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Morphological and molecular characterisation of *Longidorus patuxentensis* n. sp. (Nematoda: Longidoridae) from Maryland and California, USA

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Summary – A new needle nematode species, *Longidorus patuxentensis* n. sp., was collected along the banks of the Western Branch Patuxent River from Upper Marlboro, MD, USA, and described herein. Female body length of the new species ranges from 3.8 to 5.2 mm, with a set off lip region by depression, 77-92 μm long odontostyle, 40-53 μm long odontophore, vulva located at 46.3-50.1% and tail conoid with bluntly rounded tip. The new species has four juvenile developmental stages and no males. It looks morphologically similar to *L. breviannulatus*, *L. elongatus*, *L. martini*, *L. americanus*, *L. grandis*, *L. sabalanicus* and *L. sturhani* by having a ventrally curved to spiral body, generally similar lip region and conoid tail with a rounded terminus, but differs from these species by the odontostyle, odontophore, total stylet length and a few other characters. Phylogenetic analysis of the D2-D3 expansion segments of 28S rRNA gene sequences placed *L. patuxentensis* n. sp. in a clade with *L. litchi*, *L. fangi*, *L. jonesi*, *L. diadecturus* and *Longidorus* sp. The D2-D3 sequence of *L. patuxentensis* n. sp. was identical to that of *Longidorus* sp.5 collected from *Juglans* sp. growing in Butte County, California, USA. The D2-D3 of 28S and ITS1 rRNA and *COI* gene sequences indicated that the Maryland and California populations belong to the same species, described herein as *L. patuxentensis* n. sp.

Keywords – *COI* gene, D2-D3 of 28S rRNA gene, ITS1 rRNA gene, *Lolium arandinaceum*, morphology, morphometrics, needle nematode, new species, phylogeny, taxonomy, Upper Marlboro.

The genus *Longidorus* Micoletzky, 1922 is comprised of nematodes that are obligate ectoparasites of plants, widely distributed world-wide and associated with a large variety of plants, and considered as a major group of plant pathogens/parasites (Archidona-Yuste *et al*., 2016; Cai *et al*., 2020). It is the second most diverse and species-rich genus within the family Longidoridae Thorne, 1935 after the genus *Xiphinema* (Decramer & Robbins, 2007). The major morphological characteristics of the *Longidorus* species include a long to very long body (from 2 to >10 mm) and long total stylet (80-260 μm) (Archidona-Yuste *et al*., 2016). This group of nematodes are of importance to agriculture as parasites as well as vectors of plant viruses (nepoviruses) and cause damage to host plants due to direct feeding on root cells (Handoo *et al*., 2005; Coomans *et al*., 2012; Archidona-Yuste *et al*., 2016). Currently, only a small proportion (6.9%) of known *Longidorus* species have been reported as virus vectors (Archidona-Yuste *et al*., 2016).

Longidorus spp. parasitise a wide range of plants that include fruit and forest trees, ornamentals, grapevine, grass, and vegetable plant species (Peña-Santiago *et al*.,

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2003; Handoo *et al*., 2005; Palomares-Rius *et al*., 2010; Gutiérrez-Gutiérrez *et al*., 2011, 2013; Archidona-Yuste *et al*., 2016; Bakhshi Amrei *et al*., 2020, 2022; Tzortzakakis *et al*., 2021). Currently, the genus *Longidorus* contains more than 180 valid species. The two species *L. hyrcanus* Mobasseri, Pourjam, Farashiani & Pedram, 2023 and *L. soosanae* Pour Ehtesham, Pedram, Atighi & Jahanshahi Afshar, 2023 are the latest species added to the genus (Gutierrez-Gutierrez *et al*., 2020; Clavero-Camacho *et al*., 2021; Mobasseri *et al*., 2023; Pour Ehtesham *et al*., 2023).

During a nematode survey, an unknown *Longidorus* species with a set off laterally, slightly swollen/rounded lip region, was found from the rhizosphere of a natural grass, tall fescue, *Lolium arandinaceum* (Schreb.) Darbysh, alongside the Patuxent River, in Upper Marlboro, Prince George's County, MD, USA. Morphological and molecular examination of the recovered population revealed that it belongs to a new species, named herein as *Longidorus patuxentensis* n. sp. The rRNA and *COI* gene sequences of *L. patuxentensis* n. sp. were identical to those of an unidentified *Longidorus* sp.5 collected from *Juglans* sp. growing in Butte County, California, USA (Subbotin *et al*., 2014). This unidentified species from California is considered conspecific with *L. patuxentensis* n. sp.

The main objectives of this study were: *i*) to describe *Longidorus patuxentensis* n. sp. using integrated methods combining both morphological and molecular data; and *ii*) to reconstruct the phylogenetic relationships of this new species within the genus.

Materials and methods

Nematode samples

Twelve soil samples were collected from Upper Marlboro, Prince George's County, MD, USA, in September and October 2020. Six of the soil samples were sent to the Plant Pest Diagnostic Center, California Department of Food and Agriculture, Sacramento, CA, USA, and the remaining soil samples were analysed at the USDA, ARS, Mycology and Nematology Genetic Diversity and Biology Laboratory (MNGDBL), Beltsville, MD, USA. Nematodes were recovered from soil samples using the sieving method. The longidorid specimens were recovered from 0.25 mm and 0.84 mm openings. Specimens of *Longidorus* sp.5 recovered from the soil sample collected from the rhizosphere of *Juglans* sp. growing in Butte County, CA, USA (Subbotin *et al*., 2014) were taken for molecular analysis only.

Morphological examination

Nematodes were fixed in 3% formaldehyde solution and processed to glycerin by the formalin glycerin method (Hooper, 1970; Golden, 1990). Photomicrographs of the specimens were taken with a Nikon Eclipse Ni compound microscope using a Nikon DS-Ri2 camera. Specimens were measured with an ocular micrometer on a Leitz DMRB compound microscope. Nematodes were also observed with the low-temperature scanning electron microscopy (SEM) using the techniques described in Kantor *et al*. (2020) and Carta *et al*. (2020).

DNA extraction, PCR, sequencing and phylogenetic analysis

DNA was extracted from several specimens using the proteinase K protocol. DNA extraction and PCR were as described by Subbotin (2021). The following primer sets were used: *i*) D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) (Subbotin, 2021) for amplification of the D2-D3 expansion segments of 28S rRNA gene; *ii*) TW81 (5′-GTT TCC GTA GGT GAA CCT GC-3′) and rDNA 1.58S (5′-ACG AGC CGA GTG ATC CAC CG-3′) (Subbotin, 2021) for amplification of the ITS1 rRNA gene; and *iii*) Long-COIFmod (5′-GAT TYT TTG GDC ACC CNG ARG T-3′) and Het-CoxiR (5′-CCT AAA ACA TAA TGA AAA TGW GC-3′) (Inserra *et al*., 2021) for amplification of *COI* gene. PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen) following the manufacturer's instructions and submitted to direct sequencing at Azenta. The newly generated sequences of the new species were submitted to the GenBank database under accession numbers: ON641207 (D2-D3 of 28S rRNA gene), ON641200, ON641201 (ITS rRNA gene) and ON645204, ON645205 (*COI* gene).

The newly obtained sequences of D2-D3 of 28S and ITS1 rRNA gene sequences of the new species were aligned using ClustalX 1.83 (Thompson *et al*., 1997) with the following parameters: gap opening 5, gap extension 3 with corresponding published gene sequences of other *Longidorus* species (Handoo *et al*., 2005; He *et al*., 2005; Gutiérrez-Gutiérrez *et al*., 2013; Subbotin *et al*., 2014; Peraza-Padilla *et al*., 2017; Gharibzadeh *et al*., 2018; Archidona-Yuste *et al*., 2019; Cai *et al*., 2020; Cid del Prado Vera *et al*., 2021; Inserra *et al*., 2021 and others).

Outgroup taxa for each dataset were chosen based on previously published data. 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 3×10^6 generations for 28S rRNA gene sequence alignment and 1×10^6 generations for 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 alignments 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

Longidorus patuxentensis n. sp.
(Figs 1-3)

Measurements

See Table 1.

Description

Females

Body assuming a ventrally curved C-shape to spiral when killed by gentle heat, moderately long and thin, tapering towards anterior and posterior ends. Cuticle smooth under LM, with fine and visible transverse lines under SEM. Inconspicuous body pores visible under SEM. Lip region set off from body contour by a depression, laterally swollen/rounded, and anteriorly flattened, giving a truncate appearance to the anterior end (Fig. 2B). SEM observations revealed labial and cephalic papillae prominent, 16 papillae arranged in typical dorylamid pattern with six labial papillae in an inner circle and ten labial plus cephalic papillae in an outer circle. Amphidial fovea more or less pocket-shaped, distinctly bilobed. Stylet guiding-ring single, odontostyle about 1.8 times as long as odontophore, the latter with slightly swollen basal muscles. Pharynx about 7.5% of total body length, with its anterior part often convoluted. The positions of the pharyngeal gland nuclei are as follows: dorsal gland nuclei at 32-25%; subventral nuclei 1 68-70% and subventral nuclei 2 at 70-71%. Cardia conoid-shaped. Reproductive system didelphic-amphidelphic, the branches almost equally developed, each composed of reflexed ovary, oviductus, sphincter, tubular uterus, not well-developed ovejector, vagina and vulva in the form of a transverse slit, located slightly anterior to the middle of the body. No sperm observed in female genital tracts. Tail conoid, dorsally convex, ventrally flat, widely rounded at tip.

Male

Not found.

Juveniles

Morphologically similar to adults, but having replacement odontostyle, lacking a developed reproductive system and smaller. Only the last three stages (J2, J3, J4) were found. The separation of different juvenile stages was made based on their relative body lengths and replacement and functional odontostyle length (Table 1). With the exception of the absence of developed genital structures, the morphological features of all three juvenile stages closely resembled those of females.

Type host and locality

Rhizosphere of tall fescue, *Lolium arandinaceum* (Schreb.) Darbysh in Western Branch Patuxent River, Upper Marlboro, Prince George's County, MD, USA. The global positioning coordinates are 38.814670°N, 76.751607°W.

Other location and host

Walnut orchard, Butte, Butte County, CA, USA. Associated host, *Juglans* sp.

Type material

Holotype (one female): Slide T-796t deposited in the United States Department of Agriculture Nematode Collection, Beltsville, MD, USA. Paratype females and juveniles with the slide codes T-7871p-T-7879p for female specimens and slides T-7880p-T-7882p for the juveniles.

ZooBank ID: urn:lsid:zoobank.org:act:0C4915CC-223E-4FD5-A1DC-C18EF3F28378.

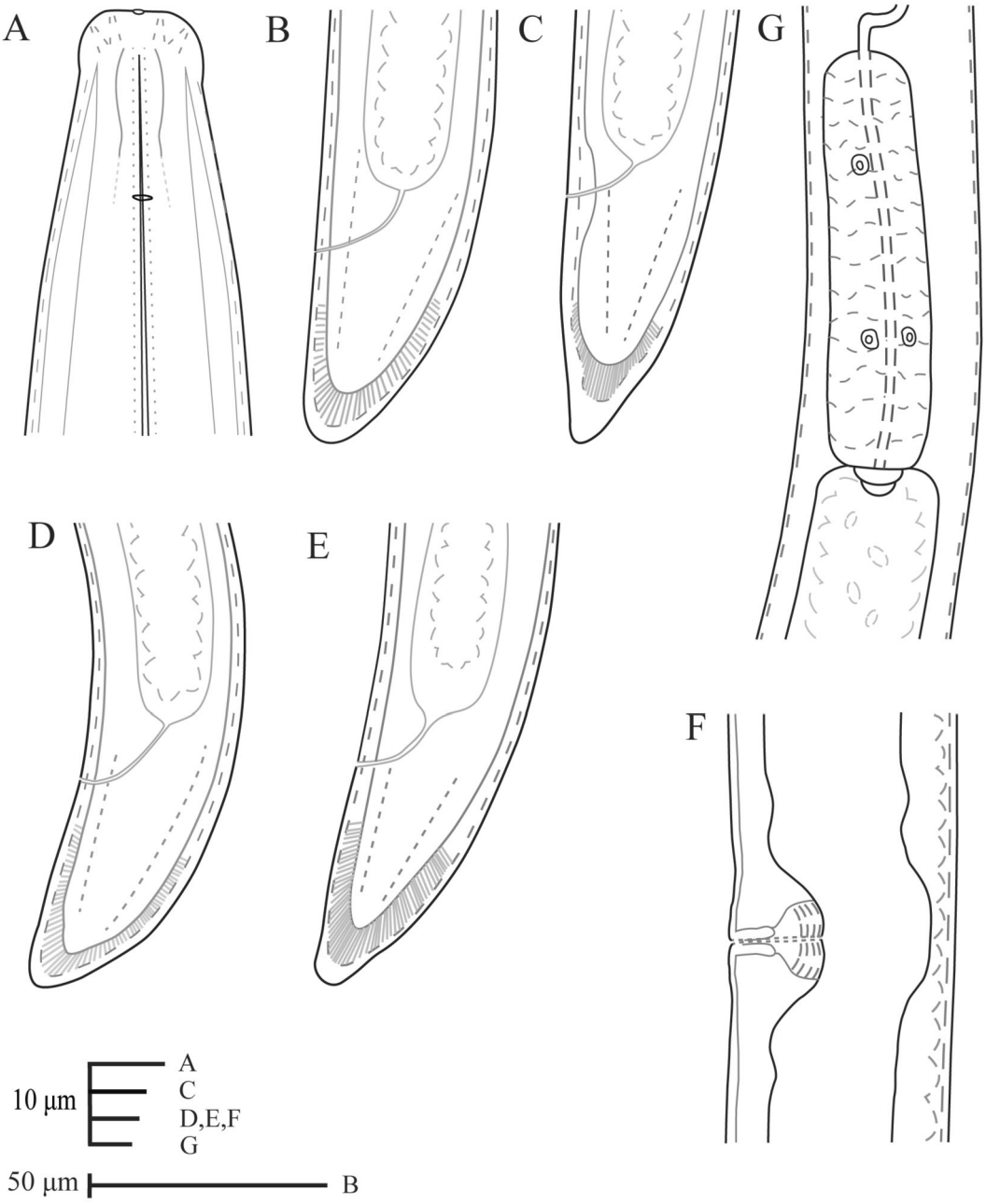

Fig. 1. Line drawings of *Longidorus patuxentensis* n. sp. A: Female anterior end; B: Female tail; C: Second-stage juvenile tail; D: Fourth-stage juvenile tail; E: Third-stage juvenile tail; F: Female vulval area; G: Female basal bulb.

Etymology

The species name is derived from the locality, Patuxent River, in Upper Marlboro, MD, USA, where the type specimens were collected.

Diagnosis and relationships

Longidorus patuxentensis n. sp. is characterised by a combination of the following morphological features in females: ventrally curved to spiral body when killed

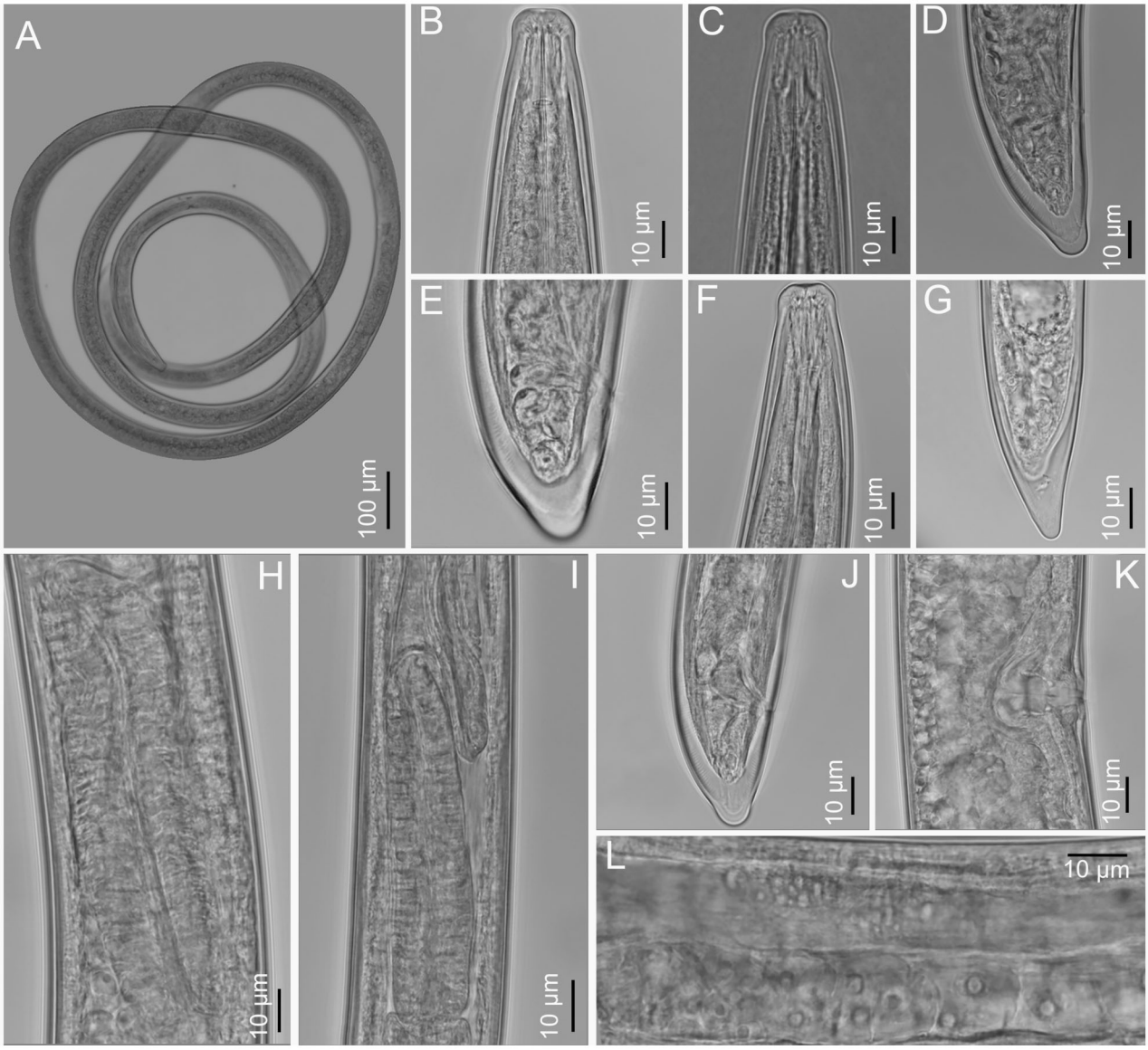

Fig. 2. Photomicrographs of *Longidorus patuxentensis* n. sp. A: Entire female; B, C: Female anterior end; D: Third-stage juvenile tail; E: Female tail; F: Fourth-stage juvenile anterior end; G: Second-stage juvenile tail; H, I: Female and fourth-stage juvenile pharyngeal bulb; J: Fourth-stage juvenile tail; K, L: Female vulval area and part of gonad. (Scale bars = 10 μm.)

by gentle heat; lip region set off from body contour by depression, slightly swollen/rounded laterally with a truncate appearance anteriorly; odontostyle 77-92 μm long; odontophore 40-53 μm long; total stylet 117-145 μm long, vulva located at 46.3-50.1% of body length; tail, conoid, dorsally convex, ventrally flat with widely rounded terminus. According to the polytomous key for the identification of species to the genus *Longidorus* by Chen *et al*. (1997), the new species has the following identification codes: A23-B23-C2-D3-E1-F23-G2-H2-I1.

Longidorus patuxentensis n. sp. looks similar to *L. elongatus* (de Man, 1876) Thorne & Swanger, 1936, from which it differs by having a lip region set off from body contour and slightly swollen/rounded laterally and having a truncate appearance anteriorly *vs* lip region continuous, slightly offset from neck contour;

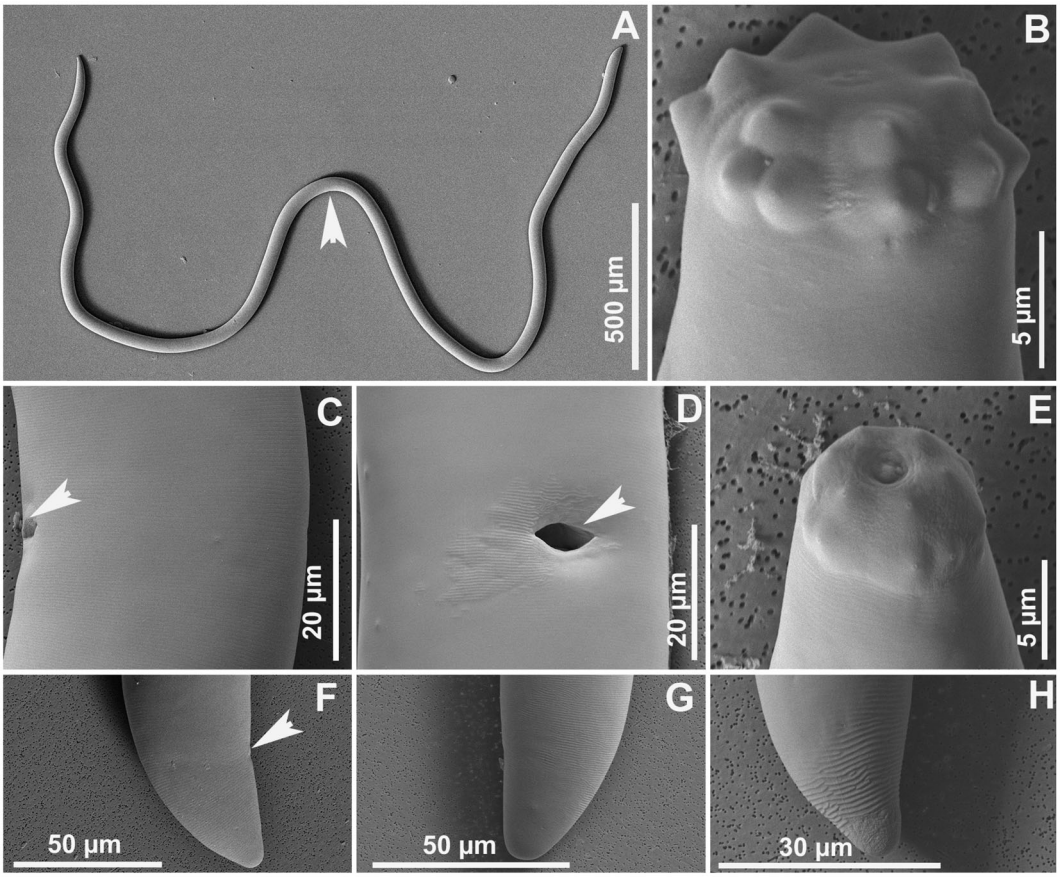

Fig. 3. *Logidorus patuxentensis* n. sp. Scanning electron microscopic images of females and juveniles. A: Entire female with arrow pointing the vulval area; B: Female anterior body region; C: Female vulval area in lateral view (arrowhead pointing towards vulva opening); D: Female vulva area in dorsal view (arrowhead pointing towards vulva opening); E: Fourth-stage juvenile anterior body region; F, G: Tail of females (with arrowhead pointing the anal opening in F); H: Tail of fourth-stage juvenile. (Scale bars: A, F, G = 10 μm; B, E = 5 μm; C, D = 2 μm.)

odontostyle length (77-92 *vs* 81-102 μm); a slightly shorter odontophore (40-53 *vs* 34-71 μm) and shorter distance from oral aperture to guide ring position (23-26 *vs* 30-36 μm) (data of *L. elongatus* after Hooper, 1961).

The new species also resembles *L. martini* Merny, 1966, from which it differs by females having a slightly longer body (3.8-5.2 *vs* 2.9-4.5 mm), wider lip region (15-17 *vs* 11-13 μm), more anteriorly situated guiding ring (23-26 *vs* 51-66 μm from anterior end), and anteriorly located vulva (V = 46-50 *vs* 52-56%).

Four other closely related species are *L. americanus* Handoo, Carta, Skantar, Ye, Robbins, Subbotin, Fraedrich & Cram, 2005, *L. sabalanicus* Asgari, Eskandari, Castillo, Palomares-Rius, 2022, *L. grandis* Ye & Robbins, 2003 and *L. sturhani* Rubtsova, Subbotin, Brown & Moens, 2001.

It differs from *L. americanus* by having shorter body in females (3.8-5.2 *vs* 5.4-9.0 mm) and shorter odontostyle (77-92 *vs* 124-165 μm), less lip region width (15-17 *vs* 26-28 μm), more anteriorly situated guiding ring (23-26 *vs* 31-43 μm from anterior end), shorter tail (29-38 *vs* 50-67 μm) and males absent *vs* present; from *L. sabalanicus* by the shape of lip region being continuous *vs* set off, higher c value (108.0-174.0 *vs* 81.0-106.0), shape of tail tip (narrower *vs* wider) and absence of males *vs* males common; from *L. grandis* by females having shorter body (3.8-5.2 *vs* 3.9-6.4 mm) and narrower lip region (15-17 *vs* 20-24 μm), more anteriorly situated guiding ring (23-26 *vs* 26-35 μm from anterior end), and lower c value (108-

Table 1. Morphometrics of *Longidorus patuxentensis* n. sp. All measurements are in μm (except L in mm) and in the form: mean ± s.d. (range).

Character	Holotype female	Paratype females	Paratype J2	Paratype J3	Paratype J4
N	1	10	3	3	8
L (mm)	4.4	4.6 ± 0.47 (3.8-5.2)	1.7 ± 0.9 (1.6-1.9)	2.3 ± 256.3 (2.0-2.6)	3.3 ± 0.2 (3.1-3.8)
a	101.1	97.3 ± 11.0 (82.3-119.3)	50.3 ± 1.2 (49.1-51.5)	52.3 ± 9.0 (41.5-63.4)	78 ± 2.7 (75.1-83.2)
b	12.9	13.3 ± 2.3 (9.9-16.2)	8.3 ± 0.9 (7.1-9.3)	11.8 ± 0.2 (11.6-12.1)	11.2 ± 1.4 (7.0-15.5)
c	115.0	138.3 ± 17.8 (108.2-173.6)	61.8 ± 13.6 (51.5-81.0)	83.2 ± 16.4 (65.0-104.8)	89.0 ± 10.1 (75.1-107.4)
c′	1.2	1.0 ± 0.1 (0.9-1.3)	1.4 ± 0.3 (1.0-1.8)	1.1 ± 0.0 (1.1-1.1)	1.23 ± 0.07 (1.13-1.32)
V%	46.3	49.2 ± 1.2 (46.3-50.1)	–	–	–
Max. body diam.	43.0	13.7 ± 0.7 (13-15)	33.5 ± 0.5 (33-34)	44.7 ± 4.8 (38-49)	42.0 ± 3.02 (38-48)
Body width at anus	30.0	31.3 ± 1.7 (29-34)	21.7 ± 2.4 (20-25)	25.7 ± 3.3 (21-28)	30.1 ± 1.6 (28.0-32.5)
Lip region width	16.5	16.3 ± 0.7 (15-17)	–	13.7 ± 0.9 (13-15)	15.5 ± 0.4 (15-16)
Lip region height	8.0	7.8 ± 0.25 (7.5-8.0)	–	5.7 ± 0.5 (5-6)	7.3 ± 0.2 (7.0-7.5)
Total stylet length	130.0	131.3 ± 9.3 (117-145)	85.7 ± 12.3 (70-100)	94 ± 17 (70-107)	115.5 ± 8.02 (105-130)
Odontostyle	80	83.6 ± 5.6 (77-92)	–	–	–
Odontophore	50	46.0 ± 4.4 (40-53)	–	–	–
Guiding ring from ant. end	26.0	24.6 ± 1.0 (23-26)	–	–	24.3 ± 1.8 (22-28)
Ant. end to pharynx	338	346.9 ± 30.3 (300-405)	–	–	282.9 ± 31.5 (219-320)
Tail length	38	33.5 ± 2.5 (29-38)	29.0 ± 6.8 (20-35)	28.3 ± 3.9 (23-32)	37.1 ± 3.1 (34-43)
J length	13.0	11.8 ± 2.1 (8-15)	–	–	8.3 ± 0.8 (7.5-9.0)
J width	18.0	18.0 ± 1.5 (16-20)	–	–	12.3 ± 0.3 (12.0-12.5)

174 *vs* 146-255); and from *L. sturhani* in having a slightly shorter body (3.8-5.2 *vs* 4.4-6.2 mm), shorter odontophore (46-53 *vs* 57-78 μm) and shorter tail (29-38 *vs* 37-44 μm).

Molecularly, *Longidorus patuxentensis* n. sp. is closely related to *L. litchii* Xu & Cheng, 1992, *L. fangi* Xu & Cheng, 1991, and *L. diadecturus* Eveleigh & Allen, 1982 in phylogenetic trees. However, it differs from *L. litchii* by having a shorter body (3.8-5.2 *vs* 5.1-6.9 mm), shorter odontostyle (77-92 *vs* 138-171 μm), guiding ring closer to anterior end (23-26 *vs* 38.5-45.5 μm) and higher c′ value (0.9-1.3 *vs* 0.61-0.79); from *L. fangi* by having a much shorter odontostyle (77-92 *vs* 122-124 μm) and the guiding ring closer to the anterior end (23-26 *vs* 69.5-86.6 μm); and from *L. diadecturus* by having a longer body (3.8-5.2 *vs* 3.3-4.0 mm), shorter odontostyle (77.0-92.0 *vs* 109-121 μm), guiding ring closer to the anterior end (23-26 *vs* 50-64 μm) and higher c′ value (0.9-1.3 *vs* 0.7-0.9).

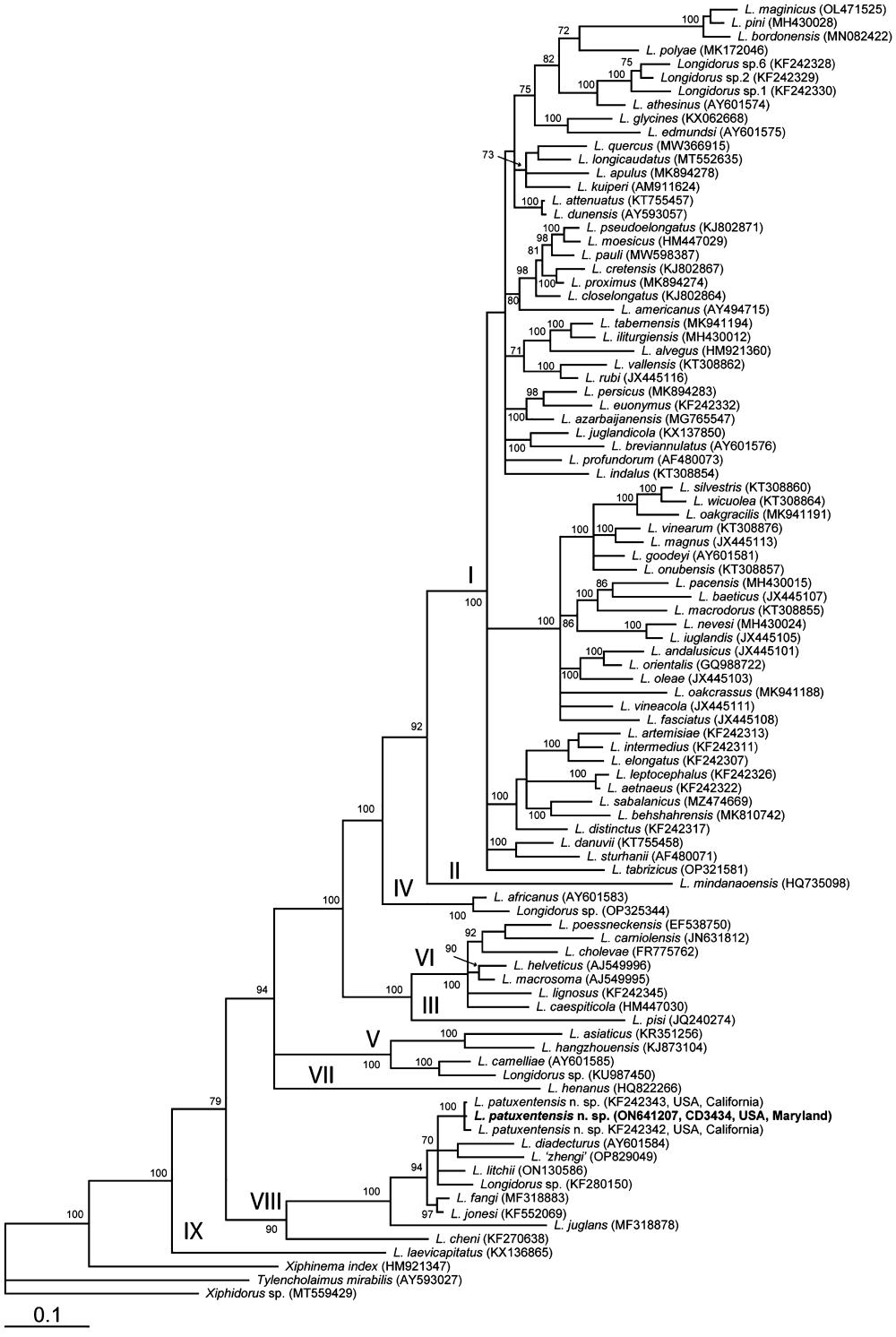

Fig. 4. Phylogenetic relationships of *Longidorus patuxentensis* n. sp. with other *Longidorus* species as inferred from Bayesian analysis of the D2-D3 of 28S rRNA gene sequences under the GTR + I + G model. Posterior probabilities greater than 70% are given for appropriate clades. New sequence is indicated in bold.

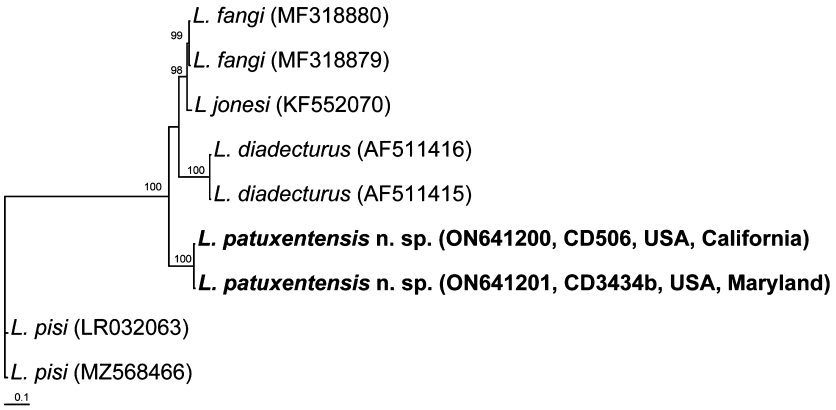

Fig. 5. Phylogenetic relationships of *Longidorus patuxentensis* n. sp. with other related *Longidorus* species from the clade VIII according to Subbotin *et al*. (2014) as inferred from Bayesian analysis of the ITS1 rRNA gene sequences under the GTR + I + G model. Posterior probabilities greater than 70% are given for appropriate clades. New sequences are indicated in bold.

MOLECULAR CHARACTERISATION

D2-D3 of the 28S rRNA gene

The D2-D3 expansion segments of 28S rRNA gene sequence alignment contained 92 sequences of *Longidorus*, including three sequences of *L. patuxentensis* n. sp. and three sequences of outgroup taxa. The alignment had 1050 characters. The phylogenetic position of *L. patuxentensis* n. sp. within the genus using this marker is given in Figure 4. The D2-D3 sequence of the Maryland population of *L. patuxentensis* n. sp. was almost identical to the sequences of the Californian population previously named as *Longidorus* sp.5 with accession numbers KF242343 and KF242343, with 0-0.2% (0-2 bp) differences observed, respectively. The California specimens was therefore considered as conspecific with the new species. In the reconstructed tree, the new species formed a clade with *L. litchii*, *L. fangi*, *L. jonesi* Siddiqi, 1962, *L. juglans* Xu, Guo, Ye, Wang, Zheng & Zhao, 2017, *L. diadecturus*, *L. cheni* Barsalote, Pham, Lazarova, Peneva & Zheng, 2018, *L. 'zhengi'* and *Longidorus* sp. with a high support value (PP = 90%). This clade corresponds to clade VIII according to Subbotin *et al*. (2014). Sequences of *L. patuxentensis* n. sp. differed from those of *L. litchii* in 3.1-3.3% (24-26 bp), *L. fangi* in 3.7-3.9% (28-30 bp), *L. jonesi* in 3.4-3.7% (26-28 bp) and *L. diadecturus* in 5.2-5.5% (40-42 bp). PCR-D2-D3-28S-RFLP diagnostic profiles with five enzymes for *L patuxentensis* n. sp. were given by Subbotin *et al*. (2014).

ITS1 rRNA gene

The two new ITS1 rRNA gene sequences of *L. patuxentensis* n. sp. from California and Maryland were 461 and 472 bp long, respectively, and differed in one nucleotide. The ITS1 rRNA gene sequence alignment included seven sequences of *Longidorus* spp. belonging to clade VIII according to Subbotin *et al*. (2014) and *L. pisi* was selected as the outgroup. The alignment had 1209 characters. The phylogenetic position of *L. patuxentensis* n. sp. in the ITS1 rRNA gene tree is given in Figure 5. Sequences of *L. patuxentensis* n. sp. differed from those of *L. fangi* in 12.1-12.3% (54-55 bp) and *L. jonesi* in 11.4-11.7% (40-41 bp).

COI *gene*

The two new *COI* gene sequences for *L patuxentensis* n. sp. from California and Maryland were 372 and 379 bp long, respectively, and were identical. The BlastX search of these *COI* sequence showed they have highest identity with those of *Longidours helveticus* Lamberti *et al*., 2001 (Kumari and Subbotin, 2012) (similarity = 79.0%, coverage = 100%) and *L. macrosoma* Hooper, 1961 (77.4%, 100%).

Discussion

In the present study, we have combined morphological and molecular approaches to examine *Longidorus* species collected from Upper Marlboro, Maryland, and Butte County, California. The convergence of evidence strongly supports the recognition of a novel needle nema-

tode species, which we propose to name *Longidorus patuxentensis* n. sp. These findings contribute to our expanding understanding of the diversity and distribution of *Longidorus* nematodes, underscoring the importance of employing methodologies that integrate morphological and molecular techniques for accurate species identification.

Our investigation also prompts an intriguing discussion within clade VIII, a group primarily composed of *Longidorus* species found and described in East Asia, including *L. litchi*, *L. fangi*, *L. jonesi*, *L. juglas*, *L. diadecturus*, *L. cheni*, formally undescribed *L. 'zhengi'* and unidentified *Longidorus* sp. Notably, among this clade, only *Longidorus patuxentensis* n. sp. and *L. diadecturus* have been documented in North America so far. However, it is imperative to consider the possibility that *L patuxentensis* n. sp. shares an Asian origin with *L. diadecturus*. The latter was initially identified in Ontario, Canada, but has recently been discovered in Hubei province, China (Barsalote *et al*., 2018). This observation raises intriguing questions regarding potential geographic connections and the dispersal patterns and evolutionary history of *Longidorus* species across different regions. It is plausible that these nematodes have undergone intercontinental migrations, potentially facilitated by natural or anthropogenic factors. To gain a comprehensive understanding of the origins and distribution patterns within clade VIII, further investigations are warranted. These future studies can shed light on the intercontinental relationships and potential migration pathways of *Longidorus* species, thereby providing valuable insights into their evolutionary dynamics.

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