

REPRODUCTION AND PARASITISM IN THE UNIONIDÆ

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THIRTY-NINE FIGURES

FIVE PLATES

CONTENTS

Introduction.....	79
The marsupium.....	81
Breeding seasons.....	87
Conglutination of embryos.....	90
Histological structure of the marsupium.....	92
Stratification of unfertilized eggs.....	93
Structure of the glochidium.....	94
Behavior and reactions of glochidia.....	98
Reactions of hookless glochidia.....	100
Reactions of hooked glochidia.....	101
The parasitism.....	103
Infections with hooked glochidia.....	104
Infections with hookless glochidia.....	107
Susceptibility of fishes to infection.....	109
Duration of parasitism.....	111
Histological changes in cyst-formation.....	111
Liberation from the cyst and post-larval stages.....	114

INTRODUCTION

The threatened extinction in the Mississippi River and its more important tributaries of those species of the Unionidæ whose shells have been taken in enormous numbers during the past fifteen years for manufacture of pearl buttons has led the United States Bureau of Fisheries to undertake an extensive investigation to determine the possibility of artificial propagation of the commercial species, and to devise such means as may be practicable of restocking depleted waters which present favorable conditions for their growth. The general direction of the investigations has been placed in the hands of the writers, who for the past three years have devoted as much time as their other duties

have allowed to the work, in certain phases of which, however, many others have collaborated.

The plan of the work has embraced, besides a thorough investigation of artificial propagation, a detailed study of the life history and ecology of the Unionidæ, emphasizing especially the geographical distribution of the group throughout the Mississippi Valley, breeding seasons and habits, physical conditions of the waters in which the different species thrive, food enemies, diseases, rate of growth, and the behavior of glochidia and fishes, as parasites and hosts, respectively. Although much yet remains to be done, results of importance have been obtained, and the entire feasibility of artificial propagation clearly demonstrated.

The writers' personal attention has been largely directed to a study of the conditions of reproduction in the group and to the parasitism of the larvæ from the point of view of their relation to artificial infection of fishes with the glochidia, while such phases of the investigation as geographical distribution, enemies and diseases and general habits have been carried on by others.

It is through the kindness of the Commissioner of Fisheries, the Honorable George M. Bowers, that we have the privilege of publishing, in advance of our detailed report, certain results which bear upon the study of reproduction and parasitism and which are briefly presented in this paper. It also gives us pleasure to express here our appreciation of the enthusiastic support which has been given us by Dr. Barton W. Evermann, Chief of the Division of Scientific Inquiry of the Bureau of Fisheries, whose unflagging interest and faith in the investigations since their beginning have been largely responsible for such success as has been attained.

As has long been known, the Unionidæ carry their young in the gills, which function as brood-pouches until the completion of the embryonic development. At the close of this period, the larva or so-called glochidium is fully formed and escapes from the egg-membrane while still within the gill. In some species the discharge of the glochidia takes place at once, although in others

they remain without further change in the brood-pouches for several months before being set free in the water.

The glochidium, long thought to be a parasite infesting the gills and known as *Glochidium parasiticum*, was proved by Carus in 1832 to be the larva of the mussel itself, although Leeuwenhoek many years before had correctly interpreted its relation. In 1866 Leydig made the important discovery that the glochidium, after leaving the parent, completes its development as a parasite on fishes.

Since an adequate review of the literature relating to the embryonic development and the parasitism of the larva has quite recently been published by Harms ('09), an historical account of the subject may be omitted here and reference had to his interesting and valuable paper.

THE MARSUPIUM

The term *marsupium* has been generally used to indicate those portions of the mussel's gills into which the eggs are received from the suprabranchial chambers after ovulation and which serve as brood-pouches for the retention and nurture of embryos and glochidia until the discharge of the latter. As no better name seems to be available, we shall employ it in this paper. Since the extent to which the gills are specialized for this purpose varies in different groups of the Unionidæ, Simpson ('00), in his Synopsis of the Naiades, has made use of the marsupium as the chief diagnostic character on which his classification is based. Those groups in which the marsupium comprises the outer or all four gills he designates as the exobranchiæ, while those in which the inner gills alone receive the eggs are distinguished as endobranchiæ. All of the European and North American species belong to the former group, while the latter contains forms that are found chiefly in Asia, Australia, Africa, Central America, and South America. As our observations have been confined to the exobranchiæ, reference will be made only to this group, the following subdivisions of which are recognized by Simpson, each distinguished by special marsupial characters.

Tetragenæ. Marsupium occupying all four gills.

Homogenæ. Marsupium occupying entire outer gills.

Heterogenæ. Marsupium occupying only posterior end of outer gills.

Mesogenæ. Marsupium occupying a specialized portion in the middle region of outer gills.

Ptychogenæ. Marsupium occupying entire outer gills which are thrown into a series of peculiar folds.

Eschatigenæ. Marsupium occupying the lower border only of outer gills.

Diagenæ. Marsupium occupying entire outer gills, but differing from that of the *Homogenæ* in that the egg-masses lie transversely in the gills.

Simpson has established another group, the *Diagenæ*, for the genus *Tritogonia* but since its marsupium is constituted by all four gills it should at least be included in the *Tetragenæ*, if not in the genus *Quadrula*, as Ortmann suggests ('09).

It will be seen from the above classification that three general conditions exist, namely one in which the marsupial adaptation, involves all four gills; one in which the entire outer gills only are utilized; and, lastly, one in which some differentiated portion of the outer gills constitutes the marsupial region.

For a complete list of the genera occurring in each of Simpson's groups, reference may be had to his *Synopsis* (*l. c.*, pp. 514-515), but since some of the types have not been adequately described and figured, we have inserted here an account of the several marsupial modifications which have come under our own observation, together with a list of the species in which we have found them.

Tetragenæ. The marsupium in these forms comprises all four gills which when gravid are distended and present a smooth pad-like appearance. It is the condition found in the genera *Quadrula* and *Tritogonia*. We have encountered it in *Tritogonia tuberculata* Branes, and in the following nine species of *Quadrula*: *ebena* Lea, *heros* Say, *lachrymosa* Lea, *metanевра* Rafinesque, *obliqua* Lamarck, *plicata* Say, *pustulosa* Lea, *trigona* Lea, and *undulata* Barnes. Fig. 4, which is drawn from a gravid female of

Quadrula ebena, illustrates the typical appearance of the marsupium in this group, although the gills, as they appear in the figure, are not as fully distended as is frequently the case. In many species of the genus the eggs and embryos are brilliantly colored, red or pink, and, when the marsupium is charged, the color shows through the transparent walls of the gills, which present a striking appearance on removing the shell.

There has been a certain amount of discussion among the conchologists as to whether, or not, the functioning of all four gills as a marsupium is a constant character in *Quadrula*, and observations have been to a certain extent conflicting. Since Simpson has made use of this feature in characterizing the group *Tetragenæ*, some importance has been attached to the apparent discrepancy in observations. Ortman (*l. c.*, p. 101), for example, states that in a dozen specimens of *Q. coccinea* only the outer gills were charged with embryos. "This would remove" he says "this species from the genus *Quadrula*, and would place it with *Pleurobema*. Baker ('98, p. 80), gives a description of the soft parts, and says, "four gills used as marsupium," but this may not be founded upon personal observations, but may have been inferred from the systematic position of the species."

While examining mussels on the upper Mississippi River in the summer of 1908, we observed a peculiarity in behavior in all of the species of *Quadrula* collected which probably accounts for the conflicting descriptions of the marsupium in this genus, and also for the fact that in some species gravid females have never been observed at all. Every species of *Quadrula* that came into our hands exhibited to a greater or less degree the habit of aborting embryos and glochidia, when taken out of the river, and if they were not opened and examined at once upon capture, they were generally found shortly afterwards to be either partially or entirely empty. Some individuals discharged the contents of their gills more readily and completely than others, the abortion involving either all four gills, or only the inner or outer ones, or, again, only a portion merely of one or more gills. In the pre-glochidial stages, when the embryos are conglutinated, the entire masses were discharged, while individuals were frequently seen in the act of abort-

ing their embryos or glochidia which were often expelled with considerable force through the exhalent siphon. This behavior was so characteristic of the genus that, in order to make a correct determination of the condition of the marsupium, it was necessary to open *Quadrulas* immediately after taking them from the water. When this was done, all four gills were invariably found to be charged on opening females which contained embryos in pre-glochidial stages, that is, at any time before normal spawning had occurred. The habit of aborting embryos, when disturbed has, been observed by us in but one species not belonging to *Quadrula*, namely, in *Unio complanatus*, which has been repeatedly seen in the act of discharging the contents of the marsupium shortly after being placed in the aquaria of the laboratory at Woods Hole, Mass. In all likelihood it occurs in other species of *Unio*, and it has been referred to by Schierholz ('88) and Latter ('91) as taking place in *Anodonta*. It is probably due, as Schierholz suggests, to imperfect aëration of the water, and if this is true, one would not expect to find it in those mussels which have a differentiated marsupium, like the *Heterogenæ*, since in such forms the respiratory and marsupial functions of the gills are not so intimately associated.

Homogenæ. The condition in which only the outer gills are utilized as a marsupium is present in sixteen genera according to Simpson. We have verified its occurrence in *Alasmidonta truncata* Wright; *Anodonta cataracta* Say, *grandis* Say, *implicata* Say; *Arcidens confragosus* Say; *Pleurobema æsopus* Green; *Symphynota complanata* Barnes, *costata* Rafinesque; and in *Unio complanatus* Dillwyn and *gibbosus* Barnes. The outer gills when filled with embryos or glochidia may be greatly distended beyond their normal dimensions, and in this condition are enormously swollen pad-like structures filling a large portion of the mantle chamber. Fig. 6 represents the marsupium of *Symphynota complanata* which may be taken as typical of the class.

Heterogenæ. In this group the marsupium occupies only the posterior portion of each outer gill, varying in extent from about one-third to two-thirds of the entire length of the latter. It is permanently differentiated, being sharply marked off from the res-

piratory portion either by a deep notch or a distinct fold and projecting further down into the mantle chamber, as it is much deeper dorso-ventrally than the non-modified anterior end of the gill. Unlike that of the Tetragenæ and Homogenæ, the marsupium in the Hertogenæ is readily recognized when empty, for, in addition to its permanent demarcation, its walls are thinner and more membranous in appearance than those of the respiratory portion, and after discharge of the glochidia it is seen as a flabby collapsed pouch. The inflation of the posterior end of the valves which is characteristic of the females in the Heterogenæ is associated with this type of marsupium, as it allows of increased space inside the shell for the accommodation of the gravid gill. Owing to this enlargement of the shell, which is absent in the males, the sexes are externally distinguishable in the Heterogenæ.

The typical condition is seen in the genus, *Lampsilis*, as shown in fig. 2, which is drawn from a gravid individual of *L. subrostratus*. The extent of the marsupial modification, however, varies in different genera and even in different species of the same genus, occupying, as already stated from about one-third to two-thirds of the length of the entire outer gill. Fig. 5 illustrates the marsupium in *L. rectus*, in which it forms the posterior third of the greatly elongated outer gill and is sharply marked off from the respiratory region by a deep notch. The last two figures indicate the two extremes of the differentiation in this group. Simpson has included fourteen genera in the Heterogenæ, only three of which, however, have come under our observation, namely *Lampsilis*, *Obovaria*, and *Plagiola*. We have recorded this type of marsupium in *Lampsilis* (*Proptera*) *alatus* Say, *anodontoides* Lea, *gracilis* Barnes, *higginsii* Lea, *lævissimus* Lea, *ligamentinus* Lamarck, *luteolus* Lamarck, *rectus* Lamarck, *subrostratus* Say, and *ventricosus* Barnes; in *Obovaria* *ellipsis* Lea; and in *Plagiola* *elegans* Lea and *securis* Lea.

Mesogenæ. This group is so designated by Simpson to include two genera, *Cyprogenia* and *Obliquaria*, in which a variable number of enlarged water-tubes in the middle region of the outer gill are specialized as the marsupium, the portion of the gill both in front and behind retaining its ordinary respiratory character. We

have encountered the condition in one species only, *Obliquaria reflexa* Rafinesque, which is represented in fig. 3. Here the distended tubes project far down below the lower border of the rest of the gill, and when gravid appear greatly swollen. The marsupial region is permanently differentiated and is easily recognized when empty. The number of tubes comprising the marsupium varies in different individuals of the species from two to eight and during the breeding season each tube is filled by a solid cord of a stiff glutinous consistency in which the embryos or glochidia are embedded.

Ptychogenæ. This group contains a single genus, *Ptychobranchus*. The marsupium occupies the entire outer gill which is thrown into a series of folds, each water-tube ending below in a swollen bulb-like enlargement, as seen in fig. 1, which is drawn from *P. phaseolus* Hildreth. Simpson (*l. c.*, p. 612) states that the number of folds varies from six to twenty. In the specimen figured there are seventeen.

Eschatigenæ. Simpson has established this group for the genus *Dromus* in which the marsupium occupies the lower border only of the entire outer gill, the ventral ends of the water-tubes being slightly distended and rounded. The condition, which has not been seen by us, would seem to differ from the marsupium of *Ptychobranchus* chiefly in the absence of the folds.

Diagenæ. Here the outer gills throughout their entire length are utilized as a marsupium, which in external appearance is quite similar to that of the *Homogenæ*. The *Diagenæ*, however, differ from other *Unionidæ* in that the embryos and glochidia are contained in peculiar cylindrical sacs which lie *transversely* in the gills, whereas in every other case the egg-masses are placed vertically in the gills. The group contains one genus, *Strophitus*. We have observed the gravid marsupium in *S. edentulus* Say, although we have not had an opportunity of determining whether the unusual position of the egg-masses is correlated with some peculiarity in the structure of the water-tubes, or not.

It is probable as Simpson concludes (*l. c.*), that the oldest type of marsupium phylogenetically is that occurring in the *Endobranchiæ* in which the inner gills only are used as brood-chambers. It is a slight transition from this condition to that existing in the

Tetragenæ with all four gills functioning for this purpose. Basing his supposition largely upon shell characters and facts of geographical distribution, he further concludes that the Homogenæ marked the next step in marsupial differentiation, while the Heterogenæ and all other groups in which a portion of the outer gills only is structurally modified for receiving the eggs are the latest product of the evolution of the Unionidæ. That this series correctly represents the phylogenetic sequence in the appearance of the marsupial modifications would seem to be borne out by the structural conditions existing in the several types, so far as we have examined them.

BREEDING SEASONS

In connection with our study of artificial propagation of freshwater mussels, we have found it necessary to collect data bearing upon the breeding seasons of a fairly wide range of species, since the records of previous observers, for North American Unionidæ at least, have been insufficient to enable us to determine the full extent of the seasons, especially in the case of some of the more important commercial species. Although our observations have been largely confined to species occurring in the upper Mississippi Valley and have been concerned primarily with species of commercial value, we have continuous records throughout the entire year for a number of important genera, and in every case the exact stage of development of the embryos has been determined by microscopic examination. Many thousands of such observations have been made, so that we are now in possession of detailed information dealing with the duration and progress of the periods of gravidity obtaining in over a dozen genera of the Unionidæ.

We have confirmed the conclusion first reached by Sterki ('95) that the North American Unionidæ, with respect to their breeding seasons, fall into two classes, the so-called "summer breeders" and "winter breeders." The latter designation, however, is not strictly appropriate, for in the species which belong to this group, the eggs are fertilized during the latter part of the summer, usually in August, and the glochidia, which are carried in a fully developed

condition in the marsupium throughout the winter, are not discharged until the following spring and summer. In fact, in some cases the close of one breeding period may overlap on the beginning of the next, as one may still find in late July a few straggling females gravid with glochidia formed in the previous autumn, while in other individuals of the species at the same time and in the same locality the eggs are passing into the gills for the next season. This seems to be true of several species of *Lampsilis*. We have encountered it in *ligamentinus*, Conner ('09) records it for *radiatus* and *nasutus*, while Ortmann ('09) states that his observations make it probable for *ventricosus* and *luteolus*. Yet, as Ortmann observes, it is generally true that an interval exists between the close of one period and the beginning of the next. This interval, however, varies in length with different species, in some extending from late spring until August, whereas in others it is of much shorter duration. It is also to be noted that the discharge of glochidia does not take place in all of the individuals of a species at the same time, but on the contrary spawning may extend over a considerable period throughout the spring and early summer (*cf.* Ortmann, *l.c.*).

In the case of the summer breeders, the eggs are fertilized during late spring and summer, and spawning as a rule is over by the end of August. In the species belonging to this group of which we have the completest records, ovulation begins in May and early June and may continue throughout July, while after the end of August gravid females have not been found.

In view of the above facts, it would seem to better accord with the actual conditions to separate the species with respect to the length of time that the glochidia remain in the marsupium, designating them as those that have a "short period" and those with a "long period" of gravidity, rather than to distinguish them as "summer breeders" and "winter breeders," respectively, for with respect to the latter neither ovulation nor discharge of the glochidia takes place in winter.

The breeding season is undoubtedly a generic character, for, so far as our observations have gone, all of the species belonging to a given genus have essentially the same period of gravidity.

Although our records in detail for each species will be published in our forthcoming report to the Bureau of Fisheries, the following lists show the distribution, with respect to the long and the short breeding seasons, of the genera which have come under our observation.

Long period of gravidity.—*Alasmidonta truncata*; *Anodonta cataracta*, *grandis*, *implicata*; *Arcidens confragosus*; *Lampsilis* (*Proptera*) *alatus*, *anodontoides*, *gracilis*, *higginsii*, *lævissimus*, *ligamentinus*, *luteolus*, *rectus*, *subrostratus* *ventricosus*; *Obovaria ellipsis*; *Plagiola elegans*, *securis*; *Strophitus edentulus*; *Symphynota complanata*, *costata*.

Short period of gravidity.—*Obliquaria reflexa*; *Pleurobema æsopus*; *Quadrula evena*, *heros*, *lachrymosa*, *metanевра*, *obliqua*, *plicata*, *pustulosa*, *trigona*, *undulata*; *Tritogonia tuberculata*; *Unio complanatus*, *gibbosus*.

Ortmann ('09) has recently published some observations on the breeding seasons of the Unionidæ of Pennsylvania, supplemented by data from Lea and Sterki, and although he merely records a species as gravid or not in a given month, his results in all essential points agree closely with ours. He includes among "winter breeders" several genera which we have not had under observation, namely, *Truncilla*, *Micromya*, *Cryprogenia*, *Ptychobranhus* and *Anodontoides*, while *Arcidens* and *Obliquaria*, which we have recorded, do not appear in his lists of "winter breeders" and "summer breeders," respectively. Although in many cases we have had the same species under observation, his records include a number that are absent from ours, while our list supplements his especially by the addition of several species of *Quadrula*, for which data have previously been quite meagre.

By examining the two sets of observations, it will be found that, whereas the *Homogenæ* and *Mesogenæ* are represented in both groups, some genera in each having the long period and others the short period of gravidity, the *Heterogenæ*, *Ptychogenæ*, and *Dia-genæ* are so-called "winter breeders" exclusively, while the *Tetragenæ* breed only in the summer.

The breeding seasons, as defined above, are based upon data col-

lected in the middle and northern sections of the United States, and in the absence of adequate records from higher and lower latitudes, it is impossible to say to what extent a colder or a warmer climate might affect the period of gravidity. That it would have some influence can hardly be doubted, although a distinction between a long and a short season will probably be found to hold true in general.

The same difference has been repeatedly stated to occur among European Unionidæ. According to Harms ('09, p. 332), for example, in *Unio* the breeding season begins early in March, or, if the weather is cold, not until the end of May. In *Anodonta*, which carries the larvæ over the winter, the eggs are fertilized about the middle of August, all of the individuals entering upon the breeding season at nearly the same time, while by the middle of October almost all the females are gravid with glochidia. *Margaritana* breeds in July and August, according to Harm's observations, and during that time produces two successive broods, from sixteen days to four weeks, according to the temperature, being required for the development of each. Although we have not determined it beyond all doubt, our records strongly indicate that the species of *Quadrula*, and possibly some other summer breeders, also spawn twice during the same seasons, first in June or July and again in July or August.

CONGLUTINATION OF THE EMBRYOS

After extrusion of the eggs from the genital aperture, they are passed along by ciliary action to the cloaca, and thence by suction according to Latter ('91, p. 53), are drawn back into the supra-branchial chambers. Here they are fertilized by spermatozoa brought in with the incoming current of water, and then pass into the water-tubes of the gills, eventually filling up those portions which function as the marsupium.

As the eggs settle down into the gills, they come into contact with the secretion formed by the glandular epithelium lining the tubes, which in many cases is of a glutinous nature, although its consistency varies in different species. It is, however, usually

quite tenacious. In a short time after entering the marsupium, the eggs become conglutinated into masses which, as the mucilaginous matrix stiffens, are molded into the exact shape of the cavity of the water-tube, of which each mass forms a cast. The masses are of course separated from each other by the intervening interlamellar junctions of the gills.

Since it is a matter of convenience to have a word to apply to these compact masses, in which the eggs or embryos are held together, whether they be plate-like, wedge-shaped, cylindrical or of some other form, we shall employ the term *conglutinate* in referring to them, as being perhaps the best one available for the purpose. They have generally been called "ovisacs" by the American systematists, but this is of course misleading, as they are not sacs in any sense, but merely masses of eggs adhering to one another by means of a cement.

The conglutinates vary greatly in size and shape in conformity with the special conditions of the marsupium existing in the different types. The commonest form is that of a flat oval plate, slightly blunter and thicker above and more pointed and thinner below. Such plates, differing, however, in size and thickness, are characteristic of *Lampsilis*, *Quadrula*, *Unio*, and many other genera. In fig. 27 two of the conglutinates of *Lampsilis ligamentinus* are represented, one from the flat side, the other on edge. In those genera in which the size and form of the water-tubes of the marsupium depart more widely from the usual condition, the conglutinates are similarly modified. In *Obliquaria reflexa*, for example, in which the marsupium consists of several elongated and distended water-tubes in the middle region of the outer gill, the conglutinates are unusually large, being slightly curved cylindrical masses of nearly uniform diameter and generally blunt at each end. Three of them are shown in fig. 26. The one on the right was taken from the most posterior water-tube of the marsupium which is not as long as the rest and its conglutinate is correspondingly shorter. The relation will be understood by reference to the figure of the marsupium in this species (fig. 3).

In those species, *e. g.*, *Unio complanatus*, in which the antero-posterior diameter of the water-tube is scarcely greater than that

of the egg, the conglomerates are very thin and as a rule are composed of but a single layer of eggs, while in others, as in the species of *Lampsilis*, the plates are much thicker, especially above, and in horizontal section show several layers. In the genus *Quadrula* the thickness is intermediate.

The amount of the matrix in the conglomerates also varies considerably in different species. In *Unio complanatus* and *Obliquaria reflexa*, the egg membranes, which seem to be of an adhesive nature, are closely pressed together in the mass on all sides, as shown in fig. 28, which is a detail drawn from one of the conglomerates of fig. 26. The glochidia are seen with the valves open but still contained within the membranes, which are closely adhering. In cases like this, it is difficult to determine whether there is any cement substances at all between the embryos, which may possibly be held together solely by means of the adhesive surfaces of the membranes. In many other cases, however, the matrix is quite evident, and the embryos, which are not so closely appressed, are simply embedded more or less loosely in a glutinous binding substance. Such is the condition in *Lampsilis* and is illustrated in fig. 24, which is a portion of one of the conglomerates of fig. 27 seen under higher magnification.

In still other species, the eggs cannot be said to form conglomerates at all, as they are merely suspended in a slimy mucus which never hardens sufficiently to enable the mass to maintain a definite form when removed from the gill. This condition is most noticeable in *Alasmidonta truncata*.

When the glochidia are fully formed, the cement, which throughout the embryonic development has held the embryos together, dissolves away, and the larvæ are discharged at the time of spawning in slimy strings of mucus which is secreted by the lining epithelium of the marsupium.

HISTOLOGICAL STRUCTURE OF THE MARSUPIUM

Figs. 30 and 31 are horizontal sections of water-tubes of the marsupium of *Quadrula ebena*, and *Lampsilis (Proptera) alatus* respectively, and, as they are drawn under the same magnification, a comparison of the size of the tubes in the two cases may be readily

made. Both contain embryos in an early cleavage stage, only a few of which, however, are represented. The actual sections show the spaces closely packed with embryos. In fig. 31, which may be taken as typical of the condition existing in the *Heterogenæ*, the tubes are not only enormously enlarged, but their walls are relatively thinner, and the whole marsupium has a more membranous appearance than in the *Quadrula* and *Unio* types.

The glandular epithelium is found chiefly on the surfaces of the interlamellar junctions, and in some species is much more highly developed and more extensive than in others, often causing the surface to be thrown into irregular folds and ridges. This is very pronounced in *Unio* and *Quadrula*. When highly magnified, as in fig. 32, the epithelium, resting upon a base of connective tissue and smooth muscle fibers, is seen to be composed of greatly swollen cells, whose vacuoles are filled with a clear mucus-like colorless fluid. Scattered among the large cells and seemingly often lying within the vacuoles, are seen several smaller and darker nuclei which are undoubtedly the nuclei of leucocytes. In fact, there can be little doubt that the epithelium becomes infiltrated with wandering blood cells from the underlying sinuses in the interlamellar junctions. Many indications are present that seem to show that these cells actually wander through the epithelium into the cavities of the water-tubes but what their ultimate fate is, if this be the case, we are as yet unable to say. Possibly they may be ingested by the glochidia and used as food.

STRATIFICATION OF UNFERTILIZED EGGS

It not infrequently happens that eggs pass into the marsupium without being fertilized, and remain there throughout the period of embryonic development, as one may find them in the same gill with fully formed glochidia. In some individuals we have found every egg in the marsupium in this condition. Such eggs have been encountered, however, only in summer breeding species and they seem to be especially common in the genus *Quadrula*, nearly every gravid female of which has been found to contain at least some unfertilized eggs. After remaining in the marsupium for

a time, such eggs generally become swollen and stratified into three distinct layers, a heavier, often pigmented, mass at one pole, a clear or hyaline intermediate zone, and a small granular cap at the lighter pole. As the eggs lie in a constant position in the gills, which are placed vertically in the normal position of the animal it cannot be doubted that the stratification is produced by gravity. It has not yet been determined whether the substances which occur in these layers are the same as would be separated out by centrifuging, or not, but this is not at all unlikely. As many of the species of mussels in which we have seen this condition, for example *Quadrula ebena*, *Q. trigona*, and *Pleurobema æsopus*, have brightly colored red or pink eggs, the stratification is quite striking, the pigments being always at the heavier pole, as it is invariably directed towards the lower border of the gill.

STRUCTURE OF THE GLOCHIDIUM

As has long been known, two well marked types of glochidia are found in the Unionidæ, one provided with stout hooks on the ventral margin of the valves, the other in quite different shape and entirely hookless. The first is characteristically parasitic on the fins and the other external parts from which scales are absent, the second upon the gill-filaments. The occurrence of the two types in the genera which we have examined is shown in the following list:

HOOKLESS GLOCHIDIA

Lampsilis
Obliquaria
Obovaria
Plagiola
Pleurobema
Quadrula
Tritogonia
Unio

HOOKEG GLOCHIDIA

Anodonta
Strophitus
Symphynota

In addition to these, there is the peculiar larva of *Lampsilis* (*Proptera*) *alatus*, which has been called "axe-head" glochidium (fig. 25). This possesses hooks which are not homologous with those of the *anodonta* type and is to be regarded as more nearly related to the hookless forms, an interpretation which is

borne out by the fact that the "axe-head" can be readily imagined as a modification of the glochidial outline seen in some species of *Lampsilis*, which like *subrostratus* show an approach to a rectangular outline. Its four hooks are so arranged that those of one valve pass inside the opposite ones, thus bringing the ventral margins close together and giving a very firm hold upon the host's tissue. In other respects, it does not show marked differences from the hookless type and the few experiments we have made with it indicate its attachment to the gills rather than the fins.

The detailed structure of each type of glochidium, as found in *Anodonta* and *Unio*, respectively, has been quite well known for many years and recently Harms ('09) has studied them with even more care. We have nothing to add to this except that we have observed concerning the larval thread (formerly erroneously termed the byssus), which we will mention, since the current accounts in zoölogical text-books and literature lead one to believe that this structure is a conspicuous feature of all glochidia, which is not the case, although it does happen to be present in the European forms which have been most studied. We find the larval thread present in the species of *Unio* and *Anodonta* which we have been able to examine with care and the thread is probably a characteristic of these genera. We have never seen any sign of such a structure in the ripe glochidia of the other genera above listed as possessing hookless glochidia, nor in the hooked forms of the genus *Symphynota*. Lillie ('95, p. 52) considers the thread a condensed excretory product, which, accepting the account of Schierholz ('88), he thinks has become an organ which is of use in bringing the glochidium in contact with the fish. This latter function is the one commonly ascribed to the thread. While we have not studied the pre-glochidial stages in the development of those species which show no thread gland in the mature glochidium (although it is important that this should be done with a view to determining whether any homologue of the thread gland is present at any time), we do not know, that repeated examination of glochidia, either ripe or well along in their development, in several species of *Lampsilis*, particularly *ligamentinus*, *rectus*, *anodontoides*, *ventricosus*, *luteolus* and *subrostratus*, and

to a lesser extent in species of the other genera mentioned, has never shown any trace of the thread which is so conspicuous a feature of the glochidium of *Unio complanatus*. We have also examined the glochidia of *Symphynota complanata* many times with the same negative results and a smaller number of observations confirm this for *S. costata*. Since many species thus have no thread in any way functional for attachment to the fish, the question arises whether the thread when present has as important a function in this respect as has been supposed. Our observations upon the glochidia of *Anodonta cataracta* confirm the descriptions of Schierholz ('88) and others who have studied the European species of *Anodonta*, as to the tangling of the glochidia into masses by means of their extruded threads, and in this genus the threads do seem effective in drawing other glochidia into contact with the fish when a single one has become attached. This is not, however, effective for the greater part of the period during which the glochidium may remain alive upon the bottom, for the threads are dissolved within a day or two and then the glochidia become entirely free from one another. When taken from the parent gill, the glochidia of *Symphynota* are entangled in a ropy mucus and this acts in a manner similar to the threads of *Anodonta*, but it is usually dissolved after a few hours in the water. In the ripe glochidium of *U. complanatus*, the threads are extruded when the glochidia are removed from the parent and placed in water. When this extrusion has taken place, the glochidia and broken egg-membranes become united into globular masses from which it is difficult to separate individual specimens, and from observing such glochidia in contact with fish, we are forced to conclude that they are not so likely to become attached to the gills or fins as they are when separated by the disintegration of the threads of mucus. The glochidia of *Lampsilis*, which when fully ripe, at once spread out into masses of entirely unconnected individuals, appear much better able to attach to the gills of fishes. Accordingly, we would consider the thread as something to be gotten rid of rather than an organ of great importance in the attachment, and this is in agreement with Lillie's interpretation of the thread as an excretory product.

There is considerable diversity in size among glochidia even from the same genus, as represented by the series of text-figures (fig. 1, A-N), all drawn to the same scale, the most striking being the difference between the two species of *Plagiola* (G and H) and between *Lampsilis rectus* and *gracilis* (K and L). Harms ('09) who has studied the exceedingly minute glochidia of *Margaritana margaritifera*, finds that they are exclusively gill parasites

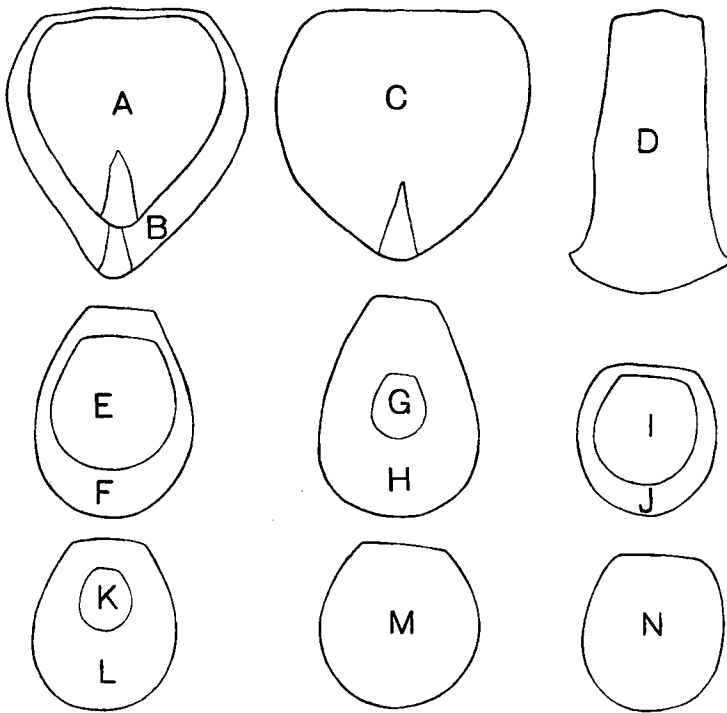


FIG. 1. Figures showing relative sizes and shapes of the shells of a series of glochidia, belonging to the following species: A, *Symphynota complanata*, 0.30×0.29 mm.; B, *S. costata*, 0.39×0.35 mm.; C, *Anodonta cataraeta*, 0.36×0.37 mm.; D, *Lampsilis* (Proptera) *alatus*, 0.41×0.23 mm.; E, *Quadrula meta nerva*, 0.19×0.18 mm.; F, *Q.*, *pustulosa*, 0.32×0.23 mm.; G, *Plagiola elegans*, 0.09×0.075 mm.; H, *P. securis*, 0.31×0.23 mm.; I, *Quadrula ebena*, 0.15×0.14 mm.; J, *Q. plicata*, 0.21×0.20 mm.; K, *Lampsilis gracilis*, 0.085×0.075 mm.; L, *L. rectus*, 0.24×0.20 mm.; M, *Obliquaria reflexa*, 0.23×0.225 mm.; N, *Unio gibbosus*, 0.22×0.19 mm.

because their small size makes attachment elsewhere impossible. The type of glochidium is constant for the genus, so far as our observations go, and in some cases the shape is also characteristic, as shown by *Symphynota* and *Anodonta* (*A*, *B* and *C*) in which the shell outline is a distinguishing feature.

BEHAVIOR AND REACTIONS OF GLOCHIDIA

At the time of spawning the glochidia, already freed from the egg-membranes and more or less loosely held together in slimy strings, are discharged at irregular intervals through the exhalent siphon. Being heavier than water, they sink rapidly to the bottom, coming to rest with the outer surface of the shell directed downward and the valves gaping widely apart. The belief was formerly general that they "swim" about by rapidly opening and closing the valves, after the manner of *Pecten*, and in spite of frequent denials by Schierholz ('88), Latter ('91), and others, the same statement is still occasionally encountered. In the recent volume on Mollusca in the *Treatise on Zoölogy*, edited by Lankester, this inexcusable error is repeated. "The glochidia," we are again informed, "swim actively by clapping together the valves of the shell," (p. 250). They are on the contrary, as is now well known, entirely incapable of locomotion and remain in the spot where they happen to fall, although it is true that they may exhibit from time to time spasmodic contractions of the adductor muscle which cause the valves to snap or wink, each contraction being immediately followed by relaxation and opening of the shell. These movements of the valves, however, are never so vigorous as to cause the glochidium to move from place to place in the water.

The glochidia remain in this helpless situation until they die, unless they happen to come in contact with the host on which they pass through the post-embryonic development as parasites. The stimulus which causes the contraction of the muscle and results in attachment to the host is in the case of hooked glochidia, a mechanical one. It may be readily imitated and glochidia of

this type made to grasp firmly the point of a needle or the edge of a piece of paper by simply touching them between the open valves. When once closed in this manner they do not relax but remain attached to the object until they die.

Electrical and chemical stimuli may also under proper conditions bring about permanent closure of the shell.

The following statement made by Latter (*l. c.* p. 56) has been frequently quoted, especially in text-books, but it has apparently never been verified or disproved. "The Glochidia," he says, "are evidently peculiarly sensitive to the odor (?) (*sic*) of fish. The tail of a recently killed Stickleback thrust into a watch-glass containing Glochidia throws them all into the wildest agitation for a few seconds; the valves are violently closed and again opened with astonishing rapidity for 15–25 seconds, and the animals appear exhausted and lie placid with widely gaping shells—unless they chance to have closed upon any object in the water (*e. g.* another Glochidium), in which case the valves remain firmly closed." Although it is not stated that the tail which caused such a commotion among the glochidia had been cut off from the fish, it is probable that such was the case. We have repeatedly tested glochidia in the same manner both with fins and gills of different fishes, and, provided that a bleeding surface is not brought in contact with the water containing the glochidia, absolutely no response on the part of the latter takes place. The result, however, is much as Latter describes if a little of the fish's blood gets into the water in the neighborhood of the glochidia, except that our experience has shown that after snapping for a few seconds they come to rest in permanent closure. It, therefore, seems possible that the contractions seen by Latter were due to the introduction of some blood with the tail of the fish, as otherwise agitation of the glochidia under similar conditions has not been observed by us.

Since the hooked and hookless glochidia, whose reactions to blood and to certain salts we have examined, behave somewhat differently, they are referred to separately below.

Reactions of hookless glochidia

The glochidia of *Unio complanatus* were used in the following experiments. When a small drop of blood of either the killifish, *Fundulus diaphanus*, or the white perch, *Morone americana*, was placed over the glochidia contained in a small amount of water in a watch-glass, the effect was immediate and very striking. Every glochidium was thrown into rapid and violent contractions, alternating with relaxations, the edges of the valves either quite or nearly touching with each snap. Where the stimulus was strongest, that is, immediately under the drop of blood, the glochidia exhibited two or three strong contractions and then remained closed, but proceeding outwards to zones of diminishing intensity, the snapping occurred intermittently for from ten to fifty seconds. Here the contractions were quite rapid at first, one or two every second, but soon the intervals became longer, until finally the activity was ended by the closure of the valves. In some cases it was observed that after the first few snaps the muscle did not completely relax and each subsequent contraction caused the valves to describe a shorter arc.

Since the hookless glochidia are essentially gill parasites and, when taken into the mouth of the fish, lodge among the gill-filaments, abrasions of the delicate epithelium covering the latter always occur and produce more or less extensive hemorrhage from the blood capillaries. It is, therefore, evident that blood exuding in the neighborhood of the glochidia must have the same effect as in our experiments, and, by causing vigorous contractions of the adductor muscle, be efficacious in bringing about a firm and permanent attachment to the filaments. And, furthermore, since glochidia of the hookless type only occasionally exhibit spontaneous contractions, and, unlike the hooked forms, respond either not at all or only quite sluggishly to tactile stimuli, the action of the blood upon them must play an important part in securing their attachment to the gills.

A series of experiments was also undertaken for the purpose of determining the reactions of the glochidia of *Unio complanatus* to solutions of several different salts, a brief account of which

may be given here. Diluted sea-water and solutions, varying in strength from 0.5 to 1.0 per cent, of NaCl, K₄Cl, KCl and NH₄Cl had exactly the same effect as fish's blood, although the intensity of the reaction varied somewhat in certain cases. Weak solutions of MgCl₂ and MgSO₄, however, as was to be expected, inhibited contractions, and glochidia, after treatment with these salts, could be killed in an expanded condition, if allowed to remain in the solutions for a sufficient length of time.

Reactions of hooked glochidia

The larvæ of *Symphynota complanata*, which are provided with stout hooks and as a rule find permanent lodgment only on the fins and other external parts of the fish, were used in studying the reactions of the hooked type of glochidium. In several respects they differ from the hookless forms. When removed from the marsupium and placed in water, they exhibit spontaneous contractions which occur at irregular and rather long intervals, and this irritability may continue in the laboratory for a day or two, or until the glochidia begin to disintegrate. Under such conditions the valves are only partially closed at each contraction of the muscle, which, moreover, is never strong enough to bring the points of the hooks into contact. It is followed at once by relaxation of the muscle and the shell remains widely open until the next snap occurs.

Hooked glochidia, in marked contrast with the behavior of the hookless forms, respond very actively to tactile stimuli, and, as has been stated, close completely and immediately when touched with any object. This reaction must be the main factor in bringing about their attachment to the fish's fins, when they are brushed over by the latter while lying on the bottom. With glochidia like those of *Symphynota complanata* the mere contact is sufficient to produce complete closure of the valves, and, whether they are exposed to the fish's blood, or not, attachment is possible as a result of the tactile stimulus alone. They do react to blood, however, and exhibit a few successive contractions, from five to fifteen, before final closure, but the way in which the response

occurs is quite different from that shown by the glochidia of *Unio complanatus* under similar conditions. Instead of being thrown into violent and rapid snapping, the valves closing and opening alternately, there is only partial recovery after each contraction, while the valves are brought closer and closer together by a series of short jerks. The final act of closing is interesting. As soon as the points of the hooks touch, the contraction of the adductor muscle becomes continuous and the hooks are slowly bent inwards against each other. Under the steady pressure exerted by the muscle, aided probably by the action of the myocytes, which have been described by Schmidt ('85 b), the spines on the outer surface are apposed and the hooks turned in completely between the valves, the margins of which are brought together, if no object intervenes. It will be readily understood that, owing to the turning in of the hooks, the spines are pressed into the fish's tissues, when attachment to the host takes place, and a firm hold is thereby secured.

When the glochidia of *Symphynota complanata* were exposed to salt solutions, the contractions produced were of the kind just described. KCl, KNO₃, and NH₄Cl in solutions of 0.5 to 1.0 per cent caused a few successive jerks, the contractions being more vigorous and closure occurring sooner with the stronger solutions. NaCl and Na₂C₂O₄ in the same strength acted less energetically, and it was necessary to use a two per cent solution to produce the same effect as was obtained with the weaker solutions of potassium and ammonium salts. A 0.5 per cent solution of CaCl₂ produced no contractions, while a 1.0 per cent solution after a latent period of fifteen minutes caused either partial or complete closure of the valves. MgCl₂ and MgSO₄, in solutions of 0.5 and 1.0 per cent, inhibited contractions, and when the glochidia were allowed to remain in them they finally died in the expanded condition. When the Mg salts, however, were used in stronger solutions closure of the valves occurred after a few spasmodic contractions.

THE PARASITISM

The only infections which we have ever observed in nature were of *Anodonta grandis*, found in the month of November, infecting the roach (*Abramis crysoleucas*), German carp (*Cyprinus carpio*), yellow perch (*Perca flavescens*), blue-gill sunfish (*Lepomis pallidus*), rock bass (*Ambloplites rupestris*) and crappie (*Pomoxis annularis*), collected from the Mississippi River at LaCrosse, Wisconsin. At that time, we handled over 25,000 fish and from the finding of these glochidia upon a majority of the fish taken at random for examination in connection with our own infections it seemed that a large proportion of the whole number were lightly infected with this glochidium. By actual count, we found from one to twenty upon a fish and they were all in about the stage shown by fig. 23. These fish had been recently collected from sloughs, similar to those in which we found many individuals of *A. grandis* with ripe glochidia, and this probably represented a typical infection under natural conditions, where we may be sure that maximum infections never obtain.

Following the methods of artificial infection as practiced since the work of Braun ('78) and Schmidt ('85), we have obtained unlimited material by confining the fish in small receptacles to which the glochidia have been added by washing from the gills of the clams. It is only necessary to see that the glochidia are so distributed in the water as to come in contact with the proper part of the fish, and in most cases, to guard against over, rather than under, infection. Active fish, such as the rock and the large-mouthed black bass, are very favorable for gill infections, since they keep the water so well agitated that the glochidia hardly settle to the bottom at all, while their strong respiratory movements draw the suspended glochidia continually against the gills. Fish like the crappie, which when undisturbed move about quietly and whose respiratory movements are less vigorous, must have the water stirred to keep the glochidia suspended, or so shallow that the fish are always near the bottom. The smaller gill slit of the crappie is another factor which makes for a very light infection in fish under two inches in length, since the glochidia

reach the gills by way of the mouth and not from the opposite direction. For fin infections, sluggish fish like the German carp need little attention, and the darters (*Etheostoma coeruleum* spec-tabile) which habitually rest upon the bottom for considerable periods, become quickly loaded with glochidia upon both fins and gills, though as we shall see, the latter appear to be particularly adapted for ridding themselves of the entire infection.

Infections with hooked glochidia

For the infections with hooked glochidia, we have used principally *Anodonta cataracta* from Falmouth, Massachusetts, the species studied by Lillie ('95). With these, we have, infected German carp under six inches in length and, unless otherwise stated, the following account refers to this combination which gives typical results. A smaller number of infections made with *Symphynota complanata* and *S. costata* upon carp and other fish are referred to in a supplementary manner. The glochidia of *A. cataracta* become attached in large numbers to the fins (Figs. 7-11 and gills of the carp. They are also found upon the other external parts which offer the condition of a soft scaleless epithelium like that of the fins; thus the region about the anus, the edge of the operculum, the lips and, in very heavy infections, even the soft area of the ventral surface between the mouth and pectoral fins may become heavily loaded. Within the mouth cavity, the gill-filaments and also the gill-bars and rakers become well covered. The glochidia which attach to these mouth parts do not remain. for, though the fish may be carrying many of their fellows upon its external parts, in about one week after the infection all glochidia have disappeared from the gill-filaments, which then become as clean as though never infected. There is some chance of a scattering of glochidia remaining upon the other internal mouth parts, for such specimens are occasionally seen well embedded and in advanced stages of their metamorphosis, but in the main, these parts also will become free of glochidia.

The general distribution upon the individual fins may be seen by reference to figs. 7-11, which show how great a proportion

of the glochidia become attached to the fin margins. If a fish is carefully watched, as its slight movements stir up the glochidia during the infection, the latter are seen continually falling upon the upper faces of the pectoral and pelvic fins. They may even be collected with a pipette and heaped upon a motionless pectoral, remaining there for some minutes without more than an occasional specimen becoming attached. The margin of the fin is so much more favorable for attachment, that it is often thickly set with glochidia, when none are found upon the fin surface, and this despite the fact that glochidia must, during infection, strike against the surfaces of the fins, many times for every time that one of them comes in contact with a fin margin. It is, therefore, the fin margin for which this glochidium is best suited, and once fastened there, it is almost certain to remain and become overgrown. When a specimen does fasten to the surface, it probably gains its hold by catching upon one of the ridges formed by the fin rays, for the hooks could hardly be used upon a perfectly flat surface. Glochidia sometimes hold to the surface of a fin by a shred of tissue, under which their hooks have caught and remain there after all their fellows (fig. 11) are completely overgrown, only to be torn off later without having caused any noticeable hypertrophy of the fin tissue. Figs. 11 and 14b show that glochidia may become overgrown either flat against the surface, or upon edge, and fig. 12 shows a young mussel leaving a surface attachment after a parasitism of seventy-four days.

The distribution of the glochidia to the several fins is determined solely by the number likely to be brought in contact with a given part of the body. Those fins which brush against the bottom are always the more heavily loaded and the numbers elsewhere depend upon the extent to which the glochidia are kept suspended on the water.

Optimum infections, as we shall term those which are close upon the limit of the number of glochidia which a fish can safely bring through the metamorphosis, often show the glochidia very closely set one after another, as in fig. 11, and several hundred may be safely carried by a fish three or four inches in length. Prolonged exposure causes so heavy an infection of the margins

(fig. 9) that the fin tissue appears unable to overgrow the mass of glochidia and they then remain attached without overgrowth for a week or more. Fig. 10 shows how, in a part of the fin having no overcrowding, normal embedding occurred; while in the more crowded areas the glochidia were still uncovered even seven days after infection. In the region of the middle upper margin of this figure, it would seem that the overgrowth might well have taken place; for many cases, like fig. 11, have been observed in which glochidia as closely set were properly embedded. The failure of overgrowth in this region is probably due to the presence immediately after infection of a greater number of glochidia, many of which have since been detached. In all cases of this kind, a smaller number will finally become embedded than in an infection where the fin has received more nearly the optimum load (figs. 7, 8 and 11), for the great majority drop off when the fin becomes so mutilated that bacterial or fungus infection sets in. These over-infections sometimes cause so much hypertrophy that the fins become lumpy and the rays so much drawn together that it is impossible for the fin to spread out normally. Often, the fins are raw and bleeding for some days and show red areas within where the blood vessels have become abnormal. The fish are likely to die from this, or from the similar injury to their gills, and these over-infections are unsatisfactory, if one wishes to bring through their parasitism the maximum number of glochidia per fish.

The steps in the implantation of the glochidium by an overgrowth of the fish's tissue may be seen in figs. 7-11 and 20-23. Figs. 7 and 20 show the glochidium three and one-half hours after the fish was removed from the infection. Most of the glochidia have bitten deep enough in from the margin to have a good hold for their hooks. The beginning of the hypertrophy appears as a faint mass of tissue, seen with its nuclei in the detailed fig. 20. At the end of twelve hours, the overgrowth is well advanced and sometimes, as fig. 21, shows different stages even in neighboring glochidia. The ragged edge of the host's tissue rises up crater-like about the glochidium, meeting above in a delicate mass, the nuclei of which are shown. Fig. 8 shows that, in twenty-

four hours, most of the glochidia are more than half covered, whether upon the edge or the surface of the fins. At the end of thirty-six hours, optimum infections of the carp show all the glochidia, which have obtained a proper attachment, well embedded, and after this the only external change is a slight increase in opacity which renders the internal structure of the glochidium less distinct. Some of our infections show embedding in as short a time as six hours (Symphynota) and Harms ('09) gives ten to twelve hours, as the time which he observed in Anodonta, so the time given for the figures is the maximum for hooked glochidia which have been well located. Glochidia upon the fin surface become embedded in a similar manner and are then in a very secure position. (Figs. 11 and 14b.)

Infections with hookless glochidia

Following these same methods of artificial infection, we have made more extensive experiments upon the parasitism of the hookless glochidia, which is the only type found in our commercial mussels. Species of the genus *Lampsilis* (*ligamentinus*, *rectus*, *anodontoides*, *ventricosus*, *subrostratus* and *luteolus*) have been the most used, but infections have also been made with several species of *Quadrula* and one of *Unio*. The list of fish employed as hosts is also more extensive and we are, therefore, able to make statements which we know to be of wider application than those made for the hooked forms.

When the same fish is used, the results for the several species of *Lampsilis* are very uniform and we can thus discuss the parasitism of this genus as a whole: but we do not find the same mussel giving uniform results with all species of fish. The glochidia of this genus have been used successfully for the infection of bluegill sunfish, yellow-perch, crappie, large-mouthed black bass (*Micropterus salmoides*), rock bass, the red-spotted sunfish (*Lepomis humilis*) and the green sunfish (*Apomotis cyanellus*). As with the hooked glochidia, the infections have all been made upon fish under six inches in length, upon which these glochidia remain in numbers only on the gill-filaments, though during infection many

become attached to and even embedded upon the fins and other external parts. Harms ('08) concludes that the hookless type persists in much greater numbers on the fins of small than of large fish and that the hooked type will survive upon the gills if large enough fish are used, and it is doubtless true that the size of the gills and fins is an important factor in determining the place of attachment for each type, since the hookless form is only adapted for holding to a delicate surface like the gill-filament, or a fine fin while the hooked seem likely to be easily torn from just such a surface. When the hookless form does once become established upon an external part, it will develop there without mishap as shown by a figure of a hooked and hookless glochidium developing side by side upon the margin of a fin (fig. 23). Within the mouth cavity, the glochidia will attach to the gill-bars and rakers if these parts are covered by a sufficiently delicate epithelium, but upon the gill-filaments they are always found in the greatest numbers. In most of our infections the filaments are the more heavily infected toward their outer ends (fig. 19), but the distribution varies somewhat with the species of fish. For example, successful infections of rock bass with *Lampsilis ligamentinus* show about seven glochidia upon the distal third of the filament to one upon the proximal two-thirds; of large-mouthed black bass about three to one; and of yellow-perch about one and a half to one; differences which are probably caused by some particular configuration of the mouth cavity which causes the glochidia to fall more upon one part of the filaments than another.

In a fish which will carry a given glochidium successfully, over-infection of the gills is easily accomplished and easily fatal, but the species of fish differ greatly in the amount of infection they are able to carry without serious mortality. In one of our most successful combinations (rock bass + *Lampsilis ligamentinus*). fish four inches in length were estimated to be carrying in the neighborhood of 2500 glochidia, an average of more than two for every filament of the gills and yet there was almost no mortality among the fish. In this case the success of so heavy an infection is perhaps explained by the distribution of the glochidia upon the gill-filaments, for we found by count that there were about seven

near the tips to one on the sides and thus the greater part of every filament was left unchanged and in full functional condition, while in other infections (large-mouthed black bass + *L. ligamentinus*), where a much greater proportion of the glochidia were upon the sides of the filaments, the mortality of the fish was very heavy, although the amount of infection was much less. A gill of the latter fish, from a lot lightly infected with these glochidia is shown in fig. 13. The number estimated for this fish, which was four inches long, being only 450, is distinctly less than the optimum.

Implantation upon the filaments occurs in a manner similar to that of the hooked glochidia upon the external parts, but much more rapidly. Figs. 15, 16, 17 and 18 show the appearance at 15 minutes, 30 minutes, 1 hour and 3 hours after infection, and our observations showing that the cyst is completed within from two to four hours, agree with what Harms ('09) has found for gill infections. The proliferation will even continue after the gill has been cut from the fish and placed in a watch glass for observation under the microscope. An immediate result of the cyst formation is the obliteration of the lamellæ upon either side of the gill-filament, which thus becomes smooth and slightly swollen in the vicinity of the glochidium. Figs. 13 and 19 show the general and detailed appearance of the cysts and the diversity in the angles at which the glochidia are attached.

The older statement that the hooked glochidia are fin and the hookless gill parasites finds, therefore, confirmation from our work though it would be better to say that the hooked attach most successfully to large strong margins like those of the fins while the hookless to soft and fine filamentous structures like the gills in fish of moderate size. The reaction of hookless glochidia to blood, with respect to the part it plays in attachment, has already been described.

Susceptibility of fish to infection

The susceptibility of different fish to infection is a matter which we think has not been sufficiently considered by any previous

investigators for we seem to have evidence that some fish are much less susceptible than others and that with such fish any considerable infection is an impossibility, the most striking instances of this being the German carp, minnows and darters.

In the case of the carp, we have a fish admirably suited for carrying the hooked glochidia of *Andonta* and *Symphynota*, but we have never been able to secure a successful infection of the gills of the carp with the hookless glochidia of the genus *Lampsilis*. The disappearance of the hooked glochidia *Anodonta* and *Symphynota* from the gills of the carp, as previously mentioned, is explicable upon the grounds given in our consideration of the reasons for the survival of the two types of glochidia upon the fins and gills respectively but any such explanation is impossible when applied to this disappearance from both gills and fins of the glochidia of *Lampsilis*.

With minnows (*Notropis cayuga* and *N. lutreusis*) two to four inches in length, we have not been able to secure any considerable infection with the glochidia in *Symphynota complanata*, for, though they will attach in large numbers during infection, they all drop from the fins and gills within a few days. The fin of these minnows, is much more delicate than that of the carp and the explanation is perhaps that so large a glochidium is easily torn away; but the large mouthed black bass has hardly a delicate fin, and for it we have records of infection where no glochidia of *S. complanata* attached during an exposure sufficient for the attachment of many to the gills. In this case, the extreme activity of the fish must be considered as a factor which might keep the hooked glochidia from attachment to the fins.

With darters, (*Etheostoma coeruleum spectabile*) one and one half to two inches in length, there appears to be an almost complete immunity against the permanent attachment of *Lampsilis* glochidia, for though they may fasten so thickly to the fins that many fish are killed within the first day after the exposure, the fish which survive this will slough off considerable portions of the fins and within a week show only the healed and regenerating parts as an indication of their recent experience. Such cases as these are of great importance and should be followed up to deter-

mine whether the simple mechanical causes of over-infection, delicacy of fin, or configuration of the mouth parts can give a satisfactory explanation; or whether the histological changes which the fish is capable of, under stimulation, by the glochidium, must be regarded as the causes of its immunity. We have not yet carried out sufficiently rigorous experiments to feel sure that the simpler explanations can be excluded. In any case, it is interesting that fish like the minnows and darters, which live close to the bottom, are not likely to become heavily infected by some of our most common glochidia.

Duration of the parasitism

The duration of the parasitism appears, to be influenced very considerably by the temperature as stated by Schierholz ('88) and Harms ('07-'09). Our records show a difference in the time, spent by a given glochidium upon the same fish, which varies with the season. For example, we have records of *Symphynota complanata* completing its metamorphosis and leaving the fish in from ten to fourteen days during December, and others in which the parasitism, begun March 25th, was continued for about forty days. *S. costata*, in an infection begun during January, remained upon the fish for upwards of seventy days. Infections with *Lampsilis glochidia* have shown a variation in the duration at the temperature of the laboratory (16°-20° C.) of from 36 to 14 days. The glochidia of *Unio complanatus* and of *Quadula plicata* in July infections gave a period of from twelve to fifteen days. In all these cases, the temperature is probably the cause of the differences, as Harms believes.

HISTOLOGICAL CHANGES IN CYST-FORMATION

As has been described, the glochidium attaches itself to the fish by closing its shell firmly over some projecting region which can be grasped between the valves, like the free border of a fin or the gill filament. In so doing, a portion of the epithelium and underlying tissue, including blood vessels and lymphatics, and

varying in amount with the extent of the "bite," becomes enclosed within the mantle space of the glochidium. This tissue early disintegrates into its cellular constituents, which are taken up by the pseudopodial processes of the larval mantle cells, and as Fausek ('95) has described, are utilized as food during the early stages of metamorphosis. In fig. 37, drawn from a glochidium six hours after attachment to a fin, the disintegrated tissue, consisting of loose epithelial cells, blood corpuscles, and fibers which lie scattered in the mantle cavity, is seen in the process of being ingested by the mantle cells. Fig. 38 shows a later stage, twenty-four hours after attachment, in which the detritus has been entirely taken up, and the mantle cells are now heavily charged with food material.

Almost immediately after attachment proliferation of the epithelium begins as the initial step in the formation of the cyst which eventually encloses the entire glochidium. The overgrowth of the larva has been described by Fausek ('95) and Harms ('07, '09) as a healing process on the part of the fish's tissues, resulting from the irritation caused by the wound. The proliferation starts around the line of constriction produced by the pressure of the edges of the valves on the epithelium, and, since the glochidium lies between and prevents the immediate closure of the lips of the wound, the extending epithelium is forced to slide up over the surface of the shell on all sides, until the free margins meet and fuse over the back of the larva, as may be understood by reference to figs. 15-18 and 20-22.

So rapid is the overgrowth, especially in the case of implantation on the gills, that it would seem that something more than the mere mechanical irritation produced by the glochidium is concerned in causing the proliferation of the epithelium. We have, therefore, carried out a series of experiments with a view to determining whether or not, a chemical stimulus is provided by the larva, and by using various methods have studied the action of glochidial extracts on the epithelium of both fins and gills. The results have been entirely negative, although the question has by no means been settled by the experiments which have been thus far attempted. By further improvements in the technique,

some of the difficulties involved in the investigation, which is still in progress, may be overcome.

The histological changes taking place in the epithelium during the formation of the cyst have been studied in this laboratory by Miss Daisy Young, and as her results will soon be published in detail, only a very brief reference will be made to the subject here. We are indebted to her for the use of six of her drawings which are reproduced in this paper in figs. 33-38, in order to illustrate the essential points involved in the cellular changes occurring during implantation.

Fig. 37 shows an early stage, 6 hours after attachment, in the formation of the cyst on the fin. The proliferation begins in the neighborhood of the constriction where several mitoses may be seen in the figure, and this seems to be the region of active growth and multiplication of cells. As the cells at this level increase in number, they appear to push those lying above them up over the outside of the shell, so that the actual covering of the glochidium is due largely to this mechanical gliding of the epithelium over its surface. Sections give no evidence at all of amitotic division, while mitoses are generally abundant in the region of active proliferation. Fig. 38 shows a case of complete implantation on a fin in 24 hours. The wall of the cyst is seen at this time to be quite thick, but it usually becomes thinner later on as the cells composing it flatten down.

In figs. 34, 35, and 36, a series of stages are represented in the formation of the cyst on gill-filaments, taken at 15 minutes, 30 minutes, and 3 hours, respectively, after attachment. Fig. 34 shows the very beginning of the proliferation and the presence of two or three mitotic figures just below the glochidium and near the raw edge of the constricted epithelium. A little coagulated blood is seen on the surface of the shell and around the lips of the wound, showing how intimately the larva comes into contact with blood at the time of attachment, as referred to above. A large mass of the fish's tissues, including portions of blood vessels, is also shown in the figure enclosed within the mantle chamber. At the next stage (fig. 35), the pushing-up of the epithelium has made considerable progress; several mitoses appear in the

section, while a few loose epithelial cells are sloughing off at the edges of the growing cyst—a not infrequent occurrence during the early stages of implantation. Fig. 36 shows the completion of the process, and the glochidium is now, after three hours in this case, entirely enclosed within its epithelial covering.

LIBERATION FROM THE CYST AND POST-LARVAL STAGES

In about one week as a rule after attachment, the wall of the cyst begins to assume a looser texture, the intercellular space becoming infiltrated with lymph, and from this time on to the end of the parasitic period there is little further change in its structure.

Before liberation of the young mussel, the valves open from time to time and the foot is extended. By the movements of the latter the cyst is eventually ruptured, its walls gradually slough away, and the mussel thus freed falls to the bottom. Fig. 33 shows an early stage in the breaking up of the cyst which is seen to be coming off in patches on one side. Portions of the wall of the cyst often adhere to the shell after liberation, while, if the young mussel has hooks, it may hang for a time by shreds of the fin in which the hooks are embedded, as seen in fig. 12.

We have not succeeded in keeping the young mussel alive in the laboratory for a longer period than six weeks. From the first they are very active and creep about in a dish by stretching out the foot, securing a hold by flattening the distal end against the bottom, and then drawing up the body after the fashion of other small lamellibranches. Fig. 29 gives an excellent illustration of the various positions assumed as they crawl about, and also shows the extent to which the shell has developed beyond the margins of the glochidial valves by the end of the first week of free life.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Gravid female of *Ptychobranchus phaseolus*. $\times \frac{3}{4}$
- 2 Gravid female of *Lampsilis subrostratus*. Natural size.
- 3 Gravid female of *Obliquaria reflexa*. Natural size.
- 4 Gravid female of *Quadrula ebena*. $\times \frac{1}{2}$.
- 5 Gravid female of *Lampsilis rectus*. $\times \frac{1}{2}$.
- 6 Gravid female of *Symphynota complanata*. $\times \frac{1}{2}$.

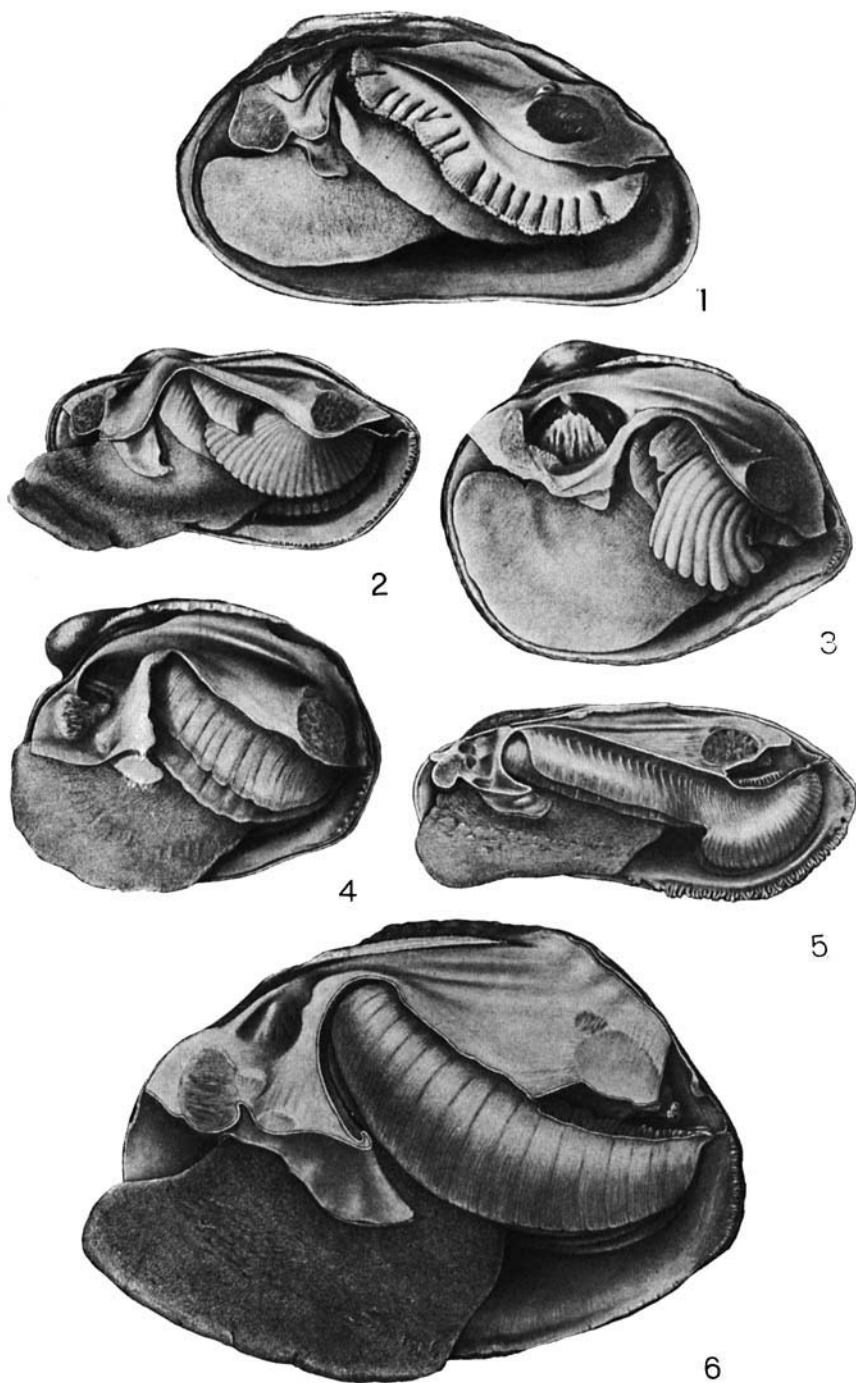


PLATE 2

EXPLANATION OF FIGURES

7 Pectoral fin of a carp, about 3 inches long, 3½ hours after infection with glochidia of *Anodonta cataraeta*. An optimum infection.

8 Ventral half of the tail of a carp, as above, 24 hours after infection. An optimum infection.

9 Tip of an over-infected fin, as above, 12 hours after infection. Showing no appreciable over-growth because of the crowding. The shadows represent glochidia upon the under surface.

10 Fin, as above, 7 days after infection. Showing the complete failure to embed in all places where the glochidia are greatly crowded. See explanation in the text p. 106 of the conditions along the upper margin.

11 Fin, as above, 36 hours after infection. Showing the complete overgrowths of all glochidia which have secured the proper attachment.

12 Young of *Symphynota costata*, leaving the fin of a carp after a parasitism of 74 days.

13 Anterior gill of a black bass infected with *Lampsilis ligamentinus*, showing distribution upon the gill as a whole and the appearance of the cysts.

14a Glochidium of *A. cataraeta* upon fin of carp. Developing normally after a shift of 90 degrees from the position first taken.

14b Two glochidia of *Anodonta cataraeta*, overgrown after 36 hours upon surface of a carp's fin.

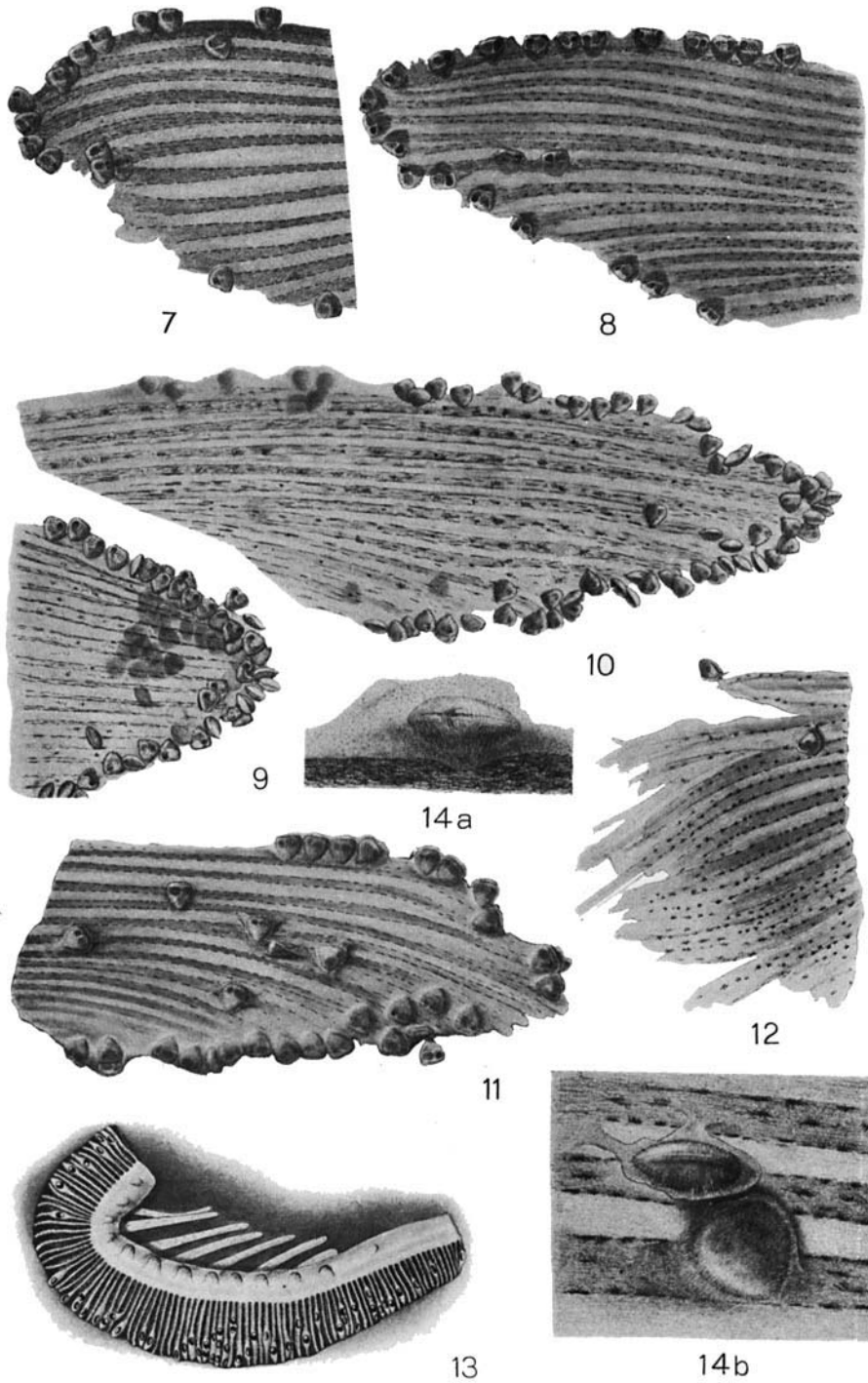


PLATE 3

EXPLANATION OF FIGURES

15, 16, 17, and 18 Stages in the formation of the cyst about a hookless glochidium (*Lampsilis ligamentinus*) upon a gill filament of the black bass. Taken at 15 min., 30 min., 1 hour, and 3 hours, after infection. The transverse lines on the filament are the lammellæ.

19 Part of a gill of black bass infected by *L. ligamentinus*. Showing the distribution and orientation of the glochidia in an infection above the optimum for this fish. Only the layer of filaments toward the observer is shown.

20 Glochidium of *Anodonta cataracta* upon fin margin of carp. 3½ hours after infection. Proliferation of the cyst just beginning.

21 Glochidia, as above, upon fin margin of carp. Showing different stages of cyst proliferation, even in neighboring glochidia.

22 Glochidia, as above, 24 hours after infection.

23 Hooked and hookless glochidia (*A. grandis* and *L. rectus*) embedded and developing on a fin margin. The former received in nature and therefore older than 28 days, which is the age of the latter.



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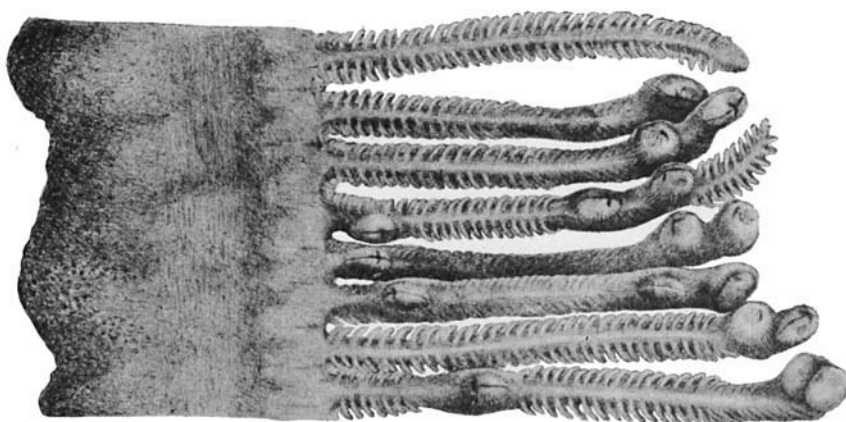
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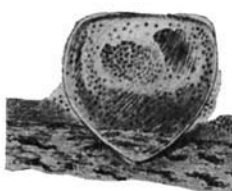
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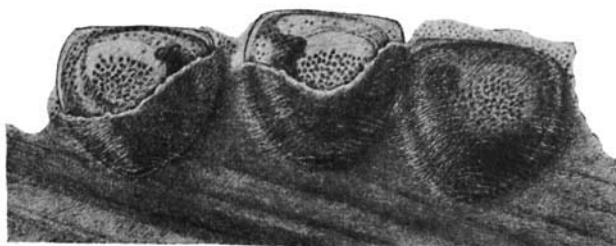
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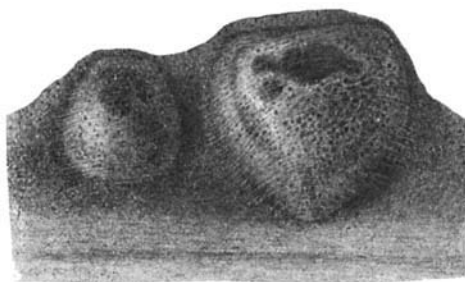
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PLATE 4

EXPLANATION OF FIGURES

24 Detail of a conglutinate of *Lampsilis ligamentinus*. The glochidia, still enclosed in the membranes, are less crowded together than those of fig. 28, and are embedded in a mucilaginous matrix.

25 Glochidium of *Lampsilis* (*Proptera*) *alatus*.

26 Three conglutinates of *Obliquaria reflexa*, removed from the marsupium $\times 2$.

27 Two conglutinates of *Lampsilis ligamentinus*, removed from the marsupium. One is shown from the flat surface, the other on edge. $\times 2$.

28 Detail of a conglutinate of *Obliquaria reflexa*, showing the membranes closely pressed and adhering together.

29 Young mussels of *Lampsilis ligamentinus*, one week after liberation from the fish, showing various positions assumed in crawling, the ciliation of the foot, and the new growth of shell.

30 Transverse section of two water-tubes of the gravid outer gill of *Quadrula ebena*, showing the glandular epithelium on the interlamellar junctions and two embryos in an early cleavage stage.

31 Transverse section of a single water tube of the marsupium of *Lampsilis alatus*, showing the much greater size of the tube than in the last figure, which is drawn under the same magnification. Several embryos in an early cleavage stage are seen in the tube.

32 Highly magnified section of a portion of the glandular epithelium lining a water-tube of the marsupium of *Quadrula ebena*, showing the large mucus cells and several nuclei of leucocytes (l) with which the epithelium has become infiltrated.

33 Section of young mussel, *Unio complanatus*, 11 days after attachment of glochidium to gill-filament, still in cyst which is beginning to break away.



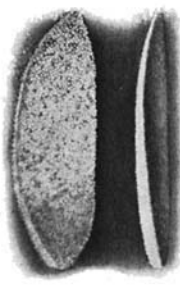
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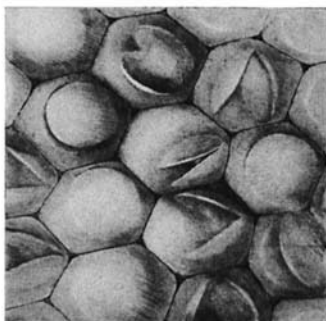
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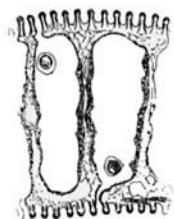
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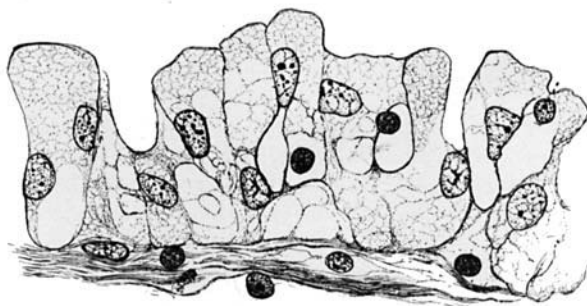
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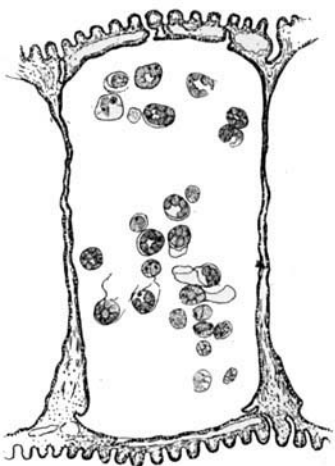
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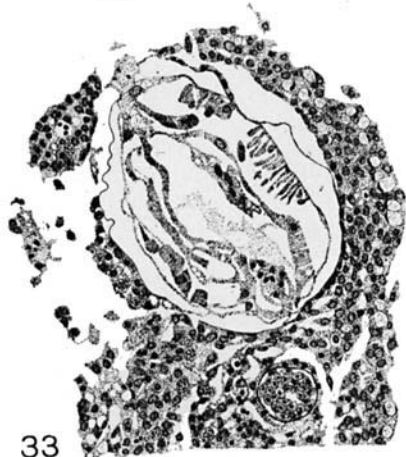
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PLATE 5

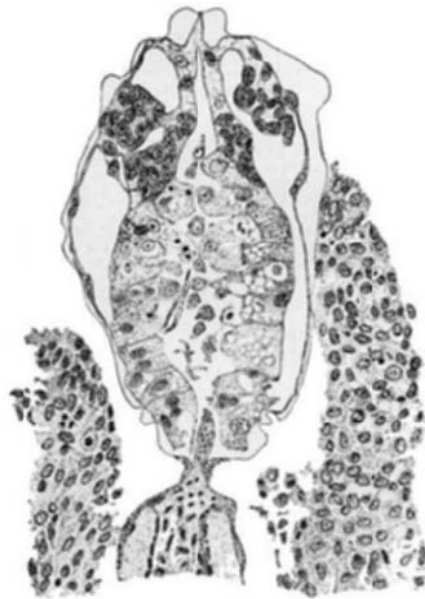
EXPLANATION OF FIGURES

34-36 Sections of glochidia of *Lampsilis ligamentinus*, taken 15 minutes, 30 minutes, and 3 hours, respectively, after attachment to gill filaments. In 34, the proliferation of epithelium is just beginning; in 35 it has made some progress; and in 36 formation of the cyst has been completed. In the first two figures several mitoses are shown in the epithelium where multiplication of cells is taking place.

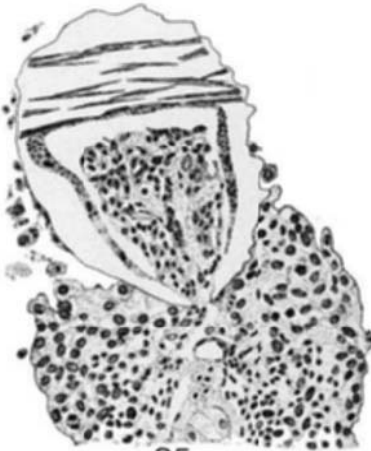
37-38 Sections of glochidia of *Symphynota complanata*, 6 and 24 hours, respectively after attachment, showing two stages in cyst-formation on fins. In 37 the cellular detritus is being ingested by the larval mantle cells, while in 38 this process has been completed. Mitosis in the cyst wall is shown in both figures.



34



37



35



36



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