Trophotylenchulus piperis n. sp. parasitic on *Piper nigrum* L. in Kerala, India (Nemata : Tylenchulidae) (1)

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SUMMARY

Trophotylenchulus piperis n. sp. parasitising roots of pepper plant, *Piper nigrum* L., from Kerala, India is described. This species is most closely related to *T. floridensis* Raski, 1957 differing by smaller size of females, males and juveniles, and by juveniles having a larger depressed area in the circumoral plate and an indented, digitate tail. Dark brown cases encircling the female, juveniles and eggs, were collected from the soil for the first time.

RÉSUMÉ

Trophotylenchulus piperis *n. sp. parasite de* Piper nigrum *L. au Kerala, Inde (Nemata : Tylenchulidae)*

Trophotylenchulus piperis n. sp., parasite des racines du poivrier, *Piper nigrum* L., au Kerala, Inde, est décrit. Cette espèce, très proche de *T. floridensis* Raski, 1957, en diffère par la plus petite taille des femelles, des mâles et des juvéniles, et chez les juvéniles par la présence d'une large zone déprimée sur la plaque circumorale et par la forme de la queue digitée et indentée. Des capsules brun-noir entourant femelles, juvéniles et œufs ont été, pour la première fois, récoltées dans le sol.

In the process of surveying for nematodes in Kerala, India specimens of *Trophotylenchulus* Raski, 1957 were found widely distributed in Calicut District on pepper plant, *Piper nigrum* L. In a preliminary report by Mohandas and Ramana (1982) these were identified as *Trophotylenchulus floridensis* Raski, 1957 but more detailed studies since then have revealed morphological differences which are judged significant setting this off as a new species separate and distinct from *T. floridensis*. A description of the new species follows.

Materials and methods

Specimens wet sieved from the soil were killed in hot water, preserved and stored in 2.5 % formalin, and later transferred to F.A.A. and dehydrated to glycerin following Cobb's slow method.

Specimens for scanning electron microscopy were transferred from F.A.A. into a graded series of ethanol (E.T.O.H.) to absolute E.T.O.H., then taken through a graded series of amyl acetate-absolute E.T.O.H. to absolute amyl acetate; a 15-60 second sonication was applied in absolute amyl acetate. After critical point drying with CO_2, specimens were mounted on stubs and coated with a 200 Å gold layer. Examination and photography was done on an I.S.I. model 35-130 DS scanning electron microscope at 10 KV.

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Trophotylenchulus piperis n. sp.

= *T. floridensis apud* Mohandas & Ramana, 1982
(Fig. 1, 2, 3)

DIMENSIONS

Females (n = 7) : L = 0.293 mm (0.254-0.325); a = 6.0 (4.5-7.9); b = 2.8 (2.5-3.1); c = 13 (12-15); c' = 2.6 (2.2-4.6); V = 76 (72-80); spear = 13-14 µm; cone = 7 µm; oesophagus = 107 µm (102-115); tail = 24 µm (18-32).

Males (n = 4) : L = 0.349-0.392 mm; a = 26-44; b = 3.5-4.6; c = 4.0-8.4; c' = 4.0-6.4; stylet = 7-11 µm; oesophagus = 92-105 µm; tail = 47-99 µm; spicules = 13-16 µm; gubernaculum = 3-4 µm; T = 36-47 %.

Second-stage juveniles (n = 18) : L = 0.287 mm (0.260-0.329 S.D. = 0.020); a = 26 (21-32 S.D. = 2.9); b = 2.9 (2.3-3.1); c = 8.8 (6.4-13.5 S.D. = 2.1); c' =

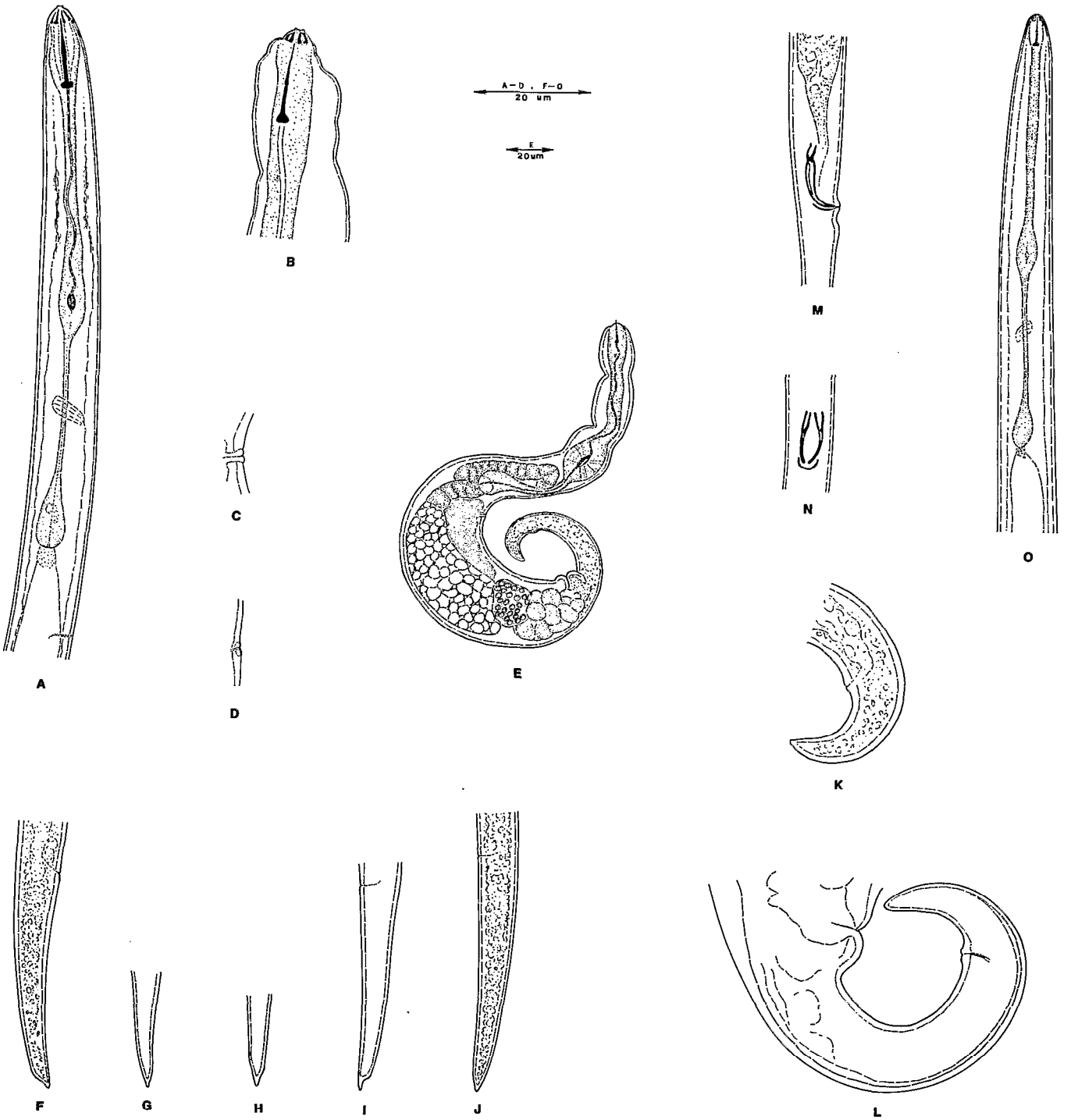

Fig. 1. *Trophotylenchulus piperis* n. sp. A-E, K-L. *Female.* A : oesophageal region; B : anterior end; C-D : two different aspects of excretory pore; E : full length; K-L : tail ends; F-J. *Second stage juveniles,* various tail shapes; M-O. *Male.* M : spicule region, lateral view; N : spicules and gubernaculum, ventral view; O : oesophageal region.

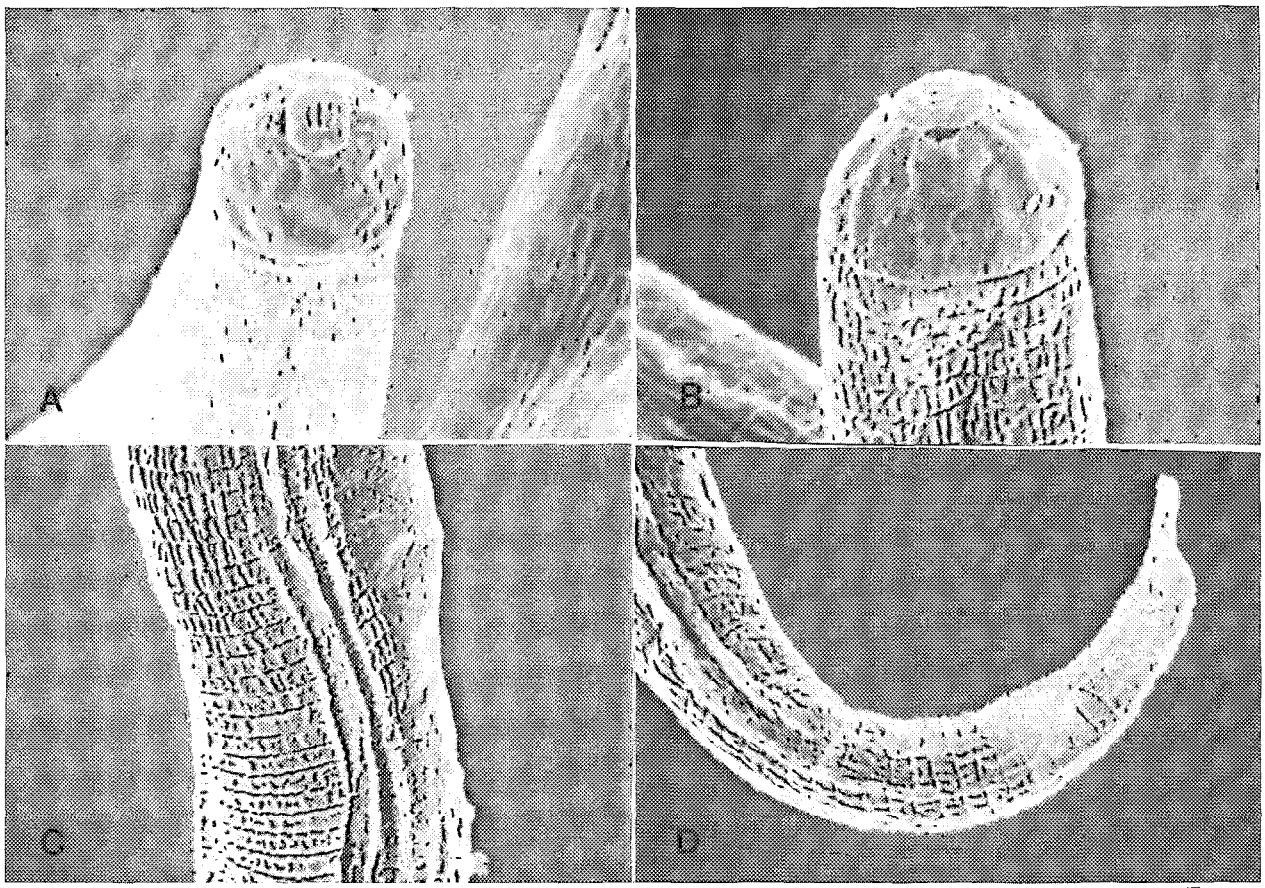

Fig. 2. *Trophotylenchulus piperis* n. sp., second-stage juveniles. Scanning electron microscope photographs. A, B : anterior end (× 6,825 and × 8,625, respectively); C : transverse annules and longitudinal bands of lateral field about midbody (× 5,290); D : tail (× 5,280).

4.5-5.7; stylet = 12.2 µm (12-13 S.D. = 0.6); dorsal gland orifice = 4-5 µm from knobs; excretory pore = 108-119 µm; oesophagus = 106 µm (93-128 S.D. = 11.5); tail = 36 µm (22-46 S.D. = 7.8); developing gonad = 6-13 µm long, about 5 µm wide.

Holotype : (Female) : L = 0.276 mm; a = 7.9; b = 2.7; c = 13.4; c' = 2.9; V = 75; stylet = ? oesophagus = 102 µm; excretory pore = 110 µm (40 %); tail = 21 µm.

Description

Female : Body spirally coiled almost twice around, markedly swollen in midportion. Swelling starts anterior to median bulb; often with two (occasionally three) constrictions anterior to median bulb (only this portion is embedded in root). Lip region distinctly set off; rounded, dome-shaped, smooth without transverse striae; about 5 µm across at base. Circumoral elevations distinct as fine, rounded protrusions on each side projecting beyond margin of lip region outline. Cephalic sclerotization weak but visible as rounded finger-like arches. Stylet delicate, difficult to follow but has rounded knobs, backwardly directed; cone about equal in length to base plus knobs. Dorsal gland opening about 9 µm from base of knobs. Lumen of oesophagus exceptionally wide. Procorpus about 5 µm across; more or less cylindrical sometimes widening gradually to median bulb which is very large (range from 12-19 µm wide × 24-35 µm long), muscular with valve about 7-10 µm long; isthmus slender then gradually widens into pyriform posterior bulb (ranges from 10-15 µm wide × 17-23 µm long). Presence or absence of oesophageal-intestinal valve not definitely established. Excretory pore slightly posterior to end of œsophagus 110-134 µm from anterior end (35-42 % of L), with well-sclerotized canal perpendicular to body wall or turns posteriad, leading to large gland located mostly ventrad to gonad immediately under excretory pore.

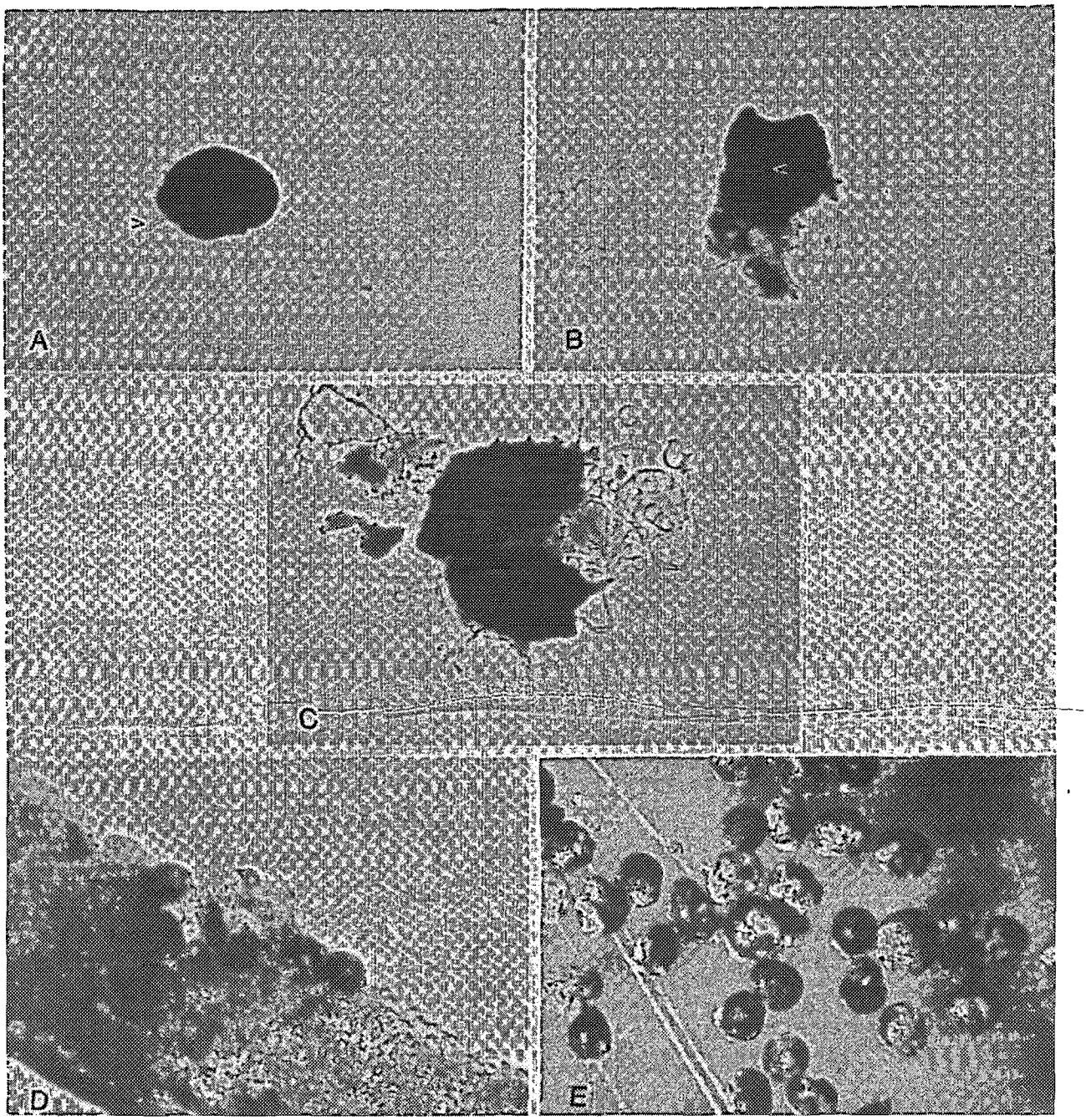

Fig. 3. *Trophotylenchulus piperis* n. sp. cases. A : arrow points to protruding female head; B : arrow points to aperture for female head; C : female, juveniles and eggs from broken case; D : cluster of cases on pepper plant roots; E : cases recovered from soil sievings.

Pore has slightly rounded margins. Gonad single, prodelphic usually extends to median bulb then by flexure turns posteriad 39-77 µm flexure again turns anteriad 33-64 µm; on one specimen a short double flexure was present near posterior bulb of oesophagus then extended to median bulb. Egg in one female measured 30 × 61 µm. Spermatheca large rounded, apparently in-line (not off-set as a pouch) with large sperms (averaging

from less than 2 to 2.5 µm). Uterus quadricolumellar; by surrounding the vagina the thick wall almost perpendicular to longitudinal axis forms a slight projection posteriad, not judged a true post-uterine sac. Vulval projections prominent rounded lips. Tail narrows abruptly at vulva then gradually to blunt rounded terminus. Anus on prominently rounded projection, rectum and internal structures not clearly visible. Terminus of some females with slight indent, usually on dorsal margin resulting in somewhat digitate shape. Body annules usually obscure, on some specimens visible on tail and at posterior portion of oesophageal region; about 2 µm wide at midbody. Maximum width at midbody 35-58 µm.

Male : Body slender, almost straight; tapers slightly anteriad, more so posteriad. Lip region continuous with body; dome-shaped as in female; smooth, without transverse striae. Circumoral elevation not clearly visible, if present too small to detect by light microscopy. Stylet very delicate, difficult to perceive. Oesophagus degenerate; median bulb slender, spindle-shaped; no valvular apparatus distinguishable; isthmus very slender, small pyriform posterior bulb. Oesophageal-intestinal valve not definitely visible. Gonad single, outstretched. Spicules well-developed, arcuate ventrally. Gubernaculum simple, rod-shaped in lateral view, U-shaped when seen dorso-ventrally. No evidence of caudal alae. Cloacal opening on slightly rounded projection. Tail ends with an acutely pointed tip sometimes projecting out slightly digitate in outline.

Second-stage juvenile : Body more or less straight when fixed, slender, tapering anteriorly, more so posteriorly. Annules average 0.8-0.9 µm wide at midbody. Lip region continuous with body, not set-off; smooth without annulation. Circumoral elevation a small distinct projection; seen by S.E.M. it is an ovoid-rounded oral plate with an inner depression; oral aperture a simple slit. Amphid apertures short, ovoid at lateral margin of oral plate. Cephalic sclerotization delicate, arched finger-like as in female. Stylet well developed, cone slightly longer (6-7 µm) than shaft plus knobs (about 5 µm). Dorsal gland orifice 4-5 µm from knobs. Procorpus cylindrical, long; median bulb forms by gradual enlargement of procorpus, more or less spindle-shaped (about 6 × 14 µm) with prominent valvular apparatus about 3 µm long. Isthmus very long, slender; posterior bulb pyriform. Oesophageal-intestinal junction appears to have bilobed valve. Excretory pore about one body width posterior to oesophagus; canal almost perpendicular to body wall then curves posteriad. Anus and rectum obscure, barely visible by light microscopy. Tail tapers gently to rounded terminus variously narrowed to a distinct projection forming a somewhat digitate outline. Genital primordium four-celled, 64-66 % from anterior end. Seen by S.E.M., transverse striae form distinct annules from smooth cephalic region almost to terminus. Lateral field obscure by light microscopy, by S.E.M. appears as two bands separated by a space resulting in four lines.

Type host and locality

Roots and soil from pepper plant, *Piper nigrum* L., in Calicut District, Kerala, India.

Type specimens

Holotype : Female, collected 20 November 1980 by K. V. Ramana, Catalogue Slide Number 2076, University of California Nematode Collection (U.C.N.C.), Davis, California.

Paratypes : 30 females, five males, 109 juveniles, same data as holotype, deposited as follows : 22 females, one male, 93 juveniles U.C.N.C., Davis, California; two females, one male, four juveniles each to United States Department of Agriculture Nematode Collection, Beltsville, Maryland; Nematode Department, Rothamsted Experimental Station, Harpenden, England; Agricultural University, Wageningen, The Netherlands; National Nematode Collection, Indian Agricultural Research Institute, New Delhi, India.

Diagnosis

Trophotylenchulus piperis n. sp. is most closely related to *T. floridensis* differing by the smaller size of female (0.29 mm *vs* 0.44), juvenile (0.26-0.33 mm *vs* 0.41-0.48) and male (0.35-0.39 mm *vs* 0.41-0.58). Morphology of anterior end of second-stage juveniles is similar to *T. floridensis* and to others of *Trophotylenchulus.* The (1983) but appears to differ by its larger depressed area in the circumoral plate. The juvenile tail also differs by the indented, digitate outline of *T. piperis* n. sp. *vs* the bluntly rounded tail of *T. floridensis.*

Comments

The new species conforms closely in life habits to *T. floridensis* and to others *of Trophotylenchulus.* The females are found singly in rounded, brittle cases completely enveloping the nematode except for a small opening closely surrounding the anterior end of the female which protrudes penetrating the host root. The case appears dark brown to black on the root but when opened and seen by transmitted light it is reddish in color. Unfortunately the female head is usually broken off in trying to extract them from the roots so entire specimens are rare. The dark cases persist in the soil, accumulating in high numbers and are readily collected in considerable numbers much like cysts of *Heterodera* spp. Juveniles also are found numerous in the soil and rarely males. In the cases enclosing females, males are rare, but juveniles range in numbers from seven to

fourteen and eggs nine to 37 found together with the adult female in individual cases.

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