SOME NEMATODES BELONGING TO THE GENUS *TYLENCHORHYNCHUS* COBB, 1913 (NEMATODA: BELONOLAIMIDAE), FROM ROMANIA

M. Ciobanu[1], G. Karssen[2] * and I. Popovici[1]

[1] *Institute of Biological Research, Department of Taxonomy and Ecology, 48 Republicii Street, 3400 Cluj-Napoca, Romania*
[2] *Plant Protection Service, P.O. Box 9102, 6700 HC Wageningen, the Netherlands*

Summary. Specimens belonging to three known species of *Tylenchorhynchus* (*T. agri*, *T. dubius* and *T. maximus*) collected from various localities in Romania were studied by light microscopy. Additional morphometrics, illustrations and data referring to their habitat are provided. The geographical distribution of *T. dubius* and *T. maximus* in Romania is broadened. A description and illustration of a previously undescribed species, based upon a single female, is given.

The nematodes belonging to the genus *Tylenchorhynchus* Cobb, 1913 are ectoparasites of roots and common in various soil types. According to Brzeski (1998), the genus is very heterogeneous, therefore requiring taxonomic revision.

Six species of *Tylenchorhynchus* have been reported so far from Romania: *T. agri* Ferris, 1963 from Maliuc (located in the Danube Delta) (Popovici, 1992); *T. clarus* Allen, 1955 from Maliuc (forest) and Enisala (wet soil) also located in the Danube Delta (Popovici, 1992); *T. cylindricus* Cobb, 1913 from grasslands near the locality Cluj-Napoca (Popovici, 1973, 1974); *T. dubius* (Bütschli, 1873) Filip'ev, 1936 from several localities: soil around *Mentha* sp. roots at Plavisevica (Andrássy, 1959), grasslands near Cluj-Napoca (Popovici, 1974), in the Tureni Gorges (Trascău Mountains), at Piatra Cloşani (Mehedinti Mountains) (Popovici, 1998) and from vegetated cliffs in various locations in the Romanian Carpathian Mountains (Popovici and Ciobanu, 1997); *T. maximus* Allen, 1955 from grassland at Piule (Retezat Mountains) (Popovici, 1993, 1998); and *T. obscurisulcatus* Andrassy, 1959 from soil around *Artemisia* sp. roots, Cerna, Dobrudscha (Andrássy, 1959).

Apart from the paper of Andrássy (1959) on *T. obscurisulcatus*, in which he provided good description and illustration, basic measurements and illustration were given for *T. cylindricus* (Popovici, 1974) but only basic measurements and short descriptions for *T. agri* and *T. clarus* (Popovici, 1992).

Specimens of *Tylenchorhynchus* not yet identified at the species level, as well as some individuals that have undergone preliminary identification and are stored in the nematode collection of the Institute of Biological Research, were studied. More morphometrics, illustrations and data referring to their habitats are provided for *T. agri*, *T. dubius* and *T. maximus* and the geographical distribution of *T. dubius* and *T. maximus* in Romania is broadened.

One undescribed species was identified in the Romanian material; the measurements and illustration are provided based upon a single female.

Data on the presence and distribution of the species have been included in the Romanian nematode fauna database. The paper is also a contribution towards an inventory of the species belonging to the genus *Tylenchorhynchus* in Romania.

MATERIALS AND METHODS

Soil samples were collected by two of us (M.C. and I.P.) during an ecological survey carried out between 1991 and 1997. Seven sites, including grasslands, vegetated cliffs, one *Populus* sp. plantation and one technogenic soil, were investigated, and information on their altitude, geographical position, plant association and soil type are given in Table I. Nematodes were extracted using the centrifugal method of de Grisse (1969), killed and preserved in a 4% formaldehyde solution heated to 65 °C, mounted in anhydrous glycerin (Seinhorst, 1959) and examined by light microscopy using an Olympus BH-2 light microscope with differential interference contrast. Photographs were taken using a digital Leica DC 300 camera.

The following papers were used for the identification of the species: Tarjan (1973); Fortuner and Luc (1987); Anderson and Potter (1991); Brzeski (1998) and Handoo (2000).

All measurements in the tables are in µm; average values and range were calculated for each measurement if more than two specimens were collected from a single site.

Plant associations' classification was used according to Coldea (1991). Soil types were classified according to the Romanian System of Soil Classification (Conea *et al.*, 1980).

* Corresponding author: g.karssen@minlnv.nl.

DESCRIPTIONS

Tylenchorhynchus agri Ferris, 1963
(Table II; Fig. 1 A-C)

Female body more or less ventrally arcuate, cuticular annulation distinct. Lateral field with outer margin crenated. Head slightly off-set, with four annules, first neck annule posterior to head slightly reduced. Stylet 19.5-20.5 µm long, basal knobs set-off from the shaft. Median bulb ovoid, basal bulb elongate pyriform, not overlapping the intestine. Epiptygma observed in one of the two females. Spermatheca relatively small, ovoid, either empty or filled with ovoid sperm, about 2.0-2.5 µm in diameter. Tail sub-cylindrical with eighteen-nineteen annules, tail terminus sub-hemispherical, smooth. Post-anal intestinal sac occupying 53.8-58.3% of the tail length. Phasmid located at the level of the seventh and the thirteenth annule posterior to anus.

Male not found.

Distribution: soil collected from *Populus* sp. plantation from Maliuc (Danube Delta), site no. 7 (Table I).

Remarks: *T. agri* was originally reported from soil collected from a field cropped with corn for 85 years located in Urbana, Illinois, U.S.A. It was also reported in Southern Europe from soil collected from a barley field located at Misrah Suffara, Malta (Larizza and Lamberti, 1995) and from the rhizosphere of *Persea americana* Mill. (avocado) from Greece (Koliopanos and Kalyviotis-Gazelas, 1979). *Tylenchorhynchus agri* was only recorded in Romania from Maliuc (in the Danube Delta) (Popovici, 1992).

It is probably a very rare species in Romania.

Tylenchorhynchus dubius (Bütschli, 1873) Filip'ev, 1936
(Table III; Fig. 2)

Female body ventrally arcuate, cuticle variously wrinkled anterior and/or posterior to vulva (observed in preserved and living specimens). Spores of *Pasteuria* sp. attached to the cuticle were observed in some specimens. Lateral field with outer margin crenated. Head from slightly to well off-set, with five-six annules. Stylet 16.5-20.0 µm long, basal knobs sloping backwards. Median bulb ovoid, basal bulb elongate pyriform, not overlapping the intestine. Body posterior to cardia with distinct fasciculi. Vulva clearly expressed outside the body contour in some females. Epiptygma observed in some females. Spermatheca rounded, empty, (except in one female, in which it was filled with ovoid sperm, about 2.5-3.0 µm in diameter). Tail cylindrical or sub-cylindrical (a tail slightly clavate was observed only in one female) with 36-66 annules, tail terminus hemispherical or sub-hemispherical, annulated. Phasmid located between the ninth and the twenty-first annule posterior to anus.

Male slightly larger than female, with broad bursa, spicules 21 µm long, gubernaculum 7 µm long.

Distribution: four locations, sites nos 1-4 (Table I), including three grasslands and one technogenic soil.

Remarks: *Tylenchorhynchus dubius* was previously reported from several locations from Romania (Andrássy, 1959; Popovici, 1974, 1998) and even from vegetated cliffs of the Romanian Carpathians (Popovici and Ciobanu, 1997). One of the habitats from which the specimens were collected was a technogenic soil under bioremediation at Rodna Veche (Rodnei Mts.), characterized

Table I. Site locations, vegetation and soil types of a nematological survey in Romania.

Site nr.	Locality	Altitude (m)	Geographical position	Plant association	Soil type
1	Fânaţele Clujului (Someşan Plateau)	350	46°45'N-23°35'E	*Jurineo transsilvanicae-Stipetum pulcherimae*	Chernozem
2	Suatu (Transylvanian Plain)	370-450	46°46'N-23°58'E	*Salvio nutantis-Festucetum rupicolae*	Not available
3	Tureni Gorges (Trascău Mts.[1])	400	46°30'N-23°41'E	*Melico-Phleetum montani*	Lithic rendzina
4	Rodna Veche (Rodnei Mts.)	525	47°25'N-24°26'E	-	Technogenic soil[2]
5	Suhardu Mic (Hăşmaş Mts.)	1450	46°43'N-25°36'E	-	Undeveloped soil
6	Muntele Roşu (Ciucaş Mts.)	1500	45°26'N-25°52'E	*Violo declinatae-Nardetum*	Cambic rendzina
7	Maliuc (Danube Delta)	12	45°13'N-29°04'E	-	Not available

[1] Mts.=Mountains
[2] Soil from mining spoil containing Pb and Zn impurities under bioremediation.

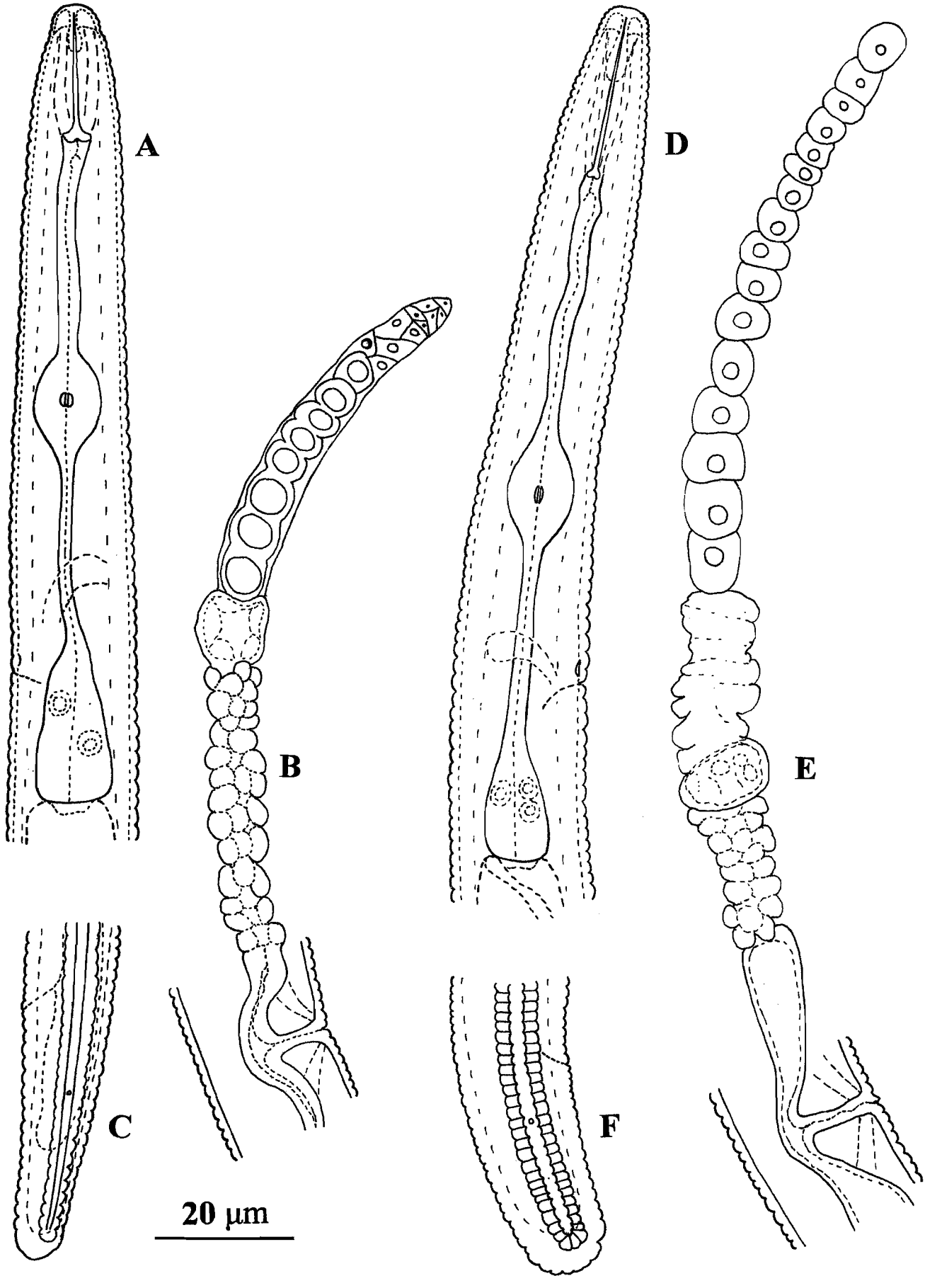

Fig. 1. *Tylenchorhynchus agri*: female; A, anterior end; B, reproductive system; C, tail. *Tylenchorhynchus* sp.: female; D, anterior end; E, reproductive system; F, tail.

Table II. Measurements and diagnostic features of *Tylenchorhynchus agri, Tylenchorhynchus maximus* and *Tylenchorhynchus* sp. (all measurements in µm).

Species:	*Tylenchorhynchus agri*		*Tylenchorhynchus maximus*	*Tylenchorhynchus* sp.
Habitat :	*Populus* sp. plantation		Cliffs vegetated by *Sedum* sp. and *Gentiana* sp.	Grassland
Locality:	Maulic (Danube Delta)		Suhardu Mic (Hăşmaş Mts.)	Muntele Roşu (Ciucaş Mts.)
n	2 ♀♀ [1]	1 ♀ [2]	2 ♀♀	1 ♀
L	726, 730	600	1490, 1490	846
a	34.8, 36.1	30.3	56.1, 51.2	38.3
b	5.6, 5.6	4.8	8.3, 8.5	6.7
c	19.2, 17.8	16.2	22.9, 26.8	27.9
c'	2.9, 2.8	3.0	3.1, ?	1.8
V%	56.2, 57.9	56.7	50.9, 53.7	58.2
Head width	7.0, 7.0		8, 7.5	7.5
Head height	3, 4		4, 3	4
Conus	11, 9.5		14, 13.5	14
Shaft	9.5, 10		10, 10	11.5
Pharynx	131, 131	125	180, 175	126
Ant. part. pharynx[3]	76, 74		109, 101	79
Post. part. pharynx[4]	55, 57		71, 74	47
Ant. part.% pharynx	58.0, 56.5		60.4, 57.8	62.8
Excretory pore	104, 111		146, 144	101
Head - vulva	408, 422		758, 800	493
Tail	38, 41	37	65, 56	30
Body width	21, 20	20	27, 29	22
Lateral field width	6, 5		9, 9	6
Anal body width	13, 15	12.5	21, ?	16

[1] Present study
[2] Popovici (1992)
[3] Measured from anterior body end to the posterior end of median bulb.
[4] Measured from the posterior end of median bulb to pharyngeo-intestinal junction.

by low nutrient resources and by high concentrations of Pb and Zn impurities (Paşca *et al.*, 1997). These data suggest that *T. dubius* has a high capacity to withstand heavily polluted environments.

Acording to Brzeski (1998), it is a very common species in arable and meadow soils, especially frequent and numerous in acidic, coarsely textured soils.

Spores of *Pasteuria* sp. attached to the cuticle were observed in two females (one collected from site no. 3 and one from site no. 4) (Fig. 2C). Such associations between the *Pasteuria penetrans* group and *T. dubius* have been reported from Belgium (Coomans, 1962), Germany (Sturhan, 1985), the Netherlands (Kuiper, 1958), Scotland (Prasad, 1971 quoted in Sayre and Starr, 1988) and the U.S.A. (Esser, 1980).

It is probably the most common species of the genus *Tylenchorhynchus* in Romania.

Tylenchorhynchus maximus Allen, 1955
(Table II; Fig. 3)

Female body C- to spiral-shaped, cuticular annulation distinct. Lateral field regularly areolated, outer margin strongly crenated. Head almost continuous, with five-six annules. Stylet 23.5-24.0 µm long, slender, basal knobs relatively small compared to stylet, sloping backwards. Median bulb oval, basal bulb pyriform, not over-

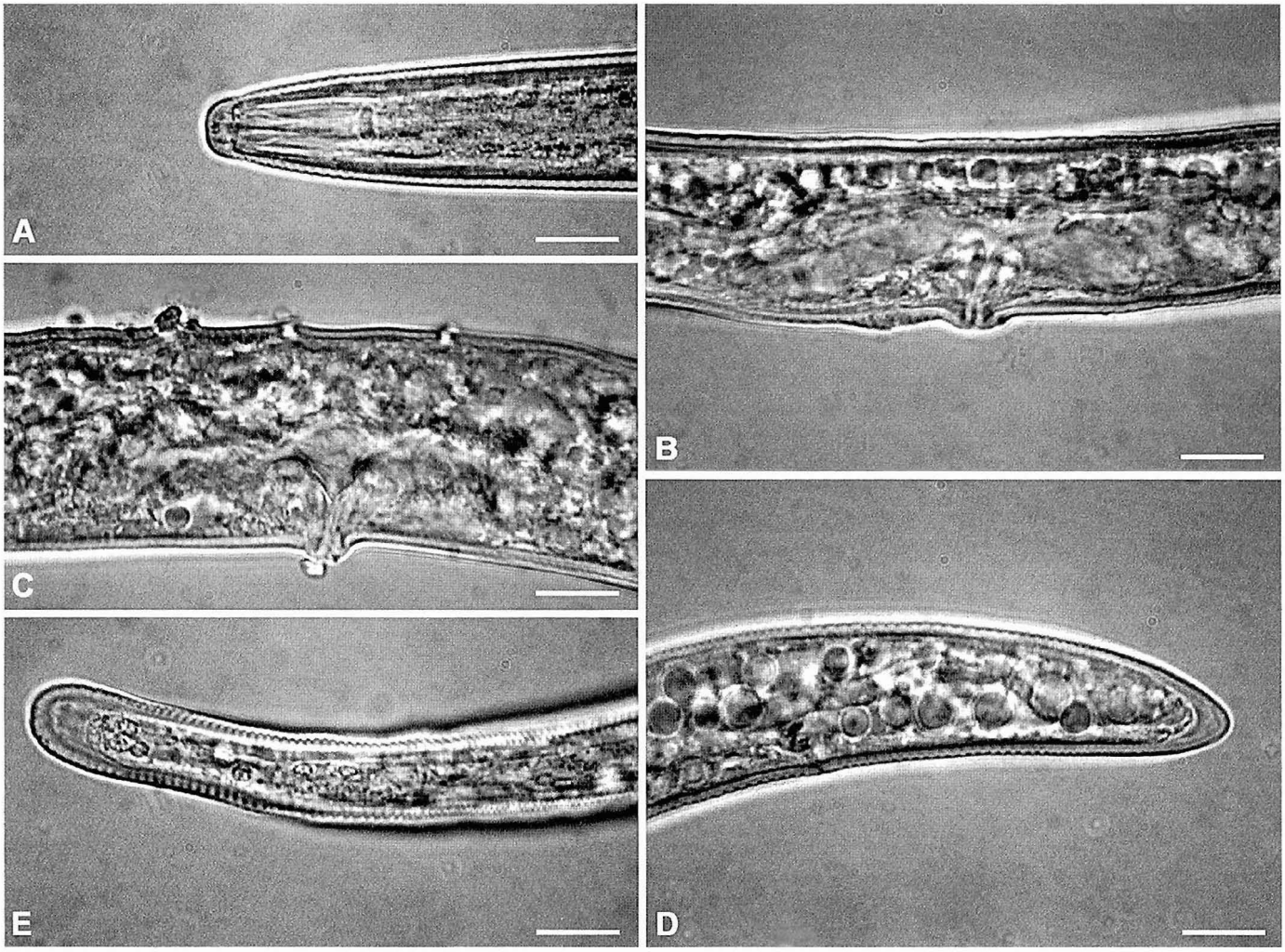

Fig. 2. *Tylenchorhynchus dubius*: female; A, anterior end; B, vulva region; C, spores of *Pasteuria* sp. attached to cuticle; D, E, posterior body region (Scale bar = 10 µm).

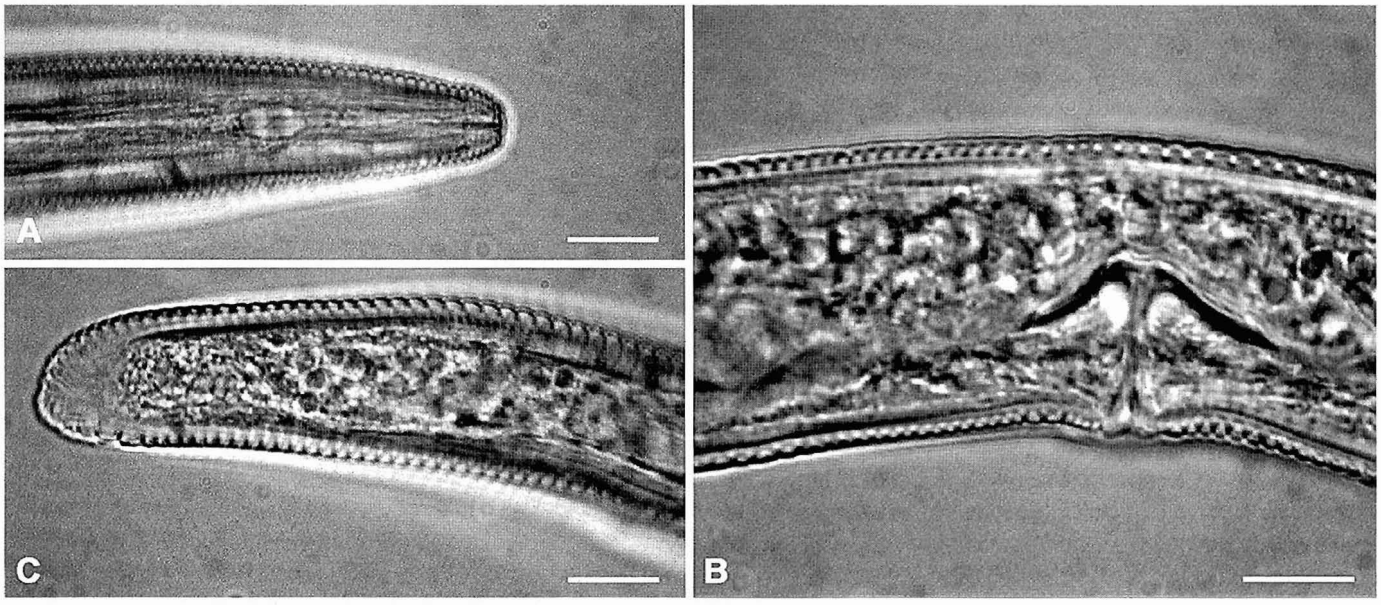

Fig. 3. *Tylenchorhynchus maximus*: female; A, Anterior end; B, vulva region; C, posterior body region (Scale bar = 10 µm).

Table III. Measurements and diagnostic features of *Tylenchorhynchus dubius* (all measurements in µm).

Habitat :	Grassland		Grassland	Grassland	Technogenic soil under bioremediation
Locality :	Fânaţele Clujului (Someşan Plateau)		Suatu (Transylvanian Plain)	Tureni Gorges (Trascău Mts.)	Rodna Veche (Rodnei Mts.)
n	3 ♀♀	1 ♂	9 ♀♀	2 ♀♀	3 ♀♀
L	754.7±33.3(718-784)	848	694.7±64.5(607-797)	821, 856	782.4±100.1(672-867)
a	34.6±3.3(30.7-36.5)	37.3	31.7±1.6(28.9-33.2)	31.7, 33.9	32.9±0.3(32.7-33.2)
b	6.2±0.2(6.0-6.4)	7.0	5.8±0.8(5.0-7.5)	6.9, 6.4	7.1±0.6(6.4-7.7)
c	17.2±1.4(16.2-18.8)	17.0	14.1±1.6(11.3-16.3)	14.8, 13.5	17.1±1.6(15.2-18.3)
c'	3.1±0.4(2.7-3.5)	2.6	2.9±0.3(2.6-3.4)	2.8, 3.3	2.6±0.2(2.3-2.7)
V%	54.8±1.7(53.0-56.3)		55.0±1.9(51.9-58.7)	52.6, 53.6	55.8±1.2(54.8-57.1)
Head width	6.8±0.3(6.5-7.0)	7.0	7.4±0.5(7-9)	7.0, 7.0	7.3±0.3(7.0-7.5)
Head height	3.4±0.4(3-4)	4	3.6±0.4(3-4)	4, 3	3.2±0(3-3)
Conus	8.6±0.4(8-9)	10.0	9.9±0.5(9-11)	9.5, 10	9.3±0.7(9-10)
Shaft	8.4±1.0(7.5-9.5)	9	8.8±0.6(8.0-10.0)	9, 9	8.2±0.3(8.0-8.5)
Pharynx	121.3±1.9(119-123)	121	120.9±11.2(106-137)	118, 135	110.6±5.6(104-115)
Ant. part. pharynx[1]	66.4±1.3(65-68)	62	65.2±6.4(58-73)	68, 76	64.5±1.3(63-66)
Post. part. pharynx[2]	54.9±2.7(52-57)	59	55.7±5.8(47-64)	51, 59	46.1±4.4(41-49)
Ant. part.% pharynx	54.7±1.6(53.8-56.6)	51.2	53.9±2.0(50.8-57.5)	57.2, 56.3	58.4±2.0(57.1-60.6)
Excretory pore	96.3±5.5(92-102)	109	97.5±7.9(85-107)	102, 111	95.9±9.7(85-102)
Head - vulva	413.9±28.7(381-432)		381.8±33.1(333-438)	432, 459	435.7±46.8(384-475)
Tail	44.0±3.5(40-47)	50	49.4±4.1(44-55)	56, 63	46.3±9.7(38-57)
Body width	21.9±1.3(21-23)	23	21.9±1.3(21-24)	26, 25	23.8±3.2(20-27)
Lateral field width	6.5±1.5(6-8)	6	6.0±0.7(5-7)	6, 8	6.3±0(6-6)
Anal body width	14.5±(13-16)	19	16.9±1.6(15-20)	20, 19	18.1±3.2(15-21)

[1] Measured from anterior body end to the posterior end of median bulb.
[2] Measured from the posterior end of median bulb to pharyngeo-intestinal junction.

lapping the intestine. Body posterior to cardia with distinct fasciculi observed in one female. Vulva expressed outside the body contour, without epiptygma. Spermatheca relatively small, rounded and empty. Tail cylindrical with 42 annules, tail terminus hemispherical, annulated. Phasmid located at the level of the seventeenth annule posterior to anus.

Male not found.

Distribution: undeveloped soil on cliffs vegetated by *Sedum* sp. and *Gentiana* sp. located at Suhardu Mic (Hăşmaş Mts.) (site no. 5 in Table I).

Remarks: *Tylenchorynchus maximus* was previously reported from subalpine grassland on rendzinic lithosol at 1,850 m above sea level located at Piule (Retezat Mountains) (Popovici, 1993, 1998). By reporting *T. maximus* from an undeveloped soil around roots of *Sedum* sp. and *Gentiana* sp., we broaden its trophic preferences, not only to meadow soils as mentioned by Brzeski (1998) but also to the "just born habitats" (Popovici and Ciobanu, 1997), with primary soils in which the early stage of natural succession is occurring.

Tylenchorynchus maximus was also collected from soil around roots of herbaceous plants and grasses on the slopes of Malika Parbat, surrounding Lake Saifulmuluk, located at 3,200 m above sea level in Pakistan (Maqbool and Shahina, 1987). These data confirm that the species is not restricted to lowland habitats and grass vegetation.

***Tylenchorhynchus* sp.**

(Table II; Figs.1 D-F, 4)

Female body C-shaped, cuticular annulation coarse

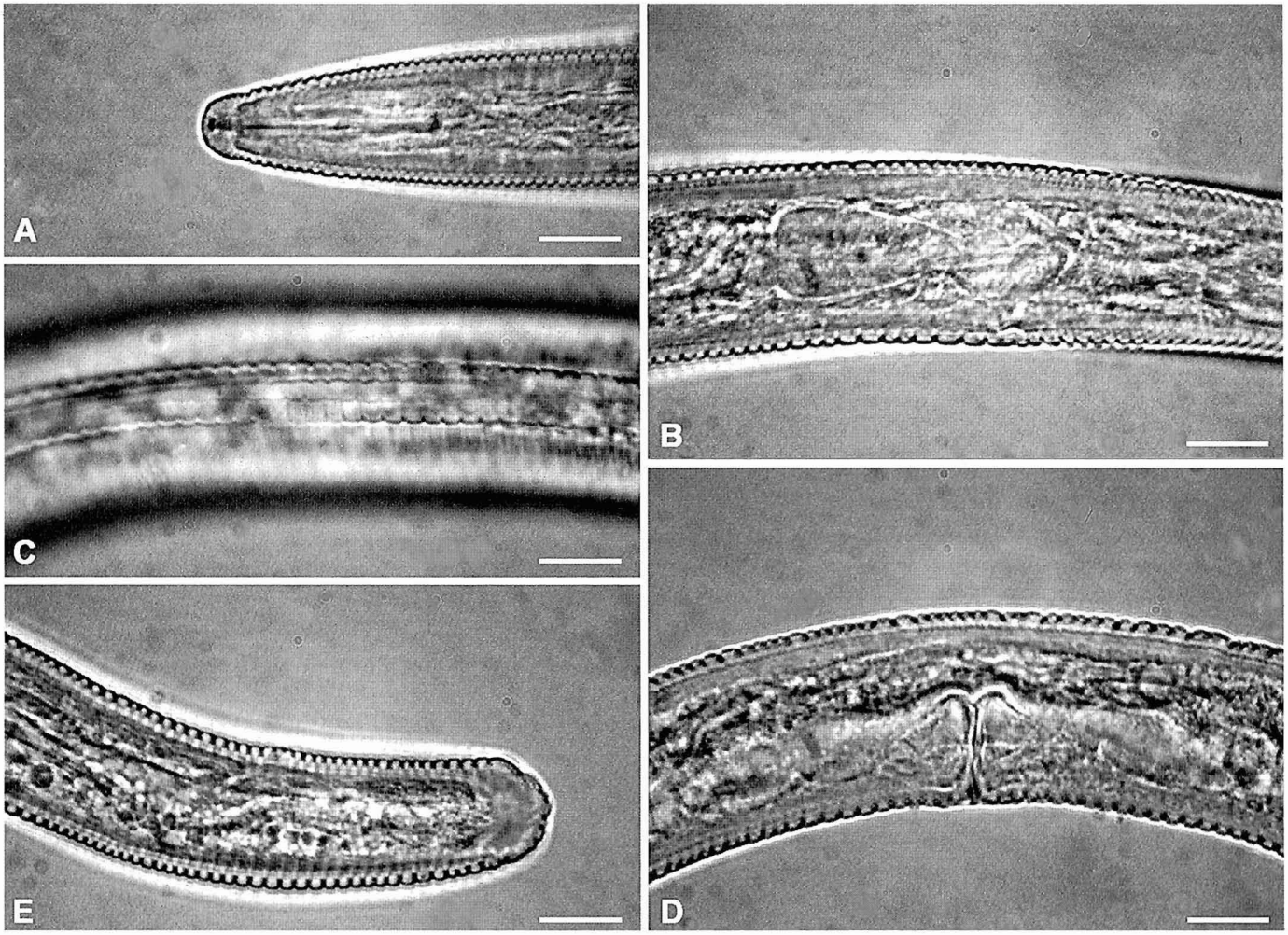

Fig. 4. *Tylenchorhynchus* sp.: female; A, anterior end; B, irregular cuticular annulation in the neck region; C, lateral field; D, vulva region; E, posterior body region (Scale bar = 10 µm).

and irregular over entire body; annules about 2.5 µm at posterior part of neck, 1.5 µm at midbody and 1 µm on ventral part of tail. Lateral field regularly areolated, outer margin strongly crenated. Head almost continuous, rounded, with six annules. Stylet slender, 25.5 µm long; basal knobs rounded, sloping backwards, about 2.5 µm in diameter, remarkably small compared to the stylet. Median bulb oval, basal bulb pyriform and rather short, not overlapping the intestine. Body with distinct fasciculi from cardia to tail. Post-anal intestinal sac absent. Vulva with distinct epiptygma. Vagina relatively deep, 12 µm long, extending inwards to more than half (54.2%) of the corresponding body diameter. Spermatheca rounded, filled with ovoid sperm, about 2.5-3.0 µm in diameter. Tail cylindrical with twenty-two annules, tail terminus hemispherical, irregularly annulated and indented. Phasmid located at the level of the seventh annule posterior to anus.

Male not found.

Distribution: grassland located at Muntele Roşu (Ciucaş Mts.), site no. 6 (Table I).

Discussion: the measurements of the single female collected come closest to *T. siccus* Sauer *et* Annells, 1981 and *T. velatus* Nobbs, 1989, both species being reported from arid regions of Australia (Sauer and Annells, 1981; Nobbs, 1989).

The specimen has the following morphological and morphometrical characteristics different from *T. siccus* and *T. velatus*: head almost continuous, vulva with single non-protruding epiptygma, shorter tail, and differences in shape with reference to head offset, and vulva with double protruding epiptygma in *T. siccus* and *T. velatus*. The Romanian specimen possess remarkably small basal knobs in comparison to its stylet and a relatively deep vagina.

ACKNOWLEDGEMENTS

The work would not have been possible without the financial support offered to the first author by the Netherlands Ministry of Agriculture, Nature Manage-

ment and Fisheries through the International Agricultural Centre in Wageningen, which is gratefully acknowledged.

The Plant Protection Service in Wageningen, the Netherlands is thanked for offering all the necessary facilities to carry out this study.

The authors are grateful to Dr. A.C. van Aelst (Wageningen University) for his help in processing the digital photographs of nematode specimens.

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Accepted for publication on 28 April 2004.