THE LIFE HISTORY OF *Phyllodistomum lohrenzi* (Loewen, 1935) (TREMATODA: GORGODERINAE)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Pure science is the foundation on which is based most, if not all, scientific investigations and progress; this is true in all fields of endeavor investigated scientifically. The writer undertook the problem of determining the life cycle of Phyllodistomum lohrenzi with a full realization of the value of pure science. His main desire was to gain knowledge of the intimate details of a trematode life cycle and the ecological relationships between a parasite, host, and the fauna of the area.

Digenetic trematodes are endoparasites of vertebrates. The adult stage or marita may be found in almost any organ, but it is in the majority of cases an inhabitant of the digestive tract. The life cycle, as the name implies, involves two or more hosts which harbor the asexual or sexual stages. The first intermediate host harbors the asexual stages which vary in complexity and number; the cercaria is, usually, the final stage that develops in this host. The second intermediate host, when it occurs in a cycle, is infected by the cercaria which transforms into a metacercaria, and the host may be almost any animal. In many cycles the second intermediate host acts as a passive transfer agent, and vegetation may be involved. The definitive host is usually a fresh water, marine, or terrestrial vertebrate which harbors the sexual or marita stage of the fluke. Life cycles within the Digenea differ mainly in the methods of infection of the hosts and in the number of intermediate hosts, commonly one or two in number but never more than three.

Thomas described the first life cycle of a digenetic trematode in 1883, that of the sheep liver fluke. Since that time, research on trematode life cycles has constituted an important branch of parasitology.

Species in the subfamily Gorgoderinae are parasites of the kidneys, ureters, and/or urinary bladder of fishes, amphibians, and reptiles. The most studied genera are Gorgodera, Gorgoderina, and Phyllodistomum. Only five cycles in the subfamily have been investigated on an experimental basis. In all of these, the first intermediate host is a fingernail clam of the family Sphaeriidae. With regard to the second intermediate host situation, we find what may be called a partial recapitulation of gorgoderid development. One individual may serve as both first and second intermediate hosts. In this type of gorgoderid cycle, there is a suppression of the free cercarial stage, and the cercaria encysts within the intermediate host. In the alternate type of gorgoderid cycle, the cercaria is freed from the first intermediate host with subsequent encystment in another host.

About 45 species have been assigned to the genus Phyllodistomum. More than half of them are American species and the others are scattered throughout the world. Many of the species have fallen as synonyms, and others still standing will, in all probability, fall when a complete review of the genus is made. A critical taxonomic study, however, will be of more value when more is known about the life cycles in this group of flukes.

A survey of Boomer Creek, in Stillwater, Oklahoma for its cercarial fauna, revealed infections in the bivalve molluscs, Musculium transversum and M. elevatum. The green sunfish, Lepomis cyanellus, and the black bullhead, Ameiurus m. melas, from this creek were infected with the

bladder flukes Phyllodistomum lohrenzi and P. caudatum, respectively. With these observations as a background and because only one other cycle in this genus was known, a series of experiments were undertaken to establish the life cycle of one or both of these flukes.

The writer felt that a problem involving a trematode life cycle would acquaint him with the morphology, taxonomy, experimental procedures, and biological relationships of stages in the cycle and fauna of the area. A comprehensive investigation of this nature was considered to be necessary as an introduction to parasitological life cycles on a research basis. Completion of this research would serve as a basis for future study.

The objectives of this problem were:

1. To describe the life cycle of Phyllodistomum lohrenzi and/or P. caudatum.

2. To prove the cycle experimentally in the laboratory.

3. To describe and illustrate the stages of the cycle.

4. To obtain data on the behavior and host-parasite relations of stages in the cycle.

5. To determine the effect of the tremetode on the host and note any pathology.

6. To determine the incidence of infection.

TAXONOMY OF THE GENUS *Phyllodistomum*

Adult digenetic trematodes of the family Gorgoderidae, subfamily Gorgoderinae are commonly called bladder flukes because of their preference for the urinary bladder of cold-blooded vertebrates as a habitat. This popular common name, however, is subject to the inaccuracies of most common names in that these flukes are not limited to the urinary bladder. Olsson (1876), according to Goodchild (1950), described *Distoma conostomum* from the esophagus and gills of *Coregonus maraena*. Nybelin (1926) stated that he always found this trematode in the urinary bladder, and attributed the abnormal habitat reported by Olsson as being due to post-mortem migration. Van Cleave and Mueller (1934) reported *Phyllodistomum superbum* from the gut of fishes. Sinitsin (1901, 1905), Nybelin (1926), Lutz (1926), Joyeux and Baer (1934), Odlaug (1937), Rankin (1939), Choquette (1947), and Goodchild (1945, 1948) observed that "bladder flukes" are not confined to the urinary bladder of fishes and amphibians but are found also in the mesonephric ducts and kidneys. Goodchild (1950) reported an infection of juvenile *Gorgoderina* sp. from the cloaca, urinary bladder, Wolffian ducts, and mesonephric tissue of a tadpole.

Gorgoderid trematodes have been reported from many fishes and amphibians. Hughes, Higginbotham, and Clary (1942), however, were the first to find a bladder fluke in a reptile and reported *Phyllodistomum cymbiforme* from the turtles, *Caretta caretta* and *Chelonia mydas*, from Egypt, Florida, and Italy.

Looss (1899) established the subfamily Gorgoderinae for the genera Gorgodera Looss, 1899 and Phyllodistomum Braun, 1899. He later (1902) erected the family Gorgoderidae for the subfamilies Gorgoderinae Looss, 1899 and Anaporrhutinae Looss, 1902.

The subfamily Gorgoderinae Looss, 1899 contains six genera: Gorgodera Looss, 1899; Phyllodistomum Braun, 1899; Gorgoderina Looss, 1902; Catoptroides Odhner, 1902; Xystretum Linton, 1910; and Macia Travassos, 1922. The subfamily Anaporrhutinae Looss, 1902 contains five genera: Anaporrhutum Ofenheim, 1900; Probolitrema Looss, 1901; Plesiochorus Looss, 1901; Petalodistomum Johnston, 1912; and Staphylorchis Travassos, 1920.

According to Nybelin (1926) the first report of a bladder fluke was that of Fabricius (1780) who described Fasciola umblae from the kidney of Salmo alpinus; Rudolphi (1819) renamed it Distoma seriale. The bladder fluke of the frog was first reported in Europe by Loschge (1785) and was named Distomum cygnoides by Zeder (1800). Leidy (1851) was the first to observe bladder flukes in American frogs. The first detailed description of American bladder flukes was given by Bensley (1897) for two varieties of Distomum cygnoides from Canada, listed as Varieties A and B.

Braun (1899) erected the genus Phyllodistomum for D. cygnoides Zeder, 1800; D. cymbiforme Rudolphi, 1819; D. patellare Sturges, 1897; D. conostomum Olsson, 1876; and designated Distomum folium Olfers, 1816 as the type species. These five species included all the trematodes known at that time to occur in the urinary bladder of fishes and amphibians. At present there are about 45 seemingly valid species described or assigned to genus Phyllodistomum.

Looss (1899) established the genus Spathidium with Distomum folium Olfers, 1816 as the type species. At the same time he established the genus Gorgodera by removing Distomum cygnoides Zeder, 1800 from the genus Phyllodistomum and designating it as type; Bensley's American varieties were included as G. amplicava and G. simplex. Recognizing the priority of Phyllodistomum over Spathidium, Looss (1901) suppressed the latter. In the same paper he described P. acceptum from two Egyptian fishes, Crenilabrus pavo and C. griseus. He noted that P. cymbiforme possessed a pharynx and, therefore, should not be included in the genus Phyllodistomum; he placed it in the genus Plesiochorus. Zschokke (1884) reported Distomum folium from Cottus gobio, Thymallus vulgaris, Trutta variabilis, and Salmo umbla. Braun (1892) reported Distomum folium from Esox lucius. Looss (1894) reported Distomum folium from the urinary ducts of Acerina cernus.

Odhner (1902) described Phyllodistomum unicum from a fish, Serranus sp.; P. linguale from an Egyptian fish, Gymnarchus niloticus; P. spatula from two Egyptian fishes, Bagrus docmac and B. bayad; and P. spatulaeforme from the eel, Malapterurus electricus. He also transferred Distomum conostomum Olsson, 1876 to the genus Phyllodistomum. Because of morphological variations, symmetrically placed testes and sharply separated anterior and posterior body regions, Odhner was hesitant about including P. spatula and P. spatulaeforme in the genus Phyllodistomum. Looss (1902) erected the genus Catoptroides to receive P. spatulaeforme and P. spatula, designating the latter as type. Odhner (1910) redescribed and provided illustrations of P. unicum Odhner, 1902; P. linguale Odhner, 1902; C. spatula (Odhner, 1902) Looss, 1902; and

C. spatulaeforme (Odhner, 1902) Looss, 1902. He also amended the generic description of Catoptroides as given by Looss (1902).

Osborn (1903a) described Phyllodistomum americanum from the tiger salamander, Ambystoma tigrinum. Later he (1903b) redescribed it on the basis of additional specimens obtained from Ambystoma punctatum from Minnesota. He also pointed out the similarity between the genera Phyllodistomum and Gorgoderina, and suggested that Gorgoderina be considered a synonym. He was of the opinion that the characters separating them were not of generic rank. He maintained that Gorgoderina translucida and Phyllodistomum americanum were transitional forms belonging to the genus Phyllodistomum.

Stafford (1904) described P. superbum from the yellow bullhead, Ameiurus n. natalis, and the yellow perch, Perca flavescens, from the St. Lawrence drainage. He stated that specimens from the perch differed from those in the bullhead in that the former were longer, the suckers spherical, and the uterus was filled with eggs arranged in rows; specimens from the bullhead had oval suckers and there was no definite arrangement of the eggs within the uterus.

Sinitsin (1905) in his study of fishes from Warsaw ponds identified Phyllodistomum folium from Carassius vulgaris, Barbus vulgaris, Gobio fluviatilis, Leuciscus rutilus, Scardinius erythropthalmus, Squalius cephalus, Idus melanotus, Aspius rapax, Abramis brama, and Blicca björkna. He compared Gorgoderina vitellilobum (Olsson, 1876) Looss, 1902 with Phyllodistomum folium (Olfers, 1816) Braun, 1899 and concluded that P. folium should be included in the genus Gorgoderina.

Linstow (1907) described P. angulatum from the fish, Lucioperca sandra, from Russia. Lühe (1909) removed it from Phyllodistomum and

placed it in the genus Catoptroides. He described C. macrocotyle from the European fishes, Carassius sp., Barbus sp., and Gobio sp. His meager description, without drawings, does not permit an analysis of species determination.

Odhner (1910) redescribed, with drawings, the species Phyllodistomum unicum, P. linguale, Catoptroides spatula, and C. spatulaeforme in a publication of the results of his expedition to the Nile in 1901. Odhner (1911) stated, in a footnote, that C. angulatum (Linstow, 1907) Lühe, 1909 and C. macrocotyle Lühe, 1909 belonged in the genus Phyllodistomum.

Cort (1912) studied the systematic position of the genera Phyllodistomum and Gorgoderina and believed them to be distinct. He concluded that P. americanum and G. translucida constituted transitional forms; that G. simplex Stafford, 1902 and G. opaca Stafford, 1902 were not distinct and, therefore, suppressed G. opaca as a synonym. He described Gorgodera minima from Rana catesbeiana and R. pipiens. Gorgodera minima, according to Goodchild (1948), was erected on bases that are of little taxonomic significance, such as its small size. He was of the opinion that adult specimens of G. minima were compared with young specimens of G. amplicava, and accordingly suppressed G. minima as a synonym of G. amplicava.

MacCallum (1917) described Catoptroides magnum and only briefly described C. aluterae. He stated that in both species the intestine formed a continuous loop and the cirrus was large and protrusible. Studying C. magnum, Travassos (1922) pointed out that the continuous loop of the intestine and protrusible cirrus excluded these species

from the genus Catoptroides. He, therefore, proposed the genus Macia to include them, with C. magnum as the type.

Zandt (1924) identified Phyllodistomum folium from Leuciscus leuciscus and L. rutilus. He believed it to be an intermediate form between P. folium (Olfers, 1816) and Catoptroides macrocotyle (Lühe, 1909).

Pearse (1924) believed that Stafford (1904) confused two species of Phyllodistomum in his original description of P. superbum. The species name superbum, therefore, was retained by Pearse for the species from the yellow perch, Perca flavescens, which more nearly fitted the original description, and P. staffordi was proposed for the other species which was found in the northern brown bullhead, Ameiurus n. nebulosus, northern black bullhead, Ameiurus m. melas, and the yellow bullhead, Ameiurus n. natalis. He described P. fausti, from the sheepshead or drum, Aplodinotus grunniens.

Following an extensive study of the literature and many of the original specimens of the European phyllodistomes, Nybelin (1926) concluded that: (1) Catoptroides macrocotyle Lühe, 1909 and Phyllodistomum macrocotyle (Lühe, 1909) Odhner, 1911 were synonyms of P. folium (Olfers, 1816); (2) C. angulatum (Linstow, 1907) Lühe, 1909 should be returned to the genus Phyllodistomum; (3) the trematode identified by Lühe (1909) and Odhner (1911) as P. folium (Olfers, 1816) was in reality an undescribed species which he named P. simile; and (4) P. folium (Olfers, 1816) as identified by Looss (1901), and P. conostomum (Olsson, 1876) Odhner, 1902 was an intermediate species which he redescribed and renamed P. pseudofolium. He also described P. megalorchis from the burbot, Lota lota, and the grayling, Thymallus

thymallus, from Sweden, and P. elongatum from the tench, Tinca tinca, from Sweden.

Ozaki (1926) erected the genus Microlecithus with M. kajika, from the urinary bladder of a Japanese frog, Polypedates buergi, as the type species.

Holl (1928) described Gorgoderina intermedia from the spotted newt, Triturus v. viridescens; he (1929) described Phyllodistomum pearsei from the blue-spotted sunfish, Enneacanthus glorious, and P. carolini from the yellow bullhead, Ameiurus n. natalis. He (1929) stated that it is possible that North Carolina was the center of distribution for phyllodistomes in North America, and that P. superbum Stafford, 1902 had extended its range through host migrations into the St. Lawrence drainage, P. fausti Pearse, 1924 into the Mississippi drainage, and P. staffordi Pearse, 1924 into both. He (1930) described P. entercolpium from a Japanese salamander, Diemictylus pyrrhogaster.

Loewen (1929) described Catoptroides lacustri from the great fork-tailed catfish, Ameiurus lacustris, from the St. Croix river in Minnesota. In his review of the species in this genus, he concluded that Phyllodistomum staffordi Pearse, 1924 should be transferred to the genus Catoptroides, and that C. angulatum (Linstow, 1907) should be returned to the genus Phyllodistomum thereby confirming the opinions of Odhner (1911) and Nybelin (1926). He (1935) described C. lohrenzi from the green sunfish, Lepomis cyanellus, from Kansas.

Layman (1930) described P. marinum from a fish, Sphaeroidas borealis, from Vladivostok, Siberia.

Harwood (1932) maintained that there were no differences between Gorgodera amplicava Looss, 1899 and G. circava Guberlet, 1920; he

considered G. circava to be a synonym of G. amplicava. Krull (1935) was in agreement with Harwood.

Ingles and Langston (1933) described Gorgoderina multilobata from frogs from California. They noted that the specimens from Rana aurora were smaller than those from R. boylii. It was their opinion that the smaller size of the flukes was due to crowding. Ingles (1936) described G. aurora, a fluke he had observed previously but had believed to be an immature specimen of G. multilobata.

Arnold (1934) upheld the concept of Looss (1902) that Catoptroides and Phyllodistomum were distinct, and described C. hunteri from the brown bullhead, Ameiurus n. nebulosus, from New York.

Yamaguti (1934) regarded the genus Microlecithus, which was established by Ozaki in 1926 for M. kajika, as a synonym of the genus Phyllodistomum, however the single whole mount and series of sections of M. kajika he studied had been artifically distorted. In this article he described Phyllodistomum parasiluri from a fish, Parasilurus asotus; P. mogurndae from a fish, Mogurnda obscura; and P. macrobrachicoli, which he secured by feeding metacercariae from the gonads of a Japanese fresh-water shrimp, Macrobrachium nipponensis, to a fish, Mogurnda obscura. He was uncertain, however, whether the latter was the natural definitive host.

Following a review of literature, Lewis (1935) concluded that the genus Catoptroides should be dropped and the species allocated to the genus Phyllodistomum. He concluded that P. pseudofolium Nybelin, 1926 was a synonym of P. folium (Olfers, 1816), that P. entercolpium Holl, 1930 was a synonym of P. patellare (Sturges, 1897), and that C. lacustri Loewen, 1926 should be placed in the genus Phyllodistomum. He did not

agree with Loewen (1929) in the belief that P. staffordi Pearse, 1929 should be placed in the genus Catoptroides.

Lynch (1936) described P. singulare from a larval salamander, Dicamptodon ensatus, from Oregon. He considered Catoptroides to be a synonym of Phyllodistomum, and was in agreement with the suggestion of Lewis (1935) that P. entercolpium was a synonym of P. patellare.

Bhalerao (1937) described Phyllodistomum shandrai from the frog, Rana tigrina, from India. He discussed the status of the genus Catoptroides and concluded that, in view of the confusion resulting from inadequate descriptions and the morphological variability of individual species, the genera Catoptroides and Phyllodistomum were synonymous. He revised the generic description of Phyllodistomum to include Catoptroides.

Olsen (1937) described Gorgoderina tanneri from the western-spotted frog, Rana pretiosa, from Utah.

Rankin (1937) described Phyllodistomum solidum from the dusky salamander, Desmognathus f. fuscus; Gorgoderina tenua from the lined salamander, Eurycea gutto lineata; and G. bilobata from the marbled salamander, Ambystoma opacum, the dusky salamander, Desmognathus f. fuscus, and the red salamander, Pseudotriton r. ruber.

Wu (1937) described Phyllodistomum sinense from the catfish, Odontobutis obscura, from China. He observed that specimens from the ureters of the toad, Bufo b. asiaticus, were smaller and had minute spines; he, however, did not ascribe these specimens to species rank but proposed to consider the toad as another host, in addition to the catfish, for P. sinense. He (1938) described P. lesteri from the metacercarial stage in the freshwater shrimps, Palaemon asperulus and

P. nipponensis, from China. He stated, as did Bhalerao (1937) and for the same reasons, that Catoptroides and Phyllodistomum were synonymous.

Pande (1937) described Phyllodistomum almorai from Rana cyanophlyctis, from India. After a comparative study of species in the genera Gorgoderina and Phyllodistomum, he concluded that the genus Gorgoderina was a synonym of the genus Phyllodistomum; this was based on the fact that species of Gorgoderina and many of the piscine species of Phyllodistomum had nonspatulate bodies. He, therefore, transferred Gorgoderina capsensis Joyeux and Baer, 1934; G. simplex Looss, 1899; G. multilobata Ingles and Langston, 1933; G. aurora Ingles, 1936; G. parvicava Travassos, 1919; G. cedroi Travassos, 1924; and G. cryptorchis Travassos, 1924 to the genus Phyllodistomum.

Steelman (1938a) described Gorgoderina schistorchis from the mud puppy, Necturus m. maculosus, and (1938b) Phyllodistomum caudatum from the black bullhead, Ameiurus m. melas, from Oklahoma.

Srivastava (1938) described Phyllodistomum lewisi from the Indian migratory fish, Belone strongylura.

Steen (1938) described Phyllodistomum undulans from the northern muddler, Cottus b. bairdi, and P. brevicecum from the mud minnow, Umbra limi, from Indiana. He was of the opinion that the possibility of considering the genus Gorgoderina a synonym of the genus Phyllodistomum could be resolved only when additional research data are made available.

Miller (1940) described Phyllodistomum lysteri from the white sucker, Catostomum commersonnii, from Canada.

Byrd, Venard, and Reiber (1940), in a study based on the excretory systems of species in the genera Gorgodera, Gorgoderina, Phyllodistomum, and Catoptroides, concluded that the excretory pattern of Catoptroides

was more like that of Gorgodera and showed distinct differences from that of Phyllodistomum. It was their opinion, therefore, that the problem of considering Phyllodistomum a synonym of Catoptroides required further detailed investigation. They also observed that the excretory patterns of Phyllodistomum and Gorgoderina were similar. The possibility, however, of considering Gorgoderina a synonym of Phyllodistomum, based on excretory patterns alone, was considered to be premature and inconclusive.

Meserve (1941) described Phyllodistomum coatneyi from the spotted salamander, Ambystoma maculatum, from Wisconsin.

Fischthal (1942) described Phyllodistomum semotili from the northern creek chub, Semotilus a. atromaculatus; P. notropidus from the Mississippi Valley common shiner, Notropis cornutus chrysocephalus; P. nocomis from the hornyhead chub, Nocomis biguttatus; and (1943) P. etheostomae from the northern greenside darter, Etheostoma b. blennioides, backside darter, Hadropterus maculatus, and the northern logperch, Percina caprodes semifasciata.

Bravo (1943) discussed the conclusions of previous workers concerning the validity of the genera Phyllodistomum, Gorgoderina, and Catoptroides. He recognized the taxonomic chaos that resulted from species descriptions based on sexually mature phyllodistomes alone; the morphological variations of reported species were so great that few distinctive morphological differences were determinable. He expressed the opinion that a system of classification based on cercarial characters, which are more distinct and more constant, would be of greater value. As an example, he pointed out that Phyllodistomum solidum passed from a gorgoderine shape to a phyllodistome shape as the worm matured.

He stated that, "because of limited knowledge of life-histories in these genera it is unwise, at present, to suppress dogmatically one of them as a synonym of the other." He stated that Gorgoderina schistorchis Steelman, 1938 and Gorgoderina tenua Rankin, 1937 possessed phyllodistome morphology and, therefore, should be placed in the genus Phyllodistomum.

Groves (1945) redescribed P. solidum Rankin, 1937 from the two-lined salamander, Eurycea b. bislineata, and the dusky salamander, Desmognathus f. fuscus; his description was quite different compared with the original.

Dawes (1946) stated that since the genus Catoptroides could not be distinguished from Phyllodistomum by generic characteristics it must fall as a synonym of the genus Phyllodistomum. He listed: (1) P. entercolpium Holl, 1930 as a synonym of P. patellare (Sturges, 1897) Braun, 1899; (2) P. carolina Holl, 1929 and P. hunteri Arnold, 1934 as synonyms of P. staffordi Pearse, 1924; (3) P. pseudofolium Sinitsin, 1905; P. angulatum Linstow, 1907; P. angulatum (Linstow, 1907) Lühe, 1909 as synonyms of P. macrocotyle (Lühe, 1909) Odhner, 1911; and (4) P. megalorchis Nybelin, 1926 as a synonym of P. simile Nybelin, 1926. It was his belief that a detailed review of the species of Phyllodistomum would show many of them to be invalid, and that Phyllodistomum folium (Olfers, 1816) would be cosmopolitan.

Manter (1947) described P. carangis from a marine fish, Caranx ruber, from Florida.

Choquette (1947) described P. lachancei from the speckled trout, Salvelinus fontinalis, from Canada.

Dayal (1949) described P. vachius from freshwater fishes from India.

Yamaguti (1951) described P. pacificum from Caranx equula from Japan.

Gupta (1951) described P. singhai from a fish, Mastacembelus armatus, from the Gomti River. This species differed from all other known species of Phyllodistomum in possessing three tubular branches in the middle of the excretory bladder which is tubular, zigzag in shape, and ends anteriorly in a sac-like structure. He (1953) described P. vittatusi from Macrones vittatus.

It has been shown in this historical review that the genera Spathidium Looss, 1899; Microlecithus Ozaki, 1926; Dendrorchis Travassos, 1926 should be considered synonyms of the genus Phyllodistomum. The position of the genus Catoptroides is not settled and the problem concerning its status requires further investigation; there are as many writers who maintain that the genus is valid as there are those who consider it a synonym of the genus Phyllodistomum. The characteristics of the excretory system in the taxonomy of these flukes and their use as a basis for maintaining Catoptroides as a genus are still doubtful. Goodchild (1943) stated that descriptions in the literature of excretory patterns of the same species or species of the same genus lacked uniformity. Because of the variations, he was of the opinion that conclusions drawn from such data were premature and indecisive.

The question of whether the genus Gorgodera is a synonym of the genus Phyllodistomum will be determined only when more detailed information is available concerning anatomical and life history studies.

A survey of the literature makes one aware that a large number of species is based on superficial characters, that anatomical variations of so-called species are unusually extreme, and that taxonomy of the

Gorgoderinae is in a confused state. Here, indeed, is a place where life cycles will, in all probability, play an important part in taxonomy. There is a fine potential opportunity to show what role life cycles and life cycle stages can play in this and other confused situations where there seems to be an endless variation in so-called species.

MATERIALS AND METHODS

The clam, Musculium transversum, was collected in the vicinity of Stillwater, Oklahoma from January 1952 to August 1953 from Country Club Pond, a stock and fish pond; Efaw Pond, a stock and fish pond on the farm of C. C. Efaw; and Boomer Creek. The clams were removed from small quantities of bottom debris which was collected with an aquatic hand net. In the laboratory, the clams were separated into lots of 15 to 20 each and placed in finger bowls. Each bowl was examined for cercariae during the morning and evening hours with the aid of a binocular, dissecting microscope. When cercariae were found in any bowl, the clams in that bowl were isolated individually to determine, by the same method, which ones were infected. All infected clams were retained individually in bowls with a small amount of pond debris. The clams were kept in pond water which was changed at least once a day, and a culture of various species of green algae and diatoms was introduced, in small amounts, at frequent intervals. During the summer months, when there were adverse room temperatures of 32° C. and above, the clams were kept in a refrigerator at 20 to 25° C. Some clams from most of the collections were dissected and examined to determine whether immature infections were present. The clams which failed to shed cercariae, after they had been under observation for approximately two weeks, were returned to the pond from which they were collected in order to preserve source material.

Parasite free clams were reared under laboratory conditions that simulated those in nature as much as possible. The method is illustrated in Fig. 1. Pans containing a layer of debris were used as habitats and were sloped in an autopsy tray. A continuous stream of pond water entered the upper end of each pan and flowed over the lower end without disturbing the habitat. The water from the pans collected in the autopsy tray and ran into a large, army type, cooking container. The water was recirculated by means of a pump in this container. The amount of water running into the pans could be regulated, and the depletion in volume due to evaporation was replaced without disturbing the clams. It was possible, in this way, to raise parasite free clams and to maintain laboratory infected ones without high mortality rates. Another method for rearing clams is illustrated in Fig. 2; air was used to oxygenate the water and keep it in motion. It was used with some success, but water and food had to be added directly to the pans which disturbed the habitat, and the clams appeared to be adversely affected.

In this fingernail clam, marsupial young are retained in brood chambers on the inner gills of the adults until they are born. Gravid individuals, collected in the field, frequently shed their young when placed in finger bowls. The culture pans were stocked with these young which were assumed to be parasite free. As a control, 25 percent of the clams were examined when they had reached a size of 5 to 8 mm. and all were free of helminth infections. Another method used for stocking the culture pans was to place adults in the pans for 2 to 3 weeks after which they were removed. The young that were born during this interim were left in the pans. The latter method was more practical in that it

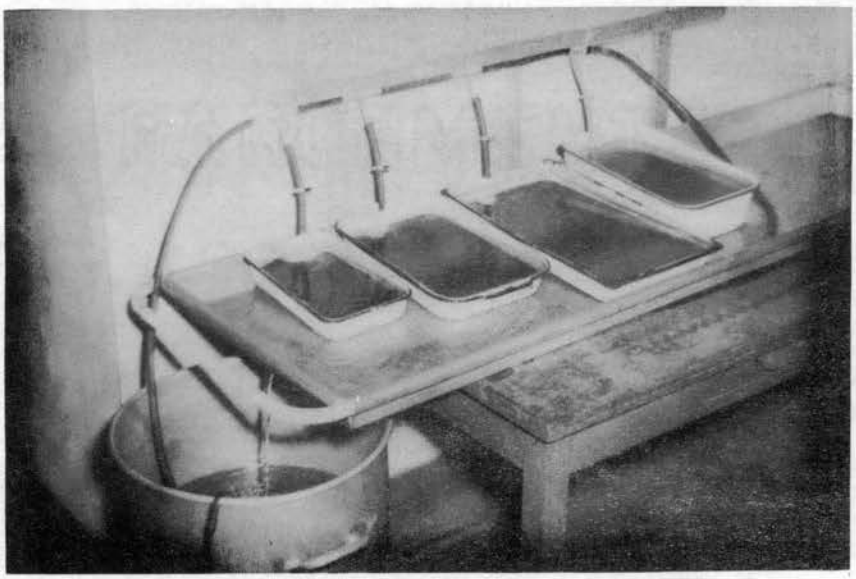


Figure 1. Photograph of apparatus showing method used to rear parasite free clams under laboratory conditions. Water was recirculated by means of a pump in the container at the left.

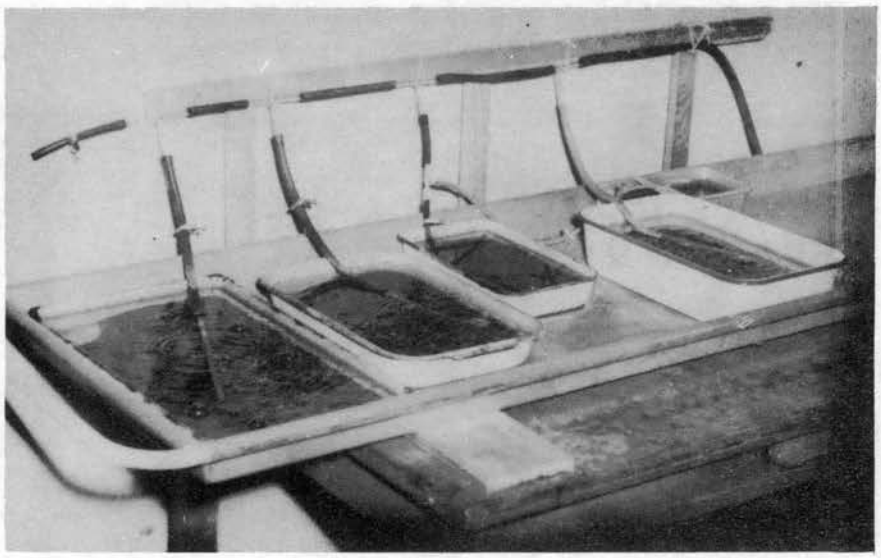


Figure 2. Photograph of apparatus showing method used to rear parasite free clams under laboratory conditions in which air was used to maintain water motion.

eliminated transferring the young clams from finger bowls to culture pans and, therefore, prevented a possible source of injury. It also reduced premature shedding of the young which was evident in adult clams kept in finger bowls.

The caddisfly larvae, Oecetis cinerascens, O. inconspicua, and Leptocella sp., were collected from Heffner Pond, a stock and fish pond on the farm of P. T. Heffner, near Stillwater, Oklahoma. Numerous collections of aquatic life and debris from this pond were free of both fingernail clams and green sunfish; the caddisfly larvae, therefore, were considered the equivalent of laboratory raised specimens. As a control, 25 percent of the larvae from each collection were teased and examined, and all were free of helminth infections. The larvae were collected from bottom debris by putting it in a large, army type, cooking container (Fig. 3) and adding water to cover it to a depth of about six inches. The larvae soon moved to the surface of the debris and the sides of the container. They were collected periodically and isolated individually in specimen jars for experimental use. The water was changed at least once a day, and the larvae were fed micro-worms, Anguillula silusiae, periodically.

Green sunfish, that were used for definitive host experiments, were collected from Steinbach Pond, a stock and fish pond on the farm of E. Steinbach, near Stillwater, Oklahoma. Numerous collections of aquatic life and debris from this pond were all free of fingernail clams; the fish, therefore, were considered the equivalent of laboratory raised specimens. As a control, 50 percent of the fish from each collection were examined for bladder flukes and all were negative for this infection. Infected green sunfish were collected from the same

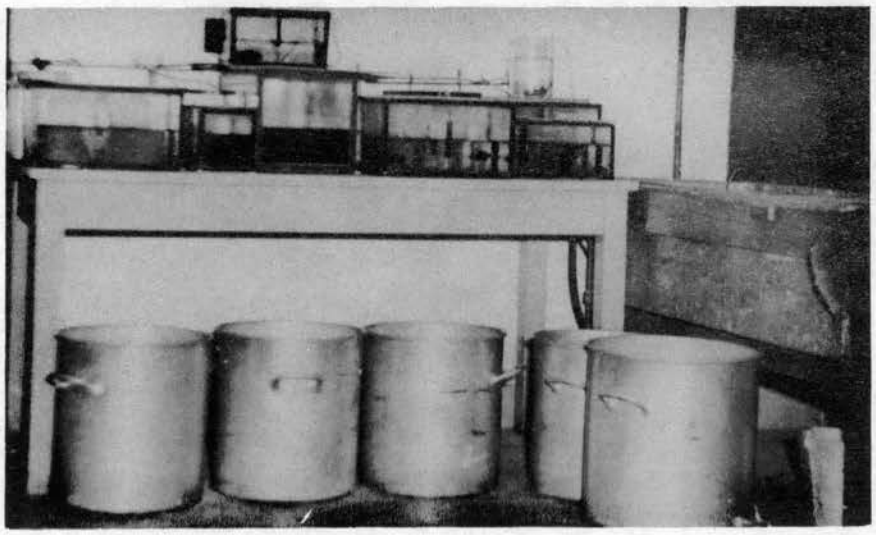


Figure 3. Photograph showing the apparatus used for maintaining fish in the laboratory. Containers at the bottom of the photograph were used to harvest caddisfly larvae.

ponds that contained infected clams. Fish for infection experiments were maintained individually in aquaria subdivided by partitions (Fig. 3). They were fed dog liver.

Both laboratory and naturally infected green sunfish were used as a source of adult flukes which produced miracidia for first intermediate host experiments. Infected laboratory cultured clams and ones collected in the field were used as a source of cercariae. However, only laboratory infected caddisfly larvae or sporocysts from laboratory infected clams were used as a source of metacercariae for all definitive host experiments. The metacercariae in both naturally infected caddisfly larvae and sporocysts in clams were observed and studied.

All stages in the life cycle, miracidium to marita, were studied, described, and illustrated. Their growth and development were determined by examining hosts, appropriate for each stage, that had been infected for known periods of time. Laboratory infected clams were dissected to obtain mother sporocysts of known age, but the age of daughter sporocysts could only be calculated.

Infected caddisfly larvae were studied to determine the place of penetration, place and method of encystment, and state of development of the metacercaria.

To secure data on the excystment, migration, and development of the trematode in the definitive host, experimentally infected fish were examined after being subjected to infection. All of the viscera and the coelom were carefully checked at each examination; organs were placed in physiological saline solution, teased, and examined for flukes under the dissecting microscope. A number of flukes from each experimental infection was fixed, stained, and mounted for study.

The stages in the life cycle were studied both alive and fixed. Intra-vitam staining with neutral red was used for morphological study of living stages which were mounted in egg albumen or methyl cellulose. These were particularily valuable in the study of the glandular detail of both the miracidium and cercaria. Representative stages in the life cycle were fixed in an alcohol-formalin-acetic acid solution or 10 percent formalin, stained with Delafield's haematoxylin or Semichon's acid carmine, and mounted in balsm. All stages, except the marita, stained poorly and fixed mounts had only limited value. Living material was used in the study of the detailed anatomy of these stages. The miracidial epidermal plates were demonstrated by the silver-line technique. To do this, miracidia, in a minimum quantity of water, were pipetted into a one percent silver nitrate solution and exposed to brilliant sunlight for 15 to 20 minutes. The miracidia were then washed three times in distilled water, mounted in glycerin or methyl cellulose, and sealed with asphaltum. This technique made the edges of the plates stand out as dark lines against a gray background.

THE LIFE CYCLE OF *Phyllodistomum lohrenzi*

The egg after being laid by the fluke is eliminated in the urine of the definitive host, *Lepomis cyanellus* Raf., hatches immediately, and liberates a free swimming miracidium. The miracidium enters the first intermediate host, *Musculium transversum* (Say), passively through the incurrent siphon, penetrates the gill, and transforms into a mother sporocyst. Each mother sporocyst produces a single generation of daughter sporocysts which averaged eight in number. Each daughter sporocyst produces numerous macrocercous cercariae. The cercaria has previously been described as *Cercaria coelocerca* Steelman, 1939. A daughter sporocyst usually contains five or six cercariae, although as many as 11 may be present. Under ordinary summer conditions, this part of the cycle is completed in 6 to 8 weeks. In addition, a daughter sporocyst may contain 1 to 6 encysted metacercariae and the significance of these will be explained.

The next stage, the metacercaria, in the life cycle is complicated by the fact that either a clam or a caddisfly larva may serve as a second intermediate host. In the infection of the clam, in which one individual may serve as both first and second intermediate hosts, there is a suppression of the free cercarial stage; the cercaria merely sheds its tail and encysts without leaving the daughter sporocyst. When the caddisfly larva is used as a second intermediate host the cercaria is shed by the daughter sporocyst and emerges from the clam through the

excurrent siphon. The cercaria is very active but unable to swim. The movements consist of vigorous lashings of the terminal part of the tail. This activity serves to attract caddisfly larvae, Oecetis cinerascens, O. inconspicua, and Leptocella sp., which ingest the cercaria. The cercaria encysts in the abdominal or thoracic body cavity of the caddisfly larva. The metacercaria is infective to the green sunfish, Lepomis cyanellus, after two days in the caddisfly larva, but whether younger ones are infective was not determined.

The green sunfish becomes infected by ingesting either infected clams or infected caddisfly larvae. Excystment of the metacercaria takes place in the stomach or in the anterior part of the small intestine of the fish. The immature fluke passes along the intestine, and, in all probability, migrates externally from the anus into the urogenital opening. They usually migrate from here to the mesonephric tubules, via the urinary bladder, where they remain for approximately 10 days before returning to the bladder. However, the migratory path of the immature fluke from the digestive tract into the excretory system is still experimentally undetermined. Immature flukes may be recovered from the urinary bladder and mesonephric tubules as soon as 24 hours after the ingestion of metacercariae. Under ordinary summer conditions, 5 to 7 weeks are required for the fluke to attain sexual maturity in the definitive host. Sexual maturity, however, is attained before growth has been completed.

Discussion

Cycles determined by Sinitsin (1905) for Gorgodera pagenstecheri, G. cygnoides, and Gorgoderina vitelliloba are inconclusive because of the absence of experimental evidence. It was claimed that in all three

of these species odonatan naiads and beetle larvae served as second intermediate hosts. He assumed that larval Epitheca sp. and Agrion sp. served, respectively, as hosts for the metacercariae of Gorgodera loossi and G. varsoviensis; he claimed that adult frogs could be infected by ingesting these intermediate hosts.

Lutz (1926) reported Cyclas sp., Pisidium sp., or Sphaerium sp. as first intermediate hosts and dragonfly larvae as second intermediate hosts of a Gorgoderina sp.

The first life cycle of a frog bladder fluke, Gorgodera amplicava, described in the U. S. was published by Krull (1933, 1935). He determined that Musculium partumeium and Helisoma antrosa were the first and second intermediate hosts, respectively, and that Rana clamitans and Rana catesbeiana were the definitive hosts. Krull (1936) listed four additional snails as second intermediate hosts for G. amplicava. He stated: "Since snails from several widely separated genera have been shown to be experimental second intermediate hosts, it may be assumed that the majority of pond snails will serve in this capacity." Goodchild (1948) extended the second intermediate hosts of G. amplicava to include tadpoles of four species of Rana, two snails, a larval urodele, and an odonatan nymph; the definitive host list was extended to include three species of Rana and one Bufo as well as a urodele.

The first detailed life history of a species in the genus Gorgoderina, G. attenuata, was described by Rankin (1939). He determined that the first intermediate host was a fingernail clam, Sphaerium occidentale; the second, tadpoles of Rana pipiens, R. clamitans, and the snail, Pseudosuccinea columella; and the definitive host was various species of Rana and the salamander, Triturus v. viridescens.

Crawford (1939, 1940) described, in less detail, the life cycle of a fluke which he designated as Phyllodistomum americanum. He determined that the first intermediate host was the pill clam, Pisidium sp.; the second, damselfly naiads, caddisfly larvae, and diving beetles; and the definitive host was the mountain toad, Bufo b. boreas and the tiger salamander, Ambystoma tigrinum.

Goodchild (1943) was the first to describe, in detail, a phyllodistome life history. He determined, for Phyllodistomum solidum, that the pill clam, Pisidium abditum, several species of Odonata, and the dusky salamander, Desmognathus f. fuscus, were the first and second intermediate and definitive hosts, respectively.

Hunt (1950) described the life cycle of Gorgodera vivata. He determined that Sphaerium simile was the first intermediate host; anisopteran and zygopteran naiads, crayfish, and larvae of Sialis sp. were second intermediate hosts; and the green frog, Rana clamitans, was the definitive host.

Lees (1953) described the life cycle of Gorgoderina vitelliloba. He determined that Sphaerium sp. and Pisidium sp. were first intermediate hosts, that the tadpole of Rana temporaria was the second intermediate host, and that various species of Rana served as the definitive hosts.

Several Europeans have postulated, on morphological similarity and from limited experimental evidence, relationships between cercariae, metacercariae, and mature phyllodistomes. On this basis, Looss (1894), Lühe (1909), and Odhner (1911) proposed that Cercaria duplicata was the larva of Phyllodistomum folium. However, in extensive feeding experiments using metacercariae of C. duplicata, on 16 species of fish several

of which were natural hosts for P. folium, Reuss (1903) was unable to obtain infections. From an analysis of the drawings made by Reuss, Goodchild (1943) was of the opinion that Reuss did not feed metacercariae but fed cercariae. Nybelin (1926) thought that C. duplicata was the larva of P. elongatum; this assumption was based on the sucker ratio, position of the genital primordium, and position of the genital pore. In the same paper, he stated that the short-tailed microcercous cercaria described by Sinitsin (1901) as the larva of P. folium was actually the cercarial stage of P. elongatum. However, he had previously, as noted above, stated that C. duplicata was the larva of P. elongatum. Finally, in an attempt to clarify the status of C. duplicata and without any evidence for the assumption, Nybelin assumed that it could possibly be the larva of P. pseudofolium.

Odhner (1911) stated that the microcercous cercaria of Sinitsin (1901) was the larval form of P. macrocotyle. In the same paper, he assumed, without experimental evidence, that Cercaria clausii was the larva of P. acceptum. Another interesting disagreement developed when Cable (1942) assumed that C. clausii was the larva of a fish trematode belonging in either the family Lepocreadiidae or Gyliauchenidae. It is evident that these assumptions, without scientific proof, have produced much confusion in relation to life cycle determinations, and, in all probability, have hindered rather than fostered the actual scientific determination of gorgoderine life cycles.

Other writers have reported on trematode life cycles in which a single animal served both as the first and second intermediate hosts, and in some encystment took place in the sporocyst. As far as the writer is aware, this is the first phyllodistome life cycle to be

reported in which the cercaria either encysts in the sporocyst to become a metacercaria or is freed from the sporocyst in the clam to encyst and become a metacercaria in a caddisfly larva.

Looss (1894) was under the impression that Cercaria micrura, a cotylocercous type, does not emerge from the sporocyst. Wesenburg-Lund (1934), working with the same species, observed cercariae and metacercariae within the same sporocyst; he determined, however, that some cercariae emerged from the sporocysts and encysted in the tissue of the snail.

Sinitsin (1901) reported that the cercaria of Phyllodistomum folium encysted within the sporocyst, but these sporocysts with encysted metacercariae emerged from the clam and were infective to fish.

Sinitsin (1911) observed that Cercaria sinuosa, C. saggitarius, C. inconstans, and C. dimorpha also encysted within the sporocyst. He stated that C. sinuosa and C. saggitarius always encysted in the sporocyst, but that in C. inconstans only the "small" cercariae had a tendency to encyst in the sporocyst and the "large" cercariae were freed. C. dimorpha, according to Sinitsin, contained dimorphic forms within the same sporocyst, "forma prodroma" and "forma postera". The "prodroma" type, which was produced in great numbers, was freed from the sporocyst and snail, and the "postera" type, which was produced in small numbers, remained and encysted within the sporocyst.

Sinitsin (1931) reported that the cotylocercous cercaria of Plagioporus virens emerged from the sporocyst and the infected snail, Fluminicola virens, then penetrated the same animal and encysted in a sporocyst. Dobrovolny (1939) questioned this behavior of the cercaria

and suggested that Sinitsin was probably dealing with two species and failed to distinguish them.

Rothschild (1937) noted a tendency toward suppression of the free-swimming cercarial stage in species of the genus Maritrema. She stated:

> In Cercaria oocysta, C. pirum, C. sinuosa, and the undescribed Cercaria A. Rothschild, 1936, the tails are shed in the sporocyst, a tailless phase follows, while the penetration glands and stylet are lost, and the cystogenous cells undergo development, and encystment then ensues.

McMullen (1937) observed that the cercariae of Plagiorchis muris and P. proximus either developed into metacercariae within the sporocysts or were freed from the sporocysts and snail and encysted in aquatic insects. He attributed the failure of some of the cercariae to emerge and encystment within the sporocyst to precocious development; he offered no suggestions regarding the factors which may cause retention of cercariae in one case and not in another. Dobrovolny (1939) reported that the same type of life cycle for Plagioporus sinitsini, a small allocread trematode from the gall bladder of fresh water fish. He stated:

> In P. sinitsini there is some evidence in support of precocious development of cercariae. It might be argued that the low incidence of infection in the snails compared to the high incidence in fish may indicate the probability of another intermediate host.

It is possible to make certain broad and loosely defined generalizations regarding life cycles in many families, however many of these generalizations break down when they are applied to a particular species. This discussion of life cycle variations in related and unrelated species adds additional weight to the statement made by La Rue (1951) which seems most appropriate:

Each species seems to play the game of parasitic life within the broad rules laid down by, and for, its family; but it appears to have developed within this code its own special rules and regulations, its own deviations from what we poor humans assume to be normal for the family.

EXPERIMENTS AND OBSERVATIONS CONCERNING THE LIFE CYCLE

The First Intermediate Host, *Musculium transversum*

Eggs in the metraterm of the sexually mature fluke contained fully developed, motile miracidia. Eggs with miracidia were obtained by placing the flukes in water, they were shed and hatched in 10 to 30 seconds. By keeping the flukes in water and subjecting them alternately to refrigeration (5 to 10° C.) and room (21 to 35° C.) temperatures they continued to shed eggs. In one fluke that did not shed eggs under the usual conditions it was observed that they hatched either in the metraterm or terminal part of the uterus. Other methods of obtaining eggs were by teasing adult flukes or by stripping an infected green sunfish causing it to void urine containing them. Eggs obtained by teasing a fluke contained larvae in all stages of development. Some of the immature miracidia continued to develop and hatched over a period of 24 hours, beyond which time the eggs were not observed.

The clams used in first intermediate host experiments were from one-third to one-half grown and measured 5 to 8 mm. in length. They were all laboratory raised and, therefore, parasite free. The clams were infected experimentally by subjecting them to numerous miracidia. The clams were exposed to the miracidia overnight in a stender dish. A total of 107 clams was exposed to miracidia, 84 of which became infected (78.5 percent); 51 of the clams died within a period of 1 to 4 weeks. Only five of the dead clams were not infected. By way of

contrast, only eight clams died in an adjoining culture pan containing numerous parasite free clams. It appears that the experimental clams were subjected to too many miracidia and many of them succumbed to the heavy infections. This method of infection was, therefore, changed and a smaller stender dish was used; one clam was placed in each dish and was exposed to only 1 to 4 miracidia. A total of 73 clams was exposed to miracidia by this method and 24 became infected (32.8 percent). Only 7 of the clams died within a period of 1 to 5 weeks and three of these were infected.

Mass infection of clams was secured by placing laboratory raised clams in an aquarium with infected green sunfish. Infected fish, to be used in the aquarium, were determined by stripping the fish and recovering the fluke eggs in the urine. Pond debris served as a habitat for the clams in the aquarium, and a screen, to prevent their disturbance, was placed about two inches above the debris. The clams were kept in the aquarium for a week or two, after which they were removed to culture pans. A total of 150 clams was placed in the aquarium, 42 of which became infected (28.0 percent). Three clams died in the aquarium and seven died in the culture pans before shedding cercariae. One of the three dead clams recovered from the aquarium was infected, as well as all seven of those that died in the culture pans.

No active penetration of the clam by the miracidium was observed. On seven occasions, the writer was fortunate enough to see the miracidium enter the incurrent siphon of a clam. The seven clams were examined from 15 minutes to two hours after the entrance of a miracidium, but the miracidia were observed in the gill tissue of only three. The

miracidium was observed penetrating the gill tissue in two of the clams that had been infected for 15 and 25 minutes, respectively. The third clam that had been infected for 70 minutes showed the miracidium deep in the gill tissue and its ciliated epidermal plates had sloughed. The passive entrance of the miracidia into the mantle cavity with the incurrent water flow is, as far as has been determined, the only method by which the first intermediate host becomes infected. Since the miracidium is devoid of sclerous penetrating aids, the successful invasion of the clam tissue must depend on muscular movement with localized gill histolysis caused by glandular secretions.

The activity of the miracidium was observed on many occasions. The miracidium showed a slow rotation on a longitudinal axis with a slight wobble and was similar to the propulsion of a paramecium. If the miracidium brushed against an object it stopped, backed up, turned, and proceeded in a new direction. Swimming of the miracidium was interrupted occasionally by brief periods during which it settled to the bottom of the dish. The miracidium remained alive at room temperature (21° C.) for 48 hours, but during the summer months when it was above 32° C. the miracidium lived for only 12 to 18 hours. At a temperature of 5 to 10° C. it remained alive for 72 hours.

Clams, Musculium transversum, were collected at various intervals over a period of 16 months and from several areas where the infection was present in fish. The number of mature infections was determined and these data are presented in Tables I and II.

It is shown from the data in these tables that the percentage of clams infected in nature varied with the habitat and the month the clams were collected. It is shown in Table I that the fish ponds had

an incidence of mature infections of approximately 2 to 6 percent, and that Boomer Creek had a low incidence.

TABLE I

SHOWING INCIDENCE OF MATURE INFECTION IN CLAMS, Musculium transversum, FROM VARIOUS COLLECTING AREAS

Collecting Area	Number of Clams Collected	Number that Shed Cercariae	Percent that Shed Cercariae
Boomer Creek	1095	15	1.37
Country Club Pond	679	13	1.91
Efaw Pond	664	39	5.87
Totals	2438	67	2.75

The Second Intermediate Hosts, Oecetis cinerascens, O. inconspicua, and Leptocella sp.

Caddisfly larvae were infected experimentally by subjecting them to cercariae in a stender dish. The cercariae were secured from infected clams kept in the laboratory. Most of the cercariae emerged from clams between 8 p.m. and midnight in numbers ranging from 17 to 86. The cercariae were most active immediately after their emergence, and movement consisted of vigorous lateral whip-like lashings of the terminal part of the tail. Such activity continued for only a short time because the cercariae became attached by the tip of the tail to either debris, the container, or other cercariae. The attached cercariae were kept in motion, however, by undulating movements of the

TABLE II

SHOWING INCIDENCE OF MATURE INFECTION IN CLAMS, Musculium transversum, IN NATURE DURING A PERIOD OF 16 MONTHS

Date Clams were Collected	Number Collected	Number that Shed Cercariae	Percent that Shed Cercariae
1952			
May	107	2	1.87
June	454	10	2.20
July	264	4	1.52
August	338	15	4.44
September	196	10	5.10
October	57	1	1.75
November	34	1	2.94
December	41	3	7.32
1953			
January	178	0	0
February	167	1	0.60
March	99	0	0
April	224	3	1.34
May	80	6	7.50
June	124	8	6.45
July	64	3	4.69
August	11	0	0
Totals	2438	67	2.75

terminal part of the tail, this activity decreased progressively with age. These cercarial movements attracted caddisfly larvae which ingested the cercariae. Cercariae incapable of tail movements were usually not disturbed by the larvae. A caddisfly larva, after ingesting one or more cercariae, withdrew into its case and would not feed for 5 to 8 minutes. Such activity was not evident in regular feeding, consequently it was assumed that there was discomfort caused by the penetration of the gut by the cercariae.

After being ingested by the caddisfly larva, the cercarial body emerged from the chamber in the anterior part of the tail while in the esophagus or crop. The tail collapsed and was digested. The cercarial body was active in the digestive tract and usually penetrated the wall of the crop, but sometimes the esophagus. Usually 5 to 10 minutes elapsed between ingestion and penetration of the wall.

The cercaria moved about in the body cavity of the caddisfly larva before encystment. It began to encyst in 5 to 7 minutes after penetration. A metacercaria 30 minutes old was surrounded by a delicate, flexible cyst wall. The cercarial stylet was shed during this period and became fixed to the inner surface of the cyst wall. The cystogenous fluid, issuing from the excretory pore, was spread and shaped into a smooth layer on the inside of the thickening cyst wall by the movement of the metacercaria. The body was in constant motion, the extremities probed the cyst wall by bending from side to side, and the anterior end was more active. Cysts two days old were delicate and flexible and their shapes could be distorted temporarily by the activity of the metacercariae. The activity of the body, however, was reduced considerably after two days of encystment. Cysts four days old were oval

and more rigid, but still possessed some flexibility. Cysts 10 days old were spherical and possessed a rigid cyst wall. Only occasional movements of the metacercariae were observed in cysts 10 days old or older.

The ingestion, penetration, and encystment of the cercariae were studied in vivo, and the data concerning these processes are presented in Table III.

It is shown in this table that about 19 percent of the cercariae ingested failed to penetrate the gut. Some of them were injured during ingestion which probably accounted for their inability to penetrate. The large, thick wall of the cercarial chamber of the tail, in all probability, prevented injury to many of the cercarial bodies by the mandibles of the larva. It also is shown that about five percent of the cercariae that penetrated the gut failed to encyst. The cysts of metacercariae in both naturally and experimentally infected caddisfly larvae were normally attached to host tissue in the abdominal or thoracic cavities.

Data showing the place of encystment and number of cysts in each caddisfly larva are given in Tables III and IV.

It is obvious from the data in these tables that the cercariae encysted in all parts of the body, but that the abdomen was the site of the heaviest concentration.

The cysts were usually attached singly, but when numerous they clumped together in distinct packets. In two experimentally infected caddisfly larvae, elongate cysts with poorly defined walls were observed in the head, in addition to the normal ones in the abdominal and thoracic cavities. In comparing data in Tables III and IV, it is shown

TABLE III

SHOWING RESULTS OF EXPERIMENTAL INFECTION OF CADDISFLY LARVAE, Oecetis sp. and Leptocella sp., BY FEEDING CERCARIAE FROM EXPERIMENTALLY OR NATURALLY INFECTED CLAMS

Host Number	Number of Cercariae Ingested	Number of Cysts Found	Location of Cysts			Cercariae that Failed to Encyst	
			Abdominal Cavity	Thoracic Cavity	Head	Number that Failed to Penetrate Gut	Number that Penetrated Gut but did not Encyst
1	3	2	2			1	
2	4	4	4			0	
3		5	3	2			
4		2	1		1		
5	5	4	3	1		1	
6	3	1	1				
7		5	3	2			
8		9	8	1			
9		13	6	5	2		
10		5	5				
11		6	4	2			2
12		8	6	2			
13		10	5	5			
14		13	9	4			
15	6	5	4	1		1	
16	4	2	2				
17	10	7	6	1		2	1
18	3	2	2			1	
19	6	6	2	4		0	
20		1	1				
21	6	4	3	1			
22		10	7	3			
23		9	7	2			

TABLE III "Continued"

Host Number	Number of Cercariae Ingested	Number of Cysts Found	Location of Cysts			Cercariae that Failed to Encyst	
			Abdominal Cavity	Thoracic Cavity	Head	Number that Failed to Penetrate Gut	Number that Penetrated Gut but did not Encyst
24		2	2			3	1
25	1	0				1	
26	5	4	3	1		1	
27		11	8	3			
28		7	5	2			
29		3	3				
30	2	1	1				1
31		9	5	4			
32		2	2				
33	7	5	4	1		2	
34	2	2	2				
Totals	67	179	129 72.06%	47 26.26%	3 1.68%	13	5

TABLE IV

SHOWING THE INCIDENCE OF INFECTION IN CADDISFLY LARVAE, Oecetis sp. and Leptocella sp., FROM VARIOUS COLLECTING AREAS

Collecting Area	Number Examined	Number Infected	Percent Infected	Analysis of Infection		Location of Cysts		
				Number Infected	Number of Cysts in Each	Abdominal Cavity	Thoracic Cavity	Head
Boomer Creek	204	2	0.98	1	2	2		
				1	3	3		
Country Club Pond	291	11	3.78	2	1	1	1	
				3	2	4	2	
				2	4	5	3	
				1	6	6		
				1	8	5	2	1
				1	10	7	3	
				1	12	7	4	1
Efaw Pond	140	19	13.57	2	1		2	
				4	2	5	3	
				4	3	7	5	
				3	4	10	2	
				1	6	5	1	
				1	7	4	3	
				2	9	11	6	1
				2	12	19	4	1
Totals	635	32	5.04	32		101	41	4
						69.18%	28.08%	2.74%

that there is little difference in the place of encystment between caddisfly larvae experimentally infected or those infected in nature. Furthermore, it is shown that experimentally infected larvae had 1 to 13 cysts each (average 5.4) and naturally infected ones had 1 to 12 cysts each (average 4.6). It will be shown later that there is apparently a limit in the number of cercariae that can encyst in a larva.

Although many experiments of a similar nature were done to establish the early part of the cycle, only the most important and significant ones will be described.

In one experiment, two clams infected in the laboratory, which were actively shedding cercariae, were put in a stender dish, containing water, with five parasite free caddisfly larvae. The clams and larvae remained together overnight. In the morning the larvae were examined, all were infected and the number of cysts in each ranged from 5 to 13. The gut of each larva contained partly digested cercariae and one had two cercariae within the body cavity that had failed to encyst.

In another experiment, seven clams infected in the laboratory, which were actively shedding cercariae, were put in a finger bowl with 20 parasite free caddisfly larvae; numerous control larvae were kept in another finger bowl. After two days had elapsed, the clams were removed to a culture pan and the caddisfly larvae were transferred to individual specimen jars. The larvae were examined 5 to 25 days after they had been subjected to infection, and seven of them died during the interval. All of the larvae became infected and the number of cysts in each ranged from 8 to 29, but those that died contained more than 15 cysts each. None of the control caddisfly larvae died, and none

contained cysts. There is, therefore, reason to believe that the size of the infection was a contributing factor in the deaths of the caddisfly larvae.

In another experiment, 30 cercariae were introduced into a stender dish containing a parasite free caddisfly larva. An hour later five cercariae had disappeared. The caddisfly larva was examined to determine the position of the ingested cercariae. Two of them had encysted in the abdominal cavity and were surrounded by a flexible, hyaline cyst wall; one cercaria was active in the thoracic cavity; one of the remaining two cercariae, which also had escaped from their tails, was active in the esophagus, and the other was penetrating the crop.

In another experiment, one caddisfly larva, that had a previous 30 day old infection, was examined soon after it had ingested four cercariae. Six larval flukes were found in the body cavity or gut; two of these in the abdominal cavity were old, encysted metacercariae with well defined cyst walls and excretory concretions in the bladder, the one in the thoracic cavity was just beginning to form a cyst wall which was exceedingly thin and fragile, the remaining two cercariae were active in the body cavity, and one in the gut was mutilated.

It was not determined what caused some cercariae to encyst in the daughter sporocyst and others to emerge from the sporocyst and the clam. No anatomical differences were observed in the metacercariae from the two sources, but the cysts were different. The cyst wall in the metacercariae found in caddisfly larvae consisted of two parts: an inner, transparent, hyaline one of metacercarial origin, and an outer, granular part, of variable thickness, probably of host origin. The cyst

wall of metacercariae from sporocysts consisted of only one layer, similar to the inner layer found in the cysts from caddisfly larvae.

A count was made of both daughter sporocysts and encysted metacercariae in three naturally infected clams. The sporocyst number was 71, 176, and 319, respectively, and the total number of encysted metacercariae in the sporocysts of each was 196, 511, and 863, respectively. This demonstrates the tremendous capacity of the sporocysts for metacercariae, and, in all probability, accounts for the large number of immature flukes found in some sunfish. Metacercariae within the sporocysts are shown in Figs. 4 and 5. An infected clam body showing a large number of sporocysts is shown in Fig. 6. It will be noted that, if maximum infections in the definitive host in nature (Table IX) are compared with experimental infections using sporocysts from clams (Table VI) and with those using infected caddisfly larvae (Table V), the maximum infections in nature compared rather well with the experimental infections using sporocysts from clams.

In order to determine the incidence and degree of infection, caddisfly larvae were collected at various intervals over a period of seven months from three areas in which the infection was known to exist. These data are presented in Table IV. They show that the percentage of caddisfly larvae infected in nature varied considerably; Efaw Pond had the highest and Boomer Creek the lowest incidence of infection.

The Definitive Host, Lepomis cyanellus

The definitive host, the green sunfish, was infected experimentally by feeding it metacercariae either from caddisfly larvae or from daughter sporocysts from clams. To prompt the ingestion of infected

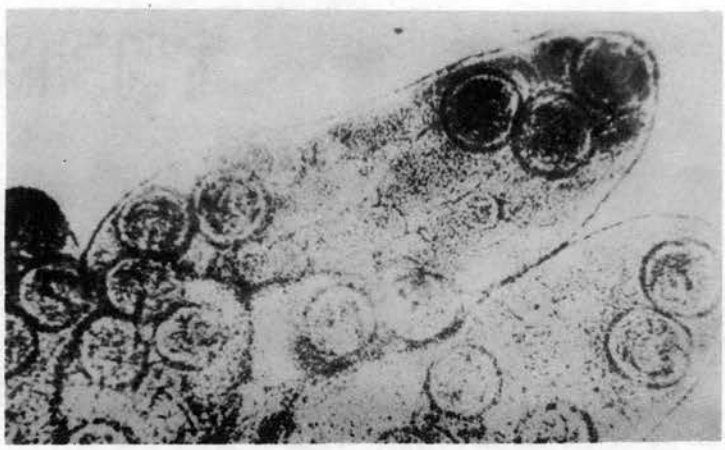


Figure 4. Photograph showing two daughter sporocysts, each with numerous encysted metacercariae.

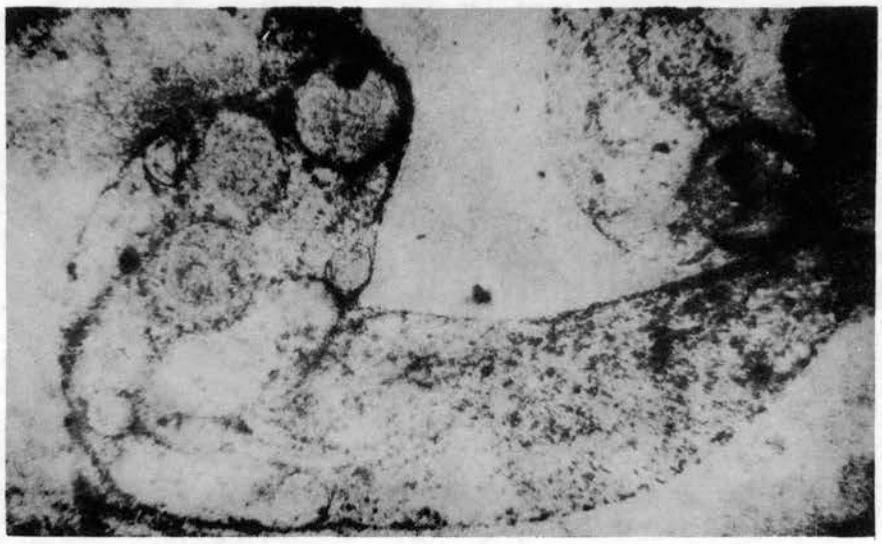


Figure 5. Photograph showing an individual daughter sporocyst with three encysted metacercariae at the upper left.

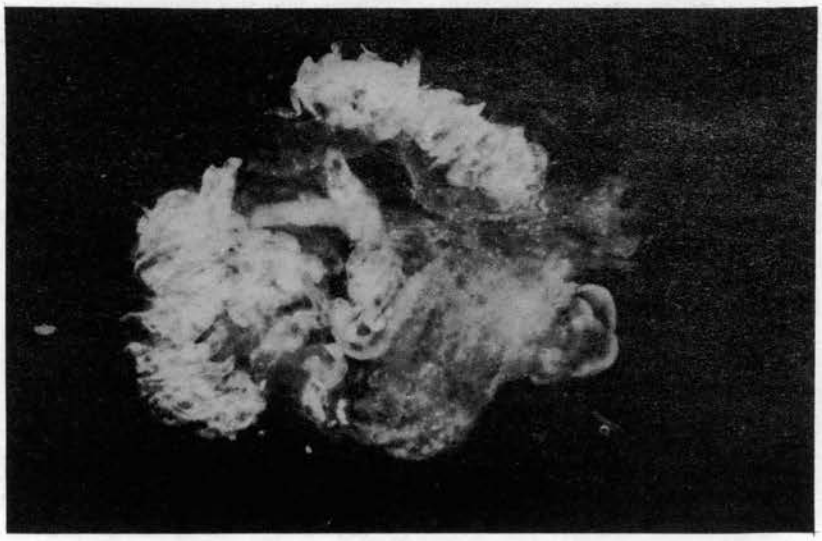


Figure 6. Photograph showing an infected clam with gills teased to show the tremendous number of sporocysts which appear to have a milky color.

material, the fish were deprived of food for one or two days prior to the time they were offered the infected material. For large fish, it was sometimes necessary to wrap the infected material in liver to induce feeding.

The results of experimental infections are summarized in Tables V and VI. It is shown that metacercariae from the daughter sporocysts excysted in the stomach of the sunfish, and that those from the caddisfly larvae normally excysted in the anterior small intestine. However, the metacercariae in cysts, up to six days of age, from caddisfly larvae excysted in the stomach of the sunfish. This is attributed to the fact that the cyst wall was not thick enough to withstand the action of the gastric juice, since cysts in both the intact intermediate hosts, except for the shell of the clam, were fed to fish. It is assumed, therefore, that the nature of the cyst wall may have something to do with the place of excystment of the metacercaria. The nature of the cyst wall in the two types of host has been described.

Experimentally infected green sunfish were examined at different intervals of time subsequent to their infection to determine the behavior of the fluke in the host. The results of the most important experiments are summarized in Tables V and VI. It is shown that immature flukes, in experimental infections up to 10 days of age, were found more frequently in the mesonephric tubules than in the urinary bladder. In infections more than 10 days old the flukes were usually confined to the urinary bladder. Consequently, it appears that the immature flukes usually migrated to the mesonephric tubules first, via the urinary bladder, and then returned to the urinary bladder. No flukes were ever found in the kidneys or coelom. Flukes recovered from the

TABLE V

SHOWING RESULTS OF EXPERIMENTAL INFECTION OF THE DEFINITIVE HOST, Lepomis cyanellus,

BY FEEDING EXPERIMENTALLY INFECTED CADDISFLY LARVAE CONTAINING METACERCARIAE

Fish Host Number	Age of Infection in the Fish	Number of Metacercariae Fed	Age of Metacercariae Fed to Fish	Number of Flukes Recovered from the Fish	Number of Flukes Not Recovered	Organ from which Flukes were Recovered: Stomach	Small Intestine	Large Intestine	Mesonephric Tubules	Urinary Bladder
1	2 hours	7	3 days	7		7 encysted				
2	2	10	10	10		10 "				
3	3	4	2	4		4 excysted				
4	3	6	6	5	1	5 "				
5	3	9	12	9		9 encysted				
6	4	7	2	7			7excysted			
7	5	11	12	11			11 "			
8	12	8	2	7	1		7 "			
9	18	13	3	12	1			12		
10	1 day	18	3	9	9				2	7
11	2 days	17	5	12	5				7	5
12	3	19	3	12	7				10	2
13	5	12	6	4	8				4	
14	8	19	5	11	8				9	2
15	9	16	2	10	6				8	2
16	10	11	4	6	5				1	5
17	11	15	2	10	5				2	8
18	12	4	9	3	1					3
19	17	18	4	8	10				1	7
20	21	10	2	6	4					6
21	25	21	12	10	11				2	8
22	30	6	2	3	3					3
23	35	17	3	9	8					9
24	45	21	3	9	12					9

TABLE VI

SHOWING RESULTS OF EXPERIMENTAL INFECTIONS OF THE DEFINITIVE HOST, Lepomis cyanellus,

BY FEEDING SPOROCYSTS CONTAINING METACERCARIAE FROM EXPERIMENTALLY INFECTED CLAMS

Fish Host Number	Age of Infection in the Fish	Number of Flukes Recovered from the Fish	Organ from which Flukes were Recovered			
			Stomach	Small Intestine	Large Intestine	Mesonephric Tubules	Urinary Bladder
1	2 hours	27	27 encysted				
2	3	88	22 "				
			56 excysted				
3	4	56	12 "	44 excysted			
4	8	92		92 "			
5	14	41		41 "			
6	18	106		24 "	82		
7	1 day	47				18	29
8	2 days	32				21	11
9	4	26				21	5
10	7	40				38	2
11	8	47				39	8
12	9	33				19	14
13	10	34				8	26
14	11	58				5	53
15	12	37				3	34
16	18	52				3	49
17	25	29					29
18	35	26					26

35 and 45 day old infections were sexually mature, and eggs were recovered from these flukes and used in first intermediate host infection experiments.

Furthermore, the data in Table V show that two day old metacercariae were infective to the definitive host, but whether younger ones are infective was not determined.

It is apparent from these data (Tables V and VI) that the flukes remain in the alimentary canal for many hours, but that they may be recovered from the urinary bladder and mesonephric tubules within 24 hours after ingestion of metacercariae. The manner in which the flukes enter the urinary bladder from the alimentary canal has been a subject of contention. Sinitsin (1901) suggested that the flukes migrated from the anal to the urogenital opening on the outside of the body. To test this hypothesis the writer fed a starved green sunfish three caddisfly larvae containing a total of eight metacercariae; four hours later, when there was little chance of regurgitation, the anal opening was stuffed with cotton. The fish was examined 26 hours after ingesting the metacercariae. Seven flukes were found in the terminal portion of the large intestine, and none was found in the urinary bladder or mesonephric tubules. Normally the flukes are found in the urinary bladder and mesonephric tubules 24 hours after metacercariae were ingested. Therefore, this experiment partially confirms the assumption that an external migration does occur. The hazards of an external migration would explain the decrease in numbers of flukes found in the urinary bladder and mesonephric tubules as compared with the number of metacercariae ingested. It is shown in Table V that experimental infections of the definitive host of 1 to 45 day duration

resulted in a loss of 102 (45.5 percent) of the 224 metacercariae fed; 122 flukes were recovered from the urinary bladder and/or mesonephric tubules.

In frogs the digestive and urogenital systems open into a cloaca and no external migration of the flukes is necessary. Rankin (1939) stated that in Gorgoderina attenuata, found in frogs and newts: "Although the exact number of cysts were not always counted, still from the number of excysted flukes recovered, it would seem as though practically every cyst survives in the definitive host."

Green sunfish, Lepomis cyanellus, were collected at various intervals over a period of 18 months and from several areas. The incidence and degree of infection was determined and these data are presented in Tables VII and VIII. Table VII shows a high incidence of infection in Efaw Pond and a comparative low incidence in Boomer Creek and Country Club Pond. Furthermore, it is shown in Table IX that the number of flukes in each naturally infected fish varied from 1 to 56; the average number was nine. Crowell (1949) reported that 17 percent of 116 centrarchid fishes examined were infected with Phyllodistomum lohrenzi. Loewen (1935) reported two of seven green sunfish infected with a total of 10 flukes, and Vernard (1940) reported 7 of 29 bass infected with 2 to 28 flukes each.

The writer examined 853 sunfish (Table VIII) collected in nature and 165 of these were infected. The flukes in all of the fish except 12 were uniform in size but various sizes were represented. In the remaining 12 there were flukes of assorted sizes, and it is apparent that these probably resulted from the ingestion of cysts at various

times. Ten of the 12 sunfish were taken from Efaw Pond in which the incidence of infection was the highest in the intermediate hosts.

TABLE VII

SHOWING INCIDENCE OF INFECTION IN GREEN SUNFISH, *Lepomis cyanellus*, FROM VARIOUS COLLECTING AREAS

Collecting Area	Number of Fish Examined	Number Infected	Percent Infected
Boomer Creek	304	25	8.20
Country Club Pond	186	27	14.51
Efaw Pond	363	113	31.12
Totals	853	165	19.34

Vernard (1940) stated:

> A bladder containing 28 *P. lohrenzi* was inflamed and considerably larger than anticipated, judging from the size of the fish from which it was removed, whereas infections of 8 flukes produced no noticeable effect.

Goodchild (1943) stated that the bladder flukes probably feed on urinary epithelium. He saw, in *Phyllodistomum solidum*, cells of an epithelial nature in sections of gut ceca. The writer studied seven fish that had from 3 to 21 flukes each. In the three fish that had more than 13 mature flukes each, the bladder wall was very thin and the flukes could be seen through it. In addition, the bladder lost its regular shape due to the presence of small papillae or protuberances in the wall, and a fluke was anchored in each. Inflammation and thinning of the bladder wall were more pronounced in these areas. Fish with light infections, less than 10 flukes, or those with immature flukes

TABLE VIII

SHOWING INCIDENCE OF INFECTION IN GREEN SUNFISH, Lepomis cyanellus, IN NATURE DURING A PERIOD OF 18 MONTHS

Date Fish were Collected	Number Examined	Number Infected	Percent Infected
1952			
February	30	1	3.33
March	93	10	10.75
April	6	1	16.67
May	3	0	0
June	50	9	18.00
July	71	14	19.71
August	104	7	6.73
September	57	2	3.51
October	96	29	30.20
November	28	6	21.42
December	29	11	37.93
1953			
January	43	16	37.20
February	11	1	9.09
March	79	23	29.11
April	15	2	13.33
May	40	12	30.00
June	52	11	21.15
July	46	10	21.73
Totals	853	165	19.34

TABLE IX

SHOWING NUMBER OF FLUKES IN NATURALLY INFECTED

GREEN SUNFISH, Lepomis cyanellus

Number of Flukes per fish	No. of Fish with this No. of Flukes	Number of Flukes per Fish	No. of Fish with this No. of Flukes
1	13	16	3
2	12	17	3
3	23	18	2
4	8	19	1
5	16	20	4
6	11	21	1
7	6	22	2
8	4	23	2
9	11	24	1
10	5	25	2
11	11	26	1
12	2	27	2
13	4	28	2
14	2	30	1
15	7	31	2
(Continued, second column)		56	1

lacked the pathogenic changes. In heavy infections of immature flukes they were attached in concentrations that allowed little or no spatial separation. Mature flukes in either light or heavy infections were never concentrated and crowding was not evident.

In conclusion, it should be reemphasized that these fish became infected, in the laboratory, by ingesting either the clam or caddisfly larvae which served equally well as second intermediate hosts. The extent to which the clam serves as an intermediate host in nature is questionable and was not investigated to any extent experimentally. It was observed, however, that the clams were active and could climb subterranean vegetation. Ingestion by the fish of such clams has been observed in aquaria. It was noted that when clams died, the valves opened and the clam body was released, and in some instances it floated slowly to the surface of the water. The fish were attracted and would ingest the dead clams. It is assumed that the metacercariae were still alive in many of these and that infection of the definitive host could take place.

Other writers have observed second intermediate hosts which, according to the habits of the definitive host, seemed unlikely to be ingested by them. Thiry (1859) concluded that the encysted larvae he observed in small, lymnaeid snails were those of a frog bladder fluke and the encysted stage of Cercaria macrocerca. Sinitsin (1905) stated that it was absurd to think that the encysted larvae observed by Thiry were those of a frog bladder fluke, since it was inconceivable that the host, Lymnaea, would be used as food by frogs. Krull (1935) stated that both terrestrial and aquatic snails are rather commonly encountered in the digestive systems of frogs. He described the life cycle of

Gorgodera amplicava in which the second intermediate host is a snail and the definitive host is a frog.

LIFE CYCLE STAGES

Miracidium

Description

Miracidium, 67 to 70 μ long by 33 to 45 μ wide, usually pyriform in shape (Fig. 7). Surface of miracidium, except for extreme ends and interepidermal plate areas, covered with vibratile cilia, about 6 to 7 μ long, each with a distinct basal granule. Backward sweep of cilia from posterior epidermal cells project beyond posterior end of miracidium, and other cilia obscure interepidermal plate areas. Epidermal plates arranged in three transverse rows with total of 16 plates (Fig. 8); six plates each in anterior and middle rows, and four plates in posterior row. Epidermal plates in anterior row, each approximately 25 μ long by 16 μ wide, roughly triangular in shape; those in middle row, each approximately 21 μ long by 16 μ wide, generally rectangular with acute corners; and those in posterior row, each approximately 19 μ long by 12 μ wide, triangular with rounded apexes. Interepidermal plate areas 2 to 3 μ in width.

Irregular internal cavity contained following structures (Fig. 7). Median sac-like gut, 26 μ long by 14 μ wide, opened anteriorly and apically. Gut filled with coarse particles which did not stain with neutral red. Pair of large, lateral, gourd-shaped glands, 39 and 35 μ long by 14 and 11 μ wide, respectively, opened on either side of gut opening. Glands filled with fine particles and stained dark red. Four

very granular cells, each 4 μ in diameter, posterior to gut and between posterior ends of paired glands. Group of small cells with large nuclei, probably representing germinal elements, occupied posterior portion of cavity. Clear vesicles, 2 to 7 μ in diameter, and opaque particles, less than 1 μ in diameter, distributed throughout cavity. No nervous system observed, although light area, only occasionally distinguished, posterior to gut may have been concentration of nervous tissue. Excretory system consisted of pair of club-shaped flame cells, one on either side, located at termination of middle-third of body. Excretory ducts followed tortuous courses anteriorly and laterally for short distance from respective flame cells, then each turned posteriorly and opened by small lateral pores on respective sides of body. Pores, between 1 and 2 μ in diameter, situated each at one of posterior corners of median epidermal plate of middle row.

Discussion

The non-operculate eggs, 35 to 46 μ by 26 to 40 μ, in the metraterm of the uterus contained fully developed, motile miracidia. When a sexually mature fluke was placed in water, eggs escaped and hatched in 10 to 30 seconds. On the basis of most reports, eggs of species in the Gorgoderinae usually contain mature miracidia before they are eliminated and usually hatch immediately in water, although Crawford (1940) reported a two day interval between egg deposition and hatching for a Phyllodistomum sp.

The miracidium was usually pyriform in shape, but by contractions and extensions it could be distorted through a wide range of shapes. These distortions were most pronounced when the miracidium was mounted in egg albumen or methyl cellulose. No definite apical papillae were

observed, although at times a protrusion, which was extended and retracted when the miracidium was active, was observed at the anterior tip of the body.

The miracidium of Phyllodistomum lohrenzi had many anatomical similarities with the miracidia of other species in the Gorgoderinae. Goodchild (1943) also reported 16 epidermal plates arranged in three transverse rows for the miracidium of P. solidum. In many respects, especially in the glandular and cellular details of the internal cavity, the miracidium of P. lohrenzi resembled the miracidium of Gorgodera amplicava. Goodchild (1948) suggested that in G. amplicava the median gut was adhesive in function and that the lateral glands were penetration glands. Germinal elements, similar to those described, were reported by Rankin (1939) and Goodchild (1943, 1948) for other species. The miracidial excretory system of P. lohrenzi appeared to be no different from other species in the Gorgoderinae.

Mother Sporocyst

Description

Mother sporocyst 24 days old, tubular with rounded ends. Anterior end embedded in clam gill tissue. Sporocyst wall 7 to 14 μ thick; consisted of admixture of cells of three types: large cells, 6 to 10 μ in diameter, with fine granular cytoplasm and large, clear nuclei, 3 to 4 μ in diameter; uniformly spherical cells, 5 μ in diameter, with hyaline cytoplasm and finely granular nuclei, 1 to 2 μ in diameter; and large, irregular cells, 8 to 11 μ in diameter, with coarse, granular cytoplasm and clear nuclei, 4 μ in diameter, these cells interspersed at regular intervals in wall. Clear vesicles conspicuous in wall. Flame cells in wall large, semilunar-shaped, and plainly visible.

Mother sporocysts six days old measured 93 to 98 μ long by 55 to 62 μ wide and were sac-like structures; in 12 days they measured 193 to 219 μ by 73 to 81 u and were somewhat tubular with rounded ends; in 24 days they had increased mainly in size without changing shape and measured 416 to 748 μ by 104 to 197 μ. Germ masses developed in germinal membrane, detached and formed daughter sporocysts in mother sporocyst. Mother sporocysts contained an average of eight daughter sporocysts which ranged in size from 45 to 65 μ long by 29 to 45 μ wide, body wall 4 μ thick, and contained masses of cells which appeared germinal in nature.

Discussion

The miracidia were carried into the clam through the incurrent siphon. They lodged between the gill lamellae and penetrated the gill tissue. The ciliated epidermal plates were sloughed which exposed the subepidermal layer of the miracidium which became the wall of the mother sporocyst. Whether epidermal plates of the miracidium were sloughed as a continuous layer or as individual units was not determined. The miracidium appeared to be firmly embedded in the gill tissue in approximately an hour after exposure.

Goodchild (1948) stated that after the miracidium of Gorgodera amplicava penetrated the clam gill tissue, "the ciliated epithelium begins to lift off the subepithelium as a continuous layer, indicating that the larva possesses a strong interepithelial plate cement which binds the outer cells together." Rankin (1939), however, stated that in the miracidium of Gorgoderina attenuata, "the clear, nonciliated areas between the epidermal plates are the exposed parts of the subepithelium over which lie the plates." Since the subepithelium becomes the wall of

the mother sporocyst, the writer assumed that the epidermal plates of the miracidium of Gorgoderina attenuata were sloughed as individual units.

A gradual flame cell change was observed in the development of the sporocyst in which the small club-shaped miracidal-type flame cell of the early mother sporocyst developed into the large semilunar-shaped flame cell of the mature mother sporocyst. A similar change was reported by Krull (1935) for the daughter sporocyst of Gorgodera amplicava and by Goodchild (1948) for both the mother and daughter sporocysts of G. amplicava. Goodchild reported that the semilunar-shaped flame cells are not limited to larval stages; he observed them in the adult of G. amplicava.

It is stated in the writer's description that large, irregular cells with coarse, granular cytoplasm and clear nuclei were found interspersed at regular intervals in the wall of the mother sporocyst. Goodchild (1948) found similar cells in the mother sporocyst of G. amplicava but he reported only three such cells for the entire sporocyst.

Daughter Sporocyst

Description

Fully developed daughter sporocyst, 1.34 to 2.86 mm. long by 0.21 to 0.44 mm. wide, tubular, ranging from slender to plump; anterior, constricted end embedded in clam gill tissue, posterior end free and rounded. Actual presence and position of birth pore as distinct structure not determined.

Sporocyst wall, 10 to 55 μ thick except at posterior end where maximum thickness of 80 μ was recorded, irregular and externally

roughened. External surface of wall covered with a thin, 3 μ, cuticula. Delicate muscle fibers located in peripheral region of wall. Cells in wall consisted of admixture of two types: more numerous, small spherical cells, 6 to 9 μ in diameter, with hyaline cytoplasm and highly refractive nuclei, 4 to 5 μ in diameter; and large irregular to subrectangular cells, 11 to 18 μ in diameter, with granular cytoplasm and clear nuclei, 7 to 10 μ in diameter. Many opaque granules, less than 1 μ in diameter and clear vesicles, 4 to 11 μ in diameter, conspicuous in wall. Large semilunar-shaped flame cells, to 41 μ in width, evident in wall. Sporocysts contained a number of germ balls, cercariae in various stages of development, and metacercariae in cysts. A sporocyst usually contained 5 to 6 cercariae, although as many as 11 were observed, and 3 or 4 encysted metacercariae, but number, when present, varied from 1 to 6 (Figs. 4 and 5). Encysted metacercariae and bodies of cercariae concentrated at ends of sporocyst and long, coiled cercarial tails filled central portion.

Discussion

The daughter sporocysts were localized in both pairs of gills and concentrated in large numbers between the inner and outer gill lamellae. The anterior ends of the sporocysts, which were imbedded in the gill tissue, probably served as holdfast organs, and the posterior ends were free in the interlamellar gill spaces. Only the exceptionally large daughter sporocysts that were attached near the upper edge of the gills extended into the mantle cavity. Rankin (1939) stated that all daughter sporocysts of Gorgoderina attenuata projected into the mantle cavity. The main body of a sporocyst was not active except for an occasional change caused by the activity of the cercariae within. Some

extension and contraction movements, however, were observed in the embedded, constricted anterior end. It is assumed that these movements served to increase the "holdfast" action of the sporocyst as it matured, by causing the anterior end to become more deeply embedded in the gill tissue.

Cellular detail of the sporocyst wall was similar to that reported by Goodchild (1943) for the daughter sporocyst of Phyllodistomum solidum. A similar change of the small club-shaped miracidial-type flame cell into the large semilunar-shaped flame cell, as previously stated for the mother sporocyst, was observed in the daughter sporocyst. Semilunar flame cells of the daughter sporocyst of species in the Gorgoderinae were reported by Thiry (1859), Looss (1894), Sinitsin (1905), Krull (1935), Vickers (1940), and Goodchild (1943, 1948).

Cercariae escaped from the daughter sporocyst into the epibranchial chamber of the gill and reached the outside through the clam's excurrent siphon. A birth pore was thought to be located terminally on the constricted end. Cercariae could be forced, by pressure, through this end of the sporocyst; the pore was, however, indescernible as a distinct structure. Goodchild (1943) recorded the birth pore of the daughter sporocyst of Phyllodistomum solidum to be located on the anterior, evertible end; Groves (1945), however, maintained that the pore was posterior. Krull (1935) and Goodchild (1948) stated that the birth pore was located anteriorly in the daughter sporocyst of Gorgodera amplicava; Rankin (1939) observed a similar position for the birth pore of the daughter sporocyst of Gorgoderina attenuata. Steelman (1938c), however, reported a posterior position for the birth pore in the daughter sporocyst that produced Cercaria raiacauda. A birth pore was not

observed by Reuss (1902, 1903) in the sporocyst that produced Cercaria duplicata. He stated that cercariae emerged from the pointed end of the sporocyst through a rupture of the wall which closed after the cercariae had escaped. Vickers (1940) did not observe a birth pore in the sporocyst that produced Cercaria macrocerca.

Cercaria

Description

Macrocercous cercaria, 0.84 to 1.06 mm. long, differentiated into three distinct parts (Fig. 12). Anterior part, one-sixth to one-eighth total length of tail, 130 to 150 μ long by 110 to 150 μ wide, bell-shaped and containing cercarial body, with narrow apical opening surrounded by a conical protuberance (Fig. 9). Middle part, 260 to 310 μ long by 140 to 200 μ wide, widest at middle and tapering anteriorly and posteriorly into anterior and terminal parts, with mass of attachment tissue for cercarial body, 6 μ long by 32 μ wide. Terminal part, 450 to 600 μ long by 30 to 90 μ wide, tapering posteriorly and terminating in an adhesive area, 49 μ long by 12 μ wide. Two types of cells in tail (Fig. 12): large, irregular cells with distinct nuclei fill middle part and smaller cells with branching protoplasmic strands scattered in terminal part. Longtitudinal muscle fibers, organized in four strands, originate in posterior area of middle part of tail and extend to posterior third of terminal part at which level fibers separate and form uniform layer that extends to end of tail. Cuticula of anterior part of tail in two layers, outer layer continuing over middle and terminal parts; cuticula of terminal part with series of concentric striae at both anterior and middle levels.

Cercarial body (Fig. 15) fusiform, 290 to 510 μ long by 70 to 130 μ wide, capable of great extension and contraction. Anterior part elongate, subcyclindrical; posterior part flattened, leaf-like. Surface of body covered with minute spines. Cuticula 2 μ thick, marked by longtitudinal and concentric striae. Sensory papillae distributed as follows: paired lateral rows of 15 each; paired dorso-lateral and paired ventro-lateral rows of eight each; 20 on oral sucker, 16 of which are around opening arranged in two concentric rings of eight each, a pair ventral at level of posterior end of stylet, and a pair ventro-medial at base of anterior third of oral sucker; six bilobed papillae in a ring around opening of acetabulum; four simple ones within cavity of acetabulum. No sensory hairs on suckers and no spines in cavities of suckers.

Stylet (Figs. 10 and 11), 13 to 15 μ long, fragile and moveable. Oral sucker subterminal, 51 to 67 μ long by 40 to 57 μ wide; acetabulum posterior to mid-body, 60 to 79 μ in diameter; ratio of oral sucker to acetabulum 1 to 1.3. Oral aperture 14 to 29 μ by 27 to 41 μ; acetabular aperture 26 to 50 μ by 37 to 53μ. Pharynx absent. Esophagus, thick-walled with small lumen, straight, or undulating when body contracted; bifurcated midway between oral sucker and acetabulum to form ceca, narrow and thin-walled, which extend posteriorly and laterally to two-thirds distance from acetabulum to posterior end. Cerebral ganglia paired at level of mid-esophagus; supraesophageal commissure visible. Unicellular, so-called penetration glands, four pairs, located in area at junction of esophagus and ceca but dorsal to them. Gland cell ducts have undulating course, curve around oral sucker, open near tip of

stylet. Genital primordia form median mass immediately posterior to acetabulum with thread of cells continuing dorsally to its anterior border.

Excretory pore median, slightly dorsal. Excretory bladder median, composed of two parts (Fig. 15): anterior one elongate, subcylindrical, thin-walled, surrounded by elongate, granular cystogenous cells whose long axes are directed radially and slightly anteriorly from long axis of bladder; posterior part short, oval, thick-walled. Bladder extends to near acetabulum; paired common collecting tubules emerge from bladder slightly posterior to its anterior termination. Each extends antero-laterally on its respective side to level of posterior third of acetabulum, each bifurcates to form main collecting tubules, each following a tortuous course, one anteriorly to level of brain and other posteriorly to posterior end of body. A total of 18 flame cells on either side: two pairs postero-lateral to oral sucker, three pairs anterior to junction esophagus and ceca, two pairs posterior to this junction but anterior to acetabulum, two pairs dorso-lateral to acetabulum, nine pairs posterior to acetabulum (five pairs lateral, two pairs submedian between acetabulum and bladder, two pairs dorsal to bladder).

Discussion

It was proved by extensive anatomical studies, infection experiments, and field observations that Cercaria coelocerca Steelman, 1939, from Boomer Creek near Stillwater, Oklahoma, was the cercaria of Phyllodistomum lohrenzi. The description of C. coelocerca reported by Steelman (1939) was extensive, therefore only the essentials of the

original description, together with the anatomy and measurements of additional detail have been presented.

It has been reported by most writers that cercariae of gorgoderid trematodes emerge from infected clams at periods which usually show no constant regularity or fixed relationship to time of day; periodicity of cercarial emergence has been reported by only a few writers. Baker (1943) reported that Cercaria steelmani escaped, in only one of 180 infected clams observed, just before midnight on seven successive days. Coil (1953) reported that C. eriensis emerged from clams between four and nine o'clock in the morning. The cercaria of Phyllodistomum lohrenzi emerged periodically from eight p.m. to midnight, in numbers ranging from 17 to 86. The number of cercariae shed depended on the size of the infection.

The cercarial body usually remained relatively inactive within its tail chamber for approximately six hours after shedding. The cercarial bodies in older specimens and those, of all ages, under pressure were more active and in many instances they freed themselves from the tail chamber; the tail remained sluggishly active in some cases for as long as an hour. The freed cercarial bodies crawled on the bottom of the container by leech-like use of the suckers. In some cases the cercarial body freed itself from its tail chamber but remained attached by a slender stipe, and died in this condition.

The writer's observations concerning the common collecting tubules of the cercarial excretory system were not in accordance with Steelman (1939). He stated: "A pair of much-convoluted lateral collecting tubules extends anteriad from front end of bladder to near level of brain before branching." It is thought that Steelman may have confused

the anterior, main collecting tubules with the common collecting tubules. The writer determined that the common collecting tubules extended only to the level of the posterior third of the acetabulum before branching.

The cercarial body cuticula was marked by longitudinal and concentric striae; the depth and number of the striae depended on the state of contraction of the cercarial body. The cuticula in the anterior and mid-section of the terminal part of the tail was wrinkled, forming a series of parallel rings, that varied in width. It was observed that these cuticular rings served as "joints" for tail motion.

Metacercaria

Description

Shape of body and position and distribution of sensory papillae as in cercaria. Longitudinal and transverse cuticular striae prominent. Two day old metacercaria 420 to 530 μ long by 90 to 140 μ wide; oral sucker 57 to 69 μ long by 49 to 62 μ wide; acetabulum 64 to 81 μ in diameter. Thirty day old metacercaria 570 to 660 μ long by 140 to 160 μ wide; oral sucker 71 to 76 μ long by 63 to 67 μ wide; acetabulum 66 to 76 μ long by 84 to 90 μ wide. Ratio of oral sucker to acetabulum 1 to 1.22 in two day metacercaria and 1 to 1.13 in 30 day metacercaria. Stylet absent, but stylet cavity prominent in oral sucker. Remnants of penetration glands and ducts usually present. Digestive and nervous systems as in cercaria. Excretory bladder (Fig. 13) club-shaped to sac-like; distended in fully developed metacercaria. Anterior part of bladder large, containing numerous rosettes of fine, refractile granules; posterior part as in cercaria. Size of both bladder and granules increased with age; two day metacercarial bladder 65 to 71 μ long by

22 to 28 μ wide, granules 3 μ in diameter; 30 day bladder 93 to 108 μ long by 46 to 74 μ wide, granules 4 to 6 μ in diameter. Cystogenous glands, surrounding bladder, much reduced and in various stages of disintegration. Excretory pore median, slightly dorsal. Primordia of genital organs somewhat more developed than in cercaria; anteriorly directed thread of cells, described for cercaria, extends more anteriorly, to bifurcation of ceca, and posterior mass more extensive and subdivided into three or four parts.

Discussion

The description of the metacercaria is based on specimens dissected from laboratory infected caddisfly larvae. Metacercariae from daughter sporocysts in clams were not used for descriptive purposes since their age could not be determined. No anatomical differences, however, were observed in the metacercariae from the two sources.

Metacercarial cysts, dissected from caddisfly larva, were spherical to oval (Fig. 13). The cyst wall consisted of two parts: an inner transparent, hyaline part of metacercarial origin, and an outer granular part, of variable thickness, probably of host origin. The growth of the metacercarial cyst was slow and not extensive. Cysts two days old averaged 140 by 170 μ; four days, 160 by 180 μ; 10 days, 180 μ; 20 days, 210 μ; 30 days, 220 μ in diameter. Cyst wall thickness (inner and outer parts, respectively) were: two days, 2 and 1 μ; 4 days, 3 and 1 μ; 10 days, 5 and 2 μ; 20 days, 6 and 8 μ; 30 days, 6 and 14 μ. The thickness of the wall in 30 day old cysts was not uniform; it varied from 13 to 22 μ. Metacercarial cysts from daughter sporocysts were oval, 150 to 180 μ by 170 to 210 μ. The cyst wall, 3 to 8 μ thick, was delicate, transparent, and hyaline.

Adult

Description

Fixed specimen (Fig. 14) 2.4 to 4.1 mm. long by 0.71 to 2.36 mm. wide; attenuated, anterior part, 0.96 to 1.72 mm. long by 0.71 to 1.10 mm. wide, sharply set off from broader, leaf-like posterior part, 1.42 to 2.41 mm. long by 1.40 to 2.36 mm. wide. Body margin either smooth or corrugated, with 5 to 8 shallow to deep postacetabular, peripheral indentations on each side. Oral sucker ventro-terminal, 400 to 600 μ long by 370 to 570 μ wide; acetabulum slightly smaller, 310 to 510 μ long by 360 to 540 μ wide, situated 440 to 840 μ posterior to oral sucker. Ratio of oral sucker to acetabulum 1.13 to 1.0. Pharynx absent. Esophagus slender, with comparatively thick walls, variable in length, straight or with a short transverse fold. Ceca thin-walled, voluminous, 50 to 90 μ wide, extend slightly beyond posterior testis. Genital pore ventral, median, midway between acetabulum and junction of ceca. Prostate gland and seminal vesicle well developed, latter 170 to 250 μ long by 160 to 200 μ wide. Cirrus absent. Testes large, anterior 420 to 610 μ long by 310 to 570 μ wide with 5 to 7 lobes; posterior 490 to 700 μ long by 470 to 640 μ wide with 6 to 9 lobes. Anterior testis opposite ovary and may alternate with it, left or right position equally represented, posterior testis back of ovary. Ovary, 220 to 420 μ long by 200 to 290 μ wide, either entire or with 1 to 4 shallow lobes. Vitellaria paired, oblong, entire or slightly lobed, at level between acetabulum and ovary, right vitellaria 100 to 140 μ long by 150 to 310 μ wide, left vitellaria 90 to 170 μ long by 140 to 280 μ wide. Mehlis' gland between vitellaria, surrounding oötype. Laurer's canal not observed. Uterine coils extensive, mainly postacetabular and intercecal.

Eggs, 35 to 46 μ by 26 to 40 μ, oval, non-operculate, embryonated in metraterm. Excretory pore slightly dorsal to posterior extremity of body. Bladder composed of two parts in tandem; anterior part elongate, thin-walled, subcylindrical, dilated posteriorly; posterior part short, wall muscular. Bladder extends anteriorly to level of ovary. Paired common collecting tubules arise from anterior end of bladder, diverge in arch-like course, extend anteriorly to posterior level of acetabulum where each bifurcates to form anterior and posterior main collecting tubules; anterior ones extend to level of oral sucker, posterior ones to near posterior end of body.

Phyllodistomum Braun, 1899 Generic diagnosis: Body usually spatulate; cuticula with or without spines. Acetabulum usually larger than oral sucker; ratio variable. Pharynx absent. Excretory bladder sac-like to Y-shaped, elongate, its pore usually subterminal and dorsal. Genital atrium usually present; genital pore ventral, between acetabulum and junction of ceca, varying slightly in position. Testes two, smooth to slightly or deeply lobed, usually oblique, one on ovarian side more posterior. Anterior testis may be anterior to ovary. Vas deferens long, seminal vesicle conspicuous, prostate gland and ejaculatory duct generally short and inconspicuous. Cirrus absent. Ovary entire or slightly lobed, usually posterior or lateral to vitellaria, rarely anterior. Oviduct relatively long, arising from dorsal surface of ovary. Ootype usually evident. Laurer's canal usually paralleling vitelline duct on opposite side of ovary and generally opening to exterior. Mehlis' gland present but indistinct. Uterine coils extensive, mainly postacetabular. Metraterm large and distinct. Vitellaria entire or lobed; common vitelline duct very short. Eggs in metraterm embryonated.

Discussion

Phyllodistomum lohrenzi (Loewen, 1935) from the urinary bladder of the green sunfish, Lepomis cyanellus, was originally described as Catoptroides lohrenzi Loewen, 1935. Bhalerao (1937) concluded that, in view of the confusion resulting from inadequate descriptions and the morphological variability within species, the genera Catoptroides and Phyllodistomum were synonymous. He revised the generic description of Phyllodistomum to include Catoptroides.

Loewen's original description was brief; the writer, therefore, has summarized and expanded it to include additional observations.

Lewis (1935) stated: "Generic characters, to be valid, must pertain to important morphological characters, well delimited and without gradational variations." Gradational variations, however, do exist with change in age, size, and state of contraction or relaxation. These gradational variations as interpreted by different writers have confused the taxonomic status of the phyllodistomes. Goodchild (1943) stated: "The chaotic taxonomic status of the sexually mature phyllodistomes is due to synonymy and homonymy. Anatomical features do not all remain constant during the maturation of members of the same species." Vernard (1940) made a study of Phyllodistomum lohrenzi from the green sunfish and bass and stated, "differences were sufficient to make the specimens appear quite distinct from P. lohrenzi until a series of intergrading specimens from Ohio green sunfish and smallmouthed bass were examined." Variations or differences due to age, size, and state of contraction or relaxation are evident only when extremes are compared but become almost nonexistent when a series of intergrading specimens in a species is examined.

A study of measurements, including those given by Loewen (1935), Vernard (1940), Crowell (1949), and the writer, indicates that there is considerable variation in the size of mature specimens of Phyllodistomum lohrenzi. There is, however, as reported by Vernard (1940) little variation in the ratio between the total size of a fluke and the size of its internal organs. Coil (1955) stated:

> Differences in size of distomes may be due to the genotype, the developmental history, the physiological environment (crowding, host differences, and nutritional condition of the host) and age.

Vernard (1940) reported that the relative state of relaxation and contraction at fixation greatly influences the morphology of P. lohrenzi. The writer has observed that relaxed specimens had a corrugated body margin, the postacetabular, peripheral indentations were shallow, the esophagus was straight or bent in a short transverse loop, the testes were lobed, and the ovary and vitellaria were smooth or slightly lobed. By way of contrast, contracted specimens had a smooth body margin, the postacetabular, peripheral indentations were deep, the esophagus was S-shaped and in some forms showed a pseudo-pharynx, the testes were deeply lobed, and the ovary and vitellaria were lobed.

Crowell (1949) reported for P. lohrenzi:

> While Loewen (1935) reported a dull, reddish-brown color and Vernard (1940) reported a light yellow or cream color, examination of living worms showed all gradations from creamy white in the smallest and most immature specimens, to a deep reddish-brown in the largest and therefore probably oldest specimens.

Another variation correlated with age is the deeper indentations in the ovary and testes of older flukes as compared with younger specimens.

Crowell (1949) suggested a possible relationship between senescence and degeneration of testes. He observed two monorchid specimens of

Phyllodistomum lohrenzi; the anterior testis was absent from two large specimens, and in another large specimen the anterior testis was decidedly atrophied. Vernard (1940) mentioned a possible degeneration of the gonads; he gave no further explanation and did not state whether testes, ovaries or both were involved. The writer found no indications of degeneration of any of the sex organs. Crowell and Vernard gave no age or measurements for the specimens that showed gonad degeneration and it is possible that they studied older specimens.

Hosts reported for Phyllodistomum lohrenzi are: the green sunfish, Lepomis cyanellus, by Loewen (1935) from the Cottonwood River, Marion, Kansas; the smallmouth bass, Micropterus dolomieu, and the largemouth bass, M. salomoides, by Vernard (1940) from Reelfoot Lake, Tennessee; the pumpkinseed, Lepomis gibbosus, and the orangespotted sunfish, L. humilis, by Crowell (1949) from the Maumee River, Grand Rapids, Ohio. The green sunfish was reported as a host from all areas of distribution given above, and the distribution was extended by Vernard (1940) to include the Olentangy and Scioto Rivers, Columbus, Ohio and by the writer to include Boomer Creek, Stillwater, Oklahoma.

The habitat of P. lohrenzi in the definitive host is both the mesonephric tubules and urinary bladder, but there is an age distribution as determined by life cycle experiments. Immature specimens up to 10 days old were found in the mesonephric tubules and bladder, older specimens usually in the bladder. The incidence and degree of infection have been presented (Tables VII, VIII, and IX).

SUMMARY AND CONCLUSIONS

The systematic position of the trematodes in the subfamily Gorgoderinae Looss, 1902 is discussed. New information on the life cycles, including larval stages, has caused present day helminthologists to believe that many of the genera and species have been established on invalid characters. The present tendency is to reduce many of them to synonymy. Indications are that the family is unnatural and will have to be revised when more life history studies have been completed.

Two new methods for rearing parasite free and laboratory infected clams, Musculium transversum, have been described.

The life history of Phyllodistomum lohrenzi (Loewen, 1935), a gorgoderid trematode from the urinary bladder of the green sunfish, Lepomis cyanellus Raf., has been completed under laboratory conditions.

In the life cycle, the miracidium enters the first intermediate host, Musculium transversum (Say), passively through the incurrent siphon, penetrates a gill, and transforms into a mother sporocyst. Each mother sporocyst produces a single generation of daughter sporocysts. Each daughter sporocyst produces numerous macrocercous cercariae. The cercaria has previously been described as Cercaria coelocerca Steelman, 1939. From the time of miracidial entrance into the clam to cercarial production, under ordinary summer conditions, requires 6 to 8 weeks in the laboratory. The cercaria infects the second intermediate host by one of two methods. (1) In this type of

infection, there is a suppression of the free cercarial stage; the cercaria sheds its tail and encysts within the daughter sporocyst. The clam, therefore, serves as both first and second intermediate hosts. (2) In this type of infection, the cercaria escapes from the daughter sporocyst and emerges from the clam through the excurrent siphon. The vigorous cercarial tail movements serve to attract caddisfly larvae, which ingest them. Oecetis cinerascens, O. inconspicua, and Leptocella sp. have been determined to be capable of serving as second intermediate hosts. Metacercariae usually encyst either in the abdominal or thoracic body cavities of the caddisfly larvae, and only occasionally in the head. The metacercaria is infective to the definitive host after two days in the caddisfly larva, but whether younger ones are infective was not determined.

The green sunfish becomes infected by ingesting either infected clams or infected caddisfly larvae. Excystment of the metacercaria takes place in the stomach or anterior part of the small intestine of the fish. The immature fluke passes along the intestine, and, in all probability, migrates externally from the anus into the urogenital opening. They usually migrate from here to the mesonephric tubules, via the urinary bladder, where they remain for approximately 10 days before returning to the bladder to complete their growth. Immature flukes may be recovered from the urinary bladder and mesonephric tubules as soon as 24 hours after the ingestion of metacercariae. Under ordinary summer conditions in the laboratory, 5 to 7 weeks are required for the fluke to attain sexual maturity in the definitive host. The mature fluke produces eggs which are extruded by the fluke, eliminated from the host, and hatch immediately in water.

All stages in the life cycle have been studied, described, and illustrated.

Each mother sporocyst produces a single generation of daughter sporocysts which average eight in number. The daughter sporocyst contains a number of germ balls, cercariae in various stages of development, and metacercariae in cysts. A sporocyst usually contains five or six cercariae, although as many as 11 were observed, and three or four encysted metacercariae, but the number, when present, varied from 1 to 6.

No anatomical differences were observed in the metacercarial body in those dissected from caddisfly larvae as compared with those from daughter sporocysts in clams, but the cysts were different. There is little development in the transformation from cercaria to metacercaria, but there is an increase in size.

A discussion of variations in the anatomy of sexually mature flukes is presented. This discussion shows that gradational variations exist with change in age, size, and state of contraction or relaxation, and these variations as interpreted by different writers have confused the taxonomic status of the phyllodistomes.

Maximum infections in the definitive host in nature compared rather well with experimental infections when sporocysts from clams were used rather than those in which infected caddisfly larvae were fed. Since the flukes in each infection were usually of a uniform size, the maximum infections demonstrate, circumstantially, that the clam is eaten by green sunfish in nature.

It was observed that fish that had more than 13 mature flukes each showed inflammation and thinning of the bladder wall. The general

effect of this pathology on the definitive host is not known. Since this fluke infects both bass and sunfish, it could have some economic importance. Small bass, especially fingerlings, would be more likely to feed on the intermediate hosts and in these the general effect of the pathology could be serious.

As in all primary investigations on a specific problem, many detailed experiments still remain to be done such as: experimental infection of other definitive hosts; seasonal variations in the cycle; detailed investigation of the pathology, particularly on very young fish, and its economic importance; the determination of the reason for some cercariae encysting within the sporocyst and others being freed; and taxonomic problems.

EXPLANATION OF PLATE

All figures, except 10 and 11, were made with the aid of a camera lucida with minor details in freehand.

Figure 7. Miracidium showing cilia and detailed internal anatomy.

Figure 8. Miracidium showing arrangement of epidermal plates.

Figure 9. Anterior part of cercarial tail showing cercarial chamber and cercarial body.

Figure 10. Stylet, ventral view.

Figure 11. Stylet, lateral view.

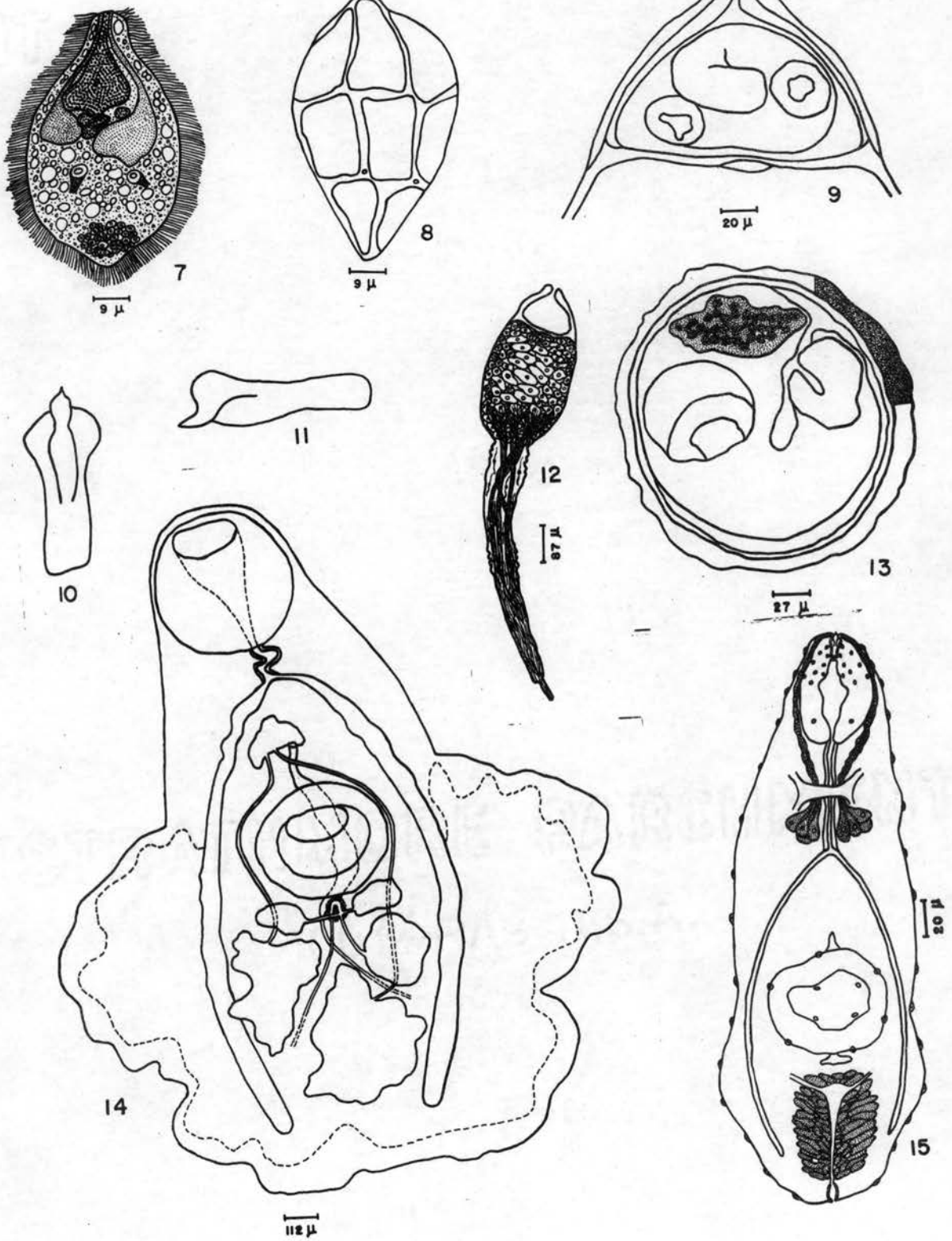
Figure 12. Cercarial tail showing parts and cellular detail.

Figure 13. Encysted metacercaria, 30 days old, showing concretions in bladder and a section of cyst wall in detail.

Figure 14. Ventral view of 45 day old fluke showing details of internal anatomy. Marginal dashed lines show extent of uterine coils.

Figure 15. Ventral view of cercarial body showing sensory papillae and details of internal anatomy.

PLATE



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VITA

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