

# Description of *Gracilacus hamicaudata* sp. n. (Nemata : Criconematidae) with biological and histopathological observations

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## SUMMARY

Obese criconematid females and larvae forming colonies were detected under the root cortex attached to the vascular cylinder of redwood roots, *Sequoia sempervirens* (D. Don) Endl. These specimens have been determined to represent a new species of Criconematidae, *Gracilacus hamicaudata* sp. n. Mature females are obese at midbody but posteriorly the body is constricted and hooked shaped with a rounded tail terminus. Annuli are conspicuous only anteriorly and posteriorly. The cephalic region has four submedian lobes and a circular oral disc. The vulval-lips protrude and the lateral field is marked with four lines. Eggs are partially embedded in a mucoid-like substance. There are giant nutritive cells formed in the parenchyma tissue of the vascular cylinder associated with the nematode colonies. These cells have dense cytoplasm with enlarged nuclei. Starch granules were found inside of these abnormal cells.

## RÉSUMÉ

*Description de Gracilacus hamicaudata sp. n.  
(Nemata : Criconematidae) et observations sur la biologie et l'histopathologie de cette espèce*

Des femelles renflées et des juvéniles d'un Criconematide ont été observés, assemblés en colonies, sous le cortex radiculaire et attachés au cylindre central de racines de *Sequoia sempervirens* (D. Don) Endl. Ils appartiennent à une nouvelle espèce, *Gracilacus hamicaudata* sp. n. Les femelles matures sont renflées dans leur partie centrale, mais la partie postérieure du corps est rétrécie et en forme de crochet, l'extrémité caudale étant arrondie. L'annélation cuticulaire n'est visible que vers l'avant et l'arrière de la femelle. La région céphalique comporte quatre lobes submédians et un disque oral circulaire. Les lèvres vulvaires sont en relief et le champ latéral comporte quatre lignes. Les œufs sont en partie entourés par une substance mucoïde. Des cellules nutritives géantes sont formées dans le tissu parenchymatique du cylindre central, en association avec la présence des colonies du nématode. Ces cellules présentent un cytoplasme dense et des noyaux agrandis. Des granules d'amidon ont été observés à l'intérieur de ces cellules modifiées.

Many workers have reported among various *Gracilacus* spp., that active vermiform larvae and immature females and males were detected in the soil. Mature obese females were observed only when the roots were washed and scrubbed.

In September 1979 during a survey of nematodes attacking forest trees, abundant larvae and a few females of *Gracilacus* were detected in soil samples. However, while dissecting roots of the Coast Redwood, *Sequoia sempervirens* (D. Don) Endl., for *Rhizonema sequoiae* Cid del Prado Vera, Lownsbery & Maggenti, 1983, colonies of *Gracilacus* spp. were found under the cortex of the roots. Most of the nematodes were attached to the stele of the root. Eggs were observed in a "gelatinous

matrix" beneath the cortex. A few reports mention the formation of colonies of females and larvae attached to the roots (Thorne, 1943; Allen & Jensen, 1950; Inserra & Vovlas, 1981) and the presence of a mucoid substance has been reported also.

Detailed morphological, anatomical and histopathological studies of this nematode were made and a description of *Gracilacus hamicaudata* sp. n. is presented here.

## Materials and methods

Larvae and females were obtained by dissecting redwood roots collected near Lake Lagunitas, Marin

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Co., California. All life stages were killed in water by heating them for a few seconds over a flame, and then they were fixed in Seinhorst's fixative (Seinhorst, 1962), processed into glycerin, and mounted in glycerin. For scanning electron microscope study, the method described by Cid del Prado Vera, Lownsbery and Maggenti (1983) was followed.

By washing redwood roots gently and dissecting carefully, it was possible to observe the presence of colonies of *Gracilacus* living under the cortex of secondary roots. These infected roots were separated and fixed in FAA for one week, dehydrated in an ethanol:butanol:distilled water series, and sectioned following the procedure reported by Cid del Prado Vera, Lownsbery and Maggenti (1983). The roots were sectioned at 8  $\mu\text{m}$  and stained with safranin and fast green. Sections were studied and photographed under Nomarski and normal microscopic illumination systems.

*Gracilacus hamicaudata* sp. n.  
(Figs 1, 2)

DIMENSIONS

*Female* (paratypes; n = 40) : L = 0.25-0.40 mm (0.34,  $\pm$  0.01); a = 8.6-19.9 (11.7  $\pm$  0.71); b = 1.9-4.3 (2.9  $\pm$  0.15); c = 17.0-28.7 (21.8  $\pm$  1.1); V = 77-87 (83  $\pm$  0.59); stylet = 73-96  $\mu\text{m}$  (85  $\pm$  1.8); excretory pore = 71-99  $\mu\text{m}$  (83  $\pm$  2.0).

*Second-stage juvenile* (n = 10) : L = 0.21-0.32 mm (0.27  $\pm$  0.02); a = 10.7-25.4 (22.5  $\pm$  2.9); b = 2.8-3.3 (2.9  $\pm$  0.1); stylet = 43-60  $\mu\text{m}$  (49  $\pm$  4.6); conus = 36-53  $\mu\text{m}$  (42  $\pm$  6.7); excretory pore = 58-80  $\mu\text{m}$  (69  $\pm$  4.9).

*Holotype* (female) : L = 357  $\mu\text{m}$ ; maximum body width 29  $\mu\text{m}$ ; a = 12.4; b = 3.2; c = 18.8; V = 80; stylet = 89; excretory pore from anterior extremity = 88  $\mu\text{m}$ ; spermatheca = 11  $\mu\text{m}$  long, 10  $\mu\text{m}$  wide.

DESCRIPTION

*Female* : Generally posterior extremity of body curved, giving a modified "C" shape; with SEM body annuli conspicuous anteriorly and posteriorly, annulus width anteriorly (calculated as the average from at least ten annuli 0.9-1.5  $\mu\text{m}$  (1.3  $\pm$  0.3), and posteriorly annulus average width 0.6-1.2  $\mu\text{m}$  (0.9  $\pm$  0.07). Annuli at midbody inconspicuous (light microscope). Cuticle thickness variable : anteriorly 0.9-2.2  $\mu\text{m}$  (1.2  $\pm$  0.16) thick; at midbody 0.6-2.2  $\mu\text{m}$  (1.5  $\pm$  0.25) thick; on posterior body 0.9-2.8  $\mu\text{m}$  (1.5  $\pm$  0.3). Lip region with four small submedian lobes, two subdorsal and two subventral and circular oral disc. Body gradually increases in width, maximum width anterior to vulval aperture. Lateral field with four incisures. Two inner incisures closer to outer incisures than to each other. Posterior

body from vulval aperture to posterior end constricted and "hook" shaped (hamate), with bluntly rounded terminus. Cephalic sclerotization weak, stylet slender, sometimes with a slight curvature; knobs with slight anterior projection, and 3.7-5.8  $\mu\text{m}$  (4.7  $\pm$  0.18) wide. Excretory pore generally at level of esophageal valve, sometimes slightly posterior, but always anterior to isthmus. Hemizonid one annulus posterior to excretory pore. Dorsal esophageal gland orifice 3.7-12.3  $\mu\text{m}$  (7.0  $\pm$  1.3) from base of stylet. Metacarpus greatly enlarged, valve 5.0-10.0  $\mu\text{m}$  (8.0  $\pm$  0.6) long and 2.0-5.0  $\mu\text{m}$  (3.2  $\pm$  0.5) in width. Isthmus slender 5.0-15.4  $\mu\text{m}$  (10.4  $\pm$  0.8) long and 1.5-4.3  $\mu\text{m}$  (2.7  $\pm$  0.2) wide. Nerve ring surrounds posterior half of isthmus. Posterior esophageal bulb slightly enlarged. Ovary outstretched, sometimes with one to three flexures; spermatheca oblong, 11.1-23.1  $\mu\text{m}$  (16.8  $\pm$  1.0) long and 10.7-18.8  $\mu\text{m}$  (14.7  $\pm$  1.0) wide. Sperm observed. Uterus usually with wide lumen, and enlarged cells. Sometimes posterior uterine cells project beyond vagina, appearing like a postuterine sac. Vulval lips rounded, protruding, lateral vulvar membranes present. Vulva from anus 25-49 (36  $\pm$  2.0) and from posterior extremity 34-65 (52  $\pm$  2.3). Anus inconspicuous, tail short with rounded terminus.

*Second-stage juvenile* : Head rounded with four submedian lobes as in female, not set off. Stylet well developed, knobs with slight anterior projection. Metacarpus slightly swollen; isthmus slightly longer than in adult female 9.2-15.7  $\mu\text{m}$  (12.6  $\pm$  1.3) long and 1.0-2.8  $\mu\text{m}$  (1.9  $\pm$  0.4) wide. Excretory pore in same position as in adult female. Genital primordia distinct, located in posterior third of body and formed by four cells. Anus indistinct. Tail terminus rounded.

TYPE SPECIMENS

*Holotype* (female) deposited in University of California Nematode Collection, Davis, USA, UCNC Slide No. 2136.

*Paratypes* : fourteen females, Slide No. 2137; five second-stage juveniles, Slide No. 2138 at UCNC, Davis, USA; and ten females, one juvenile at Colegio de Postgraduados, Centro de Fitopatología, Chapingo-Montecillos, Mexico; six females, eight juveniles at Instituto de Biología, Lab. Helminología, UNAM, Mexico; four females, five juveniles at USDA Nematode Collection, Beltsville, Maryland, USA; seven females, five juveniles at Laboratoire des Vers, Muséum national d'Histoire naturelle, Paris, France; four females at Laboratorium voor Nematologie, Landbouwhogeschool, Wageningen, Netherlands.

TYPE HOST AND LOCALITY

Redwood tree, *Sequoia sempervirens* (D. Don) Endl. Lagunitas Lake, San Rafael, Marin County, California.

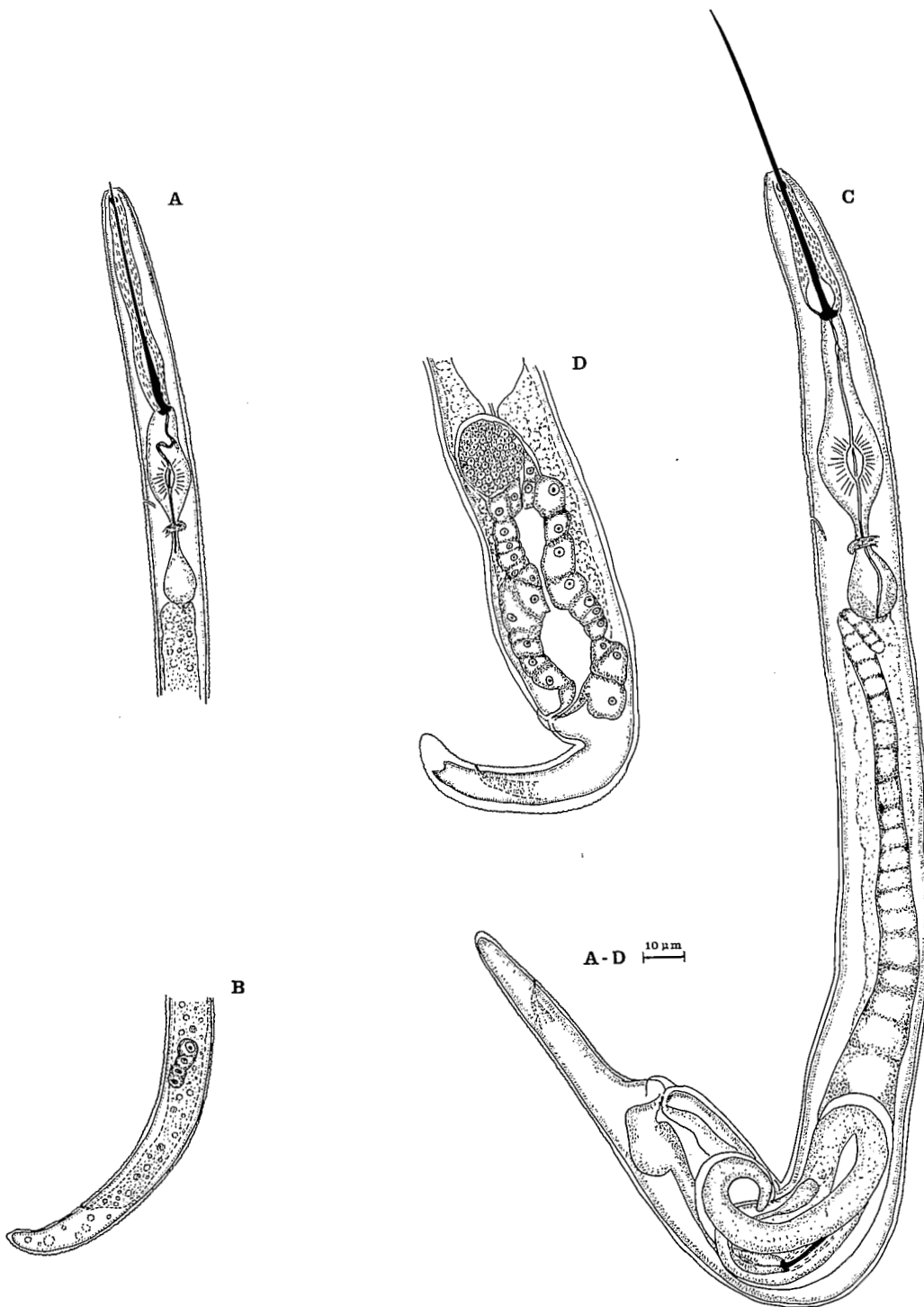


Fig. 1. *Gracilacus hamicaudata* sp. n. A-B : Second-stage larva. A : Esophageal region; B : Posterior part (lateral view) — C-D : Female. C : Female body; D : Posterior part (lateral view).

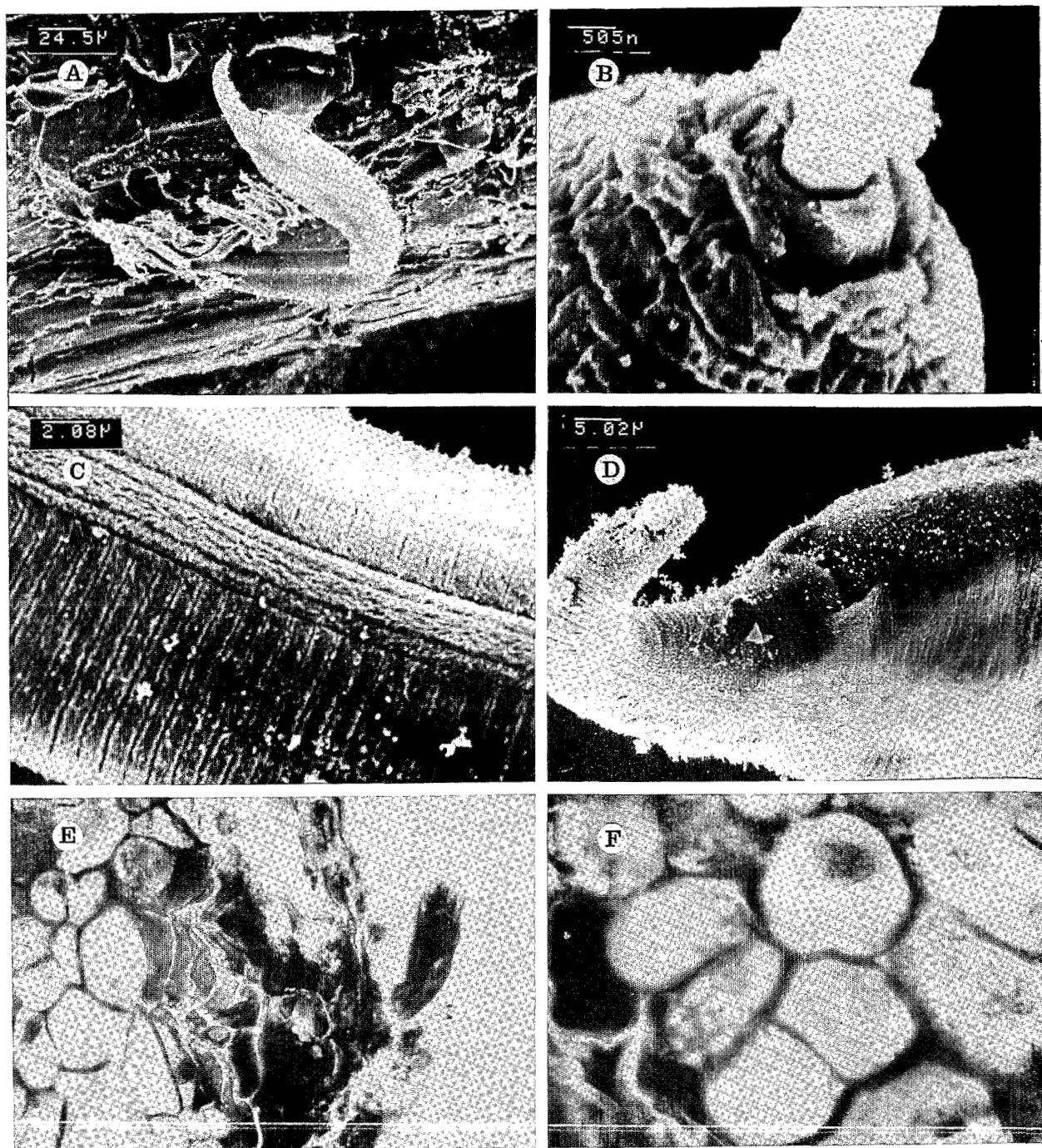


Fig. 2. — A-D : SEM of female of *Gracilacus hamicaudata* sp. n. A : Female attached to vascular cylinder; B : Face view; C : Lateral field midbody; D : Posterior end of the body. — E-F : Transversal sections of *Sequoia sempervirens* roots infected by *Gracilacus hamicaudata* sp. n.; E : Vascular cylinder, damage on pericycle and parenchyma cells; F : Parenchyma cells of vascular cylinder.

## DIAGNOSIS

*Gracilacus hamicaudata* sp. n. can be distinguished from other *Gracilacus* spp. with four lines in the lateral field by the conspicuous annulation on the anterior and posterior regions of the body and the almost complete absence of annulation at midbody (see : Raski, 1962; Huang & Raski, 1986). *G. hamicaudata* sp. n. is closest to *G. epacris* (Allen & Jensen, 1950) Raski, 1962 from which it differs by the thinner cuticle 0.6-2.8  $\mu\text{m}$  vs 2.6-4.6  $\mu\text{m}$  in mature females; it further differs by the oval shape of the spermatheca. It can also be distinguished from *G. epacris* by the distance from the anterior extremity to the base of the esophagus, *G. hamicaudata* sp. n. mean 113  $\mu\text{m}$  (90-131) vs mean 88  $\mu\text{m}$  (76-110). The distance from the anterior extremity is greater in *G. hamicaudata* sp. n. : mean distance 282  $\mu\text{m}$  (248-310) vs mean distance 215  $\mu\text{m}$  (191-258).

## BIOLOGY

*Gracilacus hamicaudata* sp. n. is also like *G. epacris* in the formation of colonies. Allen and Jensen (1950) found these colonies of females and juveniles living semi-embedded in black walnut roots, but they did not report these nematodes as being present under the cortex.

The formation of colonies under the cortex, the presence of gelatin-like material and the abnormal cells forming in infected roots has been repeated for *Cacopaurus pestis* Thorne, 1943. Thorne (1943) thought that the gelatin-like material was exudation from the root cells. However, Inserra and Vovlas (1981) found that the mucoid substance is secreted by *C. pestis* during egg laying. This observation agrees with our observation of live material.

The presence of a stylet in the fourth stage larva is also reported in *C. pestis*.

The presence of females attached to the stele or vascular cylinder were found only in the secondary roots under the cortex. Nematodes were not attached to the cortex and no portion of the nematode body was exposed outside the cortex. A mucoid substance or gelatin-like material, in which the eggs were partially embedded, was commonly observed. No males were observed in the colonies and none were recovered from roots incubated in a mist chamber. Giant cells, with giant nuclei were found in the phloem parenchyma of the vascular tissue

of Coast Redwood. These abnormal cells are ovoid or polygonal in shape with dense cytoplasm containing many starch granules. At the site where the colonies are present, the pericycle cells of the vascular cylinder increased in number, collapsed and died. Giant cells in transverse sections were 24-70  $\mu\text{m}$  ( $35 \pm 7.1$ ) long and 13-46  $\mu\text{m}$  ( $25 \pm 4.9$ ) wide. The dimensions of the nuclei were 9-19  $\mu\text{m}$  ( $14 \pm 1.4$ ) long and 9-13  $\mu\text{m}$  ( $11 \pm 1.0$ ) wide. The nucleoli were difficult to distinguish; however, in some cases one to three nucleoli were observed. The cell walls of abnormal cells, were the same thickness as normal cell walls. Normal cells, adjacent to the abnormal cells, did not have dense cytoplasm and were 20-46  $\mu\text{m}$  ( $29 \pm 4.0$ ) long and 12-32  $\mu\text{m}$  ( $19 \pm 2.7$ ) wide and the nucleus 8-15  $\mu\text{m}$  ( $10 \pm 1.0$ ) long and 5-10 ( $7 \pm 2.0$ ) wide. Only one nucleus was observed in these cells, and no starch granules were present.

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