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SYMBIOTIC RELATIONSHIPS OF THE SPHAERIID  
CLAM MUSCULIUM TRANSVERSUM

by

Peter Neal D'Eliscu

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A Dissertation Submitted to the Faculty of the

DEPARTMENT OF BIOLOGICAL SCIENCES  
WITH A MAJOR IN BIOLOGY

In Partial Fulfillment of the Requirements  
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 7 4

THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my  
direction by Peter Neal D'Eliscu  
entitled SYMBIOTIC RELATIONSHIPS OF THE SPHAERIID  
CLAM MUSCULIUM TRANSVERSUM  
be accepted as fulfilling the dissertation requirement of the  
degree of DOCTOR OF PHILOSOPHY

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SIGNED: *Philip Neal D'Elben*

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## ABSTRACT

The organisms associated with the sphaeriid clam Musculium transversum exist in several symbiotic levels. Serial sectioning, differential staining, and C<sup>14</sup>-autoradiography have shown various taxonomic groups living in phoretic, commensalistic, mutualistic, and parasitic relationships with this host clam. The phoretic bryozoan Plumatella deposits resting sessoblasts on the shell of the host. The spinose rotifer Filinia lives in the mantle cavity as a commensal. The endosymbiotic beetle Berosus is predaceous on the commensal coprozoic oligochaetes Aeolosoma and Chaetogaster. Rotifers and the parasitic nematode Seinura are captured by the predaceous fungus Endosphaerium funiculata, which lives attached to the gills of the host. Endosphaerium is therefore mutualistic, conferring on its host some resistance to a destructive parasite. The reproductive development of the fungus is closely coordinated with that of its molluscan host.

Aestivating clams show several physiological adaptations for water conservation, including the production of uric acid crystals instead of ammonia, and the secretion of hygroscopic mucous threads by pyramidal cells on the mantle. The moist environment provided by aestivating hosts also supports several components of the symbiotic

community, including juvenile oligochaetes Aeolosoma and Chaetogaster, and the resting stage of the fungus Endosphaerium. The predatory leech Glossiphonia sp. chemically detects and selects active Musculium as prey over other molluscs, including Physa virgata and Helisoma tenue.

## INTRODUCTION

Few organisms live alone. The range of long and short term interspecific relationships encompasses all taxa from the bacteria to the most advanced vertebrates. De Bary (1879) coined the term "symbiosis" to describe the "living together" of two heterospecific organisms without implying any type of mutual or unilateral dependency. Since his definition, the general relationship of symbiosis has been variously subdivided into the categories and subsets of phoresis, commensalism, mutualism, and parasitism. These categories are useful working tools, but as Cheng (1970) pointed out, they are actually areas along a gradient of dependence and interaction between two different organisms.

In more advanced forms, beneficial symbiotic relationships allow greater success for heterospecific partners functioning as a symbiotic unit than for the individual species when separated. The success and complexity of symbiotic associations depends on many factors, including morphological, physiological, chemical, and behavioral adaptations of both partners (Linicome 1963, Cheng 1970). "Symbiosis is seen to be not a special remarkable nutritional quirk of a few strange organisms, but a widespread habit which allows adaptations to overcome many common

ecological situations by combining . . . special properties" (Harley 1970, p. 65).

Although many symbiotic relationships have been documented, few investigations have established the specific levels of complexity or the energetics of interaction between symbiont and host. For this reason a study of the specific symbiotic relationships of the small freshwater clam Musculium transversum Say (Pelecypoda: Sphaeriidae) was initiated (Fig. 1). Preliminary investigations had shown the mantle cavity of this bivalve to be occupied by a taxonomically complex biological community. The micro-environment within the shell appeared to provide an ideal situation for a study of symbiosis on a microcosmic scale.

In this study the several categories of symbiotic relationships described by Cheng (1970) and Koch (1967) were interpreted with the addition of energetic considerations. Organisms associated with Musculium accidentally or incidentally, and having no obligatory or nutritionally dependent physiological relationship, were considered phoretic. Animals normally living within the mantle cavity and not invading host tissues were considered commensal. In this case it might be said that host and commensal were simply "eating at the same table." This commensal category included both facultative and obligate associations, so long as the host suffered no significant detrimental loss beyond its normal energy reserves through direct competition for



Fig. 1. The Sphaeriid Clam Musculium transversum

food or oxygen. Parasitic organisms were those considered to have energetic or nutritional dependency on the host. This dependency generally involved host tissue invasion or destruction. Within a wide range of degrees of dependency, organisms which were mutually metabolically dependent on each other were considered mutualistic.

In this study the symbiotic relationships between active Musculium and both metazoan and fungal organisms were investigated through dissection, serial sectioning, and autoradiographic methods. The interactions of phoretic, commensal, mutual, and parasitic symbionts were established by studying nutritional adaptations and partial food chains among these organisms. For several reasons, a study of aestivating clams was also initiated. Aestivating host clams may provide an environment for endosymbionts different from that of active clams. Host adaptations for aestivation may influence symbiont population dynamics and individual life forms. Aestivating clams may also provide a sanctuary for facultative symbionts, which could repopulate the habitat after the return to aquatic conditions. Studies of predation on the clam population, as well as possible mechanisms for dispersal, were also begun. Predation and dispersal were considered since these factors could also influence host population dynamics and thus indirectly affect symbiont populations.

As a taxonomic note, these studies employ the genus Musculium as a tentative classification of the molluscan host. Herrington (1962) revised the Sphaeriidae and suggested the total elimination or subgeneric designation of the genus Musculium. However, this genus is considered valid by Heard (1963, 1964, 1965a, 1965b) because of differences in siphonal, brood pouch, and shell organization. Protein analysis or chromosomal observations might help clarify this question. The genus Musculium as used in these studies includes M. transversum, M. lacustre (Müller), M. partumeium (Say), and M. securis Prime.

## METHODS AND MATERIALS

Field studies and collections of Musculium transversum were made in an oxbow pond one mile southeast of Arivaca, Arizona. Field samples were collected regularly from October, 1971, to May, 1973. The pond was formed from a tributary to the Santa Cruz River drainage system. The pond was located on the ranch lands of Mr. Fred Noon, and was designated the Noon Pond during preliminary investigations. This pond was located approximately one mile south of the Arivaca Musculium transversum location cited by Bequaert and Miller (1973). The Noon Pond has a maximum length of about 100 meters and a maximum width of about 40 meters. The maximum recorded depth was 3 meters. All of these dimensions fluctuated throughout the year, with minima occurring in February, 1972. The depth of the pond dropped to one meter at this time, with linear dimensions dropping to 60 by 20 meters. Monthly temperature and pH measurements for water immediately above the bottom substrate, 2 meters from the pond edge, are shown in Fig. 2.

Bottom sediments of the Noon Pond were composed of mud mixed with varying amounts of plant debris from the emergent and submergent plants. The southwest end of the pond was fringed by a stand of water reeds, Typha sp. Dense growths of submergent and surface Ranunculus sp. increased

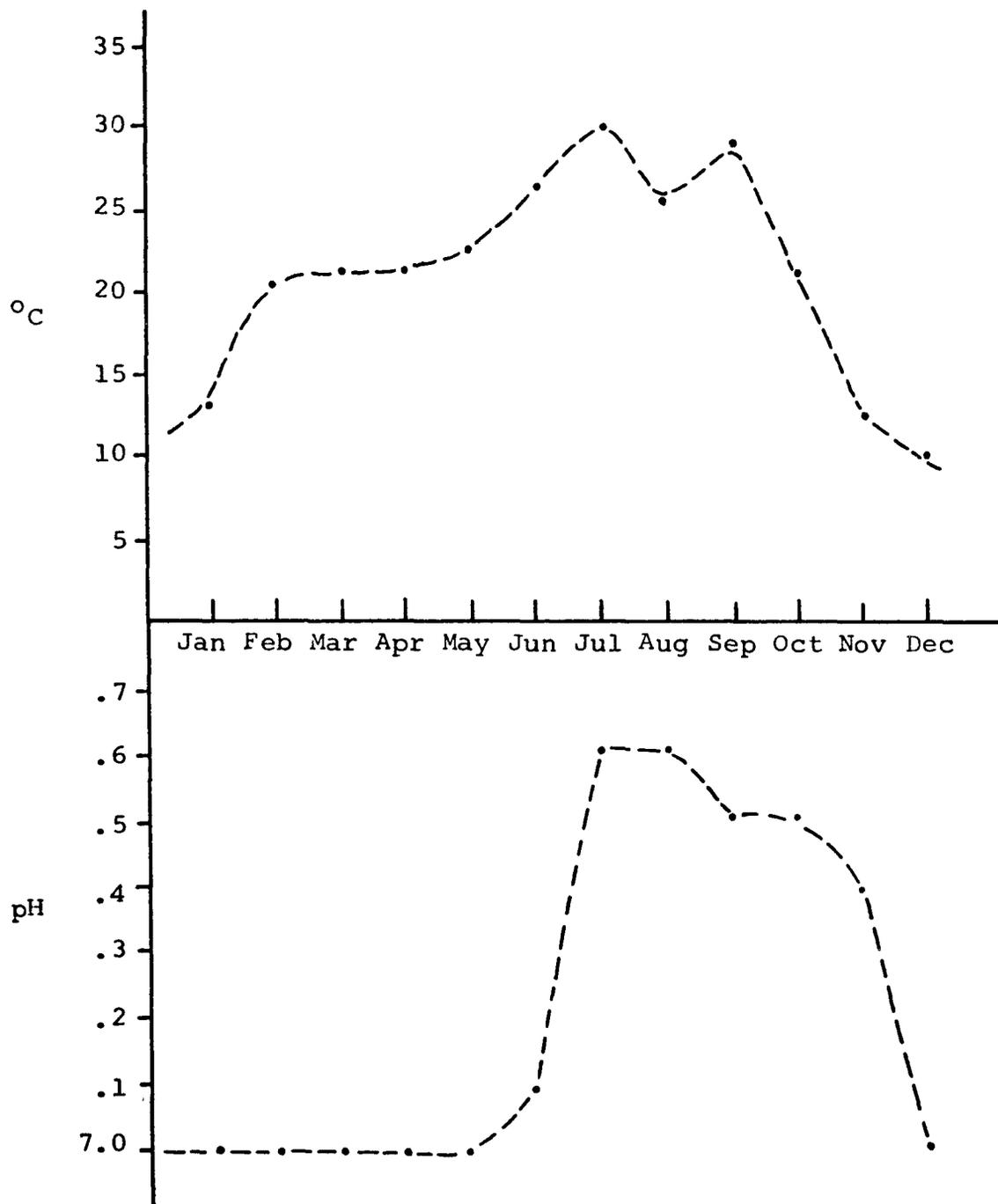


Fig. 2. Temperatures and pH of the Noon Pond October 1971 to September 1972

in size throughout the studies and covered most of the pond by May, 1973. Although the pond size fluctuated seasonally, it was considered permanent water since it supported several kinds of fish, Sonoran mud turtles, frogs, and was visited by great blue herons and various ducks. Local fishermen claimed the pond was "excellent" for blue gill and bass fishing.

Active Musculium transversum were collected in the Noon Pond through the use of a wire mesh tea strainer drawn through bottom sediments. No specific stations were established for collection. Instead, various depths were sampled from along the shore line. Temperature and pH readings were taken in nearly the same pond location each month. Samples collected from water depths greater than one meter were made by extending the wire mesh net on a set of interlocking aluminum tubes designed and constructed for just this purpose. Some samples from the deepest area of the pond were collected from 3 meters of water. Diving for samples was therefore unnecessary and possibly would have been less effective than screening due to the high turbidity of the water. In the later months of this study diving or dredging would have been restricted by the dense growth of rooted aquatic plants.

Immediately upon collection, animals were either placed in small plastic thermos containers for live transport to the laboratory, or fixed en masse in 70% ethyl

alcohol in small glass vials. Preliminary studies had shown the clams to close tightly after contacting the alcohol. As they remained tightly sealed after death, it was assumed that symbionts would remain inside individual clams even when numbers of animals were fixed at the same time. At least 25 adult clams and a variable number of juveniles were preserved in each monthly field collection.

Live clams were returned to the laboratory and maintained either in 250 ml glass culture bowls or in 5 gal. aquaria. Following several of the suggestions by Krull (1936), Thomas (1954), and Paige and Imlay (1970), the smaller containers were maintained without aeration or bottom sediments. The aquaria were aerated and contained muddy substrates from the Noon Pond. Later, clams used in leech predation experiments were maintained similarly, with the exception that bottom materials were not added. Rather, these clams were held in aerated aquaria with black, one millimeter diameter gravel so that even the smallest cream colored specimens could be easily located. In all cases the holding tanks were maintained at room temperatures ranging from 18° to 20°C.

The normal food of most sphaeriids consists of phytoplankton, bacteria, and yeasts (Gilmore 1917, Baker 1928, Krull 1936, Rodina 1948, Gale and Lowe 1971). However, the greatest success in feeding laboratory Musculium was achieved by feeding small portions of strained spinach,

carrots, and liver, obtained as commercial baby food. As Thomas (1954) pointed out, it was not clear whether this food type constituted the total intake of the laboratory animals or whether bacteria growing on the food particles were also a food source. Examination of the guts of spinach-fed clams did show green fibers assumed to be spinach particles. An additional advantage of using such deeply colored foods as spinach or carrots was the ability to see bands of food moving through the stomach digestive gland, and intestine when viewed through the thin shell with dissecting microscopes and strong back lighting.

Occasionally large "blooms" of ciliates would occur under these conditions in the larger aquaria. In these instances, the clams were carefully removed and temporarily isolated while the tank was drained of water and the sediments gently rinsed with aged water. Water aged at least 48 hours was used in all initial set-ups and for replenishment of evaporative losses. This was done to eliminate the possibility that small amounts of chlorine in the laboratory water supplies might affect fragile symbionts. Preliminary tests showed this method to be sufficient to maintain the animals and therefore Noon Pond water was not transported to the laboratory for these purposes.

Occasional field collections of aestivating clams were made by digging up peripheral pond sediments with a

small garden trowel. These samples were treated in two ways. In some cases aestivating clams were removed from the sediments in the field by coarse screening through a geological grid pan with one millimeter openings. These clams were then placed in damp paper toweling within thermos containers for transport to the laboratory. On other occasions, entire sediment samples were returned to the laboratory in plastic bags. These samples were then maintained in partially covered plastic buckets. An effort was made to keep the soil in the buckets moist, but not saturated with water. Live aestivating clams could be maintained in this manner for as long as 3 months.

#### Serial Sectioning

Clams used in thin sectioning and microdissection procedures were fixed in 70% ethyl alcohol. Although this fixative caused some contraction of the visceral mass, it proved to be the best method of fixation to prevent loss or disruption of symbiont positions within the host.

Clams were surgically removed from their shells after fixation. After further dehydration of the soft anatomy, clams were cleared in xylene, saturated, and finally embedded in paraffin blocks. The specimens were then sectioned at 7 to 9 $\mu$  on a microtome adapted to utilize "Valet Auto-Strop" razor blades as the cutting instruments. Appendix A summarizes the several staining techniques

employed after the serial sections were mounted on standard 25 x 75mm glass microscope slides. Sections were examined with a binocular microscope with magnifications ranging from 100 to 1000X. This same microscope was used in preparing many of the photomicrographs presented as figures.

#### Autoradiographic Methods

Some live clams were fed for short periods of time with Chlorella sp. which were labeled with  $C^{14}$ . The immediate source of the isotope for incorporation into algal tissue was  $C^{14}O_2$ , derived from  $NaHC^{14}O_3$  in solution. Radioactive algae were produced by growth of cultures in 250 ml Erlenmeyer flasks supplied with .01 microcurie of  $C^{14}O_2$  for one hour in indirect sunlight.

Live clams were starved for 3 to 5 days to clear the digestive tract of food before exposure to the labeled algae. Individual clams were placed with Chlorella that had been filtered from the labelling cultures and resuspended in clean 250 ml flasks filled with filtered water. Clams were removed from the experimental flasks after 15 minutes or at the first appearance of algae in the stomach, whichever came first. The passage of the green Chlorella through the digestive tract was observed through the shell when viewing the isolated clams with dissecting microscope and strong back lighting. Preliminary observations of clams during normal maintenance feeding showed that gut travel time for

the algae was quite variable and apparently not related to size or temperature.

Residual algae in the mantle cavity were removed by transferring the clams through a series of 3 water baths, allowing them to remain 10 minutes in each bath. Some clams were then immediately fixed in 70% ethyl alcohol to serve as controls. Other labelled Musculium were fixed when the algae first appeared in the hepatopancreas (10 to 60 minutes after the final water bath). A third group of clams was fixed at the first appearance of algae in the intestine, posterior to the heart (30 to 90 minutes after the final water bath). Finally, a fourth group was fixed when green algae or debris appeared in the cloaca (1 to 3 hours after the final water bath).

Suspected symbiotic organisms from the mantle cavity, gill surfaces, cloaca, and visceral organs were then removed from these labelled host clams. Some labelled clams were prepared for serial sectioning. Whole mounts or thin sections of possible symbionts were placed on glass slides. If the mounts were to be used for autoradiographic purposes, these specimens were generally left exposed, without cover slips.

In the dark room 3 cm squares of Kodak AR-10 Autoradiographic stripping film were placed over the specimens. Light proofing of the film was accomplished by wrapping individual slides in two layers of black construction paper

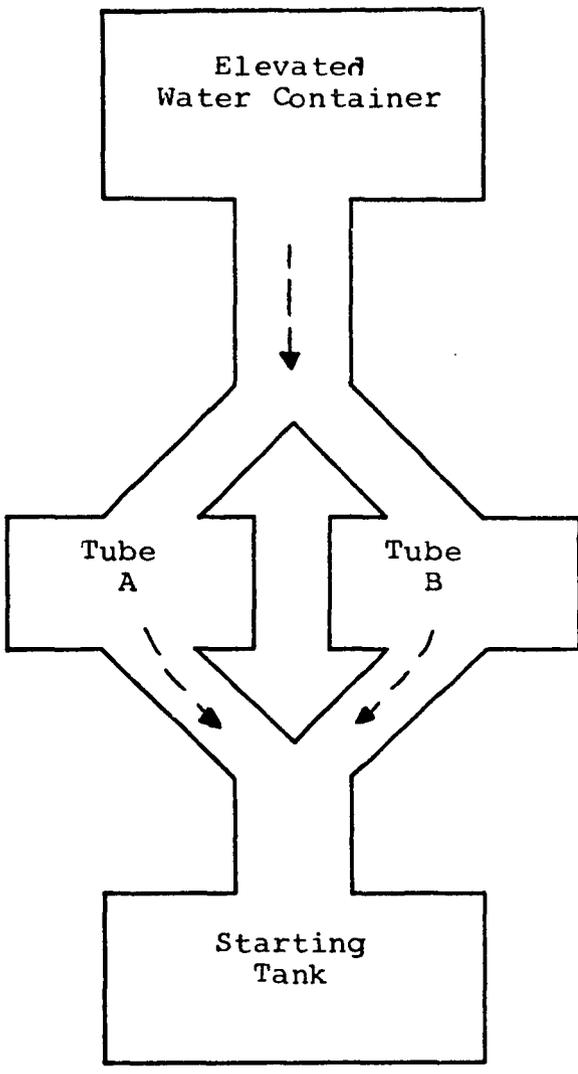
and placing them in closed wooden slide boxes. The prepared slides were incubated for 7 to 10 days at room temperatures in the laboratory. Following incubation, the film was developed as standard black and white film and the negatives examined for any significant depositions of silver grains on the film which were above those caused by minor background radiation. Ten control slides with no organisms showed only changes due to weak, random background radiation.

Mathers (1972) used a similar autoradiographic technique to track the digestive tract of the oyster Ostrea edulis. Federov and Sorokin (1967) utilized  $C^{14}O_2$  in their investigation of the respiratory metabolism of Sphaerium corneum.

#### Parallel Water Flow Tubes

A system of interconnecting plastic tubes and containers was used in testing host locating abilities of some symbionts. Figure 3 shows a schematic diagram of the apparatus. The tubing employed was pliable clear plastic with an internal diameter of 1 cm. Both the starting and holding tanks were created from small plastic bowls.

The flow rate through the parallel water flow tube system in all experiments was adjusted to 2 ml/sec. The water temperatures varied with the room temperatures, except in the experiments with predatory leeches. In the leech observations the water was held at 15°C, since preliminary



Overflow Lip

Fig. 3. Schematic Diagram of Parallel Water Flow Tube System

tests had shown them to be most active at this temperature. Choice combinations for this aquatic Y-maze were identified as tubes A and B. In most experiments involving symbionts this apparatus was used only to determine the ability of an organism to locate the host. In the leech experiments several prey-choice combinations were employed in order to determine the prey specificity of these predators.

## SYMBIOTIC RELATIONSHIPS

Parasitic and symbiotic organisms associated with freshwater bivalves have been noted by many authors since Sterki (1906) first noted the abnormal shell development of parasitized Sphaerium sp. Although most studies of bivalve symbioses have concentrated on the involvement of various sphaeriids and unionids as intermediate hosts in trematode life cycles, some investigators have considered the relationship between bivalves and other invertebrates and at least one fish.

### Introduction

The interesting reproductive parasitism of unionids by the bitterling (Rhodeus amarus) has been discussed by Wiepkema (1961) and Old (1971). The females of this small fish seasonally deposit their eggs within the mantle cavities of various unionid species, especially Unio pictorum. Until the hatching of the fry, both male and female bitterlings may defend the clam as a living incubation chamber. Although this behavior may constitute an example of seasonal commensalism, it is probably energetically parasitic when the mucus coated egg masses are large enough to disrupt the normal ciliary feeding of the host.

Chironomid midge larvae have also been found as occasional symbionts of freshwater molluscs. Chironomus sp. have been found attached to the mantle edge and within the extrapallial cavity of the unionid Anodonta cygnea (Beedham 1971). Chironomus and Metrionemus have also been observed attached to other bivalves and gastropods, including the common pond snail Lymnaea peregra (Barnard 1911, Beedham 1970).

Since many chironomid larvae are negatively phototaxic during later development, it is possible that they utilize their "hosts" only as another dark place in the environment. In this case the relationship would be considered incidental or phoretic. However, the advantages in avoiding fish predation by existing in close contact with the molluscan "host" should not be totally discounted.

Parasitic water mites of the family Unionicolidae also employ phototaxic responses in locating their bivalve hosts. Welsh (1931) found that undefined substances from host clams cause a shift from positive to negative phototaxis in dispersing unionicolids. These small acarines then move into the nearest dark aperture, usually the mantle cavity opening of a host clam

Garner (1863), Mitchell (1955, 1965), and Davids (1973) have described the reproductive parasitism of the Unionicolidae. Female mites seasonally lay their eggs between the gill lamellae and within the marsupial sacs of

their hosts. After incubation in these oxygen rich locations, hatched larvae mature and eventually emigrate from the host. There is usually little damage to the host's gill tissues. However, Humes and Jamnback (1950) found that the incubation of the mite Najadicola ingens occasionally interferes with normal marsupium development in Anodonta and Unio spp. Apparently, the deposition of mite eggs and the movements of larval stages cause many abnormal papillae to be produced on the gill surface. Furthermore, Garner (1863) stated that Unionicola sp. (as Atax sp.) forms the "pearly prominences" or pearls in some Anodontinae. This deposition of nacreous shell material indicates a physiological defense response to the tissue irritating mites.

Several authors have noted the presence of trematode larvae in freshwater clams (Appendix B). The cercariae, rediae, and sporocysts of various digenetic flukes have been described from these intermediate hosts. Definitive hosts for these parasites include a wide range of taxa; water fowl, frogs, toads, and fish, especially commercial salmonids. Man can serve as the definitive host for some bivalve mediated trematodes (Sandground and Bonne 1940).

Trematode parasites of the Sphaeriidae invade various host tissues, but are usually concentrated in the digestive gland, gonads, gills, and kidney. The infection of the digestive gland, or "hepatopancreas," in some sphaeriids appears to be extensive. Cheng and James (1960),

who observed stages in the destruction of hepatopancreatic tissues in Sphaerium striatinum, believed large numbers of clams were being killed by Crepidostomum sp. They observed that in repeated infections, many hosts died. Great fluctuations in the bivalve population size and the deposition of many empty shells suggested the possibility of Crepidostomum causing seasonal epizootic kills of Sphaerium striatinum.

Wenke (1965) and Gale (1970) found rediae and cercariae extending from the tubules in the hepatopancreas into the gonadal tissues in Musculium transversum. The distortion and evacuation of the digestive gland which they observed has also been noted in the snail Flumenicola virens, parasitized by the trematode Plagioporus virens (Porter 1970).

The growth of rediae and metacercariae of various trematodes in gonadal tissues is also devastating to some host populations. Szidat (1924, 1929) and Dollfus (1949) cited examples of gonadal atrophy in heavily infected molluscan hosts. Gonadal invasion may result in parasitic castration of the host in some cases. Cheng and James (1960) and Wenke (1965) have observed that heavily parasitized Sphaerium striatinum and Sphaerium transversum lack embryos, while similar non-parasitized clams contained embryos. Since early sphaeriid embryos are maintained in placental brood pouches within the gill cavity (D'Eliscu 1972), it is possible that this apparent castration is

actually due to branchial tissue disruption. In other words, the loss of reproductive potential is not due to the direct destruction of gonadal tissues by trematodes, but is a result of the disruption of the secondary support tissues of embryos already implanted in the gills. Cheng (1963) described some evidence supporting this possibility. He found distention, rupture, and necrosis in the gills of Musculium partumeium infected with rediae and sporocysts of Gorgodera amplicava.

Some host related modifications of parasites of sphaeriids have been observed. Kingscote (1971) suggested that when Sphaerium occidentale is used as an intermediate host by the spirochete Leptospira interrogans, the morphology and infectivity of the parasite is altered. This parasite of deer is found in the mantle cavities of both active and aestivating clams. A further modification of parasite development has been shown for the trematode Phyllodistomum bufonis infecting Pisidium adamsi (Ubelaker and Olsen 1970). Development of this parasite in young clams was significantly shorter than in older clams. Possibly the physiological defense mechanisms of young clams are not fully developed. Another possible reason for this difference in parasite growth rate is the probable higher rate of protein synthesis in young growing clams.

### Symbionts of Musculium Transversum

Microdissections and serial sectioning of Musculium transversum from the Noon Pond revealed a microenvironment occupied by a complex biological community. The flow of oxygen and food in the water current created by the clam supports many small organisms living within the mantle cavity. This generally stable environment supports many protozoa, various rotifers, two oligochaetes, a hydrophilid beetle larva, a nematode, and a unique fungus. The symbiotic levels occupied by these organisms were established through analysis of their feeding mechanisms, morphological adaptations, and by autoradiographic techniques (Table 1).

#### Rotifera

Extramarsupial juveniles and adult Musculium support several species of the rotifer genus Filinia (Fig. 4). As Edmondson (1959) pointed out, these ciliary feeders possess spiny appendages which are actually setiform extensions of the cuticle. About 33% of the clams in each monthly sample contained some Filinia within the mantle cavity. Usually these rotifers were found with their spines partially inserted between gill lamellae. The spines seem to allow Filinia temporary attachment to the surface of the gill to avoid being swept away by the branchial current of the clam.

Table 1. Autoradiographic Results for Musculium Symbionts

Symbiont	Fixation following final water bath		<u>Chlorella</u> in hepatopancreas 10-60 min.		<u>Chlorella</u> in intestine 30-90 min.		<u>Chlorella</u> in cloaca 1-3 hrs.		Number tested
	# pos	# neg	# pos	# neg	# pos	# neg	# pos	# neg	
<u>Filinia</u>	10	0	10	0	10	0	10	0	40
<u>Berosus</u>	0	5	0	5	0	5	7	2	24
<u>Aeolosoma</u>	0	10	0	10	1 <sup>a</sup>	9	8	2	40
<u>Chaetogaster</u>	0	10	0	10	0	10	8	0	38
<u>Seinura</u>	0	6	0	3	0	8	2	7	26
<u>Endosphaerium</u>	0	5	0	5	0	5	0	5	20

<sup>a</sup>Possible contamination with radioactive feces from the host clam.

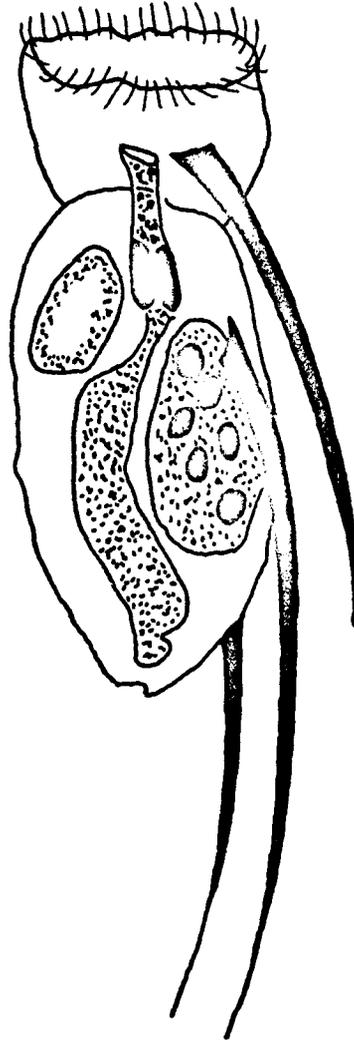


Fig. 4. The Rotifer Filinia sp., Showing Cuticular Setae;  
x 640

Filinia was seldom collected in the water or sediments outside its host. Although parallel water flow tube experiments were not performed with these small animals, the latter evidence suggests they have some means of host location.

Autoradiographic results showed that these rotifers take up labeled algae only during the initial feeding period of experimental clams (Table 1). Since Filinia does not invade tissues, and causes no significant energetic loss to the host through its coronal ciliary feeding, it probably exists as a commensal.

#### Coleoptera

Larvae of the hydrophilid beetle Berosus (probably B. peregrinus) are also mantle cavity symbionts of Musculium (Fig. 5). This interesting insect possesses spiny lateral tracheal gills on its first seven abdominal segments. Although most other aquatic hydrophilid larvae must return to the surface occasionally to respire, Berosus larvae are completely aquatic (Leech and Sanderson 1959). The tracheal gills not only allow this beetle to remain permanently submerged with its host, but their spinose shape may function in attachment or movement within the mantle cavity. Backlighted microscopic observations of larvae within host clams showed both slow, crawling and jerky, gastrotrich-like movements. The slender front legs were employed in

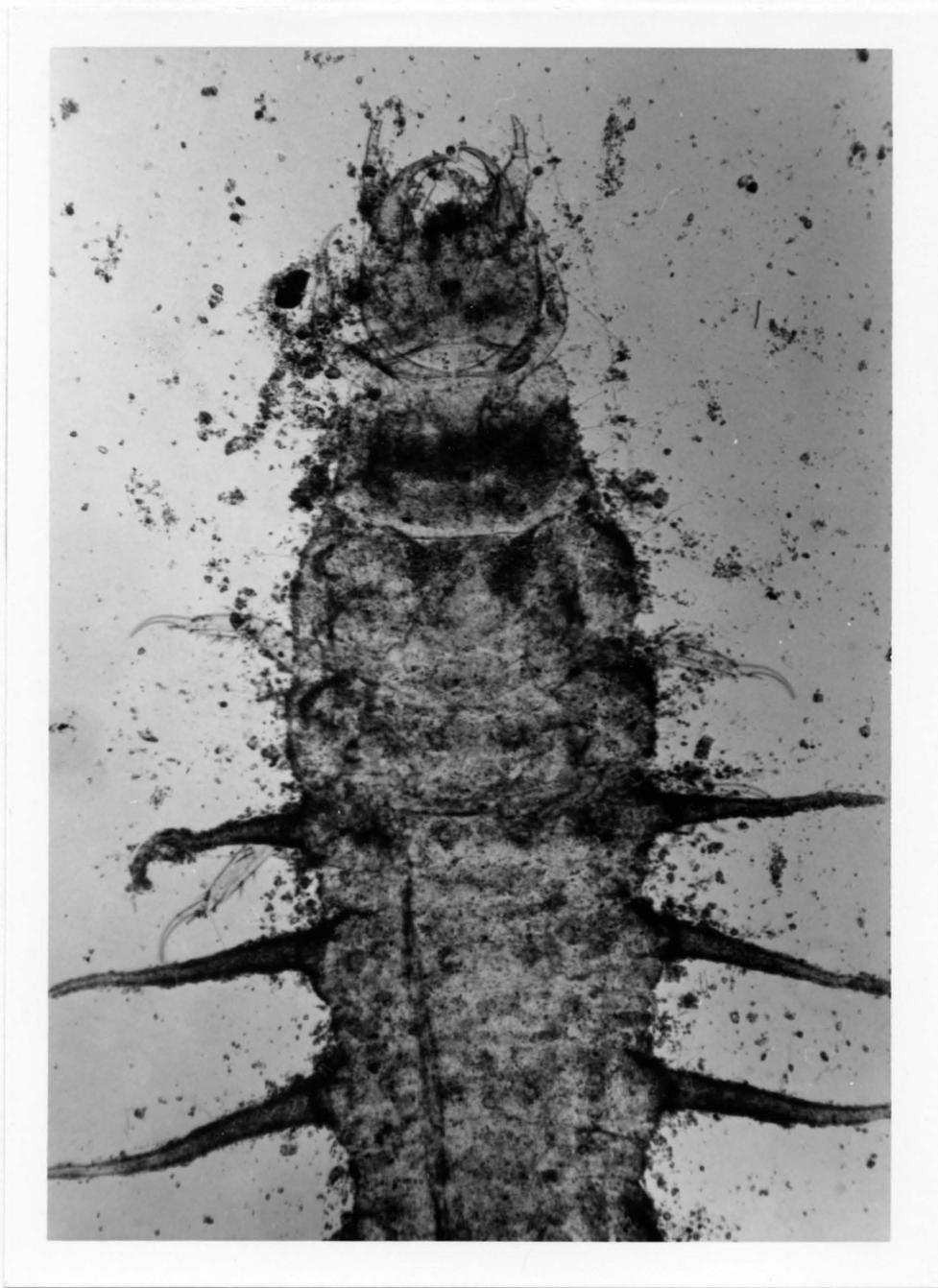


Fig. 5. Berosus sp., Showing Lateral Tracheal Gills, 2 mm

crawling, while both legs and tracheal gills were used in the jerking movements.

Leech and Chandler (1956) suggested that Berosus spp. are herbivorous, feeding on green algae. This suggestion may be based on the fact that free-living Berosus are most often found in deeper water among filamentous algae, weeds, or in marginal debris. However, Boving and Craighead (1931) and Leech and Sanderson (1959) pointed out the heavy crushing mandibular structure generally found in this genus, suggesting that this larvae is carnivorous like most other hydrophilid larvae. Berosus sp. in Musculium transversum possesses these heavy crushing mouth parts. On several occasions larvae were observed actually feeding on oligochaetes within the mantle cavity. A gut squash of a 3mm specimen revealed worm fragments and bacteria, but no intact algae. Autoradiographic results showed that Berosus took up labeled material only after radioactive debris reached the cloaca of the host (Table 1) As discussed in the following section, the oligochaetes Aeolosoma and Chaetogaster are coprozoic commensals of Musculium. These worms feed on cloacal debris of the host. Apparently, the vermivorous Berosus feeds on these oligochaetes and not on host tissues or algae. Berosus is therefore a predatory commensal of Musculium

Unlike the rotifers of the mantle cavity, Berosus maintains a symbiotic relationship with Musculium only

seasonally. Clams collected from the field contained beetle larvae only in April and May. In both these monthly samples 36% of the clams contained one larval beetle, while one 13mm Musculium in the May collection contained two small specimens. The size of symbiotic Berosus was variable in the samples, but all specimens fell within a relatively narrow range of 1.8 to 4.0mm. Many large beetle larvae did occupy larger clams, but the smallest specimens collected (1.8, 2.0, 2.1mm) were also found in larger clams. Berosus larvae larger than 4mm were collected in sediments and plant debris in May, June, and July. It appears that the larvae simply outgrow the sanctuary of the host's mantle cavity. Although the prey of free-living larvae could not be determined, it seems likely that they maintain their predatory habits, possibly shifting to larger prey organisms. No live adult Berosus were ever collected in or near the pond. However, one badly damaged dead specimen was collected in wind drift along the pond shore in October. Adult hydrophilids are strong, rapid swimmers and it is probable that adult Berosus were not collected simply because they avoided capture attempts.

Parallel water flow tube experiments showed that Berosus larvae less than 4mm long could detect and locate upstream hosts. When presented with a choice of live adults or clean shells of Musculium, 8 of 10 Berosus larvae of this size moved upstream to the hosts. Three 6mm

specimens presented with the same choice did not move from the starting tank. Apparently Berosus larvae longer than 4mm do not respond to the chemicals involved in host location by smaller larvae (Appendix D).

#### Oligochaeta

The oligochaetes Aeolosoma sp. and Chaetogaster limnaei were found as coprozoic commensals of Musculium (Figs. 6 and 7). Live specimens were found throughout the year in the mantle cavity and gill tissues. Autoradiographic results indicated they feed only on cloacal debris. Neither species seemed to invade or disrupt tissues in Musculium. Gruffydd (1965a) found some Chaetogaster feeding on the kidney cells of snails and considered them parasitic. Coker et al. (1921) reported that unionid clams occasionally contained Chaetogaster limnaei which appeared to be feeding on "juvenile mussels" (probably glochidia).

Chaetogaster and Aeolosoma appear to be commensals in Musculium at the Noon Pond. Gale (1970) found a similar commensal relationship for Chaetogaster with Musculium transversum from the Mississippi River in Iowa. Observations of Chaetogaster placed in culture dishes containing Musculium corroborate the findings of Gale. The worms moved about randomly, seemingly unaware of the presence of the host until they made accidental contact with the clam's foot. The worm would then crawl onto the surface of the



Fig. 6. Aeolosoma sp., 3 mm

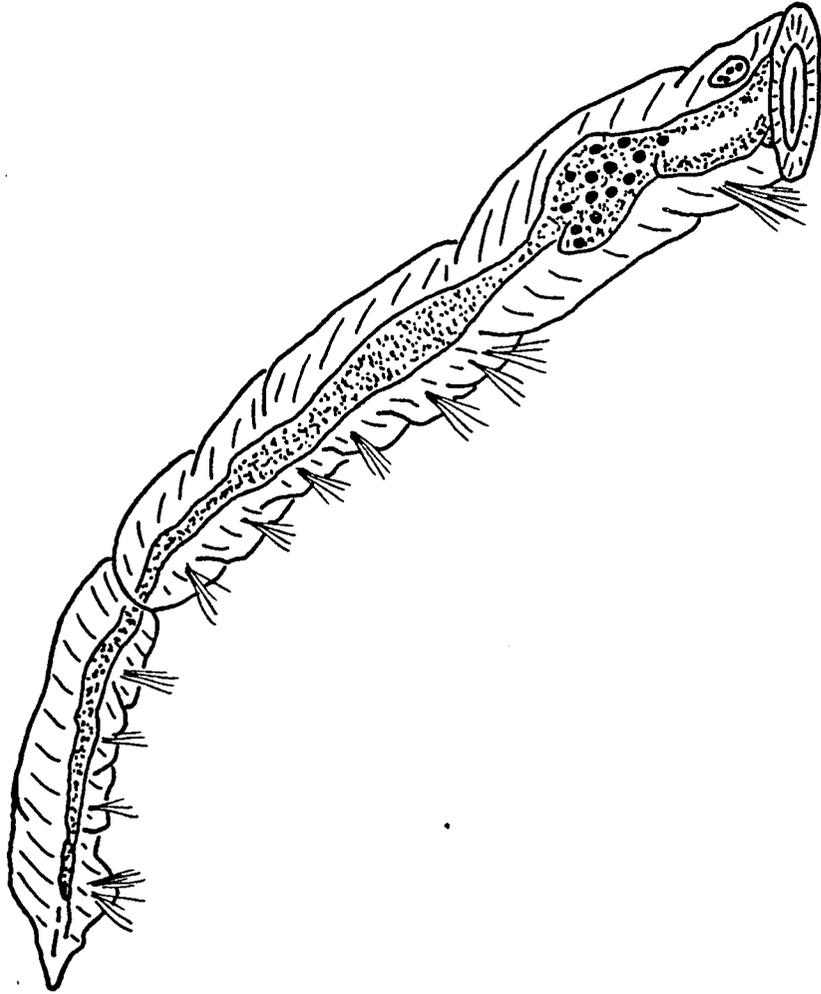


Fig. 7. Chaetogaster limnaei, Showing Ventral Setae, x 100

foot using its ventral setae in leech-like stepping movements. When the clam's foot comes in contact with the crawling worm it is quickly withdrawn, often pulling the worm inside the mantle cavity. Occasionally, the worm would be scraped off by the edge of the shell as the foot was withdrawn.

Aeolosoma entered the host in a similar manner; however, it was not accidental as in Chaetogaster. Instead, Aeolosoma appeared to locate and seek out Musculium through chemical means. Parallel water flow tube experiments with 10 Aeolosoma and 12 Chaetogaster showed marked differences in their abilities to detect and locate host clams. Presented with the choice of live adults and clean valves of Musculium in the tube system, all Aeolosoma moved rapidly to the live clams. In contrast, all but one of the Chaetogaster remained in the starting tank seemingly unaware of the potential host's presence upstream. One individual did start to move up the starting tube, but reversed direction at the branch and moved back into the starting tank. Aeolosoma appeared to detect and locate its host through chemical means, while Chaetogaster's contact with its host appeared to be accidental, probably through tactile senses alone (Appendix D).

If Aeolosoma has been reported as symbiotic in molluscs or other organisms, it is not generally known. Gruffydd (1965b) cited several reports of Chaetogaster

living within the mantle cavities of snails, consuming trematode miracidia and cercariae. If significant numbers of potential parasites were consumed, this relationship would constitute mutualistic symbiosis. Since no Musculium from the Noon Pond were infected with trematodes, this possible relationship could not be investigated further.

#### Nematoda

The style-bearing nematode, Seinura sp., is a parasite of the hepatopancreas and gonadal tissue of Musculium (Fig. 8). Only 4 to 8% of the clams in each monthly field sample were parasitized, but the active clam population maintained this parasite at these low levels throughout the year. Some heavily parasitized hosts had atrophied gonadal tissues, including ovaries, testis, and common genital ducts.

Seinura appears to disrupt large secretory cells in the hepatopancreas and gonads with its sharp anterior stylet. It probably ingests fragments of these cells. This parasite becomes radioactively positive only after the host has digested labeled algae (Table 1). It does not ingest algae deposited in the hepatopancreas, but feeds on host tissue cells. This is shown by the lack of positive radioactivity of these parasites even when they are in the hepatopancreas, surrounded by radioactive Chlorella. As shown in Table 1, radioactive Seinura were recovered only after the host had

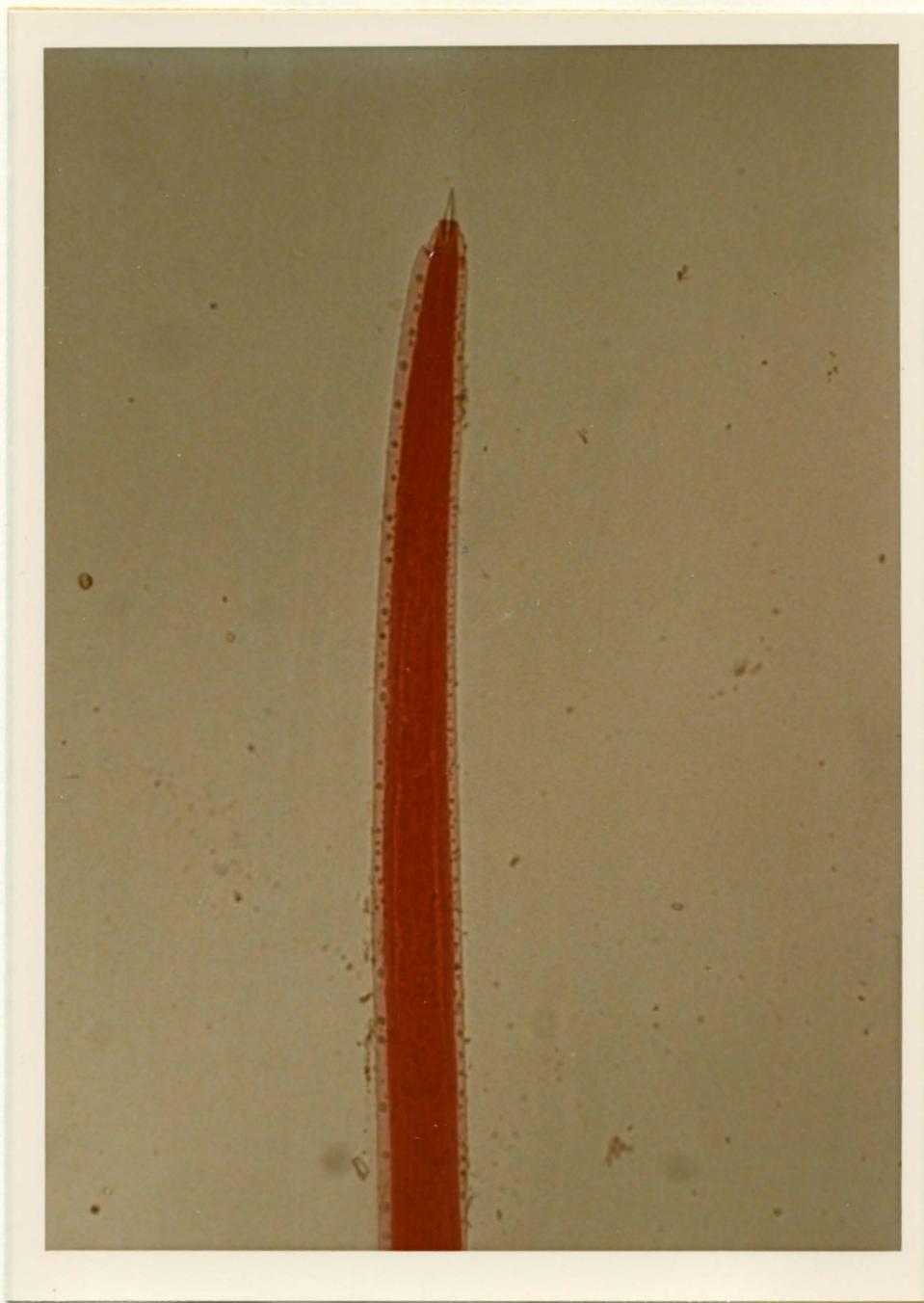


Fig. 8. Seinura sp., Showing Anterior Stylet, x 200

been exposed to labeled *Chlorella* for 1 to 3 hours and algae had begun to appear in the cloaca of the host. Close examination of the stylet and mouth parts of *Seinura* also indicated that it is adapted to a carnivorous rather than herbivorous mode of feeding.

*Seinura*, like the trematodes referred to above, may parasitically castrate its hermaphroditic host. Heavy infections of gonadal tissue observed in some individuals led to necrosis and atrophy. Although its incidence in the host population is low, the presence of this parasite is potentially devastating. The invasion of host tissues is held in check by a mutualistic fungus, discussed in the next section.

Chitwood and Allen (1959) stated that *Seinura* spp. was "usually carnivorous." It does not appear that this nematode genus has been previously reported as an endoparasite. The association between *Seinura* and *Musculium* is probably incidental, as many sediment and plant samples revealed free-living worm specimens. The fact that *Seinura* exists as a free-living carnivore outside of *Musculium* suggests that it should be termed a micropredator instead of parasite despite its invasion of visceral organs. Parallel, water flow tube experiments with larger, 2mm, *Seinura* proved inconclusive. No excitatory response was seen when a worm was given a choice between the host and clean host shells. Some worms did reach both the live *Musculium* and

the clean shells, but probably as a result of random movement and accidental discovery (Appendix D).

Seinura probably enters the reproductive tract by penetrating the walls of the nearby hepatopancreas. All clams with parasitized or degenerated gonadal regions also had infected digestive glands. It seems likely that Seinura enters the digestive tract of the host with the food. However, there is the possibility that it penetrates the external surfaces of the mantle, gills, or visceral mass and then migrates to the hepatopancreas and gonads.

Endosphaerium funiculata

The predaceous fungus Endosphaerium funiculata exists as a mutualistic symbiont with Musculium transversum (D'Eliscu 1974) (Fig. 9). This novel fungus has been found only in active and aestivating Musculium transversum from localities in California, Texas, and Arizona, including the Noon Pond. Preliminary sectioning and microdissection of Musculium from the Noon Pond revealed the existence of this new fungus. Further observations led to the subsequent description by this author of Endosphaerium funiculata as a new genus and species. According to several mycologists contacted throughout these studies, the unique biology of this new fungus may place it in a new separate family.

In the Noon Pond population 80 to 100% of the active host clams in each monthly sample harbored at least one

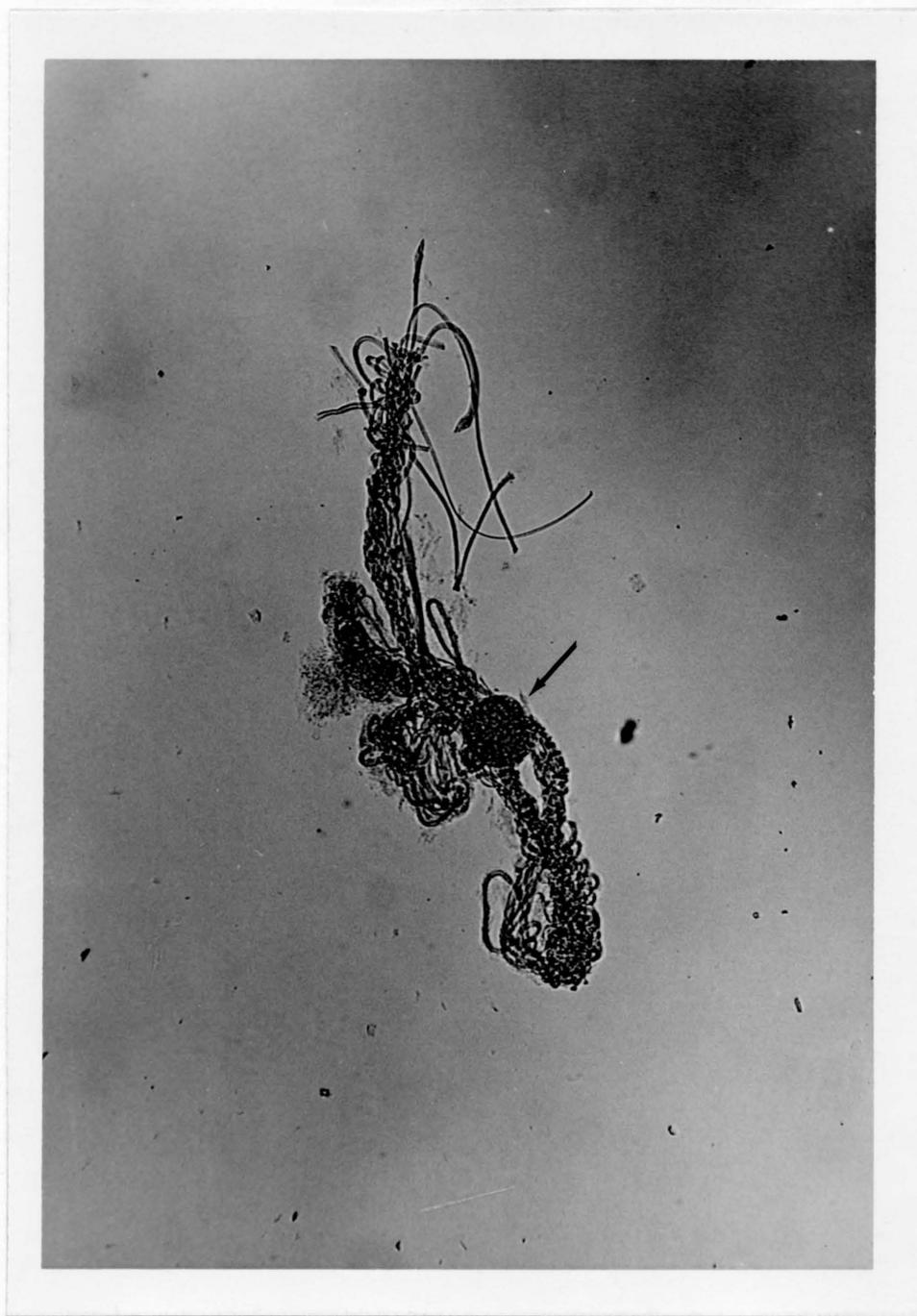


Fig. 9. Endosphaerium funiculata, Showing Thallus and Hyphal Anchor (arrow)

Endosphaerium. Only one Musculium collected from East Yegua Creek, near Lincoln, Texas, contained more than two developed fungal thalli of Endosphaerium. This Texas specimen contained three mature fungal growths.

Endosphaerium funiculata occupies the lamellar surfaces of the internal gills of Musculium. A knot-like mass of hyphae at the base of the thallus serves as an anchor, while a 200 to 950 $\mu$  rope-like hyphal cord extends into the mantle cavity. The hyphae making up the cord are unbranched and have contiguous or alternating bands of adhesive surfaces. Thin sectioning and differential staining showed no evidence of host tissue invasion by the fungus. Autoradiographic results showed no positive radioactivity in any fungi, further supporting the evidence that the host tissue is not invaded. The autoradiographic results also suggest that Endosphaerium does not take any significant amount of algal food from the host's water current.

Most clams did not show any signs of repair or tissue proliferation in the gill area normally occupied by fungi after small amounts of phenol were added to the water as a fungicide. Surgical removal of fungi from live clams produced similar results. Both experiments further demonstrated that host tissue is not invaded by the fungus.

Endosphaerium normally feeds on various rotifers, capturing them on the adhesive hyphae. Windowing of the

shell and mantle of large clams (12 to 16mm long) allowed experimental observation of the capture of small nematode vinegar eels (Anquillula sp.) and some spinose rotifers (Filinia sp.). After the prey are entangled in the adhesive hyphae, they are invaded by the rapid growth of finely branched, trophic haustoria. Some free living predatory fungi also capture rotifers and other small animals by trapping them in web-like nets or ringlets, or by sticking them on adhesive pegs (Prowse 1954, Sparrow 1960, Duddington 1962).

Since the symbiotic rotifers of Musculium become radioactive when exposed to labeled Chlorella, it was interesting that the rotifer predator Endosphaerium did not also become positive (Table 1). This discrepancy was finally explained when it was learned that for some reason rotifers were missing from the mantle community of clams used in the Endosphaerium studies. These clams had been maintained in the laboratory aquaria for 3 months and it is possible that some artificial contaminant killed the rotifers. If the rotifers had been present, it could have been expected that their capture by the fungus would have resulted in positive radioactivity of the predator. Further experiments with labeled rotifers could further clarify this predator-prey relationship.

Endosphaerium also preys occasionally on nematodes. In particular, the fungus sometimes preys on Seinura. As

discussed previously, Seinura may parasitically castrate the normally hermaphroditic host clam by invasion and disruption of its reproductive organs and ducts. While the presence of the predatory fungus probably does not establish complete refraction, it does confer an important advantage to its molluscan host in resisting this nematode parasite. Gonadal tissues and ducts from most fungus-bearing clams appeared normal. On the other hand, the few individuals from each monthly sample which did not contain fungi were invariably parasitized by Seinura. This symbiotic association is therefore mutualistic, with Endosphaerium benefitting through physical and indirect physiological support, and Musculium receiving some limited refraction to the destructive parasite. The relationship seems to be obligatory for the fungus, since it is not found free living or in any other host. Since some members of the Sphaeriidae serve as intermediate hosts for bird, amphibian, fishs, and mammalian trematodes and leptospiras, this bivalve-fungus mutualism may be important in the regulation of some vertebrate and associated parasite population levels (Yamaguti 1958, McDonald 1969, Kingscote 1971).

The reproductive biologies of both the host and mutual further indicate the complexity and interdependence of the relationship. The Sphaeriidae retain their embryos and early juveniles in marsupial brood pouches within the gills. The incubation period is seasonal, variable within

the family, and often temperature dependent (D'Eliscu 1974). Although sexual reproduction in Endosphaerium has not yet been observed, the initiation of asexual reproductive development of the fungus appears to be coordinated with the seasonal reproduction of the host clam. Microdissection and thin sectioning demonstrated that 33 of 100 late incubation juveniles of Musculium examined contained the cyst-like, spherical zoospores of Endosphaerium. Rarely, post incubation juveniles from clams not supporting fungi also contained zoospores. This suggests that the asexual transfer of the fungus may be both vertical (successive generations) and horizontal (adult to adult) within the host population.

The asexual reproductive stage of the fungus, producing zoospores from distally septate sporangia, has been found only in actively reproductive clams. Unlike some other rotifer-catching fungi, such as Zoophaqus and Sommerstoffia, Endosphaerium produces sporangia from the symbiotic thallus, rather than from modified filamentous haustoria from within the prey (Duddington, 1966).

Reproductively active fungi are usually reoriented in the gill, the hyphal cord and its developed sporangia extending into the tissues of the host's brood pouch. In addition fully developed trophic thalli without sporangia normally extend from the external surface of the gill into the mantle cavity. Non-reproductive Endosphaerium were

generally encountered in post-embryonic and a few non-reproductive adult clams. Nearly all sexually reproductive clams with fungi, however, supported thalli which extended back into the intralamellar space. No intermediate forms were encountered. This latter finding suggests a rapid reversal of the thallus orientation as a unit. If the reversal were due to a gradual resorption of the trophic thallus followed by a secondary internal growth from the hyphal anchor, intermediate forms would have probably been seen. The thallus cords of reproductive and non-reproductive fungi are indistinguishable, except for the presence of distal sporangia on reoriented hyphae. Apparently there is some reproductive initiation or release mechanism from the host clam to the fungus. Perhaps the proliferation of tissue by the host at the site of brood pouch formation somehow stimulates the reorientation of the fungus. Further observations of young adult clams could possibly better characterize the reversal dynamics.

The coordination of reproductive periods and transfer of fungal zoospores to the developing juveniles of the host ensure a mutualistic symbiosis in successive generations. More behavioral observations and some chemical analyses could perhaps better characterize the biology of this novel fungus, its unique habitat, and the mechanisms of interaction with its molluscan host.

### Bryozoa (Ectoprocta)

One incidental phoretic relationship was observed between Musculium and a freshwater bryozoan. A sessoblast or attached statoblast of Plumatella sp. was found adhering to the umbral cap of a 14mm clam collected in July (Fig. 10). This was the only example of such a relationship and probably represents the incidental use by the bryozoan of the clam shell as a stable surface in the shifting sediments of the pond.

### Ciliata

Although investigations of the protozoa inhabiting the mantle cavity of Musculium transversum were beyond the intended scope of this study, observations of ciliate morphology proved beneficial. No ciliates were found to be obligate symbionts of Musculium, except perhaps Kerona sp., which were found only on brown Hydra and Musculium mantle surfaces. Kudo (1966) and Noland (1959) also cited this cirri-bearing ciliate as a commensal on Hydra. Other ciliates identified from the mantle and gill surfaces in the present study included Onychodromus sp., Amphisiella sp., and Onychodromopsis flexilis. All of these later organisms possess both caudal and anterior setiform cirri. Kerona sp. has anterior cirri only. Similar to several other mantle cavity symbionts, these small, occasional inhabitants of the



Fig. 10. Plumatella sp., Sessoblast

microenvironment have appendages which are possibly used in locomotion or attachment.

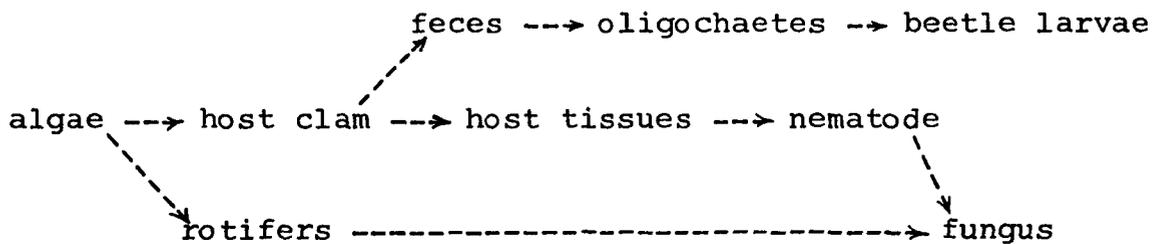
Other ciliates reported in associations with sphaeriids include Hypocomatidium sphaerii, Cepedella hepatica, and Dogiella sphaerii, all from Sphaerium corneum (Poljanskij 1926, Jarocki and Raabe 1932, Dobrzanska 1959). Kahl (1931) reported Pleuronema anodontae in several sphaeriid species. Both Thomas (1960) and Ladle (1969) noted vorticellids on the shells of sphaeriids. Similar ciliates associated with the mantle cavities of the Unionidae have been cited by Leidy (1857), Kidder (1934), and Penn (1958). Small and Antipa (1969) reported the uses of endo-commensal ciliates of mollusca as useful indicators of water quality and pollution levels.

#### Summary of Symbiotic Associations

The symbiotic organisms occupying the mantle cavity and tissues of Musculium transversum are interrelated and exist in several levels of symbiosis. Rotifers of the genus Filinia, the larval hydrophilid beetle Berosus, and the oligochaetes Aeolosoma and Chaetogaster are commensals within the mantle cavity. The predatory fungus Endosphaerium funiculata benefits the host in capturing parasitic nematodes and lives as an obligate mutual in the gill tissues of the host. The nematode Seinura is a parasite which invades and destroys digestive and reproductive

tissues. Seinura might also be considered a micropredator, since it is not an obligate parasite. However, it does not usually kill the host. Table 2 summarizes some of the partial food chains of the symbionts of Musculium transversum from the Noon Pond.

Table 2. Some Partial Food Chains of Symbionts of Musculium



It seems probable that some sort of spinose appendage or anchoring holdfast is a requirement for larger organisms living within the mantle cavity of Musculium. Chaetogaster possesses ventral bundles of setae. Aeolosoma has lateral bundles of setae. In both cases these setae are used in crawling movements. Similarly, Berosus larvae have spinose tracheal gills and Filinia sp. have long cuticular setae. Movement or attachment within the mantle cavity may

depend on these spiney projections. Endosphaerium, although not motile, is attached to the gill surface by an anchoring hyphal holdfast. Whether the strong ciliary current produced by the host or the rapidly changing shape of the mantle cavity makes these appendages necessary is not yet known. However, further investigations may show these analogous morphological adaptations in diverse taxa to constitute a case of parallel evolution.

Chemical analysis may establish the specific elements involved in distant host location by smaller Berosus larvae and Aeolosoma. As discussed below in the chapter concerning predation, it does not appear that the presence of simple ammonia is the only factor.

Beetle larvae show a marked seasonality in their symbiotic association with Musculium. So far there is no complete explanation of this. Symbiont growth and maturation, size of individual hosts or host populations, or physical and biotic parameters of the surrounding environment may contribute to symbiont population fluctuations.

## AESTIVATION

The seasonal aestivation of host clams greatly affects the dynamics, morphology, physiology, and development of some symbionts. Investigations of the aestivation of freshwater bivalves generally include studies of oxygen requirements, resistance to desiccation, and population fluctuations. In this study of symbiosis, investigations of water balance, nitrogenous waste production, and the specific changes of symbiont morphology and physiology in aestivating hosts were undertaken, because it was suspected that these have important influences on symbiont adaptations and life cycles.

### Introduction

Aestivation is an adaptive phenomenon reported in many molluscan groups. Land snails survive drought and temperature extremes by secreting a mucoid epiphragm over their shell apertures. Many aquatic pulmonates burrow into the substrate or plant debris to endure desiccation stresses.

Relatively few bivalves, however, have the ability to aestivate. Some unionid clams, such as Unio tetralasmus have been shown to survive 3 to 6 months in the moist sediments of dried ponds (Van der Schalie 1939). Adults of the sphaeriid Musculium lacustre have been found

aestivating in nearly dried mud by Vielhauer (1943), and hibernating in moist, partially frozen soil by Mitropolski (1965). In both studies, semidormant clams were quickly reactivated when placed in water. Apparently this species is both drought and frost resistant, actively feeding and growing within broad temperature and moisture ranges. When conditions approach the limits of these ranges, M. lacustre drops into a reduced metabolic state. Both Lufarov (1965) and Alimov (1967) have also demonstrated Sphaerium corneum to be a frost resistant bivalve. Boycott (1936), in describing some of the British fauna, stated that Pisidium personatum and Pisidium casertanum (as P. cinereum) frequently inhabit ditches and seasonal marshes which lack water in late summer.

The juveniles of Musculium partumeium may aestivate within the cavity of buried parental valves. In her studies of this species, Thomas (1960, 1963) also found clams surviving in frozen ground. In several studies of both active and aestivating sphaeriids, it was found that young animals were more tolerant of unfavorable conditions than adults (Alimov 1965, Bonomi and Ruggiu 1966, Gale 1970, Thomas 1960, 1963). On the other hand it would be expected that in aestivating individuals, the greater surface area to volume ratios of small clams would cause them to lose relatively more water. However, smaller clams probably do not have a

significantly greater surface area to volume ratio since the flat surfaces of their gills are not fully developed.

While several sphaeriid species aestivate as juveniles, the most successful is a species which aestivates as an adult. Sphaerium occidentale is a common inhabitant of small, temporary woodland ponds and pools. Adult aestivation is so common in this species that Herrington (1944, 1948) suggested that this clam may require a period of dormancy in order to complete its maturation. Herrington followed a population through several generations by marking and recapturing animals. He found that some individuals survived nearly three years when forced into occasional aestivation, although the normal life span for this species is little more than one year. Kenk (1949) also observed seasonally aestivating Sphaerium occidentale and Musculium partumeium (as S. truncatum) in his studies of temporary ponds in Michigan. Judd's (1966) studies of sphaeriid distributions in Ontario, Canada, also included S. occidentale in temporary ponds.

Several studies of water balance in aestivating clams have shown that S. occidentale is not especially resistant to desiccation. Edgar and Smith (1969) and Collins (1970) investigated water loss in this species at various temperatures and humidities. Both of these studies demonstrated that if these clams lose 50-55% of their body water they die. However, losses up to 40% of body water

are tolerated by most small and medium sized individuals. These figures do not appear to be significantly different from the tolerance levels of many aquatic and terrestrial molluscs. If S. occidentale shows no special tolerance for desiccation, what properties make its great success in aestivation possible? Some possible behavioral, physiological, and morphological answers are included in the following section describing aestivation in a related species of sphaeriid, Musculium transversum

#### Aestivation of Musculium transversum

Herrington (1962) considered the habitat of Musculium transversum to include temporary ponds, permanent lakes, streams, and rivers. The substrate preferences of this species are broad and include many sediment types. Only dense clay and large coarse gravel seem limiting.

In temporary water bodies and in areas of great water level fluctuation, the sediment quality may be an important factor in the biology of Musculium. In the present studies this sphaeriid was found able to aestivate facultatively in moist sediments which did not become compacted. A seasonal water fluctuation of  $\frac{1}{2}$  to 1 meter at the Noon Pond exposed various bottom substrates, but live aestivating clams were recovered only from loosely gathered sediments. Most live animals were found in substrates containing some plant debris and loose mud. It is

unlikely that this species can aestivate in soils that are dense and clay-like.

Like Sphaerium occidentale (Collins 1970), Musculium transversum does not appear to migrate across the substrate surface when confronted with decreasing water levels. It does not move along with the receding edge of water when the level drops. Instead, decreasing oxygen tensions in the warmer shallow waters appear to trigger burrowing into the sediments. This is shown by 20 experiments in the laboratory in which levels of about 2 parts per million of oxygen at 12°, 15°, 19°, and 21°C were all sufficient to elicit the burrowing response. Aestivating clams were observed at the Noon Pond throughout the year whenever the water level decreased. However, a decrease in oxygen may not be the only triggering mechanism involved.

The tolerance of Musculium transversum to low oxygen levels is probably not unusual. Jatzenko (1928) found Sphaerium corneum able to tolerate 1½ months without oxygen. Juday (1908) found Pisidium idahoense in the profundal zone of a Wisconsin lake that showed no detectable oxygen for 3 to 4 months. However, this present study is the first reported case of aestivation for Musculium transversum.

Aestivating clams were collected from the Noon Pond by digging up moist sediment samples with a small garden trowel. It was repeatedly observed that when dry, aestivating clams were placed in moist paper toweling prior to

transport or reactivated in the laboratory from sediment samples, a click or pop was heard. Collins (1970) described a similar phenomenon in Sphaerium occidentale, suggesting that the sound was ". . . not unlike that produced by a well known breakfast cereal" (p. 20). He proposes the sound originates from the rehydration of dried mucus, shell, or hinge tissues. The source of the sound was not exhaustively studied in Musculium transversum; however, it appeared that the click was produced when the hinge tissues were rehydrated. This small pop was possibly amplified by the air partially trapped within the mantle cavity space.

Careful dissections of aestivating clams showed them to exist much like terrestrial snails in aestivation. With the foot and siphons retracted within the shell and the gills pulled tightly against the visceral mass, sheets of mucus covered the entire inner surface of the mantle cavity. Generally there was only one small opening along the closed shell margin. This aperture was usually on the posterior edge in the area occupied by the siphons in active clams. This opening was presumed to be a respiratory aperture. In a few cases, external shell surfaces of clams were also coated with mucus, with some individuals nearly covered as if in a nest of mucoid filaments. The inner mucous coat and the mantle margin seal appear to function much like the epiphragm secreted across the shell aperture in aestivating land snails.

Microscopic observation of the gill surfaces showed no ciliary activity, with inactive ciliary bands flattened against the gill. Ciliary activity returned immediately however, when a drop of water was placed on the gill surface. Cardiac systolic rates in aestivating clams proved to be variable, ranging from 2 to 18 beats per minute. Individual variations in heart rate did not appear to be correlated with temperature or individual size. The systolic rate of active clams was quite regular and ranged from 30 to 60 beats per minute. Gartkiewicz (1926) obtained similar results for various Sphaerium spp.

Collins (1970) established the fact that some sphaeriids have the ability to exist anaerobically for short periods of time. It seems likely, however, that most long term aestivations of these clams involve aerobic respiration similar to that of land snails. The flow of air over moist surfaces required for this type of respiration causes a loss of water through evaporation. As suggested in the introduction to these aestivation studies, sphaeriids do not exhibit any special tolerance for desiccation. Musculium transversum does demonstrate some special morphological adaptations for replenishing and conserving fluids lost through respiratory evaporation.

Examination of thin sections and tissue squashes of the mantle of Musculium transversum reveals microscopic puncta made up of pyramidal cells. These epidermal

projections protrude from the inner and outer surfaces of the mantle flaps, even extending through the thinner areas of the shell near the umbo. From this latter evidence, Rosso (1954) proposed that these extensions of the mantle are the sources of the poorly developed prismatic shell layer of the Sphaeriidae. Collins (1970) has suggested that these "periostracal hairs" may function in oxygen uptake in both aestivating and active clams. Schröder (1907) made detailed observations of pyramidal cell structure and distribution in Musculium lacustre. He originally believed these specialized cells to be involved in secretion, but later rejected this hypothesis because the cells were not located at the mantle edge. In fact, prismatic shell deposition occurs in their absence and at locations of low density away from the umbo in Musculium transversum.

Machin (1972) has demonstrated the mucous secretions of the aestivating land snail Otala lactea to be hygroscopic. The microvilli-like structure of the epidermal sources of these secretions appear to be similar to the organization of pyramidal cells in Musculium. Observations of the mucous sheets and filaments, which seem to originate at pyramidal cells in Musculium, showed them to be highly hygroscopic. Drops of water touched by micropipette to mucous surfaces, especially in clams within a mucous nest, were quickly pulled up by capillary action. When the water contained a semivital stain in the form of methylene blue,

it was later observed to be concentrated at extended pyramidal cells on both sides of the mantle. Since the mantle is probably the site of aerial respiration in the absence of aquatic gill function, this may be a great factor in water balance. The hygroscopic function of the pyramidal cell secretions decreases the need to supply bodily fluids for the maintenance of a moist respiratory surface. Instead, water from surrounding sources in the air or sediments may be utilized. Clams which aestivate inside a mucous nest probably exist as if within a moist miniature sponge when there is enough available moisture.

A further physiological adaptation for aestivation in Musculium occurs in the removal of its nitrogenous wastes. Like most other aquatic organisms, active clams excrete most nitrogenous wastes in the form of ammonia. Two typically molluscan metanephridia or "kidneys" drain into the suprabranchial tubules of the gills, and the released ammonia is carried out the excurrent siphon with the outflow of water. It is probable that gill and mantle surfaces also release some ammonia into the surrounding water. Nitrogenous waste removal in freshwater molluscs has been thoroughly investigated by Picken (1937) and Andres and Reid (1972). The latter investigators demonstrated a modified ornithine cycle and uricolytic enzymes in several unionid bivalves. This information suggested that aestivating clams might possess the ability to produce significant

amounts of complex nitrogenous wastes such as urea or uric acid. This possibility was investigated in Musculium transversum since the production of uric acid instead of ammonia could greatly reduce the water losses related to excretion. Such a reduction in water losses would be a beneficial adaptation for aestivating clams.

Thin section and tissue squashes of aestivating and active clams were treated with an Argentaffin uric acid staining process, modified from Humason (1962). Appendix C summarizes the method used in uric acid staining.

Close inspection of the prepared slides showed that, like many aestivating land snails (Horne 1970), adult aestivating Musculium and their late marsupial embryos produce uric acid. Small crystals of uric acid are deposited in the suprabranchial tubules and in the upper half of the inner branchial spaces of both primary gills. The cells of the metanephridium also stain heavily for uric acid. The morphology of the nephric tubules in aestivating clams is somewhat modified, the entire unit becoming more flattened and convoluted. This folding suggests an increase in surface area and thus increased potential for excretory secretion. The possible effects of the uric acid crystal production on symbionts within aestivating clams is discussed in the following section.

### Symbiont Aestivation

Three components of the symbiotic microcommunity aestivate along with their host. In 100 clams of various sizes selected from field collections, the fungus Endosphaerium funiculata, the oligochaetes Aeolosoma sp. and Chaetogaster limnaei, and the predatory leech Glossiphonia sp. were found aestivating inside the mantle cavity.

Thin sections of host gill tissues showed the predatory fungus Endosphaerium funiculata to be greatly reduced in size when aestivating. The portion of the thallus normally extending from the gill surface is apparently resorbed, leaving a slightly enlarged hyphal sphere of 30 to 100 $\mu$  embedded in the gill tissues. This hyphal anchor is still positioned between gill lamellae as in active clams. The location of the fungus in the gill surface with its associated hygroscopic mucus probably provides it with necessary water. The hyphal mass is also covered by its own layers of mucus, ensuring a moist resting environment.

Endosphaerium belongs to the family Pythiaceae, which includes many fungi that are amphibious in terms of spore or hyphal dispersal (D'Eliscu 1974). The alternation between aquatic and semi-aquatic life therefore does not appear to be a great adaptive problem.

Although the resting hyphal stage of Endosphaerium is situated in the interlamellar space, it does not occupy a position high enough in the gill to come in close contact

with uric acid crystals. Therefore, this essentially dry storage of nitrogenous wastes by the host does not threaten the fungus with a potentially toxic substance. If the waste product were fluid, such as the ammonia secretions of active clams, it would probably affect the survival of the fungus through toxic or osmotic stresses.

Some embryos and postembryonic juveniles from aestivating adults contained the cyst-like zoospores of Endosphaerium. None of these embryos or juveniles contained the hyphal masses associated with aestivating developed fungi. Similar to the findings for juveniles released from active clams, these young clams eventually developed fully trophic fungal thalli when isolated in culture dishes. In 21 of 25 attempts to reactivate hyphal masses from aestivating clams, at least one trophic thallus was produced in 24 hours at 18°C. In 13 of the 21 successful experiments a thallus was produced in each of the two inner gills.

Endosphaerium funiculata aestivates in adult hosts as dormant hyphal masses, and in juveniles and embryos as encysted zoospores. Both life stages ensure the fungal symbiont of being carried over to the next active aquatic period of the host population. Aestivation within the bivalve host, rather than free-living or within some auxillary or intermediate host, also ensures temporal synchrony of both mutual and host reactivation.

A few aestivating clams contained the oligochaetes Aeolosoma sp. and Chaetogaster limnaei. Both of these worms were commonly found in the mantle cavities of active clams as commensals. Six small individuals, less than one millimeter long, of both of these species were found in thick mucous coats beneath the inner gills of aestivating adult clams. It might be suggested that the low numbers of these worms from a sample of 100 clams indicates that they are only incidental in aestivating hosts. They could become trapped in the rapid mucus production of hosts entering aestivation. However, all 12 specimens recovered from the field sample were viable when placed in water-filled culture dishes. This high level of survival possibly indicates commensal aestivation rather than chance entrapment. Neither Aeolosoma nor Chaetogaster has been previously reported as aestivating oligochaetes, even though the presence of oil globules within Aeolosoma, with the possible functions of food and moisture storage, might have suggested aestivation as a possible life stage for this worm.

Three small aestivating leeches were also found within the mantle cavities of resting inactive clams. All of the leech specimens recovered were again encapsulated in a thick mucous coat beneath the inner gills of adult clams. After reactivation, the 1 to 2 millimeter annelids were identified as juvenile Glossiphonia sp. Although Mann (1962) and Collins (1970) cite examples of leech

aestivation, this case of a prey species of mollusc providing shelter for an eventual predator was novel. The relationship of leech juveniles to aestivating clams was not pursued further, but if the association is common it probably has some effect on the population dynamics of both predator and prey in their trophic phases (see following chapter on predation).

The contributions to the aquatic community from aestivating clams may include not only leeches and oligochaetes, but other organisms as well. As mentioned in the chapter involving the symbiotic relationships of active clams, Kingscote (1971) found the deer parasite Leptospira interrogans in aestivating as well as active Sphaerium occidentale. These parasites are released into the water after a period of maturation in this intermediate host. Gut squashes of aestivating Sphaerium occidentale (Collins 1970) and Musculium transversum have revealed concentrations of live diatoms, unicellular green algae, and bacteria. It is possible that this gut flora could help repopulate the pond when the water level rises. However, since the reactivation of aestivating clams was done with unfiltered pond water from tanks near the laboratory, this was not experimentally determined.

Through burrowing behavior and mucus production, Musculium transversum creates a substrate microclimate of major importance to its survival of unfavorable conditions.

Its morphological and physiological adaptations for water balance and excretion allow it to exist in aquatic, psammobiotic, and interstitial environments. These successful adaptations for aestivation in the host also help provide a sanctuary for several types of symbiotic organisms, thus contributing to the success of symbiont populations.

## PREDATION

Several investigations have shown members of the Sphaeriidae to be included in the diets of various predatory organisms. Major predators include fish, amphibians, ducks, and leeches. Lagler (1948) found Pisidium sp. as a major component in the diet of the old squaw (Clangula hymenalis), in Lake Michigan fish studies. Hoopes (1959), Jude (1968), and Ranthum (1969) investigated the channel catfish (Ictalurus punctatus), carp (Cyprinus carpio), bullhead (Ictalurus nebulosus), and gizzard shad (Dorosoma cepedianum) as major predators on Musculium transversum in the Mississippi River. Burch and Wood (1955) found Musculium lacustre (as M. jayense) to be a major food source of the salamander Siren lacertina. Rawls (1960), Gale (1970), and Nilsson (1972) have shown diving ducks and puddle ducks to be selective predators on several species of sphaeriids. Jansen (1970) and Knutson (1970) cited examples of sciomyzid fly larvae attacking and killing sphaeriids. Knutson pointed out that the only known prey of the aquatic carnivorous larvae of Renocera spp. are sphaeriid clams. Leech predation on sphaeriid clams has been reported by Paloumpis and Starrett (1960), Mann (1962), and Gale (1970). In terms of the effects on the population dynamics of clams, leech predation appears to be the most significant.

Leech Predation on *Musculium transversum*

The leech *Glossiphonia* sp. is an active predator on adult *Musculium transversum* at the Noon Pond. On several occasions during field collections, 2 to 4 cm long specimens of this leech were found partially enclosed in adult clams. Examination of these clams after removing the worms showed portions of the visceral mass and adductor muscles to be torn or absent. Based on this preliminary evidence, several laboratory observations were made of the feeding behavior of *Glossiphonia* on *Musculium*.

When several large clams were dropped into aquaria containing single, starved leeches, feeding behavior could be observed. After apparently detecting the presence of the clams, the leech would thrash its anterior end back and forth in what seemed to be searching behavior. When one of the clams was finally discovered, the leech first attached the anterior sucker to the shell and then attached the posterior sucker. Releasing the hold of the anterior sucker, the leech arched its body toward the ventral margin of the shell. At this point the leech appeared to be "poised and ready to strike." As the clam eventually extended its foot and thus caused the valves of the shell to gape slightly, the leech would plunge the most anterior fourth of its body inside the mantle cavity.

The clam generally responded by clamping the valves tightly together, trapping the leech's body at their

margins. Within minutes, however, the leech had destroyed enough adductor muscle tissue to cause the clam to gape open. Leech attacks interrupted only seconds after the initial plunge revealed clams with disrupted adductor muscles, but intact visceral masses. This suggested that the stereotyped attack behavior includes an early destruction of the only "defensive" tissues of the prey, and then a relatively leisurely feeding on the soft visceral tissues. In some cases, prey clams were not entirely consumed at one feeding; however, return meals eventually left nothing remaining but clean valves.

Gale (1970) stated in similar observations of leech predation on Musculium transversum (as Sphaerium transversum) that it is doubtful that large leeches can successfully attack small clams since attachment of the posterior sucker appears to be an integral part of the leech's attack. It also seems likely that small leeches would be unable to attack adult clams, as they could be crushed in the snap of the valves or be unable to quickly destroy enough adductor muscle tissue.

Since the specific rates and selectivity of feeding in Glossiphonia are not known, it would be difficult to determine the effect of leech predation on the clam population. Although Paloumpis and Starrett (1960) found that some leech populations fluctuated along with small mollusc populations, direct causal relationship was not established.

The lateral searching behavior of leeches observed in the laboratory suggested some further feeding experiments. Again employing the parallel water flow tubes used in the symbiosis studies (Fig. 2), leeches were exposed to several prey-choice combinations to determine their ability to locate and distinguish prey. The results are presented in Table 3. Apparently, Glossiphonia is able to distinguish living tissue from dead tissue or shell remains. It also selectively chooses Musculium over the aquatic pond snails Physa virgata and Helisoma tenue collected from the Noon Pond. Helisoma is to some extent selected over Physa as a secondary prey choice. This selectivity suggests an ability to locate and differentiate among prey species by some specific chemical means, and not solely on the basis of CO<sub>2</sub> or ammonia production by the prey, since all the prey species presumably produce these simple waste products.

Although the effects of predation on the Noon Pond population of Musculium were not determined, it is possible that predation contributes to some fluctuation in clam numbers. Any fluctuation of the host population would also affect the populations of symbiotic organisms as well.

Table 3. Parallel Water Flow Tube Choices of Glossiphonia

Choice combinations between Tube A and Tube B		# of leeches choosing A	# of leeches choosing B
Tube A	Tube B		
<u>Musculium</u> shells	<u>Anodonta</u> Shell Fragments	10	10
<u>Musculium</u> shells	Live <u>Musculium</u> Adults	2	18*
Live <u>Musculium</u> Adults	Live <u>Musculium</u> Adults	9	11
Live <u>Physa</u> Adults	Live <u>Musculium</u> Adults	5	15*
Live <u>Helisoma</u> Adults	Live <u>Musculium</u> Adults	7	13
Live <u>Helisoma</u> Adults	Live <u>Physa</u> Adults	12	8
Tissue mass from frozen <u>Musculium</u>	Live <u>Musculium</u> Adults	3	17*

\*P < 0.05, normal approximation to binomial distribution,  $\lambda$  (10,  $\sqrt{5}$ ).

H<sub>2</sub>O flow rate of 2 ml/sec at 15°C, 20 runs each combination, with no leeches repeating a combination.

## DISPERSAL

Although discussion of the general zoogeography of the Sphaeriidae is beyond the scope of these studies, it may be useful to consider some aspects of dispersal in this group. The great world wide distribution of fingernail clams is perhaps best explained by their small size, resistance to desiccation, and the unusual behavior of attaching themselves to larger, more motile organisms. Sphaerium striatinum, for example, has been found attached to the skin of amphibious turtles (Grant and Robertson 1966). S. corneum has been shown to be more than just a phoretic symbiont in its behavior of burrowing into the toe webbing of the common toad Bufo vulgaris. The clamping of the clam's valves onto the skin of this carrier causes tissue damage which sometimes results in thickening, inflammation, and necrosis at the attachment site. In some cases, lesions, hemorrhaging, and resulting scar tissue formation may be observed (Sixl 1968).

Limited phoretic dispersal of smaller Pisidium spp. has also been observed. The frog Pseudacris triseriata has been shown to carry these clams occasionally more than 1800 feet from pond to pond. Like S. corneum, these clams become clamped to the toes of their carrier (Rees 1952, Spencer 1964). Originally mistaken for glochidia, Pisidium

variable have been found attached to the lower jaws of young trout in hatchery operations (Tanner 1954). Pisidium spp. are so small and light weight that they may be carried phoretically by corixid water bugs (Fernando 1954). As corixids are strong fliers as well as swimmers, this means of dispersal may be local or long range. Aquatic beetles may also carry Pisidium spp. for long distances (Odhner 1951). Examples of active dispersal of unionid clams by water birds have been discussed by Rees (1965), and the mechanisms involved probably apply to the Sphaeriidae as well. In this case, the clams become attached to the feathers or skin folds, or are carried along in the sediments clinging to the feet of wading birds.

The passive dispersal of freshwater bivalves by flooding, drainage channel changes, and, rarely, whirlwinds and waterspouts is probably as important as active dispersal (Boycott 1936). Since the Sphaeriidae are hermaphroditic and potentially self-fertile, any chance movement of an individual to a suitable environment may be sufficient for colonization. In addition, since individual clams and their respective symbionts constitute a symbiotic unit, any fortuitous colonization by the host may also lead to the establishment of new symbiont populations.

In field collections of organisms at the Noon Pond only one possible instance of dispersal was observed. In July, 1972, a 5 cm long Hydrophilus sp. water beetle was

collected with a single 2mm long Musculium attached to the tarsus of an anterior leg.

## SUMMARY

The metazoan and fungal symbionts of Musculium transversum are interrelated and exist in several levels of symbiosis, including phoresis, commensalism, mutualism, and parasitism. Active, non-aestivating clams support several short food chains of symbionts. Incidental phoretic bryozoans utilize the external shell surface of host clams as attachment areas for sessoblasts. Numbers of the spinose rotifer Filinia, the predaceous larval hydrophilid Berosus, and the coprozoic oligochaetes Aeolosoma and Chaetogaster are commensals within the mantle cavity. The predaceous beetle preys on both cloaca inhabiting coprozoic worms. The predaceous fungus Endosphaerium funiculata lives attached to the host's gills, and preys on both Filinia and the parasitic nematode Seinura. This nematode invades host reproductive and digestive tissues, and therefore its capture by Endosphaerium constitutes a mutualistic symbiosis. In this mutualism, Endosphaerium benefits by living in the stable environment of the mantle cavity, while the host receives some resistance to a destructive parasite. The reproductive biology of the fungus is closely coordinated with that of the host, further indicating the complexity of the relationship.

Aestivating clams show some special physiological modifications, and may also maintain some aestivating symbionts. Aestivating clams produce uric acid crystals instead of ammonia as a nitrogenous waste compound. This physiological adaptation conserves water. The production of hygroscopic mucus threads by pyramidal cells in the mantle serves as an additional water conservation mechanism. Through its adaptations for survival of dry periods, the host also provides a moist sanctuary for several components of the symbiotic community. Small oligochaetes Aeolosoma and Chaetogaster, juvenile leeches of the genus Glossiphonia, and the resting stages of Endosphaerium are supported in aestivating clams.

The close associations of symbionts and individual clams constitute a symbiotic unit. Active or passive dispersal of individual clams may also lead to colonization by their respective symbionts. Similarly, leech predation on host clams may indirectly affect symbiont populations by altering host density or size availability.

## APPENDIX A

### STAINING METHODS FOR SERIAL SECTIONS OF MUSCULIUM TRANSVERSUM<sup>a</sup>

<u>Bath</u>	<u>Time</u>
1. toluene	8 min.
2. ethyl alcohol series	16 min. (99%, 90%, 80%, 70%, @ 4 min.)
3. LiCO <sub>3</sub>	5 min.
4. H <sub>2</sub> O	3 min.
5. Alcian Blue	20 sec.
6. H <sub>2</sub> O	3 min.
7. Kornhauser's Hemalum <sup>b</sup>	3 min.
8. H <sub>2</sub> O	3 min.
9. Scott's Solution	5 min.
10. 70% ethyl alcohol	4 min.
11. Eosin B	12 sec.
12. ethyl alcohol series	6 min (95% 3 dips; 99% 6 min.)
13. toluene	3 min.

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<sup>a</sup>All stains at stock preparation after Humason (1962), except Alcian Blue used at 1% solution.

<sup>b</sup>Substitute Schiff's Reagent (5 min.), followed by bath of 0.2 N HCl (2 min.) for step 7 in observations of fungus preparations.

## APPENDIX B

### SOME TREMATODA REPORTED FROM FRESHWATER BIVALVES

#### Allocreadium isoparum

Yamaguti, S. 1958. Systema Helminthum. I. Inter-  
science Publ., New York.

#### Allocreadium spp.

Foster, T. D. and W. C. van Deventer. 1933. A compara-  
tive study of river, pool, and pond communities,  
with special reference to the sphaeriids. Trans.  
Ill. State Acad. Sci. 26(3):132.

#### Aspidogaster conchicola

Bakker, K. 1972. The relation between the parasite  
Aspidogaster conchicola (Aspidogastridae: Trematoda)  
and its host, the clam Anodonta anatina. Neth.  
Jour. Zool. 22(2):224.

Van Cleave, H. J. and C. O. Williams. 1943. Maintenance  
of a trematode Aspidogaster conchicola outside the  
body of its natural host. J. Parasitology 29:127-  
130.

#### Aspidogaster limacoides

Nagibina, L. F. and T. A. Timofeeva. 1971. True hosts  
of Aspidogaster limacoides Diesing 1834 (Trematoda:  
Aspidogastrea). Dokl. Akad. Nauk. SSSR, Ser. Bio.  
200(3):742-744.

#### Bunodera luciopercae

Cannon, L. R. G. 1971. The life cycles of Bunodera  
sacculata and Bunodera luciopercae (Trematoda:  
Allocreadidae) in Algonquin Park, Ontario. Can.  
Jour. Zool. 49(11):1417-1429.

Moravec, F. 1969. On the early development of Bunodera luciopercae (Müller, 1776) (Trematoda: Bunoderidae). Vestn. Cesk. Spolecnosti Zool. 33(3):229-237.

Bunodera sacculata

Cannon, L. R. G. 1971. Ibid.

Cercaria isospori

Dollfus, R. P. 1949. Sur une cercaire opthalmoxiphidiocerque Cercaria isospori Looss 1894 et sur la delimitation des Allocreadioidea. Ann. Parasitol. Humaine et Comp. 24(5/6):424-435.

Cercaria wabashensis

Coil, W. H. 1955. Cercaria wabashensis sp. nov., a new macrocercous Cercaria (Gorgoderinae) from western Indiana. Proc. Helminthol. Soc. Wash. 22(2):64-66.

Crepidostomum cooperi

Choquette, L. P. E. 1954. A note on the intermediate hosts of the trematode Crepidostomum cooperi Hopkins 1931, parasitic in speckled trout (Salvelinus fontinalis (Mitchill)) in some lakes and rivers of the Quebec Laurentide Park. Can. Jour. Zool. 32(6):375-377.

Wenke, T. L. 1968. Abundance of Crepidostomum and other intestinal helminths in fishes from Pool 19, Mississippi River. Iowa State J. Sci. 43:211-222.

Crepidostomum fariconis

Awachie, J. B. E. 1968. On the bionomics of Crepidostomum metoecus (Braun, 1900) and Crepidostomum fariconis (Müller, 1784) (Trematoda: Allocreadidae). Parasitology 58(2):307-324.

Crawford, W. W. 1939. Colorado trematode studies, I. a further contribution to the life history of Crepidostomum fariconis (Müller). J. Parasitology 25:379-384.

Crepidostomum ictaluri

Wenke, T. L. 1968. Ibid.

Crepidostomum illinoiense

Wenke, T. L. 1968. Ibid.

Crepidostomum metoecus

Awachie, J. B. E. 1968. Ibid.

Crepidostomum spp.

Gale, W. F. 1970. Bottom fauna of Pool 19, Mississippi River with emphasis on the life of Sphaerium transversum. Doctoral Dissertation, Iowa State University.

Cheng, T. C. and H. A. James. 1960. The histopathology of Crepidostomum sp. infection in the second intermediate host Sphaerium striatinum. Proc. Helminthol. Soc. Wash. 27(1):67-68.

Echinoparyphium aconiatum

McDonald, M. E. 1969. Catalogue of helminths reported from water fowl (Anatidae). Bur. Sport Fisheries and Wildlife, Spec. Rept. 126, 692 p.

Echinoparyphium recurvatum

McDonald, M. E. 1969. Ibid.

Echinostoma coalitum

Yamaguti, S. 1958. Ibid.

Echinostoma lindoensis

Sandground, J. H. and C. Bonne. 1940. Echinostoma lindoensis sp. nov., a new parasite of man in the Celebes with an account of its life history and epidemiology. Amer. Jour. Trop. Med. 20:511-535.

Echinostoma revolutum

McDonald, M. E. 1969. Ibid.

Gorgoderia amplicava

Cheng, T. C. 1963. Histological and histochemical studies on the effects of parasitism of Musculium partumeium (Say) by the larvae of Gorgoderia amplicava Looss. Proc. Helminthol. Soc. Wash. 30(1):101-107.

Gorgodera cygnoides

Yamaguti, S. 1958. Ibid.

Gorgodera spp.

Collins, T. W. 1967. Oxygen-uptake, shell morphology and desiccation of the fingernail clam, Sphaerium occidentale Prime. Doctoral Dissertation, University of Minnesota.

Richard, J., A. G. Chabaud, and E. R. Bryoo. 1968. Notes sur la morphologie et la biologie des trematodes digenes parasites des grenouilles du jardin de L'Institut Pasteur a Tananarive. Arch. Inst. Pasteur Madagascar 37(1):31-52.

Gorgoderina attenuata

Yamaguti, S. 1958. Ibid.

Gorgoderina vitelliloba

Yamaguti, S. 1958. Ibid.

Hypoderaeum conoideum

McDonald, M. E. 1969. Ibid.

Megalonia ictaluri

Carlson, C. A., F. P. Meyer, and C. R. Fremling. 1959. Allocreadiid flukes of mayflies of the Mississippi River. 21st Midwest Wildlife Conference, 1959.

Phyllodistomum caudatum

Yamaguti, S. 1958. Ibid.

Phyllodistomum lohrenzi

Yamaguti, S. 1958. Ibid.

Phyllodistomum solidum

Yamaguti, S. 1958. Ibid.

Phyllodistomum bufonis

Ubelaker, J. E. 1972. Life cycle of Phyllodistomum bufonis (Digenea: Gorgoderidae) from the boreal toad Bufo boreas. Proc. Helminthol. Soc. Wash. 39(1):94-100.

Phyllodistomum staffordi

Schell, S. C. 1967. The life history of Phyllodistomum staffordi Pearse, 1924 (Trematoda: Gorgoderidae Looss, 1901). J. Parasitology 53(3):569-576.

## APPENDIX C

### ARGENTAFFIN URIC ACID STAINING METHOD

1. Fix, imbed, and section normally at 7 to 9  $\mu$ .
2. Mount thin serial sections with a film of 95% ethyl alcohol.
3. Carefully deparafinize with slide upright, rinse 5 minutes in bath of 95% ethyl alcohol.
4. Treat 30 minutes at 30 C in methenamine silver solution.<sup>a</sup>
5. Rinse gently for 3 minutes in distilled water.
6. Treat 3 minutes in 8% sodium thiosulphate solution.
7. Rinse gently for 3 minutes in distilled water.
8. Dehydrate and mount coverslips with permount fluid.

Results: uric acid indicated by fine black crystalline granules.

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<sup>a</sup>Methenamine silver solution:  
silver nitrate 5% aqueous  
5.0 ml  
  
methenamine 3% aqueous  
100.0 ml

APPENDIX D

PARALLEL WATER FLOW TUBE CHOICES OF BEROSUS,  
AELOSOMA, CHAETOGASTER, AND SEINURA

Choice between live Musculium and shells

<u>Symbiont</u>	<u>Movement to life <u>Musculium</u></u>	<u>No movement</u>	<u>Movement to <u>Musculium</u> shells</u>
<u>Berosus</u> < 4 mm	8	2	0
<u>Berosus</u> > 6 mm	0	3	0
<u>Aelosoma</u>	10	0	0
<u>Chaetogaster</u>	0	12	0
<u>Seinura</u> > 2 mm	2	8	1

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