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MERMITHID NEMATODES: SEM OBSERVATIONS COMPARING HEXAMETHYLDISILAZANE AND CRITICAL POINT DRYING METHODS

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ABSTRACT

Morphological features of mermithid nematodes (Mermithidae) were studied with scanning electron microscopy, using hexamethyldisilazane-air drying in comparison with critical point drying via liquid carbon dioxide. Although general morphologic preservation of both HMDS-dried and CP-dried specimens was similar, structural features of the complex cuticle and internal organization were more easily resolved at higher magnifications in the HMDS-dried nematodes. These features include the superficial cuticular annulations, the fibrillar inner cuticle and peg-like microtrabeculae. The previously undescribed microtrabeculae are of special interest since they may facilitate an interaction of the mermithid (and perhaps nematodes in general) musculature with its body wall that, at least in part, may account for the unique thrashing locomotion so characteristic of these organisms.

INTRODUCTION

The scanning electron microscope (SEM) is a useful tool for elucidating morphologic aspects of nematodes for taxonomic studies. In preparing biological specimens for SEM, critical point (CP) drying has become the accepted standard for routine specimen drying for SEM observation (Postek, *et al.*, 1980). Eisenback (1986) compared several different techniques of preparing plant parasitic nematodes for SEM. These techniques included different fixatives in combination with different transitional fluids (liquid carbon dioxide or freon) for CP or freeze drying. He found that, for most nematode genera, glutaraldehyde fixation and freeze drying provided an adequate preservation of nematode morphology. Nation (1983), however, described another approch to specimen drying in which fixed dehydrated insects were immersed in hexamethyldisilazane (HMDS) and air dried. HMDS drying not only proved to be a satisfactory substitute for carbon dioxide critical point drying for insects, but bacteria as well (Giammara, *et al.*, 1987). HMDS drying significantly decreases the time required for specimen processing. The purpose of this study was to evaluate the applicability of HMDS drying to nematodes for SEM examination.

MATERIALS AND METHODS

Adult chironomids, host to parasitic imagocidal mermithid nematodes, were collected by light trapping at Lake Itasca, Minnesota, during the summer of 1989. Mermithids naturally emerged from their hosts in the laboratory. Isolated male and female specimens (Poinar, 1975; Johnson, personal communication), including post-parasitic larvae of an unidentified genus and of *Hydromermis,* and adults of *Lanceimermis,* were fixed in cacodylate buffered 2% glutaraldehyde for 4 days, postfixed in 1% osmium tetroxide for 30 min., and dehydrated in either an ethanol or acetone series. Acetone-dehydrated specimens were critical point dried using a Samdri 780A drier and liquid carbon dioxide as the transitional fluid. Alcohol-dehydrated specimens were immersed in HMDS for 10 min. and substantially air dried. Some specimens were also broken in half to allow study of internal features. The dried specimens were then mounted on stubs, coated with gold/palladium using a Hummer V sputter coater, and studied with an ISI DS-130 SEM using a LaB_6 electron source at an accelerating voltage of 10 kV.

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RESULTS AND DISCUSSION

COMPARISON OF HMDS- AND CP-DRIED MERMITHIDS

CPD-dried specimens of mermithids show most morphologic features characteristic of nematodes except that internal structural information was limited. The head of a CP-dried mermithid male shows such normal morphological features as cephalic papillae and mouth (Fig. 1). Shrinkage and distortion are evident in the head as well as body. Genital papillae and anal pore were also evident in CP-dried mermithid males. In both females and males, the superficial cuticular annulations (SCA) so clearly evident in transmission electron micrographs (TEM) of the body region (Batson, 1979; Poinar, 1983) are barely discernible in SEM micrographs of CP-dried specimens (Fig. 1). Cross-sectional views of CP-dried mermithids broken in two were badly fragmented and provided little information about the internal organization of these nematodes. Resolution of most structures was limited at magnifications above 4,000X.

Morphologic preservation of HMDS-dried mermithids was similar to that of CP-dried specimens except that cuticular detail and internal preservation were significantly better at higher magnifications. Although the head and body region of a HMDS-dried mermithid female in Fig. 2 exhibits some shrinkage, the specimen clearly shows the fine SCAs. The SCAs exhibit a parallel banding pattern in the body regions (Figs. 2, 3, 6). The pattern of SCAs in one mermithid species was distinctly regular (Figs. 2, 3), whereas in another it was highly irregular (Fig. 6). These initial observations suggest that SEM analysis of SCA patterns may have some taxonomic value for mermithid nematodes. In the head region, however, the pattern of SCAs becomes reoriented around the amphids so the SCAs now occur at right angles to those found in the body regions (Fig. 4) and form a circular pattern at the most anterior end (Fig. 2). Cross-sectional views of broken HMDS-dried mermithids were remarkably similar to that expected with freeze-fracture techniques. Fig. 5. shows the nematode trophosome with its numerous lipopro-

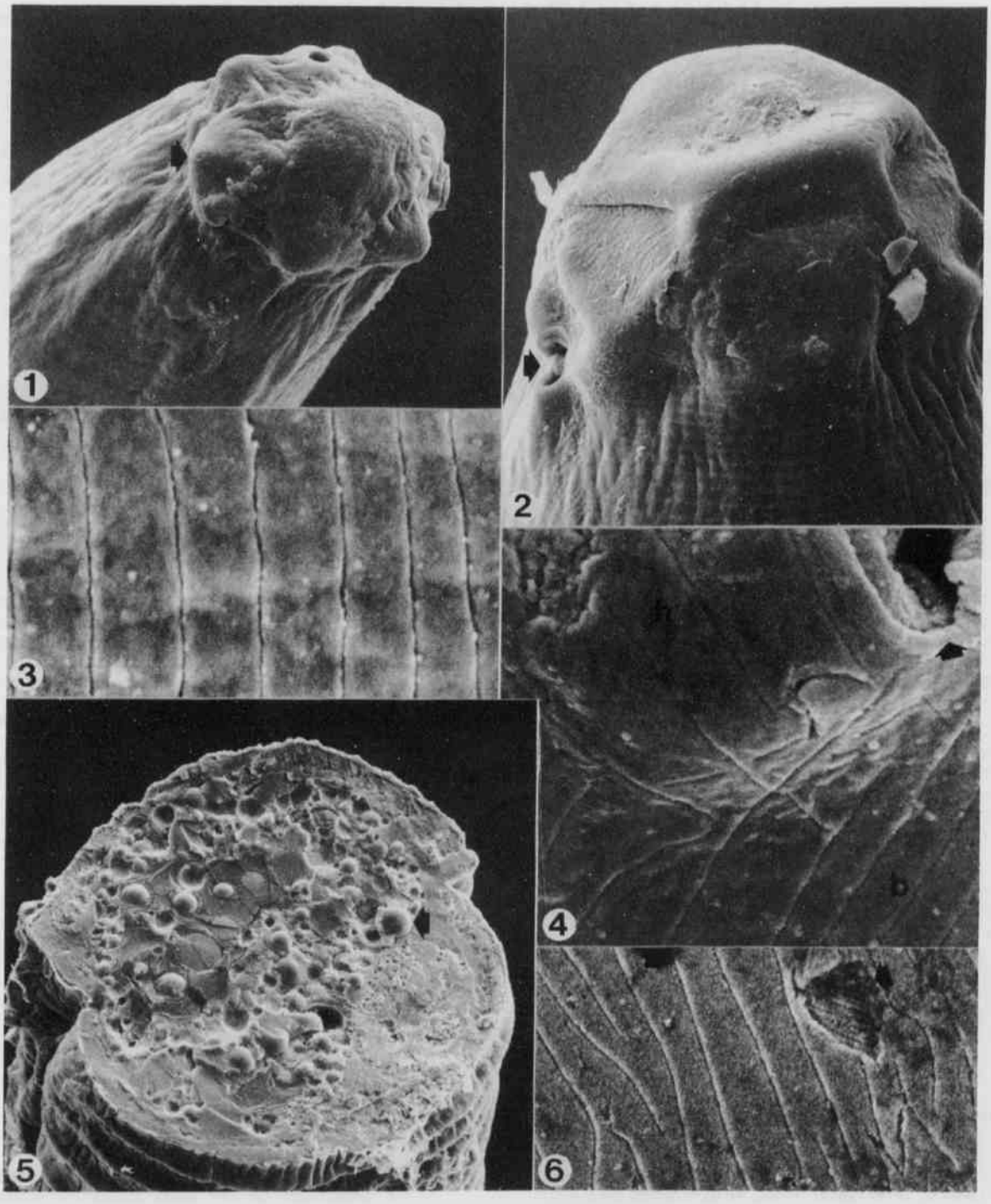

Figure 1. Head region of CP-dried *Lanceimermis* male, showing cephalic papillae (arrow) and mouth. 1,220X.
Figure 2. Head region of HMDS-dried mermithid female, showing amphid (arrow) and SCAs. 2,220X.
Figure 3. Body region of HMDS-dried mermithid, showing SCAs. 12,460X.
Figure 4. Head region of HMDS-dried mermithid, showing amphid (arrow) and transition of SCA orientation from body (b) to head (h) pattern. 13.170X.
Figure 5. Cross-sectional view of body of HMDS-dried *Hydromermis,* showing well-defined trophosome with numerous spherical lipoprotein globules (arrow). 786X.
Figure 6. Cuticle of HMDS-dried *Hydromermis,* showing exposed subcuticular fibers (arrow) and irregular SCA body pattern. 10,830X.

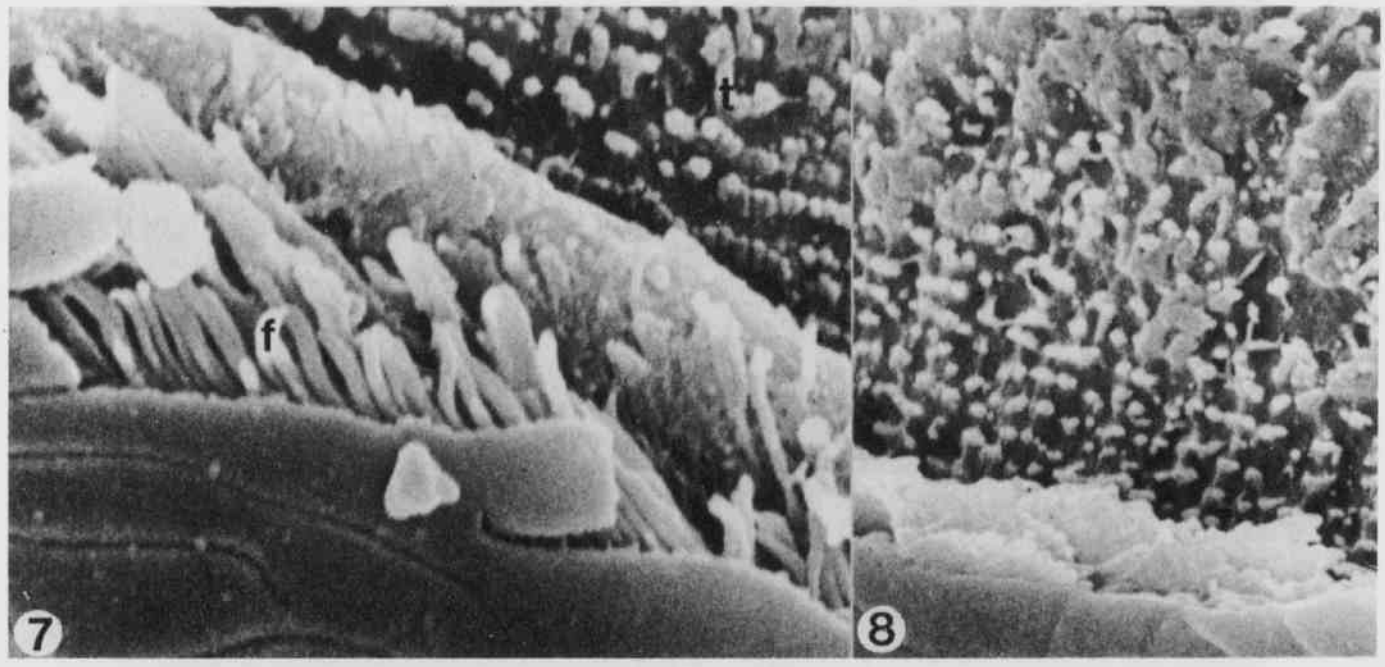

Figure 7. Torn outer body region of HMDS-dried *Hydromermis*, showing exposed fiber layers (f) of the cuticle and the numerous microtrabeculae (t) arranged in parallel series on the outer surface of a muscle band. 16,620X.

Figure 8. Same as Fig. 7, showing hypodermal remnants attached to microtrabeculae exposed beneath the cuticle. 11,400X.

tein globules as if it had been sections (Fig. 5). The unique muscle bands of the nematode were not distinct in this view. Torn cuticular surfaces of HMDS-dried mermithids (Figs. 6, 8), however, clearly demonstrated the 3-dimensional aspects of the inner fibrillar zone of the mermithid cuticle previously described by Batson (1979) from TEM micrographs. SEveral layers of fibers were evident, the individual fibers of which lie parallel one to another. All fiber layers together constituted an inner fiber belt oriented diagonally to the SCAs of the cuticle surface. Since the SCAs were perpendicular to the longitudinal axis of the nematode body, this fibrous belt is also oriented diagonally along the entire length of the nematode body.

Although general morphologic preservation of both HMDS-dried and CPD-dried specimens was similar, it is apparent that resolving the structural features of the complex cuticle and internal organization of mermithid nematodes with the scanning electron microscopy are best achieved with HMDS-dried specimens.

MICROTRABECULAE

Beneath the hypodermis, an elaborate system of minute peg-like structures covering the outer surface of the muscle bands were discovered in HMDS-dried mermithids (Figs. 7, 8). Since the presence of these structures has not been previously reported in the nematodes, including the mermithids (Batson, 1979; Poinar, 1983), we have termed these structures *microtrabeculae*.

These microtrabeculae project from the muscle surface toward the hypodermis of the body wall. They are arranged in parallel series perpendicular to the body wall and the longitudinal muscle bands (Figs. 7, 8). They appear to physically interact with the hypodermis, as suggested by tissue, presumably remnants of the hypodermis, that remains attached to some microtrabeculae (Fig. 8).

The functional role of the microtrabeculae is interesting and remains to be elucidated. The microtrabeculae however do appear to structually interact with the hypodermis (and therefore the cuticle and body wall). This could mean that they provide a structural means in which the mermithid's longitudinal musculature and body wall is integrated into a single biomechanical unit. If so, the microtrabeculae may then assume a major, previously unrecognized, role in the biomechanics of the thrashing locomotion so characteristic of the mermithids and perhaps nematodes in genera. Study of these structures and their relationship is being continued.

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