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Surface Morphology of the Advanced Third-stage Larva of *Gnathostoma doloresi* – An Electron Microscopic Study –

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Abstract

The advanced third-stage larvae of *Gnathostoma doloresi* from snakes were observed, using a scanning electron microscope. Each larva had a head-bulb which was armed with four rows of hooklets. The mouth in the head-bulb had a pair of lateral lips, on which labial papillae and an amphid were located. One pair of cervical papillae was located laterally between the 15th and 16th transverse striations. An excretory pore was visible between the 24th and 28th striations. Another pair of body papillae was laterally detected at the anterior two-thirds region. A couple of caudal papillae was clearly recognized at the extremity of the tail. These ultrastructural findings should aid in identifying gnathostomes at the larval stage.

Key words: Advanced third-stage larva, *Gnathostoma doloresi*, scanning electron microscopy.

Introduction

Gnathostoma doloresi is a nematode of wide geographic distribution in the South Pacific, and Southeast and Far East Asia. The adult is parasitic in the gastric wall of a pig or a wild boar. The advanced third-stage larva ($Ad.L_3$) encysted in salamanders in Kyushu, Japan was first discovered by Miyazaki and Ishii (1952) and Ishii (1956), who reported the morphology of this larva in detail. Subsequently, this $Ad.L_3$ was found in a newt (Hasegawa *et al.* 1981), in frogs (Hasegawa *et al.* 1982) and in snakes (Miyazaki and Kawashima 1962, Tada *et al.* 1969, Toshioka 1970, Koga and Ishii 1981, Hasegawa *et al.* 1981, Mako and Akahane 1985), in Kyushu and Okinawa, Japan. Further investigations revealed that the morphological characteristics of the $Ad.L_3$ were identical to the description given by Miyazaki and Ishii (1952). However all these morphological observations were made by light microscopy.

In the present work, the external morphology of the $Ad.L_3$ was viewed by scanning electron microscopy (SEM).

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Materials and Methods

The $Ad.L_3$ of *G. doloresi* was obtained from snakes, *Trimeresurus flavoviridis* in Amami-Oshima, Kagoshima, Japan. The larvae were placed in a glass vial and were vigorously washed three times with tap water, then were fixed in 10% formalin for one week. They were immersed in distilled water which was changed three times to remove the formalin, within the space of 24 hours. The larvae were post-fixed in 1% osmium tetroxide in Millonig's phosphate buffer (pH 7.4) for two or three hours and subsequently rinsed in the same buffer. Following dehydration in a graded series of ethanol, the specimens were critical-point dried in liquid carbon dioxide, using a Hitachi HCP-2 apparatus. The specimens were mounted on studs, coated with gold in an ion-sputtering apparatus (JEOL FC-1100) and observed under a JEOL-U3 SEM operated at 15 kV.

Results

Each larva had a clear, semispherical head-bulb at the anterior extremity, and the head-bulb had four rows of hooklets (Fig. 2). The hooklets had single-pointed blunt tips and curved posteriorly. The hooklets in the first row were much smaller than those in other rows and appeared stumpy (Fig. 3). The number of hooklets in the respective row was 37–42 (39), 37–42 (39), 31–39 (36) and 36–39 (38), posteriorly. The mouth in the head-bulb had a pair of lateral lips of equal size and of half moon shape. Each lip had a couple of labial papillae and a small amphid located between the two papillae (Fig. 4). From immediately behind the head-bulb, the body had single-pointed minute cuticular spines along the transverse striations extending to the posterior extremity (Fig. 1). The number of these striations ranged from 176 to 211. One pair of cervical papillae was located bilaterally from between the 14th and 15th striations to between the 18th and 19th, with most being between the 15th and 16th striations (Figs. 1, 2). The papillae appeared as a dome-like bulge from the tegument (Fig. 5). An ellipsoidal excretory pore (about 3.5 × 5.0 μm in size) was clearly visible on the ventral surface of the body (Fig. 6) and was located in the vicinity between the 24th and 28th striations (Fig. 1). Another pair of body papillae was detected bilaterally at the posterior one-third region of the body (Fig. 1) and was prominent from the tegument (Fig. 7). The body spines were larger and more densely distributed in the anterior area (about 3.0 μm in length) and they gradually decreased in size and number toward the tail terminal. On the ventral surface of the tail extremity, transverse striations and spines ex-

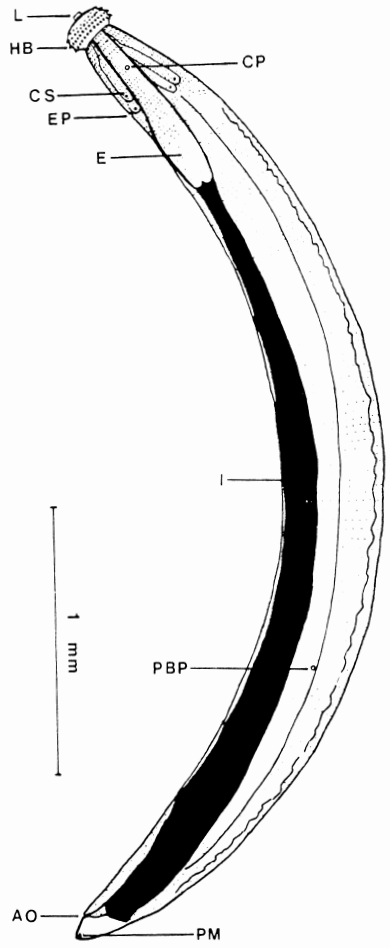


Fig. 1 Whole body of an advanced third-stage larva of *G. doloresi*. L: Lip. HB: Head-bulb. CP: Cervical papilla. CS: Cervical sac. EP: Excretory pore. E: Esophagus. I: Intestine. PBP: Posterior body papilla. AO: Anal opening. PM: Phasmid.

Fig. 2 Anterior view of the advanced third-stage larva of *G. doloresi*. CP: Cervical papilla. Scale: 33 μm.

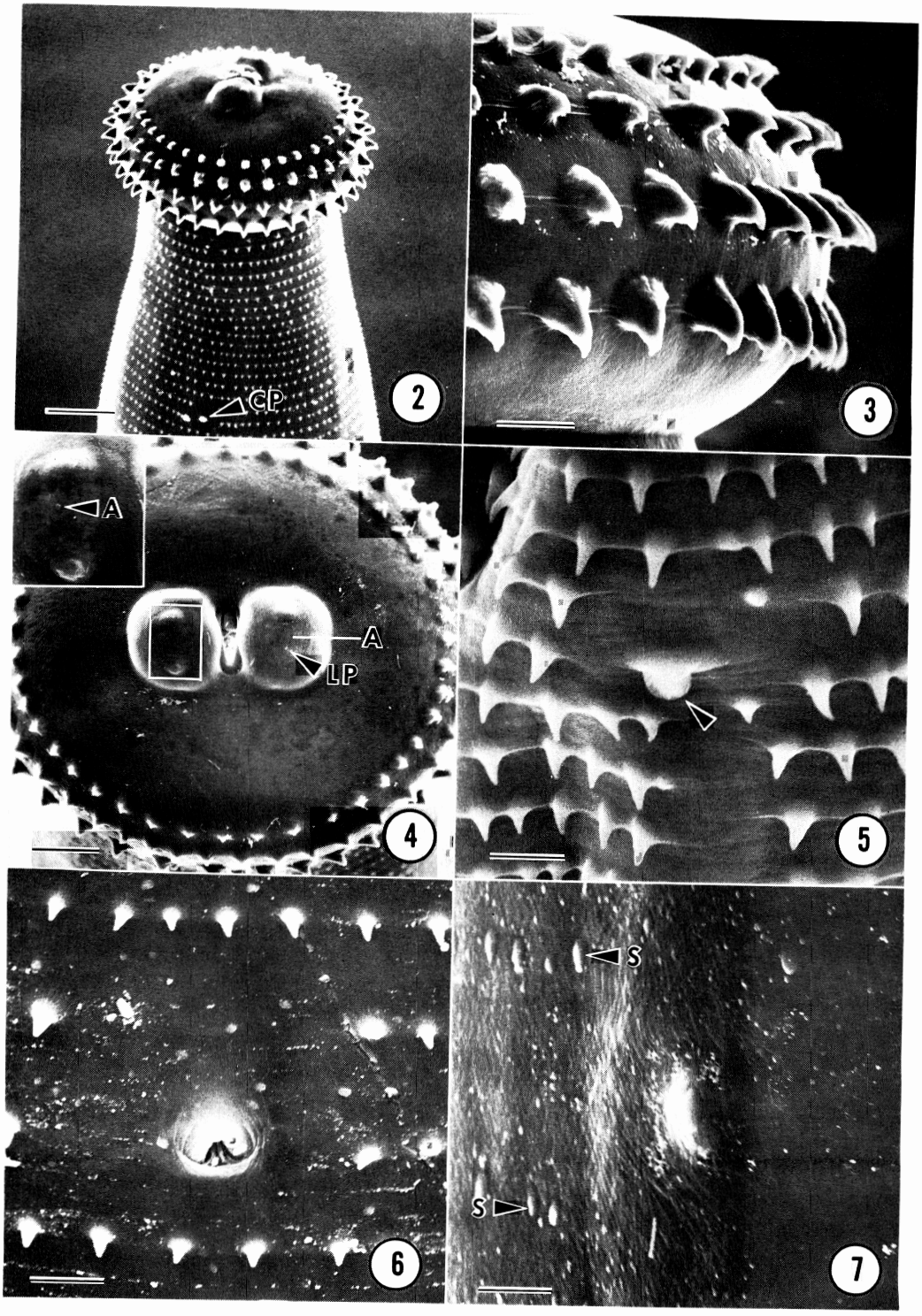
Fig. 3 Four transverse rows of hooklets on head-bulb. Each hooklet somewhat curved posteriorly. Scale: 10 μm.

Fig. 4 Frontal view of the head-bulb. An amphid and two labial papillae are seen on each lip. Scale: 20 μm. Inset: Enlarged view of the lip (× 1120). A: Amphid. LP: Labial papilla.

Fig. 5 A cervical papilla protruding from the tegument. Spines are about 3.0 μm in length. Arrow indicates the papilla. Scale: 5 μm.

Fig. 6 An oval-shaped excretory pore about 3.5 × 5.0 μm in size. Scale: 5 μm.

Fig. 7 A body papilla located at the posterior one-third region of the lateral surface of the body. Spines are about 1.8 μm in length. S: Spine. Scale: 5 μm.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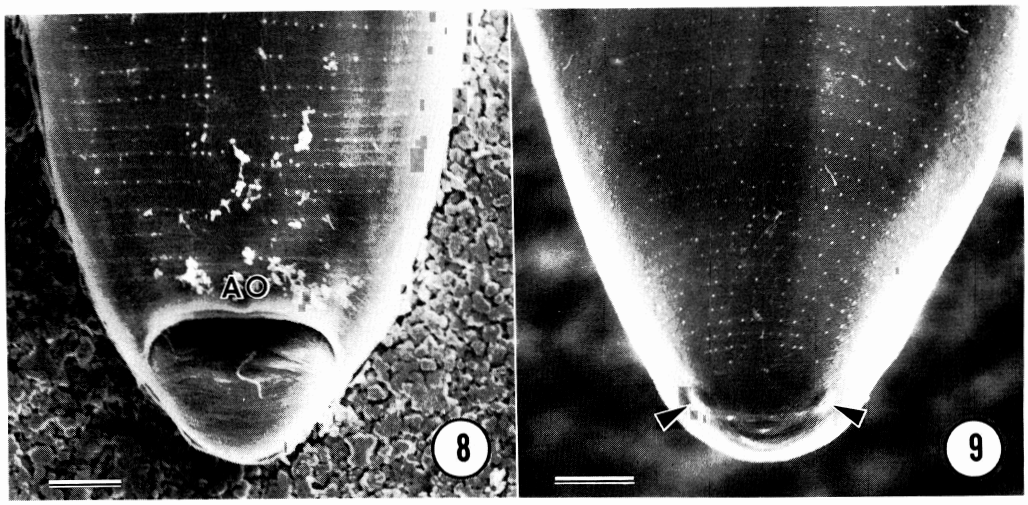

Fig. 8 Ventral view of the terminal end of a larva. Spines are absent beyond the anal opening. AO: Anal opening. Scale: 14 μm.

Fig. 9 Dorsal view of the terminal end of a larva. A pair of caudal papillae is visible (arrows). Many small spines (about 0.3 – 0.4 μm in length) are seen as dots at the extremity. Scale: 12 μm.

tended up to the anal opening (7 x 35 μm in size) but they were absent beyond the opening (Fig. 8). On the dorsal end surface, transverse striations were obscurely evident, and spines (0.3 – 0.4 μm in length) were sparsely dotted. One pair of caudal papillae (about 3.5 μm in diameter) was clearly recognized as round elevations, at the tail terminal (Fig. 9).

Discussion

Miyazaki and Ishii (1952) reported light microscopic findings of the morphological characteristics of the Ad.L_3 of *Gnathosotma doloresi*. The Ad.L_3 had four transverse rows of hooklets on the head-bulb. Each row had less than 40 hooklets. The hooklets of the first row were markedly smaller than that of the other rows. The body was entirely encircled with less than 200 transverse striations of minute cuticular spines. The spines in the posterior half body were so small that they were hardly visible. The cervical papillae were situated near the 16th transverse striation.

As for the number of hooklets, Miyazaki and Ishii (1952), Toshioka (1970) and Hasegawa *et al.* (1981) reported that each row had less than 40 hooklets, whereas Miyazaki and Kawashima (1962), Tada *et al.* (1969) and Mako and Akahane (1985) reported that the number of hooklets in each row, in most larvae was less than 40 but there were some with over 40 hooklets on the head-bulb. Our result corresponded to the latter findings.

Hasegawa *et al.* (1981) first noted the existence of the labial papillae and the amphids on the lips by sketching them on the basis of the light-microscopic findings. We obtained microphotographic evidence of the papillae and amphids. Miyazaki and Ishii (1952) stated that the cervical papillae were situated near the 16th transverse striation, while Mako and Akahane (1985) reported that the papillae were located between the 14th and 18th transverse striations. Present electron microscopic study showed the papillae mostly between the 15th and 16th striations. The location of the cervical papillae is one of the most important morphological features for identifying gnathostomes at the advanced third-stage (Miyazaki and Ishii 1952), but detection is not easy under light microscopy.

The Ad.L_3 had less than 200 transverse striations of spines over the body (Miyazaki

and Ishii 1952; Miyazaki and Kawashima 1962). However, Hasegawa *et al.* (1981) and Mako and Akahane (1985) reported that some Ad.L_3 had more than 200 transverse striations. Our findings corresponded to those in the latter case.

The excretory pore, posterior body papillae and caudal papillae, revealed in the present SEM study are hardly detectable using a light microscope. Anantaphruti *et al.* (1982) noted the posterior body papillae on the Ad.L_3 of *G. spinigerum* and Koga *et al.* (unpublished data) found these papillae on the same stage of *G. hispidum*, using SEM.

Kondo *et al.* (1984) and Koga *et al.* (1985) noted the existence of the caudal papillae on the Ad.L_3 of *G. hispidum*. These papillae might correspond to the phasmids, described by Cobb (1923) and Chitwood and Chitwood (1974).

Present scans revealed the shape and location of the labial papillae, amphids, cervical papillae, excretory pore, posterior body papillae and caudal papillae, the shapes of the hooklets on the head-bulb, and the distribution of body spines on the tail extremity.

These findings should aid in a differential identification of larval gnathostomes.

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