Meloidogyne salasi sp. n. (NEMATODA: MELOIDOGYNIDAE), A NEW PARASITE OF RICE (*Oryza sativa* L.) FROM COSTA RICA AND PANAMA[1] /

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Resumen

Se describe e ilustra el nematodo formador de nódulos radicales en arroz, Meloidogyne salasi *sp. n., encontrado en Costa Rica y Panamá. Las hembras se caracterizan por tener el cuello desplazado hacia la porción ventral del cuerpo, una protuberancia posterior y un diseño perineal ovalado, con estrías continuas y lisas. Los machos tienen los campos laterales completamente areolados, con un estilete de 18.2 µm de largo y con los fasmidios localizados posterior a la cloaca. El segundo estadio juvenil tiene una longitud promedio de 464.2 µm, una cola de 67.8 µm de largo y sus campos laterales están areolados.* M. salasi *sp. n. puede ser distinguida de las especies cercanas* M. kralli, M. acronea *y* M. graminis *por las dimensiones del cuerpo y las características del diseño perineal de las hembras, la areolación de los campos laterales en los machos, y por la longitud total y las proporciones a, b y cola/diámetro anal del segundo estadio juvenil.*

Introduction

In 1968 a root-knot nematode causing severe damage on upland rice was found in Volcán de Buenos Aires, Puntarenas, Costa Rica. The parasite was tentatively identified as a new species of *Hypsoperine* (6). Although several aspects of the biology, morphology and the pathogenicity of this nematode on rice were studied, no species description was given. In late 1979 high population densities of an undescribed root-knot nematode were found on rice, cv. C.R.1113, at La Cuesta, Puntarenas, Costa Rica (1). This nematode caused severe damage on rice under greenhouse conditions and was found to be localized on a few farms in the southeastern part of the country (9).

A root-knot nematode with characteristics similar to those described previously was found in 1975 in the province of Coclé, Panamá. Because of the severe damage caused by this nematode, farmers in this area abandoned rice production in favor of grasslands. Again, no description of the species involved was given (12).

An examination of a few perineal patterns from the population studied by Figueroa (6) and some preserved specimens from Panama, provided by Ing. Julio Lara, confirmed that the species involved is the same as the root-knot nematode found on rice at La Cuesta, Costa Rica.

Populations of this root-knot nematode from both Costa Rica and Panama have been studied cytologically (14) and found to reproduce by obligatory mitotic parthenogenesis and have a diploid chromosome number of 36.

This nematode is herein described, illustrated and named *Meloidogyne salasi* sp. n., in honor of Professor Luis Angel Salas Fonseca, the founder of Plant Nematology in Costa Rica.

Materials and methods

A culture of *M. salasi* sp. n. was established from eggs and second stage juveniles obtained from the

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type locality of La Cuesta, Costa Rica. The nematodes were increased and maintained on rice, cv. C R 1113, in a greenhouse. Nematodes from this culture were used for all morphologic and morphometric studies.

Light compound microscope (LM) studies

Galled rice roots were placed in shallow petri dishes containing distilled water and cut open. Eggs from several egg masses were selected at random with a small pipette, placed on a glass slide, ringed with Zut and covered with a coverslip. Other eggs were left overnight in the petri dish and the freshly hatched second stage juveniles were picked with a fine needle, placed in a group of ten in a drop of distilled water on a glass slide, ringed with Zut and covered with a coverslip. Fifteen to 20 minutes later the juveniles were observed and measured using a camera lucida.

Males were dissected from old galls and prepared for study using the same method as described for the juveniles.

Females were prepared by boiling galled roots in lactophenol for two minutes and dissecting them from the cooled roots. The perineal patterns were prepared according to the method described by Franklin (7) and modified by Taylor and Netscher (13). Whole females were mounted on a cavity slide and their lenght (excluding neck) and maximum body width were drawn. The females were removed from the solution and punctured in the middle of the body with a fine needle to release the internal pressure. The head and the neck were excised and mounted in a drop of lactophenol on a glass slide, covered with a coverslip and ringed with Zut. Type specimens of males and females were prepared by fixing in 3% formalin for 48 hours, transferring to lactophenol at 50°C for 24 hours, and mounting in dehydrated glycerin.

All specimens were observed under a LM using Nomarski differential interference contrast optics. Photomicrographs of males, second stage juveniles and the perineal patterns of females were taken with an Olympus OM-2 camera. Drawings of males, females and second stage juveniles were prepared with a camera lucida.

Scanning electron microscopy (SEM) studies

Males, females and second stage juveniles were processed for SEM by a modification of the techniques described by Eisenback and Hirschmann (3, 4) and Eisenback *et al.* (5).

Freshly hatched second stage juveniles were obtained by maintaining eggs in a shallow petri dish with distilled water for 18-24 hours at room temperature. The juveniles were transferred to 0.5 ml of distilled water in a BPI watch glass, chilled at 5°C for one hour and killed by adding three drops of cold (5°C) 4% glutaraldehyde solution buffered with 0.1 M sodium-cacodylate at pH 7.1. More buffered 4% glutaraldehyde was added at 24 hours intervals, three drops at a time, until a final 2% concentration was obtained. Fixation continued for an additional 72 hours at 5°C. The nematodes were washed two times in sodium-cacodylate buffer (pH 7.1), transferred after 24 hours to an small plastic chamber with a fine (15 μm diameter pores) screen on the bottom and kept for 24 hours at 5°C. Postfixation was done with 2% osmium tetroxide, buffered with 0.1 M sodium-cacodylate at pH 7.1, for 18-24 hours at room temperature. This solution was replaced with sodium-cacodylate buffer and kept at 5°C for another 24 hours. Specimens were dehydrated with a graded series of room temperature ethanol changes (5-10-20-35-50-65-80-95-100%), with 24 hours intervals per step. Another screen was placed on top of the chamber and then critical point dried with CO_2 in a Balzer drier. Dried nematodes were propped up against a hair on the surface of a stub covered with double-coated tape, coated with gold for five minutes in a Giko Engineering 1 B-2 model ion coater and viewed with an Hitachi S-450 scanning electron microscope operated at 20 KV of accelerating voltage. Photomicrographs were taken using type-55 Polaroid film.

Males were dissected from galled rice roots and treated as previously described for the second stage juveniles.

Small pieces of galled roots were fixed in a 4% glutaraldehyde solution buffered with 0.1 M sodium-cacodylate at pH 7.1. After 6-7 days whole females were dissected from the roots, washed with sodium-cacodylate buffer, post-fixed with 2% osmium tetroxide, rinsed in sodium-cacodylate buffer, dehydrated, critical point dried, mounted and photographed as previously described for the juveniles.

In describing the external morphology of the males, females and second stage juveniles, the terminology proposed by Eisenback and Hirschmann (3, 4) and Eisenback *et al.* (5) has been followed.

Species description

Meloidogyne salasi sp. n.

Females: Measurements of 50 females in lactophenol are presented in Table 1.

Table 1. Morphometrics of 50 females and 50 eggs of *Meloidogyne salasi* sp. n.

Character	Mean	Range	Standard error of the mean	Standard deviation	CV(%)
Female linear measurements (μm)					
Body length	486.3	372.0-625.0	8.92	63.10	12.9
Maximum body width	338.1	209.0-425.0	6.61	46.76	13.8
Neck length	135.1	86.0-203.0	3.25	22.99	17.0
Neck width at middle of metacorpus	63.3	43.7- 99.9	1.33	9.41	14.8
Middle of metacorpus to head end	78.2	60.9- 99.9	1.43	10.16	12.9
Metacorpus width	35.7	29.0- 41.8	0.39	2.79	7.8
Metacorpus length	35.6	30.0- 43.4	0.44	3.12	8.7
Metacorpus valve width	10.6	9.0- 13.7	0.13	0.94	8.9
Metacorpus valve length	13.7	11.5- 15.6	0.13	0.97	7.0
Stylet	10.0	8.1- 12.5	0.11	0.84	8.4
Stylet knobs height	2.1	1.5- 3.4	0.05	0.41	19.3
Stylet knobs width	3.4	2.5- 4.5	0.06	0.44	12.9
DEGO	4.9	3.4- 6.8	0.14	1.00	20.3
Excretory pore-head end	32.1	18.7- 62.5	1.45	10.26	31.9
Vulva slit length	21.9	15.9- 26.5	0.34	2.43	11.0
Anus-vulva	16.4	9.0- 24.0	0.41	2.94	17.9
Interphasmidial distance	15.2	10.6- 21.8	0.33	2.35	15.4
Female ratios					
a	1.4	1.0- 2.0	0.02	0.20	14.2
Body length/neck length	3.7	2.1- 5.8	0.12	0.85	23.1
Stylet knobs width/height	1.6	0.8- 2.6	0.05	0.36	22.1
Metacorpus length/width	1.0	0.7- 1.2	0.01	0.09	9.8
Metacorpus valve length/width	1.3	0.9- 1.5	0.01	0.11	8.7
Egg linear measurements (μm)					
Length	94.5	82.8-113.2	0.74	5.20	5.5
Width	41.1	38.2- 44.5	0.22	1.50	3.8
Egg ratios					
Length/width	2.3	1.9- 2.7	0.01	0.13	5.8

Measurements of holotype in glycerin.— Body length (excluding neck): 422 μm; maximum body width: 306 μm; neck length: 133 μm; neck width at middle of metacorpus: 43.8 μm; middle of metacorpus to head end: 71.9 μm; metacorpus width: 30.5 μm; metacorpus length: 33.6 μm; metacorpus valve width: 9.5 μm; metacorpus valve length: 12.2 μm; stylet: 10.9 μm; stylet knobs height: 2.1 μm; stylet knobs width 3.2 μm; DEGO: 4.5 μm; ratio a: 1.37; body length/neck length: 3.17; stylet knobs width/height: 1.52; metacorpus length/width: 1.10; metacorpus valve length/width: 1.28. Female as in general description. Perineal region not visible.

Description (Figures 1, 2, 3): Body pearly white, with body length (excluding neck(/maximum body width (ratio a) with an average value of 1.4 and a range of 1 to 2. Distinct posterior protuberance present (Figure 1E). Neck inserts on the ventral side of body. Its position varying from approximately even with the anterior end of body to about one third of body length ventrad to this point. Center line of neck and axis of body (straight line from middle of perineal area to the anterior most part of body) making and angle that varies between 21°C and 130°C. Cuticle distinctly annulated, often with incomplete annulations in the head and neck regions. Head region offset from body. In SEM (Figure 3A-D) the labial disc appears slightly elevated, with the rounded and relatively large prestoma located in the middle. The labial disc and the medial lips form an anchor-shaped structure, the ventral lip (determined from the position of the excretory pore) being the pointed end of it. In a few cases the ventral lip is not very pointed,

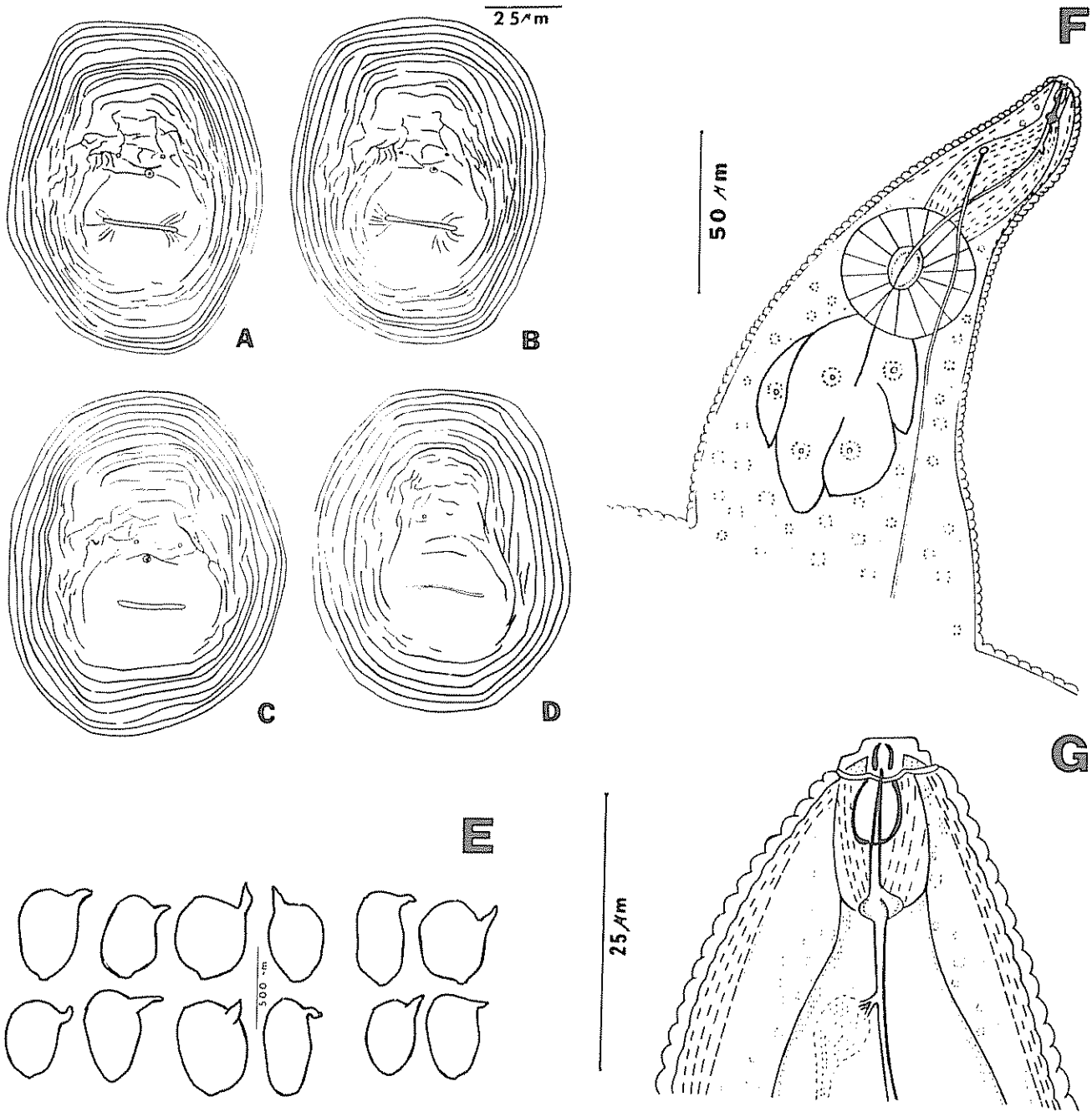

Fig 1 Females of *Meloidogyne salasi* sp n A D) Perineal patterns (A C from Costa Rica, D from Panamá). E) Outlines of body shapes F) Esophageal region (ventral). G) Cephalic region (lateral)

but the anchor-shaped structure is still recognizable (Figure 3D). Inner labial sensillae difficult to see. Head region appears as a single annule, often marked by longitudinal lines. Amphid openings clearly distinct, rectangular. Lateral lips arched, slightly larger than the ventral or dorsal sectors. The vestibule and vestibule extension are clearly distinct when observed with the LM (Figure 1G). Stylet delicate, cone usually straight, with triangular base about 1/4 of its length, tapering to a fine, pointed tip. Opening of stylet near the tip, in the anterior 1/4 of the cone Shaft with approximately the same diameter throughout and shorter than the cone Stylet knobs offset from the shaft, ovoid to almost triangular in

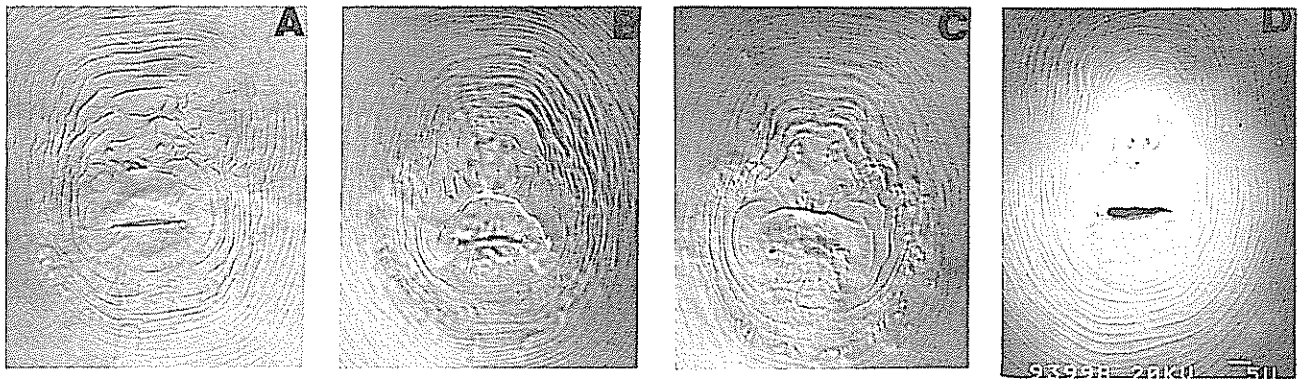

Fig. 2 Perineal patterns of females of *Meloidogyne salasi* sp. n. A–C) Light microscope photomicrographs. D) Scanning electron microscope photomicrograph.

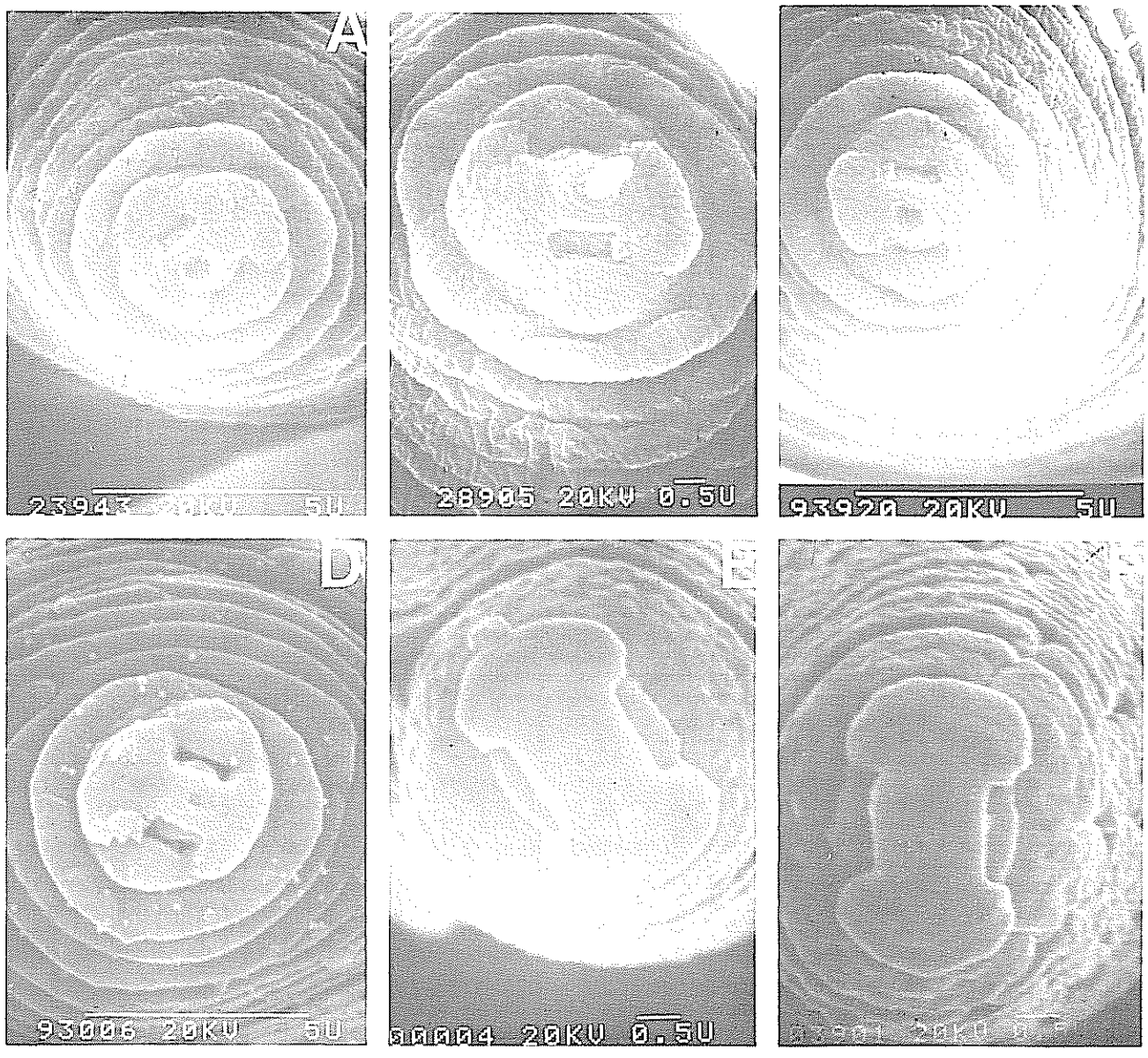

Fig. 3. Scanning electron microscope photomicrographs of face views of *Meloidogyne salasi* sp. n. A–D) Female. E–F) Second stage juvenile.

shape. Lumen of stylet in the knobs is about the same as in the procorpus, but narrows sharply in the cone. Outlet of the dorsal esophageal gland branched, with dorsal ampulla relatively large. Excretory pore position variable, about 1-1 1/2 times the stylet length behind the stylet knobs in 66% of the specimens observed. In a few females (4%) the excretory pore was about 1/2 stylet length behind the stylet knobs, while in others (6%) it was about 3 times the stylet length behind the stylet knobs. Lumen of esophagus strongly sclerotized in the procorpus and metacorpus, but very difficult to see beyond the latter. Metacorpus relatively large and rounded (Figure 1F), with a strong, oval central valve. Esophageal glands appear as a massive, globose structure with five nucleated lobes, often difficult to observe with bright field illumination but distinct with Nomarski differential interference contrast optics. Perineal pattern (Figures 1A-D, 2) oval-shaped, with fine outher striae and somewhat coarse striae in the inner portion. The striae are mostly unbroken, smooth, relatively few in numbers and far apart. Perineum with no or only one striae, and only a few in the roughly circular central area of the pattern. Vulva a transverse, smooth slit, with no or few striae coming out of its sides. Plasmids small, closely spaced. Dorsal arch high and wide, usually rectangular in shape, but somewhat square in some specimens. No evidence of lateral lines or interrupted striae where these usually are. Tail tip prominent in freshly mounted perineals.

Males: Measurements of 50 males in distilled water are presented in Table 2.

Measurements of allotype in glycerin.– Body length: 1 711 μm; maximum body width: 35 3 μm; body width at base of knobs: 16.6 μm; body width at excretory pore: 26 6 μm; body width at middle of metacorpus: 22.2 μm; excretory pore to head end: 136.7 μm; middle of metacorpus to head end: 94 5 μm; head height: 5.3 μm; head width: 10 9 μm; excretory pore to middle of metacorpus: 48.4 μm; esophageal lobe to head end: 243.2 μm; stylet: 19 μm; stylet base to head end: 21 8 μm; stylet shaft + knobs: 9 μm; stylet cone: 10 μm; stylet knobs height; 2.3 μm; stylet knobs width: 3 3 μm; DEGO: 4 4 μm; metacorpus width: 11 9 μm; metacorpus valve width: 3 4 μm; metacorpus valve length: 8.4 μm; testis: 1 034 μm; testis %: 60 4; spicules: 28 8 μm; gubernaculum: 9 μm; tail length: 13 8 μm; cloaca-phasmids: 8 4 μm; ratio a: 48.4; ratio b: 7 9; ratio c: 123 9.

Description (Figures 4, 5): Vermiform, body length variable, tapering at the anterior end (Figure 4A-B) and relatively rounded at the posterior end (Figure 4C-D). Head region slightly offset from body, bearing a variable number of incomplete annulations, with distinct head cap (Figure 5A-D). In SEM the large, rounded labial disc is slightly elevated above the medial lips, with lateral edges slightly arcuate (Figure 5C-D). Oval prestoma in the center of the labial disc, encircled by six inner labial sensillae with pitlike openings. Stoma with a slitlike opening. Medial lips wider than the labial disc, forming a continuous head cap with it, with no discernible indentations at the lateral junctions. Four cephalic sensillae appearing as slight, small cuticular depressions on the medial lips, two on each. Amphidial openings are relatively long slits below the lateral edges of the labial disc. Lateral lips almost inconspicuous, marked by short grooves that start near the lateral junction of the medial lips and the labial disc, and extend into the head region. One to three rows of short, incomplete annulations at different levels of the head region (Figure 5A-B). Frecuently the specimens have one row on one side and two or three on the opposite side. Cuticle with distinct annules, about 1.9 μm wide near the head region, 2 μm wide around the middle of the body and 1.6 μm wide near the tail. Lateral field about 6, 7.5 and 5 μm wide near the anterior, middle portion and tail areas of the body, respectively. There are basically four lateral lines in the lateral field, one at each edge on the ridge and two in the inner portion, but in some specimens five or up to six lines are visible for some distance in the middle of the body; the additional lines are fainter. Lateral fields start as two lines with crenated edges near the base of the stylet, some four to 10 body annules behind the head region, where the inner two lines appear, and continue to the posterior end, where they twist around 90°. The lateral fields are areolated in their entirety, usually corresponding with the body annulations, but in some areas, especially the middle portion, there is no correspondence (Figure 5F). Cephalic framework sclerotized, with lateral sectors slightly larger than the head cap (Figure 4A-B, 5E-F). Stylet robust, with a pointed cone, slightly longer than the shaft, with the opening near the tip and a triangular base in the basal 1/4 of its length. Stylet shaft of same diameter throughout, with ring-like structure near its base (Figure 4A-B). Stylet knobs rounded, offset from the shaft, with an ascending slope toward its base (Figures 4A-B, 5E-F). Lumen of stylet almost as wide as that of the procorpus, but narrowing at the cone. Outlet of the dorsal esophageal gland branched, with a relatively small dorsal ampulla. Procorpus two to three times as long as the muscular, elongated, oval metacorpus (Figure 4A); this with a strongly sclerotized central valve. Nerve ring encircling the short isthmus. Distinct excretory pore, with long, curved excretory duct that dissapears in the intestine. Basal lobe of esophagus overlapping ventrally the intestine, with three nuclei; anterior nucleus near beginning of lobe; posterior nucleus near the end of the lobe. Hemizonid 1-2 annules anterior to excretory pore, 1-2 annules long. Intestinal caecum extends on the

Table 2. Morphometrics of 50 males of *Meloidogyne salasi* sp. n.

Character	Mean	Range	Standard error of the mean	Standard deviation	CV(%)
Linear measurements (μm)					
Total length	1 619.0	992.0-2 093 0	40.87	289.04	17 8
Maximum body width	33.9	25 4- 41 8	0 49	3 53	10 3
Body width at base of knobs	16 8	11 8- 20.7	0 19	1 40	8 3
Body width at exc pore	26.8	23 1- 34 4	0.33	2 40	8 9
Body width at middle of metacorpus	23 6	20.1- 27 0	0 22	1.58	6 6
Exc pore to head end	156.9	88 0- 227 0	4 56	32 31	20 5
Middle of metacorpus to head end	101 7	64 0- 134 0	2 61	18 51	18 2
Head height	4.5	2.5- 5 6	0.09	0 69	15.3
Head width	10 4	7 5- 13 1	0 15	1 12	10 7
Exc pore to middle of metacorpus	55 8	18.7- 99 9	2 89	20 44	36 6
Stylet	18.2	12 1- 21 8	0 31	2 19	12 0
Stylet base to head end	20.6	15 9- 23.1	0.27	1.96	9 5
Stylet shaft + knobs	10 4	6 8- 12 5	0 20	1 43	13 7
Stylet cone	7 7	4 3- 10 3	0 17	1 22	15 7
Stylet knobs height	3 1	2 1- 4.2	0 07	0.54	17 6
Stylet knobs width	4.6	3.5- 7 5	0.09	0 66	14 1
DEGO	4 1	2 8- 5.9	0.10	0 72	17.4
Metacorpus width	12.6	8 4- 16.2	0.24	1 73	13.7
Metacorpus valve width	5.1	3.1- 7.1	0 12	0 88	17 2
Metacorpus valve length	6.8	4 8- 8.7	0.14	0 99	14 4
Testis	887.1	353 0-1 250.0	25 24	178.51	20 1
Spicules	25 8	17 5- 34.5	0 63	4 52	17 4
Gubernaculum	7 8	5.6- 11 8	0 19	1 34	17 0
Tail length	13 0	6 5- 39.0	0 65	4 66	35.7
Cloaca-phasmids	4 1	0 3- 9.9	0.35	2 50	59 6
Phasmids-tail end	8 8	4.0- 17 5	0 36	2.58	29 1
Ratios					
a	47 5	31 8- 58.1	0 92	6.51	13 6
c	132 8	46 6- 254 7	5.46	38 62	29 0
Body lenght/middle of metacorpus to head end	16 0	11 7- 21.6	0 32	2 33	14 4
Head region width/height	2 3	1 8- 3 0	0 03	0 26	11 4
Stylet knobs width/height	1 5	1.0- 3 0	0 04	0 32	21 2
Metacorpus valve lenght/width	1 3	0 7- 2.3	0 04	0 30	21 7
Percentages					
Excretory pore	9 7	6 5- 12 7	0.20	1.43	14 7
Testis	55 0	32 0- 71 6	1 11	7.90	14 3

dorsal side of the body to about the same level or below the nerve ring. Most specimens posses one outstretched testis, but a few have two testes, or the testis may be reflexed for a short distance. If two testes are present, one may be outstretched and the other reflexed, but about the same length. Sperm globular, granular. Spicules long, arcuate, typical of the genus (Figure 4C-D). In SEM each spicular tip shows one transverse opening (Figure 5H). Gubernaculum simple. Phasmids typically below the cloacal opening, with a pore-like opening. Body twists about 90° near the cloacal region

Second stage juveniles: Measurements of 50 juveniles in distilled water are presented in Table 3

Description (Figures, 3E-F, 6, 7): Body vermiform, tapering at both ends but much more so posteriorly

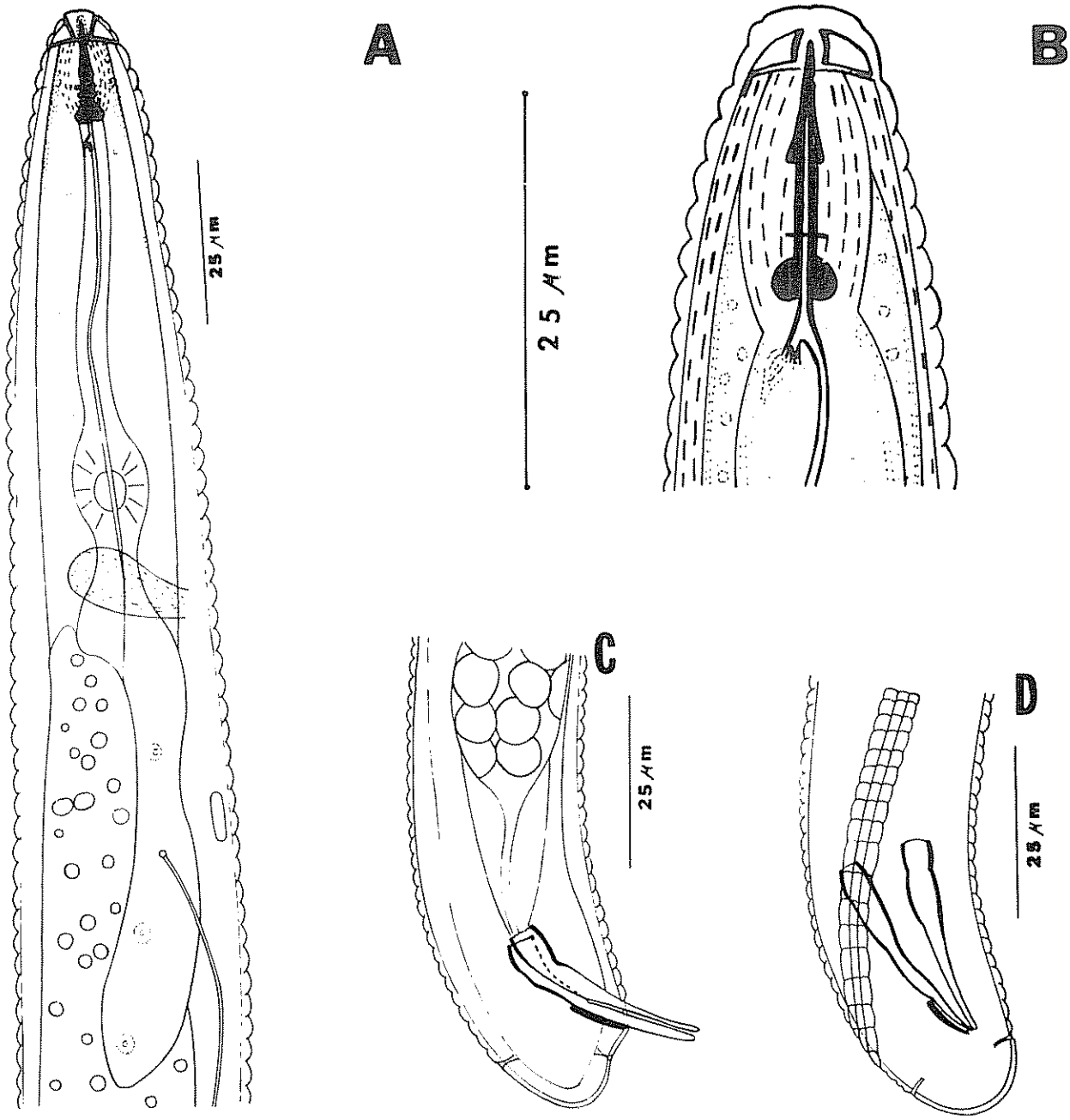

Fig. 4 Drawings of males of *Meloidogyne salasi* sp. n. A) Esophageal region (ventral) B) Cephalic region (lateral). C) Tail (lateral). D) Tail (latero-ventral).

(Figures 6, 7A, C-D). Head region slightly offset from body, with lateral sectors slightly narrower than the body, and elevated head cap. In SEM the elongated labial disc is slightly elevated above the medial lips, with lateral edges straight or almost so (Figure 3E) Oval prestoma in the center of the labial disc, encircled by six inner labial sensillae with pit-like openings. Stoma with a small slit-like opening. Medial lips crescentic in most specimens, wider than the labial disc, with no discernible indentations at the lateral junctions with it, forming a dumbell-shaped cap. In a few specimens one of the medial lips can be pointed (Figure 3F). Amphidial opening slit-like, located below the lateral edges of the labial disc. Lateral lips narrow, with straight or slightly arcuate edges, almost parallel to the lateral edges of the labial disc. Head region is smooth, without annulations. Cephalic framework weakly developed. Body dis-

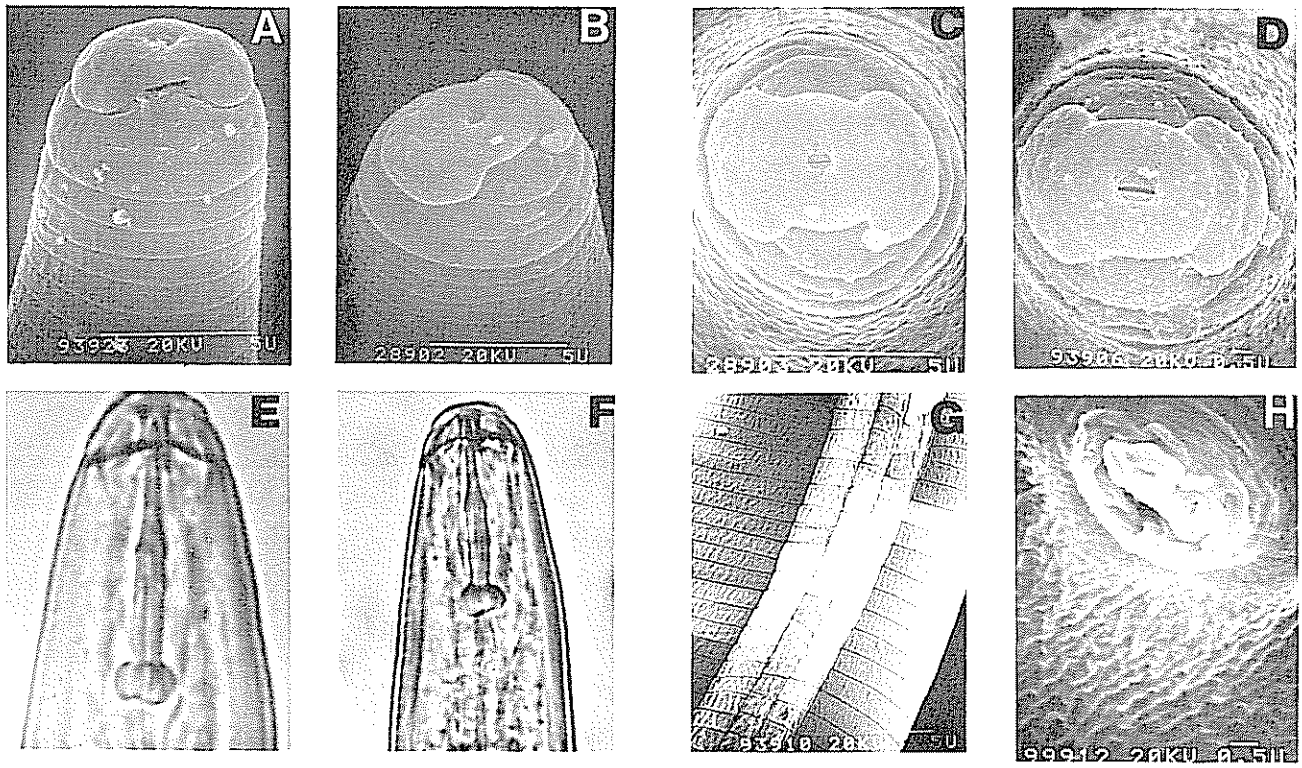

Fig. 5. Males of *Meloidogyne salasi* sp. n. A–D, G, H) Scanning electron microscope photomicrographs. E–F) Light microscope photomicrographs. A–B) Cephalic region. C–D) Face views. E–F) Cephalic region. G) Lateral field. H) Spicules showing pores on the tips.

tinctly annulated, the annulations being discernible with the LM up to the beginning of the tail terminus. Lateral fields areolated, with four lines, the two external slightly crenated (Figure 7B). They begin as two lines at about the middle of the procorpus, then three and finally four lines that continue as far as past the anus where the two central lines dissapear and the two lateral ones continue for a short distance, up to the beginning of the tail terminus. Stylet weakly developed, with small rounded knobs, one slightly larger and in a lower position than the other two (Figures 6A-B, 7A). The knobs have an ascending slope toward the shaft. A ring-like structure encircles the shaft near its base (Figure 6B). Dorsal ampulla weakly developed. Procorpus about 2 to 2 1/2 times as long as the muscular, oval metacorpus; this with a sclerotized central valve. Nerve ring encircles the narrow isthmus. Hemizonid 1-2 annules anterior to the excretory pore, about 1 annule long. Excretory pore located at about the same level or below the nerve ring, with curved excretory duct that dissapears inside the intestine. Basal lobe of esophagus rather short, with three nuclei, the anterior one located near its beginning and the posterior one near its end. The basal esophageal lobe overlapps the intestine ventrally (Figure 6A). Anal opening a small pore on the cuticle. Rectum weakly dilated. Tail relatively long, tapering to a fine, rounded, slightly clavated terminus (Figure 7C-D).

Eggs: Measurements of 50 eggs in distilled water are presented in Table 1.

Description: Eggs similar to those of other species of the genus, enclosed in a soft, highly water-soluble gelatinous matrix. Up to 2 000 eggs/egg mass have been counted on galled rice roots collected from the type locality (L. A. Salazar, unpublished data).

Diagnosis: *M. salasi* sp. n. is closely related to the recently described *M. kralli* (8), and is also related to *M. acronea* (2) and to *M. graminis* (11).

M. salasi sp. n. can be distinguished from *M. kralli* by the dimensions of the female (body length of 486 μm vs 463 μm, maximum body width of 338 μm vs 306 μm), the straight shorter stylet (10 μm vs 13.1 μm), longer excretory pore of the female (32 μm vs 15.8 μm), by the higher dorsal arch of the perineal pattern, and absence of a postero-laterally directed irregular double incisure on either side of the tail region of the perineal, longer males (1 619 μm vs 1 076 μm),

Table 3. Morphometrics of 50 second stage juveniles of *Meloidogyne salasi* sp. n.

Character	Mean	Range	Standard error of the mean	Standard deviation	CV(%)
Linear measurements (μm)					
Total length	464.4	422.0-503.0	2.59	18.35	3.9
Esophageal lobe base to head end	121.8	103.0-153.0	1.31	9.26	7.6
Maximum body width	16.2	15.3- 19.3	0.11	0.83	5.1
Body width at excretory pore	15.0	13.1- 15.9	0.09	0.64	4.2
Middle of metacorpus to excretory pore	23.6	16.8- 31.5	0.46	3.27	13.8
Middle of metacorpus to head end	56.7	50.6- 62.1	0.36	2.58	4.5
Head region width	6.2	5.0- 7.8	0.08	0.58	9.2
Head region height	3.3	1.8- 5.6	0.12	0.87	25.8
Stylet	11.4	9.2- 13.3	0.15	1.07	9.3
Stylet cone	5.2	3.7- 6.8	0.11	0.83	15.8
Stylet knobs width	2.3	1.5- 2.8	0.04	0.30	13.2
Stylet knobs height	1.5	1.0- 2.1	0.03	0.22	14.8
Stylet base to head end	14.7	12.1- 16.2	0.09	0.69	4.6
Stylet shaft	4.7	2.8- 6.2	0.09	0.70	14.8
DEGO	3.7	2.1- 5.3	0.08	0.58	15.5
Metacorpus valve length	3.9	2.8- 5.3	0.06	0.45	11.4
Metacorpus valve width	3.4	2.5- 4.3	0.04	0.33	9.7
Excretory pore to head end	80.3	71.5- 89.6	0.57	4.03	5.0
Tail length	67.8	56.5- 80.2	0.73	5.16	7.6
Tail terminus length	19.7	11.8- 26.2	0.47	3.33	16.8
Tail terminus width at beginning	5.1	3.7- 6.2	0.09	0.64	12.3
Anal width	11.8	10.7- 15.0	0.10	0.71	6.0
Anus-beginning of terminus	47.9	38.1- 58.7	0.67	4.77	9.9
Ratios					
a	28.6	23.9- 32.2	0.24	1.73	6.0
b	3.8	3.0- 4.4	0.04	0.30	7.9
c	6.8	5.9- 7.7	0.05	0.42	6.1
Tail length/anal width	5.7	4.2- 6.8	0.07	0.54	9.4
Tail length/tail terminus length	3.5	2.4- 5.7	0.08	0.58	16.7
Head region width/height	1.9	1.2- 2.8	0.05	0.37	19.3
Stylet knobs width/height	1.5	0.7- 2.1	0.03	0.25	16.7
Metacorpus valve length/width	1.1	0.6- 1.5	0.02	0.16	13.9
Percentages					
Excretory pore	17.2	16.0- 18.7	0.08	0.61	3.5

greater a and c ratios in the males (47.5 and 132.8 vs 31.7 and 117, respectively), shorter stylet cone in the male (7.7 μm vs 9.5 μm), longer excretory pore in the fields, annulations in the head region of the male (up to 4 vs 1), position of the phasmids on the male (below the cloaca vs at the level of the cloaca). Additional differentiating characters in the second stage juveniles include the body length (464 μm vs 439 μm), the smaller a and b ratios (28.6 and 3.8 vs 31 and 6.5, respectively) and the smaller tail/anal width ratio (5.7 vs 7).

M. salasi sp. n. can be distinguished from *M. acronea* by the female body length (486 μm vs 980-1 040 μm), maximum body width (338 μm 530-750 μm), shorter stylet in the female (10 μm vc 12 μm), shorter spicules of the male (25.8 μm vs 33-35 μm), longer phasmids-tail end distance (8.8 μm vs 4 μm), areolation of the lateral fields in the male, shorter second stage juveniles (464 μm vs 490 μm), smaller a, b and c ratios in the juveniles (28.6, 3.8 and 6.9 vs 32, 5.4 and 9.4, respectively), longer tail of juveniles (67.8 μm vs 49 μm) and longer tail terminus (19.7

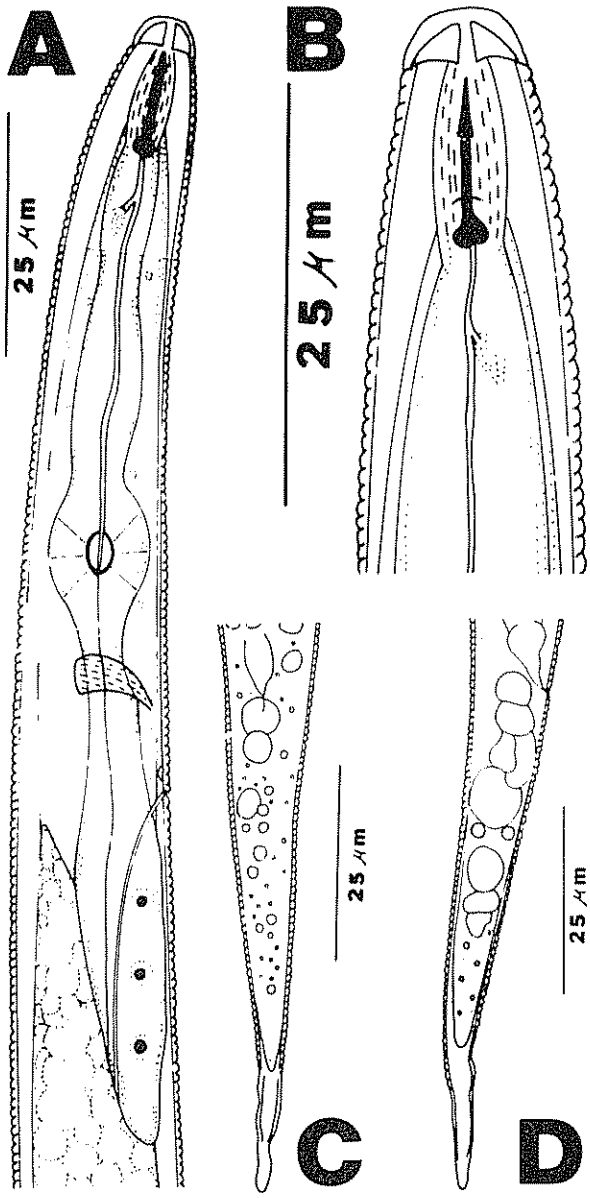

Fig. 6 Drawings of second stage juvenils of *Meloidogyne salasi* sp. n. A) Esophageal region (lateral). B) Cephalic region (lateral). C) Tail (dorsal). D) Tail (lateral).

µm vs 3.5 µm). Finally, *M. salasi* sp. n. can be differentiated from *M. graminis* by the body length of the female, maximum body width and DEGO (486, 338 and 4.9 µm vs 726, 472 and 3.7 µm, respectively), the absence of lateral lines in the perineal pattern, the fine striae in the perineal pattern. In the males by the greater a ratio (47.5 vs 43.5), the smaller c ratio (132 vs 187), the longer DEGO (4.1 µm vs 2.4 µm), the longer tail (13 µm vs 8.4 µm) and the areolation of the lateral fields. In the second stage juveniles by the length (464 µm vs 475 µm), the smaller a ratio (28.6 vs 31.7), the greater b ratio (3.8 vs. 2.3), the longer DEGO (3.7 µm vs 2.4 µm), the shorter esophageal lobe to head end distance (121.8 µm vs 200 µm), the shorter tail (67.8 µm) and the greater tail/anal width ratio (5.7 vs 4.3).

Host range: *M. salasi* sp. n. did not infect any of the plant species used in the North Carolina Differential Host test (10). Greenhouse studies conducted in Panama (12) showed that *Cynodon plectostachyus, C. dactylon, Ischaemum ciliare, Digitaria decumbens, Tripsacum laxum, Echinocloa polystachya, Leucaena leucocephala, Kazungula* sp., *Brachiaria ruziziensis, B. zuazilandensis, B. rugulosa, Panicum maximum* and *Saccharum sinensis* are poor hosts of this nematode. Field observations made by Figueroa (6) indicated that *Homolepis aturensis* is also a host for *M. salasi* sp. n. The grass *Echinocloa colonum* was found to be a host under field conditions at the type locality, in addition to rice, the type host.

Holotype (female): Isolated from greenhouse culture derived from original population obtained at La Cuesta, Costa Rica. Slide M-39, Nematode collection, Laboratorio de Nematología, Facultad de Agronomía, Universidad de Costa Rica, San José, Costa Rica.

Allotype (male): Same data as holotype. Slide M-13, Laboratorio de Nematología, Facultad de Agronomía, Universidad de Costa Rica, San José, Costa Rica.

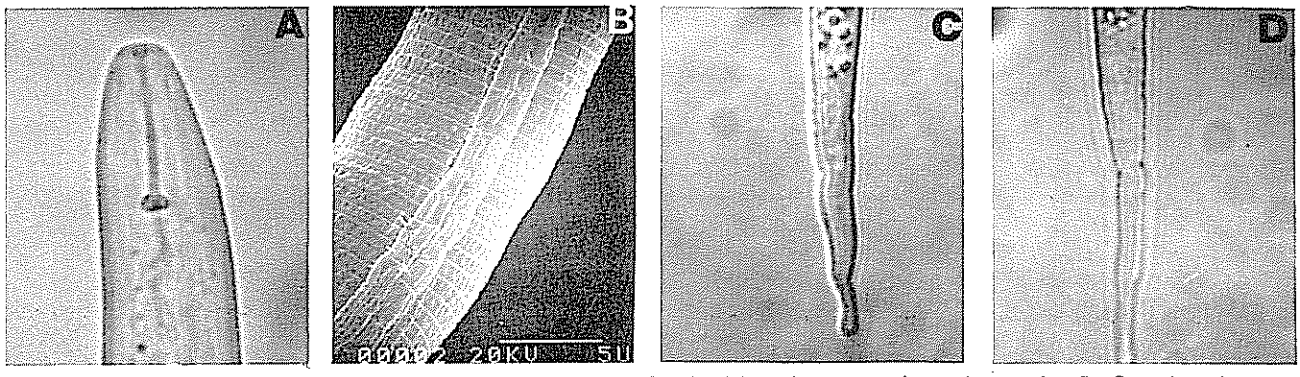

Fig. 7. Second stage juveniles of *Meloidogyne salasi* sp. n. A, C–D) Light microscope photomicrographs. B) Scanning electron microscope photomicrograph. A) Cephalic region. B) Lateral field. C–D) Tail termini.

Paratypes (males, females and second stage juveniles): Same data as holotype. USDANC, Beltsville, Maryland.

Type host and locality: Rice (*Oryza sativa* L.), cv. C.R.1113, from La Cuesta, province of Puntarenas, Costa Rica.

Summary

Meloidogyne salasi sp. n., a root-knot nematode parasite of rice (*Oryza sativa* L.) in Costa Rica and Panama, is described and illustrated. Females have the neck and head regions on the ventral side of the body, a posterior protuberance and an oval perineal pattern, with unbroken, smooth striae. Males have areolated lateral fields, the stylet is 18.2 μm long and the phasmids are located posterior to the cloaca. Second stage juveniles have an average length of 464.2 μm, a tail 67.8 μm long and areolated lateral fields. *M. salasi* sp. n. can be distinguish from the related *M. kralli, M. acronea* and *M. graminis* by the dimensions of the body and the characteristics of the perineal pattern of the females, the areolation of the lateral fields in the males, and the total length and the a, b and tail length/anal width ratios in the second stage juveniles.

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