

## ***Meloidogyne lusitanica* n. sp. (Nematoda: Meloidogynidae), a Root-knot Nematode Parasitizing Olive Tree (*Olea europaea* L.)<sup>1</sup>**

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**Abstract:** A root-knot nematode from Portugal, *Meloidogyne lusitanica* n. sp., is described and illustrated from specimens obtained from olive trees (*Olea europaea* L.). Females of the new species have a characteristic perineal pattern with medium to high trapezoidal dorsal arch with distinct punctuations in the tail terminus area. The excretory pore is located posterior to the stylet, about 1.5–2.5 stylet lengths from the anterior end. The stylet is 17.1  $\mu\text{m}$  long with pear-shaped knobs. Males have a rounded, posteriorly sloping head cap and head region not annulated. The robust stylet, 24.5  $\mu\text{m}$  long, has large, elongate knobs. Mean length of the second-stage juveniles is 449.5  $\mu\text{m}$ , stylet length 14.2  $\mu\text{m}$ , and tail length 44.1  $\mu\text{m}$ . Scanning electron microscope observations provide further details of perineal patterns and head and stylet morphology of females, males, and second-stage juveniles. *Meloidogyne lusitanica* n. sp. did not reproduce on any of the differential hosts used to separate the four most common *Meloidogyne* species. The common name “olive root-knot nematode” is proposed for *M. lusitanica* n. sp.

**Key words:** light microscopy (LM), *Meloidogyne lusitanica* n. sp., morphology, morphometrics, new species, *Olea europaea*, olive root-knot nematode, olive tree, Portugal, root-knot nematode, scanning electron microscopy (SEM), taxonomy.

There are few reports of root-knot nematodes parasitizing olive (*Olea europaea* L.). Olive was first reported as a host for root-knot nematodes by Buhner et al. in 1933, for *M. javanica* (Treub, 1885) Chitwood, 1949 by Tarjan in 1953, and for *M. incognita* (Kofoid & White, 1919) Chitwood, 1949 and *M. hapla* Chitwood, 1949 by Minz in 1961. Diab and El-Eraki, in Egypt, and Lamberti and Lownsbery, in California, found *M. javanica* associated with olive. In southern Italy, *M. incognita* was detected in only 2% of the olive groves sampled and, in other parts of Italy, *M. javanica* and *M. incognita* were found in less than 1% of the surveyed olive orchards. In Jordan, Hashim reported the presence of *M. incognita* and *M. javanica* in a few groves and nurseries. Yang and Zhong identified four species: *M. javanica*, *M. incognita*, *M. arenaria* (Neal, 1889) Chitwood, 1949, and *M. acrita* (Chitwood, 1949) Esser, Perry & Taylor, 1976 causing damage to olive in

China. In 1982 Jimenez reported the four most common root-knot nematode species infecting olive trees in Chile, and Santos recovered *M. incognita* race 2 and *M. hapla* from heavily infected olive tree roots in Portugal. Recently *M. incognita* and *M. javanica* were found in Libya associated with olive roots.

Some years ago an undescribed *Meloidogyne* species was found on olive at Cadaixo, Miranda do Corvo, Portugal, and later, on the same host, at Carvalhal de Pussos, Alvaiázere, Portugal. Preliminary reports on this nematode have been given (1,2,4,11,12). This nematode is described, illustrated, and designated herein as a new species, *M. lusitanica* n. sp. The species epithet refers to the Roman Province that included most of Portugal proper. The common name “olive root-knot nematode” is proposed. This is the first species of *Meloidogyne* to be described from Portugal.

### MATERIALS AND METHODS

Morphological and morphometric studies were made from field populations because attempts to propagate the nematode on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) and several other plants were not successful. Egg masses and adult females

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were handpicked from infected olive roots, and second-stage juveniles were hatched from egg masses in moist chambers. Males were obtained by incubating pieces of washed infected roots in petri dishes with a small amount of water; roots were rinsed periodically with water and males were collected from the washings.

*Light microscope (LM) studies:* Eggs, freshly hatched second-stage juveniles, males, and females were transferred to 2% formalin and measured immediately. Perineal patterns of females were cut from live specimens in 45% lactic acid and mounted in glycerin (14). Photographs were taken with a bright field light microscope and drawings were made with a drawing tube. In some cases differential interference contrast (DIC) was used.

*Scanning electron microscope (SEM) studies:* Second-stage juveniles, males, and females were killed and fixed in 2% glutaraldehyde solution buffered with 0.1 M sodium cacodylate buffer at 7.2 pH for 48–72 hours, at 5 C. Post fixation was done with 2% osmium tetroxide solution for 12 hours at 5 C. Specimens were dehydrated in a graded series of ethanol, critical point dried with liquid CO<sub>2</sub>, mounted on double sided adhesive tape on SEM stubs, and coated with gold (8). Perineal patterns and stylets of juveniles, males, and females were prepared as described earlier (3,7). Specimens were viewed and photographed using a JEOL 35C scanning electron microscope operating at 20 kV accelerating voltage. At least 50 specimens of each life stage were examined.

*Preparation of type material:* Females were killed and fixed in hot TAF (7 ml 37% formaldehyde, 2 ml triethanolamine, 91 ml distilled water), processed by the rapid lactophenol method (9) and mounted in lactophenol on glass cavity slides. Males and juveniles were killed and fixed in 2% glutaraldehyde at 5 C for 1 week. Specimens were subsequently washed in sodium cacodylate buffer, pH 7.2, processed through an ethanol dehydration series to 100% ethanol, then transferred to a solution of 10% glycerin in 90% ethanol, and slowly infil-

trated with glycerin at room temperature (6). These specimens were mounted in desiccated glycerin on Cobb slides. All measurements are in micrometers ( $\mu\text{m}$ ) unless otherwise stated.

## SYSTEMATICS

### *Meloidogyne lusitanica* n. sp. (Figs. 1–9)

#### *Description*

*Holotype (female in lactophenol):* Body length, 695.0; body width, 510.0; neck length, 125.0; neck width, 100.0; stylet length, 19.0; stylet knobs height, 2.5; stylet knobs width, 4.5; dorsal esophageal gland orifice (DGO) to stylet base, 5.0; head end to posterior end of metacarpus, 134.0; metacarpus length, 45.0; metacarpus width, 37.0; metacarpus valve length, 12.0; metacarpus valve width, 9.0; excretory pore to head end, 50.0  $\mu\text{m}$ ;  $a = 1.4$ ; body length/head end to posterior end of metacarpus, 5.2; stylet knobs width/height, 1.8; metacarpus length/width, 1.2; metacarpus valve length/width, 1.3; excretory pore/stylet length, 2.6. Female as in general description. Perineal region not visible.

*Female:* Morphometric data of 30 females in Table 1. Females completely enclosed by gall tissue. Body pearly white, variable in size, elongate ovoid to pear shaped, with short neck, posteriorly rounded, without tail protuberance (Fig. 1H–N). Head region not set off from body, not annulated (Fig. 1A, B). Rounded prestoma located centrally on labial disc. Pore-like openings of six inner labial sensilla surround prestoma (Fig. 2A, B). Labial disc with two bumps on ventral side (Fig. 2A), slightly raised above medial lips. Medial lips usually indented medially, often dividing into lip pairs (Fig. 2A, B). Lateral lips large, fused laterally with head region for short distance (Fig. 2A, B). Amphidial openings oval shaped, between labial disc and lateral lips. Cephalic framework weakly developed. Stylet long, easily dislodged posteriorly (Figs. 1A–G; 2C–E). Stylet cone slightly curved dorsally, widening gradu-

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

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variability (%)
<b>Linear (<math>\mu\text{m}</math>)</b>					
Body length	620.0–1,115.0	873.3	21.13	115.71	13.3
Body width	345.0–650.0	534.0	12.71	69.60	13.0
Neck length	75.0–260.0	143.7	9.19	50.36	35.1
Neck width	50.0–200.0	127.7	7.22	39.56	31.0
Body length without neck	500.0–985.0	730.7	18.85	103.25	14.1
Stylet length	16.0–19.0	17.1	0.17	0.94	5.5
Stylet knobs height	2.0–3.0	2.5	0.06	0.35	14.2
Stylet knobs width	4.0–5.5	5.0	0.07	0.37	7.5
Dorsal esophageal gland orifice (DGO) to stylet base	3.0–5.0	4.0	0.11	0.62	15.7
Head end to posterior end of metacarpus	82.5–145.0	106.0	2.45	13.42	12.7
Metacarpus length	39.0–58.5	46.5	0.79	4.36	9.4
Metacarpus width	35.0–56.0	43.4	0.83	4.53	10.4
Metacarpus valve length	10.5–13.5	12.1	0.14	0.77	6.4
Metacarpus valve width	9.0–11.0	9.8	0.11	0.63	6.4
Excretory pore to head end	28.0–60.0	44.1	1.52	8.32	18.9
Vulva slit length	16.0–26.5	20.1	0.41	2.23	11.1
Anus to vulva (center) distance	14.5–26.0	19.3	0.50	2.76	14.3
Interphasmidial distance	16.5–35.5	27.9	0.81	4.41	15.8
<b>Ratios</b>					
a	1.3–1.9	1.6	0.03	0.17	10.3
Body length/head end to posterior end of metacarpus	5.2–10.6	8.3	0.24	1.30	15.6
Stylet knobs width/height	1.6–2.5	2.1	0.05	0.26	12.8
Metacarpus length/width	1.0–1.2	1.1	0.01	0.06	5.8
Metacarpus valve length/width	1.1–1.4	1.3	0.02	0.09	6.7
Excretory pore/stylet length	1.6–3.8	2.6	0.09	0.50	19.3

ally posteriorly; shaft of same width throughout, or widening slightly near junction with knobs; knobs well developed, distinctly separate, pear shaped. Distance between stylet base and dorsal esophageal gland orifice (DGO) long (3.0–5.0). Excretory pore located posterior to stylet about 1.5–2.5 stylet lengths or 16–23 annules from anterior end.

Characteristic cuticular pattern of perineal region (Figs. 3–5), trapezoidal in shape; striae coarse, sometimes continuous, smooth to wavy, forming a medium to high, trapezoidal, dorsal arch. Ventral pattern area with fine, smooth or wavy striae. Lateral lines indicated by dorsal and ventral striae meeting at an angle, or by short lateral striae, sometimes striae discontinuous, may fork near lateral lines. Occasionally, some striae may extend laterally, forming one or two wings. Tail tip area well defined, marked by few punctuations; striae

often bending toward vulva. Perivulval region free of striae. Phasmids distinct; surface structure not apparent in SEM (Fig. 5).

*Allotype (male in glycerine):* Body length, 1,240.0; greatest body width 34.5; body width at stylet knobs, 20.0; body width at excretory pore, 27.5; head region height, 3.5; head region width, 12.0; stylet length, 24.0; stylet cone length, 13.0; stylet shaft and knobs length, 11.0; stylet knobs height, 3.0; stylet knobs width, 4.5; dorsal esophageal gland orifice (DGO) to stylet base, 4.5; head end to metacarpus valve, 106.0; metacarpus width, 9.0; metacarpus valve length, 5.0; metacarpus valve width, 3.5; head end to excretory pore, 137.5; testis length, 750.0; tail length, 11.5; spicule length, 34.5; gubernaculum length, 10.0; a = 35.9; c = 107.8; body length/head end to metacarpus valve, 11.7; head region width/height, 3.4; stylet length/body

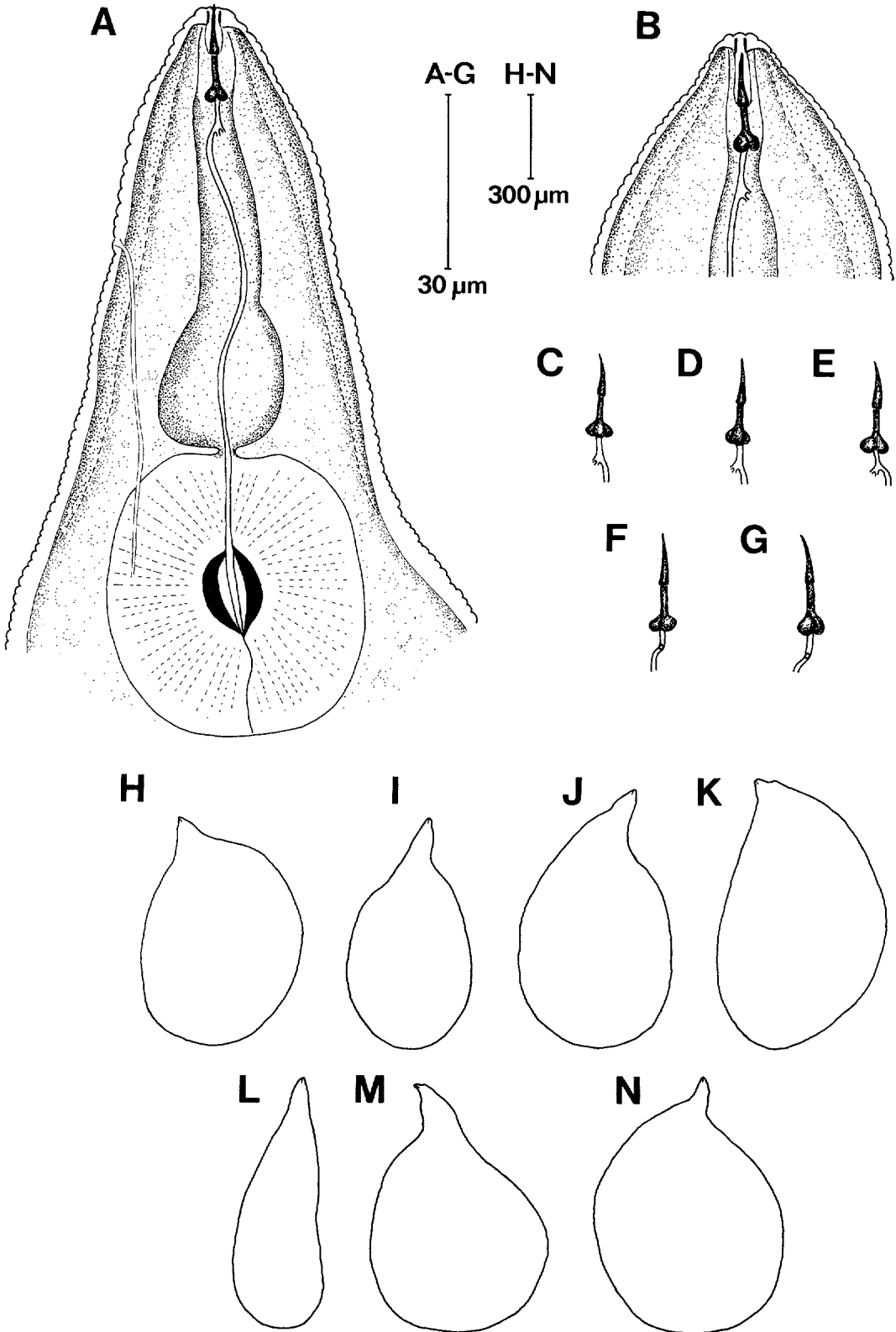


FIG. 1. Drawings of females of *Meloidogyne lusitanica* n. sp. A) Esophageal region, lateral. B) Anterior region, lateral. C-G) Stylets. H-N) Outlines of whole specimens.

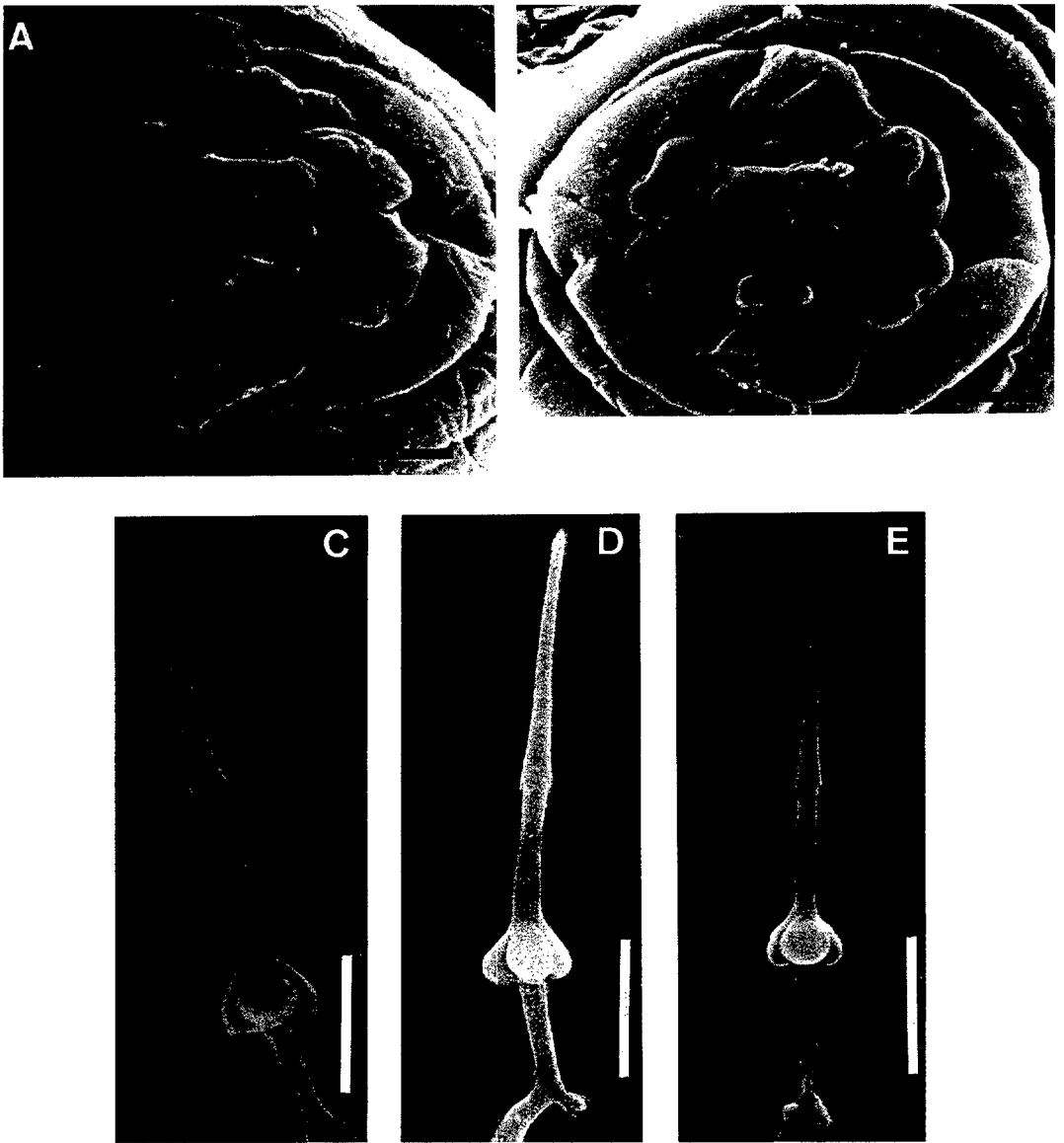


FIG. 2. SEM photographs of females of *Meloidogyne lusitanica* n. sp. A, B) Face views of head. C-E) Stylets. Scale bars: A, B = 1  $\mu$ m; C-E = 5  $\mu$ m. Arrows indicate the bumps on labial disc.

width at stylet knobs, 1.2; stylet knob width/height, 1.5; metacarpus valve length/width, 1.4; excretory pore, 11.1%; T = 60.5%. Male as in general description.

*Male:* Morphometric data of 30 males in Table 2. Body vermiform, tapering anteriorly, bluntly rounded posteriorly. Head cap in lateral view quite high and rounded, extending posteriorly onto distinctly set off head region (Figs. 6B, C; 7C, D). In SEM (face view), labial disc very large and round

(Fig. 7B), stoma slit-like located in ovoid prestomatal cavity surrounded by pore-like openings of six inner labial sensilla. Medial lips with smooth or slightly indented outer margins, fused with labial disc to form a continuous elongate structure with parallel sides. Four cephalic sensilla marked by cuticular depressions on medial lips. Amphidial apertures large, elongate slits between labial disc and lateral sectors of head region. Lateral lips absent. Head region usu-

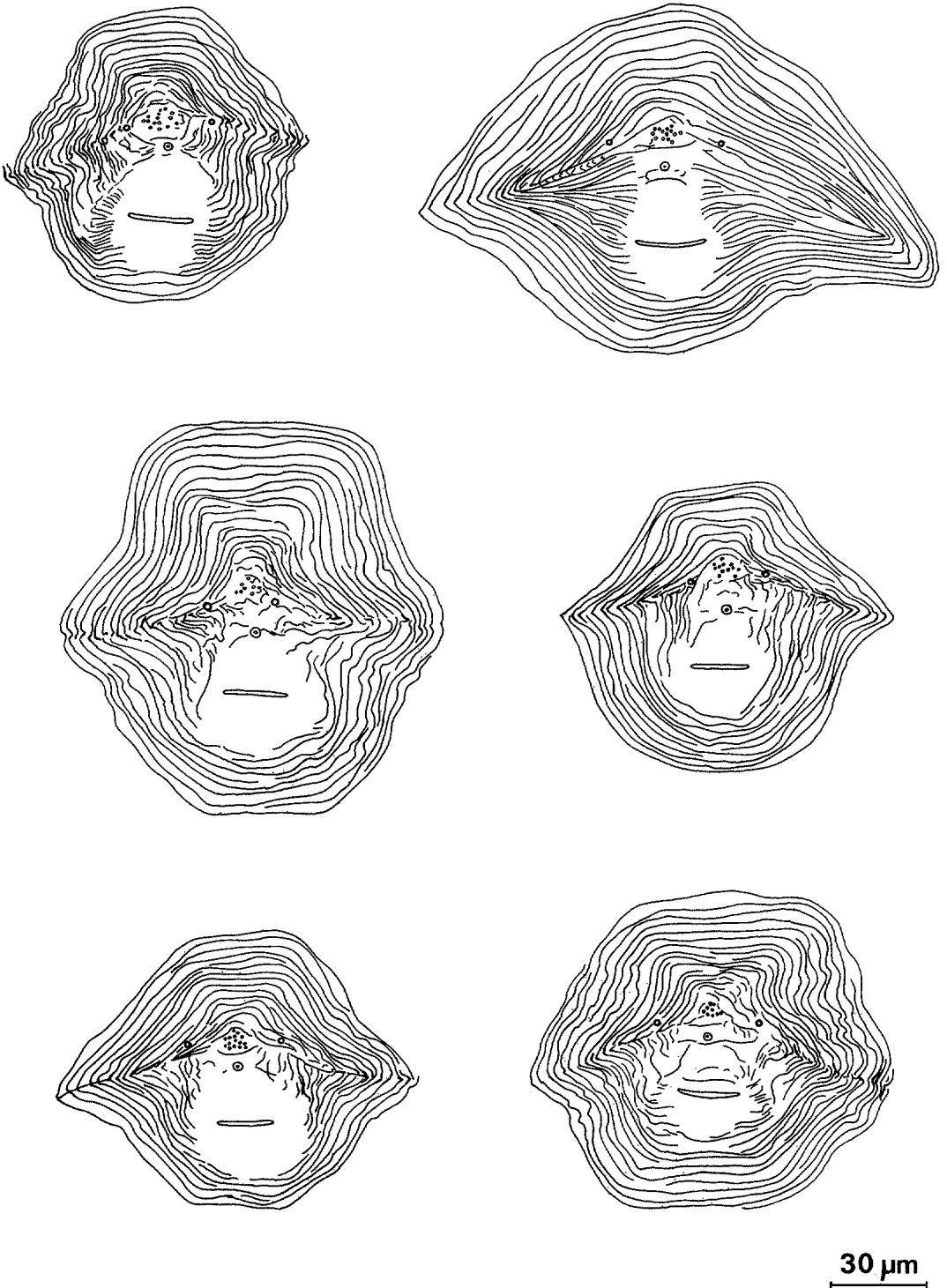


FIG. 3. Drawings of perineal patterns of *Meloidogyne lusitanica* n. sp.

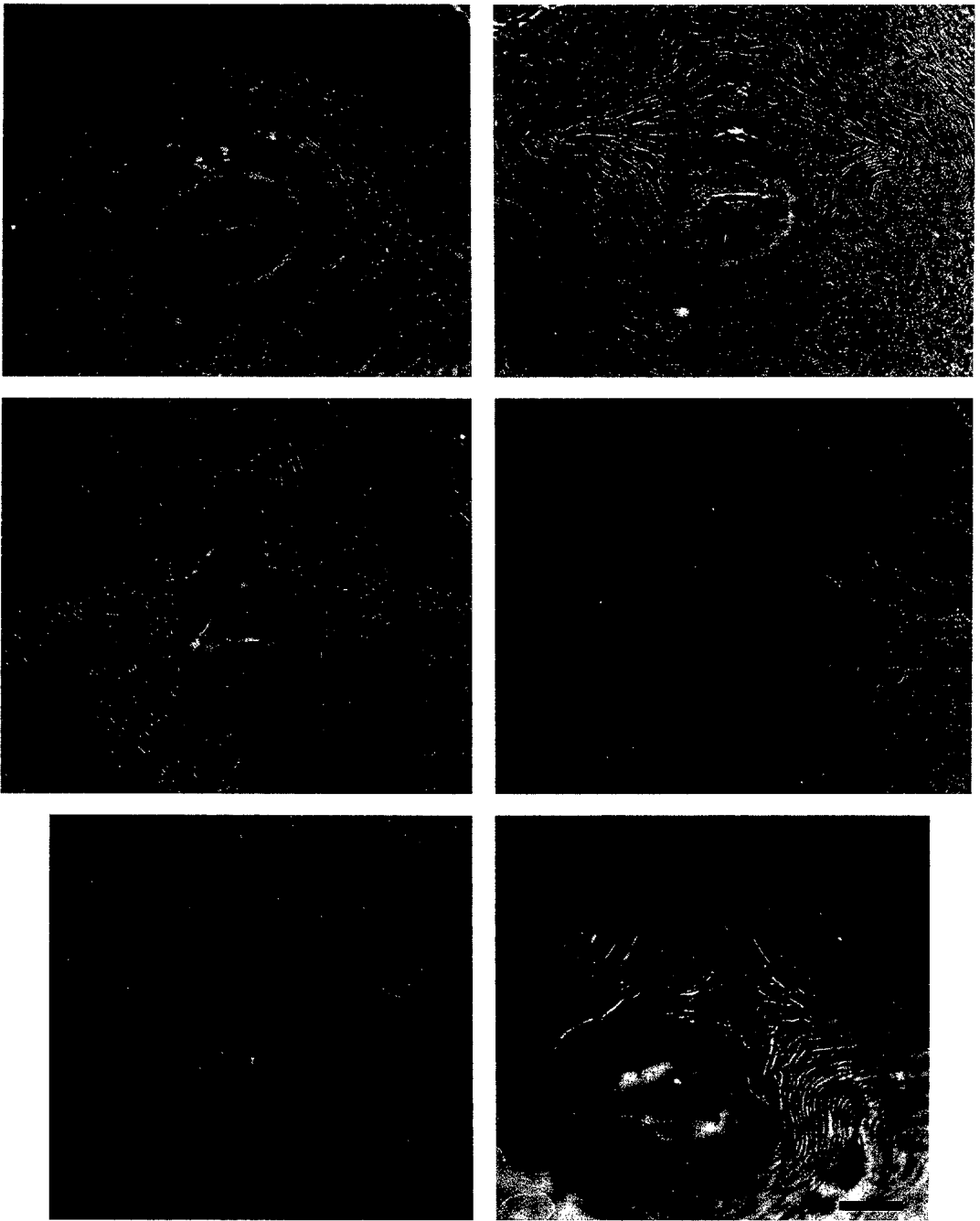


FIG. 4. Perineal patterns of *Meloidogyne lusitanica* n. sp. showing typical variation. A-E) LM photographs. F) Differential interference contrast micrograph showing punctuations in the tail terminus area. A-E same scale as F; bar = 20  $\mu$ m.

ally smooth, but may have one or two incomplete annulations. Body annules distinct. Lateral field with four incisures beginning near level of stylet base as two incisures. In LM (Fig. 6A-D), cephalic

framework moderately developed. Stylet robust, large (Figs. 6A-G; 7C-E); cone straight, pointed, gradually increasing in diameter posteriorly; stylet opening marked by very faint protuberance several

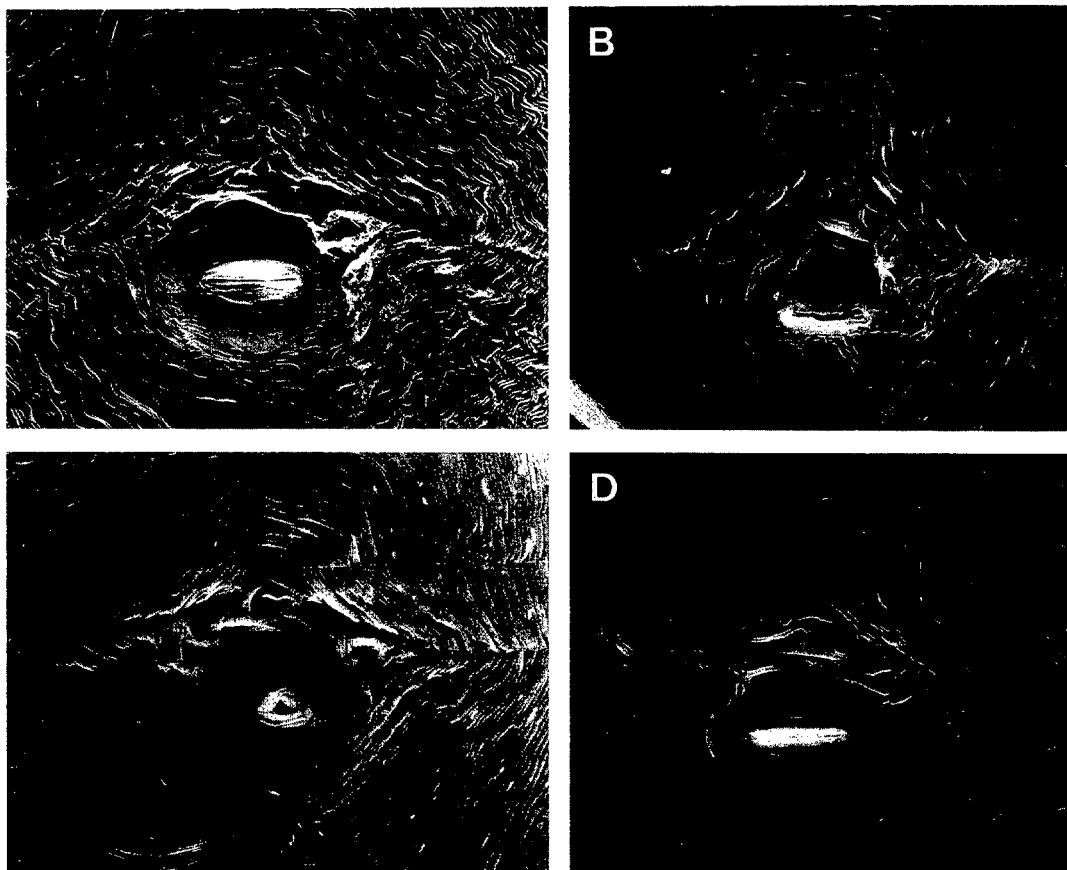


FIG. 5. SEM photographs of perineal patterns of *Meloidogyne lusitanica* n. sp. A–C same scale as D; bar = 20  $\mu$ m.

micrometers from stylet tip; shaft cylindrical; knobs elongate, pear shaped, slightly set off from shaft. Distance of DGO to stylet knob base long (4.0–6.0). Procorpus and metacarpus distinct (Fig. 6A), metacarpus elongate, oval shaped with large valve plates. Hemizonid distinct, three or four annules anterior to well-defined excretory pore. Testis usually one, sometimes two, generally outstretched. Spicules long, moderately curved ventrally; gubernaculum crescent shaped (Fig. 6H). Tail short, terminus not striated. Phasmids posterior to cloacal level (Fig. 6H, I).

*Second-stage juvenile:* Morphometric data of 30 specimens in Table 3. Body vermiform, clearly annulated, tapering more posteriorly than anteriorly. In SEM (Fig. 9D), prestoma opening ovoid, surrounded by small, pore-like openings of six inner

labial sensilla. Labial disc, medial lips, and lateral lips fused into one structure. Labial disc rounded, slightly raised above medial lips. Medial lips with crescent-shaped to rounded margins, sometimes slightly indented, suggesting subdivision into lip pairs (Fig. 9B), wider than labial disc. Cephalic sensilla not expressed externally (Fig. 9A–D). Amphidial apertures between labial disc and lateral lips. Head cap narrower than head region. Head region smooth, occasionally with one or two short, incomplete annulations. Lateral field marked by four incisures. Cephalic framework weak (Fig. 8A, E–H). Stylet long, but delicate (Figs. 8A–H, 9E, F). Stylet cone sharply pointed, increases in width gradually posteriorly; shaft cylindrical; knobs distinctly separated, pear shaped, slightly set off from shaft. Distance of dorsal esophageal gland orifice



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

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variability (%)
<b>Linear (<math>\mu\text{m}</math>)</b>					
Body length	960.0–1,980.0	1,613.2	43.83	240.08	14.8
Greatest body width	34.5–52.0	43.0	1.00	5.48	12.7
Body width at stylet knobs	16.0–23.0	20.8	0.31	1.67	8.0
Body width at excretory pore	26.0–41.5	33.5	0.68	3.72	11.1
Head region height	3.5–5.0	4.3	0.09	0.51	11.9
Head region width	12.0–14.0	12.7	0.11	0.62	4.9
Stylet length	21.0–27.0	24.5	0.26	1.41	5.8
Stylet cone length	11.0–15.0	13.6	0.20	1.11	8.2
Stylet shaft and knobs length	9.5–12.0	11.0	0.12	0.67	6.1
Stylet knobs height	2.5–3.5	3.1	0.05	0.27	8.7
Stylet knobs width	4.0–5.5	4.7	0.08	0.43	9.1
Dorsal esophageal gland orifice (DGO) to stylet base	4.0–6.0	5.0	0.12	0.66	13.1
Head end to metacarpus valve	73.0–110.5	98.3	1.71	9.38	9.5
Metacarpus width	9.0–12.5	11.2	0.21	1.16	10.3
Metacarpus valve length	4.8–7.5	5.8	0.14	0.75	13.1
Metacarpus valve width	3.0–5.5	4.0	0.11	0.58	14.4
Head end to excretory pore	130.0–207.0	169.6	3.67	20.04	11.8
Testis length	440.0–1,045.0	821.7	23.52	128.82	15.7
Tail length	10.5–15.0	12.5	0.24	1.33	10.7
Spicule length	32.0–44.5	37.9	0.53	2.92	7.7
Gubernaculum length	8.5–12.0	10.2	0.15	0.82	8.1
Phasmids to tail tip	7.0–12.0	9.2	0.26	1.43	15.6
<b>Ratios</b>					
a	26.3–45.3	37.7	0.87	4.76	12.6
c	76.8–170.5	130.2	3.92	21.48	16.5
Body length/head end to metacarpus valve	11.3–19.6	18.4	0.31	1.72	10.5
Head region width/height	2.5–3.6	3.0	0.05	0.30	9.8
Stylet length/body width at stylet knobs	1.0–1.3	1.2	0.02	0.09	7.2
Stylet knob width/height	1.3–1.8	1.5	0.03	0.15	10.1
Metacarpus valve length/width	1.1–2.0	1.5	0.04	0.24	16.2
<b>Percentages</b>					
Excretory pore	8.1–15.7	10.7	0.36	1.97	18.4
T	40.4–70.6	51.3	1.28	7.02	13.7

to stylet base, 3.5–4.5. Metacarpus ovoid with prominent valve plates. Excretory pore distinct; hemizonid one or two annules anterior to excretory pore. Tail short (39.0–50.0), conoid with rounded unstriated terminus; hyaline tail terminus distinct. Rectal dilation large. Phasmids small, always below level of anus.

*Egg* ( $n = 30$ ): Length, 107.5–144.0 (124.4; SE 1.70; SD 9.33; CV 7.5%); width, 37.0–57.0 (47.2; SE 0.76; SD 4.14; CV 8.8%); length/width ratio, 2.4–2.9 (2.7; SE 0.03; SD 0.16; CV 6.1%). Egg morphology similar to that of other *Meloidogyne* species.

#### *Type host and locality*

Roots of olive trees (*Olea europaea* L. cv. Galega) in a field near Cadaixo, Miranda do Corvo, Portugal.

#### *Type specimens*

*Holotype* (female): Isolated from olive tree roots collected from type locality. Slide no. 57/1, deposited in the Museu e Laboratório Zoológico Nematode Collection, Universidade de Coimbra, Coimbra, Portugal. *Allotype* (male): Same data as holotype. Slide no. 57/2, Museu e Laboratório

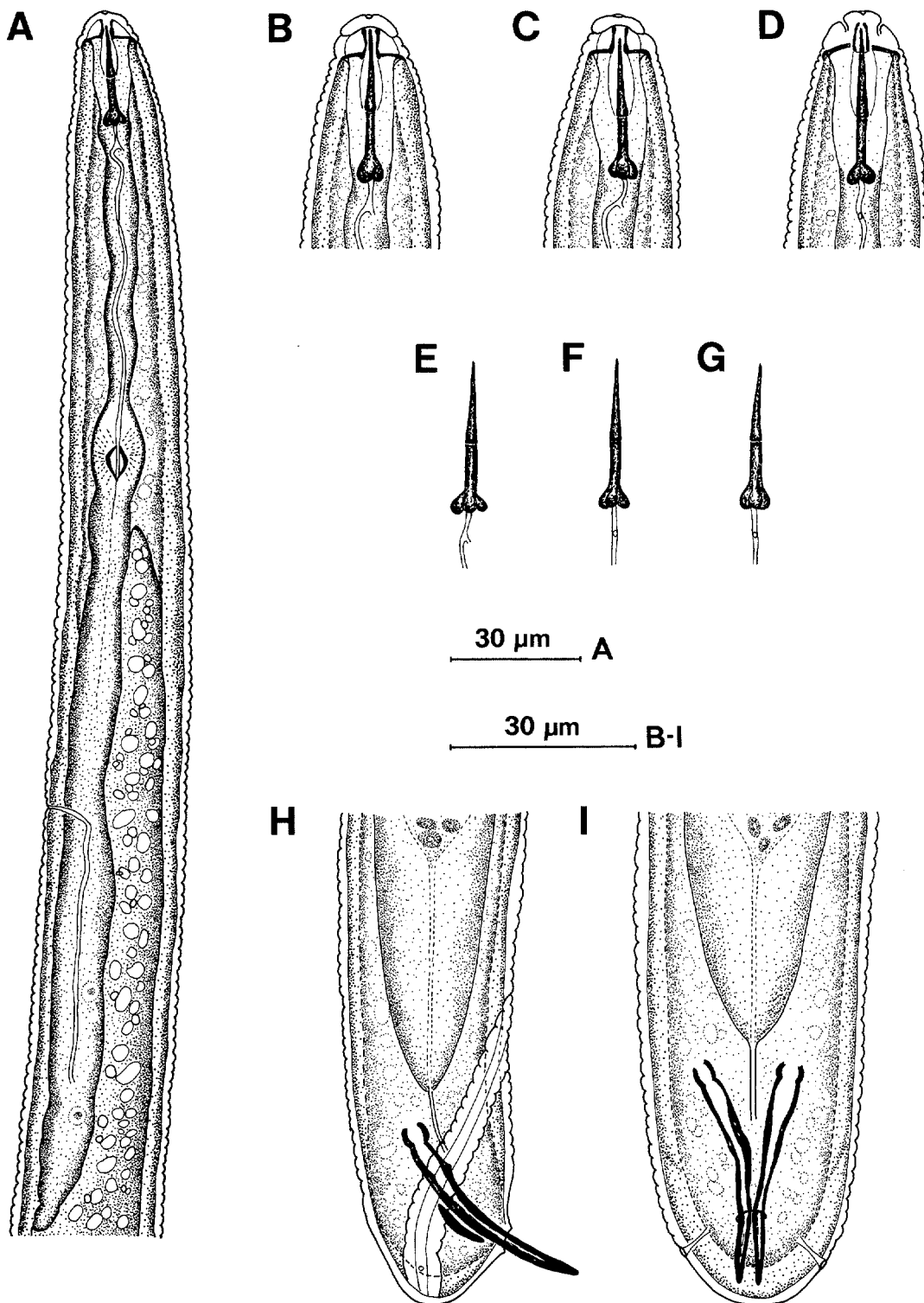


FIG. 6. Drawings of males of *Meloidogyne lusitanica* n. sp. A) Esophageal region, lateral. B-D) Anterior regions; lateral, lateral, and dorsal. E-G) Stylets. H, I) Tails, lateral and ventral.

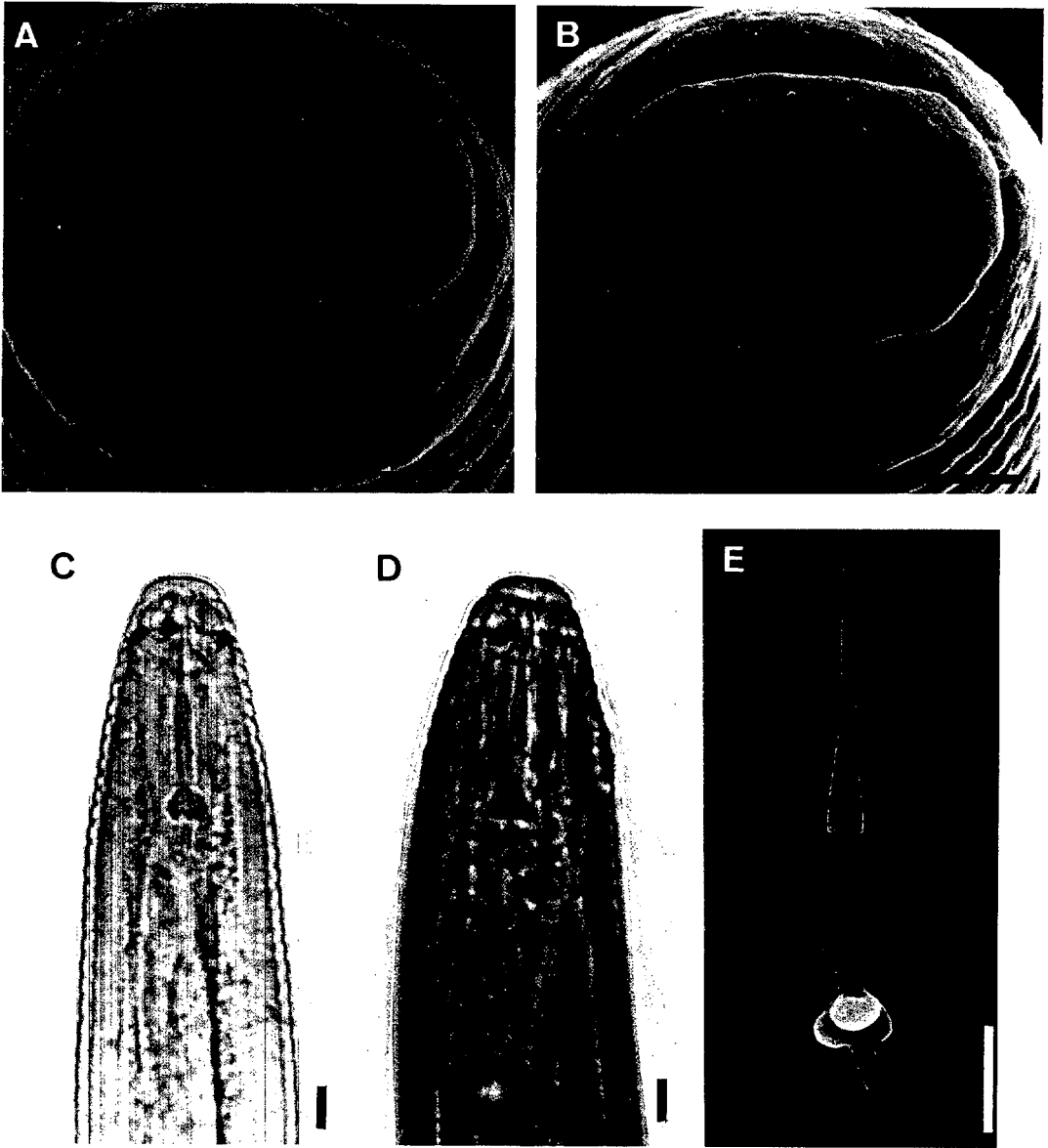


FIG. 7. Males of *Meloidogyne lusitanica* n. sp. A, B) SEM photographs of heads, face views. C, D) LM photographs of anterior regions. E) SEM photograph of excised stylet. Scale bars: A, B = 2  $\mu\text{m}$ ; C, D = 4  $\mu\text{m}$ ; E = 5  $\mu\text{m}$ .

Zoológico Nematode Collection, Universidade de Coimbra, Coimbra, Portugal. *Paratypes* (females, males, and second-stage juveniles): Same data as holotype. One female, six perineal patterns, one male and five second-stage juveniles, Rothamsted Experimental Station Nematology Collection, Harpenden, Herts., U.K. One female, six perineal patterns, one male, and six second-stage juveniles, United States Depart-

ment of Agriculture Nematode Collection (USDANC), Beltsville, Maryland, U.S.A. Females, perineal patterns, males, and second-stage juveniles in Museu e Laboratório Zoológico Collection, Universidade de Coimbra, Coimbra, Portugal.

#### *Diagnosis*

*Meloidogyne lusitanica* n. sp. can be distinguished from other species in the genus

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

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variability (%)
Linear ( $\mu\text{m}$ )					
Body length	390.0–515.0	449.5	6.44	35.29	7.9
Greatest body width	17.0–20.0	18.8	0.17	0.95	5.0
Body width at stylet knobs	11.0–12.0	11.5	0.08	0.43	3.7
Head region height	1.8–2.5	2.0	0.02	0.11	5.6
Head region width	6.0–6.5	6.3	0.05	0.25	4.1
Stylet length	13.0–16.0	14.2	0.16	0.87	6.1
Stylet shaft and knobs	5.5–6.0	5.8	0.04	0.23	4.0
Stylet knobs height	1.0–1.5	1.3	0.04	0.19	15.2
Stylet knobs width	2.0–2.5	2.2	0.04	0.19	8.7
Stylet base to head end	15.0–18.0	16.4	0.14	0.77	4.7
Dorsal esophageal gland orifice (DGO) to stylet base	3.5–4.5	3.9	0.07	0.36	9.1
Metacarpus valve length	4.0–4.8	4.4	0.05	0.25	5.8
Metacarpus valve width	3.2–3.8	3.5	0.03	0.16	4.7
Head end to metacarpus valve	55.0–73.0	63.2	0.74	4.05	6.4
Body width at excretory pore	16.0–18.5	17.2	0.14	0.75	4.4
Excretory pore to head end	78.0–102.0	90.3	0.99	5.41	6.0
Body width at anus	12.5–14.5	13.2	0.12	0.68	5.1
Tail length	39.0–50.0	44.1	0.54	2.97	6.7
Tail terminus length	10.0–14.0	12.0	0.23	1.24	10.4
Tail terminus width at beginning	5.5–7.0	6.1	0.09	0.47	7.7
Ratios					
a	20.0–27.4	24.1	0.34	1.84	7.7
c	9.1–11.4	10.2	0.09	0.50	4.9
d	3.0–3.7	3.3	0.03	0.19	5.7
Head region width/height	2.6–3.3	3.1	0.03	0.18	5.8
Stylet knobs width/height	1.5–2.1	1.8	0.04	0.19	10.6
Body length/head end to metacarpus valve	6.4–8.1	7.1	0.07	0.40	5.7
Metacarpus valve length/width	1.1–1.4	1.3	0.01	0.08	6.2
Tail length/tail terminus length	3.1–4.3	3.7	0.07	0.37	9.6
Percentage					
Excretory pore	18.0–22.3	20.2	0.22	1.21	6.0

by the following characteristics: The perineal pattern has a medium to high trapezoidal dorsal arch and distinct punctuations in the tail terminus region. Distance from the excretory pore to head end of the female is 44.1 (28.0–60.0). Head cap in the male is rounded and extends posteriorly into the head region. This male characteristic is confirmed by SEM observations which show that labial disc and median lips are fused to form elongate lip structures. Head region is often marked by one broken annulation. Second-stage juvenile head region is smooth or with one or two short broken annules. Tail shape is conoid with rounded unstriated terminus.

*Relationships*

*Meloidogyne lusitanica* n. sp. is most similar to *M. megatyla* Baldwin & Sasser, 1979 and *M. partityla* Kleynhans, 1986. However, *M. lusitanica* n. sp. differs from these species by the presence of distinct punctuations above the anus in perineal patterns, by the absence of deep indentations in the stylet knobs of the female, male, and second-stage juvenile; by the more posterior position of the excretory pore in the female, which in *M. megatyla* is 20.4 (12.1–32.3) and in *M. partityla* is 27.9 (17.3–41.0). SEM examination of the anterior views of males showed details of the head morphology which slightly resembles that of

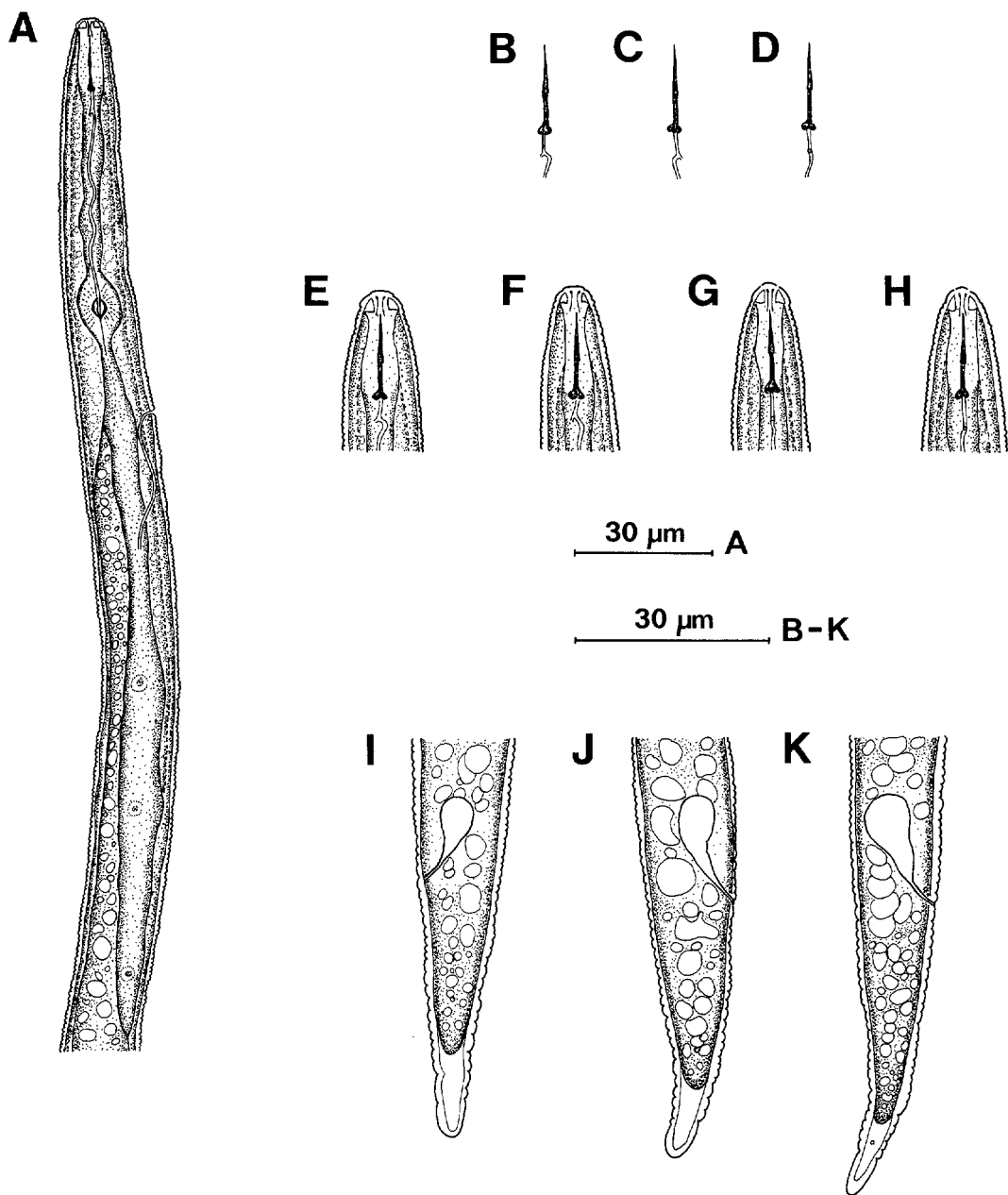


FIG. 8. Drawings of second-stage juveniles of *Meloidogyne lusitanica* n. sp. A) Esophageal region, lateral. B-D) Stylets. E-H) Anterior regions; lateral, lateral, dorsal, and ventral. I-K) Tail, lateral.

*M. arenaria* race B (5). Length of the second-stage juveniles of *M. lusitanica* n. sp. is 449.5 (390.0–511.0), making it closely related to *M. megatyla* and *M. partityla*; however, they are distinct in tail and rectum shapes. Biochemically the new species has a single esterase band at Rm 0.52 similar

to *M. arenaria* (10). *Meloidogyne lusitanica* n. sp. has also a specific and unique malate dehydrogenase phenotype (10).

*Biology*

Egg masses of *M. lusitanica* n. sp. are always large and yellowish to reddish in col-

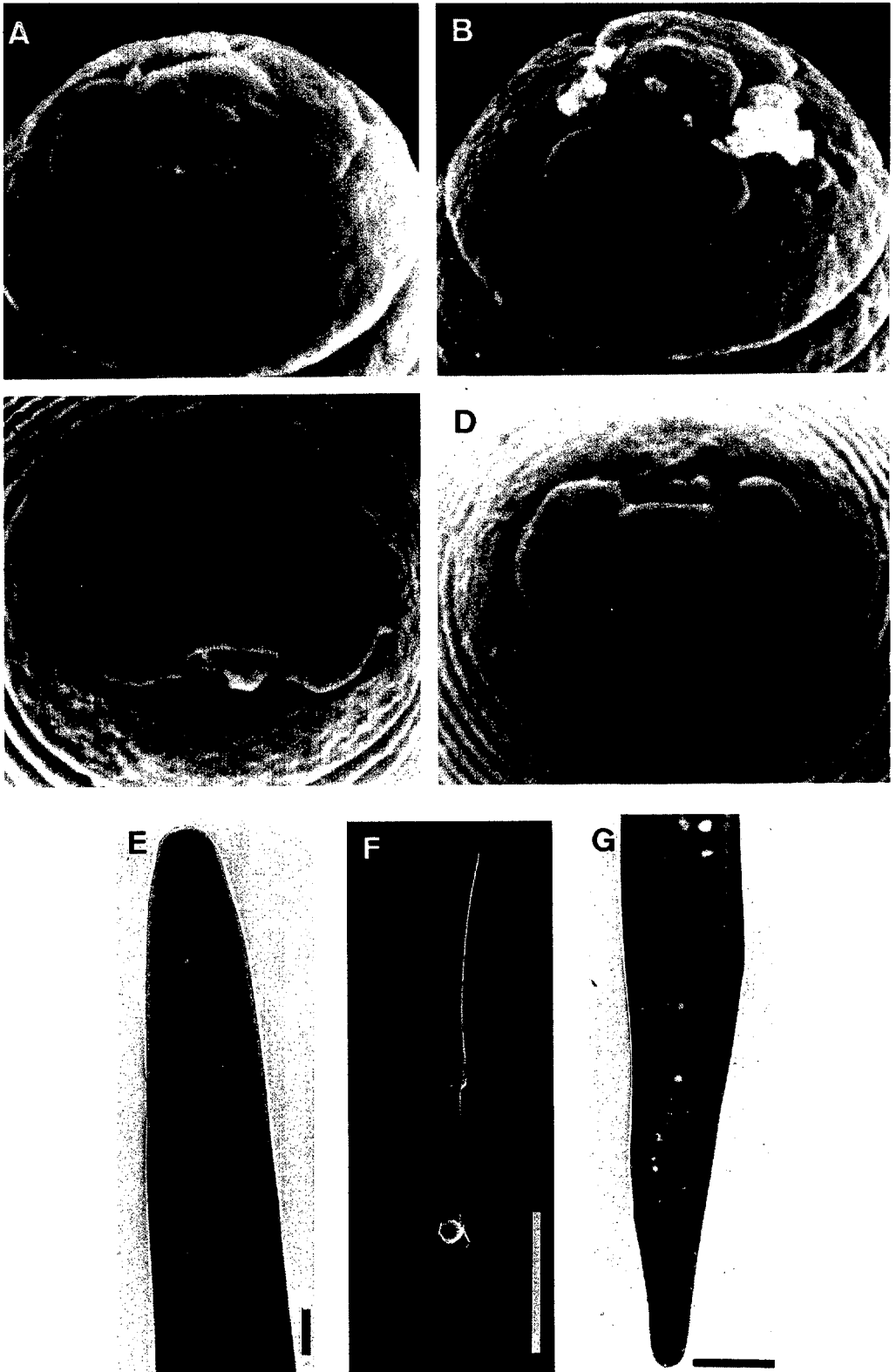


FIG. 9. Second-stage juveniles of *Meloidogyne lusitanica* n. sp. A, B) SEM photographs of heads, lateral views. C, D) SEM photographs of heads, face views. E) LM photograph of anterior region. F) SEM photograph of excised stylet. G) LM photograph of tail. Scale bars: A-C same scale as D = 1  $\mu\text{m}$ ; E, F = 5  $\mu\text{m}$ ; G = 10  $\mu\text{m}$ .

or. No females were observed to protrude from roots.

Among the host differentials (13) commonly used for identification of *Meloidogyne* species, watermelon and tomato were lightly galled but no reproduction occurred. No infection was found on tobacco, cotton, pepper, or peanut.

Geographic distribution of *M. lusitanica* n. sp. is not known, although in a survey of Portuguese olive fields this species was found in only 2 of the 90 fields sampled.

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