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Morphological and Molecular Characterization of a New Root-Knot Nematode, *Meloidogyne thailandica* n. sp. (Nematoda: Meloidogynidae), Parasitizing Ginger (*Zingiber* sp.)

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Abstract: A root-knot nematode *Meloidogyne thailandica* n. sp. was discovered on roots of ginger (*Zingiber* spp.) intercepted from Thailand in October 2002 by the U.S. Department of Agriculture Animal and Plant Health Inspection Service at the port of San Francisco. Comparison by light microscopy (LM) and scanning electron microscopy (SEM) to five other morphologically related species (*M. incognita, M. arenaria, M. microcephala, M. megatyla,* and *M. enterolobii*) revealed that the new species differs from these by one or more of the following: body, tail and hyaline tail length, shape of head, tail and tail terminus of second-stage juveniles; stylet length and shape of spicules in males; perineal pattern, stylet length and shape of knobs in females. The distinctive perineal pattern is oval to rectangular, with smooth to moderately wavy and coarse striae, and with characteristic radial structures present underneath the pattern area; the dorsal arch is high, sometimes round to rectangular, and striae in and around the anal area form a thick network-like pattern interrupted by lateral lines and large phasmids. Second-stage juveniles have a long, slender tail and long, gradually tapering hyaline tail region ending in a rounded terminus. Male spicules commonly have an acutely angled shaft with a bidentate terminus. Molecular data from the ribosomal large subunit D3 expansion segment revealed four haplotypes, two of which were unique and distinguish *M. thailandica* n. sp. from *M. arenaria, M. incognita,* and *M. javanica.*

Key words: ginger, intergenic spacer (IGS), internal transcribed spacer (ITS1), large subunit (LSU), *Meloidogyne,* morphology, new species, ribosomal DNA, root-knot nematode, scanning electron microscopy, taxonomy, Thailand.

Root-knot nematodes (*Meloidogyne* spp.) are economically important plant pathogens, and more than 90 nominal species have been described. In October 2002, a root-knot nematode was discovered on roots of ginger (*Zingiber* spp.) from Thailand that was intercepted by the Animal and Plant Health Inspection Service (APHIS) at the port of San Francisco. The importer stated that the plants were bought at a Bangkok market supplied by local nursery growers and were a variegated variety of *Zingiber* sp. The infected *Zingiber* sp. roots were sent to the U.S. Department of Agriculture (USDA) Nematology Laboratory in Beltsville, Maryland, for identification. The roots exhibited galling typical of root-knot nematode. Heavily infected roots were dark brown to black, and from each infected root area we recovered clusters of one to four root-knot nematode females with attached egg masses. All life stages of this species (juveniles, males, and females) were heavily attacked by *Pasteuria* sp. spores (Fig. 1C,E).

The taxonomy of this genus has been advanced by numerous reviews (Allen, 1952; Chitwood, 1949; Eisenback, 1985a,b; Eisenback and Triantaphyllou, 1991; Eisenback et al., 1981; Esser et al., 1976; Golden, 1976; Jepson, 1987; Karssen, 2002; Karssen and Van Hoenselaar, 1998; Sasser, 1954; Taylor, 1987; Taylor and Sasser, 1978; Taylor et al., 1955; Triantaphyllou, 1971; Triantaphyllou and Sasser, 1960; Whitehead, 1968).

Whereas the most common species of root-knot nematode in Thailand appear to be *M. incognita, M. javanica, M. hapla,* and *M. graminicola* (Toida et al., 1996), *M. microcephala* (Cliff and Hirschmann, 1984), *M. exigua,* and *M. naasi* also have been detected (Sontirat, 1981). *Meloidogyne incognita* and *M. arenaria* also have been reported on ginger in Paraná, Brazil (Santos and Lozano, 1993). Root-knot nematodes parasitizing ginger also have been reported in the following countries: *M. incognita* from Australia (Stirling and Nikulin, 1998) and China (Guo et al., 2004); *M. incognita, M. arenaria* from Belize, Central America (Bridge et al., 1996); *M. javanica* in the Islands of Mauritius and Rodrigues, Southern Africa (Lamberti et al., 1987); and *M. incognita* from Fiji (Haynes et al., 1973). *Meloidogyne incognita* is associated with ginger yield decline in Himacal Pradesh and Kerala, India (Kaur et al., 1989; Mammen, 1973, respectively) as well as Fiji (Haynes et al., 1973) and other countries. Due to morphological similarity of *M. thailandica* n. sp. to these species, it may have been overlooked in these locations on ginger in the past.

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

Materials and Methods

Morphological Characterization

Second-stage juveniles (J2) and males were recovered from infected roots, of *Zingiber* sp. that had been sent to Beltsville, Maryland, and egg masses were kept in petri dishes with a small amount of water. Second-stage juveniles (J2) were extracted from soil by sieving and Baer-

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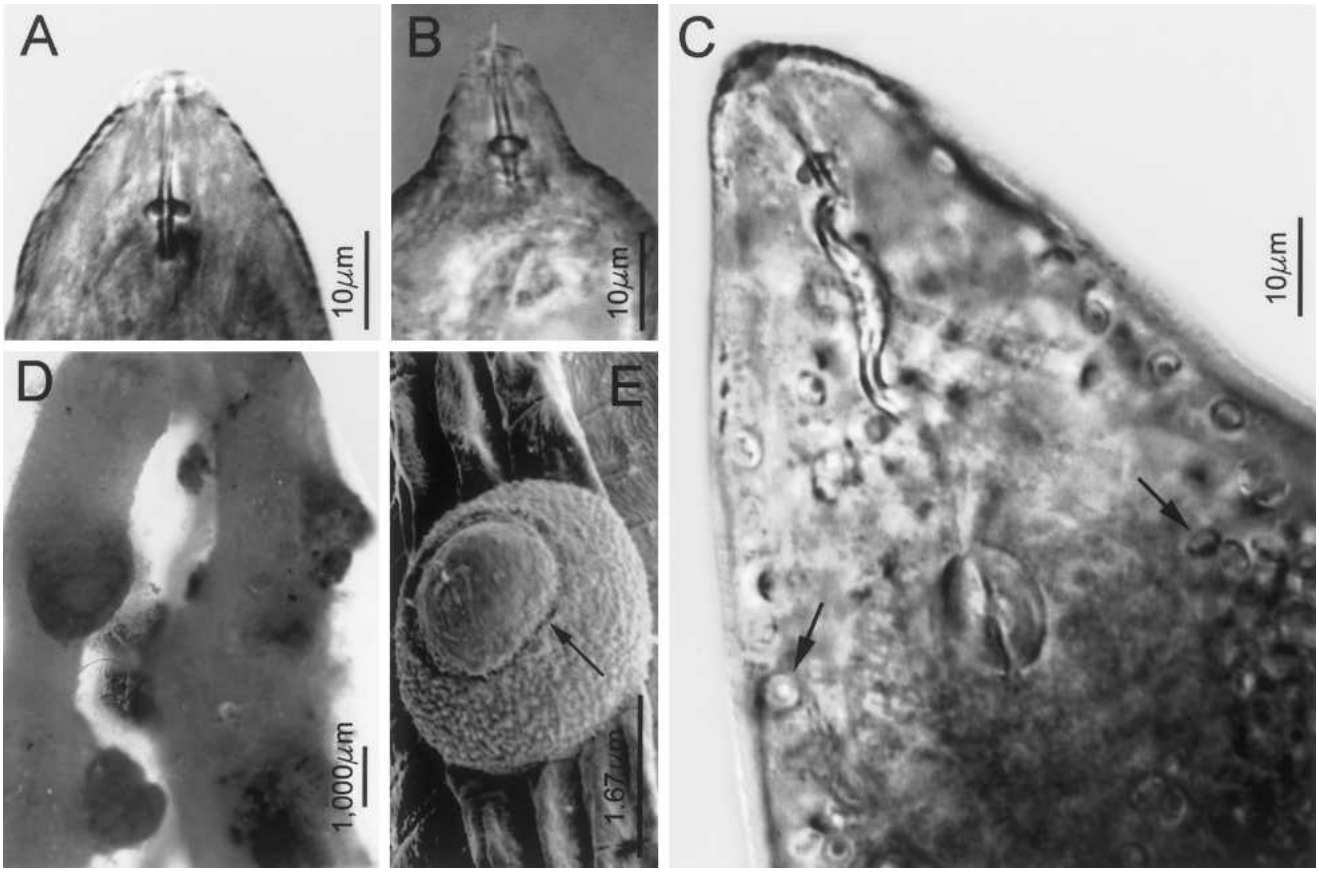

FIG. 1. *Meloidogyne thailandica* n. sp. Photomicrographs of females. A,B) Anterior ends showing stylet with backwardly slopping stylet knobs. C) Anterior end heavily attacked by *Pasteuria* sp. spores (arrows). D) Females within the galls on ginger roots. E) SEM micrograph of J2 lateral field showing *Pasteuria* sp. spore on surface (arrow).

mann funnel methods. After fixation overnight in 3% formaldehyde at room temperature, females and eggs were dissected from infected roots. Second-stage juveniles were fixed in 3% formaldehyde and processed 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. Roots and whole females were photographed using a dissecting microscope, and LM images of fixed nematodes and female perineal patterns were taken using a compound microscope. Photomicrographs of perineal patterns, J2, and males were made with a 35-mm camera attached to the compound microscope equipped with differential interference contrast (DIC) optics. Measurements of all stages were made with an ocular micrometer with measurements in micrometers, unless otherwise stated. For SEM, nematode specimens fixed in 3% formaldehyde were dehydrated in an ethanol series and critical point dried from carbon dioxide using a Samdri 780A Critical Point Drier (Tousimis Research Corp, Rockville, MD) (Wergin and Stone, 1981). Dried specimens were mounted on adhesive conductive tabs attached to Cambridge-style aluminum SEM stubs (Ted Pella Inc., Redding, CA). Stubs were transferred into a modified specimen carrier and moved to the specimen stage of an Oxford CT 1500 Cryostage operating at room temperature and sputter-coated with platinum. Coated samples were then moved to the specimen stage of an SEM and observed with an electron beam accelerating voltage of 2KV.

Molecular Characterization

DNA analysis: Nematode specimens were mechanically disrupted in groups of nine juveniles in 50 µl of extraction buffer as described by Thomas et al. (1997) and then stored in PCR tubes at −80 °C until needed. Extracts were prepared from thawed pools by incubating the tubes at 60 °C for 60 min, followed by 95 °C for 15 min to deactivate the proteinase K. Eleven microliters of the extract was used for each PCR reaction. Three different primer sets were employed for PCR. The ribosomal large subunit (LSU D3) expansion segment was amplified with W. K. Thomas-designed primers D3A 5′ -GACCCGTCTTGAAACACGGA-3′ and D3B 5′ TCGGAAGGAACCAGCTACTA-3′ using the amplification procedure of Al-Banna et al. (1997). The ribosomal intergenic spacer (IGS) was amplified with the primers 5SF 5′ -TTAACTTGCCAGATCGGACG-3′ and

18SR 5′ -TCTAAGAGCCGTACGC-3′ as previously described (Blok et al., 1997). The nuclear ribosomal internal transcribed spacer (ITS1) segment was amplified with the primers rDNA2 5′ -TTGATTACGTCCCTGCCCTTT-3′ (Vrain et al., 1992) and rDNA1.58S 5′ -ACGAGCCGAGTGATCCACCG-3′ as described previously (Cherry et al., 1997). PCR reactions contained Eppendorf MasterTaq (Brinkmann, Westbury, NY) and the buffer supplied by the manufacturer; all other components were added as described in the specific protocols for each gene. PCR products were visualized with UV illumination after ethidium bromide staining. DNA was excised from the gels and purified with the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Clean PCR products were then cloned into vector pCR2.1 from the TOPO TA cloning kit and transformed into *Escherichia coli* TOP10 cells according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Plasmid DNA was prepared with a Qiagen miniprep kit (Qiagen, Valencia, CA) and digested with EcoRI to confirm the presence of DNA insert. Double stranded DNA was sequenced with the CEQ DTCS Quick Start Kit and analyzed on the CEQ8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). Sequence was determined on both strands from all clones using M13 forward and M13 reverse primers. Four to six clones were sequenced for each gene amplified for this analysis. Sequences have been submitted to Genbank with the accession numbers AY858791 to AY858796.

DNA sequences were analyzed using BLASTN of nematode sequences contained in the EBI-EMBL parasite sequence database (http://www.ebi.ac.uk/blast2/parasites.html). Root-knot nematode sequences with highest e-values were subjected to ClustalW (Thompson et al., 1994) analysis with default parameters. The alignments were minimally gapped and required no further adjustment.

Systematics

Meloidogyne thailandica n. sp.
(Figs. 1–5)

Description (All measurements are in micrometers unless otherwise specified)

Holotype (female, in glycerine): Body length with neck 700; body width 405; neck length 301, neck greatest width 109; stylet length 13.5; stylet knob width 5; stylet knob height 2.5; dorsal esophageal gland orifice (DGO) from base of stylet 5.0; excretory pore from anterior end 38; Excretory pore/stylet length (EP/ST) ratio 2.8; body length from anterior end to posterior end of metacorpus 85, about 21 body annules from anterior end to excretory pore; cuticle thickness at neck 2.5; cuticle thickness at midbody 5.5; vulva slit length 35; distance from vulva slit to anus 20.

Female (n = 25): Measurements are listed in Table 1 and are in micrometers excluding ratios.

Body pearly white, variable in size, round to pear shaped with relatively distinct and variable-size neck, sometimes bent at various angles to body. Cephalic framework weak, hexaradiate, lateral sectors slightly enlarged, vestibule and extension prominent. Cephalids not observed. Head not offset, with labial disc; lip region with 2 annules, with first annule being slightly larger and expanded than the second. SEM observations revealed: Labial disc fused with medial lips, dumbbell shaped; lateral lips indistinct and fused with medial lips, amphidial openings elongate, located outside the indistinct fused lateral lips. Stylet strong, with rounded, posteriorly sloping knobs, cone and shaft usually straight. However, occasionally cone was slightly bent. Excretory pore distinct, generally located 1 to 3 stylet lengths posterior to stylet base. 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 oval to rectangular with smooth to moderately wavy and coarse striae and have characteristic radial structures present underneath the pattern area; the dorsal arch is high, sometimes round to rectangular, and striae in and around the anal area form a thick network-like pattern interrupted by lateral lines from each lateral side, and have a slightly sunken vulva and anus. Phasmids large and distinct.

Allotype (male in glycerine): Length = 1,380; a = 53.0; b = 7.2; c = 110; stylet length 18.0; stylet knob width 4.5; stylet knob height 3.0; excretory pore from anterior end 138; center of median bulb 77.5 from anterior end; spicule 31; gubernaculum 10.0; tail 10.

Male (n = 26): Measurements are listed in Table 2 and are in micrometers excluding ratios.

Body cylindrical, vermiform, length variable, with both long and short forms, tapering anteriorly; bluntly rounded posteriorly. Head continuous, rounded with three to four annules. In SEM (face view) labial disc is high and narrower than the head region, continuous with medial lips; medial lips extending some distance into head region; lateral lips absent; prestoma hexagonal; stomatal opening slit-like, located in large hexagonal prestoma and amphidial openings appear as long slits. Body cuticle with transverse annulation. Midbody width average 31.3. Lateral field with four aerolated incisures. Stylet robust; cone straight, pointed, knobs large, rounded. Hemizonid indistinct. Excretory pore variable in position, usually near anterior half of basal esophageal bulb, in some specimens more posterior. Median esophageal bulb large, oval shaped, measuring about 30–35 µm. Spicules arcuate, commonly with an acutely angled shaft with a bidentate tip; gubernaculum distinct, short, simple. Tail short, rounded to conoid.

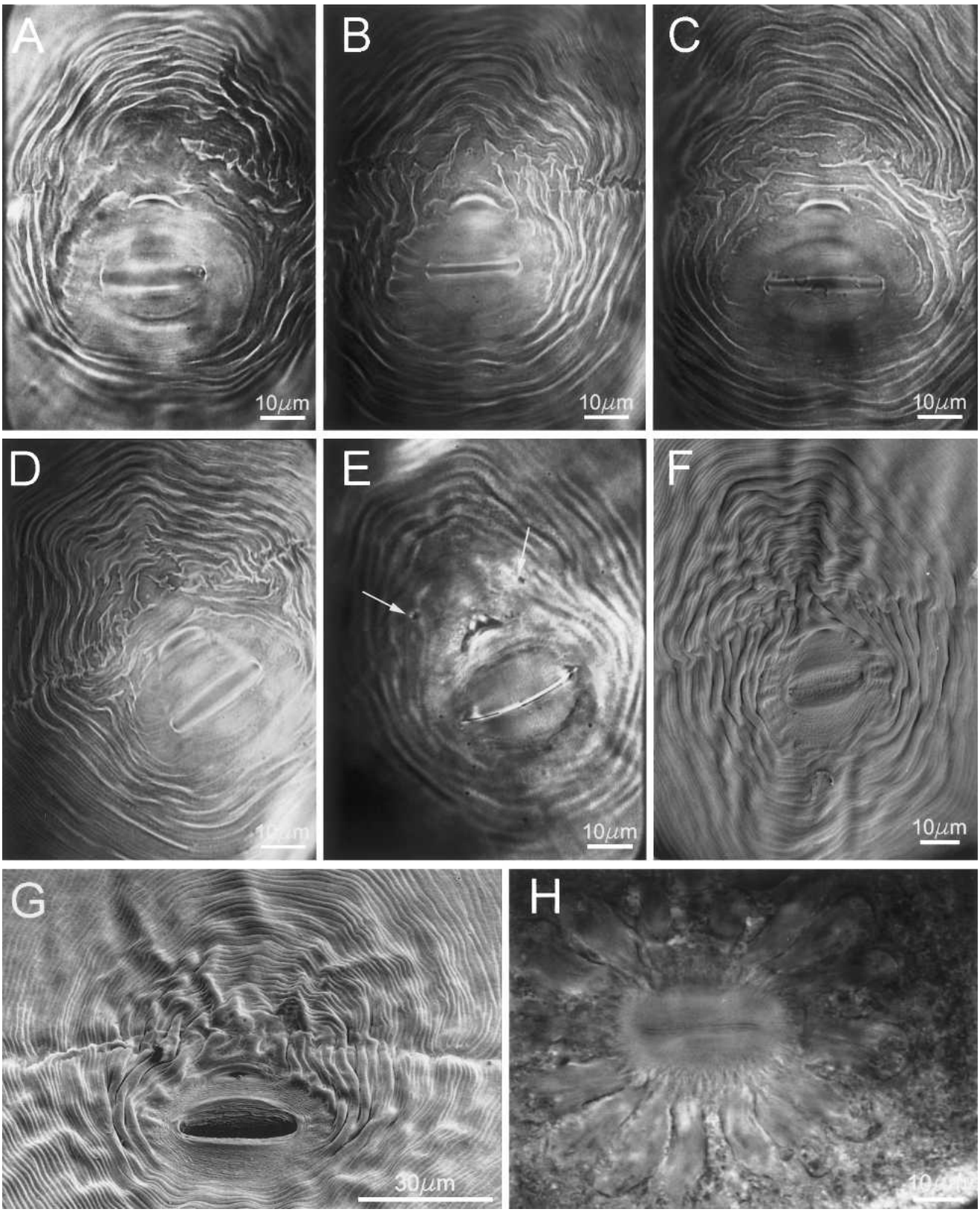

FIG. 2. *Meloidogyne thailandica* n. sp. A–G) Photomicrographs and SEM micrographs of seven female perineal patterns, respectively. H) Posterior region showing characteristic radial structures located immediately below perineal pattern.

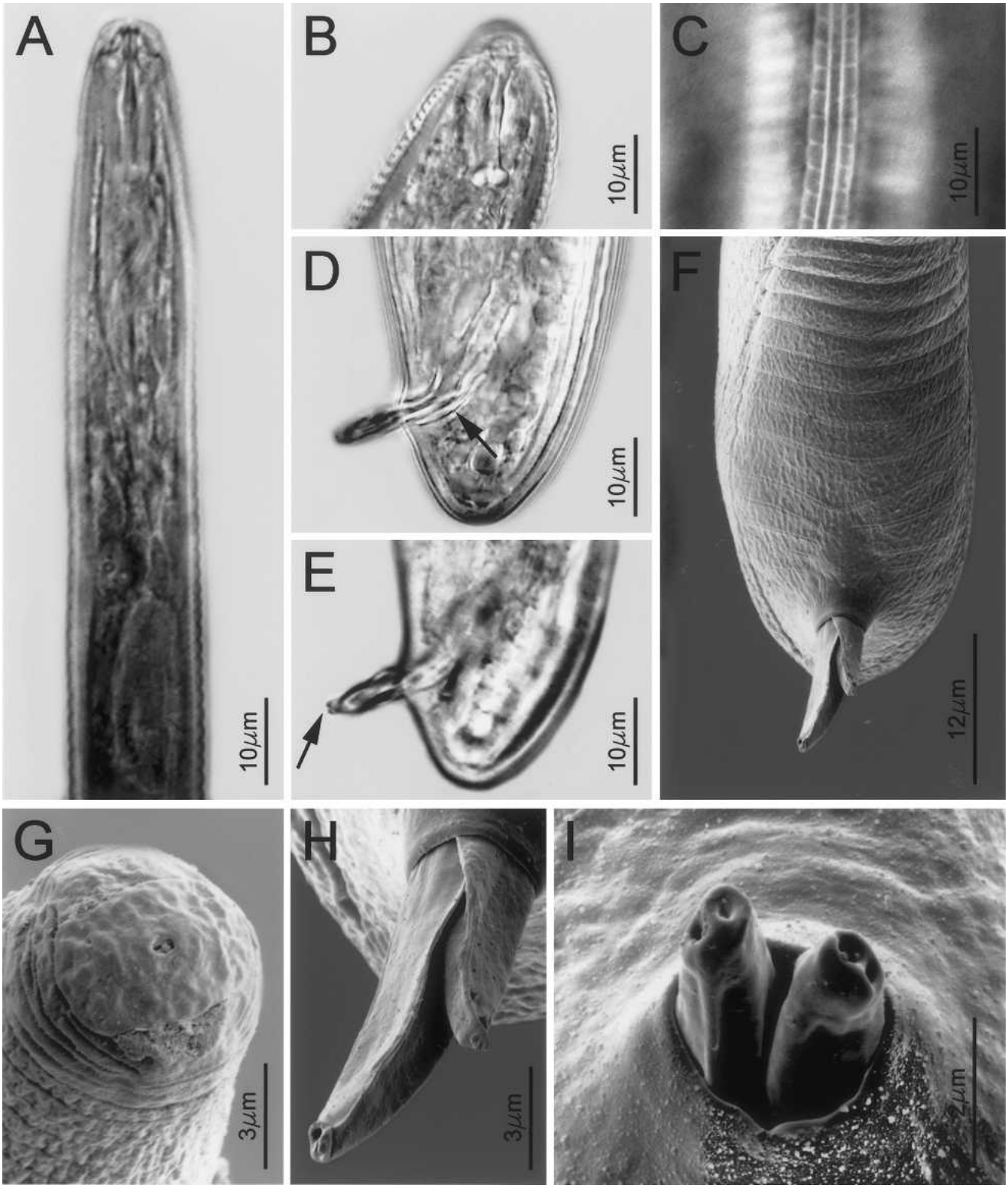

FIG. 3. *Meloidogyne thailandica* n. sp. Photomicrographs of males. A,B) Anterior regions showing outline of esophagus and stylet, respectively. C) Lateral field near mid-body showing areolation. D,E) Posterior regions (tail) showing gubernaculum (arrow) and bi-dentate spicules (arrow), respectively. F–I) SEM micrograph of males: F) Tail (lateral view) showing spicules. G) head region (face view). H, I) Cloacal region with spicules.

Second-stage juveniles (n = 25): Measurements are listed in Table 3 and are in micrometers excluding ratios.

Body small, vermiform, tapering at both extremities, but more so posteriorly. Head truncate, slightly offset with labial disc; cephalic framework weak. SEM observations confirmed two to three incomplete striations on the head and on the large post-labial annule. In SEM, stoma slit-like, located in round-shaped prestoma, sur-

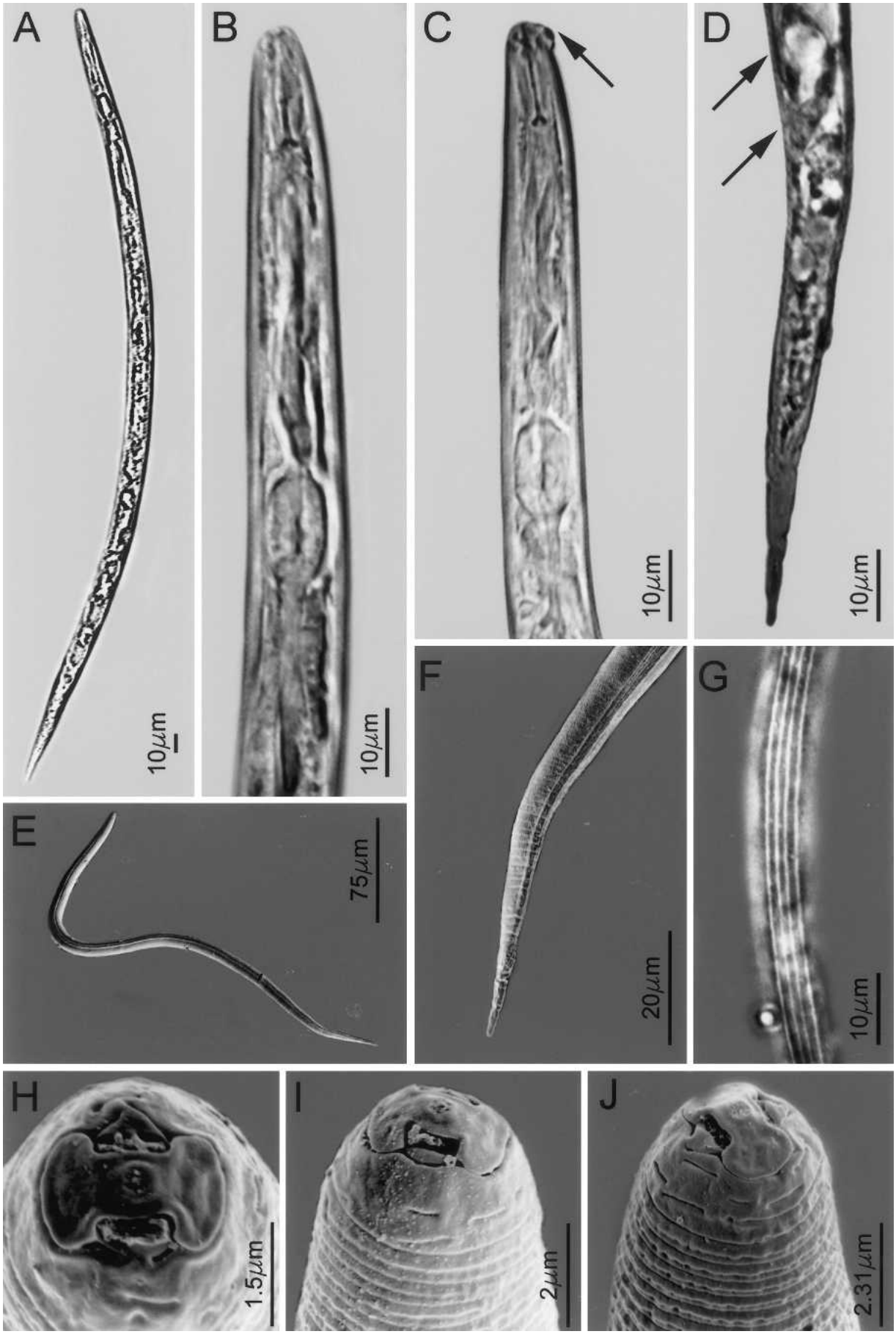

FIG. 4. *Meloidogyne thailandica* n. sp. Photomicrographs of J2. A) Whole specimen. B,C) Anterior regions. D) Posterior region (tail) anus and rectal glands (arrows). G) Lateral field at mid-body. E,F,H–J) SEM micrographs of J2: E) Whole specimen. F) Posterior region. H–J) Head regions (en face views).

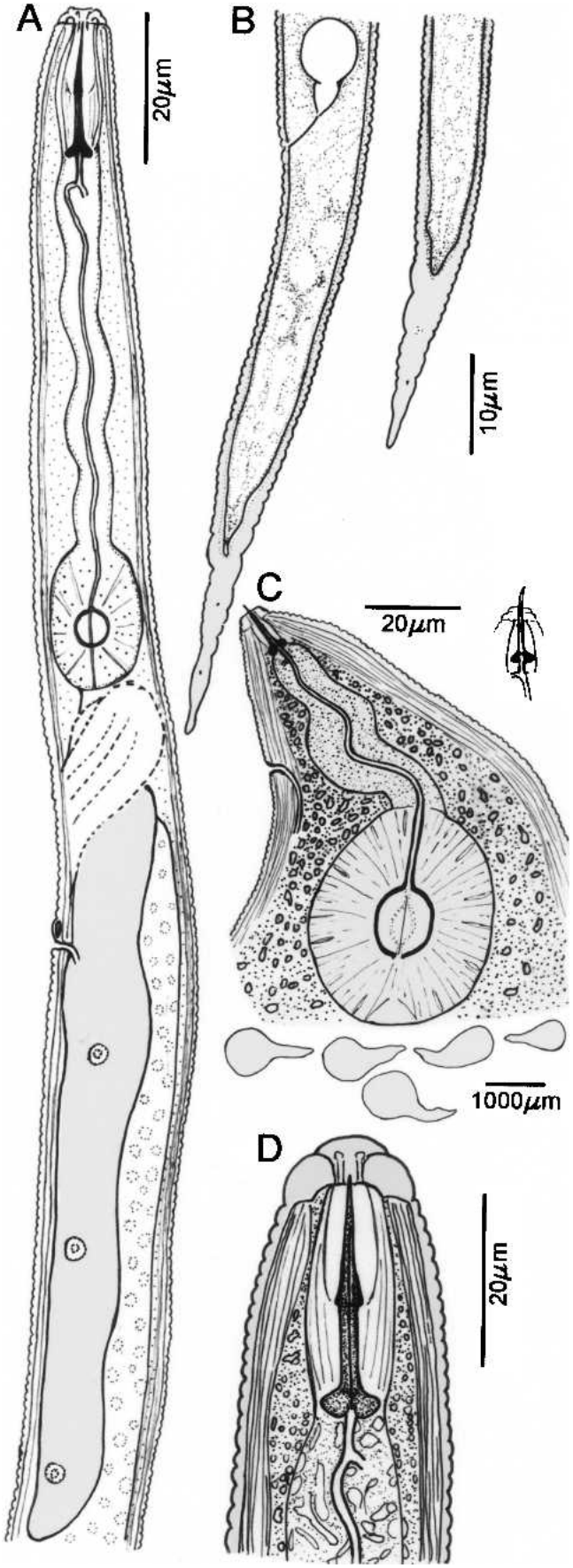

FIG. 5. *Meloidogyne thailandica* n. sp. Drawings (lateral views) of A) J2 esophagus. B) J2 tails with rectal gland. C) Female esophagus and body shapes. D) Male head region.

TABLE 1. Measurements of 25 females of *Meloidogyne thailandica* n. sp

Character	Range	Mean	Standard deviation
Linear (μm)			
Body length with neck	570–955	762	115.8
Body width	272–690	459	109.1
Neck length	118–301	232.8	60.0
Neck width	70–158	100.8	27.1
Cuticle thickness at neck	2.5–5.0	3.1	0.8
Cuticle thickness at midbody	5–15	7.9	2.7
Stylet length	12.0–15.5	13.8	1.2
Stylet knob width	4–5	4.9	0.3
Stylet knob height	1.5–3.5	2.4	0.5
DGO from base of stylet	3–5	4.0	0.9
Excretory pore from anterior end	15–47	30.6	8.4
Body length from anterior end to posterior end of metacorpus	75–125	90.6	12.5
Number of annules from anterior end to excretory pore	15–21	17	3
Vulval slit length	25.0–35.1	29.1	2.6
Vulval slit to anus distance	15–22	18.0	2.4
Ratios			
a	1.4–2.3	1.7	0.2
EP/ST	1.7–3.6	2.3	0.5

rounded by six pore-like openings of inner labial sensilla; medial lips and labial disc are bow-tie shaped; labial disc slightly rounded, raised above crescentic medial lips; lateral lips large and triangular, lower than labial disc and medial lips; amphidial openings appear as long slits located between labial disc and lateral lips. Stylet delicate, with small, posteriorly sloping rounded knobs. Cuticular annulations fine, distinct. Lateral field prominent, with four incisures. Excretory pore usually near beginning of basal esophageal bulb. Hemizonid prominent, about two annules long, 1 to 2 annules anterior to excretory pore. Phasmids indistinct. Rectal

TABLE 2. Measurements of 26 males of *Meloidogyne thailandica* n. sp.

Character	Range	Mean	Standard deviation
Linear (μm)			
Body length	950–1,510	1,240	205.2
Body width	21–48	31.3	6.0
Stylet length	17–20	18.7	1.1
Stylet knob width	4–5	4.7	0.3
Stylet knob height	2.5–3.0	2.6	0.2
DGO from base of stylet	2.5–5.0	3.7	0.9
Excretory pore from anterior end	138–170	154.6	11.7
Center of median bulb from anterior end	75–100	87.1	9.4
Spicule length	25–38	31.2	3.8
Gubernaculum length	7.5–11.0	8.9	1.2
Tail length	7.5–12.5	9.7	1.2
Ratios			
a	28.4–53.0	40.4	6.6
b	4.7–7.8	5.8	0.9
c	95.0–179.7	131.3	26.0

TABLE 3. Measurements of 25 J2 of *Meloidogyne thailandica* n. sp.

Character	Range	Mean	Standard deviation
Linear (µm)			
Body length	450–540	484	25.5
Body width	13–15	14.3	0.6
Stylet length	10–11	10.2	0.3
DGO from base of stylet	2.5–3.5	2.9	0.3
Center of median bulb from anterior end	55.0–62.5	60.6	1.8
Excretory pore from anterior end	80–110	89.2	6.7
Length from base of esophageal gland lobe to anterior end	117–170	141.5	14.8
Tail length	55–65	61.2	3.0
Hyaline tail terminus length	15–20	18.3	1.9
Ratios			
a	30.3–37.5	33.9	1.9
b	2.8–4.0	3.4	0.3
c	7.2–8.6	7.9	0.4
Head width/head height	1.7–2.4	2.0	0.2
Caudal ratio A	3.8–5.7	5.0	0.6
Caudal ratio B	6.0–13.3	8.9	1.5

glands inflated. Tail long and slender with a long, gradually tapering hyaline tail part that ends into a rounded terminus.

Type host and locality

Parasitic on roots of ginger (*Zingiber* sp.) from Thailand that were intercepted by APHIS at the port of San Francisco, California. The plants had been purchased at a Bangkok market supplied by local growers and were a variegated variety of *Zingiber* sp.

Type Specimens

Holotype (female): Isolated from roots from the type host and locality. Slide T-585t, deposited in the U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland.

Allotype (male): Slide T-586t, same data and repository as holotype.

Paratypes (Females, males, and J2): Same data and repository as holotype. Slides T-5295p-T5323p: T-5295p-T-5308p (females), T-5309p-T-5319p (males), T-5320p-T-5323p (J2). Additional paratypes will be deposited in the University of California-Riverside Nematode Collection, Riverside, California; the Nematode Collection of the Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England; Canadian National Collection of Nematodes, Ottawa, Canada; Collection Nationale de Nématodes, Laboratorie des Vers, Muséum national d'Histoire naturelle, Paris, France; Nematode Collection of the Landbouwhogeschool, Wageningen, The Netherlands; International Institute of Parasitology, CABI Bioscience, UK Centre, Surrey, England.

Diagnosis

Meloidogyne thailandica n. sp. is characterized by having a distinctive female perineal pattern that is oval to rectangular, with smooth to moderately wavy and coarse striae and with characteristic radial structures present underneath the pattern area, the dorsal arch is high and sometimes round to rectangular, and striae in and around the anal area form a thick network-like pattern interrupted by lateral lines from each lateral side and large phasmids. Female stylets 13.8 (12.0–15.5 µm) with prominent backwardly sloping knobs have an EP/ST mean ratio of 2.3 (1.7–3.6); J2s with body length of 484 (450–540 µm) have a long slender tail 61.2 (55–65 µm) with long, gradually tapering, hyaline tail region 18.3 (15–20 µm) that ends in a rounded terminus. Males with body length of 1.24 mm (0.95 mm–1.5 mm), stylet length 18.7 (17.0–20.0 µm); spicules 31.2 (25.0–38.0 µm) long with a bidentate terminus.

Relationships

Meloidogyne thailandica n. sp. is morphologically similar to *M. incognita* (Kofoid & White, 1919) Chitwood, 1949, *M. arenaria* (Neal, 1889) Chitwood, 1949, *M. megatyla* Baldwin & Sasser, 1979, *M. microcephala* Cliff & Hirschmann, 1984 and *M. enterolobii* Yang & Eisenback, 1983. From *M. incognita* it differs by having, in J2: longer body length 484 (450–540) vs. 405 (346–463); longer tail 62 (55–65) with a long, gradually tapering, broadly rounded terminus that measures 18.3 (15.0–20.0) vs. shorter tail 52 (42–63) with a shorter subacute terminus that measures 9.0 (6.0–13.5); females have shorter and straight stylet 13.8 (12.0–15.5) with prominent backwardly directed knobs vs. stylet cone distinctly curved dorsally 16 (15–17) with broadly elongate anteriorly indented knobs and EP/ST mean ratio of 2.3 vs. 1.4; in males; stylet is shorter 18.7 (17.0–20.0 µm) vs. longer stylet 24.0 (23.0–25.0 µm), spicules have a bidentate terminus vs. smooth terminus. From *M. arenaria* it differs by having, in females: a smooth perineal pattern with high dorsal arch, and around the anal area form a thick network-like pattern vs. flattened to rounded, low, dorsal arch with striae in arch indented and generally forming a shoulder on the arch; stylet not robust with narrow cone and shaft, and the shaft not increasing in width posteriorly but abruptly merging with backwardly directed stylet knobs vs robust stylet with both cone and shaft broad, shaft increasing in width posteriorly (not tapering) and gradually merging with rounded stylet knobs; in males: stylet is shorter 18.7 (17.0–20.0) vs. longer stylet 23.0 (20.0–28.0) and in J2: the hyaline tail terminus is longer 18.3 (15.0–20.0) vs. shorter hyaline tail terminus 9.0 (6.0–13.5). From *M. megatyla* it differs by having, in J2: longer body length of 484 (450–540) vs. shorter body length 416 (392–457); shorter delicate stylet 10.2 (10–11) vs. longer robust stylet measuring 14.6 (13.8–16.6); longer

tail length 62 (55–65) with long, gradually tapering terminus without enlarged annules and swelling vs. shorter tail 39.7 (31.6–45.1) with terminus having enlarged annules, sometimes forming an elongate swelling; and females have an EP/ST ratio mean of 2.3 vs. 1.2 (Baldwin and Sasser, 1979). It differs from *M. microcephala* in greater J2 body length 484 (450–540) vs. 458 (416–472), much lower b (2.8–4.0 vs. 7.4–8.9) and c ratios (7.2–8.6 vs. 9.1–13.6), in the shape and length of tail and hyaline tail terminus, tail long and slender 61.2 (55.0–65.0) with a long, gradually tapering hyaline region 18.3 (15.0–20.0) that ends into a rounded terminus vs. short and plump tail 45.4 (42.9–50.0) with an indistinct hyaline tail terminus that is characteristically set off from the rest of the body as a small finger-like projection; in females by the absence of windmill-shaped cuticular flaps around the tail terminus; and males have a longer spicule 31.2 (25–38) with a bidentate terminus vs. shorter spicule 26.6 (24.0–29.5) with a rounded terminus. *Meloidogyne thailandica* n. sp. differs from *M. enterolobii* primarily in having females with a shorter stylet 13.8 (12.0–15.5) with prominent backwardly directed knobs vs. longer stylet 15.1 (13.2–18.0) with knobs divided longitudinally by a groove so that each knob appears bisected as a pair, by the shorter excretory pore distance from anterior end 31 (15–47) vs. 63 (42–81), shorter EP/ST mean ratio of 2.3 vs. 4.0, and by the presence of a lateral line in perineal patterns; the J2 have a longer body length 484 (450–540) vs. 437 (405–473) and a shorter stylet length 10.2 (10.0–11.0) vs. 11.7 (10.8–13.0); males have a shorter body length 1.2 (0.95–1.51 mm) vs. 1.6 (1.35–1.91 mm), shorter stylet length 18.7 (17.0–20.0) vs. 23.4 (21.2–25.5), and spicules with a bidentate terminus vs. rounded terminus (Yang and Eisenback, 1983).

Molecular analysis: For the LSU D3 expansion segment, six clones consisting of four different haplotypes were obtained. Among these, six positions were found to be polymorphic. These polymorphic sites were a subset of the nine previously reported by Chen et al. (2003) to exist in root-knot nematodes. No new polymorphic sites were observed. Clone B was identical to haplotype 5, a haplotype previously found to exist in populations of *M. arenaria, M. incognita,* and *M. javanica* (Chen et al., 2003). Clones B and D differed from each other by a single nucleotide, and each differed from clone C by 2 bp and 3 bp, respectively. Clone E differed from clone B by 6 bp and was identical to *M. incognita* haplotype 6 described previously by Chen et al. (2003). None of the other *M. thailandica* n. sp. haplotypes were identical to haplotypes 3 and 4, which were also reported for *M. incognita. Meloidogyne thailandica* n. sp. did not have the 3-bp insertion at position 204 that was found to separate *M. hapla* from the apomictic species, which would place *M. thailandica* n. sp. in the latter grouping. Because the PCR amplifications in this study were performed on extracts of a pool of nine nematodes, it is not possible to say whether more than one haplotype exists within an individual of this isolate. The fact that *M. thailandica* n. sp. has both shared and unique D3 haplotypes is consistent with its identity as a separate species.

The intergenic spacer region (IGS) of nuclear ribosomal DNA has proven to be useful for distinguishing *Meloidogyne mayaguensis* from other root-knot nematodes (Blok et al. 1997). We found that all six *M. thailandica* n. sp. IGS clones representing a nine-nematode pool were identical, thus providing one unambiguous sequence for this population. Only a few nucleotides separated *M. thailandica* n. sp. from the other species. The IGS sequence from *M. thailandica* n. sp. was aligned with IGS sequences from *M. arenaria, M. javanica,* and *M. incognita,* as described in Blok et al. (1997). This 720-bp alignment yielded seven sites that differed from one or more of the other root-knot species. *Meloidogyne arenaria* showed two differences in addition to one ambiguous base (A → M); *M. javanica* had two differences plus four ambiguous bases (A → M, T → Y, A → W, and A → R); and *M. incognita* had three differences, plus one ambiguous base (A → M). Six clones were sequenced from the *M. thailandica* n. sp. population, and all were identical, allowing the assignment of a single unambiguous sequence to this isolate. The authors of the *M. mayguensis* study noted that small variations existed between clones obtained from single isolates of *M. arenaria, M. javanica,* or *M. incognita.* If we delete these ambiguities from our comparison, only two to three nucleotide changes separate *M. thailandica* n. sp. from *M. arenaria, M. javanica,* and *M. incognita.* Because the number of nucleotide differences among clones of a single isolate was similar to the number of differences between species, we cannot place confidence in the ability of IGS to discriminate these root-knot nematode species.

A 244-bp alignment of the *Meloidogyne thailandica* n. sp. ITS1 sequence with overlapping sequences from other species contained 18 parsimony informative sites. The alignment was trimmed to allow inclusion of shorter sequences from Genbank. *Meloidogyne thailandica* n. sp. differed at a single nucleotide position from the identical ITS1 sequences from *M. incognita* AF387093 and *M. arenaria* AY438554; at two nucleotide positions from *M. incognita* AY438556, *M. incognita* isolate Adel1 AF510064, and *M. arenaria* AF387092; and at three positions from *M. incognita* U96304, *M. javanica* AF387094, and *M. javanica* AY438555. The sequence *M. incognita* isolate Adel2 AF516723 differed from *M. thailandica* n. sp. at 12 positions.

The level of intraspecific variation in ITS1 was similar to the level of interspecific variation, limiting the confidence of this molecule to discriminate the root-knot nematodes compared. Thus, of the three molecules examined, the LSU D3 expansion segment was most useful for distinguishing *M. thailandica* n. sp. from the ma-

jor root-knot nematode species for which sequences were available. It is worth pointing out that the molecular comparisons parallel the morphological findings, as *M. thailandica* n. sp. shares limited morphological and molecular features with *M. incognita* and *M. arenaria* but lacks definitive molecular features of either. Sequences from *M. megatyla, M. microcephala,* and *M. enterolobii* were not available for comparison. The LSU D3 expansion segment, IGS rDNA, and ITS1 have all been used for discrimination of root-knot nematode species. However, it is increasingly clear that each molecule has limitations for a given level of taxonomic resolution. Our results highlight the importance of examining more than one molecular character to aid in the taxonomic assignment of new species, where the relationships are often unclear and closest relatives may not be readily available for comparison.

In summary, the root-knot nematode found on roots of ginger (*Zingiber* spp.) intercepted by APHIS at San Francisco from Thailand, herein referred to and described as *Meloidogyne thailandica* n. sp., is quite different from the other *Meloidogyne* spp. known to date.

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