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Morphological, Molecular, and Differential-Host Characterization of *Meloidogyne floridensis* n. sp. (Nematoda: Meloidogynidae), a Root-Knot Nematode Parasitizing Peach in Florida

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Abstract: A root-knot nematode, *Meloidogyne floridensis* n. sp., is described and illustrated from peach originally collected from Gainesville, Florida. This new species resembles *M. incognita, M. christiei, M. graminicola,* and *M. hispanica,* but with LM and SEM observations it differs from these species either by the body length, shape of head, tail and tail terminus of second-stage juveniles, body length and shape of spicules in males, and its distinctive female perineal pattern. This pattern has a high to narrowly rounded arch with coarsely broken and network-like striae in and around anal area, faint lateral lines interrupting transverse striae, a sunken vulva and anus, and large distinct phasmids. Molecular data from ribosomal IGS illustrate that *M. floridensis* n. sp. is different from the mitotic species *M. arenaria, M. incognita,* and *M. javanica.* Data from RAPDs confirm it and suggest that this new species lies in an intermediate phylogenetic position between the previous species and the meiotic species *M. hapla, M. fallax,* and *M. chitwoodi.* Differential host tests based on annual crops and on *Prunus* accessions are reported.

Key words: esterase phenotype, Florida, host range, meiotic parthenogenesis, *Meloidogyne,* morphology, new species, peach, root-knot nematode, scanning electron microscopy, taxonomy.

Root-knot nematodes (*Meloidogyne* spp.) are economically important plant pathogens, displaying marked sexual dimorphism. Males are vermiform and active. Females are pyriform or saccate and sedentary, laying eggs in a gelatinous matrix ("egg sac"). Usually only the roots are attacked, and these are induced to form characteristic galls ("knots") on many host plants. More than 80 nominal species of this genus have been described (Karssen, 2002). The taxonomy of this genus has been advanced by numerous review papers (Allen, 1952; Chitwood, 1949; Eisenback, 1985a,b; Eisenback et al., 1981; Esser et al., 1976; Golden, 1976; Golden and Birchfield, 1965; Jepson, 1987; Karssen, 2002; Karssen and Van Hoenselaar, 1998; Sasser, 1954; Taylor et al., 1955; Triantaphyllou, 1971; Triantaphyllou and Sasser, 1960; Whitehead, 1968).

Meloidogyne floridensis n. sp. was first detected by R. H. Sharpe in 1966 in Gainesville, Florida, where it parasitized the root-knot (*M. incognita* (Kofoid and White, 1919) Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949) nematode-resistant Nemaguard and Okinawa peach (*Prunus persica* (L.) Batsch) rootstocks (Sharpe et al., 1969). Later, this nematode also reproduced on Nemared (Sherman et al., 1991). This previously unnamed nematode has been referred to as (i) Nemaguard type root-knot nematode, (ii) a new nematode, and (iii) a biotype of root-knot nematode (Sharpe et al., 1969; Sherman et al., 1981; Young and Sherman, 1977). In 1982, this nematode was initially characterized as *M. incognita* race 3 (Sherman and Lyrene, 1983). However, upon further preliminary examination of this nematode via morphology, host range, and molecular characterization, this population appeared to be quite different from the known root-knot nematode species and was thought to be a new species (Nyczepir et al., 1998).

The objectives of this study were to describe this new species using light microscopy and Scanning Electron Microscopy (SEM) observations; assess the diagnostic value of morphological, molecular, and cytological characters; and present results of host-range tests on some economically important crops.

Materials and Methods

Nematode inocula: A population of *M. floridensis* n. sp. isolated from peach (cv. Nemaguard) roots from Gainesville, Florida, was increased from a single egg mass and maintained on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) at 21 °C to 30 °C in a greenhouse at Byron, Georgia. This population was distributed among the collaborators and used in the different studies reported herein.

Morphological characterization

Various stages: Second-stage juveniles (J2) and males were recovered from infected roots or egg masses kept in petri dishes with a small amount of water. Some were extracted from soil by sieving and Baermann funnel extraction. Females were dissected from infected roots after fixation overnight in 3% formaldehyde. Second-stage juveniles were fixed in 3% formaldehyde and pro-

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cessed to glycerine by the formalin-glycerine method (Golden, 1990; Hooper, 1970). Procedures used in measuring and preparing specimens were essentially those of Golden and Birchfield (1972) except some fixed females were cut and mounted in clear lactophenol solution. Photomicrographs of perineal patterns, J2, and males were made with a 35-mm camera attached to a compound microscope equipped with differential interference optics. Roots with galls were photographed under a dissecting microscope, and light microscopic photographs of fixed nematodes were taken on a compound microscope where measurements were made with an ocular micrometer. All measurements are in micrometers unless otherwise stated. For SEM, living specimens were fixed in 3% glutaraldehyde solution 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.

Cytological characterization

Nematode isolate: Nematodes were maintained on tomato cv. Moneymaker and Motelle, respectively, with and without the *Mi-1* gene conferring resistance to *M. incognita, M. arenaria,* and *M. javanica.*

Cytology: During at least four generations, white egg-mass-producing females were dissected from the roots. Preparation of slides, fixation, staining with Hoechst 33258, and microscopical observations were according to Van der Beek et al. (1998). Additionally, smeared females were stained with 4′,6-diamidino-2-phenyindole (DAPI). The smears were examined at ×1000 magnification with a UV-light microscope using an excitation filter G265, beam splitter FT395, and barrier filter LP420.

Molecular and biochemical characterization

RAPD analysis (Nematode isolates): Names and geographical origins are listed in Table 1. Each nematode isolate used in the RAPD study consisted of the progeny of a single female from a field population. The nematodes have been maintained in the INRA Antibes collection for several years and their identification frequently confirmed by isoesterase electrophoretic patterns (Dalmasso and Bergé, 1978). Several individual females were characterized to ensure that pure culture had been maintained during their repetitive propagation.

RAPD analysis (DNA extraction): For each nematode isolate, total genomic DNA was purified from 100 to 200 µl of pooled J2. Nematodes were frozen in liquid nitrogen and ground with a mortar and pestle, and total genomic DNA was extracted from the resulting powder with the phenol/chloroform procedure (Sambrook et al., 1989). Following ethanol precipitation, DNA was resuspended in TE buffer (0.01M Tris pH 8 and 0.001M EDTA) to a final concentration of 5 ng/ml and stored at −20 °C.

TABLE 1. Origin of the *Meloidogyne* spp. isolates used in this study.

Species	Isolate	Geographic origin	Experiments
M. arenaria	Ma1	Ain Taoujdate, Morocco	R[b]
	Ma2	Espiguette, France	R
	Monteux	Provence, France	H[c]
	Line 26[a]	Portugal	I[d]
M. chitwoodi	Mc1	Spijkenisse, The Netherlands	R
M. fallax	Mf1	Baexem, The Netherlands	R
M. floridensis	Msp	Gainesville, Florida	R, H, I
M. hapla	Mh1	England	R
	Mh2	La Môle, France	R
M. incognita	Mi1	Landes, France	R
	Mi2	Morelos, Mexico	R
	Calissane	Provence, France	H
	Line 19[a]	French West Indies	I
M. javanica	Mj1	Canary Islands	R
	Mj2	Turkey	R
	Higuera	Catalunia, Spain	H
	Line 23[a]	Burkina Faso	I
M. mayaguensis	Line 13[a]	Puerto Rico	I

[a] Blok et al., 1997.
[b] R = RAPD analysis.
[c] H = Host range study.
[d] I = rDNA Intergenic spacer (IGS) analysis.

Genomic DNA was diluted to a concentration of 10 ng/µl, and 17 random 10-mer oligonucleotide primers were used in RAPD experiments (Table 2). RAPD-PCR was performed as previously described (Castagnone-Sereno et al., 1994), with slight modifications as follows: Amplifications were run in a final volume of 25 µl containing 10 ng of total genomic DNA, 80 pM each of primer, dATP, dCTP, dGTP, and dTTP at 200 µM final concentration, 1× Taq incubation buffer, and 0.25 U Taq polymerase (Appligene, Graffenstaden, France). Each reaction mixture was overlaid with mineral oil. Amplification was performed with a TRIO Thermoblock thermal cycler (Biometra Inc., Tampa, FL). The cycling program was 1 minute at 94 °C, 40 cycles of 20 seconds at 94 °C, 30 seconds at 36 °C, 2 minutes at 70 °C, and a final incubation of 10 minutes at 70 °C. Amplification products were separated by electrophoresis in 1.4% agarose gels in TBE buffer at a constant

TABLE 2. Oligonucleotide primers used for RAPD analysis.

Primer name	Sequence (5′ to 3′)	% G+C
2	ATGGATCCGC	60
J10	AAGCCCGAGG	70
J20	AAGCGGCCTC	70
K04	CCGCCCAAAC	70
K07	AGCGAGCAAG	60
K09	CCCTACCGAC	70
K10	GTGCAACGTG	60
K14	CCCGCTACAC	70
K16	GAGCGTCGAA	60
K19	CACAGGCGGA	70
K20	GTGTCGCGAG	70
M10	TCTGGCGCAC	70
N10	ACAACTGGGG	60
P01	GTAGCACTCC	60
P02	TCGGCACGCA	70
P05	CCCCGGTAAC	70

current of 150 mA for approximately 3 hours, and visualized with ethidium bromide (0.5 µg/ml) under UV light.

RAPD data analysis: RAPD markers that were consistently reproduced in at least two replicate PCR reactions and that were reproducible across successive DNA extractions were used for further analysis. Consistent DNA fragments were considered as binary characters, and DNA fingerprints from each isolate were converted to a 0–1 matrix.

Phylogenetic analysis: A phylogenetic analysis was conducted using the computer program PAUP* 4.0 (Swofford, 1998) according to the following options. Characters were run unordered with no weighting, and the heuristic search algorithm was used to find the most parsimonious tree. One thousand bootstrap replicates were performed to test the support of branches for the most parsimonious tree (Felsenstein, 1985), and a 50% majority-rule consensus tree was computed.

Intergenic spacer rDNA Sequencing: Eighteen J2 were disrupted by hand with a small blade knife and transferred to microcentrifuge tubes containing 100 µl extraction buffer (Williams et al., 1992). Proteinase K was added to 60 µg/ml final concentration, and the tubes were incubated at 60 °C for 1 hour followed by 95 °C for 15 minutes, and either frozen at –20 °C or used immediately for PCR. The intergenic region between the 18S and 5S genes (IGS rDNA) was amplified using the primers and cycling conditions described in Blok et al. (1997). Each 50-µl reaction contained 10 mM Tris pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 200 µM dNTPs, 0.8 µM 5S and 18S primers, 11 µl DNA extract, and 1 unit Taq polymerase (Qbiogene, Inc., Carlsbad, CA). PCR products were separated by agarose gel electrophoresis, excised from the gel, and purified with the Qiaquick PCR Cleanup kit (Qiagen Operon, Alameda, CA). The amplicons were concentrated by ethanol precipitation for direct sequencing or cloned into pGEM-T (Promega Corp., Madison, WI) by standard methods (Maniatis et al., 1982). Plasmid DNA was prepared using the Wizard DNA Purification Kit (Promega). The sequences for *M. floridensis* n. sp. and *M. mayaguensis* Rammah and Hirschmann, 1988 Florida population have been deposited in the GenBank database (National Center for Biotechnology Information, National Library of Medicine, National Institute of Health, Bethesda, MD, http://www.ncbi.nlm.nih.gov) as AY194853 and AY194854, respectively. DNA was sequenced in both directions by cycle sequencing on an ABI 310 (Applied Biosystems, Foster City, CA). DNA sequence information was assembled using Sequencher v.4.1 (Gene Codes, Ann Arbor, MI). DNA alignments were performed with ClustalW (European Bioinformatics Institute) and manually adjusted when necessary. Cosmetic changes to the final alignment were made with MegAlign (DNASTAR, Inc., Madison, WI).

Electrophoretic analysis: Esterase b electrophoresis was used to compare *M. floridensis* n. sp. to other *Meloidogyne* spp. (i.e., M. *javanica* isolate [Mj1, Mj2, Higuera, Line 23[a]], *M. incognita* isolate [Mi1, Mi2, Calissane, Line 19[a]], *M. arenaria* isolate [Ma1, Ma2, Monteux, Line 26[a]], and *M. mayaguensis* isolate [Line 13[a]]) using standard protocols (Janati et al., 1982), and gels were stained for esterase and malate dehydrogenase activity (Esbenshade and Triantaphyllou, 1985). Additionally, *M. floridensis* n. sp. was compared to two different *M. incognita* race 3 isolates (GA-peach and SC-bean). Malate dehydrogenase and esterase phenotypes were also compared between *M. floridensis* n. sp. and *M. javanica* isolates (Mj1, Mj2, Higuera, Line 23[a]). Enzyme phenotypes for each nematode isolate were determined from replicate samples of single females. Separation of proteins was by the automated PhastSystem (Amersham Biosciences, Inc., Piscataway, NJ).

Differential Host Studies

North Carolina differential host-range test (Taylor and Sasser, 1978): Meloidogyne floridensis n. sp., *M. incognita* race 3 (GA-peach), and *M. incognita* race 3 (SC-bean) were maintained on the tomato cv. Rutgers. Eggs were extracted from galled roots with NaOCl (Hussey and Barker, 1973 , and 2,000 eggs were pipeted into depressions made in the soil around each host's hypocotyl. Treatments consisted of inoculating eight seedlings of each cultivar, and the pots were arranged in a randomized complete block on greenhouse benches. Greenhouse temperatures ranged from 21 °C to 35 °C. Plants were watered daily and fertilized as needed. After 75 days, the plants were harvested and the roots were washed in tap water and stained with phloxine B (150 mg/L for 15 minutes). Egg masses on each root system were counted and the plants rated as a host or non-host (Taylor and Sasser, 1978). A root system rating of 0 to <3 was considered a poor to non-host.

Prunus host-range test: Meloidogyne floridensis n. sp. was maintained on the tomato cv. St Pierre, lacking the *Mi* gene. It reproduces poorly on cv. Piersol, which possesses the *Mi* gene. A wide range of *Prunus* species and accessions have been evaluated with *M. floridensis* n. sp. by applying a high and durable inoculum pressure (Esmenjaud et al., 1992, 1996). From these tests, a few root-knot nematode resistant sources have been chosen to perform a differential test for comparison of their host susceptibilities to *M. floridensis* n. sp., and to one isolate each of *M. arenaria, M. incognita,* and *M. javanica* (Table 1).

All measurements are in micrometers (µm) unless otherwise specified.

Systematics

Meloidogyne floridensis n. sp.
(Figs. 1–6)

Description

Holotype (female, in glycerine): Body length with neck 700; body width 515; neck length 148; neck greatest width 75; stylet length 14.5; stylet knob width 5; stylet

TABLE 3. Differential host test for classifying *Meloidogyne* spp.[a] and races.

Meloidogyne species	Differential host[b]					
	Tobacco	Cotton	Pepper	Watermelon	Peanut	Tomato
M. floridensis	–	–/+	–	+	–	+
M. incognita (GA-peach)[c]	–	–/+	+	+	–	+
M. incognita (SC-bean)[c]	–	+	+	+	–	+

[a] + = host; – = non-host. See Taylor and Sasser (1978).
[b] Tobacco (*Nicotiana tabacum*) 'NC 95'; cotton (*Gossypium hirsutum*) 'Deltapine 16'; pepper (*Capsicum annuum*) 'California wonder'; watermelon (*Citrullus lanatus*) 'Charleston Grey'; peanut (*Arachis hypogaea*) 'Florunner'; and tomato (*Lycopersicon esculentum*) 'Rutgers'.
[c] See Taylor and Sasser (1978) for race differentiation within a species.

knob height 2.5; dorsal esophageal gland orifice (DGO) from base of stylet 6; excretory pore from anterior end 40; EP/ST ratio 2.8; body length from anterior end to posterior end of metacorpus 105; about 25 body annules from anterior end to excretory pore; cuticle thickness at neck 3; cuticle thickness at midbody 7; vulva slit length 25; distance from vulva slit to anus 17.

Female (n = 25): Measurements are listed in Table 5.

Body pearly white, variable in size, round to pear-shaped with relatively distinct variable-size neck sometimes bent at various angles to body (Fig. 1B). Cephalic framework weak, hexaradiate, lateral sectors slightly enlarged, vestibule and extension prominent. Cephalids not observed. Head not offset, with labial disc; lip region with one annule. SEM observations revealed: Labial disc fused with medial lips (Fig. 4E,F), dumbbell shaped; lateral lips indistinct and amphidial openings oval, located between labial disc and lateral lips. Stylet strong, with rounded, broad to posteriorly sloping knobs (Fig. 1A,B), cone and shaft straight. Excretory pore distinct, generally located 2 to 3 stylet lengths posterior to stylet base (Fig. 1B). Esophagus well developed with elongate cylindrical procorpus; and large, rounded metacorpus provided with heavily sclerotized valve. Body cuticle thick at midbody, thinner near anterior end of neck. Perineal pattern (Figs. 3A-I;4A-D) with high to narrowly rounded or ovoid arch, with coarse broken to network-like striae in and above anal area, faint lateral lines interrupting transverse striae, and smooth wavy lines in the outer field; perivulval region without striae, vulva and anus sunken. Phasmids large and distinct with a conspicuous phasmidial canal (Fig. 1C).

Allotype (male in glycerine): Length = 1,205; a = 34.4; b = 8.6; c = 110; stylet knob width 5; stylet knob height 3; excretory pore from anterior end 142; center of median bulb 100 from anterior end; spicule 30; gubernaculum 8.5; tail 11.

Male (n = 25): Measurements are listed in Table 6.

Body cylindrical, vermiform, length variable with both long and short forms, tapering anteriorly; bluntly rounded to clavate posteriorly. Head slightly set off, rounded to slightly truncate (Fig. 2G,H) without annulations. In SEM (face view) labial disc not raised, continuous with medial lips (Fig. 5A,B,D); medial lips extending some distance into head region (Fig. 5B); lateral lips absent; prestoma hexagonal, surrounded by six inner labial sensilla; stomatal opening slit-like, located in large hexagonal prestoma (Fig. 5D), and amphidial openings appear as long slits. Body cuticle with transverse annulation. Midbody width average 29. Lateral field with four incisures, encircles tail (Fig. 2D), outer fields aerolated (Fig. 2C,D). Stylet (Fig. 2A) robust; cone straight, pointed, knobs large, rounded, sloping posteriorly. Hemizonid prominent, about 2 annules long, located 1 annule anterior to excretory pore. Excretory pore variable in position, usually near middle of basal esophageal bulb, more posteriorly in some specimens. SEM examination of spicules confirmed nondentate tip of the spicules (Fig. 5E). Spicules arcuate, tips

TABLE 4. Differential host test using *M. floridensis* and other *Meloidogyne* species evaluated toward *Prunus* root-knot nematode (RKN) resistance sources. The evaluated plant material corresponds to the above-mentioned RKN source or has this source in its parentage.

Control material (Garfi) and RKN resistance sources	Garfi almond	Shalil peach	Alnem almond	Nemaguard peach	Myrobalan plum selections
	Plant material evaluated				
	"Garfi"	Almond × peach "GF.557"	"Alnem 1" "Alnem 88"	"Nemaguard" "Nemared", almond-peach "Garfi × Nemared"	"P.2175", "P.1079", "P.2980" (*Ma* gene)
M. arenaria	+	–	–	–	–
M. floridensis	+	+	+	+	–
M. incognita	+	–	+	–	–
M. javanica	+	+	–	+/–	–

(+) = multiplication of the RKN isolate; (–) no multiplication.

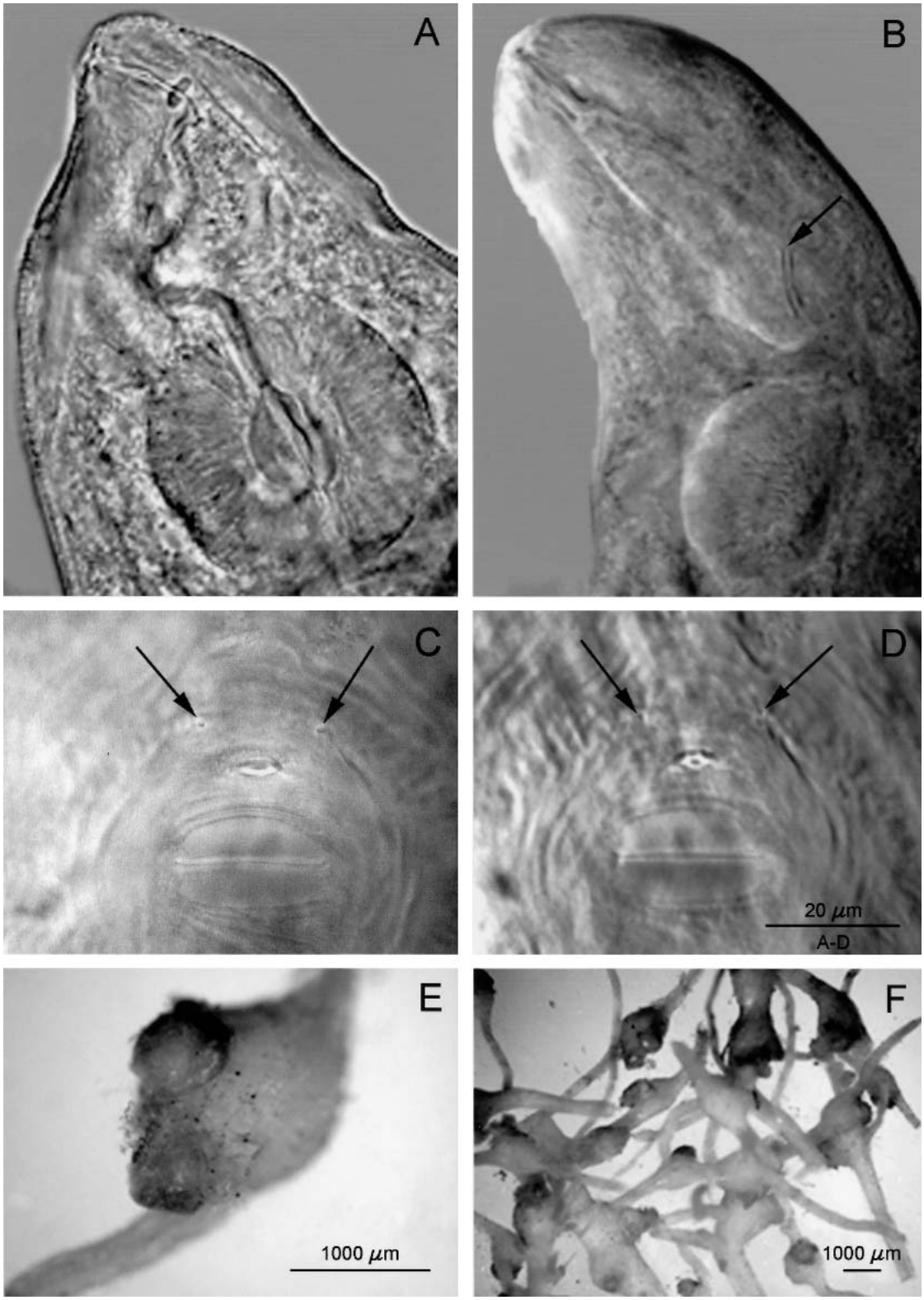

FIG. 1. *Meloidogyne floridensis* n. sp. Photomicrographs of females. A,B) Anterior end showing stylet with broad knobs, median esophageal bulb, and excretory pore (arrow). C,D) Female perineal patterns showing large phasmids (arrows). E,F) Females within the gall on peach roots with egg masses attached.

rounded (Figs. 2B,C;5D); gubernaculum distinct, short, simple (Fig. 2B). Tail short, rounded to conoid or clavate (Figs. 2B-D;6E).

Second-stage juveniles (*n = 25*): Measurements are listed in Table 7.

Body small, vermiform, tapering at both extremities

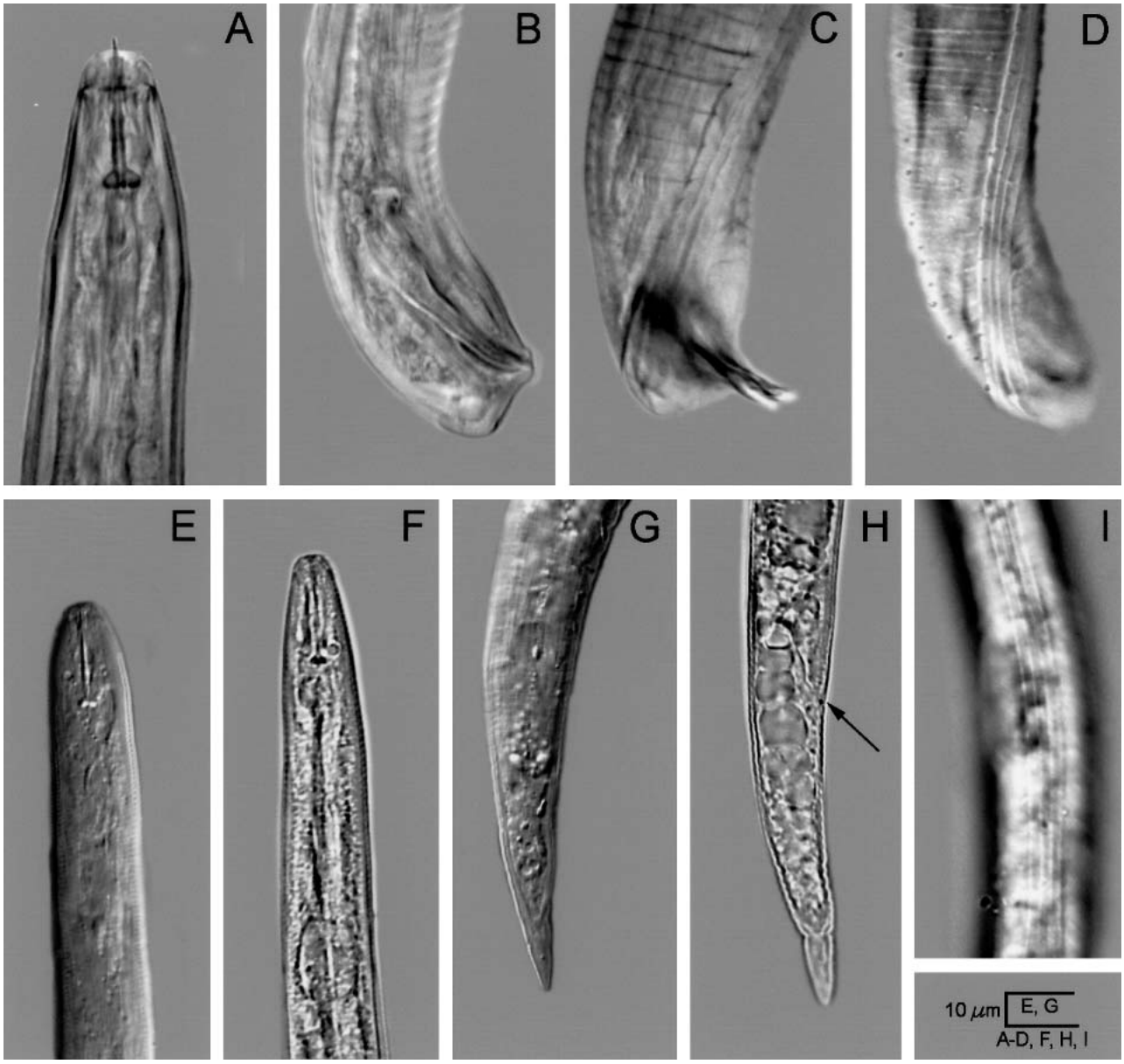

FIG. 2. *Meloidogyne floridensis* n. sp. Photomicrographs of males (A–D) and second-stage juveniles (E–I). A) Anterior region showing stylet. B–D) Posterior regions showing spicules and lateral field, respectively. E,F) Anterior region showing stylet. G,H) Posterior regions (tail) anus and inflated rectum (arrows). I) Lateral field.

but more so posteriorly (Figs. 2E-H;6A-C). Head truncate, slightly offset with labial disc; cephalic framework weak. SEM observations confirmed the absence of striations on the head and on the large post-labial annule (Fig. 6A). In SEM (Fig. 6D), stoma slit-like, located in round-shaped prestoma, surrounded by six pore-like openings of inner labial sensilla; medial lips and labial disc dumbbell shaped in face view; labial disc slightly rounded, raised above crescentic medial lips; lateral lips large and triangular (Fig. 6D), lower than labial disc and medial lips; amphidial openings appear as long slits located between labial disc and lateral lips. Stylet delicate, with small rounded knobs (Fig. 2E,F). Cuticular annulations fine, distinct. Lateral field prominent, with four incisures; some areolation, especially in anterior and posterior portion (Figs. 2I;6B,C,E). Excretory pore usually near middle of basal esophageal bulb. Hemizonid prominent, about 2 annules long, 1 to 2 annules anterior to excretory pore. Phasmids indistinct. Rectum inflated. Tail short, tapering to a bluntly rounded terminus. (Figs. 2G,H; 6B,C).

Egg (n = 25): Length 80–95 (86.5; SD 4.4); width 40–50 (44; SD 2.6); L/W ratio 1.7–2.2 (1.9; SD 0.1); egg shell hyaline without markings.

Type host and locality

Parasitic on peach roots (*Prunus persica* (L.) Batsch) at University of Florida, Experiment Station farm, in Gainesville Florida.

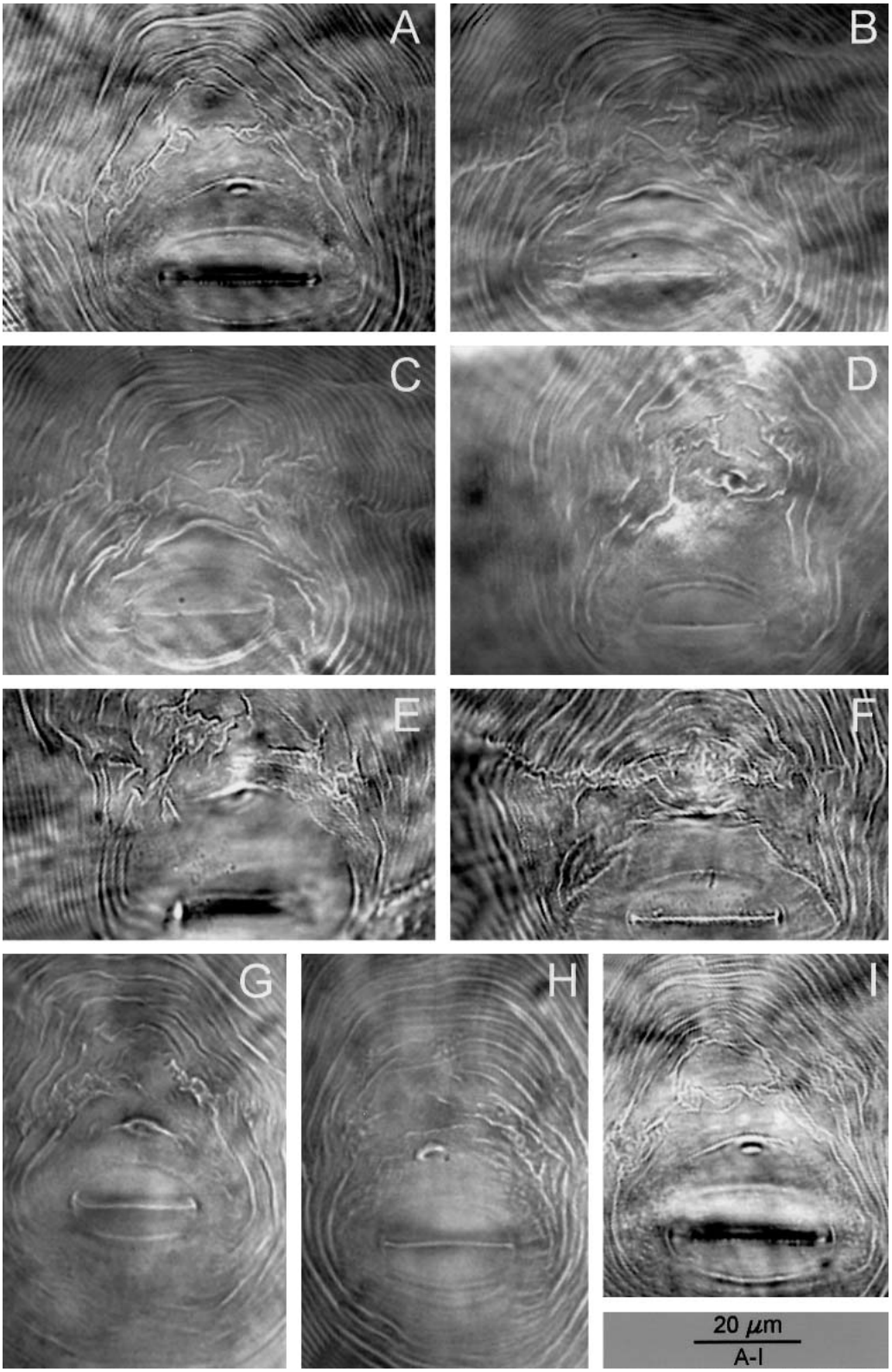

FIG. 3. *Meloidogyne floridensis* n. sp. A–I) Photomicrographs of nine female perineal patterns.

Type specimens

Holotype (female): Isolated from roots from the type host and locality. Slide T-558t, deposited in the U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland.

Allotype (male): Slide T-559t, Same data and repository as holotype.

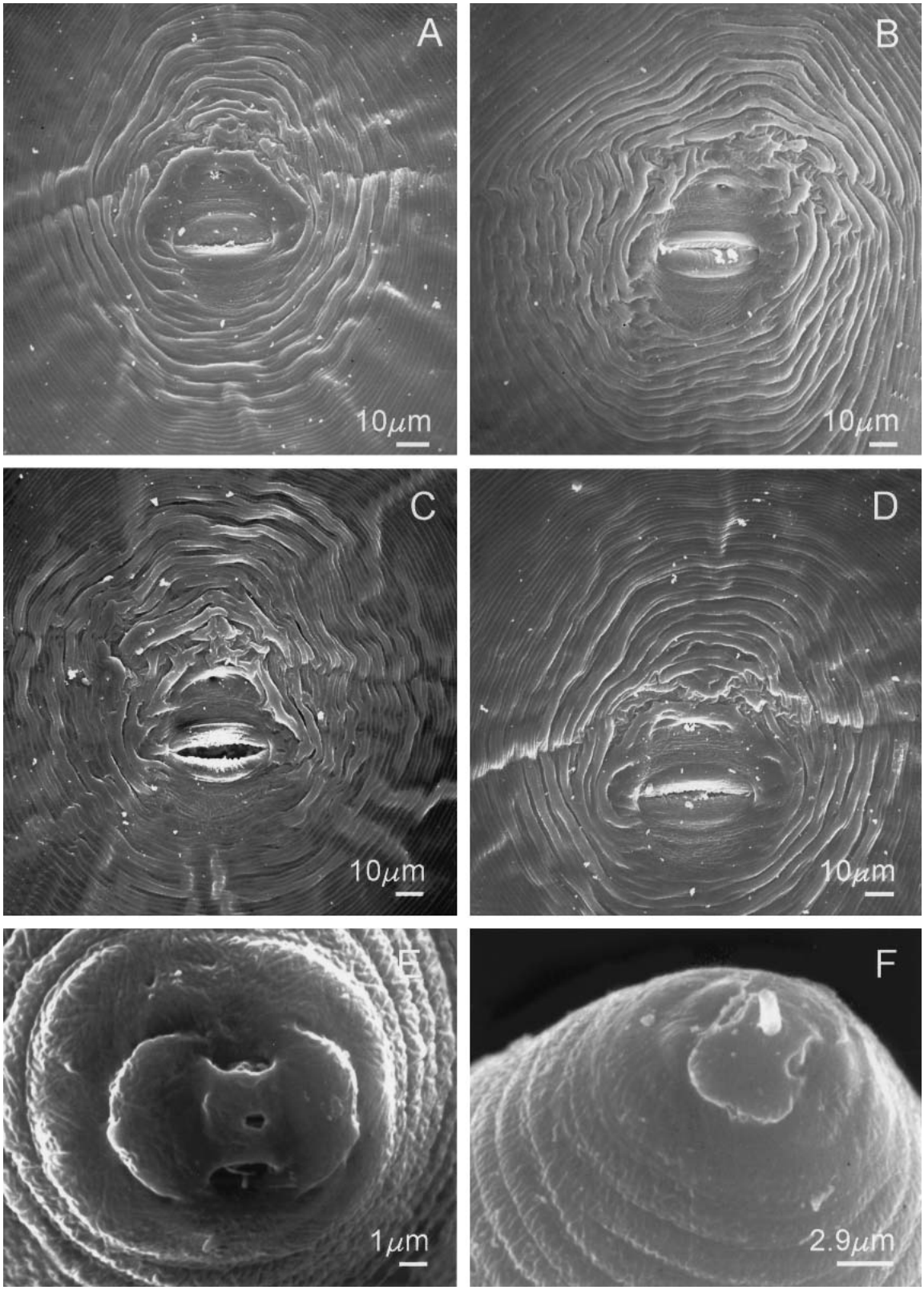

FIG. 4. *Meloidogyne floridensis* n. sp. scanning electron micrographs, females. A–D) Four perineal patterns. E,F) Face and lateral view, respectively.

Paratypes (females, males, and J2): Same data and repository as holotype. Slides T-5065p-T5096p: T-5065p-T-5083p (females), T-5084p-T-5092p (males), T-5093p-T-5094p (J2), T-5095p-T-5096p (egg masses). Additional paratypes deposited in University of California-Riverside Nematode Collection, Riverside, California;

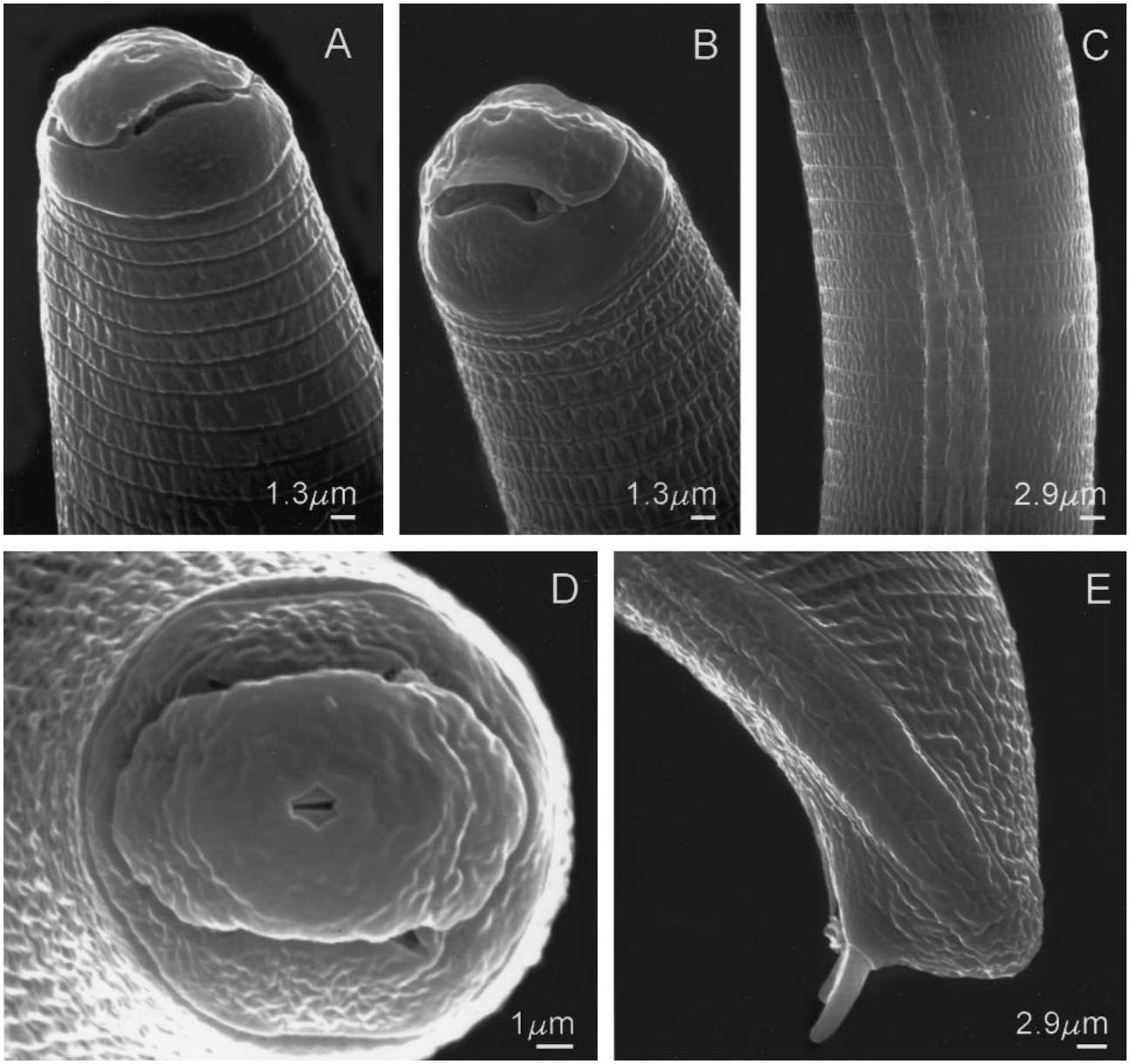

FIG. 5. *Meloidogyne floridensis* n. sp. SEM micrographs of males. A,B) Lateral view of head region. C) Lateral field. D) Enface view. E) Posterior body region (tail) showing spicules.

the Nematode Collection of the Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England; 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 Landbouwhogeschool, Wageningen, The Netherlands; Commonwealth Institute of Parasitology Collection, St. Albans, Herts., England.

Diagnosis

Meloidogyne floridensis n. sp. is characterized in having J2 with body length of 355 (310–390 μm), truncate head without annulation, stylet length of 10.1 (10–11 μm) with small rounded knobs, lateral field with 4 incisures, tail 39.4 (35–42.5 μm) long, hyaline tail terminus 9.8 (8–12 μm); female perineal pattern with a high to narrowly rounded or ovoid arch, with coarse to broken network-like striae in and above anal area, faint lateral lines interrupting transverse striae and smooth wavy lines in the outer field; prevulval region, typically without striae; vulva and anus sunken, phasmids large and distinct with conspicuous phasmidial canal. Males with both short and long forms 1,162 (564 μm–1.7 mm), stylet length of 20 (17–23 μm) with rounded posteriorly sloping knobs, spicules 28 (23–35 μm) long, and gubernaculum length 7.7 (5–10 μm).

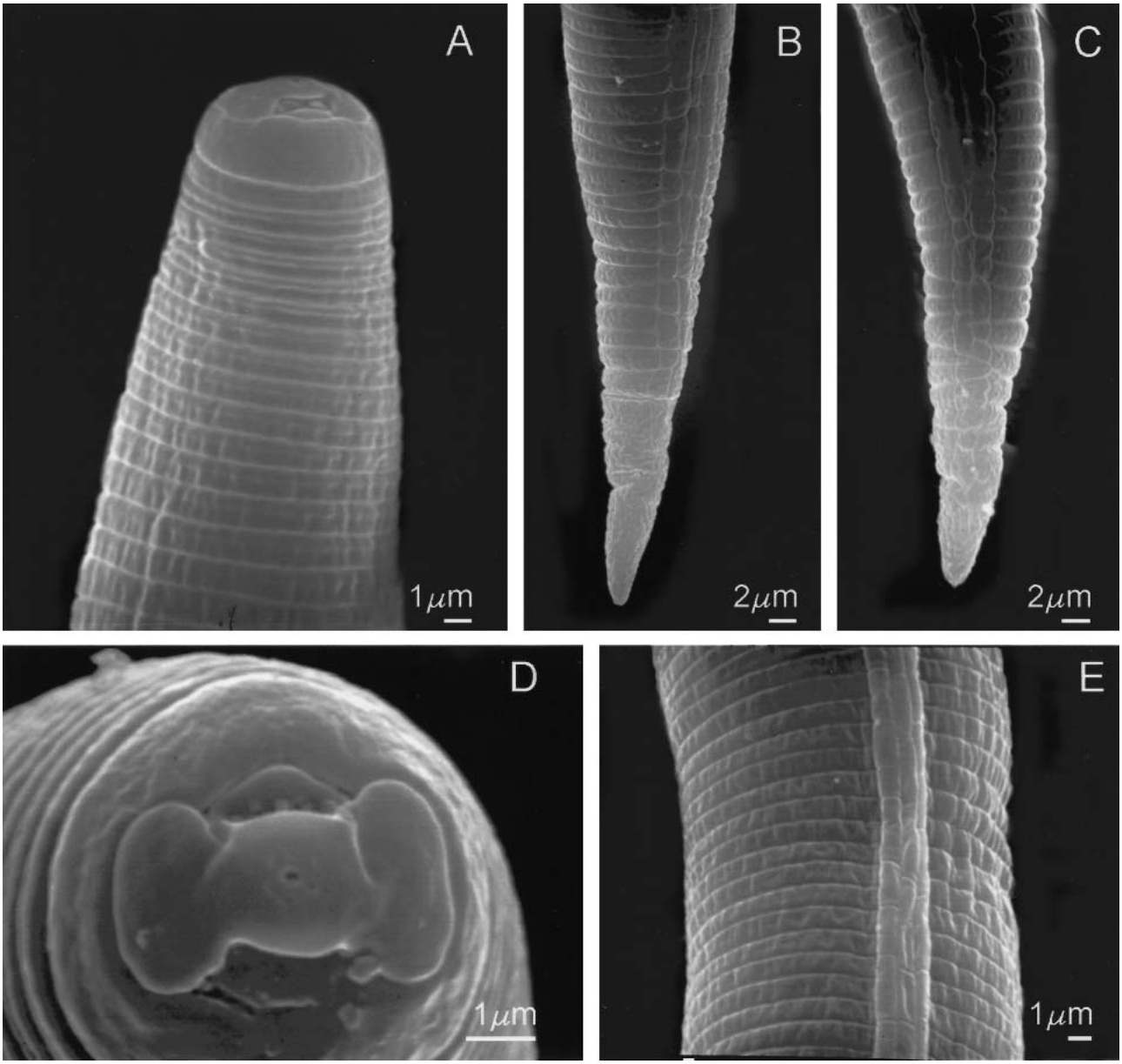

FIG. 6. *Meloidogyne floridensis* n. sp. SEM micrographs of second-stage juveniles. A) Anterior region (lateral view) showing smooth head. B,C) Posterior regions. D) En face view. E) Lateral field.

Relationships

Meloidogyne floridensis n. sp. is similar to *M. incognita* (Kofoid and White, 1919) Chitwood, 1949, *M. christiei* Golden and Kaplan, 1986, *M. graminicola* Golden and Birchfield, 1965, and *M. hispanica* Hirschmann, 1986. It differs from *M. incognita* J2 in the shape of head and tail (smooth head vs. two clear annules, shorter tail length 39.4 (35–42.5 μm) with a bluntly rounded terminus vs. longer tail 52 (42–62 μm) tapering steadily to subacute terminus); in the female EP/ST ratio 2.4 (1.6–3.7) vs. 1.4; nature of female perineal pattern (high narrowly rounded or ovoid arch with coarse to broken network-like striae in and above anal area, sunken vulva and anus and large and distinct phasmids with conspicuous phasmidial canal vs. distinct high dorsal arch with smooth to wavy striae, no sunken vulva and anus, and small, indistinct phasmids and phasmidial canals difficult to observe); in having shorter males with their stylet and spicules relatively shorter. In the host-range test, *M. floridensis* n. sp. reproduced abundantly on Nemaguard and Guardian peach vs. *M. incognita* being resistant to Nemaguard rootstock. The new species differs from *M. christiei* (Golden and Kaplan, 1986) in that *M. floridensis* n. sp. has J2 with shorter bodies, body length 355 (310–390 μm) vs. 427 (374–468 μm), in the nature of female perineal pattern (high narrowly rounded or ovoid arch with coarse to broken network-like striae vs. high, squarish arch and coarse broken striae that tend to diverge at various angles in and above anal area),

TABLE 5. Measurements of 25 females of *Meloidogyne floridensis* n. sp.

Character	Range	Mean	Standard deviation
Linear (µm)			
Body length with neck	525–890	697	96.8
Body width	356–648	491	87
Neck length	85–223	146	34.8
Neck width	50–173	86	27
Cuticle thickness at neck	1.5–6	2.8	0.8
Cuticle thickness at midbody	3–12	6.7	2.1
Stylet length	13–16	14.7	0.7
Stylet knob width	4–5.5	5	0.3
Stylet knob height	2–3	2.5	0.2
DGO from base of stylet	3.5–6	4.6	0.9
Excretory pore from anterior end	17.5–50	35	11.3
Body length from anterior end to posterior end of metacorpus	82–110	96.3	7
Number of annules from anterior end to excretory pore	14–32	22	6
Vulval slit length	21–30	26	3
Vulval slit to anus distance	15–25	19	2
Ratios			
a	1.2–1.7	1.4	0.1
EP/ST	1.6–3.7	2.4	0.8

TABLE 7. Measurements of 25 second-stage juveniles of *Meloidogyne floridensis* n. sp.

Character	Range	Mean	Standard deviation
Linear (µm)			
Body length	310–390	355	17.7
Body width	12–13.5	12.8	0.4
Stylet length	10–11	10.1	0.3
DGO from base of stylet	2.5–3	2.6	0.2
Center of median bulb from anterior end	46–55	51.4	2.3
Excretory pore from anterior end	65–83	71.4	4.9
Length from base of esophageal gland lobe to anterior end	90–160	126	21
Tail length	35–42.5	39.4	2.3
Hyaline tail terminus length	8–12	9.7	1
Ratios			
a	25–32	28	1.7
b	2.2–3.9	2.8	0.5
c	8–10.5	9	0.6
Head width/head height	1.4–2	1.9	0.2
Caudal ratio A	1.8–2.8	2.4	0.3
Caudal ratio B	2.3–4.8	3.3	0.6

female labial disc rounded, without any projections vs. labial disc indented, forming four projections, and eggs deposited in gelatinous matrix vs. inside the galls in a tubular coiled manner. *Meloidogyne floridensis* n. sp. differs from *M. graminicola* primarily in having females with a longer stylet length, 14.7 (13–16 µm) vs. 11.1 (10.6–11.2 µm), J2 with a shorter body length and tail, body length 355 (310–390 µm) vs. 441 (415–484 µm), tail 39.4 (35–43 µm) tapering to a bluntly rounded terminus vs. 70.9 (67–76 µm) tapering to a rounded to slightly clavate terminus; and males are shorter with 4 lines in lateral field vs. longer males and lateral field with 4 to 6 lines in *M. graminicola*. The new species differs from *M. hispanica* J2 in having a relatively shorter body and tail length and in the shape of tail and tail terminus, nature of female perineal pattern, in the absence of small knots in the esophageal lumen lining anterior to DGO and metacorpus valve of females and in having shorter males.

TABLE 6. Measurements of 25 males of *Meloidogyne floridensis* n. sp.

Character	Range	Mean	Standard deviation
Linear (µm)			
Body length	564–1,742	1,162	313.4
Body width	17–40	28.8	6.6
Stylet length	17–23	20.2	1.9
Stylet knob width	5–6	5.1	0.3
Stylet knob height	2.5–3.5	2.7	0.2
DGO from base of stylet	2.5–3.5	3.0	0.5
Excretory pore from anterior end	90–180	134	28.8
Center of median bulb from anterior end	63–112	88	16
Spicule length	23–35	27.8	3.4
Gubernaculum length	5–10	7.7	1.2
Tail length	6–12	9.4	1.8
Ratios			
a	31.7–56	40.5	7.9
b	4.5–8.6	6.4	1.2
c	81–217	124	35.8

Differential Host Studies

Results of the North Carolina differential host test (Table 3) showed that only watermelon (*Citrullus lanatus*) and tomato (*Lycopersicon esculentum*) were good hosts for *M. floridensis* n. sp., whereas tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*), and peanut (*Arachis hypogaea*) were non-hosts. Cotton (*Gossypium hirsutum*) was slightly infected and therefore rated as a poor host (Table 4). *Meloidogyne floridensis* n. sp. had a different host range than the two *M. incognita* populations tested (i.e., GA-peach and SC-bean isolates) and did not key out as an *M. incognita* race 3 as previously reported (Sherman and Lyrene, 1983).

Results of the *Prunus* host-range test confirmed previous reports by Sharpe et al. (1969), Sherman et al. (1981), and Sherman and Lyrene (1983): *M. floridensis* n. sp. reproduces on root-knot nematode resistant rootstocks Nemaguard and Nemared peach. Additional studies (Nyczepir and Beckman, 2000) showed that the newly released Guardian peach rootstock is also susceptible to *M. floridensis* n. sp. This is not surprising because Guardian has Nemaguard peach in its pedigree. Our data show that this species also overcomes the resistance of the Shalil peach and that of the almond

source Alnem 1 (Kochba and Spiegel-Roy, 1975, 1976) (Table 4). Thus none of the *Amygdalus* subgenus (grouping peach and almond) group would provide suitable source of resistance against this root-knot nematode. By contrast, *M. floridensis* n. sp. does not reproduce on the resistant Myrobalan plum material (subgenus *Prunophora,* grouping plums and apricot; carrying the *Ma* gene (Lecouls et al., 1997; Esmenjaud et al., 1997; Rubio-Cabetas et al., 1998). Consequently, this nematode has a major scientific interest because it can be used in *Prunus* accessions to discriminate the root-knot nematode genes from the *Amygdalus* subgenus and the *Ma* gene from the *Prunophora* subgenus.

Cytological Characterization

Oogenesis in the gonads of the *M. floridensis* n. sp. was characterized by the following sequential stages in caudal direction: the apical epithelial cell, oogonia in mitotic division, oogonia in interphase, and primary oocytes in synapsis. During prophase of the oocytes, pachynema was clearly visible as a short stage that moved quickly to coiling during diakinesis. At this stage, chromosomes could be counted: approximately 40 chromosomes were present, and in a limited number of oocytes the exact number of 36 could be fixed for 18 that were present bivalents, showing fine protein strands between the chromosomes. The oocytes developed to metaphase after having passed the spermatheca, leading by anaphase to telophase. At this stage, two sets of chromosomes were recognized. One formed the first polar body, which moved toward the egg wall and remained compact but could sometimes become interphase-like. The other set increased in size and became diffuse, sometimes interphase-like. The oocytes remained in this constellation until oviposition. The abundant reproduction of *M. floridensis* n. sp. on cv. Motelle indicates the absence of effective resistance of the *Mi-1* gene to this new root-knot species. This resistance, however, does not distinguish *M. floridensis* n. sp. from *M. incognita,* as resistance-breaking populations of *M. incognita* have been reported by several authors. It would be interesting to investigate interaction of the *M. floridensis* n. sp. with recently described resistance genes in tomato.

Cytologically, bivalent formation during meiosis of *M. floridensis* n. sp. provides evidence for a meiotic parthenogenetic pathway. The haploid chromosome number is $n = 18$ and possibly sometimes 19 or 20. In this respect, *M. floridensis* n. sp. is clearly distinct from *M. incognita,* the latter being an ameiotic parthenogenetically reproducing species. The oogenesis of *M. floridensis* n. sp., however, deviates from the meiotic parthenogenesis described by Triantaphyllou (1966) and Van der Beek et al. (1998) by the absence of a second maturation division in the female body. Activity in eggs, including cleavage divisions after oviposition, was not investigated in this study. Meiotic parthenogenesis in *Meloidogyne* spp. with one maturation division has been reported before by Van der Beek (1997) in the undescribed *Meloidogyne* species Xa with a unique isozyme phenotype different from that of *M. floridensis* n. sp.

The question of how the chromosome number is maintained under such meiotic behavior is not fully answered. The diffuse appearance of one set of chromosomes after the first maturation division points to chromosome duplication. Possibly a mechanism of endo-reduplication of first division products may occur to avoid reduction. This meiotic behavior would allow embryo development from the mature egg without fusion of nuclei. The occurrence of meiotic parthenogenesis and suppression of the second maturation division could point toward an intermediate type of parthenogenesis, in between the meiotic form with two maturation divisions and mitotic parthenogenesis.

Intergenic spacer rDNA: The IGS rDNA sequence (Fig. 7) of the new species was most similar to *M. arenaria, M. incognita,* and *M. javanica. Meloidogyne arenaria* and *M. incognita* had 8 nucleotide changes at 5 and 7 positions, respectively, representing 1.1% difference from the new species. *Meloidogyne javanica* had 9 nucleotide changes at 5 positions, representing 1.3% difference from the new species. However, *M. mayaguensis* had 162 changes at 85 positions, representing a 39% difference.

There was initial concern that this new species might be a variant of *M. mayaguensis,* a species recently discovered in the United States for the first time in Florida (Brito, pers. comm.). However, molecular and morphological evidence indicate that the peach isolate is not *M. mayaguensis.*

RAPD analyses: The 11 *Meloidogyne* isolates tested were separated based on amplification product patterns from 187 combinations of primer-DNA templates. Under the reaction conditions described, the number of amplified fragments per primer varied from 23 to 39, and their sizes ranged from 200 to 4,000 bp. Overall, 511 reproducible fragments were amplified and scored as RAPD markers. For these isolates, the RAPD profiles analyzed showed abundant polymorphisms (Fig. 8).

Using RAPD patterns alone or in combination, all the root-knot nematode isolates studied could be unambiguously identified, and visual analysis indicated that *M. floridensis* n. sp. was clearly different from all the others. From the phylogenetic analysis (Fig. 9), two groups were individualized: *M. arenaria, M. incognita,* and *M. javanica* on the one hand, and *M. hapla, M. chitwoodi,* and *M. fallax* on the other. Overall, the fact that the three latter species were more similar to one another than they were to the three obligatory mitotic parthenogenetic species (*M. arenaria, M. incognita,* and *M. javanica*) is consistent with previous RAPD studies conducted on root-knot nematodes from other origins (Baum et al., 1994; Blok et al., 1997; Castagnone-Sereno et al., 1994). This result illustrates the early

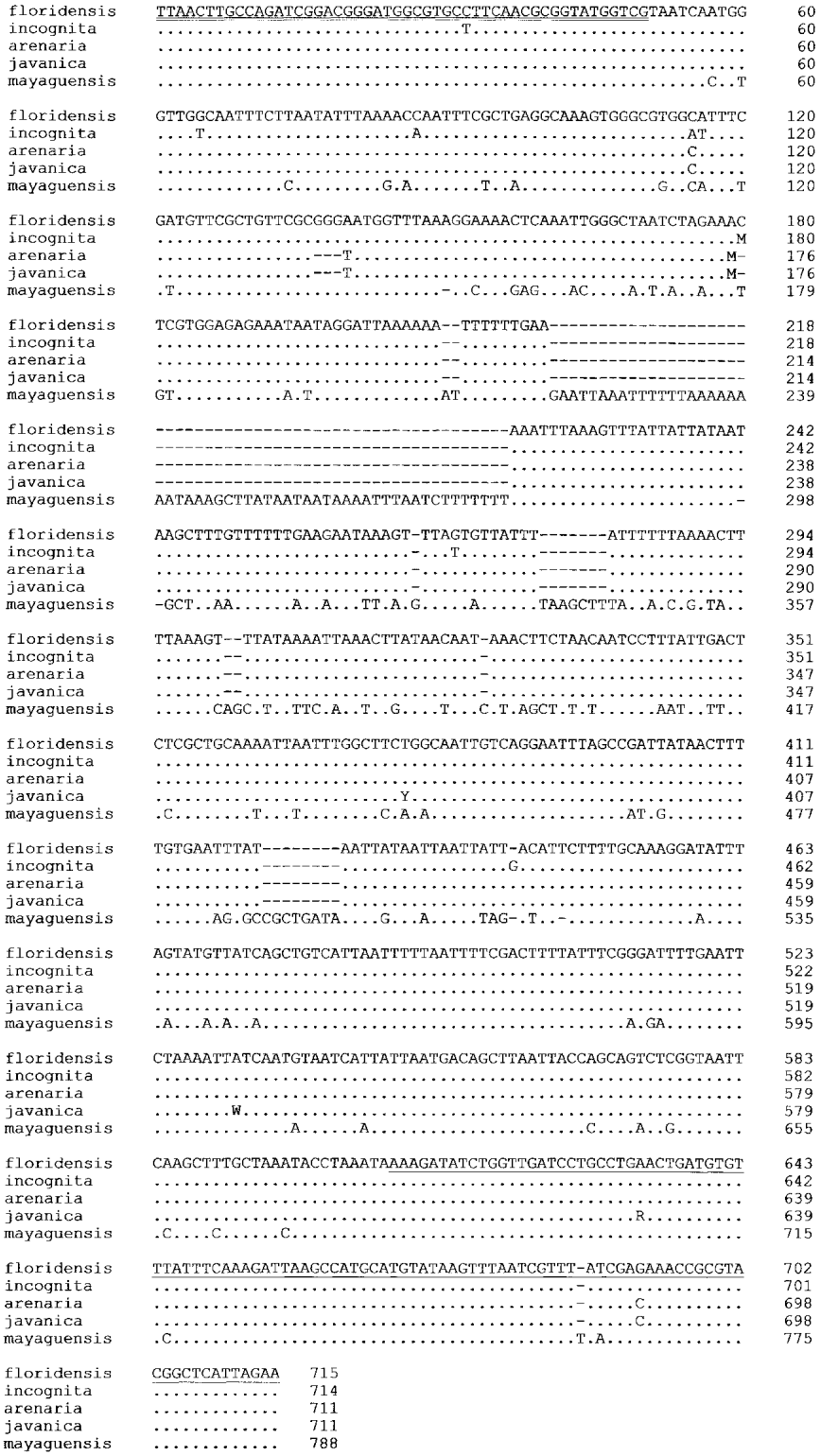

FIG. 7. Alignment of *Meloidogyne floridensis* n. sp. Intergenic Spacer (IGS) rDNA with sequences of *M. incognita, M. arenaria, M. javanica,* and *M. mayaguensis*. These sequences were not available as GenBank accessions but were obtained from Blok et al. (1997). Symbols: "." = identical nucleotide; "–" = absence of nucleotide insertion from a compared sequence. The double underline = the end of the 5S rDNA gene; a single underline = the beginning of the 18S rDNA gene.

separation of meiotic and mitotic species, and supports the hypothesis that amphimixis is the ancestral reproductive state of the genus (Triantaphyllou, 1985). The clustering of *M. floridensis* n. sp. with the meiotic species was clearly resolved, with very high bootstrap support. Therefore, it seems reasonable to hypothesize that this

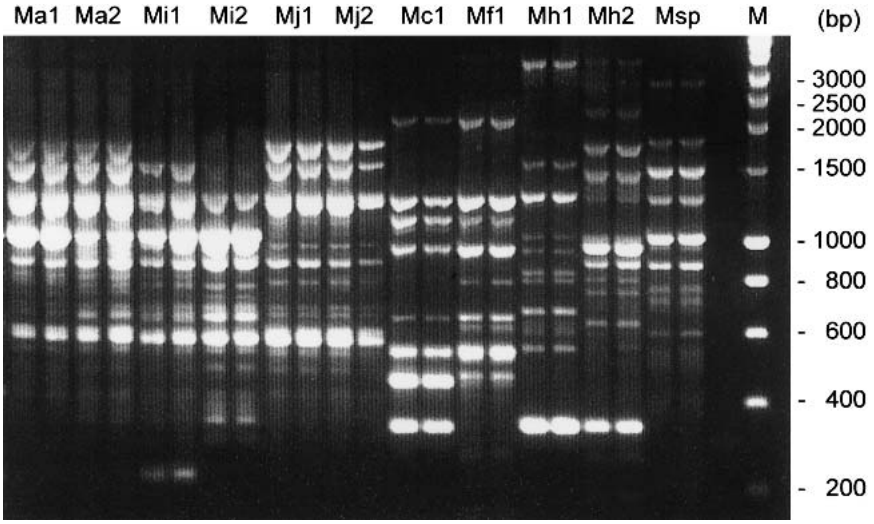

FIG. 8. Typical RAPD pattern obtained for *Meloidogyne* spp. using Primers from Table 2. Abbreviations for nematode isolates are defined in Table 1, experiment "R". PCR products were separated by electrophoresis in 1.4% agarose/TBE gels. Reproducible fragments were scored as RAPD markers. "M" represents molecular size marker.

species may reproduce by meiotic parthenogenesis and (or) amphimixis. However, further cytogenetic investigations are needed to confirm this point.

Electrophoretic analysis: Carneiro et al. (2000) have included this new species in a comparative study of enzyme phenotypes comprising major RKN species (*Meloidogyne* sp.) and have described its esterase (EST), malate dehydrogenase (MDH), superoxide dismutase (SOD), and glutamate-oxaloacetate transaminase (GOT) phenotypes. The EST phenotype of *M. floridensis* n. sp. is quite different from the other root-knot nematode species tested (Fig. 10). This atypical and unique pattern is characterized by the presence of three bands, where the central band is located at the same position as the upper band for *M. javanica*. Other enzymes exhibit banding patterns that correspond to phenotypes already reported. In particular, the MDH phenotype for *M. floridensis* n. sp. is identical to *M. javanica* and *M. incognita* (N1 type according to Esbenshade and Triantaphyllou, 1990).

In summary, the root-knot nematode found on Nemaguard peach in Florida, herein referred to and described as *Meloidogyne floridensis* n. sp., is quite differ-

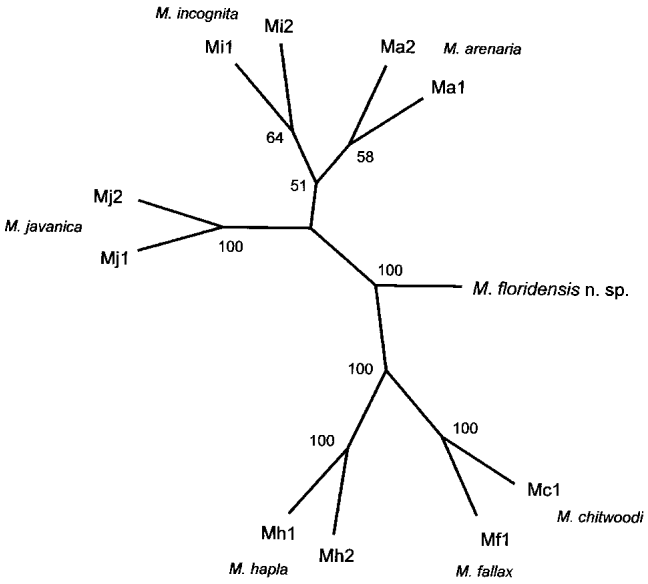

FIG. 9. Majority-rule consensus dendrogram using the maximum-parsimony algorithm bootstrapped with 1,000 replicates from a RAPD pattern 0–1 matrix for *Meloidogyne arenaria* (Ma1, 2), *M. incognita* (Mi1, 2), *M. javanica* (Mj1, 2), *M. hapla* (Mh1, 2), *M. fallax* (Mf1), *M. chitwoodi* (Mc1), and *M. floridensis* n. sp. as implemented in PAUP.

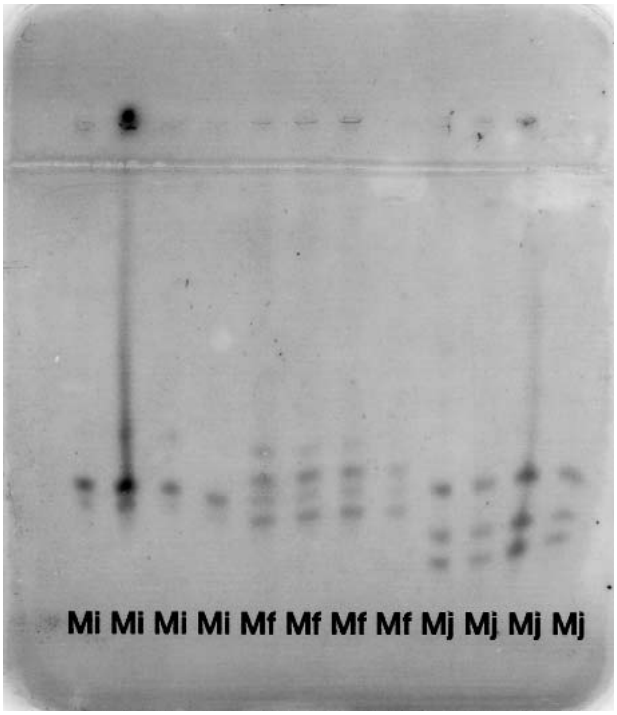

FIG. 10. Comparison of esterase dehydrogenase phenotypes of *Meloidogyne floridensis* n. sp. (Mf) with that of *M. incognita* (Mi) and *M. javanica* (Mj).

ent from the other *Meloidogyne* spp. known to date. The common name "peach root-knot nematode" is suggested.

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