

Meloidogyne enterolobii n. sp. (Meloidogynidae), a Root-knot Nematode Parasitizing Pacara Earpod Tree in China¹

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Abstract: *Meloidogyne enterolobii* n. sp. is described and illustrated from roots of pacara earpod tree, *Enterolobium contortisiliquum* (Vell.) Morong, on Hainan Island in China. The perineal pattern of the female is usually oval shaped, the striae are fine to coarse, the dorsal arch is moderately high to high and usually rounded, and the phasmids are large. The stylet knobs in females are divided longitudinally by a groove so that each knob appears as two. The mean distance of the excretory pore to the anterior end in the female is 62.9 μm . Males have a large, rounded labial disc that fuses with the medial lips to form a dorso-ventrally elongate head cap. The labial disc is slightly elevated, and the medial lips are crescent shaped. The second-stage juvenile mean body length is 436.6 μm . The lateral lips are large and triangular in face view. The tail is 56.4 μm long and narrow with a broad, bluntly rounded tip. *M. enterolobii* n. sp reproduces well on *E. contortisiliquum* and causes severe damage. Other good hosts include cotton, resistant tobacco 'NC 95,' pepper, watermelon, and tomato. **Key words:** taxonomy, morphology, host range, scanning electron microscopy.

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Pacara earpod trees (*Enterolobium contortisiliquum* [Vell.] Morong) on Hainan Island in China are seriously damaged by

a species of root-knot nematode. Preliminary identification from perineal patterns indicated the presence of *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949. In January 1981, galled roots of pacara earpod tree were obtained from the Institute of Tropical Forestry by the Chinese Academy of Forestry in Beijing, China, for a more complete characterization of this population of root-knot nematode. Additional studies conducted at North Carolina State University at Raleigh on the morphology, host range, cytogenetics, and biochemistry indicated that the population

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Table 1. Measurements of 20 females of *Meloidogyne enterolobii* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (μm)					
Body length	541.3-926.3	735.0	20.76	92.83	12.6
Body width	375.7-809.7	606.8	26.92	120.52	19.9
Neck length	114.3-466.8	218.4	16.58	74.16	34.0
Stylet length	13.2-18.0	15.1	0.30	1.35	8.9
Stylet knob height	1.9-3.1	2.4	0.06	0.26	10.8
Stylet knob width	4.1-5.6	4.9	0.09	0.39	8.0
DGO	3.7-6.2	4.9	0.17	0.78	15.9
Excretory pore to head end	42.3-80.6	62.9	2.35	10.50	16.7
Interphasmidial distance	22.2-42.0	30.7	1.07	4.78	15.6
Vulval length	25.3-32.4	28.7	0.44	1.96	6.8
Vulva-anus distance	19.7-26.6	22.2	0.39	1.76	7.9
Ratios					
a	0.97-1.94	1.25	0.05	0.23	18.4
Body length/head end to posterior end of metacarpus	5.18-9.32	7.12	0.22	0.97	13.6
Number of body annules from head end to excretory pore					
	24-48	36	1.50	6.73	18.7

Table 2. Measurements of 20 males of *Meloidogyne enterolobii* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (μm)					
Body length	1,348.6-1,913.3	1,599.8	35.76	159.91	10.0
Body width	37.0-48.3	42.3	0.80	3.56	8.4
Tail length	8.6-20.2	12.5	0.50	2.24	17.9
Stylet length	21.2-25.5	23.4	0.21	0.96	4.1
Stylet knob height	2.6-3.9	3.3	0.07	0.33	10.0
Stylet knob width	4.5-5.8	5.4	0.08	0.34	6.3
DGO	3.7-5.3	4.7	0.09	0.41	8.7
Excretory pore to head end	159.7-206.2	178.2	2.50	11.16	6.3
Spicule length	27.3-32.1	30.4	0.26	1.16	3.8
Gubernaculum length	4.8-8.0	6.2	0.21	0.96	15.5
Testis length	597.0-1,055.0	810.1	31.22	139.63	17.2
Ratios					
a	34.1-45.5	37.9	0.70	3.15	8.3
c	72.0-173.4	131.6	5.40	24.15	18.4
Body length/head end to posterior end of metacarpus	13.8-18.4	15.8	0.30	1.32	8.4
Percentages					
T	38.4-63.6	51.0	0.016	0.07	0.1

was a new species. Results of the North Carolina differential host test showed that resistant tobacco (*Nicotiana tabacum* L. cv. NC 95), pepper (*Capsicum anuum* L. [C. frutescens] cv. California Wonder), watermelon (*Citrullus vulgaris* Schrad. cv. Charleston Gray), and tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) were heavily galled and large numbers of egg masses were produced. Cotton (*Gossypium hirsutum* L. cv. Deltapine 16) was moderately galled and a moderate number of egg masses were produced. Peanut *Arachis hypogaea* L. cv. Florunner) was not galled and no reproduction occurred. The differential host test response of this new species was similar to that of *M. incognita* race 4 (5). Morphologically, however, the population was very different from *M. incognita* or any other described species of root-knot nematode. Cytogenetically this population resembles *M. incognita* race A, with $2n = 44-46$ chromosomes and mitotic parthenogenetic reproduction (8). However, unlike *M. incognita*, most oocytes of this population advance to metaphase and telophase soon after they enter the uterus and show no extended prophase stage. Similarly, biochemical studies revealed that this population has an esterase pattern (three bands of activity at $Rf = .40, .46, \text{ and } .51$) which is different from that of any other *Meloidogyne* species studied thus far (personal communication, Dr. A. C. Triantaphyllou). Because of the differences in biology and morphology to other *Meloidogyne* species, the new species, *M. enterolobii* n. sp. is described below. The common name "pacara earpod tree root-knot nematode" is suggested.

MATERIALS AND METHODS

Stock cultures of *Meloidogyne enterolobii* n. sp. were established from the galled roots of pacara earpod tree obtained from the type locality, Hainan Island, China. The nematodes were propagated and maintained by periodic subculturing on tomato seedlings (*Lycopersicon esculentum* Mill. cv. Rutgers) in a greenhouse maintained at 22–28 C. All nematodes used in morphologic and morphometric studies were from these cultures.

Light microscope studies: Eggs, freshly

hatched juveniles, males, perineal patterns, and females were prepared for light microscopy according to Hirschmann (6). Morphological observations and measurements were made within one week of slide preparation. Permanent mounts of males and second-stage juveniles were made by Thorne's method (7), and type specimens of females were prepared according to Eisenback (2). Line drawings were made with a camera lucida, and photographs were taken with a bright field light microscope.

Scanning electron microscope studies: Second-stage juveniles, males, and females were prepared for scanning electron microscopy as described previously (3, 4). At least 30 males, females, and second-stage juveniles were examined.

SPECIES DESCRIPTION

Meloidogyne enterolobii n. sp.

FEMALES: *Measurements* of 20 females in 2% formalin and perineal patterns in glycerin are listed in Table I. *Measurements* of holotype in glycerin: body length including neck 667.2 μm ; body width 414.6 μm ; neck length 264.8 μm ; stylet length 13.4 μm ; stylet knob height 2.7 μm ; stylet knob width 4.3 μm ; dorsal esophageal gland orifice to stylet base 3.7 μm ; excretory pore not visible. Female as in general description. Perineal region not visible.

Description (Figs. 1–3): Body white, pear-shaped to globular, variable in size, with prominent neck variable in size, without posterior protuberance (Fig. 1 B–F). Head region not distinctly set off from neck (Fig. 1 A). Labial disc and medial lips fuse to form head cap. Hexaradiate cephalic framework distinct but weak; vestibule and vestibule extension prominent. Cephalids and hemizonids not observed. Position of excretory pore variable, often near metacarpus. Cuticular body annulations become progressively finer posteriorly. Stylet slender; conical portion slightly curved dorsally, tapering toward tip; cylindrical shaft, posterior end often enlarged (Figs. 1 A, 2). Knobs set off from shaft, distinct from each other, and divided longitudinally by groove so that each knob appears as two. Dorsal esophageal gland orifice (DGO) 4–6 μm

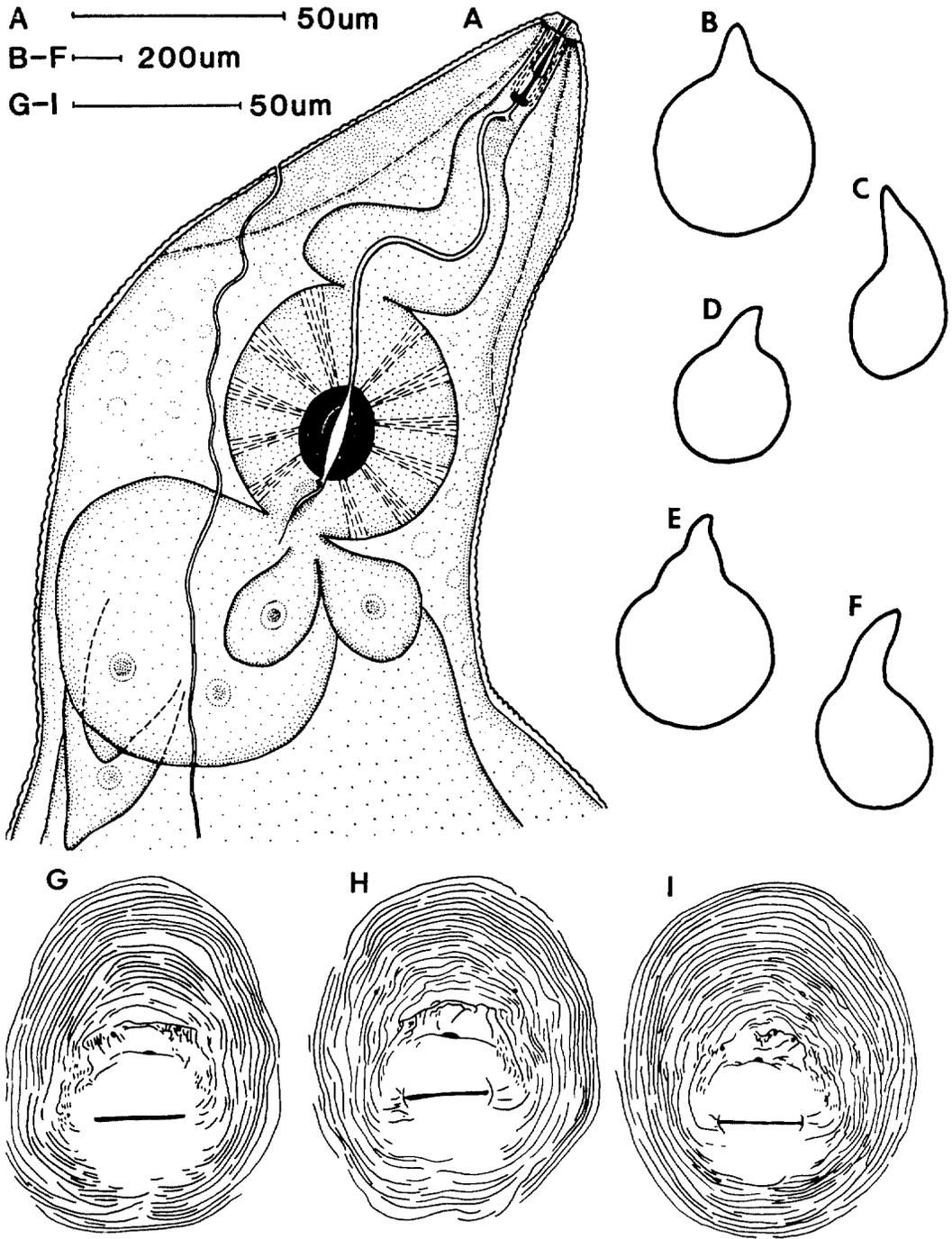


Fig. 1. Drawings of females of *Meloidogyne enterolobii* n. sp. A) Anterior portion (lateral). B-F) Outlines of whole specimens (lateral). G-I) Perineal patterns.

from base of stylet knobs; orifice branches into three channels; dorsal gland ampula large. Subventral gland orifices branched, located immediately posterior to enlarged lumen lining of metacarpus; subventral

gland ampula small but distinct. Esophageal gland comprised of one large uninucleate dorsal esophageal gland lobe; two small nucleated subventral esophageal gland lobes usually posterior to dorsal gland lobe but

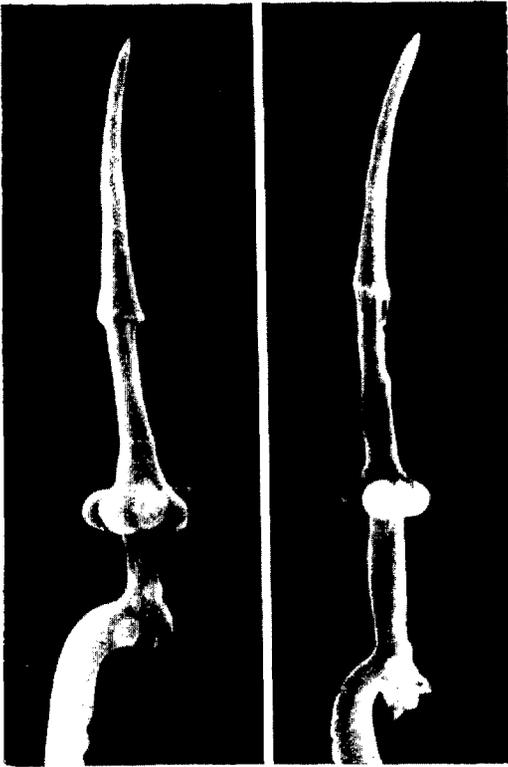


Fig. 2. SEM photographs of excised stylet of females of *Meloidogyne enterolobii* n. sp.

variable in position, shape, and size; all three lobes overlap intestine ventrally. Two small, rounded, singly nucleated esophago-intestinal cells located between metacarpus and intestine. Perineal pattern (Figs. 1

G-I, 3) usually oval shaped, with coarse and smooth striae; dorsal arch moderately high to high, often rounded, nearly square in some specimens. Lateral lines not distinct. Perivulval region generally free of striae; striae may occur on lateral sides of vulva. Striae on ventral area of pattern generally finer and smoother. Tail tip visible; phasmidial ducts large.

MALES: *Measurements* of 20 males in TAF are listed in Table 2. Measurements of allotype in glycerin: body length 1,496.4 μm ; body width 37.0 μm ; stylet length 23.6 μm ; stylet knob height 2.6 μm ; stylet knob width 4.6 μm ; dorsal esophageal gland orifice to stylet base 4.9 μm ; excretory pore to head end 165.4 μm ; tail length 14.2 μm ; spicule length 28.3 μm ; gubernaculum not visible; testis length 880.0 μm ; a = 40.0; c = 105.4; and T = 53.5%. Male as in general description.

Description (Figs. 4-6): Body translucent white, vermiform, tapering at both ends. Tail end more rounded than anterior end, twisting through 90° in heat killed specimens. In lateral view, head cap high and rounded, head region only slightly set off from body (Figs. 4 A-C, 5, 6 A). Hexaradiate cephalic framework moderately developed; vestibule and extension distinct. In SEM (Fig. 5), stoma slit-like, prestoma hexagonal, surrounded by pit-like openings of six inner labial sensilla. Labial disc and medial lips fuse, forming elongate head

Table 3. Measurements of 30 juveniles of *Meloidogyne enterolobii* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (μm)					
Body length	405.0-472.9	436.6	3.03	16.61	3.8
Body width	13.9-17.8	15.3	0.16	0.89	5.8
Tail length	41.5-63.4	56.4	0.82	4.48	7.9
Excretory pore to head end	84.0-98.6	91.7	0.61	3.34	3.6
Stylet length	10.8-13.0	11.7	0.08	0.45	3.8
Stylet knob height	1.3-1.8	1.6	0.02	0.13	8.1
Stylet knob width	2.4-3.4	2.9	0.05	0.25	8.6
DGO	2.8-4.3	3.4	0.06	0.33	9.7
Ratios					
a	24.0-32.5	28.6	0.34	1.88	6.6
c	6.8-10.1	7.8	0.12	0.65	8.3
Body length/head end to posterior end of metacarpus	6.2-6.9	6.5	0.03	0.18	2.8

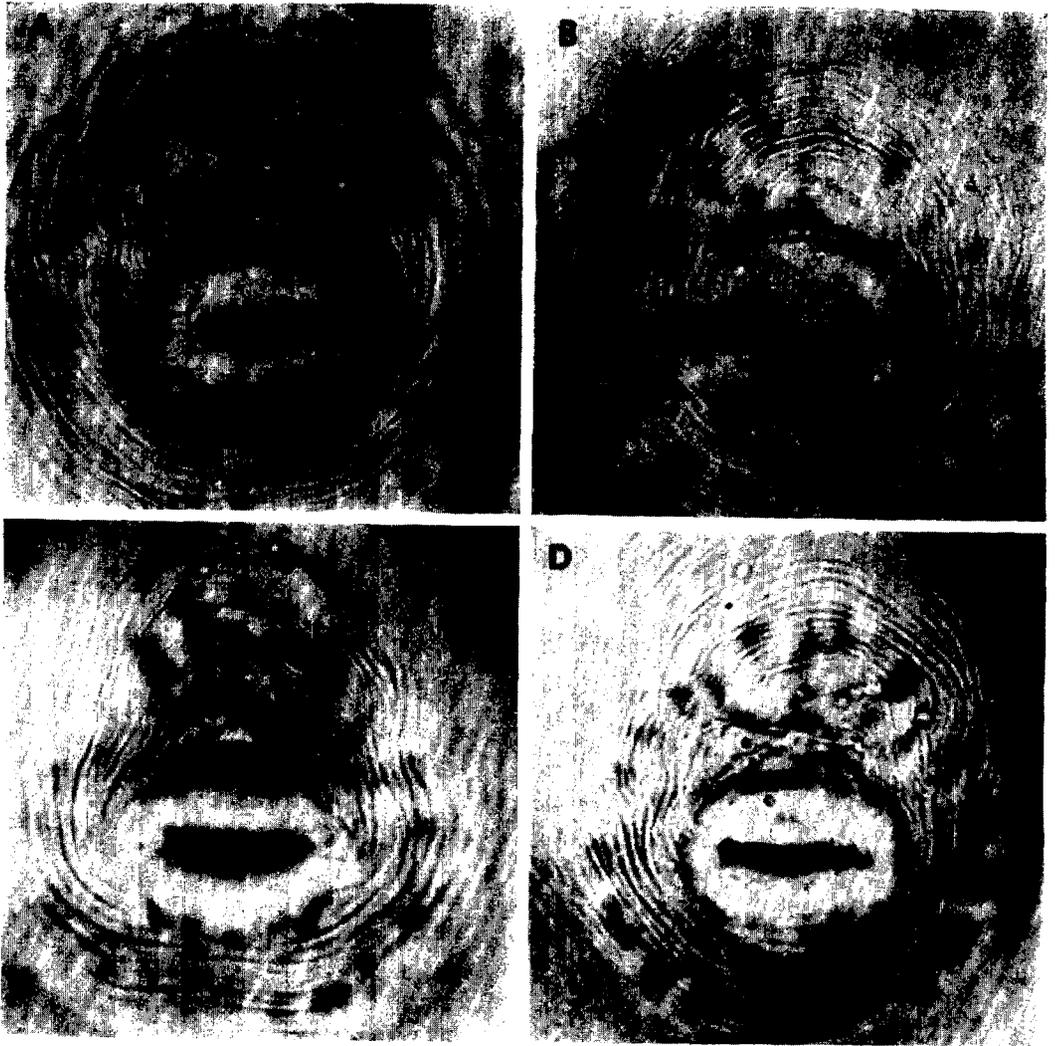


Fig. 3. LM photographs of perineal patterns of *Meloidogyne enterolobii* n. sp. showing typical variation for the species.

cap and labial disc slightly elevated above medial lips. Four cephalic sensilla marked on medial lips by shallow cuticular depressions. Amphid openings slit-like; lateral lips absent; head region not annulated; body annules distinct. Lateral field begins near level of stylet knobs as two incisures; two additional incisures start near level of metacarpus; lateral field areolated, encircles tail (Fig. 4 D-E). Stylet (Figs. 4 A-C, 6) robust; cone straight, pointed; opening located several micrometers from tip. Shaft cylindrical; knobs large, rounded, distinctly set off from shaft; in some specimens each knob is divided longitudinally by groove so that each knob appears as two but not as

pronounced as in female. Distance of GDO to stylet base long ($3.7-5.3 \mu\text{m}$), orifice branched into three channels, ampulla poorly defined. Procorpus distinct; metacarpus elongate, oval shaped with enlarged cuticular lumen lining; esophago-intestinal junction indistinct, at level of nerve ring. Gland lobe variable in length, with two nuclei. Excretory pore far from anterior end, terminal duct long. Hemizonid 2-4 annules anterior to excretory pore. One or two testes, usually outstretched. Spicules arcuate, with rounded base, single tip. Gubernaculum short and simple. Tail short and rounded. Phasmids small, pore-like, at level of cloaca.

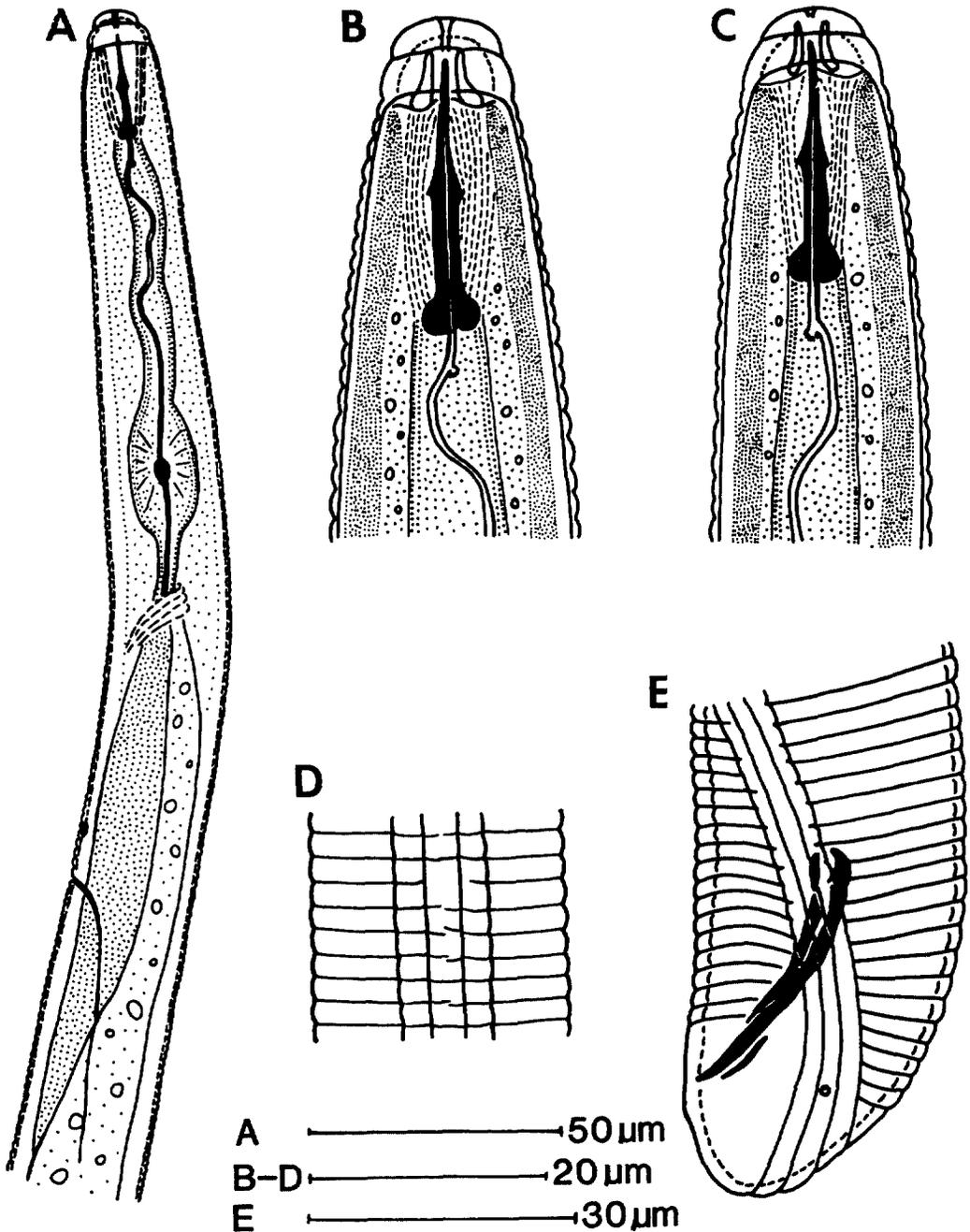


Fig. 4. Drawings of males of *Meloidogyne enterolobii* n. sp. A) Esophageal region (lateral). B-C) Head region (lateral). D) Lateral field near mid-body. E) Tail (lateral).

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

Description (Figs. 7-9): Body translucent white, vermiform, rather long, tapering at both ends with very long, narrow tail. Anterior end truncate; head region

only slightly set off from body. Vestibule and extension more developed than remainder of hexaradiate cephalic framework. In SEM (Fig. 8), stoma slit-like, located in oval-shaped prestoma, surrounded by six pore-like openings of inner labial sensilla. Medial lips and labial disc dumbbell-shaped

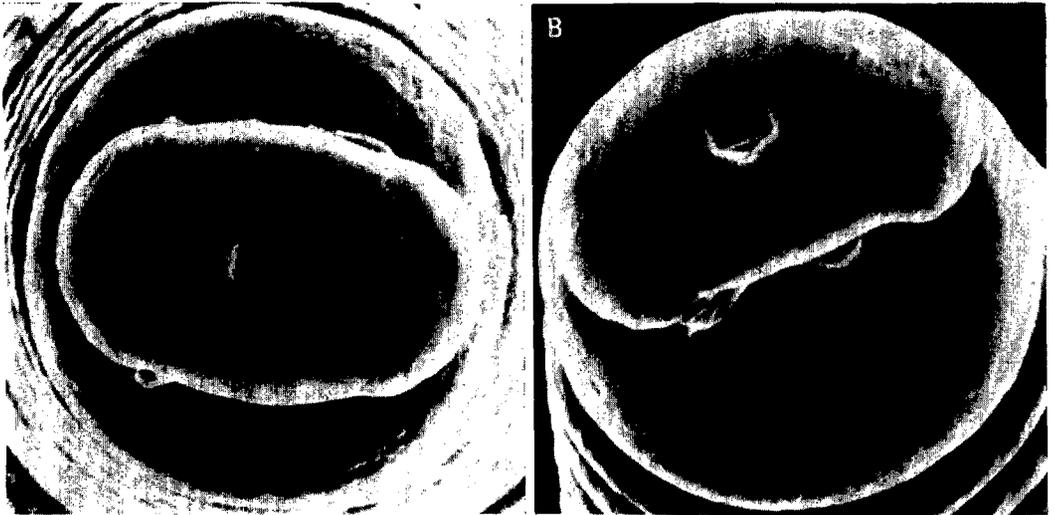


Fig. 5. SEM photographs of the head of a male of *Meloidogyne enterolobii* n. sp. A) Face view. B) Lateral view.

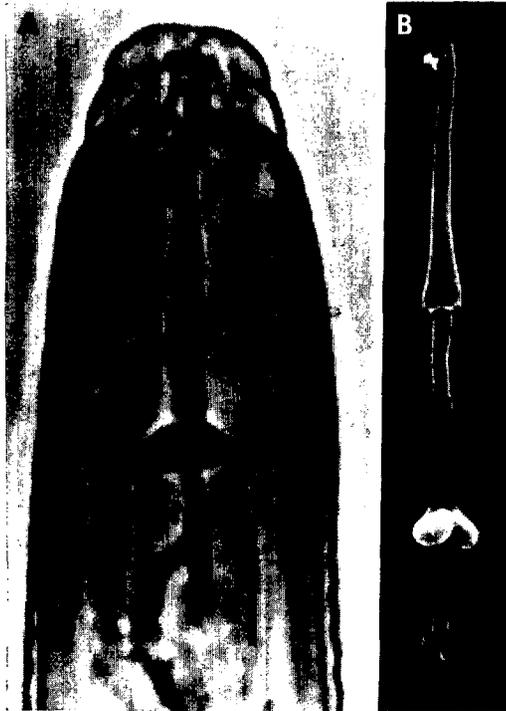
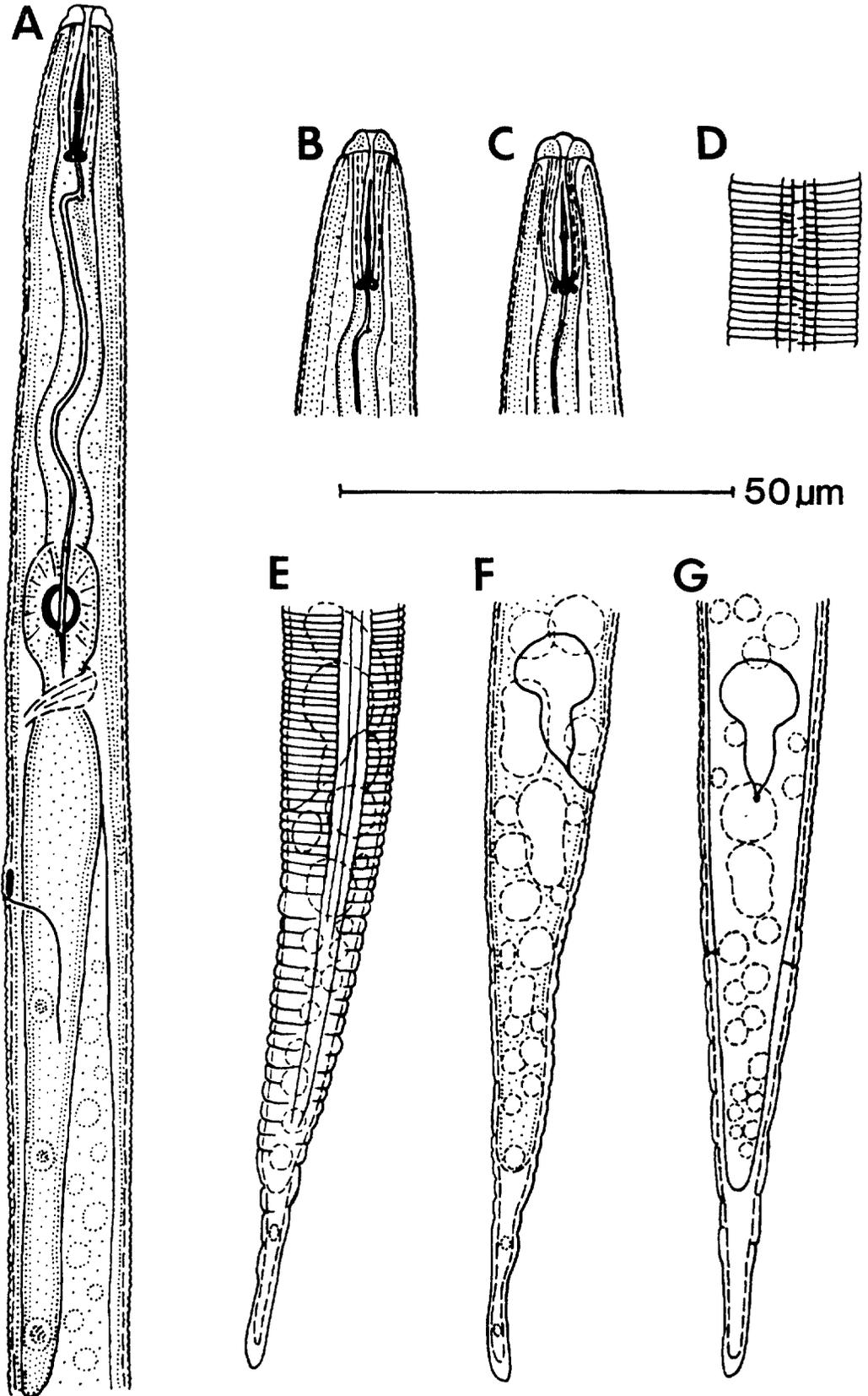


Fig. 6. Head shape and stylet morphology of a male of *Meloidogyne enterolobii* n. sp. A) LM photograph. B) SEM photograph of an excised stylet.

in face view. Labial disc rounded, raised slightly above medial lips. Lateral lips large and triangular, lower than labial disc and medial lips. Posterior edge of one or both lateral lip may fuse with the head region in some specimens. Elongate amphidial apertures located between labial disc and lateral lips. Head region not annulated; body annules distinct but fine. Lateral field beginning near level of procorpus as two lines; near metacarpus third line begins and shortly splits making four lines, running entire length of body before gradually decreasing to two lines which end near hyaline tail terminus, irregularly areolated. In LM (Figs. 7, 9), stylet delicate; cone straight, narrow, sharply pointed; shaft becomes slightly wider posteriorly; knobs large, rounded, separate from each other, set off from shaft. Distance from base of stylet to dorsal esophageal gland orifice long (2.8–4.3 μm); orifice branched into three channels; ampulla indistinct. Procorpus faintly outlined; metacarpus oval shaped with enlarged lumen lining; isthmus not clearly defined esophago-intestinal junction difficult to observe. Gland lobe variable in

Fig. 7. Drawings of second stage juveniles of *Meloidogyne enterolobii* n. sp. A) Esophageal region (lateral). B) Head region (lateral). C) Head region (ventral). D) Lateral field near mid-body. E-F) Tail (lateral). G) Tail (ventral).





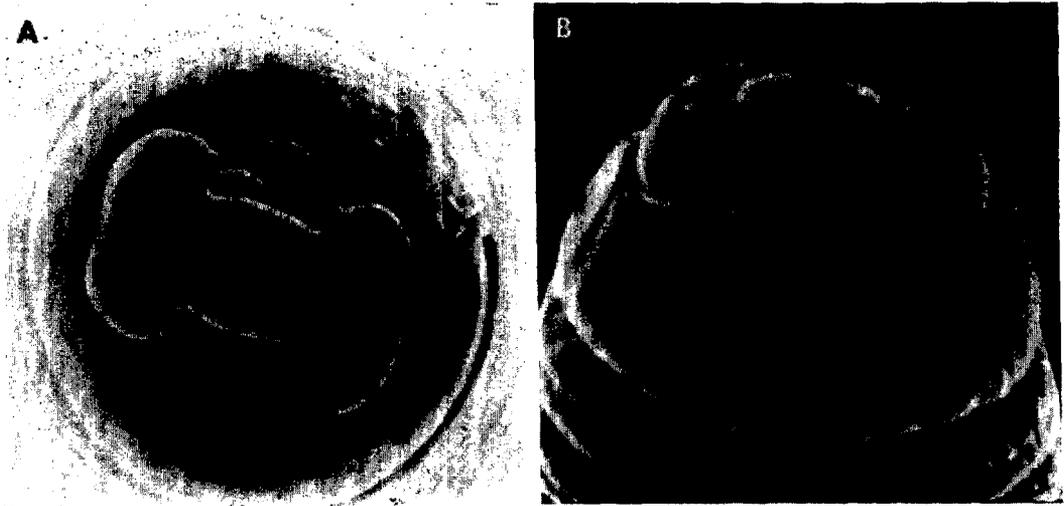


Fig. 8. SEM photographs of the head of a second-stage juvenile of *Meloidogyne enterolobii* n. sp. A) Face view. B) lateral view.

length, with three equally sized nuclei; overlaps intestine ventrally. Excretory pore distinct; hemizonid 1-2 annules anterior to excretory pore, 3-5 annules long; cuticle slightly raised over hemizonid. Tail very thin; annulations increase in size, become more irregular posteriorly. Hyaline tail terminus clearly defined; tail tip broad,

bluntly rounded. Rectum dilated. A few fat droplets may occur in hyaline tail terminus. Phasmids small, difficult to observe, located posterior to anus.

EGGS: Morphologically indistinguishable from other *Meloidogyne* species. Measurements of 20 eggs in 2% formalin: Length, 85.7-103.6 μm (mean 95.5 μm , standard error of mean 1.19, standard deviation 5.30, coefficient of variation 5.5 %); width, 33.6-41.4 μm (mean 38.2, std. error 0.51, std. dev. 2.28, coef. of var. 6.0%). Length/width ratio = 2.14-3.02 (mean 2.51, std. error 0.05, std. dev. 0.23, coef. of var. 9.1 %).

DIAGNOSIS

Meloidogyne enterolobii can be separated from other described species of the genus by the form of perineal pattern, stylet morphology, and position of excretory pore in female; morphology of the head in the male; and morphology of the head and tail in the second-stage juvenile.

The stylet of the female is characterized by knobs that are divided longitudinally by a groove. Although the distance from excretory pore to the anterior end of the female is variable, usually the distance is rather long (42.3-80.6 μm). In the male, the labial disc and medial lips fuse forming an elongate head cap and the labial disc is slightly elevated above the medial lips. In the second-stage juvenile, the medial



Fig. 9. LM photographs of second-stage juveniles of *Meloidogyne enterolobii* n. sp. A) Head region (lateral). B) Tail region (lateral).

lips and labial disc are dumbbell shaped in face view. The labial disc is rounded and raised slightly above medial lips. The lateral lips are large and triangular. Although the tail is very thin, it is relatively long (41.5–63.4 μm); the hyaline tail terminus is clearly defined.

This new species is similar in host range to *M. incognita* but differs from the latter species in many morphological characteristics. In the female, the stylet knobs are divided longitudinally by distinct grooves so that each knob appears as two; distance of the dorsal gland orifice to the stylet base is longer than in *M. incognita* (*M. enterolobii* n. sp.: 3.7–6.2 μm [4.9 μm] and *M. incognita*: 2–4 μm [3 μm]) (1); perineal pattern is usually oval shaped; dorsal arch is moderately high to high and often rounded. In the male, the large, smooth labial disc and medial lips fuse; head region is not annulated; distance of the DGO to the stylet base is longer than in *M. incognita* (*M. enterolobii* n. sp.: 3.7–5.3 μm [4.7 μm] and *M. incognita*: 1.7–3.5 μm [2.5 μm]) (1); stylet knobs in many specimens are longitudinally divided by a groove. The second-stage juvenile is longer than in *M. incognita* (*M. enterolobii* n. sp.: 405.0–472.9 μm [436.6 μm] and *M. incognita*: 360–393 μm) (1); distance of the DGO to the stylet base is longer than in *M. incognita* (*M. enterolobii* n. sp.: 2.8–4.3 μm [3.4 μm] and *M. incognita*: 2–2.5 μm) (1); head region is not annulated; lateral lips are large and triangular. Reproduction is by mitotic parthenogenesis; the somatic chromosome number is $2n = 44-46$; and most oocytes advance to metaphase and telophase soon after they enter the uterus and show no extended prophase stage. Biochemically, this new species has three major bands of esterase activity at $R_f = .40, .46, \text{ and } .51$, while *M. incognita* populations have only one major band of activity at $R_f = .47$ (5).

BIOLOGY: *Meloidogyne enterolobii* n. sp. is from a warm climate, Hainandao, Guangdong Province, China. In this area, the average temperature is 16.9 C in the coldest month and 28.3 in the warmest

month. Besides pacara earpod tree, this species also infects resistant tobacco, pepper, watermelon, and tomato and moderately infects cotton.

HOLOTYPE: Female. Isolated from greenhouse culture maintained on tomato (*Lycopersicon esculentum* Mill cv. Rutgers). Original population derived from type locality and host. Slide T-360t, USDA Nematode Collection (USDANC), Beltsville, Maryland, USA.

ALLOTYPE: Male. Same data as holotype. Slide T-361t, USDANC, Beltsville, Maryland, USA.

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

TYPE HOST AND LOCALITY: Roots of pacara earpod tree (*Enterolobium contortisiliquum* (Vell.) Morong on Hainan Island, China.

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