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Meloidogyne mayaguensis n. sp. (Meloidogynidae), a Root-knot Nematode from Puerto Rico[1]

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Abstract: Meloidogyne mayaguensis n. sp. is described and illustrated from specimens obtained from galled roots of eggplant, *Solanum melongena* L., from Puerto Rico. The perineal pattern of females is round to ovoid with fine, widely spaced striae. It has occasional breaks of striation laterally and a circular tail tip area lacking striae. The stylet, 15.8 μm long, has reniform knobs that merge gradually with the stylet shaft. Males have a high, rectangular, smooth head region, not set off from the body contour. The labial disc is continuous with the medial lips which do not slope posteriorly. The stylet, 22.9 μm long, has large rounded backward sloping knobs; the shaft is of uneven diameter. Mean body length of second-stage juveniles is 453.6 μm. The truncate head region is not annulated, and the rounded, slightly raised labial disc and the crescentic medial lips form dumbbell-shaped lip structures. The stylet, 11.6 μm long, has rounded, posteriorly sloping knobs. The slender tail, 54.4 μm long, gradually tapers to a bluntly pointed tip. Tomato, tobacco, pepper, and watermelon are good hosts; cotton and peanut are not hosts. *M. mayaguensis* n. sp. reproduces by mitotic parthenogenesis and has a somatic chromosome number of 2n = 44–45. The enzyme patterns are unique among *Meloidogyne* species.

Key words: taxonomy, morphology, new species, host range, scanning electron microscopy, *Solanum melongena* L., eggplant, *Meloidogyne mayaguensis* n. sp., root-knot nematode, Puerto Rico.

Populations of root-knot nematodes from tropical and subtropical regions of the world are maintained in the International *Meloidogyne* Project culture collection at North Carolina State University. Some of these populations identified tentatively as *Meloidogyne arenaria* were re-examined to elucidate their taxonomic status. Comprehensive studies on host range, morphology, cytogenetics, and biochemistry showed that population E180 from Puerto Rico differed from other described species, including *M. arenaria.* The differential host test response (9) of this population was similar to that of *M. incognita* race 2. Other taxonomic criteria, however, revealed no similarities to *M. incognita.* The somatic chromosome number was 2n = 44–45 (6) which deviates from that of the two cytological races of *M. arenaria* (11), and electrophoresis revealed a unique enzyme phenotype different from other *Meloidogyne* species (6). Based on these biological and morphological differences to other *Meloidogyne* species, this nematode population is described here as *Meloidogyne mayaguensis* n. sp.

MATERIALS AND METHODS

Stock cultures of *M. mayaguensis* n. sp. were established from galled roots of eggplant (*Solanum melongena* L.) obtained from the type locality in Puerto Rico. The nematodes were propagated and maintained by periodic subculturing on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) in a greenhouse at 22–28 C. All nematodes used in morphologic and morphometric studies were from these cultures.

Light microscopy (LM): Females were teased from infected root systems, hand picked, and fixed in 2% formalin. Entire females were mounted in 2% formalin, and body shape and dimensions were recorded. The anterior portion of females (neck) including the esophagus was cut off with an eye knife and mounted on slides in 2% formalin. Perineal patterns were prepared as previously described (5). At least 70 patterns were examined.

Males were obtained by incubating washed, infected root systems in moist

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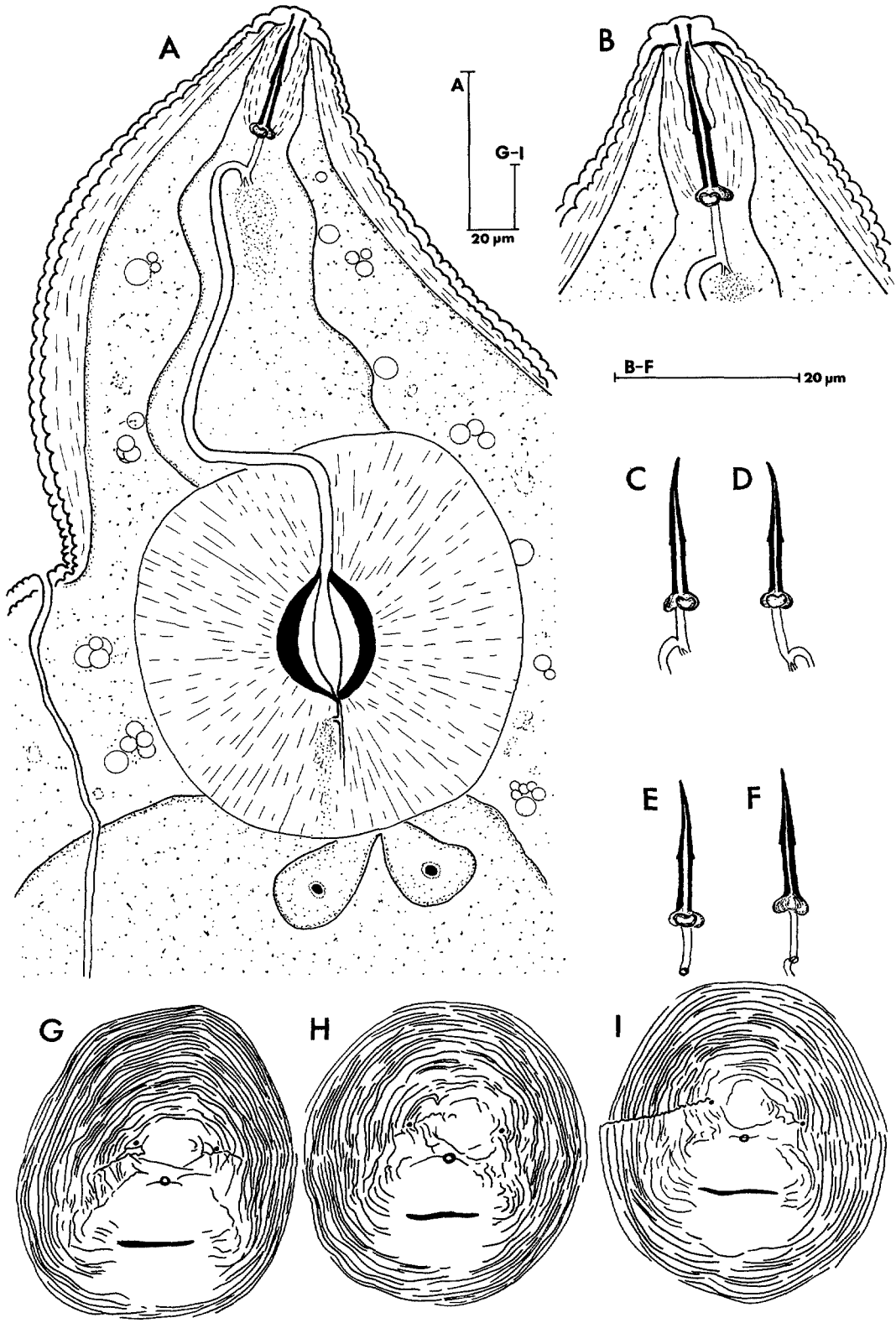

FIG. 1. Line drawings of females of *Meloidogyne mayaguensis* n. sp. A) Esophageal region (lateral). B) Cephalic region (lateral). C–F) Stylets. G–I) Perineal patterns.

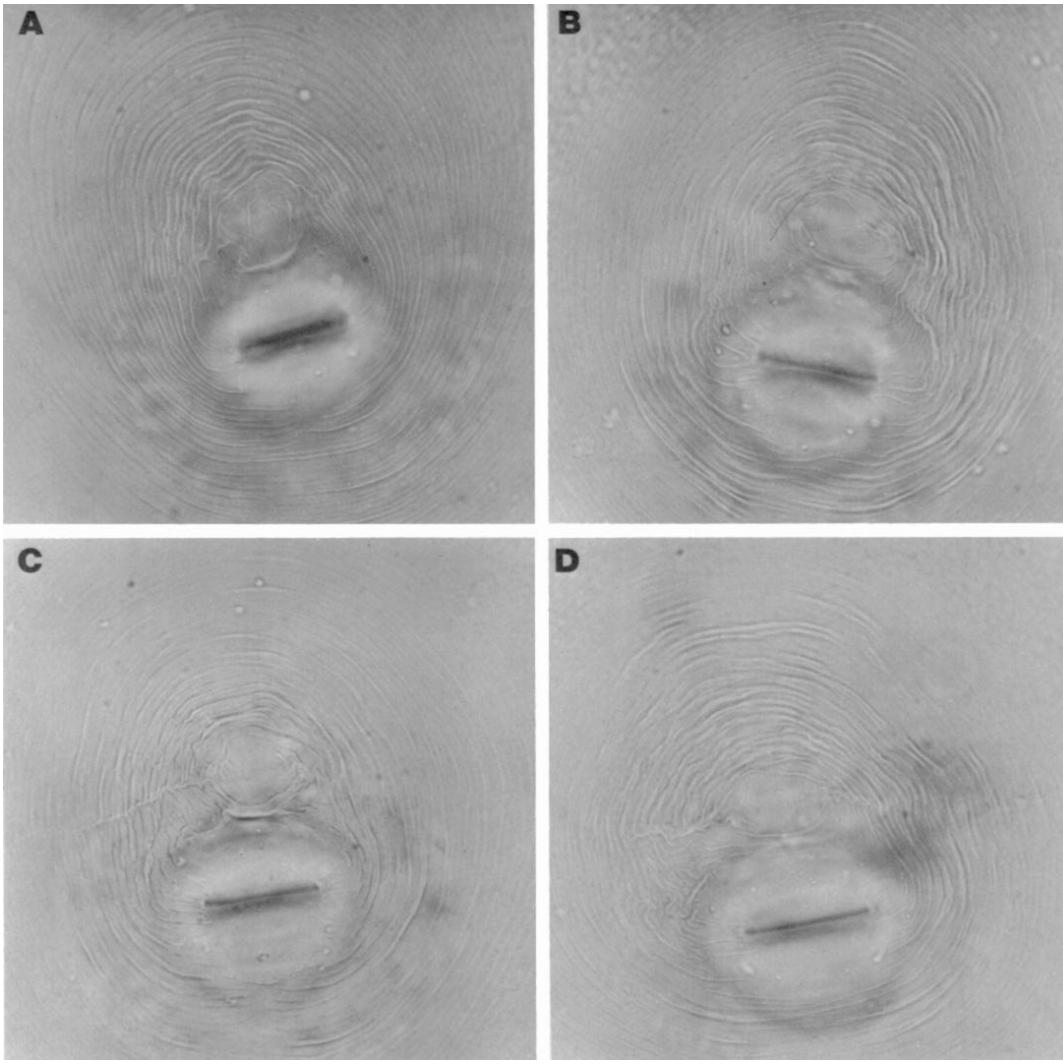

FIG. 2. LM photographs of perineal patterns of *Meloidogyne mayaguensis* n. sp. showing typical variation.

chambers. The roots were rinsed periodically with water, and males were collected from the washings. Males were killed in hot TAF and mounted in the same fixative (7). At least 100 males were examined for qualitative data. Thirty-five specimens in lateral view were used for taking morphometric data.

Second-stage juveniles (J2) were recovered from egg masses incubated in moist chambers at room temperature. They were fixed in hot TAF and mounted in the same fixative on slides (7). One hundred J2 were observed for qualitative characters. Thirty-five specimens in lateral view were examined for quantitative data.

Type specimens of all life stages were prepared according to previously described methods (8).

Line drawings were made using a drawing tube, and photographs were taken with a bright field microscope. All measurements are given in micrometers (μm).

Scanning electron microscopy (SEM): Second-stage juveniles, males, excised stylets, and spicules were processed for SEM as described previously (1–5,10). The specimens were viewed and photographed using a JEOL T 200 scanning electron microscope operating at 25 kV. At least 100 J2, 100 males, 40 male stylets, 40 female stylets, and 20 spicules were examined.

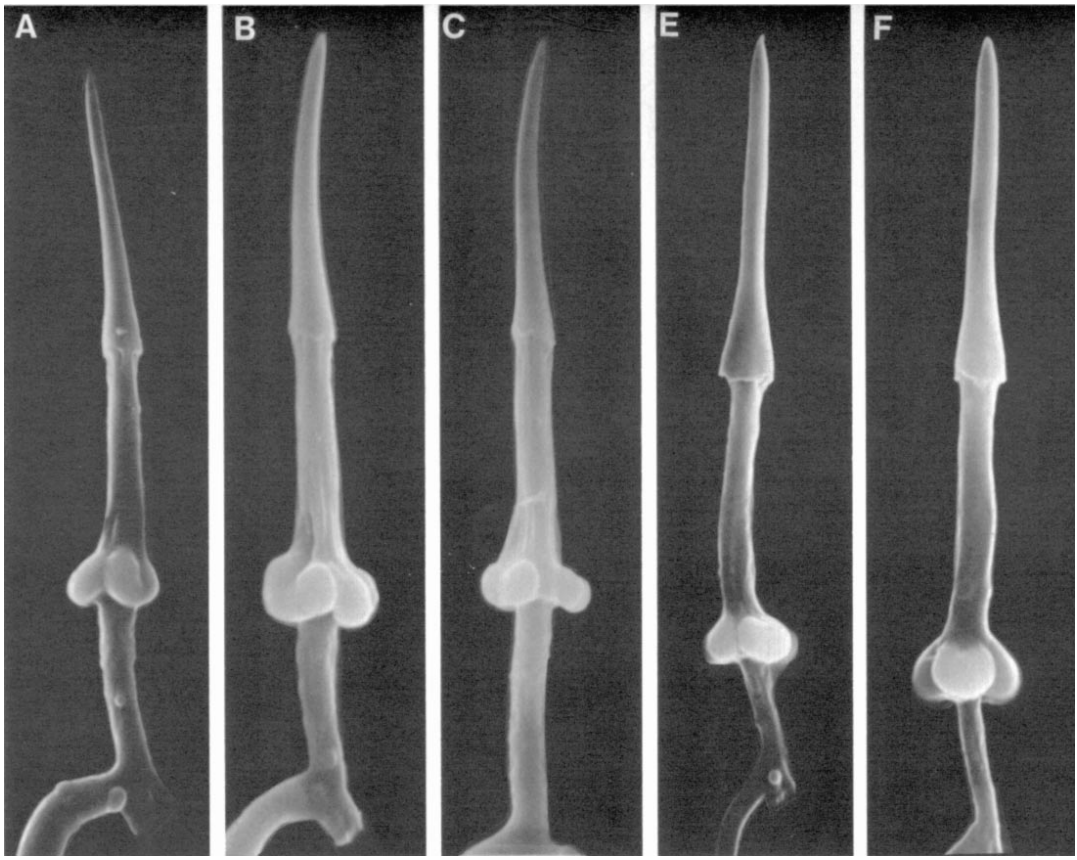

FIG. 3. SEM photographs of excised stylets of *Meloidogyne mayaguensis* n. sp. A–C) Female sytlets. E, F) Male stylets.

SYSTEMATICS

Meloidogyne mayaguensis n. sp. (Figs. 1–7)

Holotype (female in glycerin): Body length 720, length without neck 530, body width 570, neck length 190, neck width 160; stylet length 15.1, stylet knob height 2.2, stylet knob width 4.4; DGO to stylet base 6.2, excretory pore to head end 46.4; a 1.3, body length without neck/body width 0.9, body length/neck length 3.8, stylet knob width/height 2.0.

Females: Measurements of 35 females in 2% formalin (perineal patterns in glycerin) are listed in Table 1. Body white, pear-shaped and variable in size, posteriorly rounded, without tail protuberance. Cuticle finely striated at anterior neck region (Fig. 1A). Head region slightly set off from body, not annulated (Fig. 1A, B). Head cap distinct, labial disc slightly elevated. Hexaradiate cephalic framework weakly sclerotized, vestibule and vestibule extension distinct. Stylet cone pointed, as long as shaft, with slight dorsal curvature and wider than shaft at their junction (Figs. 1A–F, 3A–C). Shaft cylindrical, widening slightly near junction with knobs. Knobs reniform or transversely elongated, distinctly indented, merging gradually with shaft (Figs. 1A–F, 3A–C). Distance between stylet base and dorsal esophageal gland orifice (DGO) long (3.5–6.7), orifice triradiate. Esophagus well developed; metacorpus large, rounded, with large valve (Fig. 1A); esophageal glands large, comprising one dorsal gland and two subventral gland lobes. Two large esophago-intestinal cells near junction of metacorpus and intestine. Excretory pore usually at level of metacorpus (Fig. 1A).

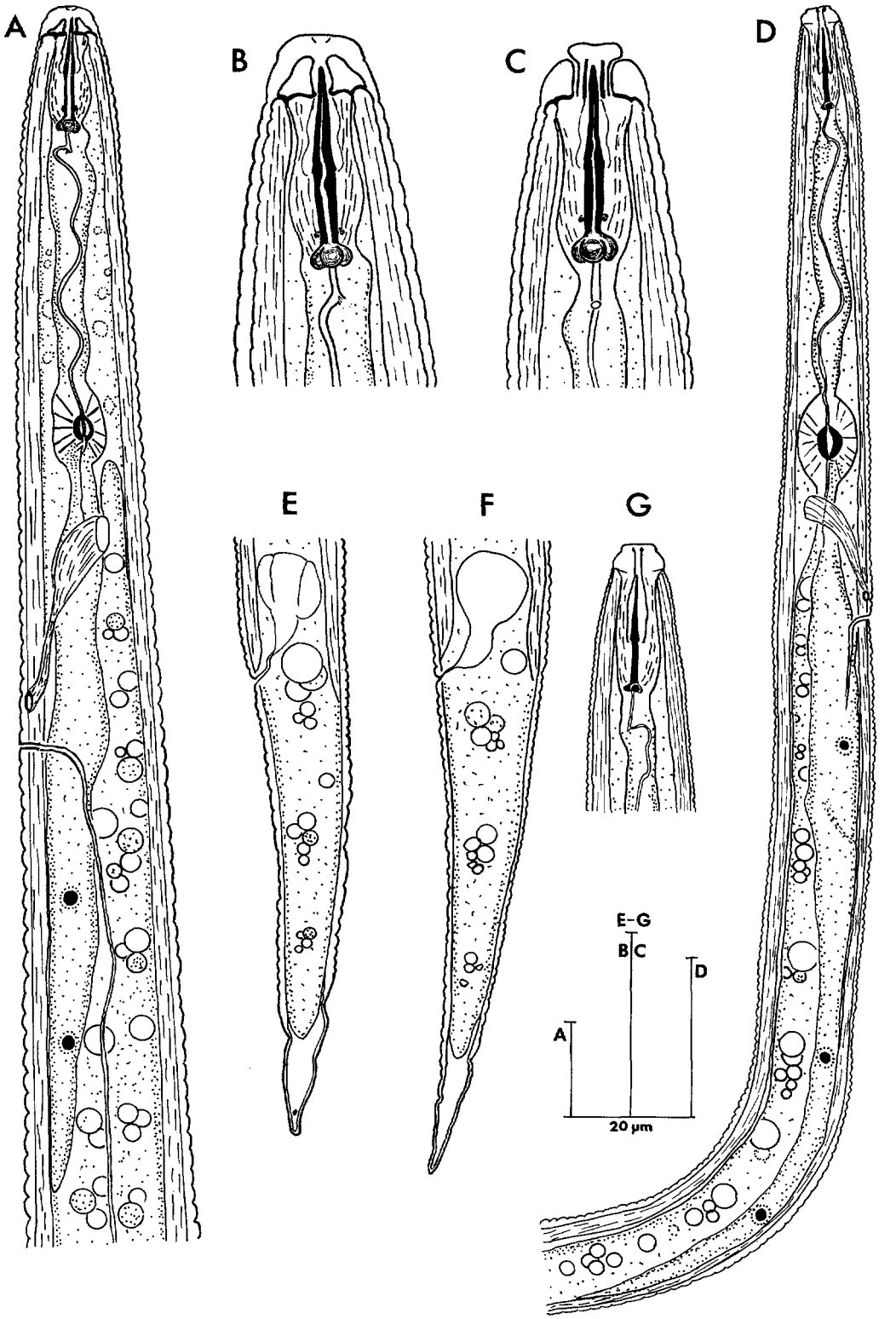

FIG. 4. Line drawings of males and J2 of *Meloidogyne mayaguensis* n. sp. A) Esophageal region of male (lateral). B, C) Cephalic regions of male (lateral, dorsal). D) Esophageal region of J2 (lateral). E, F) Tails of J2 (lateral). G) Cephalic region of J2 (lateral).

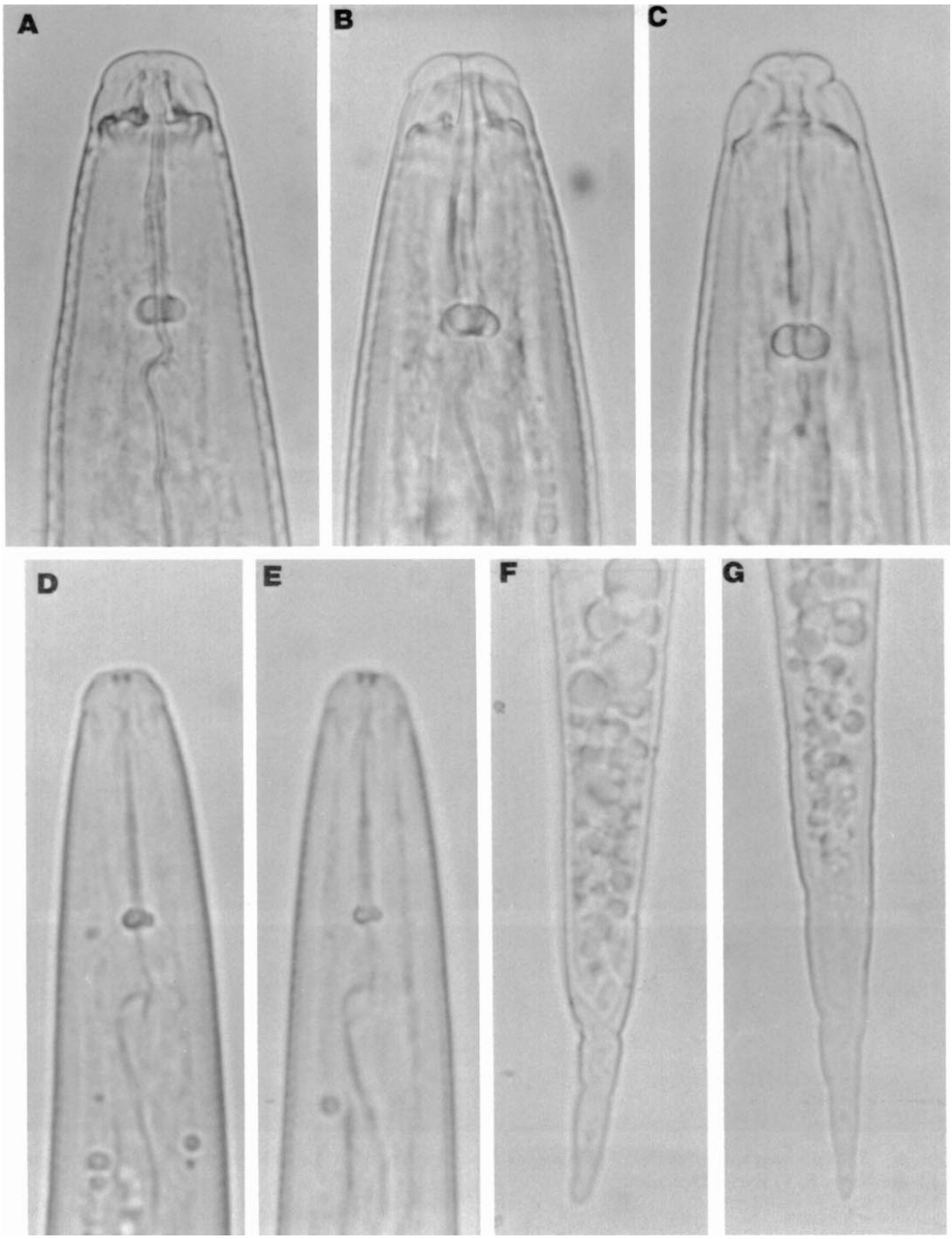

FIG. 5. LM photographs of males and J2 of *Meloidogyne mayaguensis* n. sp. A–C) Cephalic regions of males (lateral, lateral, dorsal). D, E) Cephalic regions of J2 (lateral). F, G) Tails of J2 (lateral, ventral).

Perineal patterns round to dorso-ventrally ovoid (Figs. 1G–I, 2A–D). Dorsal arch rounded, striae fine, mostly continuous, widely spaced. Ventral pattern area rounded, striae fine, closely spaced. Lateral lines seldom distinguishable, breaks in striae or a single lateral line may occur on one side of pattern at junction of dorsal and ventral arches. Tail tip area circular, free of striae. Vulva slit-like, lateral striations present.

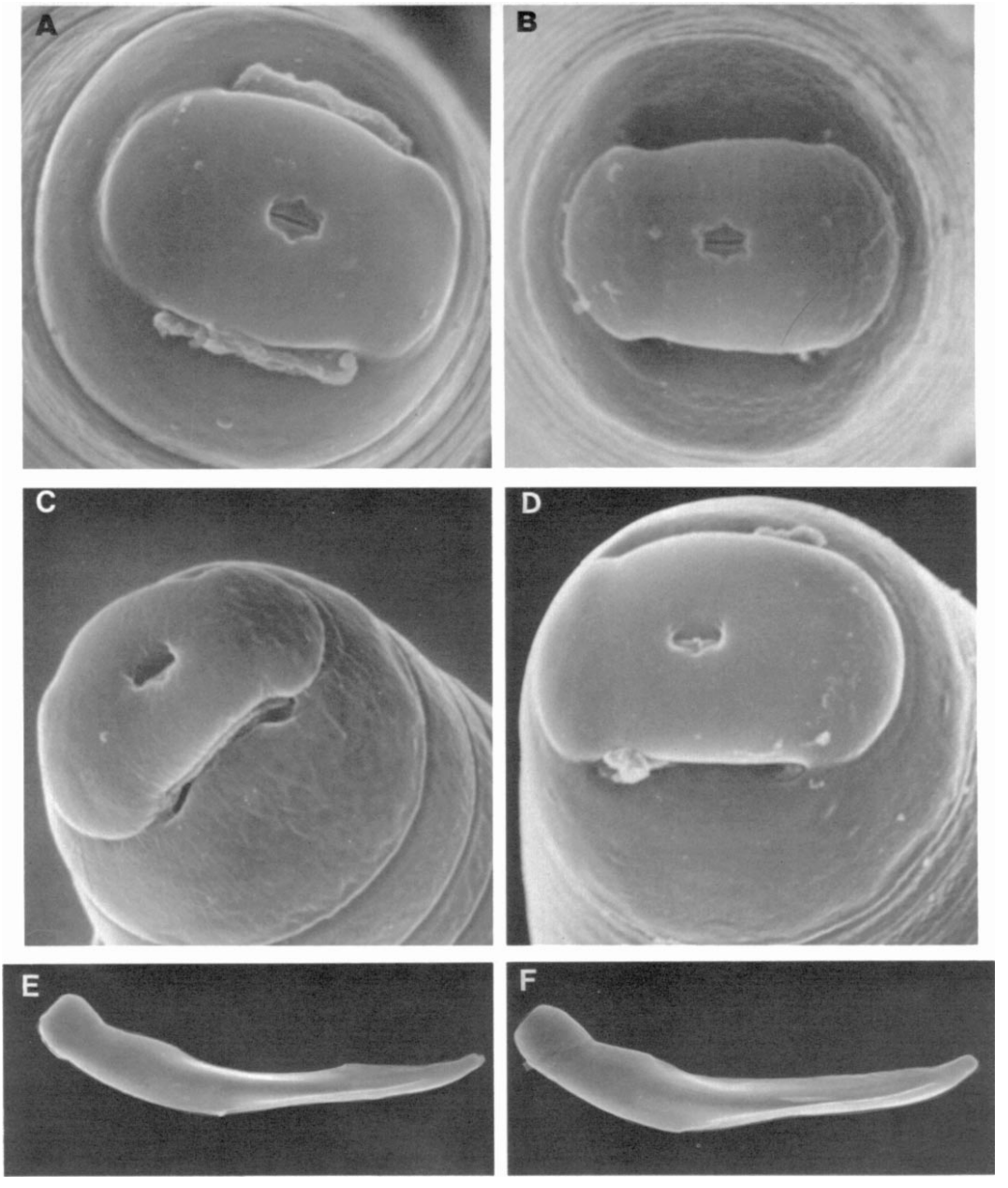

FIG. 6. SEM photographs of males of *Meloidogyne mayaguensis* n. sp. A, B, D) Face views of cephalic region. C) Lateral view. E, F) Excised spicules.

Anus covered by small cuticular fold. Phasmids small but distinct, about same distance apart as width of vulva (Fig. 1G–I).

Allotype (male in glycerin): Body length 1,450; body width 36.1, width at stylet base 19.9, width at excretory pore 28.7; head region height 7.3, head region width 12.6; stylet length 23.2, stylet knob height 3.4, knob width 5.6; DGO to stylet base 3.8, head end to metacorpus valve 97.0, excretory pore to head end 148.8, phasmids to tail end 17.0; spicule length 29.1, gubernaculum length 7.5, testis length 830, tail length 14.1; a 40.2, c 102.8, body length/head end to metacorpus valve 14.9, head region width/height 1.7, stylet knob

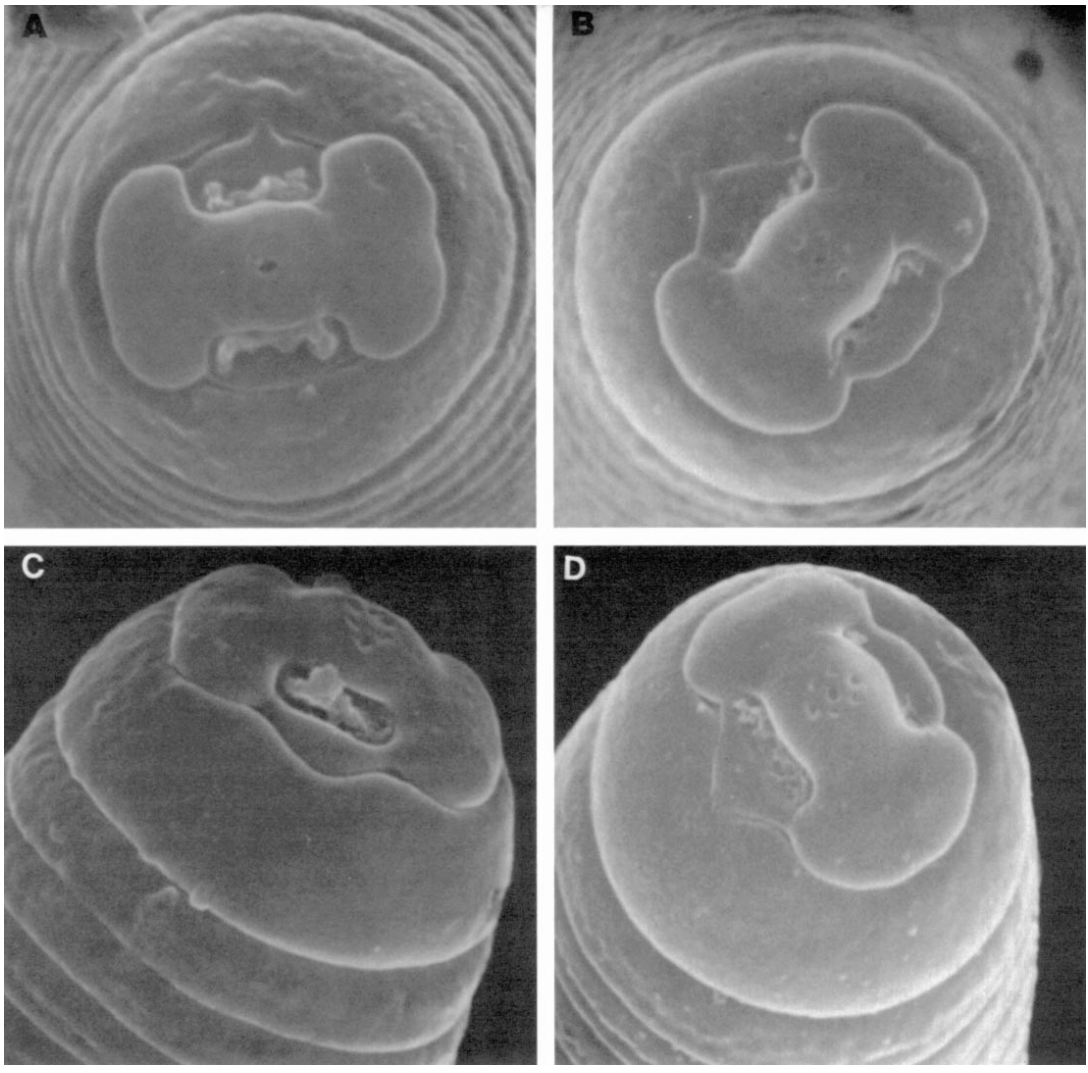

FIG. 7. SEM photographs of cephalic regions of *Meloidogyne mayaguensis* n. sp. J2. A, B, D) Face views. C) Lateral view.

width/height 1.7, excretory pore 10.3%, T 57.2.

Males: Measurements of 30 males in TAF are listed in Table 2. Body vermiform, length variable, tapering anteriorly, bluntly rounded posteriorly. Body cuticle with faint transverse annulation. Lateral field with four incisures, outer fields areolated. Head not set off, shallowly rounded to truncate (Figs. 4A, B, 5A, B). Head region high, without annulations. Head cap flat in lateral view, rectangular, or slightly rounded with rounded corners and narrower across than head region, slightly depressed at stoma. In SEM (face view) labial disc not raised, continuous with medial lips (Fig. 6A–D). Medial lips not sloping posteriorly onto head region. Lateral lips absent. Prestoma hexagonal, surrounded by six inner labial sensilla. Stoma opening slit-like. Cephalic sensilla indistinct. Amphid openings large, slit-like, below lateral edges of labial disc. Cephalic framework moderately sclerotized, vestibule and vestibule extension distinct (Figs. 4A–C, 5A–C). Stylet cone straight, gradually tapering, pointed, about half stylet length, wider than shaft at their junction (Fig. 3E, F). In LM, junc-

TABLE 1. Measurements of 35 females of *Meloidogyne mayaguensis* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variability (%)
Linear (μm)					
Body length	518.4–769.5	651.2	8.91	52.7	8.1
Body length without neck	113.4–591.3	486.2	12.69	75.1	15.4
Body width	413.1–599.4	501.0	7.48	44.2	8.8
Neck length	81.0–526.5	170.8	12.36	73.1	42.8
Neck width	81.0–234.9	160.6	5.59	33.1	20.6
Vulval slit length	20.9–30.4	26.1	0.32	1.9	7.2
Vulva–anus distance	12.7–21.1	18.4	0.25	1.5	8.0
Interphasmidial distance	18.1–29.6	23.2	0.43	2.5	10.8
Stylet length	13.8–16.8	15.8	0.13	0.8	5.0
Stylet knob height	1.7–2.5	2.1	0.03	0.2	8.8
Stylet knob width	3.9–5.2	4.3	0.04	0.3	6.1
DGO	3.5–6.7	4.8	0.14	0.8	16.6
Excretory pore to head end	25.9–86.6	48.2	2.30	13.6	28.2
Ratios					
a	1.1–1.6	1.3	0.02	0.1	9.8
Body length without neck/body width	0.2–1.2	1.0	0.03	0.2	16.0
Stylet knob width/height	1.6–2.5	2.1	0.04	0.2	10.6

tion of cone and shaft indistinct (Figs. 4A–C, 5A–C). Shaft cylindrical, diameter usually uneven, may be curved at some points, narrows distinctly at base, surrounded by ring. Stylet lumen usually wavy. Knobs large, set off from shaft, well separated from each other, rounded, sloping backward; dorsal knob base characteristically concave (Figs. 3E, F, 4A–C, 5A–C). Distance from stylet base to DGO long (3.3–5.0). Procorpus distinctly outlined; metacorpus oval with large valve (Fig. 4A). Subventral gland openings branched, located posterior to metacorpus valve. Esophageal gland lobe variable in length, with two nuclei. Esophago-intestinal junction distinct, at level of nerve ring. Intestinal caecum extending anteriorly to level of median bulb on dorsal side. Hemizonid two or three annules anterior to excretory pore. Excretory pore variable in position. Testes two, rarely one testis. Spicules slender, arcuate; spicule head cylindrical, well separated from shaft by indentation (Fig. 6E, F). Blade arcuate, with two inward-curved vela; spicule tip rounded, slightly curved ventrally with two distinct pores. Tail short, elongate conoid. Phasmids large, anterior to anus, near midspicule level.

Second-stage juveniles: Measurements of 35 J2 in TAF are listed in Table 3. Body vermiform, tapering at both ends, but more sharply posteriorly. Body cuticle with fine, distinct annulation; annules larger on posterior tail region. Lateral field with four incisures. Head region truncate, slightly set off from body (Figs. 4D, G, 5D, E). Head cap distinctly raised, narrower than head region. In SEM, labial disc rounded, slightly elevated above medial lips (Fig. 7A–D). Medial lips rectangular or crescentic, cephalic sensilla four, marked by faint depressions. Medial lips and labial disc dumbbell shaped. Lateral lips fused at right angle with medial lips, lower than medial lips, margins crescentic or triangular, may fuse with head region (Fig. 7A–C). Head region smooth, without annulations. Amphid openings large, rectangular, located between labial disc and lateral lips. Prestoma small, oval, surrounded symmetrically by six pit-like inner labial sensilla, lateral sensilla distinctly larger. Area around prestoma not recessed. Cephalic framework weakly sclerotized; vestibule and vestibule extension distinct (Figs. 4D, G, 5D, E). Stylet cone straight, pointed, about half stylet length. Knobs small, rounded, set off

TABLE 2. Measurements of 30 males of *Meloidogyne mayaguensis* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variability (%)
Linear (μm)					
Body length	1,175.0–1,742.0	1,503.0	25.90	141.9	9.4
Greatest body width	32.2–44.4	37.8	0.57	3.1	8.3
Body width at stylet base	17.4–20.5	18.9	0.14	0.8	4.0
Body width at excretory pore	25.9–31.8	29.0	0.28	1.6	5.3
Stylet length	20.7–24.6	22.9	0.15	0.8	3.6
Stylet knob height	2.4–3.7	3.0	0.05	0.3	8.9
Stylet knob width	4.3–5.6	5.0	0.06	0.3	6.8
DGO	3.3–5.0	4.1	0.08	0.4	10.2
Head end to metacorpus valve	84.8–102.0	92.1	0.78	4.3	4.6
Excretory pore to head end	147.2–180.8	166.4	1.61	8.8	5.3
Tail length	11.3–16.3	14.3	0.19	1.1	7.4
Spicule length	24.4–31.3	28.3	0.27	1.5	5.2
Gubernaculum length	6.1–9.3	7.1	0.11	0.6	8.7
Ratios					
a	31.1–49.6	39.9	0.71	3.9	9.8
Body length/head end to metacorpus valve	14.1–19.1	16.3	0.28	1.5	9.0
Stylet knob width/height	1.3–2.0	1.7	0.03	0.2	9.6
c	85.8–124.3	105.7	1.82	10.0	9.4
Percentage					
Excretory pore	9.4–14.0	11.1	0.19	1.0	9.2

from shaft, distinctly sloping posteriorly. Distance from base of stylet to DGO long (3.3–4.3). Procorpus slender, faintly outlined (Fig. 4D); metacorpus oval, valve well sclerotized. Esophago-intestinal junction obscure. Gland lobe overlapping intestine ventrally, with three nuclei, dorsal nucleus slightly smaller. Hemizonid two annules anterior to excretory pore. Tail slender, gradually tapering to bluntly pointed tip (Figs. 4E, F, 5F, G). Hyaline tail terminus distinctly set off, often containing small fat droplet at tip. Rectal dilation large. Phasmids distinct, slightly posterior to anal opening.

Eggs (50 in 2% formalin): Length 88.5–111.3 (mean 98.3, standard error of mean [SE] 0.67, standard deviation [SD] 4.77, coefficient of variability [CV] 5.0%); width 37.9–50.4 (44.0, SE 0.83, SD 5.85, CV 13.0%); length/width ratio 1.8–2.6 (2.2, SE 0.02, SD 0.16, CV 7.0%). Egg morphology similar to that of eggs of other *Meloidogyne* species. Egg shell without visible markings by LM. Egg masses small, generally 150–200 eggs per mass.

Diagnosis: Meloidogyne mayaguensis n. sp. is morphologically distinct from all other species of the genus including *M. arenaria* to which it was tentatively assigned previously. Useful diagnostic characters include the morphologies of stylet and perineal pattern of females, and head region and stylet of males.

Meloidogyne mayaguensis n. sp. superficially resembles *M. enterolobii* (12) but differs distinctly from it in the following morphological features: The stylet knobs of females are characteristically reniform and not divided so conspicuously as those of *M. enterolobii.* The dorsal knob is slightly sloping posteriad in lateral view. The rounded to dorso-ventrally ovoid perineal pattern is larger and has faint, widely spaced striae; the tail tip area is large, circular, and usually free of striae; lateral lines are usually absent. Other differences include vulval slit length (range 20.9–30.4, mean 26.1; *M.*

TABLE 3. Measurements of 35 second-stage juveniles of *Meloidogyne mayaguensis* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variability (%)
Linear (μm)					
Body length	390.4–528.0	453.6	4.80	28.4	6.3
Greatest body width	13.8–15.8	14.7	0.09	0.5	3.4
Body width at anus	10.2–12.2	10.9	0.08	0.5	4.4
Stylet length	11.1–12.2	11.6	0.05	0.3	2.4
Stylet base to head end	14.8–15.8	15.2	0.06	0.3	2.2
DGO	3.3–4.3	3.9	0.04	0.2	6.2
Head end to metacorpus valve	55.2–62.9	58.2	0.31	1.8	3.1
Excretory pore to head end	79.9–97.9	87.6	0.56	3.3	3.8
Tail length	49.2–62.9	54.4	0.60	3.6	6.5
Ratios					
a	26.4–34.7	30.9	0.32	1.9	6.1
Body length/head end to metacorpus valve	6.8–8.8	7.8	0.07	0.4	5.5
c	7.0–9.2	8.3	0.07	0.4	5.1
d	4.3–5.7	5.0	0.06	0.4	7.3
Percentage					
Excretory pore	17.8–22.3	19.4	0.17	1.0	5.1

enterolobii 25.3–32.4, 28.7), vulva anus distance (12.7–21.1, 18.4; *M. enterolobii* 19.7–26.6, 22.2), and interphasmidial distance (18.1–29.6, 23.2; *M. enterolobii* 22.2–42.0, 30.7). The high, rectangular head region of males is not set off from the body and lacks annulations. The high head cap is only very slightly demarcated. The stylet knobs are distinctly separate and not divided longitudinally by a groove; the base of the dorsal knob is concave. The stylet shaft is irregular in diameter with wavy lumen and narrows near the junction with the knobs. The J2 stylet knobs are sloping posteriad and are more amalgamated than those of *M. enterolobii.* The tail tapers gradually to the tip, and the tail terminus is not distinctly narrowed as in *M. enterolobii.*

Meloidogyne mayaguensis n. sp. has a similar host response as *M. incognita,* host race 2 (9), but it differs in morphology, cytogenetics, and biochemistry. The chromosome behavior during oogenesis resembles that of *M. arenaria* (11). Reproduction is by mitotic parthenogenesis, and the somatic chromosome number is 2n = 44–45. *Meloidogyne mayaguensis* n. sp. has an esterase phenotype (VS1-S1) identical to that of *M. enterolobii,* but has a different malate dehydrogenase pattern (N3c) (6).

Type specimens: Holotype (female)—isolated from greenhouse culture, propagated on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers), derived from original population obtained from Puerto Rico. Slide, T-428t deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland. Allotype (male)—same data as holotype. Slide, T-429t deposited in the USDANC, Beltsville, Maryland. Paratypes (females, males, J2, perineal patterns)—same data as holotype. Deposited in the USDANC, Beltsville, Maryland, and the University of California Davis Nematode Collection (UCDNC), Davis, California.

Type host and locality: Roots of egg plant, *Solanum melongena* L., sandy soil from Jobos, Isabella, northwestern region of Puerto Rico.

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