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Fine Structure of Early Third-stage and Developing Second-stage Larvae of *Gnathostoma doloresi* Reared in Cyclops

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Abstract

Fully developed early third-stage larvae of *Gnathostoma doloresi* were dissected from their cyclops intermediate hosts and examined by scanning electron microscopy. Each larva had an apical head-bulb that was armed with four rows of hooklets. The mouth was surrounded by a pair of lateral lips. Each lip bore two labial papillae and an amphid. The body was encircled by rows of transverse striations with single denticle cuticular spines which extended to the posterior end of each larva. The shape and number of cephalic hooklets, the number of transverse body striations, and the shape and location of the amphids, labial papillae, cervical papillae and posterior body papillae are described. Limited observations of second-stage larvae from cyclops revealed that they lacked a head-bulb, but had cuticular spines on their body surface.

Key words: early third-stage larva, second-stage larva, *Gnathostoma doloresi,* surface morphology

Introduction

Human cases of gnathostomiasis are normally caused by advanced third-stage larvae of *Gnathostoma spinigerum* (Miyazaki, 1960). Since 1980, three more species of *Gnathostoma* have been reported from human infections, namely *G. hispidum, G. nipponicum,* and most recently *G. doloresi* (Akahane *et al.*, 1982; Ando *et al.*, 1988; Ogata *et al.*, 1988; Nawa *et al.*, 1989). *Gnathostoma doloresi* is currently recognized as one of the most important causes of clinical disease in gnathostome infections.

There have been many reports on the fine structure of advanced third-stage larvae of *Gnathostoma* spp. by scanning electron microscopy (SEM). These include studies of *G. spinigerum* (Ishii, 1971; Ratanarapee, 1982; Anantaphruti *et al.*, 1982; Maleewong *et al.*, 1988), *G. hispidum* (Kondo *et al.*, 1984; Koga *et al.*, 1985, 1988a and 1988b), *G. nipponicum* (Ando *et al.*, 1988), and *G. doloresi,* (Koga and Ishii, 1987; Imai *et al.*, 1988). By contrast, a few workers have studied the surface ultra-structure of early third-stage gnathostome larvae from cyclops only in *G. hispidum* and *G. spinigerum* (Koga *et al.*, 1987; Maleewong *et al.*, 1988).

On second stage larvae, there are no SEM studies in any gnathostome species. The present study describes the fine structure of early third-stage and second-stage larvae of *G. doloresi* by SEM. The results obtained are compared with the description of advanced third-stage larvae from our previous report (Koga and Ishii, 1987).

Materials and Methods

Gravid, female worms, identified later as *Gnathostoma doloresi* were removed from the

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stomachs of naturally infected wild boars in Kyushu, Japan. Eggs were removed from the uteri, washed in fresh water, and allowed to embryonate and hatch over a two-week period in petri dishes containing a shallow layer of water. The dishes were kept in a 27°C incubator. Recently hatched, sheathed larvae were transferred to fresh petri dishes containing cyclops, *Eucyclops serrulatus* and *Cyclops vicinus,* that had been collected in a pond in Fukuoka. The larvae were ingested by the cyclops and subsequently underwent two molts to develop to the early third-stage. Early third-stage larvae as well as some second-stage larvae were dissected with fine needles from the pseudocoelomic cavity of infected cyclops and washed in physiological saline. The larvae were fixed in 10% formalin for one week and then soaked in five changes of distilled water over a 24 hrs period to remove the formalin. The larvae were rinsed twice in Millonig's phosphate buffer and postfixed in 1% OsO_4 for 2 hrs. The larvae were rinsed again in the same buffer, dehydrated in a graded series of ethanol, transferred to iso-amyl acetate, and critical point dried in liquid CO_2 with a Hitach HCP-2 critical point dryer. The specimens were coated with gold in an ion-sputter coating apparatus (JEOL FC-1100) and observed at 15 kV with a JEOL JSM-U3 scanning electron microscope.

Results

Early third-stage larvae measured 180 — 230 μm in length and 17 — 22 μm in width (6 larvae). The relative locations of several papillae and major organs are indicated in Fig. 1. The mouth on the head-bulb had a pair of semicircular lateral lips that were of equal size. Each lip had a pair of labial papillae and a small amphidial pore between the two papillae. Each papilla could be divided into two parts — a cephalic papilla and an outer labial papillae (Fig. 2). The head-bulb was hemispherical and had four transverse rows of hooklets (Fig. 3) containing, respectively, 39 (37 — 40), 39 (36 — 42), 37 (35 — 39) and 36 (34 — 38) individual hooklets. The hooklets in the first row appeared short and stumpy while hooklets in the third and fourth rows were sharply pointed and strongly recurved (Fig. 4). The body surface from immediately behind the head-bulb to the posterior extremity was encircled with 174 — 203 transverse striations (6 larvae) that were lined with single denticle spines. The body spines were larger and more densely distributed on the anterior end (Fig. 3) and gradually decreased in size and number towards the posterior end (Fig. 8). One pair of cervical papillae (1.9 × 0.9 μm) was located on the lateral surface of larvae. These were of the cilium type and were surrounded by a rim at their base (Fig. 5). Cervical papillae on the right side of larvae were located from between the 13th and 14th to between the 17th and 18th transverse striations, while those on the left side were found from between the 14th and 15th to between the 17th and 18th striations.

A small oval excretory pore (1.00 × 0.45 μm) was situated in the vicinity of the 22nd — 24th transverse striations on the ventral body surface (Fig. 6). The spines at this site were about 0.45 × 0.20 μm in size. Another pair of papillae were detected laterally on the posterior third of the body (Fig. 1). These posterior body papillae (postdeirids) were located at the 105—106th, 106—107th, 117—118th, 134—135th and 143—144th transverse striations. The papillae (1.3 × 0.7 μm in size) looked like the cilium type and

Fig. 1. A low magnification view of a fully developed early third-stage larva. L: Lip. HB: Head-bulb. CP: Cervical papilla. EP: Excretory pore. PBP: Posterior body papilla. A: Anus.

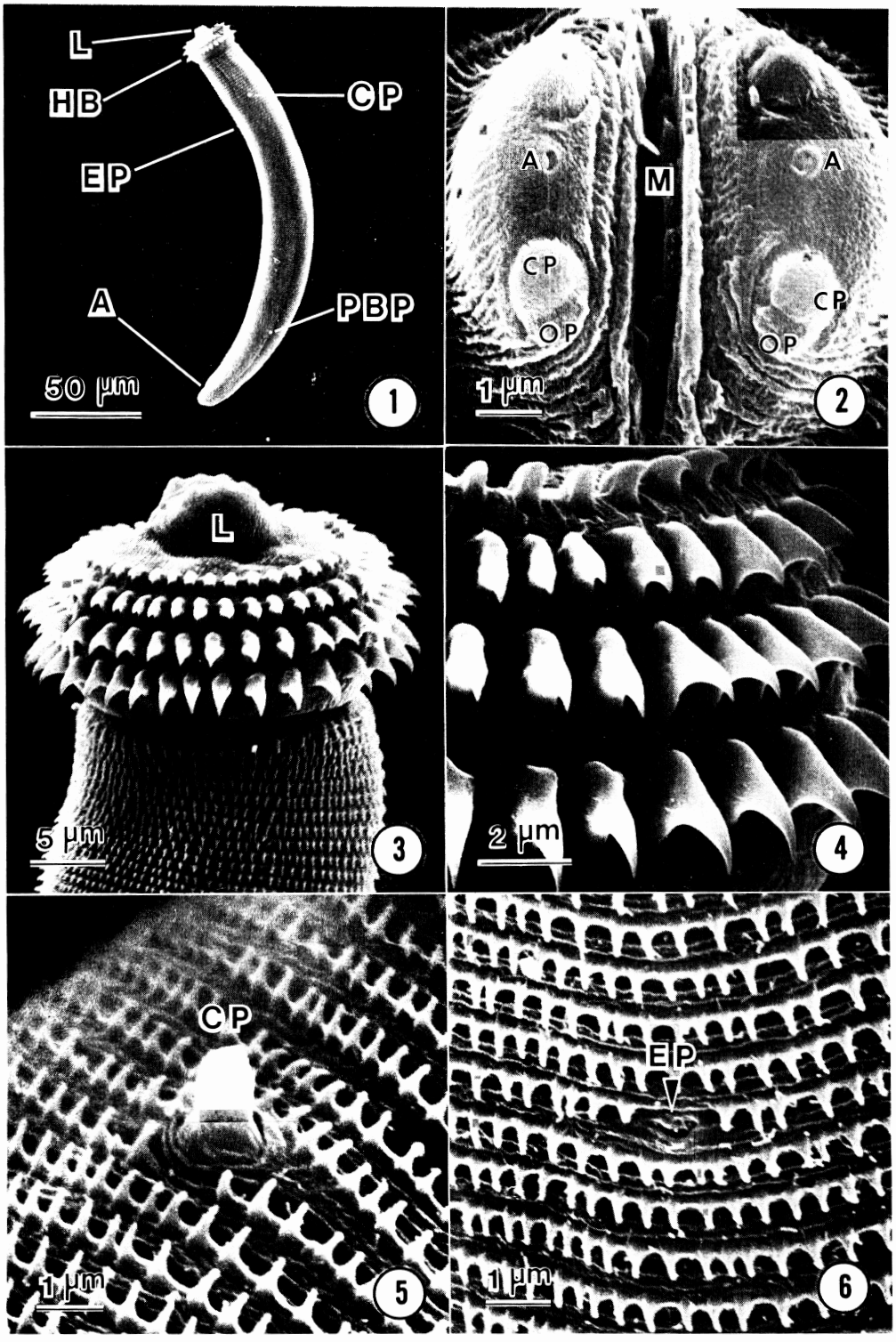
Fig. 2. Frontal view of the head-bulb. A pair of semicircular lips are present. Each lip has a pair of dome type labial papillae and an amphid. Each labial papilla consists of two parts. M: Mouth. A: Amphid. CP: Cephalic papilla. OP: Outer labial papilla.

Fig. 3. Lateral view of the head-bulb with four transverse rows of hooklets. L: Lip.

Fig. 4. Single denticle hooklets on the head-bulb. They are sharply pointed and strongly recurved.

Fig. 5. Cervical papilla resembling the cilium type. A rim surrounds the base of the papilla. CP: Cervical papilla.

Fig. 6. An excretory pore opens in the anterior region of the ventral body surface. EP: Excretory pore.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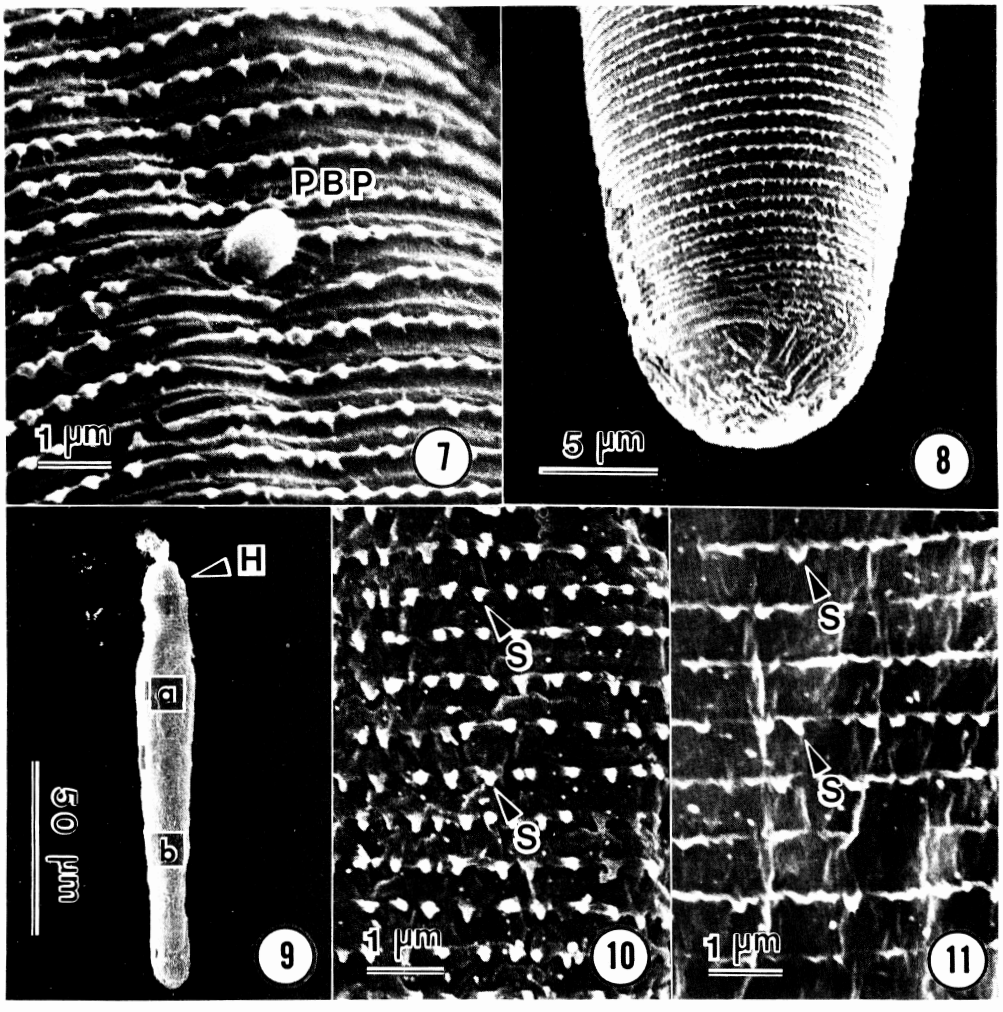

Fig. 7. Cilium-like posterior body papilla located laterally on the anterior two-thirds of the body. The papilla has a rim at its base. PBP: Posterior body papilla.

Fig. 8. Dorsal view of the terminal end of the body. Transverse striations with spines are evident.

Fig. 9. A low magnification view of a developing second-stage larva from cyclops. The larva has no head-bulb. H: Head.

Fig. 10. Enlargement of "square a" of Fig. 9. Anterior part of the body has large numbers of densely-spaced, minute, single-pointed spines. S: Spines.

Fig. 11. Enlargement of "square b" of Fig. 9. Spines are sparsely distributed. There are transverse striations having no spines at all. S: Spines.

were surrounded at their base by a rim (Fig. 7). The spines at this site were about 0.30 × 0.15 μm in size. On the dorsal surface of the tail extremity, the transverse striations and spines were clearly viewed. No caudal papillae were seen at the tail extremity (Fig. 8).

Second-stage larvae were much smaller in size (125.0 × 17.4 μm) than early third-stage larvae and lacked a cephalic bulb at their anterior end (Fig. 9). Transverse striations were visible from the neck to the tail extremity. Single-pointed spines (about 0.20 μm in length) were clearly evident along transverse striations in the anterior third of larvae (Fig. 10). The spines were re-

markably sparse in distribution on the posterior third of the body and varied in size from 0.15 μm to 0.32 μm in length (Fig. 11). On the posterior half of larvae there were sites where striations had no spines at all.

Discussion

Previous light microscopic observations of early third-stage larvae of *Gnathostoma doloresi* documented body size, number of cephalic hooklets, the existence of labial papillae, and the length of body spines (Miyazaki, 1952; Ishii, 1956; Dissamarn *et al.*, 1966). In the present study, SEM clearly revealed the shapes and locations of labial papillae, amphids, cervical papillae, an excretory pore, posterior body papillae, and the number of transverse striations.

Our measurements of larval size (180—230 × 17—22 μm) were smaller than those reported by Miyazaki (1952) (260—338 × 34—36 μm). In the present case, however, we measured dried materials. The number of hooklets on the head-bulb was almost identical to the number reported by previous workers. Based on strip sections of advanced third-stage larvae of *G. spinigerum,* Morita (1955) noted that each labial papilla consisted of two parts — a cephalic papilla and an outer labial papilla. We found a similar division of the labial papilla in this study which became invisible by SEM in more advanced third-stage larvae of *G. doloresi* (Koga and Ishii, 1987).

The cervical papillae of early third-stage larvae of *G. hispidum* were studied by SEM by Koga *et al.* (1987). These papillae had a cilium-like projection and were surrounded by a rim at their base. The same papillae were shown in early third-stage larvae of *G. spinigerum* by Maleewong *et al.* (1988), and they looked like the head of corncob. The papillae of *G. doloresi* resemble those of *G. hispidum*. By contrast, in more advanced stages of *G. doloresi,* the papillae appear as dome type one. The cervical papillae were located between the 13th — 18th striations in early third-stage larvae and between the 14th — 19th striations in advanced third-stage ones. They did not differ in locality. Whereas the papillae in early third-stage larvae of *G. hispidum* were located between the 9th — 13th striations (Koga *et al.*, 1987). *G. doloresi* was differentiated from *G. hispidum* mainly in locality of the papillae.

Posterior body papillae were described in advanced third-stage larvae of *G. spinigerum, G. doloresi* and *G. hispidum* by Anataphruti (1982), Koga and Ishii (1987) and Koga *et al.* (1988a). The same papillae were first reported in early third-stage larvae of *G. hispidum* by Koga *et al.* (1987). Observations of these structures in this study are the second record of their occurrence and precisely define their location between striations 105—144. Transverse striations and spines at the tail body were much more evident in early third-stage larvae than in advanced third-stage ones.

Second-stage larvae undergo many changes in body shape during their development in cyclops. We examined 7- to 10-day-old larvae, but it was difficult to prepare good specimens at this stage of development because larvae were very small and tended to shrink during dehydration and critical point drying. Only minimally distorted specimens were examined, and it was found that they already had spines along the transverse striations. Further studies of second-stage gnathostome larvae are needed to better elucidate details of their surface morphology.

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