Nematol. medit. (1997), 25: 73-81

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PLANT PARASITIC NEMATODES FROM UNIRRIGATED FIELDS IN ALHAMA, SOUTHEASTERN SPAIN

by
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Summary. A survey was conducted to determine the frequency and abundance of plant parasitic nematodes in unirrigated fields of Alhama region (south-eastern Spain), mainly under wheat and barley, but also food and forage legumes and olive trees. Seventy-seven fields were sampled from a total area of 38,575 ha. *Heterodera avenae* was the most widespread species in the area, followed by *Merlinius brevidens*, *Pratylenchus neglectus*, *Pratylenchus thornei*, *Meloidogyne artiellia* and *Zygotylenchus guevarai*. Twenty-three other species of plant parasitic nematodes were found. Morphometrics of *Paratrophurus acristylus*, *Paratylenchus sheri*, *P. nanus* and *P. similis* from Southern Spain are provided. *Paratylenchus israeliensis* is proposed as a new junior synonym of *P. sheri*.

The semiarid Mediterranean region is characterized by winter rainfall alternating with summer drought. The growing season is reduced to the period November to June and thus dictates that an unirrigated agricultural system is limited to the production of cereals and food or forage legumes.

Plant parasitic nematodes are an important limiting factor in the production of legumes and cereals in most temperate areas of the world (Eriksson, 1972; Griffin, 1984; Riggs and Niblack, 1993; Rivoal and Cook, 1993), but little is known about the occurrence, distribution and importance of plant parasitic nematodes on food legumes and cereals in the semiarid Mediterranean region.

Materials and methods

A survey was undertaken in unirrigated areas of Alhama region, where the soils contain sufficient available water only for growing crops during the winter-spring season (Tobar Jiménez, 1973). The cropping system in the region is rotations of wheat (*Triticum aestivum* L.), legumes [grass-pea (*Lathyrus cicera* L.), vetch (*Vicia monanthos* Desf.), lentil (*Lens culinaris* Medic.) or chickpea (*Cicer arietinum* L.)], barley (*Hordeum vulgare* L.) and fallow, but some fields are a monoculture of olive tree (*Olea europaea* L.). Sample fields were chosen to represent the whole area, according to a systematic geographical scheme. In total 77 fields were sampled from an area of 38,575 ha. Composite soil samples were collected in autumn, before the growing season, by taking cores of 3.8 cm diameter and 20 cm depth, at an approximate density of sixty-four cores per ha, representing the whole of the selected field. Wheat was the previous crop in twenty-four of the fields, barley in twenty of them, olive in four, grass-pea in three and vetch in two. The twenty-four other fields were fallow.

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Each soil sample was thoroughly mixed and nematodes were extracted from 100 ml subsamples by the revised techniques of Oostenbrink (Tobar Jiménez, 1962, 1963) and Kort (1960) for cysts, using a Fenwick can. Cyst were crushed in a homogeniser and the juveniles and eggs counted (Southey, 1970).

Identification of living promorphs was done using a stereomicroscope (Tobar Jiménez, 1988). For specific determination, using a compound microscope, nematodes were killed by applying gentle heat, fixed in 3-5% formalin and processed and mounted in dehydrated glycerine (Seinhorst, 1962). All measurements are in micrometers (µm) unless otherwise stated and given as the range (average ± standard deviation). Voucher specimens are deposited at Unidad de Nematología, Estación Experimental del Zaidín, CSIC, Granada, Spain.

Frequency of occurrence (proportion of samples in which the species was found), density [number of nematodes (eggs + juveniles for cyst nematodes) per 100 ml of soil] and relative abundance (number of specimens of each species expressed as a proportion of the total number of specimens) were calculated for each nematode species.

Results and discussion

Plant parasitic nematodes recovered in the survey, with their frequencies of occurrence, densities per 100 ml of soil, relative abundance and their associated crops, are shown in Table I.

The most widespread species was *Heterodera avenae* Wollenweber which was found in association with wheat and barley at an average density of 0.8-0.9 eggs + juveniles per g of soil. This density is less than the damage threshold (1-5 eggs + juveniles per g of soil) for wheat and barley in temperate regions (Franklin, 1951; Andersen, 1961). Although threshold levels can vary greatly with cultivar and climatic conditions, *H. avenae* does not seem to be of economic importance in the Alhama region, except in some fields with a high density (5 eggs-juveniles per g of soil) were it may be the cause of some observed damage (patches of stunted and chlorotic plants with yellow leaf tips).

Merlinius brevidens (Allen) Siddiqi and *Pratylenchus neglectus* (Rensch) Filipjev *et* Schuurmans Stekhoven were also widespread and associated with wheat, barley and olive. These associations were previously reported in the semiarid Mediterranean area by Tobar Jiménez (1973), Sikora (1988, 1988a) and Peña-Santiago (1990), but their impact on the growth and production of these crops is not known.

Pratylenchus pinguicaudatus Corbett and *Pratylenchus thornei* Sher *et* Allen were found associated mainly with barley and wheat, but occasionally with vetch. *P. thornei* has been reported on wheat, barley, vetch, chickpea, lentil and faba bean roots in Syria (Sikora, 1988; Di Vito *et al.*, 1992; Greco *et al.*, 1984). Extensive necrosis was evident in infested roots, but there were no obvious above-ground symptoms.

Meloidogyne artiellia Franklin was found in association with cereal crops and grasspea. Severe damage to faba bean, vetch and chickpea has been reported in semiarid-Mediterranean regions (Tobar Jiménez, 1973; Sikora, 1988). *M. artiellia* reproduces well on crucifers, cereals and legumes, except for oat, corn and lentil (Di Vito *et al.*, 1985). *M. artiellia* densities exceeding 1 egg/ml soil cause a considerable damage to food legumes and durum wheat (Di Vito and Greco, 1988, 1988a, 1988b). This level was reached and even surpassed in some fields (10 egg/ml of soil) in the Alhama area, with concomitant damage to cereals and food legumes.

Zygotylenchus guevarai (Tobar Jiménez) Braun *et* Loof causes damage to food legumes (Tobar Jiménez, 1973), apparently when associated with the fungus *Phyllasticta rabiei*.

Helicotylenchus tunisiensis Siddiqi and *Helicotylenchus vulgaris* Yuen were found under all the crops in the area but were not associated with any observable damage.

TABLE I - *Nematodes present in cereal-legume growing areas of Alhama region (southern Spain).*

Nematode species	Occurrence	Densities	Abundance	W	B	G	V	O	F
Heterodera avenae Wollenweber (eggs)	0.83	94	0.18	+	+				+
Merlinius brevidens (Allen) Siddiqi	0.77	53	0.10	+	+			+	+
Pratylenchus neglectus (Rensch) Filipjev *et* Schuurmans Stekhoven	0.65	63	0.12	+	+			+	+
Pratylenchus thornei Sher *et* Allen	0.40	39	0.08	+	+				+
Meloidogyne artiellia Franklin (juveniles)	0.38	21	0.04	+	+	+			+
Zygotylenchus guevarai (Tobar Jiménez) Braun *et* Loof	0.36	11	0.02	+	+		+		+
Helicotylenchus tunisiensis Siddiqi	0.30	40	0.08	+	+	+		+	+
Pratylenchus pinguicaudatus Corbett	0.30	18	0.04	+	+		+		+
Paratylenchus microdorus Andrássy	0.30	28	0.06	+	+			+	+
Paratylenchus sheri (Raski) Siddiqi	0.22	31	0.06	+	+	+		+	
Helicotylenchus vulgaris Yuen	0.21	14	0.03	+	+	+		+	+
Merlinius microdorus (Geraert) Siddiqi	0.20	12	0.02	+	+	+		+	+
Paratylenchus nanus Cobb	0.14	16	0.03	+			+	+	+
Helicotylenchus digonicus Perry	0.13	7	0.01	+	+	+		+	+
Paratrophurus acristylus Siddiqi *et* Siddiqui	0.12	8	0.02	+	+				+
Ditylenchus dipsaci (Kühn) Filipjev	0.12	9	0.02			+	+	+	+
Criconemoides informis (Micoletzky) Taylor	0.12	2	**	+	+				+
Scutylenchus quadrifer (Andrássy) Siddiqi	0.10	2	**	+	+				+
Xiphinema pachtaicum (Tulagonov) Kirjanova	0.08	1	**		+				+
Paratylenchus similis Khan, Prasad *et* Mathur	0.07	9	0.02	+		+			+
Amplimerlinius paraglobigerus Castillo, Siddiqi *et* Gómez Barcina	0.05	1	**		+				+
Macroposthonia antipolitana (De Guiran) De Grisse *et* Loof	0.05	1	**	+	+				+
Gracilacus straeleni (De Coninck) Raski	0.05	21	0.04	+	+				+
Pratylenchoides leiocauda Sher	0.03	4	0.01	+					
Paratylenchus baldacii Raski	0.03	2	**						+
Amplimerlinius magnistylus Castillo, Gómez Barcina, Vovlas *et* Navas	0.01	2	**		+				
Pratylenchus crenatus Loof, 1960	0.01	*	**						+
Gracilacus steineri (Golden, 1961) Raski, 1962	0.01	1	**	+					
Heterodera goettingiana Liebscher, 1892 (eggs)	0.01	*	**				+		

Occurrence = frequencies of occurrence; Abundance = relative abundance; Densities = Number of nematodes per 100 ml of soil.
Associated plants: W = Wheat, B = Barley, G = Grass-pea, V = Vetch, O = Olive tree, F = Fallow.
(*, densities under 0.5 nematodes or eggs per 100 ml of soil; ** values of relative abundance under 0.01).

Paratylenchus microdorus Andrássy and *Paratylenchus sheri* (Raski) Siddiqi were associated with wheat and barley in Alhama region. In Cyprus *P. microdorus* was found to be pathogenic to *Vicia sativa* L. (Philis, 1988) but in Alhama region was only found associated with cereals and olive and its pathogenicity to these plants is unknown.

Ditylenchus dipsaci (Kühn) Filipjev was found in only a few samples, but in some of them at high populations of more than 600 specimens per 100 ml of soil, a level capable of causing severe damage to food legumes (Tobar Jiménez, 1973). *D. dipsaci* is very common in the Mediterranean basin (Lamberti, 1981) and has been reported associated with cereals, grass-pea, vetch and many other hosts (Inserra and Vovlas, 1979; Sikora, 1988).

It is interesting to note that most of the species found were present also under fallow soil, which means that either they can survive under weeds or wild plants which grow during the fallow season or they have the ability to survive in the absence of a host.

Descriptions

PARATROPHURUS ACRISTYLUS Siddiqi *et* Siddiqui, 1983
(Fig. 1 A-D)

Female (n = 10): L = 0.59-0.79 (0.70 ± 0.07) mm; a = 27.2-37.8 (30.7 ± 3.1); b = 4.9-6.6 (5.8 ± 0.6); c = 16.8-19.2 (17.9 ± 1.1); c' = 2.3-2.8 (2.5 ± 0.2); V = 55.7-61.0 (57.3 ± 1.6); stylet = 21.5-23.1 (22.3 ± 0.7) µm.

Body slender, ventrally arcuate after fixation. Cuticle thick with fine annules, 1.2-1.6 µm wide at mid-body. Lateral fields one fourth as wide as body, with four smooth, equidistant incisures. Maximum body width 20.4-27.2 (23.2 ± 2.3) µm. Lip region conoid rounded or slightly truncate and continuous with body contour, 3.4-4.4 (3.9 ± 0.4) µm high x 6.2-8.5 (7.0 ± 0.8) µm wide, with four to five inconspicuous annules. Framework lightly sclerotized. Stylet very slender, conus 10.9-13.3 (12.1 ± 1.0) µm long or 51.1-59.5 (55.3 ± 3.2) as percentage of stylet length, with small rounded knobs, 3-4 µm across. Opening of the DGO at 2.5-3.5 µm from the stylet base. Median oesophageal bulb rounded 11.0-12.6 x 9.2-10.7 µm, MB = 52.2-56.5 (54.7 ± 1.4). Basal oesophageal bulb small and pyriform. Cardia well developed. Oesophagus length 116.0-127.3 (120.3 ± 3.2) µm. Excretory pore 85.5-104.5 (98.2 ± 6.2) µm from anterior end, opposite anterior portion of basal bulb. Hemizonid at level of or up to two annules anterior to excretory pore and two or three annules wide. Intestinal canals observed in some specimens. Vulva transverse, 355.5-444.6 (399.5 ± 34.3) µm from anterior end. Reproductive organs well-developed G_1 = 20.5-23.4 (21.9 ± 1.4), G_2 = 20.0-26.4 (22.7 ± 2.7). Spermatheca round, axial, sometimes filled with sperm. Ovaries outstretched with oocytes in one row. Tail cylindrical and straight, 34.0-45.6 (39.2 ± 4.3) µm long and bearing 27-34 annules; hyaline region 10.0-13.0 (11.3 ± 1.1) µm . Postrectal intestinal sac filling approximately half tail length. Tail terminus rounded to subclavate and smooth. Phasmids located at about middle of the tail.

Male (n = 3): L 0.71-0.73 (0.72 ± 0.01) mm; a = 24.9-30.0 (27.4 ± 2.5); b = 5.7-6.0 (5.8 ± 0.1); c = 14.5-17.7 (16.3 ± 1.4); c' = 2.4-2.7 (2.5 ± 0.1); stylet = 21.5-22.5 (22.0 ± 0.5) µm; spicules = 23.0-25.0 (24 ± 1.0) µm; gubernaculum = 12.3-13.0 (12.6 ± 0.3) µm.

Body C-shaped. Lip region, stylet and oesophagus as in female. Spicules arcuate and flanged distally. Gubernaculum protrusible. Tail conoid, 42.0-47.0 (44.5 ± 2.5) µm long and enveloped by bursa 63.0-84.5 (73.3 ± 10.3) µm long. Phasmids at middle of the tail, extending into bursa.

Morphology and morphometry of our specimens are similar to those given by Siddiqi and Siddiqui (1983) except for stylet length, which is longer in our population (21.5-23.1 *vs* 18-21 µm in females) and (21.5-22.5 *vs* 18-21 µm in males), longer spicules (23.0-25.0 *vs* 22.5-23 µm) and logner gubernaculum (12.3-13.0 *vs* 11-12 µm). The population described from Morocco by Castillo *et al.* (1989) shows intermediate values between the original description and our population for female and male stylet length (20-22 µm and 19-22 µm respectively) and spicules length (21-24 µm). The differences reported are considered to fall within the limits of intraspecific variation.

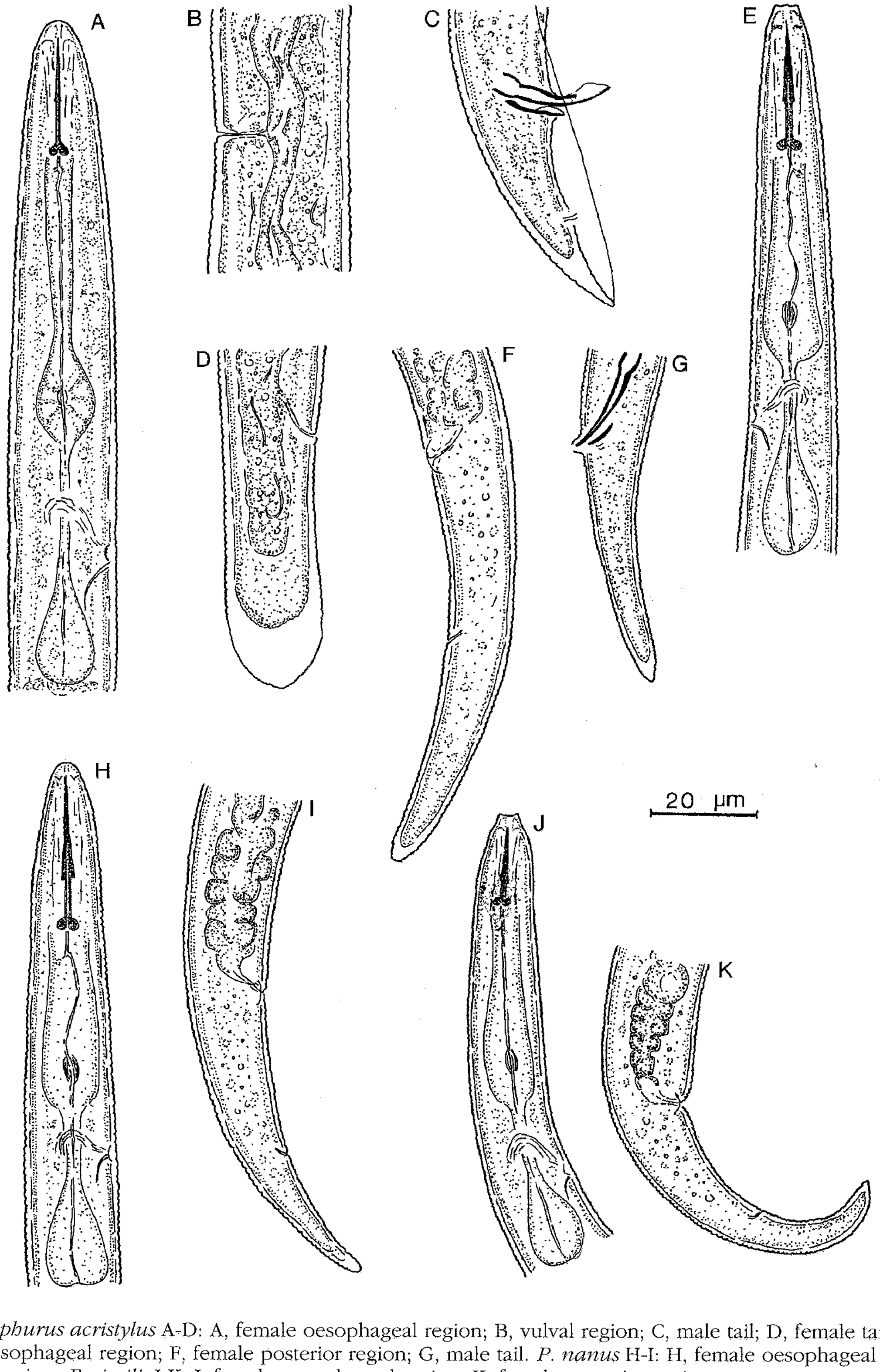

Fig. 1 - *Paratrophurus acristylus* A-D: A, female oesophageal region; B, vulval region; C, male tail; D, female tail. *P. sheri* E-G: E, female oesophageal region; F, female posterior region; G, male tail. *P. nanus* H-I: H, female oesophageal region; I, female posterior region. *P. similis* J-K: J, female oesophageal region; K, female posterior region.

PARATYLENCHUS SHERI (Raski, 1973) Siddiqi, 1986

(Fig. 1E-G)

Female (n = 12): L = 0.36-0.48 (0.42 ± 0.05) mm; a = 15.0-19.5 (17 ± 1.4); b = 3.5-4.2 (3.9 ± 0.2); c = 8.0-10.4 (9.2 ± 1.2); c' = 3.2-4.4 (3.7 ± 0.5); V = 76.5-83.3 (79.3 ± 2.8); stylet = 23.5-28.1 (25.6 ± 1.5) µm.

Body ventrally arcuate after fixation. Annules 1.3-1.4 µm wide at mid body. Lateral fields one sixth to one eight as wide as body, with four incisures. Maximum body width 20.0-32.0 (24.6 ± 4.3) µm. Lip region conoid with lateral protruding lips which give it a truncate or trapezoid appearance, 4.4-6.3 (5.5 ± 0.7) µm high x 6.8-9.5 (8.7 ± 1.1) µm wide, with inconspicuous annules. Framework with strong sclerotization. Stylet moderately robust, conus 13.0-17.4 (15.2 ± 1.5) µm long or 51.1-69 (59.7 ± 6.8) as percentage of the total stylet length. Basal knobs rounded, sloping posteriorly, 4-6 µm across. Opening of the DGO at 5-6 µm from the stylet base. Oesophagus length 86.7-111.6 (102.4 ± 8.3) µm. Excretory pore 70.0-96.0 (80.3 ± 8.8) µm from anterior end, opposite junction isthmus-basal bulb to anterior portion basal bulb. Hemizonid from one annule anterior to one annule posterior to excretory pore and two or three annules wide. Deirids four annules anterior to excretory pore. Vulva 283.0-385.0 (333.3 ± 35.5) µm from anterior end. Lateral vulval membranes rounded, 6-7 µm wide. Gonad well developed and outstretched, G_1 = 34.3-40.7 (36.7 ± 3.5). Spermatheca round, 14 µm diameter, usually with sperm 1.5 µm in diameter. Tail conoid, 38.8-47.5 (42.3 ± 4.2) µm long and bearing 31-41 (37 ± 4) annules, tapering gradually to a rounded terminus.

Male (n = 3): L = 0.37-0.43 (0.40 ± 0.03) mm; a = 20.5-21.8 (21.2 ± 0.5); b = 3.7-4.6 (4.2 ± 0.4); c = 8.8-9.3 (9.1 ± 0.2); c' = 3.6-4.8 (4.1 ± 0.5); spicules = 22.0-24.3 (23.1 ± 1.1) µm; gubernaculum 5.3-6.8 (6.0 ± 0.7) µm.

Body straight. Lateral field with four incisures. Head conical to trapezoidal, with lateral projections and inconspicuous annules. Oesophagus degenerate. Spicules slightly arcuate. Tail 40-46.5 (43.7 ± 2.7) µm long with rounded terminus, enveloped by bursa and bearing 35-44 annules.

This population resembles *Paratylenchus sheri* (Raski) Siddiqi and *Paratylenchus israeliensis* (Raski) Siddiqi in having lateral fields with four incisures, body annules distinct, head conoid with lips projecting in a rectangular outline, stylet 20-28 µm long and a strong sclerotization in the head region.

From the original description of *P. sheri* (Raski, 1973) it differs in having a longer female stylet (23.5-28.1 *vs* 20-23 µm), lower "a" value (15.0-19.5 *vs* 19-28), lower "c" value (8.0-10.4 *vs* 10-14) and shorter males (L = 0.37-0.43 *vs* 0.47 mm) with shorter spicules (22.0-24.3 *vs* 27 µm) and longer gubernaculum (5.3-6.8 *vs* 4 µm). Some of these differences are reduced or eliminated when considering the description of other populations identified as *P. sheri* (Gómez-Barcina *et al.*, 1990; Brzeski, 1995), which gave a wider range of variation for the species, but the longer female stylet (23.5-28.1 *vs* 18.3-25 µm), shorter males (L = 0.37-0.43 *vs* 0.43-0.54 mm) and shorter spicules (22.0-24.3 *vs* 24-28 µm) are still differential characters.

From *P. israeliensis* it differs only in having sperm in the spermatheca, males present and a sclerotization in head region not as strong as described by Raski (1973). However, the sclerotization is similar to that described in *P. sheri* and observed in one of its paratypes deposited in Rothamsted Experimental Station.

Differences between *P. sheri* and *P. israeliensis* were based on the stylet length, a stronger sclerotization of the head in *P. israeliensis* and different outline of lateral fields in cross section. In our opinion differences in sclerotization of the head region and outline of lateral fields are weak and not enough for differentiating species, and the values for stylet length are now overlapping between both species (18.5-25 µm for *P. sheri* and 24-28 μm for *P. israeliensis*). Therefore, we propose *P. israeliensis* as a new junior synonym of *P. sheri*.

The population described here is also considered as *P. sheri* and its shorter males with shorter spicules (L = 0.37-0.43 *vs* 0.43-0.54 mm; spicules = 22.0-24.3 *vs* 24-28 μm) merely represent an intraspecific variation.

PARATYLENCHUS NANUS Cobb, 1923
(Fig. 1H-I)

Female (n = 10): L = 0.32-0.36 (0.33 ± 0.01) mm; a = 16.8-21.5 (18.6 ± 1.5); b = 3.1-3.8 (3.4 ± 0.2); c = 11.2-15.5 (13.7 ± 1.4); c' = 1.8-2.3 (2.0 ± 0.2); V = 80.0-84.9 (83.4 ± 1.5); stylet = 24.5-29.0 (27.5 ± 1.5) μm.

Body ventrally arcuate after fixation. Cuticular annulation distinct, annules 1.3-1.6 μm wide at mid body. Lateral field with four incisures. Maximum body width 19.0-22.5 (20.8 ± 1.7) μm. Lip region conical-rounded, 3 μm high x 7 μm wide and continuous with body contour. Framework slightly sclerotized. Stylet robust, conus 15.0-19.0 (17.4 ± 1.6) μm long or 57.3-69.0 (63.2 ± 3.5) as percentage of stylet length. Stylet knobs rounded, sloping backwards, 4-5 μm across. Opening of the DGO 4-6 μm from the stylet base. Oesophagus length 86.3-100.9 (95.8 ± 5.0) μm. Ecretory pore 71.8-82.5 (76.5 ± 4.7) μm from anterior end, opposite posterior portion of isthmus. Hemizonid at level of excretory pore and two or three annules wide. Vulva transverse, 264.1-299.0 (275.3 ± 11.8) μm from anterior end. Vulval membranes rounded and 3-5 μm wide. Gonad well developed and outstretched. Spermatheca rounded, devoid of sperm. Tail conoid, 22.0-29.5 (24.3 ± 2.4) μm long and tapering gradually to a finely rounded or acute terminus.

Male (n = 1): L = 0.340 mm; a = 18; c = 11.2; c' = 2.2; spicules = 21.3 μm. Without stylet. Tail conoid with a finely rounded terminus.

Our population agrees with the description given by Cobb (1923) and our measurements are within the range of variation given by Brzeski (1995).

PARATYLENCHUS SIMILIS Khan, Prasad *et* Mathur, 1967
(Fig. 1J-K)

Female (n = 4): L = 0.26-0.34 (0.29 ± 0.03) mm; a = 12.5-17.0 (14.7 ± 2.2); b = 3.4-3.6 (3.5 ± 0.1); c = 11.6-12.5 (12.0 ± 0.5); c' = 3.3-3.8 (3.5 ± 0.2); V = 81.5-84.6 (83.2 ± 1.4); stylet = 15.8-17.5 (16.6 ± 0.8) μm.

Body ventrally arcuate forming an open C-shape after fixation. Cuticular annulation very fine but distinct, more pronounced in posterior part of body, annules up to 1 μm wide. Lateral fields with four equidistant incisures. Maximum body width 17.5-21.5 (19.8 ± 1.7) μm. Lip region conoid with lateral protruding lips which give a truncate or trapezoid appearance and with inconspicuous annules. Labial framework slightly sclerotized. Stylet robust, conus 10.5-11.7 (11.1 ± 0.6) μm long or 66-68% of the total stylet length. Basal knobs rounded, sloping laterally and 2.5-3 μm across. Opening of the DGO at 4-5 μm from the stylet base. Oesophagus length 74.7-84.0 (79.6 ± 4.3) μm. Excretory pore 59.5-69.8 (63.7 ± 4.2) μm from anterior end, opposite anterior portion of basal bulb. Hemizonid just anterior to excretory pore and three annules wide. Vulva transverse, 213.5-285.0 (244.0 ± 30) μm from anterior end. Vulval membranes rounded, 4 μm wide. Gonad well developed and outstretched, G_1 = 39.7-51.3 (47.5 ± 3.8). Spermatheca rounded, lacking sperm. Tail conoid tapering gradually to an acute-pointed terminus, 21.6-29.3 (25.7 ± 2.7) μm long.

Male: not found.

This population was originally identified as *Paratylenchus italiensis* Raski due to the acute tail terminus and female length, but we agree with the synonymization with *P. similis* proposed by Brzeski (1995). Our measurements are within the range of variation given by Brzeski (1995).

Acknowledgements. This work was carried out with the support of the training project AIR3-BM94-0356 financed by the Commission of the European Communities. The authors thank Dr. M. R. Siddiqi and Dr D. J. Hunt for the critical reading of this manuscript and their valuable suggestions.

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Accepted for publication on 6 February 1997.