

National Nematological Research Centre,
University of Karachi,
Karachi - 32, Pakistan

FOUR NEW SPECIES OF THE FAMILY HOPLLOLAIMIDAE:
(NEMATODA) WITH NOTES
ON *ROTYLENCHUS CYPRIENSIS* ANTONIOU FROM PAKISTAN

by
M. A. MAQBOOL and F. SHAHINA

During a survey of plant parasitic nematodes, several undescribed specimens belonging to the genera *Rotylenchus* Filipjev, 1936 and *Helicotylenchus* Steiner, 1945, were collected from different localities of Muzaffarabad (Azad Kashmir) and Sind in Pakistan. *Rotylenchus fragaricus* sp. n., *R. pakistanensis* sp. n., *R. alii* sp. n. and *Helicotylenchus obliquus* sp. n. are described and illustrated herein. Morphometric data of *Rotylenchus cypriensis* Antoniou, 1980, apparently a new report in Pakistan is also presented.

Soil samples were collected from various fruit orchards and vegetable fields. Nematodes were killed by gentle heat, fixed in T.A.F. and mounted in glycerine. Measurements were made by means of a disc micrometer and illustrations with the aid of a drawing tube.

ROTYLENCHUS FRAGARICUS SP. N. (Fig. 1)

Paratype females (11): L=0.79-1.00 (0.90) mm; a=24-31 (28); b=5.9-7.0 (6.4); b'=4.6-5.4 (4.9); c=74-104 (88); c'=0.5-0.6 (0.5); V=60-64 (62); O=17-22.5 (20.6); m=46-50 (48); stylet=32-34 (33) μ m.

Holotype female: L=0.97 mm; a=24; b=6.4; b'=5.3; c=88; c'=0.55; V=63; O=21.1; m=47; stylet=34 μ m.

Description

Body cylindrical, slightly tapering anteriorly, generally forms a spiral shape when relaxed by gentle heat. Cuticle with coarse annulation, 1.5-2.0 μ m wide at mid body. Lateral fields with four smooth incisures,

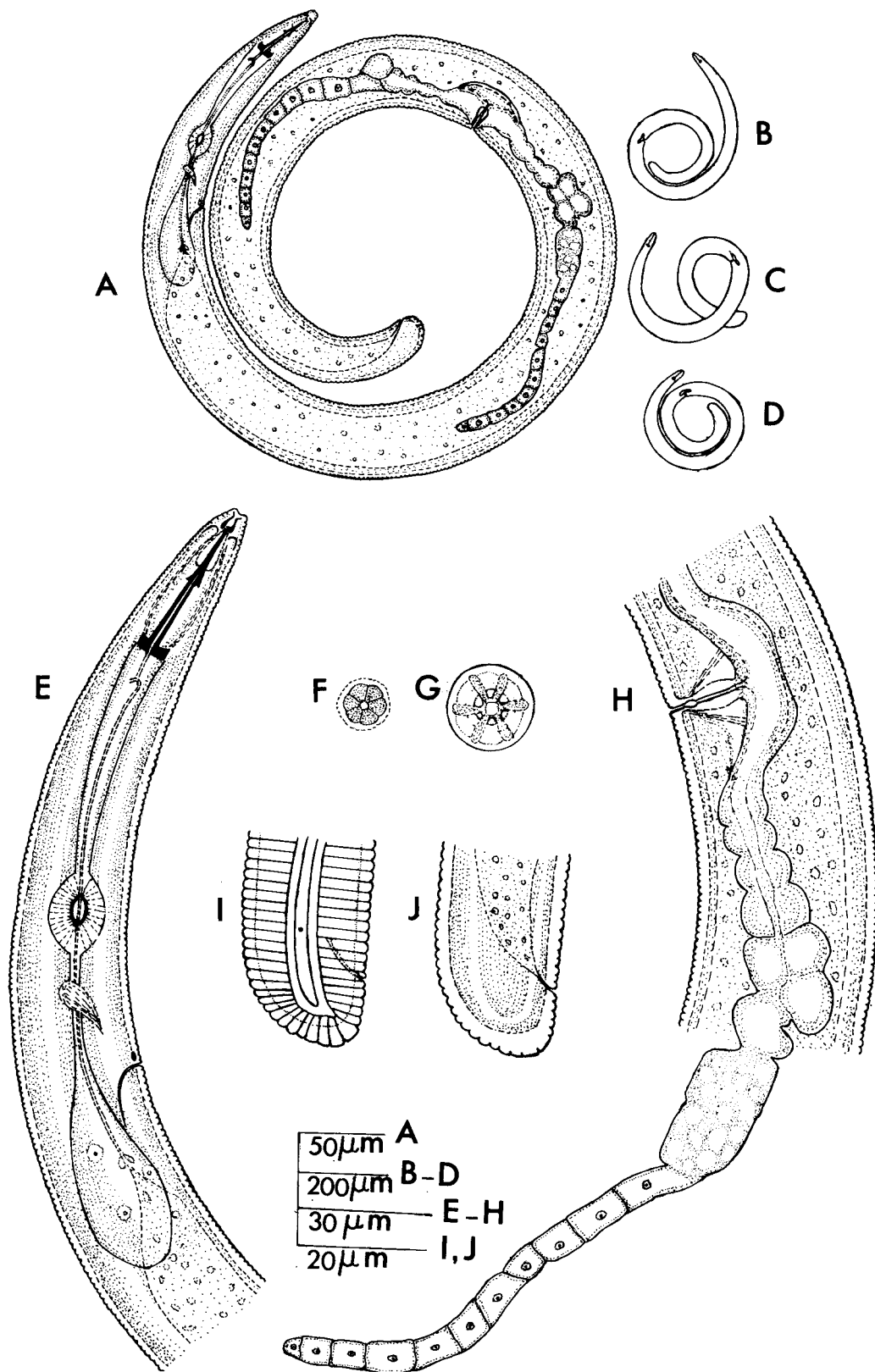


Fig. 1 - *Rotylenchus fragaricus* sp. n., A-D entire female; E, oesophageal region; F, female face view; G, female cross section through basal annule of lip region; H, vulva region and posterior gonad; I, J, tails.

not areolated except in oesophageal region. Head truncate, 9-10 μm wide at base and 6-7 μm high. Lip region slightly narrower than body width, not offset, marked by 4-5 annules. Stylet massive, basal knobs rounded posteriorly with indented anterior surfaces, 6-7 μm across. Outlet of dorsal oesophageal gland about 1/4 - 1/5 of stylet length or 6-8 μm behind stylet base. Oesophagus 163-195 μm long. Procorpus cylindrical, narrower at its junction with median bulb. Median bulb strongly developed oval, 12-13.6 μm \times 16-18 μm with a large refractive cuticular thickening at centre. Isthmus 20-22 μm long, enveloped by nerve ring. Oesophageal glands overlapping intestine mostly on dorsal side 25-30 μm or 18-23 μm annules. Excretory pore 122-135 μm from anterior end. Hemizonid prominent about 2 annules long just anterior to excretory pore. Vulva a transverse slit located posterior to mid-body. Vagina with well developed cuticular lining extending less than half into the body. Gonads symmetrical, anterior gonad 184-229 μm long and posterior gonad 176-216 μm long. Oocytes in a single row. Spermatheca offset sometimes appearing 3-4 chambered, empty non-functional. Intestine does not overlap rectum. Phasmid 1-5 annules anterior to anus. Tail short broadly rounded, 9-12 μm long and 15-20 μm wide at anus with 5-7 annules ventrally, terminus striated.

Male: Not found.

Type habitat and locality: Soil around the roots of cultivated strawberry (*Fragaria x ananassa* Duch. V. Klonmore) from Muzaffarabad, Azad Kashmir, Pakistan.

Type specimens: Collected by the authors during March, 1984. Holotype slide No. NNRC/97.1 and Paratype slide No. NNRC/97.2-97.4 (8 females) deposited in the National Nematode Collection of NNRC, University of Karachi, Karachi, Pakistan. Slide No. NNRC/97.5 (3 females) deposited in USDA Nematode Collection, Beltsville, Maryland, U.S.A.

Differential diagnosis: *Rotylenchus fragaricus* sp. n. comes close to *R. buxophilus* Golden, 1956 and *R. eximius* Siddiqi, 1964. From *R. buxophilus* it differs by the absence of longitudinal striations on basal annule, greater 'O' value, more posterior vulva and greater 'c' value; phasmid just anterior to anus and tail without any ventral projection with lesser number of annules (in *R. buxophilus* 24-28 longitudinal striation on basal annule; O=9-14; V=52-58; c=36-48 μm ; phasmid 5-15 annules above the level of anus; tail with ventral projection with 10-20 annules).

It is also distinguished from *R. eximius* in shorter body length, small stylet, shorter 'O' value, more posterior vulva and greater 'c' value (in *R. eximius* L = 1.2-1.4 mm; stylet = 38-41 μm ; O = 28-32 μm ; V = 54-55; c = 48-57).

ROTYLENCHUS PAKISTANENSIS SP. N. (Fig .2)

Paratype females (14): L = 0.55-0.70 (0.63) mm; a = 28-31 (29); b = 4.5-5.8 (5.0); b' = 3.8-4.6 (3.8); c = 44-66 (52); c' = 0.7-1.1 (0.9); V = 61-67 (64); O = 22-36; m = 51-56 (52); stylet = 24-26 (24.5) μm .

Holotype female: L = 0.68 mm.; a = 30; b = 5.4; b' = 4.3; c = 64; c' = 0.8; V = 64; O = 25; m = 51; stylet = 26 μm .

Description

Body curved ventrally to form an open spiral, tapering at both extremities. Cuticular annules distinct, 1 to 1.5 μm wide at mid-body. Lateral fields with four smooth incisures, occupying 1/4 of mid-body width, areolated only anteriorly. Head truncate, not set off, with 4 to 5 annules, 4 μm high and 6 μm wide at base. Cephalic framework sclerotized. Stylet massive, metenchium longer than telenchium, basal knobs rounded, slightly flattened anteriorly, 5.6-6.0 μm across. Outlet of dorsal oesophageal gland 1/3 - 1/4 of stylet length or 6-9 μm behind the base. Procorpus of oesophagus cylindrical. Median bulb well developed, oval, 8-10 \times 12-14 μm with a large refractive cuticular thickening at centre. Isthmus 27-29 μm , enveloped by nerve ring just behind median bulb. Oesophageal glands overlapping intestine latero-dorsally 20-25 annules or 28-32 μm . Excretory pore 108-112 μm from anterior end. Hemizonid 2 annules long just anterior to excretory pore. Vulva a transverse slit. Vagina double walled extending about 1/2 into body. Female reproductive system amphidelphic, symmetrical, spermatheca empty, ovary with a single row of oocytes. Phasmids 0-3 annules anterior to level of anus. Tail tapering, more curved dorsally with slight ventral projection 11-12 μm long or 5-7 annules.

Male: Not found.

Type habitat and locality: Specimens collected from soil around the roots of apple (*Pyrus malus* Mill.) and grapevine (*Vitis vinifera* L.) from Muzaffarabad, Azad Kashmir, Pakistan.

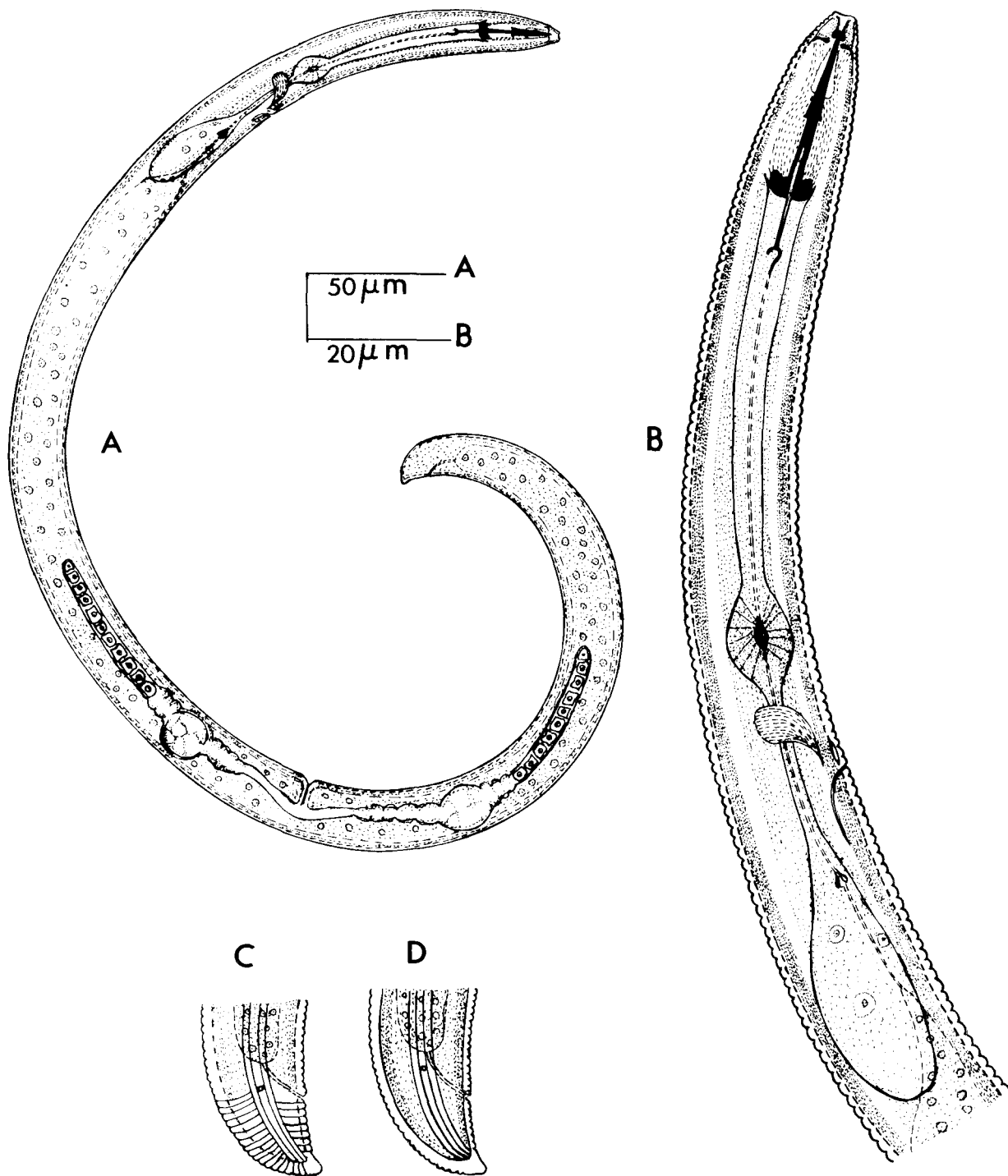


Fig. 2 - *Rotylenchus pakistanensis* sp. n., A, entire female; B, anterior region; C, D, tails.

Type specimens: Holotype slide No. NNRC/97.6 and Paratype slide NNRC/97.7-97.9 (11 females) deposited in the National Collection of NNRC, University of Karachi, Karachi, Pakistan. Slide No. NNRC/97.10 (3 females) deposited in USDA Nematode Collection, Beltsville, Maryland, U.S.A.

Differential diagnosis: *Rotylenchus pakistanensis* sp. n. is closely related to *R. devonensis* Van den Berg, 1976 and *R. unisexus* Sher, 1965. It differs from *R. devonensis* in having longer metenchium, phasmids 0-3 annules anterior to anus and tail tapering with more dorsal curvature with 5-7 annules [in *R. devonensis*: metenchium shorter ($m=43-46\%$), phasmid 7-11 annules anterior to anus and tail broadly rounded with 7-9 annules]. It differs from *R. unisexus* in having a truncate head, shorter stylet and phasmid 0-3 annules anterior to anus, and fewer tail annules (in *R. unisexus*; head hemispherical, stylet 25-29 μm , phasmids 6-7 annules anterior to anus, tail with 11 annules).

ROTYLENCHUS ALII SP. N. (Fig. 3)

Paratype females (18): $L=0.54-0.76$ (0.64) mm; $a=27-36$ (30); $b=5.0-6.9$ (5.7); $b'=4.4-5.9$ (5.1); $c=43-73$ (52); $c'=0.7-1.1$ (0.8); $V=61-65$ (62); $O=21-31$ (26); $m=50-58$ (53); stylet = 22-24 (23) μm .

Holotype female: $L=0.63$ mm; $a=27$; $b=6.2$; $b'=5.2$; $c=72$; $c'=0.7$; $V=64$; $O=21.4$; $m=57$; stylet = 22 μm .

Description

Body cylindrical, 'C' shaped; annules 1.0-1.5 μm wide near mid body. Lateral field areolated only anteriorly, occupying one-fourth of body width. Head conoid rounded, continuous 4 μm high and 7 μm wide, bearing 4-5 indistinct annules, stylet knobs rounded with flattened anterior surface 5-5.5 μm across. Outlet of dorsal oesophageal gland 5-6 μm behind stylet base. Median bulb oval 11-12 $\mu\text{m} \times 8-9 \mu\text{m}$. Isthmus 19-21 μm long. Nerve ring in the middle of isthmus. Oesophago-intestinal junction 55-68 body annules or 98-102 μm from anterior end. Excretory pore 95-105 μm from anterior end. Hemizonid 2 annules long 1-2 annules anterior to excretory pore. Vulva slightly posterior to mid body, ovaries paired with oocytes lying in a multiple row. Spermatheca spherical, filled with rounded

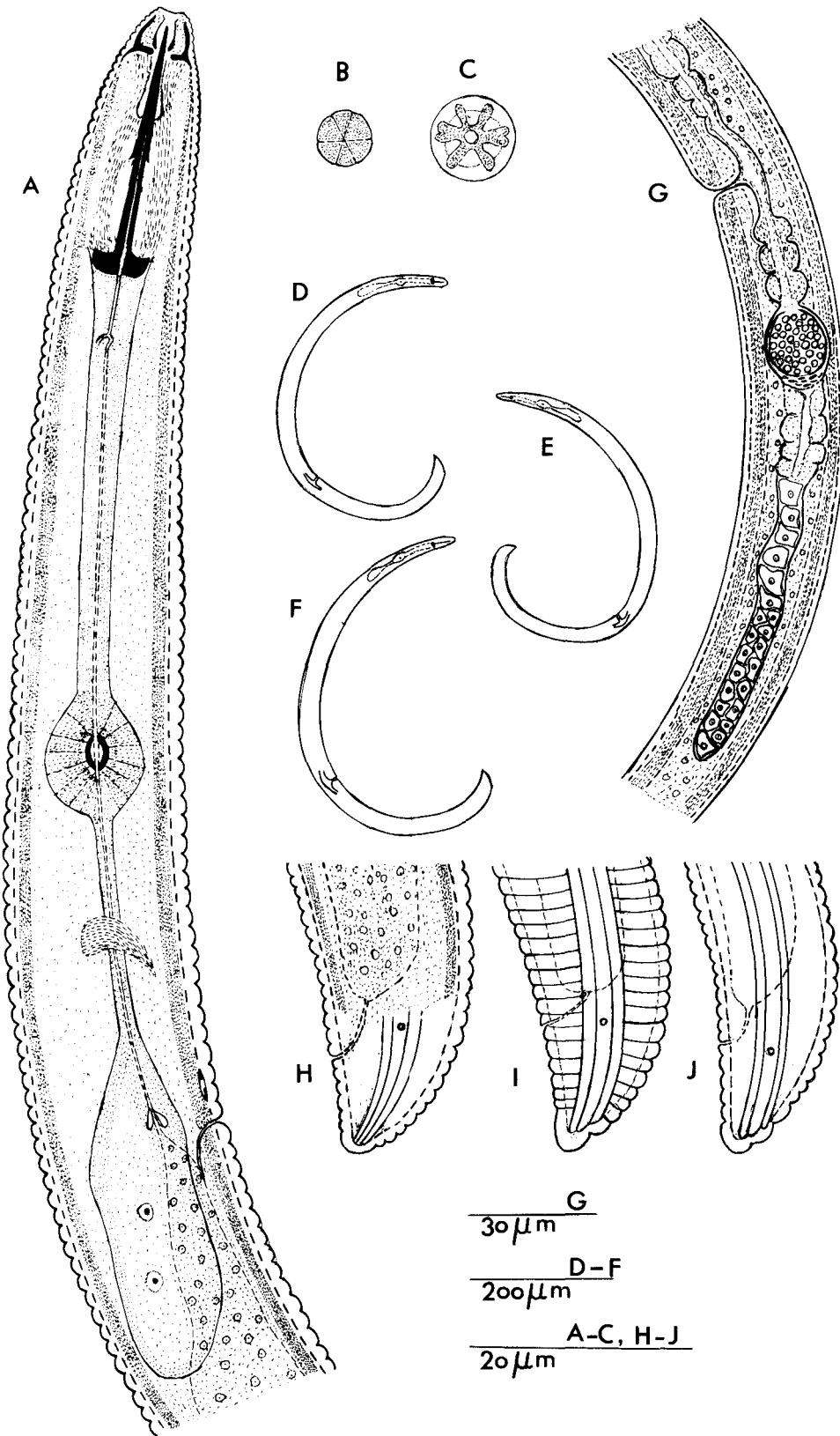


Fig. 3 - *Rotylenchus alii* sp. n., A, oesophageal region; B, female face view; c, female cross section through basal annule of lip region; D-F, whole body; G, vulva region and posterior gonad; H-J, tails.

sperms. Intestine not extending over rectum. Tail conoid to pointed, dorsally convex conoid to a short ventral projection, with 5-6 annules and 8-14 μm long. Phasmids preanal or 0-3 annules anterior to anus.

Male: Not found.

Type habitat and locality: Specimens collected from soil around the roots of apple (*Pyrus malus* Mill.) from Muzaffarabad, Azad Kashmir, Pakistan.

Type specimens: Holotype slide No. NNRC/97.11 and Paratype slide No. NNRC/97.12-97.14 (16 females) deposited in the National Collection of NNRC, University of Karachi, Karachi, Pakistan. Slide No. NNRC/97.15 (2 females) deposited in USDA Nematode Collection, Beltsville, Maryland, U.S.A.

Differential diagnosis: *Rotylenchus alii* sp. n. can be distinguished from the closely related species *R. acuspicaudatus* Van den Berg *et* Heyns, 1974 and *R. usitatus* Van Den Berg *et* Heyns, 1974. It is distinguished from *R. acuspicaudatus* by the shorter body length, smaller stylet, more posterior vulva, oval spermatheca with sperm, phasmid on tail or posterior to anus, less number of tail annules and shorter tail (in *R. acuspicaudatus* L=0.8-1.0 mm; stylet=25-28 μm ; V=54-57; spermatheca round, phasmid 7-15 annules anterior to anus 5-7 tail annules, tail 19-25 μm long). It is distinguished from *R. usitatus* by the shorter body length, smaller stylet, posterior vulva, posterior phasmid, fewer tail annules and shorter tail (in *R. usitatus* L=1.1-1.2 mm; stylet=28-29 μm ; V=55; phasmid 8-14 annules anterior, tail with 10-16 annules, 19-26 μm long).

ROTYLENCHUS CYPRIENSIS Antoniou, 1980

Females (7): L=0.67-0.69 (0.67) mm; a=27-34 (29); b=5.5-6.0 (5.9); b'=?; c=43-45 (45); c'=0.5-0.9 (0.9); V=63; O=12-18 (15); stylet=25 μm .

The specimens of *R. cypriensis* were found in the soil around roots of apple (*Pyrus malus* Mill.) from Quetta, Pakistan. Measurements of the specimens are similar to those given by Antoniou, 1980.

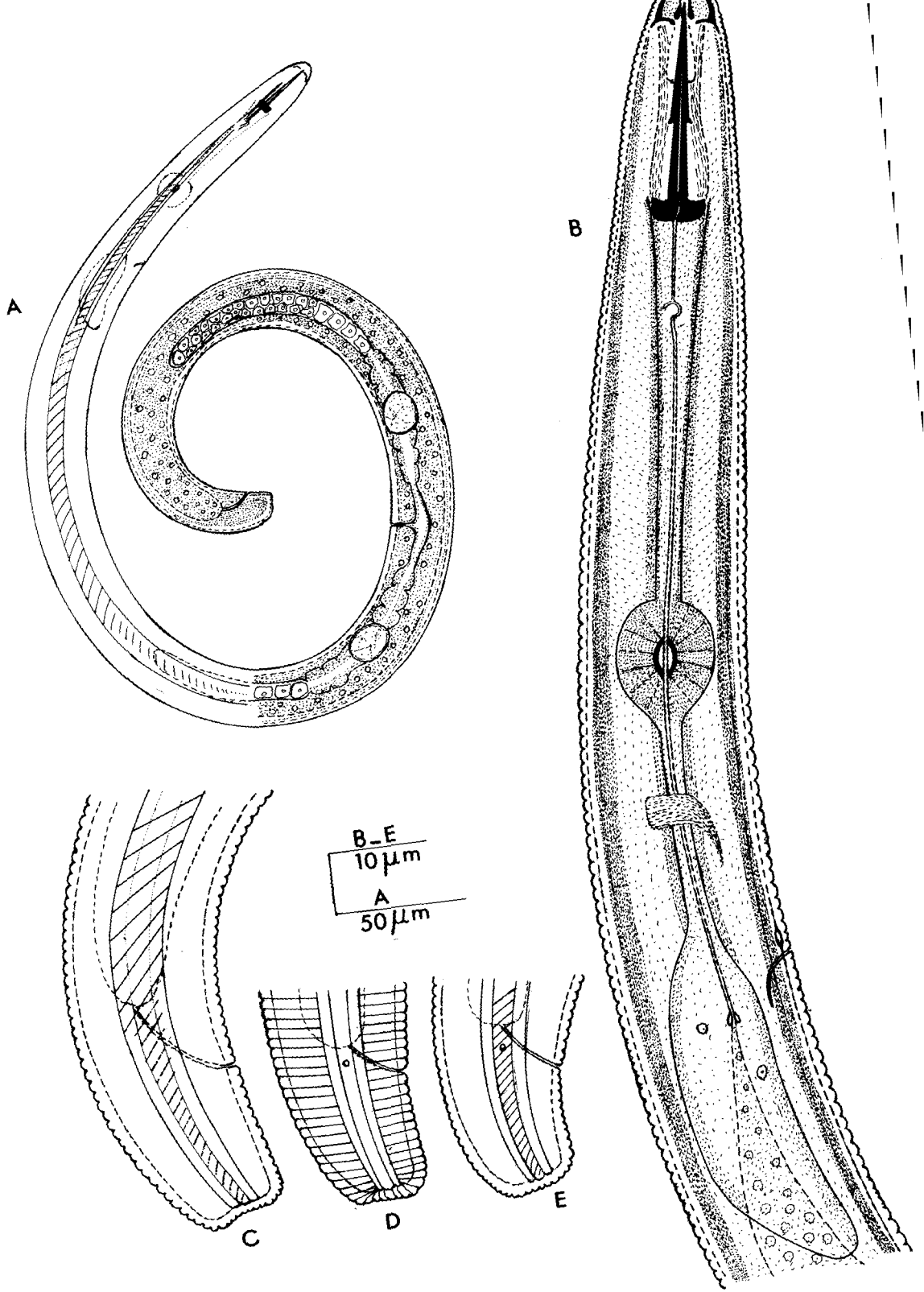


Fig. 4 - *Helicotylenchus obliquus* sp. n., A, entire body; b, anterior region; C-E, tails.

HELICOTYLENCHUS OBLIQUUS SP. N. (Fig. 4)

Paratype females (14): L=0.63-0.69 (0.66) mm; a=27-31 (29); b=5.5-6.1 (6.0); b'=4.4-5.0 (4.6); c=48-50 (49); c'=0.8-0.9 (0.9); V=61-64 (62); O=34-40 (37); m=48-51 (49); stylet=22-23 (23) μ m.

Holotype female: L=0.68 mm; a=27; b=6.6; b'=5; c=50; c'=0.8; V=62; O=34; m=48; stylet=23 μ m.

Description

Body cylindrical, tapering anteriorly, forming a spiral shape when relaxed. Cuticle striation 1-1.5 μ m wide near mid body. Lateral fields with four smooth incisures about one fourth as wide as body, not areolated but anterior and posterior region occasionally at mid body, marked by distinct oblique striae. Head hemispherical, with 4-5 indistinct annules, 7-7.5 μ m wide at base and 4-4.5 μ m high, cephalic framework lightly sclerotized. Stylet massive, metenchium slightly shorter than telenchium. Stylet knobs rounded flattened anteriorly, 5 μ m across; orifice of dorsal oesophageal gland almost one-third of stylet length from its base. Median bulb oval 15-17 μ m \times 11-13 μ m with a large refractive cuticular thickening at centre. Isthmus 20-23 μ m, encircled by nerve ring. Oesophago-intestinal junction 108-112 μ m from anterior end. Excretory pore 102-104 μ m from anterior extremity, 4-6 annules anterior to the level of oesophago-intestinal junction. Hemizonid two annules long just anterior to excretory pore. Vulva transverse slit, vagina about half body width long with single epiptygma. Gonads symmetrical, oocytes arranged in a multiple row spermatheca non-functional. Intestine does not overlap rectum. Phasmid 1-5 annules anterior to anus. Tail short 12-15 μ m long with 9-12 annules, slightly dorsally convex, cylindroid to hemispheroidal, with striated, flattened slightly depressed terminus.

Male: Not found.

Type habitat and locality: Specimens collected from soil around roots of mango (*Mangifera indica* L.) from Sind, Pakistan.

Type specimens: Holotype slide No. NNRC/31.181 and paratype slide No. NNRC/31.182-31.184 (11 females) deposited in the National Collection of NNRC, University of Karachi, Karachi, Pakistan. Slide No. NNRC/

31.185 (3 females) deposited in USDA Nematode Collection, Beltsville, Maryland, U.S.A.

Differential diagnosis: *H. obliquus* sp. n. differs from all the known species of *Helicotylenchus* in having oblique striae on lateral field over most of its length. It is close to *H. bihari* Mulk et Jairajpuri, 1974; *H. canadensis* Waseem, 1961; *H. australis* Siddiqi, 1972 on the basis of subcylindrical to hemispherical tail shape. It differs from *H. bihari* in the longer stylet, hemispherical lip region (in *H. bihari*; stylet 20-21 μm , lip region truncate). From *H. canadensis* it is distinguished by shorter stylet, hemispherical lip region (in *H. canadensis* stylet, 28-33 μm , lip region distinctly truncate). From *H. australis* it can be separated by hemispherical lip region with indistinct annules, absence of labial disc, spermatheca without sperm (in *H. australis*, lip region truncate, smooth, distinct labial disc, spermatheca packed with sperm).

S U M M A R Y

Four new Hoplolaimid species were found in soil samples collected from fruit orchards and vegetable crops in Muzaffarabad (Azad Kashmir) and Sind, Pakistan. Description and illustrations are given for *Rotylenchus fragaricus* sp. n. from cultivated strawberry (*Fragaria* x *ananassa* Duchesne), *R. pakistanensis* sp. n. from apple (*Pyrus malus* Mill.) and grapevine (*Vitis vinifera* L.), *R. alii* sp. n. from apple (*Pyrus malus* Mill.) and *Helicotylenchus obliquus* sp. n. from mango (*Mangifera indica* L.). *Rotylenchus cypriensis* Antoniou, 1980 is recorded for the first time in Pakistan.

LITERATURE CITED

- ANTONIOU M., 1980. *Rotylenchus cypriensis* sp. n. (Nematoda: Hoplolaimidae) from Cyprus. *Nematol. medit.*, 8: 137-140.
- GOLDEN A. M., 1956. Taxonomy of the spiral nematode (*Rotylenchus* and *Helicotylenchus*), and the developmental stages and host parasite relationships of *R. buxophilus* sp. n., attacking box-wood. *Maryland Ag. Exp. Sta. Bul. A-85*, 28 pp.
- MULK M. and JAIRAJPURI S. M., 1974. Nematodes of leguminous crops in India. II. Five new species of *Helicotylenchus* Steiner, 1945 (Hoplolaimidae). *Indian J. Nematol.*, 4: 212-221.
- SHER S. A., 1965. Revision of the Hoplolaiminae (Nematoda). V. *Rotylenchus* Filipjev, 1936. *Nematologica*, 11: 173-198.
- SIDDIQI M. R., 1964. *Rotylenchus eximius* n. sp. (Nematoda: Hoplolaiminae) found around almond roots in Tunisia. *Nematologica*, 10: 101-104.

- SIDDIQI M. R., 1972. On the genus *Helicotylenchus* Steiner, 1945 (Nematoda: Tylenchida), with description of nine species. *Nematologica*, 18: 74-91.
- VAN DEN BERG E., 1976. Some species of Tylenchoidea (Nematoda) from South Africa, with description of two new species. *Phytophylactica*, 8: 55-64.
- VAN DEN BERG E. and HEYNS J., 1974. South African Hoplolaiminae 3. The genus *Rotylenchus* Filipjev, 1936. *Phytophylactica*, 6: 165-184.
- WASEEM M., 1961. Two new species of the genus *Helicotylenchus* Steiner, 1945 (Nematoda: Hoplolaiminae). *Can. J. Zool.*, 39: 505-509.

Accepted for publication on 16 January 1986.