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

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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 habitats 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 (Tări Sub Carpaților), at Patra Clușani (Mehedinti Mountains) (Popovici, 1998) and from vegetated cliffs in various locations in the Romanian Carpathian Mountains (Popovici and Giobanu, 1997); *T. maximus* Allen, 1955 from grassland at Piule (Retezat Mountains) (Popovici, 1993, 1998); and *T. obscurisulcatus* Andrássy, 1959 from soil around *Artemisia* sp. roots, Cerna, Dobrușa (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), 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 DC-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 Han­ndo (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).

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**DESCRIPTIONS**

**Tylenchorhynchus agrí** 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. Epityagma 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. agrí* 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 agrí* 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. Epityagma 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 (Andrassy, 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

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**Table 1.** Site locations, vegetation and soil types of a nematological survey in Romania.

<table>
<thead>
<tr>
<th>Site nr.</th>
<th>Locality</th>
<th>Altitude (m)</th>
<th>Geographical position</th>
<th>Plant association</th>
<th>Soil type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fănațele Clujului (Someșan Plateau)</td>
<td>350</td>
<td>46°45′N-23°35′E</td>
<td>Jurino transsilvanicae-Stepetum pulcherimae</td>
<td>Chernozem</td>
</tr>
<tr>
<td>2</td>
<td>Suatu (Transylvanian Plain)</td>
<td>370-450</td>
<td>46°46′N-23°58′E</td>
<td>Salio nutantis-Festucetum rupeicolae</td>
<td>Not available</td>
</tr>
<tr>
<td>3</td>
<td>Tureni Gorges (Trascău Mts.1)</td>
<td>400</td>
<td>46°30′N-23°41′E</td>
<td>Melico-Phleum montani</td>
<td>Lithic rendzina</td>
</tr>
<tr>
<td>4</td>
<td>Rodna Veche (Rodnei Mts.)</td>
<td>525</td>
<td>47°25′N-24°26′E</td>
<td></td>
<td>Technogenic soil2</td>
</tr>
<tr>
<td>5</td>
<td>Suhardu Mic (Hașmaș Mts.)</td>
<td>1450</td>
<td>46°43′N-23°36′E</td>
<td></td>
<td>Undeveloped soil</td>
</tr>
<tr>
<td>6</td>
<td>Muntele Roșu (Ciuceș Mts.)</td>
<td>1500</td>
<td>45°26′N-25°52′E</td>
<td>Violo declinatae-Nardetum</td>
<td>Cambic rendzina</td>
</tr>
<tr>
<td>7</td>
<td>Maliuc (Danube Delta)</td>
<td>12</td>
<td>45°13′N-29°04′E</td>
<td></td>
<td>Not available</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Species:</th>
<th><em>Tylenchorhynchus agri</em></th>
<th><em>Tylenchorhynchus maximus</em></th>
<th><em>Tylenchorhynchus sp.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat:</td>
<td><em>Populus sp.</em> plantation</td>
<td>Cliffs vegetated by <em>Sedum sp.</em> and <em>Gentiana sp.</em></td>
<td>Grassland</td>
</tr>
<tr>
<td>Locality:</td>
<td>Maulic (Danube Delta)</td>
<td>Sahardu Mic (Hâmaș Mts.)</td>
<td>Muntele Roșu (Ciucăş Mts.)</td>
</tr>
<tr>
<td>n</td>
<td>2.991 1</td>
<td>2.99 2</td>
<td>1.9</td>
</tr>
<tr>
<td>L</td>
<td>726,730</td>
<td>1490,1490</td>
<td>846</td>
</tr>
<tr>
<td>a</td>
<td>34.8,36.1</td>
<td>36.1,31.2</td>
<td>38.3</td>
</tr>
<tr>
<td>b</td>
<td>5.6,5.6</td>
<td>8.3,8.5</td>
<td>6.7</td>
</tr>
<tr>
<td>c</td>
<td>19.2,17.8</td>
<td>22.9,26.8</td>
<td>27.9</td>
</tr>
<tr>
<td>c'</td>
<td>2.9,2.8</td>
<td>3.1,?</td>
<td>1.8</td>
</tr>
<tr>
<td>V%</td>
<td>56.2,57.9</td>
<td>56.7</td>
<td>58.2</td>
</tr>
<tr>
<td>Head width</td>
<td>7.0,7.0</td>
<td>8,7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Head height</td>
<td>3,4</td>
<td>4,3</td>
<td>4</td>
</tr>
<tr>
<td>Conus</td>
<td>11,9.5</td>
<td>14,13.5</td>
<td>14</td>
</tr>
<tr>
<td>Shaft</td>
<td>9.5,10</td>
<td>10,10</td>
<td>11.5</td>
</tr>
<tr>
<td>Pharynx</td>
<td>131,131</td>
<td>180,175</td>
<td>126</td>
</tr>
<tr>
<td>Ant. part. pharynx²</td>
<td>76,74</td>
<td>109,101</td>
<td>79</td>
</tr>
<tr>
<td>Post. part. pharynx⁴</td>
<td>35,57</td>
<td>71,74</td>
<td>47</td>
</tr>
<tr>
<td>Ant. part.% pharynx</td>
<td>58.0,56.5</td>
<td>60.4,57.8</td>
<td>62.8</td>
</tr>
<tr>
<td>Excretory pore</td>
<td>104,111</td>
<td>146,144</td>
<td>101</td>
</tr>
<tr>
<td>Head - vulva</td>
<td>408,422</td>
<td>758,800</td>
<td>493</td>
</tr>
<tr>
<td>Tail</td>
<td>38,41</td>
<td>65.56</td>
<td>30</td>
</tr>
<tr>
<td>Body width</td>
<td>21,20</td>
<td>27,29</td>
<td>22</td>
</tr>
<tr>
<td>Lateral field width</td>
<td>6,5</td>
<td>9,9</td>
<td>6</td>
</tr>
<tr>
<td>Anal body width</td>
<td>13,15</td>
<td>21,?</td>
<td>16</td>
</tr>
</tbody>
</table>

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 pharynxo-intestinal junction.

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

According 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).

<table>
<thead>
<tr>
<th>Habitat:</th>
<th>Grassland</th>
<th>Grassland</th>
<th>Grassland</th>
<th>Technogenic soil under bioremediation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location:</td>
<td>Fanatele Clujului (Someșan Plateau)</td>
<td>Suatu (Transylvanian Plain)</td>
<td>Tureni Gorges (Trascau Mts.)</td>
<td>Rodna Veche (Rodnei Mts.)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Range</th>
<th>Median</th>
<th>Range</th>
<th>Median</th>
<th>Range</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>398</td>
<td>391-405</td>
<td>399</td>
<td>391-405</td>
<td>398</td>
<td>391-405</td>
<td>399</td>
<td>391-405</td>
</tr>
<tr>
<td>L</td>
<td>754.7±33.3(718-784)</td>
<td>848</td>
<td>694.7±64.5(607-797)</td>
<td>821.856</td>
<td>782.4±100.1(672-867)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>34.6±3.3(30.7-36.5)</td>
<td>37.3</td>
<td>31.7±1.6(28.9-33.2)</td>
<td>31.7,33.9</td>
<td>32.9±0.3(32.7-33.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>6.2±0.6(6.0-6.4)</td>
<td>7.0</td>
<td>5.8±0.8(5.0-7.5)</td>
<td>6.9,6.4</td>
<td>7.1±0.6(6.4-7.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>17.2±1.4(16.2-18.8)</td>
<td>17.0</td>
<td>14.1±1.6(11.3-16.3)</td>
<td>14.8,13.5</td>
<td>17.1±1.6(15.2-18.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c'</td>
<td>3.1±0.6(2.7-3.5)</td>
<td>2.6</td>
<td>2.9±0.3(2.6-3.4)</td>
<td>2.8,3.3</td>
<td>2.6±0.2(2.3-2.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V%</td>
<td>54.8±1.7(53.0-56.3)</td>
<td>55.0±1.9(51.9-58.7)</td>
<td>52.6±3.6</td>
<td>55.8±1.2(54.8-57.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head width</td>
<td>6.8±0.3(6.5-7.0)</td>
<td>7.0</td>
<td>7.4±0.3(7.9)</td>
<td>7.0,7.0</td>
<td>7.3±0.3(7.0-7.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head height</td>
<td>3.4±0.1(3-4)</td>
<td>4.0</td>
<td>3.6±0.1(3-4)</td>
<td>4.3,4.3</td>
<td>3.2±0.3(3-3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conus</td>
<td>8.6±0.4(8-9)</td>
<td>10.0</td>
<td>9.9±0.5(9-11)</td>
<td>9.5,10</td>
<td>9.3±0.7(9-10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shaft</td>
<td>8.4±1.0(7.5-9.5)</td>
<td>9</td>
<td>8.8±0.6(8.0-10.0)</td>
<td>9.9,9.9</td>
<td>8.2±0.3(8.0-8.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharynx</td>
<td>121.3±1.9(119-123)</td>
<td>121</td>
<td>120.9±1.2(106-137)</td>
<td>118,135</td>
<td>110.6±5.6(104-115)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ant. part. pharynx</td>
<td>66.4±1.3(63-68)</td>
<td>62</td>
<td>65.2±6.4(58-73)</td>
<td>68,76</td>
<td>64.5±1.3(63-66)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos. part. pharynx</td>
<td>54.9±2.7(52-57)</td>
<td>59</td>
<td>55.7±5.8(47-64)</td>
<td>51,59</td>
<td>46.1±4.4(41-49)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ant. part.% pharynx</td>
<td>54.7±1.6(53.8-56.6)</td>
<td>51.2</td>
<td>53.9±2.0(50.8-57.5)</td>
<td>57.2,56.3</td>
<td>58.4±2.0(57.1-60.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excretory pore</td>
<td>96.3±5.5(92-102)</td>
<td>109</td>
<td>97.5±9.7(85-107)</td>
<td>102,111</td>
<td>95.9±9.7(85-102)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head - vulva</td>
<td>413.9±28.7(381-432)</td>
<td>381.8±33.1(333-438)</td>
<td>432,499</td>
<td>435.7±46.8(384-475)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>44.0±3.5(40-47)</td>
<td>50</td>
<td>49.4±4.1(44-59)</td>
<td>56,65</td>
<td>46.3±7.0(38-57)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body width</td>
<td>21.9±1.3(21-23)</td>
<td>23</td>
<td>21.9±1.3(21-23)</td>
<td>26,25</td>
<td>23.8±2.2(20-27)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral field width</td>
<td>6.5±1.5(6-8)</td>
<td>6</td>
<td>6.0±0.7(5-7)</td>
<td>6.8,6.8</td>
<td>6.3±0.6(6-6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anal body width</td>
<td>14.5±1.8(13-16)</td>
<td>19</td>
<td>16.9±1.6(15-20)</td>
<td>20,19</td>
<td>18.1±3.2(15-21)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 epiphtye. Spermatotheca 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 (Hășmaș Mts.) (site no. 5 in Table I).

Remarks: *Tylenchorhynchus maximus* was previously reported from subalpine grassland on rendzic 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.

*Tylenchorhynchus 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.
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 epitygma. Vagina relatively deep, 12 μm long, extending inwards to more than half (54.2%) of the corresponding body diameter. Spermheca 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 Roșu (Ciucas Mts.), site no. 6 (Table I).


The specimen has the following morphological and morphometrical characteristics different from *T. siccus* and *T. velatus*: head almost continuous, vulva with single non-protruding epitygma, shorter tail, and differences in shape with reference to head offset, and vulva with double protruding epitygma 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 Agricul
tural Centre in Wageningen, which is gratefully ac
nowledged.

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

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

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Accepted for publication on 28 April 2004.