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TEM ultrastructure of the tegumental layer of *Gymnophalloides seoi* (Digenea: Gymnophallidae)

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Abstract: A transmission electron microscopic study was performed on the ultrastructure of the tegumental layer of *Gymnophalloides seoi* (Digenea: Gymnophallidae) metacercariae and adults. The metacercariae were obtained from naturally infected oysters, *Crassostrea gigas*, and the adults from experimentally infected C3H mice. The tegumental layer generally revealed a small number of foldings, numerous small vacuoles, sines, and muscle bundles. Beneath the muscle layer, nuclei of the tegumental cells were located. There was little difference in the structure of the tegument between the metacercariae and adults. The oral sucker, having well-developed muscle layers, showed a similar structure to the ventral sucker except numerous foldings in the ventral sucker. The ventral pit was surrounded by a thin syncytial layer, where a number of microtubules and mitochondria were seen. Around the ventral pit located well-developed circular and longitudinal muscles. The results showed that the ultrastructure of the tegumental layer of *G. seoi* metacercariae and adults revealed little difference from other trematodes in general. The ventral pit, a peculiar structure of this trematode, seems to function as a sphincter or an accessory adhesive organ.

Key words: *Gymnophalloides seoi*, TEM, ultrastructure, tegumental layer, suckers, ventral pit

INTRODUCTION

Species belonging to the family Gymnophallidae have been reported as parasites in the intestine, gallbladder, or bursa of Fabricii of shorebirds (Yamaguti, 1971; Schell, 1985). In Korea, a new species belonging to this family, *Gymnophalloides seoi* Lee, Chai and Hong, 1993, was recently reported from a 66-year old patient who suffered from acute pancreatitis (Lee *et al.*, 1993). Thereafter, a highly endemic area was discovered on a southwestern coastal island of Shinan-gun, Chollanam-do (Lee *et al.*, 1994). Subsequently the oysters, *Crassostrea gigas*, naturally produced from this area were proved to be the source of infection (Lee *et al.*, 1995a). Relationship between *G. seoi* infection and diabetes mellitus was suggested (Lee *et al.*, 1995b), and medical attention is being paid on this trematode.

After the advent of the transmission electron microscope (TEM), the observation of digenean ultrastructure became possible, and there emerged the concept that the digenean tegument is a metabolically active component (Lumsden, 1975). In Korea, at least 15 species of digeneans have been recognized as human infections (Chai and Lee, 1990), and studies

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about the ultrastructure of these trematodes have been performed. However, there has been no report on TEM ultrastructure of *G. seoi*. It has a characteristic organ, the centrally located ventral pit (Lee *et al.*, 1993), and it serves as an important taxonomic key to differentiate the family Gymnophallidae (Ching, 1973). However, its function has not yet been defined. The present study was performed to observe the basic ultrastructure of the tegumental layer (tegument in general, around suckers and ventral pit) of the metacercariae and adults of *G. seoi*, with special consideration on the function of the ventral pit.

MATERIALS AND METHODS

1. Collection of metacercariae and adults of *G. seoi*

Oysters were caught from the slit at an estuary of Aphae Island, Shinan-gun, and the metacercariae of *G. seoi* were collected by artificial digestion of the oysters. The metacercariae isolated were washed three times with cold physiological saline followed by another wash with 0.1 M cacodylate buffer (pH 7.4), and fixed in 2% paraformaldehyde and 2.5% glutaraldehyde (0.1 M cacodylate buffer, pH 7.4) at 4°C for 12 hours. Metacercariae were fed to C3H mice, and adult flukes were harvested 7 days after the infection (Lee *et al.*, 1995a). They were washed in physiological saline and fixed as in the case of metacercariae.

2. TEM observation

The specimens were post-fixed in 1% osmium tetroxide for two hours. The fixed worms were dehydrated in graded series of ethanol and embedded in epon. Semithin sections of 1 μm thickness were stained with toluidine blue, and ultrathin sections of 80 nm thickness were prepared and stained with uranyl acetate and lead citrate. The stained samples were observed with TEM (1200EX-II, JEOL, Japan) at 80 KV.

RESULTS

1. Metacercariae

Tegument in general; The external surface of the metacercariae was enclosed by a syncytial layer. The matrix of the syncytial layer forming the external surface was composed of electron-dense materials. In the matrix, there were numerous small vacuoles, mitochondria, and tegumental spines (Figs. 1 & 2). The thickness of the syncytial layer was in the range of 0.6-1.2 μm. The outer surface of the tegument was even with few foldings, and located several tegumental spines at an equal space interval (Fig. 1). Beneath the internal plasma membrane, an electron-lucent basement membrane was located, the breadth of which was approximately 100 nm. Under the basement membrane located circular and longitudinal muscle bundles, and tegumental cells which had a plenty of lysosomes were present among them (Fig. 2). The average size of the tegumental spines was 8 μm in length and 5 μm in width, and the intervals between them were from 4 to 7 μm (Figs. 3 & 4).

Around oral and ventral suckers; The oral sucker of the metacercariae was circular in shape and composed of the syncytial layer, basement membrane, and muscle layer. The syncytial layer was 0.8-0.9 μm in thickness, and had a lot of vacuoles and small numbers of mitochondria. The thickness of the syncytial layer and basement membrane of the oral sucker was slightly thinner than that of the other tegument in general (Fig. 6). Among the granules several electron-dense granules were present. The granules were larger than the vacuoles. Beneath the basement membrane located bundles of circular muscles. Beneath them, well developed longitudinal muscles were present (Figs. 7 & 8). Spindle-shaped muscle cell nuclei were distributed parallel to the muscle fibers. Between the muscle layers, a tegumental cell having an electron-dense nucleus was seen (Fig. 8).

In the case of the ventral sucker, its ultrastructure was very similar to that of the oral sucker, but numerous syncytial processes and foldings of the syncytial layer were observed in the ventral sucker (Fig. 11). The parenchymal cell with an electron-dense nucleus was seen among the circular muscle bundles (Fig. 12).

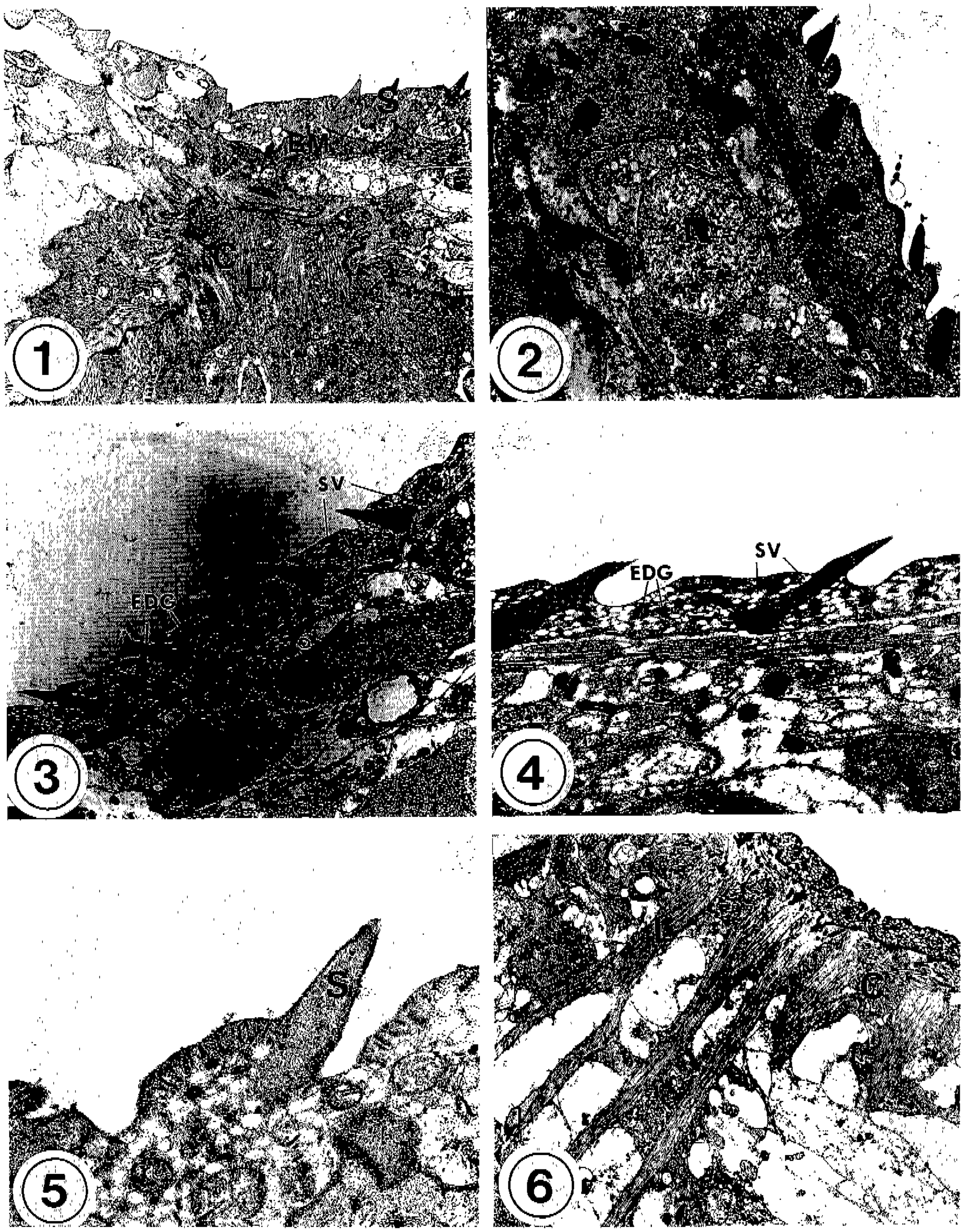

Fig. 1. The tegumental layer of a *G. seoi* metacercaria showing the spines (S), basement membrane (BM), circular (C) and longitudinal (L) muscle bundles. × 5,000. **Fig. 2.** The tegument of another metacercaria. The nucleus (N) of a tegumental cell is seen. × 6,000. **Fig. 3.** The tegument of an adult worm. Numerous small vacuoles (SV) and electron-dense granules (EDG) are seen in the syncytial layer. × 5,000. **Fig. 4.** Magnification of the tegumental layer of an adult worm. SV (small vacuole), EDG (electron-dense granules). × 10,000. **Fig. 5.** A tegumental spine (S) on the tegument. It is surrounded by the extension of external plasma membrane of the tegument. × 25,000. **Fig. 6.** The oral sucker of a metacercaria. A number of small vacuoles and well-developed circular (C) and longitudinal (L) muscle bundles are seen. × 5,000.

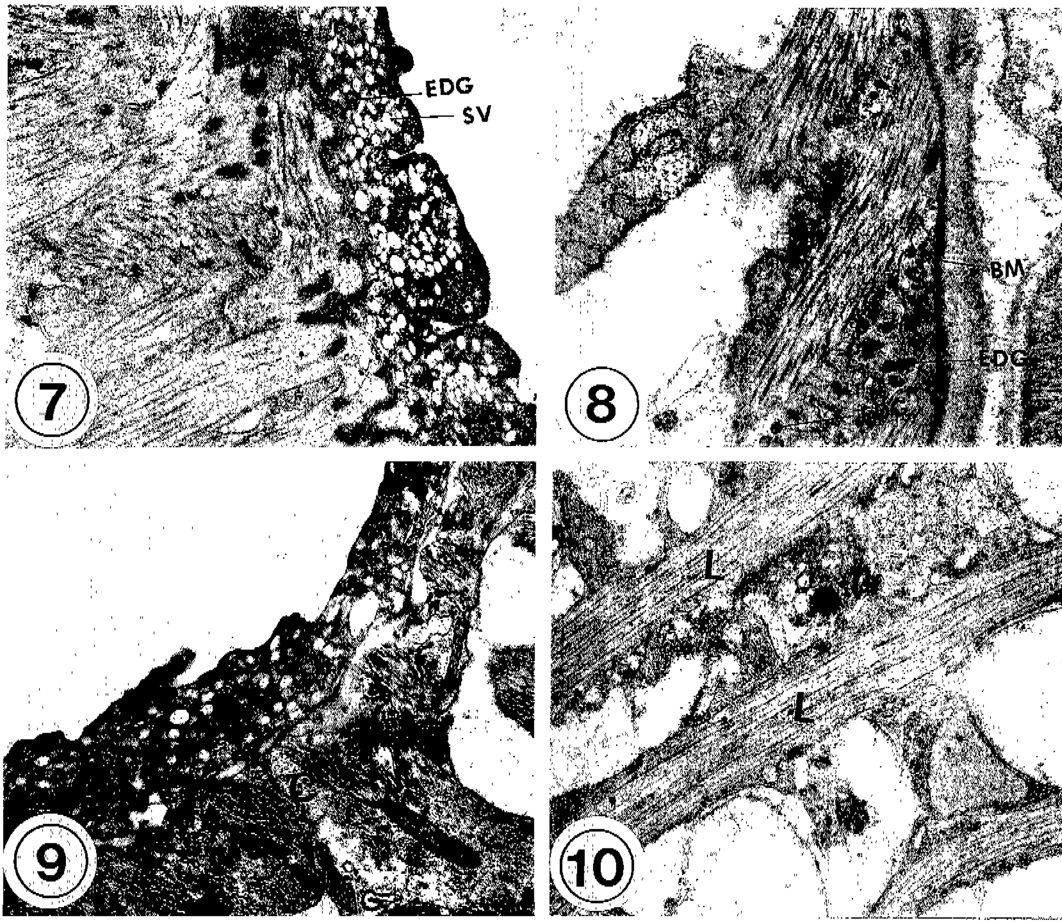

Fig. 7. The oral sucker of a *G. seoi* metacercaria. In the tegumental layer, numerous small vacuoles (SV) and electron-dense granules (EDG) are seen. × 15,000. **Fig. 8.** Deeper part of the oral sucker of metacercaria. Below the basement membrane (BM), several electron-dense granules (EDG) are seen. × 15,000. **Fig. 9.** The oral sucker of an adult worm. Circular (C) and longitudinal (L) muscles are seen. × 12,000. **Fig. 10.** Deeper part of the oral sucker of an adult worm. Well-developed longitudinal muscle bundles (L) are seen. × 12,000.

2. Adults

Tegument in general; In the case of the adults, the tegumental spines were a little longer than those of the metacercariae, and intervals between the spines were more uniform. However, no other structural difference was observed (Fig. 4). The spine was also covered with the syncytial layer, and embedded in the layer (Fig. 5).

Around the oral sucker; The compactness of the muscle layer was increased in the oral sucker of the adult worm (Figs. 9 & 10), especially in the case of circular muscles (Fig. 9), but had no remarkable difference from the metacercariae.

The ventral pit; In longitudinal sections, the lumen of the ventral pit was 3 μm wide and 20 μm long. There were some elevations of the syncytial layer toward the lumen. The syncytial layer was very thin, 0.5 μm, the thinnest among the tegumental areas of *G. seoi* (Fig. 13). In the syncytial layer, there were few vacuoles unlike in the tegumental layer. Some glycogen granules and mitochondria were observed, and numerous microtubules were distributed. The basement membrane was relatively thick, and along the basement membrane located the circular and longitudinal muscle bundles. The muscle

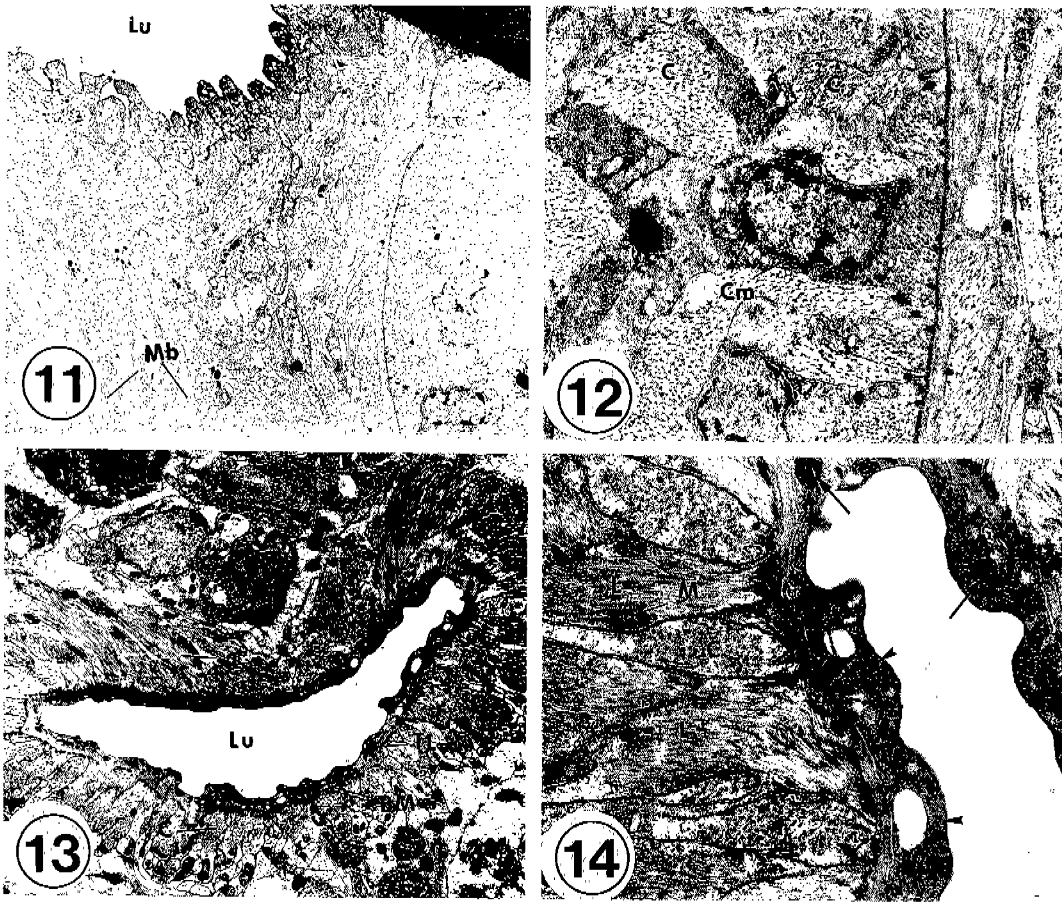

Fig. 11. The ventral sucker of an adult worm. The muscle bundles (Mb) are not apparent in comparison with those of the oral sucker. The round lumen (Lu) side is seen. × 5,000. **Fig. 12.** A tegumental cell of the ventral sucker is seen among the circular muscle bundles (C). A nucleus (N) with dense chromatins (Cm) is seen. × 15,000. **Fig. 13.** The ventral pit of an adult *G. seoi.* Circular (C) and longitudinal (L) muscles are seen. The lumen (Lu) is leaf-like, and beneath the tegumental layer (TL) located a basement membrane (BM). Below it, circular (C) and longitudinal (L) muscles are seen. × 5,000. **Fig. 14.** Magnification of the ventral pit, showing many mitochondria (arrows) and microtubules (arrowheads) in the tegumental layer. In a longitudinal muscle (L) layer, a pair of mitochondria (M) are seen. C: circular muscle. × 20,000.

bundles were well-developed, densely populated, and several mitochondria were observed at the margin of the longitudinal muscles (Fig. 14).

DISCUSSION

According to many previous studies, the spines are usually present on the tegument of digenean trematodes and their functions are believed to help the parasites to attach to the host tissue (Senft *et al.*, 1961; Lumsden, 1975). In the present study, the number of spines of *G. seoi* was increased in adult worms, but the intervals were more even in metacercariae than in adults. It seems to be a consequence of the development for an easier attachment. Except that, few morphological difference was noticed between the tegument of the adults and metacercariae. *G. seoi* had a smaller number of foldings and thinner syncytial layer in comparison with other parasites.

In *G. seoi* metacercariae and adults, there were a number of small vacuoles in the syncytial layer. Its function is not known yet, but it must participate in the absorption and secretion of some materials. The digenean tegument plays a vital role for survival of the parasites doing important functions such as absorption of nutrients, secretion or discharge of waste products (Lumsden, 1975). The tegument of aspidogastrids became long microtubules as it adapted to the host (Rohde and Watson, 1992). Similarly, in the process of adaptation there occurred the processes of the basal lamina protruding into the tegument and cross-striated processes of the basal lamina protruding into the underlying fibrous matrix (Rohde and Watson, 1992). This resulted from the fact that the aspidogastrids lived in the narrow ducts of the rectal glands of fish, and that they should firmly attach to it (Rohde and Watson, 1992). *G. seoi* had short processes in comparison to other trematodes, suggesting that the attachment of *G. seoi* to host tissue might not be so firm, instead the sucking power of its oral sucker should be very strong so as to maintain its habitat.

In addition, the morphology and distribution of the tegumental spines vary according to diverse factors including the habitat of parasites, their migratory behavior in the host, and the degree of developmental status (Lumsden, 1975). In the case of parasites migrating through the host tissue such as *Paragonimus westermani* or *Fasciola hepatica*, their spines were transformed into a different form which is suitable for migration. Such spines are more subdivided as they reach the normal habitat (Bennett, 1975). Although lacking a migration phase, *C. sinensis* which enter the bile duct through the ampulla of Vater retain spines during their larval stage, and lose the spines when they become adults (Fujino *et al.*, 1979). The digenean tegument secrets several kinds of enzymes such as phosphatase, nonspecific esterase or aminopeptidase (Erasmus and Ohman, 1963 & 1965). The granules observed from the sucker may help *G. seoi* to digest the host cells by secreting enzymes or other chemical materials.

The oral sucker of *G. seoi* was well-developed, and surrounded by big muscle bundles. This seems to enable *G. seoi* to adsorb to the villi of the host intestine strongly. The oral sucker of the metacercariae was also surrounded by the syncytial layer and a thin basement membrane. In the syncytial layer, numerous vacuoles and mitochondria were present, and several glycogen granules were observed. The syncytial layer had few foldings, and had well-developed muscle layer below it. The ventral sucker had more foldings in the syncytial layer than the oral sucker, but the developmental status of the muscle layer was poor. It might function not just as an adhesive organ but as a supporting one.

The ventral pit, a characteristic structure of *G. seoi*, appeared as a slit-like structure. It was also encircled by a thin syncytial layer. But the syncytial layer of the ventral pit had a different structure in comparison with that of the tegument or suckers. Instead of numerous granules and foldings in the tegumental layer or suckers, many mitochondria and micro-tubules were distributed throughout the syncytial layer of the ventral pit. A number of big circular and longitudinal muscle bundles were distributed below the syncytial layer, and the muscle bundles had mitochondria. In this respect, the ventral pit might function as a sphincter or an accessory adhesive organ. But TEM observations have limitations to know the function of an organ or a structure. Therefore, further studies should be performed to understand correctly the function of the ventral pit.

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=초록=

참굴큰입흡충 표피층의 투과전자현미경적 미세구조

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참굴큰입흡충(*Gymnophalloides seoi*) 피낭유충 및 성충의 표피층(일반 표피, 흡반 부위 및 ventral pit)의 미세구조를 투과전자현미경으로 관찰하였다. 피낭유충은 자연감염된 굴에서 분리하였고, 성충은 실험감염시킨 C3H 마우스에서 획득하였다. 일반적으로 표피층은 합포체의 융기 및 굴곡이 심하지 않았고, 다수의 미토콘드리아와 수많은 작은 공포들이 관찰되었으며, 근육층 아래에는 표피세포의 핵이 위치하고 있었다. 피낭유충과 성충에서 표피층의 구조적인 차이는 발견되지 않았다. 구흡반에는 잘 발달된 근육층이 분포하였고, 복흡반은 구흡반과 비슷한 구조였으나 합포체의 fold 수가 구흡반보다 많았다. Ventral pit은 합포체층의 두께가 얇았으며, 많은 수의 microtubule과 미토콘드리아가 관찰되었다. 주위에는 종주근과 윤주근 다발들이 잘 발달되어 있었다. 이상의 결과로 보아 참굴큰입흡충 표피층의 미세구조는 피낭유충과 성충 사이에 별다른 차이가 없었으며 흡충류의 일반적인 구조와 대체로 일치하였다. 이 흡충의 특징적 구조물인 ventral pit은 괄약근 또는 보조 흡착기관으로서의 역할을 수행할 것으로 생각하였다.

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