# Description of *Pratylenchus gutierrezi* n. sp. (Nematoda: Pratylenchidae) from Coffee in Costa Rica

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Abstract: A lesion nematode, Pratylenchus gutierrezi n. sp., collected from the roots of coffee in the Central Plateau of Costa Rica, is described and illustrated. Its relationships to Pratylenchus flakkensis, P. similis, and P. gibbicaudatus, the only other species of the genus having two head annules, males, or spermatheca with sperm, and an annulated tail terminus, is discussed. Other distinctive characters are its posterior vulva (mean of 80%); its prominently rounded stylet knobs, low head, and subcylindrical tail. SEM observations provide additional details of females and males, especially face views, which show for the first time sexual dimorphism.

Key words: Coffea arabica, Costa Rica, lesion nematode, morphology, nematode, new species, Pratylenchus flakkensis, P. gibbicaudatus, P. gutierrezi n. sp., P. similis, scanning electron microscopy, SEM, taxonomy.

Certain species of lesion nematodes (Pratylenchus spp.) are important parasites of coffee (Coffee spp.). In a recent excellent review of nematodes reported to occur on coffee (1), the following five Pratylenchus species were listed: P. brachyurus (Godfrey, 1929) Filipiev & Schuurmans Stekhoven, 1941; P. coffeae (Zimmermann, 1889) Filipjev & Schuurmans Stekhoven, 1941; P. goodeyi Sher & Allen, 1953; P. loosi Loof, 1960; and P. pratensis (de Man, 1880) Filipiev, 1936. Three species were represented by only one report each, with occurrences as follows: P. goodeyi in Tanzania; P. loosi in Ceylon; and P. pratensis in South India. Pratylenchus brachyurus is common on coffee in Brazil and occurs to a lesser extent in Peru and West Africa, whereas P. coffeae is the most widespread and damaging species on coffee.

Another lesion nematode recently was found parasitizing roots of coffee in the Central Plateau of Costa Rica; it was noted to be quite common in several areas of that region. Study of this nematode revealed it

to be a new *Pratylenchus* species, which is described and illustrated herein.

## MATERIALS AND METHODS

Nematodes were extracted from infected coffee roots by placing chopped or blenderized roots on filter paper over water in a Baermann funnel. Specimens were then hand picked and fixed in either hot TAF (7 ml 37% formaldehyde, 2 ml triethanolamine, 91 ml distilled water) or 3% formalin. Some were studied in fixative on temporary slide mounts; others in permanent glycerine mounts (3). Drawings and photomicrographs were made with a drawing tube or automatic 35-mm camera attached to a compound microscope equipped with a differential contrast system. For scanning electron microscopy (SEM), nematodes were processed as described by López and Salazar (4). Two slides with 16 female and male paratypes of Pratylenchus flakkensis Seinhorst, 1968 (5) were obtained from The Netherlands for examination and comparison. All measurements are in micrometers (µm) unless otherwise specified.

#### Systematics

Pratylenchus gutierrezi n. sp. (Figs. 1A–I, 2A–L, 3A–L)

Holotype (female, in glycerine): Length 481; width 19.7 at midbody; a = 24.4; b = 3.9; c = 20.5; head width 7.5; head height

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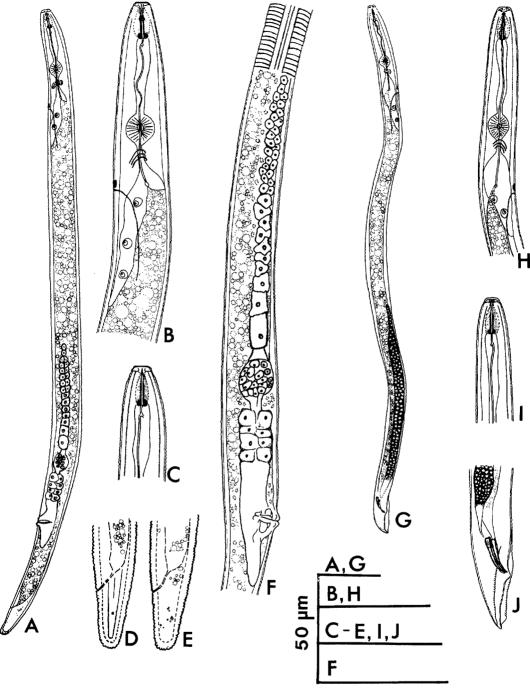


Fig. 1. Drawings of Pratylenchus gutierrezi n. sp. A-F) female. A) Entire female. B,C) Anterior region. D,E) Tail region. F) Vulva, reproductive structures, and segment of lateral field. G-J) Male. G) Entire male. H,I) Anterior portion. J) Posterior portion.

2.3; head w/h = 3.2; stylet 16.5; DGO 2.8to stylet base; stylet knob width 4.2; stylet knob height 2.3; stylet knob w/h = 1.8; center of median bulb valve 45.1 to head

end; base of esophageal gland lobe 122 to head end; excretory pore 81.3 to head end; vulva 385 to head end; V = 80%; postuterine sac 21.2; lateral field width

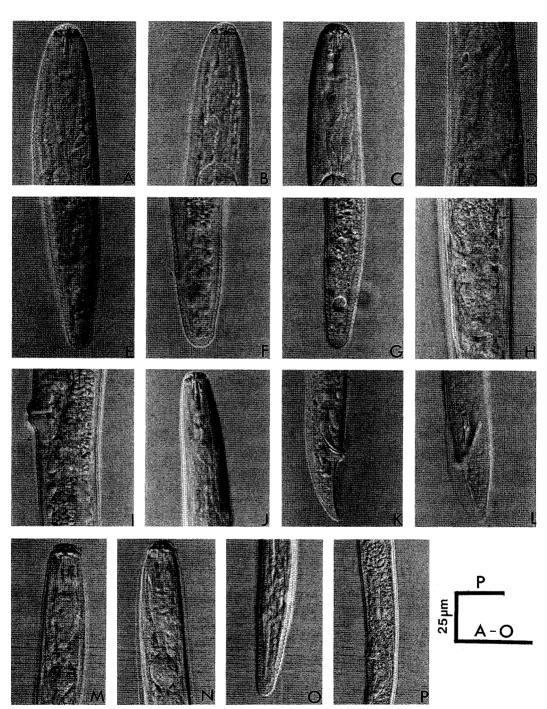


FIG. 2. Photomicrographs of two *Pratylenchus* species. A–L) *Pratylenchus gutierrezi* n. sp. A–I). Female. A–C) Anterior portion. D) Body portion posterior to median bulb showing large hemizonid and excretory pore (at arrow). E–G) Tails (in F arrow denotes anus). H) Spermatheca and vulva in ventral view (at arrows). I) Lateral view of protruding vulva and postuterine sac (at arrow). J–L) Male. J) Anterior portion. K,L) Posterior portion. M–P) *Pratylenchus flakkensis* female. M,N) Anterior portion. O) Tail region. P) Body view with spermatheca (anterior) and vulva (lower left).

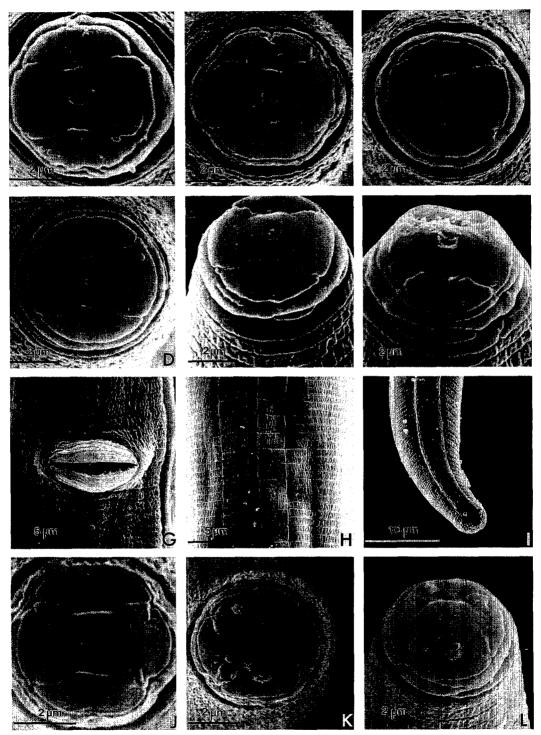


Fig. 3. SEM micrographs of *Pratylenchus gutierrezi* n. sp. A–I) Female. A–D) Face views. E,F) Face views from partial lateral angle. G) Vulva, ventral view. H) Lateral field near anterior portion. I) Tail region. J–L) Male. J,K) Face views. L) Face view from partial lateral angle.

Table 1. Measurements (µm) of females and males of Pratylenchus gutierrezi n. sp. from Costa Rica.

Character	Female ( $n = 30$ )		Male $(n = 20)$	
	Range	Mean ± SD	Range	Mean ± SD
Linear				
Body length	430.0-552.0	$500.1 \pm 27.7$	378.0-459.0	$425.2 \pm 20.8$
Body width at midbody	19.2-35.2	$25.7 \pm 3.4$	14.3-23.9	$17.7\pm2.4$
Head width	6.8-8.6	$7.7 \pm 0.5$	5.6 – 6.9	$6.3 \pm 0.3$
Head height	1.7-2.8	$2.3 \pm 0.2$	2.0 - 2.6	$2.3 \pm 0.2$
Stylet length	15.9 - 17.6	$16.8 \pm 0.5$	13.8-16.2	$15.3 \pm 0.6$
DGO to stylet base	1.9 - 4.8	$2.9 \pm 0.7$	1.6-4.1	$2.8 \pm 0.7$
Stylet knob width	3.1 - 4.9	$4.1 \pm 0.4$	2.6 - 3.5	$3.0 \pm 0.3$
Stylet knob height	1.7 - 2.7	$2.3 \pm 0.2$	1.5 - 2.2	$1.8 \pm 0.2$
Median bulb valve to head end	45.1 - 62.4	$55.9 \pm 3.8$	49.6-58.9	$53.7 \pm 2.9$
Base of esophageal gland lobe				
to head end	119.0-142.9	$128.9 \pm 6.2$	104.0-132.0	$113.3 \pm 8.8$
Excretory pore to head end	74.3 - 94.0	$83.1 \pm 4.1$	76.6 - 84.1	$79.8 \pm 2.7$
Vulva to head end	348.0-443.0	$400.0 \pm 26.3$		
Postuterine sac length	15.6 - 32.6	$22.6 \pm 5.7$		
Lateral field width	4.7 - 7.1	$5.9 \pm 1.7$	4.9 - 5.6	$5.2 \pm 0.4$
Tail length	21.1-29.2	$25.4 \pm 2.6$	17.5-21.6	$20.0 \pm 1.3$
Tail annules	18-23	$21 \pm 1.6$		
Phasmid to tail tip	12.7-19.1	$16.1 \pm 2.2$	9.5 - 16.5	$13.4 \pm 1.9$
Spicule length			15.5 - 20.7	$16.8 \pm 1.4$
Gabernaculum length			3.2 - 4.4	$3.7 \pm 0.4$
Ratios				
a	15.0 - 24.9	$19.8 \pm 2.1$	17.6 - 30.7	$24.5 \pm 3.1$
b	3.5 - 4.5	$3.9 \pm 0.3$	3. <del>4-4</del> .1	$3.8 \pm 0.2$
С	16.6 - 24.6	$19.9 \pm 2.6$	19.5-24.2	$21.5 \pm 1.2$
Head width/height	2.8 - 4.1	$3.4 \pm 0.3$	2.5 - 3.2	$2.7 \pm 0.2$
Stylet knob width/height	1.3 - 2.6	$1.8 \pm 0.3$	1.3-2.2	$1.6 \pm 0.3$
Percentages				
V%	74.1-83.8	$80.0 \pm 2.3$		

6.1; tail 23.5; tail annules 21; phasmids 16.4 to tail tip.

Female (n = 30): Measurements in Table 1. Body vermiform with some tapering at extremities. Head low, slightly offset, width over three times its height, generally rounded, heavily sclerotized, bearing two annules. By SEM (Fig. 3A-F): oral aperture ovate; labial disc and median lips fused, appearing bow-tie shaped; lateral lips large, distinct, partially fused with median lips and labial disc; amphidial apertures small, round to oval, located between labial disc and lateral lips. Stylet strong, basal knobs large, rounded. DGO near stylet base. Cuticular annules fine, width about 1 µm at midbody. Hemizonid prominent, 1-2 annules anterior to excretory pore. Median bulb distinct, valvate, occupying 3-4 sixths corresponding body width. Lateral field about 25% of body width, with four incisures forming three bands, inner band often narrower than outer two, which may show some aerolation especially in anterior and posterior portion (Fig. 3H,I); two inner incisures generally fuse anterior to anus and continue as single incisure to anal area or midtail (Fig. 1D). Vulva prominent, lips often protruding (Fig. 2I). Spermatheca round to oval, commonly with sperm (Figs. 1F, 2H). Tail subcylindrical, terminus round to bluntly rounded, coarsely annulated (Figs. 1D,E; 2E–G). Phasmids usually anterior to midtail.

Allotype (male, in glycerine): Length 393; width 13.2; a = 29.8; b = 3.7; c = 20.9; head width 6.6; head height 2.3; head w/h = 2.8; stylet 14.1; DGO 2.8 to stylet base; stylet knob width 3.3; stylet knob height 1.9; stylet knob w/h = 1.7; center of median bulb valve 47 to head end; base of

esophageal gland lobe 107 to head end; excretory pore 69.6 to head end; lateral field width 3.8; tail 18.8; phasmids 11.8 to tail tip; spicules 16; gubernaculum 3.8.

Male (n = 20): Measurements in Table 1. Essentially similar to female except smaller body size, sexual dimorphism, and different face view (Figs. 1G-J; 2K,L; 3J-L). Stylet delicate, with distinct rounded knobs (Fig. 2]). Lateral field prominent, about a third body width, incisures four. Testis single, outstretched anteriorly. Tail enveloped by crenate-edged bursa. Spicules paired, ventrally arcuate; gubernaculum small, curved. Phasmids open on bursa, generally anterior to midtail. SEM face views (Fig. 3]-L): oral aperture ovate, axial, surface raised, labial disc oblong. Median and lateral lips similar to female. By contrast to female four wedge-shaped sectors, each bearing a cephalic sensilla (Fig. 3]), corresponding in alignment to those on the median lips of female (Fig. 3B), overlap junctions (or fusion) of median and lateral lips. Amphidial apertures small, round to oval, located between labial disc and lateral lips.

# Type host and locality

Roots of Coffea arabica L. cv. Caturra, San Antonio, Naranjo County, Alajuela Province, Costa Rica. Latitude 84°25'; longitude 10°7'; 1,200 meters above sea level; premontane wet forest.

# Type specimens

Holotype (female): Isolated from roots of coffee from the above type locality. Slide T-456t, deposited in the U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland, USA. Allotype (male): Slide T-457t, same data and repository as holotype. Paratypes (females, males, and juveniles): same data and repository as holotype. Others deposited in: Coleccion de Nematodos del Laboratorío de Nematología, Universidad de Costa Rica, San José, Costa Rica; Collection of the Istituto di Nematologia Agraria del Consiglio Nazionale delle Ricerche (C.N.R.), Bari, Italy; University of California Nematode Collection (UCRNC), Riverside, California: The Nematode Collection of the Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England; Canadian National Collection of Nematodes, Ottawa, Canada; Collection Nationale de Nématodes, Laboratoire des Vers, Muséum national d'Histoire naturelle, Paris, France: Nematode Collection, Institutut voor Dierkunde, Laboratorium voor Morfologie en Systematiek der Dieren, Gent, Belgium; Nematode Collection of the Landbouwhogeschool, Wageningen, The Netherlands: Commonwealth Institute of Parasitology Collection, St. Albans, Herts.. England.

# Diagnosis

Pratylenchus gutierrezi n. sp. belongs with the other three Pratylenchus species having two head annules, males, spermatheca with sperm, and an annulated tail terminus. It is characterized especially by having 1) vulva average of 80%, 2) low head, 3) strong stylet with prominent rounded knobs, 4) subcylindrical tail and often with bluntly rounded terminus, and 5) male face with four triangular wedge-shaped sectors extending outward from labial disc.

## Relationship

This new species is morphologically close to P. flakkensis Seinhorst 1968; P. similis Khan & Singh, 1974; and P. gibbicaudatus Minagawa, 1982. It differs from P. flakkensis by: lower female head (height 1.7-2.8 vs. 2.8-4.0): rounded stylet knobs vs. knobs with "forward pointing anterior margins" (5); vulva located more posteriorly (mean of 80% vs. 76%); tail subcylindrical, tapering very little and ending in a round to often bluntly rounded terminus having coarse annulation (vs. conical tail tapering to rounded terminus having fine or indistinct annulation). Pratylenchus gutierrezi n. sp. differs from P. similis primarily in having female stylet length mean of 16.8 vs. 12, stylet knobs rounded vs. anteriorly flattened, and postuterine sac length usually one anal body width (ABW) vs. over two ABWs. It differs from P. gibbicaudatus by having vulva at mean of 80% vs. 72.8%, postuterine sac mean 23 vs. 33.5, and mean tail length of 25 vs. 31 with 21 annules vs. 30. Additionally, this new species, though resembling *P. coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941 differs markedly in having an annulated tail terminus (smooth in *P. coffeae*) and different SEM en face view of female than shown for *P. coffeae* by Corbett and Clark (2).

#### Remarks

Examination (LM) of paratype specimens of *P. flakkensis* showed the female head (Fig. 2M,N and our measurements in Table 1) to be higher than in *P. gutierrezi* n. sp. (Fig. 2A–C), and its tail being conical to a rounded terminus (Fig. 20), unlike the tail of *P. gutierrezi* (Fig. 2E–G and as described above). Though difficult to clearly show photographically, the stylet knobs of each of these two species were as described herein. A protruding vulva as noted in this new species (Fig. 2I) was not seen in these paratypes of *P. flakkensis* (Fig. 2P).

Further comments might be made on the SEM face views of *P. gutierrezi* n. sp. The amphidial apertures are not in an exact midlateral position, being shifted dorsally as suggested by Corbett and Clark (2) in several other *Pratylenchus* species. Also, in females the small surface depressions evidently of the four cephalic sensilla may be observed (Fig. 3B). In males the four wedge-shaped sectors apparently correspond to the position of the four cephalic sensilla. In males in two other populations of this nematode collected from areas near the type locality, SEM face views showed the same type of sectors. Thus, these do

not appear to be artifacts, but evidently are characteristic of the male of this species. As far as we could determine, this is the first report of SEM face views of males of a *Pratylenchus* species, although there are many papers with face views of females (2,6, et al.). Although males are rare or unknown in some *Pratylenchus* species, it could be of diagnostic value to include SEM face views of males in future species descriptive or morphological studies.

The specific epithet is named in honor of Professor Gilberto Gutiérrez Z. who has dedicated his distinguished professional career in research to improving coffee production in his native Costa Rica.

#### LITERATURE CITED

- 1. Campos, V. P., P. Sivapalan, and N. C. Gnanapragasam. 1990. Nematode parasites of coffee, cocoa and tea. Pp. 387–430 in M. Luc, R. A. Sikora, and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture. Wallingford, United Kingdom: C. A. B. International.
- 2. Corbett, D. C. M., and S. A. Clark. 1983. Surface features in the taxonomy of *Pratylenchus* species. Revue de Nématologie 6:85–98.
- 3. Golden, A. M. 1990. Preparation and mounting nematodes for microscopic observation. Pp. 197–205 in B. M. Zuckerman, W. F. Mai, and L. R. Krusberg, eds. Plant nematology laboratory manual, rev. ed. Amherst: University of Massachusetts Agricultural Experiment Station.
- 4. López, R., and L. Salazar. 1990. Microscopia electrónica de rastreo de varias poblaciones de *Meloidogyne javanica* (Nemata: Heteroderidae). Agronomía Costrarricense 14:45–54.
- 5. Seinhorst, J. W. 1968. Three new *Pratylenchus* species with a discussion of the structure of the cephalic framework and of the spermatheca in this genus. Nematologica 14:497–510.
- 6. Zeidan, A. B., and E. Geraert. 1991. *Pratylenchus* from Sudan, with the description of two new species (Nemata: Tylenchida). Revue de Nématologie 14:221-229.