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AN ANATOMICAL AND ULTRASTRUCTURAL STUDY OF THE GLOCHIDIUM OF *ANODONTA ARCAEFORMIS*

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ABSTRACT

The shape of the glochidial larvae of the freshwater clam, *Anodonta arcaeformis flavotincta* (Martens), as seen by the scanning electron microscope is triangular. Its size is about 0.40 mm x 0.47 mm. The right and left shell valves are equal in size, and are held together by a ligament. Each valve has a hook 210 μm long near the distal margin. The hook is studded with many spines on its superior face. A large area of the valve at the base of the hook also has numerous spines that become progressively smaller away from the hook.

The glochidial shell valve has two layers, and outer thin membrane bearing small processes and pits, and an inner layer with numerous holes that have their origin from the pits. The mantle cells which line the glochidial valves have many microvilli on their free surfaces. On these free surfaces open a number of micropits, presumably formed by the rupture of the protoplasmic membrane. Microvilli also cover the luminal surface of the canal through which the larval thread protrudes. The larval thread is non-cellular in structure. It varies in diameter among individuals from 1.6 μm to 5.6 μm. Two types of hair cells were observed in the mantle.

Key words: *Anodonta arcaeformis flavotincta*, glochidium, anatomy, ultrastructure.

INTRODUCTION

inside the marsupial gill pouch of the female. The larvae develop into glochidia, which is a simple bivalved larval form with attachment organs. The glochidia are eventually discharged from the marsupium of their "mother" into the surrounding water to attach to the fins, gills or lips of appropriate fish, where they encyst in the tissue, becoming ectoparasites. During the period of this parasitic phase, the glochidia pass through a metamorphic process. The metamorphosed glochidia (juvenile clams) then emerge from their cysts, drop off the fish host and take up a free-living existence, gradually developing into adults.

The structure of the glochidia of various *Anodonta* species have been described by earlier workers, *e.g.*, Surber (1912, 1915), Arey (1924, 1932a,b,c), Wood (1974a), Giusti (1973) and Giusti *et al.* (1975). Wood (1974a) made a very detailed report on the glochidium of *Anodonta cygnea* using a light microscope. Other reports on the glochidial shell of various species of *Unio, Potomida* and *Anodonta*, based on observations using the scanning electron microscope (SEM), were made by Giusti (1973) and Giusti *et al.* (1975). Recently, a much more detailed report on the structures of the glochidium of *Anodonta grandis* as seen by the SEM, was presented by Jeong (1989).

The purpose of our paper is to present SEM and transmission electron microscope (TEM) observations on the glochidium of *Anodonta* (*Anemia*) *arcaeformis flavotincta* (Martens 1905) (Eulamellibranchia, Unionidae, Anodontinae).

MATERIALS AND METHODS

Gravid mussels from the Han River were opened in a small quantity of dechlorinated tap water and their marsupia quickly excised. The marsupia were transferred to a large Petri dish containing dechlorinated tap water and then rapidly opened. The glochidia were shaken into the Petri dish and collected.

In preparation for SEM observations, the glochidia, in tap water, were fixed in a microwave

oven at 53°C. The glochidia were then dehydrated in a graded series of alcohol-amyl acetate, dried with a critical point dryer, and coated with 200 Å gold. Observations were made with a Hithachi S-450 scanning electron microscope.

In preparation for observations with the transmission electron microscope, the glochidia were fixed in a 5% glutaraldehyde-paraformaldehyde mixture for two hours, post fixed with 4% osmium tetroxide for two hours and washed with phosphate buffer (pH 7.4). To remove the calcium from their shells, the glochidia were immersed in a mixture of 5% formalin, potassium acetate and nitric acid mixture for 30 minutes, then in 5% sodium sulfate for 24 hours, and finally washed with distilled water for 24-48 hours. After being washed, the glochidia were dehydrated in a graded series of alcohol, embedded in an Epon mixture and solidified in an incubator at 38°C for two days. The specimens were sectioned with an ultramicrotome (LXB 2088), stained with uranyl acetate and lead citrate, and observed with a JEM CX I transmission electron microscope.

Voucher specimens have been deposited in the biological collections of Soonchunhyang University (catalogue no. 88-12-1) and the University of Michigan Museum of Zoology.

OBSERVATIONS

The shape of the glochidium is triangular with unequilateral valves because of the uneven development of the shell margins. Its average size is 0. 47 mm x 0.40 mm when closed (Fig. 1). The glochidial shell valves, each an identical mirror-image of the other, are held together by a ligament 130 μm in length and 6.6 μm in width (Figs. 2-5). Each valve has a hook at the apex of the valve measuring 210 μm in length. The hook is studded with many pointed spines on its superior face. At the base of the hook, the spines become progressively smaller laterally where the hook merges with the shell (Fig. 6).

The external surface of the glochidial shell valve (the pellicle) is covered with numerous small processes showing successive change in their shape and in the pattern of their distribution (Figs. 8-10). The area surrounding the ligament consists of a reticular pattern containing fine processes (Fig. 8). The reticular structures are dense on the external surface of the central region of the shell valve

10). In addition to the processes, there are a number of variously sized pits scattered over the exterior surface of the valve (Fig. 7).

The glochidial shell is about 4.4 μm in thickness, and has two layers. The outer layer is a thin membrane bearing small processes and pits, and the other is an inner layer containing numerous holes that are the origin of the pits (Figs. 11, 12). The sizes of the holes are about 1.5 μm – 6.6 μm in diameter. The holes of the internal surface do not completely perforate the shell valve (Figs. 11, 12).

The mantle cells which line the glochidial shell valves have numerous microvilli on their free surfaces (Figs. 16, 19, 20). The microvilli number about about 15 per μm^2. The microvilli also cover the luminal surface of the canal that contains the larval thread (Figs. 19, 20).

On the surface of the mantle cells, many micropits exist among the microvilli. The distance between the microvilli is about 1.5 μm (Fig. 16). The mantle is covered with an inner and an outer epithelium (Figs. 21, 22). The former is connected with the inner layer of the shell valve by small muscle bundles, and the latter have numerous microvilli on their free surface.

A larval thread, variable in diameter (1.6 μm - 5.6 μm) in different individuals, emerges from a canal located at the center of the ventral plate of the mantle

Key to abbreviations in figures

An, anterior; C, canal; ES, extrapallial space; H, hook; HC, hair cell; Ho, hole; IN, inner mantle epithelium; IS, inner shell layer; Li, ligament; LP, lateral pit; LV, left valve; M, mantle; Mu, muscle; MV, microvilli; OM, outer mantle epithelium; N, niche (pit); P, micropit; Po, posterior; RV, right valve; S, spine; T, larval thread.

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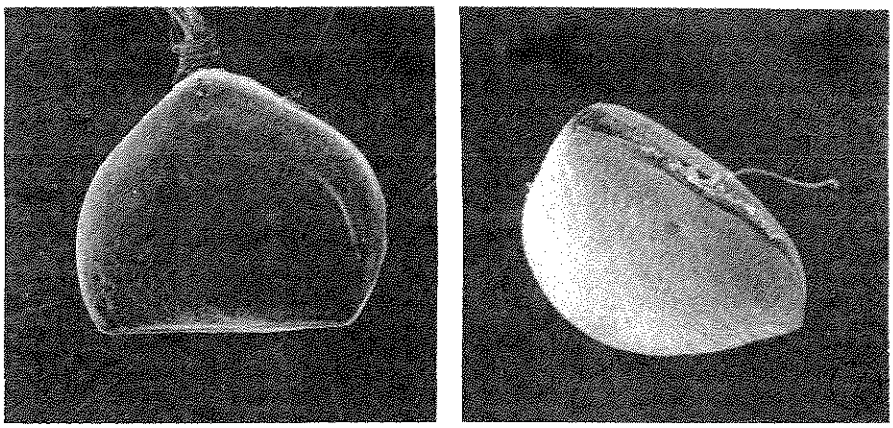
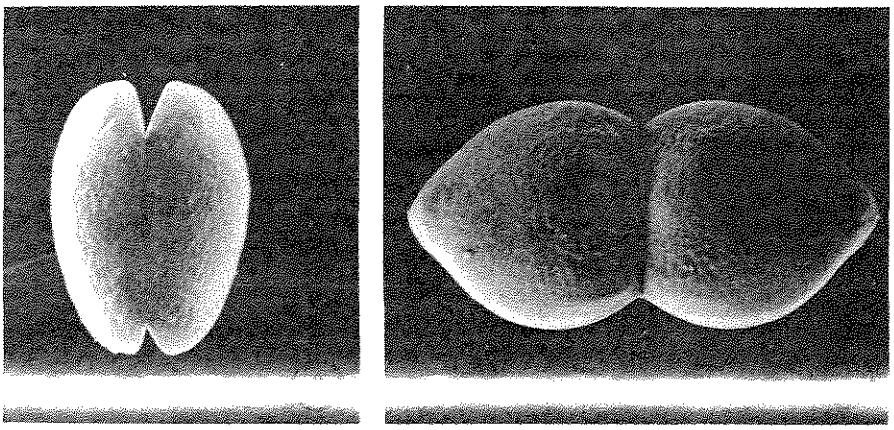
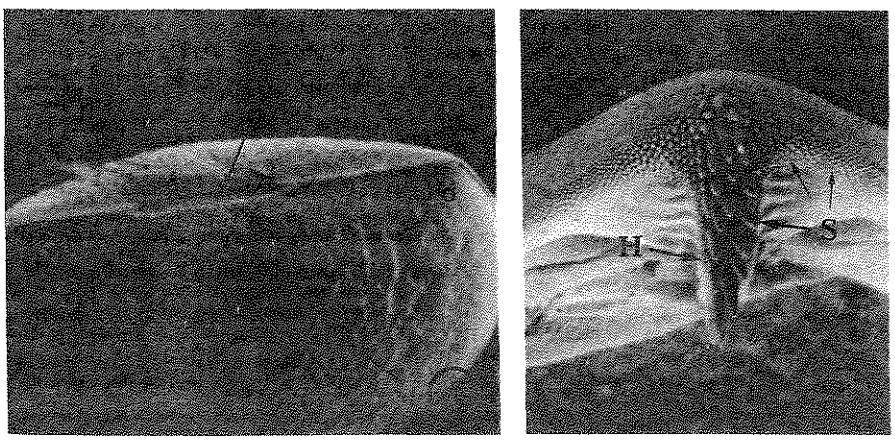
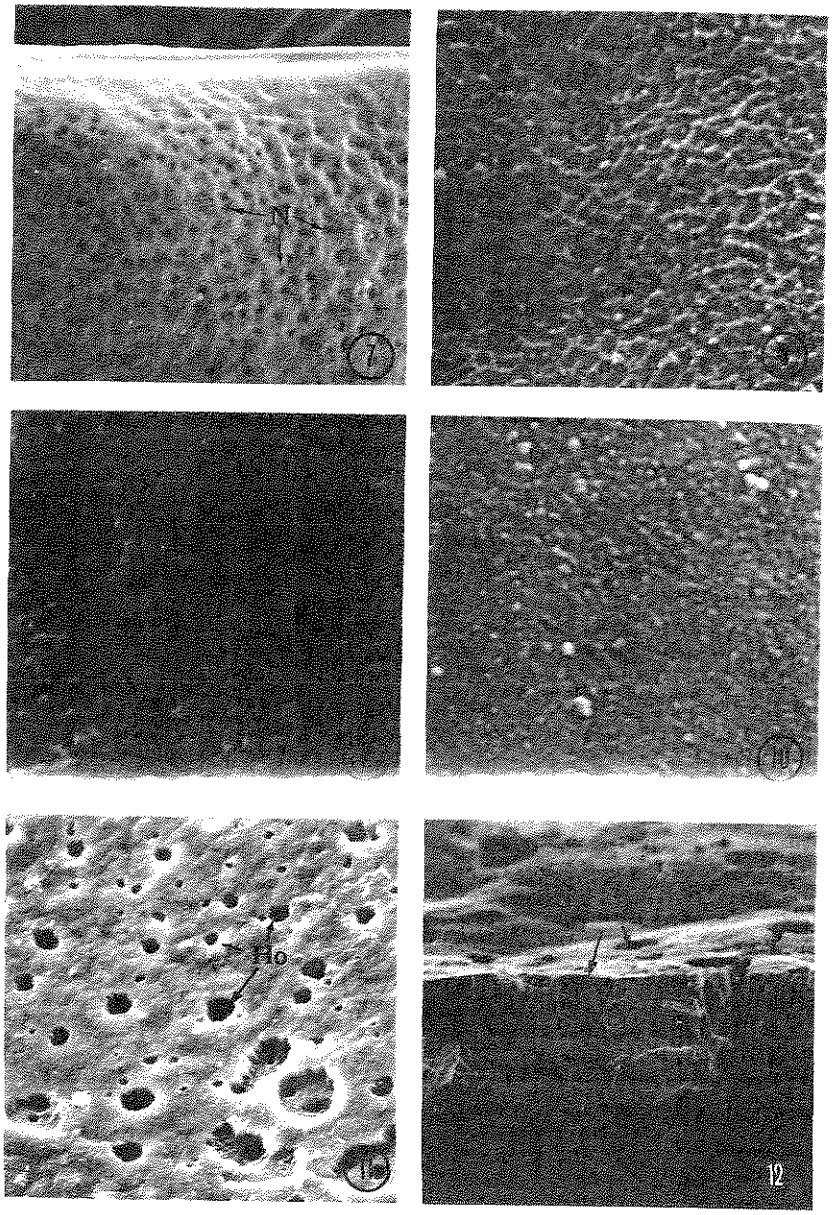






FIGS. 1-6. Glochidia of *Anodonta arcaeformis flavotincta* (cont.). FIG. 1. The triangularly-shaped shell. x85. FIG. 2. A glochidium with its valves loosely closed and with the larval thread (T) protruding. x120. FIG. 3. A glochidium in dorsal view showing the ligament. x85. FIG. 4. View of the two valves, showing their identical mirror-image shapes. x68. FIG. 5. Glochidial shell showing the ligament (Li) and surrounding area. x171. FIG. 6. The apical margin of a valve showing the hook (H) studded with numerous spines (S) of various sizes. x299.



FIGS. 7-12. Glochidia of *Anodonta arcaeformis flavotincta* (cont.). FIG. 7. The external surface of a glochidial shell valve with numerous pits (N) formed by the depressions of the external pellice over the holes of the internal crystalline layer. x278. FIG. 8. The external surface of a valve near the ligament showing the reticular pattern with its interconnecting microprocesses. x11,132. FIG. 9. The external surface of the central region of a valve showing a finer reticular structure. x11,132. FIG. 10. The external surface of a valve near the apex. The reticular structure is more dense. x11,132. FIG. 11. Internal view of a valve showing the many holes (Ho) of various sizes. The holes do not completely perforate the valve and are the origin of the pits on the pellicle. x875. FIG. 12. A view of the fractured valve showing its two layers. The inner crystalline layer has a number of holes (Ho), which are the origin of the pits on the pellicle. x2,385.

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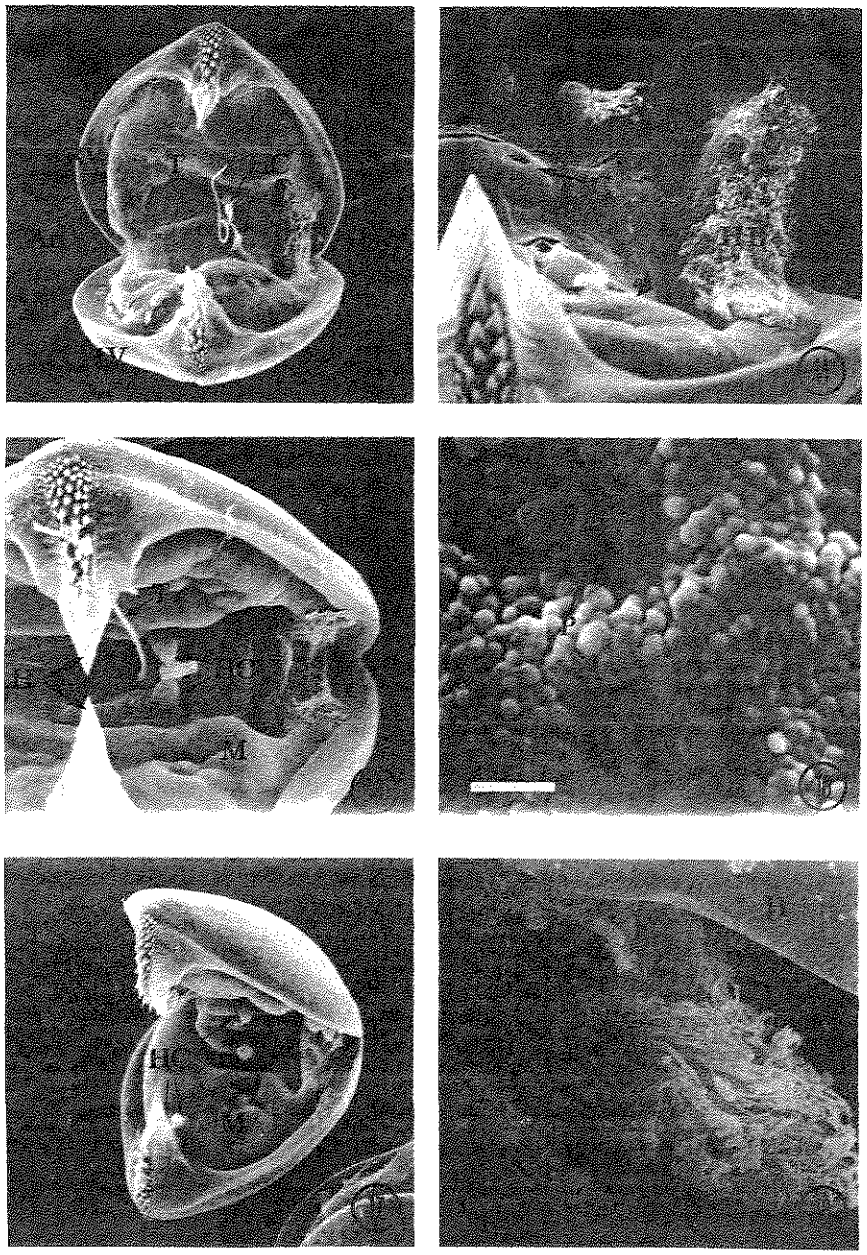
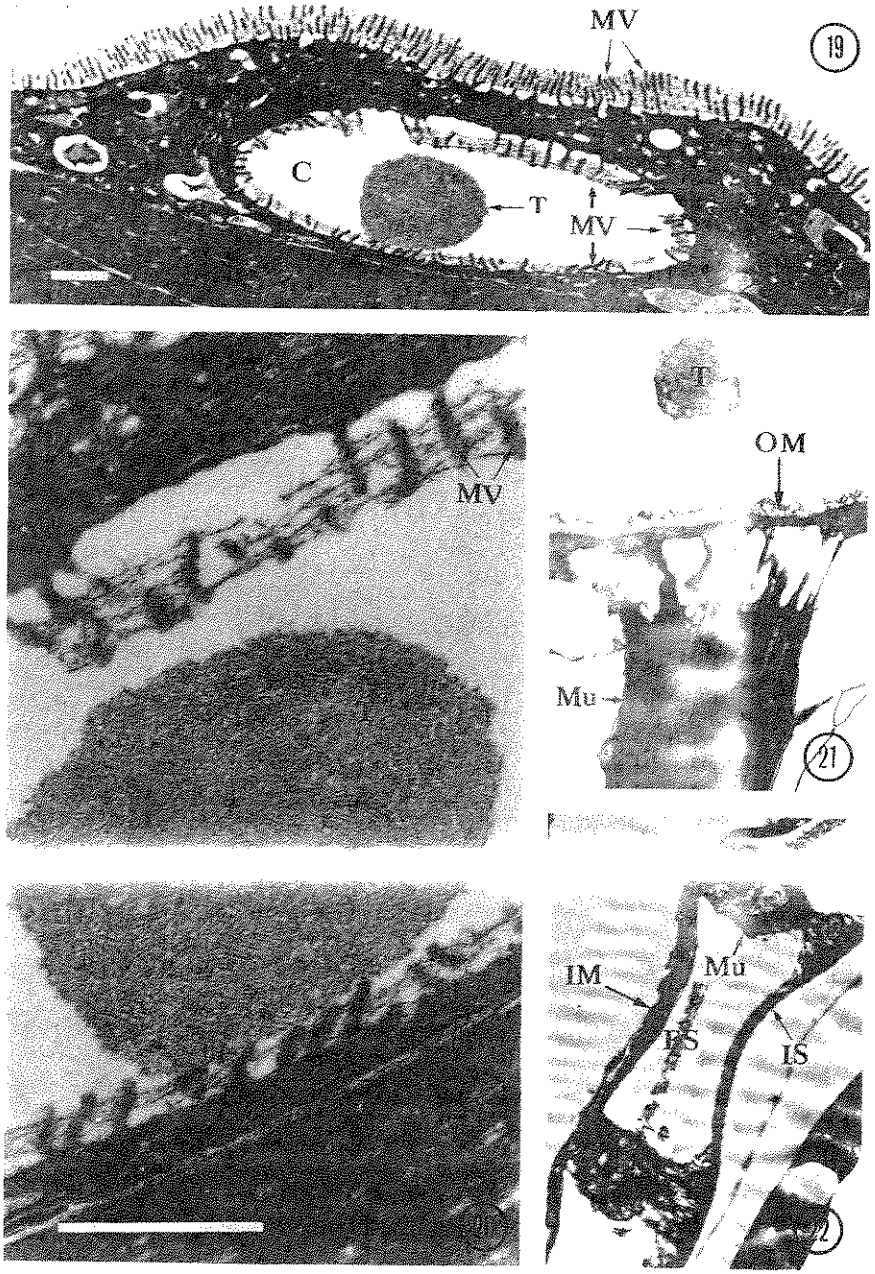


FIGS. 13-18. Glochidia of *Anodonta arcaeformis flavotincta* (cont.). Fig. 13. A glochidium with its valves opened, showing the internal organs such as mantle, larval thread (T), and hair cells (HC). x81. FIG. 14. Part of the mantle of a glochidium showing the hair cells (HC), lateral pits (LP), and the entrance of a canal (C) for the larval thread. x203. FIG. 15. Internal view of a glochidium. Note the hair cells (HC) in close proximity. x122. FIG. 16. The highly magnified external mantle surface view showing numerous microvilli (HV) and the micropits (P) among the microvilli. The bar indicates 1 μm. x8,137. FIG. 17. A glochidium showing the positions of the hair cells protruding from the mantle. x81. FIG. 18. A part of the mantle showing the two hair cells (HC) with a bunch of hairs under the hook (H). x1,139.



FIGS. 19-22. Glochidia of *Anodonta arcaeformis flavotincta* (cont.). FIG. 19. Cross-section of the mantle, showing a canal (C) containing a larval thread (T). The surface epithelium has many microvilli (MV), as does the surface of the lumen of the canal containing the larval thread. The bar indicates 1 μm. x7,540. FIG. 20. Cross-section of the larval thread in the canal shown in Fig. 19. The thread is not bounded by a membrane and its internal matrix contains only numerous microtubule-like structures. The bar indicates 1 μm. x26,634. FIG. 21. Cross section of the mantle and larval thread (T). The outer mantle epithelium (OM) is connected with the intra mantle muscle (Mu). x6,556. FIG. 22. Cross section of the inner mantle epithelium (IM) connected with the inner crystalline layer of the shell valve. x6,884.

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Two types of hair cells were observed in the mantle. One type consists of many cells grouped together in the posterior margin of the mantle that process numerous hairs covering their free surface (Fig. 14). The other type consists of a total of three hair cells per mantle. Two of the cells are positioned at the basal part of the hook in the peripheral mantle and the other cell is located very close to the ventral plate of the mantle 50 μm from the thread canal (Figs. 13-15, 17, 18). Near the posterior margin of each mantle, there is a large pit, called the lateral pit (Figs. 13, 14).

DISCUSSION

Two well marked types of glochidia have been known in the Unionidae for a long time. One type has a stout hook on the ventral margin of the valve, while the other type is entirely hookless and has a quite different shape (Lefevre & Curtis, 1910). Mussels of the genus *Anodonta* have glochidia equipped with hooks. The structure of the glochidia of various *Anodonta* species have been described by earlier researchers (*e.g.*, Harmes; 1909, Surber; 1912, 1915, Arey; 1924, 1932a,b,c, Brondiewicz; 1968, Wood; 1974a,b).

Wood (1974a) made a very detailed report on structures of the glochidium of *Anodonta cygnea* as seen with the light microscope. We have followed her terminology. Ultrastructural studies of this species were performed by Zs.-Nagy & Lábos (1969) on the adductor muscle and nervous elements, and by Giusti (1973) and Giusti *et al.* (1975) on the shells.

Our study focused on the external and internal structures of the shell and [illegible] *flavotincta* as observed with the SEM and TEM.

In specimen preparation for the SEM observations, we applied a microwave oven to fix the specimens. Hopwood *et al.* (1984) stated that the tissue fixed by using the microwave at 50°C was reasonably well preserved and certainly better than by heating alone, although there was some evidence of damage. In our study, the glochidia in tap water were fixed with a microwave oven at 53°C. When the glochidia were anesthetized with menthol before fixation, they usually secreted a large amount of mucus over the mantle surface. As well known to all SEM workers, the cleaning of the target area of observation is the main problem in the process of preparing specimens. We obtained satisfactory specimens that were free of mucus by using the microwave fixation method.

The shape of the glochidium of *Anodonta arcaeformis flavotincta* is triangular and unequilateral, as described for other species of *Anodonta* (Brondiewicz, 1968; Wood, 1974a,b; Giusti, 1973; and Giusti *et al.*, 1975). The size of the glochidium of *Anodonta arcaeformis flavotincta* is 0.47 mm x 0.40 mm.

The glochidial shell valves, which are identical but mirror images, are attached by a ligament 130 μm in length.

The external surfaces of the shell valves are covered with numerous processes similar to those reported for *Anodonta cygnea* (Giusti *et al.*, 1975) and *Anodonta grandis* (Jeong, 1989), although the patterns of distribution of the processes are different. The surfaces of the glochidial shell valves possess a number of scattered pits (Fig. 7). As mentioned for *Anodonta cygnea* (Giusti *et al.*, 1975) and *Anodonta grandis* (Jeong, 1989), the pits formed on the pellicle result from covering the holes of the internal layer of the valves.

The larval thread is positioned in the middle of the mantle, ventral center to the hinge line of the shell valves, and seems to be in a suitable position to maintain the balance of the glochidial body when pulled.

The diameters of the larval threads were believed to be uniform from their bases to their tips, between individuals and within a particular species (Jeong, 1989), but in *Anodonta arcaeformis flavotincta* the diameters of the larval threads are variable.

Wood (1974a) reported that the larval thread of *Anodonta cygnea* was a non-cellular structure of a mucoid nature that gave a positive periodic acid Schiff reaction (PAS). Our TEM results support Wood's report – the larval thread is non-cellular and is not bounded by a membrane. The matrix of the larval thread does not contain any organelles other than numerous microtubule-like structures.

Two types of the hair cells are found in the mantle of the glochidium. One type of hair cell consists of three solitarily positioned, highly specialized cells in the mantle. Each cell possesses a bunch of the protruding hairs. This type of cell presumably perceives chemical stimuli (Wood, 1974a). The function of the other type of hair cells, which are located in a group in the posterior margin near the lateral pits, is unknown.

The microvilli exiting on the free surface of the mantle and on the luminal surface of the canal, which number about 15 per mm^2, presumably function in absorption of nutrients. The micropits found among the microvilli also may absorb various materials, including nutrients, from the outside.

LITERATURE CITED

AREY, L.B. 1924. Glochidial cuticulae, teeth, and the mechanics of attachment. *Journal of Morphology*, 39: 323-335.

AREY, L.B. 1932a. Certain basic principles of wound healing. *Anatomical Record*, 51: 209-212-221.

AREY, L.B. 1932c. The nutrition of glochidia during metamorphosis. *Journal of Morphology*, 53(1): 201-221.

BRONDIEWICZ, I. 1968. On the glochidia of the genera *Unio* and *Anodonta* from the Quaternary fresh-water sediments of Poland. *Acta Palaeontologica Polonica*, 13(4): 619-630.

GIUSTI, F. 1973. The minute shell structure of the glochidium of some species of the genera *Unio, Potomida* and *Anodonta* (Bivalvia, Unionacea). *Malacologia*, 14: 291-301.

GIUSTI, F., CATAGNOLO, L., MORETTI, L. & RENZONI, A. 1975. The reproductive cycle and the glochidium of *Anodonta cygnea* L. from Lago, Trasimeno (Central Italy). *Monitore Zoologico Italiano* (N.S.), 9: 99-118.

HARMS,W. 1909. Postembryonale Entwicklungsgeschichte der Unioniden. *Zoologische Jahrbücher (Anatomie ...)*, 28: 2-386.

HOPWOOD, D., COGHILL, G., RAMSEY, J., MILNE, G. & KERR, M. 1984. Microwave fixation: its potential for routine techniques, histochemistry, immunochemistry and electronmicroscopy. *Histochemical Journal*, 16(11): 1171-1192.

JEONG, K.H. 1989. An ultrastructural study on the glochidium and glochidial encystment on the host fish. *Korean Journal of Malacology*, 5(1): 1-19.

LEFEVRE, G. & CURTIS, W.C. 1910. Studies of the reproduction and artificial propagation of fresh-water mussels. *Bulletin of the United States Bureau of Fisheries*, 30: 105-201.

SUBER, T. 1912. Identification of the glochidia of freshwater mussels. *Bulletin of the United States Bureau of Fisheries*, Document No. 771: 1-10.

SURBER, T. 1915. Identification of the glochidia of freshwater mussels. *Bulletin of the United States Bureau of Fisheries*, Appendix V to Report of the United States Commissioner of Fisheries for 1914, Document No.813: 1-9.

WOOD, E.M. 1974a. Development and morphology of the glochidium larva of *Anodonta cygnea* (Mollusca: Bivalvia). *Journal of Zoology*, London, 173: 1-13.

WOOD, E.M. 1974b. Some mechanisms involved in host recognition and attachment of the glochidium larva of *Anodonta* cygnea (Mollusca: Bivalvia). *Journal of Zoology*, London, 173: 15-30.

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ZS.-NAGY, I. & LÁBOS, E. 1969. Light and electron microscopical investigation on the adductor muscle and nervous elements in the larva of *Anodonta cygnea* L. *Annals Biologica Tihany*, 36: 123-133.

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