Malacological Review, 1993, 26: 63-70

SCANNING ELECTRON MICROSCOPE STUDIES OF THE MINUTE SHELL STRUCTURE OF GLOCHIDIA OF THREE SPECIES OF UNIONIDAE (BIVALVIA) FROM KOREA

Oh-Kil Kwon, Gab-Man Park, Jun-Sang Lee and Ho-Bok Song

ABSTRACT

The shell structures of the glochidia of *Anodonta woodiana*, *Anodonta arcaeformis flavotincta* and *Unio douglasiae* were studied with the scanning electron microscope. These glochidia were removed from adult unionids which were collected from Uiam Lake near Chun Cheon, Korea. The number and the disposition of spines and the sculpture of the shell surface were different from one species to another. We believe that the glochidial morphology will reveal characters useful for the classification of different species and for the clarification of interrelations between genera.

Key words: *Anodonta woodiana*, *A. arcaeformis flavotincta*, *Unio douglasiae*, glochidia, microsculpture.

INTRODUCTION

One of the characteristics of the Unionidae, including those of Korea, is that adult females retain their larvae (glochidia) in their demibranchs and later release them, whereupon, if successful, the glochidia attach to fish and remain there as parasites for about one month (Kwon & Choi, 1982). Wood (1974) has described the development and morphology of the glochidia of *Anodonta cygnea* Linnaeus and Lillie (1895), Lefevre & Curtis (1910) and Ney (1954) have described the glochidia of other unionids. In these papers, the descriptions of surface characteristics of the glochidia were based on the use of a light microscope. However, the scanning electron microscope (SEM) can show the details much clearer.

Using the SEM, Giusti (1973) showed that the attachment structure in both valves of the Unionidae that he studied was situated on the anterior apex and was comprised of numerous pointed spines located at the margin. He did not provide information about the closing mechanism which fastens the spines firmly into the host fish. That process is elaborated in the present work (Figs. 16, 17). The glochidial morphology and structures of *Anodonta* (Rand & Wiles, 1982), and of the species of the Tribe Alasmidontini (Clarke, 1981, 1985), have been studied by SEM with special emphasis on details of the shell valves, terminal plate and valvular (shell) pores. Yet the descriptions of the shell pores of the species have not included both external and internal shell pores, although they are probably distinctive according to species.

Our current work consists of an investigation of glochidial morphology, using SEM, with special emphasis on the structure of the ligament, the pores on the external and internal shell surfaces, the terminal plates and the spines.

MATERIALS AND METHODS

Adult mussels were collected during the period from March 15, 1987 to December 15, 1987 from Uiam Lake near Chun Cheon City, Korea. The matured glochidia were recovered from demibranchs of gravid *Anodonta (Sinanodonta) woodiana* (Lea 1834), *A. (Anemia) arcaeformis flavotincta* (Martens 1905) and *Unio (Nodularia) douglasiae* (Gray 1834) and fixed in 70% alcohol.

The alcohol-fixed specimens were transferred to vials of distilled water to remove the alcohol. Within an hour the liquid was removed from the vial with a transfer pipette and 5% potassium hydroxide (KOH) solution was added. Storage overnight (12 hrs.) in this solution generally disintegrated the tissues from the shells. Prior to dehydration, the potassium hydroxide was removed

from the sample with distilled water. After the distilled water was added the vials were capped tightly and shaken vigorously to wash the tissues out of the shells and to clean the shell surfaces (Calloway and Turner, 1978). The liquid was then removed, fresh distilled water was added, and the entire process was repeated until no debris remained.

The specimens were then dehydrated in a graded series of alcohol (five minutes in each of the following: 10%, 40%, 80%, 90%, 95% and three washes in 100% alcohol). The glochidia were then mounted on specimen stubs and viewed by SEM. Five specimens of each species were studied. Voucher specimens have been placed in the Museum of Zoology, Kangweon National University, Korea, National Science Museum, Tokyo, Japan, and the Bernice P. Bishop Museum, Honolulu, Hawaii, U.S.A.

RESULTS

The glochidia of *Anodonta woodiana, A. arcaeformis flavotincta* and *Unio douglasiae* were subtriangular and possessed two valves which are mirror images of each other. The valves of each pair are held together by a ligament (Fig. 3; see also Fig. 1 (lateral view) and Figs. 2, 9, 13 (ventral views)). Even at low magnification with SEM, it was possible to make out that the external had numerous evenly-distributed pores (Fig. 1).

At high magnification, many pores can be seen on both the external and internal shell surfaces. Species' differences were also clearly apparent. On internal shell surfaces, *Anodonta woodiana* exhibited many irregular pores and smooth surfaces (Fig. 11), whereas *Unio douglasiae* had only a few pores and smooth surfaces (Fig. 18). On their external shell surfaces, *A. woodiana* showed many regular pores (Fig. 6) with "reticulated skin" (Figs. 7, 8), *A. arcaeformis flavotincta* had a few pores and smooth surfaces (Fig. 12), and *U. douglasiae* exhibited no pores but had numerous "processes" (Fig. 19).

Numerous small and large spines were seen on the terminal plates of all three species (Figs. 4, 10, 14), as well as on their outer margins (Fig. 17). The most densely packed and smallest spines were found in *Unio douglasiae* and the longest [illegible] (Fig. 10). The large spines were centrally arranged in two uneven rows in *A. woodiana* (Fig. 4), in three uneven rows in *A. arcaeformis flavotincta* (Fig. 10), and in four rows in *U. douglasiae* (Fig. 14). There were gradients of sizes between the largest and the shortest of the large spines in all three species.

Figs. 16 and 17 show the mechanism of closing the shell and folding the margin toward the inside to fasten the spines firmly into the tissues of the host fish.

The number and size of the pores on both the internal and external shell surfaces, and the densities, sizes and arrangements of spines were drastically different between the three species. These characteristics of glochidia should be studied for their possible use in the classification of Unionidae.

DISCUSSION

Although glochidia have been studied for a long time, there are no adequate keys for their identification, and identification of the glochidial parasites recovered from the host fish is almost impossible. Our current work shows, however, that details of the fine structure of glochidia exist which can be used for positive identification of Korean species.

Unionid glochidia can be separated into three groups: "axe-head," "hookless," and "hooked" (Rand & Wiles, 1982). Specimens we studied were all "hooked" larvae, and exhibited a terminal plate (also called a hook, a tooth, a spine, or a stylet) with spines (also called protuberances, setae or microstylets) on each valve.

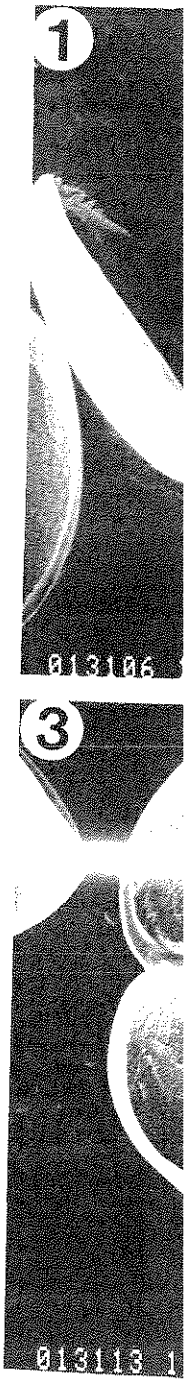


FIG. 1. Late
external shell p
and pores. FIG
Terminal plate
spines.

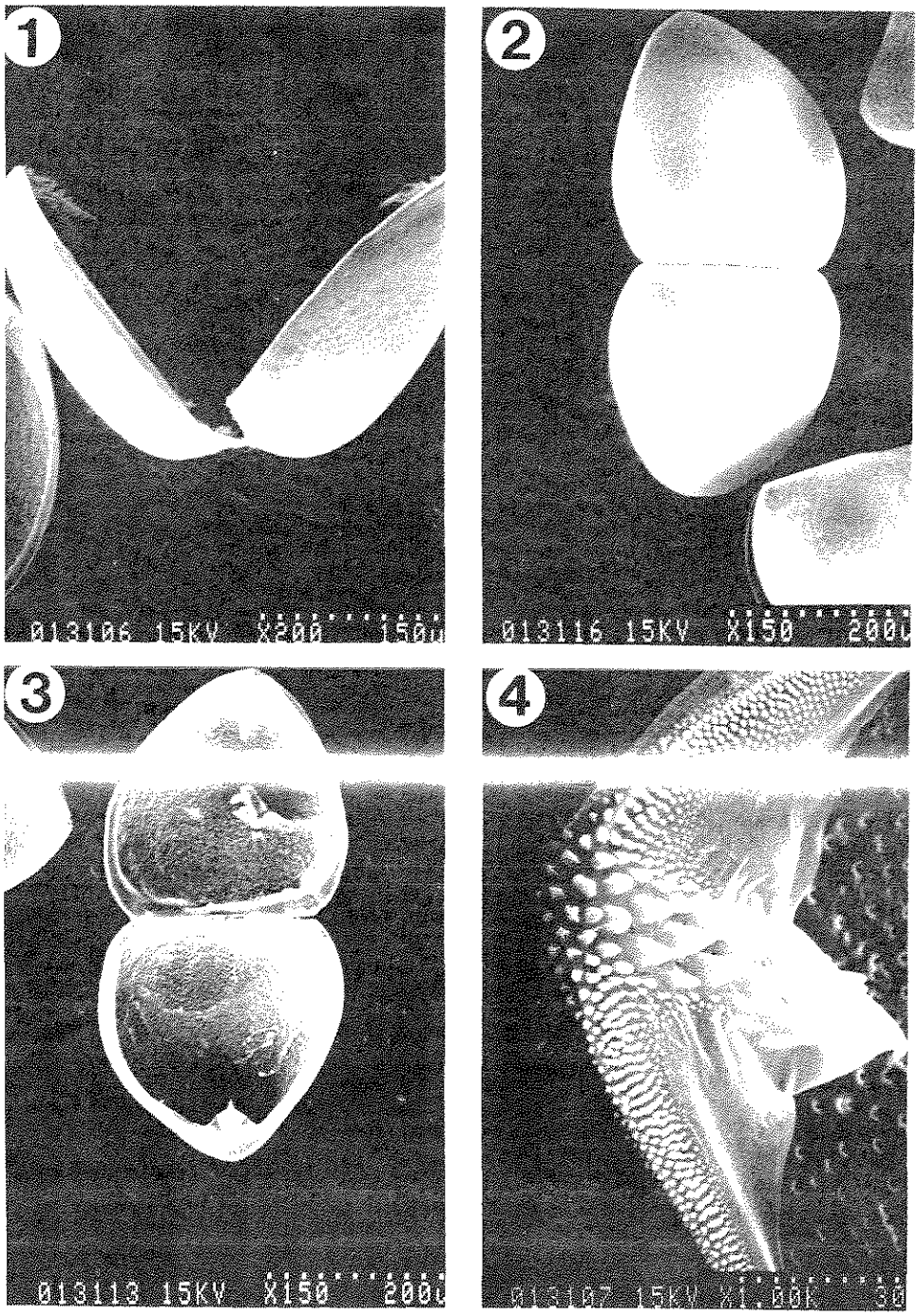
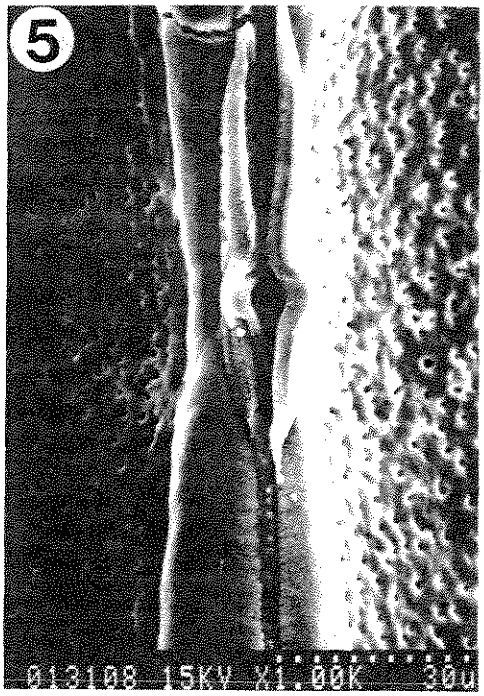
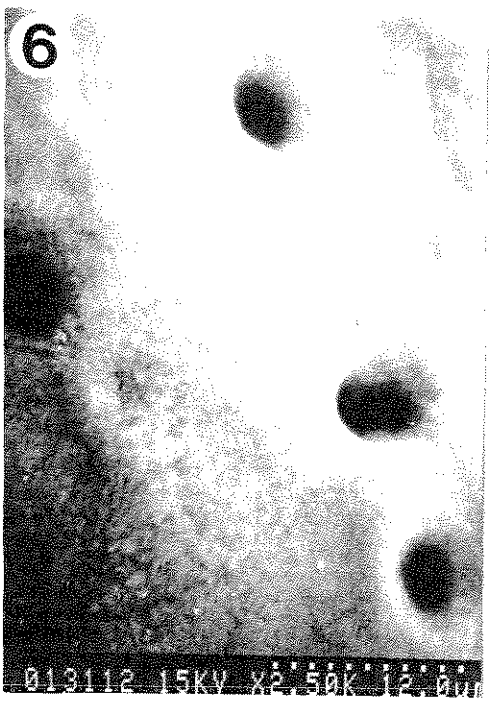
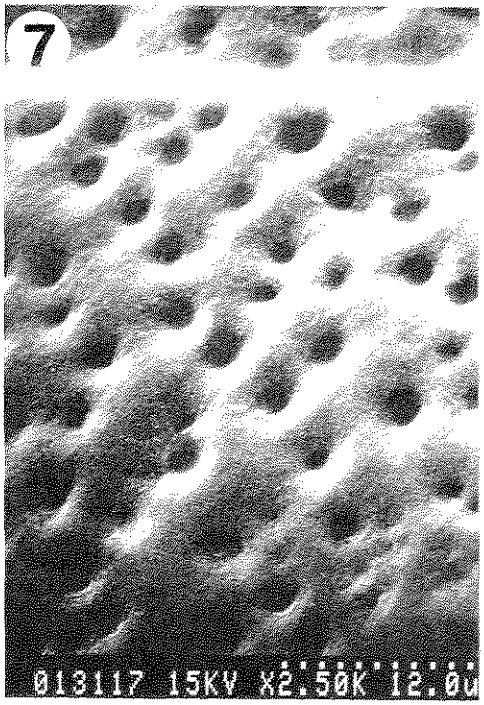


FIG. 1. Lateral view of glochidium of *Anodonta woodiana*, showing terminal plate and external shell pores. FIG. 2. Internal shell surface of *Anodonta woodiana*, showing spines and pores. FIG. 3. Ligament of *Anodonta woodiana* holding together two valves. FIG. 4. Terminal plate of *Anodonta woodiana* with distinctive arrangement of small and large spines.







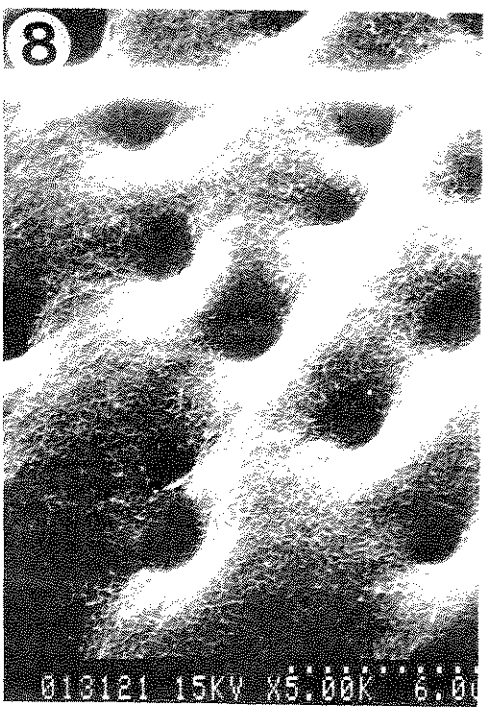
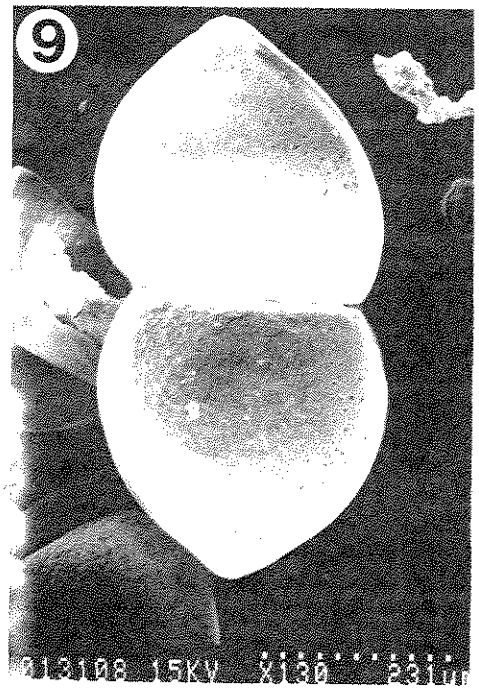
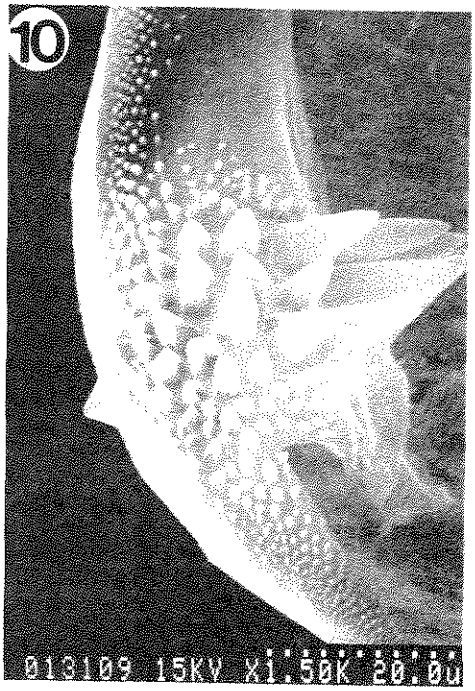


FIG. 5. Internal surface of *Anodonta woodiana*, showing rough surface and irregular pores. FIGS. 6, 7, 8. External surface of *Anodonta woodiana*, showing smooth surface and pores with "reticulated skin."

FIG. 9. Int
spines and por
spines. (Compa
ta with rough-e





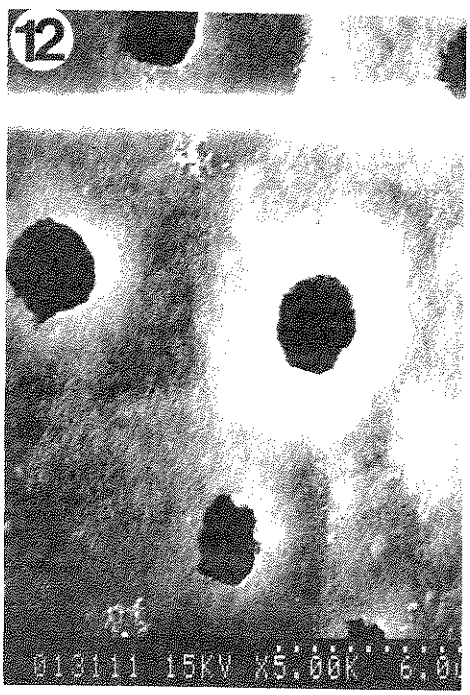
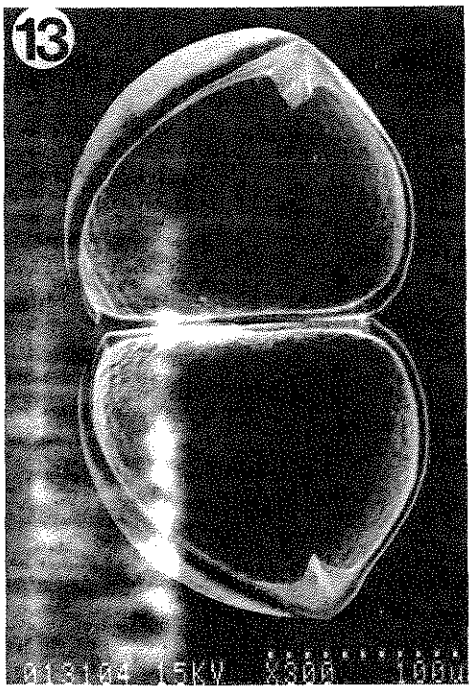
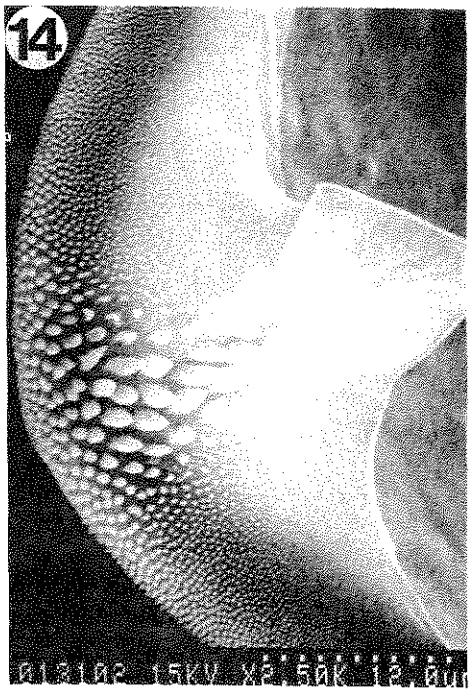
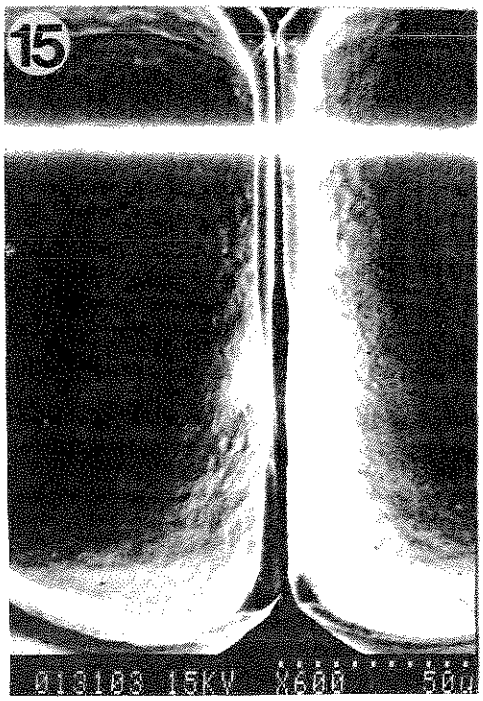
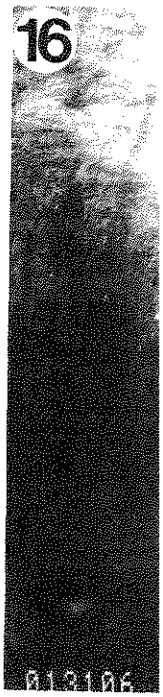


FIG. 9. Internal surface of *Anodonta arcaeformis flavotincta* with terminal plate, spines and pores. FIG. 10. Terminal plate of *A. a. flavotincta* with small and large spines. (Compare with *A. a. flavotincta*, Fig. 4). FIG. 11. Internal surface of *A. a. flavotincta* with rough-edged pores. Fig. 12. External surface of *A. a. flavotincta* with pores.









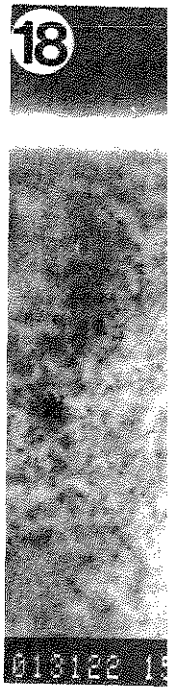
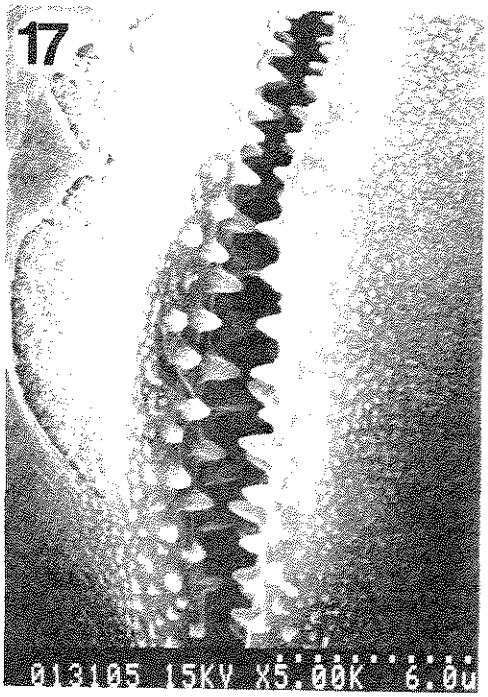
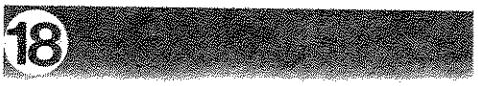
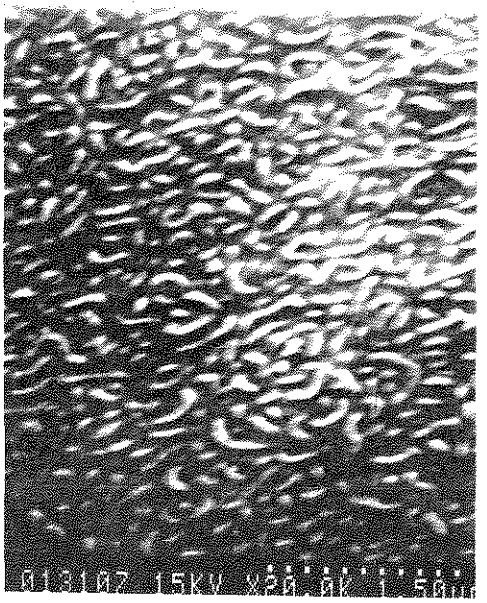


Fig. 13. Internal surface of *Unio douglasiae*, showing terminal plate with spines. Fig. 14. Terminal plate of *Unio douglasiae* with large and small spines, showing differences from that of *Anodonta woodiana* and *A. arcaeformis flavotincta*. Fig. 15. Enlargement of hinge area of *Unio douglasiae*.

Figs. 16, 17
mechanism of
pores. Fig. 19.







Figs. 16, 17. Marginal spines, on the anterior apex, of *Unio douglasiae* showing closing mechanism of glochidia. Fig. 18. Internal surface of *Unio douglasiae*, showing few and small pores. Fig. 19. External surface of *Unio douglasiae*, showing the absence of pores.

By using SEM, the morphology and the density of the pores on the shell surface were examined. Complete perforation of shell (Fig. 11) indicates that the encysted glochidia may exchange gases and obtain nutrients from host tissues, within which they are believed to be fed and undergo organogenesis (Coker *et al.*, 1921). Although some species appear not to be completely perforated, the numerous shell pores on both surfaces (Figs. 8, 18) increase the areas of the shell surface and enhance the clam's abilities to exchange gases.

There are many problems and difficulties inherent in the further study of the unionid glochidia. In addition to the characters studied here, the presence or absence of a larval thread and sensory tuft (also called sensory hairs) should be investigated. We believe that it would be useful to continue this study and to investigate other species with the scanning electron microscope.

ACKNOWLEDGEMENTS

This study was supported by the Fund of the Ministry of Education (Korean). We thank J.B. Burch for editing the manuscript.

LITERATURE CITED

AREY, L. 1924. Glockenspiel cuticles, teeth and mechanics of encyst. *Journal of Morphology*, 39: 323-335.

CALLOW, C.A.B.. & TURNER, R.I.P.. 1978. New techniques for preparing the shells of Bivalvia larvae for examination with the scanning electron microscope. *Bulletin of the American Malacological Union*, pp. 17-24.

CLARKE, A.H. 1981. The Tribe Alasmidontini (Unionidae: Anodontinae), Part I: *Pegias, Alasmidonta* and *Ancidens*. *Smithsonian Contributions to Zoology*, (326): 1-101, 32 figs.

CLARKE, A.H. 1985. The Tribe Alasmidontini (Unionidae: Anodontinae), Part II: *Lasmigona* and *Simpsonaias*. *Smithsonian Contributions to Zoology*, No. 399: 1-75, 22 figs.

COKER, R.E., CLARKE, H.W., SHIRA, A.F. & HOWARD, A.D. 1921. Natural history and propa-

[illegible] the surface shell structure of the glochidium of some species of the genera *Unio potomida* and *Anodonta* (Bivalvia: Unionacea). *Malacologia*, 14: 291-301.

KWON, O.K. & CHOI, J.K. 1982. The studies of the fresh-water mollusks in the lake Uiam and swimming-out of pre-larval fish from mussels. *Korean Journal of Limnology*, 15(1-2): 39-50.

LEFEVRE, G. & CURTIS, W.C. 1910. Reproduction and parasitism in the Unionidae. *Journal of Experimental Zoology*, 9: 79-115.

LILLIE, F.R. 1895. Embryology of the Unionidae. A study in cell lineage. *Journal of Morphology*, 10: 1-100.

RAND, T.G. & WILES, M. 1982. Species differentiation of the glochidia of *Anodonta cataracta* Say, 1817 and *Anodonta implicata* Say, 1829 (Mollusca: Unionidae) by scanning electron microscopy. *Canadian Journal of Zoology*, 60: 1722-1727.

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

Oh-Kil KWON, Gab-Man PARK, Jun-Sang LEE and Ho-Bok SONG

Department of Biology
Kangweon National University
Chuncheon, 200-701, Korea

Malacological Review, P.O. Box 3037, Ann Arbor, Michigan 48106, U.S.A.