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Tylenchulus graminis n. sp. and *T. palustris* n. sp. (Tylenchulidae), from Native Flora of Florida, with Notes on *T. semipenetrans* and *T. furcus*

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Abstract: *Tylenchulus graminis* n. sp. and *T. palustris* n. sp. are described and illustrated from broomsedge (*Andropogon virginicus* L.) and pop ash (*Fraxinus caroliniana* Mill.), respectively. *T. graminis* resembles *T. furcus* in having a distinct anus, but *T. graminis* second-stage juveniles (J2) do not have a bifid tail. *T. semipenetrans* does not have a perceptible anus. The mature female of *T. graminis* has a mucronate pointed terminus while *T. semipenetrans* has a smooth and round terminus. *T. graminis* males have wider stylet knobs and basal bulb and a longer tail than *T. semipenetrans* males. *T. graminis* J2 have a longer posterior body portion (without large fat globules) than *T. semipenetrans* J2. *T. palustris* resembles *T. semipenetrans* in having an undetectable anus but differs by the short and conoid mature female postvulval section. The male of *T. palustris* has larger stylet knobs and basal bulb than those of *T. semipenetrans* and a bluntly rounded tail terminus, which is tapered in *T. semipenetrans*. *T. palustris* differs from *T. furcus* and *T. graminis* in having an undetectable anus, by the conoid postvulval section of mature females, by the shorter and rounded tail of males, and the shorter J2 posterior body section without large fat globules. *T. graminis* and *T. palustris* are parasites of indigenous flora of Florida.

Key words: *Andropogon virginicus*, broomsedge, citrus, Florida, *Fraxinus caroliniana*, physiological race, pop ash, taxonomy, *Tylenchulus furcus*, *Tylenchulus graminis*, *Tylenchulus palustris*, *Tylenchulus semipenetrans*, scanning electron microscopy.

The genus *Tylenchulus* Cobb, 1913, presently contains two species, *Tylenchulus semipenetrans* Cobb, 1913 and *Tylenchulus furcus* Van Den Berg & Spaull, 1982 (Fig. 1A, B) (2,3,14). *T. semipenetrans* races attack dicotyledons, particularly rutaceous species, while *T. furcus* infects monocotyledons, especially *Saccharum* species (12,14).

Two tylenchulid isolates consistently found in noncultivated areas of Florida previously were identified as races of *T. semipenetrans* and named the grass and bush races (9,10,13). *Schizachyrium rhizomatum* (Swallen) Gould, *Andropogon virginicus* L. (broomsedge), and *Eremochloa ophiuroides* (Munro) Hack. (centipede grass) were hosts of the grass race (10,13). The only known hosts of the bush race were *Fraxinus caroliniana* Mill. (pop ash) and *Baccharis halimifolia* L. (saltbush) (10). Neither race attacked citrus. In comparative morphological studies, the bush and grass races were found to have sufficiently distinctive characters that warranted their elevation to species rank (10). Described here are *Tylenchulus graminis* n. sp. (the grass race) and *Tylenchulus palustris* n. sp. (the bush race). This study has particular regulatory significance in Florida, because land infested with *T. semipenetrans* cannot be certified for commercial citrus nurseries.

MATERIALS AND METHODS

Specimens of *T. graminis* and *T. palustris* were collected from broomsedge and pop ash roots at their respective type localities and near Venice and Steinhatchee, Florida.

Males and J2 were recovered from soil by Cobb's sieving and decanting method or by incubating egg masses (4,8). Entire bodies of *T. graminis* mature females were removed from broomsedge roots by high pressure water spray and collected on a 35-μm-pore sieve. Long-necked mature females of *T. palustris* were removed from pop ash roots with needles using a stereomicroscope.

Specimens for measuring, drawing, and photographing were placed on the surface

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of water agar and covered with a cover slip (6). Additional specimens were killed and fixed in lactophenol (5) or in hot aqueous 4% formaldehyde + 1% propionic acid, dehydrated in ethanol vapor, and mounted in dehydrated glycerin (7). Eggs for measurements were fixed and mounted in 2.5% formalin.

Specimens for scanning electron microscopy (SEM) were killed and fixed in formalin-propionic acid 4:1, transferred to 1% osmium tetroxide solution for 12 hours, infiltrated with Spurr's resin, and mounted on SEM specimen stubs (1). Specimens were coated with gold and observed at 5 kV accelerating voltage (1).

T. semipenetrans life stages were collected from a Florida citrus orchard, processed as described here, mounted on water agar plates, and used for comparison because of lack of paratypes. Morphological parameters of *T. semipenetrans*, *T. graminis*, and *T. palustris* were compared statistically by the Student's *t*-test. Additional comparisons were made with specimens of mature adult females, males, and J2 of the citrus and Mediterranean races of *T. semipenetrans* collected in Italy and Greece and mounted on permanent slides or SEM stubs.

Measurements of distance from genital primordium to the head end and between the excretory pore and genital primordium of J2 were obtained only from specimens fixed in lactophenol (5) because of the difficulty of observing the genital primordium in live specimens. In addition to the standard morphological characters for species of the genus *Tylenchulus*, we include measurements of the postvulval section of mature adult females as illustrated in Figure 2.

Because of variation in body cuticle thickness, the cuticle was measured ventrally and close to the excretory pore. The metacorpus position was measured from the head end to the anterior end of the metacorpus.

The following abbreviations are used in the text: body width at vulva—BWV; dorsal esophageal gland opening—DEGO; excretory pore to anterior body—EPBD; median bulb to anterior body—MEBD; postvulval section cavity—PVSC; postvulval section length—PVSL; postvulval section width—PVSW; vulva to excretory pore distance—VEPD; and standard deviation—SD. Morphological parameters are listed in the text in alphabetical order. Adult females with swollen posterior body portion are indicated in the text with the term mature adult females to distinguish them from young, nonswollen adult females. All measurements are in micrometers (μm) unless otherwise stated.

Systematics

Tylenchulus semipenetrans Cobb, 1913 (Figs. 1A; 6A, B; 7A, B, E; 8A, E; 10A; 13A; 14A, B)

Measurements of mature adult females, males, and J2 females of a population from Frostproof, Florida, are in Table 1.

Tylenchulus graminis n. sp. (Figs. 3A–C; 4A–E; 5A–D; 6C, D; 7C, F; 8B, C, F; 9; 10B)

Holotype (mature adult female in glycerine): Basal bulb length 20.0, width 14.2; body length 281.5, width 83.6; BWV 25.5; cuticle thickness 3.0; DEGO 3.9; esophagus length 103.0; EPBD 212.0; isthmus length 19.0; MEBD 55.0; metacorpus length 18.3, width 14.2; PVSC 7.1; PVSL 37.7; PVSW 12.2; stylet knob width 3.5; stylet length 12.2; tail length 12.2; VEPD 31.6. Excretory pore from anterior body end as percentage of total body length 75.3; portion of posterior body swollen as percentage of total body length 74.6; vulva from anterior body end as percentage of total body length 86.5. Ratios: a = 3.3; b = 2.7; c = 23.4.

Mature adult female: Measurements of specimens in water agar of two populations, one from the type locality, Crescent City, and the other from near Venice, Florida, are in Table 2. Body translucent white, about ⅔ of posterior portion variable, saccate. Body swollen 66.0–85.0% of total body length, 59.0–73.0% in one population from south Florida (Table 2). Entire body hook shaped, curved ventrally, widest

TABLE 1. Measurements of *Tylenchulus semipenetrans* from Florida.

Morphological characters	Mature adult females (N = 25)			Males (N = 20)			J2 females (N = 20)		
	Range	Mean	Standard deviation	Range	Mean	Standard deviation	Range	Mean	Standard deviation
Measurements in μm									
Basal bulb length	17.3–27.5	22.0	3.3	12.2–25.5	19.2	3.6	18.3–21.4	19.6	0.9
Basal bulb width	9.1–16.3	12.2	1.7	5.1–8.0	6.4†‡	0.8	7.6–9.1	8.3	0.5
Body length	312.1–465.1	389.2	43.0	346.8–380.4	362.1	9.5	333.5–384.5	363.0	14.8
Body width	61.2–114.2	85.7	12.5	10.2–12.2	11.2	0.4	12.7–13.7	13.1	0.2
BWV	21.4–30.6	25.7	2.6						
Cuticle thickness	2.9–5.6	3.7†	0.7						
DEGO	4.2–6.1	5.2†	0.3	4.5–6.1	5.1	0.3	3.0–4.0	3.7	0.3
Esophagus length	104.0–159.1	119.8	11.9	96.9–130.5	104.6	7.7	102.0–115.2	108.3	3.2
EPBD	264.9–400.4	326.9	36.3	196.8–237.6	206.8	9.4	182.5–212.1	197.7	9.6
Genital primordium anterior body end distance							184.7–211.1	197.8	9.3
Genital primordium excretory pore distance							6.1–24.4	16.5†	5.0
Gubernaculum length				3.1–4.0	3.8	0.4			
Isthmus length	11.2–30.6	19.4	4.0	22.4–40.8	30.6	4.9	24.4–31.6	27.3	1.8
MEBD	49.9–71.4	59.4	6.0	37.7–48.9	42.5	3.4	40.8–49.9	46.8	1.9
Metacorpus length	19.3–24.4	21.2	1.6				14.2–18.3	15.3	1.0
Metacorpus width	10.2–18.2	12.9	2.2				6.6–8.1	7.4	0.4
Posterior body section without large fat globules							48.9–60.1	55.3†	3.4
PVSC	1.8–7.1	4.3†‡	1.3						
PVSL	26.5–52.0	40.0‡	5.9						
PVSW	9.1–13.2	10.9†‡	1.1						
Spicule length				15.3–18.3	16.6	0.6			
Stylet knob width	3.0–3.5	3.0	0.1	0.9–1.2	1.0†‡	0.06	3.0–3.5	3.1	0.1
Stylet length	11.2–12.3	11.9	0.3	9.1–10.2	9.3	0.4	12.2–13.2	12.3	0.3
Tail length				34.6–44.8	39.9†	2.6			
Testis length				85.6–131.5	113.1	11.6			
VEPD	11.2–32.6	20.8†	5.6						

TABLE 1. Continued.

Morphological characters	Mature adult females (N = 25)			Males (N = 20)			J2 females (N = 20)		
	Range	Mean	Standard deviation	Range	Mean	Standard deviation	Range	Mean	Standard deviation
Percentages									
Excretory pore anterior body end distance as % of total body length	77.7–89.8	84.0	2.4	54.0–68.5	57.1	2.9	52.4–57.6	54.4	1.3
Genital primordium anterior body end distance as % of total body end							56.5–63.7	59.5	1.9
Portion of posterior body swollen as % of total body length	34.4–60.0	47.4†	6.8						
Testis length as % of total body length				24.6–36.1	31.2	3.1			
Vulva anterior body end distance as % of total body length	87.5–92.8	89.7	1.1						
Ratios									
a	3.5–6.4	4.5	0.8	28.4–34.7	32.1	1.5	24.8–29.1	27.4	1.1
b	2.4–4.3	3.2	0.4	2.9–3.7	3.4	0.1	3.0–3.7	3.3	0.1
c				7.7–10.1	9.0	0.5			

†‡ Symbols indicate morphological parameters of *T. semipenetrans* that differ ($P = 0.01$) from the correspondent parameters of *T. graminis* (†) or *T. palustris* (‡).

TABLE 2. Measurements of mature females of *Tylenchulus graminis*.

Morphological characters	Crescent City, Florida (N = 25)			Venice, Florida (N = 22)		
	Range	Mean	Standard deviation	Range	Mean	Standard deviation
Measurements in μm						
Basal bulb length	19.3–28.5	22.7	2.9	20.4–27.5	23.2	2.1
Basal bulb width	11.2–18.3	13.3	1.9	10.2–16.3	14.1	1.6
Body length	268.2–355.9	306.1	27.4	222.2–275.4	250.3	14.2
Body width	40.8–105.0	66.3	14.1	41.8–117.3	64.5	20.6
BWV	23.4–31.6	27.0	2.2	21.4–28.5	25.4	3.3
Cuticle thickness	1.0–4.0	2.1†‡	0.7	1.5–5.1	2.5†‡	0.9
DEGO	3.5–5.1	4.0†	0.3	3.0–4.1	3.8	0.4
Esophagus length	81.6–137.7	112.6	11.1	93.8–121.3	105.9	6.9
EPBD	202.9–285.6	233.6	22.3	171.1–219.2	197.1	12.8
Isthmus length	12.2–25.5	20.4	3.1	16.3–25.5	19.9	2.7
MEBD	44.8–71.4	55.7	6.6	43.8–59.1	50.6	4.3
Metacorpus length	15.3–21.4	18.9	2.5	16.3–24.4	19.1	2.1
Metacorpus width	11.2–20.4	13.5	2.6	10.2–17.3	13.9	2.0
PVSC	5.1–11.2	7.5†	1.1	5.1–10.2	6.9†	1.0
PVSL	34.6–45.9	40.1	4.0	29.5–39.7	33.1	2.3
PVSW	12.2–14.2	12.9†	0.7	11.2–15.3	12.1†	1.0
Stylet knob width	3.1–4.0	3.5	0.3	3.2–4.0	3.8	0.2
Stylet length	11.2–13.2	12.2	0.3	11.2–12.7	12.0	0.5
Tail length	8.1–14.2	11.8†‡	1.9 (N = 12)	8.1–12.0	10.5†‡	1.7 (N = 8)
VEPD	20.4–44.8	32.6†	6.1	13.2–30.6	20.5	3.8
Percentages						
Excretory pore anterior body end distance as % of total body length	70.3–80.3	76.2	2.6	75.6–82.6	78.7	1.5
Portion of posterior body swollen as % of total body length	66.0–85.1	72.7†‡	4.9	59.0–72.6	65.9†‡	3.9
Vulva anterior body end distance as % of total body length	82.0–89.9	86.8	1.6	84.5–88.7	86.7	1.0
Ratios						
a	2.9–7.4	4.7	1.0	2.1–6.1	4.1	1.2
b	2.1–3.9	2.6	0.3	2.1–2.7	2.3	0.1
c	19.9–32.8	26.2†‡	5.7 (N = 12)	21.9–32.3	25.9†‡	4.0 (N = 8)

†‡ Symbols indicate significant ($P = 0.01$) differences with the correspondent parameters of *T. semipenetrans* (†) or *T. palustris* (‡).

portion at excretory pore, narrowing abruptly posterior to vulva (Figs. 3A, 4D). Body annulation visible with SEM in anterior portion of body (Fig. 5A). Lip region hemispherical, not set off. Labial framework weakly sclerotized. Stylet well developed, knobs rounded, DEGO 3.5–5.1 posterior. Procorpus elongate-cylindrical, lumen lining cuticularized. Metacorpus muscular, oval or pyriform (Figs. 3A, 4A). Isthmus cylindrical-elongate, nerve ring near metacorpus. Basal bulb saccate, oblong or pyriform, dorsal gland nucleus large, 4.0 wide × 6.1 long, subventrals 3.0–4.0 in diameter (Figs. 3A, 4A). Hemizonid not observed. Excretory pore 70.0–80.0% from head apex, surrounded by 2–5 cuticular outgrowths (Figs. 4E, 5D). Nucleus of excretory cell defined, with conspicuous nucleolus 3.5–8.0 in diameter (Fig. 3A).

TABLE 3. Measurements of males and J2 females of *Tylenchulus graminis* from Crescent City, Florida.

Morphological characters	Males (N = 20)			J2 females (N = 20)		
	Range	Mean	Standard deviation	Range	Mean	Standard deviation
Measurements in μm						
Basal bulb length	21.4–34.6	27.1	3.9	18.3–26.5	21.8	2.3
Basal bulb width	8.1–12.2	9.2†	1.0	8.1–11.2	9.7	0.8
Body length	422.2–519.1	478.6	23.5	336.6–440.6	390.7	21.4
Body width	13.2–16.2	14.6	0.7	13.2–15.3	13.8	0.6
DEGO	4.0–5.1	4.6	0.5	3.1–4.0	3.5	0.3
Esophagus length	123.4–149.9	138.1	10.6	117.3–133.6	124.1	5.0
EPBD	234.6–289.6	262.8	14.7	188.7–225.4	209.6	8.2
Genital primordium anterior body end distance				225.4–295.8	251.4	16.9
Genital primordium excretory pore distance				22.4–43.8	33.5†‡	5.9
Gubernaculum length	4.1–6.1	4.9	0.5			
Isthmus length	33.5–45.9	38.6	3.1	28.5–38.7	33.1	2.8
MEBD	45.9–58.1	52.6	3.2	47.9–58.1	53.0	3.2
Metacorpus length				15.3–18.3	16.0	1.0
Metacorpus width				7.1–10.2	8.7	0.7
Posterior body section without large fat globules				58.1–76.6	69.6†‡	4.5
Spicule length	17.3–20.4	18.7	0.9			
Stylet knob width	1.6–2.1	2.0†	0.1	3.1–4.0	3.5	0.3
Stylet length	9.9–10.7	10.2	0.2	12.7–13.4	13.2	0.1
Tail length	48.9–65.2	55.6†‡	4.3	59.1–72.4	65.0	3.2
Testis length	137.7–224.4	184.4	24.0			
Percentages						
Excretory pore anterior body end distance as % of total body length	50.5–59.2	54.8	2.2	50.8–56.1	53.7	1.5
Genital primordium anterior body end distance as % of total body length				57.4–72.9	66.5	3.9
Testis length as % of total body length	28.1–48.5	38.4	4.7			
Ratios						
a	21.8–34.9	32.2	3.0	23.7–32.0	28.1	1.8
b	2.8–3.8	3.4	0.2	2.7–3.3	3.0	0.1
c	7.3–9.5	8.5	0.6	5.3–6.6	5.9†‡	0.3

†‡ Symbols indicate significant ($P = 0.01$) differences with the correspondent parameters of *T. semipenetrans* (†) or *T. palustris* (‡).

Vulval silt delimited by prominent, smooth vulval lips (Figs. 4E, 5D, 6D). Vulva-excretory pore distance 20.4–44.8, 13.2–30.6 in one population from south Florida (Table 2). Ovary single, convoluted, extending anteriorly to metacorpus in some specimens (Fig. 3A). Uterus swollen, ovate. Spermatheca spherical, filled with round sperm. Vagina uterina large, swollen. Dilator vulvae muscles evident. Anus and rectum visible in 45% of the specimens, 28% in the south Florida population (Figs. 3A; 4B, C; 5B; 6D). Body behind vulva digitate or conical, terminus pointed or peg-like, the latter being sometimes mucronate (Figs. 3A; 4E; 5B–D; 6C, D). PVSW 12.2–14.2. PVSC 5.1–11.2. Lateral field not observed. Cuticle thin, 1.0–4.0, 1.5–5.1 in the south Florida population.

Male: Measurements in water agar are reported in Table 3. Body translucent white, vermiform, slender (Fig. 3B). Body cuticle striae fine, 0.7–0.9 apart. Lateral incisures indistinct, appearing as two faint

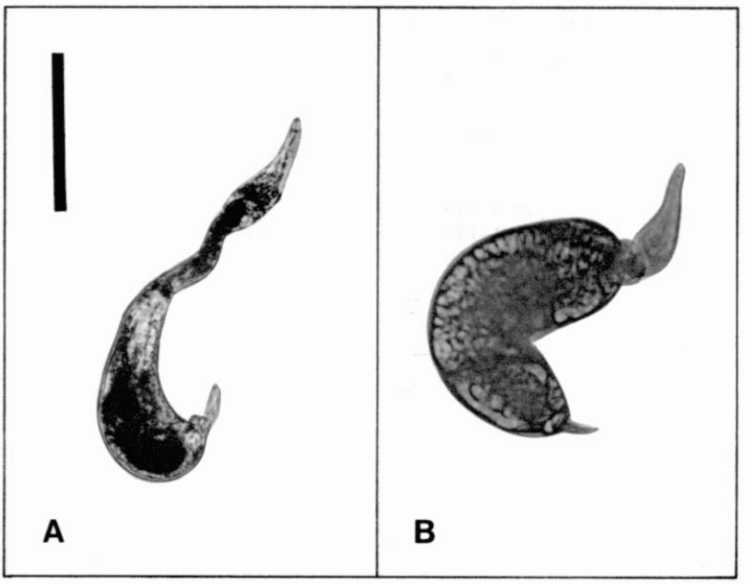

FIG. 1. Photomicrographs of adult females. A) *Tylenchulus semipenetrans.* B) *Tylenchulus furcus.* Scale bar for A and B = 120 μm.

lines. Lip region hemispherical, without distinct annulations; labial framework inconspicuous. Stylet delicate, knobs small, 1.6–2.1 wide (Figs. 3B, 7C). Procorpus degenerate, lumen distinct. Metacorpus distinct in about 70% of the specimens, musculature indistinct. Isthmus cylindrical-elongate, nerve ring near metacorpus. Basal bulb well developed, pyriform, 8.1–12.2 wide and 21.4–31.6 long (Figs. 3B, 7F). Excretory pore behind mid-body, 50.5–59.2% from anterior end. Testis single, occupying 28–48% of the body. Spicules slender, arcuate. Gubernaculum slightly curved. Caudal region elongate-conoid, 48.9–65.2 long, terminus rounded or mucronate, 48.9–65.2 long (Figs. 3B; 8B, C). Caudal alae absent.

Female second-stage juveniles: Measurements in water agar are in Table 3. Body translucent, white. Cuticle striae faint 0.8–1.0 apart. Lip region hemispherical, not set off; labial framework weak (Fig. 3C). Stylet well developed, knobs rounded, 3.1–4.0 wide; DEGO 3.1–4.0 posterior. Procorpus cylindrical. Metacorpus muscular, ovoid; basal bulb saccate, basal part obscured by short overlap of intestine. Lateral incisures indistinct, appearing as two faint lines. Excretory pore 50.8–56.1% from anterior end, 22.4–43.8 anterior to genital primordium. Genital primordium with 2–4 cells, 57.4–72.0% from anterior end. Anus visible under oil immersion in majority of live specimens, obscure or invisible in fixed specimens (Figs. 3C, 9). Hyaline posterior body portion containing few small fat globules (< 2 diameter), 58.1–76.5 long (Figs. 8F, 10B). Tail terminus round, 59.1–72.4 long.

Eggs (20 in 2.5% formalin): Length 63.0–83.0 (mean 74.0), SD 4.6. Width 30.0–34.0 (33.0), SD 3.7. Length/width ratio 1.8–2.6 (2.1), SD 0.21. Gelatinous matrix present. Egg shell not sculptured, hyaline as seen with light microscope.

Diagnosis: Mature adult females of *T. graminis* are more swollen (59–85% of body length) than those of *T. semipenetrans* (34–60%) ($P = 0.01$) (Figs. 1A, 4D) and have a shorter ($P = 0.01$) DEGO (3.5–5.1 vs. 4.2–6.1). The postvulval body section of *T. graminis* mature adult females is digitate with a pointed, sometimes mucronate terminus and an observable anus, whereas that of *T. semipenetrans* is digitate with a rounded terminus and an imperceptible anus (Fig. 6A–D). The PVSC of *T. graminis* is larger ($P =$

0.01) than that of *T. semipenetrans* (5.1–11.2 vs. 1.8–7.1) and the PVSW is wider (12.1 vs. 10.9).

Males of *T. graminis* have more highly developed ($P = 0.01$) stylet knobs than *T. semipenetrans* males (1.6–2.1 vs. 0.9–1.2 wide) (Fig. 7A–C), a wider ($P = 0.01$) and more developed basal bulb (8.1–12.2 vs. 5.1–8.0 wide) (Fig. 7E, F), and longer tails ($P = 0.01$) (48.9–65.2 vs. 34.6–44.8) (Fig. 8A, B).

T. graminis J2 have a distinct anus and rectum that are not discernible in *T. semipenetrans* J2 (Figs. 9; 10A, B); the hyaline portion of the posterior body is significantly longer ($P = 0.01$) than that of *T. semipenetrans* J2 (58.1–76.5 vs. 48.9–60.1) (Figs. 8E, F; 10A, B), and the distance between the excretory pore and genital primordium is greater ($P = 0.01$) (22.4–43.8 vs. 6.1–24.4).

Both *T. graminis* and *T. furcus* have a distinct anus and rectum and a similar body shape in all motile life stages (Figs. 1B, 4B–D) (14). These two species can be easily distinguished by the shape of the J2 tail; *T. furcus* tail is bifid (14) and *T. graminis* tail is not (Figs. 8F, 10B). Mature females of *T. furcus* examined by us have a smaller PVSW compared with *T. graminis* (8.3–10.0 vs. 11.2–14.2)

Biological characters of *T. graminis* are closer to *T. furcus* than to *T. semipenetrans*. Both *T. graminis* and *T. furcus* attack monocots (14), while *T. semipenetrans* infects only dicots.

Type host and locality: Broomsedge (*Andropogon virginicus*) collected along the shore of McKasel Lake, Crescent City, Florida. Other hosts of *T. graminis* are centipede grass and *Schizachyrium rhizomatum*.

Type specimens: Holotype (mature adult female)—isolated from roots of broomsedge collected from type locality. Slide T-430t, deposited in the United States Department of Agriculture Nematode Collection, Beltsville, Maryland. Paratypes (mature adult females, immature adult females, males and J2)—same data as holotype, deposited in Florida Department of Agriculture and Consumer Services Nematode Collection, Gainesville; United States Department of Agriculture Nematode Collection, Beltsville, Maryland; University of California Riverside Nematode Collection; University of California Davis Nematode Collection; National Collection of Nematodes, Plant Protection Research Institute, Pretoria, South Africa; Nematode Collection of the Landbouwhogeschool, Wageningen, The Netherlands; Nematode Collection of the Laboratorio Nematologia Agraria, Consiglio Nazionale delle Ricerche, Bari, Italy.

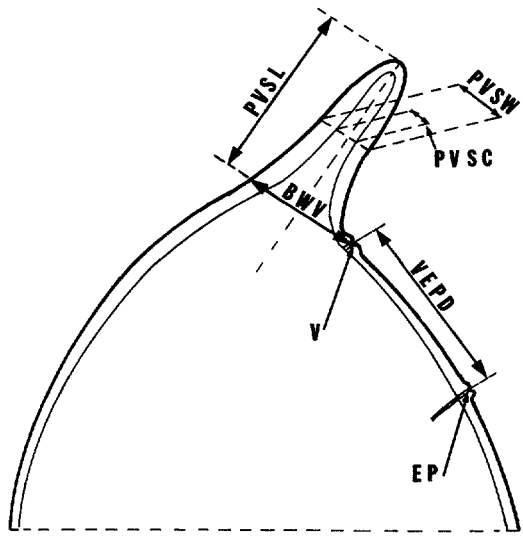

FIG. 2. Section of posterior body portion of tylenchulid adult female showing morphological parameters of postvulval section; BWV = body width at vulva. EP = excretory pore. PVSC = postvulval section cavity of body. PVSL = postvulval section length of body. PVSW = postvulval section width of body. V = vulva. VEPD = vulva excretory pore distance.

Tylenchulus palustris n. sp.
(Figs. 7D, G; 8D, G; 10C; 11A–C; 12A–F; 13B–D; 14C–F)

Holotype (mature adult female in glycerine): Basal bulb length 23.4, width 13.2; body length 284.5, width 69.3; BWV 27.5; cuticle thickness 3.0; DEGO 3.9; esophagus length 109.1; EPBD 238.6; isthmus length 23.4; MEBD 47.2; metacorpus length 19.3, width 11.2; PVSC 8.1; PVSL 28.5; PVSW 15.3; stylet knob width 3.5; stylet length 11.4; VEPD 17.4. Excretory pore to an-

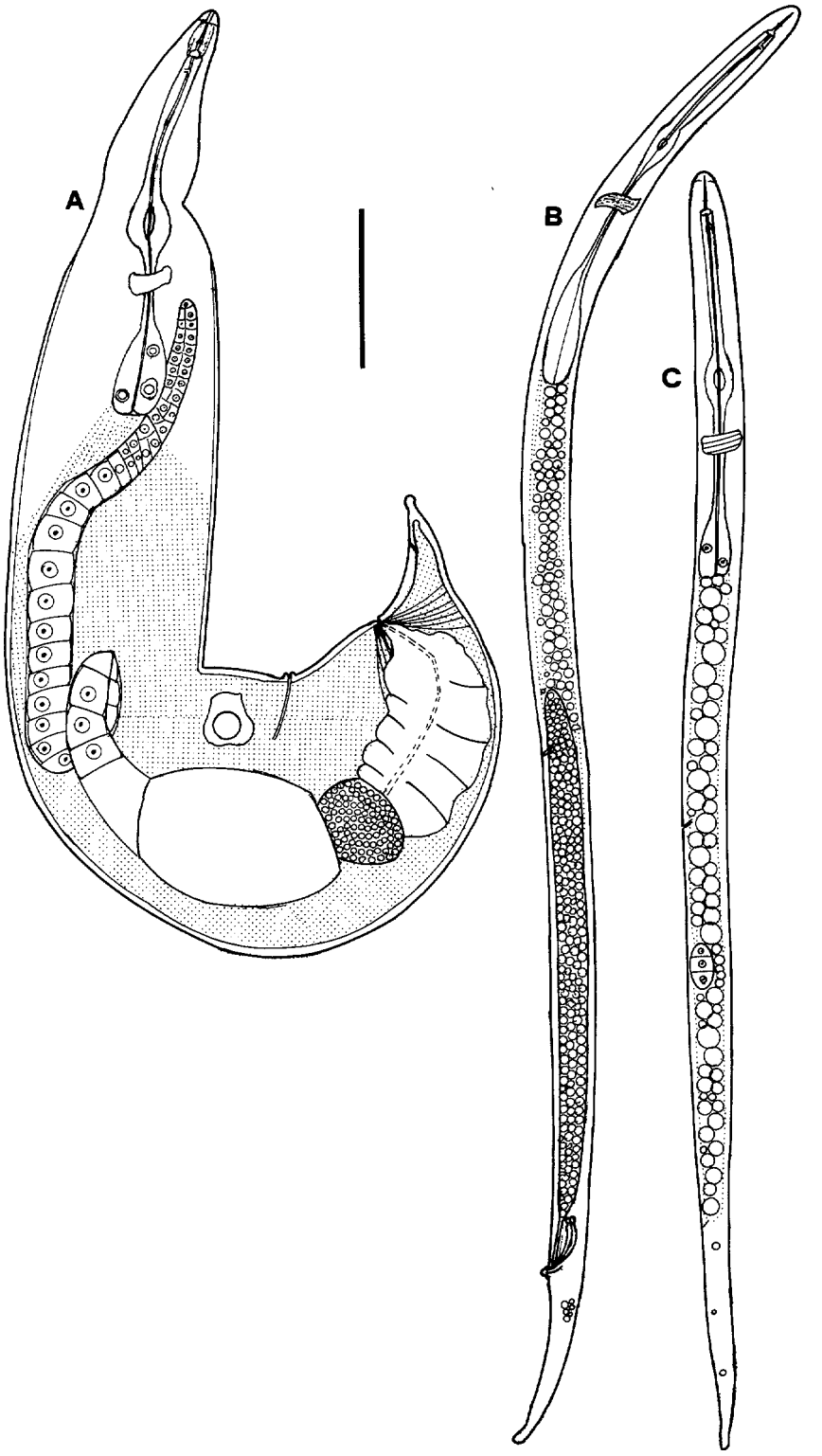

FIG. 3. *Tylenchulus graminis* n. sp. Scale bar for all figures = 50 μm. A) Adult mature female. Note the large nucleus of the excretory cell close to the excretory duct. B) Male. C) Second-stage juvenile, female.

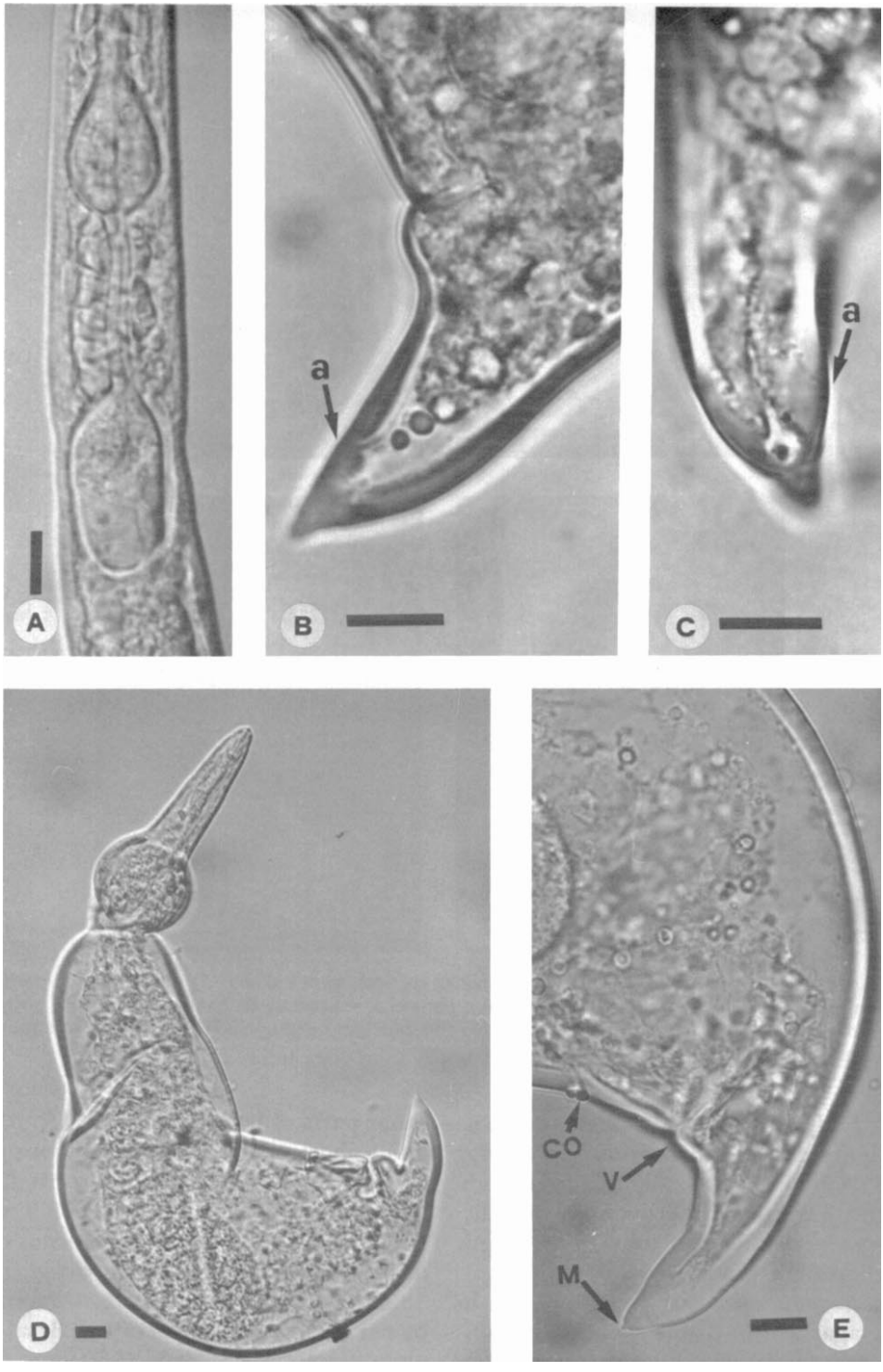

FIG. 4. Photomicrographs of *Tylenchulus graminis* n. sp. adult females. Scale bars = 10 μm. A) Anterior body portion with evident metacorpus, isthmus, and basal bulb. B, C) Postvulval sections with distinct rectum and anus (a). D) Entire body. E) Portion of posterior body with mucronate (M) postvulval section. CO = cuticular outgrowths. V = vulva.

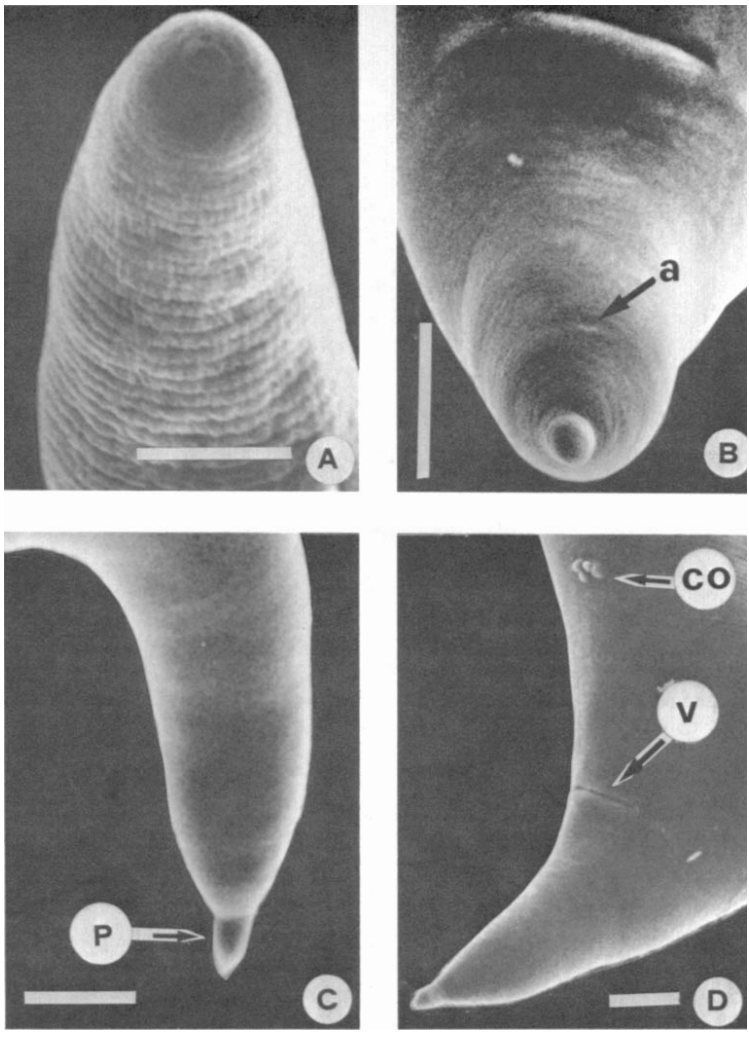

FIG. 5. SEM micrographs of *Tylenchulus graminis* n. sp. Scale bars = 5 μm. A) Anterior body portion of adult female. B) Postvulval section of mature adult female. a = anus. C, D) Shape variations of postvulval sections of adult females. Note pronounced peg (P) and cuticular outgrowths (CO) around excretory pore. V = vulva.

terior body end distance as percentage of total body length 83.8; portion of posterior body swollen as percentage of total body length 37.2; vulva anterior body end distance as percentage of total body length 89.9. Ratios: a = 4.1; b = 2.6.

Mature adult female: Measurements in water agar are in Table 4. Body translucent, white, 55% or less of posterior body portion variable, saccate, pendulum-like, widest portion between excretory pore and mid-body, narrowing abruptly after vulva (Figs. 11A, 12A). Annulation distinct with SEM near excretory pore, less evident in other parts of body (Fig. 12D). Lip region hemispherical, smooth, continuous with body. Framework weakly sclerotized. Stylet knobs well developed, 3.0–3.9 wide. DEGO 4.3–6.1 posterior. Procorpus cylindrical. Metacorpus muscular, ovoid, 11.2–18.3 wide. Isthmus elongate, surrounded by nerve ring near metacorpus. Basal bulb saccate, elongate, 19.3–28.5 long (Figs. 11A, 12B). Hemizonid not observed. Excretory pore located 80.9–87.5% from head apex, 12.2–28.5 anterior to vulva, vulva surrounded by 2–5 cuticular outgrowths (Figs. 12C, D; 13B). Excretory cell

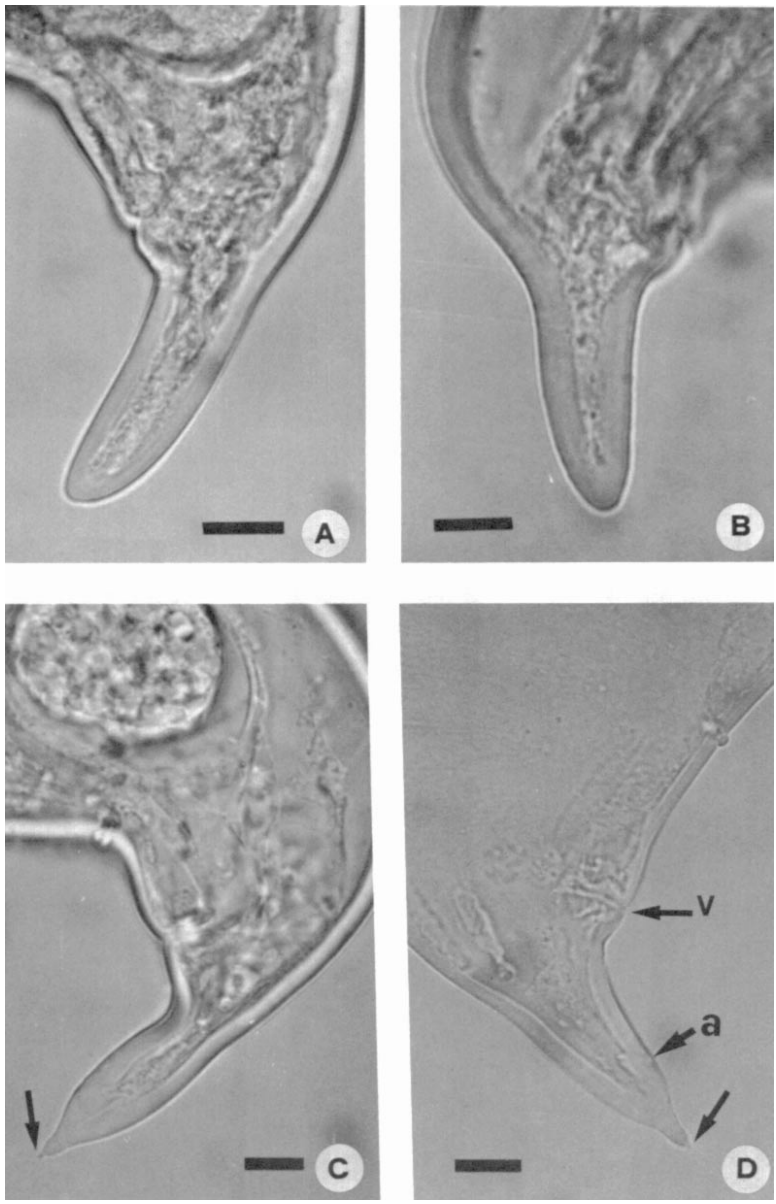

FIG. 6. Photomicrographs of *Tylenchulus semipenetrans* "Mediterranean race" from Italy and *Tylenchulus graminis* n. sp. Scale bars = 10 μm. A, B) Shape variations of mature adult female postvulval sections of *T. semipenetrans*. Note round, smooth terminus in all postvulval sections. C, D) Shape variations of mature adult female postvulval sections of *T. graminis*. Note pointed or mucronate (arrow) terminus of these postvulval sections. a = anus. v = vulva.

nucleus distinct, nucleolus prominent (Fig. 11A). Vulva slit-like, lips not sculptured (Figs. 12E; 13B, C). Ovary single, convoluted, extending anteriorly to metacorpus. Uterus swollen, ovate, followed by discrete oval spermatheca full of round sperm. Vagina uterina elongate, swollen (Fig. 11A). Anus and rectum not perceptible. Body behind vulva short, conoid, terminus round, smooth (Figs. 11A, 12E, 13B–D, 14C–F). PVSL 20.4–33.6; PVSW 11.2–17.3; PVSC 5.1–12.2. Cuticle thick, 2.5–4.4.

Males: Measurements in water agar are in Table 5. Body translucent white, vermiform, slender (Fig. 11B). Body cuticle striae fine, 0.7–0.9 apart. Lateral field ob-

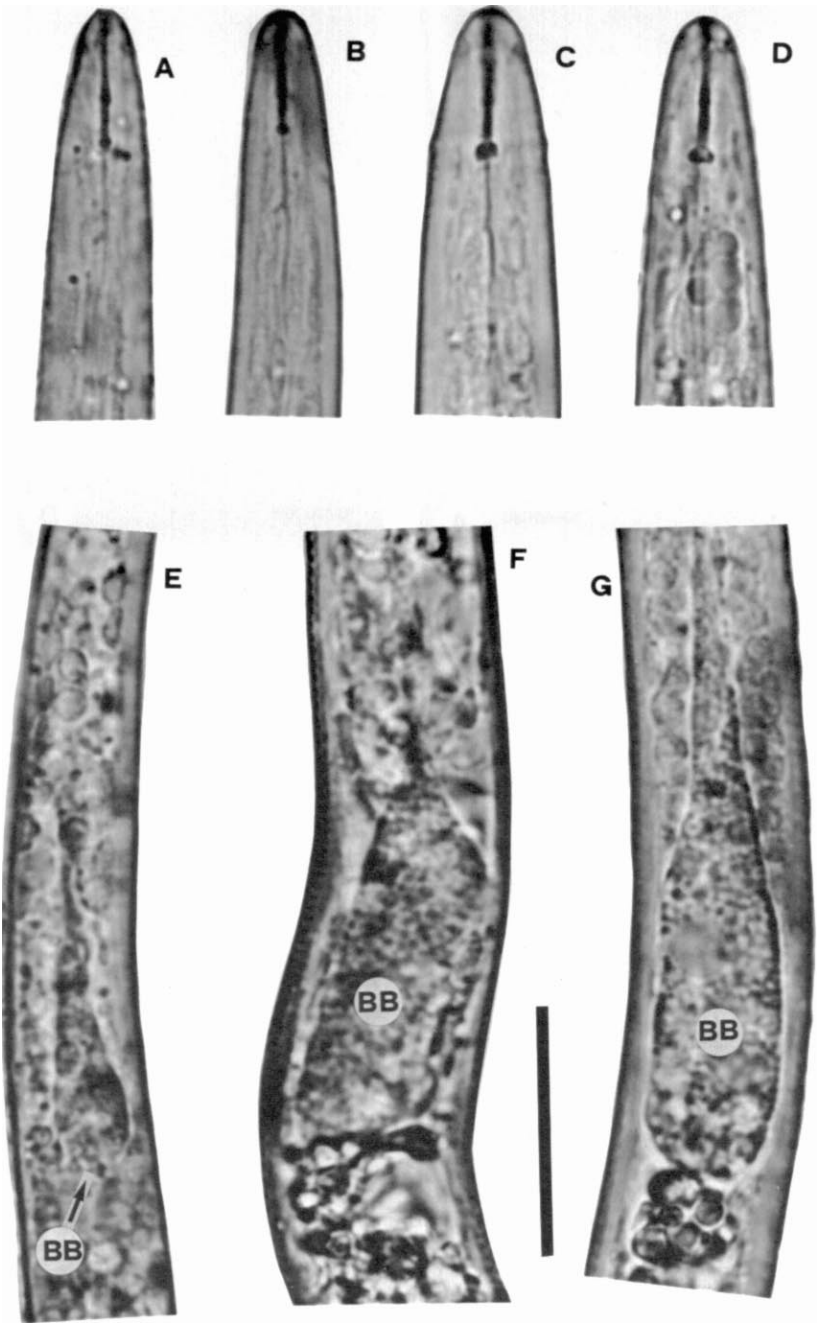

FIG. 7. Photomicrographs of morphological details of *Tylenchulus semipenetrans* (A, B, E), *Tylenchulus graminis* n. sp. (C, F), and *Tylenchulus palustris* n. sp. (D, G) males. Scale bar for all figures = 20 μm. A–D) Anterior body portion of *T. semipenetrans* (A, B), *T. graminis* (C), and *T. palustris* (D). Note the smaller stylet knobs of *T. semipenetrans* compared with *T. graminis* and *T. palustris*. E–G) Basal bulb (BB) of *T. semipenetrans* (E), *T. graminis* (F), and *T. palustris* (G). Note less developed basal bulb of *T. semipenetrans* compared with *T. graminis* and *T. palustris*.

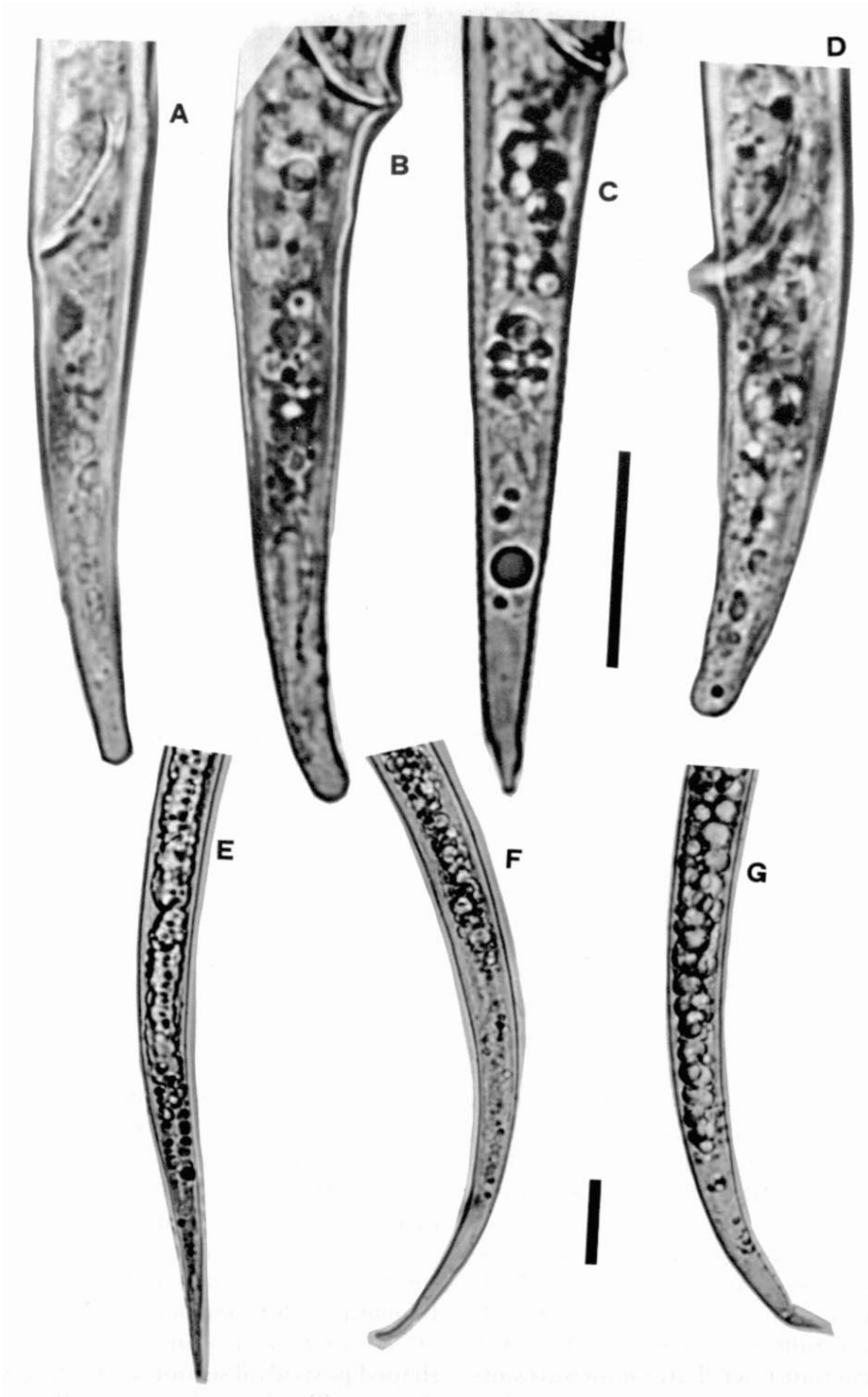

FIG. 8. Photomicrographs of morphological details of *Tylenchulus semipenetrans* (A, E), *Tylenchulus graminis* n. sp. (B, C, F), and *Tylenchulus palustris* n. sp. (D, G) males and J2 females. Scale bars = 18 μm. A–D) Male tail of *T. semipenetrans* (A), *T. graminis* (B, C), and *T. palustris* (D). Note longer tail of *T. graminis* compared with *T. palustris* and *T. semipenetrans*. E–G) Posterior body portion of J2 females of *T semipenetrans* (E), *T. graminis* (F), and *T. palustris*. (G). Note longer hyaline posterior body portion of *T. graminis* compared with *T. semipenetrans* and *T. palustris*.

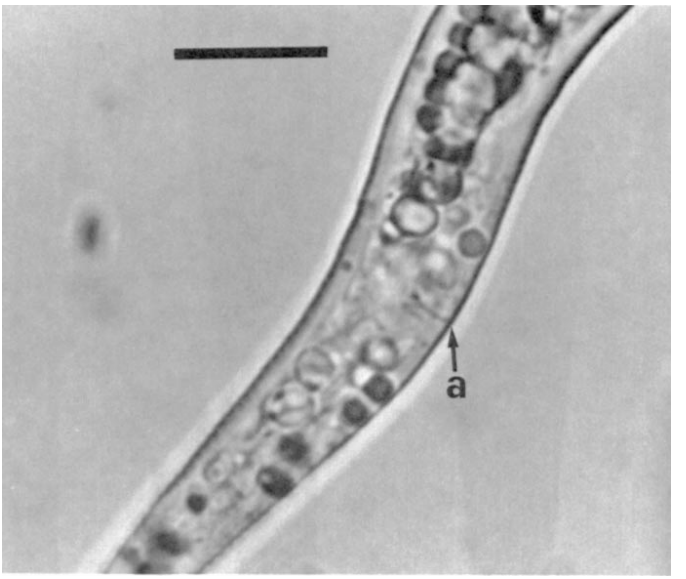

FIG. 9. Photomicrographic detail of posterior body portion of *Tylenchulus graminis* J2 female showing the rectum and anus (a). Scale bar = 10 μm.

scure, when visible, marked by two lines. Lip region hemispherical, without distinct annulation; labial framework inconspicuous. Stylet delicate, knobs rounded, 1.7–2.1 wide (Figs. 7D, 11B), DEGO 4.0–5.1 posterior. Procorpus partially degenerated, lumen distinct. Metacorpus distinct in about 72% of specimens, musculature obscure. Basal bulb well developed, pyriform, 8.1–11.2 wide, 22.4–32.6 long (Figs. 7G, 11B). Excretory pore evident, 51.3–59.8% from head apex. Testis single, occupying 25.8–39.4% of body length. Spicules slender, arcuate (Fig. 12F). Gubernaculum slightly curved. Caudal region cylindrical, terminus bluntly rounded, smooth, 33.6–43.8 long (Figs. 8D, 11B, 12F). Caudal alae absent.

Female second-stage juvenile: Measurements in water agar are in Table 5. Body translucent, white, vermiform, slightly curved ventrally when fixed (Fig. 11C). Cuticle striae indistinct. Lateral incisures obscure, appearing as two faint lines when visible. Lip region hemispherical, not set off; labial framework weak. Stylet well developed, knobs rounded, 3.0–3.9 wide (Fig. 11C), DEGO 3.4–5.0 posterior. Procorpus cylindrical; metacorpus muscular, ovoid; basal bulb saccate, posterior end slightly obscured by short overlap of intestine. Excretory pore evident, located behind midbody, 50.5–60.4 from anterior end, 2.0–25.5 anterior to genital primordium. Genital primordium with 2–4 cells, 56.3–69.0% from anterior end. Anus and rectum not observed in live or fixed specimens. Hyaline posterior body portion containing few small fat globules (< 2 diameter), 28.5–59.1 long, terminus rounded (Figs. 8G, 10C, 11C).

Eggs (20 in 2.5% formalin): Length 67.3–81.6 (mean 71.8), SD 3.4. Width 30.6–36.7 (33.1), SD 2.0. Length/width ratio 1.9–2.6 (2.1), SD 0.21. Eggs protected by a gelatinous matrix. Egg shell hyaline, unsculptured, as seen by light microscope.

Diagnosis: Tylenchulus palustris is more similar to *T. semipenetrans* than to *T. furcus.* Its mature adult females differ from those of *T. semipenetrans* in having a conoid shaped postvulval section with a large base that is unlike the digitate, roundly smooth terminus of *T. semipenetrans* (Figs. 13A–D, 14A–F). The PVSL is shorter in *T. palustris* than in *T. semipenetrans* ($P = 0.01$) (20.4–33.6 vs. 26.5–52.0), and the PVSW and PVSC are greater ($P = 0.01$) (11.2–17.3 vs.

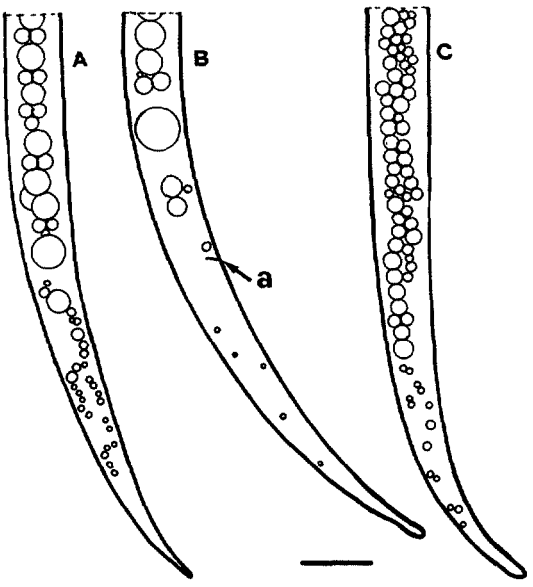

FIG. 10. Posterior body portions of *Tylenchulus semipenetrans* (A), *Tylenchulus graminis* (B), and *Tylenchulus palustris* (C) J2 females. Note presence of anus (a) and longer body posterior portion without large globules in *T. graminis* compared with *T. semipenetrans* and *T. palustris*. Scale bar = 13.4 μm.

9.1–13.2 and 5.1–12.2 vs. 1.8–7.1, respectively). *T. palustris* males have wider stylet knobs ($P = 0.01$) than *T. semipenetrans* (1.7–2.1 vs. 0.9–1.2); a more developed esophagus with a wider ($P = 0.01$) basal bulb (8.1–11.2 vs. 5.1–8.0); and a cylindrical tail with a bluntly rounded terminus which is tapered in *T. semipenetrans* (Figs. 7A, B, D, E, G; 8A, D).

T. palustris and *T. semipenetrans* J2 are indistinguishable (Figs. 8E, G; 10A, C).

T. palustris differs from *T. furcus* and *T. graminis* in having adult females that are less ($P = 0.01$) swollen posteriorly (32.3–53.8 vs. 66.0–85.1%) (Figs. 1B, 4D, 12A) in the shape of the adult female postvulval section which is conoid with a round, smooth terminus, and in having an imperceptible anus and rectum (Figs. 1B; 6C, D; 13B–D; 14C–F) (14).

Males of *T. palustris* differ from those of *T. furcus* and *T. graminis* by having a shorter ($P = 0.01$) tail (33.6–43.8 vs. 48.9–65.2) that is cylindrical with a round, blunt terminus vs. conoid with a pointed terminus in *T. furcus* and *T. graminis* (Fig. 8B–D) (14).

TABLE 4. Measurements of 25 mature adult females of *Tylenchulus palustris* from Perry, Florida.

Morphological characters	Range	Mean	Standard deviation
Measurements in μm			
Basal bulb length	19.3–28.5	23.0	2.4
Basal bulb width	9.1–20.4	12.8	3.1
Body length	271.3–410.0	321.2	32.7
Body width	65.2–99.9	78.2	11.2
BWV	20.4–35.7	25.1	3.6
Cuticle thickness	2.5–4.4	3.5‡	0.7
DEGO	4.3–6.1	5.3	0.5
Esophagus length	102.0–150.9	122.2	12.8
EPBD	229.4—328.0	272.8	32.3
Isthmus length	16.3–27.5	21.1	3.4
MEBD	48.9–78.5	59.0	6.3
Metacorpus length	16.3–25.5	20.8	2.7
Metacorpus width	11.2–18.3	14.3	2.2
PVSC	5.1–12.2	7.1†	1.3
PVSL	20.4–33.6	27.5†	3.3
PVSW	11.2–17.3	14.0†	1.7
Stylet knob width	3.0–3.9	3.2	0.3
Stylet length	11.2–12.2	11.5	0.3
VEPD	12.2–28.5	19.9	4.0
Percentages			
Excretory pore anterior body end distance as % of total body length	80.9–87.5	84.8	2.4
Portion of posterior body swollen as % of total body length	32.3–53.8	40.0‡	5.0
Vulva anterior body end distance as % of total body length	88.1–94.4	91.0	4.0
Ratios			
a	3.3–5.5	4.1	0.6
b	1.9–3.3	2.6	0.3

†‡ Symbols indicate significant ($P = 0.01$) differences with *T. semipenetrans* (†) or *T. graminis* (‡).

T. palustris J2 differ from those of *T. furcus* and *T. graminis* by having an imperceptible anus and rectum. They differ from *T. furcus* J2 in having a rounded tail tip vs. furcate, and from *T. graminis* in their shorter posterior hyaline body section (28.5–59.1 vs. 58.1–76.5) (Figs. 8F, G; 10C).

Type host and locality: Pop ash (*Fraxinus caroliniana*) collected at Aucilla Wildlife

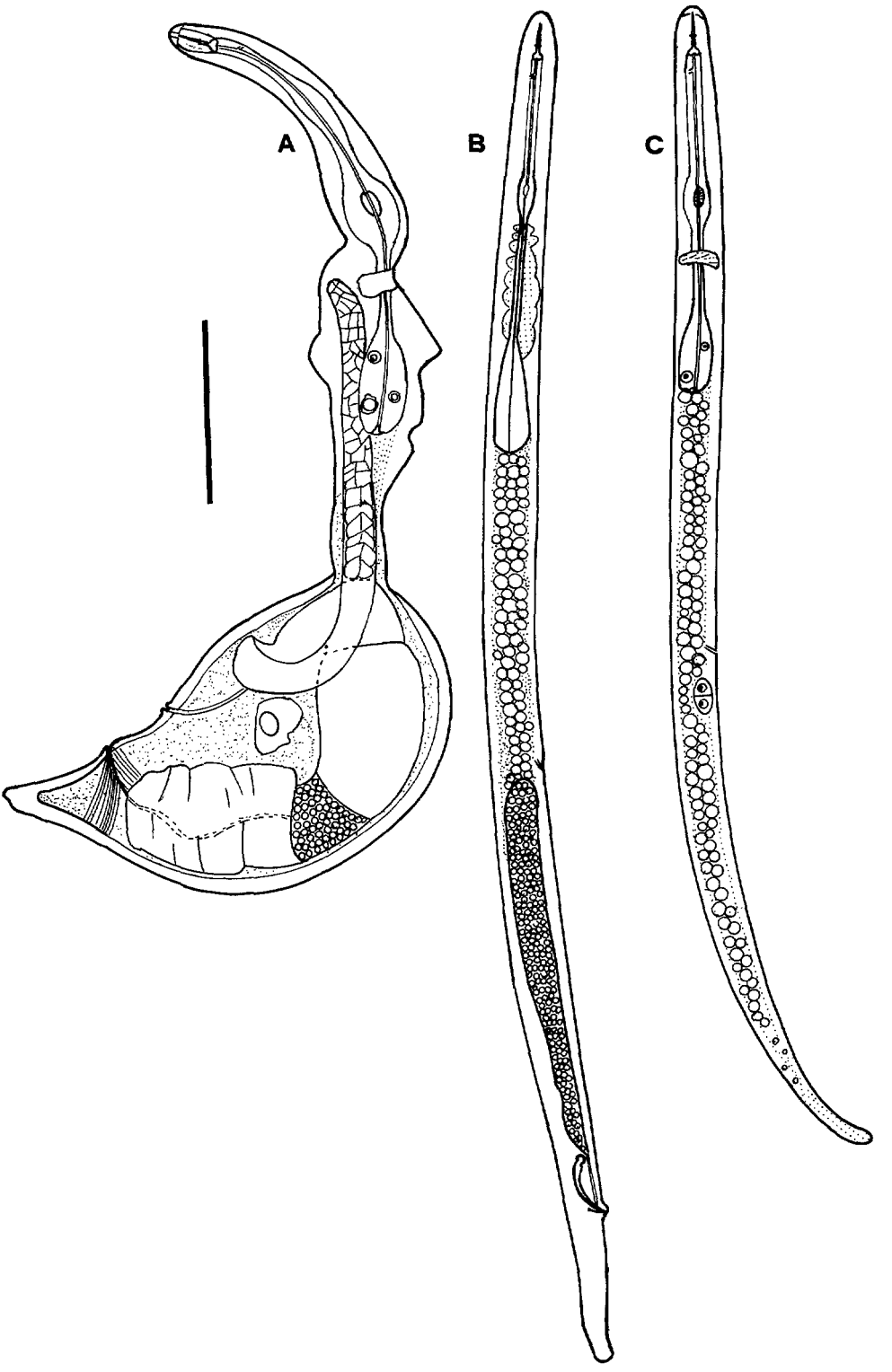

FIG. 11. *Tylenchulus palustris* n. sp. Scale bar for all figures = 51 μm. A) Adult mature female. Note large nucleus of excretory cell close to excretory duct. B) Male. C) J2 female.

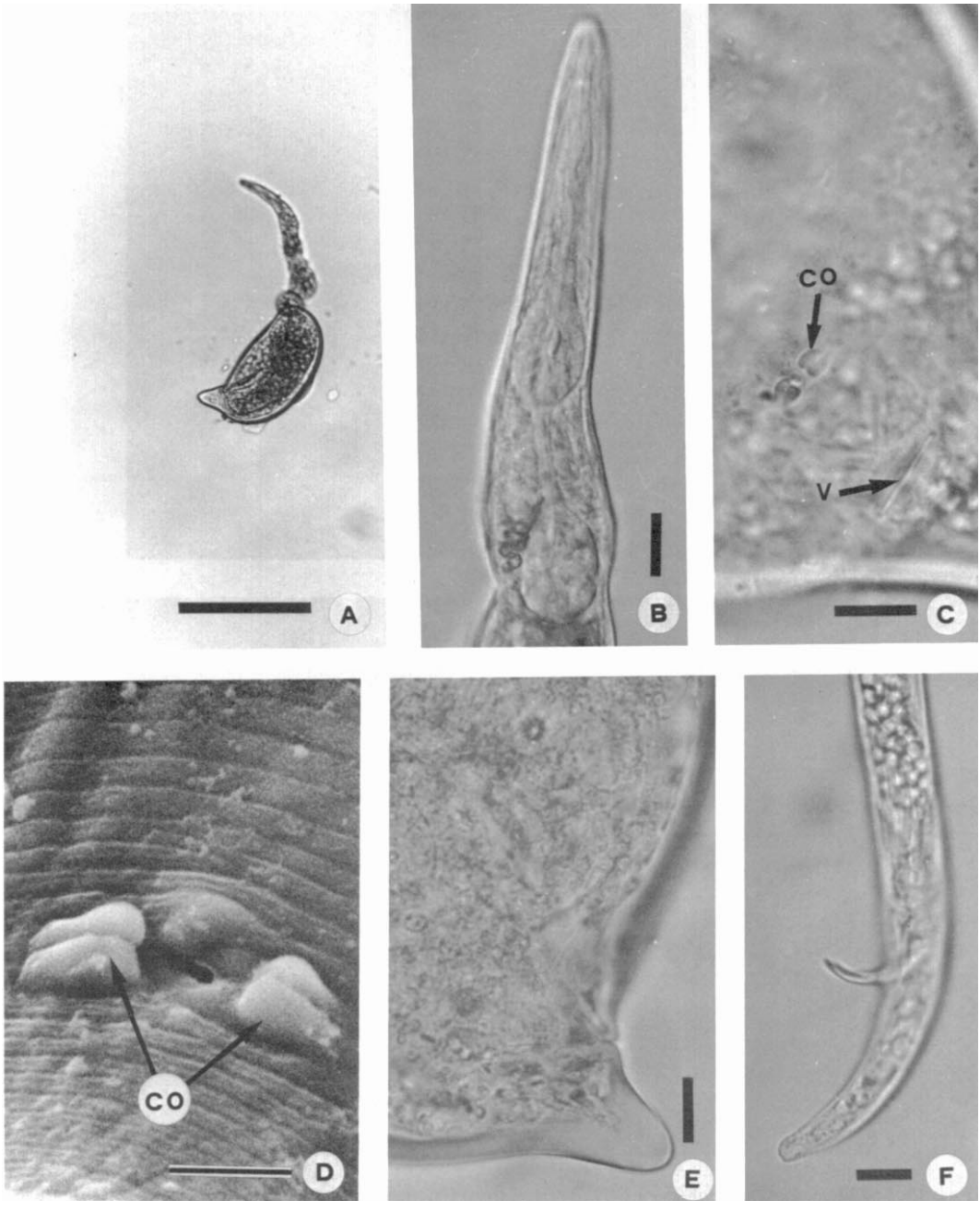

FIG. 12. *Tylenchulus palustris* n. sp. life stages. Scale bar = 129 μm in A, 5 μm in D, and 10 μm in the others. A) Mature adult female. B) Anterior body portion of adult female with evident metacorpus, isthmus, and basal bulb. C) Ventral view of mature adult female posterior body showing four cuticular outgrowths (CO) around excretory pore, and vulva (V). D) SEM micrograph of four cuticular outgrowths surrounding excretory pore. Note distinct body annulation. E) Posterior body portion of mature adult female showing short conoid postvulval section with round terminus. F) Posterior body portion of male showing cylindrical tail with bluntly rounded terminus. (Photomicrographs except for D.)

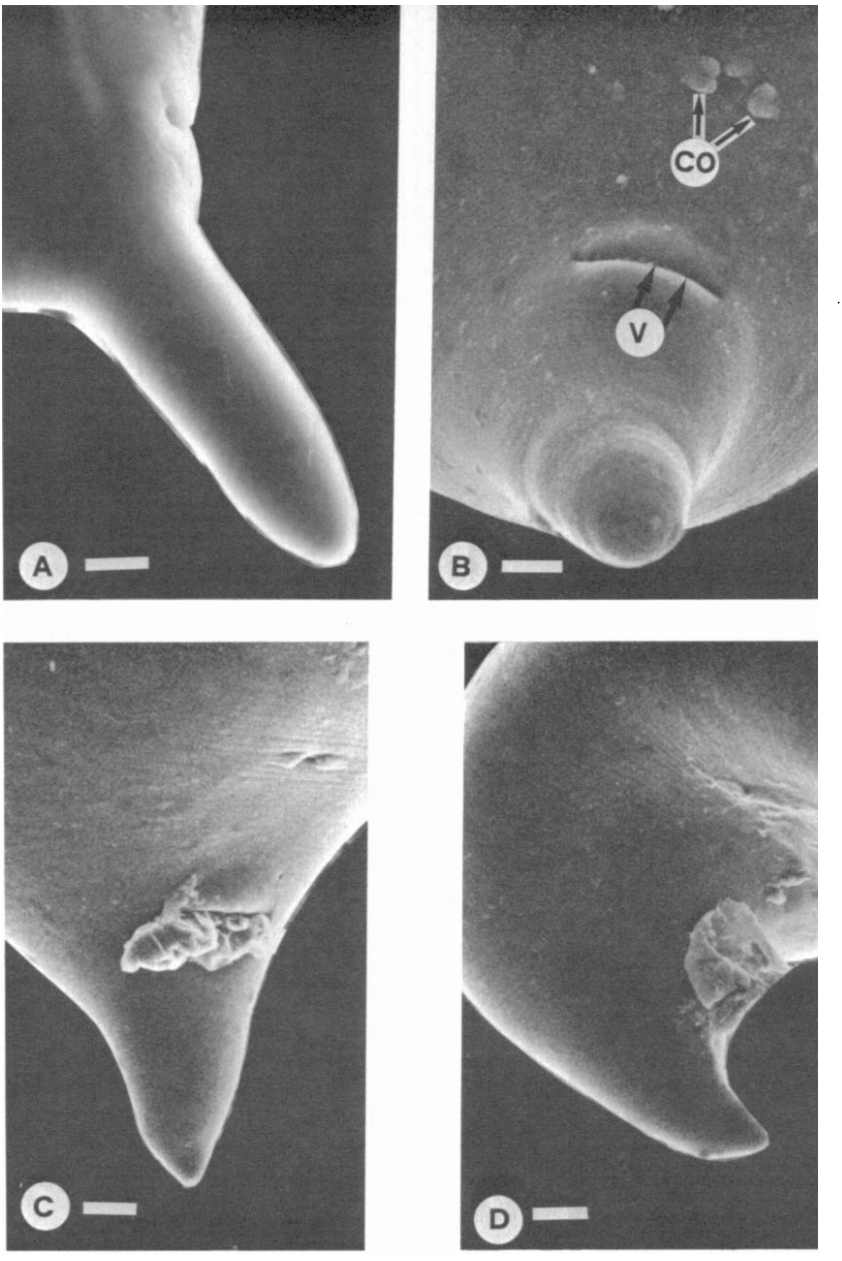

FIG. 13. SEM micrographs of *Tylenchulus semipenetrans* (A) and *Tylenchulus palustris* n. sp. (B–D) mature adult females. Scale bars = 5 μm. A) Digitate postvulval section of *T. semipenetrans*. B) Ventral view of posterior body portion of *T. palustris* showing four cuticular outgrowths (CO) around excretory pore, and vulva (V). C, D) Shape variations of conoid postvulval section of *T. palustris*.

Management Area, Perry, Florida. Another host of *T. palustris* is salt bush (*Baccharis halimifolia*).

Type specimens: Holotype (mature female)—isolated from infected roots of pop ash collected from the type locality. Slide T-431t, deposited in the United States Department of Agriculture Nematode Collection, Beltsville, Maryland. Paratypes (mature adult females, males and J2)—same data as holotype. Repositories same as for *T. graminis*.

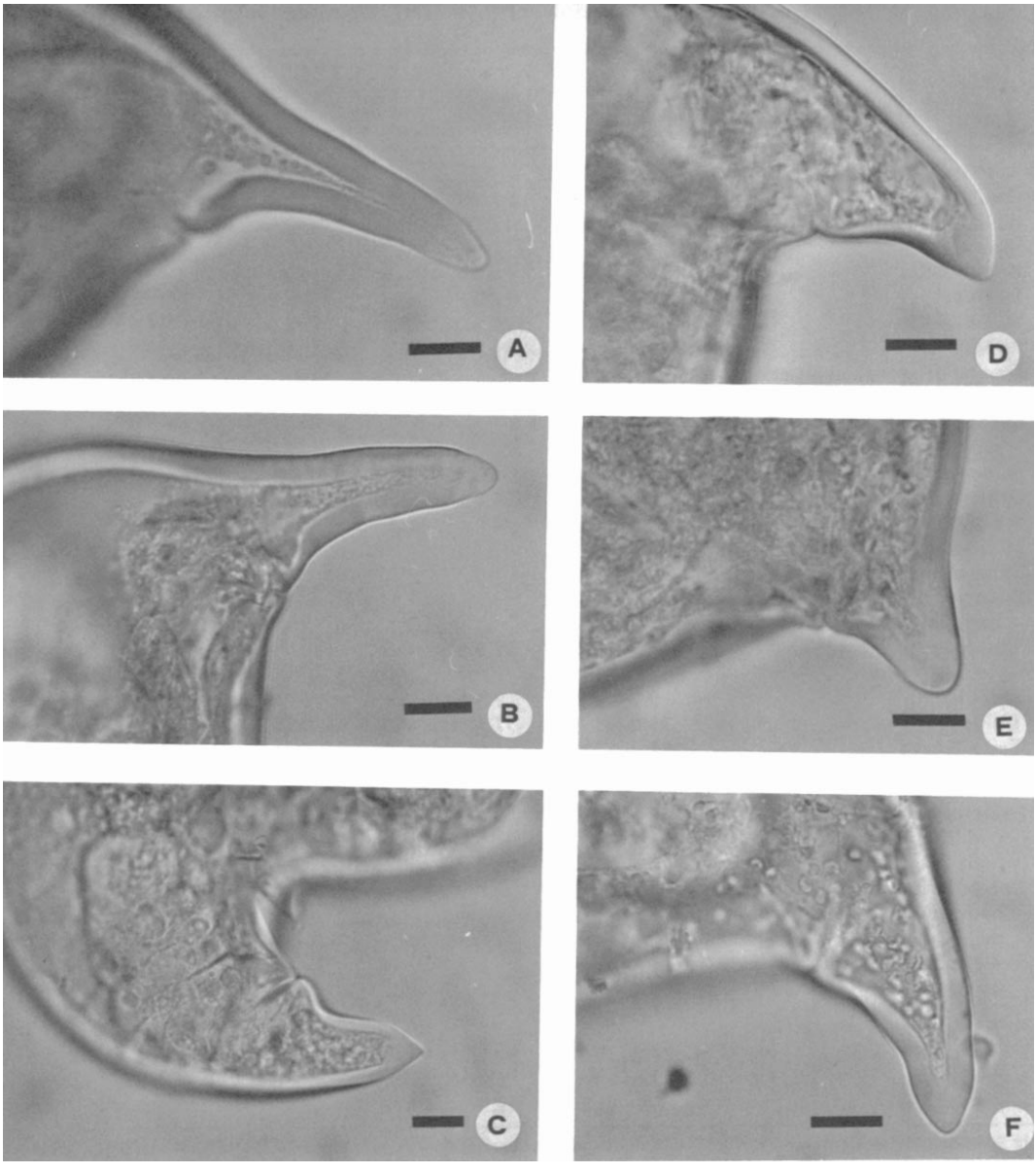

FIG. 14. Photomicrographs of posterior body portions of *Tylenchulus semipenetrans* (A, B) and *Tylenchulus palustris* n. sp. (C–F) mature adult females. Scale bars = 10 μm. A, B) Morphological variations of digitate and rounded postvulval terminus of *T. semipenetrans.* C–F) Morphological variations of convoid postvulval section of *T. palustris.*

DISCUSSION

A characteristic of the genus *Tylenchulus* as defined by Cobb (2,3) and recently by Siddiqi (12) is the lack of a functional anus. However, the morphological characters of *T. furcus* (14) and *T. graminis* indicate that a distinct rectum and anus can occur in this genus. Species that attack monocots, such as *T. furcus* and *T. graminis,* have a perceptible rectum and anus, whereas species that infect dicots, such as *T. semipenetrans* and *T. palustris,* do not.

The presence of cuticular outgrowths around the excretory pore is a peculiar characteristic of the genus *Tylenchulus* occurring in all described species (11,14).

Previously, it was believed that *T. gra-*

TABLE 5. Measurements of males and J2 females of *Tylenchulus palustris* from Perry, Florida.

Morphological characters	Males (N = 20)			J2 females		
	Range	Mean	Standard deviation	Range	Mean	Standard deviation
Measurements in μm						
Basal bulb length	22.4–32.6	27.2	2.7	18.3–22.4	20.5	1.5
Basal bulb width	8.1–11.2	9.1†	0.7	7.6–10.2	8.5	0.6
Body length	331.6–443.7	371.3	26.9	277.4–354.9	321.2	19.9
Body width	12.2–14.2	13.3	0.5	13.2–14.2	13.2	0.4
DEGO	4.0–5.1	4.5	0.3	3.4–5.0	4.0	0.3
Esophagus length	108.1–137.7	124.3	7.8	102.0–116.2	108.5	4.5
EPBD	171.3–260.1	209.9	19.3	165.2–207.0	183.0	11.1
Genital primordium anterior body end distance				167.0–214.2	181.4	12.0
Genital primordium excretory pore distance				2.0–25.5	12.8‡	6.4
Gubernaculum length	4.0–5.1	4.5	0.5			
Isthmus length	30.6–39.7	34.9	2.6	23.4–28.5	26.5	1.4
MEBD	40.8–49.9	46.0	2.5	41.8–52.0	47.1	3.2
Metacorpus length				14.2–16.3	15.3	0.8
Metacorpus width				7.1–8.1	7.8	0.4
Posterior body section without large fat globules				28.5–59.1	49.8‡	6.7
Spicule length	16.3–19.3	17.0	0.9			
Stylet knob width	1.7–2.1	1.9†	0.09	3.0–3.9	3.3	0.2
Stylet length	9.1–10.2	9.5	0.4	11.2–12.9	12.2	0.3
Tail length	33.6–43.8	37.1‡	2.7			
Testis length	87.7–153.0	124.2	19.0			
Percentages						
Excretory pore anterior body end distance as % of total body length	51.3–59.8	56.3	2.2	50.5–60.4	57.0	2.2
Genital primordium anterior body end distance as % of total body length				56.3–69.2	60.6	3.1
Testis length as % of total body length	25.8–39.4	33.3	4.2			
Ratios						
a	24.2–31.2	27.7	1.7	21.0–29.0	24.1	1.8
b	2.6–3.4	2.9	0.1	2.6–3.2	2.9	0.1
c	8.1–11.1	9.9	0.6			

†‡ Symbols indicate significant ($P = 0.01$) differences with the correspondent parameters of *T. semipenetrans* (†) or *T. graminis* (‡).

minis and *T. palustris* were *T. semipenetrans* races adapted to the noncultivated Florida flora. The morphological differences observed in these so-called "wild races" from other *T. semipenetrans* races suggest that *T. graminis* and *T. palustris* very probably were present in Florida long before *T. semipenetrans* was introduced with infected citrus plants. These findings have important regulatory significance and beneficial effects for the citrus industry in Florida, because land infested with the former "wild race" of the citrus nematode can now be certified for citrus nurseries.

The genus *Tylenchulus*, which had two species—*T. furcus* and *T. semipenetrans*, now includes four species identified in the accompanying key.

KEY TO SPECIES OF *Tylenchulus* COBB, 1913

1. Second-stage juveniles and adult females with distinct rectum and anus. Adult mature females with body

swollen posteriorly 60% or more of its total length 2

1. Second-stage juveniles and adult females without distinct rectum and anus; adult mature females with body swollen posteriorly for 58% or less of its total length 3
2. Second-stage juveniles with furcate or bifid tail tips ..... *T. furcus* Van Den Berg & Spaull, 1982
2. Second-stage juveniles with tail tapering or narrowly conoid, not furcate ..... *T. graminis* n. sp.
3. Adult mature females with conoid postvulval section with broad base; males with stylet knobs 1.6 μm wide or more, basal bulb 8.1 μm wide or more, and tail cylindrical with bluntly rounded terminus ..... *T. palustris* n. sp.
3. Adult mature females with digitate postvulval section with round terminus; male stylet knobs 1.2 μm wide or less, basal bulb 8.0 μm wide or less, tail tapering ..... *T. semipenetrans* Cobb, 1913

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