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External Morphology of the Advanced Third-stage Larvae of *Gnathostoma spinigerum:* A Scanning Electron Microscopy

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Abstract

The surface structure of advanced third-stage larvae of *Gnathostoma spinigerum* was examined by scanning electron microscopy. Larva had globular head-bulb at its apical end. This bulb was clearly distinguishable from the main body of each larva. Bulb was armed with four transverse rows of sharp hooklets. The number of hooklets in each row was 40, 43, 46 and 50, respectively. Larva possessed a pair of lateral lips on the head-bulb. Each of the lips had two labial papillae and an amphid between these papillae. Small unidentate cuticular spines were present along the entire length of larvae along their transverse striations. These cuticular striations varied in number from 225 to 256. A pair of cervical papillae was located between the 11th and 16th striations and an excretory pore was present between the 22nd and 28th striations. Another pair of papillae was detected laterally on the posterior one-third of the body. Posterior body papillae were of the domed-type and similar in structure to cervical papillae. Cuticular spines were absent behind the anus.

Key words: *Gnathostoma spinigerum*, advanced third-stage larva, surface morphology, scanning electron microscopy

Gnathostoma spinigerum Owen, 1836, is a common nematode in Southeast Asia and, Central and South America. The adult worms live in nodules of the stomach wall of feline and canine definitive hosts. Man is an unnatural host for this helminth. When accidental infections do occur, the worms often undergo extensive larval migrations and usually do not reach maturity. *G. spinigerum* was believed to be the sole cause of human gnathostomiasis for one century. Recently three other gnathostome species have been identified in human infections (*G. hispidum*, Araki and Morita, 1981; *G. doloresi*, Ogata *et al.*, 1988; *G. nipponicum*, Ando *et al.*, 1988). However, *G. spinigerum* is still the most prevalent species in human gnathostomiasis.

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The morphology of the adult and larval stages of *G. spinigerum* has been studied by light microscopy by many workers (Chandler 1925; Promas and Daengsvang 1936; Daengsvang and Tansurat 1938; Miyazaki and Umetani 1951; Miyazaki 1955, 1960; Morita 1955). Ultrastructural studies of the surface were first done by Ishii (1971) on the advanced third-stage of this species. Later studies of the larval stages of this parasite were made by scanning electron microscopy (SEM) (Ratanarapee *et al.* 1981; Ratanarapee 1982; Anantaphruti *et al.* 1982; Ratanarapee *et al.* 1988; Lamothe-Argumedo *et al.* 1989).

In this study, more detailed observations were made of the entire outer surface of the advanced third-stage larvae by SEM.

Materials and Methods

Eight advanced third-stage larvae obtained from fresh water fish *Fluta alba* in Thailand, were washed in tap water several times and then in physiological saline. They were fixed in 10% formalin for 15 days. They were then soaked for 24 h in five changes of

distilled water to remove the fixative, rinsed twice in Millonig's phosphate buffer, and postfixed in 0.5% OsO_4 for 9–10 h. The larvae were again rinsed in the same buffer, dehydrated in a graded series of ethanol, transferred to iso-amyl acetate and dried in liquid CO_2 using a Hitachi HCP-2 critical-point dryer. The specimens were coated with gold in an ion-sputter coater (JEOL FC-1100) and observed with a JEOL JSM-U3 scanning electron microscope operated at 15 kV.

Results

Advanced third-stage larvae measured 3.0–3.6 mm in length (Fig. 1). The head-bulb appeared globular and was clearly distinguishable from the body. The bulb had four rows of single-pointed hooklets (Fig. 2). As one moved anterior to posterior, the average number of hooklets in each row was 40, 43, 46 and 50, respectively (Table 1). When viewed laterally, hooklets in the first row were closely apposed to the body and were triangular with bases that were wider than either of the two sides. Hooklets in the other three rows had somewhat wider bases and higher profiles (Fig. 3). The mouth bore a pair of lateral lips (pseudolabiae) of equal size and semicircular shape. Each lip had a pair of labial papillae (6.5×8.0 μm), each of which was composed of fused cephalic and outer labial papillae (Fig. 4). Between the dome-shaped papillae on each lip, a small amphid (0.6×0.9 μm) was visible (Fig. 4, inset). The body had single-pointed, minute cuticular spines along the transverse striations from immediately behind the head-bulb to the posterior extremity. The number of these striations ranged from 225 to 256. Pairs of cervical papillae were located bilaterally from between 11th and 12th striations to between the 15th and 16th striations, with most being between the 14th and 15th striations

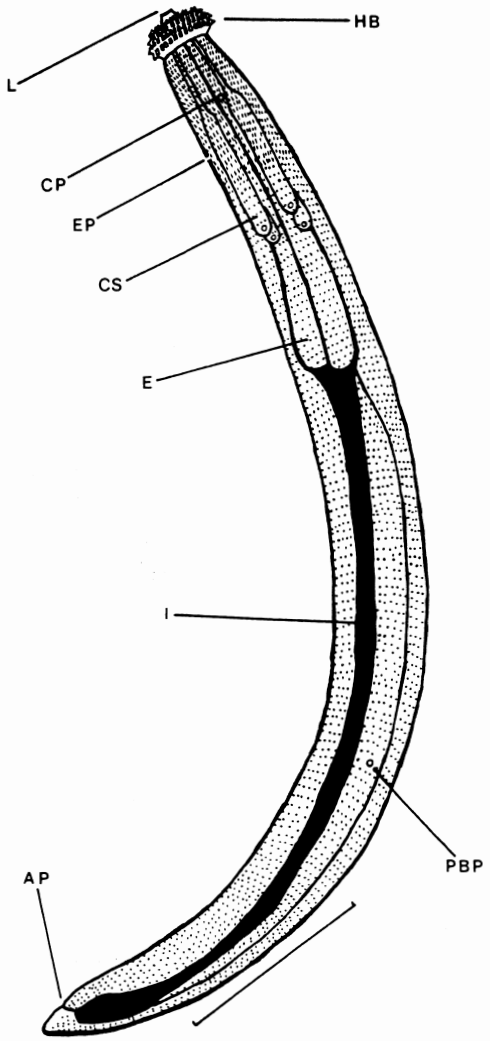

Fig. 1 Schematic diagram of an advanced third-stage larva of *Gnathostoma spinigerum*. AP: Anal pore; CP: Cervical papilla; CS: Cervical sac; E: Esophagus; EP: Excretory pore; HB: Head-bulb; I: Intestine; L: Lip; PBP: Posterior body papilla. Bar = 0.5 mm.

Fig. 2 Anterior view of an advanced third-stage larva of *G. spinigerum*. CP: Cervical papilla. Bar = 30 μm.

Fig. 3 Transverse rows of hooklets on head-bulb. Each hooklet has a wide base. Bar = 10 μm.

Fig. 4 Frontal view of the head-bulb. An amphid and a couple of labial papillae are present on each lip. A: Amphid; LP: Labial papillae. Bar = 30 μm. Inset: Enlarged view of the amphid. Arrowhead indicates the amphidial pore. ×4,500

Fig. 5 A dome-like cervical papilla is located between the 11th and 16th striations. Spines are about 2.5 μm in length. Bar = 5 μm.

Fig. 6 An ellipsoidal excretory pore opens between the 24th and 25th striations. Bar = 5 μm.

Fig. 7. A postdeirid located on the posterior one-third region of the lateral body surface. Spines are about 1.0 μm in length. S: Spines. Bar = 5 μm.

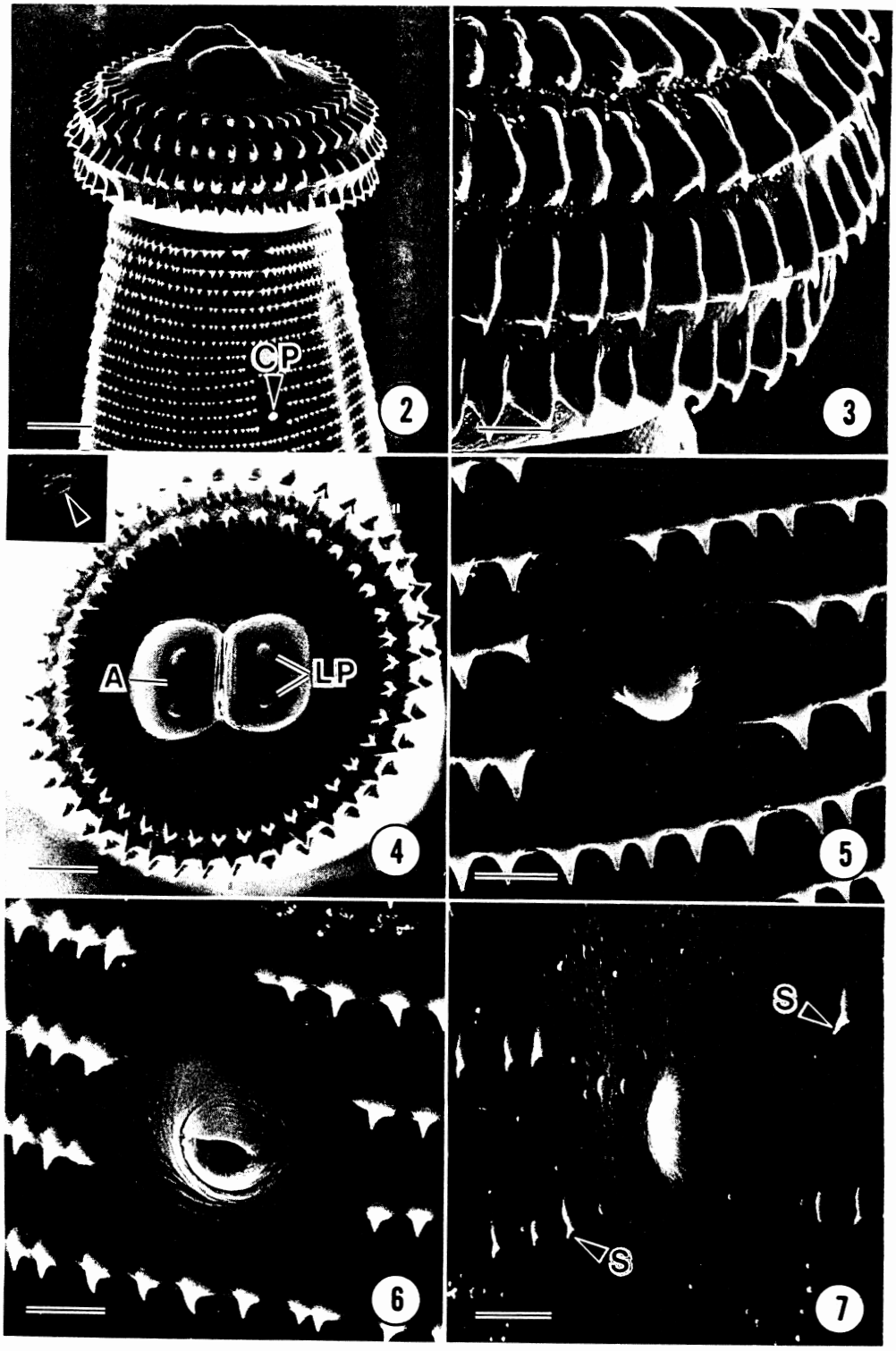
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Table 1 Morphological characteristics of advanced third-stage larvae of *Gnathostoma spinigerum*

Larva No.	No. of hooklets on head-bulb				Location of cervical papillae		Location of excretory pore	No. of body transverse striations
	I	II	III	IV	Right	Left		
1	44	41	50	52	14–15th*	15–16th*	27–28th*	251
2	40	42	51	58	14–15th	12–13th	25–26th	249
3	40	48	48	52	12–13th	13–14th	22–23rd	225
4	39	46	45	50	12–13th	14–15th	23–24th	246
5	40	44	47	48	14–15th	13–14th	25–26th	242
6	38	40	43	46	11–12th	12–13th	23–24th	256
7	40	43	43	48	13–14th	14–15th	25–26th	247
8	38	42	42	45	13–14th	14–15th	24–25th	233
Average	40	43	46	50	13–14th	13–14th	24–25th	244

*Ordinal number shows position of the transverse striation.

(Table 1). The papillae appeared as dome-like bulges from the tegument and measured about 3.5×5.5 μm (Fig. 5). An ellipsoidal excretory pore (about 7.0×6.5 μm in size) was clearly visible on the ventral surface of the body (Fig. 6) and was located between the 24th and 25th striations (Table 1). Another pair of bilateral body papillae (postdeirids) was located on the posterior one-third of the body (Fig. 1) and measured about 3.5×8.0 μm in size. All papillae were flattened, dome-shaped structures and were located between striations 137–138, 138–139, 142–143, 156–157, 164–165, 169–170 and 177–178. The body spines were larger and more densely spaced in anterior regions (about 2.0–3.3 μm in length). They gradually decreased in size to about 0.8–1.2 μm in length toward the tail. On the ventral surface of the tail, a crescent-like subterminal anal pore was evident. Spines were found as far as the

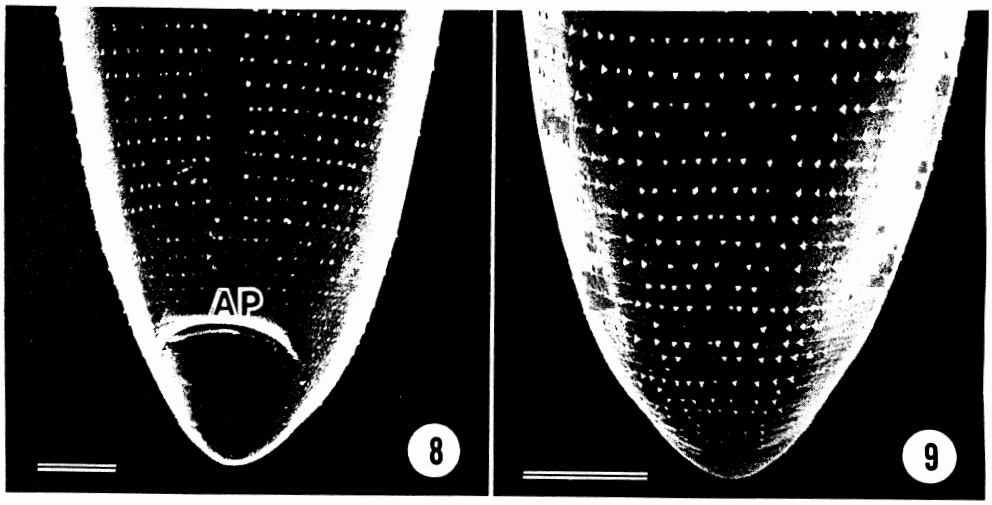

Fig. 8 Ventral surface of the terminal end of a larva. A crescent-shaped subterminal anal pore is visible. Spines are absent beyond the pore. AP: Anal pore. Bar = 20 μm.

Fig. 9 Dorsal surface of the body end. Small spines are located along the transverse striations as far as the posterior extremity of the larva. Bar = 20 μm.

anal pore but were absent beyond the pore (Fig. 8). Transverse striations reached almost as far as the terminal end of the tail and were accompanied with minute cuticular spines on the dorsal surface of the tail (Fig. 9). Caudal papillae were not recognized in this examination.

Discussion

Miyazaki and Umetani (1951) reported four rows of hooklets on head-bulbs of *Gnathostoma spinigerum* larvae with 44, 47, 50 and 52 hooklets, respectively. They also reported that some larvae had hooklets in front of the first row and behind the fourth row. Daengsvang (1968) noted that the average number of hooklets from the first to fourth row was 42, 43, 47 and 49, respectively. Two larvae had an incomplete fifth row. Anantaphruti *et al.* (1982) examined 8 larvae by SEM. The average numbers of hooklets were 43, 44, 45 and 49. They found no extra-row of cephalic hooklets. In the present study, the average numbers of the hooklets were 40, 43, 46 and 50. One larva had an incomplete extra-row of hooklets anterior to the first row, while another one had an extra-row posterior to the fourth row. There were no distinct differences in the number of cephalic hooklets. In lateral views of the head-bulb, hooklets in the first row had oblique triangular shapes with bases. Those in the remaining rows had similar shapes but had higher profiles on the surface of the head-bulb. The wide base of these hooklets is characteristic of this species.

The locations of the cervical papillae in our study were relatively constant. They were located between the 11th–16th striations (Table 1). The papillae originally reported by Miyazaki and Umetani (1951) were between the 13th and 14th striations. Anantaphruti *et al.* (1982) also described the locations of the cervical papillae. They varied from between the 7th–8th to between the 14th–15th striations. As regards the excretory pore, Miyazaki and Umetani (1951) reported that it was located between the 25th–26th striations. In the report by Anantaphruti *et al.* (1982), however the pore varied from between the 17th–18th to between the 25th–26th striations. We found the pore between the 22nd–28th striations. Some larvae observed by Anantaphruti *et al.* (1982) had cervical papillae and excretory pores situated somewhat anteriorly. During primary fixation, gnathostome larvae often invaginate their head-bulbs and drag the tegument of the upper body into the invagination. Tail shrinkage is also a common artifact. When these artifacts occur, it is impossible to determine accurate locations of the cervical papillae, excretory pores and correct number of the transverse striations. We limited our observations to specimens that were completely stretched. The number of transverse striations ranged from 225 to 256 in our study (Table 2). While worms with 206–254 striations were reported by Anantaphruti *et al.* (1982). A number of 206 appears to be outside normal values for this species.

Hitherto the positions of the cervical papillae, excretory pore, and the number and shape of respective cephalic hooklets and the number of transverse

Table 2 Morphological comparisons of advanced third-stage larvae of three *Gnathostoma* species

Gnathostoma species	No. of hooklets on head-bulb				Locations of		No. of transverse striations
	I	II	III	IV	cervical papillae	excretory pore	
1. *G. spinigerum* (fish)	44	47	50	52	13–14th	25–26th	242–287 (7 larvae)
2. *G. spinigerum* (fish)	38	39	42	45	11–14th	–	221–286 (5 larvae)
3. Present case (fish)	40	43	46	50	11–16th	22–28th	225–256 (8 larvae)
4. *G. doloresi* (snake)	39	39	36	38	15–19th	25–28th	176–211 (10 larvae)
5. *G. hispidum* (rat)	40	41	47	48	10–13th	19–20th	202–216 (10 larvae)

1. Miyazaki and Umetani (1951). 2. Otsuru and Katagiri (1958). 3. SEM study. 4. Koga and Ishii (1987): SEM study. 5. Koga et al. (1988): SEM study.

striations have become species specific characteristics of larval gnathostomes. However these morphological characteristics were difficult to detect only by light microscopy. SEM elicited satisfactory results by detecting exact surface structures. The results say that *G. spinigerum* has the largest numbers of hooklets and transverse striations in three species, while the least has *G. doloresi*. It is easy to distinguish these two species. Those numbers of *G. hispidum* however lie between two species above. That implies impossibility for differenciate this species from other two ones. However *G. hispidum* has the most anteriorly situated excretory pore in three species, which in fact gives a characteristic of this species (Table 2). To obtain accurate identifications, clear observation of well fixed specimens for SEM is important. Since three common species mentioned here have overlapping endemic areas in Asia, exact identification is needed even in larval stages.

Posterior body papillae were first documented by Anantaphruti *et al.* (1982). The shape of the papillae appeared to be of the cilium type. We found only domed type papillae. They were situated from between the 137th and 138th striations to between the 177th and 178th striations. No previous description about the position of these papillae on *G. spinigerum* has been reported.

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References

1) Anantaphruti, M., Setasubun, P., Daengsvang, S. and Vajrasthira, S. (1982): Electron microscopy of the advanced third-stage larvae of *Gnathostoma spinigerum*. Southeast Asian J. Trop. Med. Public Health, 13, 531–540.
2) Ando, K., Tanaka, H., Taniguchi, Y., Shimizu, M. and Kondo, K. (1988): Two human cases of gnathostomiasis and discovery of a second intermediate host of *Gnathostoma nipponicum* in Japan. J. Parasitol., 74, 623–627.
3) Araki, T. and Morita, H. (1981): Gnathostomiasis caused by imported loaches. Medico, 12, 5059–5063.
4) Chandler, A. C. (1925): A contribution to the life history of a gnathostome. Parasitology, 17, 237–244.
5) Daengsvang, S. (1968): Further observations on the experimental transmission of *Gnathostoma spinigerum*. Ann. Trop. Med. Parasitol., 62, 88–94.
6) Daengsvang, S. and Tansurat, P. (1938): A contribution to the knowledge of the second intermediate hosts of *Gnathostoma spinigerum* Owen, 1836. Ann. Trop. Med. Parasitol., 32, 137–140.
7) Ishii, Y. (1971): The world of scanning electron microscope (53). The surface structure of parasitic helminths. 2. Third-stage larva of *Gnathostoma spinigerum*. Igaku no Ayumi, 77, A-183–184.
8) Koga, M., Ishibashi, J., Ishii, Y. and Nishimura, T. (1988): Scanning electron microscopic comparisons among the early and advanced third-stage larvae of *Gnathostoma hispidum* and the gnathostome larvae obtained from loaches. Jpn. J. Parasitol., 37, 220–226.
9) Koga, M. and Ishii, Y. (1987): Surface morphology of the advanced third-stage larva of *Gnathostoma doloresi* – An electron microscopic study –. Jpn. J. Parasitol., 36, 231–235.
10) Lamothe-Argumedo, R., Medina-Vences, R. L., Lopez-Jimenez, S. and Garcia-Prieto, L. (1989): Hallazgo dela forma infectiva de *Gnathostoma* sp., En peces de Temascal, Oaxaca, Mexico. Anal. Inst. Bio. Univ. Nac. Auton Mexico Ser. Zool., 60, 311–320.
11) Miyazaki, I. (1955): Morphological studies on some species of *Gnathostoma* occurring in foreign lands. Fukuoka Acta Med., 46, 1237–1244.
12) Miyazaki, I. (1960): On the genus *Gnathostoma* and human gnathostomiasis, with special reference to Japan. Exp. Parasitol., 9, 338–370.
13) Miyazaki, I. and Umetani, T. (1951): Morphological description of the larval gnathostome parasitic in a fish *Ophicephalus argus* in Kyushu. Jpn. J. Clin. Exp. Med., 28, 56–59.
14) Morita, T. (1955): A study on the third-stage larva of *Gnathostoma spinigerum*. I. Morphological features. Acta Med. (Fukuoka), 25, 411–431.
15) Ogata, K., Imai, J. and Nawa, Y. (1988): Three confirmed and five suspected human cases of *Gnathostoma doloresi* infection found in Miyazaki Prefecture, Kyushu. Jpn. J. Parasitol., 37, 358–364.
16) Otsuru, M. and Katagiri, S. (1958): Notes on *Gnathostoma* collected in China (Nematode: Gnathostomatidae). Jpn. J. Parasitol., 7, 644–649.
17) Prommas, C. and Daengsvang, S. (1936): Further report of a study on the life cycle of *Gnathostoma spinigerum*. J. Parasitol., 22, 180–186.
18) Ratanarapee, S. (1982): Fine structure of *Gnathostoma spinigerum*: A comparative SEM study between an adult worm and a third-stage larva. J. Med. Assoc. Thailand, 65, 642–647.
19) Ratanarapee, S., Jesadapatanakul, S. and Mangkalanond, K. (1981): Spontaneous exit of a gnathostome through a surgical wound. Southeast Asian J. Trop. Med. Public Health, 12, 376–379.

20) Ratanarapee, S., Jesadapatanakul, S., Mangkalanond, K., Buawatana, C. and Sookpatdhee, V. (1988): SEM study on an adult worm and a third-stage larva of *Gnathostoma spinigerum* from human cases. Proc. Asia-Pacific Conf. Workshop Electron Microscopy Bangkok, 9, 571–572.