

## Three *Malenchus* species from Spain (Nemata : Tylenchidae) with a note on the amphidial opening in the genus

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**Summary** — *Malenchus acarayensis* Andrassy, 1968, *M. pachycephalus* Andrassy, 1981 and *M. ovalis* (Siddiqi, 1979) Andrassy, 1981 are reported for the first time in Spain, from soil samples collected around the roots of *Quercus rotundifolia* Lam. at Sierra de Cazorla (Southeastern Spain). The similarity of *M. ovalis* (Siddiqi, 1979) Andrassy, 1981 and *M. truncatus* Knobloch, 1976 is stressed. SEM comparisons yielded a similar sinusoidal amphid aperture in six *Malenchus* species, including the Spanish ones, differing from the straight slit in three *Malenchus* species from Tierra del Fuego, Chile.

**Résumé** — *Trois espèces de Malenchus (Nemata : Tylenchidae) et note sur la structure de l'ouverture des amphides dans ce genre* — *Malenchus acarayensis* Andrassy, 1968, *M. pachycephalus* Andrassy, 1981 et *M. ovalis* (Siddiqi, 1979) Andrassy, 1981, récoltés dans la rhizosphère de *Quercus rotundifolia* Lam., à Sierra de Cazorla, sont signalés pour la première fois dans le sud-est de l'Espagne. La similarité entre *M. ovalis* (Siddiqi, 1979) Andrassy, 1981 et *M. truncatus* Knobloch, 1976 est soulignée. Une étude comparative au MEB a permis la découverte de l'identité de forme — fente sinusoidale — de l'ouverture amphidienne chez six espèces de *Malenchus*, dont celles provenant d'Espagne, alors que cette fente est droite chez trois espèces provenant de la Terre de Feu (Chili).

**Key-words** : *Malenchus*, amphids.

Three species of *Malenchus* Andrassy, 1968 — i.e. *Malenchus acarayensis* Andrassy, 1968, *M. pachycephalus* Andrassy, 1981 and *M. ovalis* (Siddiqi, 1979) Andrassy, 1981 — are reported for the first time in Spain, from soil samples collected around the roots of *Quercus rotundifolia* Lam. at Vadillo, Sierra de Cazorla, a mountainous area in southeastern Spain. Morphometry and morphology using scanning electron microscopy are reported and compared with literature.

Specimens were killed by gentle heat and fixed in a 4 % solution of formaldehyde, then dehydrated and processed to glycerine according to the Seinhorst method. Body length and curved structures were measured with the aid of a precision curvimeter, straight structures such as maximum body width, stylet, anal body width, etc. were measured using a micrometer-scale in the eyepiece of high-power (1250 ×) microscope.

Specimens for SEM studies were processed using the method described in Zeidan and Geraert (1991).

### Study of the lip region

*Malenchus pachycephalus* and *M. acarayensis* have been studied by SEM (Figs 1, 2). The several specimens studied showed a very similar end on view. As typical for *Malenchus* the head is dorsoventrally flattened and the amphidial aperture continues from the front to the lateral side of the head. A small oral disc surrounds the dorso-ventral, oval mouth aperture; on the oral disc six small pores are distinct (Fig. 2 A : *M. pachycephalus*). The

amphidial apertures can be interpreted as very wide, straight clefts partly covered by cuticular outgrowths so that finer zigzag clefts remain. The cuticular outgrowths consist of smaller ones closer to the mouth opening, projecting from the dorsal side followed by larger ones (on the lateral side of the head) projecting from the ventral side. These cuticular outgrowths seem to be constant as they occur in about the shape and position found not only in the two species studied here, but also in *M. nanellus* Siddiqi, 1979, *M. ovalis*, *M. exiguus* (Massey, 1969) Andrassy, 1980 and most distinctly in *M. macrodorus* Geraert & Raski, 1986; in that study the amphidial apertures were, however, not as wide, suggesting they can be opened and closed [cf. *M. acarayensis* in this study (Fig. 1 A) with the same species in Geraert and Raski (1986; Fig. 12 A, B)].

The cuticular outgrowths giving a zigzag amphidial aperture are not present in the three species from Tierra del Fuego, where the amphidial aperture is a straight, slightly oblique slit.

### *Malenchus acarayensis* Andrassy, 1968

(Fig. 1)

#### MEASUREMENTS

See Table 1.

#### DESCRIPTION

*Female* : Body with  $294 \pm 21$  (262-317) annuli. Annuli 1.4-1.7  $\mu\text{m}$  wide at mid-body. Lateral field

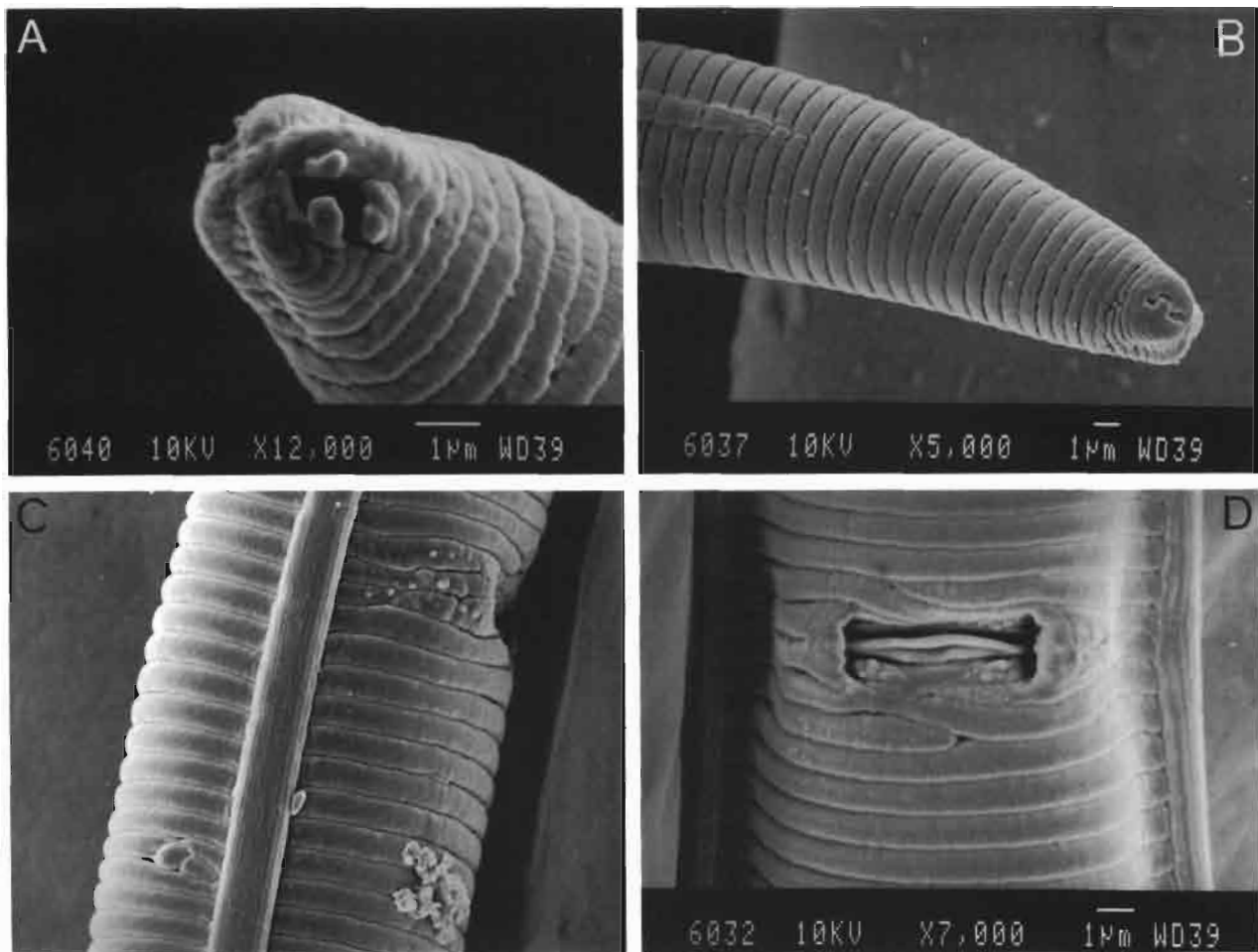
**Table 1.** Morphometric data of three *Malenchus* species (measurements in  $\mu\text{m}$ )

|                     | <i>M. acarayensis</i>         |                               | <i>M. ovalis</i> | <i>M. pachycephalus</i>       |           |
|---------------------|-------------------------------|-------------------------------|------------------|-------------------------------|-----------|
|                     | Females                       | Males                         | Females          | Females                       | Males     |
| n                   | 20                            | 5                             | 3                | 21                            | 3         |
| L                   | 353 $\pm$ 23.5<br>(305-389)   | 344 $\pm$ 23.0<br>(306-365)   | 445-475          | 404 $\pm$ 24.5<br>(366-449)   | 394-406   |
| a                   | 21.8 $\pm$ 2.3<br>(18.2-28.2) | 24.4 $\pm$ 2.6<br>(22.4-27.8) | 25-26            | 22.2 $\pm$ 1.5<br>(17.9-24.0) | 23.3-25.4 |
| b                   | 4.5 $\pm$ 0.3<br>(3.9-5.0)    | 4.5 $\pm$ 0.3<br>(4.1-5.0)    | 5.4-5.8          | 4.7 $\pm$ 0.4<br>(4.2-5.5)    | 4.4-4.7   |
| V or T              | 62 $\pm$ 2.2<br>(60-66)       | 43 $\pm$ 4.6<br>(37-48)       | 65-68            | 67 $\pm$ 0.7<br>(65-68)       | 46-51     |
| V'                  | 80 $\pm$ 1.5<br>(78-85)       | —                             | 79-80            | 79 $\pm$ 1.3<br>(77-83)       | —         |
| G 1                 | 28 $\pm$ 2.9<br>(23-34)       | —                             | 37-41            | 38 $\pm$ 6.2<br>(22-46)       | —         |
| c                   | 4.5 $\pm$ 0.5<br>(3.8-5.5)    | 3.8 $\pm$ 0.2<br>(3.6-4.0)    | 5.8-6.1          | 6.4 $\pm$ 0.4<br>5.5-7.3      | 4.9-5.4   |
| c'                  | 8.8 $\pm$ 1.4<br>(7.0-11.9)   | 9.8 $\pm$ 1.2<br>(8.2-11.0)   | 5.8-7.2          | 6.3 $\pm$ 0.5<br>(5.7-7.4)    | 7.5-8.8   |
| Stylet              | 8.5 $\pm$ 0.5<br>(8.0-9.5)    | 8.6 $\pm$ 0.7<br>(7.5-9.0)    | 10-11            | 13 $\pm$ 0.4<br>(12-14)       | 13-14     |
| m                   | 40 $\pm$ 2.0<br>(35-42)       | —                             | 26-31            | 39 $\pm$ 2.2<br>(35-43)       | 38-48     |
| MB                  | 47 $\pm$ 2.4<br>(41-50)       | 48 $\pm$ 3.2<br>(42-50)       | 49-54            | 50 $\pm$ 2.9<br>(40-53)       | 49-52     |
| Procorpus length    | 25 $\pm$ 4.3<br>(20-33)       | —                             | —                | 25 $\pm$ 2.3<br>(21-28)       | 24-26     |
| Median bulb length  | 8.3 $\pm$ 1.2<br>(7-11)       | 8 $\pm$ 0.7<br>(7-9)          | —                | 8 $\pm$ 0.6<br>(7-9)          | 7-9       |
| Basal bulb length   | 12 $\pm$ 1.4<br>(9-15)        | 11 $\pm$ 1.8<br>(9-14)        | 18-19            | 13 $\pm$ 1.0<br>(11-15)       | 13-15     |
| Excr. pore-ant. end | 62 $\pm$ 7.2<br>(50-78)       | 64 $\pm$ 4.9<br>(57-69)       | 80-84            | 81 $\pm$ 5.0<br>(71-90)       | 72-75     |
| Oesophagus length   | 78 $\pm$ 6.7<br>(70-96)       | 76 $\pm$ 6.9<br>(67-84)       | 80-84            | 87 $\pm$ 5.7<br>(71-94)       | 83-92     |
| Maximum body diam.  | 16 $\pm$ 1.8<br>(13-19)       | 14 $\pm$ 2.0<br>(11-16)       | 17.5-18          | 20 $\pm$ 1.1<br>(17-22)       | 16-17     |
| Anal body diam.     | 9 $\pm$ 1.0<br>(8-11)         | 9 $\pm$ 1.5<br>(7-11)         | 11-13            | 10 $\pm$ 0.5<br>(9-11)        | 9-10      |
| Tail length         | 79 $\pm$ 6.6<br>(69-89)       | 90 $\pm$ 7.6<br>(77-96)       | 75-80            | 63 $\pm$ 3.7<br>(56-69)       | 75-81     |
| Vulva-anus dist.    | 55 $\pm$ 4.1<br>(51-64)       | —                             | 74-82            | 70 $\pm$ 4.2<br>(60-76)       | —         |
| Tail/vulva-anus     | 1.4 $\pm$ 0.1<br>(1.3-1.6)    | —                             | 0.9-1.1          | 0.9 $\pm$ 0.07<br>(0.8-1.0)   | —         |

2-2.5  $\mu\text{m}$  wide, originating at 11-15  $\mu\text{m}$  posterior to stylet base or at 26-34 annuli from anterior end; with two lines seen with light microscopy and multiple lines (11-12) seen under SEM, and ending at about the first third of tail length. Procorpus similar to isthmus in length. Excretory pore located at the beginning of basal bulb or 50 (48-52) annuli from anterior end. Vulva sunken, under SEM shows epiptygma and small lateral

dikes. Phasmid-like structure 10-14 annuli anterior to vulva. Postvulvar uterine sac 5.5-8  $\mu\text{m}$  long. Spermatheca rounded, 11  $\pm$  2.2  $\mu\text{m}$  (7-13) wide, filled with rounded sperm.

*Male* : Always present but less numerous than females (21 %), similar to female. Cuticle annulation finer than that of female, 1.2  $\pm$  0.06  $\mu\text{m}$  (1.2-1.3) wide. Spicules ventrally curved, 15.3  $\pm$  1.5  $\mu\text{m}$  (13-16) long. Guber-



**Fig. 1.** *Malenchus acarayensis* Andrassy, 1968. SEM micrographs. A : Lip region; B : Anterior region showing beginning of lateral field; C : Lateral field at vulva, with phasmid-like structure at dorsal edge (anterior end downwards); D : Ventral view of vulva with epitygmata and lateral dikes.

naculum  $4.2 \pm 0.8 \mu\text{m}$  (3-5) long. Bursa finely crenate, adanal, 30-32  $\mu\text{m}$  long.

#### DISCUSSION

Morphology of this population fits well with original description, morphometric data are similar to those of Andrassy (1968, 1981) and Knobloch (1976), and diagnosis corresponds well with Geraert and Raski (1986).

#### *Malenchus pachycephalus* Andrassy, 1981 (Fig. 2)

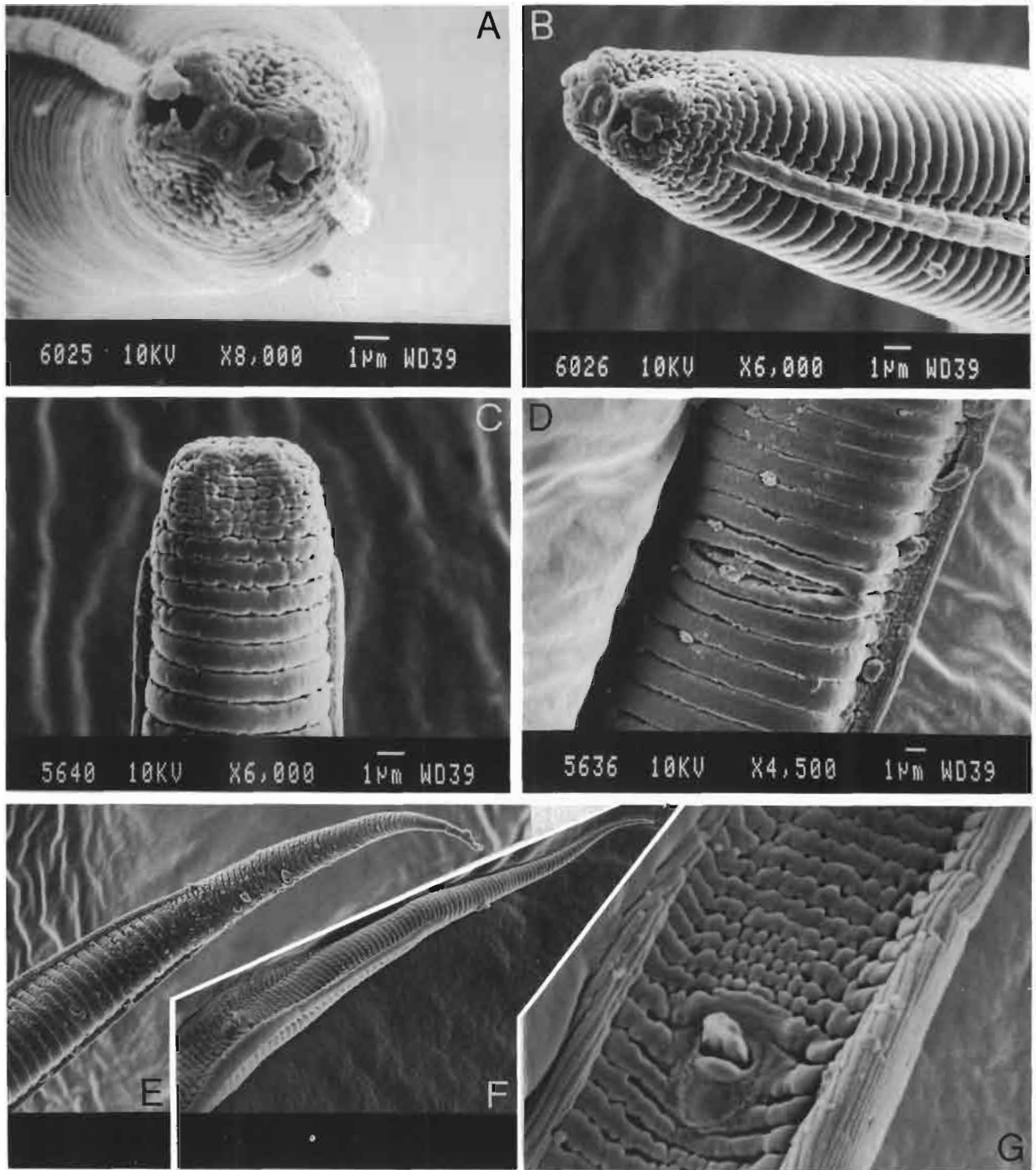
#### MEASUREMENTS

See Table 1.

#### DESCRIPTION

*Female* : Body straight or slightly ventrally curved. Cuticular annulation coarse, 2-2.2  $\mu\text{m}$  wide. Lateral

field  $2.8 \pm 0.2 \mu\text{m}$  (2.7-3.3) wide, originating at level of half of stylet or 7-11 annuli from anterior end; with two lines seen with light microscopy and multiple lines (7-9) seen with scanning electron microscopy; ending at about half of tail length. Annuli finely crenate in stylet region. Stylet strong, basal knobs amalgamated, sloping, 2-2.5  $\mu\text{m}$  wide. Dorsal gland opening at 1-1.5  $\mu\text{m}$  from stylet base. Median bulb oval, with valvae 1.5  $\mu\text{m}$  long. Deirid at level of excretory pore. Excretory pore at half of basal bulb level or 44 (42-47) annuli from anterior end. Basal bulb pyriform. Oesophagus occupying 49 (45-57) annuli from anterior end. Vulva sunken, under SEM shows epitygma but no lateral dikes; vagina perpendicular to body, 7-8  $\mu\text{m}$  long. Phasmid-like structure clearly visible with light microscopy and located at 8-11 annuli anterior to vulva. Spermatheca well developed, usually bilobed (rarely rounded), 10-18  $\mu\text{m}$  long.



**Fig. 2.** *Malenchus pachycephalus* Andrassy, 1981. SEM micrographs. A-C : Anterior end showing beginning of lateral field (A-B : male; C : female); D : Vulvar region showing epiptygmata; E-F : Female and male tail (ventral view); G : Ventral view of the cloacal region.

Postvulvar uterine sac  $9 \pm 1.4 \mu\text{m}$  (7-13) long. Vulva-anus distance similar or slightly superior to tail length, with  $35 \pm 3$  (29-37) annuli. Tail straight or slightly ventrally curved, with  $56 \pm 4$  (48-61) annuli.

*Male* : Always present but less numerous than females (12 %), similar to female. Spicules and gubernaculum ventrally curved,  $15 \pm 0.7 \mu\text{m}$  (14-15.5) long and  $3.1 \pm 0.8 \mu\text{m}$  (3-4) long, respectively. Bursa adanal, 27-37  $\mu\text{m}$  long. A male studied by SEM showed crenate and sometimes subdivided body annuli at the ventral bursa level; anterior cloacal lip slightly elevated.

#### DISCUSSION

Morphology and morphometry of this population fits well with original description, being this record unique after Andrassy (1981). However, Spanish population differs in having a slightly longer stylet  $13 \mu\text{m}$  (12-13.5) vs  $12 \mu\text{m}$ , lateral field ending at 1/2 tail length vs 1/3-1/4, head is distinctly ventro-dorsally constricted (for the type population Andrassy mentions that the head is not so strongly flattened like in other species of the genus), median bulb with distinct valve (vs without valve). We consider these characters as variability within the species.

#### *Malenchus ovalis* (Siddiqi, 1979) Andrassy, 1981

##### MEASUREMENTS

See Table 1.

##### DESCRIPTION

*Female* : Annuli 1.4-1.6  $\mu\text{m}$  wide at mid-body. Lateral field 3.5  $\mu\text{m}$  wide, originating at stylet base to 3 annuli posterior or 13-16 annuli from anterior end, two lines with light microscope, ending less than half the tail length (39-45 %). Stylet fine, delicate, knobs indistinct. Median bulb weakly developed, no valves. Deirid at level of excretory pore. Excretory pore at end of basal bulb or slightly posterior to it, or at 56 annuli from anterior end. Vulva sunken, with epiptygmata but no lateral dikes; vagina perpendicular to body, 6.5  $\mu\text{m}$  long. Phasmid-like structure 13 annuli anterior to vulva. Spermatheca bilobed, 21  $\mu\text{m}$  long. Post-vulval uterine sac about 10  $\mu\text{m}$  long. Vulva-anus distance about equal to tail length. Tail slightly ventrally curved, conical with pointed tip; annuli distinctly smaller posterior to end of lateral field.

*Male* : Not found.

##### DISCUSSION

*M. ovalis* has similar measurements, lateral field "originates about level of spear knobs", median bulb

lacks valve plates, excretory pore "eight annules anterior to two annules posterior to base of oesophagus", vulva-anus distance 0.86-1.2 times tail length. Geraert and Raski (1986) described a population from Colorado, USA, as *M. ovalis* and synonymized *M. malawiensis* (Siddiqi, 1979) Andrassy, 1981 with it.

A very similar species is *M. truncatus* Knobloch, 1976 : similar measurements and ratios, similar position of excretory pore (5-6  $\mu\text{m}$  posterior to base of oesophagus). The beginning of the lateral field is slightly more posterior than in our specimens ("from midway between stylet knobs and metacarpus"); Knobloch's drawings suggest a slightly heavier stylet and distinct valves in the median bulb. The species name *truncatus* refers to the truncate anterior end; from her Fig. 1 A a head width of 8.5  $\mu\text{m}$  can be calculated, our females have 6.5-8  $\mu\text{m}$ . The differences mentioned above are small and it is not excluded that both species are identical. In Geraert and Raski's (1986) key they are separated by the beginning of the lateral field : "at level of spear" leads a. o. to *M. ovalis*, "between spear base and end of median bulb" a. o. to *M. truncatus*; for *M. ovalis* the exact indication should be "at level of spear base".

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