New Records of *Paratylenchus* Micoletzky, 1922 from Brazil with Descriptions of Two New Species (Tylenchulidae: Nemata)¹

CHAW SHUNG HUANG² AND DEWEY J. RASKI³

Abstract: Six species of Paratylenchus Micoletzky, 1922, two of them new, are reported from the Amazon Basin of Brazil. P. colbrani is reported for the first time in Brazil. Extended ranges, along with additional measurements and descriptions, are given for P. leptus, P. salubris, and P. perlatus. P. mimulus Raski, 1975 is synonymized with P. salubris Raski, 1975. P. flectospiculus n. sp. is distinguished by four lines in the lateral field, short stylet (21-24 μ m), and spicules with distinct distal bend ventrad. P. rostrocaudatus n. sp. is distinguished by three lines in the lateral field, short stylet (15-16 μ m), and beaked tail terminus.

Key words: Amazon Basin, Brazilian Savanna, Paratylenchus flectospiculus n. sp., P. rostrocaudatus n. sp., P. colbrani, P. leptus, P. perlatus, P. salubris, P. mimulus, pin nematodes, synonymization, taxonomy.

Over the past 10 years more than 3,000 soil samples were collected from many parts of Brazil by the plant pathology group at the University of Brasilia. About 80% of the samples were taken from the vast Amazon Basin in the north and "cerrado" (savanna grassland) in the central-west and northeastern regions. Some soils were taken from agricultural lands, but the majority came from natural vegetations. *Paratylenchus* spp. including two new species identified from the samples are reported here.

MATERIALS AND METHODS

Soil samples generally were taken from the rhizosphere of the top 25 cm profile, including some root system. Nematodes were extracted by flotation-sedimentation-sieving procedures (2), normally within 72 hours after collection. Nematodes thus obtained were killed in gentle heat (60 C for 1 minute) and subsequently fixed in a glycerin-formalin-water (2-8-90) mixture. When necessary, murky mixtures were clarified by sucrose centrifugal flotation (1). In these cases, nematodes were placed back in the glycerin-formalin-water mixture soon after the sucrose was removed.

Light microscopical (LM) and morphometric studies were carried out with glycerin toto mounts prepared according to the rapid dehydration and glycerin impregnation scheme of Seinhorst (2). Nematodes in the glycerin toto mounts were used for scanning electron microscopical (SEM) studies according to the procedures of Huang and Raski (3).

Systematics

Paratylenchus colbrani Raski, 1975

A single female of this species was collected by Gilmar P. Henz from native savanna mixed vegetation, approximately 200 m east of the Colina apartment area on the campus of the University of Brasilia. The nematode measured as follows: L = $320 \ \mu\text{m}$; stylet = 16 $\ \mu\text{m}$; cone = 10 $\ \mu\text{m}$; esophagus = 72 $\ \mu\text{m}$; excretory pore = 59 $\ \mu\text{m}$ (from lip); V = 79%; tail = 24 $\ \mu\text{m}$; a = 21.3; b = 4.4; c = 13.3; c' = 3.1; lateral field = four lines.

The female was compared to paratype of *P. colbrani* Raski, 1975, University of California Nematode Collection, Davis (UCNC) slide No. 10a, from which it was indistinguishable.

This constitutes the first record of *P. colbrani* in Brazil.

Received for publication 4 March 1986.

¹ Most of this research work was performed at the University of California, Davis, where the senior author spent sabbatical leave during 1985.

² Departamento de Biologia Vegetal, Universidade de Brasilia, Brasilia, DF 70910, Brazil.

⁹ Division of Nematology, University of California, Davis, CA 95616.

We thank CNPq and FINEP of the Government of Brazil for financial support in the field work. We also thank Mrs. Sumei Y. Huang for help in specimen collection and preparation and statistical analyses of the data.

Paratylenchus leptus Raski, 1975

Specimens of this species were collected by C. S. Huang and J. E. Cares in 1981 from rhizospheres of banana, coconut palm (Cocos nucifera), and Japanese turnip (Brassica rapa) on the Solimões River bank of Xiborena Island, approximately 40 km southeast of the city of Manaus, state of Amazonas. Their measurements are as follows (14 females): $L = 188 \pm 17 (162 - 215)$ μ m; stylet = 20 ± 1.5 (18–24) μ m; V = $81.3 \pm 0.9 \ (79.2 - 82.5)\%$; esophagus = 59 ± 2 (51–63) μ m; excretory pore (from lip) = $50 \pm 4.6 (38-58) \mu m$; a = 21.9 ± 2.5 $(18.8-26.8); b = 3.2 \pm 0.3 (2.8-3.8); c =$ $17.8 \pm 4.4 (10.3 - 22.3)$; lateral field = three lines. Specimens of the Amazonian population are smaller with slightly shorter stylets than the type specimens. The differences are judged intraspecific, however, and prevailing morphological similarities identified them with P. leptus Raski, 1975. This species was first reported from soil about roots of Piper sp. near Nova Teutonia, Brazil. This report extends its range to the Amazon Basin.

Paratylenchus salubris Raski, 1975 (Fig. 1)

Three females of P. salubris Raski, 1975 were found in samples of Miconia sp. collected from Tropical Fruit and Forest Tree Experiment Station of INPA (Instituto Nacional de Pesquisas da Amazonia), approximately 45 km north of Manaus along Highway BR 174, and in rhizosphere soils of Paspalum fasciculatum from Xiborena Island, 40 km southeast of Manaus. Their measurements are $L = 234 \pm 5.8$ (231– 241) μ m; stylet = 34 \pm 3.8 (30–37) μ m; cone = $27 \pm 4.5 (23-32) \mu m$; esophagus = $71 \pm 5.5 (65 - 76) \,\mu\text{m}$; excretory pore (from lip) = $58 \pm 2.6 (55-60) \,\mu\text{m}$; a = 18.0 ± 7.7 $(12.8-26.8); b = 3.3 \pm 0.2 (3.0-3.4); V =$ 78.4 ± 1.8 (76.6-80.1)%; lateral field = four lines.

An additional 16 females were collected from rhizospheres of palm trees and unidentified Graminae near Planalto Military Command, about 76 km northeast of Brasilia along Highway Brasilia/Fortaleza, and from savanna grassland vegetations in the "PADDF" farming area, about 65 km northeast of Brasilia. Measurements of the nematodes are L = $245 \pm 25.6 (208-300)$ μ m; stylet = $36 \pm 2.2 (32-39) \mu$ m; cone = $30 \pm 3.7 (25-32) \mu$ m; esophagus = $72 \pm 3.6 (65-80) \mu$ m; excretory pore (from lip) = $58 \pm 9.2 (33-78) \mu$ m; a = $22.4 \pm 2.7 (17.6-26.4)$; b = $3.5 \pm 0.3 (2.9-4.2)$; c = $16.9 \pm 2.3 (14.8-20.0)$; V = $80.4 \pm 0.9 (78.8-82.2)$ %; lateral field = four lines.

These measurements, as well as morphology of both populations, fit well with those given for P. salubris except for the subacute tails.

Females of P. salubris were said to have bluntly rounded tail terminals, although those of the males may be acute or finely rounded (4). The blunt female tail and longer stylet are the only diagnostic characters separating P. salubris from P. mimulus (4), both found in the northeastern cacao-growing region of Brazil. Examination of the paratypes of both species (University of California Davis Nematode Collection slides no. 13e, 13k, 13n, 13u and 34k) revealed, however, that tails of both sexes of P. salubris varied from acute to subacute to bluntly rounded (Fig. 1A-C, G-I), and thus are indistinguishable from those of P. mimulus (Fig. 1D-F, J-L). Stylet length thus became the only diagnostic character separating the two species. Both the Amazonian and savanna populations possess the stylet length equivalent to that of *P. salubris* and extend its limit to $39 \,\mu\text{m}$. However, the overlapping of ranges of stylet length of P. mimulus and P. salubris raise doubts as to their separate specificities. It is concluded that their relationship can best be reflected in a single specific taxon with quite variable characteristics of tail shape and stylet length. P. salubris has page priority, and P. mimulus is hereby proposed as a synonym of P. salubris Raski, 1975, syn. P. mimulus Raski, 1975 n. syn.

Paratylenchus perlatus Raski, 1975 (Fig. 2)

Seven females were collected by Cezar Sperandio, Osmar Crestani, and Claudio



FIG. 1. A-C) Female tails of Paratylenchus salubris Raski, 1975 paratypes. D-F) Female tails of Paratylenchus mimulus Raski, 1975 paratypes. G-I) Male tails of Paratylenchus salubris paratypes. J-L) Male tails of Paratylenchus mimulus paratypes. M, N) Female anterior and posterior regions of Paratylenchus salubris 1991/2 Amazon population. Bar = 10 μ m.

Bittencourt from savanna vegetation, approximately 150 m northeast of the Colina apartment area on the campus of the University of Brasilia. Their measurements are

L = 206 ± 7.4 (194–215) μ m; a = 21.2 ± 3.8 (15.0–26.6); b = 3.6 ± 0.2 (3.4–3.7); c = 13.7 ± 1.8 (11.3–16.5); c' = 3.5 ± 1.1 (2.4–5.0); stylet = 20 ± 0.8 (19–21) μ m;



FIG. 2. SEM photographs of *Paratylenchus perlatus* Raski, 1975. A) En face view of head region. B) Lateral field, midbody. C) Tail, subventral view. D) Vulva with lateral vulval flaps. Bar represents 2 μ m in A, B; 5 μ m in C, D.

stylet cone = $15 \pm 1.1 (14-17) \mu$ m; esophagus = $58 \pm 1.9 (54-60) \mu$ m; excretory pore = $48 \pm 1.5 (45-50) \mu$ m; V = $80.7 \pm 1.6 (78.0-82.8)\%$; gonad = $84 \pm 29.4 (58-122) \mu$ m.

Measurements and morphology of females fit descriptions of *P. perlatus* Raski, 1957. The only two populations previously known for the species were associated with cacao plantations in the state of Bahia where hot and humid climate prevails and the soils are rich in organic matter. By contrast, the population reported here is associated with savanna vegetation, where the climate is mild and dry with acid lato soil very poor in organic matter. These ecological data indicate that the species is adaptable to a wide range of environmental conditions.

SEM studies (Fig. 2) of en face view of labial plate revealed four large submedian lobes extending subdorsally and subventrally but not wider than the first body annulus. Lateral lobes appeared reduced, flanked by a rectangular amphid aperture on each side. Oral opening I-shaped with four rounded papilla-like structures. Lateral field with four lines setting off three bands, center band slightly narrower than outer two. Lateral vulval flaps prominently



FIG. 3. Paratylenchus flectospiculus n. sp. A) Female anterior region. B) Male anterior region. C) Female posterior region. D) Female midbody, transverse section. E) Male tail. Bar = $10 \mu m$.

elevated, covering 3–4 body annuli. Body annuli extend to subacute tail terminus. No phasmids detected with SEM.

Paratylenchus flectospiculus n. sp. (Fig. 3)

Measurements

Holotype (female): L = 212 μ m; a = 16.5; b = 3.5; c = 13.7; c' = 2.3; stylet = 23 μ m; cone = 17 μ m; excretory pore = 53 μ m; esophagus = 61 μ m; V = 83.7%; gonad = 65 μ m. Paratypes (females, n = 7): L = 209 ± 15.2 (190-237) μ m; a = 17.7 ± 1.9 (14.8– 19.7); b = 3.7 ± 0.4 (3.2–4.5); c = 16.8 ± 4.9 (12.2–24.4); c' = 2.0 ± 0.4 (1.4–2.5); stylet = 23 ± 0.9 (21–24) μ m; cone = 17 ± 1.6 (15–20) μ m; excretory pore = 54 ± 3.2 (49–60) μ m; esophagus = 58 ± 3.2 (53– 61) μ m; V = 83.8 ± 1.6 (81.0–85.7)%; gonad = 78 ± 10 (64.8–89.0) μ m.

Paratypes (males, n = 3): L = 219 ± 28 (187-242) μ m; a = 36.2 ± 2.3 (34.6-37.8); b = 6.4 ± 0.1 (6.4-6.5); c = 18.8 ± 1.9 (17.5–20.2); excretory pore = 48 μ m; spicules 13 ± 0.6 (13–14) μ m.

Description

Females: Bodies assume fish-hook or closed "C" form after killing by gentle heat. Gravid females gradually broaden to anterior vulval lip, abruptly narrow posteriad to vulva. Head not offset, tapers anteriad, forming smooth rounded contour. Lip about one-half of neck width, annuli indistinct in LM. Submedian lobes minute but distinctly protruded. Lateral lobes slightly lower, resulting in an oral depression. Body annuli distinct, about 1 μ m wide, those in vulval region slightly wider. Cephalic framework moderately developed. Stylet well developed, cone approximately three times as long as shaft and knobs combined. Dorso-esophageal gland orifice 1-2 µm behind stylet knobs. Metacorpus of esophagus highly developed, occupying about 70% of body width, valvular apparatus crescentic, well developed. Posterior esophageal bulb pear shaped. Cardia hemispherical. Nerve ring envelopes isthmus midway. Excretory pore posterior to nerve ring. Hemizonid immediately anterior to excretory pore, about width of two body annuli. Deirid not seen. Lateral field with four lines setting off three bands visible in cross-sections, reducing to one irregular band on tail clearly visible by SEM up to third-to-last annulus. Two inner lines of lateral field may be faint. Phasmid not detected. Ovary outstretched, oogonia in single row. Spermatheca spherical or roughly so, offset, filled with globular sperms. Lateral vulval flaps pronounced, elevated, covering part of four body annuli. Tail tapers gradually, conoid, slightly curved ventrally with a subacute terminus, sometimes slightly digitate. Body annuli continued to tail terminus.

Males: Body assumes fish-hook or closed "C" form after killing in gentle heat. More slender than females. Lip about one-half width of neck. Submedian lobes barely visible. Cephalic framework very weak. Stylet and esophagus degenerated. Metacorpus and part of esophageal lumen vaguely visible. Body annuli distinct, approximately 1 μ m wide. Lateral field narrow with four lines. Tail essentially straight. Cloacal opening slightly protruded, forming a short sheath. Spicules long, protruding from body at 110–130 degrees, essentially straight except terminal one-fifth is bent ventrally at about 140 degrees. Gubernaculum small, straight. Caudal alae not observed. Tail sharply conoid, similar to that of females.

Holotype: Female, collected by Albino B. Neto in May 1984, slide no. 3034 EEB, University of Brasilia Nematode Collection.

Paratypes: Seven females and two males, distributed as follows: two males in the University of Brasilia Nematode Collection; seven females in the UCNC, Davis.

Type locality and habitat: Mixed savanna vegetation in the Experiment Station of Biology, University of Brasilia, ASA Norte, Brasilia, DF, approximately 8 km north of the campus.

Diagnosis and relationships: Among the Paratylenchus spp. with four lines in the lateral field, P. flectospiculus n. sp. is most closely related to P. salubris Raski, 1975 by virtue of body size and general body form, slightly rounded head outline, and submedian lobes only slightly protruded. It differs from P. salubris Raski, 1975 by shorter female stylet (23 μ m vs. 28 μ m or more) and spicules with distinct distal bend ventrad.

Paratylenchus rostrocaudatus n. sp. (Fig. 4)

Measurements

Holotype BR60 (female): L = 218 μ m; a = 23.4; b = 3.5; c = 12.8; c' = 3.2; stylet = 15 μ m; cone = 10 μ m; esophagus = 63 μ m; excretory pore = 50 μ m; V = 78.0%.

Paratypes BR60 (females, n = 5): L = 244 ± 20 (218-266) μ m; a = 23.3 ± 0.54 (22.8-24.2); b = 3.8 ± 0.23 (3.46-4.10); c = 13.5 ± 1.6 (11.6-15.8); c' = 2.9 ± 0.3 (2.7-3.2); stylet = 15 ± 1 (15-16) μ m; cone = 11 ± 1 (10-12) μ m; esophagus = 64 ± 4 (59-68) μ m; excretory pore =



FIG. 4. Paratylenchus rostrocaudatus n. sp. female. A) Anterior region. B) Full length. C) Posterior region. D-F) Tail termini. G) Midbody, transverse section. Bar represents 10 μ m in A, C-G; 50 μ m in B.

 $50 \pm 6 (41-55) \mu m$; V = 79.9 $\pm 1.6 (78.0-82.3)\%$.

Other collection BR40 (females, n = 8): L = 239 ± 17 (220-266) μ m; a = 22.1 ± 1.8 (20.0-25.5); b = 3.7 ± 0.23 (3.3-4.0); c = 13.6 ± 1.15 (12.3-14.9); c' = 2.9 ± 0.32 (2.5-3.4); stylet = 15 ± 1 (14-16) μ m; cone = 11 ± 1 (9-13) μ m; esophagus = $65 \pm 3 (60-69) \ \mu m$; excretory pore = 51 ± 6 (40-57) μm ; V = 79.6 ± 0.9 (78.3-80.8)%.

Description

Females: Bodies assume closed to open "C" after fixation. Head elevated, offset, truncate, anterior margin only slightly nar-

rower than base of neck, almost square in lateral view. Submedian labial lobes higher than lateral lobes, resulting in an oral depression in lateral view. Cephalic framework heavily sclerotized, elevated, width approximately 80% of neck width. Lip annuli indistinct. Body annuli less than 1 μ m wide. Stylet robust, base of cone wider than shaft. Stylet basal knobs large, anterior surface directed slightly posteriad. Dorsoesophageal gland orifice $1-2 \mu m$ behind stylet knobs. Nerve ring circumscribes long isthmus. Hemizonid between posterior isthmus and anterior part of posterior esophageal glands, two body annuli wide. Excretory pore immediately anteriad, rarely posteriad, to hemizonid. Excretory canal moderately sclerotized. Deirids not observed. Metacorpus expanded, occupying about 65% of body width, equipped with large valvular apparatus. Posterior esophageal glands in lemon-shaped bulb, cardia hemispherical. Lateral field with three incisures setting off two elevated bands. Ovary outstretched. Spermatheca oval to spherical, offset, with sparse globular sperms. Anterior vulval lip markedly higher than the posterior. Lateral vulval membrane prominent. Body significantly narrowed, curved ventrad behind vulva. Tail gradually narrows toward terminus where body annuli abruptly become invisible behind a deep constriction, resulting in a beak-shaped terminus, approximately $3 \ \mu m$ in length. Beaked tail terminus hyaline. Phasmids not seen.

Males not found.

Juveniles: Similar to females except smaller size and with obscure lateral fields.

Holotype: Female, collected by Olinda M. Martins in April 1984, slide no. 2885, University of Brasilia Nematode Collection.

Paratypes: Eleven females and six juveniles, distributed as follows: seven females and one juvenile in the UCNC, Davis; four females and two juveniles in USDA Nematode Collection, Beltsville; and three juveniles in the University of Brasilia Nematode Collection. Type localities and habitat: Holotype and some paratypes, collected by Maria do Carmo, associated with dry land rice in Fazenda Sossego, about 90 km from the University of Brasilia campus along Highway BR60 in the direction of Goiania, GO. The population is designated BR60.

The other collection was made by Sueli Gomes and Paulo Ernesto in April 1984 from a gramineous plant (common name "cabelo de porco") beside Highway BR40, 2.3 km from "JK" gasoline station, Cristalina, GO, designated herein as population BR40.

The two populations are about 100 km from each other.

Another population was collected by J. E. Cares in 1981 from unidentified Graminae in the savanna, about 500 m northeast of Colina apartment area, University of Brasilia.

Diagnosis and relationships: Paratylenchus rostrocaudatus n. sp. is most closely related to *P. leptus* Raski, 1975 and to *P. humilis* Raski, 1975 by its three-line lateral field, small size, and relatively short stylet. It differs from both by the elevated and almost rectangular head region, shorter stylet (15– 16 μ m vs. more than 18 μ m), and beaked tail terminus. It further differs from *P. humilis* by its larger size of female (218–266 vs. 170–190 μ m for *P. humilis*).

LITERATURE CITED

1. Harrison, Judith M., and C. D. Green. 1976. Comparison of centrifugal and other methods for standardization of extraction of nematodes from soil. Annals of Applied Biology 82:299–308.

2. Hooper, D. J. 1970. Handling, fixing, staining and mounting nematodes. Pp. 39-54 in J. F. Southey, ed. Laboratory methods for work with plant soil nematodes. Technical Bulletin #2, Ministry of Agriculture, Fishery & Food. London: HMSO.

3. Huang, C. S., and D. J. Raski. 1986. Some Tylenchidae found in Brazil with description of *Cucullitylenchus amazonensis* gen. n., sp. n. (Tylenchoidea: Nemata). Revue de Nématologie 9:209-219.

4. Raski, D. J. 1975. Revision of the genus *Para-tylenchus* Micoletzky, 1922 and description of new species. Part 2 of three parts. Journal of Nematology 7:274–295.