Electron Microscope Studies on the Cuticle of Swarming and Nonswarming Tylenchorhynchus martini[1]

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Abstract: Studies on the structure of the cuticle of *Tylenchorhynchus martini* swarmers with electron microscopes revealed abnormal disruptive and eruptive morphological changes in most layers. Drastic effects of swarming on cuticular structure were reflected in the partial dissolution of cortical layer and matrix and in the irregular cracking of cortical sublayers in sublateral areas of the body. The occurrence of projections in the cortex was apparently unrelated to other changes. The structure of the cuticle of nonswarmers was intact and without any morphological changes. *Key Words:* swarming, cuticle, dissolution, cuticular projection.

The swarming phenomenon in plant parasitic and free - living nematodes was discovered independently by Meyl (13) and Hollis (4). Meyl observed swarming in *Hemicycliophora typica* de Man 1921, gave it the name "Nesterbildung" and ascribed to it a sexual function. Other workers who have reported independent discovery of swarming include R. A. Chapman (1958, *personal communication*), Whitehead (16), and Esser (3).

McBride and Hollis (12) pointed out that Staniland's original use of the term "swarming" in 1957 (15) for massing of a *Rhabditis* sp. in response to light had priority, but in view of the unique character and general nature of the phenomenon investigated in Louisiana State University laboratories (4, 5, 6, 7, 8, 11, 12), it was proposed to retain for it the term "swarming" and to refer to Staniland's "swarming" as Staniland's "phenomenon".

The bulk of evidence on swarming has come from experiments with the plant parasitic nematode *Tylenchorhynchus martini* Fielding 1956. Swarming results from a stickiness of the cuticle, which has not been amenable to modification by chemicals, with the exception of trypsin, which masks the stickiness and inhibits swarming (6). The effect of trypsin is reversible; it can be removed by washing and the specimens will again swarm. Electron micrographs of *T. martini* (8) showed the presence of morphological modifications in the cuticle of swarming nematodes. Swarming specimens exhibited swelling and disruption of the external cuticle and separation of external and internal cortical layers. Poinar (14) described a cuticular infection caused by bacteria-like microorganisms (not further identified) in adults of *Thelastoma pterygoton*. He suggested that these microorganisms were capable of dissolving parts of the nematode's cuticle and establishing colonies on the cuticle surface. The purpose of this paper is to report additional studies on the fine structure of the cuticle of swarming *T. martini*, as revealed by both transmission and stereoscan electron microscopes.

MATERIALS AND METHODS

Swarming and nonswarming populations of *T. martini* were reared on rice plants in the greenhouse and extracted from infested soil by the sifting and gravity technique. Specimens were prefixed in 2% phosphate-buffered glutaraldehyde (pH 7.0) at 4 C for 2 hr, then washed in buffer solution, and fixed for 2 hr in 2% osmium tetroxide at the same temperature. After dehydration in a graded ethanol series and propylene oxide, the specimens were embedded in Araldite plastic mixture (10). Sections were cut with a diamond knife mounted in a (MT-2) Porter-Blum ultramicrotome. They were stained with a saturated aqueous solution of uranyl acetate, followed by lead citrate (2) and then studied with a HU-11A Hitachi electron microscope operated at 75 kv.

For scanning electron microscopy, nematode specimens were mounted while still alive on a 10-mm diam aluminum stub which was either uncovered or covered with a thin layer of silver base paint. Two 20- to 30-nm layers of carbon and gold palladium were evaporated onto the surface of the specimens with a Denton DV-502 high-vacuum evaporator operating at 2 to 4 X 10^{-5} Torr. Specimens

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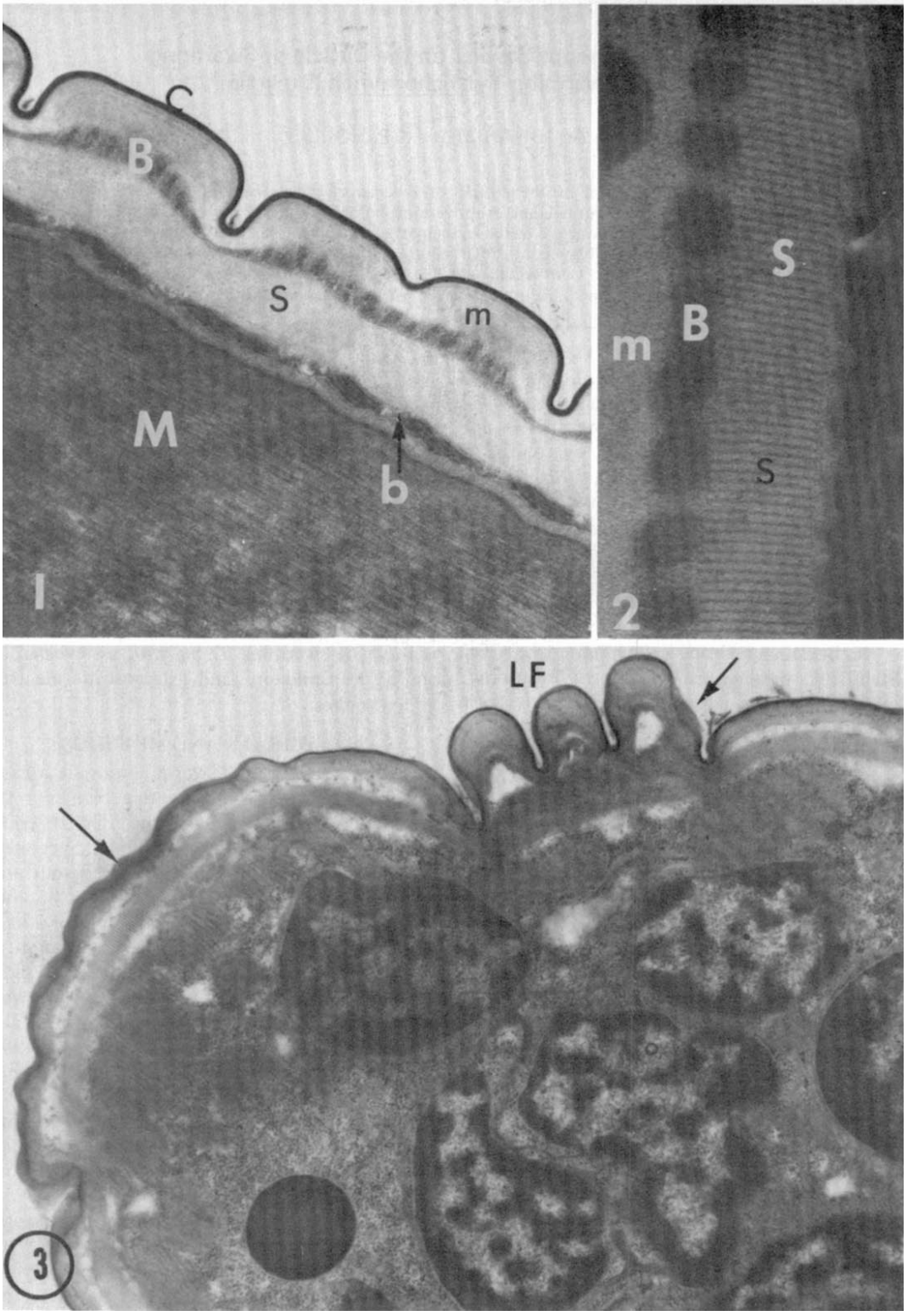


FIG. 1-3. Electron micrographs of swarming *Tylenchorhynchus martini* females. **1.** Oblique longitudinal section showing intact cuticular layers. b = basal layer; B = boundary layer; C = cortex; m = matrix; M = somatic musculature; S = striated layer (× 26,000). **2.** Enlarged portion of transverse section of the cuticle showing the boundary layer (B) and the striated layer (S) (× 53,000). **3.** Transverse section through the anterior region of the body showing lateral fields (LF) and swelling of the outer cuticular layer (arrow) (× 12,000).

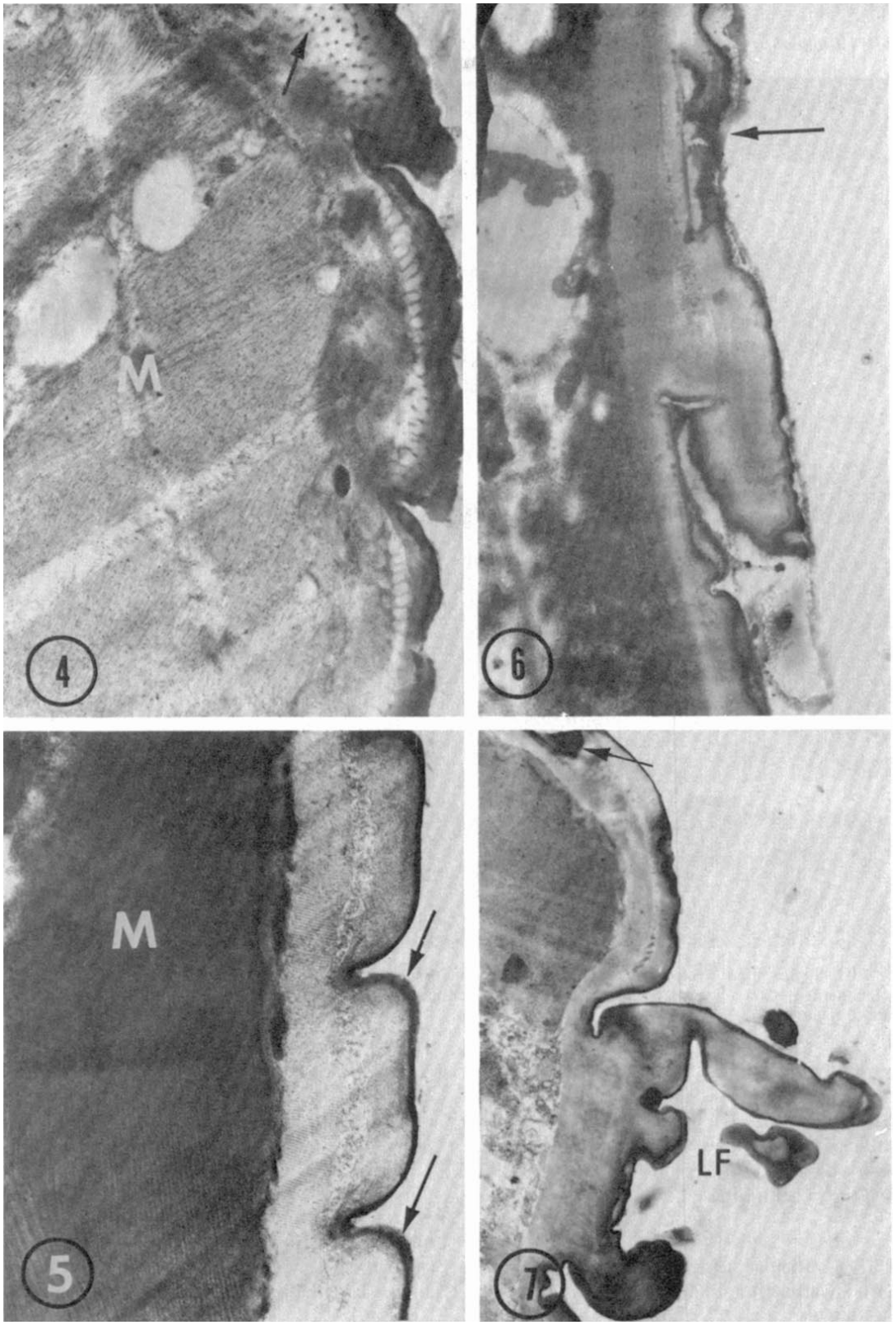


FIG. 4-7. Electron micrographs of swarming *Tylenchorhynchus martini* females. **4.** Oblique transverse section showing the boundary cuticular layer dotted with electron-dense particles (arrow). M = somatic musculature (× 30,000). **5.** Longitudinal section showing an early stage of morphological changes in the cortical layer of the cuticle (arrow) (× 23,000). **6.** and **7.** Longitudinal and transverse sections showing cuticular projection. Note rupture and dissolution of the cuticular layer (arrow). LF = lateral field (× 18,000 and × 16,000, respectively).

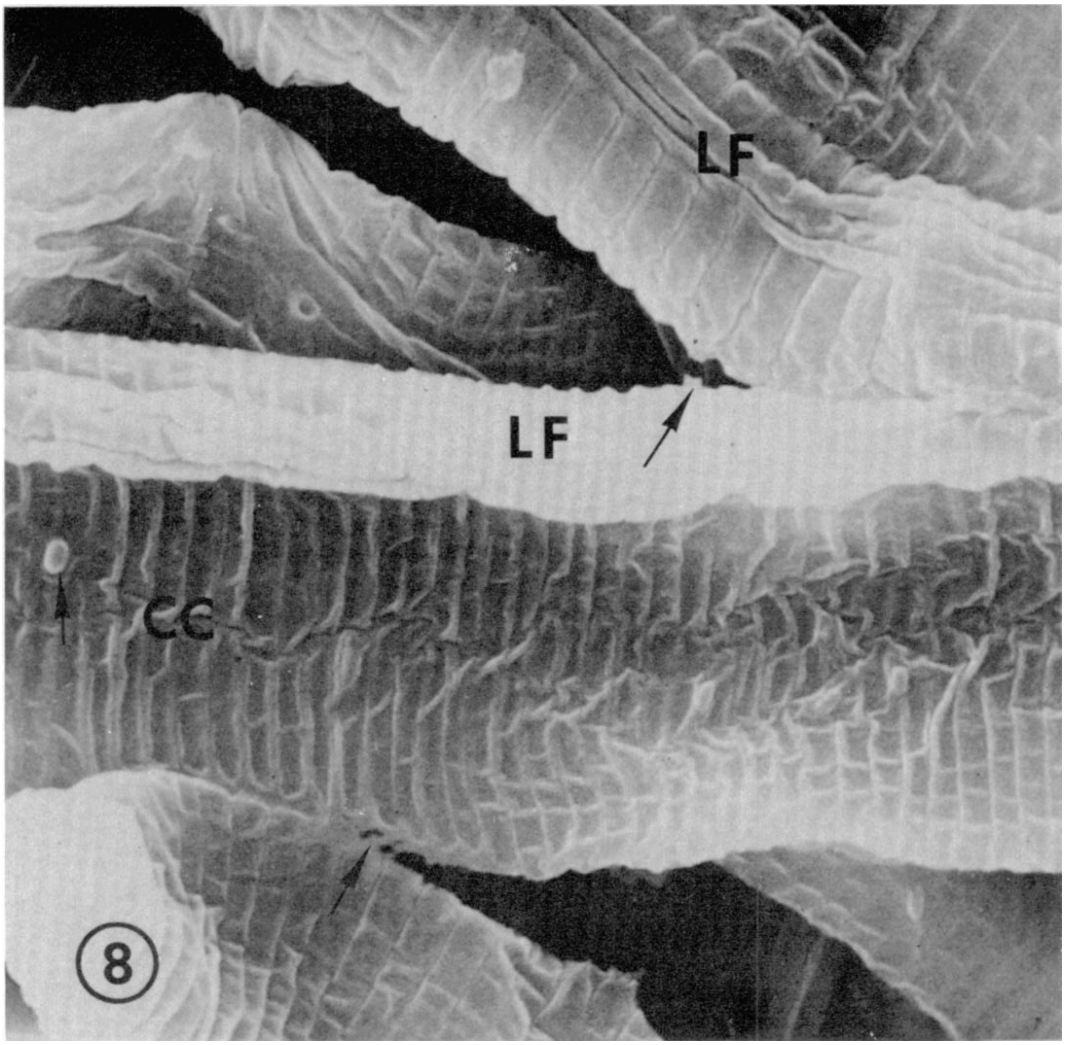


FIG. 8. Scanning electron micrograph of swarming *Tylenchorhynchus martini* showing types of cuticular attachment between swarmers, cuticular cracks and cuticular projections (arrow). CC = cuticular cracks; LF = lateral field (× 3800).

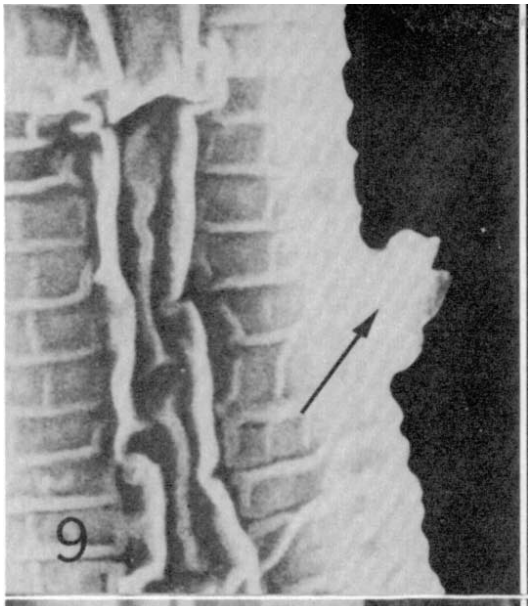
were examined with a JSM-2 stereoscan electron microscope (Japan Electron Optics Lab. Co., Ltd., Tokyo) using an accelerating voltage of 10-30 kv. Photographs were made with a Polaroid camera on Kodak type 55 P/N, or Tri-X, Ortho films.

RESULTS

The cuticular ultrastructure was the same in both swarming and nonswarming *T. martini*. Nonswarming specimens of *T. martini* exhibited normal intact cuticle without any morphological changes identical to that previously described by Ibrahim (8) (Fig. 12-14).

The cuticle of larval stages and females of *T. martini* was composed of five main layers: an outermost layer (cortex), a second layer (matrix), a boundary layer, a striated layer and a basal layer (Fig. 1, 2). The cortex consisted of

FIG. 9-14. Scanning electron micrographs of swarming *Tylenchorhynchus martini*. **9-11.** Different types of cuticular projections (arrow). LF = lateral field (× 5170, × 8790 and × 6210, respectively). **12-14.** Intact cuticular structure. **12.** Head region (× 6420). **13.** and **14.** Neck region. Note the presence of deirid (d) in the lateral field (arrow) (× 6210 and × 5690, respectively).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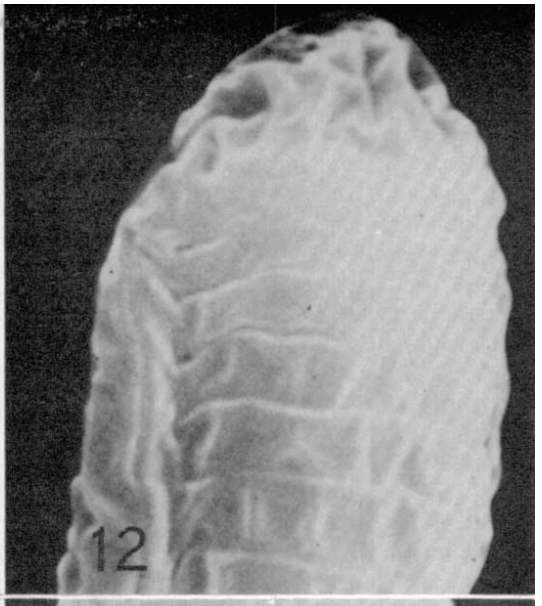
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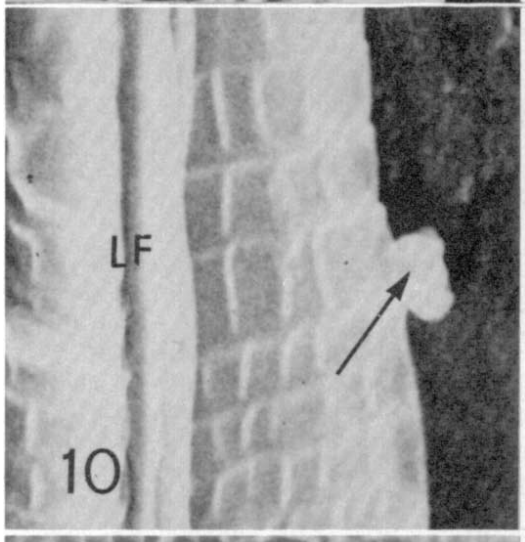
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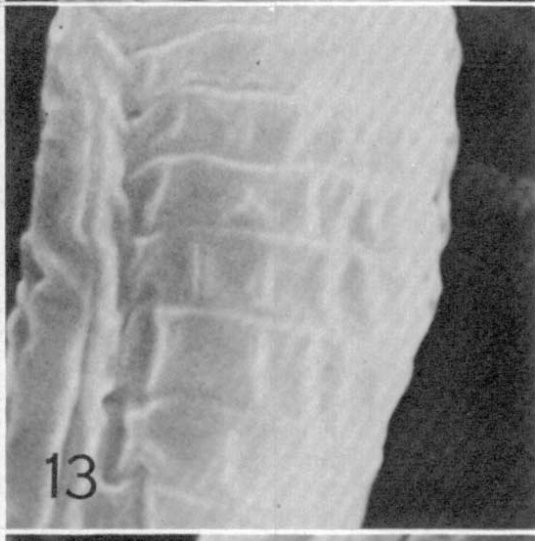
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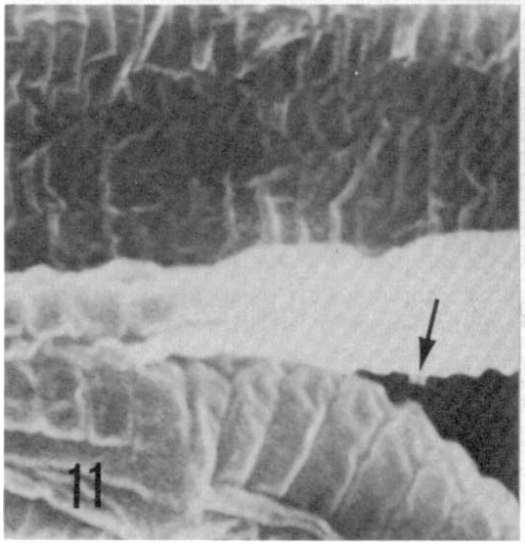
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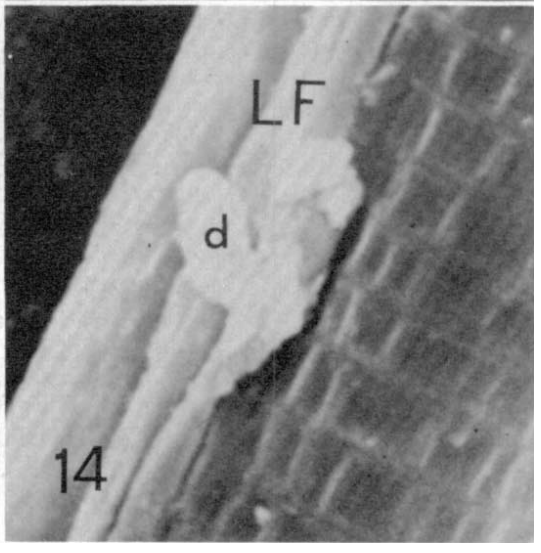
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outer osmiophilic, electron-transparent and inner osmiophilic sublayers (Fig. 6). This layer averaged 35-40 nm in thickness. Morphological changes in the cortex due to swarming appeared first as an increase in electron density of the outer and inner sublayers, then a separation of the sublayers and a swelling of the outer sublayer (Fig. 4, 6). These morphological changes first started at the edges of the interstrial regions (Fig. 5). Later changes appeared as dissolution of some parts of the cortical layer and the occurrence of randomly scattered cuticular projections, averaging 0.75 - 2.0 μm in length and 0.62 μm in thickness (Fig. 6-10). Cuticular projections which were outgrowths of both the cortical and matrix layers appeared mainly on the lateral and sublateral ridges (Fig. 7). Large numbers of these cuticular projections were observed particularly on mid- and posterior regions of the body. Proliferation of two adjacent annules above the level of the cuticular surface was observed also (Fig. 9).

Partial dissolution of the cortex and matrix probably resulted in the formation of sticky materials on the surface of the cuticle (Fig. 6, 7). Rupture and breakdown of the cuticular layers was characterized generally by the appearance of irregular cracks or grooves on the sublateral areas of the body (Fig. 8).

The matrix appeared as a homogenous layer of moderate electron density ranging from 41-72 nm in thickness at the base of an annule to about 230-260 nm midway between annules. The boundary layer, extending between the matrix and the striated layer, averaged about 156 nm in width. In longitudinal and transverse sections, the boundary layer appeared as a continuous structure of oval-to-round electron-dense or electron-transparent bodies with a few electron-dense particles scattered throughout (Fig. 1, 2, 3). In oblique section, it appeared as an electron-transparent structure dotted with electron-dense particles (Fig. 4).

The fourth cuticular layer was a striated structure averaging about 300-346 nm in thickness (Fig. 2). The striae were spaced at intervals varying from 15-18 nm and were arranged perpendicularly to the cuticular surface. The basal innermost layer appeared as a thin irregular electron-transparent structure with some electron-dense bodies embedded throughout (Fig. 1, 2, 3, 5).

There were both longitudinal and transverse ridges on the cuticular surface (Fig. 3, 8, 9). Longitudinal ridges were irregularly spaced parallel to the lateral ridges and confined between the more prominent transverse ridges which were arranged in an orderly fashion perpendicular to the lateral ridges (Fig. 9, 10). Some transverse ridges formed irregular branches before joining the lateral ridges (Fig. 9). The lateral field, averaging 3.12 μm in width, consisted of three irregular ridges extending along the lateral sides of the body (Fig. 3, 8). Between the lateral ridges, four deep lines were formed. With the exception of the cortex and the basal layer, the other cuticular layers were doubled in thickness in the lateral fields and the striated layer was replaced by two sublayers of a fibrous-like structure (Fig. 3).

Electron micrographs of specimens under the scanning electron microscope showed three principal types of cuticular attachments between swarming specimens. Swarmers adhered to each other along transverse-transverse, transverse-lateral and lateral-lateral ridges (Fig. 8, 11). Cuticular projections were seen also at the sites of attachment between swarmers (Fig. 8).

DISCUSSION

Essential features of cuticular morphology in swarming and nonswarming *T. martini* were similar to those described earlier (8). The cuticular structure of nonswarming *T. martini* resembles that of *Hirschmanniella gracilis, H. belli* and *Hemicycliophora arenaria* (9). The striated cuticular layer of *T. martini* also is similar to that described in *Aphelenchus avenae* (9) and *Criconemoides similis* (1).

There were some morphological changes in the cuticle of swarming *T. martini* not previously described. Cuticular projections have not been reported in other nematode species. Breakdown of outer layers of the cuticle seem to be a condition associated with the obvious sticky cuticle of swarming nematodes. These morphological changes are probably linked in some way to the hypodermis and the somatic muscles. The irregular and drastic character of the morphological changes in swarming *T. martini* suggest a diseased condition.

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