

SCANNING ELECTRON MICROSCOPY OF ADULT *ECHINOSTOMA MALAYANUM*

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INTRODUCTION

Many species of echinostomes can develop to maturity in the human intestine (Beaver *et al.*, 1984). The distinctive feature of echinostome unlike other intestinal flukes is that it possesses a circumoral disc harboring collar spines. The number of collar spines and their arrangement serve as one of the important morphological criterion for the classification of this family. For *Echinostoma malayanum* has 43-44 collar spines with the arrangement of, five corner spines on each side of the shoe-shape circumoral disc (3 oral and 2 aboral), two groups of lateral spines, each group consists of 11 spines arrangement in single row and dorsal spines consisting of 11 spines (6 oral and 5 aboral) (Lie, 1963). In Thailand, five species namely *Echinostoma malayanum*, *E. ilocanum*, *E. revolutum*, *Hypodermaeum conoideum* and *Episthmium caninum* had been found in man (Bhaibulaya *et al.*, 1964, 1965, 1966; Radomyos *et al.*, 1982, 1985; Yokogawa *et al.*, 1965). Their endemic areas confine mostly within the Northeastern part of the country. The prevalence of infection appears variable being 50% in Kalasin (Bhaibulaya *et al.*, 1964) and 16.4% in Khon Kaen (Phatihatakorn *et al.*, 1984). Man acquires infection by eating raw or unproperly cooked fresh water snails (*Pila* sp. and *Viviparus* sp.) and tadpoles (Radomyos, 1986).

In this study the ultrastructure of adult *E. malayanum* was examined under scanning electron microscope.

MATERIALS AND METHODS

The adult worms were collected from small intestines of white rats infected with *E. malayanum* for five and a half months. The metacercariae were obtained from natural infected *Indoplanorbis exustus*. At recovery, all worms had translucent bodies and short branched testes as described by Hadidjaja and Oemijati (1969). The worms were washed by shaking vigorously 8-10 times in normal saline solution and were fixed in hot 4% paraformaldehyde. The preparation was allowed to cool down to ambient temperature and kept in refrigerator at 4°C overnight. Thereafter, the worms were washed twice in 0.1 M phosphate buffer pH 7.4 and post-fixed in 1% osmium tetroxide in phosphate buffer for 2 hours at 4°C. After being washed again in the same buffer they were dehydrated in serial graded ethanol, transferred into amylacetate and critical-point dried in Hitachi HCP-2 apparatus. Mounting of these processed worms was performed on the specimen holders using double-sided sticky scotch tape, coated with gold. Ultrastructures of the worm were studied under Hitachi S-450 scanning electron microscope at electron accelerating voltage 15KV.

RESULTS

The ventral surface of the anterior part of adult *E. malayanum* are shown (Fig. 1). The anterior part from ventral sucker up to oral sucker appears slightly curved ventrally

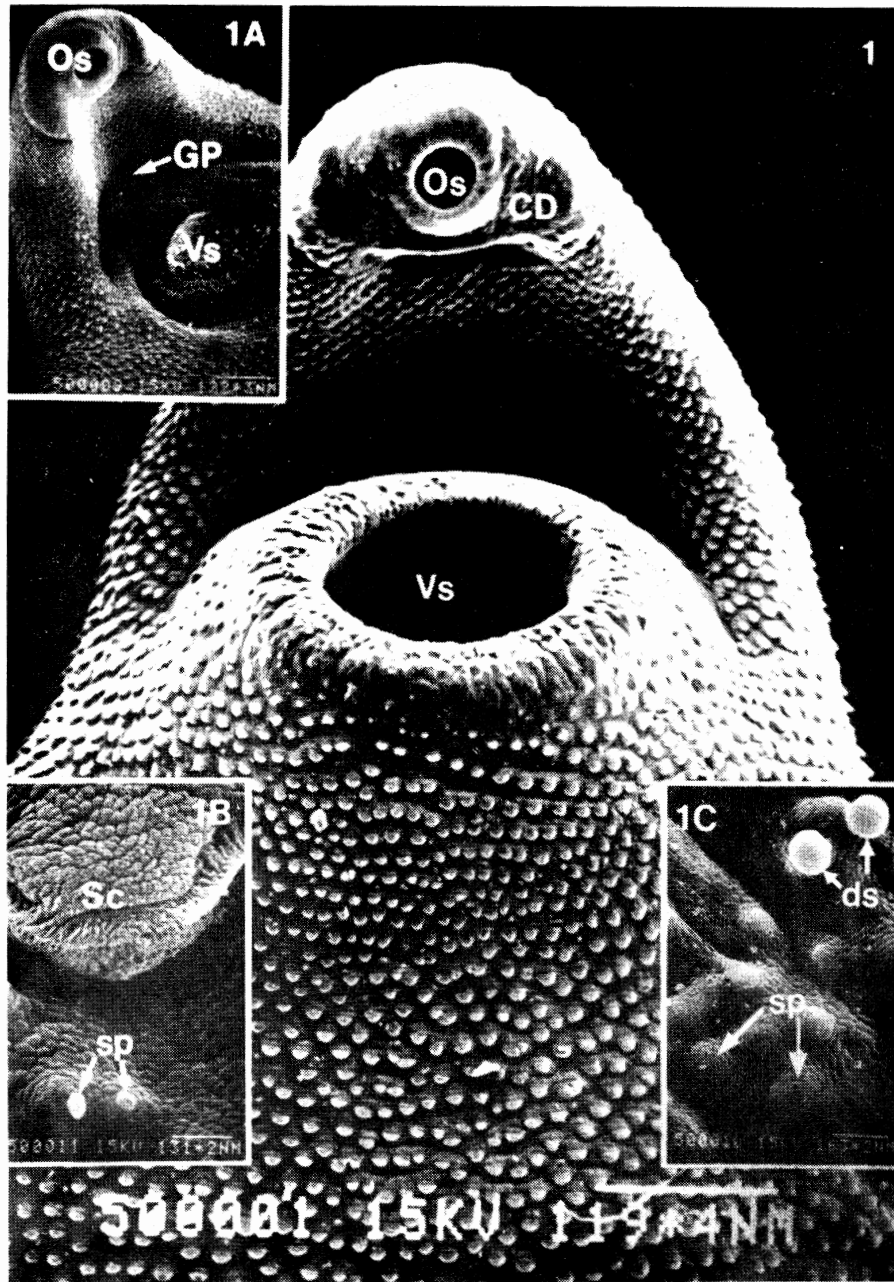


Fig. 1--The ventral side of adult *E. malayanum* showing oral sucker (Os) surrounded by circumoral disc (CD) and ventral sucker (Vs). $\times 76$.

1A. Between oral and ventral suckers, the genital pore (GP) within the valley-like pouch. $\times 120$.

1B. Triangular shape scale (Sc) at high magnification and sensory papillae (sp) in between. $\times 4,900$.

1C. Sensory papillae (sp) and dome-shaped sensory organs (ds) on circumoral disc. $\times 2,400$.

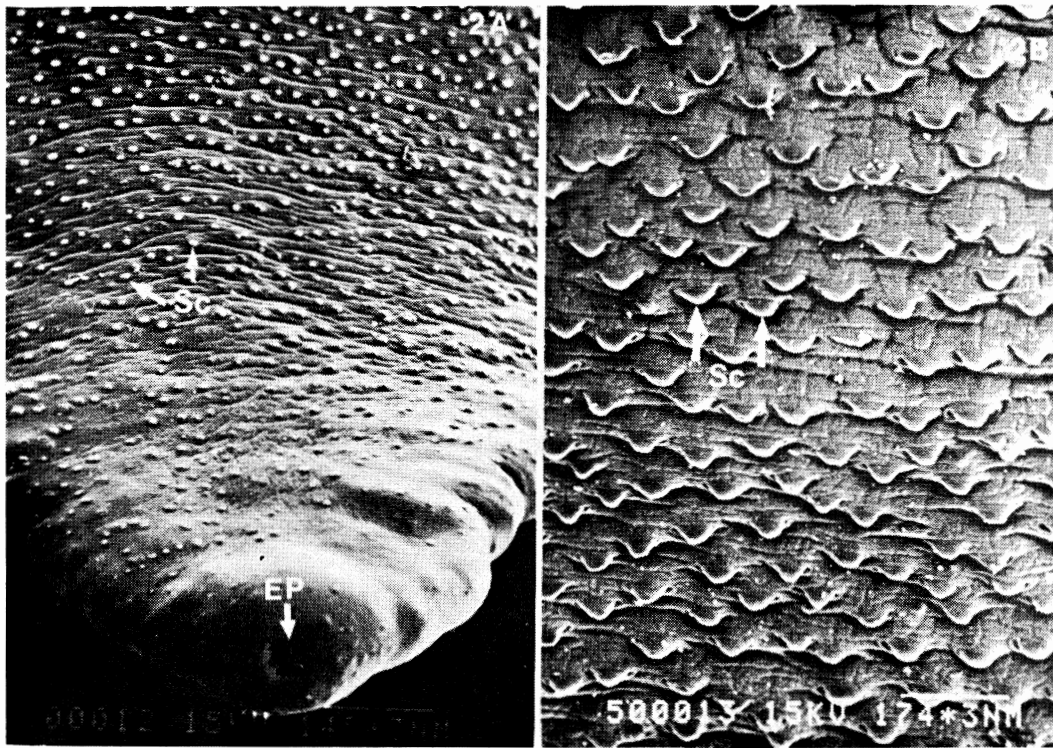


Fig. 2A. Showing the excretory pore (EP) at the terminal end and attenuated scales on the posterior part. $\times 100$.
 2B. The small scales (Sc) below the middle half of the body. $\times 200$.

forming valley-like pouch around genital openings. The oral sucker, measured $793 \times 942 \mu\text{m}$ is situated on the subterminal of the anterior, ventral side of the body. It is surrounded by horse-shoe-shaped circumoral disc. A relatively larger ventral sucker $2,727 \mu\text{m}$ in diameter, lies posterior to the oral sucker (Fig. 1 & 1A). The tegumental scales are uniformly distributed over all the ventral surface except a triangular shaped area between genital pore and ventral sucker (Fig. 1A) and around the excretory pore (Fig. 2A). On the dorsal side, the scales occur only below the circumoral disc and disappears afterwards at the same level of the upper rim of ventral sucker (Fig. 3). The shape of scales looks triangle with blunt tip and project backwards to the body. The scale is covered with tegumental layer bearing

microvilli (Fig. 1B). They are arranged alternately in transverse rows and the size is rather big in the anterior one fourth of the body (Fig. 1) then gradually decrease towards the posterior end (Fig. 2A & 2B). The scales around circumoral disc and ventral sucker are slightly smaller (Fig. 1 & 3). Unciliated sensory papillae are seen between the scales on the anterior half of the body and more densely pack on the circumoral disc as well as ventral sucker (Fig. 1, 1B & 1C). Other sensory organs are dome-shaped, spherical ones are found only on the circumoral disc (Fig. 1C). The tegument where the scales are present is covered with microvilli even though on scales (Fig. 1B & 3A). On the dorsal surface, the tegument was rough and numerous pits were observed (Fig. 3B).

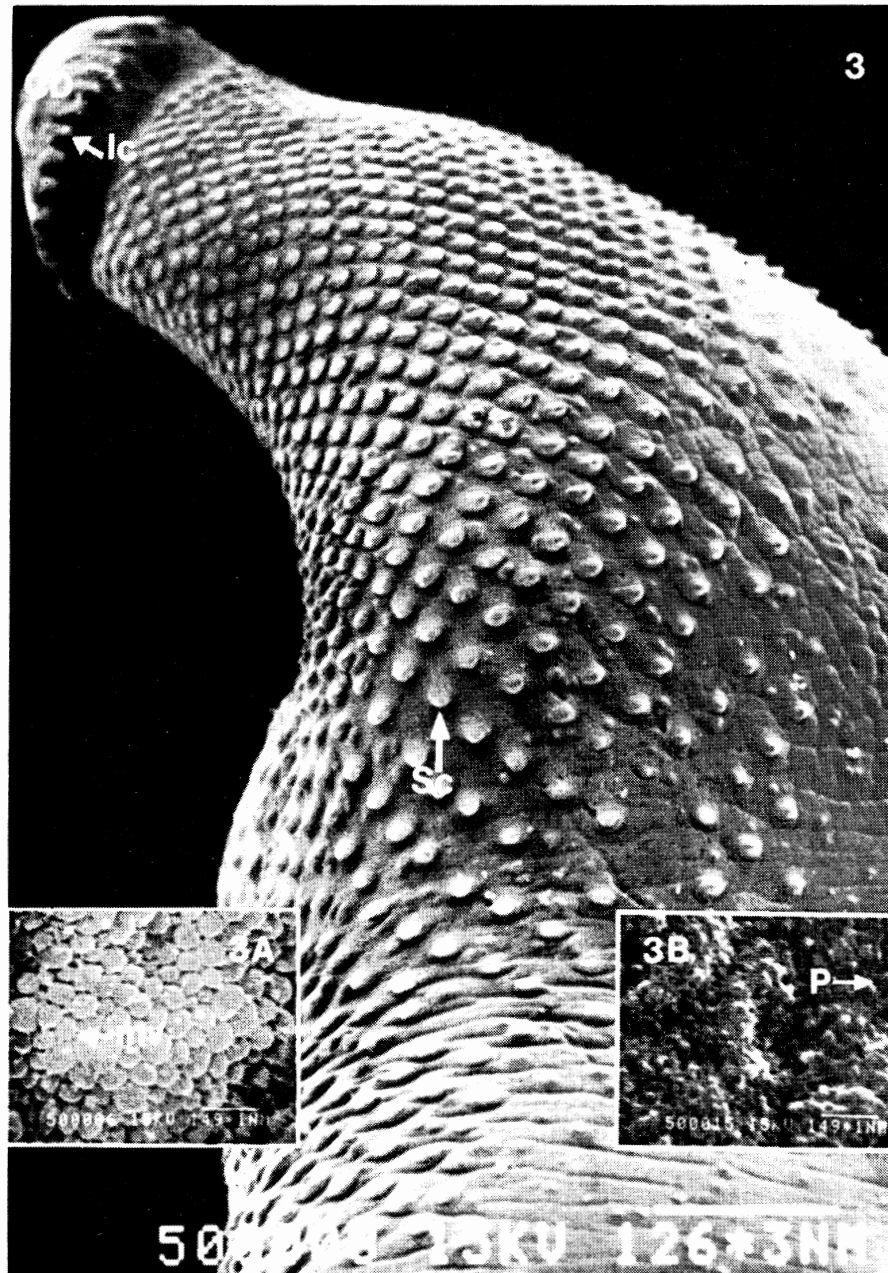


Fig. 3—Lateral side of adult *E. malayanum* showing where the scales (Sc) are absent. $\times 120$.
3A. The tegumental microvilli (mv) on the ventral side and where the scales are present. $\times 10,000$.
3B. The pitted (p) tegument on the dorsal surface. $\times 10,000$.

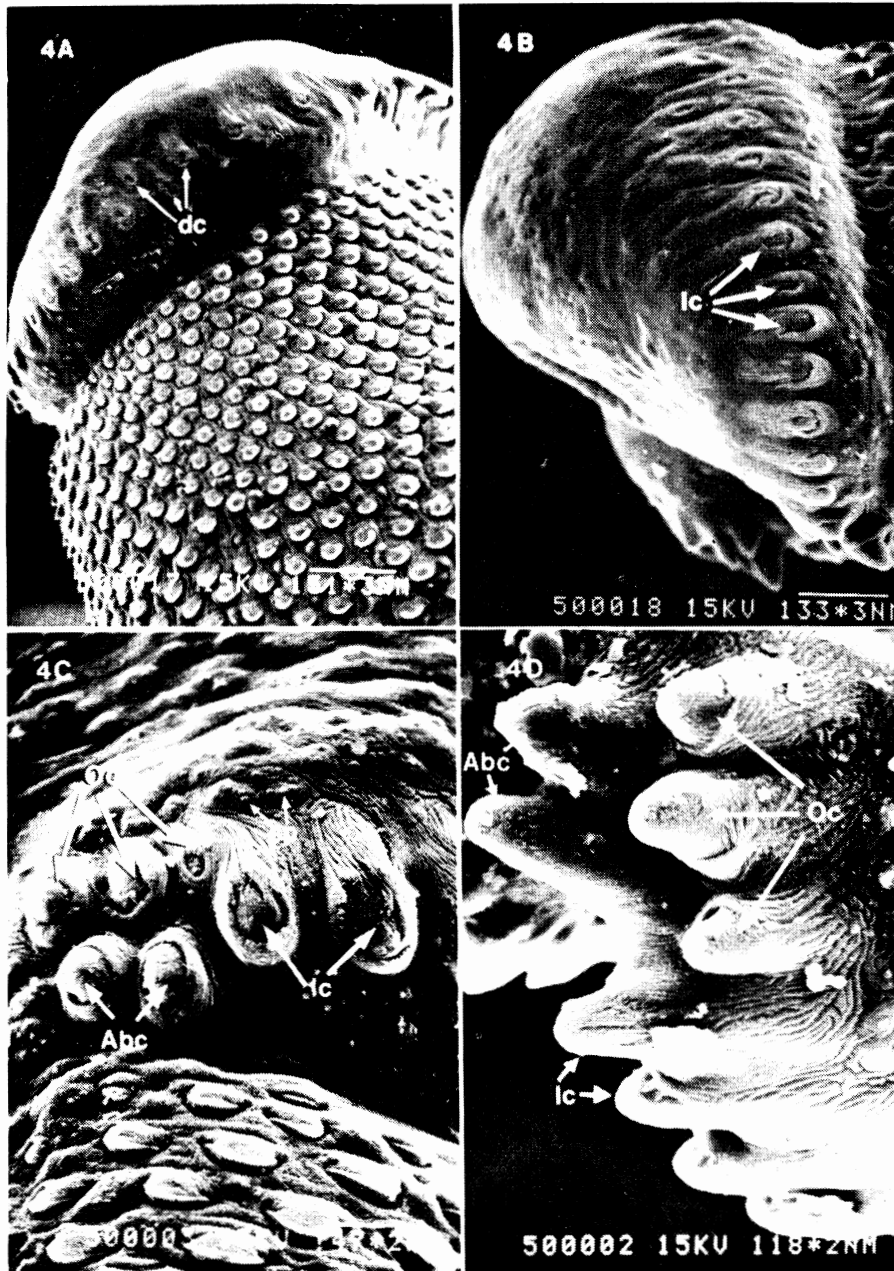


Fig. 4A. The dorsal collar spines (dc) arranged in two alternate rows, 5 oral and 6 aboral spines. $\times 300$.
 4B. The lateral collar spines (lc), 10 spines in a single row. $\times 450$.
 4C. The corner spines; 3 oral (Oc) and 2 aboral (Abc) nearby the lateral collar spines (lc) and sensory papillae (sp). $\times 1,000$.
 4D. The protruding out of the collar spines: oral (Oc), aboral (Abc) and lateral collar spines (lc). $\times 1,300$.

The circumoral disc of adult *E. malayanum* in this study had 41 collar spines. The arrangement can be divided into 3 groups, a pair of corner spines, a pair of lateral collar spines and a group of dorsal collar spines. Each side of corner spines is composed of 3 oral and 2 aboral collar spines (Fig. 4C & 4D), a total of 10 spines. Each side of lateral collar spines consists of a single row of 10 spines (Fig. 4B), total 20 spines. Dorsal collar spines are arranged in alternate rows (Fig. 4A), 5 oral and 6 aboral spines, total of 11 spines. All of the collar spines are retractable (Fig. 4C & 4D).

DISCUSSION

Fried and Fujino (1984) studied on the development of *Echinostoma revolutum* larvae which was cultivated in chick embryo and domestic chicks, under the scanning electron microscope. The tegument of the worms changed from smooth to granular and the sensory-papillae on the suckers became well-defined. However, they could study only 7-day old worms. The scanning electron micrograph of the adult worm of species of echinostome is not available.

In this study three dimension of adult *E. malayanum* contour can be observed under the scanning electron microscope. The collar spines are found in small numbers (41 spines) less than the study of Lie (1963) which had 43-44 spines. The arrangement and number of the collar spines are different in some aspects from the reports of Lie (1963) and Hadidjaja and Oemijati (1969). The corner spines are the same as Lie (1963) study but differed from Hadidjaja and Oemijati (1969) study which had 2 oral and 3 aboral spines. The lateral collar spines observed in both previous descriptions had 11 spines, but in this study only 10 spines were present. The dorsal collar spines seen in previous studies had 6 oral and 5 aboral spines, but in this study there were 5 oral and 6 aboral collar

spines. By scanning electron micrographs, three dimension can be seen at high magnification and much clearer than light microscope with only two dimension so the results are also different. The tegumental scales appear mainly on the ventral side, they may serve as the accessory structure attachment to the intestinal epithelium. The microvilli are also present on the scales so the role of the scales may be to increase the number of tegumental microvilli. In this finding, the scales around the genital atrium is absent which was similar to *Fasciola hepatica* juveniles (Bennett, 1975). The tegumental microvilli of *E. malayanum* adult are similar to *Opisthorchis viverrini* adult (Sirisinha *et al.*, 1984). In this study the microvilli are found only on the ventral surface, and the area where the scales are present. Unlike the dorsal side the portion of free of scales, where the tegument is slightly similar to schistosomes (Clegg, 1972) *S. mekongi* (Voge *et al.*, 1978) and *S. japonicum* tegument around oral sucker (Sobhon *et al.*, 1986). This part of tegument is pitted and also present in *Hypoderaeum conoideum* and *E. revolutum* (Tesana *et al.*, in the scanning electron microscopy of adults *Hypoderaeum conoideum* and *Echinostoma revolutum*, unpublished). The sensory papillae appear mainly on the ventral anterior half of the body and more densely situated on the oral and ventral suckers. These structures may be tactile nerve endings. The dome shaped nerve endings were also found by Fried and Fujino (1984) on the circumoral disc around the oral sucker of *E. revolutum* larva; their functions are unknown.

SUMMARY

Scanning electron microscopy observations of *E. malayanum* adult obtained from small intestines of infected rats was made. The number of collar spines were 41. The features observed were a pair of corner spines (3 oral and 2 aboral) total 10; a pair of lateral collar

spines (10 spines each side); total 20; dorsal collar spines (5 oral and 6 aboral) total 11. Sensory papillae were found more densely situated on the circumoral disc around the oral sucker and on the ventral sucker. Other sensory organs, dome shaped, found only on the circumoral disc. The scales appear mainly on the ventral surface. The microvilli are present on the tegument where the scales occur, while the other part of dorsal side had pitted tegument.

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