

Meloidogyne paranaensis n. sp. (Nemata: Meloidogynidae), a Root-Knot Nematode Parasitizing Coffee in Brazil

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Abstract: A root-knot nematode parasitizing coffee in Paraná State, Brazil, is described as *Meloidogyne paranaensis* n. sp. The suggested common name is Paraná coffee root-knot nematode. The perineal pattern is similar to that of *M. incognita*; the labial disc and medial lips of the female are fused and asymmetric and rectangular; the lateral lips are small, triangular, and fused laterally with the head region. The female stylet is 15.0–17.5 µm long, with broad, distinctly set-off knobs; the distance from the dorsal esophageal gland orifice (DGO) to the stylet base is 4.2–5.5 µm. Males have a high, round head cap continuous with the body contour. The labial disc is fused with the medial lips to form an elongate lip structure. The head region is frequently marked by an incomplete annulation. The stylet is robust, 20–27 µm long, usually with round to transversely elongate knobs, sometimes with one or two projections protruding from the shaft. The stylet length of second-stage juveniles is 13–14 µm, the distance of the DGO to the stylet base is 4.0–4.5 µm, and the tail length is 48–51 µm. Biochemically, the esterase (F₁) and malate dehydrogenase (N₁) phenotypes are the most useful characters to differentiate *M. paranaensis* from other species. However, the esterase phenotype appears similar to that of *M. konaensis*. Reproduction is by mitotic parthenogenesis, 3n = 50–52. In differential host tests, tobacco, watermelon, and tomato were good hosts, whereas cotton, pepper, and peanut were nonhosts.

Key words: Brazil, *Coffea arabica*, coffee, host range, *Meloidogyne paranaensis* n. sp., morphology, nematode, new species, root-knot nematode, scanning electron microscope, taxonomy.

Root-knot nematodes (*Meloidogyne* spp.) are distributed widely in coffee plantations in Brazil, where they cause great losses to the coffee farmers and the country's economy. *Meloidogyne incognita* (Kofoid & White) Chitwood, *M. exigua* Goeldi, and *M. coffeicola* Lordello & Zamith have been reported in coffee plantations of Paraná, São Paulo, and Minas Gerais States for many years either as separate or mixed populations, with fluctuations of the most dominant species (1,13). Surveys in Paraná State have shown a substantial increase in the distribution of *M. incognita* and a decrease in *M. coffeicola* (2). It is believed that *M. coffeicola* was eradicated from many plantations during the renewal of damaged coffee after the great frost of 1975 (1). Santos and Triantaphyllou (14) suggested that *Meloidogyne* spp. populations on coffee were frequently misidentified. Carneiro

(3) reported a new pathotype of *M. incognita*, which was named "biotype IAPAR." This population had been found attacking coffee in Paraná State and accounted for approximately 52% of all root-knot nematode infestations in Paraná. This nematode has been present on coffee in Brazil for many years and has been reported as "unidentified populations of *Meloidogyne* from coffee" (10,12). Based on morphological and biological differences with other *Meloidogyne* spp., this nematode population is described, illustrated, and designated herein as *M. paranaensis* n. sp.

MATERIALS AND METHODS

Stock cultures of *M. paranaensis* n. sp. derived from the original isolate from coffee (*Coffea arabica* L.) roots (Paraná State, Brazil) were maintained by periodic subculturing on tomato (*Lycopersicon esculentum* Mill. cv. Santa Cruz) in a greenhouse at 22–28 °C. All morphological and biological studies were made from these cultures. Egg masses and females were handpicked from infected tomato roots, and second-stage juveniles (J2) were hatched from egg masses in moist chambers. Males were ob-

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tained by incubating washed, infected roots in moist chambers (8). The roots were rinsed periodically with water, and males were collected from the washings.

Morphological studies: Eggs were mounted in 2% formalin and measured under light microscopy (LM). Freshly hatched J2, males, and females were relaxed and killed in hot water, transferred to 2% formalin, and measured immediately under LM. Perineal patterns of females were cut from specimens in 45% lactic acid and mounted in glycerin (15). At least 50 patterns were examined. Type specimens were prepared according to Eisenback (8). Drawings were made with a drawing tube, and photographs were taken with brightfield LM. Males, J2, and females were prepared for scanning electron microscopy (SEM) according to previously described methods (5–7). Specimens were viewed and photographed with a JEOL JSM 6301F scanning electron microscope. At least 100 specimens of each life stage were examined.

Biological studies: Electrophoresis was performed on 7% polyacrylamide gel slabs, with the technique proposed by Carneiro et al. (4). Enzyme phenotypes were named according to Esbenshade and Triantaphyllou (10). Cytological studies were made with a propionic-orcein staining method (16). A differential host test was performed with the following plants: cotton cv. Deltapine 61, tobacco cv. NC 95, pepper cv. Early California Wonder, watermelon cv. Charleston Gray, peanut cv. Florunner, and tomato cv. Rutgers (11).

SYSTEMATICS

Meloidogyne paranaensis n. sp. (Figs. 1, 2, 3, 4, 5, 6)

Holotype (female in glycerine): Body length, 684 μm ; body width, 470 μm ; neck length, 185 μm ; neck width, 211 μm , body length without neck, 423 μm ; stylet length, 16.2 μm ; stylet knob height, 2.4 μm ; stylet knob width, 4.8 μm ; dorsal esophageal gland orifice (DGO) to stylet base, 4.2 μm ;

head end to posterior end of metacarpus, 96.2 μm ; metacarpus length, 39 μm ; metacarpus width, 33.8 μm ; metacarpus valve length, 13.8 μm ; metacarpus valve width, 10.4 μm ; excretory pore to head end, 32.5 μm .

Females: Measurements of 30 females (in formalin) listed in Table 1. Body translucent-white, variable in size, elongate, ovoid to pear-shaped. Neck sometimes prominent, cuticular annulation on body finer than that on neck. Body posteriorly rounded, without tail protuberance. Head region not set off from body, not annulated (Fig. 1A,B). In SEM (Fig. 2A–C), stoma slit-like, located in ovoid prestomal cavity, central on labial disc. Pore-like openings of six inner labial sensilla surrounding prestoma. Labial disc and medial lips fused, asymmetric and rectangular, forming two straight lateral edges in face view. Lateral lips small, triangular, fused laterally with head region. Amphidial openings elongated slits between labial disc and lateral lips (Fig. 2A–C). In LM, cephalic framework weakly sclerotized, lateral sectors slightly enlarged, vestibule extension distinct (Fig. 1A,B). Anterior half of stylet cone pointed and slightly curved dorsally, posterior half conical. Shaft cylindrical, widening slightly near junction with knobs. Three large knobs tapering onto shaft (Figs. 1C; 2D,E). Distance of stylet base to DGO 4.2–5.5 μm . Esophagus with large, rounded metacarpus, valve plates large (Fig. 1A). 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 large esophago-intestinal cells near junction of metacarpus and intestine. Excretory pore at level of anterior metacarpus (Fig. 1A).

Perineal patterns variable, typically rectangular to oval shaped, dorsal arch generally high, squarish, dorsal striae varying from fine to coarse, smooth to wavy. Lateral lines mostly discontinuous, without distinct incisures, sometimes appearing as a discontinuous linear depression faintly

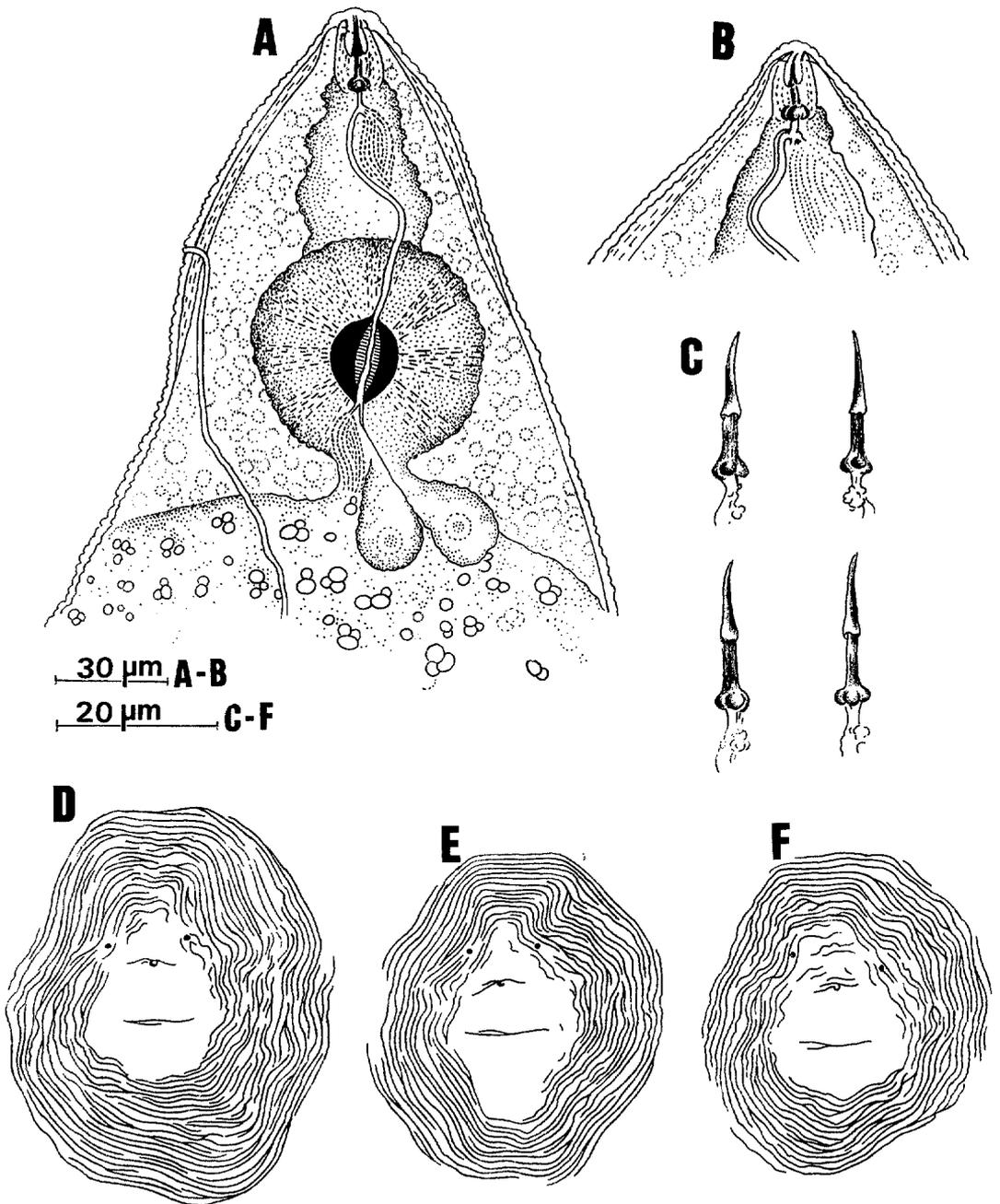


FIG. 1. Drawings of *Meloidogyne paranaensis* n. sp. females. A) Esophageal region, lateral. B) Anterior region, lateral view. C) Stylets. D-F) Perineal patterns.

marked by breaks and forks. All variants with a triangular postanal whorl. Phasmids distinct (Figs. 1D-F; 3A-F).

Allotype (male in glycerine): Body length, 1,708 μm ; greatest body width, 39 μm ; body width at stylet knobs, 19.2 μm ; body

width at excretory pore, 27.6 μm ; stylet length, 22.2 μm ; stylet knob width, 4.8 μm ; stylet knob height, 2.4 μm ; DGO to stylet base, 4.2 μm ; head end to metacarpus valve, 86 μm ; metacarpus width, 9.8 μm ; head end to excretory pore, 157 μm ;

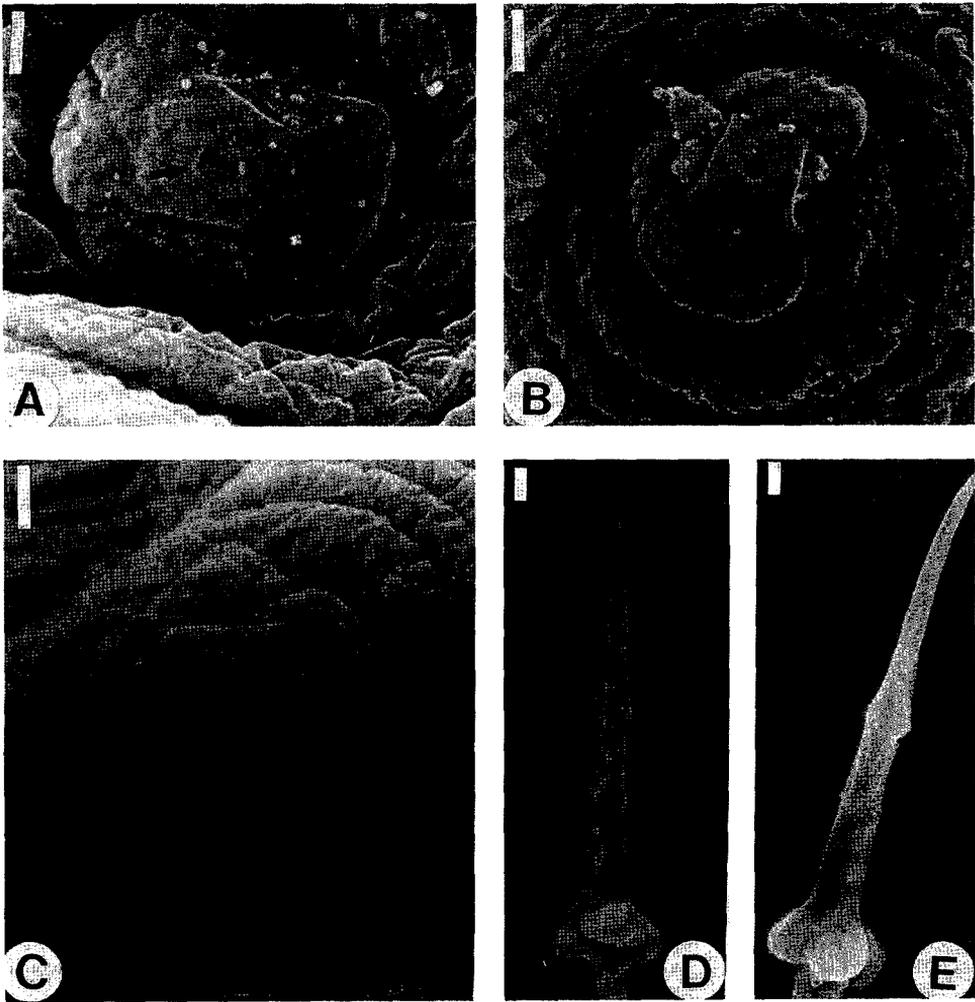


FIG. 2. Scanning electron microscopic photographs of *Meloidogyne paranaensis* n. sp. female. A–C) Face views. Arrows indicate inner labial sensilla. D,E) Excised stylets. Bars = 1 μ m.

testis length, 897 μ m; spicule length, 26 μ m.

Males: Measurements of 30 males (in formalin) listed in Table 2. Body vermiform, length variable, body tapering anteriorly, bluntly rounded posteriorly, tail arcuate, twisting through 90°. Head cap high, rounded, continuous with body contour. In LM, cephalic framework strongly developed, vestibule and extension distinct (Fig. 4A–C). Stylet robust, large (Fig. 5C–E,G), cone straight, pointed, gradually increasing in diameter posteriorly, stylet opening marked by slight protuberance several micrometers from stylet tip, shaft cylindrical, sometimes with one or two

large projections (Fig. 5E,G), knobs large, rounded, set off from shaft. Distance from stylet base to DGO 3.5–5.0 μ m. Procorpus distinct, median bulb ovoid, sometimes covered by intestinal caecum extending anteriorly. Esophago-intestinal junction at level of nerve ring, indistinct (Fig. 4A). In SEM, head cap flat, labial disc fused with median lips forming elongate, rectangular head cap. Lateral lips absent (Fig. 5A,B). Head region usually marked by a short, incomplete annulation in lateral view (Fig. 5F). Stoma opening slit-like, located in ovoid prestomatal cavity, surrounded by pit-like openings of six inner labial sensilla. Four cephalic sensilla marked by distinct

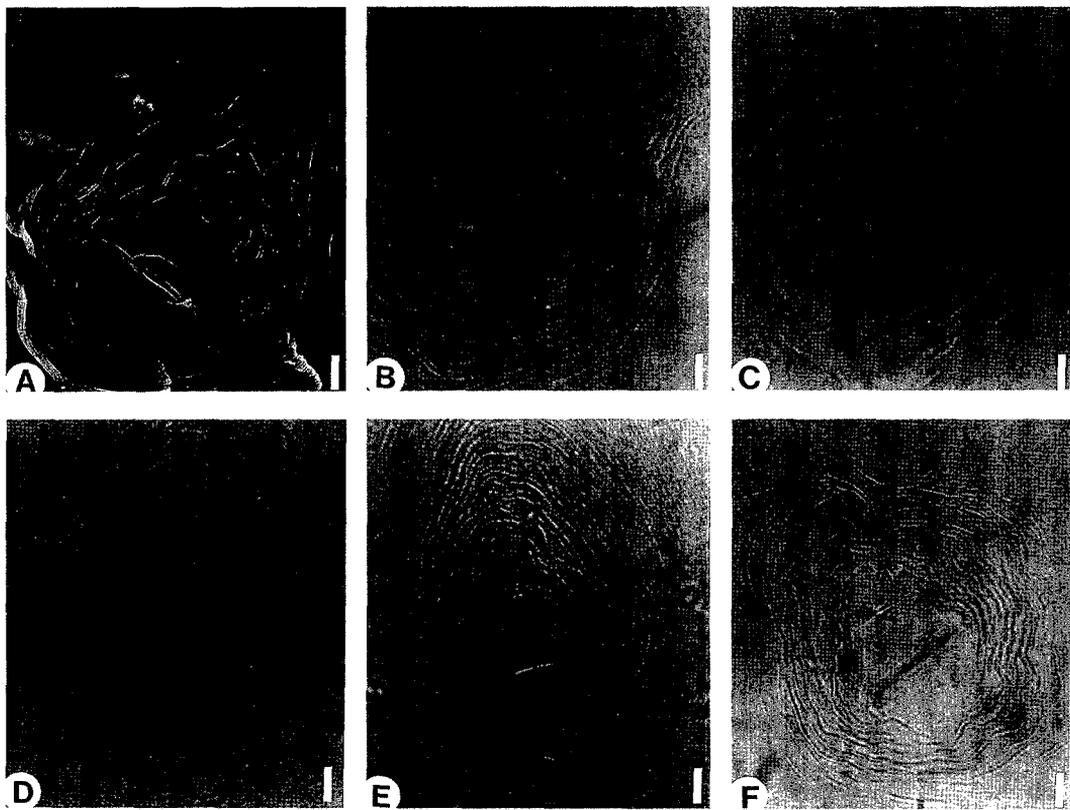


FIG. 3. Photographs of *Meloidogyne paranaensis* n. sp. perineal patterns. A) Scanning electron microscopic photograph. B-F) Light microscopy photographs. Bars = 10 μ m.

cuticular depressions on medial lips (Fig. 5B). Amphidial apertures elongate slits between labial disc and lateral sectors of head region (Fig. 5H). Hemizonid distinct, three or four annules anterior to excretory pore (Fig. 4A). Body annules large, distinct. Ar-eolated lateral field beginning near level of stylet base, usually with four incisures (Fig. 5I). Most males sex reversed with two testes, some normal with one testis. Testis(es) outstretched or distally reflexed. Spicules arcuate, gubernaculum distinct. Tail short, phasmids at level of cloaca (Figs. 4D,E;5J).

Second-stage juveniles: Measurements of 30 J2 (in formalin) presented in Table 3. Body vermiform, tapering more posteriorly than anteriorly, tail region distinctly narrowing. Body annules distinct, increasing in size and becoming irregular in posterior tail region. Lateral field with four incisures. In LM, cephalic framework weak, hexaradiate. Vestibule and vestibule

extension distinct (Fig. 4F). In SEM, stoma slit-like, located in oval prestomatal cavity, surrounded by pit-like openings of six inner labial sensilla. Labial disc and medial lips fused, forming a dumbbell-shaped structure. Labial disc rounded, slightly elevated above medial lips (Fig. 6A-C). Lateral lip sectors distinct, sometimes fused with head region and labial disc at right angle. Head region smooth, frequently with short broken annulations. Amphid openings slit-like, located between labial disc and lateral lips, often covered by exudate (Fig. 6A-C). Stylet 13-14 μ m long, delicate (Fig. 6D). Stylet cone increasing in width gradually, shaft cylindrical, knobs rounded and set off from shaft (Figs. 4F;6D,F). Distance of DGO to stylet base 4.0-4.5 μ m, orifice branched into channels. Median bulb oval. Esophago-intestinal junction obscure. Gland lobe overlapping intestine ventrally, with three

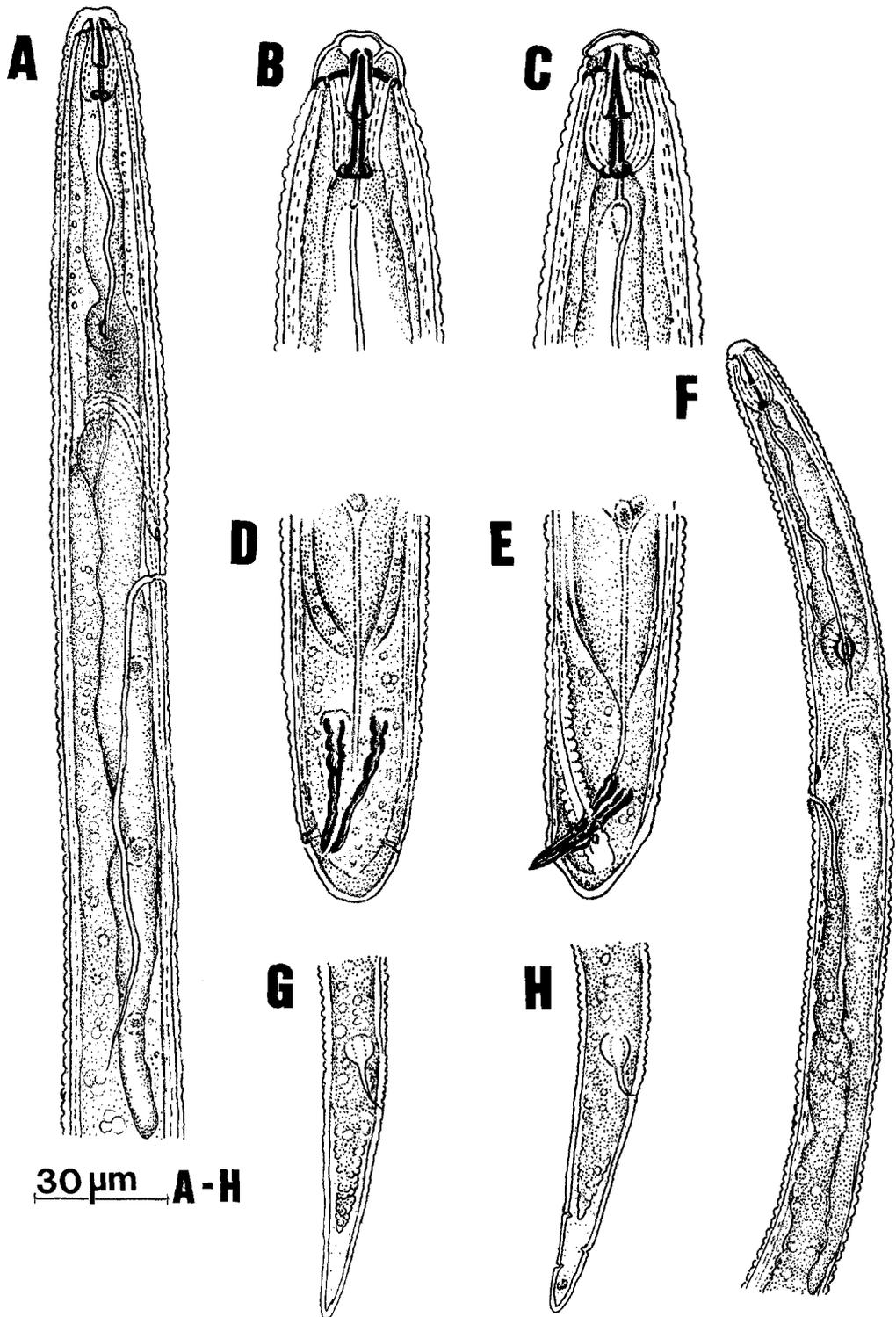


FIG. 4. Drawings of males and second-stage juveniles of *Meloidogyne paranaensis* n. sp. A) Anterior portion of male. B) Male cephalic region, dorsal view. C) Male cephalic region, lateral view. D) Male tail, ventral view. E) Male tail, lateral view. F) Anterior portion of second-stage juvenile, lateral view. G, H) Second-stage juvenile tail, lateral view.

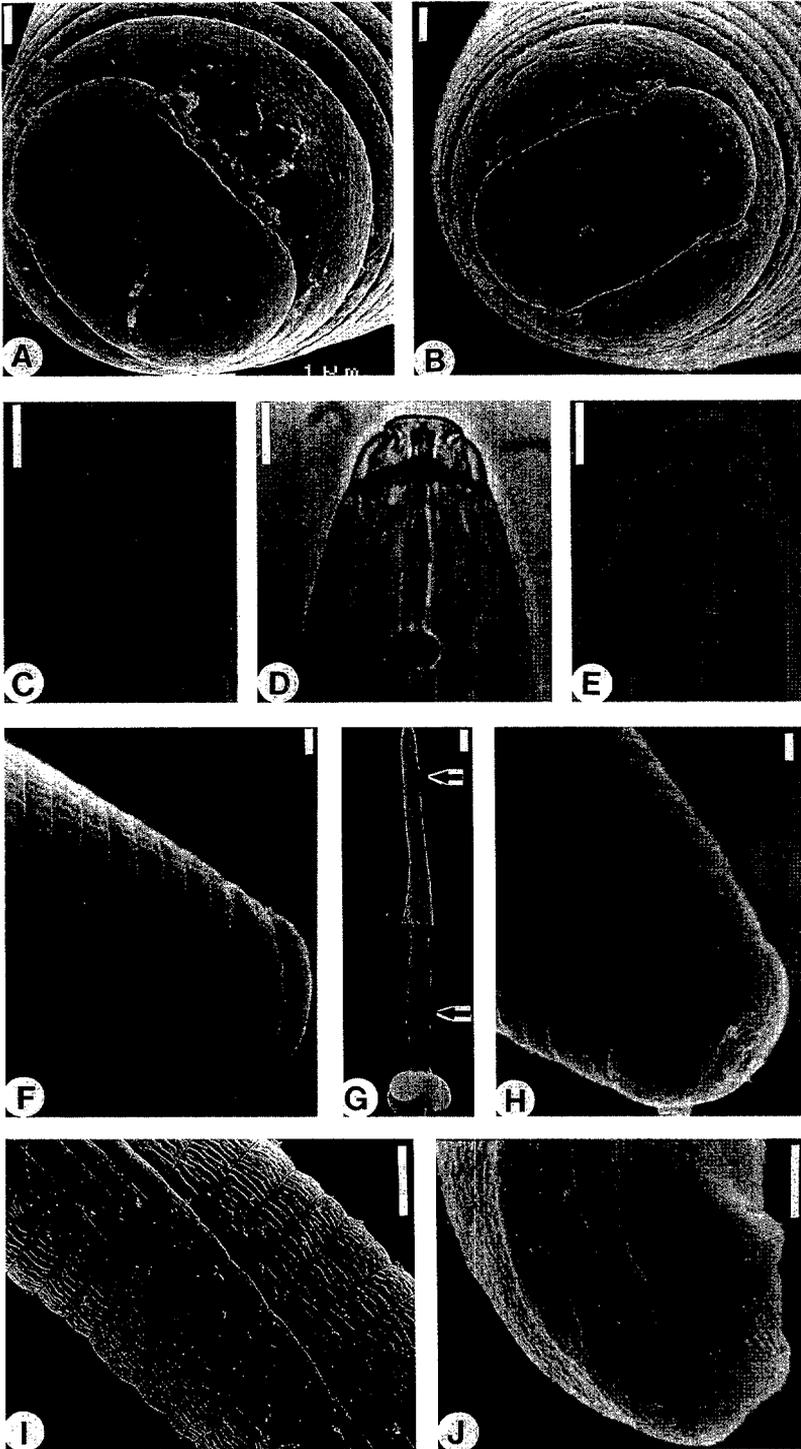


FIG. 5. Photographs of *Meloidogyne paranaensis* n. sp. males. A,B) Scanning electron microscopic (SEM) of cephalic region, face view. Arrow indicates cephalic sensillum. C–E) Light microscopic (LM) of anterior region. Arrows indicate shaft projections. F) SEM of cephalic region, lateral view. G) SEM of excised stylets. Top arrow indicates stylet opening, lower arrow indicates stylet projection. H) SEM of cephalic region, lateral view. I) SEM of lateral field. J) SEM of tail. Arrow indicates phasmid. Bars in: A,B,F,G, and H = 1 μ m; C,D,E,I, and J = 5 μ m.

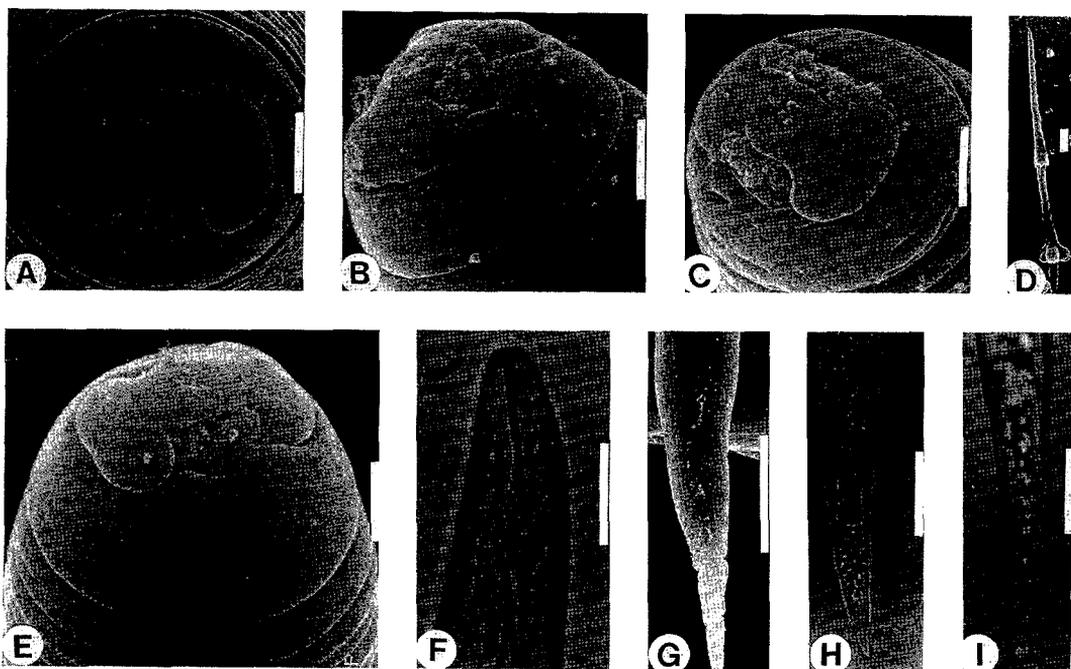


FIG. 6. Photographs of second-stage juveniles of *Meloidogyne paranaensis* n. sp. A–C) Scanning electron microscopic (SEM) photographs of anterior end, face view. D) SEM of excised stylet. E) SEM photographs of anterior end, face view. F) Light microscopic photographs (LM) of cephalic region, lateral view. G) SEM of tail. H, I) LM of tail. Bars in: A, B, C, D, and E = 1 μ m; F, G, H, and I = 10 μ m.

nuclei; hemizonid 1–2 annules anterior to excretory pore (Fig. 4F). Tail usually conoid with rounded terminus. Hyaline tail terminus distinct (Fig. 6H, I). Rectal dilatation large (Fig. 4G, H). Phasmids small, posterior to anus.

Eggs (in 2% formalin): Measurements of 30 eggs. Length: 82–106 μ m (mean 90.5), standard error of mean (SE) 0.82, standard deviation (SD) 5.32, coefficient of variability (CV) 5.6%; width: 37–51 μ m (mean 43.3, SE 0.82, SD 4.6, CV 9%); length/width ratio: 2.08–2.22 (mean 2.09, SE 0.02, SD 0.18, CV 6%). Egg morphology similar to that of other *Meloidogyne* spp.

Biological studies

Biochemistry: Characteristic esterase phenotype with one fast migrating band, F₁, and one malate-dehydrogenase phenotype, N₁.

Differential host test: Populations of *M. paranaensis* n. sp. reproduced on tobacco,

watermelon, and tomato. No reproduction occurred on cotton, pepper, and peanut.

Cytogenetics: The reproduction of *M. paranaensis* n. sp. is by mitotic parthenogenesis, karyotype $3n = 50-52$.

Type host and locality

Isolated from roots of tomato (*Lycopersicon esculentum* Mill. cv. Santa Cruz), greenhouse cultures. The isolate originated from a coffee plantation, Paranaivai, Paraná State, Brazil.

Type specimens

Holotype (female): Slide number TLQ 1996a1, and *allotype male*, slide number TLQ 1996a2, deposited in the Escola Superior de Agricultura “Luiz de Queiroz,” Departamento de Zoologia, Piracicaba, São Paulo, Brazil.

Paratypes (females, males, and second-stage juveniles): Deposited in the U.S. Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland, USA;

TABLE 1. Morphometric data of 30 females of *Meloidogyne paranaensis* n. sp.

Character	Range (μm)	Mean \pm std. errors (μm)	Standard deviation	Coefficient of variability
Body length	512-780	681 \pm 12.5	66.5	9.8
Body width	320-532	428 \pm 11.7	61.8	9.8
Neck length	140-284	212 \pm 6.36	33.7	15.9
Neck width	80-270	123 \pm 4.9	26.0	21.2
Body length without neck	340-792	481 \pm 17.1	90.6	18.8
Stylet length	15.0-17.5	16.1 \pm 0.1	0.6	3.9
Stylet knob height	2.0-3.0	2.1 \pm 0.1	0.1	12.7
Stylet knob width	4.5-6.5	5.5 \pm 0.1	0.5	8.7
Dorsal esophageal gland orifice (DGO) to stylet bases	4.2-5.5	5.0 \pm 0.1	0.3	4.9
Head end to posterior end of metacarpus	84-167	133.3 \pm 4.5	23.9	17.9
Metacarpus length	38-70	65.9 \pm 2.2	11.7	17.7
Metacarpus width	30-85	54.1 \pm 2.2	11.4	21.1
Metacarpus valve length	13.5-26.0	19.3 \pm 0.3	1.5	7.9
Metacarpus valve width	10-16	13.4 \pm 0.3	1.5	11.3
Excretory pore to head end	30-73	56.2 \pm 1.9	0.9	17.7
Vulva slit length	20-37	25.9 \pm 0.6	3.4	13.1
Anus to vulva (center) distance	15-25	20.8 \pm 0.5	2.6	12.3
Interphasmidial distance	18.1-29.6	23.2 \pm 0.4	2.5	10.8
Ratios				
Body length/body width	1.1-2.1	1.6 \pm 0.01	0.2	12.7
Body length/head end to posterior end of metacarpus	3.3-8.4	5.3 \pm 0.03	1.2	23.2
Stylet knob width/height	2.0-3.0	2.5 \pm 0.05	0.3	10.7

TABLE 2. Morphometric data of 30 males of *Meloidogyne paranaensis* n. sp.

Character	Range (μm)	Mean \pm std. error (μm)	Standard deviation	Coefficient of variability
Body length	983–2,284	1,868 \pm 52.8	284.7	15.2
Greatest body width	31–46	40.3 \pm 0.7	3.6	8.8
Body width at stylet knob	17.0–18.5	17.7 \pm 0.1	0.9	3.5
Body width at excretory pore	26–37	31.4 \pm 0.4	2.18	6.9
Stylet length	20–27	24.7 \pm 0.6	1.25	5.0
Stylet knob width	4.5–7.0	5.8 \pm 0.06	0.32	5.5
Stylet knob height	2.0–4.5	3.8 \pm 0.06	0.33	8.7
Dorsal esophageal gland orifice (DGO) to stylet base	3.5–5.0	4.6 \pm 0.1	0.5	11.0
Head end to metacarpus valve	82–107	98 \pm 1.1	5.8	5.9
Metacarpus width	8–15	11.9 \pm 0.3	1.4	11.9
Head end to excretory pore	130–205	176 \pm 3.1	16.5	9.4
Testis length	550–980	811 \pm 22.0	119	19.6
Tail length	12–23	16.6 \pm 0.6	3.0	18.3
Spicule length	22–35	26 \pm 0.5	2.9	8.2
Ratios				
Body length/body width	23.4–53.5	46.4 \pm 1.2	6.4	13.9
Body length/tail length	58–154	116 \pm 4.4	23.9	20.7
Stylet knob width/height	1.3–1.8	1.5 \pm 0.2	0.1	6.1
Excretory pore (%)	8.1–16.9	9.6 \pm 0.3	1.6	17.0

TABLE 3. Morphometric data of 30 second-stage juveniles of *Meloidogyne paranaensis* n. sp.

Character	Range (μm)	Mean \pm std. error (μm)	Standard deviation	Coefficient of variability
Body length	389–513	458 \pm 5.1	27.9	6.1
Greatest body width	15–20	15.9 \pm 0.2	1.1	6.6
Stylet length	13–14	13.5 \pm 0.2	0.9	5.3
Stylet knob height	1.0–1.5	1.3 \pm 0.01	0.2	17.9
Stylet knob width	2.0–2.5	2.1 \pm 0.01	0.2	9.7
Stylet base to head end	14–16	14.9 \pm 0.06	0.3	2.3
Dorsal esophageal gland orifice (DGO) to stylet base	4.0–4.5	4.2 \pm 0.04	0.3	5.9
Head end to metacarpus valve	53–67	58.7 \pm 0.56	3.1	5.3
Excretory pore to head end	85–98	92.1 \pm 0.6	3.2	3.5
Body width at anus	8.5–11.0	10.0 \pm 0.1	0.7	6.7
Tail length	48–51	49.0 \pm 0.15	0.8	1.7
Tail terminus length	9–10	10.1 \pm 0.1	0.7	7.3
Ratios				
Body length/body width	24.6–31.7	28.7 \pm 0.35	1.9	6.6
Body length/head end to metacarpus valve	6.6–8.9	7.8 \pm 0.1	0.5	6.5
Body length/tail length	7.6–10.7	9.3 \pm 0.1	0.6	6.7
Tail length/body width at anus	4.6–6.0	5.3 \pm 0.1	0.4	7.8
Excretory pore (%)	18.8–23.9	20.2 \pm 0.2	1.1	5.4

Museu e Laboratório Zoológico, Nematode Collection, Universidade de Coimbra, Coimbra, Portugal; Nematology Department, Rothamsted Experimental Station, Nematode Collection, Harpenden, Herts, U.K.; and University of Agriculture, Department of Nematology, Wageningen, The Netherlands.

Diagnosis

Meloidogyne paranaensis n. sp. can be distinguished from other species in the genus by combinations of the following characteristics. Females with labial disc and medial lips fused, asymmetric and rectangular; stylet 15.0–17.5 μm long, with broad, distinctly set off knobs; distance from the DGO to stylet base 4.2–5.5 μm ; perineal pattern similar to that of *M. incognita*. Males with high, round head cap continuous with the body contour; labial disc fused with the medial lips to form an elongate lip structure; head region frequently marked by an incomplete annulation; stylet robust, 20–27 μm long, usually with rounded to transversely elongate knobs, sometimes with one or two projections protruding from the shaft. Second-stage juveniles with stylet 13–14 μm long, distance of the DGO to the stylet base 4.0–4.5 μm , and the tail length 48–51 μm long. Esterase pattern (F_1) is the most useful character for differentiating this new species from other species in coffee plantation surveys in Brazil (4).

Relationships

Meloidogyne paranaensis n. sp. is most similar to *M. konaensis* (9) but differs from it in several morphological features. Females of *M. paranaensis* n. sp. have labial disc and medial lips fused, asymmetric and rectangular, forming straight lateral edges; in *M. konaensis* the labial disc is often rectangular and fused with medial lips to form a medial lip divided into distinct lip pairs (9). Males of *M. paranaensis* n. sp. differ from males of *M. konaensis* in body length (983–2,284 vs. 1,149–1,872 μm), stylet length (20–27 vs. 20–24 μm), stylet knob height (2.0–4.5 vs. 3.4–4.2 μm), stylet knobs

width (4.5–7.0 vs. 3.4–5.0 μm), head end to excretory pore (130–205 vs. 134–178 μm), and DGO to stylet base (3.5–5.0 vs. 5.9–8.4 μ). Male head cap of the two species are similar, but the medial lip of *M. konaensis* is often divided into distinct medial lip pairs (9). Male stylets of the two species are also different: *M. paranaensis* n. sp. has stylet knobs transversely elongate, broad and set off from the shaft, sometimes with one or two large projections surrounding the shaft, whereas *M. konaensis* has knobs not set off, backward sloping, merging with shaft, 6–12 large projections surrounding the shaft. The second-stage juveniles of *M. paranaensis* n. sp. differ from *M. konaensis* in body length (389–513 vs. 468–530 μm), stylet base to head end (14–16 vs. 17–19 μ), DGO to stylet base (4.0–4.5 vs. 4.2–5.9 μm), head end to metacarpus valve (53–67 vs. 65–75 μm), excretory pore to head end (85–98 vs. 89–111 μm), and tail length (48–51 vs. 49–73 μm).

Meloidogyne paranaensis n. sp. is distinct from all other described species in the genus, including *M. incognita* with which it was previously confused; however, these earlier comparisons were based only on observations of perineal patterns. *Meloidogyne paranaensis* n. sp. has a characteristic esterase phenotype (one fast migrating band, F_1), which is different from *M. incognita* (one slow band, I_1) but identical to that of *M. konaensis* and *M. querciana*; however, *M. paranaensis* can be differentiated biochemically from the latter by the MDH pattern, N_1 (10). No MDH pattern was reported for *M. konaensis*. *Meloidogyne paranaensis* n. sp. has the same differential host response as *M. javanica*.

DISCUSSION

Meloidogyne paranaensis n. sp. causes field symptoms on coffee plants such as splitting and cracking of the cortical root tissue, especially on the taproot, but it does not produce typical root-knot nematode galls on coffee. Necrotic spots occur along the roots where the females are located. Nematode feeding causes the tissue

around the giant cells to die. Symptoms on infected plants include foliar chlorosis, leaf drop, general decline, reduced growth, and often plant death. In Brazil (Paraná and São Paulo States), large coffee plantations have been damaged severely by this nematode. It is likely that *M. paranaensis* n. sp. is the same as other Brazilian populations studied by Janati et al. (12) and Esbenshade and Triantaphyllou (10). These authors (10,12) reported chromosome numbers of 50–56 from several root-knot nematode populations on coffee from various places in Brazil, Peru, and Surinam.

Currently, identification of the most agronomically important root-knot nematodes species in Brazil is made by comparison of perineal patterns (1–3,13) and by differential host tests (3). Using these two procedures, *M. paranaensis* n. sp. has been incorrectly identified as *M. incognita* (1,3, 13) for the past 22 years. Morphometric data or morphological characters observed on LM or SEM for different stages, especially for males, are not practical characters to identify species in field surveys, principally because of the lack of males and the common occurrence of mixed species in the field (1). Biochemical studies have demonstrated that major species of *Meloidogyne* can be differentiated by species-specific enzyme phenotypes (10,12), even in field surveys (4). Because of the esterase phenotype similarity (F_1) between *M. paranaensis* n. sp. and *M. konaensis*, further biochemical studies are necessary to differentiate these two species and to develop a useful method to be used in coffee plantation surveys.

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