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Surface ultrastructure of metacercaria and adult of *Gymnophalloides seoi* (Digenea: Gymnophallidae)

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Abstract: The surface ultrastructure of metacercariae and adults of *Gymnophalloides seoi*, the only known gymnophallid infecting humans, was observed by scanning electron microscopy. Metacercariae were ovoid or pyriform in shape and slightly concave ventrally. The oral sucker had two sizes of type I papillae, small and large, encircling its lip. Type I papillae were arranged in a row on both side of the body. The ventral pit had several type I papillae on its inner surface. The ventral sucker was covered with cobble-stone like cytoplasmic processes and had 6 type I papillae on its lip. The surface of the body was covered with the tegumental spines except for the ventromedian area between the two suckers. The spines at anterior body were digitated into 3-5 points, and their size decreased at posterior one third of the body. Adult worms were rhomboid or ellipsoid in shape and covered with tegumental spines except for the ventromedian area. The shape and distribution of the tegumental spines and sensory papillae were similar to those of metacercariae. However, sensory papillae arranged in a row on the ventral surface of metacercariae were not observed in adults. The ventral pit became larger and more prominent as the fluke grew. It is suggested that the ventral pit function as an additional adhesive organ to the host tissue.

Key words: *Gymnophalloides seoi*, surface ultrastructure, scanning electron microscopy, ventral pit, sensory papillae, tegumental spines, cytoplasmic process

INTRODUCTION

Gymnophalloides seoi, belonging to the family Gymnophallidae, was reported as a human intestinal trematode (Lee *et al.*, 1993), whereas other gymnophallids are generally known as parasites of shore birds such as sea gulls or oyster catchers (Yamaguti, 1971; Schell, 1985). *G. seoi* was first described from a Korean woman patient complaining of epigastric discomfort, indigestion and diarrhea, who enjoyed eating raw oysters and marine clams for many years. A small village of southwestern island of Korea was discovered to be highly endemic, where half of the inhabitants were heavily infected with *G. seoi* (Lee *et al.*, 1994). The oyster, *Crassostrea gigas*, thriving in seashore of the endemic area has been confirmed to be the second intermediate host of *G. seoi* as well as the major source of human infection (Lee *et al.*, 1995).

The ovary and testis of *G. seoi* metacercariae develop to nearly full growth. Therefore, the morphological differences of adults from metacercariae are the body size and presence of intra-uterine eggs. However, the surface

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ultrastructure may be different according to the development in the host, migratory or non-migratory. And these two stages are found in hosts of different physiology, metacercariae in the oyster and adults in mammals or possibly birds. Observation on the surface ultrastructure of the metacercaria and adult of *G. seoi*, therefore, is expected to provide some important morphological informations. Among them, ultrastructure of the ventral pit, a key structure for species differentiation of the family Gymnophallidae (Ching, 1972), could explain its possible function in the host.

MATERIALS AND METHODS

The metacercariae of *G. seoi* were isolated from the oyster, *C. gigas*, collected from the endemic area on Aphae Island, Chollanam-do, Korea (Lee *et al.*, 1995). After opening the shell, oysters were removed and digested in artificial gastric juice at 37°C for 5 minutes and the metacercariae were collected under a stereomicroscope. In order to obtain adult worms, C3H mice were orally infected with 100 metacercariae through gavage needle. Mice were killed by cervical dislocation 3 days after infection and adult worms were recovered from the intestinal contents.

All flukes were washed several times with 0.2 M cacodylate buffer (pH 7.26) and fixed in 2.5% glutaraldehyde at 4°C. After washing with the buffer, they were dehydrated through a graded series of ethanol (60%, 70%, 80%, 90%, 95% and absolute alcohol), dried in a critical point dryer and mounted on aluminum stubs. They were coated with gold by using an ion sputtering coater (Eiko IB-3) and observed with a scanning electron microscope (ISI DS-130C) at an accelerating voltage of 10 kV.

RESULTS

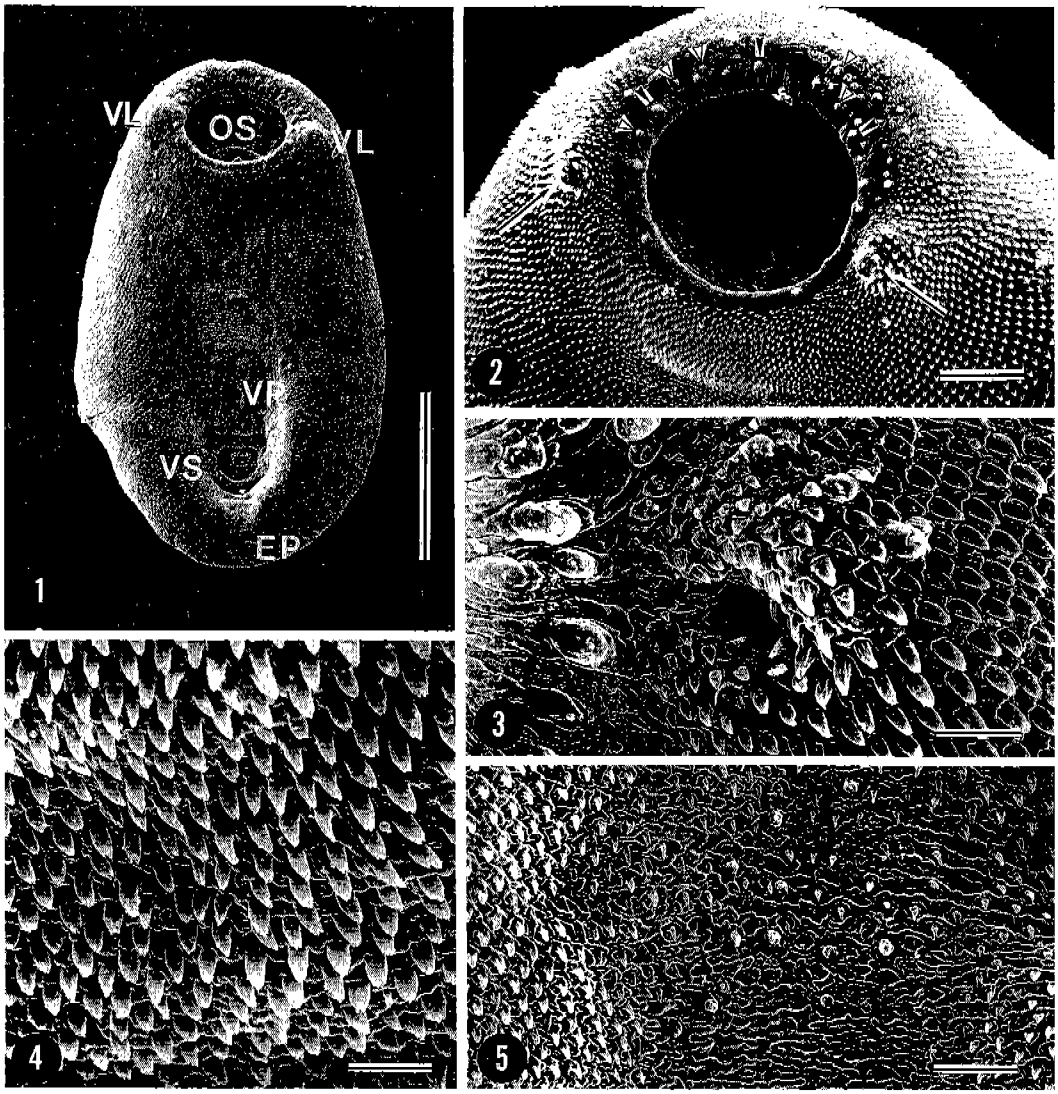
1. Metacercaria

Metacercariae were ovoid or pyriform, concave ventrally, and not encysted (Fig. 1). The oral sucker was well developed to be 2-3 times larger than the ventral sucker. The ventral sucker was posterior to the ventral pit locating at the level of the posterior two-thirds of the body. The lip of the oral sucker was devoid of tegumental spines (Fig. 2). The lateral projections, ventrolateral lips, were found on both side of the oral sucker (Fig. 3). They were elevated-dome shape, sometimes retracted and covered with 3-5 digitated tegumental spines (Figs. 2 & 3). The surface posterior to the oral sucker was densely armed with 3-5 digitated tegumental spines (Fig. 4), and no tegumental spines were observed on a median area between the oral and ventral suckers (Fig. 5). The lumen of the ventral pit was circular or transverse slit shape and its surface was not covered with tegumental spines (Fig. 6). The genital pore was hardly noticeable because it opened directly toward posterior direction. The ventral sucker was covered with cobble-stone like cytoplasmic processes, and tegumental spines around the sucker were digitated into 3-4 points (Figs. 6 & 7). The excretory pore was subterminal of the body and covered sparsely with smaller tegumental spines than those of anterior body (Fig. 8). The dorsal surface of metacercariae was covered with tongue-shaped tegumental spines with 4-5 points at the anterior (Fig. 9) and poorly developed spines at the posterior portion (Fig. 10).

The uniciliated sensory papillae (type I) were bilaterally arranged in a row throughout the entire length of a metacercaria (Fig. 1). The lip of the oral sucker had two sizes of type I papillae, small and large ones, arranging circularly around the sucker (Fig. 2). Type I large papillae formed a circle along the lip of the oral sucker, while small ones distributed mainly at dorsal side of the oral sucker. Sensory papillae were observed more frequently at dorsal side than at ventral side of the oral sucker (Fig. 2). Single type I papilla was also seen bilaterally on the inner wall of the oral sucker. Many type I papillae were distributed around and on the ventrolateral lip (Fig. 3). Type I papillae were irregularly scattered within transverse ridges between the two suckers (Fig. 5). They were also aggregated anteriorly and posteriorly to the ventral pit (Fig. 6). The ventral sucker had 6 type I papillae on its lip (Figs. 6 & 7). Type I papillae were scattered singly on the dorsal surface.

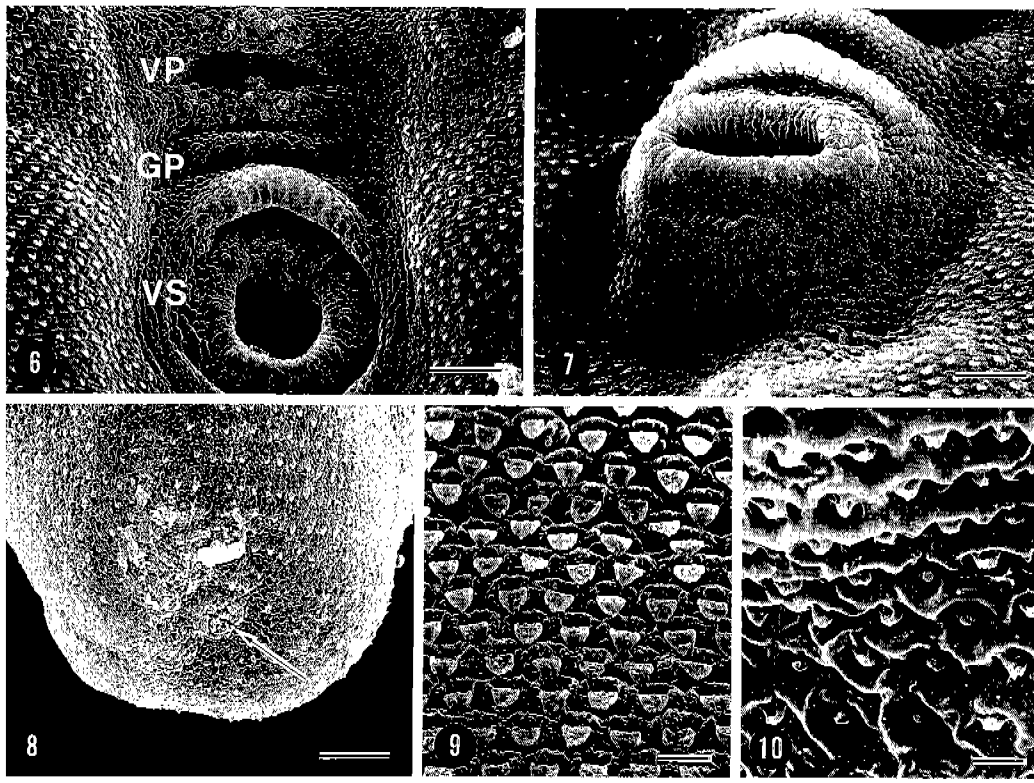
2. Adult worm

Adult worms were rhomboid or ellipsoid in



Figs. 1-5. Scanning electron micrographs (SEM) of anterior half of *G. seoi* metacercaria.
Fig. 1. Whole ventral view. EP, excretory pore; OS, oral sucker; VL, ventrolateral lip; VP, ventral pit; VS, ventral sucker. Bar = 61.9 μm **Fig. 2.** Oral sucker showing two sizes of type I papillae, small (open arrowheads) and large ones (closed arrowheads), and ventrolateral lips (arrows). Bar = 10.0 μm. **Fig. 3.** Magnification of the ventrolateral lip, showing 3-5 digitated tegumental spines and type I papillae. Bar = 2.4 μm. **Fig. 4.** Tegumental spines below the level of the oral sucker. Bar = 2.0 μm. **Fig. 5.** Tegument between the oral sucker and the ventral pit, showing median area devoid of spines and scattered type I papillae. Bar = 6.1 μm.

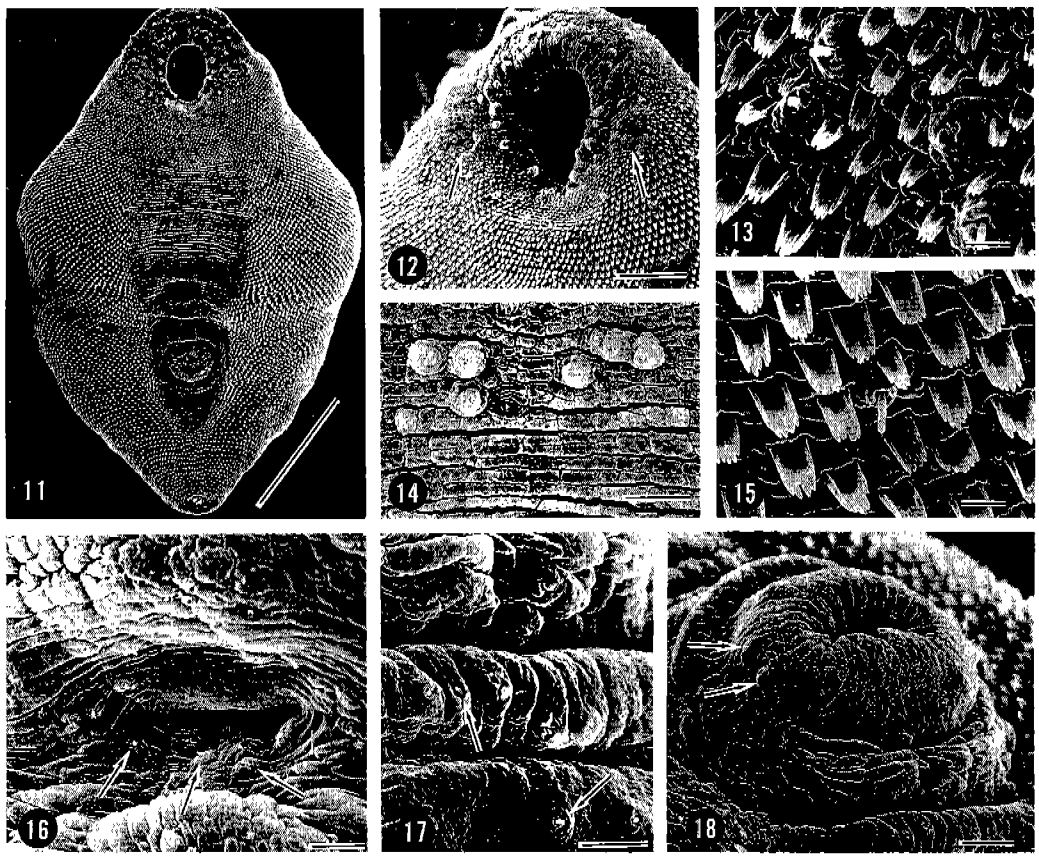
shape and covered with tegumental spines except for the ventromedian area between the two suckers (Fig. 11). The ventral pit was median at posterior level of middle one-third of the body. Transverse wrinklings were pronounced on depressed mid-median area of ventral surface. The oral sucker had a radially wrinkled lip without tegumental spines (Fig. 12). Around the oral sucker, tegumental spines were digitated into 5-6 points, the cytoplasmic processess were folded irregularly (Fig. 13). The surface anterior to the ventral pit was devoid of tegumental spines and the cytoplasmic folds were transversely wrinkled



Figs. 6-10. SEM of posterior half of *G. seoi* metacercaria. **Fig. 6.** Postero-ventral surface showing the ventral pit (VP), genital pore (GP) and ventral sucker (VS). Bar = 7.4 μm. **Fig. 7.** Ventral sucker protruded. Bar = 6.3 μm. **Fig. 8.** Terminal portion of the body showing the excretory pore (arrow). Bar = 5.3 μm. **Fig. 9.** Tongue-shaped tegumental spines on antero-dorsal surface. Bar = 1.3 μm. **Fig. 10.** Poorly digitated tegumental spines on postero-dorsal surface. Bar = 1.2 μm.

(Fig. 14). Tegumental spines on the lateral surface to the ventral pit were digitated into 6-7 points, which were larger than those around the oral sucker. Transverse cytoplasmic folds were well developed and single type I papilla was observed between the spines (Fig. 15). The ventral pit was more prominent than that of metacercariae and the lumen elliptical transversely (Fig. 16). It was covered with cobble-stone like cytoplasmic processes without tegumental spines. The surface of the ventral sucker was not covered with tegumental spines and cobble-stone like cytoplasmic processes surrounded the ventral sucker (Fig. 18). The lip of the ventral sucker was radially wrinkled and the lumen was very small compared to that of the oral sucker. Tegumental spines were digitated into 5-7 points at middle and posterior one-third of the body and the excretory pore was subterminal. Tegumental spines of the dorsal surface had 8-10 points in the anterior (Fig. 20), and 7-8 points in posterior of the body (Figs. 21 & 22). The size of spines was biggest in the middle third and smallest in the posterior third of the body. The spine density decreased posteriorly.

Two sizes of type I papillae encircled the lip of the oral sucker as seen in the metacercaria (Fig. 12). The ventrolateral lip was slightly retracted and had many type I papillae on its base. Type I papillae were embedded between the tegumental spines (Figs. 13 & 15). Two grouped type I papillae, each comprised of 5-6 papillae, were located anterior to the ventral pit (Fig. 14). Twelve to fifteen type I papillae were distributed around the border of the ventral pit (Fig. 16). On transverse cytoplasmic wrinklings between the ventral pit and ventral sucker were many type I papillae embedding in cytoplasmic ridges (Fig. 17). Type I papillae

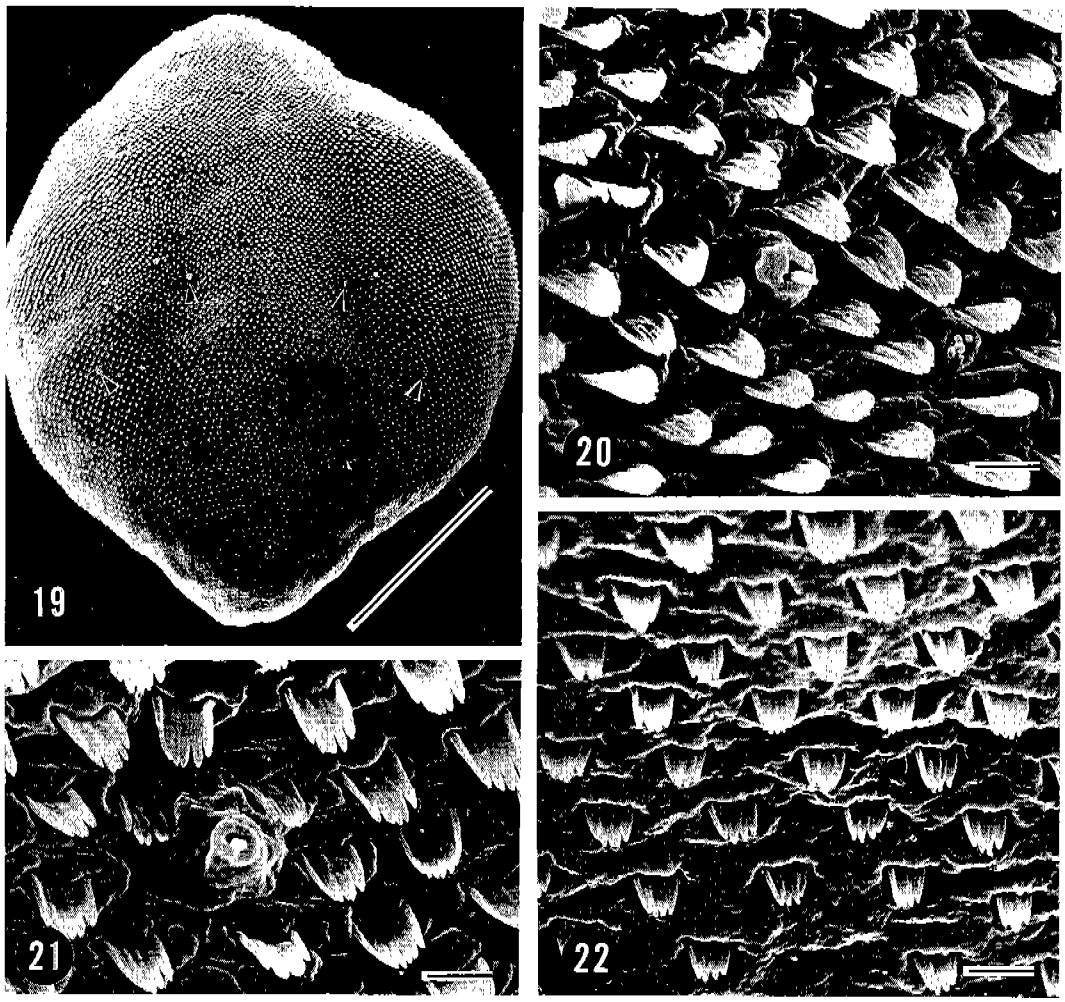


Figs. 11-18. SEM on ventral surface of a 3-day old worm. **Fig. 11.** Whole ventral view. Bar = 60.0 μm. **Fig. 12.** Oral sucker and ventrolateral lips (arrows). Many type I papillae, small and large ones, encirled the lip of oral sucker. Bar = 10.0 μm. **Fig. 13.** Tegumental spines around the oral sucker. Bar = 1.0 μm. **Fig. 14.** Surface between the oral sucker and the ventral pit showing two grouped type I papillae. Bar = 5.0 μm . **Fig. 15.** Tegumental spines at the level of the ventral pit. Bar = 1.0 μm. **Fig. 16.** Type I papillae (arrows) on inner surface of the ventral pit. Bar = 3.0 μm. **Fig. 17.** Transverse cytoplasmic wrinklings between the ventral pit and ventral sucker showing type I papillae (arrows). Bar = 10.0 μm. **Fig. 18.** Ventral sucker with type I papillae (arrows). Bar = 5.0 μm.

were distributed along the margin of the ventral sucker (Fig. 18). Many type I papillae were scattered singly all over the dorsal surface (Fig. 19).

DISCUSSION

Metacercariae and adults of *G. seoi* had similar arrangements of sensory papillae with minor difference in the linear and longitudinal arrangement on both side of the metacercaria. It is a very peculiar finding that only one kind of sensory papillae, uniciliated sensory papillae (type I), was observed on the surface of *G. seoi*, whereas most of trematodes had at least two types of sensory papillae (Bennett and Threadgold, 1975; Fujino *et al.*, 1979; Seo *et al.*, 1984). The function of type I papillae has been suggested to be tango-, rheo-, or mechanoreceptive (Hong *et al.*, 1991; Yu *et al.*, 1994). However, they were also suggested to be either chemo- or mechanoreceptive because the cilium extended through the thickness of the tegument and thus was able to respond to a variety of chemical changes in the environment (Ip and Desser, 1984). *G. seoi* metacercariae were found to be tightly sucking the epithelium and tissue of the oyster (Lee *et al.*, 1995). It is usually difficult to detach the metacercariae attaching to the bottom of the



Figs. 19-22. SEM on dorsal surface of a 3-day old worm. **Fig. 19.** Whole view showing type I papillae (arrowheads) scattered singly. Bar = 50.0 μm. **Fig. 20.** Tegumental spines with 8-10 points at anterior one-third of the body. Bar = 1.0 μm. **Fig. 21.** Tegumental spines with 6-7 points at mid-portion of the body. Bar = 1.0 μm. **Fig. 22.** Tegumental spines at posterior third of the body. Bar = 1.0 μm.

petri dish during collection of the metacercaria. Therefore, sensory papillae on the oral sucker of *G. seoi* might be developed to augment the attachment and to facilitate parasite responses to various chemical stimuli of its environment. However, it is not clear from the SEM findings how much difference in the function could be achieved from the size difference of type I papillae on the oral sucker of *G. seoi*.

Contrary to the findings of *G. seoi*, *Parvatrema timondavidi*, a member of the family Gymnophallidae, had three types of papillae: type I, type II and type III (Yu *et al.*, 1994). Many type I and type II papillae were observed around the lip of the oral sucker of *P. timondavidi*. Type III papillae, round elevations of cytoplasmic ridges with a pit in the center, were located symmetrically on the medial side of the lateral projection (ventrolateral lip) of metacercariae, and disappeared in adult worms indicating its stage-specific action during the development. The genital pore of *P. timondavidi* was equipped with grouped type I papillae, while those of *Parvatrema affinis* and *Lacunovermis macomae* had type II sensory papillae (Pekkarinen, 1984 & 1987). The genital pore of *G. seoi* is very small and opens

anterior to the ventral sucker, therefore, it is difficult to observe on routine microscopic preparations. On SEM observation, no papillae could be found around the genital pore of *G. seoi*.

It is well known that the tegumental spines have various shapes and distributions by the species of parasites. Furthermore, they are usually different in the same species of parasite according to the migratory behavior, route, developmental stages and final habitat in the host (Bennett and Threadgold, 1975; Hong *et al.*, 1991). They are more simply pointed on the surface of juveniles than adults and in migratory stages than non-migratory ones. *G. seoi* were covered with thin, pointed tegumental spines in metacercariae and broad, more pointed ones in adult worms. Therefore, the spines on metacercariae may function during their migration to their habitat, while those on adults support anchorage of the body, and enable to keep themselves from being expelled by peristaltic movement of the intestine, and facilitate absorption of nutrients by providing space between intestinal villi and parasites. Cercariae of *L. macomae* have the pointed spines, which are important for their penetration and migration in the clam tissues, whereas metacercariae have the broadened spines as they are often enclosed within membraneous cysts in marine clams (Pekkarinen, 1986).

The ventral pit, a characteristic structure of the genus *Gymnophalloides* and *Lacunovermis*, is a transverse slit without connection to internal organs (Ching, 1972). The lumen of the ventral pit of *G. seoi* varied from circular to transverse or flask shape depending on developmental stages and activities of the worm during the SEM preparation. The ventral pit is generally more prominent than the genital pore and its lumen was equipped with many type I papillae. However, the ventral pit of *Lacunovermis* recovered experimentally from a chick allantoic membrane was sometimes less pronounced than the genital pore and single sensory papilla could be found on both side of the pit (Pekkarinen and Ching, 1994). These differences accord with findings of microscopic observation and can be useful keys for differentiation of the two genera.

Up to present, the function of ventral pit was not known clearly. Ching (1972) described the ventral pit of *G. tokiensis*, a type species of the genus *Gymnophalloides*, was surrounded by strong muscle fibers. The ventral pit of *G. seoi* was observed to be a well-developed muscular structure like a sucker and many papillae were distributed around its lumen. In experimentally obtained adults of *Lacunovermis* sp., at least two sensory papillae were present on each side of the ventral pit (Pekkarinen and Ching, 1994). Transmission electron microscopic observation (TEM) of the ventral pit of *G. seoi* showed circular and longitudinal muscle bundles along the basal layer and several mitochondria at the margin of the longitudinal muscles (Seo *et al.*, 1995). From the findings of both SEM and TEM, it is suggested that the ventral pit of *G. seoi* assist its attachment to the host tissue.

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=초록=

참굴큰입흡충의 피낭유충 및 성충의 표피 미세구조

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참굴큰입흡충(*Gymnophalloides seoi*)의 피낭유충 및 성충의 표피 미세구조를 주사전자현미경으로 관찰하였다. 피낭유충은 큰 구흡반과 측면돌기를 가지고 있었으며, 크기가 다른 두 종류의 제1형 감각유두가 구흡반 구순을 따라 원을 이루며 분포하고 있었다. 또, 제1형 감각유두가 유충의 복측 양쪽으로 일렬로 길게 배열되어 있었다. 복흡반의 전방에 위치한 ventral pit는 근육성으로 잘 발달하였고 제1형 감각유두가 밀집해 있었다. 복흡반의 구순에는 제1형 감각유두가 분포하였다. 피극은 구흡반과 복흡반 사이의 복측 중앙부를 제외한 전 표피에 분포하였으며 구흡반 주위에는 3-5분지된 피극이 덮혀 있었으나 후방으로 갈수록 피극의 크기와 밀도가 감소하였다. 감염 3일된 성충은 양 말단이 뾰족한 마름모 모양이었으며 감각유두의 분포는 피낭유충에서 관찰되는 양상과 비슷하였으나 피낭유충에서 관찰된 일렬로 배열된 감각유두는 관찰되지 않았다. 피극은 피낭유충의 경우보다 크기가 더 커지고 많이 분지되었다. Ventral pit는 성충이 되면서 현저하게 발달하였으며 감각유두의 수도 증가하였다. 참굴큰입흡충의 표피 미세구조는 나경흡충과(裸莖吸蟲科)의 충체들과 차이점이 있었으며, 피낭유충과 성충의 미세구조의 차이가 뚜렷하지 않은 점은 광학현미경에서 관찰된 양상과 비슷하였다. 또, ventral pit의 모양과 감각유두의 분포로 보아 충체가 기생부위에 흡착할 때 역할을 할 것으로 생각되나 주사전자현미경 소견만으로는 정확한 기능을 추측하기 어려웠다.

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