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Morphological and Molecular Characterization of *Longidorus americanum* n. sp. (Nematoda: Longidoridae), a Needle Nematode Parasitizing Pine in Georgia

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Abstract: We describe and illustrate a new needle nematode, *Longidorus americanum* n. sp., associated with patches of severely stunted and chlorotic loblolly pine, (*Pinus taeda* L.) seedlings in seedbeds at the Flint River Nursery (Byromville, GA). It is characterized by having females with a body length of 5.4–9.0 mm; lip region slightly swollen, anteriorly flattened, giving the anterior end a truncate appearance; long odontostyle (124–165 µm); vulva at 44%–52% of body length; and tail conoid, bluntly rounded to almost hemispherical. Males are rare but present, and in general shorter than females. The new species is morphologically similar to *L. biformis, L. paravineacola, L. saginus,* and *L. tarjani* but differs from these species either by the body, odontostyle and total stylet length, or by head and tail shape. Sequence data from the D2–D3 region of the 28S rDNA distinguishes this new species from other *Longidorus* species. Phylogenetic relationships of *Longidorus americanum* n. sp. with other longidorids based on analysis of this DNA fragment are presented. Additional information regarding the distribution of this species within the region is required.

Key words: DNA sequencing, Georgia, loblolly pine, *Longidorus americanum* n. sp., molecular data, morphology, new species, needle nematode, phylogenetics, SEM, taxonomy.

Needle nematodes (*Longidorus* spp.) are an important group of plant parasites that cause serious damage to many plant species (Cohn, 1974). These slender nematodes are readily recognized from most other Dorylaims by their long (2 mm to 12 mm), rather narrow body and their elongated axial spear (odontostyle) plus an extension (odontophore) about half the odontostyle length (Hooper, 1974). Symptoms associated with *Longidorus* generally are non-specific and may include plant stunting, root tip galls, and root forking (Cohn, 1974). In addition, some *Longidorus* species are vectors of nepoviruses (Taylor and Brown, 1997). During summer 2000, a new species of the needle nematode *Longidorus* was associated with patches of stunted and cholorotic loblolly pine (*Pinus taeda* L.) seedlings in seedbeds at the Flint River Nursery (Byromville, GA) (Fraedrich and Cram, 2002). Seedlings from affected areas had poorly developed root systems that lacked lateral and feeder roots, and the nematode was found to damage root systems of loblolly pine in a controlled study. Subsequently, various plant species were evaluated as potential hosts to this needle nematode (Fraedrich et al., 2003). Slash, loblolly, and longleaf pines were good hosts. Red oak also appeared to be a host although not as suitable as pine. Small grain crops, including sorghum, sorghum-sudan hybrid, wheat, rye, and oats, were poor hosts. Yellow and purple nutsedge, common weeds at the nursery, and tomato and Cabbage, previously grown at the nursery site, were also poor hosts.

The objectives of this study were to describe this new species using light and scanning electron microscopy (SEM) observations and to assess the diagnostic value of morphological and molecular characters.

Materials and Methods

Morphological characterization: Soil samples (2.5-cm diam. to a 20-cm depth) were collected from areas that contained patches of stunted loblolly pine seedlings. Nematodes were extracted from a 200-cm^3 composite soil sample that was thoroughly but gently mixed, using the technique of Flegg (1967) with modifications by Fraedrich and Cram (2002). Nematodes were removed from Baermann funnels, and juveniles, males, and females were fixed in warm 3% formaldehyde fixative and processed to glycerine by the formalin-glycerine method (Golden, 1990; Hooper, 1970). Light microscopic images of fixed nematodes were taken on a Leica WILD MPS48 Leitz DMBR compound microscope fitted with an ocular micrometer for image measurement. For SEM, living specimens were fixed in 3% glutaraldehyde buffered with 0.05 M phosphate (pH 6.8), dehydrated in a graded series of ethanol, critical-point dried from liquid CO_2, and sputter coated with a 20 to 30-nm layer of gold-palladium.

Molecular characterization: For molecular analysis two samples were prepared: one sample contained one specimen, and the second one contained five specimens of this new longidorid nematode. Nematodes

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were transferred into 10 µl of double distilled water in an Eppendorf tube and crushed with microhomogenisator. Eight µl nematode lysis buffer (125 mM Kcl, 25 mM Tris-Cl, pH 8.3, 3.75mM $MgCl_2$, 2.5 mM DTT, 1.125% Tween 20) and 2 µl of proteinase K (600 µg/ml) were added. The tubes were incubated at 65 °C (1 hour) and 95 °C (10 minutes) consecutively. After centrifugation (1 minute; 16,000g), 4 µl of the DNA suspension was added to the PCR reaction mixture containing 10 µl 10X *Taq* incubation buffer, 20 µl 5X Q-solution, 200 µM of each dNTP (*Taq* PCR Core Kit, Qiagen, Germany), 1.5 µM of each primer (synthesized by Life Technologies, Merelbeke, Belgium), 0.8U *Taq* Polymerase (*Taq* PCR Core Kit, Qiagen, Germany), and double distilled water to a final volume of 100 µl. The D2–D3 region of the 28S gene was amplified using the primers D2A (5′- ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Rubtsova et al., 2001). DNA amplification was performed using a PTC-100 Programmable Thermal Controlled (MJ Research Inc., Watertown, MA) and consisted of 4 minutes at 94 °C and 35 cycles of 1 minute at 94 °C, 1.5 minutes at 55 °C, 2 minutes at 72 °C, and 10 minutes at 72 °C. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen Ltd., Crawley, West Sussex, UK). DNA fragments were directly sequenced in both directions using PCR primers with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, UK) according to the manufacturer's instructions. Sequences were run on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence for *Longidorus americanum* n. sp. is deposited at GenBank with accession number AY494715.

The DNA sequence of *Longidorus americanum* n. sp. was edited with Chromas 1.45. Alignment with other longidorid sequences (Rubtsova et al., 2001), including the outgroups *Xiphinema rivesi* and *Xiphinema diversicaudatum* (Y. He, unpubl. data), was performed with ClustalX 1.64 with default options (Thompson et al., 1997). Equally weighted maximum parsimony was performed using PAUP (4.0 beta version) (Swofford, 1998). Gaps were treated as missing data. Bootstrap analysis with 100 replicates was conducted to assess the degree of support for each branch on the tree.

A Bayesian phylogeny estimation (Huelsenbeck, 2000) was made with MrBayes v. 3.0, using the GTR + γ model as estimated by ModelTest (Posada and Crandall, 1998). Markov chain Monte Carlo was run for 250,000 cycles with each one hundredth cycle sampled, using a random tree as a starting point. The first 100 samples were excluded as "burn in." In PAUP, a consensus likelihood cladogram was constructed under the Maximum Likelihood option for among-site rate variation. The numbers on the majority rule consensus tree branches representing the posterior probability are given in appropriate clade in sampled trees.

Systematics

Longidorus americanum n. sp.
(Figs. 1–5)

Description

All measurements are in micrometers unless otherwise stated.

Holotype (female, in glycerine): L = 7.4 mm; a = 108.0; b = 14.9; c = 124.0; c′ = 1.4; J′ = 0.8; V% = 46; odontostyle = 143.5; odontophore = 83.0; total stylet = 226.5; anterior end to end of esophagus = 500.0; anterior end to guiding ring = 32.5; tail length = 60.0; hyaline tail length = 17.5; hyaline tail width = 22.5; lip width = 27.5; mid-body width = 69.0; anal-body width = 42.5.

Table 1. Measurements of paratype females and males of *Longidorus americanum* n. sp.

Character	Females	Males
N	27	5
L (mm)	7.00 ± 0.80 (5.40–9.00)	6.40 ± 0.40 (5.80–6.80)
a	119.6 ± 12.0 (89.5–136.6)	106.7 ± 11.7 (94.4–123.4)
b	9.0 ± 4.5 (4.6–16.8)	14.5 ± 1.6 (12.2–15.5)
c	119.1 ± 14.5 (95.0–154.6)	113.2 ± 5.9 (102.8–117.3)
c′	1.5 ± 0.1 (1.3–1.7)	1.36 ± 0.03 (1.3–1.4)
J′	0.8 ± 0.1 (0.6–1.0)	—
G1%	0.1 ± 0.0 (0.0–0.1)	—
G2%	0.1 ± 0.0 (0.0–0.1)	—
V%	48.8 ± 2.2 (44.2–51.7)	—
Odontostyle	141.9 ± 9.7 (124.0–165.0)	146.2 ± 7.7 (135.0–152.0)
Odontophore	83.0 ± 2.4 (79.2–91.4)	52.4 ± 7.0 (47.0–62.9)
Total stylet	224.4 ± 9.7 (207.1–249.7)	198.7 ± 12.1 (182.0–211.1)
Anterior end to end of esophagus	602.0 ± 104.8 (367.6–777.8)	472.7 ± 51.4 (420.0–530.0)
Guiding ring from anterior end	34.7 ± 2.4 (30.5–42.6)	34.5 ± 0.9 (33.5–35.5)
Tail length	58.9 ± 4.6 (50.8–67.0)	56.7 ± 1.4 (55.0–58.9)
ABW	40.4 ± 1.5 (36.5–42.6)	41.6 ± 0.6 (41.0–42.5)
Hyaline tail length	17.0 ± 2.2 (14.2–22.3)	15.2 ± 1.9 (13.0–18.0)
Hyaline tail width	22.6 ± 1.7 (20.3–26.4)	18.3 ± 1.1 (17.5–20.3)
Spicules	—	68.0 ± 4.5 (65.0–75.0)
Lip width	27.5 ± 0.8 (26.4–28.4)	27.0 ± 0.8 (26.9–27.5)
Body width	58.8 ± 6.4 (50.8–71.1)	60.4 ± 5.1 (54.0–67.0)

All measurements, except length, in micrometers. Means ± SD, range in parentheses.

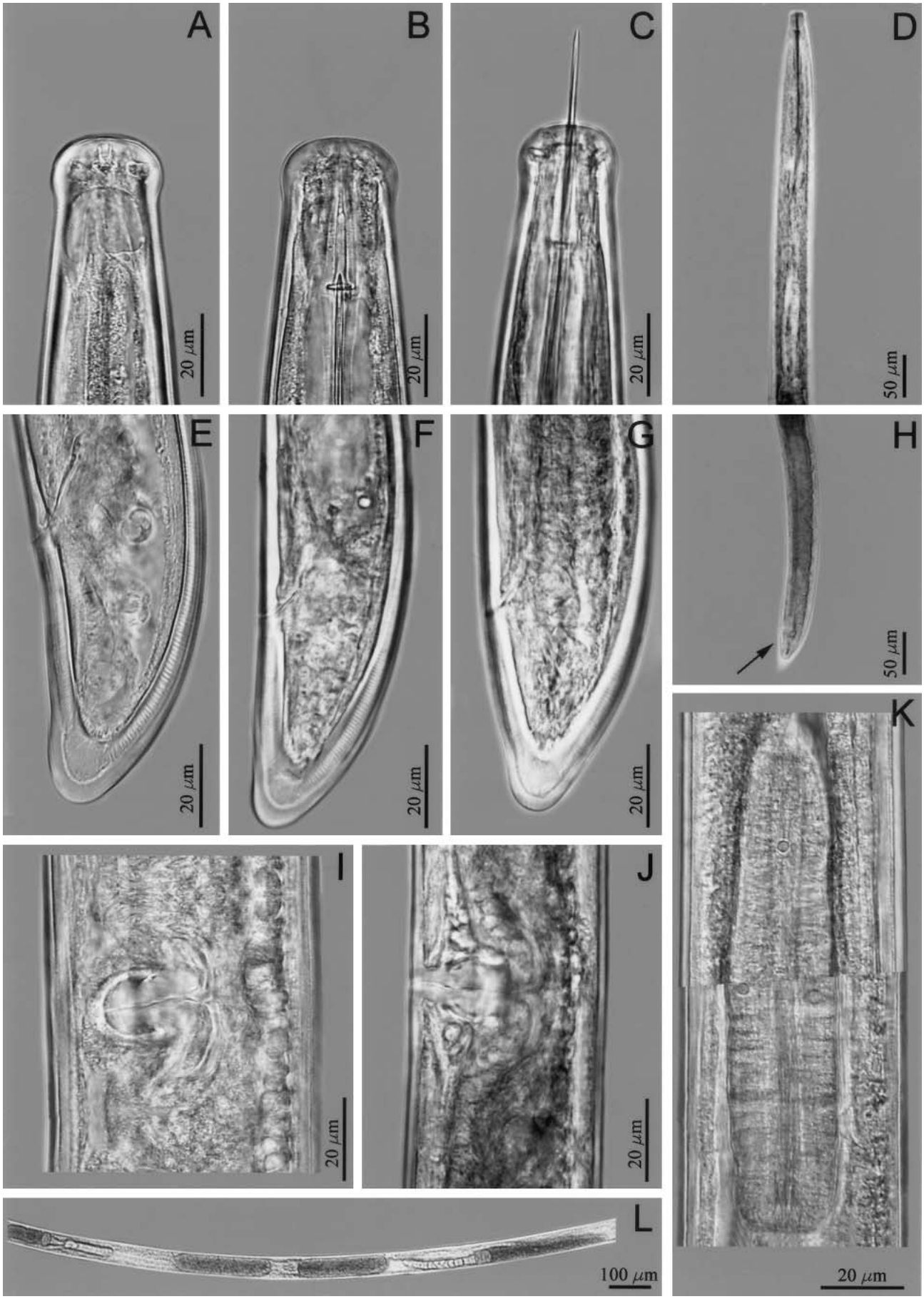

FIG. 1. *Longidorus americanum* n. sp. Photomicrographs of females. A–C) Head lateral views showing amphid, nerve ring, and stylet, respectively. D) Anterior region. E–H) Posterior regions (tail), H) showing annus (arrow). I, J) Vulval regions. K) Basal bulb of esophagus. L) Anterior and posterior branch of reproductive system.

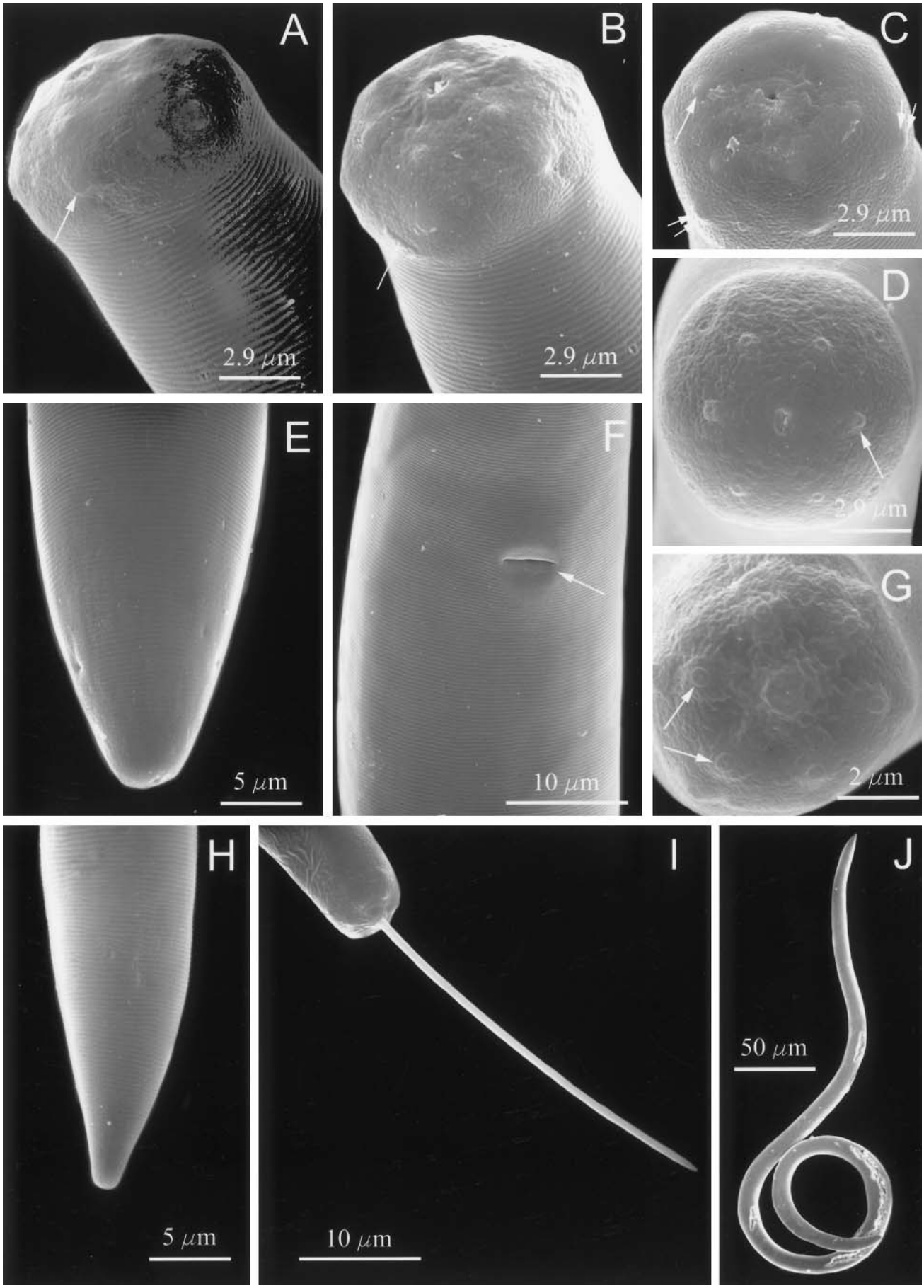

FIG. 2. *Longidorus americanum* n. sp. scanning electron micrographs of A–G) females and H–J) Juveniles. (In A) arrows point toward cephalic papillae and B) toward amphidial aperture). C,D,G) En face views of head showing cephalic papillae (arrow) and flap-like structure (double arrow). E, F) Female posterior end and vulval opening (arrow), respectively. H–J) Tail, head (with protruding stylet) and whole juvenile, respectively.

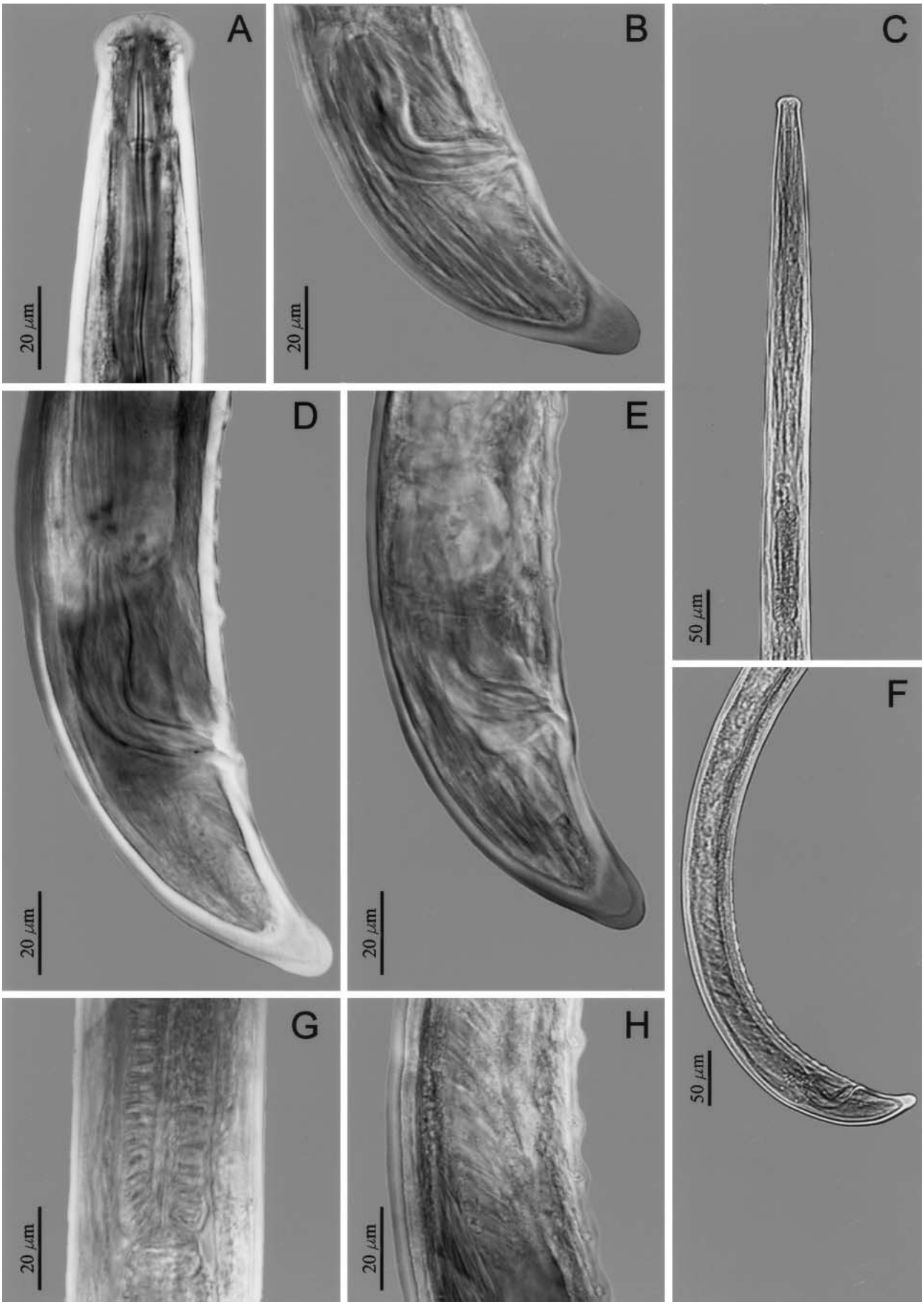

FIG. 3. *Longidorus americanum* n. sp. Photomicrographs of males. A,C) Anterior regions. B,D–F) Posterior regions showing spicules. G) Posterior part of basal bulb and cardia. H) Posterior end above spicule region.

Females (Paratypes): Measurements are listed in Table 1.

Body of heat-relaxed females assuming J or spiral shape. Cuticle containing inconspicuous pores. Cuticle along the body 2.5 µm thick, 5 µm at post-labial region and thickening toward posterior end. Lip region slightly swollen, anteriorly flattened, giving the anterior end and truncate appearance (Fig. 1, A-C). Amphids large, extending to half the distance between anterior end and guiding ring, more or less pocket-shaped, symmetrically bilobed (Fig. 1, A-B). Odontostyle long and slender, needle-like (Fig. 1, C), 2.5 µm in diam. at base, odontophore base slightly expanded. Nerve ring about two body widths (at odontophore base) posterior to the odontophore base. Esophageal bulb length about six times longer than width. Location of esophageal gland nuclei typical of the genus, i.e. dorsal nucleus at 30% to 35% and subventral nuclei at 50% to 56% of the basal bulb (Fig. 1, K). Nucleoli rounded, nucleolus of dorsal gland 2.5 µm in diam. Esophago-intestinal valve (cardia) bluntly rounded to conical. Vulva a transverse slit (Fig. 1, I, J; Fig. 2, F). Vagina perpendicular to body axis, extending to half of mid-body width or slightly less (Fig. 1, I, J). Uteri 90–125 µm in length, indistinct sphincter between uteri and oviduct. No sperm observed in female genital tracts. Ovaries paired, opposed, reflexed (Fig. 1, L). Pre-rectum 7 to 10 anal body width or 250–455 µm long. Rectum shorter than body width at anus and about 35 µm long (Fig. 1, E-G). Tail bluntly rounded to almost hemispherical (Fig. 1, E-H). Hyaline tail length slightly shorter or the same as width.

Allotype (male in glycerine): L = 6.4 mm; a = 102.1; b = 12.1; c = 117.0; c′ = 1.3; odontostyle = 152.0; odontophore = 62.9; total stylet = 214.9; anterior end to esophagus = 530.0; anterior end to guiding ring = 35.0; tail length = 55.0; hyaline tail length = 18.0; hyaline tail width = 18.0; spicule = 70.0; lip width = 27.0; mid-body width = 63.0; anal-body width = 41.0.

Male (Paratypes): Measurements are listed in Table 1.

TABLE 2. Measurement of juvenile stages of *Longidorus americanum* n. sp.

Character	J_1	J_2	J_3	J_4
N	28	10	30	20
L (mm)	1.32 ± 1.12	2.23 ± 0.38	3.70 ± 0.85	5.90 ± 0.39
	(1.11–1.60)	(1.38–3.10)	(2.45–5.02)	(5.00–6.52)
a	48.5 ± 2.1	64.4 ± 12.7	84.0 ± 16.5	101.0 ± 7.5
	(45.0–52.5)	(53.0–95.4)	(64.2–127.0)	(85.6–113.3)
b	5.4 ± 0.9	5.2 ± 0.8	7.1 ± 1.7	11.8 ± 1.2
	(3.9–7.1)	(4.3–6.5)	(5.1–12.2)	(10.0–14.2)
c	27.6 ± 2.0	40.9 ± 5.5	64.0 ± 14.0	99.1 ± 6.5
	(25.1–34.2)	(34.8–50.3)	(44.8–104.9)	(89.3–113.3)
c′	2.5 ± 0.2	2.0 ± 0.2	1.7 ± 0.2	1.5 ± 0.1
	(2.2–2.7)	(1.8–2.2)	(1.3–2.0)	(1.2–1.7)
J′	1.3 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
	(1.1–1.7)	(0.7–1.2)	(0.5–1.0)	(0.6–0.9)
Odontostyle	78.3 ± 4.1	91.2 ± 2.7	113.2 ± 8.4	137.2 ± 4.8
	(69.0–85.3)	(85.3–95.4)	(99.5–132.0)	(125.9–142.1)
Odontophore	34.8 ± 6.1	49.1 ± 7.7	56.9 ± 6.7	73.4 ± 2.5
	(24.4–44.7)	(36.5–58.9)	(38.6–67.0)	(69.0–77.1)
Total stylet	113.1 ± 5.6	140.8 ± 6.5	170.0 ± 8.7	210.6 ± 5.6
	(105.6–123.8)	(129.9–148.2)	(146.2–190.8)	(196.9–219.2)
Replacement stylet	94.7 ± 3.0	111.7 ± 5.8	127.9 ± 8.6	155.0 ± 4.6
	(89.3–101.5)	(101.5–121.8)	(113.7–148.2)	(146.2–162.4)
Anterior end to end of esophagus	248.2 ± 29.8	426.5 ± 49.7	513.5 ± 97.7	498.9 ± 42.6
	(201.0–316.7)	(345.1–488.9)	(264.7–763.0)	(397.1–558.8)
Guiding ring from anterior end	17.8 ± 0.6	24.4 ± 2.7	27.8 ± 3.1	31.0 ± 0.6
	(17.3–19.3)	(20.3–28.4)	(22.3–33.5)	(30.5–32.5)
Tail length	48.1 ± 3.9	54.1 ± 3.4	55.7 ± 4.7	59.4 ± 3.8
	(36.5–52.8)	(50.8–60.9)	(46.7–65.0)	(46.7–65.0)
ABW	19.4 ± 1.8	27.3 ± 3.4	33.6 ± 3.2	40.2 ± 1.7
	(16.2–22.3)	(24.4–34.5)	(28.4–40.6)	(36.5–42.6)
Hyaline tail length	10.7 ± 0.9	9.8 ± 1.9	12.7 ± 1.8	15.8 ± 1.3
	(9.1–12.2)	(8.1–13.2)	(8.1–15.2)	(13.2–18.3)
Hyaline tail width	8.0 ± 0.3	10.8 ± 1.4	15.3 ± 1.8	20.4 ± 0.8
	(7.1–8.1)	(8.1–12.2)	(12.2–18.3)	(18.3–22.3)
Lip width	13.7 ± 0.8	18.5 ± 1.5	22.8 ± 1.5	25.8 ± 0.6
	(12.2–16.2)	(16.2–22.3)	(20.3–25.4)	(24.4–26.4)
Body width	27.3 ± 2.4	35.1 ± 4.8	43.4 ± 6.7	58.4 ± 5.4
	(23.3–30.5)	(26.4–41.6)	(32.5–57.9)	(46.7–69.0)

All measurements, except length, in micrometers. Means ± SD, range in parentheses.

Males rare. Body shape similar to female except posterior region more strongly curved ventrally (Fig. 3, B, D–F). Morphometrics and anatomy similar to female except for structural differences in genitalia (Fig. 3, A–H) and, in general, body length shorter than females with a narrow hyaline tail. Spicules thick, lateral accessory piece inconspicious (Fig. 3, B, D–F).

Juveniles (Paratypes): Measurements are listed in Table 2.

Juveniles recorded were identified as representing J1–J4 (Fig. 4, A–H) according to the nomenclature proposed by Robbins et al. (1994). They resemble females except for undeveloped genital structures and shorter length. Body shape arcuate to J-shaped (Fig. 2, J); lip shape similar to female. Replacement odontostyle present. Tail in some specimens more bluntly to conically rounded (Fig. 2, H) and in others gradually tapers to a more bluntly rounded terminus (Fig. 4, E). Scatter plot

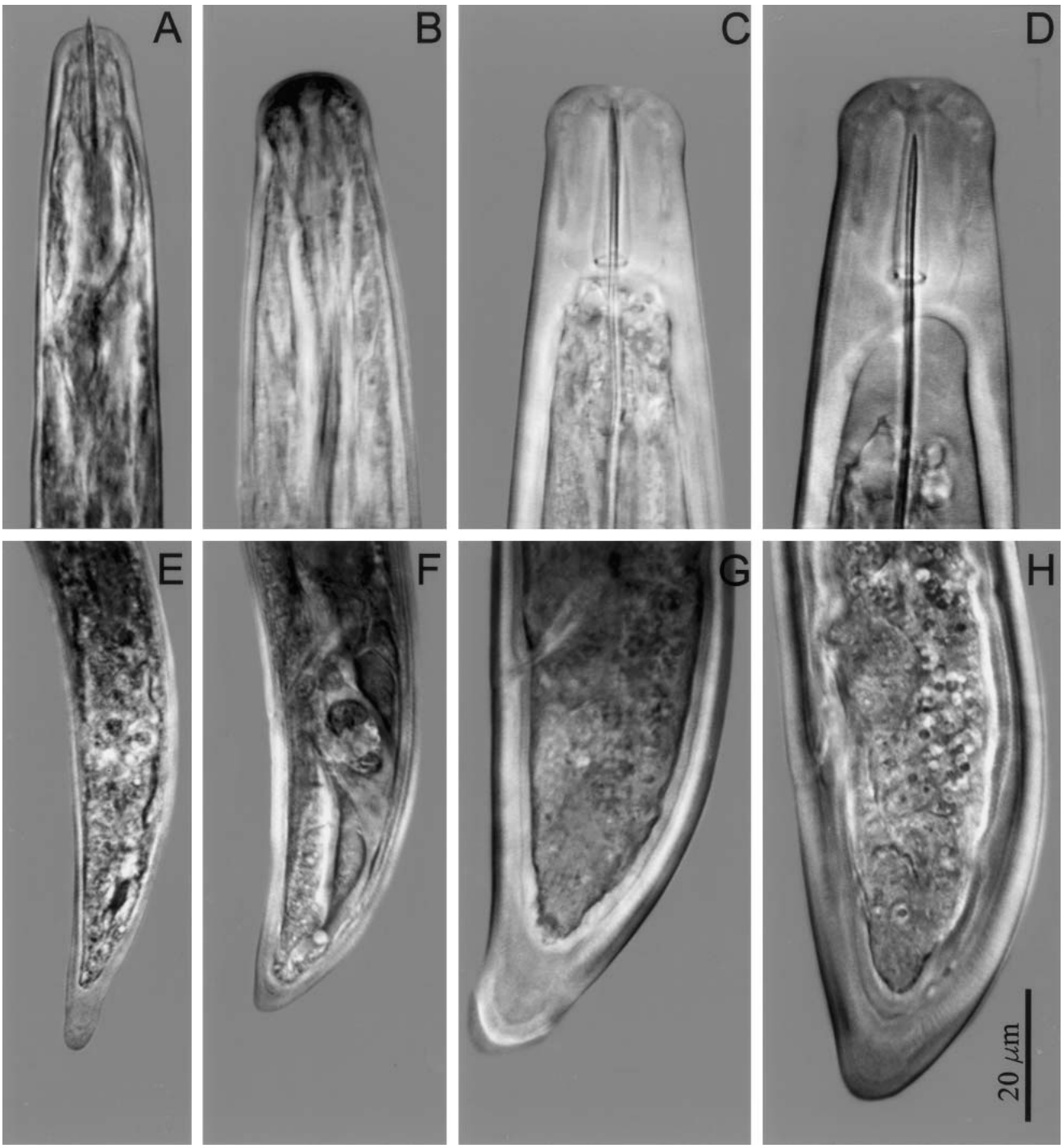

FIG. 4. *Longidorus americanum* n. sp. Photomicrographs of anterior and posterior regions of juveniles. A, E) First-stage. B, F) Second-stage. C, G) Third-stage. D, H) Fourth-stage.

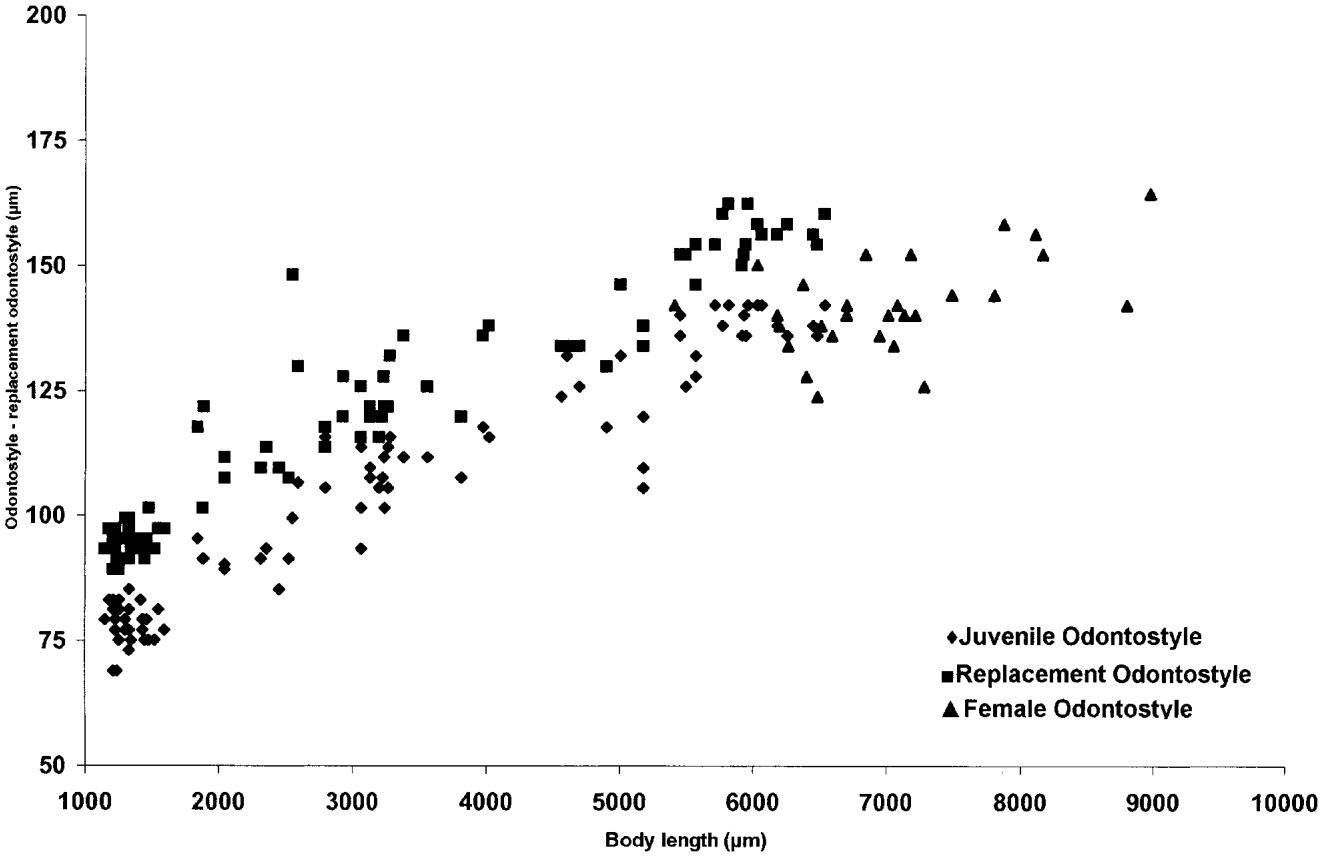

FIG. 5. Scatter plot of odontostyle length and replacement odontostyle length against body length of *Longidorus americanum* n. sp. juveniles and female paratypes.

shows a high degree of overlap between stages in odontostyle, replacement odontostyle, and body length (Fig. 5).

Type Host and Locality

Collected during summer 2000 from the rhizosphere of loblolly pine (*Pinus taeda* L.) in sandy loam soil associated with patches of stunted and chlorotic loblolly pine seedlings in seedbeds at the Flint River Nursery (Byromville, GA). The global positional coordinates are N32° 10.040 minutes; W 83° 58.337 minutes.

Type Specimens

Holotype (female): Slide T-577t, deposited in the U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland.

Allotype (male): Slide T-578t, same data and repository as holotype.

Paratypes (Females, males, and juveniles): Same data and repository as holotype. Slides T-5173p-T-5207p: T-5173p-T-5184p (females), T-5185p-T-5187p (males), T-5188p-T-5207p (juveniles). Additional paratypes deposited in the following: Nematode Collection of the Nematology Laboratory, University of Arkansas, Fayetteville, Arkansas; University of California-Riverside Nematode Collection, Riverside, California; Nematode Collection of the Nematology Department, Rothamsted

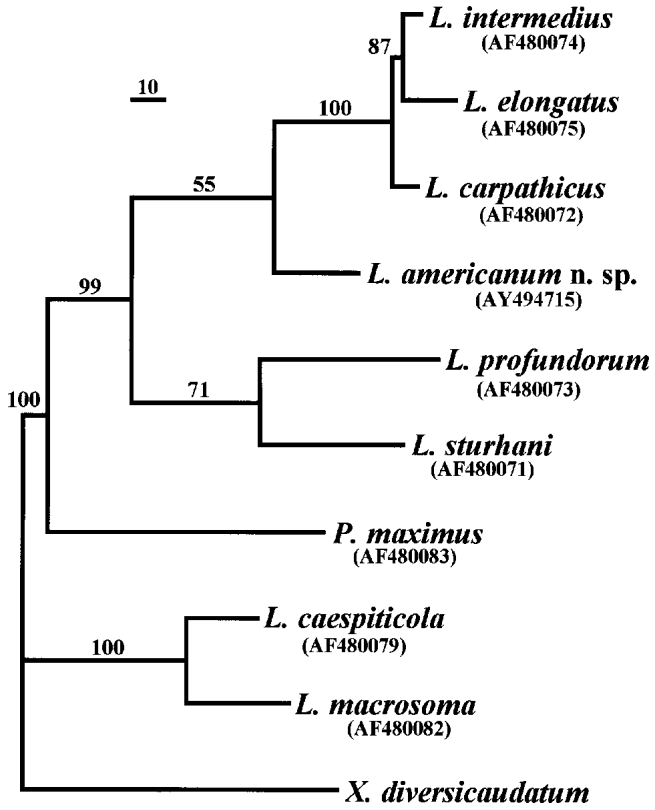

FIG. 6. Phylogenetic relationships of *Longidorus americanum* n. sp. with other Longidoridae as inferred from the parsimony analysis of the D2–D3 fragment of the 28S gene of rDNA (tree length = 481).

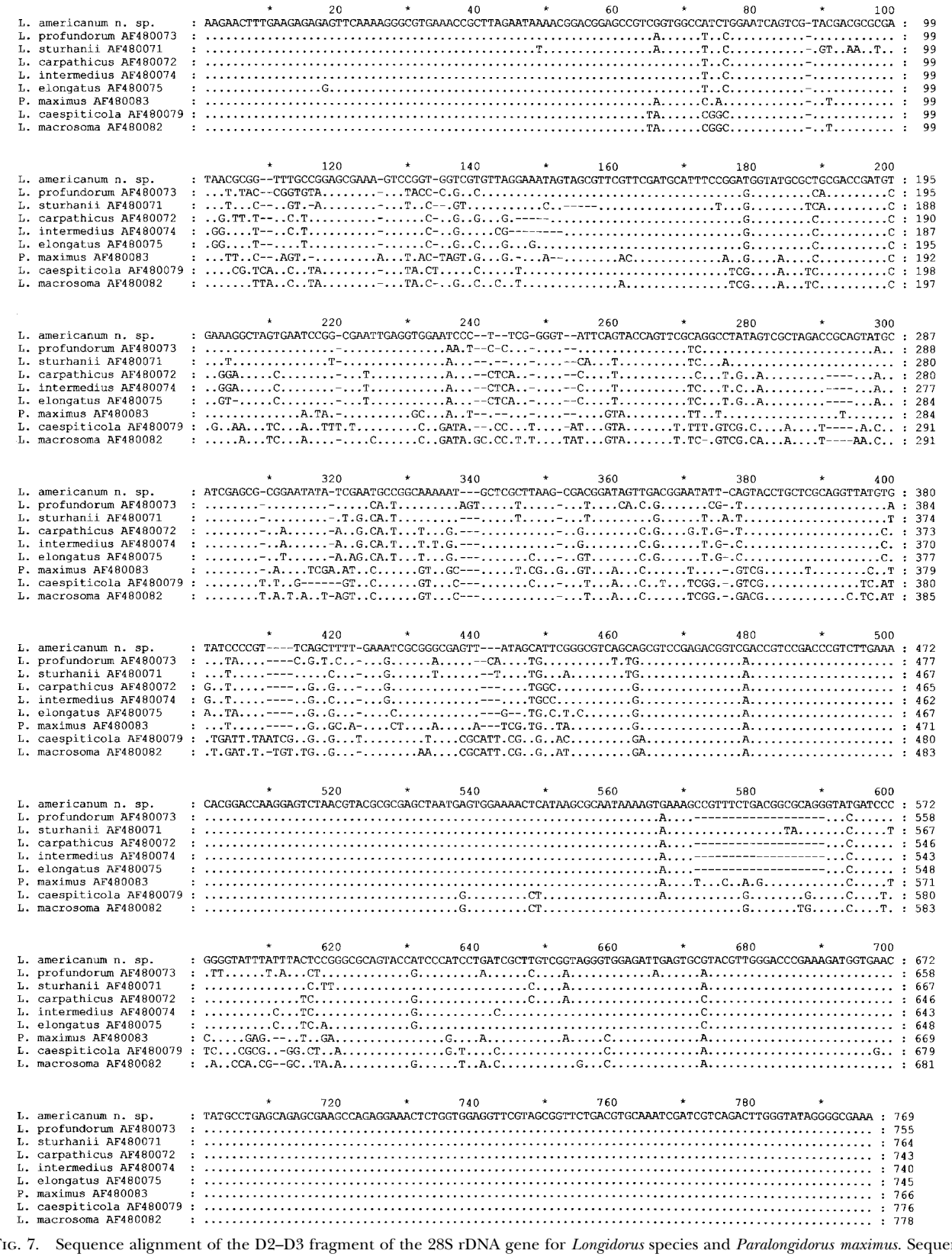

FIG. 7. Sequence alignment of the D2–D3 fragment of the 28S rDNA gene for *Longidorus* species and *Paralongidorus maximus*. Sequence gaps are indicated by dashes, and identities to *L. americanum* n. sp. are denoted by periods.

Experimental Station, Harpenden, Herts, United Kingdom; Canadian National Collection of Nematodes, Ottawa, Canada; Collection Nationale de Nématodes, Laboratoire des Vers, Muséum National d'Histoire Naturelle, Paris, France; Nematode Collection of the Landbouwuniversiteit, Wageningen, The Netherlands; Commonwealth Institute of Parasitology Collection, St. Albans, United Kingdom.

Diagnosis

Longidorus americanum n. sp. is characterized by having females with body length of 5.4–9.0 mm; lip region slightly swollen, anteriorly flattened, giving the anterior end a truncate appearance; long odontostyle that measures 124–165 µm; odontophore length 79.2–91.4 µm; total stylet 207.1–249.7 µm long; vulva located at 44% to 52% of body length; and tail conoid, bluntly rounded to almost hemispherical. Males are present, but rare, with spicule length of 65–75 µm. The polytomous code (Chen et al., 1997) for *L. americanum* is: A57-B5-C34-D3-E2-F34-G23-H12-I 2 (12).

Relationships

Longidorus americanum n. sp. is closely related to *L. biformis* Ye & Robbins, 2004, *L. paravineacola* Ye & Robbins, 2003, *L. saginus* Khan et al., 1971, and *L. tarjani* Siddiqi, 1962. It differs from *L. biformis* in having females with a longer odontostyle, 124–165 µm vs. 96.0–125.0 µm, from 12 different Arkansas locations and in the absence of supplement-like organs in females. From *L. paravineacola* it differs in having females with a longer odontostyle, 124–165 µm vs. 95.0–114.0 µm; longer tail, 51–67.0 µm which is conoid, bluntly rounded to almost hemispherical, and more than one anal body width long vs. 28.0–44.7 µm short bluntly rounded tail that is less than one anal body width long (Ye and Robinson, 2003). The proposed new species differs from *L. saginus* in that it has females with longer bodies (5.4–9.0 mm vs. 4.8–6.4 mm), longer tail (51–67 µm vs. 21–33 µm), and c′ (1.4–1.6 vs. 0.6–0.8), and in the shape of tail (tail conoid, bluntly rounded to hemispherical vs. convex conoid). Compared with *L. tarjani, L. americanum* differs in having females with a longer body (5.4–9.0 mm vs. 6.0–6.8 mm) and a shorter odontostyle (124–165 µm vs. 178–182 µm).

Molecular characterization and relationship with other species: The size of the analyzed 28S rDNA D2–D3 expansion segment from *Longidorus americanum* n. sp. was 769 bp. No sequence variation was detected between the two sequenced samples, which included a single specimen or five pooled nematodes. Sequence of the new species varied at 60 to 129 nucleotide positions from those of other longidorids (8.6% to 17.0% difference). The D2–D3 alignment for eight *Longidorus* and one *Paralongidorus* and *Xiphinema* species was 907 bp. Maximum parsimony (MP) analysis of the D2–D3 sequence alignment yielded a single parsimonious tree (Fig. 6). In this tree, *Longidorus americanum* n. sp. appeared as a sister group to the clade containing *L. carpathicus* + *L. intermedius* + *L. elongatus,* albeit with weak bootstrap support. The Bayesian inference tree based on another alignment (Fig. 7) showed a different position for *L. americanum* n. sp., placing its basal to the *Longidorus* species *sensu lato* with 100% posterior probability (Fig. 8). This position is internal to *Longidorus caespiticola* and

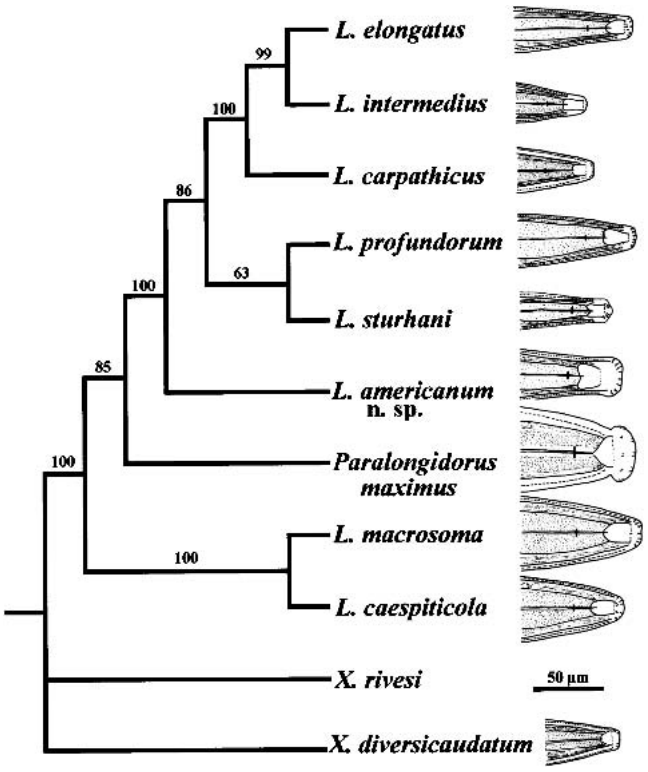

FIG. 8. MrBayes 50% majority rule consensus maximum likelihood cladogram of 2,250 trees generated from a manual alignment in PAUP. *Xiphinema* specified as outgroup, gaps removed, 250,000 generations, nst = 6, gamma = 0.35. Taxa were resized and redrawn in standardized form from various sources (Chen et al., 1997; Peneva et al., 2001; Rubtsova et al., 2001) to emphasize relative sizes and amphid foveal shapes.

L. macrosoma, two species that possess distinctly different amphids than the *Longidorus* type species and its morphologically similar relatives. Posterior probabilities and bootstrap values can be considered potential upper and lower bounds of clade reliability rather than direct comparisons (Douady et al., 2003). The differences in tree topology resulting from parsimony and Bayesian likelihood methods likely will be resolved with more taxa and/(or) sequence (Pollock et al., 2002).

Neilson et al. (2004) have included an unnamed isolate (Long-234), which was *L. americanum* n. sp. in a study of the phylogenetic relationships among most of *Longidorus* species from North America, except for *L. tarjani, L. elongatus* (de Man, 1876) Micoletzky, 1922, *L. longicaudatus* Siddiqi, 1962, and *L. sylphus* Thorne, 1936 inferred from 18S rDNA gene sequence data. This phylogenetic analysis indicated that the new species has an 8-bp difference compared to its closest relative, *L. biformis.* The other close relatives found in this study include *L. paralongicaudatus* Ye & Robbins, 2003; *L. brevi-annulatus* Norton & Hoffmann, 1971; and *L. crassus* Thorne, 1974.

In summary, the needle nematode found on loblolly pine in Byromville, Georgia, herein referred to and described as *Longidorus americanum* n. sp., is quite differ-

ent from the other *Longidorus* species known to date. The common name "pine needle nematode" is suggested.

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