

# On the morphology and distribution of *Rotylenchus agnetis* Szczygiel, 1968 (Nemata : Hoplolaimidae) in Bulgaria <sup>(1)</sup>

Vlada PENEVA \* and Sevdan NEDELICHEV \*\*

\* Institute of Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, 1113 Sofia, Bulgaria, and

\*\* University of Sofia, Laboratory of Plant Nematodes, D. Zankov Str. 8, 1421 Sofia, Bulgaria.

Accepted for publication 16 January 1991.

**Summary** — *Rotylenchus agnetis* Szczygiel, 1968 is widespread in forest nurseries in Bulgaria, occurring in different soil types. Information is given on its morphology, distribution and hosts in Bulgaria. This species shows a wide intraspecific variability. The phenomenon known as “ facultative intrauterine development ” was observed. Bulgarian populations were characterized by numerous males. *Rotylenchus aceri* Berezina, 1985 is considered a junior synonym of *R. agnetis*.

**Résumé** — *Morphologie et répartition de Rotylenchus agnetis Szczygiel, 1968 (Nemata : Hoplolaimidae) en Bulgarie* — *Rotylenchus agnetis* Szczygiel, 1968 est, en Bulgarie, une espèce fréquente en pépinières forestières et dans des sols variés. Des données sur la morphologie, la répartition et les plantes hôtes de cette espèce en Bulgarie sont présentées. *R. agnetis* présente une assez grande variabilité et le phénomène connu comme “ développement intra-utérin facultatif ” y a été observé. Les populations bulgares de cette espèce sont caractérisées par la présence de nombreux mâles. *Rotylenchus aceri* Berezina, 1985 est considéré comme un synonyme mineur de *R. agnetis*.

**Key-words** : *Rotylenchus*, morphology, development.

*Rotylenchus agnetis* was described by Szczygiel (1968) on female specimens. Later Nesterov (1979) described a *R. agnetis* population from Moldavia in which males were present. Yet, an identification key for this genus (Boag & Hooper, 1981), characterises *R. agnetis* as a species without males. Baydulova (1981) provided a further contribution on the morphology of this species from populations of *R. agnetis* from Western Kazakhstan.

Here we present observations on the morphology and distribution of *R. agnetis* from different localities and hosts in Bulgaria to supplement the available data and to clarify the taxonomic position of this species.

## Materials and methods

Nematodes were recovered from soil samples by a sieving and decanting technique, heated at 70 °C and fixed in TAF. Morphological examination was made with temporary and permanent mounts (Seinhorst, 1959; Paramonov, 1962). Specimens examined originated from the rhizosphere of fifteen seedling tree species, three herb plant species and from white poplar and raspberry near the town of Kostinbrod. The specimens were deposited in the Collection of the Institute of Parasitology, Sofia.

## *Rotylenchus agnetis* Szczygiel, 1968

(Figs 1, 2)

### MEASUREMENTS

See Table 1.

### DESCRIPTION

*Females* : Body posture upon fixation varying from a close spiral to an open C, mostly an open spiral; the posterior part tightly coiled. Head region clearly separated from the rest of the body, 4.5-6 µm high and 8-10 µm wide at the base. Basal ring with 12-24 segments formed by transverse or oblique striae. Basal and prebasal annuli often partially fused together. First body annulus very narrow. Cephalids hardly visible, observed in few specimens : anterior ones located between third and sixth annuli, and posterior ones between sixth and ninth. Metenchium almost as long as telenchium (M = 48.4-56.7). Stylet knobs rounded, anteriorly flattened to slightly concave. Orifice of dorsal oesophageal gland 5.5-7.5 µm behind stylet base (O = 16-23). Hemizonid two annuli long, just behind excretory pore; opposite the excretory pore in one specimen. Hemizonion one annulus long, 5-6 (10) annuli behind hemizonid. Dorsal lobe of oesophageal gland overlapping intestine by five or more annuli. Annuli 1.7-2.6 µm wide at mid-body.

(1) Part of the present study was reported at the Anniversary Scientific Meeting of the University of Sofia (1988).

**Table 1.** Measurements of *Rotylenchus agnetis* (males and females) from different localities and host plants in Bulgaria (in  $\mu\text{m}$  except L.).

	Manastircheto-Sandanski ( <i>Picea abies</i> )		Lazovo Dere-Velingrad ( <i>Picea abies</i> )		Cherepish-Mezdra ( <i>Pinus nigra</i> )		Kastata-Blagoevgrad ( <i>Pseudotsuga douglasii</i> )		Kostinbrod ( <i>Rubus idaeus</i> )	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
n	4	4	9	3	12	6	19	13	15	2
L (mm)	681	721	866	774	763	707	841	723	790	—
	653-730	678-748	773-960	706-879	671-826	579-812	706-950	677-806	700-890	720-790
a	27.7	34.2	30.7	33.4	31.0	34.1	31.9	35.0	26.4	—
	21.8-30.5	32.5-36.9	27.0-34.3	31.4-36.6	27.1-34.4	30.5-38.4	27.1-36.4	32.0-38.0	21.0-30.0	31.0-33.0
b	6.4	—	7.4	—	6.8	6.8	7.6	6.5	7.6	—
	6.3-6.5	5.8-7.0	7.1-7.8	6.5	6.2-7.6	6.2-7.4	6.2-8.3	6.1-7.1	5.7-8.0	6.0
b'	5.4	5.5	6.2	—	6.1	5.5	6.6	5.8	6.2	—
	4.8-5.9	5.1-5.7	5.7-6.7	5.4-6.0	5.3-7.2	4.8-6.7	5.3-7.6	5.2-6.1	5.2-7.4	5.3-5.7
c	52.3	32.1	71.2	38.7	50.9	35.4	58.5	35.9	52.1	—
	48.7-61.0	28.3-36.0	64.4-88.3	36.9-39.9	39.8-61.0	29.1-40.4	40.6-88.2	30.9-37.4	42.0-63.0	31.0-37.0
c'	0.9	—	0.6	—	0.9	—	0.9	—	0.9	—
	0.8-1.0	—	0.5-0.8	—	0.7-1.1	—	0.6-1.1	—	0.6-1.1	—
V	59.5	—	56	—	56.3	—	55.6	—	56.7	—
	57-63	—	55-59	—	52-59	—	50-60	—	53-60	—
Stylet	30.5	29	32.5	29	29.0	27	31	28	31	—
	30-31	28-30	30-36	27-30	26.5-32.0	25-28	29-34	27-31	30-33	30-32
Tail	13	23.5	12	20	15	20	15	20	16.5	—
	11-15	20.5-26.0	10-14	18-22	11-20	15-22	9-20	18-25	13-21	19-23
Ant. end excr. p.	103	106	121.5	—	106	101	105	102	109	—
	97-113	96-110	104-140	98-123	91-132	92-111	90-117	90-115	104-117	96-115
Tail annules	6-9	—	5-6	—	4-5	—	5-9	—	4-8	—
Phasmids	0-4	—	0-4	—	1-2 ant.	—	1-4 ant. to	—	2 ant. to	—
	ant. anus	—	ant. anus	—	1-2 post. anus	—	1-4 post. anus	—	1 post. anus	—
Spicules	—	26.5	—	26	—	25	—	25	—	—
	—	26-28	—	24-27	—	24-28	—	24-28	—	24-26
Gubernaculum	—	10.5	—	11	—	10	—	10	—	—
	—	10-11	—	10-13	—	9-11	—	9-11	—	9.6-10

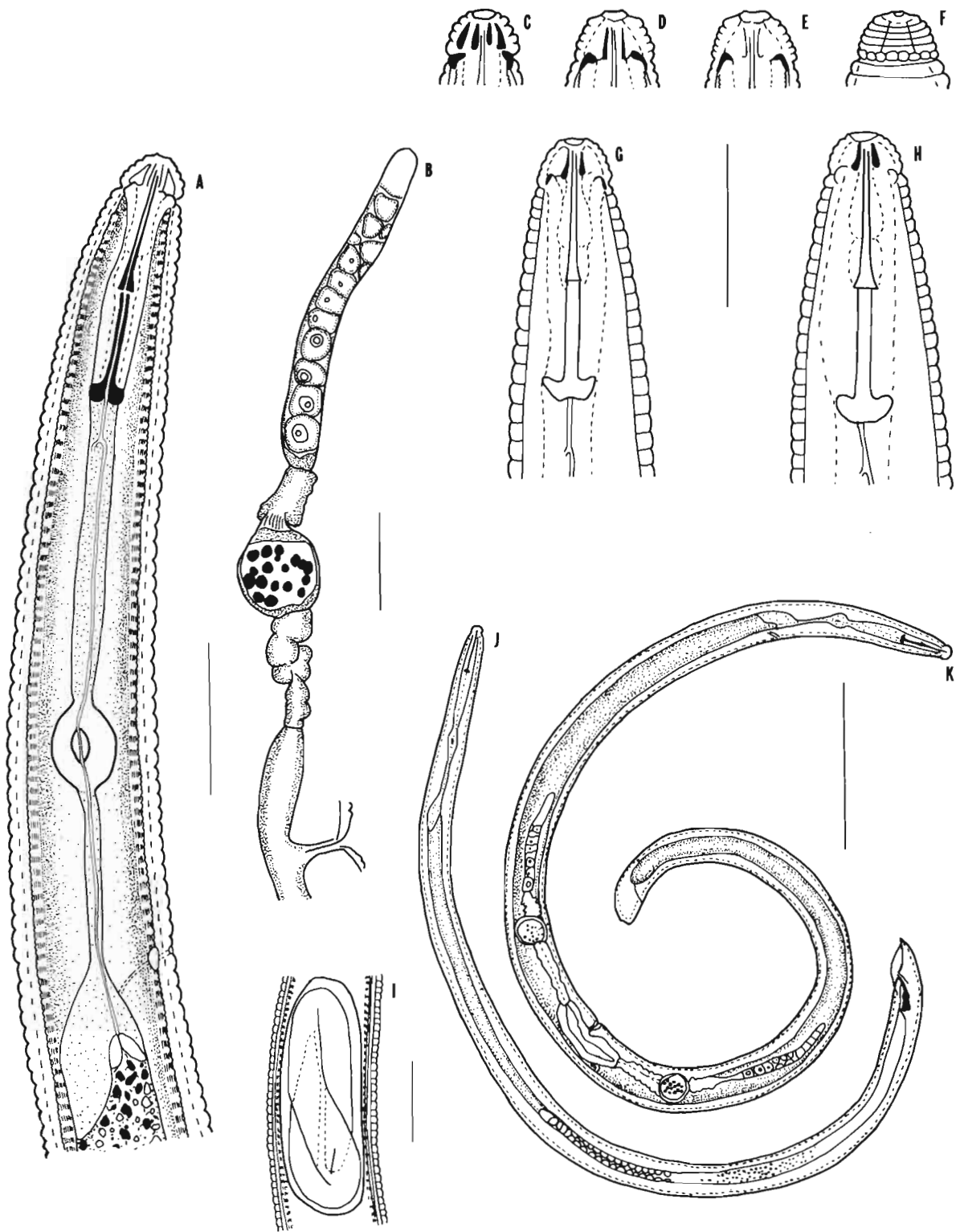
External lines of lateral field wavy. Tail bluntly conical, tapered, asymmetrical, terminus without annulation, often with large terminal annuli. In two specimens annulation continuing around the tip. Tail annuli four to nine, two specimens having eleven and twelve annuli. Phasmids one to five annuli anterior to anus or very rarely one to two posterior to anus. Caudalid observed in few specimens, five to six annuli anterior to anus. In some specimens body convex at anus level. Female reproductive system consisting of uterus, preuterine glands, spermatecae (rounded, oval, elongated) mostly filled with sperm, sphincters, oviducts and ovaries. Vulva without epiptygma.

An egg with developing larva was found in the uterus of a female in a population originating from the soil around roots of *Tilia grandifolia* (Dorostol forest nursery). The phenomenon known as "facultative intrauterine development" (Luc *et al.*, 1979; Kulinich, 1987) has not hitherto been reported in the genus *Rotylenchus*.

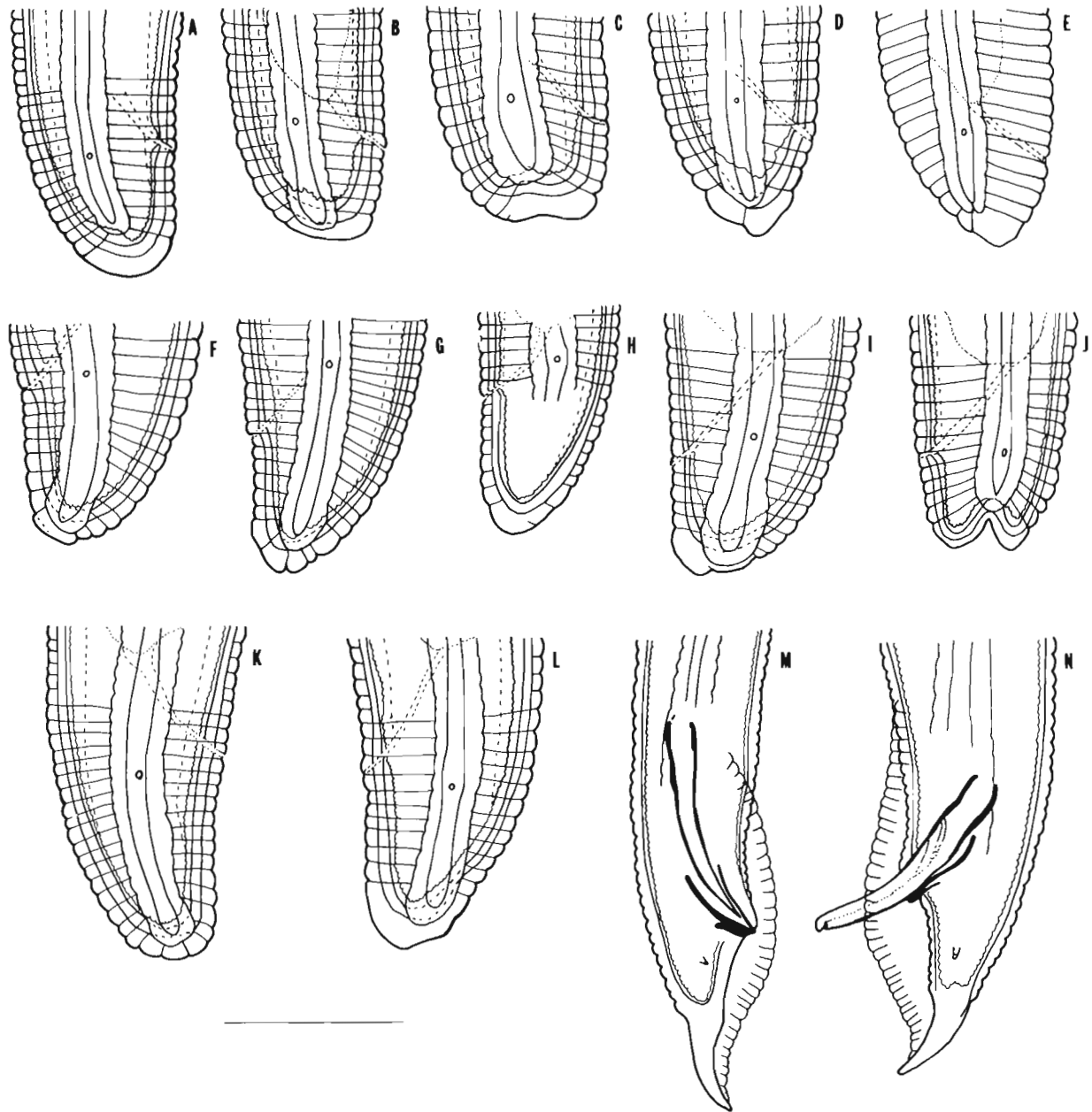
**Males :** Common. Body upon fixation curved ventrally mostly in the form of an open C, more slender than the females. Head region hemispherical, set off, 4.5-6  $\mu\text{m}$  high and 6-9  $\mu\text{m}$  wide. First body annulus as in female. Stylet shorter than in females and ranging from 25 to 30  $\mu\text{m}$ . Orifice of dorsal oesophageal gland 5-7.5  $\mu\text{m}$  behind stylet base ( $O = 18.5-24.1$ ). Oesophageal glands, hemizonid and hemizonion similar to female. Testis well-developed. Spicules slightly curved, with rounded head and weakly enlarged central part. Gubernaculum slightly protruding. Bursa enveloping tail. Phasmids on the tail or opposite cloaca.

#### DISTRIBUTION

*R. agnetis* is widespread in forest nurseries in Bulgaria occurring in more than half the nurseries studied. It was found in the rhizosphere of a large number of plant species and in different soil types (Table 2). The most numerous populations of *R. agnetis* were found in the rhizosphere of *Larix europaea* (150 specimens/100  $\text{cm}^3$



**Fig. 1.** *Rotylenchus agnetis*. Female. A : Oesophageal part; B : Female reproductive system; C, D, E, F : Head ends; G, H : Stylet regions; I : Egg with a developing larva in the uterus; K : General view. Male. J : General view. (Scale bars : A — F : 20  $\mu$ m, J, K : 100  $\mu$ m.)



**Fig. 2.** *Rotylenchus agnetis*. Female. A-M : Variations of the tail. A, J, L : Kashtata, *Pseudotsuga douglasii*; B, C : Lazovo Dere, *Picea abies*; D : Cherepish, *Pinus sylvestris*; E : Kostinbrod, *Populus alba*; F : Pomorie, *Abies cephalonica*; G : Dorostol, *Quercus rubra*; H : Manastircheto, *Picea abies*; I : Kashtata, *Pinus nigra* v. *calabrica*; K : Buzovgrad, *Pinus nigra*; Male. M : Kashtata, *Larix europea*; N : Kostinbrod, *Populus alba*. (Scale bars : A — N : 20  $\mu$ m.)

soil) and *P. douglasii* (90 specimens/100 cm<sup>3</sup>) in Kash-tata; *Tilia* sp. (108 specimens/100 cm<sup>3</sup>) in Tirnovtzi; *P. abies* (75 specimens/100 cm<sup>3</sup>) in Lazovo Dere; *C. atlantica* (51 specimens/100 cm<sup>3</sup>) and *C. deodara* (42 speci-mens/100 cm<sup>3</sup>) in Cherepish.

#### Remarks

In general, our data on morphology of *R. agnetis* fitted well within the range of variation recorded for this species (Table 3) for both quantitative and qualitative

**Table 2.** Distribution of *Rotylenchus agnetis* in the nurseries studied.

Forest nurseries	Above sea level (m)	Soil type	Soil composition	Host plants
Buzovgrad (Kazanlak)	380	cinnamonic forest	slightly loamy	<i>Pinus nigra</i>
Dorostol (Silistra)	100	leached chernozem	heavy and moderately loamy	<i>Tilia grandifolia</i> <i>Quercus rubra</i>
Zlatarevo (Parvomai)	200	alluvial	sandy loamy	fallow land after <i>Castanea sativa</i> , <i>Cerasus machaleb</i>
Kalusha (Simitli)	280	alluvial	sandy loamy	<i>Pinus nigra</i>
Kastata (Blagoevgrad)	400	alluvial	slightly loamy	<i>Pinus nigra</i> , <i>V. calabrica</i> , <i>Larix europaea</i> , <i>Syringa vulgaris</i> , <i>Pseudotsuga douglasii</i>
Lazovo Dere (Velingrad)	880	rendzinas	sandy loamy	<i>Picea abies</i> , <i>Cynodon dactylon</i> , <i>Taraxacum</i> sp.
Manastircheto (Sandanski)	300	alluvial meadow	sandy loamy	<i>Picea abies</i>
Pomorie (Nesebar)	10	leached chernozem smolnitzi	heavy loamy clayly	<i>Abies cephalonica</i>
Timovtzi (Tutrakan)	100	leached chernozem	moderately and heavy loamy	<i>Quercus rubra</i> , <i>Pyrus pyraster</i> <i>Tilia</i> sp.
Cherepish (Mezdra)	310	light grey forest	sandy loamy moderately and heavy loamy	<i>Cedrus atlantica</i> , <i>C. deodara</i> <i>Pinus nigra</i> , <i>P. sylvestris</i> <i>Portulaca oleraceae</i>
Cherna Gora (Burgas)	50	cinnamonic forest	heavy loamy	<i>Betula alba</i> , <i>Quercus rubra</i>
Chiflika (Karlovo)	350	deluvial	sandy loamy slightly loamy	<i>Pinus sylvestris</i>
Kostinbrod	600	alluvial		<i>Populus alba</i> , <i>Rubus idaeus</i>

characters such as body length, ratios a, b, b', c, V, O, M, body posture, head structure, shape of stylet knobs, structure of oesophageal glands, position of excretory pore, phasmids, characteristics of the tail. However, certain differences were observed in the average female body length values between the populations from forest nurseries Manastircheto (*P. abies*, L = 681 µm), Dorostol (*Q. rubra*, L = 748 µm, n = 4), Cherepish (*P. nigra*, L = 763 µm) and those reported by Szczygiel (1968), Nesterov (1979) and Baydulova (1981) (Table 3). Specimens from Cherepish (*C. deodara*, c = 61.6 (54-74), n = 4), Lazovo Dere (*P. abies*, c = 71.2 (64.4-88.3) and Kostinbrod (*P. alba*, c = 66.5 (57-63), n = 15) had shorter tails than the type material. Stylet length of the females studied differed from the type specimens and other European populations both in range and average values. Females from Bulgaria were characterized by a shorter stylet (26-34 µm against 32-38 µm). In males from Moldavia (Nesterov, 1979) the stylet had the same length as in females and varied from 32 to 35 µm. Males in our material possessed a shorter and

weaker stylet in comparison with the females belonging to the same population. Nesterov (1979) recorded spicule length as being between 30 and 40 µm but spicule length measured from the drawing is only 27 µm.

*Rotylenchus aceri* Berezina, 1985 has been recently described from the rhizosphere of *Acer campestre* in the North Caucasus. This species is said to be very similar to *R. agnetis*. The author considered body length (0.81-1.03 mm to 0.66-0.96 mm), distance between the stylet base and the orifice of the dorsal oesophageal gland (O = 22-27 to 18-24) and presence of males as the only distinguishing features. Examination of nine paratype females and one male specimen confirmed the original description except for O and M values (O = 19.7 (17.7-21.6) and M = 54 (51.4-56.8) in females (n = 5) and M = 54.7 in male) that are within the limits of variation known for *R. agnetis*. Also, the data reported by Baydulova (1981) on *R. agnetis* are almost identical with those given by Berezina (1985), especially with regard to the body and stylet lengths. Type population of *R. aceri* resembles two populations of *R. agnetis* from

**Table 3.** Measurements of some populations of *Rotylenchus agnetis* and *Rotylenchus aceri* (in  $\mu\text{m}$  except L).

	<i>R. agnetis</i>			<i>R. aceri</i>		
	Poland <i>Fragaria × ananassa</i> (Szczygiel, 1968) Females	Moldavia <i>Malus domestica</i> (Nesterov, 1979) Females      Males		W. Kazakhstan <i>Medicago sativa</i> (Baydulova, 1981) Females	North Caucasus <i>Acer campestre</i> (Berezina, 1985) Females      Males	
n	8	—	—	15	20	9
L (mm)	0.84 0.66-0.96	0.66-0.96	0.68-0.96	0.88 0.82-0.96	0.9 0.81-1.03	0.78 0.74-0.84
a	25.8 23-27	— 23-35	— 30-38	28 26-31	27 25.5-35.2	26.9 25-29
b	—	—	—	6.5 6.0-7.3	7.0 6.1-7.5	5.9 4.8-7.0
b'	6.0 5.1-6.8	— 5.1-6.8	— 4.8-5.9	5.9 5.3-6.2	5.8 5.2-6.6	5.2 4.4-5.9
c	54.6 46-64	— 46-64	— 48-53	46.6 42-57	66.7 51.6-88.8	33 25.4-37.5
c'	—	—	—	0.9 0.8-1.0	0.6 0.4-0.7	—
V	57.6 52-59	— 52-59	—	57 56-59	57 54-59	—
M	—	—	—	— 52-54	47 45-50	—
O	21.9 18-24	—	—	20 18-22	24.5 22-27	—
Stylet	33 32-35	— 32-35	— 32-35	33 32.5-39	36 32-39	31 31-32
Spicules	—	—	27 (35-40?)	—	—	—
Gubernaculum	—	—	—	—	—	30-38
Tail	—	—	—	—	—	11-14
	—	—	—	—	13.5 11.5-17.5	—

Bulgaria (Lazovo Dere, Kashtata). The presence or absence of males cannot be used for distinguishing the two species since *R. agnetis* males were described by Nesterov (1979). Considering the above data it is concluded that *R. aceri* Berezina, 1985 is a junior synonym of *R. agnetis* Szczygiel, 1968.

#### Acknowledgments

We are indebted to Dr. S. Subbotin for providing the type specimens of *R. aceri* for study and to Dr. Derek Brown for critical reading of the manuscript and helpful suggestions.

#### References

- BAYDULOVA, L. A. (1981). [Nematodes of the family Hoplolaimidae in Western Kazakhstan.] *Parazitologiya*, 15 : 83-86.
- BEREZINA, N. B. (1985). [A new species *Rotylenchus aceri* n. sp. (Nematoda : Tylenchida) from the rhizosphere of *Acer campestre* (L.).] *Byull. Vsesoy. Inst. Gel'mint im. K. I. Skryabina*, 41 : 89-91.
- BOAG, B. & HOOPER, D. J. (1981). *Rotylenchus ouensensis* n. sp. (Nematoda : Hoplolaimidae) from the British Isles. *Syst. Parasitol.*, 3 : 119-125.
- KRALL, E. L. (1978). [Root-parasitic nematodes. Family Hoplolaimidae. (Keys to the nematodes of plants, soils and insects. No. 5).] Leningrad, USSR, Nauka, Leningradskoe otdelenie, 424 p.
- KULINICH, O. A. (1987). [Facultative intrauterine development in phytonematodes.] *Parazitologiya*, 21 : 78-80.
- LUC, M., TAYLOR, D. T. & NETSCHER, C. (1979). On endotokia matricida and intra-uterine development and hatching in nematodes. *Nematologica*, 25 : 268-274.
- NESTEROV, P. I. (1979). [Plant-parasitic and free-living nematodes in South-Eastern USSR.] Kishinev, USSR, Shtiintza, 314 p.
- PARAMONOV, A. A. (1962). [Principles of phytohelminthology.] Volum I, Moskva, Nauka, 479 p.
- SEINHORST, I. W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica*, 4 : 67-69.
- SZCZYGIEL, A. (1968). *Rotylenchus agnetis* sp. n. (Nematoda, Hoplolaimidae) from Poland. *Bull. Acad. Pol. Sci.*, 16 : 573-575.