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Meloidogyne morocciensis n. sp. (Meloidogyninae), a Root-knot Nematode from Morocco[1]

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Abstract: Meloidogyne morocciensis n. sp. is described from specimens parasitic on peach rootstock from Morocco. This species exhibits a combination of morphological characters similar to *M. arenaria, M. incognita,* and *M. javanica.* The perineal pattern of females is oval to squarish with a moderately high to high dorsal arch, and widely spaced, smooth striae; lateral lines are absent. The stylet, 16.5 μm long, has transversely ovoid, set-off knobs. Males have a set-off, annulated head region. The large, rounded labial disc is distinctly demarcated from the crescent-shaped medial lips; lateral lips are absent. The robust stylet, 24.6 μm long, has large, rounded knobs that taper slightly posteriorly. Mean second-stage juvenile (J2) length is 401 μm. The set-off head region has incomplete annulations; the lip structures are dumbbell shaped. The stylet, 12.3 μm long, has rounded knobs that slope posteriorly. The J2 tail, 52.6 μm long, has irregularly sized annules in the posterior region and ends in a bluntly rounded tip. Tomato, tobacco, pepper, and watermelon are good hosts; cotton and peanut are not hosts. *Meloidogyne morocciensis* n. sp. reproduces by mitotic parthenogenesis and has a somatic chromosome number of 47–49. Its esterase phenotype is identical with the three-banded phenotype (A3) of *M. arenaria.*

Key words: host range, light microscopy, *Meloidogyne morocciensis* n. sp., Morocco, morphology, morphometrics, new species, peach rootstock, *Prunus persica,* root-knot nematode, scanning electron microscopy, taxonomy.

During a survey of plant-parasitic nematodes in Morocco, a population of root-knot nematode from peach (*Prunus persica* cv. Missouri) rootstock was provisionally identified as *M. arenaria* (Neal) Chitwood on the basis of a few perineal patterns. Subsequent cytological studies indicated that this population had a somatic chromosome number of 47–49 and thus resembled certain hypotriploid populations of the *M. arenaria* species complex (13; A. C. Triantaphyllou, pers. comm.). Biochemically, it also had the same esterase phenotype as *M. arenaria* (7; P. R. Esbenshade, pers. comm.). Its differential host test response, however, was similar to that of *M. incognita,* race 2, infective on tobacco, pepper, watermelon, and tomato and noninfective on cotton and peanut (8). Detailed light and scanning electron microscopy studies showed that each life stage had characteristic morphological features that were different from those of *M. arenaria, M. incognita,* and any other described *Meloidogyne* species. Because of these morphological and biological differences to other known species of *Meloidogyne,* this root-knot nematode is designated as a new species and described here as *Meloidogyne morocciensis* n. sp.

MATERIALS AND METHODS

Stock cultures of *M. morocciensis* n. sp. derived from the original population from Morocco were maintained by periodic subculturing on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) in a greenhouse at 22–28 C. Specimens from these cultures were used for all morphological and morphometric studies. To rule out that the original population consisted of a mixture of several species, single egg mass isolates were made from the original population and compared morphologically. Females and egg masses were hand picked from infected roots. Males and second-stage juveniles (J2) were obtained after incubation of infected roots or egg masses in moist chambers at room temperature.

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Light microscopy (LM): Females were fixed in 2% formalin, and their anterior portions, including the esophageal region, were severed with an eye knife and mounted in 2% formalin. Entire females were also mounted in formalin, and body shape and dimensions were recorded. Perineal patterns were cut from live egg-laying females in 45% lactic acid and mounted in glycerin. Males and J2 were fixed in hot (70–80 C) TAF (7 ml 40% formaldehyde, 2 ml triethanolamine, 91 ml distilled water) and mounted in the same fixative for observation. At least 100 specimens of each life stage were examined for qualitative characters. Thirty-five other specimens of each stage were used to obtain morphometric data. Line drawings were made using a Leitz drawing tube, and photographs were taken with a bright field light microscope. Type specimens of all life stages were prepared according to previously described methods (9).

Scanning electron microscopy (SEM): Second-stage juveniles, males, excised stylets, and spicules were processed for SEM (3,5,12). The specimens were viewed and photographed using a JEOL T 200 scanning electron microscope operating at 25 kV accelerating voltage. At least 100 J2, 100 males, 40 stylets each of females and males, and 20 spicules were examined.

All measurements are in micrometers (μm) unless otherwise specified.

Systematics

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

Description

Holotype (female in glycerin): Body length 634 (without neck 493), body width 507, neck length 141, stylet length 16.5, stylet knob height 1.9, stylet knob width 4.7, DGO to stylet knob base 4.6, excretory pore to head end 37.4; ratios—a 1.3, body length without neck/body width 0.97, stylet knob width/height 2.5.

Females: Measurements of 35 females in 2% formalin and perineal patterns in glycerin in Table 1. Body globular, pearly white, variable in size, neck prominent; posterior end rounded, without distinct protuberance. Body cuticle distinctly annulated, annuli smaller in anterior neck region (Fig. 1C). Head region set off, usually marked by incomplete annulations (Fig. 1A, C). Head cap distinct, labial disc slightly elevated. Cephalic framework weakly sclerotized; vestibule and vestibule extension distinct. Stylet cone dorsally curved (Figs. 1A–C; 3A–D); shaft cylindrical. Stylet knobs distinctly separate, set off from shaft, transversely ovoid, with or without slight anterior indentation. Dorsal esophageal gland orifice (DGO) 2.4–5.2 from base of stylet knobs. Esophageal gland lobe large; three nuclei present, indicating one dorsal gland and two subventral glands. Two esophago-intestinal cells located near junction of metacorpus and intestine. Excretory pore located between dorsal esophageal gland orifice and metacorpus (Fig. 1A).

Perineal patterns oval to squarish (Figs. 1D–F, 2). Striae coarse, widely separated, usually continuous, sometimes broken. Tail tip distinct, with or without very fine, broken striations. Fold over anus present. Vulva slit-like, usually without striae near lateral edges. Phasmids small, distinct. Dorsal arch moderately high to high, rounded to squarish, sometimes forming "shoulders." District lateral lines absent, indicated by slight interruption of striae. Sometimes lateral lines with short, vertical striae near phasmid area (Figs. 1F; 2C, D). Ventral pattern region rounded, striae smooth.

Allotype (male in glycerin): Body length 1,754, body width 38.5, width at stylet base 20.9, width at excretory pore 32.6, head region height 8.9, head region width 13.5, stylet length 24.0, stylet knob height 3.1, stylet knob width 5.4, DGO to stylet knob base 4.9, head end to metacorpus valve 100.6, excretory pore to head end 191.1, tail length 14.3; ratios—a 45.7, c 119.5, body length/head end to metacorpus valve 17.4, head region width/height 1.5, stylet knob width/height 1.7, excretory pore 10.9%.

Males: Measurements of 35 males in TAF

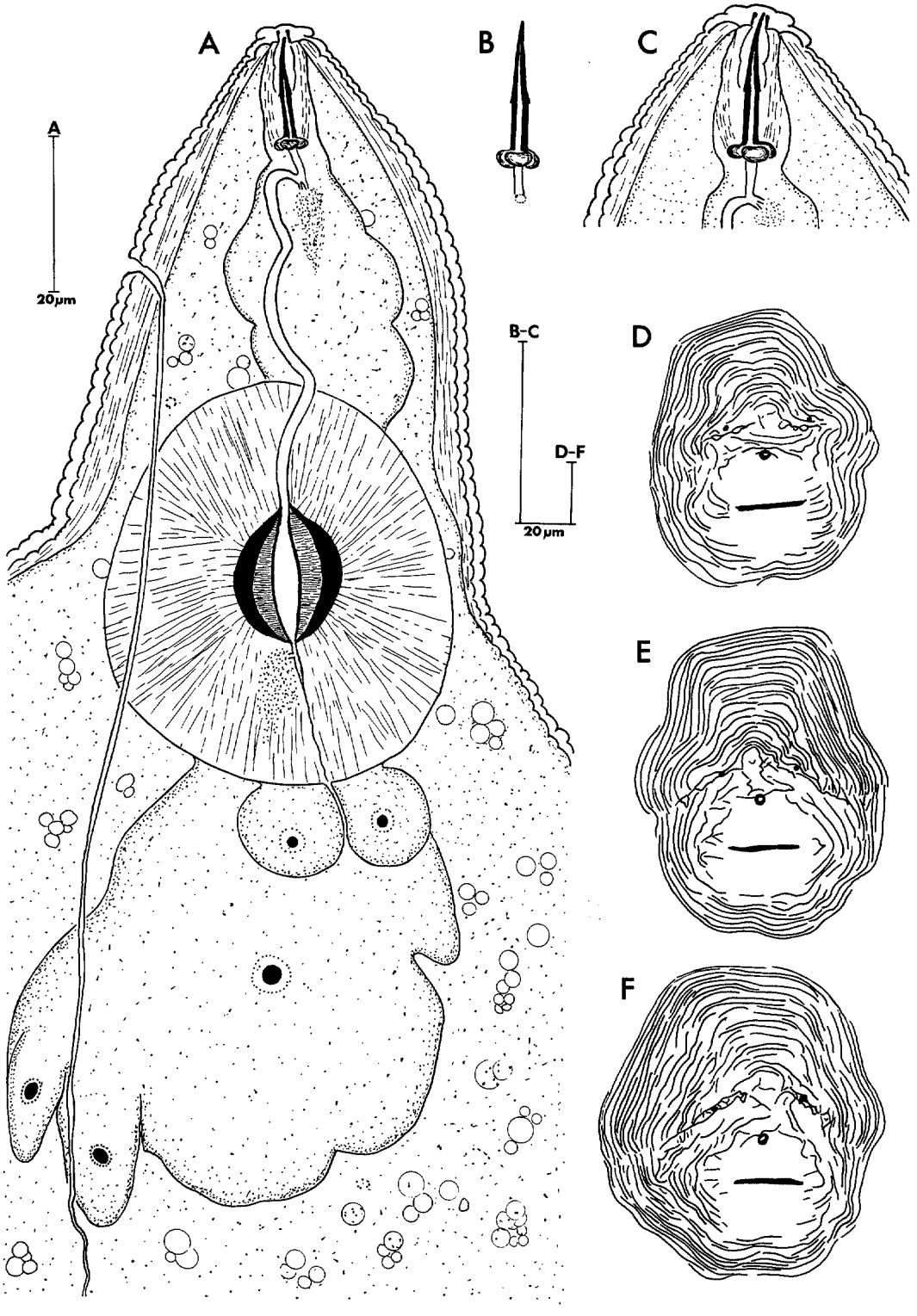


FIG. 1. Line drawings of females of *Meloidogyne morocciensis* n. sp. A) Esophageal region (lateral). B) Stylet. C) Cephalic region (lateral). D–F) Perineal patterns.

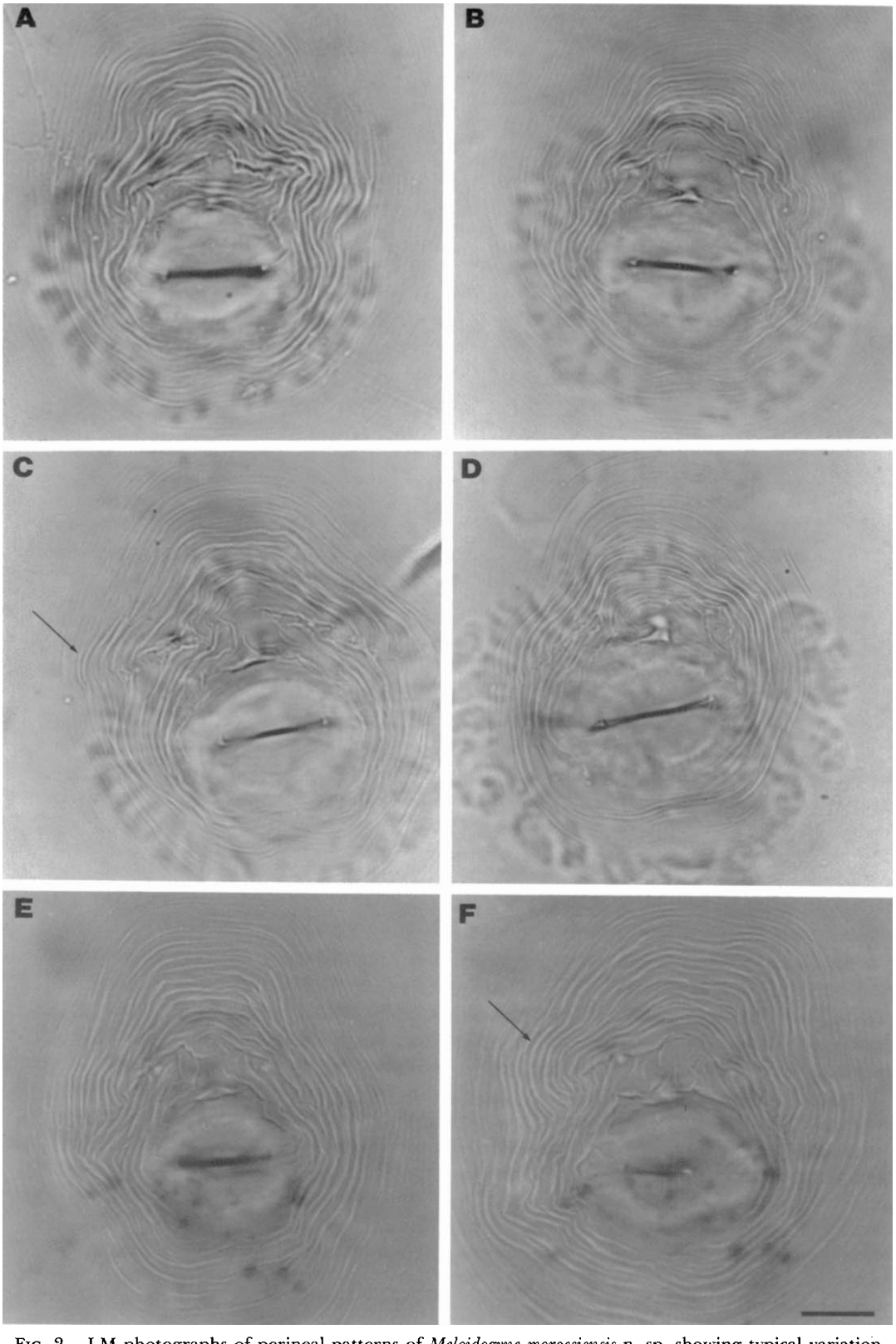


FIG. 2. LM photographs of perineal patterns of *Meloidogyne morocciensis* n. sp. showing typical variation (arrow = "shoulder"). A–E same scale as F. Bar = 20 μm.

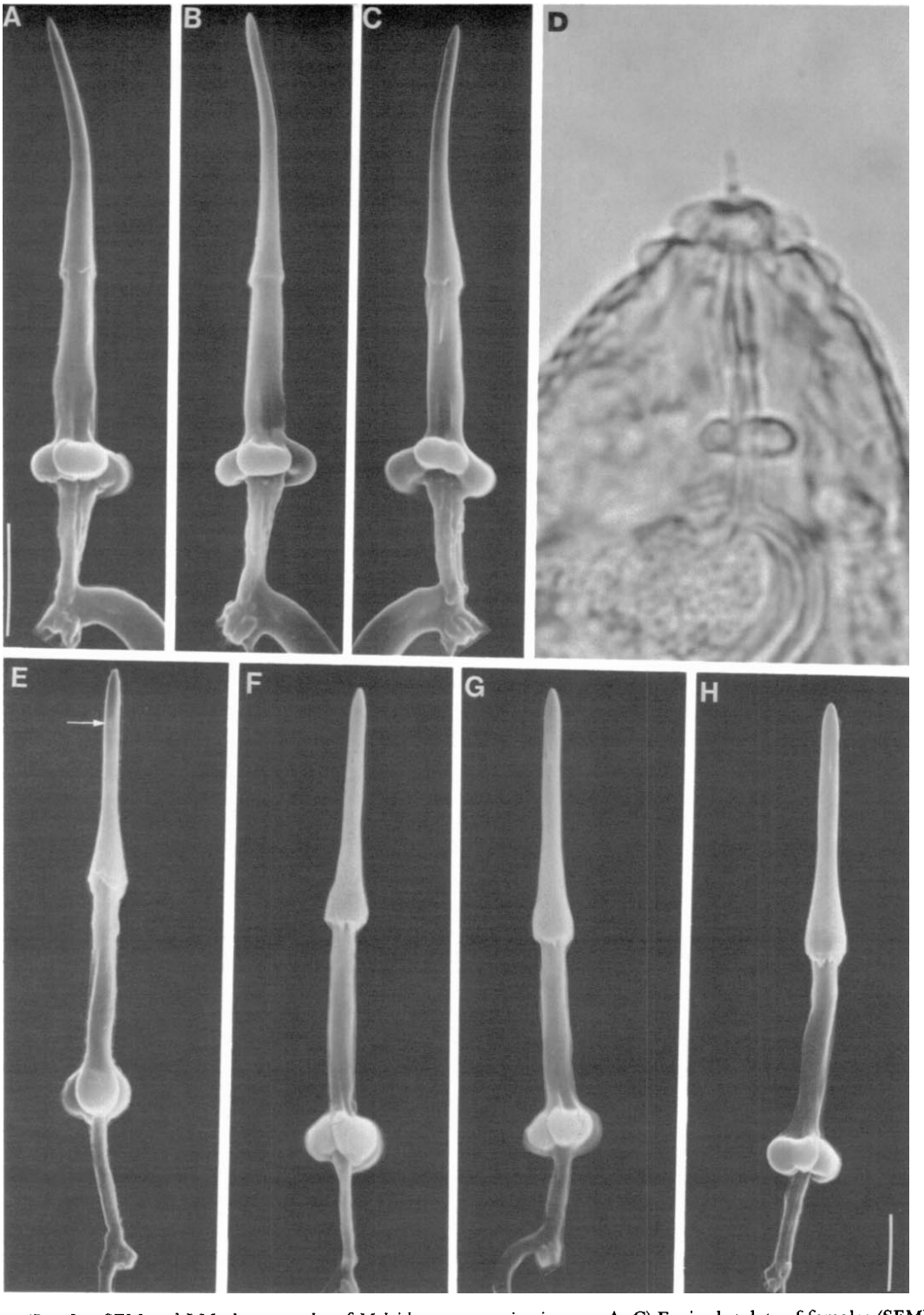


FIG. 3. SEM and LM photographs of *Meloidogyne morocciensis* n. sp. A–C) Excised stylets of females (SEM). D) Cephalic region of female (LM). E–H) Excised stylets of males (SEM) (arrow = stylet opening). B, C, same scale as A; D–G same scale as H. Each bar = 4 μm.

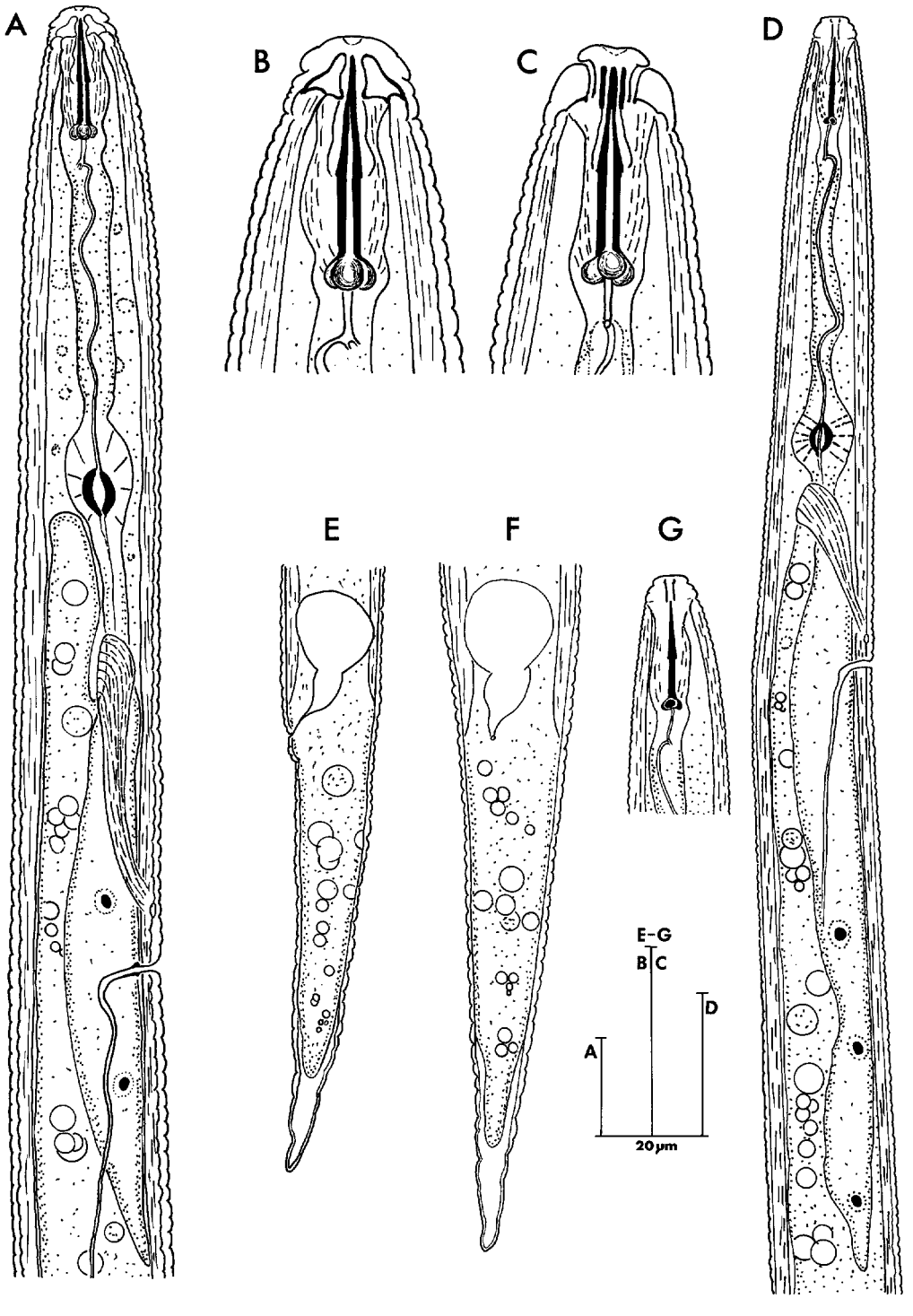


FIG. 4. Line drawings of males and J2 of *Meloidogyne morocciensis* n. sp. A) Esophageal region of male (lateral). B, C) Cephalic regions of male (lateral, dorsal). D) Esophageal region of J2 (lateral). E, F) Tails of J2 (lateral, ventral). G) Cephalic region of J2 (lateral).

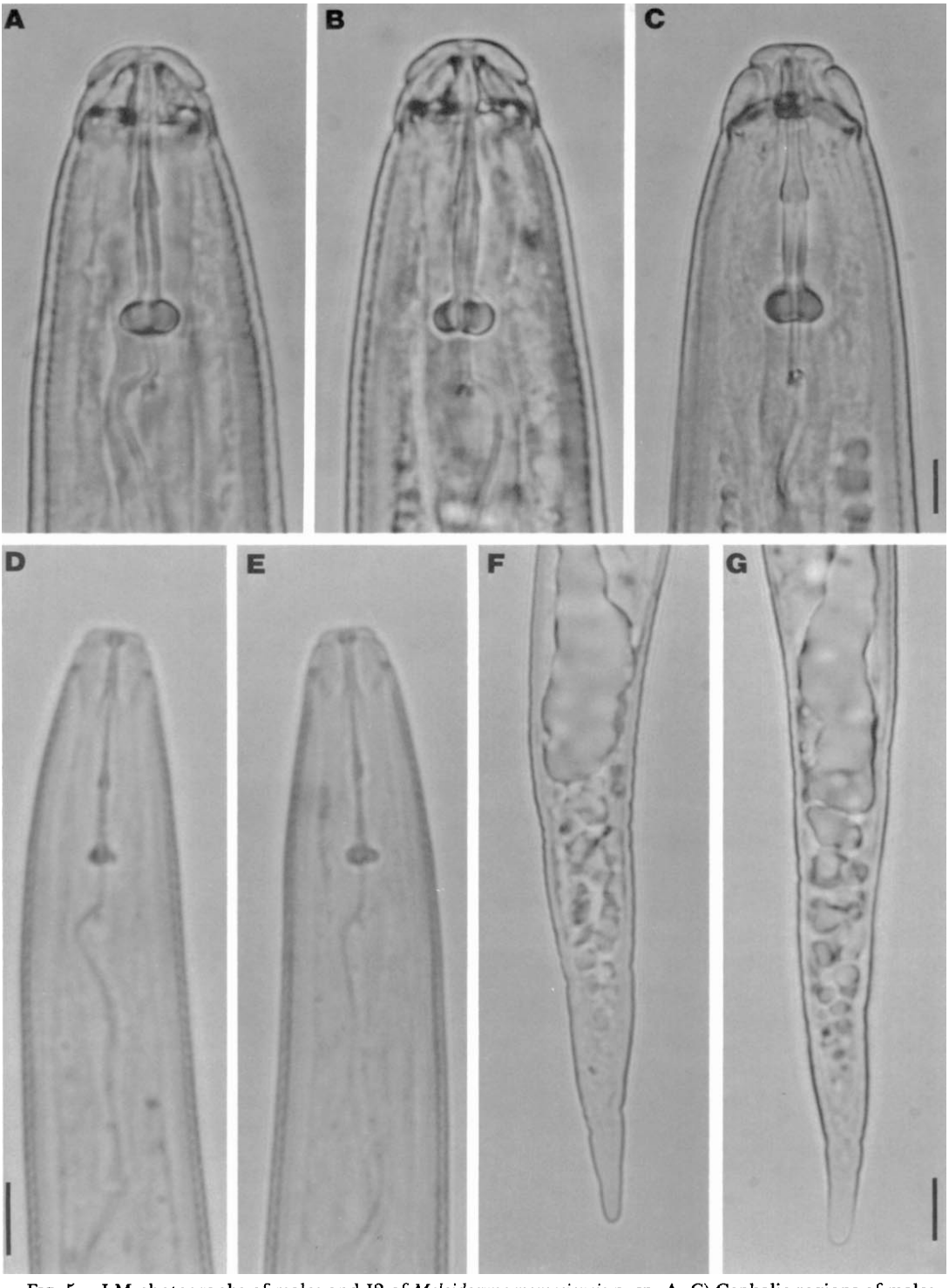
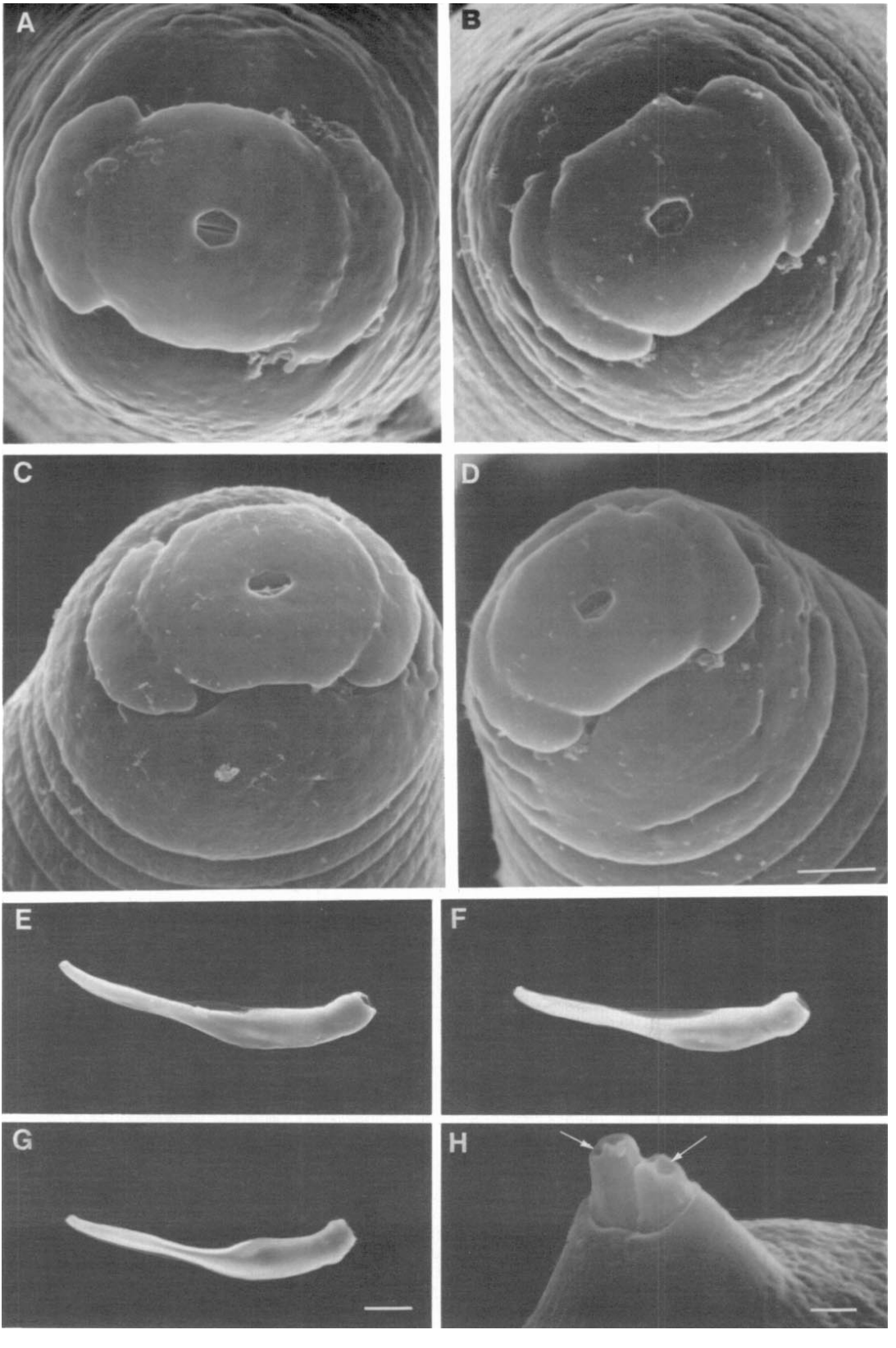


FIG. 5. LM photographs of males and J2 of *Meloidogyne morocciensis* n. sp. A–C) Cephalic regions of males (lateral, lateral, dorsal). D, E) Cephalic regions of J2 (lateral). F, G) Tails of J2 (lateral). A, B same scale as C; E same scale as D; F same scale as G. Each bar = 5 μm.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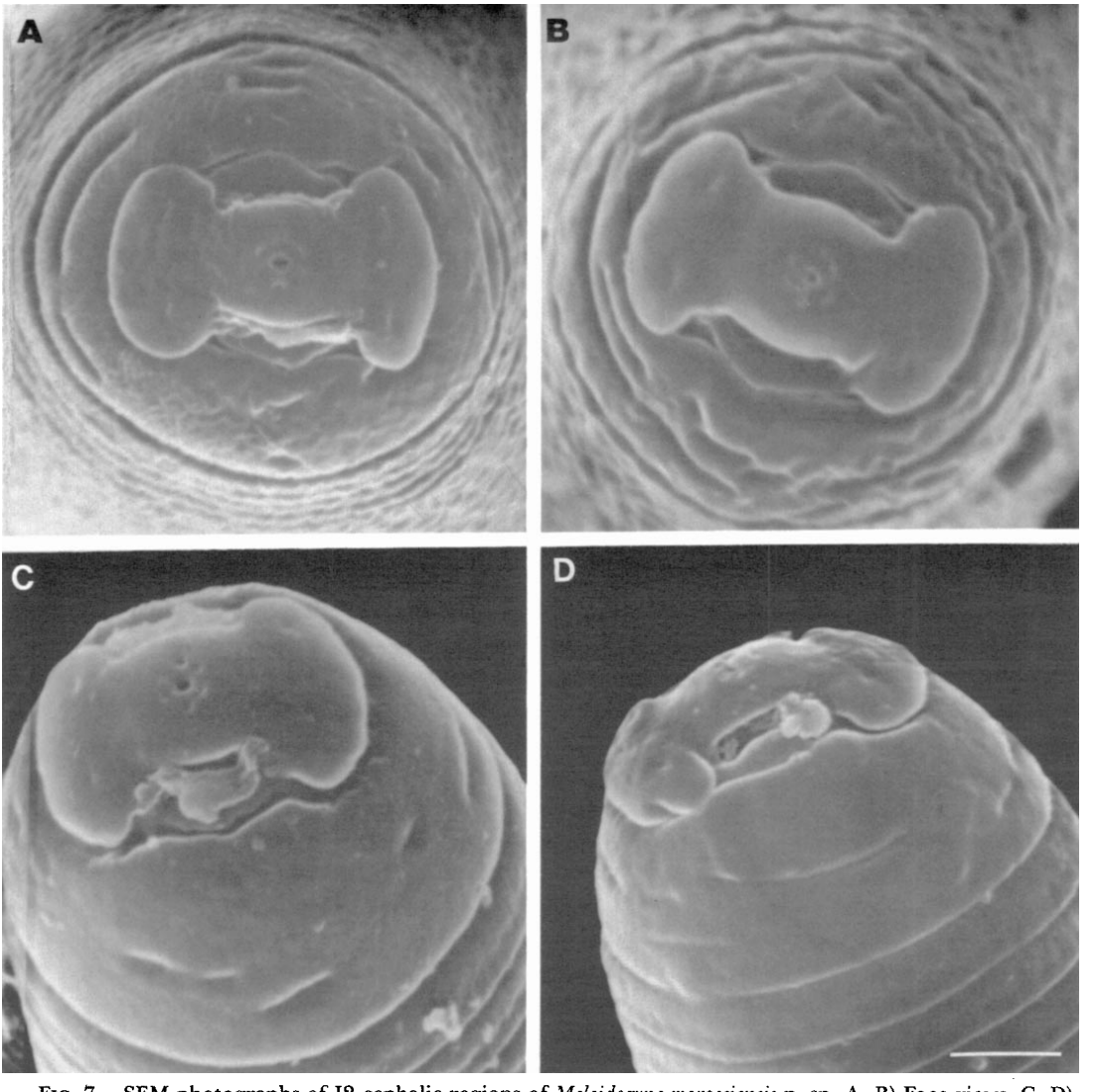

FIG. 7. SEM photographs of J2 cephalic regions of *Meloidogyne morocciensis* n. sp. A, B) Face views. C, D) Lateral views. A–C same scale as D. Bar = 1 μm.

in Table 2. Body vermiform, tapering anteriorly, bluntly rounded posteriorly. Heat-killed males assume C shape. Cuticle with distinct annulations. Lateral field with four incisures, areolated. Head region set off, with incomplete, distinct annulations, usually at medial sides (Figs. 4A–C, 5A–C). Head cap with distinct labial disc. Amphidial openings elongated slits. Cephalic framework moderately sclerotized. In SEM (face view), labial disc elevated, almost circular, distinctly separated from medial lips (Fig. 6A–D). Medial lips crescent shaped, with distinct lateral indentations at junction with labial disc. Diameter of medial lips smaller than that of labial disc. Ce-

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FIG. 6. SEM photographs of males of *Meloidogyne morocciensis* n. sp. A, B) Face views of cephalic region. C, D) Lateral views of cephalic region. E–G) Excised spicules. H) Tips of protruded spicules showing pores (arrows). A–C same scale as D, bar = 2 μm; E, F, same scale as G, bar = 5 μm; H, bar = 2 μm.

TABLE 1. Measurements of 35 females of *Meloidogyne morocciensis* n. sp.

Character	Range	Mean	SE	SD	CV (%)
Linear (μm)					
Body length	550.8–923.4	754.7	15.26	90.3	12.0
Body length without neck length	413.1–648.0	533.4	9.49	56.2	10.5
Body width	218.7–607.5	489.9	12.30	72.8	14.9
Neck length	121.5–364.5	221.0	9.74	57.6	26.1
Neck width	89.1–275.4	155.1	7.07	41.8	27.0
Vulval slit length	22.2–29.6	25.2	0.37	2.2	8.7
Vulva–anus distance	14.8–27.4	19.5	0.46	2.7	13.9
Interphasmidial distance	23.7–32.9	28.5	0.33	2.0	6.9
Stylet length	14.8–17.9	16.5	0.14	0.8	4.9
Stylet knob height	2.0–2.6	2.3	0.03	0.2	7.0
Stylet knob width	4.4–5.9	5.2	0.06	0.4	7.0
DGO	2.4–5.2	3.9	0.11	0.6	15.8
Excretory pore to head end	23.7–125.8	60.3	3.67	21.7	36.0
Ratios					
a	1.2–3.7	1.6	0.07	0.4	27.4
Body length without neck/body width	0.9–2.4	1.1	0.04	0.3	23.7
Stylet knob width/height	1.8–2.7	2.2	0.04	0.2	11.1

phalic sensilla obscure. Lateral lips absent. Stoma opening slit-like, situated below large, hexagonal prestoma (Fig. 6A–D). Inner labial sensilla indistinct, opening into prestomatal cavity. Stylet robust, large (Figs. 3E–H; 4B, C; 5A–C). Cone straight, slightly longer than shaft, tip pointed. Stylet opening situated about ¼ of cone length from tip (Fig. 3E–H). Base of cone broadened near junction with shaft. Shaft cylindrical, with same diameter along its length. Knobs set off from shaft, large, rounded, rarely slightly pear shaped and sloping posteriorly. DGO distance from base of stylet knobs 3.5–6.2. Procorpus well defined (Fig. 4A). Metacorpus oval shaped, with large valve. Esophago-intestinal junction obscure, at level of nerve ring. Gland lobe variable in length, with two nuclei. Caecum extending to level of metacorpus. Excretory pore position variable, terminal excretory duct long. Hemizonid 2–4 annules anterior to excretory pore. One or two testes, directed anteriorly, sometimes reflexed posteriorly. Phasmids pore-like, located at level of cloaca. Spicules identical (Fig. 6E–H). Head cylindrical, set off, circular cytoplasmic core opening on outward lateral side. Shaft limits indistinct. Blade arcuate, tapering towards tip. Vela clearly visible on inward side of spicule. Distance between dorsal and ventral vela wide at beginning of blade, narrowing suddenly at middle of spicule length. Blade tip slightly curved ventrally, with two pores to exterior.

Second-stage juveniles: Measurements of 35 J2 mounted in TAF in Table 3. Body vermiform, tapering at both ends, but more so posteriorly. Body annulations distinct, becoming larger and irregular in posterior tail region. Lateral field with four incisures, nonareolated. Head region slightly set off, with incomplete annulations (Figs. 4D, G; 5D, E). Head cap low, narrower than head region. In SEM, labial disc elongated, slightly elevated (Fig. 7A–D). Medial lips crescentic, with rounded corners, with or without indentations at their junction with labial disc. Cephalic sensilla distinct on medial lips. Lateral lip margins rounded to slightly triangular, positioned below labial disc and medial lips. Amphidial openings slit-like, just beneath lateral sides of labial disc. Stoma slit-like, located below circular prestoma, surrounded by six pit-like inner labial sensilla. Cephalic framework weakly sclerotized (Figs. 4D, G; 5D, E). Vestibule and vestibule extension distinct. Stylet cone straight, pointed, in-

TABLE 2. Measurements of 35 males of *Meloidogyne morocciensis* n. sp.

Character	Range	Mean	SE	SD	CV (%)
Linear (μm)					
Body length	1,296.0–1,863.0	1,620.9	26.22	155.1	9.6
Greatest body width	29.6–48.1	36.1	0.76	4.5	12.5
Body width at stylet base	18.1–22.2	20.5	0.15	0.9	4.4
Body width at excretory pore	26.6–35.9	30.4	0.36	2.1	7.1
Stylet length	22.9–25.8	24.6	0.12	0.7	3.0
Stylet knob height	2.5–3.6	3.1	0.04	0.2	7.2
Stylet knob width	4.7–6.3	5.5	0.06	0.4	6.9
DGO	3.5–6.2	4.7	0.12	0.7	15.0
Head end to metacorpus valve	89.9–103.6	97.8	0.65	3.9	3.9
Excretory pore to head end	149.5–195.4	177.1	1.79	10.6	6.0
Tail length	10.7–17.0	14.5	0.26	1.5	10.6
Spicule length	31.1–39.2	34.8	0.36	2.1	6.2
Gubernaculum length	8.1–10.7	9.4	0.13	0.8	8.1
Ratios					
a	36.5–57.5	45.2	0.77	4.6	10.1
Stylet knob width/height	1.5–2.2	1.8	0.03	0.2	8.9
c	87.6–148.5	112.9	2.51	14.9	13.2
Percentage					
Excretory pore	9.3–14.0	11.0	0.16	1.0	8.8

creases in width gradually posteriorly. Shaft cylindrical, may widen slightly posteriorly. Knobs distinctly separate, rounded, sloping posteriorly. Dorsal esophageal gland orifice distance 3–4.4. Procorpus faintly outlined. Metacorpus oval, with prominent valve (Fig. 4D). Esophago-intestinal junction indistinct, at level of nerve ring. Gland lobe variable in length, with three nuclei; dorsal nucleus smaller than two subventrals. Hemizonid two or three annules anterior to excretory pore. Tail conical, ending in bluntly rounded tip (Figs. 4E, F; 5F, G). Tail annulations become irregular, increasing in size toward tip. Hyaline tail terminus distinct. Rectal dilation large. Phas-

TABLE 3. Measurements of 35 second-stage juveniles of *Meloidogyne morocciensis* n. sp.

Character	Range	Mean	SE	SD	CV (%)
Linear (μm)					
Body length	374.4–454.4	400.8	3.67	21.7	5.4
Greatest body width	14.8–16.3	15.2	0.08	0.5	3.0
Body width at anus	10.0–11.8	11.1	0.08	0.5	4.3
Stylet length	11.3–13.3	12.3	0.09	0.5	4.1
Stylet base to head end	14.8–16.3	15.4	0.07	0.4	2.6
DGO	3.0–4.4	3.8	0.06	0.4	9.6
Head end to metacorpus valve	26.4–63.2	57.5	0.97	5.7	10.0
Excretory pore to head end	84.4–94.7	88.1	0.45	2.7	3.0
Tail length	46.6–58.1	52.6	0.46	2.7	5.1
Ratios					
a	23.6–30.7	26.4	0.24	1.4	5.5
Body length/head end to metacorpus valve	6.3–15.7	7.1	0.26	1.6	22.0
c	6.4–8.9	7.6	0.09	0.5	6.9
Tail length/width at anus	4.1–5.8	4.7	0.05	0.3	6.4
Percentage					
Excretory pore	18.6–23.4	22.0	0.20	1.2	5.2

mids obscure, ⅓ tail length posterior to anal opening.

Eggs (50 in 2% formalin): Length 84.4–101.3 (mean 93.0, standard error of mean [SE] 0.49, standard deviation [SD] 3.45, coefficient of variability [CV] 4%); width 38.5–50.3 (42.6, SE 0.32, SD 2.29, CV 5%); length/width ratio 1.7–2.6 (2.2, SE 0.02, SD 0.16, CV 7%). Morphology similar to that of eggs of other *Meloidogyne* species. Egg shell without markings in LM.

Type host and locality

Roots of peach rootstock, *Prunus persica* cv. Missouri, Ain Taoujdate, Morocco.

Type specimens

Holotype (female): Isolated from greenhouse culture, propagated on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers), derived from original population from Morocco. Slide no. T-434t, deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland. *Allotype (male):* Same data as holotype. Slide no. T-435t, deposited in the USDANC, Beltsville, Maryland. *Paratypes (females, males, J2, perineal patterns):* Same data as holotype. USDANC, Beltsville, Maryland (10 slides); University of California Davis Nematode Collection (UCDNC), Davis, California (10 slides).

Diagnosis

Meloidogyne morocciensis exhibits a combination of morphological features present in *M. arenaria, M. incognita,* and *M. javanica.* Useful diagnostic characters include the stylet morphology of females and males and the tail morphology of J2.

Relationships

The correct identification of *M. morocciensis* requires a detailed study of the distinctive morphological features of females, males, and J2. Perineal patterns of females are highly variable and can easily be confused with those of *M. arenaria* or *M. incognita* (1,6). *Meloidogyne ethiopica,* another species reported from Africa (Tanzania, Zimbabwe, and S. Africa), has been described as having perineal patterns varying from *M. arenaria* type to *M. incognita* type. However, other important diagnostic characters of *M. ethiopica,* including stylet size and stylet morphology of all life stages, are different from those of *M. morocciensis.* Stylet morphology of females of *M. morocciensis* is very similar to that of *M. javanica,* but the cone in *M. morocciensis* is curved more dorsally, and the knobs are more transversely ovoid and set off (5,6,11). Head shape of males in LM is similar to that of *M. incognita,* although the head region is more set off (6,10). In SEM, the shape of the head structures resembles that of *M. incognita.* The DGO distance, however, is longer than in *M. incognita* (3.5–6.2, 4.7; *M. incognita* 1.4–2.5, 2.1). Stylet morphology of males is similar to that of *M. arenaria,* although the stylet knobs are less rounded anteriorly and do not slope as much posteriorly (4,6). Body length of J2 is shorter than that of *M. arenaria* (374.4–454.4, 400.8; *M. arenaria* 391.6–605.2, 503.6) (1). The same is true for tail length (46.6–58.1, 52.6; *M. arenaria* 43.6–69.4, 56.0). Head shape of J2 appears similar to that of *M. incognita* (2).

Meloidogyne morocciensis n. sp. reproduces by mitotic parthenogenesis and has a somatic chromosome number of 47–49. In differential host tests it behaves as race 2 of *M. incognita* (8). Biochemically, this new species has the A3 esterase phenotype of *M. arenaria* (7). The malate dehydrogenase pattern is N1; i.e., a pattern that can also be found in *M. arenaria, M. incognita, M. javanica,* and other species. The superoxide dismutase phenotype is JA2; i.e., similar to that of *M. javanica, M. arenaria, M. cruciani,* and *M. microcephala.*

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