OBSERVATIONS ON THE POLINUCLEATE ESOPHAGEAL GLANDS OF *ROTYLENCHUS MAGNUS JAENI* CASTILLO *ET AL.*, 1994 JUVENILES WITH COMMENTS ON THE MORPHOLOGY OF A POPULATION OF *R. AGNETIS* SZCZYGIEL, 1968 FROM ITALY

N. Vovlas,[1] A. Troccoli,[1] and P. Castillo[2]

Istituto di Nematologia Agraria, Consiglio Nazionale delle Ricerche, Via Amendola 165/A, 70126 Bari, Italy,[1] Instituto de Agricultura Sostenible CSIC, Apartado 4084, 14080 Cordoba, Spain.[2]

ABSTRACT

N. Vovlas, A. Troccoli, and P. Castillo. Observations on the polinucleate esophageal glands of *Rotylenchus magnus jaeni* Castillo *et al.*, 1994, juveniles with comments on the morphology of a population of *R. anetis* Szczygiel, 1968 from Italy. Nematropica 28:231-240.

The esophagus of the juvenile stage of *Rotylenchus magnus jaeni* Castillo *et al.*, 1994 exhibits a long pharyngeal overlap and esophageal glands with seven nuclei, as reported for the adult stage of this subspecies. Nuclei of esophageal glands are about the same size, but vary in their arrangement among specimens. The large number of esophageal gland nuclei and long pharyngeal overlap of *R. magnus* suggest that this species is more evolved than the other *Rotylenchus* species which have shorter pharyngeal overlap and esophageal glands with only three nuclei. Morphometrics and morphology of an Italian population of *Rotylenchus agnetis* Szczygiel, 1968 from the rhizosphere of *Ruscus aculeatus* L. did not differ from those reported for other populations from other countries. However, females of this population showed a large annulus at the tail terminus.

Keywords: esophageal gland nuclei, geographical distribution, Hoplolaimidae, Italy, morphology, *Rotylenchus agnetis, R. magnus jaeni.*

RESUMEN

Vovlas, N., A. Troccoli y P. Castillo. 1998. Observaciones en las glándulas esofágicas polinucleadas de *Rotylenchus magnus jaeni* Castillo y cols., 1994 y morfología adicional de *R. agnetis* Szczygiel, 1968. Nematrópica 28:231-240.

El esófago de *Rotylenchus magnus jaeni*, en el estado juvenil, Castillo y cols., 1994, exhibe un sobrelapamiento faríngeo largo y glándulas esofágicas con siete nucleos, como lo que ha sido reportado para el estado adulto de estas subespecies. Los nucleos de las glándulas esofágicas, son de un tamaño similar pero su disposición varía entre espéciménes. El gran numero de nucleos en la glándula esofágica) y el largo sobrelapamiento faríngeo en *R. magnus*, sugieren que esta especie es más evolucionada que las otras especies de *Rotylenchus*, las que presentan un menor sobrelapamiento faríngeo y glándulas esofágicas, con solo tres nucleos. La morfología y caracteres morfometricos de una población italiana de *Rotylenchus agnetis* Szczygel, 1968, de la rizosfera de *Ruscus aculeatus* L, no se diferenciaron de lo reportado para otras poblaciones, en otros países. Sin embargo, las hembras de esta población, mostraron un anillo largo al termino de la cola.

Palabras claves: distribución geográfica, Hoplolaimidae, Italy, morfologia, nucleos de glándulas esofágicas.

INTRODUCTION

The arrangement of the esophageal glands in various species of the genus *Rotylenchus* shows a gradual elongation of the pharyngeal overlap (*sensu* Loof, 1991), from almost absent in *R. capitatus* (2 µm) to very long in *R. magnus jaeni* (65 µm). In the 90 nominal species of the genus, 3 regular esophageal nuclei are reported for

about 75% of the illustrated members, while no reports are available for the remaining 25% of the species. In the monograph of the genus *Rotylenchus* Filipjev, 1936, Castillo *et al.* (1994) illustrated unique characteristics of the esophageal glands in adults of *R. magnus jaeni.* The objective of the present study is to determine the number, the morphology and the location of nuclei in the esophageal glands of all juveniles stages of this species.

Rotylenchus agnetis was described from Poland by Szczygiel (1968) using female specimens. Later, Nesterov (1979) described a bisexual population from Moldavia. Baydulova (1981) gave a further contribution to the morphology of the species from populations collected in Western Kazakhstan. Peneva and Nedelchev (1992) provided several observations on the morphology and distribution of *R. agnetis* in Bulgaria, and considered *R. aceri* Berezina, 1985, a junior synonym of *R. agnetis.* Geraert and Barooti (1996) supplied additional data on its morphology, particularly with SEM illustrations, as did Scotto La Massese and Germani (1997), who described and illustrated two populations from Algeria. In the present study, the original and subsequent descriptions, and the known geographical distribution of *R. agnetis* are supplemented and amplified with data and remarks made on a bisexual population found for the first time in Italy.

MATERIALS AND METHODS

Large populations containing all life stages of the two *Rotylenchus* species were separated from soil by screening and centrifugal flotation (Coolen, 1979), killed, fixed and preserved in formaldehyde 4% + 1% propionic acid. Sixty topotype specimens (20 females, 20 males and 20 juveniles) of *R. magnus jaeni* and forty-two specimens (25 females and 17 males) of *R. agnetis* were transferred to anhydrous glycerine by Seinhorst's slow method (1959). The population of *R. magnus jaeni* originated from soil around the roots of cork trees (*Quercus suber* L.) in Santa Elena, Jaén, Spain, and that of *R. agnetis* from the rhizosphere of butcher's broom (*Ruscus aculeatus* L.), in the forest area of Monticchio's lakes, at Laghi di Monticchio, Potenza, Southern Italy. Measurements, and drawings were made for both species with *camera lucida.* Morphometric data for comparative studies of *R. agnetis* were taken from original descriptions and from the recently presented data by Geraert and Barooti (1996).

RESULTS

Rotylenchus magnus jaeni Castillo *et al.*, 1994

(topotype population; Figs. 1, 2)

The hepta-nucleate esophageal glands of *R. magnus jaeni* are a unique characteristic among the nominal species of the genus (Castillo *et al.*, 1994). The esophagus of the *Rotylenchus magnus jaeni* female shows the following morphometry: dorsal esophageal gland orifice from stylet base 7.5 ± 1.2 µm (4.0-9.0). Procorpus cylindrical (Fig. 2A), narrowing slightly at junction level. Median esophageal bulb round-oval, with valvular apparatus well developed. Esophageal glands overlap intestine dorsally for about 65 ± 19 µm (35-98) from the esophago-intestinal valve. Seven distinct and prominent esophageal gland nuclei are visible in almost all the specimens (Figs. 1D-F, 2). Frequently, six of these nuclei are clearly evident and one slightly out of focus. However, a female specimen with an extra esophageal gland nucleus (eight) was observed in the population studied (Fig. 1E). Esophagus of male does not differ from that of the female (Fig. 2C, C'). The esophagus of

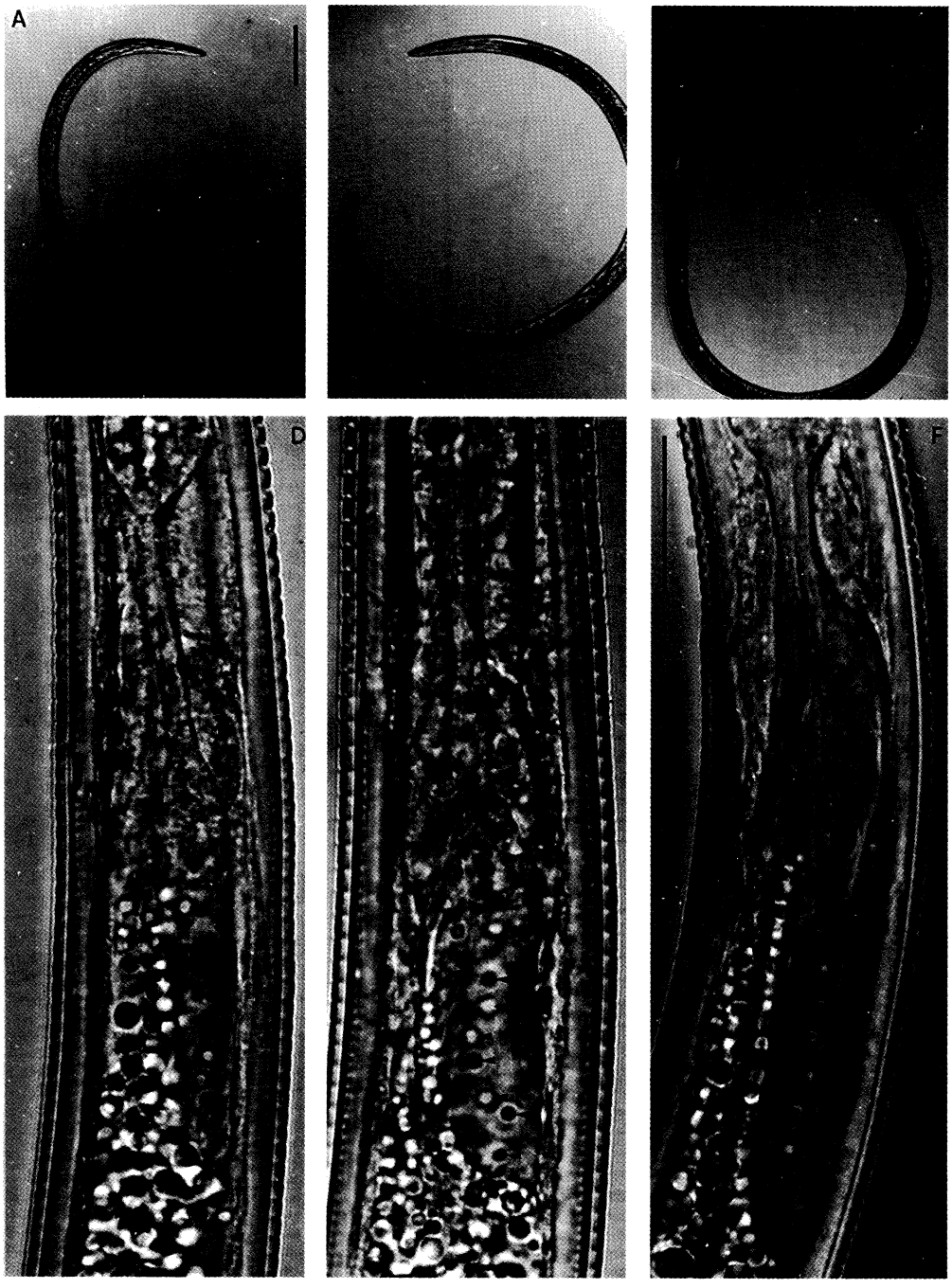
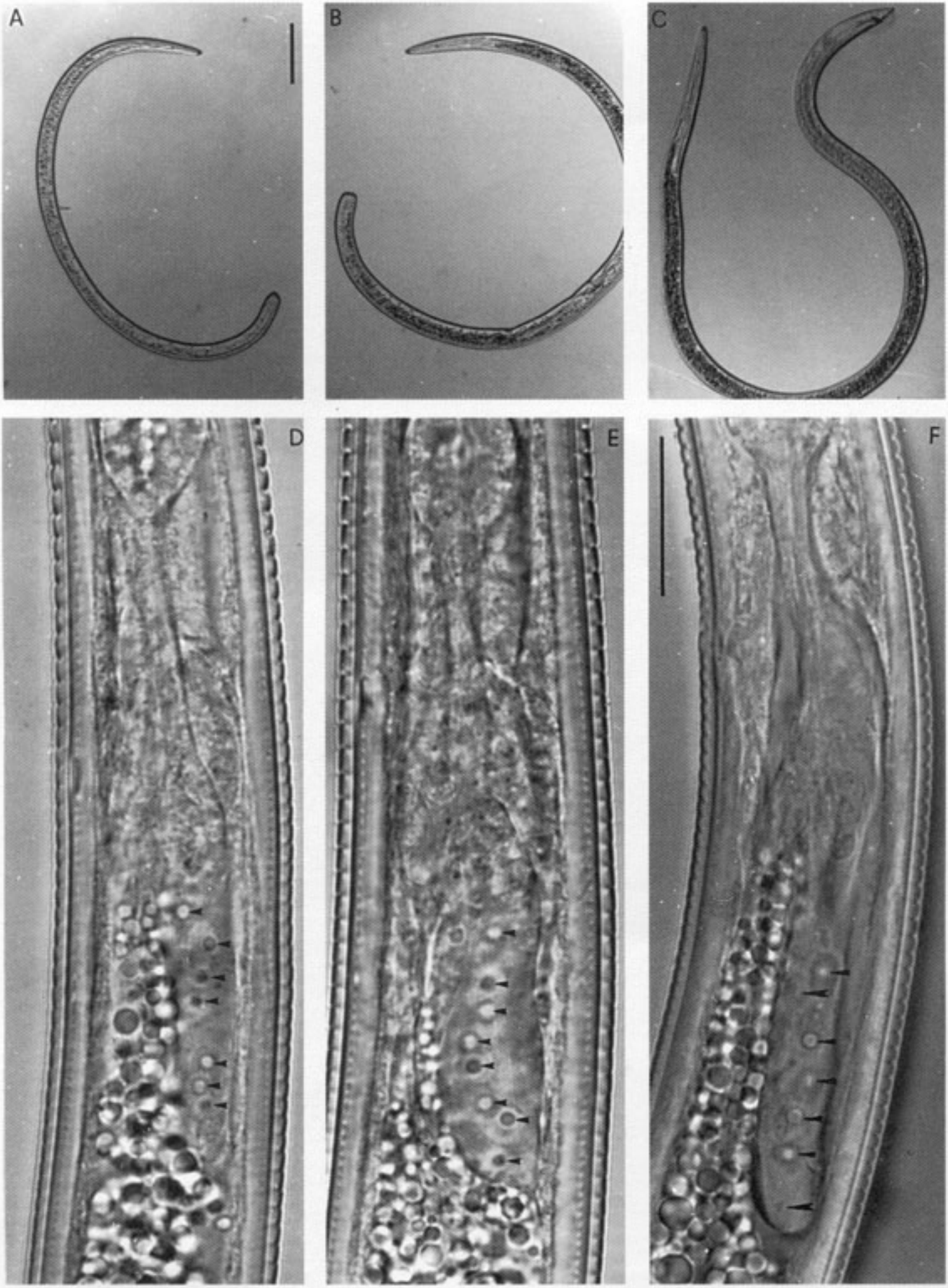


Fig. 1. *Rotylenchus magnus jaeni.* Entire bodies and polinucleate esophageal glands of fourth stage juvenile (A, D), female (B, E) and male (C, F), respectively. Arrows show the location of nuclei at different foci. (Scale bars A-C = 100 µm; D-F = 25 µm).



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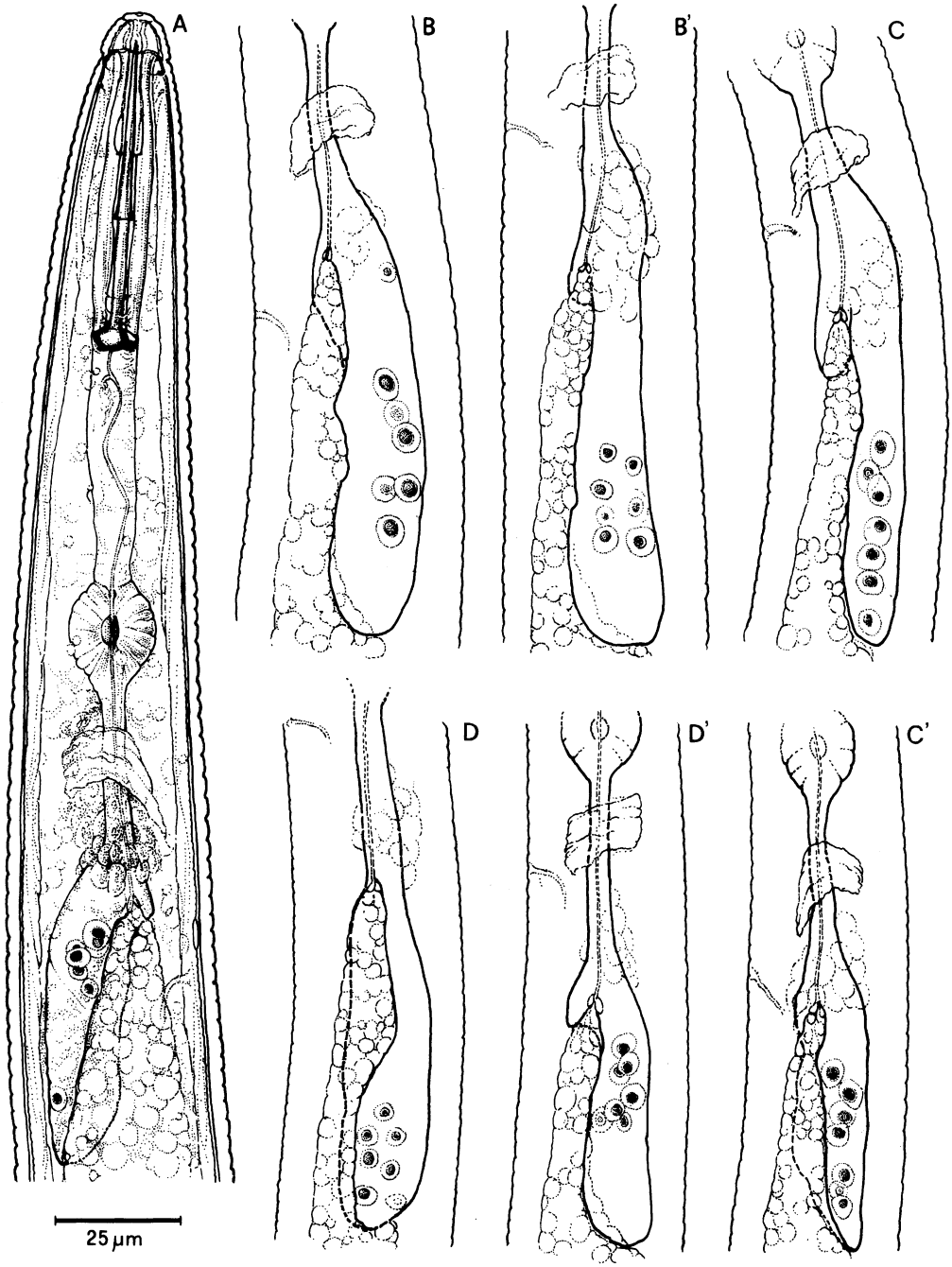


Fig. 2. *Rotylenchus magnus jaeni* anterior region (A) and esophageal glands (B-D') of females (B, B'), males (C, C') and fourth stage juveniles (D, D'), showing number and position of esophageal gland nuclei.

juvenile stages (Fig. 2D, D') has similar morphology as that of adults. Seven esophageal gland nuclei were observed in all the post-embryonic juvenile stages (2nd, 3rd and 4th stages). Esophageal gland nuclei of juveniles are similar in size but differently arranged among the specimens. In some specimens, dorsal gland nuclei are grouped in the distal portion of the gland lobe (Fig. 2B', D) whereas in others they are located in tandem, in the anterior portion of the gland (Fig. 2A, B, D').

Remarks: The presence of seven esophageal nuclei is a very peculiar characteristic and seems to be unique among Tylenchida. With the exception of *R. magnus jaeni*, such a number has only been reported by Luc *et al.* (1987) in a single aberrant specimen of *Neodolichodorus rostrulatus* (Siddiqi, 1976) Siddiqi, 1977. A similarly high number of esophageal gland nuclei is known in the genus *Hoplolaimus* Von Daday, 1907 (Hoplolaimidae), where some members of the genus show five or six esophageal nuclei instead of three. The elongation of the glands over the intestine (pharyngeal overlap) in *R. capitatus* (2 µm) to very long in *R. magnus jaeni* (65 µm) constitutes a morphocline, similar to that described by Baldwin *et al.* (1983) in the genus *Pratylenchoides*. Such a "trend" in the elongation of the esophageal glands is considered by Luc *et al.* (1987) as an evolutionary path, present in at least two families of Tylenchida: Hoplolaimidae and Pratylenchidae. The group of *Rotylenchus* species characterized by the long stylet and long pharyngeal overlap, is considered as the most evolved of the genus. Consequently, species having a very short pharyngeal overlap, such as *R. capitatus*, *R. fallorobustus*, and *R. breviglans* are considered as the least evolved. Baldwin and Bell (1981) grouped *Rotylenchus* with bulb-like esophageal glands abutting the intestine in the genus *Pararotylenchus*. However, Brzeski and Choi (1997) did not recognize the validity of this genus and synonymized *Pararotylenchus* with *Rotylenchus*.

Among *Rotylenchus* species having long pharyngeal overlap, only *R. magnus jaeni* and *R. magnus magnus* possess seven esophageal gland nuclei (Castillo *et al.*, 1994), although in the original description no mention was made of the shape and number of nuclei of the esophageal glands. Other *Rotylenchus* species with dorsal esophageal glands elongated posteriorly to the esophago-intestinal valve, such as *R. cazorlaensis*, *R. eximius*, *R. indorobustus*, *R. orientalis*, and *R. robustus* possess only three esophageal gland nuclei (Castillo *et al.*, 1994). We do not know either whether the presence of seven nuclei in the esophageal glands of *R. magnus jaeni* and *R. magnus magnus* is a consistent morphological character of the two subspecies or if it varies among *R. magnus* populations. Nevertheless, they appear to be more evolved species than the other *Rotylenchus* species because of the longer pharyngeal overlap, and larger number of esophageal gland nuclei.

Rotylenchus agnetis Szczygiel, 1968

(Italian population; Figs. 3, 4; Table 1)

Description of female: Head off-set, hemispherical in profile, with 5 annuli and an oral disc. The basal head annulus is irregularly subdivided into irregular blocks (Fig. 4A, A'). Cephalic framework heavily sclerotized. Stylet robust, about 3 times the maximum width of lip region in length; conus 50% of total stylet length; basal knobs rounded with slightly concave anterior surfaces, about 5-6 µm across (Fig. 4B-B"). Orifice of dorsal esophageal gland 3.8 ± 0.8 (2.3-5.3) µm behind stylet. Median bulb oval, extending over 5-6 body annuli. In the anterior part of its lumen, just behind

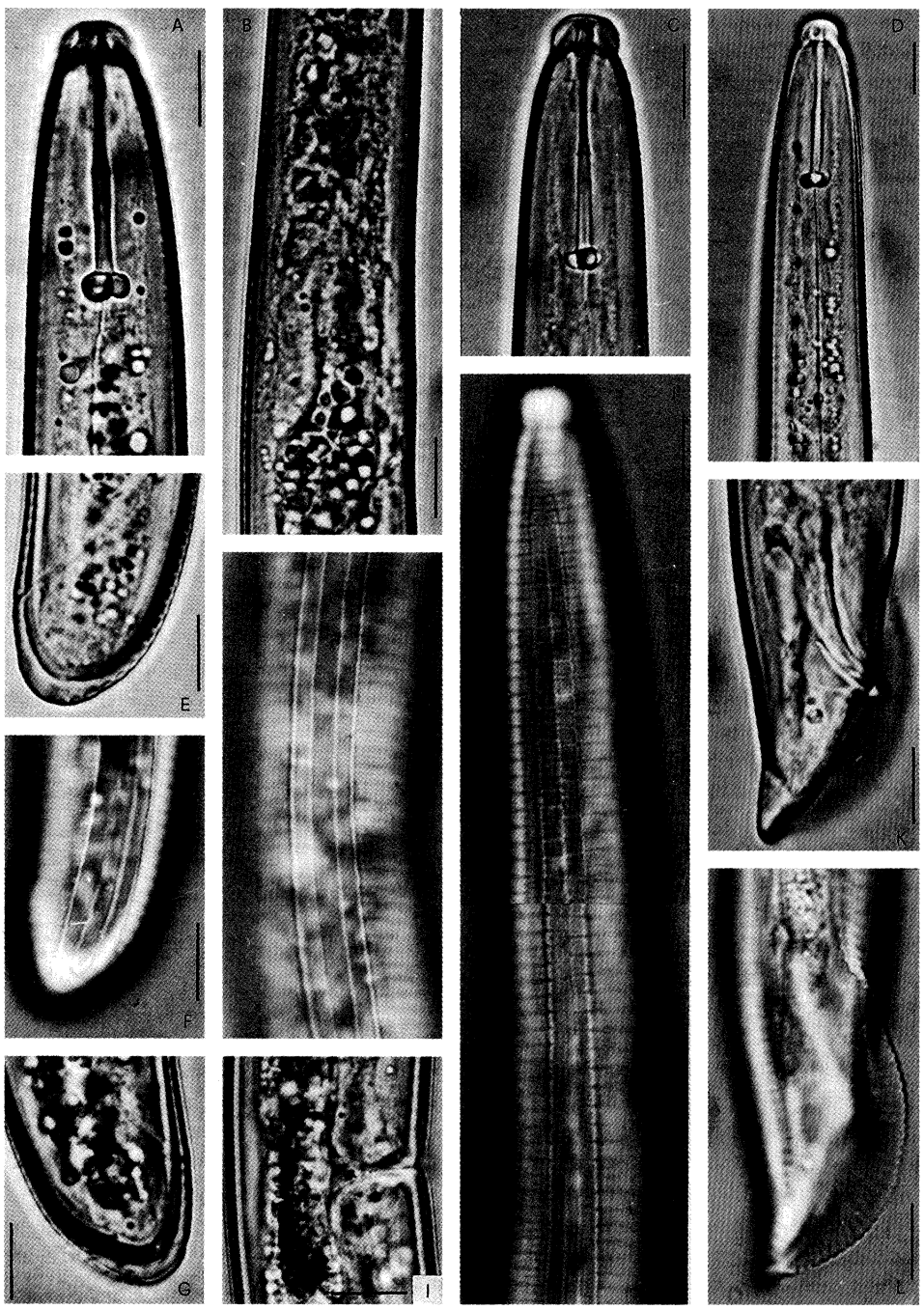
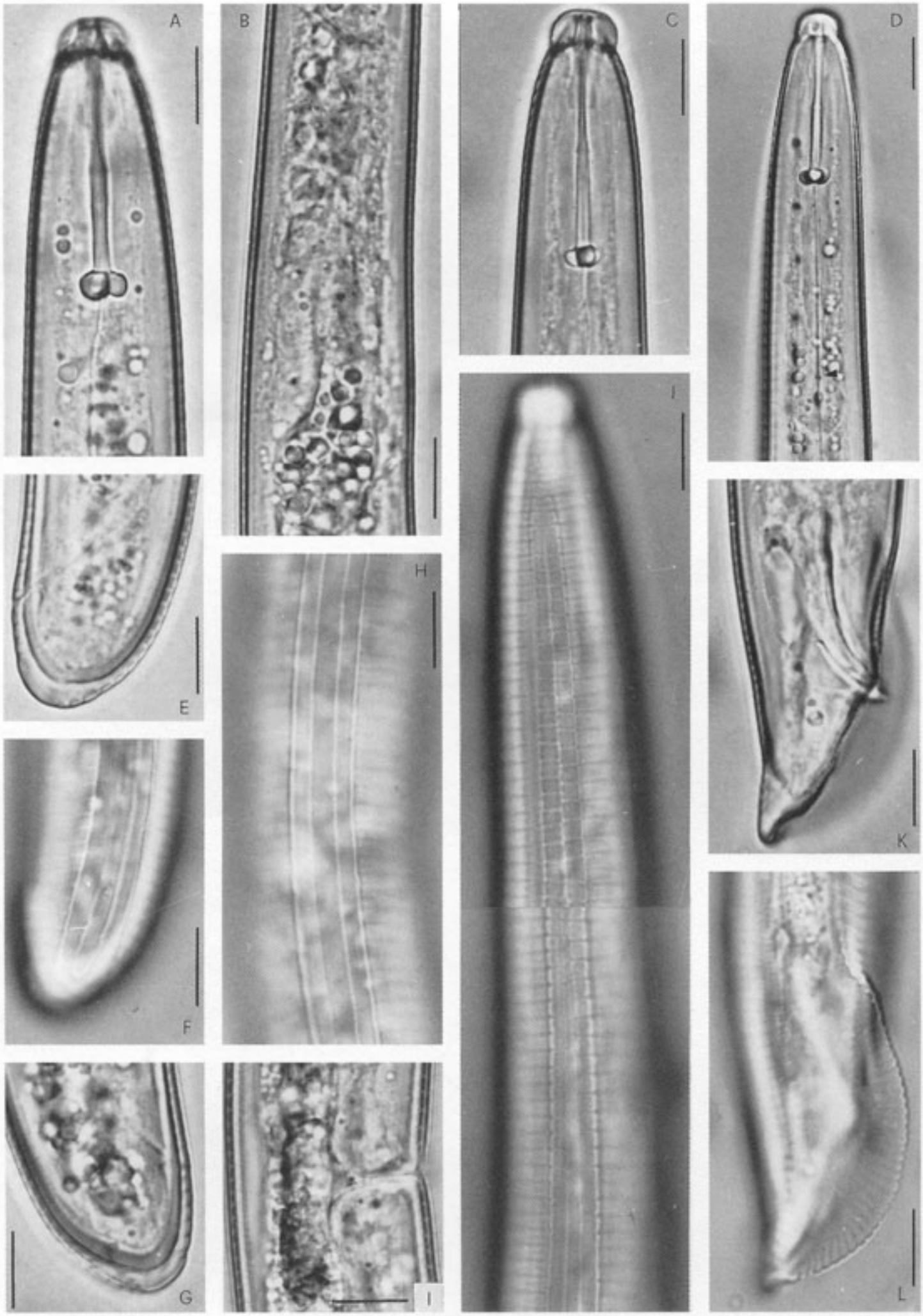


Fig. 3. *Rotylenchus agnetis* (Italian population). A: female anterior end; B: dorsal esophageal extension; C: male anterior end; D: male anterior region (small median bulb body, arrowed); E-G: female tails; H: lateral field at midbody; I: female vulval region; J: lateral field in the anterior body portion; K, L: male tails. (Scale bars = 25 µm).



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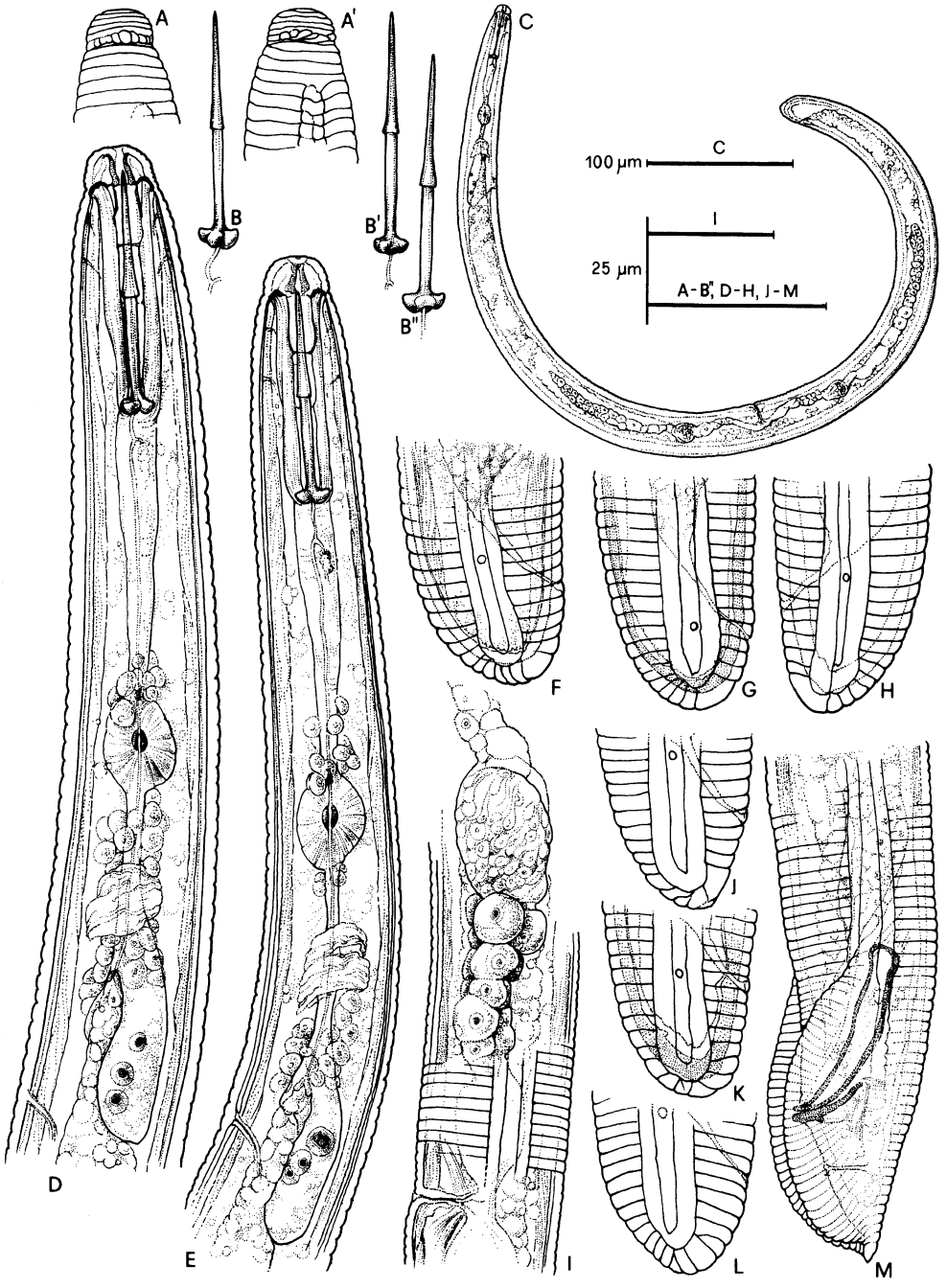

Fig. 4. *Rotylenchus agnetis* (Italian population). A, A': female lip region in lateral view; B-B": female stylets; C: entire female; D: female esophageal region; E: male esophageal region; F-H, J: typical female tails; I: female vulval region, showing spermatheca and lateral field; K, L: uncommon annulated tail terminus; M: male posterior body portion.

Table 1. Morphometrics' of an Italian population of *Rotylenchus agnetis* Szczygiel, 1968.

	females	males
n	25	17
L (µm)	0.79 ± 43.6 (0.72 – 0.86)	0.71 ± 33.6 (0.66 – 0.78)
Stylet (µm)	32.9 ± 0.9 (31.4 – 35)	30.3 ± 1.2 (27.7 – 32.2)
Conus (µm)	16.4 ± 1.3 (13 – 19.1)	15.8 ± 1.3 (13.2 – 18.5)
Knobs width (µm)	5.6 ± 0.4 (4.6 – 6.3)	5.3 ± 0.7 (4.6 – 6.6)
D.G.O. (µm)	3.8 ± 0.8 (2.3 – 5.3)	3.8 ± 0.9 (2.0 – 5.6)
Head-MB (µm)	79 ± 4.0 (68 – 87)	77 ± 4.9 (65 – 84)
Esoph. (valve) (µm)	113 ± 5.9 (101 – 123)	110 ± 7.8 (94 – 121)
Es. Overlap (µm)	24 ± 5.7 (14 – 34)	24 ± 7.0 (16 – 37)
Esoph. (tot.) (µm)	137 ± 7.1 (119 – 151)	134 ± 7.8 (113 – 146)
Excr. pore (µm)	128 ± 8.8 (105 – 142)	123 ± 7.4 (108 – 136)
Max. body w. (µm)	29.9 ± 2.3 (26.4 – 36)	25 ± 1.1 (23.8 – 28)
Annuli width (µm)	2.2 ± 0.2 (1.7 – 2.6)	2.0 ± 0.2 (1.7 – 2.2)
Tail (µm)	13.2 ± 2.6 (8.3 – 19)	22.1 ± 1.9 (19.1 – 26)
Anal body width (µm)	19 ± 1.3 (16.8 – 22.4)	15.6 ± 1.1 (13.2 – 17.2)
N. of Tail annuli	6 ± 1.1 (4 – 8)	— —
Testis (µm)	— —	334 ± 48.6 (216 – 406)
Spicules (µm)	— —	26.1 ± 1.7 (22.4 – 29.7)
Gubernaculum (µm)	— —	11.5 ± 1.0 (8.6 – 12.5)
a	26.8 ± 2.0 (23.7 – 31)	28.3 ± 1.7 (24.2 – 31.3)
b	7 ± 0.6 (5.9 – 8.3)	6.4 ± 0.4 (5.6 – 7.3)

Table 1. (Continued) Morphometrics[a] of an Italian population of *Rotylenchus agnetis* Szczygiel, 1968.

	females	males
b'	6 ± 0.5 (4.7 – 7.0)	5.3 ± 0.4 (4.5 – 6.0)
c	62.6 ± 13.2 (44.5 – 103)	32.2 ± 3.2 (25.3 – 37.4)
c'	0.7 ± 0.1 (0.5 – 0.9)	1.4 ± 0.1 (1.2 – 1.6)
G anterior (%)	22 ± 3.3 (17 – 31)	— —
G posterior (%)	22 ± 2.6 (19 – 29)	— —
V (%) or T (%)	57 ± 1.3 (54 – 60)	47 ± 7.5 (30 – 61)

[a]Mean ± S.D., range in parentheses, N = 25 females and 17 males.

the end of isthmus, a small spherical body is present (Fig. 3D), resembling the small structure described by Coomans (1962) for *R. goodeyi*. Distance from anterior end to valvular apparatus 79 ± 4.0 (68-87) µm. Pharyngeal overlap, 16-44 µm long, bearing 3 esophageal nuclei, arranged in tandem at the posterior part of the glandular lobe. Excretory pore level with, or posterior to the esophago-intestinal valve. Lateral fields with 4 equidistant lines, approximately one fourth of the body width (Fig. 3H). Anteriorly, they begin as three lines at the 7th-8th body annulus (Fig. 3J); after 25-28 annuli the central line divides to form a third band. Irregular areolation of the lateral field is present only in the anterior part of the body. Gonads paired, outstretched in opposite directions, extending for 17-31% of the body length. Tail bluntly conical, asymmetrical, with 4-8 irregular annuli (Fig. 4K, L), often with wide terminal annuli, greatly enlarged on ventral side (Figs. 3G; 4F-H, J). All the terminal annuli connect with the lateral fields. The phasmids are level with, or 2-4 annuli anterior to anus.

Male: Males as abundant as females. Body shape similar to female apart from sexual dimorphism. Bursa arising near spicule heads (Fig. 4M) and enveloping tail; coarsely annulated, moderately protruding ventrally, and extending to tail tip.

DISCUSSION

Peneva and Nedelchev (1992) and Geraert and Barooti (1996) studied extensively the morphometric variation in *R. agnetis*. In our population, the variation in tail length, number of tail annuli, and non-annulated tail ends was much more limited and almost all the female specimens possessed a large terminal annulus, appearing as a transparent bald spot mainly in ventral position (Figs. 3E, G; 4F-H). The stylet corresponds to measurements given by Szczygiel (1968) and Peneva and Nedelchev (1992), respectively, of 32-35 µm and 29-36 µm, but it is slightly shorter than the 32-39 µm reported by Berezina (1985) for *R. aceri*, a species synonymized with *R. agnetis*. It is longer than that reported by Scotto La Massese and

Germani (1997) for the two Algerian populations. DGO is also slightly shorter (2.3-5.3 µm) than all previous reports, and the bursa in males envelops tail until the tail tip, similarly to that illustrated in the Iranian [Geraert and Barooti (1996)] and Algerian [Scotto La Massese and Germani (1997)] populations *vs.* ending before the end of tail in the Bulgarian population showed in the Peneva and Nedelchev (1992) drawings.

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