A scanning electron microscopic study on Dorylaimus stagnalis Dujardin, 1845

Samina Shafqat, Irfan Ahmad, Wasim Ahmad and Anwar L. BILGRAMI

Section of Nematology, Department of Zoology, Aligarh Muslim University, Aligarh - 202002, India.

SUMMARY

Detailed observations have been made on *Dorylaimus stagnalis* with the scanning electron microscope. The cuticle on the lip region is sculptured, lips are amalgamated and provided with the usual sixteen sensilla. The longitudinal ridges (bands) on the cuticle are made up of four to seven discrete cuticular folds. The ridges which extend from the neck region to the anus/cloaca may frequently bifurcate or terminate at any point or form loops. The entire body including the ridges is finely transversely striated. In males, the posterior region has a midventral groove divisible into pre-, post- and supplement regions. Four additional rows of papillae are present on each side of the groove.

Résumé

Étude de Dorylaimus stagnalis Dujardin, 1845 en microscopie électronique à balayage

Des observations détaillées ont été faites sur *Dorylaimus stagnalis* en microscopie électronique à balayage. La cuticule de la région labiale est ornementée, les lèvres sont soudées et pourvues des seize sensilles normales. Les côtes (bandes) longitudinales sont constituées par quatre à sept replis cuticulaires peu développés. Les côtes, qui s'étendent de la région cervicale à celle de l'anus — ou du cloaque —, peuvent fréquemment être bifurquées, se terminer à n'importe quel endroit du corps, ou former des boucles. Chez le mâle, la région postérieure présente un sillon médioventral différencié en trois zones : celle des suppléments et celles situées antérieurement et postérieurement à ceux-ci. Quatre rangées supplémentaires de papilles sont présentes de chaque côté du sillon.

In recent years SEM has provided much detailed informations on the surface structure of different nematode groups (Sher & Bell, 1975; Sher, Bell & Rodriguez, 1977). Studies by Jairajpuri and Southey (1984), Baldwin (1986), Geraert and Raski (1988), and Fortuner and Luc (1987), have highlighted the utility of SEM observations in elucidating concepts in taxonomy and phylogeny.

On dorylaims, except for a few studies on longidorids (Lamberti & Martelli, 1971; Lamberti, 1975; Hogger & Estey, 1976; Eveleigh & Allen, 1982; Swart & Heyns, 1987; Coomans & Carbonell, 1987; Vinciguerra & Coomans, 1988), SEM studies have remained largely neglected. Sauer (1985) and Coomans, Vinciguerra and Loof (1990) provided scanning electron micrographs of several dorylaim genera. Although the dorylaims in general offer relatively few surface structures for observations, we studied *Dorylaimus stagnalis* Dujardin, 1845 to characterise, in particular, the longitudinal ridges, transverse striations and the caudal supplements and papillae of the males.

Materials and methods

Freshly collected *D. stagnalis* were fixed in 3 % glutaraldehyde solution for 90 min, washed in 0.05 M

Revue Nématol. 14 (4) : 511-515 (1991)

sodium phosphate buffer several times then post-fixed in 2 % osmium tetraoxide for 2 h at room temperature and finally washed again in buffer. The specimens were dehydrated in a six grade alcohol series and critical point dried using carbon dioxide as the transitional fluid. Dried specimens were mounted on stubs using a double sided adhesive tape, coated with 30 nm layer of gold and examined with a Hitachi S 2300 scanning electron microscope at 15 and 25 kV. Spicules were extracted and prepared as described by Eisenback (1986).

Results

LIP REGION

Oral aperture circular surrounded by six closely amalgamated, equal sized lips. Anterior sensilla arranged in a 6 + 4 + 6 pattern. Cephalic sensilla situated at a level between the inner and outer circlet. Each sensillum is distinctly elevated and provided with a pore. The outer and inner labial sensilla are more prominent than the cephalic ones. Cuticle on the lip region sculptured to from four to six broken concentric circumoral rings and a variable number of rings on each lip surrounding the papillae (Fig. 1 A, C).



Fig. 1. Dorylaimus stagnalis Dujardin, 1845 — Female — A, C : Anterior ends; B : Protruded odontostyle (dorso-ventral); D : Ridges at midbody; E : Ridge formation in anterior region; F, G : Ridges and transverse striations; H : Bifurcating ridges; I : Ridges forming loops; J : Vulval region; K : Female posterior region. (Bars = 5 μm in B, C; 25 μm in K.)

ODONTOSTYLE

The odontostyle is hollow, cylindroid with a smoothly rounded tip (Fig. 1 A, B). The aperture is about 13 μ m long and continues as a very narrow slit for some distance as the sleeves of the spear apparently fail to meet immediately below the aperture (Fig. 1 B).

AMPHIDS

Amphidial apertures slit-like about 5 μ m across, situated at the junction of the lip region with the body.

CUTICLE

Cuticle marked with fine transverse striations and longitudinal ridges. Transverse striations present over the entire body. The longitudinal ridges start at the neck region and gradually fade out slightly anterior to the level of anus/cloaca. Each ridge begins to form by irregular and uneven elevations of the cuticle (Fig. 1 E), gradually these elevations become uniform and grouped into longitudinal bundles forming the longitudinal ridges (Fig. 1 D). At the midbody, each ridge is about 2 µm wide and appears like a band consisting of four to seven small and discrete cuticular folds (Fig. 1 F, G). The folds forming the ridges are of variable sizes. Except for some of the outer folds, which may occasionally be relatively straight, each follows a slightly wavy pattern almost parallel to the adjacent one. The folds of the ridges may terminate abruptly or bifurcate (Fig. 1 F, G). A longitudinal ridge may not always traverse the entire length of the body but may terminate at any point. On the other hand, a ridge may also bifurcate (Fig. 1 H). Occasionally ridges bifurcate and then reunite forming loops (Fig. 1 I). Not infrequently, very small aberrant ridges may be formed at any part of the body (Fig. 2 A).

Revue Nématol. 14 (4) : 511-515 (1991)



Fig. 2. Dorylaimus stagnalis Dujardin, 1845 — A : Pre-supplement region (showing termination of ridges and one small aberrant ridge); B : Ventromedian supplements and subventral papillae; C : Posterior region showing cloacal opening and papillae; D : Single lateral papilla; E : Posterior region showing post-supplement groove and four rows of papillae; F : Spicule; G, H, I : Ventromedian supplements. (Bars = $20 \ \mu m$ in A, B, C, E, F.)

In males, the ridges begin to narrow from a region slightly anterior to the beginning of the supplements (Fig. 2 A, B).

Transverse striations are very fine, about 0.5 μ m apart at midbody and pass through the ridges (Fig. 1 G). The transverse striations generally span the distance between the ridges but may, occasionally, terminate in between. As the longitudinal ridges are elevated regions, the transverse striations generally form a shallow concavity between them (Fig. 1 D, E, F). At low magnifications the transversely striated body appears to have alternating light and dark bands (Fig. 1 D). At high magnifications the light bands themselves appear like two light bands sandwiching a dark band... the striae (Fig. 1 F, G). The margins of the interstriae region adjacent to the striae are slightly raised.

VULVA AND ANUS

The vulva is small, circular, generally less than 5 μ m in diameter, located between two longitudinal ridges (Fig. 1 J). Some amount of shrinkage was always observed in the vulval region. The anal opening is a crescent-shaped slit measuring 12-13 μ m across (Fig. 1 K).

MALE POSTERIOR REGION

On the posterior region of males are present the ventromedian supplements and an assortment of papillae. The ventral longitudinal ridges terminate well before the beginning of the supplements (Fig. 2 A) and extending from this region to the cloaca is a midventral groove

Revue Nématol. 14 (4) : 511-515 (1991)

that is clearly divisible into a pre- supplement, supplement and a post-supplement region. The pre-supplement groove (Fig. 2 A) is slightly more than 5 µm long and trench like. The margins are attenuated forming distinct edges which merge with the body anteriorly and posteriorly (Fig. 2 A, G). The ventromedian supplements which begin from just beyond the pre-supplement region are situated on a ridge within the supplement groove (Fig. 2 G, H, I). Each supplement is prominent with a centrally located papilla (Fig. 2 G, H). The papilla has a small depression but whether it is a pore or not could not be ascertained. Towards the posterior region the supplements are slightly smaller (Fig. 2 B) and the last two or three become progressively degenerate (Fig. 2 I). From about the region of the last few supplements, the margins of the groove again form an edge and continue into the post-supplement region (Fig. 2 E, I). Beyond the ventromedian supplements the groove continues posteriorly, gradually widens and merges with the body contour just anterior to the cloaca as also do the outer edges of the groove (Fig. 2 C, E).

CLOACAL OPENING

A C-shaped slit with thin anterior lip and a thick cushion-like posterior lip (Fig. 2 C).

ADANAL PAPILLAE

These papillae are paired, extremely small and about 3 μ m apart (Fig. 2 C).

OTHER PAPILLAE

In addition to the supplements, the male posterior region bears at least four rows of comparatively smaller papillae on each side. The subventral begin from anterior to the pre-supplement groove and continue up to the cloacal opening, terminating below it in a disorganized manner (Fig. 2 C, E). Of the three other rows on each side, one is lateral, one ventrosublateral and one dorsosublateral. The laterals are a consistant feature found along the entire body length, although widely spaced anterior to the supplements. In the posterior region they are more closely arranged (Fig. 2 E). The ventrosublaterals and the dorsosublaterals arise at about the region where the supplements begin and continue posteriorly, being more or less regularly arranged, towards the tail tip. The tail terminus presents a disorganized aggregation of papillae as three rows from the two sides of the body converge towards it (Fig. 2 C, E). Each papilla is button shaped, slightly more than 1 µm in diameter and with a distinct opening (Fig. 2 D).

Discussion

Although few studies are available for comparison, certain features of *D. stagnalis* appear to be quite in-

teresting. What looks like ridges in the light microscope are in effect longitudinal bands made up of discontinuous longitudinal cuticular folds. Because of the nature of the longitudinal bands (ridges) their use as a qualitative character should be judicious taking into account its extreme variability. However, provided information is available, qualitative aspects of these bands may provide a more reliable character. Further studies are needed to reveal the nature of longitudinal ridges (bands) in Dorylaimus species as well as in other dorylaims. The longitudinal ridges of Etamphidelus acucephalus and E. fueguensis are smooth and made up of a single cuticular fold, being irregularly, obliquely or transversely striated in the former species (Coomans & Raski, 1988). The sculpturing of the cuticle on lip region is also found (as seen in SEM photomicrographs) in Eudorylaimus sp., and Aporcelaimellus sp. (Sauer, 1985). The odontostyle though typically dorylaimoid, is different from that of Aporcelaimellus (Lippens et al., 1974) in that the sleeves do not appear to overlap and the aperture continues as a narrow slit for about 3/4 of its length.

The caudal region of males seems to be well adapted for a sensory and copulatory role. The ventromedian supplements are numerous and although situated in a groove, they may be fully exposed once the copulatory muscles contract pulling outwards laterally the margins of the groove. In the same context it may be tempting to suggest that when the edges of the pre- and post-supplement regions are pulled outwards they could act as grasping surfaces, performing a function somewhat analogous to the caudal alae. The numerous papillae (laterals, ventrosublaterals, dorsosublaterals and subventrals), sometimes partly referred to as body pores, are apparently glandular sensory structures of the somatic sensory system and may play a role during reproductive activities.

ACKNOWLEDGEMENTS

This research work was supported by the grants from the Indian Council of Agricultural Research, New Delhi, and the Departmental Research Support Programme of the University Grants Commission, New Delhi.

References

- BALDWIN, J. G. (1986). Testing hypothesis of phylogeny of Heteroderiderae. In : Lamberti & Taylor, C. E. (Eds). Cyst Nematodes. New York, Plenum Publishing Co. : 75-100.
- COOMANS, A. & CARBONELL, E. (1987). The status of the family Thornenematidae Siddiqi, 1969 (Nematoda : Dorylaimida). *Nematologica*, 33 : 375-386.
- COOMANS, A. & RASKI, D. J. (1988). Three new species of *Etamphidelus* Andrássy, 1977 (Nemata : Alaimidae) in Southern Chile. *Journal of Nematology*, 20 : 9-22.
- COOMANS, A., VINCIGUERRA, M.-T. & LOOF, P. A. A. (1990). Status of the genera Paractinolaimus Meyl, 1957, Trachy-

Revue Nématol. 14 (4) : 511-515 (1991)

pleurosum Andrássy, 1959 and Trachactinolaimus Andrássy, 1963 (Nematoda : Actinolaimidae) with description of Trachypleurosum venezolanum n. sp. Revue de Nématologie, 13 : 143-154.

- EISENBACK, J. D. (1985). Techniques for preparing nematodes for scanning electron microscopy. In : Barker, K. R., Carter, C. C. & Sasser, J. N. (Eds). An advanced Treatise on Meloidogyne, Vol. II. Raleigh, Dept. Pl. Pathol. & USAID : 79-105.
- EVELEIGH, E. S. & ALLEN, W. R. (1982). Description of Longidorus diadecturus n. sp. (Nematoda : Longidoridae), a vector of the peach rosette mosaic virus in peach orchards in South Western Ontario, Canada. Canadian Journal of Zoology, 60 : 112-115.
- FORTUNER, R. & LUC, M. (1987). A reappraisal of Tylenchina (Nemata). 5. The family Belonolaimidae Whitehead, 1960. *Revue de Nématologie*, 10 : 183-202.
- GERAERT, E. & RASKI, D. J. (1987). Study of some Aglenchus and Coslenchus species. Nematologica, 34: 6-46.
- HOGGER, C. H. & ESTEY, R. H. (1976). Scanning electron microscopy of a plant parasitic nematode : Xiphinema americanum. Phytoprotection, 57 : 150-154.
- JAIRAJPURI, M. S. & SOUTHEY, J. F. (1984). Nothocriconema shepherdaen. sp. with observations on extra-cuticular formation. Revue de Nématologie, 7 : 73-79.

LAMBERTI, F. (1975). Taxonomy of Longidorus (Micoletzky)

Accepté pour publication le 28 août 1990.

Filipjev and Paralongidorus Siddiqi, Hooper & Khan. In : Lamberti, F., Taylor, C. E. & Seinhorst, J. W. (Eds). Nematodes Vectors of Plant Viruses. New York, Plenum Publishing Co. : 71-79.

- LAMBERTI, F. & MARTELLI, G. P. (1971). Notes on Xiphinema mediterraneum (Nematoda : Longidoridae). Nematologica, 17 : 75-81.
- LIPPENS, P. L., COOMANS, A., DE GRISSE, A. T. & LAGASSE, A. (1974). Ultrastructure of the anterior body region in *Aporcelaimellus obtusicaudatus* and *Aporcelaimus obscurus*. Nematologica, 20: 242-256.
- SAUER, M. R. (1985). A scanning electron microscope study of plant and soil nematodes. CSIRO, Australia, 64 p.
- SHER, S. A. & BELL, A. H. (1975). Scanning electron micrographs of the anterior region of some species of Tylenchoidea (Tylenchida : Nematoda). *Journal of Nematology*, 7 : 69-83.
- SHER, S. A., BELL, A. H. & RODRIGUEZ, R. (1977). The face view of Trichodoridae. *Journal of Nematology*, 9: 254-257.
- SWART, A. & HEYNS, J. (1987). Comparative morphology of the head regions of some longidorid nematodes from South Africa using SEM. *Phytophylactica*, 19: 99-101.
- VINCIGUERRA, M. T. & COOMANS, A. (1988). Parastomachoglossa perplexa (Heyns et Argo, 1969) n. comb. with a definition of the genus. Nematologia Mediterranea, 16: 205-212.