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Meloidogyne petuniae n. sp. (Nemata: Meloidogynidae), a Root-knot Nematode Parasitic on Petunia in Brazil

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Abstract: Meloidogyne petuniae n. sp. is described and illustrated from specimens parasitic on petunia (*Petunia hybrida* L.) in Brasilia, Brazil. The perineal pattern of the female is elongate to ovoid with a high, squarish arch and widely spaced, coarse striae. The stylet of the female is 12.9–16.5 µm long and has three small, rounded knobs that are distinctly set off from the shaft. Each knob is marked by a deep longitudinal indentation posteriorly and anteriorly. In SEM the base of the shaft appears to be divided into six distinct ridges. The excretory pore opens about 15.4–53.6 µm from the head end. Males are approximately 0.8–2.2 mm long. Most specimens have a high and narrow head cap, but in some the head cap is narrow and low. The stylet of the male is 21.1–26.0 µm long and has small, rounded knobs, set off from the shaft, but not indented as in the female. Second-stage juveniles are 353–464 µm long; the labial disc is fused with the medial lips to form a dumbbell-shaped head cap; the medial lips are indented posteriorly; and the head region is marked with one to two irregular annulations. The stylet is 9.2–10.8 µm long and has rounded, posteriorly sloping knobs. The tail is slender, approximately 46.4–57.2 µm long, and has a short hyaline terminus, 10.3–13.5 µm long. The somatic chromosome number is 2n = 41 and the esterase phenotype is VS1-S1, with S1 being a weak band. The malate dehydrogenase phenotype is N1, which is unique for this species. Petunia, tomato, tobacco, pea, and bean are good hosts; pepper, watermelon, and sweet corn are poor hosts; and peanut, cotton, and soybean are non-hosts. Galls produced by this species are smaller on petunia than on tomato.

Key words: Brazil, host range, *Meloidogyne petuniae,* nematode, new species, petunia, root-knot nematode, scanning electron microscopy, taxonomy.

Petunia plants (*Petunia hybrida* L.) that were severely infected with root-knot nematodes were collected from a garden at EMBRAPA/National Research Center for Vegetable Crops, Brasilia, Brazil. Symptoms included chlorotic and stunted plants, and infected roots that were submitted to the EMBRAPA/CNPH Nematology Laboratory in 1987 contained very small galls, less than 4 mm in diameter. In comparison, galls produced on tomato roots (*Lycopersicon esculentum* Mill. 'Rutgers') were often more than twice as large.

Additional studies conducted at Virginia Polytechnic Institute and State University on the morphology, cytology, mode of reproduction, host range, and biochemistry indicated that the isolate was a new species of root-knot nematode. *Meloidogyne petuniae* n. sp. is described herein, and the common name ''petunia root-knot nematode'' is proposed.

Meloidogyne species previously described from Brazil include *M. exigua* Goeldi, 1887 (Göldi, 1887) from coffee (*Coffea arabica* L.), *M. inornata* Lordello, 1956 (Lordello, 1956a) from soybean (*Glycines max* (L.) Merr.), *M. javanica* subsp. *bauruensis* Lordello, 1956 (Lordello, 1956b) from soybean, *M. coffeicola* Lordello & Zamith, 1960 (Lordello and Zamith, 1960) from coffee, *M. lordelloi* da Ponte, 1969 (da Ponte, 1969) from *Cereus macrogonus, M. elegans* da Ponte, 1977 (da Ponte, 1977) from *Schrankia leptocarpa,* and *M. paranaensis* Carneiro et al., 1996 from coffee (Carneiro, et al., 1996). Jepson (1987) synonymized *M. elegans* and *M. inornata* with *M. incognita* (Kofoid & White) Chitwood, 1949, and *M. javanica* subsp. *bauruensis* and *M. lordelloi* with *M. javanica* (Treub) Chitwood, 1949. The four most common species of root-knot nematodes, *M. arenaria* (Neal) Chitwood, 1949, *M. hapla* Chitwood, 1949, *M. incognita,* and

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M. javanica, are widely distributed in Brazil. The last two commonly damage many vegetable and field crops.

Materials and Methods

Meloidogyne petuniae n. sp. was established from a single egg mass from galled petunia roots collected from the type locality and propagated on tomato. All nematode stages used in morphological and morphometric studies were from stock cultures maintained on tomato and petunia seedlings in a greenhouse at 22 to 28 °C.

Morphological studies: Males and second-stage juveniles (J2) were extracted from pieces of washed galled roots incubated in a moist chamber. Light microscopy (LM) observations were made from specimens killed by heat in water and transferred to 2% glutaraldehyde buffered with 0.1 M sodium cacodylic acid (pH 7.2). Females were dissected from galled roots and placed directly into fixative for at least 2 hours. Fixed specimens were always compared to live specimens mounted in 0.9% saline solution (NaCl). Females, males, and juveniles were prepared for scanning electron microscopy (SEM) according to Eisenback (1985). Perineal patterns were prepared for SEM by combining techniques described by Eisenback (1985) and Abrantes and Santo (1989). One to two-celled eggs were measured in a drop of fresh water. All LM observations were made with a bright field microscope, and at least 100 specimens of each stage were observed.

Host range test: Seedlings of petunia, tomato cv. Rutgers, tobacco (*Nicotiana tabacum* L. 'NC 95'), cotton (*Gossypium hirsutum* L. 'Deltapine 16'), pepper (*Capsicum annuum* L. 'California Wonder'), watermelon (*Citrullus vulgaris* Schard. 'Charleston Gray'), pea (*Pisum sativus* L. cultivars 'Mikado' and 'Alaska'), soybean (*Glycine max* cultivars Cristalina and Essex), bean (*Phaseolus vulgaris* L. cultivars Carioca and Red Kidney), and sweet corn (*Zea mays* L. 'Golden Cross Bantam') were transplanted as single plants into 11-cm-diam. clay pots containing 500 cm^3 of sterilized sandy loam soil and inoculated with a suspension of 300 freshly hatched J2 in 5 ml of water placed in holes around the root system. Each treatment was replicated three times and maintained in a greenhouse at 22 to 28 °C for 45 days. The roots were gently washed in water and stained with phloxine B (Dickson and Struble, 1965) to observe egg masses.

Systematics

Meloidogyne petuniae n. sp. (Figs. 1–5)

Description

Holotype (female in glycerin): Body length including neck 849 µm; body width 571 µm; neck length 190 µm; stylet length 14.5 µm; stylet knob height 1.9 µm; stylet knob width 4.0 µm; dorsal esophageal gland orifice to stylet base 3.3 µm; excretory pore to head end 35.3 µm. Perineal region not visible.

Female (Figs. 1–3): Measurements of 30 females in 2% glutaraldehyde buffered with 0.1 M sodium cacodylic acid (pH 7.2) and perineal patterns in glycerin are listed in Table 1. Body translucent white, variable in size, pear-shaped to ovoid with short neck (Fig. 1A,B), posteriorly rounded, without posterior protuberance. In SEM, stoma slit-like, located in ovoid to hexagonal prestoma, surrounded by pit-like openings of six inner labial sensilla (Fig. 2A–D). Labial disc in most specimens small, rounded, slightly raised above medial lips; labial disc and medial lips fused, dumbbell-shaped to form a narrow lip structure in face view. Medial lips crescent-shaped. Lateral lips usually triangular, sometimes crescent-shaped; partially fused with medial lips. Some females with labial disc distinctly demarcated by four small bumps. Head region slightly set off from regular body annules, rarely marked with one incomplete annulation (Fig. 2A–D). In LM, cephalic framework distinct, hexaradiate, lateral sectors enlarged. Vestibule and vestibule extension moderately sclerotized. Cephalids and hemizonids not observed. Excretory pore variable in distance from the anterior end (15.4–53.6 µm), located at or below the level of the stylet

TABLE 1. Measurements of 30 females of *Meloidogyne petuniae* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (µm)					
Body length	698–1,002	804	12.4	86.3	12.9
Body width	454–721	565	12.2	67.9	13.2
Neck length	99–405	245	14.7	90.9	38.0
Stylet length	12.9–16.5	14.3	0.30	1.6	5.5
Stylet knob height	1.8–2.5	2.1	0.07	0.4	12.3
Stylet knob width	3.5–4.8	3.9	0.11	0.6	5.7
DGO	2.4–4.2	3.3	0.09	0.5	14.7
Excretory pore to head end	15.4–53.6	33.7	1.02	7.3	14.6
Interphasmidial distance	25.8–45.1	30.5	0.78	4.26	14.0
Vulva length	20.6–34.8	26.5	0.68	3.72	14.0
Vulva-anus distance	15.5–28.4	20.5	0.53	2.89	10.1
Ratios					
a	1.3–1.5	1.4	0.01	0.05	3.6
Body length/head end to posterior end of metacorpus	9.3–11.7	10.0	0.11	0.58	5.8
Number of body annules from head end to excretory pore	14–42	22.8	1.14	6.24	27.3

base. Stylet robust, widening gradually posteriorly, cone tip straight to slightly curved dorsally (Fig. 2E). Shaft cylindrical, robust, narrower near junction with cone; knobs wide and narrow, distinctly set off from shaft with deep longitudinal indentations in the middle of each knob both posteriorly and anteriorly. These indentations dividing the shaft base into six distinct ridges (Fig. 2F). Distance of dorsal esophageal gland orifice (DGO) from the stylet base short to moderately long (2.5–4.2 µm); orifice branched into three channels; dorsal gland ampulla small. Esophageal glands large, with one large uninucleate dorsal lobe and two smaller nucleated subventral lobes usually posterior to dorsal lobe, variable in size and shape.

Perineal patterns (Figs. 1C,3) elongated to ovoid. Dorsal arch flattened to very high, sometimes squarish; striae widely spaced, coarse. Rarely present, lateral fields with broken striae on both sides; ventral striae varying from wavy to coarse striations. Tail tip well defined, with few striae. Perivulval region not striated, few striae near lateral edges of vulva. Vulva slightly sunken, surrounded by wide cuticular striae, often branched into two or more striae. Phasmids small, ducts distinct within the cuticle, surface structure not apparent. Anus distinct, sometimes within a cuticular fold, surrounded by thick cuticular layer.

Allotype (male in glycerin): Body length 1,988 µm; body width 42 µm; stylet length 21.1 µm; stylet knob height 2.4 µm; stylet knob width 4.1 µm; dorsal esophageal gland orifice to stylet base 2.1 µm; excretory pore to head end 178.2 µm; tail length 18.4 µm; spicule length 28.9 µm; gubernaculum length 6.6 µm; testis length 1,092 µm; a 47.2 µm; c 108 µm; and T 54.9%.

Male: Measurements of 30 males in fresh tap water killed by heat are listed in Table 2. Body translucent white, vermiform, robust, tapering anteriorly, bluntly rounded posteriorly; tail twisting through 90° in heat-killed specimens. Head cap high in face and lateral view, tapering anteriorly, extending posteriorly onto distinctly set-off head region (Fig. 4A–E). Hexaradiate cephalic framework sclerotized; vestibule and vestibule extension distinct. Stoma slit-like, prestoma hexagonal, surrounded by pore-like openings of six inner labial sensilla (Fig. 4A–D). In most specimens, labial disc small, rounded, slightly raised above or fused with medial lips; medial lips narrow, fused partially with labial disc. Medial lips crescent to reniform-shaped, with outer margins some-

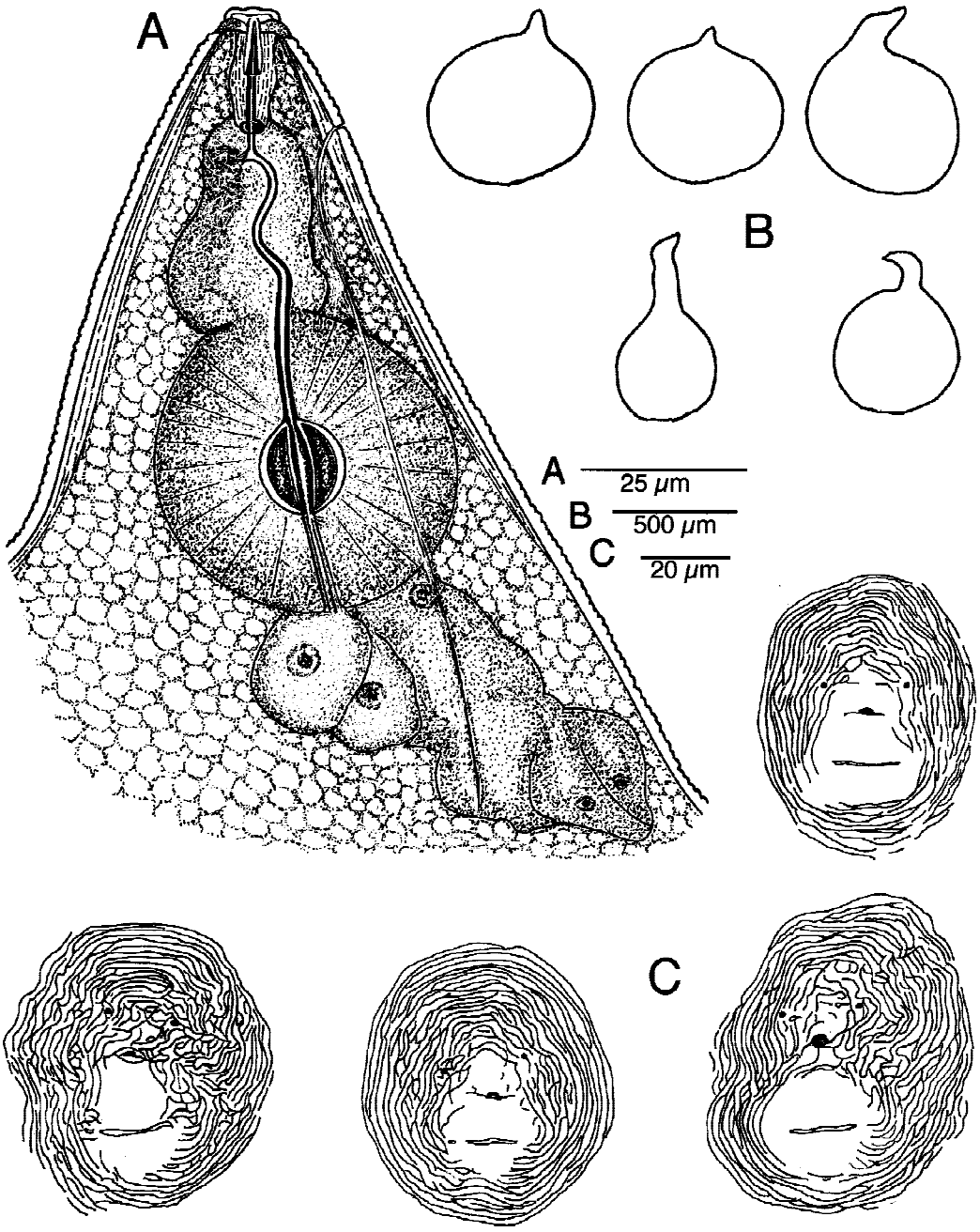

FIG. 1. Drawings of females of *Meloidogyne petuniae* n. sp. A) Anterior end (lateral). B) Outlines of whole specimens (lateral). C) Perineal patterns.

times slightly indented medially. Lateral lips, sometimes slightly demarcated by short, irregular grooves, usually almost completely fused with first head annule. Amphidial apertures wide, elongated, slit-like, located between labial disc and first head annule (Fig. 4A–D). Some specimens with large, rounded labial disc, slightly raised above medial lips. Head region smooth or marked by an incomplete annulation, distinctly set off from regular body annules. Body annules distinct. Lateral field marked with four inci-

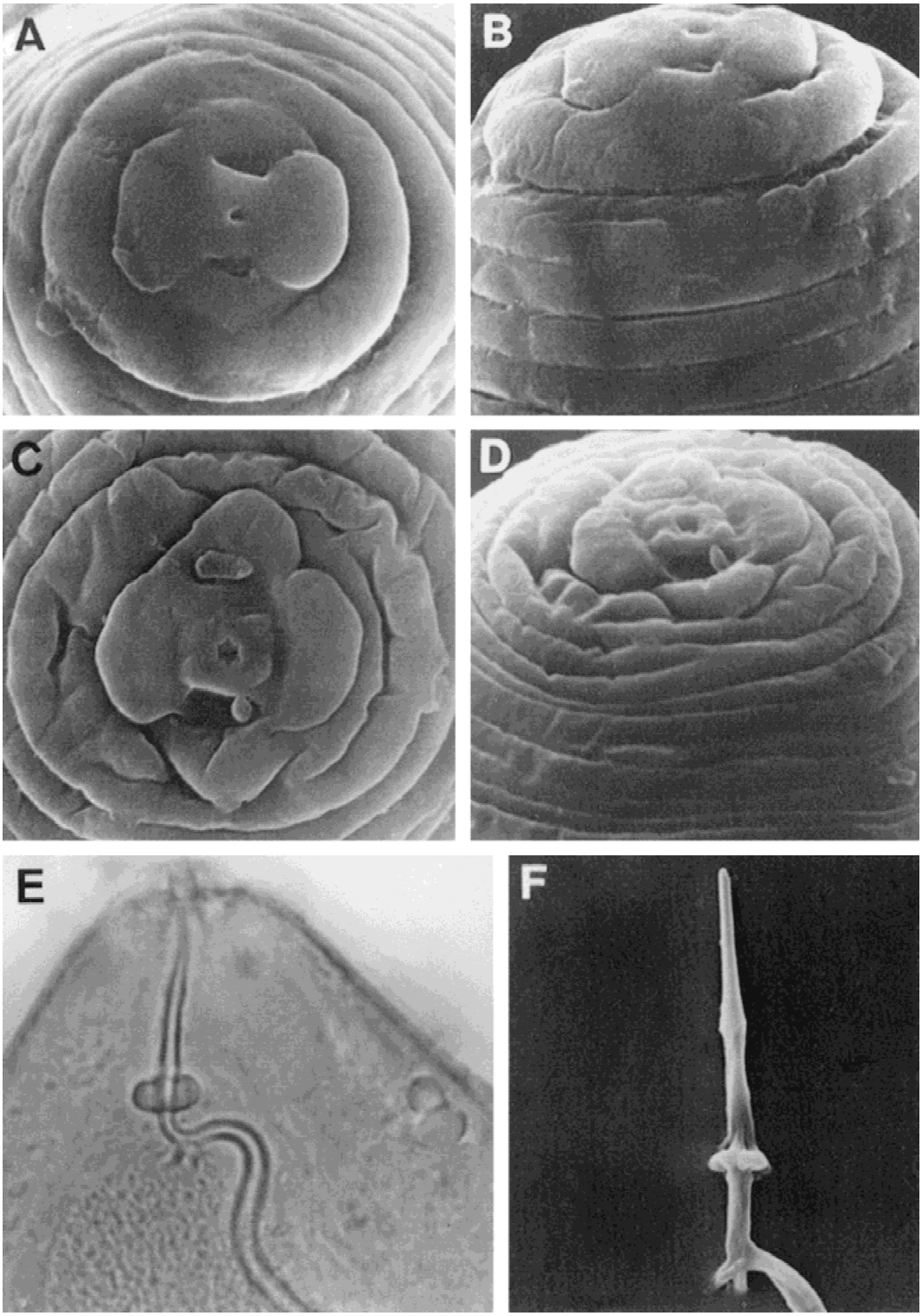

FIG. 2. Females of *Meloidogyne petuniae* n. sp. A–D) Scanning electron micrographs of anterior portion of head, face, and lateral views. E) Light micrograph of anterior end. F) Scanning electron micrograph of excised stylet.

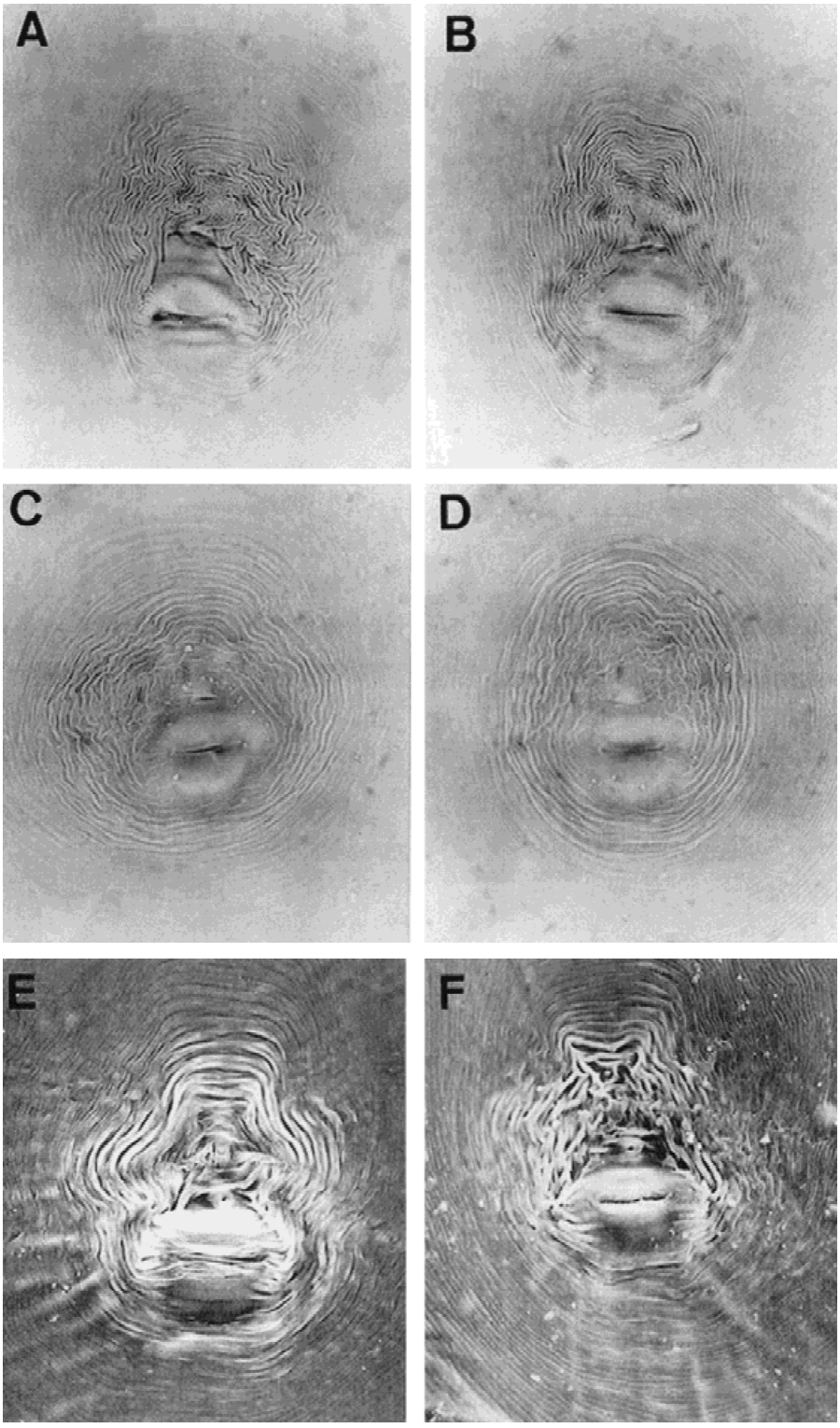

FIG. 3. Perineal patterns of females of *Meloidogyne petuniae* n. sp. A–D) Light micrographs. E,F) Scanning electron micrographs.

TABLE 2. Measurements of 30 males of *Meloidogyne petuniae* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (µm)					
Body length	849–2,202	1,904	62.71	312.2	18.8
Body width	46.0–62.4	55.2	1.01	5.1	8.6
Tail length	12.6–16.5	14.0	0.53	2.1	6.8
Stylet length	21.1–26.0	23.2	0.34	1.9	4.6
Stylet knob height	2.7–3.8	3.4	0.14	0.5	9.2
Stylet knob width	3.5–5.3	4.7	0.13	0.8	7.9
DGO	1.3–3.4	2.3	0.20	0.7	20.2
Excretory pore to head end	111–203	162	5.01	16.1	9.8
Spicule length	28.4–36.7	33.7	0.82	3.1	7.7
Gubernaculum length	5.6–8.1	7.0	0.14	0.99	10.2
Testis length	654–1,544	994	34.43	73.34	18.2
Ratios					
a	29.8–43.2	37.4	.89	2.72	9.8
c	70.6–102.4	92.2	1.81	7.9	7.8
Body length/head end to posterior end of metacorpus	14.5–20.1	17.9	0.41	1.02	8.7
Percentage					
T	47.8–76.6	55.8	.93	3.34	8.1

sures beginning near level of stylet knobs, areolated, encircling tail. Stylet slender, long (23.2 µm); cone straight, pointed, gradually increasing in diameter posteriorly; orifice located several micrometers from stylet tip (Fig. 4F). Shaft cylindrical, slender. Knobs large, wide, distinctly set off from shaft, and separated from each other; deep longitudinal indentations in the middle of each knob, posteriorly and anteriorly dividing shaft base into three distinct ridges (Fig. 4F). DGO-to-stylet-base distance variable (1.3–3.4 µm), dorsal gland duct branched into three channels, ampulla poorly defined. Procorpus distinct; metacorpus elongated, oval shape with enlarged, triradiated cuticular lumen lining; subventral esophageal gland orifices in metacorpus. Esophagointestinal junction indistinct, at level of nerve ring. Distance of excretory pore to anterior end variable (111–203 µm). Usually one testis, rarely two, generally outstretched or rarely reflexed anteriorly. Spicules robust, arcuate, with extremely thick blade, head rectangular, and sharply curved anteriorly; velum absent (Fig. 4G). Gubernaculum distinct, crescent-shaped; tail short and rounded; phasmids pore-like, at level of cloaca.

Second-stage juvenile: Measurements of 30 J2 in fresh tap water killed by heat are listed in Table 3. Body translucent white, vermiform, slender, tapering posteriorly to a sharp point (Fig. 5D). Stoma slit-like, located in oval-shaped prestoma, surrounded by pore-like openings of six inner labial sensilla (Fig. 5A,B). Labial disc rounded, slightly raised above medial lips, fused to form dumbbell-shaped lip structure; medial lips reniform. Lateral lips long, crescent-shaped, partially fused with labial disc and medial lips. Amphidial apertures elongated (Fig. 5B), located between labial disc and lateral lips. Head region high, slightly set off from body, marked by incomplete annules. Body annulation distinct, increasing in width and becoming irregularly spaced in posterior end. Lateral field beginning as a ridge near level of stylet base increasing to four lines; areolated. Cephalic framework weak (Fig. 5C). Vestibule and extension distinct. Stylet moderately sized, but delicate; stylet cone very narrow at tip and slightly pointed, gradually increasing in width posteriorly; knobs distinctly sclerotized, sloping posteriorly. DGO to stylet base moderately long (2.8–4.0 µm); orifice branched into three channels; ampulla poorly defined.

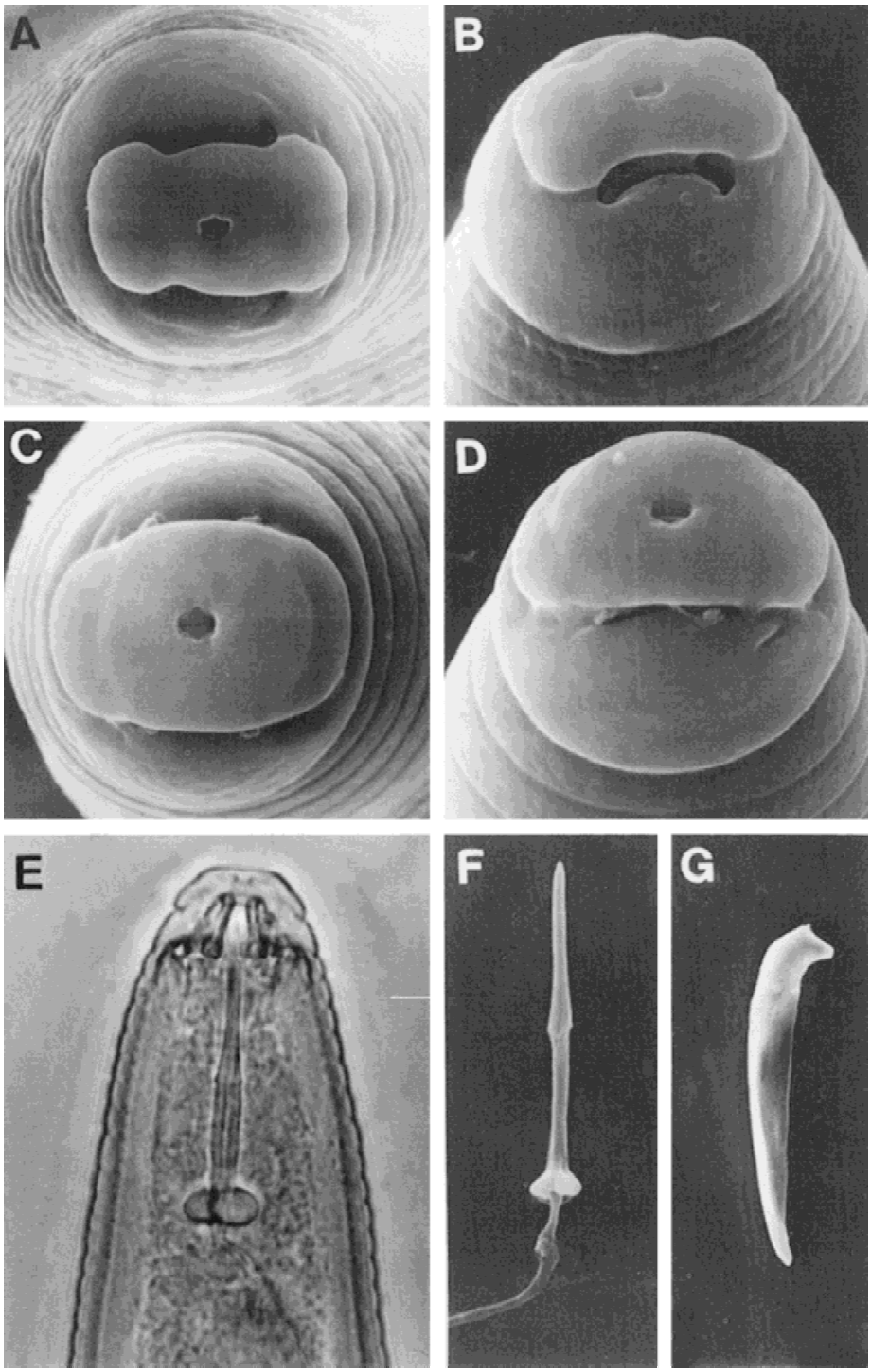

FIG. 4. Males of *Meloidogyne petuniae* n. sp. A–D) Scanning electron micrographs of anterior end of head, face, and lateral views. E) Light micrograph of the anterior end (lateral). F) Scanning electron micrograph (SEM) of excised stylet. G) SEM of excised spicule.

TABLE 3. Measurements of 30 second-stage juveniles of *Meloidogyne petuniae* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (µm)					
Body length	353–464	392	5.01	27.3	5.9
Body width	15.5–18.2	16.4	0.31	0.9	8.6
Tail length	46.4–57.2	47.0	0.64	3.7	9.6
Length of hyaline tail terminus	10.3–13.5	12.4	0.26	1.4	10.1
Excretory pore to head end	72.7–89.6	80.1	0.72	3.7	5.3
Stylet length	9.2–10.8	10.0	0.12	0.6	5.1
Stylet knob height	0.8–1.2	0.9	0.02	0.1	12.5
Stylet knob width	1.5–2.1	1.7	0.05	0.2	9.4
DGO	2.8–4.0	3.4	0.09	0.4	11.4
Ratios					
a	21.8–26.1	24.1	0.22	1.13	4.8
c	7.6–8.4	8.0	0.05	0.25	3.2
Body length/head end to posterior end of metacorpus	6.6–7.1	6.8	0.03	0.15	2.2

Procorpus distinct, metacorpus ovoid with large lumen lining; subventral esophageal glands opening posterior to triradiate lumen lining; ampulla distinct. Esophagointestinal junction indistinct, at level of nerve ring. Gland lobes variable in length. Excretory pore distinct, variable in position (72.7–89.6 µm), terminal duct long. Hemizonid distinct, anterior to excretory pore. Tail slender, ending in slightly rounded tip; annules increasing in width posteriorly; hyaline tail terminus small, length variable (10.3–13.5 µm). Phasmids small, distinct, located below level of anus; rectum dilated (Fig. 5D).

Eggs (1–2-celled in fresh tap water, n = 30): Length 75.4–98.8 µm (mean 87.0 µm, MSE 4.91 at 95% confidence interval); width 36.4–46.8 µm (41.0 ± 2.33 MSE); length/width ratio 2.07–2.11 µm (2.12 ± 0.05 MSE). Morphology indistinguishable from other *Meloidogyne* species.

Type host and locality

Roots of petunia found in the flower garden of the EMBRAPA/CNPH, National Research Center for Vegetable Crops, Brasilia, Brazil.

Type specimens

Holotype (female in glycerin): Isolated from single egg mass of greenhouse cultures maintained on petunia. Original population derived from type locality and host. U.S. Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland, USA.

Allotype (male): Same data as holotype. USDANC, Beltsville, Maryland, USA.

Paratypes (females, males, and J2): Same data as holotype. USDANC, Beltsville, Maryland, USA. University of California-Davis Nematode Collection (UCDNC), Davis, California, USA.

Diagnosis

Meloidogyne petuniae n. sp. can be distinguished from the most common species of root-knot nematodes (*M. arenaria, M. hapla, M. incognita,* and *M. javanica*) and other species in the genus by the form of the perineal pattern, the shape and morphology of head and stylet of the female; the shape and morphology of the male head, stylet, and spicule; and the head morphology of second-stage juveniles. In addition, the size and shape of galls produced by this new species on petunia compared to tomato are a useful diagnostic character for this species.

The unique perineal pattern and the morphology of the female head and stylet distinguishes this new species from all other species in the genus. The deep anterior and posterior indentations on each stylet knob and the ridges on the base of the shaft in *M.*

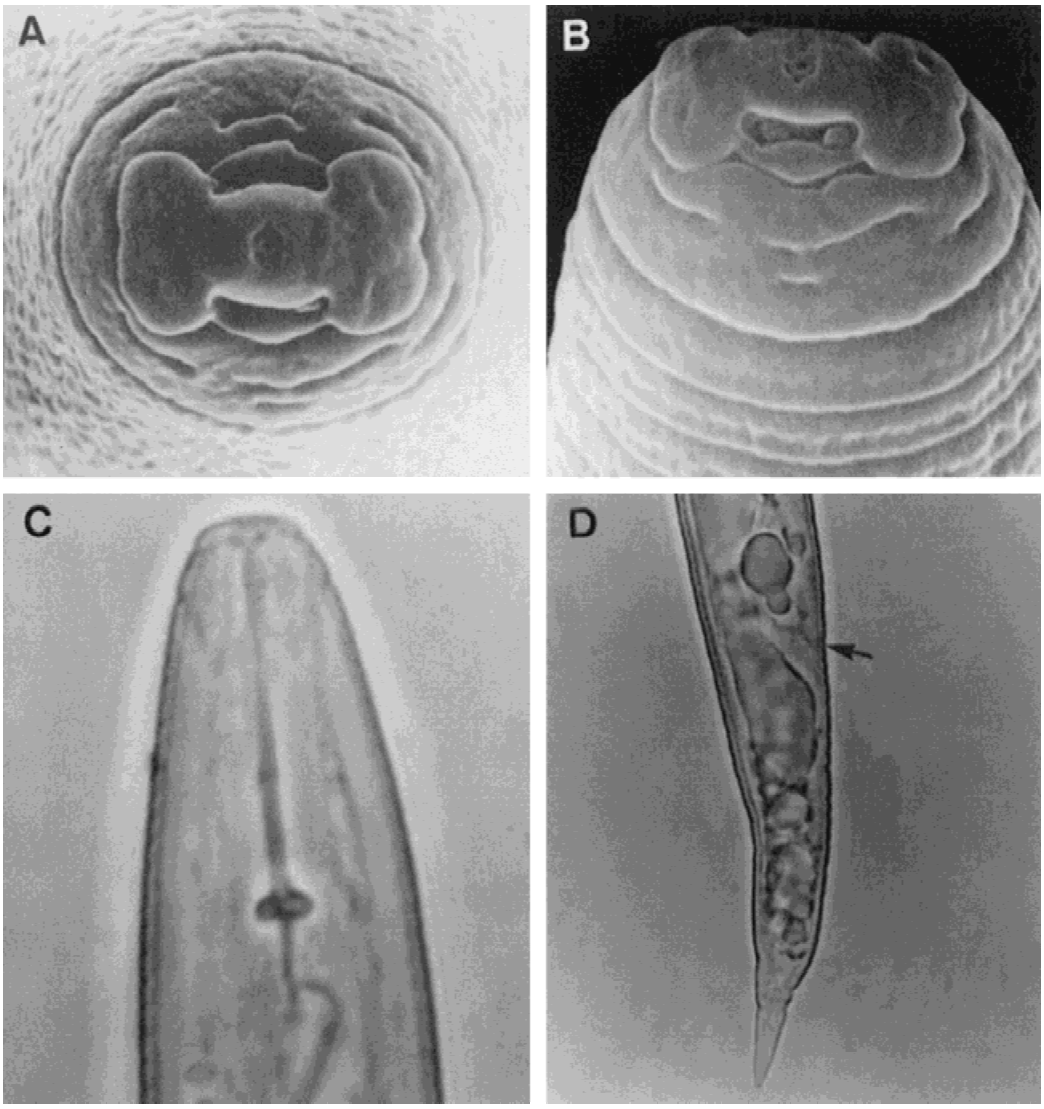

FIG. 5. Second-stage juveniles of *Meloidogyne petuniae* n. sp. A–B) Scanning electron micrographs of head, face, and lateral views. C) Light micrograph of anterior end (lateral). D) Light micrograph of tail (arrow marks anus).

petuniae n. sp. are unique for this species. The shape of the head of males of *M. petuniae* n. sp. is similar to that of *M. incognita;* however, the stylet knobs are marked by deep indentations that form ridges at the base of the shaft, similar to that of the female.

The differential host test showed that *M. petuniae* n. sp. can be easily diagnosed and differentiated from the four most common species because it does not reproduce on cotton and peanut and only slightly on pepper and watermelon. Cotton, pepper, and watermelon are good hosts for *M. incognita;* watermelon is also a good host for *M. javanica;* and pepper, watermelon, and peanut are good hosts for *M. arenaria* and *M. hapla.* Petunia was highly galled by *M. petuniae* n. sp. whereas *M. incognita* and *M. hapla* race A produced few galls. Petunia was only lightly galled by *M. javanica, M. hapla* race B, and *M. arenaria* race 2. Petunia was not galled by *M. arenaria* race 1. *Meloidogyne petuniae* n. sp. causes very small galls on petu-

nia compared to those on tomato. The four most common species, except *M. hapla,* produced large galls on petunia.

Reproduction of *M. petuniae* n. sp. occurs by obligatory mitotic parthenogenesis, and the somatic chromosome number is 2n = 41 (A. C. Triantaphyllou, pers. comm.). The esterase phenotype of *M. petuniae* n. sp. is VS1-S1, with S1 being a weak band. The malate dehydrogenase phenotype is N1 (P. R. Esbenshade, pers. comm.), which is unique for this species.

Relationships

The morphology of *M. petuniae* n. sp. is like that of *M. incognita* in many respects. The shape of the male head and the morphology of the J2 as seen with SEM are very similar to *M. incognita.* The morphology of the perineal pattern is unique to *M. petuniae* n. sp., as well as the shape of the stylet knobs and the base of the stylet shaft in males and females. The shape of the spicules is diagnostic for this species. In addition, the esterase and malate dehydrogenase phenotypes are unique for this species.

Discussion

Meloidogyne petuniae n. sp. was found for the first time parasitizing petunia, which is widespread in Brazil. *Meloidogyne petuniae* n. sp. may be indigenous in the savannah areas or it may have been introduced into Brasilia by nursery transplants of petunia or other crops such as potato, sweet potato, and yam from other Brazilian states or from other countries.

Currently, the petunia root-knot nematode is not considered economically important because it apparently has a limited distribution. However, this species can parasitize many economically important crops used in crop rotations in Brazil such as pea, bean, and corn. Potato (*Solanum tuberosum* L.), sweet potato (*Ipomoea batatus* (L.) Poir., and carrot (*Daucus carota* L.) are widely cultivated in Brazil and are good hosts. *M. petuniae* n. sp. may become important in the future because it can parasitize potato cultivars Baronesa and Bintje, sweet potato 'Roxa the Braslândia', carrot cultivars Brasilia and Nantes, pea cultivars Alaska and Mikado, bean cultivars Carioca and Red Kidney, and corn 'Golden Cross Bantam'). Soybean cultivars Cristalina and Essex are not hosts of *M. petuniae* n. sp. and thus may be used as a rotation crop if this new species is shown to be economically important.

Additional research is necessary to determine the general distribution of this nematode beyond the type locality, its pathogenic effects on various vegetable and field crops, and identification of sources of resistance. Field experiments are also needed to demonstrate the role of this nematode and possible interactions with other organisms such as fungi, bacteria, viruses, weeds, and insects.

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