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Description of *Meloidogyne pini* n. sp., a Root-Knot Nematode Parasitic on Sand Pine (*Pinus clausa*), with Additional Notes on the Morphology of *M. megatyla*[1]

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Abstract: Meloidogyne pini n. sp. is described from sand pine, *Pinus clausa,* in Georgia. The perineal pattern of the female has a large cuticular ridge surrounding a deeply recessed perivulval area. The lateral fields are marked by transverse striae. The female stylet is 14.6 μm long, and the knobs are small, rounded, and set off from the straight and narrow shaft. The excretory pore is near the level of the base of the stylet. The labial disc of the male is large, rounded, and fused with the crescent-shaped medial lips. The head region is smooth, the stylet is 20.8 μm long, and the cone is more than twice as long as the shaft. The knobs are rounded and set off from the shaft. In the second-stage juvenile, the labial disc, medial lips, and lateral lips form one smooth, continuous, ovoid head cap. Mean juvenile length is 434 μm, stylet length is 12.8 μm, and tail length is 44.4 μm. *M. pini* n. sp. also parasitizes loblolly and slash pine. Additional morphological details of *M. megatyla* are presented.

Key words: taxonomy, host range, scanning electron microscopy, *Pinus taeda,* loblolly pine, *Pinus elliotti,* slash pine, new species.

Severely stunted commercial stands of sand pine (*Pinus clausa* (Chapm.) Vasey) were observed in several locations in southeastern Georgia by U.S. Forest Service personnel. Symptoms were chlorotic, dwarfed, and tufted foliage and stunting of trees (S. W. Oak, pers. comm.). Infected root segments submitted to J. N. Sasser in 1981 had large compound galls up to 15 cm long and 3 cm in diameter (Fig. 1A). Adult *Meloidogyne* females dissected from the galls had perineal patterns different from those of any described species. Studies on the morphology and host range demonstrated that this was a new species of root-knot nematode. *Meloidogyne pini* n. sp. is described herein, and the common name "sand pine root-knot nematode" is proposed.

This is the first report of a root-knot nematode attacking *P. clausa. Meloidogyne* species previously reported on *Pinus* species include *M. arenaria* (Neal) Chitwood (11), *M. javanica* (Treub) Chitwood (2), and *M. megatyla* Baldwin and Sasser (1). An undescribed species was reported on ponderosa pine (*Pinus ponderosa* Laws) in New Mexico (10), and *M. incognita* (Kofoid and White) Chitwood has been found in soil samples from several southeastern forest nurseries (9).

Materials and Methods

Stock cultures of *Meloidogyne pini* n. sp. were established from galled roots of sand pine obtained from the type locality and propagated on sand pine seedlings. A culture of *M. megatyla,* established and maintained on loblolly pine (*Pinus taeda* L.) seedlings, was obtained from the original population used in the species description. Cultures of both species were kept in a greenhouse at 22–28 C. All nematodes used in morphologic and morphometric studies were from these cultures.

Morphologic studies: Males and second-stage juveniles (J2) were obtained from pieces of washed galled roots incubated in a moist chamber. Light microscope (LM) observations were made from specimens killed in hot TAF (8). Fixed specimens were always compared to live specimens mounted in 0.85% saline. Males and J2 were prepared for scanning electron microscope (SEM) observations as previously reported (4,5). Perineal patterns of females and excised stylets were prepared for SEM observations as described previously (6). Sty-

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TABLE 1. Measurements of 30 females of *Meloidogyne pini* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (μm)					
Body length	463.4–925.1	696.9	19.9	109.2	15.7
Body width	304.9–636.7	424.4	12.4	67.9	16.0
Neck length	83.6–273.5	167.0	9.9	54.1	32.4
Stylet length	12.8–18.4	14.6	0.2	1.3	9.1
Stylet knob height	2.3–3.7	2.8	0.1	0.3	11.3
Stylet knob width	4.1–5.6	4.7	0.1	0.4	7.7
DGO	3.2–6.3	4.6	0.1	0.7	15.1
Excretory pore to head end	3.0–32.6	15.1	1.3	7.2	47.4
Interphasmidial distance	22.6–48.6	31.3	1.0	2.3	16.9
Vulval length	16.2–26.1	21.1	0.5	2.8	13.1
Vulva–anus distance	14.5–22.9	19.2	0.4	1.9	10.1
Ratios					
a	0.8–2.2	1.7	0.1	0.3	20.5
Body length/head end to posterior end of metacorpus	5.2–11.6	8.6	0.3	1.9	21.7
Other					
Number of body annules from head end to excretory pore	2.0–13.0	7.0	0.5	2.7	37.3

lets of males and J2 were dissected for SEM by adaptation of the technique described for females. Eggs were mounted in 2% formalin. All LM observations were made within 4 hours of processing. Photographs were taken with a bright field microscope, and at least 100 specimens of each developmental stage were examined by both LM and SEM. Type specimens of J2 and males were prepared according to Thorne (12), and type specimens of females were prepared according to Eisenback (3).

Limited host range test: Seedlings of sand pine (*P. clausa* (Chapm.), Vasey, Ocala, and Choctawhatchie races), loblolly pine (*P. taeda* L.), slash pine (*P. elliotti* Engelman), blueberry (*Vaccinium* sp.), azalea (*Rhododendron* sp.), tobacco (*Nicotiana tabacum* L. cv. NC 95), cotton (*Gossypium hirsutum* L. cv. Deltapine 61), pepper (*Capsicum annuum* L. (*C. frutescens*) cv. California Wonder), watermelon (*Citrullus vulgaris* Schard. cv. Charleston Gray), and tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) were transplanted to 10-cm-d clay pots and inoculated with nematode-infested soil. Each treatment was replicated three times; sand pine served as the control. After 90 days in a greenhouse at 22–28 C, plants were washed and roots examined for galls, mature females, and egg masses.

SPECIES DESCRIPTION

Meloidogyne pini n. sp.

Females

Measurements of 30 females in 2% formalin and perineal patterns in glycerin are listed in Table 1.

Measurements (holotype in glycerin): Body length including neck = 871.1 μm; body width = 439.0 μm; neck length = 195.1 μm; stylet length = 16.1 μm; stylet knob height = 2.4 μm; stylet knob width = 4.3 μm; dorsal esophageal gland orifice to stylet base = 4.6 μm; excretory pore to head end = 8.5 μm. Female as in general description. Perineal region not visible.

Description (Figs. 1B–D, 2, 3): Body translucent white, variable in size, pear-shaped to ovoid with prominent neck sometimes twice as long as body (Fig. 1D), without tail protuberance. In SEM, stoma slit-like, located in ovoid prestoma, surrounded by pit-like openings of six inner labial sensilla. Labial disc slightly raised above lips, rectangular, indented medially on one or both sides, often marked by two or four bumps. Labial disc and medial lip dumbbell-shaped in face view. Medial lips reniform; lateral lips large, triangular, separated from medial lips and head region. Head region distinctly set off from regular body annules,

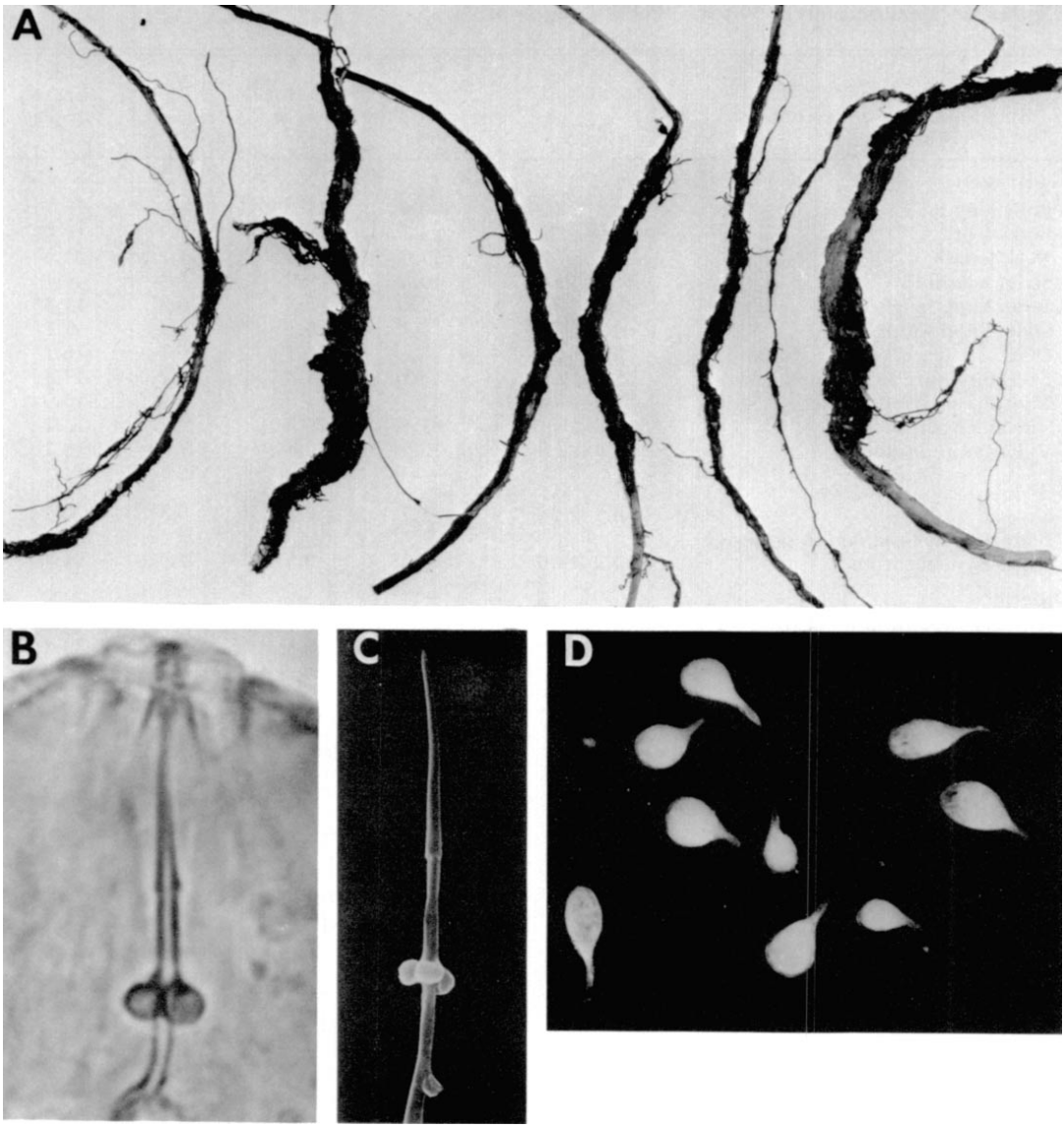

FIG. 1. Galls and females of *Meloidogyne pini* n. sp. A) Typical galls on sand pine (*Pinus clausa*) (one-half life size). B) Anterior portion of female with stylet (lateral). C) Scanning electron micrograph of excised stylet. D) Excised females showing some females with extremely long necks.

often marked with 1–2 incomplete annulations (Fig. 1B). In LM, cephalic framework weak, hexaradiate, lateral sectors slightly enlarged. Vestibule and vestibule extension prominent. Cephalids and hemizonids not observed. Excretory pore near level of stylet base. Stylet delicate; cone twice as long as shaft, tip straight or slightly curved dorsally. Shaft cylindrical; knobs rounded, set off from shaft, distinctly separated from each other. Distance of dorsal esophageal gland orifice 4.6 μm from stylet base; orifice branched into three channels; dorsal gland ampulla large. Subventral gland orifices branched, located immediately posterior to enlarged triradiate lumen lining of metacorpus. Esophageal glands with one large uninucleated dorsal lobe; two smaller nucleated subventral lobes usually posterior to dorsal lobe but extremely variable in size, shape, and position. Two small, rounded, nucleated esophago-intestinal cells located between metacorpus and intestine. Two gonads and six rectal glands as characteristic of genus.

Perineal pattern (Fig. 3) rounded to

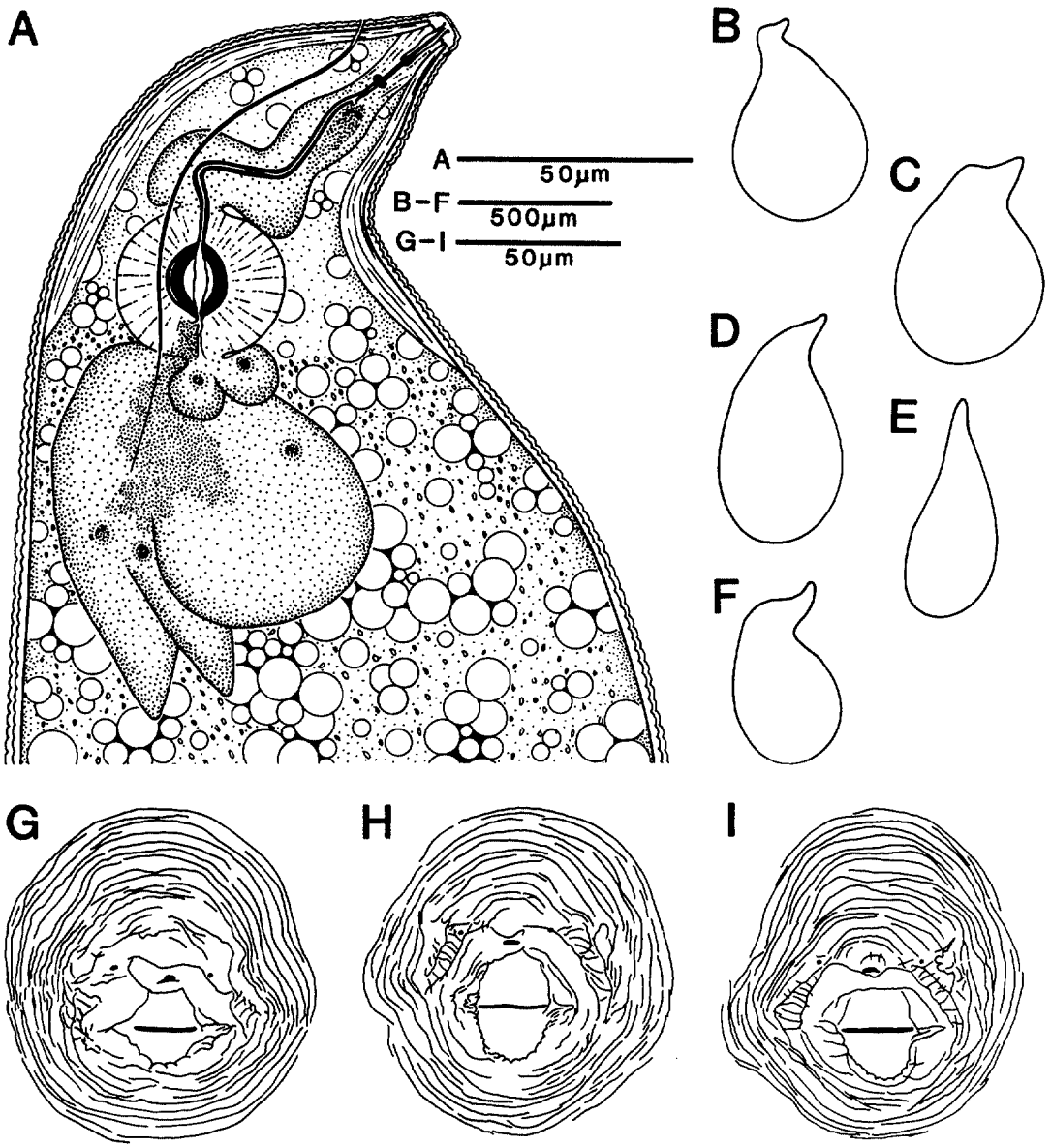

FIG. 2. Drawings of females of *Meloidogyne pini* n. sp. A) Anterior portion (lateral). B–F) Outlines of whole specimens (lateral). G–I) Perineal patterns.

ovoid, sometimes hexagonal. Striae coarse to fine. Dorsal arch flattened to high. Vulva located in depression; surrounded by wide cuticular, circular ridge; ridge often subdivided by 3–8 fine striae. Deep trough located between cuticular ridge and dorsal arch, marked by transverse striae. Perivulval region free of striae. Phasmids small, surface structure not apparent. Anus covered by cuticular flap.

Males

Measurements of 30 males in TAF are listed in Table 2.

Measurements (allotype in glycerin): Body length = 1,501.4 µm; body width = 38.7 µm; stylet length = 21.0 µm; stylet knob height = 2.7 µm; stylet knob width = 4.7 µm; dorsal esophageal gland orifice to stylet base = 4.9 µm; excretory pore to head end = 141.8 µm; tail length = 12.7 µm;

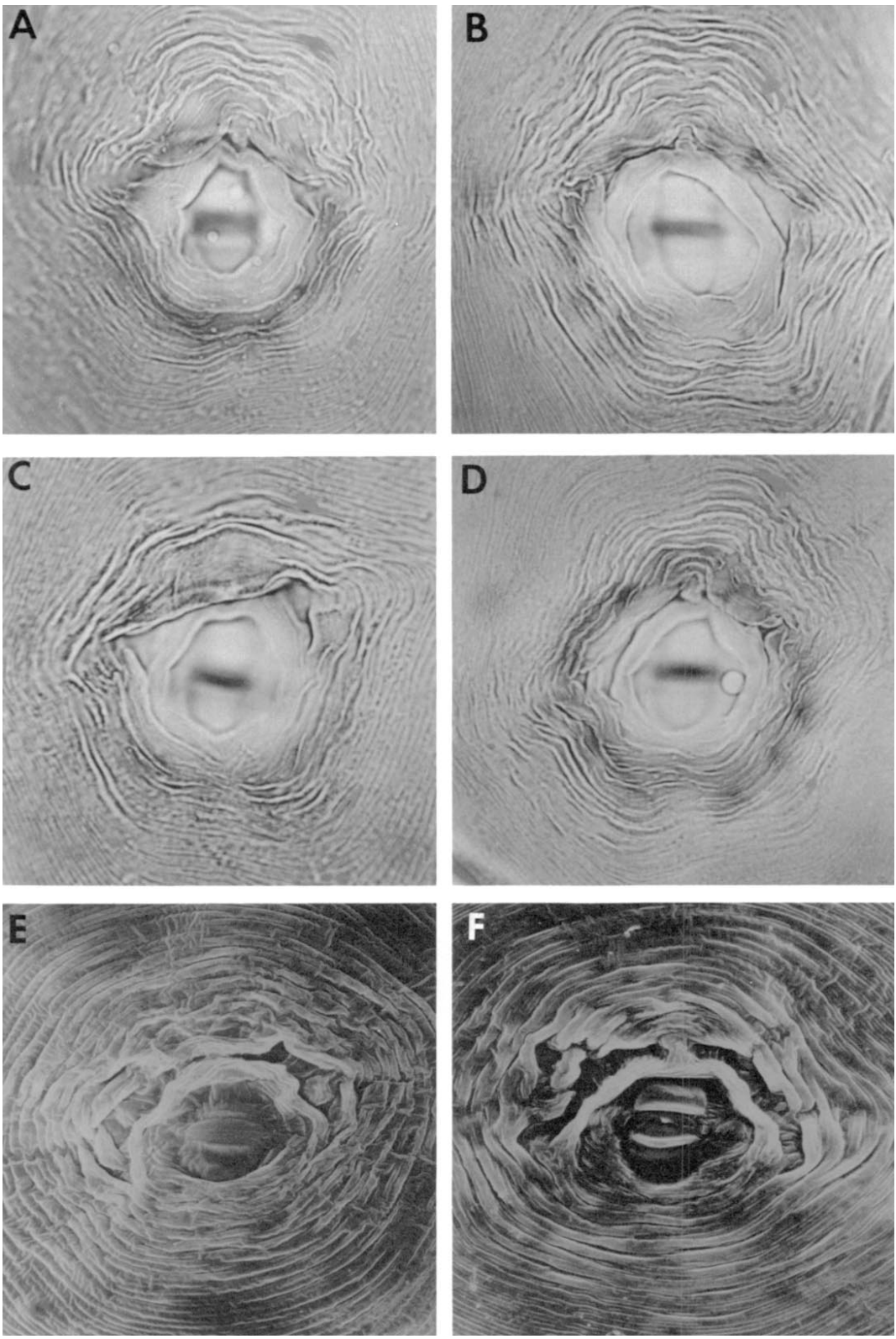

FIG. 3. Perineal patterns of females of *Meloidogyne pini* n. sp. A–D) Light micrographs. E, F) Scanning electron micrographs.

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

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (μm)					
Body length	989.6–1,637.6	1,374.5	29.0	158.7	11.6
Body width	29.0–43.2	35.3	0.6	3.4	9.7
Tail length	7.6–19.0	11.5	0.4	2.3	20.2
Stylet length	18.1–22.9	20.8	0.2	1.3	6.2
Stylet knob height	2.3–3.1	2.7	0.0	0.2	8.6
Stylet knob width	3.8–5.0	4.5	0.1	0.3	7.5
DGO	2.3–7.0	4.2	0.2	0.9	21.3
Excretory pore to head end	119.1–173.6	150.0	2.8	15.1	10.0
Spicule length	21.3–35.4	28.9	0.6	3.3	11.3
Gubernaculum length	6.5–10.2	7.8	0.2	1.0	12.8
Testis length	263.5–952.0	623.4	30.6	167.5	26.9
Ratios					
a	31.5–44.4	39.0	0.6	3.0	7.7
c	66.0–165.9	123.3	3.8	21.0	17.0
Body length/head end to posterior end of metacorpus	9.8–19.1	14.7	0.3	1.8	12.5
Percentages					
T	17.4–63.8	45.5	0.0	0.1	24.5

spicule length = 31.2 μm; gubernaculum not visible; testis length = 681.7 μm; a = 38.8; c = 118.1; and T = 45%. Male as in general description.

Description (Figs. 4A–C, 5): Body translucent white, vermiform, tapering anteriorly, bluntly rounded posteriorly, tail twisting through 90° in heat-killed specimens. Head cap low in lateral view, tapering posteriorly; head region low, slightly set off from body. Hexaradiate cephalic framework moderately developed; vestibule and extension distinct. Stoma slit-like, prestoma hexagonal, surrounded by pore-like openings of six inner labial sensilla (Fig. 5A, B). Labial disc rounded in face view, fused with medial lips. Medial lips small, crescent-shaped, sloping posteriorly. Four cephalic sensilla marked on medial lips by shallow, elongate ovoid, depressions. Amphidial apertures elongate, slit-like between labial disc and lateral sectors of head region. Lateral lips absent. Head region generally smooth, occasionally with one or two short annulations. Body annules distinct. Lateral field incisures four, two beginning near level of stylet knobs and two near level of metacorpus; lateral field areolated, encircling tail. Stylet moderate in size (Fig. 5C–E); cone twice length of shaft, straight, pointed; opening located several micrometers from tip; cone base wider than shaft; junction of cone and shaft uneven. Shaft cylindrical, posterior end wider than anterior end. Knobs large, set off from shaft, sometimes indented anteriorly. Dorsal esophageal gland orifice to stylet base variable in distance (2.3–7.0 μm), orifice branched into three channels, ampulla poorly defined. Procorpus distinct; metacorpus elongate, oval-shaped with enlarged, triradiate cuticular lumen lining; subventral esophageal gland orifices posterior to enlarged lining of median bulb, branched into several channels; ampullae distinct. Esophago-intestinal junction indistinct, at level of nerve ring. Gland lobe variable in length, with two to three nuclei. Excretory pore 119–174 μm from anterior, end, terminal duct delicate. Hemizonid 2–5 annules in front of excretory pore. Intestinal caecum extending anteriorly beyond level of nerve ring. Usually one testis, sometimes two, generally outstretched. Spicules arcuate with rounded base, single tip. Gubernaculum short and smooth. Tail short and rounded. Phasmids pore-like at level of cloaca.

Second-stage juveniles

Measurements of 30 juveniles in TAF are listed in Table 3.

Description (Figs. 4D–G, 6): Body trans-

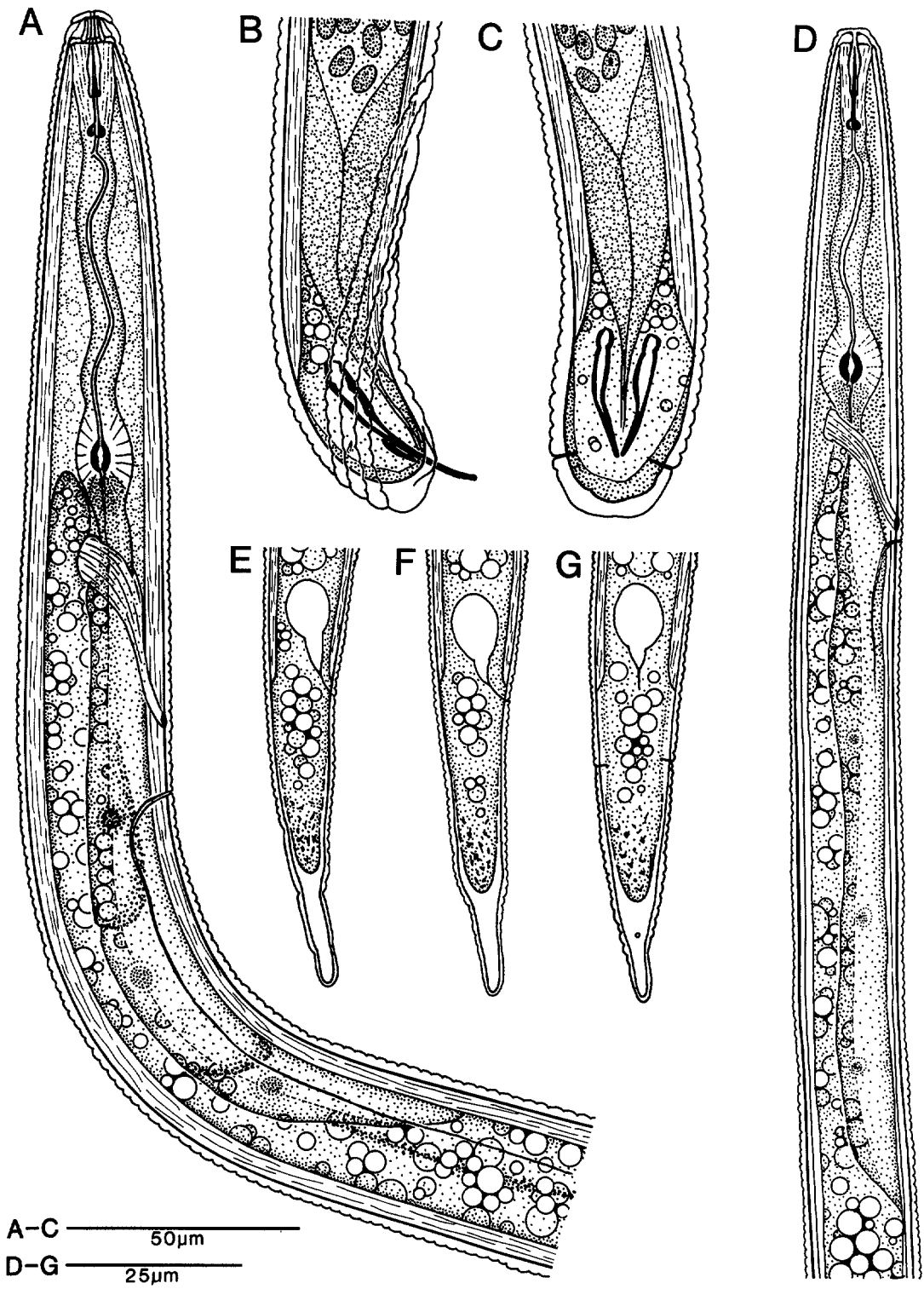

FIG. 4. Drawings of males and second-stage juveniles of *Meloidogyne pini* n. sp. A) Anterior portion of male (lateral). B) Male tail (lateral). C) Male tail (ventral). D) Anterior portion of second-stage juvenile (lateral). E, F) Tails of second-stage juveniles (lateral). G) Second-stage juvenile tail (ventral).

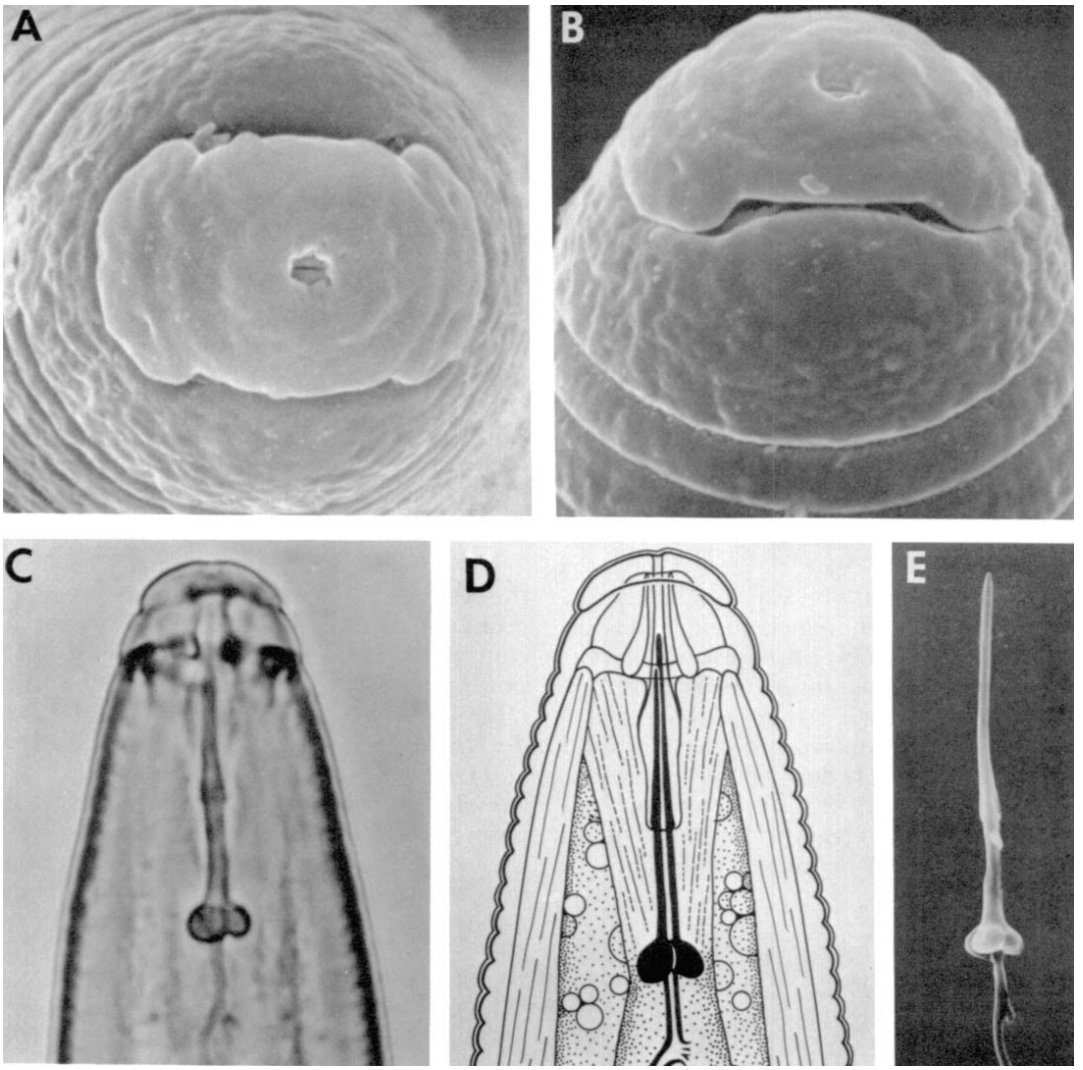

FIG. 5. Males of *Meloidogyne pini* n. sp. A, B) Scanning electron micrographs of anterior portion of head, face and lateral views. C) Light micrograph of head. D) Drawing of head. E) Scanning electron micrograph of excised stylet.

lucent white, vermiform, tapering posteriorly to a sharp point. Stoma slit-like, located in oval-shaped prestomatal depression, surrounded by pore-like openings of six inner labial sensilla (Fig. 6A, B). Labial disc rounded or rectangular, raised slightly above medial and lateral lips. Labial disc, medial lips, and elongate lateral lips fused. Amphidial apertures elongate oval, located between labial disc and lateral lips. Head region smooth, only slightly set off from body. Body annulation distinct. Lateral field begins as a ridge near level of stylet base. Two additional incisures appear near level of metacorpus, disappearing near anal opening, the outer two merging near tail terminus. Lateral field areolated. Hexaradiate framework weak by LM, vestibule and extension distinct. Stylet cone pointed, increases in width gradually; straight or slightly curved dorsally; shaft cylindrical, increasing in width posteriorly; knobs rounded and set off from shaft. Distance of dorsal esophageal gland orifice 3.1–4.4 μm; orifice branched into three channels; ampulla indistinct. Procorpus faintly outlined, metacorpus ovoid valve enlarged; subventral esophageal gland orifices posterior to valve; ampullae distinct. Esophago-intestinal junction indistinct, at level of

TABLE 3. Measurements of 30 second-stage juveniles of *Meloidogyne pini* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (μm)					
Body length	376.3–493.0	434.2	6.0	32.9	7.6
Body width	14.7–18.5	16.9	0.2	1.0	6.2
Tail length	37.0–53.4	44.4	0.7	3.6	8.1
Excretory pore to head end	64.8–91.2	80.0	1.1	5.9	7.4
Stylet length	11.4–14.1	12.8	0.1	0.7	5.1
Stylet knob height	1.5–2.1	1.7	0.0	0.1	7.8
Stylet knob width	2.6–3.4	3.0	0.0	0.2	7.0
DGO	3.1–4.4	3.7	0.1	0.4	10.8
Ratios					
a	21.8–29.1	25.7	0.3	1.9	7.2
c	7.5–11.8	9.8	0.2	0.9	9.2
Body length/head end to posterior end of metacorpus	5.4–7.9	6.8	0.1	0.5	7.4

nerve ring. Gland lobe variable in length, with three nuclei, overlapping intestine ventrally. Excretory pore distinct, hemizonid 1–2 annules anterior to excretory pore. Tail annules larger and irregular posteriorly. Rectum rarely dilated. Hyaline tail terminus clearly defined; tail tip broad, bluntly rounded; 1–2 fat droplets may occur in hyaline tail terminus. Phasmids small, indistinct, located below level of anus.

Eggs

Measurements (30 eggs in 2% formalin): Length = 87.8–117.2 μm (mean 102.9 ± 1.41 std. error of mean at 95% confidence interval); width = 41.3–52.7 μm (47.4 ± 0.52); length/width ratio = 1.77–2.51 (2.18 ± 0.03). Morphology indistinguishable from other *Meloidogyne* species.

Diagnosis: Meloidogyne pini n. sp. is morphologically distinct from all other described species in the genus. The perineal pattern and stylet morphology of the female, the shape of the male head and stylet morphology, and the J2 head and stylet morphology are useful diagnostic characters. Reproduction probably occurs by amphimixis, and the approximate somatic chromosome number is 2n = 18 (13; A. C. Triantaphyllou, pers. comm.). Biochemically, this new species has a unique phenotype of esterase activity (P. R. Esbenshade, pers. comm.).

General: The natural range of *Pinus clausa* is Florida. Sand pine occurs mainly on the Atlantic coast, less frequently along the gulf coast and inland, mainly in the north central portion of the state. In its range, sand pine occurs in light or light sandy, infertile, and slightly acidic soils which are derived from deep deposits of marine sand and clay (7).

Field observations indicate that *M. pini* n. sp. may have been introduced into Georgia by Florida nursery transplants. In one case, diseased sand pine trees obtained from a Florida source were adjacent to healthy trees obtained from a Georgia nursery (S. W. Oak, pers. comm.). *M. pini* n. sp. probably occurs within the natural range of sand pine in Florida.

Meloidogyne pini n. sp. from eastern Georgia parasitizes both Ocala and Choctawhatchie races of sand pine in the greenhouse. The North Carolina differential host plants—tobacco, cotton, pepper, watermelon, and tomato, as well as azalea and blueberry—were nonhosts. Loblolly pine and slash pine were parasitized and a few barren females developed on some of the seedlings.

Holotype (female): Isolated from greenhouse culture maintained on sand pine seedling (*Pinus clausa* (Chapm.) Vasey). Original population derived from type locality and host. Slide T-378t, USDA Nematode Collection (USDANC), Beltsville, Maryland.

Allotype (male): Same data as holotype. Slide T-379t, USDANC.

Paratypes (females, males and J2): Same

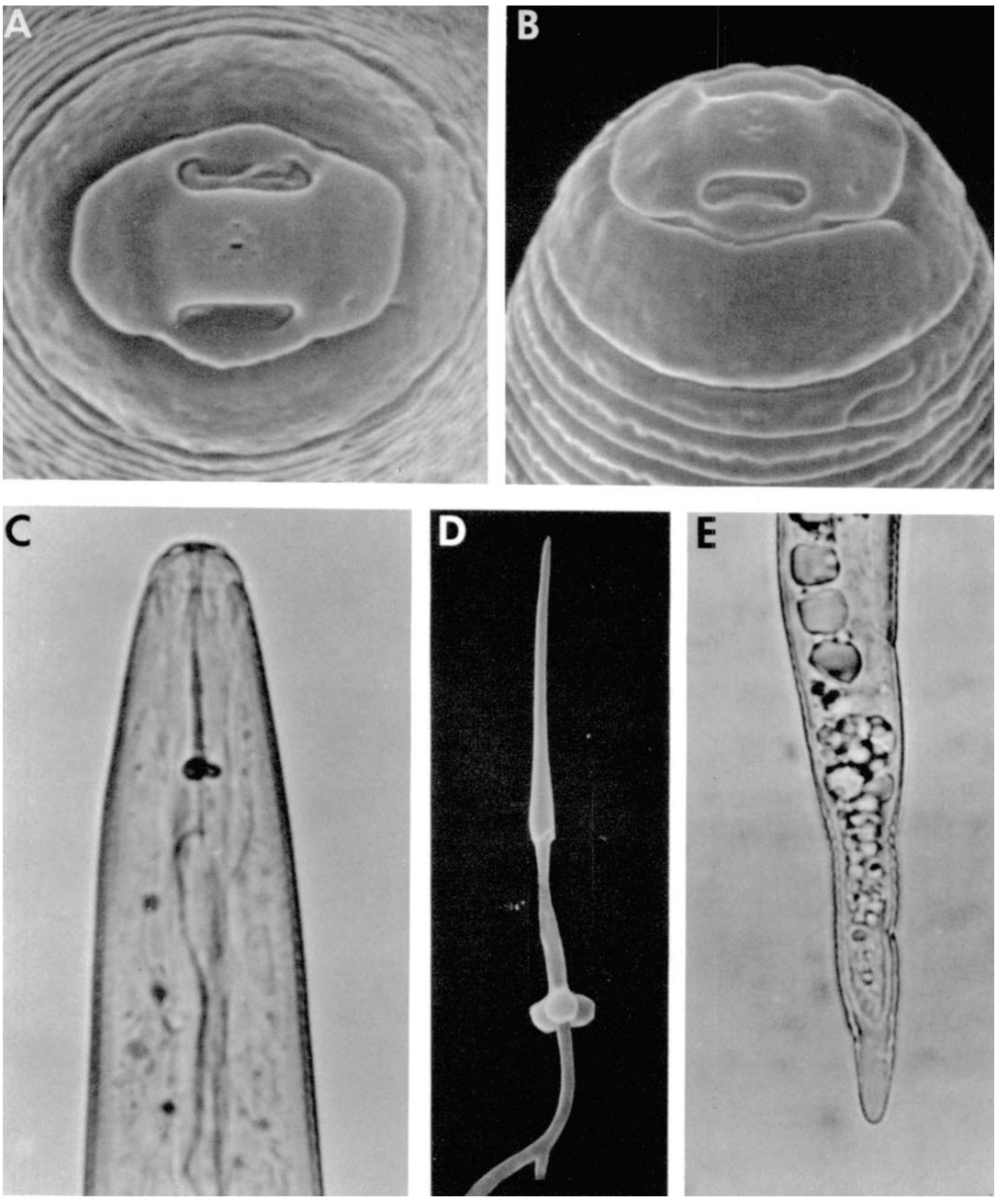

FIG. 6. Second-stage juveniles of *Meloidogyne pini* n. sp. A, B) Scanning electron micrographs of head, face, and lateral views. C) Light micrograph of head. D) Scanning electron micrograph of excised stylet. E) Light micrograph of tail.

data as holotype. USDANC, Beltsville, Maryland. University of California Davis Nematode Collection (UCDNC), Davis, California.

Type host and locality: Roots of sand pine tree (*Pinus clausa* (Chapm.) Vasey) in Tatnall County, Georgia, in a commercial plantation bordered by the Ohoopee River, Thomas Creek, and Georgia Route 121 south of Reidsville.

ADDITIONAL NOTES ON *MELOIDOGYNE MEGATYLA*

Additional morphological observations of *Meloidogyne megatyla* were made so that a more complete comparison could be made

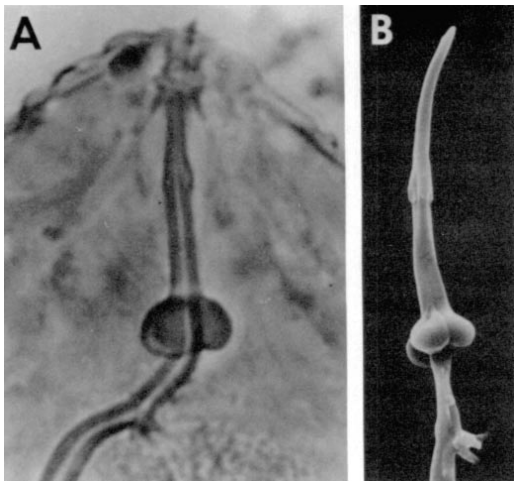

FIG. 7. Females of *Meloidogyne megatyla*. A) Light micrograph of anterior portion with stylet (lateral). B) Scanning electron micrograph of excised stylet.

with *M. pini* n. sp. Both species share common hosts, *Pinus* species, and probably have similar narrow host ranges. Light micrographs and SEM observations were not presented in the original description of *M. megatyla* (1), and a detailed morphological comparison of the two species was difficult without additional observations of *M. megatyla*.

Females: As described (1). Stylet broad (Fig. 7); cone gradually increasing in width posteriorly; shaft cylindrical or slightly wider posteriorly; junction of cone and shaft irregular. Knobs large, rounded, set off from shaft; often indented anteriorly. Perineal patterns (Fig. 8) as described.

Males: As described (1). Stoma slit-like, prestoma hexagonal, surrounded by pore-like openings of six inner labial sensilla (Fig.

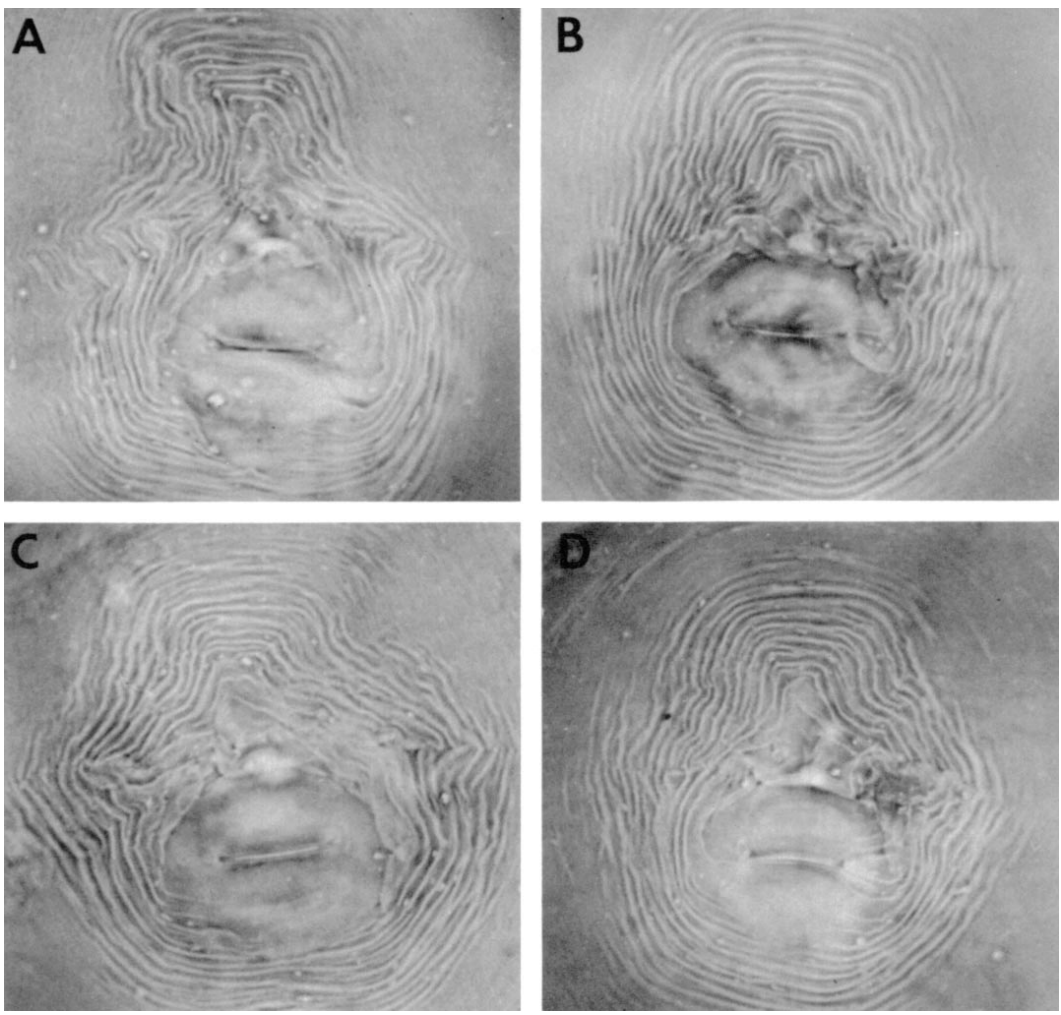

FIG. 8. Light micrographs of perineal patterns of *Meloidogyne megatyla*.

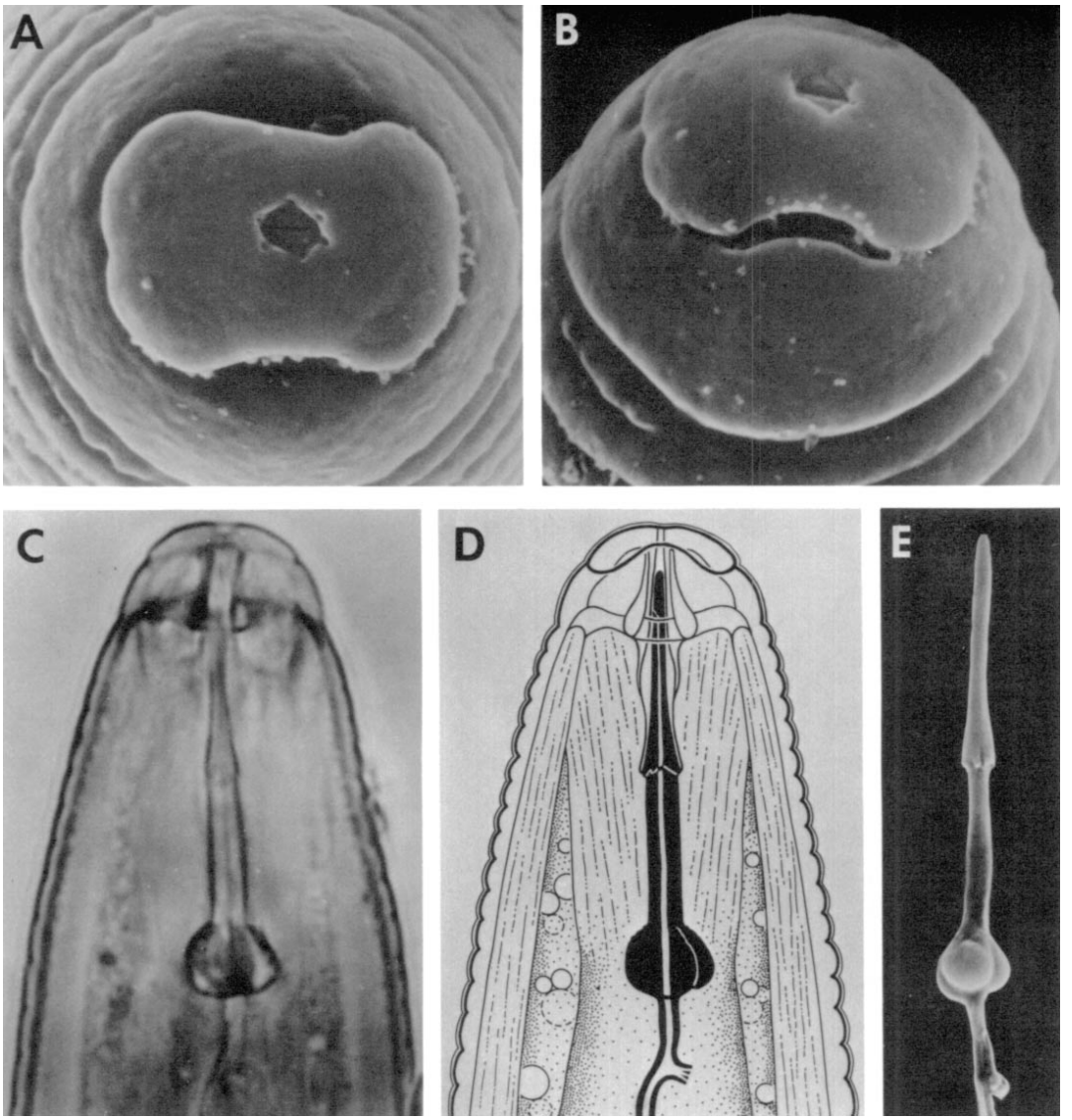

FIG. 9. Males of *Meloidogyne megatyla.* A, B) Scanning electron micrographs of anterior portion of head, face and lateral views. C) Light micrograph of head with stylet (lateral). D) Drawing of head. E) Scanning electron micrograph of excised stylet.

9A, B). Labial disc and medial lips fused, form one smooth continuous head cap. Cephalic sensilla sometimes visible on medial lips as four shallow depressions on cuticle. Slit-like amphidial apertures between labial disc and lateral sectors on head region. Lateral lips absent. Head region smooth. Body annulation distinct. Stylet (Fig. 9C–E) massive; cone straight; shaft cylindrical; knobs large, rounded, anterior margins irregular. Stylet opening located several micrometers from tip.

Second-stage juveniles: As described (1). Large slit-like stoma located in very large ovoid prestoma; surrounded by pore-like openings of six inner labial sensilla (Fig. 10A, B). Labial disc slightly raised above and fused with medial lips. Cephalic sensilla sometimes visible as four small cuticular depressions on medial lips. Medial lips sometime split into lip pairs on one or both sides. Amphidial apertures located between labial disc and medial lips. Lateral lips elongate to triangular, often fused with

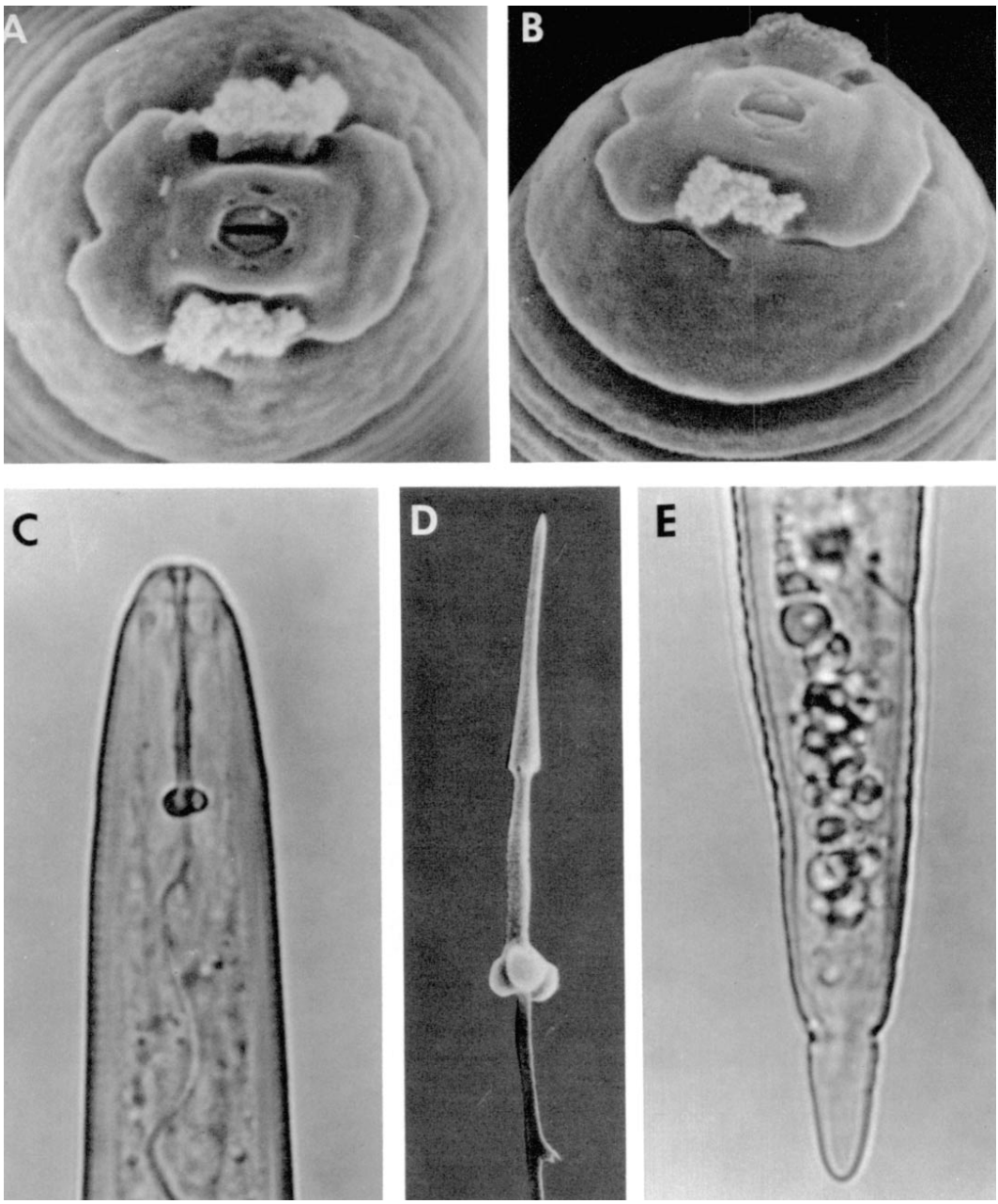

FIG. 10. Second-stage juveniles of *Meloidogyne pini* n. sp. A, B) Scanning electron micrographs of anterior portion of head, face and lateral views. C) Light micrograph of head. D) Scanning electron micrograph of excised stylet. E) Light micrograph of tail.

head region for a short distance. Head region smooth, body annulations distinct. Stylet robust (Fig. 10C, D); cone gradually increases in width, shaft cylindrical; knobs large, rounded, set-off from shaft. Tail short and broad (Fig. 10E). Hyaline tail terminus indistinct.

DISCUSSION

Perineal patterns of *M. pini* n. sp. and *M. megatyla* can be confused with patterns of other root-knot nematode species. Morphological variants of *M. pini* n. sp. appear similar to *M. arenaria* and *M. incognita* and possibly *M. javanica.* Perineal patterns of

M. megatyla are very similar to *M. incognita*, and the two species cannot be differentiated by perineal patterns alone. Reports of *M. arenaria* on slash and loblolly pine and *M. javanica* on slash pine (2,11) therefore need additional confirmation under controlled experimental conditions. Perhaps if the inoculum could have been quantified and applied with more precision, the loblolly and slash pines in our limited host range studies might have proven more suitable hosts than indicated.

Meloidogyne pini n. sp. is an economically important species. Diseased trees have been observed in commercial plantings at several locations in southeastern Georgia (S. W. Oaks, pers. comm.). Additional observations and research are necessary to determine the distribution and affect of this nematode on the cultivation of sand pine. Precise experiments are needed to demonstrate the role of the nematode in the etiology of diseases of pine and to detect interactions between the nematode and other organisms.

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