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Description of the Kona Coffee Root-knot Nematode, *Meloidogyne konaensis* n. sp.

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Abstract: *Meloidogyne konaensis* n. sp. is described from coffee from Kona on the island of Hawaii. The perineal pattern of the female is variable in morphology, the medial lips of the female are divided into distinct lip pairs, and the excretory pore is 2-3 stylet lengths from the base of the stylet. Mean stylet length is 16.0 μm , and the knobs gradually merge with the shaft. The knobs are indented anteriorly and rounded posteriorly and the dorsal esophageal gland orifice (DEGO) is long, 3.5-7 μm . The morphology of the stylet of the male is the most useful diagnostic character, with 6-12 large projections protruding from the shaft. One medial lip may be divided into distinct lip pairs. A large intestinal caecum often extends nearly to the level of the DEGO. Mean juvenile length is 502 μm , mean stylet length is 13.4 μm , and mean tail length is 58 μm . The tail may be distinctly curved ventrally and the phasmids are located in the ventral incisure about one anal body width posterior to the anus.

Key words: host range, *Meloidogyne* species, morphology, nematode, scanning electron microscopy, taxonomy.

Root-knot nematodes have been a problem on coffee in the Kona area on the island of Hawaii. Coffee trees have died in some commercial plantings. The infected and dead plants were removed from the Kona Experiment Station and replanted with rootstocks to determine if any were resistant to the species. In 1991 samples were collected to determine the identity of the species. The perineal patterns varied from those of known species. One esterase band was similar to the fast band found in several other populations from Brazil, Peru, and Surinam. Dr. Hedwig Hirschmann (pers. comm.) confirmed the unique morphology of this nematode. Dr. A. C. Triantaphyllou (pers. comm.) demonstrated that it reproduced by mitotic parthenogenesis and had $2n = 44$ chromosomes.

This nematode was detected at the Kona

Experiment Station and four privately owned farms. It has not been found in any other crops or in forested areas adjacent to coffee plantings. This species was not present in surveys of coffee plantations throughout the Hawaiian Islands. It is described herein as *Meloidogyne konaensis* n. sp. Because the nematode was discovered in the Kona area of Hawaii, and only on coffee, the common name Kona coffee root-knot nematode is suggested.

MATERIALS AND METHODS

Cultures of *M. konaensis* n. sp. were reared on tomato (*Lycopersicon esculentum* L. 'Rutgers') maintained in a greenhouse at 22-28 C. All morphologic and morphometric studies were made from these cultures. Males were obtained by incubating pieces of washed infected roots in a moist chamber, and freshly hatched second-stage juveniles were collected from egg masses incubated in a hatching chamber. Females were isolated from plant tissues with sharp forceps and placed in 0.89% saline for further preparation.

Temporary mounts of specimens that

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were gently heat-killed in water and mounted in 2% formalin were examined by light microscope (LM). Live specimens mounted in 0.89% saline were always compared to fixed material. Males and second-stage juveniles were prepared for scanning electron microscopy (SEM) (2,3), and females were prepared for LM and SEM examinations (4). Eggs were mounted in 2% formalin. All LM observations were made within 4 hours after processing, and photographs were taken with a camera mounted on a bright field microscope. At least 100 specimens of each stage were examined by LM and SEM. Type specimens were prepared as previously described (1). Drawings were made from glycerin-mounted specimens, which were compared to living and heat-relaxed nematodes.

SYSTEMATICS

Meloidogyne konaensis n. sp.
(Figs. 1–7)

Females: Measurements of 30 females are listed in Table 1. Measurements of holotype in glycerin: Body length, 990 μm ; body width, 470 μm ; neck length, 380 μm ; neck width, 110 μm ; stylet length, 16 μm ; stylet knob height, 2.8 μm ; dorsal gland orifice to stylet base, 5.0 μm ; excretory pore to head end, 47 μm .

Description (Figs. 1–4): Body translucent-white, variable in size, pear-shaped, sometimes with elongate neck 0.5–1 times body length minus neck. Neck prominent, sometimes bent at various angles to body. In LM, cephalic framework weak, hexaradiate, lateral sectors slightly enlarged, vestibule and extension prominent (Figs. 1A,2A). Excretory pore 2–3 stylet lengths posterior to stylet base. Stylet strong; cone slightly curved dorsally; shaft enlarged posteriorly; three large knobs tapering onto shaft (Figs. 1A–B,2A–B). Distance of dorsal esophageal gland orifice to stylet base approximately one shaft-length; orifice branched into three channels; ampulla large. Subventral gland orifices branched;

located immediately posterior to enlarged lumen of median bulb. Vesicles present on lumen lining in anterior portion 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, esophago-intestinal cells with nuclei attached dorsally to median bulb. In SEM (Fig. 2C–D), stoma slit-like, located in ovoid prestomatal cavity, surrounded by pit-like openings of six inner labial sensilla. Labial disc often rectangular, fused with medial lips. Medial lips divided into distinct lip pairs; lateral lips large and triangular; head annule without annulation.

Perineal patterns (Figs. 1C,3) variable in shape, striae coarse, sometimes continuous, smooth to wavy. Dorsal arch rounded to squarish. Perivulval region free of striae. Phasmids small, directly on either side and posterior to anus; surface structure not apparent in SEM (Fig. 3E–F).

Males: Measurements of 30 males are listed in Table 2. Measurements of allotype in glycerin: Body length, 1,500 μm ; body width, 37 μm ; stylet length, 22 μm ; stylet knob height, 3.7 μm ; stylet knob width, 4.6 μm ; dorsal esophageal gland orifice to stylet base, 6.9 μm ; esophagus length (center of median bulb to head end), 98 μm ; excretory pore to head end, 155 μm ; body width at stylet base, 19.8 μm ; body width at excretory pore, 24.6 μm ; tail length, 12 μm ; spicule length, 26.9 μm .

Description (Figs. 4B–L,5): Body vermiform; tapering anteriorly; bluntly rounded posteriorly; tail arcuate, twisting through 90°. Head cap high, rounded, tapering posteriorly; head region distinct from first body annule, usually without annulations. In LM, cephalic framework moderately developed, hexaradiate, lateral sectors slightly enlarged (Figs. 4,5). Vestibule and extension distinct. Stylet morphology distinct (Figs. 4G,5A–B). Stylet opening several microns from stylet tip; cone pointed, gradually increasing in diameter posteriorly, junction of cone and shaft uneven (Fig. 4G). Shaft cylindrical

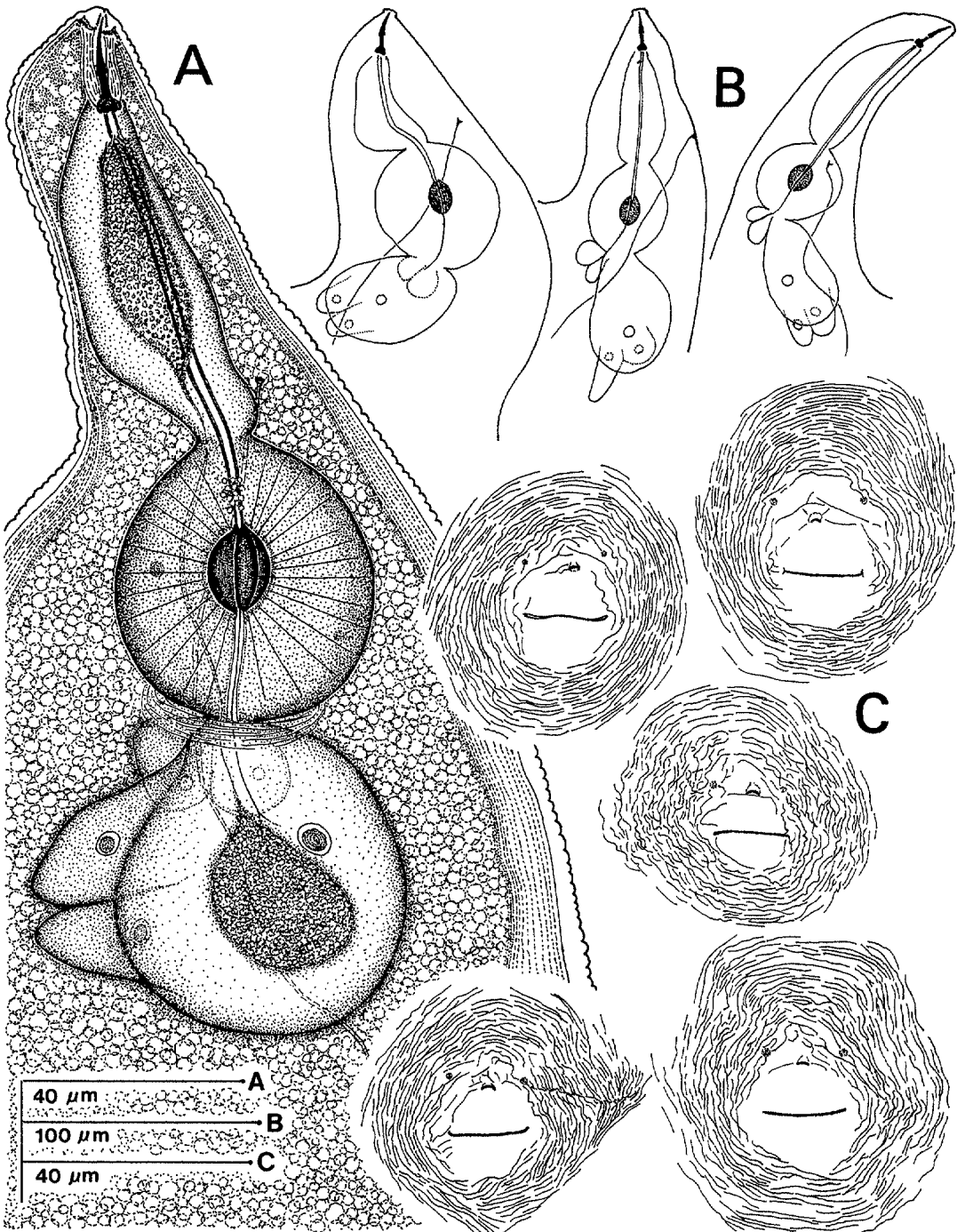


FIG. 1. (A-C) Drawings of females of *Meloidogyne konaensis* n. sp. A) Anterior portion. B) Anterior regions showing esophageal and excretory canals. C) Perineal patterns.

often slightly wider near middle with numerous large projections (Fig. 4G,5B). Knobs broadly elongate, tapering onto shaft, indented slightly anteriorly, rounded

posteriorly. Distance of dorsal esophageal gland orifice to stylet base long (6-8 μm), orifice branched into three channels, ampulla distinct. Amphids very distinct, often

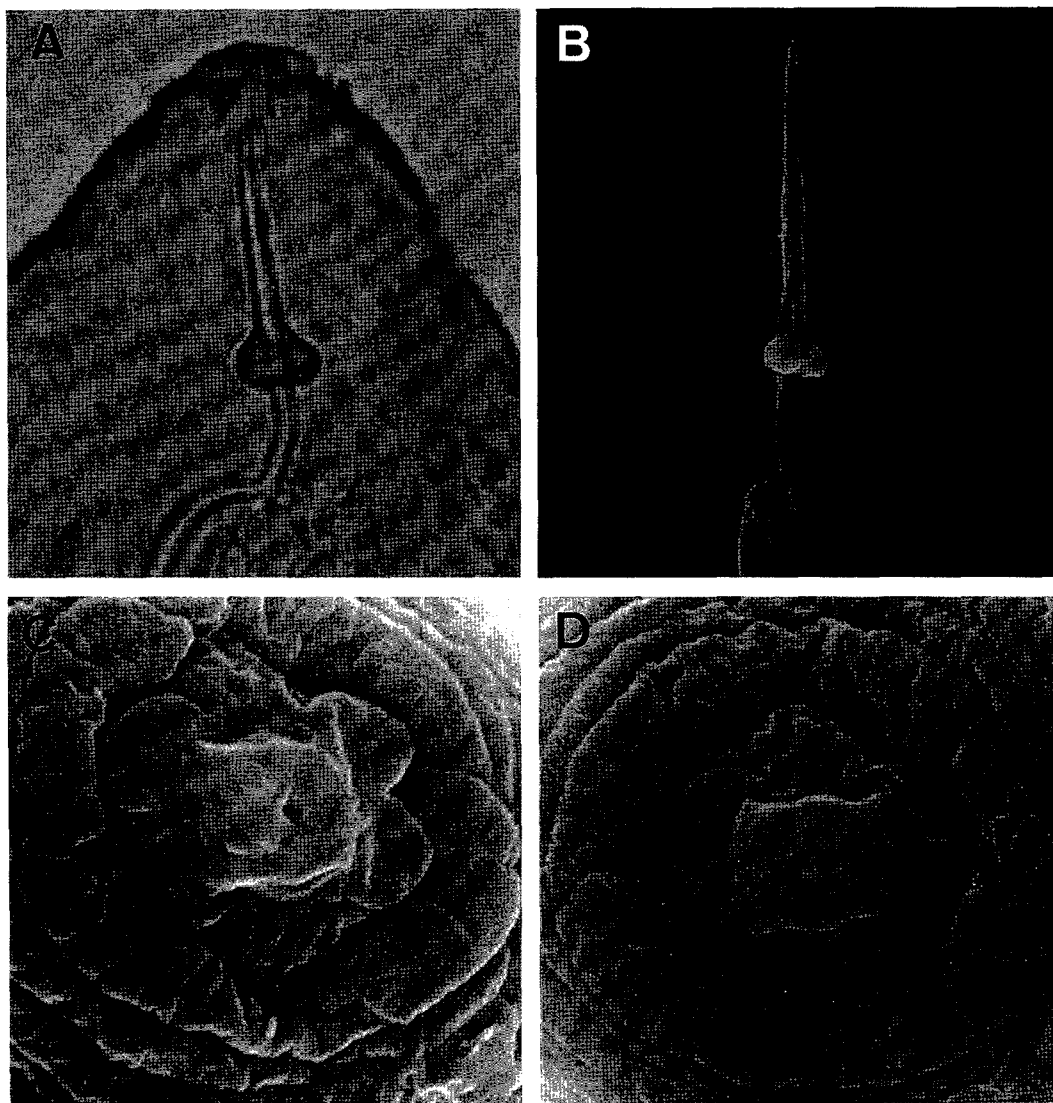


FIG. 2. (A–D) Photographs of females of *Meloidogyne konaensis* n. sp. A) LM of anterior portion with stylet (lateral). B) SEM photograph of excised stylet. C–D) SEM of face views.

producing exudates (Fig. 4E–F). 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. Three nuclei in gland lobe; lobe variable in length. Intestinal caecum extending anteriorly sometimes approaching level of dorsal esophageal gland orifice, forcing anterior esophageal ventrad. Excretory pore distinct,

6–10 annules behind hemizonid. Areolated lateral field beginning near level of stylet base usually with four incisures, in some areas one additional central incisure present (Fig. 4H). Usually two testes (sex reversed males), sometimes one testis (normal males); outstretched or anteriorly reflexed. Spicules arcuate; gubernaculum distinct. Tail short, distinct phasmids at level of cloaca.

In SEM, stoma slitlike, located in ovoid to hexagonal prestomatal cavity surround-

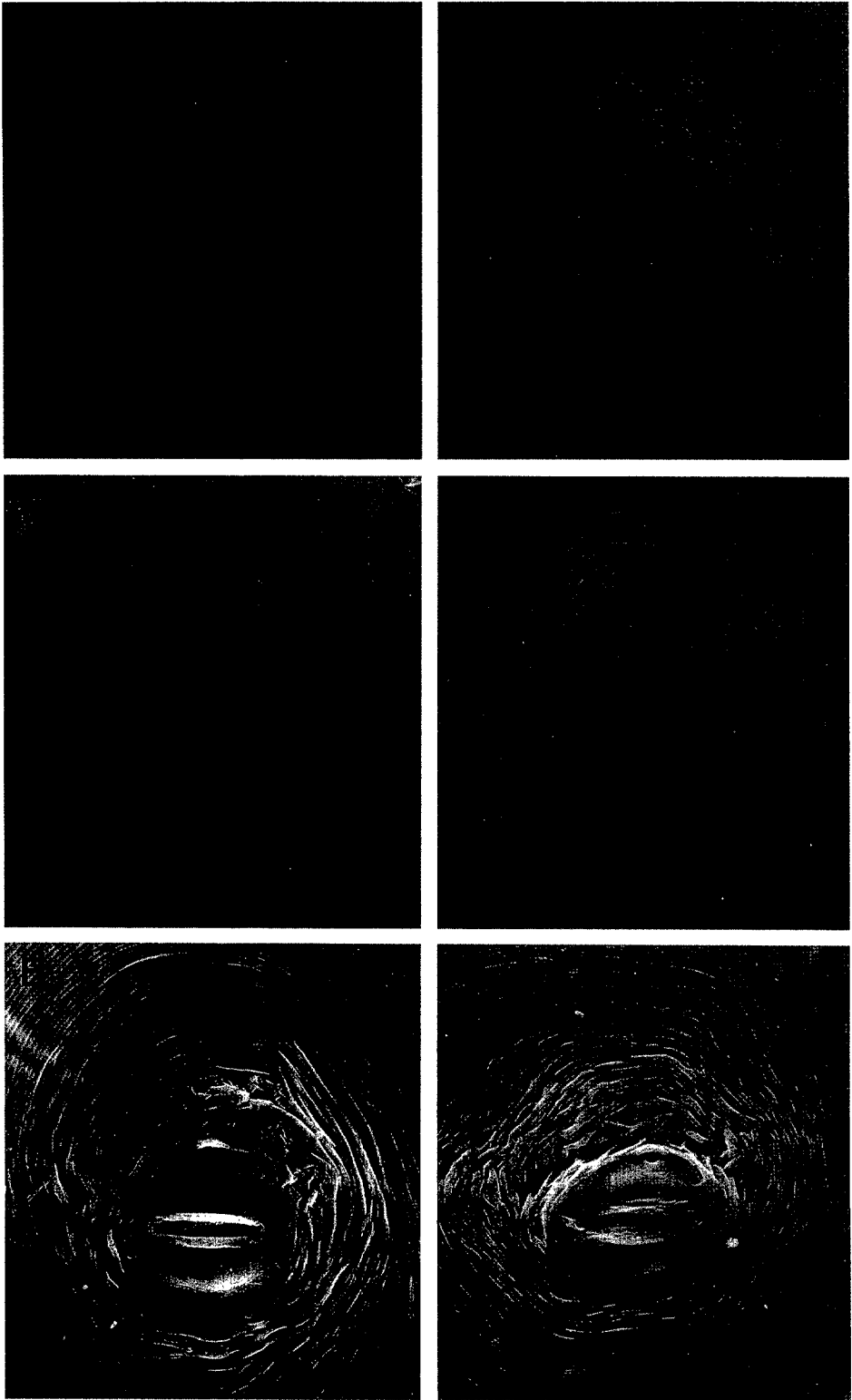


FIG. 3. (A-F) Perineal patterns of *Meloidogyne konaensis* n. sp. A-D) LM photographs showing variation typical for the species. E-F) SEM photographs.

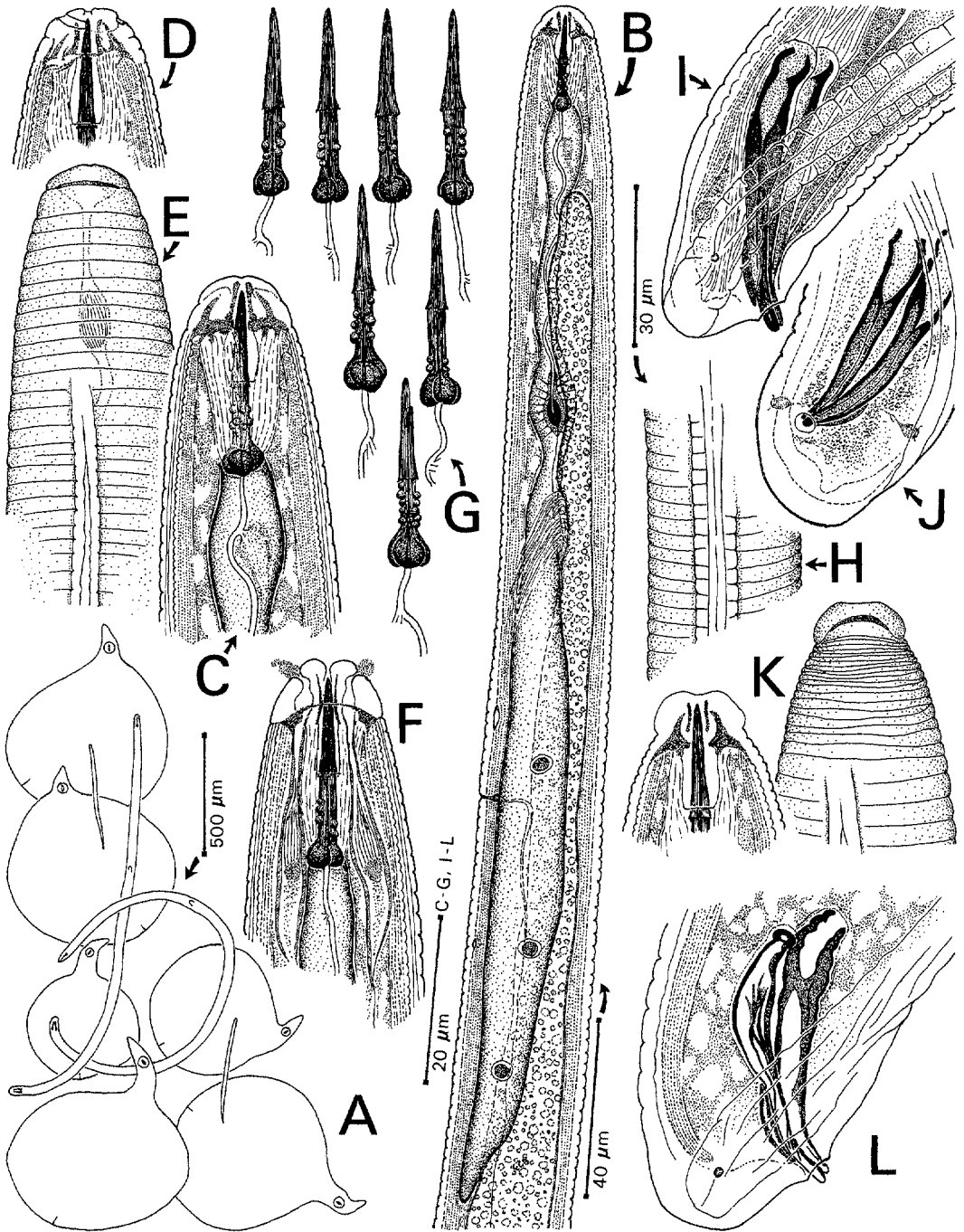


FIG. 4. (A-L) Drawings of females, males, and second-stage juveniles of *Meloidogyne konaensis* n. sp. A) Outlines of entire body of six females, two males, and two second-stage juveniles, lateral. B-F) Anterior ends of males; B-C, E, lateral; D, F, dorsal; E showing external markings and internal amphid. G) Stylets of seven males. H) Lateral field of male anterior to spicules. I) Male tail, lateral. J) Male tail turned ventrally to show phasmids. K) Abberant head region (internal and external, respectively) of sex-reversed male. L) Aberrant tail of sex-reversed male, lateral.

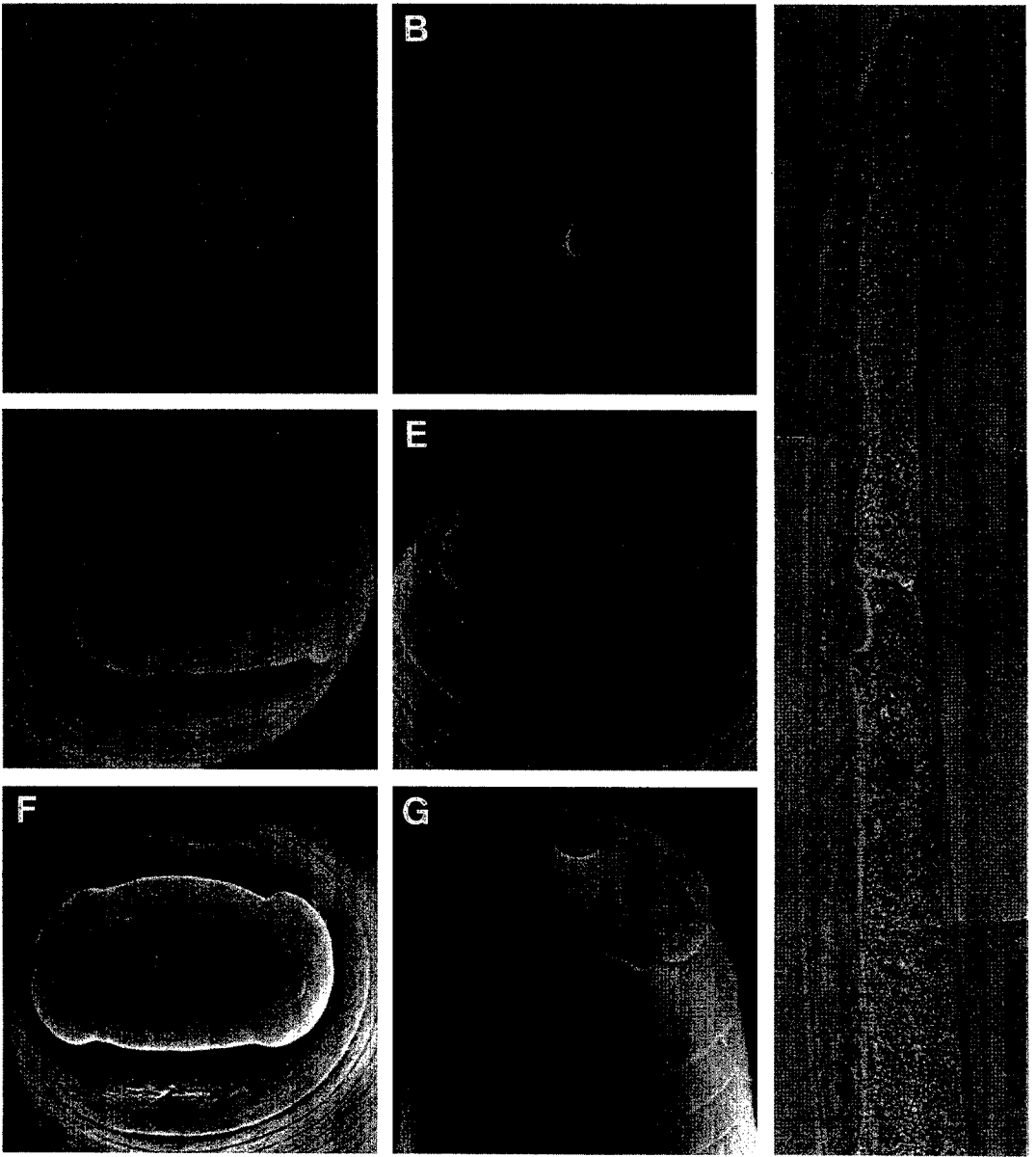


FIG. 5. (A–G) Photographs of males of *Meloidogyne konaensis* n. sp. A) LM of anterior end. B) SEM of excised stylet. C) LM composite of esophageal region. D–G) SEM of anterior end; D and F, face views; E and G, near lateral views.

ed by pit-like openings of six inner labial sensilla (Fig. 5D–G). Labial disc rounded. Medial lips often divided into lip pairs, fused with labial disc forming elongate head cap. Cephalic sensilla not clearly demarcated on medial lips. Lateral lips absent. Amphidial apertures elongate slits between labial disc and lateral sectors of

head region. Head region not annulated. Body annules distinct.

Second-stage juveniles: Measurements of 30 juveniles are presented in Table 3. Description (Figs. 4,6,7): Body vermiform, tapering more posteriorly than anteriorly. In LM, cephalic framework weak, hexaradiate. Vestibule and vestibule extension

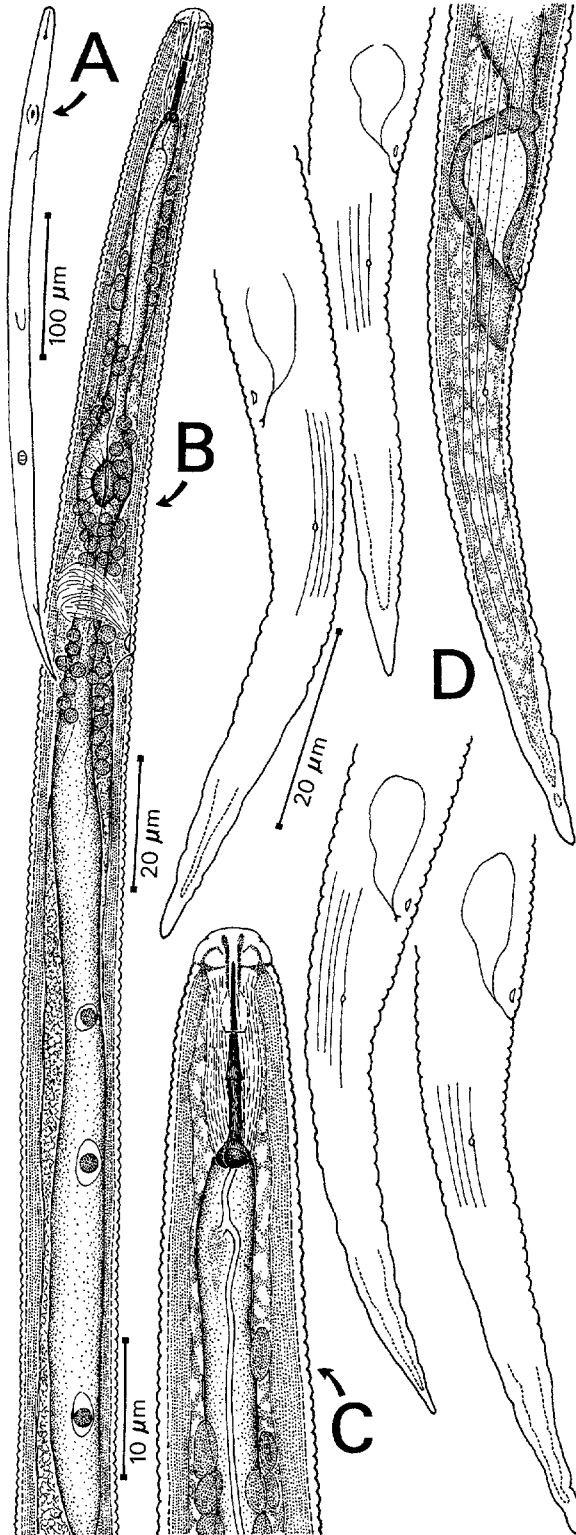


FIG. 6. (A-D) Drawings of second-stage juveniles of *Meloidogyne konaensis* n. sp. A) Outline of body. B-C) Anterior ends, lateral. D) Tails of five specimens, lateral.

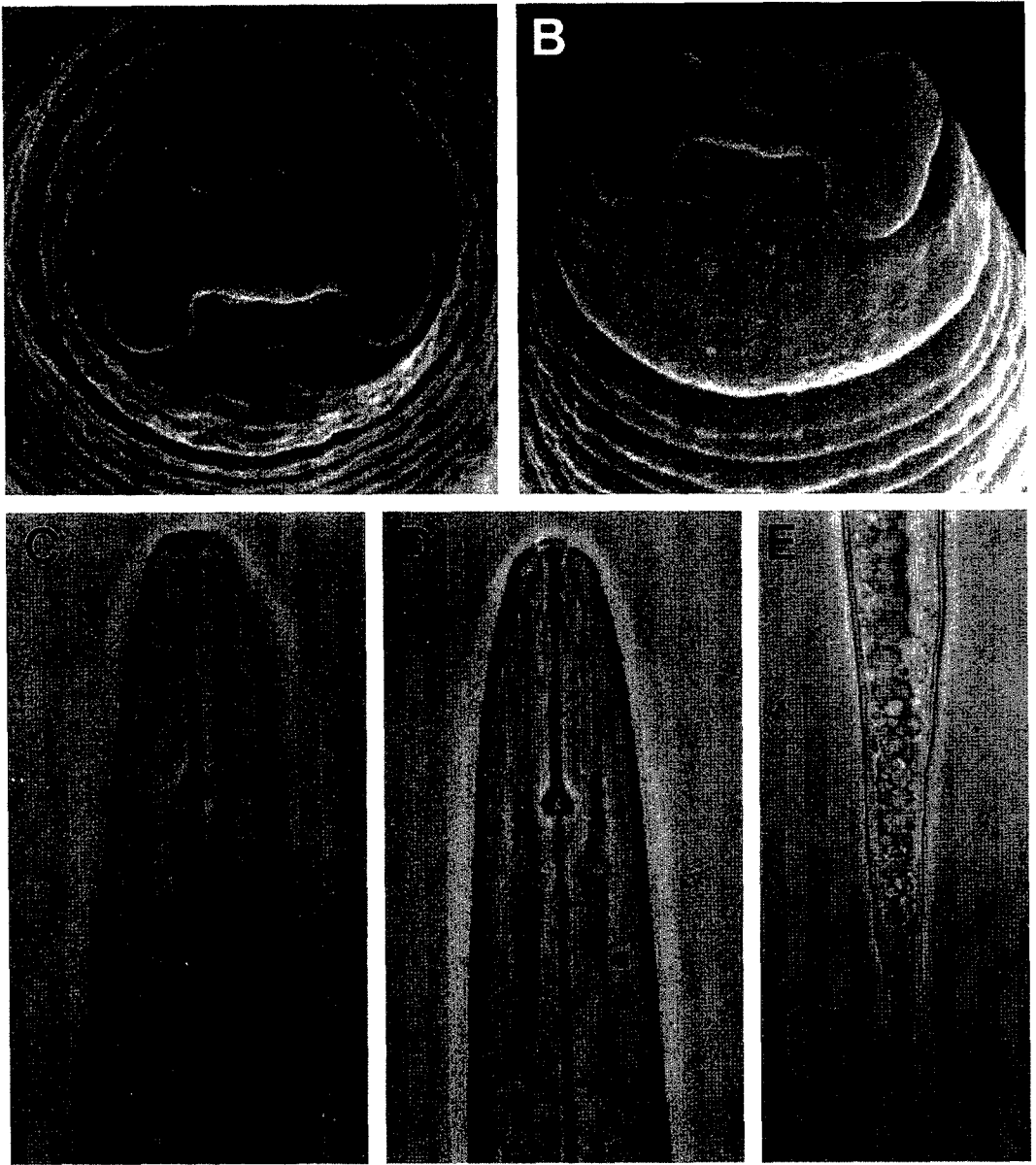


FIG. 7. (A-E) Photographs of second-stage juveniles of *Meloidogyne konaensis* n. sp., A-B) SEM of anterior end, face and lateral views, respectively. C-D) LM of anterior end, lateral and ventral views, respectively. E) Tail, lateral view.

more distinct than rest of framework (Figs. 6B-C, 7C-D). Stylet cone increasing in width gradually, shaft cylindrical to tapering posteriorly, knobs rounded and set off from shaft. Distance of dorsal esophageal gland orifice to stylet base long (4-6 μm); orifice branched into channels; ampulla indistinct. Median bulb 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 2-4 annules anterior to excretory pore. Areolated lateral field with four incisures, beginning as two near base of stylet. Tail often distinctly

TABLE 1. Measurements of 30 females of *Meloidogyne konaensis* n. sp.

Character	Range (μm)	Mean \pm std. error (μm)	Standard deviation	Coefficient of variability
Whole females				
Body length	531.8–1,510.2	991.9 \pm 43.9	240.6	24.3
Body length without neck	319.1–1,084.8	610.5 \pm 34.3	187.9	30.8
Neck length	148.9–978.4	381.4 \pm 36.8	201.4	52.8
Body width	319.5–723.2	470.1 \pm 19.4	106.0	22.6
Body length/body width	1.4–3.8	2.2 \pm 0.1	0.53	24.7
Body length without neck/body width	0.7–2.3	1.31 \pm 0.1	0.4	26.5
Excised female heads				
Stylet length	14.3–18.5	16.0 \pm 0.2	0.9	5.8
Stylet knob height	1.7–3.8	2.8 \pm 0.1	0.5	17.9
Stylet knob width	3.8–5.0	4.4 \pm 0.1	0.3	7.4
Dorsal esophageal gland orifice to stylet base	3.4–6.7	5.0 \pm 0.1	0.8	15.2
Excretory pore to head end	27.2–87.3	47.6 \pm 2.4	13.3	27.9
Perineal patterns				
Vulval length	25.2–35.3	30.5 \pm 0.5	2.5	8.2
Anus to vulva (center) distance	11.8–24.4	18.9 \pm 0.5	2.7	14.2
Interphasmidial distance	19.3–38.6	28.8 \pm 0.9	5.1	17.7

curved ventrally, annulations larger posteriorly. Hyaline tail terminus distinct. Phasmids small, in ventral incisure of lateral field, always posterior to anus.

In SEM, stoma slit-like located in ovoid prestomatal cavity, surrounded by pit-like

openings of six inner labial sensilla (Fig. 7A–B). Labial disc, medial lips, and lateral lips fused into one structure. Labial disc elevated above stoma. Medial lips with rounded margin, cephalic sensilla indistinct. Amphidial apertures between labial

TABLE 2. Measurements of 30 males of *Meloidogyne konaensis* n. sp.

Character	Range (μm)	Mean \pm std. error (μm)	Standard deviation	Coefficient of variability
Linear				
Body length	1,149–1,872	1,522 \pm 30.4	166.7	11.0
Body width	29.1–43.7	37 \pm 0.7	3.7	10.0
Stylet length	20.2–24.4	22.1 \pm 0.2	0.9	4.0
Stylet knob height	3.4–4.2	3.7 \pm 0.1	0.4	11.2
Stylet knob width	3.4–5.0	4.6 \pm 0.1	0.5	10.4
Dorsal esophageal gland orifice to stylet base	5.9–8.4	6.9 \pm 0.1	0.7	10
Esophagus length (center of median bulb to head end)	87.4–105	98.4 \pm 0.8	4.2	4.3
Excretory pore to head end	134.4–178.1	155.3 \pm 2.2	12.2	7.9
Body width at stylet base	16.8–21.8	19.8 \pm 0.2	1.2	6.1
Body width at excretory pore	23.36–25.04	24.6 \pm 0.1	1.2	7.1
Tail length	8.4–13.4	12.0 \pm 0.2	1.9	10.2
Spicule length	21.8–29.4	26.9 \pm 0.4	1.9	7.2
Ratios				
Body length/body width = a	34.5–51.1	41.2 \pm 0.7	3.8	9.2
Stylet length/body width at stylet base	1.0–1.3	1.1 \pm 0.01	0.05	4.6
Stylet knob width/height	1–1.5	1.3 \pm 0.03	0.2	14.6
Percentages				
Excretory pore	8.9–13.7	10.3 \pm 0.2	1.4	13.2

TABLE 3. Measurements of 30 second-stage juveniles of *Meloidogyne konaensis* n. sp.

Character	Range (μm)	Mean ± std. error (μm)	Standard deviation	Coefficient of variability
Linear				
Body length	468–530	502 ± 2.9	15.9	3.2
Body width	16.8–20.6	18.2 ± 0.3	1.4	7.8
Stylet length	12.6–14.3	13.4 ± 0.1	0.6	4.3
Stylet base to head end	16.8–19.3	17.4 ± 0.1	0.7	3.8
Dorsal esophageal gland orifice to stylet base	4.2–5.9	4.6 ± 0.1	0.5	11.4
Esophagus length (center of median bulb to head end)	64.7–75.6	70.7 ± 0.6	3.0	4.3
Excretory pore to head end	89–111	95.6 ± 1.1	5.8	6.1
Tail length	48.7–73.1	58 ± 1.2	6.58	11.3
Body width at anus	9.2–12.6	10.3 ± 0.2	0.9	8.3
Ratios				
Body length/body width = a	20.2–33.2	27.8 ± 0.5	2.9	11.3
Body length/esophagus length = b	6.2–7.4	6.6 ± 0.07	0.4	5.6
Body length/tail length = c	6.1–8.8	8.1 ± 0.1	0.6	7.2
Tail length/tail width at anus = d	4.7–6.9	5.6 ± 0.1	0.6	10.5
Percentages				
Excretory pore	18.7–22.0	20.6 ± 0.2	1.0	4.7

disc and elongate lateral lips may fuse with head region (Fig. 7A). Head region smooth, body annules distinct.

Eggs: Measurements of 30 eggs. Length: 89.4–104.0 μm (mean 96.9 μm ± 0.64 μm. std. error of mean at 95% confidence intervals); width: 41.6–56.2 μm (48.0 ± 0.61); length/width ratio: 1.7–2.4 (2.03 ± 0.03). Egg morphology typical for the genus. Egg masses usually protruding from root tissues, a few sometimes enclosed within root tissues.

Type host and locality

Roots of *Coffea arabica* L. from the Kona Experiment Station at Kealakekua, Hawaii County, Hawaii. Elevation 450 m. Latitude approximately 19°31', longitude approximately 156°53'.

Type specimens

Holotype (female in glycerin): Originally isolated from cultivated coffee collected from type locality June 1991 and propagated on tomato (*Lycopersicon esculentum* L. 'Rutgers'). Slide number T-5006t deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland, USA.

Allotype (male): Same data as holotype.

Slide number T-5007t deposited in the USDANC, Beltsville, Maryland, USA.

Paratypes (females, males, and second-stage juveniles): Same data as holotype. Slide numbers T-4483p through T-4492p deposited in the USDANC, Beltsville, Maryland. University of California Davis Nematode Collection, Davis, California, USA.

Diagnosis

Meloidogyne konaensis 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 tail shape and location of the phasmids of the second-stage juveniles separate this species from all other described root-knot nematodes.

Relationships

The morphology of the female and male of *M. konaensis* is very distinct. In some specimens the body shape of the female is unusual because the neck of some specimens is quite long and often bent at various angles with the body. The perineal pattern is quite variable and similar to that of *M. incognita* (Kofoid & White) Chitwood, 1949 and *M. arenaria* Chitwood,

1949. *Meloidogyne konaensis* n. sp. has been casually and incorrectly identified in the past as *M. incognita*. The morphology of the stylet of the female is similar to that of *M. arenaria*. Unlike *M. arenaria*, the medial lips are divided into distinct lip pairs in *M. konaensis* n. sp.

The most useful character is the stylet morphology of the male. In *M. konaensis* n. sp., the stylet of the male has 6–12 large projections surrounding the shaft; otherwise the stylet is similar to that of *M. arenaria*. The head cap of the male is also similar to that of *M. arenaria*; however, the medial lip is often divided into distinct medial lip pairs in *M. konaensis* n. sp. Males also have a large intestinal caecum that often extends to the dorsal esophageal gland orifice. The amphids are distinct and often produce an amphidial exudate.

The esterase phenotype of *M. konaensis* n. sp. has one fast migrating band, unlike *M. incognita*, which has one slow band, and *M. arenaria*, which has one, two, or three bands, all with a medium migration speed (5).

DISCUSSION

Meloidogyne konaensis n. sp. causes significant damage to cultivated coffee in Hawaii

(F. Zhang and D. P. Schmitt, unpubl.). The cultivar Guatemalan is the most common coffee grown in the Kona area of Hawaii. It was found to be a poor host for two populations of *M. incognita* from Hawaii.

Because the origin of the nematode is unknown, its identification is critical because it was most likely introduced to Hawaii from several other countries in the 19th century. A comparison among the populations parasitizing coffee and having an esterase phenotype with one fast band is necessary to determine the geographical origin of this new species.

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