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Scanning Electron Microscopical Findings of *Echinochasmus japonicus* Tegument

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INTRODUCTION

Echinostomatidae is a large family of flukes of birds or mammals, which is morphologically characterized by the head crown armed with collar spines (Skrjabin, 1956; Yamaguti, 1958; Rim, 1982). Out of its 8 subfamilies, Echinochasminae is distinguished by small size and dorsally interrupted collar spines. Out of the flukes of Echinochasminae, genus *Echinochasmus* has vitellaria extending to posterior margin of ventral sucker (Skrjabin and Shults, 1938; Yamaguti, 1958).

Many species of *Echinochasmus* have been known in the world, but in Korea *E. japonicus* was recorded from naturally infected ducks and experimental mice (Eom *et al.*, 1984; Chai *et al.*, 1985). *E. japonicus* was first described from experimentally infected animals by Tanabe (1926). He commented the possibility of human infection, and Ujiie (1936) demonstrated human infection experimentally. Thereafter, Seo *et al.* (1985) recorded 4 human cases of natural infection by this fluke first in Korea. Therefore, medical attention has been paid to this fluke.

Only 3 kinds of echinostomes, *Isthmiophora melis*, *Echinostoma revolutum* and *E. hortense* have been observed by SEM (Fried and Fujino, 1984; Smales and Blankespoor, 1984; Lee *et al.*, 1986) as the literature concerned. In general, the tegument of trematodes consists of cytoplasmic processes, spines and sensory papillae. The tegument is known to be equipped with physiological or biochemical functions (Lumsden, 1975). Therefore, SEM study is useful in understanding the function of tegument as well as in morphological observation for taxonomy.

However, the information for tegumental ultrastructure of *E. japonicus* is not available at present. The present study was undertaken to observe the tegumental ultrastructure of *E. japonicus* by scanning electron microscopy.

MATERIALS AND METHODS

The metacercariae of *E. japonicus* were obtained from the head portion of the southern top-mouthed minnow, *Pseudorasbora parva*, by artificial digestion technique (Chai *et al.*, 1985), which were collected in Kimhae, Kyongsangnam-do, Korea. A total of 10 albino rats of Sprague-Dawley strain was infected with 50 metacercariae respectively. They were killed at 5, 7 and 14 days after infection and the worms were recovered.

For scanning electron microscopical observation, the recovered worms were washed 3-5 times with 0.8% phosphate buffered saline and fixed in 2.5% glutaraldehyde (pH 7.2). They were dehydrated in ethanol series and in a freeze drier (Edwards). Mounted specimens were coated with gold in 30nm thickness using an ion sputtering coater (Eiko-3) and were observed by ISI Korea CL-6 or DS-130 scanning electron microscopes under accelerating voltage of 10 KV.

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RESULTS

General feature of *E. japonicus* was small, plumpy gourd-shape and ventrally concave. Anterior end of the worms was attenuated, but posterior was round-ended. The tegument of whole body was wrinkled transversely and covered with cobblestone-like cytoplasmic processes. Tegumental spines were regularly arranged on the whole ventral surface and anterior half of dorsal surface (Figs. 1 & 2 on plate).

Head crown was armed with 24 collar spines which were embedded in cytoplasmic pockets. Collar spines were extended radially around oral sucker in a row, and were interrupted at the spot of dorsomedian spine (Fig. 3 on plate & Fig. 1). High power views of the spines showed wrinkled surface and they looked like budding horns of a young stag (Fig. 4). However, four spines at both ends of head crown were found in different arrangement, i.e., the 2nd and the 4th spines from side were outstretched distinctively.

The tegument between the two suckers was covered with aspinous and cobblestone-like cytoplasmic processes. Muscular oral sucker had round swollen sensory papillae (type II) on posterior half of its lip and uni-ciliated sensory papillae (type I) on its anterior half (Fig. 5). The tegument between oral sucker and genital pore was wrinkled transversely and surfaced by cobblestone-like cytoplasmic processes with many ciliated sensory papillae (Figs. 6 & 7). Genital pore was located anterior to ventral sucker and covered with the tegument of ventral sucker origin, which was velvety with type II papillae and a hidden cirrus (Fig. 8). The ventral sucker was round and protruded ventrally. On tegument of its lip, 15-17 papillae of type II were arranged in a row (Fig. 9). The tegument from ventral sucker to genital pore was velvety (Fig. 10).

Tegumental spines were spade-shaped and distributed densely on ventral especially on ventrolateral surface and dorsal surface of anterior body. They were not found on antero-median surface of ventral side, just the tegument from oral sucker to ventral sucker, and on posterior half of dorsal surface. The shape of spines on ventral tegument was not differed by the age of worms. However, the tips of spines on dorsal surface were slightly indented into 6-7 points in the worms of 5 days age, while those of 14 days old worms were not. The sensory papillae of type I were observed among the spines. Its density was higher in ventral surface than in dorsal, and in anterior than in posterior (Figs. 11, 12, 13 & 14). Dorso-posterior surface was aspinous and was covered with cobblestone-like cytoplasmic processes (Figs. 15 & 16). Excretory pore which was covered with tegument was opened in the posterior end (Figs. 17 & 18).

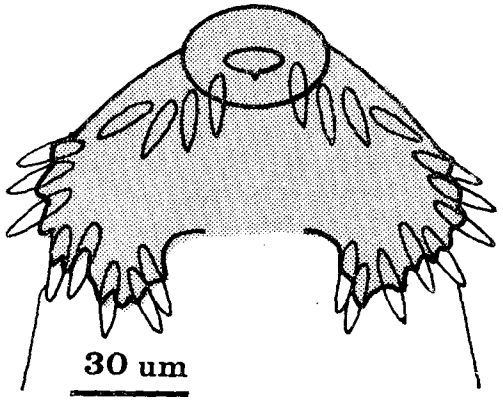

Fig. 1. Arrangement of collar spines of *E. japonicus*.

DISCUSSION

General shape of *E. japonicus* was minute and plumpy gourd-shaped with attenuated anterior and round-ended posterior body. The collar spines were arranged around oral sucker in a row as observed in other echinostomes, such as *E. revolutum*, *I. melis* and *E. hortense* (Smales and Blankespoor, 1984; Lee *et al.*, 1986). However, they were interrupted in dorso-median line. The 2nd and the 4th spines at both ends of head crown were outstretched more than others. However, whether their arrangement is distinctive and constant enough for regarding them as end group spines or not is uncertain

by the present findings.

The tegumental surface of *E. japonicus* was covered with cobblestone-like cytoplasmic processes, spade-shaped tegumental spines and two kinds of sensory papillae. The cobblestone-like appearance of cytoplasmic processes is commonly found on tegument of other trematodes. However, the spade-shaped tegumental spines may be one of unique features of this fluke. They were densely distributed on ventro-lateral tegument or dorsal surface of anterior body, but were not found between two suckers nor on posterior dorsal tegument. In case of other echinostomes such as *E. revolutum*, *I. melis* and *E. hortense*, the spines were scale shaped and densely stuck to the tegument anterior to ventral sucker (Smales and Blankespoor, 1984; Lee *et al.*, 1986).

The shape and distribution of tegumental spines of trematodes closely relate to size or shape of worms, whether they migrate or not, parasitic niche, *etc.* In case of *C. sinensis*, it has been known that the larval worms excysted in duodenum or under migration through bile duct have tegumental spines in the anterior half of body but adult worms in intrahepatic bile duct have not (Fujino *et al.*, 1979; Lee *et al.*, 1982). On the contrary, it has been known that the tegumental spines of *F. hepatica* in larval stage is sharp-pointed drill shaped but they become diverged or sawtooth shaped with 15～30 pits in adult stage(Bennett, 1975).

In this study, tegumental spines of 14 days old *E. japonicus* were larger and stouter than those of 5 days old. However, the spines on dorsal surface of 5 days old worms were indented with tips of 6～7 points. It is a reversed finding compared with that of *F. hepatica*. The shape and distribution of spines and minute body size are considered, the spines may play an important role in attaching and abrasing host tissue at intervillous spaces.

The sensory papillae on trematode tegument have been classified to 4 types by the configuration and composition. In the present study, 2 types of sensory papillae, uni-ciliated (type I) and roundly swollen(type II), were observed in *E. japonicus*. The function of type I papillae has been suggested to be tango-and/or rheoreceptive, and that of type II is supposed to be tango-and/or pressure receiving(Erasmus, 1967; Bennett, 1975; Fujino *et al.*, 1979; Seo *et al.*, 1984; Lee *et al.*, 1984 & 1986). In *E. japonicus*, type I papillae were chiefly found on ventral surface, while type II papillae were observed on the lips of oral or ventral suckers. This finding was the same as that of *E. revolutum* or *E. hortense* (Fried and Fujino, 1984; Lee *et al.*, 1986). The papillae looked like to have similar functions as proposed in other flukes.

SUMMARY

Tegumental ultrastructures of *Echinochasmus japonicus* were observed by scanning electron microscopy. The worms were recovered from albino rats which were experimentally infected with the metacercariae obtained from *Pseudorasbora parva*.

Followings are summarized findings.

1. The worms were minute and plumpy gourd-shaped with attenuated anterior and round posterior end. The tegument of whole body was wrinkled transversely and covered with cobblestone-like cytoplasmic processes.

2. Head crown was armed with 24 collar spines which were embedded in cytoplasmic pockets. The spines were arranged in a row with an interruption at dorsomedian line, however, the 2nd and the 4th spines were outstretched more than others. Oral and ventral suckers were muscular with numerous type II sensory papillae, and genital pore opened between the two suckers.

3. Tegumental spines were spade-shaped with broad base and pointed tip. They were compact in ventro-lateral tegument or dorsal surface of anterior body. They were not found between the two suckers and dorsal surface of posterior body.

4. Two types of sensory papillae, uni-ciliated (Type I) and roundly swollen sensory papillae (Type II), were observed. The type I papillae were chiefly distributed on ventral surface of

tegument and type II were on the lips of suckers.

Arrangement of collar spines, shape and distribution of tegumental spines or sensory papillae are regarded as characteristic features of *E. japonicus*.

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=국문초록=

Echinochasmus japonicus 표피 미세구조의 走査電子顯微鏡的 觀察

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李 純 炯·孫 運 睦·洪 性 台

棘口吸蟲의 하나인 *Echinochasmus japonicus*의 표피 미세구조를 관찰하기 위하여 이 연구를 실시하였다. 참붕어(*Pseudorasbora parva*)의 頭部로부터 분리, 수집한 피낭유충을 Sprague-Dawley系 흰쥐에 경구 감염시킨 후 5일, 7일 및 14일에 성충을 回收하여 고정, 건조 및 순금표면처리 과정을 거친 다음 ISI-Korea의 CL-6 및 DS-130 주사전자현미경으로 관찰하였다.

그 결과는 다음과 같다.

1. *E. japonicus*의 蟲體는 작고 통통한 표주박 모양으로 前端이 뾰족하고 後端은 둥근모양이었다. 충체의 全體表面은 가로로 불연속적인 주름이 져 있었고, 자갈모양의 원형질 돌기로 덮혀 있었다.

2. 頭冠에는 24개의 頭棘이 원형질 주머니에 박혀서 일렬로 排列하였으나, 이중 양끝에서 두번 째, 네번 째의 頭棘은 밖으로 더 벌어져 있었다. 또한 이 배열은 두관의 등쪽 정중선에서 끊어져 있었다. 口吸盤은 근육성이었고 구순에는 많은 수의 감각유두가 존재하였다. 구흡반과 복흡반 사이에는 생식공이 개구하고 있고, 동심원 모양의 복흡반은 腹측으로 약간 튀어나와 있었으며 15~17개의 Ⅱ型 감각유두가 일열로 배열되어 있었다.

3. 삼모양의 皮棘은 충체표피의 복측 앞쪽측면과 배측 전반부에 밀집되어 있었고, 구흡반에서 복흡반에 이르는 복측 정중면과 배측 후반부에는 분포하지 않았다.

4. 감각유두는 type Ⅰ, type Ⅱ 두 종류만 관찰되었으며 type Ⅰ은 복측 전반부에 주로 많이 분포하였고 type Ⅱ는 구흡반과 복흡반의 구순에만 주로 분포하였다.

이상의 결과로 *E. japonicus*의 표피 미세구조가 다른 극구흡충과 비교했을 때 몇 가지 특징적인 것을 나타냄을 알 수 있었고, 이 특징들이 다른 극구흡충류의 표피 미세구조 및 분류학적 연구에 지표로 사용될 수 있을 것으로 생각된다.

EXPLANATIONS FOR FIGURES ON PLATES

Fig. 1. Ventral view of 2-week old *E. japonicus*, OS; oral sucker, GP; genital pore, VS; ventral sucker, EP; excretory pore (×383).

Fig. 2. Dorso-lateral view of a 2-week old worm. Note the distribution of tegumental spines, concentrated on tegument of anterior body (×433).

Fig. 3. Dorso-lateral view of head portion. Note an interrupted portion of collar spines (arrow heads) (×1, 360).

Fig. 4. Magnified collar spines which look like budding horns of a young stag (×3, 490).

Fig. 5. Oral sucker (OS) with sensory papillae on its lip (P_2: type II papillae) (×2, 600).

Fig. 6. Tegument between oral sucker and genital pore, showing wrinkled cytoplasmic processes without a spine (×3, 210).

Fig. 7. Type I sensory papillae (P_1) in Fig. 6 (×9, 600).

Fig. 8. Genital pore (GP) with a cirrus (×3, 210).

Fig. 9. Ventral sucker (VS) with a characteristic arrangement of spines and type II papillae (×2, 000).

Fig. 10. Magnified cytoplasmic processes in the circled region of Fig. 9 (×15, 300).

Fig. 11. Tegumental spines of a 5 day old worm on ventrolateral surface of anterior body (×5, 000).

Fig. 12. Tegumental spines of a 14-day old worm on the same place of Fig. 11. (×3, 800).

Fig. 13. Tegumental spines of a 5-day old worm on dorso-median surface of anterior body (×4, 000).

Fig. 14. Tegumental spines of a 14-day old worm on its dorsomedian surface of anterior body (×3, 800).

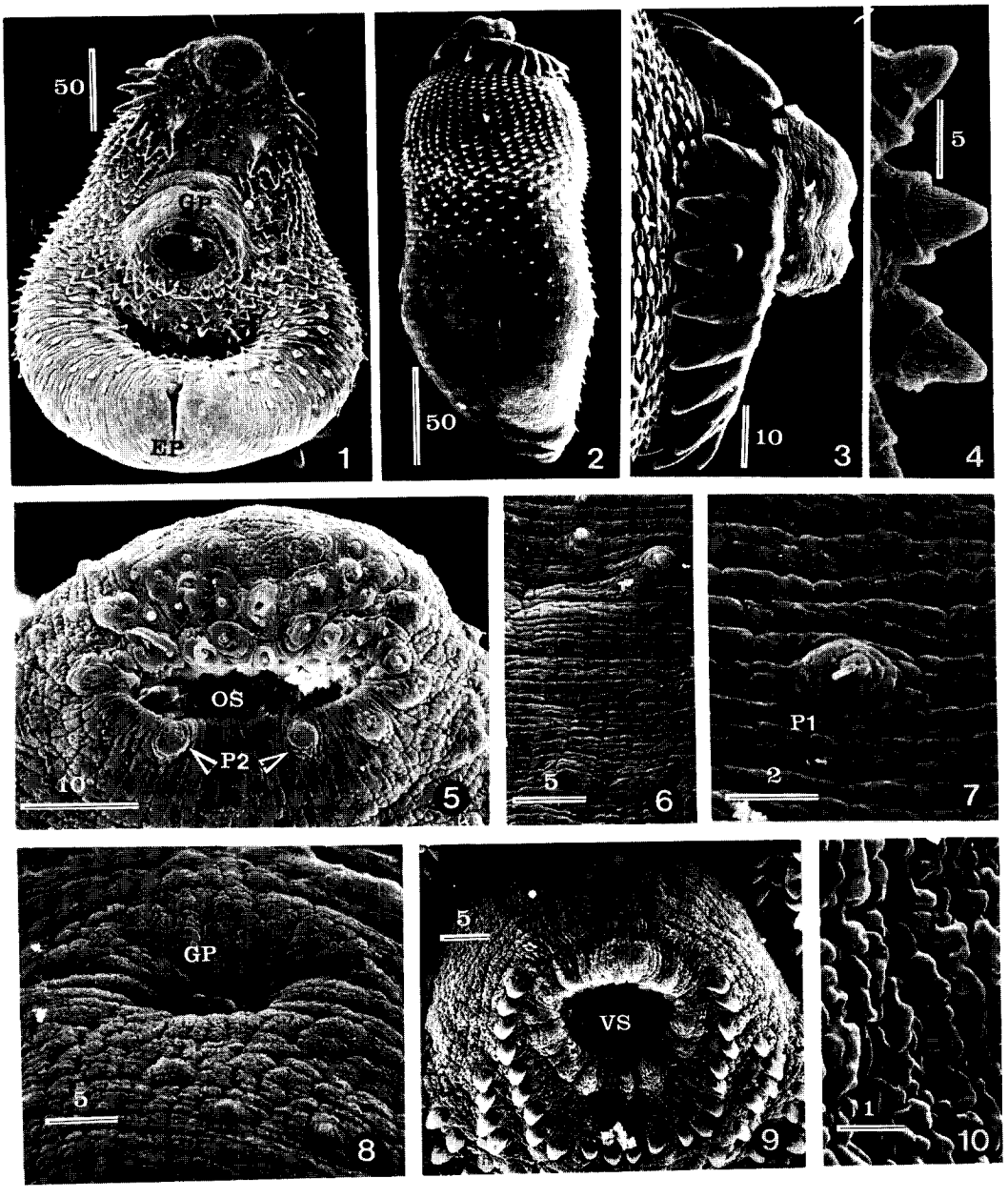
Fig. 15. Dorso-posterior tegument of a 14-day old worm without a spine (×3, 270).

Fig. 16. Magnified dorso-posterior tegument of a 14-day old worm (×9, 800).

Fig. 17. Excretory pore of a 5-day old worm (×6, 000).

Fig. 18. Excretory pore of a 14-day old worm with a few sensory papillae (×3, 200).

* Scale unit is micrometer (μm).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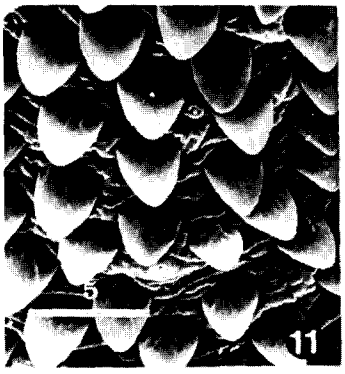
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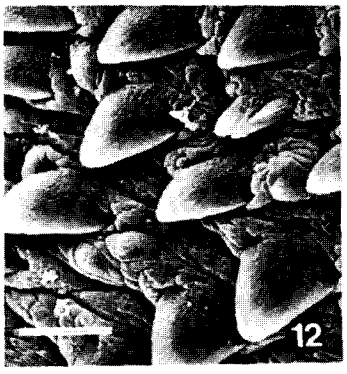
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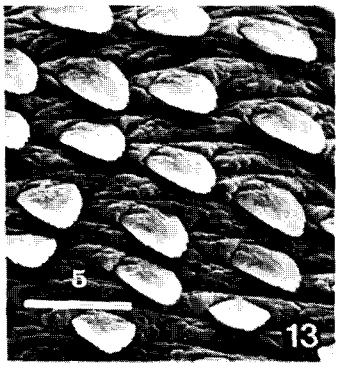
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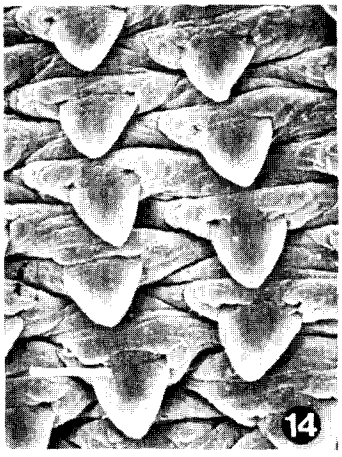
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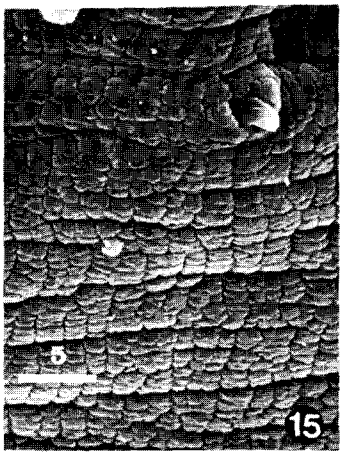
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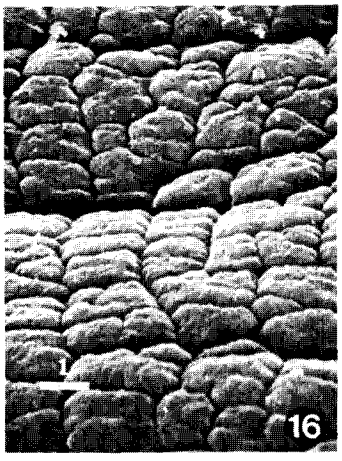
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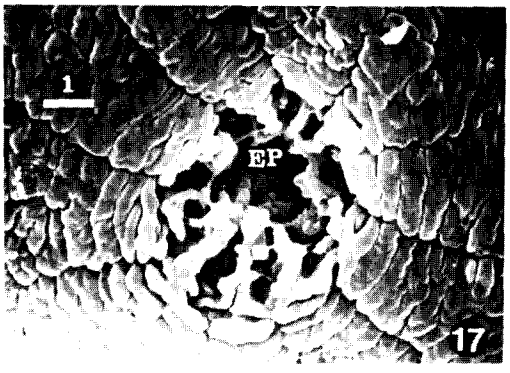
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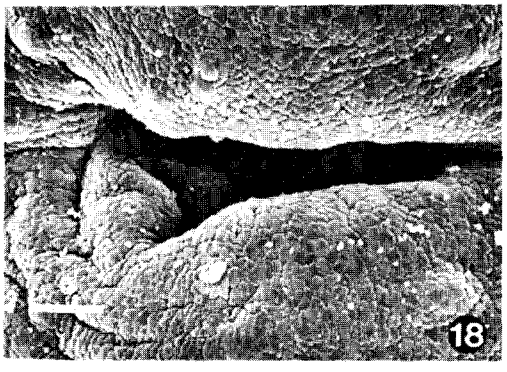
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