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Meloidogyne brasilensis n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitising tomato cv. Rossol in Brazil

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Summary – *Meloidogyne brasilensis* n. sp., Londrina and Brasilia populations, is described and illustrated from specimens from tomato cv. Rossol and pea cv. Mikado, respectively, in Brazil. Characteristically, the perineal pattern is elongated to ovoid with a flattened to very high, squarish dorsal arch with widely spaced, coarse striae. The lateral fields may have wing-like striae on one or both sides. The female stylet is 14 μm long with narrow and elongated knobs that are distinctly set-off from the shaft. The excretory pore is variable in location, but generally opens near the anterior portion of the median bulb about 50 μm from the head end. The male is 1.89 mm long and has a high head cap that slopes posteriorly. The labial disc is separated from the medial lips by a deep, rounded groove. The delicate stylet of the male is 23 μm long and has small, rounded knobs that are distinctly set-off from the shaft which has numerous small, rounded projections. Mean second-stage juvenile length is 434 μm. The juvenile head cap is highly elevated, the medial lips are crescent-shaped and unequal in size, and the head region is not annulated. The stylet is 11 μm long and has small, rounded, posteriorly sloping knobs. The tail is 53 μm long; it is marked with large, irregular annules and ends in a bluntly rounded tip. The hyaline tail terminus is short (13 μm). Reproduction occurred on NC95 tobacco, tomato, pea, and bean, whereas pepper, watermelon, peanut, cotton, corn, and soybean were not hosts.

Keywords – Brazil, new species, SEM, taxonomy.

Meloidogyne brasilensis n. sp., Londrina population, was found infecting tomato (*Lycopersicon esculentum* Mill. cv. Rossol) on the property of Fazenda João Alves, located in the city of Londrina, Paraná state, Brazil in September, 1988. Tomato cv. Rossol is considered to be resistant to *M. incognita* (Kofoid & White, 1919) Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949 (Sasser & Kirby, 1979). The Brasilia population was found infecting pea (*Pisum sativum* L. cv. Mikado) in naturally infested field soil collected from the National Research Center for Vegetable Crops (EMBRAPA/CNPH), Brasilia, about 1500 km from Londrina, named as the type locality. Symptoms included root rot, severe wilting in tomato caused by the Londrina population, and numerous galls on pea produced by the Brasilia population.

Additional research conducted at the Virginia Polytechnic Institute on nematode morphology, together with a detailed host range study, indicated that these two populations were conspecific. *Meloidogyne brasilensis* n. sp. is described herein and the common name, 'Brazilian root-knot nematode' is suggested.

This is the first report of a new species of root-knot nematode being described from tomato in Brazil. *Meloidogyne* species previously described from Brazil include *M. exigua* Göldi, 1892 from coffee, *M. inornata* Lordello, 1956a from soybean, *M. javanica* subsp. *bauruensis* Lordello, 1956b from soybean, *M. coffeicola* Lordello & Zamith, 1960 from coffee, *M. lordelloi* da Ponte, 1969 from *Cereus macrogonus*, *M. elegans* da Ponte, 1977, *M. paranaensis* Carneiro, Carneiro, Abrantes, Castagnone & Almeida, 1995 from coffee, and *M. petuniae* Charchar, Eisenback & Hirschmann, 1999 from *Petunia hybrida*. Jepson (1987) synonymised *M. elegans* and *M. inornata* with *M. incognita* and *M. javanica bauruensis* and *M. lordelloi* with *M. javanica*. The four most common species of root-knot nematodes, *viz. M. arenaria* (Neal, 1889) Chitwood, 1949, *M. hapla* Chitwood, 1949, *M. incognita* and *M. javanica* are also widely distributed in

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Brazil; the last two commonly causing damage to many vegetable and field crops.

Materials and methods

The Londrina and Brasilia populations of *Meloidogyne brasilensis* n. sp. were established from a single egg mass from galled tomato cv. Rossol and pea cv. Mikado roots collected from the cities of Londrina and Brasilia, respectively, and propagated on tomato cv. Rutgers seedlings to obtain the stock cultures. Cultures of both populations were kept in a glasshouse at 22-28°C. All nematode stages used in morphological and morphometric studies were obtained from these cultures.

Morphological studies

Males and second-stage juveniles (J2) of both populations were extracted from galled roots in a moist chamber. Light microscopy (LM) observations were made from specimens stored in a refrigerator for at least 48 h and killed by gentle heat. Fixed specimens were always compared to live specimens mounted in 0.9% saline. Females, males, and juveniles of both populations were prepared for scanning electron microscopy (SEM) according to Eisenback (1985). Perineal patterns were prepared for SEM according to the technique described by Charchar and Eisenback (2001). One and 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 were observed.

Females were measured by mounting specimens in 2% glutaraldehyde in 0.1 M cacodylic acid buffer pH 7.2 and 30 perineal patterns were placed in glycerin. Measurements of males and second-stage juveniles were made from specimens mounted in fresh tap water stored in a refrigerator for at least 48 h and killed by gentle heat. Eggs were measured by mounting specimens in fresh tap water. At least 30 specimens from each life stage were measured.

Host range test

Seedlings of tomato (*Lycopersicon esculentum* Mill. cv. Rutgers), tobacco (*Nicotiana tabacum* L. cv. NC 95), cotton (*Gossypium hirsutum* L. cv. Deltapine 16), pepper (*Capsicum annuum* L. cv. California Wonder), watermelon (*Citrullus vulgaris* Schard. cv. Charleston Gray), peanut (*Hypogea arachis* L.), pea (*Pisum sativum* L. cvs Alaska, Lincoln and Mikado), bean (*Phaseolus vulgaris* L. cvs Carioca and Red Kidney), soybean (*Glycine max* (L.) Merr. cvs Cristalina and Essex), and sweet corn (*Zea mays* L. cv. Golden Cross Bantam) were transplanted as single plants to 11 cm diameter clay pots containing 500 cm^3 of sterilised sandy loam soil and inoculated with a suspension of 300 freshly hatched juveniles of each population in 5 ml of water in holes around the root system. Each treatment was replicated three times. The plants were maintained in a glasshouse at 22-28°C. Roots were gently washed and stained with phloxine B (Dickson & Struble, 1965) to count the egg masses.

Meloidogyne brasilensis n. sp.
(Figs 1-9)

Measurements

See Table 1.

Description

Female

Body translucent white, variable in size, pear-shaped to ovoid with relatively long neck, posteriorly rounded, without tail protuberance. In SEM, stoma slit-like, located in ovoid prestoma, surrounded by pit-like openings of six inner labial sensilla. Labial disc rounded, highly raised above lips. Labial disc and medial lips dumbbell-shaped in face view. Medial lips unequal, one side bigger than other. Lateral lips elongated, narrow, triangular, separated from medial lips and head region. Head region set-off from regular body annules, often marked with several folds appearing as distinct, incomplete, transverse annulations. In LM cephalic framework distinct, hexaradiate, lateral sectors enlarged. Vestibule and extension prominent. Cephalids and hemizonid not observed. Distance of excretory pore to head end variable; in most specimens near anterior of median bulb; terminal excretory duct very long. Stylet long, robust; cone slightly shorter than shaft, tip slightly curved dorsally, widening gradually posteriorly. Shaft cylindrical, same width throughout, or widening slightly near junction with knobs. Knobs narrow, elongated, set-off from shaft, distinctly separated from each other; knobs divided by deep indentation, sloping posteriorly. Distance between stylet base and dorsal pharyngeal

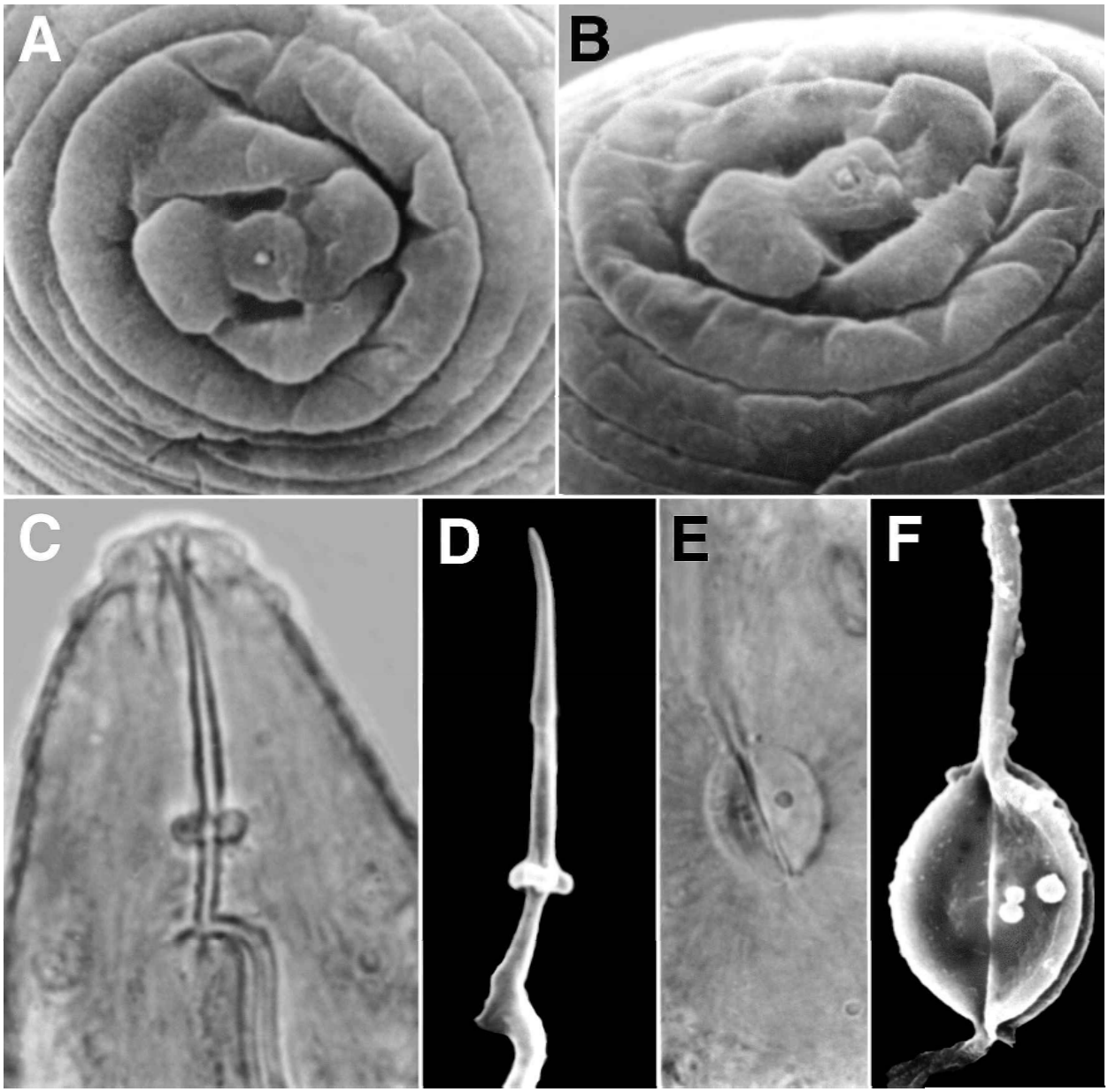

Fig. 1. *Females of* Meloidogyne brasilensis *n. sp. Londrina population. A, B: Scanning electron micrographs (SEM) of anterior end, face and lateral views; C: Light micrograph of anterior end, lateral view; D: SEM of excised stylet; E: LM of cuticular lining of excised pharyngeal metacorpus; F: SEM of cuticular lining of excised pharyngeal metacorpus.*

gland orifice (DGO) moderately long; gland orifice branched into three channels; dorsal gland ampulla large; subventral gland orifices branched, located posteriorly to enlarged triradiate lumen lining of metacorpus. Rounded granules of unknown constitution located between metacorpal lumen lining and muscles. Pharyngeal glands large, trilobed; dorsal lobe large, uninucleated; two subventral nucleated lobes variable in size, shape and position, located posterior to dorsal gland lobe. Two pharyngointestinal cells, rounded, nucleated, located between metacorpus and intestine. Two gonads and six rectal glands as characteristic of genus. Eggs (n = 30) 87 ± 2.8 (83-94) × 43 ± 2.3 (39-47) μm in size with a length/width ratio of 2.0 ± 0.07 (1.9-2.1). Perineal pattern small, rounded to oval-shaped. Dorsal arch flattened to high, squarish and widely spaced with coarse striations. Lateral fields may have wing-like striae on one or both sides. Ventral striae varying from wavy to coarse striations. Tail tip area well defined with striations in most specimens. Perivulval region not striated, often with striae near

Table 1. *Measurements of 30 females, males and second-stage juveniles (J2) of* Meloidogyne brasilensis *n. sp. (All measurements in μm and in the form: mean ± SD (range) coefficient of variation.)*

	Female		Male		J2
	Holotype	Paratypes	Allotype	Paratypes	Paratypes
L	954	763 ± 94 (601-959) 12.3	1890	1885 ± 414 (883-2489) 21.9	434 ± 34.5 (322-473) 8.0
Body diam.	617	508 ± 77 (387-728) 15.2	49	42 ± 8.5 (23-57) 20.1	17 ± 1.7 (15.6-20.8) 9.9
Neck length	277	271 ± 74 (138-442) 27.4	–	–	–
Stylet length	12.9	14.3 ± 0.9 (12.6-16.4) 6.2	19	22.8 ± 1.6 (18.9-25.2) 7.2	11.0 ± 0.7 (9.7-12.2) 5.9
Stylet knob height	1.9	1.8 ± 0.3 (1.3-2.5) 16.5	2.6	2.4 ± 0.4 (1.3-3.4) 17.7	0.8 ± 0.14 (0.4-1.1) 18.1
Stylet knob width	3.9	3.7 ± 0.5 (2.9-4.6) 13.4	3.9	3.9 ± 0.4 (2.9-4.6) 10.7	1.6 ± 0.19 (1.3-2.1) 12.4
DGO	5.0	4.0 ± 0.6 (2.9-5.0) 14.1	2.6	2.7 ± 0.6 (1.7-4.2) 20.7	3.0 ± 0.3 (2.5-3.4) 10.5
Excretory pore to ant. end	64.5	50 ± 11 (35-76) 21.9	221	186 ± 30.5 (127-250) 16.4	88 ± 8.0 (73-108) 9.1
Interphasmidial distance	–	23.7 ± 2.5 (18.1-29.0) 10.4	–	–	–
Vulva length	–	23.9 ± 1.8 (19.3-27.1) 7.7	–	–	–
Vulva-anus distance	–	19.9 ± 1.9 (15.5-23.2) 9.7	–	–	–
Body annules from ant. end to excretory pore	37	38.3 ± 9.6 (20-56) 25	–	–	–
Tail length	–	–	18	17.3 ± 2.1 (13.4-22.7) 12.2	53 ± 3.9 (43-58) 7.4
a	1.6	1.5 ± 0.1 (1.3-1.6) 3.7	38.3	44.8 ± 4.9 (35.8-55.1) 11.0	25.1 ± 1.7 (20.7-27.7) 6.9
b′	8.5	8.0 ± 0.5 (7.2-8.7) 5.6	21.3	19.3 ± 3.4 (10.5-25.3) 17.5	7.2 ± 0.2 (6.3-7.4) 3.3
c	–	–	104.8	109 ± 23.2 (52.5-167.7) 21.3	8.2 ± 0.2 (7.5-8.6) 2.1

Table 1. *(Continued).*

	Female		Male		J2
	Holotype	Paratypes	Allotype	Paratypes	Paratypes
T	–	–	46.3	45.0 ± 5.3 (32.8-57.3) 11.8	–
Spicule length	–	–	33.5	31.6 ± 3.3 (25.2-36.1) 10.6	–
Gubernaculum length	–	–	6.5	7.4 ± 0.8 (5.9-8.4) 10.2	–
Testis length	–	–	875	831 ± 254 (334-1337) 30.5	–
Hyaline tail terminus	–	–	–	–	13.1 ± 1.6 (10-16) 11.8

lateral edges of vulva. Phasmidial ducts distinct, phasmid surface structure not apparent in SEM. Anus distinct, often covered by a thick cuticular fold.

Male

Moderately long nematodes; body tapering anteriorly, bluntly rounded posteriorly; posterior end twisting through 90° in heat-killed specimens. Head cap high in lateral view, extending posteriorly on to distinctly set-off head region. Head region tapering posteriorly, distinctly set-off from body. Hexaradiate cephalic framework well sclerotised; vestibule and extension distinct. Prestoma large, hexagonal, stoma opening slit-like, located in large, hexagonal prestomatal cavity, surrounded by pore-like openings of six inner labial sensilla. In SEM (face view), labial disc rounded, very large, distinctly raised above medial lips. Medial lips narrower than labial disc, outer margins crescent-shaped. Labial disc and medial lips partially fused to form elongated lip structure. Four cephalic sensilla marked on the medial lips by shallow depressions. Amphidial apertures narrow, elongated, slit-like; located between labial disc and lateral sectors of head region. Lateral lips absent. Head region smooth, annulation absent. Body annules large, distinct. Lateral field with four incisures, two beginning near level of stylet knobs and two near level of metacorpus; lateral field areolated, encircling tail. Stylet delicate, slender; cone straight, pointed, gradually increasing in diameter posteriorly, opening located several micrometres from tip. Shaft cylindrical with small, rounded projections near middle of shaft. Stylet knobs small, very narrow, set-off from shaft; sloping posteriorly. Dorsal pharyngeal gland orifice to stylet base of variable length, dorsal gland duct branched into three channels, gland ampulla distinct. Procorpus distinctly outlined, metacorpus elongated, oval-shaped with enlarged tri-radiate cuticular lumen lining; subventral pharyngeal gland orifices branched, located posteriorly in metacorpus. Pharyngo-intestinal junction weak. Gland lobe variable in length, with two or three nuclei visible. Excretory pore distinct, variable in position, terminal duct long. Hemizonid slightly anterior to excretory pore. Intestinal caecum extending anteriorly on dorsal side at level of metacorpus. Usually one testis, rarely two; outstretched, or reflexed anteriorly. Spicules long with a thick blade, slightly arcuate with single tip, long and wide head with an indistinct velum. Gubernaculum distinct, crescent-shaped. Tail short and rounded. Slit-like opening of phasmids in lateral field at cloacal level.

Second-stage juvenile

Body long, slender, tapering anteriorly but more so posteriorly; tail region distinctly narrowing. Body annules distinct, increasing in size and becoming irregular in posterior tail region. Lateral field starting at middle of procorpus and extending nearly to tail tip; four incisures, usually areolated. Stoma slit-like, located in oval-shaped prestomatal depression, surrounded by pore-like openings of six inner labial sensilla. Head cap high, narrower than head region. In SEM, labial disc rounded, raised above medial lips by a rounded groove. Medial lips with outer mar-

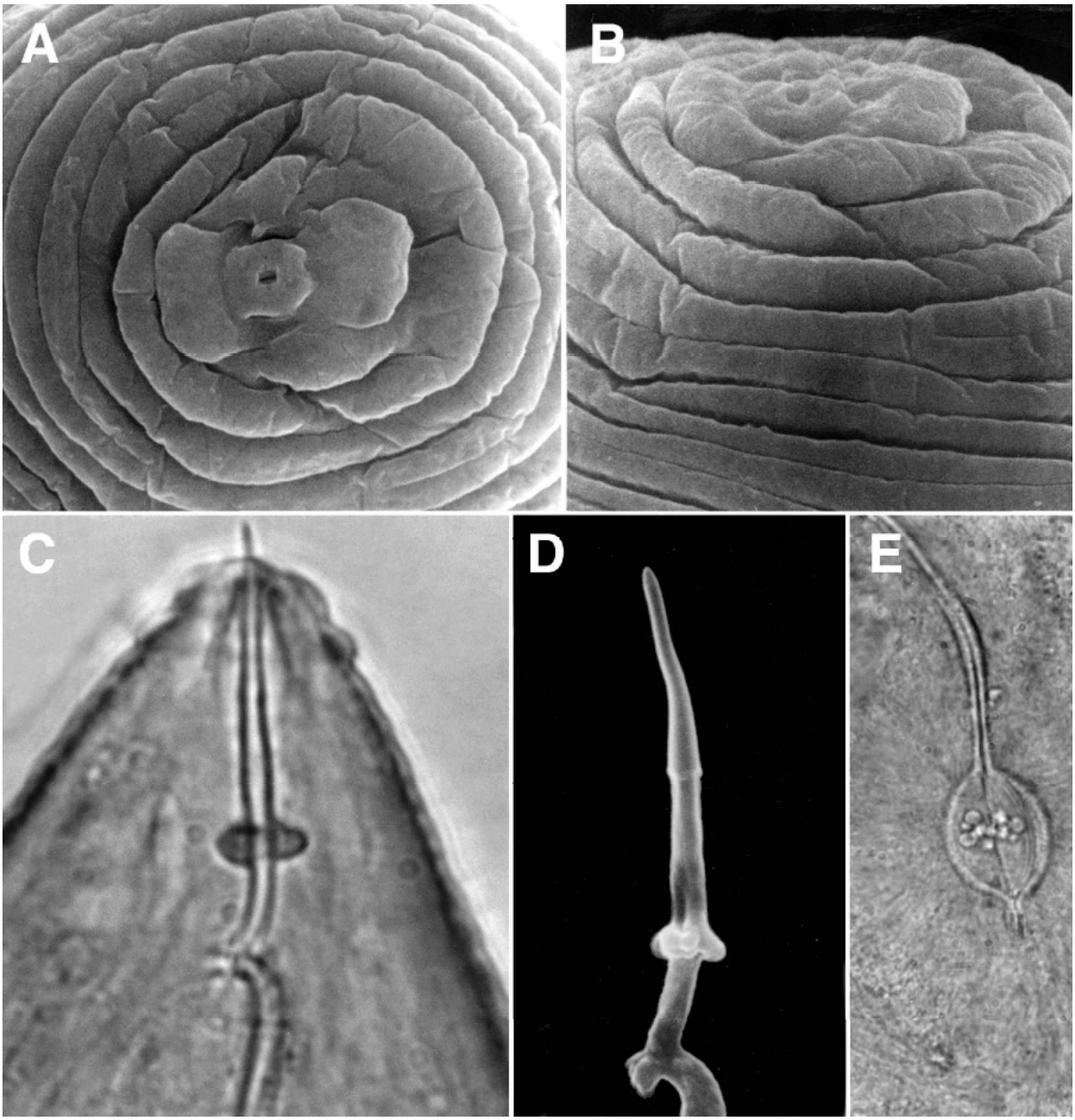

Fig. 2. *Females of* Meloidogyne brasilensis *n. sp. Brasilia population. A, B: Scanning electron micrographs (SEM) of anterior end, face and lateral views; C: Light micrograph of anterior end, lateral view; D: SEM of excised stylet; E: LM of cuticular lining of excised pharyngeal metacorpus.*

gins crescent-shaped and unequal, one side wider than the other. Medial lips and labial disc dumbbell-shaped. Lateral lips distinct, lower than medial lips, margins crescent-shaped, may fuse slightly with head region. Head region smooth, without annulation. Amphidial apertures elongated, located between labial disc and lateral lips. Head region high, distinctly set-off from body. Hexaradiate framework weakly sclerotised in LM, vestibule and extensions distinct. Stylet moderately long, but delicate, stylet cone sharply pointed, shaft cylindrical, may widen slightly posteriorly; knobs rounded. Distance of dorsal pharyngeal gland orifice to stylet base moderately long; orifice branched into three channels; ampulla weakly defined. Procorpus faintly outlined, metacorpus ovoid with

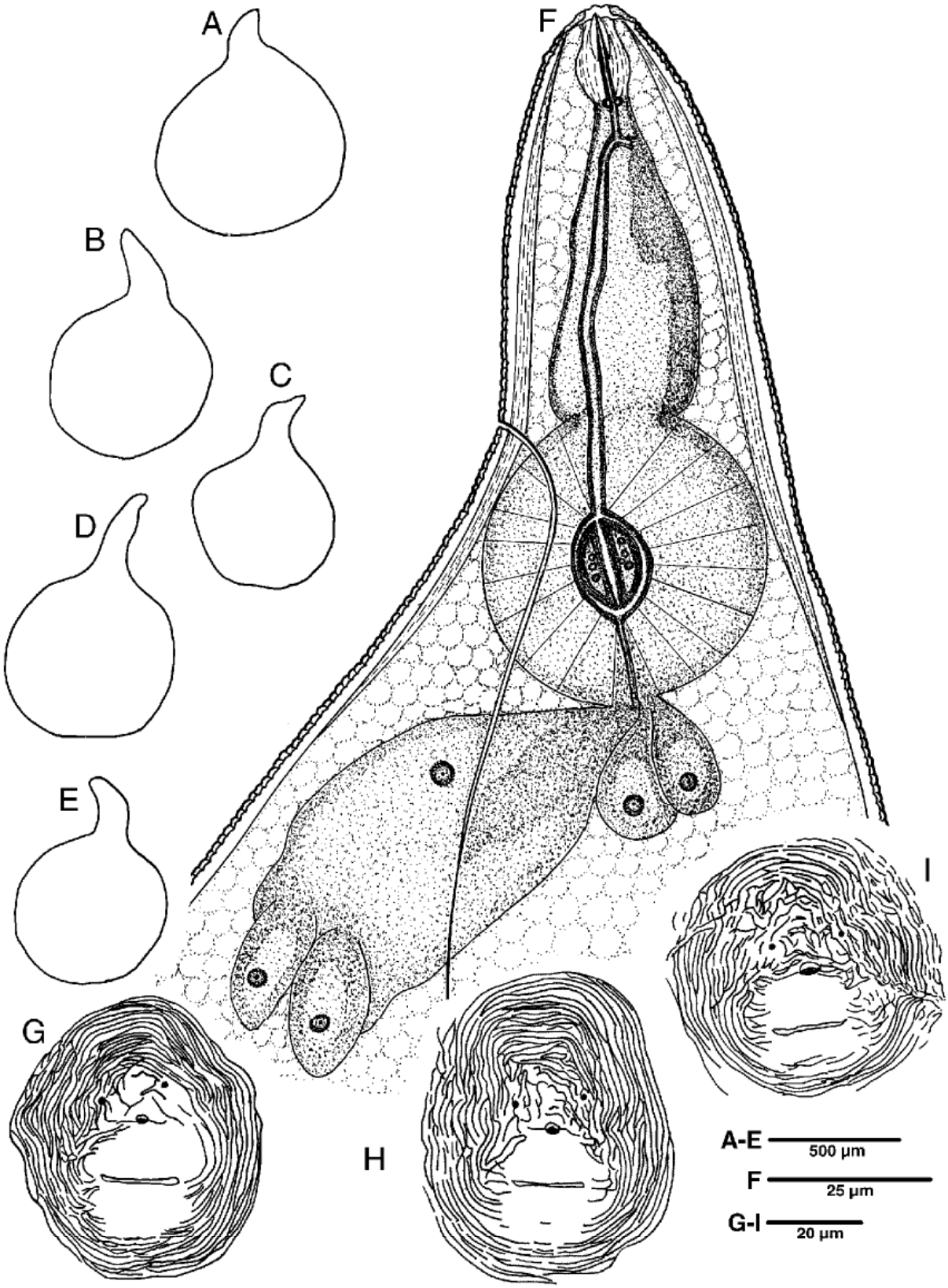

Fig. 3. *Drawings of females of* Meloidogyne brasilensis *n. sp. A-E: Outlines of whole specimens, lateral view; F: Anterior end, lateral view; G-I: Perineal patterns.*

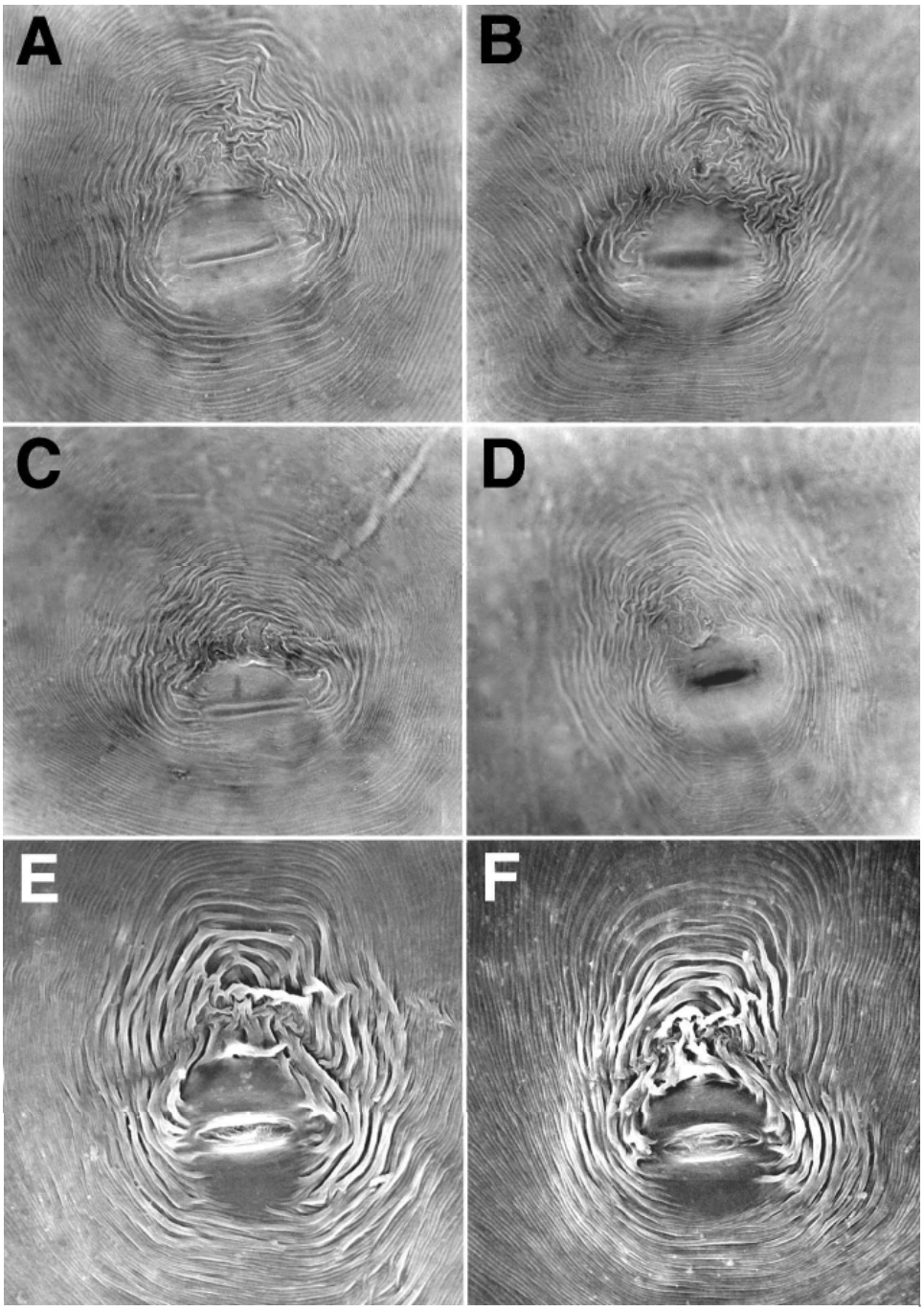

Fig. 4. *Perineal patterns of females of* Meloidogyne brasilensis *n. sp. A-D: Light micrographs; E-F: Scanning electron micrographs.*

distinct lumen lining; subventral pharyngeal gland orifices posterior to valve; ampullae distinct. Pharyngo-intestinal junction weak, at level of nerve ring. Pharyngeal gland lobe variable in length with three small nuclei. Excretory pore distinct, variable in position, terminal duct very long. Hemizonid distinct, located anterior to excretory pore. Tail slender, ending in bluntly rounded tip. Hyaline tail end short. Rectal dilation large. Phasmids small, located posterior to anus.

Type host and locality

Tomato (*Lycopersicon esculentum* Mill.) cv. Rossol found on the property owned by Fazenda João Alves, Londrina, Paraná state, Brazil.

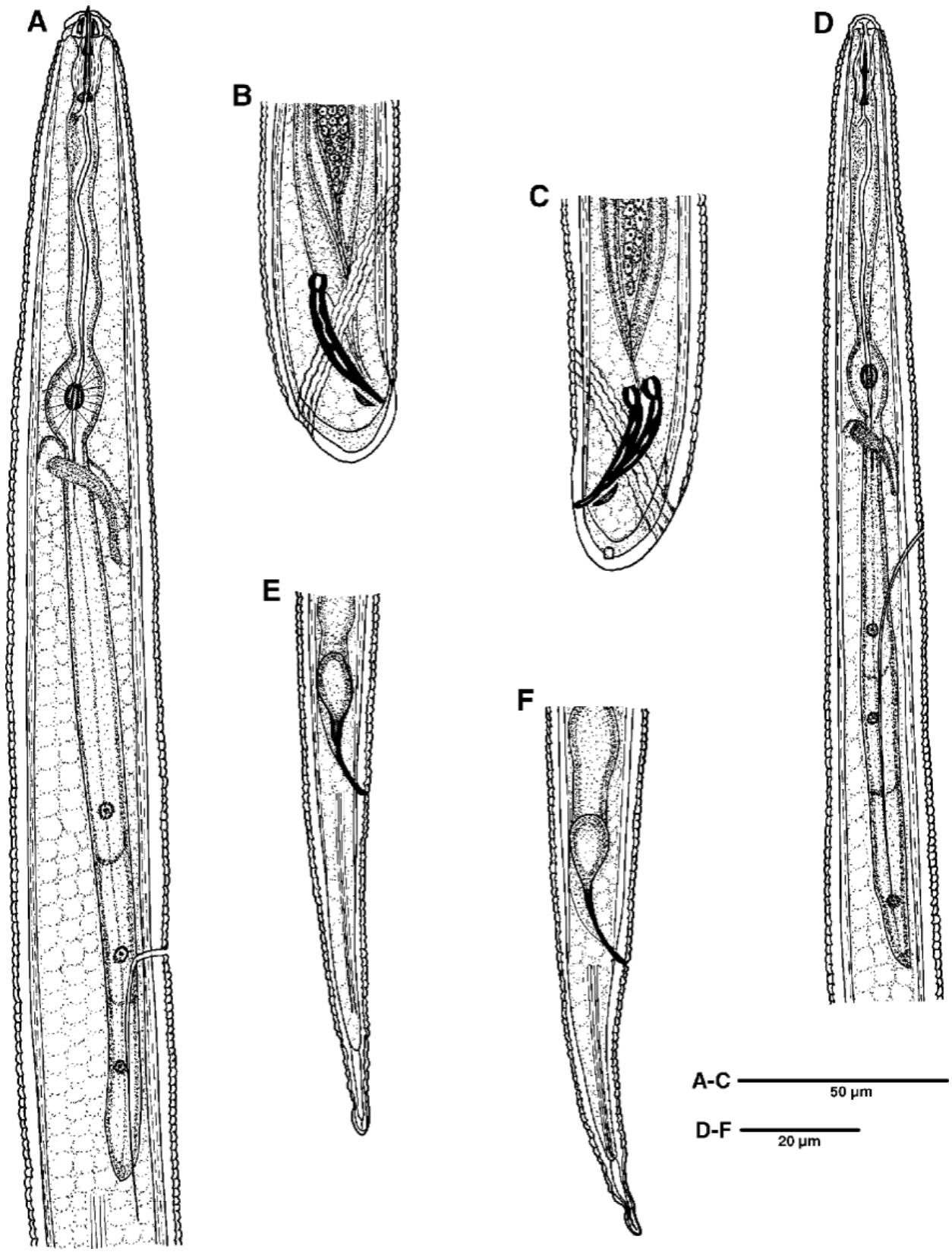

Fig. 5. *Drawings of males and second-stage juveniles of* Meloidogyne brasilensis *n. sp. A: Anterior end of male, lateral view; B, C: Male tails, lateral views; D: Anterior end of second-stage juvenile, lateral view; E, F: Tails of second-stage juvenile, lateral views.*

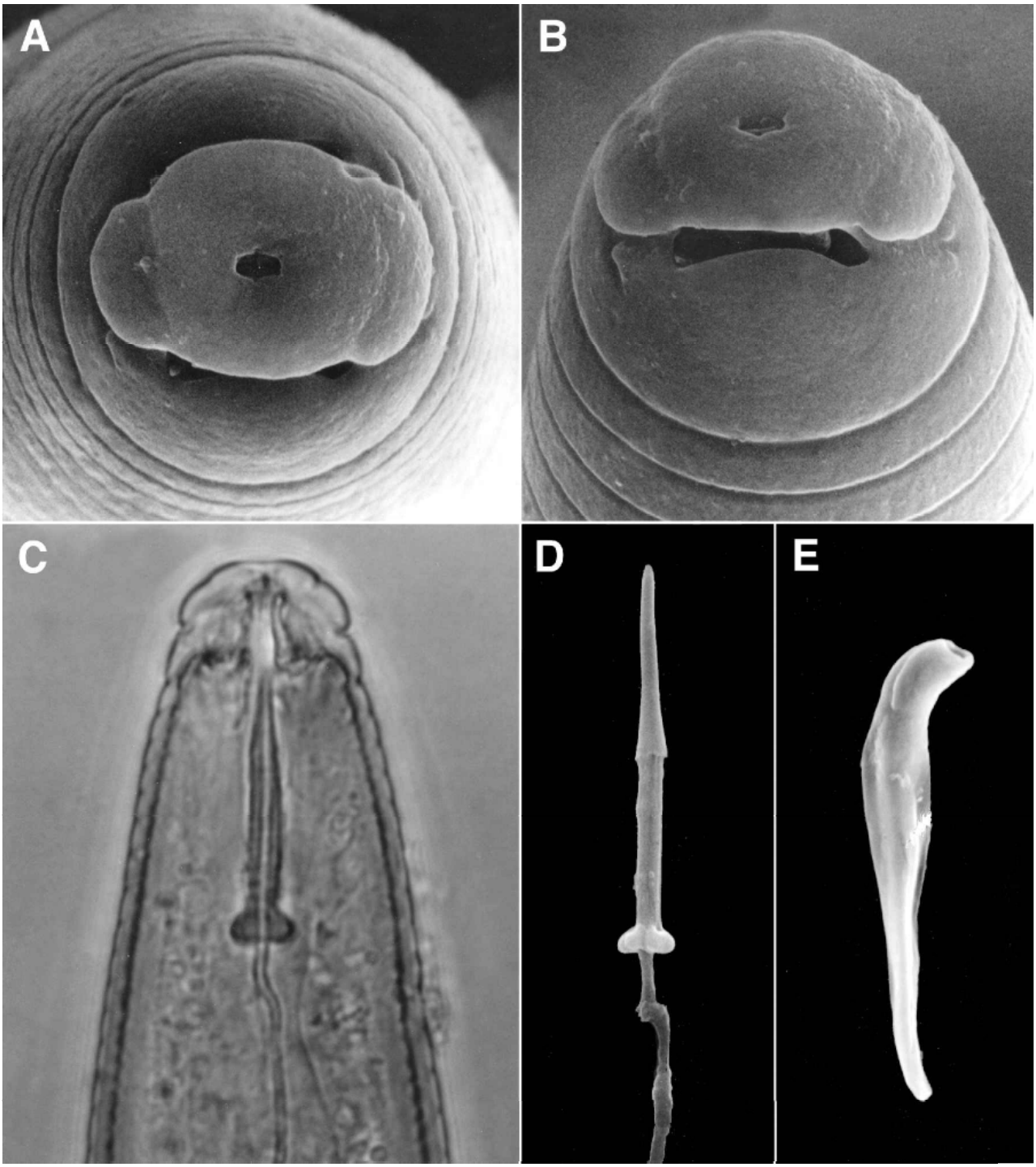

Fig. 6. *Males of* Meloidogyne brasilensis *n. sp. Londrina population. A, B: Scanning electron micrographs (SEM) of anterior end, face and lateral vews; C: Light micrograph of anterior end, lateral view; D: SEM of excised stylet; E: SEM of excised spicule.*

Other population and host

M. brasilensis n. sp. was also found infecting pea (*Pisum sativum*) in Brasilia, about 1500 km away from the type locality.

Type material

Holotype female isolated from single egg mass of glasshouse cultures maintained on tomato cv. Rutgers. Original population derived from type locality and host.

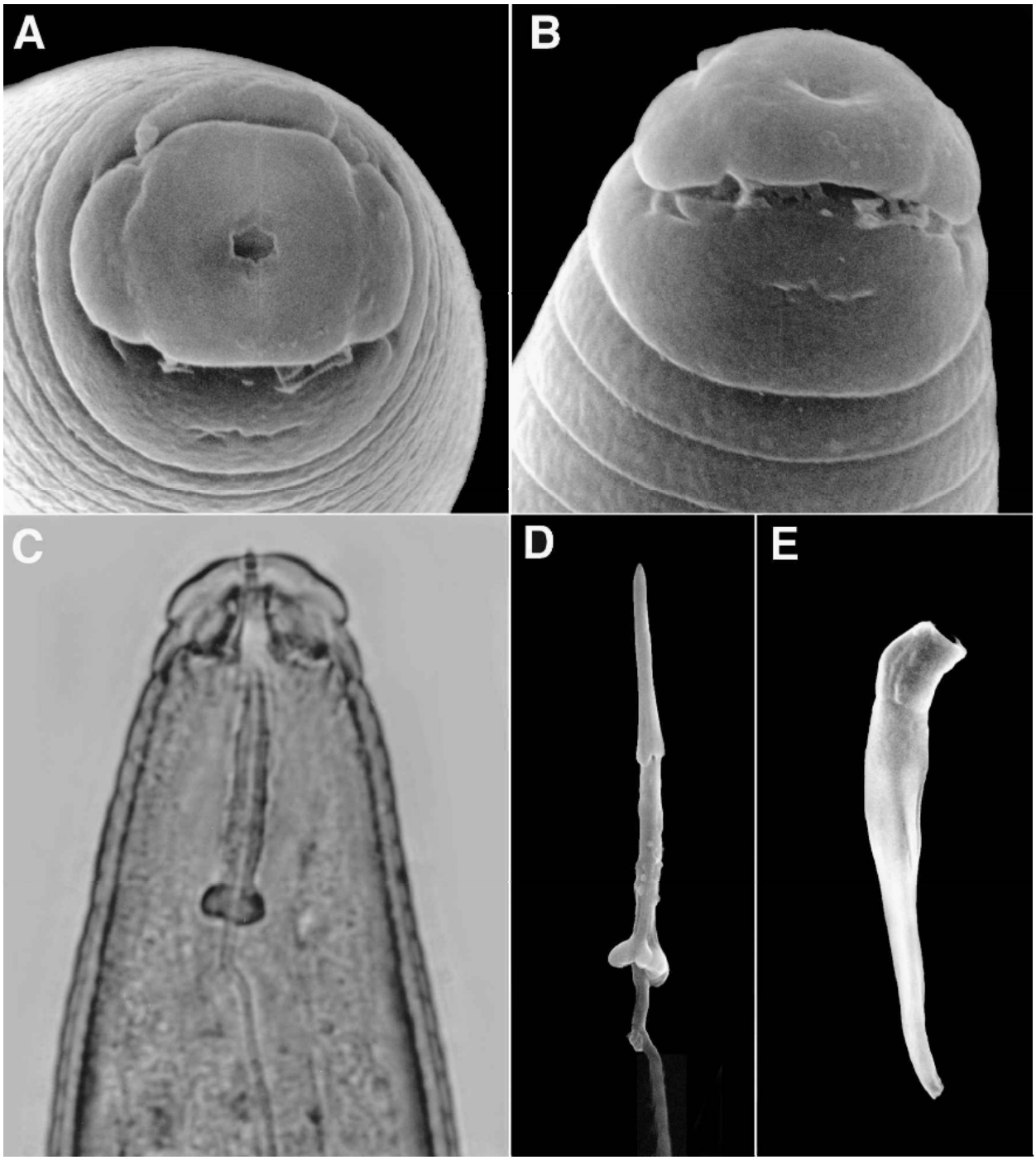

Fig. 7. *Males of* Meloidogyne brasilensis *n. sp. Brasilia population. A, B: Scanning electron micrographs (SEM) of anterior end, face and lateral views; C: Light micrograph of anterior end, lateral view; D: SEM of excised stylet; E: SEM of excised spicule.*

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

Diagnosis and relationships

Meloidogyne brasilensis 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 (see Eisenback *et al.*,

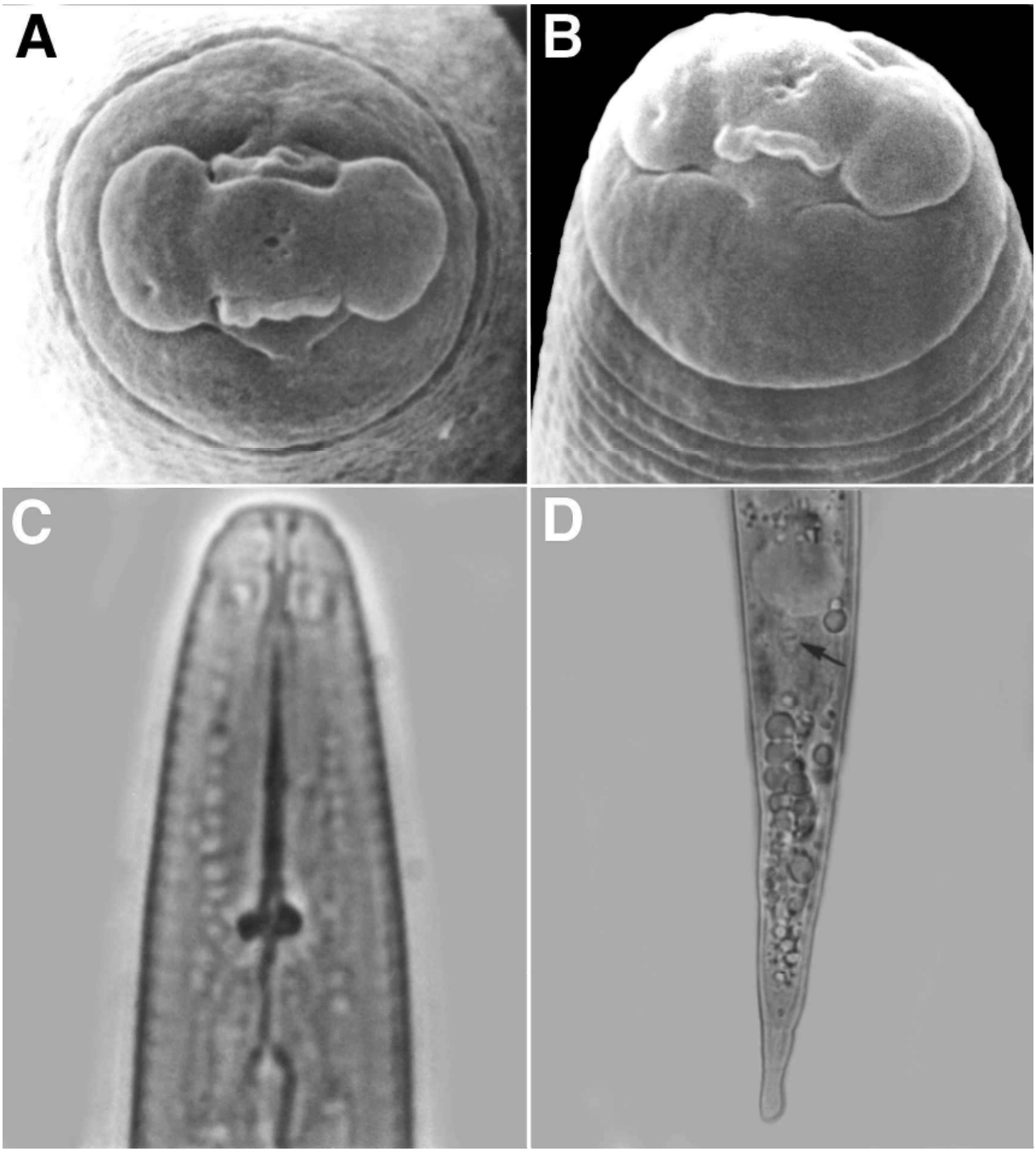

Fig. 8. *Second-stage juveniles (J2) of* Meloidogyne brasilensis *n. sp. Londrina population. A, B: Scanning electron micrographs of head, face and lateral views; C: Light micrograph (LM) of head, lateral view; D: LM of tail, lateral view (arrow indicates rectum).*

1981) by the shape of the perineal pattern, the shape and morphology of head and stylet, and the position of the excretory pore of the female; the shape and morphology of the male head and stylet; and the head morphology of second-stage juveniles.

The external morphology of *M. brasilensis* n. sp. is different from the four most common and from all the other species in the genus. The lateral lips of females are elongated, crescentic to triangular, thus differing from other species. The stylet knobs of female and male of *M. brasilensis* n. sp. are smaller than those observed in the four most common species. Rounded granules of unknown constitution (Figs 1E, F; 2E; 3F) seen between the three rays of the lumen lining of the median bulb

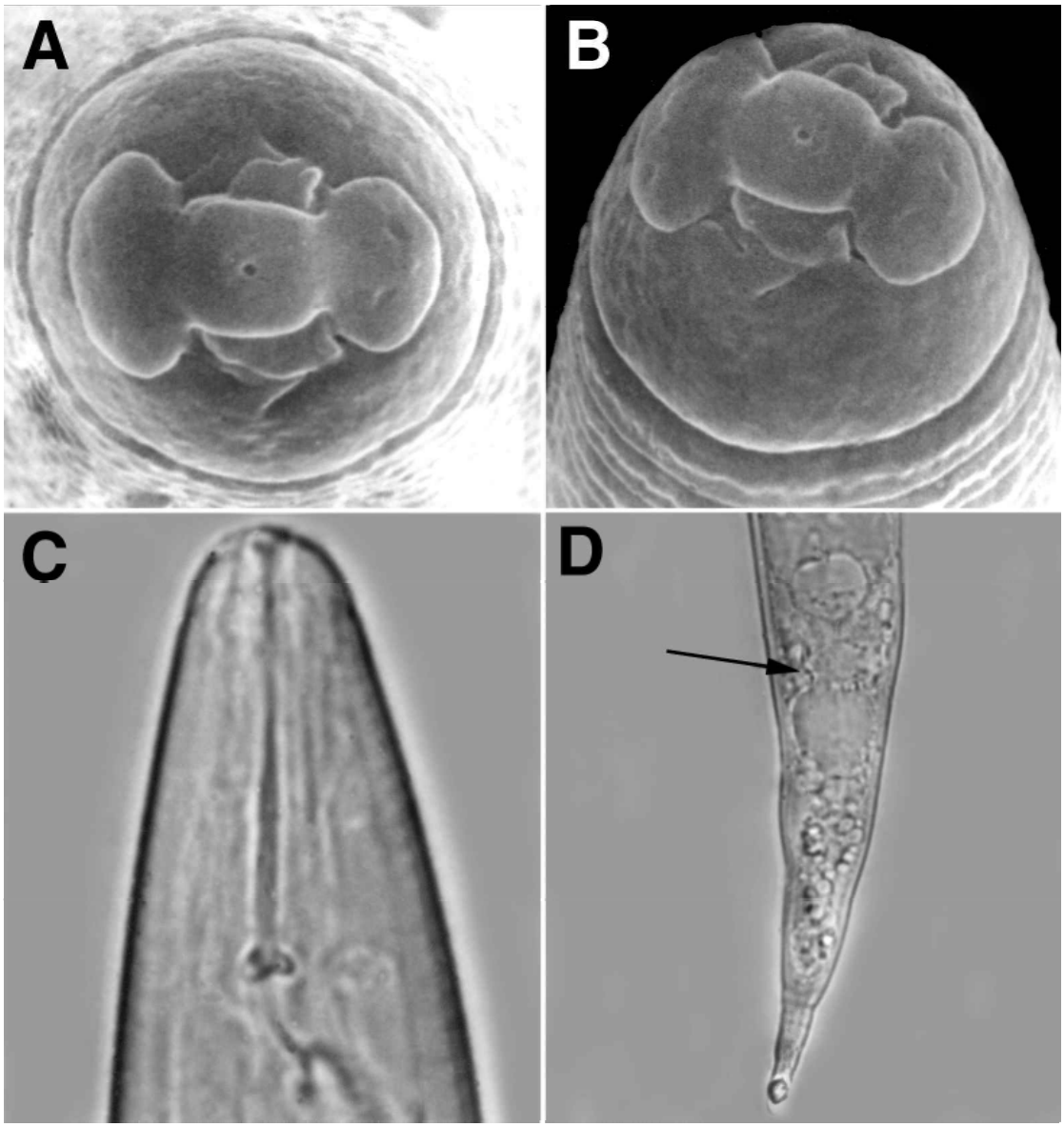

Fig. 9. *Second-stage juveniles (J2) of* Meloidogyne brasilensis *n. sp. Brasilia population. A, B: Scanning electron micrographs of head, face and lateral views; C: Light micrograph (LM) of head, lateral view; D: LM of tail, lateral view (arrow indicates rectum).*

of *M. brasilensis* n. sp. have not been observed in other species of root-knot nematodes. Although the distance from the excretory pore to the anterior end of the female is variable in most root-knot nematode species, the distance in this new species is long (35-76 μm), the pore opening between the stylet base and median bulb.

The labial disc of most males of *Meloidogyne* species is fused with the medial lips (Eisenback & Hirschmann, 1980; Jepson, 1987), but in *M. brasilensis* n. sp. is distinctly elevated and separated by a deep rounded groove. Males of *M. brasilensis* n. sp. do not have annulations on the head region. The head region is very high and distinctly set-off from body. The stylet of *M. brasilensis* n. sp. is delicate with small knobs; small projections occur near the middle of the shaft. These characters have also been observed in *Meloidogyne konaensis* Eisenback, Bernard & Schmidt, 1994, where they are much larger than in *M. brasilensis* n. sp.

In SEM, the medial lips of the second-stage juveniles are small and unequal in size. The tail tip of *M. brasilensis* n. sp. varies from bluntly rounded (Fig. 7D) to sharply pointed (Fig. 8F).

The Londrina and Brasilia populations of *M. brasilensis* n. sp. do not reproduce on pepper, watermelon, peanut,

corn, soybean or cotton and a host range test can easily differentiate this species from the four most common root-knot nematodes. Pepper, watermelon, and cotton are good hosts for *M. incognita*; watermelon is a good host for *M. javanica*, and pepper, watermelon, and peanut are good hosts for *M. arenaria* and *M. hapla*. Likewise, *M. brasilensis* n. sp. reproduces on tomato cv. Rossol which is resistant to *M. incognita*, *M. javanica* and *M. arenaria*.

Remarks

Meloidogyne brasilensis n. sp. Londrina population was found parasitising tomato cv. Rossol in Paraná state, Brazil. Tomato is widely cultivated in Brazil and this variety is resistant to *M. incognita* and *M. javanica*. Unfortunately Rossol tomato is very susceptible to *M. brasilensis* n. sp. The Brasilia population of *M. brasilensis* n. sp. was found in Brasilia, about 1500 km away from the type locality, parasitising pea cv. Mikado. *M. brasilensis* n. sp. may be indigenous in southern Brazil and might have been introduced into Brasilia by nursery transplants of other crops such as potato, sweet potato and yam.

The Brazilian root-knot nematode, *M. brasilensis* n. sp., is the first new species to be described from tomato in Brazil. This new species may be a very serious agriculture problem in Brazil because it parasitises several field crops that are used extensively in crop rotation programmes in Brazil. Another population of *M. brasilensis* n. sp. was found about 1500 km away parasitising pea in Brasilia. Varieties of soybean (cvs Cristalina and Essex) were not infected by the *M. brasilensis* n. sp. population found in Paraná state, but the Brasilia population reproduced slightly, indicating that rotation with soybean may be useful to suppress the Londrina, but not the Brasilia, population. The Brasilia population, based on its ability to reproduce on pea varieties during the spring and winter in the glasshouse, was more sensitive to cold than the Londrina population. In summer tests, both populations reproduced well on pea and bean varieties.

Additional research is necessary to identify resistance in tomato for this new species. Field experiments to demonstrate the role of this nematode as a pathogenic agent alone or in concert with other organisms are lacking. Likewise, field surveys are needed to assess the distribution of *M. brasilensis* n. sp. beyond the present localities and to determine its effects (economic loss) on other vegetable and field crops.

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