Meloidogyne cruciani[1] n.sp., a Root-knot Nematode from St. Croix (U.S. Virgin Islands) with Observations on Morphology of This and Two Other Species of the Genus[2]

R. García-Martinez,[3] A. L. Taylor,[4] and G. C. Smart, Jr.[4]

Abstract: Meloidogyne cruciani n.sp. infecting tomato (*Lycopersicon esculentum* Mill.) in the U.S. Virgin Islands is described and illustrated. *M. cruciani* is distinguished from other species of the genus by having punctations around the anus of the female and by the second-stage juveniles possessing tri-lobed esophageal glands which are longer than most other species, with their posterior end at about 46.4% of the body length. The esophageal glands of the immature and adult females are contained in five separate lobes. *Key words:* taxonomy, morphology, renette cell, host range, esophageal glands, new species *Meloidogyne areniaria, Meloidogyne incognita.*

Journal of Nematology 14(3):292-303. 1982.

During a survey of plant-parasitic nematodes in the U.S. Virgin Islands, an undescribed species of *Meloidogyne* was recovered from tomato roots. Specimens of the nematode were established at the University of Florida on 'Rutgers' tomato plants. Single egg mass isolations were made and one isolate was selected for taxonomic and morphologic studies reported herein.

MATERIALS AND METHODS

Egg masses dislodged from 'Rutgers' tomato roots were teased apart and the exposed eggs were placed in water and left overnight at 28–29 C. Newly-hatched second-stage juveniles were mounted in 2% formalin on ringed slides and were immediately measured and drawn using a camera lucida (13). Males were dissected from large root-galls and egg masses and prepared as for the juveniles. Females were dissected from roots and placed in 2% formalin. The posterior ends were excised, transferred to 45% lactic acid, cleaned of debris, trimmed to the perineal pattern, and mounted in glycerin. The anterior ends of females were prepared by fixing and staining whole females in lactophenol-cotton blue (12), after which the anterior ends were excised and mounted in glycerin. All type material was prepared using the same technique as for the female anterior ends.

Photomicrographs of perineal patterns were made using Nomarski interference contrast optics. In preparation for scanning electron microscopy (SEM), females were killed and fixed in 2.5% glutaraldehyde solution with phosphate buffer for 12 h, transferred to 2% osmium tetroxide for 24 h at 8 C, dehydrated in an ethanol series (10–100%) for 15 min in each concentration, critical point dried, mounted, coated with gold, and examined. A modification of the techniques developed by Eisenback et al. (10) was used to extract stylets of females, males, and second-stage juveniles for SEM observations.

A differential host test (19) was conducted on the following plants: sweet corn (*Zea mays* L. var. rugosa Bonaf. cv. Silver Queen), cotton (*Gossypium hirsutum* L. cv. Delta Pine), peanut (*Arachis hypogaea* L. cv. Florunner), pepper (*Capsicum annum* L. cv. California Wonder), strawberry (*Fragaria* × *ananassa* Duch. cvs. Albritton and Florida 90), sweet potato (*Ipomoea batatas* [L.] Poir. cvs. Allgold and Porto Rico), tobacco (*Nicotiana tabacum* L. cv. NC 95), watermelon (*Citrullus lanatus* [Thunb] Matsum. and Nakai cv. Charleston Gray) and tomato (*Lycopersicon esculentum* Mill. cv. Rutgers). Seedlings were transplanted into steam-sterilized, sandy soil in 10-cm clay pots and inoculated with 5,000 eggs. Each treatment was replicated five times. Inoculum was prepared by shaking egg masses in a 1% NaOCL solution for 2

Received for publication 23 April 1981.

[1]Species name is derived from the type locality, St. Croix, U.S. Virgin Islands. The common name "Crucian root-knot nematode" is proposed. R. Garcia-Martinez is the sole authority for this new species (Article 50, International Code of Zoological Nomenclature).

[2]Florida Agricultural Experimental Stations Journal Series, No. 3031. Portion of a dissertation submitted by the senior author in partial fulfillment of the requirements for the Ph.D. Degree in Nematology, University of Florida, Gainesville, FL 32611. This work was supported in part by a grant from the United States Department of Agriculture, USDA Research Agreement No. 12-4-7001-1107, to G. C. Smart, Jr.

[3]Nematologist, University of Florida, Agricultural Research Center, P.O. Drawer 1210, Live Oak, FL 32060.

[4]Adjunct Professor and Professor, Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611. The authors thank R. P. Esser and D. W. Dickson for reviewing the manuscript and examining specimens of this new species.

min (15). The plants were grown for 60 days in a greenhouse maintained at 22–26 C, and then the roots were washed and examined for galls and egg masses.

Meloidogyne cruciani

FEMALES (21): Length = 426.0–1,121.8 μm (mean 787.5 μm, 95% confidence interval ± 86.2); body width = 315.7–770.0 μm (505.1 μm ± 61.5); a = 1.2–2.1 (1.5 ± 0.1); stylet length = 11.4–16.2 μm (14.2 μm ± 0.6); stylet knob height = 2.1–2.9 μm (2.4 μm ± 0.1); stylet knob width = 3.8–5.1 μm (4.5 μm ± 0.2); dorsal esophageal gland orifice to base of stylet knobs = 3.2–5.1 μm (3.9 μm ± 0.2); excretory pore to anterior end = 25.7–45.1 μm (32.2 μm ± 2.2); center of median bulb to anterior end = 65.4–93.3 μm (78.3 μm ± 3.4); vulva slit length = 20.0–25.7 μm (23.2 μm ± 0.7); vulva slit to anus = 15.9–20.3 μm (18.1 μm ± 0.6); interphasmidial distance = 25.7–36.8 μm (30.3 μm ± 1.2).

HOLOTYPE (female): Length = 949.9 μm; body width = 536.4 μm; a = 1.8; stylet length = 12.7 μm; stylet knob height

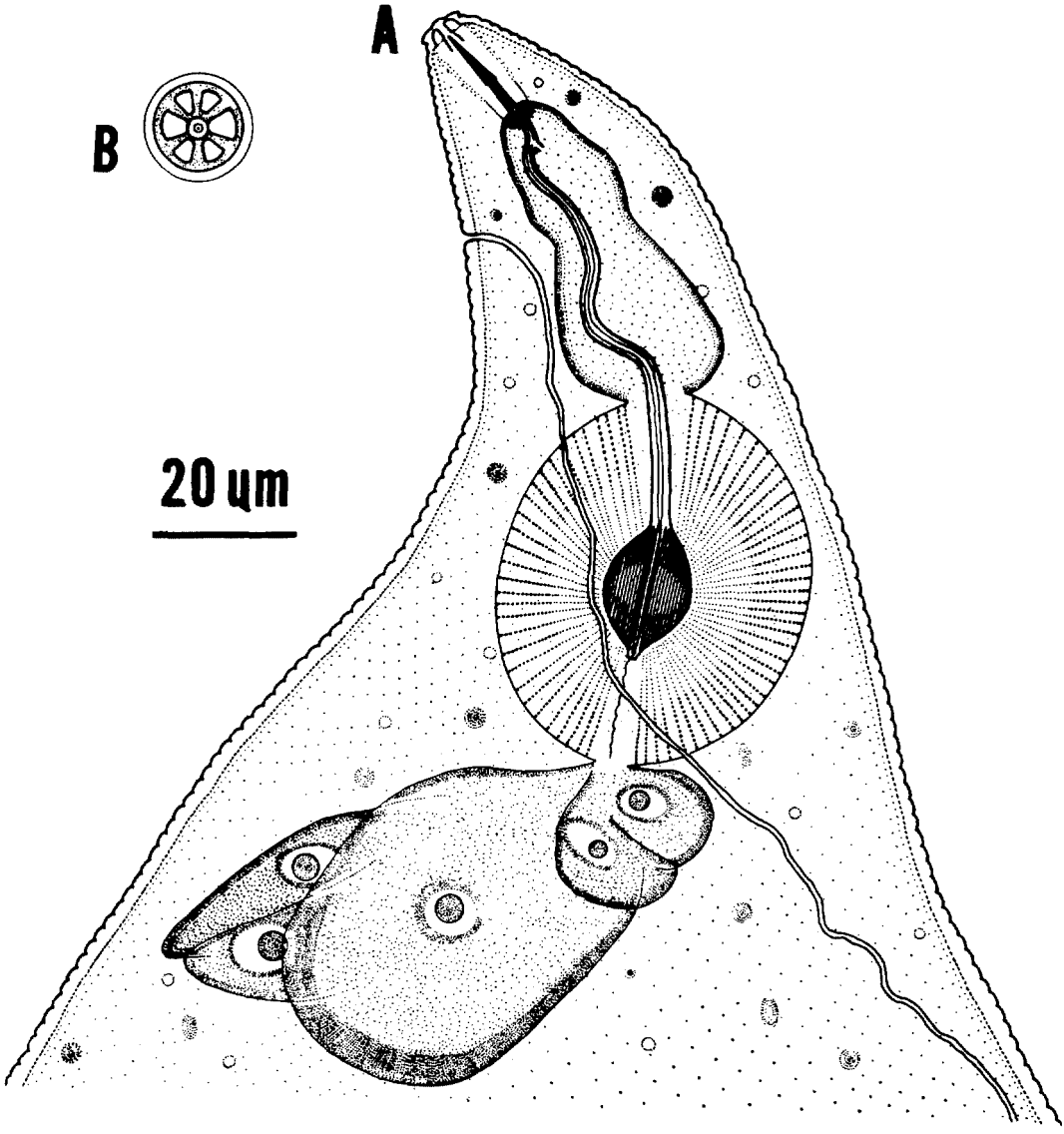


Fig. 1. Female of *Meloidogyne cruciani* n.sp. A) Anterior region. B) Face view showing cephalic framework.

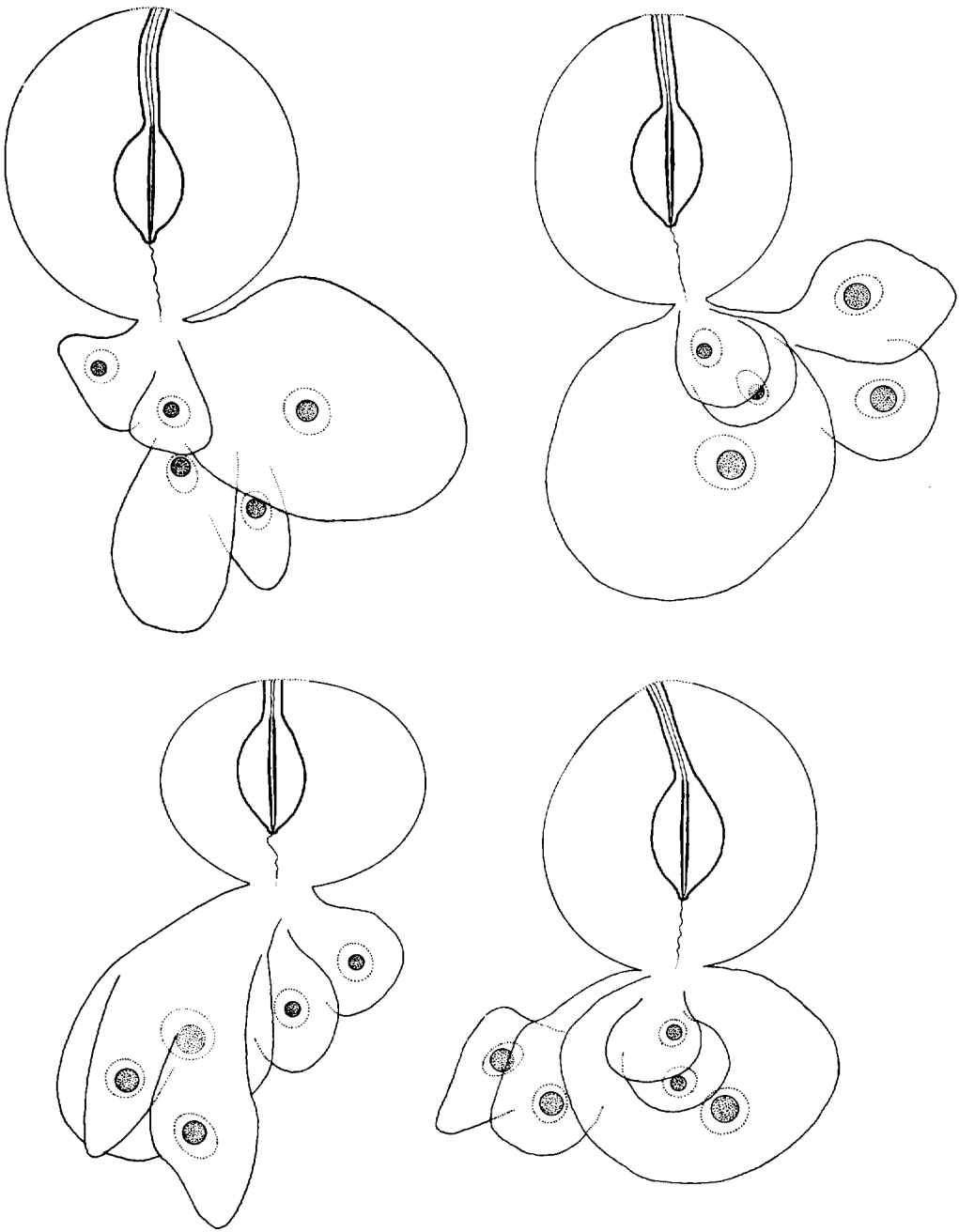
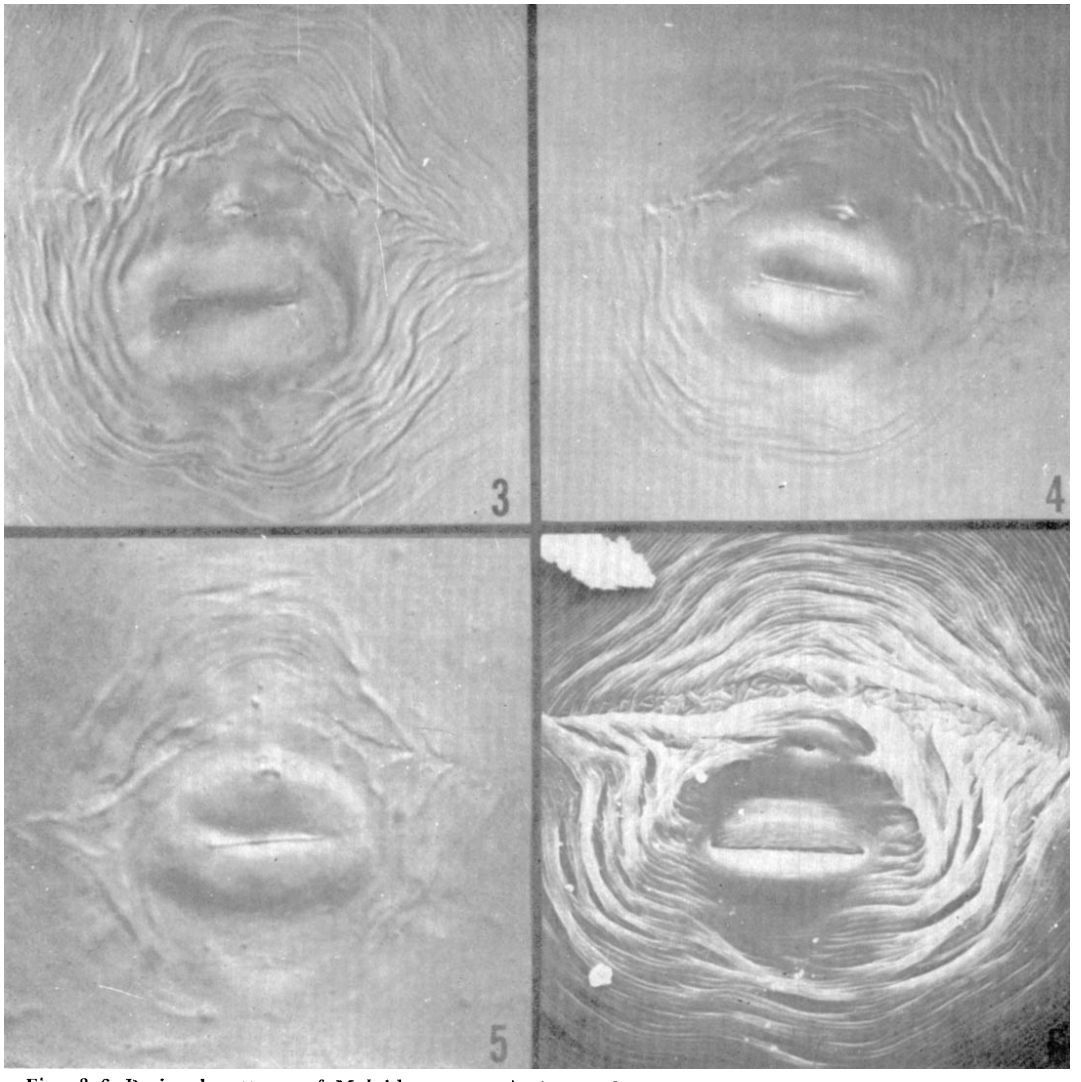


Fig. 2. Variations in size and shape of esophageal glands.

= 2.7 μm; stylet knob width = 4.4 μm; dorsal esophageal gland orifice to base of stylet knobs = 4.1 μm; excretory pore to anterior end = 31.7 μm; center of median bulb to anterior end = 93.3 μm; vulva slit length = 23.8 μm; vulva slit to anus = 20.0 μm; interphasmidial distance = 31.7 μm.

Description: Females white, pear-shaped to globular, without prominent posterior protuberance (Fig. 8). Neck tapers, curving gently (Figs. 1A, 8). Head set off slightly with labial cap and one or two cephalic annules. Labial or cephallic sensillae not observed. Amphidial openings oval, inconspicuous. Cephalic framework with lateral sectors larger than ventral or dorsal sectors. Stylet robust, with rounded knobs. Excretory pore about one stylet length from base of stylet knobs, variable in exact position; excretory duct easily seen throughout anterior region terminating in a uninucleate gland (Fig. 9). Esophageal lumen between base of stylet knobs and valve of median bulb well sclerotized with an average width of 2.4 μm. Prominent metacorpus with strongly sclerotized valve. Esophageal glands consisting of five distinct nucleated lobes (Figs. 1A, 2). One lobe always larger than other four. Perineal pattern (Figs. 1A, 3–7) with subcuticular punctations (stippling) almost surrounding anus on lateral and



Figs. 3–6. Perineal patterns of *Meloidogyne cruciani* n.sp. 3–5) Photomicrographs showing subcuticular punctations. 6) Scanning electron micrograph.

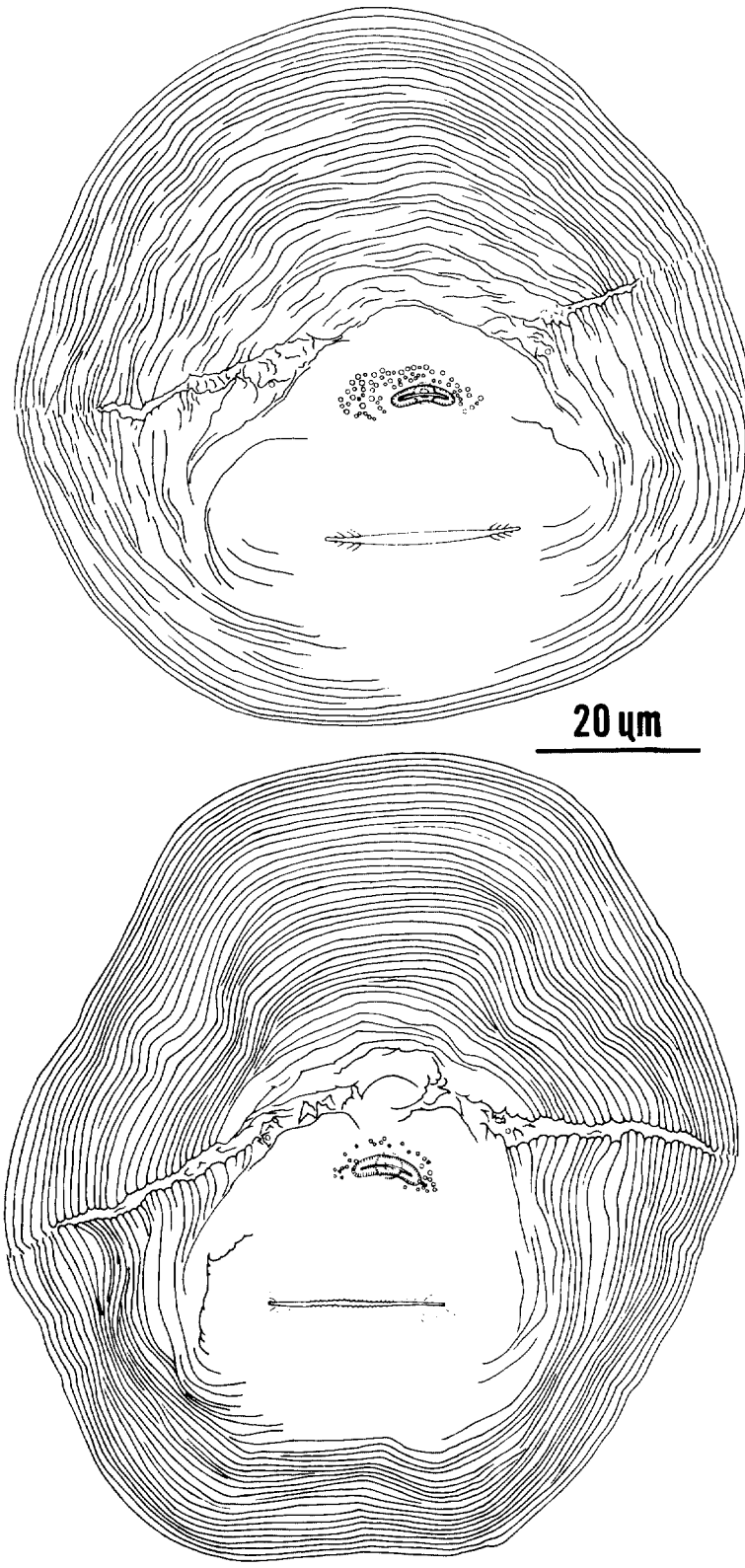


Fig. 7. Typical perineal patterns of *Meloidogyne cruciani* n.sp.

posterior sides (Figs. 3, 4, 7). Striae deep, wavy, sometimes broken. Lateral field fairly deep with distinct phasmids. Phasmidial ducts often visible. Vulva lips faintly serrated, margins with very fine striae. Tail terminus indistinct.

MALE (25): Length = 1,160–1,620 μm (mean 1,378.8 μm, 95% confidence interval ± 52.3); body width = 23.2–46.3 μm (33.8 μm ± 2.4); stylet length = 19.4–24.1 μm (22.0 μm ± 0.5); stylet base to anterior end = 21.3–26.3 μm (24.4 μm ± 0.5); stylet knob height = 2.9–3.8 μm (3.3 μm ± 0.1); stylet knob width = 4.1–6.0 μm (5.2 μm ± 0.2); dorsal esophageal gland orifice to base of stylet knobs = 3.2–7.9 μm (4.9 μm ± 0.4); center of metacorpus valve to anterior end = 66.7–118.1 μm (88.9 μm ± 4.1); excretory pore to anterior end = 127.3–189.5 μm (149.2 μm ± 5.8); anterior end of testis to posterior end = 489.4–1,127.0 μm (823.4 μm ± 60.1); spicule length = 28.7–38.0 μm (31.3 μm ± 0.9); gubernaculum = 6.7–11.1 μm (8.8 μm ± 0.5) phasmid to posterior end = 8.3–22.9 μm (16.7 μm ± 1.4); a = 31.9–71.2 (43.2 ± 3.7); c = 89.2–238.2 (132.6 ± 13.1); c′ = 0.3–0.7 (0.5 ± 0.1); 0 (distance from dorsal esophageal gland orifice to base of stylet knobs, expressed as % of stylet length) = 14.3–36.8 (22.6 ± 1.9); T% (distance from anterior end of testis to posterior end, expressed as % of body length) = 39.8–79.7 (60.1 ± 4.7).

ALLOTYPE (male): Length = 1,440 μm; body width = 29.9 μm; stylet length = 22.8 μm; stylet base to anterior end = 24.8 μm; stylet knob height = 3.5 μm; stylet knob width = 4.8 μm; dorsal esophageal gland orifice to base of stylet knobs = 4.9 μm; center of metacorpus valve to anterior end = 97.4 μm; excretory pore to anterior end = 139.2 μm; anterior end of testis to posterior end = 933.8 μm; spicule length = 28.7 μm; gubernaculum = 8.1 μm; phasmid to posterior end = 18.4 μm; a = 48.0; c = 151.3; c′ = 0.4; 0 = 21.5; T% = 64.9.

Description: Body long, vermiform, tapering at both ends (Fig. 10A). Head set off with two annules and distinct head cap. Labial or cephalic sensillae not observed. Cephalic framework with lateral sectors larger than ventral or dorsal sectors; ends of framework slightly forked when viewed laterally. Stylet robust, with rounded knobs sharply tapering into stylet shaft. Amphidial glands prominent posterior to stylet knobs. Cephalids not observed. Metacorpus poorly developed, slightly larger than procorpus with well-sclerotized valve. Esophageal glands consisting of three distinct nucleated lobes. Excretory pore prominent (139.1 μm from anterior end). Hemizonid 3.5 annules anterior to excretory pore. Excretory duct long, terminating in sac-like unicellular gland. Lateral fields begin anteriorly as two lateral lines near stylet knobs and become four near metacorpus. There may be three

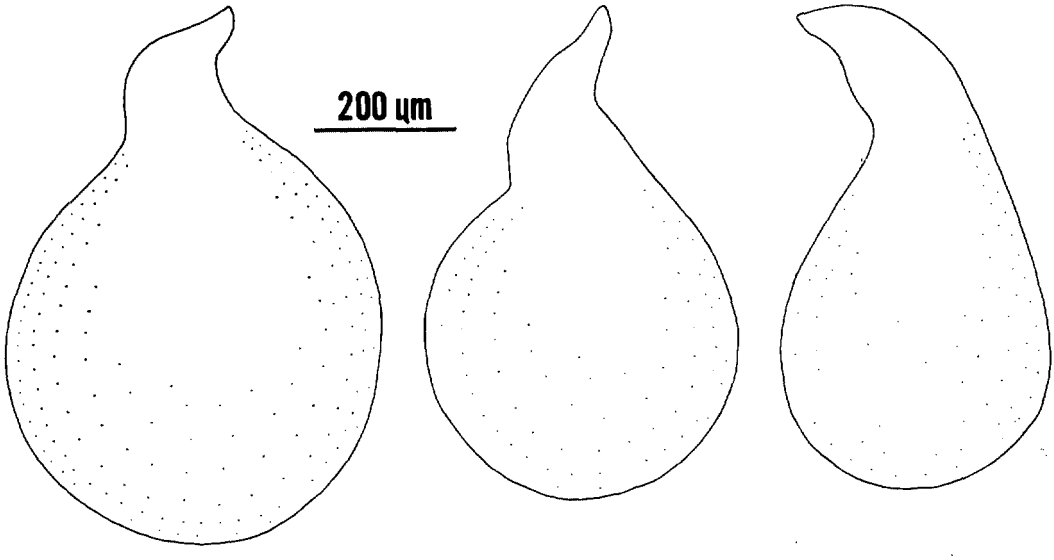


Fig. 8. Outlines of females of varying sizes and shapes.

less pronounced lines between four main ones. There is anastamosis of the lateral lines in posterior end of body. Body twists anteriorly to the spicules. Testis predominately one, two occasionally. Spicules slightly arcuate, tips rounded (Fig. 10D). Gubernaculum with fine serrations on cuneus (Fig. 10D). Phasmids 5.9 μm anterior to cloaca.

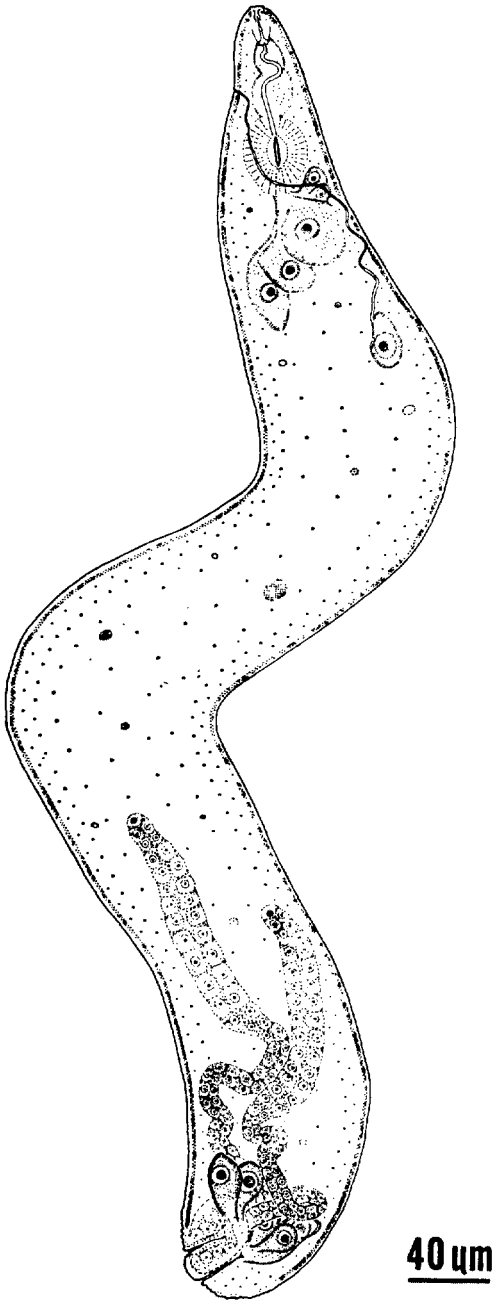


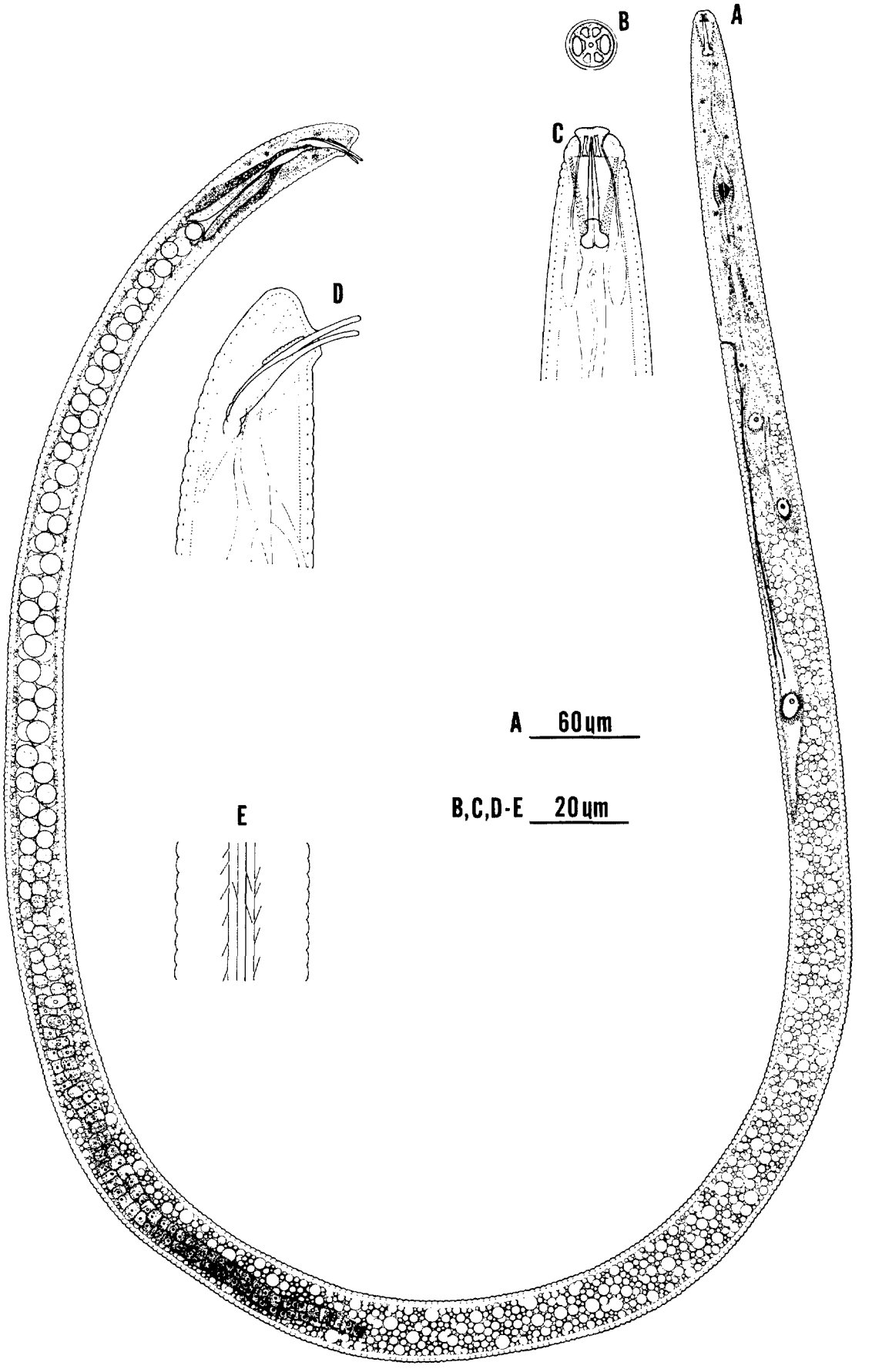
Fig. 9. Young adult female of *Meloidogyne cruciani* n.sp.

SECOND-STAGE JUVENILES (20): Length = 418.6–479.8 μm (mean 435.3 μm, 95% confidence interval ± 8.7 μm); width = 14.6–18.7 μm (17.2 μm ± 0.5); stylet length = 9.8–12.1 μm (10.6 μm ± 0.2); stylet base to anterior end = 14.3–17.6 μm (15.2 μm ± 0.4); stylet knob height = 1.1–1.6 μm (1.4 μm ± 0.1); stylet knob width = 2.1–2.7 μm (2.3 μm ± 0.1); dorsal esophageal gland orifice to base of stylet knobs = 3.2–3.9 μm (3.5 μm ± 0.1); center of metacorpus valve to anterior end = 51.7–61.9 μm (57.8 μm ± 1.4); distance from cardia to anterior end = 69.5–86.7 μm (76.3 μm ± 2.1); distance from posterior end of glands to anterior end = 190.4–250.4 μm (202.0 μm ± 6.4); excretory pore to anterior end = 74.6–103.2 μm (88.1 μm ± 3.4); genital primordium to posterior end = 148.2–175.5 μm (163.5 μm ± 4.3); phasmid to posterior end = 34.9–43.8 μm (39.2 μm ± 1.2); tail length (anus to posterior end) = 41.3–51.7 μm (46.6 μm ± 1.3); tail width (at anus) = 9.8–13.0 μm (11.2 μm ± 0.4); a = 22.9–29.8 (25.4 ± 0.8); b = 5.0–7.1 (5.8 ± 0.2); b′ = 1.8–2.4 (2.2 ± 0.1); c = 8.6–10.5 (9.4 ± 0.3); c′ = 3.7–4.6 (4.2 ± 0.1); 0 = 28.9–37.9 (33.1 ± 0.9).

Description: Body vermiform, tapering slightly anteriorly and much more posteriorly (Fig. 11A). Head set off slightly with one annule; head cap with weakly visible cephalic framework, lateral sectors larger than ventral or dorsal sectors. Labial or cephalic sensillae not observed. Stylet robust, rounded knobs slanting posteriorly. Cephalids not observed. Amphidial glands prominent, posterior to stylet knobs. Esophagus extremely long, posterior extremity of glands average 46.4% of total body length. Metacorpus well developed with well-sclerotized valve. Esophageal glands contained in three distinct nucleated lobes, each with distinct chromocenter. Excretory pore position variable; always posterior to

→

Fig. 10. Male of *Meloidogyne cruciani* n.sp. A) Entire specimen. B) Face view showing cephalic framework. C) Anterior portion. D) Tail (lateral view). E) Lateral field.


A
B
C
D
E
A 60µm
B,C,D-E 20µm

esophago-intestinal valve. Hemizonid 2–4 annules anterior to excretory pore. Excretory duct long, terminating in sac-like unicellular gland. Lateral fields originate as two lines one stylet length posterior to base of knobs, becoming four near metacorpus. Two inner lateral lines terminate near phasmids and outer two terminate near tail terminus. Genital primordium seen easily in two-cell stage. Rectum dilated. Phasmids small and difficult to see; one anal body width posterior to level of anus. Tail gradually tapering, with annules disappearing near hyaline area. Tail terminus notched, with smooth, bluntly conoid tip.

HOLOTYPE (whole female): Orig-

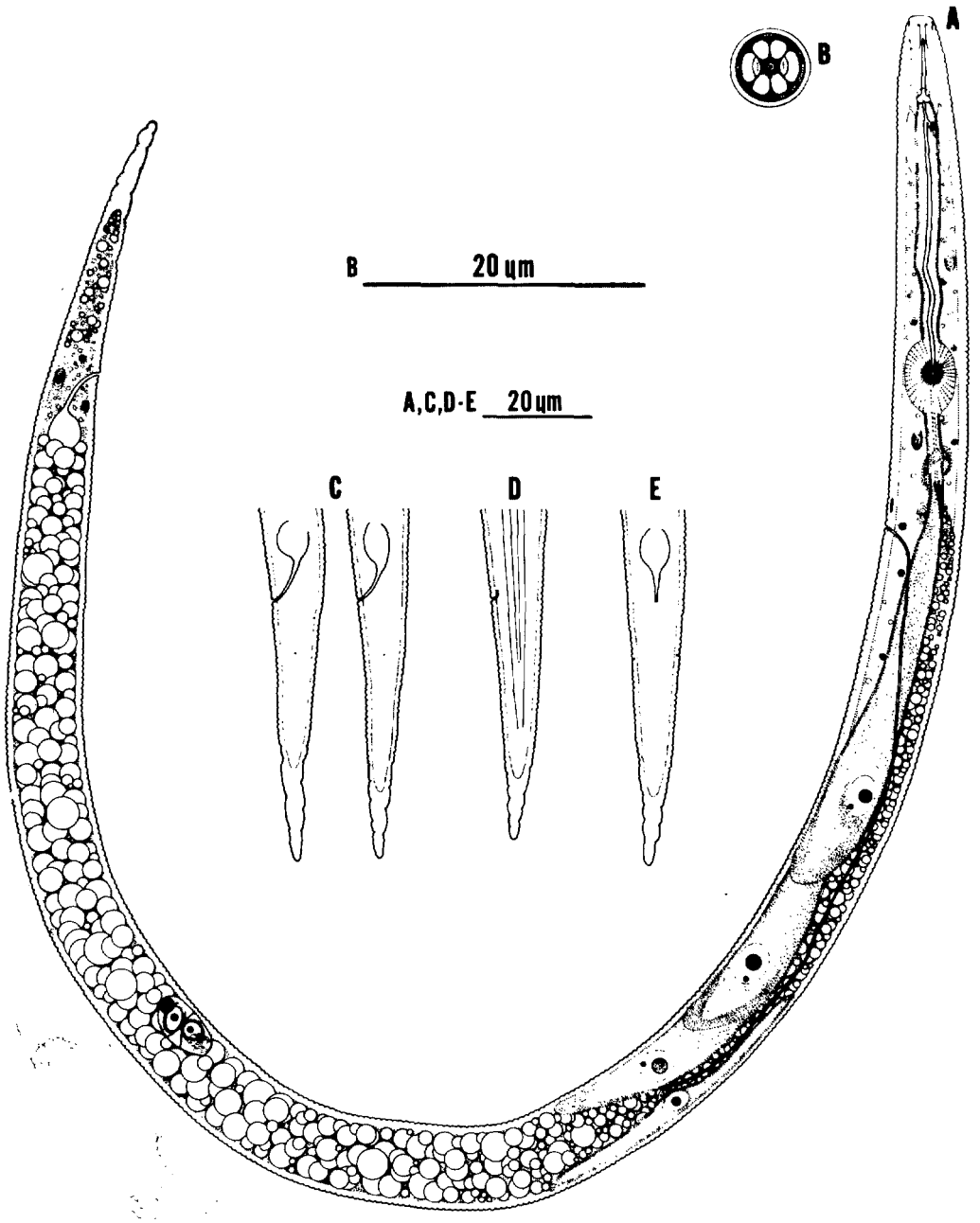


Fig. 11. Larvae of *Meloidogyne cruciani* n.sp. A) Entire specimen. B) Face view showing cephalic framework. C) Tails (lateral view). D) Lateral field at tail region. E) Tail (ventral view).

inally recovered in tomato roots from the Agricultural Community Gardens, St. Croix, U.S. Virgin Islands, in September 1977 and grown subsequently on Rutgers tomato in an isolated greenhouse. (Slide T-333t, USDA Nematode Collection, [USDANC]), Beltsville, Maryland, USA.

ALLOTYPE (male): Isolated from Rutgers tomato roots cultured in a greenhouse and established from type locality. Slide T-334t, USDANC, Beltsville, Maryland, USA.

PARATYPES: Females (whole mounts, perineal patterns), males, and second-stage juveniles. Same data as allotype. USDANC, Beltsville, Maryland; Laboratorie voor Nematologie, Binnehaven, Wageningen, The Netherlands; Nematology Department, Rothamsted Experimental Station, Harpenden, Herts, England; Canadian National Collection of Nematodes, Ottawa, Canada; Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida; and Entomology and Nematology Department, University of Florida, Gainesville, Florida.

DIAGNOSIS: *Meloidogyne cruciani* differs from other published descriptions of species of the genus by its perineal pattern with punctations around the anus. The only other species reported to have punctations in the perineal area is *M. hapla* Chitwood, 1949 (3), but the punctations of *M. hapla* are in the area of the tail terminus. Some perineal patterns of *M. cruciani* resemble those of *M. javanica* in having prominent lateral lines but differ in (i) the pronounced lateral lines of *M. cruciani* do not extend as far as those of *M. javanica;* (ii) the range of the second-stage juveniles' body length distinctly separate them; and (iii) the second-stage juveniles of *M. cruciani* differ from most other species of the genus in possessing extremely long, distinctly tri-

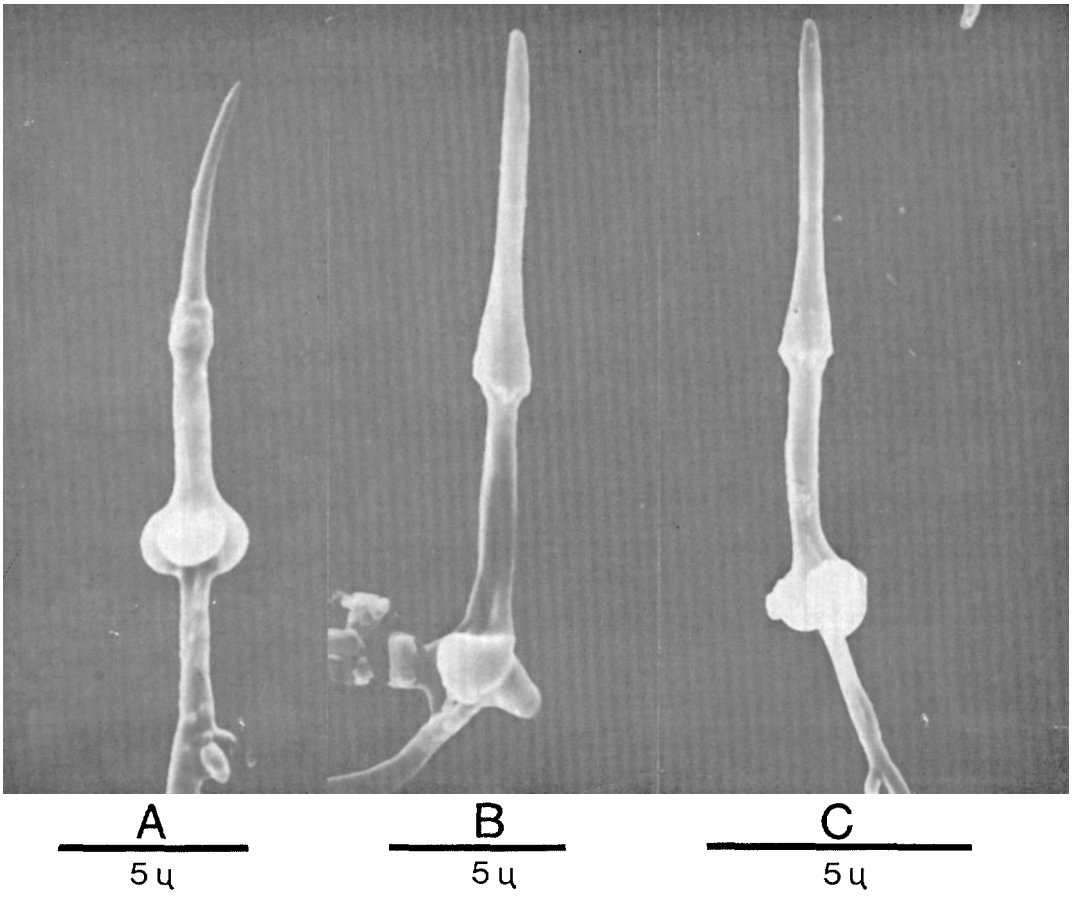


Fig. 12. SEM photographs of excised stylets of *Meloidogyne cruciani* n.sp. A) Female stylet. B) Male stylet. C) Second-stage juvenile stylet.

lobed esophageal glands. Female and male stylets (Fig. 12 A, B) as viewed with the SEM are distinctly different from the four most common species (11). SEM of the stylet of the second-stage juvenile (Fig. 12C) is included in the description, even though SEM of larval stylets has not been used before.

GENERAL DESCRIPTION: In the study of this species, several morphological characters which have not been illustrated and described for all species of the genus were noted. These are not presented as specific characters; some may be characters common to all species of the genus. As noted, some were indeed seen on two other species, but neither time nor available material permitted an extensive study of other species. These morphological characters are the following: 1) The presence of a "guiding apparatus" around the stylet of males and larvae (Figs. 10C, 11A). These rings are often associated with the order Dorylaimida in the class Adenophore (1), but have been reported in the Secernentea (2,17,21). 2) Three distinct lobes of the esophagi of males and second-stage juveniles of this species (Figs. 10A, 11A). Original illustrations of *M. hapla* show the males and second-stage juveniles as having three lobed esophageal glands, but other descriptions and illustrations of other species of this genus examined report one single esophageal lobe with three nuclei. Postinfection studies of *Meloidogyne naasi* (18) and *M. incognita* (20) illustrate the parasitic second-stage juveniles as having three-lobed esophageal glands. This indicates that the second-stage juveniles of these species might also have three-lobed esophageal glands. 3) Inside each nucleus, a small chromocenter is present beside the nucleolus in each lobe of the esophagi of the second-stage juveniles (Fig. 11A). This has not been reported for other species of this genus. 4) The esophageal glands of the females (Figs. 1A, 2) consist of five separate and distinct lobes. We have found that females of *M. incognita* and *M. arenaria* also have five-lobed esophageal glands. 5) A uninucleate gland (renette-type) excretory system in females, males, and second-stage juveniles. Observation of this excretory gland in adult females is possible before enlargement (Fig. 9). This uninucleate type of excretory system, which was first called a renette cell-type by Cobb (6), has been shown in other genera of the Secernentea (4,5,7,8,9,16). 6) The gubernaculum of the males (Fig. 10D) has fine serrations on the cuneus; this condition was also present in males of *Verutus volvingentis,* a new genus described by Esser (14).

In the host-differential test, peanut, strawberry, and cotton were not hosts. Tomato, watermelon, sweet potato, tobacco, corn, and pepper were hosts. Based on these results, *Meloidogyne cruciani* seems to have a similar host range as that of *M. incognita* Race 2. One other plant, cabbage (*Brassica oleracea* L. cv. Greenback), was also found to be a suitable host.

TYPE HOST AND TYPE HABITAT: tomato, *Lycopersicon esculentum* Mill., roots.

TYPE LOCALITY: Agricultural Community Gardens, College of the U.S. Virgin Islands, St. Croix, U.S. Virgin Islands.

LITERATURE CITED

1. Andrássy, I. 1976. Evolution as a basis for the systematization of nematodes. London: Pitman Publishing Ltd.

2. Baldwin, J. G., and H. Hirschmann. 1976. Comparative fine structures of the stomatal region of males of Meloidogyne incognita and Heterodera glycines. J. Nematol. 8:1-17.

3. Chitwood, B. G. 1949. Root-knot nematodes. I. A revision of the genus Meloidogyne Geoldi 1887. Proc. Helminthol. Soc. Wash. 16:90-104.

4. Cobb, N. A. 1891. Strawberry-bunch (a new disease caused by nematodes). Agr. Gaz. N.S.W. 2: 390-400.

5. Cobb, N. A. 1893. Nematodes, mostly Australian and Fijian. Macleay Memorial Volume, Linn. Soc. N.S.W. (Sidney) 252-308.

6. Cobb, N. A. 1913. *In* The Helminthological Society of Washington meeting. Terms amphid and renette introduced. Science, N.S. 27:498-499.

7. Cobb, N. A. 1914. Citrus-root nematode. J. Agr. Res. 2:217-230.

8. Cobb, N. A. 1915. Tylenchus similis, the cause of a root disease of sugar cane and bananas. J. Agr. Res. 4:561-568.

9. Cobb, N. A. 1917. A new parasitic nematode found infesting cotton and potatoes. J. Agr. Res. 11: 27-33.

10. Eisenback, J. D., H. Hirschmann, and A. C. Triantaphyllou. 1980. Morphological comparison by LM and SEM of Meloidogyne female head structures, perineal patterns, and stylets. J. Nematol. 12: 300-313.

11. Eisenback, J. D., H. Hirschmann, J. N. Sasser, and A. C. Triantaphyllou. 1981. A guide to the four most common species of root-knot nematode.

North Carolina State University.

12. Esser, R. P. 1973. A four minute lactophenol fixation method for nematodes. Plant Dis. Reptr. 57:1045-1046.

13. Esser, R. P., V. G. Perry, and A. L. Taylor. 1976. A diagnostic compendium of the genus Meloidogyne (Nematoda: Heteroderidae) Proc. Helminthol. Soc. Wash. 43:138-150.

14. Esser, R. P. 1980. Taxonomy and biology of Verutus volvingentis n. gen. n.sp. (Tylenchida: Nemata). Proc. Helminthol. Soc. Wash., in press.

15. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of Meloidogyne spp., including a new technique. Plant Dis. Reptr. 57:1025-1028.

16. Perry, V. G., H. M. Darling, and G. Thorne. 1959. Anatomy, taxonomy and control of certain spiral nematodes attacking blue grass in Wisconsin. University of Wisconsin, Research Bulletin 207.

17. Roman, J., and H. Hirschmann. 1969. Embryogenesis and postembryogenesis in species of Pratylenchus (Nematoda: Tylenchida). Proc. Helminthol. Soc. Wash. 36:164-174.

18. Siddiqui, I. A., and D. P. Taylor. 1979. The biology of Meloidogyne nassi. Nematologica 16:133-143.

19. Taylor, A. L., and J. N. Sasser. 1978. Biology, indentification and control of root-knot nematodes (Meloidogyne species). North Carolina State University Graphics. pp. 101-103.

20. Triantaphyllou, A. C., and H. Hirschmann. 1960. Post infection development of Meloidogyne incognita Chitwood. 1949. Ann. Inst. Phytopathologique Benaki 3:1-11.

21. Yuen, P. H. 1967. Electron microscopical studies on Ditylenchus dipsaci (Kuhn). I. Stomatal region. Can. J. Zool. 45:1019-1033.