Curran Benz 1996

ANNUAL REPORT

SURVEY OF METAZOAN SYMBIONTS OF EIGHT FRESHWATER MUSSEL SPECIES (UNIONIDAE) COLLECTED IN KENTUCKY LAKE, TENNESSEE[1]

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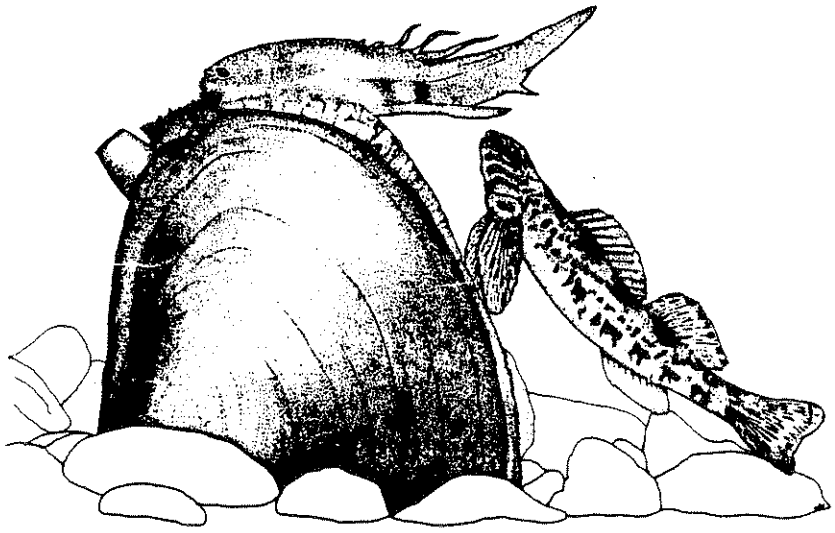
26 December, 1996



[1] This is the third annual report for the Tennessee Wildlife Resources Agency contract No. 7A-4-11062-4-00 entitled, "Qualitative survey of metazoan inhabitants of freshwater mussels, TN River."

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Acknowledgements

We thank A. D. McKinney (Tennessee Wildlife Resources Agency) for overseeing the collection and shipping of mussels, D. Hubbs and R. Todd (both Tennessee Wildlife Resources Agency) for collecting and shipping mussels, Dr. J. N. Caira (The University of Connecticut) for facilities used throughout the laboratory analyses, P. R. Cislo (The University of Connecticut) for assistance with several of the statistical tests, T. A. Tarpley (Tennessee Aquarium and Southeast Aquatic Research Institute) for rendering the figure which appears on the inside cover page, and the Southeast Aquatic Research Institute and Tennessee Aquarium for facilitating the study and donating GWB's time to the project.

Abstract

This report presents third-year progress of an ongoing study of metazoan associates of freshwater mussels (Unionidae) in Kentucky Lake (Tennessee). A total of 500 unionid mussels (Unionidae) comprising eight species (*Amblema plicata*, *Elliptio cressidens*, *Fusconaia ebena*, *F. flava*, *Megalonaias nervosa*, *Quadrula metanevra*, *Q. pustulosa*, *Q. quadrula*) have been examined to date (152 examined during the project's third year). Mussels have been collected from three general regions in Kentucky Lake (Tennessee River mile *circa* 88, TN river mile 168, and TN river mile *circa* 197) between May 1994 and July 1996. Thirteen taxa of symbionts have been found associating with the sampled unionids, together representing six phyla (Platyhelminthes, Nematoda, Annelida, Mollusca, Tartigrada, and Arthropoda), 12 families, and at least 13 genera and species. Most of the collected symbionts were parasites, and together these represented four taxa (*Aspidogaster conchicola*, *Cotylogaster occidentalis*, *Cercaria filicauda*, and *Unionicola* sp.).

Aspidogaster conchicola was found infecting all eight unionid species and was the most common flatworm collected. Overall *A. conchicola* prevalence values for the studied mussel species ranged from 14 to 73 percent and corresponding overall mean intensity values ranged from 1.0 to 7.8 worms per mussel. To date, the greatest number of *A. conchicola* found infecting one mussel was 40. All *A. conchicola* were collected from the renal or pericardial chambers. Histological examination of *A. conchicola in situ* did not reveal any pathologies associated with infections. Of the eight studied mussel species, only *Megalonaias nervosa* was found to be infected with *A. conchicola* at significantly different levels regarding the various collections sites, with none of the sampled *M. nervosa* from the 168 river mile location being infected and 33 percent of *M. nervosa* from the *circa* 88 river mile area being infected. For six of the eight studied unionid species there was no significant difference in the number of *A. conchicola* infecting the kidney versus the pericardial chamber. In *F. ebena* infections of the kidney were significantly more common, while in *Q. metanevra*, infections in the pericardial chamber were more common.

A parasitic mite, *Unionicola* sp., was collected from five of eight studied unionid species. Overall *Unionicola* sp. prevalence values for the eight studied mussel species ranged from 0 to

94.6 percent and corresponding overall mean intensity values ranged from 0 to 8.4 mites per mussel. To date, the greatest number of *Unionicola* sp. found infecting one mussel was 46. All mites (adults and larvae) were collected from the demibranchs, and histological examinations of infected mussels did not reveal any pathologies associated with mite infections. Prevalence and intensity values regarding mite infections were significantly different for some mussel species regarding the three collection areas.

All parasite taxa collected to date in this ongoing study exhibit one or two host life cycles. Those with two host life cycles utilize fishes as definitive hosts. None of the parasites collected in this study have been reported to infect humans. While we can't assess with certainty the potential for parasites collected in this study to cause survival or health related problems for mussels in the wild or those confined under aquaculture conditions, the implications of the relatively high prevalence of *A. conchicola* and *Unionicola* sp. infections as well as the relatively high parasite burdens sometimes associated with *A. conchicola*, *Unionicola* sp., and *Cercaria filicauda* infections in the studied unioind species warrants further study.

INTRODUCTION

The southeastern United States is the center of biodiversity for the freshwater mussel family Unionidae. This regional unionid fauna has historically consisted of at least 269 species, together representing 91 percent of North America's freshwater mussels (Neves *et al.*, *in press*). Within the Southeast, the states of Alabama and Tennessee historically have possessed the richest unionid faunas (175 and 132 species respectively) (see Neves *et al.*, *in press*). Tennessee's unionids have resided in all of its five major drainages, where ideal aquatic habitats for this diverse fauna have existed.

Throughout North America, Unionidae is experiencing an unprecedented decline in both numbers of species and numbers of individuals due to various environmental factors which all seem associated with human activity (Marking and Bills, 1979; Schmidt *et al.*, 1989; Stolzenburg, 1992; Williams *et al.*, 1992). Within Tennessee, 20 species of freshwater mussels are federally listed as endangered species (Hatcher, 1995). Because of the commercial importance of some unionids (e.g., see McGregor and Gordon, 1992) and due to the potential use of unonids as valuable indicator organisms capable of detecting minute levels of aquatic toxicants (e.g., see Jacobson *et al.*, 1993), recent state, federal, and private conservation efforts have focused on the management, propagation, and culturing of native freshwater mussels. To ensure the applied success of these efforts, basic natural history information about unionids is needed to allow resource managers and biologists to properly care for captive individuals and to facilitate the monitoring of wild populations and the identification of species in need of management.

Parasites can play important roles in wild and captive populations. Although some parasite species routinely act as agents of disease in natural settings, most parasites coexist with their hosts by establishing levels of infection which do not threaten the viability of host populations. In natural environments, the transmission stage of a parasite's life cycle often represents a limiting phase that ultimately regulates parasite population levels. Environmental factors which increase the probability that parasite transmission will be successful can cause

abnormal increases in parasite loads that may ultimately alter the overall pathogenicity of said parasite species. Wild host populations can sometimes be adversely impacted by parasites under unusual environmental circumstances which facilitate parasite transmission. However, it is in semi-closed and closed-captive environments where parasites (especially those with direct life cycles) are most efficient at infecting hosts and in developing burdensome populations which ultimately can overwhelm the capacity for host species to support themselves under the pressures of parasitism.

A thorough understanding of the overall impact of parasitism on wild unionid populations does not exist. Because of this, it is impossible to evaluate the potential significance of parasitism regarding the captive maintenance of unionids, and it is also impossible to contemplate in advance appropriate methods to control parasitic infections.

The literature dealing with the parasite fauna of freshwater mussels is dominated by geographically scattered parasite records associated with taxonomic accounts that generally lack temporal scope. Many of these reports have documented the presence of parasites without supplying data concerning seasonal prevalence, intensity of infection, or quantitative estimates of total parasite burdens. Some studies, however, have focused more closely on the natural parasite burdens of unionids (e.g., Kelly, 1899; Najarian, 1955; Hendrix and Short, 1965; Flook and Ubelaker, 1972; Danford and Joy, 1984). Most recently Duobinis-Gray *et al.* (1991) and Vidrine and Wilson (1991) have reported prevalence and mean intensity data respectively for a species of parasitic flatworm in Kentucky and for some parasitic mites in Tennessee that infect some species of unionids.

This report presents 30 month's results of an ongoing study designed to gain information about the parasites naturally occurring in wild unionid populations via a survey of the metazoan associates of several species of freshwater mussels within the Tennessee River system. In addition to documenting parasite presence, this study was designed to provide preliminary information regarding overall parasite burdens, parasite prevalences, and parasite intensities, as well as preliminary information regarding histopathology associated with parasitic infection. This report does not present all of the results gathered to date regarding this ongoing multi-year project. Readers interested in results obtained during the first two years of this study should consult Curran and Benz (1994, 1995).

MATERIALS AND METHODS

A total field sample of 500 unionid mussels (152 mussels during the project's third year) comprising eight species have been collected for this study between May 1994 and July 1996 by Tennessee Wildlife Resources Agency (TWRA) biologists in Kentucky Lake (an impoundment of the Tennessee River; see Figure 1). Field samples were gathered as a series of ten collections from three positions within Kentucky Lake (TN river mile *circa* 88, TN river mile 168, and TN river mile *circa* 197)(see Figure 1, Table I).

Tennessee Wildlife Resources Agency biologists identified the collected mussels and placed them in individual plastic bags or in bags containing batches of one species collected on the same date and at the same location. Species identification labels were included in the field sample bags and a data sheet providing information about location of the collection, date, collectors' names, and the approximate water depth of collection was included with each mussel shipment. Bagged mussels were placed on ice in an insulated container and were shipped to the laboratory (University of Connecticut) within two days of capture. Once received at the laboratory, mussels were stored in a refrigerator (6.5°C) and were examined within 14 days of their arrival.

In the laboratory, 486 mussels (see Table I) were examined using the following procedure (see Figures 2 and 3 for an anatomical reference to the major organs of unionids): water from the collection bag and the outside of the shell was examined for organisms using a dissection microscope. Next the shell was opened using a blunt scalpel and the mantle liquid was drained into a petri dish and examined for organisms. Lastly, the soft tissues were examined under low magnification, dissected and re-examined under low magnification for symbionts. Soft tissues examined included those of the mantle, foot, gills, digestive gland, stomach, intestine, kidney, gonad, and pericardium. All symbionts were collected and the exact location of each was recorded. Along with these examinations, each examined mussel was sexed. To do so, wet mount preparations of squashed gonad tissue were examined for the presence of sperm or glochidia/eggs using a compound microscope. Mussels whose sex could not be identified using this method were deemed "undetermined" regarding sex and were excluded from subsequent analyses requiring an identified sex.

Mussels sampled during the last five field collections were shipped to the laboratory bagged in batches rather than individually. Because of the possibility of cross contamination of external symbionts between batched individuals which would bias survey results, the external shells of batched mussels were not examined quantitatively.

Mussels from the first five collections were individually weighed using an electronic balance. The following weights (g) were recorded: total weight (*i.e.*, shell and soft tissues), soft weight (*i.e.*, total weight of all soft tissues including the retractor and adductor muscles), and shell weight (*i.e.*, weight of shell without retractor and adductor muscles). Because of a problem which rendered the balance useless, morphometrics (maximum length and maximum width of the right valve [cm]) rather than weight were recorded for mussels gathered during the last five field collections. All mussel shells were individually marked and stored.

Metazoan symbionts collected from the examined mussels were fixed, preserved, identified, and stored using standard laboratory techniques (e.g., see Pritchard and Kruse, 1982). While some symbionts were identified to the level of species, higher level taxa (e.g., genus, family) were used where appropriate to identify others.

A total of 14 mussels comprising four species were selected for histological examination (2 *Amblema plicata*; 2 *Fusconaia ebena*; 9 *Quadrula pustulosa*; 1 *Q. quadrula*). Examinations of these mussels differed from those discussed above in that the organs mentioned above were excised using a scalpel and examined under low magnification prior to relaxation and then fixation in Bouin's fixative or 10 percent buffered formalin. Fixed organs were later transferred to 70 percent ethyl alcohol. Portions of each organ to be histologically examined were isolated and then dehydrated through a graded series of alcohols, cleared with xylene, and embedded in paraffin wax using standard histological techniques. Thin tissue sections were cut (12μm serial intervals) using a rotary microtome, and were subsequently stained with Gill's hematoxylin and eosin, and permanently mounted on slides using standard histological techniques. Mounted tissue sections were examined using bright-field microscopy.

Symbiont prevalence and density indices were calculated for each of the three most common groups of collected symbionts. Symbiont prevalence is defined as the percentage of individuals associated with the symbiont in a given host population (or sample), and was calculated as follows:

$$P_{yx_t} = \frac{C_{yx_t}}{N_{yx_t}} \times 100$$

where:

P_{yx_t} = the prevalence of symbiont species x in host species y at time t,

C_{yx_t} = the number of hosts of species y found associated with symbiont species x at time t,

and

N_{yx_t} = the total number of host species y examined for symbiont species x at time t.

Symbiont density (sometimes referred to as intensity) is defined as the mean number of symbionts found with associate hosts in a given host population (or sample), and was calculated as follows:

$$D_{yx_t} = \frac{\sum_{1}^{N_y} A_{yx_t}}{N_{yx_t}}$$

where:

D_{yx_t} = the mean density of symbiont species x associated with host species y at time t,

$\sum_{1}^{N_y} A_{yx_t}$ = the sum of the total number of symbiont individuals of species x collected from each individual species y host examined at time t,

and

N_{yx_t} = the total number of host individuals of species y associated with symbiont species x at time t.

To determine whether parasite burdens differed among the unionid species inhabiting the three collection localities in this study, nested analysis of variance models (ANOVA) were constructed from the parasite prevalence and mean intensity data collected for each of the eight species of unionids. Locality was nested in the host species variable of the models. Significance was assessed at $\alpha = 0.05$. Separate models were constructed for *Unionicola* sp. and *Aspidogaster conchicola* data.

An analysis of variance model was used to examine whether *Aspidogaster conchicola* was found more often in the kidney versus the pericardial chamber of infected unionids. Significance was assessed at $\alpha = 0.05$.

Analysis of variance models were used to separately examine whether parasite prevalence and parasite mean intensity differed among individual unionids of different shell lengths. For these analyses, data from the eight examined host species were lumped and separate analyses were carried out for *Unionicola* sp. and *A. conchicola*. Significance was assessed at $\alpha = 0.05$.

Analysis of variance models were used to separately examine whether parasite prevalence and parasite mean intensity differed among unionids of various total weights. For these analyses, data from the eight examined host species were lumped and separate analyses were carried out for *Unionicola* sp. and *A. conchicola*. Significance was assessed at $\alpha = 0.05$.

Analysis of covariance models (ANCOVA) were conducted to separately determine whether prevalence and mean intensity values for parasites infecting unionids were different in male versus female unionids. In these models, maximum shell length was used as the covariant parameter. For these analyses, data from the eight examined host species were lumped and separate analyses were carried out for *Unionicola* sp. and *A. conchicola*. Significance was assessed at $\alpha = 0.05$.

Analysis of covariance models were conducted to separately determine whether prevalence and mean intensity values for parasites infecting unionids was different between male and female unionids. In these models, total mussel weight was used as the covariant parameter. For these analyses, data from the eight examined host species were lumped and separate analyses were carried out for *Unionicola* sp. and *A. conchicola*. Significance was assessed at $\alpha = 0.05$.

Chi-square tests were performed to determine whether the two most common parasite taxa (*i.e.*, *Aspidogaster conchicola* and *Unionicola* sp.) were independently distributed among

individual mussels of each respective host species. The number of observed occurrences of each of the four possible results (*i.e.*, no parasites; aspidogastrid only; unionicolid only; aspidogastrid and unionicolid together) was compared to the number of expected occurrences. The probability of either parasite taxon (aspidogastrid or unionicolid) occurring in a mussel was assumed to be equal to its observed overall prevalence in that species of mussel. The probability of either parasite taxon not occurring in a particular mussel species was calculated as 1 minus the observed overall prevalence in that species of mussel. Expected chi-square values were calculated by multiplying the sample size by the probability that one of the four possible combinations would occur (*i.e.*, no parasites; aspidogastrid only; unionicolid only; aspidogastrid and unionicolid together). The calculated chi-square values for each possible combination were totaled for each of the eight mussel species examined and compared to tabled critical values for the chi-square statistic ($\alpha = 0.05$, $df = n-1$). In instances when calculated chi-square values were less than or equal to tabled critical values, the presence of the two parasite taxa in mussels was considered to be independent. When calculated chi-square values were greater than tabled critical values, the presence of the two parasite taxa in mussels was considered to be related.

Statistical Analysis Systems© (SAS) software was used throughout this study to perform the ANOVA, ANCOVA, and chi-square analyses outlined above. In instances when the results of any of the aforementioned statistical tests are not mentioned in this report, the reader is hereby advised that test results identified nothing of significance.

RESULTS

Thirteen taxa were found in association with the 486 unionids examined to date in this ongoing study. Together, these symbionts represented six phyla (Platyhelminthes, Nematoda, Annelida, Mollusca, Tartigrada, and Arthropoda), twelve families, and at least thirteen genera and species (Table II). Arthropoda was the most taxon rich of the six phyla noted in this study, and was represented by five genera. Platyhelminthes was represented by three species, all of which were internal parasites of mussels. Nematoda was represented by one genus and Tartigrada was represented by a single species. Two individual oligochaetes represented the only annelids found, and two individual molluscs (a snail and a fingernail clam) were found within the incurrent siphons of separate mussels (Table II). Nematodes, annelids, and tartigrades were always found on the

external surface of their unionid associates. Four of the five arthropod taxa were insects found externally on unionids. The fifth arthropod taxon was a parasitic mite (*Unionicola* sp.) which was found internally (Table II). By far, the two most common symbiotic taxa were *Aspidogaster conchicola* and *Unionicola* sp., both of which were internal parasites (see Table II).

Aspidogaster conchicola (see Figure 4) was collected from all of the eight unionid species examined in the study. A total of 923 *A. conchicola* was collected from the 486 unionids sampled (see Table II). Four-hundred and twelve (44.6 percent) were collected from the kidneys of their hosts, while 511 (55.4 percent) were collected from the pericardial cavities of their hosts. Of the 923 *A. conchicola* collected, 21 were juveniles. In *Fusconaia ebena*, *Aspidogaster conchicola* was found to infect the kidney significantly more often than the pericardial chamber ($p = 0.0001$), while in *Quadrula metanevra* this worm was found significantly more often in the pericardial chamber than in the kidney ($p = 0.0106$). No statistical difference was noted for the remaining six mussel species regarding kidney versus pericardium as location sites of this parasite.

Overall prevalence of infection values for *Aspidogaster conchicola* ranged from 14.3 percent in *Megalonaias nervosa* ($n = 35$) to 73.2 percent in *Quadrula quadrula* ($n = 56$) (see Table III). For seven of the eight species of mussels examined, no significant difference in the prevalence of infection with *A. conchicola* was found among the three study areas. A significant difference in the prevalence of *A. conchicola* between the *circa* 88 river mile area and 168 river mile location was found in *Megalonaias nervosa* ($p = 0.0330$). Regarding this result, thirty-three percent of the *Megalonaias nervosa* ($n = 15$) collected from the *circa* 88 river mile area were infected with *A. conchicola*, but this parasite was not found in any of the *M. nervosa* ($n = 20$) collected from the 168 river mile location.

Overall mean intensity values for *A. conchicola* infections were low, ranging from 1.0 ± 0.0 to 2.7 ± 2.5 in seven of the eight species of unionids examined to date in this study (see Table IV). The overall mean intensity of *A. conchicola* infections was highest in *Quadrula quadrula*, with infections averaging 7.8 ± 8.1 worms (see Table IV). No significant differences in mean intensity were found for *A. conchicola* infections in any of the eight species of unionids examined among the three study areas ($p = 0.1919$). Seasonal variation in *A. conchicola* prevalence and mean intensity values was not casually apparent, however, lack of a temporally robust sample prevented statistical testing for possible seasonal effects.

Histological sections revealed no pathologies associated with *Aspidogaster conchicola* infections (see Figure 5). This parasite was always found in either the pericardial or renal cavities, and no encapsulated *A. conchicola* individuals have been observed to date in this study.

A total of 953 *Unionicola* sp. was collected from five of the unionid species examined (see Table II). *Unionicola* sp. adult and larval stages (nymphs) were always found on the demibranchs of unionids. *Elliptio cressidens* (n = 16) and *Fusconaia flava* (n = 18) did not harbor mite infections. Only one of 215 *Fusconaia ebena* examined was infected with unionicolids, and this individual harbored only a single mite. Prevalence for *Unionicola* sp. was highest (94.6 percent) in *Quadrula quadrula* (n = 56), and nearly as high (92.9 percent) in *Quadrula pustulosa* (n = 71)(Table V). Only 28.2 percent of the *Quadrula metanevra* (n = 39) examined were infected by mites (Table V). A significant difference in the prevalence of *Unionicola* sp. infecting *M. nervosa* was found between the *circa* 88 river mile area and 168 river mile location ($p = 0.0103$). Seventy-three percent of the *M. nervosa* (n = 15) collected from the *circa* 88 river mile area were infected with *Unionicola* sp., while 95 percent of *M. nervosa* (n = 20) collected from the 168 river mile location harbored these parasites. A significant difference in the prevalence of *Unionicola* sp. infection between sampling locations was also found regarding the mussel species *Quadrula pustulosa*. Unionicolids were found at a significantly lower prevalence in *Q. pustulosa* at the *circa* 197 river mile area (prevalence = 70 percent) than at the other two river areas (prevalences both = 100 percent)($p = 0.003$). A significant difference in the mean intensity of *Unionicola* sp. infections between study locations was found for *Q. pustulosa*, with this parasite being found in significantly higher numbers at the 168 river mile location ($p = 0.001$)(see Table VI). Seasonal trends in *Unionicola* sp. prevalence and mean intensity were not casually apparent, however, lack of a robust temporal sample prevented statistical testing for possible seasonal effects.

Individuals of six of the eight mussel species examined in this study (*i.e.*, *Amblema plicata*, *Fusconaia ebena*, *Megalonaias nervosa*, *Quadrula metanevra*, *Q. pustulosa*, and *Q. quadrula*) were often simultaneously infected with both of the parasites *Aspidogaster conchicola* and *Unionicola* sp. (see Table VII). The chi-square analysis used to assess the dependence of multiple infections only denoted infections of *A. conchicola* and *Unionicola* sp. in *Fusconaia ebena* to be dependent upon one another (Table VII).

In addition to *A. conchicola* and *Unionicola* sp., two other internal parasites were collected from unionids. A second species of aspidogastrid, *Cotylogaster occidentalis*, internally infected several unionids (see Figure 4, Table II). One *C. occidentalis* was found in an *Elliptio cressidens* and one other in a *Quadrula pustulosa* collected at the 168 river mile location. *Cotylogaster occidentalis* was also found in *Quadrula pustulosa* collected at the *circa* 88 and *circa* 197 river mile areas (see Table II) – a single worm found in each of two *Q. pustulosa* collected at the *circa* 88 river mile area, and a single worm found in a *Q. pustulosa* collected at the *circa* 197 river mile area.

The digenean family Gorgoderidae was represented by one taxon, which was found in three unionid species examined (infections found in: one *Amblema plicata* collected at the *circa* 88 river mile area; one *Quadrula metanevra* collected at the *circa* 197 river mile area; and five *Quadrula pustulosa*, one collected at the *circa* 88 river mile area, one collected at the 168 river mile location, and three collected at the *circa* 197 river mile area)(see Table II).

Gorgoderid infections consisted of large numbers of larval stages (sporocysts). Sporocysts (Figure 6) were densely packed within the digestive gland, gonad, and viscera surrounding the foot of infected mussels. Identification of these parasites was based on the morphological characters exhibited by the rhopalocercous cercariae developing within each sporocyst (see Figure 6). These cercariae resembled those of *Cercaria filicauda*, a species described by Fishthal (1951) from study material collected from unionids in Illinois.

DISCUSSION

Nine of thirteen taxa collected in association with the 486 unionids examined in this study (four insect taxa, one oligochaete, one fingernail clam, one snail, one nematode, and one tartigrade) are common members of aquatic benthic communities. We consider these taxa to have been coincidentally associated with the sampled unionids and regard them as commensals or accidental associates that have no detrimental impact on unionid populations. Four taxa collected to date throughout this study (*Aspidogaster conchicola*, *Cotylogaster occidentalis*, *Unionicola* sp., and *Cercaria filicauda*) were found inside unionids, seemingly indicating more intimate relationships with these mussels. Each of these taxa are generally considered parasitic.

Aspidogaster conchicola was the only internal parasite collected from all eight of the unionid species we examined from Kentucky Lake. Recently, Duobinis-Gray *et al.* (1991) examined 219 mussels (ten species) from 17 locations in Kentucky Lake (12 locations in Kentucky and 5 locations in Tennessee). Nine of ten unionid species that they examined were infected with *A. conchicola*, and overall they reported a prevalence of 28.8 percent and an overall mean intensity of 2.5 *A. conchicola* per mussel (Duobinis-Gray *et al.*, 1991). Regarding the four unionid species shared by the present study and that of Duobinis-Gray *et al.* (1991), the latter authors reported a prevalence of 29.1 percent and a mean intensity of 1.9 for *A. conchicola* in *Amblema plicata*, a prevalence of 100 percent and a mean intensity of 1.0 in *Fusconaia ebena*, a 7.4 percent prevalence and a 5.2 mean intensity in *Megalonaias gigantea* (= *M. nervosa*), and a 45 percent prevalence and a 2.4 mean intensity in *Quadrula quadrula*. These prevalence and mean intensity values are all respectively equal to or lower than those reported herein for the same host taxa (see Tables III and IV).

In a similar examination of unionids conducted in West Virginia, Danford and Joy (1984) surveyed 500 unionids (22 species collected from a total of 32 localities) for aspidogastrid parasites and found seven species harboring *A. conchicola*. Overall, Danford and Joy (1984) reported a prevalence of 9.4 percent for this infection and they provided prevalence and intensity values for two of the unionid species we also report on herein. Regarding these two species, Danford and Joy (1984) reported a prevalence of 7 percent and a mean intensity of 4.0 for *A. conchicola* in *Amblema plicata*, and a prevalence of 2 percent and a mean intensity of 2.0 in *Quadrula pustulosa*. These values are lower than those we report herein for these two unionid species collected from Kentucky Lake (see Tables III and IV).

Aspidogaster conchicola is a common and widespread parasite of molluscs in North America that sometimes occurs at relatively high prevalence levels in wild populations (e.g., see Hendrix *et al.*, 1985). Unlike many parasites, *A. conchicola* shows little phylogenetic mediated host specificity within Mollusca (*i.e.*, it does not seem to be restricted to particular natural taxonomic groups of freshwater mussels or snails). Historically, *A. conchicola* has been collected from at least 85 unionid species, as well as from four species of gastropods (Hendrix *et al.*, 1985). These parasites live in the pericardial and renal cavities of their mollusc hosts (see Figure 5) where

it is thought that they consume blood cells and large particulate matter suspended in the hemolymph (Halton, 1972).

Pauley and Becker (1968) were the first to describe pathology induced by *Aspidogaster conchicola* in unionids. In examining heavy *A. conchicola* infections in the pericardial and renal cavities of *Anodonta californiensis* and *A. oregonensis* they observed distention and distortion of the renal cavities with associated renal metaplasia (*i.e.*, reduction of the columnar epithelium and loss of cilia). Fibrosis of the connective tissue underlying the damaged epithelial tissue was noted, but no evidence of hemocytic infiltration was observed in the damaged epithelium of these two unionid species. In *Gonidea angulata*, they reported no damage to the pericardial or renal tissues, but the parasite was found to be encapsulated by host tissues in areas outside the pericardial and renal cavities, including in the muscle and connective tissue of the foot, in hemolymph vessels, in digestive tubules, and in the intestinal lumen. Using various staining techniques they determined that the capsules lacked collagen and consisted of elongate fibroblasts, and that they were similar to capsules known from marine bivalve/parasite interactions (Cheng and Burton, 1965). Each capsule contained a living parasite, and often also contained host hemocytes. Pauley and Becker (1968) concluded that metaplasia in pericardial and renal tissues of the two *Anodonta* spp. was a chronic condition caused by heavy infections of *Aspidogaster conchicola*, while encapsulation was an acute response to this parasite when located in unusual sites within *Gonidea angulata*.

Bakker and Davids (1973) reported similar damage associated with *Aspidogaster conchicola* infection of the pericardial and renal epithelia of *Anodonta anatina*. They attributed the observed damage to the influence of the parasite's ventral sucker on the host epithelium rather than to the feeding activity of the parasite.

Huehner and Etges (1981) described the encapsulation of *Aspidogaster conchicola* in the pericardial and renal tissues of several unionid species from Ohio (*Anodonta marginata*, *A. grandis*, *Lampsilis siliquoidea*, *L. ventricosa*, *Lasmigona complanata*, and *Quadrula quadrula*). They found encapsulated parasites in regions of their unionid hosts outside the pericardial and renal cavities, especially in regions dorsal to the digestive gland and anterior to the pericardial chamber. The capsules contained host cells and either a single living worm, a moribund worm, or a mass of embryonated eggs. In two instances they observed three recently hatched juvenile

worms and empty egg shells within capsules. Huehner and Etges (1981) suggested that encapsulation by the host occurred most often when *A. conchicola* strays into areas outside of the pericardial or renal cavities, and that encapsulation is probably a contributing factor to the mortality of this parasite.

Based on limited histological evidence, Gentner (1971) speculated that *Aspidogaster conchicola* feeds on host blood cells. Huehner *et al.* (1989) suggested that the ventral disc of *A. conchicola* secretes digestive enzymes that allow it to feed on pericardial and renal epithelia of unionid hosts. The secretion of digestive enzymes from the ventral disc combined with mechanical feeding using a muscular bucal sucker might provide a mechanism to create the damage described by Pauley and Becker (1968) and Bakker and Davids (1973). Interestingly, Rohde (1975) and Rohde and Sandland (1973) provided evidence that another species of aspidogastrid feeds on host epithelial tissues.

Histological examination in this study provided no evidence supporting the notion that the ventral disc of *Aspidogaster conchicola* is secretory and destructive to epithelial tissue as proposed by Huehner *et al.* (1989). We also did not find any evidence of renal metaplasia or damage to the pericardial epithelium in any of the unionids that we histologically examined, nor did we find any encapsulated *A. conchicola* in these mussels (although one of us [SC] has observed this phenomenon in *Anodonta implicata* collected from northeastern North America).

The life cycle of *Aspidogaster conchicola* is not yet entirely understood. It is thought to be a one host life cycle which takes place within a molluscan host (see Figure 7). Adults worms live in the pericardial and renal cavities of their unionid hosts where they release embryonated operculated eggs which probably leave the pericardial/renal complex through the nephridiopore and get shunted to the external aquatic environment via the excurrent siphon. Bakker and Davids (1973) showed that these eggs do not hatch while in the aquatic environment, but instead they hatch only after entering a unionid. How the eggs infect the new unionid host and how the infection is manifested in the pericardial chamber of the host is as yet unclear. Bakker and Davids (1973) suggested that the eggs hatch on the demibranchs and the cotylocidia (larvae) then migrate through the nephridiopore to the kidney. These larvae then develop in the kidney and move to the pericardial chamber when they mature (Bakker and Davids, 1973). A second hatching possibility might be that the eggs require digestive enzymes for hatching to occur, and thus must be

swallowed and pass through the gut of the unionid host (Huehner and Etges, 1972; 1977; Rohde, 1975). The cotylocidium then migrates through the intestine, out the anus to the nephridiopore, into the suprabranchial chamber, then to the renal cavity, and finally into the pericardial chamber where it develops to adulthood.

A plausible but never demonstrated alternative sequence of events in which the eggs never leave the original host mussel has also been proposed (Williams, 1942). In this scenario, eggs released in the pericardial or renal cavities of a unionid hatch in place rather than exit the host. There they develop directly within the host along side their parent worms. This particular scheme provides an efficient mechanism for autoinfection that might ultimately result in large numbers of parasites within hosts.

Our data did not support the observation that juvenile worms mature in the kidney of the host and then move to the pericardial chamber as reported by Bakker and Davids (1973). We encountered only 21 juvenile worms to date throughout this study, and these young worms were found in both the kidneys and pericardial chambers of unionids. We also found many adult *Aspidogaster conchicola* in the kidneys of all eight examined species of unionids, and furthermore, in *Fusconaia ebena* adult *A. conchicola* were found to infect the kidney significantly more often than the pericardial chamber.

Given our uncertain understanding of the life cycle of *Aspidogaster conchicola*, its documented ability to cause disease (see Pauley and Becker, 1968; Bakker and Davids, 1973), and its typical high prevalence and sometimes high intensity in wild unionid populations as documented herein and in previous studies (e.g., Kelly, 1889; Duobinis-Gray *et al.*, 1991), we believe that *A. conchicola* has some potential to detrimentally impact the survivorship of captive mussel populations. Our view regarding this matter is founded on the premise that in closed or semi-closed aquaculture systems with high densities of potential hosts, transmission rates could become abnormally elevated and result in unnaturally high parasite burdens that might be associated with debilitating diseases. The wide host range of this parasite also should facilitate interspecific transmission in polyculture situations. Studies of the consequences of heavy *Aspidogaster conchicola* burdens regarding unionid survival should be encouraged. If results of these studies reveal this parasite to significantly affect mussel survival, research on the efficacy of various antihelminthics in controlling these platyhelminths might become worthwhile.

Throughout this study the aspidogastrid *Cotylogaster occidentalis* was found much less commonly than its relative *Aspidogaster conchicola*. *Cotylogaster occidentalis* is thought to complete its life cycle directly within a unionid host in a fashion similar to *A. conchicola*. Unlike *A. conchicola*, however, *C. occidentalis* is known to develop and live in the intestine of the freshwater drum, *Aplodinotus grunnions* (see Figure 8)(see Dickerman, 1948). Fish become infected when they feed on infected molluscs (see Dickerman, 1948). Despite this ability to exploit a vertebrate host which is relatively more vagile than a unionid, *C. occidentalis* typically occurs in much lower prevalence in unionid communities than does *A. conchicola* (e.g., see Table II). *Cotylogaster occidentalis* is also found in low prevalence in freshwater drum populations (see Simer, 1929; Dickerman, 1948). It is interesting to note that *C. occidentalis* and *A. conchicola* each seem to rely on a different mode of transmission (*i.e.*, the colonization of new hosts) to fulfill their life cycles. *Aspidogaster conchicola* relies on transmission via the liberation of an embryonated egg while *C. occidentalis* liberates a ciliated, free-swimming larva. These two transmission modes seem significantly different, however, studies are needed to determine how they might affect the realized prevalence levels of these two closely related species.

Larval gorgoderid infections (Gorgoderidae) indentified to date in this study have consisted of one species, *Cercaria filicauda*; one of nine gorgoderid rhopalocercous cercarial types known from freshwater bivalves in North America (see Fishthal, 1951; Flook and Ubelaker, 1971). Generic and specific designation for digeneans is based on adult characters. However, it is possible to assign a family identity to cercaria based on larval characteristics. When only larval stages of digeneans are found, (as in this study), researchers categorize unknown cercaria in the genus *Cercaria* and assign a species epithet based on either physical characteristics of the larvae or on host affiliations. *Cercaria filicauda* is a member of the subfamily Gorgoderinae and is characterized by having a long filament attached posteriorly to a corrugated transformable tail (see Figure 6). Without exception, members of the Gorgoderinae have two host life cycles (Figure 9) that utilize a unionid as the intermediate host and a fish or toad (depending on the particular gorgoderid species) as the definitive vertebrate host (Fishthal, 1951). Within the intermediate host, a single gorgoderin miracidium develops into a mother sporocyst, which in turn produces the second generation of daughter sporocysts asexually. It is within these second generation sporocysts that the rhopalocercous cercariae develop (see Figures 6 and 9). These

cercariae have no free-living existence, and they transform into metacercariae by encysting within the sporocysts. Sporocysts containing metacercariae exit the unionid host through the excurrent siphon and are eaten by the definitive vertebrate host. Metacercariae break free in the stomach of the vertebrate and migrate to the urinary bladder where they take up residence as adults (Figure 9).

Due to the low levels of prevalence reported here for gorgoderin infections, and because a definitive vertebrate host is required for these digenes to produce future parasite generations, we feel that these parasites could not significantly impact a cultured mussel population existing in a closed or semi-closed system without the presence of definitive hosts. However, it might be possible for very heavily infected mussels captured in the wild and transferred into captive systems to henceforth be compromised by these infections.

A unionicolid mite (*Unionicola* sp.) was another important parasite found infecting unionids from Kentucky Lake in this study. The prevalence and mean intensity values reported herein from six of the eight unionid species that were infected with *Unionicola* sp. are similar to those reported by Vidrine and Wilson (1991) for unionids collected from the Stone and Duck rivers in central Tennessee in 1956 and 1962. From the Stone River, they reported that 20 of the 29 species of unionids examined harbored *Unionicola* infections, with an overall prevalence of 58.2 percent. From the Duck River, they reported that 13 of 20 species of examined unionids were infected with *Unionicola*, with an overall prevalence of 59 percent. Fourteen *Unionicola* species were collected from mussels gathered in the Stone River and 11 species were associated with mussels taken from the Duck River. While we are not sure how to interpret these relatively high levels of unionicolid species richness as compared to our results in Kentucky Lake, it could be suggested that greater relative levels of habitat diversity within sampled sections of the Stone and Duck rivers might be responsible for the greater species richness reported by Vidrine and Wilson (1991).

Members of the genus *Unionicola* parasitize freshwater mussels worldwide (Cook, 1974). In revising the genus *Unionicola*, Vidrine (1986) presented descriptions of species found in North America. A typical unionicolid life cycle (see Figure 10) as described by Mitchell (1955), begins when the adult female oviposits between the gill filaments or on the mantle of a mussel using her highly modified genital spines. In one to three weeks, larvae hatch from the deposited eggs.

These larvae either leave the mussel through the excurrent siphon or they enter a dormant period and remain encysted for up to a year without further development. During spring, larvae free in the surrounding environment re-enter unionids and encyst in epithelial tissue of the mantle around the incurrent siphon or in the gills. Once encysted, this larva is known as a nymphochrysalis. An active, predaceous, sexually immature deutonymph emerges from the nymphochrysalis after several weeks and leaves the mussel for a temporary free-living existence. After feeding extensively in the water column on plankton, the deutonymph returns to a unionid host to complete its development into an adult. The deutonymph enters the mussel and encysts in the gill lamellae forming an imagochrysalis. The imagochrysalis persists for a period of a few days before the adult mite emerges from it. Soon after emergence, mites reach sexual maturity and mating occurs in the unionid host. Most unionicolid species spend the majority of their adult lives in the water column, and occupy unionids for shorter periods to mate and deposit eggs.

Different unionicolid species have been reported to cause different types of damage to their hosts, and in turn they illicit different pathological responses. Some unionicolids are reported to ingest host cells and thus cause feeding associated disease in unionids (e.g., see Baker, 1976, 1977). Baker (1976) showed that *U. intermedia* can attach to the gills of mussels using its pedipalps, and its tarsa can become deeply imbedded in the gill tissue of its host. This produces tissue damage resulting in leukocytic infiltration (*i.e.*, edema and inflammation of the area below the damaged epithelium by host blood cells). As mentioned above, additional damage to mussel hosts can be caused by female mites during oviposition (see also Mitchell, 1965). Macroscopic observations of dissected unionids from this study did not reveal any apparent damage caused by unionicolid mites on the gills. Histological examination of the gills of three species of unionids (*Quadrula quadrula*, *Q. metanevra*, and *Q. pustulosa*) revealed no leukocytic infiltration under gill tissue inhabited by adult mites. Our observations suggest that individual unionicolids may not always significantly harm their unionid hosts, and it is difficult for us to predict whether these mites could ever establish high enough densities within cultured mussel populations to cause reduced mussel survivorship.

SUMMARY

To date, examination of 500 unionid mussels representing eight species (*Amblema plicata*, *Elliptio cressidens*, *Fusconaia ebena*, *F. flava*, *Megalonaias nervosa*, *Quadrula metanevra*, *Q pustulosa*, *Q. quadrula*) collected from three general locations in Kentucky Lake (Tennessee River mile *circa* 88, TN river mile 168, TN river mile *circa* 197) between May 1994 and July 1996 revealed:

1) sampled mussels to be associated with six metazoan phyla (Platyhelminthes, Annelida, Nematoda, Mollusca, Tartigrada, Arthropoda), 12 families, and at least 13 genera and species.
2) approximately 90 percent of the collected symbionts belonged to parasitic taxa.
3) *Aspidogaster conchicola* was the most commonly collected parasitic flatworm – this parasite has been found to infect all mussel species examined.
4) parasitic mites, *Unionicola* sp., were commonly collected from five of the eight studied unionids.
5) *Aspidogaster conchicola* and *Unionicola* sp. were the only taxa in this study that achieved infection prevalence values greater than 10 percent.
6) no significant pathologies associated with parasitic infection have been identified to date in studied mussels.

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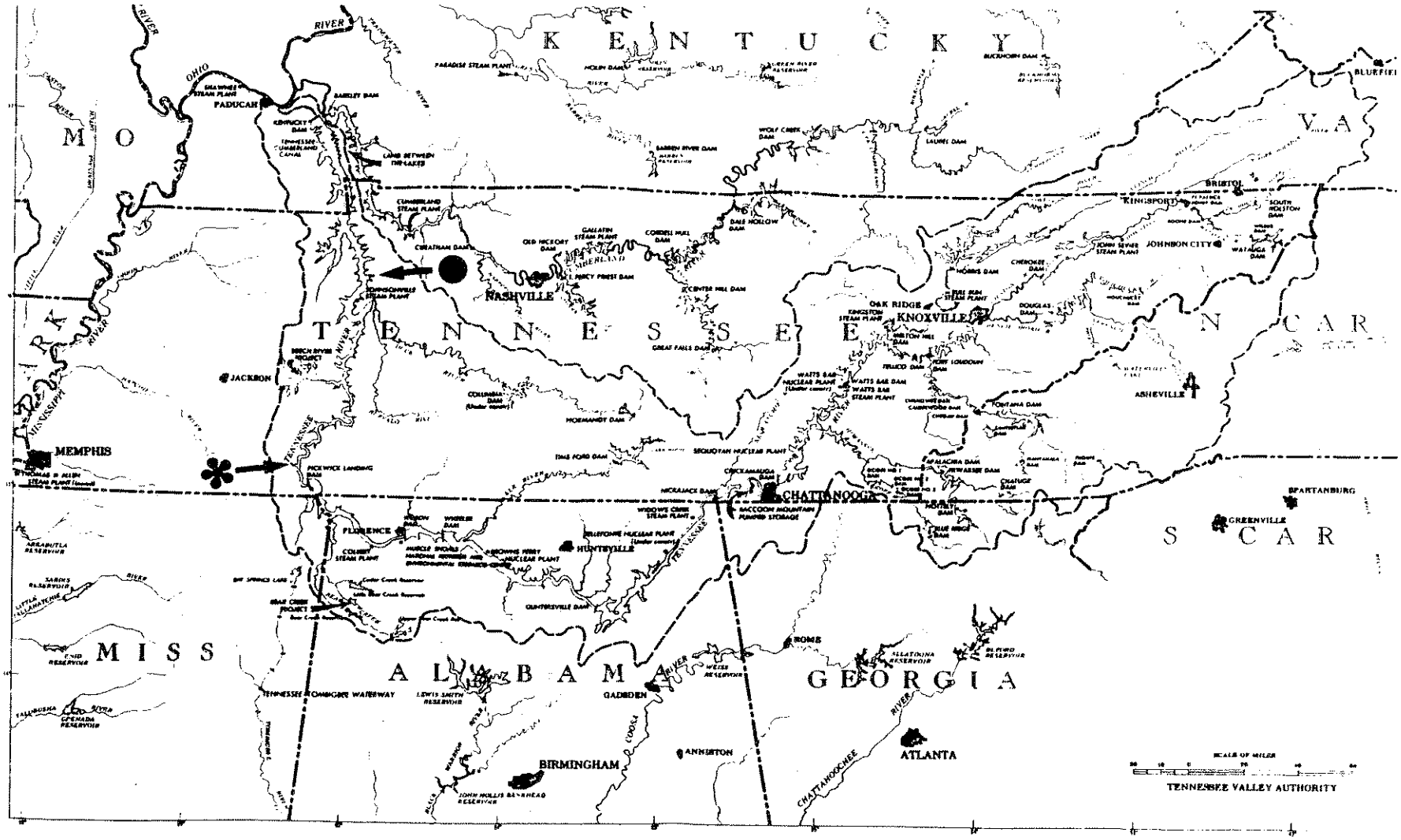

Figure 1. Map of Tennessee region showing the 110 mile reach of Kentucky Lake (between Tennessee River miles 87.4 [solid dot] and 197.6 [asterisk]) where mussels have been sampled for this study (TN river mile *circa* 88, river mile 168, and river mile *circa* 197).

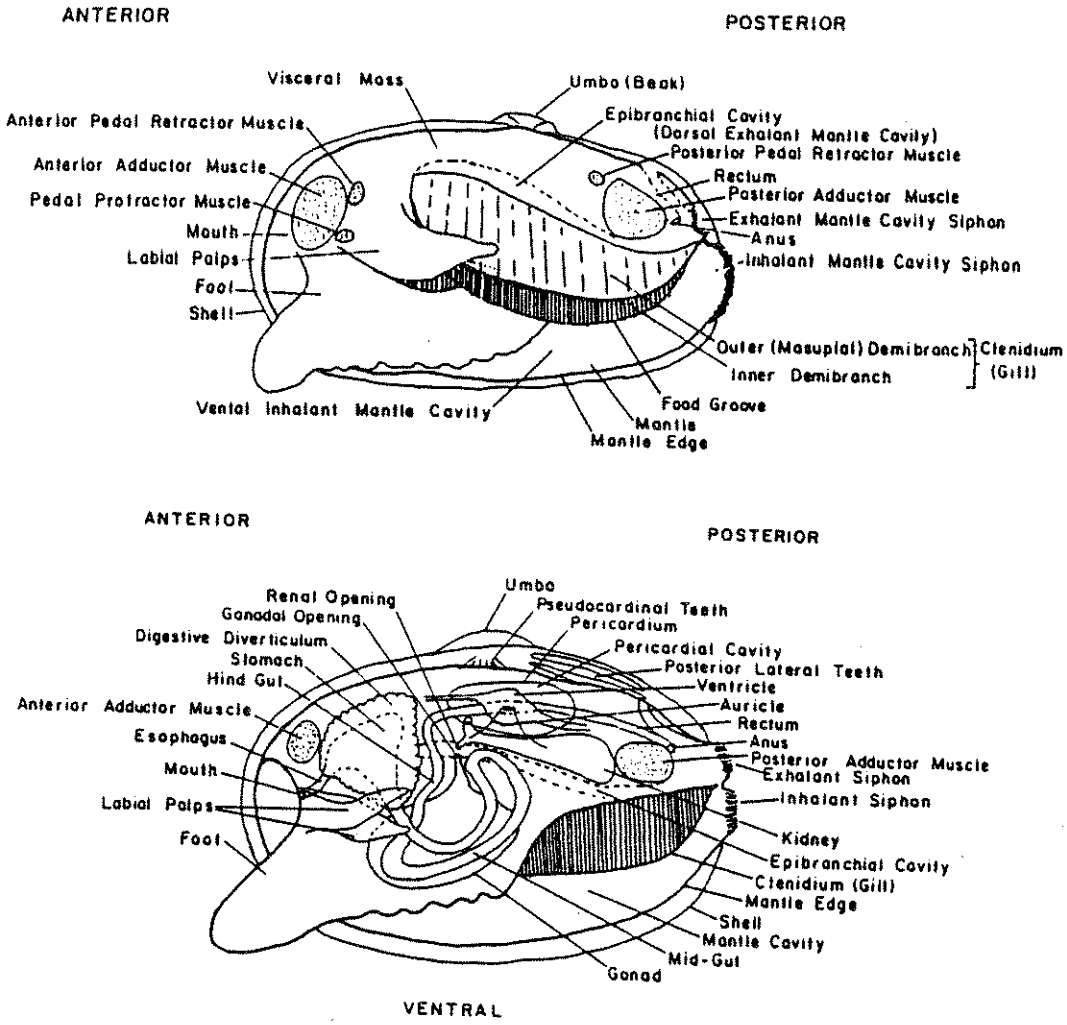

Figure 2. General external (top) and internal (bottom) anatomy of a unionid bivalve. Figure modified from McMahon, 1991.

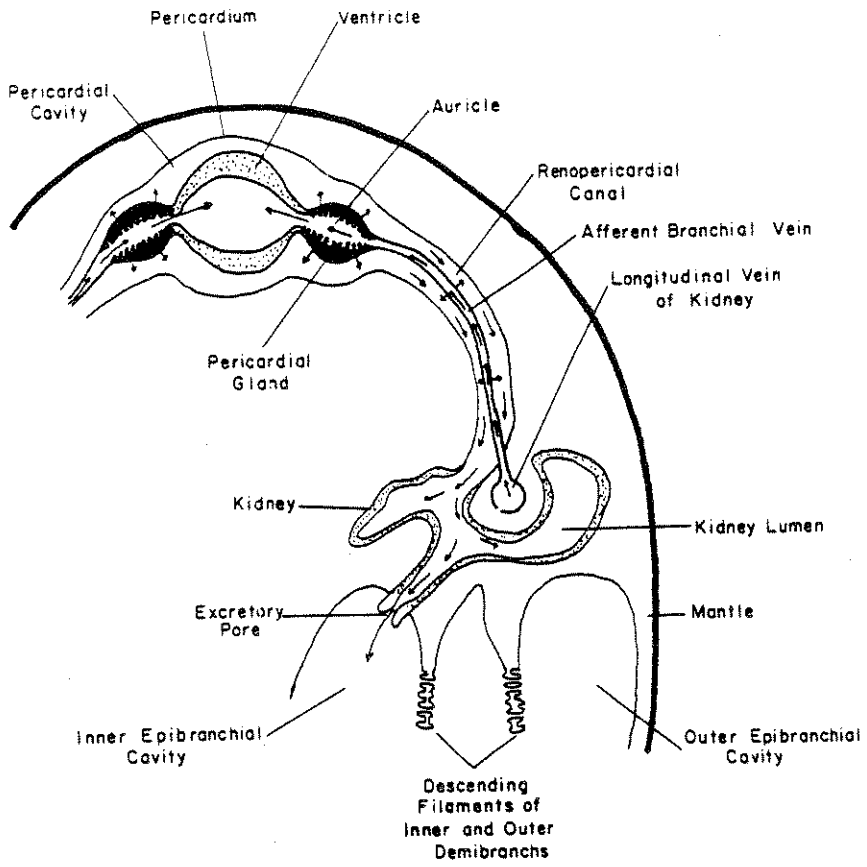

Figure 3. Diagram of anatomical features of the excretory system of a typical freshwater bivalve. Arrows indicate water excretion pathways. Figure modified from McMahon, 1991.

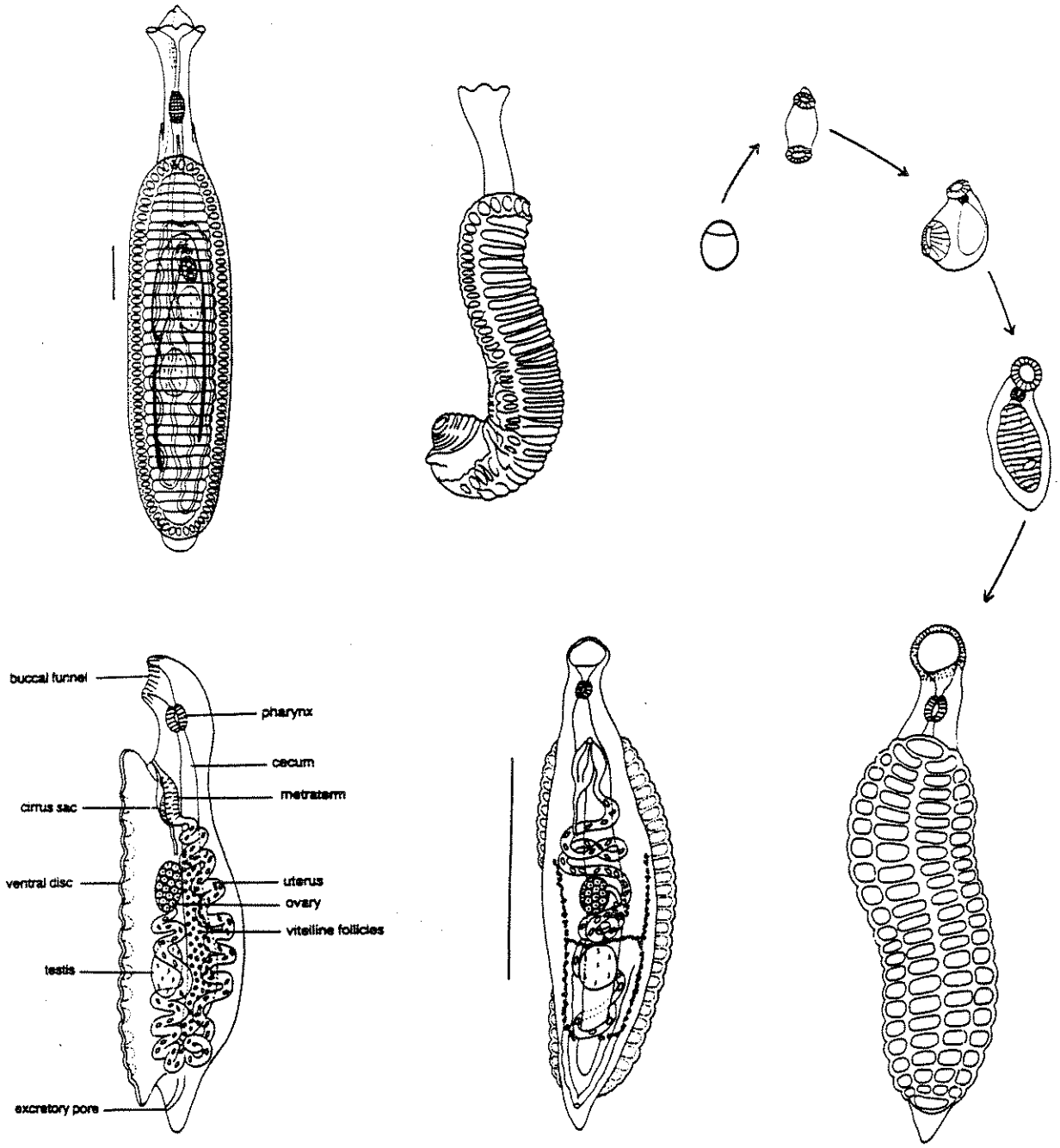

Figure 4. Aspidogastrids found in unionid mussels. Bottom, *Aspidogaster conchicola* (left to right); adult lateral view, adult dorsal view, adult ventral view. Top left and middle, *Cotylogaster occidentalis* adult ventral view (left), adult lateral view (middle). Top right, *A. conchicola* immature forms. Note four longitudinal rows of loculi in adult *A. conchicola* and three in adult *C. occidentalis*. All scale bars = 1.0 mm. Figure modified from Schell, 1985.

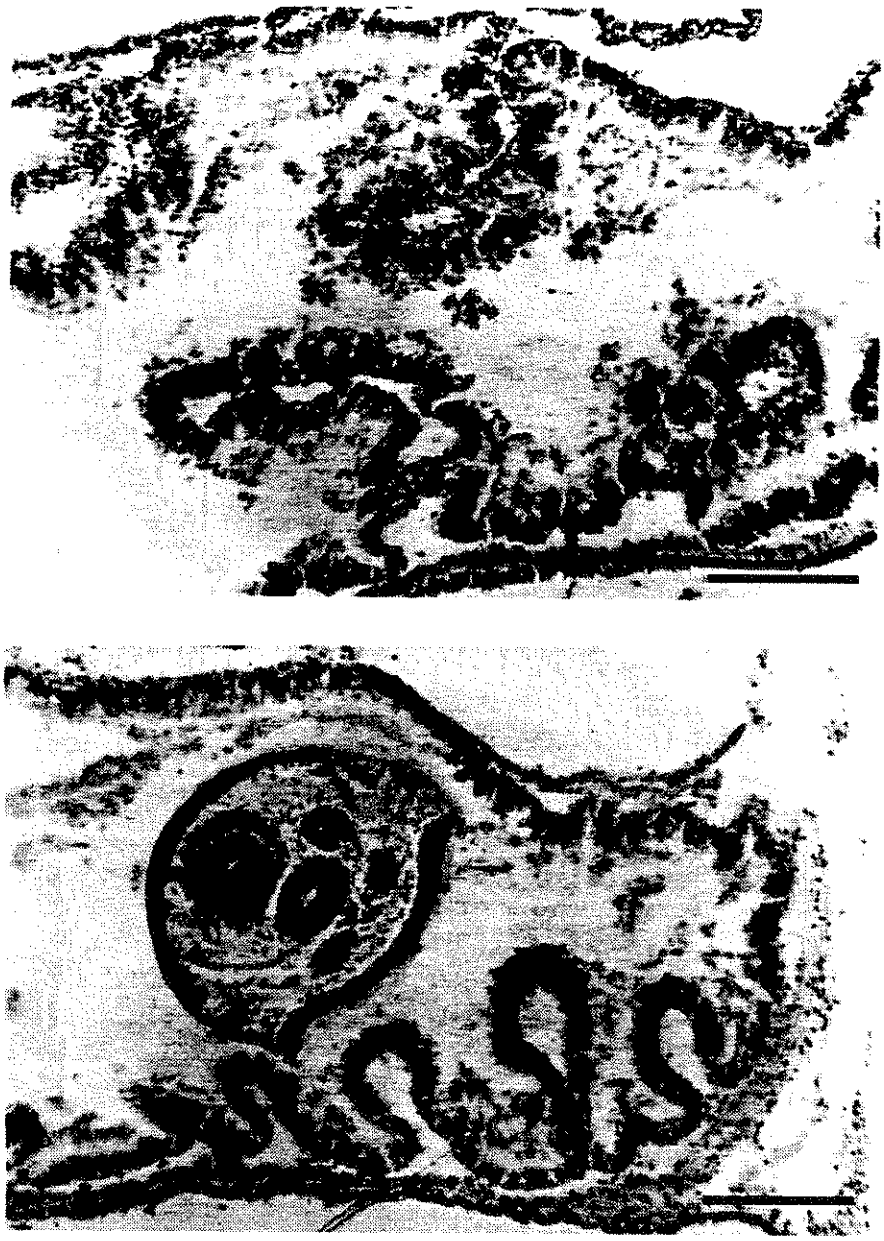

Figure 5. Kidney cross sections (12 μm thick) of *Quadrula pustulosa* stained with Gills hematoxylin and eosin. Top: uninfected kidney showing typical renal epithelium. Bottom: kidney infected with *Aspidogaster conchicola*. Note parasite in center of renal lumen and typical appearing renal epithelium. All scale bars = 550 μm.

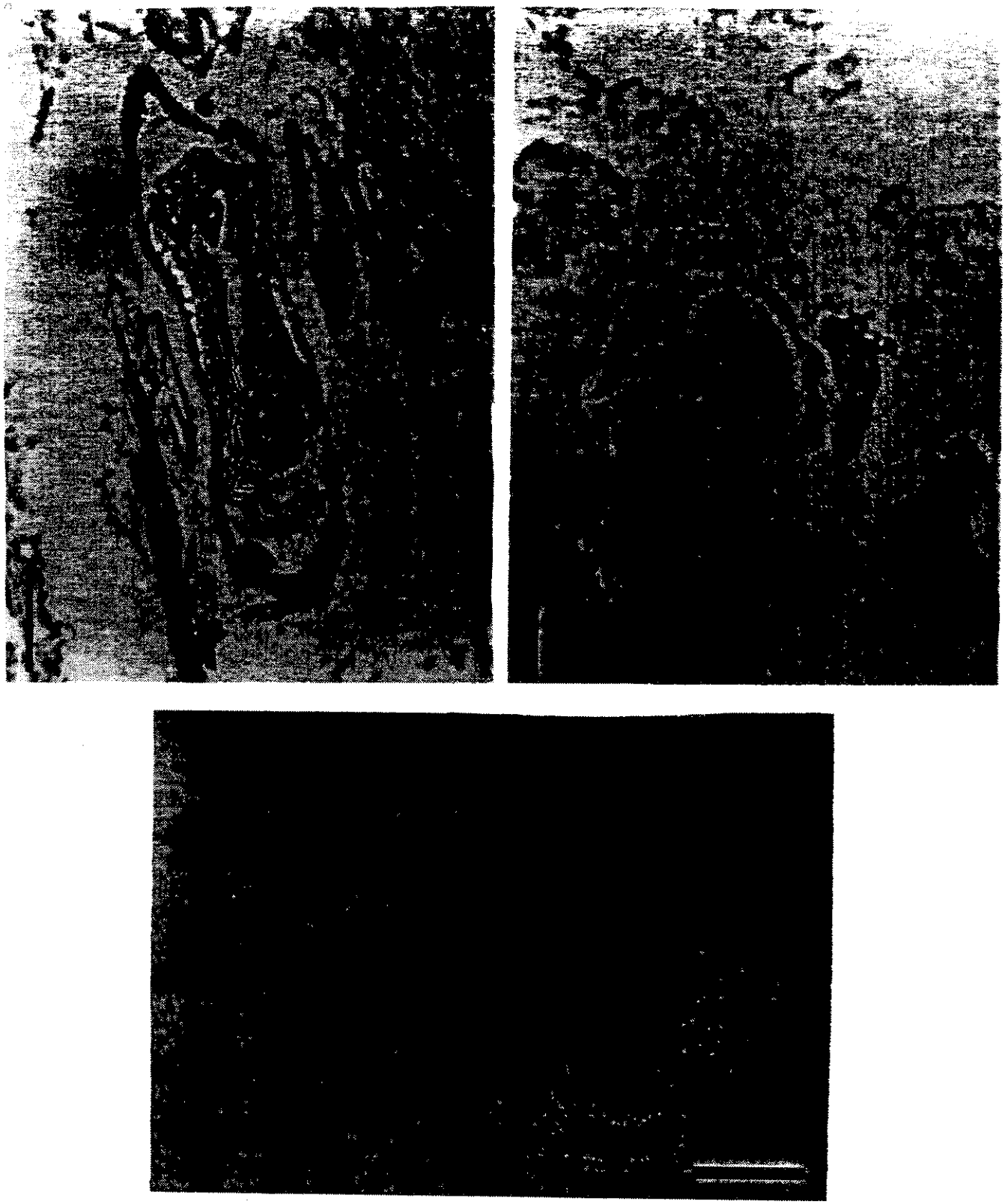

Figure 6. *Cercaria filicauda* (Trematoda: Gorgoderidae) from *Quadrula pustulosa*. Top left: longitudinal section (12 µm, H&E) of daughter sporocyst (containing one visible cercaria) embedded in visceral foot of mussel. Scale bar = 108 µm. Top right: cross section (12 µm, H&E) of daughter sporocyst (containing one visible cercaria) embedded in viscera of mussel. Scale bar = 200 µm. Bottom: cercaria removed from daughter sporocyst found in viscera. Scale bar = 133 µm. Note corrugated tail section of cercaria which is especially visible in longitudinal and cross sections of daughter sporocysts.

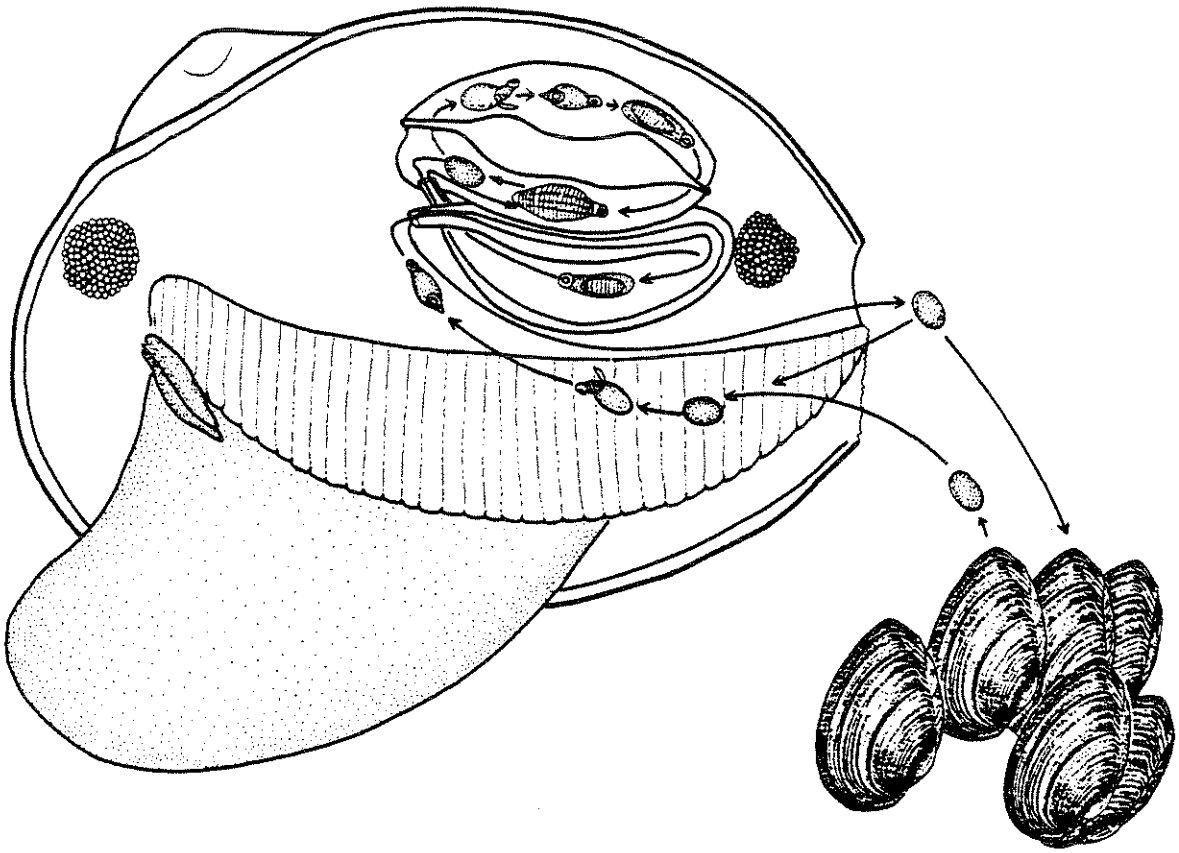

Figure 7. Possible life cycle of *Aspidogaster conchicola* (Trematoda: Aspidogasteridae). Adult worms live in the pericardial chamber or kidney of the unionid host. Operculate eggs containing embryos are shed by adult worms, and might follow one of three general developmental pathways. Via the first, eggs hatch and larvae develop into adults within the kidney or pericardial chamber. In this manner individual mussels might become infected with ever greater numbers of worms by a process known as autoinfection. Through the second possible pathway, eggs pass out of the kidney and into the suprabranchial chamber via the excretory pore. Subsequently, they are ejected into the external environment via the excurrent siphon. Liberated eggs become infective when they are inhaled by a mussel through the incurrent siphon. Inhaled eggs become trapped on the gills where they hatch, liberating larvae which move through the excretory pore into the kidney. Worms develop into mature adults in the kidney or pericardial chamber. Via the third possible life cycle pathway (not depicted in this illustration) an embryonated egg which has entered the branchial chamber is swallowed and passes through the gut of the unionid host. This stimulates the egg to hatch and the cotylocidium then migrates through the intestine, out the anus and into the excretory pore to ultimately mature in the kidney or pericardial chamber. It should be noted that none of these three pathways have been conclusively documented (see text for further details).

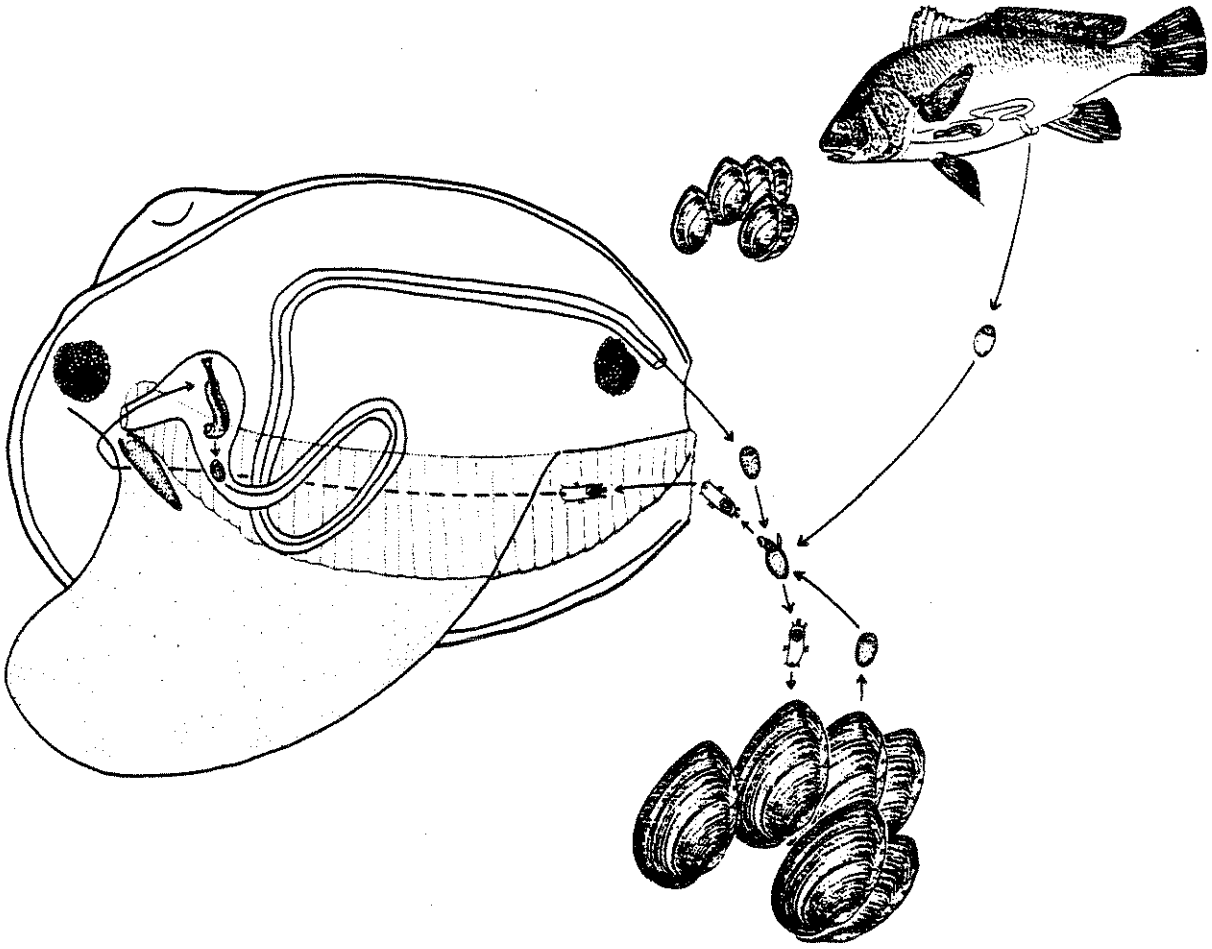

Figure 8. Life cycle of *Cotylogaster occidentalis* (Trematoda: Aspidogasteridae). Adult worms live in stomach or intestine of unionid host. Operculate eggs containing embryos are shed by adult worms and pass out of the mussel with feces via the anus. Free in the environment, the egg hatches and liberates a ciliated larva (cotylocidium) which actively seeks and infects a unionid host by entering the incurrent siphon. Larvae pass from the gills to the mouth, where they enter the digestive tract and mature. Freshwater drum presumably become infected with worms by eating infected molluscs. In the fish host, *C. occidentalis* matures in the intestine. Gravid worms have been collected from fish hosts (see Dickerman, 1948), and we assume that eggs passed from fish hosts will hatch to liberate larvae that can infect molluscs. Because eggs must develop in the external environment for several days before hatching, it is likely that autoinfection is not a highly developed component of this species' life cycle.

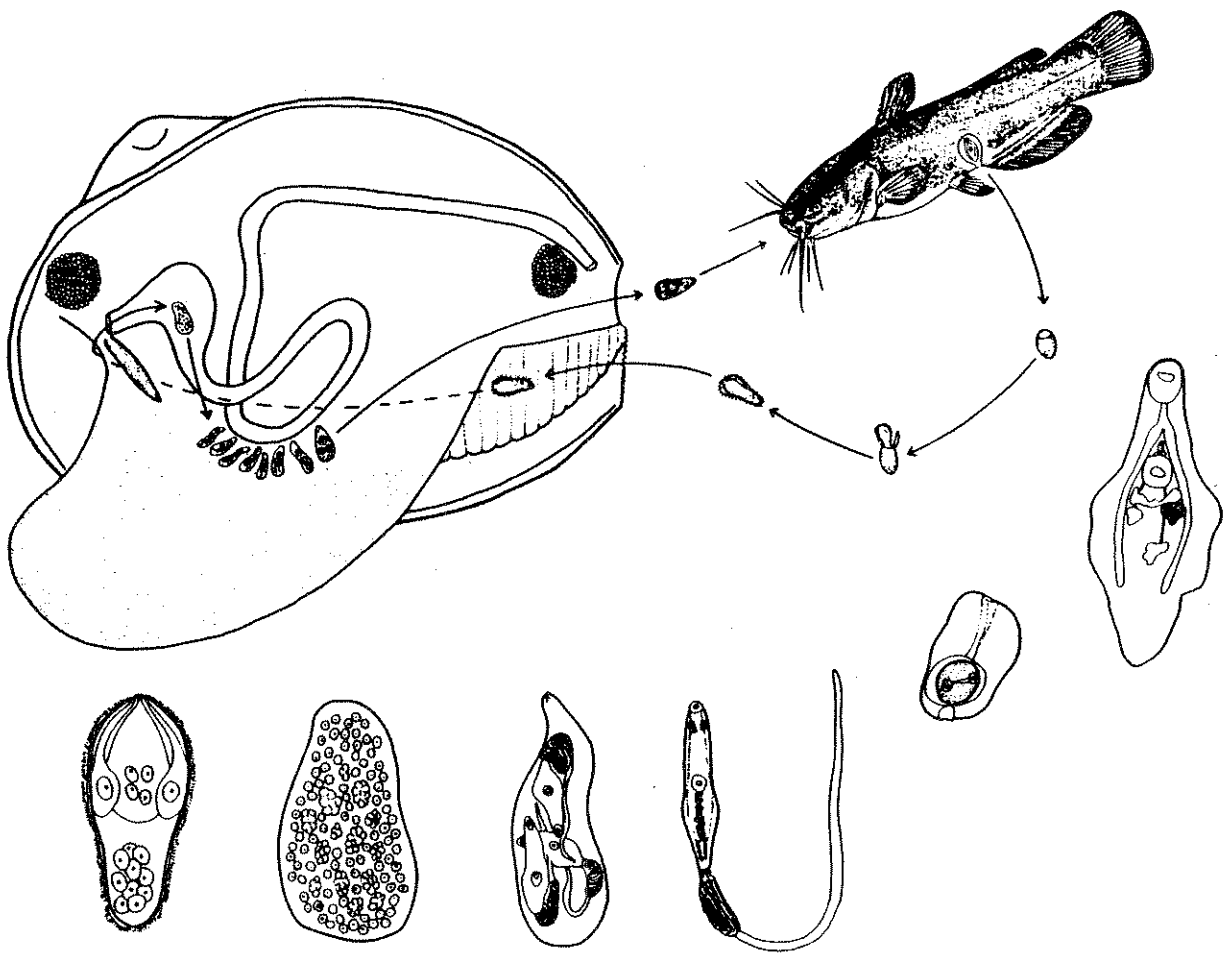

Figure 9. Generalized life cycle of species within Gorgoderinae (Trematoda: Digenea). Adult worms live in fish urinary bladder. Embryonated, operculate eggs are released into environment via urogenital opening. Free swimming miracidium hatches from egg and enters unionid via incurrent siphon. Miracidium travels into digestive system and develops into mother sporocyst. Mother sporocyst migrates to gonads/viscera and develops into many daughter sporocysts. Rhopalocercariae (typically 3-6) develop within daughter sporocyst. Cercariae transform into metacercariae within daughter sporocysts. Daughter sporocysts containing metacercariae migrate out of mussel and are eaten by fish host. Ingested parasites migrate to urinary bladder and develop into mature worms. Bottom illustrations depict various stages (not drawn to scale) in the aforementioned life cycle (left to right): miracidium, mother sporocyst, daughter sporocyst containing three cercariae, cercaria, metacercaria, adult worm. Bottom illustrations modified from Fischthal, 1951; Hoffman, 1967; and Schell, 1985.

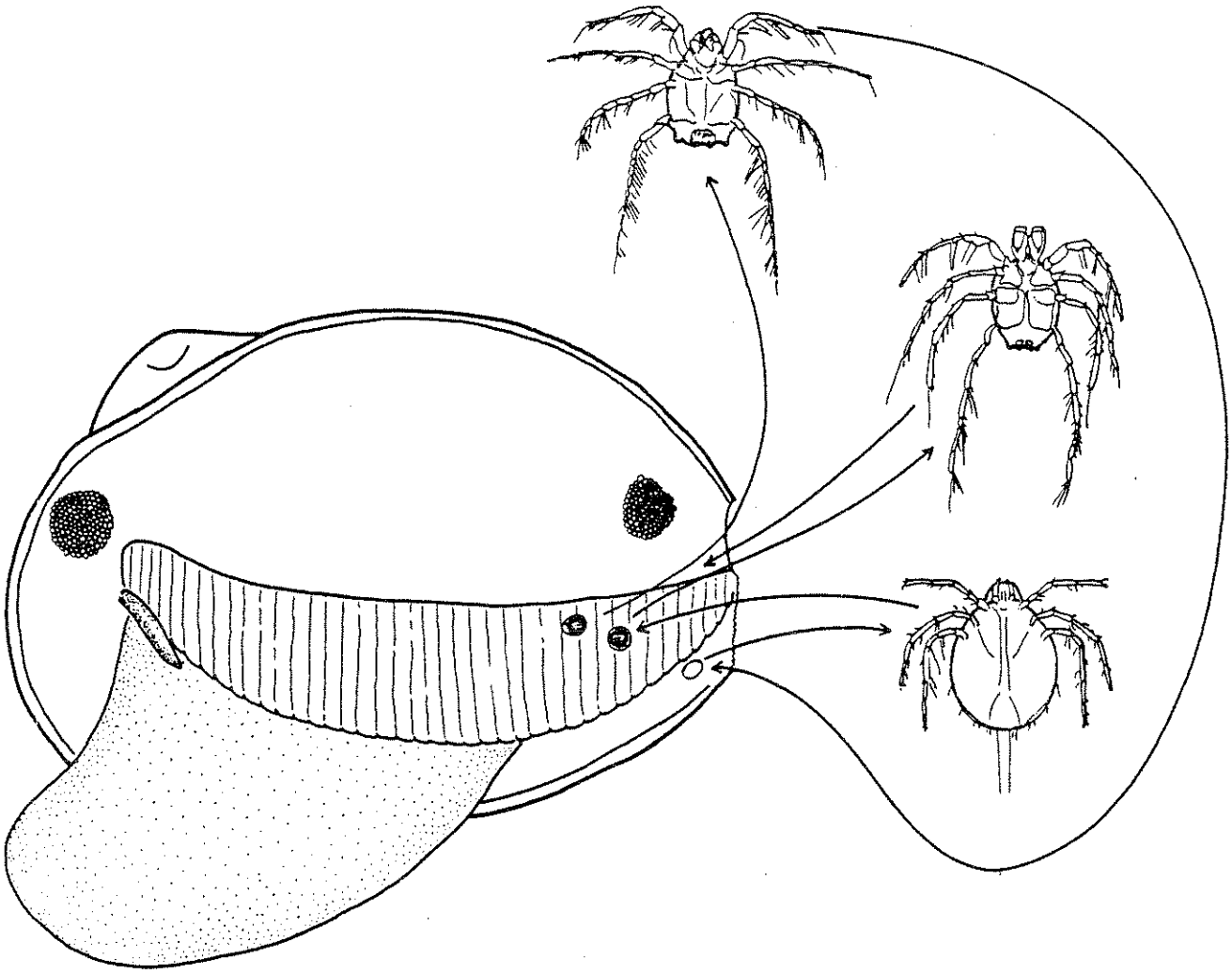

Figure 10. Generalized unionicolid (Acarina: Unionicolidae) life cycle. Adult female (top) enters unionid and deposits eggs in mantle near incurrent siphon or in the gill filaments. In several weeks eggs hatch and larvae (bottom right) leave mussel to live free in environment. During spring, larvae enter mussels and encyst in epithelial tissues of the gills or mantle. Encysted larvae are known as nymphochrysales. In several weeks a larva (deutonymph; middle right) hatches from the nymphochrysalis and leaves the mussel. After feeding in the water column on plankton, the deutonymph returns to a mussel and encysts in the gill tissues forming an imagochrysalis. In several days an adult emerges from the imagochrysalis. Soon after emerging as adults, mating occurs on the gills. Most unionicolid species spend the majority of their adult lives in the water column.

Table I. Numbers of unionids collected and examined for metazoan symbionts from three regions in Kentucky Lake (Tennessee).[1]

	Tennessee River Mile Locations										
	circa 88						168	*circa* 197			
	Date										
Unionid Species	05/04/94	05/23/94	02/17/95	06/05/95	12/14/95	07/09/96	04/19/95	07/26/94	12/06/94	12/14/95	TOTALS
Amblema plicata	-	13	4	18	-	-	-	-	-	1	36
Elliptio cressidens	-	-	-	-	-	-	16	-	-	-	16
Fusconaia ebena	10	14	20	20	49	24	24	14	19	21	215
Fusconaia flava	-	14	4	-	-	-	-	-	-	-	18
Megalonaias nervosa	-	-	-	15	-	-	20	-	-	-	35
Quadrula metanevra	-	-	-	-	-	-	-	14	18	7	39
Quadrula pustulosa	-	-	12	-	22	-	17	-	-	20	71
Quadrula quadrula	7	13	20	8	6	-	-	-	-	-	56
TOTALS	17	54	60	61	77	24	77	28	37	51	486

[1] Numbers in table do not include 14 mussels which were used in histopathology studies (see text for details).

Table II. Metazoan symbionts collected in association with eight species of mussels (Unionidae) sampled from three areas in Kentucky Lake (Tennessee River miles *circa* 88, 168, and *circa* 197) between May 1994 and July 1996. Asterisks denote trematode infections consisting of many daughter sporocysts and cercariae.

Unionid Species	Sample Site [1] (date)	Metazoan Symbiont	Location on/in Host	Number of Associates Collected
Amblema plicata	87.4 (06/05/95)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	25
		Unionicola sp.	gill filaments	10
	88.1 (02/17/95)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	11
		Unionicola sp.	gill filaments	8
		Dorylaimus sp.	external shell	2
		larval chironomid	external shell	1
		larval gorgoderid	viscera	1*
	89.0 (05/23/94)	*Unionicola* sp.	gill filaments	56
		Aspidogaster conchicola	pericardial cavity and lumen of kidney	24
		Dorylaimus sp.	external shell	13
		larval chironomid	external shell	3
		larval trichopteran	external shell	1
	197.6 (12/14/95)	*Unionicola* sp.	gill filaments	3
Fusconaia ebena	87.1 (12/14/95)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	103
	87.4 (06/05/95)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	25
	88.1 (05/04/94)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	12
		Dorylaimus sp.	external shell	7
		Unionicola sp.	gill filament	1
	88.1 (02/17/95)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	54
		larval chironomids	extenal shell	9
		Dorylaimus sp.	external shell	5
	88.1 (07/09/96)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	34
	89.0 (05/23/94)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	43
		Dorylaimus sp.	external shell	10
		larval ceratopogonid	external shell	1
		tartigrade	external shell	1
	168.0 (04/19/95)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	51
	197.6 (07/26/94)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	36
		Dorylaimus sp.	extenal shell	4
	197.6 (12/06/94)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	28
		oligochaetes	external shell	2
		Dorylaimus sp.	extenal shell	1
	197.6 (12/14/96)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	20

Table II. continued.

Unionid Species	Sample Site [1] (date)	Metazoan Symbiont	Location on/in Host	Number of Associates Collected
Fusconaia flava	88.1 (02/17/95)	*Dorylaimus* sp.	extenal shell	9
		Aspidogaster conchicola	pericardial cavity and lumen of kidney	3
		larval chironomid	extenal shell	1
	89.0 (05/23/94)	*Dorylaimus* sp.	collection bag wash and external shell	11
		larval chironomids	external shell	1
Megalonaias nervosa	87.4 (06/05/95)	*Unionicola* sp.	gill filaments	93
		Aspidogaster conchicola	pericardial cavity and lumen of kidney	5
	168.0 (04/19/95)	*Unionicola* sp.	gill filaments	169
Quadrula metanevra	197.6 (07/26/95)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	17
		Dorylaimus sp.	collection bag wash and external shell	5
		unionicolid water mites	gill filaments	4
	197.6 (12/04/94)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	13
		Unionicola sp.	gill filament	5
		Dorylaimus sp.	external shell	2
		larval chironomids	external shell	2
		larval gorgoderids	viscera	1*
		larval trichopteran	external shell	1
		larval ephemeropteran	external shell	1
		snail	incurrent siphon	1
		fingernail clam	incurrent siphon	1
	197.6 (12/14/96)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	5
		Unionicola sp.	gill filaments	3
Quadrula pustulosa	87.1 (12/14/96)	*Unionicola* sp.	gill filaments	89
		Aspidogaster conchicola	pericardial cavity and lumen of kidney	24
		Cotylogaster occidentalis	intestine	1
	88.1 (02/17/95)	*Unionicola* sp.	gill filaments	52
		Aspidogaster conchicola	pericardial cavity and lumen of kidney	28
		larval gorgoderids	viscera	1*
	168.0 (04/19/95)	*Unionicola* sp.	gill filaments	265
		Aspidogaster conchicola	pericardial cavity and lumen of kidney	11
		Cotylogaster occidentalis	intestine	1
		larval gorgoderids	viscera	1*
	197.6 (12/14/96)	*Unionicola* sp.	gill filaments	37
		Aspidogaster conchicola	pericardial cavity and lumen of kidney	18
		larval gorgoderids	viscera	3*
		Cotylogaster occidentalis	intestine	1

Table II. continued.

Unionid Species	Sample Site [1] (date)	Metazoan Symbiont	Location on/in Host	Number of Associates Collected
Quadrula quadrula	87.1 (12/14/96)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	15
		Unionicola sp.	gill filaments	14
	87.4 (06/05/95)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	27
		Unionicola sp.	gill filaments	24
	88.1 (05/04/95)	*Unionicola* sp.	gill filaments	21
		Aspidogaster conchicola	pericardial cavity and lumen of kidney	15
		Dorylaimus sp.	collection bag wash and external shell	10
		larval chironomids	external shell	1
		larval trichopterans	external shell	1
	88.1 (02/17/95)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	97
		Unionicola sp.	gill filaments	79
		Dorylaimus sp.	collection bag wash and external shell	6
	89.0 (05/04/94)	*Aspidogaster conchicola*	pericardial cavity and lumen of kidney	158
		Dorylaimus sp.	collection bag wash and external shell	38
		Unionicola sp.	gill filaments	37
		unionicolid larvae	external shell and internal wash	4
		larval chironomids	external shell	3
	197.6 (12/14/96)	*Unionicola* sp.	gill filaments	4
		Aspidogaster conchicola	pericardial cavity and lumen of kidney	2

[1] Tennessee River mile location.

Table III. Prevalence of infection (P) for *Aspidogaster conchicola* from eight species of unionids collected from three areas in Kentucky Lake, Tennessee. N = number of unionids examined.

	Tennessee River Mile Locations																			
	circa 88												168		*circa* 197					
	Date																			
	05/04/94		05/23/94		02/17/95		06/05/95		12/14/95		07/09/96		04/19/95		07/26/94		12/06/94		12/14/95		OVERALL	
Unionid Species	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P
Amblema plicata	-		13	77%	4	25%	18	61%	-		-		-		-		-		1	0%	36	61%
Elliptio cressidens	-		-		-		-		-		-		16	35%	-		-		-		16	35%
Fusconaia ebena	10	70%	14	79%	20	85%	20	75%	49	63%	24	79%	24	60%	14	64%	19	53%	21	67%	215	68.8%
Fusconaia flava	-		14	0%	4	80%	-		-		-		-		-		-		-		18	16.7%
Megalonaias nervosa	-		-		-		15	33%	-		-		20	0%	-		-		-		35	14.3%
Quadrula metanevra	-		-		-		-		-		-		-		14	64%	18	53%	7	71%	39	64.1%
Quadrula pustulosa	-		-		12	83%	-		22	59%	-		17	47%	-		-		20	50%	71	59.2%
Quadrula quadrula	7	57%	13	100%	20	65%	8	75%	6	50%	-		-		-		-		2	50%	56	73.2%

Table IV. Mean intensity, standard error, and range of infection for *Aspidogaster conchicola* from eight species of unionids collected from three areas in Kentucky Lake (Tennessee River) Tennessee. N = number of unionids examined.

	Tennessee River Mile Locations										
	circa 88						168	*circa* 197			
	Date										
	05/04/94	05/23/94	02/17/95	06/05/95	12/14/95	07/09/96	04/19/95	07/26/94	12/06/94	12/14/95	OVERALL
Unionid Species	N I[1]	N I	N I	N I	N I	N I	N I	N I	N I	N I	N I
Amblema plicata	-	13 2.4±8.4 (1-4)	4 4.0±0.0 (4)	18 2.3±1.6 (1-5)	-	-	-	-	-	1 0	36 2.7±1.8 (1-7)
Elliptio cressidens	-	-	-	-	-	-	16 1.7±0.8 (1-3)	-	-	-	16 1.7±0.8 (1-3)
Fusconaia ebena	10 1.7±0.9 (1-3)	14 3.9 ±2.5 (2-8)	20 3.2±2.0 (1-8)	20 1.8±1.1 (1-5)	49 3.4±3.7 (1-15)	24 1.8±1.1 (1-5)	24 3.4±3.1 (1-14)	14 3.7±2.7 (1-10)	19 2.8±2.4 (1-7)	21 1.4±0.6 (1-3)	215 2.7±2.5 (1-15)
Fusconaia flava	-	14 0	4 2.3±2.5 (1-8)	-	-	-	-	-	-	-	18 1.0±0.0 (1)
Megalonaias nervosa	-	-	-	15 1.0±0.0 (1)	-	-	20 0	-	-	-	35 1.0±0.0 (1)
Quadrula metanevra	-	-	-	-	-	-	-	14 1.9±1.5 (1-5)	18 1.2±0.4 (1-2)	7 1.0±0.0 (1)	39 1.4±1.0 (1-5)
Quadrula pustulosa	-	-	12 2.8±2.2 (1-8)	-	22 1.9±1.3 (1-5)	-	17 1.4±1.1 (1-4)	-	-	20 1.6±0.9 (1-4)	71 2.0±1.5 (1-8)
Quadrula quadrula	7 13±10.5 (1-40)	13 12±10.5 (1-40)	20 7.5±8.1 (1-32)	8 4.5±1.9 (2-7)	6 5.0±5.3 (1-11)	-	-	-	-	2 2.0±0.0 (2)	56 7.8±8.1 (1-40)

[1] I = mean intensity of infection ± SD, range of intensity of infection in parentheses.

Table V. Prevalence of infection (P) for *Unionicola* sp. from eight species of unionids collected from three areas in Kentucky Lake, Tennessee. Adult and nymph mites are grouped together. N = number of unionids examined.

	Tennessee River Mile Locations																			
	circa 88												168		*circa* 197					
	Date																			
	05/04/94		05/23/94		02/17/95		06/05/95		12/14/95		07/09/96		04/19/95		07/26/94		12/06/94		12/14/95		OVERALL	
Unionid Species	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P
Amblema plicata	-		13	92%	4	100%	18	28%	-		-		-		-		-		1	100%	36	61.0%
Elliptio cressidens	-		-		-		-		-		-		16	0%	-		-		-		16	0.0%
Fusconaia ebena	10	1%	14	0%	20	0%	20	0%	49	0%	24	0%	24	0%	14	0%	19	0%	21	0%	215	0.47%
Fusconaia flava	-		14	0%	4	0%	-		-		-		-		-		-		-		18	0.0%
Megalonaias nervosa	-		-		-		15	73%	-		-		20	95%	-		-		-		35	88.6%
Quadrula metanevra	-		-		-		-		-		-		-		14	29%	18	21%	7	43%	39	28.2%
Quadrula pustulosa	-		-		12	100%	-		22	100%	-		17	100%	-		-		20	70%	71	92.9%
Quadrula quadrula	7	71%	13	100%	20	95%	8	88%	6	100%	-		-		-		-		2	100%	56	94.6%

Table VI. Mean intensity, standard error, and range of infection for *Unionicola* sp. from eight species of unionids collected from three areas in Kentucky Lake, Tennessee. Adult and nymph mites are grouped together. N = number of unionids examined.

	Tennessee River Mile Locations																	
	circa 88										168		*circa* 197					
	Date																	
	05/04/94		05/23/94		02/17/95		06/05/95		12/14/95		07/09/96		04/19/95		07/26/94		12/06/94		12/14/95		OVERALL	
Unionid Species	N	I[1]	N	I	N	I	N	I	N	I	N	I	N	I	N	I	N	I	N	I	N	I
Amblema plicata	-		13	4.7±2.1 (2-8)	4	2.0±0.8 (1-3)	18	2.0±1.2 (1-4)	-		-		-		-		-		1	0	36	3.5±2.1 (1-8)
Elliptio cressidens	-		-		-		-		-		-		16	0	-		-		-		16	0
Fusconaia ebena	10	1.0±0.0 (1)	14	0	20	0	20	0	49	0	24	0	24	0	14	0	19	0	21	0	215	0.0±0.0 (1)
Fusconaia flava	-		14	0	4	0	-		-		-		-		-		-		-		18	0
Megalonaias nervosa	-		-		-		15	8.4±4.2 (4-18)	-		-		20	8.7±9.5 (2-46)	-		-		-		35	8.4±7.8 (2-46)
Quadrula metanevra	-		-		-		-		-		-		-		14	1.0±0.0 (1)	18	1.3±0.5 (1-2)	7	1.0±0.0 (1)	39	1.1±0.3 (1-2)
Quadrula pustulosa	-		-		12	4.0±1.7 (3-7)	-		22	3.7±3.9 (1-20)	-		17	16.6±13.8 (2-40)	-		-		20	2.6±1.6 (1-5)	71	6.6±9.1 (1-40)
Quadrula quadrula	7	4.2±4.4 (2-12)	13	2.8±1.4 (1-5)	20	4.0±1.7 (2-7)	8	3.4±0.5 (3-4)	6	2.3±1.0 (1-4)	-		-		-		-		2	2.0±0.0 (2)	56	3.3±1.8 (1-12)

[1] I = mean intensity of infection ± 1SD, range of intensity of infection in parentheses.

Table VII. Chi-square comparison of observed frequency distribution (occurrence) and expected frequency distribution of two symbiont taxa (*Aspidogaster conchicola* and *Unionicola* sp.) in eight species of unionids.

Mussel Species: Symbiont Species Assemblage	Observed Frequency (number of mussels inhabited)	Expected Frequency (number of mussels inhabited)	χ^2
Amblema plicata[1]			
(n=36)			
None	6	5.4756	0.05022196
Worm Only	8	8.5644	0.03719436
Mite Only	9	8.5644	0.02215536
Worm and Mite	15	13.3956	0.19216006
Elliptio cressidens[2]			
(n=16)			
None	12	10	0.4
Worm Only	6	6	0.0
Mite Only	0	0	n/a
Worm and Mite	0	0	n/a
Fusconaia ebena[3]			
(n=215)			
None	68	63.92724	0.25947271
Worm Only	148	140.96776	0.35080645
Mite Only	0	3.15276	3.15276
Worm and Mite	1	6.95224	5.09607853
Fusconaia flava[4]			
(n=18)			
None	15	14.994	n/a
Worm Only	3	n/a	n/a
Mite Only	0	n/a	n/a
Worm and Mite	0	n/a	n/a
Megalonaias nervosa[5]			
(n=35)			
None	2	3.41943	0.58921561
Worm Only	2	0.57057	3.58110333
Mite Only	28	26.57557	0.07634835
Worm and Mite	3	4.43443	0.46400314

Table VII. continued.

Mussel Species: Symbiont Species Assemblage	Observed Frequency (number of mussels inhabited)	Expected Frequency (number of mussels inhabited)	χ^2
Quadrula metanevra[6] (n=39)			
None	10	10.0303164	9.1631E-05
Worm Only	19	17.9716836	0.05883893
Mite Only	4	3.9394836	0.00092962
Worm and Mite	7	7.0585164	0.00048511
Quadrula pustulosa[7] (n=71)			
None	1	2.056728	0.54293716
Worm Only	5	2.984272	1.36152448
Mite Only	29	26.911272	0.16211737
Worm and Mite	37	39.047728	0.10738627
Quadrula quadrula[8] (n=56)			
None	1	0.795424	0.05261513
Worm Only	1	2.172576	0.63285909
Mite Only	15	14.212576	0.04362591
Worm and Mite	38	38.819424	0.01729690

[1] $\chi^2_{Total} = 0.30173174$; $\chi^2_{critical} = 7.815$ (df = 3, $\alpha = 0.05$).

[2] χ^2_{Total} = n/a; $\chi^2_{critical}$ = n/a (df = 3, $\alpha = 0.05$).

[3] $\chi^2_{Total} = 8.85911769$; $\chi^2_{critical} = 7.815$ (df = 3, $\alpha = 0.05$).

[4] χ^2_{Total} = n/a; $\chi^2_{critical}$ = n/a (df = 3, $\alpha = 0.05$).

[5] $\chi^2_{Total} = 4.71067042$; $\chi^2_{critical} = 7.815$ (df = 3, $\alpha = 0.05$).

[6] $\chi^2_{Total} = 0.06034529$; $\chi^2_{critical} = 7.815$ (df = 3, $\alpha = 0.05$).

[7] $\chi^2_{Total} = 2.17396528$; $\chi^2_{critical} = 7.815$ (df = 3, $\alpha = 0.05$).

[8] $\chi^2_{Total} = 0.74639704$; $\chi^2_{critical} = 7.815$ (df = 3, $\alpha = 0.05$).