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Scanning Electron Microscopy on the First Stage Larvae of *Dracunculus medinensis*

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Abstract

The surface ultrastructure of the first stage larvae of *Dracunculus medinensis* was examined with a scanning electron microscope. Four cephalic papillae, one conical papilla, and two amphidial openings were observed around the mouth situated at the center of the anterior extremity. The excretory pore, 0.12 × 0.81 μm, and the anus, 0.14 × 0.6—1.3 μm, were situated on the ventral midline at 65 μm and approximately 330 μm from the anterior extremity, respectively. The phasmidial openings, 0.6 × 1.8 μm, having anterior and posterior lips, were located on both lateral lines, just posterior to the anus. The mean number of transverse striations on the cuticle was 254. The lateral lines were observed distinctly as an interruption of the annulations.

Key words: *Dracunculus medinensis,* first stage larva, morphology, scanning electron microscopy

Introduction

Following an initial study on the morphology and life cycle of *Dracunculus medinensis* by Fedchenko (1870), several studies on the morphology of the parasite (Blacklock and O'Farrell, 1919; Brackett, 1938; Kobayashi *et al.*, 1986; Moorthy, 1937, 1938; Southwell and Kirshner, 1938) have been done. However, there have been few ultrastructural studies on the larva, with the exceptions of the phasmid by Muller *et al.* (1970) and Muller (1971). In *D. ophidensis* (Brackett, 1938) and *D. oesophageus* (Desportes, 1938), the head structure of the embryo was described under a light microscope (LM). This paper describes the ultrastructure of the first stage larvae of *D. medinensis* by scanning electron microscopy.

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Materials and Methods

An adult female was removed from a patient and this is the first human case of dracunculiasis in Japan (Kobayashi *et al.*, 1986), and this was preserved in glycerol-70% ethanol. First stage larvae were obtained from the uteri of the female and brought to distilled water through a degraded series of 70%, 40%, and 10% ethanol for one day each. The larvae were treated with 1% pepsin in 0.7% HCl solution (pH 3.0) for 30 min at 37°C and with phosphate-buffered trypsin solution (2000NF/ml at pH 7.0) for 30 min at 37°C to remove mucous from the specimens. After several rinses with a phosphate buffer (pH 7.4), the larvae were fixed with 1% osmium tetroxide in the phosphate buffer (pH 7.4) for 12 hr at 4°C. They were then dehydrated with ethanol, substituted by isoamyl acetate, and dried in a critical point drying apparatus. After gold-palladium coating, the specimens were observed with a Hitachi S-550 scanning electron microscope (SEM). These larvae were used for

measurements and demonstrating fine structures.

First stage larvae were also obtained from sediment in a bottle preserving Guinea worms removed from a patient in Nigeria in 1986 (offered by the Meguro Parasitological Museum, Specimen No. 19490). The larvae, preserved in 10% formalin, were washed several times in distilled water and treated following the same procedures mentioned above. These specimens were used to confirm the head structures and to determine the degree of shrinkage.

Results

The first stage larvae were cylindrically elongated, and the anterior extremity was rounded. The cuticular surface was striated. The tail was filiform and tapered to a tip (Fig. 1).

The length of the larvae was 500 μm, the average of five specimens ranging from 473 μm to 536 μm. The length of the body part and tail was 330 μm (323—341 μm) and 184 μm (171—197 μm), respectively. Annulations (Fig. 4) were observed on the cuticular surface starting from 2.7 μm (2—3.3 μm) at the posterior to the anterior extremity to 111 μm at the posterior to the anus (Fig. 8). The width of the worm was 8.5 μm at the anterior part (at the tenth annulation), 11.3 μm at the middle (at the center of the annulated part), and 2.5 μm at the end of the annulation.

The mouth, showing a slit-like opening, 0.07 $\times$ 1.8 μm, was located at the center of the anterior extremity (Fig. 2). Around the mouth, four hemi-spherical single papillae, about 1 μm in diameter, were situated in a circle, 4.1 μm in diameter. There was a conical papilla, 0.7 μm in diameter and 0.65 μm in height, at the dorsal side of the mouth. The openings of the amphids, about 0.5 μm in diameter, were located on either sides of the mouth, and were 0.7 μm apart from the edge of the mouth. The circumferences of the amphidial openings were slightly raised (Fig. 2).

The excretory pore was situated on the ventral midline, 65 μm from the anterior extremity and between the 45th and 46th annulations. The size of the flattened opening was 0.12 $\times$ 0.81 μm (Fig. 3).

The anus, 0.14 $\times$ 0.6—1.3 μm in size, opened on the ventral midline approximately 330 μm from the anterior extremity (Figs. 5, 6).

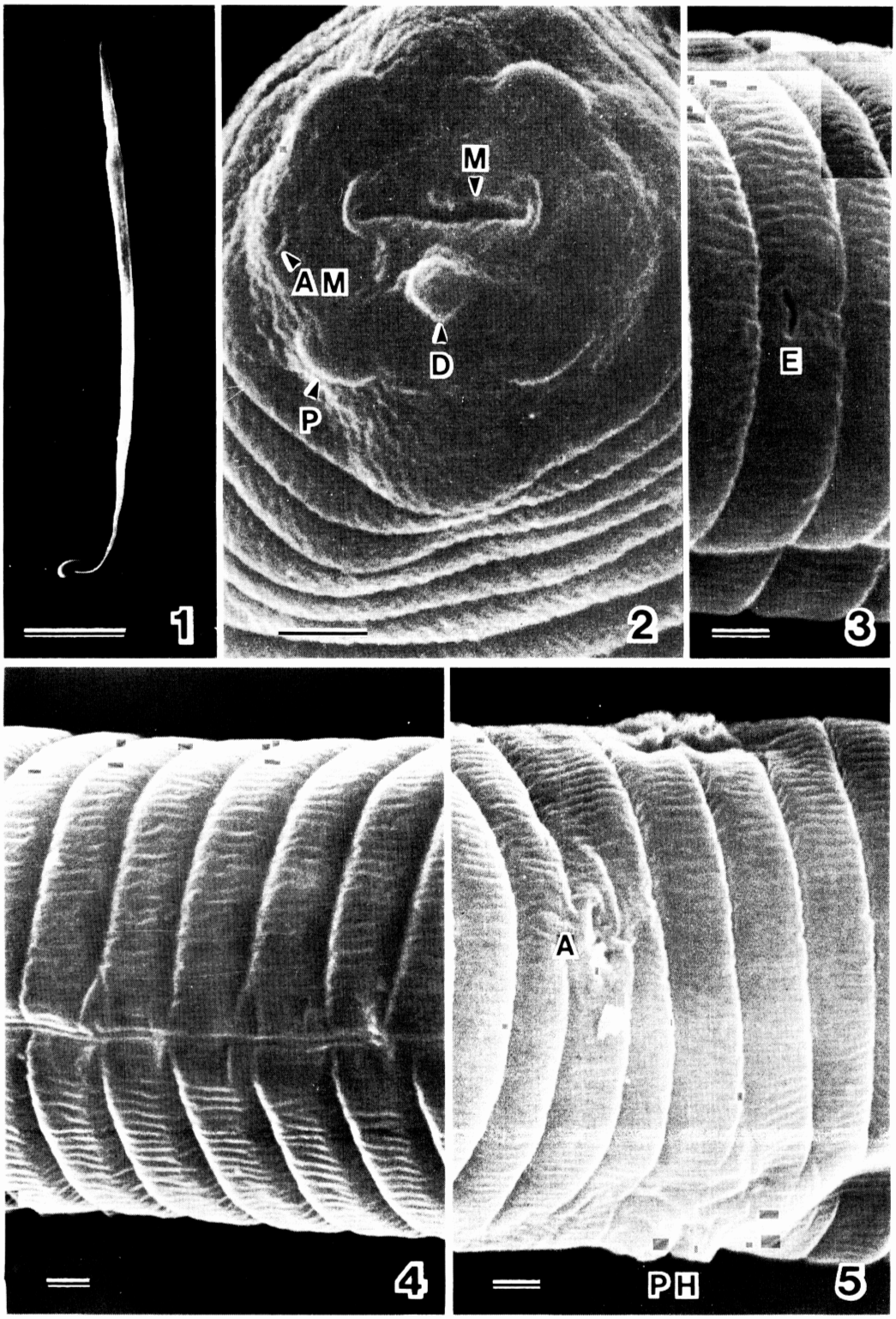
The phasmids opened on either side of the lateral lines, 3.9 μm posterior to the anus (Fig. 5). Their openings, 0.6 $\times$ 1.8 μm in size, were expanded bilaterally, and forming the small anterior and posterior lips (Fig. 6).

The posterior third (67.1 μm) of the tail had no annulations (Fig. 7), and the anterior part of that region showed a strand-like structure (Figs. 8, 9). The posterior part was smooth, and the tip was round (Fig. 10).

The total number of annulations was 254 (ranging from 235 to 268). The distances between the two adjacent annulations at the anterior, middle, and posterior parts of the worm's body were 1.4 μm (1.2 μm—1.6 μm), 2.1 μm (2.0 μm—2.2 μm), and 1.2 μm (1.1 μm—1.3 μm), respectively.

The lateral lines (Fig. 4) were observed as interruptions of the annules.

Figs. 1—10. Scanning electron micrographs of the first stage larvae of *Dracunculus medinensis*. 1. The whole worm of the first stage larva. Scale: 100 μm. 2. Head showing mouth (M), dorsal denticle (D), four large papillae (P), and amphidial openings (AM). Upside is ventral. Scale: 1 μm. 3. The excretory pore (E), 0.12 $\times$ 0.81 μm, is situated 65 μm from the anterior extremity on the ventral midline. Scale: 1 μm. 4. Annulations and lateral line in the anterior middle part of the body. The distance between the adjacent two annulations is 1.76 μm. Scale: 1 μm. 5. An anus (A), 0.14 $\times$ 0.6 μm, opens on the ventral side and two phasmids (PH), 0.6 $\times$ 1.8 μm, open on both the lateral sides, two-third of the worm's length from the anterior extremity. Scale: 1 μm. 6. A phasmidial opening (PH), 5.0 μm posterior to the anus (A), having small anterior and posterior lips. Scale: 1 μm. 7. Posterior part of the tail. Scale: 10 μm. 8. The portion of the tail shifting from the striated part to that of the strand-like structure. The surface construction differs distinctly between the former and the latter. Scale: 1 μm. 9. Strand-like structure. Scale: 1 μm. 10. Tail tip, almost bare. Scale: 1 μm.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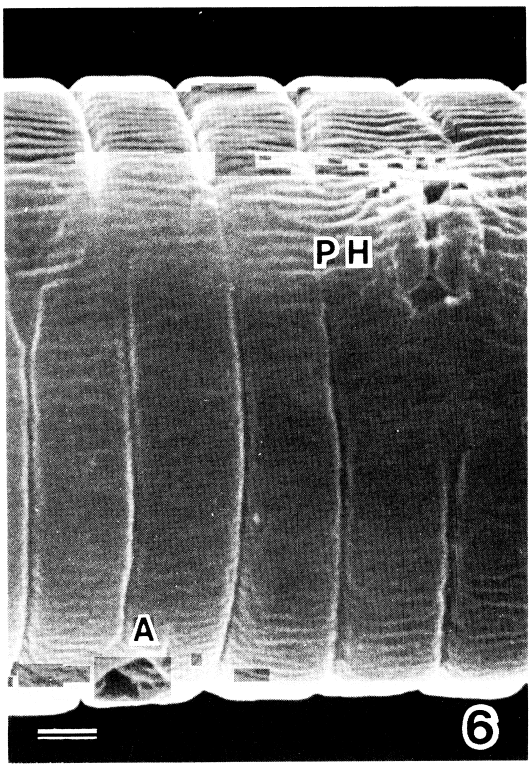
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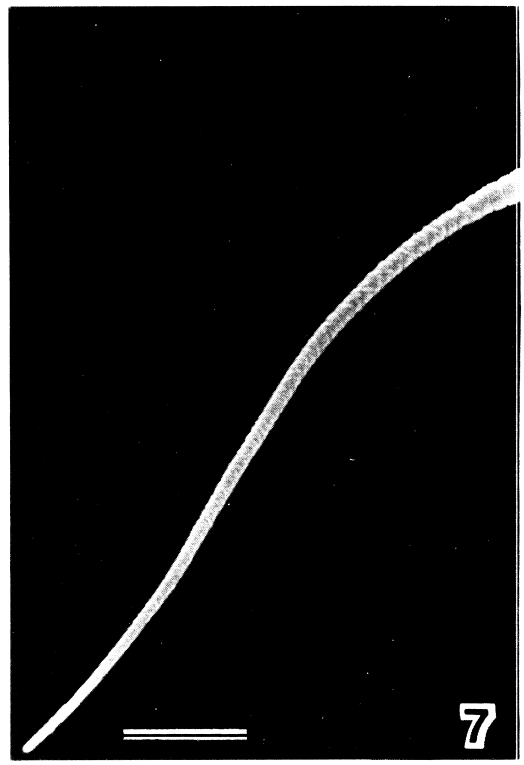
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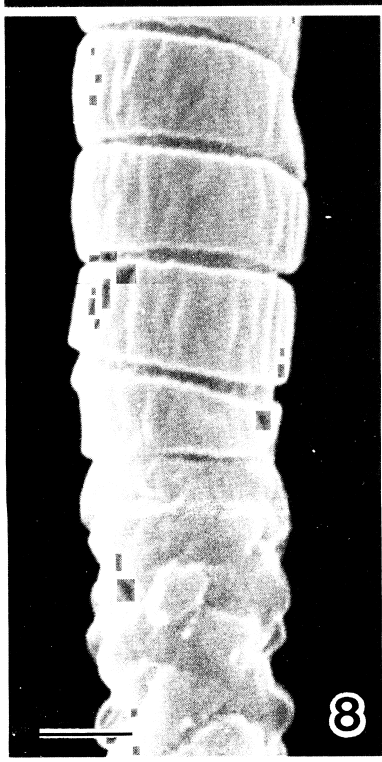
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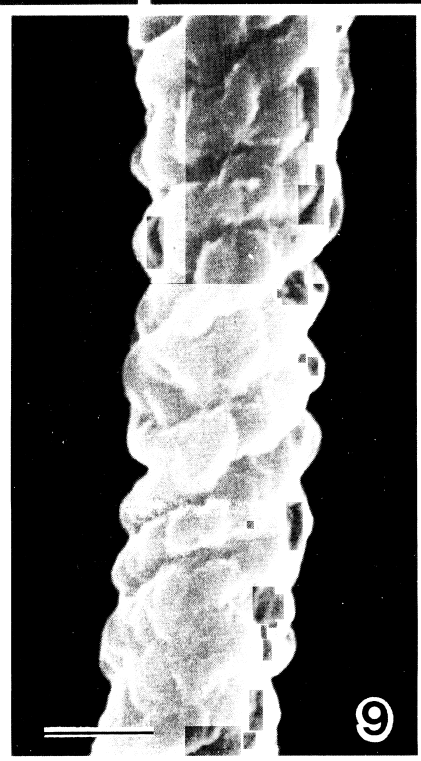
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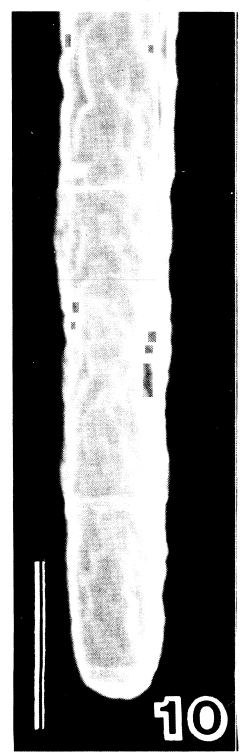
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Discussion

The reported lengths of the first stage larvae of *Dracunculus medinensis* varied between 490—737 μm (Fedchenko, 1870; Blacklock and O'Farrell, 1919; Moorthy, 1938; Southwell and Kirshner, 1938). In the Japanese case, Kobayashi *et al.* (1986) reported that the mean length of the embryo was 654 μm (530—710 μm) that was measured under LM. In this study, however, the mean length of the larvae measured under SEM was 500 μm, which was significantly different from that of Kobayashi *et al.* (1986). To determine the cause of the difference, some first stage larvae derived from Nigeria were measured by the LM and others by the SEM. The worm lengths were 580 μm (517—632 μm) under the LM and 408 μm (373—437 μm) under SEM. Since the measurements did not coincide with each other, the specimens that had been observed under the SEM were measured directly under the LM. These values agreed with each other. It is possible that the difference is a result of the shrinkage of the parasite through the preparative process for SEM, i.e. treatment with enzymes, dehydration, and critical point drying.

Fedchenko (1870) observed a conical prominence at the dorsal side of the mouth, and Moorthy (1938) described a dorsal denticle, six papillae (two each of interno-laterals, interno-ventrals, and interno-dorsals) on an internal circle, four double papillae on an external circle around the mouth, and amphidial openings situated posterior to the interno-lateral papillae. The conical papilla at the dorsal side of the mouth in our study was identical to both the conical prominence described by Fedchenko (1870) and the dorsal denticle described by Moorthy (1938). In our observations, however, the six papillae on the internal circle reported by Moorthy (1938) were not observed. Furthermore, four large single papillae were observed at the same place where he described four double papillae as being located. The same structures were observed on the head of the larvae derived from Nigeria. The double papillae reported by Moorthy (1938) may be internal structures undetectable by SEM. The exact number and the position of papillae on the head could not be confirmed under the LM.

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