한국패류학회지 : 제 7 권 1 호
Korean J. Malacol.
Vol. 7(1) : 76-86, 1991

A Scanning Electron Microscopic Study of the Glochidial Encystment on the Host Fish (2)

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= 國文要約 =

Glochidium larva의 附着으로 인한 宿主魚類의 被囊形成過程에 關한 走射電子顯微鏡的 硏究(2)

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Anodonta fukudai(대칭이)의 glochidium larva를 *Acheilognathus yamatsutae*(줄납자루)에게 실험실 내에서 人工感染시켜 숙주인 줄납자루의 상피조직이 그 glochidium larva를 에워싸 被囊을 形成하는 過程과 피낭 내에서 變態過程을 거친 幼貝가 피낭을 이탈하는 과정을 走射電子顯微鏡(SEM)을 이용하여 관찰하였다.

Glochidium larva는 宿主魚類의 표피 중 비늘로 덮혀 있지 않은 부분이면 쉽사리 부착이 가능했고 숙주는 피낭을 형성하였으나 실험 편의상 가장 많이 부착하는 지느러미에 부착한 glochidium larva들을 중심으로 觀察을 계속하였다.

줄납자루는 自然的인 好宿主 중의 하나임에도 불구하고 그 上皮細胞들이 glochidium larva를 중심으로 初期被囊(early cyst)을 형성하는데는 21~25시간을 소요하였다.

이는 열대어종인 guppy를 숙주로 사용한 實驗的 感染에서 3~4 시간 밖에 소요되지 않은 것에 비하면 상당히 긴 시간이었다. 宿主의 被囊 내에서 變態過程을 거친 幼貝가 被囊으로부터 移脫을 시작하는 시기는 감염 후 12일부터였으며 14일이면 대다수 이탈 하였는데 일단 被囊이 일부분 터지기 시작하면 移脫은 被囊形成過程보다 신속히 완료되었다.

숙주로부터 이탈한 幼貝는 활발하게 발을 움직이며 활동을 하나 貝殼만은 아직 완전히 변태되지 않아 중정도의 退行性變化를 일으킨 hook와 spine들을 가지고 있었고, 첫번째 成長線이 形成되어 있었다.

被囊形成에 참여하는 宿主의 上皮細胞들은 주로 주변으로부터 이동하여 온 細胞들이나 被囊形成過程이 상당히 느린 점을 感案하면 傷處部位의 세포들이 增殖하여 참여한 것들도 많이 있을 것으로 思料된다.

被囊形成過程 중의 세포들은 연속적인 移動過程에서 끊임없이 그 모양들이 변하였고 被囊이 완전히 형성되어 안정된 기간인 며칠간만을 제외하고는 全 期間을 통하여 被囊을 이루고 있는 細胞들과 이웃한 細胞들 表面의 微細構造는 세포들이 계속 安靜을 잃고 있음을 보여주었다.

Received November 11, 1991

INTRODUCTION

The genus *Anodonta* is representative of the freshwater clams in Korea. The young larvae of the fresh-water mussels pass their early stages of the development inside the marsupial gill pouches of the female.

Here they develop into simple bivalves, so called glochidium and discharged from the marsupial gills for further processes of development in the tissue of the proper host fishes.

The glochidia discharged from the gills attach to the fins or gills or any other surfaces of appropriate fishes where they become encysted in the tissue. During this parasitic stage, the glochidia take processes of the metamorphosis and become independent as juvenile clams.

This investigation was conducted to observe the successive stages of glochidial encystment following the glochidial attachment to the host fishes and to observe the excystment processes occurred when the metamorphosed glochidium, a new juvenile mussel, escapes from its cyst.

MATERIALS AND METHODS

The fresh-water mussel used in this study was *Anodonta fukudai*, subfamily Anodontinae, family Unionidae, order Eulamemllibranchiata.

Several gravid mussels were collected from a stream of the Yesan Water Reservoir located in Yesan, Choongnam Province. For this experimental infection of the glochidia to the host fishes, many individuals of a host fish, *Acheilognathus yamatsutae* Mori, Cyprinidae, were also caught from the same stream. The collected animals were kept in the aquariua separated by the species.

At experiment a gravid mussel from the aquarium in which the living mussels were kept, was opened in a small quantity of dechlorinated tap water and the marsupia were quickly cut loose. The marsupia were transferred into a large petri dish containing dechlorinated tap water and then rapidly cut opened. The glochidia were shaken out into the petri dish and collected well without any pieces of tissue of the mussel.

For the artificial infection of the glochidia to the host fish, many of the glochidia collected were transferred to the bottom of a fish globe containing 15 cm deep dechlorinated tap water at room temperature. The host fishes from the aquarium were allowed to swim around in the fish globe containing lots of glochidia on the bottom. Sometime after the fishes were introduced, the glochidia moved up one by one to the fishes by snapping their valves. To help the glochidia get more chances of attachment to the fishes, the water was shaken sometimes. The fishes infected with around 5 to 10 glochidia within 5 minutes were transferred to the beakers 200 ml in capacity of 2 fishes per beaker. Two fishes per every 30 minutes were sacrificed to examine successive stages of early encystment for 48 hours. From this time, the rest of infected fishes were transferred to a fish globe and 2 or 3 fishes were caught once every 24 hours until the metamorphosed juvenile clams in the host tissues completely dettached from the host fishes.

The fishes were sacrificed in 2.5% glutaraldehyde solution and their fins or some other parts of the fishes infected with the glochidia were carefully removed from the fishes under the stereoscope. The specimens were washed

with phosphate buffer solution, dehydrated in a graded series of alcohol-amyl acetate mixture, and dried with the critical point dryer.

The specimens completely dried up were stored in dust-free containers until the gold coating was applied and were observed with the scanning electron microscope(ISI-SS4D).

OBSERVATIONS

The shape of the glochidium with two identical shell valves is apparently triangular and its average size is 0.47 mm × 0.42 mm when closed(Fig. 1, 2).

When the glochidia approach to their host fishes, they buoyed up themselves and moved toward their hosts by snapping their bivalves. The hooks of the glochidium hold and interlocked the tissues of the host such as the fins, the gills, the lips, the wall of the buccal cavity, and sometimes the scales. The most favorable part for the glochidial attachment was the fins.

Once a glochidium attaches to a certain part of the host tissue, it did not move to another part for better choice but remain at there throughout its parasitic phase.

But, sometimes some of the glochidia already attached to the host tissue failed to maintain continual attachment and they dettached from the host soon or later after attachment.

The process of encystment was progressed very slowly. The visible change of epithelial cells, migration of the cells, was detectable from 2 to 4 hours after attachment(Fig. 3). Around 20 hours after attachment, the glochidia were mostly encysted(Fig. 5-9), and from 21 to 25 hours after attachment all of the glochidia attached were roughly encysted except some individuals of those unstably attached in the beginning(Fig. 6, 10).

It was observed that the epithelial cells of the host tissue, the fins in this case, migrated toward the attached glochidia in wave and began to cover it(Fig. 2, 3). At this stage, the epithelial and connective tissue became loose. The cells lost their mutual connections and became separated a little(Fig. 5, 7, 8, 22~24).

The shapes of the cells participating in the encystment process changed from time to time during migration and the outer surface structures of the cells were considerably changed so that the ridges of the cell surfaces were became irregular or reduced(Fig. 21~24).

After an early cyst was formed during a day, the cyst wall became thickened with several layers of the epithelial cells which were either migrated from other part of the fin or proliferated in the cyst(Fig. 23).

The epithelial cells covering the late cysts retained the stable forms of the cells shapes with normal surface structures but, these conditions were soon disturbed again due to the excystment activities, from the inside of

Abbreviations

CB, cell border	JC, juvenile clam
EC, epitheial cell	M, mantle
G, glochidium	S, spine
GL, growth line	T, larval thread
H, hook	VM, visceral mass

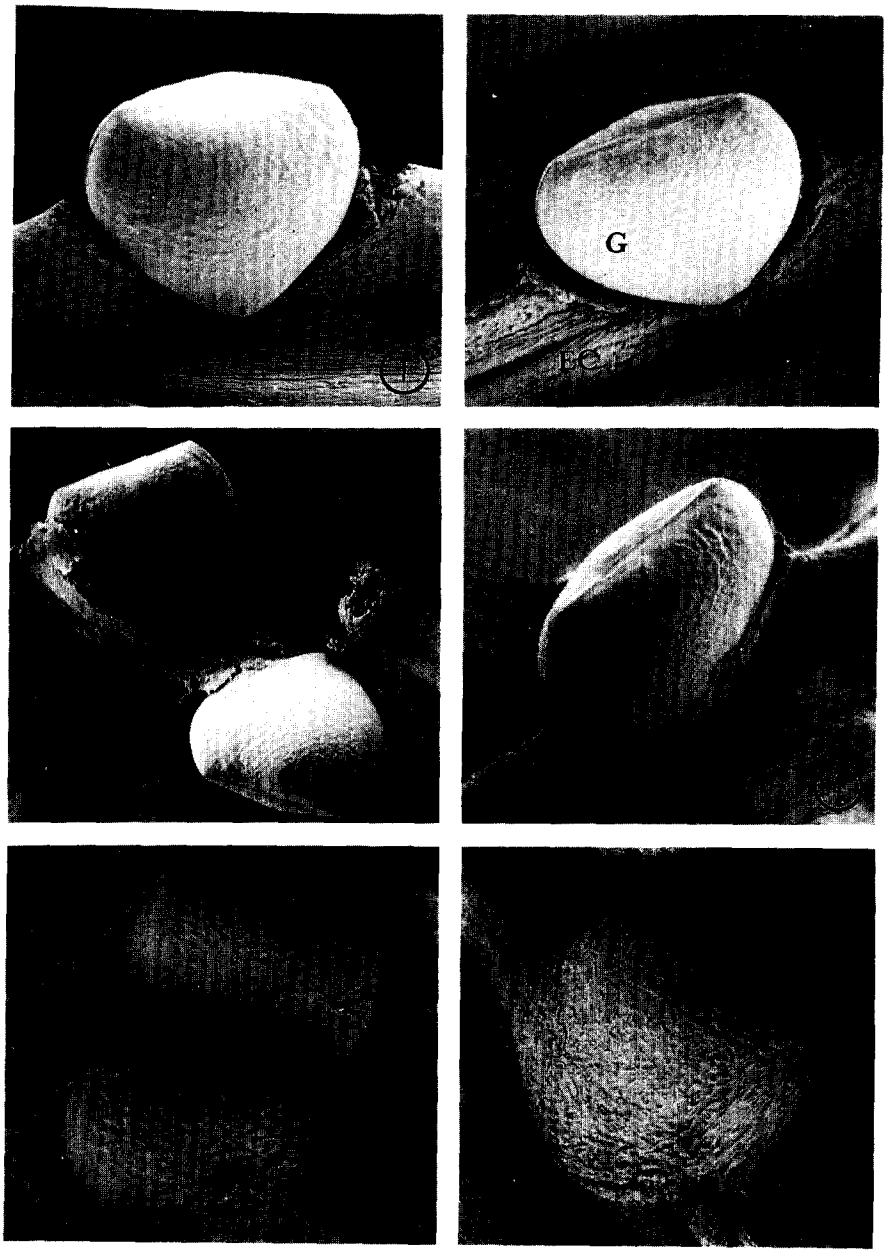

Fig. 1-6. Early stages of encystment.
Fig. 1. A glochidium attached to the fin of the host fish. Thirty minutes after attachment. ×100
Fig. 2. A glochidium(G) attached to the fin. One hour after attachment. ×100
Fig. 3. Two glochidia attached to the fin. Four hours after attachment. ×70
Fig. 4. Twelve hours after attachment. ×100
Fig. 5. Twenty hours after attachment. ×100
Fig. 6. An early cyst showing a glochidium completely encysted. ×100

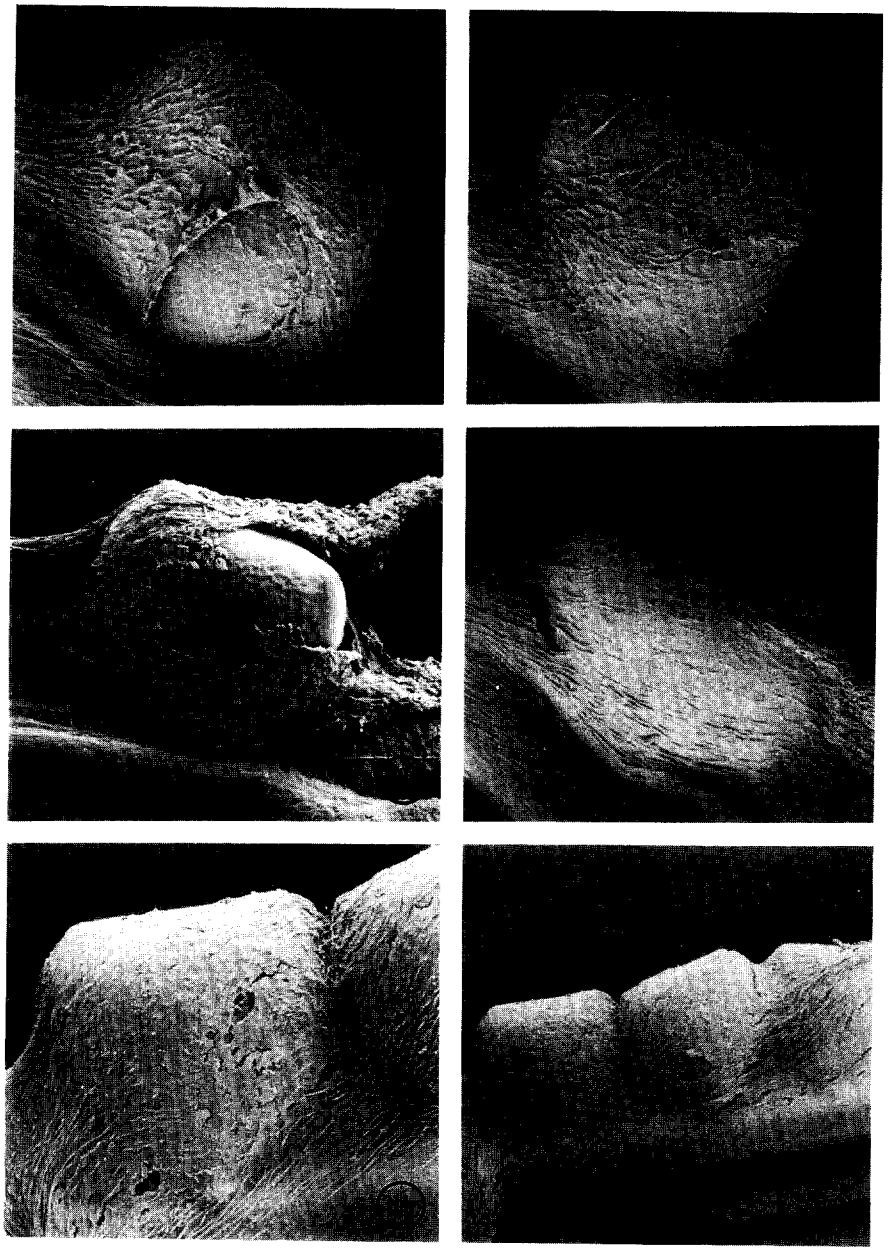

Fig. 7-9. A glochidium mostly encysted.
Fig. 7. Twenty one hours after attachment. ×100
Fig. 8. Twenty two hours after attachment. ×100
Fig. 9. Twenty three hours after attachment. ×100
Fig. 10-12. Early cyst stages. The epithelial cells roughly cover all over the glochidia.
Fig. 10. Twenty four hours after attachment. ×100
Fig. 11. Twenty seven hours after attachment. ×100
Fig. 12. Three glochidia encysted. Twenty seven hours after attachment. ×50

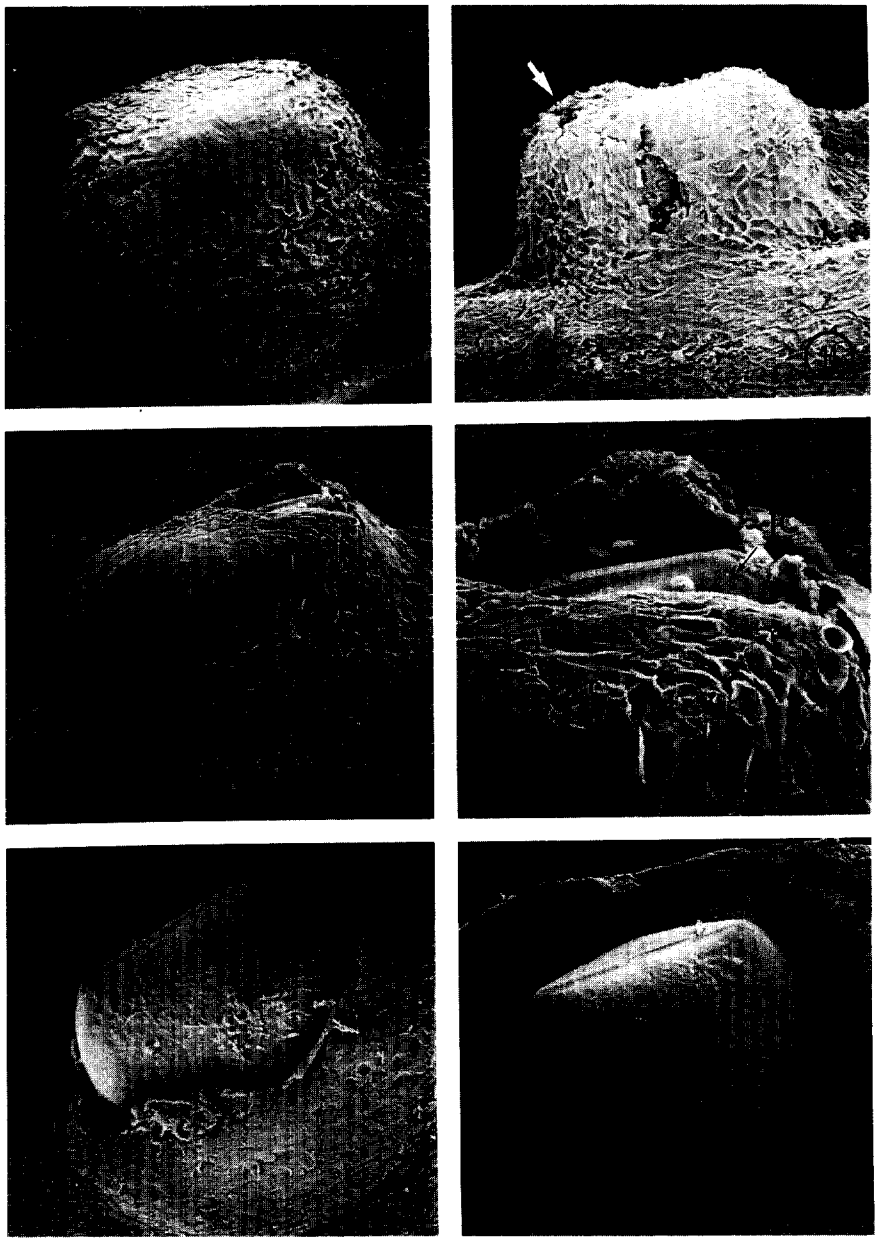

Fig. 13. A late cyst stage showing the epithelial cells in almost stable forms. ×100

Fig. 14-20. Successive stages of excystment(dettachment)

Fig. 14. A cyst a litle bit teared(arrowed). Twelve days after attachment. ×100

Fig. 15. A cyst teared a little shows the hinge part of the juvenile clam. Twelve days after attachment. ×100

Fig. 16. Enlarged view of the indicated part in Fig. 15. The hinge part of juvenile clam(JC) is seen. Twelve days after attachment. ×300

Fig. 17. A juvenile clam emerging from the cyst. Fourteen days after attachment. ×100

Fig. 18. A juvenile clam emerged from the cyst just before dettachment. Fourteen days after attachment. ×100

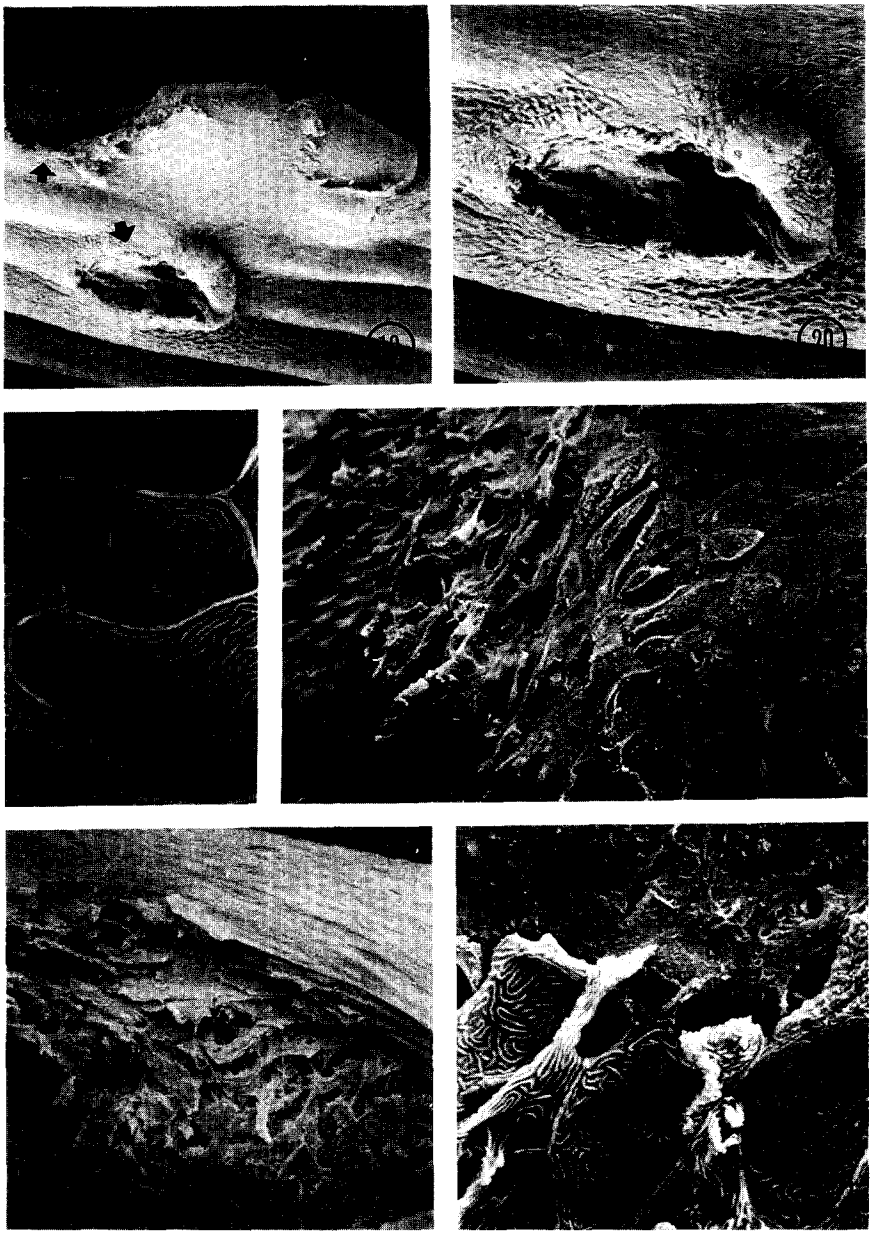

Fig. 19. A fin with two glochidia emerging from their cysts and two cysts opened(arrowed) because of the dettached juvenile clams. Fourteen days after attachment. ×100

Fig. 20. Enlarged view of the cyst opened, the indicated part in Fig. 19. The escaped(dettached) juvenile clam remained a deep injury on the fin the the host. ×300

Fig. 21-24. The changes of the epithelial tissue of the host

Fig. 21. The normal epithelium of the fin showing the well arranged surface ridges and the clear cell borders(CB). ×2,000

Fig. 22. The epithelial cells(EC) migrating toward the glochidium(G). The shapes of the cells in motion changed a lot. × 450

Fig. 23. A cyst wall with the cells several layered. Two days after attachment. ×50

Fig. 24. A cyst wall with the epithelial cells showing the unstable shapes and surface structures. ×1,300

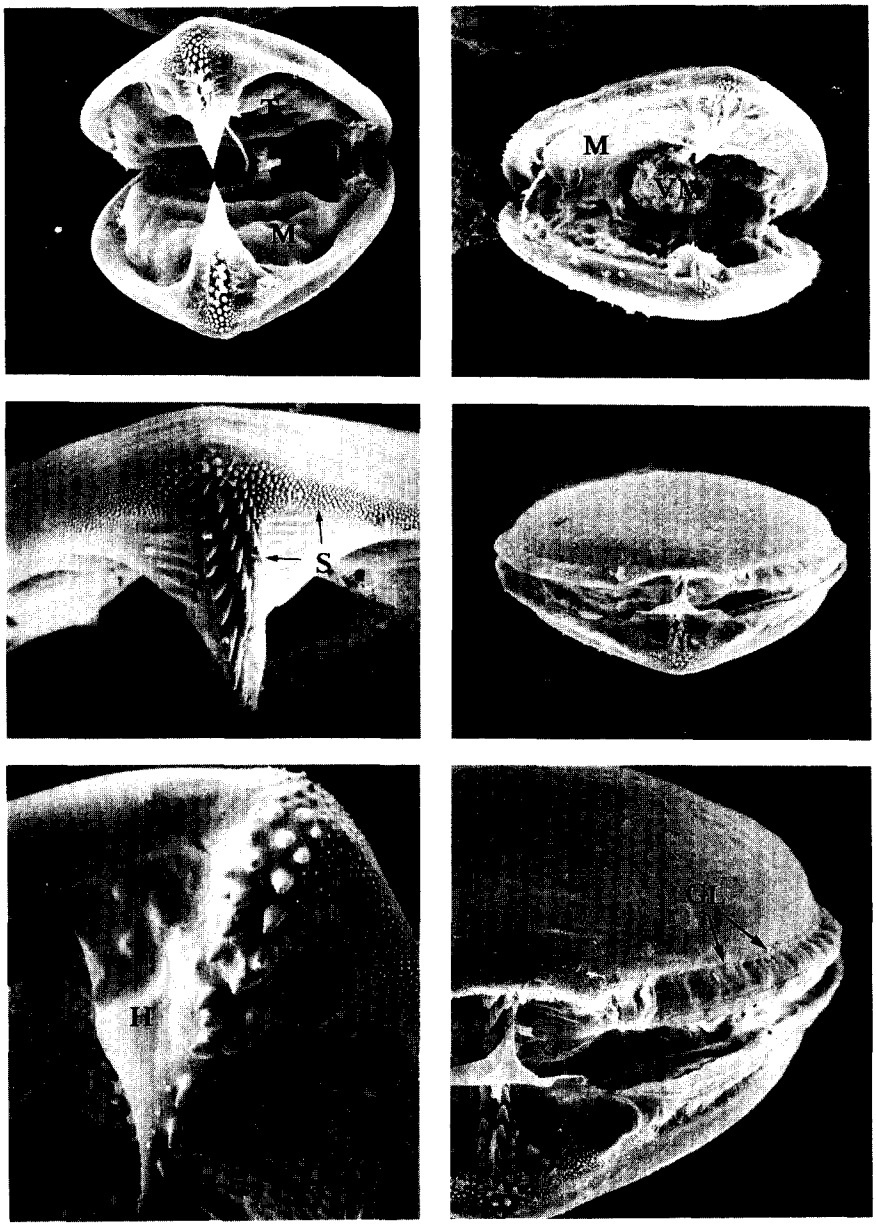

Fig. 25-30. Comparison of the juvenile clam with the glochidium in part.

Fig. 25. A glochidium isolated from the marsupial gill shows a typical form with the hooks(H), the larval thread(T), the hair cells(HC), and the mantle(M). ×100

Fig. 26. A juvenile clam right after dettachment showing a small visceral mass at center and the mantles attached to the valves. ×70

Fig. 27. A hook(H) of a normal glochidium studded with numerous spines(S) in various sizes. ×200

Fig. 28. A juvenile clam with the valves closed. The margines of the shell valves got grown. ×70

Fig. 29. A hook(H) of the juvenile clam showing the spines(S) degenerated much during the parasitic phase. ×400

Fig. 30. An enlarged view of a part of the juvenile clam in Fig.28. The first growth line(GL) is clearly visible along the margines of the valves. ×150

the cyst, by the metamorphosed juvenile clam warming up to escape from the cyst.

The process of excystment was visually detectable from 12 days after attachment and most of the cysts were emptied from 13 to 15 days after attachment with some exception at room temperature(17~20°C).

When the cyst was opened by the juvenile clam, the first sign detected was a little tear of the cyst wall covering the hinge and marginal zones of the juvenile clam(Fig. 14~16). And then, the juvenile clam pushed up its covering(the epithelial cells) and gradually emerged its whole shell from the cyst(Fig. 17~20). The dettached juvenile clams left deep injuries on the host tissues respectively which may be healed later(arrowed in Fig. 19 and Fig. 20). No cells of the host epithelium, which were still attached to the juvenile clam emerging from the cyst, were observed.

The most juvenile clams escaped from the cyst were a little bigger than the glochidia and they were still possessed of the hooks even though much degenerated(Fig. 26~30). The first growth line was appeared on the shell valves of the juvenile clam when observed right after dettachment(Fig. 28, 30).

DISCUSSION

The mussels belonging to the Unionidae have very specialized larval form, so called glochidium, with or without hooks on the apex of the larval shell valves for easy attachment to the host.

In *Anodonta fukudai* used for the present study, the glochidium larva is mature by October at the latest and remains in the demibranchs following the cold seasons until next April to May. When the glochidium is released from the demibranchs to begin its parasitic phase, the larval thread, adductor muscle, sensory hair cells and hooked shells all play an important roll in the host attachment.

The glochidium which has left the demibranchs of "mother" reaches the host fish without taking any planktonic food, but uses the small amount of materials from the digestion of the epidermal cells of the fish (Harms, 1909; Arey, 1932b).

Even though there are a little varieties in the period of time of parasitic phase by the species of the host, the new juvenile clam eventually falls to the bottom and starts its life.

At the final stage of the parasitic phase, the shells of the glochidium usually degenerate a little, especially the spines of the hooks, while the first growth line is formed along the margines of the shells and the juvenile tissues considerably develop. These results support the report of Wood(1974).

The question that immediately arise is that why such a long period of "gestation" in the mother gills and of "quiescence" attached to the host body? It is really difficult an answer. Giusti *et al.*(1975) suggested a hypothesis supposed to be the most reasonable one that spending a given period of time attached to the surface of the fish is necessary to keep the glochidium itself safe before reaching the age when the autonomous life is possible and the fragile glochidium shell has greater chance of maintaining its integrity even after heavy waves and current movement. They also proposed another possible hypothesis meaning that the parasitism is secondary to a simple phenomenon of phoresis due to the need of using fish to reach and colonize other niches in the same area.

The above hypotheses may be partly ac-

ceptable, but the glochidium may not have a such parasitic phase without getting supplied certain vital materials for the process of the metamorphosis from the host fish.

In present study, the glochidium of *Anodonta fukudai* took long period of time(21~25 hours), for the early encystment in the fins of *Acheilognathus yamatsutae*, despite it is commonly known as one of the favorable natural hosts, and around 14 days for completion of parasitic phase.

When the glochidium of the same species was artificially infected to the guppy in the laboratory, it took only 3~4 hours for early encystment and metamorphosis was completed usually within 10 days. According to the another experiment undertaken by Jeong (1989) with *Anodonta grandis* and the guppy, the period of time taken for early encystment and for complete metamorphosis was also short as above.

Whichever host the glochidium took introduced above it used to go through all the processes of metamorphosis without any relation between the period of time of parasitic phase. This fact strongly support the present author's opinion that the glochidium should pass its parasitic phase in the host tissue to intake certain vital materials for the metamorphosis.

On the method of cyst formation, some earlier researchers such as Young(1911), Lefevre and Curtis(1912) and some others mentioned that direct proliferation of the cells of the host tissue provided the material for the cyst that encloses the attached glochidium.

Opposing the opinions of the above workers Arey(1932a, 1932c) and Jeong(1989) asserted that the process of cyst formation is one of cell migration whereby neighboring host cells assemble and actively push forward over the invader until wound is closed and the glochidium is covered.

It may be considerable that in case of the period of time for encystment is very long like in the present study, the cells participating in the encystment may also involve lots of the cells supplied by direct proliferation from near surroundings.

When a metamorphosed juvenile clam takes the procedures for dettachment(excystment), the clam may secrete certain enzymes, hydrolytic or proteolytic or both, to make itself isolated from the epithelial cells of the host fish covering it all over for a long. According to the present study, the materials probably secreted by the juvenile clam seemed to inhibit the approach of the epithelial cells of the host considering that there were no host cells still attached to the juvenile clam which is emerging from the cyst were observed.

The relationship between the host and the glochidium should be studied more in morphological and immunohistochemical or any other possible measures.

SUMMARY

A scanning electron microscopic study on the glochidial encystment and excystment of *Anodonta fukudai* on *Acheilognathus yamatsutae*, a common natural hostfish, was conducted.

The glochidium easily attached to the unscaled surfaces of the host fish such as the fins, lips, and the wall of the buccal cavity. For this study, the fins infected with the glochidia were mainly observed in a series.

The process of encystment was slowly progressed, for 21~25 hours for the early cyst and for 2~4 days for the thick walled cyst.

The process of excystment was visually detected on the 12th day since the attachment was occurred. The first visible sign was a little tear of the cyst wall covering the hinge and marginal zones of the juvenile clam and once the little sign was appeared the progress of emerging and dettachment of the juvenile clam from the host was finished relatively in short time.

During the process of the encystment, the cells participating in covering the attached glochidium were seemed mainly supplied by migration from the surroundings.

The shapes of the cells migrating and covering the glochidium were considerably changed and the surface structures of the cells lost their normal pattern of the surface ridges. The unstable forms of the cells were observed almost all throughout the period of the glochidial attachment. No cells of the host epithelium, which were still attached to the juvenile clam emerging from the cyst, were observed.

The most juvenile clams escaped from the cysts were a little bigger than the glochidia and they were still possessed of the glochidial hooks even though much degenerated. The first growth line was appeared on the shell valves of the juvenile clam when observed right after dettachment.

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