SCANNING ELECTRON MICROSCOPIC STUDY OF MAMMOMONOGAMUS LARYNGEUS

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Abstract. Surface structures, particularly numerous body sculptures of the nematode *M. laryngeus* recovered from cattle in Cuba were studied by scanning electron microscopy. New morphological structures in form of “granulations” were found of the mouth capsule. The structure of cuticle in region of vulva opening and of bursa cuticle was described in detail. It was found that even older preserved material of helminths can be used for SEM studies.

*Mammomonogamus laryngeus* (Railliet, 1899) (Syngamidae) is a parasite of upper respiratory tracts of cattle, sheep, goat, buffalo and man. It is distributed in subtropical and tropical regions. Its morphology was studies by numerous authors (Chapin 1925, Buckley 1934, Vaz 1935, Van den Berghe 1937, Ryzhikov 1949, Caballero and Vogelsang 1950, Da Silva 1961, Bwangamoi 1968, Mauri et al. 1971), but a more detailed analysis of morphological characters of this nematode was published only in the papers by Graber et al. (1971, 1972) and Mejia (1978). In spite of a rather wide knowledge of morphological characters of *M. laryngeus*, SEM studies revealed some morphological elements which can be detected only by means of this method.

New results of SEM studies of adult specimens of *M. laryngeus* from Cuban cattle are presented in this paper.

MATERIAL AND METHODS

A sample of 1—2-year-old material preserved in Barbagallo solution (7.5 g of NaCl, 1 liter of distilled water, 30 ml of 40 % formol) was used for SEM. Also entire male and female bodies and isolated mouth capsules were preserved.

The material was processed using a simple method without coating (Malick and Wilson 1975), but instead of recommended thiocarbohydrazide, more easily available thiosemicarbazide (TSC) was applied.

The material was prefixed in 3.5 % glutaraldehyde in 0.1 M cacodylate buffer and shortly washed in 0.1 M cacodylate buffer. After postfixation in 1 % water solution of OsO4 and TSC repeated three times the material was dehydrated in a graded alcohol series up to absolute ethylalcohol and then slowly dried in a vacuum drier. The initial prevacuum of about 10^-1 torr (2—3 h) was gradually increased up to 10^-3 torr. At this vacuum the material was dried for 24 h. The ready samples were then observed and photographed using a JEM U3 scanning electron microscope operated at accelerating voltage of 25 kV.

RESULTS

The arrangement of surface morphological structures on the head end of *M. laryngeus* males and females in principle conforms to hitherto published descriptions of this species. The mouth opening is surrounded by six papillae protruding above the surface of the cuticular rim around the mouth opening (Figs. 1,2). The papillae divide the cuticular rim into six distinct fields (festons) and fuse caudally with head cuticle at the
sites of their localization. An elevation of head end visible in light microscope approximately in the first third of mouth capsule (Scheme) appears in the SEM as a line separating the apical part of head with smooth cuticle from the caudal part with transversely striated cuticle (Fig. 1). (This and following Figs. apply to Plate I and II.)

On the bottom of mouth capsule (Fig. 2) there are 8 teeth with rounded tips and the same number of ribs supporting the capsule. The teeth form the basis of ribs (Figs. 3, 5).

An interesting morphological detail, hitherto undescribed in the literature, was observed on the surface of mouth capsule. In the light microscope appeared small spines arranged in form of a chessboard on the capsule surface (Fig. 3), but with radial arrangement at its margins (Fig. 5). However, after isolation of the mouth capsule from nematode body and examination in SEM it was found that irregular granular structures are involved, projecting above the capsule surface and possessing a depression in the centre (Fig. 4). SEM observations of a longitudinal section through the mouth capsule showed that the central depressions of granules run into hollow canals which are situated more deeply and directed to the interior of the capsule (Fig. 6) where they open without any apparent morphological formations. This “granulation” of the mouth capsule was observed in all nematodes examined (1794 specimens), in spite of their different degree of clearness.

The cervical papillae of *M. laryngeus* are large, rounded and distinctly prominent above the body surface. Their cuticle bears transverse striations well visible along the whole body length in males. In females, the cuticular striations are less conspicuous, particularly in postvulval region (Figs. 7, 13).

The posterior part of male body terminates by bursa copulatrix separated from body by a deep distinct groove (Fig. 8). The bursa itself is rough. It is covered with a wrinkled cuticle which seems to be covered by small spines if it is observed under a light microscope. Fig. 9 shows the inner side of bursa with cloaca and ribs. The wrinkled granular sculpture of cuticle on the inner side of bursa is visible at higher magnification (Figs. 10, 11).

The cuticle in the region of vulva opening (Fig. 13) appears as an imprint of the inner side of bursa at higher magnification (Fig. 12). Copulation of male and female is shown in Fig. 14.

**DISCUSSION**

The method of scanning electron microscopy has not yet been applied in the studies of morphological structures in members of the genus *Mammomonogamus*. In the present study it was used for the elucidation of some morphological details in *M. laryngeus*.

As it was mentioned above, light-microscopic observations of *M. laryngeus* revealed spine-like structures on the surface of mouth capsule which appear like granules in the
SEM. Similar structures have been described only in one member of *Mammomonogamus, M. okapiæ*. Van den Berghe (1937) recovered and described this nematode from a free-living ruminant, related to giraffe, *Okapia johnstoni* from Belgian Congo. In the characterization of its head end the author writes that "... the capsule is rough and armed with spines (épines)". Since there was no comparative material available, it cannot be stated whether or not these structures are identical with those described in this paper, though it is suggested by some circumstances. A consultation with Dr. Graber and Dr. Mejia by letter and examination of original material of *M. laryngeus* from Railliet’s collection and of *M. nasicala* indicate that these structures probably occur in all members of the genus *Mammomonogamus*. It is therefore necessary to revise the available material of mammomonomagamids and in case that the granular structures on the mouth capsule are present in all hitherto described species they should be included in the characteristics of this genus.

As to the function of these structures, it is probable that they participate in the digestive process in the mouth capsule of the nematode, particularly in digesting solid components originating from the mucous membrane of laryngopharynx of the host. It is possible that also the individual variability in the intensity of visibility is related just with the momentary activity of these structures. The real structure and function of these granules can be determined with certainty after the studies of their ultrastructure and biochemistry are carried out.

Another new information obtained in SEM studies of *M. laryngeus* is the fact that the cuticle in female is not smooth at the site where male bursa copulatrix is attached, but it is shaped according to the sculpture of the cuticle in inner side of bursa. This is wrinkled, covered with small knots and in the light microscope the bursa seems to be armed with minute spines. It is possible that also in case of *M. okapiæ*, the only species of *Mammomonogamus* in which the spines on bursa were recorded, these are due to a characteristic formation of the cuticle as it was observed by us in *M. laryngeus*.

In conclusion it should be mentioned that 1—2-year-old material preserved in Barbagallo solution was used in our studies. As it is evidenced by the results obtained, even this material can be used for SEM observations, but it should be considered that the photomicrographs need not be always perfect.

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ИЗУЧЕНИЕ НЕМАТОДЫ MAMMOMONOGRAMUS LARYNGEUS ПОД СКАНИРУЮЩИМ ЭЛЕКТРОННЫМ МИКРОСКОПОМ

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Резюме. Поверхностные структуры, особенно многочисленные складки тела нематоды *M. laryngeus* от крупного рогатого скота Кубы изучались под сканирующим электронным микроскопом. Обнаружены новые морфологические образования в форме "гранулирован" на ротовой капсуле. Уточнено описание структуры кутикулы в области отверстия ульвы и кутикулы бурсы. Обнаружено, что даже и старый консервированный материал гельминтов можно использовать для исследования с помощью СЭМ.
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Fig. 1. Head end of *M. longicollis* (SEM, 150×).
Fig. 2. Mouth capsule (apical view) (SEM, 230×).
Fig. 3. Part of mouth capsule with teeth (t) and ribs (r) and surface granulation (photomicrograph orig., 2.5×45).
Fig. 4. Surface of mouth capsule with granulation (detail) (SEM, 2000×).
Fig. 5. Part of head end with cuticle (c) and mouth capsule with radial arrangement of granules (w) (photomicrograph orig., 2.5×45).
Fig. 6. Wall of mouth capsule with distinct canals directed from granules to inner part of capsule (SEM, 2400×).
Fig. 7. Tail end of female (SEM, 550×).
Fig. 8. Bursa copulatrix of *M. laryngeus* (lateral view) (SEM, 300 x).

Fig. 9. Bursa copulatrix with cloaca and ribs (ventral view) (SEM, 300 x).

Fig. 10. Sculpture of inner wall of bursa copulatrix (SEM, 1000 x).

Fig. 11. Sculpture of bursa cuticle (detail) (SEM, 4000 x).

Fig. 12. Sculpture from the region of vulva opening (detail) (SEM, 2000 x).

Fig. 13. Vulva opening (SEM, 120 x).

Fig. 14. Copulation (SEM, 170 x).