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Meloidogyne trifoliophila n. sp. (Nemata: Meloidogynidae), a Parasite of Clover from Tennessee[1]

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Abstract: Meloidogyne trifoliophila n. sp. is described from white clover collected at Ames Plantation, Fayette County, Tennessee. The perineal pattern is rounded, with long, smooth striae and rounded arch, and without distinct lateral lines or perivulval striae. The female stylet is 12.6–15.5 µm long, the excretory pore is level with or up to one stylet length posterior to the stylet knobs, and the vulva is subterminal. The posterior terminus is weakly protuberant. The male lateral field is composed of approximately eight repeatedly broken or forked incisures. The male stylet is 17.0–18.9 µm long, the stylet knobs are rounded and sloping, gradually merging with the shaft, and the head region consists of one large annule. Second-stage juveniles are 357–400 µm long, with a stylet length of 11.9–13.6 µm and one head annule. The tail tapers to a slender tip. This new species is similar to *M. graminicola* and *M. triticoryzae* but differs from them in perineal pattern and lateral field morphology, and numerous morphometric characters.

Key words: clover, clover root-knot nematode, *Meloidogyne graminicola, Meloidogyne trifoliophila, Meloidogyne triticoryzae,* nematode, new species, root-knot nematode, scanning electron microscopy, taxonomy, Tennessee, *Trifolium* spp.

During a survey of nematodes in a tall fescue pasture, a root-knot nematode was collected that resembled *Meloidogyne graminicola* Golden & Birchfield, 1965. This isolate proved to be a parasite of white clover (*Trifolium repens* L.), also growing in the pasture (Kimmons et al., 1990) and was demonstrated to be a successful parasite on all tested *Trifolium* spp. (Bernard, 1989). The morphology of this nematode placed it in Group 11 of Jepson (1987), but it did not fit any of that group or other known species of *Meloidogyne.* It is herein described as *M. trifoliophila* n. sp. Because of its ability to parasitize *Trifolium* spp., we suggest the common name, clover root-knot nematode.

Materials and Methods

Cultures of *M. trifoliophila* were reared on ball clover (*T. nigrescens* L.) in the greenhouse in autoclaved soil to eliminate *M. incognita* (Kofoid & White) Chitwood, a minor constituent of the original field collection. Ball clover has sometimes been reported as a host of *M. incognita* (Pederson and Windham, 1989; Windham and Pederson, 1992) but was highly resistant to the contaminating isolate originally found in the field collection (Bernard and Jennings, unpubl.). All the morphological statements in this paper were made from these cultures. Males were collected by dissecting galls, and juveniles were collected by incubating eggs in water at room temperature. For permanent slides, nematodes were fixed in hot (80 °C) 4% formalin and then processed to glycerin with Seinhorst's rapid method (Seinhorst, 1959). Measurements and drawings were made from nematodes permanently mounted in glycerin. Photographs with light microscopy (LM) were made within 4 hours after nematode extraction from root tissue. Males and females were prepared for scanning electron microscopy (SEM) as previously described (Eisenback and Hirschmann, 1980a,b).

Systematics

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

Females: Measurements of the holotype and 20 paratype females are listed in Table 1. Body milky white, oval to pear-shaped, neck usually short, not bent; vulval region not protuberant (Fig. 1A,B). Cephalic framework weakly developed; lip region low,

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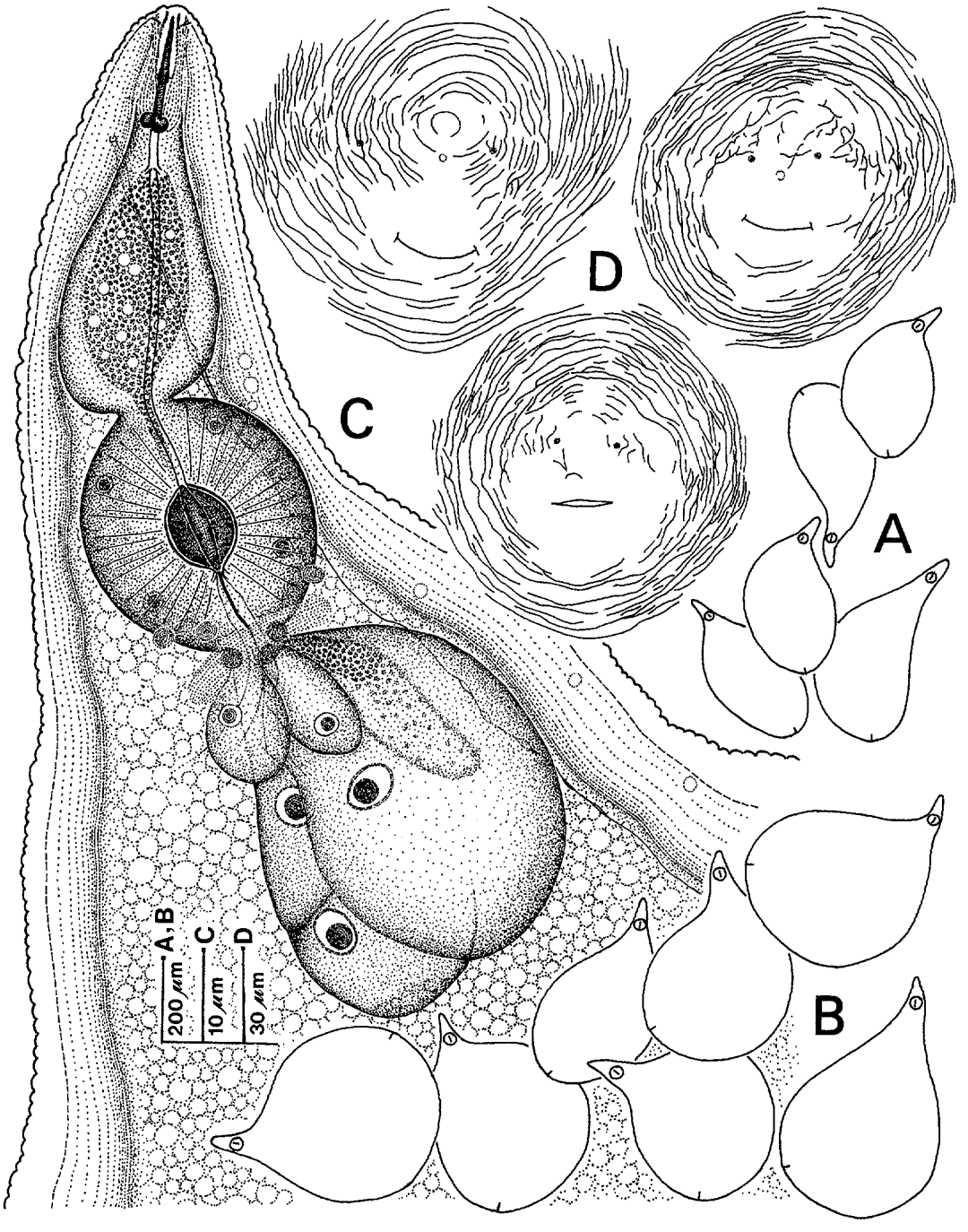

FIG. 1. Drawings of females of *Meloidogyne trifoliophila* n. sp. A) Outlines of young females. B) Outlines of fully expanded females. C) Anterior region. D) Perineal patterns.

rounded (Figs. 1C,2A). In SEM (Fig. 2C,D), labial disc obscure, fused with medial lips. Medial lips divided into distinct lip pairs, each lip bilobed. Lateral lips distinct, triangular. Head annule not clearly demarcated, anterior annules sometimes with bead-like extensions (Fig. 2C,D). Excretory pore level with stylet knobs or up to one stylet length

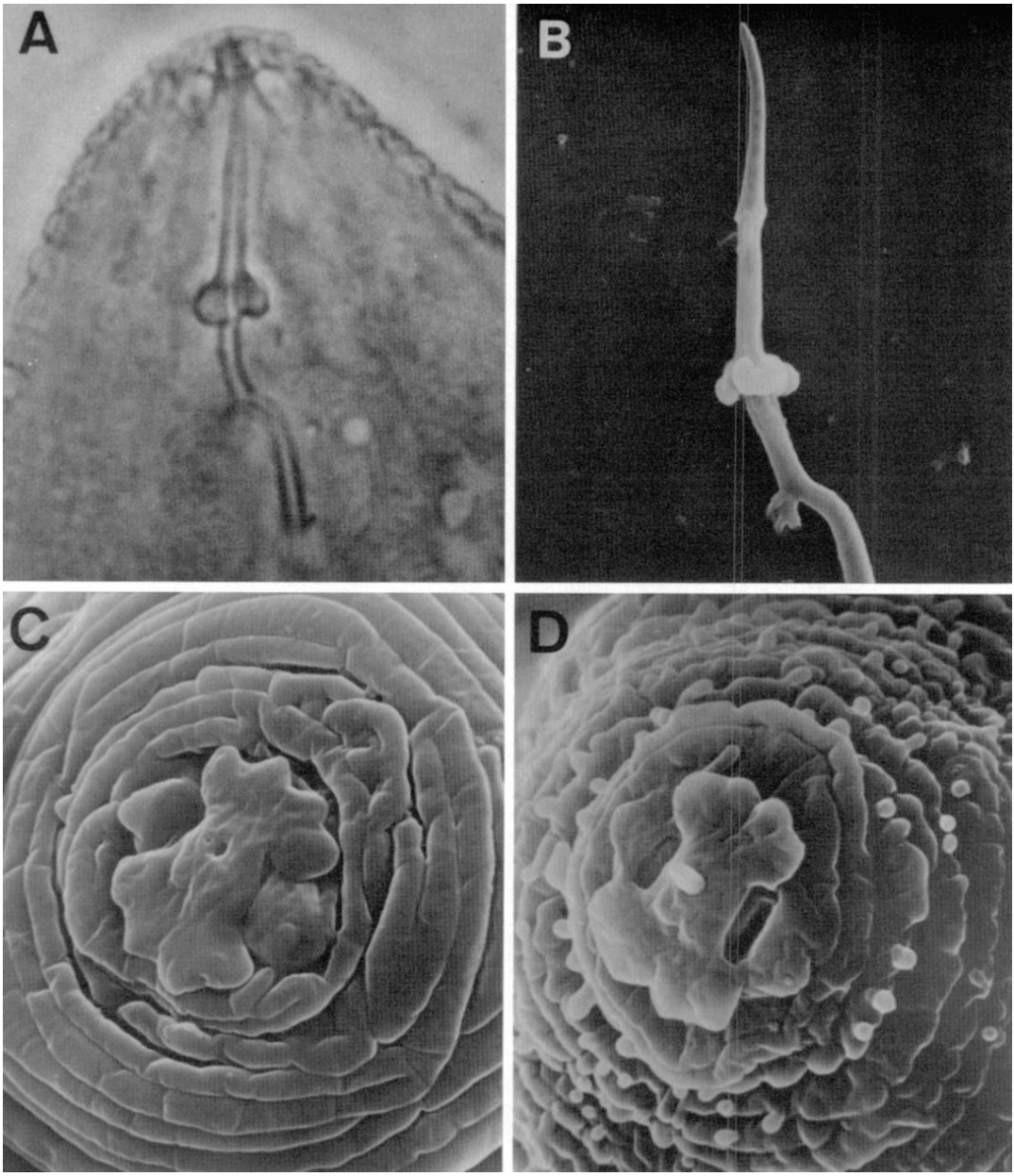

FIG. 2. Photographs of females of *Meloidogyne trifoliophila* n. sp. A) LM of anterior region. B) SEM of excised stylet. C,D) SEM of face views.

posterior to knobs; excretory canal visible well past esophageal glands. Stylet cone curved dorsally (Fig.2B), knobs bilobed, with only slight tapering onto stylet shaft (Figs. 1C;2A,B). Distance of dorsal esophageal gland orifice (DGO) to stylet knobs less than one shaft length. Dorsal gland ampulla large. Vesicles not observed along lumen in anterior metacorpus. Dorsal esophageal gland large, approximately twice the volume of the smaller subventral glands (Fig. 1C). Two esophago-intestinal cells attached dorsally to metacorpus. Vulva of mature females offset from long body axis (Fig. 1B).

Perineal patterns (Figs. 1D,3,4) typically rounded; striae fine, smooth to slightly wavy, infrequently forked. Dorsal arch smoothly rounded, in SEM with thickened striae pos-

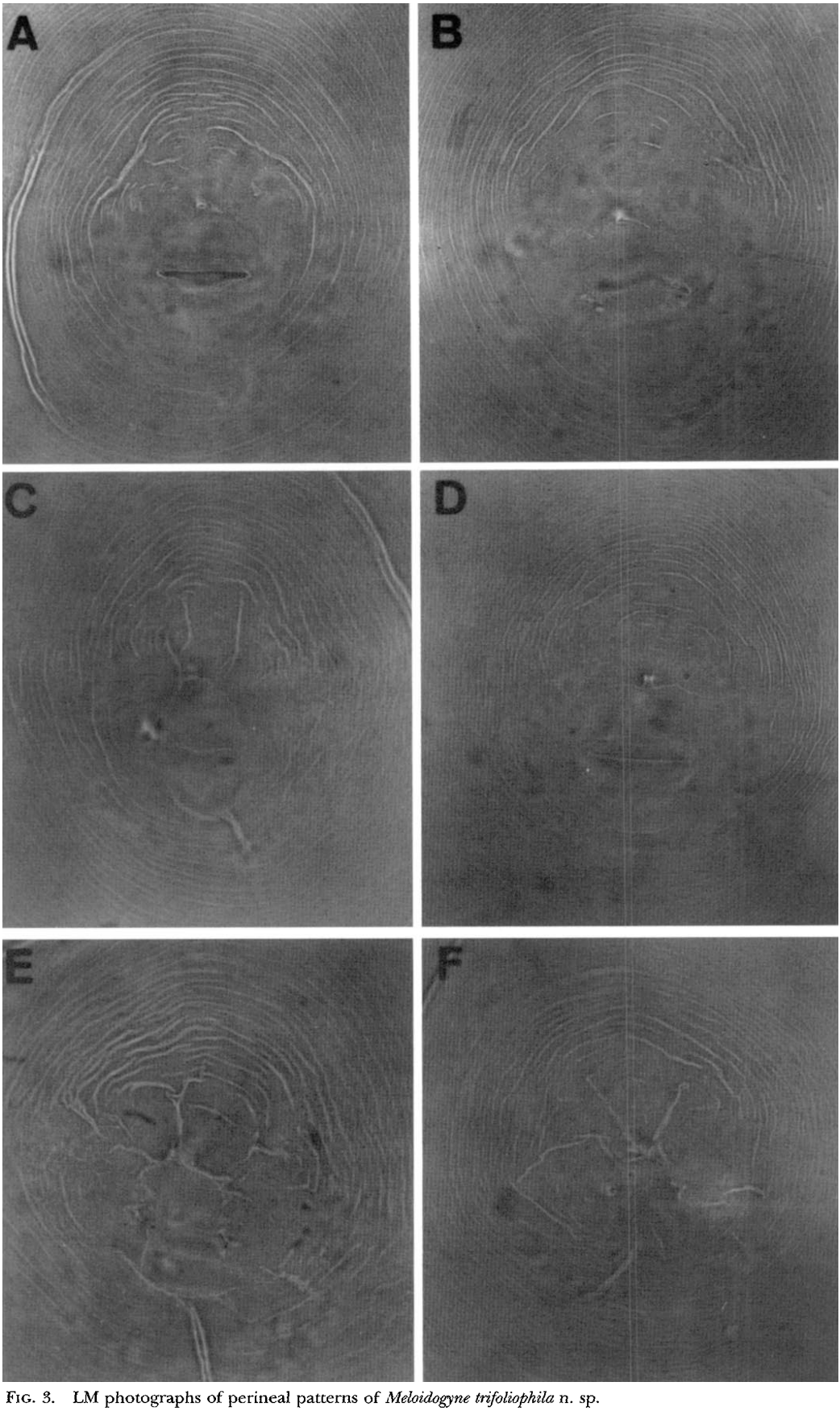

FIG. 3. LM photographs of perineal patterns of *Meloidogyne trifoliophila* n. sp.

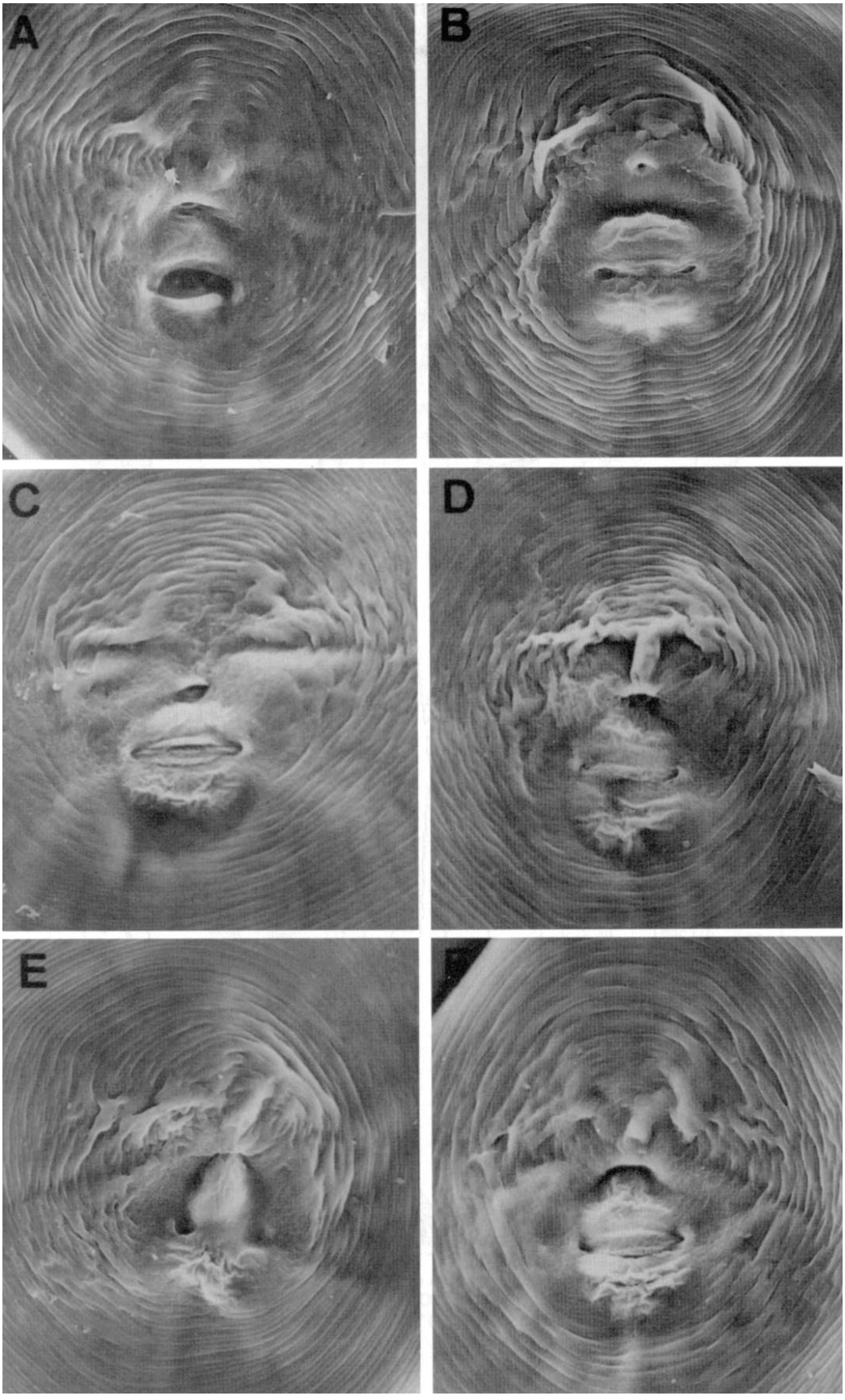

FIG. 4. SEM photographs of perineal patterns of *Meloidogyne trifoliophila* n. sp.

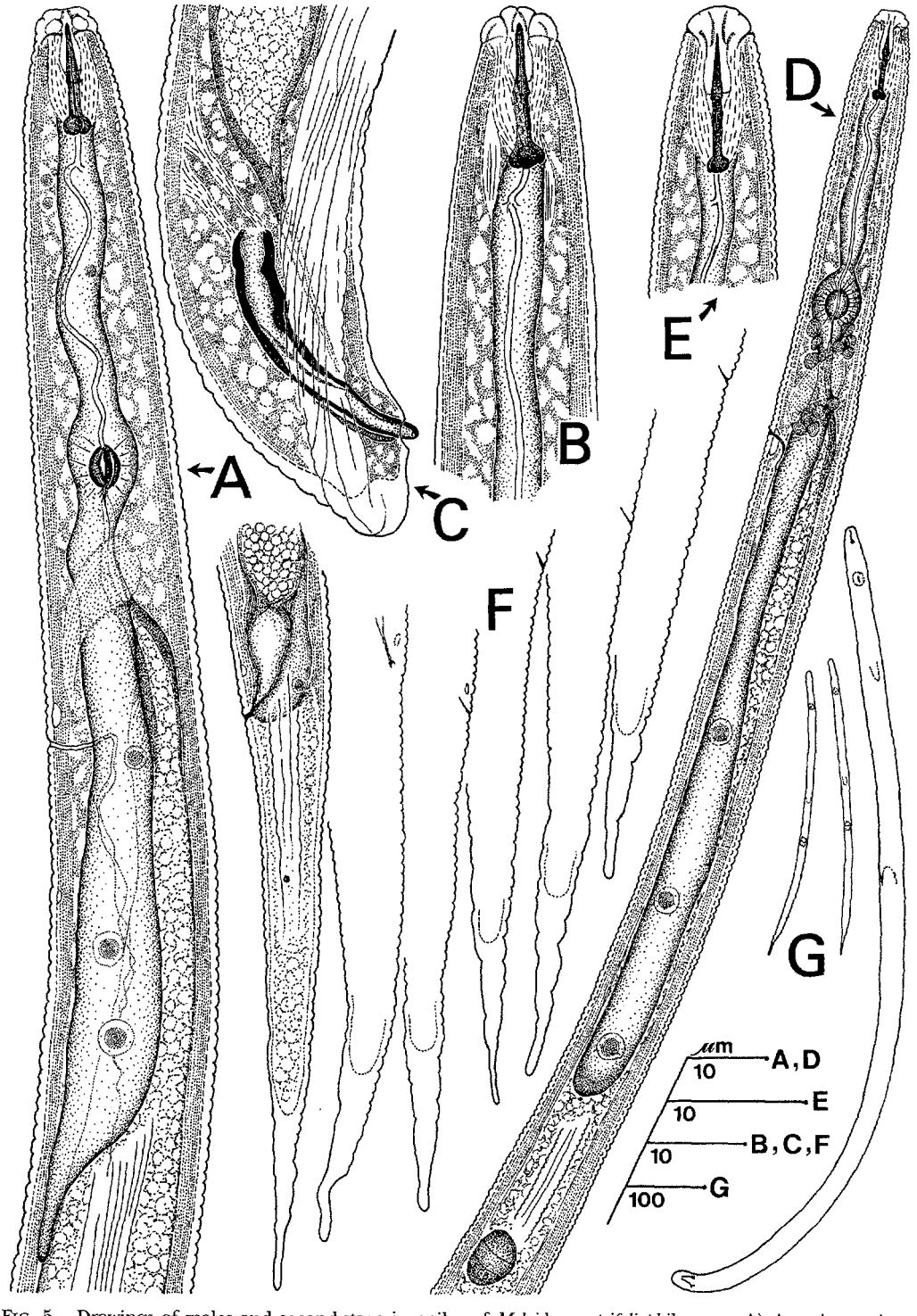

FIG. 5. Drawings of males and second-stage juveniles of *Meloidogyne trifoliophila* n. sp. A) Anterior region of male, lateral view. B) Anterior end of male, dorsal view. C) Male tail, lateral view. D) Anterior region of second-stage juvenile. E) Anterior end of second-stage juvenile. F) Tails of second-stage juveniles. G) Outlines of male and two second-stage juveniles.

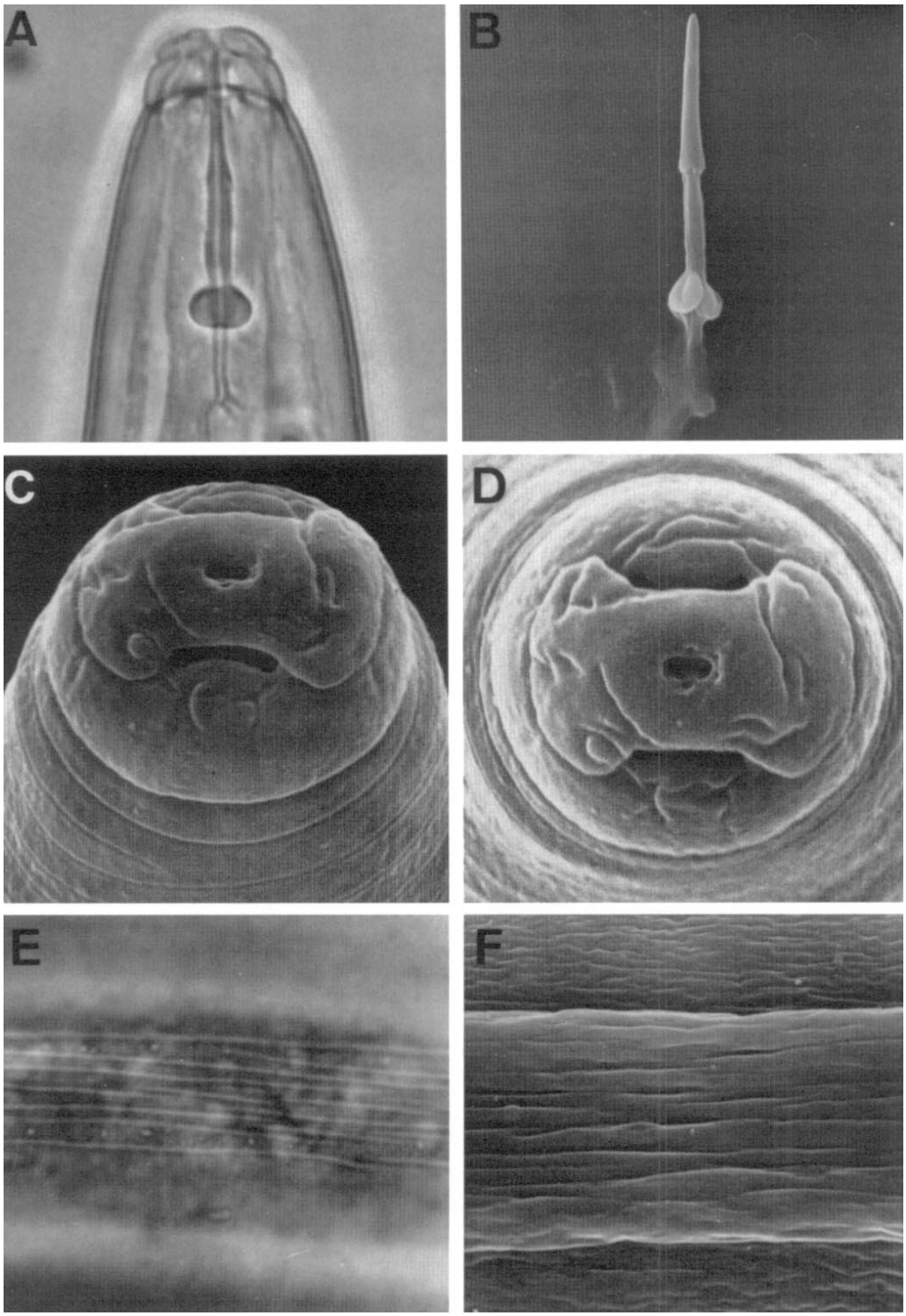

FIG. 6. Photographs of males of *Meloidogyne trifoliophila* n. sp. A) LM of anterior end. B) SEM of excised stylet. C, D) SEM of face views. E) LM of midbody lateral field. F) SEM of midbody lateral field.

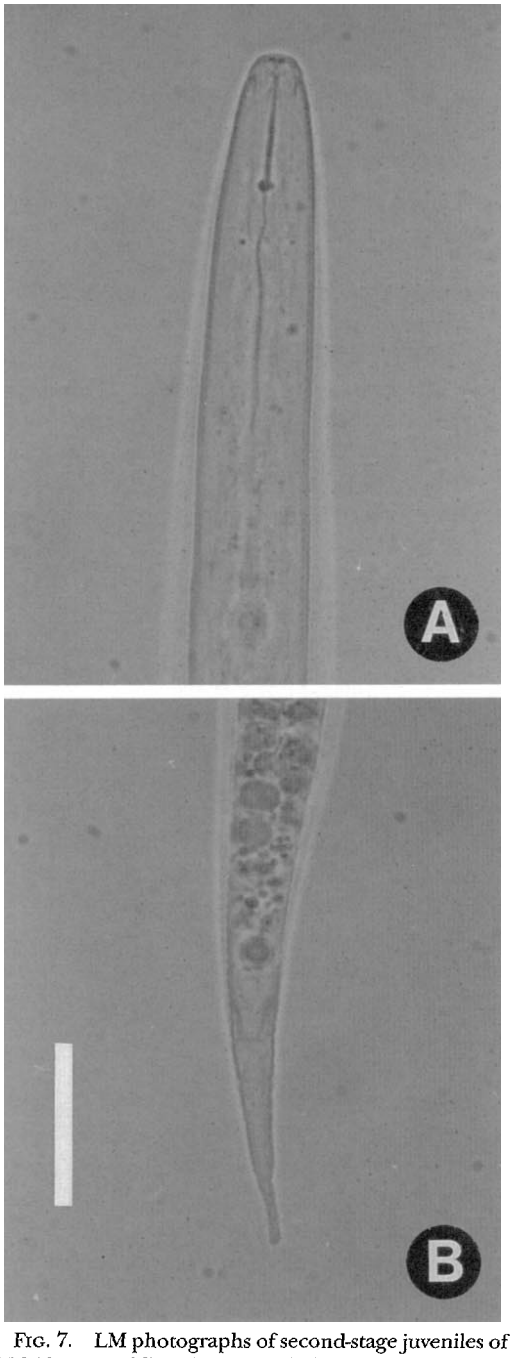

FIG. 7. LM photographs of second-stage juveniles of *Meloidogyne trifoliophila* n. sp. A) Anterior end, lateral view. B) Tail. Scale bar = 15 µm.

terior to anus in phasmid region. Lateral field obscure or absent in LM, sometimes indicated by a few short, forked striae (Fig. 1D); in SEM, lateral field indicated by weak depression and forking of striae (Fig. 4). Perivulval region usually free of striae. Phasmids posterior to anus or rarely at anus level.

Males: Measurements of 20 paratype males are listed in Table 2. Body vermiform, tapering anteriorly; tail conoid, bluntly rounded, twisting 90°. Head cap high, rounded-truncate (Figs. 5A,B;6A); head region distinct from first body annule, without annulation or with a few short, irregular lines (Fig. 6C,D). In SEM, stoma slitlike, prestoma narrowly oval and surrounded by pits of inner labial sensilla (Fig. 6C,D). Labial disc shape irregular, partially demarcated by short grooves; medial lips fused on each side, rounded, with several straight and curved grooves possibly associated with cephalic sensilla. Lateral lips weakly demarcated, not protuberant. Amphid apertures elongate slits. Vestibule and vestibule extension distinct (Fig. 5A,B). Stylet robust, cone bluntly pointed at tip, widening at junction with shaft; shaft cylindrical, smooth, slightly narrowed anteriorly (Fig. 6A,B). Knobs oval, indented longitudinally, tapering onto shaft, rounded posteriorly. Mean distance of dorsal gland orifice to stylet base 4.5 µm. Dorsal gland ampulla indistinct. Procorpus thick, almost as wide as metacorpus (Fig. 5A). Esophago-intestinal junction at level of nerve ring. Esophageal gland lobe long, with three nuclei—one nucleus level with the excretory pore, the other two usually in posterior half of lobe (Fig. 5A); occasionally, one of the posterior nuclei in anterior half of lobe just behind anteriormost nucleus. Anterior intestinal caecum absent. Excretory canal prominent, twisted irregularly, visible to end of gland lobe. Excretory pore 1–2 annules behind hemizonid; hemizonion about 13 annules behind hemizonid. Lateral field not areolated, composed of numerous frequently broken or forked incisures, appearing as about eight lines in LM (Figs. 5A,6E), appearing more numerous in SEM (Fig. 6F). In posterior region, incisures more broken, with the posteriormost incisures merging with the lateral field terminus (Fig. 5C). Spicules weakly capitate, the distal two-thirds nearly straight; gubernaculum weakly curved in lateral view (Fig. 5C). Phasmids not seen.

TABLE 1. Measurements of the holotype and 20 females of *Meloidogyne trifoliophila* n. sp.

Character	Holotype	Paratypes			
		Range	Mean	SE	SD	CV (%)
Linear (µm)						
Body length	491	475–626	537	12.1	50.1	9.3
Body length without neck	371	364–495	422	10.6	43.6	10.3
Body width	365	237–429	375	12.7	52.4	14.0
Neck length	120	89–172	114	5.5	22.6	19.8
Stylet length	13.3	12.6–15.5	14.1	0.26	0.94	6.7
Stylet knob height	1.8	1.5–2.3	1.9	0.07	0.26	13.7
Stylet knob width	2.4	1.9–2.9	2.4	0.07	0.25	10.4
DGO to stylet knobs	4.2	3.0–4.4	3.8	0.14	0.51	13.4
Head end to metacorpus valve	64.6	61.6–68.9	64.8	0.66	2.41	3.7
Head end to excretory pore	23.7	17.0–26.2	22.6	0.72	2.59	11.5
Vulval slit length	—	20.0–34.7	26.3	0.59	3.22	12.2
Vulva-anus distance	—	12.4–29.1	19.2	0.69	3.73	19.4
Interphasmidial distance	—	14.1–23.8	17.9	0.42	2.31	12.9
Angular (degrees)						
Vulval angle from median	54	9–65	41.2	4.89	17.64	42.8
Ratios						
a	1.4	1.3–2.1	1.5	0.05	0.22	14.7
Body length without neck/body width	1.0	1.0–1.6	1.1	0.04	0.17	15.5
Stylet knob width/height	0.8	0.7–1.0	0.8	0.03	0.10	12.5

Second-stage juveniles: Measurements of 20 juveniles are given in Table 3. Body slender, tapering to an elongated tail (Fig. 5G). Cephalic framework weak, vestibule and vestibule extension distinct (Fig. 5E) but not strongly sclerotized except at anterior terminus (Fig. 7A). Stylet slender, cone weakly expanding at junction with shaft, knobs rounded and tapering. Distance from stylet knobs to dorsal gland orifice less than shaft length. Ampulla obscure. Metacorpus broadly oval, valve large and heavily sclerotized (Fig. 5D). Gland lobe very long, nearly reaching genital primordium, nuclei equally spaced in the posterior half (Fig. 5D). Esophago-intestinal junction at level of nerve ring. Excretory pore slightly behind nerve ring, opening through hemizonid.

TABLE 2. Measurements of 20 paratype males of *Meloidogyne trifoliophila* n. sp.

Character	Range	Mean	SE	SD	CV (%)
Linear (µm)					
Body length	915–1,207	1,077	17.6	80.5	7.5
Greatest body width	26.8–39.0	33.5	0.78	3.57	10.7
Stylet length	17.0–18.9	18.0	0.13	0.57	3.2
Stylet knob height	1.9–3.0	2.5	0.07	0.32	12.8
Stylet knob width	2.0–3.1	2.6	0.08	0.35	13.5
DGO to stylet knobs	3.4–6.5	4.5	0.20	0.86	19.1
Head end to metacorpus valve	58.2–77.6	68.6	1.3	5.8	8.5
Head end to excretory pore	97–123	112	1.7	7.4	6.6
Tail length	7.3–11.6	9.7	0.36	1.34	13.8
Spicule length	27.2–33.5	30.0	0.55	2.07	6.9
Gubernaculum length	7.3–9.7	8.7	0.27	0.82	9.4
Ratios					
a	26.5–35.8	32.3	0.53	2.44	7.6
c	89–151	115	4.75	17.8	15.5
Stylet knob width/height	0.68–1.26	1.05	0.03	0.14	13.3
Percentage					
Excretory pore	9.0–11.4	10.3	0.16	0.69	6.7

TABLE 3. Measurements of 20 paratype second-stage juveniles of *Meloidogyne trifoliophila* n. sp.

Character	Range	Mean	SE	SD	CV (%)
Linear (µm)					
Body length	357–400	379	4.4	15.2	4.0
Greatest body width	11.0–14.3	12.9	0.29	0.99	7.7
Body width at anus	8.7–12.9	10.1	0.31	1.07	10.6
Stylet length	11.9–13.6	12.7	0.20	0.52	4.1
DGO to stylet knobs	2.9–4.6	3.6	0.26	0.62	17.2
Head end to metacorpus valve	44.6–53.8	49.8	1.1	3.4	6.8
Head end to excretory pore	65.5–76.1	70.1	1.3	3.8	5.4
Tail length	60.6–87.3	69.9	2.5	8.7	12.4
Hyaline region of tail	15.1–19.3	16.4	1.5	0.6	9.2
Ratios					
a	26.9–33.6	29.4	0.66	2.29	7.8
Body length/head-valve length	7.0–8.4	7.7	0.15	0.45	5.8
c	4.2–6.2	5.5	0.19	0.66	12.0
c′ (Tail length/width at anus)	5.7–8.6	6.9	0.24	0.82	11.9
Percentage					
Excretory pore	16.8–19.4	18.4	0.29	0.87	4.7

Rectum inflated. Lateral field with four incisures, not areolated. On tail, lateral field with two incisures not fusing terminally. Phasmids small, distinct, slightly offset ventrally within the lateral field, about one-third tail length from anus (Fig. 5F). Caudalid sometimes visible, three annules anterior to anus. Tail elongate-conoid, tapering to a slender, terminal, digitiform process (Figs. 5F,7B). Hyaline region of tail 15–19 µm long.

Type host and locality

Roots of *Trifolium repens* L. (white clover) in a mixed clover-tall fescue pasture, Ames Plantation, Fayette County, Tennessee.

Type specimens

Holotype (female in glycerin): Collected from a white clover plant maintained in the greenhouse, derived from a collection made at the type locality, July 1987; Carol Kimmons, collector. Slide number T-522t deposited in the U.S. Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland, USA.

Paratypes (females, males, second-stage juveniles, and perineal patterns): Same data as holotype. Slide numbers T-4587p through T-4602p deposited in USDANC, Beltsville, Maryland. Additional paratypes deposited in the University of California Riverside Nematode Collection, Riverside, California, USA.

Diagnosis

Meloidogyne trifoliophila n. sp. can be distinguished from other *Meloidogyne* spp. by the following combination of characters: perineal pattern round, striae long and smooth, no distinct lateral field; vulva offset from long body axis, slightly protuberant; lateral field of male with eight or more frequently forked or broken incisures; second-stage juveniles short, stylet slender, tail elongate-conoid with a digitate tip.

Relationships

The tail morphology of the second-stage juvenile of *M. trifoliophila* n. sp. places it in Jepson's Group 11 (Jepson, 1987), which includes *M. graminicola* Golden & Birchfield, 1965 (Golden and Birchfield, 1965, 1966; Mulk, 1976), *M. naasi* Franklin, 1965 (Franklin, 1965, 1973), *M. oryzae* Maas, Sanders, & Dede, 1978 (Maas et al., 1978) and *M. ottersoni* (Thorne, 1969) Franklin, 1971 (Thorne, 1969). A recently described

species, *M. triticoryzae* Gaur, Saha, & Khan, 1993 (Gaur et al., 1993) also belongs to this group. The following characters and morphometrics of *M. trifoliophila* n. sp. separate it from the other Group 11 species: female body length 475–626 µm (330–480 µm in *M. triticoryzae*); perineal pattern round, striae smooth, dorsal arch without prominent interruptions or ridges (in *M. graminicola,* perineal pattern dorsoventrally elongated with prominent ridges and angled striae in the dorsal arch); female stylet length 12.6–15.5 µm (10.6–11.2 µm in *M. graminicola,* 14–18 µm in *M. oryzae,* 10–12 µm in *M. ottersoni*); female excretory pore one stylet length or less behind stylet knobs (more than one stylet length in *M. graminicola,* anterior to stylet knobs in *M. naasi,* 2–3 stylet lengths behind knobs in *M. oryzae*); female interphasmidial distance 14.1–23.8 µm (8–11 µm in *M. triticoryzae*); male stylet length 17.0–18.9 µm (16.2–17.4 µm in *M. graminicola,* 19–20 µm in *M. oryzae,* 14–16 µm in *M. ottersoni*); male with eight lateral field incisures (four in *M. naasi, M. ottersoni,* and *M. triticoryzae*); second-stage juvenile length 357–400 µm (415–484 µm in *M. graminicola,* 418–465 µm in *M. naasi,* 500–615 µm in *M. oryzae,* 430–500 µm in *M. ottersoni*); second-stage juvenile stylet length 11.9–13.6 µm (11.2–12.3 µm in *M. graminicola,* 13–15 µm in *M. naasi* and *M. ottersoni,* 14–15 µm in *M. oryzae*); dorsal gland orifice 2.9–4.6 µm from the stylet knobs (2.8–3.4 µm in *M. graminicola,* 2–3 µm in *M. naasi* and *M. triticoryzae,* 2.5 µm in *M. oryzae* according to drawing); length of hyaline region of tail 15.1–19.3 µm (20–22 µm in *M. graminicola,* 21–23 µm in *M. naasi,* 23 µm in *M. oryzae* and *M. ottersoni*).

Discussion

Root-knot nematode J2 in Jepson's J2 Tail Shape Group 11 are defined as having a tapering tail with a long, narrow hyaline portion and a marked clavate terminus. *Meloidogyne trifoliophila* J2 tails rarely are clavate. However, illustrations in Jepson (1987) of Group 11 tails clearly show linear, as well as clavate, termini. Therefore, J2 of *M. trifoliophila,* which have tails similar to many Group 11 tails illustrated in Jepson (1987), should be placed in Group 11.

Species in Group 11 typically are parasites of grasses, especially rice, wheat, and barley. In a host range study (Bernard and Jennings, unpubl.), *Meloidogyne trifoliophila* n. sp. rarely parasitized most grasses and did not establish itself on rice, wheat, or barley. This new species may have been misidentified in the past as *M. graminicola,* which has been reported to parasitize many dicotyledonous hosts (Jepson, 1987; Windham and Pederson, 1992). Close examination of isolates within this group is required to establish their specific identities.

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