Development and life cycle of *Neodolichodorus rostrulatus* (Siddiqi, 1976), with observations on the copulatory plug (Nematoda : Tylenchina)

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SUMMARY

The life cycle of *Neodolichodorus rostrulatus* is described, from egg laying to formation of adults; its duration is sixteen to seventeen weeks; detailed observations on the embryonic development are reported. *N. rostrulatus* has been demonstrated to be strictly ectoparasitic on tomato. The glands which in the male produce the cement of the copulatory plug observed on the mated females are described and illustrated in some detail.

RÉSUMÉ

Développement embryonnaire et cycle biologique de Neodolichodorus rostrulatus *(Siddiqi, 1976), et observations sur le bouchon copulatoire (Nematoda : Tylenchina)*

Le cycle biologique de *Neodolichodorus rostrulatus* est décrit depuis la ponte jusqu'à la formation des adultes; sa durée est de seize à dix-sept semaines; des observations détaillées sur le développement embryonnaire sont rapportées. Il a été démontré, sur tomate, que *N. rostrulatus* est strictement ectoparasite. Une étude détaillée et abondamment illustrée concerne les glandes du mâle produisant la matière du bouchon copulatoire observé chez les femelles fécondées.

A population of *Neodolichodorus rostrulatus* (Siddiqi, 1976) from Senegal was used for a redescription of the species including males and the various juvenile stages (Luc, Coomans & Sarr, 1987). Observations on the development and life cycle of the species are reported here as well as some details on the production and function of the copulatory plug.

Materials and methods

Fifty samples of 100 juveniles of second stage of *N. rostrulatus* were picked out of a large population extracted from soil by elutriation (Seinhorst, 1962). This population, cultivated in glass house on tomato, originates from a tomato field, at Tiaroye, Senegal (Luc, Coomans & Sarr, 1987). Each of these 50 samples was inoculated on a two-week old tomato plant (cv. Roma), growing in a plastic pot, in 1 000 cm^3 autoclaved soil and placed in a glass house, at 27° ± 2.

Each week, two pots were used : nematodes were extracted from soil by elutriation; half of the root system of the two tomato plants was placed in a mistifier (Seinhorst, 1950), the second half being stained with acid fuchsin in boiling lactophenol (Franklin, 1949).

To study the embryonic development, eggs were obtained by placing gravid females in a Syracuse watch glass containing 3 cm^3 of distilled water. Fourteen one-celled eggs were selected and each of them placed in a drop of distilled water on a cavity slide. The slides were kept in a moist chamber (humidity *ca* 80 %) at 25°. Observations were made every hour during the first twelve hours, and every 12 h during the remaining period.

Results

LIFE CYCLE

Eggs are usually laid (Fig. 1A) at the two-cell stage, more rarely at the one- or four-cell stage. The fourteen one-celled eggs selected to study the embryonic development presented a granular content with a hyaline crescent-shaped area at each extremity; they measured 120 (117 — 142) × 39 (33 — 46) µm (Fig. 1A).

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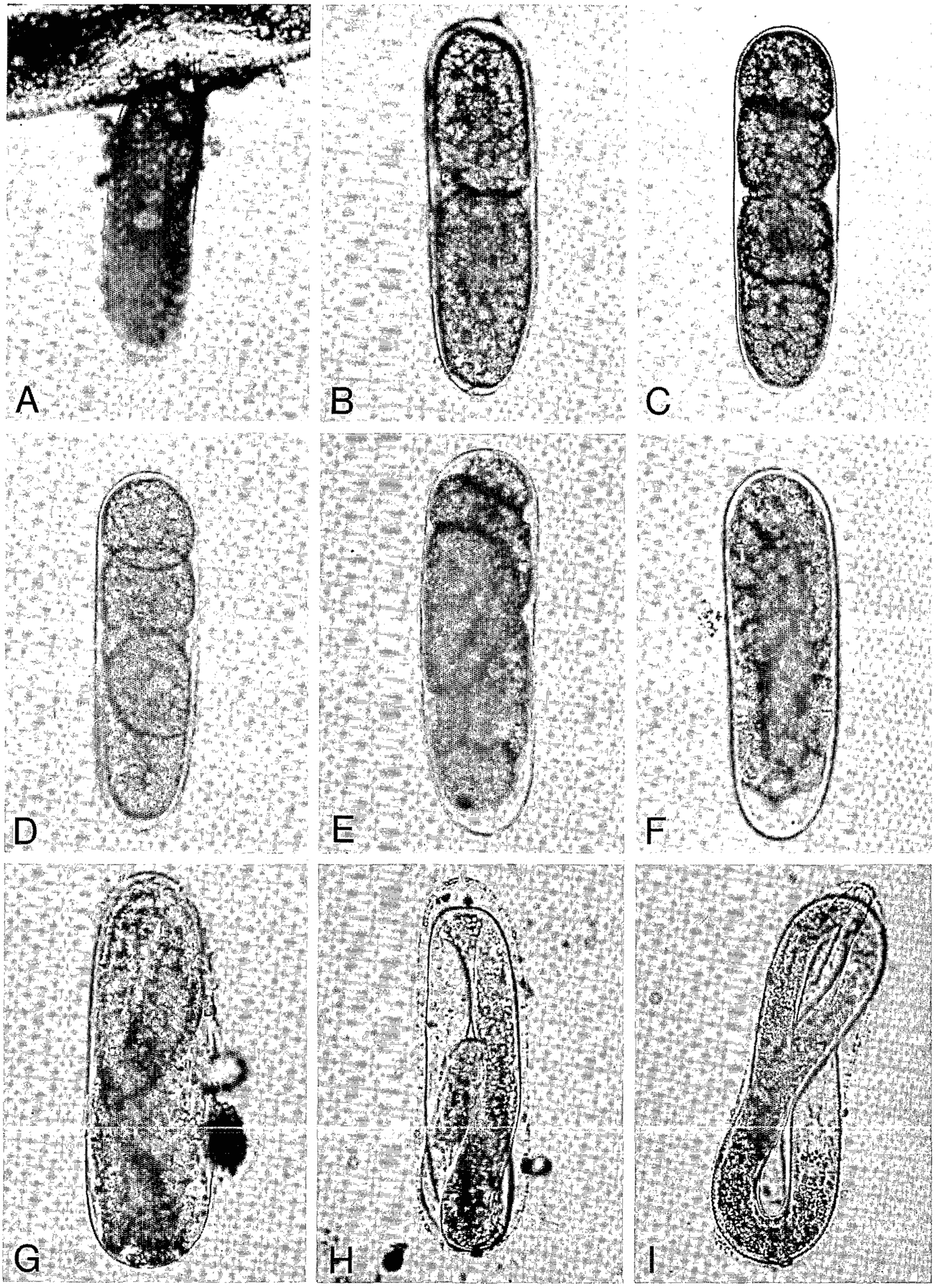

Fig. 1. Development of *Neodolichodorus rostrulatus* A : Egg-laying; B : Two-cell stage; C : Four-cell stage; D : Five-cell stage; E : Eight-cell stage; F : Gastrula stage; G : Early pretzel stage; H : Late pretzel stage (first stage juvenile, without stylet); I : Second stage juvenile just before hatching.

When deposited at the one-cell stage, the two-cell stage (Fig. 1B) appears 2 h after egg-laying and the four-cell stage (Fig. 1C) 12 h after egg-laying. Then, the cell divisions become asynchronous (five-cell stage, Fig. 1D) and rapidly lead to the eight-cell (Fig. 1E) and morula stage. The gastrula stage can be recognized between the 3rd and the 4th day. At that moment the embryo is clearly divided into two areas, differing in their translucent *vs* opaque content (Fig. 1F). The first stage juvenile, folded inside the egg shell, appears at the 7th day (Fig. 1G, H) and moults after one day to the second stage (Fig. 1I). The latter is folded eight-shaped inside the egg shell before it hatches at day 9 of 10.

The second moult, leading to the third juvenile stage, takes place from day 15 to day 21 after inoculation and the third moult, leading to the fourth juvenile stage, occurs from day 28 to day 35 after inoculation. The fourth moult begins during the seventh week and lasts about four weeks so that the first adults can be observed during the eleventh week after inoculation.

From an initial inoculum of 100 J2, 65 reached the adult stage. The adults may survive at least seven weeks in the soil.

Mating takes place during the first days following the exsheatment of the adults. After mating the vulva is sealed off by a copulatory plug (see below). J2 of the second generation are first recorded in the soil about six weeks after the adults of the first generation start to appear. This indicates that there is a time lapse of 4-5 weeks between mating and egg laying. The complete life cycle takes about sixteen to seventeen weeks : four weeks for the pre-embryonic period, ten days for the embryonic development (till hatching of the second stage juveniles) and eleven weeks for the post-embryonic development.

N. rostrulatus reproduces readily on tomato. It is a strict ectoparasite : no specimen of any stage could be extracted from the roots using a mistifier or could be observed in stained roots.

Observations on the copulatory plug

Although a direct proof is not available, indirect evidence indicates that the substance forming the copulatory plug is produced by the male. Indeed, as shown in Figs 2 and 3A-C the posterior region of the *vas deferens* is very large and contains numerous granules. Cross sections show the *vas deferens* so large as to compress the intestine in that area (Fig. 2, 1-6). At first the wall of the duct is finely granular and the cells appear vacuolar (Fig. 2, 1 & 2); then large, refractive granules appear especially in the ventral and lateral parts of the wall of the *vas deferens* (Fig. 2, 3-6). The last of these granules can be seen between the proximal parts of the spicules (Fig. 2, 7). It is supposed that these granules contain the substance which is evacuated in the cloaca and then used as a cement to hold the mating pair in copula. Afterwards, the secretion is retained by the female as a large, yellowish mass (copulatory plug) which seals off the vulva (Figs 3D-F & 4). Similar plugs have been observed in *Pelodera strongyloides* (Chitwood, 1929; Wagner & Seitz, 1984), *Cephalobus* sp. and *Acrobeles* sp. (Chitwood, 1929), *Bunostomum trigonocephalum* (Threlkeld, 1941), *Acrobeloides* sp. (Jairajpuri & Azmi, 1977), *Scutellonema cavenessi* (Demeure, Netscher & Quénéhervé, 1980) and *Desmodora schulzi* (Vincx, 1983). In *Croconema mammillatum*, Steiner and Hoeppli (1926) described a « copulatory ring » observed in mated females; it differs from the copulatory plug as being situated above the vulva of which the opening remains free, and encircling the body.

Jensen (1986) illustrates in *Theristus (T) copulatus* a peculiar cap around the vulva which strongly recalls a copulatory plug.

Chitwood (1929) describes in *P. strongyloides* two lateral ejaculatory glands alongside the posterior part of the *vas deferens* and opening into the cloaca, which he considers to produce the adhesive cement deposited on the female at copulation. These glands are similar in position and appearance to those observed in *N. rostrulatus* (compare ej.1 in Fig. 3 of Chitwood & Chitwood, 1950 with our Fig. 2), except that they are more developed and more incorporated into the wall of the *vas deferens* in *N. rostrulatus*. Also in *D. schulzi*, Vincx (1983) describes in the male a « glandular organ » opening in the cloaca and occupying a similar position along the *vas deferens*.

According to Wagner and Seitz (1984) the primary function of the plug is the cementing of the mating pair, but the secretion could also prevent bacteria and fungal spores to enter the vagina. Sudhaus (1976) and Wagner and Seitz (1984) postulate a bactericidal effect of the plug. At least in *P. strongyloides* the bursa acts as a mould for the copulatory substance (Wagner & Seitz, 1984).

When, after copulation, both partners become detached, the cement only adheres to the female cuticle. A possible explanation for this phenomenon was proposed by Wagner and Seitz (1984) : a secretion produced by epidermal glands of the female and excreted through cuticular canals would enhance the adhesion of the cement to the female's cuticle, a mechanism comparable to the principle of a two component glue. Such glands of which the existence is hypothetical could not be located with certainty in *N. rostrulatus* females. On the other hand, Chitwood (1929) reported the possibility of a second copulation in *P. strongyloides* after a male redissolved a portion of the previously deposited plug (called « copulatory saccus » by Chitwood). If so, the male (at least of that species) may possess release glands that dissolve the cement, allowing to free himself from the female.

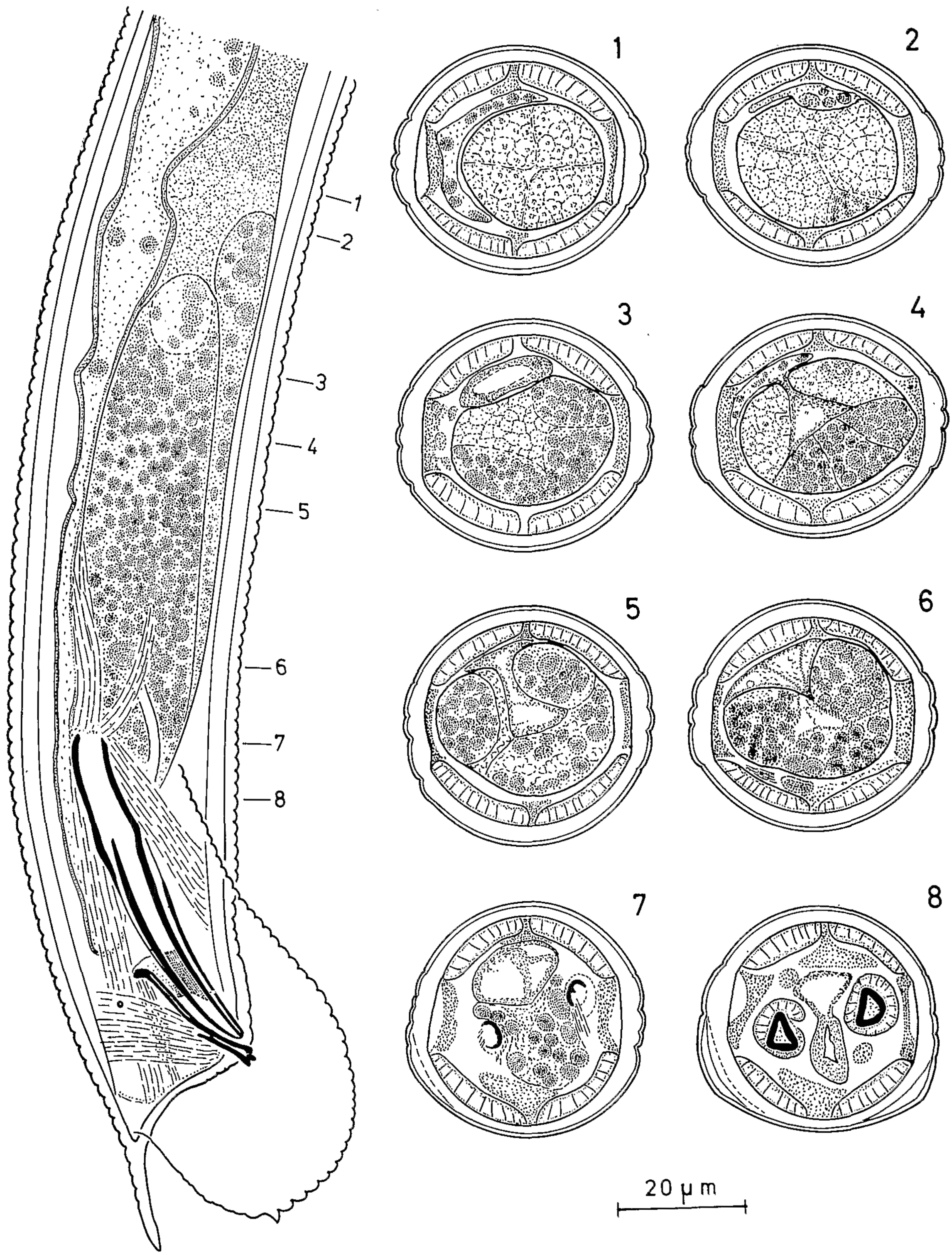

Fig. 2. Hind part of male *N. rostrulatus* with cross sections at levels indicated, showing the glandular area that probably produces the copulatory plug.

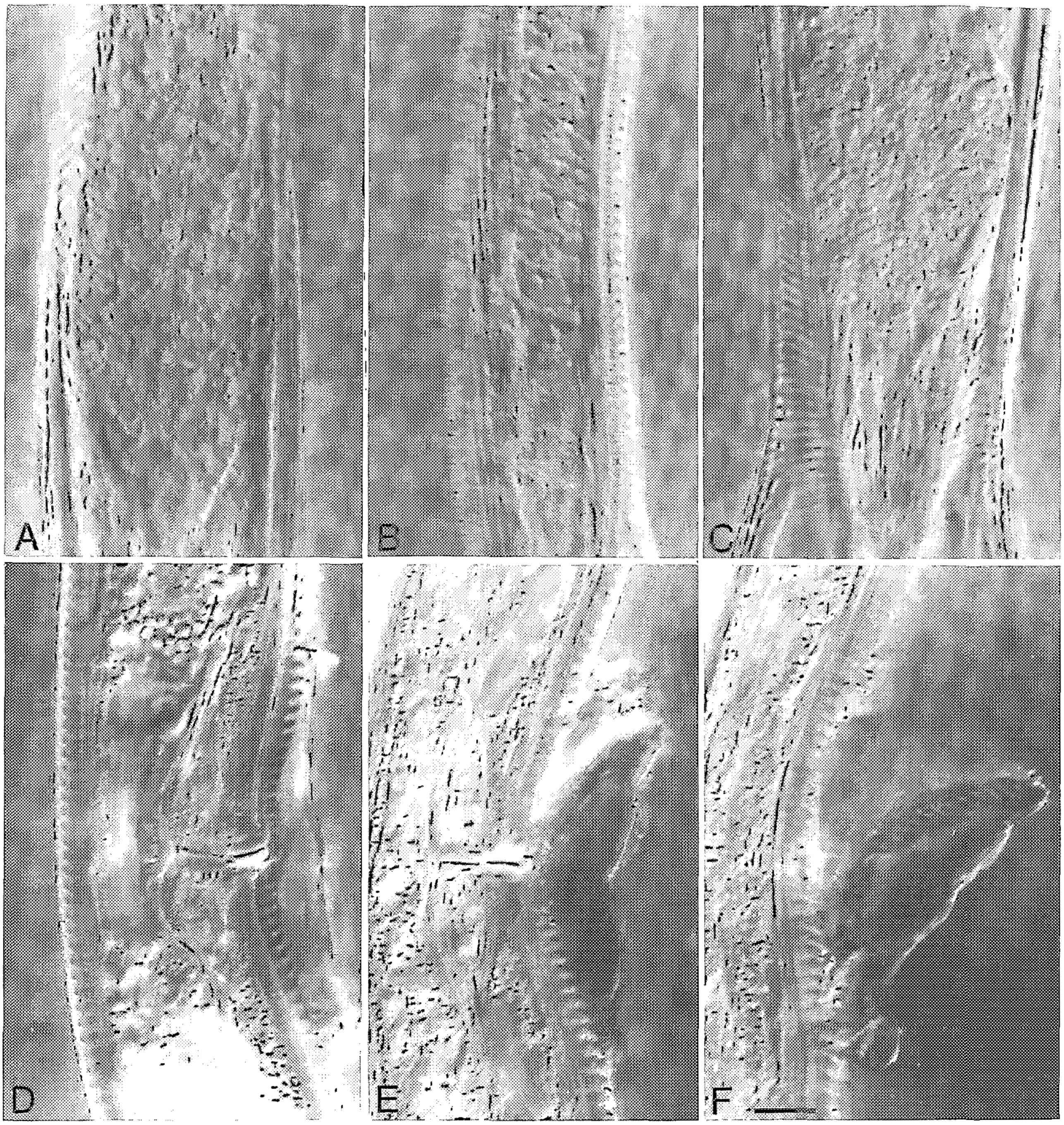

Fig. 3. A-C : Hind part of male *N. rostrulatus* in dorsal, lateral and ventral view respectively, showing large secretory granules. D-F : Copulatory plug on the vulva area of fertilized females (in C partly detached). Bar represents 10 μm.

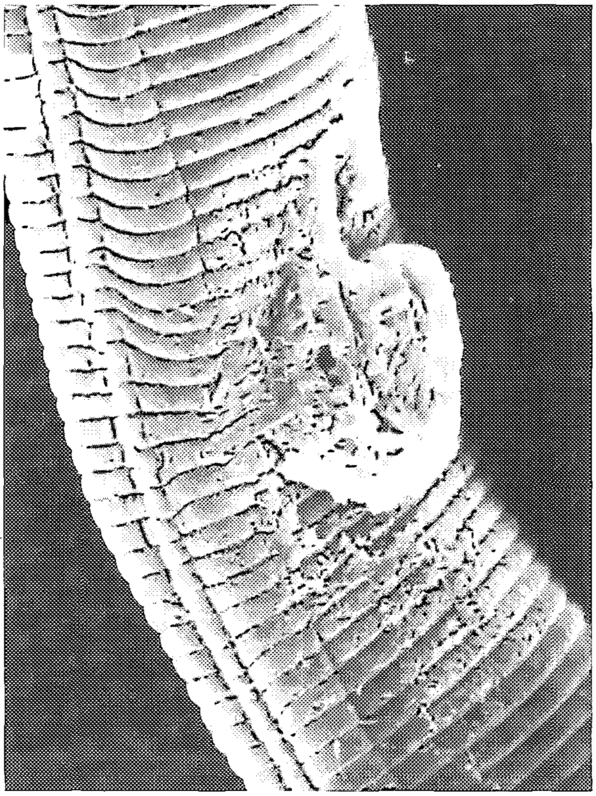

Fig. 4. Scanning electron micrographs of the copulatory plug on the vulva area of *N. rostrulatus* females.

ACKNOWLEDGEMENTS

The authors are indebted to the National Foundation for Scientifical Research, Brussels, Belgium, for supporting part of the research.

REFERENCES

CHITWOOD, B. G. (1929). Note on the copulatory sac of *Rhabditis strongyloides* Schneider. *J. Parasitol.*, 15 : 282-283.

CHITWOOD, B. G. & CHITWOOD, M. B. (1950). *An introduction to Nematology*. Baltimore, University Park Press, 213 p.

DEMEURE, Y., NETSCHER, C. & QUÉNÉHERVÉ, P. (1980). Biology of the plant-parasitic nematode *Scutellonema cavenessi* Sher, 1964 : reproduction, development and life cycle. *Revue Nématol.*, 3 : 213-225.

FRANKLIN, M. T. (1949). A quick method of demonstrating nematodes of the genus *Aphelenchoides* in leaves. *J. Helminth.*, 23 : 91-93.

JAIRAJPURI, M. S. & AZMI, M. J. (1977). Reproductive behaviour of *Acrobeloides* sp. *Nematologica*, 23 : 202-212.

JENSEN, P. (1986). The nematode fauna in the sulphide-rich brine seep and adjacent bottoms of the East Flower Garden, NW Gulf of Mexico. II. Monhysterida. *Zool. Script.*, 15 : 1-11.

LUC, M., COOMANS, A. & SARR, E. (1987). Redescription of *Neodolichodorus rostrulatus* (Siddiqi, 1976) Siddiqi, 1977 (Nematoda : Tylenchina). *Revue Nématol.*, 10 : 29-37.

SEINHORST, J. W. (1950). De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaaltje (*Ditylenchus dipsaci* (Kühn) Filipjev). *Tijdschr. Plziekt.*, 50 : 291-349.

SEINHORST, J. W. (1962). Modification of the elutriation technique method for extracting nematodes from soil. *Nematologica*, 8 : 117-128.

STEINER, G. & HOEPPLI, R. (1926). Studies on the exoskeleton of some Japanese marine nemas. *Arch. Schiffs-u. Tropenhyg.*, 30 : 547-576.

SUDHAUS, W. (1976). Vergleichende Untersuchungen zur Phylogenie, Systematik, Ökologie, Biologie und Ethologie der Rhabditidae (Nematoda). *Zoologica*, 43, Heft 125 : 1-229.

THRELKELD, W. Z. (1941). Notes on copulation of certain nematodes. *Virginia J. Sci.*, 2 : 31-34.

VINCX, M. (1983). Redescription and ontogeny study of *Desmodora schulzi* Gerlach, 1950. *Biol. Jb. Dodonea*, 51 : 171-179.

WAGNER, G. & SEITZ, K. A. (1984). Funktionsmorphologische Untersuchungen an Vagina, Vulva, Vulvapfropf und Vulva - assoziierter Hypodermis bei *Pelodera strongyloides* (Nematoda : Rhabditidae). *Nematologica*, 29 (1983) : 190-202.

Accepté pour publication le 8 septembre 1986.