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Morphological and molecular characterisation of populations of *Longidorus longicaudatus* Siddiqi, 1962 and *L. americanus* Handoo, Carta, Skantar, Ye, Robbins, Subbotin, Fraedrich & Cram, 2005 from Florida, USA

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Summary – Two populations of needle nematode, *Longidorus longicaudatus* Siddiqi, 1962, are described from *Quercus hemisphaerica*, *Q. nigra* and *Q. virginiana* from north Florida, USA. These populations are characterised morphologically by females having a body shorter than 3800 μm, a rounded or slightly flattened lip region, an amphidial fovea pouch-like often with two symmetrical lobes, an odontostyle 99-110 μm long, a conoid tail ending in a bluntly pointed terminus, ranging values of ratio c′ greater than 2, and males very rare. The polytomous code for these populations is A34, B23, C2, D23, E2, F12, G12, H6, I12, J1, K6. Although the morphology and morphometrics of these two populations fit the original description of *Longidorus longicaudatus*, Florida specimens have greater diameters of lip region, mid and anal body than those of the five type specimens used for the description of this species. The Florida *L. longicaudatus* is similar to *L. paralongicaudatus*, but differs from the paratypes of this species in having smaller and greater values of ratios c (53.8 (43.8-64.5) *vs* 79.2 (61.9-103.5)) and c′ (2.4 (2.1-2.9) *vs* 1.8 (1.5-2.0)), respectively, and longer tail (60 (53-67) *vs* 46 (36-53) μm). Molecular characterisation of one of the two Florida *L. longicaudatus* populations was made based on the D2-D3 of 28S rRNA, ITS1 rRNA and *COI* gene sequences. The results of the ITS1 rRNA gene sequence analysis indicated that it is genetically different from *L. paralongicaudatus*. A few specimens of a needle nematode associated with *L. longicaudatus* were identified morphologically and molecularly as the pine needle nematode, *L. americanus*. This detection is a new record of the occurrence of the pine needle nematode in Florida.

Keywords – *COI* gene, D2-D3 of 28S rRNA gene, ITS rRNA gene, morphology, morphometrics, oak tree, pine needle nematode, *Quercus hemisphaerica*, *Quercus nigra*, *Quercus virginiana*, taxonomy.

Oak trees (*Quercus* spp.) are listed as potential hosts of needle nematodes (*Longidorus* spp.) in several widely separated geographic areas of Europe (Roca *et al*., 1986; Krnjiać *et al*., 2000; Archidona-Yuste *et al*., 2019) and the USA (Thorne, 1974; Robbins & Brown, 1991; Ye & Robbins, 2003, 2004). These hardwood forest trees are also reported as hosts of needle nematodes in Florida, where longidorid specimens collected from the rhizosphere of live oak (*Q. virginiana* Mill.) in the central part of the state were used by Siddiqi (1962a) for the description of *L. tarjani* Siddiqi, 1962. Other longidorids were detected by Esser (1990) on the same host in Gainesville, in the northern part of the state, and identified as *L. longicaudatus* Siddiqi, 1962 (Siddiqi, 1962b), a species described only morphologically. There is no information on the molecular characteristics of any needle nematodes on Florida's oaks. A nematode survey was conducted in 2017-2019 in hardwood forests of north-central Florida to obtain *Longidorus* specimens. Samples were collected mainly from laurel (*Q. hemisphaerica* Willd.), live, and water (*Q. ni-*

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gra L.) oaks. The bulk of the samples was from live and water oaks in a suburban natural area of Gainesville and from laurel oaks in another site five miles distant from the first location. These sites were found to be infested by a needle nematode that was morphologically different from *L. tarjani*. Morphological comparisons of these populations with other species described in the literature indicate that these longidorids were morphologically close to *L. longicaudatus* as reported by Esser (1990) and *L. paralongicaudatus* Ye & Robbins, 2003, a species well characterised morphologically and molecularly. The original description of *L. longicaudatus* lacks important diagnostic characters such as the shape of the fovea, a character that was not elucidated in the report by Esser (1990). Ye & Robbins (2003) provided the morphometrics, without molecular data, of a longidorid population (Long-16) that they identified as *L. longicaudatus* from an undetermined locality. There is a need to clarify the taxonomic status of these longidorids in the hardwood trees of the southeastern USA. During our study, a few specimens of a needle nematode with longer body and fitting the morphology of *L. americanus* Handoo, Carta, Skantar, Ye, Robbins, Subbotin, Fraedrich & Cram, 2005 (originally described as '*L. americanum*', the incorrect ending having its gender changed in Fraedrich *et al*., 2005) were also found associated with one of these *L. longicaudatus* populations.

The main goal of the present study was to provide: *i*) morphological description and conclusive identification of these Florida needle populations; *ii*) molecular characterisation and phylogenetic relationships of one of these populations using the D2-D3 expansion fragments of 28S rRNA, ITS1 rRNA and *COI* gene sequences; and *iii*) information on the morphology and molecular characteristics of the Florida population of *L. americanus*.

Materials and methods

Nematode populations

Nematode populations used in this study were obtained from 50 soil samples collected under the canopy of associated live and water oak trees in a suburban area of Gainesville, Florida, and from laurel oak at another nearby locality (Table 1). Presence of pine roots at these localities cannot be excluded since a slash pine (*Pinus elliottii* Engelm.) tree was growing 10 m away from the oak trees. Samples consisted of moist soil and clusters of feeder roots collected under the canopy of these oak trees at a depth of 10-15 cm. The surface of the soil was covered by oak leaves that prevented the growth of weeds. Nematodes were extracted from soil using the centrifugal flotation method (Jenkins, 1964). Specimens were hand-picked with an eyelash, transferred into a Syracuse watch glass and sorted according to their body size and tail shape. At both localities, the needle nematodes consisted mainly of specimens with a conoid tail *sensu* Chen *et al*. (1997) with a bluntly pointed terminus. Three specimens (two females and one fourth-stage juvenile (J4)) with larger body and a rounded tail terminus were also detected in two samples from the first locality. All the extracted needle nematodes were used for morphological and molecular analyses.

Light microscopic study and morphological identification

Live adult specimens were hand-picked in tap water, immobilised by gentle heating and mounted in water agar on a slide for measurements and photographs using a modified Esser's technique (Esser, 1986). Additional specimens were hand-picked and processed and mounted

Table 1. Species and populations of needle nematodes characterised in the present study.

Species	Location	Host	Sample code	GenBank accession number			Source
				D2-D3 of 28S rRNA	ITS rRNA	*COI*	
Longidorus americanus	USA, Florida, Gainesville	*Pinus elliottii*	N19-00401-2, CD2930	MT552636	–	–	R.N. Inserra
L. longicaudatus	USA, Florida, Gainesville	*Quercus nigra* + *Q. virginiana*	N19-00401-1, N17-00933, CD2529	MT552634, MT552635	MT552637	MT547993	R.N. Inserra
L. longicaudatus	USA, Florida, Gainesville	*Q. hemisphaerica*	N19-009005	–	–	–	R.N. Inserra

in glycerin on permanent slides (Hooper, 1970). Measurements of specimens were made using a Nikon (Optiphot) ocular micrometer and drawings with a drawing tube, using a Leica (Wild MPS 46/520) compound microscope. Photographs were taken with a Zeiss compound microscope, AXIO Scope A1 equipped with Nomarski interference and an AxioCam ICc5. Measurements taken are those suggested by Ye & Robbins (2004), Palomares-Rius *et al*. (2010) and Peneva *et al*. (2013) for *Longidorus* species.

DNA EXTRACTION, PCR AND SEQUENCING

DNA was extracted from several specimens of each sample using the proteinase K protocol. DNA extraction, PCR and cloning protocols were used as described by Tanha Maafi *et al*. (2003). The following primer set was used for PCR: the forward D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and reverse D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) primers (Subbotin *et al*., 2013) for amplification of the D2-D3 expansion segments of 28S rRNA gene, the forward TW28 (5′-GTT TCC GTA GGT GAA CCT GC-3′) and reverse rDNA5.8S (5′-ACG AGC CGA GTG ATC CAC CG-3′) primers (Cherry *et al*., 1997) for amplification of the ITS1 rRNA gene and the forward Long-COIF-mod (5′-G ATT YTT TGG DCA CCC NGA RGT-3′) and reverse Long-COIR-mod (5′-GCH ACY ACR TAR TAR GTR TCR TG-3′) for amplification of *COI* gene. The PCR products were purified using QIAquick (Qiagen) Gel or PCR extraction kits and submitted for direct sequencing or cloned using pGEM-T Vector System II kit (Promega). Sequencing was conducted at Quintara Biosciences. The newly obtained sequences were submitted to the GenBank database under accession numbers: MT547993 (partial *COI* gene of *L. longicaudatus*), MT552634, MT552635 (D2-D3 of 28S rRNA gene of *L. longicaudatus*), MT552636 (D2-D3 of 28S rRNA gene of *L. americanus*), MT552637 (ITS1 of rRNA gene of *L. longicaudatus*) and MT559429 (D2-D3 of 28S rRNA gene of *Xiphidorus* sp.).

PHYLOGENETIC AND SEQUENCE ANALYSIS

The newly obtained sequences of the D2-D3 of 28S rRNA and ITS1 genes were aligned using ClustalX 1.83 (Thompson *et al*., 1997) using the following parameters: gap opening – 5, gap extension – 3 with corresponding published gene sequences (Ye *et al*., 2004; Handoo *et al*., 2005; He *et al*., 2005; Gutiérrez-Gutiérrez *et al*., 2013; Subbotin *et al*., 2013; Gharibzadeh *et al*., 2018; Archidona-Yuste *et al*., 2019; Cai *et al*., 2020a, b and others). Outgroup taxa for each dataset were chosen based on previously published data. Ambiguously aligned segments were eliminated from the ITS rRNA gene sequence alignment with Gblocks Server, version 0.91b (Castresana, 2000). All sequence alignments were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) under the GTR + G + I model. BI analysis for each gene was initiated with a random starting tree and was run with four chains for 7.0×10^6 generations for 28S rRNA gene sequence alignment and 1.0×10^6 generations for the ITS1 rRNA gene sequence alignment. Two runs were performed for each analysis. The Markov chains were sampled at intervals of 100 generations. After discarding burn-in samples (10%), a 50% majority rule consensus tree was generated. Posterior probabilities (PP) in percentage are given on appropriate clades. Sequence analysis of alignment was performed with PAUP* 4b10 (Swofford, 2003). 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.

Results

NEMATODE POPULATIONS

The characteristics of the Florida populations of *L. longicaudatus* and *L. americanus* are described hereunder.

Longidorus longicaudatus Siddiqi, 1962
(Figs 1-3)

MEASUREMENTS

See Tables 2, 3.

DESCRIPTION

Female

Body cylindrical, C-shaped or arcuate when relaxed. Lip region clearly expanded. Cuticle 3 μm thick in postlabial region, 2-2.5 μm along body and 4-5 μm in tail region posterior to anus. Guiding ring 4.5-5.0 μm in diam. Lateral pores not visible in anterior and central part of body. Amphidial aperture assumed to be pore-like, but not visible using a light microscope. Amphidial fovea pouch-like with two symmetrical lobes. Lobes indistinct in some specimens. Odontostyle slender, 1.9-2.0 μm in diam. at

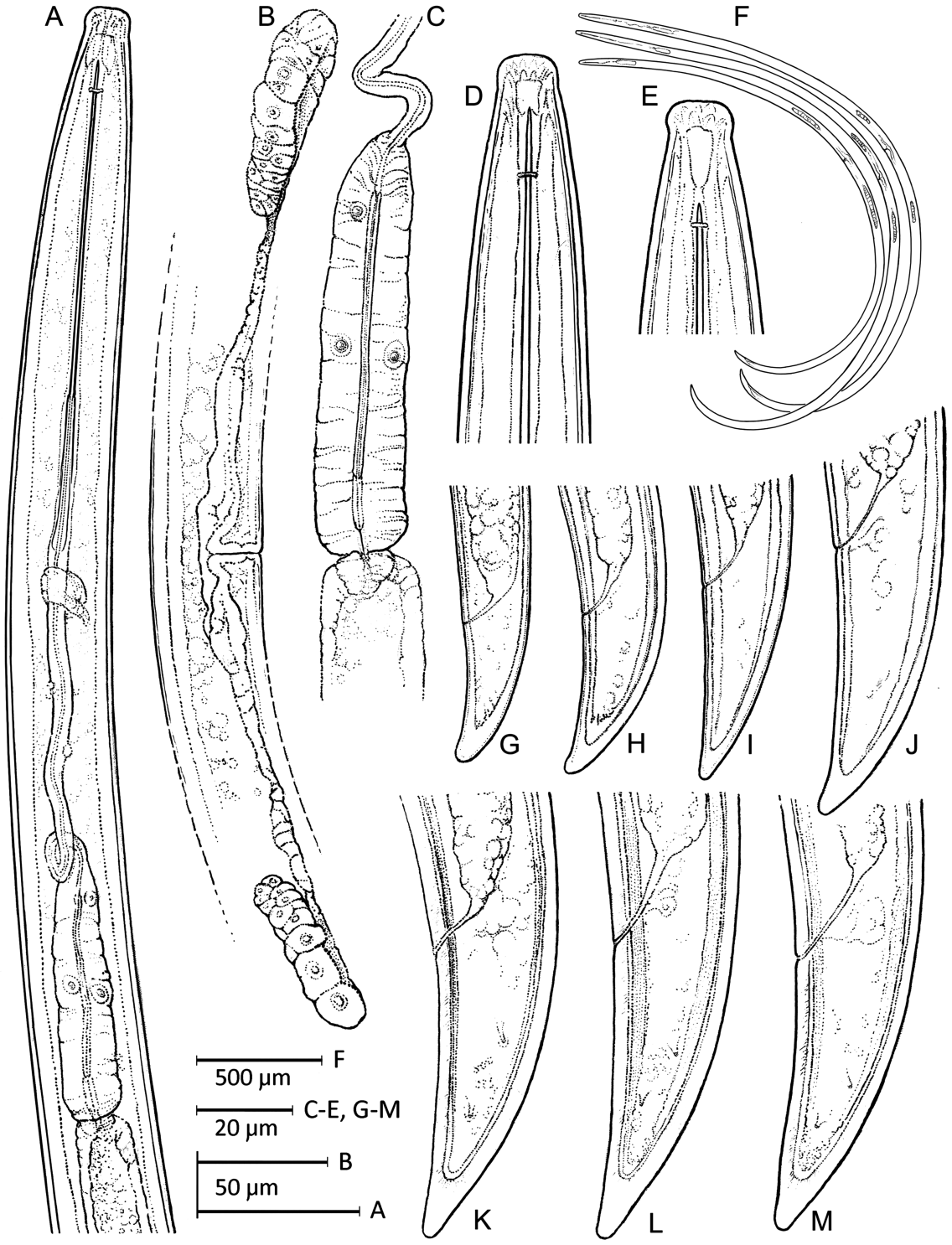

Fig. 1. Line drawings of Florida *Longidorus longicaudatus* A: Pharynx of female; B: Genital tract; C: Pharyngeal bulb; D, E: Anterior end showing amphidial fovea; F: Body habitus after fixation; G-J: Tail shape of first- to fourth-stage juveniles; K-M: Tail shape variation in female.

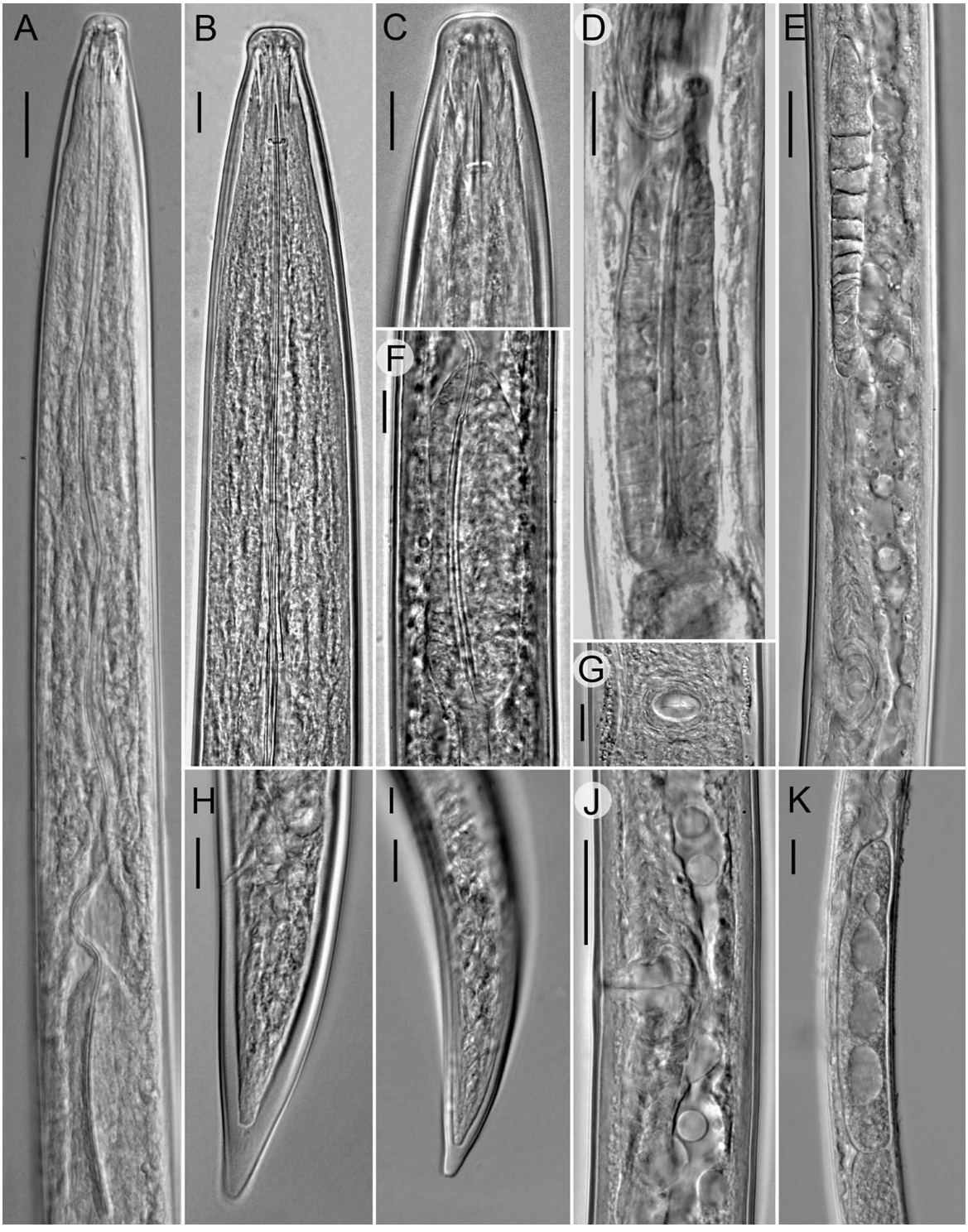

Fig. 2. Photomicrographs of Florida *Longidorus longicaudatus* female. A: Pharynx; B: Anterior end showing odontostyle; C: Anterior end showing amphidial fovea; D, F: Pharyngeal bulb; E: Reflexed anterior ovary and oviduct; G: Ventral view of vulva H, I: Tail shape variation; J: Vagina and uteri; K: Intra-uterine egg.

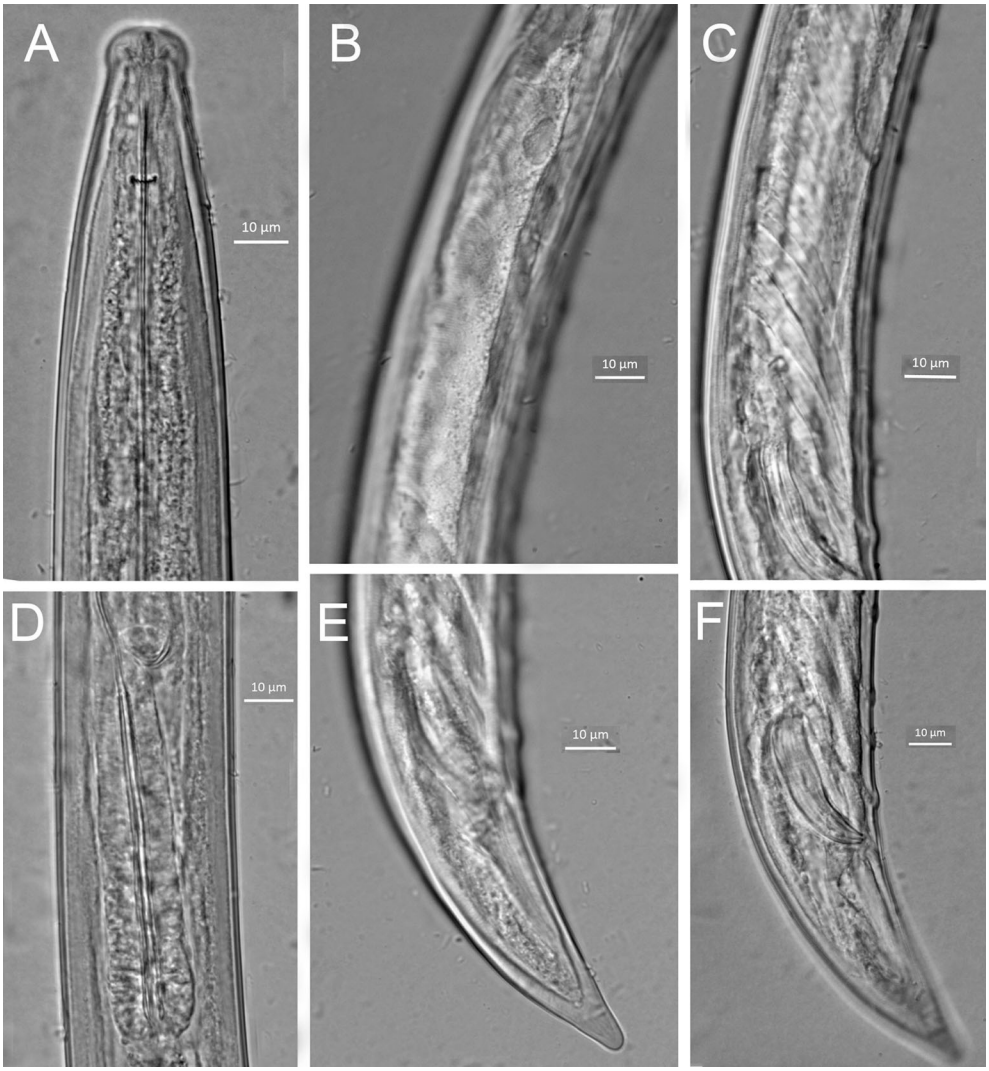

Fig. 3. Photomicrographs of Florida *Longidorus longicaudatus* male. A: Anterior end; B: Distal end (germinal zone) of testis; C: Posterior body showing spicules and supplements; D: Pharyngeal bulb; E, F: Posterior end showing spicules (masked by copulatory muscles in E), supplements and tail.

base. Nerve ring 14 μm wide, located 10 μm posterior to base of odontophore. Pharyngeal bulb, cylindrical about four times longer than wide with a semicircular or dome-like cardia, 16 μm in diam. at base and 5-6 μm high. Location of dorsal and ventrosublateral pharyngeal nuclei at 22-32 and 41-70% of basal bulb length, respectively. Rectum shorter than body diam. at anus and about 0.5-0.9% of this diam. Lateral chord observed in 5% of specimens, 7 μm wide. Vulva a transverse slit located in an ovoid depression of cuticle when observed in ventral view. Vagina extending to half of corresponding body diam. and thick-walled in its proximal portion. Uteri, oviducts and reflexed ovaries obscure in 40% of specimens and variable in length. No sperm observed. Tail elongate-conoid ending in a bluntly pointed tip and bearing two pairs of lateral pores.

Male

At sampling site ratio of male/female was 1: 60. Body shape similar to female but with shorter body and tail, smaller ratio c′ and body diam. Tail slightly curved. Spicules thick and slightly curved. Lateral accessory

Table 2. Morphometrics of females of two populations of *Longidorus longicaudatus* from Florida compared with those of *L. longicaudatus* and *L. paralongicaudatus*. All measurements are in μm and in the form: mean ± s.d. (range).

Character	*Longidorus longicaudatus*				*L. paralongicaudatus*
	Population N19-00401 from water oak Gainesville, FL	Population N19-00905 from laurel oak Gainesville, FL	Siddiqi, 1962b	Pop. Long 16 (Ye & Robbins, 2003)	Paratype (Long 137) (Ye & Robbins, 2003)
n	25	15	5	5	26
L	3317 ± 292 (2636-3751)	3038 ± 204 (2518-3373)	2250-3000	3001 ± 0.21 (2282-3350)	3620 ± 0.33 (3001-4370)
a	77 ± 6.1 (63.3-89.3)	75.3 ± 3.9 (66.6-83.9)	73-80	77.2 ± 7 (69.5-85.1)	89.1 ± 7.6 (74.6-107.7)
b	9.3 ± 1.2 (7.5-11.9)	8.7 ± 0.6 (8-9.8)	7-8.2	9.9 ± 1.3 (8.6-12)	11.1 ± 2.4 (7.4-16.3)
c	53.8 ± 4.7 (43.8-64.5)	50.8 ± 4.5 (44.8-60.8)	40-50	48.1 ± 3.7 (42.7-52.3)	79.2 ± 10.6* (61.9-103.5)
c′	2.4 ± 0.1 (2.1-2.7)	2.5 ± 0.1 (2.3-2.9)	2.8-3.2	3.0 ± 0.2 (2.8-3.4)	1.8 ± 0.2* (1.5-2.0)
V	45.1 ± 1.5 (42.7-48.6)	45.7 ± 1.1 (44-48.8)	44.0-47.6	46.1 ± 1.9 (44.3-48.1)	46.4 ± 2.2 (43.1-50.6)
Vulva to head	1497 ± 137.6 (1207-1800)	1389 ± 94.7 (1144-1540)	–	–	–
Vulva to tail terminus	1820 ± 172.3 (1430-2083)	1648 ± 120.4 (1374-1835)	–	–	–
Anterior ovary length	109 ± 34.3 (90-233)	196 ± 92.9 (89-352) (12)	–	–	–
Anterior oviduct length	210 ± 34.4 (158-290)	290 ± 1224 (114-550) (10)	–	–	–
Entire anterior genital tract	318 ± 99.3 (198-634)	473 ± 208 (218-903) (10)	–	–	(165-540)
G (anterior)	9.5 ± 3.1 (5.6-19.8)	16.3 ± 6.2 (7-26.7) (10)	–	7.2 ± 1.2 (6.3-9.4)	7.4 ± 2.8 (5.0-15.5)
Posterior ovary length	112 ± 42.1 (78-267)	246 ± 134.5 (52-483) (10)	–	–	–
Posterior oviduct length	205 ± 68.4 (120-366)	290 ± 132.4 (150-602) (9)	–	–	–
Entire posterior genital tract	318 ± 99.3 (198-634)	516 ± 243.7 (202-1017) (9)	–	–	(160-430)
G (posterior)	9.5 ± 3.1 (5.6-19.8)	18.8 ± 8.2 (9.8-33.6) (8)	–	6.3 ± 0.9 (5.0-7.6)	6.7 ± 1.9 (4.6-13.3)
Odontostyle length	104 ± 3.2 (99-110)	103 ± 2.3 (99-106)	92-100	99 ± 4.4 (96-104)	104 ± 3.4 (96-114)
Odontophore length	56 ± 2.4 (49-59)	58 ± 2 (54-65)	46-51	50 ± 3.6 (46-54)	60 ± 2 (57-65)
Odontostyle + odontophore length	160 ± 4.8 (148-168)	161 ± 3 (155-166)	–	150 ± 6.7 (142-158)	164 ± 3.8 (156-173)
Pharynx length	361 ± 39.6 (304-457)	347 ± 23.2 (303-376)	–	–	–
Anterior end (ae) to guiding ring (gr) distance	26 ± 1.1 (24.2-28.7)	24.5 ± 0.8 (23.0-26.0)	21-24	24.5 ± 0.7 (24.0-25.0)	24.6 ± 1.3 (20.3-26.4)
Pharynx bulb length	87 ± 3.1 (81-95)	81 ± 3.8 (76-88)	–	–	(75-93)

Table 2. (Continued.)

Character	*Longidorus longicaudatus*				*L. paralongicaudatus*
	Population N19-00401 from water oak Gainesville (FL)	Population N19-00905 from laurel oak	Siddiqi, 1962b	Pop. Long 16 (Ye & Robbins, 2003)	Paratype (Long 137) (Ye & Robbins, 2003)
Pharynx bulb diam.	20.5 ± 1.4 (18.0-24.5)	20.0 ± 1.8 (16.0-23.5)	–	–	– (16-20)
Tail length	62 ± 2.9 (56-67)	60 ± 4.6 (53-67)	66	63 ± 3 (59-67)	46 ± 4.2 (36-53)
Tail hyaline region	12.3 ± 1.1 (10.0-14.8)	11.7 ± 1 (10.0-13.3)	12	12.4 ± 2.2 (10.0-16.0)	13.3 ± 1.5 (10.2-16.2)
Tail hyaline region diam.	–	6.0 ± 0.3 (5.4-6.8)	–	–	–
Body diam. at:					
lip region (lw)	14.7 ± 0.6 (13.4-16.3)	13.9 ± 0.3 (13.0-14.4)	12**	13.8 ± 0.4 (13.0-14.0)	14.7 ± 0.9 (13.2-16.2)
guiding ring (rw)	21.9 ± 0.9 (20.2-24.0)	20.5 ± 0.9 (18.8-21.8)	–	–	–
mid-body	43.1 ± 3.1 (37.2-51)	40.3 ± 1.3 (38-42.5)	33**	38 ± 2.6 (35.5-40.5)	40.6 ± 2.2 (34.5-44.7)
anus	25.6 ± 1.5 (22.2-28.7)	23.3 ± 1 (21.3-24.7)	18**	20.5 ± 1 (20.0-22.0)	26.3 ± 1.7 (22.3-29.4)
d (ae-gr/lw)	1.8 ± 0.1 (1.6-1.9)	1.7 ± 0.1 (1.6-1.8)	–	–	–
d′ (rw/lw)	1.5 ± 0.1 (1.4-1.6)	1.4 ± 0.1 (1.3-1.5)	–	–	–
Guiding ring diam.	4.9 ± 0.1 (4.5-5.0)	4.7 ± 0.3 (4.2-5.0)	–	–	4
DO to anterior bulb margin	14.2 ± 1.7 (12.0-18.0) (17)	14 ± 2 (10.4-18.0) (13)	–	–	–
DN to anterior bulb margin	21.4 ± 1.4 (20.0-25.0)	24.7 ± 3.5 (17.0-30.0) (10)	–	–	–
SO to anterior bulb margin	72 ± 3 (66-76)	67 ± 2.9 (62-70) (10)	–	–	–
$SN_{1,2}$ to anterior bulb margin	49 ± 6.6 (38-62)	50 ± 9.8 (42-56) (2)	–	–	–
DO%	16.2 ± 1.9 (14.0-20.0)	17.2 ± 2.8 (12.0-23.6) (13)	–	–	–
DN%	24.2 ± 1.6 (21.9-28)	27.4 ± 4.9 (19.9-34.6) (8)	–	–	(26.3-33)
SO%	80.6 ± 7.4 (52.3-88.2)	80.7 ± 2.3 (78.0-85.0) (10)	–	–	–
$SN_{1,2}$%	56.8 ± 8.2 (41.3-69.6)	61.7 ± 11.6 (53.5-70.0)	–	–	(46.0-63.0)

Abbreviations as defined by Palomares-Rius *et al*. (2010) and Peneva *et al*. (2013).
* Indicates characters of *L. paralongicaudatus* that differ from those of *L. longicaudatus* populations.
** Indicates characters of *L. longicaudatus* paratypes that differ from other *L. longicaudatus* populations and *L. paralongicaudatus*.

Table 3. Morphometrics of Florida *Longidorus longicaudatus* male and first- to fourth-stage juveniles (J1-J4). All measurements are in μm and in the form: mean ± s.d. (range), unless stated otherwise.

Character	Male	J4	J3	J2	J1
n	1	5	1	2	1
L	2731	2014 ± 91.1 (1868-2094)	1221	967 ± 39.9 (938-995)	784
a	85	63.4 ± 2.3 (61.0-65.6)	58.1	38.9 ± 0.3 (38.7-39.1)	37.8
b	8.2	6.1 ± 0.3 (5.7-6.4)	6.4	5.8 ± 0.1 (5.7-5.9)	4.8
c	53.1	37.8 ± 2.1 (36.0-40.8)	27.4	29.2 ± 0.1 (29.2-29.3)	35.2
c′	1.8	2.7 ± 0.4 (2.3-3.3)	3.1	2.3 ± 0.1 (2.2-2.4)	2.2
V	–	–	–	–	–
Genital tract (or testis) length	920	–	–	–	–
G or T	33.6	–	–	–	–
Odontostyle length	100	84 ± 3.2 (80-89)	73	62	54
Odontophore length	56	50 ± 2 (48-53)	40	–	36
Odontostyle + odontophore length	156	135 ± 3.7 (128-138)	113	–	91
Replacement odontostyle	–	100 ± 1.4 (98-102)	86	70 ± 0.6 (69-70)	62
Pharynx length	330	329 ± 19.9 (300-354)	188	165 ± 3.5 (163-168)	161
Anterior end (ae) to guiding ring (gr)	27.2	20.6 ± 0.5 (20.0-21.2)	14.8	12.3 ± 1.3 (11.4-13.3)	11.4
Pharynx bulb length	81	71 ± 4.2 (65-77)	53	–	48
Pharynx bulb diam.	17.5	16.0 ± 1.2 (14.8-17.5)	17.3	–	12.8
Tail length	51	53 ± 2 (51-56)	44	33 ± 1.4 (32-34)	31
Tail hyaline region	11.5	9.0 ± 1.3 (7.1-9.9)	5.5	8.4 ± 4.2 (5.0-11.8)	7.9
Tail hyaline region diam.	–	5.4 ± 0.5 (4.9-5.9)	3.9	6.2 ± 1.7 (5.0-7.5)	5
Body diam. at:					
lip region (lw)	14.8	12.5 ± 0.5 (12.0-13.0)	10.0	9.2 ± 1 (8.5-10.0)	7.9
guiding ring (rw)	22.3	20.6 ± 0.5 (20.0-21.2)	14.8	12.5 ± 0.7 (12.0-13.0)	11.4
mid-body	32.1	31.7 ± 0.7 (30.6-32.6)	21.0	24.8 ± 1.2 (24.0-25.7)	20.7
anus	28.7	18.3 ± 1.8 (17.0-21.3)	14.0	15.4 ± 0.1 (15.3-15.5)	13.5
d (ae-gr/lw)	1.8	1.6 ± 0.05 (1.6-1.7)	1.7	1.3 ± 0.3 (1.1-1.5)	1.4
d′ (rw/lw)	1.5	1.4 ± 0.1 (1.3-1.7)	1.4	1.3 ± 0.1 (1.3-1.4)	1.4

Table 3. (Continued.)

Character	Male	J4	J3	J2	J1
Guiding ring diam.	4.6	4.0 ± 0.1 (3.9-4.2)	4.0	–	–
DO to anterior bulb margin	13.0	9.9 ± 1.2 (8.0-11.0)	5.0	–	–
SO to anterior bulb margin	71	56 ± 2.3 (54-59)	43	–	–
DO%	16.0	13.9 ± 1.7 (12.3-16.1)	9.3	–	–
DN%	–	–	–	–	–
SO%	87.1	78.8 ± 4.3 (71.7-82.9)	53.4	–	–
$SN_{1,2}$%	–	–	–		–
Spicule length	41.5	–	–	–	–

Abbreviations as defined in Palomares-Rius *et al*. (2010) and Peneva *et al*. (2013).

pieces not visible in our specimen. Two adanal pairs of supplements preceded by six spaced pairs.

First- to fourth-stage juveniles (J1-J4)

Juvenile stages separated using functional and replacement odontostyle lengths. Body habitus sickle-shaped. Tail conoid *sensu* Chen *et al*. (1997) with a subdigitate (J1) or bluntly pointed (J2-J4) terminus. Body and tail shape of J2-J4 like those of female.

NOTES

The first population was collected from feeder roots of live and water oaks in suburban Gainesville, FL, USA (latitude 29°66′75.38″ N, longitude 82°38′95.80″ W). The second population from laurel oak was collected from a nearby natural area (latitude 29°63′49.87″ N, longitude 82°37′08.06″ W). Voucher specimens consisting of ten females of these populations were mounted on glass slides and deposited in the nematode collection at the Istituto per la Protezione Sostenibile delle Piante, CNR, Bari, Italy; an additional five females of each were deposited at the United States Department of Agriculture Nematode Collection, Beltsville, MD, USA, and WaNeCo, Plant Protection Service, Wageningen, The Netherlands.

The Florida populations of *L. longicaudatus* are characterised morphologically by females having a body shorter than 3800 μm, an anterior body tapering distinctly ending in a rounded or slightly flattened lip region, a pouch-like amphidial fovea, often with two symmetrical lobes, an odontostyle 99-110 μm long, a conoid tail, range values of ratio c′ greater >2 and males very rare. The polytomous code (Chen *et al*., 1997; Loof & Chen, 1999; Peneva *et al*., 2013) for these populations is A34, B23, C2, D23, E2, F12, G12, H6, I12, J1, K6. These codes reflect the range values of each character across the two populations. Twelve percent of the specimens in the population from live oak and 20% of those of the population from laurel oak had a code A3. One specimen out of 25 in the first population had a code B3, the other specimens (34) in both populations had a code B2.

Although the two Florida longidorid populations differed from *L. longicaudatus* in having larger lip region, mid-body and anal body diam., we consider these populations to be conspecific with *L. longicaudatus* because of the variability of these characters. The Florida *L. longicaudatus* is mostly very similar to *L. paralongicaudatus* paratypes from which it differs in having smaller and greater values of ratio c (53.8 (43.8-64.5) *vs* 79.2 (61.9-103.3)) and c′ = 2.4 (2.1-2.9) *vs* 1.8 (1.5-2.0), respectively, and longer tail 60 (53-67) μm across the two populations *vs* 46 (36-53 μm). These two species, however, have a similar polytomous code. The polytomous code for *L. paralongicaudatus* is A345, B23, C12, D2, E2, F12, G12, H56, I12. Furthermore, our Florida populations cannot be well differentiated morphometrically from other populations identified as *L. paralongicaudatus* by Ye & Robbins (2003) and having average values of c′ greater than 2.0. These populations include: Long-26, 72, 91, 93 (Arkansas), Long 34 (from Tennessee), Long 237 and Long 238 (both from Georgia).

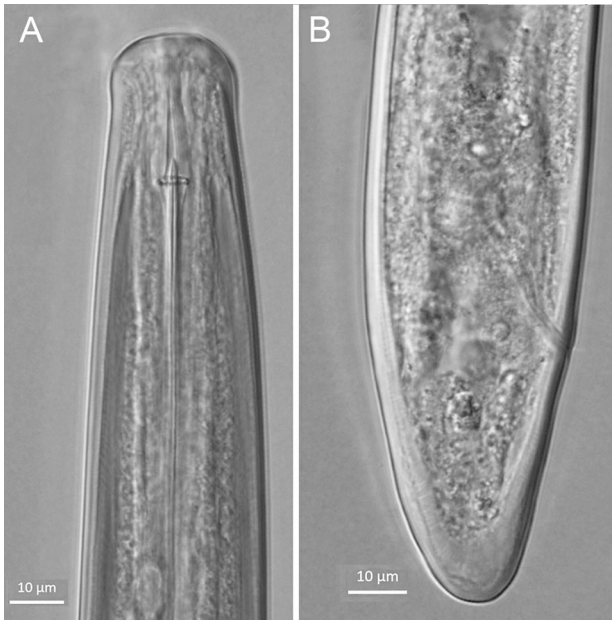

Fig. 4. Photomicrographs of *Longidorus americanus* from Florida. A: Female anterior end showing guiding ring and portion of odontostyle; B: Female posterior end showing tail shape.

Longidorus americanus Handoo, Carta, Skantar, Ye, Robbins, Subbotin, Fraedrich & Cram, 2005
(Fig. 4)

MEASUREMENTS

See Table 4.

DESCRIPTION

Female and *J4*

Body shape and morphometrics of the two specimens of this species found associated with *L. longicaudatus* did not differ from those reported in the original description of *L. americanus*.

MOLECULAR CHARACTERISATION AND PHYLOGENETIC RELATIONSHIP OF FLORIDA *L. longicaudatus* AND *L. americanus* WITH OTHER SPECIES

D2-D3 of the 28S rRNA gene

The D2-D3 of the 28S rRNA gene sequence alignment contained 82 sequences of *Longidorus*, including two new sequences of Florida *L. longicaudatus*, and three sequences of outgroup taxa, including a new sequence of *Xiphidorus* sp. collected in mountain areas of southern Peru. The alignment was 947 bp in a length. The phylogenetic position of *L. longicaudatus* within the genus is given in Figure 5. Florida *L. longicaudatus* formed a clade with *L. kuiperi* Brinkman, Loof & Barbez, 1987 and *L. apulus* Lamberti & Bleve Zacheo, 1977 with moderate support (PP = 92%). Two clones of this gene in *L. longicaudatus* differed by 7 bp (1%). Florida *L. americanus* clustered with the Georgia population of this species, their sequences differing by 4 bp (0.5%).

ITS1 rRNA gene

The ITS1 rRNA gene sequence of *L. longicaudatus* contained 959 bp. The ITS1 rRNA gene sequence alignment showed many ambiguous aligned positions and only conservative regions obtained with Gblock were included in the analysis. The alignment included 23 sequences and was 420 bp in length. The phylogenetic position of Florida *L. longicaudatus* within the genus is given in Figure 6 and shows *L. longicaudatus* grouping (PP = 86%) with *L. euonymus* Mali & Hooper, 1974, *L. persicus* Esmaeili, Heydari, Archidona-Yuste, Castillo & Palomares-Rius, 2017 and *L. azarbaijanensis* Gharibzadeh, Pourjam & Pedram, 2018.

COI gene

The *COI* gene sequence for Florida *L. longicaudatus* contains 331 bp. Blast search of its Florida *COI* sequence showed high similarity with those of *L. carpetanensis* Arias, Andres & Navas, 1986 (similarity = 78.9%, coverage = 94%), *L. pini* (78.1%, 97%), and *L. alvegus* Roca, Pereira & Lamberti, 1989 (78.5%, 93%).

Discussion

The diagnostic morphological characters for the described Florida populations with elongate conoid tails from oak trees agree to a significant degree with those reported by Siddiqi (1962b) in the original description of *L. longicaudatus* using a population collected from an unknown host in Edmunds, SC, USA. The amphidial fovea in these Florida populations was pouch-like with two symmetrical lobes and indistinct in some specimens. A pouch-like fovea with no lobes is shown by Siddiqi (1962b) in a drawing of a South Carolina specimen observed in lateral view in the original description of *L. longicaudatus*. However, Chen *et al*. (1997) did not consider this drawing as enough evidence of the shape of the fovea for this

Table 4. Morphometrics of female and fourth-stage juvenile (J4) of *Longidorus americanus* from Florida compared with the original description. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Florida population		Original description (Handoo *et al*., 2010)	
	Female	J4	Female	J4
n	1	1	27	10
L	8685	5846	7000 ± 0.80 (5400-9000)	5900 ± 0.39 (5000-6520)
a	154	115.7	119.6 ± 12 (89.5-136.6)	101.0 ± 7.5 (85.6-113.3)
b	17.3	13.1	9.0 ± 4.5 (4.6-16.8)	11.8 ± 1.2 (10.0-14.2)
c	162.6	107.4	119.1 ± 14.5 (95.0-154.6)	99.1 ± 6.5 (89.3-113.3)
c′	1.4	1.3	1.5 ± 0.1 (1.3-1.7)	1.5 ± 0.1 (1.2-1.7)
V	50.3	–	48.8 ± 2.2 (44.2-51.7)	–
Vulva to anterior end	4375	–	–	–
Vulva to tail terminus	4310	–	–	–
Anterior ovary length	257	–	–	–
Anterior oviduct length	451	–	–	–
Entire anterior genital tract length	708	–	–	–
Posterior ovary length	300	–	–	–
Posterior oviduct length	453	–	–	–
Entire posterior genital tract length	753	–	–	–
G (anterior)	8.1	–	–	–
G (posterior)	8.6	–	–	–
Odontostyle length	151	138	142 ± 9.7 (124-165)	137 ± 4.8 (126-142)
Odontophore length	76	69	83 ± 2.4 (79-91)	73 ± 2.5 (69-77)
Odontostyle + odontophore length	228	207	224 ± 9.7 (207-250)	211 ± 5.6 (197-219)
Replacement odontostyle length	–	148	–	155 ± 4.6 (146-162)
Pharynx length	500	444	602 ± 104.8 (368-778)	499 ± 42.6 (397-559)
Pharynx bulb length	165	132	130 (from figure)	–
Pharynx bulb diam.	27.7	21.0	20.0 (from figure)	–
Anterior end-guiding ring (ag)	30.1	30.2	34.7 ± 2.4 (30.5-42.6)	31 ± 0.6 (30.5-32.5)
Tail length	53.4	54.4	58.9 ± 4.6 (50.8-67.0)	56.4 ± 3.8 (46.7-65.0)
Tail hyaline region	18.8	11.0	17.0 ± 2.2 (14.2-22.3)	15.8 ± 1.3 (13.2-18.3)
Body diam. at:				
lip region(lw)	26.7	26.2	27.5 ± 0.8 (26.4-28.4)	25.8 ± 0.6 (24.4-26.4)
guiding ring (rw)			30.0	–
mid-body			50.5	58.8 ± 6.4 (50.8-71.1)
anus			42.0	40.4 ± 1.5 (36.5-42.6)
d (ag/lw)	1.2	1.1	–	–
d′ (rw/lw)	1.4	1.1	–	–
Guiding ring diam.	6.0	–	–	–
DO to anterior bulb margin	17.8	12	–	–
SO to anterior bulb margin	128	101	–	–
$SN_{1,2}$ to anterior bulb margin	94	66	–	–
DO%	10.7	9.1	–	–
DN%	–	–	–	–
SO%	77.5	76.7	–	–
$SN_{1,2}$ %	55.6	50.4	–	–

Abbreviations as defined in Palomares-Rius *et al*. (2010) and Peneva *et al*. (2013).

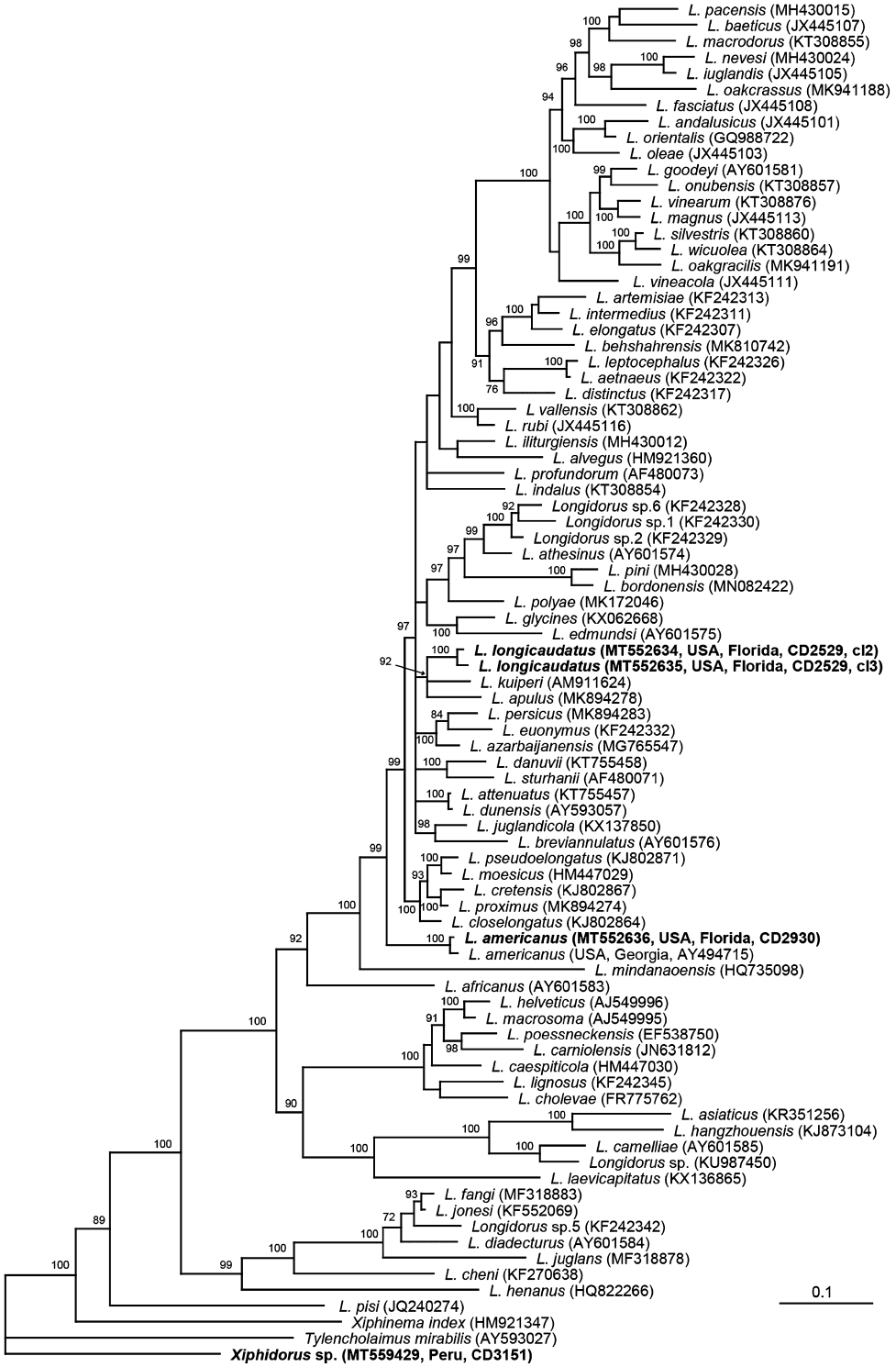

Fig. 5. Phylogenetic relationships of Florida *Longidorus longicaudatus* and *L. americanus* with other *Longidorus* species as inferred from Bayesian analysis using the D2-D3 region of the 28S rRNA gene sequences under the GTR + I + G model. Posterior probabilities greater than 70% are given for appropriate clades. New sequences are indicated in bold.

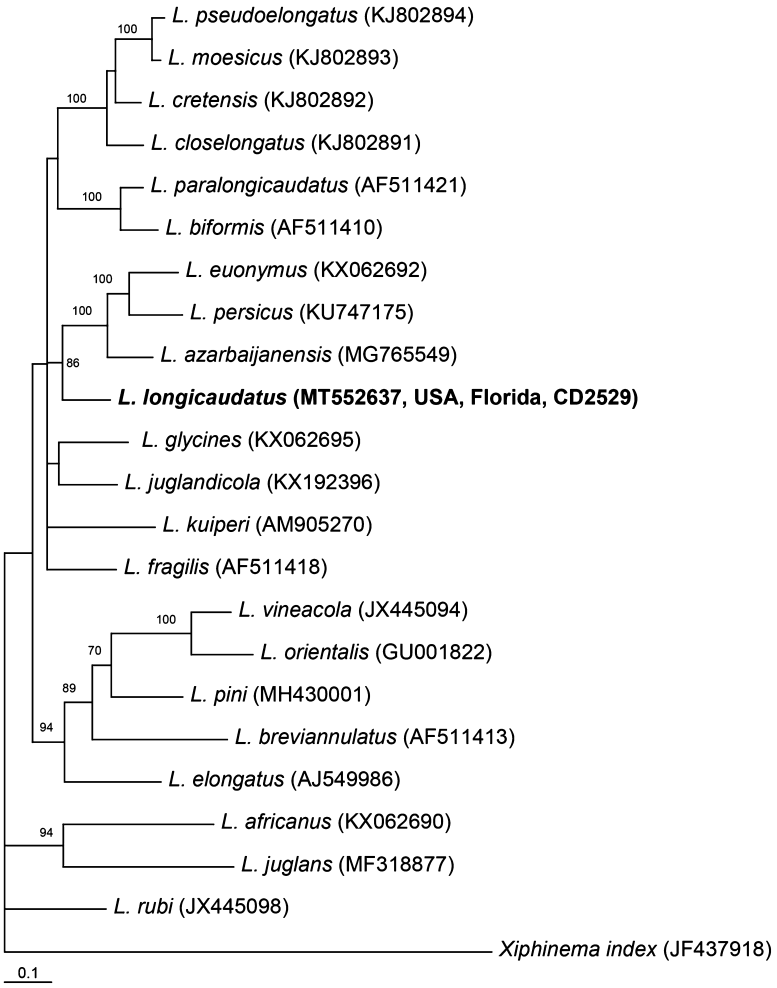

Fig. 6. Phylogenetic relationships of Florida *Longidorus longicaudatus* with other *Longidorus* species as inferred from Bayesian analysis using the ITS1 rRNA gene sequences under the GTR + I + G model. Posterior probabilities greater than 70% are given for appropriate clades. The new sequence is indicated in bold.

species. Some allometric characters, such as the lip region, mid-body and anal body diam., were greater in the Florida populations than in the five type specimens used for the description of this species. The polytomous code, A3, B1, C2, D23, E?, F1, G1, H6, I1, suggested by Chen *et al*. (1997) for *L. longicaudatus*, reflects the smaller values of these characters and the insufficient representation of the shape of the fovea in the original description. Greater morphometric values of these characters were reported also by Ye & Robbins (2003) for the population Long 16 that they identified as *L. longicaudatus* from an undetermined host and locality. These differences may be due to the fixation and mounting methods of the specimens in these studies. We are aware that all the identifications of *L. longicaudatus* made in the past by Esser (1990) and later by Ye & Robbins 2004, and those in this study, are based only on morphological characters without support of molecular data since no DNA sequence of populations of *L. longicaudatus* from the type locality in South Carolina, USA, are available. However, we consider these longidorid populations as *L. longicaudatus* until DNA sequences of the topotype populations are obtained for comparison.

The results of morphological analysis showed that *L. paralongicaudataus* is a heterogeneous species and some populations having values of $c' > 2$, as used by Ye & Robbins (2003) in the original description of this species, do not differ from the Florida *L. longicaudatus* and may

be considered representatives of this species. The heterogeneity in the morphological characters of the *L. paralongicaudatus* populations was emphasised by Ye & Robbins (2003) who stated that "*L. paralongicaudatus* populations to be quite variable; the reasons for these variations are not known but could be due to geographical differences, host differences, or even small specific differences.". The available DNA sequences of *L. paralongicaudatus* include only those of the 18S rRNA gene (AY283160) of the type population (Long-137) and ITS1 rRNA region (AF511421) of another Arkansas population (Long 143), both having ratios c′ values < 2 (Neilson *et al*., 2004; Ye & Robbins, 2004; Ye *et al*., 2004). Another 18S and ITS1 rRNA sequence (KJ934123) of a nematode identified as *L. paralongicaudatus* from turf grasses collected in North Carolina was published by Zeng *et al*. (2012). This nematode had a shorter body length (2265-2837 μm) and shorter tail length (32.0-34.3 μm). Although the 18S rRNA gene sequence of this sample is rather similar to that of the type population (Long-137), the ITS1 region is very different from the ITS1 of the population under code Long 143. Thus, sequence analysis clearly indicates molecular heterogeneity of species presently identified as *L. paralongicaudatus* and the presence of several cryptic species in the studied samples.

The ITS1 sequences of *L. paralongicaudatus* paratypes from Arkansas and another North Carolina population are different from those of *L. longicaudatus* from Florida and, in the phylogenetic tree using the ITS1 rRNA gene, clustered in different clades. The lack of molecular information for other *L. paralongicaudatus* populations from other geographical areas complicates the verification of their taxonomic identity. Molecular data are needed for these populations to verify whether their sequences match those of Florida *L. longicaudatus* or those of *L. paralongicaudatus*. The molecular data that we provide in this study for Florida *L. longicaudatus* facilitate future studies on the identification of the heterogeneous populations of needle nematodes closely similar morphologically to both *L. longicaudatus* and *L. paralongicaudatus* occurring in hardwood forests in the south-eastern states of the USA.

The phylogenetic analysis of Florida *L. longicaudatus* using the *COI* sequence showed high similarity with those of *L. carpetanensis*, *L. pini* and *L. alvegus*. These three species share with *L. longicaudatus* females the tail shape and c′ ratio values close to, or greater than 2 (1.6-2.2, 2.2-2.7 and 2.2-2.9, respectively). By contrast, the species that clustered with *L. longicaudatus* in the phylogenetic trees using the D2-D3 of 28S rRNA (*L. kuiperi* and *L. apulus*) and ITS rRNA genes (*L. euonymus*, *L. persicus*, and *L. azarbaijanensis*) have females with a different tail shape and smaller average c′ ratio values than *L. longicaudatus* (<1.5 *vs* >2.3).

In our survey, *L. longicaudatus* populations were consistently detected in soil samples consisting of feeder roots and soil collected from the three oak species mentioned above. Needle nematode densities ranged from 30-40 specimens per kg of soil. Specimens of the dagger nematodes *Xiphinema setariae* Luc, 1975 and *X. georgianum* Lamberti & Bleve-Zacheo, 1979 were also associated with the needle nematodes.

The D2-D3 of 28S rRNA gene sequence of *L. americanus* from Florida is very similar to that reported for the original population of this species from Georgia (Handoo *et al*., 2005). The detection of *L. americanus* in Florida is a new state record and suggests that this nematode occurs outside the boundary of Georgia where the nematode was found to damage loblolly pine (*Pinus taeda* L.) seedlings (Fraedrich *et al*., 2005). The small number of specimens detected indicates that the nematode does not feed on oak roots and was associated with roots from the slash pine growing nearby the oak trees. These observations may suggest that *L. americanus* may also feed on slash pines.

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