

## ***Meloidogyne californiensis* n. sp. (Nemata: Meloidogyninae), Parasitic on Bulrush, *Scirpus robustus* Pursh<sup>1</sup>**

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**Abstract:** *Meloidogyne californiensis* n. sp. is described and illustrated from bulrush *Scirpus robustus* in California. LM and SEM studies revealed that this species differs from other known species in the genus *Meloidogyne* especially by the prominent posterior cuticular protuberances in the female, the distinct shape of the perineal pattern which is marked by one prominent stria in the perineum, indistinct lateral lines, many broken discontinuous striae on both sides of the arch, and the excretory pore being located posterior to stylet base. Second-stage juveniles 448–628  $\mu\text{m}$  long, stylet length 11–13  $\mu\text{m}$ , stylet delicate, with small knobs sloping posteriorly, cephalic region with 2 or 3 annuli, and inflated rectum. Males vary greatly in size (712–1,952  $\mu\text{m}$ ), stylet length 18–28  $\mu\text{m}$  (mean 22  $\mu\text{m}$ ), cephalic region slightly set off the body with two or three annuli, spear heavy with massive rounded knobs, lateral field marked by four areolated incisures as seen by SEM.

**Key words:** *Meloidogyne californiensis*, root-knot nematode, scanning electron microscopy, new species, *Scirpus robustus*, bulrush.

Bulrush plants (*Scirpus robustus* Pursh) with large white root galls were collected in 1979 from the edge of a freshwater stream of Pomponio Beach (Half Moon Bay), California. The galls contained large numbers of different developmental stages, including adult females and egg masses of an unknown *Meloidogyne* sp. Morphological studies proved that this species is different from others in the genus *Meloidogyne*, and it is described herein as *M. californiensis* n. sp.

### **MATERIALS AND METHODS**

Specimens used in this study were from cultures originally obtained from Pomponio Beach, California, and maintained on bulrush in a greenhouse. Root galls, containing adult females were washed and fixed in cold 1% acid fuchsin lactophenol. Mature females were teased from the roots and mounted in glycerin jelly on aluminum slides, covered with a cover slip, and sealed with zut. Posterior ends of other females were cut off and perineal sections were mounted in glycerin. Large glass rod supports were used to support the cover slip because the perineal area has prominent protuberances. Heads of females were

mounted in glycerin, and eggs were mounted in water.

Second-stage juveniles and males were obtained from the same cultures of bulrush grown in the greenhouse. Extraction from fresh infected root galls was carried out in a mist chamber. After 24 hours juveniles and males were collected. Males were also recovered from soil by wet sieving followed by Baermann funnel extraction. Juveniles and males were hand picked into water in a glass cavity slide and killed with heat. Some specimens were temporarily mounted in water; others were fixed and mounted in F.A.A. or were processed into glycerin for permanent mounts (3).

**Preparation of juveniles and males for SEM study:** Second-stage juveniles and males were killed by heat in a glass cavity slide and fixed in F.A.A. They were dehydrated in an ethanol series starting with 10% and 20% for 30 minutes each, then transferred to 30%, and left overnight. These were then transferred through 40%, 50%, and 60% ethanol for 30 minutes each. They remained in 60% ethanol overnight. The following day they were transferred to 75%, 80%, 95%, and 100% ethanol. After dehydration, specimens were taken gradually (25%, 50%, 70%, 90%) through a series of dilutions of amyl acetate in absolute ethanol to 100% amyl acetate. Nematodes were ultrasonicated for 1 minute in 100% amyl acetate, critical point dried, and mounted

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on stubs with an aluminum foil support. Specimens were coated with gold (400–500 Å) and viewed with a Cambridge Mark II scanning electron microscope using an accelerating voltage of 10 kV.

*Preparation of adult females for SEM study:* Fresh root galls were collected from the bulrush culture, washed, killed, and fixed in 10% buffered neutral formalin. Females were then dissected out and transferred to 2.5% formalin for 3–4 hours and subsequently transferred to F.A.A. Those females maintaining their shape were dehydrated and critical point dried as described for males and juveniles. Females were mounted on stubs by using double coated scotch tape and coated with gold (400–500 Å).

In the description,  $b''$  is calculated by dividing the body length by the length from anterior body extremity to the center of metacarpus and  $T'$  by dividing body length by testis length.

All measurements are given in micrometers ( $\mu\text{m}$ ) unless otherwise designated.

## TAXONOMY

### *Meloidogyne californiensis* n. sp. (Figs. 1–7)

*Females in glycerin jelly (36):* Body length (without neck) 264–492 (mean  $325 \pm$  standard deviation 59.68); neck length 72–140 ( $94 \pm 20.59$ ); body width 168–403 ( $226 \pm 60.69$ ); neck width 33–56 ( $43 \pm 7$ ); cuticle thickness in midbody 2–6 ( $3 \pm 0.58$ ).

*Excised female heads in glycerin (22):* Stylet length 12–18 ( $14 \pm 2.12$ ); stylet knob width 2–3 ( $2.3 \pm 0.29$ ); stylet knob height 1–2 ( $1.5 \pm 0.31$ ); dorsal esophageal gland orifice to stylet base 3.6–6 ( $4.3 \pm 0.68$ ); anterior extremity to stylet base 12–20 ( $15 \pm 2.16$ ); stylet base to excretory pore 6–19 ( $11 \pm 3.8$ ); anterior extremity to excretory pore 19–33 ( $24 \pm 4.7$ ); anterior extremity to end median bulb valve 65–94 ( $72 \pm 8.6$ ); metacarpus length 23–38 ( $29 \pm 4.28$ ); metacarpus width 22–29 ( $24 \pm 2.53$ ).

*Perineal patterns in glycerin (18):* Vulval width 19–27 ( $23 \pm 2.90$ ); interphasmidial width 15–25 ( $18 \pm 2.95$ ); distance from

anus to vulva (center) 12–23 ( $17 \pm 2.93$ ); distance from anus to center of imaginary line between phasmids 4–8 ( $5.5 \pm 1.19$ ).

*Holotype female in glycerin:* Body length with neck 628; body width 364; L/W ratio 1.72; neck length 128; neck greatest width 76; stylet 13; stylet knob width 2; stylet knob height 1; dorsal esophageal gland orifice from stylet base 4; metacarpus length 38; metacarpus width 28; valve length 14; valve width 10; excretory pore from anterior end 26; about 20 body annuli from the anterior extremity to excretory pore, vulval slit to anus, lateral view 22; cuticle thin, maximum thickness at midbody 5.

*Description of female (Figs. 1, 2):* Body pearly white, globular, pear shaped, sometimes saccate, with prominent posterior protuberances; neck distinct, tapering anteriorly, directed oblique to longitudinal axis of body. Stylet delicate, basal knobs rounded, sloping posteriorly. Head annuli, two or three, not set off from neck. Labial framework weakly developed. Excretory pore located posterior to stylet, about two stylet lengths, or 16–26 annuli, from anterior end. Cuticle thin, 2–6 at midbody. Neck striation distinct, body striation fine except around vulva. Perineal pattern (Figs. 1, 2) with two posterior cuticular protuberances on each side of vulva. Lateral lines indistinct, many with wavy broken striae marking lateral areas of pattern, arch low, rounded, with spaced broken striae; ventral region marked with smooth discontinuous striae. Anus covered dorsally by prominent fold, phasmids large, conspicuous. Tail area with a prominent whorl.

*Males in water (33):* Body length 712–1,952 ( $1,362 \pm 358$ ); stylet 18–28 ( $22 \pm 2.7$ );  $a = 28$ –45 ( $35 \pm 5.7$ );  $b = 10$ –12 ( $11 \pm 0.44$ );  $c = 65$ –140 ( $100 \pm 32$ ); DEGO from stylet base 2–6 ( $4 \pm 1.09$ ); anterior extremity to esophago-intestinal valve 102–128 ( $111 \pm 9.44$ ); anterior extremity to excretory pore 110–171 ( $127 \pm 20$ ); anterior extremity to esophageal gland end 188–265 ( $213 \pm 24.38$ ); body width 24–42 ( $35 \pm 4.81$ ); tail 7–23 ( $13 \pm 3.73$ ); spicules 20–40 ( $28 \pm 5.59$ ); gubernaculum 5–9 ( $8 \pm 1.67$ ); testis length 290–990 ( $670 \pm$

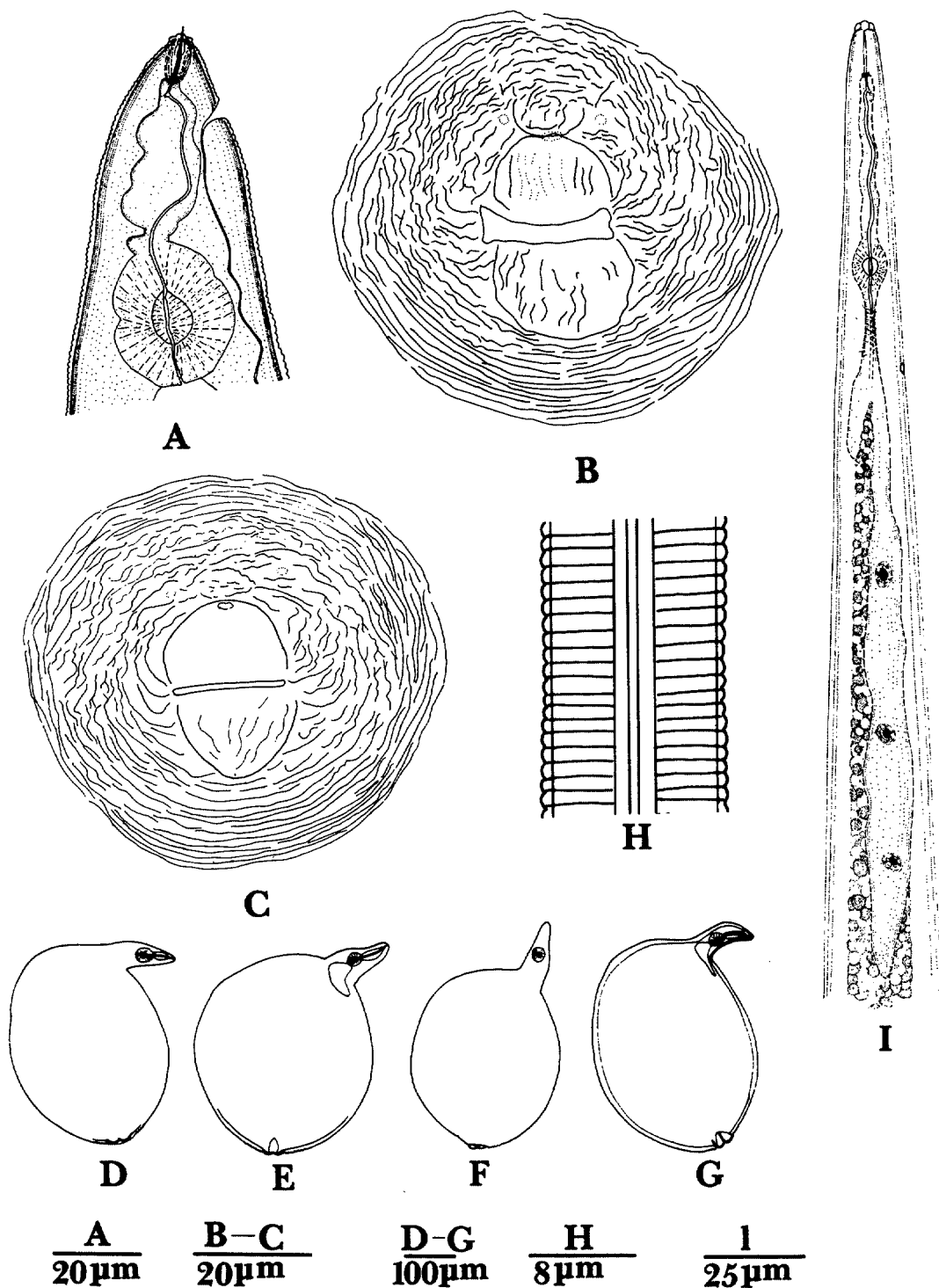


FIG. 1. *Meloidogyne californiensis* n. sp. A-G) Female. A) Anterior part of the body. B, C) Perineal pattern. D-G) Adult female. H) Lateral field. I) Anterior body region, second-stage juvenile.

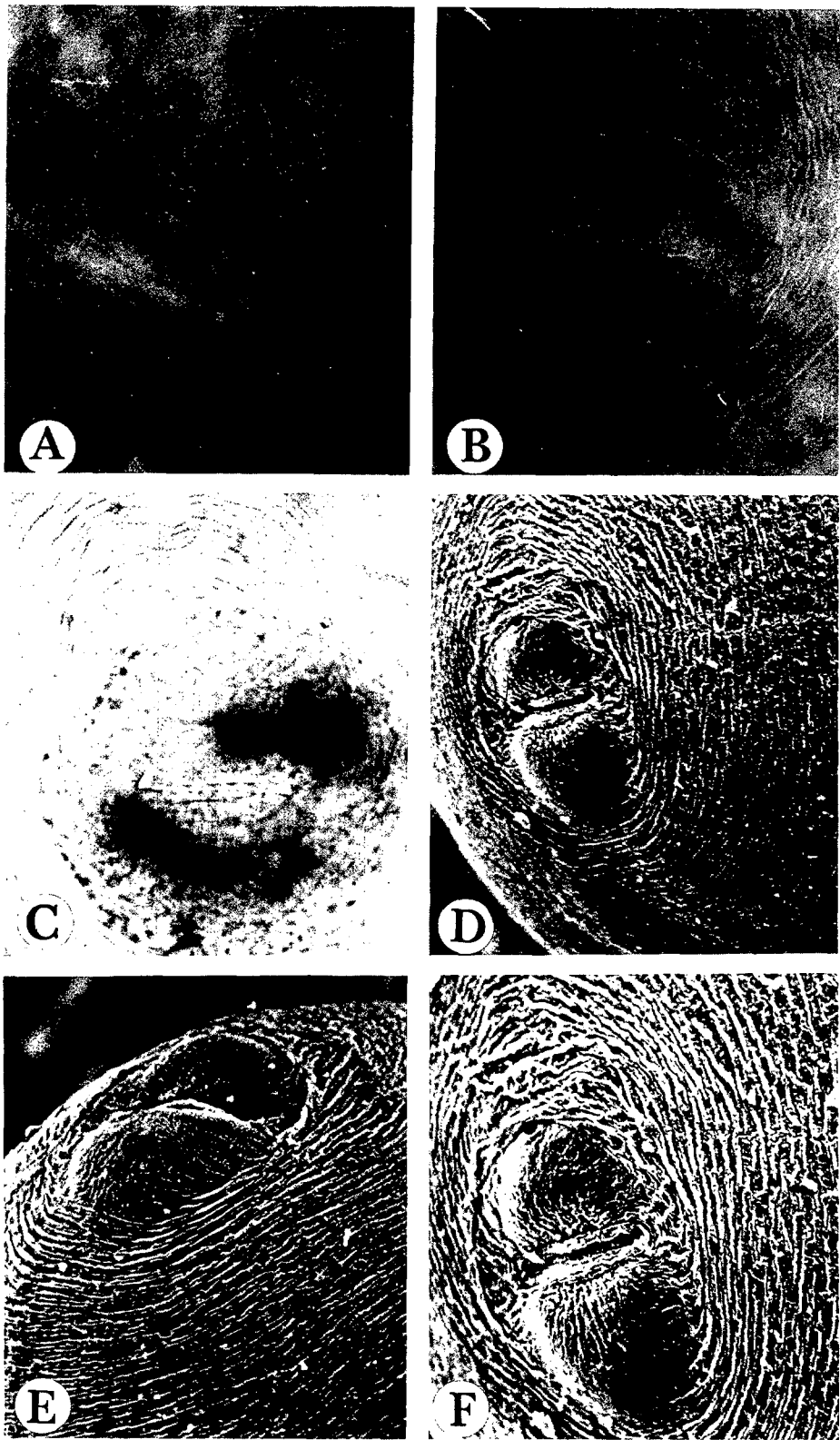


FIG. 2. *Meloidogyne californiensis* n. sp. female. A, B) LM photomicrographs of perineal patterns. C-F) Scanning electron micrographs of perineal region.

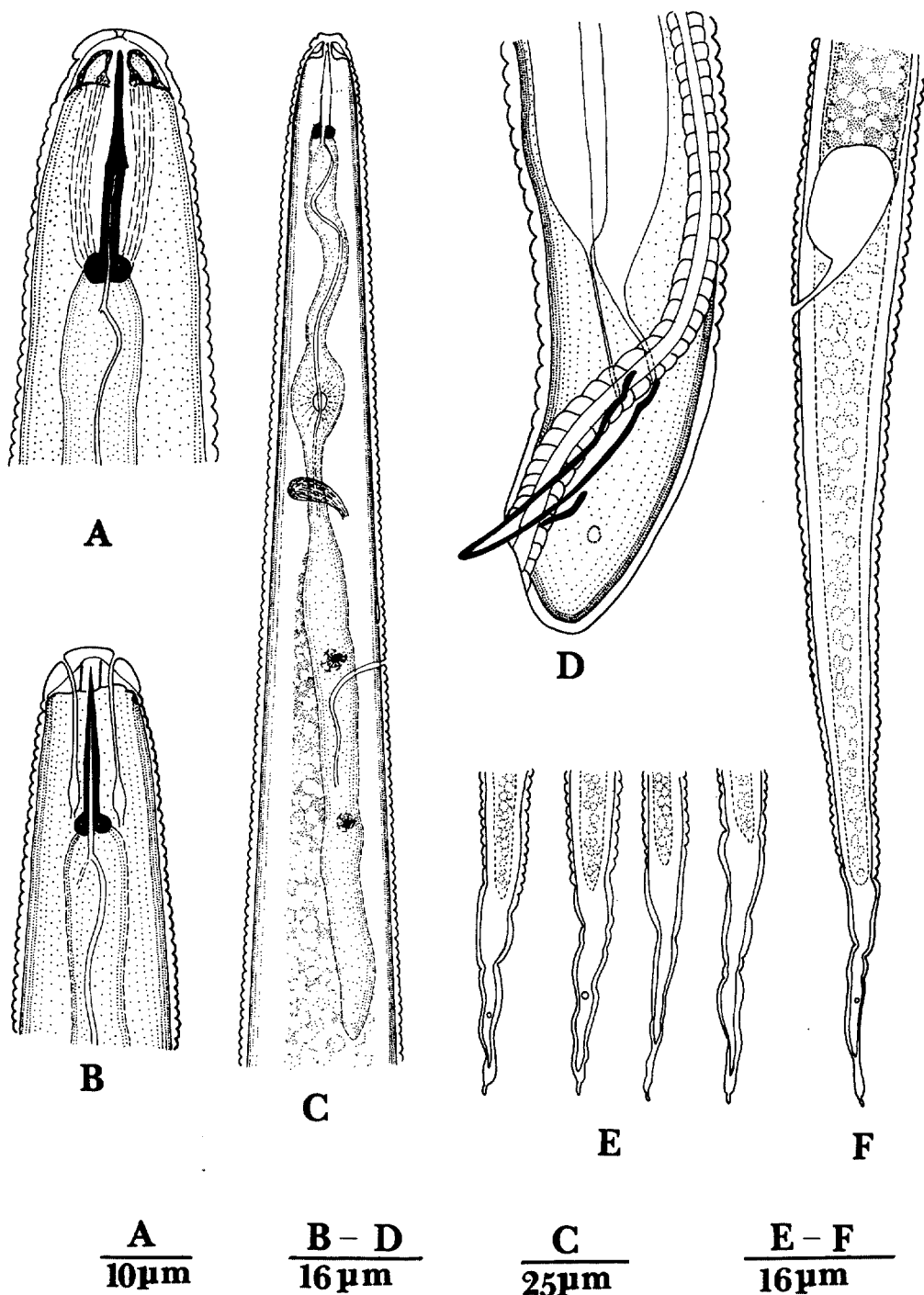


FIG. 3. *Meloidogyne californiensis* n. sp. A-D) Male. A) Cephalic region (lateral). B) Cephalic region (dorsal). C) Anterior body region (lateral). D) Tail (lateral). E, F) Second-stage juvenile tail termini and tail.

233); phasmids to tail tip 10-17 ( $13 \pm 2.78$ ;  $T = 36-65$  ( $51 \pm 8$ ).

*Allotype male in glycerin:* Body length 1,095; stylet 19; stylet knob height 3; stylet knob width 4; stylet base to anterior body

end 21;  $a = 31.08$ ;  $b'' = 13.67$ ;  $c = 97.70$ ;  $c' = 63.62$ ; dorsal esophageal gland orifice to stylet base 4; anterior extremity to center of metacarpus 80; excretory pore 126 from anterior extremity; hemizonid 114

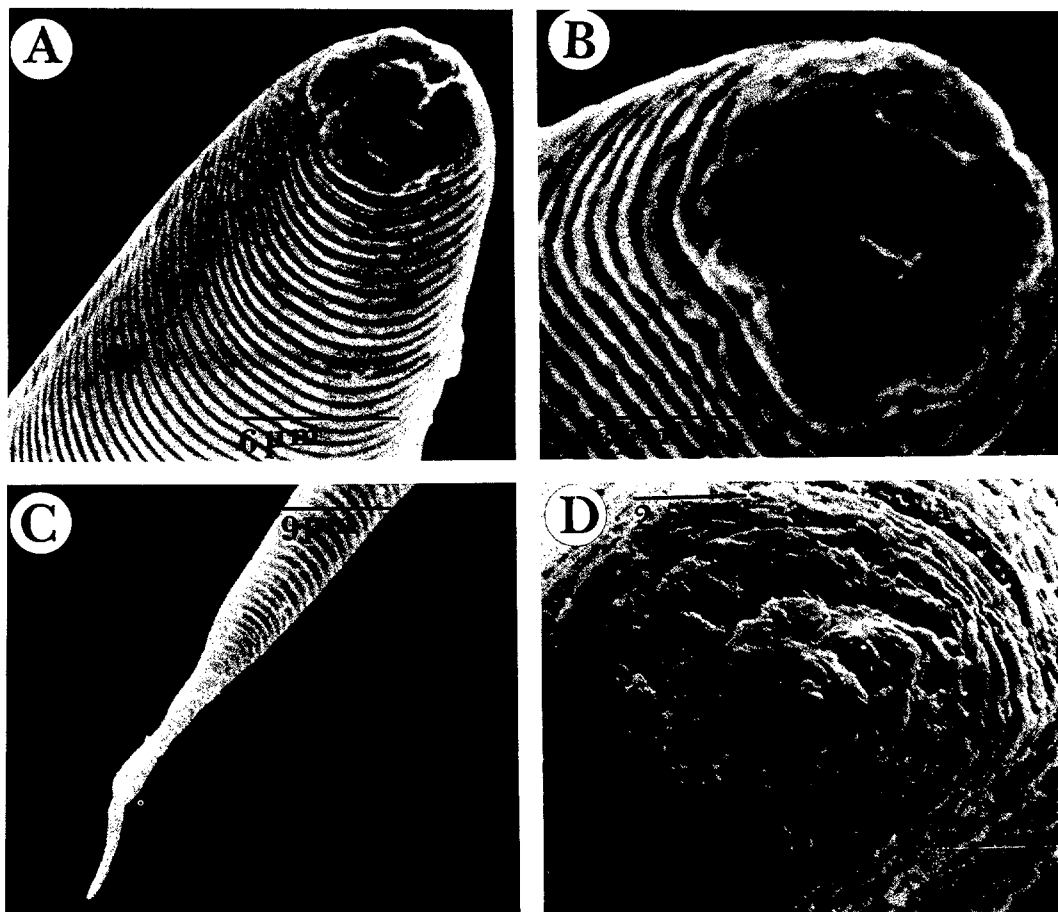


FIG. 4. Scanning electron micrographs of *Meloidogyne californiensis* n. sp. A, B) Juvenile face view. C) Second-stage juvenile tail. D) Female face view.

from anterior extremity; hemizonid length 5; hemizonid to excretory pore 8; termination of esophageal gland 237 from anterior extremity. Body annulation 2 wide. Body width 35; testes length 654; spicules 20; gubernaculum 5; anal body width 17; tail 11 long; phasmids to tail tip 11 (at the level of cloaca).  $T' = 1.672$ ,  $T = 59.79$ .

**Description of male (Figs. 3, 6, 7):** Body vermiform, slender, tapering, rounded at both extremities. Head slightly set off, with two or three annuli; spear heavy with massive rounded knobs sloping posteriorly. Lateral field marked with four areolated incisures. Incisures begin 12 annuli from lip region, with LM outer bands only, with or without areolation. Lateral field bands three, equal in width, 0.2 of body width. Body cuticle coarsely annulated, annuli

about  $2\ \mu\text{m}$  wide. Hemizonid anterior to excretory pore, about three body annuli long. Phasmids at level of cloaca. Spicules arcuate, gubernaculum as illustrated (Fig. 3D). Body length varying, with long slender males more than twice as long as the short ones. Long males possess two testes, short males one.

**Second-stage juveniles in F.A.A. (35):** Body length (L)  $448\text{--}628$  ( $559 \pm 29.76$ ); stylet length  $11\text{--}13$  ( $12.06 \pm 0.63$ );  $a = 32\text{--}34$  ( $32.9 \pm 0.89$ );  $b'' = 8\text{--}10$  ( $9 \pm 0.81$ );  $c = 6\text{--}7$  ( $6.2 \pm 0.21$ ); anterior extremity to excretory pore  $77\text{--}91$  ( $86 \pm 4.74$ ); body width  $16\text{--}18$  ( $17 \pm 0.80$ ); tail length  $82\text{--}98$  ( $88.6 \pm 4.24$ ); hyaline region to tail tip  $16\text{--}30$  ( $24 \pm 4.37$ ). Length from anterior extremity to end of metacarpus valve  $55\text{--}70$  ( $61 \pm 4.82$ ); metacarpus length  $12\text{--}14$

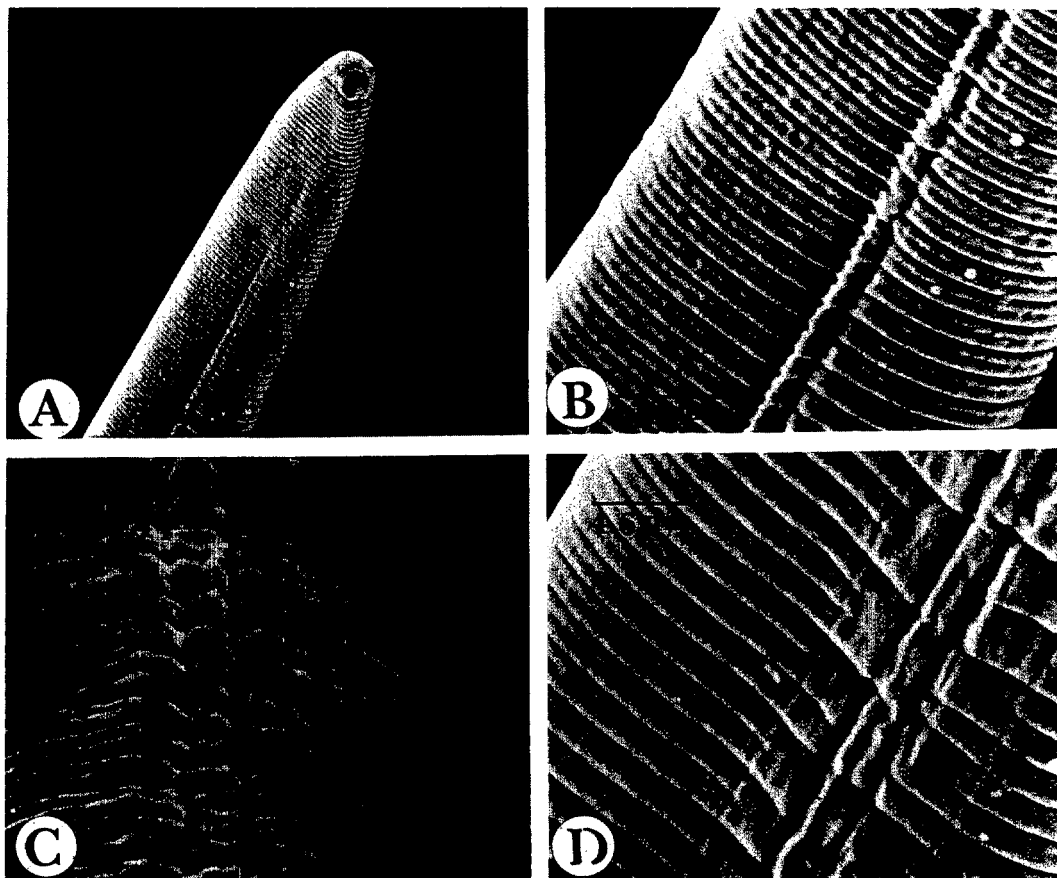


FIG. 5. Scanning electron micrographs of *M. californiensis* n. sp. second-stage juvenile. A) Anterior body region. B) Lateral field at anterior region. C) Lateral field posterior to two incisures area. D) Lateral field at midbody.

( $12.7 \pm 0.83$ ); metacarpus width 6–9 ( $8 \pm 0.78$ ); anterior end to esophageal gland end 173–264 ( $222 \pm 29.10$ ); anterior extremity to hemizonid 74–88 ( $79 \pm 17.64$ ). Lateral field begins 29–30 annuli posterior to lip region.

*Description of juveniles (Figs. 1, 3, 4, 5):* Body vermiform, tapering slightly anteriorly and pronounced posteriorly. Cephalic region slightly set off, with two or three annuli, cephalic framework weak. Stylet delicate, knobs small, sloping posteriorly. Dorsal esophageal gland orifice about 2–3 from stylet base. Lateral field begins 29–30 annuli posterior to lip region; anteriorly begins with two areolated incisures, as seen with SEM; then four areolated incisures along most of body; no areolation seen with LM; occupies 0.25–

0.32 of body at midbody. Outer two bands wider than middle band. Hemizonid two body annuli anterior to excretory pore, about three body annuli long. Esophago-intestinal valve inconspicuous, posterior to level of excretory pore. Rectum inflated, phasmids small, conspicuous, located midway between hyaline area and tail tip. Tail shape consistent, terminal part of tail deformed, terminus pointed, tip with mucro-like projection.

*Eggs in water (29):* Length 95–124 ( $112 \pm 7.99$ ); width 34–56 ( $48 \pm 5.77$ ). Egg shell hyaline, without visible markings.

*Type host:* Bulrush, *Scirpus robustus*, collected by T. Watson of the California Department of Food and Agriculture on 11 April 1979.

*Type locality:* Pomponio Beach (Half

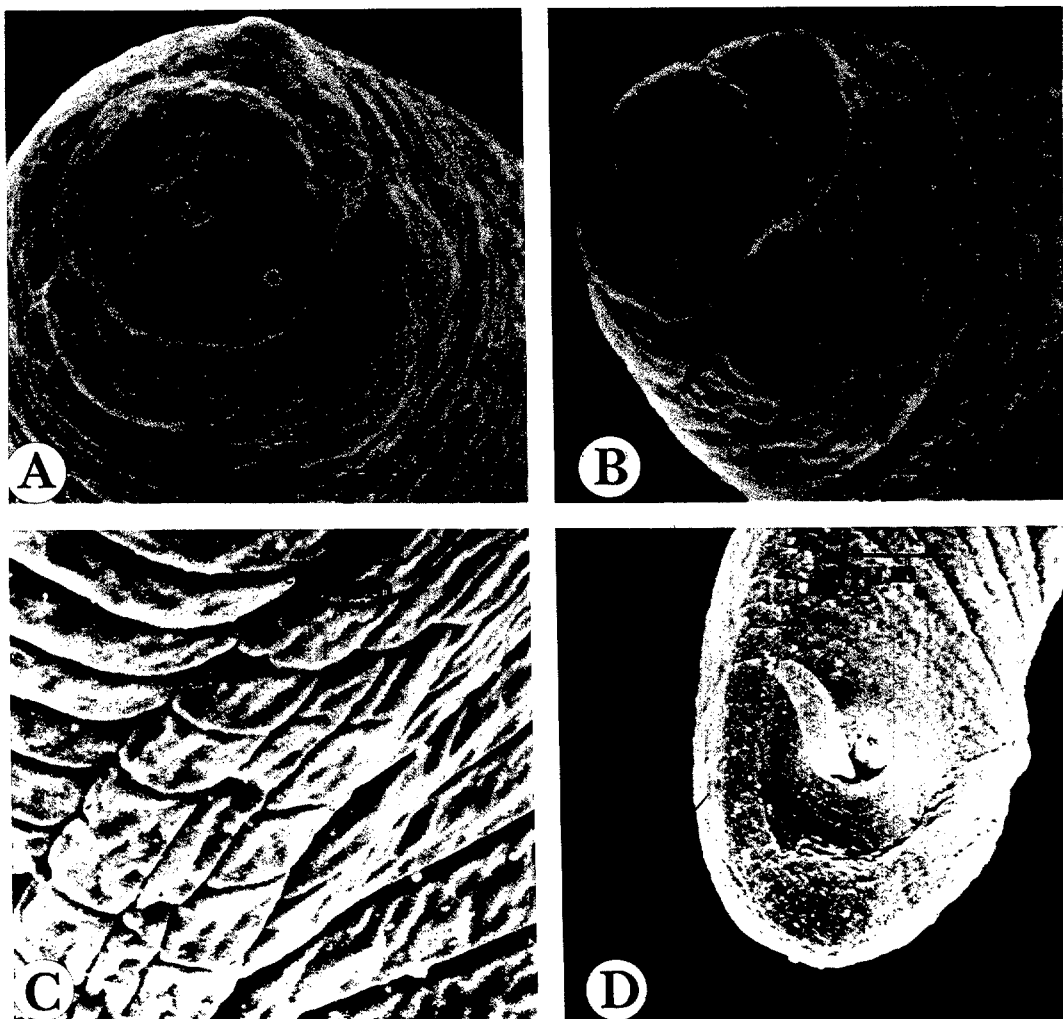


FIG. 6. Scanning electron micrographs of *M. californiensis* n. sp. long male. A, B) Face view. C) Aerolated lateral field. D) Posterior body region (tail).

Moon Bay) California. At the edge of a fresh water creek, as well as from completely submerged plants in the water. *M. californiensis* was found with a *Hirschmanniella* sp., which is described elsewhere in this issue (p. 147) as a new species.

#### Type designations

**Holotype (female):** Type slide no. 1998 from culture grown in the greenhouse on bulrush, originally obtained from type locality, deposited in University of California, Davis Nematode Collection (UCDNC).

**Allotype (male):** Same data as holotype, slide no. 1999.

**Paratypes:** One female, 30 males, 25 per-

ineal sections, 25 second-stage juveniles. Same data as holotype. One female, 9 perineal sections, 26 males, and 11 second-stage juveniles deposited in UCDNC; 5 perineal sections, 1 male, 4 second-stage juveniles, United States Department of Agriculture Nematode Collection, Beltsville, Maryland; 3 perineal sections, 1 male, and 4 second-stage juveniles, Nematode Collection of the Landbouwhogeschool, Wageningen, The Netherlands; 4 perineal sections, 1 male, and 4 second-stage juveniles, the Nematode Collection of the Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England; 4 perineal sections, 1 male and 3



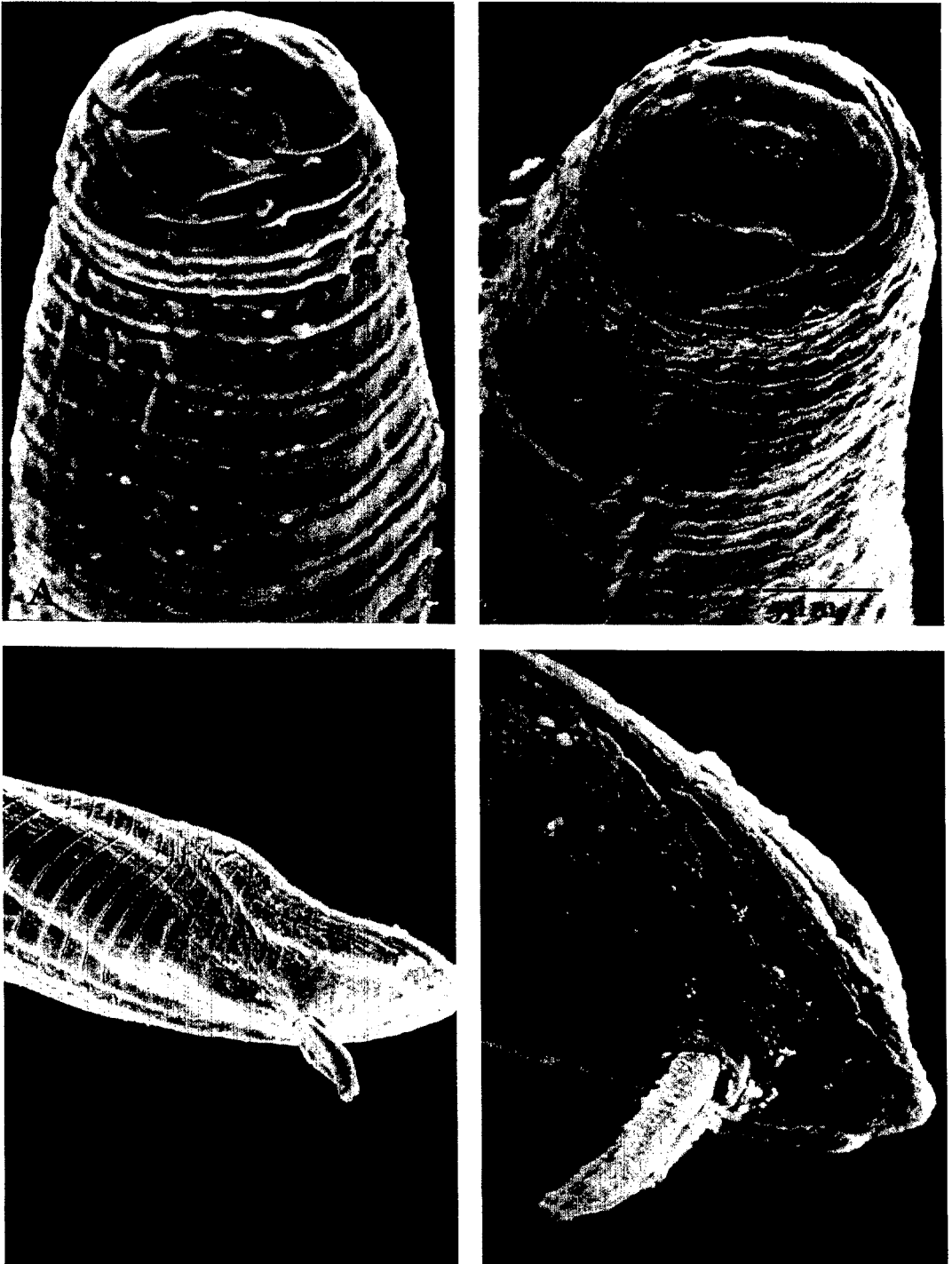


FIG. 7. Scanning electron micrographs of *M. californiensis* n. sp. short male. A, B) Face view. C, D) Posterior body region (tail).

second-stage juveniles, Muséum National d'Histoire naturelle, Collection Nationale de Nématodes, 61 Rue de Buffon, 75005 Paris, France.

*Diagnosis:* *Meloidogyne californiensis* n. sp. differs from all other described species of root-knot nematodes by its distinct perineal pattern with two prominent protu-

berances bordering the vulva. *Meloidogyne californiensis* n. sp. could be related to *M. ottersoni* (Thorne, 1969) Franklin, 1971 and *M. spartinae* (Rau and Fassuliotis, 1965) Whitehead, 1968. The length of the second-stage juvenile of *Meloidogyne californiensis* n. sp. is 448–628 (559), making it closely related to the above species.

However, *M. californiensis* n. sp. larvae differ from *M. ottersoni* which have undilated rectums (4). *M. californiensis* n. sp. juveniles have a larger “a” value and smaller stylet than *M. ottersoni*; larval tail shapes also differ. The female of *M. californiensis* n. sp. differs from *M. ottersoni* in the position of the excretory pore, which is far forward and opposite to the spear base in *M. ottersoni*, and the shape of perineal pattern. In *M. californiensis* n. sp. the excretory pore is located on the 16–25th annulus behind the lip region, i.e., about two stylet lengths. Second-stage juvenile of *M. californiensis* n. sp. are shorter, 448–628 (559), than juveniles of *M. spartinae*, 612–912. The “a” value in *M. californiensis* is 32–33.8 versus 43–65 in *M. spartinae*; also the “c” value is smaller, 5.9–6.5 versus 7–9 in *M. spartinae* (2,5). *M. californiensis* n. sp. has a smaller stylet, 11–13 versus 14–17 in *M. spartinae*.

#### DISCUSSION

*M. californiensis* n. sp. galls were obtained from bulrush roots at the edge of a fresh water creek as well as from completely submerged plants; bulrush plants some distance from the creek had no galls. Galls produced on bulrush by this species of *Meloidogyne* have a distinct shape. Terminal galls are peanut shaped, clubbed, or spindle shaped. Nonterminal galls sometimes are curved or spiral shaped like those produced by *M. naasi* (1), but the galls are not accompanied by root branching. Large numbers of females and immature stages may be found in a single gall. Sometimes up to 30 or 40 adult females were present in a single gall. When large numbers of females are found in one gall, most of the egg masses are laid inside the gall, but when there are one or a few females, they are

laid outside the galls. The egg masses are always large and yellowish in color; they contain an average of 500 eggs/mass. Males are often found in the soil or coiled inside the gall. It was observed that giant males were numerous in the soil in January. They were at least twice as long as the normal males which are observed throughout the year. The large males have two testes, indicating sex reversal (normal short males have one testis). SEM photomicrographs showed that there are distinct differences in face view and tail tip morphology between the short males and long males (Figs. 6, 7). In the long male face view, the labial disc surrounding the stoma opening is rounded and elevated above the subdorsal and subventral fused lips and the tail terminus is broad and rounded. In the short male face view, the labial disc is very small and is not distinctly raised above the subdorsal and subventral fused lips; this gives the lip cap a continuous elongated shape. The shape of the tail is much narrower and pointed, rather than broad and rounded as in long males.

Comparative studies concerning the length of *M. naasi* and *M. hapla* males and the variation of face views were also done using SEM. There are no differences in the face view or tail tips between the long and short males of these two species.

A host-range test was conducted on the following economically important crops: tomato, *Lycopersicon esculentum* Mill (VF 145); barley, *Hordum vulgare* L. (Hannchen); barley (Wasco); wheat, *Triticum aestivum* L. (D6301); wheat (Anza); Sudan grass; rice, *Oryza sativa* L. (S-6); sweet corn, *Zea mays* L.; alfalfa, *Medicago sativa* L.; broad beans, *Vicia faba* L.; red clover, *Trifolium pratense* L.; and sugarbeet, *Beta vulgaris*. All soil from the tested plants was screened and the roots were incubated in a mist chamber; no *Meloidogyne* juvenile stages were recovered. The tomato test was repeated several times, as were the rice and barley tests; at no time were galls observed or nematodes recovered. Although *Meloidogyne californiensis* n. sp. did not infect or reproduce on any of the tested plants, it

might infect and reproduce on plants closely related to the bulrush, *Scirpus robustus*.

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