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Surface ultrastructure of *Metagonimus miyatai* metacercariae and adults

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Abstract: A scanning electron microscopic study was performed to observe surface ultrastructures of excysted metacercariae and adults of *Metagonimus miyatai*. Metacercariae were collected from the scale of the pale chub (*Zacco platypus*), and adult flukes were harvested 1-4 weeks after infection to rats. In excysted metacercariae, the oral sucker was devoid of tegumental spines and had type I and type II sensory papillae. Anteriorly to the ventral sucker, spines were dense and digitated into 5-7 points, whereas near the posterior end of the body spines were sparse and digitated into 2-3 points. In one-week adults, 7 type II sensory papillae were arranged around the lip of the oral sucker, and at inner side of the lip one pair of small and two pairs of large type I sensory papillae were seen on each side. The distribution of tegumental spines was similar to that of metacercariae, but they were more differentiated with 9-11 pointed tips. In two- to four-week old adults, the surface ultrastructure was nearly the same as in one-week old adults, however, sperms were frequently seen entering into the Laurer's canal. Conclusively, the surface ultrastructure of *M. miyatai* was generally similar to that of *M. yokogawai*, however, differentiation of tegumental spines and distribution of sensory papillae around the oral sucker were different between the two species, which may be of taxonomic significance.

Key words: *Metagonimus miyatai*, surface ultrastructure, developmental stages, tegumental spines, sensory papillae

INTRODUCTION

After long debates on the species of the genus *Metagonimus* (Takabayashi, 1953; Ito, 1964; Saito, 1984), *Metagonimus miyatai* was reported as a new species (Saito *et al.*, 1997). The validity of *M. miyatai*, however, should be studied further until it is fully recognized. In this connection, a genetic study using polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) analysis revealed that *Metagonimus* Miyata type (= *M. miyatai*), together with *M. takahashii*, are genetically distinct species from the type species, *M. yokogawai* (Yu *et al.*, 1997).

Surface ultrastructure of trematodes has been studied to understand not only their morphological and functional characteristics but also taxonomic significance of each species; *Cryptocotyle lingua* (Køie, 1977),

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Heterophyes aegualis (Taraschewski, 1984), *M. yokogawai* (Lee *et al.*, 1984), *Neodiplostomum seoulense* (Lee *et al.*, 1985), *Heterophyopsis continua* (Hong *et al.*, 1991) and *Heterophyes nocens* (Chai *et al.*, 1992). These studies have revealed that their surface ultrastructures vary by species especially in terms of size, shape, number and distribution of tegumental spines and sensory papillae, and differentiation patterns of cytoplasmic processes according to developmental stages.

Surface ultrastructure of *M. miyatai*, however, has not been reported. The present study was performed to observe the surface ultrastructure of *M. miyatai* metacercariae and adults, and results were compared with those reported for *M. yokogawai* (Lee *et al.*, 1984).

MATERIALS AND METHODS

Metacercariae of *M. miyatai* were collected from the scale of the pale chub (*Zacco platypus*) caught from Umsong-gun, Chungchongbuk-do, by artificial digestion technique. Two hundred metacercariae were fed orally to each of 5 Sprague-Dawley rats. The rats were killed at 1, 2 and 4 weeks post-infection and adult flukes were recovered. Metacercariae were excysted in trypsin solution (Tris buffer, pH 8.0) at 38°C. Excysted metacercariae and adults were washed several times with 0.2 M cacodylate buffer (pH 7.3) and fixed in 2.5% glutaraldehyde solution at 4°C. After washing with phosphate-buffered saline, they were dehydrated through a graded series of ethanol (60%, 70%, 80%, 90%, 95% and 100%), dried in a critical point dryer, and mounted on aluminum stubs. They were coated with gold using an ion sputtering coater (IB-3, Giko Engineering Co., Japan) and observed with a scanning electron microscope (ISI DS-130C, Korea) at an accelerating voltage of 10 kV.

RESULTS

1. Excysted metacercariae

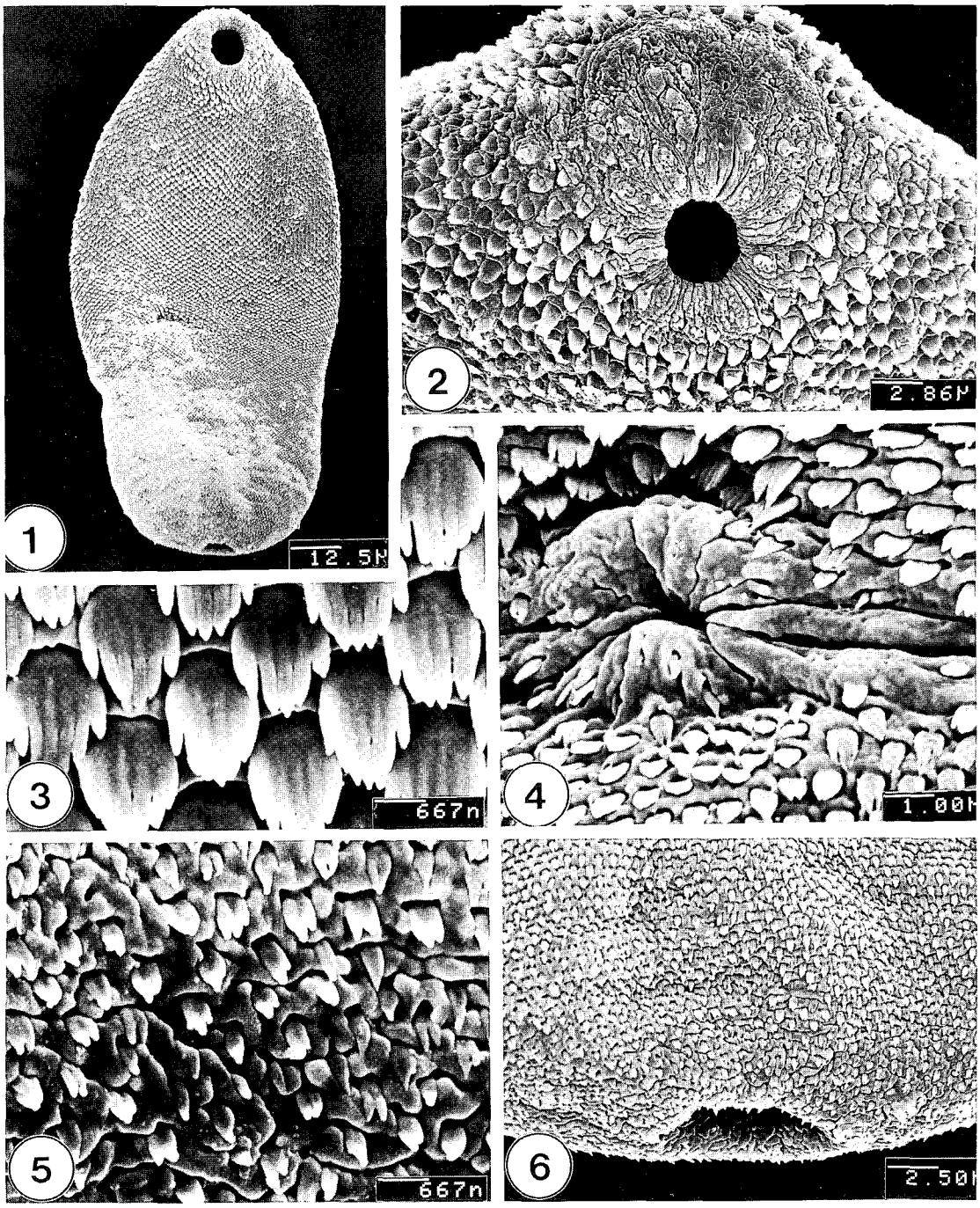
Excysted metacercariae was leaf-like, ventrally concave, ovoid or pyriform in shape (Fig. 1). The oral sucker was big, locating near the anterior end of the body (Fig. 2). The ventral sucker was positioned in the right middle field of the body, facing toward the oral sucker. The whole body surface was covered with flat cytoplasmic processes, numerous tegumental spines (Figs. 1-3) and sensory papillae. The lip of the oral sucker was devoid of spines (Fig. 2) and had single or grouped type I sensory papillae (= ciliated knob-like swellings) and 7 type II sensory papillae (= round swellings of the tegument). Several type I papillae were also seen on the lip of the ventral sucker (Fig. 4). Tegumental spines anterior to the ventral sucker were dense and digitated into 5-7 points (Fig. 3). However, the number of points decreased laterally and posteriorly (Fig. 5). Near the posterior end of the body excretory pore was opened (Fig. 6), spines were sparsely distributed and digitated only into 2-3 points.

The dorsal surface was also densely and regularly covered with tegumental spines. Spines were, however, short, blunt, and 5-6 pointed anteriorly, and 6-8 pointed in the middle portion of the body. Toward the posterior half of the body, spines were converted into single-pointed peg-like structures, and the distance between spines was increased. Type I single or clumped (groups of 2-3) papillae were sparsely distributed.

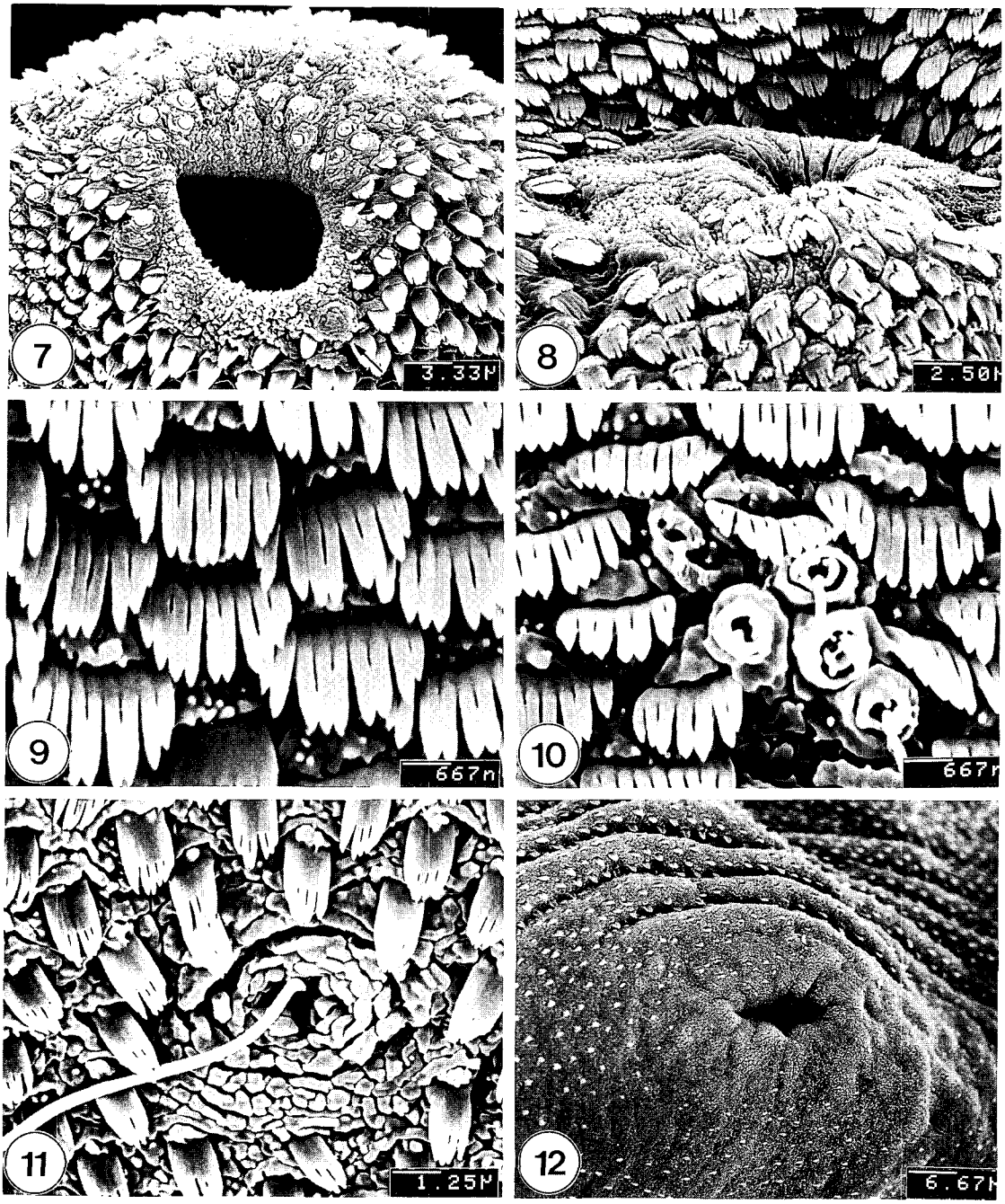
2. Adult flukes

One-week old worms: The body was similar to metacercariae in shape, but became much enlarged, and covered with knob- or cobble-stone-like cytoplasmic processes, and tegumental spines (Figs. 7-12). On the lip of the oral sucker 7 type II sensory papillae were seen (Fig. 7), and at the inner side of the lip one pair of large and two pairs of small type I sensory papillae were seen on each side. The ventral sucker became thicker than in metacercariae, and located at the right side of the anterior body. Around the ventral sucker (Fig. 8), type I papillae, 3-4 in number, were distributed.

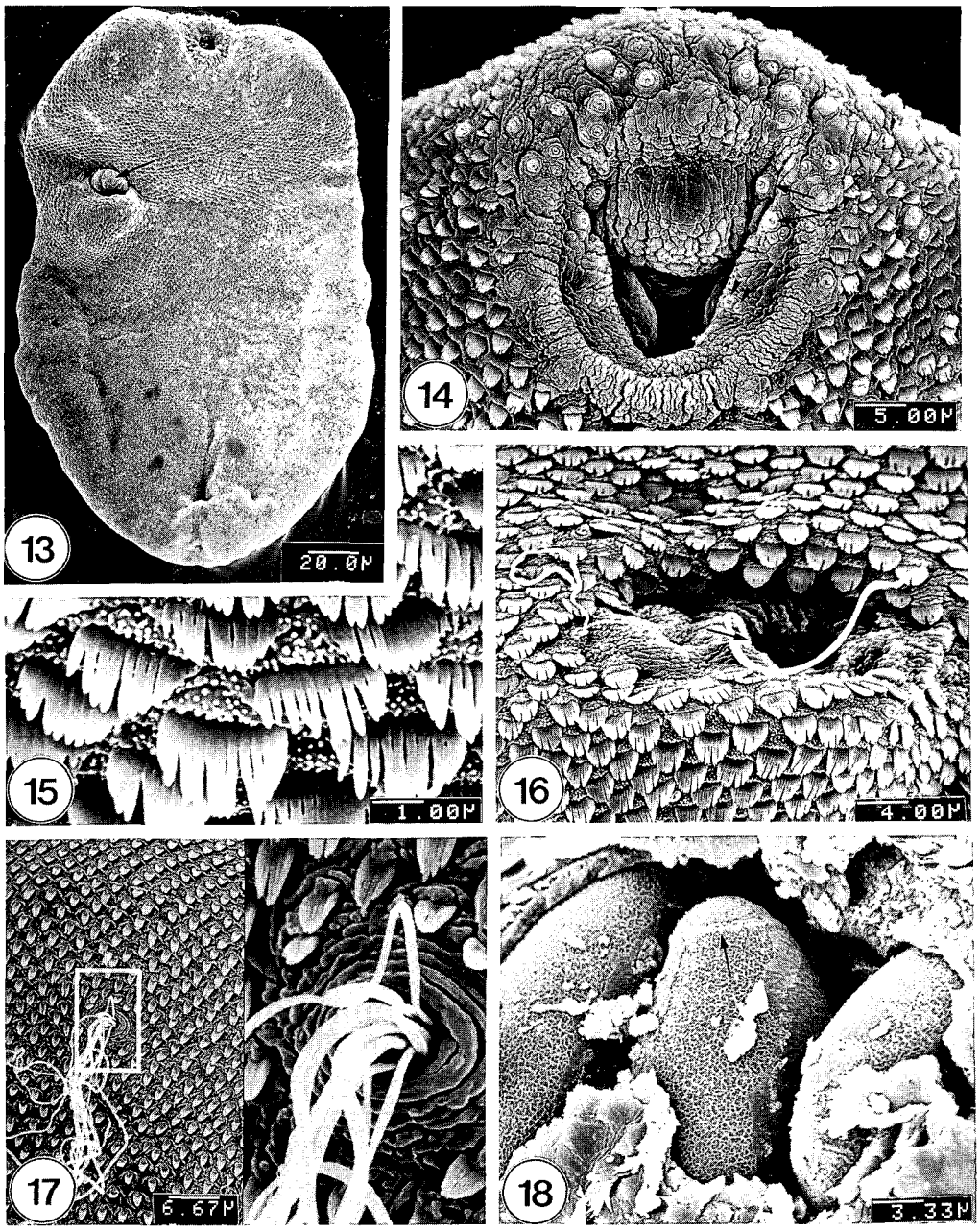
The distribution of spines was very similar to that of metacercariae, but the space between spines became a little wider (Figs. 9-11) than in metacercariae. The anterior part of the ventral surface was covered densely with



Figs. 1-6. SEM view of metacercariae of *Metagonimus miyatai*.
Fig. 1. Ventral view of an excysted metacercaria, showing oral, ventral suckers, and an excretory pore. **Fig. 2.** Tegument around the oral sucker. Note distribution of sensory papillae on the lip of the oral sucker. **Fig. 3.** Tegumental spines on the ventral surface between oral and ventral suckers. Digitations of spines are 5-7 pointed. **Fig. 4.** Tegument near the ventral sucker. Several uniciliated papillae are seen. **Fig. 5.** Tegument of the posterior ventral surface. Two- to three-pointed peg-like spines are seen. **Fig. 6.** Tegument around the excretory pore. Spines are sparsely distributed.



Figs. 7-12. SEM view of one-week old adults of *Metagonimus miyatai*.
Fig. 7. Tegument around the oral sucker. Note distribution of type I and type II (arrows) sensory papillae on the lip of the oral sucker. **Fig. 8.** Tegument near the ventral sucker, on which a genital pore (arrow) is seen. **Fig. 9.** Tegumental spines just below the oral sucker. The spines are broad, and having 9-11 pointed tips, and knob-like projections are seen on the cytoplasmic processes. **Fig. 10.** Tegumental spines on the ventral surface between oral and ventral suckers. The spines have 7-11 pointed tips, and 5 uniciliated type I sensory papillae are seen. **Fig. 11.** Dorsal tegument near the Laurer's canal. A sperm is seen entering into the canal, which is surrounded by several layers of cytoplasmic projections. Tegumental spines are 5-6 pointed. **Fig. 12.** Tegument around the excretory pore. The tegumental surface is severely wrinkled, and spines are sparsely distributed.



Figs. 13-18. SEM view of two- and four-week old adults of *Metagonimus miyatai*.
Fig. 13. A whole worm, ventral view, showing prominent oral and ventral suckers, and the cirrus (arrow) protruding from the genital pore. **Fig. 14.** The oral sucker showing many sensory papillae and tegumental spines. The lip is equipped with 7 type II papillae, and at inner side of the oral sucker one pair of large (arrows) and two pairs of small (arrow heads) type I papillae are seen on each side. **Fig. 15.** Tegumental spines between oral and ventral suckers. Spines are short but broad, having 10-11 pointed tips, and numerous knob-like projections are seen between spines. **Fig. 16.** Tegument near the ventral sucker, where two sperms are escaping from the genital pore (arrow). **Fig. 17.** Dorsal tegument around the Laurer's canal (left). Many sperms are seen entering into the canal, which is surrounded by several layers of sphincter-like structures (right). Tegumental spines are 5-6 pointed (right). **Fig. 18.** Three intrauterine eggs are seen after etching the middle portion of the dorsal surface, ovoid to ellipsoid in shape and showing a prominent operculum (arrow) and relatively smooth shell surface.

large and 9-11 pointed spines, and with many type I sensory papillae (Figs. 9-10). On the posterior surface, spines were sparse, with 2-6 points.

On the dorsal surface, spines were well differentiated into 10-11 points anteriorly, but became less pointed posteriorly. The Laurer's canal was seen with a sperm entering into the canal (Fig. 11). The opening of the Laurer's canal was surrounded by several layers of sphincter-like structures. The distribution of spines around the Laurer's canal was moderate. The excretory pore was anus-shape (Fig. 12), and slightly depressed. The pore was completely devoid of spines, and at a little remote areas a few spines were seen. The surface around the excretory pore was wrinkled irregularly.

Two- and four-week old worms: The general feature of the worm (Fig. 13) was not different from that of one-week old adults. Around the lip of the oral sucker, 7 large type II sensory papillae were observed, and at inner side of the lip one pair of large and two pairs of small type I sensory papillae were characteristically seen on each side (Fig. 14).

Tegumental spines (Fig. 15) were distributed over the whole body surface except areas of oral and ventral suckers. The ventral sucker was located on the right side of the anterior part of the body, with many wrinkles on its lip. Sperms were seen escaping from the genital pore (Fig. 16). Around the ventral sucker, numerous 9-11 pointed spines (Fig. 15), and several type I sensory papillae were observed. On the surface near the posterior end, spines were sparsely distributed, and pointed simply.

On the dorsal surface, the degree of spine differentiation was nearly the same as on the ventral surface. The Laurer's canal was clearly recognized, and many sperms were seen entering into the Laurer's canal (Fig. 17). By cracking of the mid-dorsal surface, intrauterine eggs were visualized (Fig. 18); they were ovoid to ellipsoid in shape, with an operculum and relatively smooth shell surface.

DISCUSSION

As is a common feature of all kinds of trematodes, the whole tegument of *M. miyatai*, metacercariae and adults, was covered with cytoplasmic processes, together with tegumental spines and sensory papillae. According to their development, the body size became bigger, flat cytoplasmic processes in metacercariae differentiated into knob- or cobblestone-like projections in adults, digitations of tegumental spines increased, and interspace between spines became wider.

The surface ultrastructure of *M. miyatai* was generally similar to that reported for *M. yokogawai* (Lee *et al.*, 1984). However, a few differential features were observed in the morphology and distribution of tegumental spines and sensory papillae. In metacercariae of *M. yokogawai*, tegumental spines on the ventral surface of the anterior body had 7-8 pointed tips, but in *M. miyatai*, the number of tips was only 5-7, a less differentiated feature. In adults of *M. yokogawai*, however, digitations of well differentiated tegumental spines were 7-9 points (Lee *et al.*, 1984), whereas in *M. miyatai* adults, 9-11 pointed spines were popularly seen. Also the dense distribution of spines was limited from anterior to middle surfaces of the body in *M. yokogawai* (Lee *et al.*, 1984), but extended more posteriorly in *M. miyatai*. The type III papillae (round swelling of cytoplasmic ridges) observed at inner side of the lip of the oral sucker in 4-week old adults of *M. yokogawai* (Lee *et al.*, 1984) was not seen in one- to four-week old adults of *M. miyatai*. Instead, one pair of large and two pairs of small type I papillae were characteristically seen at inner side of the lip of the oral sucker in *M. miyatai* adults.

The tegumental surface of trematodes is known to have physiological or biochemical functions essential for their existence and thriving (Lumsden, 1975). In larval trematodes, the tegument is generally flat or cobblestone-like, but as they mature, it is differentiated into a more fine and velvety one (Lee *et al.*, 1985; Hong *et al.*, 1991). This modification of the surface structure is regarded as a consequence to suffice the increase of nutrient requirements by parasites in the host body (Bennett and Threadgold, 1975; Fujino *et al.*, 1979; Font and Wittrock, 1980; Lee *et al.*, 1984).

The size, shape and distribution of tegu-

mental spines are different by species of parasites as well as developmental stages, habitats, and migratory behaviors (Fujino *et al.*, 1979; Bennett and Treadgold, 1980; Font and Wittrock, 1980; Lee *et al.*, 1989). In the case of *Clonorchis sinensis*, it was reported that larval flukes which excysted in the duodenum have double or triple-pointed tegumental spines at anterior half of the body, but spines gradually disappear as the worms grow to be adults, and full-grown adults become compeletely devoid of spines (Fujino *et al.*, 1979; Lee *et al.*, 1982). On the contrary, it was shown in larval worms of *Fasciola hepatica* that tegumental spines of single-pointed form metamorphose into multipointed ones just prior to the entry into the bile duct (Bennett and Threadgold, 1975). Conversion of simple spines into serrated ones during developmental stages is also known in *Neodiplostomum seoulense* (Lee *et al.*, 1985) and *Paragonimus iloktsuenensis* (Lee *et al.*, 1989). These developmental changes were thought to help flukes adapt to the changing environment in the host (Bennett and Threadgold, 1975).

The function of ciliated type I papillae has been suggested to be tango- and/or rheo-receptive, when their morphology and distribution in various trematodes were considered (Erasmus, 1967; Morris and Threadgold, 1967; Bennett and Threadgold, 1975; Fujino *et al.*, 1979; Lee *et al.*, 1984; Seo *et al.*, 1984). On the other hand, Ip and Desser (1984) suggested that ciliated sensory papillae might be either chemo- and/or mechano-receptive because this type sensory papillae extended through the whole thickness of the tegument and had a free sensory cilium.

Spines were lacking on the lip of the oral sucker in excysted metacercariae and adults of *M. miyatai*. In the cercarial stage of *Metagonimus* spp., however, it was reported that about 30 hook-like spines were present on the oral sucker (Fujino *et al.*, 1976). The disappearance of oral spines seems to be due to their loss after attachment at and penetration into the fish intermediate host.

The presence of sawtooth or brush-shaped tegumental spines seem to be one of characteristic features of the family Heterophyidae. Tegumental spines of *H. nocens*, for example, were in most cases multidigitated with 12-17 points (Chai *et al.*, 1992). Similar features were reported in *C. lingua* (Køie, 1977), *H. aequalis* (Taraschewski, 1984), *M. yokogawai* (Lee *et al.*, 1984) and *H. continua* (Hong *et al.*, 1991). However, the number of points and its changing patterns according to worm development appear to be different by species of parasites. In the case of *H. continua*, for example, the number of points was increased from 10-14 in metacercariae to 15-17 in adults (Hong *et al.*, 1991), and from 5-7 (metacercariae) to 9-11 (adults) in *M. miyatai* (this study), whereas the number of points remained nearly unchanged between metacercariae and adults in *M. yokogawai* (Lee *et al.*, 1984).

It was interesting to note in this study that the function of the Laurer's canal opened on the dorsal surface should be an important route to receive sperms in *M. miyatai*, as many sperms were seen entering into the Laurer's canal. Similarly in *C. sinensis*, the Laurer's canal was suggested to be a copulatory organ receiving sperms (Jeong, 1983). Also interesting was to see the morphology of intrauterine eggs of *M. miyatai*, by cracking the dorsal surface of adult flukes, which were oval to ellipsoid in shape, with an operculum, and relatively smooth shell surface showing less prominent muskmelon patterns.

Conclusively, the surface ultrastructure of *M. miyatai* was generally similar to that of *M. yokogawai* (Lee *et al.*, 1984), however, differences were observed in the digitation and differentiation patterns of tegumental spines and distribution of sensory papillae in metacercariae and adults, which may be of taxonomic significance.

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초록=

미야타흡충 피낭유충 및 성충의 표피 미세구조

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미야타흡충 (*Metagonimus miyatai*) 피낭유충 및 성충의 표피 미세구조를 주자전자현미경으로 관찰하였다. 피낭유충은 피라미 (*Zacco platypus*)의 비늘에서 분리하였고, 성충은 흰쥐에 피낭유충을 감염시킨 후 1-4주에 소장으로부터 회수하였다. 탈낭한 피낭유충의 전체적인 외형은 요코가와흡충과 큰 차이가 없었으며, 복흡반은 충체 중앙으로부터 오른쪽에 위치하였다. 구흡반의 구순에는 제I형 감각유두 및 제II형 감각유두가 여러 개 관찰되었다. 복흡반 앞쪽에는 끝이 5-7분지된 피극들이 빽빽하게 덮혀 있었으며, 충체 후반부의 배설공 부근에는 2-3분지된 피극으로 덮혀 있었다. 감염 1주된 성충의 경우, 구흡반의 구순에는 제II형 감각유두 7개가 분포하고 있었으며, 구흡반 내벽에는 큰 제I형 감각유두가 1쌍씩, 작은 제I형 감각유두가 2쌍씩 좌우측에서 각각 관찰되었다. 피극의 분포는 피낭유충의 경우와 비슷하였으나, 분화도는 증가하여 9-11분지된 피극들이 관찰되었다. 감염 2-4주된 성충의 표피 미세구조는 감염 1주된 성충과 대동소이하였으나, 정자가 충체 배측 표면으로 개구된 Laurer's canal로 들어가고 있는 것이 관찰되었다. 이상의 결과를 종합할 때, 미야타흡충의 표피 미세구조는 요코가와흡충과 전반적으로 비슷하였으나 피극의 분포 및 분화도, 구흡반 주변의 감각유두 분포 등에 다소 차이가 있음을 알 수 있었으며 이러한 차이가 분류학적으로 의미있는 것일 가능성이 제시되었다.

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