Species differentiation of the glochidia of *Anodonta cataracta* Say, 1817 and *Anodonta implicata* Say, 1829 (Mollusca: Unionidae) by scanning electron microscopy

THOMAS G. RAND[1] AND MICHAEL WILES
Department of Biology, Saint Mary's University, Halifax, N.S., Canada B3H 3C3

Received October 9, 1981

RAND, T. G., and M. WILES. 1982. Species differentiation of the glochidia of *Anodonta cataracta* Say, 1817 and *Anodonta implicata* Say, 1829 (Mollusca: Unionidae) by scanning electron microscopy. Can. J. Zool. **60**: 1722–1727.

Morphology of shell valves, shell valve terminal plates, marginal and plate protuberances, sensory tufts, and valvular pore was studied for usefulness in congeneric differentiation of glochidia of *Anodonta cataracta* Say, 1817 and *A. implicata* Say, 1829 from two lakes in Nova Scotia. Investigation was with a Cambridge S150 scanning electron microscope operated at 10 kV. Shell valve and terminal plate structures and spatial and size frequency distributions of plate protuberances allow discrimination of these two species of *Anodonta*. The tubercle-like protuberances probably aid attachment to fish hosts, sensory tufts aid in detection of host and environmental stimuli, and valvular pores may function in nutrition and respiration of glochidia. Possible function of valvular pores was inferred from the observation that they penetrate mantle and valves completely.

RAND, T. G., et M. WILES. 1982. Species differentiation of the glochidia of *Anodonta cataracta* Say, 1817 and *Anodonta implicata* Say, 1829 (Mollusca: Unionidae) by scanning electron microscopy. Can. J. Zool. **60**: 1722–1727.

La morphologie de certaines structures a fait l'objet d'une étude détaillée chez *Anodonta cataracta* Say, 1817 et *A. implicata* Say, 1829 en deux lacs de Nouvelle-Ecosse, afin d'établir l'utilité de ces structures dans la différenciation congénérique des glochidies; ce sont: les valves de la coquille, les plaques terminales des valves, les protubérances sur les plaques et en bordure, les touffes sensorielles et les pores valvulaires. Un microscope électronique à balayage Cambridge S150 opéré à 10 kV a servi à l'étude. Les valves de la coquille et les structures des plaques terminales de même que la répartition et la distribution de fréquence des tailles des protubérances des plaques permettent de distinguer les deux espèces d'*Anodonta*. Les protubérances semblables à des tubercules favorisent probablement l'attachment au poisson-hôte; les touffes sensorielles servent probablement à repérer l'hôte et à détecter les stimulus du milieu; les pores valvulaires jouent sans doute un rôle dans la nutrition et la respiration des glochidies puisqu'ils traversent entièrement le manteau et les valves.

[Traduit par le journal]

Introduction

In a statistical study of shell valve morphometrics of freshwater Nova Scotian unionid glochidia, Wiles (1975) showed that the larvae of the two species *Anodonta cataracta* Say, 1817 and *A. implicata* Say, 1829 are morphologically similar when viewed with the light microscope. However, in this study it was established that the glochidial shell valves of these two species are morphometrically distinguishable by a comparison of mean length with mean breadth.

Morphological descriptions of glochidia available in the literature (Coker *et al.* 1921; Surber 1915) are inadequate for identification of species, allowing only the differentiation of genera.

The use of the scanning electron microscope (SEM) has become increasingly important in describing surface structures of parasites, yet there is only one previously published study on the surface characteristics of glochidia, that by Atkins (1979) on *Hyridella (Hyridella) drapeta* (Iredale), of the family Hyriidae. Glochidia in this family are rather different from those in the Unionidae.

[1]Present address: Bermuda Aquarium, Museum and Zoo, P.O. Box 145, Flatts Smith's 3, Bermuda.

The current work consists of an investigation of glochidial morphology and structures using SEM, with special emphasis on shell valves, shell valve terminal plates, marginal and plate protuberances, sensory tufts, and valvular pores. The functions of protuberances and pores are discussed and the morphological differences between glochidia of two species of *Anodonta* are shown.

Materials and methods

Advanced glochidia were recovered from marsupia of gravid *A. cataracta* and *A. implicata* from Shubenacadie (44°58′ N; 63°34′ W) and Ponhook (44°55′ N; 64°00′ W) lakes, Hants County, Nova Scotia. Adult clams were collected during the period November 8, 1970 to August 8, 1974, and were expeditiously fixed and stored in cold 70% isopropyl alcohol.

Identification of adults was based on shell valve morphology and on comparisons of specimens with the line drawings of Athearn and Clarke (1962), Clarke and Berg (1959), Johnson (1946), Matteson (1948), and Ortmann (1911). Shell valve length to breadth ratios were compared and tested statistically for significant differences.

Excised pieces of marsupia from the alcohol-fixed specimens of each clam species were secondarily fixed for 2 h in 1.5% osmium tetroxide in a 0.1 *M* phosphate buffer, pH 7.4.

0008-4301/82/071722-06$01.00/0

TABLE 1. Comparison of mean length and breadth measurements of advanced marsupial glochidia of *A. cataracta* and *A. implicata*

Species	No. measured	Mean length	SD	Mean breadth	SD	df	t	P
A. cataracta	209	382.00	27.15	383.11	26.44	416	0.423	0.6
A. implicata	84	345.82	16.55	345.48	13.98	166	0.144	0.5

TABLE 2. Comparison of the mean lengths of the large protuberances on the terminal, ventral plates of *A. cataracta* and *A. implicata*

			Approximate t-test statistics			Wilcoxon two-sample (Mann–Whitney) test		
Species	No. measured	Mean length (μm)	SD	t_s'*	P	U_s†	t_s	P
A. cataracta	29	15.64	±3.500	5.68	0.001	784.5	6.87	0.001
A. implicata	26	11.16	±2.284					

*Because of heteroscedasticity, the t_s' value was calculated using the approximate t-test formula: $t_s' = \pm(\bar{Y}_1 - \bar{Y}_2) - (\mu_1 - \mu_2)/\sqrt{(S_1^2/n_1) + (S_2^2/n_2)}$.

†U_s was calculated as C or $(n_1 \cdot n_2) - C$, whichever was greater, using the formula $C = [n_1 \cdot n_2 + n_2(n_2 + 1)/2] - \sum^{n_2} R$, where n_1 is the size of the larger sample (= 29). Then $t_s = U_s - (n_1 \cdot n_2/2)/\sqrt{n_1 \cdot n_2(n_1 + n_2 + 1)/12}$.

Glochidia were later freed from maternal tissues by shaking, collected in Pasteur pipettes, placed into Reichart "flo-thro" specimen holders, and dehydrated in a graded ethanol series. Ethanol was replaced by acetone and critical drying was through liquid CO_2 in a Sorvall critical-point drying apparatus. The glochidia were mounted on SEM specimen stubs with conductive silver paint, coated with approximately 100 nm of gold with a water-cooled, Nanotech gold sputtering unit (model SEMPREP 2) and viewed in a Cambridge S150 SEM with lanthium hexaboride illumination and operated at 10 kV.

Ilford F.P.4, 70-mm roll film was used for photomicrography as this type produces excellent results with the Cambridge S150 SEM.

Results

Description of glochidia

Anodonta cataracta Say, 1817

Glochidia are subtriangular in lateral view (Fig. 1). Mean length is not significantly different from mean breadth (Table 1). Externally, ventral ends of the valves are tapered but rounded. The external lateral surface of each valve is finely sculptured and penetrated by pores 1.83 ± 0.25 μm in mean diameter. Each pore is surrounded by a wall 1.93 ± 0.08 μm thick. The mantle has pores through it 1.87 ± 0.51 μm in mean diameter (Fig. 2). The coefficient of dispersion index (based on the use of the Poisson distribution) for the mantle pores indicates that they are distributed uniformly over the mantle. The ventral valve margin is ribbed (Fig. 3) and armed externally with small protuberances 2.03 ± 0.86 μm in mean length. The ventral margins are also ribbed and armed internally (Fig. 4). At the ventral margins of both valves there is a prominent triangular plate 129.97 ± 10.72 μm long (Fig. 4) which is widest proximally and tapers distally to a sharp point. This plate is armed with large protuberances (Table 2). The largest of these are arranged into three rows proximally but only the central row extends to near the distal tip of the plate. There are also small protuberances of 2.47 ± 0.58 μm mean length on the plate and valve margins, and there is a discontinuity in lengths between the large and small ones. There are three sensory tufts lying adjacent to the distal tip of the triangular plate. A larval thread was seen in all specimens of this species.

Anodonta implicata Say, 1829

The glochidia are subtriangular in lateral view (Fig. 5). Their length is the same as their breadth (Table 1). The ventral end of the shell is tapered but noticably less rounded than in *A. cataracta*. The external lateral surface of each valve is finely sculptured and has pores 1.69 ± 0.37 μm in mean diameter (Fig. 6). The pore walls average 0.95 ± 0.20 μm thick and are significantly thinner (t = 5.71, df = 21) than those of *A. cataracta*. The mantle has pores through it 1.75 ± 0.46 μm in mean diameter. The coefficient of dispersion index for the mantle pores indicates that they are distributed uniformly in this tissue. The external surfaces of both valves are ribbed and armed ventrally with small protuberances with a mean length of 2.03 ± 0.86 μm. The other margins of the valves are also ribbed and armed. There is an armed triangular plate 78.83 ± 7.64 μm long in this species. The plate is in a similar position to and of similar shape as that of *A. cataracta*.

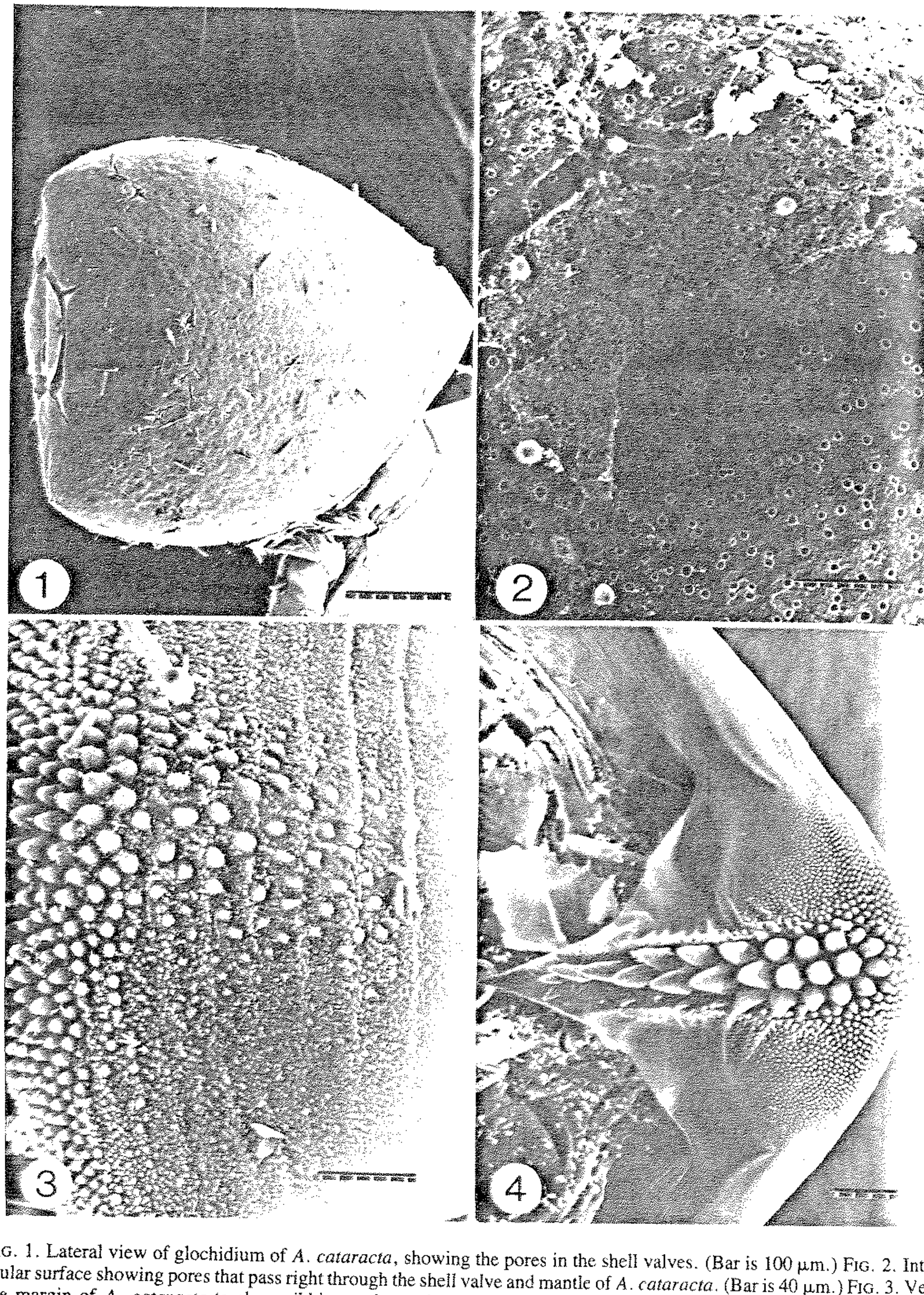
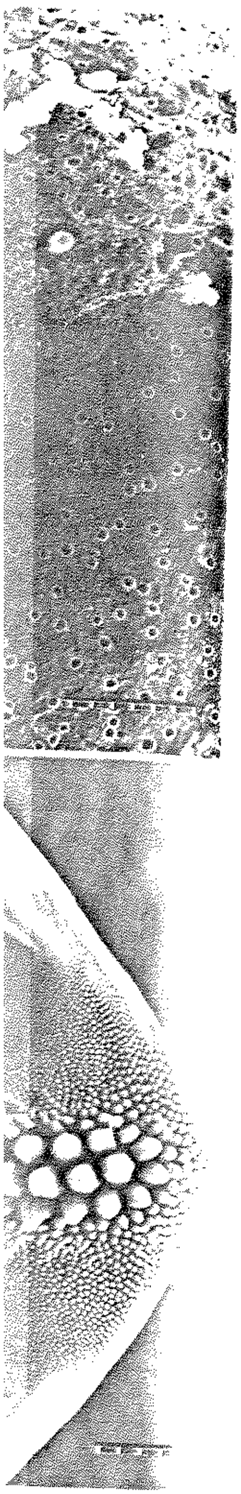


FIG. 1. Lateral view of glochidium of *A. cataracta*, showing the pores in the shell valves. (Bar is 100 μm.) FIG. 2. Internal valvular surface showing pores that pass right through the shell valve and mantle of *A. cataracta*. (Bar is 40 μm.) FIG. 3. Ventral valve margin of *A. cataracta* to show ribbing and protuberances on the external valvular surface. (Bar is 10 μm.) FIG. 4. Terminal, ventral plate of *A. cataracta* with its distinctive arrangement of large protuberances in three major rows. (Bar is 20 μm.)



100 μm.) FIG. 2. Interna
is 40 μm.) FIG. 3. Ventra
(Bar is 10 μm.) FIG. 4
hree major rows. (Bar is

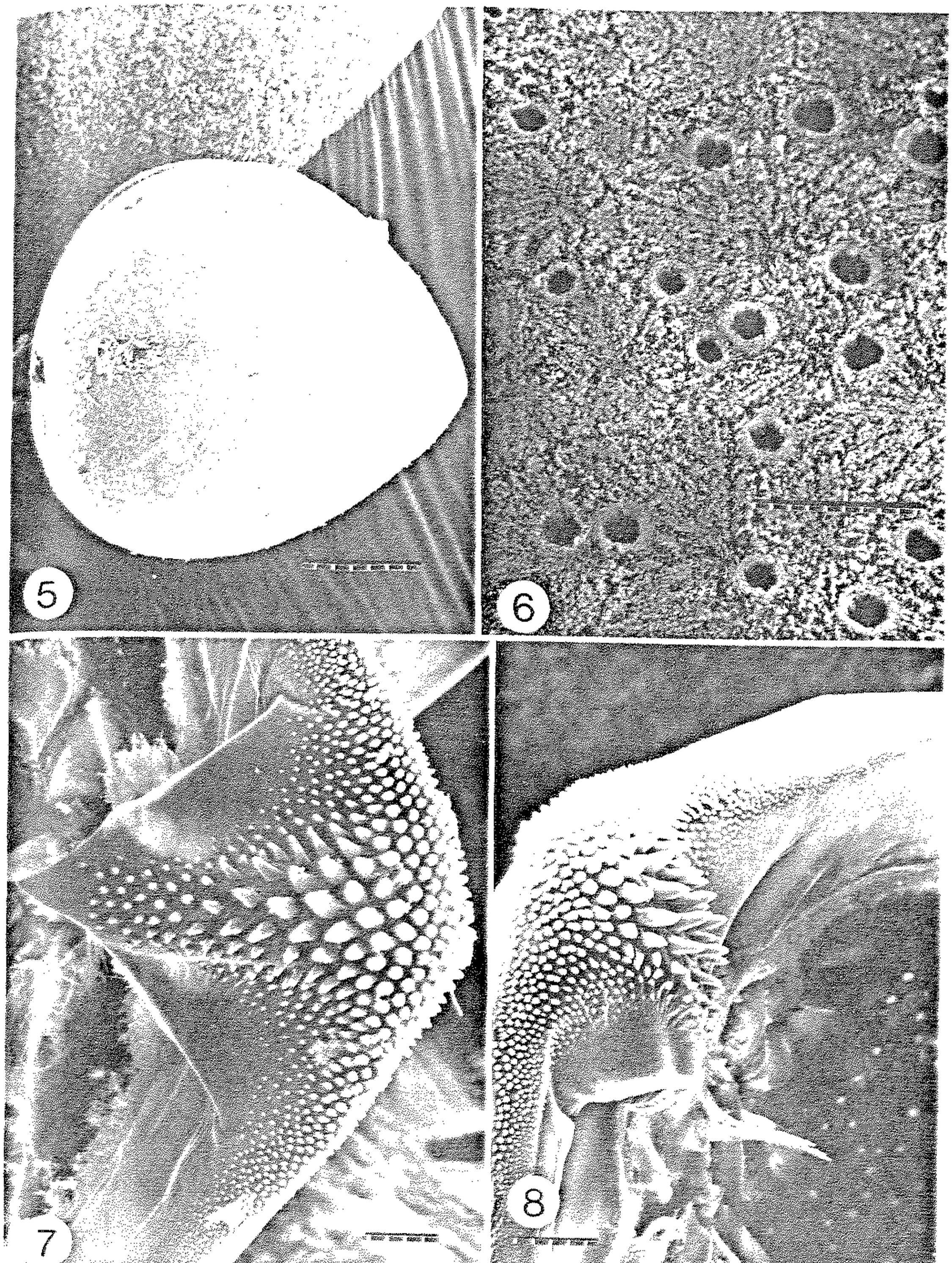


FIG. 5. Lateral view of glochidium of *A. implicata*. (Bar is 100 μm.) FIG. 6. Ventral valve margin of *A. implicata* to show valvular pores on the external surface. (Bar is 10 μm.) FIG. 7. Terminal, ventral plate of *A. implicata* to show how the arrangement and size of the large protuberances differ from those on *A. cataracta*. (Bar is 20 μm.) FIG. 8. Sensory tufts associated with the mantle cavity of *A. implicata*. (Bar is 20 μm.)

However, the arrangement of the large protuberances is different from that in *A. cataracta* (Fig. 7) and they are significantly shorter (Table 2). The largest protuberances are centrally arranged in four uneven rows and do not extend as far distally as they do on the plate of *A. cataracta*. There is a gradient of sizes between the largest and shortest of the large protuberances on the plate, in contrast to the discontinuity between long and short ones in *A. cataracta*. There are three sensory tufts lying adjacent to the distal tip of each triangular plate (Fig. 8). Although thousands of specimens taken from several different adults were carefully examined, no glochidium of this species was seen with a larval thread.

Discussion

Glochidia were studied in Europe as early as 1695 by Leeuwenhoek (Coker *et al.* 1921). They were first extensively studied in the United States by Lillie (1895) and later by Lefevre and Curtis (1910*a*, 1910*b*, 1912). Although these parasites have been known for a long time, identification of glochidia to species within a genus is still a formidable problem where congenerics occur. Most reports of glochidia recovered from their hosts are presented as *Glochidium* sp. (Margolis and Arthur 1979) which is an incomplete nomenclature. This practice tells nothing about the identity of the parent clam and many taxonomic problems remain unsolved for glochidia (Fryer 1970). Additionally, because there is often considerable diversity in size and shape of glochidia even within one genus (Lefevre and Curtis 1912; Ortmann 1919), there are no adequate keys for identification of species of glochidia. Although Surber (1912) constructed a key to glochidia based on dimensions and overall shape, these characteristics are inadequte for use at the species level.

Unionid glochidia are separable into "axe-head," "hookless," and "hooked" groups, all easily distinguishable. *Anodonta* produces hooked larvae which have on each valve a terminal plate (also called a tooth, or a hook or a spine in the literature). In the present study we show that the prominent terminal plate is an important taxonomic feature, as are its protuberances.

Detailed morphology of terminal plates and of their protuberances (variously called spines or setae in the literature) was studied for *Anodonta* glochidia by Lillie (1895), Lefevre and Curtis (1912), Coker *et al.* (1921), and Cope (1959), yet none of these workers realized the taxonomic significance of these structures. Now, as we show here, diagnostic differences are evident between mean lengths of terminal plates and of large protuberances and between spatial and length distribution frequency patterns in *A. cataracta* and *A. implicata* by use of SEM.

Presence of four pairs of presumed sensory tufts in the mantle of *Anodonta* glochidia was reported by Lillie (1895), but whether they exist in all species is unknown. We prove the presence of four tufts on each valve in both *A. cataracta* and *A. implicata*, three being located near the terminal plate and one close to the ventral margin of the adductor muscle. That these tufts are sensory organs was proposed by Lillie (1895) on structural and histochemical grounds. Our observations on their specific locations near terminal plates and adductor muscle provide support to Lillie's hypothesis.

Presence of pores on external valvular walls of glochidia was reported by Lillie (1895), Lefevre and Curtis (1912), Surber (1912), Coker *et al.* (1921), Cope (1959), and Atkins (1979), who gave no details of either surface structure or possible function of these pores. The present study shows, however, that the pores pass right through both valvular walls and mantle tissue and that mean pore diameters on the external valvular surfaces and the pattern of their spatial distribution over the mantle tissues were the same in both species. Complete perforation of valve walls and mantle by these pores indicates that encysted glochidia may exchange respiratory gases between their own tissues and those of the host and obtain nutrients from host tissues, within which they are believed to feed and undergo organogenesis (Coker *et al.* 1921).

Presence of a larval thread in *Anodonta* glochidia was reported by Lefevre and Curtis (1912) and Coker *et al.* (1921). Lefevre and Curtis regarded this structure as a characteristic of this genus although they apparently never examined *A. implicata* to further confirm their theory. Although its exact function is still not known, the thread is thought to entangle other larvae into a mass to enhance the probability of multiple infections of a single potential host with which one or all larvae come into contact (Lefevre and Curtis 1912). The present study suggests that the larval thread in *A. implicata* is probably absent, which may be evidence for ecological isolation of *A. cataracta* from *A. implicata*.

This study provides evidence for morphometrical, morphological, and possible ecological differences between *A. cataracta* and *A. implicata* glochidia. In view of the many problems which still exist in attempting to differentiate species within a genus, it would be both interesting and rewarding to expand this study and examine other species of unionid glochidia using SEM.

Acknowledgements

We thank Mr. Paul Nitishin, SEM technician, Biology Department, University of New Brunswick in Fredericton, for technical advice and assistance in preparing specimens for microscopic examination and Mr. Peter Wilkinson, Anatomy Department, Dalhousie University in Halifax, for assistance in the use of the Cambridge S150 SEM.

ATHEARN, H. D., and A. H. CLARKE. 1962. The freshwater mussels of Nova Scotia. Bull. Natl. Mus. Can. **183**: 11–41.

ATKINS, L. 1979. Observations on the glochidial stage of the freshwater mussel *Hyridella (Hyridella) drapeta* (Iredale) (Mollusca: Pelecypoda). Aust. J. Mar. Freshwater Res. **30**: 411–416.

CLARKE, A. H., and C. O. BERG. 1959. The freshwater mussels of central New York. Mem. Cornell Univ. Exp. Stn. No. 367.

COKER, R. E., H. W. CLARK, A. F. SHIRA, and A. D. HOWARD. 1921. Natural history and propagation of freshwater mussels. Bull. U.S. Bur. Fish. **37**: 77–181.

COPE, O. B. 1959. New parasite records from stickleback and salmon in an Alaska stream. Trans. Am. Microsc. Soc. **75**(2): 157–162.

FRYER, G. 1970. Biological aspects of parasitism of freshwater fishes by crustaceans and molluscs. Symp. Br. Soc. Parasitol. **8**: 103–118.

JOHNSON, R. I. 1946. *Anodonta implicata* Say. Occas. Pap. Mollusks Mus. Comp. Zool. Harv. Univ. **1**: 109–116.

LEFEVRE, G., and W. C. CURTIS. 1910*a*. Experiments in the artificial propagation of freshwater mussels. Bull. U.S. Bur. Fish. **28**: 615–626.

——— 1910*b*. Reproduction and parasitism in the Unionidae. J. Exp. Zool. **9**: 79–115.

——— 1912. Studies on the reproduction and propagation of freshwater mussels. Bull. U.S. Bur. Fish. **30**: 109–201.

LILLIE, F. R. 1895. The embryology of the Unionidae. J. Morphol. **10**: 1–100.

MARGOLIS, L., and J. R. ARTHUR. 1979. Synopsis of the parasites of fishes of Canada. Bull. Fish. Res. Board Can. No. 199.

MATTESON, M. P. 1948. Life history of *Elliptio complanatus* (Dillwyn, 1817). Am. Midl. Nat. **40**: 690–723.

ORTMANN, A. E. 1911. A monograph of the najades of Pennsylvania. Mem. Carnegie Mus. **4**: 279–347

——— 1919. A monograph of the Naiades of Pennsylvania. Mem. Carnegie Mus. **8**: 1–384.

SURBER, T. 1912. Identification of the glochidia of freshwater mussels. U.S. Bur. Fish. Doc. No. 771.

——— 1915. Identification of the glochidia of freshwater mussels. U.S. Bur. Fish. Doc. No. 818.

WILES, M. 1975. The glochidia of certain Unionidae (Mollusca) in Nova Scotia and their fish hosts. Can. J. Zool. **53**: 33–41.