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Longidorus ferrisi n. sp. from California Citrus

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Abstract: In October 1999, the authors received fixed specimens of a species of *Longidorus* from Howard Ferris found about the roots of a citrus tree in Oakville, Napa County, CA. After determining it to be new a species, we requested additional specimens. The samples contained roughly equal numbers of males and females. *Longidorus ferrisi* n. sp. is most similar to *L. elongatus*, but can be distinguished by a greater c-ratio (111-187 *vs* 73-141), a lesser ć (0.7-1.1 *vs* 1.0-1.3), a more offset head, a more posterior guide ring (35-40 *vs* 30-33 μm), the presence of sperm in the uterus in mature females, and the approximate 1:1 ratio of females to males. Other similar species include *L. artemisiae, L. crassus, L. glycines*, and *L. milanis*. *Longidorus ferrisi* n. sp. differs from *L. artemisiae* by a lesser a-ratio (74-102 *vs* 109-155), a lesser ć value (0.7-1.1 *vs* 1.0-1.6), a more posterior guide ring (35-40 *vs* 27-34 μm), a longer odontostyle (91-108 *vs* 84-98 μm), a wider lip region (16-19 *vs* 14-17 μm), wider mid-body (53-69 *vs* 41-52 μm), and longer spicules (57-65 *vs* 39-49 μm). The new species differs substantially from *L. crassus* by its lip shape and the presence of males, and differs from *L. glycines* by a shorter body (4.33-5.97 *vs* 6.14-8.31 mm), a lesser ć value (0.7-1.1 *vs* 0.9-1.4), a narrower lip region (16-19 *vs* 20-23 μm), wider mid-body (53-69 *vs* 39-57 μm), longer spicules (53-69 *vs* 45-53 μm), and fewer supplements (7-11 *vs* 11-17). *Longidorus ferrisi* n. sp. differs from *L. milanis* by a longer body (4.33-5.97 *vs* 3.00-4.90 mm), a greater c value (111-187 *vs* 86-130), a wider mid-body (53-69 *vs* 43-56 μm), a different head shape, and longer spicules (53-69 *vs* 41-54 μm). The nuclear 18S ribosomal DNA sequence of this species revealed that this species is unique with respect to all sequenced *Longidorus* species.

Key words: 18S rDNA, California, Citrus, DNA sequencing, *Longidorus ferrisi* n. sp., molecular phylogeny, morphology, needle nematode, new species, taxonomy.

In October 1999, the authors received fixed specimens of an undescribed species of *Longidorus* found about the roots of a citrus tree in Oakville, Napa County, CA from Howard Ferris, described herein as *L. ferrisi* n. sp. After determining it to be a new species, we requested additional specimens. A second sampling was made on 10 January 2000. The original samples had been heat relaxed and killed by gently heating the samples and fixing in 2% formalin. The second sampling was killed and fixed in 2% formalin and 6% NaCl so that molecular studies could be made as well. The samples both contained roughly equal numbers of males and females. The nuclear 18S ribosomal DNA sequence of the new species was deposited in GenBank as accession number AY283163 and identified as *L.* sp. California: Long-161 (Neilson et al., 2004). Morphological relationships were determined by comparing the new species data to that of species in the polytomous key (Chen et al., 1997) and an update of the key compiled by the senior author (unpublished). Molecular relationships were inferred from 18S sequences to investigate phylogenetic relationships with other previously sequenced *Longidorus* species.

Materials and Methods

All specimens from the first sample were transferred to glycerin by a modification of Seinhorst's rapid method (1959) and permanently mounted on 25 x 75 mm glass microscope slides. A portion of the second samples was kept in 6% NaCl for molecular study and the remaining specimens were fixed in 2% formalin and treated as the specimens in the first sample. Specimens of this species were reported in Neilson et al. (2004) as Long-161 with an 18S rDNA sequence deposited in GenBank as accession number AY283163. Permanent specimens were measured and morphological observations made using a Nikon Optiphot-2 compound microscope with Nomarski interference contrast at powers up to x1,000. Drawings were made using CorelDRAW (Pedram et al.,

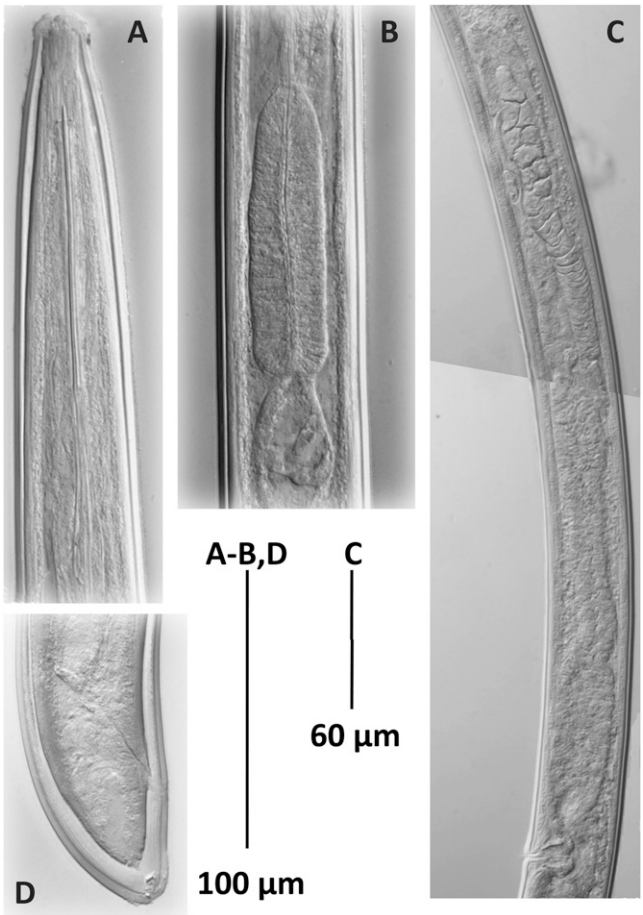

Fig. 1. *Longidorus ferrisi* n. sp. Holotype female. A) Anterior. B) Esophageal bulb. C) Anterior genital region. D) Tail.

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2008). Measurements of body length and distance to the vulva were made using a Nikon drawing tube, while all other measurements were calculated using an ocular micrometer. All measurements are in micrometers unless expressed otherwise. Morphological data is expressed as a mean ± standard deviation (range).

DNA extraction, PCR, and DNA sequencing on near full length 18S rDNA of *L. ferrisi* n. sp. were prepared as described by Neilson et al. (2004). The sequence was deposited in the GenBank database and was compared with those of the other nematode species available in the GenBank sequence database using the BLAST homology search program. DNA sequences from GenBank were aligned by Clustal W (http://workbench.sdsc.edu, Bioinformatics and Computational Biology group, Dept. Bioengineering, UC San Diego, CA). The model of base substitution was evaluated using MODELTEST (Posada & Crandall, 1998; Huelsenbeck & Ronquist, 2001). The Akaike-supported model, the base frequencies, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. Bayesian phylogenetic analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck & Ronquist, 2001) running the chain for 1 × 106 generations and setting the "burn-in" at 1,000. We used the Markov Chain Monte Carlo (MCMC) method within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) using the 50% majority rule.

Systematics

Longidorus ferrisi n. sp.
(Figs. 1-4)

Measurements: See Tables 1-2.

Description: Females: Body spiral to J shaped upon heat relaxation, tapering toward both ends, cuticle appears

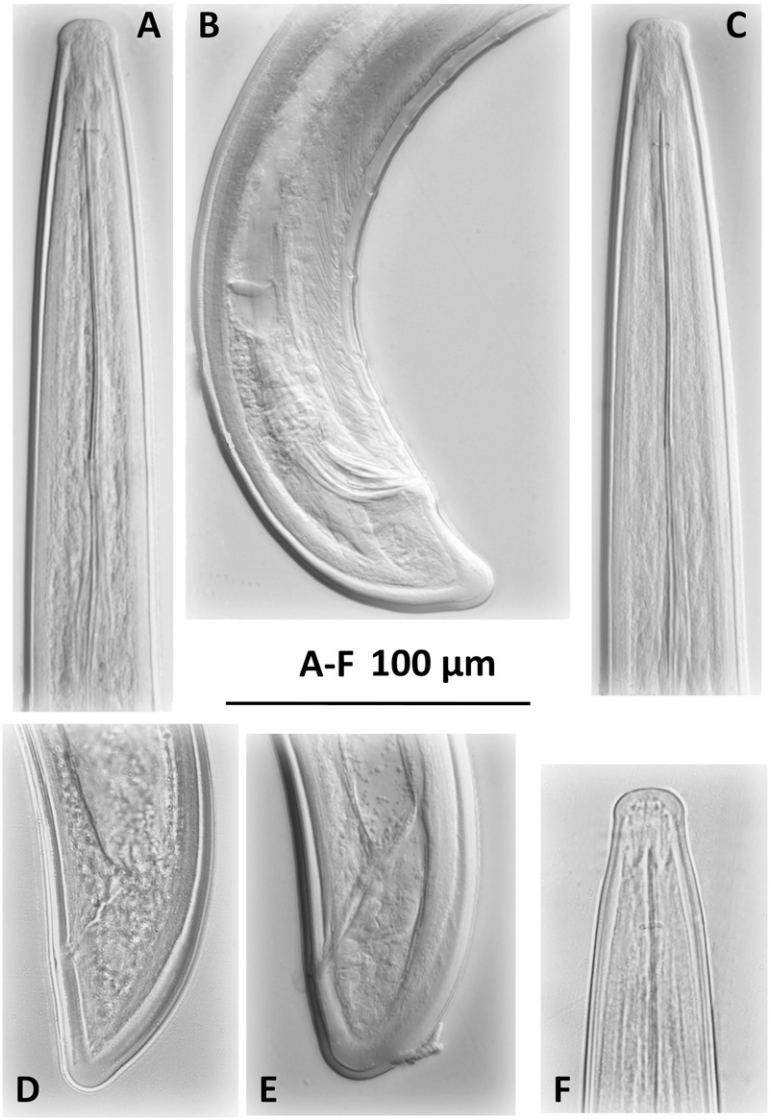

Fig. 2. *Longidorus ferrisi* n. sp. A-B. Allotype male. A) Anterior. B) Tail with supplements. Paratype females. C-F. C) Anterior. D-E) Tails. F) Anterior showing amphid pouch.

smooth as seen by light microscopy, 3 to 4 µm thick along the body, 8 to 10 µm thick near tail terminus. Lateral hypodermal chords visible throughout the length of the body. Head terminus flattened with rounded sides that appear slightly offset to parallel for about 1/3 the distance to the guide ring where the body starts tapering posteriorly (expanding). Head region 16 to 19 µm wide. Amphidial pouches symmetrically bilobed, deeply indented, extend about 65 to 70 % of the distance to the guide ring. Odontophore long and slender, odontophore base not flanged. Guide ring about 6 µm in width. Nerve ring posterior to the odontophore base. Esophagus dorylaimoid with cylindrical basal esophageal bulb, 71-102 µm long, 22-28 µm wide, with the normal arrangement of one dorsal (15-22%) and two subventral nuclei (SV1 45-54%, SV2 50-56%). Cardia conoid at the junction of the esophageal bulb. Reproductive system amphidelphic, didelphic, with reflexed ovaries. Anterior reproductive system 568-893 µm long, posterior reproductive system 507-828 µm long. Vulva only seen laterally. Vagina perpendicular to the body axis with thickened cuticular lining, extending to half the body width. Anterior uteri 203-500 µm long, posterior uteri 238-453 µm long. Sperm found in all uteri observed. An indistinct sphincter between uteri and swollen end of oviducts. Prerectum 254-457 µm long. Tail short, conoid dorsally and less so ventrally, with rounded terminus. Hyaline length of tail region about ½ the hyaline width.

Males: Morphologically similar to female, more strongly curved tail. Spicules well developed, arcuate. Supplements

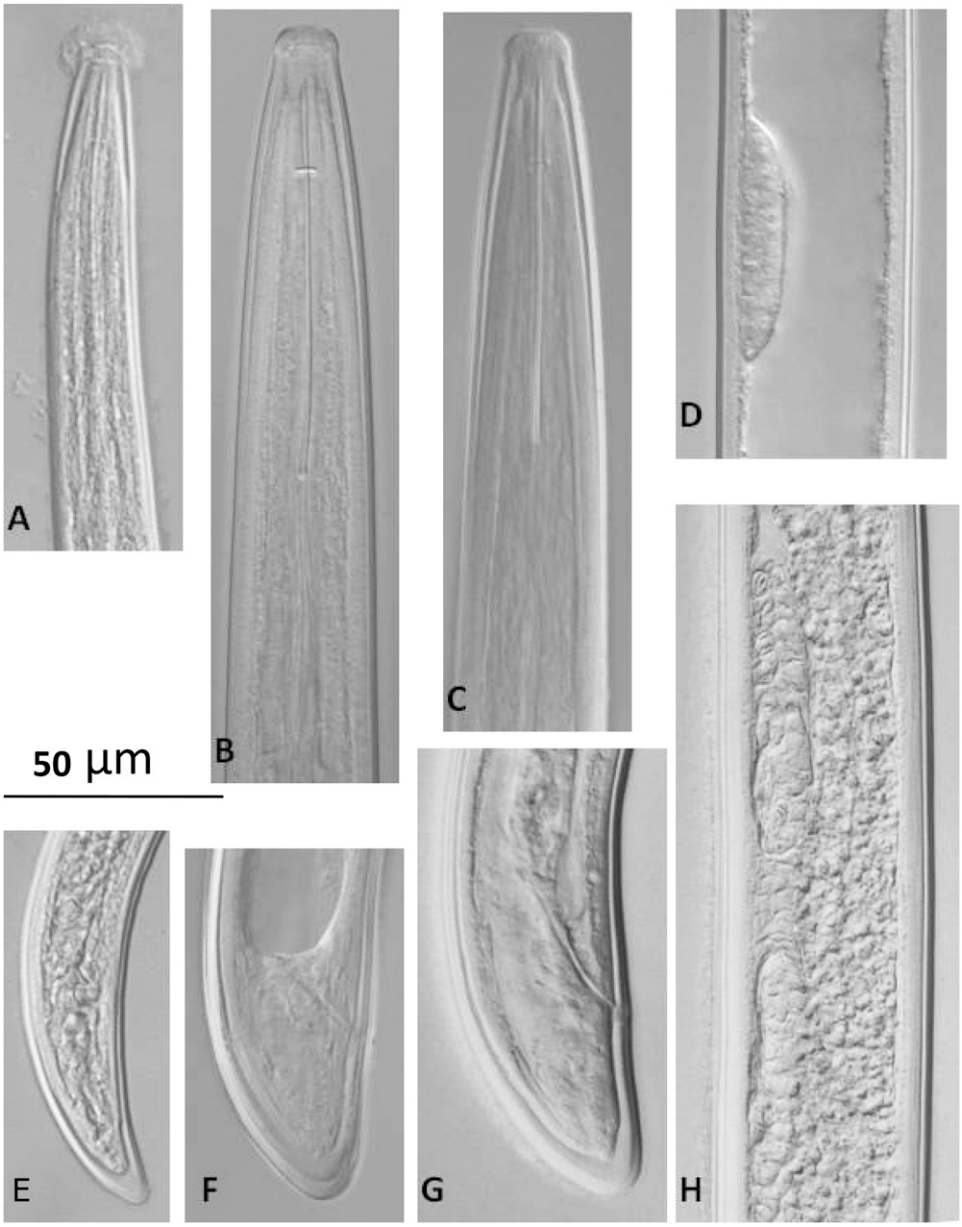

Fig. 3. *Longidorus ferrisi* n. sp. juveniles. A, E) J2 anterior and tail. B, F) J3 anterior and tail. C, G) J4 anterior and tail. D) J3 genital primordial. H) J4 genital primordial.

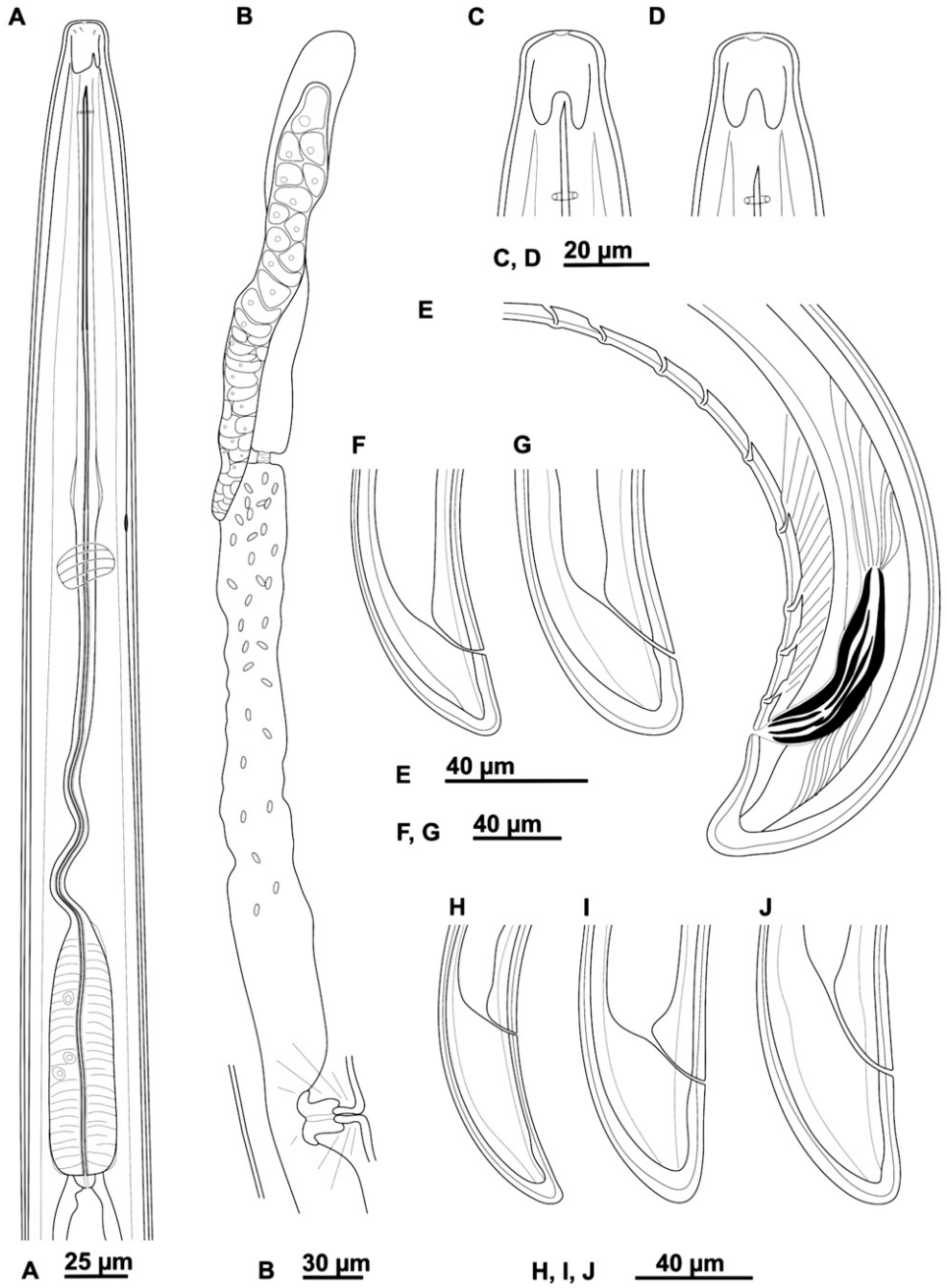

FIG. 4. *Longidorus ferrisi* n. sp. drawings. A) Holotype anterior to esophageal-intestional juncture with some intestine. B) Holotype anterior genital region. C, D) Paratype female amphids. E) Allotype male tail with supplements. F, G) Paratype female tails. H) J2 Tail. I) J3 tail. J) J4 Tail.

an adanal pair, a ventromedian series of seven to 11. Tail one anal body width or less in length, dorsally convex, ventrally concave, terminus bluntly rounded.

Juveniles: Morphologically similar to adults, but smaller. The presence of four juvenile stages is assumed although the first-stage juvenile with the replacement odontostyle embedded in the odontophore base was not found. Replacement odontostyles of J2 observed were well posterior to the odontophore base and correlated well with the odontostyle length of the following J3 stage. The replacement odontostyle of J3 correlated well with the odontostyle length of the J4 stage. The J4 stage replacement odontostyle length correlated well with the odontostyles of adult females and males.

Type locality and habitat

Sandy loam soil over gravel around citrus tree (Mandrin orange), small backyard planting, Oakville, Napa County, California. Collected October 1999 and 10 January 2000 by Howard Ferris from University of California, Davis. Global positional system coordinates N38° 25.950; W122° 25.334.

TABLE 1. Morphometrics of *Longidorus ferrisi* n. sp. females, males, and holotype ♀ from California citrus.

Character*	Paratypes ♀♀	Paratypes ♂♂	Holotype ♀	Allotype ♂
N	24	17	1	1
Body length (L) (mm)	5.14 ± 0.47 (4.33-5.97)	4.82 ± 0.41 (4.10-5.55)	5.47	4.20
a	86 ± 7.3 (73.5-102)	87 ± 7.0 (69-100)	87	83
b	12.9 ± 2.0 (10.4-18.9)	12.0 ± 1.0 (10.4-14.4)	12.0	10.2
c	137 ± 19 (111-187)	122 ± 11.1 (105-144)	150	109
c'	0.9 ± 0.1 (0.7-1.1)	0.9 ± 0.1 (0.9-1.0)	0.9	1.0
V or T (%)	52 ± 3.0 (44-59)	48 ± 4.2 (39-55)	53	54.4
Distance from anterior end to guide ring	36 ± 1.4 (35-40)	37 ± 2.0 (33-41)	38	32
Odontostyle length	102 ± 4.0 (91-107)	102 ± 3.8 (91-110)	99	99
Odontophore length	73.5 ± 3.3 (69-81)	70 ± 2.9 (65-75)	72	65
Total stylet length	175 ± 4.8 (164-183)	172 ± 2.6 (164-175)	171	164
Lip width	18 ± 0.7 (16-19)	19 ± 1.4 (16-22)	19	18
Body width	60 ± 3.6 (53-69)	56 ± 2.6 (51-61)	63	51
Anal body width	42 ± 2.7 (39-48)	43 ± 2.1 (41-49)	43	41
Tail length	38 ± 5.0 (31-51)	40 ± 3.0 (35-47)	38	39
Distance anterior end to vulva (mm)	2.65 ± 0.23 (2.32-3.14)	-	2.89	-
Hyaline tail length	13 ± 2.4 (10-18)	11 ± 1.4 (8-14)	14	10
Spicule length	-	60 ± 2.5 (53-63)	-	57
Number of supplements + adanal pair	-	9 ± 1.5 (7-11)	-	9
Anterior genital tract length	687 ± 90 (568-893)	-	731	-
Posterior genital tract length	684 ± 93 (508-826)	-	691	-
Distance from anterior end to esophagus-intestine junction	403 ± 40.1 (300-463)	402 ± 31.0 (357-402)	455	414
J' (Hyaline length/hyaline width)	0.5 ± 0.1 (0.4-0.7)	0.5 ± 0.1 (0.5-0.7)	0.6	0.6

*All measurements in µm unless noted otherwise.

Type specimens

Holotype female slide number T-637t and Paratype (Allotype) male T-582p deposited in the Nematology Laboratory Collection, USDA, ARS, Beltsville, Maryland. Two paratype females and two paratype males are deposited in each of the following collections: University of California, Riverside; University of California, Davis; CABI Bioscience, UK Centre, Surrey, UK; Department of Nematology, Agricultural University, Wageningen, the Netherlands; and the Canadian National Collection of Nematodes. All remaining paratype material is deposited in Nematology Laboratory, USDA, ARS, Beltsville, Maryland.

Etymology

Named for Howard Ferris, who found it and supplied the specimens, and GPS coordinates.

Diagnosis

Longidorus ferrisi n. sp. is an amphimictic species with approximately equal numbers of males and females. Females are characterized by a body length of 4.33-5.97

TABLE 2. Morphometrics of *Longidorus ferrisi* n. sp. juveniles from California citrus.

Character*	J2	J3	J4
n	2	2	19
Body length (L) mm	2.00, 2.03	4.43, 3.68	4.29 ± 0.33 (3.68-4.83)
a	66, 67	87, 72	88 ± 6.5 (72-99)
b	10.3, 8.3	14.5, -	11.9 ± 1.2 (10.3-14.1)
c	43, 53	109, 82	107 ± 15.1 (70-136)
c'	1.9, 1.9	1.0, 1.1	1.0 ± 0.1 (0.8-1.3)
Distance from anterior end to guide ring	21, 23	33, 27	31 ± 2.0 (26-35)
Odontostyle length	61, 63	75, 75	88 ± 3.1 (83-93)
Odontophore length	45, 47	55, -	59 ± 4.2 (53-67)
Total stylet length	106, 110	130, -	147 ± 3.8 (142-154)
Replacement odontostyle length	77, 75	91, 87	99 ± 4.1 (89-106)
Lip width	10, 10	16, 14	16 ± 0.9 (14-16)
Body width	31, 31	51, 51	49 ± 3.3 (43-57)
Anal body width	24, 20	41, 41	39 ± 2.1 (37-43)
Tail length	47, 39	41, 45	41 ± 5.0 (33-53)
Hyaline tail length	22, 24	12, 10	20 ± 4.0 (10-24)
Distance from anterior end to esophagus-intestine junction	195, 244	305, -	363 ± 30.0 (317-434)
J' (Hyaline length/hyaline width)	2.2, 2.0	2.0, 2.0	2.0 ± 0.3 (1.5-2.4)

*All measurements in µm unless noted otherwise.

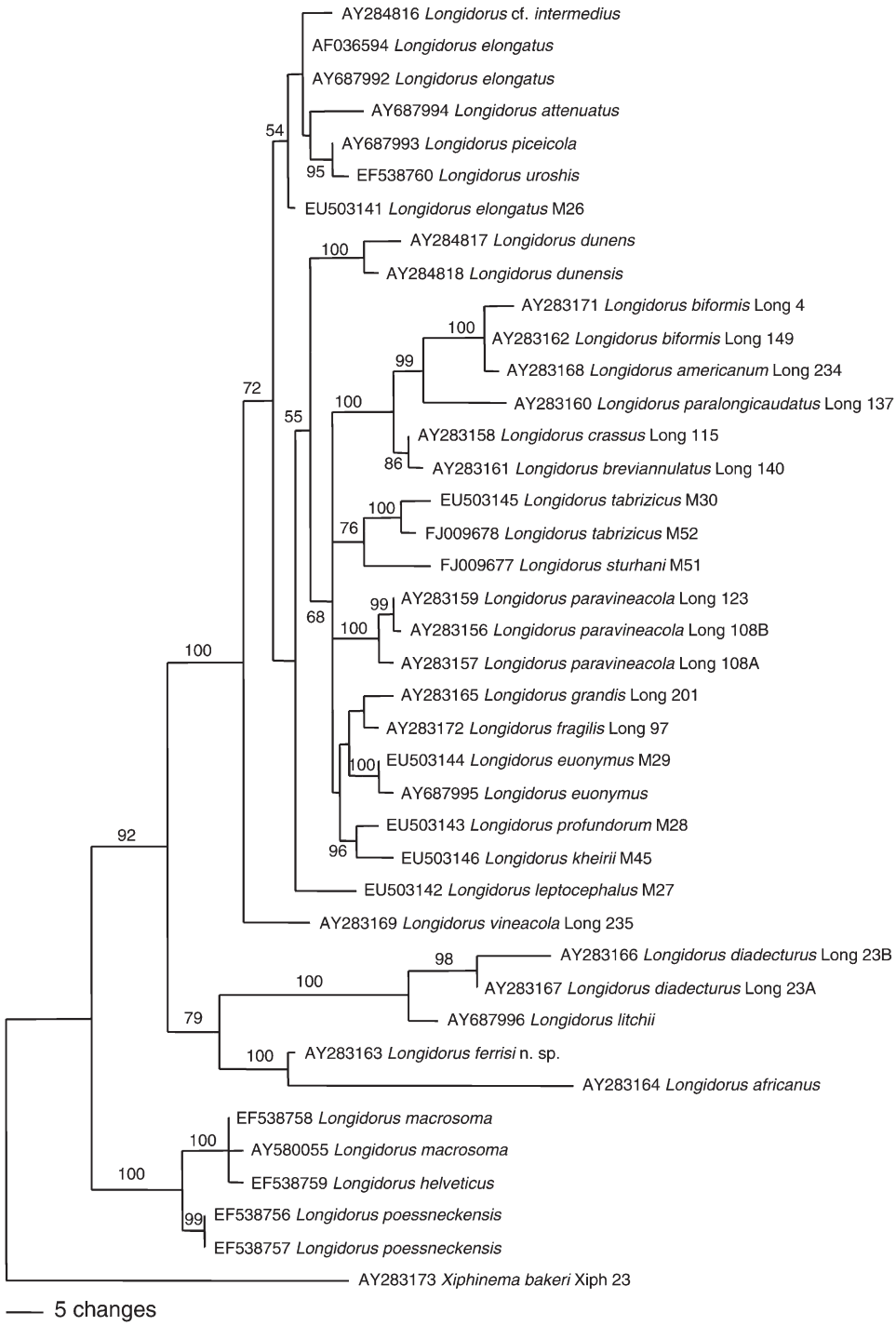

FIG. 5. The 10001st Bayesian tree inferred from 18S under GTR+I+G model (lnL=5000.5371; freqA=0.2746; freqC=0.2108; freqG=0.2597; freqT=0.2549; R(a)=1.3095; R(b)=3.5348; R(c)=1.844; R(d)=0.9033; R(e)=5.8123; R(f)=1; Pinva=0.7544; Shape=0.7679). Posterior probability values exceeding 50% are given on appropriate clades.

mm; an odontostyle length of 91-108 μm; a distance from anterior end to guide ring of 35-40 μm, expanded, wide, and flattened head end, a broadly rounded conoid tail, ć values between 0.7-1.1; and mature females with numerous sperm in the uteri. Males are characterized by a body length of 4.23-5.71 mm, an odontostyle length of 91-108 μm, a distance from anterior end to guide ring between 34-41 μm; expanded, wide, and flattened head end, a broadly rounded conoid tail, ć values between 0.8- 1.0, and spicules 57-65 μm in length. The code for the new species is: A34-B3-C3-D3-E2-F23-G12-H2-I2.

Morphological relationships

Longidorus ferrisi n. sp. is most similar to *L. elongatus* (de Man, 1876) Thorne & Swagger, 1936 but can be distinguished by a greater c ratio (111-187 *vs* 73-141), a lesser ć (0.7-1.1 *vs* 1.0-1.3), a more offset head; a more posterior guide ring (34-40 *vs* 30-33 μm), the presence of sperm

in the uterus in mature females, and the approximate 1:1 ratio of females to males. Other similar species include *L. artemisiae* Rubtsova, Chizov & Subbotin, 1999, *L. crassus* Thorne, 1974, *L. glycines* Ye & Robbins, 2004, and *L. milanis* (Krnjaic, Lamberti, Krnjaic, Agostinelli & Radicci, 2000) Roca, 2006. *Longidorus ferrisi* n. sp differs from *L. artemisiae* by a lesser a ratio (74-102 *vs* 109-155), a lesser ć value (0.7-1.1 *vs* 1.0-1.6), a more posterior guide ring (34-40 *vs* 27-34 µm), a longer odontostyle (91-108 *vs* 84-98 µm), a wider lip region (16-19 *vs* 14-17 µm), a wider mid-body (53-69 *vs* 41-52 µm) in females, and longer spicules (57-65 *vs* 39-49 µm). The new species differs readily from *L. crassus* by its lip shape and the presence of male,; and differs from *L. glycines* by a shorter body (4.33-5.97 *vs* 6.14-8.31 mm), a lesser ć value (0.7-1.1 *vs* 0.9-1.4), a narrower lip region (16-19 *vs* 20-23 µm), wider mid-body (53-69 *vs* 39-57 µm) in females, longer spicules in males (57-65 *vs* 45-53 µm), and fewer supplements (7-11 *vs* 11-17). *Longidorus ferrisi* n. sp. differs from *L. milanis* by a longer body (4.33-5.97 *vs* 3.00-4.90 mm), a greater c value (111-187 *vs* 86-130), a greater body mid-body width (53-69 *vs* 43-56 µm), a different head shape in females, and longer spicules (57-65 *vs* 41-54 µm). In addition, this new species has unique 18S rDNA sequence compared to all available *Longidorus* species in GenBank.

Distribution

Only known from the type location.

Molecular relationships

The 1760-bp 18S rDNA of *Longidorus ferrisi* n. sp. had a nucleotide frequency of 27.5% A, 25.5% T, 21.0% C, and 26.0% G. A Blastn search of the sequence revealed that this is a unique species from all available *Longidorus* 18S sequences on GenBank. Figure 5 presents a phylogenetic tree including all those species using *Xiphinema bakeri* Williams, 1961 as the outgroup. Species in Fig. 5 with Long and Xiph codes were collected from America (Neilson et al. 2004) and M codes from Iran (Pedram et al., 2008) in our previous projects. *Longidorus ferrisi* n. sp. is in a clade with *L. africanus*, a species collected from vineyard in San Diego County, California with 100% support. The species differ morphometrically and morphologically in that *L. ferrisi* n. sp. is amphimictic and has a longer body length. This clade comes closest to a 100% supported monophyletic clade comprised of *L. diadecturus* Eveleigh & Allen, 1982 and *L. litchi* Xu & Cheng, 1992. These two species have the guide ring located at the mid-odontostyle, which easily differentiated from *L. ferrisi* n. sp.

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