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MELOIDOGYNE ULMI SP. N., A ROOT-KNOT NEMATODE FROM ELM

by

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Summary. A root-knot nematode, *Meloidogyne ulmi* sp. n. found on elm plants in Tuscany (Italy), is described and illustrated. *M. ulmi* sp. n. can be distinguished among *Meloidogyne* species with similar second stage juvenile tail lengths (24.2-37.5 μm in *M. ulmi* sp. n.) by a combination of characters: in the female stylet slender (12.0-15.7 μm long), moderately curved, knobs rounded or transversely ovoid, slightly concave anteriorly, perineal patterns oval with dorsal arch low to medium high, tail end area well delimited with punctations in some specimens, lateral field indistinct or marked by folds, sometimes by lateral lines at one or both sides; in the male lateral field areolated, usually with four, frequently with five to seven lines, labial cap shallowly rounded, one fifth to one fourth as high as the postlabial head region and a little narrower than the same region, stylet 17.5-22.9 μm long, with knobs rounded to pear shaped, more or less backwardly sloping; in the second stage juvenile lateral field areolated, usually marked by four, sometimes, five or six, lines, stylet short (8.5-11.1 μm long), hemizonid posteriorly adjacent or posterior to the excretory pore, tail conical, tapering to a finely rounded, almost pointed terminus or broader and rounded at the tip, cuticular constrictions along the hyaline part. Under SEM females, males and second stage juveniles show a labial disc set off from the medial lips. The only host plants so far known for *M. ulmi* sp. n. are *Ulmus chenmoui* and *U. glabra*.

During 1995-1998 galled root samples containing root-knot nematodes were collected at San Rossore (Pisa, Italy) from slowly declining *Ulmus chenmoui* trees. The plants, previously imported from the Netherlands as three year old plants, were growing on experimental plots of the Istituto per la Patologia degli Alberi Forestali, CNR, Florence, Italy. The fine feeder roots of the trees had small rounded galls while the older roots had larger knots (Fig. 1A). The galls contained mature females (Fig. 1B), often embedded in a capsule of suberized tissue with egg masses partly appearing at the root surface. Very often a few males were coiled around a single female into the egg sac. Subsequently, populations of a root-knot nematode, apparently the same species, were found at Mantignano (Florence, Italy) on *U. chenmoui* Cheng and *U.*

glabra Huds. plants imported from the Netherlands and used as rootstock of *Ulmus* hybrid selections.

The nematode observed in both localities differs from other known *Meloidogyne* species and is here designated and described as *Meloidogyne ulmi* sp. n.

Materials and methods

All observations were made on the original populations from elm roots. Adult females were removed intact from fresh roots, second stage juveniles and males were extracted by incubating in water fresh samples of infected roots with adhering soil. Some roots were stored overnight in a water solution of cellulase (305

µg/100 ml) to make the extraction of females easier.

For light microscopy (LM) studies, adult females were fixed in hot TAF and cut in half; the anterior regions were directly mounted in TAF (Courtney *et al.*, 1955) or in glycerol, the posterior regions were transferred to 45% lactic acid and mounted in glycerol (Taylor and Netscher, 1974). Males and second stage juveniles were fixed in hot TAF and mounted in TAF (Jepson, 1987) or glycerol after processing by the glycerol-ethanol method (Seinhorst, 1959). Some males, females and second stage juveniles were fixed in FP 4: 1 and mounted in lactophenol with cotton blue stain. Permanent mounts of female anterior and posterior regions, males and second stage juveniles were made in glycerol. Drawings were made with a drawing tube, photographs and measurements were taken with a LM. All measurements are in µm. For each character mean, standard deviation and range were calculated.

For scanning electron microscopy (SEM) freshly collected females, males and juveniles were fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 h and post-fixed in osmium tetroxide 2% overnight at 4 °C. Perineal patterns were cut from the females in glutaraldehyde. Specimens were dehydrated in a seven-graded ethanol series, critical point dried, mounted on stubs, sputter coated with gold. The nematodes were examined with a Philips SEM.

***MELOIDOGYNE ULMI* sp. n.**

(Table I; Figs. 1-8)

Description

Measurements of holotype female, allotype male, female, male and second stage juvenile paratypes are in Table I.

Female: body pear shaped, rounded, rarely saccate, neck straight to somewhat arcuate, var-

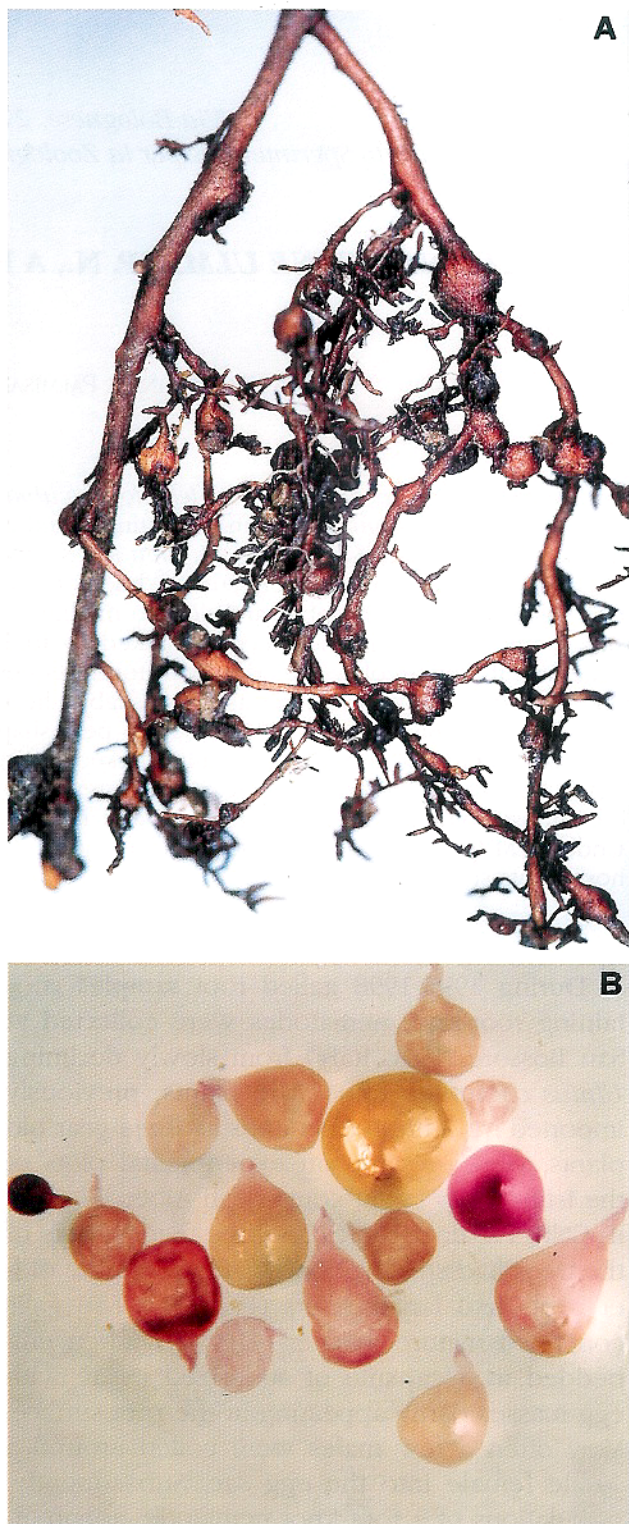


Fig. 1 - *Meloidogyne ulmi* sp. n.: A, elm roots with root galling; B, females.

TABLE I - *Measurements of Meloidogyne ulmi sp. n.*

	Holotype ♀	Allotype ♂	Paratypes 30 ♀♀	Paratypes 30 ♂♂	Juveniles 30
Body length	702.4	1508.9	770.9±140.4 (568.4-1043.4)	1461.7±189.9 (1052.7-1776.3)	412.6±20.6 (372.7-460.4)
Max. body width	576.5	38.7	618.3±151.6 (357.3-1006.9)	36.9±4.3 (26.6-48.4)	14.2±1.8 (12.1-18.2)
Body width at stylet knobs		18.2		16.7±1.3 (14.5-19.4)	7.6±1.0* (6.1-9.7)
Body width at excretory pore		30.3		27.6±2.6 (23.0-31.5)	12.4±1.8* (9.7-16.9)
Body width at anus		20.6		20.7±4.1 (14.5-31.5)	8.4±1.0* (7.3-10.9)
Head height		4.8		5.5±0.5 (4.8-6.1)	3.4±0.3 (3.0-4.2)
Head width		10.9		10.4±0.5 (9.7-10.9)	4.8±0.2 (4.2-5.2)
Neck length	126.4		164.9±67.0 (58.2-381.6)		
Stylet length	13.3	18.8	14.2±1.0 (12.0-15.7)	19.4±1.2 (17.5-22.9)	10.0±0.8 (8.5-11.1)
Conus length	7.3	10.9	7.2±0.8* (5.9-8.5)	10.8±1.0 (9.1-13.1)	5.2±0.4 (4.6-6.1)
Anterior end to stylet base		22.4		22.2±0.9* (20.6-24.2)	14.3±1.0 (12.1-15.7)
Stylet knobs height	2.4	2.4	1.8±0.6* (1.1-3.9)	2.4±0.1 (2.0-3.0)	1.2 constant
Stylet knobs width	4.8	4.2	3.5±0.7* (2.6-5.2)	3.9±0.4 (3.0-4.8)	2.4±0.1 (1.8-2.4)
D.G.O.	4.8	6.1	4.6±0.8 (3.3-6.5)	6.3±0.8 (4.8-8.5)	3.6±0.4 (3.0-4.8)
Anterior end to median bulb center		92.0		89.0±9.3 (65.3-108.9)	55.9±3.2 (47.2-60.7)
Anterior end to median bulb base		101.6		98.6±9.8 (76.2-118.6)	62.2±3.6* (53.2-66.6)
Median bulb length	38.7	20.6	42.6±6.5 (32.7-58.8)	19.3±3.0 (15.0-27.8)	12.6±1.2 (10.4-16.9)
Median bulb width	37.5	12.1	40.9±7.0 (31.3-59.0)	11.4±1.6 (7.3-16.9)	7.9±0.7 (6.7-9.7)
Median bulb valve length	13.3	6.1	12.4±1.0 (11.1-14.4)	6.0±0.9 (4.2-7.3)	3.9±0.5 (3.3-4.8)
Median bulb valve width	10.9	3.6	9.7±1.4 (7.2-12.4)	4.2±0.6 (3.6-6.1)	3.6±0.2 (3.0-4.2)
Anterior end to excretory pore	26.6	162.1	32.3±7.8 (15.7-45.1)	147.1±18.8 (96.8-188.8)	72.2±5.1 (60.1-77.4)
Excretory pore to median bulb base		60.5		48.7±12.1 (20.6-76.2)	11.6±3.8 (4.8-19.4)
Oesophageal glands overlapping intestine length		146.4		138.6±19.4* (96.8-162.1)	74.9±17.6* (43.6-105.3)
Tail length		10.9		11.5±1.1 (8.5-13.3)	31.3±3.1 (24.2-37.5)
Hyaline tail terminus length					8.2±1.8 (4.8-12.7)

TABLE I - *Continued.*

	Holotype ♀	Allotype ♂	Paratypes 30 ♀♀	Paratypes 30 ♂♂	Juveniles 30
Anterior end to genital primordium center					253.7±13.2* (217.8-269.8)
Testis length		637.7		716.1±167.0 (324.3-976.5)	
Spicule length		35.1		33.8±1.9 (30.0-37.5)	
Gubernaculum length		7.3		9.0±0.9 (7.3-9.8)	
Vulval slit length	21.2		22.0±2.9 (17.0-28.7)		
Vulva - anus distance	18.2		19.0±1.9 (15.0-22.2)		
Interphasmidial distance	21.2		19.2±3.8 (13.7-28.9)		
Level of phasmids to vulva	26.6		25.1±4.2 (15.7-39.2)		
Level of phasmids to anus	8.5		6.9±2.2 (2.6-15.7)		
Phasmids - posterior end		10.9		8.5±2.0 (4.8-11.5)	
a	1.2	39.0	1.3±0.3 (1.0-2.4)	39.7±4.0 (32.9-46.3)	29.5±3.4 (22.3-35.5)
m	54.6	58.1	51.8-5.1* (43.5-62.1)	55.7±2.5 (51.7-60.0)	50.3±3.8 (44.4-57.1)
b		13.4		13.1±1.1* (11.3-15.1)	5.7±0.3 (5.3-6.4)
b'		5.8		5.8±0.5* (5.0-6.9)	3.0±0.5 (2.3-4.4)
b''		16.4		16.3±1.5 (12.0-19.1)	7.4±0.3 (6.7-8.3)
b1		14.9		15.0±1.2 (11.4-17.7)	6.6±0.3* (6.0-7.4)
c		138.6		128.3±19.2 (87.0-172.9)	13.3±1.2 (11.5-16.6)
c'		0.5		0.6±0.1 (0.5-0.8)	3.7±0.5 (2.5-4.7)
T		42.3		48.7±9.7 (27.9-70.9)	
Head height/width		0.4		0.6±0.1 (0.4-0.6)	0.7±0.1 (0.6-0.9)
Stylet knobs width/height	2.0	1.8	2.1±0.7 (1.3-3.5)	1.7±0.2 (1.3-1.9)	2.0±0.1 (1.5-2.0)
Median bulb length/width	1.0	1.7	1.0±0.1 (0.8-1.4)	1.7±0.4 (1.3-2.8)	1.6±0.2 (1.4-2.0)
Excretory pore/body length percentage		10.8		10.1±0.9 (8.7-12.6)	18.0±1.1* (15.2-20.4)
Caudal ratio A					2.1±0.5 (1.2-3.0)
Caudal ratio B					3.3±0.9 (1.7-5.3)

* n=23. All measurements in μm .

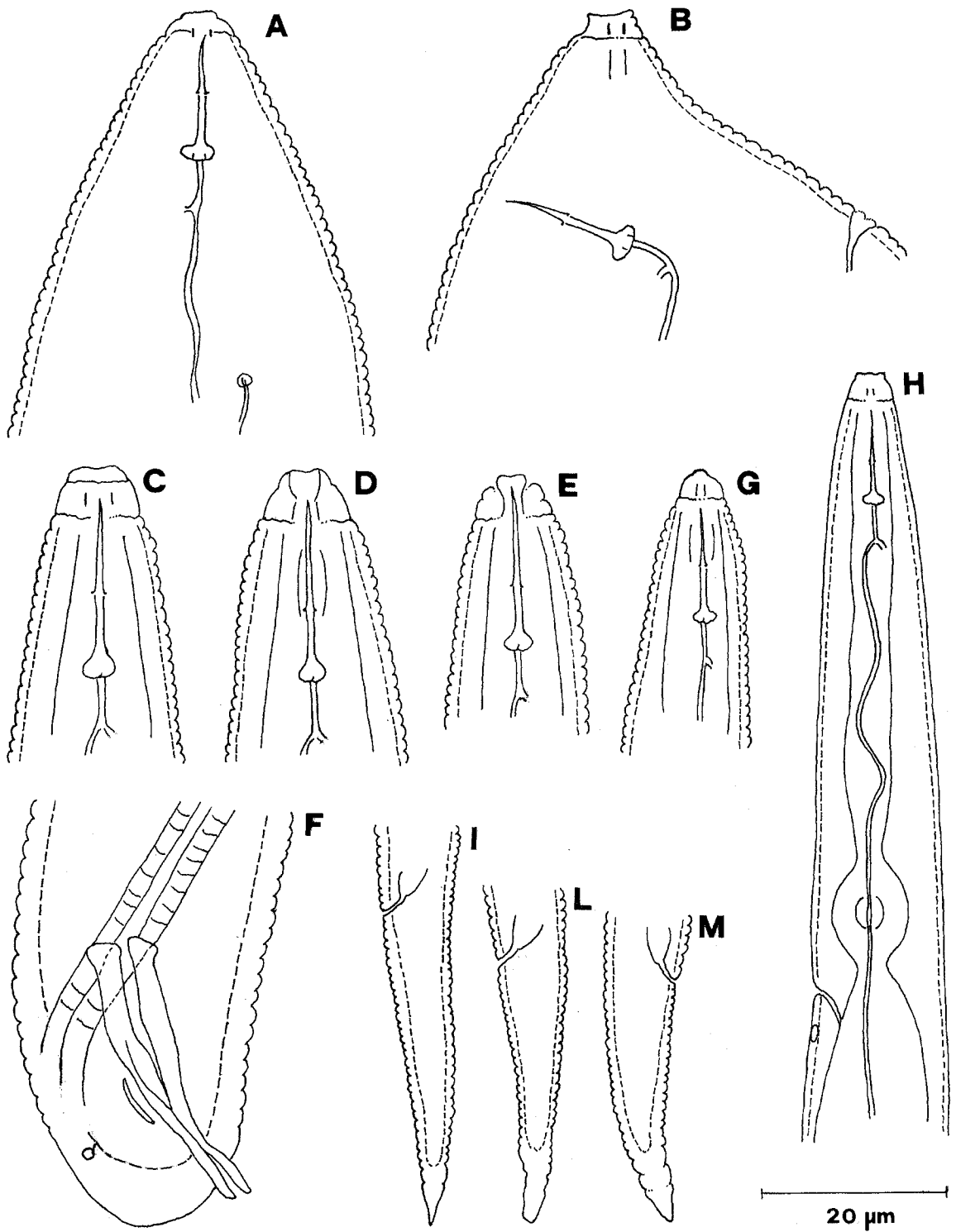


Fig. 2 - *M. ulmi* sp. n.: A and B, female anterior region, ventral and lateral; C-E, male head end, lateral and ventral; F, male tail, lateral; G and H, second stage juvenile head end, ventral and anterior region, lateral; I-M, second stage juvenile tail shape variation, lateral.

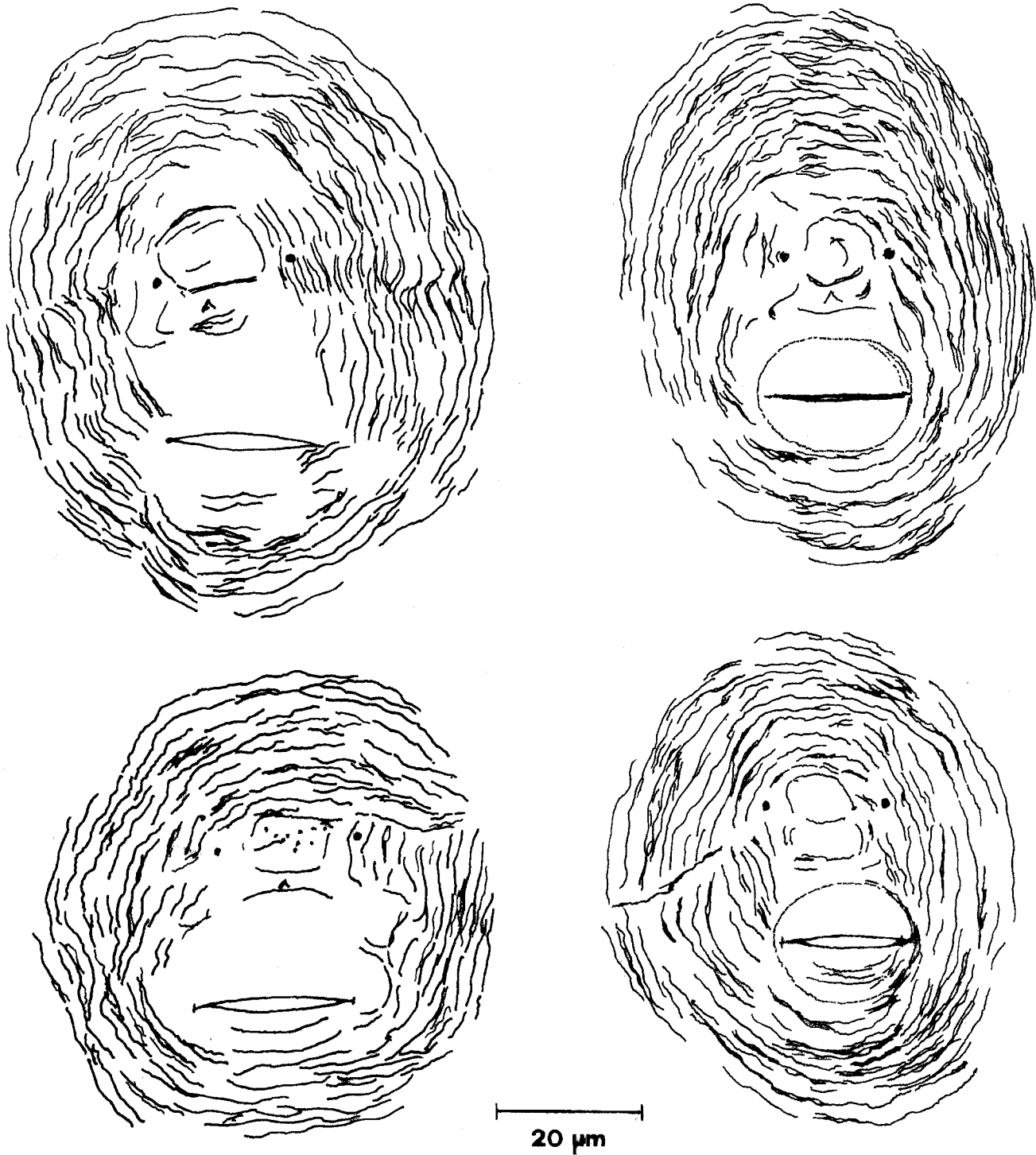


Fig. 3 - Females of *M. ulmi* sp. n.: perineal patterns.

ible in length. Head region set off from regular body annules, cephalic framework weakly sclerotized, head cap distinct, under LM postlabial region in some specimens appears marked by two annulations. Under SEM prestoma situated centrally on the labial disc surrounded by six inner labial sensilla. Labial disc round to square, set off from the lips, medial lips partly divided, on the postlabial region apparently two incomplete annules. Lateral lips small but distinct. Stylet cone slender, moderately curved, shaft slightly widened towards the junction with the knobs, knobs rounded to transversely ovoid, slightly concave anteriorly. Excretory pore variable in distance from the anterior end, located behind the stylet base. Some vesicles often visible along the metacarpus lumen near the valve. Perineal patterns oval, dorsal arch flattened to medium high, round or somewhat square, dorsal and ventral striae varying from fine and smooth to relatively coarse. Tail end area well delimited, square to oval, sometimes with punctations or marked by a few lines. In a few specimens some lines from the dorsal arch traverse the perineum and the space between anus and vulva. Lateral field indistinct or marked by folds, sometimes by lateral lines at one or both sides. Anus within a cuticular fold, phasmids conspicuous, interphasmidial distance about the same or smaller than the vulva width. Vulva with edges finely serrated, located on a slightly prominent area usually devoid of striae.

Male: body slightly tapering anteriorly, bluntly rounded posteriorly, posterior portion twisted. Body annules width 2.2-2.9 μm . Lateral field usually with four, frequently with five to seven lines in the mid body region, beginning as two lines at about the stylet base, ending as two or three lines at the tail tip, outer bands clearly, inner bands faintly, areolated; areolation more pronounced in the posterior body region, in some specimens one or two lines appear broken in the middle of the field. Head slightly set off from body contour, labial cap shallowly round-

ed, one fifth to one fourth as high as postlabial head region and a little narrower than the same region; postlabial annule under LM smooth or marked by two annulations. Under SEM, prestoma ovoid situated centrally on the labial disc, surrounded by six inner labial sensilla, labial disc rounded, prominent and set off from the medial lips which appear almost completely divided, crescent shaped. Four conspicuous cephalic sensilla present on the medial lips. Lateral lips absent, sometimes vestigial, apparently fused with the postlabial annule. Amphidial openings large, located at the lateral sides of the disc. Three or four sometimes faintly marked incomplete annules are visible on the postlabial head region. Stylet of medium size, cone slender, knobs rounded to pear shaped more or less backwardly sloping. Excretory pore variable in position. Hemizonid 0-7 annules anterior to excretory pore. Testis monorchis, generally outstretched, in two specimens germinal zone reflexed, reflexed part 1.4-1.8 body widths long. Tail bluntly rounded to conical rounded, rarely with a small knob at the tip. Spicules straight to slightly ventrally arcuate, terminus blunt. Gubernaculum slightly crescent shaped. Phasmids about at the same level or slightly posterior to the cloaca.

Males are frequent.

Second stage juvenile: body moderately long, straight or slightly ventrally arcuate when dead. Body annulations small but distinct. Lateral field usually with four, sometimes five or six lines at mid-body, beginning as two lines near the level of stylet base, ending as two or three lines at hyaline tail terminus, outer bands areolated. Head region slightly set off from body, truncate in lateral view. Cephalic framework weakly sclerotized. Under SEM labial annule dumbbell shaped, labial disc ovoid, distinct from the medial lips, medial lips fused but slightly distinct indicating two lips, with four cephalic sensilla, lateral lips small, crescent shaped, amphidial apertures appearing as large, elongate slits between labial disc and lateral lips. Postlabial annule slightly marked by a transverse stria. Sty-

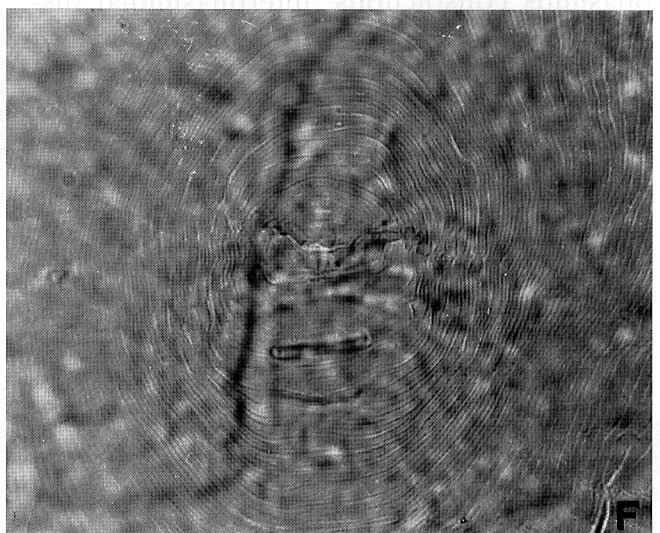
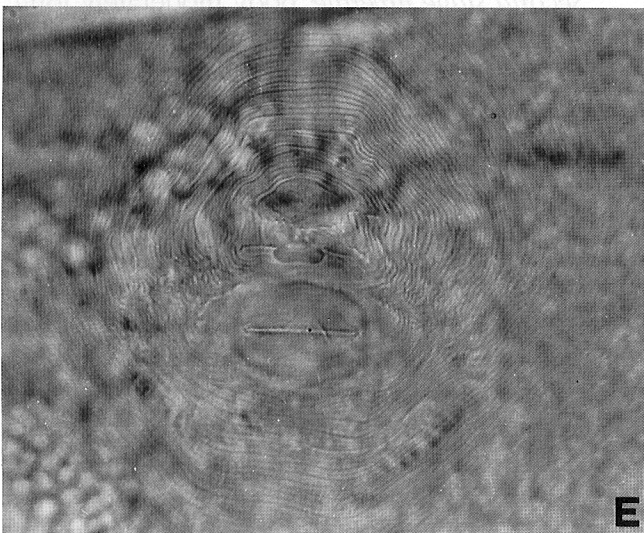
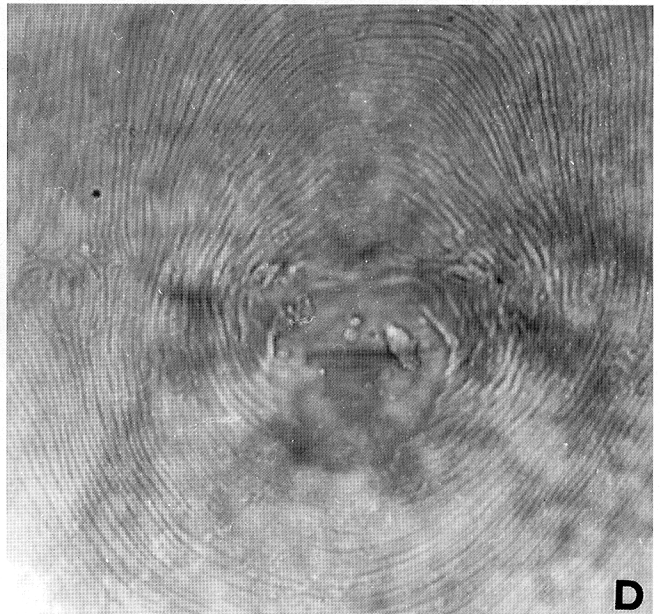
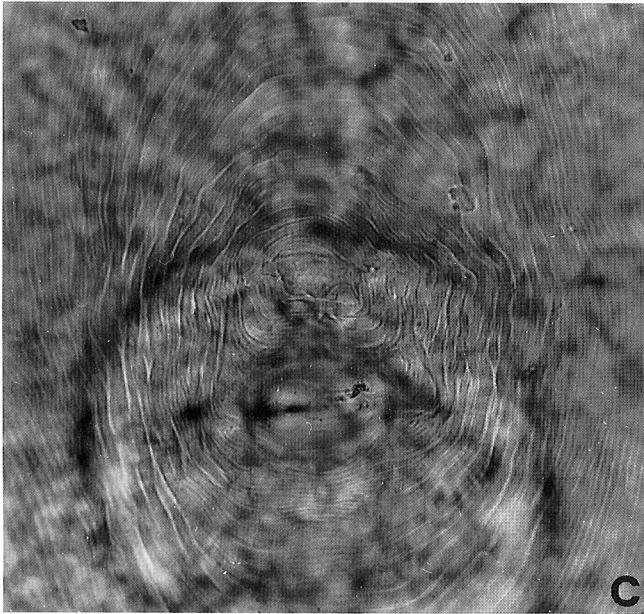
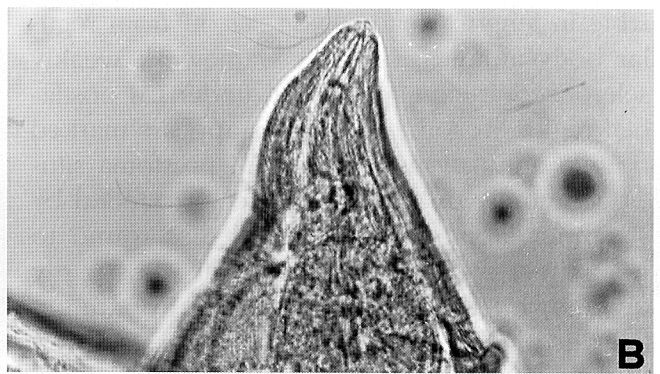
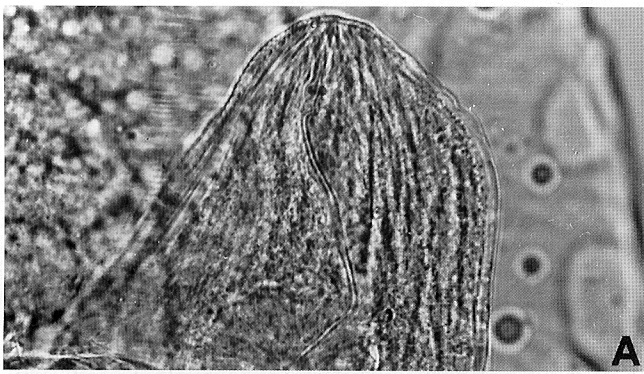


Fig. 4 - LM photomicrographs of females of *M. ulmi* sp. n.: A and B, anterior regions; C-F, perineal patterns.

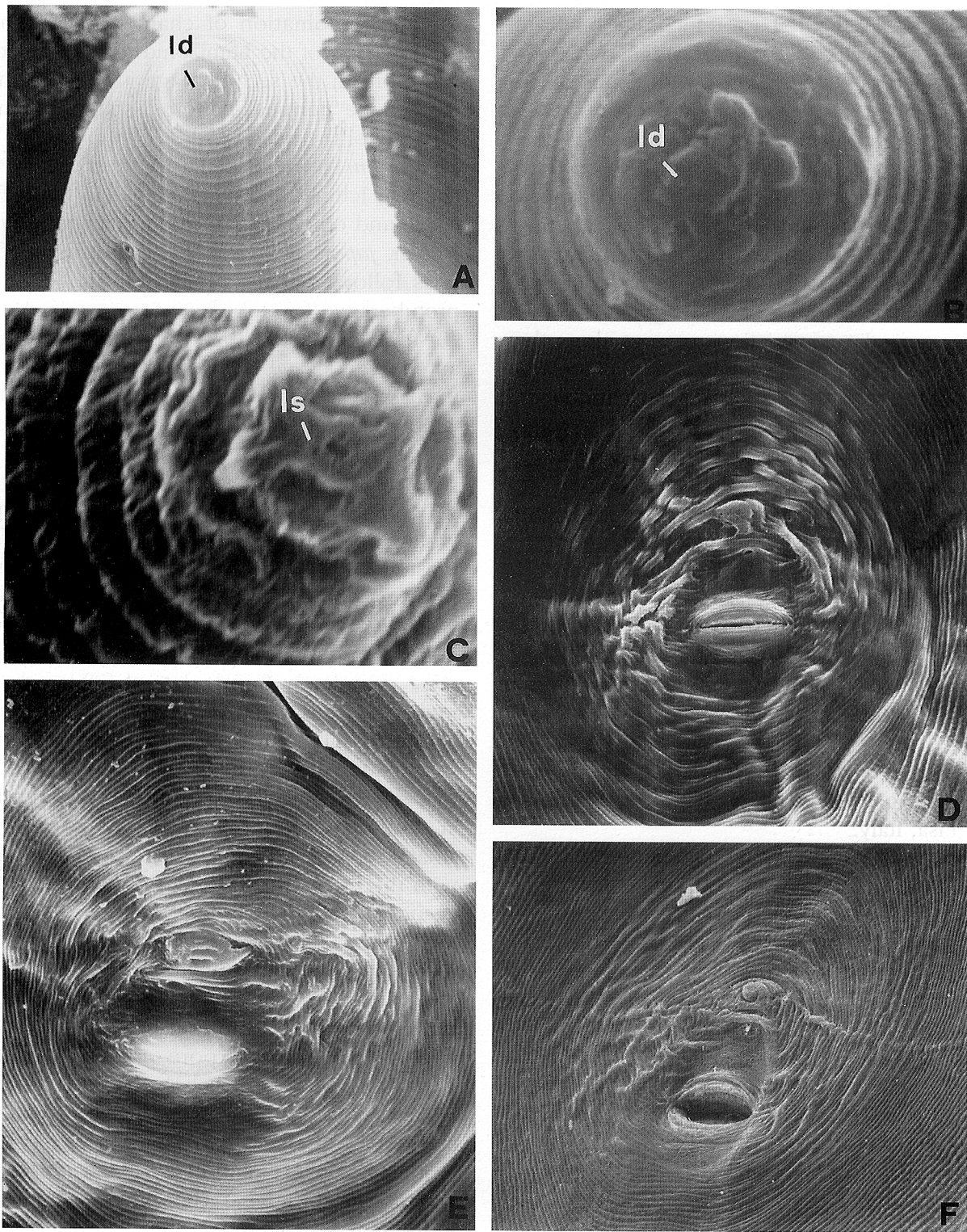


Fig. 5 - SEM photomicrographs of females of *M. ulmi* sp. n.: A-C, head ends, latero-ventral x 1,310, face view x 5,000 and 7,400; D-F, perineal patterns x 710, x 810, x 885 (ld=labial disc; ls=inner labial sensilla).

let delicate, short, knobs rounded, set off from the shaft. Pharynx with faintly outlined procorpus, metacarpus oval shaped with pronounced valve. Excretory pore variable in position. Hemizonid distinct, sometimes extending over 3 body annules, 0-4 annules posterior to excretory pore. Rectum inflated seen in some specimens. Tail of medium length, conical, tapering to a finely rounded almost pointed terminus or broader and rounded at the tip, rarely constricted at about two thirds of its length or bent at the tip; cuticular constrictions present along the hyaline terminus, the first one generally located at its anterior end; hyaline region variable in length, delimited at the anterior end by a widely rounded or narrower and tapering hypodermis outline. Phasmids difficult to observe, in some specimens posterior to anus, 11-22 μm from tail end.

Eggs (1-2 celled; n=40): length 100.2 ± 8.1 (82.3-115.0) μm ; width 37.4 ± 3.7 (31.5-48.4) μm ; length/width ratio 2.7 ± 0.3 (2.2-3.2); (embryonated; n=40): length 106.2 ± 7.6 (95.6-118.6) μm ; width 39.7 ± 4.6 (32.7-49.6) μm ; length/width ratio 2.7 ± 0.3 (2.0-3.5).

Type host and locality. Roots of *Ulmus chenmoui* Cheng found in a plantation at San Rossore, Pisa, Italy.

Type specimens. Holotype female, allotype male, 26 female, 26 male and 24 second stage juvenile paratypes in the nematode collection of the Istituto Sperimentale per la Zoologia Agraria, Florence, Italy; 2 female perineal patterns and heads, 2 male and 3 second stage juvenile paratypes at the USDA Collection, Beltsville, Maryland, USA; 2 female perineal patterns and heads, 2 male and 3 second stage juvenile paratypes at the Commonwealth Institute of Parasitology, St. Albans, U.K.

Diagnosis and relationships. Several *Meloidogyne* species have relatively short second stage juvenile tails falling in the same range as *M. ulmi* sp. n. (24.2-37.5 μm). Among these species, *M. ulmi* sp. n. can be distinguished by the fol-

lowing combination of characters: tail shape and position of the hemizonid relative to excretory pore in the second stage juvenile, form of the female perineal patterns, shape and morphology of the male head.

M. ulmi sp. n. is similar to *M. mali* Itoh, Ohshima *et* Ichinohe, 1969 described in Japan from apple and other hosts including *Ulmus davidiana* var. *japonica* (Toida, 1979), but differs from it as follow: female without distinct posterior protuberance (protuberance present in *M. mali*), female stylet with knobs rounded slightly anteriorly concave (in *M. mali* backwardly sloping), typical perineal patterns lacking distinct lateral lines (in *M. mali* lateral field marked by single or double lines), distance between the phasmids about the same or smaller than the vulva width (in *M. mali* larger than the vulva width); male labial cap shallowly rounded, almost as wide as the postlabial region (narrower in *M. mali*), under SEM lateral lips absent or vestigial (in *M. mali* lateral lips apparent), medial lips almost completely divided (in *M. mali* almost completely fused), lateral field with four incisures, but additional incisures frequently present (only four incisures in *M. mali*), tail shorter (tail length 28-44 μm in *M. mali*); second stage juvenile, as seen with SEM, with labial disc set off from the medial lips, lateral lips small (in *M. mali* labial disc apparently fused with the medial lips, lateral lips well developed according to Okamoto and Yaegashi, 1981), stylet shorter (12-15 μm in *M. mali*), lateral field areolated with four, sometimes five or six, incisures (in *M. mali* not areolated, only four incisures observed), position of the hemizonid relative to the excretory pore not known for *M. mali*. Considerable morphological differences in perineal patterns and second stage juvenile tail shape were noticed by Okamoto *et al.* (1983) among some populations of *M. mali*. Similar variation in these characters has been observed in the new species too.

M. ulmi sp. n. also resembles *M. suginamiensis* Toida *et* Yaegashi, 1984 a parasite of

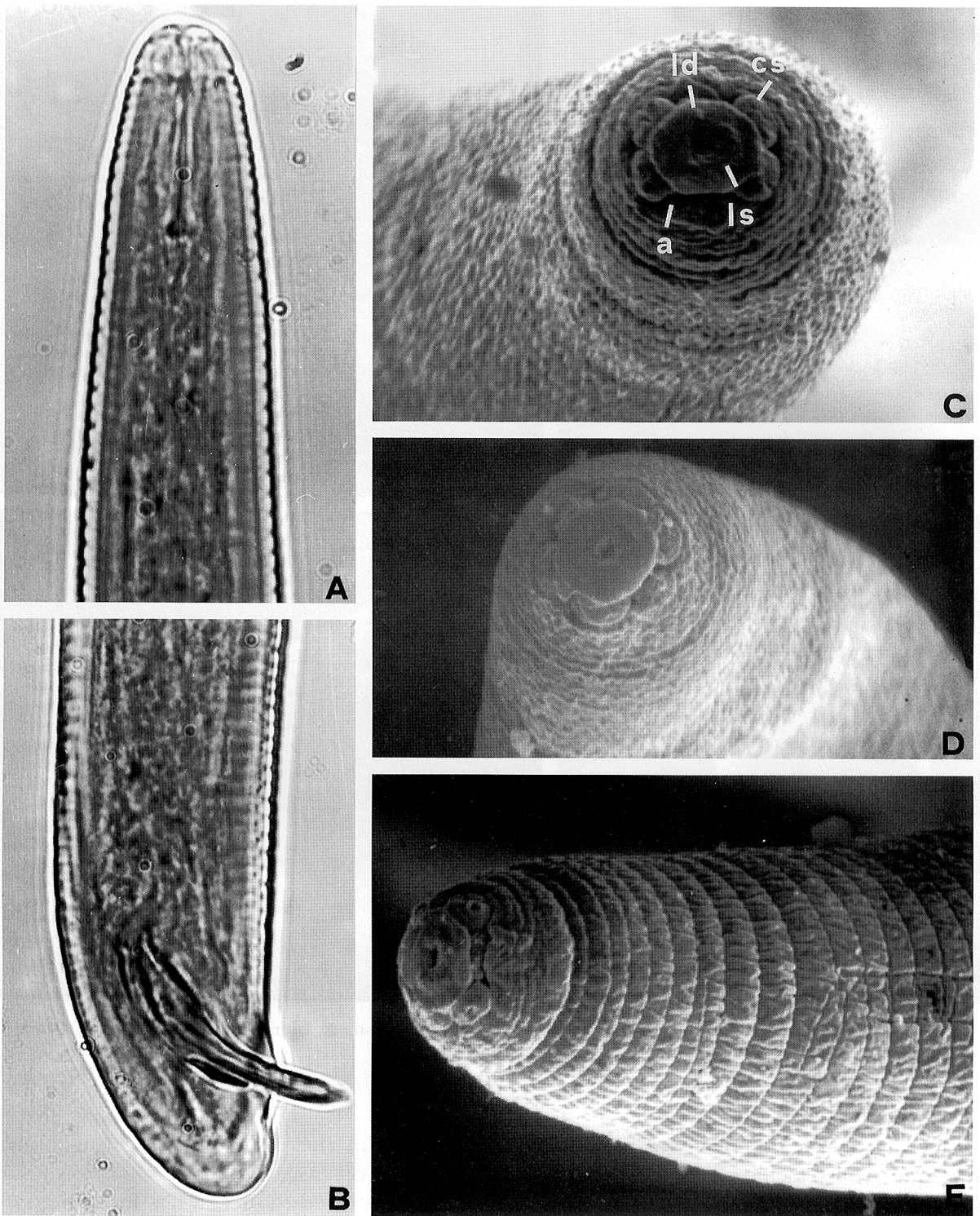


Fig. 6 - LM (A and B) and SEM (C-E) photomicrographs of males of *M. ulmi* sp. n.: A, anterior region, lateral; B, posterior region, lateral; C and D, head regions, face view x 4,020; E, anterior region, lateral x 3,540 (a = amphid; cs = cephalic sensilla; ld = labial disc; ls = inner labial sensilla).

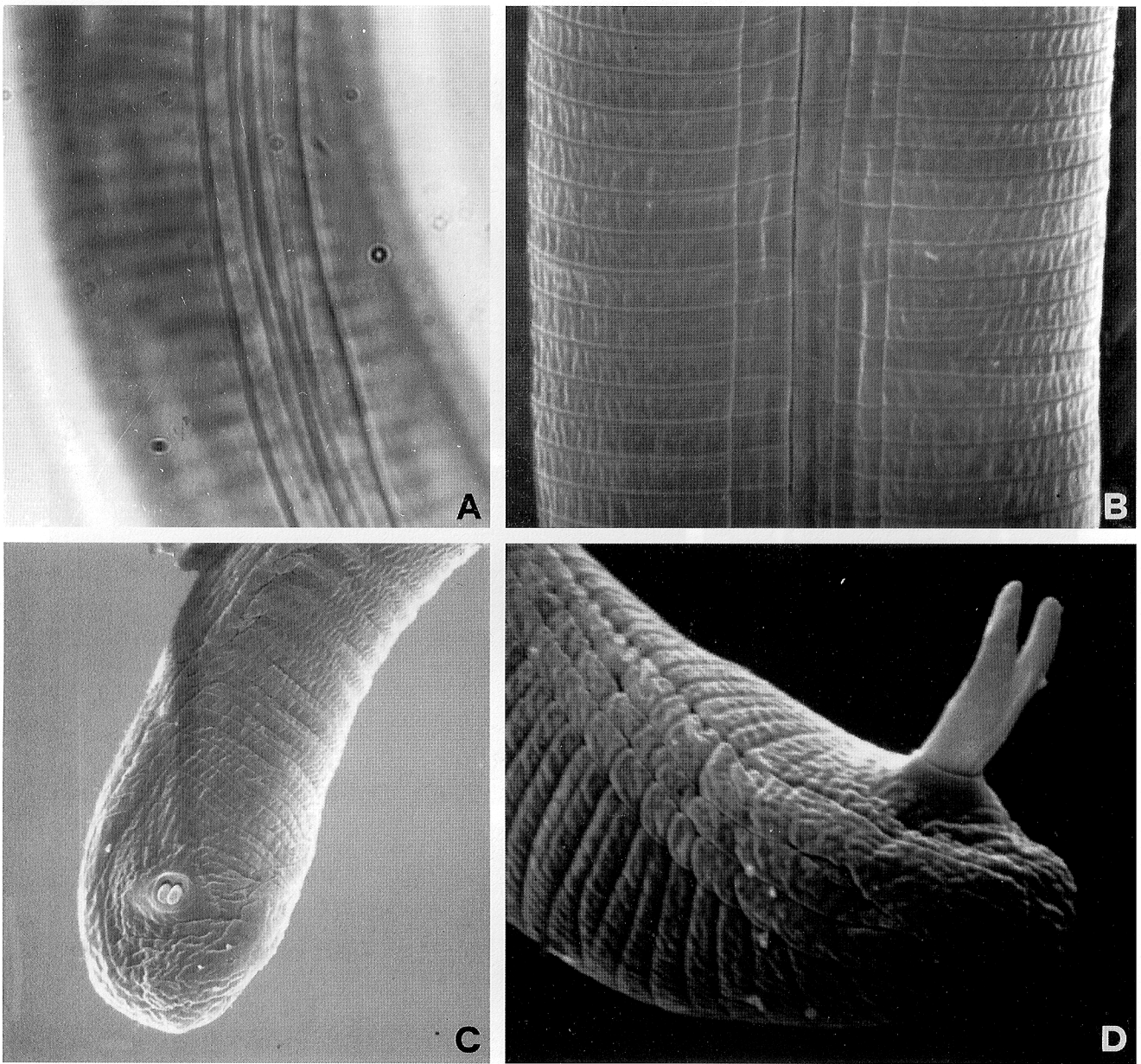


Fig. 7 - LM (A) and SEM (B-D) photomicrographs of males of *M. ulmi* sp. n.: A, lateral field at mid-body; B, lateral field at mid-body x 2,500; C and D, tails, ventral x 1,550 and lateral x 2,400.

mulberry and a number of vegetables, weeds and woody plants including *U. davidiana* var. *japonica* (Toida and Yaegashi, 1984) and *M. vandervegtei* Kleynhans 1988, described from an unidentified woody plant in Natal, South Africa. From *M. suginamiensis* the new species

differs in the female having the labial disc marked off from the lips as seen under SEM (labial disc fused to medial lips in *M. suginamiensis*), perineal pattern with tail terminus area clearly delimited (less distinct in *M. suginamiensis*), interphasmdial distance about the

same or shorter than the length of vulval slit (same distance greater than the vulval slit in *M. suginamiensis*); by the male having a labial cap, as seen in lateral view, almost as wide as post-labial region of head (narrower in *M. suginamiensis*), under SEM a labial disc distinctly offset from the medial lips (labial disc apparently fused to the medial lips in *M. suginamiensis*), lateral lips absent or vestigial (clear in *M. suginamiensis*), hemizonid anterior to excretory pore (posterior in *M. suginamiensis*), tail shorter (13-19 μm in *M. suginamiensis*); by the second stage juvenile with lateral lips, as seen under SEM, small (well developed in *M. suginamiensis*), shorter stylet (12-15 μm long in *M. suginamiensis*), tail more slender (somewhat stouter in *M. suginamiensis*), hyaline tail part longer (3-5 μm in *M. suginamiensis*).

M. ulmi sp. n. differs from *M. vandervegtei* in the female having a shorter stylet (16.4-20.1 μm in *M. vandervegtei*), perineal patterns usually with few lines around the vulva and between anus and vulva (many striae present in the perivulval area and between anus and vulva in *M. vandervegtei*); in the male having a shorter stylet (21.7-26.9 μm in *M. vandervegtei*), shorter tail (11.8-21.5 μm in *M. vandervegtei*), and shorter spicules (34.9-44.1 μm in *M. vandervegtei*); in the second stage juvenile with a longer body (313-388 μm in *M. vandervegtei*), hemizonid conspicuous posterior to the excretory pore (indistinct, anterior to the excretory pore in *M. vandervegtei*) and hyaline tail area delimited (indistinct in *M. vandervegtei*).

Some females in *M. ulmi* sp. n. have perineal patterns resembling those of *M. hapla* Chitwood, 1949 in general shape and punctations on the tail terminus area. However, in the new species the examined specimens appeared distinguishable from *M. hapla* by the greater DGO and longer spicules of the male (respectively 2.7-5.4 μm and 21.6-28.1 μm in *M. hapla*), by the hemizonid posterior to the excretory pore (anterior in *M. hapla*), shorter tail (33-48 μm in *M. hapla* according to Whitehead, 1968; 48.2-69.8 μm ac-

ording to Jepson, 1987) and shorter hyaline tail terminus (11.7-18.9 μm in *M. hapla* according to Jepson, 1987) of the second stage juvenile. Furthermore, the head morphology of second stage juvenile and males of *M. ulmi* sp. n., as seen by SEM, differs from that of *M. hapla* (Eisenback and Hirschmann, 1979; 1980). The second stage juveniles have a labial disc apparently offset from the medial lips (fused with the medial lips, although elevated in some populations of *M. hapla*), the males have a labial disc distinctly set off from the medial lips which appear almost completely divided, cephalic sensilla conspicuous (labial disc and medial lips fused, medial lips undivided, cephalic sensilla not expressed externally of faintly visible in *M. hapla*).

Discussion

The only host plants currently known for *M. ulmi* sp. n. are *U. chenmoui* and *U. glabra* which are as rootstocks for *Ulmus* hybrid selections. The nematode has probably been introduced to San Rossore (Pisa, Italy) and Mantignano (Florence, Italy) in nursery transplants from the Netherlands. Attempts to propagate the nematode on tomato in the glasshouse have so far failed. Additional research is necessary to determine the distribution of *M. ulmi* sp. n. beyond the mentioned localities and its possible pathogenic effects on vegetables and field crops.

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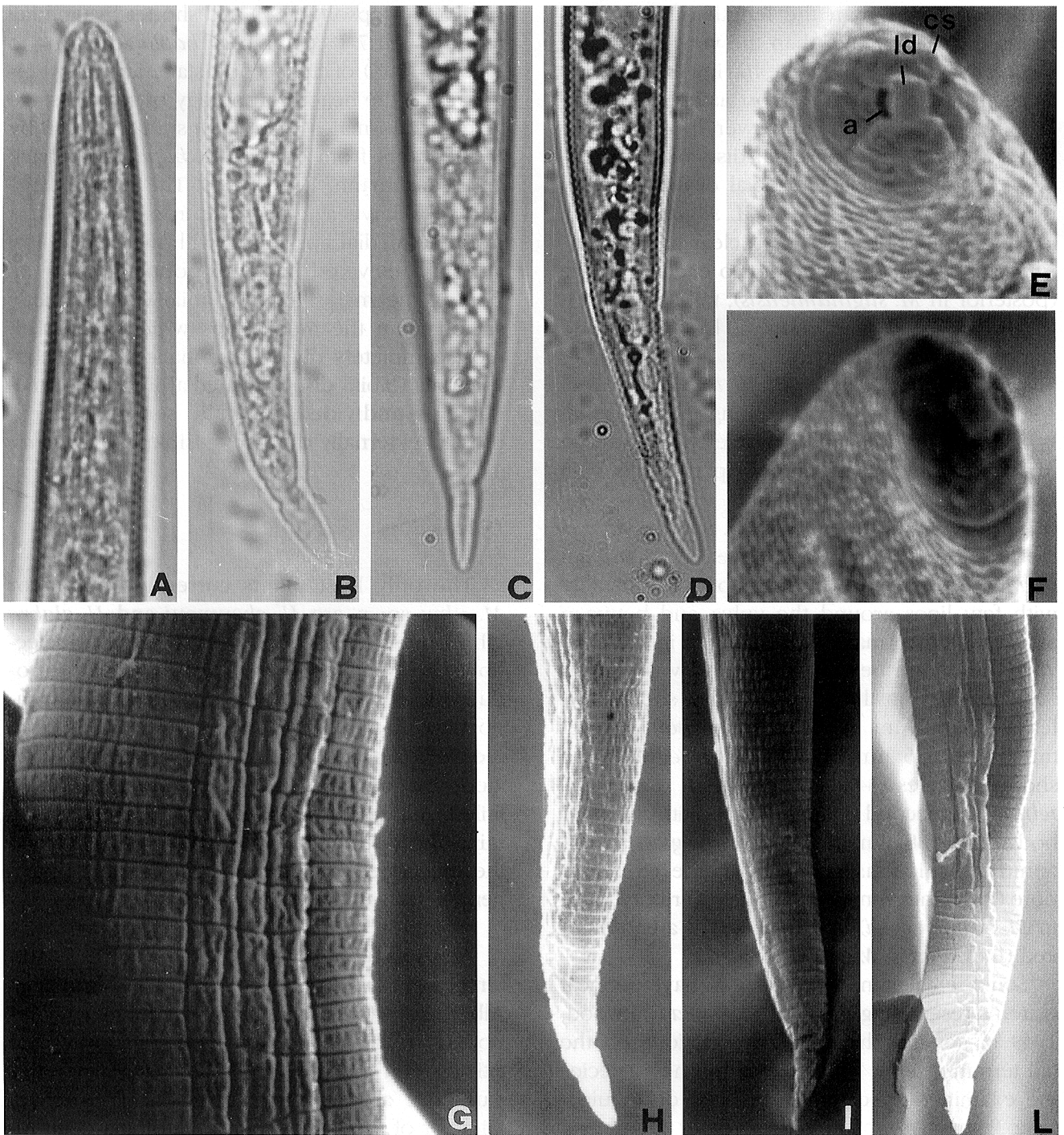


Fig. 8 - LM (A-D) and SEM (E-L) photomicrographs of second stage juveniles of *M. ulmi* sp. n.: A, anterior region; B-D, tail shape variation, B and D lateral, C ventral; E and F, head end regions, face view x 7,050 and x 6,020; G, lateral field x 4,580; H-L, tail shape variation, lateral x 2,200, ventral x 1,850 and lateral x 1,850 (a = amphid; cs = cephalic sensilla; ld = labial disc).

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