

## Description of *Rotylenchus graecus* n. sp. from Greece (Nematoda: Hoplolaimidae)

N. VOVLAS AND A. TROCCOLI<sup>1</sup>

**Abstract:** *Rotylenchus graecus* n. sp. is described and illustrated from a bisexual population found in Filippias, Epirus, northwestern Greece, in the rhizosphere of ivy (*Hedera helix* L.) and giant reed (*Arundo donax* L.) grown on the edge of irrigation canals. *Rotylenchus graecus* n. sp. is characterized by a lip region with five annules, stylet length of 29–32  $\mu\text{m}$ , vulva position at 53–65%, female tail pointed-conoid with 15–23 annules, and bursa with regularly annulated margins enveloping tail. The new species differs from all other *Rotylenchus* species with conoid tails in having a greater number of tail annules. *Rotylenchus graecus* n. sp. appears closely related to *R. acuspicaudatus* Van den Berg & Heyns, 1974, but differs from it primarily by the greater number of female tail annules (15–23 vs. 13–16), stylet length (29–32 vs. 25.7–28.3  $\mu\text{m}$ ), and the less ventrally protruding bursa, which has smooth posterior margins rather than the finger-like appearance of the posterior bursal annules in *R. acuspicaudatus*.

**Key words:** Greece, Hoplolaimidae, morphology, nematode, new species, *Rotylenchus graecus*, spiral nematode, taxonomy.

Soil samples collected on the edge of irrigation canals at Filippias, Epirus, Greece, from the rhizosphere of ivy (*Hedera helix* L.) and giant reed (*Arundo donax* L.) contained a bisexual population of a *Rotylenchus* species with pointed-conoid female tail (1), apparently similar to *R. acuspicaudatus* Van den Berg & Heyns, 1974. Further observations with light and scanning electron microscopy indicated that our specimens should be assigned to a new species primarily because they have a greater number of tail annules than any other known *Rotylenchus* spp. (1). This new taxon is described as *Rotylenchus graecus* n. sp. The specific name refers to the geographic origin epithet (Latin *graecus* = from Greece).

### MATERIALS AND METHODS

Specimens used in this study were extracted from soil samples with magnesium sulphate centrifugal flotation (2). Specimens for light microscopy were killed with gentle heat, fixed in a 4% solution of formaldehyde + 1% propionic acid, and processed to glycerine with Seinhorst's rapid method (3). Wergin's methods (6) were

used for the preparation of specimens for SEM observations and observed at 5–10 kV at accelerating voltage. Abbreviations used are defined in Siddiqi (4). All measurements are in micrometers ( $\mu\text{m}$ ) unless otherwise stated.

### SYSTEMATICS

#### *Rotylenchus graecus* n. sp. (Table 1, Figs. 1,2)

**Holotype (female in glycerine):** L = 904; maximum body width = 44; anal body width = 25; esophagus length = 145; esophagus overlapping intestine = 20; excretory pore from anterior end = 128; stylet length = 30; tail length = 18; V% = 56; G<sub>1</sub> in length = 205; G<sub>2</sub> = 205; a = 20; b = 7.2; b' = 6.2; c = 50; c' = 0.9; lateral field = 5.6; tail annules = 18.

**Paratype females in glycerine (n = 20):** Measurements in Table 1.

#### Description

Body shape slightly curved to C-shaped. Body annules  $1.4 \pm 0.1$  (1.2–1.7) wide. Lip region with five annules, hemispherical in profile,  $10 \pm 0.5$  (9–11) wide,  $6 \pm 0.5$  (4.9–7.3) high (Figs. 1A, 2A,B). Lateral field with four longitudinal incisures  $6.0 \pm 0.7$  (4.9–7.3) wide, one-sixth of body width. Cephalic framework strongly developed. Stylet robust with rounded basal knobs 6

Received for publication 14 February 1995.

<sup>1</sup> Nematologists, Istituto di Nematologia Agraria, Consiglio Nazionale delle Ricerche, via G. Amendola 165/A, 70126 Bari, Italy.

TABLE 1. Morphometric data of paratypes of *Rotylenchus graecus* n. sp.

Morphological characters <sup>a</sup>	Females (n = 20)			Males (n = 10)		
	Mean	SD	Range	Mean	SD	Range
Measurements in $\mu\text{m}$						
Body length	884	61	771-976	847	29	804-829
Maximum body width	41	4.9	32-50	36	3.0	32-42
Anal body width	22	4.9	19-26	22	1.1	20-24
Esophagus length	144	5.5	132-153	141	5.8	133-153
Excretory pore from anterior end	122	8.0	104-137	119	4.9	12-28
Stylet length	30	0.9	29-32	29	0.6	28-30
Tail length	18	3.1	14-23	36	4.1	28-42
Spicules	—	—	—	41	1.1	40-43
Gubernaculum	—	—	—	17	0.6	16-18
Percentages						
V or T	56	2.5	53-65	50	9.8	41-67
G <sub>1</sub>	22	2.4	17-28	—	—	—
G <sub>2</sub>	22	1.7	20-27	—	—	—
Ratios						
a	22	2.0	18-25	23	1.8	21-26
b	7.2	0.4	6.3-7.9	7.0	0.3	6.5-7.4
b'	6.2	0.3	5.6-6.7	6.0	0.3	5.3-6.4
c	50	6.9	39-65	24	2.7	21-30
c'	0.8	0.1	0.5-0.9	1.6	0.2	1.3-1.9

<sup>a</sup> Abbreviations defined in Siddiqi (1986).

$\pm 0.5$  (5.3-7.6) wide; conus  $13.8 \pm 0.7$  (12.9-15.2). Orifice of dorsal esophageal gland  $7.5 \pm 1.2$  (6-11) from stylet base. Procorpus cylindrical, narrowing slightly at junction with the median esophageal bulb. Median esophageal bulb broadly oval at  $84 \pm 5.6$  (67-91) from anterior end, valvular apparatus well developed (Fig. 2B). Excretory pore level with or slightly anterior to esophago-intestinal junction. Nerve ring enveloping isthmus at the middle. Esophageal glands with three nuclei (Fig. 2B), overlapping intestine dorsally, overlap length  $21 \pm 4.7$  (17-37). Cardia well developed. Reproductive system with two equally developed genital branches, G<sub>1</sub> anterior =  $197 \pm 26$  (148-262); G<sub>2</sub> posterior =  $197 \pm 20$  (161-243). Vulva slightly posterior to mid-body, with distinct double epitygma (Figs. 1C, 2E). Ovaries with single row of oocytes, except in multiplication zone; spermatheca spherical (Fig. 1C), 19-21  $\mu\text{m}$  in diameter, filled with round sperms 3-4  $\mu\text{m}$  in diameter. Uterus quadricolumellar; phasmids 1-8 annules,  $5.2 \pm 2.8$  (2-11) anterior to anus,  $23 \pm 3.6$  (18-27) from tail terminus. Tail conoid-

rounded with  $19 \pm 2.3$  (15-23) annules on ventral side (Figs. 1F, 2C,M).

*Allotype (male in glycerine)*: L = 869; maximum body width = 36; anal body width = 24; esophagus length = 143; esophagus overlapping intestine = 22; excretory pore from anterior end = 125; stylet length = 30; tail length = 37; T% = 67; a = 24; b = 7.2; b' = 6.1; c = 23; c' = 1.5; lateral field = 5.9; spicules = 42; gubernaculum = 18.

*Paratype males in glycerine (n = 10)*: Measurements in Table 1.

*Description*

Males common (20-40% of the total population). Body ventrally arcuate (Fig. 2F) and showing no marked sexual dimorphism. Body annules  $1.4 \pm 0.1$  (1.2-1.5) wide. Lip region hemispherical in profile, with five annules, measuring  $9.7 \pm 1.2$  (6.6-11) wide  $5.8 \pm 0.4$  (5.0-6.3) high. Lateral field with four longitudinal incisures  $5.4 \pm 0.7$  (4.6-6.9) wide. Genital tube outstretched,  $424 \pm 92$  (346-584) long with single row of spermogonia, except in the multiplication zone. Tail conical, bursa en-

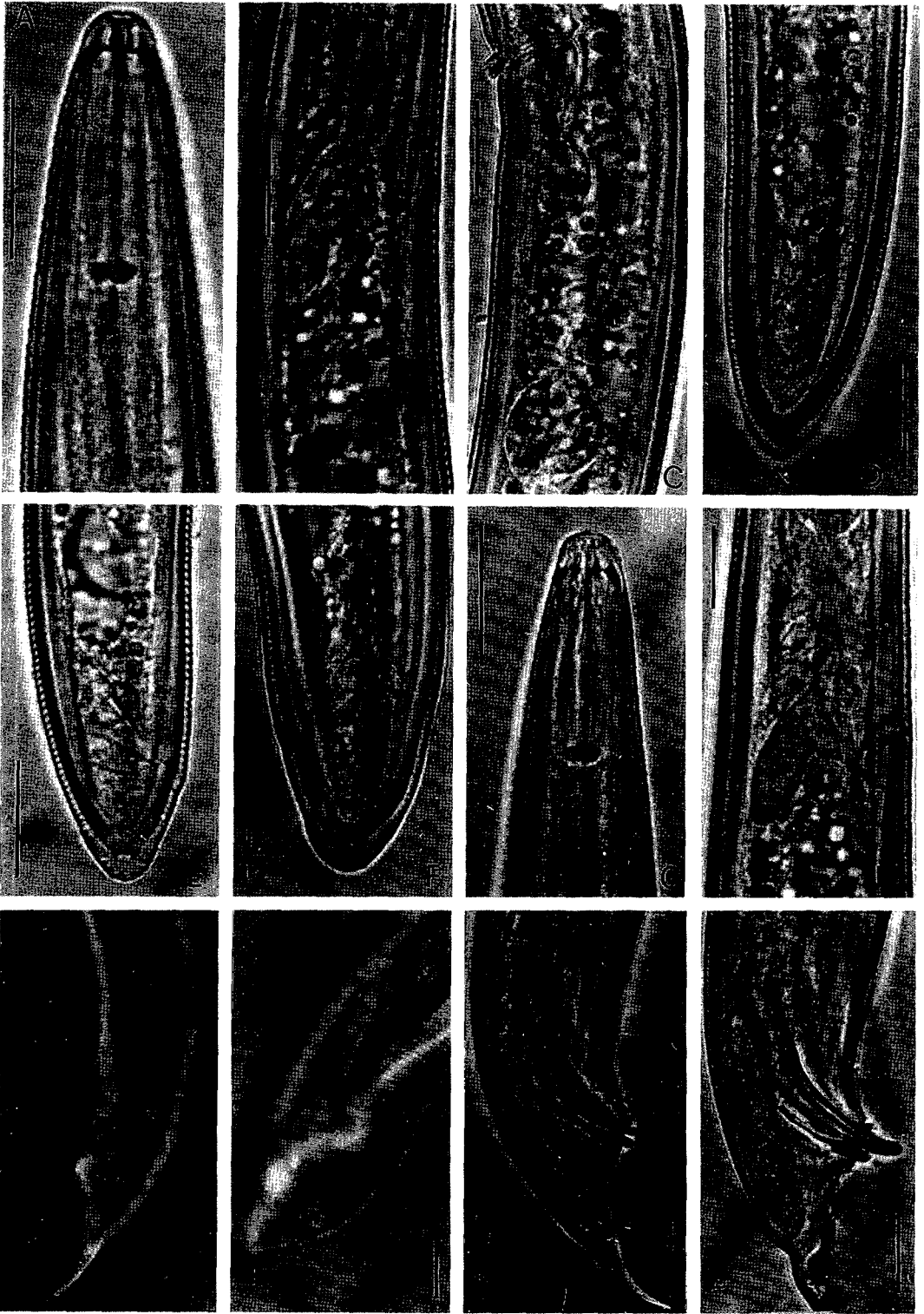


FIG. 1. Photomicrographs of *Rotylenchus graecus* n. sp. female (A-F) and male (G-L) (scale bar = 20  $\mu$ m). A) Anterior body portion. B) Esophageal region. C) Vulval region. D-F) Caudal regions. G) Anterior body portion. H) Esophageal basal portion. I-L) Caudal regions at different focus.

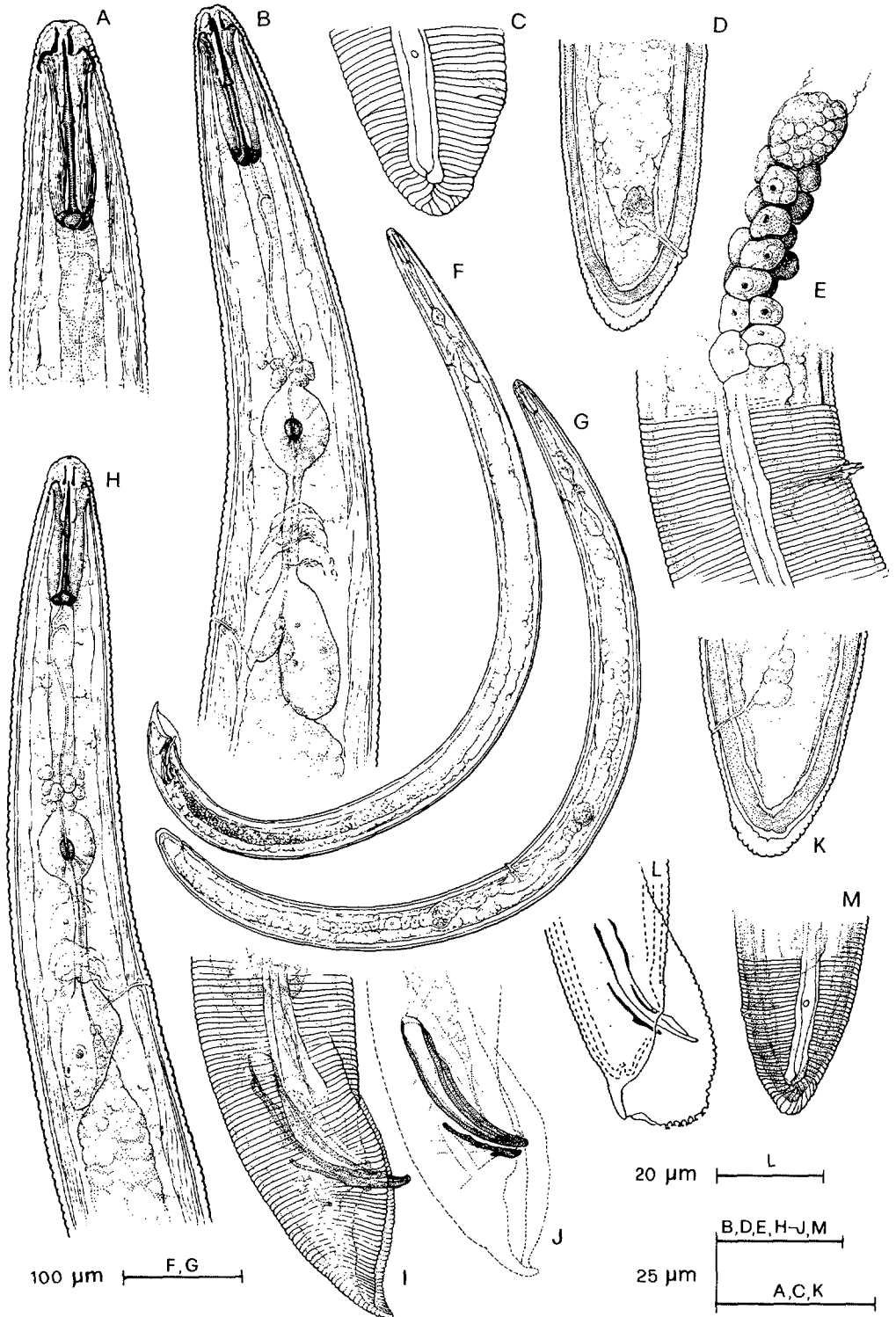


FIG. 2. *Rotylenchus graecus* n. sp. A,B) Female anterior and neck region. C,D,K,M) Female caudal regions. E) Female mid-body region. F,G) Entire male and female. H) Male neck region. I,J) Male caudal regions. L) Male tail of *Rotylenchus acuspicaudatus* (after Van den Berg and Heyns, 1974, for comparison).

veloping tail, moderately protruding ventrally. Spicules and gubernaculum ventrally curved (Fig. 2I,J), strongly developed.

#### *Type host and locality*

Holotype female, allotype male, and paratypes from a population extracted from soil samples collected in July 1993 on the edge of irrigation canals at Filippias, Epirus, Greece, in the rhizosphere of ivy (*Hedera helix* L.) and giant reed (*Arundo donax* L.).

#### *Type designations*

Holotype female, allotype male, and additional paratypes deposited in the authors' nematode collection at Istituto di Nematologia Agraria, Consiglio Nazionale delle Ricerche, Via G. Amendola 165/A, 70126 Bari, Italy. Paratypes (female and male on glass slides) deposited at the University of California, Davis; USDA Nematode Collection, Beltsville, Maryland; Entomology and Nematology Department, Rothamsted Experimental Station, Harpenden, England; Museum National d'Histoire Naturelle, Paris, France; and Nematode Collection of the Department of Nematology, Landbouwniversiteit, Wageningen, The Netherlands.

#### *Diagnosis*

*Rotylenchus graecus* n. sp. is a member of the *Rotylenchus* species group with conoid female tail (1) but is distinguished from all other species in the group by the greater number of tail annules in the female and by the absence of fringe-like annules on the posterior part of the bursa (5). It is characterized by a lip region with five annules, stylet length of 29–32, vulva position at 53–65%, conoid female tail with 15–23 annules, and the less ventrally pro-

truding bursa, which has smooth posterior margins.

#### *Relationships*

*Rotylenchus graecus* n. sp. is close to *R. acuspicaudatus* Van den Berg & Heyns, 1974 in general morphology of females and males. However, females of *R. graecus* can be differentiated from *R. acuspicaudatus* by the greater number of tail annules (15–23 vs. 13–16), regularly conical tail shape (ventrally directed in *R. acuspicaudatus*), and longer stylet of 29–32 (25.7–28.3 in *R. acuspicaudatus*).

Males of *R. graecus* n. sp. can be differentiated from those of *R. acuspicaudatus* by the size, shape, and annulation of the bursa. Caudal alae of *R. graecus* are arcuate, with a width of about 36% of the anal body width, whereas those of *R. acuspicaudatus* are much wider, with a width of 93% of the anal body width. In addition, in *R. acuspicaudatus* the posterior bursal annules possess a unique characteristic "fringe-like" appearance (5) (Fig. 2L).

#### LITERATURE CITED

1. Castillo, P., N. Vovlas, A. Gomez-Barcina, and F. Lamberti. 1993 (publ. 1994). The plant-parasitic nematode *Rotylenchus* (a monograph). *Nematologia Mediterranea* 21 (Suppl.):1–200.
2. Coolen, W. A. 1979. Methods for extraction of *Meloidogyne* spp. and other nematodes from roots and soil. Pp. 317–329 in F. Lamberti and C. E. Taylor, eds. *Root-knot nematodes (Meloidogyne species)*. Systematics, biology, and control, New York: Academic Press.
3. Seinhorst, J. W. 1966. Killing nematodes for taxonomic study with hot f. a. 4:1. *Nematologica* 12: 178.
4. Siddiqi, M. R. 1986. *Tylenchida*. Parasites of plants and insects. London: CAB.
5. Van den Berg, E., and J. Heyns. 1974. South African Hoplolaiminae. 3. The genus *Rotylenchus* Filipjev, 1936. *Phytophylactica* 6:165–184.
6. Wergin, W. P. 1981. Scanning electron microscopic techniques and applications for use in nematology. Pp. 175–204 in B. M. Zuckerman and R. A. Rohde, eds. *Plant-parasitic nematodes*, vol. 3. New York: Academic Press.