Description of Meloidogyne christiei n. sp. (Nematoda: Meloidogynidae) from Oak with SEM and **Host-Range Observations**

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Abstract: Meloidogyne christiei n. sp. is described and illustrated from turkey oak (Quercus laevis) in Sanlando Park, Altamonte Springs, Florida. This new nematode species has a distinctive perineal pattern commonly with a high, squarish arch and coarse broken striae which tend to diverge at various angles, especially in and above the anal area. Female labial disc is indented, forming four points or prongs, unlike other species. Eggs are deposited inside the gall in a tubular, coiled manner. Vaginal muscles are exceptionally prominent and dense. SEM observations provided further detail of the perineal pattern and details of the head of females, males, and second-stage juveniles. Galls on the root commonly occur singly, but sometimes in small clusters, and appear as discrete nodules on the side of the root and without adjacent swelling. In general, only one female is found in each gall but occasionally two are present. In greenhouse tests, citrus, tobacco, cotton, pepper, watermelon, peanut, and tomato were not hosts. This nematode occurs throughout central Florida commonly on Q. laevis, the only known host.

Key words: taxonomy, morphology, root-knot nematode, new species, SEM ultrastructure, Quercus laevis.

There are few reports of root-knot nematodes (Meloidogyne spp.) on oak (Quercus spp.). In 1965 Goodey et al. (5) referred to three reports of root knot on oak, the earliest being in 1908 and the most recent in 1948. Gardner (1) published a root-knot host list of about 100 plants in 1926 in which Q. agrifolia Nee and Q. suber L. were included. In 1979 M. querciana Golden, 1979 was described (2) on pin oak (Q. palustris Muenchh.) from Virginia; and in host tests, red oak (Q. rubra L.) and American chestnut (Castanea dentata (Marsh.) Borkh.) were both hosts of this species but none of nine other plants tested were susceptible. In a parallel test, pin oak was not a host for any of three populations of M. incognita (Kofoid & White, 1919) Chitwood, 1949 (2).

Recently an undescribed *Meloidogyne* species causing unusual galls on roots was found on turkey oak (Q. laevis Walt.) at Sanlando Park in Altamonte Springs, Florida, and later on the same host throughout Central Florida. This nematode is described and illustrated herein, and results of limited host tests are given. A preliminary report on this nematode has been given (4).

MATERIALS AND METHODS

Specimens were obtained from collections of soil and roots from Quercus laevis in Sanlando Park, Altamonte Springs, Florida. Second-stage juveniles and males for examination were usually recovered from fresh infected roots kept in petri dishes with a small amount of water. Some were recovered from soil by sieving and Baermann funnel extraction. Females and eggs were later dissected from these roots after fixation for 12 hours or more in 3% formaldehyde solution at room temperature.

The procedures used in preparing, measuring, and drawing specimens were essentially as those used by Golden and Birchfield (3) except that some fixed females were cut and mounted in clear lactophenol solution. Photomicrographs of perineal patterns were made on 9 × 12-cm Polaroid positive-negative film with an automatic camera attached to a compound microscope equipped with an interference contrast system; those of infected roots and whole females were made with an automatic 35-mm camera attached to a dissecting microscope. For scanning electron microscopy, living specimens of males, fe-

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males, and second-stage juveniles were fixed in 3% glutaraldehyde solution with 0.05 M phosphate buffer (pH 6.8) dehydrated in a graded series of ethanol critical-point dried from liquid CO_2 , sputter-coated with a 20–30-nm layer of goldpalladium, and examined with a scanning electron microscope (SEM). Some females in glycerine were also examined with SEM.

To evaluate the host range of the rootknot nematode population from Q_i laevis, 10 replicate 15-cm clay pots per plant species containing young plants of the Meloidogyne North Carolina differential host test (6) and Citrus limon (L.) Burm. f. were randomized on a greenhouse bench. Plants were grown in a steam sterilized potting medium of Astatula fine sand: peat moss: vermiculite (2:1:1; pH = 6.7) and subsequently infested with 1,000 juveniles (J2). To obtain inoculum, excised galls were mechanically crushed with mortar and pestle containing tap water. Inoculum was added to soil in each pot over 3 successive days. The experiment was harvested 90 days after pots were infested, and roots were examined for galls. Root systems were then aerated in tap water (25 C) for 72 hours and juveniles collected on a $35-\mu$ m-pore sieve. All measurements are in micrometers (μ m) unless otherwise stated.

Systematics

Meloidogyne christiei n. sp. (Figs. 1-40)

Female (50): Length 523–779 (mean 637, standard deviation [SD] 69); width 352-623 (470, SD 70); a = 1.1–2.0 (1.4, SD 0.2); stylet 13–15.3 (13.9, SD 0.7); dorsal esophageal gland orifice (DGO) 3.0-4.7 (3.8, SD 0.6) from base of stylet; excretory pore from anterior end 9–28 (17, SD 4.7); center of median bulb 53–88 (64, SD 8.8) from anterior end; vulval slit length 18–26 (23, SD 2.2); distance from vulval slit to anus 18–28 (22, SD 2.4).

Holotype (female): Length 681; width 508; a = 1.3; stylet 14.2; DGO 3.5 from base of stylet; excretory pore 16.8 from anterior end; center of median bulb 68 from anterior end; vulval slit length 25; distance from vulval slit to anus 21.

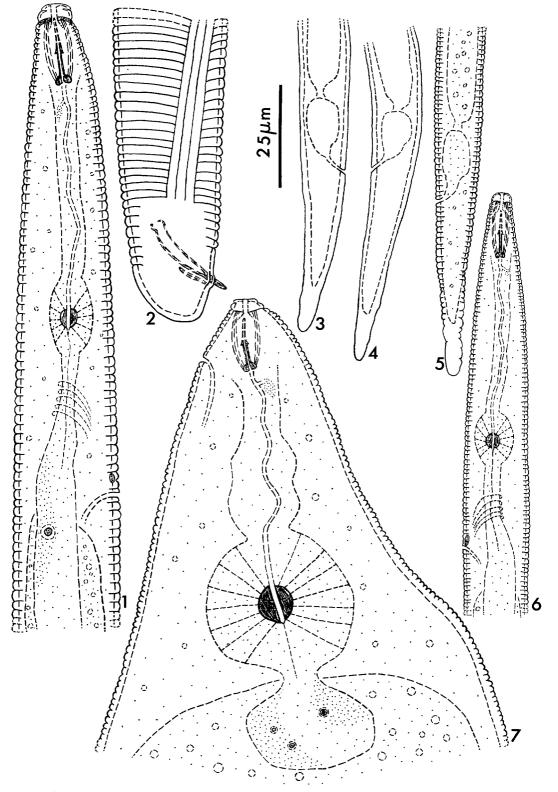
Body pearly white, globular to pearshaped, often with slight posterior protuberance, neck distinct situated anteriorly, commonly off center from a median plane with terminal vulva (Figs. 25, 40). Esophageal and anterior region usually as illustrated (Fig. 7). Head cephalic framework weak, offset from neck but variable in shape, bearing usually one annule, labial disc having four projections or prongs (Figs. 17–19). Excretory pore distinct, generally located at a level near base of unprotruded stylet. Stylet small, strong, dorsally curved, knobs rounded, sloping posteriorly. Perineal pattern (Figs. 8-16, 20-24) usually with high, squarish arch, striae widely spaced, coarse, broken, which tend to diverge at various angles especially in and above anal area. Beneath the pattern and vulva slit a large circular area of exceptionally dense, prominent vaginal muscles usually present. Commonly no egg sac occurs outside gall on root; instead eggs deposited in a tubular, coiled egg sac within the gall (Figs. 37–39).

Male (27): Length 1,019–1,495 (1,227, SD 98); a = 29-48 (38, SD 3.9); b = 5.2-7.1 (6, SD 0.6); c = 121-308 (160, SD 43); stylet 17.1–18.9 (17.8, SD 0.5); DGO 3.5–5.3 (4.1, SD 0.5) from base of stylet; center of median bulb 64–78 (71.4, SD 4.5) from anterior end; spicules 24–26 (25, SD 2.5); gubernaculum 6.5–8.9 (7.3, SD 0.6); tail 3.5–10 (8.2, SD 1.6).

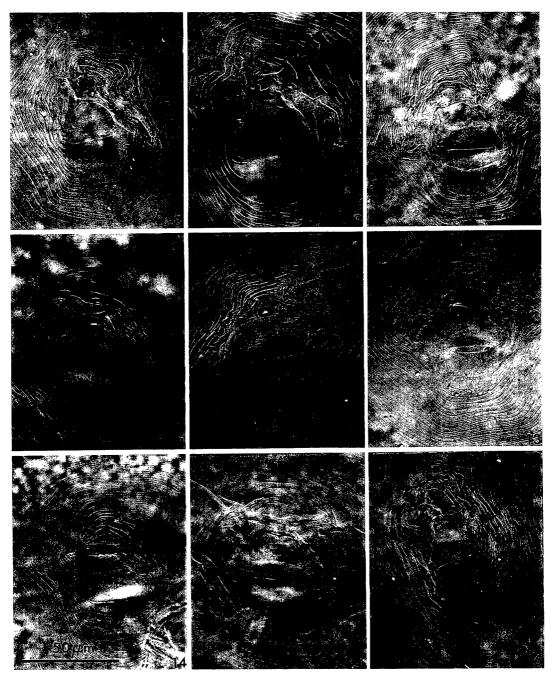
Allotype (male): Length 1,235; a = 31; b = 5.8; c = 176; stylet 17.1; DGO 3.5 from base of stylet; center of median bulb 64 from anterior end; spicules 25.4; gubernaculum 8.2; tail 7.

Body slender, vermiform, tapering slightly at both extremities. Head slightly offset, with large labial disc, and a prominent post labial annule not divided into annules (Figs. 1, 26, 27). Body annules distinct; midbody width averaging 32. Lateral field (Figs. 2, 29) with four incisures, forming three bands, center one slightly smaller, not areolated. Stylet, knobs, excretory pore, and other portions of anterior region appear essentially as illustrated (Fig. 1). Testis one. Spicules rather short, arcuate, with rounded tips (Figs. 2, 28). Tail rounded.

Second-stage juveniles (57): Length 374– 468 (427, SD 21); a = 21-30 (26.5, SD 2.2); b = 1.9-2.5 (2.2, SD 0.2); c = 9-11 (10, SD 0.5); stylet 10.6–11.8 (11.4, SD 0.3); DGO 3.5–4.7 (4, SD 0.3) from base of stylet; center of median bulb 52–62 (57, SD



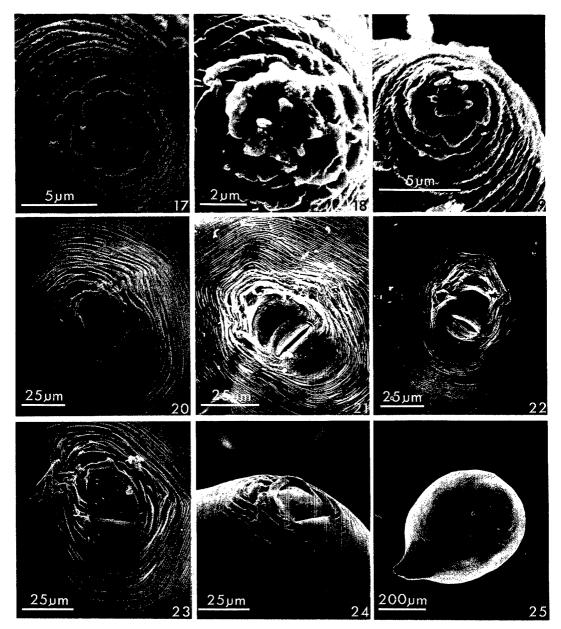
FIGS. 1-7. Drawings of *Meloidogyne christiei* n. sp. 1, 2) Male, anterior and posterior regions. 3-6) Second-stage juveniles, posterior and anterior regions. 7) Female, anterior region.



FIGS. 8-16. Photomicrographs of nine perineal patterns of Meloidogyne christiei n. sp.

2.3) from anterior end; head width to head height ratio 2.2–2.5 (2.4, SD 0.1); body width 14–21 (16, SD 1.4); length from base of dorsal esophageal gland to anterior end 171–226 (196, SD 18); tail length 35–50 (42, SD 2.8); hyaline tail terminus 9–14 (12, SD 1.3); caudal ratio A 1.6–3.1 (2.5, SD 0.3); caudal ratio B 2.8–6.2 (4, SD 0.7).

Body small, vermiform, tapering at both extremities but much more so posteriorly. Head essentially not offset, with weak cephalic framework, labial disc and large post

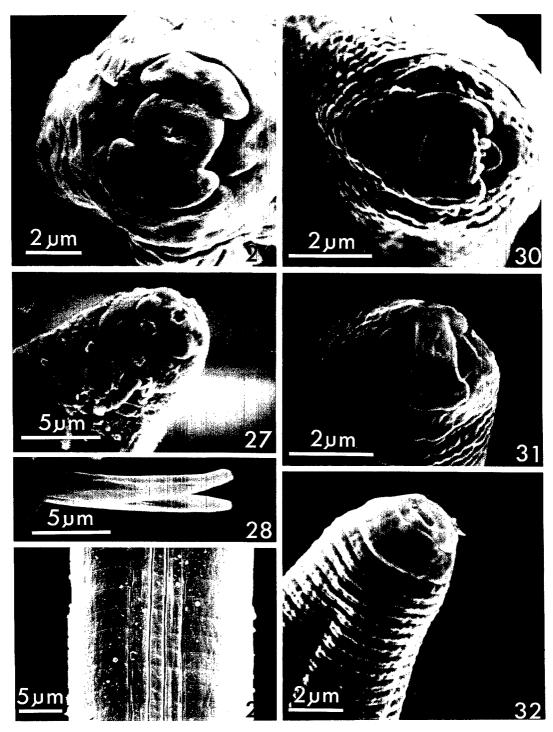


FIGS. 17-25. SEM micrographs of females of *Meloidogyne christiei* n. sp. 17-19) View of head region. 20-24) Perineal patterns. 25) Whole female.

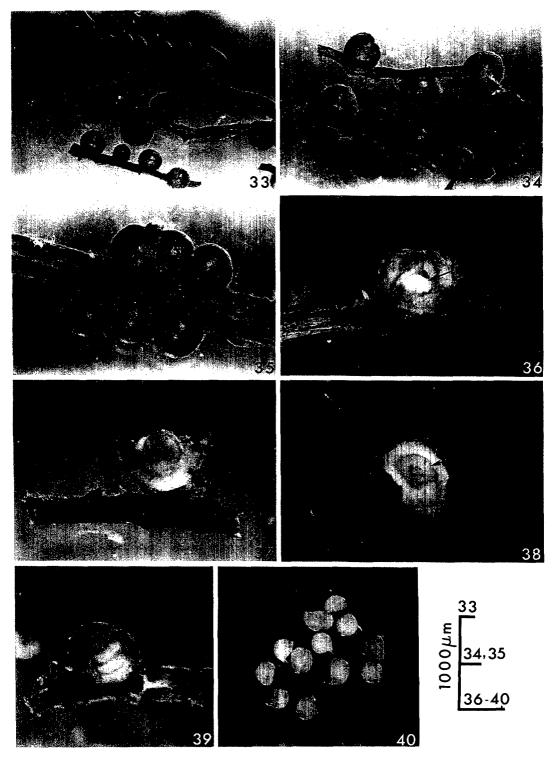
labial annule without striations (Figs. 6, 30– 32). Cuticular annulation very fine, measuring ca. 1 at midbody. Lateral field prominent and about one-third body width, with four incisures, not areolated. Stylet, knobs, hemizonid, excretory pore, and anterior portion commonly appearing as illustrated (Fig. 6); phasmids small, located in anterior half of tail. Rectum inflated. Tail tapering to a bluntly rounded terminus (Figs. 3–5). Eggs (30): Length 101-124 (114, SD 7.2); width 47-68 (60, SD 5); L/W ratio 1.7-2.3 (1.9, SD 0.2). Egg shell hyaline, without visible markings, when observed with optical microscopy.

Type designations

Holotype (female): Collected by David T. Kaplan, 13 November 1984 at Sanlando Park in Altamonte Springs, Florida. Slide



FIGS. 26-32. SEM micrographs of males and second-stage juveniles of *Meloidogyne christiei* n. sp. 26-29) Males. 26, 27) Heads. 28) Spicules. 29) Lateral field. 30-32) Heads, second-stage juveniles.



FIGS. 33-40. Photomicrographs of specimens of *Meloidogyne christiei* n. sp. on, or isolated from, roots of turkey oak. 33-35) Intact galls on roots. 36) Female (arrow) in partially dissected gall. 37-39) Tubular, coiled egg sacs (arrows) posterior to female (not visible) in partially dissected galls. 40) Intact females.

T-419t, United States Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland.

Allotype (male): Slide T-420t. Same data as holotype. USDANC, Beltsville, Maryland.

Paratypes (males, females, second-stage juveniles, eggs and infected type host roots):

USDANC, Beltsville, Maryland; University of California Davis Nematode Collection, Davis, California; The Nematode Collection of the Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England; Canadian National Collection of Nematodes, Ottawa, Canada; Collection Nationale de Nématodes, Laboratoire des Vers, Muséum national d'Histoire naturelle, Paris, France; Nematode Collection, Instituut voor Dierkunde, Laboratorium voor Morfologie en Systematiek der Dieren, Gent, Belgium; Nematode Collection of the Landbouwhogeschool, Wageningen, The Netherlands; Commonwealth Institute of Parasitology Collection, St. Albans, Herts., England.

Type host and locality: Roots of Quercus laevis Walt. in Sanlando Park in Altamonte Springs, Florida.

Diagnosis

Meloidogyne christiei n. sp. is distinctive as described and illustrated herein especially by the shape of the perineal pattern, the presence of four projections on female labial disc, prominent and dense vaginal muscles, and deposition of eggs in a tubular coiled manner. The perineal pattern sometimes is similar to two other species: 1) M. artiellia Franklin, 1961, but among other differences, the second-stage juveniles of this species have a short blunt tail 24.5 μ m long and stylet of 14.7 μ m; and 2) M. ardenensis Santos, 1968, but in this species the female stylet length averages 17 μ m, the male stylet is 22 μ m, and the excretory pore of second-stage juveniles is located usually 3–5 annules anterior to hemizonid.

SEM examination of specimens confirmed and showed much greater detail of structures observed with the light microscope. The unusual labial disc of the female was well resolved, and the nature of the perineal pattern was more clearly revealed. Anterior views of second-stage juveniles and males showed details of the amphidial openings, lips, and labial disc, and the absence of striations on the large post labial annule; other views of the male showed the lateral field and nondentate tips of the spicules.

In greenhouse host range studies, M. christiei n. sp. did not produce galls nor reproduce on six standard differential host plants or C. limon. Although widely distributed throughout mixed stands containing Q. falcata in Florida, the nematode has been detected only on *Q. falcata* roots and only in Florida. The type host (Spanish oak) is the only known host for this nematode. The galls caused by this nematode are distinctive and even suggestive of nitrogenfixing nodules in appearance (Figs. 33-35). They occur as discrete nodules, commonly singly but sometimes as a cluster (Fig. 35) on the side of the root and without adjacent swelling. Generally only one female per gall is found (Fig. 36), but occasionally two are detected. Of particular interest is the tubular, coiled manner in which the eggs are deposited by the female within the root. This, and other facets of this species, needs detailed investigation. This manner of egg deposition may be associated with the dense, prominent vaginal muscles of the female.

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