Meloidogyne hispanica n. sp. (Nematoda: Meloidogynidae), the 'Seville Root-Knot Nematode'

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Abstract: Meloidogyne hispanica n. sp. is described and illustrated from specimens obtained from peach rootstock, Prunus persica silvestris Batsch, from the Seville district of Spain. The perineal pattern of the female is oval shaped to rectangular with low dorsal arch and often widely spaced lateral lines with fringe-like striae. The stylet, 14.1 μ m long, has broad, distinctly set off knobs. Males have a high, rounded head cap that slopes posteriorly. Labial disc and medial lips are fused to form elongate lip structures. The robust stylet, 23.5 μ m long, has large, rounded knobs that are slightly set off from the shaft. Mean second-stage juveniles length is 392.6 μ m. The truncate head region is generally not annulated. The distinctly rounded and raised labial disc and the crescent-shaped medial lips form dumbbell-shaped lip structures. The stylet, 11.1 μ m long, has rounded, posteriorly sloping knobs. The slender tail, 46.4 μ m long, has large irregular-sized annules in the posterior region and ends in a bluntly rounded tip. Tomato was a good host; tobacco, pepper, and watermelon were poor hosts; cotton and peanut were nonhosts. Meloidogyne hispanica n. sp. reproduces by mitotic parthenogenesis and has a somatic chromosome number of 2n = 33-36. The

Key words: taxonomy, morphology, new Meloidogyne species, host range, scanning electron microscopy, Prunus persica silvestris.

A population of *Meloidogyne* from Seville, Spain, designated as "Spain 1" was first studied biochemically by Dalmasso and Bergé (1). It was similar in certain enzyme patterns to *M. incognita* with which it was grouped. Triantaphyllou (8) subsequently found that the same population differed from *M. incognita* in the behavior of its chromosomes during oogenesis.

Additional research conducted at North Carolina State University on the morphology, cytology, mode of reproduction, host range, and biochemistry revealed several unusual features peculiar to this population (designated as 468-Spain in the IMP collection), indicating that it was probably a new species. Results of the North Carolina differential host test showed that only tomato was a good host. Tobacco, pepper, and watermelon were very slightly infected. No reproduction occurred on cotton and peanut. This host response is somewhat similar to host race 2 of M. incognita or M. arenaria, but this population is distinct in several other respects from those and all other root-knot nematode species. Cytological studies indicated that reproduction is by mitotic parthenogenesis and the chromosome number is 2n = 33-36(8). Biochemical investigations revealed that this population has a characteristic esterase phenotype (S2-M1) different from that of other Meloidogyne species (6).

On the basis of its biological and morphological differences from other *Meloidogyne* species, this nematode is described here as *Meloidogyne hispanica* n. sp. The common name "Seville root-knot nematode" is suggested.

Several populations obtained through the International *Meloidogyne* Project are very similar to *M. hispanica* n. sp. with respect to cytological, biochemical (6), and preliminary morphological data. These include populations E 428 from Portugal (2n = 36), E 19 from Korea (2n = 38), and E 73 from the Fiji Islands (2n = 36). Population E 428 with approximately the same chromosome number, the same host range, and biochemistry was studied extensively morphologically and found to be identical with *M. hispanica* n. sp.

MATERIALS AND METHODS

Stock cultures of *M. hispanica* n. sp. derived from the original isolate from Seville,

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Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variability (%)
Linear (µm)					
Body length	570.0 - 1,180.0	830.0	27.46	150.4	18.1
Body width	330.0-740.0	503.0	19.88	108.9	21.6
Neck length	140.0-440.0	232.7	13.10	71.7	30.8
Neck width	60.0-200.0	129.8	5.78	31.6	24.4
Vulval slit length	20.0 - 25.4	23.5	0.23	1.2	5.3
Vulva–anus distance	17.2 - 22.6	19.0	0.23	1.3	6.7
Interphasmidial distance	16.7 - 28.1	22.1	0.51	2.8	12.7
Stylet length	13.6 - 14.6	14.1	0.05	0.3	1.9
Stylet knob height	2.1 - 2.8	2.5	0.03	0.2	7.1
Stylet knob width	4.1 - 5.1	4.6	0.04	0.2	5.1
DĠO	2.8 - 4.0	3.2	0.06	0.3	10.6
Excretory pore to head end	11.9-71.1	30.5	2.24	12.3	40.3
Metacorpus length	39.5-63.2	48.8	0.88	4.8	9.8
Metacorpus width	39.5 - 52.6	45.5	0.72	3.9	8.6
Metacorpus "valve" length	14.1 - 20.5	15.6	0.25	1.4	8.7
Metacorpus "valve" width	11.5 - 13.8	12.6	0.10	0.5	4.1
Ratios					
а	0.9 - 2.2	1.7	0.05	0.3	17.0
Body length/neck length	2.5 - 5.9	3.7	0.14	0.8	21.0
Stylet knob width/height	1.6 - 2.2	1.9	0.03	0.2	7.7
Metacorpus length/width	0.9 - 1.2	1.1	0.01	0.1	4.8
Metacorpus "valve" length/width	1.1-1.5	1.2	0.02	0.1	7.1

TABLE 1. Measurements of 30 females of Meloidogyne hispanica n. sp.

Spain, were maintained by periodic subculturing on tomato (Lycopersicon esculentum Mill. cv. Rutgers) in a greenhouse at 22–28 C. Nematodes from these cultures were used for all morphological and morphometric studies. Egg masses and adult females were handpicked from infected roots. Second-stage juveniles were hatched from egg masses in moist chambers. Males were obtained by incubating washed, infected roots in moist chambers; the roots were rinsed periodically with water, and males were collected from the washings.

Light microscope (LM) studies: Eggs, freshly hatched second-stage juveniles, males, females, and perineal patterns were prepared for study according to previously described methods (7). Morphological observations and measurements of all life stages were completed within 24–48 hours after slide preparation. At least 100 specimens of each life stage were examined.

Scanning electron microscope (SEM) studies: Second-stage juveniles, males, excised stylets, and perineal patterns of females were processed for SEM as described previously (2-5). The specimens were viewed and photographed using an ETEC Autoscan scanning electron microscope operating at 20 kV accelerating voltage or a JEOL T 200 operating at 25 kV. At least 100 second-stage juveniles, 100 males, 50 perineal patterns, and 50 excised stylets of females were examined.

Preparation of type material: Females were fixed in FA (formalin 10 ml, glacial acetic acid 10 ml, distilled water 80 ml) for 12– 24 hours. Following fixation they were transferred to hot (near boiling) lactophenol for 3–5 minutes and were processed further through a four-step graded series of lactophenol-glycerin solutions with increasing amounts of glycerin into 100% glycerin in an oven at 60 C (10 minutes at each step). Once in 100% glycerin, the females were transferred to a desiccator; after 24–48 hours they were mounted in desiccated glycerin on Cobb slides.

Males and second-stage juveniles were fixed in 2% glutaraldehyde in the cold (7 C) for 2–3 days and washed in sodium cacodylate buffer (pH 7.4) for 24 hours in the cold. They were then placed in a solution of 1.5% glycerin in water (to which a crystal of copper sulfate had been added to prevent fungal growth) and were slowly infil-

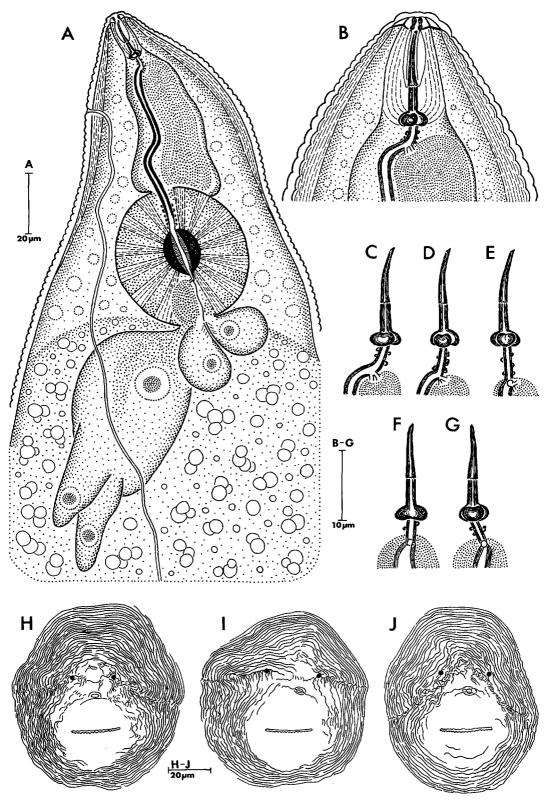


FIG. 1. Line drawings of females of *Meloidogyne hispanica* n. sp. A) Esophageal region (lateral). B) Cephalic region (lateral). C–G) Stylets (lateral, lateral, ventrolateral, ventral, dorsal). H–J) Perineal patterns.

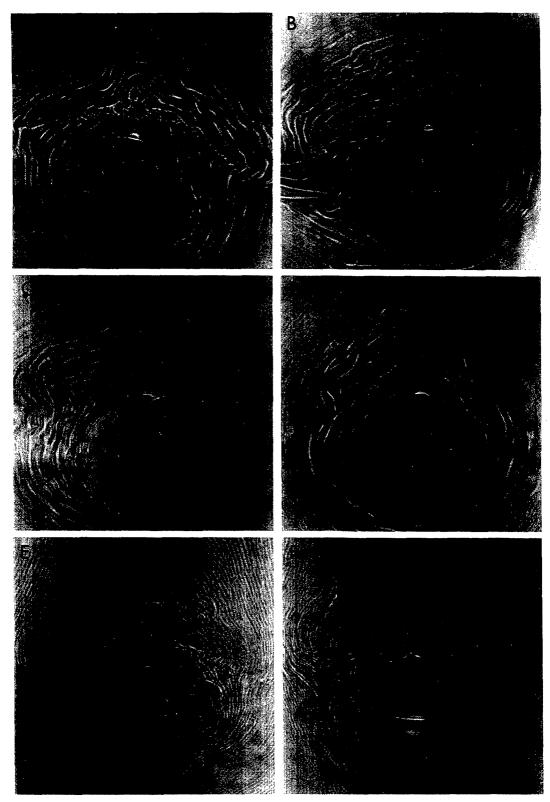


FIG. 2. LM photographs of perineal patterns of Meloidogyne hispanica n. sp. showing typical variation.

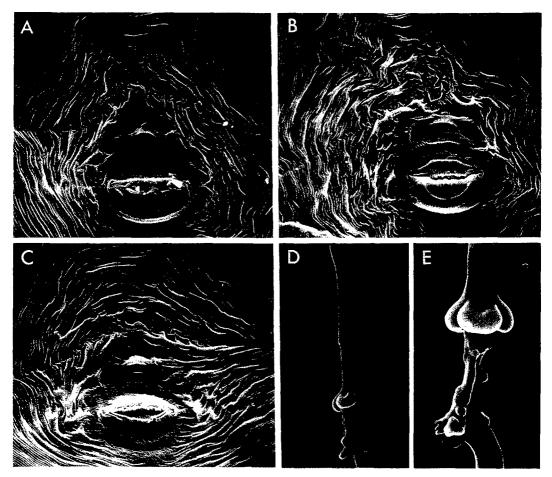


FIG. 3. SEM photographs of females of Meloidogyne hispanica n. sp. A-C) Perineal patterns. D, E) Stylets.

trated with glycerin in an oven at 34 C. All glycerin-processed specimens were kept for at least 48 hours in a calcium chloride desiccator and then were mounted in desiccated glycerin on Cobb slides.

Systematics

Meloidogyne hispanica n. sp. (Figs. 1–8)

Females

Measurements of 30 females in 2% formalin (perineal patterns in glycerin) are listed in Table 1.

Measurements of holotype in glycerin: Body length including neck 965 μ m; body width 620 μ m; a 1.6; neck length 215 μ m; neck width 100 μ m; body length/neck length 4.5; stylet length 13.9 μ m; stylet knob height 2.1 μ m; stylet knob width 4.1 μ m; stylet knob width/height 2.0; DGO to stylet base 4.0 μ m; excretory pore to head end 33.2 μ m.

Description: Body ivory colored, ovoid to globular shape, characteristically with long neck, posteriorly rounded, without tail protuberance. Head region not set off from body, not annulated (Fig. 1A, B). Head cap broad, squarish. First 8–10 annules behind head region smaller than remaining body annules. Hexaradiate cephalic framework weakly sclerotized, lateral sectors slightly enlarged, vestibule and vestibule extension distinctly sclerotized. Stylet small, easily dislodged posteriorly (Figs. 1C-G, 3D, E). Stylet cone slightly curved dorsally, widening gradually posteriorly. Shaft of same width throughout, or widening slightly near junction with knobs. Stylet knobs broad with indented anterior margins, distinctly separate, usually set off from shaft, sometimes slightly sloping posteriorly. Distance

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variability (%)
Linear (µm)					
Body length	1,340.6-1,990.0	1,677.6	30.75	168.4	10.0
Greatest body width	32.4-47.4	41.1	0.63	3.5	8.4
Body width at stylet knobs	18.3 - 21.4	20.3	0.13	0.7	3.6
Body width at excretory pore	28.8-38.6	33.6	0.42	2.3	6.9
Head region height	7.0-8.1	7.6	0.05	0.3	3.9
Head region width	11.8 - 13.7	12.8	0.09	0.5	3.8
Stylet length	21.7-24.3	23.5	0.11	0.6	2.6
Stylet base to head end	23.7-27.8	26.0	0.17	0.9	3.6
Stylet shaft and knobs	9.9-11.5	10.6	0.08	0.4	4.1
Stylet knob height	2.9-3.5	3.2	0.03	0.2	4.8
Stylet knob width	5.1 - 6.1	5.6	0.06	0.3	5.5
DĠO	1.4 - 3.6	2.5	0.08	0.5	18.2
Head end to metacorpus "valve"	86.9-104.9	95.3	0.84	4.6	4.8
Excretory pore to head end	148.6 - 254.1	181.5	3.70	20.3	11.2
Tail length	10.7 - 16.2	13.3	0.32	1.7	13.1
Phasmids to tail end	10.3-19.4	14.4	0.42	2.3	16.1
Spicule length	31.1-33.7	32.1	0.14	0.8	2.4
Gubernaculum length	7.4 - 9.3	8.3	0.09	0.5	5.8
Testis length	650.0-1,150.0	808.4	20.73	113.6	14.0
Ratios					
а	31.4-61.4	41.2	1.12	6.1	14.8
с	98.2-172.6	128.1	3.47	19.0	14.8
Body length/head end to					
metacorpus "valve"	14.7 - 20.1	17.6	0.26	1.4	8.2
Head region width/height	1.6 - 1.8	1.7	0.01	0.1	2.9
Stylet knob width/height	1.5 - 2.0	1.7	0.02	0.1	5.9
Percentages					
Excretory pore	8.2-18.1	10.9	0.30	1.7	15.1
T	32.7-62.2	48.7	1.47	8.2	16.8

TABLE 2. Measurements of 30 males of Meloidogyne hispanica n. sp.

between stylet base and dorsal esophageal gland orifice (DGO) short to moderately long (2.8–4.0 μ m), gland orifice branched into three channels; dorsal gland ampulla large; subventral gland orifices branched, located posteriorly to enlarged triradiate lumen lining of metacorpus ("valve") (Fig. 1A). Esophageal lumen lining with small knots anterior to DGO and metacorpus "valve." Esophageal glands large, trilobed, dorsal lobe largest; two subventral lobes variable in size, shape, and position, located posteriorly to dorsal gland lobe. Esophago-intestinal cells two, near junction of metacorpus and intestine. Excretory pore position variable, close to head end, in most specimens at level of DGO; terminal excretory duct very long.

Perineal patterns oval shaped to rectangular (Figs. 1H–J, 2, 3A–C). Dorsal arch generally low, some patterns with higher squarish arch. Dorsal striae vary from fine and wavy to coarse. Ventral pattern area generally with fine, smooth striae, may form ventral wings on one side (Fig. 2B, F). Lateral lines distinctly forked, frequently spaced widely, with fringe-like striae between lines. Tail tip area well defined, free of striae. Perivulval region not striated, rarely striae near lateral edges of vulva. Vulval edges slightly crenate. Phasmidial ducts distinct, no phasmid surface structure apparent in SEM.

Males

Measurements of 30 males in TAF are listed in Table 2.

Measurements of allotype in glycerin: Body length 1,770 μ m; body width 35.2 μ m; head region height 7.5 μ m; head region width 12.0 μ m; stylet length 23.7 μ m; stylet knob height 3.0 μ m; stylet knob width 5.4 μ m; DGO to stylet base 2.8 μ m; head end to metacorpus "valve" 98.8 μ m; excretory

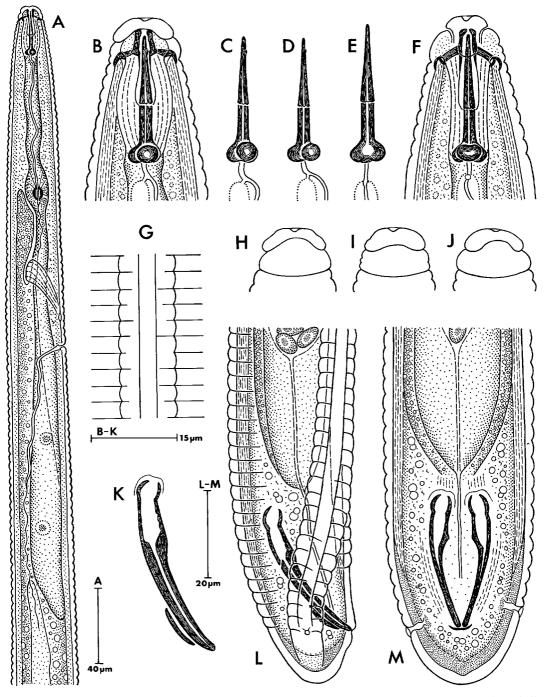


FIG. 4. Line drawings of males of *Meloidogyne hispanica* n. sp. A) Esophageal region (lateral). B, F) Cephalic regions (lateral, dorsal). C-E) Stylets (lateral, lateral, ventral). G) Lateral field near mid-body. H-J) Shape of head regions (lateral). K) Spicule and gubernaculum (lateral). L) Tail (lateral). M) Tail (ventral).

pore to head end 177.8 μ m; tail length 13.8 μ m; spicule length 31.8 μ m; gubernaculum length 7.9 μ m; testis length 800 μ m; a 50.3; c 128.3; body length/head end to meta-corpus "valve" 17.9; head region width/

height 1.6; stylet knobs width/height 1.8; excretory pore 10.1%; T 45.2.

Description: Generally large nematodes, rather robust; body tapering anteriorly, bluntly rounded posteriorly; tail twists

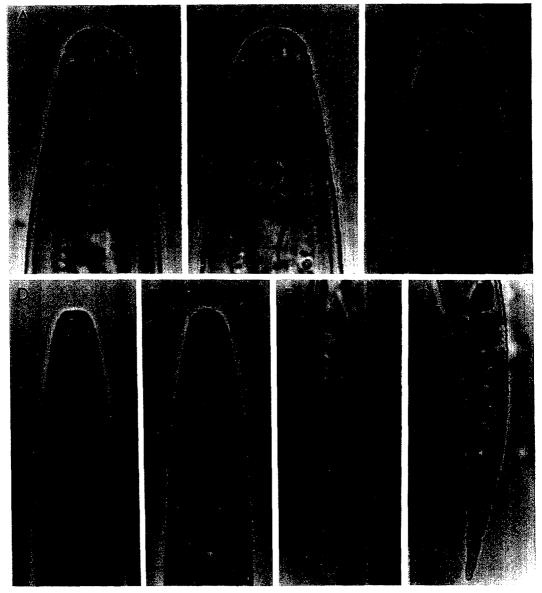
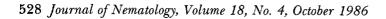


FIG. 5. LM photographs of males and second-stage juveniles of *Meloidogyne hispanica* n. sp. A-C) Cephalic regions of males (lateral, lateral, dorsal). D, E) Cephalic regions of second-stage juveniles (lateral, dorsal). F, G) Tails of second-stage juveniles (lateral; arrow indicates anus).

through 90 degrees in heat-killed specimens. Body annules large, distinct. Lateral field with four incisures, rarely with a fifth central, broken incisure, faint areolation in outer fields throughout (Fig. 4G); on tail region areolation extends into central field (Figs. 4L, 6E). Lateral fields bulge out slightly at anterior beginning near level of stylet base. Head cap in lateral view high and rounded extending posteriorly onto distinctly set off head region (Figs. 4B, F, H–J, 5A, B). In SEM (face view), labial disc very large, wider than long axis of medial lips, elongated, and slightly raised (Fig. 6A– D). Medial lips narrow, crescent shaped with smooth outer margins. Frequently, distinct indentations at junction of labial disc and medial lips. Labial disc and medial lips fused to form elongate lip structures extending posteriorly onto head region. Occasionally, slight dorso-ventral asymmetry of lip structures. Lateral lips generally indicated or fully formed. Head region usually smooth but may have up to



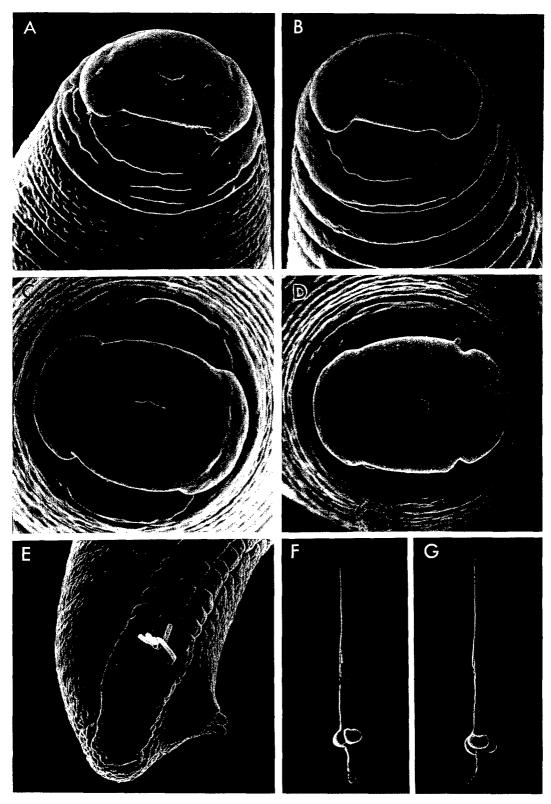


FIG. 6. SEM photographs of males of *Meloidogyne hispanica* n. sp. A, B) Lateral views of cephalic region. C, D) Face views. E) Tail (lateral). F, G) Stylets.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variability (%)
Linear (µm)					······
Body length	356.4-441.4	392.6	2.64	18.7	4.8
Greatest body width	13.4-15.8	14.5	0.08	0.5	3.7
Body width at excretory pore	13.1 - 15.0	13.9	0.06	0.4	2.9
Body width at anus	10.2 - 11.7	10.8	0.05	0.4	3.3
Head region height	2.4 - 3.1	2.7	0.02	0.2	6.5
Head region width	4.8 - 5.7	5.4	0.02	0.2	2.9
Stylet length	10.4-11.9	11.1	0.04	0.3	2.7
Stylet base to head end	14.0 - 15.6	14.7	0.05	0.4	2.4
Stylet shaft and knobs	4.7 - 5.6	5.0	0.03	0.2	3.6
Stylet knob height	1.2 - 1.6	1.4	0.01	0.1	7.0
Stylet knob width	2.3 - 2.8	2.6	0.02	0.1	5.0
DĠO	2.2 - 3.4	2.8	0.04	0.3	9.6
Head end to metacorpus "valve"	50.2 - 61.1	55.6	0.31	2.2	4.0
Metacorpus "valve" length	4.3 - 4.8	4.6	0.02	0.1	2.8
Metacorpus "valve" width	3.8 - 4.4	4.1	0.02	0.1	3.8
Excretory pore to head end	73.9-86.0	80.1	0.38	2.7	3.3
Tail length	41.1 - 53.4	46.4	0.40	2.8	6.1
Ratios					
а	24.6 - 30.9	27.1	0.17	1.2	4.5
Body length/head end to					
metacorpus "valve"	6.3-8.0	7.1	0.04	0.3	4.2
c	7.7-9.4	8.5	0.05	0.4	4.3
d .	3.8-5.0	4.3	0.04	0.3	6.5
Head region width/height	1.7 - 2.3	2.0	0.02	0.1	6.9
Stylet knob width/height	1.6 - 2.2	1.8	0.02	0.1	7.1
Percentage					
Excretory pore	19.0 - 21.7	20.4	0.09	0.64	3.1

TABLE 3. Measurements of 50 second-stage juveniles of Meloidogyne hispanica n. sp.

three incomplete annulations. Prestoma large, hexagonal. Stoma opening slit-like, located in large, hexagonal prestomatal cavity. Six inner labial sensilla small pits, opening at edge of, or into, prestomatal cavity. Cephalic sensilla distinct. Amphidial apertures large, elongate slits. Cephalic framework well sclerotized, vestibule and vestibule extension distinct (Figs. 4B, F, 5A-C). Stylet robust, large (Figs. 4B-F, 5A-C, 6F, G); cone straight, pointed, gradually increasing in diameter posteriorly; stylet opening marked by slight protuberance several micrometers from stylet tip, shaft cylindrical, knobs large, rounded, slightly set off from shaft, rarely sloping posteriorly. Distance of DGO to stylet knob base short (1.4–3.6 μ m). Dorsal gland duct branched into three channels, gland ampulla poorly defined. Procorpus distinctly outlined (Fig. 4A); metacorpus elongate, oval shaped with large "valve." Subventral gland openings branched, located posteriorly to metacorpus "valve." Esophagointestinal junction distinct. Gland lobe variable in length with two equal-sized nuclei variable in position. Intestinal caecum extends anteriorly on dorsal side to level of metacorpus. Excretory pore variable in position, terminal duct long, curved, ending in excretory cell with large nucleus near right lateral chord. Hemizonid 1-5 annules anterior to excretory pore. Usually one testis, rarely two testes, outstretched, or reflexed anteriorly. Sperm large, rounded, granular. Spicules long, slender, slightly arcuate with single tip (Fig. 4K-M). Gubernaculum crescent shaped. Tail short, elongate conoid (Figs. 4L, M, 6E). Phasmids at level of cloaca, with slit-like openings in SEM.

Second-stage juveniles

Measurements of 50 juveniles in TAF are listed in Table 3.

Description: Body moderately long, slender, tapering anteriorly but more so posteriorly; tail region distinctly narrowing.

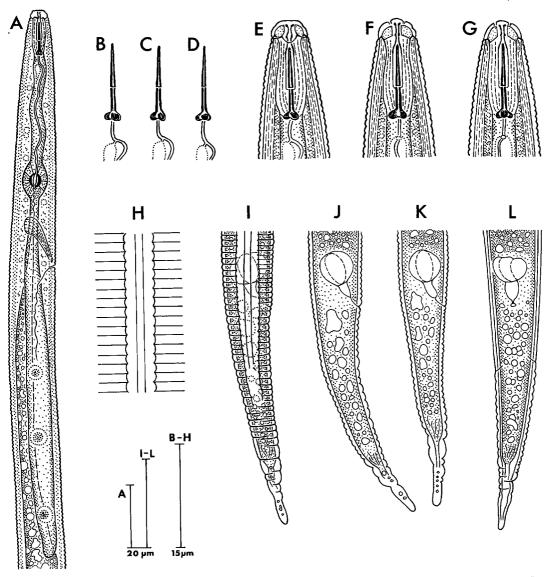


FIG. 7. Line drawings of second-stage juveniles of *Meloidogyne hispanica* n. sp. A) Esophageal region (lateral). B-D) Stylets (lateral). E-G) Cephalic regions (lateral, ventral, dorsal). H) Lateral field near mid-body. I-L) Variations in tail morphology (I-K lateral, L ventral).

Body annules distinct, increase in size and become irregular in posterior tail region. Lateral field starts approximately at middle of procorpus and extends to near phasmid, 4.6–5.3 μ m wide, with four incisures, not areolated, outer lines crenate (Fig. 7H). Head region truncate, distinctly set off from body (Figs. 5D, E, 7E–G). Head cap low, narrower than head region. In SEM labial disc rounded, distinctly raised (Fig. 8). Outer margins of medial lips crescent shaped to rounded, smooth. Medial lips and labial disc dumbbell shaped. Lateral lips fused at right angle with medial lips, lower than medial lips, margins rounded to slightly triangular, may fuse with head region. Head region smooth, occasionally with 1–2 short, broken annulations. Prestoma oval, stoma slit-like, large. Inner labial sensilla pit-like, large, opening on la-

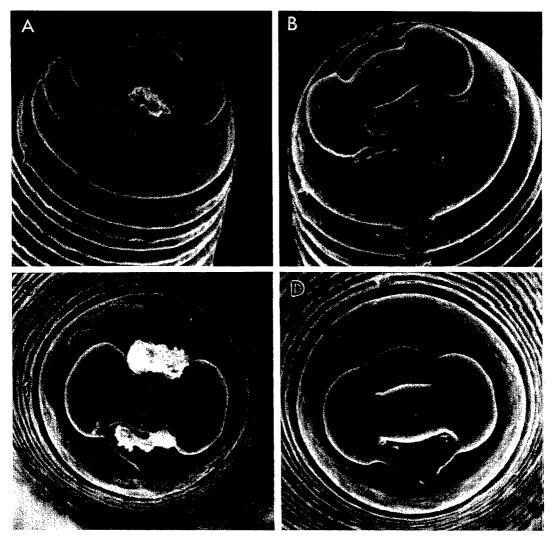


FIG. 8. SEM photographs of cephalic regions of second-stage juveniles of *Meloidogyne hispanica* n. sp. A, B) Lateral views. C, D) Face views.

bial disc, symmetrically arranged around prestoma. Area around prestomatal opening not recessed. Cephalic sensilla indistinct. Cephalic framework weakly sclerotized (Figs. 5D, E, 7E–G). Vestibule and vestibule extension distinct. Stylet moderately sized, but delicate (Figs. 5D, E, 7B– G). Stylet cone sharply pointed, increases in width gradually posteriorly; shaft cylindrical, may widen slightly posteriorly; knobs robust, distinctly separated, rounded, sloping posteriorly. Distance of dorsal esophageal gland orifice to stylet base moderately long $(2.2-3.4 \ \mu m)$; dorsal gland ampulla poorly defined. Procorpus faintly outlined, metacorpus ovoid with prominent "valve"; isthmus not clearly outlined (Fig. 7A). Esophago-intestinal junction indistinct, at level of nerve ring. Esophageal gland lobe variable in length with three distinct nuclei, about equal in size. Hemizonid 1–2 annules anterior to excretory pore. Tail slender, ending in bluntly rounded tip (Figs. 5F, G, 71–L). Posterior tail region, with large annules of variable size, frequently appears knobby; hyaline tail terminus indistinct. Rectal dilation large, filled with matrix material. Phasmids obscure, short distance posterior to anal opening.

Eggs

Measurements of 50 eggs in 2% formalin: Length 80.0-105.0 μ m (mean 91.5 μ m, standard error of mean 0.85, standard deviation 6.01, coefficient of variability 6.6%); width 37.0-52.0 μ m (mean 42.4 μ m, standard error of mean 0.52, standard deviation 3.70, coefficient of variability 8.7%); length/width ratio 1.7-2.6 (mean 2.2, standard error of mean 0.03, standard deviation 0.24, coefficient of variability 11.1%).

Description: Egg morphology similar to that of eggs of other Meloidogyne species. Egg shell without visible markings by light microscopy.

Diagnosis

Meloidogyne hispanica n. sp. can be distinguished from *M. incognita* and other described species of the genus by the following morphological characteristics: The stylet knobs of the female are not as deeply indented as those of most populations of M. incognita, and the stylet cone is only slightly curved. The perineal pattern has distinct lateral lines often with fringes. The head cap in the male is very high and rounded and extends posteriorly onto the head region. This characteristic is confirmed by SEM observations which show that labial disc and medial lips are fused to form elongate lip structures. Distinct indentations are frequently present at the junction of labial disc and medial lips. In SEM, the labial disc of second-stage juveniles is rounded and the area around the prestomatal cavity is not recessed as in M. incognita. Also, the pit-like inner labial sensilla open out on the labial disc at a greater distance from the prestomatal opening. The stylet knobs are robust and distinctly slope posteriorly. The posterior tail region has large, irregular-sized annules resulting in a characteristically knobby appearance. The hyaline tail terminus is indistinct.

Cytologically, *M. hispanica* n. sp. is similar to the diploid race of *M. arenaria* and differs from the diploid race of *M. incognita* in that prophase I is not prolonged and the chromosomes do not clump together (8).

Biochemically, M. hispanica n. sp. has a characteristic esterase phenotype (S2–M1), different from that of other Meloidogyne species (6).

Type specimens

Holotype (female): Isolated from greenhouse culture, propagated on tomato (Lycopersicon esculentum Mill. cv. Rutgers), derived from original population obtained from Spain. Slide T-421t, deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland.

Allotype (male): Same data as holotype. Slide T-422t, deposited in the USDANC, Beltsville, Maryland.

Paratypes (females, males and second-stage juveniles): Same data as holotype. Deposited in the USDANC, Beltsville, Maryland, and the University of California Davis Nematode Collection (UCDNC), Davis, California.

Type host and locality

Roots of peach rootstock, Prunus Persica silvestris Batsch, peach orchard, Seville district (Andalusia), Spain.

LITERATURE CITED

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