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Meloidogyne megatyla n. sp., a Root-Knot Nematode from Loblolly Pine[1]

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Abstract. Meloidogyne megatyla n. sp. is described from *Pinus taeda* in North Carolina. Stylet knobs are distinctively high in proportion to width, giving an especially massive appearance to the knobs of larvae and males. Mean larval length is 416 μm and stylet length is 14.6 μm. The perineal pattern is composed of smooth striae, with a high arch, and is often somewhat rectangular. The relationship of *M. megatyla* to other *Meloidogyne* species is unclear, although a comparison is made with *Meloidogyne incognita* and *Meloidogyne mali*. Galling was slight; only about 50 eggs were produced per egg mass, and under greenhouse conditions a single generation may take more than 10 weeks. *Meloidogyne megatyla* n. sp. did not reproduce on any of the differential hosts commonly used to distinguish among *Meloidogyne* species. *Key Words:* taxonomy, host range.

A soil sample from a residential lawn in Bladen County, North Carolina, was referred by the North Carolina State Plant Disease and Insect Clinic to Dr. K. R. Barker's laboratory for assay of nematodes. Distinctive juveniles (larvae) were collected which were referred to the senior author for specific identification. Additional soil and root samples were collected from the original site, and a population of *Meloidogyne* sp. was isolated from roots of loblolly pine (*Pinus taeda* L.). This nematode appeared to be morphologically different from other species of *Meloidogyne*, particularly with respect to the perineal pattern and the large stylet knobs of second-stage juveniles and males. Furthermore, its host response was different from that of other *Meloidogyne* spp. It is therefore designated a new species, *Meloidogyne megatyla* n. sp. (megas Gr. large and tylos, Gr. knob), and described below. A common name is suggested: "pine root-knot nematode."

Meloidogyne spp. have been previously reported in association with pine (2, 5, 12, 13), and the histopathology of an undescribed root-knot nematode species has been investigated on *Pinus ponderosa* Laws (10, 11).

MATERIALS AND METHODS

Stock cultures of *M. megatyla* n. sp. were established on loblolly pine seedlings with egg masses collected from the type locality and kept in the greenhouse at 22–28 C. All studies were made from specimens collected from those cultures and subcultures; morphological and morphometric studies were made from temporary slide preparations. Juveniles and males were generally examined within a few hours of killing with heat, and fixing and mounting in 2% formalin. Live specimens were also examined for comparison with fixed material.

Female head mounts were prepared by puncturing the posterior body ends in a drop of aqueous 1.5% NaCl and excising the heads in 2% formalin for mounting on slides. Perineal patterns were cut in 1.5% NaCl, cleaned in 45% lactic acid, and mounted in glycerin for observation.

Type males and juveniles were killed by heat and then prepared by Golden's method as described by Hooper (8) except that picric acid was omitted from the fixative. Type females were prepared as described by Hirschmann and Riggs (7).

Egg masses were removed from pine roots and individual eggs were recovered by vigorously stirring for 3 min in about 100 ml water containing 1.05% NaOCl. The eggs were collected and thoroughly rinsed with tap water on a 26 μm (500-mesh) sieve. They were then mounted in 2% formalin for examination; only one- and two-celled eggs were measured.

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Seedlings of loblolly pine and the following differential hosts were transplanted to 10-cm clay pots and inoculated with 8000 eggs per pot: corn (*Zea mays* L. cv 'Minn A401'), cotton (*Gossypium hirsutum* L. cv 'Deltapine 16'), peanut (*Arachis hypogaea* L. cv 'Florrunner'), pepper [*Capsicum annuum* L. (*C. frutescens*) cv 'California Wonder'] strawberry [*Fragaria chiloenis ananassa* Duch. cv 'Albritton'), sweet potato [*Ipomoea batatas* (L.) Poir. cv 'Allgold' and 'Puerto Rico'], tobacco (*Nicotiana tabacum* L. cv 'NC 95'), tomato (*Lycopersicon esculentum* Mill. cv 'Rutgers'), and watermelon (*Citrullus vulgaris* Schard. cv 'Charleston Gray'). Each treatment was replicated five times. Inoculum was prepared as an aqueous suspension of NaOCl-treated eggs from egg masses recovered from stock cultures on pine. After 84 days at greenhouse temperatures of 22–28 C, roots were washed and carefully examined for galls and egg masses.

SPECIES DESCRIPTION

ORDER: Tylenchida Thorne, 1949, Superfamily Heteroderoidea Filipjev and Schuurmans Stekhoven, 1941 (Golden, 1971), Family Meloidogynidae Skarbilovich, 1959 (Wouts, 1973), Subfamily Meloidogyninae Skarbilovich, 1959, Genus *Meloidogyne* Goeldi, 1887, *Meloidogyne megatyla* n. sp.

FEMALES: *Measurements of 25 females in 2% formalin.*—Body length (without neck): 406.0–690.8 μm (mean 559.68 μm, 95% confidence interval ± 31.67; body width: 210.6–491.4 μm (357.84 μm ± 32.05); neck length: 52.9–154.4 μm (103.04 μm ± 10.63); neck width: 37.3–112.0 μm (80.8 μm ± 7.89); bulb length: 32.3–56.0 μm (44.44 μm ± 2.85); bulb width: 31.1–58.5 μm (41.98 μm ± 2.90); a: 1.13–2.23 (1.61 ± 0.11).

Measurements of 20 excised female heads in 2% formalin.—Stylet length: 14.0–17.7 μm (16.43 μm ± 0.48); stylet base to head end: 14.9–25.0 μm (19.52 μm ± 1.35); stylet knob height: 2.2–3.5 μm (2.90 μm ± 0.15); knob width: 3.9–5.9 μm (4.99 μm ± 0.23); dorsal gland orifice to stylet knobs: 3.8–7.3 μm (5.04 μm ± 0.45); excretory pore to head end: 12.1–32.3 μm (20.44 μm ± 2.88); number of annules from excretory pore to head: 8–16 (11.25 ± 0.96).

Measurement from 30 perineal patterns in glycerin.—Vulval width: 21.2–28.2 μm (25.21 μm ± 0.75); perivulval (region surrounding vulva which is relatively free of striae, extending posteriorly to anus) height: 26.0–43.4 μm (35.47 μm ± 1.65); perivulval width: 29.9–49.8 μm (42.2 μm ± 1.62); interphasmidial distance: 12.9–22.5 μm (17.3 μm ± 1.09); anus (center) to vulva (center) distance: 15.2–23.4 μm (18.9 μm ± 0.77); perivulval height expressed as a ratio over perivulval width: 0.7–1.0 (0.84 ± 0.04).

Description (Fig. 1, 2).—Female white, variable in size, pear-shaped with gradually tapering neck and posteriorly rounded with no tail protuberance. Cuticle with maximum thickness about 10.0 μm and finely striated; cephalids not observed. Head slightly offset with two or three annules. Lip cap dorsoventrally elongate; amphidial openings apparently small and oval; six inner labial sensillae and four cephalic sensillae. Cephalic framework approximately hexaradiate with slightly enlarged lateral sectors. Stylet dorsally curved with large rounded knobs. Dorsal gland orifice branched into three channels; subventral gland orifices immediately posterior to medium bulb valve and also branched. Dorsal gland ampulla very large; gland lobe indistinct. Excretory pore often near level of stylet knobs but position highly variable. Two gonads as characteristic of genus.

Perineal pattern composed of deep, smooth, rarely broken coarse striae superimposed over fine, sometimes wavy striae. Dorsal arch high and broad, flattened dorsally, and coarse striae may diverge anteriorly (outward) as they approach single lateral lines. Striations interrupted but otherwise little altered at lateral line. Phasmidial ducts faintly visible, little or no phasmid surface structure. Anus covered by cuticular flap; tail terminus indistinct. Vulva generally surrounded by smooth perivulval region.

Measurements of holotype in glycerin.—Body length (without neck): 540 μm; body width: 480 μm; neck length: 140 μm; neck width: 110 μm; bulb length: 41.4 μm; bulb width: 38.0 μm; a: 1.13; stylet length: 15.0 μm; dorsal esophageal gland orifice to stylet knob: 4.6 μm; excretory pore to head end: 27.6 μm; number annules from excretory

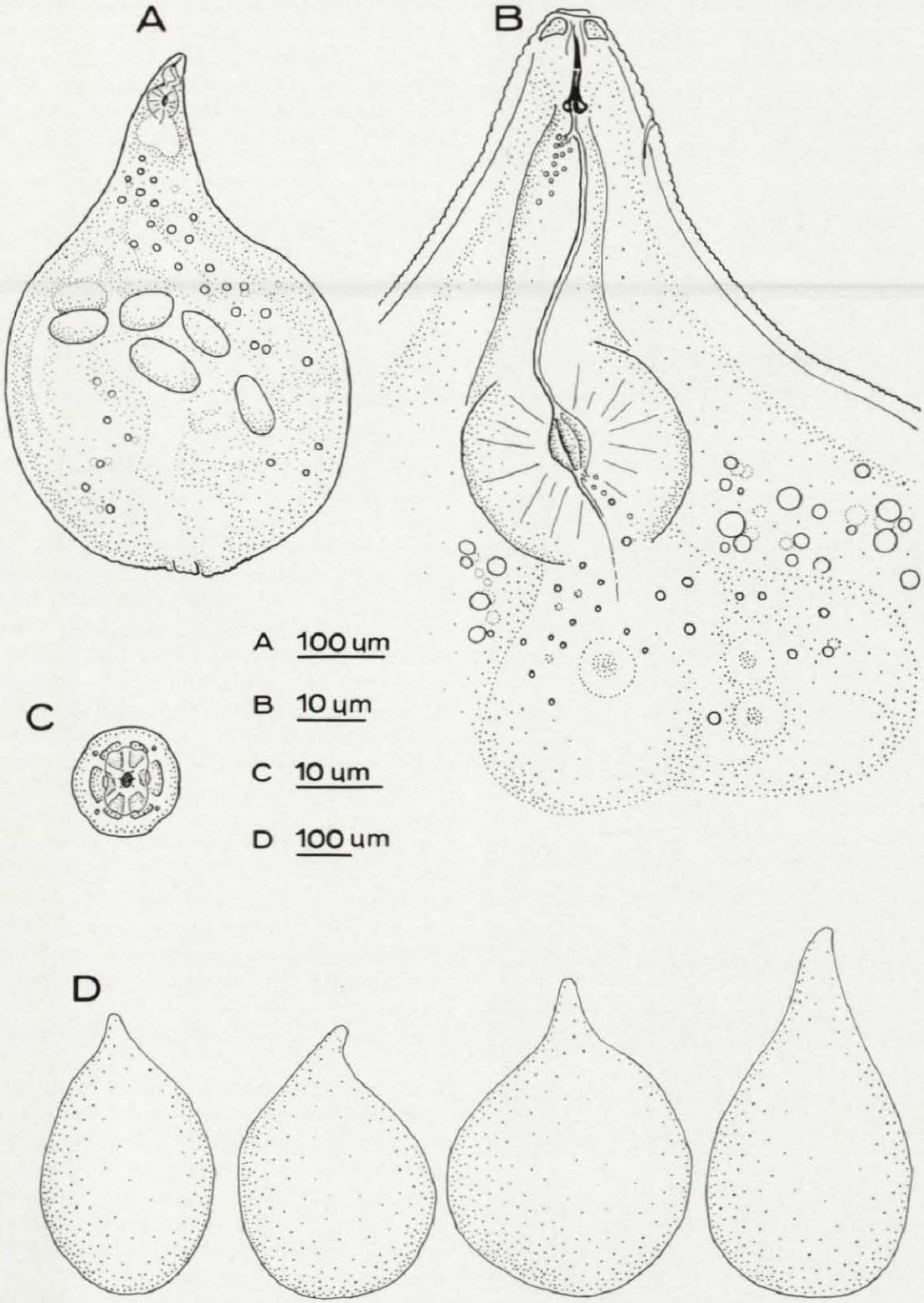
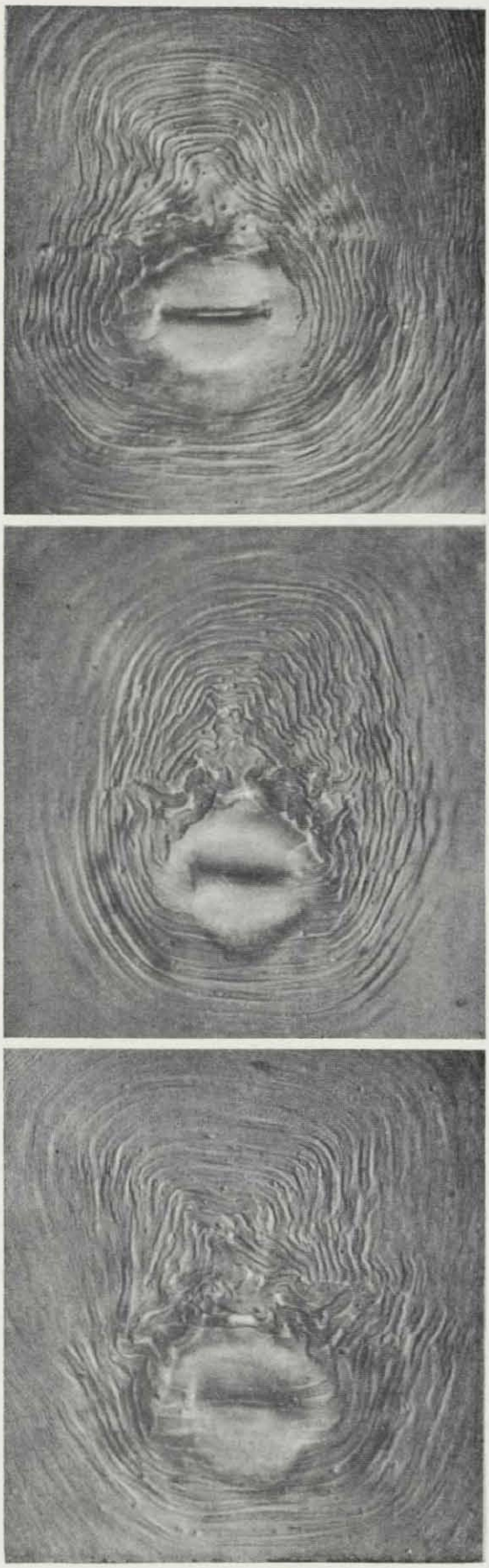


FIG. 1 (A–D). Drawings of females of *Meloidogyne megatyla* n. sp. *A)* Entire specimen (lateral). *B)* Anterior portion (lateral). *C)* Face view showing lip cap and framework. *D)* Outline of specimens of varying size and shape.



pore to head end: 11; anus to vulva distance (lateral view): 22.9 μm; cuticle thickness: 9.40 μm. Female as in general description. Body slightly flattened posteriorly with large neck region. Reproductive system relatively clearly defined with nine eggs.

EGGS: *Measurement of 30 eggs in 2% formalin.*—Length: 88.5–102.4 μm (mean 95.74 μm, 95% confidence intervals ± 1.26); width: 43.1–56.7 μm (48.3 μm ± 1.21); length/width ratio: 1.7–2.2 (2.2 ± 0.04).

Description.—Not distinctive from eggs of other *Meloidogyne* spp. Egg masses small with generally less than 50 eggs per mass.

MALES: *Measurement of 20 males in 2% formalin.*—Body length: 960–1545 μm (mean 1312.6 μm, 95% confidence interval ± 68.39); body width: 30.3–42.4 μm (35.3 μm ± 1.26); stylet length: 21.7–25.5 μm (23.9 μm ± 0.54); stylet base to head end: 22.2–28.3 μm (25.7 μm ± 0.54); stylet knob height: 3.9–5.1 μm (4.5 μm ± 0.16); stylet knob width: 4.2–6.3 μm (5.2 μm ± 0.18); stylet knob plus shaft length: 11.4–13.1 μm (12.3 μm ± 0.22); dorsal esophageal gland orifice to stylet base: 4.2–6.3 μm (5.2 μm ± 0.25); esophagus length (center of metacorpus valve to head end): 106.1–149.5 μm (128.5 μm ± 7.11); anterior end of testes to tail end: 616–1182 μm (829 μm ± 67); spicule length: 29.3–36.9 μm (33.7 μm ± 1.4); phasmid to tail end: 6.1–12.1 μm (9.4 μm ± 0.79); a: 30.9–42.0 (37.2 ± 1.67); b: 10.9–17.4 (15.1 ± 0.69); T% (distance from anterior end of testis to tail end, expressed as % of body length): 44–82% (63% ± 4.4%); excretory pore % (distance from excretory pore to head end as % of body length): 8.1–14.7% (10.5% ± 0.61).

Description (Fig. 3).—Body vermiform, tapering and rounded at both ends. Heat-killed specimens curve concavely on ventral side and tail twists through 90°. Lateral field with four lines, spaced approximately equally throughout most of lateral field; areolation and crenation indistinct anteriorly but very pronounced posteriorly. Anteriorly, outer lines originate at about level of stylet knobs, inner lines begin very close together, or as a single line at about

FIG. 2. Female perineal patterns showing variation typical for *Meloidogyne megatyla.*

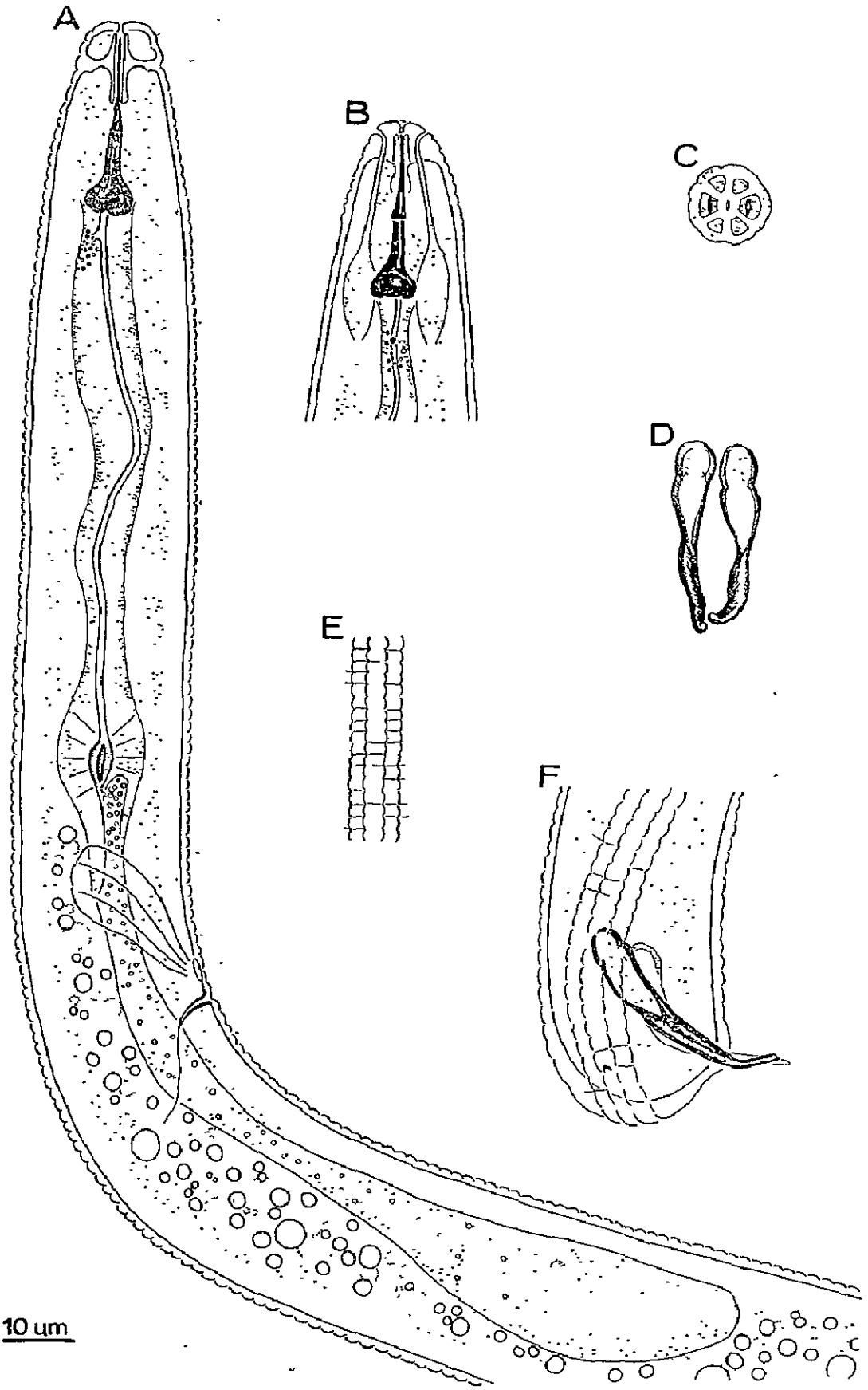


FIG. 3 (A–F). Drawings of males of *Meloidogyne megatyla* n. sp. *A)* Anterior portion (lateral curvature of specimen for convenience in illustrating). *B)* Cephalic region (dorsal). *C)* Face view showing cephalic framework. *D)* Spicules (dorsal). *E)* Lateral field. *F)* Tail (lateral).

midregion of procorpus. Occasionally four lateral lines separated by faint intermediate lines. Head usually with three annules dorsally and two ventrally. Labial disc not pronounced; amphidial openings apparently small and elongate, and amphidial cheeks indistinct. Labial and cephalic sensillae as described for female. Cephalic framework moderately heavy and hexaradiate with lateral sectors only slightly enlarged. Stylet heavy with massive rounded knobs. Amphidial glands broadest near level of knobs. Cephalid not observed. Orifice of dorsal gland duct branched as in female, but gland ampulla small and poorly defined. Metacorpus of esophagus oval and only slightly broader than procorpus, valve large and well-developed with branched ducts of subventral gland orifices near its base. Poorly defined esophago-intestinal junction at level of nerve ring; intestinal caecum extends anteriorly to level of subventral gland orifices. Gland lobe variable in length, but more than 100 μm long with two distinct nuclei. Excretory pore pronounced with hemizonid located immediately anterior. Spicules arcuate; gubernaculum not clearly observed. One testis or two testes. Tail short with cloaca opening generally very far posterior, near level of phasmids.

Measurements of allotype in glycerin.—Body length: 1367 μm; body width: 34.0 μm; stylet length: 22.0 μm; stylet base to head end: 25.3 μm; stylet knob height: 4.3 μm; stylet knob width: 5.2 μm; stylet knob plus shaft length: 11.4 μm; dorsal esophageal gland orifice to stylet base: 6.0 μm; esophagus length: 92.0 μm; excretory pore to head end: 145.5 μm; anterior end of testis to tail end: 970 μm; spicule length: 30.0 μm; phasmid to tail end: 10.0 μm; a: 40.2; b: 14.9; T%: 80.0%; excretory pore %: 10.6%. Male as in general description, although glycerin preparation may have resulted in slight shrinkage, particularly of esophagus.

SECOND-STAGE JUVENILES: *Measurements of 23 juveniles in 2% formalin.*—(Table 1). Description (Fig. 4).—Body veriform, tapering slightly anteriorly and more

TABLE 1. Measurements of 23 second-stage juveniles of *Meloidogyne megatyla* n. sp.

Character	Range	Mean	95%	99%	Standard deviation	Coefficient of variability (%)
Linear (μm)						
Body length	392.6–457.1	416.42	± 7.78	± 10.57	17.99	4.3
Body width	14.7– 17.9	16.24	± 0.32	± 0.44	0.75	4.6
Stylet length	13.8– 16.6	14.61	± 0.28	± 0.38	0.65	4.5
Stylet base to head end	16.4– 17.7	16.95	± 0.18	± 0.24	0.41	2.4
Stylet knob height	2.1– 2.5	2.26	± 0.04	± 0.06	0.10	4.5
Stylet knob width	2.5– 3.4	3.02	± 0.10	± 0.14	0.24	8.0
Dorsal esophageal gland orifice to stylet base	4.2– 5.9	5.09	± 0.21	± 0.28	0.48	9.5
Esophagus length (center of valve to head end)	52.7– 63.5	58.57	± 1.01	± 1.37	2.33	4.0
Excretory pore to head end	76.2– 97.3	83.06	± 1.83	± 2.49	4.24	5.1
Genital primordium to tail end	130.1–184.0	154.06	± 4.73	± 6.43	10.94	7.1
Phasmid to tail end	18.4– 41.0	32.55	± 2.21	± 3.01	5.12	15.7
Tail length (anus to tail end)	31.6– 45.1	39.77	± 1.27	± 1.72	2.93	7.4
Tail width (at anus)	10.5– 16.4	11.97	± 0.54	± 0.73	1.25	10.4
Ratios						
a	21.9– 28.8	25.70	± 0.80	± 1.08	1.84	7.2
b	6.7– 7.8	7.11	± 0.14	± 0.18	0.31	4.4
c	9.5– 13.5	10.52	± 0.38	± 0.52	0.88	8.4
d	2.5– 3.9	3.34	± 0.14	± 0.19	0.32	9.5
Percentages						
Genital primordium to tail end expressed as % of body length	32.2– 43.0	37.02	± 1.10	± 1.49	2.54	6.9
Excretory pore to head end expressed as % of body length	18.0– 23.3	19.97	± 0.46	± 0.63	1.07	5.4

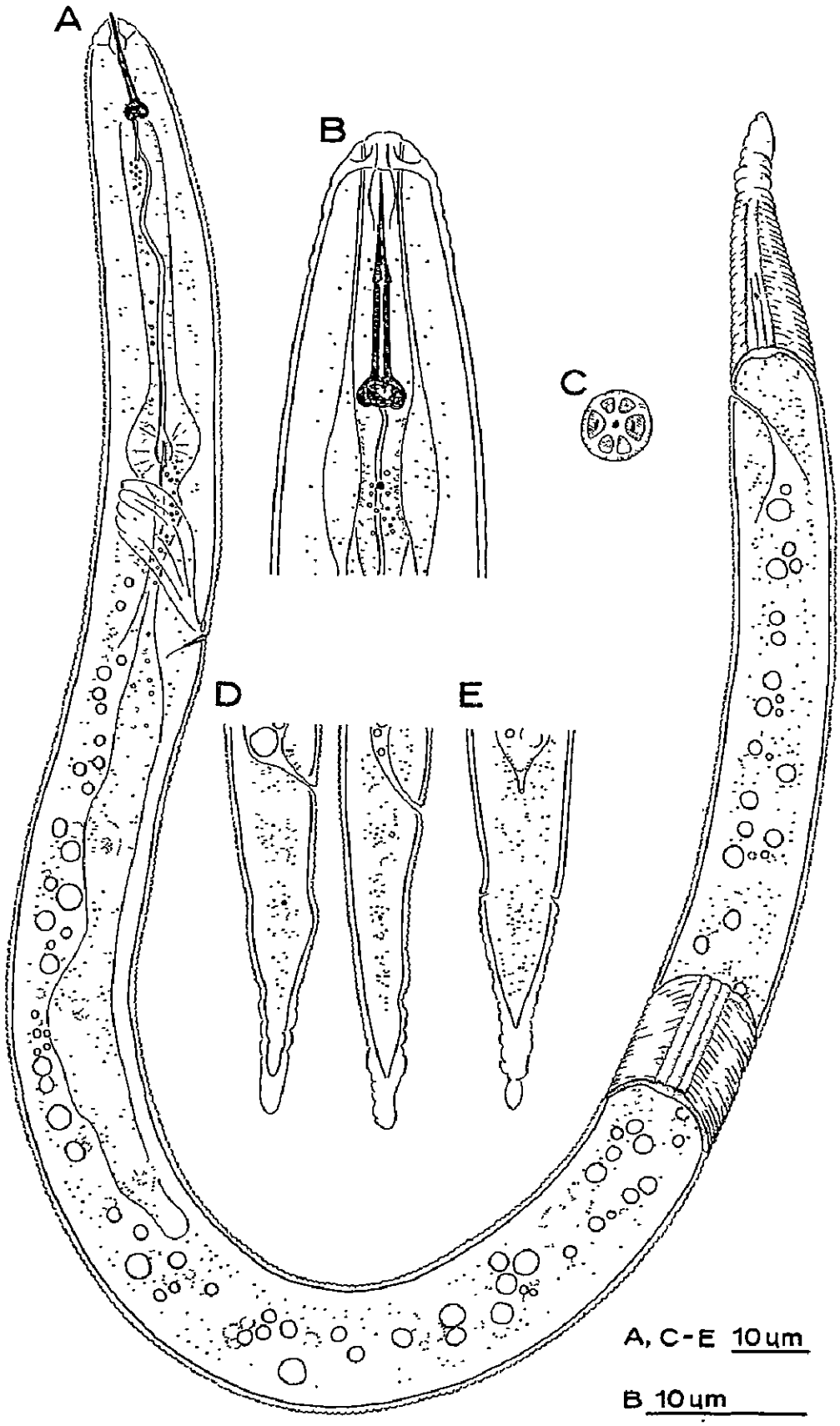


FIG. 4 (A–E). Drawings of larvae of *Meloidogyne megatyla* n. sp *A)* Entire specimen (lateral curvature of specimen for convenience in illustrating). *B)* Cephalic region (dorsal). *C)* Face view showing cephalic framework. *D)* Tails (lateral). *E)* Tail (ventral).

pronouncedly posteriorly. Heat-killed specimens straight or curved slightly concavely on ventral side. Lateral field with four lateral lines similar to those of male, except anterior origin may be at level of posterior half of procorpus, and lines terminate 4–5 μm from tail tip rather than encircling it. Head slightly offset, usually with three annules dorsally and two ventrally. Labial disc not pronounced, amphidial openings apparently small and oval. Labial and cephalic sensillae as described for adults. Cephalic framework hexaradiate with lateral sectors slightly enlarged. Stylet robust with large rounded knobs. Amphidial gland broadest near level of dorsal orifice. Cephalids not observed. Metacorpus about twice the diameter of procorpus. Poorly defined esophago-intestinal junction posterior to nerve ring; small intestinal caecum. Gland lobe variable in length with two distinct subventral gland nuclei and one less distinct dorsal gland nucleus. Tail gradually tapering; terminus with enlarged annules, sometimes smooth at tip or forming an elongate swelling. Rectum not inflated.

DIAGNOSIS: Meloidogyne megatyla n. sp. is distinct from other *Meloidogyne* species. Therefore, a comparison of the perineal pattern with that of *Meloidogyne incognita* Chitwood, 1949 (3), and the juveniles with *Meloidogyne mali* Itoh *et al.*, 1969 (9), is not intended to imply a close phylogenetic relationship among these three species. The perineal patterns of *M. megatyla* and *M. incognita* both have a high dorsal arch and irregular single lateral lines. Although the perineal pattern of *M. incognita* has been described as highly variable, it has been noted to be composed generally of wavy broken coarse striae which form a high arch, and dorsal and ventral coarse striae that tend to fork as they approach lateral lines (3, 6, 14). Although *M. megatyla* also has a high arch, the furrows are generally smooth and seldom broken in the dorsal region [approximately zone 4 (6)]. The truncate pattern, which is characteristic of coarse striae in the dorsal arch, begins close to the anus. Striae may fork as they approach the lateral lines. In certain individuals, "wings" tend to occur at the lateral lines (zone 3), and in some specimens the dorsal arch (zone 4) is nearly as broad as the ventral sector (zone 2). Many patterns are dorsally and ventrally flattened and are somewhat rectangular, as are patterns of *Meloidogyne brevicauda* Loos.

Juveniles of *M. megatyla* are similar in size to those of *M. mali*. However, they are distinct with respect to tail length (mean 31 μm in *M. mali*, mean 39.8 μm in *M. megatyla*) and tail width (mean 8.5 μm in *M. mali*, mean 12.0 μm in *M. megatyla*); in addition, stylet knobs of juveniles and males are much larger in *M. megatyla* than those illustrated for *M. mali* (9).

Holotype.—Female. Isolated from *P. taeda* seedling, cultured in greenhouse and established from type locality. Collected January, 1978. Slide No. 34, University of California Nematode Collection, Riverside, California.

Allotype.—Male. Same date as for female. Slide No. 35, of same California collection.

Paratypes.—Females (whole mounts, perineal patterns, head mounts), males, larvae. Same data as holotype; same California collection, as well as USDA Nematode Collection, Beltsville, Maryland, and Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida.

Type host and type habitat.—Loblolly pine, *Pinus taeda* L., roots.

Type locality.—Property surrounding residence of Mrs. Wallace Baxley, U.S. Highway 701, Elizabethtown, North Carolina, 28337, about 2 m from north side of house.

BIOLOGY: Galling was slight and proliferation of ectomycorrhiza generally surrounded the immediate vicinity of infection. A single root tip was often infected with several females, even when overall infection of a plant was light. Reproduction on pine was very slow under greenhouse conditions (apparently a single generation may take more than 10 weeks), and we rarely observed more than 50 eggs in a given egg mass. Males might be produced only under specific conditions which we have not yet defined; only on one occasion were large numbers recovered from a greenhouse culture.

No signs of nematode infection were observed on the differential hosts: corn, cotton, peanuts, pepper, strawberry, sweet potato, tobacco, tomato, watermelon. It has not yet been determined whether conifers other than *P. taeda* are susceptible.

DISCUSSION

Some morphometrics initially included in this study were later deleted because of necessary subjectivity in their determination or extreme variation. For example, tail length was not included for males because the cloacal opening is far posterior, and a localized tail tip is not evident on the rounded posterior. Although distance of the phasmid to the posterior terminus in males was included, similar difficulties are apparently reflected in the large range for this measurement. Length of the gubernaculum was omitted. It may be present but so weakly sclerotized that we were unable to resolve it with confidence. In females, males, and juveniles, position of the esophageal gland nuclei was too variable to be of value. Location of the excretory pore relative to the anterior end of the female was included because it is a commonly-used measurement among *Meloidogyne* spp., but it is highly variable in *M. megatyla*. Neck length of the female is also a commonly-used measurement among *Meloidogyne* spp., but the posterior origin of the neck may be difficult to precisely determine.

Measurement of stylet length is sometimes considered to be a problem because the tip may be obscure; some authors have suggested substituting a measurement from the base of the stylet knobs to the anterior end of the specimen (6). In the present study both measurements are included, and among juveniles, variability is less for the latter measurement than for stylet length *per se*. However, in females and males the reverse is true, perhaps because stylets are larger, and the stylet may have been fixed in varying degrees of protraction.

Electron microscope observations have demonstrated that the amphidial cheek, first described by Cobb (4), is the outermost part of the lateral lip which is separated from the remainder of the lip region by the slitlike amphidial opening (1). Although Cobb used the lateral cheek as a basis for placing root-knot nematodes in a distinct genus, Whitehead's (15) illustrations suggest considerable variation in cheek size among *Meloidogyne* spp. Reduction of cheeks in *M. megatyla* probably should not preclude its placement in the genus *Meloidogyne*. Scanning electron microscope observations are needed to further elucidate apparent variation of cheeks among species of *Meloidogyne*.

Meloidogyne megatyla did not reproduce on any of the annuals used as differential hosts. In addition, we noted that it has a long life cycle on pine, and that a given female apparently produces relatively few eggs. It might be expected that *Meloidogyne* species of relatively long-lived hosts, such as pine, would have narrower host ranges than populations of species which must be adapted to ecosystems with seasonally changing flora. Many successive nematode generations on a given host could result in a high level of parasitic specialization. Furthermore, *Meloidogyne* species of long-lived hosts might not be subject to as strong a selection pressure for short generations and production of large numbers of eggs, as are other species.

The geographic distribution of *M. megatyla* is not known, although *Meloidogyne* populations with similarly proportioned stylet knobs have been collected from *P. taeda* in Florida (unpublished observations). The relationship between *M. megatyla* and an undescribed *Meloidogyne* species collected from *Pinus ponderosa* in New Mexico (10, 11) has not been determined, although the host-parasite relationship of *M. megatyla* on *P. taeda* seems to be similar to that reported for the undescribed species of *P. ponderosa*.

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