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Tylenchulus palustris Parasitizing Peach Trees in the United States

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Abstract: Most morphological characteristics of three populations of a *Tylenchulus* sp. from peach roots in Alabama, Arkansas, and Georgia did not differ from those of *T. palustris* paratypes. However, some mature females differed slightly from those of *T. palustris* paratypes from Florida. These mature females were more swollen in the posterior portion of their bodies, and they possessed digitate postvulval body sections with round rather than conoid termini. These morphological variants had a wide postvulval section core (PVSC), as do *T. palustris* paratypes; they did not differ from the paratypes in other characteristics. Second-stage juveniles and males were less morphologically variable and were not different from the paratypes. No males were found in populations from Alabama and Georgia. The *Tylenchulus* sp. from three peach sites was determined to be *T. palustris*. This is the first report of *T. palustris* on an economically important crop.

Key words: Alabama, Arkansas, citrus nematode, Georgia, morphological variability, peach, *Prunus persica,* scanning electron microscopy (SEM), systematics, *Tylenchulus palustris, Tylenchulus semipenetrans.*

Rutaceous species and a limited number of other dicotyledonous plants are infected by *Tylenchulus* spp. in the United States. *Diospyrus lotus* L., *Mikania batatifolia* DC., *Olea europaea* L., and *Vitis vinifera* L. are reported as hosts of *T. semipenetrans* Cobb, 1913, whereas *Fraxinus caroliniana* Mill. and *Baccharis halimifolia* L. are infected by *T. palustris* Inserra et al., 1988 (5,7). For several years a *Tylenchulus* species has been detected in diagnostic samples collected from peach (*Prunus persica* (L.) Batsch.) in the United States (8). Populations from Alabama and Georgia were tentatively identified as *T. semipenetrans,* whereas a population from Arkansas was considered distinct from *T. semipenetrans* in several morphological characteristics of both adult females and males (8). In 1988 a cooperative study was conducted in order to describe the morphological characteristics of these populations and to determine their taxonomic status.

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Materials and Methods

Three *Tylenchulus* populations infecting peach roots were studied. The first population was collected from a peach orchard in Georgia and is now maintained in peach culture in a greenhouse at the USDA laboratory in Byron, Georgia. The second population was from a peach orchard in Clanton, Alabama, and the third was from a peach orchard in Batesville, Arkansas. Peach trees in the latter two sites were grafted on 'Lovell' rootstock.

Adult males and second-stage juveniles (J2) were extracted from the soil by Cobb's sieving and decanting method (2). Entire adult female bodies were teased from peach roots with needles.

Live specimens for measuring, drawing, and photographing were placed on the surface of water agar under a cover slip (3). Additional specimens were killed and fixed in hot aqueous 4% formaldehyde + 1% propionic acid, dehydrated in ethanol vapor, and mounted in dehydrated glycerin (4).

Scanning electron microscopy (SEM) specimens 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 stubs (1). Specimens were coated with gold and observed at 5 kV acceleration voltage.

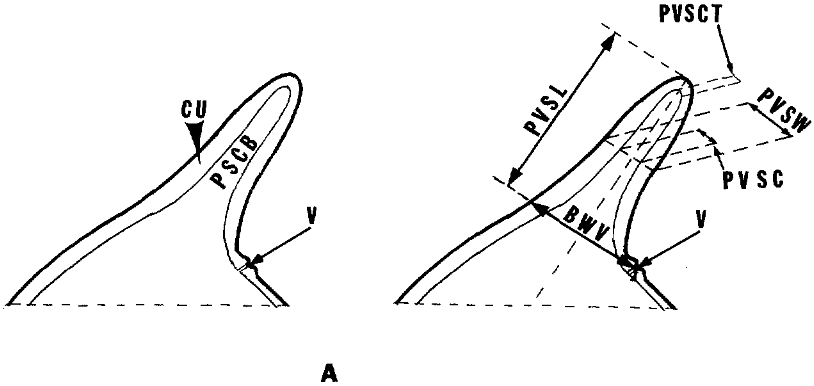

FIG. 1. Schematic representation of postvulval body portions of a tylenchulid adult stage. A) Selected morphological structures. CU = cuticle. PSCB = postvulval body section core. V = vulva. B) Selected parameters. BWV = body width at vulva. PVSC = width of postvulval section core. PVSCT = width of postvulval body core at terminus. PVSL = postvulval section length of body. PVSW = postvulval section width of body. V = vulva. Broken line in body core indicates body axis.

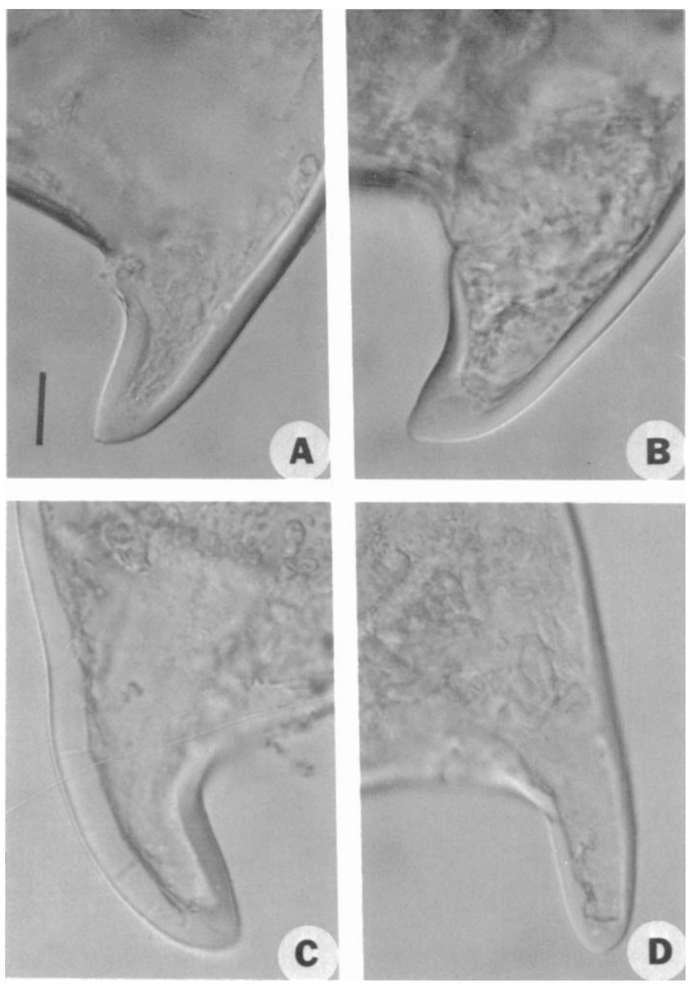

FIG. 2. Photomicrographs of posterior bodies of mature females of *Tylenchulus palustris* and *Tylenchulus* sp. from peach. A, B) *T. palustris* paratypes. C, D) *Tylenchulus* sp. from peach in Georgia. Note slight morphological variations between the two populations. Scale bar for A–D = 10 μm.

TABLE 1. Morphometrics of mature adult females of *Tylenchulus* sp. from peach.

	Alabama (N = 8)				Arkansas (N = 25)				Georgia (N = 25)			
Morphological characters†	Range	Mean	SD	CV	Range	Mean	SD	CV	Range	Mean	SD	CV
					Measurements in μm							
Basal bulb length	19.6–27.4	23.5	2.67	11.3	19.6–33.0	22.7	2.71	11.9	24.0–30.4	26.8	2.08	7.7
Basal bulb width	12.0–15.7	13.6	1.07	7.8	11.7–16.6	13.5	1.64	12.1	12.0–18.4	14.4	1.50	10.3
Body length	261.6–308.7	276.5	17.31	6.2	224.4–316.5	265.9	24.79	9.3	253.8–382.4	326.5	32.23	9.8
Body width	44.1–70.6	49.6	10.82	21.8	39.2–76.4	57.6	9.60	16.6	44.1–73.5	54.8	7.49	13.6
BWV	21.6–29.4	25.1	2.55	10.1	20.6–30.4	25.0	2.42	9.6	26.4–33.6	29.0	2.30	7.9
Cuticle thickness	1.0–3.1	2.0	0.85	40.7	1.0–3.2	1.9	0.70	36.1	1.5–4.9	2.7	0.96	34.8
DEGO	4.4–4.9	4.7	0.18	3.8	4.4–5.0	4.6	0.25	5.4	4.6–5.6	4.9	0.25	5.1
Esophagus length	112.7–129.4	118.9	6.00	5.0	111.7–128.0	116.7	3.90	3.3	125.4–144.0	132.8	5.25	3.9
EPBD	199.9–257.7	223.3	19.65	8.8	162.7–251.9	211.6	24.81	11.7	202.9–299.2	259.7	28.07	10.8
Isthmus length	20.6–25.5	23.9	3.00	12.5	19.6–24.5	21.9	1.48	6.7	22.5–32.0	25.5	2.64	10.3
MEBD	49.0–53.9	52.2	1.79	3.4	49.0–57.8	52.1	2.11	4.0	54.9–66.4	60.3	3.38	5.6
Metacorpus length	17.6–21.6	19.3	1.48	7.6	17.0–23.5	20.0	1.92	9.6	16.8–23.2	20.5	1.59	7.7
Metacorpus width	11.8–19.6	15.2	2.82	18.5	11.7–17.6	14.5	1.88	12.9	13.7–20.0	16.3	1.16	7.1
PVSC	8.4–11.8	9.9	1.37	13.7	6.4–11.8	8.7	1.13	12.8	7.2–12.8	9.8	1.83	18.6
PVSCT	3.8–5.9	4.7	0.66	14.2	2.5–6.8	4.4	0.83	18.7	3.2–5.8	4.4	0.59	13.2
PVSL	28.4–45.0	34.1	5.38	15.7	27.4–43.1	34.5	4.94	14.2	34.2–50.4	38.8	8.46	21.8
PVSW	12.7–14.7	14.0	0.70	5.0	11.8–16.7	13.5	0.87	6.4	13.2–18.4	15.1	1.53	10.0
Stylet knob width	3.6–4.0	3.9	0.05	1.3	3.4–4.0	3.7	0.16	4.3	3.4–4.8	4.0	0.22	5.5
Stylet length	11.8–12.8	12.4	0.37	3.0	10.8–11.8	11.4	0.37	3.2	12.6–13.6	13.1	0.34	2.6
VEPD	14.7–25.4	18.2	4.52	24.8	9.8–36.2	19.5	5.08	25.9	13.6–40.0	25.2	6.38	25.2
					Percentages							
Excretory pore anterior body end distance as % of total body length	75.2–84.6	80.6	2.98	3.7	74.1–85.9	79.4	3.04	3.8	76.2–82.1	79.8	2.02	2.5
Portion of posterior body swollen as % of total body length	59.0–83.7	74.8	8.61	11.5	43.2–85.0	67.3	11.34	16.8	37.6–70.0	51.6	8.12	15.7
Vulva anterior body end distance as % of total body length	82.6–89.8	87.1	2.41	2.7	83.0–91.0	86.8	2.03	2.3	84.8–90.0	87.6	1.39	1.5
					Ratios							
a	4.4–7.4	5.7	1.09	19.0	3.1–6.8	4.6	0.79	17.1	4.2–7.2	6.0	0.77	12.8
b	2.2–2.5	2.3	0.13	5.8	1.8–2.7	2.2	0.22	9.8	1.8–2.8	2.4	0.23	9.5

† All abbreviations explained in the text.

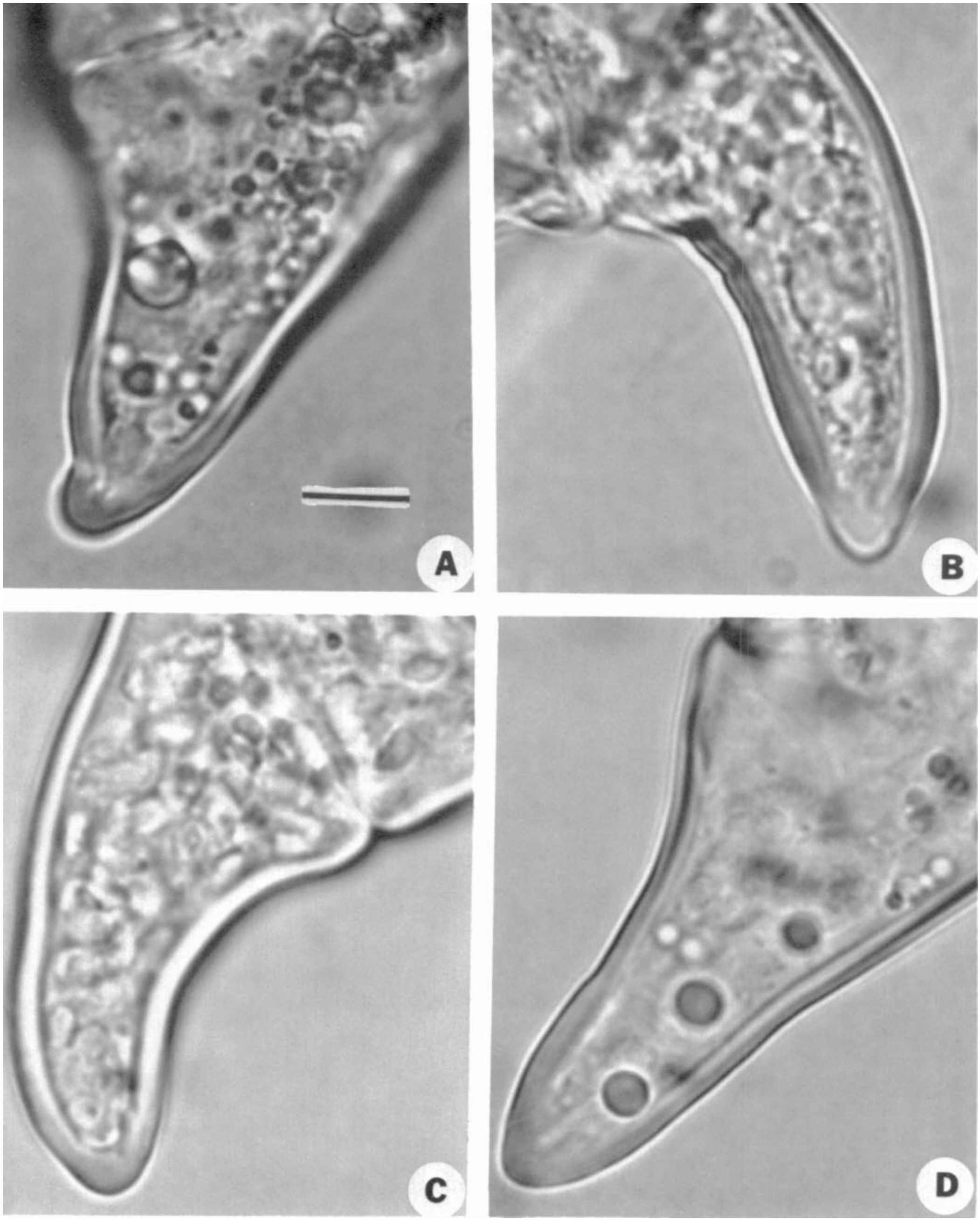

FIG. 3. Photomicrographs of posterior bodies of mature *Tylenchulus* sp. females from peach. A, B) Arkansas population. C, D) Alabama population. Scale bar for A–D = 8 μm.

Preliminary observations indicated that the *Tylenchulus* populations from peach might be *T. palustris.* Therefore, the peach populations were compared with the paratypes of this recently described species (6). The same morphological parameters reported in the original description of *T. palustris* (6) were evaluated in this study. The posterior portion of the adult female body delimited proximally by the vulva and distally by the body terminus was termed the postvulval section of body (Fig. 1A, B). The body organs covered by the cuticle in the postvulval section were termed the postvulval section core of body (Fig. 1A). The following abbreviations were used previ-

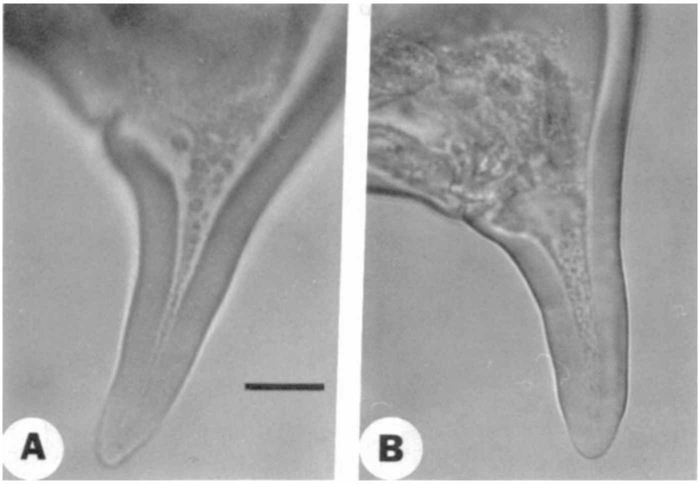

FIG. 4. Photomicrographs of posterior bodies of mature *Tylenchulus semipenetrans* females. A, B) Females from a Florida citrus orchard. Note that terminus of postvulval body core is narrow and inconspicuous compared to those of *T. palustris* paratypes and *Tylenchulus* sp. from peach (Figs. 2, 3). Scale bar for A, B = 10 μm.

TABLE 2. Morphometrics of adult males of *Tylenchulus* sp. from peach in Arkansas and *T. palustris* from pop ash in Florida.

	Arkansas (N = 15)				Florida (N = 20)‡			
Morphological characters†	Range	Mean	SD	CV	Range	Mean	SD	CV
	Measurements in μm							
Basal bulb length	18.6–29.0	24.3	2.65	10.9	22.4–32.6	27.2	2.7	9.9
Basal bulb width	8.3–10.0	9.0	0.66	7.3	8.1–11.2	9.1	0.7	7.6
Body length	338.1–407.7	363.1	21.00	5.7	331.6–443.7	371.3	26.9	7.2
Body width	12.2–14.7	13.6	0.75	5.5	12.2–14.2	13.3	0.5	3.7
DEGO	3.8–5.9	4.8	0.44	9.1	4.0–5.1	4.5	0.3	6.6
Esophagus length	105.8–123.5	114.4	5.08	4.4	108.1–137.7	124.3	7.8	6.2
EPBD	176.4–237.2	207.7	15.55	7.4	171.3–260.1	209.9	19.3	9.1
Gubernaculum length	4.4–5.9	4.8	0.33	6.7	4.0–5.1	4.5	0.5	11.1
Isthmus length	23.5–33.3	28.1	3.26	11.5§	30.6–39.7	34.9	2.6	7.4
MEBD	42.1–46.1	44.8	1.48	3.3§	40.8–49.9	46.0	2.5	5.4
Metacorpus length	10.8–15.7	12.9	1.89	14.7§	Not reported			
Metacorpus width	4.9–6.9	5.7	0.64	11.0§	Not reported			
Spicule length	16.7–19.6	18.0	0.80	4.4	16.3–19.3	17.0	0.9	5.2
Stylet knob width	1.4–2.1	1.7	0.17	9.9	1.7–2.1	1.9	0.09	4.7
Stylet length	9.1–10.3	9.7	0.34	3.5	9.1–10.2	9.5	0.4	4.2
Tail length	31.4–42.1	37.3	3.26	8.7	33.6–43.8	37.1	2.7	7.2
Testis length	91.1–157.8	113.6	18.50	16.2	87.7–153.0	124.2	19.0	15.2
	Percentages							
Excretory pore anterior body end distance as % of total body length	52.2–60.1	57.1	1.95	3.4	51.3–59.8	56.3	2.2	3.9
Testis length as % of total body length	25.4–41.0	31.1	4.22	13.5	25.8–39.4	33.3	4.2	12.6
	Ratios							
a	23.8–29.5	26.6	1.82	6.8	24.2–31.2	27.7	1.7	6.1
b	2.9–3.4	3.1	0.16	5.3	2.6–3.4	2.9	0.1	3.4
c	8.6–11.7	9.7	0.80	8.3	8.1–11.1	9.9	0.6	6.0

† All abbreviations explained in the text.
‡ Morphometrics of *T. palustris* from pop ash were obtained from the original description (6).
§ N = 8.

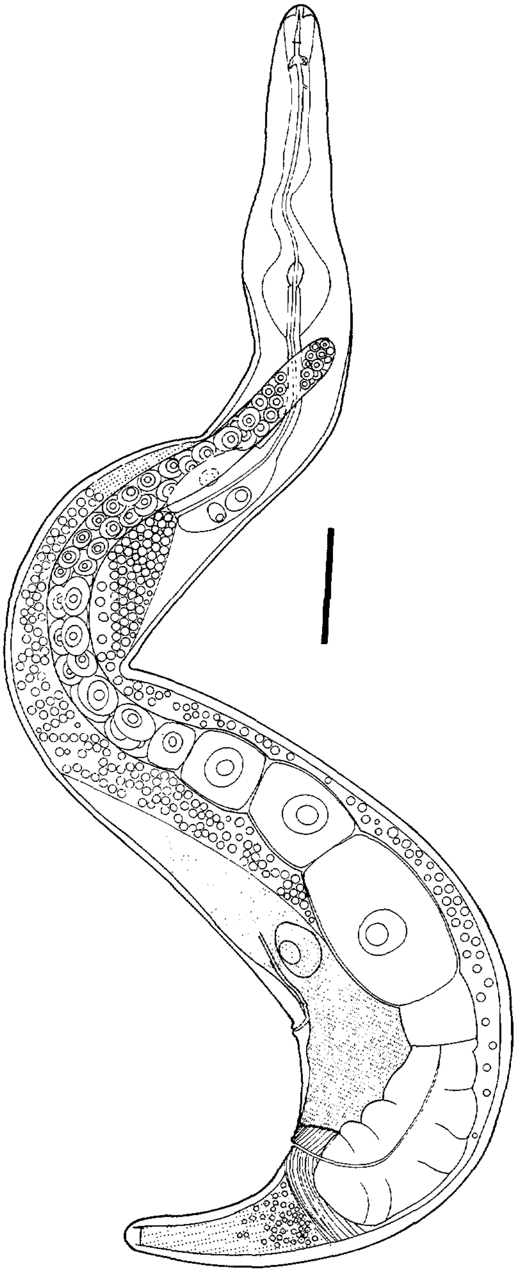

FIG. 5. Mature *Tylenchulus* sp. female from peach in Georgia. Note that >70% of body is swollen posteriorly. Postvulval section is digitate; its terminus is smooth and round, and its body core is larger than that of *T. semipenetrans.* Scale bar = 26 μm.

ously (6): BWV, body width at vulva; DEGO, dorsal esophageal gland opening; EPBD, excretory pore to anterior body end distance; MEBD, median bulb to anterior body end distance; PVSC, width of postvulval section core of body (Fig. 1B); PVSL, postvulval section length of body (Fig. 1B); PVSW, postvulval section width (Fig. 1B); VEPD, vulva to excretory pore distance; CV, coefficient of variation; SD, standard deviation. The width of postvulval body core at terminus, PVSCT (Fig. 1B), was not reported in the original description of *T. palustris* and has been added here for its important diagnostic value. This parameter was also measured in 25 *T. palustris* paratypes and in 25 specimens of *T. semipenetrans* from a Florida citrus orchard. Because perceptible rectum and anus were observed in some J2 from peach, *T. palustris* J2 from the type locality in Florida were collected and examined live in water agar plates for these two characters which were not previously reported. Selected data for adult females and J2 were subjected to Student's *t*-test to interpret morphological comparisons. All measurements are in micrometers (μm) unless otherwise indicated. Selected parameters of *T. palustris* paratypes and some important diagnostic characters of adult female and J2 paratypes (6) are reported in the text.

RESULTS AND DISCUSSION

Measurements of the three *Tylenchulus* populations from Alabama, Arkansas, and Georgia are reported in Tables 1–3. The postvulval section of mature adult females from the three localities was not different in shape and width from that of *T. palustris* paratypes (6) (Figs. 2A–C; 3A–D). The PVSC (illustrated in Fig. 1B) values for the three populations from Alabama, Arkansas, and Georgia (6.4–12.8) (Table 1) were not different from those of *T. palustris* (5.1–12.2) (6). The PVSCT (Fig. 1B) values of the three populations (2.5–6.8) (Table 1) also were similar to those of *T. palustris* paratypes (3.1–8.8). The PVSCT values of *T. semipenetrans* (1.0–3.9) were smaller (P = 0.01) than those of *T. palustris* and of the three populations from peach (Fig. 4A, B). Less than 54% of the body was swollen in some mature females from peach (Table 1) as is the case with *T. palustris* paratypes (6). However, more than 60% of the body

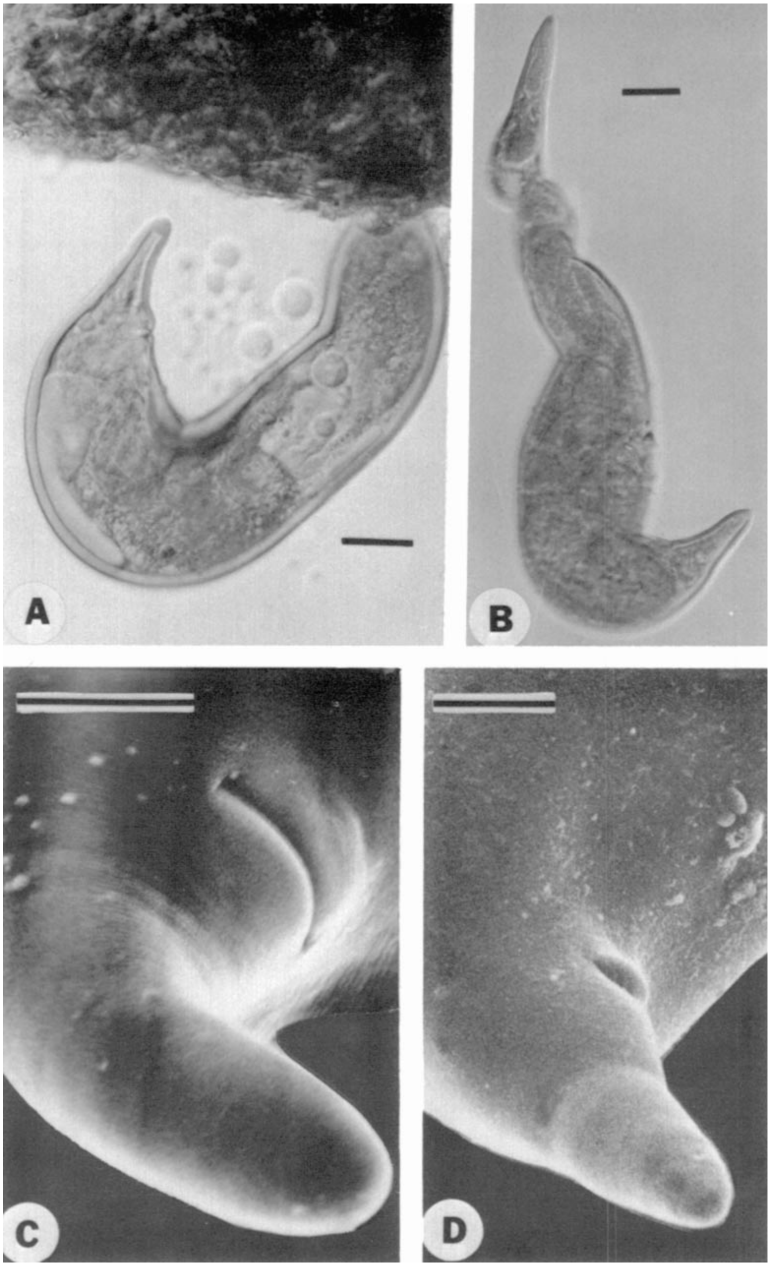

FIG. 6. Mature females of *Tylenchulus* sp. from peach and *T. palustris* from pop ash. A, B) Photomicrographs of specimens from peach in Georgia. C) SEM micrograph of a specimen's posterior body from peach in Georgia. D) SEM micrograph of posterior body of *T. palustris* from pop ash at the type locality in Florida. Note that the postvulval sections are digitate in *Tylenchulus* sp. from peach and conoid in *T. palustris* from pop ash. Scale bars = 25 μm for A, B; 15 μm for C, D.

was swollen posteriorly on most mature females from Alabama and Arkansas and on some mature females from Georgia (Table 1). The body shape and the morphological characteristics of these mature females with more than 60% of their body swollen are illustrated (Figs. 5; 6A, B). Body terminus of some of these females was rounded and smooth (Figs. 2D, 5, 6C). However, these specimens always had wide PVSC as do *T. palustris* paratypes. In contrast, *T. semipenetrans* swollen females have narrower PVSC (Fig. 4).

Among populations from peaches, males

FIG. 7. Photomicrographs of adult males of *Tylenchulus* sp. from peach and *T. palustris* from pop ash. A, C) *T. palustris* paratype. B, D) Specimen from peach in Arkansas. Males from the two populations are morphologically similar. Scale bars = 20 μm for A, C; 18.6 μm for B, D.

were detected only in the Arkansas population; they represented 5% of the soil population. Males comprised 5–10% of *T. palustris* populations from native flora of Florida and 17% of *T. semipenetrans* from the citrus orchard of Florida. Some *T. palustris* populations from native Florida habitats also lack males. Morphological characters of adult males from the Arkansas population did not differ from those of *T. palustris* paratypes (Table 2). Stylet knobs were pronounced (Fig. 7A, B); tails, 31.4–42.1 long, were cylindrical with a bluntly rounded terminus (Fig. 7C, D) similar to *T. palustris* paratypes.

Morphometrics of J2 from Alabama, Arkansas, and Georgia (Table 3) did not differ from those of *T. palustris* paratypes (6). The body lengths of J2 from Alabama, Arkansas, and Georgia populations (313.6–398.8) were slightly longer than those of *T. palustris* J2 from the type locality (277.4–354.9) (6). Rectum and anus were visible in about 60% of the live J2 from the three peach localities (Fig. 8) and in about 70% of those from the type locality in Florida. Tail lengths and c values of J2 from peach (37.2–55.8 and 6.3–10.3) did not differ from those of *T. palustris* paratypes (45.0–50.9 and 7.2–8.0) (6).

TABLE 3. Morphometrics of second-stage juveniles of *Tylenchulus* sp. from peach.

Morphological characters†	Alabama (N = 15)				Arkansas (N = 15)				Georgia (N = 15)			
	Range	Mean	SD	CV	Range	Mean	SD	CV	Range	Mean	SD	CV
Measurements in μm												
Basal bulb length	20.6–24.5	22.2	1.17	5.2	19.6–23.5	21.0	1.16	5.5	21.5–30.0	24.3	2.21	9.0
Basal bulb width	8.3–10.8	9.2	0.86	9.3	7.8–9.8	8.7	0.57	6.5	7.8–9.8	8.9	0.66	7.4
Body length	322.4–373.4	349.2	16.40	4.6	315.6–364.6	334.4	11.05	3.3	313.6–398.8	369.1	24.53	6.6
Body width	13.7–15.2	14.2	0.48	3.3	12.7–14.2	13.3	0.43	3.2	12.7–14.7	13.6	0.64	4.6
DEGO	4.4–4.9	4.7	0.17	3.5	3.7–4.8	4.2	0.36	8.6	3.8–4.7	4.2	0.26	6.2
Esophagus length	111.7–126.4	118.9	3.97	3.3	102.0–113.7	110.0	5.59	5.0	106.7–134.2	122.1	7.13	5.8
EPBD	179.3–214.6	197.5	10.60	5.3	180.3–208.7	190.0	6.92	3.6	165.6–222.5	202.2	15.12	7.4
Genital primordium anterior body end distance	190.1–232.3	216.4	13.45	6.2	200.0–229.3	209.1	7.65	3.6	205.8–251.8	234.2	14.61	6.2
Genital primordium excretory pore distance	4.9–25.5	18.8	5.64	29.9	11.8–28.4	19.0	4.81	25.2	20.6–49.0	32.0	9.56	29.8
Isthmus length	26.5–34.3	30.5	1.96	6.4	22.5–30.3	25.6	1.99	7.7	27.4–36.2	32.9	2.66	8.0
MEBD	46.0–52.3	49.3	1.82	3.6	44.1–50.0	46.7	1.54	3.3	42.1–54.8	47.3	3.37	7.1
Metacorpus length	14.7–18.6	16.7	0.92	5.5	13.7–17.6	16.2	1.03	6.3	15.7–18.6	17.3	0.84	4.8
Metacorpus width	8.3–10.8	8.9	0.85	9.5	7.8–8.8	8.1	0.44	5.4	7.8–9.8	8.3	0.61	7.3
Posterior body portion without large fat globules	25.5–52.9	44.0	8.57	19.4	33.3–52.5	44.3	8.58	19.3	19.6–55.0	38.6.	9.84	25.4
Stylet knob width	2.9–3.5	3.1	0.16	5.3	3.0–3.8	3.1	0.22	7.2	2.9–3.4	3.0	0.12	4.0
Stylet length	12.0–12.7	12.5	0.20	1.6	11.0–12.5	11.7	0.36	3.1	12.7–13.5	12.9	0.24	1.8
Tail length	40.2–55.8	49.0	4.23	8.6	43.1–51.0	47.3	2.37	5.0	37.2–52.9	46.2	4.81	10.4‡
Percentages												
Excretory pore anterior body end distance as % of total body length	55.3–58.0	56.5	0.87	1.5	55.3–58.7	56.7	1.12	1.9	51.3–57.2	54.7	1.78	3.2
Genital primordium anterior body end distance as % of total body length	57.0–64.3	61.9	2.03	3.2	60.0–64.7	62.4	1.60	2.5	66.6–61.1	63.4	1.74	2.7
Ratios												
a	22.5–27.2	24.5	1.51	6.1	23.8–26.9	25.0	0.99	3.9	20.1–29.4	26.0	2.99	11.5
b	2.7–3.2	2.9	0.13	4.5	2.8–3.2	3.0	0.12	4.0	2.6–3.1	2.9	0.26	9.0
c	6.3–8.1	7.1	0.67	9.3	6.3–7.9	7.0	0.43	6.1	7.0–10.3	8.0	1.05	13.1‡

† All abbreviations explained in the text.
‡ N = 10.

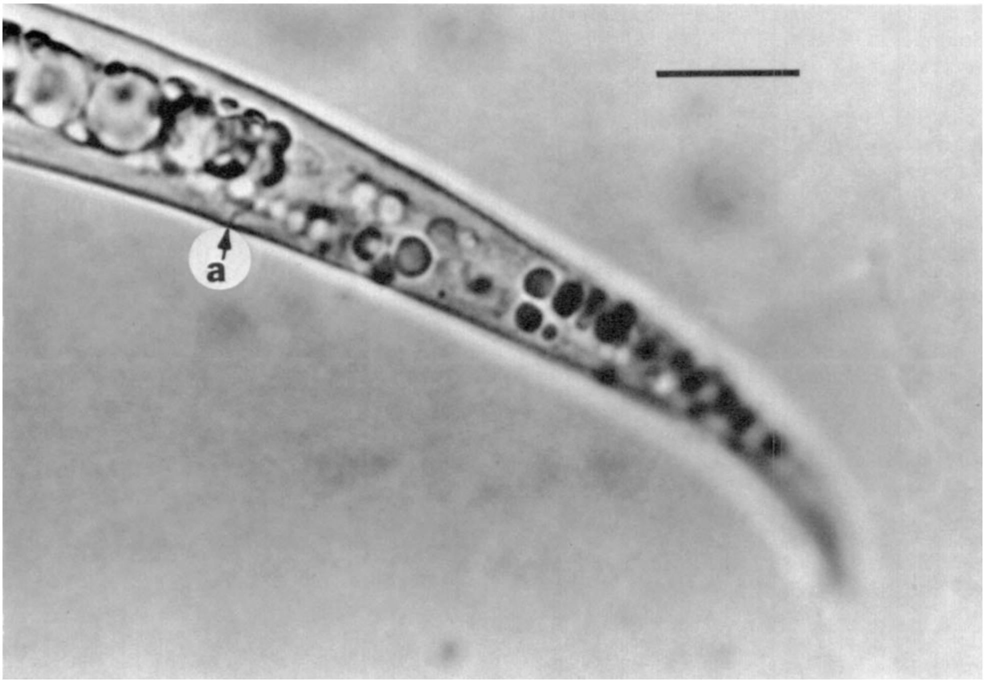

FIG. 8. Photomicrograph of *Tylenchulus* sp. J2 from peach in Arkansas showing rectum and anus (a). Scale bar = 9 μm.

We conclude that the populations from peach are *T. palustris*. This is the first report of this nematode on an economic crop. The results of this study suggest that the examination of several life stages (e.g., swollen females, adult males, J2) is necessary for a correct identification of *Tylenchulus* species. Relevant characters for *T. palustris* identification include shape of mature female posterior body, tail shape, length of male and J2, and male stylet knob width.

Tylenchulus palustris density was lower in the Alabama peach orchard than in the Arkansas peach orchard (0.3 vs. 1.5 J2/cm^3 soil), and only a few adult females were found attached to peach roots in the Alabama population (Table 1). Peach trees at the Alabama site were also infected with *Meloidogyne* sp. and *Criconemella xenoplax* (Raski) Luc & Raski, 1981. In the Arkansas peach orchard, *T. palustris* was associated only with *C. xenoplax*. The lack of males in the samples from Alabama and Georgia may have reflected lower nematode population densities than in the Arkansas site. The percentage of males in the population may increase as population density increases.

The occurrence of *T. palustris* in peach orchards in different geographical areas may be due to several factors. Very probably the nematode was disseminated with infected propagative material from the same infected peach source. We cannot, however, exclude the possibility that peach trees were infected by *T. palustris* populations from other plants present in the infested sites.

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