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Studies on the Head Structure of *Spirometra erinacei* (Cestoda, Pseudophyllidea) Plerocercoid by New Stretching-Fixation Method

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ABSTRACT. Development of new fixation method for maintaining the head region of *Spirometra erinacei* kept stretched in aqueous chemicals was worked out, and the external morphology of the well-stretched head of plerocercoid was studied under light and scanning electron microscopy.

The stretching method for plerocercoid head was most effective when specimen was placed in 10% ethanol Ringer solution for 5 minutes after soaking several hours in 0.1% chloretone Ringer solution.

In the plerocercoid head, the bothria and bothridia were clearly found on the dorsal and ventral surfaces, and a frontal pit was also found on the apical end in the same manner as that of some diphyllobothriid cestodes, *Diphyllobothrium ditremum, D. dendriticum* and *D. vogeli.* Morphological appearance of microtriches on the surface of frontal pit periphery were quite different from those of other parts of the head surface.

Key words : diphyllobothriid cestode — *Spirometra erinacei* — plerocercoid head — stretching-fixation — SEM observation

The adult worms of *Spirometra erinacei* (Rudolphi, 1918) Faust, Campbell and Kellogg, 1929 belonging to genus *Diphyllobothrium* are very common intestinal cestodes of cats, dogs and wild felines in broad areas in Orient, and practically all over the world. Although larvae (spargana) of *S. erinacei* are predominantly found in the cutaneous or subcutaneous tissues of amphibians, reptiles, avians and mammals, they are parasitic in humans through ingestion of infected animals. Human infestations with the plerocercoid (sparganosis) often occur in Asian countries such as Japan, Korea, Southern part of China, Vietnam, Thailand, Indonesia and other. The morphological details of the head region of *S. erinacei* plerocercoid have not yet been clarified since the plerocercoid head inevitably shrinks when the specimen was immersed in various types of fixatives.

We developed an advanced fixation technique for maintaining the plerocercoid head kept in the stretched state using aqueous chemical solutions, and the external structure of the well-stretched head was studied with a light microscope and a scanning electron microscope.

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MATERIALS AND METHODS

The plerocercoids of *S. erinacei* were obtained from the striped snakes, *Elaphe quadrivirgata* (Boie) captured in Okayama Prefecture, Japan. The plerocercoids without external damage in Ringer solution were cut transversely about 3 cm long from the anterior end under a binocular dissecting microscope. The plerocercoid head was stretched in ethanol Ringer solution (10% ethanol in Ringer solution) and chloretone Ringer solution (0.1% chloretone in Ringer solution).

For light microscopy, the specimens were interposed between two slides and fixed overnight in 70% alcohol and stained with Delafield's hematoxylin. For scanning electron microscopy, the specimens were fixed in 2.5% gultaraldehyde and 1.0% osmotic acid, and dehydrated through graded alcohol series and dried in a carbon dioxide critical point apparatus, followed by platinum palladium coating and then studied with Hitachi S-570 scanning electron microscope.

RESULTS

I. Stretching procedure for the plerocercoid head

An appreciable number of anesthetic methods using aqueous chemical agents for platyhelminthic animals namely tubellarians, trematodes and cestodes have been described by Kaplan (1969).[1)] Among them, 10% ethanol solution, 2.5% potassium chloride, 0.1% chloretone and the lowest concentration of menthol were employed for head stretching of *S. erinacei* plerocercoid, in addition to low-temperature treatment method. The separated use of the respective agents did not give any marked morphological changes in the head stretching procedure.

It is evident from the results of various trials done with those 4 agents, 2 of 10% ethanol and 0.1% chloretone were probably the most effective for the head stretching.

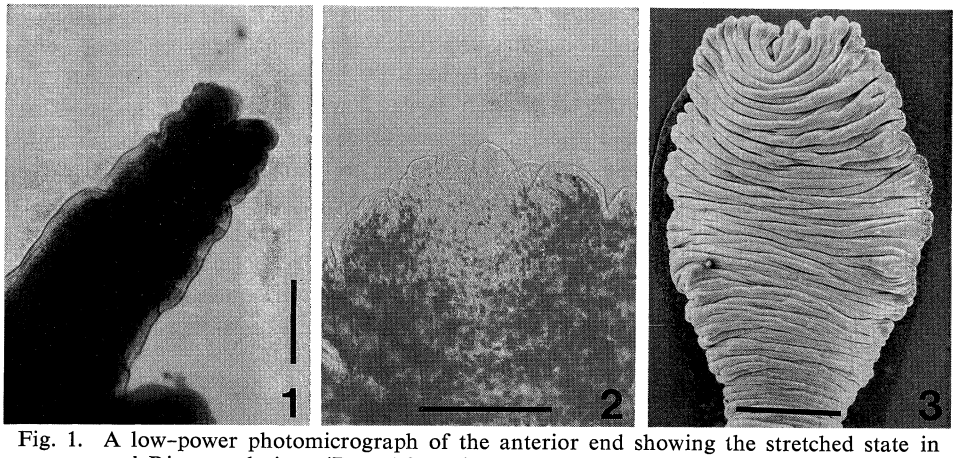

Fig. 1. A low-power photomicrograph of the anterior end showing the stretched state in warmed Ringer solution. (Bar=1.0 mm)

Fig. 2. An enlarged photomicrograph of the anterior end showing the contracted state after direct fixation in 70% alcohol, dorso-ventral view. (Bar=0.3 mm)

Fig. 3. A low-power SEM picture of the anterior part showing the contracted state after direct fixation in 2.5% glutaraldehyde, dorso-ventral view. (Bar=1.0 mm)

It is well known that the head of *S. erinacei* plerocercoid maintains expansive and contractile movement in Ringer solution (Iwata, 1962),[2] and the movement can be stimulated in warmed Ringer solution (Fig. 1). When specimens were first placed into 0.1% chloretone Ringer solution, the head movement became very slow in 30 minutes, then the movement was completely suspended within a few hours in chloretone Ringer solution.

The specimens were then carefully taken out from the foregoing solution and rapidly replaced with 10% ethanol Ringer solution, since the apical ends of specimens showed shrinkage after they were transferred to common fixatives (Figs. 2 and 3). The shape of the specimens gradually varied in 10% ethanol Ringer solution. The head region stretched very slowly towards the anterior side and the apical end became almost circular with anterior tongue-like expansion. 5 minutes later, the apical end of the specimens treated with 10% ethanol Ringer solution showed no more shrinkage in several fixatives (Fig. 4).

II. External morphology of the plerocercoid head

The external structure of the well-stretched head is shown in Figs. 4 and 5. Anterior end of the head had a rounded image with a spoon-like shape under light and scanning electron microscopy. In the vicinity of the apical end, the slightly protuberant portions were observed bilaterally on the dorsal and ventral surfaces (Fig. 5). The protuberant extent extended over about 0.3 mm from the apical end and it was obviously confirmed as bothridium under the condition of incomplete development. The protuberant portion was identical to the region intensely stained with Delafield's hematoxylin. The concave portion existed between two bothridia on dorsal and ventral surfaces could be developed into bothrium in near future.

The frontal pit was clearly recognized on the apical top region (Fig. 5), and the opening of the pit was about 8 μm in diameter (Fig. 6). The whole surfaces of the head region were densely covered with microtriches. The shape of microtriches were similar at any portion with an exception of the frontal pit periphery having microtriches of plump form (Figs. 7 and 8). The

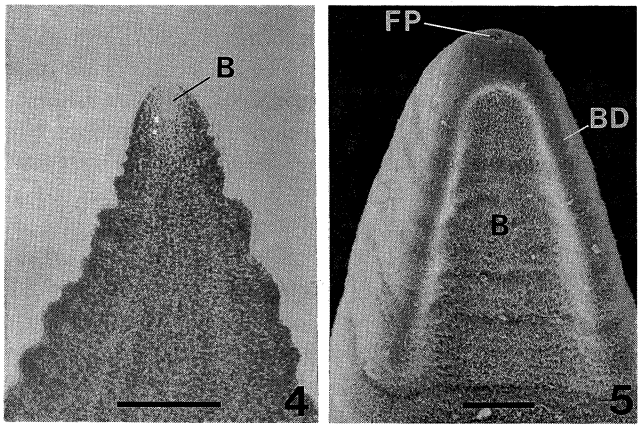

Fig. 4. A low-power photomicrograph of the anterior part treated by new stretching-fixation method, dorso-ventral view. (Bar=0.4 mm)

Fig. 5. A low-power SEM picture of the anterior end showing well-stretched head, dorso-ventral view. (Bar=50 μm) B : bothrium, BD : bothridium, FP : frontal pit.


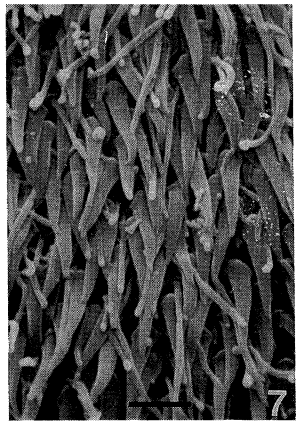
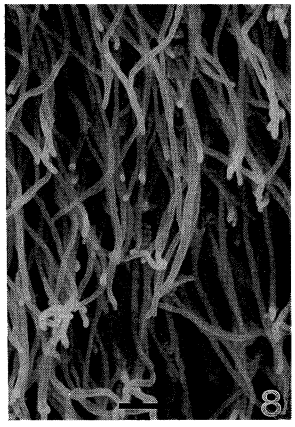

Fig. 6. An enlarged SEM picture of the head-end showing a frontal pit (arrow), dorso-ventral view. (Bar=10 μm)
Fig. 7. Enlargement of microtriches around the frontal pit. (Bar=1.0 μm)
Fig. 8. Enlargement of microtriches on the head surface except peripheral areas of the frontal pit. (Bar=1.0 μm)

microtriches around the frontal pit, on the other hand, were much wider and thicker in the basal portion than those in whole surfaces (Fig. 7), and judging from its shape the frontal microtriches may belong to so-called conoid-type.

DISCUSSION

In general, plerocercoids of *S. erinacei* are about 10 to 20 cm long having milk white color, and the head region reminds a pin-point. Although a large number of studies dealt with morphology of *S. erinacei* plerocercoids have been reported, to our best knowledge there is no head morphology. The reason was that the anterior end of the plerocercoid head constantly shrank when placed in conventional fixatives. Kwa (1972)[3] has reported histochemistry of the scolex of *S. erinacei* plerocercoids, the apical end seemed to be shrank due to fixation. Yamane *et al.* (1974)[4] showed the head region of *S. erinacei* plerocercoid by scanning electron microscopy that neither bothria and bothridia nor frontal pit appeared on the apical end. It is probable that the head region of their specimens also shrank according to the strong influence of fixing solution.

The purpose of the present investigation is to contrive effective fixation technique for maintaining the plerocercoid head in the stretched state, and to identify external morphology of the head region. In order to confirm better fixation technique, the above described 4 chemicals have individually and repeatedly used. As a results, both 0.1% chloretone Ringer solution and 10% ethanol Ringer solution were probably the most effective for stretching the head.

The operational order of chemicals is of major importance, and it turned out to be a failure if two chemicals were used in wrong order. Therefore, it is suggested to use 0.1% chloretone, then apply 10% ethanol and fixative; otherwise this new method would not contribute any results. By the new head stretching procedure it is obvious that more detailed studies on the head region of *S. erinacei* plerocercoids will be developed.

The morphological appearance of the well-stretched head region of *S. erinacei*

plerocercoids treated by the new method is more nearly comparable to those of the plerocercoid head of other diphyllobothriid cestodes, *Diphyllobothrium dendriticum* (Nitzsch, 1824), *D. ditremum* (Creplin, 1825) and *D. vogeli* Kuhlow, 1953 (Halvorsen, 1970[5]; Andersen, 1975[6]; Gustafsson and Vaihela, 1981[7]). The plerocercoids of the above 3 species are elongated, chalky or spindle-shaped, and measure 10 to 20 mm. There exists the bothria, bothridia and a frontal pit on the surface of the apical end, and pseudosegmented appearance which resulted from slight contraction of individual is recognized on the body surface. These plerocercoids fixed with Susa fixative or Bouin's fluid (Gustafsson and Vaihela, 1981[7]) and formol-saline (4% formal in 1.0% saline) or Bouin's fluid (Halvorsen, 1970[5]; Andersen, 1975[6]), the head region of each specimen kept relatively rigid form. However, the apical end of *S. erinacei* plerocercoids is shrank with in the above fixatives. Therefore, it is observed that the head region of *S. erinacei* plerocercoids is much shrank as compared with plerocercoids of preceding species.

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