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Scanning Electron Microscopic Comparisons Among the Early and Advanced Third-stage Larvae of *Gnathostoma hispidum* and the Gnathostome Larvae Obtained from Loaches

MASATAKA KOGA[1)], JUNKO ISHIBASHI[1)], YOICHI ISHII[1)], AND TAKESHI NISHIMURA[2)]

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Abstract

Morphological characteristics between the early and advanced third-stage larvae of *Gnathostoma hispidum* were compared by scanning electron microscopy. Differentiation was made by the shape of the hooklets on the head-bulb, the cervical and posterior body papillae. The phasmidial openings were found at the tail extremity of the advanced third-stage larvae. Two types (small and large types) of gnathostome larvae obtained from loaches were also compared with the above early and advanced third-stage larvae. The small type larvae (*G. hispidum*) were similar in morphology to the early third-stage larvae and the large type ones closely resembled the advanced third-stage larvae. The large type larvae, unidentified yet, were considered to be the advanced third-stage larvae of *G. hispidum* judging from their morphological characteristics.

Key words: *Gnathostoma hispidum*, third-stage larvae, scanning electron microscopy

Introduction

Gnathostoma has two phases in the third-stage, those are the early third-stage and the advanced third-stage. However, between these two stages, no distinct morphological differences are recognized light-microscopically. These two larvae are usually distinguished by a body length, about 0.5 mm for the early third-stage larvae and about 3.0–4.0 mm for the advanced ones.

We studied the surface morphological characteristics of the early and advanced third-stage larvae of *Gnathostoma hispidum*, electron-microscopically.

Recently, human gnathostomiasis has occurred chiefly in the south and west districts of Japan (Tsuji 1981, Araki 1984, Morita *et al.* 1984). All these patients had eaten live loaches, a fresh water fish imported from China. Examination of the loaches in the city markets in Japan revealed a large number of gnathostome larvae (Nishimura *et al.* 1981, Akahane and Mako 1984, Koga *et al.* 1985). Two types of larvae were recovered, one was about 0.8 mm in body length (small type) and the other about 3.0 mm long (large type). Most (98%) of the larvae belonged to the small type. The small type was regarded as the early third-stage and the other as the advanced third-stage, judging from the body length. The small type larvae were transmitted experimentally to a pig *via* rats and reached the adult stage (Akahane *et al.* 1982). The adult worms were identified as *G. hispidum*. The large type still remains unidentified. We compared these larvae from loaches with the early and advanced third-stage larvae of *G. hispidum* by scanning electron microscopy (SEM).

1) *Department of Parasitology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan*
2) *Department of Medical Zoology, Hyogo College of Medicine, Nishinomiya 663, Japan*

古賀正崇　石橋純子　石井洋一
（九州大学医学部寄生虫学教室）
西村　猛（兵庫医科大学医動物学教室）

Materials and Methods

The early third-stage larvae from cyclopoids (C, E.L_3) were the same materials as used previously (Koga *et al.* 1987). Loaches were purchased from city markets in Fukuoka and Osaka, Japan. The gnathostome larvae obtained from the loaches were allotted to two groups, small type (L, E.L_3) and large type (L, Ad.L_3). The advanced third-stage larvae were obtained from rats previously infected with the small type larvae (*G. hispidum*) from the loaches. All small type larvae readily become the advanced third-stage in rats (R, Ad.L_3) two or three months after the infection.

The larvae were placed in a glass vial and were vigorously washed three times with tap water. They were then fixed in 10% formalin for one or two weeks and immersed in distilled water which was changed three or four times to remove the formalin, within 24 hrs. The larvae were post-fixed in 1% osmium tetroxide in Millonig's phosphate buffer (pH 7.4) for two or three hrs 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 JSM-U3 SEM operated at 15 kV.

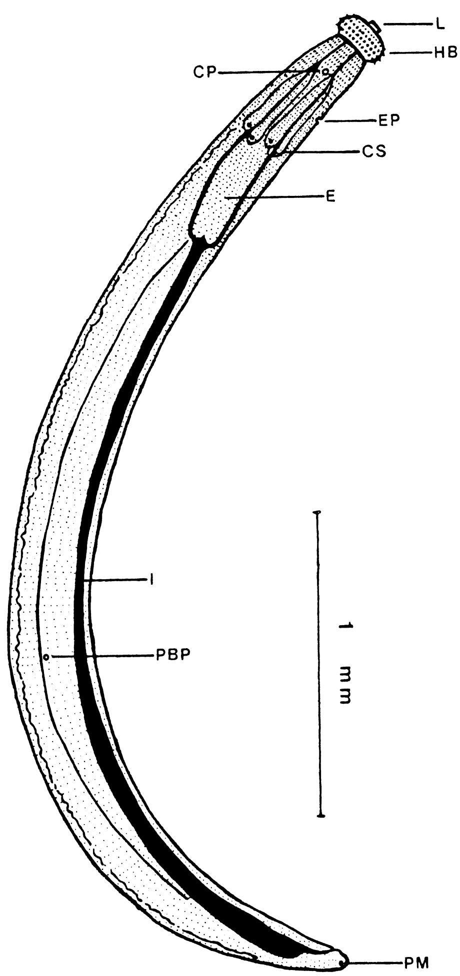


Fig. 1 A schematic diagram of an advanced third-stage larva of *G. hispidum*. L: Lip. HB: Head-bulb. CP: Cervical papilla. EP: Excretory pore. CS: Cervical sac. E: Esophagus. I: Intestine. PBP: Posterior body papilla. PM: Phasmid.

Results

A schematic diagram of the whole body of a *G. hispidum* larva is shown in Fig. 1. The positions of several papillae and organs are indicated. The body and alimentary tract average sizes are shown in Table 1 in the C, E.L_3, L, E.L_3, L, Ad.L_3 and R, Ad.L_3. All these larvae had semispherical head-bulbs at their apical ends and the bulbs were armed with four rows of hooklets. The number of the hooklets in each row is shown in Table 2. The shape of the hooklets in the third row changed in these four kinds of larvae. Those in earlier stage (C, E.L_3) were sharp and crooked posteriorly and they looked like a tusk (Fig. 2). The hooklets in the R, Ad.L_3 each had an oblique pyramid-like base with a short sharp claw at its distal tip (Fig. 14). Those of L, E.L_3 seemed to be intermediate between those of C, E.L_3 and R, Ad.L_3 (Fig. 6). The hooklets of L, Ad.L_3 were practically identical with those of R, Ad.L_3 (Fig. 10).

The bodies were wholly encircled with transverse striations of minute single-pointed spines which exist from immediately behind the head-bulb to the posterior extremity (Fig. 1). The number of these striations is shown

Table 1 Comparison on the sizes of gnathostome larvae at several stages

	Body length (mm)	Body width (mm)	Esophagus length (mm)	Intestine length (mm)	Intestine / Esophagus
C, E. L_3	0.530	0.049	0.191	0.285	1.50
L, E. L_3	0.813	0.079	0.268	0.469	1.75
L, Ad. L_3	2.500	0.220	0.606	1.617	2.67
R, Ad. L_3	3.700	0.300	0.840	2.710	3.22

C, E.L_3: Early third-stage larvae from cyclopoids.
L, E.L_3: Small type larvae from loaches.
L, Ad.L_3: Large type larvae from loaches.
R, Ad.L_3: Advanced third-stage larvae from rats.

Table 2 Comparison on the morphology of gnathostome larvae from cyclopoids, loaches and rats

	No. of hooklets on head-bulb				Location of		No. of transverse striations
	I	II	III	IV	cervical papillae	excretory pore	
C, E. L_3	37	36	38	43	9-13th*	19-20th*	175-217
L, E. L_3	36	40	42	45	10-13th*	19-20th*	184-214
L, Ad. L_3	40	41	41	44	10-12th*	19-20th*	183-201
R, Ad. L_3	40	41	47	48	10-13th*	19-20th*	202-216

* Ordinal number shows position of the transverse striation.

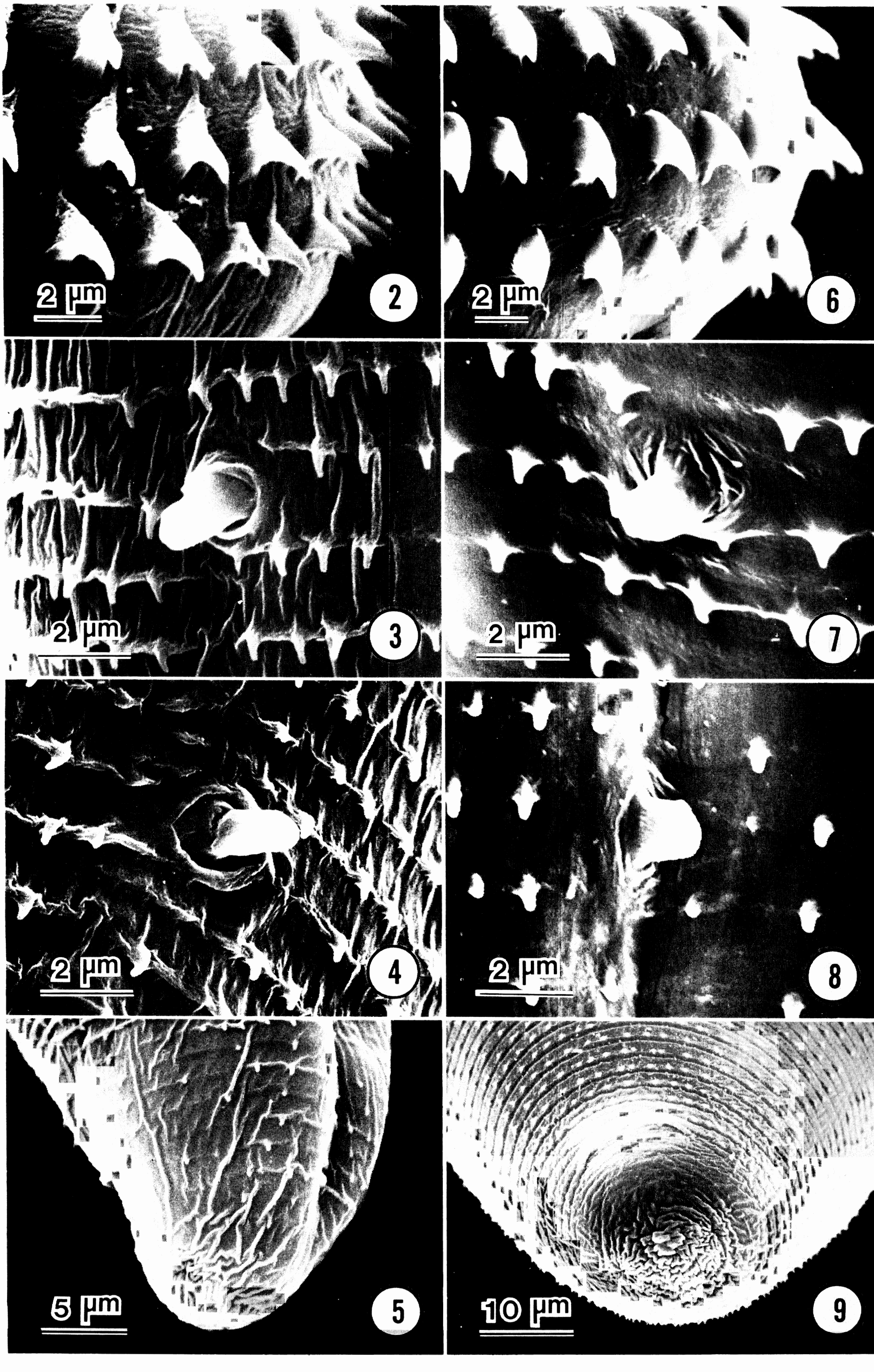
in Table 2.

One pair of cervical papillae was located bilaterally between the striations of the 9th and 13th (Table 2). The papilla in C, E.L_3 was of cilium type and was surrounded by a rim at the base (Fig. 3). The cervical papilla of R, Ad.L_3 appeared as a dome-like bulge on the tegument (Fig. 15). A cilium-like projection was seen in L, E.L_3 and it resembled the papilla of C, E.L_3 (Fig. 7). L, Ad.L_3 had bulbous cervical papilla which seemed to be intermediate between those of L, E.L_3 and R, Ad.L_3 (Fig. 11).

An ellipsoidal excretory pore was situated in respective larva in the vicinity of the 19–20th striations (Table 2). Another pair of posterior body papillae was located bilaterally at the anterior two-thirds of the body (Fig. 1). The papillae markedly changed their shape according as the larval stage. The posterior body papilla of C, E.L_3 had a cilium-like projection, and the bulbous base seemed to lie in a socket (Fig. 4). That of R, Ad.L_3 was flattened and a dome-like (Fig. 16). The papilla of L, E.L_3 had a cilium with a rim at the base (Fig. 8). A dome-like body papilla was seen in L, Ad.L_3 (Fig. 12) and it was quite similar to that of R, Ad.L_3.

Figs. 2–5 The early third-stage larvae from cyclopoids.
Fig. 2 The second to the fourth transverse rows of hooklets on the head-bulb.
Fig. 3 A cilium-like cervical papilla. The papilla is surrounded by a rim at its base.
Fig. 4 A posterior body papilla. It lies in a socket and protrudes like a cilium from the socket.
Fig. 5 Lateral view of the tail terminal. The phasmid is invisible at the extremity.

Figs. 6–9 The small type larvae from loaches.
Fig. 6 Hooklets of the second to the fourth rows on the head-bulb.
Fig. 7 A cilium-like cervical papilla. The rim at its base is vestigially present.
Fig. 8 A posterior body papilla. It has a rim at its bottom.
Fig. 9 Ventral view of the tail end. The phasmids are obscure.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At the tail extremity, the caudal papillae (phasmids) were not observed in earlier stages, i.e. in C, E.L_3 and L, E.L_3 (Figs. 5 and 9). The phasmids were visible at the posterior end in L, Ad.L_3 (Fig. 13). In R, Ad.L_3, they were clearly recognized in the form of round elevations (Fig. 17).

Discussion

Hitherto the advanced third-stage larva of gnathostomes has been differentiated from the early third-stage larva by the length of the body and the hosts from which the larva was obtained. In the present study, several morphological differences were revealed between the early and advanced third-stage larvae of *Gnathostoma hispidum*. Regarding shapes of the hooklets, they changed from a sharply crooked tusk to an oblique pyramid with a small claw at the distal tip. The cilium-like cervical papillae became dome-like ones. The markedly protruded cilium-type posterior body papillae became a dome-like bulge. The caudal papillae not visible in the early third-stage became evident in the advanced third-stage. These caudal papillae corresponded to the phasmids described by Cobb (1923) and Chitwood and Chitwood (1974). The intestine of the early third-stage larva was short compared to its esophagus, that is about 1.50 times the length of the esophagus. The intestine of the advanced one was longer by about 3.22 times over the esophagus (Table 1). These findings might be an index for a differential identification of these two stages. There were no differences in the number of hooklets in each row, the location of the cervical papillae and the excretory pore, and the number of the transverse striations on the body surface between the two stages of the larvae.

The L, E.L_3 were as small as the early third-stage larva from cyclopoids. The shapes of the hooklets, the cervical papillae and the posterior body papillae were similar to those of C, E.L_3. No differences were found in the number of the hooklets and of the transverse striations, and in the locations of the cervical papillae and of the excretory pore among C, E.L_3, L, E.L_3 and R, Ad.L_3 (*G. hispidum*).

The large type larvae from loaches, though the species is unknown, had almost the same shape of hooklets as those of R, Ad.L_3. The cervical papillae were intermediate between those of L, E.L_3 and R, Ad.L_3. The posterior body papillae completely assumed a domed-type as that of R, Ad.L_3. The phasmids were visible at the tail extremity. Length of the intestine was 2.67 times that of the esophagus (Table 1). This suggests that L, Ad.L_3 had almost reached the advanced third-stage larvae. The L, Ad.L_3 showed no differences in the number of hooklets in each row, the locations of cervical papillae and excretory pore, and the number of body transverse striations from those of C, E.L_3, L, E.L_3 and R, Ad.L_3 (Table 2). In the case of R, Ad.L_3, however, the head-bulbs sometimes had incomplete hooklet row either in front of the first row or in rear of the fourth row.

Miyazaki and Umetani (1951) reported characteristics of the advanced third-stage larva of *G. spinigerum* light-microscopically, and Koga and Ishii (1987) reported the morphology of

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Figs. 10–13 The large type larvae from loaches.
Fig. 10 The second to the fourth hooklet rows on the head-bulb.
Fig. 11 A bulbous cervical papilla.
Fig. 12 A dome-shaped posterior body papilla. Arrow indicates the papilla.
Fig. 13 Posterior view of the tail extremity. A pair of phasmids is seen bilaterally. Arrows indicate the papillae.

Figs. 14–17 The advanced third-stage larvae from rats.
Fig. 14 Hooklets of the second to the fourth rows on the head-bulb.
Fig. 15 A dome-shaped cervical papilla. Arrow indicates the papilla.
Fig. 16 A dome-shaped posterior body papilla. Arrow indicates the papilla.
Fig. 17 Dorsal view of the tail terminal. A pair of phasmids is clearly visible (arrows).

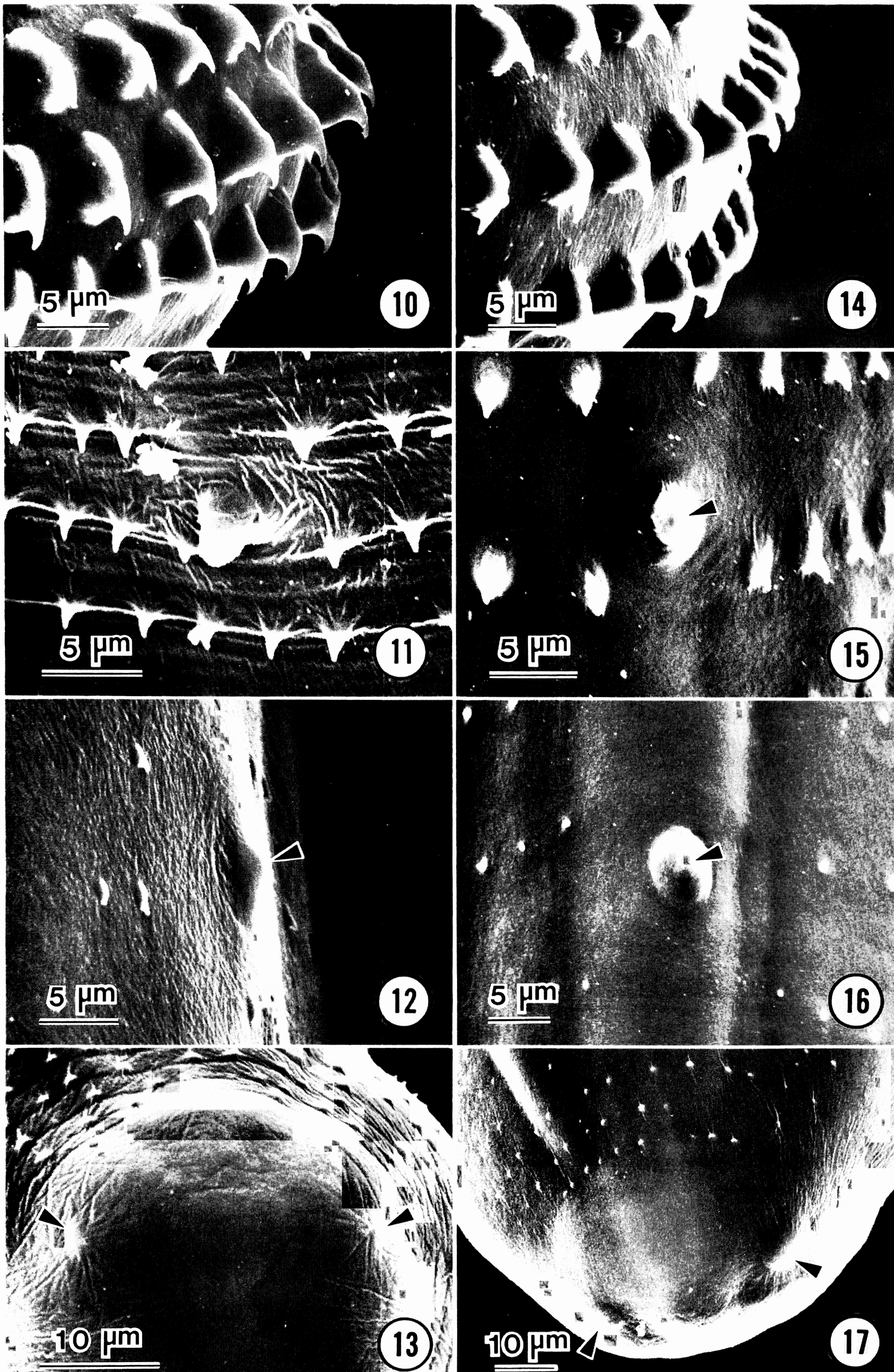
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Table 3 Comparison on the morphology among three *Gnathostoma* species larvae at the advanced third-stage

	No. of hooklets on head-bulb				Location of		No. of transverse striations
	I	II	III	IV	cervical papillae	excretory pore	
*G. spinigerum** (fish)	44	47	50	52	13-14th‡	25-26th‡	242-287
G. doloresi† (snake)	39	39	36	38	15-19th‡	25-28th‡	176-211
G. hispidum (rat)	40	41	47	48	10-13th‡	19-20th‡	202-216

* Miyazaki and Umetani (1951) † Koga and Ishii (1987)
‡ Ordinal number shows position of the transverse striation.

the same stage of *G. doloresi*, using SEM. The results are summarized in Table 3. These three gnathostomes are common and widely distributed in Southeast and Far East Asia. The three species in Table 3 can be readily differentiated in this stage, by the number of hooklets, the locations of cervical papilla and excretory pore, and the number of transverse striations. The location of cervical papilla of *G. hispidum* was similar to that of *G. spinigerum*. Location of the excretory pore of the *G. hispidum* differed from those of the other two species. *G. hispidum* resembles *G. doloresi* with regard to the number of body transverse striations.

Compared the characteristics of L, Ad.L_3 with those of other species, the L, Ad.L_3 was practically identical with *G. hispidum*. We consider that this L, Ad.L_3 might be the advanced third-stage larvae of *G. hispidum*. Akahane and Mako (1987) reported the morphological features of the large type gnathostome larvae (L, Ad.L_3) from loaches. They stated that the larvae seemed to be the advanced third stage ones of *G. hispidum*, judging from the shape and number of the hooklets and number of the nuclei in an intestinal epithelial cell. Further investigation is needed to elucidate why the early and advanced third-stage larvae of *G. hispidum* cohabit in the same host.

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