxa and was 781 bp in length. Phylogenetic positions of *X. luci* and unidentified *Xiphinema* sp. D from Mexico are

presented in Figure 6A. Maximal sequence diversity of *X. americanum* group from clade I was 3.8%.

COI *gene of the* Xiphinema *non*-americanum *group*

The sequence alignment contained 30 sequences of 24 species of *Xiphinema* non-*americanum* group and two outgroup taxa and was 373 bp in length. Sequence of *X. ficusi* sp. n. formed a clade with those of *X. coxi* Tarjan, 1964 and *X. belmontense* Roca & Pereira, 1992 and differed from them by 16.9% (59 bp) and 19.9% (67 bp), respectively. Phylogenetic relationships of *X. ficusi* sp. n. with selected species of *Xiphinema* non-*americanum* group are given in Figure 5B. Phylogenetic positions of other *Xiphinema* spp. (sp. A., sp. B, sp. C) and *X. basiri* from Mexico are presented in Figure 5B.

COI gene of the Xiphinema americanum *group*

The sequence alignment contained 42 sequences of 13 species of *X. americanum* group from clade I *sensu* Orlando *et al.* (2016) and two outgroup taxa and was 371 bp in length. Phylogenetic positions of three unidentified *Xiphinema* spp. from Mexico are presented in Figure 6B. Intraspecific sequence diversity for *Xiphinema* sp. D varied from 0.8 to 10.4%.

Molecular characterisation of *Ca. Xiphinematobacter*

The study revealed the presence of *Ca. Xiphinematobacter luci* in *X. luci* and *Ca. Xiphinematobacter* sp.15 in *Xiphinema* sp. D. Intraspecific diversity of partial 16S rRNA gene sequences for *Ca. Xiphinematobacter* sp.15 varied from 0 to 2.3%. Phylogenetic relationships within *Ca. Xiphinematobacter* are given in Figure 7. Kim *et al.* (2014) proposed a threshold of 98.65% 16S rRNA gene sequence similarity for species demarcation of bacterial species. Orlando *et al.* (2016) extrapolated this threshold for the partial sequence similarity of this gene for *Ca. Xiphinematobacter* and estimated it as 97.7%, which was used for bacterial species delimiting in the present study.

Endosymbiotic bacteria of the genus *Ca. Xiphinematobacter* were only found in representatives of the *X. americanum* group. Vandekerckhove *et al.* (2000) described three endosymbiont bacterial species: *Ca. Xiphinematobacter americani*, *Ca. X. rivesi* and *Ca. X. brevicolli* inside three species: *Xiphinema americanum sensu stricto*, *X. rivesi* and *X. brevicolle*, respectively. Recently, Myers *et al.* (2021) described *Ca. X. luci* inside *Xiphinema luci*. Additionally, still undescribed *Ca. Xiphinematobacter* species have been found by several authors (Lazarova

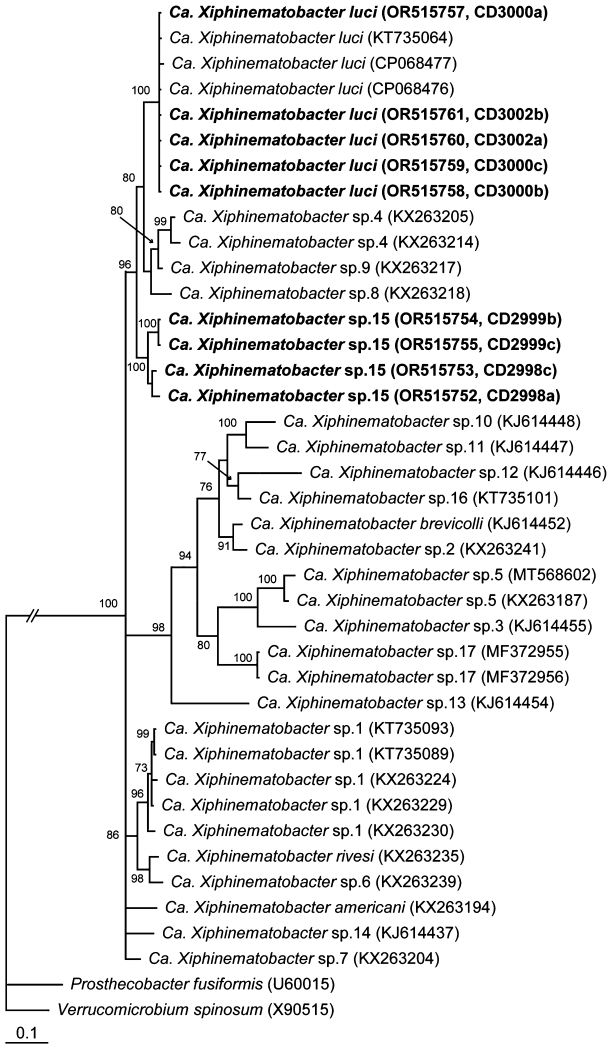

Fig. 7. Phylogenetic relationships within *Candidatus Xiphinematobacter*. Bayesian 50% majority rule consensus tree as inferred from 16S rRNA gene sequence alignment under a GTR + I + G model. New sequences obtained in this study are in boldface.

et al., 2016; Orlando *et al.*, 2016; Palomares-Rius *et al.*, 2016; Mobasseri *et al.*, 2019). Orlando *et al.* (2016) reported a high level of specificity for host-endosymbiont relationships between nematodes and bacteria and showed that each nematode species has its own unique bacterial species. In several cases, two or more bacterial species were found for one nematode species. In the present study, we determined that putative species, *Xiphinema* sp. D. carried *Ca. Xiphinematobacter* sp.15 with rather high intraspecific 16S rRNA gene sequences diversity compared to other species of this genus.

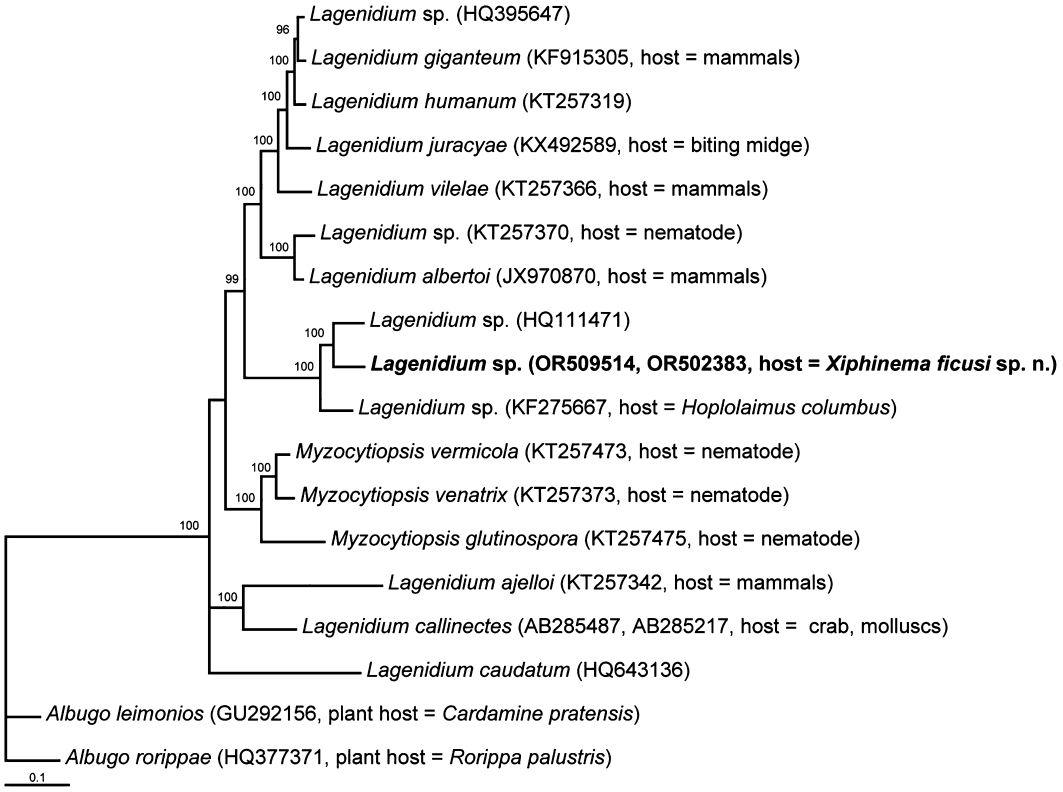

Fig. 8. Phylogenetic position of *Lagenidium* sp. parasitising *Xiphinema ficusi* sp. n. within the *Lagenidium*/*Myzocytiopsis* clade. Bayesian 50% majority rule consensus tree as inferred from the ITS rRNA and D2-D3 of 28S rRNA gene sequence alignment under a GTR + I + G model. New sequence obtained in this study is in boldface.

Molecular characterisation of *Lagenidium* sp.

Sporangia of an unidentified oomycete were found inside bodies of several females of *Xiphinema ficusi* sp. n. Molecular identification of this oomycete using the D2-D3 of 28S rRNA and ITS rRNA gene sequences revealed that it belongs to the genus *Lagenidium*. Phylogenetic position of this species within other *Lagenidium* spp. and *Myzocytiopsis* spp. as inferred from the ITS rRNA and D2-D3 of 28S rRNA gene sequences alignment is presented in Figure 8. Sequence of *Lagenidium* sp. from *X. ficusi* sp. n. formed a clade with those of *Lagenidium* sp. from Taiwan and *Lagenidium* sp. infected *Hoplolaimus columbus* Sher, 1963 from India deposited by Gokte-Narkhedkar *et al.* (data unpubl.).

Oomycete species from the genera *Lagenidium* and *Myzocytiopsis* are mainly parasites of nematodes, mammals and other organisms (Newell *et al.*, 1977; Esser & Schubert, 1983; Glockling *et al.*, 2000; Blackwell *et al.*, 2014; Mendoza *et al.*, 2016; Spies *et al.*, 2016; Osman *et al.*, 2018; Grover & Barkoulas, 2021). To the best of our knowledge, this is the first report of *Lagenidium* sp. infecting *Xiphinema*.

Biogeographical notes

Biogeographically, *Xiphinema* species presently found in Mexico can be divided into two groups: cosmopolitan and endemic. In the cosmopolitan species group, *X. basiri* is distributed in tropical regions, *X. luci* is reported from several warm and tropical locations (Coomans *et al.*, 2001), and *X. index* originated in the Near and Middle East and has been distributed across the world with domesticated grapevine plant material (Nguyen *et al.*, 2019). On the other hand, *X. ficusi* sp. n., *X. tropicale*, and four unidentified *Xiphinema* sp. A, sp. B, sp. C and sp. D, are found in Mexico and are probably in the group of local endemic species. We suggest that the isolated valley region surrounded by ridges of the Sierra Madre

Mountains in Mexico might be one of the world centres of diversity for the genus *Xiphinema*.

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