Four new species of *Gracilacus* Raski, 1962 (Criconematoidea : Nemata)

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SUMMARY

Four new species of *Gracilacus* Raski, 1962 are described from Brazil. *Gracilacus longilabiata* sp. n. is distinguished by highly offset and expanded lips in both sexes, four lines in lateral field, small size of female (216-315 μ m) and by male without stylet. *Gracilacus brasiliensis* sp.n. is distinguished by four lines in lateral field, small size (250 μ m), short stylet (62 μ m) and vulva 70 %. *Gracilacus colina* sp.n. is distinguished by its dotted, cuticular ornamentations only in cesophageal region, three lines in lateral field and long stylet (72 μ m). *Gracilacus punctata* sp.n. is distinguished by four lines in lateral field, short stylet (48 μ m), dotted cuticular ornamentations over entire body.

Resumé

Quatre nouvelles espèces de Gracilacus Raski, 1962 (Criconematoidea : Nemata)

Quatre nouvelles espèces de *Gracilacus* Raski, 1962, provenant du Brésil, sont décrites. *Gracilacus longilabiata* sp.n. se distingue par une région labiale expansée et très séparée chez les deux sexes, un champ latéral à quatre lignes, la faible taille des femelles (216-315 μ m) et l'absence de stylet chez le mâle. *Gracilacus brasiliensis* sp.n. se distingue par un champ latéral à quatre lignes, une faible taille (250 μ m), un stylet court (62 μ m) et une vulve à 70 %. *Gracilacus colina* sp.n. se distingue par des ornementations cuticulaires punctiformes limitées à la région œsophagienne, un champ latéral à trois lignes et un long stylet (72 μ m). *Gracilacus punctata* sp.n. se distingue par un champ latéral à quatre lignes, un stylet court (48 μ m) et des ornementations cuticulaires punctiformes sur tout le corps.

Out of the more than 3 000 samples collected in Brazil over the last ten years, mostly from the Amazonian basin in the north and the "cerrados" (closed savannas) in the Centro-west and Northeastern regions of that country, many were found to contain members of Paratylenchidae. Four species of *Gracilacus* Raski, 1962 were identified as new to science, descriptions of which follow.

Procedures similar to those described by Huang and Raski (1986) were followed to prepare the specimens for light and scanning electron microscopical (SEM) studies. Measurements were made on specimens in glycerin impregnated permanent mounts.

Gracilacus longilabiata sp.n.

(Figs 1 & 2)

MEASUREMENTS

Females (paratypes; n = 12) : L = 0.28 ± 0.03 (0.22-0.32) mm; $a = 22.4 \pm 4.5$ (15.1-28.6); $b = 3.0 \pm 0.4$ (2.6-3.9); $c = 12.6 \pm 2.7$ (10.2-16.4); c = 12.6

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 \pm 2.7 (10.2-16.4); V = 78.2 \pm 1.0 (76.4-80.0) %; stylet = 64 \pm 3.4 (58-71) µm; cone = 57 \pm 2.4 (54-62) µm; excretory pore = 70 \pm 10 (45-80) µm; œsophagus = 95 \pm 14 (66-108) µm.

Males (paratypes; n = 4) : L = 0.30 \pm 0.03 (0.26-0.33) mm; a = 37.3 \pm 4.2 (32.6-40.9); c = 12.0 \pm 1.9 (10.0-13.9); tail = 25 \pm 2.6 (22-27) µm; excretory pore = 64 \pm 0.5 (60-67) µm; spicules = 16 \pm 0.6 (15-16) µm.

Juveniles 2d or 3d stage (paratypes; n = 6): $L = 0.24 \pm 0.02 (0.22-0.26) \text{ mm}$; $a = 18.7 \pm 1.8 (16.6-18.5)$; $b = 3.0 \pm 0.3 (2.5-3.3)$; c = 16.4 (one only); tail = 14 µm (one only); stylet = 40 $\pm 0.8 (39-41)$ µm; cone = 34 $\pm 1.4 (32-35)$ µm; excretory pore = 62 $\pm 2.8 (59-67)$ µm; cosophagus = 80 $\pm 4.9 (72-87)$ µm.

fuveniles, 4th stage (paratypes; n = 2) : L = 0.24-0.26 mm; a = 17.5-18.2; b = 3.2-3.3; excretory pore = 59-62 µm; œsophagus = 72-87 µm.

Holotype (female) : L = 0.30 mm; a = 25.0; b = 2.9; c = 14.1; c' = 3.0; V = 80.0 %; stylet = 66 μ m; cone = 58 μ m; excretory pore = 79 μ m; esophagus = 104 μ m.

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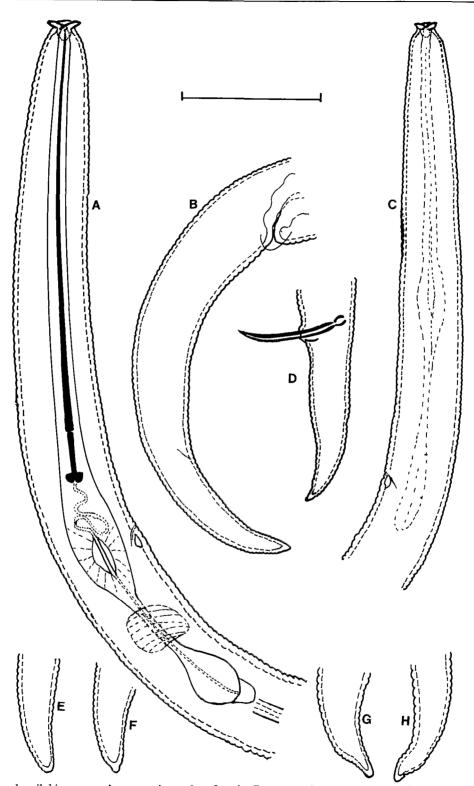


Fig. 1. Gracilacus longilabiata sp. n. A = anterior region, female; B = posterior region, female; C = anterior region, male; D = posterior region, male; E-H = various types of tails, females. Bar represents 20 μ m for A-H.

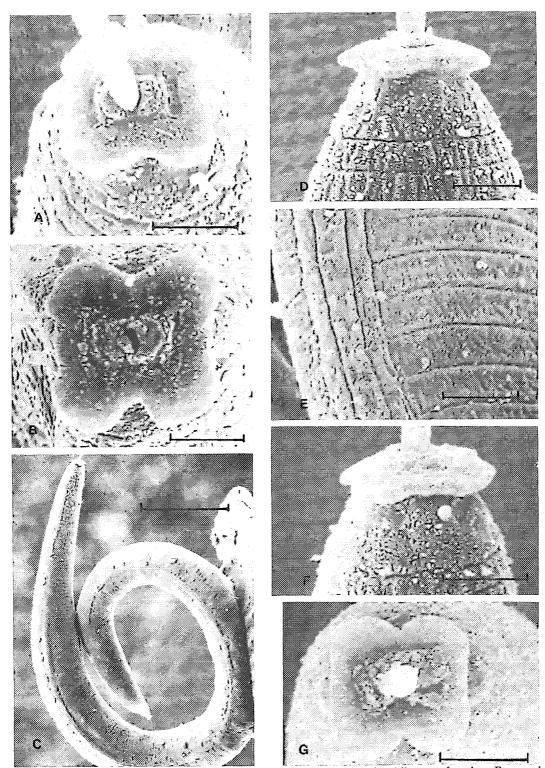


Fig. 2. Gracilacus longilabiata sp. n. SEM photographs A = cephalic region of female, oblique *en face* view; B = cephalic region of a second female, *en face* view; C = lateral view of a juvenile, probably third-stage; D = cephalic region of the female in A, lateral view; E = lateral field, female, near vulva; F = cephalic region of the juvenile in C, lateral view; G = cephalic region of the juvenile in C, *en face* view. Bar represents 2 μ m for A, B, D, E, F, G; 20 μ m for C.

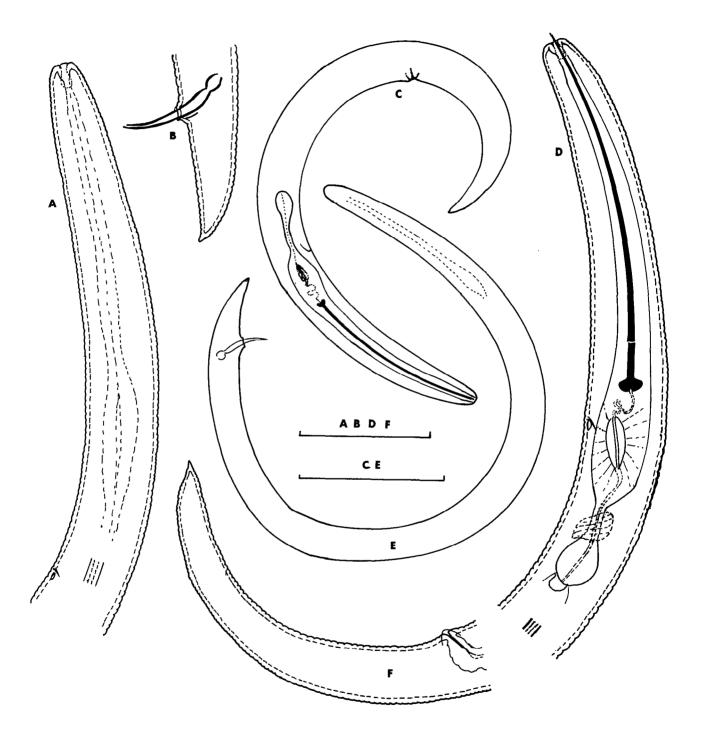


Fig. 3. Gracilacus brasiliensis sp.n. A = anterior end, male; B = tail and spicules, male; C = full length, female; D = anterior end, female; E = full length, male; F = posterior end vulva-terminus, female. Bar in A, B, D, F = 20 μ m; in C, E = 50 μ m.

DESCRIPTION

Females : Bodies assume open or closed " C " after fixed. Lips markedly offset, submedian lobes pronounced, expanded. Lateral lobes much more reduced. As revealed by SEM, labial plate consists of four large submedian lobes, symmetrically arranged. Two reduced lateral lobes may be continuous with or slightly separated from submedian lobes. Amphidial aperture rectangular. Cephalic papillae not seen. Neck region highly reduced, making expanded lips more pronounced. Body annuli distinct, about 1 µm. Cephalic framework moderately developed. Stylet well-developed, basal knobs pronounced, anterior surface flat. Dorsal cesophageal gland orifice about 1-3 µm below stylet knobs. Median œsophageal bulb well-developed, widest portion about one half of body width. Valvular apparatus crescentic, pronounced. Excretory pore immediately anterior to hemizonid, in region of median bulb, rarely close to nerve ring. Lateral field four lines, two internal ones vague in light microscopy. SEM revealed three ridges in the lateral field, the middle one wider than outer two. Oogonia single row. Spermatheca offset, global to oblong, filled with spherical sperm cells. Anterior vulval lip slightly higher than posterior. Lateral vulval flaps elevated, equivalent to at least four body annuli. Vagina sigmoid. Body tapers gradually after vulva. Tail termini conoid to subacute, smooth. Post-uterine sac not observed. Phasmid not seen with light microscopy or SEM.

Males: Bodies more slender than females, assume "C" form after fixed. Lip markedly set off, neck very reduced, submedian lobes expanded, lateral lobes protruded, smaller than submedians. Body annuli distinct, less than 1 μ m. Cephalic framework weak. CEsophagus reduced, only visible as vague outline. Valvular apparatus not seen. Stylet not present. Excretory pore immediately anterior to hemizonid, located near end of œsophagus. Lateral field four lines. Spicules strong, slightly curved ventrally. Cloacal opening protruded slightly, forming small penial sheath. Tail tapers gradually to subacute terminus.

Juveniles: Bodies of all juvenile stages similar to those of females, except tail termini more bluntly rounded. Fourth-stage juveniles have no stylet, œsophagus reduced but clearly defined, no valvular apparatus, anterior portion of lumen somewhat dark, sclerotized. Other juvenile (not precisely determined) stages carry normal œsophagus and stylet. Under SEM, a third juvenile appeared to have labial plate similar to female. Lateral field of juvenile much less clear.

TYPE SPECIMENS

Holotype : Female, slide no. 2898, University of Brasilia Nematode Collection.

Paratypes : Eight females, four males and five juveniles, deposited in the University of California Nematode Collection (UCNC) Davis.

TYPE LOCALITY AND HABITAT

Holotype was collected by Olinder Maria Martins from rhizosphere of banana on the left-hand side of Highway Brasilia/Padre Bernado, 18 km from Brazlandia, Brasilia-DF. From the same locality, some paratypes were also collected by Albino B. Neto and Paulo Ernesto, from unidentified Graminae and from "jaboticabeira". From a second locality, along Hwy BR 40, 3 km from the gas station " JK " near the city of Cristalina, Goias, five more paratypes were collected by Maria do Carmo Drintig from unidentified Graminae.

DIAGNOSIS AND RELATIONSHIPS

Among species with four lines in the lateral fields, Gracilacus longilabiata sp. n. and G. capitata Adams & Eichenmuler, 1962, are the only ones known to have expanded lips. Females of G. longilabiata sp. n. are distinguished from those of G. capitata by smaller size $(0.28 \pm 0.30 (0.22-0.32) \ \mu m vs \ 0.41-0.51 \ mm)$. Males of G. longilabiata sp. n. differ from those of G. capitata in highly offset, expanded lips (vs slightly offset, lobed), no stylet (vs weak stylet), longer, more slender tail.

Gracilacus brasiliensis sp. n.

(Fig. 3)

MEASUREMENTS

Females (paratypes; n = 2): L = 0.22-0.25 mm; a = 14.7-19.6; b = 2.4-2.5; c = 10.9-12.0; c' = 3.2-3.6; V = 70.0-70.3 %; stylet = 58-62 µm; cone = 49-54 µm; excretory pore = 60-65 µm; œsophagus = 87-103 µm; tail = 18-23 µm.

Male (paratype) : L = 0.31 mm; a = 25.9; c = 12.4; spicules = 17 μ m; tail = 25 μ m; excretory pore = 78 μ m.

Juveniles (paratypes; n = 16) : L = 0.24 \pm 0.02 (0.19-0.30) mm; a = 20.4 \pm 2.1 (16.5-26.0); b = 3.0 \pm 0.2 (2.8-3.4); c = 12.5 \pm 1.6 (10.9-14.8); stylet = 38 \pm 2.3 (33-40) µm; cone = 32 \pm 2 (28-35) µm; excretory pore = 60 \pm 7.5 (54-67) µm; oesophagus = 78 \pm 5.3 (66-89) µm; tail = 20 \pm 2.5 (16-22).

Holotype (female) : L = 0.24 mm; a = 19.8; b = 2.4; c = 10.8; c' = 3.8; V = 71.8 %; stylet = 62 µm; cone = 52 µm; excretory pore = 65 µm; oesophagus = 100 µm; tail = 22 µm.

DESCRIPTION

Females : Bodies assume "C" form after fixed. Body annuli delicate but distinct. Lip region not offset,

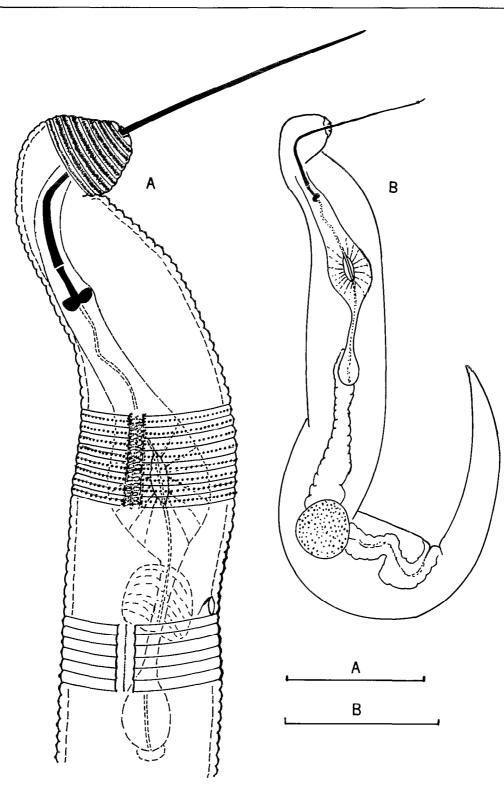


Fig. 4. Gracilacus colina sp. n. A = anterior portion, female. B = full length, female. Bar in A = 20 μ m; in B = 50 μ m.

continuous from body forming smooth conic contour. with truncate anterior surface. Submedian lobes slightly protruded, forming small oral depression in lateral view. Cephalic framework well-developed. Stylet slender, elongated, slightly curved. Cone more than six times length of shaft and knobs combined. Stylet knobs large, anterior surface inclined posteriad. Metacorpus large, occupying about 70 % of body width. Valvular apparatus large, its length about equivalent to largest width of metacorpus. Isthmus short, Hemizonid at the level of anterior part of metacorpus. Excretory pore immediately preceding hemizonid. Lateral field four lines, two inner ones lighter than others. Gonad outstretched, about 50 µm in length. Spermatheca oval, offset, filled with globular sperms. Vulva slightly elevated from body. Lateral vulval membranes present, occupying about 4-5 body annuli. Anus very obscure. Tail conoid, terminus sharply pointed, with clear area of variable size. Body annuli distinct to tail terminus.

Male : More slender than females. Lip region similar to that of females. Cephalic framework less developed. Oesophagus rudimentary, only vague contour of procorpus and metacorpus seen. Stylet lacking. Positions of hemizonid and excretory pore equivalent to those of females. Body annuli more visible than those of females, especially in tail region. Lateral field four lines. Gonad about 110 μ m. Cloacal opening elevated, forming a short penial sheath. Spicules well-developed, slightly curved ventrally, protruding from body at about 120°. Gubernaculum about 2 μ m. Tail similar to female.

Juveniles : Genital primordia indicated that all of the juveniles obtained are of second — or third —, more likely third-stages. Bodies resemble those of females except sexual differentiation. Tail termini less acute, sometimes gradually taper to rounded ends.

TYPE SPECIMENS

Holotype : Female, slide no. 984A, University of Brasilia Nematode Collection.

Paratypes : Two females, one male, 17 juveniles deposited in UCNC Davis.

TYPE LOCALITY AND HABITAT

Holotype and paratype females collected by Antonio Apoliano dos Santos in 1979 from " cerrado", approximately 500 m north of Colina apartment area on the campus of the University of Brasilia, ASA Norte, Brasilia-DF, associated with mixed vegetation, including *Erytroxillium tortuosum*. Other paratypes, males and juveniles, were collected by Paulo Brioso and Gilmar Henz in 1983 beside Hwy BR 40 approximately 100 km from city limit of Brasilia. Still other paratypes (juveniles only) were obtained by Osmar Crestani in 1983 from " cerrado " near Lake Feia, about 20 km west of the city of Formosa, State of Goias. All paratypes are associated with " cerrado " mixed vegetation.

DIAGNOSIS AND RELATIONSHIPS

Gracilacus brasiliensis sp. n. is most closely related to G. mira Raski, 1962 because of four-lined lateral fields, excretory pore anterior to median oesophageal valve, presence of lateral vulva membrane and almost pointed tail termini. G. brasiliensis sp. n. is distinguished from G. mira by smaller size (0.25 mm vs 0.33 mm), shorter stylet (62 μ m vs 77 μ m) and vulva more anteriorly positioned (70 % vs 83 %).

Gracilacus colina sp. n.

(Fig. 4)

Measurements

Holotype (female) : L = 0.27 mm; a = 9.2; b = 2.6; c = 8.6; c' = 3.9; V = 69.3 %; stylet = 72 μ m; cone = 64 μ m; oesophagus = 102 μ m; excretory pore = 78 μ m; tail = 31 μ m.

DESCRIPTION

Female : Only one gravid female was found. Body swollen anterior to vulva, ventrally curved sharply around vulva assuming form of fishing hook. Lip region not offset, continuous with body contour. Submedian lobes protruded, forming small oral depression. Cephalic framework well-developed. Body annuli distinct. Transverse striae anterior to excretory pore ornamented with single row of cuticular warts, regularly spaced. Annuli posterior to oesophagus appear to be smooth without ornamentations. Neither is lip region ornamented. Lateral field three lines, center one being extremely vague. Where the transverse annuli are ornamented, the longitudinal lines in lateral fields substituted by clusters of irregular cuticular warts. Stylet well-developed with pronounced basal knobs whose anterior surface is smooth, slightly inclined posteriad. Oesophagus prominent, widest part of median bulb occupying 65 ° of body width. Nerve ring at level of excretory pore. Hemizonid immediately anterior to excretory pore. Ovary outstretched, extending to level of median bulb. Oogonia arranged in multiple rows. Spermatheca spherical, offset, filled with globular sperms. Anterior vulval lip elevated. Lateral vulva flaps small. Anus obscure. Body posterior to anus tapers gradually, ending in finely rounded terminus. Lateral field gradually disappears after anus. Body annuli distinct to terminus.

Males and juveniles : Not found.

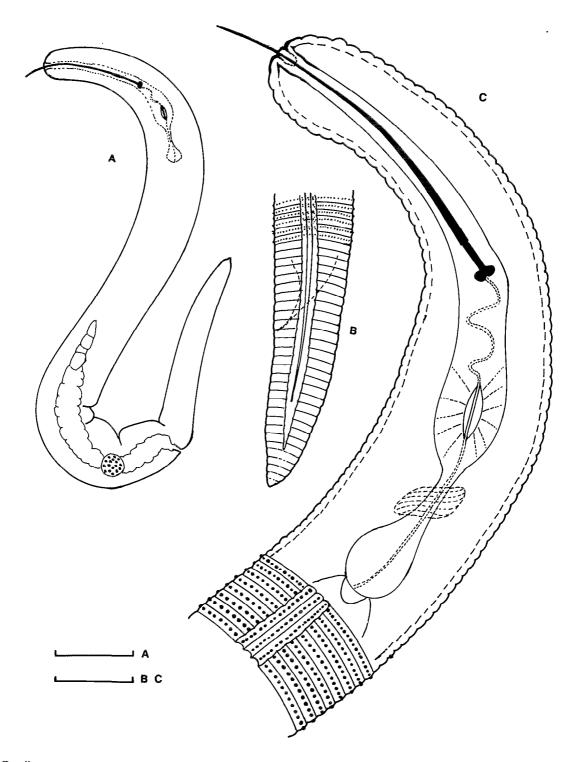


Fig. 5. Gracilacus punctata sp. n. A = full length, female; B = tail, female; C = anterior end, female. Bar represents 30 μ m for A; 10 μ m for B, C.

TYPE AND ONLY SPECIMEN

Holotype : Female, slide number 1574, University of Brasilia Nematode Collection.

TYPE LOCALITY AND HABITAT

The specimen was collected by Claudio Bittencourt da Silva in 1980 from " cerrado ", approximately 500 m from the Colina apartment area on the campus of the University of Brasilia. The nematode was associated with unidentified native vegetation.

DIAGNOSIS AND RELATIONSHIPS

Gracilacus colina sp. n. is characterized by small size of 0.27 mm, lip region rounded with coarse annuli, stylet 72 μ m, body annuli ornamented with single row of cuticular warts found anterior to excretory pore, lateral field with three lines setting off two bands, lateral vulval membranes small, tail finely rounded.

Gracilacus colina sp. n. is most closely related to Gracilacus punctata sp. n. and Gracilacus mutabilis (Colbran, 1969) Raski, 1976 by virtue of dotted cuticular ornamentations. G. colina sp. n. differs from both the other species by the lateral field having three lines (vs four lines), longer stylet (72 μ m vs approximately 50 μ m), vulva more anterior (69 % vs approximately 80 %) and by cuticular ornamentations located only in oesophageal region (vs entire body of all of swollen portion). Gracilacus colina sp. n. is also closely related to G. idalima Raski, 1972 from which it differs by total lack of cuticular ornamentation on females of G. idalima even in grossly swollen gravid stage.

Gracilacus punctata sp. n. (Figs 5 & 6)

MEASUREMENTS

Female (paratype) : L = 0.34 mm; a = 23; b = ?; c = 10.4; c' = 4.4; V = 74.8 %; stylet > 38 μ m (broken).

Holotype (female) : L = 0.31 mm; a = 14.1; b = 3.2; c = 14.3; c' = 2.6; V = 75.7 %; stylet = 48 µm; cone = 43 µm; excretory pore = 45 µm; oesophagus = 85 µm.

DESCRIPTION

Females : Obese females assume "S" or "W" forms after fixed. Lip region continuous from body forming smooth dome with first 3-4 body annuli. Submedian lobes protruded, resulting in small oral depression. Cephalic framework well-developed. Body annuli coarse, averaging 1.6 μ m, those in stylet region slightly

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coarser. Every body annulus dotted with reflective spherical warts, arranged in single row with regular intervals around body, except in lateral fields where warts are irregularly clustered. SEM showed that cuticular warts are elevated spheres sitting on ridge of every annulus. Lip free of cuticular warts. Lateral field commences in stylet basal knob region, beginning with two lines but quickly followed by three ridges and four deep lines. Lateral field visible to last 5-6 tail annuli. Body annuli interrupted at edge of lateral field. Stylet robust and elongated, cone more than $7.5 \times$ length of shaft and knob combined. Median oesophageal bulb occupies about 1/3 body width, with large crescentic valvular apparatus. Isthmus short. Excretory pore anterior to isthmus. Vulva indented, anterior vulval lip more elevated. Lateral vulval flaps not observed. Spermatheca rounded, significantly offset, filled with globular sperms. Tail subacute. Phasmid not seen.

Males and juveniles : Not found.

TYPE SPECIMENS

Holotype female, slide no. 2003B, University of Brasilia Nematode Collection.

Paratype: One female, slide no. 2002B6, deposited in UCNC Davis. Two more mature female cuticles were also collected, intact but devoid of body contents. One was used for SEM studies, the other deposited with paratype.

TYPE LOCALITY AND HABITATS

Holotype and paratypes collected by C. S. Huang and J. E. Cares in 1981 from tropical rain forest in northern edge of Fruit and Forest Experiment Station, INPA (Instituto Nacional de Pesquisas da Amazonas), Manaus, State of Amazonas. The mixed vegetations were not identified.

DIAGNOSIS AND RELATIONSHIPS

Gracilacus punctata sp. n. is characterized by its small size (0.31-0.34 mm), stylet of 48 µm, coarse body annuli (averaging 1.6 µm) with strong spherical warts covering the entire female body length, warts arranged in a single row excepting lateral field where they are irregularly clustered, lateral field with four lines forming three bands, lateral vulval membranes lacking and tail subacute.

Gracilacus punctata sp. n. is most closely related to G. colina sp. n. by virtue of the prominent punctations on the cuticle of gravid females. It differs by a shorter (48 μ m for G. punctata sp. n. vs 72 μ m for G. colina sp. n.), lateral field with four lines (vs three lines) and cuticular ornamentations covering entire body length (vs ornamentation only on anterior end in G. colina). G. punctata sp. n. also resembles Gracilacus mutabilis

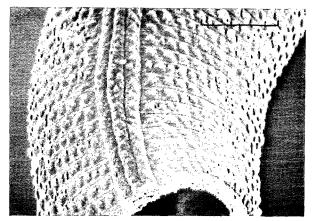


Fig. 6. Gracilacus punctata sp. n. SEM of female cuticle in ∞ sophageal region. Bar represents 5 μ m.

(Colbran, 1969) Raski, 1976 in general body form; lateral field with four lines. However, *G. punctata* sp. n. differs by lateral field which remains four-lined with irregular clusters of cuticular warts, whereas on *G. mutabilis* lateral field has four lines in unswollen portion and three bands of minute tubercles in swollen portion of body. *G. punctata* sp. n. also shows relationship with *G. mira* Raski, 1962 from which it differs by total lack of cuticular punctations in *G. mira*. In addition, the stylet of *G. punctata* sp. n. is shorter (48 µm vs 70-85 µm); body annuli much more coarse (total annuli

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in oesophageal region = 48 for *G. punctata* sp. n., more than 100 for *G. mira*) and lateral vulval membrane absent (moderately developed in *G. mira*).

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