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# Morphological Features in Cross Section of Early and Advanced Third-stage Larvae of *Gnathostoma nipponicum*

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**Abstract**

Morphological features of early and advanced third-stage larvae of *Gnathostoma nipponicum* in cross section were examined by light microscopy. A cross section of intestine of the advanced third-stage larvae (AdL3) was composed of 10 to 14 cells with 0 to 4 large nuclei. The features of parts other than the intestine in cross sections of *G. nipponicum* AdL3 were similar to those of three other species, *G. spinigerum, G. doloresi* and *G. hispidum,* already described by other workers. Morphological features in cross section of early third-stage larvae (EaL3) of *G. nipponicum* were fundamentally the same as that of AdL3 except that both the intestine and genital organ in EaL3 were undeveloped.

**Key words:** *Gnathostoma nipponicum,* larva, cross section

## Introduction

Three native and one imported *Gnathostoma* species occur in Japan. Almost all of the Japanese human cases of gnathostomiasis in the past have been associated with *Gnathostoma spinigerum.* Since 1980, however, *G. hispidum* was found in loaches imported from foreign countries such as mainland China and is also known to cause gnathostomiasis in humans (Akahane *et al.*, 1982; Demitsu and Aizawa, 1985). Human cases caused by the other two species have only recently been reported in Japan; two cases of *G. nipponicum* infection from ingestion of raw native Japanese loaches in Mie Prefecture (Ando *et al.*, 1988) and ten cases of *G. doloresi* infection from ingestion of raw fresh water fish in Miyazaki Prefecture (Ogata *et al.*, 1988; Nawa *et al.*, 1989). Thus, all four species of *Gnathostoma* occurring in Japan have been shown to cause zoonotic infection.

The advanced third-stage larvae (AdL3) of these four species were distinguishable by a difference in the number of transverse rows and features of hooklets on the head bulb (Miyazaki, 1960). Morphological features of AdL3 of *G. spinigerum* in cross section were described by Morita (1955) and morphological differences in cross sections of AdL3 of three species, *G. spinigerum, G. doloresi* and *G. hispidum,* were compared by Akahane *et al.* (1986).

In the present study, cross sections of *G. nipponicum* AdL3 are described to facilitate the identification of sections of *Gnathostoma* species in human cases.

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## Materials and Methods

AdL3 of *G. nipponicum* were obtained from the muscle of loaches or mice experimentally infected for more than 128 days with early-third stage larvae (EaL3) which were obtained from eggs as described by Ando *et al.* (1989). AdL3 (1.5–2.0 mm long, 120–150 $\mu$m wide) and EaL3 (520.2 $\mu$m long, 51.4 $\mu$m wide) in copepods were fixed in 10% formalin solution, washed with Pipes buffer overnight, processed by dehydration and embedded in acrytron E (Mitsubishi Rayon, Japan). Serial sections of 3 $\mu$m were cut with a glass knife, stained with hematoxylin and eosin, and examined by light microscopy.

## Results

A lateral view of the AdL3 whole body of *G. nipponicum* is shown in Fig. 1. A larva was

morphologically divisible into the lips, head bulb and body. The lips consisted of one oblong-shaped process on each side and each lip had two labial papillae with an amphid between them. The head bulb had three transverse rows of hooklets with oblong bases which increased in number posteriorly. From immediately behind the head bulb to the posterior extremity, the body was encircled by more than 200 transverse rows of minute cuticular spines.

The inside of the head bulb consisted of four ballonets, a part of an esophagus and muscle of ballonet (Fig. 2). The four ballonets were connected with corresponding cervical sacs hanging down in the pseudocoel. The anterior part of the cervical sac was narrow (Fig. 3), the posterior part of it was wide (Fig. 4) and the bottom of each cervical sac had a cell with a nucleus. The mouth opened between a pair of lips followed by a club-shaped esophagus which was divided into anterior muscular and large posterior glandular parts. The nerve ring surrounded the anterior part of the esophagus (Figs. 3 and 10).

The body wall was composed of cuticle, hypodermis and musculature. The cuticle was transversely striated and covered with minute spines. The hypodermis bulged into the pseudocoel at four places to form four longitudinal ridges termed ventral median cord, dorsal median cord and lateral cords. Lateral cords, especially, projected conspicuously into the pseudocoel (Fig. 6) except in the posterior end (Fig. 8). One or 2 nuclei existed at the border of each cell. Musculature consisted of polymyarian coelomyarion muscle cells and developed more on the ventral side than on the dorsal side (Fig. 6). The number of muscle cells in one-fourth of the circumference of the section was 10 to 13.

The intestine began from the posterior end of the esophagus and ended in the anus with a subterminal opening (Fig. 12). The intestine was a simple straight tube formed by one-layer of columnar cellular epithelium packed with dark-brown-colored granules in the protoplasm. The circumference of the intestine was composed of 10 to 14 cells throughout its length (Fig. 6). Cells in cross section had 0 to 4 nuclei whose average size was $3.8 \times 5.0\ \mu$m.

The genital organ was not fully developed in either sex. In the female, it was like an elongated football hanging down in the pseudocoel on the ventral side at the middle of the intestine (Figs. 7 and 11), and in the male, it consisted of a pair of incomplete copulatory spicules (Fig. 9).

On the other hand, the intestine of EaL3 was undeveloped and looked glandular in structure. Therefore, the number of cells composing the intestine and nuclei in the cells was undistinguish-

Fig. 1. Lateral view of advanced third-stage larva of *Gnathostoma nipponicum* (1.7mm long).

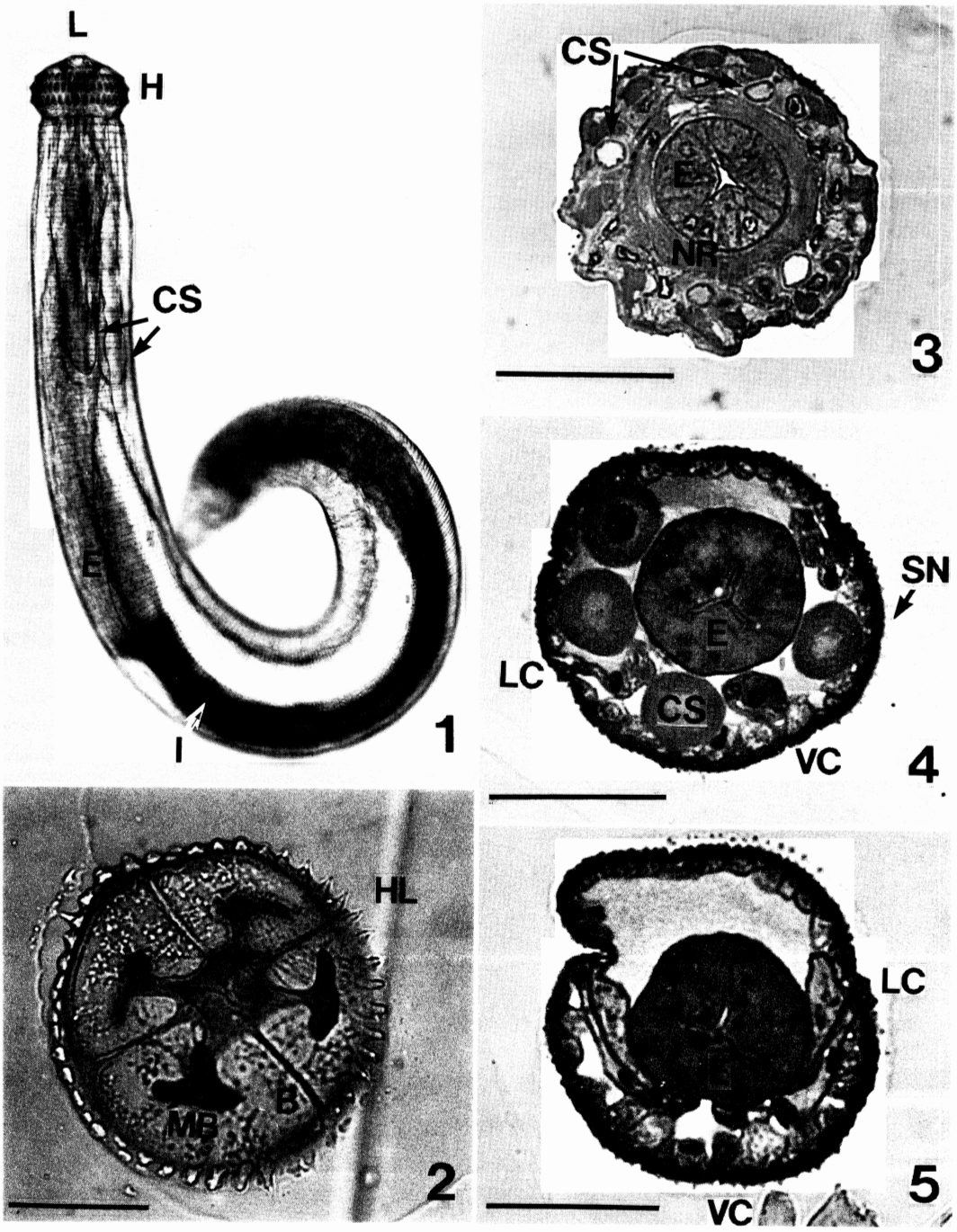
Figs. 2–9. Transverse sections (2–9) and longitudinal sections (10–12) of advanced third-stage larvae and transverse section (13) of early third-stage larvae of *Gnathostoma nipponicum* (bar indicate 50 $\mu$m).

2. Head bulb.
3. Nerve ring.
4. Posterior cervical sacs.
5. Posterior esophagus.
6. Middle part of the intestine.
7. Genital organ of the female.
8. Posterior part of the intestine.
9. Spicule of the male.
10. Anterior part of the body.
11. Middle part of the body of the female.
12. Posterior part of the body.
13. Middle part of the intestine.

**Abbreviations**

A: anus, B: ballonet, C: cloca, CS: cervical sac, E: esophagus, GO: genital organ, H: head, HL: hooklet, I: intestine, L: lip, LC: lateral cord, MB: muscle of ballonet, NR: nerve ring, P: pseudocoel, R: retractor, SN: spine, SP: spicule, VC: ventral median cord.

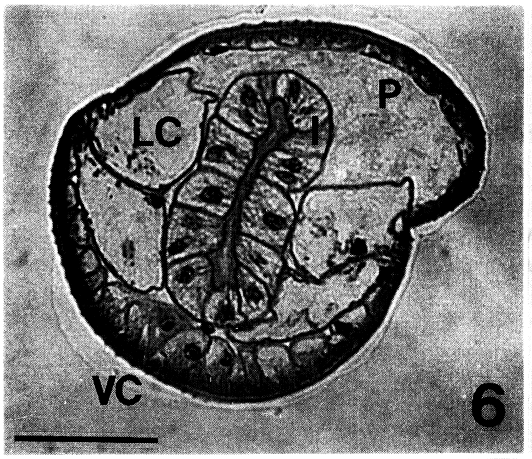
able in many sections (Fig. 13). But in one section, the intestine consisted of 5 or 6 cells with large nuclei. The lateral cord projected into the pseudocoel and the musculature of the body wall was more developed on the ventral side than on the dorsal side. However, the degree of development was less than in AdL3. The spicules were not seen in sections of four EaL3. Morphological

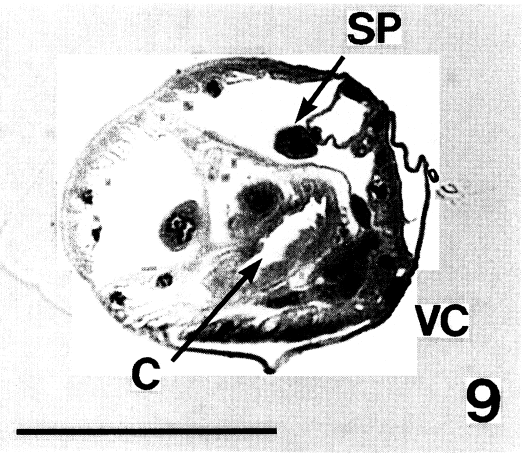


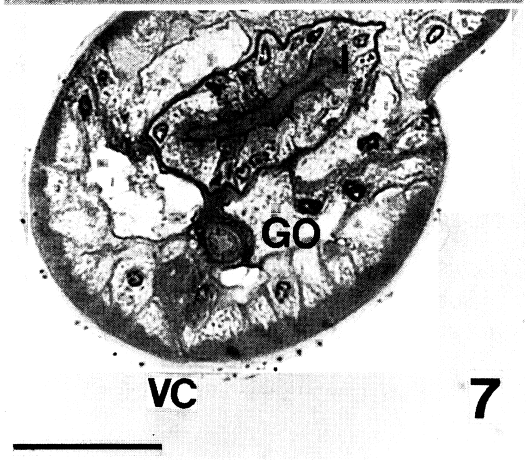
features of other organs, such as the head, esophagus and cervical sac, were fundamentally the same as those of AdL3.

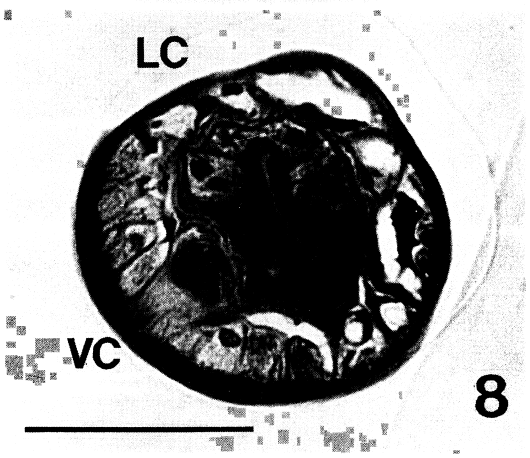
## Discussion

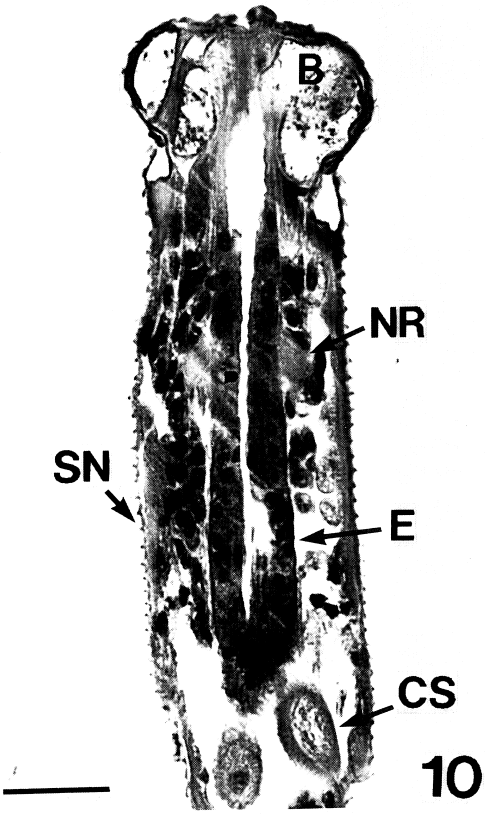
The number of nuclei in the epithelial cells of the intestine were 3 to 7 nuclei per cell in *G.*

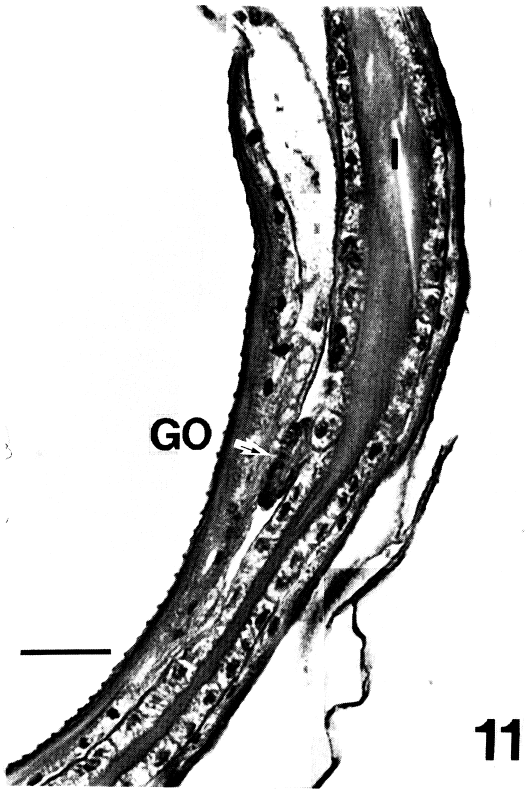


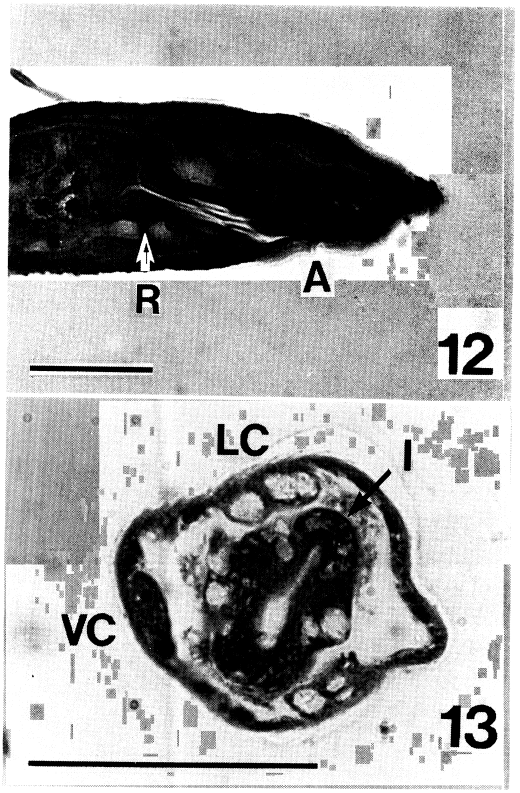






*spinigerum,* mainly 2 nuclei in *G. doloresi* and usually 1 nucleus in *G. hispidum* (Akahane *et al.*, 1986). While, in *G. nipponicum* 0 to 4 large nuclei, mainly 1 nucleus (about 50%), were found in the sections of the intestine. However, as we observed a few cells which had 5 nuclei per cell in the intact intestine (not cross sections), 5 nuclei per cell may be occasionally found in cross sections of the intestine in the future even though we could not find any cells with 5 nuclei in cross section in these observations.

Other morphological features of AdL3 cross sections from *G. nipponicum* were very similar to AdL3 of *G. spinigerum* described by Morita (1955). Akahane *et al.* (1986) also reported that there were almost no morphological differences among AdL3 of *G. spinigerum, G. doloresi* and *G. hispidum* except in the structure of the intestine. Therefore, there are no morphological differences in cross sections among the four species of *Gnathostoma* except in the structure of the intestine.

The most conspicuous differences in *G. nipponicum* from the other 3 species were the number of epithelial cells of the intestine and the presence of large nuclei in the cells. These differences are helpful in identifying cross sections of larvae from human cases.

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