Description of the Blueberry Root-knot Nematode, Meloidogyne carolinensis n. sp.[1]

J. D. EISENBACK[2]

Abstract: Meloidogyne carolinensis n. sp. is described from cultivated highbush blueberry (cultivars derived from hybrids of *Vaccinium corymbosum* L. and *V. lamarckii* Camp) in North Carolina. The perineal pattern of the female has a large cuticular ridge that surrounds the perivulval area, and the excretory pore is near the level of the base of the stylet. The stylet is 15.9 μm long and the knobs gradually merge with the shaft. The head shape and stylet morphology of the male are quite variable. The typical head and four variants, as well as the typical stylet and two variants, are described. The labial disc, medial lips, and lateral lips of second-stage juveniles are fused and in the same contour. The head region is not annulated. Mean juvenile length is 463.7 μm, stylet length is 11.9 μm, and tail length is 42.5 μm. *Key words:* taxonomy, morphology, new *Meloidogyne* species, host range, scanning electron microscopy.

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A biological study of an undescribed species of root-knot nematode parasitizing cultivated and wild blueberries in North Carolina was conducted in 1967 (6). Observations on the morphology, cytology, mode of reproduction, and host range revealed many unusual features peculiar to this species. Differences in morphology were observed in shape of the perineal pattern and location of the excretory pore in the female. Cytological studies indicated that the mode of reproduction was obligatorily amphimictic and the haploid chromosome number was 17 or 18. Host range studies showed that only blueberry (*Vaccinium* sp.) and azalea (*Rhododendron* sp.) were good hosts. A few mature females developed with some reproduction possible on beet (*Beta vulgaris* L.), radish (*Raphanus sativus* L.), cabbage (*Brassica oleracea* var. *capitata* L.), carrot (*Daucus carota* var. *sativa* D.C.), and tomato (*Lycopersicon esculentum* Mill.). Juveniles developed abnormally up to the fourth stage on cucumber (*Cucumis sativis* L.), muskmelon (*Cucumis melo* var. *reticulatus* Naud.), watermelon (*Citrullus vulgaris* Schrad.), squash (*Cucurbita pepo* L.), corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), and oats (*Avena sativa*), and there was no reproduction. Eggplant (*Solanum melongena* L.), tobacco (*Nicotiana tabacum* L.), pepper (*Capsicum frutescens* L.), cotton (*Hibiscus esculentus* L.), sweet potato

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[2]Research Associate, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27650.

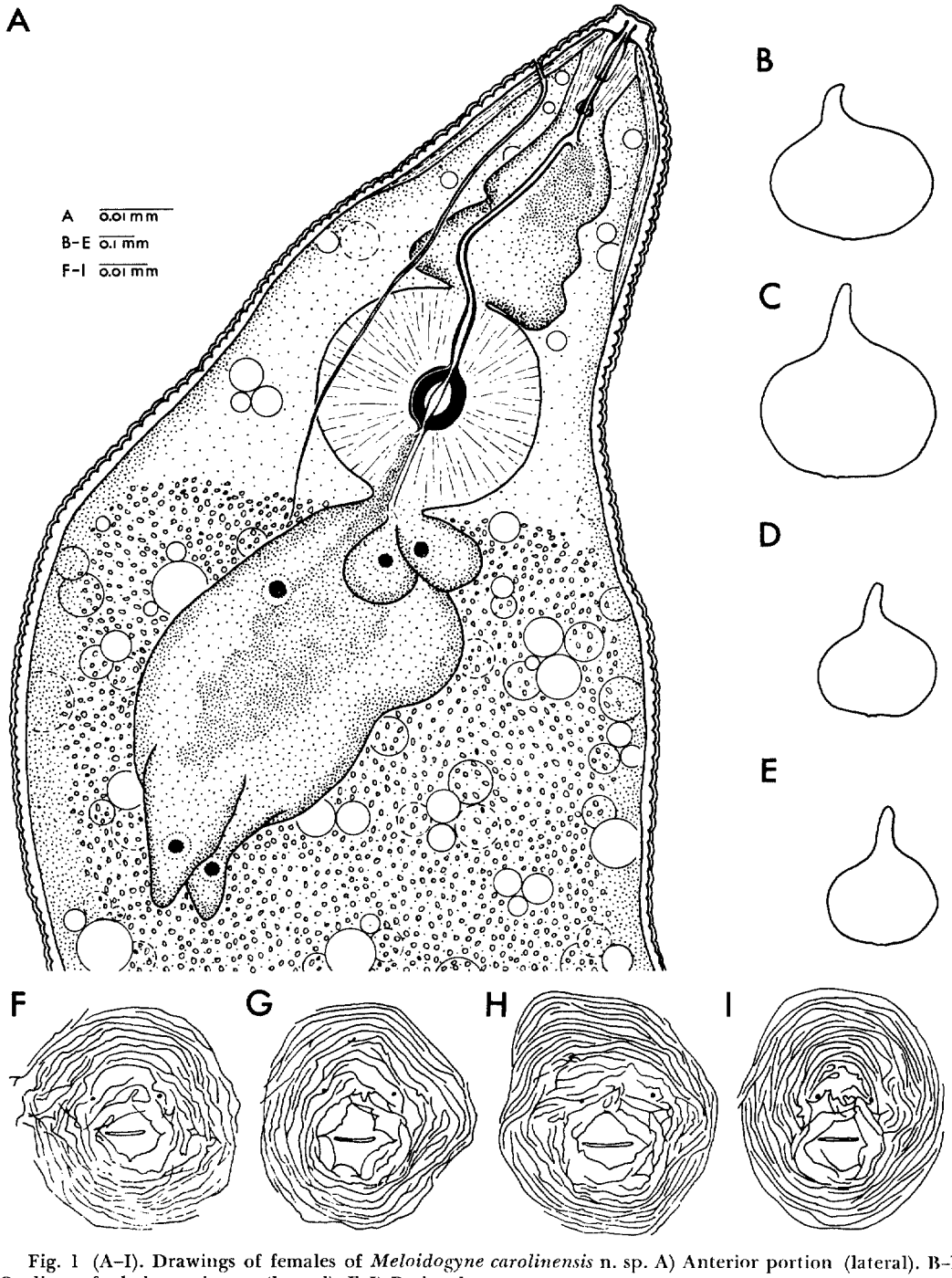


Fig. 1 (A–I). Drawings of females of *Meloidogyne carolinensis* n. sp. A) Anterior portion (lateral). B–F) Outlines of whole specimens (lateral). F–I) Perineal patterns.

Table 1. Measurements of 30 females of *Meloidogyne carolinensis* n. sp.

Character	Range (μm)	Mean ± std. error (μm)	Standard deviation	Coefficient of variability
Whole females				
Body length	373.5–690.3	508.6 ± 13.71	75.5	14.8
Body length without neck	297.0–507.6	377.9 ± 10.71	58.7	15.5
Neck length	71.0–236.7	130.6 ± 6.81	37.3	28.6
Neck width	68.4–174.6	96.2 ± 3.83	21.0	21.8
Body width	315.0–593.1	444.4 ± 13.51	74.0	16.7
Body length/body width	1.9– 5.6	4.0 ± 0.15	0.8	20.8
Body length without neck/body width	0.9– 1.5	1.2 ± 0.03	0.1	12.5
Excised female heads				
Stylet length	14.9– 16.9	15.9 ± 0.10	0.6	3.4
Stylet knob height	1.9– 2.8	2.3 ± 0.03	0.2	7.4
Stylet knob width	3.3– 4.4	4.0 ± 0.04	0.2	5.9
Dorsal esophageal gland orifice to stylet base	2.4– 5.9	4.6 ± 0.14	0.8	17.3
Excretory pore to head end	8.6– 28.2	13.9 ± 0.66	3.6	26.1
Perineal patterns				
Vulval length	14.9– 23.7	20.2 ± 0.34	1.8	9.2
Anus to vulva (center) distance	5.6– 24.2	16.4 ± 0.62	3.4	20.7
Interphasmidial distance	14.0– 34.7	27.8 ± 0.81	4.4	16.0

Table 2. Measurements of 30 males of *Meloidogyne carolinensis* n. sp.

Character	Range (μm)	Mean ± std. error (μm)	Standard deviation	Coefficient of variability
Linear				
Body length	1,253.7–1,980.0	1,487.5 ± 26.56	145.5	9.8
Body width	32.1– 44.5	36.6 ± 0.49	2.7	7.3
Stylet length	17.4– 21.9	20.0 ± 0.21	1.1	5.6
Stylet knob height	2.3– 3.0	2.7 ± 0.03	0.2	5.4
Stylet knob width	3.6– 5.2	4.4 ± 0.06	0.3	7.6
Dorsal esophageal gland orifice to stylet base	1.9– 5.2	4.0 ± 0.13	0.7	18.4
Esophagus length (center of median bulb to head end)	75.5– 98.0	86.8 ± 1.13	6.2	7.2
Excretory pore to head end	126.3– 180.9	151.8 ± 2.53	13.9	9.1
Body width at stylet base	15.7– 19.1	17.5 ± 0.17	0.9	5.2
Body width at excretory pore	25.6– 31.3	28.7 ± 0.30	1.7	5.8
Tail length	4.9– 10.4	7.4 ± 0.26	1.4	19.1
Spicule length	27.0– 35.3	31.7 ± 0.44	2.4	7.6
Ratios				
Body length/body width = a	32.3– 49.9	40.7 ± 0.64	3.5	8.6
Stylet length/body width at stylet base	0.9– 1.3	1.2 ± 0.01	0.1	7.2
Stylet knob width/height	1.5– 1.93	1.67 ± 0.01	0.02	7.8
Percentages				
Excretory pore	9.6– 12.4	11.0 ± 0.00	0.01	9.8

Table 3. Measurements of 30 second-stage juveniles of *Meloidogyne carolinensis* n. sp.

Character	Range (μm)	Mean ± std. error (μm)	Standard deviation	Coefficient of variability
Linear				
Body length	416.7 –515.7	463.7 ± 4.89	26.8	5.8
Body width	13.4 – 17.6	15.1 ± 0.17	0.9	6.3
Stylet length	10.9 – 13.1	11.9 ± 0.11	0.6	5.3
Stylet base to head end	13.6 – 15.6	14.6 ± 0.08	0.5	3.1
Dorsal esophageal gland orifice to stylet base	3.0 – 4.7	3.9 ± 0.08	0.5	11.5
Esophagus length (center of median bulb to head end)	53.1 – 78.2	60.0 ± 0.82	4.5	7.5
Excretory pore to head end	73.4 – 86.1	80.4 ± 0.65	3.6	4.4
Tail length	34.7 – 49.0	42.5 ± 0.71	3.9	9.2
Body width at anus	9.7 – 11.8	10.8 ± 0.09	0.5	4.6
Ratios				
Body length/body width = a	27.2 – 36.3	30.7 ± 0.41	2.2	7.3
Body length/esophagus length = b	5.74– 8.61	7.76 ± 0.56	0.01	4.2
Body length/tail length = c	9.65– 13.25	10.94 ± 0.95	0.02	9.1
Tail length/tail width at anus = d	3.2 – 4.9	3.9 ± 0.07	0.4	9.2
Percentages				
Excretory pore	16.0 – 19.0	17.5 ± 0.00	0.01	8.2

(*Ipomoea batatas* [L.] Lam.), strawberry (*Fragaria chiloensis* [Duch.] var. *ananassa* Baily), elm (*Ulmus americana* L.), maple (*Acer saccharinum* L.), and coffee (*Coffea arabica* L.) were poor hosts, and juveniles usually did not progress beyond the second stage. Few nematodes penetrated bean (*Phaseolus vulgaris* L.), lima bean (*Phaseolus lunatus* L.), soybean (*Glycine max* [L.] Merr.), or cowpea (*Vigna sinensis* [Turner] Savi). The nematode was found in 24 of 63 North Carolina commercial blueberry plantings sampled in 1967 and is probably indigenous to that state. It parasitized highbush, lowbush (*V. angustifolium* Ait.), and creeping blueberry (*V. crassifolium* Andr.), as well as several cultivars of cultivated blueberry. A species description has not been published, and the species name proposed by Fox has remained unofficial (5,7, 10). The purpose of this study is to describe this nematode as the "blueberry root-knot

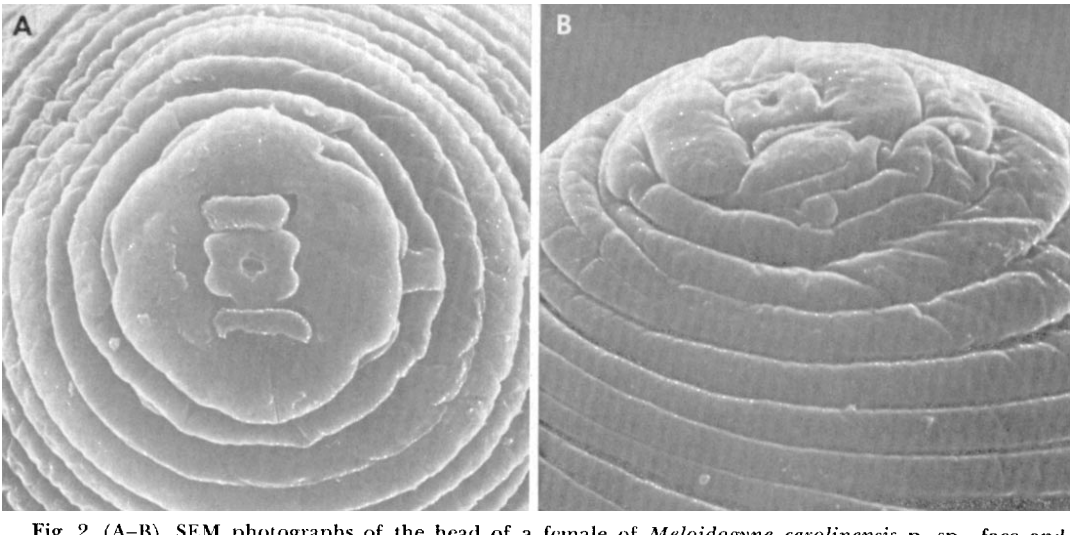


Fig. 2 (A–B). SEM photographs of the head of a female of *Meloidogyne carolinensis* n. sp., face and lateral views, respectively.

nematode," *Meloidogyne carolinensis* n. sp., as suggested by Fox.

Root-knot nematodes have been found associated with cultivated highbush blueberry (cultivars derived from hybrids of *Vaccinium corymbosum* L. and *V. lamarckii* Camp.) for many years. *Meloidogyne* spp. on blueberry may have been identified incorrectly as *M. incognita* (1,8) or listed as unidentified species (9,12).

MATERIALS AND METHODS

Three mature highbush blueberry plants (cv. Wolcott) heavily infected with *M. carolinensis* n. sp. were collected from the type locality (farm near Charity, North Carolina) and maintained in a greenhouse at 22–28 C. All morphologic and morphometric studies were made from these cultures.

Males and second-stage juveniles were obtained by incubating pieces of infected washed roots in a moist chamber. Light microscope (LM) observations were made from temporary mounts of specimens that were gently heat-killed in water and mounted in 2% formalin. Fixed material was always compared to live specimens mounted in 0.85% saline. Males and second-stage juveniles were prepared for scanning electron microscopy (SEM) as previously reported (2,3). Females were prepared for LM and SEM observations of heads, perineal patterns, and stylets as described earlier (4). Stylets of males and juveniles were dissected for SEM by adapting the technique developed for removal of the

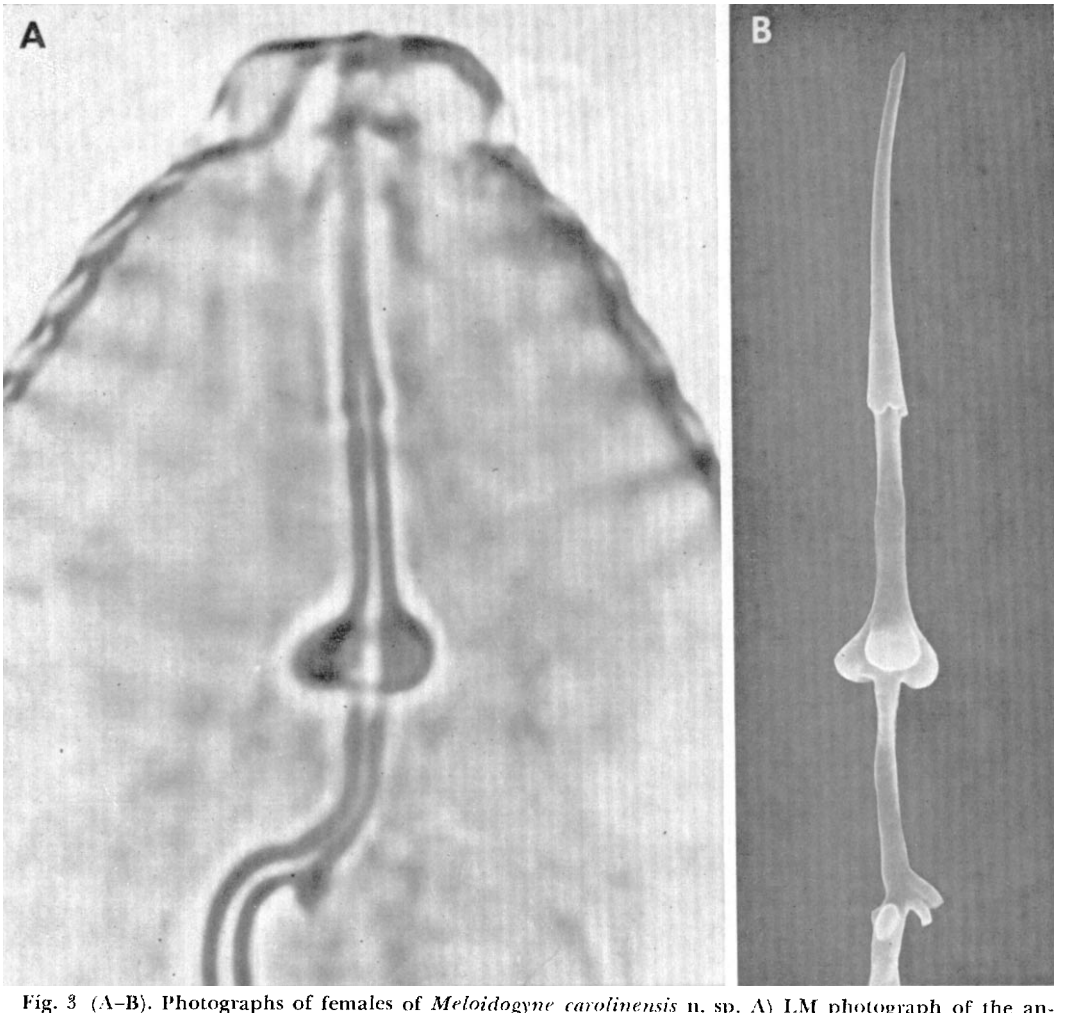
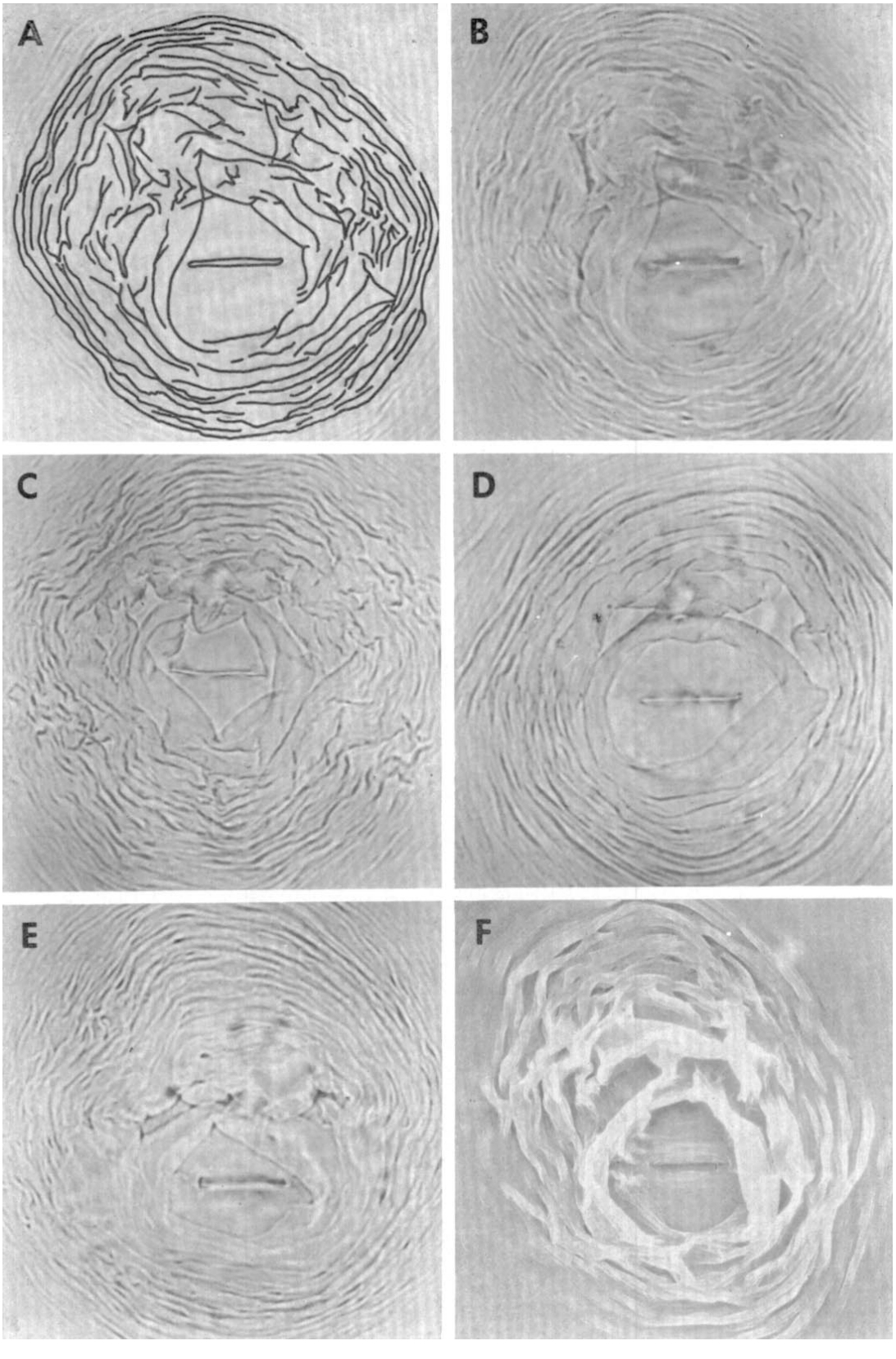


Fig. 3 (A–B). Photographs of females of *Meloidogyne carolinensis* n. sp. A) LM photograph of the anterior portion with stylet (lateral). B) SEM photograph of an excised stylet.


A
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stylets of females. Eggs were mounted in 2% formalin. All LM observations were made within 4 h after processing, and photographs were taken with a bright field microscope. At least 100 specimens of each stage were examined by LM and SEM.

Type specimens were prepared as for SEM (2), except that after dehydration in 100% ethanol, the specimens were transferred into a solution of 10% glycerin in 90% ethanol. The ethanol was allowed to evaporate slowly at room temperature until the specimens were infiltrated with glycerin.

SPECIES DESCRIPTION

Meloidogyne carolinensis n. sp.

FEMALES: *Measurements* of 30 females are listed in Table 1. Measurements of holotype in glycerin: Length, 566.7 μm; body width, 426 μm; body neck length, 124.7 μm; neck width, 110.0 μm; stylet length, 16.4 μm; stylet knob height, 2.4 μm; dorsal gland orifice to stylet base, 4.9 μm; excretory pore to head end 10.0 μm.

Description (Figs. 1–4): Body translucent-white, variable in size, pear-shaped to ovoid, width often greater than length without neck. Neck prominent, cuticular annulations on body finer posteriorly. Body posteriorly flattened, with slight protuberance. In SEM (Fig. 2), stoma slit-like, located in ovoid prestomatal cavity, surrounded by pit-like openings of six inner labial sensilla. Raised labial disc, separated from lips by a deep groove. Labial disc often rectangular, may be indented medially on one or both sides, often marked by two or four bumps. Lateral and medial lips often fused, forming one structure. Head region set off, with 2–3 annulations. In LM (Fig. 3A), cephalic framework weak, hexaradiate, lateral sectors slightly enlarged, vestibule and extension prominent. Excretory pore anterior to stylet base. Stylet delicate; cone slightly curved dorsally; shaft enlarged posteriorly; knobs tapering onto shaft, separate, rounded posteriorly. Distance of dorsal esophageal gland orifice to stylet base approximately one shaft-length; orifice branched into three channels; ampulla large. Sub-ventral gland orifices branched; located immediately posterior to enlarged lumen of median bulb. Esophageal gland with one large dorsal lobe with one nucleus; two small nucleated subventral gland lobes, variable in shape, position, and size, usually posterior to dorsal gland lobe; two small rounded, esophageal-intestinal cells with nuclei attached to dorsal lobe between median bulb and intestine.

Perineal pattern (Figs. 1F–I, 4) rounded to hexagonal; striae coarse, sometimes continuous, smooth to wavy. One large nearly continuous cuticular ridge surrounds perivulval region; coverslip causes ridge to fold inward making anus appear below a cuticular flap. Perivuval region free of striae. Phasmids small, directly on either side of anus; surface structure not apparent in SEM (Fig. 4F).

MALES: *Measurements* of 30 males are listed in Table 2. Measurements of allotype in glycerin: Body length, 1,324.2 μm; body width, 34.7 μm; stylet length, 20.9 μm; stylet knob height, 2.5 μm; stylet knob width, 4.2 μm; dorsal esophageal gland orifice to stylet base, 4.2 μm; esophagus length (center of median bulb to head end), 86.4 μm; excretory pore to head end, 150.7 μm; body width at stylet base, 16.3 μm; body width at excretory pore, 29.2 μm; tail length, 6.8 μm; spicule length, 28.1 μm.

Description (Figs. 5A–C, 6–10): Body vermiform, tapering anteriorly, bluntly rounded posteriorly, tail twisting through 90°. Head shape extremely variable. Typically, head cap low, rounded, tapers posteriorly; head region narrower than first body annule. In SEM (Fig. 6), stoma slit-like, located in ovoid to hexagonal prestomatal cavity surrounded by pit-like openings of six inner labial sensilla. Labial disc rounded, slightly raised near prestoma. Rounded medial lips fused with labial disc forming elongate head cap. Four cephalic sensilla marked by cuticular depressions on medial lips. Amphidial apertures appear as

Fig. 4 (A–F). Perineal patterns of *Meloidogyne carolinensis* n. sp. A–E) LM photographs showing variation typical for the species. F) SEM photograph showing the raised cuticular ridge surrounding the perivulval area. Figs. A and B are photographs of the same pattern, except the striae have been traced in ink in Fig. A.

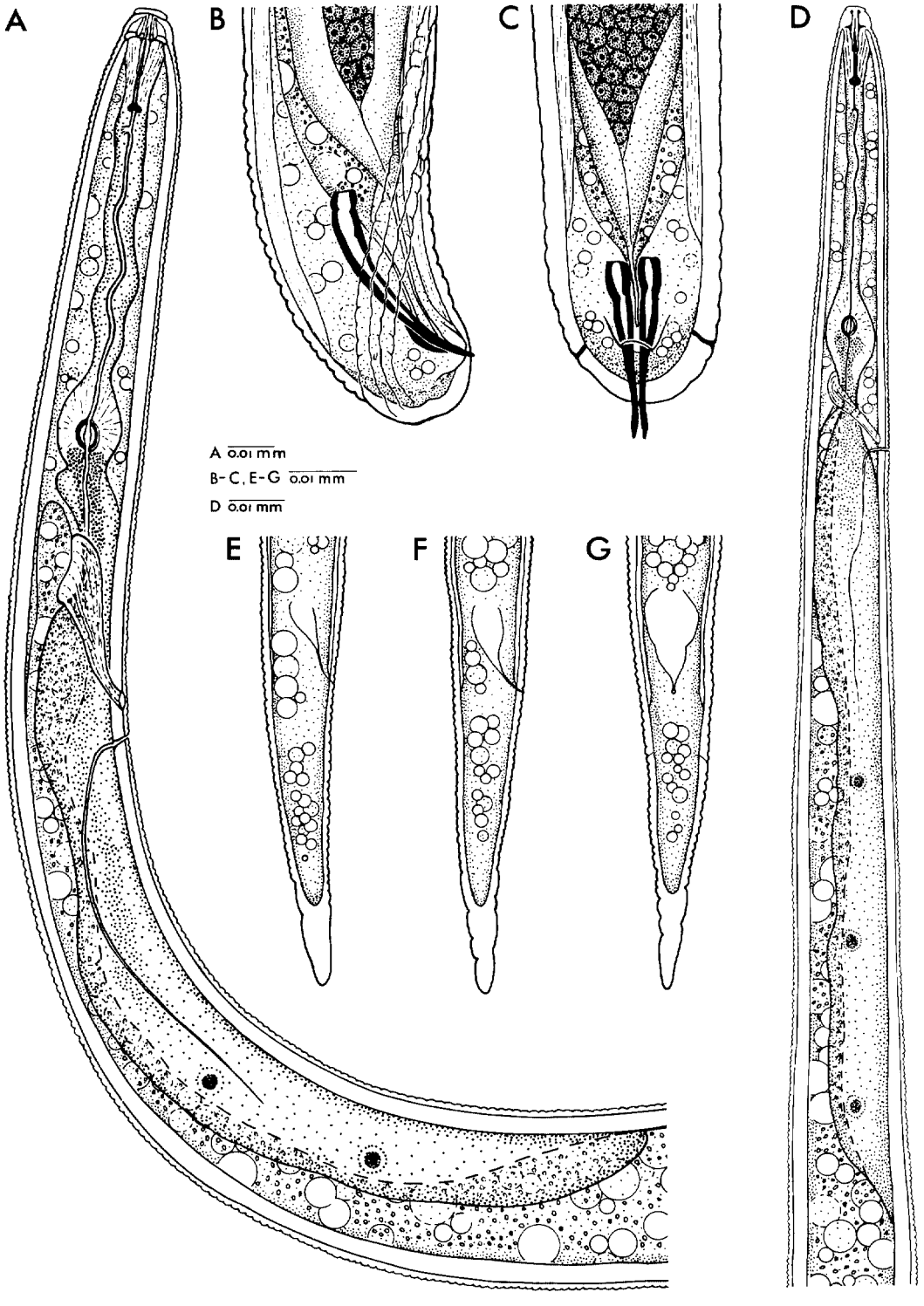

Fig. 5 (A–G). Drawings of males and second-stage juveniles of *Meloidogyne carolinensis* n. sp. A) Anterior portion of male (lateral). B) Male tail (lateral). C) Male tail (ventral). D) Anterior portion of second-stage juvenile (lateral). E–F) Juvenile tails (lateral). G) Juvenile tail (ventral).

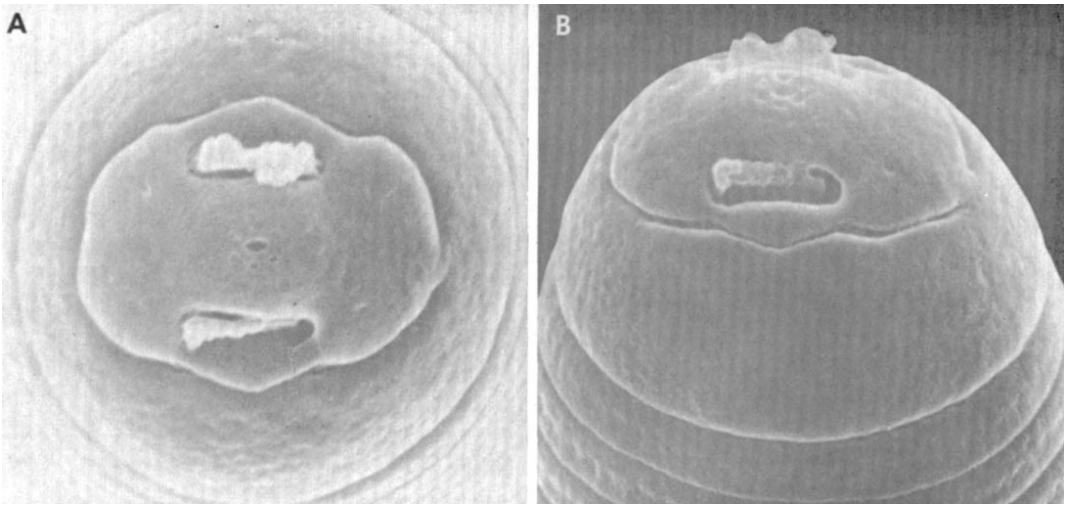

Fig. 6 (A–B). SEM photographs of the head of a male of *Meloidogyne carolinensis* n. sp., face and lateral view, respectively.

elongate slits between labial disc and lateral sectors of head region. Lateral lips absent. Head region not annulated. Body annules distinct. Lateral field with four incisures, areolated, beginning near level of stylet base.

In LM (Fig. 7A), cephalic framework moderately developed, hexaradiate, lateral sectors slightly enlarged. Vestibule and extension distinct. Stylet morphology extremely variable (Figs. 7, 8, 10). Typical stylet opening marked by slight protuberance several microns from stylet tip; cone pointed, gradually increasing in diameter

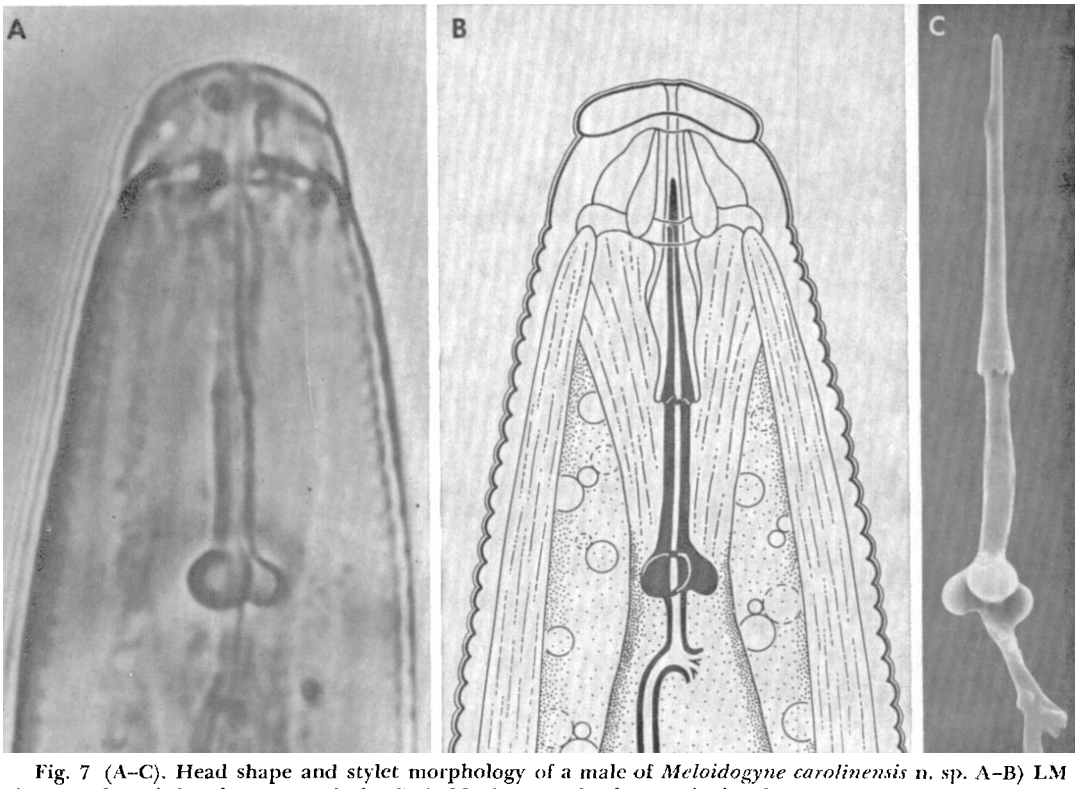

Fig. 7 (A–C). Head shape and stylet morphology of a male of *Meloidogyne carolinensis* n. sp. A–B) LM photograph and drawing, respectively. C) SEM photograph of an excised stylet.

posteriorly, junction of cone and shaft uneven (Fig. 7). Shaft cylindrical, often slightly wider near middle. Knobs broadly elongate, set off from shaft, indented slightly anteriorly, rounded posteriorly. Distance of dorsal esophageal gland orifice to stylet base moderately long, orifice branched into three channels, ampulla indistinct. Procorpus distinct, median bulb ovoid, triradiate lining of enlarged lumen of median bulb thinner than in female. Subventral gland orifices posterior to lining of median bulb, branched. Esophago-intestinal junction at level of nerve ring, indistinct. Two nuclei in gland lobe; lobe variable in length. Intestinal caecum extending anteriorly near level of medium bulb. Excretory pore distinct. Hemizonid 3–4 annules anterior to excretory pore. Usually one testis, sometimes two testes; outstretched or anteriorly reflexed. Spicules arcuate; gubernaculum distinct. Tail short, phasmids at level of cloaca.

Variants (Figs. 8–10): More than 50% of all males exhibit distinct morphological variation in head morphology. Differences exist in height and shape of head cap and width of head region relative to width of first body annule. Variant 1 (Figs. 8A, 9A), similar to typical male except head cap much higher, more rounded. Variant 2 (Figs. 8B, 9B), one or both medial lips lower

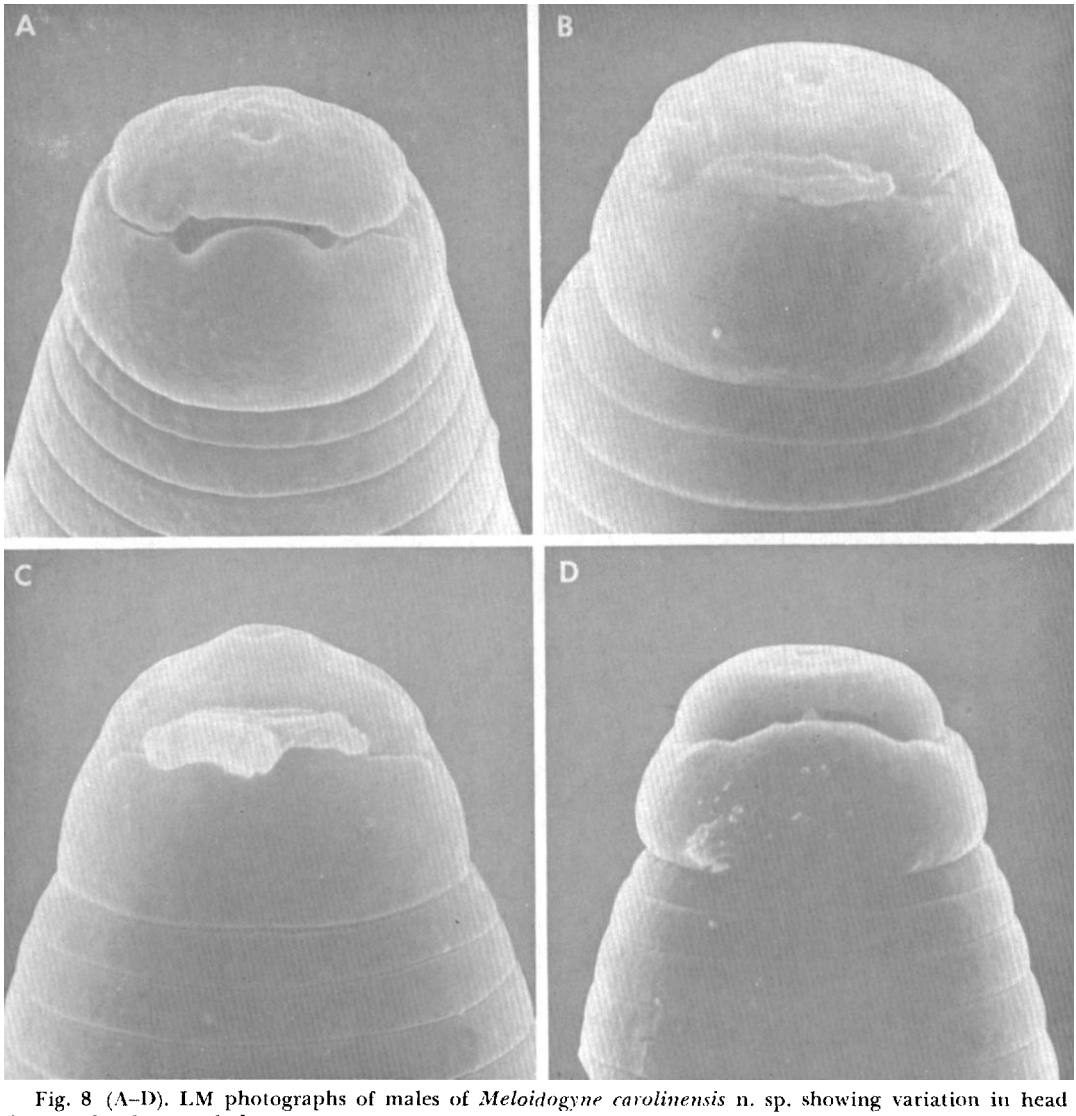

Fig. 8 (A–D). LM photographs of males of *Meloidogyne carolinensis* n. sp. showing variation in head shape and stylet morphology.

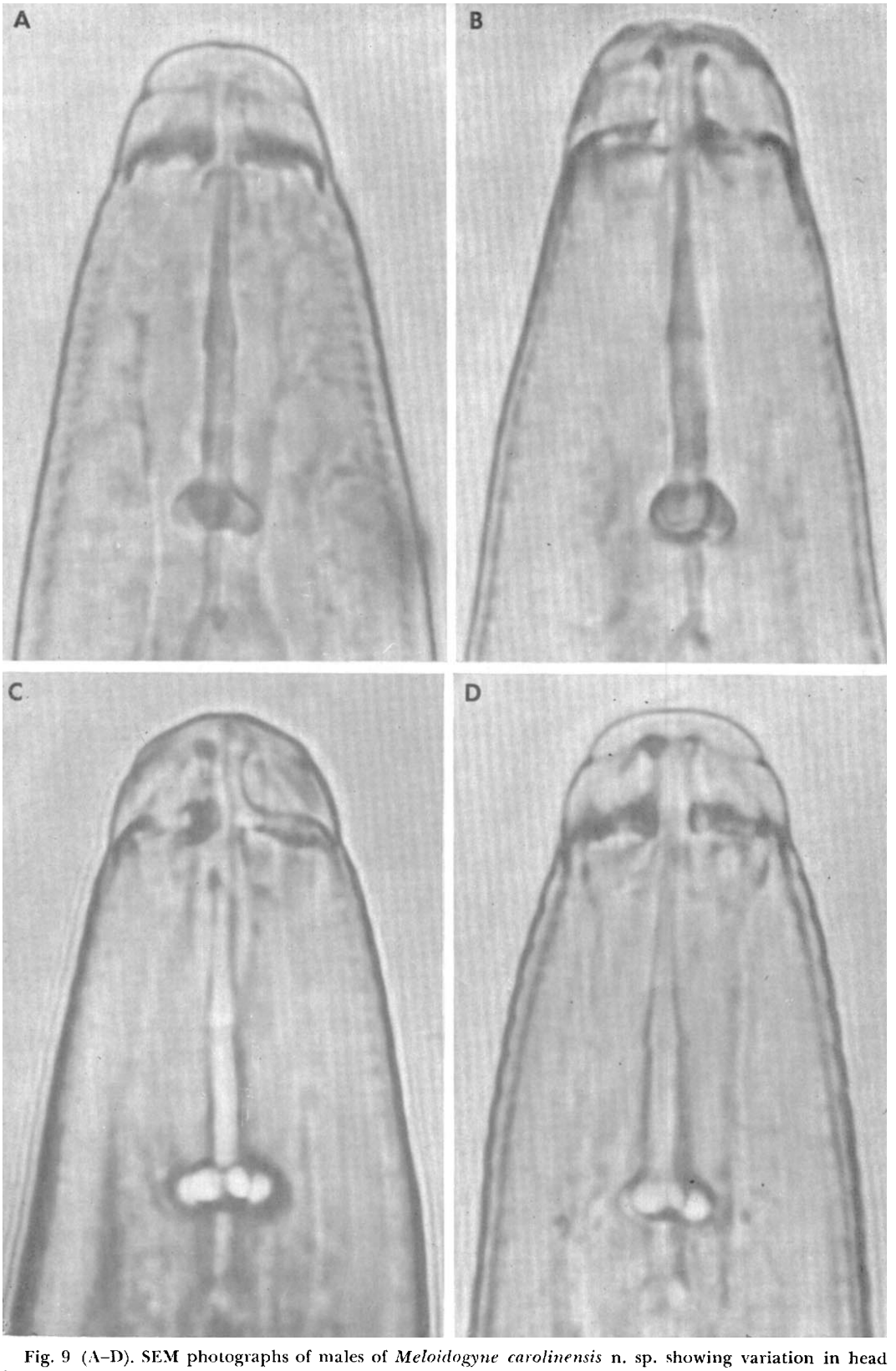

Fig. 9 (A–D). SEM photographs of males of *Meloidogyne carolinensis* n. sp. showing variation in head shape.

than labial disc. Variant 3 (Figs. 8C, 9C), labial disc distinctly elevated near prestomatal cavity. Variant 4 (Figs. 8D, 9D), head cap narrower than in typical male; head region wider than first body annule. Stylet morphology also variable (Figs. 8, 10). Variant 1 (Figs. 8B, 10B), stylet much longer, knobs larger, more rounded, tapering anteriorly onto shaft. Variant 2 (Figs. 8C–D, 10C), knobs anteriorly indented, each knob often appears as two; knobs taper onto shaft. Stylet morphology cannot be correlated to differences in head morphology.

SECOND-STAGE JUVENILES: *Measurements* of 30 juveniles are presented in Table 3.

Description (Figs. 5D–G, 11–12): Body vermiform, tapering more posteriorly than anteriorly. In SEM (Fig. 11), stoma slit-like located in ovoid prestomatal cavity, surrounded by pit-like openings of six inner

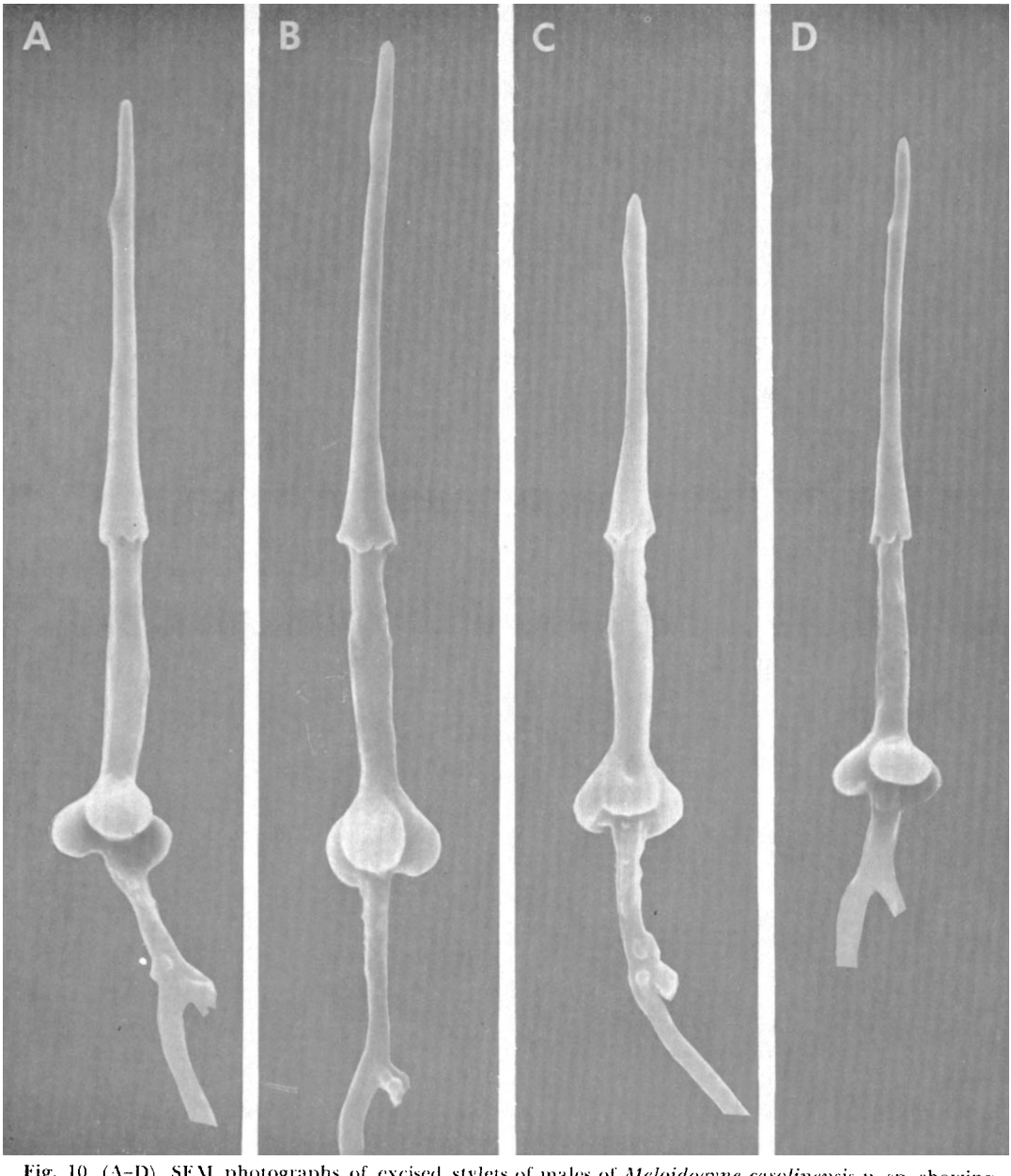

Fig. 10 (A–D). SEM photographs of excised stylets of males of *Meloidogyne carolinensis* n. sp. showing variation in stylet morphology.

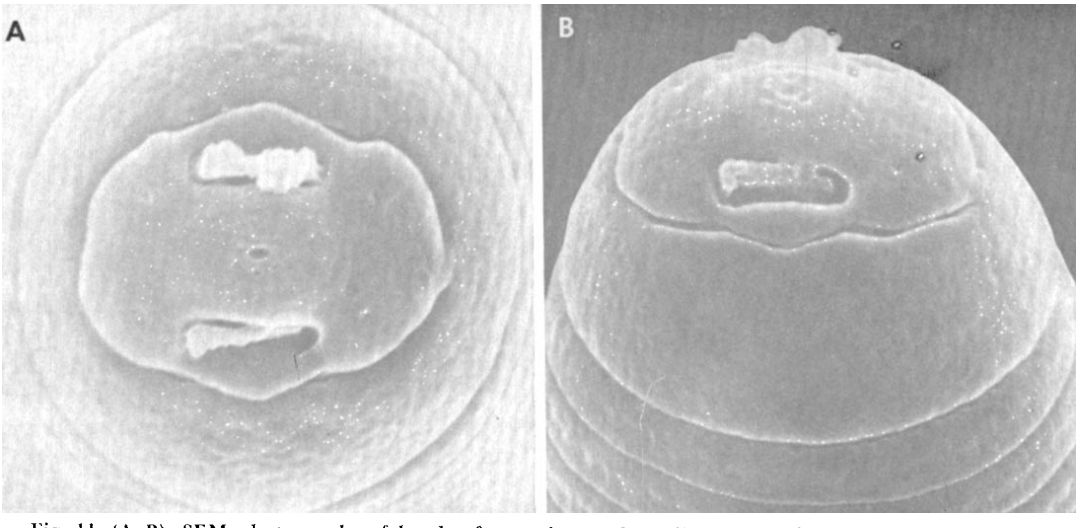

Fig. 11 (A–B). SEM photographs of heads of second-stage juveniles of *Meloidogyne carolinensis* n. sp., face and lateral views, respectively.

labial sensilla. Labial disc, medial lips, and lateral lips fused into one structure. Labial disc elevated above stoma. Medial lips with rounded margins, cephalic sensilla distinct. Amphidial apertures between labial disc and lateral lips. Head region smooth. Body annules distinct. Lateral field with four incisures, areolated. In LM (Fig. 12), cephalic framework weak, hexaradiate. Vestibule and vestibule extension more distinct than rest of framework. Stylet cone increases in width gradually, shaft cylindrical, knobs rounded and set off from shaft. Distance of dorsal esophageal gland orifice to stylet base long; orifice branched into channels; ampulla indistinct. Median bulb

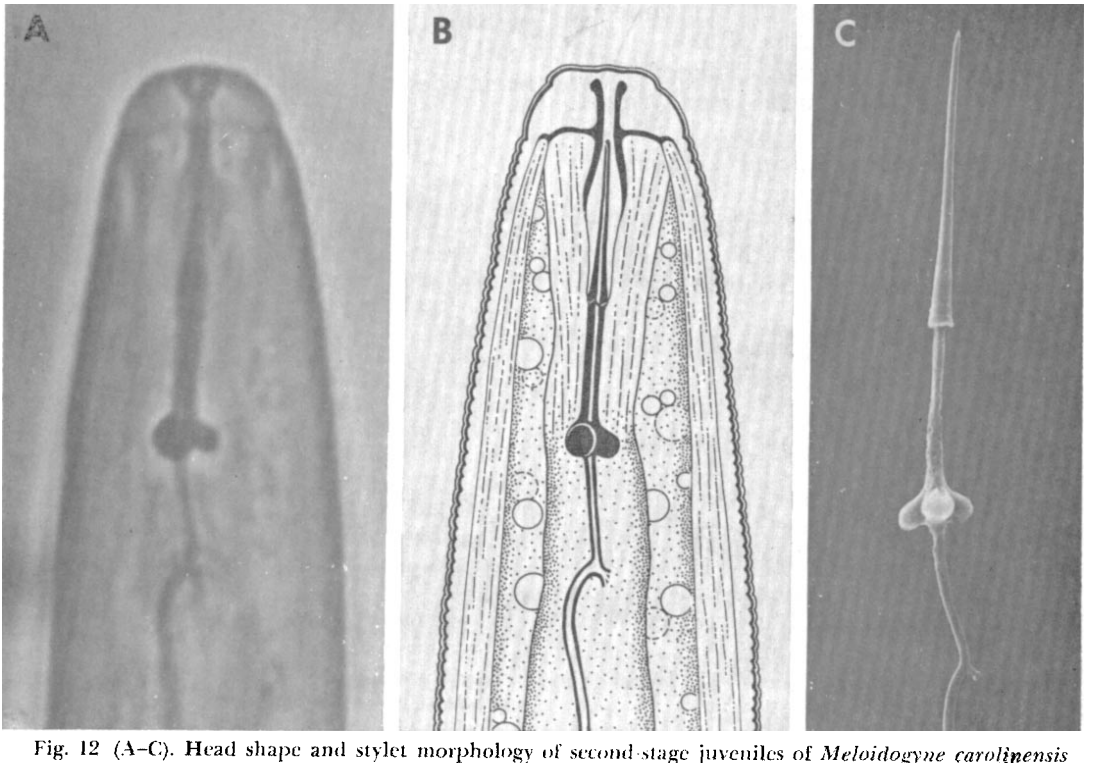

Fig. 12 (A–C). Head shape and stylet morphology of second-stage juveniles of *Meloidogyne carolinensis* n. sp. A–B) LM photograph and drawing, respectively. C) SEM photograph of an excised stylet.

ovoid; triradiate lining strongly sclerotized; subventral gland orifices branched, located immediately posterior to enlarged lumen of median bulb. Esophago-intestinal junction indistinct, at level of nerve ring. Gland lobe of variable length with three nuclei. Excretory pore distinct; hemizonid 1–2 annules anterior to excretory pore. Tail annulations larger and more irregular posteriorly. Hyaline tail terminus distinct. Phasmids small, always below level of anus.

EGGS: *Measurements* of 30 eggs. Length: 79.6–104.2 μm (mean 90.7 μm ± 1.10 std. error of mean at 95% confidence intervals); width: 34.0–41.6 μm (37.1 ± 0.38); length/width ratio: 1.9–3.0 (2.5 ± 0.05).

Description: Egg morphology typical for the genus. Egg masses usually enclosed within root tissues. Sometimes eggs not present, although gelatinous matrix very large.

DIAGNOSIS: *Meloidogyne carolinensis* n. sp. is morphologically distinct from all other described species in the genus. The perineal pattern and stylet morphology of the female, the shape of the male head and stylet morphology, and the head and stylet morphology of the second-stage juvenile are useful diagnostic characters.

HOLOTYPE: Female. Isolated from cultivated highbush blueberry cv. Wolcott (cultivars derived from hybrids of *Vaccinium corymbosum* L. and *V. lamarckii* Camp) collected from type locality and maintained in a greenhouse. Collected June, 1980. Slide T-356t. USDA Nematode Collection (USDANC), Beltsville, Maryland, USA.

ALLOTYPE: Male. Same data as holotype. Slide T-357t. USDANC, Beltsville, Maryland, USA.

PARATYPES: Females, males, and second-stage juveniles. Same data as holotype. USDANC, Beltsville, Maryland. University of California Davis Nematode Collection (UCDNC), Davis, California, USA.

TYPE HOST AND LOCALITY: Roots of cultivated highbush blueberry cv. Wolcott (cultivars derived from hybrids of *Vaccinium corymbosum* L. and *V. lamarckii* Camp) from the Frank Blanchard farm on state Highway 11 near Charity, North Carolina.

DISCUSSION

The blueberry root-knot nematode was the first *Meloidogyne* species found to be obligatorily parthenogenetic. Along with *M. microtyla, M. megatyla,* and other amphimictic species, *M. carolinensis* n. sp. is not considered to be successful agriculturally because it has a limited host range and an isolated distribution (11). Even though the parasitic ability of this species is quite restricted, the morphological variability of the male is striking. The characteristic perineal pattern and the narrow host range distinguishes *M. carolinensis* n. sp. from all other species. The morphology of the stylet of the female and second-stage juvenile and the head morphology of the second-stage juvenile are generally stable. The most variable characters are the head shape and stylet morphology of males. The five distinct types of male head shapes and three different types of stylets are not correlated: any particular head shape may possess any type of stylet. Apparently, distinct gaps occur between the morphological variants, and *M. carolinensis* n. sp. may be a polymorphic species with respect to morphology of the male. If that is true, then perhaps this species could be a useful tool in the study of the genetics of root-knot nematodes.

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